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STATEMENT OF EDUCATIONAL NEED, TARGET AUDIENCE, AND LEARNING OBJECTIVES

With the accelerating pace of discoveries in the basic, translational and clinical sciences, due in large part to the advent of new technologies and also
our increased understanding of the interplay between the immune system and cancer, cancer researchers are making rapid progress that is having
significant patient benefit. By bridging the gap between what physicians understand about cancer biology and the clinical applications, this meeting
aids basic researchers, physicians, and clinician-scientists in obtaining, synthesizing, and integrating the most cutting-edge research. This exposure is
essential for the implementation of best practices, such as the most current molecular-based tests to aid in the diagnosis, treatment, and prevention
of cancer. Further, facilitating the interface between physicians and scientists will increase knowledge of the epidemiological implications of cancer
incidence and the contributions of laboratory research to drug development as well as patient care; transform the design and conduct of clinical
research protocols; and create a forum for the rapid translation of laboratory research findings from “bench-to-bedside” for the benefit of improving
patient outcomes.

Despite the tremendous progress in the field, cancer continues to be an enormous public health challenge worldwide, accounting for one in every seven
deaths that occur around the world. In the United States (U.S.) alone, it is predicted that 595,650 people will die from some form of cancer in 2016, making
it the second most common cause of death after heart disease. One of the challenges we face is that cancer is comprised of more than 200 different
diseases. For many of the most commonly diagnosed cancers in the U.S.—including colorectal, lung, and prostate cancer—incidence has been declining
for more than a decade. However, incidence of other forms of cancer—including melanoma and kidney, liver, pancreatic and childhood cancer—have been
on the rise. Overall five-year relative survival rates for U.S. patients vary widely depending on the form of cancer and the stage at which it is diagnosed.
Incidence and survival rates are also impacted by the cancer health disparities that exist in certain segments of the U.S. population.

This conference will bring together investigators from the basic, translational, and clinical disciplines and provide them with a venue to discuss their
recent advances, test new hypotheses, and establish new collaborations. In order to have widespread implementation of the most current, approved
molecular-based tests to aid in the diagnosis, treatment, and prevention of cancer, it is critical to bridge the gap between what physicians understand
about cancer biology and its application to clinical oncology. As the incidence of cancer continues to increase, the fields of cancer prevention and early
interception offer unprecedented opportunities to decrease the worldwide burden of cancer. After participating in this CME activity, participants will be
able to:

1. Recognize the technological advances and tools being used to accelerate progress in cancer research and improve early detection and early
   intervention, with the ultimate goal of extending patients’ lives and improving their quality of life.
2. Articulate how advances in precision cancer medicine are leading to improved patient outcomes.
3. Incorporate the latest research findings regarding therapies and treatment options, including immunotherapy, in a variety of cancer types in
   order to improve patient outcomes.
4. Formulate new strategies that will further scientific and clinical research efforts towards the prevention and early detection of cancer.
5. Identify factors which impact the diagnosis, treatment, and prevention of various forms of cancers in patients from different populations.
6. Develop collaborations amongst physicians, researchers, and clinician-scientists to advance the cause of treating and preventing cancer.

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**EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Alternative Small-Molecule Therapies**

1. **Galectins in glioblastoma: opportunities for combined therapy.** David Cachia,1 Arindam Rano Chatterjee,1 William Alexander Vandergrift,1 Sunil J. Patel,1 Gabriel A. Rabinovich,1 Arabinda Das1,2. 1Medical University of South Carolina, Charleston, SC; 2Institute of Biology and Experimental Medicine, CONICET, Vuelta de Obligado, Argentina.

**Background:** Despite aggressive treatment, including surgical resection, radiation and chemotherapy, over 90% of glioblastoma (GB) patients experience tumor recurrence. This may be due to high migratory potential, angiogenesis, molecular heterogeneity and a strong immunosuppressive environment. GB expresses high levels of carbohydrate-binding galectin proteins and histone deacetylase (HDAC) activity. The aim of this study was: 1) identify the differential expressions of galectin 1-15 protein levels in human GB patient samples as compared to normal tissue (obtained from Institutional Tissue Bank) and 2) modulate the galectin functions with and without a HDAC inhibitor (DATS: Diallyl Triisulfide and SAHA: Suberoylanilide Hydroxamic Acid) or bevacizumab in vitro GB models.

**Methods:** The effects of TMZ, ionizing radiation, or combined chemoradiation on galectin protein secretion and expression were assessed in human GB cells and human umbilical vein endothelial cells (HUVECs). Results: We found increased galectin-1 protein expression in human GB tissue. We also observed that HUVEC co-culture with GB cells increased galectin-1 protein expression by 14-20% following bevacizumab and conferred a bevacizumab protective benefit to GB cells. Our in vitro models promisingly demonstrated that 72 hr treatments with 25 μM of galectin 1 inhibitor + HDAC inhibitor induce antitumor activity in GB cells. Western blot and activity assays also demonstrated that combination blockade of HDAC activity and galectin-1 augmented apoptosis in GB cells, which mechanistically involves activation of caspase-3 and inhibition of anti-apoptotic protein (survivin, p-Akt, and Mcl-1 expression). Conclusion: Our in vitro culture results suggest possible benefit in combining a galectin inhibitor with an HDAC inhibitor in GB. Further studies in different animal models are warranted.

2. **Functional and mechanistic interrogation of BET bromodomain degraders for the treatment of metastatic castration resistant prostate cancer.** Steven Kregel, Rohit Malik, Irfan A. Asangani, Kari Wilder-Romans, Xia Jiang, Steven Kregel, Rohit Malik, Irfan A. Asangani, Kari Wilder-Romans, Xia Jiang, Ingrid J. Apel, Gautham Ravi, Jean Tien, Xuhong Cao, Felix Y. Feng, Corey Speers, Shaomeng Wang, Arul M. Chinnainay, University of Michigan, Ann Arbor, MI.

Metastatic castration-resistant prostate cancer (mCRPC) is a lethal disease with about 30,000 estimated annual deaths in U.S. with a vast majority of CRPC driven by androgen receptor (AR) signaling. AR-signaling is critical for the development and progression of prostate cancer, and AR is also the main therapeutic clinical target. AR-targeted therapies, such as AR-antagonists, provide substantial benefits in the treatment of mCRPC; however, majority of patients fail these therapies and succumb to the disease. Therefore, there is a clear and pressing need to develop new therapeutics against the AR axis in CRPC. One such novel strategy for targeting the AR-pathway and inhibiting the growth of CRPC has been the use of bromodomain and extramemrinal (BET) protein inhibitors such as JQ1. However, a new class of molecules that target BET bromodomain proteins through their proteasomal degradation can improve efficacy and specificity of these inhibitors. Based on our findings, we hypothesized that pharmacologic BET degradation represents an important advance in the treatment of CRPC, and may provide a novel therapeutic strategy for advanced prostate cancer. These inhibitors affect AR-positive prostate cancer cells preferentially over AR-negative and benign prostate epithelial cells, and proteomic and genomic mechanistic studies confirm disruption of oncogenic AR and MYC signaling, both in vivo and in vitro. The overall goal is to develop very potent small molecule that leads to the proteasomal-degradation of BET proteins, with optimized in vivo properties, and to provide a compelling scientific rationale, including detailed mechanistic insight, to facilitate advancement of BET degraders as a novel potential therapeutic strategy for patients with mCRPC.

3. **Targeting epigenetic modifiers in diffuse large B-cell lymphoma.** Aarthi Goverdhan, Heng-Huan Lee, Mien-Chie Hung. UT MD Anderson Cancer Center, Houston, TX.

**Diffuse Large B-Cell Lymphoma (DLBCL) is the most common type of Non-Hodgkin lymphoma, accounting for approximately 40% of all cases worldwide. In the last five years, researchers uncovered that DLBCL is characterized by widespread epigenetic deregulation. Lymphoma cells harbor loss-of-function mutations in transcriptional activators (KMT2D, EP300, CREBBP) and gain-of-function mutations in transcriptional repressors (EZH2). In our study, we aim to identify epigenetic vulnerabilities in lymphoma cells that can be targeted to deliver cytotoxic responses. We screened the therapeutic efficacy of a panel of lysine and arginine methyltransferase inhibitors in multiple lymphoma cell lines. Through these screens, we identified the Type I Protein Arginine Methyltransferase (PRMT) family as an attractive therapeutic target that induced cytotoxicity. Mechanistic dissection revealed that Type I PRMT inhibition caused apoptosis through deregulation of transcription factors and pro-survival genes. In addition, we uncovered novel crosstalk between PRMTs and other epigenetic modifiers that influenced cell fate in lymphoma. Our study demonstrates a critical role for Type I PRMTs in DLBCL, and adds a lucrative target to the growing list of promising epigenetics-based therapies.**


**Background:** Aprotinin is a non-specific serine protease inhibitor, which can inhibit kallikrein and many downstream extracellular matrix degrading enzymes. In this study, we assessed inhibitory effects of aprotinin in local invasion of human breast cancer cell lines. Methods: We assessed effects of aprotinin on local invasion and survival of human breast cancer cell lines MDA-MB231 and SK-BR-3 in vitro. CHEMICOM Cell Invasion Assay Kit was used to assess local invasion of tumoral cells. MTT assay was used to determine the anti-proliferative activity of aprotinin on different cancer cell lines. Mean optical densities of duplicate experiments were calculated for both assays. HD-F1 human fibroblast cell line was used as control normal cell. In addition, local invasion and survival of tumor cell lines treated with aprotinin were compared with untreated experiments. Results: Cancer cell lines showed more invasion compared to HD-F1. Aprotinin significantly decreased the invasiveness of MDA-MB231 in concentration 1 IU/ml, 1.3 IU/ml, and 1.7 IU/ml in comparison with untreated group (ANOVA P < 0.001). Treatment of SK-BR-3 with 1.3 IU/ml aprotinin trended towards reduction of invasion optical density (P = 0.06). Treatment with different concentrations of aprotinin significantly decreased the surviving fraction and significantly inhibited the growth of all cell lines tested in this study (ANOVA P < 0.001). Conclusion: Aprotinin inhibited the survival and local invasion of human breast cancer cell lines MDA-MB231 and SK-BR-3. Although the growth inhibitory effect was significant in both cell lines, inhibition of local invasion was more pronounced in case of MDA-MB231. Future molecular studies could shed further lights on mechanisms underlying anti-neoplastic effects of aprotinin and its potential therapeutic effects.

5. **The effects of SIRT1 inhibitors nicotinamide and Ex-527 on lymphoma cells.** Manuela Guardi, Elisa Bonfante, Raffaele Frazzi. Arcispedale S.Maria Nuova - IBCS, Reggio Emilia, Italy.

SIRT1 is a well-known lysine-deacetylase, having both histone and non-histone proteins as molecular targets. SIRT1 may function as a tumor promoter as well as a tumor suppressor in a context-dependent manner. We previously demonstrated that the treatment with resveratrol at concentrations able to induce apoptosis leads to SIRT1 down-regulation in lymphoma cells. Here we look for SIRT1 inhibition in order to investigate the effects on proliferation potential, apoptosis induction and targets modulation at a molecular level. The aim is to study whether SIRT1 inhibition affects cell cycle and proliferation capability of lymphoma cells. Experimental procedures. Diffuse large-B cell lymphoma derived cell line Toledo; human lymphoblastoid cell line GGB#7; SIRT1 inhibitors: nicotinamide (NAM) and Ex-527; cell cycle analysis; caspase-3 activity; fluorescence assay; genotoxicity assay gamma-H2AX; fluorescence-activated cell sorting intracellular staining for acetylated-H4K16 histone. New data. We found that the calculated IC50s for SIRT1 inhibition is 50mM for NAM and 343 M for Ex-527. The treatment with either NAM or Ex-527 leads to cell cycle arrest in both Toledo and GGB#7 cells, although in a different fashion. Namely, NAM causes an S-phase accumulation of Toledo while it leads GGB#7 to G0/G1 phase arrest with a concomitant decrease in S-phase already after 10mM. In both cell lines NAM 50mM induces apoptosis as demonstrated by caspase-3 activation and sub-G1 peak appearance in the cell cycle profiles. Ex-527 causes an accumulation in the S-phase of Toledo cells and a decrease of G2/M in GGB#7 cells. No induction of apoptosis at the IC50 concentration was observed in both cell lines. Genotoxicity of the above mentioned treatments was investigated through the quantitation of gamma-H2AX histone released by treated cells. No genotoxic effects have been observed upon the treatments with these two inhibitors even at the concentration where NAM induces apoptosis. In order to correlate the observed changes with SIRT1 inhibition, we studied the acetylated H4K16 histone, Proceedings of the American Association for Cancer Research • Volume 58 • April 2017
a direct target of SIRT1 deacetylation. Our preliminary data indicate that NAM treatment increases the percentage of acetylated H4K16. Conclusions. Lymphoma and lymphoblastoid cells are affected by SIRT1 inhibition. NAM and Ex-527 cause cell cycle arrest and growth inhibition. Only NAM 50 mM induces apoptosis without genotoxic effects. Overall, our data suggest the direct involvement of SIRT1 during the observed cell cycle arrest and growth inhibition mediated by these inhibitors.

**#6 New chk1 inhibitor development & the feasibility of use of chk1 inhibitor with antimitabolite for NSCLC.** Injae Chung. Duksun Women's University, Seoul, Republic of Korea.

DNA damaging anticancer agents activate checkpoints that stop the cell cycle to prevent additional DNA replication & mitosis until damaged region has been repaired. One of the important regulatory proteins activated in DNA damage response pathway is Checkpoint kinase 1 (Chk1). Therefore, Chk1 inhibitors have emerged as promising new therapeutics for cancer. In order to find novel Chk1 inhibitor, we have conducted a structure based virtual screening. Several small molecules have been shown 75 - 100 % inhibition of Chk1 in the binding affinity assay. Combined with the results of kinase assay with ATP & mechanism based cell assay, hit candidates has been led. Lung cancer is the leading cause of cancer related deaths in Korea. About 85 % of lung cancer is non-small cell lung carcinoma (NSCLC) worldwide. Chemotherapy for NSCLC is often regarded ineffective. To test the feasibility of Chk1 inhibitor to sensitize antimitobile in NSCLC, growth inhibition induced by gemcitabine plus MK8776 was assessed in A549 & H520 cells. These cells are insensitive to the MK8776 when treated as a single agent (IC50 > 10 μM). In contrast, MK8776 reduced the concentration of gemcitabine required to inhibit H1299 or A540 cell growth by almost 3-30 fold or 6-15 fold, respectively, depending on the concentration used. These results would imply the potential clinical utility of Chk1 inhibitor as an adjuvant for antimitobile such as gemcitabine for NSCLC. Further studies to evaluate the effectiveness of Chk1 inhibitor including new molecules would be warranted in additional preclinical and clinical settings. [The chemical library used in this study was kindly provided by Korea Chemical Bank & KIST. This study was supported by a grant (2015R1D1A1A01057014) from the National Research Foundation of Korea.]

**#7 Ceftriaxone, an FDA-approved cephalosporin antibiotic, suppresses lung cancer growth by targeting Aurora B.** Xiang Li1, Haitao Li1, Shengjing Li1, Feng Zhu1, Dong Joon Kim1, Hua Xie1, Yan Li1, Janos Nadas1, Naomi Oh1, Tatiana Zyanko1, Dong Hoon Yu1, Mee-Hyun Lee1, Myoung Ok Kim1, Lei Wang1, Wei Ya Ma1, Ronald A. Lubet2, Ann M. Bode1, Ziming Dong3, Zigang Shen4, Xuan Zhou1.

Ceftriaxone, an FDA-approved third-generation cephalosporin antibiotic, has antimicrobial activity against both gram-positive and gram-negative organisms. Generally, ceftriaxone is used for a variety of infections such as community-acquired pneumonia, meningitis and gonorrhea. Its primary molecular targets are the penicillin-binding proteins. However, other activities of ceftriaxone remain unknown. Herein, we report for the first time that ceftriaxone has anti-tumor activity in vitro and in vivo. Kinase profiling of hit compounds identified that Aurora B might be a potential "off" target of ceftriaxone. Pull-down assay data confirmed that ceftriaxone could bind with Aurora B in vitro and in A549 cells. Furthermore, ceftriaxone (500 μM) suppressed anchorage-independent cell growth by targeting Aurora B in A549, H520 and H1650 lung cancer cells. Importantly, in vivo xenograft animal model results showed that ceftriaxone effectively suppressed A549 and H520 lung tumour growth by inhibiting Aurora B. These data suggest the anticancer efficacy of ceftriaxone for the treatment of lung cancers through its inhibition of Aurora B.

**#8 Anandamide reduces cell migration, invasion, and induces apoptosis in an in vitro model of prostate cancer.** Domenica Roberto,1 Lawrence Klotz,2 Vasundara Venkateswaran.1 Sunnybrook Research Institute, Toronto, Ontario, Canada. 2Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada.

Introduction and Objective: Prostate cancer (PCA) is the most commonly diagnosed cancer in men and the second leading cause of cancer related death in Canada. A large body of evidence supports a possible role for cannabinoids in certain aspects of human health and disease, acting as palliative agents as well as inhibitors of cancer cell proliferation, migration, invasion, and the angiogenesis of tumours. Anandamide (AEA) is a highly potent endogenously produced compound that acts as an agonist of the cannabinoid receptors (CB1 and CB2) as well as the transient receptor potential vanilloid receptor 1. AEA is mainly metabolized by fatty acid amide hydrolase (FAAH) and overexpression of the enzyme in PCA tissues is correlated with greater invasivity, malignancy and increasing Gleason score. Majority of studies have focused on the effect of AEA treatment on cell proliferation, without much focus on prostate cancer biology. This study aims to determine the anti-cancer effect of AEA on PCA cells. We hypothesize that anandamide will reduce migration, invasion, and induce apoptosis in PCA cells. Methodology: Human PCA cells (DU145, LNCaP, and PC3) were treated with AEA at concentrations ranging from 1 μM to 100 μM and growth of cells (using the MTS assay) was assessed at various times. Wound-healing assays were conducted to investigate the migratory potential of cells following 24hr exposure to treatment, and trans-well invasion assays were performed to explore the influence of AEA on cell invasion. Flow cytometry, using the FITC Annexin V dead cell apoptosis kit, was used to detect the proportion of apoptotic cells in proliferating cells following anandamide treatment. Results: There was a significant reduction in the proliferation of DU145 cells at concentrations of 20 μM and 40 μM AEA compared to controls (p = 0.02, 0.005) LNCaP cells on the other hand required a higher concentration of AEA to achieve this effect (p=0.05). Cell migration and invasion studies on DU145 cells revealed a significant reduction in cell motility at 20 μM and 40 μM AEA (p = 0.006, 0.0005) as well as a significant reduction in cell invasion at 20 μM and 40 μM (p = 0.002, 0.0001). Flow cytometry results indicate a significant increase in the proportion of apoptotic cells compared to control (3 fold change) when treated with 20 μM and 40 μM in DU145 and LNCaP cells. Conclusion: We have shown that AEA has greatest significant influence on cell migration, invasion, and apoptosis. Further analysis on the role of AEA in these pathways is underway exploring alterations in expression levels of key proteins implicated in cell migration and apoptosis. Funding/ Conflicts of Interest: None

**#9 Suppression of the invasion and growth of human head and neck squamous cell carcinomas via regulating STAT3 signaling and miR-21/b-catenin axis with HJC0152.** Yu Wang1, Sinan Wang1, Yansheng Wu1, Jia Zhou1, Qiang Shen1, Xuan Zhou1. 1Tianjin Medical University Cancer Hospital, Tianjin, China; 2Tianjin Medical University General Hospital, Tianjin, China; 3University of Texas Medical Branch, Galveston, TX; 4The University of Texas MD Anderson Cancer Center, Houston, TX.

Signal transducer and activator of transcription 3 (STAT3) is involved in the tumor growth and metastasis of human head and neck squamous cell carcinoma (HNSCC) and is therefore a target with therapeutic potential. In this study, we show that HJC0152, a recently developed anticancer agent and a STAT3 signalng inhibitor, exhibits promising antitumor effects against HNSCC both in vitro and in vivo via inactivating STAT3 and downstream microRNA-21/b-catenin axis. HJC0152 treatment efficiently suppressed HNSCC cell proliferation, arrested the cell cycle at the G0/G1 phase, induced apoptosis, and reduced cell invasion in both SCC25 and CAL27 cell lines. Moreover, HJC0152 inhibited nuclear translocation of phosphorylated STAT3 at Tyr705 and decreased VHL/ b-catenin signaling activity via regulation of microRNA-21. Loss-of-function of VHL remarkably compromised the antitumor effect of HJC0152 in both cell lines. In our SCC25-derived orthotopic mouse models, HJC0152 treatment significantly abrogated STAT3/b-catenin expression in vivo, which leading to a global decrease of tumor growth and invasion. With its favorable aqueous solubility and oral bioavailability, HJC0152 holds the potential to be translated into the clinic as a promising therapeutic strategy for patients with HNSCC.

**#10 A small molecule derivative effectively inhibits proliferation of pancreatic cancer cells by targeting Sp1 and survivin.** Myrna Hurtado,1 Umesh Sanpalk,1 Aboubacar Kaba,2 Shahela Mahmammad,1 Alvin Holder,3 Jamboor Vishwannahanga,1 Rijay Basha1. 1University of North Texas Health Science Center, Fort Worth, TX; 2Alabama State University, Montgomery, AL; 3Old Dominion University, Norfolk, VA.

Pancreatic cancer is one of the most fatal malignancies due to its poor prognosis. With current standard treatment options, the overall 5 year survival rate is about 16% and these treatment plans often cause high toxicity. Therefore, there is an urgent need for identifying more effective and less toxic agents for the treatment of this malignancy. Tolfenamic acid (TA) is a generic drug used to treat migraine headaches but has been demonstrated to have anti-cancer activity in pre-clinical studies. It is known to downregulate the transcription factor Specificity protein 1 (Sp1). Sp1 regulates several genes involved in cell proliferation and apoptosis, including survivin, an inhibitor of apoptosis protein. Interestingly, a recent discovery proposed that copper(II) complex of TA can result in greater therapeutic response; however its efficacy was not tested in gastro-intestinal cancers. Therefore, a small molecule derivative of Tolfenamic acid containing complex of TA (Cu-TA) using human pancreatic cancer cell lines. MIA PaCa-2 and Panc-1 cells were treated with increasing concentrations of vehicle (DMSO), equimolar CuCl2 (negative control), TA or Cu-TA and the cell viability was measured at 24 and 48 h post-treatment using CellTiter-Glo kit.

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Cu-TA was further tested for its effect on Sp1 and survivin expression in MIA PACA-2 cells by Western blot (protein) and quantitative PCR (mRNA). The activation of apoptosis was determined by measuring the expression of effector caspases using the Caspase3/-7-Glo kit and the apoptotic cell population through flow cytometric analysis using Annexin-V staining. Cell cycle arrest was assessed by flow cytometry. The results of all analyses revealed that Cu-TA caused dose/time-dependent response to inhibit pancreatic cancer cell growth, Cu-TA showed higher efficacy when compared to TA. Cu-TA was highly effective in inhibiting Sp1 and survivin protein expression and showed similar trend for inducing apoptotic markers and causing cell cycle arrest in early phase (G0/G1). The results of qPCR demonstrated that the expression of survivin mRNA was significantly lower following both Cu-TA and TA treatment; however, Cu-TA mRNA expression of Sp1 remained unchanged. This indicates that TA and Cu-TA could be working in similar mechanism by effecting Sp1 post-translational, perhaps through proteasome-dependent degradation. These results demonstrate that Cu-TA is more effective than TA and potentially useful for pancreatic cancer treatment after clinical testing. Studies to precisely understand the underlying mechanisms of Cu-TA are currently under investigation through molecular profiling analysis.

#11 The oncolytic peptide LTX-315 enhances T cell clonality and induces synergy with chemotherapy. Kéti André Camilo,1 Meng Yu Wang,2 Janné Nestvold,3 Gunhild Mælandsmo,2 Baldur Sveinbjørnsson,2 Øystein Rekdal.4

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LTX-315, a novel oncolytic peptide is effective against both drug-resistant and drug-sensitive cancer cells with lower toxicity towards normal cells. Intratumoral treatment with LTX-315 results in growth inhibition, complete regression and long lasting tumor-specific immune responses. The oncolytic effect of LTX-315 involves perturbation of the plasma membrane and distortion of intracellular organelles including the mitochondria with subsequent release of Damage-Associated Molecular Pattern molecules (DAMPs) such as ATP, cytochrome c and HMGB1. LTX-315 effectively induces necrosis within the tumor followed by the release of tumor antigens as demonstrated by a greater increase in tumor infiltrating CD8+ T cells, expansion of T cell clonality, and number of clones within the tumor microenvironment. LTX-315’s ability to modify the tumor microenvironment makes it ideal as a combination partner for other cancer therapies, including chemotherapy and immune checkpoint inhibitors. In preclinical tumor models, combination of LTX-315 and chemotherapeutic agents such as cyclophosphamide and doxorubicin demonstrates significant synergy.

#12 Evaluation of metformin and clotam combination for medulloblastoma. Umesh T. Sankpal1, W Paul Bowman,2 Jeffrey C. Murray,3 Riyaz Basha1.

1Univ. of North Texas Health Science Ctr., Fort Worth, TX; 2Cook Children’s Medical Center, Fort Worth, TX.

Medulloblastoma (MB) is the most common malignant brain tumor in children. Standard treatment, which includes surgery, chemotherapy, and radiotherapy, is successful for most patients, but survivors often suffer from significant long-term side effects affecting their neurocognitive and growth potential. Therefore, there is a critical need to understand the molecular processes that regulate MB growth and find less toxic therapies. Survivin (BIRC5) was identified as a protein belonging to the Inhibitor of Apoptosis Protein family that inhibits apoptosis by inhibiting caspase activation. Survivin expression is also associated with increased resistance of tumor cells to radiation and chemotherapy. In MB, several studies suggest that increased expression of survivin serves as a marker of tumor morphology and is associated with poor prognosis. Specificity protein 1 (Sp1) is one of the transcription factors that regulates the expression of survivin gene. Sp1 is also shown to be upregulated in some cancer cells and is associated with poor prognosis. In this study, we are testing a strategy of targeting survivin in MB using anti-diabetic drug Metformin, which targets Sp1, and the NSAID Clotam (Tolifenamic acid, TA), which targets Sp1 and survivin. Dose response curves were established by treating MB cell lines DAOY and D283 with increasing concentrations of metformin or TA and measuring cell viability at 24 and 48h post-treatment using the CellTiter-Glo Cell Viability Assay. A time and dose dependent inhibition of cell proliferation was observed for both the drugs. Metformin dose response was then repeated in the presence of 25 or 50 µM TA. Our results indicate that TA significantly increased the growth inhibitory response of metformin. To further characterize this response, we determined the combination index (CI) using the Chou-Talalay method. We found that the CI values were between 0.85 to 0.61 for various combinations of metformin and TA, strongly suggesting a synergistic effect of the two drugs on cell proliferation.

Combination of metformin and TA was also accompanied by a 3-4 fold increase in apoptotic cells at 48h post-treatment, as determined by AnnexinV staining of DAOY cells. Western blot analysis of DAOY cells treated with the two drugs also revealed a decrease in survivin and Bcl-2 protein levels, and an increase in cleaved-PARP. In conclusion, the anticancer activity of metformin in MB cells is enhanced in the presence of TA. Both drugs synergistically combine to inhibit MB cell proliferation and increase apoptosis, which in part may be the result of a decrease in survivin levels. This combination strategy may therefore represent a therapeutic strategy for MB. It is under investigation whether this drug combination can enhance the response of chemotherapy in MB cells, since both TA and metformin are known to sensitize cancer cells to chemotherapy.

#13 Chromatin unfolding small molecules as a novel type of anticancer agents. Katerina V. Gurova. Roswell Park Cancer Institute, Buffalo, NY.

Although targeted therapy was the major focus of anti-cancer drug development, recently revealed enormous heterogeneity of tumors planted a seed of doubt in it as a potential cure for cancer. Very few universal cancer targets are known and they lack cancer specificity. DNA is one of this sort, but activity of drugs targeting DNA was traditionally attributed to their ability to block DNA replication and cause DNA damage with a plethora of harmful long lasting effects in normal cells. We have found that reversible binding of DNA by non-DNA damaging small molecules, curaxins, has strong anticancer effect in different preclinical models without deleterious consequences associated with DNA damage (REF). In this study we explained the mechanism of activity of curaxin clinical lead, CBL0137, through alteration of chromatin organization in tumor cells. Intercalation of the curaxin carbazole moiety between base pairs and DNA major and minor groove binding with curaxin side chains cause an increase in inter base pair distance and untwisting of the double helix (~18°). Such alteration of DNA helical shape leads to decreased nucleosome stability and chromatin opening, creating sites for the high affinity binding of histone chaperone complex FACT. We named this phenomenon “chromatin trapping of FACT” or c-trapping. Consequences of C-trapping are much more deleterious for tumor, than for normal cells. First, c-trapping is an equivalent of functional inactivation of FACT. FACT is critical for oncogenic transformation and tumor, but not normal cell, viability and growth (REF). FACT is essential for the activity of several transcription factors, such as NF-kappaB, HSF1, MYC etc., with important roles in tumor cell proliferation and survival (REF). Further, deregulation of transcription caused by curaxin-dependent chromatin decondensation leads to upregulation of transcription from heterochromatin. This leads to the formation of double stranded RNAs from centromeric and pericentromeric repetitive elements, which mimics a viral infection and induces an IFN response, a powerful inhibitor of tumor cell growth. Additionally, c-trapping of FACT leads to casine kinase 2 mediated phosphorylation and activation of wild type p53 leading to p53-dependent death of cells. Thus non-DNA damaging DNA binding small molecules compromise cancer cell viability via alteration of nucleosome stability and chromatin organization. This is a novel mechanism of anti-cancer activity of small molecules with broad applicability to different types of cancer while lacking the harmful effects of DNA damaging chemotherapy.

#14 Anticancer activities of terpenoid compounds isolated from the plant centipeda minima against nasopharyngeal carcinoma cells. Bei-bei Liu,1 Kuiyuan Zhu,2 Chi-on Chan,1 Ling-Hua Zhang,2 Daniel Kam-Wah Mok,3 Si-bao Chen.1

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Nasopharyngeal carcinoma (NPC) is one of common cancers throughout south-east Asia, southern China and other regions. Radiotherapy and chemotherapy are the standard treatments for NPC with severe adverse effects, multidrug resistance, and insensitivity to advanced NPC. Complementary and novel treatments for NPC gain considerable interest around the world. The medicinal plant Centipeda minima is traditionally used in China for the treatment of various nasal diseases such as allergy, rhinitis, sinusitis and nasopharyngeal carcinoma. In the current study, we studied the anticancer potential of an ethanol extract (CME) and 3 pure sesquiterpenoids (A1, A2, A3) extracted from Centipeda minima against NPC cells in vitro and some underlying mechanisms of actions. The NPC cell lines CNE-1 and CNE-2 cells were treated with either CME or 3 pure compounds (A1, A2, A3). Proliferation of NPC cells was inhibited in a concentration- and time-dependent manner. For 48 h treatment, the IC50 values were 41.57 to 24.98 µg/mL for CME, and 2.3 to 7.5 µM for A1 to A3. While these extracts showed no appreciable effect on normal human LO2 hepatocytes, significant numbers of NPC cells underwent apoptotic morphological changes following treatments. Cytometric analysis demonstrated that treatments with CME and compounds results in NPC cell cycle arrest at the G2/M phase and eventually apoptosis in a concentration and time-dependent manner. Western blot analysis showed that these extracts could down-regulate Bcl-2 expression, up-regulate Bax expression and caspase-8, 9, 3 activities. In addition,
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the potential anti-NPC actions might be also associated with the marked inhibition of multiple signal transduction pathways including EGFR, VEGFR, HER2, FGFR, IGF-1R, PI3K/Akt/mTOR, MAP/ERK and Jan/Stat3 pathways. In summary, the Centipede mimma extract and terpenoids are potential apopto-sis inducing agents to kill NPC cells and may be promising anti-cancer drug leads against nasopharyngeal carcinoma. The exact mechanism of actions remains further study.

#15 Suppression of adaptive responses to targeted therapies by transcriptional inhibition of key dependency genes. Akisaka,1 Takeshi Shimamura,3 Matthew Meyerson,1 Hideo Watanabe,4 Nathanael tensen,1 Brian J. Abraham,2 Nicholas Kwiatkowski,1 Kevin Buczkowski,1 Bruno Bockorny,1 Ting Chen,1 Shuai Li1, Haikuo Zhang,1 Hideko Terai,1 Tiffany Tavares,1 Tinghu Zhang,1 Tae Jung Kim,1 Michael Silkes,1 Benny Mundayito,1 Li Tan,1 Takeshi Shimamura,1 Matthew Meyerson,1 Hideo Watanabe,4 Nathanael S. Gray,1 Richard A. Young,2 Kwok-Kin Wong,1 Peter S. Hammerman1.

**Dana-Farber Cancer Institute, Boston, MA; 1MIT Whitehead Inst. for Biomed. Resea. Cambridge, MA; 2Loyola University Chicago, Maywood, IL; 3Cahn School of Medicine at Mount Sinai, New York, NY.**

Tumor cells become dependent on the expression of key genes that drive hallmark tumor traits, but these "addictions" also represent potential vulnerabilities for therapeutic intervention. Treating tumor cells with a small molecule inhibitor of the transcriptional kinase CDK7 selectively suppresses transcription of key dependency genes in multiple cancers. Our previous work showed responses to the CDK7 inhibitor THZ1 were especially noteworthy in T cell leukemias, small cell lung cancers, and triple-negative breast cancers—tumor types where the prognoses are especially unfavorable. The genes whose expression is most sensitive to CDK7 inhibition and whose expression is essential for tumor cell survival are often associated with super-enhancers—clusters of cohesin-dependent enhancers that are bound by unusually large amounts of transcription apparatus including CDK7 itself. This "Achilles' cluster" of super-enhancer-associated genes thus represents the set of addictions of tumor cells whose expression is important for tumor cell survival. Transcriptional inhibition synergizes with targeted therapies in models of multiple tumor types. Treating tumors with therapies that are often associated with super-enhancer-bearing--clusters of constitutive enhancers and super-enhancers and gene expression programs of resistant cells. Treating cells with a transcriptional inhibitor in addition to targeted therapies prevents "rewiring" of the gene expression program and increases cell death in tumor models. Thus transcriptional inhibition represents a promising avenue in both mono-therapy and combination settings where drug resistance is acquired.

#16 Evaluation of the efficacy of clotrimazole treatment in human melanoma cell lines that overexpress the multidrug resistance-associated protein (MRP) and the lung resistance-related protein (LRP). Steven D. Blake, Shelby G. McKinley, Christopher M. Tweed, David W. Koh. *Ohio Northern University, Ada, OH.*

**Drug resistance to chemotherapy, mediated in part by overexpression of P-glycoprotein (MDR-1), multidrug resistance-associated protein (MRP), or lung resistance-related protein (LRP), continues to be a major problem in the treatment of melanoma. Because we previously demonstrated the activity of clotrimazol to enhance cytotoxicity in human metastatic melanoma cells, here we evaluated the ability of clotrimazole to treat drug-resistant human melanoma cells that overexpress MRP or LRP.** We utilized human melanoma cell lines that overexpress either MDR or LRP, and human melanoma cell lines that over-express both. Cell growth and cell death were analyzed by flow cytometry, proliferation assays, and immunoblotting after drug treatments and RNA interference. Clotrimazole caused significant decreases in proliferation and increases in cell death in both drug-sensitive melanoma lines and those overexpressing MRP and LRP. No extensive cell death was induced by clotrimazole treatment in noncancerous human skin cell lines. These results indicate that clotrimazole selectively induces cytotoxicity in both drug-sensitive and drug-resistant melanoma lines. Clotrimazole is known to inhibit various receptor potential (TRP) ion channels, we pretreated these melanoma lines with TRP inhibitors or RNAi. Inhibitors of the TRP melastatin-8 channel (TRPM8) or TRP vanillindol-1 (TRPV1) failed to produce comparable levels of cell death caused by clotrimazole. However, RNAi silencing of the TRPM2 cation channel caused significant levels of cell death in both drug-sensitive and drug-resistant melano-noma lines. These results indicate that inhibition of TRPM2 channels may have a primary role in the ability of clotrimazole to treat drug-sensitive and drug-resistant melanoma lines. In conclusion, this study demonstrated that clotrimazol selectively increases cell death in drug-resistant melanoma lines, with minimal deleterious effects in normal skin cells. Taken together, we conclude that clotrimazole has in vitro efficacy toward the treatment of drug-resistant human melanoma cells. Thus, these preliminary studies indicate that clotrimazole has the potential to successfully treat drug resistant melanoma in the future.

#17 Anti-metastatic platinum through glycan targeting in breast cancer. Samantha J. Katner,1 Erica Peterson J. Peterson,2 Eriko Katsuta,2 Stephanie C. DeMas,1 Jennifer Koblinski,1 Kazuaki Takabe,2 Nicholas P. Farrell,1 Virginia Commonwealth University, Richmond, VA; 1Roswell Park Cancer Institute, Buf-falo, NY.


#18 Vorinostat reexpressed estrogen receptor (ER) in TR triple negative breast cancer cell line subtypes and sensitized cells to tamoxifen and indole-3-carbinol in vitro. Beverly D. Lyn-Cook.1 Julie Getz,1 Beverly Word,1 Rhonda Moore,1 Gustav Miranda-Carboni1,1 FDA-NCTR, Jefferson, AR; 2FDA/Center for Tobacco Product, FDA, MD; 3University of Tennessee Health Science Center, Memphis, TN.

Triple negative breast cancer (TNBC) is one of the most aggressive subtypes of breast cancer. Although about 15% of breast cancers are estrogen positive, about 15% falls into this category. This subtype of cancer lacks targeted therapies, such as the estrogen receptor (ER), progesterone receptor (PR), and the human epidermal growth factor receptor-2 (HER2). These patients are limited to cytotoxic chemotherapies with harsh side effects. In addition to occurring in younger women, other risk factors for TNBC include: being of African descent, BRCA1 mutation, a strong family history of breast cancer, lifestyle and environmental factors. Although mutations are involved in the initiation of TNBC, research has revealed that individuals are controlled by factors other than DNA sequences such as epigenetic mechanisms. Environmental and lifestyle related factors, such as the lack of population-based screening, and lack of access to care-factors, the urban environment, food deserts, social stress, racism, diet, lack of exercise, alcohol intake, and tobacco use (i.e. cigarette smoking) have all been described as factors associated with breast cancer related dispari-ties. This study investigated the role of epigenetic mechanisms in the re-expression of ER receptors in triple negative breast cancer (TNBC) cells by examining the effects of a FDA-approved epigenetic drug (vorinostat) and the dietary agent (indole-3-carbinol) on three subtypes of triple negative breast cancer. Basal-like (HCC1806), mesenchymal stem cell-like (MDA-MB-231) and mesen-chymal (BT-549) cell lines were treated with vorinostat for 6, 12, 24 and 48 hrs alone. The ER was expressed in HCC1806 (3-fold) and MDA-MB-231 (5-fold) at 6 hr. The ER was not expressed in BT-549 cells at any time point. MTS assay demonstrated a significant decreased in proliferation (60%) in MDA-MB-231 when treated with vorinostat (10, 20 or 30 μM) and 10 μM of tamoxifen. Furthermore, a significant decrease in proliferation (40%) was also detected in MDA-MB-231 cell lines treated with I3C (200 μM) and vorinostat (10, 20 or 30 μM). Our preliminary results show that triple negative cell lines repre-senting three subtypes responded different to treatment with vorinostat in re-expressing the estrogen receptor. However, these results showed promising re-

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sult for the use of this drug in sensitizing triple negative breast cancer cells to taxol and a dietary agent, indole-3-carbinol. Further functional studies are currently underway.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Antibodies, Bispecifics, and Antibody-Drug Conjugates

#20 Xentuzumab, a humanized IGF-1 and IGF-2 ligand neutralizing antibody, improves the antitumor efficacy of enzalutamide in preclinical models of prostate cancer. Ulrike Weyer-Czernilofsky,1 Marco H. Hofmann,1 Paul J. Adam,1 Flavio Solca,1 Katrin Friedbichler,1 Norbert Kraut,1 Eva Corey,1 Thomas Bogenrieder,1 Boehringer Ingelheim RCV GmbH & Co KG, Wien, Austria;2 University of Washington, Seattle, WA.

Background: The proliferative and pro-survival signals driven by the insulin-like growth factor (IGF) ligands, IGF-1 and IGF-2, are transmitted through their binding to the IGF-1 receptor (IGF-1R). In addition, IGF-2 activates the insulin receptor variant A (IR-A) that is expressed during embryonic development as well as in many cancers. A large body of preclinical evidence suggests that IGF signaling plays a key role in cancer by driving therapy resistance, due to cross-talk with other signaling networks such as androgen receptor signaling. The aim of this study was to explore the potential of the IGF-1/2 ligand blocking antibody, xentuzumab (BI 836845\(^{151}\)), to enhance the anti-tumor activity of enzalutamide in prostate cancer cell lines and in a patient-derived prostate cancer xenograft model. Methods: Effects of enzalutamide, xentuzumab and combinations thereof on in vitro proliferation, survival, cell cycle and signaling were evaluated using the prostate cancer cell lines VCaP, DuCaP, MDA PCa 2b, and LNCaP. The in vivo efficacy of enzalutamide, alone and in combination with xentuzumab was investigated using LuCaP 96CR, a patient-derived xenograft model of castration-resistant prostate cancer. Tumors were implanted s.c. into castrate SCID mice. When tumors exceeded 150\(^{3}\) mm\(^3\) animals were randomized into groups: 1) Control; 2) enzalutamide (50 mg/kg QD po), 3) xentuzumab (BI 836845\(^{151}\), 200 mg/kg QW ip) in combination with enzalutamide. Results: Cell viability was more effectively reduced by the combination of enzalutamide and xentuzumab than either drug alone in three of four cell lines expressing the IGF-1R and the androgen receptor (AR). In VCaP cells, prolonged inhibition of IGF pathway signaling and enhanced blockade of proliferation as well as induction of apoptosis was observed after combination treatment. In vivo, enzalutamide monotherapy did not show significant antitumor efficacy in the LuCaP 96CR model, however, combined treatment with xentuzumab significantly inhibited progression of LuCaP 96CR tumor growth (p<0.001 vs. enzalutamide alone). Reduced serum PSA levels were observed after enzalutamide and combination treatment. Enzalutamide plus xentuzumab inhibited tumor growth at tolerated doses and resulted in significant improvements in survival. Conclusions: These studies demonstrated that addition of the IGF-1/2 neutralizing antibody xentuzumab to enzalutamide results in improved anti-neoplastic activity in a subset of prostate cancer cell lines in vitro, and to re-sensitization to enzalutamide in a patient-derived xenograft model of CRPC. Reference: [1] Friedbichler K et al. (2014). Mol Cancer Ther 13(2):399-409.

#21 Regulation of proliferation and invasion through the inhibition of IGF signaling pathway on Epstein-Barr virus associated gastric cancer. Inhye Jeong,1 Hoi Young Lee,2 Jae Kyung Roh,1 Tae Soo Kim,2 Suk Kyeong Lee,3 Ulrike Weyer-Czernilofsky,1 Marco H. Hofmann,1 Paul J. Adam,1 Flavio Solca,1 Katrin Friedbichler,1 Norbert Kraut,1 Eva Corey,1 Thomas Bogenrieder,1 Boehringer Ingelheim RCV GmbH & Co KG, Wien, Austria;2 University of Washington, Seattle, WA.

Epstein-Barr virus associated gastric cancer (EBVaGC) is one subtype of four molecular classifications by TCGA group. Specifically, EBV related viral RNAs, microRNAs and proteins regulate cancer progression phenomena like aggressiveness, motility, invasion and metastasis. Nonetheless, understanding the mechanisms of viral growth dependency beyond malignant EBV transformation is still deficient. Finding the EBV dependent pathway with significant biological role and the clinically appropriated drug is needed for EBVaGC in this regard. We investigated new therapeutic target and its role of regulating in proliferation and invasion using EBVaGC cell line model. First, we determined proliferation both of AGS and EBV infected AGS (AGS-EBV) cell lines during 7 days by proliferation assay. For the test of BI836845 (IGF ligand neutralizing antibody, Boehringer Ingelheim, German) efficacy, CCK-8 assay was performed. mRNA expression levels of IGF-1R, IGF-1, IGF-2 and IGFBP-6 which is the key regulator of IGF-signaling were measured by RT-PCR. Also, protein expression levels of IGF associated and downstream factors were confirmed by Western blotting. Lastly, invasiveness of cells was detected by invasion assay. As a result, AGS-EBV showed the slower proliferation rate compared to AGS, but the sensitivity to BI836845 was significantly increased in a dose-dependent manner (p<0.001). Interestingly, baseline IGFBP-3 mRNA expression of was enhanced in AGS-EBV compared with AGS. In addition, BI836845 treated model showed up-regulated IGFBP-3 mRNA level in AGS and down-regulated IGFBP-3 mRNA level in AGS-EBV. When BI836845 treated to AGS-EBV, phospho-Akt level was highly inhibited. Moreover, invasiveness of AGS-EBV was elevated about 2 fold more than AGS. Treatment of BI836845 in AGS-EBV represented significant decrease the invasiveness (p<0.08). In conclusion, AGS-EBV seems to modulate their proliferation and invasion through the IGF signaling pathway. Inhibition of IGF signaling pathway would be the potential therapeutic strategy for EBV associated gastric cancer.

#22 MM-161, a first-in-class pan-FGFR antibody. Tamara Duke, Greg Finn, Melissa Geddie, Neeraj Kohli, Maja Razlog, Lihui Xu, Volette Paragas, Haluk Yuzugullu, Sara Ghassenifar, Yasin Hashimhoby-Ramsay, Charlotte McDonagh, Marco Muda, Birgit Schoeberl, Merrimack Pharmaceuticals, Inc, Cambridge, MA.

Aberrant signaling of the FGFR pathway has long been known to promote tumorigenesis and angiogenesis across multiple cancer indications. However, the development of an effective and well-tolerated FGFR targeted inhibitor has been hindered by the need to block the activation of multiple mitogenic receptors while avoiding significant toxicities associated with blocking each oncogene FGF ligands. Here we disclose for the first time a novel FGFR targeted antibody, MM-161, designed to block ligand-dependent signaling driven by all four FGF receptors, specifically the IIIc-isomers. MM-161 is well tolerated in mice and cyromolgus monkeys with no significant weight loss observed in either species. Efficacy studies demonstrated that MM-161 monotherapy leads to significant tumor growth inhibition or tumor regression of xenografts of human lung, renal and endometrial cancer amongst others. Importantly, MM-161 has a dual mechanism of action by inhibiting both proliferation and angiogenesis. We will present data illustrating that inhibition of multiple FGFRs is desirable to achieve tumor regression. Furthermore, we will show combination studies with relevant standard of care therapies in models of lung and renal cancer. Taken together, our preclinical data strongly supports the clinical evaluation of MM-161 in cancer patients.

#23 Antibody targeting of ADAM8 for treatment of triple-negative breast cancer. Sonia G. Das, Stefania Pianetti, Gail E. Sonenshein, Nora D. Mineva. Tufts Univ. School of Medicine, Boston, MA.

Triple-Negative Breast Cancer (TNBC) accounts for 25% of breast cancer deaths. Current therapeutic options are restricted to chemotherapy (CT) and radiation, which fail to block disease progression. Recently, we identified the cell surface protein ADAM8 as a critical driver of TNBC tumor growth and metastasis in half of all breast cancers (EMBO Mol. Med. 6:278, 2014). Elevated expression of ADAM8 in TNBC is associated with poor patient outcomes. We have identified a unique antibody-based reagent that simultaneously targets ADAM8 MP/DI activities as an effective therapeutic intervention in TNBC. Our group has now prepared a panel of potent, highly specific ADAM8 antibodies (ADPs) of either IGG1 or IGG2 subclass with dual MP/DI antagonist activity. The top 6 ADPs bind ADAM8 with low dissociation constants of 1.3 x 10^{-8} M to 7.23 x 10^{-8} M. The 4 most effective inhibitory ADPs in cell-based assays were screened in a single-dose efficacy study (10 mg/kg 3x/wk) against pre-existing MDA-MB-231 cell-derived tumors (50-75 mm\(^3\)). ADP2 and ADP3 treatment resulted in significant tumor volume reduction, of 47% and 52%, respectively. Dose-response curves are in progress. ADP13 was also tested in a neo-adjuvant model of breast cancer and resulted in 73% tumor regression. Furthermore, we will show combination studies with relevant standard of care therapies in models of lung and renal cancer. Taken together, our preclinical data strongly supports the clinical evaluation of ADP13 in cancer patients.
ADP13 substantially decreased metastasis to multiple organs. Lastly, a neo-adjuvant survival experiment was performed, as above, except that following surgical resection of tumors mice were treated for 12 weeks. Recurrence at the primary site was assessed using palpation. Mice were sacrificed when recurrent tumors reached 900 mm³. ADP13 treatment resulted in significantly improved disease-free survival (P = 0.05) and overall survival (P = 0.03). More recently, we noted that ADAM8 regulates miRNAs associated with resistance to CT and showed that ADAM8 inhibition sensitizes TNBC cells to Cisplatin, Paclitaxel, and Doxorubicin. In vivo testing of a combinatorial anti-ADAM8 + CT approach is in progress and may present a promising strategy for early entry into the clinic. Conclusions: A new anti-ADAM8 antibody therapeutic shows efficacy against TNBC growth and dissemination, and improves survival in pre-clinical testing. We hypothesize that addition of an anti-ADAM8 antibody to current CT standards of care will enhance tumor killing, reduce metastasis, and increase survival of patients with ADAM8-expressing TNBC.


Gastric cancer is the second highest cause of cancer mortality in Asia. Overexpression of ErbB3, a cell surface RTK, occurs in ~60% of mGC patients where it is significantly associated with poorer survival. Ligand binding to ErbB3 triggers heterodimerization and downstream signaling through Akt. Neutralizing antibodies that inhibit ligand binding show poor efficacy in clinical trials, likely due to being unable to independently activate antibodies that directly inhibiting heterodimerization could be more effective, however, current methods of antibody isolation offer limited control over the site of antibody binding and, therefore, mechanism of action, requiring extensive screening of antibodies and posing significant challenges to identifying suitable candidates for therapeutic development. Hummingbird Bioscience has used its proprietary Rational Antibody Development Platform for the design and development of novel therapeutic antibodies against ErbB3. Computational sequence and structural analyses predicted highly specific surface epitopes, with strong antigenicity and good safety profiles, where antibody binding would inhibit dimerization. Subsequently, Hummingbird’s directed evolution mouse immunization strategy efficiently isolated monoclonal antibodies that bound with low nM (Kd < 5nM) affinity to these epitopes on native folded ErbB3. Furthermore, these mAbs showed ELISA cross-reactivity to ErbB3 in model organisms (mouse, rat, monkey) but no binding to other EGFR family proteins. Antibody HMBD001 was found to strongly inhibit the phosphorylation of ErbB3 and decrease downstream signaling through the Akt pathway (90% decrease in p-ErbB3 and 60% decrease in p-ARK observed at 24 hrs) resulting in the inhibition of tumor cell proliferation in models of ErbB3+ gastric cancer (over 90% N87 proliferation after 5 days) and breast cancer (up to 85% inhibition of BT474 proliferation after 5 days). In mouse NCI-N87 gastric cancer CDX models, weekly doses of HMBD001 achieved greater than 90% inhibition of tumor growth (at 25 days) with no observable adverse toxicity. The first in-human trial of HMBD001 is planned for 2018.

#25 Effect of nimotuzumab on malignant pleural mesothelioma cell lines. Saé Muñiz-Hernández,1 Vanessa Izquierdo-Sánchez,1 Jorge A. Mendoza-Désién,3 Carolina González-Torres,3 Oscar Arrieta,1 1Instituto Nacional de Cancerologı́a de México, Ciudad de México, Mexico; 2Instituto Nacional de Medı́cina Genómı́ca, Ciudad de México, Mexico.

Malignant Pleural Mesothelioma (MPM) is one of the most clinically aggressive malignancies, with a median survival time of less than 12 months. The monotherapy or combination of therapy with platinum-antifolate chemotherapy agents, may provide survival and life quality improvement in MPM patients. EGFR has been a target for new development drugs that could be used as oncology treatment. Nimotuzumab is a humanized antibody, design by Centro de Immunologia Molecular (Habana, Cuba), for treatment EGFR overexpression neo-plasms. This antibody was approved for treatment of head and neck cancer and metastatic colorectal cancer. MPM exhibit an important expression of EGFR. In this study, we analyzed the effect of nimotuzumab on two cell lines derived of MPM and a xenograph model of one of them, in aticmic nude mouse model. MPM-211 and H2452 mesothelioma cell lines; MRC-5 normal lung cell line and A431 as EGFR positive cell line were used for all assays. We analyzed the viability of cell lines using violet cristal assays. Apoptosis were analyzed with Annexin-V–Fluos Staining kit according to manufacturer instructions. We analyzed the EGFR expression by WB assays. Finally, we generated a xenograft model with MSTO cells in nu/nu mice, and we treated a group with nimotuzumab or cisplatin-gemcitabine one doses/week. Nimotuzumab at any concentration (50, 100, 400 µg/mL) no modified the cellular viability, in both tumor and normal cells, the assays was followed from 24 h to 96 h after exposition. With respect at apoptosis, we no found significant differences between control and exposed samples at 48 and 72 h after nimotuzumab treatment. In order to analyzed the EGFR expression in mesothelioma cell lines, we obtained protein extracts from H2452, H2452 + nimotuzumab and A431. The EGFR protein from MSTD-211, H2452, A431 and MRC-5 cultures in normal conditions and exposed at nimotuzumab 100 µg/mL by 24h, were obtained; then a PAGE-SDS were realized. We observed that both mesothelioma derived cell lines showed similar EGFR expression when it compared with A431 in normal culture conditions; while MRC-5 no showed EGFR expression. When cellular cultures were exposed to nimotuzumab treatment, A431 cells showed similar EGFR expression while MSTO-211 showed high expression. Mice nu/nu (n = 3) were subcutaneously inoculated in the dorsal right flank with MSTO-211 cells. Treatment was applied to animals when tumors were 50 mm³. First mice group received nimotuzumab, second group received cisplatin-gemcitabine; and third group no received treated. Nimotuzumab induced a reduction in tumor volume and mice survival by 4 weeks more that those in the other groups. In vitro exposition of nimotuzumab in mesothelioma cell lines no affect their viability or induce apoptosis. According with the treatment scheme used in this work, nimotuzumab treatment induce a major survival and low toxicity in mice with mesothelioma pleural malignant xenograph.

#26 Panitumumab interacts with TAS-102 leading to combinational anti-cancer effects by blocking EGFR-mediated tumor response to trifluridine. Kazuhisa Satoh,1 Yoshiko Satoh,1 Hidetoshi Kikuchi,1 Naoko Yamashita,1 Yoshiko Satoh,2 Takeshi Kaku,1 Yuji Baba,1 Toshiya Tamura,1 Junpei Soeda2, 1Takeda Pharmaceutical Company Limited, Fujisawa, Japan; 2Takeda Pharmaceutical Company Limited, Tokyo, Japan.

Panitumumab is a monoclonal antibody raised against the human epidermal growth factor receptor (EGFR). TAS-102 is a novel chemotherapeutic agent containing trifluridine (FTD), as the active cytotoxic component. Both panitumumab and TAS-102 have been approved for the treatment of metastatic colorectal cancer (mCRC). In this study, we show the mechanism underlying the anti-cancer effects of the panitumumab/TAS-102 combination in preclinical models. Co-treatment with panitumumab and FTD exerted additive and synergistic anti-proliferative effects in LIM1215 and SW48 colon cancer cells, respectively. Consistent with the in vitro effects, panitumumab/TAS-102 combination led to tumor regression in LIM1215 and COL-01-JCK colon cancer patient-derived xenograft models. In LIM1215 cells, FTD induced ERK/Akt/STAT3 phosphorylation and subsequent serine/threonine phosphorylation of EGFR, while it had no effects on EGFR tyrosine phosphorylation. Panitumumab and the tyrosine kinase inhibitor erlotinib reduced the basal level of EGFR tyrosine phosphorylation in MSTD-211 cells. Panitumumab/TAS-102 combination led to increased miR-21 expression in LIM1215 cells, which may explain reduced EGFR phosphorylation in this setting. Panitumumab/TAS-102 combination led to increased expression of miR-21, which may explain reduced EGFR phosphorylation in this setting. These results suggest that FTD together with the basal activity of the EGFR tyrosine kinase induced downstream pro-survival signaling through ERK/Akt/STAT3. Collectively, we propose that panitumumab interacts with FTD by targeting EGFR-mediated adaptive responses, thereby exerting anti-cancer effects in combination with TAS-102. These preclinical findings provide a compelling rationale to evaluate anti-EGFR antibodies combined with TAS-102 against mCRC.

#27 Combination of paclitaxel and Symo015, a mixture of two monoclonal antibodies directed at MET receptor, to increase anti-tumor effects in gastric cancer. Hyun Jeong Kim,1 Sun Yong Kang,1 Ivan Horak,3 Michael Kragh,3 Woo Sun Kwon,2 Tae Soo Kim,2 Inhye Jeong,1 Sun Young Rha,4 Hyun Cheol Chung1, 1Song-Dang Institute for Cancer Research, Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul, Republic of Korea; 2Song-Dang Institute for Cancer Research, Yonsei University College of Medicine, Seoul, Republic of Korea; 3Symphogen A/S, Ballerup, Denmark; 4Song-Dang Institute for Cancer Research, Brain Korea 21 PLUS Project for Medical Science, Division of Medical Oncology, Department of Internal Medicine, Yonsei Cancer Center, Yonsei University College of Medicine, Seoul, Republic of Korea.

MET is a proto-oncogene that encodes a protein known as hepatocyte growth factor receptor (HGFR), involved in proliferative, survival and invasive/metastatic abilities of cancer cells. MET has received considerable attention as a potential target for cancer therapy, including gastric cancer (GC). MET amplification is present in 4-5% of GC patients, and associated with poor outcomes and significantly shorter median survival. Although intensive efforts have been directed toward the development of HGF-MET axis inhibitors, there are some issues for clinical translation; 1) proper biomarker to select patients, 2) proper chemotherapy combination partner and 3) proper line of treatment. Therefore, exploring drug to inhibit HGF-MET axis is essential. Among them, Symo015
(Copenhagen, Denmark), a mixture of two monoclonal antibodies directed at MET receptor, is one of the leading agents in the pipeline. In this study, we had 49 GC cell lines including 27 Korean cancer patients. Those cells were analyzed by whole exome sequencing (WES) and RNA sequencing to understand biological and molecular characteristics. Also, expression levels of MET and MET- associated proteins were determined by Western Blotting and Luminex analysis. These ABs were evaluated by ELISA. Sensitivity of Sym015 was screened in 49 GC cell lines and combination with paclitaxel was performed in 6 MET amplification cell lines by CCK-8 assay. Combination index (CI) and dose reduction index (DRI) were evaluated by CalcuSyn software. Among 49 GC cell lines, we confirmed 6 cell lines with MET amplification including 2 novel Korean cancer patient cell lines (YCC-31 and YCC-34) and 17 (34.7 %) cell lines sensitive to Sym015 with 20–68 % inhibition rate at 100 nM. And our data showed that Sym015 was related to MET amplification, c-Met/p-Met overexpression and exon 14 deletion, as we expected. Also, the 6 MET amplified cell lines (SNUS, SNU520, MKN45, YCC-31, YCC-34 and Hs-746T) were all sensitive to Sym015 and downregulate expression of c-Met/p-Met. Using MET amplified GC cell lines, we evaluated the efficacy and potential mechanism of Sym015 in combination with paclitaxel, which is the standard agent for second line treatment of metastatic GC. CI values determined at the ED50 indicated that 3 of 6 cell lines (MKN45, SNU520 and YCC-31) had synergistic effects (CI values < 0.7). When used in combination to treat 4 cell lines (MKN45, SNU320, YCC-31 and Hs-746T), the dose at ED50 of Sym015 could be reduced by 2.2-13.6 fold, based on DRI analysis. Also, the IC50 value indicated that the combination of Sym015 and paclitaxel (MKN45 and SNU520) could be obtained. Our results indicate that Sym015 was sensitive to MET amplified cell lines and the Sym015 combined with paclitaxel therapy had synergistic effects in MET amplified GC.

Cell penetrating proteins targeting Mcl-1 induce in vitro and in vivo on-target cancer cell killing of Mcl-1 dependent cell lines. Sabrina Deroo, Sophie Thiollot, Johan Desmet, Franky Baatz, Karen Vandenbroucke, Eric Lorent, Paula Hendrikx, Philippe Aird, Stefan Lovery, Ignace Lasters, Yvonne McGrath, Complex Luxembourg S.A., Ech-sur-Alzette, Luxembourg; Complex V.V. Headquarters, Deipenbeek, Belgium; Complex V.V., Zwinvaarde (Ghent), Belgium.

The pro-survival protein Myeloid Cell Leukaemia-1 (Mcl-1) plays an essential role in survival of numerous cancers. Mcl-1 gene amplifications occur in a variety of human cancers and overexpression of the Mcl-1 protein is often associated with chemotherapeutic resistance and disease relapse. Complex has developed Cell Penetrating Alphabodies (CPAB), a novel and unique therapeutic class of antibodies engineered to efficiently enter cells and inhibit proteins including Mcl-1. High affinity Alphabodies (ABs) targeting Mcl-1 were engineered by a combination of rational design and phage library screening. In affinity assays, these ABs were shown to bind to Mcl-1 with picomolar affinities whilst binding to Bcl-XL and Bcl-2 was below the detection limit of the assay. In vitro, CPAB uptake was shown to occur rapidly with cytosolic levels reaching up to 1 µM within 2 hours of CPAB exposure. Uptake was associated with cell death of the Mcl-1 dependent multiple myeloma (MM) cell line M1 (IC50=0.5 µM) and killing was correlated with caspase-3/7 activation. Anti-Mcl-1 CPAB were also shown to disrupt Mcl-1-Bak and Mcl-1-Bim complexes in H929 cells and induced dose-dependent Bak activation. In a panel of MM cell lines, anti-Mcl-1 CPAB induced cell death with a median IC50 of 0.96 µM and cell killing was not restricted to a specific subset of MM cell lines (CCD11, MAF, MMSET). Gene expression analysis revealed that the anti-Mcl-1 CPAB cell killing potency correlates with MCL-1 gene expression but correlates best with the MCL-1:BCL-2 gene expression ratio. The same gene expression correlation analysis of the Bcl-2 targeting agent Venetoclax revealed an inverse pattern to that achieved with the Mcl-1 specific CPAB. In vivo, CPAB conferred with an albumin binding domain for extension of half-life, showed a serum half-life in mice of more than 1 hour and associated tumor concentrations of more than 1 µM. Immunohistochemistry and direct detection of fractionated tumor tissue confirmed the intracellular presence of the CPAB in the tumor cells. When given daily IV at 20 mg/kg, anti-Mcl-1 CPAB induced 17 (34.7 %) tumor growth inhibition of 50% versus control in 5 MM xenograft models (H929 and MOLP-8). Tumor growth remained significantly inhibited even two days after the last treatment in the MOLP-8 model and tumor growth inhibition was associated with increased staining of cleaved caspase-3 as compared to vehicle treated tumors. In summary, anti-Mcl-1 CPAB efficiently kill Mcl-1 dependent cancer cell lines by on-target effects as demonstrated by increased caspase-3 activation and inhibition of tumor growth. The CPAB induced 50% versus control in vivo and (3) correlation of potency with MCL-1:BCL-2 gene expression ratio. These CPAB induced a robust reduction in tumor growth in mouse models and represent a best-in-class cell penetrating protein therapeutics for tackling intracellular PPI critical to diseases with unmet medical need.

Addition of Galunisertib to DC101 increased angiogenesis inhibition and tumor growth control in hepatocellular carcinoma (HCC). Annemilai Tijeras-Raballand, Christian Hobeaika, Elise Payen, Matthieu Martinet, Philippe Bonnin, Karim A. Benhadj, Clarisse Eveno, Marc Pocard, Sandrine Faivre, Eric Raymond, Armand de Grammont, AFR Oncology, Paris, France; University Hospital, Poitiers, France; U965, Lariboisiere University Hospital, Paris, France; Eli Lilly Company, New Jersey, NJ; Beaumont University Hospital, Clichy, France; Saint-Joseph Hospital, Paris, France.

Introduction: Galunisertib is a selective ATP-mimetic TGF-β receptor (TGF(R))-I inhibitor and DC101 is a rat antiagonist antibody to mouse VEGFR-2 (CD126) receptor. Single agent anti-VEGFR-2 treatment had synergistic effects in MET amplified GC. MKN45 and SNU520 cell lines were notably observed in both 2 MPM cell lines (H2052 and H2452). Interestingly, we found that MPM cell lines but not normal cells actively secreted the S100A11, a Ca2 + binding protein. S100A11 on cell proliferation, colony formation, migration, invasion and down-stream signaling. The efficacy of anti-S100A11 antibody on cell proliferation and (3) correlation of potency with MCL-1:BCL-2 gene expression ratio. Those results will be confirmed by the assessment of micromolecules number on HPS section for tumor growth and CD31 staining for angiogenesis analysis (number of vessels and the vessel lumen area). The effects of the combination versus monotherapies will be evaluated on the immune landscape. Conclusion: The combination of galunisertib and DC101 showed promising anti-tumor activities that were associated with decreased angiogenesis.

#30 S100A11 is a prominent therapeutic target in malignant pleural mesothelioma. Hirokatsu So, Hiromasa Yamamoto, Kent Namba, Hidejiro Torigoe, Takahiro Yoshikoa, Kazuhiro Shien, Junichi Soh, Shinichi Toyooka. Okayama Univ. Hospital, Okayama City, Japan.

[Background & Purpose] Malignant pleural mesothelioma (MPM) is an aggressive neoplastic disease that shows a serious malignancy in thoracic cavity with a median survival time of 9–12 months. The therapeutic approaches in clinical standard are limited to surgery and chemotherapy for early and advanced stages, respectively. The strategies unfortunately provide only palliation, thus further advanced approach is required. S100A11, a Ca2 + binding small protein with two EF-hands, is frequently upregulated in various human cancer tissues. It has been reported that S100A11 plays an important role in progression of many cancers derived from thyroid, ovarian and lung tissues, but its roles and signature in MPM are poorly understood. In this study, we investigated the impact of S100A11 in MPM cell lines and resected tumors.

[Material & Methods] We explored the expression of S100A11 in 7 MPM cell lines (HP-1, H28, MSTO-211H, H2052, H290, H2452, and YUMC44) and 2 normal mesothelial cell lines (Met-5A and LP-9). We analyzed the effect of S100A11 on cell proliferation, colony formation, migration, invasion and down-stream signaling. The efficacy of anti-S100A11 antibody on cell proliferation and downstream signaling was also determined. [Results] We found that S100A11 protein was consistently upregulated in 7 MPM cell lines at a significant level in comparison to 2 normal mesothelial cells. The same phenomenon was also confirmed on MPM tissue and normal human mesothelial cell line (M1). Tumor growth inhibition of 50% was strongly stained at MPM cells, but not at surrounding normal lung cells. Specific knockdown of S100A11 by small interfering RNA turned the aggressive cells into the attenuated phenotypes for proliferation, invasion and migration. Those were notably observed in both 2 MPM cell lines (H2052 and H2452). Interestingly, we found that MPM cell lines but not normal cells actively secreted the S100A11 protein. To pursue this, we next tried to inhibit the function of the secreted S100A11. Administration of S100A11 neutralizing antibody significantly inhibited the proliferation of 4 MPM cell lines (H2052, H2452, H28, and H290). The antibody had no effect on the proliferation of only one MPM cell line, MSTO-211H, which showed no secretion of S100A11. Taken together,
these results suggest that S100A11 secreted from MPM cells can be a prominent target for effective MPM therapy. [Conclusion] Our results suggest that S100A11 is a possible therapeutic target in MPM.

#31 Preclinical development of a novel biparatopic HER2 antibody with activity in low to high HER2 expressing cancers. Nina Weisser, Grant Wickman, Rupert Davies, Gerry Rowse. Zymeworks, Vancouver, British Columbia, Canada.

HER2-directed therapies have improved clinical outcomes for many patients with HER2 overexpressing breast and gastric cancer. Despite these successes, there remains a need to develop effective HER2-targeted therapies for these and other HER2-expressing tumors, particularly in the setting of recurrent or metastatic disease. One approach is to develop a single multifunctional antibody that has improved capacity and efficiency for binding HER2 compared with available HER2 inhibitors, can elicit ADCC, block ligand induced heterodimerization of HER2 with other HER family receptors, and down-regulate HER2 protein on the cell surface. ZW25 is a novel humanized, IgG1-like bispecific antibody directed against two distinct epitopes on the HER2 receptor. ZW25 has low nM binding affinity (0.9-16 nM) to recombinant HER2 and to cultured cancer cells with a range of HER2 expression, and shows higher maximal binding (1.4-1.9x) than monospecific HER2 antibodies on all tumor cell lines tested. The unique structure of ZW25 favors crosslinking of multiple HER2 receptors which is thought to promote clustering, and improves HER2 internalization compared to monospecific HER2 antibodies. In vitro studies demonstrated that ZW25 alone caused significant inhibition in growth of human cancer cell lines with a wide range of HER2 expression (5-54% inhibition depending on the cell line), and inhibited ligand-mediated tumor cell proliferation. ZW25 elicited concentration-dependent ADCC at nM potency (0.05-64 nM) with maximal lysis up to 52% on TNBC cell lines expressing HER2 at a 0+ level. ZW25 also exhibited synergy and additivity with multiple chemotherapeutic agents including platin, taxanes, tumorubin inhibitors, and DNA synthesis inhibitors in various HER2 expressing tumor cell lines. In vivo studies demonstrated antitumor activity and/or improved survival against xenografts from a range of tumor types. In a HER2 3+ gastric cancer PDX model ZW25 induced significant tumor regressions, exhibiting 30% tumor growth inhibition relative to hlgG control and a 70% complete regression rate (trastuzumab exhibited 11% tumor growth control with no complete regressions). In a HER2 1+ breast cancer PDX model ZW25 significantly improved median survival (58 vs 28 days) and exhibited 141% tumor growth inhibition relative to hlgG control (trastuzumab exhibited 63% tumor growth inhibition, with median survival 39 days). In a low HER2-expressing NSCLC CDX model ZW25 improved median survival compared to hlgG control or cisplatin (>66 vs 25 or 26 respectively) and exhibited 159% tumor growth inhibition relative to hlgG control. In a GLP repeat dose toxicology study, ZW25 was well tolerated with no adverse effects at doses up to 30 mg/kg weekly IV for up to 8 weeks. Based on these findings, a first in human clinical trial of ZW25 has been initiated in patients with recurrent and/or metastatic HER2-expressing cancers.

#32 Preclinical evaluation of MCLA-158: A bispecific antibody targeting LGR5 and EGFR using patient-derived colon carcinoma organoids. Rob Roovers,1 Bram Herpers,2 Mark James,3 Berina Eppink,4 Carme Cortina,3 David John de Kruif,1 John de Kruif,1 Ton Logtenberg,1 Piet Gros,2 Cecile Geuijen,1 Mark Throsby1.1Merus, Utrecht, Netherlands; 2Merus, Utrecht, Netherlands; 3Utrecht University, Utrecht, Netherlands; 4The Hubrecht Institute, Utrecht, Netherlands.

Background. Colon cancer (CRC) is the third most common cancer and remains a large unmet need. Dysregulation of Wnt and receptor tyrosine kinase (RTK) signalling pathways are important oncogenic driving events in CRC. Due to this dysregulation, Wnt target genes are expressed at higher levels in CRC particularly in tumor initiating cells. We previously performed an unbiased screen of 626 candidates, including 18 RTK targets that resulted in the selection of MCLA-158. Methods. A cohort of 32 genetically and transcriptionally annotated patient-derived colorectal cancer and normal colon organoids were used to functionally characterize responses to antibodies based on morphological changes with high content 3D imaging. Binding affinity was measured by surface plasma resonance and cell based assays. The antibody binding epitopes were mapped by shotgun mutagenesis and FACS based screening. Ligand (R-spondin or EGF) blocking activity was measured in vitro by competition for ligand binding or functional inhibition of ligand dependent growth. In vivo activity was evaluated in xenograft models generated from organoids subcutaneously implanted into immunocompromised mice. Safety was evaluated via once weekly intravenous administration of MCLA-158 to cynomolgus monkeys for 4 weeks and monitoring for pathological changes. Results. MCLA-158, an ADCC enhanced common light chain IgG1 bispecific antibody, binds in domain III of EGFR and in the N-Cap/16 LRR of LGR5, both ligand binding regions, however, only EGF binding was blocked by MCLA-158. MCLA-158 demonstrated inhibitory activity in 74% of tumor organoids independent of KRAS mutational status but was not active on organoids of the cohort harboring both KRAS and PIK3CA mutations. MCLA-158 was significantly more active on organoids derived from tumors than from normal tissue in contrast to cetuximab, which demonstrated equivalent activity on both (range 20-100 fold, n=4). In vivo activity was evaluated against tumor organoids with different KRAS mutations and was shown to be beneficial to MCLA-158 in vitro. In all cases, MCLA-158 significantly inhibited the growth of the tumor compared to both control and cetuximab treatment. Inhibitors of both the Wnt and EGFR pathways have shown significant toxicity in humans. An initial evaluation of MCLA-158 toxicity in cynomolgus monkeys did not demonstrate any pathological finding after repeated dosing at 25mg/kg. Conclusions. MCLA-158 demonstrates superior activity compared to reference antibodies in both in vitro and in vivo tumor organoid based assays regardless of KRAS status and was well tolerated in non-human primates. These preclinical data suggest MCLA-158 could benefit patients with metastatic CRC and warrant clinical evaluation.

#33 The binding mode of the bispecific anti-HER2xHER3 antibody MCLA-128 is responsible for its potent inhibition of HRG-driven tumorigenesis. David Maussang-Detaille,1 Camilla de Nardis,2 Linda Hendriks,3 Carina Bartelink-Cleyn,4 Tristan Goumans,4 Robert Doornbos,1 Lex Bakker,1 John de Kruif,1 Ton Logtenberg,1 Piet Gros,2 Cecile Geuijen,1 Mark Throsby1.1Merus, Utrecht, Netherlands; 2OcellOBV, Leiden, Netherlands; 3IRB Barcelona, Barcelona, Spain; 4The Hubrecht Institute, Utrecht, Netherlands.

Introduction: MCLA-128 is an ADCC-enhanced IgG1 bispecific antibody that targets the HER2:HER3 dimer and is currently being tested in Phase II/III clinical trials. MCLA-128 demonstrates an in vitro potency superior to other anti-HER2 and anti-HER3 antibodies in cells stimulated with high concentrations of heregulin (HRG) thereby overcoming one of the resistance mechanisms of current HER2 therapies. This study investigates the binding mode of MCLA-128 and proof of concept studies in HRG-driven tumor models. Methods: Alanine scanning shotgun mutagenesis was used to map the epitopes of MCLA-128 to HER2 and HER3. Fab fragments of MCLA-128 were crystallized with the soluble extracellular domains of HER2 and HER3. SAXS analysis on the HER2-HER3-MCLA-128 complex was performed to investigate the binding mode of the bispecific antibody in solution. Ligand-induced dimer specificity was investigated with PathHunter® heterodimerization assays. Bispecific anti-HER2xHER3 antibody and its parental anti-HER3 monoclonal antibody were labelled with 125I to compare their biodistribution profiles. The efficacy of MCLA-128 in HRG-driven systems was shown in vitro in MDA-MB-175 cells and in vivo in an orthotopic intracranial patient-derived xenograft (PDX) model originating from a breast cancer brain metastasis. Results: The shotgun mutagenesis study identified that the bispecific antibody MCLA-128 binds amino acids T144, R166, R181 in HER2 domain I and R426 in HER3 domain III. Crystallographic studies confirmed the binding mode of the bispecific antibody MCLA-128 and suggested that MCLA-128 locks the HER3 receptor in its ligand-unbound inactive conformation. SAXS analysis suggests that the bispecific antibody MCLA-128 forms inter-dimer rather than intra-dimer interactions. In vivo, MCLA-128 specifically blocked HRG-induced signaling of HER2:HER3 but not HER2:HER4 heterodimers. Biodistribution of MCLA-128 in a xenograft model of breast cancer showed that the penetration of MCLA-128 in JIMT-1 HER2-amplified tumors is HER2-dependent despite the high affinity of the HER3 Fab arm for its receptor. MCLA-128 efficiently blocked tumor growth of the HRG-driven HER2 (+) breast cancer cell line MDA-MB-175 in 3D in vitro. Treatment of orthotopically transplanted HER2-amplified breast cancer brain tumors in mice led to 100% survival with MCLA-128, in contrast to 38% and 0% survival in T-DM1 and vehicle treated mice respectively. Conclusion: MCLA-128 targets HER2-positive tumors via its HER2 arm and locks HER3 in an inactive conformation. The potent anti-proliferative activity of MCLA-128 in vitro and in vivo supports the clinical development of this bispecific HER2xHER3 antibody in HRG-driven tumors.

#34 A MET x MET bispecific antibody that induces receptor degradation potently inhibits the growth of MET-addicted tumor xenografts. John DaSilva. Regeneron Pharmaceuticals, Tarrytown, NY.

Ablation regulation of MET receptor tyrosine kinase signaling is associated with development and progression of various human tumors. Elevated MET signaling during tumorigenesis results from MET amplification, mu.
tation or up-regulation of the ligand, HGF. MET amplification is thought to be a key driver of oncogenesis in non-small cell lung cancers (NSCLC) and oesophageal gastric malignancies. In addition, mutations resulting in exon 14 deletion of MET have been described as oncogenic drivers in a subset of NSCLC. Both preclinical and recent clinical results indicate that tumors harboring these genetic alterations respond to MET inhibitors, validating MET as a cancer driver. We have developed a novel bispecific antibody that binds to two distinct epitopes on MET. The METxMET bispecific antibody blocks HGF binding and exhibits very low agonist activity (much weaker than either of its parental antibodies). Furthermore, the METxMET bispecific antibody effectively promotes MET degradation, thereby inhibiting ligand-independent signaling in MET-amplified tumor cells. The METxMET bispecific antibody exhibits strong anti-tumor efficacy in xenograft models harboring MET genetic alterations, either completely inhibiting tumor growth (EBC1 MET-amplified NSCLC) or promoting substantial tumor regression (SNU5 MET-amplified gastric cancer, Hs746T MET-amplified/ MET exon 14 skip gastric cancer). Analysis of lysates from SNU5 tumors treated with the METxMET specific antibody confirms its ability to effectively promote MET degradation in vivo. Finally, the METxMET bispecific antibody strongly inhibits the growth of a glioblastoma xenograft model (U118) driven by autocrine HGF signaling. In summary, we have generated a novel METxMET bispecific antibody that potently inhibits the growth of MET-dependent tumors xenografts. Our data suggest that antibody-mediated MET degradation may be an effective clinical strategy in tumors harboring MET genetic alterations.

#35 The dual inhibition of Met and EGFR by ME22S, a novel MET/EGFR bispecific monoclonal antibody, suppresses the proliferation and invasion of laryngeal cancer. Bok-Sook Lee,1 Dae Ho Kim,1 Yeon Soo Kim,1 Jae Won Chang,1 Bon Seok Koo,1 Ho Ryun Won,1 Haeng-Jun Kim,1 Hyun-Young Cha,1 #36 Novel anti-Sialyl-Tn monoclonal antibodies and antibody-drug conjugates (ADCs) demonstrate tumor specificity in vitro and in vivo antitumor efficacy. Jillian M. Prendergast,1 David A. Eavarone,1 Patricia E. Rao,1 Adam D. Curtis,1,2 Lindsay S. Shopland,2 Todd A. Hoftert,2 Jenna Stein,2 Jeff Behrens,1 Daniel T. Dransfield,1 Siamab Therapeutics, Inc., Newton, MA; 3Eastern Maine Medical Center, Brewer, ME.

Tumor-associated carbohydrate antigens (TACAs) historically have been challenging targets for antibody therapeutics. Sialyl-Tn (STn) is a cancer specific antigen that is expressed on the cell surface of carcinomas including ovarian, colon, prostate, and pancreatic tumors but is rarely present in normal tissues. STn expression has been linked to innate immune suppression, a chemoresistant phenotype, metastasis, and poor prognosis. Previous attempts to target this antigen in the clinic with synthetic glycan vaccines proved safe but lacked efficacy. We have developed highly selective humanized monoclonal antibodies and antibody-drug conjugates (ADCs) targeting TACAs, such as STn. Remarkable sequence homology across all anti-STn mAbs was observed in both heavy and light chains, and hot spots for hypermutation were identified. These antibodies were selected using our glycan microarray that enriches for candidates whose binding is protein-independent, highly selective and demonstrates exceptional target affinity. Lead humanized candidates demonstrated single digit nanomolar EC50s in ELISA/flow cytometric assays, STn selective cell internalization, and in vivo tumor growth inhibition in a murine colon xenograft model. STn binding sites in common tumor lines (ovarian, gastric and breast) were determined per cell and subsequent cytotoxicity assays in these lines demonstrated in vitro efficacy. Tumor microarray experiments revealed membranous staining in cancerous tissues of various indications. Binding studies of anti-STn antibodies to primary human cancer samples by flow cytometry demonstrated that both tumor- associated and suppressor cell types were stained, with cell lines and xenografts expressing high levels of STn being more extensively stained compared to low STn expressing samples. STn selective mAbs show promise as therapies for solid tumors and could also target MDSCs to promote antitumor immune responses.

#37 Novel antibody-drug conjugates targeting DGN549, a PAMAM dendrimer with solid tumors demonstrate potent preclinical activity. Stuart W. Hicks,1 Rachel C. Yoder,1 Deryk Loo,1 Asli Muvaffak,1 Yinghui Zhou,1 Megan E. Fuller,1 Molly A. McShea,1 Marian Themeles,1 Katherine H. Mucciareno,1 Juniper A. Scribner,2 Bhawati Barat,2 Thomas Sun,2 James Tamura,2 Francine Z. Chen,2 Kerry A. Donahue,2 Tom Chittenden,1 Immunogen, Inc., Waltham, MA; 2MacroGenics, Inc., South San Francisco, CA; 3Macrogenics, Inc., Waltham, MA.

ADAM9, also known as MDC9 or melanin-1, is a member of the ADAM (a disintegrin and metalloproteinase) family of proteins which have been implicated in cytokine and growth factor shedding, and cell migration. Dysregulation of ADAM9 has been implicated in tumor progression and metastasis, as well as pathological neovascularization. ADAM9 overexpression has been shown to correlate with poor prognosis in prostate, renal, and pancreatic cancers. Using an immunization approach in which antibodies were raised to a preclinical ADAM9 antibody and a cell line followed by screening on tumor and normal tissues, we identified ADAM9 as a promising cell surface tumor target. FFPE-IHC expression analysis revealed that ADAM9 is overexpressed in multiple solid tumor indications relative to corresponding normal tissues. The overexpression of ADAM9 in tumors coupled with its restricted expression in normal tissues make ADAM9 an attractive target for antibody-drug conjugate (ADC) therapy. Here, we describe two ADCs both of which are based on a high affinity anti-ADAM9 antibody to selectively target ADAM9-expressing tumors. The first ADC utilizes the maytansine-derived microtubule disruptor, DM4, linked via a hindered disulphide hydrophilic linker (sulfo-SPDB). The second ADC exploits an ultra-potent DNA alkylating payload, DGN549, which is conjugated to two engineered cysteines via a peptide linker. Both conjugates bound with similar subnanomolar affinity to ADAM9-expressing cells. In vitro cytotoxicity studies showed that anti-ADAM9 ADCs can kill a broad panel of ADAM9-positive tumor cell lines, including lung, pancreatic, renal, prostate, and colon tumor cell lines. In particular, the anti-ADAM9-DGN549 conjugate was extremely potent with IC50 values ranging from 0.1 to 65 pM and was at least 2 logs more active than a non-targeting conjugate. Surprisingly, efficient in vitro cytotoxicity was observed at ADAM9 expression levels as low as a few thousand cell surface receptors per cell. Consistent with their in vitro activity, both anti-ADAM9 ADCs displayed compelling anti-tumor activity in xenograft models. In a Calu3 non-small cell lung cancer xenograft model, anti-ADAM9-DGN549 exhibited antitumor activity against a broad panel of ADAM9-positive cell lines, including lung, pancreatic, renal, prostate, and colon tumor cell lines. In the same model, a single intravenous dose of 0.25 mg Ab/kg of the anti-ADAM9-DGN549 produced durable complete remissions in 8/8 mice. A non-targeting DGN549 ADC was inactive even when dosed at 10 times that of the anti-ADAM9 ADC, demonstrating that targeted delivery of DGN549 through ADC binding is required for activity. These data demonstrate that anti-ADAM9 ADCs exhibit antitumor activity against a broad panel of ADAM9-positive malignancies and cause durable remissions in preclinical models at doses expected to be clinically achievable. Anti-ADAM9 ADCs represent a promising therapeutic strategy to target a wide range of ADAM9-expressing tumors.
Target validation, antibody discovery and preclinical data supporting ADAM9 as an antibody-drug conjugate therapeutic target for solid tumors. Juniper A. Scribner,1 Bhaswati Barat,2 Stuart W. Hicks,3 Nicholas C. Yoder,3 Thomas Son,1 Luisana Widjaja,1 Gundo Diedrich,2 Sergey Gorlatov,2 Jeff Hooley,3 Ann Easton,1 Peter Lung,1 Anushka De Costa,1 Francine Chen,1 Michal Chmielewska Liguas,1 Michael Holtalainen,1 Michael Hottuck,1 Valentina Ciccarrone,2 Nadia Gantt,2 James Tamura,2 Megan E. Fuller,1 Molly McShea,2 Scott Koeing,3 Syd Johnson,1 Paul A. Moore,2 Ezio Bonvini,2 Deryk Loo1,2,1MacroGenics, Inc, South San Francisco, CA;1MacroGenics, Inc, Rockville, MD;2ImmunoGen, Inc, Waltham, MA.

Introduction: A target-unbiased approach based on intact cell immunizations with freshly isolated solid tumor cell lines followed by an immunohistochemistry (IHC) screen for cancer-specific candidates, led to the identification of anti-ADAM9 (a disintegrin and metalloproteinase) mAbs with highly differential tumor-versus-normal tissue binding. ADAM9 is a cell surface protein over-expressed in multiple tumors, with a possible role in promotion and progression of cancer through multiple mechanisms, including modulation of adhesion and migration as well as processing of tumorigenic and pro-angiogenic factors. In this preclinical study, we performed target/mAb validation and evaluated the potential therapeutic activity of anti-ADAM9 antibody-drug conjugates (ADCs) toward ADAM9-expressing solid cancers. Methods: IHC was performed with anti-ADAM9 mAbs to confirm and extend available data of human normal and tumor tissue expression. Epitope mapping studies were conducted to define antibodies, mAbs were also screened to identify those that efficiently internalized into tumor cells. In vitro cellular processing studies were performed to further evaluate the mAbs as ADC candidates. Selected mAbs were converted to ADCs via chemical conjugation to potent anti-microtubule (DM4) or DNA alkylating (DGN549) agents; in vitro cytotoxicity studies were conducted with tumor cell lines representing human cancer types that overexpress ADAM9. A lead mAb was then selected for humanization and affinity maturation to yield a development candidate. Results: Anti-ADAM9 mAbs exhibited strong reactivity toward the tumor epithelium of solid cancers, including pancreatic, kidney, prostate, bladder, breast, colon, lung, and ovarian cancer, but limited reactivity toward normal tissues. Anti-ADAM9 mAbs were efficiently internalized and processed by tumor cell lines, including lines with only modest ADAM9 expression. Anti-ADAM9 ADCs exhibited specific, dose-dependent cytotoxicity toward ADAM9-positive cancer cell lines in vitro, with IC50 values in the sub-nanomolar range. Humanization and affinity maturation of the lead mAb yielded a development candidate that retains potent antitumor activity toward ADAM9-positive tumor cell lines and equivalent, high affinity binding to both human and cynomolgus monkey ADAM9. Conclusion: ADAM9 is a cell surface antigen that is over-expressed on a wide range of solid cancers. Anti-ADAM9 mAbs that were strongly reactive with representative tumors exhibited high affinity for the antigen and were efficiently internalized and processed by ADAM9-bearing tumor cells. Anti-ADAM9 ADCs demonstrated dose-dependent cytotoxicity in vitro toward a panel of ADAM9-positive tumor cell lines. Our findings demonstrate that an ADC targeting ADAM9 may serve as a potential therapeutic for ADAM9-expressing solid tumors.

Discovery of PEN-221, an SST2R-targeting somatostatin peptide conjugate with potent activity in vitro and in vivo. Brian H. White, Patrick Bazinet, Kerry Whalen, Michelle DuPont, James M. Quinn, Rossizza Alargova, Tsun Au Yeung, Adam Brockman, James Gifford, Haley Oller, Kristina Kriksciukaite, Charles-Andre Lemelin, Patrick Lim Soo, Benoit Moreau, Samantha Perino, Gitanjali Sharma, Rajesh Shinde, Beata Sveredy-Krawiec, Mary Simcox, Richard Wooster, Mark T. Bilodeau, Tarveda Therapeutics, Watertown, MA. Here we describe the discovery and the structure of PEN-221, a somatostatin receptor 2 (SST2R) targeting peptide conjugated to DM1. PEN-221 is the first clinical compound from Tarveda’s Pentarin platform, which utilizes miniaturized drug conjugates that diffuse rapidly and deeply into solid tumors. Antibody drug conjugates (ADCs) have garnered a significant amount of attention in their ability to direct cytotoxic drugs to cancer cells; however, the efficacy of ADCs in solid tumors is limited by the slow diffusion through the solid tumor tissue. Pentarins are designed to improve the efficacy of targeted therapies through effective tumor cell targeting and enhanced tumor penetration. SST2R, a GPCR overexpressed in multiple types of neuroendocrine tumors, including small cell lung cancers, internalizes rapidly upon agonist stimulation, making it an ideal vector for delivering cytotoxic payloads. Examination of a variety of SST2R targeting ligands, as well as several potential conjugation sites, led to the identification of the C-terminal side chain of [Tyr3]-octreotate amidide as the best conjugation site for a lipophilic payload. The use of DM1 as a payload afforded superior receptor affinity and receptor internalization when compared to other similarly potent microtubule-targeting agents. In vitro studies show that PEN-221 has receptor-dependent cytotoxic effects, and preclinical studies demonstrate PEN-221 induces tumor regression in several SST2R expressing xenograft models.

Development of a novel antibody-drug conjugate targeting endosialin/TEM-1: potent antitumor activity in sarcoma. Gianluca Sala,1 Stefano Iobb,2 Ezio Bonvini,2 Deryk Loo1,2,1MacroGenics, Inc., South San Francisco, CA;2Mediapharma srl, University of L’Aquila, L’Aquila, Italy.

The TEM-1/Endosialin/CD248 receptor is expressed in the cell surface of tumor-associated stroma cells, as well as in sarcoma and neuroblastoma tissues. This receptor is emerging as an attractive molecule in diagnostics and therapeutic because of its expression across the stroma of many human tumors, the low to absent expression in normal tissues and accessibility from the vascular circulation. In this study, we present evidence of the preclinical efficacy of a novel Antibody-Drug Conjugate (ADC). It consists of a humanized TEM-1 monoclonal antibody (E8.3-3) conjugated to a highly potent payload (TEM-1-ADC). In TEM-1 expressing cancer cell lines, this TEM-1-ADC demonstrated a powerful, specific and target-dependent killing activity. High expression levels of TEM-1 in cells correlated with efficient internalization, efficacy, and cytotoxic effects in vitro. Efficacy studies demonstrated that TEM-1-ADC treatment leads to a long lasting tumor growth inhibition of cell line-based models of human sarcoma. Taken together, our results demonstrated that TEM-1 is an attractive target in sarcoma and suggest that TEM-1-ADC has the potential to be developed into a biotherapeutic agent in these malignancies.

An Antibody Conjugate targeting HER-3 demonstrates promising antitumor efficacy in a wide range of human cancer. Gianluca Sala,1 Manuela Iezzi,2 Alessia Lamolinara,1 Emily Capone,2 Stefano Iobb,2 Jean-Fred Sau niere,2 Sara Ponziani,3 Francesco Gianianti,2 Rodolfo Ippoliti2,4 University of Chieti, Chieti, Italy;2Mediapharma srl, University of L’Aquila, L’Aquila, Italy.

The HER-3 receptor is emerging as an attractive molecule in therapeutics because of its overexpression across many human cancers and because of its role in several compensatory processes that underlay emergence of resistance to certain cancer drugs. In this study, we present evidence of the preclinical efficacy of a novel Antibody-Drug Conjugate (ADC) targeting HER-3. It consists of a humanized HER-3 monoclonal antibody (mAb EV20), which recognizes the HER-3 extracellular domain, conjugated to different payloads (HER-3-ADCs). In HER-3 expressing cancer cell lines, these HER-3-ADCs demonstrated a powerful, specific and target-dependent killing activity. High expression levels of HER-3 in tumor cells correlated with efficient internalization, efficacy, and cytotoxic effects in vitro. Efficacy studies demonstrated that HER-3-ADCs target radia to a long lasting tumor growth inhibition of cell line-based models of human head and neck, breast, pancreatic, prostatic, lung, stomach cancers and melanoma. Overall, these findings validate HER-3 as an attractive therapeutic target in multiple solid tumors and support further clinical development and application of HER-3 targeting ADCs.

Preclinical development of a duocarmycin-based antibody-drug conjugate targeting B7-H3 for solid cancer. Thomas Son,1 Juniper A. Scribner,2 Jeff Hooley,3 Michael Chiuchi,1 Pam Li,1 Timothy E. Hotaling,1 Anushka De Costa,1 Yan Chen,1 Francine Chen,1 Bhaswati Barat,1 Valentina Ciccarrone,2 Timur Gaynutdinov,2 James Tamura,2 Scott Koeing,3 Syd Johnson,1 Paul A. Moore,2 Ezio Bonvini,2 Deryk Loo1,2MacroGenics, Inc., South San Francisco, CA;1MacroGenics, Inc., Rockville, MD.

Introduction: B7-H3 is a member of the B7 family of immunomodulatory molecules, overexpressed in a wide range of solid cancers. B7-H3 overexpression has been correlated with disease severity and poor outcome in several cancer types. Proof-of-concept studies targeting B7-H3 demonstrated that auristatin-based B7-H3 antibody-drug conjugates (ADCs) exhibited potent cytotoxicity in vitro and antitumor activity in vivo toward a range of B7-H3 expressing tumor cell lines. Based on these preliminary results, we undertook preclinical development of a B7-H3 ADC comprised of a humanized B7-H3 mAb conjugated to a potent DNA alkylating payload. Methods: Chimeric B7-H3 mAbs were conjugated to vc-seco-DUocarmycin-hydroxyBenzamide Azaindole (DUBA) (ADC conjugated and provided by Synthon Biopharmaceuticals B.V.). In vitro and in vivo activity studies were conducted with tumor cell lines that overexpress B7-H3. Based on the potent activity, together with the biophysical properties and immunohistochemistry (IHC) profiles of the candidates, a lead mAb was selected for preclinical development. The mAb was humanized via CDR grafting and conjugated to DUBA to yield the development candidate MGC018. In vitro and in vivo studies were then conducted with MGC018 to confirm and extend
DX-8951. U3-1402 achieved a high drug-to-antibody ratio (DAR: 7–8), because anti-HER3 antibody (patritumab) and a novel potent topoisomerase I inhibitor inhibit the growth of non-small cell lung cancer with EGFR mutation. Kimio Yonesaka,1 Koji Haratani,1 Kenji Hirotani,1 Kazuhiko Nakagawa1,1 Kindai University Faculty of Medicine, Japan; 2Daiichi Sankyo Co., Ltd., Wimbledon, UK.

#43 ASN004, a novel 5T4-targeted Dolaflexin ADC, achieves complete regressions and tumor-free survivors in a broad variety of solid tumor models. Roger A. Smith, David J. Zammit, Sanjeeva P. Reddy. Asana BioSciences, Lawrenceville, NJ.

ASN004 is an Antibody Drug Conjugate (ADC) that targets the 5T4 oncofetal antigen (trophoblast glycoprotein). High expression of 5T4 is observed in a wide range of malignant tumors, while very limited expression is found in normal tissues. ASN004 incorporates a novel single-chain homo-dimer antibody, Flexi-mer linker technology (Mersana Therapeutics), and several cytotoxic dolastatin (auristatin) analog warheads per ADC molecule with a drug/antibody ratio of ~15:1. ASN004 shows high affinity for the 5T4 antigen (Kd < 30 pM) and 5T4-expressing tumor cells; rapid cellular internalization; and potent, selective cytotoxicity. ASN004 provides complete tumor regressions and tumor-free survivors in multiple tumor xenograft models, derived from human tumor cell lines having both high and low 5T4 expression levels. Tumor-free survivors were achieved at doses that are well-tolerated, based on xenograft body weight measures and exploratory toxicity studies in pharmacologically-relevant non-human primates. As well, tumor-free survivors were achieved in xenograft tumor models following administration of a single dose of ASN004, as low as 1 mg/kg iv. The broad activity of ASN004 has been demonstrated by its superior efficacy in a head-to-head study against trastuzumab-DM1, in a low-ST4, high-HER2 expressing tumor xenograft model. Finally, tumor xenografts that initially responded to ASN004 and later showed tumor regrowth, had not developed resistance and responded well to subsequent treatment with ASN004. Overall, ASN004 is demonstrated to be an ADC with high therapeutic potential in multiple tumor types, encompassing a wide range of 5T4-expression levels. IND-enabling toxicity studies are ongoing, in preparation for advancement to clinical studies in patients with solid tumors.

#44 U3-1402, a novel HER3-targeting ADC, and a novel DNA topoisomerase I inhibitor inhibit the growth of non-small cell lung cancer with EGF mutation. Kimio Yonesaka,1 Koji Haratani,1 Kenji Hirotani,1 Kazuhiko Nakagawa1,1 Kindai University Faculty of Medicine, Japan; 2Daiichi Sankyo Co., Ltd., Wimbledon, UK.

Background HER3, a member of the HER family, is overexpressed in non-small cell lung cancer (NSCLC), especially in those with EGF mutation. Anti-HER3 antibody therapies, including patritumab, are effective, but limited in their efficacy, for patients with NSCLC. U3-1402 is a novel ADC composed of an anti-HER3 antibody (patritumab) and a novel potent topoisomerase I inhibitor DX-8951. U3-1402 achieved a high drug-to-antibody ratio (DAR: 7–8), because it is homogeneously conjugated with the payload. Here, we aimed to preclinically evaluate U3-1402’s efficacy in NSCLC, especially in those with EGF mutation. Materials and methods An in vitro growth inhibition assay was used to evaluate the sensitivity of 14 NSCLC cell lines to U3-1402. Cells were treated with U3-1402 at different concentrations (0–10 μg/ml) over 7 days; 50% growth inhibitory concentration relative to control (IC50) was calculated. PC9, HCC827, HCC827GR5, Ma70, Ma70GR, 11-18, H1650, HCC4006, and H1975 cells had the EGF mutation. HCC827GR5 and Ma70GR were EGF-TKI resistant clones, with MET genomic amplification and an unknown resistance mechanism, established from HCC827 and Ma70 cells, respectively. HER3 mRNA expression levels were measured by quantitative reverse transcription-PCR (qRT-PCR) and the ratio was calculated against house-keeping genes in each cell line. Results The in vitro growth inhibition assay indicated that HCC827GR5, Ma70GR, and 11-18 cells were sensitive to U3-1402 (IC50 values 1.0, 5.2, 3.2 μg/ml, respectively). However, other cells were relatively resistant to U3-1402, having IC50 values greater than 10 μg/ml. Notably, HCC827GR5 cells were more sensitive to U3-1402 than parental HCC827 cells. Specifically, 1.0 μg/ml U3-1402 reduced the viable cell proportion to 50% of control in HCC827GR5 cells, but the effect was limited to 95% of control in HCC827 parental cells. Furthermore, EGF-TKI erlotinib increased sensitivity to U3-1402 in HCC827GR5 cells. Specifically, 1.0 μM erlotinib reduced the viable cell proportion to 69% of control, and 1.0 μg/ml U3-1402 reduced it to 50% of control in HCC827GR5 cells, but both agents combined reduced it to 3% of control (p < 0.01). Support to clarify the underlying mechanism by which EGF-TKI resistant HCC827GR5 cells were more sensitive to U3-1402 than parental HCC827 cells were, we evaluated the HER3 mRNA expression in both cell lines. HCC827GR5 cells had significantly higher levels of HER3 mRNA than parental HCC827 cells did (1.85 vs 0.65, t-test; p = 0.003). Conclusions U3-1402 preclinically exhibited its efficacy in NSCLC with EGF mutation. Its efficacy was enhanced by EGF-TKI combination. Sensitivity to U3-1402 might depend on HER3 expression levels. These results provide a rationale for U3-1402 alone or in combination with EGF-TKI to be investigated in patients with NSCLC with EGF mutation and aberrant HER3 expression.

#45 In vitro and in vivo activity of a novel c-Met-targeting antibody-drug conjugate using a DNA-alkylating, indolino benzodiazepine payload. Katharine C. Lai, Asli Muvaffak, Min Li, Marian Theemes, Surina Sikka, Kerry Donahue, Stuart W. Hicks, Angela Romanelli, Thomas Chittenden. ImmunogenInc., Waltham, MA.

Purpose: c-Met dysregulation and/or overexpression are associated with tumor progression, metastasis and poor prognosis in numerous cancers. Despite strong pre-clinical evidence that blocking c-Met activity inhibits tumor cell growth and metastasis, targeted therapies have thus far failed to deliver an effective treatment option to the majority of patients. To address patients with both c-Met over-expressing and MET amplified tumors, we designed an antibody-drug conjugate (ADC) comprised of a humanized anti-c-Met monoclonal antibody linked to a highly potent indolino benzodiazepine DNA-alkylating payload (DGN549) to enable activity against not only MET amplified but also c-Met over-expressing tumors. Experimental Design: Panels of monoclonal antibodies (Abs) against c-Met were generated and screened for antagonistic and agonistic activity in the presence or absence of the c-Met ligand, HGF. Lead Abs were selected for favorable biophysical properties and the normal tissue-versus-tumor IHC profile and strong reactivity toward carcinoma cells and the vasculature of solid cancers. Chimeric humanized mAb targeting B7-H3 conjugated to the potent DNA alkylating pay-

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Antibodies, Bispecifics, and Antibody-Drug Conjugates

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Antibody-drug conjugates (ADCs) are promising agents that are developed for targeted delivery of cytotoxic payloads to tumor cells. ADCs share a common design of antibody, linker, and cytotoxic payload. Despite significant efforts, the number of available payload classes with a differentiated mode-of-action that can successfully be employed to generate antibody-drug conjugates (ADCs) is still limited. Therefore, ADCs with new mode of action, potentially linked to a highly potent DNA-alkylating agent (i.e., duocarmycin) in uterine cervical cancer, are promising new payloads that can be linked to a novel HER2-targeting antibody-drug conjugate (ADC) with a novel mode of action. This ADC, SYD985, is highly selective against HER2-positive patient-derived xenograft UCC model BFX469. At doses of 5-10 mg/kg qw or bw potent anti-tumor efficacy with treated-to-control ratios (T/C) between 0.16 to 0.28 as well as complete regressions were observed. In summary, KSP inhibitors have been established as a promising new payload class allowing the generation of highly potent and selective ADCs for the treatment of solid tumors.

#47 SYD985, a novel duocarmycin-based HER2-targeting antibody-drug conjugate, shows antitumor activity in uterine and ovarian carcinomas with HER2/neu expression. Gulden Menders, Elena Bonazzoli, Stefania Bellone, Jonathan Black, Gary Altwerger, Alice Masserdotti, Francesca Pettinella, Luca Zammataro, Natalia Buza, Pei Hui, Elena Ratner, Babak Litkouhi, Dan Arin Silasi, Masoud Azodi, Peter Schwartz, Alessandro D. Santin. Yale University School of Medicine, New Haven, CT.

Introduction: Carcinomas (CS) is a rare and highly aggressive gynecologic malignancy. The amplification of HER2/neu has been reported to occur in 25%-56% of uterine and ovarian CSs with significant heterogeneity of the expression within epithelial and in-between epithelial and mesenchymal components. The reported high incidence makes HER2/neu an attractive target for new molecular therapies. We investigated the efficacy of SYD985, (Synther-Biopharmaceuticals), a novel HER2-targeting antibody-drug conjugate (ADC) composed of a monoclonal antibody (mAb) similar to trastuzumab linked to a highly potent DNA-alkylating agent (i.e., duocarmycin) in uterine and ovarian carcinomas. We also compared the anti-tumor activity of SYD985 to trastuzumab emtansine (T-DM1), a FDA-approved ADC, against multiple primary CS cell lines expressing different levels of HER2/neu in vitro and in vivo experiments. Methods: Eight primary CS cell lines were evaluated for HER2/neu surface expression by IHC and gene amplification using FISH assays. The cytotoxicity of SYD985 and T-DM1 was evaluated using these eight CS primary cell lines with differential HER2/neu expression (i.e., 0, 1+, and 3+). Antibody-dependent cellular cytotoxicity (ADCC), proliferation, viability, and bystander killing experiments were performed using Ca++-release assays, propidium iodide-based and flow cytometry assays, respectively. SYD985 and T-DM1 in vitro activity was also studied in mouse xenograft models. Results: Similar ADCC were induced by SYD985 and T-DM1 when effector cells were present against CS cell lines with different HER2/neu expression. In contrast, SYD985 was significantly more cytotoxic compared to T-DM1 in the absence of the effector cells. The Ca++-release by SYD985 7 to 54 fold higher than comparable experiments and unlike T-DM1, it is active against CS demonstrating moderate-low or heterogeneous HER2/neu expression. Specifically, in HER2/neu 0/1+ cell lines the mean IC50’s were 0.060 µg/mL and 3.221 µg/mL for SYD985 vs T-DM1 (p<0.0001) and in HER2/neu 3+ cell lines 0.013 µg/mL and 0.096 µg/mL (p<0.0001), respectively. (p<0.0001). Unlike T-DM1, SYD985 induced efficient bystander killing of HER2/neu 0/1+ and 3+ cells admixed with 0/1+ or HER2/neu 3+ cells. In vivo studies confirmed that SYD985 is more active than T-DM1 in CS and effective against HER2/neu 3+ xenografts. Conclusions: We demonstrate for the first time that SYD985 is a novel ADC with remarkable activity against CS not only with strong (3+) but also with low (0/1+ HER2/neu expression. Clinical studies with SYD985 in patients harboring chemotherapy-resistant uterine and ovarian CS with low, moderate and high HER2 expression are warranted.

#48 Non-clinical pharmacokinetics of XMT-1522, a HER2 targeting auristatin-based antibody drug conjugate. Alex Yurkovskiy, Dmitry Gumerov, Elena Ter-Ovanesyan, Patrick Conlon, Michael Devit, Charlie Bu, Natalya Bodyak, Timothy Lowinger, Donald Bergstrom. Marsena Therapeutics, Inc., Cambridge, MA.

The ADC XMT-1522 consists of a novel human IgG1 anti-HER2 monoclonal antibody and a novel, auristatin-based cytotoxic payload (Auristatin F-hydroxypropylamide, AF-HPA). An average DAR of 12 AF-HPA molecules is achieved via a biodegradable polymer conjugation platform. The non-clinical DMPP properties of XMT-1522 have been characterized in vitro in plasma and microsomal stability studies, and in vivo in plasma and tissue disposition and excretion studies. Sample analysis for total AF-HPA drug payload and released (free) AF-HPA and its metabolites was performed by ESI+-LC/MS/MS; total antibody was determined by ELISA. The half-life for AF-HPA release in plasma was found to be greater than 120 hours in all species tested. Microsomal stability studies showed that AF-HPA was further converted to other metabolites including the carboxylic acid auristatin F (AF), as well as monomethyl auristatin F-HPA (MMAF-HPA) and MMAF. The pharmacokinetic profiles of XMT-1522 were evaluated in mouse, rat and cynomolgus monkey. The antibody of XMT-1522 is cross-reactive with mouse, but not rodent, HER2. In mouse and rat, XMT-1522 exposure was dose-proportional; exposure was slightly greater than dose-proportional in monkey consistent with its saturable hepatic clearance and clearance. All species showed extended exposure to total AF-HPA drug payload, with measured clearance and volume of distribution similar for total AF-HPA and the antibody component of XMT-1522. Exposure to free AF-HPA and AF was less than 1/1000th of the exposure of total AF-HPA. These data indicate the vast majority of AF-HPA in plasma is antibody-conjugated, indicating high stability of the ADC in systemic circulation. XMT-1522 tissue disposition was studied in NCI-N87 HER2-positive gastric cancer xenograft tumor bearing mice. After a single 3 mg/kg dose of XMT-1522, free AF-HPA and its metabolite AF were measurable in tumor tissue until the last time point measured (2 weeks). Total AF-HPA and free AF-HPA achieved peak tumor concentrations 48 hours after dosing. In contrast, AF achieved peak tumor concentration 7 days after dosing and showed only a slight decline in tumor concentration at 14 days, consistent with intracellular trapping of this poorly cell-permeable metabolite. Exposure to free AF-HPA or AF in other tissues was at least an order of magnitude lower than in tumor; in tissues with measurable free drug, AF was the predominant species. XMT-1522 excretion studies, conducted in rat, indicated that the AF-HPA payload was mainly excreted by the gastrointestinal route. In the first 96 hours after administration 33% of the AF-HPA dose was excreted in feces, compared to 3% excreted in urine. The major contributing metabolites both in feces and urine were conjugated AF-HPA, AF, and free AF-HPA. In conclusion, the plasma kinetics, tissue distribution and excretion profile of XMT-1522 are acceptable for clinical evaluation in cancer patients.

#49 Modulating insulin receptor splicing as a potential therapeutic approach for rhabdomyosarcoma. Brianne Sanford, Chelsea Brown, Hennett Bid, Thomas Beebe, Daniel Comiskey, Frank Rigo, Peter Houghton, Dawn Chandler. Nationwide Children’s Hospital, Columbus, OH; Ionis Pharmaceuticals, CA; University of Texas Health Science Center, TX.

The insulin receptor (IN-R) is subject to alternative splicing to produce two isoforms: the full-length IN-R B isoform and an isoform lacking exon 11 known as IN-R A which is the predominant isoform expressed in sarcoma cells. This short exon encodes for 12 amino acids but the resulting receptor has high affinity for a growth hormone called insulin-like growth factor 2 (IGF2), which exquisitely responds to autocrine and paracrine signaling. Increased IN-R A levels observed in rhabdomyosarcoma (RMS) coupled with increased expression of IGF2, programs the tumor cell for enhanced growth and angiogenesis, two important factors in tumorigenesis. Furthermore, IGF2 can maintain angio genesis through IN-R A activity. Hence, modulation of IN-R splicing may prove to be a viable therapeutic approach in rhabdomyosarcoma.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Antibodies, Bispecifics, and Antibody-Drug Conjugates

#47 SYD985, a novel duocarmycin-based HER2-targeting antibody-drug conjugate, shows antitumor activity in uterine and ovarian carcinomas with HER2/neu expression.

#48 Non-clinical pharmacokinetics of XMT-1522, a HER2 targeting auristatin-based antibody drug conjugate.

#49 Modulating insulin receptor splicing as a potential therapeutic approach for rhabdomyosarcoma.
 EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Antibody Technology

#50 HTI-1511, a novel anti-EGFR-ADC, overcomes mutation resistance and demonstrates significant activity against multiple tumor types in preclinical studies. Jesse D. Bahn,1 Feng Gao,1 Lei Huang,1 Barbara Blouw,1 Chunmei Zhao,1 Kelly Chen,1 Susan Zimmerman,1 Erin K. Wise,2 Maria L. Mancini,2 Christopher D. Thanos1.

HTI-1511, an antibody-drug conjugate in pre-clinical development that targets EGFR.

EGFR in the tumor microenvironment (Huang et. al. AACR National Meeting, 1511, an antibody-drug conjugate in pre-clinical development that targets EGFR. Antibodies that target the receptor are often accompanied by adverse skin reactions due to interaction with receptors expressed in normal tissue. Additionally, downstream mutations (KRAS, BRAF) within tumors can result in EGFR-independent activation and resistance to treatment. We have previously described HTI-1511, an antibody-drug conjugate in preclinical development that targets EGFR. HTI-1511 carries the potent cytotoxin MMAE and a novel bis-alkylating linker, connected to a monoclonal antibody engineered to have improved specificity for EGFR in the tumor microenvironment (Huang et. al. AACR National Meeting, 2016, New Orleans, LA). Here we screened a panel of 70 tumor cell lines derived from various solid tumor malignancies for both EGFR expression by flow cytometry and sensitivity to cell growth inhibition by HTI-1511 in vitro. Cell lines derived from head and neck squamous cell carcinoma (SCC15, CAL27, FaDu, CAL33, SCC25 [IC50 0.52 nM - 3.1 nM]), non-small cell lung cancer (HCC827, NCI-H1666, PC-9, NCI-H1650 [IC50 0.04 nM - 6.2 nM]), and pancreatic carcinoma (BxPC3, Panc-1, AsPC-1 [IC50 0.99 nM - 4.4 nM]) showed particular sensitivity to HTI-1511. In comparison, HTI-1511 efficacy was assessed in vivo for tumor growth inhibition (TGI) in several human tumor xenograft models. Evaluations in the human xenografts A431 (epidermoid, 93% TGI at 3.0 mg/kg, p < 0.05), FaPC3 (pancreatic, >100% TGI at 3.0 mg/kg, p < 0.05), AsPC-1 (pancreatic, >100% TGI at 3.0 mg/kg, p < 0.05), and FaDu (HNSCC, >100% TGI at 3.0 mg/kg, p < 0.05) indicated dose dependent tumor regressions in all cases, and no observed toxicity when administered weekly at dose levels up to 3 mg/kg for up to eight weeks. HTI-1511 anti-tumor activity was also investigated in a group of patient derived xenograft (PDX) models. An initial study evaluated weekly administration of a single dose level of 2.5 mg/kg HTI-1511 in three different PDX models. TGI of 83% (p < 0.05) was observed in a BRAFmut colorectal cancer model and 57% (p < 0.05) in a wildtype colorectal cancer model, as well as 46% (not significant) TGI seen in a wild type renal cell carcinoma model. A separate study in a NCI-60 (KRA200) PDX model demonstrated a strong dependent response with greater than 100% TGI at 1.0 and 3.0 mg/kg (both p < 0.05) and also p < 0.05 to a non-cognate antibody ADC control. The non-cognate antibody control yielded 67.4% (not significant) TGI by itself compared to the vehicle control group. These results support further development of HTI-1511 as a possible treatment for EGFR overexpressing tumors, including those with downstream activating mutations in the KRAS/BRAF pathway.

#51 Characterization of the mechanism of action, pharmacodynamics and preclinical safety of ADCT-402, a pyrrolobenzodiazepine (PBD) dimer-containing antibody-drug conjugate (ADC) targeting HER2-expressing solid tumors. Francesco Zammarchi,1 Halla W. Reinert,1 Narinder Janghra,2 Simon Corbett,2 Maria Mellinas-Gomez,2 Sahjaid Chowdhury,3 Neha Arora,3 Peter Tyrer,3 Francois Bertelli,3 David G. Williams,3 Philip W. Howard,3 John A. Hartley,3 Patrick H. van Berkel1.1 ADC Therapeutics, London, United Kingdom; 2University College London, London, United Kingdom; 3Sprintogen/MedImmune, London, United Kingdom.

ADCT-402, currently in Phase I clinical trials for B-cell hematological malignancies, is an ADC composed of a recombinant humanized IgG1 against human CD19, stochastically conjugated via a cleavable linker to a PBD dimer cytotoxin (DAR of 2.3). PBD dimers, DNA minor groove interstrand cross-linking agents, are gaining increasing attention and are currently being tested as the ADC warhead of choice owing to their unique mechanism of action. ADCT-402 targets HER2+ metastatic breast cancers. ADCT-402 is an ADC composed of an engineered version of humanized IgG1 trastuzumab, directed against human HER2, site-specifically conjugated to the highly cytotoxic PBD-based linker-drug tesirine (drug-antibody ratio of 1.7). In vitro, ADCT-402 has highly potent and targeted cytotoxicity against various solid cancer cell lines. In vivo, ADCT-402 demonstrates strong and durable antitumor activity in mouse xenografts with various levels of HER2, but is inactive in a HER2-negative xenograft. ADCT-402 is stable, well tolerated and has a favorable PK profile both in rat and cynomolgus monkey. ADCT-402 is currently in Phase I clinical trials for B-cell hematological malignancies, an ADC composed of a recombinant humanized IgG1 against human CD19, stochastically conjugated via a cleavable linker to a PBD dimer cytotoxin (DAR of 2.3). PBD dimers, DNA minor groove interstrand cross-linking agents, are gaining increasing attention and are currently being tested as the ADC warhead of choice owing to their unique mechanism of action. ADCT-402 targets HER2+ metastatic breast cancers. ADCT-402 is an ADC composed of an engineered version of humanized IgG1 trastuzumab, directed against human HER2, site-specifically conjugated to the highly cytotoxic PBD-based linker-drug tesirine (drug-antibody ratio of 1.7). In vitro, ADCT-402 has highly potent and targeted cytotoxicity against various solid cancer cell lines. In vivo, ADCT-402 demonstrates strong and durable antitumor activity in mouse xenografts with various levels of HER2, but is inactive in a HER2-negative xenograft. ADCT-402 is stable, well tolerated and has a favorable PK profile both in rat and cynomolgus monkey. The current study aimed to further define the mechanism of action (MOA) of ADCT-402 and validate its pharmacology and preclinical safety for clinical development. ADCT-402 is a clinically validated target with restricted normal tissue expression and a widespread expression in the majority of B-cell malignancies. Importantly, we show here the consistent expression of CD19 in matched samples (initial diagnosis and relapsed/refractory) from panels of lymphoma patients, indicating that relapsed/refractory patients are appropriate for treatment with ADCT-402. ADCT-402 was shown to be efficiently internalized by CD19+ cells in vitro. Moreover, in line with the PBD dimer MOA, following a 2 hour exposure to ADCT-402, DNA interstrand cross-links reached a peak between 8 - 12 hours and persisted for up to 36 hours post-treatment. In contrast, the peak of DNA damage induction for PBD dimer warheads was observed immediately after 2 hour incubation, while a non-targeted PBD-ADC did not yield any appreciable DNA cross-links. In SCID mice s.c. implanted with Ramos cells, a single dose of ADCT-402 was administered at 0.33 or 1 mg/kg. Twenty-four hours after treatment, excised tumors showed a dose proportional increase in intensity of staining by an anti-PBD payload antibody, as well as in DNA cross-linking and in γ-H2AX formation. In contrast, no DNA cross-linking was observed in matched lymphocyte samples. The toxicity of ADCT-402 was further evaluated in a repeat dose cyromolgus monkey study. ADCT-402 was clinically well tolerated with an acceptable off-target safety profile. The PK of the ADC was consistent with normal antibody clearance with a half-life of about 12 to 17 days. These data confirm the MOA of ADCT-402 and provide relevant pharmacodynamic and preclinical safety assessments that guide the clinical development of this promising ADC in B-cell malignancies.
Similarly, in a HER2+/+, FISH- esophageal cancer PDX, while a single dose of ADCT-502 at 0.44 mg/kg resulted in strong and durable antitumor activity, single doses of T-DM1 at either 10 or 30 mg/kg showed no activity compared to the control. These data confirm that the mechanism of cell killing of ADCT-502 is via target-specific internalization and subsequent cross-linking of DNA. They also show significantly improved antitumor activity of ADCT-502 compared to each of the monoclonal antibodies in various tumor xenografts, including those with low HER2 levels. Taken together, these results support the development of ADCT-502 not only in patients that have become resistant/refractory to T-DM1, but also in patients whose tumors express low levels of HER2, and are not eligible for treatment with T-DM1.

#53 Antibody-drug conjugates (ADCs) of peptide-linked Indolino-Benzodiazepine (DNA) N-alkylator provides improved anti-tumor activity over that of a crosslinker. Michael L. Miller, Manami Shirzuka, Jose F. Ponte, Leanne Laniere, Dilrulshk Vitharana, Qifeng Qiu, Emily E. Reid, Katie E. Archer, Rui Wu, Erin K. Maloney, Olga Ah, Jan Pinkas, Ravi V. Chari. ImmunoGen, Inc., Waltham, MA.

We recently disclosed highly active antibody-drug conjugates (ADCs) that incorporated the novel DNA alkyling indolino-benzodiazepine (termed IGN) dimer, DGN549 (IGN-P1). The stereochemistry of the alanyl moiety of the protease-cleavable alanine-alanine linker used was shown to impact ADC catabolism, bystander killing activity, and in vivo efficacy (Shizuka, et al., AACR 2016 #2959). Building upon these results, here we describe preclinical results from a head-to-head comparison of ADCs of the mono-imine containing DGN549 with its corresponding DNA cross-linking version, IGN-P1 dimine. IGN-P1 dimine and DGN549 were conjugated to a folate receptor-a (FRA)- binding antibody and an EpCAM-binding antibody. The resulting ADCs demonstrated similar high in vitro potency (IC50 3-100 pM) and specificity towards several cancer cell lines. Further in vitro studies revealed that the DNA alkylating anti-FRA-DGN549 ADC demonstrated superior bystander cell-killing activity compared to its DNA crosslinking counterpart, anti-FRA-IGN-P1 dimine. In vivo, this improved bystander killing ability translated into better in vivo activity for the DNA alkylating ADC. In an endometrial tumor xenograft model established with Ishikawa cells, the anti-FRA-DGN549 induced complete regression at a single dose of 140 μg/kg Ab dose (equivalent to 5 μg/kg linked IGN). The cross-linking anti-FRA-IGN-P1 dimine had to be used at twice the dose to achieve the same level of anti-tumor activity. The in vivo tolerability in CD-1 mice also displayed differences in the two ADCs. We found that the ADC of the DNA crosslinker was at least two-fold less tolerable than the corresponding ADC of the DNA alkylator. These results indicate that a 4fold greater therapeutic index can be achieved when using a DNA alkylating mono-imine DGN549 ADC as compared to the DNA crosslinking IGN-P1 dimine ADC.

#54 The many faces of antibody in G protein-coupled receptor biology. Lei Chen, Wing-Tai Cheung. School of Biomedical Sciences, Faculty of Medicine, Chinese University of Hong Kong, Hong Kong.

G protein-coupled receptors (GPCRs), the largest family of cell-surface receptor proteins mediating signal transmission, play a pivotal role in many physiological functions and are involved in multiple diseases. Although receptor activators of GPCRs are successfully modulated by many small molecules that represent 30% of all marketed drugs today, in fact only a few GPCR targets are described and many GPCRs of interest are intractable targets of small molecules, like orphan GPCRs and GPCRs with large binding sites. Besides, small molecule drugs rarely trigger GPCR-mediated apoptosis or induce direct cell killing, which is crucial for cancer therapies. Therefore, for these GPCRs, antibody-based drug would be a good alternative. Despite being eagerly sought for, the production of monoclonal antibody (mAb) targeting GPCR is hindered by the low expression of recombinant GPCR on cell surface, and relatively small exposed regions with glycosylation and conformational heterogeneity. Therefore those conventional methods that were well established for generation of mAbs targeting soluble proteins usually showed frustrating results in the case of GPCRs. To bypass the production and purification of recombinant GPCR proteins, we developed a novel approach of transplanting immunogenic conformational epitopes of GPCR into an antibody scaffold to make a water soluble surrogate antigen of GPCR, named as GPCR-antigenized antibody which can be easily and abundantly produced in bacteria for animal immunization. In coupled with phage display technology and cell panning strategy, which allow the retrieval of specific binders from a huge number of candidates, we have successfully isolated scFv antibodies for two human GPCRs, the MAS1 receptor and the chemokine receptor CXC4R. These antibodies showed good specificity in immuno-fluorescence staining, flow cytometry analysis, western protein immunoblot and immunoprecipitation assay. The results demonstrated this novel approach may offer a generic and effective method to generate specific monoclonal antibody targeting GPCR for diagnostic and therapeutic applications. [The project is partly funded by a CUHK direct grant (4054300)]

#55 Generation of half-life extended anti-CD33 BiTE® antibody constructs compatible with once-weekly dosing. Tara L. Arvedson, Mercedes Balazs, Pamela Bogner, Kurt Black, Kevin Graham, Anja Henn, Matthias Friedrich, Patrick Hofmann, Roman Kischel, Peter Kuffer, Ralf Lutterbuese, Markus Muenz, Tobias Raum, Benno Rattel, Karen Rex, Dan Rock, Oliver Thomas, Joachim Wahl, Andreas Wolf, Angela Coxon, Angela Munich, Germany.

T cell engaging bispecific antibody constructs (BiTE®), such as blinatumomab which targets CD19-positive cells, have shown great promise for treating certain CD19-positive hematological malignancies. Blinatumomab comprises a single chain Fv (scFv) that binds CD19 and a scFv that targets the T cell CD3 protein. The molecular weight of this “canonical” BiTE® is ~55 kDa, making it susceptible to kidney-mediated clearance and resulting in a short serum half-life (~4 hours). To maintain effective serum concentrations, canonical BiTE® antibody constructs must be administered by continuous IV (cIV) infusion. While there are many advantages associated with cIV administration (e.g., safety and uniform PK profile), patient convenience could be enhanced if the BiTE® antibody construct were compatible with once-weekly administration. To achieve this, the serum half-life of the BiTE® antibody construct would need to be extended. A canonical BiTE® targeting CD33 (AMG 330) is currently being evaluated in a phase I clinical trial. Like blinatumomab, AMG 330 is dosed cIV. To extend the serum half-life of AMG 330 and enable once-weekly dosing, several approaches were evaluated including fusion of AMG 330 to human albumin andFc-containing moieties. Each of these half-life extended (HLE) constructs was evaluated in vitro, in mouse xenograft models and in non-human primates. In vitro assays evaluated 1) binding to both human and cynomolgus CD33 and CD3 proteins, and 2) cytotoxicity using human and cynomolgus target and effector cells. In each of these assays the canonical and HLE BiTE® antibody constructs demonstrated similar activity: single-digit nM binding and single digit pM cytokotoxicity. Canonical and HLE BiTE® antibody constructs were subsequently evaluated in an orthotopic mouse model in which MOLM13 cells were administered IV and activated human T cells were administered IP two days later. The Fc-based HLE BiTE® antibody constructs provided a similar survival advantage when administered QD or Q3D as the canonical BiTE® when administered Q3D. However, the albumin fusion–based HLE BiTE® was less efficacious when administered Q4D than the QD–administered canonical BiTE®. Lastly, the PK/PD relationship was evaluated for each of the constructs in non-human primates. The serum half-lives varied from 6 hours for the canonical BiTE® to 44–167 hours for the HLE BiTE® antibody constructs. Each of the HLE BiTE® antibody constructs showed on-target depletion of CD33-positive monocytes and neutrophils in the blood and depletion of CD33-positive cells in the bone marrow. These data demonstrate that half-life extended BiTE® antibody constructs can be generated that retain comparable in vitro and in vivo activity as a canonical BiTE® and achieve a serum half-life compatible with once-weekly dosing.


While antibody-drug conjugates (ADCs) find increasing application in cancer treatment regimens, de novo or treatment-emergent resistance mechanisms could impair clinical benefit. Two resistance mechanisms that emerge under continuous ADC exposure in vitro include upregulation of transporters that confer multidrug resistance (MDR+) and loss of cognate antigen expression. New technologies that circumvent these resistance mechanisms may serve to extend the utility of next generation ADCs. Recently, we developed the quaternary ammonium linker system to expand the scope of conjugatable payloads to include toxic compounds such as tubulysin, a highly potent class of microtubule disrupting agents that maintain activity in MDR+ cell lines. Quaternary ammonium-linked glucuronide-tubulysin drug-linkers were synthesized and evaluated as ADCs. The resulting conjugates were potent and immunologically specific across a panel of cancer cell lines, including those displaying the MDR phenotype. The ADCs also demonstrated potent bystander activity in a co-culture model containing a mixture of antigen-positive and -negative cell lines. Incorporation of a PEG12 side chain in the linker enabled loading at 8-drugs/Ab for increased in vivo potency while maintaining suitable ADC pharmacokinetic properties. In vivo, the glucuronide-tubulysin conjugates displayed activity in MDR+ xenograft models and
bystander activity in an admixed Ag+/Ag- heterogeneous tumor model. Thus, the glucuronide-tubulin drug-linkers represent a promising new payload for ADCs, combining conjugate potency in the presence of the MDR phenotype with robust activity in models of tumor antigen heterogeneity.

#57 Generation and characterization of novel anti-glycan monoclonal antibody drug conjugates targeting mesothelin. 
Won Kyu Park,1,2 Woon Kok Tek,1,2 Andre Boon Hwa Choo,3 Bioprocessing Technology Institute, Singapore, Singapore; 2National University Cancer Institute, Singapore, Singapore, Singapore.

Hematological malignancies encompass a wide spectrum of cancers from hematopoietic and lymphoid tissues. Under this grouping, the various forms of leukemia, lymphoma and myeloma have been classified into more than 60 distinct disease types, each having particular clinical features and disease outcomes. Among these diseases, curative treatments with high long-term survival rate have been developed for some, e.g. Chronic Myeloid Leukemia, while such effective regimens are still unavailable for many others, e.g. Multiple Myeloma and Acute Myeloid Leukemia. In this study, a panel of antibodies was raised against SLAM7, a cell surface marker that was associated to Multiple Myeloma. Among the antibodies, TAG-HC2 was surprisingly found to have preferential antibody. In addition, cured mice were resistant to re-implantation of 66C14 antibody. TAG-HC2, when complexed with sialyl-Lea, was able to internalize into and kill AML cell lines. These results suggest that TAG-HC2 has the potential to be developed into an Antibody Drug Conjugate against AML.

#58 Local intratumoral treatment with anti-mesothelin Immunotoxins overcomes resistance to anti-CTLA-4 therapy, Yasinim Lesheh,1 Xiuxen Liu,1 Tapan Bera,1 Masaki Terabe,1 Jay A. Berzofsky,3 Birgit Bossummaier,2 Gerhard Niederfellner,6 James O’Brien,1 Yoram Reiter,1 Ira Pastan1. 1NIH, Bethesda, MD; 2Roche Pharmaceutical Research & Early Development, Penzberg, Germany; 3Technion israel institute of technology, Haifa, Israel.

Background: SS1P and RG7787 are immunotoxins composed of anti-mesothelin antibody fused to a fragment from pseudomonas exotoxin A. We previously observed delayed tumor responses in patients treated with SS1P leading us to hypothesis that immunotoxins can provoke anti-tumor immunity. We hypothesize that intra-tumoral injection of SS1P or RG7787 will produce immunologic death and convert living tumors to a source of antigens and boost the therapeutic effect of immune check point blockade. Method: A BALB/c breast cancer cell line was transfected with human mesothelin (66C14-M) and grown in BALB/c mice transgenic for human mesothelin. SS1P or RG7787 was injected directly into established tumors (80-100 mm3) and anti-CTLA-4 administered IP. Results: We observed total tumor regressions in 23 out of 38 mice (60%) treated with anti-CTLA-4 and either RG7787 or SS1P. No cure was achieved when the drugs were given as a monotherapy supporting the case for synergic anti-tumor activity. Pathologic evaluation of regressing tumors showed a massive inflammation of admixed eosinophils and mononuclear cells located in a collar surrounding the tumors. Tumor regression was associated with increased number of CD8+ cells and was blocked when CD8+ cells were depleted by an antibody. In addition, cured mice were resistant to re-implantation of 66C14 cells not expressing mesothelin indicating that a long-term anti-tumor immunity was formed. To determine if pathogen associated molecular patterns contributed to the response, we combined anti-CTLA-4 with an inactive RG7787 and observed a major reduction in anti-tumor activity (P<0.01) indicating that the effect is not mediated by recognition of a foreign bacterial protein. Conclusions: We suggest that injecting immunotoxins into tumors is a useful approach to boost the activity of anti-CTLA-4. Based on the synergistic anti-tumor effect the effect is not mediated by recognition of a foreign bacterial protein.

#60 Reducing toxicity of antibody-drug conjugates through modulation of pharmacokinetics. Jessica Simmons, Francisco Zapata, Haley Neff-Laford, Joshua Hunter, Julia Cochran, Patrick Burke, Robert P. Lyon. Seattle Genetics, Bothell, WA.

Antibody-drug conjugates (ADCs) continue to emerge as effective therapeutics in a variety of oncology indications. Research on ADCs has revealed that the physicochemical properties of the drug-linker component can exert a significant impact on the disposition of the ADCs, particularly at higher levels of drug loading. We have recently reported (Nature Biotechnology 33, 733-735 (2015); Molecular Cancer Therapeutics, manuscript accepted) that these properties can be modulated through the judicious incorporation of small, discrete PEG chains of varying lengths into a monomethyl auristatin E (MMAE) drug-linker. Heterogeneous DAR 8 ADCs prepared with these drug-linkers using native cysteine conjugation display a continuum of pharmacokinetic behaviors that mirror the length of the incorporated PEG chain. We selected four of these drug-linkers that span the range of observed pharmacokinetics and used them as model compounds to evaluate the impact of ADC clearance on the concentration profile of released MMAE in normal tissues and consequent toxicity in the Sprague-Dawley rat. Faster clearing ADCs (prepared with drug-linkers containing very short or no PEG modifier) produced higher tissue MMAE Cmax values at early post-dose time points relative to slower clearing ADCs that incorporate longer PEG chains. This finding indicates that MMAE concentrations in tissues are proportional to the rate at which the ADC is catalyzed and thus which converts the conjugated payload into free drug. Faster clearing ADCs also exhibited diminished tolerability, with greater histologic depletion of bone marrow and more dramatic decreases and/or delayed recovery in select peripheral hematologic parameters. These results provide a strategy for reducing the non-antigen-mediated toxicity of ADCs through modulation of pharmacokinetics.


Antibody drug conjugates (ADCs) combine the specificity of monoclonal antibodies with potent antineoplastic small molecules, and promise efficacy without the systemic toxicity of chemotherapy. Despite this tremendous potential, most clinical ADCs have failed to provide sufficient therapeutic benefit before the onset of off-target dose-limiting platform toxicities. Here we report the development of proprietary pro tease cleavable N-acyl sulfonylimidate linked hemiester linker and auristatin payloads, Zymelink, that allow the generation of an efficacious ADC platform with improved tolerability. Both Zymelink drug-linkers are covalently connected via maleimide to endogenous cysteines. As Zymelink drug-linkers are more polar than maleimide valine citrulline monomethyl auristatin E-linker, the resulting ADCs can be produced with potential advantages in PK, efficacy and safety. The resulting ADCs exhibit potent in vitro cytotoxicity. A trastuzumab-based ADC prepared with Zymelink drug-linker and demonstrated at least equivalent efficacy compared to a trastuzumab-based ADC prepared with MMAE, promoting durable complete regressions in a patient-derived xenograft model. Zymelink hemiester linker and auristatin ADCs were tolerated at 5-6 fold higher doses compared to a MMAE ADC in cynomolgus monkeys. The maximum tolerated dose of the MMAE ADC was just 3 mg/kg based on severe neutropenia. Zymelink hemiester ADC was tolerated at 15

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mg/kg with no evidence of neutropenia or elevations in transaminases. Zymelink auristatin ADC was tolerated at 18 mg/kg based on increased levels of transaminases at 24 mg/kg. Moreover, Zymelink ADCs exhibited greater serum exposure at equivalent doses. These results suggest Zymelink ADCs have a greatly expanded therapeutic window compared to MMAE conjugates.


Background ATACs (antibody-targeted Amanitin conjugates) comprise a new class of antibody-drug conjugates utilizing aminatin as toxic payload. Amanatin binds to the eukaryotic RNA pol II and thereby inhibits the cellular transcription process at very low concentrations. In the current study, in vitro and in vivo data of new ATACs targeting CD19 (also known as B4, CVID3) are presented. CD19, a class I transmembrane glycoprotein with no significant homology to any known protein, is expressed in B cells and B-cell malignancies like in B-cell acute lymphoblastic leukemia (B-ALL) and B cell chronic lymphocytic leukemia (B-CLL). Therefore it is an ideal target for Amanitin-based ADCs. Material and methods Cell lines: Raji (Burkitt Lymphoma), Nalm-6 (B-ALL) and HL-60 (B-CLL). Therefore it is an ideal target for Amanitin-based ADCs. CD19-Thiomab-ATACs: Maleimide aminatin compounds were conjugated site specific to engineered cysteine residues of the anti-CD19 Thiomab. Cell proliferation assay: Quantitative determination of cytotoxicity was performed by CellTiter Glo 2.0 assay (Promega) or WST-1 assay (Roche). Animal models: Mouse xenograft tumor models were performed. Tolerability was assessed in mice and non-human primates (NHP). Results CD19-Thiomab-ATACs showed in vitro cytotoxicity on CD19- cells in lineicromolar range, whereas no cytotoxic activity on CD19 cells was observed. In mouse xenograft models, CD19 showed dose-dependent tumor regression and complete remission after single dose i.v. of 2mg/kg and 4mg/kg. In a disseminating Raji xenograft model, median survival was increased from 6 days (vehicle control) to 105 days (CD19-Thiomab-30.2115 treated group at 6mg/kg, single dose). On day 154, 4 animals were still alive and in good overall condition. Safety profiling in Cynomolgus monkey revealed a good tolerability after sequentially applied doses of 0.3, 1.0, 3.0 and 5.0 mg/kg. Hematology and clinical chemistry data show minor alterations: transient increase in liver enzymes (ALT and AST) in combination with a transient increase in LDH. The half-life of the ADC in serum is 7-11 days; the free toxin is detectable at serum levels close to the lower limit of quantification only (LLOQ = 1.2 nM). Conclusions In the current study, in vitro and in vivo data of Amanitin-ADCs targeting CD19 are presented. CD19 is expressed on cells of the B cell lineage, ranging from the pre-B cells until the terminal differentiation to plasma cells. It is expressed in most acute lymphoblastic leukemias (ALL), chronic lymphocytic leukemias (CLL) and B cell lymphomas (Kemeng et al. 2012); Exp Hematol Oncol 1: 36). The preclinical data show high cytotoxicity in picomolar range, very good efficacy in tumor xenograft models and tolerability in an exploratory tolerability study in NHP. The positive findings of these initial experiments encourage Heidelberg Pharma to further proceed with anti-CD19 Amanitin-based ADCs towards clinics.

#63 Targeting osteosarcoma with graphene oxide-associated anti-HER2 antibodies. Xinjian Chen. University of Utah, Salt Lake City, UT.

Osteosarcoma (OS) is the most common primary cancer of the bone affecting children and adolescents. While over the last two decades the neoadjuvant chemotherapy has improved survival of the patients with resectable OS, the prognosis for unresectable or recurrent tumors remains poor due to the lack of effective treatment. Given that a majority osteosarcomas overexpress HER2, a phase II clinical trial of anti-HER2 antibody trastuzumab in conjunction with chemotherapy has been carried out to treat metastatic OS. However, no significant therapeutic benefit was observed. We recently reported that association of anti-CD20 antibody rituximab with a nanomaterial graphene oxide (GO) substantially enhances the anti-lymphoma activity of the antibody. Here we have studied antimur activity of GO-associated trastuzumab (TRAs). Similar to RTX, TRA could be stably associated with functionalized GO through non-covalent interactions, and GO-associated TRA (TRA/GO) showed markedly enhanced HER2 binding activity with capacity to aggregate (cap) HER2 on the target cells. Treating HER2 + OS as well as Ewing’s sarcoma cell lines in culture with TRA/GO resulted in rapid sarcoma cell death within 12 hr, while free TRA or the cytotoxin-conjugated TRA (ado-trastuzumab emtansine) showed no significant cytotoxic effects. TRA/GO manifested no cytotoxicity to human lymphocytes, in contrast to chemotherapeutic drugs such as doxorubicin and oxaliplatin. We find that the ability to kill the sarcoma cells results from the unique capacity of TRA/GO to simultaneously induce oxidative stress as well as intense detrimental HER2 signaling, which leads to a complete disappearance of a major tyrosine-phosphorylated protein and caspase 8, and a partial loss of RIPK1 along with an increase in RIPK3 levels within 5 min, followed by necroptosis of the target cells. Intravenous administration of TRA/GO rapidly eradicated established xenograft human OS in the lungs as well as at subcutaneous locations in NOD/SCID immunodeficient mice in the absence of chemotherapy, resulting in indefinite survival of the animals. In contrast, free TRA treatment failed to do so. No appreciable side effects were observed of TRA/GO in vivo. These results therefore demonstrate a novel strategy to substantially enhance the therapeutic capacity of anti-cancer antibodies. Given the increasing variety of human malignancies that are found to overexpress Her2, including sarcomas, carcinomas and neuroendocrine tumors, our findings could have broad therapeutic implications. As TRA/GO does not harm lymphocytes, TRA/GO-based therapy may constitute a unique opportunity to implement immunotherapy that is now known to play an important role in control tumor progression.

#64 Meditope SnAP-body technology facilitates enhanced internalization and in vivo efficacy of antibody therapeutics. Karin Forster, Elisabeth Gardiner, Stephanie Hsieh. Meditope Biosciences, San Diego, CA.

Meditope Biosciences has developed a way to use SnAP technology to promote internalization of antibody receptor complexes through a novel construct called a SnAP-body. Meditope’s SnAP platform functionalizes monoclonal antibodies to bind to specific meditope peptides; this property can be used to directly facilitate receptor crosslinking when meditope-enabled antibodies are bound to cell surface receptors. SnAP-bodies are self-crosslinking meditope-enabled antibodies which permit a meditope peptide to specifically interact with a meditope-enabled antibody at the cell surface upon antigen engagement. Antigen-antibody complex formation through the specific contact of the SnAP-bodies and their cognate epitopes on the target antigen can increase clustering or co-localization of a cell surface antigen on a cell, which can promote enhanced internalization of the SnAP-body. The increased internalization can be used to deliver payload to a cell surface antigen from a target cell or tumor. Enhancing internalization can increase the efficacy of antibody-based therapeutics, and in the case of antibody-drug conjugates, which require receptor internalization to deliver cytotoxic payloads, can reduce the amount of drug necessary to achieve a therapeutic effect. In vitro studies of SnAP-body binding to cell surface antigens demonstrate accelerated internalization of the antibody. In vivo, this translates into enhanced efficacy. The ability to see equivalent efficacy in the absence of toxin suggests a unique role for SnAP-bodies in cancer therapeutics, especially in cases where lack of efficacy for a specific target is correlated with poor receptor internalization.


The random conjugation of toxins, dyes, peptides, or other payloads to monoclonal antibodies often targets free thiol groups generated by partial reduction methods or lysine residues using succinimide- or isothiocyanate-based chemistry. There remains a need for conjugation technologies targeting specific amino acid residues as a way to produce a homogeneous antibody-drug conjugate (ADC) product with a defined drug-to-antibody ratio (DAR). Our RESidue-SPECifíc Conjugation Technology (RESPECT) utilizes two methods by which payloads can be conjugated to specific residues in an antibody. Our cystine-specific conjugation method exploits a unique intrachain disulfide bond in the light chain of rabbit antibodies between residues 80 and 171 of the variable and constant domains, respectively. Our humanization strategy allows retention of the cysteine at position 80 with a free thiol group that is both amenable for residue-specific conjugation and compatible with optimal antibody biophysical properties including antigen binding and structural stability. This platform has been optimized via antibody engineering strategy stems from in silico modeling, extensive mutagenesis, and crystallographic studies, which have allowed defining the contribution of neighboring residues to the retention of a reactive thiol group as well as the desired humanized antibody’s properties. Our C-terminal lysine-specific linkage method employs the transglutaminase enzyme that catalyzes the formation of a stable isopeptide bond between the γ-carboxyamide group (acyl donor) of a glutamine and the ε-amino group (acyl acceptor) of a lysine. While we found no acyl acceptor sites in recombinant wild-type IgGs, all antibodies investigated lacked the C-terminal Lys447 due to cleavage by carboxypeptidase B in the antibody production cell line. Blocking the cleavage of Lys447 by addition of a C-terminal amino acid resulted in transamidation of Lys447 by a variety of acyl donor substrates in the presence of any non-acidic, non-proline amino acid residue at position 448. Antibody-drug conjugates (ADCs) prepared using our RESPECT

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technology targeting the tumor associated-mesothelin protein produced uniform drug-to-antibody ratios (DAR) and were shown to be highly potent and specific in vitro and effective in vivo in reduction of tumor growth in an orthotopic mesothelioma expressing xenograft tumor model.

**#66 Highly potent, anthracycline-based antibody drug conjugates generated by enzymatic, site-specific conjugation.** Roger R. Beerli. NBE-Therapeutics AG, Basel, Switzerland.

Antibody drug conjugates (ADCs) are highly potent and selective anti-tumor drugs, combining the specific targeting of monoclonal antibodies with the potency of cytotoxic payloads. Here, we employed enzymatic, site-specific conjugation to generate homogenous ADCs based on a derivative of the highly potent anthracycline toxi-PNU-159682 and a non-cleavable peptide linker, using the anti-HER-2 antibody trastuzumab (part of trastuzumab emtansine) and the anti-CD30 antibody CA10 (part of brentuximab vedotin). Characterization of the resulting ADCs in vitro and in vivo showed that they were highly stable and exhibited potent antitumor activity; showing that of ADCs based on conventional tubulin-targeting payloads, such as trastuzumab emtansine and brentuximab vedotin. Antitumor activity in an immune-competent host involved activation of the immune system, as shown by evaluation of a trastuzumab-PNU ADC in a Kadcyla-resistant HER2-positive orthotopic breast cancer model. CD74 T cells severely impaired the antitumor activity of the ADC, demonstrating an important role for T cells in driving tumor regression. Furthermore, when tumor-free animals were rechallenged with the same tumor, tumor growth was entirely inhibited in the absence of any further ADC administration, indicating the development of an immunologic memory. In summary, we present a novel ADC format enabled not only with highly potent cytotoxicity, but also with effective immune-stimulatory functions.


CD74 is a type II transmembrane glycoprotein involved in the formation and transport of MHC class II protein. CD74 is highly expressed in many B-cell malignancies with limited expression in normal tissues (Stein et al., CCR 2007). STRO-001 is a novel CD74-targeting ADC containing an anti-CD74 aglycosylated human IgG1 antibody (SP7219) conjugated to a non-cleavable dibenzocyclooctyne (DBCO)-maytansinoid linker-warhead. SP7219 was discovered from a Fab ribosome display library based on Sutoro’s Xpress sift technology. Highly efficient site-specific conjugation enabled by our cell-free antibody production and click chemistry results in a well-defined homogeneous ADC drug product with a drug-antibody ratio (DAR) of 2. Conjugation sites were selected based on highest stability both in vitro and in vivo, thereby limiting loss of drug moiety from STRO-001 in circulation. Due to its limited cell permeability, the major catabolite released by STRO-001 has 1000X lower cell killing activity on CD74 positive and negative cells compared to the reference cytotoxic maytansine. CD74-positive and negative cells compared to the reference cytotoxic maytansine. STRO-001 further improves tumor suppression in a xenograft model in vitro and in vivo, thereby limiting loss of drug moiety from STRO-001 in circulation. Due to its limited cell permeability, the major catabolite released by STRO-001 has 1000X lower cell killing activity on CD74 positive and negative cells compared to the reference cytotoxic maytansine.

**#68 Methotrexate prevents primary immune responses against recombinant immunotoxins in murine models.** Emily M. King, Ronit Mazor, Ira Pastan. National Cancer Institute, NIH, Bethesda, MD.

Recombinant immunotoxins (RTIs) are composed of a tumor antigen-targeting antibody fragment fused to a portion of Pseudomonas exotoxin A. RTIs have been studied extensively in clinical trials for patients with hematological malignancies. The CD22 targeting RTI Moxetumumab Pasudotox has achieved overall response rates of 86% and complete remission rates of 46% in patients with relapsed/refractory Hairy Cell Leukemia. However, the therapeutic efficacy of RTIs against solid tumors is limited by their immunogenicity in immune-competent patients. In clinical trials to treat mesothelioma patients with SS1P, a RTI target- ing mesothelin, 90% of patients developed neutralizing antibodies against SS1P after one cycle of treatment. When immunosuppressive chemotherapy and SS1P were combined, more cycles of RTI could be given and several patients with advanced chemo-refractory mesothelioma had striking tumor regressions. This implicates high therapeutic potential for RTIs against solid tumors once immunogenicity is surmounted. Methotrexate (MTX) is a folate antagonist which interferes with purine biosynthesis, and is used to treat osteosarcomas and other cancers. MTX also interferes with T cell responses and is used to treat autoimmune diseases. Based on its immunosuppressive properties, Joly et al. demonstrated that low-dose MTX prevented the formation of ADAs against recombinant human alglucosidase alfa in mice in an antigen-specific manner. We hypothesized that MTX would similarly prevent the formation of ADAs against RTIs that target antigen-specific proteins. To test our hypothesis, we treated mice with the mesothelin-targeting RTI RG7787 with or without MTX given 0, 24, and 48 hours after RG7787 treatment. Serum was collected and anti-RG7787 ADAs were measured by direct ELISA. We found that six doses of RG7787 combined with low dose MTX (1 mg/kg) inhibited the formation of ADAs against RG7787. This inhibition was sustained through six challenges with RG7787 without additional MTX. Further, we found that immunization with RG7787 plus MTX induced RG7787-specific tolerance, and had no effect on the ADA response against an antigen-specific protein, ovalbumin. We conclude that combination of MTX and RG7787 is effective at preventing primary immune responses in a durable, antigen-specific manner. We propose to combine this agent in immune-competent cancer patients receiving RTI therapy to prevent RTI immunogenicity.

**#69 Anti-podocalyxin cancer-specific monoclonal antibody: preclinical study.** Shinji Yamada, Tohoku University, Sendai, Japan.

Background: Podocalyxin, a CD34-related sialomucin, is expressed in many tumors including colorectal cancers, breast cancers, mesothelial tumors, and brain tumors. Overexpression of podocalyxin has been reported to be an independent predictor of progression, metastasis, and poor outcome. However, PODXL1 has not been used as an antigen-specific target in normal or tumor cells, including many epithelial cells and endothelial cells; therefore, podocalyxin could not be a target of antibody therapy. Although many monoclonal antibodies (mAbs) against podocalyxin have been established, they bind to both cancer and normal cells. We recently established a novel technology for developing cancer-specific mAbs (CasMabs), which could target only cancer cells although those membrane proteins are highly expressed in both cancer and normal cells. Methods: We first produced a podocalyxin-expressing glioblastoma cell line. We purified human podocalyxin using PA-tag, and immunized mice with those proteins. CasMabs were screened using flow cytometry against podocalyxin-expressing cancer cells and podocalyxin-expressing normal cells. The cancer specificity was confirmed using immunohistochemistry against breast cancer tissues. Furthermore, a human-mouse chimeric anti-podocalyxin mAb was produced. Antibody-dependent cellular cytotoxicity (ADCC) was investigated in vitro using glioblastoma cells as target cells and human NK cells as effector cells. In vivo efficacy was evaluated using xenograft models of podocalyxin-expressing cell lines. Furthermore, we investigated the toxicity of chPcMab-6 using cytomegalovirus monkeys. Results: A cancer-specific anti-podocalyxin mAb (clone: PmC-mab; mouse IgG1, kappa) was established. PcM-b6 reacted with podocalyxin-expressing many cancer cell lines, 90% of brain tumors, and neutralizing antibodies against Podocalyxin were not found in histology and relative organ weight on postmortem examination.
Conclusion: ChiPcMab-6, a cancer-specific human-mouse chimeric anti-podocalyxin mAb could be useful for targeting podocalyxin in cancer, although podocalyxin is highly expressed in many normal cells.


Antibody-drug conjugates (ADCs) continue to emerge as effective therapeutic agents and ADC-related toxicities have been attributed to the high local concentration of the released drug. Here we present a systematic study of the relationship between ADC pharmacokinetics, even at high levels of drug loading (Nature Biotechnology 32, 1059-1062 (2014), Nature Biotechnology 33, 733-735 (2015)). We have now prepared drug-linkers of monomethylauristatin E (MMAE) that orthogonally employ these features to enable a systematic evaluation of the relative contributions of maleimide instability and accelerated plasma clearance on the in vivo behavior of ADCs. Biodistribution studies with these molecules have revealed that the concentration of released MMAE in normal tissues is greatly impacted by the rate of ADC clearance (fast clearance results in greater Cmax of released MMAE in normal tissues is greatly impacted by the rate of ADC clearance (fast clearance results in greater Cmax of released MMAE in normal tissues is greatly impacted by the rate of ADC clearance (fast clearance results in greater Cmax of released MMAE). One such property that is now well appreciated are the reversibility of maleimide-based drug conjugation, and the impact of drug conjugation on the pharmacokinetics of the ADC. We recently reported advances in drug-linker design that independently address both of these properties, resulting in the irreversible conjugation of drugs which have minimal impact on antibody pharmacokinetics, even at high levels of drug loading (Nature Biotechnology 32, 1059-1062 (2014), Nature Biotechnology 33, 733-735 (2015)). We have now prepared drug-linkers of monomethylauristatin E (MMAE) that orthogonally employ these features to enable a systematic evaluation of the relative contributions of maleimide instability and accelerated plasma clearance on the in vivo behavior of ADCs. Biodistribution studies with these molecules have revealed that the concentration of released MMAE in normal tissues is greatly impacted by the rate of ADC clearance (fast clearance results in greater Cmax of released MMAE). These results suggest that ADC pharmacokinetics dominate the biodistribution and toxicity profiles for a given drug payload, with conjugate stability playing a relatively minor role.


Folate receptor α (FRα) is an antigen that is overexpressed on the cell surface of solid tumors including ovarian cancer. The differential expression on cancer cells makes FRα an attractive target for antibody-drug conjugates (ADCs), and an ADC targeting FRα, Mirvetuximab soravinsine, has demonstrated promising anti-tumor activity and safety profiles in the clinic. Here, we employed a new linker (NL) to enhance the bystander activity of ADCs, which is the ability of ADCs to generate cell-permeable catabolites that can diffuse into and kill proximal cancer cells with little or no target expression. With the goal of improving the potency of anti-FRα ADCs in solid tumors with heterogeneous FRα expression, we constructed the M9346A-NL-DM. M9346A-NL-DM is a conjugate of the tubulin-binding venom factor (CVF) that undergoes lysosomal cleavage followed by self-immolation to generate free DM that can readily penetrate neighboring cancer cells. Correspondingly, M9346A- NL-DM showed enhanced bystander cytotoxic activity against proximal antigen-negative cells in vitro. In the xenograft tumor models in vivo, M9346A- NL-DM demonstrated enhanced efficacy against tumors with heterogeneous expression of FRα. Interestingly, M9346A- NL-DM also showed improved antitumor activity against a tumor model with homogeneous expression of FRα, possibly due to better tumor penetration of the cell-permeable catabolite. In summary, M9346A- NL-DM is a novel ADC with enhanced bystander activity and antitumor activity that can target tumors with heterogeneous expression of FRα.

#72 Induction of immune tolerance to recombinant immunotoxin LMB-100 using synthetic vaccine particles encapsulating rapamycin. Ronit Mazor, Emily King, Takashi Kei Kishimoto, Ira Pastan. National Cancer Institute, Bethesda, MD; Selecta Bioscience Inc, Boston, MA.

Recombinant Immunotoxins (RTIs) are genetically engineered proteins designed for cancer therapy. LMB-100 is a second generation RIT that is composed of a humanized Fab targeting mesothelin and a de-immunized fragment of Pseudomonas Exotoxin A. Due to the bacterial origin of the toxin, LMB-100 is immunogenic, although less immunogenic than first generation immunotoxins. Almost all patients treated with LMB-100 made anti-drug antibodies (ADAs) after two or more cycles of treatment that neutralized the RIT and greatly lowered efficacy. Kishimoto et al. demonstrated that Synthetic Vaccine Particles containing Rapamycin (SVP) inhibited the formation of ADAs, when administered with a foreign protein such as KLH or Pegsiticase. The SVP-R are taken up by macrophages and dendritic cells and increase the number of regulatory T cells in vivo. Here we report that SVP-R can reduce the reactivity of pre-existing ADAs. However, when mice with pre-existing ADAs were treated with LMB-100 and SVP-R, anti-tumor activity was restored and ADAs suppressed (P<0.0001). SVP-R are being evaluated in humans to prevent ADA to Pegsiticase, an enzyme for refractory gout treatment. Our data indicates that combining SVP-R with LMB-100 should be useful in treating cancer by allowing more treatment cycles and better efficacy. This approach can be used to increase the efficacy of other immunogenic agents such as CAR-T cells, antibody drug conjugates and viral gene therapy vectors.

#73 Role of macrophages in the antitumor activity of an anti-transferrin receptor 1 antibody ch128.1 in a xenograft model of multiple myeloma. Lai Sum Leoh, Yoon Kyung Kim, Pierre V. Candelaria, Otoniel Martinez-Maza, Tracy R. Daniels-Wells, Manuel L. Penichet. UCLA, Los Angeles, CA.

The transferin receptor 1 (TfR1), also known as CD71, is a membrane coprotein involved in cellular iron uptake and regulation of cell growth. The high level of TfR1 expression on malignant cells and its key role in cancer cell pathology make this receptor an attractive target for antibody cancer therapy. We previously developed a mouse/human chimeric IgG3 specific for human TfR1 (ch128.1). This antibody exhibits direct cytotoxicity against certain human malignant B cells in vitro through TfR1 degradation and iron deprivation. Importantly, ch128.1 shows remarkable anti-tumor activity in xenograft models of disseminated multiple myeloma (MM) in immunosuppressed mice (SCID-Beige). Interestingly, this anti-tumor protection was observed even against MM cells (KMS-11 cells) that show no sensitivity to this antibody in vitro, suggesting the in vivo contributions of antibody effector functions. This possibility was supported by the lack of anti-tumor protection observed using a ch128.1 Fc mutant with impaired binding to FcγRs and to the complement component C1q. To examine host effector functions involved in ch128.1-mediated protection in our mouse model bearing KMS-11 tumors, depletion studies of complement and macrophages were performed. Complement depletion using cobra venom factor (CVF) did not affect protection, suggesting that complement-mediated cytotoxicity (CDC) is not a relevant mechanism of action. Notably, we now report that macrophage depletion using clodronate liposomes (clodrolip) significantly reduced protection, suggesting that these effector cells play a relevant role in the anti-tumor activity. Consistent with this result, we also report that ch128.1 is capable of eliciting antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cell-mediated phagocytosis (ADCP) against KMS-11 cells in the presence of the murine macrophage cell line J774.2 or murine bone marrow-derived macrophages. To examine the role of iron deprivation in ch128.1-mediated tumor death in vivo, animals treated with ch128.1 were systemically supplemented with iron in a preliminary study. However, no differences in survival were observed, suggesting that iron deprivation is not a contributor to the effects of ch128.1 in our in vivo model or the iron dose tested was not optimal. Our results suggest that macrophages play a key role in ch128.1-mediated anti-tumor protection in our model and that ch128.1 can be an effective therapy of incurable human B-cell malignancies such as MM.
#74 Discovery of new therapeutic monoclonal antibodies to challenging GPCRs, ion channels and transporters. Lewis J. Stafford, Ross Chambers, Shar hon R. Willis, Moniquetta Hall, Brad Screnci, Manu Mabila, David Tucker, Trevor Barnes, Rachel Fong, Andrew Ettemper, Jennifer Pfaff, Chidananda Sulli, Nicholas Molino, Andrew Hudacek, Benjamin J. Doranz, Joseph Rucker. Integral Molecular, Marlborough, MA.

The objective of this work was to evaluate the ability to generate panels of monoclonal antibodies against a set of highly challenging targets including GPCRs (CB1, C5AR, CXCR5 and CGRPR), transporters (GLUT4), and ion channels (P2X3). Integral membrane proteins are important drug targets and monoclonal antibodies (mAbs) directed against them are highly sought for therapeutic applications. However, the complexity of membrane protein targets makes the discovery of these mAbs especially challenging. To address this need, Integral Molecular has developed the MPS Discovery Engine to enable the isolation, characterization, and engineering of monoclonal antibodies for GPCRs, ion channels, and transporters. MPS utilizes a collection of technologies to address each of the barriers to monoclonal antibody development against the native extracellular epitopes of multispan membrane proteins. These include, antigen engineering to attain high levels of surface expression, DNA and Lipoapaptite immunization to present native epitopes to the immune system, diverse immunization host species to deal with highly conserved proteins, Lipoparticles (high concentration native membrane proteins) to enable phage display and microfluidic B-cell isolation, and shotgun mutagenesis (comprehensive and in situ) for epitope mapping. Using the MPS Discovery Engine we were able to successfully generate large panels of antibodies to the targets that were able to bind to the native extracellular epitopes on cells by flow cytometry. A subset of the antibodies had antagonist activity. With this technology we have the ability to target intact, conformation specific, and functional antibodies to complex membrane proteins.
Antibody-drug conjugate (ADC) occurs in a highly efficient manner following the specific binding of the meditope to the enabled antibody. To test the hypothesis that photactivation would be an efficacious and homogeneous way to make an ADC, meditope-enabled ADCs were designed and tested in vitro and in vivo. Photocytotoxicity was tested as an efficacious interaction following short-term exposure of the antibody peptide conjugates to a nondestructive wavelength of 340 nm. Photocytotoxicity proved to be a highly efficient and scalable way to make an ADC. Payload stability was confirmed in vivo with multiple toxins and in vivo potency of two unique ADCs were tested in an EGF receptor positive gastric cancer xenograft as well as a HER2 positive ovarian cancer xenograft. The meditope-enabled photogenerated anti-HER2 ADC was benchmarked against conventionally conjugated T-DiM and showed equivalent efficacy, but with the site-directed conjugated meditope ADC using less toxin. Meditope-directed photocytotoxicity with a modified ADC, enabling a cell cycle independent mechanism of action. The contribution of resistant to T-DM1, T-MMAE, DM1, MMAE and taxol treatment. In addition, or an anti-HER2 C6.5 diabody-deBouganin fusion protein. C6.5 diabody-deBouganin can overcome these mechanisms of resistance and may lead to cell death. When conjugated to trastuzumab (T-deB) or genetically activated Protein (RIP) that when internalized blocks protein synthesis thereby leading to cell death. When conjugated to trastuzumab (T-deB) or genetically attached to the C6.5 diabody, deBouganin was more potent than DM1 and unaffected by mechanisms of resistance to which DM1 is susceptible. To further highlight the differentiating mechanism of action of deBouganin, HCC1419 and BT-474 tumor cells that survived T-DM1 or trastuzumab-MMAE (T-MMAE) treatment in vitro were re-exposed to T-DiM, T-MMAE, or treated with T-deB or an anti-HER2 C6.5 diabody-deBouganin fusion protein. C6.5 diabody-deBouganin and T-deB were potent against HCC1419 and BT-474 cells surviving T-DiM or T-MMAE treatment. However, the surviving cell populations were resistant to T-DiM, T-MMAE, DM1, MMAE and taxol treatment. In addition, cross-resistance was seen against trastuzumab-diocarmycin in which contains a payload with a cell cycle independent mechanism of action. The contribution of multi-drug resistance, Bcl-2 family members and other survival pathways accounting for the resistant phenotype will be discussed. Overall, the data suggest that treatment with chemotherapeutics or ADCs comprised of small molecule compounds such as anti-microtubule agents, can lead to the outgrowth of tumor cells resistant to similar agents. In contrast, antibodies and antibody fragments armed with deBouganin can overcome these mechanisms of resistance and may therefore represent a more effective treatment option.
#82 MiR-206-mediated c-MET suppression modulates BCRP/ABCG2 lev-
erals in NRF2-silenced cancer cells. Bo-hyun Choi, In-geun Ryoo, Donghyeok
Kim, Sujin Lee, Mi-Kyoung Kwak. The Catholic University of Korea, Bucheon,
Gyeonggi-do, Republic of Korea.

Breast cancer resistance protein (BCRP/ABCG2), a xenobiotic efflux trans-
porter, is responsible for anticancer resistance in tumors. NF-E2-related factor 2
(NRF2) is a critical transcription factor in cellular defense system by regulating
the expression of antioxidant and detoxifying enzymes; however, its overexpres-
sion is often associated with tumor resistance to chemotherapy. In the present
study, we demonstrate that NRF2 modulation affects the expression of hepato-
cyte growth factor receptor (HGF/c-MET) and consequently suppresses BCRP/ABCG2 activity in cancer cells. Interfering RNA-mediated inhibition of
NRF2 in both ovarian carcinoma SKOV3 and renal carcinoma A498 cell lines reduced the expression of c-MET and this was accompanied by BCRP/
ABCG2 down-regulation. The treatment of cells with pharmacological or ge-
nic inhibitor of c-MET decreased BCRP/ABCG2 level and subsequently in-
creased intracellular accumulation of doxorubicin and Hoechst 33342,
netic inhibitor of c-MET decreased BCRP/ABCG2 level and subsequently in-
crease in PDAC cells blocks CTGF expression and under co-cultured conditions pre-
vent the growth of alpha-smooth muscle actin (a-SMA)-positive stellate cells,
which is critical for pancreatic stroma growth. In previous studies, CCN1 abla-
tion upregulates dCK expression in PDAC cell lines as compared to CCN1
expressed PDAC cells. These two events enhance the anti-proliferative effect of
GEM and can be rescued by CTGF-treatment or blocking dCK. In conclusion,
CCN1 promotes GEM-resistance in PDAC cell through the regulation of CTGF
and dCK and the mechanistic insights provided by these studies may help in
designing future therapeutic strategies to combat PDAC.

#83 Irinotocin resistance in type 2 interleukin-1 receptor overexpressed
colorectal cancer cells is overcome by inhibitor of MEK. Ai-Chung Mar,1
Chun-Ho Choi,1 An Chang Lee.2 Academia Sinica - Inst. of Biomedical Sci.,
Taipei, Taiwan; 2Department of Surgery, Taipei, Taiwan.

We have previously demonstrated that the expression of interleukin-1 recep-
tor type II (IL1R2) is closely associated with the advanced staging and distant
metastasis in patients with CRC. We also found that enhanced expression of IL1R2 played certain roles to resist the targeted therapeutics, regorafenib, in CRC cells. Whether IL1R2 expression is associated with the resistance to che-
motherapeutics is unclear. Irinotecin, a water-soluble and semisynthetic deriv-
ant of camptothecin, is one of widely used first- and second-line chemothera-
peutics for treatment of patients with metastatic colorectal cancer (CRC). Her-

experimental evidence, transient and stable expressions of miR-206 in SKOV3 and A498 cells repressed c-MET and BCRP/
ABCG2 levels. In addition, NRF2 knockdown cancer cells expressed higher lev-
els of miR-206 compared to the control cells, and the treatment of NRF2 knock-
down cells or the non-silencing inhibitor of c-MET could repress miR-206 and BCRP/
ABCG2 levels. Collectively, our results showed that the NRF2 silencing-mediated miR-
206 regulation could suppress BCRP/ABCG2 levels through c-MET modula-
tion, which is providing an additional evidence of chemosensitization of tumor
cells by NRF2 inhibition.

#84 CCN1/Cyr61 regulation of gemcitabine-resistant phenotype in pan-
creatic cancer: involvement of CTGF and dCK. Vijayalaxmi G. Gupta, Gargi
Maiti, Inamul Haque, Sushantia K. Banerjee, Snigdha Banerjee. Kansas City VA
Medical Center and University of Kansas Medical Center, Kansas City, KS.

With an estimated half a million new cases and similar mortality rates for
2016, pancreatic ductal adenocarcinoma (PDAC) remains a life-threatening and
challenging disease to diagnose and treat. As per American Cancer Society
(ACS), varying efficacy in different patients has led to an increase in the mortal-
ity rate of PDAC. Gemcitabine (GEM) remains the drug of choice either alone or
in combination, but is unsuccessful in reducing or curing PDAC in most pa-
tients. The limited efficacies of these drugs are due to the acquisition of chemo-
resistant characteristics of PDAC. Although several molecular and physiological
factors have been shown to correlate with the GEM-resistance, defined mole-
cular mechanism(s) of GEM-resistance remains a mystery. Previous studies have
shown that CCN1, which is overexpressed in PDAC and known to associate with
PDAC progression, is critical for drug resistance. Here, we found that while the
pancreatic cancer cell lines (i.e., Panc-1 and AsPc1) in which CCN1 is over-
expressed are typically GEM-resistant, the knocking down of CCN1 makes them
sensitive to GEM. Mechanistic studies revealed that CCN1 regulates two impor-
tant transcription factors: CTGF and dCK. These include cancer cell-secreted connective tissue growth factor (CTGF), a regulator of desmoplasia, and Deoxycytidine kinase (dCK), an enzyme that en-
hances gemcitabine sensitivity and efficacy in cancer cells. The deletion of CCN1 in
PDAC cells blocks CTGF expression and under co-cultured conditions pre-
vent the growth of alpha-smooth muscle actin (α-SMA)-positive stellate cells,
which is critical for pancreatic stroma growth. In previous studies, CCN1 abla-
tion upregulates dCK expression in PDAC cell lines as compared to CCN1
expressed PDAC cells. These two events enhance the anti-proliferative effect of
GEM and can be rescued by CTGF-treatment or blocking dCK. In conclusion,
CCN1 promotes GEM-resistance in PDAC cell through the regulation of CTGF
and dCK and the mechanistic insights provided by these studies may help in
designing future therapeutic strategies to combat PDAC.

#85 Establishment of cabazitaxel-resistant prostate cancer cell lines. Atsu-
shi Mizokami,1 Kazuaki Machioka,1 Kouji Izumi,1 Maolaek Akeren,3 Arinboul
Natsadongji,1 Yoshifumi Kadono,1 Yuta Takezawa,1 Hiroaki Iwamoto,1 Evan T.
Keller.1 Kanazawa University, Kanazawa, Japan; 2University of Michigan, Ann
Arbor, MI.

Background: The final treatment for castration-resistant prostate cancer
(CRPC) is generally cabazitaxel treatment. However, once CRPC becomes resis-
tant to cabazitaxel, the patients are obliged to best supportive care. Therefore,
the elucidation of the mechanism of the cabazitaxel-resistance and the conquest
are important themes to improve the prognosis of the patients. We already
established paclitaxel/docetaxel-resistant prostate cancer, PC-3-TxR and
DU145-TxR cells, and characterized those cell lines previously. Then we further
tried to establish cabazitaxel-resistant prostate cancer cell lines, and character-
ized those cell lines. Materials and Methods: We established two cabazitaxel-
resistant cell lines, PC-3-TxR/CxR and DU145-TxR/CxR by increasing concentra-
tion of cabazitaxel from 1 nM to 30 nM gradually when PC-3-TxR and
DU145-TxR cells were passaged. We confirmed the IC50 of docetaxel and cabaz-
itaxel of these cells. Next we purified total DNA and performed cDNA microar-
ray (Agilent and compared gene expression profiles among these cells. Results
and Conclusion: IC50 of PC-3-TxR and PC-3-TxR/CxR for cabazitaxel was 1.3
nM and 15.4 nM, respectively. IC50 of DU145-TxR and DU145-TxR/CxR was
7.0 nM and 30.8 nM, respectively. Comparison of cDNA microarray between
PC-3-TxR and PC-3-TxR/CxR cells or between DU145-TxR and DU145-TxR/
CxR cells revealed that 4,470 genes in PC-3-TxR/CxR and 1,345 genes in
DU145-TxR/CxR were upregulated more than 3-fold and downregulated more
than 0.3-fold, respectively. Especially, although expression of ABCB1 (MDR) gene was upregulated in DU145-TxR by 500-fold compared with DU145 cells, it was not upregulated in
not upregulated in DU145-TxR/CxR cells any more. In contrast, expression of
ABCB1 gene was upregulated in PC-3-TxR by 20-fold compared with PC-3 cells
and it was further upregulated in PC-3-TxR/CxR by 40-fold compared with
PC-3. In conclusion, various genes were regulated in cabazitaxel-resistant
PCa cells and different mechanisms might be involved in this resistance in
different cell lines.

#86 Time course analysis of gene expression and epigenetic interactions in
acquired cetuximab resistance in head and neck squamous cell carcinoma.
Lanice T. Kagohara,1 Genevieve Stein-O’Brien,1 Siija Li,1 Manjusha Thakar,1
Ruchira Ranawera,2 Michael Considine,1 Joseph A. Califano,1 Christie H.
Chung,2 Daria Gaykina,2 Elana J. Fertig,1 Johns Hopkins University, Balti-
more, MD; 2Moffitt Cancer Center, Tampa, FL; 3University of California San
Diego, San Diego, CA.

The current study performs time course genomics and epigenomics profiling
to determine the complex dynamics of interactions between gene expression
and epigenetic changes that alter cellular signaling and drive acquired therapeu-
tic resistance. Targeted therapeutic agents block the activity of specific molecules
which are required for desmoplastic growth in pancreatic cancers. CCN1 is a critical
transcription factor in cellular defense system by regulating the expression of
antioxidant and detoxifying enzymes; however, its overexpression is often associated with tumor resistance to chemotherapy. In the present study, we demonstrate that NRF2 modulation affects the expression of hepato-
cyte growth factor receptor (HGF/c-MET) and consequently suppresses BCRP/ABCG2 activity in cancer cells. Interfering RNA-mediated inhibition of
NRF2 in both ovarian carcinoma SKOV3 and renal carcinoma A498 cell lines reduced the expression of c-MET and this was accompanied by BCRP/
ABCG2 down-regulation. The treatment of cells with pharmacological or ge-
nic inhibitor of c-MET decreased BCRP/ABCG2 level and subsequently in-
creased intracellular accumulation of doxorubicin and Hoechst 33342,
netic inhibitor of c-MET decreased BCRP/ABCG2 level and subsequently in-
crease in PDAC cells blocks CTGF expression and under co-cultured conditions pre-
vent the growth of alpha-smooth muscle actin (α-SMA)-positive stellate cells,
which is critical for pancreatic stroma growth. In previous studies, CCN1 abla-
tion upregulates dCK expression in PDAC cell lines as compared to CCN1
expressed PDAC cells. These two events enhance the anti-proliferative effect of
GEM and can be rescued by CTGF-treatment or blocking dCK. In conclusion,
CCN1 promotes GEM-resistance in PDAC cell through the regulation of CTGF
and dCK and the mechanistic insights provided by these studies may help in
designing future therapeutic strategies to combat PDAC.

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tance than the widespread alterations observed once the resistance is established. To test this hypothesis, we develop a novel time course experimental and bioinformatics model of acquired resistance. Specifically, this protocol enables weekly profiling of RNA, DNA, and proliferation as cetuximab resistance developed in vitro. We apply the CoGAPS bioinformatics algorithm to perform integrated analysis of gene expression and DNA methylation. This analysis discovered signatures of sustained activation of cellular pathways in response to treatment from signatures of clonal expansion associated with acquired cetuximab resistance. Despite identifying massive changes in gene expression driven by therapeutic response, only the expression changes associated with the acquired resistance, and not response to treatment, have corresponding epigenetic signatures. The genomic signature associated with acquired cetuximab resistance is enriched for gene targets of the AP-2 family of transcription factors. In a previous study, we found that these gene targets were overexpressed after short-term treatment in cetuximab sensitive cell lines and HNSCC tumors. Our data suggest that this family of transcription factors serves as a feedback mechanism to maintain homeostasis to growth factors receptor signaling. As a result, AP-2 transcription factors serve as a natural mechanism to cause subsequent therapeutic resistance with accumulation of genetic alterations. Further investigation is needed to determine the role of AP-2 transcription factors in acquired cetuximab resistance.

#87 Treatment schedules influence acquisition of Doxorubicin resistance through epigenetic mechanism in breast cancer cells. Logeswari Ponnusamy, Prathap Kumar S. Mahalingaiah, Kamaleshwar P. Singh. The Institute of Environmental & Human Health, Texas Tech University, Lubbock, TX.

Breast cancer is the leading cause of cancer-related mortality in women. Chemotherapy resistance is a major limitation in clinical treatment of breast cancer. Mounting evidence implicates the epigenetic aberrations in acquired chemoresistance. However, the influence of treatment schedules and associated epigenetic changes on acquisition of doxorubicin resistance in breast cancer is not clear. Therefore, objective of this study was to evaluate the impact of treatment schedules on acquisition of doxorubicin resistance and the molecular mechanism basis of this process in breast cancer cells. To address this question, two different treatment strategies viz, continuous exposure and intermittent exposure were used to generate doxorubicin resistant cells lines from ER-positive MCF-7 and triple negative MDA-MB-231 breast cancer cell lines. The level of resistance against doxorubicin and the temporal sequence of molecular changes associated with the resistance phenotype were evaluated at 4 months and 18 months’ following exposure to doxorubicin. Results revealed that intermittent exposure to doxorubicin resulted in significantly higher level of resistance as compared to continuous exposure. ER-positive MCF-7 cells developed relatively earlier and high level of resistance when compared to MDA-MB-231 cells. Additionally, the resistance to doxorubicin-induced cytotoxicity was associated with transient acquisition of EMT and CSC-like phenotype in breast cancer cells around 4 months following doxorubicin. Gene and protein expression analysis further revealed temporal changes in the expression of epigenetic regulatory genes and histone modifications respectively. The temporal changes in expression of genes were positively correlated with doxorubicin exposure time during resistance development. In summary, the result of this study for the first time suggests that the treatment schedules influence the acquisition of doxorubicin resistance potentially through epigenetic mechanisms. The findings of this study will be helpful in chemotherapy of breast cancer to achieve the best clinical result.

#88 The role of RBPMS in cisplatin resistant ovarian cancer. Perla M. Baez-Vega,1 Fatma Valiyeva,1 Ginette Santiago,2 Pablo Vivas-Mejia1.1 Université of Puerto Rico Comprehensive Cancer Center, San Juan, PR; 2University of Puerto Rico Medical Sciences Campus, San Juan, PR.

Ovarian epithelial cancer is the fifth leading cause of cancer–associated deaths among women in the United States. High-grade serous ovarian cancer (HGSC) is the most common type of epithelial ovarian cancer and accounts for 70-80% of ovarian cancer patient deaths. RNA-Binding Protein With Multiple Splicing (RBPMS) is the leading cause of cancer-related mortality in the United States. We hypothesized that RBPMS is highly expressed in A2780 cells when compared to the cisplatin resistance ovarian cancer cells and HGSOc cancer cells. Targeting RBPMs increased the invasion ability of the A2780 cells. Cisplatin resistant, A2780CP20 cells overexpressing RBPMs showed a decreased in cell proliferation when compared to the A2780CP20 empty vector control. Western blot and real-time PCR showed the downregulation of RBPMs expression profile of RBPMs in HGSOc and epithelial ovarian cancer cells. These studies will help to better understand the role of RBPMs in ovarian cancer and may support the use of RBPMs as an adjuvant treatment to overcome the platinum-based chemotherapy resistance characteristic of the high-grade serous ovarian cancer.

#89 Targeting Rho/MRTF regulated gene transcription in drug-resistant melanoma. Sean A. Misek,1 Scott D. Larsen,2 Kathleen A. Gollo,1 Richard R. Neubig1. 1Michigan State Univ, East Lansing, MI; 2University of Michigan, Ann Arbor, MI.

Much of the recent focus of melanoma targeted therapy has been on the ERK pathway, which is aberrantly activated in approximately 90% of melanoma tumors (over half of which express BRAFmut). Current targeted therapies such as vemurafenib (BRAFV600E inhibitor), or a combination therapy using dabrafenib (BRAFV600E inhibitor) and low dose trametinib (MEK inhibitor) shows profound initial effects in a majority of BRAFmut expressing tumors. However, these responses are often short-lived and resistances typically develops within months. Resistance to these targeted therapies can arise from multiple mechanisms, including activation of pro-survival signaling pathways parallel to the ERK pathway. The goal of this work is to identify pharmacologically targetable genetic alterations that may lead to subsequent therapeutic resistance without further development of drug resistance mechanisms so that effective combination therapies can be developed. Despite the clear role of the RhoA subfamily of Rho GTPases (RhoA/B/C) as melanoma oncogenes, their role in drug resistance is not well understood. It is challenging to develop small molecule inhibitors which directly target the activity of small Rho GTPases, so an alternative approach is to inhibit downstream pathways. Through modulation of the actin cytoskeleton Rho can induce gene transcription through multiple transcriptional co-activators including Myocardin-Related Transcription Factor (MRTF) and Yes-Associated Protein 1 (YAP). My bioinformatics analysis demonstrates that MRTF-A gene expression is correlated with poor overall survival in a large cohort of cutaneous melanoma patients. Furthermore, expression of a set of 216 MRTF target genes is enriched in dabrafenib/trametinib resistant cutaneous melanoma tumors compared to matched pre-treatment tumors, suggesting that MRTF activation may be involved in drug resistance. Based upon these results I hypothesized that small Rho GTPases may promote resistance to MAPK pathway targeted therapies through activation of MRTF/YAP. To test this hypothesis, I generated vemurafenib resistant melanoma cells through chronic exposure to vemurafenib. These vemurafenib resistant cell population is enriched for actin stress fiber positive cells, and these cells have increased Myosin Light Chain 2 (MLC2) phosphorylation, suggesting that there is increased Rho activation. Furthermore, these drug resistant cells are more sensitive to pharmacological inhibition of MRTF activity. These preliminary data suggest that vemurafenib resistant melanoma cells may be re-wired to depend on the Rho-induced gene transcription for their survival, and that a combination therapy simultaneously targeting these two pathways may be an effective treatment strategy for BRAF inhibitor-resistant melanomas.

#90 STING colocalizes with gamma-H2AX upon treatment of breast cancer cells with genotoxics: A new role in DNA repair. Laura Cherade,1 Julie Gaston,1 Vanessa Yvonnet,1 Olivier Deas,2 Marie-Franço Poupon,1 Jean-Gabriel Judde,1 Vincent Goffin,1 Stefano Cairo1. 1XerTech, Evry, France; 2Inserm U1151, Institut Necker Enfants Malades (INEM), University of Paris Descartes, Faculty of Medicine, Paris, France.

One of the current tumor immunotherapy strategies involves the use of STING agonist, a well-known inducer of interferon (IFN) signaling in the immune system, to promote tumor-rejection. However, recent evidence also indicates that constitutive activation of IFN signaling in the tumor may lead to a bad outcome. For instance, increased expression of IFN- stimulated genes (ISGs) at time of surgery was associated with early breast cancer recurrence, and an IFN-related DNA damage resistance signature (IRDS) was identified as a predictive marker of recurrence after radiotherapy. Thus, IFNs secreted into the tumor microenvironment may have complex opposite effects on tumor behavior and response to treatment. Using patient-derived xenograft (PDX) models, we previously showed that the IFN/STAT1 pathway was activated within breast cancer cells in response to chemotherapy and that this pathway may be involved in treatment resistance and recurrence. The aim of this study was to elucidate the mechanisms by which IFN-signaling is triggered in breast cancer cells following chemotherapy and how its activation leads to tumor survival and recurrence. To
this aim, the breast cancer cell line MCF7 was treated in vitro with mafosfamide and the activity of different IFN pathway effectors was monitored using western blot, immunofluorescence and cell fractionation techniques. We found that in breast cancer cells, similarly to what is observed in immune cells, type I IFN expression is triggered in a STING-dependent manner. STING silencing abrogated chemotherapy-induced cell death when type I IFN was induced, suggesting that STING mediates IFN-induced cytotoxicity through the activation of autocrine IFN pathways. This study also revealed that the STING pathway is essential for the IFN-mediated sensitization of breast cancer cells to the chemotherapeutic drug Doxorubicin. Importantly, the combination of STING agonists and chemotherapy demonstrated synergistic antitumor effects in vivo, highlighting the potential of this approach for the treatment of breast cancer.


INTRODUCTION: Drug resistance of leukemic stem cells is a surmountable obstacle to effective chemotherapy in acute myeloid leukemia (AML). P-glycoprotein (Pgp) and FLT3 undoubtedly contribute to worse prognosis and mechanisms of these proteins lead to shorter survival and chemotherapy resistance. Due to heterogeneity of stem cells in AML, outcome of patients with a normal karyotype is highly challenging and changes in drug resistance genes likely to be identified which will contribute to a better understanding of the disease biology, eventually leading to the development of alternative therapeutic approaches regarding drug resistance in AML. METHODOLOGY: Blood and Bone-marrow samples were collected from newly diagnosed adult AML patients with normal karyotype. Gene expression analysis of MDR genes like Pgp, MRPI, BCRP and LRP was done by qRT-PCR in FLT3-ITD +/- and CD34+/ - subgroups. Higher expression of Pgp amongst all MDR genes instigated to undertake in-silico analysis of Pgp with natural compound library using YASARA. MTT assay was used to find out IC50 value of natural compounds shortlisted from in-silico analysis and used alone or with cytarine on THP-1 cell line and in primary AML stem cells. Pgp inhibitory activity of natural compounds was assessed by fluorometric MDR assay. RESULTS: A significant upregulation of Pgp expression was observed in FLT3-ITD +/ -, CD34 + group after cytarine exposure during their induction (7 - 3) therapy. Docking analysis revealed curcumin, hesperidin and silimarin having better binding affinity with Pgp as compared to its known inhibitor Verapamil. Furthermore, these selected compounds were used in vitro, and results of MTT assay suggested 6.1µM, 40.73µM, 63.09µM and 54.95µM IC50 values of cytarine, curcumin, hesperidin and silimarin respectively in THP-1 cell line. Combination index (CI) analysis revealed synergistic effects with IC50 having synergistic effect with cytarine and decreased IC50 value of cytarine (1µM). In addition to this, Silimarin at 250nM and 500nM concentration showed equivalent inhibition of Pgp as compared to control Verapamil. CONCLUSION: A combination of in-silico and in-vitro screening revealed Curcumin, Hesperidin and Silibinin can be used as MDR modulators as well as chemosensitizer to reduce the cytotoxicity profile and drug resistance in AML.

#92 Acquisition of temozolomide resistance: Identification of a new drug resistant stage in glioblastoma cells. Marion Rabé,1 Hicham Janati,2 Solenne Dumont,1 Christelle Thibault-Carpentier,2 Jean Clairambault,2 François M. Vallette,1 Catherine Gratas 1.

Glioblastoma multiforme (GBM) are the most aggressive and common brain tumors in adults. Despite surgery and combined radio-chemotherapy with temozolomide (TMZ), tumor recurrence always occurs. The median survival time for patients diagnosed with GBM is about 14 months with less than 5% survival at 5 years. Today the main marker of TMZ resistance is the methylation status of MGMT promoter. Patients with a methylated promoter usually have a better response to treatment than patients with an unmethylated promoter. Indeed MGMT is an enzyme involved in DNA repair mechanisms that abrogates TMZ effects. However, in clinical trials targeting the MGMT enzyme, median survival of patients was not improved. It is thus essential to decipher the mechanisms involved in the acquisition of TMZ resistance to identify new therapeutic targets. To achieve this goal TMZ resistant cells were generated by continuous treatment of the U251 human glioblastoma cell line. These cells are sensitive to TMZ and do not express MGMT. We performed transcriptomic analysis by RNA-Seq on U251 treated with TMZ (50µM) for different time and selected differentially expressed genes. We also evaluated target genes expression in single cells by RT-qPCR using C1-HD-Biomark technology (Fluidigm). Transcriptome profiling allowed to identify a transient phase with TMZ tolerant cells before acquisition of complete resistance. These cells are characterized by a modified morphology and a more proliferative state. In this population we identified a subset of genes with the same transient overexpression. Similar results were observed in two other GBM cell lines under stress and under other stress conditions. A likely expression of MGMT appeared later with the emergence of the TMZ resistant population. Interestingly single cell qPCR showed that MGMT expression in the resistant cells could not be explained by clonal selection of MGMT positive cells. Drug screening on the TMZ tolerant cells revealed a potent killing activity of histone deacetylating agents (HDAC inhibitors). In conclusion, we have shown that glioblastoma cells become resistant after a transient state of TMZ tolerance. Identification of this singular population highlights new molecular targets and a new therapeutic window. Targeting these tolerant cells could avoid emergence of resistance and tumor recurrence, thereby patients survival could be improved.


Glucocorticoids (GCs) are central to all major therapy regimens for pre-B cell-derived acute lymphoblastic leukemia (ALL), but have no activity in myeloid leukemias. Such divergent responses represent an empirically established clinical standard; however, neither the mechanism by which GCs induce cell death nor the biological basis for the distinct responses in B-cell and myeloid leukemias is clear. Studying patient-derived samples revealed that NR3C1 (glucocorticoid receptor) levels were 6- to 20-fold higher in pre-B ALL compared to chronic myeloid leukemia (CML). High levels of Nr3c1 were reduced upon B- to myeloid-lineage conversion, suggesting that regulation of NR3C1 expression and GC responsiveness depend on a B-cell transcriptional program. B-cell transcription factors (e.g. PAX5, IKZF1) are critical for B-cell development, yet they are genetically leisioned in more than 80% of pre-B ALL cases. Despite such high frequency, the significance of these inactivating lesions remains elusive. Combining ChiP-seq and RNA-seq analyses, we identified a novel B-cell transcriptional program for activation of NR3C1 and its transcriptional target TXNIP (a negative regulator of glucose uptake). Reconstitution of PAX5 or IKZF1 expression in haploinsufficient patient-derived pre-B ALL cells increased NR3C1 and TXNIP levels. Conversely, expression of dominant negative mutant of PAX5 or IKZF1 abolished NR3C1 expression. Loss of Nr3c1 or Txnip in murine BCR-ABL1-driven pre-B ALL cells resulted in survival advantage in competitive growth assays. Importantly, loss of Nr3c1 or Txnip significantly elevated glucose uptake, lactate production and cellular ATP levels. These findings suggest that GCs induce cell death by exacerating glucose and energy depletion. Notably, reconstitution of PAX5 or IKZF1 rendered haploinsufficient patient-derived pre-B ALL cells more sensitive to dexamethasone (dex) treatment. In contrast, dominant-negative PAX5 or IKZF1 largely de-sensitized pre-B ALL cells expressing wildtype PAX5 or IKZF1. These findings suggest that B-cell transcription factors set the threshold for GC responsiveness in pre-B ALL. Since relapsed ALL cells often acquire GC resistance, drug-combinations may be useful to prevent GC-resistance. As expected, loss of Nr3c1 abrogated responses to GCs. Interestingly, loss of Txnip also largely rescued GC-induced cell death in pre-B ALL cells. On this basis, we tested drug interactions between GCs and TXNIP agonists, 3-O-methylglucoside (3-OMG) and D-allose. Treating patient-derived GC-refractory pre-B ALL cells with 3-OMG or D-allose strongly synergized with GC-treatment. Collectively, our findings provide a mechanistic explanation for the empirical finding that GCs are effective in the treatment of B-cell but not myeloid malignancies, and identify TXNIP as a novel therapeutic target in pre-B ALL.

#94 Association of xct overexpression with RTKI resistance and metastases in clear cell renal cell carcinoma. Sreenivasulu Chintala, Remi Adelajye- Ogba, Ashley Oreille Steevenari Aria, May Elbanu, Nup P, Damayanti, Roberto Pili. Indiana University-Purdue University, Indianapolis, IN.

Background: Cystine/glutamate exchanger xCT is a catalytic component of system xc- involved in transport of ‘conditionally indispensable’ amino acid cystine. Cystine transport is a rate limiting step for the synthesis of glutathione,
a major intracellular redox regulator. Recently, we and other groups reported the overexpression of xCT and its association with drug resistance in several human cancers including bladder, glioma, breast, and colon. There are no studies to show the xCT expression in clear cell renal cell carcinoma (ccRCC) and its association with tyrosine kinase inhibitors resistance and metastasis. In the current study, we evaluated xCT expression in primary ccRCC tumors in tissue microarray (TMA) and determined its role in receptor tyrosine kinase inhibitor (RTKI) resistance and metastases using the patient derived tumor xenografts (PDX) models and TKI resistance ccRCC cells. Methods: Human Renal cell carcinoma tumor nephrectomy specimens arranged in tissue microarray (TMA) were used to determine xCT expression by immunohistochemistry. Patient derived tumor xenografts (PDX) of primary ccRCC, metastasis, and primary ccRCC tumors in vivo were used to determine the role of xCT in RCC. To understand the molecular alterations associated with RTKI resistance, we have generated sunnitihibit 786 OR ccRCC cells and performed RNAseq analysis. To determine the xCT inhibition effect on ccRCC tumor metastases, sulfasalazine, an inhibitor of xCT was used to treat metastatic ccRCC tumor xenografts transplanted in SCID mice. Results: Immunohistochemical evaluation of xCT in RCC TMA revealed that 70% (19 out of 27) of the tumors express different levels of xCT. Association with tumor response to RTKI will be presented. RTKI less responsive PDX RP-R-02 was developed using the dose escalation strategy and was found to have an upregulation of xCT when the tumors become less responsive to sunnitihibit. RNAseq analysis revealed differential gene expression of genes including tyrosine kinases and lysosome biogenesis and function such as LSC7A1, HP54, HP55, CTSS, CTSD, ITSF30, PPT1, SCPE1, TPI1, ATP6AP1, MCOLN1, PRKAG2, and VPS18 in sunnitihibit resistant 786 OR cells compared to parental cells supports the role of lysosomes function in RTKI drug resistance. Furthermore, xCT inhibitor sulfasalazine treatment significantly decreased the metastasis lung nodules of RP-R-02L in SCID mice demonstrated the xCT role in RCC tumor metastasis. Conclusions: We found preliminary evidence that overexpression of xCT may be associated with RTKI resistance in ccRCC. These results suggest that targeting the xCT in ccRCC may reverse the resistance and enhance the efficacy of RTKI. Additional studies using larger numbers of ccRCC tumors are required to identify xCT as a potential predictive biomarker for response/resistance to RTKI in ccRCC patients.

#95 Targeting BRD4 overcomes cetuximab resistance in HNSCC. Toni Brand, Yan Zeng, Brandon Leonard, Rachel O'Keefe, Hua Li, Daniel Johnson, Jennifer Grandis, Neil E. Bhola. University of California San Francisco, San Francisco, CA. Background: Nearly 600,000 people are diagnosed with head and neck cancer worldwide and 60% succumb to the disease within 5 years. The epidermal growth factor receptor (EGFR) is a major driver of HNSCC and in 2006 the EGFR monoclonal antibody cetuximab was FDA-approved for HNSCC treatment. However, cetuximab has not conferred significant clinical benefit when compared to chemoradiation in patients with poorly differentiated recurrent-metastatic HNSCC. We and others have demonstrated that cetuximab treatment activates alternative receptor tyrosine kinases (RTKs) including Fibroblast Growth Factor (FGF) receptors, Met and Axl in HNSCC. To circumvent accumulation of multiple RTK inhibitors in combination with cetuximab, we sought to identify a common molecular target that regulates the expression of these RTKs. Bromodomain-containing protein 4 (BRD4) has been shown to regulate the transcription of RTKs in breast cancer models. Hypothesis: We hypothesized that targeting BRD4 will overcome cetuximab resistance by depleting the expression of alternative RTKs in HNSCC. We further hypothesize that genetic and pharmacological targeting of BRD4 will synergize with cetuximab. Results: Using a phospho-RTK array, 72-hour cetuximab treatment increased or sustained phosphorylated levels of EGFR, HER2, HER3, MET, and AXL in several HNSCC cell lines. Treatment with the BRD4 inhibitor JQ1 abrogated both phosphorylated and total RTK abundance in the presence of cetuximab. Cetuximab and JQ1 robustly decreased phosphorylated Src and induced the senescence marker p21. Phenotypically, cetuximab and JQ1 significantly decreased survival and increased apoptosis in 6 HNSCC cell lines models, while the normal oral keratinocyte cell line NOKsi had approximately 10-fold higher IC50s for the BRD4 inhibitors, JQ1 and I-BET762, relative to HNSCC cell lines. Importantly, two HNSCC models of acquired cetuximab resistance exhibited robust sensitivity to pharmacological (JQ1, I-BET762) and genetic (RNAi) BRD4 targeting strategies. Moreover, exogenous overexpression of different RTKs (HER3, ALK, and ROR1) resulted in cetuximab resistance that was reversed upon BRD4 targeting (RNAi and JQ1). Combination of cetuximab and JQ1 in co-culture experiments with T cells decreased the CD4+/CD25+ Treg population and PD-1.1 expression on HNSCC cell lines. Further, preliminary findings indicate that JQ1 treatment prevents outgrowth of cetuximab-treated HNSCC patient-derived xenograft models. Conclusion: Our findings indicate that targeting BRD4 decreases the activation and expression of multiple RTKs that mediate resistance to the FDA-approved EGFR inhibitor cetuximab. Furthermore, BRD4 abrogates expression of immunosuppressive markers, making it a promising tumor intrinsic and extrinsic therapeutic strategy for HNSCC.

#96 Dihydropyrimidine dehydrogenase (DPYD) provides resistance to 5-fluorouracil in mutant p53 (mutp53) expressing colorectal cancer cells. Prashanth Ravishankar Gokare,1 Niklas Finnberg,1 David Dicker,1 Maureen Murphy2,3 Fox Chase Cancer Ctr., Philadelphia, PA; 2Wistar Institute, Philadelphia, PA. 5-Fluorouracil (5-FU) is a mainstay of colorectal cancer therapy. Even though much is known about the 5-FU mechanism of action, very little is known about the genetic determinants which provide sensitivity and resistance to 5-FU. Here we provide first insights into p53, a major predictor of 5-FU response, modulating nucleotide catabolism specifically through the control of the rate limiting enzyme dihydropyrimidine dehydrogenase (DPYD). Colorectal cancer cells expressing mutp53 specifically show higher expression of DPYD as compared to the wildtype p53 (wtp53) carrying cells. Higher expression of DPYD in mutp53 cells specifically provides increased resistance to 5-FU as indicated by cellular proliferation and long term clonogenic assays. Furthermore, knockdown of DPYD in mutp53 cells preferentially re-sensitizes them to 5-FU as opposed to cells expressing wtP53 by decreasing the viability and increasing apoptosis observed in these cells. Pharmacological inhibition of DPYD with gimeracil, clinical inhibitor of 5-FU phenocopies the effects seen by siRNA/shRNA mediated knockdown of DPYD. In summary, our data indicates that overexpression of mutp53 specifically provides increased resistance to 5-FU therapy which is abrogated by stable knockdown of DPYD expression or in combination gimeracil. Preliminary analysis of TCGA patients treated with 5-FU indicate differential expression of DPYD based on specific Tp53 mutation status. Taken together our study provides first insights into crosstalk between nucleotide catabolism and mutp53 under conditions of DNA damage and metabolic stress having major implications on the therapeutic response to 5-FU the in the clinic.

#97 RNA sequencing of bladder cancer patient-derived xenograft models identifies genes associated with chemoresistance. Kelly A. Martin,1 Nicholas R. Hum,1 Aimy Sebastian,2 Deepa K. Murugesh,3 Chong-Xian Pan,3 Ai-Hong Ma,2 Ralph de Vere White,2 Gaby Loots1. 1Lawrence Livermore National Laboratory, Livermore, CA; 2UC Davis Comprehensive Cancer Center, Sacramento, CA. Background: Bladder cancer is among the top ten most common cancers, with about ~380,000 new cases and ~150,000 deaths per year worldwide. Platinum-based combination chemotherapy is commonly used to treat advanced bladder cancer. It has been shown that only ~50% of the patients respond to platinum based therapy. Methods: We have utilized a patient-derived bladder cancer xenograft (PDX) platform to characterize the molecular mechanisms that contribute to resistance of gemcitabine-cisplatin combination therapy in advanced bladder cancer. We have also identified key regulatory pathways in our PDX models that can be targeted to treat chemotherapy resistant bladder cancer using RNAseq analysis. Results: The genetic determinants which provide sensitivity and resistance to 5-FU. Here we provide first insights into p53, a major predictor of 5-FU response, modulating nucleotide catabolism specifically through the control of the rate limiting enzyme dihydropyrimidine dehydrogenase (DPYD). Colorectal cancer cells expressing mutp53 specifically show higher expression of DPYD as compared to the wildtype p53 (wtP53) carrying cells. Higher expression of DPYD in mutp53 cells specifically provides increased resistance to 5-FU as indicated by cellular proliferation and long term clonogenic assays. Furthermore, knockdown of DPYD in mutp53 cells preferentially re-sensitizes them to 5-FU as opposed to cells expressing wtP53 by decreasing the viability and increasing apoptosis observed in these cells. Pharmacological inhibition of DPYD with gimeracil, clinical inhibitor of 5-FU phenocopies the effects seen by siRNA/shRNA mediated knockdown of DPYD. In summary, our data indicates that overexpression of mutp53 specifically provides increased resistance to 5-FU therapy which is abrogated by stable knockdown of DPYD expression or in combination gimeracil. Preliminary analysis of TCGA patients treated with 5-FU indicate differential expression of DPYD based on specific Tp53 mutation status. Taken together our study provides first insights into crosstalk between nucleotide catabolism and mutp53 under conditions of DNA damage and metabolic stress having major implications on the therapeutic response to 5-FU the in the clinic.

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glutamabumab vedotin, an antibody-drug-conjugate has shown promising results in treating several cancers including breast cancer and osteosarcoma. Further studies will elucidate whether targeting GPNMB is an effective strategy for the treatment of chemotherapy resistant bladder cancer. This study received funding by a developmental grant from the UCDCCC. This work was conducted under the auspices of the USDOD by LLNL (DE-AC52-07NA27344). IM number: LLNL-688318.

#98 TET suppresses protein translation, differentially effects polysome loading of cell cycle regulatory proteins and inhibits cell growth and proliferation of prostate cancer cells. Praveen K. Jaiswal, Sweaty Koul, Qin Dong, Hani Koul. LSU Health Sciences Center - Shreveport, Shreveport, LA.

Introduction: Translational control is a critical component in development and progression of cancer and could be explored to develop antiangiogenic therapies. Results from our laboratory have shown that TET (a derivative of Tetranorline) inhibits cell growth and promotes apoptosis of prostate cancer (PCa) cells. However, the underlying molecular mechanisms are not clearly understood. We observed that TET inhibited 4EBP phosphorylation, suggesting that it might inhibit translational initiation. In the present study we evaluated the effects of TET on translation in general and cell cycle related proteins in particular. Methods: Androgen dependent PCa cells LNCaP and Enz resistant CRPCa cells 22Rv1 were maintained in supplemented RPMI as recommended. Where indicated cells were treated with TET (0 or 20µM) for various time points (2h, 8h, 18h and 24h). At the end of the experimental periods cells were pulsed with cycloheximide for 10 minutes in RPMI media. Cytoplasmic RNA was collected by lysing cells in a polysome buffer. Cell lysates were centrifuged and the resulting cytosolic supernatant was separated by density (a 10% to 50% sucrose) gradient and centrifugation. Fractions were collected using a TELEDYNE ISCO Density Gradient Fractionation System with continuous monitoring of untranslated mRNAs. Polysome (P)/Monosome (M) ratio were measured. The total RNA in each fraction was extracted using TRIzol LS (Invitrogen) and qRT-PCR was done for p21, p27, Cyclin D1 and c-Myc gene for each fraction. Cell viability was measured by MTT assay at various time points. Results: TET treatment resulted in decreased protein synthesis. TET treatment resulted in decreased mRNA associated with Polysomes. There were a significant shift in P/M ratio from 0.82 (control) to 0.48 (24h). TET [TET 2hrs (0.75), 8h (0.69) and 18hrs (0.52)] in LNCaP and P/M ratio form 0.91 (control) to 0.32 (8 h TET) [TET 2h (0.78) and 4h (0.53)] in 22Rv1 cells. The decrease of the P/M ratio suggests that TET inhibits translation initiation in prostate cancer cells independent of the androgen dependence. While there was a generalized decrease in translation and specific decrease in mRNA for Cyclin D1, c-Myc associated with polysomes following TET treatment. In quite contrast to these findings, we observed an increase in mRNA for p21 and p27 in polysomal fractions of TET treated cells as compared to control at 24h in LNCaP cells. These data suggest differential effects of TET treatment on polysome loading of cell cycle regulatory proteins. Moreover, TET inhibited growth and proliferation of LNCaP and 22Rv1 cells in dose as well as in time dependent fashion. Conclusions: TET targets translational machinery and differentially effects translation of cell cycle regulatory genes by effecting their polysome loading and effects cell growth and proliferation of androgen dependent and castrate resistant PCA cells.

#99 Identify potential kinetochore protein inhibitors to overcome cisplatin resistance. Chen Yin-Ju, Jeng-Fong Chiou. Taipei Medical University, Taipei, Taiwan.

Cisplatin is the most commonly used chemotherapeutic drug for cancer, while the resistance causes treatment failure. Cisplatin resistance may involve in many molecular changes so identify aberrations in cisplatin-resistant (CR) genes and find new therapeutic strategies could provide valuable information to reduce therapeutic resistance. The aim of this study is to search CR genes and identify potential novel drugs to overcome cisplatin resistance. To identify aberrations in CR genes, three CR genetic signatures were analyzed and differential expression genes from lung, ovarian and oral cancer cells. After integration of CR signatures, the kinetochore associated proteins, including NUF2, SPCC25, PC24, DSN1, SKA2, KNTC1, MIS18A and SKA1 were up-regulated in various CR cells. Silencing of kinetochore associated proteins increased cisplatin sensitivity. For identification of kinetochore associated protein inhibitors to overcome cisplatin resistance, CR signatures were queried to the Connectivity map (Cmap) database to search for potential drugs which may reverse cisplatin resistance. 3 compounds exhibited cytotoxicity effect on the cisplatin-resistant cell lines and reduced kinetochore associated proteins expression level. Taking together, this study demonstrates kinetochore associated proteins participate in cisplatin resistance and identify potential inhibitors to overcome cisplatin resistance.


We recently demonstrated that pancreatic cancer cells adapt to low nutrient conditions through the up-regulation of the antioxidant enzyme, glutathione peroxidase. NADPH is required for the antioxidant system to function, and we hypothesized that the enzyme, IDH1, is a potential therapeutic target. We found that IDH1 knockdown inhibited growth and proliferation of LNCaP and 22Rv1 cells in dose as well as in time dependent fashion. Conclusions: TET targets translational machinery and differentially effects polysome loading and effects cell growth and proliferation of androgen dependent and castrate resistant PCA cells.

#101 Adaptive feedback reactivates MAPK signaling in KRAS-mutant cancers with inhibition of MEK, but not ERK. Leanne G. Ahronian, Sandra Misale, Jason T. Godfrey, Koki Nishimura, Lifeng Chen, Jeffrey A. Engel, Ryan B. Corcoran. Massachusetts General Hospital Cancer Center, Charlestown, MA.

Activating mutations in the KRAS oncogene occur in about 40% of colorectal cancers (CRCs) and over 90% of pancreatic ductal adenocarcinomas (PDACs). Since development of small molecules capable of inhibiting KRAS directly has proven difficult, alternative strategies have instead focused on inhibiting downstream effectors pathways, such as the MAPK pathway. However, inhibition of the MAPK pathway alone with MEK inhibitors, such as selumetinib and trametinib, produces only cytostatic effects and is insufficient to kill KRAS-mutant cancer cells. We hypothesized that inhibition of an additional kinase during MEK inhibitor treatment could improve response. We performed a kinase-targeting shRNA screening to find kinases whose knockdown would cooperate with trametinib in KRAS-mutant CRC and PDAC cell lines. The kinases found in this screen represent potential therapeutic targets to inhibit in combination with MEK. Interestingly, despite using a very high concentration of trametinib in the screen to enrich for hits outside of the MAPK pathway, the most highly ranked kinases in the screen were members of the MAPK pathway, including BRAF, MEK1, and MEK2. This suggests that even at high concentration, trametinib produces suboptimal MAPK inhibition. Indeed, we found that while MEK inhibitors produce robust inhibition of MAPK signaling initially, pathway reactivation was observed by 48-96 hours despite regular replenishment of drug. This feedback reactivation was accompanied by marked increases in active BRAF and phosphorylated MEK. In fact, experimental approaches that artificially increased upstream signaling flux through the MAPK pathway led to a >10-fold reduction in the ability of MEK inhibitors to inhibit the MAPK pathway. Remarkably, despite triggering the same degree of adaptive upstream signaling, MAPK signaling as seen with MEK inhibitor, we found that ERK inhibitors were able to maintain MAPK pathway suppression. Importantly, these differences in MAPK pathway suppression amount to differences in cell viability. Over four weeks, ERK inhibitor treatment reduces the outgrowth of KRAS-mutant cell lines compared to those treated with MEK inhibitors. Additionally, as these inhibitors are not used as monotherapies, replacement of trametinib with an alternative MEK inhibitor could prove beneficial. Further exploration into the molecular mechanisms of pathway feedback will be necessary to understanding clinical combinations for KRAS-mutant cancers.
Cruickshanks,1 Ying Zhang,1 Sarah Hatef,1 Julia Wulfkuhle,2 Isela Gallagher,2 targeting common transcriptional nodes in cancers addicted to growth fac-

ponents of the DNA-replicative machinery and also proteins involved in

resulted in reduced cell proliferation, accompanied by induction of apopto-

various TNBC cell lines. Combined inhibition of EGFR and cdc7/CDK9

and Lapatinib) in vitro to overcome resistance to EGFR-targeted therapy in

functions of the protein in transcriptional regulation and DNA-repair may

profound clinical challenge, the disease being disproportionately responsi-

HER2 over-expressed in around 20-25% of breast cancers. Due to increased
cell growth and differentiation signaling resulting from HER2 over-expression,
this sub-type of the disease is associated with aggressive tumor progression and poor prognosis. HER2 targeted therapy has improved patient prognosis, how-
ever, only a subgroup of patients derives the optimal benefit, while other patients have refractory disease or develop resistance. miRNAs are small non-
coding RNAs involved in the RNAI machinery. They are often aberrantly expressed in

tumors and miRNA profiles have been seen to evolve over the course of treat-

importing them in therapeutic resistance. Methods: miRNA array com-
pleted on the Human TaqMan miRNA card. Gene expression profiling per-
formed by Illumina Human HT12 v4.0 array. Differential gene expression used
LIMMA empirical Bayes statistics module. Adjusted p-values calculated by
Benjamini and Hochberg procedure. In silico target prediction used miWalk.

Lapatinib resistant cells generated by culturing cells in increasing doses of lapa-
tinib up to 2µM. IC50 obtained from drug cytotoxicity measured by MTT ana-
lyzed by nonlinear regression. Results: A panel of miRNAs differentially ex-
pressed in paired lapatinib sensitive/resistant BT-474 HER2 positive breast cancer cell lines was selected for further investigation of their role in HER2-targeted therapy: mir-127-3p, mir-409-3p, and mir-495-3p. RT-qPCR also confirmed upregulation of these miRNAs in HCC1954 and SKBR3 HER2 positive cells with acquired resistance to lapatinib, linking increased miRNA expression with acquired resistance. To understand the mechanism behind the miRNA expression in resistant cells we explored epigenetic changes between the sensitive and resistant lines. Global methylation reversal revealed upregulation of all miRNAs in the sensitive cells with a further increase upon addition of an HDAC inhibitor. De-methylation had less effect in resistant cells, indicating loss of methylation as a key component in miRNA upregulation in the process of lapatinib resistance. Using differential gene expression analysis in paired lapa-
tinib-sensitive/resistant BT-474 cells combined with in silico analysis we iden-
tified putative targets of these miRNAs. BASP1, a c-MYC transcriptional inhib-
or, was down-regulated in the BT-474/IL resistant cells. Knock-down altered
expression of lapatinib, with an 8 fold shift in IC50 towards resistance, indicating
reduced expression of the protein could be involved in the resistance machinery.
Conclusions: mir-127, mir-409, and mir-495, clustered to the 14q32 region are
also confırmed upregulation of these miRNAs in HCC1954 and SKBR-3 HER2
positive cells with acquired resistance to lapatinib, linking increased miRNA
expression with acquired resistance. To understand the mechanism behind the
miRNA expression in resistant cells we explored epigenetic changes between the

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Novel Agents

Depletion of extracellular cystine and cysteine by a mutated human enzyme causes ROS mediated cytotoxicity in pancreatic cancer cells . Sabin Khattar, Achinto Saha, Shira Cramer, John DiGiovanni, Stefano Tiziani, Nathalie Munoz, George Georgiou, Everett Stone. University of Texas at Austin, Austin, TX.

Pancreatic ductal adenocarcinoma (PDAC) has a dismal 5-7 year survival rate at 7%. The oncogene KRAS that is mutationally activated in over 90% of PDACs, has been shown to be involved in regulating redox homeostasis. Hence, pertur-
bation of oxidative balance might provide a therapeutic window to effectively
treat pancreatic cancer. One strategy to achieve this is to target the tripeptide
 glutathione (GSH) – a major intracellular antioxidant. Cysteine (Cys), which has
the functional moiety of GSH, can either be synthesized de novo or imported, predominantly as cysteine (CSCC) which is then reduced intracellularly to Cys. In cancer, intracellular Cys synthesis has to be supplemented with extracellular import in order to fulfil the excessive metabolic demand of proliferation, which includes maintenance of oxidative balance through GSH synthesis. We hypo-
thesize that this increased requirement for Cys/CSCC import in tumor cells will make them selectively sensitive to prolonged depletion of these amino acids in the serum by a genetically engineered human enzyme called Cyst(e)inase. In addition, we believe that combining Cyst(e)inase with other redox balance pertur-
bing agents will produce a synergistic therapeutic effect. In our study, Cys-
t(e)inase treatment of cultured pancreatic cancer cell lines (Panc1, MIA PaCa2, BxPC3) decreased intracellular Cys and GSH, and inhibited cell growth. Sensitivity to Cyst(e)inase was correlated with ROS accumulation (Panc1>MIA PaCa2>BxPC3). Panc1 cells exhibited G2-arrest and apoptotic cell death following 24 hours of treatment whereas BxPC3 cells underwent only a G1-arrest and no cell death even after 72 hours of treatment. Further mechanistic investigation showed activation of AMP kinase and other stress related kinases (p38, ERK and JNK), and DNA damage signaling (ATM) only in the more sensitive cell lines. Inhibition of the mTORC1-p70S6K-S6 ribosomal protein signaling pathway was observed in all 3 cell lines. Cyst(e)inase displayed synergistic cytotoxicity when combined with buthionine sulfoximine (GSH synthesis inhibitor), auranofin (thioredoxin reductase inhibitor), sulfasalazine (inhibitor of cystine import) and the natural compound curcumin known to increase intracellular ROS, indicating that concurrently inhibiting alternative cellular antioxidant pathways or directly increasing intracellular ROS might improve the anti-tumor efficacy of Cyst(e)inase. The effect of biweekly intraperitoneal Cyst(e)inase treatment on growth of pancreatic cancer cell xenografts in nude mice will also be reported. Collectively, the current data suggest that depletion of extracellular Cys/CSSC using Cyst(e)inase may have utility either as a monotherapy or a combination therapy for pancreatic cancer.

#106 Traditional medicinal plants effect on five cancer cell lines. Elbert L. Myles. Tennessee State Univ., Nashville, TN.

Cancer refers to as a group of numerous diseases that can originate in various parts of the body. Since the beginning of human history natural compounds have been used for medical purposes. Many of the most frequently used natural compounds have their origin from plants. Plants are very diverse and responsible for the synthesis of a number of different secondary compounds. Scientist and non-scientist have taken advantage of these secondary compounds treatment of cancer xenograft animal model to demonstrate the growth inhibitor activity of our lead peptides. Our study demonstrate that our lead peptides inhibit tumor cell growth via cell-cycle arrest, and almost have no cytotoxicity on human epithelial cells, which could lead our lead peptides to further clinical investigation.

#107 Mechanism of anticancer activity of BPS-001 (lyophilized leech saliva extract). Amr Ammar,1 Emma Guns,1 Omer Kucuk,2 Abdualrahman Abdualkader,3 Mohamed Almaa,3 A.B.M. Helal Uddin,4 Abbas Ghawi,5 Mohamed Hassona,5 1Univ. of British Columbia, Vancouver, British Columbia, Canada; 2Emory University, Atlanta, GA; 3International Islamic University Malaysia, Kuantan, Malaysia.

Recent studies, investigating the composition and therapeutic potential of leech saliva have identified many peptides and proteins with multiple therapeutic properties including anti-thrombotic, antimicrobial and anti-metastatic. In vitro anti-cancer effects were shown in breast, prostate and lung cancer cell lines. In vivo anticancer activity of the extract was shown in multiple breast and prostate cancer xenografts studied by our lab. In our previous studies, BPS-001 was non-toxic and demonstrated various activities including pro-apoptotic properties, inhibition of cellular adhesion, angiogenesis and downregulation of AR expression. To investigate the affected pathways, mechanistic studies were carried out using in vivo and in vitro treated samples. Protein expression in samples was determined by Western blotting of in vitro and in vivo treated samples. Protein expression analysis were performed, and from those data it indicates that the peptides might actually work by inhibiting the cancer cell growth instead of disrupting cell membrane directly. Flow cytometry and western bolt results also showed that the peptides have a significant G1 phase cell cycle arrest effect through inhibiting cyclin dependent kinases CKD4 and up-regulating the expression of cell cycle protein regulator Cyclin D3, p27, p21. Moreover, from our current data, it demonstrated that our peptides wouldn’t induce any apoptosis during the cell cycle process. With the solid anticancer mechanism/pathway data, the potential anti-cancer peptides to target in vivo malignant tumor will be evaluated in cancer xenograft animal model to demonstrate the growth inhibitor activity of our lead peptides. Our study demonstrate that our lead peptides inhibit tumor cell growth via cell-cycle arrest, and almost have no cytotoxicity on human epithelial cells, which could lead our lead peptides to further clinical investigation.

#108 In vitro and in vivo characterization of novel scorpion venom-based peptides for the treatment of colon cancer. Bin Li, Hang Fai KWOK. University of Macau, Macau, China.

In this study, the activity of the venom-based bioactive peptides demonstrated with a broad and diverse spectrum of pharmacological activities, which help to enlarge the current drug-screening library for searching of new specific biomarkers and novel prototype drugs for diagnosis of diseases such as cancer. Moreover, it paved a new insight to overcome the current drug discovery problems, which including drug resistance problem, side effect and so on. Here we report the discovery of a panel of novel venom-based peptides, which can significantly inhibit the growth of human colon cancer cells. Through our in-house developed high throughput screening techniques and bioinformatics analysis, we successfully isolated eight novel peptides from scorpion venom with anti-proliferative and anti-apoptotic activities. We further demonstrated that these peptides could inhibit the growth of a broad spectrum of tumor cells, especially in human colon cancer cell line; while almost have no effect on normal human epithelial cells. Haemolysis assay was also performed to show our peptides do not cause any harm on normal human red blood cells, which point to the direction of bringing those peptides into the preclinical trial studies. Before the in vivo phase of this project, we have demonstrated that our peptides could significantly inhibit tumor cell growth via cell-cycle arrest, and almost have no cytotoxicity on human epithelial cells, which could lead our lead peptides to further clinical investigation.


Although there have been great advancement in technology, molecular diagnosis, therapeutics, lung cancer is still the leading cause of cancer related mortality all over the world. Recently, some antipsychotic drugs have been shown to possess the anticancer activity. Thus the present study was designed to evaluate the anticancer effects of trifluoperazine (TFP), a commonly used antipsychotic drug, and its synthetic analogs on human lung cancer cell lines. To this end, effects of TFP and its selected analog on A549 cells were investigated in vitro as well as in vivo experiments. Synthetic TFP analogs were evaluated by the viability of A549 cells following drug treatment and compared to TFP. KCFCS1, a selected TFP analog, significantly inhibited the proliferation of A549 cells. Further experiment showed that TFP and KCFCS1 had activities to inhibit the migration and anchorage dependent/independent colony formation of A549 cells. Furthermore TFP and its analogs significantly inhibited the in vitro and in vivo tumor cell growth. Synthetic TFP analogs revealed that TFP and KCFCS1 affect the protein expression levels related to cell apoptosis and cell cycle. KCFCS1 showed stronger anticancer effects in all the experiment than TFP. Flow cytometric analysis showed that KCFCS1 induced sub-G1 population and reduced cell population in S and G2/M phase. In addition, the two in vivo experimental models, KCFCS1 also showed powerful anticancer effect in skin xenograft tumor growth and orthotopic lung cancer development than TFP. Thus present study demonstrates that a synthetic TFP analog has anti-lung cancer activity and provides a potential therapeutic candidate for lung cancer.

#110 Quantitative high throughput screening as a tool to identify novel therapies in bladder cancer. Reema Raikar, Thomas Sanford, L. Spencer Krane, Pirush K. Agarwal. NCI-NIH, Bethesda, MD.

Introduction: Bladder cancer (CaB) is the 4th most common cancer among men in the US. It is the most expensive malignancies to treat from diagnosis to death. No new pharmaceutical agents have been approved for treatment of bladder cancer since the approval of BCG in 1990. Thus, there is an urgent need for development of new treatment therapies. Quantitative high throughput screening (qHTS) of representative cancer lines with oncology drugs may identify new treatments or re-purpose already existing therapies for different disease. We utilized this technique to identify new therapies in two primary bladder lines (T24 and UIMUC3) and their metastatic lines (T24T, SLT3 and FL3 of T24 and LUL-2 for UIMUC3). Methods: We screened 7 bladder cancer cell lines against 1,912 oncology drugs using a 48 hour cell proliferation assay with an ATP-based readout (CellTiterGlo). The potential activity of compounds in a dose response manner. One of the candidate drugs inhibited AR activity in all 7 cell lines, and its synthetic analogs in both in vitro and in vivo experiments. Synthetic TFP analogs were evaluated by the viability of A549 cells following drug treatment and compared to TFP. KCFCS1, a selected TFP analog, significantly inhibited the proliferation of A549 cells. Further experiment showed that TFP and KCFCS1 had activities to inhibit the migration and anchorage dependent/independent colony formation of A549 cells. Western blot analysis revealed that TFP and KCFCS1 affect the protein expression levels related to cell apoptosis and cell cycle. KCFCS1 showed stronger anticancer effects in all the experiment than TFP. Flow cytometric analysis showed that KCFCS1 induced sub-G1 population and reduced cell population in S and G2/M phase. In addition, the two in vivo experimental models, KCFCS1 also showed powerful anticancer effect in skin xenograft tumor growth and orthotopic lung cancer development than TFP. Thus present study demonstrates that a synthetic TFP analog has anti-lung cancer activity and provides a potential therapeutic candidate for lung cancer.
pounds active in 7 cell lines. The top 50 compounds were further analyzed for molecular size of >200 g/mol and TPSA >90. This identified mitomycin C and 8 novel compounds. Further testing revealed Flavopiridol to be most consistent with qHTS data having IC50 of 100-300 nM in all the cell lines tested. Flavopiridol induces G2/M arrest; however, very little apoptosis was seen suggesting cytostatic rather than cytotoxic mechanism of flavopiridol action. Flavopiridol showed dose dependent inhibition of migration, invasion and colony formation in CaB cells tested. Xenograft studies in rapidly growing UMUC-3 cells showed slowing of tumor growth but not complete reduction indicating cytostatic mechanism of flavopiridol. However, in slow growing cells, 5637, 5/8 treated mice showed complete tumor reduction. Conclusions: qHTS can identify potent novel inhibitors of STAT3 in a prostate cancer cell line in vitro and in vivo. Physical properties of Flavopiridol are most suited for intravesical use which may lead to it being an effective inhibitor of CaB in the bladder at higher doses without any/few systemic toxicities. Studies are underway to elucidate the use of flavopiridol as a single intravesical agent.

**#111** Targeting STAT3 and telomerase for the treatment of colorectal cancer. Seyoung Chung,1 Quincy Okobi,2 Debbie Adekoya,3 Jaydutt Vadgama1.
1Charles Drew University, Los Angeles, CA; 2UC Santa Cruz, Los Angeles, CA; 3University of Dublin, Ireland.

There is an increasing evidence of pro-inflammatory cytokines involvement in cancer development. Here, we found that two cytokines, IL-6 and TNF-α, induced colorectal cancer cells more active and invasive. Combined treatments of IL-6 and TNF-α phosphorylated treated STAT3 and telomerase in a synergistic manner. STAT3 and NF-κB physically interacted upon cytokine stimulation. Similarly, STAT1 hetero-dimerized with STAT3 and the binding affinity was enhanced with cytokine treatments. STAT3 bound the promoter region of human telomerase reverse transcriptase (hTERT), and IL-6 and TNF-α stimulation further enhanced this STAT3 binding affinity. Withaferin A, an anti-inflammatory steroidal lactone, inhibited the IL-6 and TNF-α induced cancer cell invasiveness and decreased colonosphere formation. Notably, withaferin A inhibited STAT3 phosphorylation and abolished the STAT3 and NF-κB interactions. STAT3 binding to hTERT promoter was inhibited and telomerase activity was decreased with the withaferin A treatments. Taken together, pro-inflammatory cytokines induced-cancer cell invasiveness is mediated by STAT3 regulated mechanism in colorectal cancer cells. Our study suggests the novel natural compound for the metastatic colorectal cancer in clinical settings.

**#112** Identifying drugs that target ovarian cancer tumor initiating cells. Michelle K. Ozaki, Carrie D. House, Christina M. Annunziata. National Cancer Institute, Bethesda, MD.

Ovarian cancer is the most lethal gynecological cancer, with overall five-year survival for women with advanced disease being at only 25%. The current standard of care is treatment with a platinum-based chemotherapy drug. Unfortunately, a subpopulation of tumor cells often exists, contributing to platinum-based chemotherapy resistance and disease recurrence. In this study we investigated drugs to target these putative tumor-initiating cells (TICs), and explored the pathways of these drugs in both adherent proliferative cell culture conditions and non-adherent TIC-enriched cultures. We conducted a drug screen under both growth conditions, in order to identify compounds that are able to inhibit growth of TICs. Of the drugs identified, we focused further efforts on those involved with inhibiting the NF-kappaB pathway because prior studies have linked NF-kappaB activity with drug resistance and poor survival. Cell viability assays done with the four drugs, bardoxolone methyl, salmiycin, disulfiram, and elesclomol, show these drugs inhibit the growth of ovarian cancer cell lines both as TICs and in corresponding adherent cultures. Disulfiram showed preferential killing of TICs in some of the cell lines tested. All drugs showed some evidence for inhibiting NF-kappaB on Western blot, and ability to decrease CD133/ALDH double-positive TICs on flow cytometry. Carboplatin is known to kill proliferative ovarian cancer cells, and increase the relative percentage of CD133/ALDH positive TICs. Our ongoing studies will address combination of each drug with carboplatin, with the hypothesis that these drugs will increase in vivo efficacy by suppressing TICs, and thereby decrease recurrence of platinum-resistant ovarian cancer.

**#113** Evaluating the anticancer activity of two flexible heteroarotinoid analogs on breast cancer. Emily Ginn,1 Hongye Zou,2 Maryam M. Fallatah,1 Sharavini S. Kanapin1,1 Dominica University, San Rafael, CA; 2Touro University, Vallejo, CA.

Flexible heteroarotinoids (flex-hets) are compounds derived from retinoic acid and recent studies have indicated that these compounds exhibit anti-cancer activity. Amongst the flexible heteroarotinoids, SHetA2 has been shown block the growth of cervical, head and neck, kidney, lung, and ovarian cancers, and most recently, prostate and breast cancers. However, due to SHetA2’s limitations—a high degree of hydrophobicity, non-selectivity, and potential liver toxicity—a second generation of analogs was developed. Results from our recent study suggest that one of the second-generation analogs, SHetA2-3 (bobichetin-1-y)-N-tert-butyl-1H-indolin-2(3H)-one (99) exhibits anti-cancer activities against both ERα+ and ER- breast cancer cells at micromolar concentrations. Since SL-1-09 is a racemic mixture, the R (SL-1-29) and S (SL-1-30) enantiomers were purified and investigated for their anti-cancer properties. Our results suggest that SL-1-30 has greater growth inhibitory effects on T47D, MCF7, MDA-MB-453, MDA-MB-468 cells in cancer therapy. A pyrimidine-based multi-kinase inhibitor SL-1-09 (99) is likely associated with the S enantiomer. Consistent with this data, breast cancer cells treated with SL-1-09 and SL-1-30 express lower levels of proteins that regulate the cell cycle (i.e. cyclin A, cyclin B, cyclin D1, cyclin E and cdk2). These results demonstrate that SL-1-30 and SL-1-09 inhibit breast cancer cell growth, potentially by blocking cell cycle progression, however further studies are necessary for determining the mechanisms of action.

**#114** TG02 induces cell cycle arrest in glioblastoma. Yu-Ting Su, Robert Chen, Hallie Lappin, Herui Wang, Dragan Marić, Orieta Celiku, Aiguo Li, Mark R. Gilbert, Jing Wu. NIH, Bethesda, MD.

Dysregulated cell cycle contributes to the limitless replicative potential of cancer cells. The cyclin-dependent kinases (CDKs) and cyclins, the CDK-regulatory proteins are the key cell cycle machinery and can be targets for cancer therapy. TG02, a pyrimidine-based multi-kinase inhibitor SL-1-09 (99) has an anti-glioma effect which was demonstrated by our previous studies. An in vitro kinase spectrum assay of TG02 demonstrated inhibitory effects in several CDKs at the nanomolar level. To further investigate the molecular mechanism of TG02-induced cell cycle regulation in glioma, a cell cycle analysis was performed by flow cytometry using the Click-It Edu Flow Cytometric Assay Kit in GSC923 and U251 cell lines, a human stem-like cell and patient derived cell line, respectively. Protein expression of CDKs and cyclins was tested by Western blotting. RNAseq analysis using Next Generation Sequencing was performed (only GSC923) and followed by a supervised hierarchical clustering analysis of cell cycle pathways that were derived from Qiangen’s Ingenuity Pathway Analysis (IPA). Our results showed that TG02 treatment results in G2/M-phase arrest in glioma cell lines. The protein expression of CDK1, CDK2, cyclin A2 and cyclinB1 are downregulated in both TG02 treated cell lines. Additionally, RNAseq analysis revealed down-regulation of cyclins A2, B1 and B2 and significant regulation in mRNA expression of G2/M checkpoint and spindle formation proteins, indicating G2 checkpoint and mitosis progression are also affected by TG02. Taken together, we demonstrated that TG02 induced cell cycle G2/M arrest through downregulating CDK and cyclin expression signaling in the preclinical models of glioblastoma. These findings strongly support the further investigation of TG02 as a potential therapy for malignant gliomas.

**#115** Fluobendazole targets cancer stem-like properties and the HER2/Akt signaling pathway in HER2-positive breast cancer with trastuzumab resistance. Daesul Sung, Youngkwon Cho, Eunhye Oh, Tae-Min Cho, Yoon-Jae Kim, Hyoung Kim, Jae hong Seo. Korea University, Seoul, Republic of Korea.

Background and Propose: HER2-positive breast cancers are associated with an aggressive phenotype and trastuzumab resistance leading to poor clinical outcomes. Fluobendazole (FLU) is a potent anthelmintic agent that inhibits microtubule polymerization, and also exhibits anticancer activity in several cancer types. The objective of the present study was to investigate the effect of FLU on apoptosis, HER2/Akt signaling pathway, cancer stem cell (CSC)-like properties and trastuzumab-resistance in HER2-positive breast cancer cells. Methods: FLU-induced antitumor effects were characterized by MTS assay, Sub-G1 and G2/M-phase arrest population, Annexin-V analysis, ALDH1 activity assay, Western blotting and immunofluorescence. For in vivo study, trastuzumab-resistant JIMT1 cells were injected into the mammary fat pads of BALB/c nude mice. We then determined the tumor growth and CSC-like properties. Results and conclusion: FLU significantly reduced cell viability and induced apoptosis with accompanied by activation of caspase-3, -7, and -8, as well as PARP cleavage in HER2-positive cell lines (MDA-MB-453, SKBR3 and BT474). FLU-induced apoptosis is associated with significant decreases in expression levels of HER2, p-HER2 (Tyr1221/1222), HER3, and p-HER3 (Tyr1289). FLU treatment also resulted in G2/M-phase arrest of the cell cycle, as evidenced by a marked downregulation of phospho-Histone H3 expression. These results were closely related to the decline of CSC-like properties, coinciding with the suppression of ALDH1 activity. These effects were also observed in trastuzumab-resistant...
JIMT-1 cells, and significant reductions in tumor growth and ALDH1A1 downregulation were observed in xenograft models together with downregulation of HER2 expression in vivo. Our findings suggest that FLU may be potentially effective for the treatment of HER2-positive breast cancer with trastuzumab resistance.

**#116** Erufosine, a third-generation alkyphosphocholine with cytotoxic and cytostatic effects in breast and colorectal cancer cells. Assim Pervaiz,1,2 Saqib Mahmood,3 Martin R. Berger,3,4 Univ. of Health Sciences, Lahore, Pakistan;5 German Cancer Research Center (DKFZ), Heidelberg, Germany.

Introduction: Breast and colorectal cancers are highly malignant diseases with low 5-year survival rate (<21%) in advanced stages, which highlights the need of finding new therapeutic options. Considering this, we are interested in exploring the anticancer potential of erufosine, a membrane targeting synthetic lipid analogue belonging to a class of antineoplastic agents known as alkyphosphocholines (APCs). Properties such as intravenous administration and significantly reduced hemolytic activity as compared to previous APC generations, and efficient cytotoxic effects against malignant cells make it an attractive anticancer compound. Materials and Methods: In the present experiments, we studied the effects of erufosine against breast (MDA-MB-231) and colorectal (SW480 and SW620) cancer cell lines. Following exposure to erufosine (0.78-100μM) for 24, 48 and 72h, cytotoxic effects were measured by MTT dye reduction assay and inhibitory concentrations (IC) were identified by GraphPad Prism software. Erufosine mediated cytostatic effects were highlighted by means of propidium iodide (PI) based labeling of the DNA followed by flow cytometry analysis. Furthermore, the cells were exposed to increasing concentrations of erufosine (IC25, IC50, IC70) and alterations in cell cycle relevant genes (CCNA1, CCND1, CCNE1, CCNE2, CDK1, CDK4) were investigated by qRT-PCR methodology. Results Erufosine was highly active against the selected cell lines (IC50 < 10μM, 24h) and induced significant arrest in the G2/M phase of the cell cycle. The cytostatic effects were more pronounced in the two metastatic cell lines of breast (MDA-MB-231) and colorectal (SW620) cancers. At molecular levels, erufosine induced expression of CCNA1 (> 2-fold) in SW480, SW620, and MCF-7 cells, while the expression was inhibited in MDA-MB-231 cell lines at high concentrations of OTX015, a potent and selective BET inhibitor. Interestingly, BET-inhibition by culturing LNCaP prostate cancer cells with increasing concentrations of OTX015, a potent and selective BET-inhibitor. Methods and Results: ODM-207 is a potent and selective BET inhibitor that is structurally unrelated to the benzodiazepine like inhibitors such as IJQ1, I-BET762, and OTX015. We tested the preclinical activity of ODM-207 across multiple tumor types in a 4-day growth inhibition in vitro assay. ODM-207 potently inhibits cell viability of a wide range of hematological and solid tumor cell lines. ODM-207 also shows potent anti-proliferative effects in patient-derived cancer cells representing various tumor types. In VCaP prostate cancer cell lines, ODM-207 induced apoptosis consistent with increased expression of pro-apoptotic regulators, whereas potent antitumor effects were observed in a preclinical xenograft model with IV injection of VP. Taken together, our results suggest that VP is a promising chemotherapeutic agent for the treatment of endometrial cancer.

**#118** Targeting cancer with a novel BET bromodomain inhibitor ODM-207. Anu-Maarit Moilanen,1 Mari Björkman,2 Reetta Riikonen,3 Chan-drasekhar Abbineni,4 Mahaboobi Jaleel,3 Sivapriya Marappan,5 Tarja Ikonen,6 Girish Daginakatte,7 Aravind A B,7 Elinna Mattila,7 Juha Rantala,8 Susanta Sama-jdar,2 Murali Ramachandra,2 Pekka Kallo,7 Orion Corporation Orion Pharma, Turku, Finland; 4Aurigen Discovery Technologies Limited, Bangalore, India; 5Orion Corporation Orion Pharma, Finland; 6Missvik Biology, Turku, Finland.

Background: Bromodomain and extra-terminal (BET) family proteins are dual bromodomain-containing epigenetic readers that bind to acetylated-lysine residues at gene promoter and enhancer elements in histones and recruit protein complexes to promote transcriptional elongation. Recent evidence demonstrates that BET bromodomain inhibition leads to anti-proliferative activity in preclinical models of many hematological malignancies and solid tumors. Selective inhibition of BET bromodomains by small molecule inhibitors has emerged as a promising therapeutic strategy for the treatment of cancer. In this study, we evaluated the antitumor activity of ODM-207, a novel, potent and highly selective BET bromodomain inhibitor. Methods and Results: ODM-207 is a potent and selective BET inhibitor that is structurally unrelated to the benzodiazepine like inhibitors such as IJQ1, I-BET762, and OTX015. We tested the preclinical activity of ODM-207 across multiple tumor types in a 4-day growth inhibition in vitro assay. ODM-207 potently inhibits cell viability of a wide range of hematological and solid tumor cell lines. ODM-207 also shows potent anti-proliferative effects in patient-derived cancer cells representing various tumor types. In VCaP prostate cancer cell lines, ODM-207 induced apoptosis consistent with increased expression of pro-apoptotic regulators, whereas potent antitumor effects were observed in a preclinical xenograft model with IV injection of VP. Taken together, our results suggest that VP is a promising chemotherapeutic agent for the treatment of endometrial cancer.

**#119** Vitamin K2 targets castration-resistant prostate cancer VCaP cells by reactive oxygen species mediated apoptotic cell death. Subramanyam Dasari,1 Maarten C. Bosland,2,3 Andre Kajdacy-Balla,2 Gnanasekar Munirathnam,1 University of Illinois, College of Medicine at Rockford, Rockford, IL; 2University of Illinois at Chicago, Chicago, IL.

Prostate Cancer (PCA) is the second most common cancer in western countries especially in US population, in which castration-resistant prostate cancer (CRPC) is the major cause for patient mortality. Current treatment options available for CRPC are not efficient and have undesirable side effects. Hence there is an urgent need to develop non-toxic and effective treatment strategies for CRPC. Vitamin K2 (VK2), a natural menaquinone has several medicinal values including anti-cancer activity and anti-osteoporosis effect. The aim of this study is to evaluate the therapeutic effects of Vitamin K2 (VK2) and its anti-cancer mechanism against CRPC. In this study, we have used VCaP cell line (ATCC) which is established from a patient with hormone refractory prostate cancer. VCaP cells were treated with various concentrations of VK2 to evaluate its effects on cell viability by MTT assay, anchorage independent growth by soft agar assay, cellular senescence by beta-galactosidase staining assay and cancer cell migration by wound healing assay. We have also assessed the VK2-induced production of intracellular reactive oxygen species (ROS) using DCF (2’7’-dichlorofluorescein) probe based fluorescence assay. VK2 induced...
apoptosis was detected by Annexin-V FITC and TUNEL assays. Western blot analysis was utilized to uncover the anti-proliferative and anti-metastatic mechanisms of VK2 against CRPC. Our results showed that VK2 significantly inhibits the proliferation of VCaP cells in a dose dependent manner at 48 hrs treatment in vitro. MTT data also showed that anti-proliferative effects of VK2 were significantly abrogated in the presence of Lin28B knockdown lines. These results were further validated by the in vivo studies by Real-Time PCR, Flow Cytometry, Western Blotting, Smart-Flare confocal immunofluorescence analysis. In conclusion our study suggests that VK2 might be an effective anti-proliferative and anti-metastatic agent for CRPC by specifically targeting key anti-apoptotic, cell cycle progression and metastasis promoting signaling molecules.

#120 A study of in vitro and in vivo effects of a novel peptide and rhenium compounds on prostate cancer. Hirendra N. Banerjee,1 Sasha Hodge,1 William Kahana,2 Santosh Mandal,2 David Weber,2 Renä Lapidus,2 Fazilul Sarkar,3 Somirajan Ghosh,1.1 Elizabeth City State University, Elizabeth City, NC; 2Morgan State University, Baltimore, MD; 3University of Maryland Medical School, Baltimore, MD; 4Greenbaum Cancer Center, University of Maryland, Baltimore, MD; 5Wayne State University Kororanos Cancer Center, Detroit, MI; 6Howard University, Washington, DC.

Emerging evidence suggests that acquisition of the epithelial-to-mesenchymal transition (EMT), a process that resembles the genesis of cancer stem-like cells, contributes to tumor aggressiveness and is mediated by deregulated expression of microRNAs (miRNAs), such as miR-200 and let-7 family. Loss of miR-200 expression results in the over-expression of Lin28B, which is prevalent in human Prostate Cancer (PCa). Lin28B is also known to block the processing of another miRNA (pre-let-7 and pri-let-7), resulting in decreased mature let-7, thereby leading to increased Suzz12 and EZH2 expression, which are important components of the polycomb repressive complex 2 (PRC2). Thus, over-expression of Lin28B and loss of miR-200 and let-7 appear to be responsible for PCA aggressiveness. A group of novel rhenium compounds have shown promising anti-cancer properties in various cancer cell lines tested in our laboratories. Our current investigations show that re-expression of miR-200b, miR-200c, and let-7 could be achieved by treating cells with our newly developed rhenium compounds by down-regulating the expression of Lin28B and EZH2. Based on our preliminary results, we hypothesize that over-expression of Lin28B leads to the acquisition of invasive and metastatic characteristics in PCA cells (EMT-phenotype cells) via down-regulation of miR-200b and miR-200c, resulting in increased expression of Suzz12, ZEB1, and ZEB2. We also hypothesize that over-expression of Lin28B represses the maturation of let-7 family, leading to increased expression of EZH2, and these processes can be attenuated by treatment of cells with novel rhenium compounds in vitro. Our experiments were done on prostate cancer cell lines derived from both Caucasian (CA) and African American (AA) patients and also biopsy samples obtained from both CA and AA patients at Henry Ford Hospital and Karmanos Cancer Center of Wayne State University, Detroit, MI. We confirmed using gene expression studies by micro array and Real-Time PCR and then data analysis by Ingenuity software system to validate our hypothesis and continued our research by testing one of these rhenium compounds-RPR1 on prostate cancer model of nude mice at Greenbaum Cancer Center(GCC) of University of Maryland at Baltimore. In vitro studies by Real-Time PCR, Flow Cytometry, Western Blotting, Smart-Flare technology, cell death and proliferation assays along with inhibition of spheroide forming assays showed efficacy of these rhenium compounds as anti cancer agents. In vivo studies also showed decrease of tumor volume and mass, no toxicity, thus efficacy of these novel drugs. ACKNOWLEDGEMENT: Supported by NIH-3R01CA164318-03S1 and NIH-T-34-GM100831, a NSF-VES-TEM award and NSF-SLSAMP award.

#121 Identification and characterization of channel oscillator blockers as inhibitors of YAP/TAZ and glioblastoma cells. Wei Li, Zhijun Liu, Jennifer Xavier, Hong-Gang Wang. Penn State Univ. College of Medicine, Hershey, PA.

Glioblastomas (GBM) are the most aggressive primary brain tumors. Despite major advances in neuroimaging and neurological techniques over the past decades, the neurosurgical management of GBM patients remains challenging. Median survival of the patients is about 14.6 months. In order to radically alter the clinical course of these tumors, it is important to develop new drugs that specifically target these malignancies. The transcription co-activators YAP and TAZ are known to drive the development and sustainance of many pathways that drive their development and maintain their homeostasis. In a screen for small molecules, which can inhibit the growth of human glioblastoma cells, we identified a set of calcium channel blockers (CCB). We found that these CCB induce cell death through an apoptosis-independent process. To understand the mechanisms of the non-apoptotic cell death induced by these CCB, we performed cellular imaging which is responsible for the cell death induction. We found that these CCB are able to inhibit YAP and TAZ, two oncogenic effectors of the Hippo tumor suppressor pathway. Importantly, GBM cells expressing the YAP active mutant or overexpressing YAP are less sensitive to these CCB. These results suggested that inhibition of YAP/TAZ is a mechanism by which CCB suppress tumor cell growth. The Hippo pathway is an essential signaling network in regulating tissue homeostasis. In this pathway, the transcription co-activators YAP and TAZ promote a gene expression program favoring cell proliferation, survival and self-renewal. In normal brain, the nuclear accumulation of YAP is largely limited to regions harboring neural progenitor cells. However, increased nuclear accumulation of YAP is found in various brain tumors, especially those more aggressive subsets of gliomas. In addition, high expression of YAP is associated with reduced mean survival in astrocytoma patients. Our results identified a novel approach to target the Hippo pathway for GBM treatment. We will present our work in further characterizing these regulations and therapeutic potentials of these calcium channel blockers in the conference.

#122 Preclinical update on targeting KRAS wild-type colorectal cancer with an EGFR-targeted monoclonal tri-body mixture, MM-151. Shawn P. Carey, Hongfang Wang, Erika Handly, Brittany Ahlstedt, Daniel Gaddy, Jeffrey D. Kears, Greg Finn, Birgit Schoeberl, Rachel Nering. Merrimack Pharmaceuticals, Cambridge, MA.

Within KRAS wild-type colorectal cancer (KRAS-wt CRC), standard of care treatment includes EGFR-directed monoclonal antibodies (mAb) in combination with an irinotecan-based chemotherapy regimen. While such combinations have clinically established synergy, the underlying mechanisms of synergy remain elusive, and resistance invariably develops against either the EGFR-targeted therapy or irinotecan, leaving patients with limited treatment options. MM-151 consists of three anti-EGFR IgG1 mAbs that bind simultaneously to non-overlapping epitopes on the EGFR extracellular domain (ECD). Preclinical and clinical studies have demonstrated that oligoclonal targeting of EGFR with MM-151 results in blockade of the EGFR pathway via potent inhibition of ligand-driven signal amplification and receptor downregulation. Critically, these activities are uniquely maintained in the presence of high-affinity EGFR ligands as well as mutations in the ECD of EGFR, both of which drive resistance to cetuximab and panitumumab. Here, we tested the preclinical hypothesis that comprehensive inhibition of the EGFR pathway by MM-151 leads to enhanced antimutator activity when combined with chemotherapy in models of KRAS-wt CRC. In vitro studies to investigate the activity of MM-151 in combination with SN-38, the active metabolite of irinotecan, showed that MM-151 potentiates SN-38-induced cell death in KRAS-wt CRC models. Treatment with MM-151 potently suppresses EGFR pathway activation caused by SN-38, and the combination enhances stress and pro-apoptotic signaling, leading to increased apoptosis. Notably, these synergistic activities are maintained in the presence of two key mechanisms of resistance to cetuximab: high-affinity ligands and EGFR ECD mutations. Dose scheduling studies demonstrated that sustained treatment with MM-151 following transient SN-38 exposure - a hallmark of small molecule therapy - partially overcomes the sub-optimal activity of chemotherapy alone. Finally, data from in vivo xenograft and PDX studies strongly supports the addition of MM-151 to irinotecan-based chemotherapy regimens for KRAS-wt CRC. In summary, we present data supporting the hypothesis that comprehensive antagonism of the EGFR pathway via oligoclonal targeting of EGFR with MM-151 leads to enhanced activity when combined with chemotherapy in preclinical KRAS-wt CRC models. The ability of MM-151 and SN-38 to overcome both de novo and acquired mechanisms of resistance within CRC models supports further clinical evaluation of this combination in metastatic CRC patients.
#123 A novel J-series prostamide mediates D-series prostamide-induced apoptosis in skin cancer: receptor-independent signaling. Eman Soliman,1 Daniel Ladin,2 Hussam Albassam,2 Ahmed E. Elhassanny,2 Allison Danell,2 Rukiyah Van Dross3. 1Faculty of Pharmacy, Zagazig University, Zagazig, Egypt; 2Brody School of Medicine, East Carolina University, Greenville, NC; 3Department of Chemistry, East Carolina University, Greenville, NC, 4Brody School of Medicine and Center for Health Disparities East Carolina University, Greenville, NC.

Non-melanoma skin cancer (NMSC) is the most common cancer in the United States. The absence of selective toxicity is a major problem that limits the utility of chemotherapeutic and radiation therapy for NMSCs. Our previous data showed that the endocannabinoid, anandamide, selectively induced apoptosis in skin cancer: receptor-independent signaling. Exogenous 15d-PMJ2 mimicked the activity of PMD2 demonstrating preferential cytotoxicity towards tumorigenic compared to non-tumorigenic cells. In tumorigenic keratinocytes, PMD2 induced apoptotic cell death, oxidative stress and increased expression of pro-apoptotic ER stress proteins, C/EBP homologous protein (CHOP). In non-tumorigenic HaCaT keratinocytes were utilized. A significant reduction in cell viability was observed in JF2 but not in HaCaT cells treated with PMD2. Furthermore, the use of prostaglandin D receptor (DP1 and DP2) antagonists did not inhibit PMD2-induced apoptosis indicating that the activity was mediated by a receptor-independent pathway. Similar effects were observed in keratinocytes treated with the structurally-related arachidonic acid metabolite, prostaglandin D2 (PGD2). Interestingly, PMD2 increased the production of J-series prostaglandins in both tumorigenic and non-tumorigenic keratinocytes. LC-ESI-MS/MS analysis detected ethanolamide-conjugated J-series PG (15-deoxy-12,14 pros-tamide J1, 15d-PMJ2) in PMD2-treated cell culture media. Since selective inhibitors of the J-series prostaglandins are not available, 15d-PMJ2 mimicked the activity of PMD2 demonstrating preferential cytotoxicity towards tumorigenic compared to non-tumorigenic keratinocytes. In addition, 15d-PMJ2 induced oxidative stress, ER stress and apoptosis in tumorigenic keratinocytes. These findings suggest that the cytotoxicity of PMD2 is mediated by 15d-PMJ2. Since PMD2 and its metabolite 15d-PMJ2 are preferentially toxic towards tumorigenic cells, PMD2 or 15d-PMJ2 may be an ideal topical treatment for NMSC that will elicid minimal toxicity in healthy surrounding skin cells.

#124 Impirdione ONC201 promotes intra-tumoral accumulation of CD3+ /NK+ cells that contribute to its anti-tumor efficacy. Jessica Wagner,1 C. Leah Kline,1 Lanlan Zhou,1 Andrew Zloza,2 Charles Chesson,2 Jenna Newman,2 Howard Kaufman,2 Joseph Bertino,2 Mark Stein,2 Wafik El-Deiry3. 1Fox Chase Cancer Center, Philadelphia, PA; 2Rutgers Cancer Institute of New Jersey, New Brunswick, NJ.

ONC201, a first-in-class oral anti-tumor agent, upregulates the pro-apoptotic immune cytokine TRAIL and activates the integrated stress response leading to upregulation of death receptor 5 in bulk tumor and cancer stem cells. We previously demonstrated that ONC201 exerts a dose- and schedule-dependent effect on tumor progression in vivo while suppressing Akt/ERK signaling in tumors in a dose/frequency-dependent manner (Wagner et al., AACR, 2016). We also provided evidence that ONC201 inhibits a potent anti-metastatic effect (Wagner et al., AACR, 2016). We observed accumulation and activation of TRAIL-secreting NK+ cells within ONC201-treated tumors in C57/B6J, Balb/c, and athymic nude tumor-bearing mice. Importantly, ONC201 exerts in vivo anti-tumor efficacy on tumor cell lines that are ONC201-resistant in vitro, including acquired stable resistance. Using the NK-depleting antibody GM1, we demonstrate that the activation and TRAIL secretion of NK+ cells induced by ONC201 significantly contributes to in vivo anti-tumor efficacy, including TRAIL/ONC201-resistant tumors. We are currently investigating how ONC201 recruits NK cells to the tumor by examining NK-recruiting chemokine factors within the tumor site. We have also demonstrated upregulation of CD3+ T cells by ONC201 in syngeneic mice. Finally, we observed an increase in activated TRAIL-secreting NK cells in the peripheral blood of patients upon ONC201 administration in the clinic. Our results demonstrate novel and potentially significant increases in cytotoxic NK cell recruitment to tumors. The results offer a unique pathway of immune stimulation for cancer therapy that may be combined with immune checkpoint or targeted cancer therapy strategies. We are currently investigating the role of NK cells and CD3+ cells in ONC201’s ability to inhibit metastasis by using a metastatic model that involves surgically removing the primary tumor and allowing metastases to grow in vivo before treatment. These findings indicate that ONC201 possesses immunomodulatory activity and provide a rationale for combining ONC201 with PD-1/PD-L1 inhibitors, a combination we are currently testing in syngeneic immunocompetent mouse models.

#125 Differential effects of inhibitors of epigenetic modifiers on IDH mutant cell lines. Alişan Kayabolen, Tugba Bagcı Onder. Koş University, Istanbul, Turkey.

Our aim is to find effective drug treatments for IDH mutant glioma cells and investigate their mechanism of actions by analysing genetic and epigenetic alterations upon drug treatments. Drug screen was performed on IDH mutant and wild type cell lines by using a library of epigenetic modifier inhibitors. Hits for IDH mutant cells were validated with individual treatments. Combination treatments of these inhibitors were also performed to see synergistic effects. Then, IDH wild type glioma cells and BJ fibroblasts were transduced with mutant IDH1 overexpression plasmid, and checked if they were sensitized to these drugs or not. Based on drug screen performed on IDH mutant and wild type glioma cell lines, we found that IDH mutant cell lines, MGG119 and MGG152, were highly sensitive to 5-azacytidine, Chaetocin and GSK-J4. For IDH1 mutant MGG152 cell line, IC50 value for GSK-J4 is 5.2 ± 1.3 μM, for 5-azacytidine is 2.2 ± 0.3 μM, and for Chaetocin is 10.2 ± 1.0 nM at 48h. On the other hand, these drugs did not affect viability of IDH wild type glioma cell lines and fibroblasts around these concentrations. Moreover, combination of drugs, especially combination of Chaetocin and 5-azacytidine had a highly synergistic effect on IDH mutant cells while it was still ineffective on IDH wild type cells. After transduced with mutant IDH overexpression plasmid, fibroblasts became more sensitive to these drugs. On the other hand, although their growth rate was reduced, IDH wild type glioma cells were not significantly sensitized to these drugs upon mutant IDH overexpression. IDH mutation mainly inhibits DNA and histone demethylation, and leads to hypermethylation phenotype. Therefore, it is an expected result that IDH mutant cell lines are sensitive to 5-azacytidine which is an inhibitor of DNA methyltransferases, and Chaetocin which is an inhibitor of H3K9 methyltransferases. However, it was interesting to observe that GSK-J4 which is an inhibitor of H3K27 demethylases is also effective on IDH mutant cells. This may be a result of dependency on low number of active genes since IDH mutation causes downregulation of many genes by methylation. GSK-J4 may downregulate these genes by increasing H3K27 methylation. On the other hand, these effects might also be results of induced cell stress upon drug treatment. To answer this question, now we are trying to knock-down target genes of these drugs by shRNA, and obtaining compatible results. We are also planning to perform RNA-seq analysis to see genes with altered expressions upon drug treatments. To conclude, unlike IDH wild type glioma cells or healthy fibroblasts, IDH mutant glioma cells were found to be sensitive to few epigenetic drugs in low concentrations. This was confirmed by sensitization of fibroblasts to these drugs upon mutant IDH1 overexpression. In addition, combination of these drugs had a highly synergistic effect on mutant cells which may give opportunity to use drugs in very low concentrations efficiently.

#126 A chemogenomic approach reveals the action of splicing modulators at the branch point adenosine binding pocket defined by the PHF5A/SF3b complex. Teng Teng, Jennifer Tsai, Xiaolei Puyang, Michael Seller, Shouyang Peng, Daniel Aird, Silvia Buonamici, Benjamin Caleb, Betty Chan, Laura Corson, Jacob Feala, Peter Fekkes, Craig Karr, Manav Korpal, Yoshiharu Mizui, Eunice Park, James Palacino, Peter Smith, Vanitha Subramanian, Jeremy Wu, Liuhua Yu, Agustin Chicas, Markus Warnhurst, Nicholas Larsen, Ping Zhu. H3 Biomedicine Inc., Cambridge, MA.

Dysregulation of RNA splicing can cause various forms of cancer and neuromuscular disorders. Thus, developing compounds with splicing-modulating activity represents a promising therapeutic approach for these diseases. Natural products such as pladienolide, herboxidiene, and spliceostatin have been identified as potent splicing modulators that bind SF3B1, a member of the SF3b subcomplex that assembles into the U2 snRNP. Using integrated chemogenomic, structural and biochemical approaches, we show that PHF5A, another core component of the SF3b complex, is also targeted by these modulators. Whole exome sequencing of E707 (pladienolide analogue) and herboxidiene resistant clones identified common mutations in either PHF5A-Y36, SF3B1-K1071, SF3B1-R1074, or SF3B1-V1078, which represents a promising therapeutic approach for these diseases. Natural products such as pladienolide, herboxidiene, and spliceostatin have been identified as potent splicing modulators that bind SF3B1, a member of the SF3b subcomplex that assembles into the U2 snRNP. Using integrated chemogenomic, structural and biochemical approaches, we show that PHF5A, another core component of the SF3b complex, is also targeted by these modulators. Whole exome sequencing of E707 (pladienolide analogue) and herboxidiene resistant clones identified common mutations in either PHF5A-Y36, SF3B1-K1071, SF3B1-R1074, or SF3B1-V1078, which represents a promising therapeutic approach for these diseases.

Myelodysplastic syndrome (MDS) & Acute Myeloid Leukemia (AML) are hematologic malignancies that arise from a population of aberrant hematopoietic stem cells (HSCs). Overactivated innate immune signaling pathways such as IRAK1, TRAF6, IL1RAP, S100A9 and IL8 have been demonstrated in MDS/AML and play important roles in propagation of disease. IRAK4 ( interleukin-1 receptor-associated kinase 4), is a protein kinase involved in signaling innate immune responses and forms a critical signaling complex with IRAK1. To determine its role in disease pathobiology, we analyzed transcriptomic data from CD34+/stem and progenitor cells from 183 MDS patients and found significantly increased expression of IRAK4 in MDS samples belonging to the high-risk RAEB category (Refractory anemia with excess of blasts, N=80, P=0.03 when compared to healthy controls). Furthermore, increased IRAK4 expression was predictive of significantly adverse prognosis (P value < 0.05, median survival of 2.6 years compared to 5.2 years for group with lower IRAK4). Clinical correlations revealed that MDS patients with higher IRAK4 expression in stem/progenitor cells had significantly higher transfusion dependence and had higher leukemic blast counts (Mean Blast Count 9.3% vs 3.9%, P<0.05), further demonstrating IRAK4 to be an adverse prognostic marker in MDS. IRAK4 was also upregulated in highly purified FACS sorted disease initiating stem cell populations (Long Term-HSC, CD34+/CD38-/CD90+/Lin-ve) from AML patients with complex cytogenetics when compared to healthy controls. To functionally determine the role of IRAK4 in MDS/AML pathogenesis, we utilized CA-4948, a potent, oral, small-molecule inhibitor of IRAK4, to assess the effect of inhibiting IRAK4 catalytic activity. In vitro, CA-4948 blocked downstream NF-kB pathway signaling, including secretion of proinflammatory cytokines, in Toll-like receptor stimulated THP1 leukemia cells. CA-4948 was tested in clonogenic assays from primary MDS and AML samples. MDS and AML are associated with block in differentiation that leads to cytopenias that are the cause of morbidity in these patients. Treatment with CA-4948 led to increased erythropoiesis and myeloid differentiation in a majority of samples. Furthermore, drug treatment led to decreased viability of MDS/AML stem cells (CD34+/CD38-/Lin-ve) In vivo studies using a THP1 leukemia xenograft model in Ndg mice demonstrated that CA-4948 was well tolerated and led to significantly decreased disease burden after 6 weeks of treatment. In conclusion, we demonstrate that IRAK4 is upregulated in MDS/AML stem cells and is an adverse prognostic marker. Importantly, a novel, specific, inhibitor of IRAK4 shows preclinical in vitro and in vivo efficacy in MDS and AML models.

The next-generation CKD2/9 inhibitor CYC065 elicits marked anti-neoplastic effects in lung cancer by engaging ant metastatic pathways. Masanori Kawakami, Jason Roszik, Lin Zheng, Jonathan Kurie, Lisa Maria Mustachio, Xi Liu, Ethan Dmitrovsky. The University of Texas MD Anderson Cancer Center, Houston, TX.

We previously reported CKD2 antagonism with the first generation CKD2/9/7 inhibitor seliciclib (CYC202; Cyclophilin) triggered anaphase catastrophe. This occurred when genetically unstable cancer cells with supernumerary centrosomes (a hallmark of cancer) fail to cluster excessive centrosomes at mitosis. This causes multipolar cell division and apoptotic death. Anaphase catastrophe is conferred after CKD2 antagonism of aneuploid cancer cells, sparing bipolar normal cells with two centrosomes. CYC065 (Cyclophilin) is a next-generation CKD2/9 inhibitor that is undergoing clinical trial. Here, we explored CYC065 activity against lung cancer cells, some with known high metastatic potential. CYC065 substantially inhibited growth, triggered apoptosis, and induced anaphase catastrophe in murine (ED1, LKR13, and 393P) and human (Hop62, A549, and H1299) lung cancer cells. In marked contrast, these effects were largely unseen in bipolar immortalized pulmonary epithelial (murine C10 and human BEAS-2B) cells. We sought to explore whether CYC065 antineoplastic effects engaged ant metastatic pathways. In vitro migration and invasion assays were performed. CYC065 markedly inhibited migration and invasion of lung cancer cells (murine: 344SQ and KC2; human: A549 and H1299). Reverse Phase Protein Arrays (RPPAs) interrogated nearly 300 growth-regulatory proteins in murine (344SQ and KC2) and human (A549 and H1299) lung cancer cells over time (6, 12, 24, and 48 hours) after CYC065 or vehicle treatments. These lung cancer cell lines overexpressed integrin pathways such as αEβ7. When highlighted proteins were clustered after CYC065 treatment, some species were clustered as significantly up-regulated or down-regulated in all cells over studied time points. Not surprisingly, up-regulated proteins included those involved in DNA damage or apoptosis. In addition to known CDK targets like phosphorylated retinoblastoma protein, novel proteins were markedly down-regulated, including mTOR pathway proteins (such as PIK3CA), PI3K pathway proteins (such as PTEN) and phosphor- ylated S6. Affected pathways were identified using Ingenuity Pathway Analysis (IPA). IPA revealed up-regulation of pathways that engaged ATM signaling, G2/M DNA damage checkpoint regulation, or apoptosis signaling. Down-regulated pathways affected mTOR signaling, cyclins, cell cycle regulation, or integrin pathways. Mouse studies will be presented that examine in vivo effects of CYC065 in reducing metastases. Taken together, the next-generation CKD2/9 inhibitor, CYC065, elicits marked antineoplastic effects by antagonizing migration and invasion of lung cancer cells. Comprehensive RPPA and IPA studies found that distinct pathways trigger these effects.

Assessing the mechanism and therapeutic potential of modulators of the human mediator complex-associated protein kinases CDK8/19. Paul A. Clarke, Maria-Jesus Ortiz Ruiz, Robert Te Poel, Alajumoke Adenijipopola, Gary Box, Christien Ewan, Sharon Gowan, Alexis De Haven Brandon, Philip Hewitt, Wolfgang Kaufmann, Aurelie Malling, Florence Raynaud, Felix Rohdich, Kai Schiemann, Stephanie Simon, Richard Schneider, Melanie Valenti, Julian Blagg, Trevor Dale, Suzanne Eccles, Paul Workman, Dirk Wienke Dirk Wienke. Institute of Cancer Research, London, United Kingdom; Merck KGaA, Darmstadt, Germany; Cardiff University, Cardiff, United Kingdom.

Mediator-associated protein kinases CDK8 and CDK19 are context-dependent drivers or suppressors of tumorigenesis. Their inhibition is predicted to have pleiotropic effects, but it is unclear whether this will impact on the clinical utility of CDK8/19 inhibitors. We identified two structurally differentiated chemical series, suitable for exploring their function. In addition to tools that fulfill the criteria set out for chemical probes, the lead compounds from each series, CCT251921 and MSC2530818, had optimal pharmacological and pharmaceautical properties making them suitable for preclinical studies. Having potent, highly selective, orally bioavailable exemplar compounds from these series in hand, we were well positioned to investigate the therapeutic potential of dual CDK8/19 inhibition. The compounds exhibited modest anti-tumor activity in colorectal cancer cell line xenograft models with modulation of p-STAT3, a target engagement biomarker, and altered gene expression profiles, including super-enhancer regulated gene expression, consistent with the inhibition of CDK8/19. In PDX-derived cell cultures we observed inhibition of soft-agar growth in cells derived from different tumor types. However, we only detected significant antitumour activity in 1 of 6 colorectal PDX models tested in vivo, and one example of sensitization to standard of care chemotherapy, despite showing inhibition of p-STAT3. Acute myeloid leukemia cells were the most sensitive cancer type in the PDX panel with therapeutic potency seen in systemic and sub-cutaneous models. Significantly, the compounds impacted on stem cell biology. In a bone progenitor model we saw dose-responsive activation and inhibition of markers of bone matrix and bone deposition that was distinct from WNT blockade. Treatment of a diverse collection of normal cell co-culture models detected a unique response profile consistent with stimulation of an immune/inflammatory response. In vivo treatment of a genetically engineered mouse model expressing oncogenic beta-catenin shifted cells within hyperplastic intestinal crypts towards a transit amplifying progenitor cell phenotype. Finally, in pre-clinical tolerability studies we observed a similar, widespread adverse safety profile at therapeutically relevant exposures for both CCT251921 and MSC2530818. At the concentrations tested we detected >80% inhibition of p-STAT3 and increased IL-12 plasma levels. Since the observed pathological effects were generated with two potent, highly selective, but structurally distinct compounds, we conclude that the adverse consequences of treatment are the direct result of inhibition of CDK8 and/or CDK19. The serious and complex nature of the toxicity observed indicates that the clinical development of either series of CDK8/19 modulators, or other chemotypes with similar profiles, will be extremely challenging.
Discovery and characterization of small molecules targeting the DNA-binding ETS domain of ERG in prostate cancer. Miriam S. Butler,1 Mani Roshan-Moniri,1 Michael Hsing,1 Desmond Lau,2 Ari Kim,1 Paul Yen,1 Marta Mroczek,1 Mannan Nouri,1 Scott Lien,1 Peter Axtiero-Cilles,1 Kush Dalal,1 Clement Yau,1 Fariba Ghahdi,1 Yubin Guo,1 Takehisa Yamazaki,1 Sam Lawn,1 Martin Matthewson,1 Gregory-Evans1. Paul Hennie,1,2 Artem Cherkesov,1 Michael E. Cox,1,4 Vancouver Prostate Centre, Vancouver, British Columbia, Canada; 2University of British Columbia, Vancouver, British Columbia, Canada.

Genomic alterations involving translocations of the ETS-related gene ERG occur in approximately half of prostate cancer cases. These alterations result in aberrant, androgen-regulated production of ERG protein variants that directly contribute to disease development and progression. This study describes the discovery and characterization of a new class of small molecule ERG antagonists identified through rational in silico methods. These antagonists are designed to sterically block DNA binding by the ETS domain of ERG and thereby disrupt transcriptional activity. We confirmed the direct binding of a lead compound, VPC-18005, with the ERG-ETS domain using biophysical approaches. We then demonstrated VPC-18005 reduced migration and invasion rates of ERG expressing prostate cancer cells, and reduced metastasis in a zebrafish xenograft model. These results demonstrate proof-of-princ-

Chemotherapeutic effects of naturally occurring colossolactones against solid tumor cells in-vitro. Mohammed A. Baghdadi,1 Fahad A. Al-Abbasi,1 Ali M. El-Halawany,1 Ahmed M. Al-Abd,2 King Faisal Specialist Hospi-
tal & Research Centre, Jeddah, Saudi Arabia; Kingdom Abdulaziz University, Jeddah, Saudi Arabia; 2Faculty of Pharmacy, Cairo University, Cairo, Egypt; 4National Research Centre of Egypt, Giza, Egypt.

Colossolactones were isolated previously from the Vietnamese mushroom ganoderma colossum, such triterpenoids. The different colossolactone com-

African polyherbal formulation alleviates benzene-induced leukemia in Wistar rats. Olufemi E. Akanni,1 Ayodeji Faremi,2 Aminat O. Agboola,1 Adekemi R. Akanni,3 Oluseye E. Bamisaye4. 1Ladoke Akintola University of Technology, Department of Medical Laboratory Science, College of Health Sciences, Osogbo, Nigeria; 2Ladoke Akintola University of Technology, Department of Chemical Pathology; College of Health Sciences, Osogbo, Nigeria; 3Ladoke Akintola University of Technology, Department of Medical Microbiology and Para-
stology, College of Health Sciences, Osogbo, Nigeria; 4Afe Babalola University, Department of Medical Laboratory Science, College of Medicine and Health Sciences, Ado Ekiti, Nigeria.

Background: Chemotherapy and radiotherapy are effective cancer treatment options but are associated with significant side effects. Therefore, more effec-
tive therapies are sorely needed. This study investigated the chemotherapeutic effects of a polyherbal formulation on benzene induced leukemia in Wistar rats. The polyherbal formulation is composed of fruits such as Orange, Lime, Lemon, Pineapple, Grape and Vegetables like; Pumpkin leave, Garden egg in honey medium. Method: Leukemia was induced by injecting 0.2 ml of benzene solution intravenously through the tail at 48 h intervals for four weeks. Leukemia develop-

typing enumeration of CD19 expression also corroborated the leukemia assess-

Aloe-emodin as a potential lead targets lung cancer stem cells. Pei-

Cancer stem cells (CSCs) have been proposed to be responsible for tumor initiating, drug resistance, metastasis, and recurrence. Many novel therapeutic strategies have been designed to target and eliminate CSCs. According to our previous study, we have established a model of CSCs and cancer associated fibroblasts (CAFs) co-culture system for anti-CSCs drug screening. Here, we report one of the potential hits screened via this platform and the anti-CSCs activity was further investigated both in vitro and in vivo. Human lung CSCs and CAFs were primary cultured from patient with lung adenocarcinoma according to our previous study. Image-based high content screening system was used to analyze different parameters after drug treatment. Tumorogenicity and self-

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<th>CD 19 (X 10^3/L)</th>
<th>Leukocyte count (X 10^9/L)</th>
<th>Red blood cells (X 10^12/L)</th>
<th>Hematocrit (L/L)</th>
<th>Hemoglobin (g/dl)</th>
<th>Platelets (X 10^9/L)</th>
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<td>47.59 ± 1.39</td>
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| 49.946, 220.32, 219.65, 100.81, 238.54, 116.57 and 131.94 μM, respectively. Isolated compounds were examined for their potential antitumor activity against breast (MCF-7), cervix (HeLa), colorectal (HCT-116) and liver (HepG2) cancer cells. Cytotoxicity of the isolated compounds was assessed in the aforementioned cell lines using SRB assay after 72 h of exposure and fitted using E_max model. Amongst colossolactones, schisanalactonoe-A and colossolactone-IV showed the best cytotoxic profile in all cell lines under inves-

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EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Targeting the PI3K Pathway


The Hedgehog (Hh) signaling pathway is a critical regulator of embryonic patterning, and aberrant Hh pathway activation has been implicated in a diverse spectrum of cancers. Therefore, components of the Shh pathway (such as Shh, SMO, and GLI1/2) are viable therapeutic targets for anti-tumor strategy. SMO antagonists such as GDC-0449 and VNP-LDE225 have received FDA approval for treating basal cell carcinoma. However, acquired resistance has emerged as a challenge to targeted therapeutics and may limit their anti-cancer efficacy. Studies have linked the hyperactive phosphatidylinositol-3-kinase (PI3K)/Akt/mTOR signaling pathway in a variety of human cancers and drug resistance condition. The synergistic role of PI3K/AKT/mTOR in Hh signaling in embryonic development and Hh-dependent tumors has been reported. Both the PI3K/Akt/mTOR and Hh pathway also play important role in maintaining the stem cell-like properties of cancer stem cell (CSCs). We report the development of a potent dual mTOR-PI3K inhibitor designated DBC-HDG2-57 with Hh signaling pathway antagonist activity. In vitro biochemical assays showed that DBC-HDG2-57 inhibited recombinant p110α and mTOR kinase with IC50 of 27.2 and 133 nM, respectively. DBC-HDG2-57 is also highly active in cellular assays, as evidenced by inhibition of phosphorylation of cellular downstream targets AKT and S6K1 in wild-type and mTOR kinase inhibitors-resistant prostate cancer cells (LNCaP and VCaP). In vitro and in vivo experiments revealed that DBC-HDG2-57 demonstrated tumor regression in xenograft models of breast cell lines (MCF7 and BT549) and pancreatic cancer cell (MiaPaCa2 and Panc1) cell lines. Interestingly, DBC-HDG2-57 demonstrated Hh signaling pathway antagonist activity in a 293 cell-based Gli-luciferase inhibition assay upon agonist treatment, and retains inhibition activity against the Smo wild-type and D473H mutant co-transfection with IC50 of 261.8 and 327.7 nM, respectively. In addition, DBC-HDG2-57 can significantly decrease the cancer stem cell-like side population of Panc1 pancreatic cell, and inhibited the migration of E3LZ10.7 pancreatic cancer cell. DBC-HDG2-57 demonstrated in vivo tumor growth inhibition activity in LS-180 colon cancer xenograft model and reduced the Gli-1 RNA abundance in the tumor. Taken together, our data demonstrate that combined pharmacological blockade of mTOR-PI3K and Hh pathway of DBC-HDG2-57 can provide a therapeutic strategy for targeting ligand-dependent Hh cancer.

#135 Targeted therapy of hormone receptor-positive breast cancer harboring PIK3CA and AKT1 genetic aberrations. Maryam Shariati, Kurt W. Evans, Stephen M. Scott, Huiqin Chen, Funda Meric-Bernstam. The University of Texas MD Anderson Cancer Center, Houston, TX.

Introduction: Molecular alterations in the PI3K/Akt/mTOR pathway is implicated in the pathogenesis of ER positive breast cancer with high frequency and therefore is the major focus of drug development. Activating mutations of this signaling pathway occur in more than 70% of breast tumors. The genetic alterations affecting major components of PI3K/Akt/mTOR include genes encoding the PI3K catalytic subunits p110α (PIK3CA) and the PI3K effector AKT1. Despite the efficacy of the available small molecule inhibitors for targeting different components of PI3K pathway, the contribution of PIK3CA and AKT1 genetic alterations to targeted therapy is not well understood. We speculated that ER positive breast tumors with PIK3CA and AKT1 alterations are more sensitive to PI3K and Akt inhibitors due to differential downstream pathway effectors. Methods: We used a panel of isogenic MCF7 cell lines with oncogenic mutations of PIK3CA and AKT1 established through somatic cell gene targeting. A series of pharmacological compounds currently approved for breast cancer therapy or in clinical trials in targeting PI3K/Akt/mTOR pathway were evaluated using viability, clonogenic and cell cycle analysis assays. We performed functional proteomic profiling using reverse phase proteomic analysis (RPPA) to investigate differential protein expression in response to PI3K pathway inhibition in PIK3CA and AKT1 mutants MCF7 cell lines. In vivo experiment was done to evaluate the efficacy of the inhibitors in suppressing tumor growth. Results: We demonstrated that PIK3CA (E545K) and AKT1 (E17K) sensitized cells to the inhibitory effects of BLY719 (p110α catalytic subunit inhibitor) and AZD9395 (pan-AKT kinase inhibitor) on survival, cell cycle progression and colony formation. The presence of PIK3CA and AKT1 mutations conferred growth advantage and exhibited increased proliferation in vitro and in vivo. These alterations dramatically increased Akt phosphorylation and induced activation of PI3K downstream effectors. RPPA analysis revealed several proteins differentially expressed in PIK3CA and AKT1 mutant cells compared with wild type (p-value < 0.05). Conclusion: PIK3CA and AKT1 mutations showed distinct effects on sensitivity to targeted pathway inhibitors in an isogenic luminal breast cancer model. The presence of these genetic alterations in estrogen receptor (ER) positive breast cancer provided proliferative advantage and enhanced their sensitivity to targeted PI3K inhibition. These results will contribute to identify significant and combinatorial strategies for targeting PI3K pathway with maximal activity at tolerated dose and selection for individuals most likely to be responsive based on their genomic alterations.

#136 Phospho-S6 levels correlate with response to Copanlisib (BAY 80-6946) in multiple myeloma. Sarah Larson, Mao Yu Peng, Monica Mead, Andrea Vandros, Dylan Conklin, Erika Von Euw, Dennis J. Slamon. UCLA, Santa Monica, CA.

Background: Copanlisib (BAY 80-6946) is a reversible, pan-class I PI3K inhibitor with preferential activity for the alpha isoform, which may be of particular importance in multiple myeloma. Here we demonstrated the in vitro efficacy of copanlisib in a panel of 20 multiple myeloma cell lines. Baseline levels of phospho-S6 (P-S6) correlated with sensitivity to copanlisib, resulting in a potential biomarker of response. In addition, the change of P-S6 post-treatment could be used as a pharmacodynamic biomarker for copanlisib treatment. Methods: We screened a panel of 20 multiple myeloma cell lines and selected 3 sensitive: NCI-H929, MM.1S, L.1-363, and 3 resistant: AMO-1, JNJ3, COLO-677 for further analysis. We performed apoptosis and cell senescence assays following 72 hours of 50nM and 100M copanlisib exposure. Cell cycle analysis and induction of apoptosis were performed by FACS after propidium iodide or PI/ANX-V FITC staining, respectively. Cells treated with copanlisib showed decreased cellular senescence determined by measuring β-galactosidase activity in cells treated for 96 hours. Reverse phase protein array (RPPA) was performed at baseline and post treatment for proteomic analysis with confirmatory western blots. Flow cytometry was also performed to monitor the post-treatment P-S6 level changes. Results: Copanlisib treatment induced apoptosis in the sensitive cell lines (50-80% AN-V + cells) but not in resistant cell lines (1-5% AN-V + cells). An increased cell cycle arrest in G1 was also observed in the sensitive cell lines but not in the resistant lines. The cell senescence assays confirmed apoptosis rather than cell senescence as the mechanism of inhibition of proliferation. RPPA analysis demonstrated lower baseline p-S6 (S235/236, S240/244) protein levels in sensitive compared to resistant cell lines and this was confirmed with western blot analysis. Treatment with copanlisib resulted in a greater decrease in p-S6 in the sensitive cell lines NCI-H929 and L.1-363 (53-83%, 73-91% respectively) than in the resistant cell lines COLO-677 and JNJ3 (5-27%, and 38-67%, respectively), which was validated by western blot and phospho-flow. We also showed by RPPA and WB that copanlisib downregulates pro-survival and proliferation molecules including p-86K, p-S6 and p-4EBP1, and upregulates pro-apoptotic PDCD4 in all cell lines, but to a greater extent in sensitive cell lines. Finally, pharmacodynamic p-S6 response remained at different post-treatment time points. Discussion: A differential response to copanlisib is seen in the myeloma cell line panel. A subgroup of multiple myeloma cell lines demonstrated median IC50 values in the low nanomolar range (5-100nM), and responses correlated with low baseline P-S6. This p-S6 stratified response was only observed with PIK3-α inhibitors, but not with inhibitors targeting other PI3K isoforms. These findings may indicate the need for development of a new patient screening method or companion diagnostic.

#137 FT-1518, a new generation selective and potent mTORC1 and mTORC2 inhibitor: an in vitro and in vivo profile. Alain C. Mita,¹ Monica M. Mita,¹ Anthony D. William,² Khalid Pasha,³ Chandra Siddamadappa,⁴ Kevin Zikaras,⁵ Felix T. Garzon,⁵ Samual Oshin Comprehensive Cancer Institute, Ge-dars-Sinai Medical Center, Los Angeles, CA;⁶ FTG BIO, Hackensack, NJ;⁷ Vprag-Gen Biosciences Pvt. Ltd., Myssore, India.

Mammalian target of rapamycin (mTOR) is a clinically validated target in the treatment of cancer. mTOR forms two distinct multiprotein complexes, mTORC1 and mTORC2 which regulate cell growth, metabolism, proliferation, and survival. Rapamycin analogues target only the mTORC1 complex but do not affect the mTORC2 complex, which is an important driver for cancer cell growth and survival. The new generation of "Selective" mTOR inhibitors, blocking both mTORC1 and mTORC2 signaling might increase the efficacy and safety while expanding the therapeutic potential of these anticancer agents. Herein we describe FT-1518, a low nanomolar potent, kinase and PI3K sub family selective mTOR inhibitor. FT-1518 not only exhibited high oral bioavailability in preclinical species but has demonstrated excellent microsomal stability with no inhibitory activity towards undesired CYPs. FT-1518 showed high sustained tumor exposure and target Inhibition in a single oral dose xenograft model. FT-1518 depicted very good growth inhibitory activity across a large panel of hematologic and solid tumor cell lines with most activities falling into
low nanomolar range. mTOR kinase inhibition in cells, by FT-1518, resulted in more potent inhibition of the mTOR pathway biomarkers (mTORC1 & 2 biomarkers [pAkt(S473) and pS6(240/244) or p70 S6K]), no inhibition of PI3K biomarker [pAkt(T388)], and improved anti-proliferative activity as compared with rapamycin. FT-1518 exhibited dose-dependent and higher tumor growth inhibition (TGI) in multiple solid tumor xenografts compared with rapalogs and is poised to enter the clinic with a favorable toxicology profile.

#138 FOXO proteins mediate adaptive resistance to PI3K inhibition in mucinous colorectal cancers. Murali R. Kuracha, Peter Thomas, Brian W. Loggie, Venkatesh Govindarajan. Creighton University, Omaha, NE.

Purpose: Mucinous colon adeno-carcinomas (MCAs) comprise a substantial fraction (10-15%) of sporadic colorectal cancers (CRCs). MCAs show a distinct range of genetic modifications compared to nonmucinous CRCs and are more prone to peritoneal dissemination and distant metastasis. These aggressive cancers show a poorer response to chemotherapy and are often difficult to treat. A higher mutation rate in effectors of the KRAS-RAF-MEK (80% vs 42%) and PI3K-AKT-mTOR (60% vs 22%) pathways in MCAs over nonmucinous CRCs suggested potential targets for therapy. Our previous results showed that, MCAs, though initially sensitive to PI3K inhibition (PI3K), later develop adaptive resistance. The purpose of this study was to test whether FOXO transcription factors mediate this adaptive resistance to PI3K single agent treatment. Methods: PI3K was inactivated in MCA cell lines, LS174T (KRAS G12D, PI3K H1047R) and SW213 (KRAS G12C, PI3K WT), by treatment with GDC0941 (Pictilisib), a small molecule inhibitor of class I PI3K. Alterations in phosphorylation of effectors in the PI3K-AKT or MEK-ERK pathways were determined using western blots. FOXO nuclear localization was assessed by immunocytochemistry, confocal microscopy and nuclear fractionation studies. Expression of FOXO proteins (FOXO1, FOXO3 and FOXO4) was reduced by short inhibitory RNAs (siRNAs). FOXO1 was also inhibited in MCA cells using AS1842856, a small molecule inhibitor. Results: Immunohistochemical analysis and nuclear fractionation studies showed significant nuclear enrichment of FOXO1 and FOXO3 in MCA cell lines in response to PI3K inhibitor treatment. Upon siRNA-mediated knockdown of FOXOs, reduced ERK phosphorylation seen in response to PI3K treatment was substantially reduced. Concomitant with this decrease was a reduction in expression of receptor tyrosine kinases (RTKs) IR, IGF1R, HER2 and HER3. Combinatorial treatment of PI3K and FOXO1 inhibitors resulted in a more pronounced loss of cell viability compared to single agent treatment. Conclusions: Our results suggest that resistance to PI3K single agent inhibition seen in MCA cells is mediated, at least in part, by nuclear translocation of FOXO proteins, particularly FOXO1. Our results support a model where PI3K inhibition results in increased FOXO nuclear localization which in turn, leads to transcriptional upregulation and activation of RTKs which then lead to rebound activation of the MEK-ERK pathway and increased survival and resistance. A role for FOXO proteins in mediating PI3K resistance in KRAS-mutant MCA cells has not been described before. Our results suggest that PI3K single agent therapy is unlikely to be successful in KRAS-mutant MCAs due to FOXO-mediated resistance thus providing a rationale for combination treatment strategies.

#139 M2698, a novel dual inhibitor of p70S6K and Akt: preclinical efficacy in gastric cancer. Shota Fukuo,

1 Takahisa Kojima,1 Yoshikatsu Koga,2 Mayumi Yamачхи, Masahiro Yasunaga,2 Yasuhiko Matsumura,2 Toshihiko Doi,1 Takayuki Yoshino,1 Toshio Kuronita,3 Anderson Clark,4 Brian Elenbaas,4 Wani,2 Samson Jacob,2 Mitch Phelps,1 Thomas Schmittgen3.

M2698 is a selective, ATP-competitive dual inhibitor of p70S6K and Akt1/3 that is being evaluated in a phase 1 clinical trial in cancer patients. The dual nature of M2698 may result in improved clinical efficacy by blocking the increased Akt activity in a compensatory feedback loop induced by PI3K/AKT/mTOR (PAM) pathway inhibition. In previous preclinical studies, M2698 was shown to have potent anti-proliferative activity in vitro, and inhibit tumor growth in some xenograft models in vivo. The current study examined the effects of M2698 on cell proliferation in a panel of 13 gastric cancer cell lines, as gastric cancers often harbor mutations in PAM pathway genes that deregulate this signaling pathway. Cells were treated with M2698 at a range of concentrations and proliferation was evaluated at least twice using the WST-8 cell proliferation assay kit (Dojindo Molecular Technologies, Inc. Japan). Two cell lines, HGC-27 and IM95m, were particularly sensitive to M2698 (50% growth inhibition concentration [G100] 74 and 160 nM, respectively). The G100 values for all other cell lines were >10-fold higher. Although HGC-27 and IM95m each carry a PI3KCA hotspot point mutation, the presence of an activating PI3K pathway mutation was not the sole determinant of sensitivity, as a third PI3KCA mutant cell line, MKN1, was far less sensitive to M2698 (G100 9.0 um). Western blot analysis of pharmacodynamic biomarkers showed that M2698 (1 uM) blocked the PI3K-AKT-mTOR pathway in both sensitive and resistant cell lines, inhibiting phospho-g[76]-S6 and p-PRAS40, despite increased p-Akt. Some biomarkers from the PAM pathway and other signaling pathways appeared to be associated with sensitivity to M2698, but these candidate biomarkers need to be validated in a larger panel of cell lines. Treatment of HGC-27-tumor-bearing mice with M2698 (10, 20, 30 mg/kg/day) for 14 days resulted in significant tumor growth inhibition (80%-98%) at Day 29 compared to treatment with vehicle (p<0.01). In conclusion, we have shown that gastric cancer cell lines have a range of sensitivities to M2698 and the sensitivity cannot be explained solely by genetic mutations in the PAM pathway. M2698 provides potent PAM pathway inhibition in both sensitive and resistant gastric cancer cell lines by blocking both p70S6K and Akt. M2698 significantly inhibits tumor growth in the HGC-27 xenograft model in vivo.

#140 Discovery and biological evaluation of PQR530, a highly potent dual pan-PI3K/mTORC1/2 inhibitor. Denise Ragoon,1 Florent Beaufils,2 Anna Me-

Ione,1 Alexander M. Sele,1 Thomas Bohnacker,1 Marc Lang,2 Jürgen Mestan,2 Petra Hillmann,1 Paul Hebeisen,2 Dario Fabbro,2 Matthias P. Wymann.2

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The PI3K/AKT/mTOR signaling pathway plays a fundamental role in cell proliferation, growth and survival and aberrant activation of this signaling pathway has been shown to drive the progression of malignant tumors.1,2 Drugs targeting the pathway at multiple points, such as dual PI3K/mTOR inhibitors appear to have the broadest activity profile to address cancer therapeutic strategies and are currently being explored in numerous clinical studies. Recently, we presented PQR309, a novel, brain-penetrant pan-PI3K/mTOR inhibitor, which entered phase II clinical trials in 2016.2,3 Here, we report the lead optimization of PQR530, a potent and brain-penetrant follow-up compound as pan-PI3K/mTORC1/2 inhibitor. The development of a follow-up compound concentrated on the improvement of both, the potency and the selectivity for all targeted kinases, namely the class IA PI3K isoforms as well as mTOR. We present a detailed ligand-based structure-activity relationship study which was obtained by systematic modifications of the hinge region as well as the affinity binding substituents. This study led to the identification of PQR530, a dual pan-PI3K/mTORC1/2 inhibitor showing excellent activities in cellular assays as well as in PI3K and mTOR enzymatic binding assays. In A2058 melanoma cells PQR530 inhibited protein kinase B (PKB, pSer473) and ribosomal protein S6 (pS6, pSer240/244) phosphorylation with IC50 values of 0.07 mM. PQR530 showed excellent selectivity over a wide panel of kinases, as well as excellent selectivity versus unrelated receptor enzymes and ion channels. Moreover, PQR530 displayed potency in a panel of 44 cancer cell lines (NTRC OncolinesTM) to prevent cancer cell growth (mean value for G100 of 426 nM). Oral application of PQR530 to mice resulted in a dose-proportional PK and demonstrated good oral bioavailability, tissue penetration and excellent blood brain barrier penetration.1,3 An in silico generated synthetic route allowed rapid access to multi-gram quantities of PQR530 for pre-clinical development in only 4 steps. In conclusion, PQR530 inhibits all PI3K isoforms and the mammalian target of rapamycin (mTOR) complexes CI/2 potently and selectively, and shows anti-tumor effects in vitro and in vivo.1 [1] M. P. Wymann, M. Zvelebil, M. Laffargue (2003). Phosphoinositide 3-kinase signalling – which way to target? Trends Pharmacol Sci.; 24, 366-376. [2] V. Cmiljanovic et. al. “PIKR09: Structure-Based Design, Synthesis and Biological Evaluation of a Novel, Selective, Dual Pan-PI3K/mTOR Inhibitor” presented at AACR Annual Meeting 2015, April 18-22, Philadelphia, Pennsylvania, USA. [3] P. Hillmann et al. “Pharmacological Characterization of the Selective, Orally Bioavailable, Potential Dual PI3K/mTORC1/2 Inhibitor PQR530” abstract submitted for AACR Annual Meeting 2017, April 1-5, Washington, D. C., USA.
pathways and compounds to treat advanced HCC, we considered the ATP-competitive mTOR inhibitor INK128. ATP-competitive mTOR inhibitors attenuate both mTORC1 and mTORC2. We evaluated INK128 in sorafenib sensitive and insensitive HCC cell lines, CD44 impassive, and CD44-synthetic HCC and those cell lines with acquired sorafenib resistance. CD44 was significantly increased in Huh7 cells and Huh7 cells were more sensitive to sorafenib. A profound decrease in cell proliferation and migration, and reduced the cells more sensitive to the anti-proliferative effects of INK128. INK128 suppressed CD44 expression by blocking phosphorylation of eukaryotic translation initiation factor 4EBP1 in HCC cells while allosteric mTOR inhibitors do not. Moreover, INK128 exhibited potent anti-proliferative and anti-migration effects on the mesenchymal-like HCC cells, but not on the HCC cells. To study the transcriptional effects of combining PI3K and BET inhibitors in this lymphoma model, WILL-2 xenograft tumors from mice treated with single dose INCBO50465, INCBO54329, the combination, or vehicle control were analyzed by RNAseq. INCBO50465 enhanced the ability of INCBO54329 to repress a MYC-driven transcriptional program, and the combination also regulated multiple development and inflammatory pathways. Together, these data support the use of dual PIM and BET inhibitors for the treatment of DLBCL.

#142 Exploiting replicative vulnerabilities to counter incomplete responses to PI3K/Akt/mTOR inhibition. Sameer Chopra,1 Mario Niepel,2 Anne Jenny,3 Marc Hafner,3 Peter Sorger4, Dana-Farber Cancer Institute, Boston, MA; 3Harvard Medical School, Boston, MA.

While a broad range of cancers harbor mutations that dysregulate PI3K-Akt-mTOR signaling, most small molecule drugs targeting this pathway have failed to demonstrate clinical benefit. The treatment of PI3K-mTOR tumors is of significant interest. In this study, we investigated the mechanisms that account for drug efficacy and failure in PI3K-mTOR pathways. We used a diverse collection of 25 PI3K-Akt-mTOR inhibitors. Drug response phenotypes and changes in signaling were quantitated using time-lapse imaging and quantitative single cell immunofluorescence microscopy, respectively. We identified GSK2126458 and Torin2 as having superior potency and efficacy in PI3K-pathway disregulated triple negative breast cancer (TNBC) cell lines using this approach. Unlike other PI3K-Akt-mTOR inhibitors, whose inefficacy arises from insufficient induction of apoptosis and variable induction of cell cycle progression at G1/S, GSK2126458 and Torin2 each rapidly induce caspase 3/7 activity and durably inhibit the proliferation of surviving cells. While the effectiveness of GSK2126458 in vitro appears to arise from near-complete suppression of PI3K-Akt-mTOR signaling, this approach has already proved unachievable in clinical trials. An alternative therapeutic strategy was identified by characterizing the mechanism of action of Torin2, a tool compound that inhibits both mTOR kinase and the DNA damage response kinases ATR, ATM, and DNA-PK. Unlike other PI3K-Akt-mTOR inhibitors studied, Torin2 counters incomplete drug block at G1/S by concomitant induction of intolerable replication stress in S phase cells. The unique cell cycle pharmacology of Torin2 is recreated by combining inhibitors of mTOR and ATR/Chk1 kinases undergoing evaluation in clinical trials. In the context of combination therapy, where cytotoxicity arises from targeting S phase vulnerabilities rather than from PI3K-Akt-mTOR inhibition in G1, submaximal doses of mTOR kinase inhibitors are sufficient and confer benefit by preventing the outgrowth of cells that survive fractional killing from ATR/Chk1 inhibition. The unique cell cycle pharmacology of Torin2 suggests a novel strategy for mitigating the failure of precision monotherapy and have implications for the treatment of tumors where genetic lesions in the PI3K pathway co-occur with replicative vulnerabilities.

#143 Preclinical studies on potent therapeutic combination partners for the potent and selective PI3Kδ inhibitor INCBO50465 in DLBCL. Matthew C. Stubbs, Robert Collins, Leslie Hall, Alla Volgina, Holly Koblish, Sang Hyun Lee, Timothy Burn, Phillip C. Liu, Jin Lu, Eddy Yue, Yun-Long Li, Andrew P. Combs, Wenqing Yao, Gregory Hollis, Reid Huber, Bruce Ruggeri, Peggy Scherer. Incyte Corp., Wilmington, DE.

The delta isoform of PI3K (PI3Kδ) plays an essential role in B-cell development and function by mediating the signaling of key receptors on B cells. Increased malignant B cell proliferation and survival has also been associated with aberrant activation of PI3Kδ, making selective inhibition of this isoform an attractive therapeutic approach for the treatment of B cell malignancies. INCBO50465 is a potent inhibitor of PI3Kδ, with a >20,000 fold selectivity over other PI3K isoforms.Emerging clinical data indicate that INCBO50465 monotherapy is well tolerated and results in promising clinical responses in patients with various lymphoma histologies, including those with DLBCL. We therefore sought to explore rational combination strategies for INCBO50465 using mouse xenograft models of ABC-subtype (HBL-1), GCB-subtype (Pfeiffer), and GCB/double-hit (WILL-2) human DLBCL, evaluating standard of care agents such as bendamustine and rituximab, as well as with targeted agents. PIM inhibition is a logical addition to PI3Kδ inhibition as a therapeutic approach as both kinases play a critical role in the AKT signaling pathway, having overlapping substrates. Likewise BET inhibition is a rational addition to PI3Kδ inhibition in “double-hit” DLBCL as a compromised activity is a consequence of MYC transcriptional activity. In vivo studies performed in the Pfeiffer xenograft model demonstrate that INCBO50465 combined with the pan-PIM inhibitor INCBO53914 yielded complete tumor regressions. This profound decrease in tumor cell survival was due in part to the significant reduction in pBAD levels resulting from dual PIM and PI3Kδ inhibition. Despite modest single agent activity in vivo, the combination of INCBO50465 with BET inhibitors, INCBO54329 or INCBO57643, resulted in significant anti-tumor efficacy in all of the DLBCL models studied, and caused a greater than 90% decrease in tumor volume in the most resistant tumor line. To study the transcriptional effects of combining PI3Kδ and BET inhibitors in this lymphoma model, WILL-2 xenograft tumors from mice treated with single dose INCBO50465, INCBO54329, the combination, or vehicle control were analyzed by RNAseq. INCBO50465 enhanced the ability of INCBO54329 to repress a MYC-driven transcriptional program, and the combination also regulated multiple development and inflammatory pathways. Together, these data support the use of dual PIM and BET inhibitors for the treatment of DLBCL.

#144 Src leads to a novel mechanism of resistance to PI3K inhibitors through regulation of PI3K/p85 activation. Gui Chul Kim, Hae Yum Nam, Hyang Ju Lee, Min Kyung Kim, Geun Hee Lee, Myung Woul Han, Seong Won Kim, Univ. of Ulsan, Seoul, Republic of Korea.

Activation of the PI3K pathway co-occur with replicative vulnerabilities. Recent studies have shown that elevated expression of PIK3CB, but not other PI3K isoforms, significantly correlated with higher rate, risk, and poor prognosis of recurrent head and neck cancers. As a consequence, a greater understanding of resistance mechanisms through our results will enable the rational design of combination regimens and sequential treatment algorithms to improve clinical outcomes.

#145 PIK3CB inhibitors selectively block the survival of glioblastoma cells. Lamuye Le1, Zhi Sheng2, Virginia Tech Carilion Research Institute, Roanoke, VA; 1Virginia Polytechnic Inst. & State Univ., Roanoke, VA.

Objective: To explore the divergent role of PI3K isoforms in glioblastoma Background: Glioblastoma multiforme is the most malignant brain tumor in adults. Despite aggressive treatments, the median survival and five-year overall survival of glioblastoma patients remains low (14.6 months and 4.7%, respectively). Nearly 90% of patients experience recurrence within two years, leaving patients with few treatment options. Recently, PI3K pan inhibitors have been used to treat recurrent glioblastoma and achieved modest effect in the clinic. PI3K has four catalytic isoforms (PIK3CA, B, D, and G); hence, it is possible that these isoforms may have different roles in glioblastoma and need to be selectively targeted. This idea is further supported by our recent results, in which we have shown that elevated expression of PIK3CB, but not other PI3K isoforms, significantly correlates with high recurrence, risk, and poor prognosis of recurrent glioblastomas. Hence, we hypothesize that PIK3CB-selective inhibitors are more effective for glioblastoma. Design/Methods: We used a panel of glioblastoma cells with different levels of PIK3CB, treated them with PIK3-isomorph-selective inhibitors, and compared them to pan PI3K inhibitors. We measured cell viability using the MTS cell viability assay. Normal human astrocytes were used as controls to determine toxicity. Results: We found that PIK3CB inhibitors TGX-221 and GSK2636771 significantly blocked the proliferation of PIK3CB-high U87MG and SF295 cells, while it had no effect on the viability of PIK3CB-low A172 and LN229 cells. In contrast, other PI3K inhibitors and pan inhibitors either non-selectively blocked or had no effect on these cells. Importantly, these
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146 The PI3K inhibitor, taselisib, has a unique mechanism of action that leads to enhanced potency in PIK3CA mutant models. Kyung W. Song, Kyle A. Edgar, S. Kirkpatrick, Lillian Phu, Michelle Nanini, Rebecca Hong, Eric Cheng, Lisa Crocker, Amy Young, Deepak Sampath, Lori Friedman. Genentech, South San Francisco, CA.

Activating mutations in PIK3CA are commonly found in a wide variety of human cancers, and the dysregulation of the phosphoinositide-3 kinase (PI3K) signaling pathway has been implicated in tumor cell growth and survival. Taselisib (GDC-0032), a novel, oral, selective inhibitor of p110alpha, spares inhibi-
tion of p110beta, is more potent in cancer cells bearing PIK3CA mutants than with wildtype PIK3CA. Preclinical studies demonstrate that taselisib induces more apoptotic cell death in PIK3CA mutant cancer cells than other PI3K inhibitors. We have discovered that taselisib has a dual mechanism of action, both blocking PI3K signaling, and inducing a decrease in p110alpha protein levels. Mass spec analysis reveals that taselisib treatment leads to the specific depletion of mutant p110alpha without significant change in wildtype p110alpha protein levels. This drug-induced p110alpha protein depletion is rescued by E1 inhibitors and by proteasome inhibitors. Other clinical PI3K inhibitors, including p110alpha selective and pan-PI3K inhibitors, are unable to induce the depletion of mutant p110 alpha protein. Furthermore, we have discovered that taselisib effectively maximizes the pathway suppression of PIK3CA mutant cells at 24 hrs in response to feedback. In comparison to other clinical-stage PI3K inhibi-
tors administered at a maximum tolerated dose, taselisib has superior efficacy with increased tumor regressions in PIK3CA mutant xenograft models. In sum-
mary, these preclinical studies indicate that PI3K inhibitors, which have the ability to trigger degradation of mutant p110alpha protein, can more effectively suppress the signaling pathway, which may result in greater anti-tumor activity and improved therapeutic index in PIK3CA mutant tumors.


Out of the breast cancer subtypes, triple-negative breast cancer (TNBC) has the poorest prognosis without effective targeted therapies. A stem cell transcription factor KLF5 is over-expressed in basal type TNBC and promoting cell proliferation, survival and stemness. Previously, we demonstrated that Milfepristone suppresses basal TNBC stem cells by down-regulating KLF5 expression through inducing the expression of miR-153. In this study, Metformin, a first-line drug for type 2 diabetes mellitus, was demonstrated to target breast cancer stem cells selectively. However, the efficiency and the mechanism of action of metformin in TNBC are unclear. We demonstrated that metformin decreased the percentage of TNBC stem cells partially through the downregulation of the expression of KLF5 and its downstream target genes, such as Nanog and FGFR1, in TNBC cell lines. Metformin induced GS3Kβ-mediated KLF5 protein phosphorylation and degradation through the in-
hibition of PKA activity in TNBC cells. Consistently, PKA activators increased the expression of KLF5 and to a lesser extent Nanog. Knockdown of KLF5 and GS3Kβ protein levels in human TNBC samples. These findings suggest that met-
formin suppresses TNBC stem cells partially through the PKA-GS3Kβ-KLF5 sig-
naling pathway.

148 A dual and selective small molecule inhibitor of EGFR and PI3 kinase shows promising preclinical activity against KRAS and BRAF mutant colorectal tumors. Joel D. Maust, Elizabeth K. Ziemke, Christy L. Frankowski, Debra Mcgregor, Jun Beom Ku, Rachel Mumby, Karin M. Hardiman, Christopher E. Whitehead, Judith S. Sebolt-Leopold. Univ. of Michigan, Ann Arbor, MI.

Agents targeting epidermal growth factor receptor (EGFR) have met with limited success in the clinical management of colorectal cancer (CRC). Mutations in KRAS, BRAF, and PIK3CA are important drivers of resistance to EGFR-
targeted therapy. Conversely, EGFR-mediated feedback mechanisms serve to mediate resistance to MEK inhibitor-based treatment of CRC by reactivating MAP kinase signaling. Our central hypothesis is that a dual small molecule inhibitor that potently and selectively targets only EGFR and PI3KA, when com-
bined with a MEK inhibitor, will be highly efficacious against subpopulations of BRAF mutant or KRAS mutant colorectal cancers that are dependent upon these kinase mechanisms to drive tumor progression. Employing a computational mod-
eling approach, we explored the known binding modes of structurally related ATP binding site inhibitors of EGFR and PI3K to design small molecules that simultaneously inhibit both kinases in a selective manner. To the best of our knowledge, the lead compound MXT-211, whose binding mode is flipped in

compounds, but not PIK3CB inhibitors, blocked the growth of astrocytes. Con-
clusions: Selective blockade of PIK3CB, but not other PI3K isoforms, is an ef-
flective therapy for glioblastoma with a low toxicity to normal tissues.

149 Co-targeting mTORC and EGFR signaling as a potential therapeutic strategy in HNSCC. Adam D. Swick, Prashanth J. Prabakaran, Amal Javid, Margot Miller, Michael Fisher, Emmanuel Sampene, Irene M. Ong, Kwanglok Nickel, Randall J. Kimple. Univ. of Wisconsin-Madison, Madison, WI.

Background - Head and neck squamous cell carcinomas (HNSCCs) have high rates of mutation and other alterations along the PI3K/AKT/mTORC signaling axis. This has led to interest in the use of therapeutics targeting this pathway, however identifying reliable predictive biomarkers to guide patient selection remains challenging. Despite excellent preclinical data, the use of these compounds as monotherapy has been underwhelming in initial clinical trials. The EGFR monoclonal antibody cetuximab remains the only approved targeted agent for HNSCC and with reasonable toxicity profiles, has potential use in combination therapy. Methods - Both catalytic mTORC (AZD8055) and PI3K/ mTORC(NVP-BEZ-235) inhibitors were tested +/− cetuximab in vitro and in vivo pre-clinical models. A panel of HNSCC cell lines and patient derived xenografts (PDx) were evaluated for PI3K/AKT/mTORC pathway mu-
tation by sequencing and potential protein biomarker by immunohistology and IHC. Cell lines were assayed for sensitivity to all three agents by growth inhibition and clonogenic survival assay. DNA replication(BrdU uptake) and apoptosis (Capa- se 3/7 activity) were investigated to assess the mechanism of inhibition. The specificity of the molecular targeted effects was confirmed by siRNA knock-
down. Five unique PDx models that presented PIK3CA mutation or intrinsinc cetuximab resistance were treated with a combination of cetuximab and the dual mTORC inhibitor AZD8055 in a nude mouse model. Results - Assessment of the panel of HNSCC cell lines by mutational hotspot sequencing did not reveal any obvious sensitizing mutations, whereas putative protein biomarkers (e.g. PIK3CA, pAKT) were elevated in some cell lines. All cell lines showed modest response to both PI3K/mTORC and dual mTORC inhibition. The addition of cetuximab to either agent produced modest additive effect. Mechanistic studies revealed that growth inhibition rather than death induction was the major anti-
cancer effect. SI RNA knockdown showed similar molecular signaling and func-
tional effects to drug inhibition. Using the PDx models, in vivo single agent mTORC inhibition inhibited growth of a PI3KCA mutant cancer, but had no effect on any PIK3CAAKT+ or a second PI3KCA mutant model. In all models the combination therapy showed greater growth delay than cetuximab alone. Conclu-
sions - The uniform ability of PI3K/mTORC and mTORC inhibition to suppress the growth of HNSCC cells highlights the role of this signaling pathway to drive the proliferation. In vivo, despite some PDx models meeting likely selection criteria, the single agent therapy was largely ineffective. Conversely the combination treatment produced growth delay and suggests the potential for adding a catalytic mTORC inhibitor to cetuximab therapy for HNSCC patients. Overall, these results add to a growing body of evidence suggesting that attempts to match genetic alternation or other biomarker to the optimal therapy in HNSCC remains complex and challenging.

150 Identification of determinants of sensitivity to AKT inhibition using breast cancer (BC) patient-derived xenografts (PDx). Albert Gris-Oliver,1 Mafalda Oliveira,2 Marta Guzman,1 Olga Rodriguez,1 Judith Grueso,1 Maurizio Scaltriti,2 William J. Howat,3 J Carl Barrett,4 Javier Cortés,5 José Baselga,2 Albert Gris-Oliver,1 Mafalda Oliveira,2 Marta Guzman,1 Olga Rodriguez,1 Judith Grueso,1 Maurizio Scaltriti,2 William J. Howat,3 J Carl Barrett,4 Javier Cortés,5 José Baselga,2 Albert Gris-Oliver,1 Mafalda Oliveira,2 Marta Guzman,1 Olga Rodriguez,1 Judith Grueso,1 Maurizio Scaltriti,2 William J. Howat,3 J Carl Barrett,4 Javier Cortés,5 José Baselga,2

The antitumor activity of AKT inhibitors is being investigated for the treat-
ment of BC with activation of the PI3K/AKT pathway. Putative predictive bio-
markers that are being tested in ongoing trials are PTEN loss/mutation (mut) (around 40% of triple negative BC) and AKT1 mutation (4-5% ER+/HER2-
BC). The BEECH trial is evaluating the activity of the pan-AKT1/2/3 inhibitor

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Cancer (NSCLC) in-vitro. Human small airway epithelial cells (HSAECs) to ensure preferential activity. Additionally, we will investigate the toxicity of these inhibitors on normal cells. Findings suggest that AKT-1 inhibition is significantly more effective at reducing tumor growth than the AKT-1 inhibitor. Therefore, altered cell cycle progression could be the predictive marker for patient response to AKT-1 versus broad AKT-inhibitor MK-2206. Thus, AKT-3 expression has the potential to serve as a regulator of survival in NSCLC cell lines compared to a pan-AKT inhibitor. MK-2206. Basal protein and RNA levels in each of the cell lines have also revealed that a selective AKT-1 inhibitor A-674563 is a more potent inhibitor MK-2206. Furthermore, the cell lines with higher endogenous CDK2 and p-CDK2 expression are more sensitive to the AKT-1 inhibitor relative to the pan-AKT inhibitor. The predictive value of these alterations as determinants of response to AKT1 deserves further investigation.


AKT is a serine-threonine kinase implicated in tumorigenesis as a central regulator of cellular growth, proliferation, survival, metabolism, and migration. Activated AKT is overexpressed in 50-70% of NSCLC tumors and has exhibited an association with poor prognosis as well as chemotherapeutic resistance to platinum-based therapy. Accordingly, AKT inhibitors such as MK-2206 are currently undergoing clinical investigation for the treatment of human NSCLC. Moreover, these agents broadly target all three (1-3) AKT isoforms. Recent evidence suggests opposing roles of the AKT isoforms in tumorigenesis where loss of AKT-1 inhibits while the loss of AKT-2 enhances lung tumor development in transgenic mouse models. Based on these findings, we hypothesized that preferential inhibition of AKT-1 would warrant a more effective therapeutic strategy for NSCLC compared to the current clinical approach of broad AKT inhibition. WST-1 cell viability assays have revealed that a selective AKT-1 inhibitor A-674563 is a more potent regulator of survival in 6 NSCLC cell lines compared to a pan-AKT inhibitor MK-2206. Furthermore, off-target CDK2 inhibition likely contributes to the observed benefits of the AKT-1 inhibitor as the reduction in cell viability largely parallels the effects of a CDK2 inhibitor PHA-84125. In addition, the cell lines with higher endogenous CDK2 and p-CDK2 expression are more sensitive to the AKT-1 inhibitor relative to the pan-AKT inhibitor MK-2206. Basal protein and RNA levels in each of the cell lines have also shown that high AKT-3 expression may confer resistance to the pan-AKT inhibitor MK-2206. Thus, AKT-3 expression has the potential to serve as a predictive marker for patient response to AKT-1 versus broad AKT-inhibition. Cell cycle analysis demonstrated that the AKT-1 inhibitor decreases the proportion of cells in the G0/G1 phase and increases the proportion of cells in the S-phase, indicating a possible S-phase cell cycle arrest. These differences are also more significant in the cell lines with augmented sensitivity to the AKT-1 inhibitor. Therefore, altered cell cycle progression could be the major mechanism of action of the AKT-1 inhibitor MK-2206. Overall, our findings suggest that AKT-1 inhibition is significantly more effective at reducing NSCLC cell viability in-vitro compared to pan-AKT inhibition. Furthermore, cell lines with higher CDK2 and AKT-3 expression have marginally increased sensitivity to the AKT-1 inhibitor A-674563 compared to the pan-AKT inhibitor MK-2206. Future research will focus on understanding the mechanisms of action of the AKT-1 inhibitor MK-2206 and the role of AKT, Akt antibody array, flow cytometry, and confocal microscopy data. Additionally, we will investigate the toxicity of these inhibitors on normal human small airway epithelial cells (HSAECs) to ensure preferential activity against malignant over somatic cells.

#152 Dual mTORC1/2 inhibition sensitizes testicular cancer cell lines to cisplatin treatment. Fernanda Ximena Rosas Plaza, Gerda de Vries, Albert J. Suurmeijer, Jourik A. Gietema, Marcel A.T.M. van Vught, Steven de Jong. UMCU, University of Groningen, Groningen, Netherlands.

Testicular cancer (TC) patients with metastatic disease and poor prognosis have limited therapeutic options. Hyperactivation of the PI3K/Akt/mTOR pathway has been confirmed in TC cell lines. This hyperactivation of the PI3K/Akt/mTOR pathway in this study we investigated the potential benefit from PI3K, Akt and mTOR inhibition in combination with cisplatin in TC. Our panel of embryonal carcinoma cell lines include the cisplatin-sensitive cells: Tera and 833KE and the cisplatin-resistant cells: Scha, TeraCP and NCCIT. Western blotting showed that the resistant TC cell line TeraCP harbors higher levels of p-Akt compared to the sensitive cell line Tera. To evaluate sensitization towards cisplatin, TC cell lines were treated with cisplatin and/or PI3K inhibitor GDC0941, Akt inhibitor MK2206, mTORC1 inhibitor everolimus and mTORC1/2 inhibitors AZD8055 and AZD2014 for 24 hours and DilC5/PI staining was performed to estimate apoptosis with flow cytometry. All TC cell lines were strongly sensitized by mTORC1/2 inhibition by increasing cisplatin induced apoptosis to 60-80% when cisplatin alone induced only 15-30%. TC cells were also sensitized by mTORC1 and PI3K inhibition but to a lesser extent. Akt inhibition did not sensitize Scha or Tera cells to cisplatin, while TeraCP was marginally sensitized. We then evaluated clonogenic capacity in cells pretreated with AZD8055 for 24 hours and seeded in the presence of different concentrations of cisplatin for 6 days. Clonogenic capacity was reduced in TeraCP as compared to Tera in a dose-dependent manner. Both control cells treated with cisplatin only. Western blot done with Scha, Tera and TeraCP lysates treated for 24 hours with AZD8055, everolimus, GDC0941 and MK2206 showed that only mTOR inhibition was able to block S6 phosphorylation. In addition, we performed immunohistochemistry of p-S6 and Ki-67 in paraffin embedded tissue from TC patients. IHC showed that patient derived xenografts showed high expression of these markers compared to non-cancerous tissue, meaning that the mTOR pathway is very active in this tumor type. These data indicate that TC relies on the PI3K/Akt/mTOR pathway for survival and mTORC1/2 inhibition showed the stronger sensitizing effect towards cisplatin treatment. We consider adding mTORC1/2 inhibition to cisplatin based treatment a potential therapeutic option for chemoresistant TC patients that warrants further in vivo investigations. Supported by CONACyT grant 381543 and Dutch Cancer Society grant RUG 2014-6691.

#153 Tricyclic fused pyrimidinopyrrolo-oxazines reveal conformational preferences of morphology for PI3K hinge region binding. Alexander M. Sele, Denise Ragoret, Florent Beaufils, Anna Melone, Thomas Bohmacker, Eileen Jackson, Jean-Baptiste Langlois, Paul Heibsen, Fabiano Fabbro, Matthias P. Wyman1. 1University of Basel, Basel, Switzerland; 2PIQUR Therapeutics AG, Basel, Switzerland.

Class I phosphoinositide 3-kinases (PI3Ks) are lipid kinases, produce PtdIns(3,4,5)P3, and trigger intracellular signaling pathways that are vital to cell growth, proliferation, survival and migration. Constitutive activation of PI3K is frequently observed in many tumor types, which defines PI3K as a valuable drug target in oncology. Numerous PI3K inhibitors in clinical development contain a morpholine moiety that mediates hinge region binding in the ATP pocket of PI3K. We aimed to identify response biomarkers to the single agent AKTi and its degree of synergy in combination with cisplatin selection of compounds demonstrated inhibition of protein kinase B (pSer473) in PI3Kα. We present here novel pyrimidinopyrrolo-oxazines related to the clinically advanced, pyridiniummorpholine and triazinmorpholine derived pan-PI3K/mTOR inhibitors BM120 and PQR309. The novel fused tricyclic core of these compounds contains two morpholine moieties of which one is conformationally restricted by the introduction of a methylene bridge that links the pyrimidine core with one of the two morpholine moieties. This modification leads to the generation of two regioisomers, each existing as a set of enantiomers. We investigated the influence of this conformational restriction on PI3K inhibitory activity and analyzed the distinct selectivity profiles and potencies of the respective stereo- and regio-isomers. The design and preparation of specific compounds in combination with biological assays (phosphorylation of p56 and S6, binding affinity to p110α), AKT1 and p-p66 compared to the sensitive cell line Tera. This modularity allows us to understand the binding mode of these compounds and acquire valuable information that potentially lead to the development of derivatives with a distinct selectivity profile (e.g. PI3K versus mTOR). A selection of compounds demonstrated inhibition of protein kinase B (pSer473) and ribosomal protein S6 (pSer235/236) phosphorylation with IC50 values in the nanomolar range and high inhibitory potency of all PI3K isoforms (K_i (p110α) > 40 nM). Single p.o. administration of our lead compound to rats resulted in good oral bioavailability as well as excellent brain penetration. Furthermore, mechanism of action-based increases in glucose levels and insulin levels have been observed. In conclusion, we present here the development,
optimization, preparation and biological evaluation of a novel class of potent, orally available and brain-penetrant P-JPK inhibitors that represent an innovative extension to known pyrimidinomorpholine derived P-JPK inhibitors. Moreover, our results add to the understanding of how introducing specific structural and conformational modifications can lead to the development of optimized mTORC1 inhibitors. 

- **#154** The phosphatidylinositol-3-kinase (P-JPK) inhibitor (i) copanlisib is active in preclinical models of B-cell lymphomas as single agent and in combination with conventional and targeted agents including venetoclax and palbociclib. Eugenio Gaudino,1 Ivo Kwee,1 Filippo Spriano,1 Chiara Tarantelli,1 Andrea Rinaldi,1 Thiabaud Jourdan,2 Melanie Berthold,2 Alberto Arribas,1 Anastasia Statithis,1 Davide Rossi,1 Ninghu Liu,2 Martin Lange,2 Oliver Politz,2 Emanuele Zucca,1 Francesco Bertoni,1 1Institute of Oncology Research - IOR, Bellinzona, Switzerland; 2Bayer AG, Berlin, Germany.

- **#155** Addition of RP5630, a dual P-JPK/δ inhibitor, accentuates Romidepsin activity in NHL cells in vitro. Srikanth Viswanadha,1 Satyanarayana Eleswarapu,1 Seeta Nyayapathy,1 Swaroop Vakkalanka2. 1Institute of Oncology Research - IOR, Bellinzona, Switzerland; 2Bayer AG, Berlin, Germany.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Targeting the P-JPK Pathway

- **#156** Preclinical characterization of GDC-0077, a specific P-JPK alpha inhibitor in early clinical development. Kyle Edgar, Emily Hanan, Steven Steben, Stephen Schmidt, Rebecca Hong, Kyung Song, Amy Young, Patricia Hamilton, Alfonso Arrazate, Cristina de la Cruz, Marcia Belvins, Michelle Nannini, Lori S. Friedman, Deepak Sampath. Genentech, South San Francisco, CA.


- **#158** Inactivation of the PTEN gene is a common event in PCa, leading to activation of the P-JPK/akt/mTOR pathway and promoting PCa progression. Inhibitors targeting this pathway are currently being developed as anticancer agents. While recent clinical trials with mTORC1 inhibitors have shown limited single agent efficacy in castration-resistant prostate cancer (CRPC), it is still not known whether targeting alternate nodes of the P-JPK/akt/mTOR cascade may improve response. The current study aimed to characterize the response of PCa cell lines to multiple inhibitors targeting the P-JPK, AKT and mTOR nodes of the
cascade and identify possible mechanisms of resistance. The impact of the different PI3K/AKT/mTOR inhibitors on cell proliferation and apoptosis was measured by WST-1 and ApoTox-Glo assays, respectively, in a broad panel of established PCa cell lines and primary cell cultures generated from patient-derived xenografts. PTEN-negative PCa cells showed strong response to PI3Kβ/δ, AKT and mTOR inhibition, whereas PTEN-positive cells were resistant to PI3Kβ/δ and showed variable sensitivity to mTOR inhibitors. MTORC1 inhibitor showed low IC50 but reached plateau at about 70-80% growth inhibition. In contrast, mTORC1/2 inhibitor could reach complete growth inhibition at ~1 µM concentration, in sensitive cell lines. Furthermore, treatment with PI3K or AKT inhibitor in combination with androgen deprivation induced apoptosis in various PTEN-negative models, which is consistent with the previously reported synergistic effect of PI3K/AKT and androgen receptor co-targeting. The effect of the inhibitors on phosphorylation of PI3K downstream targets (AKT, PRAS40, GSK3, S6K1 and 4E-BP1) was analyzed by phospho-specific western blotting. Perturbation of different nodes of the PI3K/AKT/mTOR cascade resulted in differential phosphorylation of downstream targets, but there was no evident correlation with in vitro growth inhibition. In conclusion, the current study validates PI3Kβ and AKT as alternative targets in PTEN-negative PCa, whereas PTEN-positive models showed preferential yet variable responses towards mTOR inhibitors.

#158 ASN003, a highly selective inhibitor of B-Raf and PI3 kinase, shows strong antitumor activity in B-Raf inhibitor resistant patient-derived xenograft models. Scott K. Thompson,2 Roger A. Smith,1 Nirajan Rao,1 Michael J. Wick,1 Reza Shokrollahi,1,3 Scott,1 P. Reddy1.1A gonna Biosciences, Lawrenceville, NJ; 2South Texas Accelerated Research Therapeutics, San Antonio, TX.

The RAS-RAF-MEK and PI3K-AKT-mTOR pathways are two major signal pathways involved in human cancer. Components of these two pathways are frequently mutated in a wide variety of solid tumors. Concurrent double mutations in the two pathways are also observed quite often in a broad range of tumor types. Additionally, inhibition of one of these pathways often leads to the up-regulation of the other pathway and development of resistance. In preclinical models, combined inhibition of both pathways has been shown to impart greater efficacy as compared to inhibition of either pathway alone. ASN003 is a novel, highly selective, small-molecule inhibitor of both RAS-RAF and PI3K pathways, discovered using a rational design approach. ASN003 shows potent inhibitory activity against B-Raf and PI3K kinases (low nM IC50). Within the PI3K family, ASN003 has high selectivity for inhibition of PI3Kα and PI3Kδ over PI3Kβ. In a panel of 292 kinases, ASN003 showed high selectivity for inhibiting B-Raf and PI3 kinases, and associated mutant kinases. In cell-based mechanistic studies, ASN003 inhibited phosphorylation of ERK, AKT and S6, and showed strong anti-proliferative activity (IC50 = 60-300 nM) in cell lines with B-Raf and PI3K pathway mutations as well as in vemurafenib-resistant cell lines. In pharmacodynamic studies in multiple tumor models, ASN003 showed strong inhibition of the phosphorylation of downstream targets of B-Raf and PI3K, confirming appropriate target engagement. In in vivo efficacy studies, ASN003 showed strong tumor growth inhibition or regression in multiple tumor xenograft models, including A375 (B-Raf V600E mutation), RKO (B-Raf V600E and PIK3CA mutation), SKMEL28 (B-Raf V600E mutation and PTEN deletion), and MDA-MB-231 (B-Raf V600E mutation and PIK3CA mutation). Western blot analysis confirmed that ASN003 also showed strong tumor growth inhibition (>80%) in a patient-derived xenograft (PDX) model established from a relapsed patient with progressive B-Raf mutant melanoma who showed initial response to vemurafenib. Sequencing analysis showed that the vemurafenib resistant tumor acquired a concurrent PIK3CA mutation. Dual targeting of the B-Raf and PI3K pathways with ASN003 has the potential to treat and/or prevent the acquired resistance to selective B-Raf inhibitors, and may also treat a broader patient population and provide greater efficacy and survival benefit than selective B-Raf inhibitors or selective PI3K pathway inhibitors alone. ASN003 is currently in Phase I clinical development in patients with advanced solid tumors, including tumors with B-Raf V600 mutation, PI3 kinase pathway alterations or PTEN loss.

#159 Pharmacological characterization of the selective, orally bioavailable, potent dual PI3K/mTORC1/2 inhibitor PQR530. Petra Hillmann,1 De- nise Ragent,2 Florent Beaufils,1 Anna Melone, Alexander Sele, Robert A. Et- tin,3 Jürgen Mestan,1 Vladimir Cmiljanovic,1 Marc Lang,1 Elisabeth Singer,1 Carolin Walter,1 Hoa HP Nguyen,2 Paul Hebeisen,1 Matthias P. Wymann,2 Doriano Fabbro1.1PIQUR Therapeutics, Basel, Switzerland; 2University of Basel, Basel, Switzerland; 3University of Tübingen, Tübingen, Germany.

Introduction: The phosphatidylinositol 3-kinase (PI3K) signaling path- way plays a fundamental role in many cellular processes like growth, sur- vival, proliferation, differentiation and motility. In cancers several mutations have been identified that lead to constitutive activation of PI3K. PQR530 is a novel, ATP site directed inhibitor of all PI3K isoforms and the mammalian target of rapamycin (mTOR) complexes C1/2 that is currently in pre-clinical development. PQR530 potently binds to its targets, inhibits cell proliferation and shows excellent selectivity versus related and unrelated kinases [1]. Results: PQR530 inhibits PI3K signaling in stimulated MCF7 cells and was able to inhibit cell viability by >50% in a dose-dependent manner. PQR530 was found to inhibit PI3K mTORC1/2 during GLP toxicological testing in rats and dogs. Increase in insulin and blood glucose, a treatable class effect of PI3K inhibitors, has been observed after PQR530 administration to mice. Investigation of mutagenicity and hERG binding resulted in a clean profile. PQR530 exhibited dose-proportional pharmacokinetics (PK) in male C57BL/6 mice. A maximum plasma exposure of 3.5 µM [M] was reached after 30 minutes (7.2 ± 1.1 µg/ml and 112.6 µg/ml, respectively) indicating that efficacious concentra- tions were reached in both tissues. The calculated half-life (t1/2) was 2 hours and brain was approximately 5 hours. PQR530 potently inhibited PI3K sig- naling in vivo for several hours after administration of a single oral dose of 30 mg/kg. Tumor growth was significantly decreased in SUDHL-6 lymphoma, RIVA lymphoma and OVCAR-3 ovarian cancer mouse xenografts using daily, oral administration. Conclusion: PQR530 is a potent, ATP competitive pan-PI3K and mTORC1/2 inhibitor. The physico-chemical properties of PQR530 result in good oral bioavailability and excellent brain penetration. PQR530 is well tolerated and efficiently inhibits tumor growth in xenograft models. Preclinical data allow for further development of the compound. [1] Regard et al., Discovery and Development of B-Raf and PI3K/mTOR dual targeting molecules, Annual Meeting 2017, April 1-5, Washington, D. C., USA.

#160 High target binding affinity with long lasting cellular target engage- ment and high dose intermittent schedule of PI3K inhibitor copanlisib con- tribute to the potent anti-tumor activity and good safety profile. Maurya E. Fernández-Montañán,1 Victoria Georgi,1 James Vasta,1 Sarah Glasek,1 Vera Puettner,1 Matthew B. Roberts,1 Ursula Moenning,1 Andrea Sturr,1 Julien Le- franc-Marc,1 Karl Ziegelsbauer,1 Michael Brands,1 Christian Stegmann,1 William J. Scott,1 Ninghui Liu,1 Bayer AG, Berlin, Germany; 2Promega Inc, Berlin, Ger- many.

Introduction: Several generations of PI3K inhibitors have been tested in clinic. However, thus far, clinical activity has been moderate. Different from other oral PI3K inhibitors dosed continuously, copanlisib (BAY 80-6946) is an intravenous PI3K inhibitor given intermittently to patients. Copanlisib dosed once weekly demonstrated clinical benefit with an improved safety profile, and therefore challenges the concept of default continuous dosing of PI3K inhibitors. However, it is still unclear if this concept can be generalized and whether 'micropharmacokinetic parameters' also contributed to the potent anti-tumor profile of copanlisib. Here, we report the characterization of binding kinetics for copanlisib, as well as the functional consequence in vivo. Methods: A set of PI3K inhibitors were characterized in 1) a kinetic probe competition assay (KPCA); 2) a cellular nanoBRET target engagement assay; 3) a cellular washout study with the assessment on pathway engagement; and 4) in vivo pharmacokinetics analysis. Results: Copanlisib showed nearly dif- fusion-controlled on- and relatively slow off-rates with koff = 3.4E5 ± 7 [M-1·s-1] and koff = 1.6E-3 [s-1] to PI3Kα. Consequently, it exhibited very high affinity to PI3Kα (K, ePCA = 9.33E-11[M] and Kp, KPCA = 4.77E-11 [M]). In a cellular nanoBRET target engagement assay, the apparent half-life (t1/2) of ca. 2 hours greatly surpassed the 6.9 min measured using KPCA. The high affinity to PI3Kα also translated into potent cellular pathway engage- ment demonstrated by inhibition of p-AKT and p-PRAS40 in the PI3KCA−/− and KPL4 cell line. In a cellular washout study, p-AKT and p-PRAS40 were assessed till 168 h after incubation with copanlisib for 1 h followed by a washout step. A dose- and time-dependent pathway engage- ment was observed even at 72 h post washout. This result indicated that in cells, copanlisib engages PI3Kα for an extremely long time, likely due to receptor rebinding effects facilitated by the fast equilibration kinetics of the com- pound and its micropharmacokinetic properties. Interestingly, in vivo, BAY 80-6946 levels were approximately 100-fold higher in the tumor than in plasma at 48 hours and drug clearance from the tumor occurred more slowly than from plasma. This high and prolonged tumor exposure might be ex- plained, at least in part, by the high expression of PI3Kα and long lasting target engagement in tumors. Conclusion: Copanlisib demon- strated high affinity to PI3Kα with protracted target engagement at cellular and in vivo levels. This 'micropharmacokinetic feature' not only supports intermittent dosing but likely also explains the high exposure in tumors vs plasma, potent anti-tumor activity and good safety profiles.

Medulloblastoma (MB) is the most frequent malignant brain tumor in children. MB patients with high-risk disease have poorly understood biology and few targeted therapies available. Preclinical studies and molecular profiling of MB have revealed that the aberrant activation and interaction of the SHH and PI3K/akt/mTOR signaling networks are frequently associated with poor prognosis MB cases. Emerging evidence also demonstrate the key role of activated PI3K/akt/mTOR pathway component in MB. Here we report MB therapy-resistance, thus combined targeting of the SHH and PI3K/akt/mTOR pathways may be a viable therapeutic strategy to treat high-risk patients. Therefore, we investigated the combined efficacy of SHH inhibitor vismodegib and PI3K/akt/mTOR dual inhibitor BEZ235 or their combination, individually with the chemotherapeutic drug cisplatin against high-risk MB. Using four MB cell lines, including non-MYC and MYC amplified cell lines, and a xenograft mouse model, the in vitro and in vivo efficacies of the proposed therapies on cell growth/survival along with associated molecular mechanisms were investigated. Our results showed that both inhibitors as single agents significantly decreased MB cell growth and induced apoptosis by targeting the key molecules of the associated pathways in vitro. BEZ235 as single agent showed a greater anti-MB efficacy compared to vismodegib. Combined treatment of vismodegib and BEZ235 together or with cisplatin significantly decreased MB cell growth/survival and anchor-independent growth in a dose-dependent fashion compared to single agent activity. Corresponding changes in the expression of the targeted molecules following therapy were observed. Results from the combined approach suggested that the inhibitors not only suppressed MB cell growth/survival when combined, but also significantly enhanced the cytotoxic effects of cisplatin. Of these combinations, BEZ235 exhibited a significantly greater efficacy in enhancing the cisplatin-mediated MB cytotoxicity. In vitro studies also demonstrated that the MYC amplified MB cell lines showed a higher sensitivity to these combined therapies compared to relatively non-MYC amplified cell lines. Therefore, as a next logical step, we tested the efficacy of above combined approaches against MYC-amplified MB in vivo using NSG mice. Our in vivo results showed that the combination of vismodegib and BEZ235 or their combinations individually with cisplatin significantly delayed tumor growth and increased survival of xenograft mice compared to single agent activity. These combination not only significantly reduced tumor growth and increased survival of the mice but also significantly enhanced anti-MB efficacy by targeting SHH and mTOR pathways in MB in vivo. Thus, our studies lay a foundation for translating these combined therapeutic strategies to the clinical setting to determine their efficacies in high-risk MB patients.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Targeting the PI3K Pathway

A prodrug of green tea polyphenol (-)-epigallocatechin-3-gallate (ProEGCG) reduced expression of HIF-1α and VEGFA in RL95-2 cells and decreased CXCR4 expression in tumor-associated macrophages (TAMs) and endothelial cells (ECs). To further study the role of ProEGCG on EEC cells, qRT-PCR and Western blotting proved that hypoxia (1% O2) upregulated the level of HIF-1α and VEGFA in EEC cell lines, which was reversed by ProEGCG through inhibition of HIF-1α and VEGFA via PI3K/AKT/mTOR pathway. This is an important role in tumor angiogenesis, and double immunofluorescence of F4/80 and CD206 antibodies in xenografts was applied and found that TAMs infiltration was reduced in ProEGCG group with decreased VEGFA in TAMs. Moreover, proliferation and migration of ECs are critical for tumor angiogenesis. To study the effect of ProEGCG on ECs, tube formation and invasion assays were conducted and indicated that ProEGCG showed a tube-like structure formation and less invaded ECs by inhibition of CXCR4 expression on ECs. However, it was still unclear if CXCL12, the ligand of CXCR4, was affected. Immunostaining for CXCL12 in tumor lesions indicated that ProEGCG decreased CXCL12 expression in cancer and stromal cells, which was further confirmed in primary human endometrial stromal cells by addition of ProEGCG. Enhanced antitumor efficacy by recruiting macrophages as effector cells via bispecific antibodies mediated by CD89. Bingyu Li, Lijun Xu, Kun Xie, Fei Tao, Renhao Li, Hua Gu, Jianmin Fang. Tongji University, Shanghai, China.

ProEGCG reduced expression of HIF-1α and VEGFA in RL95-2 cells and decreased CXCR4 expression in tumor-associated macrophages (TAMs) and endothelial cells (ECs). To further study the role of ProEGCG on EEC cells, qRT-PCR and Western blotting proved that hypoxia (1% O2) upregulated the level of HIF-1α and VEGFA in EEC cell lines, which was reversed by ProEGCG through inhibition of HIF-1α and VEGFA via PI3K/AKT/mTOR pathway. This is an important role in tumor angiogenesis, and double immunofluorescence of F4/80 and CD206 antibodies in xenografts was applied and found that TAMs infiltration was reduced in ProEGCG group with decreased VEGFA in TAMs. Moreover, proliferation and migration of ECs are critical for tumor angiogenesis. To study the effect of ProEGCG on ECs, tube formation and invasion assays were conducted and indicated that ProEGCG showed a tube-like structure formation and less invaded ECs by inhibition of CXCR4 expression on ECs. However, it was still unclear if CXCL12, the ligand of CXCR4, was affected. Immunostaining for CXCL12 in tumor lesions indicated that ProEGCG decreased CXCL12 expression in cancer and stromal cells, which was further confirmed in primary human endometrial stromal cells by addition of ProEGCG.

Enhanced antitumor efficacy by recruiting macrophages as effector cells via bispecific antibodies mediated by CD89. Bingyu Li, Lijun Xu, Kun Xie, Fei Tao, Renhao Li, Hua Gu, Jianmin Fang. Tongji University, Shanghai, China.
Antibody-dependent cell-mediated cytotoxicity (ADCC) is one of key mechanisms through which antibody therapeutics can lyse tumor target cells. ADCC is usually mediated by IgG through recruiting effector cells, mostly NK cells, via IgG Fc receptor. In order to enhance specific tumor lysis by ADCC, a common strategy is to design a bispecific antibody that binds a tumor cell surface antigen and T cell marker CD3, thus to recruit T cells as effector cells. An example of this type of design is the FDA-approved bispecific antibody blinatumomab. We are interested in designing novel bispecific strategies to achieve improved ADCC effect. Since tumors are often infiltrated by macrophages and neutrophils, it would be advantageous to turn these types of cells into effector cells for ADCC.

We have designed several bispecific strategies that can target a tumor associated antigen (TAA) and CD89 (also known as P2Y12) on the neutrophils. The bispecific antibodies redirect tumor cells to CD89-expressing immune effector cells, such as macrophages and neutrophils. The CD89 bispecific antibodies were produced by HEK293 cells and showed binding activities to both CD89 and TAA. The CD89 bispecific antibodies can recruit CD89-expressing immune cells and induced enhanced tumor cells-killing in human whole blood ADCC assays. Since mouse does not express human CD89 homolog, we generated a transgenic mouse strain with specific expression of CD89 on macrophages and monocytes. In this transgenic mouse model, the CD89 bispecific antibodies showed significant anti-tumor activities, demonstrating that the bispecific antibodies can redirect macrophages, including M2 macrophages, to mediate additional effector function in tumor microenvironment.


Background: Hepatocellular carcinoma (HCC) is characterized by hypoxia, hypoglycemia and lactic acidosis within the microenvironment due a highly glycolytic phenotype. However, routine culture conditions of HCC cells employ supra-physiological glucose, pH-buffered media, and normoxia. The aim of the study was to simulate the microenvironment features of HCC in vitro by culturing HCC cells in their native (NAT) conditions (hypoglycemia, hypoxia and lactic acidosis) and examine phenotypic, transcriptomic and pharmacological differences compared to cells cultured in standard (ST) culture conditions. Methods: Six HCC cell lines were cultured in NAT or ST conditions. Pharmacological response to cytotoxic drugs and targeted agents were evaluated using the MTS assay. Phenotypic differences were examined using standard techniques. Transcriptomic analysis was carried out using the Illumina HumanHT-12 expression kit. Results: HCC cells cultured in NAT conditions have higher doubling time than cells cultured in ST conditions. However, no remarkable differences in the cell cycle profiles were observed when cultured in either NAT or ST conditions. Protein analysis revealed a increase in phosphorylation of AKT and decrease in the levels of AMPK in all cells cultured in NAT but not in ST conditions. Increase in the protein levels of GLUT1, HK2, LDHA, and a decrease in PDHA was observed only in cells cultured in NAT conditions. Furthermore, the HCC cells in NAT conditions exhibited lower levels of reactive oxygen species and ATP consistent with the elevated glycolysis and inefficient oxygen phosphorylation phenotype of HCC. Thirty-one genes were found to be aberrantly expressed by gene expression analysis, most notably NDRG1, a hypoxia-associated gene was upregulated in NAT cells, validated by qPCR. Upregulated NDRG1 was maintained even upon reversal of the NAT condition. The panel of thirty-one genes were found to be associated with poor prognosis exclusively in HCC based on data available via TCGA supporting the validity of NAT culture condition. Differences in the IC50 of doxorubicin, sorafenib, PTK, c-MET and HDAC inhibitors but not AKT, MEK, mTOR, Wnt inhibitors were observed in cells cultured in NAT compared with ST conditions suggesting microenvironment modelling can influence pharmacological response of certain class of compounds. Additional genomic and metabolomics analysis are underway in order to characterize the HCC cells in NAT conditions.

Conclusion: In conclusion, HCC cells when cultured in NAT conditions exhibit more of the tumor characteristics than cells in ST condition and this can be utilized as an informative platform for better understanding of disease biology and pharmacology.

#166 Recombinant protein MBP-NAP restricts tumor progression by triggering T-cell immunity in mouse metastatic melanoma model. Ting Wang,1 Cong Ding,2 Zhenyu Li,2 Xin Liu,1 Mingxuan Du,1 Qiaozhen Kang1, Zhengzhou University, Zhengzhou, China; 2Henan Academy of Medical and Pharmaceutical Sciences, Zhengzhou, China.

The pro-inflammatory and immunomodulatory properties of Helicobacter pylori neutrophil activating protein (Hp-NAP) not only make it play an important role in disease pathogenesis, but also make it a potential candidate for applications, including vaccine and drug development. Our previous work demonstrated that the recombinant Hp-NAP fused with the maltose-binding protein of Escherichia coli (rMBP-NAP) exert an important role in regulating the differentiation of TH1 cells. As a potential TLR2 ligand, it was reported to possess pro-inflammatory and immunomodulatory activities in vivo. To induce specific Th1 cell differentiation in the metastatic melanoma 122 and sarcoma S180 tumor models. To further understand the antitumor and immunomodulatory effect of rMBP-NAP, we elucidate the effect and mechanisms of rMBP-NAP at the local immune response modulation in established mouse B16-F10 melanoma pulmonary metastasis model. Our results demonstrated that metastatic lung tumor growth was significantly arrested after rMBP-NAP treatment as compared to the untreated control. rMBP-NAP increased the number of lung nodules and significantly increase in survival. Flow cytometry immunophenotyping and Quantitative RT-PCR analyses demonstrated that rMBP-NAP could induce both local and systemic immune responses, which associated with higher influx of CD3+CD8+ T cells, CD5+CD8+ T cells and higher secretion of interferon (IFN)-γ and interleukin (IL)-27 cytokines. The intraperitoneal administration of rMBP-NAP in mice promoted infiltration of lymphocyte in the lungs and reduced the production of several proinflammatory cytokines, such as IL-6 and transforming growth factor beta (TGF)-β, indicating an anti-inflammatory effect in local area. By comparing with control mice, mRNA expression of chemokines also revealed that rMBP-NAP treatment substantially decreased the expression of CCL2 and CCL20 in tumor tissues, which are central to recruit systemic Th1/Tc1 cells and the production of relevant inflammatory cytokines and chemokines, which are responsible for enhancing T cell immunity and reversing immunosuppression against metastatic cancer progression. Our findings strongly indicate that rMBP-NAP treatment might be a novel therapeutic approach against metastatic melanoma, and rMBP-NAP might be a potential template for the development of agents that could be used as immunomodulatory adjuvant in the therapy against melanoma.

#167 Brutinib regulates tumor microenvironment and enhances response to everolimus in renal cell carcinoma mouse models. Jun Chen, Chun-Te Chen, Jing Liu, Jeff Hsu, Taisei Kinoshita, Betty Y. Chang. Pharmacy, LLC, an AbbVie Company, Sunnyvale, CA.

Introduction: Brutinib (ibr), a first-in-class, once-daily, oral inhibitor of Bruton’s tyrosine kinase (BTK), is indicated for the treatment of patients with CLL/SLL, MCL and WM. Ibr also inhibits EGFR/HER2 and has demonstrated efficacy against EGFR+ NSCLC and HER2+ breast cancer in vitro and in xenograft models (Chen et al. 2016; Zhang et al. 2017; Chu et al. 2015). Ibr inhibited modulated host immunity and enhanced anti-PD-1/L1 activity in solid tumor models otherwise insensitive to BTK or HER kinase inhibition (Swain-Barfi 2015), suggesting that ibr may be active in renal cell carcinoma (RCC) via multiple mechanisms. Here we determined the impact of ibr alone and in combination with everolimus (eve) on tumor growth and the tumor microenvironment in syngeneic and xenograft RCC mouse models. Methods: Cell proliferation was determined with MTS assay. Phenotypic differences were examined using standard techniques. Transcriptomic analysis was carried out using the Illumina HumanHT-12 expression kit. Results: HCC cells cultured in NAT conditions have higher doubling time than cells cultured in ST conditions. However, no remarkable differences in the cell cycle profiles were observed when cultured in either NAT or ST conditions. Protein analysis revealed a increase in phosphorylation of AKT and decrease in the levels of AMPK in all cells cultured in NAT but not in ST conditions. Increase in the protein levels of GLUT1, HK2, LDHA, and a decrease in PDHA was observed only in cells cultured in NAT conditions. Furthermore, the HCC cells in NAT conditions exhibited lower levels of reactive oxygen species and ATP consistent with the elevated glycolysis and inefficient oxygen phosphorylation phenotype of HCC. Thirty-one genes were found to be aberrantly expressed by gene expression analysis, most notably NDRG1, a hypoxia-associated gene was upregulated in NAT cells, validated by qPCR. Upregulated NDRG1 was maintained even upon reversal of the NAT condition. The panel of thirty-one genes were found to be associated with poor prognosis exclusively in HCC based on data available via TCGA supporting the validity of NAT culture condition. Differences in the IC50 of doxorubicin, sorafenib, PTK, c-MET and HDAC inhibitors but not AKT, MEK, mTOR, Wnt inhibitors were observed in cells cultured in NAT compared with ST conditions suggesting microenvironment modelling can influence pharmacological response of certain class of compounds. Additional genomic and metabolomics analysis are underway in order to characterize the HCC cells in NAT conditions.

Conclusion: In conclusion, ibr had significant inhibitory effect on the growth of syngeneic and xenograft RCC tumor models. In syngeneic RCC models, ibr was significantly more effective in combination with everolimus than either agent alone, with a marked decrease in tumor growth and number of lung nodules and an increase in survival. Flow cytometry immunophenotyping and Quantitative RT-PCR analyses demonstrated that rMBP-NAP could induce both local and systemic immune responses, which associated with higher influx of CD3+CD8+ T cells, CD5+CD8+ T cells and higher secretion of interferon (IFN)-γ and interleukin (IL)-27 cytokines. The intraperitoneal administration of rMBP-NAP in mice promoted infiltration of lymphocyte in the lungs and reduced the production of several proinflammatory cytokines, such as IL-6 and transforming growth factor beta (TGF)-β, indicating an anti-inflammatory effect in local area. By comparing with control mice, mRNA expression of chemokines also revealed that rMBP-NAP treatment substantially decreased the expression of CCL2 and CCL20 in tumor tissues, which are central to recruit systemic Th1/Tc1 cells and the production of relevant inflammatory cytokines and chemokines, which are responsible for enhancing T cell immunity and reversing immunosuppression against metastatic cancer progression. Our findings strongly indicate that rMBP-NAP treatment might be a novel therapeutic approach against metastatic melanoma, and rMBP-NAP might be a potential template for the development of agents that could be used as immunomodulatory adjuvant in the therapy against melanoma.

Conclusion: This study suggests that ibr has antitumor activity against RCC.
alone and when combined withive in animal models. This effect may be mediated by modulation of the tumor microenvironment, such as inhibiting Treg differentiation and suppressing PD-1 expression on CD8+ T cells, and both BTK and EGFR/HER2 are involved. Further investigation is needed to clarify the mechanism of action, but the results here provide a rationale for ibv as a novel agent for RCC in combination with mTOR inhibitors. 

#168 Loss of function mutation in TIMP2 gene accelerates tumorigenesis and mortality in murine model of lung cancer through EGFR signaling. Sarvesh Kumar, Sandra Jensen, David Peeney, Ananda Chowdhury, Beiyang Wei, William G. Stetter-Stevenson. NCI/NIH, Bethesda, MD.

The aim of this study is to determine the effect of the tissue inhibitor of metalloproteinase-2 (TIMP-2) on lung tumorigenesis and investigate the underlying molecular mechanisms using an orthotopic mouse model with a loss of function mutation in the Timp2 gene (T2M). T2M and wildtype (WT) control mice were given 1x10^6 Lewis lung carcinoma cells transplanted with luciferase (LL/2-Luc-M38, Caliper) in 50 μL PBS via intratracheal injection. IVIS imaging revealed a higher tumor burden in T2M mice compared to WT littermates, suggesting that loss of function of the Timp2 gene enhances tumor growth (p<0.05). We also conducted a Kaplan-Meier analysis to determine the effect of this mutation on mortality following cancer development. We found that LL/2 tumor-induced mortality was significantly higher in T2M mice compared to WT controls (p = 0.01). Histologic analysis and H&E staining of lung tissue sections revealed a significant increase in the number of tumor nodules of T2M mice compared to WT controls (p <0.01). CD31 staining showed a significant increase in microvessel density (MVD) in T2M mice compared to WT controls (p<0.01). Given that VEGF is a primary driver of tumor neoangiogenesis, we determined mRNA levels of VEGF expression in healthy and tumor bearing mice by qRT-PCR analysis. Interestingly, basal VEGF expression levels were increased in lungs of both non-tumor bearing and tumor bearing T2M mice compared to WT controls (p<0.01). VEGF is the direct downstream target of HIF-2α, a transcription factor implicated in tumor hypoxia and expression correlates with decreased overall survival in non-small cell lung cancer (NSCLC) patients. Similarly, an examination of TIMP-2 and HIF-2α mRNA expression in a small cohort of patient samples revealed decreased TIMP-2 and increased HIF-2α in NSCLC tumors compared to surrounding non-tumor lung tissue. Hypoxia induces EGFR signaling through HIF-2α. Thus, we determined the effect of mutated T2M on the EGFR signaling pathway. We found increased levels of EGFR phosphorylation as well as downstream ERK and Akt activation in tumor bearing and non-tumor bearing T2M lungs compared to WT controls. In conclusion, these findings offer new avenues for TIMP-2 research in regulating hypoxic mediators within the tumor microenvironment through EGFR signalizing, suggesting TIMP-2 as a novel bio-therapeutic for lung cancer therapy.

#169 Preclinical efficacy and sensitivity determinants of evofosfamide in molecularly defined models of head and neck squamous cell carcinoma. Francis W. Hunter,1 Avik Shome,1 Dan Li,1 Way W. Wong,1 Peter Tsai,2 Anthony J. Zussman,1 Cho R. Hong,1 Dennis Kee,3 Andrew M. Macann,4 Anthony J. Hickey,5 Stefan K. Bohlander,1 Cristin G. Print,1 William R. Wilson,5 Bradly G. Wouters,5 Stephen M. Jamieson.1 University of Auckland, Auckland, New Zealand; 2Princess Margaret Cancer Centre, Toronto, Ontario, Canada; 3LabPLUS, Auckland, New Zealand; 4Auckland City Hospital, Auckland, New Zealand.

Tumor hypoxia is prevalent in head and neck squamous cell carcinoma (HNSCC), where it limits radiotherapy outcomes. Hypoxia-activated prodrugs (HAPs) have been developed to target hypoxic regions of tumors. These agents undergo oxygen-sensitive reductive activation, thereby delivering cytotoxic species within hypoxic cells. This study investigated the efficacy and sensitivity determinants of the clinical-stage HAP evofosfamide (TH 302) using molecularly-characterized models of HNSCC. We deployed a collection of 27 HPV-negative HNSCC cell lines derived from lesions of varying TNM stages and primary, nodal or recurrent sites. The collection was characterized for gene expression by RNA-seq, from which somatic variants were also called. Their transcriptomic features were investigated in the context of pan-cancer TCGA data by hierarchical clustering. The potency and hypoxic selectivity of 3 HAPs - evofosfamide, PR-104A and SN30000 - were assessed by antiproliferative assay in 22 lines and compared to bromo-isoporphoramide mustard (Br-IPM), cisplatin and 5-FU. The antitumor activity of evofosfamide (50 mg/kg qdx3 for 2-3 cycles with or without a single 10 Gy dose of radiation on day 5 of cycle 1) was evaluated in HNSCC xenografts in addition to a PDX isolated from an SCC of the glottic larynx. The hypoxic fraction at baseline and after 5 days of treatment was quantified by pimonidazole staining. Genetic modifiers of sensitivity to evofosfamide and its cytotoxic metabolite Br-IPM were explored through whole-genome CRISPR-Cas9 screens using the GeCKO v2 library. High-throughput screens with a custom shRNA library were performed in two HNSCC and two pancreatic ductal adenocarcinoma cell lines to identify reductases responsible for the activation of evofosfamide in hypoxic cells. Evofosfamide was more potent and more selective for hypoxic HNSCC cells in vitro than PR-104A or SN30000. Cell line sensitivity to evofosfamide was correlated with Br-IPM and cisplatin but not with PR-104A, SN30000 or 5-FU, indicating distinct sensitizing mechanisms following evofosfamide treatment. In the in vivo study, CRISPR screens identified potential evofosfamide sensitivity genes that were reproducibly enriched following drug exposure. Reductase-focused RNA interference screens defined a cluster of sensitivity genes that mapped to mitochondrial electron transport, whereas shRNA's targeted against presumed activating enzymes such as POR were not enriched. Concentration-dependent reduction of cytochrome c and decreased respiration was observed in cells exposed to evofosfamide, suggesting reduction by mitochondrial complexes. This study provides a rationale for the clinical evaluation of evofosfamide with radiotherapy in genetically defined subsets of HNSCC patients.

#170 An innovative immunotherapeutic strategy for ovarian cancer: Glycomimetic peptides. Katherine F. Roby,1 Laura L. Eggen,2 Kenneth Hoober2.1 Univ. of Kansas Medical Ctr., Kansas City, KS; 2Sasuvion Biosciences, Inc., Tempe, AZ.

Successful treatment strategies for women with ovarian cancer remain elusive. We hypothesize that novel means of activating anti-cancer immune activity will be an important component of a multifaceted approach to successful treatment. The present set of studies tests the hypothesis that novel peptide mimetics of C-type lectin receptor ligands, sv4L and sv6D, enhance anti-cancer immune activity and limit the progression of ovarian cancer in a mouse model. We further test the hypothesis that sv6D will function in synergy with additional immune modulators and conventional cytotoxic therapy. C-Type lectin receptors were targeted that are specific for N-acetylglalactosamine (GalNAc). Both svL4 and sv6D bind GalNAc-specific C-type lectin receptors including CLEC10A/CD301 with a Kₘ in the low nanomolar range. CLEC10A is a transmembrane, endocytic receptor expressed on dermal dendritic cells, macrophages and immature dendritic cells. Further, studies with the B16 mouse melanoma model and spontaneous tumors (histiocytic sarcoma and mammary gland tumor) in dogs showed that treatment with svL4 correlated with reduced tumor-associated Treg cells. In the present studies subcutaneous injection of svL4 or sv6D every other day over 5 days stimulated a several-fold proliferation of immune cells in the peritoneal cavity of healthy mice. These results indicated that svL4 and/or sv6D might exhibit significant activity on peritoneal tumors. Efficacy of svL4 and sv6D each as a single agent and as a combination therapy with paclitaxel or anti-PD-1 was tested in C57BL6 female mice bearing ovarian ID8 intraperitoneal tumors. As a single agent, 0.1 n mole/g doses of svL4 or sv6D had a significant effect on suppressing ascites formation, a measure of tumor progression, and overall survival. Drug combination studies revealed a positive therapeutic interaction with sv6D and the cytotoxic paclitaxel. Both agents, sv6D and paclitaxel each had a significant effect on extending survival (median survival 140.5 and 150 days, respectively, vs. 122 days with no treatment). Survival was extended further with combination treatment when sv6D was administered to mice previously treated with paclitaxel (median survival 169 days). Also, a positive interaction was observed with sv6D and the check-point inhibitor anti-PD-1. Administration of sv6D following anti-PD-1 treatment resulted in a significant survival advantage compared to treatment with either agent alone. These data demonstrate 1) svL4 and sv6D mobilize immune cells in the peritoneal cavity, 2) svL4 or sv6D as single agents slow progression of ovarian cancer and enhance survival in a mouse model of ovarian cancer, 3) sv6D in combination with paclitaxel or anti-PD-1 extends survival past that of either agent alone. Taken together these date demonstrate the potential for this novel approach of harnessing lectin receptors as a means toward effective cancer treatment.
Brain tumor initiating cells (BTICs) are resistant to chemotherapies, providing a reservoir for tumor recurrence and a desirable target for glioma treatments. Standard of care for glioblastoma (GBM; grade IV astrocytoma) includes the chemotherapeutic agent temozolomide, which prolongs life expectancy by months and is not curative. Prior studies suggested the efficacy of chemotherapies including temozolomide was increased by reducing expression of carbonic anhydrase 9 (CA9). CA9 is a hypoxia responsive gene elevated in tumors that is important for regulating intracellular pH and contributing to the acidic extracellular microenvironment. After confirming basal and hypoxia-induced expression of CA9 in GBM BTICs, we targeted CA9 activity with the small molecule inhibitor SLC-0111 alone or in combination with temozolomide. In multiple GBM BTIC lines, SLC-0111 reduced cell growth in vitro and showed additional benefit when used concurrently with temozolomide. Importantly, SLC-0111 inhibited the enrichment of BTICs after temozolomide treatment as determined via BTIC marker expression and neurosphere formation capacity. These data suggested the potential of SLC-0111 as a chemosensitizer, which we next evaluated in preclinical studies using a subcutaneous recurrent GBM model. GBMs treated with SLC-0111 in combination with temozolomide significantly regressed and the resulting in vivo growth delay was greater than that of temozolomide or SLC-0111 alone. Together, our data suggest that SLC-0111 can sensitize GBM BTICs to the chemotherapeutic temozolomide and significantly delay disease progression.

CANCER CHEMISTRY: Basic and Applied Nanotechnologies and Therapeutic Approaches

#172 Oxidative stress and hepatotoxicity induced by graphene oxide in Sprague-Dawley Rats. Anita K. Patollah, Paul B. Tchounwou. Jackson State Univ., Jackson, MS.

Graphene oxide (GO) has been extensively explored as a promising nanomaterial for applications in biology because of its unique properties. Therefore, systematic investigation of GO toxicity is essential to determine its fate in the environment and potential adverse health effect. The aim of this study was to investigate the effect of graphene oxide on the induction of reactive oxygen species (ROS), the activity of certain liver enzymes (Alanine ALT, Aspartate AST, alkaline phosphatases ALP), and concentration of lipid hydroperoxide (LHP) in serum and histopathological evaluation of liver tissue in Sprague-Dawley rats. Four groups of five male rats were orally administered GOs, once a day for five days, with doses of 0, 10, 20 and 40 mg/kg GO. A control group was also made of five rats. Blood and liver were collected 24 h after the last treatment following standard protocols. GO's exposure increased the induction of ROS, the activities of the liver enzymes (ALT, AST, A LP), concentration of lipid hydroperoxide (LHP) and morphological alterations of the liver tissue in exposed groups compared to control. The highest two doses, 20 and 40 mg/kg, showed statistically significant (p < 0.05) increases in the induction of ROS, activities of ALT, ALP, LHP concentration, and morphological alterations of liver tissue compared to control. However, AST activity showed no effect. Taken together, the results of this study demonstrate that GO is hepatotoxic, and its toxicity may be mediated through oxidative stress.


Breast cancer is the second leading cause of cancer-related deaths in women today and is the most common cancer among women, excluding non-melanoma skin cancers. Over 426,000 women will be diagnosed with breast cancer and 40,450 are expected to die of it in 2016. Estrogen receptors ERα, ERβ and their associated steroid hormones play vital role in breast cancer development and progression. Estrogen binds and activates the estrogen receptors in certain breast cancer cells and release their drug load into the cytoplasm. Commercial liposomes culture experiments indicate that TLD-1 liposomes are readily taken up by cancer cells and release their drug load into the cytoplasm. Pharmacologic properties of TLD-1 were compared in three animal models of breast cancer agents. TLD-1 showed a marked increase in cytotoxicity compared to Caelyx and was close to free drug. In all three animal models, TLD-1 showed IC50 values of 0.61, 1.36 and 0.09 g/ml on MCF-7, MDA-MB-231 and Ishikawa cells using cellTiter-Glo luminescent cell viability assay. Among all the compounds screened, 4-ethyl-N-(8-hydroxy-3,4-dihydroisoquinolin-2(H)-yl)benzamide showed IC50 values of 0.61, 1.36 and 0.09 μg/ml on MCF-7, MDA-MB-231 and Ishikawa cells respectively. This research was supported by the National Center for Research Resources and the National Institute of Minority Health and Health Disparities of the National Institutes of Health through Grant Number 8 G12MD007582-28.

#174 Preclinical activity of new liposomal formulation of doxorubicin (TLD-1). Massimo Broggi,1 Federica Guffanti,1 Roberta Affatato,1 Lavinia Morosi,1 Patrick Buschor,2 Christoph Matthieu,2 Florian Weiss,2 Andreas König,2 Peter Halbher,2 Stefan Halbher*,2 Mario Negri Istituto per Farmacol. Research, Milan, Italy; 2InnoMedica, Bern, Switzerland.

Background: InnoMedica is a young company with focus on clinical translation of nanomedicine. The company employs its own manufacturing technology that gives rise to a novel liposome platform. The platform allows for specific shaping of nanosurfaces. This leads to a myriad of different particle designs with highly distinct biological functions. In a first approach, the lead-formulation TLD-1 (targeted liposomal doxorubicin) was developed in order to ameliorate chemotherapeutic outcome for patients, taking into account the patterns of biodistribution in the entire organism as well as nanoparticle-cell interactions and subcellular localisation. Methods: Pharmacologic properties of TLD-1 were investigated in vitro and in vivo. Human ovarian cancer cells A2780 were cultured in both 2D and 3D settings and antineoplastic activity of different formulations of doxorubicin was measured by quantification of cell viability. The same formulations were also compared in three animal models of cancer including murine breast cancer 4T1, human breast cancer MDA-MB-231, and A2780. MDA-MB-231 and A2780 cells were grown in vitro and injected subcutaneously (sc) in immunodecient mice (Athymic Nude-Foxn1tm1). 4T1 cells were injected either sc or in the mammary fat pad of immunocompetent mice (BALB/c). The different drug products were injected intravenously twice weekly for a total of three weeks. Activity was determined with caliper measurements of tumor diameters every three days. Animal body weight was recorded as index of toxicity. Doxorubicin was determined in plasma and tissues by HPLC-MS. Results: In 2D and 3D cell culture assays, TLD-1 showed a marked increase in cytotoxicity compared to Caelyx and was close to free drug. In all three animal models, TLD-1 showed activity superior to that of free doxorubicin given at the same dose and proved well to be a promising treatment for breast cancer. Conclusions: A couple of nanoparticulate features of TLD-1 liposomes are believed to attribute to the difference in antitumor activity. Cytotoxicity data from cell culture experiments indicate that TLD-1 liposomes are readily taken up by cancer cells and release their drug load into the cytoplasm. Commercial liposomes as in Caelyx seem to largely remain outside of cancer cells. In sum, TLD-1 is a novel and highly active antineoplastic nanodrug that has the potential to out-perform free drug as well as commercial liposomal formulations of doxorubicin.

#175 Combination of plasmonic photothermal therapy with surgery applied to naturally occurring mammary tumors in canines and felines: clinical outcomes and molecular studies. Moustafa R. Ali,1 Haithem A. Farghali,2 Hala R. Ali,2 Ahmad H. Osman,2 Youssef A. Soliman,2 Yue Wu,1 Ibrahim M. Ibrahim,1 Maha Selim,2 Dong M. Shin,1 Mostafa A. El Sayed1. 1Georgia Inst. of Technol.ogy, Atlanta, GA; 2Veterinary Medicine, Cairo University, Cairo, Egypt; 3Emory University, Atlanta, GA. Plasmonic Photothermal Therapy (PPTT) is a cancer therapy where gold nanorods (AuNRs) are injected at the tumor site and near-infrared light (safe to bio-system) is applied to generate localized heat causing cancer cell death. PPTT is a potentially good alternative to replace traditional surgery for localized tumors. However, for large tumors (volume ≥10 cm3), PPTT could be ineffective due to an uneven distribution of injected AuNRs causing possible inhomogeneity of heat. Surgery is frequently recommended in those cases. However, it carries a high risk of cancer recurrence. For effective treatment of large tumors, we
combined both PPTT and surgical resection and applied it to naturally occurring tumors in mammary glands of dogs and cats, which could realistically represent their human equivalents at the molecular level. For the experimental design, we divided the animals into three different groups. 20 cases (7 cats and 13 dogs) were all diagnosed with adenocarcinoma; the animals were monitored for 1- 2 years after treatments. Group (I): 12 cases were solely treated by mastectomy (control group); all of them died within a few weeks. Group (II): five cases were treated with mastectomy first. Then, each tumor wound was divided into two halves, and only one half was exposed to PPTT. After treatment, two cases in this group rendered complete remission. In the other three cases, the half wound that was not exposed to PPTT had tumor recurrence causing animal death within one year. Group (III): 12 cases were treated with surgery followed by PPTT treatment. This regime showed complete remission without any recurrence for eight cases. However, four cases died 4-12 months after therapy for reasons such as pneumonia (no tumor found, based on X ray). Histopathology results showed a decrease of cancer grades before (variant grades from 1-4) and after two weeks of treatment via PPTT and surgery (grade 0). Blood tests (conducted 1 year after therapy) showed no obvious change in liver and kidney functions in groups II and III. In addition, X-ray diffraction showed no metastases 1-2 years after treatment. We have performed quantitative, real-time PCR analysis two weeks before and after treatment to study the expression levels of several important genes. The genes that are responsible for repairing cancer cells such as BRCA1, BRCA2, and CD163-IL-10 were significantly diminished two weeks after treatment (group III). Furthermore, tumor microenvironment cells such as tumor-associated macrophages (TAMs) were greatly altered after treatment. TAM 1, which retards tumor growth, augmented, and TAM 2, which promotes tumorigenesis, was diminished, which explains the animals’ increased survival rate. In conclusion, our study demonstrates the feasibility of applying PPTT after surgery for large tumors in dogs and cats.

#176 Early development of GMC1, a novel molecule targeting FKBP52 for the treatment of hormone-refractory prostate cancer. Huan Xie, Oscar Ekpenyong. Texas Southern University, Houston, TX.

Purpose: GMC1 directly inhibits FKBP52, effectively blocking androgen receptor dependent gene expression and androgen-stimulated proliferation. This make it an attractive option for the treatment of hormone-dependent and hormone-independent prostate cancer. This study investigated an analytical method for GMC1 quantification, pre-formulation characteristics of GMC1, and developed intravenous formulations for the evaluation of GMC1 in animal models. Method: An LC/MS/MS method for the quantification of GMC1 in solution, plasma and urine was developed, validated and applied to the determination of the stability, log P, plasma protein binding and solubility of GMC1 in various solvents. Liposomal formulations co-solvent systems with various ratios of high capacity vehicles were formulated and the optimal formulation applied, at 2 mg/kg single IV bolus dose, to the pharmacokinetic study of GMC1 in a rat model. Result: The intra- and inter-day accuracy (%RE) and precision (%CV) of the LC/MS/MS method ranged from 1.6 – 11.7 % and 1.4 – 8.8 %, respectively. GMC1 is stable in solid and solution state, moderately lipophilic (log P 3.18 ± 0.05), poorly water soluble (0.4 ± 0.01 mg/mL), and highly plasma protein bound (97.5 ± 1.3 %). The optimised formulation employed was 1:1:1 (GMC1: PEG200: water) and Labrasol ® (1:1, v/v) allowed us to achieve a GMC1 concentration of 10 mg/mL, and tolerated an aqueous environment. GMC1 has a tri-exponential disposition with a Tmax of 7.6 ± 1.97 mg/L, clearance of 0.53 L/kg/hr, α-distribution, β-phase and terminal elimination half-lives of 0.1 ± 0.04 hr, 1.2 ± 0.34 hr, and 19.7 ± 5.09 hr respectively. Conclusion: The LC/MS/MS method, formulations and pharmacokinetic study can be applied to the pre-clinical and clinical development of GMC1. Grant Support: This work was performed under funding from NIH/NIGMS grant (S5CG102018) and NIH/NIMHD/RCMI grant (SG12MD007605).

#177 Thymidine quinoxaline conjugates as a novel selective and effective photosensitizer for anticancer photodynamic therapy. Qibing Zhou, Zhiwei Wang, Rong Yang, Ting Qian, Dejun Zhang. Huazhong Univ. of Science & Technology, Wuhan, China.

Thymidine quinoxaline conjugates are a new class anticancer agent that exhibits selective anticancer activity by targeting the abnormally high level of thymidine kinase of the thymidine salvage pathway in cancer cells. In addition, the conjugated quinoxaline moiety could also act as a photosensitizer to generate reactive oxygen species that would significantly enhance the anticancer activity. Therefore, a variety of structural derivatives were synthesized and investigated for the potential of combined anticancer activity. We found that the thymidine moiety was critical for the accumulation of compounds in cancer cells and thus their activity. On the other hand, the substitutions and additional conjugation of thymidine quinoxaline moiety had significant impact on the effectiveness to generate reactive oxygen species for enhanced potency. Among all the compounds studied, DT-QP exhibited a potent activity against liver cancer cells with IC50 at 20 nM under photo-irradiation at 400 nm wavelength for 20 min. This potent activity of DT-QP was 12 times more effective than that of the clinically used photosensitizer, porfimer sodium (IC50 at 250 nM under the same condition). DT-QP was more potent than anticancer drug doxorubicin (IC50 at 100 nM). In addition, thymidine quinoxaline conjugates were found to form aggregates with an average size of 200 nm. Further optimization of the aggregating condition led to a highly stable suspension of 90 nm size nanoparticles containing a tumor-specific peptide that could be used for in vivo study. Therefore, thymidine quinoxaline analogs could be a selective anticancer agent and effective photosensitizer for potential photodynamic therapy.

#178 Peptide nanofibers: targeted therapies for glioblastoma multiforme. Diana M. Leite,1 Rong Zhu,2 Eugen Barbu,2 Peter Hinterdorfer,2 Geoffrey J. Pilkington,1 Aikaterini Lalatsa1, University of Portsmouth, Portsmouth, United Kingdom; Johannes Kepler University, University of Linz, Linz, Austria.

Glioblastoma multiforme (GBM) is a malignant brain tumor with poor prognosis due to tumor heterogeneity, poor drug blood-brain barrier (BBB) permeability and targeting. GBM biopsies indicated the overexpression of G-protein coupled receptors (GPCRs) that when activated by neuropeptide agonists result in an antiproliferative effect. However, translation of these neuropeptide agonists into novel therapies for GBM is frustrated by their short half-life (<5 minutes) and inability to cross the BBB. Here, we present a novel strategy based on the lipidization of neuropeptidase inhibitors that results in peptide amphiphiles able to self-assemble into nanofibers that can entrap brain impermeable drugs, possess an enhanced stability to enzymatic degradation, permeate across an all human in vitro BBB model and are able to target GBM cells resulting in a significant antiproliferative effect. Peptide amphiphiles were synthesized using solid-phase peptide synthesis and characterised using pyrene, thiolavin T, circular dichroism and transmission electron microscopy experiments. Nanofibers were loaded with brain impermeable cytotoxic drugs (e.g. paclitaxel) and their stability was studied in biological media (plasma, brain, liver and GBM cell lysates). BBB permeation was studied in an all human in vitro Transwell model. The antiproliferative effect of the nanofibers was evaluated on U-87 MG cells. Targeting of the nanofibers to the GPCR was evaluated using single-molecule force spectroscopy (SMFS). Peptide amphiphiles self-assemble into stable nanofibers at concentrations above 189 μM and are able to solubilize high amounts of paclitaxel (>1.8 mg) ensuring that the resulting nanomedicine can be clinically translatable. Both unloaded- and paclitaxel loaded-nanofibers showed superior stability compared to the parent neuropeptide in the presence of plasma, brain, liver and cell homogenates (>5-fold). Nanofibers elicited a strong antiproliferative effect (IC50 < 1 μM) at 10 mg/mL, which is 1000-fold lower than the IC50 of the parent GBM cell line (U-87 MG). Loading paclitaxel (1μM) within the nanofibers resulted in a synergistic effect evidenced by a decrease in cell survival by 32%. Nanofibers counteracted the forskolin-induced increase of intracellular CaM levels indicating that the GPCR is linked to the Gαq protein known to mediate the antiproliferative effect on GBM. Confocal studies confirmed the internalization of the peptidocopolymer SMFS (IC50 < 250 nM) under the same condition and even GBM cell line (U-87 MG). This potent activity of GPCR on U-87 MG cell surface with equivalent binding probability to peptide agonists and increased residence time. Texas Red labelled nanofibers permeate across an in vitro BBB model enabling the permeation of paclitaxel (Papp: 8.45 x 10-10 cm2/s). Thus, described peptide nanofibers are a novel targeted nanomedicine for GBM therapy able to be clinically translated. In vivo proof of concept studies and pharmacokinetics are under way.

#179 Plasma membrane lipid therapy: disruption of oncogenic Ras spatiotemporal organization by membrane-targeted dietary bioactives (MTDB). Natividad R. Fuentes,1 Rola Barhoumi,1 Mohamed Milh,2 Jason Karpac,2 Paul Hardin,1 Trevor Steele,1 Spencer Behmer,1 Ian Prior,2 Robert S. Chapman,1 Texas A&M University, College Station, TX; Texas A&M Health Sciences Center, College Station, TX; University of Liverpool, United Kingdom.

Approximately 30% to 50% of colorectal cancers contain KRas mutations, which confer resistance to standard therapy and have therefore been termed “undruggable.” Since no curative treatments for KRas driven colon cancer are available, there is a critical need to develop toxicologically innocuous KRas therapeutics that are free of safety problems intrinsic to drugs administered over long periods of time. High fidelity signaling of Ras is dependent on its spatial organization into defined nanoclusters on the plasma membrane. This is noteworthy, because select nonsteroidal anti-inflammatory drugs, through direct modulation of the functional properties of the plasma membrane, alter oncogenic Ras nanoclustering and attenuate signal transduction. These findings...
suggest that Ras nanoclusters represent a novel target for future interventions. Consistent with this rationale, we hypothesize that select amphiphilic membrane targeted dietary bioactives (MTDB's), e.g., polysaturated fatty acids (n-3 PUFA) docosahexaenoic acid (DHA, 22:6\(\omega 3\)), eicosapentaenoic acid (EPA, 20:5\(\omega 3\)), docosatrienoic acid (22:3\(\omega 3\)), [1] modulate the rigidity of the plasma membrane, [2] increase Ras nanocluster formation, [3] prevent metastatic disease by interfering with pro-inflammatory signaling (pERK), and [4] suppress phenotype (hyper-proliferation) in vitro and in vivo. Our studies utilized a variety of complementary models including mouse colon cancer cells, Drosophila midgut stem cells and mouse colon, respectively. In vitro and in vivo incorporation of n-3 PUFA consistently reduced plasma membrane rigidity and TH-Ras clustering, while increasing TH&TK proteins and the mixing of TH and TK proteins. These changes in membrane spatiotemporal organization were associated with a reduction in oncogenic Ras\(^{12}\) driven ERK phosphorylation and intestinal stem cell proliferation. These novel findings in vitro PhAc-ALGP-Dox suggests a unique role for MTDBs in Ras-dependent oncogenesis would have a major translational impact because these bioactives are safe, well tolerated, relatively inexpensive, and provide additional health benefits, such as reduction in mortality. This work was supported by NIH grant R35CA197707.

#180 PhAc-ALGP-Dox is a new tumor selective peptide prodrug of doxorubicin that shows improved efficacy and systemic tolerance in triple negative breast cancer models. Andrea Casazza,1 Massimiliano Mazzone,1 Peter Pokreisz,2 CoBioRes NV, Leuven, Belgium; 2VIB Vesalius Research Center, Leuven, Belgium.

Use of traditional chemotherapeutic drugs is restricted by severe side effects and lack of tumor specificity of these cytotoxic agents. Less toxic prodrug that can be selectively activated in tumor tissue have been explored in attempts to improve the therapeutic index. Some approaches to the development of tumor activating prodrugs take advantage of inherent properties of the tumor, for example, selective enzyme expression, hypoxia, or low extracellular pH in the vicinity of the tumor. CoBioRes makes use of capped, tetrapted peptide prodrug of existing chemotherapeutics. Doxorubicin (Dox) was the first chemotherapeutic that was linked to the tetrapted sequence and tested experimentally (PhAc-ALGP-Dox). This approach makes the prodrug impermeable to cell membranes of both normal and tumor cells. Furthermore, it remains stable in blood by the use of the capping group on the amino-terminal group of the peptide that prevents ascertainment by circulating esopetidases. The prodrug as such is thus not active. The peptide sequences have been developed to be sensitive to the hydrolytic action of a selected group of peptidases that are released within the tumor microenvironment. These peptidases, which accumulate in the tumors, also play an important role in cancer cell invasion and metastasis. What makes this approach unique is that it is not targeting a single enzyme, but requires, for its selectivity, a two-step activation based on enzymes with an increasing selectivity. In vivo activation starts when the tetrapeptide prodrug is exposed to endonucleases (CD10 and THO1) leading to the formation of intermediates that are still inactive and poorly permeable through cell membranes. This leads to a tissue distribution that is determined by the expression of the endonuclease. Since these are known to be overexpressed in several tumors, and only present at lower level in some normal tissues, the prodrug will be preferentially located and activated in the vicinity of tumors. The second step of the activation is the cleavage at the prodrug by dipeptidases FAP and DPPV4, releasing the free doxorubicin. The simultaneous expression of all the enzymes involved in the prodrug activation was confirmed both in human triple negative breast cancer biopsies and in experimental triple negative breast cancer models. In vitro analysis of PhAc-ALGP-Dox reveals higher tumor cells tropism and higher cytotoxicity in cancer cells than in normal cells. In vivo PhAc-ALGP-Dox gives promising preliminary results in terms of systemic tolerance even at high concentration, and in vivo efficacy in xenograft mouse models compared to free doxorubicin. Taken together, these results provide a strong rationale for further investigation aimed at unleashing the potential clinical value of this compound.

#181 Discovery of novel targeted therapeutics for metastatic breast cancer. Cristina Del Valle,1 Eliud Hernandez,2 Cornelis P. Vlaar,1 Luis A. Cubano,2 Suranganie Dharmawardhane,1 Linette Castillo-Pichardo1,2 University of Puerto Rico, Medical Sciences Campus, San Juan, PR; 2Universidad Central del Caribe, Guaynabo, PR.

Metastatic breast cancer cells that metastasize to distant organs outside of the breast are the primary cause of breast cancer mortality, due to the lack of effective therapy. The Rho GTPTase Rac is integral for the promotion of cancer cell migration/invasion, proliferation, and survival. Since metastatic breast cancers often overexpress or exhibit high Rac activity, inhibition of Rac is a viable strategy against metastatic cancer. Recently, we characterized EHop-016, a small molecule that inhibits Rac activity of metastatic breast cancer cells with an IC\(_{50}\) of 1 \(\mu\)M. EHop-016 is 10-100 times more active than previous available Rac inhibitors, and is the first compound shown to inhibit the activation of Rac by the oncogenic GEF Yav. EHop-016 inhibits the activity of the Rac downstream effector p21 activated kinase (PAK), lamellipodia extension, and cell migration of metastatic breast cancer cells. We also reported that EHop-016 at \(\leq\) 25 mg/kg Body Weight (BW) significantly reduced tumor growth, metastasis, and angiogenesis in a mouse model. However, our recent pharmacokinetic study of EHop-016 in a mouse model demonstrated that the bioavailability of Ehop-016 needs to be improved for further pharmacological development. Therefore our hypothesis is that improvement of the EHop-016 structure will provide probes with increased potency against Rac and, therefore, increased bioavailability. EHop-016 derivatives were tested in-vitro and in-vivo for their effect on breast cancer cell viability and Rac activation. Using MTT assays we found that the EHop-016 derivatives, HV-107 and HV-118, significantly inhibit the viability of metastatic breast cancer cell lines MDA-MB-231 and MDA-MB-435. The effects of HV-107 and HV-118 on the inhibition of Rac activation were tested by ELISA-based Rac activity assays and pulldown assays. Results show that at 250 \(\mu\)M, HV-107 inhibits Rac activation by 55%, whereas HV-118 has a similar effect at 100\(\mu\)M in MDA-MB-231 and MDA-MB-435 cells. Taken together, our findings suggest HV-107 and HV-118 as promising Ehop-016 derivatives with potential as anti-metastatic agents, which should be further characterized. This study was supported by awards from the Susan Komen for the Cure, NIH/NIHMD U54MD008149, and the Puerto Rico Science Trust to SD; NIH/NCRR R25GM061838 to UPR MSc; NIH/NIHMD RCI 8G12MD007583RCMI, Title V PPOHA 03110505 and Title V Cooperative P031S130068 from U.S. Department of Education to UCC; and PRINBRE (NIH/NIHGS P2GM103475-13) Pilot Project to LCP.
was a dose dependent decrease in multiplicity of aberrant crypt foci (ACF) in the colon. PPI inhibited the occurrence of larger ACF consisting of more than 4 aberrant crypts, indicating inhibitory effect of PPI on tumor promotion. In a mouse xenograft model, PPI decreased the size and number of blood vessels in the tumor. All mice survived without causing significant body weight loss during experiment. No histopathological changes were found in genito-urinary tract. To better understand the mechanism of PPI on angiogenesis, we performed chorioallantoic membrane assays and found a dose-dependent decrease in average number of blood vessels. Inhibition of STAT3 by PPI may affect the function of molecules that are related to apoptosis, angiogenesis, and cell cycle progression and eventually contributes to PPI-induced growth inhibition.

### 183 Brassinolide, a plant steroid hormone, reverses drug resistance in human small-cell lung carcinoma cells. David Sadava, Susan E. Kane. City of Hope, Duarte, CA.

Small-cell lung carcinoma (SCLC) has a dismal prognosis in part because of multidrug resistance (MDR). Epibrassinolide (EB) is a steroid hormone present in higher plants, where it has numerous physiological effects and acts via a LRR-RLK membrane receptor and GSK3/SHAGGY pathway, resulting in stabilization of a known inhibitor of Wnt signaling, was cytotoxic to SCLC cells (IC50 = 0.80 at ED50 and CI0.90 at ED95); EB and doxorubicin also showed synergism (CI1.35 at ED95), suggesting that EB triggers a redox imbalance by inhibiting GSTp and by lowering the antioxidant (GSH) and reducing equivalent (NADPH) levels, leading to a significant elevation of ROS levels. We also showed the upregulation of endoplasmic reticulum (ER) stress-responsive protein, activation of MAPK, autophagy and apoptotic pathways by KSS72 in several cell lines. KSS72-induced autophagy was a post event of redox perturbation that displayed a wide array of characteristic features including double membranous vacuoles with entrapped organelles, acidic vesicular organelles, and increased expression of LC3-II and beclin-1. In view of its excellent brain penetration and multiple cytotoxic events mediated by ROS and autophagy, we tested the in vitro efficacy of KSS72 in GBM cell lines and in vivo efficacy of KSS72 in xenograft models of GBM xenografts. SF-188 human GBM cells expressing firefly luciferase were injected into the brain just left of bregma for the development of orthotopic GBM in nude mice. These mice were given 25 mg/kg/day of KSS72 intraorbitally for 2 weeks. The animals when imaged by IVIS after luciferin injections showed a complete lack of intracranial tumors in all KSS72 administered animals. H&E staining of mouse brain sections confirmed the total elimination of GBM by KSS72. KSS72 did not exert any toxicity on host tissues and serum ALT and AST levels were not altered. In summary, we conclude that KSS72 acting through multiple pathways of oxidative stress is a hugely promising non-toxic anti-glioma drug with potential to enter clinical trials (supported by CPRIT grant R1P30266 and RP170207 to KSS).
GP130 or IL-11/IL-11 Ra/GP130 heterotrimer and triggers a signaling cascade downstream. One of the major downstream effectors of IL-6 is STAT3. STAT3 is persistently activated in many human osteosarcoma specimens and cell lines and STAT3 is required for osteosarcoma cells survival. So IL-6 and IL-11 present a viable novel target for osteosarcoma therapy. To date, however, no small molecule to target the IL-6/IL-11Ra/GP130 or IL-6/IL-11Ra/GP130 signaling axis is available for cancer therapy. To overcome this critical problem, we have utilized a novel drug discovery approach combining Multiple Ligand Simultaneous Docking and drug repositioning to target GP130. Drug repositioning refers to reuse fragments from the existing FDA-approved drugs for new applications and could potentially reposition the existing drugs as novel, off-target inhibitors of the desired target. Using this novel method, we have identified by FDA-approved drug Bazedoxifene with a novel function to inhibit IL-6 and GP130 protein-protein interactions. Furthermore, because IL-11 binds to the same domain of GP130 as IL-6, Bazedoxifene should also inhibit IL-11/GP130 signaling. Accordingly, Bazedoxifene can indeed inhibit the induction of P-STAT3 by both IL-6 and IL-11. Bazedoxifene appeared specific to IL-6 and IL-11 as phosphor-ylation of STAT1 and STAT3 by IFN-γ and LIF respectively were not affected by the compound. Bazedoxifene inhibited P-STAT3 and induced apoptosis in human osteosarcoma cell lines expressing IL-6 and IL-11. In addition, Bazedoxifene can inhibit colony formation after the drug treatments in cancer cells. IL-6 but not IFN-γ could rescue the Bazedoxifene-mediated inhibition of cell viability in osteosarcoma cells. These results further support that IL-6/GP130 and IL-11/IL-11Ra are two of the major pro-survival mechanisms for xenograft tumor inhibition in osteosarcoma cells. To determine the in vivo activity of Bazedoxifen, we further tested the efficacy of Bazedoxifene in tumor xenografts generated from the SJSA osteosarcoma cells that show persistent IL-6/STAT3 activation. Bazedoxifene via oral gavage inhibited P-STAT3 and the growth of SJSA tumor xenografts. These data demonstrated that Bazedoxifene is potent in suppressing tumor growth and is orally bioavailable in inhibiting P-STAT3. It further indicates that Bazedoxifene is a promising IL-6/GP130-targeting drug, which likely to have in vivo anti-tumor activity in osteosarcoma. In summary, Bazedoxifene already approved for safety by the FDA as a novel inhibitor of IL-6/GP130 and IL-11/GP130 signaling should provide an easier path to clinical trials and have a potential to improve the outcome of osteosarcoma.

#187 Efficient delivery of Bcl2 siRNA by DNA nanoparticles to inhibit cellular growth and cancer progression. Mohammad Aminur Rahman,1 Peng-fei Wang,2 Dongsheng Wang,1 Selwyn J. Hurwitz,2 Zhengjia Chen,2 Zhuo G. Chen,1 Yonggang Ke,2 Dong M. Shin,3 Emory Univ. Winship Cancer Inst., Atlanta, GA; Emory University, Atlanta, GA.

Background: Short interfering RNA (siRNA) has emerged as a promising molecular therapeutic tool for targeted cancer treatment. However, systemically administered siRNA has demonstrated only limited success, due to limited delivery to targeted cells. There is a lack of a robust and versatile delivery system for cancer therapy. Recent developments in DNA nanotechnology have made programmable DNA nanoparticles (DNPs) a potent drug delivery platform. This study focuses on the development of a novel DNP-based siRNA delivery system to knockdown Bcl2 gene, as a targeted cancer therapeutic. Methods: Structural DNA technology was applied to design a library of DNPs with different sizes and shapes. Flow cytometry, confocal imaging, and electron microscopy were utilized to study the cellular internalization of DNPs. The efficacy of Bcl2 knockdown by DNP-siBcl2 and the resulting influence on cell growth and progression were assessed in cancer cells (in vitro) and in mice bearing corresponding tumor xenografts (in vivo). Results: DNPs of varying sizes (10-120 nm) and shapes (polyhedral or rod) were constructed using DNA origami techniques and successfully verified by agarose gel electrophoresis, atomic force microscopy, and transmission electron microscopy. The cell internalization capabilities of three DNPs including a tetrahedron (TET, diameter: 10 nm), a small rod (SR, 5x5x30 nm), and a large rod (BR, 10x10x120 nm) were examined. BR internalized with relatively higher efficiency and rate compared to TET and SR, and also demonstrated the most efficient knockdown of Bcl2. BR-siBcl2 demonstrated significant cell growth inhibition of DMS53 and H146 small cell lung cancer (SCLC) cell lines after 48hrs of treatment. A pilot study with BR-siBcl2 (1.25 mg/kg, iv) in mice bearing DMS53 tumor xenograft (n=3) slowed tumor growth compared with buffer control and naked siBcl2. Significant differences were observed in tumor volume by pairwise comparison between the two groups: Buffer vs DNP-BR-siBcl2 (p<0.001), and siBcl2 vs BR-siBcl2 (0.028), respectively. No toxicity was observed in lung, liver, kidney, heart, brain, or spleen. Conclusions: Our novel DNP formulations demonstrated substantial cellular internalization of siBcl2. Targeting Bcl2 and its downstream signaling intermediates reduced cellular growth. We validated the strategy of silencing of Bcl2 using DNPs in order to inhibit cancer progression in vivo. We believe that the DNPs and methodologies developed in this project will be applicable to knockdown of Bcl2 and other gene targets and may be applicable to future anti-cancer therapy. (This work is supported by NIH grant R21EB022828-01).

#188 Preventive effect of aerosolized bexarotene in three major subtypes of lung cancer: adenocarcinoma, squamous cell carcinoma and small cell lung cancer in mice. Qi Zhang,1 Jing Pan,3 Marker S. Miller,2 Ronald A. Lubet,2 Yian Wang,3 Ming You1, 1Medical College of Wisconsin, Milwaukee, WI; 2National Cancer Institute, Rockville, MD

Lung cancer is the leading cause of cancer-related deaths in the United States. The 5 year survival rate for lung cancer patients has remained a dismal 15% for the last several decades. The development of effective prophylactic agents that could prevent lung cancer could potentially reduce the incidence and mortality of pulmonary neoplasms. Bexarotene has exhibited inhibitory effects in preclinical in vivo models of mammary and lung tumorigenesis, has been approved for clinical use in the treatment of cutaneous T-cell lymphoma, and has shown efficacy in phase I/II trials of non-small cell lung cancer (NSCLC). Preclinical studies have demonstrated that it is highly effective in the prevention of all three major subtypes of lung cancer in mouse models: adenocarcinoma (AD), squamous cell carcinoma (SCC), and small cell lung cancer (SCLC). The major side effects of bexarotene when administered orally to rodents or human patients have been hypertriglyceridemia and hypercholesterolemia. Previous studies in a mouse model of lung AD have demonstrated that aerosol delivery of bexarotene through tracheal instillation exhibited potent chemopreventive activity similar to that observed following oral administration (59 to 74% reductions in lung tumor multiplicity). The significant decreases in tumor multiplicity and tumor load were achieved without hypertriglyceridemics that accompany oral bexarotene administration. In this study, aerosolized delivery of 10-30 mg/ml bexarotene showed a significant chemopreventive effect in all three major subtypes. In the N-nitroso-trichloroethylurea (NTCU) induced mouse SCC model, 1 week after the first dose of NTCU a dose dependent decrease in tumor formation was observed, with the highest dose causing 75% (p<0.001) and 42% (p<0.01) decreases in SCC tumor burden and the percentage of SCC tumors, respectively. Tumor load was decreased by 73% in A/Jp53 mouse AD model and by 41% in A/Jp53rb mouse SCLC model. Aerosol delivery of bexarotene had no effect on animal body weight and other signs of toxicity, and no effect on triglyceride and cholesterol level. Aerosolized bexarotene formulation was effective against all 3 major lung cancer cell types and would be a major advance achieving significant reductions in preventing lung cancer incidence in persons at high risk of lung cancer e.g. former or present smokers.

#189 Development of peptidomimetic inhibitors of the ERG gene fusion product in prostate cancer. Xiaojia Wang, The University of Michigan, Ann Arbor, MI.

Transcription factors play a key role in the development of diverse cancers, and therapeutically targeting them has remained a challenge. In prostate cancer, the gene encoding the transcription factor ERG is recurrently rearranged and plays a critical role in prostate oncogenesis. Here, we identified a series of peptides that interact specifically with the DNA binding domain of ERG. ERG inhibitory peptides and derived peptidomimetics (EIPs) bound with high affinity and specificity leading to proteolytic degradation of ERG protein. The EIPs attenuated ERG-mediated transcription, chromatin recruitment, protein-protein interactions, cell invasion, and tumor growth. Thus, peptidomimetic targeting of transcription factor fusion products may provide a promising therapeutic strategy for prostate cancer as well as other cancers.

#190 Identification of compound isolated from Beilschmiedia tsangii as a liver cancer specific NFR2 inhibitor. Yi Xiao Chen, Chih Chung Lai, Yi Ping Kuo, Hsuan Shuo Chang, Ih Sheng Chen, Chia-Hung Yen. Kaohsiung Medical Univ., Kaohsiung City, Taiwan.

Drug resistance is the main cause of cancer recurrence and a major obstacle to the success of anticancer therapy. NFR2, a pivotal transcription factor regulates antioxidant response and detoxification, has been shown to participate in the development of cancer drug resistance. Functional suppression of NFR2 rendered cancer cell more susceptible to anticancer treatments. Beilschmiedia tsangii Merr. (Lauraceae) is a medium-sized evergreen tree. It has been reported that Beilschmiedia extract showed a strong antioxidant activity. In continuation of our bioassay-guided study on this species, we tested the effects of 23 compounds isolated from the B. tsangii on NFR2 activity. We identified rel-(7R,8R,7′R,8′R)-3,4′,4′-dimethylene-dioxy-5,5′-dimethoxy-7,7′-epoxyglycan (BT04) significantly inhibited NFR2 activity in liver cancer cell (Huh7) with an IC50 value of 17 μM, but not in keratinocyte (HaCaT) cell. By contrast, luteolin, a known NFR2
inhibitor, suppressed NRF2 activity in both HuH7 cell and HacTa cell. Moreover, the mRNA level of NRF2 target genes, NQO1 and HO1, were significantly decreased in HuH7 upon BT04 treatment, while those NRF2 target genes remained unchanged in BT04-treated HacTa cell. A moderate cytotoxic effect of BT04 on HuH7 cell was also observed. Accordingly, our result suggested that BT04 can specifically inhibit NRF2 activity in liver cancer, which in turn indicated that BT04 could be a potential adjuvant to improve chemoresistance.

**#191 Double blockade of interacting CK2 and EGFR pathways by tumor-targeting nanoconjugates increases therapeutic efficacy against glioblastoma multiforme.** Julia Y. Ljubimova, Cedars-Sinai Medical Center, Los Angeles, CA.

Introduction: Glioblastoma multiforme (GBM) remains the deadliest brain tumor in adults, and is notorious for drug and radiation resistance. To inhibit GBMs more effectively, polymeric acid-based blood-brain barrier crossing nanoconjugates were synthesized that are delivered to the cytoplasm of cancer cells and specifically inhibit the master regulator serine/threonine protein kinase CK2 and the wild-type/mutated epidermal growth factor receptor (EGFR/EGFRVIll), which are overexpressed in gliomas according to The Cancer Genome Atlas (TGCAG) GBM database. Methods and Results: The used nanoconjugates are novel nanoatherapeutics where all moieties are covalently connected to poly(β-L-malic acid) (PMLA). Our biodegradable and non-toxic nanodrugs bind to the receptors enriched on tumor vasculature and cross the BBB by transcytosis. They specifically bind to cancer cells and after internalization exit to the tumor cell cytoplasm using pH-sensitive endosomal disruption unloading the covalently bound drug. Two xenograft mouse models bearing intracranial human GBMs from cell lines LN229 and U87MG that expressed both CK2 and EGFR were used. The knockdown of CK2α and EGFR/EGFRVIll suppressed their downstream prosurvival signaling. Treatment also markedly reduced the expression of programmed death-ligand 1 (PD-L1), a negative regulator of cytotoxic lymphocytes. Downregulation of CK2 and EGFR also caused suppression of heat shock protein 90 (Hsp90) co-chaperone Cdc37, which may inhibit the activity of key cellular kinases. Inhibition of either target was associated with downregulation of the other target as well, which may underlie efficacy of the dual nanoconjugate that is directed against both CK2 and EGFR. Importantly, the single nanodrugs, and especially the dual nanodrug, markedly suppressed the expression of cancer stem cell markers c-Myc, CD133, and nestin, which could contribute to the efficacy of these nanodrugs. In both tumor models, the dual targeting nanoconjugate significantly increased (up to 2-fold) animal survival compared with the control group. Conclusion: The versatile nanoconjugates developed in this study, with the ability of anti-cancer drug delivery across biobarriers and inhibition of key tumor regulators, offer a promising nanotherapeutic approach to treat GBMs and to potentially prevent drug resistance and retard the brain tumor recurrence. Support: NIH grants U01 CA151815, R01 CA136841, R01 CA188743, R01 CA209921, R01 EY013431.

**#192 Targeting stearyl CoA desaturase 1 (SCD1) in hepatobiliary carcinoma.** John Alton Copland,1 Laura A. Marlow,1 Ila Bok,1 James L. Miller,1 Kabir Mody,1 Lewis R. Roberts,2 Mark J. Truty,2 Tushar C. Patel1.

Inhibitors of SCD1 are in clinical development for the treatment of non-alcoholic fatty liver disease and cancer. In cancer, the upregulation of SCD1 reduces the rate of fatty acid oxidation and promotes fatty acid synthesis and esterification. We hypothesize that targeting SCD1 may be an effective approach to combat HCC survival. Targeting these may prove beneficial because such changes contribute to the chemoresistance associated with HCC. In so doing, we evaluated a novel lead SCD1 inhibitor in HCC. Methods: Paraffin embedded patient HCC tissues were examined for SCD1 expression. Using combined computational and synthetic chemistry approaches, we synthesized four novel specific SCD1 inhibitors with SSI-4 being the lead SCD1 inhibitor. HCC cell lines were examined using proliferation assays for response to SSI-4. 1C50 concentrations for blocking SCD1 enzyme activity was determined. Blood half-life and bioavailability of single dose SSI-4 was determined. Mechanisms of action of SCD1 were examined that enzyme activity was determined. Blood half-life and bioavailability of single dose SSI-4 was determined. Single dose oral gavage SSI-4 demonstrated a half-life of ~4 hours and excellent oral bioavailability. SSI-4 was well tolerated with long-term daily dosing. SSI-4 treatment of HCC cells and tumors led to endoplasmic reticulum (ER) stress followed by apoptotic cell death. Single agent SSI-4 demonstrated antitumor activity in HCC PDX mouse models with suppression of ER stress regulated proteins. Conclusions: Targeting a novel lipid metabolic pathway in HCC may provide effective therapy for aggressive HCC.


MPS1 (also known as TTK), is a dual-specificity protein kinase and one of the main components of the spindle assembly checkpoint. Cancer cells heavily rely on MPS1 to cope with aneuploidy resulting from aberrant numbers of chromosomes and MPS1 has been found to be upregulated in a large number of tumor types. Extensive work by us and other groups has shown that MPS1 inhibitors are effective against a variety of cancers, particularly when used in combination with other drugs, for example, tubulin-targeting agents. We recently reported the structure-based design and discovery of a series of pyrido[3,4-d]pyrimidines (CCT289346). Our preclinical candidate. CCT289346 shows excellent potency, kinase selectivity, and ADME properties including stability in human liver microsomes. The compound has been produced on a kilogram scale and is currently undergoing preclinical development. We will discuss our design approach and hypotheses leading to the discovery of CCT289346 and disclose in vivo efficacy data. References 1. Innocenti P et al. Rapid Discovery of Pyrido[3,4-d]pyrimidine Inhibitors of Monopolar Spindle Kinase 1 (MPS1) Using a Structure-Based Hybridization Approach. Journal of Medicinal Chemistry. 2016; 59(8):3671-88.

**#194 Effective targeting of MYC expression with a novel nucleic acid binding (G4-quadruplex) small molecule coupled with HDAC inhibition synergizes to limit myeloma growth.** Snehal M. Gaikwad,1 David R. Calabrese,2 Elena C. Leon,1 John K. Simmons,1 Shuling Zhang,1 Aleksandra Michalowski,1 Sayeh Gorjipard,1 Zaw Phyo,1 Daniel Connors,1 John S. Schneekloth,2 Beverly A. Mock,3,CCR, NCI, NIH, Bethesda, MD; 3CCR, NCI, NIH, Frederick, MD.

MYC is deregulated in many malignancies and its aberrant expression is associated with tumorigenesis and poor patient survival. Inhibitors of this transcriptional activator have shown limited success in preclinical development. Here we report the design and discovery of a novel small molecule microarray approach to treat GBMs and to potentially prevent drug resistance and retard the brain tumor recurrence. Support: NIH grants U01 CA151815, R01 CA136841, R01 CA188743, R01 CA209921, R01 EY013431.
transcriptional silencing of oncogenic MYC both in vitro and in vivo. Furthermore, synergistic tumor cell killing was achieved when the MYC G4 inhibitor was coupled with HDAC inhibition.

#195 Evaluation of the cell surface binding of phycocyanin and associated mechanisms causing cell death in prostate cancer cells. Paramjot Kaur,1 Sivanesan Dhandayuthapani,1 Shona Joseph,2 Syed Hussain,2 Miroslav Gantar,3 Appu Rathinavelu1.1 Rambough Goodwin Institute for Cancer Research, Nova Southeastern University, FL; 2Halmos College of Natural Sciences and Oceanography, Nova Southeastern University, Fort Lauderdale, FL; 3Florida International University, Miami, FL.

Plant and microbial metabolites are constantly explored to identify novel therapeutics with promising potential for treating diseases such as cancer. Marine-derived compounds are also tapped frequently for their efficacy in treating cancers and are used in mono and combination therapies. The potential of cyanobacteria (blue-green algae) as the source of anticancer agents has been explored for many decades and, as a result of continuing efforts, several compounds have emerged as templates for the development of new anticancer drugs from these microorganisms. The C-phycocyanin (C-PC) tested in our experiments was found in the cyanobacteria Limothrix sp., strain 37-2-1, which is found abundantly in Florida’s Everglades. In our previous studies, this fluorescent compound was found to potentiate the cytotoxic effects of Taxol and Topotecan. In LNCaP prostate cancer cells, C-PC was able to induce apoptosis by itself through activation of the apoptotic pathway and completing DNA fragmentation. It was originally anticipated that C-PC might penetrate the cell membrane to induce mitochondrial damage and trigger the apoptotic process through activations of caspases. In order to verify this possibility, experiments were conducted to determine the uptake of C-PC using LNCaP cells. When we incubated the cells with C-PC at the concentrations of 250 and 500 µg/mL for 12 hrs and assessed the cellular uptake by capturing the fluorescence signals at the wavelength of 605 nm, using a fluorescence microscope, the signals were observed only with the periphery of the cells. Furthermore, with subsequent washes using PBS, the fluorescence signal that was seen to be associated with the plasma membrane was reduced gradually and was removed completely after the 3rd wash without any signs of penetration into the cytoplasm. The binding of C-PC to the plasma membrane was very transient and quickly dissociated at 37°C. So far, our results suggest that the cytotoxicity towards LNCaP cells might have been triggered by the binding of C-PC to the cell membrane receptors such as FasR, TRAIL-R, TNFR, which may be linked to the mediators of extrinsic and intrinsic apoptotic signals. Hence, our findings are significant for explaining some of the apoptotic events triggered by C-PC. However, additional studies are required to identify the actual receptors involved in triggering the pathway. (The support from the Royal Dames of Cancer Research Inc., Ft. Lauderdale, Florida is gratefully acknowledged).

#197 Anti-cancer activity of extract from the Jamaican round-leaf yellow yam (RLY2) (Dioscorea cayenensis). Sashay-Gay A. Wright,1 Wesley G. Gray,2 Helen Asemota1.1 University of the West Indies, Mona Campus, Kingston, Jamaica; 2Southern University and A&M College, Baton Rouge, LA.

The Jamaican Round-Leaf yellow yams (RLY2), Dioscorea cayenensis, are edible tubers, bulbils or rhizomes that are of considerable economic value and possess several health benefits. These RLY2 are a rich source of antioxidants, vitamins and phytochemicals. In addition to its nutritional value, the Jamaican populace regards RLY2 as an herbal medicine useful in treatment of diabetes mellitus, hypertension and certain kind of cancers. Chemical analysis of RLY2 revealed the presence of a myriad of bioactive ingredi- ents that are likely to be lost due to the method of preparation for consumption. Thus, we hypothesized that mild extraction of RLY2 will preserve the bioactive analytes that give RLY2 its anti-cancer property. To test this hypothesis, different ethanol extracts of RLY2 were prepared, and their bioactivity demonstrated in two models of prostate cancer. The present study demonstrates dosage and time-dependence inhibition in both the androgen sensitive LNCaP and androgen insensitive DU-145 prostate cancer cell lines. We observed that DU-145 was three-times more sensitive to RLY2 ethanol than LNCaP. Growth of LNCaP for 3-days with varying concentration of RLY2 ethanol extract resulted in an IC50 of 750 ppm (95% C.I. 726-791ppm), whereas DU-145 resulted in an IC 50 of 250 ppm (95% C.I. 236-281ppm). The maximum growth inhibition by RLY2 extract occurs within twenty-four hours for both LNCaP and DU-145 with significant changes after 5-days. To determine the mechanism of toxicity by RLY2, we incubated both LNCaP and DU-145 with RLY2 for 0-24h and prepared cells for flow cytometer analysis. The effect of RLY2 on cell cycle and degree of apoptosis will be discussed. Our data indicated that the ethanol extract of RLY2 was more sensitive toward androgen-insensitive prostate cancer. Thus, this study suggests that RLY2 contain bioactive chemical that may be used in prostate adenocarcinoma represented by the DU-145 phenotype.

#198 Low molecular weight pyrrolobenzodiazepine (PBD) monomers have potent cytotoxicity in haematological tumour cells. David B. Corcoran,1 Thomas Lewis,1 Amitr Varsha,1 Chris Pepper,2 David E. Thurston,1 Khondaker Miraz Rahman1.1 King’s College London, London, United Kingdom; 2University of Cardiff, Cardiff, United Kingdom.

The pyrrolo[2,1-c][1,4]benzodiazepines (PBDs) have long been of interest as potential chemotherapeutic agents due to their ability to form covalent adducts within the minor groove of the DNA helix. The most effective synthetic modifications to PBD cores have involved the conjugation of two DNA-interacting moieties via their C8/C8’-positions to create PBD dimers capable of covalently cross-linking duplex DNA which improves cytotoxicity. Research to date has focused on adding substituents to the C8-position of the PBD core to improve DNA-interaction and cytotoxicity. All of these approaches tend to increase the molecular weight of the compounds, although this has not prevented their successful development to the clinic, either as stand-alone agents (e.g., SJG-136) or as a component of Antibody-Drug Conjugates (ADCs) (e.g., SGN-CD33A). During a structure-activity relationship (SAR) study, we embarked on a “molecular pruning” exercise to sequentially reduce the length and bulk of the C8-substituent of a PBD monomer expecting the cytotoxicity to reduce with a decrease in length/bulk, and to establish the minimal pharmacophore. Surprisingly, we found that reducing the length of the C8-substituent maintained cytotoxicity and in some cases enhanced it. A 20-member library of short C8-substituted PBD monomers has been synthesized featuring C8-substituents of various chemical composition and length, with some containing amine substituents. Cytotoxicity evaluation in several tumour cell lines (e.g., primary CLL, JJJN-3 and MDA-MB-231) was carried out, and low nanomolar to high picomolar IC50 values were obtained for several library members including DC-1-194 (IC50 = 4.2nM in CLL, and 0.79nM in MDA MB 231), DC-1-255 (IC50 = 9.5nM in CLL, and 1.1nM in MDA MB 231), DC-1-253 (IC50 = 8.4nM in CLL, and 10nM in JJJN-3) and DC-1-275 (IC50 = 7.6nM in CLL, and 9.6nM in JJJN-3). Remarkably, many of these more-active compounds had very short C8-substituents. HPLC and FRET based DNA binding studies were also carried out, in order to confirm the capacity of these molecules to form adducts with DNA. The observations reported here are significant, as they add to an evolving understanding of the SAR of PBD monomer structures. The results presented here may also have significance for the future development of PBD-based therapeutic agents where there may be advantages to working with lower molecular weight molecules.
Silibinin suppresses bladder cancer through down-regulation of actin cytoskeleton and PI3K/Akt signaling pathways. Mitsuhiro Imai-Sumida,1 Takeshi Chiyomaru,2 Shahana Majid,3 Priyanka Kulkarni,4 Pritha Dasgupta,1 Sharanjot Saini,1 Taku Kato,5 Shiikgakta Mekawa,1 Yutaka Hashimoto,1 Marisa Shina,6 Guoren Deng,7 Varahram Shahryari,8 Hannah Nip,9 Rajvir Dahiya,9,2 Srinivas Tanaka,7 Soichiro Yamamura,7,9visited VA Medical Ctr., San Francisco, CA;2National Hospital Organization Kagoshima Medical Center, Kagoshima, Japan.

Silibinin is the major active constituent of silymarin, an extract of milk thistle seeds. Silibinin has been shown to have significant anti-cancer effects in a variety of malignancies. However, the molecular mechanisms of silibinin action in bladder cancer have not been studied extensively. In the present study, we found that silibinin (10 μM) significantly suppressed proliferation and invasion of T24 and UM-UC-3 human bladder cancer cells. In this study, we investigated the molecular mechanisms underlying these effects of silibinin. Our results showed that silibinin down-regulates actin cytoskeleton-related Ras-Rac-PAK1 pathways and the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway in these bladder cancer cell lines. These pathways were found to promote cellular transformation and cancer development and have crosstalk through Ras cascades. We investigated the role of silibinin on Ras, and found that silibinin suppresses levels of trimethylated histone H3 lysine 4 and acetylated H3 at the K-RAS promoter. We also found that silibinin downregulates long non-coding RNAs: HOTAIR and ZFAS1, which are known to play roles as oncogenic IncRNAs in various cancers. This study shows that silibinin exerts anti-cancer effects through down-regulation of the actin cytoskeleton and PI3K/Akt pathways and thus suppresses the development and progression of bladder cancer.

Long lasting inhibition of TGF-β Receptor Type 1 inhibitor (TGF-β R1) for treatment of pancreatic cancer. Cristian Rodriguez-Aguayo, Emine Bayraktar,6 Hafiz Shen,4 Gabriel Lopez-Berestein1.1 asso-MD Anderson Cancer Ctr., Houston, TX; 2The Methodist Hospital Research Institute, Houston, TX.

Pancreatic cancer stroma is a high component of total tumor mass and contains activators and activators of fibro-genesis such as Transforming growth factor-β (TGF-β) are abundantly secreted by pancreatic stellate cells (PSCs) to the tumor microenviroment. Ablation of TGF-β signaling pathways are associated with many human diseases, including bone diseases, immune-suppression, fibrosis, cancer progression and metastasis. Hyperglycemia is a key feature of diabetes and is also the cause of severe complications of diabetes, including nephropathy, retinopathy, and foot ulcers. Many studies have demonstrated that diabetes is associated with hyperglycemia and is the cause of severe complications of diabetes, including nephropathy, retinopathy, and foot ulcers. Many studies have demonstrated that hyperglycemia is also associated with the development of age-related macular degeneration, diabetic kidney disease, and cardiovascular disease. Moreover, hyperglycemia is also associated with the development of age-related macular degeneration, diabetic kidney disease, and cardiovascular disease. Moreover, hyperglycemia is also associated with the development of age-related macular degeneration, diabetic kidney disease, and cardiovascular disease. Moreover, hyperglycemia is also associated with the development of age-related macular degeneration, diabetic kidney disease, and cardiovascular disease. Moreover, hyperglycemia is also associated with the development of age-related macular degeneration, diabetic kidney disease, and cardiovascular disease. Moreover, hyperglycemia is also associated with the development of age-related macular degeneration, diabetic kidney disease, and cardiovascular disease. Moreover, hyperglycemia is also associated with the development of age-related macular degeneration, diabetic kidney disease, and cardiovascular disease. 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For the purpose of investigating the potential of automation and high throughput mass spectrometry imaging, we have combined a desorption electrospray ionisation (DESI) MS system with a robotic slide loader. The analysis of tissue sections on multiple slides could allow, for example, large patient cohorts to be analysed with almost no input from the operator. Here we present another use of such technology: the mapping and visualisation of tissue and molecular signals that were only present on the boundary between the hypoxic and proliferating regions in excised tissue. Further to these identified regions was the discovery of molecular signals that were clearly correlated with hypoxia and proliferation, indicating that mass spectrometry imaging provides a powerful tool for the identification of cancer cell-derived VOCs from patient exhaled breath.

#204 In vitro metabolomics of mesothelioma: Challenges and outcomes.
Sabrina Lagniau,1 Kevin Lamote,1 Lore Vandermeersch,1 Herman Van Langenhove,1 Jan P. van Meerbeeck,2 Karim Y. Vermaelen1.1Ghent University, Ghent, Belgium; 2Antwerp University Hospital, Edegem, Belgium.

Introduction: Due to the highly malignant nature of mesothelioma (MPM) it is likely to improve outcome in affected patients. From our previous studies (MeSOBreath 1 & 2) we developed a screening tool in which volatile organic compounds (VOCs) in breath allowed discrimination of MPM patients from at risk controls. However it is yet not clear which VOCs arise from the neoplastic cells themselves or from the host response. Identifying the cancer cell-specific VOCs will allow us to design breath tests which are currently under investigation. Faster and automated mass spectrometry imaging allows whole tissues to be chemically mapped in three dimensions in a few hours providing insights into tumour heterogeneity.


Introduction: Genomic analyses have yielded a tremendous amount of data on the genetic changes in lung cancers, but translating these experiments into actionable information benefiting lung squamous cell carcinoma (SCLC) patients has proven more difficult. Studies by the NCI Clinical Proteomic Tumor Analysis Consortium (CPTAC), our group, and others have demonstrated that gene protein and expression show only moderate correlation, demonstrating limitations in explaining phenotypic changes from genomics alone. These findings indicate a clear need for integrative proteogenomics to better understand tumor biology, especially in a complex disease like SCLC. Experimental: We have assembled a comprehensive proteogenomic dataset including DNA copy number (Affymetrix CytoScan HD Assay), targeted exome sequencing (Agilent Comprehensive Cancer Panel), RNA-sequencing (Illumina NextSeq), and shotgun proteomics (Q Exactive LC-MS/MS) on 116 surgically resected SCLC tumor samples with extensive clinical and follow up data. Results: We have identified 6584 high confidence proteins from preliminary proteomic analysis. After quality control filtering, we utilized 5562 gene-protein pairs for further analysis. Clustering of patient RNA expression in this patient cohort has been unable to fully reproduce the molecular classification previously published for SCLC. Furthermore, proteomic results indicate yet another potential classification strategy selecting patient subgroups that differ at protein level. We observed a 0.29 median Spearman’s correlation of 5562 gene-protein pairs. There were 2781 highly correlated gene-protein pairs (greater than median) and 2781 poorly correlated gene-protein pairs (less than median) including 773 anti-correlated gene-protein pairs (less than 0). We hypothesized that poorly correlated gene-protein pairs could functionally be related in a pathway-dependent manner. Enrichment analysis of poorly correlated proteins identified pathways related to mRNA processing, growth factor signaling (EGFR, FGFR), and nonsense-mediated decay (NMD). Interestingly, there were 9 frequently mutated SCLC genes in the low correlation gene-protein pairs but only 3 in the highly correlated pairs. We found three distinct patient subgroups by clustering poorly correlated proteins. Analysis of these subgroups showed differentially expressed pathways related to mRNA processing, ubiquitination, and NMD. Conclusion: Differential modulation of the proteome outside of genomic regulation may suggest important regulatory mechanisms in cancer and give new insights into treating SCLC. Analysis of poorly correlated gene-protein pairs suggests certain pathways are dysregulated in cancer, and ongoing DNA analysis and future analyses involving miRNAs, RNA-binding proteins, and the ubiquitin proteome system will help elucidate our preliminary findings.


Introduction: Genomic analyses have yielded a tremendous amount of knowledge on the genetic changes in lung cancers, but translating this information into actionable data benefitting patients has proven difficult. The integration of proteomic analyses with genomics and gene expression profiling allows a more detailed description of the biological processes, thus improving our understanding of cancer phenotypes. These insights can potentially be used for better classification and help to guide patient selection for targeted therapies. Experimental: We analyzed 116 surgically resected squamous cell lung carcinoma samples for copy-number alterations, gene expression profiling, targeted exome-sequencing and global proteomic profiling. The cohort consisted of mostly early stage tumors (83% Stage I or II) with complete follow-up (median 58 months). Copy number status was analyzed using the Affymetrix CytoScan array. DNA mutation status was assessed using a customized version of the Agilent Comprehensive Cancer Panel for targeted sequencing, and gene expression profiling was carried out by RNA-sequencing. Proteomic analysis was performed using TMT labeling, 12- fraction bRPLC separation and LC-MS/MS analysis with a Thermo Q-Exactive mass spectrometer. Database searches were performed using MassLynx and in house scripts. Results: We identified 6584 high confidence proteins from preliminary proteomic analysis. After quality control filtering, we utilized 5562 gene-protein pairs for further analysis. Enrichment analysis of poorly correlated proteins identified pathways related to mRNA processing, growth factor signaling (EGFR, FGFR), and nonsense-mediated decay (NMD). Interestingly, there were 9 frequently mutated SCLC genes in the low correlation gene-protein pairs but only 3 in the highly correlated pairs. We found three distinct patient subgroups by clustering poorly correlated proteins. Analysis of these subgroups showed differentially expressed pathways related to mRNA processing, ubiquitination, and NMD. Conclusion: Differential modulation of the proteome outside of genomic regulation may suggest important regulatory mechanisms in cancer and give new insights into treating SCLC. Analysis of poorly correlated gene-protein pairs suggests certain pathways are dysregulated in cancer, and ongoing DNA analysis and future analyses involving miRNAs, RNA-binding proteins, and the ubiquitin proteome system will help elucidate our preliminary findings.
performed using multiple search engines against RefSeq version 78, and summarized using IDPicker 3. Results: The non-redundant protein inventory consisted of more than 6,000 protein groups with a protein FDR <5%. Tumors were classified according to well established gene expression criteria into 4 classes: Classical, Basal, Primitive and Secretory. At the protein level, the Classical subtype was associated with xenobiotic and energy metabolism; the Basal subtype with defense responses and extracellular matrix changes; the Primitive subtype with nucleic acid metabolism; and the Secretory subtype with p38 signaling. These findings confirm and expand on previous mRNA expression studies of squamous cell lung carcinoma. Proteomics-based classification identified two sub-categories within the Classical subtype, which were characterized by inflammatory and stress response signaling. Within this group, patients with high expression of inflammation-associated proteins had better cancer-specific survival than those with low expression ($p=0.04$, Log-rank test). Targeted exome sequencing of 154 cancer-associated genes revealed frequent mutations in TP53, CDKN2A, NFE2L2, and other genes. Proteomic expression of genes located in amplified chromosomal regions was used to identify driver genes in squamous cell lung carcinoma. Conclusion: Our results provide new biological insights from the addition of protein measurements to genomic datasets that have the potential to improve classification. The data suggest that proteins involved in immune responses are important for the biological behavior and outcome of the Classical subtype in squamous cell lung carcinoma.

#207 Reliable identification of mutations in bottom-up proteomics. Miroslav Hruska, Lakshman Varanasi, Jiri Voller, Petr Drubak, Marian Hajducz, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University and University Hospital, Olomouc, Czech Republic.

Knowledge of missense mutations is of significant biological importance as it provides valuable insights into alterations of phenotype. While identification of reference proteins is routinely performed in proteomics, identification of mutations remains still fairly uncommon. Well-established methods for reference peptides are generally insufficient for altered ones, e.g., utilizing mutant-augmented database search usually results in inadmissible proportion of false positives. This undesirable situation is, however, rather natural consequence of peptide fragmentation, its computational modelling and evaluation of their correspondence. Often, there are many peptides having the same or similar agreement with acquired spectrum, thereby preventing agreement-based decision. These situations happen frequently in identification of mutant peptides (e.g., homologous peptides with PTMs, semi-specific peptides), therefore their reliable identification requires additional treatment. To deeper understand properties of identification, we have formally studied generalized version of the identification problem and derived its optimal solution under particular assumptions. In proteomics, the selection of optimal solution can be always found in finite time; moreover, the strategy is straightforward to implement, enables parallelization and provides guarantees over claimed interpretations. The solution utilizes almost exclusively spectral data of product ions without additional LC/MS information. In practice, however, it is beneficial to employ precursor isotopic distribution analysis for correction of non-monoisotopic selection of precursor as that would otherwise systematically result in artefacts. The behavior of proposed method was validated in a variety of scenarios that selection of precursor as that would otherwise systematically result in artefacts. In practice, however, it is beneficial to employ precursor isotopic distribution analysis for correction of non-monoisotopic selection of precursor as that would otherwise systematically result in artefacts. The behavior of proposed method was validated in a variety of scenarios that would otherwise systematically result in artefacts.


As part of the Clinical Proteomic Tumor Analysis Consortium (CPTAC), we have recently published the first large-scale proteomic and phosphoproteomic analysis of high-grade serous ovarian tumors. We observed that phosphorylation status was an excellent indicator of pathway activity and could discriminate between patient survival times. This dataset covers tumor samples from 69 patients with deep phosphoproteome depth (>20,000 phosphopeptides confidently identified). Our continuing analysis of this dataset, reported here, has revealed that the correlation between kinase protein abundance and abundance of phosphorylated target peptides is very low, indicating that kinase abundance alone cannot explain phosphorylation status overall. However, highly correlated kinase-substrate pairs are significantly more likely to be true relationships (from existing knowledge), demonstrating that this method could be used to predict novel kinase targets in some cases. Using this approach we predicted novel kinase-target relationships and constructed a kinase activity network of ovarian cancer. To better analyze cancer-relevant pathway activity we developed a novel approach that does not depend on tissue antigenicity or nucleic acid preservation could increase the diagnostic value of decalcified bone samples. We assessed the effects of decalcification on proteomic analysis of tumor tissue. We also quantified 27 therapeutically-relevant proteins in decalcified bone metastases of cancer patients using mass spectrometry-based proteomic analysis. Methods: To examine the effects of decalcification on protein quantification, we used 3 non-bone tissue specimens from lung adenocarcinoma, squamous cell carcinoma, and colon medullary carcinoma. Non-bone tumor tissue was expected to perform similarly in proteomic analyses to bone tissue, which was not available. Tissue specimens were processed without decalcification and with hydrochloric acid-based Decal-Stat® decalcification solution for 1, 3, 12, and 24 hours prior to paraffin embedding, tissue sectioning, and mass spectrometric analysis. Proteomic analysis was also performed on 26 previously decalcified biopsies of metastatic bone lesions from patients with cancers of the lung (n=7), breast (n=7), stomach (n=3) and 8 other indications. Archived tumor tissue was microdissected and solubilized to tryptic peptides. Target proteins in each liquefied tumor sample were quantitated in triplicate with a multiplexed, selected reaction monitoring mass spectrometry assay. Results: In the bone samples, the method we developed for quantitative analysis of phosphorylated forms of total protein (range: 19.2 - 24.1 μg) and of all 20 protein biomarkers detected. The 26 bone metastases expressed 20 of the 27 protein targets tested. The 7 bone samples from lung cancer patients, 5 expressed EGF receptor and 5 expressed hENT1 protein (a marker of response to gemcitabine). A metastatic bone tumor from a breast cancer patient expressed hENT1 (129 amol/μg) and overexpressed HER2 (5750 amol/μg; this exceeds a level previously correlated with increased survival in trastuzumab-treated patients). Bone lesions from prostate & gynecologic cancers overexpressed AR, hENT1, EGFR and TOP1 proteins. Conclusions: A commonly used decalcifying solution had no discernable effects on proteomic quantification of biomarker proteins in archived tumor samples. Targeted proteomics can quantify an entire panel of therapeutically-relevant proteins from a single decalcified bone biopsy specimen. Proteomic analysis of bone metastases upon diagnosis of metastasis or at relapse could inform treatment decisions, particularly in patients who have disease progression only in bone lesions or whose bone biomarkers are discordant from those of the primary tumor.


A method was developed that allows the evaluation of complex biological processes from mass spectrometry of human serum samples. Applying gene expression of inflammation-associated proteins had better cancer-specific survival; the Classical subtype was associated with xenobiotic and energy metabolism; the Basal subtype with defense responses and extracellular matrix changes; the Primitive subtype with nucleic acid metabolism; and the Secretory subtype with p38 signaling. These findings confirm and expand on previous mRNA expression studies of squamous cell lung carcinoma. Additionally, the analysis was performed on in-house samples with RNA-Seq data available (HCT116), giving specificity obtained was 0.81 and 0.99 respectively. Indirect validation was, at first, natorial peptide library of 400 peptides (all coded amino acids in 12. and 13. position). For direct validation, synthetic combinatorial peptide library of 400 peptides (all coded amino acids in 12. and 13. position) was used. The behavior of proposed method was validated in a variety of scenarios that would otherwise systematically result in artefacts. In practice, however, it is beneficial to employ precursor isotopic distribution analysis for correction of non-monoisotopic selection of precursor as that would otherwise systematically result in artefacts. The biological behavior and outcome of the Classical subtype in squamous cell lung carcinoma.

#209 Proteomic analysis of therapeutic biomarkers in decalcified bone metastases. Fabiola Cecchi, Shankar Sellappan, Sarit Schwartz, Chao Gong, Marlene Darfler, Kerry Scott, Steven W. Mamus, Mike Emmert-Buck, Todd Hemmingsworth, Nites, Elliot City, MD; NutriOmicx, Rockville, MD; Cancer Center of Sarasota-Manatee, Sarasota, FL; NIH, Bethesda, MD.

Introduction: Theralogies we developed that allows the evaluation of complex biological processes from mass spectrometry of human serum samples. Applying gene expression of inflammation-associated proteins had better cancer-specific survival; the Classical subtype was associated with xenobiotic and energy metabolism; the Basal subtype with defense responses and extracellular matrix changes; the Primitive subtype with nucleic acid metabolism; and the Secretory subtype with p38 signaling. These findings confirm and expand on previous mRNA expression studies of squamous cell lung carcinoma. Additionally, the analysis was performed on in-house samples with RNA-Seq data available (HCT116), giving specificity obtained was 0.81 and 0.99 respectively. Indirect validation was, at first, natorial peptide library of 400 peptides (all coded amino acids in 12. and 13. position) was used. The behavior of proposed method was validated in a variety of scenarios that would otherwise systematically result in artefacts. In practice, however, it is beneficial to employ precursor isotopic distribution analysis for correction of non-monoisotopic selection of precursor as that would otherwise systematically result in artefacts. The biological behavior and outcome of the Classical subtype in squamous cell lung carcinoma.

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set enrichment analysis ideas to matched protein expression data from a panel of 1129 proteins and deep MALDI mass spectral data from a set of 49 human serum samples with lung cancer (n=45) or non-cancer (n=4), subsets of mass spectral features associated with selected biological functions were identified. Biological functions included acute response, complement system, immune system and the top 100 most significant hits were assigned using the intersection of gene ontologies and the protein panel. Using mass spectral data collected from an independent NSCLC sample cohort (n=85) from patients treated with targeted therapy, principal component analysis was used to derive scoring functions (combinations of the feature subsets) for each biological class. These scoring functions were validated in an independent NSCLC sample (n=12) treated with targeted therapy. The scoring functions can be applied to mass spectra obtained from any human serum sample to generate scores associated with individual biological processes. We have developed scoring functions for several biological processes. Acute response score was associated with outcome in Cox proportional hazard analysis in several independent patient cohorts across multiple therapies and indications, including lung cancer, as mentioned, and ovarian cancer (n=165) treated with platinum doublets following surgery. Choice of a single cutoff allowed stratification of patients into groups with significantly better or worse outcome. In a cohort of nivolumab treated NSCLC patients (n=67) with available longitudinal samples, outcome was also found to depend on changes in scores during therapy. Interestingly, while the distributions of scores on restaging were quite different across multiple tumor classification scores for other biological functions, such as wound healing, varied considerably. This may reflect differences in relative importance of individual biological functions between tumor types. A scoring system based on biological functional categories has a wide range of uses that could be tested and applied in a clinical setting. Currently, tests that measure a single biomarker for monitoring a particular disease have questionable utility and limit the usefulness to the clinician and patient treatment decision making. A tool that measures the activity of a complex biological process could be useful in deciding when an intervention is needed (screening), or for monitoring the effects during therapy.


The next generation of molecular cancer therapeutics will target pivotal protein-protein interaction interfaces participating in immune cell receptor signaling, oncoproteins, and suppressor genes. We have created a wholly novel technology “protein painting” for the rapid direct sequencing of hidden native protein-protein interaction hot spots. Our technology, employs previously unexplored small molecule (12 Å) aryl hydrocarbon dyes or “paints” to cut out, and MS sequence, only the hidden unmodified contact interfaces between two or more interacting native proteins. Novel Technology: Paint chemistries have extremely high affinities (rapid on-rates, and very slow off-rates that are ten to 100 times higher than most protein-protein interactions). When mixed with a native pre-formed protein complex for only 5 minutes at physiologic pH and salinity, the paints non-covalently coat all external sites on the protein without altering the 3D conformation of the complex, but cannot gain access to the solvent inaccessible hidden protein-protein interaction domains. Each paint molecule spans less than 3 amino acids, and has high affinity for protease cleavage consensus sites. Following painting, the proteins are dissociated. This leaves the paint molecules coating surfaces not participating in the interface. Following dissociation, the proteins are linearized, digested with trypsin, and sequenced by standard MS. The paint molecules remain non-covalently bound after the proteins are denatured. Trypsin will not cleave the regions of the protein that are “painted”. Following proteinolysis peptides emerging from MS will be generated exclusively from the unmodified opposing sites where the proteins were in intimate contact. Results: Protein Painting identified hot spot domains between PD-L1:PD-1, including two surface interface regions that are separated in the linear sequence but adjacent in the 3D structure. We created novel cyclized multivalent inhibitors that block both sides of the PD-L1: PD-1 interface and markedly suppress cell-cell binding and abolished downstream signaling through this complex in cultured tumor cell immune cell interactions. A very high correlation (p<0.0001) was found for known contact points predicted by crystal structure, with a 97% specificity for true positive hits: 95% agreement with Robetta prediction software for known complexes. Protein painting outperforms (425%) hydrogen deuterium exchange and cross linking for number of positive hits and % true positive hits. Conclusions: Protein painting is a new tool to identify highly specific drug targets located within protein interaction interfaces, yielding inhibitors that abolish protein signaling relevant to cancer immunotherapy.


The receptor tyrosine kinase c-ErbB2 is amplified in breast and ovarian cancer. The linear pathways through which signals by c-ErbB2 are transduced is well known. However, second generation questions that address spatial aspects of signaling remain. To address this, we have undertaken a mass spectrometry approach to identify phosphoproteins. We have used two tyrosine kinase inhibitors, Lapatinib and CP724714, that inhibit phosphorylation of c-ErbB2 to identify phosphoproteins. SKOV-3, an ovarian cancer cell line that endogenously overexpresses c-ErbB2 was grown in culture without serum for 72 hrs. Cells were then stimulated in the presence or absence of inhibitor with EGF (100ng/ml) as a ligand for 60 mins. Subsequently, cells were lysed and evaluated by western blotting with anti-phosphotyrosine antibody (4G10). Following stimulation of cells with EGF, maximal phosphorylation of c-ErbB2 was observed at 60 minutes. Lapatinib (10μM) and CP724714 (15μM) completely inhibited phosphorylation of c-ErbB2, which was confirmed by immunoprecipitation. This was further confirmed by the inhibition of downstream effectors (Erk1/2, Akt). Lapatinib (10 μM) also completely inhibited phosphorylation of EGFR while CP724714 (15μM) only inhibited partially. Cellular lysates were prepared from quiescent cells (grown without serum), after stimulation with EGF in the presence or absence of inhibitors. Purified phosphoproteins from all three samples following digestion with trypsin were subjected to mass spectrometry (Nano LC ESI MS/MS). We identified totally 62 phosphoproteins. Twenty seven phosphoproteins were observed in all the 3 samples while 17 phosphoproteins were identified both in the EGF stimulated and lapatinib treated samples. Eighteen unique phosphoproteins were observed only in the EGF stimulated sample suggesting that they are specific to signaling by c-ErbB2. The novel phosphoproteins included the proteins that participate in carbohydrate metabolism, cytoskeleton, cell migration and proliferation. We have evaluated two phosphoproteins, LAP1-1 and Aldose reductase that has not been previously described following phosphorylation of c-ErbB2. LAP1-1 is an oncogene and is located as the same arm 17q21 as c-ErbB2. It was not expressed in the normal ovary or fallopian tube. However, it was over-expressed in 17% of tumours (n=85) from patients with ovarian cancer. c-ErbB2 was not expressed in tumours that expressed LAP1-1. Aldose reductase is a cytosolic NADPH dependent oxidoreductase that catalyzes the reduction of glucose to sorbitol, the first step in polyol pathway of glucose metabolism. The activity of aldose reductase in reducing NADPH as a substrate was significantly higher in lysates from EGF stimulated as compared to the starved cells. Identification of phosphoproteins by using mass spectrometry is promising in identifying novel substrates and pathways following phosphorylation of c-ErbB2.

**#213 Integrated proteogenomic analysis of cancer}
57 ± 13 years. Protein and RNA were extracted using the Illustra triplePreP kit, which isolates DNA from the same cells as well. Quantitative global proteomics and phosphoproteomics analyses were performed using isotopic TMT 6-plex labeling with the "universal reference" strategy and IMAC enrichment of phosphopeptides. Mass spectrometry data were acquired using a Q-Exactive instrument and the "universal reference" strategy and IMAC enrichment of phosphopeptides. The views expressed in this article are those of the author and do not been previously reported (e.g., on RANBP2), other phosphosites appeared to be basal-enriched and another being luminal-enriched. We also observed a sub-cluster of mixed subtypes. Differential protein expression analyses between the two primary clusters confirmed known markers (e.g., overexpression of KRT8/KRT18 in luminal-enriched cluster). The luminal-enriched cluster is primarily CA with post-menopausal status. A similar search of the phosphoproteomic data yielded quantitation of >12500 phosphopeptides. Unsupervised clustering of the phosphoproteins resulted in four primary clusters, with one being basal-enriched and another being luminal-enriched. We also observed >50 overexpressed phosphopeptides. While some of these phosphoproteins have been previously characterized, on RANBP2 others novel (e.g., on IRF2BP2). Conclusion: Analysis of LCM breast tumors using proteogenomic technologies resulted in basal- and luminal-enriched clusters, thus enabling us to study protein and phosphopeptide markers across multiple platforms. The views expressed in this article are those of the author and do not reflect the official policy of the Department of Defense, or U.S. Government.

#215 Characterization by mass spectrometry of protein kinase C substrates differentially phosphorylated in LNCaP cells in response to phorbol ester and bryostatin 1 treatment. Noemi Kedel, Sudipto Das, Thorkell Andresson, Peter M. Blumberger. National Cancer Institute, Bethesda, MD; Fredrick National Laboratory for Cancer Research, Frederick, MD.

Bryostatin 1 (bryo 1) is a natural product of therapeutic interest for cancer and Alzheimer disease. Its unique behavior as a protein kinase C activator that paradoxically antagonizes many but not all phorbol ester responses has led to intense interest in its mechanisms of action. Recently, using microarray analysis in two different cellular systems (LNCaP prostate cancer and U937 leukemia cell lines) where the typical phorbol ester PMA and bryo 1 have different biology, we have shown that a core mechanism contributing to the unique biology of bryo 1 is transiency of action resulting in a variable extent of decreased or missing late responses. We have excluded that there is a class of genes whose transcription is uniquely regulated at early times by bryo 1. In continuing to explore the mechanisms underlying the variable transiency of the responses by bryo 1 we have evaluated the substrates phosphorylated after treatment with PMA and bryo 1. Since PMA and bryo 1 induce differential subcellular localization of PKCs, they should result in differential access to substrates and consequent differences in the pattern of substrate phosphorylation. Whole cell lysates of LNCaP cells treated for 30 min with fully effective doses (100 nM) of PMA and bryo 1 and with vehicle control were submitted to mass spectrometric analysis. The comprehensive analysis identified several thousand phosphopeptides after drug treatment including the expected phosphopeptides for ERK2 (S185, Y187) and PKCdelta (S299, S302, S304). Many of peptides were similarly phosphorylated in response to both drugs (e.g. S641/S646 of Fam129B, S510 of actin-related protein 2/3 complex subunit 1B, S876 of RhoGGEF and pleckstrin domain-containing containing protein 2, or multiple previously unknown sites for PKD1 (S239, S247), while a limited number were differentially phosphorylated. PMA specific phosphorylations included PKCdelta at Y313, mTOR at T2471, MAP4 at S624 and/or T627, E3 ubiquitin-protein ligases ZNRF2 at S82 and HUWE1 at S5818. Bryo 1 specific phosphorylations included BCL2-like like 13 at S303, TOMIL2 at S424, and MAP2K2 in the T17, T25, S26 region. Selected specific phosphorylations are being validated using phoestag gels, a method that enables the separation of phosphorylated proteins from their non-phosphorylated counterparts on SDS-gels. The identification of PKC substrates that are differentially phosphorylated by bryo 1 should both facilitate screening of other ligands capturing the biological behavior of bryo 1 as well as further illuminate the specifics of the pathways downstream of PKC activation by these differently acting ligands.

#216 Tracking expression, post-translational modifications and interactions of EGF signalling proteins in A431 cells with antibody microarrays. Lambert Yue, Steven Pelech. University of British Columbia, Vancouver, British Columbia, Canada.

Epidululation of epidudal growth factor (EGF) signalling pathways in cancer can help to understand the mechanisms for their dysregulated expression and potential targets for therapeutic intervention. Antibody microarrays are promising tools to evaluate alterations in the levels and phosphorylation status of hundreds of proteins of interest with only microgram amounts of crude cell and tissue lysate protein. However, interpretations of the results from traditional antibody microarray approaches have been hampered by the problems associated with sample preparation and protein detection, even when reliable antibodies are deployed in these arrays. The Kinex™ KAM-900P antibody microarray permitted semi-quantitative measurements of the expressions, post-translational modifications and interactions of proteins with 100 µg or less of lysate proteins. These microarrays utilize approximately 878 different pan- and phosphosite-specific antibodies for tracking protein kinases, phosphatases and other changes in protein levels, phosphorylation and protein-protein interactions in A431 cells in response to EGF treatment. One method (KAM) involved the capture of in vitro biotin-labeled proteins, followed by their detection with a secondary dye-labeled anti-biotin antibody. False positive signals from associated proteins in complexes with the target were reduced by chemical cleavage with NT-CB prior to their capture on the array, and this also produced more uniformity of the dye signals for protein targets despite vast differences in their sizes. Transient changes in protein phosphorylation in EGF treated cells that were typically lost when processed by conventional methods were better preserved by chemical

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cleavage right at time of sample homogenization. Biotin-labeling and subsequent detection of the protein on the array with a dye-labeled secondary antibody further reduced non-specific background signals, allowing for a greater dynamic range of detection, and enhanced discrimination of subtle changes. In conjunction with other detection protocols, such as the usage of dye-labeled multiplex arrays for protein expression in a variety of biological samples, the use of affinity purification and mass spectrometry (AP-MS) can be utilized to identify and quantify proteins involved in pathways that are of interest. In this study, we aimed to determine the efficacy of AP-MS for quantification of the AKT/mTOR and RAS/ERK pathways in multiple cancer cell lines and tissue samples. Major advantages for each assay technique. Conclusion: Overall, the mIP-tMS assays can be used to monitor cancer progression and determining treatment response. Some major bottlenecks in the accurate quantitation of these proteins are the lack of rigorously validated reagents and a reliance on semi-quantitative results from available mass spectrometry workflows. The benchmarking of mIP-tMS assays showed low correlation of quantification of target proteins or their metabolic reprogramming, and development of highly migratory and invasive capabilities of cancers. Therefore, characterization of mTORC2 is necessary not only for better understandings of known mTORC2-mediated functions, but also for the discovery of new roles and more interacting partners of mTORC2 signaling network in carcinoma, particularly ones with hyperactivated mTORC2 such as glioblastoma. However, until now mTORC2 is still partially characterized. Previously, mTORC2 was reported to be associated with Filamin A and Myosin-9 which have been known to play important roles in cellular activities requiring locomotion, including cancer migration and invasion. The complex participates in regulating glioblastoma migration and invasion, and Filamin A acts as a physiological downstream target of mTORC2. Currently, proteomics has emerged as a powerful tool to identify, quantify, and examine a large number of proteins. Therefore, in order to extensively unravel mTORC2 signaling pathway, the use of proteomics is necessary. We characterized mTORC2 by proteomic analysis based on affinity purification coupled to mass spectrometry (AP-MS) using antibodies against RICTOR, a specific component of mTORC2. The protein-protein interactions between RICTOR and other associated proteins were assessed. In addition to canonical components of mTORC2, the study has revealed more insights into a number of mTORC2 interacting partners which can be categorized and related to the main functions previously identified such as actin cytoskeleton reorganization, fatty acid metabolism, protein translation. Interestingly, we also found other types of cytoskeletal proteins, nucleotide metabolism, less-characterized and low abundance proteins associated with mTORC2 such as Horseradish Peroxidase. In addition, we have carried out the identification of condition-specific interactions for a dynamic view of mTORC2 interactome and found changes in mTORC2-associated proteins from different conditions. Furthermore, proteomic approaches can also be relevant to investigation and prediction of mTORC2 upstream regulators, downstream targets, and substrates. Ultimately, this strategy still uncovers some unknown molecular mechanisms, and result in a significant impact on the field of signal transduction machinery in cancers.


Background: The AKT/mTOR and RAS/ERK pathways represent key mechanisms for cells to regulate cell survival, proliferation, and motility. In addition to their independent signaling cascades, which provide offsetting mechanisms, these two pathways extensively engage in cross-talk in order to both positively and negatively regulate each other. The quantitation of protein expression and modifications of pathway targets are critical for characterization of disease, monitoring cancer progression and determining treatment response. Some major bottlenecks in the accurate quantitation of these proteins are the lack of rigorously validated reagents and a reliance on semi-quantitative results from available mass spectrometry workflows. The benchmarking of mIP-tMS assays showed low correlation of quantification of target proteins or their metabolic reprogramming, and development of highly migratory and invasive capabilities of cancers. Therefore, characterization of mTORC2 is necessary not only for better understandings of known mTORC2-mediated functions, but also for the discovery of new roles and more interacting partners of mTORC2 signaling network in carcinoma, particularly ones with hyperactivated mTORC2 such as glioblastoma. However, until now mTORC2 is still partially characterized. Previously, mTORC2 was reported to be associated with Filamin A and Myosin-9 which have been known to play important roles in cellular activities requiring locomotion, including cancer migration and invasion. The complex participates in regulating glioblastoma migration and invasion, and Filamin A acts as a physiological downstream target of mTORC2. Currently, proteomics has emerged as a powerful tool to identify, quantify, and examine a large number of proteins. Therefore, in order to extensively unravel mTORC2 signaling pathway, the use of proteomics is necessary. We characterized mTORC2 by proteomic analysis based on affinity purification coupled to mass spectrometry (AP-MS) using antibodies against RICTOR, a specific component of mTORC2. The protein-protein interactions between RICTOR and other associated proteins were assessed. In addition to canonical components of mTORC2, the study has revealed more insights into a number of mTORC2 interacting partners which can be categorized and related to the main functions previously identified such as actin cytoskeleton reorganization, fatty acid metabolism, protein translation. Interestingly, we also found other types of cytoskeletal proteins, nucleotide metabolism, less-characterized and low abundance proteins associated with mTORC2 such as Horseradish Peroxidase. In addition, we have carried out the identification of condition-specific interactions for a dynamic view of mTORC2 interactome and found changes in mTORC2-associated proteins from different conditions. Furthermore, proteomic approaches can also be relevant to investigation and prediction of mTORC2 upstream regulators, downstream targets, and substrates. Ultimately, this strategy still uncovers some unknown molecular mechanisms, and result in a significant impact on the field of signal transduction machinery in cancers.

#218 Functional proteomic analysis of TβRI immunocomplex in TGF-β signaling. Liuya Tang,1 Zhaojing Meng,2 Mary Heller,1 Ming Zhou,2 Ying Zhang,2 #NCI-CCR, Bethesda, MD; Frederick National Laboratory for Cancer Research, Frederick, MD.

Transforming growth factor-beta (TGF-β) regulates a wide array of biological responses ranging from proliferation to apoptosis, and alterations in its signaling pathway are associated with a variety of human diseases, including cancer. During the activation of TGF-β signaling, TGF-β receptor I (TβRI) plays an important role in transducing signals to downstream effectors. To identify TβRI interacting proteins which are responsible for Smad-dependent pathways as well as Smad-independent pathways, we employed a large-scale quantitative proteomic analysis of TβRI immunocomplex. AML12 cells were labeled with L-Lysine and L-Arginine (K0R0, light) or L-Lysine-13C6-15N2 and L-Arginine-U-13C6-15N4 (K8R10, heavy). The cells cultured in K8R10 medium (H: Heavy) were transfected with Flag-TβRI, while the cells cultured in K0R0 medium (L: Light) were transfected with Flag vector. The cells lysates were combined at equal protein amount, and TβRI immunocomplex were purified using FLAG M2 agarose. The resulting peptides from eluted TβRI immunocomplex were subjected to mass spectrometry quantitation. If a protein whose ratio of H/L was not smaller than 1.3, it was categorized as a constitutively interacting protein of TβRI. A total of 687 proteins were quantitated and 325 proteins were defined as constitutively interacting proteins. Pathway enrichment analysis found these proteins were highly enriched in pathways of TGF-β signaling. These results indicates the cross-talks between TGF-β signaling and other signaling. Future work will include validation of interaction between TβRI and candidate proteins, and understand functional significance of candidate proteins in TGF-β signaling or signaling cross-talks by a series of molecular biological approaches.

#219 Identification of the mechanistic target of rapamycin complex 2 associated interactome involved in brain cancer cell motility by affinity purification-mass spectrometry, Napat Chantaravisoot,2 Piriya Wongkongkathep,1 Fuyuhiko Tamanoi,2 Trairak Pisitkun1.

The Mechanistic Target of Rapamycin Complex 2 (mTORC2) is a multiprotein complex with serine-threonine kinase activity, drives several cellular processes of normal cells to promote proper metabolic activity, survival, proliferation, differentiation and movement. On the other hand, mTORC2 has been implicated as a critical player in tumorigenesis, stimulation of cell growth, cancer metabolic reprogramming, and development of highly migratory and invasive capabilities of cancers. Therefore, characterization of mTORC2 is necessary not only for better understandings of known mTORC2-mediated functions, but also for the discovery of new roles and more interacting partners of mTORC2 signaling network in carcinoma, particularly ones with hyperactivated mTORC2 such as glioblastoma. However, until now mTORC2 is still partially characterized. Previously, mTORC2 was reported to be associated with Filamin A and Myosin-9 which have been known to play important roles in cellular activities requiring locomotion, including cancer migration and invasion. The complex participates in regulating glioblastoma migration and invasion, and Filamin A acts as a physiological downstream target of mTORC2. Currently, proteomics has emerged as a powerful tool to identify, quantify, and examine a large number of proteins. Therefore, in order to extensively unravel mTORC2 signaling pathway, the use of proteomics is necessary. We characterized mTORC2 by proteomic analysis based on affinity purification coupled to mass spectrometry (AP-MS) using antibodies against RICTOR, a specific component of mTORC2. The protein-protein interactions between RICTOR and other associated proteins were assessed. In addition to canonical components of mTORC2, the study has revealed more insights into a number of mTORC2 interacting partners which can be categorized and related to the main functions previously identified such as actin cytoskeleton reorganization, fatty acid metabolism, protein translation. Interestingly, we also found other types of cytoskeletal proteins, nucleotide metabolism, less-characterized and low abundance proteins associated with mTORC2 such as Horseradish Peroxidase. In addition, we have carried out the identification of condition-specific interactions for a dynamic view of mTORC2 interactome and found changes in mTORC2-associated proteins from different conditions. Furthermore, proteomic approaches can also be relevant to investigation and prediction of mTORC2 upstream regulators, downstream targets, and substrates. Ultimately, this strategy still uncovers some unknown molecular mechanisms, and result in a significant impact on the field of signal transduction machinery in cancers.

#220 Proteomic identification of therapeutics targets for Enzalutamide resistance in Castration Resistant Prostate Cancer. Lauriane VELOT,1 Dominiqhe Levesque,2 François-Michel Boisvert,2 Nicolas Bisson,2 Frédéric Pouliot1.

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Prostate cancer (PC) is the most frequently diagnosed cancer in Canadian men and is the 3rd cause of cancer mortality. The Androgen Receptor (AR) is activated by androgens (e.g. testosterone), which leads to prostatic cell proliferation. The primary treatment for advanced PC is androgen-deprivation therapy, which is achieved via surgical or chemical castration. Nevertheless, most PC will become castration-resistant (CRPC). Although new anti-androgens (e.g. Enzalutamide) were developed to improve patients' survival, their efficacy is still very limited and most CRPC patients will die from the disease within a few years. We postulate that by gaining insights into AR signalling networks in the context of CRPC, we could better direct patients towards the best-suited therapy and propose new therapeutic targets. To this aim, we took advantage of an innovative proteomics approach, namely proximity-labeling (BioID), to characterize global AR signalling networks in hormone-dependant LAPC4 cells. We identified 45 AR-associated proteins in non-stimulated cells, 35 of which were not previously...
reported. Upon androgenic stimulation, the AR signalling network increased to 320 proteins, including 278 (253 were novel) that were restricted to androgen-stimulated cells. Enzalutamide treatment resulted in a loss of 259 proteins from the network when compared to stimulated cells. As expected, this reproduced quite faithfully the status of non-stimulated LAPC4 cells. Interestingly, we identified 4 potential network sensitive proteins. These are interesting targets that may be relevant for the acquisition or the prediction of Enzalutamide resistance. Hence, they could become in time alternative therapeutic targets for CRPC treatment, to be used when Enzalutamide fails.

#221 Integrated functional proteomics of MET/VEGFR inhibitors reveals complex mechanism of action of foretinib in NSCLC. Natalia J. Sumi, Bin Fang, Lily L. Rix, Muhammad Ayaz, Fumi Kinose, Eric A. Walsh, Steven A. Eschrich, Harshani R. Lawrence, John M. Koomen, Eric B. Haura, Uwe Rix. Moffitt Cancer Center, Tampa, FL.

BACKGROUND: Foretinib (FORE) and cabozantinib (CABO) are two MET/VEGFR inhibitors with similar chemical structures. CABO is FDA-approved for medullary thyroid and renal cancer; in addition, it is in clinical trials for treatment of non-small cell lung cancer (NSCLC). Through an unbiased viability screen we have observed potent cellular activity of FORE, but not CABO, in several NSCLC cell lines. We have previously shown that most NSCLC cell lines are insensitive to MET or VEGFR inhibition, suggesting off-target activity of FORE in these cells. The aim of this project is to identify the mechanism of action of FORE in NSCLC and design an optimized combination therapy. METHODS: Cellular viability assays were done using CellTiter-Glo, cell cycle analysis by flow cytometry. Western blotting was performed to evaluate the induction of apoptosis through PARP1 and caspase cleavage, as well changes in signaling. We synthesized FORE and CABO analogues and performed differential quantitative chemical and phosphoproteomics to determine the target kinase profile and pathway effects in NSCLC cells. Changes in gene expression upon drug treatment were measured by RNA-seq. RNAi in combination with pharmacological inhibition was performed to interrogate targets and pathways. RESULTS: FORE showed greater potency in NSCLC cell lines than CABO with regard to inhibition of viability and induction of apoptosis. FORE decreased phosphorylation of AKT and ERK. Chemical and phosphoproteomics revealed several kinases, such as MEK and MAP4K5, to bind preferentially by FORE over CABO that differentially affect the adherence junction and MAPK signaling pathways. Target validation showed differential inhibition of MEK1/2 and MAP4K5. Cellular validation with RNAi in combination with pharmacological inhibitors suggested that MEK1/2, MAP4K5 and IGF1R are involved in the mechanism of action of foretinib in NSCLC cells. RNA-seq pathway analysis furthermore suggested regulation of chromatin organization and Wnt pathway signaling by foretinib. CONCLUSION: Our results suggest that the difference in the efficacy between FORE and CABO is related to polypharmacology of FORE, which simultaneously targets IGF1R, MEK1/2 and MAP4K5. This difference results in divergence in signaling pathway inhibition and induces distinct effects in NSCLC. The establishment of FORE targets and signaling pathways can lead to optimized combination therapy for NSCLC and for identification of new actionable kinases in lung cancer cells.

#222 Protein network mapping of bladder cancer: tumor compartment and microenvironment analysis. Kimberly A. Hodge, Andrew Hau, Marielenia Pierobon, Donna Hansel, Emanuel Petricoin. Moffitt Cancer Center, Tampa, FL.

BACKGROUND: The bladder尤其是肿瘤-基质相互作用。膀胱癌上皮细胞和基质之间有相互作用，对肿瘤的发生和发展至关重要。我们使用了肿瘤和基质之间的信号网络来理解肿瘤的发生和发展。

#223 Targeting critical signaling nodes using multiplexed antibody based phosphopeptide enrichment with iMRM validation. Charles L. Farnsworth, Yiying Zhu, Matthew Stokes. Cell Signaling Technology, Danvers, MA.

Introduction: A challenge for biomedical researchers is to develop assays to analyze complex systems that interrogate whole cellular signaling networks. Here we show the results of employing antibody based immunoenrichment arsenal of enrichment to determine changes in phosphorylation upon kinase inhibitor treatment. Human cancer cell lines, including gastric and breast adenocarcinomas were treated with kinase inhibitors and cellular extracts were digested with trypsin and peptides were purified. A pool of phosphorylation dependent site-specific antibodies was employed to enrich for sites of phosphorylation. Following antibody based enrichment these peptides were identified and quantified using liquid chromatography/tandem mass spectrometry (LC-MS/MS). Phosphopeptides were identified using SEQUEST, differences in abundance were determined by employing label-free MS1 based quantification using Progenesis from Non-LinCear Dynamics. We also show validation data for a number of the antibodies employed using immuno–multiple reaction monitoring, or iMRM. Employing a QExactive mass Spectrometer (Thermo Scientific) we developed calibration curves for a number of the targeted tryptic phosphopeptides using combinations of heavy and light peptides with endogenous cancer cell line peptides as a background matrix. Experimental Results: Cell lines were treated with DMSO or inhibitors to which the cell lines should be sensitive/insensitive. Following peptide purification, a pool of antibodies were multiplexed and used to enrich for phosphopeptides derived from signaling proteins across a number of signaling pathways. The results show significant changes in abundance for many proteins/sites, including activation loop tyrosine residues of tyrosine kinases, changes in sites on proteins in the AKT/PDK pathway, sites in the MAPK pathway, and other critical regulators of cellular signaling such as sites on the ErbB family members. Targeted LC-MS/MS methods using iMRM were used to confirm these results and show the promise of using such targeted strategies for quantifying changes in phosphorylation consistently across many samples. Conclusion:The strategy of multiplexing antibodies for immuno-enrichment allows for simultaneous detection and quantification of hundreds to thousands of known phosphorylation sites that have been shown to be important nodes of regulation. Targeted LC-MS/MS methods such as iMRM will allow researchers to confidently profile changes in both total levels as well as phosphorylation site occupancy for critical signaling proteins.


Reliable immunodetection of proteins is a critical step in the discovery of novel biomarkers. It is often performed manually. The immunodetection protocol consists of multiple steps including blocking of nonspecific binding sites, incubation using primary and secondary antibodies and extensive washing between steps that easily introduce bias and errors. The quality of results depends on multiple subjective and objective factors such as the qualification and technical skills of the personnel performing the assay and the accuracy of temporal and temperature control, especially during the immunodetection step. BlotCycler, automated western blot processor, uses fluidic control system that allows to
eliminate the variability associated with immunodecoration and to achieve higher sensitivity by optimized washing procedure. We used blotCycler to analyze critical variables for immunodecoration including temperature, incubation time and reagent mixing. Relatively small changes in duration and temperature of incubation significantly affect not only the intensity of signal but also the specificity of antibody-antigen interactions with antigen. Automated immunodecoration at 4°C significantly improve the specificity of detection and sensitivity that allows reproducible detection of low expressing proteins. Automated processing using blotCycler allows optimization and control of critical variables leading to improved reproducibility and specificity of immunodecoration and should be routinely used for biomarker candidate discovery and confirmation, reliable protein quantification, primary antibody specificity testing, and optimization of primary and secondary antibodies concentrations.

#225 Chemoproteomic evaluation of target engagement in clinical samples. Tyzoan K. Nomanbhoy, ActivX Biosciences, Inc., La Jolla, CA.

One of the challenges during the clinical development of kinase inhibitors is understanding whether or not a compound is actually binding the kinase of interest, in the appropriate tissue, during the course of compound treatment. While compound concentration in blood can be determined from pharmacodynamics, there are very few approaches that directly measure whether or not the compound is binding the target kinase. Herein, we apply a chemoproteomics platform (KiNativ R) using a desthiobiotin ATP acyl phosphate probe (ATP probe), to monitor target engagement in clinical samples. As a proof of concept, we demonstrate that compound can be added to whole blood, after which proteins are labeled for 2 h with biotin-ATP, lysed with the ATP probe, and analyzed by mass spectrometry to determine whether or not compound treatment blocks the probe-labeling of the target. We successfully applied this approach in the analysis of both the reversible JNK inhibitor CC-930, as well as the covalent BTK inhibitor Brutinib (Imbruvica®). We then extended the study to monitor the inhibition of BTK by Brutinib in PBMCs isolated from patients undergoing drug treatment. Finally, we demonstrate that in addition to profiling kinases in PBMCs isolated from whole blood, this method can also be used to profile kinases in solid tumors. Thus, the chemoproteomics approach described here could be applied as a general method to monitor target engagement for inhibitors developed against a variety of different kinases in clinical samples.

#226 Verification of prostate cancer genetics biomarker candidates at protein level using PRISM-SRM. Hui Wang,1 Yuqian Gao,1 Athena Schepmoes,1 Gyorgy Petrovics,2 Jennifer Cullen,2 Thomas Fillmore,2 Tujio Shi,1 Wei-Jun Qian,1 Richard Smith,2 Brandi Weaver,1 Robin Leach,1 Ian Thompson,1 Sudhir Srivastava,1 Jacob Kagan,2 Albert Dobi,1 Karin Rodland,1 Shiv Srivastava,1 Tao Liu,1 Pacific Northwest National Laboratory, Richland, WA; University of the Sciences University of the Health Sciences, Bethesda, MD; University of Texas Health Science Center at San Antonio, San Antonio, TX; National Cancer Institute, Bethesda, MD.

Mass spectrometry (MS) based targeted proteomics such as selected reaction monitoring (SRM) provides an antibody-independent strategy for sensitive, specific and multiplexed verification of genetics biomarker candidates at the protein level. In order to identify a panel of proteins with the potential to discriminate between aggressive and indolent forms of prostate cancer and predict prostate cancer progression, we have selected 52 protein candidates from existing prostate cancer genetics data sets and validated cancer drivers, and performed quantitative proteomics analysis in tumor and control tissue samples using the highly sensitive PRISM (high-pressure, high-resolution separations coupled with intelligent selection and multiplexing)-SRM approach. PRISM-SRM assays have been developed for the 52 prostate cancer biomarker candidates including: prostate cancer progression associated genes, prostate cancer associated genes that were up-regulated in transcriptions studies, and other cancer-related genes (including the ERG or ETV1 isoforms). Two sets of tissue samples were analyzed using PRISM-SRM with heavy isotope-labeled synthetic peptides as internal standards: 1) 10 high Gleason-score (7-9) primary prostate tumors and 10 benign prostate hyperplasia (BPH) tissues (OCT-embedded specimens); and 2) 10 primary tumors from patients showing metastatic progression, 10 primary tumors from patients who showed biochemical recurrence (BCR), and 10 primary tumors from patients with no BCR or metastatic progression after more than 10 years of follow-up after radical prostatectomy (FFPE whole mount prostate specimens). Overall, PRISM-SRM analyses of all the patient tissue samples enabled the detection of 48 out of 52 biomarker candidates, suggesting extremely low level of expression of the remaining 6 genes (HXC6, OSTP, TWST1, and ERG8); in comparison regular LC-SRM can only detect 21 of these candidates at the protein level. In the 10 high Gleason-score tumors and 10 BPH controls, 13 proteins were found differentially abundant with P<0.05. In the 10X10X10 FFPE sample analysis, there were three proteins discriminating between “metastatic progression” and “no progression” tumors, one protein discriminated between BCR and “no progression” tumors, and four proteins discriminated between metastatic progression and BCR tumors showing a trend towards differences between metastatic and BCR tumors. These results will be further evaluated, individually and in panels, in independent, larger cohort for their potential prognostic applications. In summary, PRISM-SRM provides a highly sensitive method for quantification and rapid screening of multiple potential biomarker candidates at the protein level. This approach holds great potential for rapidly translating genomics-based discovery candidates into protein-based biomarkers.

#227 Mass spectrometry-based proteomics analysis of the non-small lung cancer secreteme. Emmanuel K. Cudjoe, Tareq Saleh, David A. Gewirtz, Adam M. Hawkridge. Virginia Commonwealth University, Richmond, VA.

Non-small cell lung cancer (NSCLC) is the more common subtype (~80%) of lung cancer, a leading cause of cancer death worldwide. NSCLC has one of the lowest 5-year relative survival rates due to a combination of late stage diagnosis and treatment failure. Autophagy, a stress response mechanism in which cancer cells recycle organelles, and proteins to generate the necessary nutrients and metabolic intermediates for survival, is thought to be one of the principal mechanisms of treatment resistance and relapse in cancer. Due to its relatively refractory nature to chemotherapy and radiation, long-term goals is to identify molecular signatures and pathways of autophagy that can be targeted to improve the sensitivity of NSCLC to chemotherapy and radiation treatments. As a function of p53 expression, irradiation and functional autophagy status in NSCLC will be discussed. The novel functional and potential diagnostic value of these differentially secreted proteins for distinguishing between the autophagic responses of NSCLC to IR. The novel functional and potential diagnostic value of these differentially secreted proteins for distinguishing between the autophagic responses of NSCLC to IR. The novel functional and potential diagnostic value of these differentially secreted proteins for distinguishing between the autophagic responses of NSCLC to IR.
cancer (PCa) and 35 with CRPC, were measured. Results: The proteome analysis identified 12 candidates of secreted cell membrane proteins as new biomarkers. The proteome analysis indicated that not only matured GDF15, but pro-peptide as well as fragments (GDPP) are released from prostate cancer cells. Patients’ serum was analyzed for mature and pro-peptide GDF15 using ELISA and immunomunoprecipitation mass spectrometry. The results showed that the secreted level of GDPP-1, one of the processing forms of GDPP, was significantly higher in CRPC than those in BPH and untreated PCa (P<0.01). ROC analysis also showed that the AUC of GDPP-1(0.86) was higher than that of matured GDF15 (0.76). When the cutoff value of GDPP-1 was set at 4.0 ng/mL, there was also showed that the AUC of GDPP-1(0.86) was higher than that of matured GDF15 (0.76). When the cutoff value of GDPP-1 was set at 4.0 ng/mL, there was a significant difference of overall survival (OS) in CRPC patients between those with more than 4.0 ng/mL compared to those with less than 4.0 ng/mL of GDPP-1, whereas there was no significant difference of OS measurable by PSA in CRPC patients. These data suggest that GDPP-1 may be a novel biomarker for CRPC. Conclusion: GDPP-1 shows potential as a novel biomarker for CRPC.

#229 Establishing reference intervals of human urine proteome for monitoring physiological and pathological changes. Wenchuan Leng,1 Xiaotian Ni,2 Changqing Sun,3 Anna Malovannaya,4 Yi Wang, Jun Qin.1 National Center for Protein Sciences (The PHOENIX Center, Beijing), Beijing, China; 2 East China Normal University, Shanghai, China; 3 Tianjin Baodi Hospital, Tianjin, China; 4 Baylor College of Medicine, Houston, TX.

Urine as a true non-invasive sampling source holds great potential for biomarker discovery. But the lack of method for profiling urine proteome in high throughput and systematic evaluation of variations in urine proteomes based on large number of population have been the two major obstacles for finding new biomarkers from urine. Due to low throughput, only limited number of urine samples can be measured in discovery phase of biomarker studies, which make it hard to determine whether proteins differentially expressed between groups represent actual differences between control and disease states or just physiological variations among individuals. So, candidate biomarkers often fail in validation phase. Here, we report a streamlined workflow with capacity of measuring 8 urine proteomes per day per MS at the coverage of more than 1500 proteins. With this workflow, we systematically evaluated variations in 497 human urine proteomes from 167 apparently healthy donors, allowing us to evaluate day to day and inter-personal variation in human urine proteome. Then personal and pan-human reference intervals (RIs) of urine proteome were established based on this large-scale dataset. We demonstrated that RIs can be used to monitor physiological changes by detecting transient outlier proteins, such as trans-continental travel and common flu. And it was also found that if the underlying cause is physiological variation, outlier proteins will fall back to the normal range in the follow up measurement, as exemplified in the intercontinental travel case. Persistent outlier proteins may be indicative of non-physiological conditions. These results indicate that periodical measurements of a person’s urine proteome could establish a personal health archive that would be valuable for detecting future health issues. Furthermore, we proposed a complete novel strategy dependent on RIs-based algorithm for biomarker discovery and validation to screen for diseases, which were exemplified by analyzing 154 urine proteomes from patients with 7 types of cancers. The algorithm can distinguish normal people from cancer patients with specificity of 95% and sensitivity of 85%. This study paves a way to use urine proteome for health monitoring and disease screening.

#230 Proteomic approaches in the discovery of novel drug targets or potential biomarkers in breast cancer. Ioanna-Maria Orfanou, Theodoros Karampelas, George Mermelkas, Konstantinos Vougas, Constantin Tamvakopoulos. Biomedical Research Foundation Academy of Athens, Athens, Greece.

The aim of this study is the detection, identification and quantification of known and new candidate cell membrane receptors that are overexpressed in Breast Cancer (BC) cells. In order to achieve this goal we have developed State-of-the-Art Proteomic approaches. These novel membrane target receptors can be used either as biomarkers or for the design of targeted drugs against aggressive disease subtypes with poor prognosis and therapeutic outcomes, such as Triple Negative BC (TNBC) and HER2 overexpression BC. For our study, four well characterized BC epithelial cell lines were selected: HCC-1954 and SKBR3 (HER2 overexpressing), MDA-MB-231 (TNBC) and MCF-10A (benign control). We employed a combination of subcellular fractionation and membrane enrichment protocols and combined those with quantitative, tandem Mass-Spectrometry (MS) based Proteomics. The discovery phase of our approach included: the GelC-MS/MS technique, where protein fractions were first separated by 1D gel electrophoresis followed by In-Gel enzymatic digestion prior to identification by a high resolution Orbitrap mass analyser. A complementary approach for known proteins associated with BC that cannot be readily detected in the discovery phase, such as GPCR receptors, was based on nano LC-MS/MS analysis. Proof of Concept experiments, with the use of a Triple Quadrupole (QqQ) instrument, were based on the detection of standard peptides derived from the protein of interest after following an in solution trypptic digestion protocol. Using GelC-MS/MS approach we identified the well-known BC involved receptors, EGFR and HER2, as well as other potential protein targets in concordance with the literature (e.g. TFR1, EPHA2, GPCR5A). We further confirmed these results by Western blot analysis. Certain GPCRs expected to be present in the cell lines tested were not detected in the studies used on the GelC-MS/MS analysis, thus we proceeded to the more sensitive and quantitative nanoLC-MRM approach. As a paradigm target we chose the GnRH receptor, an established target known to be expressed in BC. The quantitative nanoLC-MRM approach revealed GnRHR significant expression in the MDA-MB-231 BC line and in the WPE-NB26-3 Prostate cancer cell line (used as an overexpressing GNRHR positive control). Our results indicate that the strategy of combining and applying various Proteomic approaches into selected cell lines for the identification of new protein targets will add more information to the genetic and histological classification of the tumor in order to achieve our goal, which is a holistic view of a patient’s molecular profiling that can ultimately lead to treatment in the most effective way.

PREVENTION RESEARCH: Diet and Cancer

#231 The polyphenolic plant lignan secoisolaricresinol diglucoside reduces mammary tumor growth, possibly via inhibition of local inflammatory signaling. Claire G. Lineberger,1 Laura W. Bowers,2 Nikki A. Ford,3 Emily L. Rossi,1 Bruce K. Kimler,2 Carol J. Fabian,2 Stephen D. Hursting1. 1The University of North Carolina at Chapel Hill, Chapel Hill, NC; 2The University of North Carolina at Chapel Hill, Austin, TX; 3The University of Kansas Medical Center, Kansas City, KS.

Background: Secoisolariciresinol diglucoside (SDG) is a polyphenolic plant lignan found in flax and sesame seeds as well as legumes, whole grains, fruits and vegetables. It is metabolized by the gut bacteria into two major enterolignans: enterolactone (E NL) and enterodiol (END). These enterolignans have been associated with reduced breast cancer risk and progression in population studies as well as decreased tumor growth in preclinical models of breast cancer. Methods: The impact of SDG supplementation on tumor growth in a mouse model of basal-like breast cancer was examined. C57BL/6 mice were fed a control diet (10% kcal from fat) or control diet with SDG supplementation (100 mg/kg food) for eight weeks, then both groups were orthotopically injected with E0771 mammary tumor cells. An inflammatory signaling qPCR array (Qiagen) was performed on mammary tissue distal to tumor. Tumors were stained by immunohistochemistry (IHC) for Ki67 to measure proliferation levels and phospho-p65 to determine inflammatory signaling pathway activation. Tumors and mammary tissue were also stained for F4/80 to quantify macrophage infiltration. Serum level of hormones, adipokines, and cytokines were measured via lumiex assay (Bio-Rad). Results: SDG supplementation significantly decreased tumor weight (p<0.05). SDG did not affect body weight or body fat percentage but did significantly decrease expression of F4/80, CRP, and other pro-inflammatory markers in the mammary tissue. IHC staining revealed no difference in tumor proliferation; however, SDG supplementation did reduce inflammatory signaling in the tumors, indicated by a significant decrease in phospho-p65 staining. However, serum cytokine levels were not significantly different between the groups. Tumors are currently being stained by IHC for cleaved caspase 3 to measure levels of apoptosis. In addition, cell culture experiments will be conducted to define the impact of ENL treatment on protumorigenic cross-talk between tumor cells, adipocytes, and macrophages. Specifically, the effects of conditioned media from adipocyte/macrophage co-cultures (with +/- ENL treatment) on mouse E0771 and human MDA-MB-231 tumor cell proliferation, migration and invasion will be examined. Conclusions: SDG supplementation reduced mammary tumor growth in association with SDG’s effects on local, but not systemic, inflammatory signaling.

#232 Modification of dietary sugar on the chemotherapeutic potential in breast cancer. Yan Jiang, Pattrea R. Rhea, Lorenzo Cohen, Peiyiing Yang. UT MD Anderson Cancer Ctr., Houston, TX.

Background: The estimated cost of breast cancer (BCa) care in the U.S. is over 16 billion dollars yearly, more than any other cancer. Thus, identifying contributors to BCa development and barriers in BCa treatment is a continuing focus of BCa research. The per capita consumption of sugar in Americans has surged to...
The impact of omega-3 fatty acid supplementation on obesity-induced inflammatory signaling within the breast tumor microenvironment. Duan K. Quach,1 Brittany Harlow,2 Laura Wininka,3 Andrew Brenner,4 Murali Beoram,5 Stefano Tiziani,6 Lucy Longfelder,1 Gloria Galvan,1 Christopher Jolly,1 Produced inflammatory signaling within the breast tumor microenvironment. Apoptotic gene array and microarray analysis were used to determine gene expression profile. Results: Among six human BCa cells tested, a glucose treatment (30 mM) promoted the growth of MDA-MB-231 and SUM159 cells after 10 days by 2.5 to 3 fold, respectively. In contrast, T47D, BT474, MDA-MB-468 and MCF-7 cells did not respond to glucose stimulation. Glucose treatment abrogated doxorubicin-induced cell apoptosis in triple negative Aga-6 and omega-3 fatty acids at ratios of 46:1, 20:1, 10:1 and 1.3:1 for 24 hours. The V4 region of the 16S rRNA gene was sequenced using the HiSeq 2500 system. Alpha diversity measures including number of OTU (operational taxonomic units) and Shannon index were calculated for each sample. Mixed ANOVA models with a random effect accounting for twin pair status, were used to examine the association of alpha diversity with polyp status. Results: Alpha diversity measured by Shannon index and number of unique OTUs was inversely associated with obesity and high beef consumption and positively associated with yogurt consumption. Surprisingly, we found that fecal microbiota alpha diversity was higher in subjects with adenomas and hyperplastic polyps compared to subjects with no polyps. When restricted to subjects with colonoscopy in the 3 years prior to stool collection, differences were stronger and statistically significant (mean OTUs for subjects with adenoma, hyperplastic polyps and no polyps = 908, 850, and 801, respectively; Padenoma vs. no = 0.017, Phyperplastic vs no = 0.007; Plinear trend = 0.018). In order to determine whether diet changes may have played a role, we examined dietary change from food frequency questionnaires collected 15 years apart. We found that subjects with no polyps increased yogurt consumption by 50% over the 15 year period, whereas subjects diagnosed with adenomas and hyperplastic polyps increased their yogurt consumption more (140% and 130%, respectively). Conclusion: Participants diagnosed with adenomas and hyperplastic polyps had higher fecal microbiota alpha diversity compared to those without polyps. The timing of measurement of fecal microbiota years after colonoscopy obscures the causal relationship between adenoma and fecal microbiome. One possibility is that subjects may have increased their yogurt consumption after a polyp diagnosis, resulting in increased fecal alpha diversity, compared to subjects who were not diagnosed with polyps. Alternatively, polyp removal may alter microbial diversity. A third possibility is that polyp susceptibility is associated with an outgrowth of deleterious bacteria, still present after polyp removal, reflected as increased alpha diversity. Twin comparisons will be presented.

#235 Probiotics ameliorate Porphyromonas gingivalis-promoted pancreatic cancer progression in oncogenic Kras transgenic mice. Ming-Shiou Jan,1,2 Wan-Ting Chen,1 Yu-Jen Chen,1 Chia-Wei Lin,3 Wen-Wei Chang,1 Chung-Hung Tsai,1 Jia-Shiou Peng,2 Li-Jin Hsu3. The University of Texas at Austin, Austin, TX;1 UT Health San Antonio MD Anderson, San Antonio, TX;2 The START Center for Cancer Care, San Antonio, TX.

Periodontal disease has been shown to play important roles in the pathogenesis of many chronic diseases, such as cancer. Periodontitis is a chronic inflammatory disease affecting the tissues that support the teeth, and is associated with increased risks of several systemic diseases, including diabetes, cardiovascular disease, and cancer. The mechanisms underlying these links are not fully understood, but gut microbiota and diet appear to play a role.

Periodontitis results in chronic inflammation, which can lead to the development of periodontal disease. In turn, periodontal disease can contribute to the progression of cancer. This is because the inflammatory response associated with periodontal disease can stimulate the growth of tumors, promote angiogenesis, and suppress the immune system, all of which can facilitate cancer progression.

In a recent study, researchers investigated the role of probiotics in preventing the progression of pancreatic cancer induced by Porphyromonas gingivalis in oncogenic Kras transgenic mice. They found that probiotics were able to reduce the incidence of pancreatic tumors and improve survival rates in these mice. This study provides evidence that probiotics may have potential as a therapeutic strategy for preventing the progression of pancreatic cancer.

Conclusion: Probiotics may have potential as a therapeutic strategy for preventing the progression of pancreatic cancer.
oral-smear of P. gingivalis. These probiotic strains have been demonstrated to have the efficacy of either anti-inflammatory or enhancement of cell-mediated immunity, respectively. Our results demonstrated that oral administration of the probiotics significantly suppressed oncogenic Kras-induced pancreatic cancer development and reversed P. gingivalis-stimulated cancer deterioration in mice. Stronger expression of EMT markers and pancreatic cancer tissues was observed in Kras12461; Pdx-1-Cre transgenic mice treated with the probiotics. Our results suggest that the microbiota profile is very important for pancreatic cancer development. The manipulation of microbiota for enhancing antitumor immunity may be an emerging strategy for cancer prevention.


Preclinical and epidemiological data suggest that a unique feature of lipogenesis in cancer cells which has received limited attention is the accumulation of monounsaturated fatty acids (MUFA) which are largely derived from saturated fatty acids (SFA) by the action of stearyl-CoA desaturase-1 (SCD-1). Activation of SCD-1, a delta-9 fatty acid desaturase, is considered to be an important factor in the development of obesity and several types of cancer including breast cancer. However, no data are available on how changes in SCD-1 activity induced by potential chemopreventive agents relate to established biomarkers of breast cancer risk. To address this issue, we measured the activity of SCD-1, expressed as the ratio of palmitoleic acid (C16:1n7) to palmitic acid (C16:0) (SCD-16) and oleic acid (C18:1n9) to stearic acid (C18:0) (SCD-18) in plasma samples of post-menopausal women enrolled in our recently published clinical trial (Sandhu N, et al Cancer Prev Res 9:275, 2016) designed to test the individual and combined effect of the antiestrogenRaloxifene and the omega-3 preparation Lovaza on breast density, a validated biomarker of breast cancer risk. We observed that daily administration of Lovaza (1.860 mg eicosapentaenoic [EPA] + 1.500 mg docosahexaenoic [DHA]) significantly reduced SCD-1 activity, an effect which was sustained for the two-year duration of the trial. Raloxifene, on the other hand, did not significantly alter SCD-1 activity in our subjects. SCD-1 activity was positively correlated with BMI (for SCD-16, r=0.45, p<0.01; for SCD-18, r=0.23, p<0.01) and paralleled changes in BMI in the same direction over the two years of the study. These findings support the role of this enzyme in the development of obesity. Importantly, decreasing levels of SCD-1 were found to be associated with a progressive reduction in breast density in obese women (BMI≥30) (for SCD-16: r=0.47, p<0.01; for SCD-18: r=0.36, p<0.05). No correlation between breast density and SCD-1 was observed in non-obese subjects (r=-0.02 for SCD-16 and 0.04 for SCD-18). Our results suggest that BMI-related factors play an important role in the reduction of breast density by omega-3 fatty acids. They also indicate that SCD-1 may be a useful biomarker in future clinical trials testing the benefit of nutritional interventions in reducing obesity associated breast cancer risk.

#237 Increased NSCLC tumorigenesis in mice fed a high fat diet is associated with increased plasma IGF-1 levels and PD-1 expression in CD4+ tumor-infiltrating lymphocytes. Regan M. Memmott,1 Krista Pearman,2 Joell Gillis,3 Tony Tullo,2 Valerie Wong,2 Benjamin Singer,3 Kristin Lastwika,3 Franco D’Alessio,3 Phillip Dennis,2 Jeffrey William Norris,2 1University of Arizona Phoenix, Phoenix, AZ; 2Midwestern Univ. - Glendale Campus, Glendale, AZ; 3Johns Hopkins Medical Institute, Baltimore, MD; 4Northwestern University, Evanston, IL, 5Fred Hutchinson Cancer Research Institute, Seattle, WA; 6Johns Hopkins University - School of Medicine, Baltimore, MD; 7Astra-Zeneca, MD.

Lung cancer is the leading cause of cancer-related mortality worldwide, and 85% of lung cancer cases are associated with tobacco use. Activating mutations in K-Ras have been identified in ~25% of tobacco-associated lung adenocarcinomas. Using mouse models of K-ras-driven lung tumorigenesis, we previously demonstrated that deletion of the IGF-1 gene or reduction of systemic IGF-1 levels using the anti-diabetic drug metformin markedly reduced tumor burden. Preclinical and clinical studies suggest that diet composition is the best predictor of IGF-1 levels. Therefore, we hypothesized that diets high in fat or carbohydrate would promote lung tumorigenesis by increasing systemic IGF-1 levels. To assess the effect of diet on systemic IGF-1 levels, 9 week old C57Bl/6j and A/J mice were fed standard cereal, high-carbohydrate, or high-fat (HFD) diets for 12 weeks. Compared to control feed control mice, plasma IGF-1 and insulin levels were increased in both strains of mice fed a HFD, but not in mice fed a diet high in carbohydrate. This was not due to obesity, as only the C57Bl/6j mice fed a HFD had an increase in body weight. We then investigated the effect of HFD on lung tumorigenesis using two mouse models. In the first, C57Bl/6j 1245-124 mice, which harbor a mutation in K-ras, were fed either cereal diet or HFD for 10 weeks following weaning. Lung tumor burden in the mice fed HFD was increased 2.7-fold compared to littermates fed cereal diet. In the second model, the tobacco carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1 butanone (NNK) was given by intraperitoneal injection to A/J mice beginning at 6 weeks of age. This carcinogen causes lung tumor development by inducing K-ras mutations. After 3 weekly injections of NNK, the mice were randomized to cereal diet or HFD for ten weeks. Mice fed HFD had a 60% increase in lung tumor burden. In both models, there was no relationship between the weight of the mice and lung tumor burden. Immunohistochemical analysis of the proliferation marker Ki-67 showed no significant difference in expression between tumors from mice fed a HFD or a cereal diet. Therefore, we evaluated tumor-infiltrating lymphocytes (TIL) from mice on both diets. Interestingly, both immunohistochemical analysis and flow cytometry demonstrated a 50% reduction in the number of TIL in mice fed HFD compared to mice fed a cereal diet. Additionally, HFD was associated with a 2-fold increase in CD4+ TIL, and the CD-4 staining intensity in these lymphocytes was significantly greater than in mice fed a cereal diet. These results may suggest that a high fat diet increases lung tumorigenesis by increasing systemic IGF-1 levels and by creating an immune-permissive tumor microenvironment.

#238 Evidence of early colorectal cancer risk and prevention pathways in the fecal microbiome of colonoscopy patients: associations with diet and circulating adipocytokines. Carrie R. Daniel,1 Kristi L. Hoffman,2 G S. Raju,3 Samir M. Hanash,1 Diane S. Hutchinson,2 Nadim J. Ajami,3 Richard G. Fowler,1 glendale, AZ; 2Midwestern Univ. - Glendale Campus, Glendale, AZ; 3Fred Hutchinson Cancer Research Institute, Seattle, WA; 4Johns Hopkins University - School of Medicine, Baltimore, MD; 5Astra-Zeneca, MD.

Accumulating evidence suggests that the gut microbiome’s role in early colorectal cancer etiology extends beyond the pro-carcinogenic activities of specific pathogens and is largely influenced by the wider microbial community of commensal bacteria. To identify early microbiome-related pathways and potential dietary intervention targets, we conducted an epidemiologic study among cancer-free colonoscopy patients at known and varied risk of colorectal neoplasia. Sporadic patients undergoing colonoscopy screening provided consent and fasting blood. Polyps, if found, were removed at colonoscopy and ~1 month later (prior studies show the effect of the colonoscopy prep dissipates within this time period), eligible patients provided a stool sample via mail kit (n=47). Patients completed comprehensive dietary assessments and clinicopathologic factors, including screening history, were abstracted from electronic medical records. We characterized the fecal microbiome via whole genome shotgun sequencing (Illumina HiSeq) and circulating blood adipocytokines via Luminex multiplex assays (Millipore). Majority of the patients were female and recently diagnosed with precancerous polyps, primarily tubular and sessile serrated adenomas. Polyp patients were more likely to be obese and to not consume alcohol, but otherwise similar by age, diet composition, and other risk factors to patients with normal colonoscopy findings. We observed no effects of time interval between colonoscopy and fecal sample collection, or of recent polyp diagnosis/removal, on microbial alpha or beta diversity. Correlation networks between species revealed that Fecalibacterium and Roseburia hominis, known butyrate-producers. Among recurrent patients (n=14), Bacteroides fragilis co-occurred with Bilophila wadsworthia (r=0.6; P=0.02). Higher intake of fiber and/or overall diet quality, as defined by the Healthy Eating Index, was associated with several bacteria largely linked to butyrate production (e.g., Bifidobacterium animalis, F. prausnitzii, Roseburia intestinalis, Coprococcus eutactus, Eubacterium eligens). These same dietary factors were inversely correlated with Blautia hydrogenotropha, an acetogen, and other bacteria implicated in inflammation and colorectal cancer (e.g., B. fragilis). Biologically plausible associations between microbiota composition and potential pathways important to cancer risk (e.g., one-carbon, lysine, carbohydrate and fatty acid metabolism) with dietary factors and circulating adipocytokines involved in immunity, inflammation, and glucose metabolism support the functionality of these diet-microbe relationships.

#239 Associations of polysaturated fat, bile acids, and gut microbiota on colorectal adenoma risk. Tengteng Wang, Nicole M. Brown, Amber N. McCarthy, Robert S. Sandler, Temitope O. Keku. University of North Carolina at Chapel Hill, Chapel Hill, NC.

Background: Intestinal microbiota, and their metabolites are increasingly recognized as important players in the genesis of colorectal adenomas and cancer. Epidemiological studies have also shown that polysaturated fat intake is associated with
increased risk of colorectal adenomas. This study examined the association between adherent gut microbiota and mucosal bile acids, and total polyunsaturated fat intake in relation to colorectal adenomas in a case-control study. Methods: Participants were 217 adenoma cases and 218 adenoma-free controls who underwent screening colonoscopies and completed food frequency questionnaires. The mucosal adherent microbiota was characterized by bacterial 16S rRNA sequencing. Global metabolomics of colonic mucosal tissue was conducted by gas chromatography and mass spectrometry on a subset of samples. Genus level microbiota and bile acids were compared between cases and controls using t-test with correction for multiple comparison. Logistic regression and Pearson correlations were used to estimate adjusted odds ratios (ORs) and 95% confidence intervals (CIs), and correlation coefficients to assess the association among microbiota abundance, bile acids concentration, and total polyunsaturated fat intake. Results: Adenoma subjects had a significantly higher bacterial abundance of Sphingomonas, Marinomonas and Ralstonia, but lower abundance of Adlercreutzia compared to controls. The concentration of eleven bile acids (Chenodeoxycholate, Cholate, Deoxycholate, Glycochenodeoxycholate, Glycocholate, sulfated 7-ketodeoxycholate, 12-dehydrocholate, Tauroch- nodeoxycholate, Taurodeoxycholate, Taurinoholate 3-sulfate, and Tauroursodeoxycholate) were significantly elevated in cases compared to controls. Subjects with increased abundance of Sphingomonas and Ralstonia were significantly more likely to have adenomas (OR 2.17, 95% CI 1.31-3.57; OR 1.19, 95% CI 1.02-1.38) respectively. Subjects with increased abundance of Adlercreutzia were less likely to have adenomas (OR 0.44, 95% CI 0.21-0.93). The multivariable adjusted OR for high consumption of Adlercreutzia relative to the reference category adjusted for age, sex, race, and BMI was 0.46 (95% CI 0.27-0.80). The correlations between bacterial diversity and eleven bile acids were also significantly differ by control status. Conclusion: The interaction of the gut microbiota, bile acids and consumption of polyunsaturated fat are associated with colorectal adenoma risk.

#240 Dietary patterns and risk of colorectal neoplasia in Puerto Rican Hispanics: A case control study. Jylyann Perez-Mayoral,1 Sachely Julian,1 Marievieslle Soto-Salgado,2 Michael J. González,4 Marcia Cruz-Correia,4 University of Puerto Rico Comprehensive Cancer Center, San Juan, PR;2 University of Puerto Rico Medical Sciences Campus, San Juan, PR.

Colorectal cancer (CRC) is the leading cause of cancer death in Puerto Rico and the second cause of death in the US. It is well accepted that modifiable lifestyle habits such as diet, contribute to CRC development. Diet could reduce up to 70% of CRC cases. Dietary patterns vary according to race/ethnicity. Puerto Rican Hispanics (PRH) have been shown to have a distinct diet compared to US non-Hispanic Whites (NHW). According to the BRFFS, only 17.7% of PRH and 23.4% of NHW consume fruit and vegetables five times per day. The objective of this study was to describe the association of specific food groups consumption with the risk of colorectal neoplasia (CRN; polyps and/or CRC) in PRH. The validated Colon Cancer Family Registry food frequency questionnaire was administered to healthy individuals (controls) and individuals with CRN (cases) recruited through the Puerto Rico Colorectal Cancer Registry (PURIFICAR). Models adjusted by age, gender, educational attainment and family history of CRC were fitted to estimate the odds ratios (OR) with 95% confidence intervals (CI) through a polytomous logistic regression models comparing cases and controls. A total of 577 subjects were included in the study (controls=254; polyps=50; CRC=273). The frequency of consumption of fruits, low fat dairy products, eggs, pasta/rice, non-fried chicken, and seafood were significantly different between controls and CRN cases (p<0.05). Adjusted models showed that a higher consumption of fruits (OR[p<0.05] 0.29), and full-fat dairy products (OR[p<0.05] 0.22) were associated with decreased risk of polyps (p<0.05). A frequency of consumption of 2-4 times per week, when compared to ≤1 per week, of full-fat dairy products (OR[4 times per week=0.51], seafood (OR[4 times per week=0.50]), and red meats (OR[4 times per week=0.56]) was associated with a decreased risk of CRC (p<0.05). Our results showed that a higher consumption of fruits and full-fat dairy products significantly reduce the risk of polyps in PRH. In addition, consumption of seafood, full-fat dairy, and red meats up to 4 times per week may reduce the risk of CRC in PRH. Educational interventions for cancer prevention in PRH should incorporate nutritional information tailor to our community.

#241 Vimentin offers a potential target for the prevention of ovarian tumor progression and recurrence. Aparna Yellapa,1 Janice M. Bahr,7 Sameer Sharma,1' Pincas Bitterman,2 Sanjib Basu,2 Animesh Barua1.1 Rush University Medical Center, Chicago, IL;2 University of Illinois at Urbana-Champaign, Urbana-Champaign, IL.

Background: Aggressive rates of progression and frequent recurrence are the main causes of high rates of death due to ovarian cancer (OVCA). Vimentin, an intermediate filament protein, expressed by ovarian mesenchymal cells may be involved in OVCA metastasis and recurrence. Ashwagandha (Withania somnifera, ASH, a natural product) has shown to improve tumor-associated stress against several malignancies. Objectives: The goals of this study were to examine whether (1) OVCA progression is associated with vimentin expression; and whether (2) OVCA progression and vimentin expression can be prevented by dietary supplementation of Ashwagandha (ASH) in laying hen model of spontaneous OVCA. Methods: Clinical study: Clinical trial on laying hens with normal ovaries (n=10), benign tumors (n=5) and malignant ovarian epithelial cancers at early (n=7) and late stages (n=16) were collected from patients and examined for vimentin expression. Prospective study: 4-year old laying hens with normal ovaries or ovaries with early stage OVCA were supplemented with 2% dietary ASH root powder or basal diet (24 hens in each group containing 18 healthy, 6 with early stage OVCA). Hens were provided with feed and water ad libitum for 120 days and euthanized thereafter. Tumor stages were recorded; serum, normal or cancer ovaries were collected. Expression of vimentin both in clinical and preclinical treated or untreated specimens was examined. Statistical differences in the intensity of vimentin expression among different pathological and ASH supplemented groups were determined using ANOVA and t-tests. Results: Vimentin was localized in the nucleus and cytoplasm of normal ovarian, benign and malignant cells. Compared with normal and benign tumors, the intensity of vimentin expression was significantly lower (P<0.0001) in early stage OVCA and increased remarkably in late stage OVCA. Similar to OVCA patients, intensity of vimentin expression was lower in hens with early OVCA than normal hens, and increased significantly (P<0.0001) in hens with late stage OVCA. As compared to healthy hens, tumor incidence and progression as well as vimentin expression decreased significantly in hens treated with 2% ASH. Conclusions: Results of the present study suggest that vimentin expression increased during tumor metastasis. ASH treatment reduced OVCA metastasis by inhibiting vimentin expression. Therefore, vimentin may represent a potential target for ASH to prevent OVCA progression and metastasis. Support: Support: R21CA187309-02.

#242 The anti-tumor effect and the impact on gut microbiota of gynepenosides Rd and Rb3 in ApoMin/+ mice. W.L. Wendy Hsiao,1 Guoxin Huang,1 Imran Khan,1 Lei Chen,1 Xiaoang Li1. 'State Key Lab of Quality Research in Chinese Medicine, Macau Univ of Sci. & Tech, Macau; China;2 Rutgers University, Piscataway, NJ.

Gut commensal microbiota (GM) is closely associated with health and diseases, including cancer. In our previous studies, we have demonstrated that Gynostemma pentaphyllum saponins (GPs) exert antitumor effects in both athymic and ApoMin/+ mice. Our recent study also showed that GPs exhibited prebiotic effect by up regulating the beneficial bacteria and improving the epithelial microenvironment of the gut. Gypenoside IV (Rb3) and gypenoside VII (Rd) are the two constituents of GPs. The Rd exhibits stronger antitumor effect than Rb3 in an in vitro cell model system established in our lab. Our aim is to confirm the antitumor effect of these two compounds, as well as their impact on GM in the ApoMin/+ mouse model. In the study, single daily dose of Rb3 or Rd at 20mg/kg, and solvent control were orally fed to the mice for 8 weeks, started at 6 weeks of age before the appearance of spontaneous intestinal polyps. The fecal microbiome was analyzed by enterobacterial repetitive intergenic consensus (ERIC)-PCR and 16S rRNA gene pyrosequencing technique. We found that Rd was more effective than Rb3 in reducing polyp formation, and improving the intestinal lining by up-regulating anti-inflammatory cytokines IL-4 and IL-10. Rd, not Rb3, might facilitate intestinal tissue repair by enhancing M2 marker while suppressing M1 markers expression. Moreover, Rd treatment greatly enhanced the ratio of Bacteroides vis Firmicutes, while profoundly reduced the abundance of cancer-cachexia-associated bacteria, such as Parabacteroides goldsteinii (22.5%), Dysgonomonas wimpennyi (48.5%) and Blautia cocoides (36.77%). These findings suggested that the level of antitumor effect of the gypenosides might be correlated with its ability to modulate the gut microbiota from dysbiosis to symbiosis. This study was supported by Macau Science and Technology Development Fund 015/2014/A1.

#243 Diet and exercise-induced weight maintenance, alone and in combination with a whole tumor cell vaccine, delays mammary tumor growth and reduces MDSC accumulation. Yitong Xu, William J. Turbitt, Andrea M. Mastro, Connie J. Rogers. Pennsylvania State University, University Park, PA.

Obesity and physical inactivity increase breast cancer risk, while the prevention of weight gain by diet and exercise can be protective. Numerous biological mechanisms have been proposed to explain the beneficial effects of weight maintenance, however few studies have examined the immune response to energy balance. We have previously shown that diet and exercise-induced weight maintenance (WM) achieved via a 10% restriction in calories and access to voluntary running wheels in combination with a whole tumor cell vaccine (VAX) significantly reduced mammary tumors. The present study extends this work to evaluate tumor growth progression after six months of WM and VAX treatment, alone and in combination. Mice were randomly assigned to four experimental groups: 1) WM, 2) WM + VAX, 3) diet and exercise-induced weight gain (WNG), and 4) diet and exercise-induced weight gain + VAX. A tumor cell vaccine (VAX) that had previously been shown to have antitumor effects in our mouse model was used to treat established tumors. This study was conducted to determine whether dietary and exercise-induced WM delays tumor progression, and whether VAX treatment delays tumor progression. The present study extends previous work by evaluating diet and exercise-induced WM, alone and in combination with VAX, in a mouse model where dietary and exercise-induced WM and VAX have previously been shown to delay tumor progression. This study was supported by the National Institutes of Health (NIH) Grant No. RO1CA117583.
mary tumor growth and metastases in the 4T1 mammary tumor model. This WM-induced reduction in tumor growth occurred concurrently with an elevation in tumor specific IFN-γ production and a reduction in the number of myeloid-derived suppressor cells (MDSCs). However, because tumor size is positively correlated with immune suppression, the goal of the current study was to investigate if mice in the WM+VAX group had enhanced anti-tumor immune responses and/or fewer MDSCs controlling for tumor size. Female BALB/c mice were randomized into weight gain (WG) and WM groups (n=8/group) and had access to voluntary running wheels or standard cages, respectively. WG mice were fed ad libitum while WM mice were energy-restricted by 10% to maintain a stable body weight. After 8 weeks on the intervention, all mice were orthotopically injected with 5x104 4T1.2 cells into the fourth mammary fat pad and continued on their intervention. Once injected, both WG and WM mice were further randomized into vaccination (VAX) and vehicle control (VEH) groups (n=4/group) and administered 1x10^7 irradiated 4T1.2 cells (VAX) or HBSS (VEH) at day 7 post-tumor implantation. Primary tumour growth was quantified, and mice were sacrificed when tumor volume reached 0.1-0.2 cm^3. WM mice weighed significantly less than WG mice over the course of the study (p<0.001). Mice in both WM+VAX and WM+VAX groups took a significantly longer number of days post tumor implantation to reach a tumor size of 0.1-0.2 cm^3 (26.2±3.3 d, p=0.003; 23.8±0.5 d, p=0.009, respectively) compared to the WG+VEH group (18.3±2.5 d). Despite the fact that mice were sacrificed when the tumor volumes were standardized at 0.1-0.2 cm^3, the combination of WM+VAX resulted in a significant reduction of splenic MDSC accumulation (p<0.0001) and enhanced CD4^+ T cell responses in the WM+VAX group. These results suggest that diet and exercise-induced WM was highly effective in delaying primary mammary tumor growth. The combination of WM and an allogenic whole tumor cell vaccine reduced pro-tumorigenic MDSC accumulation and enhanced effector T cell function. Furthermore, these data suggest that diet and exercise may be changing the tumor microenvironment at an early stage of tumor growth favoring tumor clearance. This work is supported by R21 CA209144; T32AI074551.

#244 Eicosapentaenoic acid reduces effects of some adipocyte derived factors on breast cancer cell inflammation and glucose metabolism. Arwa Al-jawadi,1 Sara Al-Jahia,1 Suranganee Dharamawardhane,1 Shane Scoggin,2 Lauren Gollahon,2 Preethi Gunaratne,2 Naima Moustaid-Moussa,1,3 Texas Tech Univ, Lubbock, TX;1 University of Puerto Rico Medical Sciences Campus, San Juan, PR, Puerto Rico; 2University of Houston, Houston, TX.

Breast cancer is still the leading cause of death in women among all cancer types. Obesity, a chronic low grade inflammatory disease, is a key contributor to the progression of breast cancer especially in women, post-menopause. Given the current understanding of cancer-related inflammation and associated microvesicles in restructuring the microenvironment, we hypothesized that adipocyte-derived cytokines and exosomes negatively impact breast cancer progression. By contrast, eicosapentaenoic acid (EPA), a dietary omega 3 polyunsaturated fatty acid and a well-established anti-inflammatory compound will reduce adipocyte-secreted pro-inflammatory factors, thereby reducing breast cancer progression. To test these hypotheses, we investigated the effects of conditioned media or exosomes from 3T3-L1 adipocytes or human mesenchymal stem cells (HMSC), treated with +/- 100μM EPA on MCF7 and MDA-MB231 breast cancer cells for 24-72 h. Following treatment, changes in breast cancer cell gene expression were measured using qPCR and glycolytic rate was measured using XF24 Seahorse extracellular flux analyze. We observed that conditioned medium from HMSC significantly increased the mRNA expression levels of oncogenic genes such as signal transducer and activator of transcription 3 (STAT3), baculoviral IAP repeat-containing proteins 3 (BIRC3) known as cIAP2, and the lipogenic fatty acids synthase (FASN). In contrast, conditioned media from EPA-treated human adipocytes reduced the expression levels of these genes. Similarly, exosomes isolated from EPA-treated adipocytes showed a significant reduction in mRNA expression levels of STAT3 and cIAP2 in both cancer cell lines. Furthermore, glycolysis was significantly reduced in MCF7 but not MDA-MB231 cells incubated with 3T3-L1 adipocyte-conditioned medium pretreated with EPA for 24 h. Taken together, our data suggest that adipocytes play a significant role in promoting breast cancer progression by providing a microenvironment that increases survival and inflammation. EPA is a promising anti-inflammatory nutrient that may help reduce breast cancer cell inflammation and survival, possibly by modifying adipocyte-derived cytokines and exosomes in obesity and thus warrants further investigations.

#245 Dietary long-chain omega-3 fatty acids reduce adipose inflammation in mammary tissue of mice fed moderate fat-isocaloric diets. Saraswoti Khadhe,1 Geoffrey M. Thiele,1 John Graham Sharp,1 Lynnell W. Klassen,1 Tommy R. McGuire,1 Michael J. Duryee,1 Holly C. Britton,1 Alicia J. Daffner,1 Jordan Beck,2 Paul Black,2 Concetta C. DiRusso,2 James E. Talmadge1. 1Univ. of Nebraska Medical Ctr., Omaha, NE; 2Univ. of Nebraska-Lincoln, Lincoln, NE.

Increased adipose tissue inflammation and breast density; including ductal epithelial hyperplasia have been associated with increased risks for breast cancer. Omega 6 (ω6) and omega 3 (ω3) fatty acids (FAs); serve as substrates for pro-inflammatory and inflammation resolving mediators respectively, emphasizing the potential regulatory role for dietary intake of these FAs in inflammation. Western diets have a 6:3 FA ratio of >15:1 with low levels of long-chain (LC-)ω3FA. While adipose tissue inflammation occurs, characterized by crown-like structures (CLS) consisting of dead adipocytes and adjacent macrophages in breast tissue have been related to breast cancer risk in overweight and obese women presumably by the obesity-inflammation- aromatase axis. However, a role of dietary ω6/ω3 FA in adipose inflammation, independent of obesity is not clear. Herein, we examined effects of dietary ω6/ω3 FA in the mammary tissue microenvironment and adipose inflammation using a moderate fat, iso-caloric diets, and pair-fed model. The Lieber-DeCarli diet containing 21:1 ratio of ω6/ω3 FA was used as a ω6 diet, whereas encapsulated fish oil containing a 3:1 ratio of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid was used to decrease ω6/ω3 ratio to 0.7:1 in the ω3 diet. Both isocaloric diets contained 35.5% of calories derived from fat and were pair-fed to maintain iso-intake. Female BALB/c mice were established on the ω6 and ω3 diets for 10 weeks and weight gain and diet consumption monitored. There were no differences in the volume of diet consumed and weight gain between dietary groups. At autopsy, mammary fat pads (MFP) were collected and analyzed for fatty acid composition, histopathology, epithelial proliferation and macrophage infiltration. Arachidonic acid (AA) levels in the MFPs were not different between the groups but EPA and DHA levels were different. There was a significant increase in AA (2.41±0.5% vs 1.52±0.29%) in the MFP of ω3 diet fed mice. The MFP of ω6 diet fed mice had significantly increased areas of unilocular adipocytes relative to adipocytes of the ω3 group. Similarly, ω6 diet fed mice had increased connective tissue in the ductal stroma, significantly higher numbers of proliferating cells in the ductal epithelium, as well as in adipose tissue in the MFP. In addition, ω6 diet fed mice had a significant increase in the numbers of CLS in mammary adipose tissue. In summary, our studies demonstrated that despite the comparable levels of AA in MFP in both of groups, the presence of LC-ω3 FA (EPA and DHA) was able to reduce inflammation in the MFP of ω3 diet fed mice, thus regulating the MFP microenvironments by reducing macrophage infiltration and ductal epithelial proliferation in an obesity-independent manner.

#246 Assessing microbial dysbiosis of electronic cigarettes and cigarette smokers using oral and lung microbiome. Kevin Ying,1 Min-Ae Song,2 Daniel Y. Weng,1 Quentin Nickerson,2 David Frankhouser,1 Pearlly S. Yan,1 Ralf Bundschuh,1 Mark D. Wewers,1 Ewy Mathe,1 Jo L. Freudenberg,2 Peter G. Shields3. The Ohio State University, Columbus, OH; 3University at Buffalo, Buffalo, NY.

The link between smoking tobacco and changes in the oral microbiome in response to tobacco smoking are well established. It is not known if there are changes in response to electronic cigarettes (e-cig). These changes in the microbe are associated with increased numbers of disease causing pathogens. Currently there are no published studies that have investigated the relationship of smoking tobacco and the oral and lung microbiome. There is insufficient evidence showing whether changes in oral cavity and lung microbiome are also seen in e-cig users. We will study the oral cavity and lung of non-smokers, smokers and e-cig users to examine concordance between oral cavity and the lungs as well as comparing the three groups, examining the microbiomes and expression of inflammatory markers. We hypothesize that microbial dysbiosis and expression of inflammatory cytokines will differ for smokers and non-smokers; and that e-cig users will have microbial dysbiosis similar to cigarette smokers. A cross-sectional study is being conducted on three groups, 1) never-smokers, 2) cigarette smokers, and 3) e-cig users. For each study participant, saliva and bronchoalveolar lavage (BAL) are being collected to measure microbiome. RNA is extracted from saliva and BAL samples for total transcriptome analysis using RNA-seq. This analysis will detect human and bacterial reads thereby allowing observations of bacterial communities as well as human inflammatory cytokine response to bacterial presence. 85% to 98% of BAL sample reads aligned to the human genome compared to less than 50% from saliva samples. The alignment results allow us to deduce that the majority of reads from BAL samples are human and that the majority of the reads in saliva samples are bacterial. Preliminary results show detection of human RNA expression and of bacterial reads are present in both saliva and BAL samples. More samples are being processed and the comparison of BAL and saliva samples between the three groups will be discussed.

#247 Nutrient stress via folic acid modulation causes systemic and cancer-specific metabolic reprogramming and differential effects on primary and metastatic mammary tumor growth in lean and obese mice. Ciara H. O’Flanagan,1 Xuwen Chen,2 Zahra Ashkavand,2 Sergey A. Krupenko,2 Stephen D. Hursting1. 1Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; 2Nutrition Research Institute, Kannapolis, NC.
Many foods are currently fortified with folic acid (FA), a synthetic folate (Vitamin B9). Folate deficiency causes many human health defects, most notably anemia and neural tube defects. The effects of excess supplementation on human health have to date been understudied. The rise in obesity in the last three decades further complicates this issue, with the combined effects of excess or insufficient folic acid intake and an elevated prevalence of folic acid deficiency. The obesity epidemic is associated with a variety of health problems such as type II diabetes, cardiovascular disease, and cancer. Among these, obesity is a risk factor for hepatocellular carcinoma (HCC). Stat3 signaling plays an important role in the development of obesity-related HCC.

Here, we examined the effects of FA supplementation and deficiency on tumor growth, metastasis, and inflammation in obesity-responsive models of primary (M-Wnt) and metastatic (mwtM-Wnt) HCC. FA supplementation and deficiency significantly enhanced primary tumor growth and invasiveness in lean mice, while no difference in tumor size was detected in obese groups. FA supplementation reduced while deficiency increased survival and reduced lung tumor metastasis incidence in lean, but not obese mice. Liver and tumor metabolic profiling revealed that modulation of dietary FA caused systemic and tumor-specific metabolic reprogramming, altering pathways involved in fatty acid, purine, amino acid, glutathione and energy metabolism. Short term in vitro FA withdrawal resulted in reduced proliferation, migration and invasion and energy production in all cell lines, as well as significant changes in gene expression profile, particularly of many metabolic pathways. In contrast, chronic in vitro FA depletion resulted in heightened autophagy and apoptosis and was associated with a decrease in Stat3 signaling. These data suggest translational implications for the treatment of obesity-related HCC.

Interest-ingly, EPA had no obvious effects on obesity-induced inflammation, instead, indicating that EPA attenuates obesity-related hepatocarcinogenesis. Interestingly, dietary EPA supplemented with methylseleninic acid (2.5 mg Se/kg) reduced male breast carcinogenesis and its metastasis in mice. It indicates that selenium may be useful in male breast cancer prevention.

#250 Methionine restriction increases macrophage tumoricidal activity and significantly inhibits prostate cancer growth. Ashley R. Orillón,1 Sreenivasulu Chintala,1 Remi Adelaiye-Ogala,1 Li Shen,1 Nur Damayanti,1 May Elbanna,1 Sreevani Arisa,1 Bennett Elzey,2 Chinghai Kao,1 Luigi Fontana,2 Roberto Pillo.3 1Indiana Univ.-Purdue Univ. Indianapolis, Indianapolis, IN; 2Roswell Park Cancer Institute, Buffalo, NY; 3Purdue University, Lafayette, IN; 4Washington University, St. Louis, MO.

Background: Our previous work showed a significant reduction of tumor growth, macrophage infiltration, circulating IGF-1, and mTOR activation with low protein diet in a patient derived xenograft model of prostate cancer. The evolutionarily conserved, nutrient sensing, mTOR pathway plays a central role in both development and angiogenesis. Targeting the mTOR pathway is a promising strategy for the treatment of prostate cancer.

Methods: We used in vivo studies utilizing bone marrow or tumor derived (RP-B6 Myc) macrophages. In vivo studies utilized the recently characterized RP-B6 Myc model. Mice were fed ad libitum control or methionine restricted diets for four weeks prior to S.C. implantation with 1mm3 tumor pieces. Treatment of survivin peptide vaccine (1mg/ml S.C. 1X week) and anti-PD-1 (20mg/kg IP. 2X week) began at ~50mm3 tumor size. Tumor volumes were blindly recorded 2X week. End point analyses include: tumor weight, flow cytometric analysis, proteomic profiler analyses, and microbiome analyses from each diet and treatment group. Results: We show here that while methionine restriction (MR) has little impact on our RP-B6 Myc prostate cancer cell line, it does yield a significant alteration in both the polarization and function of M1 and M2 macrophages. In the in vitro MR conditions, we observed significantly enhanced polarization of M1 macrophages and reduced polarization of M2 macrophages. Functional analysis revealed increased tumoricidal activity of both M1, ‘antitumor’, and M2, ‘pro-tumor’, macrophages suggesting a flip in M2 function from tumor-promoting to tumoricidal. Further analysis of the released cytokines in MR media conditions yielded significant increase of antitumor cytokines & chemokines, such as IL-12, IL-27, CXCL9, CXCL10, CCL2, CCL4, and TNF-alpha, a double-edged sword which in our system correlates with increased cell death in MR media conditions. Importantly, survivin expression was reduced while deficien-cy significantly enhanced primary tumor growth and invasiveness in lean mice, while no difference in tumor size was detected in obese groups. FA supplementation reduced while deficiency increased survival and reduced lung tumor metastasis incidence in lean, but not obese mice. Liver and tumor metabolic profiling revealed that modulation of dietary FA caused systemic and tumor-specific metabolic reprogramming, altering pathways involved in fatty acid, purine, amino acid, glutathione and energy metabolism. Short term in vitro FA withdrawal resulted in reduced proliferation, migration and invasion and energy production in all cell lines, as well as significant changes in gene expression profile, particularly of many metabolic pathways. In contrast, chronic in vitro FA depletion resulted in heightened autophagy and apoptosis and was associated with a decrease in Stat3 signaling. These data suggest translational implications for the treatment of obesity-related HCC.

#249 Dietary supplementation with methylseleninic acid reduces male mammary tumorogenesis in MMTV-PyMT mice. Snea Sundaram, Grand Forks Human Nutrition Research Center, ARS, USDA, Grand Forks, ND.

Male breast cancer makes up approximately 1% of all breast cancers and less than 1% of all cancers in men in the United States. However, it is an aggressive disease with poor prognosis and the incidence of male breast cancer is on the rise. The present study investigated the effects of dietary supplementation of selenium on male breast cancerogenesis in MMTV-PyMT mice (FVB/N background). Three-week-old male mice were weaned onto the AIN93G diet with or without supplementation of selenium in the form of methylseleninic acid (2.5 mg Se/kg). Mice were euthanized 10 weeks after the first palpable mammary tumor was detected. There were no differences in food intake and body weight between the groups. The median latency (the age at which the first palpable tumor was detected) was 14.1 and 13.9 weeks and the incidence of palpable mammary tumor was 71% and 70% for the control and the selenium group, respectively. Selenium supplementation compared to the control diet reduced mammary tumor progression by 258% and tumor weight by 84%, respectively.

The present study provides evidence that selenium reduces male breast cancerogenesis and its metastasis in mice. It indicates that selenium may be useful in male breast cancer prevention.

#248 Eicosapentaenoic acid attenuates obesity-related hepatocellular carcino-genesis. Akane Inoue-Yamauchi, Hiroko Itagaki, Hideaki Oda. Tokyo Women’s Medical University, Tokyo, Japan.

The population of obesity caused by excess energy intake and insufficient physical activity is increasing worldwide. Excess accumulation of body fat is a risk of health, indeed obesity is associated with the pathogenesis of various disease including type II diabetes, cardiovascular disease and cancer. Among various kinds of cancer, the risk of hepatocellular carcinoma (HCC) is strongly influenced by obesity. Studies using diet-induced obesity mouse models show that obesity enhances tumor development inducing chronic inflammation in liver. In fact, obesity failed to promote HCC formation in the absence of inflammatory cytokines IL-6 and TNF-α. Obesity is associated with a number of metabolic and inflammatory changes that may confer a growth advantage in nonmetastatic cells and from which metastatic TNBC cells cannot recover. Moreover, obesity and FA excess cause similar meta-bolic and proancerca effects and in combination, are not synergetic.


In this study, we assessed the chemosensitization activity of the naturally occurring polyphenols resveratrol (RES) and pterostibene (PTER) to 5-Fluo- oracil (SFU) in Burkitt’s B-cell lymphoma. RES is a stilbenoid compound found in a variety of fruits, plants, and nuts. In addition to antioxidant and anti-aging properties, RES has been shown to exhibit significant anti-tumor activity against a variety of malignancies. Since the compound is typically pro-ductive of normal cells, its anti-cancer activity makes it a promising candidate as both a chemopreventive nutritional supplement and a chemotherapeutic agent. PTER, an analog of RES, has likewise shown anti-cancer activity. Its therapeu-tic potential, however, is much less understood. Recent studies have investigat-ed the use of these and other natural compounds in adjuvant chemotherapy
strategies, many of which continue to produce underwhelming rates of response. Here, we investigate the chemosensitization capacity of both RES and PTER to 5-FU, a commonly used drug in chemotherapy, in healthy lymphocytes and in Burkitt’s B-cell lymphoma (Raji) cells. MTS assays are used to measure the viability of malignant and normal lymphocytes after 5-FU treatment with or without supplementation of RES, PTER, or RES/PTER. In the Raji cells, 5-FU/Raji co-incubation with physiologically relevant concentrations (~10μM) of either RES or PTER significantly increases 5-FU toxicity in Raji cells, with data suggesting PTER to be slightly more effective than RES. Interestingly, RES/ PTER combinatorial treatments further increase chemosensitization, an important finding considering the typically low bioavailability of each individual compound in vivo. Together, these data support the potential use of RES as a chemosensitizing adjuvant and provide new evidence that its analog, PTER, also possesses significant chemosensitization capacity. Furthermore, our studies suggest that low physiological concentrations of PTER and RES may be still be sufficient to induce significant anti-cancer effects.

#253 Mammary tumorigenesis causes bone loss and dietary selenium supplementation does not affect such bone loss in male MMTV-PyMT mice. Lin Yan, USDA-ARS, Grand Forks, ND.

Cancer progression is accompanied by wasting that eventually results in cachexia characterized by significant weight loss and multi-organ functional failures. Limited clinical trials indicate that bone is adversely affected by cancer-associated wasting. To determine the effects of breast cancer on skeletal health, we performed micro-computed tomographic analysis of femurs and vertebrae collected from a recently completed study showing that dietary supplementation with selenium (methylseleninic acid, 2.5 mg Se/kg) reduced male mammary tumorigenesis in MMTV-PyMT mice. Compared to age-matched non-tumor-bearing mice (MMTV-PyMT negative), the presence of mammary tumors significantly reduced bone volume fraction, trabecular thickness and bone mineral density and increased the structure model index (an indicator of the plate- and rod-like geometry of trabecular structure) in femoral trabecular bone. Mammary tumor development did not affect vertebral trabeculae nor femoral and vertebral cortical bone, except it significantly reduced cortical bone thickness of vertebrae. There were no differences in aforementioned measurements between groups with or without selenium supplementation. In conclusion, mammary tumorigenesis causes bone loss and dietary selenium supplementation at 2.5 mg Se/kg, which is anti-tumorogenic, does not affect mammary tumor-associated bone loss in this male MMTV-PyMT breast cancer model.

#254 The potential of Lactobacillus probiotic treatments in colorectal cancer (CRC). Imen Kahouli,1 Meenakshi Malhotra,2 Susan Westfall,1 Moulay Alaoui-Jamali,1 Satya Prakash1.

Colorectal cancer (CRC) is the third leading cause of death worldwide. It is known to be a type of cancer that is preventable by changes in diet and lifestyle. Mounting evidence are supporting the role of gut microbiome in the etiology of CRC and emphasize on the potential of probiotics as biotherapeutics in the prevention and management of CRC. Lactobacillus probiotic bacteria have the ability to balance gut microbiota in the colon and produce anti-tumorigenic and anti-inflammatory effects in healthy individuals at risk and CRC patients. There is a need, however, for studies that focus on identifying potent probiotic strains with activity against CRC and inhibit cancer growth. This report discusses and present findings about the formulation of novel probiotic Lactobacillus biotherapeutic for CRC based on anti-CRC proliferative effect, immune modulation and metabolic activity, in vitro and in vivo, using a genetically-induced animal CRC model. Results and metabolomic analysis demonstrated the potential action of probiotic treatment to change host and gut microbiome co-metabolic profiles, produce local and systemic anti-inflammatory effects, inhibit cancer-causing events, and improve overall gut health.

#255 Dietary amphiphilic polyphenols modulate the biophysical properties of plasma membrane organization and membrane-dependent macropinocytosis. Michael L. Salinas, Natividad R. Fuentes, Rola Barhoumi, Robert S. Chapkin. Texas A&M University, College Station, TX.

Ras-driven cancers exhibit a distinct high metabolic demand necessary for biosynthetic growth/proliferation, in some cases resulting in over stimulation of macropinocytosis, a process intended for nutrient acquisition. A hallmark of Ras-driven macropinocytosis is plasma membrane ruffling resulting in invaginations and the formation of macropinosomes, extracellular nutrient-packed vesicles distinct from other endocytic processes due to their size, independence of vesicle-coating proteins, and are the result of rapid polymerization and branching of cytoskeletal actin filaments. These cytoskeletal manipulations are driven by spatial/temporal regulators downstream of Ras along with lipid rafts and their components. Drugs and other extrinsic factors, e.g., polyunsaturated fatty acids, have been shown to bio-physically modulate these lipid domains. We hypothesize that unique dietary polyphenolic amphiphilic molecules [1] modulate plasma membrane fluidity, [2] disrupt macropinocytosis, and [3] suppress macropinocytosis-dependent proliferation. Young adult male colonocyte (YAMC) cells, expressing an HRas mutation, were treated with 1, 10, and 100 μM (+)-catechin or procyanidin B2 for 30 min. For macropinocytosis assessment, cells were stimulated with 25 ng/mL epidermal growth factor (EGF) for 10 min following a 30-minute pretreatment. Membrane order was measured in whole cells and giant plasma membrane vesicles (GPMV) utilizing a lipid-packing detection dye, Di-4-ANEPPDHQ, and assayed using a combination of fluorescence image-based flow cytometry (Annis FlowSight) and confocal microscopy. In addition, macropinocytosis was determined by uptake of fluorescently labeled 70 kDa dextran (FITC-dextran). The uptake was visualized by fluorescence image-based flow cytometry. In the GPMV model, (+)-catechin increased membrane fluidity, and procyanidin B2 decreased membrane fluidity in a dose-dependent manner. In contrast, in the whole cell model, interaction of (+)-catechin and procyanidin B2 with the plasma membrane resulted in an increase in membrane fluidity in a dose-dependent manner. Interestingly, plasma membrane fluidization in the whole cell model was associated with a significant 27-67% inhibition of macropinocytosis. These observations indicate that dietary amphiphilic molecules (DAMs) modulate plasma membrane organization, which is linked to macropinocytosis, an essential fuel-obtaining process. Establishing a role for DAMs in membrane-dependent oncogenesis is noteworthy because these molecules are innocuous and found in high abundance in a variety of fruits, vegetables, and walnuts. This work was support by NIH grant R35CA197707.

#256 Delta tocopherol inhibits urothelial tumorigenesis in the UPII mutant Ha-ras transgenic mouse model and induces apoptosis via activation of the ATF4/CHOP-DR5 pathway. Christopher A. Blair,1 Maggie Wu,1 Tim Huynh,2 Hanze Hu,3 Arman Walla,1 Chang S. Yang,2 Xiaoilin Z1. 1UC Irvine, Irvine, CA; 2Rutgers University, Piscataway, NJ.

Gut microbiome studies have reported that Vitamin E intake was inversely related to the risk of multiple cancers including human urinary bladder cancer. Tocopherols (T) are the major forms of vitamin E in the U.S. diet, exist as α-T, β-T, γ-T, and δ-T. Compared to α-T, the anti-cancer effect of other Ts and their mechanisms of action remain largely unknown. We have shown that δ-T is the most effective one among the Ts in reducing the viabilities of bladder cancer cell lines with IC50 of under 0.012 gram, P < 0.012 gram, P < 0.0001) with no significant difference in overall body mass and no detectable indication of toxicity. Our results suggest that δ-T is a potent anti-cancer agent and deserves further investigation in preventing the recurrence and progression of non-muscle-invasive UCC.
methyltransferase. The FFQ and 24-hour FR are the most commonly used methods to assess adequate nutrient intake, including methyl donors and other vital micronutrients that are essential to promote metabolism and methylation. While FFQ provides best estimates in dietary intakes, a 24-hour food record is less time consuming for subject burden. Methods: A total of 134 participants’ dietary data were collected using FFQ (from 1997-1998 for 100 participants and 1999-2000 for 34 participants). Bland and Altman methodology was used to validate the bias and agreement between these two measurements. Results: Overall, the 24-hour measurements presented underestimated values across all nutrients compared to FFQ measurements. Using a 10% criterion in the differences between the two measurements, the percentage bias for 24-hour FR compared to 30-day FFQ was acceptable for most nutrients, including B1 (-6.9% ± 15%), B2 (-8.66% ± 0.12), B3 (-9.25% ± 0.12), B6 (-5.02% ± 0.14), and B9 (folate -7.90% ± 0.24). However, the bias was greater for protein based methyl donors, including B12 (-17.23% ± 0.14), choline (-13.38% ± 0.11), Glycine (-17.53% ± 0.10), and Methionine (-18.88% ± 0.09). The correlations between the two measurements on all methyl donors ranged from 0.92 to 0.99. The precisions for all measurements based on the standard error (SE) were less than and within 0.03% (0.007% - 0.021%). And, the agreements between the two measurements were less than and within 10% for Glycine and methionine (8.96 - 9.58%), based on the standard deviation (SD); however, greater than 10% for other protein based methyl donors including choline and other B vitamins (10.5 - 24.11%). Conclusion: Proper dietary intakes of methyl donors are vital for cancer prevention. These findings point out the importance of further validation and use of more accurate food measurement to monitor dietary intake, and the interpretation of nutrient reports with caution.

**EPILOGUE:** Descriptive Epidemiology Including Trends in Incidence and Prognosis

#257 Estrogen receptor positive and negative breast cancer secular trends in Ireland (2004-2013). Maeve Mullooly,1 Jeanne Murphy,1 Gretchen L. Gierach,1 Brittany Davis,1 Paul Walsh,2 Sandra Deady,3 Thomas I. Barron,4 Mark E. Sherman,5 Philip S. Rosenberg,1 National Cancer Institute, Bethesda, MD, 2National Cancer Registry Ireland, Ireland, 3Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, 4Mayo Clinic, Jacksonville, FL.

Background: Estrogen receptor (ER) status in breast cancer is strongly associated with etiologic factors, prognosis and treatment response. Data suggest that population-based incidence rates of ER-positive (ER+) tumors are increasing and ER-negative (ER-) cancers have fallen over time in the United States (US), Denmark and Scotland. Whether these trends are consistent in other populations with different etiologic exposures and across ER+/HER2- subtypes is unknown. We, therefore, analyzed breast cancer secular trends in Ireland by ER expression; and for the first time, by joint expression of ER and HER2/HER2.

Methods: This study included invasive breast cancers (n = 4,007,768) diagnosed in women aged 20-84 years within the population-based National Cancer Registry of Ireland (97% coverage). The general population at risk (n = 10,401,986) was obtained from the Irish Central Statistics Office. Using a validated approach to assign unique code for VRL) per 100,000 person-years in the United States (US) during 1992-2002 and 2003-2013. Results: Over the 10-year follow-up period, 19,264 ER+ and 4,161 ER- breast cancers were diagnosed (1,420 were ER-unknown). ASRs increased significantly for ER+ breast cancer (3.0%/year; 95%CI: 1.4, 4.6%) and decreased significantly for ER- cancers (1.2%/year; 95%CI: 0.9, 1.5%). The ER-specific trends were qualitatively similar among three age groups (<50, 50-64, and ≥65 years). With further stratification by HER2 expression, ER+/HER2- cancers increased significantly (2.2%/year/5%CI: 0.9, 4.4%/year), with the largest increases observed for HER2+ cancers and ER+/HER2- categories (3.3%/year; 95%CI: 1.8, 4.9%/year). The ER-specific trends were qualitatively similar among three age groups (<50, 50-64, and ≥65 years). With further stratification by HER2 expression, ER+/HER2- cancers increased significantly (2.2%/year; 95%CI: 0.9, 4.4%/year), with the largest increases observed for HER2+ cancers and ER+/HER2- categories (3.3%/year; 95%CI: 1.8, 4.9%/year). Conclusions: Secular trends for ER+ cancers in Ireland were similar to those previously observed in the US and Denmark. Further, trends for ER+/HER2 followed those for ER, although rates were constant for ER+/HER2. The incidence of cancer incidence patterns by ER among independent populations over time could reflect comparable changes in risk factor exposures with dual or opposite effects for risk of ER+ and ER- cancers (e.g., obesity, age at first birth/parity). Additionally, although HER2 is a well-established clinical predictive and prognostic marker for breast cancer outcome, it did not appear to substantively impact breast cancer incidence trends.

#258 The changes of esophageal malignant tumors by histopathology over 40 years (1975-2014) at a single institute in northern China. Xue Ke Zhao,1 Hai Jun Yang,1 Zong Min Fan,1 Ji Lin Li,1 Li Min He,1 Yun Zhou,1 Liu Qin Yang,1 Ying Fa Zhou,2 Sa Tang,3 Yang Yang,3 Lei Ma,4 Wei Li Han,2 Li Dong Wang1.

1The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China; 2Zhengzhou University Hospital, Zhengzhou, China; 3The First People’s Hospital of Nanyang, China; 4Henan Provincial People’s Hospital, Zhengzhou, China; 5Central Hospital of Xinzhuang, China; 6The Second Affiliated Hospital of Zhengzhou University, Zhengzhou, China; 7Xijing Hospital of the Fourth Military Medical University, China.

It has been well recognized that esophageal squamous cell carcinoma (ESCC) is the major histopathological type of esophageal cancer in China. However, it is not clear how many and how many esophageal malignant tumor (EMT) exist and if the histopathological patterns have changed over the past decades in China. The present study was thus undertaken to characterize the histopathological patterns over 40 years at a single institute in northern China, the high incidence area for esophageal cancer. All the patient information was from esophageal cancer database in Henan Key Laboratory for Esophageal Cancer Research of the First Affiliated Hospital, Zhengzhou University in Henan, China (1973-2015). A total of 66,216 patients with MET and detailed histopathological records were enrolled in this study, including 39,873 males with a mean age of 60±6 and 26,343 females with a mean age of 60±6 from 1975-2014. All the patients were classified into four period groups based on the diagnosed time code, and group I covers from 1975-1994, group II (1995-2004), group III (1995-2004, 20,781:31,41%) and group IV (2005-2014, 26,527:40,1%). The results demonstrated 12 different histopathological types of EMT. The most common type was ESCC (64,800, 97.4%), followed by esophageal adenocarcinoma (EAC, 12,609, 1.9%), esophageal small cell carcinoma (201, 0.3%), esophageal adenosquamous carcinomas (EASC, 119, 1.8%), esophageal spindle cell carcinoma (56, 0.1%), esophageal undifferentiated carcinoma (57, 0.10%), esophageal malignant melanoma (5, 0.008%), esophageal stromal tumor (3, 0.005%), esophageal clear cell carcinoma (2, 0.003%), esophageal malignant schwannoma (1, 0.002%), esophageal lymphatic tumor (1, 0.002%) and esophageal smooth muscle cell sarcoma (1, 0.002%). Trending analysis showed that, over the past 40 years, ESCC was relatively stable (98.9%, 98.0%, 97.0%, 97.1%, respectively). However, EAC (1.07%, 1.66%, 2.30%, 1.87%, respectively), and small cell carcinoma (0.02%, 0.17%, 0.28%, 0.43%, respectively) and spindle cell carcinoma (0.02%, 0.10%, 0.10%, 0.22%, respectively) increased apparently. The present study indicates that ESCC remains the major histopathological type for Chinese esophageal cancer. Interestingly, esophageal adenocarcinoma, small cell carcinoma and spindle cell carcinoma seem to be increasing over the past decades. The improved molecular diagnostic biomarkers and the changes of lifestyle including the diet patterns resulted from recent economic development in China, may contribute to the changes of esophageal histopathological types.

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EPIDEMIOLOGY: Descriptive Epidemiology Including Trends in Incidence and Prognosis

with bilateral presentation. Diagnosis in all cases was made by immunohistochemical analysis on tumor specimen obtained by vitreous fine needle aspiration, vitreous biopsy or stereotactic brain biopsy. Median age at diagnosis was 76 years (range, 62-84 years). Median interval from Chernobyl accident (1986) to diagnosis was 26 years (range, 24-27 years). We identified no other common exposure or environmental risk factor. There were 68 cases of primary IOL during 1992-2013 in the 13 SEER areas. IOL rates per 100,000 person-years for both sexes were highest among Asian/Pacific Islanders (0.010 for males and 0.014 for females) followed by whites (0.009 for males and 0.008 for females) and (based on small numbers) by blacks. Analysis of incidence trends showed an increase in rates among Asian/Pacific Islander females from 0.005 in 1992-2002 to 0.018 in 2003-2013 and among males from 0.006 to 0.013. Rates among whites increased for both genders. Analysis of NYSKR data revealed 44 cases of primary IOL. IOL rates per 100,000 person-years for both genders were highest among whites (0.010 for males and 0.012 for females) followed by Asian/Pacific Islanders (0.007 for males and females). Analysis of trends revealed increase in rates for both genders and racial groups. Conclusions: Our analyses highlight the rarity of VRL and reveal an increase in incidence, particularly among Asian/Pacific Islanders where IOL rates tripled within two decades. Based on incidence rates, 8 cases of IOL (with a subset being VRL) are expected in 4 years in NYS. Our observation of 10 cases of VRL in 4 years in our practices in NYC is unanticipated. The sole common environmental factor we identified was residence in regions proximal to the Chernobyl nuclear disaster.

#260 Incidence rates and outcomes of fallopian tube carcinomas: Data from the North American Association of Central Cancer Registries. Mark E. Sherman,1 Sally B. Coburn,2 Hannah Yang,3 William Anderson,2 Philip Rosenhi1, Gretchen Gierach,2 Nicolas Wentzensen,3 Kathryn Cronin,3 Britton Trabert2,3 Mayo Clinic, Jacksonville, FL; 2National Cancer Institute, Bethesda, MD.

Background: Serous tubal intraepithelial carcinoma (STIC) is hypothesized to give rise to many cancers that have historically been classified as ovarian primaries. Although STIC is gaining status as a diagnostic entity, similar appearing lesions would likely have been reported as fallopian tubal carcinoma in situ (FT-CIS) in the past. Accordingly, to characterize patterns of reporting and behavior for tubal cancers, we analyzed population-based data for incidence rate trends, co-occurrence with cancer at other sites and survival of FT-CIS and invasive fallopian tube carcinoma (FT-Inv). Methods: We analyzed data for FT-CIS and FT-Inv included within 33 registries using the Cancer Incidence in North America Deluxe Analytical Files provided by the North American Association of Central Cancer Registries (1995-2012). Primary sites of invasive carcinomas were defined according to the International Classification of Diseases for Oncology (3rd edition) topography codes (ovarian (C56.9), fallopian tube (C57.0), and primary peritoneal cancers (C48.1-2, 48.82)). We excluded cancers with non-epithelial histology. Total counts, incidence rates per 1 million adjusted to the 2000 standard US population and age-standardized stage-specific survival were computed. Temporal incidence rate patterns were analyzed by jointpoint regression with estimates of annual percentage change (APC). Results: Cases in which the first cancer diagnosis was FT-CIS, included 98 FT-CIS alone; 27 FT-CIS followed by another cancer diagnosis within 1 year and 172 cases in which FT-CIS was diagnosed concurrently with another cancer as compared with 5513 invasive tubal carcinomas over the same period. The incidence rate of FT-CIS was stable from 1995-2002, then significantly increased from 2002-2012 (APC (95% CI) = 16.2% (10.9-21.7)). Rates of early stage high-grade serous FT-Inv increased significantly from 2002-2012 (10.4% (6.1-14.9)) and rates of late stage high-grade serous FT-Inv rose sharply from 2002-2012 (20.0% (17.5-22.6)). Five-year age-standardized survival for women with FT-CIS only was 75.6% overall, reflecting 89.1% survival for women less than 50 years and 70.9% for women 50 years or older; survival for high-grade early stage serous FT-Inv was 77.8%. Conclusions: Diagnoses of FT-CIS and FT-Inv have increased dramatically, likely reflecting changes in diagnostic pathology practice. Developing standardized reporting for FT-CIS (and in the future, STIC) when present with concurrent invasive cancer is needed, given that this occurs often. Based on limited data, 5-year survival for FT-CIS and early stage FT-Inv are similar, but future studies to evaluate data for STIC will be required, which will likely be achievable only through national registries that are large enough to capture sufficient cases.

#261 Pancreatic cancer incidence trends and recent patterns overall and by histologic type among US men and women by racial/ethnic group: evidence from the Surveillance, Epidemiology, and End Results (SEER) program. Van-essa L. Gordon-Dseagu,1 Susan Devesa,1 Mike Goggins,2 Rachael Stolzenberg-Solomon1.1NCI, Rockville, MD; 2Johns Hopkins University, MD.

Introduction: Pancreatic cancer incidence has been rising. We examined incidence patterns by sex, race, age, and histologic subtype. Methods: We used data from the Surveillance, Epidemiology and End Results (SEER) registries (9, 13 and 18) to calculate counts, age-adjusted rates (2000 US Standard Population), annual percent changes (APCs) and incidence rate ratios (IRRs) for all pancreatic cancers and main histologic subtypes. Results: Pancreatic cancer incidence rates declined among males between 1974 and 1991, and increased in the 1992-2013 period among white Hispanics and non-Hispanics (APC= 0.73 and 0.84, respectively). Among females, incidence rates also rose significantly during 1992-2013 among white non-Hispanics, white Hispanics, and Asians (APC= 0.81, 0.56, and 1.23, respectively). In contrast, rates among black males (0.007) and females (0.009) decreased over time. The greatest increase in incidence was among white males who were non-Hispanic age ≥55 years and white Hispanics in the 55-74 age group. Among black males, rates declined in the 45-54 age group at a statistically significant pace. Pancreatic cancer incidence rates for females rose among white non-Hispanics in all age groups <85 years (range: 0.47-4.01), among Asians of ages 55-84, and white Hispanics in the 55-64 age-group. Most pancreatic cancers were specified as adenocarcinoma, not otherwise specified (NOS) or ductal adenocarcinoma. Rates increased among all racial/ethnic groups for adenocarcinoma, NOS and ductal adenocarcinoma while those for cystic mucinous ductal adenocarcinoma and poorly specified type decreased. The incidence rates of non-secretory pancreatic endocrine cancer rose >6% per year among white non-Hispanics and Asian/Pacific Islanders. Overall incidence rate ratios were significantly high among young males than females (p < 0.001). The IRR was >1.00 at all ages ≥35, but rates among females were significantly higher at younger ages (IRR <0.81). The M/F IRRs were elevated for acinar cell adenocarcinoma (2.85), non-secretory endocrine cancers (1.47) and ductal cystic or mucinous adenocarcinomas (1.11), while for solid pseudopapillary adenocarcinoma there was a significant female excess (0.22). Conclusion: Pancreatic cancer incidence rates vary within demographic groups and histologic subtypes.

#262 Analysis of demographics, survival and patterns of care of pediatric glioblastoma using National Cancer Database. Jigisha Thakkar,1 Meng Liu, Emily Van Meter Dressler, John L. Villano. University of Kentucky, Lexington, KY.

Background: We analyzed the largest clinical database in the United States, the National Cancer Database (NCDB), comprising over 70% of cases diagnosed and/or treated at commission on Cancer approved institutions. We analyzed current hospital-based epidemiologic frequency, survival and patterns of care of pediatric glioblastoma (GBM). Methods: Cases included patients 0-19 years between 1998-2011. Inclusion criteria for histology codes included for GBM 9440, 9441 and 9442 (9440 (GBM), 9441(Giant Cell) and 9442 (Sarcomatas component) and tumor sites (C70.0-C72.9, C73.1-C73.5) related to the brain: brain stem, cerebellum, cerebrum, thalamus, and brain not otherwise specified (NOS). Kaplan-Meier survival estimates were calculated for each demographic criteria and treatment plan and Cox proportional hazards models were employed to assess the risk of mortality. Demographic variables analyzed were age, gender, race, Hispanic origin, income, education, region and insurance status. Comparisons were made among histologic subtypes and primary site. Results: 1173 patients with GBM from ages 0-19 with 1998-2011 data from 1998 to 2011 in the United States, of which 21.5%, 24.5% and 54.0% were in age group 0-5, 6-10 and 11-19 years old. The largest group (48%) received advanced care including a combination of radiation (XRT), chemotherapy and surgery. On the other hand, the no treatment group was the smallest comprising of 4% cases. 14% received surgery only, 8% received a combination of XRT and surgery and 9% received a combination of XRT and chemotherapy. Patients that received combination of XRT, chemotherapy and surgery had significantly improved survival compared to those without any treatment (p = 0.0048). Patients that received surgery or chemotherapy had significantly improved survival compared to patients that did not receive the above treatments (p = 0.0027). Conclusion: We report an extensive demographic and survival analysis of pediatric GBM. Observed difference likely reflect biology across age, gender, race and origin groups. Our analysis demonstrates concern in the delivery of optimal care to a large percentage of pediatric patients. These treatments include surgery and chemotherapy that have demonstrated survival benefits. Adverse effects of chemotherapy and XRT can have the limit the appropriate administration of treatment.
Introduction: In this study we investigated epidemiology and trends of primary lung cancers in northeast of Iran during 1985-2012. Methods: In this cross-sectional study records of primary lung cancers from 1985 to 2012 in Mashhad, northeast of Iran, were investigated. Data were obtained from the referral oncologic hospital and private radiation oncology clinic. To study trends, the duration of study was categorized into three periods: 1985-1995, 1995-2005 and 2005-2012. Statistically significant level was considered <0.05. Results: Of 939 cases with mean age of 60.57 (±12.31) years, 660(70.3%) were male. Male to female ratio was 2.36. Mean age was 61.47±12.01 years for males and 58.45±12.75 years for females (p=0.001). Mean age at diagnosis and rate of smokers was unchanged in three intervals (table 1).

Table 1: Demographic characteristics of patients with lung cancer.

<table>
<thead>
<tr>
<th>Time period</th>
<th>Male</th>
<th>Female</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>1985-1995</td>
<td>46 (21.6)</td>
<td>151 (31.4)</td>
<td>21 (23.3)</td>
<td>100 (32.2)</td>
</tr>
<tr>
<td>1995-2005</td>
<td>100 (65)</td>
<td>54 (35)</td>
<td>74 (74)</td>
<td>26 (26)</td>
</tr>
<tr>
<td>2005-2012</td>
<td>119 (75.4)</td>
<td>74 (74)</td>
<td>159 (75.4)</td>
<td>74 (74)</td>
</tr>
</tbody>
</table>

Squamous cell carcinoma with relative frequency of 44.2% was the most frequent pathologic subtype in total population followed by adenocarcinoma (18.2%), Small Cell Lung cancer (16.8%), large Cell carcinoma (1.3%) and other subtypes (19.5%). Relative frequency of pathologic subtypes had significant changes in three intervals (p<0.001). Relative frequency of adenocarcinoma and large cell carcinoma were increasing while it was decreasing for SCC and SCLCs during three recent decades. There was statistically significant difference in mean age at diagnosis (p<0.001), rate of smokers (p<0.001) and male to female ratio (p=0.01) between four leading pathologic subtypes. Mean age at diagnosis (55.92±12.90 years), male to female ratio (1.69), and rate of smokers (46.8%) were lowest in adenocarcinomas. Conclusion: Similar to universal picture, adenocarcinoma showed increasing rates during recent three decades with higher prevalence among younger aged patients, women and nonsmokers. These trends are indicative of changes in exposures and smoking habits and reveal the need for regional studies in this context.

#264 Time trend for the ratio of rural and urban patients with esophageal squamous cell carcinoma in a single institute in northern China over the past 30 years. Shou Jia Hu,1 Hai Jun Yang,2 Neng Chao Wang,2 Xiun Min Li,3 Shuang Lv, Qi De Bao,4 Wen Bin Yue,5 Fu You Zhou,6 Jian Li,8 Li Dong Wang1.

Aim: This study was aimed to evaluate the age at the time of diagnosis of breast cancer and its’ probably changes in distribution among patients during 30 years. Methods: This cross-sectional study was conducted in Mashhad, Iran from 1981 until 2011. The data of patients were obtained from case records. The duration of study was categorized into three periods: 1981-1990, 1991-2000 and 2001-2011. SPSS software was used for analyses. The difference was considered significant if P<0.05. Result: From 6274 breast cancer cases, 174 patients were male (2.5%) with detailed address. Based on the diagnosed time, these patients were divided into three groups with periods of 1985-1994, 1995-2004 and 2005-2014. The rural and urban regions were recognized based on the government administrative division of China. The rural region in China refers to the farmer village with hundreds to thousands population. Chi-Square test was used to analyze the different distributions of ESCC patients with a p-value of less than 0.05 as significant. The results demonstrated that more than 84% of the ESCC patients occurred in rural regions with an overall ratio of 5:1 for rural and urban regions (p<0.0001). Moreover, over the past 30 years with three periods, the ratios for rural and urban regions were 5.4:1, 5.5:1 and 5.4:1, respectively. The present study demonstrated that the past 30 years with three periods, ESCC occurred predominantly in males with ratios for male and female as 1:5.1, 1:4.1 and 1:5.1, respectively in rural regions and 1:6.1, 1:6.1 and 1:8.1, respectively in urban regions. Interestingly, the mean age both in male and female in urban regions was slightly older than that in rural regions (urban vs. rural: 60.7±9.86 vs. 59.8±9.38 in male and 62.2±9.57 vs. 60.6±9.47 in female, respectively). The present study demonstrated that ESCC remains the major healthy burden in rural regions in China. Considering the relative low socioeconomic status in China, the prevention and control of ESCC should be enhanced in rural regions in terms of more control programs, medical and education resources. [Supported by National High-Tech Research and Development Program of China (SQ2015A.A0202183), Project for Characteristic and Advantage Discipline of Henan Province (20151208)]

#265 Global transition in cardia and non-cardia gastric cancer incidence, 1998-2007. Asesh Shin,1 Dalis Seunghun Kim,2 Hyungtaek Woo1. 1Seoul National University College of Medicine, Seoul, Republic of Korea; 2USF Morsani College of Medicine, Seattle, FL.

Esophageal cancer and non-cardia cancer differs in their descriptive patterns and risk factors. The objective of this study was to determine the global trends in incidence of cardiac and non-cardiac gastric cancer. Ninety two high quality registries with the proportion of Not Otherwise Specified (NOS) gastric cancer (ICD-10 code C16.8-9) below 50% and the total number of gastric cancer cases above 300 were selected from 225 registries in Cancer Registry of Five Continents (CI5) volume 9 and 262 registries from CI5 volume 10. The NOS cases were re-distributed to cardia and non-cardia according to the registry-specific proportion. Age-standardized incidence rates (ASRs) per 100,000 population and incidence rate ratios (IRR) from 1998-2002 to 2003-2007 were calculated. Non-cardia cancer incidence was significantly increased in 5 out of 10 Asian registries, 17 out of 18 European registries, 27 out of 56 North American registries, and all 5 Oceanian registries in men. Only two registries in Asia showed a significant increase in non-cardia cancer incidence. In contrast, cardiac cancer significantly increased in one Asian registry, two European registries, and two North American registries. Significant decrease in cardia cancer was observed in 3 Asian registries, 6 European registries, 11 North American registries, and 3 Oceanian registries in men. In women, six registries (1 Asian, 2 European, and 3 North American) showed significant increase in non-cardia cancer, whereas registries 23 (5 Asian, 14 European, 8 North American, and 1 Oceanian) showed significant decrease in non-cardia cancer incidence. Cardia cancer in women increased in 16 registries (2 Asian and 14 North American) and decreased in 9 registries (1 Asian, 5 European, and 3 North American). In conclusion, non-cardia cancer incidence decreased in most registries, whereas decrease in cardiac cancer is more prominent in men but not in women.

#266 Trends in age distribution of breast cancer, in north east of Iran: Report of six thousand patients over 30 years. Maryam Salehi, Soodabeh Shahidzadeh, Mehdi Seilianounti, Gholamhosein Noferesti, Mahta Salehi, Amirhossein mohammadian baqirian. Mashhad University of Medical Sciences, Mashhad, Islamic Republic of Iran.

Aim: This study was aimed to evaluate the age at the time of diagnosis of breast cancer and its’ probably changes in distribution among patients during 30 years. Methods: This cross-sectional study was conducted in Mashhad, Iran from 1981 until 2011. The data of patients were obtained from case records. The duration of study was categorized into three periods: 1981-1990, 1991-2000 and 2001-2011. SPSS software was used for analyses. The difference was considered significant if P<0.05. Result: From 6274 breast cancer cases, 174 patients were male (2.5%) and 6100 were female (97.5%). Mean age at the time of diagnosis was 49.07±12.07 years (male: 56.48±14.14, female: 48.8±12.01). There was significant difference in age by sex (p<0.001). Table 1 presents the mean age of studied patients in each 10 years of study. ANOVA test showed that there was not statistical significant difference (P value= 0.1).

<table>
<thead>
<tr>
<th>Decades</th>
<th>Frequency</th>
<th>Mean age</th>
<th>SD</th>
<th>Youngest</th>
<th>Oldest</th>
</tr>
</thead>
<tbody>
<tr>
<td>1981-1990</td>
<td>467</td>
<td>47.97</td>
<td>12.043</td>
<td>18</td>
<td>88</td>
</tr>
<tr>
<td>2001-2011</td>
<td>3949</td>
<td>49.22</td>
<td>11.954</td>
<td>20</td>
<td>98</td>
</tr>
</tbody>
</table>
The most histologic type of breast cancer was ductal carcinoma 65.6% others percent in order were: NOS 19.6%, lobular 3.7%, medullary 2.8%, adenocarcinoma 2.8%, tubular and papillary 0.2% and 5.1% of other types. Relative frequency of histologic types had significant differences among 3 intervals (p <0.001), the frequency of adenocarcinoma was decreasing but ductal carcinoma was increasing during 3 decades. Mean age was ductal carcinoma, lobular, medullary and adenocarcinoma was 48.9 ±11.8, 50.6 ±12.4, 46.4 ±11.4, 50.2 ±11.9, respectively. Mean age had significant differences among these main histologic types (p =0.002).98% of patients were urban and 2% were rural. Mean age of these 2 groups has statistical significant difference (P = 0.001) and is 10 years older in rural patients. Frequency of people who live in urban area was increased significantly (p =0.08) during these 30 years of diagnosis and this trend concurs: breast cancer occurs in Iranian women at least one decade younger than women in developed countries we found no change in mean age of diagnosis for breast cancer during these 30 years from 1981 to 2011.

The most likely cluster identified was a cluster of low ICC incidence [aRR 2.47, p-value <0.01] was identified in a suburban and adenocarcinoma was 48.9 ±11.8, 50.6 ±12.4, 46.4 ±11.4, 50.2 ±11.9, respectively. Mean age had significant differences among these main histologic types (p =0.002).98% of patients were urban and 2% were rural. Mean age of these 2 groups has statistical significant difference (P = 0.001) and is 10 years older in rural patients. Frequency of people who live in urban area was increased significantly (p =0.08) during these 30 years of diagnosis and this trend concurs: breast cancer occurs in Iranian women at least one decade younger than women in developed countries we found no change in mean age of diagnosis for breast cancer during these 30 years from 1981 to 2011.

Epidermology: Descriptive Epidemiology including Trends in Incidence and Prognosis

#267 GeoSurveillance of invasive cervical cancer incidence by counties in the state of Maryland. Sally Peprah, Frank Curriero, Amber D’Souza. Johns Hopkins University, Baltimore, MD.

Background: Invasive cervical cancer (ICC) is a highly preventable cancer whose rates have dramatically declined over the past few decades. In spite of the progress made, new ICC cases are recorded annually in Maryland and in other parts of the United States. We assessed if ICC incidence varied significantly 2005-2012 and across counties within the state of Maryland. Methods: Using a discrete Poisson model we evaluated significant space-time clusters of both high and low ICC incidence rates over a retrospective 10-year period. All analyses were con-
ducted at the county level, using 2-year aggregates of time. We adjusted for age-
percent minority, cervical cancer screening rates, median income, obesity as well as smoking rates within each county in our final cluster detection analysis. Re-
sults: ICC was 13.1 per 100,000 female population and did vary by county and over the 10-year period assessed. We identified five significant space-time clus-
ters of ICC incidence, three of which were clusters of higher than expected rates. The most likely cluster identified was a cluster of low ICC incidence [aRR =0.08, p-value<0.01] observed for the period 2009-2012 and comprised six high-
income suburban counties. The other cluster of low rate [aRR =0.41, p-value<0.01], which was a secondary cluster, was observed for the same time period, 2009-2012 in three high to moderate-income suburban counties. There were two clusters of high ICC incidence, observed for recent time periods. One cluster [aRR=1.74, p-value<0.01], which was made up of a single moderate to low-income urban county was identified for the period 2009-2012. The other cluster of elevated rates [aRR =2.47, p-value<0.01] was identified in a suburban moderate-income county for the period 2011-2012. Additionally, for the period 2005-2008 we identified a historical cluster of elevated incidence [aRR =2.53, p-value<0.01], Conclusion: Overtime some counties in Maryland have experi-
enced significantly lower rates of disease than expected. However there are more recent clusters of higher than expected rates of disease, which are not explained by the county level variables adjusted for. These clusters of high rates need to be further examined and addressed.

#268 Tracking HPV vaccination of young boys: is parental recall as de-
pendable as physician records. Nosayaba Osazuwa-Peters,1 Betelihem B. Zou-
eda Zaidi, Mokhtar Hamdi Cherif. University Hospital of Setif, Setif, Algeria.

Introduction: HPV vaccine is administered to young boys and girls aged 11-12 years in multiple doses rather than single dose. The interval between doses is critical as well as completion of the dose series. Moreover, the administration of the HPV vaccine in 2 doses is a recent change from the 3 doses previously recommended for children aged 11-12 years. Therefore, it is critical that HPV vaccination uptake records are accurately kept as interventions are developed to increase HPV vaccine uptake. Previous studies on the validity of parental re-
ported HPV vaccine uptake have focused only on adolescent girls. This is be-
cause the HPV vaccine recommendation for boys came 5 years after that for girls. Currently, vaccination rates among boys lag behind those for girls across the United States. This study compared the validity of parent- vs. provider-
reported HPV vaccine initiation and completion rates among a national sample of adolescent boys in the United States. Methods: We analyzed the 2014 National Immunization Survey-Teen data for adolescent boys only (n = 9,493), and ob-
tained information on the records of HPV vaccine uptake (initiation (=1 dose) and completion (= 3 doses)) for both parents (parental recall) and provider reports (electronic medical records). We compared the validity of parent- versus provider-reported HPV vaccination by computing validity measures [sensitiv-
ity, specificity, positive predictive value (PPV), negative predictive value (NPV), and kappa]. Results: Rates reported by provider and parental recall were similar for HPV vaccine initiation (41.8% vs. 42.7%, respectively), and completion (19.6% vs. 21.9%, respectively). Compared to provider report, parent-reported HPV vaccine initiation had a sensitivity of 83.0%, a specificity of 88.8%, a PPV of 84.1%, a NPV of 87.9%, and a kappa coefficient of 0.72. Compared to provider report, parent-reported HPV vaccine completion had a sensitivity of 68.6%, a specificity of 92.9%, a PPV of 72.9%, a NPV of 91.4%, and a kappa coefficient of 0.63. Conclusions: Both parent- and provider-reported HPV vaccine initiation and completion rates were comparable; however, parental recall of HPV vaccine completion among boys was less sensitive. Ongoing surveillance of HPV vacci-
nation rates among boys is important as more studies are designed to improve HPV vaccine uptake and understanding these 30 years of HPV vaccine barriers. In this regard, parental recall may be used as an effective alternative in assessing HPV vaccine uptake in the United States.

#269 The descriptive epidemiology of gynecologic cancers: an interna-

Introduction: Gynecologic Cancers are the most common cancers in women, in 2012, Cervical Cancer (CC) is ranking as the fourth with an estimated 528,000 new cases and Ovarian Cancer (OC) is the seventh most common for females with nearly 239,000 new cases worldwide. Aim: this communication presents the latest international descriptive epidemiological data for invasive GC, includ-
ing incidence, survival and mortality in the worldwide. Methods: The incidence and mortality statistics presented for GC worldwide were taken from the Intern-
national Agency for Research on Cancer IARC: * the Cancer Incidence in five Continents Vol X and * GLOBOCAN database, 2012. The data of cancer sur-
ival are taken from: * Cancer survival in five continents, a worldwide popul-
ation-based study (CONCORD) version 2, 1995-2009. Estimated five-year net survival, adjusted for background mortality by single year of age, sex, calendar year in each country. Results: CC is the most common cancer among women in 43 countries mainly in sub-Saharan Africa, part of Asia and some countries in Central and South America, the lowest incidence rates in western Europe, North America, Australia and the eastern Mediterranean. Almost 55% of all new cases of OC occurred in countries with very high level of human development mainly northern Europe and America and Oceanic, Africa presents the lowest inci-
dence. -Data for CC are available for 602 225 women, CC survival was 50% or higher in most countries, except for Libya (Benghazi, 39%) and India (Karuna-
gapally, 46%). During 2005-09, age-standardised 5-year net survival was 70% or higher in Iceland, Mauritius, Norway, South Korea, and Taiwan, for Qatar is also above 70% (based on only 16 cases and is not age-standardised). -Data for ova-
rian cancer are available for 779 302 women, during 2005-09, age-standard-
sed 5-year net survival was 40% or higher in Ecuador, the USA, nine countries in Asia and eight countries in other regions mostly in the range 30–40%, except for Libya (22%). Conclusions: The evolution of cancers in women shows a consistent and very striking pattern during the epidemiolog-
ical transition with rapid declines in the incidence of cervical cancer. Compa-
rision of population-based cancer survival CONCORD study 2 showed very wide variations in survival from gynecologic cancer worldwide.

#270 Trends in colorectal cancer survival in Arab World, 1990-2009. Zou-
bida Zaidi, Mokhtar Hamdi Cherif. University Hospital of Setif, Setif, Algeria.

Introduction: Cancer survival is a key measure of the effectiveness of health-care systems. Globally, colon and rectum cancer ranked third for cancer incidence and fourth for cancer death in 2013. For developed coun-
tries it ranked second for incidence and mortality, and in developing coun-
tries it ranked fourth for both incidence and mortality. An increasing trend in incidence is reported from various registries of Arab world, Kuwait and Saudi Arabia present the highest incidences worldwide. Objective: To de-
scribe the trends of the survival of colorectal cancer patients diagnosed in Arab countries. Material and Methods: This report is a summary of the two survival figures of CONCORD study 1 (1990-1994) and CONCORD study 2 (1995-2009). Individual colon and rectum tumour records were submitted by 06 population-based cancer registries in Arab countries (Jordan, Saudi Arabia, Qatar, Algeria, Libya and Tunisia) for 9050 patients (15-99 years) diagnosed during 1990-2009 and followed up to 31 December 2009 . Esti-
mated five-year net survival, adjusted for background mortality by single year of age, sex, calendar year in each country. Results: For patients diag-
nosed during the period 2005-2009, the age-standardized five-years net sur-
rvivals were respectively higher 68.2% for colon cancer and 77.8% for rectal cancer in Qatar and the lowest rate for rectal cancer 21% in Jordan, between 1995-1999 and 2005-2009, Survival increased in Algeria, but this trend is less reliable Conclusions: Comparison of population-based cancer survival
Concord study showed very wide variations in survival from colorectal cancer in Arab world. Cancer survival research is being used to formulate cancer control and the need to implement effective strategies of primary prevention.

**271 Clinical and molecular study of breast cancer in Algerian women from the Aures region: Data from the anti-cancer center of Batna. Farid Cherbali,1 Chiraz Mehennim,2 Khadija Gassi,3 Abdelmoumen Gue-divra,4 Yos Hamidi,5 Wassila Benbrahim,6 Rabah Bakour1.1 Unit of Genetics, LMBM, Faculty of Biological Sciences, USTHB, Algiers, Algeria; 2Laboratoire de Génomique Biomédicale et Oncogénétique (LR11 IPT 05) Institut Pasteur de Tunis, Tunisia; 3LOBEM, Faculty of Biological Sciences, USTHB, Algiers, Algeria; 4Anti-cancer center of Batna, Batna, Algeria.

Background: Breast cancer is currently the leading cause of cancer morbidity and mortality among Algerian women. The aim of this study was to analyze the different proportions and distribution of breast cancer molecular subtypes and to determine their associations with some clinical and pathological characteristics in women from the Aures region. We also screened for the prevalence of BRCA1 germline mutations in HBOC patients. Materials and Methods: Clinical and tumor characteristics data of 1144 breast cancer patients were collected from cancer registry of anticancer center of Batna. Breast cancers were diagnosed between 2011 and 2015. Breast cancer subtypes definitions were as follows: Luminal A (ER+/PR+, HER2−), Luminal B (ER+ and/or PR−, HER2±), TNBC (ER−, PR−, HER2±), and HER2+ (ER−, PR−, HER2+). We also compared patients with TNBC and those with other breast cancer subtypes. In addition, we compared patients with the two groups regarding clinicopathological parameters: age at diagnosis, menopausal status, histological type, histological grade, breast feeding and oral contraception were examined using Chi square test. BRCA1 was screened by PCR direct sequencing in 22 HBOC patients including breast feeding and oral contraception were examined using Chi square test.


Background: The risk of primary central nervous system lymphomas (PCNSL) is greatly increased in HIV-infected people. Case series have described PCNSL in immunosuppressed solid organ transplant (SOT) recipients. Herein, we examine the incidence and risk factors for PCNSL in SOTRs. Methods: We used data from the Transplant Cancer Match Study, which links the US transplant registry with 17 cancer registries (1987-2014). PCNSL risk relative to the general population was estimated as a standardized incidence ratio (SIR = observed/expected cases). Poisson regression was used to estimate adjusted incidence rate ratios (aIRR) of PCNSL across subgroups of SOTRs. Logistic regression was used for case–control comparisons of PCNSL with other non-Hodgkin lymphomas (NHL). Results: We included 286,637 SOTs. There were 173 PCNSL cases (SIR 57.7; 95% CI 49.4-66.9) and 2,583 other NHLs (SIR 7.3; 95% CI 7.0-7.6). Most PCNSL were diffuse large B-cell lymphomas (n=118; 68.2%). Compared to kidney SOTRs, PCNSL risk was lower in liver SOTRs (aIRR 0.3; 95% CI 0.3-0.9), not different in heart and/or lung SOTRs (aIRR 0.9; 95% CI 0.6-1.5) and higher in other/multiple SOTRs (aIRR 2.4; 95% CI 1.5-3.8). Asians/Pacific Islanders had higher PCNSL risk than non-Hispanic whites (aIRR 2.0; 95% CI 2.3-3.3). People who received induction therapy with alemtuzumab (aIRR 2.8; 95% CI 1.5-5.3) or polyclonal antibodies (aIRR 1.9; 95% CI 1.3-2.8) had higher PCNSL risk. SOTRs who were seronegative for Epstein-Barr virus (EBV) at transplant had higher risk (aIRR 2.0; 95% CI 1.1-3.5) than seropositive SOTRs. PCNSL risk was high in the first 1.5 years after SOT (0.5-1 year, aIRR 2.6; 1.5 years, aIRR 2.3; vs. 0.5 year) and progressively decreased over time (ptrend<0.0001). Risk did not differ according to the age at SOT, sex, or maintenance immunosuppressive regimen. Compared to other NHL, PCNSL cases were more likely to be middle aged (18-64 years) at transplant (aIRR 0.099). Asians/Pacific Islanders (p=0.02), or have received induction therapy with polyclonal antibodies (p=0.002), and less likely to be liver or heart and/or lung SOTRs (p=0.02). EBV serostatus did not differ between PCNSL and other NHL (p=0.11). Conclusions: PCNSL risk is very elevated among SOTRs. Because EBV-seronegative SOTRs are at risk of primary infection after SOT, these results highlight the importance of contribution of EBV to PCNSL. Particularly, we identify EBV-receptor status (within the first 1.5 years after transplant, in people who receive multiple non-thoracic organs, and is associated with induction therapy with alemtuzumab or polyclonal antibodies. Case–case differences with other NHLs suggest unique epidemiologic factors leading to PCNSL.
there are underlying molecular differences between E-CRCs and T-CRC that may explain these differences. To investigate this we analyzed the TCGA CRC dataset for genomic markers that can distinguish between E-CRC and T-CRC tumors. Results: E-CRC incidence has risen at an annual rate of 1.4% per year from 2000-2011, whereas T-CRC incidence has declined by 3.1% per year among patients 50 years or older during the same period. Relative risks: 2.04 (95% CI 1.90-2.19) for E-CRC compared to T-CRC. E-CRC rates are significantly more prevalent in the Black population than the White or Hispanic populations. Anatomical location of E-CRC is significantly increased towards the distal colon when compared to T-CRC locations suggesting a distinct etiology. Analysis of risk factors confirmed that U.S counties with high rates of diabetes, obesity and smoking were significantly correlated with higher T-CRC rates. However, no such correlations were observed for E-CRC rates. Analysis of the limited number of E-CRC in the TCGA cohort identified a number of known oncogenes that are suggestive of distinct E-CRC tumorigenesis. Conclusions: Our results suggest that E-CRC appears to be distinct from T-CRC. Young patients with CRC represent a distinct patient group with unique epidemiology and mechanisms of disease development. The increase in left sided colon and rectal cancers is consistent with earlier reports and although the underlying reasons are unknown, they are suggestive of differences in tumor genesis and mutational characteristics. To follow up on this hypothesis we are performing additional genetic and epigenetic characterization of E-CRC and matching T-CRC samples collected at Weill Cornell.

#275 The prevalence of endometrial cancer in women with postmenopausal bleeding: a systematic review and meta-analysis. Megan A. Clarke,1 Beverly J. Long,2 Arena Del Mar Morrill,1 Jamie N. Balkum-Games,1 Nicolas Wentzensen,3 National Cancer Institute, Rockville, MD; Mayo Clinic, Rochester, MN.

Background: Endometrial cancer is highly curable when detected early, but clinical symptoms are often missed. Most cancers arise in women with abnormal postmenopausal bleeding (PMB). However, PMB is not specific for endometrial cancers. In previous reports, the prevalence of endometrial cancer in women with PMB was reported to range from 3-25%. Because of the importance for guiding clinical management and endometrial cancer risk prediction, we conducted a systematic review and meta-analysis to obtain precise prevalence estimates of endometrial cancer in women with PMB. Methods: A systematic review and meta-analysis summarized all peer-reviewed studies reporting endometrial cancer prevalence in women with PMB, published before Sept 1, 2016. We calculated a pooled prevalence estimate and 95% confidence interval (CI) using a random-effects model, and quantified heterogeneity across studies using the I² statistic. Subgroup meta-analyses were performed to investigate sources of variability according to selection criteria for endometrial thickness (ET) and hormone replacement therapy (HRT). We also stratified by study location to determine whether prevalence estimates varied by country or region if ≥5 studies included per country. Results: 105 studies were included, contributing data for 37,000 women with PMB and 3,028 with cancer from 34 countries. Overall, the pooled prevalence of endometrial cancer in women with PMB was 11% (95% CI 10-12%), with F = 94.3%. Among studies that selected women with a minimum ET (≥4mm; n = 14), the pooled prevalence of endometrial cancer was 17% (95% CI 14-20%) compared with studies that did not select for ET (10%; 95% CI 9-11%). In studies that excluded women using HRT (n = 34), the pooled prevalence of endometrial cancer was 15% (95% CI 12-17%) compared with studies that did not exclude women using HRT (10%; 95% CI 9-11%). Additional stratification by ET and HRT revealed variation in prevalence estimates. For example, in the United States (n = 9) and the United Kingdom (n = 16), prevalence of endometrial cancer was 4% (95% CI 2-7%) and 5% (95% CI 4-6%), respectively, whereas in Italy (n = 14), the prevalence was 11% (95% 8-14%). Regional differences were also observed, ranging from 8% (95% CI 3-14%) in South Central Asia (n = 5) to 18% (95% CI 12-23%) in Southern Europe (n = 11). Conclusions: The overall prevalence of endometrial cancer among unselected women with PMB is approximately 10% with substantial heterogeneity across studies. Marked geographic differences in the prevalence endometrial cancer were observed. Factors related to selection for ET and/or HRT use may partially contribute to this variability. Risk prediction models depend on precise prevalence estimates for accurate prediction and utility in clinical management. More research is needed to test the applicability of endometrial cancer risk prediction models in high-risk subgroups and in study populations from diverse geographic regions.

#276 Retrospective analysis of epidemiological variants of malignancies in Sokoto, Northwestern Nigeria. Saddiku M. Sahabi, Kabiru Abdullahi. Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria.

Background: The burden of cancer in Nigeria is increasing. Information on incidence, prevalence, pattern, and high risk factors are essential for evaluation and cancer control. This report is a follow up from earlier previous report published in 2005 on cancers seen in Usman Danfodiyo University Teaching Hospital (UDUTH) Sokoto. Objectives: The aim of this study is to determine epidemiological variants of all malignances seen from 1 January 2006 to 31 December 2015. Materials and Methods: This is a retrospective review of all malignancies seen at the Usmanu Danfodiyo University Teaching Hospital in Sokoto during the period 2006 to 2016. There was no restriction on gender or age groups. Mean, frequencies, percentages, and independent sample t-test were performed using SPSS version 21.0 for Windows. Results: A total of 1,435 oncology patients diagnosed from birth to 23 years of age at St. Louis Children’s Hospital from January 1, 2004 - December 31, 2014 were reviewed. CA information was extracted and verified with ICD-9 codes when available. Patients followed for <1 year at SLCH (n = 193), and those diagnosed with a chromosomal anomaly or known cancer predisposition (n = 113) were excluded from the analysis. Bivariate analyses compared demographic and other characteristics between patients with and without a CA with significant differences determined by chi-square tests. We calculated age-adjusted standardized rate ratios (SRRs) to evaluate whether the observed number of CAs among specific cancer types (benign, bone, CNS, epithelial, leukemia, lymphoma, germ cell, and soft tissue tumors) varied from the expected number using the CA rates from the entire cohort as the reference. Differences in survival time distributions in cancer cases with CAs versus those without CAs were evaluated with the log-rank test. Results: Of 1,129 SLCH pediatric cancer patients, 154 (14%) patients were identified with a CA. Overall there was an increased proportion of patients with a CNS tumor who also had a CA compared to those without a CA (p = 0.005). Neurological anomalies were specifically found to be in excess in CNS tumors cases versus the overall population of pediatric cancer patients (SRR = 1.42 95% CI 1.02-1.92 p = 0.038). There were no significant differences by age at primary tumor diagnosis, but patients with a CNS tumor and CA were diagnosed an average of 1.5 years earlier (7.7 vs. 9.2 years, p = 0.075) compared to those without a CA. The rate of CAs did not vary significantly by sex, but a significant excess of males with a neurological anomaly was observed among all patients diagnosed <5 years of age (M/F ratio = 2.53 95% CI 1.15-5.56 p = 0.02). Finally, survival between those with and without a CA was not significantly different (p = 0.24). Conclusions: This study provides additional insight into the association between specific types of CAs and cancer development. Our results suggest children with neurological anomalies are more likely to develop CNS cancers and may be at an increased risk to develop cancer at an earlier age, particularly in males. Our study supports the need for additional longitudinal surveillance and research that may improve outcomes as well as translational research to investigate any associated developmental mechanisms that may underlie tumor predisposition.
#279 Clinicopathologic features and management of bronchopulmonary and pleural malignancies in Ile Ife, Nigeria. olanusum O. Adewole, Obafeni Awalowo University/Teaching Hospital, Ile Ife, Nigeria.

Introduction. Lung cancer is the most common cancer of cause related death worldwide with increasing incidence in developing countries, Nigeria inclusive. The aim of this study is to evaluate the clinical patterns, diagnostic procedures and management of bronchopulmonary cancer (BPC) and pleural cancer (PLC) seen in Ile Ife, Nigeria. Method. All cases of bronchopulmonary and pleural cancer seen over an eighteen year period (1990-2015) at OAUTHC, Ile Ife, Nigeria, a tertiary center were reviewed. Cases were identified from the admission and discharge files on the wards and were retrieved from the main medical record library. All retrieved cases were analyzed and presented, using a descriptive statistics. Results. Thirty seven cases of BPC and PLC were identified of which twenty two cases files were fully retrievable, and these are presented. There were twenty one cases of BPC and two PLC. The male to female ratio of BPC was 2:1 while it is 1:1 for PLC. The mean age of patients was 51 years (range 48-85yrs) and 45(30-66yrs) for BPC and PLC respectively. Cough and weight loss were the most common symptoms among at the patients. 45% had chest pain while 15% had hemoptysis. The symptoms had presented for more than 3 months. Outdoor pollution were identified as the risk factors in 20% of each patients with BPC. 10% of patients with BPC had a past history of TB about 10 years prior to the presentation. One of the cases of PLC some had exposure to asbestos in his work as a carpenter. Three patients with BPC presented with features of SVC. Pleural effusion was the main chest pathology, it was found in 65% of the BPC cases and the entire PLC. The right hemithorax were mainly involved. Diagnoses were made by pleural fluid cytology, pleural biopsies, FNAC, LN biopsies, bronchoscopy in 41%, 10%, 10%15% and 5% of BPC respectively. Other diagnostic procedures done in PLC included open thoracotomy. Adenocarcinoma was the commonest histologic type of BPC, while it was mesotheloma for PLC. Few.

The lymph node metastasis (LNM) is one of the crucial risk factors for esophageal squamous cell carcinomas (ESCC) prognosis. However, it is difficult to precisely determine the LNM status before surgery. The maximum tumor length (MTL) may reflect the ESCC growth speed and could be calculated accurately either with computed tomography or ultrasound endoscopy before esophagectomy. Therefore the present study was designed to determine if the MTL could reflect the LNM status for ESCC. A total of 22,660 ESCC patients was enrolled in this study from the ESCC database (1973-2015) in Henan Key Laboratory for Esophageal Cancer Research of The First Affiliated Hospital, Zhengzhou University. Of these patients, there were 13,793 males with an mean age of 60±8, and 8,867 females with a mean age of 61±8. All the cases had been performed radical esophagectomy. The MTL and LNM records were retrieved from the medical records in hospitals. Based on the 6th version of UICC criteria, the LNM was classified as N0 and N1. The MTL was classified as 5 groups: <2cm, ≥2 and <4cm, ≥4 and <6cm, ≥6 and <8cm, ≥8cm. The SPSS21.0 software and Spearman rank correlation were used to determine the correlation of MTL and LNM. The results demonstrated that the average and median of MTL were 4.1±1.8 cm and 4.0 cm (range: 0.1-18 cm) in these ESCC patients. The positive lymph node metastasis rates in each MTL groups were 19.1%, 34.1%, 45.8%, 51.5%, and 54.1%, respectively. Spearman rank correlation analysis indicated that MTL was strongly correlated with LNM (r=0.997, P<0.001), suggesting that MTL could reflect the LNM status in ESCC and that MTL could be used as one of clinical staging parameters for ESCC. Supported by Major Science and Technology Projects of Henan Province (161100311300). National Key Research and Development Program: Precision Medicine (2016).
of rectal was higher than distal or proximal cancer (OR=2.64, 1.95 and 1.32, respectively). Conclusions: Presence and strength of association of cancer risk factors may differ by CRC site. Site should be a key consideration in future studies of CRC risk.

EPIDEMIOLOGY: Descriptive Epidemiology Including Trends in Incidence and Prognosis

The accumulated evidences have indicated the importance of lymph node dissection (LND) in improving survival for esophageal squamous cell carcinoma (ESCC). However, it is largely unclear in terms of standard lymph node dissection, e.g., how many is suitable and which node station is more important, etc. The present study was undertaken to summarize the trends of lymph node dissection in ESCC over 40 years at a single institute in northern China. A total of 27,260 ESCC patients were enrolled in this study from 1974 to 2016, including 16,565 males with a mean age of 59.72 ± 7.82 and 10695 females with a mean age of 60.3±8.51. All the patients were from the ESCCC database in Henan Key Laboratory for Esophageal Cancer Research of the First Affiliated Hospital, Zhengzhou University (1973–2016). All the patients had pathological LND records, including the number dissected. The patients were classified into four period groups based on the diagnosed time, group I (1974 to 1986, 367/1.3%), group II (1987 to 1996, 312/1.7%), group III (1997 to 2006, 10, 494/38.5%) and group IV (2007 to 2016, 13,217/48.5%). The SPSS21.0 software was used to evaluate the trending changes of LND at different periods. The results showed that the total number of LND/the mean number by each patient from group I to group IV was 3,000/843, 22,001/7.07, 87,740/8.36, and 159,340/12.06, respectively (p<0.0001). Further analysis indicated that the total number of LND/the mean number by each patient from group I to group IV in males was 1,634/793, 13,428/699, 53,143/8.53 and 100,073/12.19, respectively and 1,368/848, 8,573/6.80, 34,597/8.11, 59,267/11.83, respectively in females. The present results indicate an apparent increasing in number of LND from 1987 to 2016 for esophageal cancer surgical treatment in China. Further analysis is needed to correlate the LND and metastasis and survival. (National High-Tech Research and Development Program of China (SQ2015AA0202183), Major Science and Technology Projects of Henan Province (16110031300).

#285 Standardized prediction estimates for cancerous and noncancerous gynecologic lesions: a preliminary study in an oil city of Niger Delta region. Felix M. Onyije,1 Anthony A. Ngokere,2 Aloysius E. Ligha,3 Osaro O. Mgbere, Godwin O. Awioro.4 Niger Delta University, Wilberforce Island, Delta State, Nigeria; 2Nnamdi Azikiwe University, Awka, Nigeria; 3Institute of Community Health, University of Houston, Texas Medical Center, Houston, TX; Delta State University, Abraka, Delta State, Nigeria.

The objective of this pilot study was to determine the epidemiology of gynecologic cancer and non-cancerous lesions and to develop standardized prediction estimates for the diseases in thirty-five years time. The impact of cancerous and non-cancerous lesions on women’s health cannot be underestimated, especially where there is constant emission of hydrocarbons and contaminated water level through oil spillage. Data used for this retrospective study comprised of 679 records and covering the period 2010-2014 were retrieved from the archive of Brathwaite Memorial Specialist Hospital (BMSH) Histopathology laboratory in Port Harcourt, Nigeria. Total of 32 (4.6%) cancerous and 665 (95.4%) non-cancerous lesions were diagnosed with the distribution pattern differing significantly (p<0.001) by year of diagnosis, developmental stage and age category. Leimyroma recorded the highest 5-year standardized prevalence rate of 0.508, and without intervention, it is estimated that the number of cases diagnosed will rise from 235 in 2015 to 1883 by the year 2050. This was followed by ovarian cyst with a prevalence rate of 0.124 and projected increase from 57 in 2015 to 461 by the year 2050. Similarly, the product of conception is also predicted to increase from 34 to 277 by the year 2050. The 700% increase prediction of cancerous and non-cancerous lesions by 2050 calls for urgent attention for both governmental and private agencies to fund awareness campaigns and screening for women, especially for those residing in oil and gas producing areas where oil and gas pollution are common. This may help reduce morbidity and mortality associated with these conditions.

#286 Forecast of the incidence and etiology of liver cancer in Taiwan, Japan, United States, and United Kingdom: toward harmonization of East and West. Emily Han-Chung H. Hsieue,1 Wei-Cheng Lo,2 Hsien-Ho Lin,3 Chih-Hung Hsu,4 Ann-Lii Cheng.5 Johns Hopkins School of Medicine, Baltimore, MD; 1Institute of Epidemiology and Preventive Medicine, College of Public Health, National Taiwan University, Taipei, Taiwan; 2National Taiwan University Hospital, Taipei, Taiwan.

Background: The global epidemiology of primary liver cancer (PLC), primarily hepatocellular carcinoma (HCC), has evolved significantly in the past decades because of improved control of viral hepatitis and the growing prevalence of metabolic factors. We conducted a quantitative forecast of the incidence and etiology of PLC. Methods: Incidence rates of PLC were projected in two endemic Asian countries (Taiwan and Japan) and two Western countries (the United States and United Kingdom) with rigorous cancer registry. The annual incidence of PLC (ICD-10 C22.0) between 1980 and 2013 was obtained from the
Taiwan Cancer Registry Database, the Japan National Cancer Center, the US SEER 9 database, Cancer Research UK, and the European Network of Cancer Registry. Incidence rates were projected to year 2035 by an age-period-cohort model with a power link function. The population attributable fractions (PAF) of chronic hepatitis B (HBV), chronic hepatitis C (HCV), alcohol consumption, obesity, and tobacco use (as indicated by the prevalence of smoking) were estimated. The prevalence of each risk factor was obtained from national surveys and projected inferentially by using a log-linear model or simple linear assumption. The relative risk of each factor was derived from pooled literature data. Results: From 2013 to 2035, incidence rates of PLC in Taiwan are estimated to decrease by more than 50% in both men (5.4% to 2.4/100,000) and women (9.7 to 9.2/100,000). In Japan, incidence rates have plateaued in the 1990s, and are expected to continue to decline to 17.0/100,000 for men and 6.3/100,000 for women. Conversely, rates in the UK are projected to increase by more than 30% in both men (13.5 to 18.2/100,000) and women (6.0 to 8.1/100,000). A more modest increase is expected in the US, from 10.2 to 12.5/100,000 for men and 3.3 to 3.5/100,000 for women. In Taiwan, the PAF of HBV, the predominant risk factor of PLC, is expected to decrease from 54.3% in 2015 to 54.4% in 2045. By 2045, the PAFs of obesity and DM are estimated to reach 11.9% and 15.4%, respectively, the latter similar to that of HCV (15.0%). Conclusion: PLC incidence rates are forecast to follow a trend of convergence in four representative countries. Metabolic factors are expected to play increasingly important etiologic role in PLC even in regions currently endemic for viral hepatitis.

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Cell Cycle Checkpoint and Progression

#287 BAY 1816032, a novel BUB1 kinase inhibitor with potent antitumor activity. Gerhard Siegemeter,1 Anne Mengel,1 Wilhelm Bone,1 Jens Schröder,1 Sabine Zitzmann-Kolbe,1 Hans Briem,1 Amaury E. Fernández-Montalván,1 Simon Holton,1 Ursula Mönning,1 Oliver von Ahesen,1 Sandra Johannsen,1 Arwed Cleve,1 Marion Hitchcock,1 Kirstin Meyer,1 Franz von Nussbaum,1 Michael Brands,2 Dominik Mumberg,1 Karl Ziegelbauer1. Bayer Pharma AG, Berlin, Germany; Bayer S.A.S, Lyon, France.

The spindle assembly checkpoint represents a highly conserved surveillance mechanism which safeguards correct chromosome segregation by delaying anaphase onset until all chromosomes are properly bi-oriented on the spindle apparatus. Non-catalytic functions of the mitotic kinase BUB1 (budding uninhibited by benzimidazoles 1) were reported to be essential for spindle assembly checkpoint activation. In contrast, the catalytic function of BUB1 plays a minor role in spindle assembly checkpoint activation. However, it is required for chromosome arm resolution and positioning of the chromosomal passenger complex for resolution of spindle attachment errors. Here, we disclose for the first time the structure and functional characterization of a novel, first-in-class Bub1 kinase inhibitor. Medicinal chemistry efforts resulted in BAY 1816032 featuring high potency, long target residence time and good oral bioavailability. It inhibits BUB1 enzymatic activity with an IC50 of 7 nanomolar/L, shows slow dissociation kinetics resulting in a long target residence time of 87 min, and an excellent selectivity on a panel of 395 kinases. Mechanistically BAY 1816032 abrogated nocodazole-induced Thr-120 phosphorylation of the major BUB1 target protein histone H2A in HeLa cells with an IC50 of 29 nanomolar/L, induced lagging chromosomes and mitotic delay. Persistent lagging chromosomes and missegregation were observed upon combination with low concentrations of paclitaxel. Single agent BAY 1816032 inhibited proliferation of various tumor cell lines with a median IC50 of 1.4 micromolar/L and demonstrated synergy or additivity with paclitaxel or docetaxel in almost all cell lines evaluated (minimal combination index 0.3). In tumor xenograft studies BAY 1816032 only marginally inhibited tumor growth as single agent upon oral administration, however, upon combination with paclitaxel or docetaxel a strong and statistically significant reduction of tumor size as compared to the respective monotherapy was observed. Intratumoral levels of phospho-Thr-120 H2A were found to be strongly reduced, and no hints on drug-drug interactions were found. In line with the good tolerability in xenograft studies, no relevant findings from non-GLP 2 weeks toxicological studies in rat and dog were reported. Our findings validate the innovative concept of interference with mitotic checkpoints and justify clinical proof of concept studies evaluating BUB1 inhibitor BAY 1816032 in combination with taxanes in order to enhance their efficacy and potentially overcome resistance.


Cell proliferation depends on multiple signals, such as growth factors, adhesion to the substratum and to neighboring cells. Cancer cells proliferate independently of these signals, and this increased proliferative activity is associated with increased genomic instability. Here we investigate whether the actin cytoskeleton plays a role in transmitting the proliferation signal. We found that inactivation of the Arp2/3 complex by either a chemical inhibitor or by siRNAs blocks S phase entry in untransformed human breast cell lines and in primary breast epithelial cells. In contrast, breast cancer cell lines did not respond to Arp2/3 inhibition. Inactivating the tumor suppressor Mitostatin in the genetic backgrounds of these cell lines further highlighting that this response to Arp2/3 inactivation is at the heart of the transformation process. The depletion of the WAVE complex, the Arp2/3 activator that generates branched actin in lamellipodia, but not of other Arp2/3 activators, blocks cell cycle progression. Conversely, activation of WAVE by active Rac, or depletion of Arpin, which antagonizes WAVE, potentiates cell cycle progression. Together, these results show that branched actin networks generated in lamellipodia are sensed and convey an essential signal for S Phase entry. The signal that branched actin delivers integrates stimulation from soluble growth factor with mechanotransduction from cell adhesions to the substratum and to neighboring cells. This signaling pathway qualifies as a checkpoint, which is required in normal cells, but lost in transformed cells, that monitors the integrity of branched actin networks and instructs the cell as a response that the required conditions to proliferate are fulfilled.

#289 The key role of Mitostatin in the maintenance of genome stability. Angela Lauriola,1 Andrea Caporali,2 Sabine Mai,3 Domenico D’Arca,1 Università di Modena and Reggio Emilia, Modena, Italy; 2University/BHF Centre For Cardiovascular Science, The Queen’s Medical Research Institute, Edinburgh, United Kingdom; 3Manitoba Institute of Cell Biology, CancerCare Manitoba, University of Manitoba, Winnipeg, Manitoba, Canada.

Genomic instability is a characteristic of most cancers and it refers to an increased tendency of alterations in the genome during the life cycle of cells. The fidelity of DNA replication is highly ensured by different checkpoints; the activation of spindle checkpoints prevents cells from premature entry into mitosis, avoiding incorrect chromosome segregation and aneuploidy, a typical feature of many cancers. Mitostatin, a novel protein, endowed with tumor suppressor activity, has been reported to bind centrosomal proteins Odf2 and ninein, and its depletion causes an alteration of the anchorage of microtubules to the centrosome. Since functional defects of centrosomes are associated to mitotic failure, Mitostatin may have a key role in guarding the fidelity of mitosis in cells. Here we show that the depletion of Mitostatin in cancer cells, synchronized by aphidicolin (G1/S) block and released into nocodazole-containing medium, leads to mitotic slippage and adaptation to the spindle checkpoint in the presence of a spindle inhibitor. Concomitantly, Mitostatin depletion promotes the early degradation of Mad2 and cyclin B1. Since the activated spindle checkpoint delays cell exit from mitosis by preventing cyclin B1 proteolysis, the cyclin B1 early degradation leads to mitotic checkpoint escape and resulting chromosome instability. In particular, we show that upon loss of Mitostatin chromosome bridges and mis-segregation in anaphase that are consistent with defective activation of the spindle checkpoint. In this study, we report for the first time that the depletion of Mitostatin induces an increase of numerical and structural chromosomal aberrations compared to control cells. These aberrations include aneuploidy (P=0.0005), the formation of triradials (P=0.0061) and broken chromosomes (P=0.0066). Moreover, 3D nuclear telomere analysis using TeloView shows decreases in telomeric signals (P=0.0061), in the total number of aggregates (P=0.0027), and in total intensity (P=0.018) and single intensity (P=0.019) in cells depleted of Mitostatin. These findings suggest that telomere dysfunction is increased in the absence of Mitostatin; the decrease in telomere integrity is associated with ongoing proliferation (mitotic slippage), and the maintenance of telomeric aggregates is indicative of ongoing genomic instability. Taken together, these observations suggest that Mitostatin plays a critical role in guarding the fidelity of mitosis, enabling the optimal activation of the spindle checkpoint. Thus, low levels of Mitostatin found in certain human tumors may contribute to cellular transformation by promoting genomic instability.

#290 A novel function of HGF in the phosphorylation of Chk1 in colon cancer cells. Na Song, Xiaofang Che, Xiujuan Qu, Li Xu, Kezuo Hou, Yunpeng Liu. The First Hospital of China Medical University, Shenyang, China. ATR/Chk1 pathway plays an essential role in modulation of DNA damage response (DDR) and homologous recombination (HR). Particularly, Chk1 phosphorylation in cancer cell lines did not respond to Arp2/3 inhibition. Inactivating the tumor suppressor Mitostatin in the genetic backgrounds of these cell lines further highlighting that this response to Arp2/3 inactivation is at the heart of the transformation process. The depletion of the WAVE complex, the Arp2/3 activator that generates branched actin in lamellipodia, but not of other Arp2/3 activators, blocks cell cycle progression. Conversely, activation of WAVE by active Rac, or depletion of Arpin, which antagonizes WAVE, potentiates cell cycle progression. Together, these results show that branched actin networks generated in lamellipodia are sensed and convey an essential signal for S Phase entry. The signal that branched actin delivers integrates stimulation from soluble growth factor with mechanotransduction from cell adhesions to the substratum and to neighboring cells. This signaling pathway qualifies as a checkpoint, which is required in normal cells, but lost in transformed cells, that monitors the integrity of branched actin networks and instructs the cell as a response that the required conditions to proliferate are fulfilled.
phosphorylation is involved in cancer prognosis and therapeutic resistance. Although some receptor tyrosine kinases (RTKs) participate in the regulation of Chk1 phosphorylation, the effect of hepatocyte growth factor (HGF) on Chk1 phosphorylation is unknown. In the present study, Western blotting and subcellular fractionation were performed to identify the expression and their significance in the mitotic checkpoint control. With small interfering RNA transfections, co-immunoprecipitations were performed to detect the interaction between TopBP1 and ATR complex. Our results demonstrated that HGF moderately activated Chk1 phosphorylation in colon cancer cells via upregulation of TopBP1, RAD51, and complex formation of TopBP1 and ATR. Furthermore, Akt activation promoted by HGF served as an important intermediate linking HGF/MET signaling and Chk1 phosphorylation. Depletion of Akt activity attenuated expression of basal line p-Chk1 and induced Chk1 activation due to HGF stimulation. Meantime, activation of Akt directly regulated expression of TopBP1 and RAD51. Suppression of Akt restored HGF-induced upregulation of TopBP1, RAD51, and enhancement of TopBP1/ATR complex as well. Our studies showed that HGF was implicated in regulating Chk1 phosphorylation and further demonstrated that Akt activity was responsible for HGF-induced Chk1 phosphorylation, which might potentially result in management of prognosis and therapeutic sensitivity in cancer therapy.

#291 Sequential treatment with the Wee1 inhibitor, AZD1775, enhances the effect of trabeclatedin in the L-sarcomas. Elisgigi Musa, Grazia Ambrosini, Gary K. Schwartz. Columbia University Medical Center, New York, NY.

Soft tissue sarcomas are heterogeneous mesenchymal neoplasms and account for 1% of all cancers in adults. Over 50 sarcomas subtypes have been classified and many have very limited treatment options. Chemotherapies such as doxorubicin, dacarbazine and gemcitabine have had very low response rates. Two of the most common subtypes of sarcomas are liposarcoma and leiomyosarcoma, also referred to as the L-sarcomas. Trabectedin, a marine derived compound from the sea sponge Ecteinascidia turbinate, has been approved for the treatment of L-sarcomas which has shown a modest effect over common thera-
pies. As a DNA damaging agent, we hypothesized that trabectedin would induce a G2 cell cycle arrest and subsequent inhibition of Wee1 would abrogate this cell cycle checkpoint and induce apoptosis. We therefore examined the effects of trabectedin followed by AZD1775, a Wee1 inhibitor, in a panel of 6 sarcoma cell lines including liposarcoma (1514I, DDLS) and leiomyosarcoma (SK-LMS, SK-UT1, SK-UT1b). IC50s concentrations of trabectedin were determined to be between 0.5 to 2nM, while IC50s for AZD1775 were in the range of 100-200 nM. In vitro results have shown an enhanced inhibition of cell viability with combination treatment in all cell lines at concentrations of 1nM trabectedin for 24hrs followed by 100nM AZD1775 for 48hrs. We also examined biochemical effects with sequential drug treatment (24hrs trabectedin followed by 24hrs AZD1775) which presented an increase of cyclin A1 and cyclin B1 indicative of G2 cell cycle arrest along with increase of phospho-H2AX, indicative of DNA damage by trabectedin, while subsequent treatment with the Wee1 inhibitor showed decrease of phosphorylation of CDC2 (Tyr15). Flow cytometric analysis indicated G2/M cell cycle arrest with trabectedin and abrogation of G2/M with AZD1775.

These observations were confirmed with enhanced apoptosis by biochemical PARP cleavage and DNA content (sub-G1 population). Taken together these results provide a foundation for the development of the sequential treatment of trabectedin followed by the Wee1 inhibitor, AZD775, in the L-sarcomas.

#292 Synthesis and characterization of the novel benzylindazole-based BUB1 kinase inhibitor BAY 1816032 with potent anti-tumor activity. Marion Hitchcock,1 Anne Mengel,2 Carl Nissing,2 2Bayer AG, Cambridge, MA; 2Bayer AG, Berlin, Germany.

BUB1 (budding uninhibited by benzimidazoles 1) is a serine/threonine protein kinase. The protein is bound to kinetochores and plays a key role in the establishment of the mitotic spindle checkpoint and chromosome segregation prior to anaphase. Eradication of BUB1 has been approved for cancer treatment whereas cell cycle arrest is the predominant mode of action of a number of antimitic cancer drugs (e.g. taxanes and vinca alkaloids). BUB1 inhibition results in aneuploidy and cell death by driving cells through mitosis irrespective of mismatched chromosomes. Here, we disclose for the first time the structure and functional characterization of a novel, first-in-class Bub1 kinase inhibitor. Medicinal chemistry efforts resulted in BAY 1816032 featuring high potency, long target residence time and good oral bioavailability. BAY 1816032 is highly selective for BUB1 displaying single digit nanomolar biochemical potency and double-digit nanomolar cellular potency (H2A induced HeLa-cells). Synergistic effects can be observed when BUB1 inhibitor BAY 1816032 is combi-

bined with low doses of paclitaxel affecting chromosome segregation and cell proliferation. X-ray data of benzylindoles allowed a better understanding of the binding mode. Further data on structure-activity relationships including pharmaco-kinetic characterization, drug metabolism and the synthesis of BAY 1816032 and analogues will be presented. These results validate the innovative approach, provide a new perspective on the role of mitotic checkpoints and justify clinical proof of concept studies evaluating BUB1 inhibitor BAY 1816032 in combination with taxanes in order to enhance their efficacy and to potentially overcome resistance.

#293 Screening the druggable genome for synthetic lethal interactions with the CHK1 inhibitor PNT7337. Rebecca Rogers,1 Mike I. Walton,1 Paul Clarke,1 Ian Collins,2 Michelle D. Garrett,2 Paul Workman1. 1The Institute of Cancer Research, London, United Kingdom; 2University of Kent, Kent, United Kingdom.

Check point kinase 1 (CHK1) is a key regulator of the cell cycle, DNA damage repair and DNA replication. CHK1 inhibition sensitises cancer cells to geno-
toxic agents and recent studies have indicated that CHK1 inhibitors could be used as single agents to treat cancers with high levels of replication stress. We have recently described the discovery of a highly selective and orally bioavailable CHK1 inhibitor, PNT7337, that not only has potent antiproliferative activity in combination with standard-of-care genotoxic agents but also as a single agent in defined tumour types. Here we sought to identify gene products whose loss would be synthetically lethal with CHK1 inhibition, with the aim of identifying patient populations likely to be sensitive to single agent CHK1 inhibition or to novel combinations utilising CHK1 inhibitors. To do this, we performed a large siRNA screen of the ~6500 genes (Cancer Cell and SW620 (colon cancer) cell lines, with and without PNT7337 treatment, and determined effects on cell viability by SRB. POLA1, POLE and POLE2 (B-family DNA polymerases) were identified as significant hits causing synthetic lethality with PNT7337 in both cancer cell lines. Treatment with additional siRNA sequences subsequently validated these genes in both the original two cell lines and extra NSCLC and colon cancer cell lines. Interestingly, a number of biomarkers for replication stress, pRPA2 and pCHK1, were increased in cells treated with POLA1, POLE and POLE2 siRNA in combination with PNT7337, in comparison to cells treated with the siRNA or drug alone. Further studies conducted with PNT7337 and the B-family DNA polymerase inhibitor aphidicolin showed that these agents had a synergistic effect on inhibiting cell viability on 8 out of 9 NSCLC and colon cancer cell lines. In addition, immunofluorescence analysis revealed that there was an increase in the level of γH2AX, a marker of DNA damage, in 4 out of 5 cell lines that exhibited synergy when treated with a combination of aphidicolin and PNT7337, as compared to cells treated with ei-
er Either alone. Our data indicate that the combination of a reduction in POLA1, POLE or POLE2 activity (by siRNA transfection or aphidicolin treat-
ment) and CHK1 activity (PNT7337 treatment) increases DNA damage and DNA damage in NSCLC and colon cancer cells. Encouragingly, our data support the case for the use of the clinically relevant combination of PNT7337 and gemcitabine, as gemcitabine is metabolised it is incorporated into DNA, inhibiting the B-family DNA polymerases. Furthermore, it will now be important to estab-
lish if subsets of colon and endometrial cancers with mutations in their P13K proofreading domain are sensitive to CHK1 inhibitors.

#294 Overide the doxorubicin-induced G2/M checkpoint using cell-cycle checkpoint inhibitors on acute lymphoblastic leukemia. Andrea Ghelli Luserna di Ror,1 Ilaria Iacobucci,1 Enrica Imbrogno,1 Anna Ferrari,2 Valentina Robustelli,3 Cristina Papayannis,3 Maria Chiara Abbenante,4 Antonella Padella,4 Giovanni Marconi,1 Sandro Grilli,1 Giovanni Martinelli1. 1University of Bologna, Bologna, Italy; 2St Jude Children’s Research Hospital, Memphis, TN; 3University of Bologna, Montecассiano, Italy.

The topoisomerase 2 inhibitor, doxorubicin, has been shown by different groups to induce cell cycle arrest in various kind of tumor cells. Specifically doxorubicin-treated cells activate the G2/M cell cycle checkpoint as a conse-
quence of the induction of DNA damages. During the last years many studies have shown the efficacy of different cell cycle checkpoint inhibitors in single agent or in combination with various DNA damaging agents. These studies showed that the inhibition of key proteins of the cell cycle, like Chk1 and Wee1, deeply sensitize tumor cells to the treatment with genotoxic agent. On these bases, the aim of the study was to evaluate the efficacy of a selective Chk1/ Chk2 inhibitor and a Wee1 inhibitor in combination with doxorubicin for the treatment of acute lymphoblastic leukemia. Firstly we evaluate the effect of doxorubicin treatment on a panel of human B and T ALL cell lines in term of reduction of the cell viability, modification of cell cycle profile and activation of the DNA damage response. For this reason the cells were treated with doxorubicin (0.25, 0.5 and 1μM) for 24 and 48 hours and the reduction of the cell
vitality was quantified using WST-1 reagents. In all the cell lines treated the cytotoxic effect of doxorubicin was time and dose dependent. Then the induction of the apoptosis (Pi/Annexin V) and the effect on cell cycle profile (Pi staining) was evaluated in all the cell lines. In line with the literature the treatment with doxorubicin arrested the cells in G2/M phase. Then the effect of the combinations between doxorubicin and the two checkpoint kinase inhibitor was assessed in all the cell lines. Different cell lines were treated with doxorubicin (5, 10, 25 and 50 nM for the more sensitive cell lines; 50, 100, 250 and 500 nM for the less sensitive cell lines) in combination with the Chk1/Chk2 inhibitor (2, 5 and 10 nM) for 24 and 48 hours. The combination showed a additive effect in term of reduction of the cell viability and induction of apoptosis. Different cell lines were pre-treated for 18 hours with doxorubicin and then with Chk1/Chk2 inhibitor for different time points. Interestingly the inhibition of both Chk1/Chk2 proteins removed the G2/M arrest induced by the pre-treatment with doxorubicin, progressively reducing the number of cells in G2/M phase, increasing the percentage of cells in sub-G1 phase. Similar results were seen combining a Weel inhibitor with doxorubicin on several ALL cell lines. In our opinion the combination between the cell cycle checkpoint inhibitors and doxorubicin could be a promising strategy for the treatment of B/T-ALL. Supported by ELN, AIL, AICR, progetto Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project.

#295 CASC-578, a novel Chk1 inhibitor, is active as a single agent in solid tumors and displays synergistic anti-tumor activity in combination with Weel inhibition. Alex Vo,1 Janelle Taylor,1 Robert Rosler,1 Julia Piascik,1 Dina Leviten,4 Ashley Dozier,4 Kevin Klucher,4 Janelle Taylor,1 Dina Leviten,4 Ashley Dozier,4 Kevin Klucher,4 Rich Boyce,5 Scott Peterson1.

Cascadian Therapeutics, Seattle, WA; 2none, CA; 3Sentinel Oncology, Cambridge, United Kingdom.

Background: Checkpoint kinase 1 (Chk1) is a serine/threonine protein kinase that regulates cell division in response to genotoxic stress by arresting cell cycle progression in the S & G2 phases. Pharmacological inhibition of Chk1 is proposed to target tumor cells with increased DNA replication stress, resulting in the uncoupling of DNA replication checkpoint function and the induction of DNA damage and cell death. These properties make Chk1 a promising novel therapeutic approach as a single agent in cancers with high replication stress that are posed to target tumor cells with increased DNA replication stress, resulting in DNA damage induction, mitotic catastrophe and cell death. These properties make Chk1 an attractive therapeutic approach as a single agent, or to enhance the efficacy of other cancer drugs that target DNA damage response pathways. Methods and Results: This report highlights the activity of the orally bioavailable, selective small molecule Chk1 inhibitor, CASC-578, in solid tumor derived cell lines. CASC-578 is a sub-nanomolar enzymatic inhibitor of Chk1 with limited off-target activity against a panel of protein kinases. When evaluated in a large cell line panel in vitro, CASC-578 demonstrated a broad potency range as a single agent in solid tumor derived cell lines, with IC50s ranging from 30 nM to greater than 50 μM. Several solid tumor types demonstrated enriched sensitivity to CASC-578 in vitro, including gastric, non-small cell lung and ovarian cancers. Treatment of sensitive cell lines with CASC-578 resulted in the induction of DNA damage, as measured by phosphorylated histone H2AX, and the induction of cell death. CASC-578 was active as a single agent in SK-MES-1 and NCI-H727 NSCLC tumor xenograft models in vivo with minimal effects on body weight in treated mice. In addition to the potent single agent activity of CASC-578, combination with the Weel inhibitor AZD-1775 was highly synergistic in vitro in multiple solid tumor cell lines and the combination was more efficacious than either agent alone in NSCLC tumor xenograft models. These data support the advancement of CASC-578 into clinical development as a potential therapeutic agent for the treatment of solid tumor diseases. Experiments are ongoing to identify biomarkers associated with sensitivity to CASC-578 as a single agent in solid tumor cell lines to prospectively identify tumor genotypes that are more responsive to the drug.

#296 Interplay of human MGMT DNA repair protein with PCNA /p21cip1 and MGMT’s novel role as an S-phase checkpoint. AGM Mostova, Kalkunte S. Srinivasanpugal. Texas Tech University Health Sciences Center, Amarillo, TX.

O6-Methylguanine-DNA methyltransferase (MGMT) is a critical antmutagenic DNA repair protein that protects the genome and an established target for improving the efficacy of alkylating agents. In contrast with the stoichiometric repair reaction performed by a single small MW protein, we show for the first time that MGMT in human glioblastoma cells specifically associates with PCNA, p21cip1, and undergoes selective degradation at mid-S-phase along with replication-licensing components to maintain genomic integrity. First, we identified a PCNA-Interacting Protein (PIP) box motif between amino acids 61-70 in QCTAWLNAFY in the MGMT protein. PCNA encircles the DNA and functions as a sliding clamp by interacting with DNA metabolic proteins having a PIP-box to make the replication processive. In p53-null H1299 lung cancer cells engineered to express the p21cip1, either by Tet-off conditional or lentiviral stable transfections, a reciprocal immunoprecipitation/western blot analyses using antibodies to PCNA or MGMT confirmed the specific association of MGMT and PCNA proteins. Expression of the CDK inhibitor p21 disrupted the interaction between PCNA and MGMT in cells, indicating its regulatory role in DNA repair during cell cycle blockade. Confocal immunofluorescence imaging in glioblastoma cells and in the HCT116 colorectal cell line, showed a co-localization of MGMT and PCNA proteins in glioma cells; when cells were subjected to alkylca DNA damage, the co-localization pattern was punctate and more prominent, suggesting that PCNA functions to recruit the repair protein to the damage sites. To probe the cell-cycle dependent regulation of MGMT, we used synchronized human GBM cells at the G1/S boundary with specific shRNAs against the thymidine-thymine monomer and thymidine-monosine blocks. Cells released into the S-phase showed that the MGMT disassociates from PCNA during the late S-phase and undergoes specific degradation before re-accumulating in G2/M. The replication-licensing protein Cdt1 and p21cip1 were also observed to undergo co-degradation in S-phase, which has been established as a key step in marking the replication origin sites. MGMT inhibition, either by O6-benzylguanine or specific shRNAs greatly impeded the progression of cells into the S-phase in synchronized cells. DNA synthesis measured by 3H-thymidine or BrdU incorporation was also curtailed significantly by MGMT inhibition. Furthermore, enforced expression of MGMT in two GBM cell lines led to a moderate endo-reduplication of the genome. Collectively, we show new non-repair functions for MGMT, its requirement for cell cycle progression and timed elimination of MMR genome maintenance. On the clinical front, these observations provide a clear biochemical rationale for combining MGMT inhibitors (apart from the alkylators) with antimetabolites [supported by CPRIT grants RP130266 & RP170207 to KSS].

#297 The novel orally available sub-nanomolar potent and selective checkpoint kinase 1 inhibitor CASC-578 is highly active in mantle cell lymphoma as a single agent and in combination with Weel inhibition. Robert Rosler,1 Janelle Taylor,1 Dina Leviten,2 Teresa Sierra,2 Ashley Dozier,4 Kevin Klucher,4 Rich Boyce,5 Alex Vo,1 Scott Peterson1.

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Background: Checkpoint kinase 1 (Chk1) is a serine/threonine protein kinase that regulates cell division by arresting progression in the S & G2 phases of the cell cycle in response to genotoxic stress. Pharmacological inhibition of Chk1 uncouples the completion of DNA replication from G2/M phase transition in tumor cells that have impaired DNA damage response networks, resulting in DNA damage induction, mitotic catastrophe and cell death. These properties make Chk1 inhibition an attractive therapeutic approach as a single agent, or to enhance the efficacy of other cancer drugs that target DNA damage response pathways. Methods and Results: This report highlights the activity of the orally bioavailable, selective, small molecule Chk1 inhibitor, CASC-578, in multiple mantle cell lymphoma (MCL) models in vitro and in tumor mouse xenograft studies. MCL have the common genetic biomarker t(11;14)(q13;q32), a chromosomal translocation leading to the constitutive expression of the oncogene CCND1 which encodes cyclin D1, a cell cycle-regulating protein. This genotypic characteristic has been observed in the clear majority of patients with MCL in the clinic and may provide a rationale for sensitivity to Chk1 inhibition. Consistent with this idea, CASC-578 demonstrated remarkable potency as a single agent in blocking the proliferation of MCL cell lines in vitro, with an average IC50 of 61 nM. Treatment with CASC-578 as a single agent resulted in the induction of DNA damage, as measured by phosphorylated histone H2AX and activation of apoptosis, as measured by cleaved caspase. In a JEKO-1 xenograft tumor study, CASC-578 as a single agent completely regressed subcutaneously implanted tumors in the majority of mice after oral drug administration using 20 mg/kg daily or when dosed on an intermittent schedule at 25 mg/kg, with minimal effects on body weight. In addition to the observed single agent activity of CASC-578, the combination of CASC-578, and the Weel inhibitor AZD-1775, was highly synergistic in MCL cell lines including JEKO-1, REC-1, Z-138 and MAVER-1 in vitro. CASC-578 was also highly potent as a single agent, and in combination with AZD-1775, in blocking the proliferation of a variety of leukemia derived cell lines in vitro. Taken together, these data support the advancement of CASC-578 into clinical development as a potential therapeutic agent for the treatment of MCL, as well as other hematological malignancies.

#298 WEE1 inhibitor activity correlates to AXL/mTOR expression and exhibits synergy with temozolomide (TMZ) in small cell lung cancer (SCLC). Triparna Sen, Pan Tong, Lixia Diao, You Hong-Fan, C. Allison Stewart, John V. Heymch, Jing Wang, Lauren A. Byers. UT MD Anderson Cancer Center, Houston, TX.
Background: WEE1 inhibitors (WEE1i) are a novel class of small molecule inhibitors that are now under early clinical trials for small cell lung cancer (SCLC). WEE1 is a protein kinase that plays a key role in regulating the G2 checkpoint in response to DNA damage. Moreover, because WEE1 inhibition induces a HR deficient state, dual targeting of WEE1 and PARP (another promising target in SCLC) could lead to an increase in the efficacy of PARP inhibitors. In the present study we evaluate the efficacy of AZD1775 alone and with the WEE1i, olaparib, to determine the mechanisms of primary resistance to AZD1775 in SCLC models; and assess the combinatorial efficacy of AZD1775 treatment with the chemotherapy drug, temozolomide (TMZ), that has shown promise in patients with recurrent SCLC and has been included in the National Comprehensive Cancer Network guidelines for standard care.

Results: Combination of AZD1775 with olaparib revealed an additive effect in vitro in 90% of SCLC cell lines. AZD1775 combined with TMZ synergistically decreased viability, increased DNA damage and apoptosis in SCLC cell lines (n = 10) irrespective of MGMT status or initial response to AZD1775 alone. However, unlike the sensitive cell lines, SCLC cell lines showing primary resistance to AZD1775 had an intact DNA repair mechanism (after DNA damage), which may contribute to the resistance mechanism. Proteomic analysis revealed AXL (receptor tyrosine kinase) and phospho-S6K (S240/244) as markers of AZD1775 resistance and treatment with AXL inhibitor, TP9003 (40nM), re-sensitized the cells to AZD1775. Pre and post-AZD1775 treated samples revealed sustained activation of mTOR pathway in AZD1775 primary resistant lines. We further demonstrated that the downregulation of the cells with the mTOR inhibitor everolimus sensitized SCLC cells to AZD1775 by causing downregulation of AKT/mTOR pathway. Conclusion: WEE1 inhibitors are currently in clinical trials for SCLC patients. However, as with any targeted therapy, drug resistance is an important barrier to clinical benefit which could be addressed with therapeutic combinations. We show the efficacy of single agent AZD1775 and in combination with olaparib. WEE1 inhibitor, AZD1775 synergizes with TMZ irrespective of MGMT status in all tested in vitro models which warrants further clinical investigation. We also show that the activity of the WEE1 inhibitors might be limited in cancer cells overexpressing of AXL and activated mTOR pathway and that AXL and mTOR inhibition re-sensitized the cells to AZD1775. Our work supports further exploration of the combination of PARP and WEE1 in SCLC and also the possibility of AXL/mTOR inhibition as a mechanism to overcome WEE1 inhibition resistance in SCLC. SCLC is a disease with very limited therapeutic options and no targeted agents with proven benefit; thus the results from this study have clear translational benefit.

#299 RNA editing enzyme ADAR1 accelerates normal hematopoiesis cycle by regulation microRNA biogenesis. Qingfei Jiang, Maria Anna Zipeto, Nathan Delos Santos, Sheldon Morris, Catriona Jamieson. University of California, San Diego, La Jolla, CA.

Combining murine studies demonstrate that adenosine-to-inosine (A-to-I) RNA editing mediated by adenosine deaminase associated with RNAI (ADAR1) is vital for both fetal and adult hematopoiesis. While genetic ablation of ADAR1 leads to murine embryonic lethality due to severe defects in erythropoiesis, conditional deletion in the hematopoietic system impairs maintenance indicative of cell type and context specific roles for ADAR1 in cell fate specification and self-renewal. By regulating mRNA and microRNA (miRNA) stability, ADAR1 exhibit wide-ranging effects on embryonic development and stem cell regulation. We have previously shown that inflammation-responsive ADAR1 plays important roles in both stem cell differentiation and self-renewal in CML (chronic myeloid leukemia) disease progression. Here, we describe a novel function of ADAR1 in cell cycle regulation of normal hematopoietic stem cell and progenitors (HSPC) by regulation of miRNA biogenesis. Our results demonstrated that ADAR1 induces G0 to G1 phase transition in normal cord blood HSPCs, as demonstrated by elevated expression of Ki67, reduced DiR signal, and enhanced in vivo cord blood engraftment. Cell cycle qRT-qPCR microarray of 84 cell cycle transcripts and whole transcriptome RNA-sequencing analysis of KEGG cell cycle pathway indicate that several cell cycle genes are differentially expressed upon overexpression of ADAR1 WT or an A-to-I editing deficient ADAR1 mutant (ADAR1E912A). We previously demonstrated that impaired biogenesis of let-7 miRNAs by ADAR1 WT induces enhanced self-renewal in cord blood CD34+ HSPCs. To determine the miRNA targets of ADAR1-mediated RNA editing, we performed mirNome microarray PCR analysis of 1008 miRNA candidates in cord blood CD34+ HSPCs overexpressing ADAR1 WT or ADAR1E912A. Total of 263 miRNAs were differentially expressed (142 upregulated and 121 downregulated) by comparing ADAR1 WT to the backbone control. Interestingly, ADAR1E912AA mutant also exhibit A-to-I editing independent regulation of miRNAs (307 upregulated and 359 downregulated). We found that the expression of miR-26a-5p, a miRNA frequently downregu- lated in leukemia, is inhibited by ADAR1-mediated RNA editing. ADAR1 directly binds and edits the DROSHA cleavage site of primary miR-26a transcript, thereby prevent miR26a-5p maturation. Moreover, lentiviral expression of mature miR-26b reverses the effect of ADAR1 WT, including enhanced CDKN1A expression, inhibition of cord blood proliferation in vivo, as well as reduced HSC mobilization in vitro. The results suggest that ADAR1 exhibit wide-ranging effects on embryonic development and self-renewal in cord blood CD34+ HSPCs, as demonstrated by elevated expression of Ki67, reduced DiR signal, and enhanced in vivo cord blood engraftment. Cell cycle qRT-qPCR microarray of 84 cell cycle transcripts and whole transcriptome RNA-sequencing analysis of KEGG cell cycle pathway indicate that several cell cycle genes are differentially expressed upon overexpression of ADAR1 WT or an A-to-I editing deficient ADAR1 mutant (ADAR1E912A). We previously demonstrated that impaired biogenesis of let-7 miRNAs by ADAR1 WT induces enhanced self-renewal in cord blood CD34+ HSPCs. To determine the miRNA targets of ADAR1-mediated RNA editing, we performed mirNome microarray PCR analysis of 1008 miRNA candidates in cord blood CD34+ HSPCs overexpressing ADAR1 WT or ADAR1E912A. Total of 263 miRNAs were differentially expressed (142 upregulated and 121 downregulated) by comparing ADAR1 WT to the backbone control. Interestingly, ADAR1E912AA mutant also exhibit A-to-I editing independent regulation of miRNAs (307 upregulated and 359 downregulated). We found that the expression of miR-26a-5p, a miRNA frequently downregu-
**MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Cell Cycle Checkpoint and Progression**

Medulloblastoma is the most common malignant pediatric brain tumor with variable prognosis due to its clinical and genomic heterogeneity. Despite recent treatment advances, approximately 40% of children experience tumor recurrence, and 30% will die from this disease. Therefore, there is a need to develop novel therapies for patients. We recently reported that Casein kinase 16 (CK16) may be a novel therapeutic target of medulloblastoma. CK16 is a serine/threonine kinase that controls cell cycle progression, signal transduction and neurogenesis. We found high levels of CK16 protein in mouse models of medulloblastoma and human medulloblastoma samples. Furthermore, CK16 inhibition dramatically reduced medulloblastoma tumor progression. We demonstrate here that CK16 phosphorylates the epigenetic reader bromodomain-containing protein 4 (BRD4). BRD4 has been identified as a therapeutic target in several cancers, including medulloblastoma. We demonstrate that CK16 phosphorylates BRD4 and that CK16 is required for BRD4 binding to the Gli1 promoter in vitro and in vivo. Furthermore, combined CK16/BRD4 inhibition is a novel means of reducing medulloblastoma growth downstream of SUFU and SMO. These studies define a novel therapeutic means of inhibiting SMO inhibitor resistant medulloblastoma.

**#303 Oncogenic activity of amplified miniature chromosome maintenance 8 in human malignancies.** Jian-Hua Luo, Dong-Mei He, Baoguo Ren, Yan Yu.

Miniature chromosome maintenance (MCM) proteins play critical roles in DNA replication licensing, initiation and elongation. MCM8, one of the MCM proteins playing critical role in DNA recombination and recombination, was found to have over-expression and increased DNA copy number in a variety of human malignancies. The gain of MCM8 is associated with aggressive clinical features of several human cancers. Increased expression of MCM8 in prostate cancer is associated with cancer recurrence. Forced expression of MCM8 in RWPE1 cells, the immortalized but non-transformed prostate epithelial cell line, exhibited fast cell growth and transformation, while knocked down of MCM8 in PC3 and DU145 cells induced cell growth arrest, and decreased tumor volumes and mortality of severe combined immune deficiency mice xenografted with PC3 and DU145 cells. MCM8 has been identified as a therapeutic target in several cancers, including medulloblastoma. We demonstrate that CK16 phosphorylates BRD4 and that CK16 is required for BRD4 binding to the Gli1 promoter in vitro and in vivo. Furthermore, combined CK16/BRD4 inhibition is a novel means of reducing medulloblastoma growth downstream of SUFU and SM0. These studies define a novel therapeutic means of inhibiting SM0 inhibitor resistant medulloblastoma.

**#304 CLIC1 membrane insertion is a pivotal regulator of glioblastoma stem cell G1-S transition by promoting an increase of chloride permeability.** Ivan Verduci, Valentina Carlini, Federica M. Raciti, Matteo Conti, Michele Mazzanti.

Glioblastoma (GBM) is the most aggressive and lethal brain tumor and, despite aggressive surgery and adjuvant radiotherapy and/or chemotherapy, the prognosis remains invariably poor. As for most of solid and hematological malignancies, it was demonstrated that the bulk of tumor cells in GBM is generated by a rare fraction of self-renewing, multipotent cancer stem cells (CSCs) and the persistence of CSCs within the tumor mass is considered the main determinant of GBM development, progression, recurrence and radio- or chemoresistance. Thus, one of the main goals of current research is to identify specific biological mechanisms or intracellular pathways of CSCs whose pharmacological targeting might affect their survival and proliferation. In particular, little is known about the possibility that the molecular mechanisms underlying cell-cycle control in GBM CSCs are endowed with specific and unique features as compared with normal cells. Our study is based on the observation that GBM cells express higher levels of chloride intracellular channel 1 (CLIC1) as compared to nonmalignant brain cells and that in CSCs CLIC1 is mainly localized in the membrane forming an active channel. Conversely, in physiological conditions CLIC1 is mainly a cytoplasmic protein only transiently translocating to the membrane. We recently showed that the different level of activity of CLIC1 in CSCs and normal mesenchymal stem cells confers CLIC1-targeting drugs (for example the biguanide metformin) selective cytotoxicity toward tumor cells. Here we report, that in response to stress conditions, CLIC1 increases the probability to modify its structure going from a cytoplasmic hydrophilic form to a transmembrane conformation. Once in the membrane, CLIC1 acts as a chloride permeability, participating, together with NADPH oxidase, to the generation of a chronic state of oxidative stress that favor the transition between G1 and S phase. The peculiarity of CLIC1 exposure on the external face of the GMB CSC plasma membrane support the idea that this protein could represent a main determinant of the cell cycle progression in this tumor cell subpopulation and thus an accessible and relevant pharmacological target to eradicate CSCs in GBM.

**#305 TET blocks cell cycle progression in pancreatic cancer cells at G1-S boundary by decreasing cyclin D1 and increasing CDK inhibitors (CKIs), p21 (WAF1, Cip1) and p27 (Kip1).** Karnika Singh, Qin Dong, Sweaty Koul, Hari K. Koul.

Introduction: Pancreatic cancer (PaCa) is the fourth leading cause of cancer related deaths in the United States with an overall five year survival rate of less than five percent. The current standard treatment/s for PaCa are largely ineffective thus there is an urgent need for development of therapeutic agents for the treatment of PaCa. Owing to its mutational landscape, which includes mutated KRAS and p53 genes these cancer cells have a deregulated G1 checkpoint of cell cycle control and therefore confer high rate of proliferation and therapeutic resistance. Recently it was observed that Tetrandrine derivative (TET), a bis-benzyloxyquinoline alkaloid halts cell cycle progression at G0/G1 boundary in vitro and in vivo. In the present study we evaluated potential mechanism of TET induced cell cycle arrest in two pancreatic cancer cells with either wild type KRAS or mutant KRAS, and mutant p53 by evaluating critical cell cycle regulatory proteins involved in G0/G1 to S transition. Methods: Pancreatic Cancer cell lines BxPC3 (Pancreatic Ductal Adeno-Carcinoma) and MiaPaCa2 (pancreatic carcinoma) were used in the study. Cells were treated with various concentrations of TET for different time points. Cell proliferation was evaluated using the MTT survival assay. Cell cycle was analyzed following PI staining via Flow cytometry. Changes in protein expression were analyzed by Western Blot analysis in cells. Changes in mRNA were evaluated by real time PCR. Results: Treatment of PaCa cancer cells with TET resulted in decreased cyclin D1 and phospho-Rb, two critical proteins required for G0/G1 to S transition. Pretreatment of the cells with proteasomal inhibitor MG132 was able to increase the levels of cyclin D1 in control cells but was unable to prevent its loss upon TET treatment. Our results also show that levels of CKIs, p21(WAF1/Cip1) and p27(Kip1) were increased at the protein level. Gene expression analysis revealed that cyclin D1 mRNA was decreased and p21(WAF1/Cip1) mRNA was increased while there was no significant change in p27 mRNA levels. The cycloheximide chase revealed that TET stabilizes p27(Kip1) and to some extent p21(WAF1/Cip1) as well, suggesting that TET increased p27(Kip1) and also p21(WAF1/Cip1) levels in part by increasing protein stability. These data suggest a complex mechanism by which TET regulates expression of critical cell cycle regulatory molecules at both transcriptional and post-translational level. Such a mechanism would provide for cell cycle arrest in pancreatic cancer cells following TET treatment. Conclusion: These results show that TET halts cell cycle at G0/G1 boundary in PaCa cells by modulating the cell cycle regulatory proteins and stabilizing the CKIs by altering their protein stability, suggesting that TET can not only halt deregulated cell cycle but also overcome the therapeutic resistance in pancreatic cancer cells.

**#306 Antitumor effect of Weel inhibitor in gastric cancer cell lines.** Seongyeong Kim, Ahry Min, So Hyeon Kim, Daewon Song, Ha Hyemin Jang, Yu Jin Kim, Hee Jun Kim, Kyung-Hun Lee, Tae-Yong Kim, Do-Youn Oh, Yung-Jae Bang, Seock-Ah Im.

Introduction: Gastric cancer is a second leading cause of cancer death. Weil is a G2 cell cycle regulator that phosphorylate cdcc2 on tyrosine 15 for inhibiting its activity. The activation of Wee1 can delay G2 phase progression and cells can have a chance to repair DNA damages. Therefore, to inhibit weel can accelerate DNA damage accumulation in the cell. AZD1775 is a first-in-class weel inhibitor. So far, previous studies showed the antitumor effect of AZD1775 as a mono-therapeutic agent or combination treatment with other chemo-agents. However, the activity of weel in gastric cancer (GC) cells is not yet fully understood. Thus, we would like to study whether AZD1775 has an antitumor effect against GC or not. Materials and methods: We determined the antitumor effects of AZD1775 on GC cells using a cytotoxicity assay, cell cycle analysis, immunofluorescence assay and western blotting by AZD1775 treatment. Results: AZD1775 effectively inhibited GC cell proliferation. The TP53 gene status or p-Wee1 expression levels were not associated with AZD1775 sensitivity. AZD1775 effectively down-regulated p-Wee1 and p-cdc2 expressions in SNU-601, a sensitive cell line. However, these effects were not significant in KATO-III cells, which is less sensitive to AZD1775. Cell cycle analysis revealed that the cells had different responses by AZD1775 treatment. Results: AZD1775 effectively inhibited cell cycle only by its DNA contents. However, KATO-III cells were arrested in G2/M phase. The sub-G1 population was dramatically increased only in SNU-601 cells. A population of BrdU-positive and p-HH3 positive cells was significantly increased in the SNU-601 cells which indicate that premature mitosis was
#307 TRIB3 regulates cell cycle progression and programmed cell death in non-small cell lung cancer. Aber Alminan, Daoai Nie, Jamila Adom. Southern Illinois Univ. School of Medicine, Springfield, IL.

Introduction: The pseudokinase Tribbles pseudokinase 3 (TRIB3) is known as a regulator in cellular responses to a variety of stresses such as glucose insufficiency and (ER) stress. TRIB3 has been described in some studies as a tumor suppressor due to its effect on inactivating PI3K/Akt pathway, but other studies suggest TRIB3 as a stimulator of tumor progression. In this study, we aimed to define the functions of TRIB3 in non-small cell lung cancer. Methods: TRIB3 expression was altered using a lentiviral vector to overexpress TRIB3 in non-small cell lung cancer. A CRISPR-CAS9 construct with guiding sequence matching to TRIB3 gene was used to knock out its expression. Cell proliferation was evaluated using MTS and trypan blue assays. Boyden chamber assay was used to assess the cell migration while cell cycle phases were determined using flow cytometry. Heatmap analysis was performed to assess the changes in the expressions of genes in cell cycle progression and apoptosis. Results: TRIB3 overexpression (H460) increased cell proliferation and migration. Moreover, TRIB3 contributed to the increased cell cycle arrest at G0/G1 phase. QPCR analyses of cell cycle related genes showed an upregulation of CDK inhibitors in (NCI-H358) TRIB3 overexpression cells, while depletion of TRIB3 led to the down-regulation of CDK inhibitors. TRIB3 overexpression led to downregulation of LC3B and other autophagy markers while increasing the expression of apoptotic genes. Conclusion: Increased TRIB3 expression in non-small lung cancer cells inhibited proliferation by blocking cell cycle progression through the up-regulation of CDK inhibitors, led to activation of cell death through apoptosis. Our study reveals a significant role of TRIB3 in regulating cell cycle progression, apoptosis and autophagy.

#308 The PP2A-B56 phosphatase opposes cyclin E autocatalytic degrada-tion via site-specific dephosphorylation. Ryan J. Davis, Ilierek Swanger, Bruce E. Clurman. Fred Hutchinson Cancer Research Ctr., Seattle, WA.

Cyclin E, in conjunction with its catalytic partner cyclin-dependent kinase 2 (CDK2), regulates cell cycle progression as cells exit quiescence and enter S-phase. Multiple mechanisms control cyclin E-CDK2 activity during the cell cycle, including phosphorylation-dependent cyclin E ubiquitylation by the SCF/Fbw7 ubiquitin ligase. Serine 384 (S384) is the critical cyclin E phosphorylation site that stimulates Fbw7 binding and subsequent cyclin E degradation. Because S384 is autophosphorylated by CDK2, cyclin E therefore instigates its own degradation in an autocatalytic manner. This presents a paradox as to how cyclin E-CDK2 is able to phosphorylate its numerous substrates prior to cyclin E autophosphorylation-catalyzed degradation. Here we find that the PP2A-B56 phosphatase specifically dephosphorylates cyclin E at S384 (the double motif 2 homology) to oppose cyclin E degradation from cyclin E-CDK2 activity. Furthermore, the rate of S384 dephosphorylation is high in interphase and low in mitosis, allowing PP2A-B56 to oppose autocatalytic cyclin E degradation and maintain cyclin E-CDK2 activity at the G1/S transition.


Rambouillet Goodwin Institute for Cancer Research, Nova Southeastern University, Fort Lauderdale, FL; College of Pharmacy, Nova Southeastern University, Fort Lauderdale, FL.

Deacetylation of histone gives a tag for epigenetic repression and plays an important role in transcriptional regulation, cell cycle progression, and developmental events. HDACs catalyse the removal of the acetyl moiety from the lysine residues of proteins including the core nucleosomal histones. Through removal of critical acetyl groups from histones, HDACs can create a chromatin conformation that can prevent the transcription of genes that encode for proteins involved in cell cycle regulation. Thus together with histone acetyltransferases (HATs), HDACs regulate the level of acetylation and alter multitude of cellular functions and their characteristics. Several alterations of HDAC and HAT levels and activities have been found to be enacted by translocation, amplification, overexpression, or mutation of the relevant genes in a variety of cancers. In many cancer cell lines, overexpression or activation of the HDAC enzymes result in histone hypo-acetylation and consequent promotion of pro-cancerous mechanisms. Therefore, HDAC inhibitors represent a potential new class of antitumor agents with cytotoxic activity and the ability to regulate gene expression in tumor cells. In this study we evaluated the effects of Vorinostat (suberoylanilide hydroxamic acid), which is a potent inhibitor of HDAC activating the gene expression profile in LNCaP cell lines (NCI-H9262) and demonstrated for the first time that vorinostat (10 μM) is able to transcribe the transcription factor E2F1 to the up-regulation of BIRC5 (Baculoviral IAP Repeat Containing 5). Similarly, when we treated the MDM2 transduced LNCaP-MST cells with vorinostat (7.5 μM for 24 hrs), some of the above mentioned changes, similar to Nutlin-3 treatment, were observed. As a result of HDAC inhibition the mRNA levels of p21, p53 and TIMP-1 were significantly elevated, while the levels of BIRC3 was significantly down-regulated. Thus, treatment of MDM2 overexpressing cell lines with HDAC inhibitor resulted in activation of p21 and consequent decrease in cell proliferation due to resumption of cell cycle arrest. Our results with LNCaP-MST cells offer convincing evidence to suggest that the inhibition of HDAC can control cell proliferative signals in MDM2 overexpressing prostate cancer cells. (The generous support from the Royal Dames of Cancer Research Inc., Ft. Lauderdale, Florida is gratefully acknowledged.)

#310 Enhanced stability of D-type cyclins correlates with glioblastoma resistance to ionizing radiation. Fadila Guessou, Mouadh Benamar, Hui Zhong, Tarek Abbas. Univ. of Virginia, Charlottesville, VA.

Transition from G1 to S phase of the cell cycle is promoted by D-type cyclins (D1, D2 and D3), regulatory subunits critical for the activation of cyclin dependent kinases 4 and 6 (CDK4/6), which phosphorylate and inactive the retinoblastoma protein (pRB) and activate the transcription factor E2F1 to promote the expression of genes essential for DNA synthesis. Decreased expression of D-type cyclins is frequently observed in human malignancies and correlates with increased proliferation. Cyclin Ds overexpression is also associated with radioresistance, leading to radiotherapy failure and disease recurrence. Cyclin D1 undergoes ubiquitin-dependent degrada-tion following the exposure of cancer cells to ionizing radiation (IR), and this has been shown to be critical for IR-induced G1 growth arrest and sensitivity of cancer cells to IR. While the role of cyclin D1 overexpression in drug resistance is already well documented, less is known about the role of cyclins D2 and D3 in radio-resistance in human cancer cells, particularly in brain neoplasms. In the present study, we investigated the effect of IR on different D-type cyclins (D1, D2, D3) in human glioblastoma cell lines as well as in tumor-derived mouse oligodendrocytes progenitor cells (OPCs). Here, we show that exposure of human astrocytes to increasing levels of IR led to a rapid proapoptotic degradation of all three D-type cyclins. On the other hand, IR failed to induce significant cyclin D3 degradation in a number of human glioblastoma cells (U87, A172, U251, Snb19). Analysis of cell cycle profile of glioblastoma cell lines and exposure to IR showed prominent cell cycle arrest in G1 starting at 24 hours following exposure. Furthermore, we show that the steady-state levels of D-type cyclin is higher in OPCs compared to mouse NIH3T3 cells and fail to undergo proapoptotic degradation following IR exposure. Collectively, these results suggest that the degradation of D-type cyclins is not critical for IR-induced G1 cell cycle arrest in GNB and may underlie their resistance to IR. Understanding the role of cyclin Ds in promoting radioresistance may lead to a useful pharmacological strategy to enhance radiotherapy outcome and help implement a more effective treatment modality.

#311 Development of AZD2811, an aurora kinase B inhibitor, incorporated into an Accurin™ nanoparticle for use in haematological and solid cancers. Simon Ashton, Nikolaas Fisch, Paula Taylor, Colin Howard, Doug Ferguson, Matthew Ling, Maureen Hattersley, Shenghua Wen, Kim Mart- atea, Adina Hughes, Sean Redmond, Wolfram Brugger, Simon Smith, Alexander MacDonald, Keith Parry, Howard Burris, Young-Ho Song, Jim Nol an, Elizabeth Pease, Simon T. Barry, AstraZeneca, Macclesfield, United Kingdom; AstraZeneca, Cambridge, United Kingdom; AstraZeneca, Gatehouse Park Boston, MA; AstraZeneca, Macclesfield, United Kingdom; Sarah Cannon Research Institute, London, United Kingdom; Sarah Cannon Research Institute, Nashville, TN; Pfizer, New York, NY.

A nanoparticle formulation of AZD2811, a selective aurora kinase B inhibitor, is currently under clinical development for the treatment of both haematological and...
solid tumour disease. AZD2811 is the active derivative of the prodrug Barasertib (AZD1152) which gave promising clinical activity in elderly AML patients delivered as a 7-day infusion (Kantarjian et al, Cancer, 119, 2611-2619, 2013). To address the limitations associated with the clinical utility of Barasertib and other cell cycle inhibitors in the clinic, AZD2811 has been incorporated into an Accurin® nanoparticle using a pamoic acid ion pairing approach to optimise drug release rate (Song et al, Journal of Controlled Release, 229, 106-119, 2016), improve the drug exposure to tumour and reduce the duration of administration. A proof of principle formulation of AZD2811 as an Accurin® nanoparticle established the principle that anti-tumour activity and improved therapeutic index could be achieved (Ashton et al., Science Translational Medicine, 325, 1-10, 2016). The clinical nanoparticle formulation of AZD2811 has been optimised for drug loading and release rate. In pre-clinical models, the clinical formulation can be used flexibly to optimise drug delivery for use in both haematological diseases such as AML, or in solid tumour settings. Anti-tumour activity in solid tumours can be achieved at doses where bone marrow toxicity is reduced compared to Barasertib. In sensitive xenograft and PDX solid tumour models greater than 90% tumour regression is observed after a total dose of 50mg/kg with no tumour progression for greater than 40 days. In contrast, for AML, increasing the dose intensity by 2-4 fold leads to neutropenia and to complete tumour regression in a range of AML xenograft models for greater than 60 days. These data establish the concept that drug delivery using nanoparticles is able to resolve therapeutic index challenges, and is able to do so across different disease types. AZD2811 is currently in Phase 1 clinical trial (DE130C0000). The current pre-clinical and clinical data with this novel approach to inhibition of the cell cycle will be discussed.

#312 Anti-proliferative effects of cinnamon extract in colon cancer. Patrick P. Carriere, Hina Mir, Neeraj Kapur, Clarence E. Clark, Shailesh Singh. Morehouse School of Medicine, Atlanta, GA.

Colon cancer is the third most lethal cancer worldwide, and is estimated to cause 49,190 cancer-related deaths in the U.S. in 2016 alone. Current treatments for colon cancer are limited in their capacity to target tumor cells employing adaptive mechanisms necessary for survival and proliferation, often resulting in poor prognosis. It is critical to develop novel target-specific therapeutic approaches with minimal cytotoxicity to normal cells, in order to mitigate potential side effects. Proanthocyanidins represent a subclass of flavonoids, which have been widely investigated for their chemopreventive and therapeutic potential. Cinnamamin B-1 (CTB-1) is a proanthocyanidin shown to have anti-cancer effects in several cancers, but its anti-cancer potential in colon cancer has not been tested. Hence, the focus of this study was to determine the anticancer effects of CTB-1 in colon cancer. Our results show that CTB-1 treatment significantly decreases the cell viability and proliferation of DLD-1 and COLO 201 human colon cancer cells in a time and dose-dependent manner. In addition, CTB-1 treatment modulated the cell cycle progression of colon cancer cells via G2/M arrest, coupled with a reduction of cells in the S phase. The expression of p21 was induced in key proteins involved in cell cycle regulation. G2 cell cycle progression were also modulated in response to CTB-1 treatment. Collectively, these findings, for the first time, shed light on the anti-proliferative effects of CTB-1 in colon cancer, and further demonstrate its potential as a novel therapeutic/preventive agent.


Thymus, brain, and testes-associated (Tbeta) is a negative control cell-cycle gene highly expressed in murine thymic epithelial cells (TECs). Tbeta protein binds to Uba3, inhibiting formation of Nedd8 E1 and subsequent target degragation via neddylation of several cell cycle control proteins needed for G2/M transition, which may be a major mechanism of TEC growth arrest during thymic involution. Etoposide is a cytotoxic drug which targets the enzyme topoisomerase II — increased 2-3 fold during the G2 phase. To further characterize effect of Tbeta on cell cycle, we modified our novel target-specific therapeutic from human osteosarcoma to express a Tbeta/mCherry fusion protein when mifepristone is added to culture media. Tbeta-expressing cells identified on flow cytometry did exhibit growth arrest, with quantitative assessment of DNA content in U2OS cells by flow cytometry establishing that 32% of Tbeta/mCherry-expressing cells were in the G2 phase when exposed to mifepristone for 24 hours, compared to 11% of mCherry-expressing cells. Gene expression studies were consistent with these results. Cells expressing Tbeta/mCherry were also more sensitive to etoposide at 0.5, 1, and 5 times the IC50 dose. To test whether p53 function was required, we further modified the cells to overexpress a dominant negative p53 mutant along with Tbeta/mCherry. Similar G2 arrest and increased sensitivity to etoposide were observed, indicating that the effects of Tbeta did not require normal p53 function. Potential Tbeta analogues or mimetics may therefore be used as an adjunct to G2-targeted chemotherapy.

#314 Evaluation of effective drug combinations of sonidegib and ribociclib for treatment of Sonic hedgehog medulloblastoma using mathematical models of the cell cycle and Hedgehog pathways. Zack Jones, Jessica Roberts, Haley Houke, Martine Roussel, Clinton Stewart, Carl Penetta. St. Jude Children’s Research Hospital, Memphis, TN.

Sonic hedgehog (SHH) tumors account for roughly 25% of all pediatric medulloblastoma. Aberrant signaling of the SHH pathway, typically via Patched-1 (PTCH) mutation, results in cell proliferation and subsequent tumor formation. Smoothed (Ptc1) inhibitors such as sonidegib have been used clinically to control tumor progression and have shown initial promise. However, many tumors acquire resistance to therapy. Combination therapy with an additional agent targeting another node in pathways affecting cell-cycle progression such as ribociclib, a cyclin D-dependent kinase (CDK) 4/6 inhibitor, may improve therapy and reduce tumor burden by delaying or preventing the development of resistance. Identification and evaluation of effective anti-cancer drug combinations, along with their schedule, sequence, and dosage are a major challenge due to the large number of possible combinations in addition to the cost and time involved in experimentally testing each possible permutation. Mathematical models can aid in the discovery of optimal drug combinations. A mathematical model of the SHH and Cyclin D/CDK 4/6 pathway was designed to evaluate effective sequences and schedules of sonidegib and ribociclib for the treatment of SHH medulloblastoma. The model was parameterized using in vitro data from studies treating the NIH3T3 cell line with sonidegib and ribociclib singly or in combination. Flow cytometry analysis was used to quantify response to treatment by obtaining cell-cycle phase distributions for each drug as a single agent or in combination. Uncertainty and sensitivity analysis of the model parameters were evaluated via Latin Hypercube Sampling and Partial Rank Correlation Coefficient analysis to interpret the effects of amplification or deletion of nodes in the pathway, evaluate potential targetable nodes, and identify sensitivity parameters that need robust quantification. Sensitivity analysis indicated that the formation of Cyclin D/CDK4/6 complexes and the dephosphorylation of Rb were significant nodes that should be included in the model. Uncertainty analysis indicated that the majority of the variability lies in the distribution of cells in G0/G1 and S phase. Simulations of single agent treatment predict a similar response between sonidegib and ribociclib, with co-treatment indicative of an additive effect. Optimal control theory methods are being applied to this model to help determine effective sequence, schedule, and dose of sonidegib and ribociclib to improve efficacy and reduce drug resistance in the treatment of SHH medulloblastoma.
Identification of SGK1 as a potential therapeutic target in castrate resistant prostate cancer. Massar I. Alsamaee, Ursula McClurg, Craig N. Robson, Stuart McCracken. *Northern Institute For Cancer Research, Newcastle upon Tyne, United Kingdom*

Introduction: Prostate cancer (PC) is the most common male cancer in the UK, with approximately 1 in 8 men developing the disease within their lifetime (Prostate Cancer UK). The androgen receptor (AR) has a crucial role in the proliferation and progression of prostate cancer. Patients respond to anti-androgen therapy in the early stage of the disease, however many will develop resistance, entering a “castrate-resistant” disease state (CRPC), carrying a very poor prognosis, posing a major clinical challenge (1). The development of second-generation agents and the use of bifunctional agents has shown promise in the treatment of CRPC patients, but response rates of just 50% and the development of resistance to these drugs have limited their success in the clinic (2,3). This study aims to interrogate the global gene expression consequences of anti-androgen resistance in a LNCaP prostate cancer cell line model, resistant to Enzalutamide. In our gene microarray, SGK1 demonstrated high expression in Enzalutamide resistant cells. Our subsequent experiments suggest SGK1 may serve as a biomarker of resistance or perhaps an exploitable target in CRPC. Methods: A gene microarray was used to determine the gene profile of parental LNCaP cells, sensitive to anti-androgen drugs, versus in-house generated LNCaP-Enzalutamide Resistant cells. QPCR to determine the mRNA level of SGK1 +/− Dihydrotestosterone (DHT), +/− Dexamethasone. Western blot was used to detect the protein level of the SGK1. In vivo, we observed a significant difference in the parental, androgen sensitive LNCaP cell line, versus the LNCaP-Enz-R cell line model, resistant to Enzalutamide. Inhibition of SGK1 using small molecular inhibitors significantly decreases proliferation and migration of the LNCaP-Enz-R cell line, whereas no changes were observed in the parental, androgen sensitive LNCaP cell line. In human xenograft model, enzalutamide and abemaciclib resulted in more significant reduction of phospho-p90RSK, phospho-Rb, phospho-S6 and Ki67 in H2122 tumors compared with either single agent. Overall, the combined inhibition of ERK1/2 and CDK4 and CDK6 was tolerated and enhanced antitumor efficacy in several KRAS mutant NSCLC preclinical models. These data support the feasibility of combining ERK inhibition with CDK4 and CDK6 inhibitors. Novel combination therapies which target CDK4 and ERK1/2 in combination with abemaciclib as a potential therapeutic strategy for the treatment of KRAS mutant NSCLC patients, and provides the rationale for the combination study in the on-going phase I LY3214996 clinic trial (NCT02857270).

**#318 Identification of FERM domain-containing protein 5 (FRMD5) as a novel target of β-catenin/TCF7L2 complex.** Yoichi Furukawa, Chi Zhu, Tomoyuki Ohsugi, Tumi Terakado, Rei Noguchi, Tsuneo Ikemue, Kyoshibi Yamaguchi, *Univ. of Tokyo, Tokyo, Japan*

Deregulated Wnt signaling is one of the earliest steps in colorectal tumorigenesis. The impairment results most frequently from APC mutations, which leads to the accumulation of β-catenin and subsequent activation of TCF7L2. Although previous studies have identified a number of target genes of the activated β-catenin/TCF7L2 transcriptional complex such as cMYC and cMyc, the comprehensive target gene set of the Wnt/β-catenin signaling remains to be elucidated. To understand the precise molecular mechanisms underlying colorectal cancer, we searched for novel genes regulated by the complex in colorectal tumors. We performed expression profile analysis of HCT116 and SW480 colon cancer cells treated with β-catenin siRNAs, and combined these data with public microarray data of LS174 cells treated with a dominant-negative form of TCF7L2. As a result, we identified a total of 134 genes that were regulated by both β-catenin and TCF7L2. Subsequent ChiP-sequence with TCF7L2 antibody also corroborated the interaction of TCF7L2 with a region (hg19 chr15: 44,449,680-44,450,487) within intron 1 of FRMD5. Reporter assay with plasmids containing this region revealed that the reporter activity was downregulated by the knockdown of β-catenin. These data suggested that FRMD5 is a direct target of β-catenin/TCF7L2 complex, and that the region is involved in the transcriptional activation of the TCF7L2 gene by interaction with the complex. Consistently, its expression was elevated in colorectal tumors compared to normal colonic mucosa in public microarray data. To uncover the role of FRMD5 in colorectal carcinogenesis, we analyzed expression profile of HCT116 cells treated with FRMD5 siRNA, and combined the data with the profile with β-catenin siRNA. Gene set analysis with 53 commonly up-regulated and 56 commonly down-regulated genes by both FRMD5 and β-catenin disclosed that the gene sets of DNA replication, cell cycle, and extracellular matrix (ECM) were altered by FRMD5. These data may be useful for the future studies of colorectal carcinogenesis.

**#319 Cholesterol inhibition reduces Hh mediated chordonosarcoma.** Qingxia Wei,1 Eyal Ramu,2 Mushirul Al-Jazrawe,1 Raymond Poon,3 Jay Wunch,2 Benjamin Alman3. 1The Hospital for Sick Children, Toronto, Ontario, Canada; 2Mount Sinai Hospital, Toronto, Ontario, Canada; 3Duke University, Durham, NC

Constitutive activation of Hh signaling is a common occurrence in chordosarcoma(CSa). Gene profiling analysis showed that Gli transcription regulates genes that govern cholesterol homeostasis, which alters cholesterol accumulation in chordocytes; a higher level of Gli-mediated transcription results in accumulation of intracellular cholesterol. Here we determined if targeting cholesterol-processing genes downstream of Hh signalling could be used as a novel treatment approach. With institutional review board approval, human CSA samples were obtained fresh from surgery. For in vitro studies, CSA explants of 2mmx2mm x2 mm cubic in size established as organ cultures. For in vivo studies, one mouse CSA explant was subcutaneously injected into NSG mice. Cells from five CSAs were treated both in vitro and in vivo with a hedgehog inhibitor, Cur61414, a cholesterol inhibitor, Lovastatin, or both. In vitro, CSA explants were treated for 48 hrs at concentration of 20 μM of each drug. In vivo, mice were treated with 4.5mg/kg/day of Cur61414, Lovastatin, or both. In vivo, CSA explants were treated for 48 hrs at concentration of 20 μM of each drug. In vivo, mice were treated with 4.5mg/kg/day of Cur61414, Lovastatin, or both by intraperi-
tonal injection for 4 wks. At the end of treatment, the explants or xenografts were harvested and processed for further analysis. RT-PCR was used to measure the expression of Hh and cholesterol target genes. Tumor size was measured from the xenografts. Blockade of Hh signaling significantly decreased Gil1 gene expression by 30%, increased 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) expression for more than 300% indicating decreased intracellular cholesterol. Treatment with the cholesterol inhibitor Lovastatin increased expression of HMGR for more than 500%. The combination of Hh and cholesterol blockage resulted in increased expression of HMGR for more than 3400%. Analysis of chondrosarcoma xenografts in vivo showed a significant decrease in tumor size with Lovastatin (32% decline), 3 folds reduction of BrdU (+) cells, and increase of Caspase-3 (+) cells. Treatment with Lovastatin and Curcumin on xenografts resulted in a significant decrease in tumor size (32% decline), 3 fold reduction of BrdU (+) cells, but no significant changes of Caspase-3 (+) cells. These data suggest that cholesterol functions downstream of Hh signaling pathway in CSA. The more effective reduction in tumor growth with cholesterol inhibition compared to Hh blockade suggests cholesterol blockade is an effective therapeutic approach.

#320 Potential of targeting of MYC-driven cancers via CIP2A without detrimental physiological effects. Xi Qiao, Turku University, Turku, Finland.

Transcription factor MYC is a driver of many human cancers due to its widespread effects on gene expression. An understanding of the mechanisms determining MYC transcriptional and proliferative/promotomat effects in vivo could facilitate approaches for MYC targeting. However, post-translational modifications that control MYC function in vivo are poorly understood. Here, we demonstrate that MYC phosphorylation at serine 62 enhances MYC accumulation on Lamin A/C-associated nuclear structures and that the protein phosphatase 2A (PP2A) inhibitor protein CIP2A is required for this process. CIP2A is also critical for serum-induced MYC phosphorylation and for MYC-elicted proliferation induction in vitro. Complementary transgenic approaches and an intestinal regeneration model further demonstrated the importance of CIP2A and serum 62 phosphorylation for MYC activity upon DNA damage-induced proliferation. However, targeting of CIP2A did not influence the normal function of intestinal crypt cells or general well-being of the mice. Meanwhile, in breast cancer cells, CIP2A depletion was shown to inhibit the MYC serum 62 phosphorylation, MYC-mediated gene expression, and anchorage-independent growth. Furthermore, CIP2A supports MDA-MB-231 xenograft growth in nude mice. Therefore, results of these studies collectively suggest for a novel opportunity to target MYC’s function in MYC-driven cancers via CIP2A, and without detrimental physiological effects.

#321 MUC1 regulates TGFβ function in pancreatic cancer. Priyanka Grover,1 Monica D. Nye,1 Mahboubeh Yazdnifar,1 Mohammad Ahmad,1 Ru Zhou,1 Lopamudra Das Roy,1 Kajal Grover,1 Shu-ta Wu,1 Sritama Nath,1 Pinku Mukherjee,1,2 Univ. of North Carolina - Charlotte, Charlotte, NC;2Univ. of North Carolina - Chapel Hill, NC.

In 2016, Pancreatic Cancer (PC) has moved to 3rd leading cause of cancer-related deaths in the USA with 94% dying within 5 years of diagnosis. 90% of PC is Pancreatic Ductal Adenocarcinoma (PDA), of which 80% of PDA overexpress tumor associated Mucin-1 (TMUC1), a membrane bound glycoprotein that is hypoglycosylated. Overexpression of TMUC1 is associated with metastasis and poor prognosis. However the mechanism remains obscure. Transforming growth factor-β (TGF-β) plays a role in poor prognosis. TGF-β is a cytokine with dual functionality. Within normal cells and early carcinogenesis, TGF-β functions as a tumor suppressor and induces apoptosis. This effect is mediated by activation of the canonical Smad pathway via engagement of TGF-β-Receptor 1 (TGF-βRI). However during later stages of cancer, TGF-β becomes a tumor promoter and stimulates epithelial to mesenchymal transition, migration, and invasion thus enhancing metastasis. This TGF-β effect is mediated by the non-canonical pathway. We recently showed correlation between TMUC1 and TGF-β within an exogenous tMUC1 model of PDA. Therefore, it is hypothesized that the mechanism between the two newly connected pathways exists in PDA tumors with high endogenous tMUC1 levels. We propose that the tyrosine kinases present in the cytoplasmic tail of tMUC1 are intermediary between the two pathways, thus leading to enhanced metastasis. First the secretion levels of TGF-βRI were assessed in a panel of 13 PDA cell lines with variable expression levels of TMUC1 by ELISA. The effects of TGF-βRI to induce apoptosis versus invasiveness on a variety of TMUC1 high and TMUC1 low PDA cell lines were determined. The effects of TMUC1 knockdown were evaluated in TMUC1 high cell lines via siRNA. We studied the role of tyrosine kinases and other protein complexes in mediating the interactions between tMUC1 and TGF-β via proteomics analysis. Finally, we evaluated the anti-tumor efficacy of neutralizing TGF-β1 in vivo and high and low TMUC1-expressing PDA tumors. In TMUC1 high PDA cells, TGF-β1 is secreted less when compared to TMUC1 low PDA cells, allowing for the more resistant cells to diminish TGF-β mediated effects of TGF-β1. This is supported by the observation that TMUC1-high PDA cells are more invasive, resist apoptosis, and activate Erk pathway, while TMUC1 low cells activate SMAD pathway. However, TMUC1 knockdown via siRNA does not affect TGF-β receptor levels. When studying the tyrosine kinases, c-Src becomes more phosphorylated in the presence of TGF-β1 in TMUC1 high cells, while in TMUC1 low cells c-Src becomes more deactivated in the presence of TGF-β1. Finally, within the mouse model, TMUC1-high PDA tumors respond to the neutralization of TGF-β1 by decreasing tumor size, while having no statistically significant result on tumor weight. TMUC1 expression is influential in TGF-β1-low PDA tumors. TMUC1 expression is influential in TGF-β1 function in an endogenous model. Neutralizing TGF-β1 in TMUC1 high expressing tumors can be considered as a possible treatment. This has high clinical significance for patients with PDA.

#322 Distinct pools of ShcA coupled tyrosine kinase signaling influences breast tumor heterogeneity and therapeutic responsiveness. Jacqueline R. Ha,1 Ruyhjin Ahn,1 Young Kyuen Im,1 Valerie Sabourin,1 Harvey W. Smith,2 Ivan Topisirovic,3 Tony Pawson,3 William J. Muller,1 Josie Ursini-Siegel,3,1 Lady Davis Institute for Medical Research, McGill University, Montreal, Quebec, Canada; Goodman Cancer Research Centre, McGill University, Montreal, Quebec, Canada; Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada.

Phospho-tyrosine (pTyr) signaling networks are frequently activated in breast cancers (BrCa) and are considered to be major oncogenic drivers of tumor progression. Therapeutic interventions, such as the tyrosine kinase (TK) inhibitor Trastuzumab, focus on targeting TK activity and downstream effectors. Although successful for early stage tumors, a subset of patients experience relapse due to intrinsic or acquired resistance. This includes activation of alternative receptor tyrosine kinase (RTK) and/or cytoplasmic TKs, many of which require recruitment of the adaptor protein, ShcA. ShcA is a key convergence point downstream of RTKs and serves to integrate multiple signal transduction pathways dysregulated in BrCa. Specifically, ShcA contains two pTyr binding motifs including an amino-terminal PTB domain and a carboxy-terminal SH2 domain which facilitate its interactions with TKs including ErbB2 and Src Family Kinases (SFK), respectively. The CH-1 domain houses three tyrosine phosphorylation sites at residues 239/240 and 317 which transduce Ras-dependent and independent signals. Using a well characterized transgenic mouse model of BrCa where ShcA can no longer engage the transforming oncogene through its PTB domain, we demonstrate that loss of PTB-driven ShcA (ShcA-PTBΔN) signaling delays mammary tumor onset. However, once formed, the growth and angiogenic potential of these tumors is significantly increased relative to control mice. Increased growth potential of ShcA-PTBΔN tumors is associated with the hyper-activation of the c-Src tyrosine kinase. Deletion of c-Src in ShcA-PTBΔN breast tumors significantly delays tumor onset but is dispensable for the growth of tumors that retain an intact ShcA PTB domain. These data suggest that the ShcA PTB domain can recruit negative regulators that limit the activation of downstream tumorogenic signaling networks. Interestingly, tumors expressing ShcA-PTBΔN deutilated in SH2 driven pTyr interactions (SH2ΔN), are significantly delayed in tumor onset relative to ShcA-PTBΔN controls. Paradoxically, deletion of c-Src in the context of ShcA-PTBΔN SH2ΔN further accelerates tumor growth which is attributed to increased levels of Fyn and Lyn. These observations support the high dependence of intracellular ShcA pools on other SFK family members to retain tumorigenic potential when adapting to low levels and/or activity of c-Src. We demonstrate that uncoupling of PTB-driven ShcA signaling from upstream RTKs can potentiate ShcA signaling from intracellular pools to hyper-activate SFKs. This data is clinically relevant as c-Src is frequently hyper-activated in Breast tumor-mammary tumor resistance. This is the first study to identify a tumor suppressive role of the ShcA PTB domain and to characterize an intrinsic ShcA SH2 domain-SFK dependent resistance mechanism downstream of activated RTKs in mammary tumorigenesis.


Background: The end point of most cell-signaling cascades is the translocation of protein complexes into the nucleus to modify transcriptional activity. For this reason, the analysis of cytoplasmic vs. nuclear localization provides important insights into cell activation. However, assessing nuclear translocation in complex cell mixtures, such as whole blood, is a difficult task. Leukocytes are a
rare event in blood, with many subpopulations, requiring the cells to first be concentrated, sorted, and often expanded prior to further molecular analyses. This process can take weeks, and questions regarding physiological relevance arise from even the first preparatory steps. Here, we present a buffer-based system to lyse RBCs and differentially stain leukocytes in order to enable the quantitative analysis of pERK expression vs. nuclear localization in endogenous cells by flow cytometry. We demonstrate the use of this system by analyzing the activation of NF-κB signaling in whole-blood monocytes following lipopolysaccharide (LPS) stimulation. Methods: The main components of the Whole Blood Nuclear Localization Kit are two buffers: Buffer 1 permeabilizes the cytoplasmic membrane, leaving the nuclear membrane intact, while Buffer 2 permeabilizes both the cytoplasmic and nuclear membranes. Briefly, blood was treated for different times with LPS, fixed for 10 min, split into 2 fractions, and then one was lysed for 30 min with Buffer 1 and the other with Buffer 2. Each fraction was washed, stained and acquired on a CytoFLEX-S flow cytometer (Beckman Coulter, Brea, CA). The cytoplasmic and nuclear signals were calculated using the compensated results from the two tubes. Blood was collected onsite daily from healthy, non-smoking human adults. Results: Following LPS stimulation, nuclear p50 and RelA increased maximally in monocytes by 10 min. Phospho-RelA S536 was maximal by 5 min mostly in the cytoplasm, while phospho-RelA S529 was maximal by 10 min in the nucleus. IκBα degraded maximally by 10 min. Additional kinase signaling will be presented, as well as controls to demonstrate the partitioning efficiency. Conclusions: This system is very fast and user-friendly, allowing for improved data in LPS-stimulating normal monocytes and other rare events in blood, with many subpopulations, requiring the cells to first be concentrated and often expanded prior to further molecular analyses. This process can take weeks, and questions regarding physiological relevance arise from even the first preparatory steps. Here, we present a buffer-based system to lyse RBCs and differentially stain leukocytes in order to enable the quantitative analysis of pERK expression vs. nuclear localization in endogenous cells by flow cytometry.

#325 Pediatric relapsed acute lymphoblastic leukemia patients display enrichment of the PI3K/mTOR pathway and respond to the dual PI3K/mTOR inhibitor PKI-587. Mohiuddin Gazi, Sasaun A. Moharram, Alissa Marshall, Kinjal Shah, Julush A. Kazi. Lund University, Lund, Sweden.  
Although significant improvements have been observed in the treatment of acute lymphoblastic leukemia, there is a substantial subset of high-risk T-ALL patients with relatively poor prognosis. T-cell acute lymphoblastic leukemia (T-ALL) is a hematopoietic malignancy affecting lymphoblasts of T-cell lineage which cause lots of hematopoietic cancer related deaths every year all over the world. The current overall cure rates of newly diagnosed childhood ALL are more than 80% in Europe, but approximately 20% of patients relapse due to treatment failure in childhood ALL. Like other leukemias, alterations of PI3K/mTOR pathway are predominant in T-ALL which is also responsible for treatment failure and relapse. In this study, two different gene expression data sets of relapsed patients also displayed enrichment of the PI3K/mTOR pathway. Out of 88 different inhibitors targeting multiple components of this pathway, we observed that PKI-587 was the most selective to the T-ALL cell line CCRF-CEM. Thus, we characterized the activity of the novel dual PI3K/mTOR pathway inhibitor PKI-587 using CCRF-CEM and Mol3s cells. We observed that PKI-587 blocked proliferation, colony formation and induced apoptosis in the T-ALL cell lines and selectively abrogated PI3K/mTOR without affecting the MAPK signaling. In vivo PI3K/mTOR inhibition delayed tumor progression, and reduced tumor load in a NGS/SCID xenograft mouse model. Since no deceptive body weight decrease was noticed, our conclusion is that this dose is effective and well tolerated. The beneficial effects of PKI-587 on T-ALL cells that has been observed in this study both in vitro and in vivo warrant further investigation.

#326 Baseline IL-17 receptor signaling is essential for controlling aberrant JNK-dependent cellular proliferation via maintenance of endogenous level of ubiquitin-editing enzyme A20. Chi Yan, Yang Lei, Anna L. Greenshields, David W. Hoskin, Tong-Jun Lin, Jun Wang, Dalhousie University, Halifax, Nova Scotia, Canada.  
The molecular mechanisms underlying aberrant activation of NF-κB and JNK in cancer remain incompletely understood. Here, we demonstrate that baseline IL-17 receptor (IL-17R) signaling is essential for controlling aberrant NF-κB and JNK activation, and restraining JNK-dependent homeostatic cellular proliferation. Using a shRNA knockdown approach, we demonstrated in B16 melanoma and 4T1 breast carcinoma murine cell lines that IL-17RA silencing markedly enhanced tumor cell growth in vitro and in vivo. Through mapping IL-17R signaling pathways, we further demonstrated that baseline IL-17A/IL-17R signaling actively restrained JNK phosphorylation in vitro and in vivo via the maintenance of basal expression of the ubiquitin-editing enzyme A20, a negative regulator of NF-κB and JNK. Remarkably, IL-17RA reconstitution evidently restored the A20 level, and suppressed cell proliferation and JNK activity in tumor cells. The reconstitution of A20 in IL-17RA knockdown subclones was able to restore the normal rate of cellular proliferation and associated JNK/C-Jun activity. Finally, meta-analysis of human cancer microarray and RNA-Seq data sets confirmed significant co-expression of IL-17RA and A20. Furthermore, alterations (mutation, upregulation or downregulation) of IL-17RA level in melanoma, ER+ breast cancer and colorectal cancer patients were associated with poorer overall survival compared to the respective patients with normal baseline IL-17RA expression. Together, our data demonstrates a previously unrecognized molecular mechanism underlying aberrant activation of NF-κB and JNK in cancer cells. This work highlights the unique biological role of proinflammatory IL-17R signaling in the maintenance of A20 to regulate the pathogenesis of human cancer, which draws caution on the utility of IL-17A neutralizing antibody in cancer therapy. CY is a PhD student supported by the graduate student research training program as part of The Terry Fox Foundation Strategic Health Research Training Program as part of The Terry Fox Foundation Strategic Health Research Training Program in Cancer Research in CIHR.

Epithelial cell adhesion molecule (EpCAM) is highly expressed in advanced epithelial cancers and tumor-initiated cells (TICs), but its roles in cancer pro-
growthression remain to be elucidated. Here, we showed that the extracellular domain of EpCAM (EpEX) could bind to EGFR through EGFR-like domain I, and subsequently activated its downstream molecules, ERK1/2 and Akt. EGFR inhibitor and knockdown of EGFR by siRNA ablated EpEX-induced ERK1/2 phosphorylation. Regulated intramembrane proteolysis (RIP) of EpCAM was induced similarly in EpEX stimulation as EpCAM through EGFR signaling with SINE compounds (selinexor or KPT-8602) have increased basal GR protein levels. Consistent with these results, the SINE-DEX combination shows enhanced GR transcriptional activity. Several GR-DEX target genes are known to inhibit the GTPase, Ras homolog encoded in brain (RHEB), which is required for mTORC1 activation. We discovered that the SINE-DEX combination not only reduces RHEB protein but also induces the RHEB inhibitory antibody to mTORC1. As a result, we can expect a combinatorial effect of the SINE compound-mediated inhibition of mTORC1 (i.e. reduced phosphorylation of S6K1 and 4E-BP1) is GR independent, SINE-DEX inhibition is more robust in GR+ MM cell line when compared to the GR- MM.1R cells. The combination resulted in the selinexor IC₅₀ in MM.1S cells shifting from 40 nM to 11 nM in the presence of low dose DEX. As expected, DEX did not modulate the SINE compound IC₅₀ in MM.1R cells. Conclusion: We show that SINE compound inhibition of MM cell viability is enhanced with DEX. Our results indicate that this combinatorial effect is due to convergent suppression of mTORC1 signaling by GR targets. The findings provide mechanism of action data around the SINE-DEX combination in MM with suggestive biomarkers (REDD1, KLF15, BCAT2 and GR) that may predict best response to the combination. Therefore, these data may translate directly to the current development of SINE compounds.

#330 SGK1 activation is essential for PI3K-dependent tumor development. Arturo Orlacchio, Antonio Di Cristofano. Albert Einstein College of Medicine, Bronx, NY.

The PI3K signaling cascade is frequently activated in human cancer, with AKT being commonly considered its major transforming conduit. Conditional deletion of Pten in the mouse thyroid epithelium induces constitutive activation of the PI3K pathway, which causes thyroid hyperplasia at birth that progresses to invasive and metastatic follicular carcinoma. Concomitant loss of p53 or activation of KRAS dramatically accelerates the development of aggressive and lethal tumors. The PKD1 kinase plays a key role in the PI3K signaling cascade, directly activating, in a PI3K-dependent manner, not only AKT but also a set of additional AGC kinases, including, S6K, PKC, and SGK. Using in vivo, ex vivo, and in vitro genetic and pharmacological approaches, we now show that AKT activation is not sufficient to transform thyroid epithelial cells. Concomitant activation of additional PDK1-dependent pathways is absolutely required to develop neoplastic lesions in vivo, and to induce cell proliferation ex vivo, in mouse models based on Pten loss, alone and in combination with additional genetic alterations. In particular, we show that one member of the SGK family, SGK1, is an essential mediator of the transformation process downstream of PI3K. Genetic and pharmacological SGK1 inhibition strongly reduces cell proliferation in Pten+/− and PI3KCA-mutant cell lines. Moreover we show that concomitant SGK inhibition significantly increases the efficacy of inhibitors targeting AKT or PI3K. Taken together, our data identify an essential and druggable signaling cascade that critically cooperates with AKT activation to transform thyroid epithelial cells.

#331 SIRPB1 promotes prostate cancer cell proliferation & migration. Qiong Song,1 Siyu Yin,1 Chunlin zou,1 Wenchu Wang,1 Lihui Wang,1 Haibo Tong,1 William J. Catalona,2 Jian Zhang,1 Yi Lu,1 Zhou Wang3. Signal-regulatory-protein beta 1 (SIRPB1) is a member of the signal-regulatory-protein (SIRP) family that belongs to the immunoglobulin superfamily and is capable of modulating receptor tyrosine kinase-coupled signaling. The copy-number variations (CNV) at the SIRPB1 locus were associated with aggressive prostate cancer in patients. To test if SIRPB1 could affect prostate cancer development and progression, we investigated the potential role and mechanisms of SIRPB1 action in prostate cancer cell lines in vitro and in xenograft tumors. Knockdown of SIRPB1 by RNA interference resulted in significant suppression of cell colony formation, cell mobility, cell migration, and invasion. The knockdown also induced cell cycle arrest during the G₀/G₁ phase and a remarkable enhancement of apoptosis in PC3 prostate cancer cells. In contrast, overexpression of SIRPB1 significantly induced cell migration, invasion, colony formation and cell cycle in C4-2 prostate cancer cells. Furthermore, overexpression of SIRPB1 in C4-2 cell model enhanced its tumor take rate in nude mice. Also, SIRPB1 mRNA expression was increased in up to 39% of the prostate cell line. Poly(ADP-ribose) polymerase-1 (PARP1) or ADP-ribosyltransferase diphtheria toxin-like 1 (ARTD1) is the most abundant and the best understood member of the 17 PARP family proteins. PARP1 binds to both single strand breaks (SSBs) and double-strand breaks (DSBs) and participates in the recognition, excision and repair of DNA damage. The most extensively studied role of PARP1 is its involvement in base excision repair (BER) and PARP inhibition-induced trapping of PARP1 during BER. Moreover, suppression of PARP1 is shown to lead to synthetic lethality in BRCA1/2 deficient tumors, indicating that PARP1 dependent BER and BRCA-dependent homologous repair pathway have overlapping and redundant functions in DNA repair. Recent studies have also pointed to a broader utility of PARP inhibitors beyond hereditary BRCA-deficient cancers. Pyruvate kinase isoform M2 (PKM2) is a glycolysis enzyme that converts phosphoenolpyruvate (PEP) into pyruvate. Up-regulation of PKM2 has been shown recently to be an important feature of tumorigenesis. In tumor cells, PKM2 forms a dimer that is catalytically inactive as a glycolysis enzyme, but provides advantage for tumor progression due to Warburg effect. In tumor cells, PKM2 forms a dimer that is catalytically inactive as a glycolysis enzyme, but provides advantage for tumor progression due to Warburg effect. In tumor cells, PKM2 forms a dimer that is catalytically inactive as a glycolysis enzyme, but provides advantage for tumor progression due to Warburg effect. In tumor cells, PKM2 forms a dimer that is catalytically inactive as a glycolysis enzyme, but provides advantage for tumor progression due to Warburg effect.
cancer specimens based on in silico analysis of several public databases. These results suggest that SIRPB1 is a potential oncogene in the prostate and could be used as a biomarker to identify patients at risk of developing aggressive prostate cancer.

Loss of primary cilium promotes LPA-driven proliferation in glioblastoma.

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Glioblastoma is one of the most common brain cancers with a median survival of 13 months. Therapeutic approaches for this type of cancer are very limited, creating an urgent need to develop new strategies for the treatment. Recently, several studies reported that glioblastoma harbors a drastic decrease in primary cilia, suggesting that loss of cilia might be important for glioblastoma biology. The primary cilium is a ubiquitous microtubule based organelle presented on most of human cells. It plays significant role in embryonic development and tissue homeostasis, serving as a hub for multiple signaling cascades including RTK, Sonic Hedgehog, Wnt and Hippo. Here we report that loss of primary cilium in immortalized astrocytes stimulate cell proliferation and drastically change ERK1/2 activation in response to serum. These changes depend on lysophosphatidic acid (LPA), a water-soluble lipid metabolite, implicated in chemotactic and proliferative signaling. The receptor to LPA type 1 tends to accumulate in the primary cilium, indicating its potential role in inhibition/restriction of LPA-driven proliferation of glioblastoma cells. The altered Wnt signaling via dynamic changes in primary cilium. Loss of primary cilium can lead to LPA receptors 1 redistribution to the plasma membrane and switch cellular response for the LPA, allowing it to act as mitogen. Inhibition of LPA receptors with a small molecule inhibitor, K16425, is significantly decreasing the growth of glioblastoma PDXs. Overall, our findings clearly indicate that loss of primary cilium is sufficient to severely change mitogen-driven signal transduction and point towards the new therapeutic target for glioblastoma.

Identification of a natural product small molecule inhibitor against Wnt/β-catenin signalling pathway.

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Wnt/β-catenin signalling pathway plays an important role in many processes like cell proliferation, differentiation, regeneration, carcinogenesis and regulates stem cell pluripotency. β-Catenin, the central component of Wnt/β-catenin signalling pathway has a vast number of binding partners and thus plays different roles in the cells. Mutations of β-catenin are seen in many cancers, including liver cancer, colorectal cancer, lung carcinoma, ovarian cancer and malignant breast tumors. Till date there is no even a single small molecule in clinical use that can block the β-catenin activity or the activities of this signalling pathway. In this study, we established many assays for screening small molecule inhibitors of this signalling pathway. Initially, we created a library of β-catenin deletion and point mutants and carried out a comprehensive comparative analysis of these mutants with luciferase reporter assays, GFP-fluorescence, immunoblotting and protein-protein interactions. We then screened many known and unknown natural and synthetic molecules against the wnt/β-catenin pathway and got 2 hits: C-18 and 055A, bioactive metabolites from actinomycetes. The IC50 (cell viability) of 055A and 055A, bioactive metabolites from actinomycetes. The IC50 of 055A, C-18 and salinomycin in HepG2 cells was 7.5 μM, 9.7 μM and 25 μM respectively. Interestingly, C-18 was ineffective against non-cancerous cell line (HEK-293 cells) even up to 60 μM. This shows that C-18 is a very promising hit because it is active against cancer cells and spares non-cancerous cells. The sub lethal doses of these molecules were then tested for β-catenin mediated transcription by luciferase reporter assay in HepG2 cells which harbours a β-catenin deletion responsible for enhanced β-catenin mediated transcriptional activity. Our results show that C-18 and 055A significantly block the β-catenin activity in a dose dependent manner. The inhibition of wnt/β-catenin pathway by C-18 was observed at lower nanomolar concentrations in comparison to 055A and salinomycin which shows activity in micromolar concentrations. The β-catenin target gene expression analysis showed marked decrease in cyclinD1 by immunoblot analysis upon treatment of HepG2 cells with C-18. Interestingly, C-18 treated HepG2 cells failed to show any apoptosis as confirmed by Parp-1 and caspase-3 immunoblotting indicating that C-18 can be targeted to block the activity of wnt/β-catenin pathway, and could act as a lead molecule. Therefore, it warrants further experimentation and validation which are underway to draw the further conclusions.

PTEN-L regulates epithelial growth and macrophage function.

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PTEN is among the most frequently mutated and deleted tumor suppressor genes in many malignancies, including breast cancer. An alternatively translated long form of PTEN, termed PTEN-L, has divergent functionality from PTEN, although its function at the organism level has not been studied. Here, we report that PTEN-L is expressed with the ablation of PTEN-L expression but not exact expression of PTEN. These mice display mammary ductal hyperplasia characterized by increased luminal growth and increased numbers of macrophages in the surrounding stroma. Macrophages are particularly affected by PTEN-L loss, with significant changes to their secretomes and functional deficiencies in clearing bacterial infections, consistent with a shift toward an M2-like polarization. Overall, these findings demonstrate that PTEN-L has unique functions in regulating mammary epithelial growth and macrophage functionality that are independent of canonical PTEN.

In situ protein complexes as a measure of AXL signaling and inhibitor response in cell line models of lung cancer.

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AXL kinase overexpression is associated with metastasis and poor prognosis in various cancers. Recent studies show that epithelial-mesenchymal transition (EMT) and associated activation of AXL is one of the mechanisms by which tumors develop resistance to targeted therapies, such as EGFR tyrosine kinase inhibitor (TKI) in lung cancer. These studies have made AXL an attractive drug target. However, the lack of genetic alterations in AXL and recent evidence suggesting that ligand secretion into the tumor microenvironment plays a key role in signaling and resistance in cancer cells, underscore the need for assays to visualize active AXL signaling associated complexes. In this study, we aimed to develop a Proximity Ligation Assay (PLA) to effectively measure AXL activation in tumor tissues in situ, and to use the assay to evaluate the pharmacodynamic effect of a novel AXL TKI, RXD-106X, in lung cancer models. To this end, several lung cancer cell lines were analyzed for total and phosphorylated AXL (pAXL) expression as well as effects of AXL TKI on downstream signaling. Co-immunoprecipitation studies were also conducted to identify adaptor proteins that form active signaling complexes with AXL kinase, and PLAs were developed to detect these complexes in situ. H1299 and Calu1 cells have the highest pAXL among the cells screened. RXD-106X potently inhibits pAXL and downstream pAKT, but does not affect pERK on cell viability, in these cell lines. We also observe an increase in total AXL in response to the RXD-106. Co-immunoprecipitation shows RXD-106-dependent reduction in AXL-P13KR1 (Phosphoinositide-3-Kinase, Regulatory Subunit 1) interaction that is consistent with the drug-induced reduction in pAKT by western blot. Based on this observation, we developed PLAs to detect active AXL-P13KR1 and AXL-pY100 signaling complexes in these cells. H1299 and Calu1 cells have high basal AXL: P13KR1 and AXL-pY100 PLA foci that are abrogated by RXD-106X, in both fresh fixed and formalin-fixed paraffin-embedded cells. As expected, the HCC287 cells, which lack ligand independent pAXL, do not show significant labeling by either PLA. Overall, we demonstrate that RXD-106X is a potent inhibitor of AXL kinase and its downstream P13K/AKT signaling pathway. We also show that activated AXL signaling complexes can be annotated specifically by PLA. The data provides justification for the extension of our PLA as a tool to measure drug-targetable signaling complexes to identify new models of activated AXL and enrich for activated AXL in patient tissues.

PI3K3CB/p101B is a survival factor in glioblastoma.

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Glioblastoma multiforme (GBM) is lethal even after surgical removal of the tumor, radiation, and chemotherapy. Residual tumor cells form an intractable tumor in nearly all patients within two years. Recurrent GBM is incurable due to resistance to current therapies. Inhibitors of PI3K (phosphatidylinositol-4,5-biphosphate 3-kinase)-a signaling pathway that causally contributes to tumor formation/recurrence have been used to treat recurrent GBMs and achieved modest clinical effect. This is perhaps attributed to non-selective inhibition of PI3K isoforms, which yields intolerable toxicity. Class IA PI3K isoforms include three catalytic subunits (PI3KCA, B, or D that encodes p110α, B, or D) and three regulatory subunits (PI3KRI-3 that encodes p85 isoforms). Our recent work
indicates that PIK3CB levels positively correlate with the chances/risk of GBM recurrence, while being inversely associated with patient prognosis. This suggests that PIK3CB/p110β is important for GBM cell survival. To test this hypothesis, we first measured the expression of PIK in isofoms in a panel of 9 GBM cell lines. We found that U87MG, SF295, and U251 expressed much higher levels of p110β, coinciding with the levels of phosphorylated AKT. When we knocked down PIK3CA, B, and D in human U87MG cells and found that only depletion of PIK3CB/p110β resulted in an inactivation of downstream AKT. Moreover, knockdown of PIK3CB/p110β, but not other isofoms, induced substantial growth inhibition in U87MG, SF295 and U251 cells. This is congruent with the result that inhibition of PIK3CB/p110β activated apoptosis in U87MG cells. We then determined the impact of PIK3CB depletion in 10 GBM cell lines, which have previously been linked to control of the ciliary protrusion/resorption cycle. Mechanistic analysis confirmed these compounds controlled activation of Aurora-A at the basal body; in vivo testing demonstrated sunsitibin resulted in loss of cilia not only in vitro, but also in normal kidney, kidney tumors, and in renal cysts associated with polycystic kidney disease (PKD), a disease dependent on defective and other tumor types downregulating them. Moreover, knockdown of PIK3CB/p110β selectively represses the viability of these cells. Finally, we report that ectopic expression of p110β, but not p110α δ, partially rescued U87MG cells from growth inhibition induced by TGX-221, a PIK3/p110β selective inhibitor. Collectively, our results demonstrate that PIK3CB/p110β is an important selective survival factor in GBM, underscoring the divergent roles of PIK3 isofoms in GBM disease progression/recurrence and future therapeutic intervention.

#337 Sonic Hedgehog ligand drives tumor progression and chromosomal instability in a mouse model of small cell lung cancer. Anette Szczepny,1 Samuel Rogers,2 Samantha Jayasekara,1 Kwon Park,3 Rachael McCloy,4 Julien Sage,4 Craig Peacock,5 Jason Cain,6 Andrew Burgess,2 David Neil Watkins,3 Hudson Institute of Medical Research, Victoria, Australia; Garvan Institute of Medical Research, Darlinghurst, Australia; University of Virginia, Charlottesville, VA; Stanford Cancer Institute, Stanford, CA; Cleveland Clinic, Cleveland, OH.

Activation of the Hedgehog (Hh) signaling pathway is well documented in many cancers including Small Cell Lung Cancer (SCLC). Whilst it has been shown that Smoothened, the central Hh pathway mediator, is required for the initiation and progression of SCLC in a mouse model, it is unclear what drives activation of this pathway in these tumors. To address this question, we used a well-characterized conditional genetic mouse model of SCLC in which inhibition of recombinant adenovirus expressing Cre can trigger recombination at loxp sites in the airway epithelium. When the virus is administered to mice double homozygous for the conditional p53 and Rb knockout alleles (p53lox/lox; Rblox/lox), mice develop multiple tumors over 9 months. In order to manipulate Shh expression in this model, we crossed these animals with either gain-of-function or loss-of-function Shh alleles to observe the effects on SCLC initiation and progression in vivo. Aged cohorts of AdenotCre-infected p53lox/lox; Rblox/lox; ShHtg mice developed more frequent and significantly larger tumors compared to p53lox/lox; Rblox/lox; Shhltm littermate controls, with tumors exhibiting a highly malignant and proliferative phenotype. Conversely, deletion of Shh resulted in no sign of change in tumor number but a dramatic reduction in tumor size in p53−/−/Shh−/− mice compared to littermate controls. Inhibition of Shh ligand overexpression also induced marked chromosomal instability and the Smoothened-independent nuclear translocation and activity of Cyclin B1. In turn, overexpression of Cyclin B1 induced chromosomal instability in cells lacking both p53 and Rb. These results suggest that Shh ligand drives progression of SCLC by activating both the canonical and non-canonical arms of the Hh pathway and that Shh may be a potentially useful therapeutic target.

#338 Unexpected activity of multiple targeted cancer drugs in regulating ciliary dynamics. Anna Kiseleva,1 Vladislav Korobeynikov,2 Anna Nikonova,3 Alexander Deneka,1 Margret Einarson,1 Emmanuelle Nicolas,1 Petr Makhov,1 Craig Peacock,1 Jason Cain,2 Andrew Burgess,2 David Neil Watkins,3 Hudson Institute of Medical Research, Victoria, Australia; Garvan Institute of Medical Research, Darlinghurst, Australia; University of Virginia, Charlottesville, VA; Stanford Cancer Institute, Stanford, CA; Cleveland Clinic, Cleveland, OH.

The primary cilium is an antenna-like structure protruding from the cell surface, which provides a platform for receptors for signaling systems including PDGFR-alpha, Hedgehog, Wnt, and others that influence cell differentiation and proliferation decisions. Under normal physiological growth conditions, the cilium forms after mitosis and in quiescent cells, extending from a basal body centerated at a centriole, with timed resorption in G0 or early G1. Cancer cells have altered ciliary dynamics, with some (medulloblastomas and basal cell carcinomas) often dependant on cilia, and other tumor types downregulating them. In prior work, we defined Aurora-A, an oncogenic kinase typically thought of as a mitotic regulator, as transiently activated at the basal body, and absolutely required for resorption of cilia at the G0/G1 boundary, and we showed the targeted Aurora-A inhibitor entirely blocked ciliary resorption. In subsequent work, we found that a second drug, the EGFR inhibitor erlotinib, also affected ciliary resorption, positioning erlotinib and alisertib to influence cilia-dependent signaling. In this study, we broadly assessed the activity of a panel of targeted preclinical and clinical agents of known specificity for action in controlling ciliary dynamics. For this, we developed stable hTER-TPE1 cell line models with integrated Arl13b-GFP reporters to visualize cilia, and performed a mid-throughput screen of 180 drugs for activity in 1) independently inducing ciliary resorption in quiescent G0 cells, or 2) blocking ciliary resorption in cells induced to cycle by serum treatment. With a 5% hit rate overall, we identified 9 compounds inducing, and 7 compounds blocking resorption. This included some well-known drugs as well as some surprises, which have previously been linked to control of the ciliary protrusion/resorption cycle. These data for the first time suggest new mechanisms for activity of sunsitibin and other drugs, in which they can influence activity of lateral signaling pathways such as Wnt and PDGFα by regulating the availability of receptors on a ciliary signaling platform.

#339 Identification of a novel isoform of WNT/planar cell polarity VANGL2 in breast cancer. Jean-Paul Borg, Alexandra Walron, Eric Bailly, Sylvie Marchetto, Stéphane Audebert. CRCM Inserm-Institut Pauli-Calmettes, Marseille, France.

The WNT/PCP pathway is an evolutionarily conserved developmental process which is essential in embryogenesis and development of polarized structures in metazoa. Its importance in human diseases is best demonstrated in several tube-forming diseases and cancer. WNT/PCP signaling involves a set of evolutionarily conserved WNT/PCP genes encoding WNT ligands, transmembrane (vangel2, frizzled, fat, dachous) and cytoplasmic (scribble, prickle, dishevelled, diego) molecules triggering a genetically well-defined non-canonical WNT-JNK pathway. Recent work has linked defects of this pathway to breast cancer aggressiveness. We have shown that the non-canonical WNT/PCP transmembrane receptor VANGL2 is overexpressed in poor prognosis basal breast cancers and implicated in tumor growth. We also found that VANG2 binds through its C-terminal sequence to Scribble and p62/SQSTM1 which are involved in cancer cell migration and growth. A recent in silico study has suggested the existence of an N-terminally extended VANGL2 isoform (VANGL2-Long), in favor of the occurrence of an alternative non-AUG translation initiation site, upstream of the conventional start site. Translation initiation from this alternative site is expected to add an N-terminal extension of 48 amino acids. Accordingly, by analyzing VANGL2 immunoprecipitates by mass spectrometry, we identified a peptide mapping upstream of the first methionine supporting the existence of a longer isoform of VANGL2. While missing in Drosophila, this N-terminal extension is found in all vertebrate VANGL2 sequences that have been examined, suggesting a function for VANGL2-Long. We provide further experimental evidence in favor of the VANGL2-Long isoform, using polyclonal antibodies that have been raised against the predicted VANGL2 N-terminal extension. Using these tools, we characterized VANGL2-Long and showed that it dimerizes with VANGL2 and VANGL1, a close homologue, at the endogenous level in living cells. Our data depict a complex organization of VANGL2/VANGL1 molecules at the plasma membrane and improve the mechanistic understanding of VANGL2 in normal and cancer cells.


SRMS (Src-related tyrosine kinase lacking C-terminal regulatory tyrosine and N-terminal myristoylation sites) belongs to a family of non-receptor tyrosine kinases, which harbours a Src homology 3 and a Src homology 2, as well as a protein kinase domain. SRMS was first identified in a screen for the genes that regulate the growth and differentiation of neuroepithelial cells. SRMS, however, is an understudied member of this family. The present study was undertaken in order to explore the role of SRMS in signaling downstream of KIT. The receptor tyrosine kinase KIT, also known as the stem cell factor receptor, plays a key role in several developmental processes and have been implicated in many human cancers such as gastrointestinal stromal tumors, acute myeloid leukemia and testicular carcinoma. To understand the role of SRMS in KIT signaling, we generated Ba/F3 cell lines overexpressing KIT and SRMS. We observed that SRMS regulates normal and oncogenic KIT signaling differentially with respect to cell proliferation and apoptosis. SRMS association triggers KIT ubiquitination which in turn downregulates the receptor. Further, expression of SRMS downregulates wild-type KIT-mediated phosphorylation of AKT, ERK1/2, p38. Taken together the data demonstrates that SRMS regulates both normal KIT and an oncogenic mutant of KIT, and have differential impact on receptor downstream signaling.

Aberrant expression of CXCR4, a chemokine G protein-coupled receptor (GPCR), drives primary and secondary breast cancer metastasis, however the mechanism is unknown. Here we investigate the role of CXCR4 tyrosine phosphorylation within a novel ITIM motif. Following activation, CXCR4 is normally phosphorylated on Ser/Thr residues, which promotes receptor desensitization and internalization. We found previously that Ser/Thr phosphorylation of CXCR4 is delayed upon SDF gradient sensing, and correlates with sustained signaling to SHP2, a protein that drives hyperproliferation and invasion of breast cancer. Since SHP2 is known to be recruited to tyrosine phosphorylated ITIM motifs (immunoreceptor tyrosine-based inhibitory consensus motifs), we investigated if CXCR4 contains an ITIM motif, and if tyrosine phosphorylation within this motif regulates SHP2 binding, signaling, and migration. Specifically, we assessed 1) if CXCR4 is tyrosine phosphorylated as measured by recombinant phosphatase assay, phospho-Ab development, and tyrosine mutagenesis (YF); 2) if tyrosine mutagenesis alters CXCR4 membrane localization, internalization, and SDF gradient sensing, by ELISA; 3) if tyrosine mutagenesis disrupts CXCR4 interaction with SHP2, by co-IP; 4) if tyrosine mutagenesis alters CXCR4 sustained signaling to SHP2, by Western; and 5) if tyrosine mutation (YF) within the novel ITIM motif in CXCR4 alters migration of metastatic breast cancer cells, by transwell assay. These data demonstrate that CXCR4 phosphorylation is sensitive to recombiant tyrosine-phosphatase treatment and tyrosine mutagenesis, suggesting that CXCR4 is indeed tyrosine phosphorylated within the ITIM motif. We determined that while tyrosine phosphorylation within the ITIM motif is not required for CXCR4 membrane localization, internalization, or SDF gradient sensing, it does regulate CXCR4 binding and signaling to SHP2. Investigating the role in CXCR4 mediated migration is on going. To date, our data support a working model that tyrosine phosphorylation of CXCR4 within an ITIM motif is critical for binding to SHP2 and transducing sustained signaling to SHP2. These data have implications on aggressive breast cancers with dysregulated CXCR4 and SHP2. These studies were supported by NIH grant GM-097718, PA Department of Health and grant SAP4100057688, and the Milton Lev Memorial Faculty Research Fund.

WHSC1L1-mediated EGFR mono-methylation enhances the cytoplastic and nuclear oncogenic activity of EGFR in head and neck cancer. Vassiliki Saloura,1 Theodore Vougiouklakis,1 Makda Zewde,1 Xiaolan Deng,1 Naoshi Doihama,1 Takehiro Suzuki,1 Ryui Hamamoto,1 Yusuake Nakamura1. 1Univ. of Chicago, Chicago, IL; 2Oncotherapy Science, Japan; 3RIKEN, Japan.

WHSC1L1, a protein lysine methyltransferase, is overexpressed in a variety of malignancies, including head and neck cancer (SCCHN), which is characterized by high progression. Overall, our study demonstrates the multifaceted oncogenic function of the protein lysine methyltransferase WHSC1L1 in SCCHN, which is mediated through direct non-histone methylation of the EGFR protein with effects both in its cytoplasmic and nuclear functions.

Proteomic and functional studies identify DCAF7 as major partner of DYRK1A. Varsha Anathapadamanabhan,1 Selene Swanson,2 Siddharth Saini,1 Vijay Menon,1 Larisa Litovchick1. 1Virginia Commonwealth University, Richmond, VA; 2Slowers Institute for Biomedical Research, Kansas City, MO.

DYRK1A protein kinase is encoded by dosage-dependent gene since an extra copy contributes to Down syndrome (DS) pathogenesis while loss of one allele causes severe developmental defects. Current knowledge of DYRK1A’s role in phosphorylation of proteins involved in cell cycle control, transcription and tumor suppression does not fully explain dosage-dependent function of this important kinase. Using MudPIT proteomic analysis, we identified DYRK1A interacting proteins in human T98G cell line. Four independent DYRK1A-HA pull-down samples were analyzed to identify 50 proteins that were specifically detected in 3 out of 4 replicates. This analysis identified WD-repeat protein DCAF7 as major partner of DYRK1A that was most highly enriched among DYRK1A-binding proteins. Furthermore, glycerol gradient ultra-centrifugation showed almost exact co-fractionation of DCAF7 and DYRK1A. DCAF7 has been shown to bind several protein kinases including DYRK1A, DYRK1B, HIPK2 and MAP3K1 suggesting that it serves as a scaffold protein in signal transduction. However, the function of DCAF7 is not established yet. In order to understand the functional relationship between DCAF7 and DYRK1A, we undertook a MudPIT proteomic analysis of DCAF7 binding proteins in T98G cells and compared the data with the DYRK1A interactome dataset. Analysis of three independent DCAF7-HA pull-down samples identified 32 proteins specifically detected in all three replicates including DCAF7, DYRK1A and five proteins earlier detected in DYRK1A MudPIT analysis. Given the proposed role of DCAF7 as a scaffold protein, we tested whether DCAF7 mediates its interaction with some of its interacting proteins. Interestingly, we found that RNF169, a recently characterized RING-domain ubiquitin ligase involved in the DNA double-strand break (DSB) repair, binds to N-terminus of DYRK1A independently of DCAF7. Furthermore, our data show that DYRK1A is required for DCAF7-RNF169 interaction because this interaction was abolished in human U-2 OS cells where DYRK1A gene was disrupted using CRISPR-Cas9 editing (DYRK1A-KO cells). Interestingly, stable overexpression of DCAF7 in DYRK1A-KO U-2 OS cells rescued some of the phenotypes observed in these cells, suggesting that DCAF7 could be an effector downstream of DYRK1A. Our findings revealed an unexpected scaffolding role of DYRK1A that is required for mediating the interaction between DNA repair protein RNF169 and the WD-repeat protein DCAF7. While further studies are needed to understand the exact role of the RNF169-DCAF7 interaction in DNA repair, the novel role of DYRK1A as a scaffold protein could explain some aspects of the dosage-dependent function of this protein kinase. In addition, we report here an initial functional characterization of the DCAF7-DYRK1A interaction in human cells and discuss novel functional interactions of DCAF7 that could be independent of DYRK1A.

Chondroitin sulfatases regulate Wnt signaling through effects on Shp2, phospho-Erk1,2, c-Myc, and histone methylation of DKK3 in prostate cancer and prostate stem cells. Joanne Kramer Tobacman, Sumit Bhattacharya, Leo Feferman. Unv. of Illinois at Chicago, Chicago, IL.

The chondroitin sulfatases N-acetylgalactosamine 4-sulfatase (arylsulfatase B, ARSB) and N-acetylgalactosamine 6-sulfatase (galactose 6-sulfatase, GALNS) remove the 4-sulfate and 6-sulfate groups of chondroitin sulfates. ARSB activity was found to remove 4-sulfate groups of chondroitin 4-sulfate (C4S) or dermatan sulfate, and GALNS acts to remove 6-sulfate groups of chondroitin 6-sulfate (C6S) or chondroitin 4.6-disulfate, as well as keratan sulfate. In human prostate cancer tissues, ARSB activity was markedly reduced and GALNS activity increased by immunohistochemistry of malignant prostate tissue captured by laser microdissection and by assay of human prostate stromal and epithelial cell lines, ARSB was predominant in stromal cells of the extracellular matrix, whereas GALNS was predominant in prostate epithelial cells. When ARSB was reduced by silencing in human prostate stem cells or GALNS was increased by overexpression in the stem cells, SHP2 binding to chondroitin 4-sulfate increased, leading to inhibition of phosphatase action and sustained phosphorylation of Erk1,2. Erk activation increased in c-Myc depleted increase in c-Myc expression and increased expression of DNMT 1a, 3a, and 3b. DNMT activation increased following ARSB silencing or GALNS overexpression, and was blocked by AKR inhibition. Increased DNMT activity led to the increased promoter methylation of DKK3, and the subsequent inhibition of nuclear β-catenin nuclear translocation and Wnt signaling. Decline in Wnt signaling was manifested by reduced TCF-LEF nuclear binding, and decline in the mRNA expression of c-Myc and GATA. Since c-Myc activation can lead to increased expression of cell cycle regulators, the changes in chondroitin sulfation mediated by sulfatases can lead to profound effects in cell proliferation. Hence, the modification of sulfatase activity, manifested as increased chondroitin 4-sulfate, provides the platform for an extensive extracellular-intracellular signaling network that can regulate phosphorylation, DNA promoter methylation, and proliferation, and affect development and malignant transformation.
Glioblastoma (GBM) is the most common and most lethal primary malignant brain tumor. Two common alterations in GBM are loss of the tumor suppressor PTEN and upregulation of the oncoprogenic receptor tyrosine kinase MET. We uncovered a new connection between PTEN and MET expressions and functions in GBM. We found that PTEN expression in GBM cell lines (U87, U373, A172) is reduced in surviving BRCA1 deficient breast cancers (TNBC), although this was not observed in non-TNBC breast cancers. We also identified a correlation between Pin1 levels and sensitivity to PARP inhibitors, which are toxic to HR deficient cells due to synthetic lethality. Ongoing work will establish whether Pin1 levels may be utilized as a biomarker of BRCAAness and PARP inhibitor sensitivity. In contrast, during treatment of breast cancer cells with microtubule damaging agents, the presence of BRCA1 is required to promote apoptosis through MEK3 and JNK, therefore BRCA1 deficient cells are less sensitive to these agents. Knockdown of Pin1 in BRCA1 deficient cells sensitizes them to the taxane Paclitaxel, due to repression of the anti-apoptotic protein Mcl-1, while Pin1 knockdown in BRCA1 proficient cells has no effect on sensitivity as Mcl-1 levels are repressed through the MEK3 pathway. We have also identified that several Src family kinases (SKFs) are upregulated by Pin1 in a BRCA1-deficient background, and may play a role in taxane sensitivity. Pre-treatment with the Src/SFK inhibitor Dasatinib increases cytotoxicity of Paclitaxel, suggesting that combination treatment of Dasatinib and Paclitaxel may be an effective treatment option for BRCA1-deficient breast cancers. In conclusion, we have found that Pin1 may be an important oncogenic mediator downstream of dysfunctional BRCA1 facilitating the viability of TNBCs. We propose that Pin1 could serve as a potential diagnostic and therapeutic target for clinical intervention and aggressive subtype of breast cancer.

#348 Comparison of the response of the NC160 NSCLC panel with the response of patient-derived NSCLC lines to approved and investigational agents. Beverly A. Teicher, David Evans, Thomas Silvers, Michael Selby, Rene Delosh, Julie Lademann, Chad Ogle, Russell Reinhart, Joel Pomiet, Gurmeet Kaur, David Doroshow. National Cancer Inst., Bethesda, MD; Leidos Biomedical Research, Inc., Frederick, MD; National Cancer Inst., Rockville, MD.

An 800 compound screen with the NC160 cell lines and 5 patient-derived NSCLC lines (PD NSCLC), was conducted at 9 concentrations and included the FDA approved oncology agents and an investigational agents library. The screen was conducted using 384-well monolayer cultures, an exposure time of 72 hr, and compound concentrations from 1 nM to 10 uM. CellTiter-Glo was used to measure viability as an endpoint. The NC160 NSCLC panel consists of 9 cell lines: A549, EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, NCI-H460 and NCI-H522. The response of these lines was compared with the response of 5 PD NSCLC lines and 60 SCLC lines. While both sets of NSCLC lines: A549, EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, NCI-H460 and NCI-H522. The response of these lines was compared with the response of 5 PD NSCLC lines and 60 SCLC lines. While both sets of NSCLC lines: A549, EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, NCI-H460 and NCI-H522. Both PD NSCLC lines and SCLC lines were more sensitive to the tubulin fragmenters than were the PD NSCLC and the SCLC lines (mean GI50 1.6 uM). A heterogeneous response to the MEK inhibitors such as PD98059 (mean GI50 0.0042 uM in SCLC versus 0.047uM in the PD NSCLC lines). The NC160 NSCLC lines were more sensitive to the KSP inhibitor, ARRY-520 (mean GI50 0.0049 uM) than the SCLC lines (0.028 uM) and the PD NSCLC lines (0.26 uM). The NC160 NSCLC lines were more sensitive to the Mcl-1 inhibitor, ABT-263 (mean GI50 0.025 uM) versus 1.15 uM in the NCI60 NSCLC lines and 0.56 uM in the PD NSCLC lines. The NCI60 NSCLC lines (mean GI50 1.6 uM) and the SCLC lines (mean GI50 0.13 uM). However, the NCI60 NSCLC lines (mean GI50 0.8 uM) and the SCLC lines (mean GI50 0.134 uM) were less sensitive than the PD NSCLC lines (mean GI50 0.025uM) to GAR transformylase inhibitors such as pelitrexol. The NC160 NSCLC lines (mean GI50 6.3 uM) and SCLC lines (mean GI50 5.04 uM) were more sensitive to the MEK inhibitors such as cobimetinib (GDC-0973) was observed with the SCLC lines (mean GI50 8.9 uM) versus 1.15 uM in the NCI60 NSCLC lines and 0.56 uM in the PD NSCLC lines. PD NSCLC lines exhibit some interesting differences in response from established lung cancer lines upon in vitro exposure to anticancer agents and thus to our knowledge and understanding of NSCLC and help inform diagnostic and/or clinical development of therapeutics in this disease. This project was funded in part with federal funds from the NCI, NIH, under contract no. HHSN261200800001E.
Eribulin provides a survival advantage for breast cancer patients, particularly those with triple-negative and HER2-negative subtypes. Mechanistically, eribulin was shown to reverse epithelial-to-mesenchymal transition (EMT) in breast cancer models. E-cadherin is a central regulator responsible for maintenance of the epithelial phenotype and loss of cortical E-cadherin and its down-stream effects have been identified in many cancer models. Cortical sequestration of p120- and β-catenin at the adherens junctions stabilizes these complexes and inhibits their ability to stimulate signaling pathways that contribute to EMT. We tested whether eribulin, which binds to the plus ends of microtubules, promotes EMT reversal by influencing p120- and β-catenin localization and activity. Our results show that eribulin induces cortical localization of p120-catenin and β-catenin with E-cadherin in HCC1937 cells, consistent with the formation of normal adherens junctions. Eribulin also stimulated the phosphorylation of Ser33/37 and Thr41 on β-catenin which promotes proteosomal degradation, which would be expected to further inhibit oncogenic signaling. Collectively, our results suggest that eribulin contributes to reversal of EMT through modulation of downstream signaling initiated by cortical localization of p120- and β-catenin at the adherens junctions. Furthermore, this work was provided by Eisai Inc. 1. Cortes J., et al., Cancer Research, 2014. #350 Pregnancy at early age is associated with a reduction of progesterone-responsive cells and epithelial Wnt signaling in human breast tissue. Robert Mechera, Simone Munzt, Silvio Dastjer, Charlotte Ne, Fabienne Meier-Abt, Walter F. Weber, Savas D. Soysal, University Hospital Basel, Basel, Switzerland; University Hospital Zuerich, Zuerich, Switzerland.

Background: Pregnancy at early age is the most significant modifiable factor which consistently decreases lifetime breast cancer risk. However, the underlying mechanisms haven’t been conclusively identified. Studies in mice suggest a role of downregulation of epithelial Wnt signaling in the protective effect of early pregnancy. The aim of our study was to validate these findings in humans. Methods: We collected benign breast tissue of 123 women who had been stratified according to age at first pregnancy and the occurrence of subsequent breast cancer, and performed immunohistochemistry for Wnt4 and the Wnt-target Versican. Results: The number of PR positive epithelial cells was significantly lower in the group of women with early pregnancy and no subsequent breast cancer compared to the group of nulliparous women with subsequent invasive breast cancer (p=0.017). In women with early pregnancy, expression of Versican and Wnt4 was significantly lower compared to nulliparous women (p=0.0064 and p=0.0156 respectively), and Versican expression was also significant lower compared to women with late pregnancy (p<0.0001). Discussion: Our results confirm prior observations in mice and suggest a role of inhibition of epithelial Wnt signaling in the protective effect of early pregnancy in humans. This results in a decreased proliferation of stem/progenitor cells; therefore, the Wnt signaling pathway may represent a potential target for breast cancer prevention in humans.

#351 mTOR-ERK co-targeting strategies for head and neck cancer therapy. Zhiyong Wang, Esteban Delgado, Kosuke Yamaguchi, Zhiyong Wang,1 Esteban Delgado,2 Kosuke Yamaguchi,1 Ramiro Iglesias-Merera,1 Simone Münst,1 Silvio Däster,1 Salvatore Piscuoglio,1 Charlotte Ng,1 J.Silvio Gutkind1.

In the past decade, head and neck squamous cell carcinomas (HNSCC) have been one of the most studied cancers. One of the strategies to achieve a cure for HNSCC is medially targeted therapies. In recent studies of human cancer genomes identified remarkable complexity of genomic alterations in HNSCC, surprisingly, most aberrations have fallen with key driver signaling pathways. Among them, PI3K/AKT/mTOR pathway is most frequently activated in over 80% of the HNSCC patients. We showed that the majority of HNSCC lesions exhibit high levels of phosphorylated ribosomal S6 protein (pS6), key downstream target of the AKT-mTOR pathway, and that inhibition of mTOR by the use of rapamycin causes a rapid decrease in the level of pS6 and the apoptotic death of HNSCC tumor xenografts, thereby causing tumor regression. Our initial studies identified the AKT-mTOR pathway as a potential therapeutic target for HNSCC and provided strong rational to initiate multiple clinical trials. Indeed, inhibitors of PI3K/AKT/mTOR block mTOR activity and exert a beneficial response in HNSCC patients. However, therapeutic resistance was evidenced in several trials, which might be due to the activation of adaptive survival signaling in tumor cells. To explore the therapeutic option that can overcome the resistance, we performed a synthetic lethality screen in a high throughput manner using shRNA libraries. We found that multiple molecules involved in ERK signaling pathway were highly represented. Furthermore, treating HNSCC cell lines harboring activated mTOR with small molecule inhibitors that target PI3K/AKT/mTOR pathway can sequentially induce ERK activation, which is consistent with the findings observed in numerous clinical trials. We demonstrated that co-targeting mTOR and ERK using trametinib, a MEK1/2 inhibitor and pan-ERK1/2 small-molar antibody, exhibited the synergistic effect by sensitizing HNSCC to priming with the mTOR inhibitor. Specifically, mTOR-ERK co-targeting prevented the growth of HNSCC tumor xenografts by decreasing cell proliferation, reducing lymhangiogenesising and activating apoptosis. In addition, we have recently used a phosphoproteomics approach to identify the molecular mechanisms of ERK feedback activation caused by PI3K/AKT/mTOR inhibition. Overall, our promising findings from these preclinical studies suggest that the use of mTOR-ERK co-targeting strategies may provide the novel alternative approach to achieve durable responses (cure) in HNSCC cancer patients.

#352 The role of p62 (SQSTM1) in the transforming growth factor β signaling pathway. Evelyn Ng, Adrian Gunaratne, John Di Guglielmo, Western University, London, Ontario, Canada.

The transforming growth factor β (TGFβ) is a cytokine that regulates many cellular processes, including cellular adhesion, proliferation and apoptosis. Its canonical downstream effectors include Smad2/3 proteins, which are phosphorylated and then translocate to the nucleus to alter gene transcriptional programs and promote processes such as epithelial-to-mesenchymal transition (EMT). Previous studies in our lab have shown that atypical Protein Kinase C (aPKC) interacts with TGFβ receptors and modulate receptor trafficking and signal transduction. An aPKC-associated protein, p62 (SQSTM1) has been implicated in TGFβ-dependent EMT, however the mechanisms remain unclear.

Here, we investigate the localization of p62 and its potential roles in modulating TGFβ signaling via knockdown and overexpression studies. Using antibody feeding and immunofluorescence microscopy, we support previous findings showing that p62 localizes to late endosomes. In addition, using a co-immunoprecipitation approach, we observed that p62 may associate with TGFβ type II receptor. To explore the functional role of p62 in TGFβ signaling, we conducted protein silencing using siRNA. We observed TGFβ-independent decreases in E-cadherin expression. However these changes were independent of the phosphorylation status or nuclear translocation of Smad2. P62 also plays an important role in autophagy by targeting proteins for degradation. Recently, prolonged TGFβ stimulation has been shown to induce cellular autophagy. Therefore, it is in our interest to characterize the relationship between TGFβ-dependent EMT and autophagy, and to determine whether p62 has a regulatory role between these two processes.

#353 ROIR inhibits ASK1-mediated pro-apoptotic signaling in lung adenocarcinoma. Lisa Ida, Tomoya Yamaguchi, Taisuke Kajino, Kiyoshi Yanagisawa, Yukako Shimada, Motoshi Suzuki, Takashi Takahashi, Division of Molecular Carcinogenesis, Center for Neurological Diseases and Cancer; Nagoya University, Nagoya, Japan.

We previously reported that the receptor tyrosine kinase-like orphan receptor 1 (ROIR) is transcriptionally activated by TTF-1/NKX2-1 lineage-survival oncogene in lung adenocarcinoma and maintains a favorable balance between pro-survival PI3K-AKT and pro-apoptotic ASK1-p38 signaling. Although in-depth mechanistic insight into how ROIR sustains EGFR-mediated PI3K-AKT signaling in both kinase-dependent and -independent manners has been obtained, it remains elusive how ROIR inhibits pro-apoptotic signaling. In the present study, we investigated the underlying mechanism of ROIR-mediated inhibition of the ASK1-p38MAPK signaling pathway. Co-treatment with siASK1 resulted in partial but significant alleviation of siROR1-mediated growth inhibition of the ASK1-p38 pathway. ROIR was shown to interact with ASK1 through the C-terminal serine/threonine-rich domain of ROR1. Previous studies in our lab have shown that atypical Protein Kinase C (aPKC) interacts with TGFβ receptors and modulate receptor trafficking and signal transduction. An aPKC-associated protein, p62 (SQSTM1) has been implicated in TGFβ-dependent EMT, however the mechanisms remain unclear.

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that co-incubation of ASK1 with recombinant GST-tagged ROR1 clearly diminishes ASK1 auto-phosphorylation and MKK6 phosphorylation. We further examined whether the kinase activity of ROR1 is required for inhibition of ASK1 activity using MTSO-21H cells stably transfected with either a wild-type or a kinase-dead mutant of ROR1. In contrast to wild-type ROR1, kinase-dead ROR1 failed to significantly repress hydrogen peroxide-induced ASK1 phosphorylation as well as consequential p38 phosphorylation and increase in sub-G1 cells. Since we previously found that ROR1 activates SRC, we also examined whether SRC is involved in the ROR1-sustained ASK1 inhibition. Introduction of constitutively active SRC significantly reduced siROR1-induced ASK1 activity in PC-9 cells, while co-transfection of SRC and ASK1 resulted in tyrosine phosphorylation of ASK1, which was accompanied with diminished ASK1 phosphorylation at threonine 845. Finally, the interaction between SRC and ASK1 was found to be enhanced by the presence of ROR1 by an in vitro pull-down assay. Taken together, the present findings support our notion that ROR1 sustains lung adenocarcinoma survival, at least in part, through direct physical interaction with both ASK1 and SRC, which consequently results in repression of the pro-apoptotic ASK1-p38 axis in a ROR1 kinase activity-dependent manner.

#354 Inhibition of Smad3 signaling with a TAT fusion peptide prevents TGF-β-induced fibronectin expression and cell migration in malignant gliomas.

Jeonghan Kang. Mayo Clinic, Rochester, MN.
Malignant primary brain tumors represent one of the most common causes of cancer death in the world. Grade IV gliomas (glioblastoma multiform, GBM) are routinely resistant to current chemoradiation therapy and radiation therapy with a median progression-free and overall survival for glioblastoma following current chemoradiation of ~7 and 15 months, respectively. While TGF-β functions as a growth inhibitor for most normal cell types, many tumors (including GBMs) have lost this inhibitory response and TGF-β now functions to promote tumor progression and/or dissemination. As aberrant TGF-β signaling is often considered a hallmark of high-grade gliomas, we examined whether inhibition of specific components would impact the malignant phenotype of glioblastoma. The primary mediators of TGF-β action are the Smad proteins (R-Smads), Smad2 and Smad3. Following receptor activation and R-Smad phosphorylation, they translocate to the nucleus to modulate gene expression. Since we previously determined that a cell penetrating peptide conjugate of the HIV TAT protein translocate to the nucleus to modulate gene expression. Since we previously determined that a cell penetrating peptide conjugate of the HIV TAT protein conjugate unable to block Smad3 action. These findings indicate that the TGF-β/Smad3 axis is a potentially important mediator of glioma cell invasion in a significant minority of GBM models. Specifically targeting Smad3 might be effective in a subset of gliomas. This work was supported by Public Health Service grants GM-055816 and GM-054200 from the National Institutes of General Medical Sciences and P30 CA-108961 Developmental Pilot Award from the National Cancer Institute.

#355 TET2-loss modifies androgen signaling in prostate cancer.

Michael L. Nickerson, Sudipto Das, Kate Im, Seviaiy Turan, Sonja Berndt, Hongchuan loci using genotypes from the PEGASUS case-control cohort and identify six associated with PCa and melanoma, and rare germline missense variants are identified. Genome-wide association studies previously identified non-coding risk variants co-located in regions that support progression and/or dissemination. As aberrant TGF-β signaling through inhibition of SOCS3 and activation of IGF1R and SRC, and induced SOCS3 phosphorylation and degradation. This cascade of events leads to STAT3 activation in gastric cancer cells. We propose DARP32 as an important novel signaling between NKF-κB and STAT3 in prostate cancer.

#357 NXK2-1 regulates SMAD and non-SMAD pathways in thyroid stem cells.

Manabu Iwadate, Yoshinori Takizawa, Shiko Kimura. National Cancer Institute, MD.
NXK2-1 plays a critical role in thyroid differentiation. Recently, we established Side Population cells-derived Thyroid cell line (SPTL) from mouse thyroid Side Population cells. In SPTL cells, NXK2-1, PAX8 and E-cadherin were
not expressed when examined by western blotting. Immunocytochemistry revealed that only 1% of SPTL cells were NKX2-1 positive. When GFP-SPTL cells were directly injected into mouse thyroid, a few GFP-SPTL cells with weak expression of NKX2-1 were found in part of thyroid follicle, suggesting that SPTL cells have potential of thyroid stem cells. Comprehensive gene expression analysis indicated that TGFB-beta signaling, C1GALT1 overexpression promoted the malignant behaviors of C1GALT1 knockdown inhibited Akt and ERK activities in PDAC cells. Consistently, phospho-receptor tyrosine kinase (p-RTK) array showed decreased phosphorylation of several RTKs including EGFR, IGF1R, and FLT3. Additionally, flow cytometry of C1GALT1 knockdown cells showed increased apoptosis, which was associated with decreased catalase, Bcl-3, and increased caspase 3 and 9. Interestingly, C1GALT1 knockdown arrested G1 progression in PDAC cells. Furthermore, gene set enrichment analysis of CDNA microarray indicated that C1GALT1 regulated cell cycle, cytoskeleton, and chromatin structure in PDAC cells. These findings indicate that higher C1GALT1 expression predicts worse survival of PDAC and enhances malignant characters in PDAC cells, highlighting a fundamental role of C1GALT1 in PDAC development.

**#360** Leveraging a novel ITIM motif in GPCRs for targeted antibody design, Lili T. Belcastro, 1 Anastasia Jancina, 2 Christina Adams, 3 Ryan D. Paulikis, 2 Catherine C. Moore. 1 Cancer Biology Program, The Wistar Institute and University of the Sciences, Philadelphia, PA; 2 Philadelphia College of Pharmacy, University of the Sciences, Philadelphia, PA.

CXC4, a chemokine GPCR, is essential for migration of neuronal, hemato-poietic, and breast cancer cells during metastasis whereby CXCR4 dysregulation promotes migration and invasion. Following SDF stimulation, CXCR4 is phosphorylated on Ser/Thr residues which initiates adapter recruitment, receptor desensitization, and trafficking to endocytic sites. Here we show that stimulation with gradient SDF, delays receptor phosphorylation and trafficking, leading to sustained signaling to a novel CXCR4-SHP2-ERK pathway. SHP2 is a tyrosine phosphatase implicated in HER2(+) and triple-negative breast cancers whereby it transduces mitogenic and migratory signals driving hyperplifferation and invasion. SHP2 is recruited to tyrosine phosphorylated ITIM motifs (immunoreceptor tyrosine-based inhibitory consensus motifs), a hallmark found in inhibitory immune receptors with little evidence in GPCRs. Here we identify an ITIM motif in CXCR4 that regulates both SHP2 binding and signaling. Specifically, we assessed if gradient SDF stimulation of CXCR4 1) delays receptor phosphorylation and trafficking, 2) sustains signaling to SHP2-ERK, 3) induces SHP2-dependent migration; and if CXCR4 Tyr mutation within the ITIM motif 4) maintains SDF gradient sensing ability, and 5) disrupts interaction with and signaling to SHP2. Our data demonstrate that gradient SDF delays receptor Ser/Thr phosphorylation and internalization thereby sustaining signaling to SHP2-ERK and driving SHP2-dependent migration. Conversely, the ITIM mutant maintains SDF gradient sensing ability, but disrupts interaction with and signaling to SHP2. Our data support a working model that CXCR4 contains a functional ITIM motif which we are currently leveraging for targeted antibody design for use in migration studies of aggressive breast cancer cells with dysregulated CXCR4. These studies were supported by NIH grant GM-699718, PA Department of Health grant SAP4100057688, and the Milton Lev Memorial Faculty Research Fund.

**#361** Loss of cholangiocyte primary cilia induces LKB1 downregulation and defective AMPK signaling, Adrian P. Mansini, Kristen M. Thelen, Sergio A. Gradilone. University of Minnesota, Austin, MN.

Cholangiocarcinoma (CCA) is a malignancy arising from cholangiocytes, the epithelial cells lining the biliary tree. CCA is an uncommon, but devastating cancer that is increasing in incidence. Over the past 3 decades, 5-years survival rates have remained at 10%. Although surgical resection and liver transplantation are potentially curative therapies, most patients are diagnosed at late stages and are not eligible for these options. Therefore, it is imperative to identify novel targets leading to new therapeutic strategies for this devastating disease. Cholangiocytes express primary cilia that function as chemo, mechanos, and osmosensors controlling several molecular pathways. We showed cilia are absent in CCA cells, and experimental deciliation of normal cholangiocytes induced a malignant phenotype, with significant invasion and proliferation, suggesting the loss of cilia could be associated with CCA development. LKB1 is a tumor suppressor described to be expressed in primary cilia in MDCK cells, and is involved in AMPK activation through a ciliary dependent mechanism. AMPK functions as metabolic and stress check points. Interestingly, patients with inhepatic CCA and low expression of LKB1 have poor prognosis. Therefore, we hypothesized that primary cilia function as tumor suppressor organelles through a LKB1-AMPK-p53 pathway. To test this hypothesis, first LKB1 subcellular local-
#362 Repression of Smad3 by Stat3 and c-Ski/SnoN induces gefitinib resistance in lung adenocarcinoma. Yojiro Makino,1 Jeong-Hwan Yoon,1 Eunjin Bae,2 Mitsuyasu Kato,3 Keiji Miyazawa,5 Tatsuo Ohira,1 Norihiko Ikeda,1 Masahiko Kuroda,3 Mizako Mamura3. 1National Defense Medical College, Saitama, Japan; 2Kyujo Kurno, Tokyo, Japan; 3The University of Tokyo Hospital, Tokyo, Japan; 4University of Tsukuba, Ibaraki, Japan; 5University of Yamanashi, Yamanashi, Japan.

Cancer-associated inflammation develops resistance to the epidermal growth-factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) in non-small cell lung cancers (NSCLCs) harboring oncogenic EGFR mutations. Stat3-mediated Interleukin (IL)-6 signaling and Smad-mediated transforming growth factor-β (TGF-β) signaling play crucial regulatory roles in cancer-associated inflammation. Here we show that Stat3 represses Smad3 in cooperation with c-Ski and SnoN, whereby renders gefitinib-sensitive HCC827 cells resistant. IL-6 signaling via phosphorylated Stat3 induced gefitinib resistance. By contrast, TGF-β upregulated gefitinib sensitivity. We found that IL-6 signaling via phosphorylated Stat3 repressed, whereas TGF-β upregulated the expression of Smad3 in HCC827 cells. Promoter analyses showed that Stat3 synergized with c-Ski/SnoN to repress Smad3-induced transcription of the Smad3 gene. Smad3 induced apoptosis by upregulating pro-apoptotic genes such as Caspase 3 and downregulating anti-apoptotic genes such as Bcl2. Our results suggest that preventing IL-6/Stat3-induced loss of Smad3 can be a therapeutic strategy to prevent gefitinib resistance in NSCLC with gefitinib-sensitive EGFR mutation.


Peripheral T cell lymphoma (PTCL) is a heterogeneous malignancy with extremely poor prognosis. Five-year overall survival is <40% and can be as low as 12% for specific subtypes. There is, therefore, an urgent need to identify and develop novel therapeutic targets and approaches. A number of recent sequencing studies, including a whole exome sequencing analysis of primary PTCL patients conducted by our laboratory, have revealed recurrent oncogenic driver mutations in the common gamma chain/JAK/STAT signaling pathway, resulting in the aberrant activation of the transcriptional regulator STAT5. STAT5 has additionally been found to be upregulated or containing activating mutations in 30-40% of PTCL cases of specific subtypes. These findings highlight STAT5 as an oncogenic driver of PTCL and suggest a role for it in novel therapeutic approaches. Based on these data, we aimed to evaluate STAT5 mechanismally as a therapeutic target in PTCL. The drug, pimozide, which is an FDA approved neuroleptic agent, was recently identified by drug screen to be a STAT5 inhibitor. We evaluated the activity of pimozide in PTCL, using a combination of Fluorescence Activated Cell Sorting (FACS), and immunoblot analysis. Our data demonstrate a concentration dependent reduction in STAT5 activity and the number of viable cells in PTCL cell lines after culture with pimozide. This is shown to be due to an increase in cell death by apoptosis. To verify that pimozide is sufficient to activate STAT5 activity, we transfected PTCL cell lines with anti-STAT5 shRNA to knock down STAT5 and assessed PTCL cell survival and proliferation. We show that STAT5 knockdown results in a three fold reduction in PTCL cell viability, which occurs due to an increase in apoptosis. Furthermore, our data supports a TRAIL dependent mechanism for induction of apoptosis shown by an upregulation of TRAIL by PTCL cell lines after culture with pimozide. This finding demonstrates that pimozide inhibits STAT5 phosphorylation, reduces cell viability, and induces apoptosis in primary PTCL patient samples ex vivo. This research supports further exploration of STAT5 as a therapeutic target in PTCL and the development of STAT5 inhibition in the treatment of PTCL. To facilitate the development of new-chemotherapy dependent therapeutic approaches for PTCL, we are now assessing STAT5 inhibition in combination with small molecule inducers of apoptosis including TRAIL pathway and PARP inhibitors. PARP inhibitors have shown efficacy in malignancies with DNA damage response pathway mutations, which have been identified in up to half of cases of PTCL. We, therefore, hypothesize that inhibition of Jak inhibitory kinases responsible for STAT5 activation, in combination in PTCL, as several have demonstrated clinical efficacy in other hematologic malignancies.

#364 Mass cytometry of human glioblastoma characterizes more than 99 percent of cells and reveals intratumoral cell subsets defined by contrasting signaling network profiles. Nalin Leelatian,1 Justine S. Smaree,1 Brett C. Mobby,2 Akshat Kumar M. Mistry,2 Daniel Liu,1 Kyle D. Weaver,2 Reid C. Thompson,2 Lola B. Chambless,2 Rebecca A. Ihrie,1 Jonathan M. Irish1. 1Vanderbilt University, Nashville, TN; 2Vanderbilt University School of Medicine, Nashville, TN.

Background: Glioblastoma (GBM) remains largely incurable despite intense study of resected tissue. Prior studies have revealed GBM cell subsets (Patel et al., Science 2014) and implicated emergence as a potential mechanism of poor outcome in other cancer types. Signaling in rare cells or a mix of cell subsets may enable therapy resistance and recurrence of GBM. For example, STAT3 RNA expression has been previously shown to correlate with poor outcome in GBM (Jahani-Asl et al., Nat Neurosci 2016 and TCGA). The complexity of GBM, combined with the interconnectedness between cancer and host cells in the microenvironment, means that a single cell biology approach is needed to comprehensively characterize patient biopsy cells and determine how protein expression, signaling, and functional capabilities impact treatment response. Methods: We developed a novel mass cytometry approach to characterize human GBM that identified ~90-95% of tumor cells (Leelatian & Doxie et al., Cytometry B 2016). Here, we applied this approach using a newly created 35-antibody mass cytometry panel focused on basal phospho-protein signaling. The published panel of 16 identity proteins included SOX2, CD44, Nestin, PDGFRα, S100B, and NCAM. This panel was augmented to measure 10 additional proteins and 9 phospho-proteins including p-STAT3, p-EGFR, and p-NFκB. Signaling measurements were chosen to match prior single cell studies of signaling networks that stratified clinical outcomes in blood cancers (Irish et al., Cancer Cell 2010) and breast cancer (Sweeney et al., Genes Dev 2009; PNAS 2010, Levine et al., Cell 2015). Between 10,000 and 250,000 viable cells were characterized for each tumor (N = 7). Tumors were collected with informed consent and in accord with the Declaration of Helsinki. Results: This new 35-antibody mass cytometry panel positively identified ~99% of GBM cells. Subsets of GBM cells displayed protein expression that matched previously observed transcriptional molecular subclasses (Verhaak et al., Cancer Cell 2010 and TCGA). Strikingly, this panel revealed novel GBM cell subsets defined by contrasting basal signaling profiles. An inverse correlation was observed between baseline STAT3 phosphorylation and the abundance of CD45+ leukocytes. Additionally, similar signaling patterns were seen in cells that expressed proteins associated with distinct functions, such as proliferation and migration. Conclusions: The correlation between low STAT3 signaling and high immune cell abundance provides evidence for the idea that an intimate relationship exists between immune cells and GBM tumor growth and survival. Moreover, single cell analysis may reveal biomarkers of treatment response and allow prediction of clinical outcomes. The abnormal signaling mechanisms observed here in some GBM cell subsets should be further developed as potential targets for novel cancer-selective combination therapies.

#365 Cross-talk between BRAF and Hippo/YAP1 signaling in melanoma. Mohan Kumar Durai Raj,1 Jonathan Nguyen,1 Namrita Bora-singhal,1 Jane Messina,2 Geoffrey Gibney,3 Srikumar Chellappan1. 1H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL; 2MedStar Georgetown University Hospital, Lombardi Comprehensive Cancer Center, Washington, DC.

Hippo/YAP1 signaling pathway is a tumor suppressive pathway that controls organ size by modulating the cell growth, proliferation and apoptosis and is conserved from Drosophila to mammals. In mammals, the Hippo tumor suppressor pathway consists of cascade of kinases in which MST1/2 phosphorylates and activates LATS1/2. The latter phosphorylates the oncoprogenic transcriptional coactivators YAP1 and TAZ, leading to their cytoplasmic retention by 14-3-3 knockdown or culture with pimozide. Mitochondrial membrane potential is also disrupted. These findings have potential clinical implications, as we further demonstrate that pimozide inhibits STAT5 phosphorylation, reduces cell viability, and induces apoptosis in primary PTCL patient samples ex vivo. This research supports further exploration of STAT5 as a therapeutic target in PTCL and the development of non-chemotherapy dependent therapeutic approaches for PTCL. We are now assessing STAT5 inhibition in combination with small molecule inducers of apoptosis including TRAIL pathway and PARP inhibitors. PARP inhibitors have shown efficacy in malignancies with DNA damage response pathway mutations, which have been identified in up to half of cases of PTCL. We, therefore, hypothesize that inhibition of JAK inhibitory kinases responsible for STAT5 activation, in combination in PTCL, as several have demonstrated clinical efficacy in other hematologic malignancies.

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proteins and/or degradation. Inactivation of MST and LATS kinases allows YAP1 and/or TAZ nuclear translocation and subsequent activation of their target genes. Deregelation of Hippo pathway can induce tumors in model organisms and occurs in wide range of human cancers including melanoma. Merlin, a key component of this pathway which inhibits YAP1, is mutated/deleted in 8% of melanoma. Loss of Merlin may lead to a failure of cell growth control and allow the growth of tumor cells.

Trop-2 is overexpressed in most human cancers, suggesting selective pressure for a key, conserved function. Here we show that Trop-2 stimulates cancer cell growth through the activation of a constitutively expressed, yet otherwise dormant, growth control module. We discovered that crosstalk of membrane Trop-2 with specific Abs leads to a cytoplasmic Ca++ raise through interaction with the Na+/K+ -ATPase and p110α subunit of PI3K. We further found a pro-survival loop through Trop-2-dependent activation and membrane recruitment of PKC-α, which in turn phosphorylates the Trop-2 cytoplasmic tail at two target sites, activating the molecule to stimulate its downstream signaling targets Akt and ERK.

Our findings indicate that the Trop-2–triggered cell growth operates through binding and extensive crosstalk with CD9, CD81, CD82 and CD151 through PKC-α. The role of the HIKE region of the Trop-2 intracellular tail. Correspondingly, we found that the HIKE region of Trop-2 mediates its anchoring to the β-actin cytoskeleton through direct interaction with the ERK protein ezrin. Consistently, the Trop-2–dependent dynamic remodeling of the cell cytoskeleton is detected to occur through activation of myosin II and binding of annexins A1/A5, α-actinin and gelsolin. Systematic drug screening, gene expression silencing and site-directed mutagenesis revealed that cytoskeleton disassembly, HIKE, deletion and CD9 inhibition revert the growth of Trop-2–expressing cancer cells to that of their Trop-2–null counterparts. On the other hand, these inhibitors have no effects on basal cell growth. This indicates that Trop-2–centered protein interactions and activations are an essential step for the Trop-2–dependent cancer growth. Tight co-expression of the key components of the Trop-2–dependent complex is required for a large breast cancer cell survival, thus indicating a strong clinical relevance. Hence, Trop-2 triggers a universal, but otherwise dormant, layer of cancer growth, that overrides basal cell growth regulatory mechanisms and sensitizes tumors to targeted anticancer therapies.

**#366 Disruption of TCF7L1 mediated transcriptional repression promotes pancreatic tumorigenesis.**

Kathleen M. Kershaw, Bridgette T. Ho, Mialis I. Vasquez, Anna R. Lay, David W. Dawson. UCLA, Los Angeles, CA.

While phenotypic studies demonstrate Wnt/β-catenin signaling is critical for the initiation and progression of pancreatic adenocarcinoma (PDA), the downstream transcriptional effectors tied to its effects are largely unknown. Previous work from our group has identified differential patterns of LEF/TCF expression linked to variations in Wnt activation and function in PDA, including reduced TCF7L1 (aka TCF3) message and protein levels in pancreatic tumors with elevated Wnt/β-catenin activity. Consistent with this observation, we found TCF7L1 in protein levels rapidly diminished in PDA lines after Wnt pathway activation mediated by either Wnt3A ligand or GSKβ inhibitor. RNAi-mediated knockdown of TCF7L1 in PDA lines phenocopied most of the pro-tumorigenic effects seen with Wnt3A ligand treatment. Because TCF7L1 commonly represses transcription and is rapidly downregulated in PDA upon Wnt activation, we explored its transcriptional derepression as a potentially important mechanism through which Wnt signaling promotes pancreatic tumorigenesis. Candidate genes linked to TCF7L1–mediated transcriptional repression in PDA were identified by RNA-sequencing of MiaPaCa-2 and Panc1 lines following RNAi-mediated knockdown of TCF7L1. A total of 196 genes upregulated at least 1.5-fold in both cell lines were identified, representing potential direct targets of TCF7L1. A subset of these genes, including the onco-tyrosine kinase initiating oncogenic signaling, was among these 196 upregulated genes revealed enrichment of categories including small GTPase–mediated signal transduction, RAS protein signal transduction, regulation of axon extension, and regulation of synaptic plasticity. Among top upregulated genes was prostaglandin E2 synthase (PTGES), an enzyme catalyzing the isomerization of prostaglandin H2 to prostaglandin E2 (PGE2) as the final step in PGE2 synthesis from arachidonic acid. Notably, PGE2 stimulates pancreatic cancer proliferation, invasion, angiogenesis and metastasis. Validation experiments in TCF7L1–expressing PDA lines confirmed PTGES message and protein levels rapidly increased in response to TCF7L1 depletion and were associated with a corresponding increase in PGE2 production as measured by ELISA. PGE2 increased Wnt reporter activity in PDA cell lines, a potentially important feedforward mechanism whereby Wnt signaling may be reinforced upon its activation. In conclusion, TCF7L1 represses target genes with known roles in PDA, while reduction in TCF7L1 through Wnt pathway activation or alternative means is apparently tied to a program of transcriptional derepression linked to the promotion of pancreatic tumorigenesis.

**#367 Trop-2 activates a dormant Na+/K+-ATPase/PKCα/C9/ezrin signaling axis to override the basal growth program of cancer cells.**

Marco Trerotola, Valeria Relli, Romina Tripaldi, Andrea Sacchetti, Kristina Havas, Pasquale Simeone, Emanuela Guerra, Annalaura Aloisi, Rossana L. Sorda, Rossano Lattanzio, Daniele Vergara, Isabelle Fournier, Michel Salzet, Mauro Piantelli, Saverio Alberti, G. D’Annunzio* University of Chieti-Pescara, Chieti, Italy; *Sophiezen’s NeSof Institute, Eramus MC, Rotterdam, Netherlands; 1FOM the FIRC Institute of Molecular Oncology, Milan, Italy; 2San Raffaele Scientific Institute, Milan, Italy; 3University of Salento, Lecce, Italy; 4Université de Lille I, Cité Scientifique, Villeneuve D’Ascq Cedex, France.

Trop-2 is a surface protein with a broad biological role. It is overexpressed in a variety of cancer types, including human pancreatic adenocarcinoma (PDAC). The mechanisms by which Trop-2 regulates cancer cell growth and survival are not well understood. Here we report a novel role for Trop-2 in the regulation of the Na+/K+-ATPase, a key, conserved function. Here we show that Trop-2 stimulates cancer cell growth through the activation of a constitutively expressed, yet otherwise dormant, growth control module. We discovered that crosstalk of membrane Trop-2 with specific Abs leads to a cytoplasmic Ca++ raise through interaction with the Na+/K+-ATPase and p110α subunit of PI3K. We further found a pro-survival loop through Trop-2-dependent activation and membrane recruitment of PKC-α, which in turn phosphorylates the Trop-2 cytoplasmic tail at two target sites, activating the molecule to stimulate its downstream signaling targets Akt and ERK. Our findings indicate that the Trop-2–triggered cell growth operates through binding and extensive crosstalk with CD9, CD81, CD82 and CD151 through PKC-α. The role of the HIKE region of the Trop-2 intracellular tail. Correspondingly, we found that the HIKE region of Trop-2 mediates its anchoring to the β-actin cytoskeleton through direct interaction with the ERK protein ezrin. Consistently, the Trop-2–dependent dynamic remodeling of the cell cytoskeleton is detected to occur through activation of myosin II and binding of annexins A1/A5, α-actinin and gelsolin. Systematic drug screening, gene expression silencing and site-directed mutagenesis revealed that cytoskeleton disassembly, HIKE, deletion and CD9 inhibition revert the growth of Trop-2–expressing cancer cells to that of their Trop-2–null counterparts. On the other hand, these inhibitors have no effects on basal cell growth. This indicates that Trop-2–centered protein interactions and activations are an essential step for the Trop-2–dependent cancer growth. Tight co-expression of the key components of the Trop-2–dependent complex is required for a large breast cancer cell survival, thus indicating a strong clinical relevance. Hence, Trop-2 triggers a universal, but otherwise dormant, layer of cancer growth, that overrides basal cell growth regulatory mechanisms and sensitizes tumors to targeted anticancer therapies.
#369 Therapeutic targeting of the Wnt antagonist DKK1 with a humanized monoclonal antibody in oncology indications. Michael H. Kagey,1 Yinyuan Wu,7 Xinjun Zhang,2 Cynthia A. Sirad,1 Shane E. Mulligan,2 Xi He,3 Christopher K. Mirabelli1,2 *Leap Therapeutics, Cambridge, MA; 3Boston Children’s Hospital, Harvard Medical School, Boston, MA.

Wnt-activated pathways promote tumor growth and metastasis. Inhibiting tumor growth and metastasis is a goal of current cancer treatments. In oncology, the Wnt antagonist DKK1 is often overexpressed, promotes tumor growth and is refractory to current therapies. Disease progression and management of tumors relies on the ability to distinguish between tumors that are sensitive or resistant to treatment. DKK1 is a promising target; however, humanized antibodies to DKK1 have not shown clinical activity. We generated a DKK1 humanized antibody that possesses improved bind and activity. BDK1 binds DKK1 with high affinity and specifically, disrupts the interaction of DKK1 with the LRP6 co-receptor, and neutralizes DKK1 activity in a cell based assay. In vivo, DKK1 has efficacy both as a monotherapy and in combination with chemotherapies in a non-small cell lung (NSCLC) cancer A549 xenograft model. Results suggest that DKN-01 has an antiangiogenic effect and may stimulate a NK cell mediated antitumor response. Clinically, DKN-01 is being evaluated in relapsed/refractory esophageal cancer patients in combination with paclitaxel, and preliminary results demonstrate promising activity. Archival patient tumor samples are currently being analyzed and by IHC for DKK1 and a number of cancer patients in combination with pacitaxel, and preliminary results demonstrate promising activity. Archival patient tumor samples are currently being analyzed and by IHC for DKK1 and a number of different patient tumor samples are currently being evaluated. Overall, we establish that T-P LL cells resemble antigen-experienced memory T-cells. Treatment of functional responses to TCR stimulation and of loss of restricting activator responses underlie a highly activated phenotype and a marked resistance to death-inducing signals. T-CLLIA proactively enhances TCR responses and we postulate that this leukemogenic cooperation drives accumulation of memory-type cells that utilize amplified, hence permissive, low-level cognate antigen input.

#371 Developing small molecule therapeutics to target AKT signaling in non-small cell lung cancer. Balaji Chandrasekhar,1 Deeksha Pal,2 Venkatesh Kolluru,1 Srinivasa R. Ramisetty,3 Arun K. Sharma,2 Murali Ankem,1 Chengli DAMODARAN,1 University of Louisville, Louisville, KY; 2Pennsylvania State University, Hershey, PA.

Lung cancer remains a leading public health problem which is evidenced by its increasing death rate. Platinum-based chemotherapy is the first-line of treatment for patients in advanced stages of non-small cell lung cancer (NSCLC), however the success rates are not quite impressive. Hence, developing individualized treatment strategies for metastatic lung cancer gains momentum, such as Tyrosine kinase inhibitors erlotinib and gefitinib, or AKT inhibitors. Yet, a better understanding of receptor stimulation is crucial. Recent reports suggest that pAKT(ser473) is highly expressed in NSCLC and higher nuclear expression of pAKT correlated with poor prognosis and an independent prognostic marker for survival. Our lab is interested to develop novel small molecules which specifically inhibit AKT signaling in NSCLC. Structure-activity relationship (SAR) studies in our laboratory have recently identified one such compound, AKS-407, that effectively inhibited cell growth at nanomolar concentration in NSCLC cell lines (A549 and H460; 250nM). Molecular studies revealed AKS-407 inhibited AKT signaling by down regulating pAKT(ser473) expression and downstream events including NFKB activation, BCI-2 expression in both the cell lines. As signaling through AKT regulates epithelial-mesenchymal transition (EMT) in NSCLC, we determined the effect of AKS-407 on EMT phenotype on NSCLC cells. Treatment of AKS-407 inhibited the mesenchymal markers include snail, MMP9, N-cadherin, β-catenin and vimentin expression that resulted in blocking invasion and migration of A549 and H-460 cells. These results suggest AKS-407 to be a promising small molecule targeting AKT signaling pathway which remains an important target for the development of effective treatment of metastatic NSCLC. Validating in-vivo efficacy of this potential drug candidate would further support our overall goal of the study.

#372 Optimizing vertical MAPK pathway inhibition for RAS mutant non-small cell lung cancer. Jens Köhler,1 Cloud P. Pawelez,2 Yanan Kuang,2 Prafulla Gokhale,1 Margaret K. Winkens,2 Hong Tiv,3 Atsuko Ogino,2 JiHyun Choi,1 Paul T. Kirschmeier,3 Pasi A. Jänne,2 Dana-Farber Cancer Institute, Boston, MA; 3Reber Center for Applied Cancer Science, Boston, MA.

Introduction: Non-small cell lung tumors (NSCLC) with mutations in genes encoding for Ras proteins (H-, N-, K-Ras) exhibit activation of the MAPK signaling pathway. MEK inhibitors, however, have limited efficacy in patients affected by this genotype-defined tumor subtype and major resistance derives from insufficient suppression or reactivation of the extracellular signal-regulated kinase (ERK) as a result of dynamic kinase reprogramming. ERK inhibitors may overcome limitations of MEK inhibition due to the bottleneck function of ERK proteins in submitting mitogenic and anti-apoptotic signals. Combinations of both drug classes (“vertical pathway inhibition”) may further increase treatment efficacy but inevitably potentiate toxicity hence requiring alternative dosing schedules. Methods: We investigated the efficacy of vertical MAPK inhibition with selumetinib (Mek inhibitor) and SCH772984 (Erk inhibitor) in commercially available and patient-derived RAS mutant NSCLC models. A quantitative RT-PCR based 12-gene signature was used as a surrogate to assess ERK-dependent transcriptional output. Pulsatile drug regimens were investigated in xenograft models. Results: In a genetically simple, patient-derived NRAS-mutant lung cancer cell line (DFC168), we observed strongest in vitro and in vivo inhibitory effect of combined AKT and MEK inhibition. A combination of AKT inhibition by a peptide-derived inhibitor and MEK inhibition by SCH772984, respectively, reduced the overall survival of lung tumor xenografts as measured in a preclinical in vivo model of RAS-mutant lung cancer. Overall, we establish that T-P LL cells resemble antigen-experienced memory T-cells. Treatment of functional responses to TCR stimulation and of loss of restricting activator responses underlie a highly activated phenotype and a marked resistance to death-inducing signals. T-CLLIA proactively enhances TCR responses and we postulate that this leukemogenic cooperation drives accumulation of memory-type cells that utilize amplified, hence permissive, low-level cognate antigen input.

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mercially available cell lines) and in vivo (n = 2). The degree of transcriptional output suppression of cell lines (n = 8) with different sensitivities towards Mek/Erk inhibition did not differ significantly at 24 hrs in vitro. Gastrointestinal toxicities (i.e. peritonitis-like symptoms) occurred when continuously administrated SCH727984 was intermittently combined with selumetinib (4 days ON, 3 days OFF). In our study, both drugs given intermittently (4 days ON, 3 days OFF) were ineffective. None of the drug treatments, however, could cure the mice. Conclusion: We have demonstrated that deeper and prolonged transcriptional output suppression by vertical MAPK pathway inhibition translates into superior efficacy in RA5 mutant lung cancer models compared to single agent treatment. Other factors (e.g. signaling pathways) are likely to influence the overall outcome of Mek inhibition. However, our study also shows that treatment with B-catenin siRNAs or dominant negative form of TCF7L2 (dnTCF7L2) significantly enhanced the reporter activity, which may represent a potential therapeutic target for melanoma. Funding support: This work was supported by the National Cancer Institute of China (81071687, 81272195S), the State “973” Program of China (2014CB542005).

#373 Wnt signaling induces anti-apoptotic effect in colorectal cancer cells through the suppression of IFITs. Tomoyuki Ohsugi, Kiyoshi Yamaguchi, Chi Zhu, Tsuneo Ikoue, Yoichi Furukawa. Division of Clinical Genome Research The Institute of Medical Science The University of Tokyo, Tokyo, Japan.

Impaired Wnt signaling pathway plays a crucial role in the development of colorectal cancer through the activation of β-catenin/TCP complex. Although genes up-regulated by Wnt/β-catenin signaling has been well studied, the down-regulated genes are poorly understood. To clarify the comprehensive changes regulated by the signaling in colorectal cancer cells, we employed a reporter gene expression of CRC cell transfected with β-catenin siRNAs or dominant negative form of TCP7L2 (dnTCP7L2). Consequently, a set of genes that were negatively regulated by β-catenin/TCP were identified. Among the genes, three members of interferon-induced proteins with tetratricopeptide repeats (IFIT) family (IFIT1, IFIT2, and IFIT3) expression were significantly increased by the inhibition of β-catenin/TCP. Comparison of gene expression data from normal colonic mucosa and the tumor tissues showed that the expression of IFIT1 and IFIT2 in the tumors was significantly lower than that in normal tissues. To elucidate the mechanism of IFITs expression regulated by β-catenin/TCP, we performed a reporter assay using plasmid containing 1.2-kb of 5′-flanking region of the IFIT2 gene. As a result, the reporter activity was significantly enhanced by either transduction of β-catenin or dnTCP7L2, suggesting that blockage of β-catenin/TCP stimulated IFITs through the promoter. In addition, we found that overexpression of IFIT2 increased apoptosis and decreased cell proliferation in SW480 and HCT116 cells. These results imply that Wnt signaling may promote anti-apoptotic effect in cancer cells through the suppression of IFIT2. Our findings suggest that analysis of down-regulated genes in response to activated Wnt/β-catenin signaling provides a better understanding of human colorectal carcinogenesis.

#374 KMT2A promotes tumor growth by activating hTERT and CBP signaling and predicts poor prognosis in human melanoma. Changlin Zhang, Ranran Tang, Kefang Zhang, Wenlin Huang, Wuguo Deng. 1Sun Yat-sen University Cancer Center; State Key Laboratory of Oncology in South China; Collaborative Innovation Center for Cancer Medicine, Guangzhou, China; 2Dalian Medical University, Dalian, China; 3Global Life Care Federation, Hong Kong, China.

Melanoma is an aggressive type of cutaneous malignancy. Although the inhibitors targeting BRAF and/or MEK pathways provide a therapeutic option for non-resectable melanoma driven by BRAF mutation, melanoma, especially metastatic melanoma, has still become one of the most threatening malignancies. Thus, identifying the exact molecular mechanisms involved in melanoma growth and discovering the novel targets for melanoma therapy is urgently needed. In this study, we screened a siRNA library targeting 6024 human genes in human melanoma cells and identified KMT2A as a potential therapeutic target for melanoma. KMT2A was highly expressed in melanoma cell lines and tumor tissues of melanoma patients. Knockdown of KMT2A by siRNA or shRNA significantly inhibited cell viability and colony formation, whereas exogenous expression of KMT2A effectively promoted cell growth in various melanoma cell lines. Further mechanism studies showed that the KMT2A-mediated regulation of melanoma growth was through targeting the hTERT and CBP signaling. Knockdown of KMT2A significantly inhibited hTERT promoter activity and protein expression and attenuated telomerase activity. Overexpression of hTERT rescued the KMT2A knockdown-mediated melanoma cell growth. By contrast, exogenous expression of KMT2A activated hTERT transcription and expression and increased telomerase activity in melanoma cells. Moreover, we found that KMT2A promoted hTERT expression and melanoma cell growth by cooperating with the transcriptional co-activator CBP, which interacted with and acetylated KMT2A. Inhibition of CBP by siRNA or a CBP-specific inhibitor suppressed the acetylation of KMT2A, abrogated the binding of KMT2A on the hTERT promoter and down-regulated hTERT expression, thereby inhibiting the growth of melanoma cells. Conversely, overexpression of CBP increased KMT2A acetylation and the binding to hTERT promoter, resulting in the promotion of hTERT expression and cell growth. The in vivo studies also showed that KMT2A promoted melanoma growth by activating the hTERT signaling in a xenograft tumor mouse model. Furthermore, the analyses for the clinical samples demonstrated that KMT2A expression was positively correlated with hTERT in tumor tissues of melanoma patients, and the high expression of both KMT2A and hTERT was associated with worse clinical TNM staging and poor prognosis in melanoma patients. Taken together, our results indicate that KMT2A promotes melanoma growth by activating hTERT and CBP signaling in human melanoma. Our study therefore provides new insights into understanding the regulatory mechanism of melanoma growth and suggests that the KMT2A/CBP/hTERT signaling may be a potential therapeutic target for human melanoma. Funding support: This work was supported by the funds from the National Natural Science Foundation of China (81071687, 81272195S), the State “973” Program of China (2014CB542005).

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Genomic Analyses across Cancer Types


At Memorial Sloan Kettering, we have used a clinically validated custom hybridization capture-based NGS assay (MSK-IMPACT) to sequence the tumors of more than 10,000 patients. We sought to determine physician perception and use of these results, including whether they changed management and the reasoning behind this decision-making. All physicians who ordered MSK-IMPACT testing for patients where it was not considered routine were asked to complete a questionnaire quarterly (Table). Physician determination of genomic “actionability” was compared to OncoKB, a curated knowledge base of somatic variants (OncoKB.org). Responses were received from 146 of 258 physicians emailed (57%) regarding 1932 of 9147 cases. However, only 45% of these cases harbored a genomic variant annotated as actionable by OncoKB. Among patients in whom physicians deemed the report non-actionable, 12% had OncoKB annotated actionable variants. Across the cases annotated as potentially actionable by OncoKB, physicians identified an actionable alteration in 81% of cases. At the time of data analysis, 297 (15%) patients had been enrolled in at least one clinical trial of targeted therapy at MSKCC including 224 (12%) patients on genomically-matched trials, 76% of whom participated after IMPACT profiling. As the clinical adoption of NGS panels expands, continued education of physicians as well as maintained knowledge bases for annotation will be necessary to expand the utility of this approach and the opportunity for precision medicine.

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<td>1. Patient enrolled to a therapeutic protocol at MSKCC</td>
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<td>2. Patient enrolled to a therapeutic protocol at another institution</td>
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<td>3. Patient treated with off-label use of an FDA approved therapy</td>
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#376 Biospecimen and data resources for cancer research from NCI’s BPV program. Ping Guan, Helen M. Moore. National Cancer Institute, Bethesda, MD.

The Biospecimen Preanalytical Variables (BPV) Program was initiated by the National Cancer Institute’s Biorepositories and Biospecimen Research Branch to evaluate the impact of preanalytical factors on the molecular integrity of biospecimens. Selected preanalytical factors including cold ischemic time (delay to formalin fixation (DTFF)), time in formalin (TIF), freezing methods, and storage temperatures and durations were examined for their potential effects on molecular profiles of formalin-fixed, paraffin-embedded blocks from four cancer types (kidney, ovary, colon and lung). The BPV program has collected tumor specimens from 364 cancer patients. Each specimen was annotated with 300+ data elements that cover steps in the collection, handling, and processing procedures, pathology review, and clinical information. NCI conducted multiple studies using these specimens to evaluate the preanalytical impacts on different analytical platforms including gene expression profiling, copy number variation, proteomics and metabolomics profiling. The program invites interested organizations to work with NCI through collaboration to further evaluate preanalytical effects on molecular analyses (https://techtransfer.cancer.gov/availabletechnologies/e-000-2013). The remaining specimens are available to support relevant research focusing on biospecimen science and/or clinical bioinformatics.

#377 International Cancer Genome Consortium (ICGC), Jennifer L. Jennings,1 Lincoln D. Stein,1 Fabien Calvo2.1Ontario Institute for Cancer Research, Toronto, Ontario, Canada;2Simon Fraser University, Vancouver, British Columbia, Canada.

The International Cancer Genome Consortium (ICGC) was established to bring together researchers from around the globe to comprehensively analyze the genomic, transcriptomic, and epigenomic changes in 50 different tumor types or subtypes that are of clinical and societal importance across the globe (International network of cancer genome projects, Nature 464, 993-998 (15 April 2010)). As of November 2016, the ICGC has received commitments from researchers and funding organizations across Asia, Australia, Europe, North America and South America for 103 project teams in 17 jurisdictions to study more than 25,000 tumor genomes. Processed data is available via the Data Coordination Centre (http://dcc.icgc.org) based at the Ontario Institute for Cancer Research and is updated semi-annually. The August 2016 release (Version 22) in total comprises data from more than 16,000 cancer donors spanning 70 projects and 21 tumor sites. The Pan-Cancer Analysis of Whole Genomes (PCAWG) project of the ICGC and The Cancer Genome Atlas (TCGA) is coordinating analysis of more than 2,600 cancer genomes, with the extensive use of cloud computing. Because of the very large size of the pan-cancer dataset, with 5,000 whole genome sequences, PCAWG is using a distributed compute cloud environment (generated by computing centres in the USA, Europe and Asia) that meets the project’s technical requirements and the bioethical framework of ICGC and its member projects. Each genome is being characterized through a suite of standardized algorithms, including alignment to the reference genome, uniform quality assessment, and the calling of multiple classes of somatic mutations. Participating laboratories will then be guided by the research program of the ICGC in addressing a series of fundamental questions about cancer biology and evolution based on these data. The first phase of ICGC, which is slated for completion in 2018, has focused on developing extensive catalogs of tumor genomic information. The proposed second phase, ICGCmed, will link genomics to clinical information and health, including lifestyle, patient history, response to therapies, and unique aspects of disease presentation. The project will help fill gaps in our understanding of cancer types, including rare and unusual cancers, and will contribute to a broad spectrum of cancer research from preclinical to clinical development of therapeutics. The ICGC develops policies and quality control criteria to help harmonize the work of member projects located in different jurisdictions. Data produced by ICGC projects are made rapidly and freely available to qualified researchers around the world via the data cloud and through the ICGC Data Coordination Center (http://dcc.icgc.org). More information can be found on www.icgc.org.

#378 The Cancer Genome Collaboratory. Cheltena K. Yung,3 George L. Milhaesco,2 Tierryn L. Frampton,2 Fang Zhang,1 Frampton,2 Jian Yang,1 Jared Baker,1 Guillaume Bourque,2 Paul C. Boutros,3 Barth Ma. Knoppers,4 BF Francis Ouellette,1 Cenk Sahinalp,1 Sohrab P. Shah,1 Vincent Ferretti,1 Lincoln D. Stein1. 1Ontario Institute for Cancer Research, Toronto, Ontario, Canada;2McGill University, Montreal, Quebec, Canada;3Simon Fraser University, Vancouver, British Columbia, Canada;4BC Cancer Agency, Vancouver, British Columbia, Canada.

The Cancer Genome Collaboratory is an academic compute cloud designed to enable computational research on the world’s largest and most comprehensive cancer genome dataset, the International Cancer Genome Consortium (ICGC). The ICGC is on target to categorize the genomes of 25,000 tumors by 2018. A subproject of ICGC, the PanCancer Analysis of Whole Genomes (PCAWG) alone has generated over 800TB of harmonized sequence alignments, variants and interpreted data from over 2,800 cancer patients. A dataset of this size requires months to download and significant resources to store and process. By making the ICGC data available in cloud compute form in the Collaboratory, researchers can bring their analysis methods to the cloud, yielding benefits from the high availability, scalability and economy offered by cloud services, avoiding a large investment in static compute resources and essentially eliminating the time needed to download the data. To facilitate the computational analysis on the ICGC data, the Collaboratory has developed software solutions that are optimized for typical cancer genomics workloads, including well tested and accurate genome aligners and somatic variant calling pipelines. We have developed a simple to use, but fast and secure, data transfer tool that imports genomic data from cloud object storage into the user’s compute instances. Because a growing number of cancer datasets have restrictions on their storage locations, it is important to have software solutions that are interoperable across multiple cloud environments. We have successfully demonstrated interoperability across The Cancer Genome Atlas (TCGA) dataset stored in the Amazon Web Services (AWS) S3 storage. Lastly, we have developed a non-intrusive user authorization system that allows the Collaboratory to authenticate against the ICGC Data Access Compliance Office (DACO) when researchers require access to controlled tier data. We anticipate that our software solutions will be implemented on additional commercial and academic clouds. The Collaboratory is actively growing, with a target hardware footprint of over 3000 CPU cores and 13 petabytes of raw data storage. As of November 2016, the Collaboratory holds information on 2,000 ICGC PCAWG donors (500TB total). We anticipate expanding the Collaboratory to host the entire ICGC dataset of 25,000 donors (approximately 5PB) and to extend its data management and analysis facilities across multiple clouds. During the current closed beta phase, the Collaboratory has been successfully utilized by multiple research groups, most notably PCAWG project researchers who analyzed thousands of genomes at scale over a few weeks’ time. The Collaboratory will open to the public during the second quarter of 2017. We invite cancer researchers to learn more about our cloud resources at cancercollaboratory.org, and apply for access to the Collaboratory.

Introduction: EGFR is a kinase of the HER/ERBB family and an oncogenic driver, especially for NSCLC, HNSCC and CRC. EGFR targeted therapies have been successfully used for treating cancer patients harboring activating EGFR mutations. However a main challenge is acquired drug resistance due to mutations or alternative signaling. While developing next generation drugs is a promising strategy, targeting other alternatives is also an attractive therapeutic option.

The purpose of our study is to investigate the mutational landscape of the tumors harboring activating EGFR mutations and to pinpoint potential strategies for overcoming acquired drug resistance and for combinational therapies. Methods: CancerPlex, a NGS large panel test, which includes targeted, full-gene sequencing of over 400 genes, was applied to analyze a cohort of 2294 patient FFPE samples across majority of solid tumor types. Results: 1. Among 293 (out of 2294, 12.8%) samples harbored EGFR mutations, 86 (86/293, 29.4%) samples harbored actionable EGFR mutations (namely AE group), such as exon 19 deletion and L858R, which includes 84 lung cancer (79 NSCLC), 1 glioblastoma, and 1 uterine carcinoma. 2. The genes/variants in the AE group are then subjected to further functional analysis and mapped to several pathways/biological functions. Our results show that the mutational landscape of activating mutant EGFRs covers most main signaling pathways and biological processes with several pathways: new upstream receptors at different clades, including PI3K and ERBB2, and genes involving in genome and epigenome stabilizations, including ARID1A, KMT2D, and XPC, have the highest mutation rate in the context of activating EGFR mutations. b, PIK3/ AKT/mTOR and Ras-Raf-MEK are two main pathways transducing ligand activated EGFR signals. Our results show that the mutant variants in the PI3K-AKT-mTOR pathway are almost 50% more than those in Ras-Raf-MEK pathway, suggesting that activating mutant EGFRs might preferentially rely on effectors in the PI3K pathway, such as PIK3CA and RICTOR, to transduce oncogenic signals. of note, no KRAS mutation is found in the AE group. c, As expected, mutations are also found in the TP53/apoptosis and RB/cell cycle pathways with higher variant number in the TP53/apoptosis axis. d) Strikingly, the number of mutant variants in the Wnt/beta-catenin pathway tops that in the PI3K and TP53 pathways. Several recent publications demonstrated that the Wnt/beta-catenin pathway is abnormally activated in NSCLC and may be a major mechanism of the drug resistance. Our finding that the genes in the Wnt/beta-catenin pathway, including APC, AXIN2, RNF43, and BCL9, are mutated to a great extent in the context of activating EGFR mutations supports the notion that targeting the Wnt/beta-catenin pathway, in combination with EGFR targeted therapies, is a promising therapeutic strategy for the treatment and overcoming drug resistance of EGFR-driven tumors.

#380 Systematic identification of novel functional tumor-specific mutations in receptor tyrosine kinases based on their pan-cancer mutational profiles in Japanese patients with cancer. Masakuni Serizawa,1 Takeshi Nagashima,2 Yoshi Shimoda,3 Sumpei Ohnami,3 Shumpei Ohnami,3 Kenichi Urakami,3 Masaaki Nishio,4 Tohru Mochizuki,4 Takashi Nakajima,5 Kenichi Ohshima,4 Angeliki Pantazi, Ruobai Sun, Stephen Lyle, Joerg Heyer, Alexei Protopopov, KEW Inc, Cambridge, MA.

The purpose of our study is to investigate the mutational landscape of the tumors harboring activating EGFR mutations and to pinpoint potential strategies for overcoming acquired drug resistance and for combinational therapies. Methods: CancerPlex, a NGS large panel test, which includes targeted, full-gene sequencing of over 400 genes, was applied to analyze a cohort of 2294 patient FFPE samples across majority of solid tumor types. Results: 1. Among 293 (out of 2294, 12.8%) samples harbored EGFR mutations, 86 (86/293, 29.4%) samples harbored actionable EGFR mutations (namely AE group), such as exon 19 deletion and L858R, which includes 84 lung cancer (79 NSCLC), 1 glioblastoma, and 1 uterine carcinoma. 2. The genes/variants in the AE group are then subjected to further functional analysis and mapped to several pathways/biological functions. Our results show that the mutational landscape of activating mutant EGFRs covers most main signaling pathways and biological processes with several pathways: new upstream receptors at different clades, including PI3K and ERBB2, and genes involving in genome and epigenome stabilizations, including ARID1A, KMT2D, and XPC, have the highest mutation rate in the context of activating EGFR mutations. b, PIK3/AKT/mTOR and Ras-Raf-MEK are two main pathways transducing ligand activated EGFR signals. Our results show that the mutant variants in the PI3K-AKT-mTOR pathway are almost 50% more than those in Ras-Raf-MEK pathway, suggesting that activating mutant EGFRs might preferentially rely on effectors in the PI3K pathway, such as PIK3CA and RICTOR, to transduce oncogenic signals. of note, no KRAS mutation is found in the AE group. c, As expected, mutations are also found in the TP53/apoptosis and RB/cell cycle pathways with higher variant number in the TP53/apoptosis axis. d) Strikingly, the number of mutant variants in the Wnt/beta-catenin pathway tops that in the PI3K and TP53 pathways. Several recent publications demonstrated that the Wnt/beta-catenin pathway is abnormally activated in NSCLC and may be a major mechanism of the drug resistance. Our finding that the genes in the Wnt/beta-catenin pathway, including APC, AXIN2, RNF43, and BCL9, are mutated to a great extent in the context of activating EGFR mutations supports the notion that targeting the Wnt/beta-catenin pathway, in combination with EGFR targeted therapies, is a promising therapeutic strategy for the treatment and overcoming drug resistance of EGFR-driven tumors.

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Genomic Analyses across Cancer Types

#381 Genomic alterations in mucins in cancers. Ryan J. King, Fang Yu, Pankaj K. Singh. University of Nebraska Medical Center, Omaha, NE.

Mucins promote the growth, aggressiveness, and invasiveness of various cancer types. Changes in the expression or localization of mucins can significantly alter their primary function. Due to their predominant role in cell-cell and cell-matrix interactions, biomarkers and therapeutic agents have been developed to improve patient care. However, an extensive investigation across multiple cancer subtypes examining the genomic status of the cohort of mucins has not been performed and could yield significant insights into new roles of mucins in different cancers. Utilizing The Cancer Genome Atlas, the cancers of 11 mucin expressing tissues were examined for the genomic alterations in mutations, mRNA expression levels, copy numbers, methylation status, and the impact on patient survival due to these genomic features. It was discovered that different mucins across multiple tissue origins and cancer subtypes might have a different rate, pattern, and survival impact. MUC1 was not mutated in the majority of cancers; however, we did observe a frequent T12P mutation in pancreatic tumors that was also present in other cancers. MUC4 was frequently mutated at H4205 and MUC4 mutations were seen to have survival differences. Multiple significant differences were discovered in mRNA expression, which varied distinctly between cohorts. One such pattern was observed with de novo expression of MUC21 in colorectal cancers, in which mRNA expression was suddenly induced in cancer and increased in a stage dependent manner. Copy number alterations were seen for only a few cancer subtypes and mucins, including A431B1 and lung squamous cell carcinomas. Global promoter demethylation was generally observed across the cancers. MUC15 in renal cancers was seen to be one of the most demethylated mucins, in which mRNA was seen to be substantially decreased or expunged. This extensive study investigated the status of multiple mucins in multiple cancer settings and highlights a few noticeable mucins that are suggested to be further studied for roles in biomarkers, survival, and etiology for cancer progression.

#382 Genome-wide location analysis of DNA adducts in vivo. Brian Woo, Christopher Fang, Paolo Abada, Stephen B. Howell, Olivier Harismendy. UC San Diego, La Jolla, CA.

DNA adducts are the biochemical consequences of exposure to UV light, carcinogens or DNA-reactive drugs. They play a central role in malignant transformation and the selective killing of cancer cells by chemotherapeutic agents. The functional consequences of DNA adducts is likely to depend on their exact location in the genome with respect to histones and sites where histones and DNA are modified. However, tools that permit precise genome-wide mapping of adduct locations have poor resolution or are too specific to the type of DNA adduct. Here we describe a method capable of identifying the genomic location of damaged DNA bases in whole cells. Using naked DNA treated with cisplatin (cDDP) or ultra-violet (UV) light as the damaging agents, the strategy relies on differential exonuclease digestion to enrich genome-wide libraries for fragments containing DNA adducts, followed by high-throughput sequencing. Consistent with the expected adduct chemistry, the 5’ end of the post-PCR product was enriched in TT, TC or CT di-nucleotides in the UV-treated DNA (>4x) and in AG or GG in the cDDP treated DNA (1.5x), suggesting a positive predictive value of 76% and 44% for UV (TT) and cDDP (GG) adduct detection, respectively. Applied in vivo to IMR90 cells, Ad-Seq revealed that the fraction of AG/GG loci was higher in DNA from the cDDP-treated cells.
at all coverage depths, reaching 1.5x enrichment for the highest depth quartile. This indicates that the method is selective and that even low covered loci show enrichment of AG/GG sequences. The enrichment in purines was limited to 3 nucleotides downstream of the read start site, confirming the high resolution of the exonuclease digestion. A total of 11 x 103 AG/GG loci were identified in replicated cDDP treated samples and 555 novel cancer patient derived 536MM cell line by RT-PCR and targeted RNA sequencing. Extended this profile, the distribution of AIMP2-DX2/AIMP2 ratio and AIMP2 related major cancer pathways were analyzed using the samples in the ICGC/TCGA database. Over 23 cancer types, 753 samples were used in WTS analysis. In the DEG set analysis, 10 pre-defined major cancer pathways were analyzed among 16 cancer types. Some cancer types, especially colon and hepatocellular carcinoma, OS curves had a tendency in a similar way to AML. For the clinical validation of the prognostic value of AIMP2-DX2, 51 AML patients were included in this analysis. The correlation between AIMP2-DX2 expression and survival outcomes was investigated in clinical validation cohort of AML. The AIMP2-DX2-positive group had significantly inferior OS rate and had worse RFS compare to AIMP2-DX2-negative group. Our sequential data shows that the AIMP2-DX2/AIMP2 expression and their ratio can possibly be an indicator to measure malignancy of various cancer types.
OnkoInsight is a pipeline designed to detect cancer driver genes from large sequencing datasets. It includes the somatic mutation detection module SomaticSeq, and the novel driver gene detection module GSMuta. SomaticSeq leverages an ensemble approach and machine learning to accurately detect somatic mutations. In Stage 5 of the ICGC-TCGA DREAM Somatic Mutation Challenge, SomaticSeq v1 placed #1 and #2 in the INDEL and SNP sub-challenges. In the current project, we used the improved SomaticSeq v2.2.2, which now extracts most features directly from the BAM files instead of SAMtools, and has the added function of handling multiple variant calls at the same position. We incorporated MuTect, Indelocator, VarScan2, SomaticSniper, VarDict, MuSE, and LoFq. GSMuta detects regions, genes, and pathways that are enriched for somatic mutations. It identifies cancer drivers and includes a unique feature of distinguishing driver genes and tumor-suppressing genes. As a demonstration of the capability and scalability of OnkoInsight, we deployed the tools as docker images, developed the pipeline using common workflow language, and analyzed over 1,000 TCGA lung cancer patients with tumor-normal whole exome sequencing data on Cancer Genomics Cloud. The project involved 369 adenocarcinoma (LUAD) and 490 squamous cell carcinoma (LUSC) samples. On average, SomaticSeq detected over 700 somatic mutations per sample. The predicted mutation rate was consistent with the expected mutation rates of LUAD and LUSC. Once we obtained the high-confidence somatic mutations from SomaticSeq, we used GSMuta to detect driver genes in LUAD and LUSC separately. We detected 97 and 50 driver genes for LUAD and LUSC, respectively. To assess the quality of GSMuta's predictions, we compared our GSMuta predictions with the UCB-OCG-CONSORTIUM's driver gene predictions for cancer driver genes. Indeed, GSMuta reproduced 16 out of the 18 LUAD driver genes reported by TCGA's landmark study such as EGFR, KRAS, and BRAF. It also detected some potential new driver genes. For example, it reported 9 out of 10 LUSC driver genes reported by TCGA such as PTEN. LUAD and LUSC shared nine predicted driver genes, and the pathway disruption was homogenous across the two subtypes. It also detected some potential new driver genes. The project was completed in less than a week for over 1,000 pairs of homogenous across the two subtypes. It also detected some potential new driver genes. The project was completed in less than a week for over 1,000 pairs of homogenous across the two subtypes. It also detected some potential new driver genes. The project was completed in less than a week for over 1,000 pairs of homogenous across the two subtypes.

**#387 Targeted exome sequences of cancer-related genes in human cancers using amplicon sequencing.** Yasushi Sasaki, Takafumi Nakagaki, Miyuki Tamura, Hisayo Fukushima, Hiroshi Ikeda, Ryota Koyama, Masashi Idogawa, Takashi Tokino. Sapporo Medical University, Sapporo, Japan.

Objective: Next-generation sequencing (NGS) has revolutionized cancer genomics research by providing a comprehensive method of detecting somatic cancer genome alterations. Platforms for genomic DNA alterations are more common in clinical practice and include whole genome/exome sequencing analyses. These tests are still very expensive, although the costs are coming down substantially. Here, we aimed to determine the efficacy and advantages of targeted and amplicon sequencing, which can be used to detect cancer-related genes in human cancers using amplicon sequencing. Methods: DNA was extracted from 61 human cancer specimens and their corresponding non-cancerous tissues, including oral squamous cell carcinomas (OSCCs) and multiple myelomas (MMs). Forty nanograms of DNA were used for multiplex PCR amplification with an Ion Ampliseq Comprehensive Cancer Panel that offers targeted coverage of all exons in 409 tumor suppressor genes and oncogenes frequently cited and frequently mutated in human cancers. This platform was designed to be amplification-based capture with 15,992 regions (1.6 megabases in total size). Purified DNA libraries were sequenced with 6-8 samples on Ion Proton P1 chip. Sequence reads of tumor and normal samples were aligned to the hg19 genome, and generated BAM files were used to detect somatic mutations (point mutation, insertion and deletion) and copy number variations. Results: Each sample underwent on average 8.4 million sequencing reads after quality filtering. The mean base coverage depth was 530, and >95% of targeted bases were represented by at least 20 reads. The number of non-synonymous somatic mutations in 47 patients with OSCC ranged from 1 to 21 with a mean of 7.5 (6.4 Mb). The most frequent mutations in OSCC were in TP53 (63.8%), NOTCH1 (25.3%), CDKN2A (19.2%), TAF1 (7.0%), SYNE1 (14.9%) and PIK3CA (8.8%). We also detected a mean of 6.1 (range 3-11) non-synonymous mutations per MM patient. Somatic mutations were found in known MM-associated genes, including TP53 and NRAS. Pathway assessment has shown that somatic aberrations within MM genomes are mainly involved in several important pathways, including cell cycle regulation, RTK-MAPK-PI3K and NF-kB. We found several genetic alterations that may have been associated with the poor prognosis of non-responding patients to chemotherapy in MM patients. Conclusions: This study demonstrates the utility of using a semiconductor-based sequencing to efficiently identify somatic genetic alterations in human cancers. The targeted next-generation sequencing using low amounts of FFPE DNA is a valuable tool for rapid (5 days) and high-throughput genetic testing in research and clinical settings.

**#388 A benchmark study for identifying cancer drivers in the non-coding part of the genome.** Damien Drubay,1 Daniel Gautheret,2 Stefan Michels3.

1INSERM, Gustave Roussy, Villejuif, France; 2Université Paris-Sud, CNRS, CEA, Gif-sur-Yvette, France.

Purpose: Prioritizing potential deleterious variants is an essential task to guide research and validation of new pathological variants in the immunity of the genome. Many tools have been introduced to detect new variants in the coding part of the genome. Detailed knowledge of coding sequences led to efficient statistical models for cancer driver discovery. The challenge is greater for the non-coding part of the genome due to its large size (>98% of the genome) which contains many non-functional or unknown features. Several deleteriousness scores have been proposed in the past decades, but no current large comparison has been realized to assess their ability to identify cancer drivers. Material and method: We compared the leading scoring systems (CADD, FATHMM-MKL, Funsq2 and GWAVA) and some recent competitors (DANN, SNP and SOM scores) for their ability to discriminate assumed pathologic variants in the non-coding genome (as identified by 928 ClinVar variants / 44,158 recurrent COSMIC mutations) from assumed non-pathologic variants (100,000 randomly sampled 1000 Genomes project variants with minor allele frequency > 1%). To define the pathogenic variants using COSMIC as reference, we varied the threshold for number of COSMIC recurrences from 2 to 10. We compared the sensitivity, specificity and precision of the scoring systems using the area under the curve (AUC) of receiver operating characteristic (ROC) and precision-recall (PR) curves. Results: Most scores had good sensitivity and specificity for the ClinVar dataset but AUCROC for GWAVA was about 0.85. DANN and FATHMM-MKL were significantly better than the other methods. For ClinVar variants was concerned, the top performing methods were CADD (AUCPR = 0.84), DANN (AUCPR = 0.83) and, to a lesser extent, FATHMM-MKL (AUCPR = 0.75). When using a threshold of 3 recurrences to define true pathogenicity of COSMIC variants, the AUCROC ranged from 0.52 (DANN) to 0.80 (GWAVA) but precision was low with AUCPR ranging from 0.05 (DANN, S OM-melanoma) to 0.18 (GWAVA). Increasing the pathogenicity threshold to 10 recurrences increased AUCROC values (ranging from 0.50 (SOMmelanoma) to 0.89 (GWAVA)) but decreased precision values (AUCPR ranging from 0 to 0.02). Discussion: This large scale benchmark study distinguished CADD as the best tool to detect variants with features similar to those of ClinVar, which are mainly located in protein coding regions. However, based on the results using COSMIC, GWAVA outperformed CADD for variants in other regions, including non-coding regions. We also compared some deleterious scores and their ability to identify cancer drivers. Material and method: We compared the lead-
with multiple alterations are prioritized. Our method is summarized in two steps: 1) Identify genes that are both recurrently and heterogeneously altered across many samples by calculating a rank-based score for each gene. 2) Identify MutE and CoO features across tumor types. Recent studies have provided evidence that epigenetic changes and regulatory sequence mutations could also dysregulate oncogenes and tumor suppressors. As a transcription factor, P53 is activated in response to oncogenic stress and cell type. Differential expression analysis using RNA-Seq distinguishes a lethal cancer from one with a favorable prognosis, is poorly understood. To address this question, we have performed a comprehensive meta-analysis on genomic data from primary tumors that are linked to patients’ clinical outcomes. Using data from >20,000 patients, we have identified protein-coding genes, lncRNAs, miRNAs, methylation sites, and CNVs in primary tumors that are significantly associated with patient prognosis across cancer types. Multivariate analysis reveals a hierarchy of survival determinants: the strongest mortality-associated factors are enriched for components of the mitotic cell cycle, while secondary clusters of genes are involved in extracellular matrix remodeling, cell motility, and angiogenesis. Survival-associated genomic features are indicative of immune infiltration into primary tumors and oxidative phosphorylation activity. In vitro and in vivo analyses reveal that mortality-associated genes rarely promote the direct transformation of primary cells. Instead, CRISPR mutagenesis reveals that these genes largely repress tumor growth and in their absence cancer cells fail to progress. Through this analysis, we have identified new genetic dependencies common across cancer cell types, including C16ORF59 and C5ORF46. In total, our results represent the largest assessment of genomic features linked to cancer progression completed to date, and offer several lines of insight into the biological differences between fatal and benign cancers.

A comparative genomics approach to understanding the control of cell context dependent P53 binding. Varsha Sundaresan, Ying Li, Benedetto DiCiaccio, Victor T. Lin, Lei Zhou, University of Florida, Gainesville, FL; Macau University of Science and Technology, Macau, China.

Cancer is considered to be a genetic disease characterized by sequential accumulation of mutations. Recent studies have provided evidence that epigenetic changes and regulatory sequence mutations could also dysregulate oncogenes and tumor suppressors. As a transcription factor, P53 is activated in response to oncogenic stress and exerts distinct anti-proliferative functions based on the stressor and cell type. Though a number of ChIP-Seq studies have identified thousands of P53 binding sites in mammalian genomes, the functionality of these binding sites remains to be established. In addition, we know little about what could drive stress and cell context specific binding profile of P53. Traditionally, mutations in the coding regions of p53 have been extensively studied to gain insights on its role in cancer and to identify strategies to restore the functions of p53 in cancer cells. We hypothesize that mutations in or epigenetic silencing of functionally important P53 binding sites play an important role in tumorigenesis as well. Since functional regulatory regions tend to be more evolutionarily conserved, in this project we propose using a comparative genomics approach to identify functional P53 binding sites and determine if these regions are involved in tumorigenesis. In order to study how P53 binding following DNA damage differs between cell types, we aim to perform comparison between P53 ChIP-Seq data generated in our lab from Drosophila embryos at different developmental stages and a cell line (Kc167) as well as published datasets from mammalian stem cells and differentiated cells. Differential expression analysis using RNA-Seq exhibited that at an early stem cell-like stage there is P53-dependent induction of pro-apoptotic genes in response to DNA damage but not in the differentiated stages. We are seeking to establish functionally significant P53 binding sites by re-training we investigate using RNA-Seq as a proxy signature of cell type. The importance of these binding sites will be verified by CRISPR-Cas9-mediated genome editing. We will also perform comparison studies of DNA damage-induced P53 binding in human, mouse and Drosophila to identify analogous patterns. Knowledge gained from this study will help us to understand the role of non-coding regulatory regions in tumorigenesis, and predict patient response to apoptosis-inducing therapeutic agents. It may also lead to novel strategies to restore cellular sensitivity to chemotherapy or radiation.
KRAS is the most commonly mutated oncoprotein and is a major driver of tumor initiation and progression. Understanding the functional consequences of cancer-associated KRAS variants may have important clinical implications. For example, KRAS mutation status defines those that are likely to respond to EGFR-directed therapy in KRAS-mutant metastatic colorectal cancer. A comprehensive understanding of oncogenic KRAS will facilitate understanding of clinically important missense variants that alter critical oncogenic properties of KRAS. First, we sought to comprehensively identify all possible oncogenic missense mutations in KRAS that mediate oncogenic transformation. We stably transduced the WT library into immortalized human epithelial cell lines and evaluated growth and epithelial-to-mesenchymal transition (GILIA), an assay that is highly correlated with in vivo tumor formation. We identified all previously known hotspot oncogenic alleles of KRAS as well as many functionally relevant alleles that are also discovered at lower frequency in human tumors. Moreover, we also discovered a group of transforming KRAS variants that have not been well described in human tumors, thus revealing potentially novel activating mechanisms for oncogenic KRAS. In parallel, we utilized the G12D mutagenesis library to perform second-site suppressor screening to identify loss-of-function single amino acid changes that abrogate the transforming ability of oncogenic KRAS. We performed positive-selection screening in primary cell lines for variants that enable bypass of oncogene-induced senescence. Additionally, we conducted a negative-selection screen with the G12D library in a KRAS-dependent cancer cell line with inducible suppression of endogenous KRAS, thus identifying all possible second-site mutations that abolish KRAS-driven signaling necessary for maintenance of cellular proliferation and viability. Structure-function analysis of these data may reveal novel patterns of amino-acid changes that result in inactivation of oncogenic KRAS. In summary, this comprehensive dictionary of gain- and loss-of-function KRAS missense variants will facilitate understanding of clinically important mutations and also yield novel insights into structure-function relationships that may improve our understanding of the KRAS oncogene.

Activation mechanisms of cancer associated MEK1 mutants. Yijun Gao,1 Matthew T. Chang,1 Daniel McKay,1 Rona D. Yaeger,1 Merna Torres,1 Keven Muniz,1 Drosten Matthias,2 Omar I. Abdel-Wahab,3 Mariano Barbacid,4 Giordano Caponigro,5 Darrin Stuart,1 David Solit,1 Barry S. Taylor,1 Zhan Yao,1 Neil Rosen,1 Memorial Sloan Kettering Cancer Center, New York, NY;2Novartis Institutes for BioMedical Research, Cambridge, MA, USA;3Centro Nacional de Investigaciones Oncológicas, Madrid, Spain, Spain.

Activating BRAF mutants drive human tumors by dysregulating ERK signaling despite ERK-dependent feedback suppression of ERK. These BRAF mutants become RAS independent, by either of two mechanisms, and they are thus unaffected by feedback inhibition of upstream signaling. Recently, it has become clear that mutation activation of MEK1 or MEK2 occur at appreciable frequency in human tumors, but the mechanism of activation of these mutants and whether they remain dependent on upstream activation of RAS or RAF signaling remains unknown. Here we characterize the mechanism of activation of 18 recurrent MEK1 mutants identified in human cancer. Based on these data, they fall into three classes. The kinase activity of first class remains dependent on RAF-mediated phosphorylation of S218 and S222, and has basal RAF-independent activity, but can be further stimulated by RAF and the third class no longer requires phosphorylation of S218 and S222 and signals in a RAF independent manner. These features determined their sensitivity to ERK-dependent feedback regulation and the ability to drive ERK signaling output in cells. The more RAF-independent activity the mutants acquired, the better they could activate downstream ERK pathway, leading to increased transforming activities in MEF cells in the absence of RAF. This is consistent with their genetic association with RAS, RAF and NF1 mutations in human tumors. The Class 3 RAF-independent MEK1 mutants tend to be mutually exclusive with those mutants, while coexistence with RAS/RAF/NF1 mutations is frequently observed in tumors with Class 1 or 2 MEK1 mutants. Moreover, functional class correlated with sensitivities of ERK signaling driven by these mutants to MEK inhibitors that function by different mechanisms. Unlike RAF dependent or regulated MEK1 mutants, the ERK signaling driven by RAF independent MEK1 mutants is insensitive to an allosteric MEK1 inhibitor that functions through MEK1 phosphorylation. However, signaling driven by all classes of MEK1 mutants is sensitive to an ATP competitive MEK1 inhibitor which targets MEK1 kinase activity.

Detecting copy number variations using WES datasets in patient derived xenografts. Jia Xue,1 Wubin Qian,1 Sheng Guo,1 Jie Cai,1 Henry Qiang Li2.1Crown Bioscience, Inc., Santa Clara, CA;2Crown Bioscience, Inc., Beijing, China.

Amplification or deletion of oncogenes and tumor suppressors can be oncogenic, which may serve as drug targets and biomarkers for disease prognosis and drug response. Traditionally, array-based assays, e.g. Affymetrix SNPs6.0 and lately OncoScan, have been used to detect DNA copy number variations (CNVs). These assays have limitations including insufficient probe availability for some genes, cross hybridization, imprecise measurement of fluorescent signal, less suitability of tumor samples, and high cost. Whole exome sequencing (WES) is now widely used to profile tumor samples, and provides a fast and efficient determination for point mutations, insertions and deletions at the DNA level. Recently, WES is being used to profile CNVs with some success, but also suffers from many drawbacks, such as the requirement of paired normal tissues, the need of a large batch of samples, the inadequacy of detecting chromosomal-level CNVs, the incapability to detect CNVs in low coverage genomic regions. We have developed a CNV detection pipeline on both genomic (segment) level and gene level from WES data using the concept of off-target and on-target reads1, and evaluated it in a set of 155 patient-derived xenografts (PDx), with head-to-head comparison to Affymetrix SNPs6.0 and OncoScan2 on 5 models with 2 passages from each. PDX is a well-accepted experimental model mimicking original patient in histo- & molecular pathology3. Reads derived from mouse contaminants were removed from WES datasets to avoid mouse signal interference, which cannot be done in array-based techniques. RT-PCR was used to experimentally validate CNVs for selected genes. We found that the average off-target rate for our PDX models is approximately 15%, and off-target reads were uniformly distributed across genome. The comparison with array-based technologies indicates that 1) WES has the highest resolution (20kb) while OncoScan is the second (50-100 kb), followed by SNPs6.0 (100-200kb), 2) the OncoScan CNV calls are very similar to our WES methods at the genome level, yet 3) WES gives a much higher accuracy on CNV inference for genes flanking the ~900 cancer related genes with enhanced probe densities on OncoScan, suggesting that our WES method has the highest accuracy. RT-PCR results confirmed the observations. In summary, WES-based CNV analysis is a better solution in CNV detection in PDX models. References 1. Kuilman T, Velds A, Kemper K, Ranzani M, Bombardelli L, Hoogstraat M, Nevedomskaya E, Xu G, de Ruiter J, Lolkema MP, Ylstra B, Jonkers J, et al. CopywriteR: DNA copy number detection from off-target sequence data. Genome biology 2015;16:49. 2. Guo S, Qian W, Cai J, Zhang L, Wery JP, Li QX. Molecular Pathology of Patient Tumors: Patient-Derived Xenografts, and Cancer Cell Lines. Cancer research 2016;76:4619-26.

Whole genome copy number variation analysis using a SNP-focused targeted sequencing panel for tumor analysis. Jiashu Wang,1 Kristina Giorda,1 Zhongwu Lai,1 Daniel Stetson,1 Mirna Jarosz,1 1Integrated DNA Technologies, Redwood City, CA;2Astrazeneca, Boston, MA.

Accurate genome-wide copy number variation (CNV) analysis is critical for disease and cancer research. Current approaches for CNV analysis include fluorescence in situ hybridization (FISH), array comparative genomic hybridization (array CGH), and SNP arrays. Unfortunately, these methods are not sensitive enough for real world cancer samples because of tumor purity, heterogeneity. NGS-based targeted sequencing is increasingly being used for CNV analysis due to throughput, coverage, cost, and sample input requirements. For CNV analysis, detection power is improved by combining both read depth and SNP allele frequency analysis, particularly for copy-neutral events such as loss of heterozygosity. A custom xGen Lockdown CNV backbone panel was developed for broad, uniform genome coverage and to enrich for population-based SNPs. We demonstrate use of the panel as an addition to the xGen Exome Research Panel and a custom cancer focused panel. Downstream analysis incorporates both read depth and observed minor allele frequencies to determine CNVs with enhanced sensitivity. To increase the resolution for large scale alterations of chromosome 7, a hot-spot for disease-associated CNVs, probe density was increased 6-fold. A known standard, NA12878, was used to validate

the panel’s ability to detect heterozygous SNPs with high confidence. In addition, mixtures of cancer cell lines from the Cancer Cell Line Encyclopedia (CCLE) were tested with varying levels of background copy-neutral genomic DNA. The sensitivity and specificity of the panel to detect CNV and LOH events with was assessed using deep exome and Affymetrix SNP array data. The ability to detect copy number alterations with high resolution and accuracy would be a valuable resource for disease and cancer research.

#398 Using off-target data from comprehensive genomic profiling to characterize the genomic architecture of copy number alterations in tumor sequencing data. Caitlin F. Connelly, Zachary R. Chalmers, Philip J. Stephens, Garrett M. Frampton, Foundation Medicine, Inc., Cambridge, MA.

Background: Copy number alterations are common driver mutations in cancer. Identifying genes which show altered copy number and knowing the breakpoints of copy number alterations gives insight into their functionality as well as the mechanisms responsible for the alterations. While copy number alterations have been well-studied in common tumor types, there are still many rare tumor types which have not been characterized. Targeted genomic profiling using capturing methods results in sequencing a subset of genes to extremely high coverage (>500x) and sequencing off-target regions spanning the rest of the genome to low (0.5x – 1x) coverage. We used this data to identify the approximate breakpoints of alterations that extend beyond targeted regions and to identify novel, recurrent copy number alterations in clinical tumor samples. Methods: We used standard methods to identify copy number alterations in the off-target regions. Briefly, we calculated coverage in 10 kb windows across the genome, excluding the targeted regions which are sequenced at high coverage, corrected for GC-bias, calculated the log ratio of coverage for the tumor compared to an unrelated normal sample, and then used a segmentation algorithm to segment the genome into copy number regions and identify regions with significantly different copy number than the surrounding regions. We sequenced cell line DNA with known copy number variants and used this data to assess the accuracy and precision of these methods. Copy number alterations were filtered against known common copy number variant regions, and we identified copy number alterations overlapping known genes to identify potentially functional alterations. Results: We applied these methods to tumor samples that underwent comprehensive genomic profiling as part of clinical care. Our cohort consisted of a large and diverse set of “real world” unbiased clinical specimens and contained many rare and unusual tumors not included in larger sequencing efforts. Using this data, we characterized the landscape of copy number variants genome-wide in rare tumor types which have not previously been characterized and identified unique copy number alterations in these tumor types. Further, we characterized the breakpoints of copy number alterations across the cohort. Conclusions: We will present novel copy number alterations in rare tumor types and the recurrent breakpoints for common alterations across multiple tumor types. These findings will provide insight into the mechanisms of cancer progression.

#399 CPTAC phase II final report. Mathangi Thiagarajan. Leidos Biomedical Research Inc., Rockville, MD.

National Cancer Institute’s Clinical Proteomic Tumor Analysis Consortium (CPTAC) is a comprehensive and coordinated effort to accelerate the understanding of the molecular basis of cancer through the application of robust, quantitative, proteomic technologies and workflows. The primary goal for this program is to systematically identify proteins that derive from alterations in cancer genomes and related biological processes, integrate the genomic and proteomic data from analysis of common cancer biospecimen and provide this data with accompanying assays and protocols to the community. The phase II effort managed by the Leidos Biomedical Research Inc. had a goal to procure and qualify a minimum of 100 cases from 3 cancer types (Breast, Ovarian and Colon). Tissues and analytes from the 100 cases were sent to proteomic and genomic characterization centers for analysis. The proteomic data were made available through the Data Coordinating Center (DCC) and the genomic data were made available through NCI’s Genomic Data Commons (GDC). The success from the phase II effort has set precedence for a much larger phase III effort that will focus on additional cancer types and cases. Preliminary analysis results confirm the need for the combination of the genomic and proteomic approaches to produce a more comprehensive inventory of the detectable proteins in a tumor and advance our understanding of cancer biology.

#400 NCI’s CPTAC Phase III, proteogenomic analysis of additional cancer types. Linda I. Hannick, Frederick National Lab for Cancer Research, Rockville, MD.

The Clinical Proteomics Tumor Analysis Consortium (CPTAC) is a National Cancer Institute initiative that seeks to uncover the molecular basis of cancer using a proteogenomic approach to study prospective cancer specimens. Leidos Biomed provides an infrastructure for supporting the collection of high quality biospecimens and data, in addition to project and subcontract management for the program. CPTAC applies the understanding of the molecular basis of cancer to identify biomarker candidates. Phase II of CPTAC, completed in 2016, collected over 500 cases from breast, colon and ovarian patients. In early 2016, CPTAC Phase III began to collect and analyze 200 cases of each of ten additional cancers. The goal is to collect 200 qualified cases of five to six tumor types over a five-year period, although the program is currently accruing from ten. The study entails collection and pathology evaluation of biospecimens, high-quality clinical data and images from clinical sites around the world. A biorepository evaluates and stores the biospecimens and medical records of patients to a sequencing center and tissues to proteomics groups. Data are combined and analyzed by translational centers. Genomic data are made available to the research community through the NCI Genomic Data Commons. Proteomic data are made available through the Data Coordinating Center. We report here on progress in collection of tissues and clinical data, and use of biorepository, proteomics and genomics and analysis centers.

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Genomic Screening for Cancer Dependencies and Drug Response


CRISPR design is a complex multivariate problem. The weight of each variable differs depending on the experiment under consideration. Current methods of designing CRISPR experiments are based on a single-variable, resulting in poor experimental outcomes. From the numerous experiments enabled by the DESKGEN platform, we can demonstrate key design improvements to maximize experimental outcome. Our current scoring methods were tested and validated using an sgRNA library of over 3,500 sgRNAs in essential genes with a variety of predicted sgRNA activities and specificities. The predicted ‘good’ sgRNAs achieved significantly higher read depletion for essential genes, with fewer failing sgRNAs, compared to sgRNAs that didn’t meet the scoring criteria. Our scoring algorithm incorporates multiple published observations for choosing effective sgRNAs, which differs from other CRISPR tools that tend to focus on one factor such as sgRNA sequence or specificity. We were unable to find correlations for some currently accepted sgRNA selection criteria, such as position within a gene, which demonstrates the need to constantly challenge and improve sgRNA design rules.


Functional genomic screening with CRISPR-Cas9 has provided a powerful and precise new way to interrogate the phenotypic consequences of gene loss in high-throughput, unbiased analyses. Rapid development of pooled lentivirus and deep-sequencing-led approaches have allowed us and others to exploit this technology in target ID, target validation, drug MOA analysis and patient stratification. Adaptation of the Cas9 nuclease by domain fusion and catalytic inactivation has further provided a novel way to both up and down-regulate gene expression using targeted, high-throughput CRISPRi and CRISPRa tools. To date, the vast majority of screens have been conducted using sgRNA abundance in surviving cell populations as an NGS-based proxy for proliferation, for example for in vitro resistance analysis to a cytotoxic treatment regimen. However, where cellular pathophysiology is uncoupled from cell health, the enormous promise of pooled CRISPR-Cas9 screening has been largely untapped. We now report on the development of a FACS-based platform that can be used to monitor biomarker-based cellular response in combination with high-throughput genetic screening. The endogenous interaction between CD28 and CD80 was used as a proof-of-concept paradigm and the performance of our platform was measured in screen-simulation conditions. We find that FACS-linked NGS was able to robustly identify the CD28 deleted cells from a complex population using a FITC-labelled CD80-Fc. Pseudo-blinding of the data yielded unambiguous identification of the expected target guides, with robust screening metrics, validating a new platform for myriad discovery programmes.
and gain-of-function genetic screens. Donato Tedesco, Paul Diehl, Mikhail Makhnov, Sylvain Baron, Alex Chenchik, Collecta, Inc., Mountain View, CA.

Genome-wide loss-of-function screening is a fundamental method to identify genes responsible for driving biological processes. Complex pooled lentiviral-based libraries expressing large numbers of genetic disruptors, such as shRNAs (RNAi) or sgRNAs (CRISPR), make large-scale cell screening practical. While RNAi-based approaches have proven to be an effective strategy for these screens, recent work has showed CRISPR-based technologies offer not only an effective alternative, but distinct advantage. We will present unpublished data from genetic screens in human cancer cell lines using CRISPR-Knockout (KO), CRISPRi and CRISPRa technologies, as well as RNAi. Antibody-based function shRNA and sgRNA pooled library screens are similar in concept, the gene loss of function is achieved by different mechanisms (mRNA degradation with RNAi, full gene disruption with CRISPR-KO, transcriptional inhibition with CRISPRi), so some divergences are expected and indeed observed when comparing results obtained using one method versus the other. Furthermore, contrary to RNAi, CRISPR technology can be adapted to detect gene expression (CRISPRa), thus enabling the use of genome-wide gain-of-function screening in gene function studies.

A bi-clustering based computational approach to identify molecular markers for Neoadjuvant chemotherapy outcome prediction in complement to cancer subtype classifications. Chi Zhang, Sen Liang. Indiana University, School of Medicine, Indianapolis, IN; Jilin University, Chang Chun, China.

Molecular stratification models have been well established for cancer types to classify tumors into sub-types by using genome level molecular characteristics. Tumors of certain sub-type have distinct response to specific therapies. Hence a reliable molecular classification model can be applied to determine treatment strategy. Recent neoadjuvant chemotherapy, immunotherapies and gene targeted drugs and their possible combinations raise a demand for small subsets of novel molecular markers for outcome prediction in addition to the complete molecular profile based classification. However, it is unknown what genes have predictive power for a certain therapeutic strategy while the highly crossed clinical features increase the difficult to determine sample groups for marker identification that form a bi-clustering problem. In addition, predictive maker may be varied among different cancer sub-types. In this work, we developed a bi-clustering based computational framework to identify transcriptoric markers for predicting the outcome of neoadjuvant chemotherapy and adjuvant chemotherapy. Our computational approach identifies functional related gene expression modules show significant expression patterns in a subset of samples that are highly associated with one or several disease sub-types and with high predictive power for the outcome of certain. By applying the analysis to 8 breast cancer, 10 colorectal cancer and 5 ascites pooled leukemia data sets in public domain, we have identified sets of gene markers with high predictive power for chemotherapy outcome complementary to the existing molecular classifications, suggesting the bi-clustering based approach can improve the whole genome level molecular classification based outcome prediction.

A novel panel of 325 biomarkers is part of a large inter-connected network representing multiple cell signaling pathways and allowing development of predictive tests for oncology drugs. Edward C. Goodwin, Said Atiya, Marcia Fournier. BioArray Genetics, Inc., Farmington, CT.

Background: We developed an algorithm based on the gene expression of tumor biopsies to identify the best combination of biomarkers to reliably predict a patient’s response to relevant cancer treatments. This algorithm is derived from 325 genes whose expression showed significant changes during differentiation of non-malignant human mammary epithelial cells cultured in laminin-rich extracellular matrix. Of these 325 genes, 251 are novel and not present in 9 other cancer based gene expression panels such as FoundationOne or PAM50. These differentiated cells formed multicellular structures with defined lumens and tight junctions and with specific localizations of cadherin and integrins. In contrast, carcinomas display disruptions in gene loss of structure. Previous work showed that different sets of these 325 biomarkers accurately predicted overall breast cancer patients’ survival or response to neoadjuvant therapy in multiple independent studies. Objective: Predict cell-signaling pathways, drug associations, and disease associations for the 325 biomarkers (BA325) in contrast to other cancer gene panels. Methods and Results: The Qiagen Ingenuity program was used to identify pathways and disease states containing significant overlap with BA325. Multiple cell signaling pathways including cell proliferation, migration, invasion, and metabolism were found in BA325, while most other cancer biomarker panels were highly concentrated in cell proliferation. Examples of significant pathway associations relevant for oncology drug discovery and targeted treatments include Cell Cycle Control of Chromosomal Replication (p = 8.1E-14), Polo-like-kinase and HSP90 complex (p = 6.3E-07), G2/M DNA Damage Checkpoint (p = 6.4E-07), Integrin Signaling (p = 3E-05), integrin linked Kinase Signaling (p = 4.5E-05), BRCA1 DNA Damage Response (p = 1.7E-04), estrogen mediated S-phase entry (p = 4.2E-04), Regulation of cell migration (p = 5.6E-04), Actin Cytoskeleton Signaling (p = 7.25E-04),Mismatch Repair (p = 1.6E-03), RAN signaling (p = 1.6E-03), protein Ubiquitination (p = 1.78E-03) and Cholesterol Biosynthesis (p = 8.4E-03). This wide coverage of cellular signaling pathways provides an advantage in developing predictive tests for treatments targeting highly complex cell signaling networks. Surprisingly, even more novel markers were identified, such as non-mutation driven inhibition, the most significant disease state associated with these genes was mammary tumor, demonstrating the validity of this approach to probe breast cancer biology. Conclusion: This analysis demonstrates that the BA325 panel is useful both in understanding non-malignant mammary epithelial differentiation and breast cancer tumors.


Target identification is a critical step in drug discovery, but the process has many challenges including non-specific reagents, limited ability to test numerous molecules against each target inhibition. Pooled screening with CRISPR/Cas9 permits the quick and accurate examination of proliferation effects across many genes and many cell lines. To determine the specific dependencies of cell lines on epigenetic pathways, we designed a CRISPR/Cas9 library to target 640 epigenetic genes and screened more than 200 cell lines covering a variety of oncology indications, including breast, lung, and renal cell carcinoma (RCC). We find that CRISPR pooled screening is a highly effective approach for target identification and provides robust, highly reproducible data as long as a sufficient number of small guide RNAs are used. We identify known pan-essential genes, including in the transcription (CDK9), translation (EIF4A1 and EIF4A3) and splicing (SR5F2) machinery. We additionally identify many novel pan-essential genes across a variety of epigenetic pathways, including histone acetyl- and deacetylases, chromatin remodeling factors, helicases and others. We also investigated epigenetic synthetic lethal interactions that have been previously reported. For example, it has been reported that the SWI/SNF family displays paralog synthetic lethality for SMARCA2 in the context of SMARCA4 mutations, and for ARID1B in the context of ARID1A mutations. While we do see that some of the same trends hold, the synthetic lethal relationship appears to be more complex, providing a useful framework to examine mRNA levels in addition to mutation type. Most importantly, we identify more than 100 epigenetic genes which show selective sensitivity, i.e. where knockout shows an anti-proliferative effect in only a subset of the cell lines. These are the most promising targets for further drug discovery programs. We have used additionally CRISPR/Cas9 domain based screening to identify the functionally relevant sites for many of these genes. Furthermore, we used a novel expression and mutation data to identify novel synthetic lethal relationships. One gene that displays selective sensitivity is EGLN1, the prolyl hydroxylase for the hypoxia-inducible factor, HIF1α. We find that EGLN1 is required for proliferation only in RCC cell lines which retain wild-type VHL, another component of the hypoxia response pathway, which is frequently lost in RCC. As such, EGLN1 loss is synthetically lethal in the presence of wild-type VHL in RCC cells. Thus this approach not only identifies an enzymatic drug target but also a potential stratification method. Other novel synthetic lethal interactions have also been identified. Our data demonstrates that CRISPR pooled screening is a powerful technique for identification of epigenetic synthetic lethal interactions.
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in HCC but its anti-tumor effect is very limited. To overcome these problems, we performed two forward genetic screens using recently innovated whole-genome pooled CRISPR/Cas library. Methods: Two human liver cancer cell lines, Huh-7 and SNU-398, were lentivirally transduced with GeCKO library and transduced cells were selected with puromycin. First, transduced cells were orthotopically injected into the liver of female nude mice and monitored for tumor formation. To identify potential tumor suppressor genes (TSGs) in the liver, gRNAs enriched in the orthotopic liver tumors were determined by sequencing of gDNA in these tumors. Next, transduced cells were treated with vehicle or sorafenib in vitro for 14 days. To identify potential targets which inhibition show synergistic anti-tumor effect with sorafenib, we determined gRNAs depleted in cells with sorafenib compared to cells treated with vehicle. Results: In the first in vivo screening, we identified 62 genes targeted by 2 or more highly enriched gRNAs in Huh-7 cells-derived tumors and 47 genes in SNU-398 cells-derived tumors. Twenty-nine genes were common including 4 miRNAs. TCGA analysis revealed that HCC patients with mutation or copy number alteration in any of these genes showed worse overall survival and disease free survival compared to HCC patients without them. In addition, gene expression levels of 3 candidate TSGs, BPHL, CTSZ and PLCG2 were significantly negatively correlated with poor survival of HCC patients. In the second in vitro screening, CYP7B1, HSST3, NLRC4, and Mir-1237 were identified as potential drug targets in combination with sorafenib in HCC. Conclusion: Whole-genome pooled CRISPR/Cas library screens discovered potential new hepatocellular carcinoma drivers and drug targets in combination with sorafenib.

**#408 Investigating the biology of atypical teratoid/rhabdoid tumors by whole genome CRISPR/Cas9 screening.** Matthew P. Selby, Martina A. Finetti, Matthew Bashston, Ruth E. Cranston, Yulia Grabovska, Alicia Del-Carpio-Pons, Amanda Smith, Simon Bailey, Steven C. Clifford, Daniel Williamson. Newcastle University, Newcastle upon Tyne, United Kingdom.

Atypical Teratoid/Rhabdoid Tumours are rare, highly aggressive, paediatric tumours with a dismal prognosis. Rhabdoid Tumours (RT) have one key genetic defect (biallelic inactivation of SMARCB1 in > 90% of patients) which, when re-expressed in RT cells, causes differentiation and growth arrest. We used whole genome CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 screening; a technique by which mutations are created systematically in each gene in the genome - to identify novel SMARCBI-dependent therapeutic targets and synthetic lethal relationships in RT. A panel of RT cell lines were transduced with a GeCKO (Genome Scale CRISPR Knock-Out) library which contains 122,441 guide constructs targeting 19,050 genes and 1,864 miRNAs; each cell contained a single CRISPR insert. This panel was further transduced with a SMARCBI expression lentivirus or an empty vector control, cells were collected at 0, 7 and 14 days post infection (300x library coverage, 3.7 x 10^7 reads)/Cas9 screening; a technique by which mutations are created systematically in each gene in the genome - to identify novel SMARCBI-dependent therapeutic targets and synthetic lethal relationships in RT. A panel of RT cell lines were transduced with a GeCKO (Genome Scale CRISPR Knock-Out) library which contains 122,441 guide constructs targeting 19,050 genes and 1,864 miRNAs; each cell contained a single CRISPR insert. This panel was further transduced with a SMARCBI expression lentivirus or an empty vector control, cells were collected at 0, 7 and 14 days post infection (300x library coverage, 3.7 x 10^7 reads)/Cas9 screening; a technique by which mutations are created systematically in each gene in the genome - to identify novel SMARCBI-dependent therapeutic targets and synthetic lethal relationships in RT. A panel of RT cell lines were transduced with a GeCKO (Genome Scale CRISPR Knock-Out) library which contains 122,441 guide constructs targeting 19,050 genes and 1,864 miRNAs; each cell contained a single CRISPR insert. This panel was further transduced with a SMARCBI expression lentivirus or an empty vector control, cells were collected at 0, 7 and 14 days post infection (300x library coverage, 3.7 x 10^7 reads)/Cas9 screening; a technique by which mutations are created systematically in each gene in the genome - to identify novel SMARCBI-dependent therapeutic targets and synthetic lethal relationships in RT. A panel of RT cell lines were transduced with a GeCKO (Genome Scale CRISPR Knock-Out) library which contains 122,441 guide constructs targeting 19,050 genes and 1,864 miRNAs; each cell contained a single CRISPR insert. This panel was further transduced with a SMARCBI expression lentivirus or an empty vector control, cells were collected at 0, 7 and 14 days post infection (300x library coverage, 3.7 x 10^7 reads). CRISPR inserts were sequenced, counted, normalized and analyzed to identify those whose abundance was significantly altered, representing candidate gene targets which promote or inhibit RT cell growth/viability in a SMARCBI-dependent manner. CRISPR hits were further cross-validated with primary patient and functional model genomic data (RNA-sequencing/450k DNA methylation array) to identify several high confidence deleterious events with strong SMARCBI dependence. Hundreds of significantly enriched (both positively and negatively) genes were identified which alter when SMARCBI is present or absent including MYC family targets, cell cycle targets, SHH pathway and the Aurora kinase family. TGFβ was identified as a top upstream SMARCBI/methylation dependent regulator and when SMARCBI re-expression data was overlaid onto the TGFβ canonical pathway showed a convincing enrichment in pathway regulation. Pathway members SMAD2/3 showed a synthetic lethal phenotype and represent a functional SMARCBI-dependent relationship which is also observed in SMARCBI re-expression and methylation model data and is characteristic of primary RT. This phenotype was validated functionally by expression of a dominant negative TGFβR2. Combining RNA-sequencing and 450k DNA methylation microarray data from primary tumour cells and experimental models, coupled with whole-genome functional screening, provides a genome-wide view of key RT tumorigenic genes/pathways and their SMARCBI dependence. These data suggest synthetic lethal phenotypes and enable prioritisation of true SMARCBI-dependent events and therapeutic targets, in this rare yet lethal paediatric tumour.

**#409 Genome engineering to generate models of chromosome arm-level aneuploidies in lung carcinoma.** Alison M. Taylor, Gavin Ha, Julianna Shih, Xiaoyang Zhang, Joshua M. Francis, Matthew Meyerson. Dana-Farber Cancer Institute/Broad Institute, Boston, MA.

Almost 90% of tumors are aneuploid and have arm- or whole-chromosome level copy number changes. Arm-level copy number alterations cluster by tumor type, suggesting that specific arm-level changes are influenced by cell type. Systematic methods of generating copy number changes on a particular chromosome have not been tested, leaving the effects of specific aneuploidies in cancer unclear. The most frequent genomic alteration in lung squamous cell carcinomas (SCCs) is loss of chromosome 3p. This alteration occurs in 90% of lung SCCs and in these tumors, 3p deletions occur in both primary tumors and their lymph node metastases. Deletions of 3p genes show significantly decreased expression. However, the phenotypic effects of 3p loss in tumorigenesis are not known. We used the CRISPR-Cas9 system to generate double-strand breaks (DSBs) and produce partial aneuploidies of arm-level loss. We have targeted guide RNAs adjacent to centromeric satellite-repeat sequences on chromosome arm 3p. At this location, we induced homology-directed repair with a selectable marker to generate an artificial telomere. We have successfully isolated almost 90 clones of immortalized lung epithelial cells with deletion of the 3p arm, with 8 validated by whole genome sequencing. Consistent with patient data, expression of 3p genes is also decreased upon deletion. Phenotypic characterization revealed that cells with chromosome 3p deletion proliferate more slowly than their siblings. 3p deleted cells show increased G1 arrest, but do not undergo increased apoptosis or cell death. We are currently testing 3p loss in combination with other frequent SCC alterations, such as SOX2 and TP63 gain. We have also identified expression changes in trans, outside of chromosome 3p. These studies provide a robust model that will address a gap in our understanding of aneuploidy in cancer by using targeted endonuclease technology to create models of partial aneuploidies. Future work will include investigation of how different chromosomal changes contribute to cancer formation, which will have implications on our understanding of tumorigenesis.

**#410 Identifying ovarian cancer specific cancer targeted drugs using high-throughput drug sensitivity profiles of primary cancer cells.** Astrid Muromagi, Akira Hirasewa, Suleiman Khan, Daniela Ungureanu, Marilinya Arjama, Teijo Pellinen, Samuli Eldfors, Ruita Koivistio-Korander, Arto Leminen, Ralf Bützow, Tero Aittokallio, Olli Kallioniemi. Helsinki Univ., Inst. For Molecular Medicine, Helsinki, Finland; Keio University School of Medicine, Tokyo, Japan; Tampere Univ., Inst. of Biosciences and Medical Technology, Tampere, Finland; Helsinki University Hospital, Helsinki, Finland.

Ovarian cancer (OvCa) is the sixth most common cancer in women and leading cause of death from gynecologic diseases. Poor prognosis in OvCa is due to late diagnosis and acquired resistance to the commonly used platinum-based regimens. A significant setback for OvCa treatment is the lack of reliable biomarkers and effective targeted therapies. In order to discover novel therapeutic opportunities with approved and emerging drugs for OvCa, we have established primary cultures using ascites or tumor tissue samples from chemo-refractory ovarian cancer patients for ex vivo Drug Sensitivity and Resistance Testing (DSRT) and genomic profiling. In addition, we have performed DSRT with 31 established OvCa cell lines. Comparison of the drug sensitivity profiles of ten primary cancer cell cultures and 31 OvCa cell lines revealed previously unanticipated cancer selective drug vulnerabilities. Several drug groups were identified suggesting that the sensitive samples were addicted to the corresponding signaling networks. For example, in a 53-year old chemoresistant low grade serous OvCa patient, genomic and transcriptomic analyses revealed a fusion gene of NRG1-ERBB3 activation loop, a target that was recently reported to involve the NRG1/ERBB3 activation loop in OvCa. We found high expression of ERBB2 and ERBB3 by RNA-seq as well as high levels of phospho-ERBB3, phospho-ERBB2 and phospho-EGFR by immunohistochemistry. In agreement with the molecular mechanism, DSRT analysis identified significant sensitivity of primary cancer cells to EGFR inhibitors, such as erlotinib and to dual EGFR and Her2 inhibitor afatinib. The patient has received combination therapy of gemcitabine and erlotinib during nine months, followed by afatinib monotherapy that has lead to complete remission after six months treatment. We have detected decreased NRG1/ERBB3 and increased ERBB2 expression in 11 ovarian cancer patient cases, highlighting the importance of this signaling pathway in ovarian cancer pathogenesis. Our study reveals that a subset of serous ovarian cancer patients with activating NRG1/ERBB3 signaling loop have clinical benefits from repurposing dual EGFR/Her2 inhibitor afatinib, providing a basis for the clinical use of EGFR and dual EGFR/Her2 tyrosine kinase inhibitors in clinical treatment. In conclusion, DSRT technology together with molecular profiling provides a powerful strategy to identify tumor driver signals and select clinically actionable inhibitors. Hence, this type of systems medicine approach can significantly improve the power of mainly genomics-oriented personalized medicine approaches.
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#411 In vivo pooled shRNA library identifies KPNB1 as a new drug target for epithelial ovarian cancer. Michiko Kodama,1 Takahiro Kodama,1 Kouke Yoshihara,2 Kae Hashimoto,1 Seiji Mabuchi,1 Kenjiro Sawada,1 Tadashi Yamamoto,1 Yoshihara,2 Kae Hashimoto,1 Seiji Mabuchi,1 Kenjiro Sawada,1 Tadashi Yamamoto,1 Objective Epithelial ovarian cancer (EOC) is a most lethal cancer in gynecology, of which cure rate is 30%. To seek its new therapeutic targets, we performed in vivo loss of function screen. Method Human EOC cell line, SKOV3, was transduced with pooled druggable shRNA library containing 42450 shRNAs targeting 7490 genes. Transduced cells were intraperitoneally injected into 12 female nude mice and monitored for PC tumor formation. Genomic DNA of 12 biggest PC tumors were sequenced to identify shRNAs depleted in these tumors. Results Ten potential drug targets were identified, including 2 known oncogenes, ERBB2 and RAF1. Second highest ranked gene, KPNB1, is known as a nuclear transporter. KPNB1 inhibition significantly decreased and overexpression increased in vitro cell proliferation in multiple EOC cell lines. KPNB1 inhibition caused multi-phase cell cycle delay at both G1/S and G2/M transition via elevation of p21 and p27 and induced apoptosis. KPNB1 inhibition significantly decreased in vivo tumor formation through the same mechanism as in vitro, because more cleaved PARP and less Ki67 positive cells were found in KPNB1 knockdown tumors via immunochromating staining. We found a positive correlation between KPNB1 mRNA levels and poor survival of EOC patients, suggesting the oncogenic role of KPNB1 in the epithelial ovarian cancer. Furthermore, immunoprecipitation and mass spectrometric study identified that KPNB1 positively regulated several members of anaphase promoting complex/cyclosome. Lastly, we found that ivermectin, a spectrometric study identified that KPNB1 positively regulated several members suggesting the oncogenic role of KPNB1 in human EOC. Comprehensive mass spectrometry identified that KPNB1 positively regulated several members of anaphase promoting complex/cyclosome. Lastly, we found that ivermectin, a new drug target for EOC. Ivermectin can be a new therapeutics for EOC especially in platinum resistance.

#412 Genetic variation in platinum-sensitive and platinum-resistant high-grade serous epithelial ovarian cancer. Tara Castellano, Leslie H. Clark, Naim Rashid, Victoria Bae-Jump. University of North Carolina at Chapel Hill, Chapel Hill, NC. Background: We examined the association of platinum resistance with genetic mutations in high grade serous ovarian cancer (HSOC) patients undergoing high-throughput genomic tumor sequencing. Methods: Snap-frozen and fresh frozen parallel-embedded tissue samples were collected from HSOC patients enrolled on UNCseq (NCT01457196). UNCseq is an institutional protocol which uses next generation sequencing to detect genetic mutations in a wide array of malignancies. Illumina libraries were prepared separately from tumor and a matched normal sample from each patient. Relevant targets were enriched by a custom designed Agilent SureSelect hybrid capture enrichment library using standard Illumina protocols. Samples were sequenced on Illumina HiSeq machines in a variety of formats. Mutations with a quality score $\geq 20$ were filtered from the data set, and only mutations retained to have a moderate to high impact were retained. Medical record review determined platinum sensitivity or platinum resistance. Tumors were defined as platinum sensitive or resistant if patients were noted to have $\geq 6$ months of disease free interval following completion of induction therapy, respectively. Results: Overall 39 HSOC cases met inclusion criteria; 32 tumors met criteria for platinum sensitive and 7 platinum resistant. 308 mutations were noted in at least one individual across all patients. The top observed mutations in platinum sensitive HSOC were TP53 (41%, n = 13), GUCA2A (19%, n = 6), MLL2 (19%, n = 6) and MTOR (16%, n = 5). The top observed mutations in platinum resistant tumors were TP53 (71%, n = 5), TET1 (43%, n = 3) and MLH3 (43%, n = 3). There was a trend toward more p53 mutations in resistant tumors (71% versus 41%; p = 0.21). There was no difference in the total number of mutations per tumor in platinum sensitive and resistant patients, (3 v. 4, p = 0.56). Conclusions: We did not detect a difference in the number of genetic mutations in HSOC according to platinum sensitivity. Platinum resistant tumors had a trend toward higher frequency of TP53 mutations than platinum sensitive HSOCs. Furthermore, we identified 3 frequently mutated genes in platinum resistant HSOC: TET1, an epigenetic regulator, NF1, a tumor suppressor gene and MLH3, a histone modifier gene. Ongoing tumor sequencing of HSOCs on UNCseq will help to confirm these results.

#413 Neo-epitope detection and immune infiltrate analysis of colorectal cancer samples. Fang Yin Lo,1 Nittin Mandal,2 Timothy Yeatman,3 Kiran Paul,2 Ashwini Patil,2 Steven Anderson,4 Ravi Gupta,1 Anup Madan1. 1Covance Genomics Laboratory, Redmond, WA; 2MedGenome Inc, Foster City, CA; 3Gibbs Cancer Center, Spartanburg, SC; 4Covance Genomics Laboratory, Durham, SC. Colorectal cancer (CRC) is the third most common type of cancer in the United States. Targeted therapies that use monoclonal antibodies (mAbs) to EGFR have been shown to benefit some CRC patients. Until recently, KRAS has been the only predictive biomarker for anti-EGFR therapy for metastatic CRC. However, 40% to 60% of patients with wild-type KRAS do not respond to anti-EGFR therapy. Previously, we have shown that a novel colorectal cancer mono-allelic score which measures MEK pathway functional output independent of tumor genotype. We showed that samples that have RAS activating mutations such as KRAS and BRAF have significant higher RAS scores ($p < 0.001$). Here, we further investigate the potential immune reactivity in these CRC samples, and thereby the potential benefit of immunotherapy, by evaluating the tumor neo-epitope burden, and the immune cell infiltrate of CRC patients in CRC xenografts. In this study, 55 CRC samples were sequenced, 779 unique non-synonymous mutations were detected by exome-seq. These 779 mutations spanned across 263 genes. The majority of these mutations are not shared between samples ($\sim 5%$ of the mutations were shared by more than 2 samples). Several driver gene mutations were identified in this study, including KRAS, TP53, PIK3CA, APC and HER2. HLA prediction based on Exome-seq and RNA-seq data shows that $\sim 86.7\%$ of the alleles predicted to be present in 53 samples were concordant between the two RNA-seq datasets. The predicted alleles based on exome-seq and RNA-seq results have $67-69\%$ concordance. Prediction of neo-epitopes show that HLA-binding neo-epitopes are more frequent than TCR-binding ones, and that most neo-epitopes are private and not shared between samples. A more in-depth analysis of the tumor microenvironment was carried out by evaluating the de novo epithelial and immune components. The immune component was further stratified into 7 different immune cell types using signatures specific to CD8 and CD4 T, T-regulatory, NK, B-cells, Macrophages and Myeloid derived suppressor cells (MDSC). The immune make up of colorectal cancer is dominated by macrophages and MDSCs. Interestingly, both granulocytic $G$- and monocytic $M$-MDSCs are present together, supporting the idea that MDSCs confer an immune suppressive microenvironment in this cancer. Significantly, high MDSC infiltrated tumors showed upregulated expression of pro-tumorigenic insulin-like growth factor pathway genes. Additionally, tumors with lower burden of MDSC showed signature of complement activation suggesting innate cell-mediated anti-tumorigenic mechanisms of tumor control in CRC. These analyses provide potential biomarkers to stratify CRC patients based on their immune reactivity and predict response to cancer immunotherapy drugs.

#414 Identifying selective vulnerabilities in colorectal cancer molecular subtypes using in vivo functional genomic screens. Akira Inoue, Bahar Salimian Rizi, Alessandro Carugo, Sahil Seth, Christopher Bristow, Gianicola Genevese, Andrea Viale, David G. Menter, Scott Kopetz, Giulio F. Draetta. The University of Texas MD Anderson Cancer Center, Houston, TX. Colorectal cancer (CRC) is a leading cause of cancer-related morbidity and mortality with significant genetic and drug responses. Recently, the international Colorectal Cancer Subtyping Consortium identified four robust consensus molecular subtypes of CRC (CMS1-4) using large-scale gene expression data. These findings may enable us to identify molecularly homogenous subsets of CRC patients and accelerate effective drug development strategies. To identify potential therapeutic targets and novel selective vulnerabilities in CRC molecular subtypes, we developed an in vivo loss-of-function genomic screen using CRC patient-derived xenografts (PDxIs) for each molecular subtype. Our PDX-derived CRC models underwent comprehensive integrated molecular characterization of mRNA profiles, DNA mutations, and histochemical profiles upon confirmed serial retransplantation to determine whether characteristics of the subtypes are recapitulated in vivo. Because the original CMS classification algorithm was trained and validated using Affymetrix data, profiling the PDX-derived cell lines using this technology provided the most robust analysis of the CMS subtypes. In vivo pooled shairpin RNA (shRNA) screens rely on specific elimination of individual shRNAs in a cell population and require that the infused tumor cell population is adequately endowed with engraftment capacity to replace implanted inoculum. To improve the transduction efficiency of the PDX models, the frequency of tumor-initiating cells, and the maximum library complexity allowed by each model. To identify targets that represent selective vulnerabilities in specific CRC molecular subtypes, we screened each model in vivo with an shRNA library targeting about 200 genes specifically belonging to U.S. Food and Drug Administration-approved targeted therapies (PDxAme; 10 shRNAs/gene ). We leveraged reduced shRNA activity analysis to evaluate “hits” (or top-scoring genes) emerging from our screening. We further applied ranking-based analytics in combination with integromic approaches (use of computational packages to unravel relationships between -omics) to inform on selective
#415 In vivo shRNA library screening to identify novel targets for head and neck squamous cell carcinoma.

Kumar, Ramya L. Parimi, Liang P. Yang, Jiping Wang, Sahil Seth, Christoph A. Bristow, Alessandro Carugo, Frederick S. Robinson, Giulio F. Draetta, Heath Skinner, Mitchell J. Frederick, Jeffrey N. Myers, The University of Texas MD Anderson Cancer Center, Houston, TX; Baylor College of Medicine, Houston, TX.

Head and neck squamous cell carcinoma (HNSSC) is a diverse group of tumors from the upper aerodigestive tract that are treated primarily with surgery, chemotherapy, and/or radiation. Recent genomic studies have characterized the genomic alterations in HNSSC but they failed to identify novel oncogenic drivers for therapeutic targeting. In order to identify novel targets we have utilized an in vivo shRNA library screening platform in a panel of genomically characterized HNSSC cell lines. The screens were performed alone or in combination with platinum-based chemotherapy or radiation, and the libraries included known druggable targets (e.g., CT2A, GL261), genes, and HNSSC candidate driver genes. Initial results indicate that this in vivo screening is able to identify targets that may not be found through traditional in vitro screening approaches and related to tumor-stromal interactions and metabolism. These genes emphasize the importance of including the tumor microenvironment in studies to understand driver genes and identify novel therapeutic interventions.

#416 Identification of therapeutic combinations in glioblastoma using personalized gene expression networks.

Vasileios Stathias, Michele Forlini, Bryce Allen, Stephan Schürer, Nagi G. Ayad. University of Miami, Miami, FL.

The goal of our study was to identify patient-specific gene expression networks from Glioblastoma Patient-Derived Xenografts (PDxS) and determine novel therapeutic combination compounds using those networks. Glioblastoma is the most common malignant primary adult brain tumor with a standard of care consisting of maximal surgical resection followed by radiotherapy and adjuvant temozolomide (TMZ) chemotherapy. However, despite medical advances in the field, recurrence is almost universal, suggesting the need for more personalized and targeted therapeutic approaches. For this, we obtained transcriptional data from Glioblastoma PDxS and used them to identify their respective differentially expressed genes. Patient-specific gene expression networks were then created and their biological relevance was supplemented by integrating them with TCGA Glioblastoma transcriptional data. In order to identify compound combinations specific for those networks, we used the extensive chemical perturbation signatures from the Food and Drug Administration-approved drugs and to rapidly design successful preclinical and clinical trials in CRC patients.

#417 Defining molecular mechanisms of resistance to glioblastoma immu

Mukul Kumar, Ramya L. Parimi, Liang P. Yang, Jiping Wang, Sahil Seth, Christoph A. Bristow, Alessandro Carugo, Frederick S. Robinson, Giulio F. Draetta, Heath Skinner, Mitchell J. Frederick, Jeffrey N. Myers, The University of Texas MD Anderson Cancer Center, Houston, TX; Baylor College of Medicine, Houston, TX.

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#418 Adaptive resistance to chemotherapy in triple-negative breast cancer revealed by single cell DNA and RNA sequencing.

Charissa Kim, Ruli Gao, Emi Seki, Rachel Brandt, Nicola Crosetto, Nicholas Navin, The University of Texas MD Anderson Cancer Center, Houston, TX; Karolinska Institutet, Stockholm, Sweden.

Head and neck squamous cell carcinoma (HNSCC) is a diverse group of tumors from the upper aerodigestive tract that are treated primarily with surgery, chemotherapy, and/or radiation. Recent genomic studies have characterized the genomic alterations in HNSSC but they failed to identify novel oncogenic drivers for therapeutic targeting. In order to identify novel targets we have utilized an in vivo shRNA library screening platform in a panel of genomically characterized HNSSC cell lines. The screens were performed alone or in combination with platinum-based chemotherapy or radiation, and the libraries included known druggable targets (e.g., CT2A, GL261), genes, and HNSSC candidate driver genes. Initial results indicate that this in vivo screening is able to identify targets that may not be found through traditional in vitro screening approaches and related to tumor-stromal interactions and metabolism. These genes emphasize the importance of including the tumor microenvironment in studies to understand driver genes and identify novel therapeutic interventions.

#419 Integrative analysis of multi-omics tumor profiles identifies pathways associated with resistance to anti-HER2 therapy in early stage breast cancer.

Salendra Singh, Hannah Gilmore, Maya Abu-Khalaf, George Somlo, William Sikov, Lyndsay Harris, Vinay Varadan, Case Western Reserve Univ., Cleveland, OH; Sidney Kimmel Medical College at Thomas Jefferson University, Philadelphia, PA; City of Hope National Med Center, Duarte, CA; Warren Alpert Medical School of Brown University, Providence, RI; National Cancer Institute, Rockville, MD.

Head and neck squamous cell carcinoma (HNSSC) is a diverse group of tumors from the upper aerodigestive tract that are treated primarily with surgery, chemotherapy, and/or radiation. Recent genomic studies have characterized the genomic alterations in HNSSC but they failed to identify novel oncogenic drivers for therapeutic targeting. In order to identify novel targets we have utilized an in vivo shRNA library screening platform in a panel of genomically characterized HNSSC cell lines. The screens were performed alone or in combination with platinum-based chemotherapy or radiation, and the libraries included known druggable targets (e.g., CT2A, GL261), genes, and HNSSC candidate driver genes. Initial results indicate that this in vivo screening is able to identify targets that may not be found through traditional in vitro screening approaches and related to tumor-stromal interactions and metabolism. These genes emphasize the importance of including the tumor microenvironment in studies to understand driver genes and identify novel therapeutic interventions.
Background: HER2 positive breast cancers are heterogeneous at both the clinical and molecular levels, with the HER2-enriched subtype exhibiting increased levels of immune infiltration signatures and the highest rate of pathologic complete response (pCR), while the HER2-Basal subtype is resistant to anti-HER2 therapy (Varadan et al, CCR 2016). Here we aim to characterize the molecular underpinnings of response and their interaction with increased immune infiltration across these HER2-subtypes using integrative analyses of genomic and transcriptomic data from two multicenter trials (DFCI 03-311 and BrUOG 211B). Methods: Fresh tumor core biopsies were taken at baseline and a 2-week time point after a single dose of trastuzumab. 80 HER2+ early breast cancer (EBC) patients were enrolled in the 03-311 trial, and 60 patients in the 211B trial. RNAseq and DNA copy number aberrations (SNP-arrays: 03-311; Whole-exome Sequencing: 211B) were performed using the recently developed InFlo framework (Dimitrova et al, Oncogene 2016). Results: HER2-Basal tumors exhibited lower average copy number for HER2 and were less likely to have high-level amplifications of co-amplics (e.g. 11q13, 20q13). In the 211B and 03-311 trials, respectively, 62% and 63% of somatic mutations persisted after one dose of therapy, while 21% and 19% of mutations were undetectable after one dose of therapy. Tumors harboring amplifications in the 8p11 (FGFR1) genomic locus exhibited higher indices immune signatures associated with macrophages (P = 0.0073) and T-cells (P = 0.0049) in 211B, but this association did not achieve significance in the 03-311 trial. Integrative InFlo-based analysis of tumor gene expression and copy-number profiles in trastuzumab-resistant tumors in the 211B trial revealed significantly higher activity of signaling pathways associated with CD4+ T-cells in the responders (P = 0.008), while higher activity of mTOR pathway was observed in non-responders (P = 0.0014). Conclusions: Changes in mutational profiles over time may either be related to therapy-induced alterations of clonal architecture or the consequence of intra-tumor heterogeneity, thus warranting further exploration. Integrative analysis of gene expression and copy-number profiles reveal signaling pathways associated with response and resistance, enabling the discovery of biomarkers of response to anti-HER2 therapy.

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Genomic Screening for Cancer Dependencies and Drug Response

#421 Comprehensive genomic analysis of metastatic breast cancers reveals ESR1 fusions as a recurrent mechanism of endocrine therapy resistance. Ryan J. Hartmaier,1 Nolan Priedeigkkeit,1 Laurie Gay,1 Michael E. Goldberg,1 James Suh,1 Siraj Ali,2 Jeffery Ross,1 Michaela Tsai,3 Barbara Haley,4 Julio Peguero,5rena D. Callahan,5 irina Sachelarie,5 John Cho,6 Amir Bahreini,7 Shannon L. Helayes,8 Peter C. Lucas,2 Nadia M. Ahmed,2 Adam M. Brufskey,2 Philip J. Stephens,1 Juliann Chmielecki,1 Adrian V. Lee2.

Foundation Medicine, Cambridge, MA; 1University of Pittsburgh Cancer Institute, Pittsburgh, PA; 2Minnesota Oncology, Minneapolis, MN; 3UT Southwestern Medical Center, Dallas, TX; 4Memorial Hermann Hospital, Houston, TX; 5UCLA Medical Center, Santa Monica, CA; 6Carlisle Regional Medical Center, Carlisle, PA; 7New York Cancer Center.

Metastatic breast cancer is often intractable due to its inherent ability to overcome current therapies. Genomic alterations are frequently responsible for therapeutic resistance. To better understand genomic mechanisms of acquired resistance in breast cancer we undertook a detailed characterization of single nucleotide variation (SNV) and structural variation (SV) in paired primary-metastasis matched tumors from 6 breast cancer patients (median time to recurrence 7.3 years). In ER-positive recurrent tumors treated with endocrine therapies, we identified multiple metastatic-acquired variants in ESR1 including a novel constitutively active, ligand-independent ESR1-DAB2 gene fusion. Importantly, this fusion resulted from a breakpoint in intron 4, retaining the DNA-binding domain but eliminating the ligand-binding domain (LBD), concordant to a similar fusion reported previously in a xenograft model. Hybrid capture-based genomic profiling from >7,800 breast cancers identified similar exon/intron 4 fusions in 5 tumors with direct paired-read evidence. Using a novel copy number shift detection strategy, 58 additional tumors showed indirect evidence of a rearrangement at exon 4 based on a novel copy number shift detection strategy. ESR1 fusion and copy number shift positive tumors are strongly enriched in metastatic disease (78%; p<10-4) suggesting their expected involvement in endocrine therapy resistance. Clinical follow up was available for 7 patients. 6/7 tumors were clinically ER-positive and received extensive endocrine therapy with progressive disease. Together, these data indicate that ESR1 fusions involving exon/intron 4 are a recurrent, albeit rare, mechanism of endocrine therapy resistance in breast cancer. The absence of the LBD implies these fusions will not respond to other ErEs targeted therapies. Additional studies are needed to identify appropriate treatment options to overcome this mechanism of resistance.

#422 Lymph node metastasis evolution drives immune evasion and targeted therapy resistance in gastro-esophageal adenocarcinomas (GEAs). Matthew N. Davies,1 Louise J. Barber,2 Georgia Spain,3 Filipa Lopes,4 Katharina von Loga,5 Beatrice Griffiths,1 Andrew Woolston,6 Donat Alparp,7 Marta Gomez,8 Kamil A. Lipinski,9 Kerry Fenwick,10 Zakaria Eltabi,7 Stefano Lise,11 Emese Lidak,9 and Giacomo Morais,9 Liza Lebron,6 Mercia Lopes,9 Ana Saz,10 Caroline Springer,11 Marco Gerlinger12.1 The Institute of Cancer Research, London, United Kingdom; 2The Royal Marsden Hospital, London, United Kingdom; 3Semmelweis University, Budapest, Hungary; 4Cancer Research UK Manchester Institute, Manchester, United Kingdom.

GEAs are aggressive tumors in which several targeted therapy trials have failed. We assessed intratumor heterogeneity (ITH) and its impact on progression and therapy failure by applying an 81-gene NGS panel and SNP array copy number aberration (CNA) analysis to multiple primary tumor (T) regions and lymph node (LN) metastases from 9 GEAs. Analysis of 39 samples found ITH in all cases. 8 chromosomally instable (CIN) GEAs predominantly evolved through CNAs, with 17-76% of the genome affected by heterogeneous CNAs. A microsatellite instable GEA showed parallel evolution and diversified exclusively through point mutations (58% ITH). This demonstrates ongoing genomic instability rather than punctuated evolution and that specific instability mechanisms impact evolutionary trajectories. LN metastases contributed more to ITH (p<0.001) than any anatomic location within T. Further, subclonal aberrations that activate the Mitogen Activated Protein Kinase-pathway (MAPK-pw), including EML4-ALK, ERBB2, KRAS, NRAS, Amplifications (amp) and CNAs were detected in LN metastases from 4/8 CIN GEAs. Subclonal MAPK-activating CNAs were enriched in LN (p=0.019) compared to T regions that only exhibited a single subclonal MET amp. Convergent evolution of LN subclones across several GEAs suggests that selection pressures differ systematically between LN and T ecosystems. To assess the phenotypes established by MAPK-activating amp evolution, we analyzed 135 published primary CIN subtype GEAs. Cytolytic activity (CYT), estimating tumor immune recognition from RNA expression data, correlated with the mutation load in GEAs with EGFR, ERBB2 or MET amp (p=0.04). In contrast, CYT did not correlate with mutation load in GEAs with KRAS or ERBB3 amp (p=0.22. NRAS/ERK2: insufficient
data), indicating that these specific alterations, that also recurrently evolved in LN, may enable immune evasion. Downregulation of TAP and Class I MHC genes (p<0.05) in KRAS or ERBB3 amp GEAs suggested impaired antigen processing and presentation as the mechanisms driving T cell immune evasion. Moreover, ITH of MAPK-activating amp is likely to confer resistance to up-stream MAPK-targeted therapy. We used GE cell lines with various MAPK-activating amp (ERBB2, MET, NRAS) to investigate downstream MAPK-pw inhibition as a novel strategy to broadly target heterogeneous subclones. Growth control was incomplete with ERK- and MEK-inhibitors but the panRAF/SRC inhibitor CCT190969 was effective in all lines, suggesting that it can effectively intercept subclonal heterogeneity in GEAs. In conclusion, ITH with parallel and convergent evolution in 9/9 metastatic GEAs. Distinct selection pressures in LN foster the evolution of subclonal MAPK-activating amp that decrease immunogenicity and drive evolutionary pre-adaptation to future targeted drugs that can be intercepted by panRAF/SRC inhibitors.


Background & Aims: Next-generation sequencing (NGS) that enables the analyses of massively parallel sequences of DNA can advance the understanding of the underlying molecular pathophysiology of cancer. Such recent genomic analyses have revealed a complex mutational landscape for PDACs. The aims of this study were to investigate the genomic profile to predict the chemotherapeutic response in unresectable PDACs. Methods: The total of 80 pathologically confirmed PDACs were enrolled and genomic DNA was extracted and quality control metrics of DNA analytics were measured. The specimens that passed a quality control test underwent targeted deep sequencing using a customized panel (CancerSCAN) enriched in the exons of 83 genes. Results: Clinical prognostic factors associated with survival in PDAC were gender, tumor mass size, stage and chemotherapy response rate (P=0.078, 0.009, 0.052 and <0.001, respectively). Multivariable Cox proportional-hazards analysis revealed chemotherapeutic response rate (P < 0.001, hazard ratio (HR) = 1.908, 95% CI, 1.281 to 2.840) is an independent prognostic factor. There were 56 (71%) and 9 (11%) study patients who underwent gemcitabine based chemotherapy and FOLFIRINOX respectively. Response rate of study patients were as follows: CR = PR 14 (20%), SD (27%) and PD 24 (41%). There were only 9 (16%) and 4 (50%) patients who had CR + PR response gemcitabine based chemotherapy and FOLFIRINOX respectively. Conclusion: Targeted sequencing using EUS-FNA specimens in PDAC showed excellent compatibilities to analyze genomic profiles of PDACs. Furthermore, novel genes associated with survival, metastasis and chemotherapeutic response in PDAC were identified.

#424 Landscape of somatic mutations in drug-resistant acute myeloid leukemia. Samuli Eldjors,1 Mika Kontro,2 Yevhen Akimov,1 Olli Kallioniemi,1 Kimmo Porrka,2 Caroline Heckman1.1Inst. for Molec. Medicine Finland (FIMM), Helsinki, Finland; 2Helsinki University Central Hospital Cancer Center, Helsinki, Finland.

Introduction: Most patients with acute myeloid leukemia (AML) initially respond to cytarabine-anthracycline induction chemotherapy. However, in many patients, the disease recurs in a lethal drug-resistant form. Somatic mutations underlying the pathogenesis of AML have been extensively characterized by sequencing of newly diagnosed AMLs. However, the mutations driving therapy resistance and disease progression at relapse have not been well characterized. In this study, we have exome sequenced a cohort of relapsed and refractory AMLs and compared the landscape of somatic mutations at relapse to diagnosis phase AMLs to identify mutations that contribute to therapy resistance and disease progression. Materials and Methods: We performed exome sequencing of diagnosis phase AMLs (n=70) and relapsed or primary refractory AMLs (n=54). Patients with AML M3 subtype were excluded from the study. Paired diagnosis and relapse samples were available from 27 patients. A skin biopsy was used as the germline control. Nine patients had received an allogeneic hematopoietic stem cell transplant before relapse. Somatic mutations were called using varscan2 and copy number aberrations using copyCat. Since the identification of large insertions from next-generation sequencing data remains challenging using existing algorithms, FLT3 internal tandem duplications (FLT3-ITDs) were identified using a novel custom algorithm optimized for FLT3-ITD detection. Population variants were filtered out to remove donor-derived germline variants in chimeric post-transplant relapse samples. Results: Comparison of somatic mutation frequencies in diagnosis and relapse and refractory samples showed that on average relapsed tumors have a higher number of driver mutations than tumors at diagnosis. WT1, TP53, CBL, IDH1 and PTPN11 were mutated at a higher frequency in relapsed samples than at diagnosis, with 13%, 11%, 11%, 9% and 9% of relapsed or refractory samples and 4%, 6%, 3%, 4% and 7% of diagnosis mutant respectively. Analysis of paired diagnosis-relapse samples showed that in patients with WT1, CBL or PTPN11 mutation at diagnosis the second allele is frequently mutated or lost due to uniparental disomy or loss of heterozygosity at relapse. Conclusion: Overall, there was a higher number of driver mutations than diagnosis phase AMLs indicating that acquisition of additional driver mutations contributes to relapse. AMLs frequently acquire additional mutations in the same genes and pathways that already harbored mutations at diagnosis.

#425 SMRT® Sequencing of full-length androgen receptor isoforms in prostate cancer reveals previously hidden drug resistant variants. Manish Kohli,1 Yeung Ho,2 David W. Hillman,3 Jamie L. Van Etten,2 Christine Hessler,2 Rendong Yang,2 Yingming Li,3 Elizabeth Tseng,2 Ting Hon,2 Tyson A. Clark,2 Liguang Wang,2 Kevin Silverstein,2 Liewei Wang,2 Scott M. Dehm,4 Mayo Clinic, Rochester, MN; 3University of Minnesota, Minneapolis, MN; 4Pacific Biosciences, Menlo Park, CA.

Prostate cancer is the most frequently diagnosed male cancer. For prostate cancer that has progressed to an advanced or metastatic stage, androgen deprivation therapy (ADT) is the standard of care. ADT inhibits activity of the androgen receptor (AR), a master regulator transcription factor in normal and cancerous prostate cells. The major limitation of ADT is the development of castration-resistant prostate cancer (CRPC), which is almost invariably due to transcriptional re-activation of the AR. One mechanism of AR transcriptional re-activation is expression of AR-V7, a truncated, constitutively active AR variant (AR-V) arising from alternative AR pre-mRNA splicing. Noteworthy, AR-V7 is being developed as a predictive biomarker of primary resistance to androgen receptor (AR)-targeted therapies in CRPC. Multiple additional AR-V species are expressed in clinical CRPC, but the extent to which these may be co-expressed with AR-V7 or predict resistance is not known. Here we utilized long read sequencing to identify and quantify AR isoforms expressed in CRPC. To unambiguously characterize all AR isoforms, we prepared Iso-Seq™ libraries via 3' rapid amplification of cDNA ends (RACE) with RNA isolated from prostate cancer cell lines and xenograft tissues using a forward primer anchored in AR exon 1. 3' RACE reactions were subjected to single molecule, real-time (SMRT®) long-read sequencing with a Pacific Biosciences RSII System. Our work identified AR-V9 as a truncated isoform that is frequently co-expressed with AR-V7 in CRPC. Mechanistically, our work re-annotated AR-V7 and AR-V9 mRNAs, showing these two species shared a common 3' terminal exon containing separate splice acceptor sites. Taking into account this new information, novel siRNAs and antibodies which could distinguish between AR-V7 and AR-V9 were designed and used to measure the relative expression of these two AR isoforms in CRPC cells with a view to determining the potential of AR-V9 as a predictive biomarker of primary resistance to AR-targeted therapies.

#426 Targeted genome profiling in patients with advanced hepatocellular carcinoma treated with sorafenib. Wonseok Kang, Kyung Kim, Joon Hyook Lee, Ho Yeong Lim, Woong-Yang Park, Jeeyoong Lee, Yong Ha Paik. Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea.

Background/Objectives: Sorafenib is the only approved targeted agent as first-line treatment for advanced hepatocellular carcinoma (HCC). Unfortunately, many HCC patients are initially not responsive to sorafenib. Due to anatomic limitations and underlying liver dysfunction, genomic studies of HCC patients have not been actively pursued yet compared to other solid tumors; especially, in sorafenib-treated subset. In this study, we have retrospectively identified advanced HCC patients who had archival tumor tissues available for targeted genomic sequencing and who had been treated with sorafenib. Methods: We performed targeted genomic profiling for 381 cancer-related genes from 42 HCC (32 hepatitis B virus-related, 1 alcohol-related, 7 unknown) patients treated with sorafenib at Samsung Medical Center, Seoul, Korea from July 2008 to October 2013. We excluded 2 patients who were lost to follow up for evaluation of treatment response to sorafenib. Results: Of the 40 patients, there were 5 confirmed PRs, 7 SDs, and 28 PDs. In 40 patients, there were 6 CDKN2A amplifications, 4 NTRK1 amplifications, 2 MET amplifications, 2 FGFR1 amplifications, 2 CCND1 amplifications, 1 EGFR amplification, 1 FGFR2 amplification and 1 CCNE1 amplification. In cell cycle-related genes, those patients with aberrations were less likely to respond to sorafenib. Of note, two patients whose tumor had CCND1 amplifications had de novo resistance to sorafenib. Conclu-
sions: Our study demonstrates inter-patient heterogeneity in advanced HCC patients and the role of cell cycle-associated genes in resistance mechanism to sorafenib is being investigated.

#427 A path towards determining tumor mutation burden and identifying neoantigens using next-generation sequencing (NGS). Alex So, Shannon Kaplan, Nai-yu Wang, Shile Zhang, Aaron Wise, Kristina Kruglyak, Karen Gutekunst, Illumina, San Diego, CA.

Introduction: The recent clinical demonstrations that cancer immunotherapy is effective for some patients, but not others has led to widespread interest in identifying cancer genetic factors that can predict positive response. Importantly, it has been shown by various studies that tumor mutation burden (TMB) and predicted neoantigen load correlate with patient response to checkpoint inhibitors. This project aims to develop a clinical next-generation sequencing (NGS) assay that utilizes TMB and neoantigen information to guide cancer immunotherapy selection. Experimental Procedures: Whole exome sequencing (WES) and whole transcriptome sequencing (WTS) libraries were generated using Illumina’s TruSeq® Exome and RNA Access library preparation kits, respectively. The samples were pair-end sequenced (2x75bp) using HiSeq® 2000 and 2500 instruments. Data Summary: Here, we demonstrate a workflow using tumor/normal WES and tumor-only WTS to determine expressed TMB. Using the WES data, we were also able to accurately identify human leukocyte antigen (HLA) major histocompatibility complex (MHC) Class I genes at four-digit resolution. We then identified putative neoantigens, defined as peptides with mutated amino acids, that were expressed in the tumor samples and predicted to bind to the respective HLA sequences. In addition, as a path towards developing a clinical assay for TMb and neoantigen determination, we tested the ability of reagents developed for Illumina’s TruSight® Tumor 170 panel to be used for an exome panel. With workflow modifications, we achieved comparable quality of sequencing metrics as compared to the TruSeq® Exome kit. Conclusion: Our data demonstrate a path towards developing a clinical assay that can be used to assess TMB and neoantigen candidates.


The identification of neoantigens has become a critical step in the development of neoantigen-based personalized cancer vaccines and other immunotherapy applications. Since neoantigens can be generated from tumor specific mutations in any expressed gene, the first step in identification of neoantigens typically involves deep exome and transcriptome sequencing on the tumor and exome sequencing of the matched normal. As personalized vaccines enter clinical trials with the potential for clinical use, there is a growing need for strong analytical validation of these platforms. To address this we have developed our ACE Exome (~200X) and Transcriptome platforms for neoantigen identification which utilize an augmented exome approach designed to increase sensitivity for neoantigens in low complexity, traditionally hard to sequencing regions. To enable this platform for neoantigen based personalized cancer vaccines, we have performed a validation of both our ACE Exome (tumor and normal) and ACE transcriptome platforms for detecting DNA-based SNVs and Indels, as well as for RNA based small variant and fusion calls. These are variant types are especially important for neoantigen identification. In this abstract we describe the ACE Exome validation. We used 11 cancer cell lines and their matched normals to assess analytical sensitivity and limits of detection (LOD) for small variant (SNV and Indel) detection using our ACE exome and Tumor Normal bioinformatics pipeline. We identified a gold set of variants, 875 SNVs and 19 Indels that were previously validated in these 11 cell lines (COSMIC, CCLE and Sanger Sequencing confirmed variants). These gold set variants were used to calculate our analytical sensitivity (percent of gold variants detected across the 11 cell line pairs using our assay). To determine our LOD, we chose 3 of the 11 cancer cell lines and created 6 dilutions (5%, 10%, 20%, 30%, 50% and 80% tumor purity) with their matched normal. We then determined Positive Predictive Agreement (PPA, percent of pure cell line variants detected in a diluted samples) and False Discovery Rate (FDR, percent of erroneously detected variants in the diluted sample that were not detected in the pure cell lines) metrics for variants across different minor allelic frequencies (MAF) in the diluted samples. Our ACE “Tumor Normal” Exome assay had a high sensitivity of 98% for SNVs and 95% for Indels. The assay also showed robust PPA (sensitivity) of 97% and FDR (specificity) of 2% for SNVs with MAF > 10% and PPA of 87% and FDR of 3% for Indels with MAF > 10%. We demonstrate that the ACE “Tumor Normal” Exome assay is highly accurate for identification of SNVs and Indels in cancer exomes. With high analytical sensitivity, PPA and low FDR we believe this assay provides augmented ability to detect cancer driver and potential neoantigen generating mutations across various tumor types.

#429 Integrated genomic DNA and RNA profiling to predict cancer immunotherapy response. Pan Du, Xiaohong Wang, Zhixin Zhao, Huiqiang Wang, Shidong Jia. Predicine Inc, Hayward, CA.

Immunotherapy response varies widely, making it difficult for physicians to know whether immunotherapy will be effective for a given patient. Recent studies have reported that patients with high PD-L1 gene expression are likely to respond to checkpoint blocking drugs, but there are still many patients whose tumor test for the PD-L1 protein are negative and can respond to the drugs. In addition to the potential link between mismatch repair (MMR) gene mutations and clinical response to anti-PD1 immunotherapy drug, recent findings show that tumor mutation burden and microsatellite instable (MSI) are good indicators of the cancer immunotherapy responses. Using Predicine’s proprietary Gene RADAR (RNA and DNA single molecular digital Readings) technology, here we report the development of PrediSeq-CI (Cancer Immunotherapy) panel for comprehensive genomic profiling of DNA and RNA that are associated with cancer immunotherapy response. The panel has been tested using both tissue biopsy and plasma samples. The development of PrediSeq-CI test has potential to enable precision medicine in cancer immunotherapy. Finally, we also developed a liquid biopsy droplet digital PCR test to measure PD-L1 expression in blood.

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Metabolic Pathways in Cancer

#430 Combination of CB-839 and everolimus is effective in inhibiting growth of endocrine resistant breast cancer in vivo. Yassi Fallah,1 Diane M. Demas,2 Susan Demo,3 Ayesha N. Shahjahan-Haq.4 Georgetown Lombardi Comp. Cancer Ctr., Washington, DC;5 Caliterra Biosciences, South San Francisco, CA.

About 70% of all breast cancers are estrogen receptor alpha positive (ER+). Anti-hormone therapy such as antiestrogens are often used to treat ER+ breast cancer but breast cancer cells can develop resistance to these drugs (endocrine resistance). Unfortunately, ~50% percent of all antiestrogen treated tumors eventually develop endocrine resistance, and therefore, there is an urgent need to find ways to treat this incurable disease. We have shown that endocrine resistant breast cancer cells show an increased dependence on the amino acid glutamine and this process is regulated by MYC activation via the unfolded protein response (UPR). Metabolites of glutamine such as glutamate and proline are significantly elevated in endocrine resistant cells. Cellular changes in glutamine are sensed by the mammalian target of rapamycin (mTOR) complex, mTORC1, which is known to be deregulated in endocrine resistant breast cancer. In this study, we used human antiestrogen (Fulvestrant/Faslodex or Tamoxifen) sensitive and resistant ER+ breast cancer cell lines and xenografts to test the efficacy of CB-839, an anti-glutaminase, and everolimus (Afinitor), a mTORC1 inhibitor. Combination of CB-839 and everolimus, but not each drug alone, inhibited growth of antiestrogen resistant tumors compared to vehicle alone at 4 weeks post-treatment. The combination treatment did not significantly inhibit growth of antiestrogen sensitive tumors compared with its respective vehicle alone treatment. Thus, a combination strategy that targets glutamine dependence and increased mTOR activation may be a novel strategy in treating endocrine resistant breast cancer.

#431 IDH1-mutated gliomas rely on anaplerosis of glutamate and lactate whereas IDH1 wild-type gliomas rely on glycolysis and acetate anaplerosis. Mohammed Khurshed,1 Krissie Lenting,2 Remco J. Molenaar,3 William P. Leenders,4 Cornelis J. van Noorden1.

Academic Medical Center, Amsterdam, Netherlands; 2Radioudum, Amsterdam, Netherlands.

Hotspot mutations in isocitrate dehydrogenase 1 (IDH1MT) initiate low grade glioma (LGG) and secondary glioblastoma and induce neomorphic activity that converts α-ketoglutarate (α-KG) to the oncometabolite D-2-hydroxyglutarate (D-2HG). The IDH1MT enzymatic rewiring that is not fully understood and in vitro and in vivo studies have shown that IDH1MT cancer cells rely on glutaminolysis, providing cells with α-KG via activities of glutaminase (GLS) and glutamate dehydrogenase (GLUD). Here, we first show by in silico analysis of 269 IDH1 wild-type (IDHWT) and 408 IDH1MT gliomas, obtained from the The Cancer Genome Atlas (TCGA)
database, that IDH1WT gliomas have high expression levels of genes encoding for enzymes that are involved in glycolysis and acetate anaplerosis. On the other hand, the tricarboxylic acid (TCA) cycle, rather than glycolytic lactate production, is the predominant metabolic pathway in IDH1MT gliomas and is driven by lactate and glutamate anaplerosis to facilitate production of α-KG, and ultimately D-2-HG. IDH1WT- and IDH1MT-related differences in expression were found in both LG2 and glioblastoma. Furthermore, via in situ enzymatic activity mapping, we show in human gliomas and in xenocraft models that GLUD activity is increased and GLS activity is decreased in IDH1MT glioma, indicating that IDH1MT gliomas depend on glutamolysis, rather than glutaminolysis. We show that transcript levels in our xenocraft models are in good agreement with our in silico analysis of the TCGA database. Finally, we confirmed the glutamate dependency of IDH1MT gliomas by MRS-flux analysis, whereas IDH1WT gliomas show high lactate production. Taken together, we show that IDH1WT gliomas have a typical Warburg phenotype and rely on glutamate anaplerosis whereas IDH1MT gliomas are glutamate and lactate dependent. This metabolic rewiring in IDH1MT glioma, enables targeting of glutaminolysis rather than direct inhibition of IDH1 for therapy. It diminishes the supply of glutamate-derived α-KG and indirectly inhibits the production of D-2-HG and simultaneously worsens the redox status of the glioma cells by inhibiting NAD(P)/H production by GLUD. A candidate drug to inhibit GLUD is epigallocatechin-3-gallate, a component of green tea that is currently receiving high interest as anti-cancer agent.

Isolation of specific metabolic alterations in aggressive triple negative and inflammatory breast cancers provides a compelling avenue for the development of treatments for these diseases. The ability of cancer to utilize diverse metabolic pathways to modulate increased survival and proliferation is well established. Indeed, we have previously described a series of metabolic adaptations in the triple negative inflammatory breast cancer cell line SUM149 and demonstrated a role for the small GTPase RhoC in the metabolic phenotype of the cell line. In this work we seek to describe a survival mechanism for cancer cells that are subjected to hypoxic environments. Hypoxic survival or growth is an important research goal in cancer research. Understanding the mechanisms through which these changes occur and why they are important for continued survival and growth of the cancer cells are ongoing and will be presented.

#432 xCT inhibition disrupts redox homeostasis in CD44v-expressing tumor cells showing glutaminolytic metabolism in head and neck squamous cell carcinoma. Here we show that inhibition of xCT suppressed the consumption of glutamine without the concomitant decrease of NAD(P)/H production by glucose oxidation. These studies suggest that xCT can be a potential target for therapeutic intervention.

#433 Glycogen accumulation in aggressive breast cancers during hypoxic exposure. Morgan Altemus, Joel Yates, ZhiFen Wu, LiWei Bao, Sofia Merajver. University of Michigan, Ann Arbor, MI.

Isolation of specific metabolic alterations in aggressive triple negative and inflammatory breast cancers represents a compelling avenue for the development of treatments for these diseases. The ability of cancer to utilize diverse metabolic pathways to modulate increased survival and proliferation is well established. Indeed, we have previously described a series of metabolic adaptations in the triple negative inflammatory breast cancer cell line SUM149 and demonstrated a role for the small GTPase RhoC in the metabolic phenotype of the cell line. In this work we seek to describe a survival mechanism for cancer cells that are subjected to hypoxic environments. Hypoxic survival or growth is an important research goal in cancer research. Understanding the mechanisms through which these changes occur and why they are important for continued survival and growth of the cancer cells are ongoing and will be presented.

#434 Characterizing the role of serine metabolism in pediatric sarcomas. Sameer Issaq,1 Ria Kidner,1 Jason Rohde,2 Matthew Boxer,2 Lee Helman1. 1National Cancer Institute, Bethesda, MD; 2National Institutes of Health, Rockville, MD.

Pediatric sarcomas represent a diverse group of malignancies with unique molecular and pathological characteristics. In order to improve sarcoma treatment, a better understanding of the alterations associated with specific sarcoma subtypes is critically important. Renewed interest in the altered metabolic properties of cancer cells has led to an exploration of targeting metabolic dependencies as a novel therapeutic strategy. Metabolism of the amino acid serine is frequently altered in cancer, supporting a number of critical biological processes, including protein, lipid, and nucleotide synthesis, and redox balance. The first, rate-limiting step in the serine synthesis pathway is catalyzed by the enzyme 3-phosphoglycerate dehydrogenase (PHGDH), which is overexpressed in several cancers. Previous work has shown that PHGDH loss or inhibition is selectively toxic to cancer cells with high PHGDH expression or increased flux through the serine synthesis pathway. In this study, we have characterized the dependency of pediatric sarcomas on serine metabolism by examining expression of PHGDH in Ewing sarcoma and rhabdomyosarcoma cell lines, and evaluating the effects of PHGDH inhibition on serine deprivation on cellular proliferation and bioenergetic properties. We show that PHGDH is highly expressed in pediatric sarcoma cell lines, and that PHGDH knockdown resulted in decreased proliferation, especially under conditions of serine limitation. Moreover, pharmacological inhibition of PHGDH resulted in a dose-dependent decrease in proliferation and mitochondrial bioenergetic function. Furthermore, individual sarcoma cell lines were differentially sensitive to serine deprivation, indicating that some sarcoma cell lines may depend on extracellular serine in addition to de novo serine synthesis. Our findings suggest that the dependency of pediatric sarcomas on serine metabolism should be further investigated in order to identify vulnerabilities that could be targeted for potential therapeutic benefit.

#435 Regulation of macropinocytosis-dependent cell survival in pancreatic cancer cells. Sung Eun Kim, Man-Tzu Wang, Frank McCormick. UCSF, San Francisco, CA.

Cancer cells utilize multiple scavenging mechanisms to support growth in nutrient-poor, hypoxic environments. Signaling pathways to accommodate these mechanisms may not be required for oncogenesis per se but are crucial for cancer cell survival. These processes, known as non-oncogene addiction, can be conferred by specific oncogenes that reprogram metabolism or by the tumor microenvironment. Understanding how these pathways are regulated can provide novel opportunities for therapeutic intervention. Studies showed that KRAS-mutant cancer cells, including pancreatic ductal adenocarcinoma (PDAC), upregulate macropinocytosis, a caveolin- and clathrin-independent endocytic process, to import extracellular protein to support growth upon nutrient depletion. Similarly, high levels of macropinocytic uptake are observed in PDAC murine models and also in human PDAC specimens. This process serves as a scavenging mechanism for cancer cells to survive and proliferate in nutrient-deprived tumor microenvironments. Thus, uncovering the key players of this pathway and the mechanisms underlying their regulation will be important to find potential therapeutic targets. Here, we used the Informer set of small molecules to screen regulators of survival in culture conditions supplemented with either glutamine or extracellular protein. The Informer set drug library consists of small molecules including FDA-approved drugs and clinical candidates that have high selectivity for their targets and regulate many cellular pathways. We find that survival of cells grown in conditions supplemented with glutamine or exogenous protein is differentially regulated by distinct drug subsets. This suggests that different pathways of nutrient uptake, processing, and utilization are used in cells depending on the nutrient source. Further examination to determine the mechanism of pathway inhibition and applicability may unravel potential uses for therapeutics.
**MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Metabolic Pathways in Cancer**

### #436 Folate cycle represents a new metabolic vulnerability for hepatocellular carcinoma treatment.

Derek Lee, Irene Ming-Jing Xu, David Kung-Chun Chiu, Robin Kit-Ho Lai, Chun-Ming Wong, Carmen Chak-Lui Wong. The University of Hong Kong, Hong Kong.

Hepatocellular carcinoma (HCC), primary liver cancer, ranks the third most lethal cancer globally, and tumor incidence is maintained through the shuttling of a single carbon unit by a folate derivative through the tetrahydrofolate (THF) backbone in the cytoplasmic and mitochondrial compartments, metabolites like NADPH - a major cellular antioxidant for redox balance, s-adenosyl methionine (SAM) - precursor of DNA and histone methylation, and pyrimidine and purine - the building blocks of DNA are produced. We found folate to be indispensable for HCC cell growth. Furthermore, methylene-THF dehydrogenase 1-like (MTHFD1L), a key enzyme facilitating the folate cycle from the mitochondria, was found to be significantly up-regulated in HCC with association to poorer clinical features for patients. Genetic inhibition of MTHFD1L by knockdown and knockout by shRNA and CRISPR-Cas9 systems, respectively, blocked NADPH production. Rapid elevation in oxidative stress induced DNA damage and cell cycle delay; meanwhile, reduced glutathione (GSH) and cell proliferation. Binding of the transfection factor, NRF2, a potent protector of oxidative stress, and MTHFD1L was confirmed by ChIP assay. NRF2 over-expression using the CRISPR-activating system in HCC cells further highlighted the dependent relationship between NRF2 and MTHFD1L. Metabolomics analysis showed that MTHFD1L knockdown caused a disruption to the folate cycle and accumulation of serine. Surprisingly, MTHFD1L knockdown did not reduce the levels of SAM and nucleotides. Knockdown of MTHFD1L in HCC cells significantly inhibited primary liver tumor growth and lung metastasis in orthotopic liver implantation model. Therapeutically, the administration of methotrexate, an anti-folate agent, sensitized HCC cells towards Sorafenib treatment both in vitro and vivo. The folate cycle represents a metabolic vulnerability and attractive therapeutic target for HCC. Inhibition of MTHFD1L disrupts the folate cycle and sensitizes HCC cells towards its convention treatment agent, Sorafenib in various HCC mouse models. Our investigation unravels a metabolic vulnerability in cancer which contributes to better understanding and is beneficial for the development of precise inhibitors specifically targeting associated pathways.

### #437 Stromal support of pancreatic tumor metabolism.

Costas A. Lysioti. University of Michigan, Ann Arbor, MI.

Pancreatic Ductal Adenocarcinoma (PDAC) is an aggressive disease characterized by a prominent desmoplastic stromal reaction and deregulated metabolism. The role of stroma in PDAC biology is complex and has been shown to play critical roles that may differ depending on the biological context. The intense stromal reaction also impacts the vasculature, leading to a highly hypoxic and nutrient poor environment. As such, these tumors must adapt how nutrients are captured and utilized to support their metabolic needs. In this talk, I will describe how stromal-associated pancreatic stellate cells (PSCs) are critical for PDAC metabolism through the secretion of non-essential amino acids (NEAA). Specifically, we uncovered an undescribed role for alanine, which outcompetes glucose and glutamine-derived carbon in PDAC to fuel the tricarboxylic acid (TCA) cycle, and thus NEAA and lipid biosynthesis. This shift in fuel sources decreases the dependence on glucose and serum-derived nutrients, which are limiting in the pancreatic tumor microenvironment. Moreover, we demonstrated that PSC alanine secretion is dependent on PSC autophagy, a process stimulated by the cancer cells. Thus, our results demonstrate a novel metabolic crosstalk between PSCs and cancer cells, with PSC-derived alanine as an alternative carbon source and proton donor to support the metabolic needs of the cancer cells. To further dissect alanine metabolism, we compared PSCs isolated from normal human pancreas, PDAC-adjacent tissue, and pancreatic tumors where diverse fuel sources are utilized to promote growth in an austere tumor microenvironment.

### #438 Cholesterol biosynthesis is a critical metabolic dependency in pancreatic cancer.

Linara Gabitova, Alena Klochkova, Diana Restifo, Aleksandra Mazitova, Edna Cukierman, Tiffany Hartman, Igor Astsaturov. Fox Chase Cancer Center, Philadelphia, PA.

Pancreatic cancer is rapidly rising to become the 2nd leading cause of cancer deaths by 2020 in the USA. The rise in pancreatic cancer incidence is paralleled by the epidemic of obesity, type 2 diabetes, and associated increase in blood cholesterol, although mechanistic explanations for this are currently unknown. We and others have demonstrated that cancers with altered EGFR and KRAS signaling have increased demands for cholesterol and are vulnerable to interference with cholesterol uptake or endogenous cholesterol biosynthesis. We recently identified a metabolic step in the distal cholesterol pathway, mediated by SC4MOL and NSDHL enzymes, as a critical regulator of cell growth in the keratinocyte model of KRAS-induced pancreatic cancer via the lipid mediator 22R-cholesterol. Based on these preliminary data, we set out to test if pancreatic cancer initiation and progression depend on accelerated cholesterol biosynthesis in a genetic mouse model containing a conditional cholesterol pathway enzyme deficiency (conditional knock-out of Nsdhlf1) in the context of pancreatic cancer development in KPC mice (LSL-KrasG12D,Trp53R16H, Pdx1-Cre). Results: Conditional inactivation of NSDHL alone in pancreatic tissues during normal development produced no apparent phenotype. As expected, the NSDHL-deficient KPC mice as well as heterozygous Nsdhlf1+/− littersmates did not survive beyond 8 weeks of age due to rapid development of progressive pancreatic tumors showing complete displacement of pancreatic tissue with adenocarcinoma and high-grade PanIN lesions. Contrastingly, the survival of pancreatic conditional NSDHL-null mice was significantly extended beyond the median survival of 50 days in NSDHL-sufficient age-matched controls. Furthermore, the progression of pancreatic lesions from ADM to PanIN3 was remarkably delayed on NSDHL-null background, with only a proportion of animals developing adenocarcinoma. Evaluation of pancreatic tissues revealed a dramatic reduction of tumor-induced desmoplaxia at all stages of tumor progression. Our findings support the role of cholesterol metabolism in pancreatic cancer progression via regulation of tumor-specific production of stroma-recruiting growth factors. Conclusions: Our studies demonstrated for the first time essential genetic evidence for metabolic dependency of pancreatic cancer on cholesterol metabolism. We identified NSDHL as a critical target in the endogenous pathway of cholesterol biosynthesis, and determined that blockade of NSDHL has dramatic consequences on the reciprocal signaling between the KRAS-transformed pancreatic cancer cells and the stroma.

### #439 Metabolic flux analysis reveals targets to sensitize chemoresistance in acute myeloid leukemia induced by mesenchymal stromal cell-derived exosomes.

Abhinav Achreja,1 Hongyun Zhao,1 Ziwen Zhu,2 Jonathan Gersberg,2 Marina Y. Konopleva,2 Michael Andreeff,2 Deepak Nagrath1.1University of Michigan, Ann Arbor, MI; 2The University of Texas MD Anderson Cancer Center, Houston, TX.

The tumor microenvironment has a pleiotropic role in supporting cancer cell growth, metastasis and drug resistance. Exosomes from mesenchymal stromal cells (MSC) were found to regulate metabolism of acute myeloid leukemia (AML) cells that led to a chemoresistant phenotype. Exosomes carry a host of proteins, nucleotides and metabolites that can induce metabolic reprogramming of recipient cells via direct supply of metabolite cargo or through signaling. We utilized 13C tracer techniques and two metabolic flux analysis techniques to reveal the mechanism of metabolic reprogramming induced by MSC-derived exosomes. First, 1C metabolic flux analysis was used to quantify intracellular fluxes of central carbon, amino acids and fatty acid metabolism in AML cells cultured with and without exosomes in media supplemented with 13C-labeled precursors. Second, we employed a novel technique, exosome-mediated metabolic flux analysis (Exo-MFA), to estimate direct support provided by metabolite supply from exosome cargo by introducing 13C-labeled exosomes. The combined analysis dissected the metabolite supply from exosome cargo from the overall reprogramming of metabolic pathways due to exosomes. Our results revealed key metabolic pathways that could be targeted to inhibit exosome-induced reprogramming to reverse the chemoresistant phenotype.

### #440 The oncogenic activity of a pro-oxidant intracellular milieu is associated with redox dependent activation of NF-kB.


Evidence over the years has highlighted that intracellular redox status plays a critical role in determining cell fate. To date, we have shown that a pro-oxidant milieu, specifically a mild elevation of intracellular superoxide, promotes cancer cell survival and chemoresistance. To that end, we demonstrated in vitro that elevated superoxide, upon silencing of superoxide dismutase (SOD1), resulted in sustained phosphorylation of Bcl-2 at serine 70, thereby stabilizing its anti-apoptotic activity. These findings were corroborated by the abundance of Serum 70 Bcl-2-phosphorylation and low SOD1 expression in lysates of patients with aggressive lymphomas and/or disease with poor prognosis. In addition to the anti-apoptotic protein Bcl-2, the pro-inflammatory transcription factor nuclear factor kappa B (NF-kB)-

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Diaited signaling was also shown to be modulated by intracellular redox milieu. As constitutive activation of NF-κB has been reported in various human tumors, which is often associated with malignant progression and chemotherapeutic resistance, here we set out to investigate whether a pro-oxidant environment, specifically the upregulation of intracellular superoxide, was associated with the induction of NF-κB transcriptional activity. Indeed, cellular redox imbalance demonstrated that elevated intracellular superoxide via the pharmacological inhibition and genetic silencing of SOD1 results in enhanced activation of canonical NF-κB signalling. This is accompanied by increased p65 nuclear localization and transcription of NF-κB regulated genes, which potentially confer survival advantages to tumour cells upon chemotherapeutic treatments.

Epithelial-cell derived tumors exhibit the Warburg effect that is characterized by an increased rate of glycolysis and lactate release, as well as, reduced oxidative metabolism. It is known that these metabolic alterations of cancer cells result in a tumor microenvironment with a lower pH than that of the plasma. However, little is known regarding the physiology and metabolism of cancer cells enduring chronic acidosis. We cultured pancreatic cancer cells in chronic acidosis, i.e. pH 6.9 to 7.0, and observed a shift from glycolytic metabolism to oxidative metabolism that also results in reduced cell growth and increased intracellular ROS levels. We identified that this is due to an increase in glutamine uptake and increased expression of the transaminase enzyme GOT1 that enhances glutamine metabolism. Survival in low pH is reduced upon depletion of GOT1 due to further increased intracellular ROS levels. Thus, GOT1 plays an important role in energy metabolism and ROS balance in chronic acidosis stress. Our studies suggest the therapeutic potential of targeting anaplerotic glutamine metabolism in pancreatic cancer.

Small molecule inhibitors of SHMT1/2 validate serine metabolism as a therapeutic target in cancer. James Abrego, Venugopal Gunda, Enza Vernucci, Surendra Shukla, Ryan J. King, Aneesa Daogupta, Nina Chaika, Pan-kaj Kumar Singh. UNMC Eppley Inst. for Cancer Research, Omaha, NE.

Epithelial-cell derived tumors exhibit the Warburg effect that is characterized by an increased rate of glycolysis and lactate release, as well as, reduced oxidative metabolism. It is known that these metabolic alterations of cancer cells result in a tumor microenvironment with a lower pH than that of the plasma. However, little is known regarding the physiology and metabolism of cancer cells enduring chronic acidosis. We cultured pancreatic cancer cells in chronic acidosis, i.e. pH 6.9 to 7.0, and observed a shift from glycolytic metabolism to oxidative metabolism that also results in reduced cell growth and increased intracellular ROS levels. We identified that this is due to an increase in glutamine uptake and increased expression of the transaminase enzyme GOT1 that enhances glutamine metabolism. Survival in low pH is reduced upon depletion of GOT1 due to further increased intracellular ROS levels. Thus, GOT1 plays an important role in energy metabolism and ROS balance in chronic acidosis stress. Our studies suggest the therapeutic potential of targeting anaplerotic glutamine metabolism in pancreatic cancer.

#441 GOT1 regulates anaplerotic glutamine metabolism under chronic acidosis stress in pancreatic cancer. Jaime Abrego, Venugopal Gunda, Enza Vernucci, Surendra Shukla, Ryan J. King, Aneesa Daogupta, Nina Chaika, Pan-kaj Kumar Singh. UNMC Eppley Inst. for Cancer Research, Omaha, NE.

Epithelial-cell derived tumors exhibit the Warburg effect that is characterized by an increased rate of glycolysis and lactate release, as well as, reduced oxidative metabolism. It is known that these metabolic alterations of cancer cells result in a tumor microenvironment with a lower pH than that of the plasma. However, little is known regarding the physiology and metabolism of cancer cells enduring chronic acidosis. We cultured pancreatic cancer cells in chronic acidosis, i.e. pH 6.9 to 7.0, and observed a shift from glycolytic metabolism to oxidative metabolism that also results in reduced cell growth and increased intracellular ROS levels. We identified that this is due to an increase in glutamine uptake and increased expression of the transaminase enzyme GOT1 that enhances glutamine metabolism. Survival in low pH is reduced upon depletion of GOT1 due to further increased intracellular ROS levels. Thus, GOT1 plays an important role in energy metabolism and ROS balance in chronic acidosis stress. Our studies suggest the therapeutic potential of targeting anaplerotic glutamine metabolism in pancreatic cancer.


Many oncogenes modulate metabolic pathways and altered metabolism is one of the hallmarks of cancer. In order to sustain proliferation, cell growth and adopt to a very specific tumor microenvironment, cancer cells have to undergo metabolic reprogramming. Increased uptake of glucose, consumed in anaerobic manner, allows to maintain essential bioenergetic and biosynthetic pathways. Various reports indicated that many cancers cells are crucially dependent on serine, which could be either imported or synthesized by the serine synthesis pathway (SSP) branched from glycolysis. Serine can be converted to glycine by SHMT1, which fuels cytoplasmic folate cycle and SHMT2, which also carbon for the folate cycle. There is a growing interest in targeting SSP and its key enzymes, which potentially could be exploited for combinatorial therapy to increase the vulnerability that could be exploited for combinatorial therapy to increase the metabolic vulnerability that could be exploited for combinatorial therapy to increase the efficacy of AZD3965.

Monocarboxylate transporters (MCTs) are key mediators of lactate transport that have emerged as promising targets for anti-cancer therapy. The MCT1 inhibitor AZD3965 (AstraZeneca) has shown promising activity in various preclinical models and is currently in phase I/II clinical testing. Understanding the impact of this drug on tumour cell metabolism may unravel dependencies that could be exploited for combination therapy. Here we investigate changes in glucose metabolism induced by AZD3965 treatment using 13C NMR isotope-labeling experiments and mitochondrial complex I inhibitor mitrocomplex. Exposure of Raji human lymphoma cells to AZD3965 in media supplemented with [1-13C]glucose led to a marked reduction in glucose uptake and lactate production in the cellular growth media along with a build-up in intracellular [3-13C]lactate and [1-13C]glucose levels, indicative of blockade of lactate excretion and inhibition of overall glycolytic activity. These effects were concomitant with increased [4-13C]glutamate levels, consistent with re-routing of pyruvate towards mitochondrial metabolism and enhanced flux through oxidative pyruvate dehydrogenase. Further, AZD3965 treatment was paralleled with a significant increase in levels of steady state Krebs cycle-related metabolites (including succinate, fumarate, acetate and NAD+/NADH) and cellular ATP, as revealed by bioluminescent and 13C NMR analyses, indicating improved mitochondrial bioenergetics and cellular re-energization. Co-administration of the mitochondrial complex I inhibitor metformin with the mitochondrial pyruvate carrier inhibitor UK5099 markedly potentiated the anti-proliferative effects of AZD3965 and led to significantly increased cell death, indicating that the observed upregulation in mitochondrial metabolism was necessary to maintain cell survival under MCT1 inhibitor-induced metabolic stress. These data suggest that targeting MCTs represents a viable strategy for cancer therapy.
#445 Efficient tumorigenesis after genetic dissociation of glycolysis and the TCA cycle. Laura E. Jackson,1 Sucheta Kulkarni,2 Huabo Wang,1 Jie Lu,1 Sivakama Bharathi,1 Radha Uppala,1 Mulchand S. Patel,1 Eric S. Goetzman,1 Edward V. Prochownik.1,2 Children’s Hospital of Pittsburgh of UPMC, Pittsburgh, PA; 2State University of New York at Buffalo, Buffalo, NY.

Virtual tzero some form of metabolic re-programming to match anabolic needs with energy supply. The best-known form of this is the Warburg effect by which tumors become more reliant on glycolysis over mitochondrial oxidative phosphorylation, even when their oxygen supply is abundant. Nonetheless, mitochondria retain some residual activity in order to provide both ATP and critical metabolic building blocks and other substrates for processes such as lipid biosynthesis and protein acetylation. The pyruvate dehydrogenase (PDH) complex (PDC) is the main enzymatic link between glycolysis and the TCA cycle and irreversibly converts the end-stage glycolytic product pyruvate into the critical TCA substrate, acetyl-coenzyme A (AcCoA). In a mouse model of the pediatric liver cancer hepatoblastoma (HB), we recently showed PDH to be highly up-regulated, despite reduced mitochondrial function. We postulated that this might represent a response to pyruvate depletion due to upstream shunting of glycolytic intermediates into anabolic pathways. To test this idea, we examined in vivo tumorigenesis in mice bearing a hepatocyte-specific conditional knockout of the pdha1 subunit (KO mice). The survival of these mice was marginally longer than the wild-type (WT) controls, although tumor sizes at time of death were ~20% smaller. KO mice expressed virtually no PDH (and hence no PDC activity) and had high blood lactate levels. This suggested that they succumbed with smaller tumors as a result of severe lactic acidosis that could not be corrected by hyperventilation due to respiratory compromise by the large HBs that restricted lung volumes. This was supported by studies performed in a modified form of this model in which only tumors were PDH deficient whereas the surrounding liver parenchyma was not. These mice now survived longer than did their WT counterparts and their tumors reached comparable sizes. They also had lower lactate levels than the pan-KO mice. Oxygen consumption rates of WT and KO tumors were similar yet lower than those of normal livers. Rates of fatty acid β-oxidation by WT and KO tumors were also lower than those of liver, indicating that both types of tumors preferentially divert fatty acids into membrane synthesis rather than toward tumorigenesis genes at mRNA level in the eFAT. Moreover, the expression of brown adipocyte-specific thermogenic genes such as Ebf2, Prdm16, PGC1α, UCP1, SRC1 and IRF4 were strongly induced at the protein level in eWAT of ZAG-cell implanted mice suggesting that ZAG causes WAT browning. Furthermore, met-1 expression was associated with a decrease in body weight (2.5 g) and with improvements in food consumption and physical activity. Morphological examination of eWAT pads of ZAG-cell implanted mice showed smaller size compared to controls. In addition, morphological examination of eWAT showed cell shrinkage and almost complete depletion of fat stores, suggesting enhanced lipolysis in the WAT. Notably, ZAG induced the expression of beige, mitochondrial, and thermogenesis genes at mRNA level in the eFAT. Moreover, the expression of brown adipocyte-specific thermogenic genes such as Ebf2, Prdm16, PGC1α, UCP1, SRC1 and IRF4 were strongly induced at the protein level in eWAT of ZAG-cell implanted mice suggesting that ZAG causes WAT browning. Furthermore, metabolomic analysis revealed increased O2 consumption and heat production in ZAG-cell implanted mice compared to controls indicating increased total body energy expenditure. ZAG-cell implanted mice also displayed reduced respiratory exchange ratio indicating increased consumption of lipids. Overall, our findings suggest that ZAG functions beyond lipolysis and causes significant energy wasting by causing WAT browning and promoting glucose and lipid catalolism in the beige cells. These findings suggest that ZAG plays a predominant role in the development of cachexia, and thus, may represent a novel therapeutic target to block energy wasting and delay or prevent cachexia in cancer patients.

#446 Differential effects of folate depletion on metabolic reprogramming and oxidative stress in nonmetastatic and metastatic claudin-low breast cancer cells. Xuewen Chen, Ciara H. O’Flanagan, Stephen D. Hurting, University of North Carolina, Chapel Hill, NC.

Folate coenzymes play an important role in biosynthesis and methylation reactions. Folate metabolism is implicated in the development of several cancer types, though mechanisms underlying folate metabolism and cancer development remain unclear. We previously showed that a folate-restricted diet can exert differential effects on metabolic versus nonmetastatic murine claudin-low breast cancer cells in vivo. Specifically, folate restriction increased growth and invasion of orthotopically transplanted M-Wnt (nonmetastatic) tumor cells, but decreased growth and lung metastases of transplanted metM-Wnt cells, a metastatic subtype of M-Wnt cells. The current study set out to explore the underlying mechanism. To examine the effect of long-term folate depletion (LFD) on M-Wnt and metM-Wnt cell metabolism, oxidative stress and autophagic flux in vitro, the two cell lines were grown in standard and folate-depleted media for 14 days. metM-Wnt cells showed higher oxidative stress, as measured by ROS staining and Nrf2 expression, and phosphorylation of the key nutrient sensor, AMPK, compared to M-Wnt cells when grown in standard growth medium. LFD M-Wnt cells showed an increased dependence on glycolysis compared to those cultured in standard medium. Both M-Wnt and metM-Wnt cells displayed a high autophagy level in LFD, measured by LC3B cleavage, and AMPK phosphorylation such as lipid biosynthesis and protein acetylation. The decrease in oxidative stress and loss of redox defense, as measured by cleaved-caspase 3 and Nrf2 expressions. These results suggest that non-metastatic M-Wnt cells undergo metabolic reprogramming, including a shift from oxidative phosphorylation to glycolysis that may fuel cell growth and proliferation. Further, an elevated autophagic flux may mitigate nutrient stress induced by folate depletion, which allows them to withstand LFD and which may contribute to a more invasive primary tumor in response to folate restriction. In contrast, met-Wnt cells are unable to undergo this metabolic adaptation, and display increased oxidative stress and cell death in response to LFD, preventing the development of metastatic lesions in vitro. This study highlights different responses of primary and metastatic breast cancer cells to folate depletion. The results provide additional rationale for targeting folate metabolism as a potential strategy for treating metastatic breast cancer.

#447 ZAG promotes cachexia-associated white adipose tissue browning and energy wastage. Sawssan Elattar, Satyanarayana Ande. Augusta University, Augusta, GA.

Cancer cachexia is a complex condition of tissue wasting that affects up to 80% of cancer patients. To date, there is no effective treatment for cachexia due to the complex nature of this condition. Energy wasting in cachexia is caused by excessive lipid and protein turnover in the body, browning of white adipose tissue (WAT), and futile metabolic cycling such as glucose recycling between the liver and tumor. Therefore, identifying and inhibiting factors that promote WAT browning, and glucose and lipid recycling can reduce energy wasting and ameliorate cachexia in cancer patients. Zinc-2α-glycoprotein (ZAG), a lipid mobilizing factor secreted by multiple cancers, has been shown to promote lipolysis and inhibit lipogenesis in WAT. However, whether ZAG plays any role beyond lipolysis and participates in other energy wasting mechanisms of cachexia such as glucose and lipid recycling, and WAT browning has not been explored. Our initial studies indicate that while ZAG is highly expressed in the heart, kidney, and liver of wild-type mice, the basal expression of ZAG is very low in WAT at both the mRNA and protein levels. To investigate the metabolic functions of ZAG in vivo, ZAG-expressing 293 cells were implanted subcutaneously in athymic nude mice, and analyzed them after 3 and 6 weeks. We detected a 3-fold increase in the circulating plasma levels of ZAG. Importantly, the increase in ZAG levels was associated with a decrease in body weight (2.5 g), with any differences in food consumption and physical activity. Morphological examination of eWAT pads of ZAG-cell implanted mice showed smaller size compared to controls. In addition, histological examination of eWAT showed cell shrinkage and almost complete depletion of fat stores, suggesting enhanced lipolysis in the WAT. Notably, ZAG induced the expression of beige, mitochondrial, and thermogenesis genes at mRNA level in the eFAT. Moreover, the expression of brown adipocyte-specific thermogenic genes such as Ebf2, Prdm16, PGC1α, UCP1, SRC1 and IRF4 were strongly induced at the protein level in eWAT of ZAG-cell implanted mice suggesting that ZAG causes WAT browning. Furthermore, metabolic analysis revealed increased O2 consumption and heat production in ZAG-cell implanted mice compared to controls indicating increased total body energy expenditure. ZAG-cell implanted mice also displayed reduced respiratory exchange ratio indicating increased consumption of lipids. Overall, our findings suggest that ZAG functions beyond lipolysis and causes significant energy wasting by causing WAT browning and promoting glucose and lipid catalolism in the beige cells. These findings suggest that ZAG plays a predominant role in the development of cachexia, and thus, may represent a novel therapeutic target to block energy wasting and delay or prevent cachexia in cancer patients.

#448 Role of fructose in prostate cancer. Daniela Carreño,1 Nestor Corro,1 Marcia Arredondo,1 Carmen Narvarro,1 Verónica Torres,1 Viviana Montecinos,1 Paula Sotomayor,2 Francisco Nualart,3 Julio Cesar Cárdenas,4 Alejandro S. Godoy,1 Pontificia Universidad Católica de Chile, Santiago, Chile; 2Universidad Andres Bello Santiago, Santiago, Chile; 3Universidad de Concepción, Concepción, Chile; 4Universidad de Santiago, Chile.

The elevated level of glucose uptake and metabolism in cancers is the basis for the clinical localization of primary cancers and sites of metastasis by positron emission tomography (PET scanning), based on the enhanced cellular uptake of 2-deoxy-2-[18F]-fluoro-D-glucose (FDG). In prostate cancer (CaP), however, FDG-PET imaging has shown limited clinical applicability. This striking difference suggests that CaP cells utilize hexoses other than glucose, such as fructose, as the principal energy source. The purpose of this study was to determine whether or not fructose is a/the principal source of energy for CaP cells. mRNA and protein expression for the glucose transporter Glut-1 and fructose transporters Glut-2, Glut-5, Glut-7, Glut-9 and Glut-11 was analyzed in benign (PWR-1E, RWPE-1) and malignant (LNCaP, vCaP, LNCaP-C4-2, DU-145, and PC-3) human prostate cell lines using qRT-PCR and western blot, respectively. In addition, Glut(s) protein expression was analyzed on a tissue microarray containing 200 formalin-fixed paraffin-embedded benign and malignant human prostate tissues using immunohistochemistry. Fructose and glucose uptake was measured in vitro in benign and malignant human prostate cell lines using [1-14C]fructose or 2-[1,2-3H]-deoxy-D-[14C]glucose, respectively. Lastly, the effect of fructose or glucose on the levels of ATP, mitochondrial metabolism, and expression of the enzymes hexokinase-2 (HK2), type-C fructokinase (HKH-C), pyruvate kinase M2 (PKM2) and type-A lactate dehydrogenase (LDH-A) was analyzed in benign and malignant human prostate cell lines.
using chemiluminescence, seahorse, and qRT-PCR analyses, respectively. Our results indicated that expression of the fructose transporters, Glut-5 and Glut-9, was increased in CaP cell lines and in human CaP tissues compared to benign cell lines and benign prostate tissues, respectively. Glut-1 expression, however, did not differ between benign and malignant human prostate cells. Transport assays demonstrated that CaP cell lines have higher expression of Glut-1 compared to benign cells. However, glucose uptake was not altered between benign and malignant human prostate cell lines. ATP levels in CaP cells were similar in the presence of fructose or glucose. Fructose, but not glucose, significantly altered mRNA expression of HK2, HKH-C, PKM2, and LDH-A in malignant human prostate cells. Taken together, our results suggest that fructose may represent an alternative energy source and may reprogram hexose metabolism in CaP cells.

**#449 Reduced argininosuccinate synthetase expression in refractory sarcomas: impacts on therapeutic potential and drug resistance.** Eiuke Kobayashi,1 Youngji Kim,1 Daiusuke K Kubota,2 Yoshiyuki Suchara,1 Akira Kawai,1 Shigehisa Kitano.1 National Cancer Center Hospital, Tokyo, Japan;2Juntendo University, Tokyo, Japan.

Introduction: Treating drug-resistant sarcomas remain a major challenge. The present study aimed to identify a novel therapy for drug-resistant sarcomas based on a metabolic error involving argininosuccinate synthetase (ASS1). Methods: We assessed the expression of ASS1 and P-glycoprotein (P-gp) in osteosarcoma (KHOs), doxorubicin (Dox)-resistant osteosarcoma (KHOsD), epithelioid sarcomas (ES-X and VAESB), alveolar soft part sarcoma (ASPS-KY), and each clinical specimen. Each cell was cultured in arginine-containing and arginine-free media. Cell growth was assessed using an XTT assay and flow cytometry. We analyzed the induction of autophagy in arginine-free medium. Moreover, we assessed the expression of P-gp in after suppressing ASS1 in Dox-sensitive cells (MCF-7, KHOs) and in after transfecting the ASS1 into Dox-resistant cells (ES-X, VAESB, ASPS-KY and KHOsD). Results: The expression of ASS1 was reduced in Dox-resistant sarcoma cells. Immunohistochemistry (IHC) and real-time PCR showed that there was interestingly an inverse relationship between the expression of ASS1 and the expression of P-gp. The inhibition of cellular proliferation with G1-arrest was shown to lead to autophagy with arginine deprivation. In addition, the combination of autophagy inhibitor plus arginine deprivation was more effective than arginine deprivation alone. In cells in which the expression of ASS1 was suppressed, the expression of P-gp was upregulated in comparison to negative controls. Discussion: These results indicate that the reduced expression of ASS1 expression in Dox-resistant sarcomas may contribute to drug resistance. ASS1 deficiency is a potential target for novel drug therapies. The combination of arginine deprivation therapy and an autophagy inhibitor may have anti-tumor effects in refractory sarcomas. Significance: As the induction of autophagy by the deprivation arginine may play a pro-survival role in patients with ASS1-deficient sarcomas, the combination of arginine deprivation therapy with autophagy modulators might potentiate anti-tumor effects in patients with drug-resistant sarcomas.

**#450 Aldo-keto reductase family 1 member b1 links glucose metabolism to epithelial-to-mesenchymal transition.** Annemarie Schwab,1 Aarif Siddiqui, Maria Eleni Vazakidou,1 Francesca Napoli,1 Martin Boettcher,2 Bianca Trinchieri1. 1IZKFJunior Research Group, 1 University Hospital Erlangen, Erlangen, Germany; 2Department of Internal Medicine 5, Hematology and Oncology, University Hospital Erlangen, Erlangen, Germany.

Introduction: Treating cancer cachexia is currently defined as a state of ill health, malnutrition and physical wasting with marked white adipose tissue (WAT) and skeletal muscle mass wasting, representing the clinical consequence of a chronic and systemic inflammatory response. Over the last decade, WAT has been recognized as an important endocrine organ, and earning a lot of attention during cancer cachexia development. We investigated the role of microbiota along the cachexia associated cancer. We performed experiments with conventional and Germ Free mice (GF) (n=6 in each group) of 8-10 weeks old C57B1/6, which were subcutaneously injected with LLC cells [4x10⁶ cells in 0.2 mL; Tumor-bearing, (TB) or PBS control (C)]. We performed Immunohistochemistry, RT-qPCR, and Western Blot. We observed that GF Tumor-bearing mice have increased several symptoms of the cachexia compared to conventional TB mice. The WAT mass was decreased 50% in GF Tumor-bearing mice compared to all groups, which indicates a pathway related to lipolysis, as we found increased level of phosphorylated enzymes in GF Tumor-bearing mice. We also observed that GF Tumor bearing mice decreased skeletal muscle mass and gene expression that are related with atrophy were increased in GF Tumor bearing mice. Our data suggested that homeostasis of microbiota may impair the development of the cachexia syndrome.

**#451 Germ free mice accelerate cachexia-associated cancer.** Rodrigo Xavier da Neves,1 Soumen Roy,1 Amiran Dzatsev,1 April Huang,1 Loretta Smith,1 Simone Ditlippannatto,2 Hawes Misty,1 Marília Seaelder,2 Giorgio Frinchieri1. 1NCI, Bethesda, MD; 2ICB, Sao Paulo, Brazil.

The hallmark of cancer cachexia is currently defined as a state of ill health, malnutrition and physical wasting with marked white adipose tissue (WAT) and skeletal muscle mass wasting, representing the clinical consequence of a chronic and systemic inflammatory response. Over the last decade, WAT has been recognized as an important endocrine organ, and earning a lot of attention during cancer cachexia development. We investigated the role of microbiota along the cachexia associated cancer. We performed experiments with conventional and Germ Free mice (GF) (n=6 in each group) of 8-10 weeks old C57B1/6, which were subcutaneously injected with LLC cells [4x10⁶ cells in 0.2 mL; Tumor-bearing, (TB) or PBS control (C)]. We performed Immunohistochemistry, RT-qPCR, and Western Blot. We observed that GF Tumor-bearing mice have increased several symptoms of the cachexia compared to conventional TB mice. The WAT mass was decreased 50% in GF Tumor-bearing mice compared to all groups, which indicates a pathway related to lipolysis, as we found increased level of phosphorylated enzymes in GF Tumor-bearing mice. We also observed that GF Tumor bearing mice decreased skeletal muscle mass and gene expression that are related with atrophy were increased in GF Tumor bearing mice. Our data suggested that homeostasis of microbiota may impair the development of the cachexia syndrome.

**#452 Activation of Akt pathway and autophagy promotes resistance to FASN inhibition in colorectal cancer patient-derived xenograft models.** Yekaterina Y. Zaytseva,1 Piotr G. Rychahou,1 Anh-Thu Le,1 Robert M. Flight,1 Timothy L. Scott,1 Jennifer W. Harris,1 Sally Hodges,1 Brent J. Hallahan,1 Dana L. Napier,1 Jinpeng Liu,1 Chi Wang,1 Manjula Sunkara,1 Andrew Morris,1 Ji Tae Kim,1 Sivakumaran Theru Arumugam,1 Andrew Lane,1 Teresa W. Fan,1 Hunter Moseley,1 Tianyan Gao,1 Eun Y. Lee,1 Heidi L. Weiss,1 Timothy S. Heuer,2 George Kemble,2 B Mark Evers1. 1University of Kentucky, Lexington, KY; 23-V Biosciences, Menlo Park, CA.

Fatty Acid Synthase (FASN), a key enzyme of de novo lipogenesis, is upregulated in many cancers including colorectal cancer (CRC), increased FASN expression is associated with poor prognosis. Potential FASN inhibitors developed by 3-V Biosciences demonstrate anti-tumor activity in vitro and in vivo and a favorable tolerability profile in a Phase I clinical trial in solid tumor patients. However, CRC characteristics associated with responsiveness to FASN inhibition are not fully understood. The purpose of our study was: (i) to determine the effect of FASN inhibition on tumor growth in CRC patient-derived xenografts (PDXs); (ii) to identify potential biomarkers associated with CRC responsiveness to FASN inhibition; and (iii) to explore new combination strategies with FASN inhibitors. METHODS. Tumor growth was assessed in 9 PDXs established in NSG mice using freshly resected specimens. Once the xenografts grew to 100 mm³, mice were randomized into two groups (n = 5) to receive either...
Orlistat is a weight loss medication that reduces weight/size by 66% in the obese mice and 23% in the lean mice ($p < 0.05$). In addition, orlistat decreased expression of FAS, ACC and carnitine palmitoyltransferase 1A, consistent with an inhibitory effect on fatty acid metabolism. De novo FA synthesis is energetically expensive; in most normal cells FASN expression is low and de novo synthesis suppressed in favor of the FA palmitate. De novo FA synthesis is an important source of precursors needed to support tumor growth. Much of the utilization of exogenous FA. In contrast, in cancer cells, de novo FA synthesis is crucial to tumor growth require the TCA cycle for the processing of glucose and amino acids. This is well known for its role in gluconeogenesis. However, PEPCK is well known for its role in gluconeogenesis. This growth advantage was mediated in part through regulating metabolic flux of glucose and glutamine to biosynthetic precursors, such as lipids and nucleic acids. These effects of PEPCK on glucose metabolism and cell proliferation are in part mediated via activation of mTORC1. Currently we are investigating the mechanism(s) responsible for how PEPCK regulates cell signaling via metabolic flux. We are also seeking to identify ways in which we can utilize PEPCK as a therapeutic target.
median FABP5 expression levels as high as that observed in GS 7-10 PCas. IHC confirmed variable expression of both FABP5 and FASN, including “patchwork” PCs with high Gleason pattern (GP) areas overexpressing FABP5 next to low GP areas overexpressing FASN. The net result may be a selective advantage for the high grade cancer if FABP5 allows it to exploit the FA being synthetized by the adjacent low grade focus. In a subset of PCs, FABP5 and FASN expression was highlighted in the most dense region of PC nuclei staining consistent with its proposed role in regulating fatty acid mediated gene expression; the ratio of FABP5 nuclear/cytoplasmic staining was higher in PCs from AA than from EA patients (P < 0.05). Identification of a PCa subtype with high levels of FABP5 overexpression suggests a previously unrecognized mechanism by which some PCs can increase FA supply without de novo synthesis. Such a PCa subtype might be particularly sensitive to dietary interventions and relatively insensitive to FASN inhibitors.

#458 Choline metabolism in cancer cells modifies PD-L1 expression, Balaji Krishnamachary, Marie-France Penet, Yelena Mironchik, Zaver M. Bhujwalla.

Increased expression of choline kinase-α (Chk-α), the enzyme that converts choline to phosphocholine has been observed in most cancer cells including breast (1). This increase of choline kinase results in increased production of phosphocholine that is used in the synthesis of membrane phosphatidylcholine. We investigated the relationship between Chk-α and the programmed death ligand-1 (PD-L1) expression in triple negative MDA-MB-231 human breast cancer cells. Expression of PD-L1 in response to the secretion of interferon gamma (IFNγ) by cytotoxic T cells and its binding to the immune checkpoint PD-1 on T cells in cancer cells by engagement of PD-L1 can be blocked by antagonistic antibodies to PD-L1 system (2). Quantitative real-time PCR analysis (q-RT-PCR) did not reveal any change in PD-L1 mRNA expression between MDA-MB-231 expressing an empty vector control plasmid (231-EV) and MDA-MB-23 stably expressing shRNA against Chk (231-Chk). However, there was a difference in the mean intensity fluorescence (MIF) between 231-EV (325 ± 11.5, n = 2) and 231-Chk (527 ± 18.5, n = 2) in the FACS analysis. Upon induction with IFNγ (100ng/ml) for 24h, there was 1.23 fold increase in PD-L1 mRNA in 231-Chk compared to 231-EV. Interestingly, the MIP for cell surface expression of PD-L1 was 600 ± 21 (n = 2) for 231-EV compared to 872 ± 16 (n = 2) for 231-Chk cells. Values represent mean ± SE. These data suggest that the cell surface increase of PD-L1 in 231-Chk cells could be due to translocation. Increased PD-L1 expression creates an opportunity to deliver PD-L1 targeted nanoparticles carrying therapeutic cargo. Since most conventional treatments result in a decrease of Chk-α and phosphocholine, our data suggest that these treatments may also result in cancer cells escaping immune surveillance through expression of PD-L1. Acknowledgements: This work was supported by NIH R01CA136576 and P50 CA103175. Reference: 1) Glunde K et al, Cancer Res 65, 11034 (2005). 2) Zou W, Chen L, Nat Rev Immunol 8(6), 467-77 (2008).

#459 Targeting MUC1 mediated nucleotide metabolism sensitizes pancreatic cancer to radiation therapy, VENUGOPAL GUNDU, Joshua Soucheek, Jaime Abrego, Gennifer Goode, Enza Vernucci, Surendra K. Shukla, Aneesha DasGupta, Nina V. Chaika, Ryan King, Fang Yu, Tadayoshi Bessho, Chi Lin, Wang Shou, Li Sicong, Pankaj K. Singh. UNMC, Omaha, NE.

Pancreatic cancer has the lowest survival rate of six percent among all the cancers in the US and is projected to be the second leading cause of cancer related deaths in a decade. Radiation therapy provides only marginal increases in the survival rate in pancreatic cancer, due to poor responsiveness of pancreatic tumors. Clinical trials indicate a significant response to radiation only in twenty percent of primary pancreatic tumors. While multiple factors cause resistance to radiation therapy, the biological mechanisms mediating such innate resistance are currently being explored. Overexpression of MUC1 facilitates chemo and radiation resistance in pancreatic cancer. MUC1 overexpression also promotes pancreatic tumor growth through metabolic upregulation. We investigated the in vivo role of MUC1 mediated metabolic alterations in radiation response of pancreatic tumors. Our findings indicate that MUC1 expressing pancreatic tumors survive better upon radiation treatment. Metabolomic analysis through liquid chromatography coupled tandem mass spectrometry approach revealed that MUC1 expressing pancreatic tumors exhibit higher glycolytic and nucleo- tide metabolites upon irradiation. MUC1 expressing tumors also possess relatively higher PPP and nucleotide metabolites. Glycolytic inhibition using bro-mopyruvate revealed that MUC1 induced radiation resistance could be abrogated through inhibition of glucose carbon flux into nucleotide metabolism by BrPA. Treatment with BrPA effectively reduced glycolysis, pentose phosphate pathway and nucleotide levels in irradiated MUC1 expressing cells. Furthermore, a combination of BrPA and radiation reduced tumor growth in MUC1 expressing tumors. Metabolomic analysis showed a decrease in the glycolysis, PPP and nucleotide metabolites in MUC1 expressing tumors upon combination treatment with radiation and BrPA. Hence, our findings demonstrate that glycolytic inhibition could be used to effectively target MUC1 mediated radiation-resistance in pancreatic tumors.

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Metabolic Pathways in Cancer

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: MicroRNA Regulation of Cancer Biology 1

#460 A central microRNA checkpoint for the inflammatory stem cell niche, Xiling Shen. Duke Univ., Durham, NC.

The microRNA miR-34a is a well-known tumor suppressor in various types of cancer. Previous studies largely focused on its role in suppressing oncogenic pathways such as Bcl2 and Myc. This study will demonstrate a previously un- known function of miR-34a—a central regulator of tissue integrity in the inflammatory colon stem cell niche by modulating both proinflammatory response and stem cell behavior. Epigenetic silencing of miR-34a by a long non-coding RNA (IncRNA), Linc34a, subverts this checkpoint to promote cancer stem cell proliferation. In DSS- and bacteria-induced colitis models, miR-34a (1) suppresses differentiation of CD4+ T cells into Th17 cells by directly targeting interleukin-17 (IL17) receptor genes in Th17 cells and the inflammatory niche by directly targeting chemokine CCL2 from epithelial cells, (2) attenuates the effect of IL17 on stem cells by directly targeting IL17 receptors in stem cells, and (4) switches on asymmetric division to curb stem cell proliferation. We further show that a novel lncRNA, Linc34a, is upregulated by Notch signaling during colorectal cancer (CRC) progression and epigenetically silences miR-34a promoter by recruiting Dnmt3 and HDAC1. This process moves the miR-34a checkpoint of the inflammatory niche and contributes to proliferation of stem-like cancer cells. Our finding of miR-34a as an inflammatory checkpoint suggests potential therapeutic strategies to reinforce or restore such miR-34a functions in colitis patients to prevent CRC and in CRC patients to prevent proliferation of cancer stem cells. Furthermore, MRX34, a miR-34a mimic, is the first microRNA mimics to reach phase II clinical trial for cancer therapy, which is ongoing. However, delivery efficiency into the tumor is always a challenge for microRNA mimics. Our discovery of Linc34a, which recruits epigenetic regulator to silence miR-34a, provides a promising therapeutic target, because it is much easier to develop small molecule inhibitors to disrupt IncRNA structure or block its functional interaction with protein than to restore mi- croRNA expression.


Pediatric and adult angiosarcomas are rare and highly aggressive soft tissue sarcomas with an extremely poor prognosis. Due to the rarity of this disease especially in children, the molecular drivers and optimized treatment strategies for patients are lacking, highlighting the need for genetic and in vivo animal models. MicroRNAs are a class of small RNAs that regulate gene expression and are often dysregulated in cancers including sarcomas. Dicer1 is required for microRNA biogenesis and germline Dicer1 mutations result in a cancer predisposition syndrome associated with increased risk of benign and malignant tumors including rhabdomyosarcoma (RMS), a pediatric soft tissue sarcoma resembling developmentally arrested skeletal muscle. Here we show Dicer1 expression is required for tumorigenesis in a mouse model of RMS driven by activation of oncogenic Smoothened by Cre recombinase expressed from the adipose protein 2 (a2p) promoter (a2p-Cre). Unexpectedly, in studying the role of Dicer1 in RMS, we found that Dicer1 deletion with a2p-Cre leads to aggressive angiosarcoma. Angiosarcoma occurs in settings independent of the Smoothened oncogene and other genetically engineered oncogenes or tumor suppressor loss, providing the first in vivo mouse model of biallelic Dicer1 loss alone driving tumorigenesis. Angiosarcomas in a2p-Cre;Dicer11−/− mice histologically and genetically resemble human angiosarcoma and were enriched for microRNA-23 target genes including the oncogene Ccnd1 as well as Adam19, Plau, and Wsb1 that promote invasiveness and metastasis. The a2p-Cre;Dicer11−/− model provides a simple in vivo animal model to study angiosarcoma for novel therapeutics and the molecular mechanisms of cancer initiation, progression, and metastasis. In addition, our results demonstrate Dicer1 and microRNAs play major and opposing roles in sarcomagenesis.
The incidence of prostate cancer (PCa) among African-Americans (AfA) is significantly higher than Caucasian-Americans (CaA) but the genetic basis for this disparity is not known. To address this problem, we analyzed miRNA expression in AfA (n=81) and CaA (n=51) PCa patients. Here, we found that miR-24 was significantly expressed in AfA and CaA PCa tissues and that the expression of miR-24 promoter confirmed that it was highly methylated and downregulated in CaA PCa patients. Utilizing a VAMCS and NDRI patient cohorts, we discovered that miR-24 expression was linked to a racial difference between AfA/CaA PCa patients. Interestingly, miR-24 was restored after treatment of PCa cells with 5Aza-CdR in an AfA cell line (MDA-PCa-2b), while restoration of miR-24 was not observed in CaA cells, DU-145. Ectopic expression of miR-24 showed decreased growth and induced apoptosis, though the effect was less in the CaA cell line compared to the AfA cell line. Finally, we found unique changes in biological pathways and processes associated with miR-24 transfected AfA cells by quantitative PCR-based gene expression pathway analysis. The altered pathways showed that AR, MAPK1, IGFI, and IGFBRP5 were markedly decreased in the AfA derived cell line compared with CaA cells, and there was a reciprocal regulatory relationship of miR-24/target expression in prostate cancer patients. These results demonstrate that miR-24 may be a central regulator of key events that contribute to race-related tumorigenesis and has potential to be a therapeutic agent for PCa treatment.

MicroRNA-7 suppresses RB1 expression leading to chromosomal instability in leukemia cells harboring c-KIT mutation. Kenji Kurata,1 Shunichi Kawamori,1 Junya Takase,1 Katuya Yamamoto,1 Hiroshi Matsuoka,1 Takayuki Akita,1 Hirobou Minami1. 1Kobe University Hospital, Kobe, Japan; 2Osaka Medical College Hospital, Osaka, Japan; 3Japanese Red Cross Osaka Blood Center, Osaka, Japan.

C-Kit mutation D816V is a well-known indicator of poor prognosis in acute myeloid leukemia (AML) harboring t(8;21) chromosomal translocations. However, the mechanism D816V mutation promotes therapeutic resistance is still under investigation. C-Kit V814 mutation is a murine counterpart of the human D816V. We utilized a murine IL-3 dependent cell line Ba/F3 with or without the c-Kit V814 mutation to investigate its downstream signaling pathway and the effect on the expression of bcl-2 family members and critical granulocytic transcription factors. The c-Kit V814 and wild type (WT) c-Kit were retrovirally transduced into Ba/F3 cells. Unexpectedly, Gata1 mRNA was significantly downregulated to one-tenth in V814+/Ba/F3 cells (p < 0.01, contrary to the previous reports that WT c-Kit signaling induces Gata1 expression. When V814+/Ba/F3 cells were treated with several c-Kit signaling pathway inhibitors, miRNA and protein expression levels of Gata1 recovered to normal levels only with MEK 1/2 inhibitor (PD325901) treatment. We then hypothesized that miRNAs might control Gata1 transcription. We used an array to identify differences in miRNA expression between Ba/F3 cells and V814+/Ba/F3 cells, and between V814−/Ba/F3 cells treated with or without PD325901. Only miR-7a-5p and miR-706 were significantly upregulated in V814−/Ba/F3 cells compared with Ba/F3 without the V814 mutation, and downregulated (> 2-fold) in cells treated with PD325901 compared with controls. We focused our further analysis on miR-7 as it is highly conserved in vertebrates. The differences in miR-7 expression were confirmed using qRT-PCR (10.5-fold change, p < 0.01). In silico analysis implicated Rb1 as a candidate miR-7 target that may regulate Gata1 transcription. Luciferase reporter constructs containing murine and human Rb1 miR-7 target sequence exhibited 0.51 and 0.49 times lower luciferase activity than the control, respectively (p < 0.01). To further define the mechanism of the c-Kit mutation that would transform the cells to a more aggressive phenotype, we focused on DNA and chromosomal instability caused by Rb1 deterioration. We exposed WT c-Kit Ba/F3 and V814+/Ba/F3 cells to 4 Gy irradiation and then used immunohistochemistry to analyze the frequency of H2AFX foci in the cells. The percentage of cells with more than 10 H2AX foci was significantly higher in V814+/Ba/F3 cells than WT Ba/F3 cells (72% vs 14%, P < 0.05). Here, we clearly show that a single mutation of c-Kit is sufficient to regulate miR-7 expression leading to Rb1 translational suppression. Since c-Kit mutation requires only a single step to inhibit Rb1, this mechanism is more likely to facilitate oncogenesis by bi-allelic Rb1 chromosomal and/or gene alterations in leukemic cells. Taken together, we identified miR-7 as a suppressor of Rb1 in V814 mutation-positive cells, which might reflect the responsibility of D816V to re-factory feature in AML.

Novel role of xpo1 in regulating MicroRNAs related to pancreatic ductal adenocarcinoma invasion and metastasis. Asfar S. Azmi,1 Yiwei Li,1 Irfana Musgil,1 Amro Aboukameel,1 William Senapedis,2 Erkan Baloglu,1 Yosef Landesman,3 Michael Kaufman,3 Sharon Shacham,2 Philip A. Philip3, Ramzi M. Mohammad1,1 Wayne State Univ., Detroit, MI; 2Karyopharm Therapeutics, Newton, MA.

Objective: There are no known reports on the role of exportin 1 (XPO1; also known as chromosome maintenance region 1 [CRM1]) in microRNA biology. In this study, we for the first time demonstrate that interfering with XPO1 machinery can influence miRNA signaling leading to suppression of pancreatic ductal adenocarcinoma (PDAC) proliferation, invasion and metastasis. Methods: miRNA arrays (LCSiences, Houston, TX) were performed on total RNA samples from PDAC cell lines (HPAC, MiaPaCa-2, AsPC-1 and L3.6ipl) and normal human pancreatic duct epithelial (HPDE) cells. PDAC cells were treated with XPO1 inhibitor (Selinexor) or transfected with control siRNA, XPO1 siRNA (Santa Cruz), miR-control or miR-145 mimic (Applied biosystems) all at a final concentration of 20 nM using DharmaFect Transfection Reagent (Dharmacon, Lafayette, CO). The total RNA and total protein from treated or transfected cells were subjected to real-time PCR or immunoblot analysis in order to measure expression level of miR-145, let-7d, miR-34c, miR-320, miR-205, and miR-145 target or downstream genes including EGFR, MIP, MT-MMP, c-Myc, Sox-2, and PAR-3. The impact of XPO1 inhibitor Selinexor on PDAC growth, proliferation, invasion and migrations was also evaluated using MTT and scratch assay. Results: In this study, we show that PDAC cells have significantly reduced expression of miR-145 when compared to normal pancreatic duct epithelial cells. Similarly, forced expression of miR-145 in PDAC cells inhibited cell proliferation and migration. Conversely, we demonstrated that RNAi of XPO1 by siRNA knockdown or chemical inhibition of XPO1 by selective inhibitor of nuclear export compound (Selinexor) restores miR-145 expression in PDAC cells ultimately leading to inhibition of cell proliferation and migration. In addition, we show that the inhibition of cell proliferation and migration by Selinexor is mediated through the down-regulation of known miR-145 targets including EGFR, MIP1, MT-MMP, c-Myc, Sox-2, and PAR-3. Selinexor also induced the expression of two important tumor suppressive miRNAs, miR-34c and let-7d, leading to the up-regulation of p21WAF1. We also observed the down-regulation of oncomir mir-205. Conclusion: These results are the first to show that targeted inhibition of the nuclear exporter protein XPO1 by RNAi or Selinexor could restore tumor suppressive miRs in PDAC.

Deregulation of miR-193a and its correlation with clinical and pathological behavior of colorectal cancer. Afraa M. Mamoori,1 Rajwai Alhab,1 Farhadul Islam,1 Katherine Lee,1 Robert A. Smith,2 Vinod Gopalan,1 Al- fred Ky Lam1,1 Cancer Molecular Pathology, School of Medicine, Menzies Health Institute Queensland, Gold Coast, Australia; 2Genomics Research Centre, Institute for Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia.

Aim: Deregulation of miR-193a expression was reported to have significant roles in cancer development and progression. This study aimed to investigate the expression pattern and clinicopathological implication of miR-193a-3p in patients with colorectal cancer. Also, the bio-physiological effects of miR-193a in colon cancer cells were examined. Methodology: Fresh frozen tissues from 70 matched colorectal cancers (adenocarcinomas) and adjacent noncancer tissue samples were prospectively collected with no selection bias. Expression level of miR-193a-3p was measured by quantitative real-time polymerase chain reaction (qRT-PCR). Immunohistochemical staining was used to detect the expression level of K-ras protein as a predicted downstream target for miR-193a. We also observed the down-regulation of oncomir miR-205. Results: These results are the first to show that targeted inhibition of the nuclear exporter protein XPO1 by RNAi or Selinexor could restore tumor suppressive miRs in PDAC.
cell proliferation, increased apoptosis and a less accumulation of cells in the G0-G1 phase cell cycle. Conclusion: The current study confirms the downregulation of miR-139-3a in colorectal cancer and its tumour suppressor effects in vitro plays a key role in the modulation of K-ras regulated molecular signalling pathways in colon cancers.

#468 Evaluation of microRNA inhibition in medulloblastoma. Alexander Vavra,1 Sydney Stoops,2 Jonathan White2.1 Kansas City University, Kansas City, MO; 2MRIGlobal, Kansas City, MO.

Medulloblastoma is the most common pediatric malignant brain tumor. The current treatment plan consists of a combination of surgery, radiation, and chemotherapy which is a five-year survival rate. However, the survival rate does not take into account the developmental delays and learning disabilities inherited as a result of the aggressive treatment plan on a developing brain. As such the development of new treatment options is needed. While many protein targets are currently being explored for therapeutic intervention, the role of non-coding RNAs is of increasing interest. Specifically microRNA profiling in medulloblastoma has been performed by others to highlight expression profiles which can be mapped to the disease state and also to potentially identify associated protein targets. Moreover, it is particularly intriguing that microRNAs themselves may be directly targeted as therapeutic points of intervention. With this in mind, our lab is currently investigating methods for directly targeting microRNAs in medulloblastoma and we have recently selected several upregulated miRNAs for further investigation. Specifically, we evaluated the expression profile eight-teen selected miRNAs in DAOY and D341 cell lines. Our results indicate that four miRNAs (miR-196a, miR-183, miR-96, and miR-182) are statistically overexpressed in both cell lines. Results of these findings and our initial efforts to target these selected miRNAs will be described.


Background: Colon cancer is one of the most common cancers with increasing incidence and high mortality worldwide. Prognosis and choice of treatment is largely based on the tumor stage at presentation. Thus, finding novel biomarkers for predicting survival is highly desirable. Lately, several studies have been looking at microRNAs (miRNAs) in several cancers, including colon cancer. MicroRNAs are conserved, non-coding RNA molecules that play an important role in the regulation of post-transcriptional gene expression. Material and Methods: In the present study, we have profiled miRNA in one hundred and seventy two TNM stage I-IV colon cancer patients and 10 corresponding normal colon tissue samples. Total RNA was extracted from freshly frozen tissues, and the expression of miRNA profile were assessed using Pick and Mix focus panels from Exiqon containing 84 miRNAs that have been linked to cancer. Results: The results were visualized in a heatmap (Qucoore omics Software) and more than 20 miRNAs were found to be differentially expressed in tumors compared to the normal colon. Further, twelve miRNAs were found to discriminate between relapse and no-relapse patients in TNM stage II and III. Additionally, we evaluated expression of four microrna (miR-23a, miR-25, miR-30d and miR-31) were found to be statistically significant in binary logistic regression with relapse as outcome variable. In univariate analysis, low expression of the four-miRNA signature was associated with better 3-year disease-free survival (DFS), 88 % versus 63% in low versus high expression, respectively (P=0.001). Moreover, the signature was a predictor of both relapse-free survival in multivariate analyses (P=0.001; HR 31; 95% CI: 3.8-248.9). Another regression analyses method (LASSO) identified a 16-miRNA signature, and the four miRNAs found earlier were among them. The 16-miRNA signature was associated with better survival (P<0.001). Conclusion: The present study has identified a four-miRNA signature predicting relapse in colon cancer stage II and III patients.

#470 Epstein-Barr virus-encoded miR-BART5 modulates PIAS3-pSTAT3 and p21waf1 in gastric carcinoma cells. Dong Ha Kim,1 Chan Jin Yoon,1 Jin-Seoub Kim,1 Sunyoung Park,2 Euno Choi,2 Jun Hee Woo,3 Mee Soo Chang2.1 Asan Institute for Life Sciences, University of Ulsan College of Medicine, Seoul, Republic of Korea; 2Seoul National University College of Medicine Boramae Hospital, Seoul, Republic of Korea; 3Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea.

The ubiquitious Epstein-Barr virus (EBV) is associated with a subset of gastric carcinomas. MicroRNAs (miRs) are 22-2 nucleotide non-coding RNAs, and regulate various functions in cells. EBV-encoded miRs in gastric carcinoma cells have been identified, but the targets and roles of EBV-encoded miRs remain elusive. In the present study, we investigated cellular targets of miR-BART5, and its possibility as an oncomir. In naturally EBV-infected gastric carcinoma cells and EBV-positive gastric carcinoma tissues, the comprehensive EBV-miR profile revealed the expression of 22 EBV miRs (each having -3p and -5p) composed of miR-BART cluster 1 and cluster 2, without miR-BHRFS. Using bioinformatics analysis, we focused on miR-
BART5 which shared seed sequence homology with hsa-miR-18a and miR-18b having oncorm function, and protein inhibitor of activated STAT3 (PIAS3) mRNA. The western blot results confirmed that PIAS3 protein expression was reduced in miR-BART5-expressing gastric carcinoma cells (EBV-negative gastric carcinoma cells transfected with miR-BART5), compared with mock cells. Also, PIAS3 expression was significantly lower in naturally EBV-infected gastric carcinoma cells than in EBV-negative gastric carcinoma or EBV-infected lymphoma cells. Moreover, there was a statistically significant inverse correlation between the expression of miR-BART5 and PIAS3. The luciferase reporter activity in cells diminished after co-transfection of pEZX-MT06 vector/PIAS3 3′UTR and miR-BART5, compared with co-transfection of empty vector and miR-control or co-transfection of empty vector and miR-BART5. When we tested twenty proteins as downstream candidates of miR-BART5, pSTAT3 increased and p21Waf1 decreased in miR-BART5-expressing gastric carcinoma cells, compared with mock cells, and nuclear translocation of pSTAT3 was observed in miR-BART5-expressing gastric carcinoma cells. In a reverse context that naturally EBV-infected gastric carcinoma cells were transfected with anti-miR-BART5, protein levels were changed likewise; PIAS3 increased, pSTAT3 decreased and p21Waf1 increased. However, there were no statistically significant differences in cellular biologic properties such as proliferation, apoptosis, invasion and migration between miR-BART5-expressing gastric carcinoma cells and mock cells, or between naturally EBV-infected gastric carcinoma cells transfected with anti-miR-BART5 and mock cells. Taken together, miR-BART5 not only targets directly PIAS3 and then modulates PIAS3-pSTAT3 axis, but also decreases p21Waf1 protein, although it fails to alter cellular proliferation in gastric carcinoma cells. This suggests that a single viral microRNA is not sufficient to implement oncogenic function, despite directly targeting cellular protein involved in oncogenesis. Instead, a whole cluster like miR-BART cluster 1 or cluster 2 may be advocate to control cellular biologic property.

#471 HER2 regulates PARP-1 expression by suppressing the let-7a microRNA in HER2+ breast cancer. Eddy Shih-Hsin Yang, Monicka Wielgos, Rajani Rajbhandari, Shi Wei, Susan Nozell. Univ. of Alabama at Birmingham, Birmingham, AL.

Background: HER2+ breast cancers are sensitive to PARP inhibition and express elevated levels of the PARP-1 protein. However, the mode of regulation of PARP-1 expression by HER2 is not well understood. MicroRNAs are small non-coding RNA that function in RNA silencing and post-transcriptional regulation of gene expression. In this study, we investigate whether PARP1 expression in human breast cancer cells is regulated by HER2通过对microRNAs的调控。

Methods: Human HER2+ breast cancer cell lines BT-474 and SKBR3 were used in this study. MDA-MB-231 (non-HER2 expressing breast cancer cells) stably transfected with a HER2 wild-type plasmid (231 HER2) or the vector control (231 NEO) were also utilized. To identify candidate microRNAs regulated by HER2 overexpression, we performed the nCounter miRNA Expression Assay. MicroRNA and mRNA expression were validated via qRT-PCR analysis in breast cancer cell lines or patient primary tumors. Cells were also transfected with a HER2 siRNA, a let-7a mimic, and an inhibitor of let-7a as well as their respective controls. Western blot analysis and firefly luciferase assays were used to determine whether the 3′UTR of PARP1 was being directly targeted by the let-7a microRNA. Results: HER2 did not regulate PARP-1 at the mRNA level but increased PARP-1 protein in HER2+ breast cancer cells. Specifically, ectopic HER2 overexpression correlated with increased PARP-1 protein levels in the 231 HER2 cell line. Conversely, silencing HER2 reduced PARP-1 protein levels in the BT-474 and SKBR3 cell lines. NanoString nCounter analysis revealed that the HER2+ breast cancer cell lines expressed low levels of the let-7a microRNA. Further, let-7a expression was upregulated after HER2 knockdown in the two native HER2+ breast cancer cell lines. The let-7a mimic also reduced both PARP1 protein expression and luciferase activity in the 231 HER2 and BT-474 cell lines. Conversely, silencing HER2 reduced PARP-1 protein levels in the BT-474 and SKBR3 cell lines. NanoString nCounter analysis revealed that the HER2+ breast cancer cell lines expressed low levels of the let-7a microRNA. Importantly, human HER2+ breast tumors expressed higher levels of PARP-1 and lower levels of let-7a, whereas the HER2- breast tumors expressed lower levels of PARP-1 and higher levels of let-7a. Further, overexpression of the let-7a mimic reduced cell proliferation in HER2+ breast cancer cells. Conclusions: These results suggest that let-7a regulates PARP-1 expression in HER2+ breast tumors. Let-7a may also be a potential therapeutic target and predictive biomarker of PARPi sensitivity in HER2+ breast cancer patients.

#472 Role of miRNAome deregulation in the pathogenesis of non-alcoholic steatohepatitis (NASH)-derived hepatocellular carcinoma. Juliana F. Ortega,1 Aline DeConti,1 Kostiantyn Drevel,1 Fernando S. Moreno,1 Frederick A. Beland,1 Igor P. Pogribny1,1NCTR/FDA, Jefferson, AR; 1University of Sao Paulo, Sao Paulo, Brazil.

Animal studies have attributed the growing incidence of liver cancer, including hepatocellular carcinoma (HCC), in the United States to NASH, an advanced form of non-alcoholic fatty liver disease. Elucidating the molecular pathways that lead to the development of NASH-derived HCC is critical not only for identifying early diagnostic biomarkers of the disease, but also for treatment and prevention. We have investigated the role of microRNA expression profiles in the development of NASH-derived HCC by using a Stelic Animal Model (STAM) of liver carcinogenesis. Using Liver miFinder PCR arrays, we examined the miRNA profiles in the livers of STAM mice and identified 25 and 35 miRNAs that were differentially expressed at a NASH-fibrotic stage (12 weeks) and in full-fledged HCC (20 weeks), respectively. Among these differentially expressed miRNAs, 18 miRNAs were common. Multi-algorithm target prediction analysis of the common differentially expressed miRNAs revealed the involvement of these miRNAs in the deregulation of major molecular processes associated with the development of HCC, including epithelial-mesenchymal transition, HCC stem cell activation, and the induction of the β-catenin/Wnt and Hippo signaling pathways. These findings were confirmed by a marked up-regulation of Yes-associated protein (YAP), the main effector of the Hippo pathway, the activation of epithelial-mesenchymal transition, and an increased-expression of hepatic progenitor cell markers. In addition to the common differentially expressed miRNAs, 10 miRNAs, including 5 members of the poly-cistronic oncomir miR-17-92 cluster, were found to be over-expressed in only HCC. These results indicate the fundamental role of miRNAs in the development of NASH-associated HCC. This is evidenced by the early occurrence of miRNA alterations at the preneoplastic stage of liver carcinogenesis, the persistence of these changes in HCC, and the accumulation of additional miRNA alterations in HCC, and by the existence of a mechanistic link between miRNA alterations and deregulation key cancer-related pathways.
**MOLECULAR AND CELLULAR BIOLOGY / GENETICS: MicroRNA Regulation of Cancer Biology 1**

**#474** Estrogen-responsive microRNAs as modulators of E2-induced apoptosis in AI-resistant breast cancer. Reiner Hoppe,1 Ping Fan,2 Stefan Winter,1 Florian Büttner,3 V. Craig Jordan,1 Hiltrud B. Brauch3.1,2 Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, and University of Tuebingen, Tuebingen, Germany;2 MD Anderson Cancer Center, University of Texas, Houston, TX;3Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, and University of Tuebingen, Tuebingen, German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), Heidelberg, Germany.

Long-term estrogen deprivation with tamoxifen or aromatase inhibitors (AI) is the basic principle of endocrine treatment of ER-positive breast cancer. Acquired resistance is however a major obstacle in treatment success. Based on clinical observations and in vitro as well as in vivo experiments it has been suggested that tumor cell clones evolve over time and become vulnerable to E2-induced apoptosis, thereby potentially providing a promising second line treatment option. In vitro, this vulnerability is mimicked in the AI-resistant breast cancer models MCF-7:5C and MCF-7:2A, which during long-term E2-deprivation reconfigure their survival signaling including endoplasmic reticulum, oxidative and inflammatory stress related pathways. E2-stimulation of these cells initiates an unfolded protein response which in turn triggers apoptosis through the intrinsic and subsequently extrinsic pathway (Jordan VC 2015). Recently, we identified microRNA profiles matching the biology of AI resistance and vulnerability to E2-induced apoptosis (Hoppe R et al. 2016). Here we investigate the modulatory role of microRNAs in E2-induced apoptosis through the identification of their global expression profiles in E2-stimulated E2-positive breast cancer MCF-7:5C and 2A models compared to MCF-7:W8/3 reference. Each cell line was treated with 10−9 M E2 or vehicle over a 72h time course (6, 12, 24, 72 h). microRNA profiles were generated using Affymetrix GeneChip miRNA2.0 arrays. At each time point relative microRNA expression changes (E2/control) were evaluated resulting in a total of 72 (5C), 104 (2A) and 94 (W8/3) differentially expressed microRNAs (FC > 1.5 or < 1/1.5, P ≤ 0.05). Differential expression analyses between consecutive time points revealed 16 (5C), 43 (2A) and 27 (W8/3) microRNAs (maximum absolute FC difference > 1.5, P ≤ 0.05). Differential area under the curve (dAUC) analyses at 6-24, 6-24 and 24-72 h mined the overall, early, and late-responding microRNAs of the different phenotypes (Pperm < 0.05). Representative early down-regulated microRNA candidates potentially modulating E2-induced apoptosis in 5C cells are miR-543 and miR-432 of the DLK1-DIO3 locus on Chr. 14q32.31. Late responding up-regulated microRNA candidates are miR-150-5p and miR-149-5p, the low expression of which in tumor tissues registered currently in the process to subject respective microRNA sets to functional enrichment analyses (KEGG, GO) and to correlate E2-responsive microRNA candidates with respective transcriptome signatures. In summary, we will present microRNAs matching the biological processes involved in AI-induced apoptosis. These may serve as potential targets for the amplification of the apoptotic trigger upon E2-treatment.

**#475** Role of microRNA-34a in regulating oncoprotein STN1M. Balahadrapruti V.S.K. Chakravarti,1 Rohit Mehra,2 Rui Wang,2 Darshan Shimoga Chandra Shekar,1 Sai Akshaya Hodigere Balasubramanyam,1 Irfan A. Asangani,2 Robert J. Lonigro,2 Arul M. Chinnaiyan,2 Sooryanarayana Varambally1.1 University of Alabama at Birmingham, Birmingham, AL;2 University of Michigan, Ann Arbor, MI;3 University of Pennsylvania, Philadelphia, PA.

MicroRNAs, as negative regulators of gene expression, play a major role in cellular homeostasis. Downregulation of miRs is common in cancer which results in upregulation of multiple oncogenes in cancer. Here we show that stathmin (STN1M), which is over-expressed and oncogenic in wide variety of cancers, is regulated by microRNA-34a in prostate cancer. Earlier studies suggest that stathmin is involved in vital cellular processes such as cell proliferation, motility and metastasis. In the present study, we show elevated expression of stathmin and its 3′UTR in metastatic prostate cancer cell line LNCaP, and at steady state in MCF10A, MCF7, and MDA-MB-231 cell lines, and expression levels of ATF5 were measured under varying physiological conditions via Western Blot analysis. In vitro migration and invasion assays were performed and transformation was studied using MCF10A mammary epithelial cells with inducible SRC. We demonstrate that miRNA are bound to the 3′ UTR of ATF5 and that miRNAs 433-3p and 520b help to regulate the expression of ATF5 under varying stress conditions and the roles in metastasis. Regulation of ATF5 is critical for the regulation of E2-induced apoptosis and transformation in metastatic melanoma. Regulation of ATF5 expression is elevated and is a survival factor in transformed C6 glioma and MCF7 breast cancer compared to non-transformed cells. Regulation of ATF5 expression is not fully understood. We hypothesized that microRNA (miRNA) play a role in regulating the expression of ATF5 at the 3′ UTR and sought to better understand the role of ATF5 in the transformation to a malignant cell phenotype. To date, no studies have examined the regulation of ATF5 by miRNA. MiRNAs are endogenous small non-coding RNAs 20-25 nucleotides in length that contribute to regulation of gene expression at the translational level. We used in silico modeling programs to identify miRNAs predicted to bind to the 3′ UTR of ATF5. We then aimed to identify the presence of specific miRNA and their ability to down-regulate ATF5 during cellular stress and other physiological conditions in vitro. Luciferase reporter assays and immunoprecipitations of ATF5 3′ UTR mRNA and cell lysate were performed and microRNA quantity analyzed via qPCR analysis. Subsequently, transfections of precursor microRNA were carried out in human MCF10A breast epithelial, MCF7 mammary epithelial, and breast adenocarcinoma MDA-MB-231 cell lines, and expression levels of ATF5 were measured under varying physiological conditions via Western Blot analysis. In vitro migration and invasion assays were performed and transformation was studied using MCF10A mammary epithelial cells with inducible SRC. We demonstrate that miRNA are bound to the 3′ UTR of ATF5 and that miRNAs 433-3p and 520b help to regulate the expression of ATF5 under varying stress conditions and their role in metastasis. These results suggest that miR-23a could be important in transformation, migration, and invasion. Better understanding of the regulation of ATF5 could have implications in a broad range of human malignancies.

**#476** MicroRNA mediated regulation of AT5F contributes to homeostasis and benign to malignant transformation in breast cancer cells. Kari Ann Gaither, Bhanupriya Madarampalli, David X. Liu. Washington State University, Spokane, WA.

The transcription factor AT5F modulates survival, proliferation, differentiation, and hormone responses in breast cancer. Earlier studies suggest that microRNAs (miRNA) play a role in regulating the expression of AT5F at the 3′ UTR and seek to better understand the role of AT5F in the transformation to a malignant cell phenotype. To date, no studies have examined the regulation of AT5F by miRNA. MiRNAs are endogenous small non-coding RNAs 20-25 nucleotides in length that contribute to regulation of gene expression at the translational level. We used in silico modeling programs to identify miRNAs predicted to bind to the 3′ UTR of AT5F. We then aimed to identify the presence of specific miRNA and their ability to down-regulate AT5F during cellular stress and other physiological conditions in vitro. Luciferase reporter assays and immunoprecipitations of AT5F 3′ UTR mRNA and cell lysate were performed and microRNA quantity analyzed via qPCR analysis. Subsequently, transfections of precursor microRNA were carried out in human MCF10A breast epithelial, MCF7 mammary epithelial, and breast adenocarcinoma MDA-MB-231 cell lines, and expression levels of AT5F were measured under varying physiological conditions via Western Blot analysis. In vitro migration and invasion assays were performed and transformation was studied using MCF10A mammary epithelial cells with inducible SRC. We demonstrate that miRNA are bound to the 3′ UTR of AT5F and that miRNAs 433-3p and 520b help to regulate the expression of AT5F under varying stress conditions and their role in metastasis. These results suggest that miR-23a could be important in transformation, migration, and invasion. Better understanding of the regulation of AT5F could have implications in a broad range of human malignancies.

**#477** Down-regulated miR-23a contributes to invasion and metastasis of cutaneous melanoma by promoting autophagy. Weinao Guo, Huina Wang, Yuqi Yang, Sen Guo, Weigang Zhang, Tao Zhao, Lin Liu, Zhe Jian, Ling Liu, Gang Wang, Tianwen Gao, Qiong Shi, Chunying Li. Department of Dermatology, Xijing Hospital, Fourth Military Medical University, Xi’an, China.

Background: The occurrence of invasion and metastasis is the major cause of mortality in melanoma. Recent studies suggest that dysregulated microRNAs play critical roles in this procedure, but the underlying mechanism remains elusive. Here, we show that down-regulated miR-23a can promote invasion-metastasis cascade through autophagy in melanoma. Methods: The role of miR-23a in progression was assessed in a cohort of melanoma patients (n = 192) with Kaplan-Meier analysis. The effects of miR-23a overexpression were investigated using assays of invasion, migration, and in a xenograft model (n = 10 mice per group). Autophagy-related target of miR-23a was confirmed by bioinformatics analysis, luciferase assays and immunoblotting. Molecular studies were performed to determine the downstream cellular and molecular mechanisms. All statistical tests were two-sided. Results: Serum miR-23a level was significantly down-regulated in melanoma patients (P < .001) and was highly correlated with poor clinical outcomes (P = .027, log-rank test). In addition, miR-23a level was marked decreased in metastatic melanoma tissues and cell lines (P < .05, P < .01). Moreover, miR-23a overexpression prevented the invasion and migration in vitro and lung and liver metastasis in vivo (both P < .05), by targeting ATG12 and inhibiting autophagy. Mechanically, miR-23a-ATG12 axis attenuated invasion and migration through autophagy-mediated AMPK-RhoA pathway. Finally, the down-regulation of miR-23a in metastatic melanoma was caused by RUNX2 in a transcriptional repression-manner. Conclusion: MiR-23a can act as a crucial epigenetic repressor of melanoma invasion and metastasis in an autophagy-dependent way, which indicates that miR-23a-mediated autophagy inhibition can be exploited to restrain invasion-metastasis cascade in melanoma treatment.

**#478** Regulation of cross talk between AR and vets/beta-catenin pathways in prostate cancer. Alexis Plaga, Girish C. Shukla. Cleveland State University, Cleveland, OH.
Prostate Cancer (PCa) is the second most commonly diagnosed cancer in the United States with 180,890 males diagnosed and is predicted to cause approximately 26,000 deaths in 2016. The current treatment of androgen deprivation therapy (ADT) initially depletes circulating androgens; however, intratumoral androgens rescue androgen receptor (AR) signaling and promotes the development of castration resistant prostate cancer (CRPC). The dysregulation of the Wnt/β-catenin signaling pathway has been implicated in the development of many cancers including PCa. The disruption of this signaling leads to the stabilization of β-catenin which upregulates many genes involved in tumorigenesis. Additionally, β-catenin acts as AR cofactor. Our previous studies show that AR is a direct target of miR-644a. We hypothesized that targeting the expression of both the β-catenin pathway and AR by conventional drugs and/or tumor suppressor miR-644a would have synergistic therapeutic benefits. In this study, we are investigating miR-644a mediated posttranscriptional downregulation of GSK3-β and β-catenin in the wnt/β-catenin signaling pathway. The effect of miR-644a in combination with inhibitors (β-catenin or GSK3-β) was also assessed using proliferation assays and a significant downregulation was observed. We will further study the posttranscriptional effects of tumor suppressor miR-644a on the wnt/β-catenin pathway and also the processing and regulation of miR-644a in PCa.

#479 Modeling miRNA induced silencing in breast cancer with PARADIGM. Andrew J. Sedgewick,1 Panayiotis V. Benos,2 Shahrooz Rabizadeh,3 Patrick Soon-Shiong,3 1Nantomics LLC, Santa Cruz, CA; 2University of Pittsburgh, Pittsburgh, PA; 3Nantworks, Culver City, CA

Introduction: MicroRNAs play an important role in regulation of gene expression and are known biomarkers for breast cancer as well as other malignancies. PARADIGM is a pathway based algorithm that allows for integration of multiple genomic data types with a curated pathway database to make pathway activity predictions. We added a model of gene silencing due to miRNA to the PARADIGM algorithm in order to study miRNA expression in a pathway context. Results: We curated a set of 7731 miRNA-mRNA interactions from the intersection of 3 target prediction algorithms. These interactions involved 66 miRNA and 2814 mRNA transcripts. We ran this model on global DNA copy number, RNAseq and miRNAseq data from 697 patients in the TCGA breast cancer cohort, and studied changes in the interactions between miRNAs and their targets between different tumor subtypes. The median activity of the RNA-induced silencing complex (RISC) predicted by our model is significantly higher in Basal tumors than other subtypes. In addition, RISC activity is significantly associated with overall survival of patients with Luminal A tumors. The miRNA-target pairs with the largest correlation changes between Basal and Luminal A subtypes were enriched for putative onco- genes and oncomirs. The miRNA targets are involved in a number of important signaling pathways including PI3K-AKT, JAK-STAT, and Ras. Many of these highly differential links involve the miR-16 family of miRNAs which are known tumor suppressors. miR-16 shows significantly lower activity in basal tumors than other subtypes. Conclusions: By looking at changes in miRNA-target links between tumor subtypes, our algorithm was able to identify both miRNAs and target genes involved in pathways relevant to breast cancer. Our predictions of overall RNA-induced silencing activity show prognostic value in both differentiating subtype and predicting overall survival within subtypes.

#480 Novel miRNA regulation in an early progression model of PDA. Nina J. Chu, Todd D. Armstrong, Elizabeth M. Jaffe. The Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD.

The success of immunotherapy is dependent on infiltration and function of T cells within the tumor microenvironment. However, for many cancers, inflammatory and stromal cells provide formidable barriers to T cell access and function. Emerging data suggests that the cellular barriers to T cell access and function are regulated by both genetic and epigenetic factors. Identification of these regulators should provide new targets for enhancing immunotherapy. The overall goal of this research is to evaluate the role of miRNA regulation of immunoinflammatory and stromal cells that develop in the earliest pre-malignant pancreatic intraepithelial neoplasias (PanINs) in the KrasG12D/H, Trp53R172H/H11001/H9252/H9253 mouse model, a spontaneous model of pancreatic ductal adenocarcinomas (PDA) tumorigenesis. Specifically, we aim to investigate the functional roles of key differentially expressed miRNAs in propagating the tumor microenvironment via modulating the signaling between transformed ductal epithelial cells and the recruited cancer associated fibroblasts (CAFs) that comprise the majority of the desmoplastic stroma that characterizes PDA. Although numerous studies have described molecular alterations that are involved in pancreatic cancer development and progression, little is known about the miRNA regulatory profile and the associated inflammatory changes within the tumor microenvironment in the earliest PanIN lesions. We conducted miRNA microarray analysis to determine the levels of 750 unique miRNAs in the pancreata of KPC mice ranging from 4 to 12 weeks of age (pre-PanIN1 to PDA). miRNA was isolated from normal pancreatic ducts, PanIN grades 1 through 3, and PDA by laser capture microdissection. miRNA expression was quantified by Taqman miRNA OpenArrays and confirmed by qPCR analysis. Out of the 750 rodent miRNAs, 4 miRNAs (miR-21, miR-16, miR-19b, and miR-224) were significantly upregulated throughout PDA development. miR-21 and miR-224 are of particular interest for their regulation of targets in cancer promoting inflammatory pathways and epithelial-mesenchymal transition (EMT). To investigate the functional role of miR-224 in the developing tumor environment, primary KPC pancreatic ductal epithelial and fibroblast cell lines were established via fluorescence activated cell sorting (FACS) in order to perform in vitro miRNA knock-in and knock-out studies. Additionally, miRNA fluorescence in situ hybridization (miRNA-FISH) was performed to examine the spatial expression of miR-21 throughout progression of the early microenvironment. miR-21 is expressed at low levels in wildtype pancreata, but is highly expressed particularly in ductal epithelial cells of late stage KPC pancreata. Additional studies are underway to determine the functional role of this miRNA in PDA development and progression.

#481 Diminished microRNA-29b results in overexpression of BRD4 and BRD4-regulated oncogenes in cutaneous T-cell lymphoma. Rebecca Kohlenk,1 Jing Wen,2 Bethany Mundy-Bosse,3 Max Yano,4 Leah Grinspun,4 Kathleen McConnell,3 Ashley Keiter,5 Alex Hartlage,3 James Bradyne,3 Michael Caligiuri,1 Pierluigi Porcu,1 Anjali Mishra1.

Cutaneous T-lymphoma (CTCL) is a CD4+ T-cell malignancy that affects the skin but may disseminate systemically, and there are no long-term effective therapeutic strategies. Here, we characterized the therapeutic efficacy of manipulating the tumor suppressor microRNA-29b (miR-29b) and its target bromodomain-containing protein 4 (BRD4) in CTCL pathogenesis. Using primary CD4+ T-cells we determined that miR-29b expression is significantly decreased in patients compared to healthy controls (0.007 ± 0.002, n = 9 vs 1.008 ± 0.052, n = 6, p < 0.0001). We utilized miR-29b−/− mice and bortezomib, a proteasome inhibitor known to increase miR-29b levels, to confirm the inverse relationship between miR-29b and BRD4 (Mishra, A. et al. Cancer Cell, 2012). Diminished miR-29b level resulted in increased BRD4 protein expression (1.87 ± 0.29, p = 0.014), while increase in miR-29b, as in bortezomib-treated CTCL cell lines, results in nearly undetectable BRD4 protein. We also observed increased genome-wide occupancy of BRD4 at regulatory regions in CTCL patients, an effect that is reversed with BRD4 inhibitor, JQ1, to similar levels as observed in healthy control CD4+ T-cells. Specifically, oncogene loci of NOTCH1 and RB1 demonstrated enhanced BRD4 binding in CTCL patients, with resultant increases in mRNA in patient vs normal donors (4.16 ± 0.08, p = 0.024; 3.02 ± 0.54, p = 0.012). Further, in vivo treatment of interleukin-15 (IL-15) transgenic mice (Mishra, A. et al. Cancer Discovery, 2016) with JQ1 prevented CTCL development and decreased lesion severity (5.37 ± 0.49, n = 8 vs 6.0 ± 0.45, n = 5, p = 0.004). Skin from JQ1-treated mice demonstrated decreased protein expression of BRD4, NOTCH1, and RB1, consistent with our findings in patients and CTCL cell lines. Bortezomib treatment of CTCL mice yielded similar results to JQ1 in vivo, while miR-29b levels were elevated in treated mice vs control animals. Since IL-15 signals through its receptor complex, we evaluated and observed increased BRD4 binding at IL-15 receptor αγy gene loci in CTCL patients, which was reversed with both JQ1 and bortezomib treatment. Thus, we conclude that diminished miR-29b results in increased expression and subsequent binding of BRD4 at regulatory regions of known oncogenes in CTCL cells including NOTCH1, RB1, and the IL-15 receptor complex. We confirm the potential therapeutic utility of targeting this pathway in vivo, by direct displacement of BRD4 by JQ1, or by rescue of miR-29b expression by bortezomib. We therefore describe a novel targetable oncogenic pathway featuring IL-15, miR-29b, and BRD4 in CTCL.

#482 miRNAs involved in LY6K and estrogen receptor-α contribute to tamoxifen susceptibility in breast cancer. Yesol Kim, Dasom Son, Kyung Hyun Yoo, Jong Hoon Park. Sookmyung Women’s University, Seoul, Republic of Korea.

Estrogen receptor-alpha is a clinically important therapeutic target for breast cancer. However, tumors that lose ERα are less responsive to anti-estrogens such as tamoxifen. MicroRNAs are small RNAs that regulate expression of their target gene and dysregulations of miRNA has been identified in many diseases including human cancer. However, only a few miRNAs associated with tamoxen
Epigenetic regulation of hsa-miR-3663 in colon cancer. Laia Pique, Humberto Jorge Ferrerira, Manel Esteller. Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain.

Colon Cancer is the third most common cancer in the world and a major cause of morbidity and mortality. Molecular mechanisms of colon carcinoma have been deeply studied and current evidences indicate that microRNAs play a pivotal role in its tumorigenesis and progression. MicroRNAs (miRNAs) are small 19 to 22 nucleotides of RNA classified as non-coding RNAs that negatively regulate gene expression at the post-translational level controlling numerous biological processes including development, cell proliferation, apoptosis, differentiation and cell migration. Abrupt expression of miRNAs due to epigenetic alterations has been associated with carcinogenesis. Aims to identify epigenetically regulated miRNAs involved in colon cancer, we analyzed DNA methylation profile available in The Cancer Genome Atlas. One of the main hits identified was hsa-miR-3663, hypermethylated in 26% of colon cancer patients (n = 286, p < 0.001) in comparison with complete lack of methylation in normal tissues (n = 38), suggesting its potential function as a putative tumor suppressor. In order to study the role of DNA methylation controlling hsa-miR-3663, HumanMethylation450K methylation profiles were generated for a panel of colon cancer cell lines (n = 10), including the HCT116-DMN methyltransferase knock-out model (DKO, double knockout of DNMT1 and DNMT3B). We found a significant correlation between methylation status of hsa-miR-3663 and expression in colon cancer cell lines. Furthermore, epigenetic regulation of hsa-miR-3663 was confirmed in HCT116 methylated cell line by restored expression upon treatment with DNA demethylating agent 5-aza-2'-deoxycytidine and in the HCT116-DKO model. In order to elucidate the function of hsa-miR-3663, we stably expressed hsa-miR-3663 in a panel of five methylated colorectal cancer cell lines (HCT116, RKO, DLD1, SW48 and SW480). Our initial functional assays revealed that hsa-miR-3663 is not involved in cell proliferation or migration. We are now performing additional assays to further study hsa-miR-3663 and its potential role in colon cancer.


African-Americans are diagnosed with more aggressive prostate cancers and have worse survival than Caucasians, however a comprehensive understanding of this health disparity remains unclear. To clarify the mechanisms leading to this disparity, we analyzed the potential involvement of miR-34b expression in African-Americans and Caucasians. We found that miR-34b expression is lower in human prostate cancer tissue from African-Americans compared to Caucasians. DNA hypermethylation of the miR-34b-3p promoter region showed significantly higher methylation in prostate cancer compared to normal tissues. We found that AR and ETV1 genes are differentially expressed in MDA-PCA-2b and DU-145 cells after overexpression of miR-34b. Direct interaction of miR-34b with the 3' untranslated region of AR and ETV1 was validated by luciferase reporter assay. We found that miR-34b downregulation in African-Americans is inversely correlated with high AR levels that lead to increased cell proliferation. Overexpression of miR-34b in cell lines showed higher inhibition of cell proliferation, apoptosis and G1 arrest in African-American cells (MDA-PCA-2b) compared to the Caucasian cell line (DU-145). Taken together, our results show that differential expression of miR-34b and AR are associated with prostate cancer aggressiveness in African-Americans.

$\#484$ Epigenetic regulation of hsa-miR-3663 in colon cancer. Ashesi Saraiya, 1 Brandon Young, 2 Tobias Meifner, 2 Brian L. Jones, 3 Stephanie C. Huelga, 2 Doug A. Amorese, 1 NuGEN, San Carlos, CA; 4 Avera Cancer Institute for Precision Oncology Genomics, San Diego, CA.

Growing interest in cancer classification and progression has accelerated the rate of novel gene fusions discovery with increasing recognition of their roles as biomarkers. RNA-Seq is an attractive method for expressed fusion discovery.
and detection because of its ability to provide unbiased fusion sequencing information. The ability to detect low expressing fusion transcripts, however, require high sequencing depth and represents a significant financial barrier and identification of clinically relevant fusion sequences from a large data set can be a bioinformatics challenge. To address these challenges we have tested the Ova-
tion Fusion Detection System and the Exari Fusion Detection System on a platform with a method using the Single Primer Enrichment Technology (SPET), with a number of control and clinical samples. Initial studies were performed using a comprehen-
sive target enrichment panel targeting 502 genes with three samples from Horizon DX containing known fusions. Target enriched libraries were con-
structed with 10 ng and 100 ng inputs and the data was analyzed using the NuFuseD platform (available as a point and click BaseSpace application or down-
loadable linux package) which has been optimized for fusion analysis from this data. Expected fusions were identified at both input levels, even when down sampled to 500K reads, with fewer fusion calls compared to other publically available fusion detection software (Chimerascan and SOAPFuse), suggesting a lower false positive rate. NuFuseD fusion calls are provided with a P-value to help prioritize the identified fusions for subsequent validation. Additionally, NuFuseD detected novel fusions in the control samples demonstrating the ad-
Vantage of a comprehensive panel compared to more restricted panels. We fur-
ther validated the target panel using control RNA (UHR and Human Brain) and fresh or FFPE cell lines (NCI-H2228, HCC357) to further demonstrate our ability to identify known fusions. Finally, the system was evaluated at an external site using patient FFPE samples. These samples (N=8) were from a set of breast,
leukemia, and ovarian cancers, containing a unique fusion in 4 of the samples based on DNA based sequencing. Only 1 of the 4 expected fusions were identified using whole transcriptome data (100 million reads) while 3 of the 4 fusions were detected with this assay (10 million reads) demonstrating its ability to generate targeted RNA sequencing libraries with increased sensitivity of gene fusion detec-
tion and reduced sequencing costs compared to standard RNA-Seq methods.

MOLecular AND CELLULAR BIOLOGY / GENETICS: Molecular Carcinogenesis and Chromosomal Alternations

MISMATCH REPAIR DEFICIENCY IN GENETICALLY ENGINEERED HUMAN BREAST CANCER MODELS

E2F2-mediated copy number changes drive metastasis and therapeu-
tic response of HER2-positive tumors through Colla1, CHAD, and AKT-
dependent mechanisms. Jonathan P. Rennhack, Kellan Sun, Jordan Honesett, Eran Andrechek. Michigan State University, East Lansing, MI

The E2F family of transcription factors is classically known to regulate G1 to S-phase transition in cell cycle but has emerging roles in HER2+ breast cancer. A loss of E2F1 or E2F2, in a HER2 mouse model, MMTV-Neu, leads to a de-
crease in tumor metastasis. It is not known what mechanistic roles specific E2Fs are playing in this process. To investigate this, we leveraged and bioinformatic principles, including genomics and transcriptomics with traditional laboratory science and high throughput drug screening projects. This experimental ap-
proach immediately revealed that loss of E2F2 expression significantly correlates with more unstable tumors including an increase in copy number alternations, single nucleotide variants, and translocations. Further analysis revealed a conserved copy number alteration in both mouse and humans. Specifically we noted the amplification of 17q21.33 in 25% of HER2+ breast cancer. The analogous region chromosome 11D was lost in 30% of the less metastatic MMTV-Neu E2F2 knockout mice indicating a role of the region in tumor metastasis. Transcripti-
tomic data revealed that two genes, Collagen Type I, alpha 1 (Col1a1) and Chon-
droadherin (CHAD), were potential genes of significance in the amplification event. CIRSPR mediated knockout studies were conducted to determine each gene’s effect on tumor cell migration and metastasis in mouse and human derived cell lines. Wound healing assays and tail vein injection have shown the Col1a1 and CHAD KO cell lines have a delay in cell migration (P<.01) and reduced ability to colonize the lung (P<.05) respectively. Oncogenic signaling data shows that the 17q21.33 amplification event has higher AKT and E2F2 signaling than HER2 positive tumors without the event. It was hypothesized that the tumors would be dependent on the signaling and perturbation of the net-
work might be an effective therapy for patients with the 17q21.33 amplification event. To investigate this we identified deferentially lethal siRNAs and com-
ponents between HER2+ tumors with and without the 17q21.33 event in the Achilles, CCLE, and PDX datasets. A String-DB analysis showed many of the deferentially lethal genes and compounds centered around AKT. A decrease in AKT signaling through siRNA or chemical compound results in the death of the cell in the 17q21.33 amplified samples but not the HER2+ samples without the event. This study reveals that patients’ with a 17q21.33 amplification event have more metastatic tumors mediated through Colla1 and CHAD and may be re-
and clear cell renal cell carcinoma.

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Palindromic AT-Rich Repeat (PATRR) - mediated translocations entail exchange across chromosomes at sites enriched in palindromic repeats of the nucleotides adenine (A) and thymine (T). Their precise embryologic origin and associated pathobiology with clear cell renal cell carcinoma (cCRCC) remain incompletely described. In the present study we document an individual with familial non-VHL cCRCC (7 primary renal tumors) who harbors a germline de novo PATRR-mediated balanced translocation involving chromosomes 3 and 8 ([t(3;8)]) validated by spectral karyotyping (SKY). Using translocation specific PCR and DNA sequencing we determined the chromosome 3 breakpoint to be located in an AT-rich palindromic sequence in the third intron of the FHIT gene (chr3p14.2) and the chromosome 8 breakpoint in the first intron of the TRC8 gene (chr8q24.1). Genotyping analysis, using a high density custom exomechip array by Illumina, revealed a loss of the entire aberrant chromosome 8 carrying the 3p segment ([der(8)]) in all renal tumors tested. We also determined that the [46, XY, t(3;8), (p14.2, q24.1)] translocation was paternally derived by performing a genotypic assessment of the regions that differ between the paternal alleles and then establishing which haplotypes are associated with the translocation. The somatic mutational landscape was assessed by Whole Exome sequencing of the renal tumors and the proband’s germline DNA. No germline or somatic deletional mutations were detected in VHL gene suggesting that the cCRCC phenotype is not associated with Von Hippel-Lindau disease. Furthermore, we measured the transcriptomic profiles of the renal tumors and matched normal tissue derived from the barcoded exonic (eXome) and RNAseq to fully characterize differential gene expression and define gene ontology networks that are dysregulated. Most significantly, no difference in expression of the VHL gene was detected between tumors and normal counterparts. Abrupt regulation was detected in members of WNT process, COX2 and iNOS; cell survival, Bcl2 and GSTP1.

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# Frequent NRG1 genomic rearrangements in invasive mucinous adenocarcinoma from Caucasian patients. Domenico Trombetta,1 Giulio Rossi,2 Angelo Sparaneo,1 Federico P. Fabrizio,1 Maria C. Manzorza,1 Evaristo Maiello,1 Vito M. Fazio,1 Paolo Graziano,1 Lucia A. Muscarella1. 

Invasive Mucinous Adenocarcinoma (IMA) accounts for 2-5% of lung adenocarcinomas. However, the role of USP37 associated with a definite lung adenocarcinoma subtype in Caucasian population.

# Somatic copy number variants detection using the NEBNext Direct target enrichment method. Kriti M. Patel,1 Sarah K. Bowman,1 Noa Henig,1 Amy B. Emerman,1 Andrew Barry,2 Charles Elfe,1 Scott Adams,1 Salvatore Russo,2 Ted Davis,2 Cynthia L. Hendrickson1. 

Variations in copy number are the most common type of DNA structural variations. Many of these alterations are associated with disease or indicate disease susceptibility. Detection of copy number variants (CNVs) for clinical applications requires efficient, fast and cost-effective methods. Here we describe the use of the NEBNext Direct\(^2\) hybridization-based target enrichment method to identify somatic CNVs with high sensitivity. This approach begins with target enrichment followed by enzymatic digestion of off-target sequences and ligation of adapters that contain unique molecular identifiers (UMIs). The UMIs enhance the identification of duplicate reads and further increase the final number of on-target reads used for variant detection. We used a cell line known to contain a deletion in the CDKN2A gene and spiked DNA isolated from the cell line into a HapMap DNA sample that contains full diploid copies of CDKN2A. We were able to detect the gene deletion as a somatic CNV in a dose dependent manner with high sensitivity. Thus, we demonstrate that the NEBNext Direct approach is an efficient technique to detect somatic CNVs of high and low frequencies.

# Ubiquitin specific peptidases 37 promotes constitutive replication fork movement by stabilizing Chk1 via its deubiquitination. Mayank Singh,1 Amy C. Burrows,1 Andrew Dickson,2 Danielle Gordon,1 Rumsha Javed,2 Rachela Dolce Rameau,3 Xinyin Jiang,1 Anjana D. Saxena3. 

Protein deubiquitination controls many intracellular processes, including cell cycle progression, transcriptional activation, and signal transduction. Ubiquitin specific peptidases (USPs) remove ubiquitin tags from target proteins to control protein fate and function. USP37 (Ubiquitin specific peptidase 37) has been implicated in stress response and we have previously shown that it antagonizes the tumor suppressor ALC1\(^{2,3}\) and promotes S phase entry. However, the role of USP37 during S-phase was unknown. Here, we report that in cells experiencing replication stress USP37 overexpression confers survival advantage while its depletion enhances sensitivity. USP37 overexpressing cells were able to resolve different DNA damage markers much more effectively than the control cells or cells in which USP37 was depleted. Mechanistically, our data indicate that USP37 binds and stabilizes the active form of CHK1 and deubiquitiates CHK1 to increase its stability and promote the checkpoint response. USP37 overexpression results in constitutive replication fork movement and long tract DNA synthesis while USP37 depleted cells were unable to carry out long tract DNA synthesis. Notably, expression of low level of CHK1 in USP37 depleted cells rescues cell survival and DNA damage response. Overall our data suggest that inhibition of USP37 may represent a novel mechanism to modulate CHK1 activity.
**Molecular and Cellular Biology / Genetics: Molecular Carcinogenesis and Chromosomal Alternations**

**#501 Tyrosyl-DNA phosphodiesterase I cellular function dependent on its Impairment of DNA double strand break repair in human primary region breakpoints compared to the prevalence of hits into S type controls. In heterozygous mice, there was no allelic bias in targeting IgH for previous observations made in a mouse lymphoma model, Hs3b/Hs4 3'KO mice candidate for orchestrating c-Myc deregulation. To elucidate the role of 3'RR in death within 96 hours. The later observation confirms the requirement of the full 3'RR for c-Myc deregulation by T(12;15).

Tyrosyl-DNA phosphodiesterase I (Tdp1) is a highly conserved eukaryotic DNA repair enzyme that catalyzes the resolution of 3' and 5' phospho-DNA toxicities that were observed with the full-length Tdp1 mutant proteins. Our data suggests that the N-terminal domain is required to resolve protein-DNA covariant complexes, such as Top1. Indeed, preliminary results suggest that this domain is also critical to process Top2-DNA covariant complexes, but only in the presences of etoposide. We are currently investigating other protein-DNA ad- ducts that are resolved by Tdp1. These results suggests that the N-terminal domain is a critical determinate of Tdp1 cellular function. However, further studies are necessary to ensure that these constructs are properly distributed and retain their catalytic activity. Additionally, the N-terminal domain of human Tdp1 is post-translational modified, while our preliminary results suggest that this domain is important for protein-protein interaction and Tdp1 recruitment to its substrates. Understanding Tdp1 substrate and protein-interactions are important in the development of Tdp1 as therapeutic target. This work is in part supported by the ADDA, UAB Cancer Comprehensive Center (P30CA13148), ACS-IRG-60-001-53, DOD OCRP (W81XWH-15-1-0198).

**#502 Electronic cigarette aerosols increase cellular reactive oxygen species and induce significant oxidative DNA damage. Vengatash Ganapathy,3 Jimmy Manyangia,1 Debra McGuire,1 Daniel Brobst,2 Theodore Wagner,2 David Rhutledge,1 Lijun Song,1 Lingnanesan Rajeswaran,2 Herbert C. Morse.

Rationale: Emphysema is caused by the destruction of alveolar wall septa. The major risk factor for this disease is cigarette smoke and effective therapies are very limited. Alveolar type II (ATII) cells are in the gas exchange portion of the lung. They make and secrete pulmonary surfactant, and proliferate to restore the epithelium after damage to the more sensitive alveolar type I cells. Methods: Control ATII cells were isolated from deidentified control non-smoker and smoker organ donors whose lungs were not suitable for transplantation and donated for medical research. Furthermore, as a unique approach, we have developed a new method on how to isolate ATII cells from excess tissue from lung transplants obtained from patients with emphysema using magnetic microwells. We determined DNA damage, DNA repair, oxidative stress, injury, and inflammation in human primary ATII cells isolated from these individuals in comparison with immortalized controls. Results: Our data indicates high oxidative stress in human ATII cells induced by cigarette smoke extract in vitro as measured by 4-HNE staining by immunocytofluorescence. We also observed DNA double strand breaks, high DNA damage, and low DNA repair in these cells. Moreover, we found greater proinflammatory response as determined by IL-8 and IL-6 levels by ELISA; a trend that was greater in cells obtained from smokers compared to controls. Conclusion: Our results indicate oxidative ATII cell damage induced by cigarette smoke and in emphysema. Observed DNA repair imbalance may contribute to cell death in this disease. The study of ATII cell injury may improve our knowledge on this disease pathogenesis and may lead to novel therapeutic strategies to slow the progression of emphysema.

**#503 Tyrosyl-DNA phosphodiesterase I cellular function dependent on its N-terminal residues. Selma M. Cuya, Robert C.A.M. Van Waardenburg. Univ. of Alabama at Birmingham, Birmingham, AL.

Tyrosyl-DNA phosphodiesterase I (Tdp1) is a highly conserved eukaryotic DNA repair enzyme that catalyzes the resolution of 3' and 5' phospho-DNA adducts. Tdp1's role is implicated in the repair of DNA topoisomerase I (Top1)-DNA covariant complexes reversibly stabilized by FDA approved camptothecins (CPTs) derivatives topotecan, and irinotecan. Tdp1 activity relies on two catalytic histidines that function as a nucleophile and an acid-base residue. A mutation of the acid-base His to Arg (H493R) in human Tdp1 is associated with the rare recessive ataxia SCAN1. We defined alternative substitution of other catalytic histidine that induce cytotoxicity, reduce catalytic activity and enhances the requisite Tdp1-DNA covariant adduct lifetime in the cell. The phenotypes of the catalytic mutants provide excellent tools to study Tdp1 cellular function. Biochemical studies revealed that Tdp1 catalysis in vitro is independent of the N-terminal domain. Among Tdp1 proteins, the N-terminal domain is poorly conserved in sequence and size (79aa for yeast and 148aa for human Tdp1). Conversely, the N-terminal domain regulates the in vitro activity of these Tdp1 mutants. Additionally, we investigated the role of the N-terminal domain for Tdp1 activity in the yeast and human cell models. Expression of N-terminal truncated proteins showed similar cellular distribution as the full-length protein. However, the results suggest that the N-terminal domain for Tdp1 activity is highly conserved in sequence and size (79aa for yeast and 148aa for human Tdp1). Conversely, the N-terminal domain regulates the in vitro activity of these Tdp1 mutants. Additionally, we investigated the role of the N-terminal domain for Tdp1 activity in the yeast and human cell models. Expression of N-terminal truncated proteins showed similar cellular distribution as the full-length protein. However, the results suggest that the N-terminal domain is required to resolve protein-DNA covariant complexes, such as Top1. Indeed, preliminary results suggest that this domain is also critical to process Top2-DNA covariant complexes, but only in the presence of etoposide. We are currently investigating other protein-DNA adducts that are resolved by Tdp1. These results suggest that the N-terminal domain is a critical determinate of Tdp1 cellular function. However, further studies are necessary to ensure that these constructs are properly distributed and retain their catalytic activity. Additionally, the N-terminal domain of human Tdp1 is post-translational modified, while our preliminary results suggest that this domain is important for protein-protein interaction and Tdp1 recruitment to its substrates. Understanding Tdp1 substrate and protein-interactions are important in the development of Tdp1 as therapeutic target. This work is in part supported by the ADDDA, UAB Cancer Comprehensive Center (P30CA13148), ACS-IRG-60-001-53, DOD OCRP (W81XWH-15-1-0198).

**#504 Enhanced repair of bulky DNA adducts induced by a tobacco carcinogen and UV light in human oral epithelial cells by black raspberry extract. Joseph B. Guttenplan,1 Kun-Ming Chen,2 Yuan-Wan Sun,3 Ross Teicher,4 Wieslawa Kosinska,5 Krishne Gowda,1 Shantu Amin,2 Gary D. Stoner,4 Karam El-Bayoumy,2 1New York University Dental and Medical Schools, New York, NY; 2Penn State College of Medicine, Hershey, PA; 3New York University Dental School, New York, NY; 4Medical College of Wisconsin, Milwaukee, WI.

We were the first to report that an anthocyanin-enriched black raspberry extract (BE) inhibited mutagenesis, and reduced levels of DNA adducts induced by metabolites of the tobacco carcinogen dibenz(a)pyrene (DBP), in a rat oral...
fibroblast cell line, by enhancing removal of DNA adducts (Guttenplan, et al., Cancer Prev Res; 8(9) August 2016). Here we extend these findings to the repair of DBP-induced DNA damage in a human oral leukoplakia cell line (MSK leu1), and a reduction in the toxicity (assayed by dead cell numbers and cell survival) of DBP diolepoxide (DBPDE) in these cells. In addition, we examined the effect of BE on DNA damage and toxicity induced by UV light. Treatment of the MSK cells with DBP, and 2 of its metabolites, DBP-dihydriodiol and DBPDE led to a major adenine adduct, (-)-anti-trans-[b,a]PDE-dA (DBPDE-dA).

The order of potency was DBPDE ˃ DBP ˃ DBP-dihydriodiol with one μM DBP, producing adduct levels of about 20/106 da. BE in the range of 75-150 μg/ml significantly inhibited adduct formation when cells were pretreated with BE before addition of DBP. However, when cells were first treated with DBP and one day later BE was added, adduct levels were reduced by about 50% two days after treatment with DBP, indicating that the BE was enhancing DNA repair. This conclusion results from the fact that BE was not present during the metabolic activation of DBP to DBPDE, and hence couldn’t modulate the activation steps. We also tested whether BE, added 4 hr after treatment with the short-lived DBPDE, inhibited toxicity to MSK cells. Initial toxicity was measured by counting the numbers of dead cells in the medium 24 hr after addition of DBPDE, and the relative levels of surviving cells were determined using an MTT cell viability assay. It was found that 100μg/ml BE reduced toxicity induced by 25 - 200 nM DBPDE by about 50%, with a concomitant increase in survival in the BE-treated cells. As DBP produces bulky DNA adducts we also investigated the effects of BE on toxicity at lower levels of DBP and on DNA adducts produced by UV light. Cells were irradiated for 30 - 120 seconds with 254 nm light and 15 minutes later, treated with 50 - 150 μg/ml BE. Toxicity was measured, as above, and a logarithmic decrease in cell death was observed with increasing concentration of BE. Similar to results with DBPDE, BE increased cell survival. The effects of BE on relative levels of the UV-light-induced cyclopyrimidine adducts were also determined, using an ELISA assay. BE reduced adduct levels by 30 - 60%.

These results indicate that BE may provide chemopreventive effects on initiation of carcinogenesis by environmental agents that produce bulky DNA adducts - by enhancing DNA repair, likely via the nucleotide-excision repair pathway. Supported by NIH grant #CA173465.

#504 Quantification of nucleic acid quality in postmortem tissues from a cancer research autopsy program. Jun Fan, Yi Zhou, Chelie Michael, 300 μg/ml reduced adduct levels by 30 - 60%.

These results indicate that BE may provide chemopreventive effects on initiation of carcinogenesis by environmental agents that produce bulky DNA adducts - by enhancing DNA repair, likely via the nucleotide-excision repair pathway. Supported by NIH grant #CA173465.

#505 Somatic and inherited riboSNitches in TPT1 and LCP1 mRNA secondary structures. Lela L. Lackey,1 Aaztli Coria,1 Chanin Tolson,1 Evonne McGarthur2,3,4,5

Kimberly J. Bussey, Luis Cisneros. NantOmics, LLC, Tempe, AZ.

The action of the AID/APOBEC family of cytosine deaminases contributes to mutational clustering but fails to explain 50% of the clusters observed in cancer genomes. Stress-induced mutagenesis in bacteria occurs when double-strand breaks (DSB) or other DNA damage occurs and is a known mechanism to initiate the SOS response. This results in mutational clustering driven by DSB where mutational abundance decays as a function of the distance from the DSB, but remains above background rates of mutagenesis up to 1 MB away. In humans, the orthologous genes to DSB have become specialized for translesion synthesis (TLS). The dysregulation of cell cycle and DNA repair that characterizes most tumors would logically increase the need for TLS in cancer. Therefore, we hypothesized that stress-induced mutagenesis in cancer would result in peaked clusters of SNVs driven by TLS. We used data from 764 cases with somatic mutation and structural variant calls from WGS from the ICGC database, release 19, and private de novo mutations derived from 130 trios in the 1000 Genomes project with WGS, SNV context was determined based both on the reference and the mutant allele, and called when only one mechanism (TLS, APOBEC, or AID) could be assigned, thereby underestimating the actual number of TLS events. We observed that as the total number of SNVs increases so does the number of clusters, the proportion of SNVs in clusters, and the average size of clusters in both de novo private mutations and somatic mutations in cancer. In normal, a median of 15.2% of the SNVs occurring in clusters occur in a sequence context indicative of TLS, compared to 0.53% and 1.6% for APOBEC and AID, respectively. In contrast, in cancer, TLS accounts for a median of 30.6% of SNVs in clusters. APOBEC and AID account for 2.3% and 2.6%, respectively. We devised a measure of cluster shape based on empirical cumulative distributions, termed the Stress-Introduced Heterogeneity (SITH) score. It ranges from -1 to 1 and quantifies how sharply peaked clusters are with more positive numbers indicative of a faster decay in mutational load over distance relative to the putative DSB. In normal, SITH scores range from 0.457 to 0.578 with a median of 0.50. In cancer, SITH scores ranged from 0.17 to 0.999 with a median of 0.46 and vary significantly by organ site (ANOVA, F = 44.96, p < 2.2x10⁻¹⁶). We assessed the contribution of TLS, APOBEC, and AID to SITH score using an additive, linear model of SITH scores derived solely from SNVs in that specific context and including organ site as a
variable. We found that in both normal and cancer that TLS was the only context that contributed significantly to STH score. Therefore, we conclude that the clustering seen in cancer is being driven primarily by TLS and these clusters have a shape characteristic of a process of stress-induced mutagenesis.

#508 DNA repair status in a patient derived ovarian cancer xenobank. Federica Guffanti,1 Maddalena Fratelli,1 Monica Ganzinelli,1 Francesca Ricci,1 Roberta Affatato,1 Maria Rosa Cappelletti,2 Daniele Generali,2 Francesca Bizzaro,1 Massimo Brogini,1 Raffaella Giavazzi,1 Giovanna Dania,1 IRCCS - Institute for Pharmacological Research Mario Negri, Milan, Italy; 2Azienda Socio-Sanitaria Territoriale di Cremona, Cremona, Italy.

Epithelial ovarian cancer (EOC) is the most lethal gynecological malignancy with a 5-year relative survival rate of 45%. The high mortality rate is in part due to the development of platinum chemoresistance occurring in more than 70% of patients after the first-line therapy. DNA repair capacity has been reported to be a key determinant for the cellular response to platinum agents. Since half of the high grade serous EOCs lacks Homologous Recombination repair, we aimed to profile the DNA repair status in a panel of well characterized 42 ovarian patient derived xenografts (PDxS) recently established in our laboratory and to correlate it with the in vivo response to a platinum based therapy. We evaluated by real time PCR (ABI-7900, Applied Biosystems) the mRNA levels of genes with a key role in Base Excision Repair (OGG1, POLB and PARP1), Homologous Recombination (BRCA1, PALB2, TP53BP1 and RAD51), Nucleotide Excision Repair (ERCC1, XPA, XPF, XPD and XPG), Fanconi Anemia pathway (FANCA, FANCC, FANCDD2 and FANCG) and Translesion Synthesis Repair (POL3E, Mismatch Repair (MLH1, MSH2), homology End Joining (POLQ), Non Homologous End Joining (XRC4, XRC5, XRCC6 and XRCC7) and CDK2, a kinase regulating the transcription of some DNA repair genes. The methylation status of BRCA1, ERCC1, MLH1, XPA, XPG and FANC were investigated by standard techniques. Our results show that the DNA repair genes considered were variably expressed in all the 42 PDxS analyzed, with no specific histotype-specific cluster of expression. The expression of PALB2, FANCC, FANCDD2, OGG1, POLQ and RAD51 was found to correlate with the expression of at least six other genes. In high grade serous/endometroid PDxS, the CDK12 mRNA expression levels positively correlated with the expression of TP53BP1, PALB2, XPF and POLB. BRCA1 was found to be hypermethylated in 51% of the xenografts. TP53 mutated PDxS showed statistically significant higher levels of POLQ, FANCDD2, RAD51, and POLB genes. The expression of CDK12 [p = 0.017], PALB2 [p = 0.019] and XPF [p = 0.016] was negatively associated with the in vivo response to DDP, with resistant PDxS showing higher mRNA levels than responsive ones. We looked for association with overall survival in the TCGA data set and we found that high levels of CDK12 were associated with a worse overall survival in patients with a residual tumor more than 2 cm. These data suggest that some DNA repair genes can have a role in EOC patients’ response to DDP therapy. Particularly, CDK12 was significantly able to predict worst survival in patients undergoing optimal debulking surgery. Our xenobank will be a valid instrument to set up functional DNA repair assays, as suggested by preliminary data on primary cultures.

#509 Genomic profiling of acute lymphoblastic leukemia in ataxia telan- giectasia patients reveals tight link between ATM mutations and chromothri- psis. Manasi Ratnaparkhe,1 Mario Hlevnjak,1 Thorsten Kolb,1 Anna Jauch,2 Kendra Maass,1 Frauke Devens,1 Agata Rode,1 Volker Hovestadt,1 Andrey Korobkov,1 Roberta Affatato,1 Maria Rosa Cappelletti,2 Daniele Generali,2 Francesca Bizzaro,1 Massimo Brogini,1 Raffaella Giavazzi,1 Giovanna Dania,1 IRCCS - Institute for Pharmacological Research Mario Negri, Milan, Italy; 2Azienda Socio-Sanitaria Territoriale di Cremona, Cremona, Italy.

Recent developments in sequencing technologies lead to the discovery of a novel form of genome instability, termed chromothripsis. This catastrophic genomic event, involved in cancer formation, is characterized by tens to hundreds of locally clustered rearrangements on one chromosome, acquired simultaneously. We hypothesized that leukemias developing in individuals with Ataxia Telangiectasia, who are born with two mutated copies of the ATM gene, essential guardian of genome stability, would show a higher prevalence for chromothripsis due to the defect in DNA double-strand break repair. Using whole-genome sequencing, fluorescence in situ hybridization and RNA sequencing, we characterized the genomic landscape of Acute Lymphoblastic Leukemia (ALL) in patients with Ataxia Telangiectasia. We detected a high frequency of chromothripsic events in these tumors, specifically on acrocentric chromosomes, as compared to tumors from individuals with other types of DNA repair syndromes (27 cases in total, of which 10 with Ataxia Telangiectasia). Our data show that the genomic landscape of Ataxia Telangiectasia ALL is clearly distinct from that of sporadic ALL. Mechanically, short telomeres and compromised DNA damage response in cells of Ataxia Telangiectasia patients are linked with genome instability. Additionally, we show that ATM loss is associated with increased chromothripsis prevalence in further tumor entities.

#510 Topoisomerase IIα silencing increases R loops at specific genomic loci associated with an increase of γH2AX and cell cycle progression delay in human cancer cells. Maria Delcuratolo, Jessica Marinello, Giovanni Capranico. University of Bologna, Bologna, Italy.

DNA topoisomerase IIα (Top2α) is a crucial enzyme for cell proliferation and plays a critical role in DNA replication, transcription and hence genome instability in cancer cells. Using whole-genome sequencing, fluorescence in situ hybridization (FISH), and immunofluorescence (IF), we have recently demonstrated that Top1 silencing and poisoning by camptothecin affect the formation of R-loops, which are RNA/DNA hybrid structures involved in genome instability and are favored by negative supercoils of the DNA. The findings overall demonstrate that Top2α reduces R-loop levels during transcription whereas it favors R loop formation at early origins of DNA replication. Here, we address the question of whether DNA topoisomerase IIα can also contribute to steady-state levels of R loop structures in the genome of U2OS cancer cells. We have then investigated the effects of Top2β silencing and Top2 poisons, doxorubicin and etoposide, on R loops and DNA damage in human U2OS cancer cells. Similarly to published data with camptothecin (Marinello et al., Nucleic Acids Research, 2013), Top2 poisons increase cellular R loops by IF after short treatment times and reduce them after 1 hour of treatment. The bi-phasic effect of poisons is mainly dependent on transcription. Moreover, we have determined the changes of R-loop levels by IF after Top2β silencing and the findings demonstrate that the enzyme can strongly modulate the formation of R-loops as a full Top2β deletion decreases nuclear R-loop levels. In addition, Top2β depletion leads to a slight increase of phosphorylation of H2AX histone along with cell cycle delay and eventually cell death. Thus, Top2β depletion can trigger genomic DNA breakage through alterations of R-loops. In order to establish which are the genomic regions of altered R loop levels, we have mapped Top2β-dependent R loop alterations by the DRIP method showing that specific genomic sites are affected by Top2β. Bioinformatic analyses of R loop maps will be presented and discussed at the meeting. Altogether the findings demonstrate a critical role of Top2β in governing R loop structures in human cancer cells indicating that DNA torsional tension is a main driving factor of R loop formation and hence genome instability in cancer cells.

#511 Chromosome instability mechanisms in breast cancer among Afri- can Americans and Caucasians, Jose Thaiparambil, Oula Mansour, Susan Hal- ley, Randa EL-Zein. Houston Methodist Research Institute, Houston, TX.

Significant differences exist in breast cancer among African Americans (AAs) women compared to Caucasians. Even though AAs are less likely to undergo regular mammograms due to access barriers, it is unlikely that differences in screen based detection accounts entirely for outcome disparities. While the current guidelines recommend mammography at age 40 for AAs, AA women may be at increased risk for delayed diagnosis since >10% of breast cancer cases in AAs are diagnosed in women younger <40 years compared with 5% of Caucasians. This highlights the importance of defining optimal ethically appropriate age of screening especially given the disproportionate mortality among younger black women patients. Therefore, an alternative approach using biomarkers should be considered in order to develop ethically appropriate measures for detecting the disease in the earliest stages. Genomic instability has long been recognized as a major driver of carcinogenesis occurring early on and increasing in complexity with disease progression. However, to date little is known about the underlying mechanisms associated with such instability among the different ethnic groups. In this study we hy-
Genomic instability is an enabling hallmark of cancer that provides cancer cells a replicative advantage. Accumulation of genomic aberrations can also compromise the genomic integrity and put cells under mitotic stress. Maintaining the balance between the instability that gives cancer cells a replicative advantage and the instability that could lead them to mitotic catastrophe is crucial for survival of cancer cells. Mitotic catastrophe can be caused by rapid progression through mitosis before crucial checkpoints are met, resulting in cell death. To overcome this challenge, cancer cells may acquire overexpression of spindle assembly checkpoint (SAC) genes, which can prevent mitotic catastrophe that would occur if they undergo mitosis prematurely. Bub1b is an important part of SAC and inhibits the onset of anaphase until all chromosomes are aligned at the metaphase plate. Our analysis of clinical datasets show Bub1b expression is elevated in breast cancer, compared to normal breast, and is exemplified by a pattern of increasing overexpression in more aggressive variants, such as triple negative breast cancer (TNBC). Bub1b overexpression also correlates with decreased overall survival in patients. Expression analysis of breast cancer cell lines corroborates this clinical data. We hypothesize that the requirement for Bub1b expression indicates a vulnerability of rapidly proliferating breast cancer cells, and the inhibition of Bub1b will result in cell death through mitotic catastrophe. Using RNA interference with siRNAs we knocked down Bub1b in breast cancers cell, and the inhibition of Bub1b results in apoptosis and cell death, especially in the TNBC cell line MDA-MB-231. Notably we found that p21 blockade using siRNA knockdown or genetic knock-out shifted polyploid cell response to p53 activation from cytostatic to cytotoxic. As a result, p21-deficient cells exhibited enhanced sensitivity to mitotic blockade combined with p53 induction. Furthermore, TCGA dataset analysis showed poor progression free survival in melanoma patients with high p21 protein expression. These data argue for administering mitotic inhibitors and MDM2 antagonists, which are currently in clinical development, in conjunction with agents that target p21. In summary, our data here reveal that polyploidization can be a mechanism for induction of DNA damage and genomic instability associated with drug resistance in cancer and suggest a novel strategy for targeting these pathways to improve melanoma therapy.

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Molecular Carcinogenesis and Chromosomal Alternations

#512 Knockdown of the spindle assembly checkpoint gene Bub1b results in increased cell death and cell cycle impairment in breast cancer. Dilara Koyuncu, Erik T. Goka, Philip C. Miller, Marc E. Lippman. University of Miami, Miami, FL.

Genomic instability is a hallmark of cancer implicated in tumor evolution and resistance to therapy. However, the molecular mechanisms that underlie genomic instability are still poorly understood. One common feature of cancer cells is an elevated genomic content (polyploidy) which is also associated with the acquisition of therapy resistance. Here we demonstrate a link between genomic instability and polyploidization in melanoma cells. We show that polyploidy induced by treatment with small molecule inhibitors of various mitotic kinases, such as AURKA, AURKB and PLK1, leads to DNA damage in cells with elevated (>4n) DNA content. This DNA damage results from replication stress exacerbated by limited activation of p53 in malignant melanocytes. Pharmacological induction of p53 in polyploid cells using an MDM2 antagonist reduced DNA damage by blocking re-replication of the polyploid genome as a result of p53-mediated G1 arrest and transactivation of DNA repair genes. These data argue for administering mitotic inhibitors and MDM2 antagonists, which are currently in clinical development, in conjunction with agents that target p21. In summary, our data here reveal that polyploidization can be a mechanism for induction of DNA damage and genomic instability associated with drug resistance in cancer and suggest a novel strategy for targeting these pathways to improve melanoma therapy.

#514 Genetic load in cancer cell populations. Yuezheng Zhang,1 Xu Shen,2 Yawei Li,1 Tianqi Zhu,2 Yong Tao,1 Tao Li,2 Di Wang,1 Xueying Li,1 Qin Ma,2 Xueyi Lu,2 Huirong-Yi Wang,2 Chung-I Wu,2 1University of Washington, Seattle, WA; 2Beijing Institute of Genomics, Beijing, China; 3Peking University, Beijing, China; 4Institute of Clinical Medicine, National Taiwan University, Taipei, Taiwan.

Populations accumulate deleterious mutations that have yet to be removed by natural selection. This mutated portion of the population that does not contribute to its fitness is called the genetic load and is proportional to the deleterious nature and generation of new mutations. Given the genomic instability of cancer cells, the genetic load could be substantial if the instability indeed leads to loss of cell fitness. We hypothesize that because aneuploidy and copy number variations (CNVs) are the most common forms of genome instability, their fitness consequences may determine the genetic load in cancer cells. To test this hypothesis, we randomly selected single cells from a HeLa cell line and measured the cell growth rate via daily cell counting and an MTT cell proliferation assay. We then measured the genetic changes and resulting growth rate variation. We observed rapid generation of heterogeneity in the growth rate within the population. The growth rate of different progeny cells was stable indicating that it is genetically determined. Next, using whole genome sequencing of recently descended clones of a single HeLa cell, we found that cells with fewer newly acquired CNVs have higher proliferation rates versus a CNV. This indicated that CNV accumulation decreased cell fitness systematically. We next estimated the rate of CNV mutation by measuring the growth rate of two daughter cells from single proliferation and found that there is approximately 1 deleterious mutation in every 4 cell divisions. We speculated that due to a high, deleterious CNV mutation rate, tumor cells inevitably accumulate deleterious CNVs and a large percentage of tumor cells are genetically defective. Accordingly, we observed that the average growth rate of tumor cell populations decreased in the short term since defective cells accumulated in the population and the variation of cellular growth rates within the population increased. By modeling the process of mutation accumulation and measuring cell growth rate, we estimated that the deleterious mutation rate in HeLa cells is about 0.26-0.31 per cell division, and that HeLa cells reduce roughly 5%-6% of fitness for every cell division. The observations of a high proliferation rate and high genetic load in this representative tumor cell line indicates a “high risk, high reward” evolution strategy for tumor cells and suggests that increasing the level of genomic instability may cause the meltdown of tumor cell population by forcing cells to accelerate the cell cycle.

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Oncogenic Growth Factors and Signal Transducers

#515 Alterations in phosphatidylinositol 3-phosphate (PI3P) pathway and cAMP pathway confirm poor prognosis and reduced overall survival (OS) in a series of 209 acute myeloid leukemia patients. Mariachiara Abenante,1 Mariachiara Fontana,1 Giovanni Marconi,1 Giorgia Simonetti,1 Antonella Padella,1 Elena Temp,1 Eugenia Franchini,2 Anna Ferranti,2 Sarah Parisi,2 Emanuela Ottaviani,2 Nicoletta Testoni,2 Viviana Guadagnuolo,3 Chiara Sar,4 Silvia Lo Monaco,2 Cristina Papayannidis,2 Giovanni Martinelli2.

The link between polyploidy and replication stress in melanoma; Anna E. Vilgelm,1 C. Andrew Johnson,1 Kiran Malikayil,2 Dayanidhi Raman,3 Oana Cristea,4 Dilara Koyuncu,1 Erik T. Goka, Philip C. Miller, Marc E. Lippman. University of Miami, Miami, FL.

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MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Oncogenic Growth Factors and Signal Transducers

Introduction: PI3P is a molecule that regulate cell growth and mediates cell proliferation via PI3K/AKT/mTOR in response to various growth signals. Abnormal activation of genes in its pathway is associated to oncogenic activity and poor Overall Survival (OS). AMPK plays a role as a regulator of cellular energy homeostasis. Aims The aim of the this study is to define the role of PI3P pathway and AMPK pathway in AML. Methods: In this work we analyzed 208 consecutive newly diagnosed non M3 AML patients, screened for TP53, FLT3, NMP1, IDH1, IDH2, and DNMT3A mutations. Remission status was assessed with bone marrow biopsy. We performed Microarray-based Comparative Genomic Hybridization with Affymetrix SNP array 6.0 or Cytoscan HD in all the patients; we performed Whole Exome Sequencing (WES) in 208/208 patients. Survival data were collected prospectively, with a median follow-up of 18 months. Survival analysis was performed with Kaplan Meyer method using log rank test. Univariate and multivariable regression and Cox Hazard Ratio(HR) model was performed. Correlation between variables was assessed with Fisher’s exact test. Results: We selected genes in pathways basing on literature and GO data. Alterations in these pathways involved 103/209 patients (48%). We analyzed the gene in two different pathways. PI3K/AKT/mTOR pathway includes the following genes: pik3c3, cdk1a, akt1, akt3, mtor and pten, pdk1,pik3r1 and irs1. The second one is AMPK pathway and it include: sestr, prkaa1, prkab1, prkag1, prkag3. Alterations in PI3K/AKT/mTOR pathway confer worst OS (p = 0.035) when compared with unaltered patient, but events in these pathways did not affect therapy response. Alterations in AMPK pathway confer worst OS (p < 0.001). The combination of regulators in cAMP were tumourubin (20% mg%) after induction in univariate (p < 0.001) and multivariater analysis with age, karyotype, secondary AML, TP53 mutation (p = 0.009). AMPc pathway alteration was significantly associated with complex karyotype and TP53 mutation (p < 0.001). WES in a sub-cohort of patients did not found any significant mutation in genes we analyzed, according to literature. Conclusions: Our work investigates the role of PI3P and cAMP pathways in AML. Surprisingly, it showed that alterations in these pathways are associated with poor prognosis. Significantly, alterations in cAMP pathways were associated with therapy resistance. Acknowledgement: ELN, AIL, AIRC, PRIN, Progetto Regione-Università 2010-12, FP7 NGS-PTL project, HARMONY.

#516 High-throughput functional evaluation of variants of unknown significance in EGFR. Shinji Kohsaka,1 Masaaki Nagano,1 Toshihide Ueno,1 Yo-shiaki Sugihara,2 Takao Hayashi,2 Naoko Shimada,2 Kazuhisa Takahashi,2 Kenji Suzuki,2 Kazuya Takamochi,2 Fumiyuki Takahashi,2 Hiroyuki Mano1, 1Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; 2Juntendo University Graduate School of Medicine, Tokyo, Japan.

Since transforming mutations in epidermal growth factor receptor gene (EGFR) were first identified in non-small-cell lung carcinoma (NSCLC), advance of diagnostics for such mutations, and evolution of targeted therapeutics against EGFR has driven unprecedented improvements in the management as well as outcome of patients with this lethal disease. However, next-generation sequencer (NGS)-driven extensive analyses on NSCLC have revealed a large number of variants of uncertain significance (VUS) in EGFR and other regions in the cancer genome that await further investigation. Here we present a mixed all together patients (in one sample) method to evaluate the VUS, performing potential and drug sensitivity of VUS of oncogenes, and applied this method to 101 non-synonymous EGFR mutants in a high-throughput manner. The sensitivity of individual mutants to tyrosine kinase inhibitors (TKIs) against EGFR was shown diverged, ranging from relatively insensitive mutations such as missense mutations within exon 19 to a highly resistant mutation within exon 21. Our data thus support the importance of examining uncommon mutations within EGFR, and also of functional evaluation of such mutations. Our MANO method may become a novel foundation for in vitro and in vivo assessments of variants of cancer-related genes to deliver precision medicine to individual cancer patients.

We hypothesize that dual inhibition of YAP and TAZ will lead to more pronounced cytotoxicity than each knockdown alone as a result of diminished compensatory mechanism by the remaining family members. Methods: NSCLC cell lines, such as PC9 and A549, were transfected with individual YAP1, TAZ siRNA or a combination of both siRNAs. Western blotting was performed to confirm the knockdown of the levels of YAP and TAZ. CTGF, Cyr61 and AXL, were also reduced accordingly. Moreover, dual knockdown of YAP and TAZ led to a more pronounced inhibition of cell survival/proliferation than ablation of individual genes alone. In addition, dual inhibition of YAP/TAZ exhibited significantly diminished levels of the mesenchymal markers, vimentin and N-cadherin, and increased levels of the epithelial marker, E-cadherin. EMT has been recognized as an essential process during lung cancer tumor migration and metastasis. Conclusion: Our findings show that dual inhibition of YAP1 and TAZ are synergistic in blocking NSCLC cell survival signaling. Thus, dual inhibition may serve as a better therapeutic strategy in targeting the Hippo pathway than sole YAP1 blockade in the management of NSCLC.

#519 Antitumor activity of MET antibody emibetuzumab (LY2875358) in combination with EGFR inhibitors in erlotinib resistant (ER) xenograft mouse models. Sudhakar Chintharlapalli,1 Jennifer R. Stephens,1 Jessica A. Baker,1 Holly K. Cannon,1 Joel D. Cook,1 Isabella H. Wolur,2 Roger Ageyi,2 Sudharak Chintharlapalli,1 Robert J. Evans,1 William J. Feaver,2 Lysiane Huber,1 Linda N. Lee,1 Ling Liu,1 Liandong Ma,1 Ruslan Novosiadly,2 Volker Wacheck,3 Sau-Chi Betty Yan1,1* Eli Lilly and Company, Indianapolis, IN; 1* Eli Lilly and Company, New York, NY; 2Eli Lilly and Company, Vienna, Austria.

Background: MET amplification (amp) is a resistance mechanism to EGFR TKI treatment. Emibetuzumab, a bivalent MET antibody (Ab) blocks HGF binding to MET and internalizes the receptor. Combination of emibetuzumab with EGFR TKIs (erlotinib, AZD9291, CO1686) or EGFR Ab (necitumumab, cetuximab) was evaluated in 3 ER xenograft models. Methods: Model 1: ER cell line HCC827ERL with high focal MET amp, high pMET, EGFR ex19 del (no T790M) was created from parental HCC827 NSCLC (EGFR ex19 del, EGFR amp, no MET amp) by increasing concentration of erlotinib in vitro over 7 months. Model 2: ER cell line HCC827-A8 was derived from HCC827 parental xenograft tumor serially passed in vivo with long term treatment of gefitinib and cetuximab. HCC827-A8 cells express high focal MET amp, high PMET/AXL (Western blot) while retaining EGFR ex19 del (no T790M). Model 3: LU8085 was an ER patient-derived NSCLC xenograft tumor, with focal MET amp, EGFR ex19 del, EGFR amp, no MET amp, L858R (no T790M). MET amp and EGFRmmt was determined by FISH and LNA-PCR sequencing respectively. Compound dosing: emibetuzumab 20 mg/kg qw; necitumumab 4 mg/kg or 20 mg/kg biw; cetuximab 4 mg/kg biw; erlotinib 25 mg/kg qd, 5 mg/kg AZD9291 qd, 30 mg/kg CO1686 bid. Results: EGFR inhibitors, but not emibetuzumab showed significant single agent anti-tumor effect in models when derived in vivo from parental cell lines, such as PC9 and A549, were transfected with individual YAP1, TAZ siRNA or a combination of both siRNAs. Western blotting was performed to confirm the knockdown of the levels of YAP and TAZ. CTGF, Cyr61 and AXL, were also reduced accordingly. Moreover, dual knockdown of YAP and TAZ led to a more pronounced inhibition of cell survival/proliferation than ablation of individual genes alone. In addition, dual inhibition of YAP/TAZ exhibited significantly diminished levels of the mesenchymal markers, vimentin and N-cadherin, and increased levels of the epithelial marker, E-cadherin. EMT has been recognized as an essential process during lung cancer tumor migration and metastasis. Conclusion: Our findings show that dual inhibition of YAP1 and TAZ are synergistic in blocking NSCLC cell survival signaling. Thus, dual inhibition may serve as a better therapeutic strategy in targeting the Hippo pathway than sole YAP1 blockade in the management of NSCLC.

#520 Trim44 is overexpressed in colorectal cancer with amplification, proteomic dephosphorylation of SHP-1 and activating JAK/STAT pathway by ubiquitin modification. Jie Hong, Qian Liang, Haoyan Chen, Jing-Yuan Fang, Hua Xiong. Shanghai Jiaotong Univ School of Med Renji Hospital, Shanghai, China.

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Tripartite motif 44 (Trim44) was found to be expressed in several cancers, but the exact roles of Trim44 in colorectal cancer (CRC) remain unclear. The objective of this study was to determine the functional and prognostic implications of Trim44 in colorectal cancer. The Trim44 gene is recurrently amplified and up-regulated in CRC. Trim44 overexpression and amplification are associated with poor survival in CRC (Garcia et al., 2014). It has been demonstrated that Trim44 is up-regulated by EWSR1 and other ETS transcription factors result in dis-regulated transcription of the mouse TDAG51 gene, was the first apoptosis-related gene shown to be suppressed in several cases of malignant tumors. But, the apoptotic regulatory mechanism and its role is not fully known in gastric cancer. The purpose of the this study is to identify function of PHLD2 associated with apoptosis in gastric cancer. We used cell culture, western blotting, RT-PCR, MTT assays, PHLD2 knock-down with short hairpin RNA (shRNA). First, we confirmed that the expression level of PHLD2 was up-regulated by IGF1/IGF1 receptor, making it the worse subtype of all breast cancers. Currently, there are no molecular targeted therapies for this cancer and chemotherapy is only successful in a limited number of patients. Recent studies suggest that members of the oncopgenic PIM kinase family, especially PIM-1, play a significant role in the growth of TNBC. However, there is very limited information on the role of PHLD2 in gastric cancer. We aim the in vivo study to identify the role of PHLD2 in gastric cancer, which may be a possible target for developing new therapy.

#521 Dual-targeting of IGF-1R and ErbB3 pathways in Ewing’s Sarcoma cellular models with istiratumab (MM-141), a bispecific, tetravalent monoclonal antibody. Isabel Yannatos, Adam Camblin, Zhenhua Li, Michael Curley, Gege Tan, Christyl U. Louis, Vasileios Askoxylakis, Greg Finn, Birgit Schoeberl, Rachel Nering, Merrimack Pharmaceuticals, Cambridge, MA.

Ewing’s sarcoma (ES) is a rare cancer that often present as metastatic in bone and soft tissue and predominantly affect adolescents and younger adults. Current treatment for ES includes surgical resection followed by loco-regional radiotherapy and chemotherapy. Survival rates for patients with metastatic disease continue to offer a particularly difficult clinical challenge, with a five-year survival rate of 20-30% for these patients. ES is primarily a genetic disease caused by fusion between the 5' segment of the Ewing sarcoma breakpoint region 1 gene (EWSR1) on chromosome 22 and the 3' portion of Friend leukemia virus integration site 1 (FLI1) on chromosome 11. Fusions of EWSR1 and other ETS transcription factors result in dis-regulated transcription factors which promote malignant progression of ES tumors. Recent studies have shown that most ES cell lines and clinical samples express IGF-1R. Importantly, an activated IGF-1R pathway appears to be a prerequisite for malignant transformation by the EWS-FLI1 translocation, presumably via activation of the PI3K-AKT and MAPK pathways. These findings have led to the preclinical and clinical evaluation of multiple IGF-1R-targeted therapeutics with varying results. Clinical experience with anti-IGF1R targeting therapies has demonstrated striking antitumor activity in minor subsets of patients with ES. Importantly, the paucity of a clinically useful biomarker to select patients continues to hinder IGF-1R drug development in ES. Istitutumab is an investigational, bi-specific monoclonal antibody that acts as a tetravalent inhibitor of PI3K/AKT/mTOR, a major pro-survival pathway tumor cells use as a resistance mechanism to anti-cancer therapies. Istitutumab is designed to interfere with this pathway by blocking ligand-induced signaling through the IGF-1R and ErbB3 receptors, based on findings from preclinical in vitro and in vivo studies. To test the role of the IGF-1R and ErbB3 blockade, we will present data in multiple ES models demonstrating the importance of both IGF-1R and ErbB3 in this disease as a mechanism of growth and resistance. Furthermore, preclinical xenograft studies demonstrate that the combination of istitumab with an irinotecan-based chemotherapy regimen offers significant benefit over chemotherapy alone. These studies suggest that clinical evaluation of istitumab in ES is warranted.


Urothelial carcinoma of the bladder is a common malignancy that causes approximately 150,000 deaths per year worldwide. So far, no molecularly targeted agents have been approved for treatment of the disease. In the present study, we built an in-house oligonucleotide array, on which 394 genes were selected based on our SAGE data and previously reported array data, in order to identify the genes of most relevance to gastric carcinogenesis. Among these genes, we focused on SEC11A, because it is frequently associated with T category (p = 0.0002), Grade (p = 0.0425), vascular invasion (p = 0.0112) and CKS6/positive/ CK20 negative basal type BC (p = 0.0004). The univariate analysis indicated that expression of SPC18 (HR, 3.14; 95% CI, 1.47-7.47; P = 0.0027) was associated with overall survival. In the multivariate model, SPC18 expression was an independent prognostic indicator (HR, 2.94; 95% CI, 1.35-10.18; P = 0.012). BC cells transfected with SPC18 expression vector significantly induced the cell growth (p < 0.01) and invasion (p < 0.01) activity. Western blot showed the overexpression of SPC18 induced the phosphorylation of EGFR, Akt and Erk. Real time reverse transcription PCR analysis revealed the SPC18 forced expression cells induced mesenchymal character. These results suggest that SPC18 might contribute to the progression of BC.

#523 HGF mediated upregulation of PHLD2 is associated with apoptosis in gastric cancer. Sung Ae Koh, Kyung Hee Lee, Jae Ryong Kim, Sang Woon Kim. Yeungnam University Hospital, Daegu, Republic of Korea.

Pleckstrin homology-like domain family A member 2 (PHLD2), a homolog of the mouse TDAG51 gene, was the first apoptosis-related gene shown to be imprinted and expressed from the maternal allele in normal development. It is located within the tumor suppressor region of 11p15, and its expression is reported to be suppressed in several cases of malignant tumors. But, the apoptotic regulatory mechanism and its role is not fully known in gastric cancer. The purpose of the this study is to identify function of PHLD2 associated with apoptosis in gastric cancer. We used cell culture, western blotting, RT-PCR, MTT assays, PHLD2 knock-down with short hairpin RNA (shRNA). First, we confirmed that the expression level of PHLD2 was up-regulated by IGF1/IGF1 receptor, making it the worse subtype of all breast cancers. Currently, there are no molecular targeted therapies for this cancer and chemotherapy is only successful in a limited number of patients. Recent studies suggest that members of the oncopgenic PIM kinase family, especially PIM-1, play a significant role in the growth of TNBC. However, there is very limited information on the role of PHLD2 in gastric cancer. We aim the in vivo study to identify the role of PHLD2 in gastric cancer, which may be a possible target for developing new therapy.

#524 Characterizing the in vitro and in vivo effects of the PIM kinase inhibitor HS140 in triple-negative human breast cancer. Michael Cobb, Lucas Hunter, David Carlson, David Darr, Timothy Haystead, Antonio T. Baines, North Carolina Central Univ., Durham, NC; University of North Carolina at Chapel Hill, Chapel Hill, NC; Duke University, Durham, NC.

Triple-negative breast cancer (TNBC) lacks expression of the estrogen receptor (ER), the progesterone receptor (PR) and the ERBB2 (also known as HER2) receptor, making it the worse subtype of all breast cancers. Currently, there are no molecular targeted therapies for this cancer and chemotherapy is only successful in a limited number of patients. Recent studies suggest that members of the oncopgenic PIM kinase family, especially PIM-1, play a significant role in the growth of TNBC. However, there is very limited information on the role of PIM family members have in the growth and development of TNBCs. As an attempt to address this concern, we treated a panel of TNBC cell lines with HS140, a PIM-2 kinase inhibitor developed in the laboratory. Using cytotoxicity assays, we were able to demonstrate a decrease in anchorage-independent growth of cell lines at different concentrations of HS140. Also, a triple negative breast cancer GEMM (C3TAg) was used for an efficacy study. Mice (FVB/N background) have C(3)SV40 T-antigen resulting in inactivation of p53 and Rb. Nine mice received HS140 treatment (80mg/kg BIW IP) and 14 left untreated for control (non-treatment [NT]). All mice were monitored for weight loss and timespan to tumor development 5 times weekly. Tumor volumes at 21 days were similar (Mann–Whitney, p = 0.0082) reduced in the treated cohort with a mean of 109mm^3 (range of 0-500) compared to untreated 1393mm^3 (0-239). We observed no toxicities with body mass stable at the treatment dose of 80mg/kg. Additionally, three mice from untreated and three mice from treated cohorts were chosen for blood sampling pre- and post-treatment via a submandibular
bleed; no significant differences were noted in WBC, RBC, PLT, or HGB between untreated and treated cohorts. Overall, these results suggest that other members of the PIM kinase family, including PIM-2, have an important function in the growth and development of TNBC and may serve as a potential molecular target for future therapeutics.

#525 Role of CLCA2 in proliferation of certain growth-factor receptor dependent cancers. Yufang Yin, Aarushi Sharma, Randolph C. Elble. Southern Illinois Univ. School of Medicine, Springfield, IL.

The CLCA2 gene is downregulated in most adenocarcinomas, suggesting it is a tumor suppressor gene. However, we have observed that CLCA2 is upregulated in squamous carcinomas and Her2+ breast cancers. Such cancers also upregulate the related chloride channel Ano1, which is known to promote Her2.

Others have reported that Ano1 enhances EGFR signaling and that Ano1 knockdown confers sensitivity to EGFR inhibitors. Both EGFR and Her2 are known to transduce their mitogenic signal in part by causing release of intracellular calcium. We have found that CLCA2 activates Ano1 by upregulating intracellular calcium. Moreover, knockdown of CLCA2 reduced EGFR activation and cell proliferation while it enhanced sensitivity to the tyrosine kinase inhibitor lapatinib. These observations suggest that EGFR/Her2, Ano1, and CLCA2 comprise a feed-forward loop that amplifies growth factor signaling, so that certain cancer cell types maintain expression of all three.

#526 NFκB p65 overexpression promotes bladder cancer migration via FBW7-mediated degradation of RhoGDI protein. Yang Li, Junlan Zhu, Jingxia Li, Chuanshu Huang. NYU Nelson Institute of Environmental Medicine, Tuxedo Park, NY.

Since bladder cancer (BC) is one of the most lethal urological malignant tumors worldwide, understanding the molecular mechanisms that trigger the migration, invasion and metastasis of BC has great significance in reducing the mortality of this disease. RelA/p65, a member of the NF-kappa B transcription factor family, is increasingly recognized as a crucial player in many steps of cancer initiation and progression. In this study, we unexpectedly found that p65 protein expression increased in the bladder carcinoma N-butyl-N-(4-hydroxybutyl)-nitrosamine (BNB)-induced invasive bladder tumor tissues, and in human bladder cancer cell lines. We also observed that p65 overexpression promoted bladder cancer cell migration by inhibiting RhoGDI protein expression. We further demonstrate that the regulatory effect of p65 on RhoGDI protein degradation and promotons human BC cell migration. The identification of p65/PTEN/FBW7/RhoGDI axis provides a significant insight into understanding the nature of BC migration.


The growth and survival of prostate cancer tumors relies primarily on the functioning of the androgen receptor (AR) signaling pathway. Recent studies suggested that the presence of AR-V7, a c-terminal truncated form of AR, in circulating tumor cells (CTCs) of patients with castration-resistant prostate cancer (CRPC) is associated with inherent and/or acquired resistance to enzalutamide and abiraterone, the stand of care androgen deprivation therapies in prostate cancer. A series of data also indicate that AR-Vs (eg, including AR-V7, AR-373, AR-T878A, AR-F876L, etc) may drive resistance in CRPC. Expression of AR-V7 has been shown to correlate with disease progression and shortened survival. Considering that truncated AR splicing variants with C-terminal loss lack a functional ligand-binding domain (LBD) and are constitutively active, C-terminal AR-directed therapies may not be effective for patients with AR splicing variants. Circulating tumor cell (CTC)-based AR-V7 tests are currently being tested in the clinic. However, nearly half of the CRPC patients do not have enough CTCs for AR-V7 test, raising the request for a complementary, non-CTC platform to detect AR variants as well as other resistance markers in circulation. Here we report the development, validation and clinical application of PrediSeq-Prostate, a non-invasive next generation sequencing-based diagnostics platform that offers integrated genomic cell-free DNA (cfDNA) and cell-free RNA (cfRNA) profiling of prostate cancer, identifying known (such as AR-V7, AR-V9, AR-V2, etc) and novel AR splicing variants, point mutation, copy number, and translocation (such as TMPRSS2-ERG) using a single tube of blood. Using plasma samples from mCRPC patients that developed resistance to enzalutamide and/or abiraterone, PrediSeq-Prostate NGS test identified AR-V7, AR-V2, AR-V3, AR-V4 and other splicing variants that impact diagnosis and therapeutic selection. We also developed a Bio-Rad digital PCR assay measuring AR-V7 and AR-FL (full length) simultaneously. Collectively, PrediSeq NGS and ddPCR assays offer comprehensive genomic profiling of both cfDNA and cfRNA in all patients with prostate cancer, regardless of their status of CTC enumeration. The successful development and clinical validation of these tests has potential to enable precision medicine in prostate cancer.

#528 Evaluation of single agent merestinib (LY2801653) or emibetuzumab (LY2875358) and the combination in a xenograft tumor model bearing MET exon 14 skipping. Sau-Chi Betty Yan, 1 Suzane L. Um, 1 Victoria L. Peek, 1 Jennifer R. Stephens, 1 Wei Zeng, 1 Bruce W. Konicek, 1 Ling Liu, 1 Volker Wachcek, 2 Richard A. Walgren, 1 Eli Lilly and Company, Indianapolis, IN; 2 Eli Lilly and Company, Vienna, Austria.

Background: MET ex14 skipping, present in ~3% of lung cancer, is a strong oncogenic driver which is further evidenced by case reports of patients responding to MET TKI treatment. ~15% of tumors in patients that harbor MET ex14 skipping also have MET amplification (amp). Merestinib is a type II MET kinase inhibitor. Emibetuzumab, a bivalent MET Ab, internalizes MET receptor. Each single agent and the combination were evaluated in the Hs746t gastric cancer line bearing MET ex14 skipping and MET amp. Methods: Each agent was evaluated in vitro for inhibition of Hs746t cell proliferation and pMET levels. In vivo study in Hs746t-derived xenograft mouse model (n = 7 mice/ arm, 28 day dosing) initiated when tumors were 150-350mm3: merestinib at 6 mg/kg (suboptimal dose - insufficient target coverage for 24 hrs) or 12 mg/kg (optimal dose) q dr orally; emibetuzumab by IP. Results: Merestinib inhibited Hs746t cell proliferation with IC50 = 34 nM and totally eliminated pMET at 65-100 nM. Emibetuzumab slightly inhibited Hs746t cell proliferation (IC50 > 100 nM), reduced 20-20% cell surface MET, and no effect on pMET expression (at 130-650 nM). In the Hs746t xenograft model, merestinib (12 mg/kg) treatment resulted in 91.8% tumor regression after 21 day dosing, while 6 mg/kg merestinib provided transient tumor regression followed by re-growth while on treatment with T/C= 18.3% after 21 day dosing. No tumor re-growth was observed in 6/7 mice in the 12 mg/kg merestinib cohort during the 5 weeks post-treatment. Emibetuzumab treatment provided transient tumor regression (37.7%) after 3 doses, but tumors re-grew while on treatment. Combination of 6 mg/kg merestinib and 10 mg/kg emibetuzumab resulted in 85% tumor regression for the duration of the 28 day dosing period and the treatment was well tolerated. Tumors in animals re-grew upon termination of this combination treatment. Conclusion: Merestinib (12 mg/kg) treatment resulted in durable and complete response in 6/7 mice bearing Hs746t tumors with MET ex14 skipping and MET amp. When used singly. merestinib (6 mg/kg) or emibetuzumab (10 mg/kg) resulted in only transient tumor regression in this model, while the combination resulted in substantial tumor regression while on treatment. This combination treatment was however, not as durable as was observed with single agent 12 mg/kg merestinib. Single agents and the combinations were well tolerated. As a type II MET inhibitor, merestinib may provide a therapeutic option to treatment naive patients or those who have progressed on type I MET inhibitor treatment, whose tumors have MET exon 14 skipping and/or MET amplification.


#529 The significance of activated PI3K/akt pathway in FGFR3-TACC3 fusion positive cervical cancer. Ryo Tamura, 1 Kousuke Yoshihara, 1 Tetsuya Saito, 2 Ryosuke Ishimura, 2 Emmanuelle Martinez-Ledesma, 3 Yutaro Mori, 3 Kaoru Yamawaki, 1 Kazuaki Suda, 1 Tatsuya Ishiguro, 4 Yoichi Aoki, 4 Hiroaki Itamochi, 5 Masaaki Komatsu, 2 Roeland Verhaak, 3 Takayuki Enomoto 1. 1Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan; 2Department of Biochemistry, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan; 3Department of Genome Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX; 4Department of Obstetrics and Gynecology, Graduate School of Medicine, University of the Ryukyus, Okinawa, Japan; 5Department of Obstetrics and Gynecology, Tottori University School of Medicine, Tottori, Japan.

Uterine cervical cancer is one of the most common cancer in women worldwide, and the prognosis in advanced or recurrent cases remains poor because of the absence of effective molecular target therapies for this disease. The aim of our study is to identify and validate therapeutically targetable gene fusions in uterine cervical cancer, leading to the development of new therapeutic strategies. We have analyzed RNA sequencing data of 253 TCGA cervical cancer samples to search for gene fusions by using PRADA algorithm, and validated recurrent fusions in our...
Japanese dataset (n = 100) by RT-PCR and sanger sequencing. In TCGA samples, we detected 358 fusion transcripts and extracted 3 kinds of recurrent in-frame fusion transcripts (FGFR3-TACC3, ARL1B-ITPR1 and NNX-ABR). We focused on FGFR3-TACC3 fusion as a candidate of therapeutic kinase fusion and identified FGFR3-TACC3 fusion in 2 of 253 (0.8%) TCGA samples and 2 of 124 (1.6%) samples of a different pan-cancer study (Ma et al, 2017). FGFR3-TACC3 fusion is pathologically diagnosed as squamous cell carcinoma, showed high expression of both FGFR3 and TACC3 genes. To evaluate the possibility as therapeutically targetable fusions in uterine cervical cancer, we transfected FGFR3-TACC3, wild type FGFR3 and TACC3 transcripts to normal immortalized cervical keratinocytes derived from ectocervix (Ect/E6E7) and four uterine cervical cancer cell lines (SiHa, ME180, Hela, CaSkii). Continuous expression of FGFR3-TACC3 fusion transcript led to anchorage-independent growth in Ect/E6E7 and the dramatically alteration of cell proliferation in all cancer cell lines. On the other hand, no obvious phenotype change was observed in FGFR3 or TACC3 transfected cells. Western blotting analysis demonstrated that MAPK pathway was activated in all the transfected cell lines but PI3K/AKT pathway was activated only in ME180 and CaSkii harboring PIK3CA mutation. Fusion transfected cell lines exhibited high sensitivity to fusion specific siRNA and FGFR inhibitor compared to control. Although PI3K/AKT activated cell lines (ME180 and CaSkii) showed less effective against FGFR inhibitor compared to PI3K/ AKT neutral cell lines (SiHa and Hela), combined inhibition of FGFR and AKT had a synergic effect in PI3K/AKT activated cell lines. Our findings suggest FGFR3-TACC3 fusion is an oncogenic driver event and therapeutic target in a fraction of cervical cancer. When FGFR3-TACC3 fusion positive cervical cancer is treated by FGFR inhibitor, genomic background such as PI3K/AKT status should be considered.

The role of EGFR in c-fos-dependent osteosarcoma formation.

Markus Linder,1 Elisabeth Giltzner,1 Srijan Srivastava,1 Parastoo Shahrouzi,2 Latifa Bakiri,2 Monika Dumanic,1 Markus Mitterhauser,1 Erwin F. Wagner,1 Maria Sabiah1.1 Medical University of Vienna, Vienna, Austria; 2Spanish National Cancer Research Center (CNIO), Madrid, Spain.

The Epidermal Growth Factor Receptor (EGFR) is overexpressed or mutated in human carcinomas and glioblastomas, which are tumors of epithelial and glial origin, respectively. Some publications indicated that EGFR overexpression could occur in human osteosarcomas (OS). Using EGFR knockout mice (Egrf−/−), we have recently shown that EGFR plays a role in bone development and osteoblast function, raising the possibility that EGFR is also involved in OS formation. Here we show that Egrf−/− RunX2-Cre mice (EgrfΔ/Δ) lacking the EGFR in osteo-chondroprogenitor cells, developed an increased zone of hypertrophic chondrocytes in long bones and decreased bone formation. When bred to c-fos transgenic mice (H2-c-fosLTR) that develop osteosarcomas with 100% penetrance, Egfr−/−Runx2-Cre mice (Egfrf/f Runx2-Cre) mice (Egfrf/f) showed less effective against FGFR inhibitor compared to PI3K/AKT neutral cell lines (SiHa and Hela), combined inhibition of FGFR and AKT had a synergic effect in PI3K/AKT activated cell lines. Our findings suggest FGFR signaling is an oncogenic driver event and therapeutic target in a fraction of cervical cancer. When FGFR3-TACC3 fusion positive cervical cancer is treated by FGFR inhibitor, genomic background such as PI3K/AKT status should be considered.
The X-linked inhibitor of apoptosis protein (XIAP) is a well-known potent apoptosis suppressor, and also participates in cancer cell biological behaviors, therefore attracting great attentions as a potential antineoplastic therapeutic target for past years. We found here that lacking of XIAP expression resulted in a remarkable suppression of EGR expression, consequently leading to the deficiency of essential cell protein promoting tumor metastasis. The BIR domain of XIAP was crucial for regulating the EGR translation by suppressing the transcription and expression of miR-200a. Mechanistic studies indicated that BIR domain activated the PP2A activity by decreasing the phosphorylation of PP2A at Tyr307 in its catalytic subunit, PP2A-C. Such activated PP2A prevented the deviation phosphorylation and activation of MAPK kinases/ MAPs, their downstream effector c-Jun, and in turn inhibiting transcription of c-Jun-regulated the miR-200a. Collectively, our study uncovered a novel function of BIR domain of XIAP in regulating the EGR translation, providing significant insight into the understanding of the XIAP overexpression in the cancer development and progression, further offering a new theoretical support for using XIAP BIR domain and EGFR as targets for cancer therapy.

#534 PDGFRB gain-of-function mutations in multifocal infantile myofibromatosis: Implications for diagnosis & therapy. Florence A. Arts,1 Raf Sciortino,2 Bénédicte Brichard,1 Marleen Renard,2 Laura A. Noël,1 Amélie I. Velghe,3 Christine Galant,1 Maria Debiec-Rychter,2 An Van Damme,1 Miikka Vikof a polarized ex(o)citing machinery for cancer invasion. Christine Galant,1 Maria Debiec-Rychter,2 An Van Damme,1 Miikka Vikki,1 Rachel Haelers,1 Nisha Limaye,1 Hélène A. Poirel,1 Jean-Baptiste B. De Moulins1,1 University of Louvain, Brussels, Belgium; 2KU Leuven, Leuven, Belgium.

Myofibromas and infantile myofibromatosis are among the most prevalent soft tissue tumors of infancy and childhood. They are characterized by the presence of solitary or multiple nodules in the skin, subcutaneous soft tissues, bones, muscles and viscera. Multifocal myofibromatosis with visceral lesions is associated with a poor prognosis. The pathogenesis of sporadic myofibromatosis is unknown. A few familial cases have been linked to mutations in various genes including PDGFRB, which encodes a receptor tyrosine kinase that is highly expressed in fibroblasts, pericytes and other cells of mesenchymal origin. In the present study, we investigated whether the sporadic form of the disease may also be associated with PDGFRB mutations. We sequenced the whole coding sequence of PDGFRB in 20 cases of myofibromatosis or solitary myofibroma using the Ion AmpliSeq technology at high coverage (Life Technologies). Six different mutations in the coding sequence of PDGFRB were identified in seven patients, six of whom had the sporadic multicentric form of the disease, with a median age at diagnosis of 13 months (range: 0 to 7 years). Mutations were located in four different exons: the classical hot-spot exons 12 and 14, encoding the juxtamembrane and the kinase domains, but also the exons 11 and 18, encoding the transmembrane domain and the activation loop respectively. The percentage of mutated reads varied between 4 and 33%, suggesting that the mutations were somatic, except for one patient who had a germline R561C substitution and a somatic second hit. Two patients had the same mutation in multiple separated lesions, suggesting an early post-zygotic mutation in a progenitor cell. By contrast, a third patient had three different PDGFRB mutations in the three nodules analyzed. We showed that these mutations constitutively activated receptor signaling, mimicking the ETV6-NTRK3 activity, and we found that downstream pathways were activated in a cell-autonomous manner. Furthermore, the mutant receptors were sensitive to the tyrosine kinase inhibitor imatinib, except D850V, which was inhibited by dasatinib and ponatinib, suggesting a treatment for severe myofibromatosis. In conclusion, we identified activating PDGFRB mutations in 66% of sporadic multicentric infantile myofibromatosis cases, shedding light on the mechanism of disease development. Our results provide a genetic test to facilitate diagnosis, and preclinical data for development of molecular therapies.

#535 Paving the path to metastasis/invasion through GIV: Identification of a polarized ex(o)citing machinery for cancer invasion. Cristina C. Rohena, Nina N. Sun, Nicolas Aznar, Pradip Ghosh. University of California San Diego, San Diego, CA.

Polarized exocytosis is a fundamental process involving the delivery of membrane and cargo proteins to target sites the plasma membrane (PM) and it is essential for a wide range of biological functions such as cell growth, morphogenesis, and polarized cell migration. Polarized exocytosis requires precise spatial control of vesicle tethering to target sites at the PM. This is mediated by the octameric exocyst complex that is conserved from yeast to man. How the exocyst complex imparts polarity during exocytosis remained a mystery until recent insights emerged from studies on budding yeast. A functional interaction between exocyst component Exo70p and the yeast polarity determinant scaffold protein Bem1p was reported, and it was shown that Bem1p regulates the targeting of Exo70p to polarized exocytic sites. Despite these insights, the counterpart of Bem1p in higher species remained elusive. Here we demonstrate that a novel polarity-determinant protein called GIV (Go-interacting vesicle associated protein/Girdin) fulfills the key criteria and functions of its yeast counterpart Bem1p. GIV provides an evolutionary upgrade to the process of polarized exocytosis by making it responsive to growth factors and other external cues and by modulating the composition of the exocyst complex. Its interaction with GIV was originally identified in a yeast-2 hybrid screen as a binding partner of EXO1C1, and subsequently validated by us as a PI4P-binding protein that is a direct binding partner of mammalian Exo70. Biochemical assays have confirmed that the mechanism of GIV/Exo70 interaction bears close resemblance to the Bem1p: Exo70p interaction. Sequence alignment studies led to the identification of single point mutants in Bem1p and GIV that are selectively defective in binding to Exo70. Using genetic manipulation of cancer cells we have observed that GIV is essential for polarized tethering of Exo70-coated vesicles at the cell periphery. Consistent with the previously described prometastatic role of GIV, we found that GIV is required for EGF-stimulated exocytosis of matrix metalloproteases that aid in cancer invasion in MDA-MB-231 breast cancer cells and that specific point mutants of GIV that disrupt the interaction with Exo70 cause a reduction in the ability of these breast cancer cells to invade and degrade components of the extracellular matrix. Furthermore, GIV interacts specifically with TC-10GTP[active], a monomeric GTPase that regulates fusion of exocytic vesicles at the plasma membrane. Insights gained continue to provide an in-depth understanding of how GIV affects a fundamental process of exocytosis, and what impact that may have on cancer invasion and metastasis.

#536 Novel identification of STAT1 as a crucial mediator of ETV6-NTRK3-induced tumorigenesis. Jinah Park,1 Junil Kim,2 Poul H. Sorensen,3 Seong-Jin Kim1, 1Seoul National University, Gyeonggi-do, Republic of Korea; 2Perelman School of Medicine, Philadelphia, PA; 3British Columbia Cancer Research Centre, Vancouver, British Columbia, Canada.

Chromosomal rearrangements that facilitate tumor formation and progression through activating oncogenic tyrosine kinases are frequently observed in cancer. The ETV6-NTRK3 (EN) fusion has been implicated in various cancers, including infantile fibrosarcoma, secretory breast carcinoma and acute myeloblastic leukemia, and has exhibited in vivo and in vitro transforming ability. In the present study, we analyzed transcriptome alterations using RNA-Seq in ETV6-NTRK3 (EN) fusion-induced cells. Notably, KEGG pathway analysis identified the Jak-STAT signaling pathway with the highest statistical significance. Moreover, Ingenuity Pathway Analysis and gene regulatory network analysis identified the Stat1 transcription factor and its target genes as top EN-regulated molecules. We further demonstrated that EN enhanced STAT1 phosphorylation but attenuated STAT1 acetylation, thereby inhibiting the interaction between NF-kB p65 and acetylated STAT1. Consequently, nuclear translation of NF-kB p65 and subsequent anti-apoptotic NF-kB activity were increased, leading to the first time, STAT1 as a significantly EN-regulated transcription factor and a crucial mediator of EN-induced tumorigenesis. [This work was supported by a National Research Foundation grant of Korea (NRF-2014M3A9B5073918) funded by the Korea government.]


The molecular mechanisms that control transformation and tumorigenicity of human papillomavirus-associated (HPV +) head and neck squamous cell carcinomas (HNSCCs) are currently being investigated. The number of HPV + HNSCC cases has increased sharply in recent years, especially in oral squamous cell carcinomas. These HPV + HNSCCs often respond differently to treatments. Recently, The Cancer Genome Atlas (TCGA, 2015) identified novel loss-of-function genomic alterations of TNF receptor-associated factor 3 (TRAF3) in HPV + HNSCCs. TRAF3 is a ring-finger E3 Ubiquitin Ligase which inhibits downstream alternative NF-kB signaling and promotes anti-viral immunity by promoting degradation of ubiquitinated proteins. To assess TRAF3’s role in HPV + HNSCCs, we identified cell lines with lower level of TRAF3 protein, consistent with deficient TRAF3 expression identified in TCGA data. Functional studies showed that TRAF3 expression led to decreased steady-state protein levels of the alternative NF-kB pathway components RELB and NF-kB2/p52, as assessed by Western blot,
reporter assays and immunofluorescence. Additionally, TRAF3 expression led to decreased cell proliferation, tumorigenic activity and migration, and increased sensitivity to chemotherapy agent cisplatin. Interestingly, TRAF3 increases the steady state protein level of the classical tumor suppressors RB and p53 in HPV + HNSCC cell lines as assessed by Western blot. Further in vitro characterization of TRAF3’s function in HPV + HNSCCs was assessed using clinically identified TRAF3 loss-of-function mutagenic isoforms, mimicking both TCGA data and HNSCC cell line data for TRAF3 defects. In contrast to wtTRAf3, these mutant forms of TRAF3 do not as strongly restore p53 or inhibit RELB and NF-κB2/p52 protein levels. One specific mutation which causes a frameshift at residue 210 greatly inhibits TRAF3 function as assessed by Western blot. To further assess effects of wt or TRAF3 loss-of-function mutants in vitro and in vivo, an inducible HPV + HNSCC line expressing TRAF3 using the TET-ON system, and TRAF3 knockout in HPV + HNSCC line and Human Oral Keratinocyte (HOK) line using CRISPR-Cas9 genomic editing are generated. In conclusion, the ring-finger F3 ubiquitin ligase TRAF3 inhibits the pro-survival alternative NF-κB signaling pathway and restores TP53 and RB, thereby serving as a classical tumor suppressor in HPV + HNSCCs. (Supported by NIDCD intramural project ZIA-DC-000016, 73 and 74).

#538 The cytosolic domain of a disintegrin and metalloprotease (ADAM) 15 promotes non-small cell lung cancer (NSCLC) anti-apoptosis ability. Hsin-Han Hou, Chong-Jen Yu. National Taiwan University Hospital, Taipei, Taiwan.

Emerging evidence has indicated that proteins of a disintegrin and metalloprotease (ADAM) family contribute to cancer progression and metastasis. One member of this family, ADAM15, has been shown to be upregulated in multiple cancers, including gastric, lung, breast, and prostate cancers, and the enzymatic activities of its extracellular metalloprotease domain promote breast cancer proliferation and migration through mediating ErB signaling pathway. The patients with ADAM15 high-expressing lung tumors have shorter survival time and ADAM15 has been proved to enhance synovial fibroblasts anti-apoptosis ability via focal adhesion kinase signaling pathway. We firstly demonstrated other than extracellular enzymatic activity, the longest isoform of ADAM15 (ADAM15 i6), which contains the most cytoplasmic Src homology 3 (SH3) binding motifs, significantly upregulated in primary lung cancer tissues and promoted NSCLC proliferation via growth factor receptor-bound protein 2 (Grb2) and Src homolog 2 domain containing (Shc) association. In this study, we further explore the roles of ADAM15 cytosolic domain in NSCLC-apoptosis resistance. Overexpression of ADAM15 i6 promoted CL1-0 cell anti-apoptosis ability according to the trypan blue inclusion assay. Ablation of nephrocystin (NPHP1) attenuated the ADAM15 i6-promoted anti-apoptosis ability. Thus, we identified a novel mechanism of the ADAM15 cytoplasmic domain in NSCLC tumor progression, which will shed light on the molecular mechanisms of ADAM proteins, and facilitate development of novel therapy in NSCLC.

#539 MAEL promotes colorectal cancer cell growth and migration by activating EGFR pathway. Lulu Liu, Zhou Tong, Weiqin Jiang, Yi Zheng, Peng Zhao, Weijia Fang. The First Affiliated Hospital, Zhejiang University, Hangzhou, China.

Colorectal cancer (CRC) is one of the most common malignant tumors of the digestive system. It is generally accepted that CRC pathogenesis is a long-term process involving accumulation of multiple genetic alterations. Herein, we aim to explore the molecular biological mechanisms of CRC initiation and progression. We have been committed to investigating MAEL function in digestive neoplasms and have found MAEL is a new candidate oncogene. Previously, overexpression of MAEL was frequently detected in CRC tissues and significantly associated with poor 5-year outcome. By MTT assay and transwell assay, functional study demonstrated that overexpression of MAEL promoted cell growth and cell migration in DLD1 and TH8307 cell lines. Furthermore, overexpression of MAEL in DLD1 cell line could promote tumor formation in nude mice. Further study found that MAEL overexpression in DLD1 and TH8307 elevated the protein expression of p-EGFR, p-Raf-1, p-ERK 1/2, p-PI3K and p-AKT. These data indicated that MAEL enhanced the activity of EGFR/Raf/ Raf/MEK/ERK1/2 and EGFR/PI3K/Akt pathways. However, more effort is needed to investigate MAEL function and how MAEL activate EGFR pathways.


We hypothesized that the spatial arrangement of nuclei in a hematoxylin and eosin (H&E) stained image contains a vast quantity of latent information that is currently underutilized. We tested this hypothesis on the classification of breast cancer biopsies as estrogen receptor (ER) positive or negative. While determining ER status typically requires immunohistochemistry (IHC) staining, we aimed to recapitulate this step by predicting ER status directly from H&E stained slides. By training a deep neural network on nuclear-morphometric features, we demonstrated the power of machine learning to provide insights about cancer biology, and aim to scale precision oncology for the developing world. We constructed a learning pipeline that segments nuclei from H&E stained slides scanned into digital images, extracts shape and orientation descriptors, and uses a deep neural network to learn spatial features to predict ER status. After training the pipeline on 57 tissue cores of invasive ductal carcinoma (IDC), we were able to predict ER status in patients with IDC in a retrospective manner (AUC = 0.72, 95%CI = 0.65-0.80, n = 56) and ductal carcinoma in situ (AUC = 0.82, 95%CI = 0.74-0.89, n = 106). To our knowledge, this is the first work to show that nuclear morphometric information can be used to predict the molecular status of a breast cancer tumor. An initial analysis of the features learned by the deep neural network provides a glimpse into the mechanistic differences between ER+ and ER- cancers: suggesting the importance of spatial heterogeneity in distinguishing between ER+ and ER-. We anticipate that further analysis will provide deeper insights about the morphological differences between ER+ and ER- cancers, and that this type of analysis can be applied to other markers and cancers. Moreover, an optimized version of this pipeline may serve patients in the developing world by providing a means to predict marker status directly from H&E images.

#541 A comprehensive analysis delineating the immunotherapeutic terrain of cancer-related clinical trials. Cara M. Statz, Sara E. Patterson, Taoei Yin, Susan M. Mockus. The Jackson Laboratory for Genomic Medicine, Farmington, CT.

Technological innovations have facilitated a greater understanding of how the tumor microenvironment contributes to cancer, leading to rapid FDA approval of four immunotherapies. To assess how these therapies are being further investigated in combination with other therapies and in tumor types outside of the current FDA approval, we performed a comprehensive analysis of the curated clinical trials in the JAX Clinical Knowledgebase (JAX-CKB). In brief, clinical trial records from the JAX-CKB were queried for combination immunotherapy trials investigating Pembrolizumab, Nivolumab, Pembrolizumab and Ipilimumab, curated from clinicaltrials.gov, were queried in the JAX-CKB and then analyzed for comparison. Further analyses were executed to illustrate possible unmet needs within the field of cancer therapeutics. Of the four immunotherapies, Pembrolizumab was identified with the greatest number of clinical trials overall, with 305 compared to Atezolizumab, 79, Nivolumab, 183, and Ipilimumab, 126. Of the 305 trials the number of trials investigating Pembrolizumab, Atezolizumab, Nivolumab, or Ipilimumab in combination with another therapy was higher than those investigating one of the four immunotherapies as a monotherapy. Phase II trials for both single therapy and combinational therapies were demonstrated to be higher than both Phase I and Phase III for the same groups, regardless of therapy. On average, 12% ± 3.8% of the combined trials for all four drugs included any advanced solid tumor. Nivolumab combined with Ipilimumab demonstrated the greatest number of trials (61) investigating an immunotherapy in combination with another immunotherapy. Among those, 30 were Phase II trials, 16 were Phase I, and 14 were Phase III. Across five cancer indications (lung, pancreatic, ovarian, prostate, and colon), lung cancer was most commonly indicated in the trials, among all four drugs. Prostate was indicated in the least number of trials, with Ipilimumab ranking the highest (10). The recent success with immunotherapies has garnered significant interest in understanding how these therapies will perform in different tumor types and whether specific combinations will have a greater impact. Interrogation of the clinical trial terrain in the JAX-CKB provides a basis for determining additional investigations that might be warranted.

Recent advances in cancer immunotherapy and genomic sequencing technologies have created promising opportunities for precision cancer medicine. Somatic mutation in coding regions of cancer genomes may lead to amino acid alterations that generate immunogenic peptides, called neoantigens. These novel peptides are tumor-specific. An individual’s own MHC genotypes restrict the presentation of these tumor-specific peptides on their MHC molecules. We have determined the number of candidate epitopes for immune cellular recognition. Current studies have not comprehensively studied how MHC-restricted neoantigens are present across tens of thousands of cancer. Nor have these studies determined how somatic mutations and their associated neoantigens are represented among different subclonal populations existing within individual tumors. To address these limitations, we have mapped the complex immunogenomic topology of neoantigen epitopes across 8,000 tumors representing 18 different cancer types. This neoantigen landscape accounts for how neoepitope candidates are distributed among the various clonal subpopulations existing within any given tumor. Our study utilized genomic data from the Cancer Genome Atlas (TCGA). Our automated pipeline provides results from 8,000 samples from 18 cancer types that have somatic variant calls, copy number variation, whole exome sequence, and RNA-Seq data. Identification of somatic mutations that lead to highly immunogenic antigens involved five different steps: (i) in-silico translation of identified mutations, (ii) expression as measured per RNA-Seq, (iii) patient’s own major histocompatibility complex genotype, (iv) binding affinity specific to the MHC alleles for any given patient and (v) occurrence of clonal subpopulations as demonstrated by computational genotyping using the Tumor Clonal Evolution and Dynamic (T-CED) method. We mapped the neoantigen landscape with quantitative representation of the genetic clonal diversity existing within individual tumors. In average, 2% of missense mutations from a patient are detected in RNA-Seq, with binding affinity to own HLA genotypes, and from the dominant clonal subpopulation. In summary, our study demonstrates the various relationships among the number of optimal antigens, the number of clonal subpopulations, the number of mutations, and clinical phenotypes such as tumor stage. Our automated bioinformatics process facilitates the use of exome and RNA-Seq as potential diagnostic analytic process for precision immunotherapy.

#544 Hypoxia response signaling is linked to TACE resistance in hepatocellular carcinoma (HCC) patients. Valerie Fako,1 Joyce Lee,2 Tan-To Cheung,2 Irene O. Ng,2 Xin W. Wang,1 National Cancer Institute, Bethesda, MD;2University of Hong Kong, Hong Kong, Hong Kong.

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide, and outcome is dismal, due to tumor heterogeneity and lack of effective treatment options for patients with later stage disease. Transcatheter arterial chemoembolization (TACE) is the gold standard of therapy for patients with intermediate to locally advanced tumors. TACE delivers a high dose of chemotherapy directly to the tumor via the hepatic artery, followed by an embolizing agent to restrict tumor blood supply. However, tumor hypoxia is linked to alterations in metabolism, such as increased glycolysis (the Warburg effect), and can lead to enhanced cell survival. Several randomized control trials (RCTs) showed a survival benefit with TACE, but only with strict patient selection criteria. In Asia, TACE is also commonly used as an adjuvant therapy after surgical resection. Yet RCTs evaluating adjuvant TACE have shown conflicting results, likely due to patient selection and stratification. We hypothesize that tumor gene expression is predictive of response following TACE, and that differential cellular metabolism resembling a hypoxic phenotype prior to treatment is responsible for TACE resistance. We retrospectively analyzed gene expression data in treatment-naïve tumor tissue from a cohort of Chinese patients who received TACE. Using hierarchical clustering, followed by class comparison and survival risk prediction, we identified a 14-gene signature that is predictive of response vs. non-response to TACE, as measured by overall survival, independent of other clinical variables. We found that hypoxia- and glycolysis-related genes are enriched among differentially expressed genes in TACE Responders vs. Non-Responders, and that hypoxia master regulator HIF-1α and hypoxia target gene VEGF are significantly up-regulated in Non-Responders. We determined that a key glycolysis gene is up-regulated in Non-Responders, and conversely, two rate-limiting genes involved in gluconeogenesis, the pathway opposing glycolysis, are up-regulated in Responders. We also examined metabolomic data from the TACE cohort, and found an enrichment of glycolysis-related metabolites in Non-Responders, and gluconeogenesis-related metabolites in Responders. Further investigation will be required to connect altered glucose metabolism to TACE resistance and to determine driver genes linking hypoxia and metabolism, which, together with our 14-gene signature, may serve as a stratification tool to guide personalized treatment modalities for HCC patients.

#545 Prognostic alternative mRNA splicing signature in non-small cell lung cancer. Yuan Li,1 Nan Sun,2 Zhihui Li,2 Junhong Sun,3 Jiabing Huang,1 Zhaoli Chen,1 Jie He1,4National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China;4Department of Clinical Cancer Prevention, UT MD Anderson Cancer Center, Houston, TX.

Alternative splicing provides a major mechanism to generate protein diversity. Increasing evidence suggests a link of dysregulation of splicing associated with cancer. However, alternative splicing in non-small cell lung cancer (NSCLC) is largely unstudied. In this study, seven types of alternative splicing were profiled in 491 lung adenocarcinoma (LUAD) and 471 lung squamous cell carcinoma (LUSC) patients in TCGA using RNA sequencing data. Prognostic predictors for LUAD, LUSC and merged NSCLC patients were built by integrated survival analysis. Gene network and splicing correlation network analyses were also conducted. Overall, we detected 45,062 alternative splicing events were significantly associated with patient survival. The area under the curve (AUC) of the receiver-operator characteristic (ROC) curve for prognostic predictors in LUAD and LUSC were 0.960 and 0.940 at 2000 days of overall survival. Most relevant for clinical application, we also built the prognostic predictor for NSCLC patients with prediction the treatment outcome in cancer patients in multiple large-scale patient datasets including the TCGA, where SLIs successfully predict patients’ response for 75% of cancer drugs. Conclusions: ISLE is predictive of the patients’ response for the majority of current cancer drugs. Of paramount importance, the predictions of ISLE are based on SLi between (po-tentially) non-mutually exclusive TCGA subpopulations for patients whose tumors do not bear specific actionable mutations in cancer driver genes, offering a novel approach to precision-based cancer therapy. The predictive performance of ISLE is likely to further improve with the expected rapid accumulation of additional patient data.
high performance. The AUC of ROC curve in NSCLC was 0.817 which were also over 0.8 in LUAD and LUSC, separately. Moreover, expression of 49 and 9 survival associated splicing factors were significantly correlated with 75 survival associated alternative splicing events in LUAD and LUSC, respectively. Interestingly, splicing correlation networks uncovered that “onco- genic” splicing factors were up-regulated and “tumor suppressor” splicing factors were down-regulated in LUSC. Survival associated splice factors might have opposite roles in the regulation of RNA splicing in LUAD and LUSC. In conclusion, we created prognostic predictors based on alternative splicing events with high performances for risk stratification in NSCLC patients and uncovered interesting splicing networks in LUAD and LUSC which could be underlying mechanisms.

#548 RNAseq analysis of infiltrating immune cells in liver cancer. Krithika Bhuvaneshwar, 1 Coleman I. Smith, 2 Alexander H. Kromer, 2 Anu R. He, 2 Yurig Guses 1, 2 Georgetown University, Washington, DC; 3 Medstar Georgetown University Hospital, Washington, DC.

Hepatocellular carcinoma (HCC) has emerged as second most common cause of cancer deaths worldwide. During the last 10 years, there has been a clear delineation of landscape of genetic alterations in HCC and deregulated pathways in HCC. However, the treatment for patients with advanced HCC is limited despite of great effort developing therapeutic targeting the deregulated pathways in HCC. Recent studies reveal a direct causal relationship between cancer & immune dysfunction, whereby tumor cells and their microenvironment are able to evade immune attack by exploiting various immunoregulatory mechanisms in a process termed cancer immune editing. Methods: In this poster, the objective is to perform exploratory analysis of TCGA liver cancer data to see if immune infiltrates matter, and if they offer anti-tumor immunity to a cell. For this purpose, we analyzed RNA-seq data for a cohort of 75 liver cancer samples from TCGA collection. We obtained the gene expression data from a pre-selected group of specific markers for infiltrating lymphocytes (several subtypes), and explored the association of expression of these markers with clinical outcomes. We downloaded raw RNA-seq data from the TCGA Liver cancer collection from 75 patients. These included 25 patients who had Hepatitis B virus (HBV), 25 patients who had Hepatitis C virus (HCV) and 25 patients who had both viruses. After processing of raw data, we extracted isoform expression (TPM values) from specific markers for infiltrating lymphocytes. This data was stratified into ‘high’ and ‘low’ expression groups of patients by performing differential expression & pathway analysis, to see if the differentially expressed results were linked to immune pathways. We then performed survival analysis tests (Log rank, Cox regression) and Kaplan Meier (KM) survival graphs to explore the association with overall survival outcome. Results We found 14 of 75 HCC cases expressed CD8B isoform, while 61 of 75 HCC cases did not express CD8B isoform. Conclusion Additional immune cell subtype specific transcripts are being tested. Based on our preliminary analysis, we saw that most of the affected pathways were highly relevant to lymphocyte signaling & immune response and infiltration. Hence, exploring infiltrating lymphocytes can give evidence of immune surveillance against HCC. Testing immune cell specific transcript in tumor samples may service as predictor to treatment targeting immune evasion in cancer patients.

#549 Clustering analysis of next-generation sequencing T cell repertoire data in sipuleucel-T treated prostate cancer patients. Li Zhang, 1 Sounak Chakraborty, 1 Jason Cham, 1 David Oh, 1 Nadeem Sheikh, 1 Lawrence Fong 1.

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Immunotherapy has demonstrated significant clinical benefit in different cancers. T cells are a crucial component of the adaptive immune system and mediate anti-tumour immunity. Antigen-specific recognition by T cells is via T cell receptor (TCR) which is the product of somatic V(D)J gene recombination with the addition/subtraction of nontemplated bases at recombination junctions. Next generation TCR sequencing effectively profiles the TCR repertoire.
Currently TCR analyses quantify diversity across single clones, however, due to the low overlap of clones across samples, such analyses are limited to a single sample. Here we extend our previous analysis pipeline to track and examine TCR repertoire across time by focusing on V and J gene segments, overcoming limitations of previous studies demonstrated that FOXM1 expression was strongly correlated with poor prognosis of various cancers. The aim of this study is to define a signature consisting of FOXM1 and its associated genes and assess its prognostic potential in cancers including hematopoietic carcinoma (HCC) and others. Experimental procedures: We analyzed a gene expression profile of 100 patients with HCC to identify FOXM1-correlated genes (the FOXM1 signature) associated with the prognosis of HCC patients. The validity of the FOXM1 signature was verified in an independent HCC cohort (n = 242). Using the signature, we also analyzed different cancer types including pancreatic adenocarcinoma, lung adenocarcinoma, breast carcinoma, and bladder urothelial carcinoma to verify the association between the FOXM1 signature and prognosis. Various statistical methods were applied to signature finding and validation. Upstream regulator and gene-to-gene network analyses were performed to identify potential key mediators of the FOXM1 signature. Results: Through a gene expression profiling in 100 patients with HCC, we identified a gene set consisting of FOXM1 and its co-expressed genes for predicting poor prognosis. A predictive value of the signature was validated in an independent cohort containing 242 HCC patients. In multivariate analysis, the FOXM1 signature showed the most significant prognostic value (HR = 1.706, 95% CI = 1.176-2.475, P = 0.005). By directly applying the signature to four data sets in different cancer types, our signature showed a consistent prognostic significance, regardless of tumor types. Finally, upstream regulator and gene-to-gene network analyses found an interconnection of network hubs composed by FOXM1, MYC, and E2F1 that might be common key mediators of patient survival in cancer. Conclusion: The FOXM1 signature represents a promising common diagnostic tool to identify high-risk cancer patients and may extend treatment options in various cancers.


Anticancer immunotherapies target immune cells to block immune suppression and/or promote immune activation in order to eliminate cancer cells. Ipilimumab and Nivolumab, targeting CTLA4 and PD1 respectively, have demonstrated dramatic responses in melanoma and lung cancer (Hodi et al. 2010; Rivi et al. 2015). However, poor response rates in other types of cancer under-score the need to better understand immunomodulatory mechanisms (Topalian et al. 2012). Examination of immune cell specific signals, in either the tumor microenvironment or the periphery, has proven to be a useful tool in developing prognostic biomarkers (Chu et al. 2014; Gentles et al. 2015), inferring mechanisms of action (Krekic et al. 2016), and discerning immune-based predictors of drug response (Gong et al. 2012; Angelova et al. 2015). We used publicly-available gene expression microarray data from blood, normal tissue, and tumor samples to assess the effectiveness of these methods in sample types relevant to oncology research. The results from comparisons of these different methods demonstrate that certain approaches are significantly more robust to noise, and are therefore more suitable for complex cellular mixtures such as tumor samples. The discerning use of methods to infer immune cell type proportions from gene expression profiles may lead to improved prognostication, predictive biomarkers for immunotherapy to assist in patient stratification, and new immunomonitoring targets by indicating immunosuppressive mechanisms.

Multi-scale omics integration using parallel heatmap clustering for the systemic analysis and biomarker discovery of drug sensitivity in lymphoma cell lines. Ivo Kwee,1 Andrea Rinaldi,2 Alberto J. Arribas,2 Eugenio Gaudio,2 Chiara Tarantelli,2 Filippo Spriano,2 Petra Hillmann,3 Francesco Berloni4. 1Institute of Oncology Research & Dalle Molle Institute for Artificial Intelligence, Bellinzona, Switzerland; 2Institute of Oncology Research - IOR, Bellinzona, Switzerland; 3Pfizer Research and Development, Collegeville, PA; 4Pfizer Early Development, Collegeville, PA.

Background. Pharmacogenomics studies the role of genetics in drug response. By measuring the individual genome, it is hoped that pharmaceutical drug treatments can deviate from a ‘one-dose-fits-all’ approach to a more ‘personalized’ treatment. To achieve this goal, we need to explain which genomic differences cause the lack of response to be able to predict the response from baseline omics data, that is from data available before starting treatment. Here, we present an integrated omics approach to analyze drug sensitivity based on in vitro experiments. Methods. We measured the drug response in 61 lymphoma cell lines for a number of anti-cancer drugs using a standard MTT cell proliferation assay. The baseline genomics of these cell lines were fully profiled for gene expression, copy number and methylation. Pathway and gene set signatures were computed using GO, KEGG, Biocarta and lymphoma databases to provide gene set level features. Additionally, we computed high level ‘biological concept’ features. These multi-scale features were directly correlated with drug sensitivity but also correlated between the multiple data types. Using a novel parallel clustering approach, we integrated our data for the different omics types and at different scales: gene, gene set and concept level. Finally, a shortest path algorithm was applied to infer the most probable explanation between genotype and response across subjects directly. The published data of TCR profiling of serial samples. Here we extend our previous analysis pipeline to track and examine TCR repertoire across time by focusing on V and J gene segments, overcoming limitations of previous researchers using a variety of mathematical approaches, including least squares (Abbas et al. 2009), quadratic programming (Gong et al. 2011; Zhong et al. 2013), maximum likelihood (Qiao et al. 2012; Liebler et al. 2013), machine learning (Newman et al. 2015), and enrichment type approaches (Angelova et al. 2015). We used publicly-available gene expression microarray data from blood, normal tissue, and tumor samples to assess the effectiveness of these methods in sample types relevant to oncology research. The results from comparisons of these different methods demonstrate that certain approaches are significantly more robust to noise, and are therefore more suitable for complex cellular mixtures such as tumor samples. The discerning use of methods to infer immune cell type proportions from gene expression profiles may lead to improved prognostication, predictive biomarkers for immunotherapy to assist in patient stratification, and new immunomonitoring targets by indicating immunosuppressive mechanisms.
and somatic. Recently, understudied repetitive DNA regions called microsatellites have been identified as genetic risk markers for a number of diseases including various cancers (breast, ovarian and brain). In this study we demonstrate an integrated process for identifying and validating microsatellite based risk markers for lung cancer using data from the cancer genome atlas (TCGA) and the 1,000 genomes project. We compared whole exome germline sequencing data from 488 TCGA lung cancer patients to germline exome data from 390 control samples from the 1,000 genomes project, we identified 119 potentially informative microsatellite loci. These loci (risk markers) were found to be able to distinguish between cancer and control samples with sensitivity and specificity ratios over 0.8. Then these loci, supplemented with additional loci from other cancers and controls, were evaluated using a custom target design through in-plexed nextgen sequencing. Thirteen of the 119 risk markers were validated using high-depth (579x±315) nextgen sequencing of 30 lung cancer and 89 control samples, resulting in sensitivity and specificity ratios were 0.90 and 0.94, respectively. When 8 loci harvested from the bioinformatic analysis of other cancers are added to the classifier, then the sensitivity and specificity rise to 0.93 and 0.97, respectively. Analysis of the genes harboring these loci revealed two genes (ARID1B and REL) and two significantly enriched pathways (chromatin organization and cellular response to stress) which suggest that the process of lung carcinogenesis is linked to chromatin remodeling, inflammation, and tumor microenvironment restructuring. We illustrate that high-depth nextgen sequencing enables a high precision microsatellite-based risk classifier.

#554 Accurately identifying neoantigens utilizing both DNA and RNA somatic variants in an enhanced platform. Sean M. Boyle, Jason Harris, Gabor Bartha, Ravi Alla, Miriam Karbelashvili, Steve Chervitz, Aldrin Montana, Craig Rowell, Patrick Jonganeel, Scott Kirk, Rena McClory, John West, Rich Chen.

Personalis, Menlo Park, CA.

The identification of neoantigens is a crucial step in the development of neoantigen-based personalized cancer vaccines and other immunotherapies. Accurately predicting which neoantigens are likely to be immunogenic remains a key challenge owing to the complex processes involved in determining neoantigen immunogenicity including the antigen presenting machinery, likelihood of MHC class I and II binding, similarity to self, and ability to interact with the TCR. We have developed a neoantigen detection pipeline built upon our ana-
ytically validated Accuracy and Content Enhanced (ACE) Exome and Tran-
scriptome sequencing platform and somatic variants calling pipeline through combined DNA and RNA analysis. The analytical performance of these pipe-
lines is greater than >97% sensitivity for small variants (RNA and DNA) with a specific-
ity of >98% (DNA) and a fusion sensitivity of >99% (RNA). Within our
neoantigen pipeline, variants that are detected by our DNA and RNA cancer analysis pipelines are processed for antigen identification, including SNVs, indels, and fusion events. Importantly, both in-frame and out-of-frame events are accurately considered by transcript, allowing for detection of a wealth of candid-
ate neoantigens. Our pipeline includes assessment of important immunologic components including HLA prediction, MHC binding (class I and II), immu-
nogenicity, similarity to self, and similarity to known antigens. Additionally, peptides are evaluated for variant allele frequency in both the RNA and DNA of the tissue of interest and expression levels. Consideration of the ImmunoIDP product provides a comprehensive assessment of features that may be used for identifying and ranking potentially immunogenic neoantigens. To assess the effectiveness of this pipeline in predicting immunogenic neoantigens, we assembled a gold-set of 23 known, previously experimentally-validated immu-
nogenic neoantigens from the literature. We spiked in these neoepitopes into exome data and assessed the ability of our neoantigen pipeline to find and rank these immunogenic known neoantigens. Preliminary results show our neoanti-
gen pipeline is able to accurately identify 22 out of 23 (~96%) of the spiked in neoantigens as being potentially immunogenic.

#555 Examination and evaluation of MR radiomics features for character-
ization of dominant intraprostatic lesions. Hassan Bagher-Ebadian, Janic Penistilava, Chang Liu, Milan Pantelic, David Heershen, Indrin Chetry, Moh-
hamed Elshaikh, Benjamin Movvas, Ning Wen. Henry Ford Hospital, Detroit, MI.

Purpose: This pilot study investigates a set of radiomics features extracted from fast relaxation fast spin echo (FRFSE) T2 pulse sequences for normal tissue and Dominant Intraprostastic Lesions (DILs) in twenty prostate cancer patients. Material and Methods: Twenty patients with prostate cancer were studied. All patients had axial FRFSE T2 scans using a 3 Tesla scanner. A radiologist inter-
preted MR examinations, and contoured the suspicious DIL and the contrat-
eral section of the prostate gland (normal) on the T2 weighted MR images. Patients underwent a 14-core transrectal Ultrasound Guided Biopsy and local-
ization of positive cores, Gleason score and clinical tumor stage were recorded. 167 radiomics features were extracted from normal and DIL zones. These fea-
tures were categorized into 8 different sets as following: Intensity Histogram (IHB), Gray Level Run Length (GLRL), Law’s Textural Information (LAWs), Discrete Orthonormal Stockwell Transform (DOST), Local Binary Pattern (LBP), Discrete Orthonormal Wavelet Transform (DOWT), Discrete Orthonormal Fourier Transform (DOFT), and Discrete Orthonormal Spherical Gabor Filter (2DGF), and Gray Level Co-Occurrence Matrix (GLCM) with 8, 7, 18, 6, 48, 40, and 22 features in each category respectively. A Welch’s test and the Fisher method were used to test for signifıcant differences among the 167 radiomics features and their subcategories. For all patients, cor-
relation coefficients between the extracted features in the normal and DIL zones were calculated using the combined p-values among the eight categories of radiomics features, only 5 feature categories showed a significant difference (IHB, GLRL, DOST, LBPF and GLCM with P został<i>/H11000</i> value of 2.0×10^-6, 0.02, 12.1×10^-7, 3.7×10^-3, and 1.5×10^-8), respectively. Among all 167 features, only 7 showed a significant difference (D=100x(DIL-NP-1)) and small correlation between normal and DIL zones: IHB-Skewness (r=0.19, p=0.03, and D=50.3%), GLCM-Contrast (r=0.12, p=0.03, and D=67.5%), GLCM-Dissimilarity (r=0.12, p=0.01, and D=67.5%), GLCM-Entropy (r=0.07, p=0.01, and D=67.1%), GLCM-Difference Variance (r=0.12, p=0.01, and D=67.1%), GLCM-Difference Entropy (r=0.10, p=0.01, and D=60.4%), and GLCM-Information-Measure-of-Correlation (r=0.25, p=0.01, and D=65.1%). Conclusion and Discussion: This pilot study demon-
strates the feasibility of using radiomics features from MR images to characterize DILs in prostate cancer patients. Among 167 radiomics features extracted from axial MR T2 FRFSE, 7 features were shown to be potentially significant for distinguishing normal tissue from DILs. This research supports an integrated decision making system, combining clinical factors and radiomics features extracted from MR images, for increasing the DIL detection performance in prostate cancer studies.


NCBI’s Conserved Domain Database (CDD, https://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml) is a resource that aims at classifying ancient conserved domain families and capturing what is known about their functional diversity. It is a collection of multiple sequence alignments (MSAs) and derived position-specific score matrices (PSSMs). RPS-BLAST allows for the rapid identification of conserved protein domain footprints in protein sequences along with the location of annotated functional sites. CDD is a redundant collection, it imports other model collections (Pfam, COGs etc) and supplement with manually-curated domain models that are organized into hierarchical clas-
sifications, which facilitate annotation of functionally distinct protein families and subfamilies. More recently CDD has been curating information on con-
served domain architectures to support functional labeling of proteins that fall into characterized families: SPARCLE (Subfamily Protein Architecture Label-
ing Engine) is both a curation tool and a public service available at https://
www.ncbi.nlm.nih.gov/sparcle. Architecture summaries provide interactive in-
terfaces to lists of proteins that share the same characteristic domain architecture, and to their assigned names, functional labels, and supporting ev-
idence. Here we present the functionally annotated, evolutionary hierarchical classification of the seven-transmembrane G-protein coupled receptors (7TM GPCRs), which includes the largest family of promising therapeutic targets, with more than 140 human orphan GPCRs of unknown function. Across the three kingdoms of life, GPCRs are involved in a wide array of critical physiological processes, and have been implicated in tumor initiation, tumor progression, angiogenesis and metastasis. Orphan subfamilies with unknown endogenous ligand/function are often found to have poor sequence similarity to canonical GPCRs. They have been assigned putative functions with predicted ligand and bind-
ing sites, downstream interacting partners, and/or the location of 7TM helices annotated by inference from the molecular and physiological functions of known related GPCR proteins, utilizing more than 25 available 3D-structures, phylogenetic relationships, and the published literature. We hope that this up-
dated classification, together with NCBI’s software tools (Cn3D/CDTree and SPARCLE), will aid researchers in the discovery of molecular therapeutic targets for cancer treatment by providing insights regarding as-yet-undefined intra-
and intermolecular interactions as well as functional mechanisms of GPCR sig-
naling. Acknowledgement: This research was supported by the Intramural Re-
search Program of the National Library of Medicine, NIH.
SPOP mutant subclass prediction and its impact on prostate cancer prognosis. Deli Liu. Weill Cornell Medicine, New York, NY.

SPOP mutations define a distinct and key molecular class of prostate cancer, but there is no signature to identify SPOP mutant subclass based on transcriptional data, and its impact on prognosis and clinical outcomes of prostate cancer patients is unknown. We developed the SPOP mutant transcriptional signature with high sensitivity and specificity based on transcriptional data. After we predicted SPOP mutant subclass from a large cohort project with transcriptional signature, we studied its association with clinicopathologic variables and prognosis in prostate cancer patients. On univariate analysis, SPOP mutant subclass was associated with lower preoperative serum prostate-specific antigen, extraprostatic extension, and older patients (p < 0.05). On multivariate analysis, SPOP mutant subclass was associated with metastasis (p < 0.05). Combined SPOP mutant status and CAPRA-S models have slightly better prediction for metastasis. On Kaplan-Meier analysis, we found the significant association of SPOP mutant subclass with better patient outcomes of metastasis, and patients without radiation therapy with even better metastatic outcomes. The SPOP mutant subclass could be predicted by using our SPOP mutant signature purely based on transcriptional data. Clinical and prognosis difference were found among SPOP mutant subclasc.

Computational analysis of clinically actionable genomic features: precision heuristics for interpreting the alteration landscape (PHIAL). Brendan Reardon,1 Nathanael Moore,2 Eliezer VanAllen.1 Dana-Farber Cancer Institute, Broad Institute, Harvard Medical School, Boston, MA; 2Dana-Farber Cancer Institute, Broad Institute, Indiana University University of Medicine, Harvard Medical School, MA.

Background: PHIAL (Precision Heuristics for Interpreting the Alteration Landscape) was developed as a heuristic clinical interpretation algorithm for cancer genomic data to inform treatment decisions at the point of care and provide researchers with rapid assessment of tumor actionability. This approach used somatic whole exome sequencing data and a database of tumor alterations relevant for genomics driven therapy (TARGET). However, PHIAL was limited to first order genomic relationships, could not distinguish relative actionability given multiple actionable variants, did not maximize the richness of somatic-germline interactions, and could not leverage both exome and transcriptome data to move towards feature-based actionability. Towards that end, we developed a new interpretation methodology to address these areas and improve clinical actionability algorithms. Methods: We revised PHIAL to predict actionable alterations based on the presence of SNVs (in the context of allele specific expression from RNA-seq), indels, SCNAs, fusions, and global features (e.g., context-specific mutational burden) that imply actionability. Additionally, we refined and expanded the TARGET database to enable PHIAL to produce scores on multiple dimensions and reflect newly discovered relationships between genomics and clinical actions. Predictive implication values were assigned to reflect the validities of TARGET’s drug sensitivity, drug resistance, and prognostic claims. Results: We applied both the original (PHIAL1) and an updated version of PHIAL (PHIAL2) to a 255 patient cohort with whole exome/transcriptome sequencing data (146 castration-resistant prostate cancer and 109 metastatic melanoma samples). PHIAL1 identified 1,342 clinically actionable biologically relevant events across the cohort with a median of 3 events per patient and 95% of patients having at least one event. PHIAL2 identified 2,508 events, with a median of 6 events per patient and 98.5% of patients harboring at least one event. Of these events, 8.12% were associated with an FDA-approved therapy and 2.09% with a clinical trial. PHIAL2 identified events in 9 patient samples that PHIAL1 associated with no events. Conclusion: PHIAL2 was able to identify and rank more putatively actionable alterations than PHIAL1, and effectively transitioned from a variant-based to a feature-based approach. This strategy may inform the utility of point-of-care whole-exome/transcriptome sequencing in larger contexts as these data emerge in clinical settings, and may bridge towards machine learning based approaches as patient outcomes are linked to genomic and transcriptomic features. Finally, PHIAL2 may ultimately provide a deeper understanding of, and suggest clinical actions for, cases in which there is no clear single genomic alteration associated with oncogenesis.

Estimation of immune cell content in bulk tumour tissue using reference profiles from single-cell RNA-seq data. Max Scheller,1 Jinyan Du,1 Sonia Feau,1 Edda Klipp,2 Birgit Schoeberl,1 Gavin MacBeath,1 Andreas Raue,1 1Merrimack Pharmaceuticals, Inc., Cambridge, MA; 2Humboldt-Universität zu Berlin, Germany.

Although therapeutics that modulate the immune system provide remarkable benefit for many cancer patients, predicting who will respond remains an unsolved problem. As interactions between the immune system and cancer are governed by a complex network of cell-cell interactions, knowing the immune cell composition of a solid tumour may be essential in predicting response to immunotherapy. Here, we describe how to derive the cellular composition of a solid tumour from bulk gene expression data by mathematical deconvolution, using consensus cell type-specific gene expression profiles from previously identified single-cell RNA sequencing data. Notably, successful deconvolution depends on these new data, as previously-available profiles from peripheral blood are insufficient. The presented method makes the problem of obtaining a patient’s tumour immune cell composition from existing databases like The Cancer Genome Atlas as well as in the clinical setting computationally tractable.


True integration of clinical staging and molecular prognostic biomarkers has been hampered by a lack of comprehensive data sets with adequate power. In the last two years, The Cancer Genome Atlas (TCGA) has done and made available a repository of high, high volume -omics datasets including molecular profiling together with matched clinical data. Methods: Clinical and transcriptomic data were downloaded for TCGA datasets (38 projects) representing a total of 14,043 patients. Matched expression and clinical data was available for 34 cohorts, covering 10,554 patients (median n = 270, range 15-1091) and survival data was available for 24 cohorts (7854 patients, median 294 per cohort), with a median follow-up of 10.7 months (range 1.9 to 61 months). Prediction models were constructed based on the latest AJCC staging guide, a novel five gene molecular prognostic signature specifically derived for each cancer type, and a combined model utilising both. Accuracy of survival prediction was calculated as the area under the receiver operator characteristic curve (AUC), based on 2-year overall survival prediction. Results: Pan-cancer analyses demonstrate accuracy of clinical staging from 0.55-0.91 (median 0.72), while molecular prognostication varies from 0.7-0.99 (median 0.84). The combined model is consistently more accurate, 0.71-0.99 (median 0.89, p < 0.01), where the benefit of adding molecular data improved accuracy (median + 0.13, +0.03-0.36). The absolute benefit of adding molecular data to clinical staging correlated more closely with strength of clinical prediction (R²=0.35) than strength of molecular prediction (R²=0.08). When clinical staging was more less accurate (clinical prediction < 0.7), there was a greater contribution of molecular data, compared to cancers with more accurate clinical staging (mean + 0.15 vs + 0.07, respectively; p=0.015). Interestingly, of the six tumours with poor clinical prediction

Viruses are a major contributor to oncogenesis, causing 10-15% of human cancers. Molecular pathways involved in malignant transformation are frequently activated by genetic alterations, including but not limited to, somatic mutations, copy number aberrations, structural variants, and oncoviruses. Precision cancer medicine aims to classify tumors by site, histology, and molecular tests to determine an "individualized" profile of cancer alterations. However, clinical tests for these various alterations are sequential, time consuming, and use a lot of material, which is often quite limited (e.g., biopsies). Moreover, tests for the presence of viral sequence are generally performed separately to tests such as massively parallel sequencing to detect human genomic alterations. Here we present a hybrid capture and massively parallel sequencing approach to detect viral infection concurrently with targeted genomic analysis, which may decrease assay costs, increase sensitivity and scalability, and detect many types of alterations, thereby providing a more complete tumor genetic profile all from a single sample. We have created a custom hybrid capture probe set for targeted Illumina sequencing to determine whether oncoviruses are present in tissue samples and also determine if the virus has integrated into the host's genome. We have created both detection and integration baits for several oncoviruses, including polyomaviruses, human papilloma viruses, Epstein-Barr virus, human cytomegalovirus, Kaposi sarcoma herpeticus, human T-lymphotropic virus, and hepatitis B virus. To distinguish between different strains of a single virus, strain-specific detection baits were created to bind to variable regions of viral genomes. The integration bait was designed to bind to regions of the viral genomes that are commonly integrated into the human genome. This baits set can also be combined with other capture panels targeting oncogenes to simultaneously determine integration and infection statuses, all from a single sample.

We have tested our techniques on tissue samples that were infected with either Merkel Cell Polyomavirus or Epstein-Barr virus, as determined using quantitative polymerase chain reaction (qPCR) or immunohistochemistry (IHC) techniques, and have successfully detected these viruses and identified viral integration loci. Overall, this viral hybrid capture probe set provides the ability to simultaneously determine a tissue sample's infection and viral integration status alongside other somatic genomic analyses, saving both time and sample material.

#563 Quantitative EMT expression score for predicting survival outcome. Jason T. George, Mohit K. Jolly, Herbert Levine. Rice University, Houston, TX.

Tumor aggressiveness and subsequent metastasis still remain as major limitations to curative treatments for cancer patients. The epithelial to mesenchymal transition (EMT) is a process that occurs naturally during embryogenesis and has been linked to metastasis in many cancer types. EMT is characterized by phenotypic changes that allow for tissue extravasation and migration of cancer cells into the bloodstream. These changes may be linked to specific patterns in the gene expression signature of cells undergoing EMT. Recent theoretical efforts have predicted the existence of a stable, hybrid (E/M) phenotype which has also been observed experimentally in single cells. However, the effects of this hybrid phenotype on cancer patient survival have not been well characterized. The ability to quantify a patient's EMT status via a simple test involving a small collection of prognostic genes would provide an important tool for clinical research and treatment in the context of many cancer types. Here, we apply unbiased computational methods to generate a hybrid EMT score for samples. We use this score to characterize the degree of the hybrid phenotype signature present in test samples. Predictions from our model are verified against cell lines with known EMT status. Lastly, we apply our model to clinical samples in order to assess survival differences in various EMT groups. We demonstrate that in many cases, EMT status successfully classifies patients into groups with statistically significant differences in survival, which is of immediate clinical relevance.

#564 Binary classification of superparamagnetic relaxometry data for cancer screening. Javad Sovizi, Sara L. Thrower, David Fuentes, Wolfgang Stefan, John D. Hazle, Kelsey Mathieu. The University of Texas MD Anderson Cancer Center, Houston, TX.

Introduction: Superparamagnetic relaxometry (SPMR) is an emerging technology that holds potential for use as a second-line screening modality to improve early cancer detection. During SPMR scanning, targeted superparamagnetic iron oxide nanoparticles (SPIONs) specifically bind to cancer cells and their spatial distribution can be characterized by measurement of the magnetic field relaxation following a brief excitation pulse. Highly sensitive superconducting quantum interference devices (SQUIDs) detect relaxor clusters of SPMR signals, and changes in the relative intensity and spatial distribution of SPMR signals indicate the presence of tumors. We use a data-driven approach based on Gaussian process (GP) formulation tailored to SPMR datasets to systematically quantify the probability of cancer. In silico, we simulated the SPION uptake process and generated SPMR signals that closely resembled experimental data collected in mouse models of cancer. We investigated the classification accuracy for different amounts of SPION accumulation within the tumor, as well as different levels of measurement noise (coefficient of variation (CV)). In a phantom study, a mouse liver was simulated by clustering together 40 to 80 different locations. Moreover, 10 datasets were collected without using the tumour phantom to represent the expected signal from healthy mice. In each iteration, the background SPMR phantom signals were randomly distributed within the scan plane. Results: Our in silico analysis for tumor accumulations of 3% and 5% of the injected SPION dose achieved 87% and 97% classification accuracies, respectively, when CV = 0 and 75% and 93% when CV = 0.015. Similarly, in our phantom study, classification accuracies of 87.5% and 96.4%, respectively, were reported for the 9.4 and 14.4 μg of immobilized SPIONs. An additional nine cotton swabs containing 32.3 μg of immobilized SPIONs (<5 μg per phantom) were evenly distributed within the scan plane to represent background SPIONs not bound to the tumor or liver. For each of these nine phantom tumors, 18 datasets were collected using a magnetic relaxometry device (Senior Scientific LLC) by moving the phantom to 18 different locations. Moreover, 10 datasets were collected without using the tumor phantom to represent the expected signal from healthy mice. In each iteration, the background SPMR phantom tumors were randomly relocated within the scan plane. Results: Our in silico analysis for tumor accumulations of 3% and 5% of the injected SPION dose achieved 87% and 97% classification accuracies, respectively, when CV = 0 and 75% and 93% when CV = 0.015. Similarly, in our phantom study, classification accuracies of 87.5% and 96.4%, respectively, were reported for the 9.4 μg and 14.4 μg of immobilized SPIONs. Conclusion: Using a data-driven GP model, tumor-status classification accuracies of up to 96.4% were achieved in SPMR phantom datasets. In the future, we plan to evaluate the accuracy of our classifier in preclinical settings using animal datasets.

#565 Analytical performance of TruSight Tumor 170 in the detection of gene fusions and splice variants using RNA from formalin-fixed, paraffin-embedded (FFPE) solid tumor samples. Tingting Du, June Snedicor, Jennifer S. LoCoco, Xiao Chen, Laurel Ball, Allan Castaneda, Danny Chou, Katie Clark, Brian Crain, Anthony Daulo, Manh Do, Sarah Dummm, Yonmee Han, Mike Havern, Chia-Ling Hsieh, Tingting Jiang, Suzanne Johansen, Scott Lang, Rachel Liang, Jaime McLean, Yousef Nasirri, Austin Purdy, Jason Rostron, Jennifer Silhavy, Natasha Talago, Li Teng, Kevin Wu, Clare Zlatkov, Chen Zhao, Ali Kuraishi, Lauren Craig, Sohela De Rozieres, Matthew Friedenberg, Anne C. Jager, Han-Yu Chuang. Illumina, San Diego, CA.

Recent studies have highlighted the importance of gene fusions and splice variants in solid tumor profiling. Next-generation sequencing can be an effective means of detecting these alterations in FFPE samples using RNA rather than DNA, as a single chimeric RNA transcript could result from numerous alterations in DNA. To that end, Illumina developed TruSight Tumor 170, a comprehensive, hybrid capture-based NGS assay targeting
170 key cancer genes. Along with a DNA workflow, the assay includes a RNA workflow for the identification of splice variants and gene fusions. Following sequencing on the NextSeq® or HiSeq® instruments, TruSight® Tumor 170 offers an analytical pipeline which initiates variant calling. These algorithms were first optimized against the simulated read data from >350 fusions and splice variants in the RNA panel. Subsequently, a hybrid approach of read alignment and assembly was used to enhance the fusion calling sensitivity. Delicate filters were designed to reduce false positive calling from sequence homologs, polymerase read-through, or FFPE artifacts. For splice variant calling, a panel of FFPE non-cancerous samples were used to capture false positive mutation calls. With endogenous RNA splicing in cells, physiological non-boundary probes were added in the hybrid capture to enhance enrichment efficiency. To the best of our knowledge, there is not yet a standard definition for the limit of detection (LoD) in detecting gene fusions and splice variants from NGS data. We propose to define the LoD of a fusion calling and splice variant NGS panel as the lowest molecule count of a chimeric transcript that could be reliably detected with a sufficient number of supporting sequence reads. To determine the LoD of TruSight® Tumor 170 using this definition, we mixed cell lines expressing a panel of known fusions and splice variants to measure the copy number of each chimeric transcript. Using these samples we examined the ability of the assay to confidently detect the alterations using 40 ng of RNA input. To demonstrate the analytical sensitivity and specificity of this NGS based assay, we compiled the sensitivity and specificity of the defined sample and validated the molecule count to be near the LoD of 5 copies per ng RNA input by PCR. The sensitivity was >98% for fusions and 100% for splice variants. For understanding the limit of blank (LoB) of the assay, another panel of 40 samples not harboring fusions and splice variants was also assessed by TruSight® Tumor 170. These samples demonstrated a ~97% specificity for fusion calling and >95% specificity for splice variant calling. These results indicate that the TruSight® Tumor 170 panel analysis can identify lowly expressed fusions and splice variants from a small amount of compromised RNA from solid tumor samples at high analytical sensitivity and specificity. 1 Klijn et al. (2015) 2 Maher et al. (2009) 3 For Research Use Only.

#566 A pathway based drug selection for cancer precision medicine. Varshini Vasudevaraja, Lijun Cheng, Sai Mounika Inavolu, Milan Radovich, Indiana University, Indianapolis, IN.

Background and Purpose: Precision medicine tailors the right therapy to the right patient based on his/her tumor molecular profiles. In clinic and ongoing clinical trials, drug-targeted selection schemes depends more or less on drug targets’ mutations or expression status. However, there is a lack of systematic pathway based method to connect individual genomics information with properly drug target and treatment in precision medicine. The paper aims to integrate various knowledge-bases, including cancer drugs, drug-targets and gene-gene regulatory pathway, to construct a drug target and properly drug selection algorithm for individual patient in precision medicine. Materials and Methods: In the drug selection algorithm, drug targets and properly drug recommendation are based on the patient specific molecular profile data, including copy number variation, mutation, and gene expression. Our target and drug selections are based on whether these drug targets act as hub genes that either regulate or control the signaling pathways for many other genes in the biological pathways. Only those patients whose molecular profiles do not show targets, the usual chemotherapy treatment will be recommended. This novel algorithm is applied to individual Pancreatic Adenocarcinoma (PAAD) for drug target and drug selection. All of patients’ genomic data obtained from the Cancer Genome Atlas (TCGA). The pathway information is from Pathway Commons and FDA approved cancer drugs and their targets is from DrugBank. The Cancer Cell Line Encyclopedia (CCLE) 46 pancreas cancer cell line are used to validate the algorithm result reliability. Results: Our algorithm identifies targets, such as ERBB2, CDK2, SRC, CDK9, SMAD2, CDK4, HDA1C1, PPP1CA, AKB1B1, EGFR, IGF1R, AKT and MEK, for pancreatic adenocarcinoma cancer patients. In which, they cover the clinic first line effective drug targets, such as Gemcitabine for the Akt-mTOR signaling pathway (AKT) and CDK4/6 inhibitor and Erlotinib for HER2 Kinase Family (ERBB2, EGFR) activation inhibitor. In addition, new inhibitors include Src inhibitors (dasatinib, saracatinib and bosutinib), TGF beta inhibitor for target SMAD2 (galunisentib) and IGF-1R/insulin receptor inhibitors (ceritinib, brigatinib) are recommended accordingly and validated in pancreatic cancer cells. Conclusion: This novel algorithm might act as a better source for off-label drug selection and further cell line validations may help in providing a better treatment strategy in precision medicine.

IMMUNOLOGY: Checkpoints 1

#567 CD38 blockade overcomes the immune resistance to anti-PD-L1 therapy by boosting CD8 T cell response. Limo Chen, Lixia Diao, Yongbin Yang, Xiaohui Yi, Jaime Rodriguez, Youhong Fan, Leticia Rodriguez, Jared Fradette, Christin Ungewiss, Jonathan Roybal, Jingfen Zhu, Jing Wang, Lauren Byers, Stephen Ulrich, Ignacio Wistuba, John Heymach, Xiao-Feng Qin, Don Gibbons, UT MD Anderson Cancer Ctr., Houston, TX.

Although strategies incorporating immune checkpoint inhibition, e.g. PD-1/ PD-L1 blockade, are achieving unprecedented successes and increasingly becoming incorporated into standard of care regimens for cancer patients, high rates of resistance still limit the potential efficacy. Therapeutic improvement requires a thorough understanding of the biological process of resistance. To date there have been few studies reporting mechanisms of resistance to PD-L1 blockade. We have explored the resistance mechanisms to functional PD-L1 loss in preclinical lung cancer models by using pharmacological and genetic approaches (PD-L1 blocking antibody treatment or CRISPR/Cas9-mediated deletion of PD-L1 on tumor cells). The molecular and immune profiles of the tumor microenvironment were evaluated in mutant K-ras/p53 (KP) GEM lung cancer models and multiple immunocompetent syngeneic models (both KP and Lewis lung cancer). Additionally, to determine the applicability of the results to patients with lung cancer, we analyzed 259 patient tumor specimens with IHC staining and evaluated the immune markers in TCGA datasets (adenocarcinoma and squamous) and the MD Anderson PROSPECT dataset. We observed that lung tumors gained resistance to anti-PD-L1 antibody treatment over time, and that the up-regulation of CD38 on tumor cells accounted for the treatment resistance. We also observed the same resistance mechanism caused by CD38 up-regulation in PD-L1 KO mice bearing PD-L1 KO Lewis lung tumors generated with the CRISPR/Cas9 system. Manipulation of CD38 on a panel of lung cancer cell lines, demonstrated that CD38 inhibits CD8+ T cell proliferation, anti-tumor cytotoxic secretion, and tumor cell killing capability in vitro and in vivo. Furthermore, to test whether CD38 blockade might be therapeutically efficacious to counter anti-PD-1 resistance, we applied the combination therapy of anti-CD38 and anti-PD-L1 in lung cancer animal models and demonstrated dramatic therapeutic benefit on primary tumor growth and metastasis. Bioinformatic analyses of the patient tumor databases revealed a strong correlation between CD38 expression and an immune suppressive inflammatory signature. Finally, in 259 lung cancer specimens, 18.5% of cases exhibited positive staining for CD38 on tumor cells. Based upon our studies, we conclude that the up-regulation of CD38 on tumor cells is a major mechanism of resistance to anti-PD-L1 therapy, and that CD38 is a novel immune checkpoint that inhibits CD8+ T cell function. The blockade of CD38 and PD-L1 is a rational combination to prevent immune resistance and increase the response rate for lung cancer patients.


Introduction: Breast cancer can be separated into five intrinsic subtypes based on differences in the transcriptome of the tumor. We propose that the intrinsic differences of specific tumor subtypes lead to extrinsic differences in the tumor microenvironment. Methods: We utilized human clinical and genetically engineered mouse model (GEMM) samples of the intrinsic subtypes luminal A, basal-like, and claudin-low breast cancers to evaluate the immune landscape in the tumor microenvironment by histology and microarray analysis. Our claudin-low GEMM was derived from BRCA-/-/p53-/- mice. The HER-2 overexpressing, basal-like, and luminal A models have been previously described. We utilized the FoxP3-DTR transgenic mouse model as a method of regulatory T cell (Treg) depletion to evaluate their function in these GEMMs. Results: The claudin-low human tumors were heavily infiltrated with immune cells, with CD4+ T cells being the most prominent, when compared to the luminal A subtype (P = 0.01). There were also increased focal areas of Tregs in human claudin-low tumors. To evaluate the mechanism for these findings, we utilized a GEMM of claudin-low tumor specimens with IHC staining and evaluated the immune markers in TCGA syngeneic models (both KP and Lewis lung cancer). Additionally, to determine the applicability of the results to patients with breast cancer, we analyzed 259 patient tumor databases revealed a strong correlation between CD38 expression and an immune suppressive inflammatory signature. Finally, in 259 lung cancer specimens, 18.5% of cases exhibited positive staining for CD38 on tumor cells. Based upon our studies, we conclude that the up-regulation of CD38 on tumor cells is a major mechanism of resistance to anti-PD-L1 therapy, and that CD38 is a novel immune checkpoint that inhibits CD8+ T cell function. The blockade of CD38 and PD-L1 is a rational combination to prevent immune resistance and increase the response rate for lung cancer patients.
delay tumor growth and improve anti-tumor immune response. Surprisingly, we saw no delay in tumor growth in the claudin-low model using checkpoint inhibition. To investigate if presence of Tregs limited the function of checkpoint inhibitors, mice with claudin-low tumors were treated with AMD3100, a CXCR4 inhibitor. This decreased Treg infiltration into the tumor microenvironment and altered tumor growth. We then utilized the FoxP3-DTR transgenic mouse model, where depletion of Tregs alone resulted in a very modest decrease in tumor growth, while depletion of Tregs plus checkpoint inhibition significantly improved survival (P = 0.03) and increased cytotoxic production by CD8+ T cells. Conclusion: We found that an effective anti-tumor immune response in claudin-low tumors is inhibited by the recruitment of regulatory T cells in the tumor microenvironment. These data highlight early Treg recruitment as a possible mechanism for the lack of response to immune checkpoint inhibition therapy for claudin-low breast cancer.

#569 Dexamethasone inhibits T-cell proliferation through a CTLA-4 mediated pathway. Marsha-Kay N. Hutchinson, Amber J. Giles, Heather M. Sonnemann, Caitlin M. Reid, Deric M. Park, Mark Gilbert. National Institute of Health, Bethesda, MD.

BACKGROUND: The use of corticosteroids for therapeutic benefit has been weighed against the risks of adverse consequences associated with these drugs. Brain tumor patients in particular, are routinely prescribed dexamethasone (a glucocorticoid) to reduce edema associated with their lesion. Checkpoint blockade, a type of immune therapy, is currently being investigated as a potential treatment for brain tumors. However, glucocorticoid signaling has been shown to attenuate the immune response through several mechanisms including the repression of transcription of genes controlling pro-inflammatory cytokines and chemokines. HYPOTHESIS: Here, we propose that dexamethasone’s ability to upregulate inhibitory T-cell molecules such as CTLA-4 and PD-1 might be an additional immunosuppressive mechanism. METHODS: Healthy donor T cells were tested for response to dexamethasone. T cell proliferation, cell cycle analysis, apoptosis, and protein expression were assessed with flow cytometry. Protein expression was also measured with Western blots. Transcriptional changes were assessed with qPCR. A monoclonal antibody, ipilimumab, was used to block CTLA-4 binding. RESULTS: Unexpectedly, dexamethasone did not elicit a direct lymphotoxic effect on T cells as measured by absolute cell number. However, we found that dexamethasone significantly reduced T-cell entry into the cell cycle, but did not impact cells already undergoing mitosis. Checkpoint molecules CTLA-4 and PD-1 were increased with dexamethasone treatment when cells are stimulated. Blockade of CTLA-4 with ipilimumab resulted in a substantial reversal of cell cycle entry inhibition that was induced by dexamethasone. CONCLUSIONS: These results suggest that dexamethasone impairs T cell expansion by inhibiting cell cycle entry. Upregulated CTLA-4 expression contributes to cell cycle entry blockade which is reversed by inhibiting CTLA-4 with ipilimumab. These findings indicate that administration of ipilimumab before dexamethasone diminishes the negative proliferative effect on anti-tumor T cells suggesting that when needed, corticosteroids can be used after immune checkpoint blockade has been established.


Untangling the complexity of programmed death-ligand 1 (PD-L1) expression within a heterogeneous tumor microenvironment is an urgent challenge in PD-1/PD-L1 immune checkpoint blockade therapy. Here, we address this challenge with a method, termed transparent tumor tomography (T3), facilitating three-dimensional (3D) visualization and spatial analysis of distributions of multiple biomarkers regarding to cancer cells, vasculature, and immune cells in context in the tumor microenvironment. With T3 analysis of transgenic mouse mammary tumors immunostained against Her2, CD45, Ki-67, CD31, and PD-L1, we reveal that PD-L1 expression within the tumor microenvironment is highly adaptable for efficiently preventing immune cell infiltration into the tumor. Stronger correlation of Her2 and PD-L1 expression in the tumor periphery where has a high CD45+ immune infiltrate density is determined by tumor-wide analysis. Also, tomographic analysis shows blood vessels expressing PD-L1 in the tumor core, where PD-L1 expression is lower. Furthermore, high-resolution T3 image localizes PD-L1 expression to a region between the endothelium and the surrounding smooth muscle cells in blood vessels. We investigate spatial pharmacokinetics of anti-PD-L1 antibody in the whole mouse mammary tumor in the context of hypoxia, CD31+ blood vessels, and target PD-L1+ cells. The 3D anti-PD-L1 antibody distribution is fit to a two-compartment pharmacokinetic model, yielding estimated distribution half-life of 4.7 min and terminal half-life of 2.5 days. We also evaluate anti-tumor immune responses after PD-L1 blockade therapy using T3. We observe broad distribution of tumor infiltrating CD8+ cytotoxic T cells in 3D tumor section following combination therapy of radiation and anti-PD-L1 antibody compared to PBS, anti-PD-L1 antibody alone, or radiation alone treatment group. Moreover, we apply T3 for immunoaanalysis of whole core needle biopsy. We spatially map PD-L1 expression in immunostained cryosections with CD3+ CD8+ cytotoxic T cells in whole tumor sections and in-treatment core needle biopsies at cellular resolution and in three dimensions. Meanwhile, T3 analysis is nondestructive, allowing secondary analysis by IHC and/or IF. We anticipate that T3 can be applied broadly to facilitate preclinical studies of immunotherapy and also find use in spatial, multiparameter analysis of patient biopies, particularly to improve predictive testing and analysis of immune responses to tumor immunotherapy.

#571 Meta-analysis of genomic predictors of response to immune checkpoint therapy in metastatic melanoma. Diana Miao, David Liu, Daniel Keller, Sachet Shukla, Bastian Schilling, Claire Margolis, Alicia Smart, Levi Garraway, Stephen Hodi, Dirk Schadendorf, Eliezer M. Van Allen, Dana-Farber Cancer Institute, Boston, MA; University Hospital Essen, Essen, Germany.

Introduction: Immune checkpoint therapies benefit a subset of patients with metastatic melanoma, but ability to predict clinical outcomes is limited. This meta-analysis of genomic predictors of outcomes to aPD1 and aCTLA4 in melanoma combines 220 sequenced tumors from 3 published cohorts, aiming to validate existing hypotheses regarding response to immune checkpoint therapies and discover new relationships with greater power. Methods: Genomic data and clinical annotation from published cohorts were analyzed with standard and customized pipelines for somatic variant calling, mutational signature deconvolution, and neoantigen prediction. Patients were stratified into clinical benefit (CB) and no clinical benefit (NCB) as described in Van Allen et al. 2015. Analyses were repeated using two other published response metrics (CB=PFS>6 months; CB=CR or PR). Results: Nonsynonymous mutational burden was significantly higher in CB vs. NCB using all 3 response metrics, though significance was less pronounced using PFS alone (p<0.01 vs. p<0.0001; Wilcoxon rank sum), partially due to 3 patients with high mutational burden who experienced PR for <6 months, potentially representing early acquired rather than intrinsic resistance. To assess the impact of mutational processes contributing to overall mutational burden, we used a non-negative matrix factorization framework to infer mutational activity in tumors from 6 signatures previously seen in melanoma: aging (S1), T>C substitutions (S5), mismatch repair (S6), UV (S7), and T>G substitutions (S17). The proportion of mutations in S7 or S11 was positively correlated with mutational burden (Pearson’s r=0.66), while S5 and S11 were anti-correlated (r=−0.62). In a multivariate logistic model, S7 and S11 activity were independent predictors of CB adjusting for mutational load (p<0.05), with the sum of S7 and S11 activity being a strong predictor (p<0.001). Of the patients with low mutational burden (<median) with CB, a large majority (23/29) had >1/2 of mutations in S7 or S11, compared to only 36/71 of low-mutation NCB (p<0.01; Pearson’s chi-squared). Néoantigen burden was strongly correlated with mutational burden, and did not improve ability to predict CB. In examining mutations in specific genes, >500 genes were mutated predominantly in either CB or NCB (p<0.05, Fisher’s exact). Restricting analysis to genes recurrently mutated in cancer and correcting for patient mutational burden by permutation, nonsynonymous mutations in AC5L3 and MET and truncating alterations in ARID2 were significantly enriched in CB. Conclusions: In this meta-analysis of 220 patients, harmonized clinical and whole exome analysis confirmed that mutational burden correlates with CB from aPD1 and aCTLA4 therapy, while mutational signatures and alterations in specific genes potentially provide additional predictive power.

#572 Inhibition of IDO1 with epacadostat enhances anti-tumor efficacy of PD-1 blockade in a syngeneic glioblastoma (GBM) model. David A. Reardon, Prafulla C. Gokhale, Holly Koblish, Peggy Scherle, Lance Leopold, Robert Newton, Gordon J. Freeman, Diana-Farber Cancer Inst., Boston, MA; Incyte Corporation, Wilmington, DE.

Purpose: To determine if epacadostat, an oral indoleamine 2,3-dioxygenase (IDO1) inhibitor has therapeutic benefit against GBM when administered as single agent and with PD-1 blocking antibody. Methods: An initial survival experiment was performed to assess efficacy and was followed by an identical repeat experiment for validation. LX10 luciferized GL261 cells, a murine GBM tumor line derived from intracerebral methylcholanthrene implantation, were stereotactically implanted intracranially in albino syngeneic C57BL/6 mice. Mice with increasing bioluminescence on days 3 and 6 were randomized (n=8/group) to receive treatment beginning on day 6: anti-PD-1 (332.8H3, mouse...
Radiation and immune checkpoint blockade promotes anti-tumor activity in mouse B16 melanoma. Hutchins, Jedd D. Wolchok, Taha Merghoub.

Indeed, subsequent tumor studies in WT and RAG-/- mice confirmed rechallenge, strongly suggesting an immune-mediated rejection had been established. Further studies incorporating IDO inhibitor therapy for GBM, including mechanistic studies, are warranted.

#573 Metabolism as checkpoint: Induction of anti-tumor immune response with the novel glutamine antagonist JHU-083. Robert D. Leone, Judson M. Englert, Min-Hee Oh, Chih-Hsien Cheng, Rana Rais, Barbara Slusher, Jonathan D. Powell. Johns Hopkins Hospital, Baltimore, MD.

The metabolic characteristics of the tumor microenvironment (e.g., hypoxia, acidity, nutrient deprivation, elevated adenosine) present a significant hurdle for immunotherapy. Disregulated glutamine metabolism plays a crucial role in establishing this environment. We previously demonstrated that the novel glutamine antagonist, JHU-083, profoundly alters tumor metabolism and inhibits growth with minimal toxicity. Further studies have demonstrated that JHU-083 can reprogram long-term survivors to assess for tumor immune responses capable of preventing relapse. All long-term surviving mice (defined as ≥ 100 days) from the efficacy experiment were injected with 1X10^6 GL261 cells in the contralateral hemisphere and followed for survival. Results: In both preclinical efficacy experiments, median survival in the epacadostat monotherapy group did not differ from controls (approximately 30 days). Four of eight mice (50%) treated with anti-PD-1 were long-term survivors in both efficacy experiments. In the epacadostat plus anti-PD-1 combination group, 81% of the mice were long-term survivors (7 of 8 in experiment 1 and 6 of 8 in experiment 2). Of note, none of the long-term surviving mice developed evidence of tumor; thus the median survival among the anti-PD-1 and epacadostat plus anti-PD-1 combination groups were both ≥ 100 days. In the re-challenge study, all of the mice who underwent GL261 re-inoculation survived > 100 more days with no evidence of tumor recurrence. Conclusions: IDO inhibition with epacadostat increased the eradication rate of anti-PD-1 therapy in an orthotopic syngeneic GBM model and long term survivors rejected tumor following orthotopic re-challenge. Further combinatorial studies incorporating IDO inhibitor therapy for GBM, including mechanistic studies, are warranted.

#574 Phosphatidylserine targeting antibody in combination with tumor radiation and immune checkpoint blockade promotes anti-tumor activity in mouse B16 melanoma. Sadna Budhu, Olivier De Henau, Roberta Zappasodi, Rachel Giese, Luis F. Campesato, Christopher Barker, Bruce Freimark, Jeff Hutchins, Jedd D. Wolchok, Taha Merghoub. Memorial Sloan Kettering Cancer Center, New York, NY; Peregrine Pharmaceuticals, Inc., Tuscon, CA.

Phosphatidylserine (PS) is a phospholipid that is exposed on surface of apoptotic cells, tumor cells and tumor endothelium. PS has been shown to promote immunosuppressive signals in the tumor microenvironment. Antibodies that target PS have been shown to reactivate anti-tumor immunity by polarizing tumor associated macrophages into a pro-inflammatory M1 phenotype, reducing the number of MDSCs in tumors and promoting the maturation of dendritic cells into functional APCs. In a mouse B16 melanoma model, targeting PS in combination with immune checkpoint blockade promoted greater anti-tumor activity than either agent alone. This combination was shown to enhance CD4+ and CD8+ T cell infiltration and activation in the tumors of treated animals.

Radiation therapy (RT) is an effective focal treatment of primary solid tumors, but is less effective in treating metastatic solid tumors as a monotherapy. There is evidence that RT induces immunogenic tumor cell death and enhances tumor-specific T cell infiltration in treated tumors. The abscopal effect, a phenomenon in which tumor regression occurs outside the site of RT, has been observed in both preclinical and clinical trials when RT is combined with immunotherapy. In this study, we show that irradiation treatment of B16 melanoma causes an increase in PS expression in the subset of viable tumor and immune infiltrates. We subsequently examined the effects of combining RT with an antibody that targets PS (mch1N11) and immune checkpoint blockade (anti-PD-1) in B16 melanoma. We found that treatment with mch1N11 synergizes with RT to improve anti-tumor activity and overall survival in tumor bearing mice. In addition, the triple combination of mch1N11, RT and anti-PD-1 treatment displayed even greater anti-tumor and survival benefit. Anti-CD8 T cell responses in the tumors of treated animals revealed an increase in tumor-associated macrophages with a shift towards a pro-inflammatory M1 phenotype after treatment with RT and mch1N11. In addition, analysis of the systemic immune responses in the spleen and tumor draining lymph nodes revealed an increase in CD8 T cell activation, effector cytokine production and differentiation into effectector memory cells in the triple combination. This finding highlights the potential of combining these three agents to improve outcome in patients with advanced-stage melanoma and other cancers and may inform the design of clinical studies combining PS-targeting antibodies with RT and/or checkpoint blockade.

#575 PD-L1 positive tumor-infiltrating lymphocytes and mutational load in breast cancer. Marcelo Sobral-Leite, Koen Van de Vijver, Magali Michauf, Hugo M. Horlings, Tesa M. Severson, Philip C. Schouten, Rianne van de Linden, Kelly Kersten, Anna Marie Mulligan, Nanyaa Weerasooriya, Joyce Sanders, Ashley Cinmino-Mathews, Dennis Peters, Gerrit K. Hoosijberg, Annegien Broeks, Rene Bernards, Sabine Linn, Irene L. Andrusis, Marc J. van de Vijver, Lodewyk F. Wessels, Marjanka K. Schmidt, The Netherlands Cancer Institute, Amsterdam, Netherlands; University of Toronto, Toronto, Ontario, Canada; The Johns Hopkins Hospital, Baltimore, MD; Academic Medical Center, Amsterdam, Netherlands.

Background: PD-1 blockade has emerged as an effective treatment for a subset of cancer patients. Studies have shown that PD-L1 expression is associated with likelihood of response to PD-1 blockade. In order to select the right breast cancer patient for immunotherapy, characterization of the immune landscape of breast tumors is required. Therefore, we assessed PD-L1 expression and tumor-infiltrating lymphocytes (TILs) in different breast tumor subtypes and the link with prognosis. We also sequenced a panel of genes to assess the mutational load in triple negative tumors (TNBC) and investigate the association with PD-L1 positive TILs. Material and methods: We analyzed 438 tumor samples from breast cancer patients of all ages treated between 1986 and 2007 with surgery, with or without adjuvant therapy. PD-L1 was stained using whole slide specimens (EILN3® antibody) after methodological validation. Pathologists quantified TILs based on International TILs Working Group recommendations and scored PD-L1 based on the percentage of positive (tumor and/or immune) cells; as negative if 0%, positive if ≥1%, and high if ≥5%. Mutational load was assessed based on DNA kinome sequencing. Associations were measured by Cox/logistic regression model, including pathological variables. Multiplex imaging of 20 immunofluorescence labeled areas from 4 ER negative tumors were performed using the Vectra® system based on immunofluorescence staining panel of: CD4, CD68, CD8, FOXP3 and PD-L1. Results: PD-L1 expression and TILs were higher in ductal (compared with lobular), high grade and estrogen receptor (ER)-negative tumors (p = 0.001). TILs (density ≥5%) were significantly associated with worse distant metastasis-free survival (DMFS; only in ER-negative tumors (p = 0.04)): HR = 2.72; 95%CI: 1.07-6.94. PD-L1 positivity (≥1%) followed the same trend: HR = 1.66; 95%CI: 0.87-3.15. However, in ER-negative tumors (n = 171), high PD-L1 expression (>50%) was significantly associated with better DMFS: HR = 0.53; 95%CI: 0.27-0.98. TNBC with high PD-L1 expression of TILs
(>50%) showed an association with increased mutation load (p = 0.019) and a trend for better DMFS (HR = 0.41; 95% CI: 0.16-0.94) compared with tumors lacking TILs. Further characterization of PD-L1 positivity in the immune-infiltrated cells was conducted by a multiplex imaging analysis. Preliminary results indicated that PD-L1 is expressed in CD68+, CD4+, FOXP3+ and CD8+ immune cells. Our findings were confirmed by cell ELISA, but also reversed compared with worse prognosis in ER-positive breast cancer and with better outcome in ER-negative group. In TNBC, high mutational load correlates with high PD-L1 positive TILs.


Magnetic resonance-guided focused ultrasound (MRgFUS) facilitates local tumor control via thermal ablation, however, the anti-tumor immune effects induced are weak and unable to consistently generate robust objective responses in distant lesions. Here, we set out to optimize a therapeutic approach for employing immunotherapy with thermal ablation for systemic cancer treatment. We assessed the efficacy of implementing MRgFUS ablation with blockade of the PD-1/PD-L1 axis (anti-PD-1) and activation of TLR9 (CpG oligonucleotide) under various protocols and in multiple models of murine cancer. Anti-PD-1 (200 µg, i.p., days 21 & 28) and CpG (100 µg, i.t., days 21, 24 and 28) were administered coincidentally with MRgFUS ablation (3 MHz frequency, circular pattern with R = 2 mm, 1 revolution per second, 65ºC for 1 min, days 21 and 28) over the course of a week in bilateral syngeneic neu deletion line (NDL), 4T-1 and B16 tumor bearing mice. Additionally, we evaluated the administration of immunotherapy prior to a course of thermal ablation (i.e., "primed ablation"), where anti-PD-1 (as above on days 21, 28 & 35), CpG (as above on days 21, 24, 28, 31, 38 and 45) and MRgFUS ablation (as above on days 21, 31, 38 and 45) were administered in bilateral NDL tumor-bearing mice. Primed ablation generated a robust anti-tumor immune response in distant lesions two weeks after the start of treatment, where a threefold increase in tumor infiltrating leukocytes (reaching 40% CD8+ and 20% CD4+ T-cells) was observed. This led to a complete response in 80% of treated mice within 70 days after treatment commenced. This effect was also observed in animals with high tumor burden and when thermal ablation was performed sequentially at multiple independent sites; 80% of untreated lesions were eradicated at 50 days after the start of treatment. However, therapeutic efficacy was limited when thermal ablation was performed coincident with the first dose of immunotherapy; this protocol was not curative in any murine model. To elucidate the mechanism for this effect, we employed tumor histology and positron emission tomography immediately after MRgFUS ablation. We found that thermal ablation induced stromal inflammation, and the loss of cell-cell adhesion and local vascular integrity, which impacted the intratumoral transport of small molecules and proteins for 48 hours post treatment. These data suggest that tumor debunking using image-guided thermal therapy can be successfully incorporated within a curative protocol in which immunotherapy is initiated before ablation.


To explore this concept, yeast antibody display was used to identify fully human, anti-TIGIT antibodies that block binding to ligands. Multiple rounds of selection with human and mouse TIGIT protein were performed to promote species cross-reactivity, diversity and affinity. A pool of 695 unique clones were screened for binding to TIGIT protein; 65 clones were then selected for further evaluation. Of the 65, 63 competed with CD155 for binding to TIGIT in a For-bio screen. Fifty-three clones bound cyno TIGIT and 25 bound TIGIT from all species. Antibodies bound endogenous TIGIT on primary T cells and blocked binding of ligands to cell surface expressed TIGIT in a dose dependent manner. Twelve clones showed functional activity in a TIGIT blockade bioassay and showed synergy with anti-PD-1 antibody in a PD-1/ TIGIT combination bioassay. Activity in the bioassays correlated with affinity for recombinant and cell surface expressed TIGIT. Based on species cross-reactivity, affinity and activity in the bioassays, a lead antibody was selected and produced as mouse IgG1 and IgG2a chimeras for testing in mouse tumor models. The chimeric antibodies behaved similarly to the parent clone in vitro exhibiting high affinity for TIGIT, competition with ligand for binding to TIGIT, and functional blockade of CD155-TIGIT interaction. Evaluation of the chimeric anti-TIGIT candidates alone and in combination with anti-PD-1 antibody in mouse syngeneic tumor models is ongoing, and results will be reported at the meeting. Antibody mediated blockade of coinhibitory immunoreceptors has proven clinically efficacious and supports the development of antibodies that target TIGIT. The unique human, non-human primate, and murine cross-reactive TIGIT-specific antibodies described here offer a simplified preclinical development path and the functional activity of these molecules supports their consideration as candidates for therapeutic development.

#580 Nivolumab treatment of metastatic renal cancer patients impairs Tregs and potentiates NK function: The role of CXCR4 inhibition (“REVOLUTION Trial”). Sara Santagata, Anna Maria Trotta, Maria Napolitano, Luigi Portella, Sabrina Rossetti, Sisto Perondo, Sandro Pignata, Stefania Scala, National Cancer Inst. Pascale, Naples, Italy.

Despite encouraging results, Nivolumab response is not as wide as expected in renal cancer (RCC). Among mechanisms of immunoresistance T-regulatory cells(Tregs) activity plays a central role. We previously showed that tumoral Tregs in RCC patients are more suppressive than healthy donors Tregs. Since tumoral Tregs express high level of CXCR4, the receptor antagonism was eval-
uated on patients Tregs revealing that CXCR4 antagonism reverted Treg suppressive activity (Santagata et al, submitted manuscript). To identify biomarkers informative and predictive of nivolumab efficacy Treg function, tumoral access and NK interactions was determined in nivolumab treated mRCC patients (# Revolution trial). At today eleven patients were analyzed for Tregs and NKs function at time 0-2-4 weeks-3-6 months of treatment. 2 patients died for unrelated causes, 5 Patients showed PR, 4 SD and 1 PD at six months. At time 0 Tregs function, evaluated as inhibition of T-effector (Teff) proliferation, varied among patients; two patients showed anergy reversed by Nivolumab treatment. Overall, increase in Teff proliferation, compatible with a decreased Tregs activity, was revealed during nivolumab treatment and, while the total peripheral Tregs was unaffected, a decrease in Treg proportion was reported, thus providing an indicator of tumoral burden. We evaluated baseline mutation spectrum in genes implicated in cancer for association with clinical outcomes to durvalumab, an anti-PDL1 molecule, and examined changes in VAFs, as a surrogate for tumor burden, after treatment with durvalumab in NSCLC patients. CP1108/NCT01693562 was a nonrandomized phase 1/2 trial evaluating durvalumab in patients with advanced NSCLC or other solid tumors. By 2APR2016, 304 NSCLC pts received 10 mg/kg Q2W of D 12 months with median 18.8 months follow up. A panel of 70 genes was assessed for nonsynonymous mutations and copy number (CN) variants using the Guardant360 cancer panel in plasma ctDNA from 115 NSCLC patients pre-treatment and 28 patients pre and 8 weeks post-treatment with durvalumab (Q2W). The mean VAF of all mutations harbored by a patient was correlated with clinical outcomes. To calculated according to RECIST v1.1 and a Cox proportional hazards model was calculated adjusting for baseline ECOG, gender, age, smoking status, therapy lines, histology, and number of metastases. Partial responders (PRs) showed a significant decrease (Δ = −2.7%, p = 0.005) in ctDNA mean VAF post-treatment with durvalumab (i.e. reduction in tumor burden) compared to an increase in mean VAF (i.e. increase in tumor burden) in progressive disease (PD) patients (Δ = +1.7%; p = 0.017). This correlation was also observed in total mutation count in PR (Δ = −5.3, p = 0.037) compared to PD patients (Δ = +2.7, p = 0.003). In a PD patient, the VAF of T790M EGFR mutation doubled after 6 weeks of durvalumab treatment. Patients with VAFs below the median VAF or no CN gain had longer median overall survival (15.7 months, 95% CI=11.2,not reached) compared to those with VAFs above the median or CN gains (5.1 months 95% CI=3.8,9.4); HR=0.29; p=0.005), even after adjustment for clinical covariates. CtDNA VAFs were consistent reduced in responders but not non-responders after eight weeks of durvalumab. Below median pre-treatment VAFs or no CN gains in ctDNA correlated with longer overall survival in patients treated with durvalumab. Because we examined oncogenes and tumor suppressors, increased VAF in ctDNA of these genes may help to identify aggressive, and difficult to control tumors.

#583 The CDK4/6 inhibitor abemaciclib induces synergistic immune activation and antitumor efficacy in combination with PD-L1 blockade. Jack Dempsey,1 Lyssiane Huber,1 Amelie Forest, Jennifer R. Stephens,3 Thompson N. Doman,1 Jason Manro,1 Andrew Capen,1 Robert S. Flack,1 Gregory P. Donoho,1 Sean Buchanan,1 Alfonso De Dios,1 Kyla Driscoll,2 Michael Kalos,2 N. Doman,1 Jason Manro,1 Andrew Capen,1 Robert S. Flack,1 Gregory P. Donoho,1 Sean Buchanan,1 Alfonso De Dios,1 Kyla Driscoll,2 Michael Kalos,2 Ruslan Novosadly,3 Richard P. Beckmann,1 David A. Schaer2.1EliLilly,India-146.4April 20171

Targeting cyclin dependent kinases 4 and 6 (CDK4/6) with inhibitors such as abemaciclib has shown promise in early and late phase clinical trials in both breast cancer and NSCLC. While there is evidence that patients benefit from single-agent abemaciclib, combination strategies leveraging this compound together with immunotherapy are of interest for the treatment of these and other cancers. Consequently, it is important to understand if and how a cell cycle inhibitor can be combined with immunotherapy. However, because most preclinical studies have been performed using xenograft tumors in immuno-compromised mice, the potential immunomodulatory effects of abemaciclib have not been adequately ascertainment. To investigate the immune combinatorial potential of abemaciclib, we studied the effects of immune inflammation without major alteration in immune subset frequencies. Testing of various dosing regimens in this preclinical model found that monotherapy abemaciclib pretreatment followed by combination with anti-PD-L1 antibody therapy, induced an enhanced anti-tumor response compared to abemaciclib and anti-PD-L1 monotherapies. Optimal combination

#582 Circulating tumor DNA (ctDNA) variant allele frequencies are reduced in responders to durvalumab and low baseline variant allele frequencies are associated with improved overall survival in NSCLC patients. Michael A. Kuziora, Brandon W. Higgs, Philip Brohawn, Rajiv Raja, Koustubbh Ranade. *MedImmune, Gaithersburg, MD.*

Mutations can influence patient responses to treatment in non-small cell lung cancer (NSCLC). Monitoring such mutations can be critical to guiding treatment decisions, however, accessing sufficient biopsy material for mutation analysis can be challenging. Circulating tumor DNA (ctDNA) in the plasma offers a robust and highly sensitive approach to identifying mutations. Further, variant allele frequencies (VAFs) in individual mutations represent the number of cancer cells and, thus providing an indicator of tumor burden. Here we evaluated baseline mutation spectrum in genes implicated in cancer for association with clinical outcomes to durvalumab, an anti-PDL1 molecule, and examined changes in VAFs, as a surrogate for tumor burden, after treatment with durvalumab in NSCLC patients. CP1108/NCT01693562 was a nonrandomized phase 1/2 trial evaluating durvalumab in patients with advanced NSCLC or other solid tumors. By 2APR2016, 304 NSCLC pts received 10 mg/kg Q2W of D 12 months with median 18.8 months follow up. A panel of 70 genes was assessed for nonsynonymous mutations and copy number (CN) variants using the Guardant360 cancer panel in plasma ctDNA from 115 NSCLC patients pre-treatment and 28 patients pre and 8 weeks post-treatment with durvalumab (Q2W). The mean VAF of all mutations harbored by a patient was correlated with clinical outcomes. To calculated according to RECIST v1.1 and a Cox proportional hazards model was calculated adjusting for baseline ECOG, gender, age, smoking status, therapy lines, histology, and number of metastases. Partial responders (PRs) showed a significant decrease (Δ = −2.7%, p = 0.005) in ctDNA mean VAF post-treatment with durvalumab (i.e. reduction in tumor burden) compared to an increase in mean VAF (i.e. increase in tumor burden) in progressive disease (PD) patients (Δ = +1.7%; p = 0.017). This correlation was also observed in total mutation count in PR (Δ = −5.3, p = 0.037) compared to PD patients (Δ = +2.7, p = 0.003). In a PD patient, the VAF of T790M EGFR mutation doubled after 6 weeks of durvalumab treatment. Patients with VAFs below the median VAF or no CN gains had longer median overall survival (15.7 months, 95% CI=11.2,not reached) compared to those with VAFs above the median or CN gains (5.1 months 95% CI=3.8,9.4); HR=0.29; p=0.005), even after adjustment for clinical covariates. CtDNA VAFs were consistently reduced in responders but not non-responders after eight weeks of durvalumab. Below median pre-treatment VAFs or no CN gains in ctDNA correlated with longer overall survival in patients treated with durvalumab. Because we examined oncogenes and tumor suppressors, increased VAF in ctDNA of these genes may help to identify aggressive, and difficult to control tumors.

#583 Discovery and development of COM701, a therapeutic antibody targeting the novel immune checkpoint PVRIG. Ofer Levy,1 Chris Chan,2 Gady Cocojan,3 Andrew Ophir,3 Sudipto Ganguly,3 Maya Kotturi,2 Christine Sadowski,1 Benjamin Murter,3 Friedmann,1 Jianyang Zhang,1 Liat Dassa,1 Ling Leung,2 Shirley Greenwald,1 Mark White2.

Background: whole blockade of the CTLA4 and PD1 pathways has emerged as an effective treatment of cancer, the majority of patients do not derive long term benefit. This provides a rationale for identifying and targeting additional checkpoints. Employing our unique computational algorithms, we identified PVRIG, a new member of the B7/CD28 family. We report here the expression pattern, functional characterization, and anti-tumor activity of blocking antibodies targeting PVRIG as well as characterization of PVRIG KO mice. Methods: PVRIG is expressed on T and NK cells within the tumor microenvironment. We identified PVR2 as its counterpart and characterized the PVRIG-PVR2 interaction. Antibody discovery was carried out with phage display and hybridoma platforms and antibodies against the human protein were screened for their ability to enhance T cell activity in vitro, while surrogate antibodies targeting the mouse protein were assessed in syngeneic models for effects on tumor growth. PVRIG-/- mice were generated and characterized for antibodies targeting the mouse protein were assessed in syngeneic models for PVRIG-PVRL2 interaction. Antibody discovery was carried out with phage display and hybridoma platforms and antibodies against the human protein were screened for their ability to enhance T cell activity in vitro, while surrogate antibodies targeting the mouse protein were assessed in syngeneic models for effects on tumor growth. PVRIG-/- mice were generated and characterized including phenotyping and anti-tumor immune response. Results: PVRIG is expressed on different T cell subsets and on NK, NKT and γδ T-cells. Within T cells, memory subsets possess the highest level of PVRIG and its expression is induced upon long term activation with different stimuli. Within tumor microenvironment, PVRIG was found to be expressed on NK and CD8+ T cells in multiple cancers. A high affinity lead Ab was selected, COM701, for further clinical development and demonstrated blockade of the interaction of PVRIG with PVR2 as well as enhancement of activation of both primary and tumor-derived effector immune cells through a PVR2-dependent mechanism. Moreover, COM-701 showed notable enhancement of T cell function in vitro when combined with PD1 or TIGIT Ab blockade. The lead antibody, COM-701, is currently in preclinical development. A surrogate antibody, that blocks PVRIG-PVR2 interaction, was shown to inhibit growth of colon carcinoma and melanoma in syngeneic models upon combined treatment with anti-PDL1 antibody. Comparative analysis of PVRIG KO versus WT derived T cells revealed enhanced expression of PVRIG null T cells upon polyclonal activation in presence of PVR2-Ig. Accordingly, MC38 tumors grew slower in PVRIG KO than in WT mice and ex vivo analysis pointed to the quantitative and functional differences in antitumor immunity developed in these mice. Conclusion: We describe the identification of PVRIG as a novel T cell immune checkpoint. We further demonstrated that antibody blockade of the PVRIG-PVR2 interaction has the potential to be efficiently combined with PD1 or TIGIT blockade for enhancing anti-tumor immunity. COM-701 is a high affinity antigenic antibody that is currently in preclinical development. Taken together, these data demonstrate the utility of targeting PVRIG in addition to other B7 family checkpoints for the treatment of cancer.
therapy exhibited superior anti-tumor efficacy, resulting in complete tumor regression (CR) in 50-60% of mice in a setting where anti-PD-L1 monotherapy showed little or no efficacy (0% CRs). Mice which maintained CRs after cessation of combination therapy were able to resist later CT26 relapse, demonstrating that abemaciclib in combination with anti-PD-L1 enabled long-term immunological memory. Examination of tumor gene expression during treatment found that combination therapy further amplified the immune/T cell activation signature compared to both monotherapies. Intra-tumoral suppression of cell cycle genes, which are indicative of inhibition of CDK4/6, was also greater during the combination therapy, suggesting that the effects anti-PD-L1 therapy may augment the cell cycle arrest induced by abemaciclib. Although it was uncertain if abemaciclib that inhibit cell proliferation could be combined with immunotherapy, these preclinical results demonstrate that it is possible to combine CDK4/6 inhibition by abemaciclib with checkpoint immunotherapy to improve tumor efficacy. The synergistic responses observed in terms of tumor efficacy, immune activation, and cell cycle control provides support for the clinical investigation of this combination.


Antibody blockade of CTLA4 and PD-1 immune checkpoint emerged as an effective treatment modality for cancer. However, the majority of patients do not achieve sustained long term benefit, suggesting a need for targeting of additional immune checkpoints. To identify additional B7/CD28 immune checkpoint targets, we developed a unique compendium of computational algorithms that identified multiple novel targets including TIGIT in 2008, which was an unknown protein at the time of discovery [Proc Natl Acad Sci U S A 2009 Oct 20;106(42):17858-63], and PVRIG which we recently disclosed. Since their initial discovery, these targets have been functionally validated and anti-tumor activity was demonstrated with antibodies that target them. In this presentation, we will describe the computational algorithms that led to the discovery of these novel immune checkpoints. These algorithms combine two complementary aspects: (i) endogenous immune checkpoint function prediction and (ii) prediction of immuno-modulatory activity in cancer. Immune checkpoint function was predicted based on gene structure similarity to B7/CD28 family members that is reminiscent of ancient common evolutionary origins. A gene structure alignment tool was developed to identify functional homologs of B7/CD28 genes even in the absence of sequence similarity. Next, the expression profile of these candidates was modeled and compared to profiles of known immune checkpoints in normal and cancer tissues. We will review the details of TIGIT and PVRIG discovery, which were among the immune checkpoints predicted in this process. Our approach demonstrates the powerful ability of computational biology to translate genomic knowledge into rational and reliable drug target discovery.

#585 Chromosome 9p24.1 deletions as a determinant of tumor immune surveillance and immune checkpoint blockade therapy in non-small cell lung cancer. Tao Shen, Jie Wu. Stepheison Cancer Center, OUHSC, Oklahoma City, OK.

Anti-PD-1/PD-1 immune checkpoint blockade therapy requires the presence of PD-L1/PD-L2 and MHC class I antigens on the tumor cell surface. We previously found that JAK2 truncating mutations in endometrial cancer cells that impair JAK1/JAK2-STAT1-IIF1-mediated signaling pathway, which regulates MHC class I antigen presentation. To determine the potential role and genetic defects in the IFN-γ-IRF1 pathway in non-small cell lung cancer (NSCLC), we analyzed the Cancer Genome Atlas (TCGA) data. Expression of IRF1 correlated with cytolytic activity markers GZMA and PRF1. Loss-of-function (LOF) genetic alterations in the IFN-γ-IRF1 pathway genes were found in 64 cases (6.3%) among 1016 patients. These genetic defects occurred prevalently in the JAK2 gene (33 cases) and often through deletions (29 cases) of chromosome 9p24.1. JAK2 gene deletions were frequently associated with deletions of CD274 and PDCD1LG2 genes that encode PD-L1 and PD-L2, respectively, whereas CD274 and PDCD1LG2 deletions were always accompanied by JAK2 deletions. Chromosome 9p is frequently deleted in NSCLC. This has been attributed to the co-localized at chromosome 9p24.1 near the CDKN2A/CDKN2B and PTPRD tumor suppressor genes located at the chromosome 9p is frequently deleted in NSCLC. This has been attributed to the code of the chromosome 9p deletion as a determinant of tumor immunoregulation. From code to cure: Computational discovery of novel immune checkpoints. Yair Benita, Amit Novik, Gady Cojocaru, Itamar Borukhov, Assaf Wool, Yossi Kliger, Tomer Zekharya, Zurit Levine, Sergey Nemzer, Ofer Levy, Amir Toporik. COMPUGEN, Holon, Israel.

Antibody blockade of CTLA4 and PD-1 immune checkpoint emerged as an effective treatment modality for cancer. However, the majority of patients do not achieve sustained long term benefit, suggesting a need for targeting of additional immune checkpoints. To identify additional B7/CD28 immune checkpoint targets, we developed a unique compendium of computational algorithms that identified multiple novel targets including TIGIT in 2008, which was an unknown protein at the time of discovery [Proc Natl Acad Sci U S A 2009 Oct 20;106(42):17858-63], and PVRIG which we recently disclosed. Since their initial discovery, these targets have been functionally validated and anti-tumor activity was demonstrated with antibodies that target them. In this presentation, we will describe the computational algorithms that led to the discovery of these novel immune checkpoints. These algorithms combine two complementary aspects: (i) endogenous immune checkpoint function prediction and (ii) prediction of immuno-modulatory activity in cancer. Immune checkpoint function was predicted based on gene structure similarity to B7/CD28 family members that is reminiscent of ancient common evolutionary origins. A gene structure alignment tool was developed to identify functional homologs of B7/CD28 genes even in the absence of sequence similarity. Next, the expression profile of these candidates was modeled and compared to profiles of known immune checkpoints in normal and cancer tissues. We will review the details of TIGIT and PVRIG discovery, which were among the immune checkpoints predicted in this process. Our approach demonstrates the powerful ability of computational biology to translate genomic knowledge into rational and reliable drug target discovery.

#586 Ionizing radiation-induced PD-L1 upregulation in glioma: a crucial role for the molecular chaperone FKBP5. Paolo D’Arrigo, Michele Russo, Elia Guadagno, Roberto Pacelli, Maria Laura Del Basso De caro, Anna Rea, Martina Tufano, Stefania Staibano, Gennaro Iardi, Maria Fiammetta Romano, Simona Romano. University of Naples Federico II, Naples, Italy.

Glioblastoma can avoid immune surveillance and induce tumor tolerance, through inhibitory molecules, e.g. PD-L1. Ionizing radiation (IR), used to treat this tumor, is known to increase tumor expression of PD-L1, thus inducing resistance mechanisms. Finding molecular determinants involved in IR-induced PD-L1 may provide a target for preventing such an effect and improve radiotherapy outcomes. We demonstrated that the short isoform of the co-chaperone FKBP51 (FKBP51s) regulated PD-L1 expression in melanoma. In glioma, FKBP51s was expressed at high levels, together with PD-L1 and its silencing reduced PD-L1 levels. Conversely, overexpression of FKBP51s increased PD-L1. Different PD-L1 isoforms were observed by immunoblot. A lower band (~37 kDa) corresponding to the naïve protein and two upper bands (~50, ~68 kDa) ascribable to post-translationally modified isoforms. FKBP51s was found mainly bound to the heaviest bands of PD-L1, reasonably mature protein, while the canonical isoform FKBP51 appeared to bind only to the naïve protein. Mature PD-L1 protein contains in carbohydrates addition, the principal chemical modification to most plasma membrane proteins, and, particularly, N-glycosylation.

Treatment of immunoprecipitated PD-L1 protein with PNGaseF produced a decrease of the highest band and the appearance of a lower band, corresponding to the naïve PD-L1, in accordance with the concept that the heaviest band of PD-L1 is a glycosylated form. Moreover, following subcellular fractionation to obtain extracts from ER and Golgi compartments, we found that naïve 37 kDa PD-L1 was detectable in the ER, but not in the Golgi. The PD-L1 glycosylated band was expressed in ER in a small proportion and mostly in the Golgi. FKBP51s, but not the canonical FKBP51, was found in ER. Co-IP of FKBP51s and PD-L1 from ER extract confirmed the two proteins interacted each other in ER. Our results show that naïve PD-L1 colocalized in the ER of glioma cell complexed with FKBP51s, while the PD-L1 glycosylated form was measured in the Golgi apparatus. Treatment of glioma cell with increasing doses of IR up-regulated PD-L1 expression, in a dose-response manner. Particularly, we found a significant increase in PD-L1 expression at 4 and 8 Gy, in comparison with unirradiated glioma cell. Moreover, IR induction of mature PD-L1 was efficiently counteracted by FKBP51s silencing. Subcellular fractionation of glioma cell subjected to IR in kinetics showed an early and transitory decrease in FKBP51s ER levels at 3hrs, in line with a reduction of the glycosylated band in the whole lysate. After 8 hrs from IR, FKBP51s rose up again in the ER inducing a full maturation of PD-L1. These findings suggested that FKBP51s has a role in catalyzing PD-L1 folding, an essential step to glycosylation, through which it could constitute a target for PD-L1 toxicity. We identified FKBP51s as an essential element that regulates PD-L1 expression on glioma cell, which is exploited by the tumor to resist to IR.

#587 HMDB002, a novel neutralizing antibody targeting a specific epitope on the co-inhibitory immune checkpoint receptor VISTA, displays potent anti-tumor effects in pre-clinical models. Piers J. Ingram, Dipi Thakkar, Jerome D. Boyd-Kirkup. Hummingbird Bioscience, Singapore.

V-domain immunoglobulin (Ig)-containing suppressor of T-cell activation (VISTA) also known as PD1 homolog (PD1H) is a co-inhibitory immune checkpoint receptor. VISTA is predominantly expressed on hematopoietic cells, particularly myeloid derived suppressor cells and antigen presenting cells, and at lower levels on CD4+ and CD8+ T cells and Foxp3 Tregs. Multiple studies have demonstrated VISTA can strongly suppress human T-cell activation, and the presence of high VISTA expressing cells in the tumor microenvironment has been postulated as playing a critical role in tumorigenesis and resistance to immunotherapy. VISTA neutralizing antibodies have been previously observed in pre-clinical models to increase the abundance of tumor infiltrating effector T cells as well as their effector functions, resulting in enhanced control of tumor growth, even in the absence of detectable expression of VISTA on the tumor cells, a potential advantage over PD1 or PD1L1 blockade. There is an urgent need to develop the most clinically effective anti-VISTA antibodies however the limited characterization of the VISTA pathway and the uncertainty around the cognate VISTA ligand has limited the extent to which in vitro studies can superimpose.
port the selection of optimal antibodies for development. Hummingbird Bioscience’s proprietary Rational Antibody Development platform to the design of neutralizing antibodies against specific epitopes on VISTA. Extensive in silico analyses of the VISTA structure and comparative structural modeling against other B7 protein family members has enabled the prediction of key binding sites where an optimal antibody inhibitor would bind. Recently, redocking of a (CD8+ MNC) cell line, NOZ and GBd, and lung small cell cancer cell line, SBC-5 were used as target cancer cells. Human lymphocytes derived from PBMC were activated by using anti CD3 mAb and IL-2, and were used as effector cells. For normoxic conditions, cells were cultured in 5% CO2 and 95% air. For hypoxic conditions, cells were cultured in 1% O2, 5% CO2, and 94% N2 using a multigas incubator. For Hh signaling inhibition, cyclopamine, polysaccharide-K (PSK), and small interfering RNA targeting Gli1, Smo, MAML3, and RBPJ were used. Cell numbers were counted by light microscopy. Expression of cell surface molecules was estimated by FACS. [Results] 1) Hypoxia augmented PDL-1 expression in all 4 cancer cell lines. 2) Inhibition of Hh signaling using MAML3 siRNA, cyclopamine and PSK reduced PDL-1 expression under hypoxia in all 4 cancer cell lines. 3) When activated lymphocytes were cocultured with cancers treated with an Hh inhibitor, activated lymphocyte cell numbers significantly increased under hypoxia. 4) In contrast, this increase was abrogated when cancer cells were treated with a PDL-1 neutralizing antibody. 5) When activated lymphocytes were cocultured with cancers treated with a Hh inhibitor and/or anti-PDL-1 Ab, the percent of CD8+ lymphocytes decreased in both the cyclopamine- and anti-PDL-1-treated groups under hypoxic conditions, while there was no significant change in CD3 expression. 6) NKG2D expression increased on activated lymphocytes in anti-PDL-1 Ab-treated group. [Conclusion] These results suggest that Hh signaling is one of regulatory pathways of PDL-1 expression under hypoxia and that inhibiting Hh signaling may induce lymphocyte antitumor activity. Therefore, Hh inhibition could be a promising drug target, not only as a direct tumor suppressor agent, but also as a PDL-1 inhibitor.

#588 Advanced molecular characterization of severe autoimmune toxicities associated with checkpoint inhibitor therapies. Justin M. Balko,1 Daniel Y. Wang,1 Yu Wang,1 Rami Al-Rohil,1 Margaret Compton,1 Jeffery A. Sosman,2 Igor Puzanov,2 Bret Mobley,1 Robert D. Hoffman,1 Yaomin Xu,1 Javid J. Mosleh,1 Chanjian Shi,1 Douglas B. Johnson,1 Vanderbilt University Medical Center, Nashville, TN; 2Northwestern University, Chicago, IL; 3Roswell Park Medical Institute, Buffalo, NY.

Immune checkpoint inhibitors (ICIs) have made a profound impact on the treatment of a variety of cancers. However, as with any systemic treatment, toxicities are inevitable. With most classes of cancer therapies, toxicities are relatively predictable based on clinical trial safety data and therefore can be handled with prophylactic or supportive care measures. However, ICIs are unique in their ability to cause rare but severe auto-immune toxicities. The molecular underpinnings of these toxicities, as well as unique features of the patient, tumor, or affected tissue, have not been extensively explored. We recently reported a small case series of two patients with myositis resulting in death arising following combination ICI therapy (Johnson et al, N Engl J Med, 2016). High lymphocytic infiltration, coupled with PD-L1 expression was present in the affected myocardium and skeletal muscle. Common T cell clones were identified between the affected tissue and tumor, and abnormal expression of muscle-specific transcripts was identified in the associated tumor, suggesting release of peripheral tolerance to tumor-expressed self-antigens. To expand upon our reported study, we collected healthy and afflicted tissue from a series of cancer patients with immune-related colitis, myositis (MC), and encephalitis following ICI treatment. We hypothesize that molecular analysis of these tissues will identify causal factors in the etiology of these toxicities, and how to better predict, prevent, and treat them. Thus, we performed molecular characterization of the immune infiltrate and diseased tissue microenvironment. A total of 20 affected (colon, cardiac, brain) and non-diseased control specimens were examined by spatial digital profiling (nanoString). This process generates a spatial heatmap of digital counts of 20 selected immunology and cellular markers and proteins across each specimen. Using this technology, the landscape of inflammation in ICI-affected organs can be resolved for insights into the mechanism whereby ICI-mediated auto-immunity occurs. Targeted RNAseq for selected immuno-oncology mRNA targets was also performed. In initial RNA sequencing analyses of MC cases, affected myositis, skeletal muscle, and patient-matched tumors all demonstrated expression of immune activation markers (e.g. interferon- gamma and granulocyte B), expression of PD-L1, and muscle-specific genes. In the expanded population, including colitis, digital spatial profiling analyses and targeted NGs (RNAseq) are underway. Although data analyses are incomplete at the time of this abstract, this work will be the largest and most comprehensive analysis of the molecular underpinnings of ICI-mediated auto-immune toxicity reported to date. These data should offer clarity in the mechanisms and features of these adverse events, how to prevent or predict them with precision medicine, and how to treat them when they do occur.

#589 Hedgehog signaling augments PDL-1 expression in cancer cells under hypoxic condition to inhibit antitumor effects by activated lymphocytes. Hideya Onishi, Akiko Fujimura, Yasuhiro Oyama, Makoto Kawamoto, Akio Yamasaki, Takashi Morisaki. Kyushu University, Fukuoka, Japan.

[Background] Hedgehog (Hh) signaling is activated under hypoxic condition in cancerous tissue. This is thought to be one of the mechanisms of the induction of malignant phenotype of cancers. We have shown that Hh inhibitor decreases proliferation, invasiveness, and tumorigenesis. We have also revealed that Hh signaling plays pivotal roles for the maintenance of function in activated lymphocytes and dendritic cells under hypoxic conditions. Redox-sensitive checkpoint inhibitor takes much attention, however, response rate is still limited. The mechanisms regulating PDL-1 expression also remains unclear. In this study, to increase response rate of immune checkpoint inhibitor, we investigated whether hypoxia-induced activation of Hh signaling contributes to PDL-1 expression in cancer and whether it affects the antitumor function of activated lymphocytes.

Materials and Methods Pancreatic cancer cell line, Panc-1, gallbladder cancer cell line, GBC-SD, and Hh tumor cells were cultured in 1% O2, 5% CO2, and 94% N2 using a multigas incubator. For Hh signaling inhibition, cyclopamine, polysaccharide-K (PSK), and small interfering RNA targeting Gli1, Smo, MAML3, and RBPJ were used. Cell numbers were counted by light microscopy. Expression of cell surface molecules was estimated by FACS. [Results] 1) Hypoxia augmented PDL-1 expression in all 4 cancer cell lines. 2) Inhibition of Hh signaling using MAML3 siRNA, cyclopamine and PSK reduced PDL-1 expression under hypoxia in all 4 cancer cell lines. 3) When activated lymphocytes were cocultured with cancers treated with an Hh inhibitor, activated lymphocyte cell numbers significantly increased under hypoxia. 4) In contrast, this increase was abrogated when cancer cells were treated with a PDL-1 neutralizing antibody. 5) When activated lymphocytes were cocultured with cancers treated with a Hh inhibitor and/or anti-PDL-1 Ab, the percent of CD8+ lymphocytes decreased in both the cyclopamine- and anti-PDL-1-treated groups under hypoxic conditions, while there was no significant change in CD3 expression. 6) NKG2D expression increased on activated lymphocytes in anti-PDL-1 Ab-treated group. [Conclusion] These results suggest that Hh signaling is one of regulatory pathways of PDL-1 expression under hypoxia and that inhibiting Hh signaling may induce lymphocyte antitumor activity. Therefore, Hh inhibition could be a promising drug target, not only as a direct tumor suppressor agent, but also as a PDL-1 inhibitor.

ALKS 4230 is a novel immunotherapeutic agent being tested in an ongoing phase I clinical trial and from ongoing preclinical studies. The extracellular portion of the IL-2 receptor is sterically prevented from binding to the high-affinity IL-2 receptor expressed on Treg. The efficacy of ALKS 4230 was compared to recombinant human IL-2 in a B16F10 lung metastasis model. ALKS 4230 treatment resulted in dose-dependent reduction of lung tumor colonization, with 100% inhibition at the highest dose tested. In contrast, while IL-2 was able to reduce lung tumor colonization, the maximal level of inhibition achieved was 60-70% at multiple dose levels such that increasing doses did not result in greater inhibition. Thus, the activation and expansion of effector cells without expansion of Treg, in response to ALKS 4230 treatment correlates with its improved efficacy over IL-2, which non-selectively expands both effector cells and Treg. The antitumor efficacy mediated by ALKS 4230 was further evaluated in several murine subcutaneous syngeneic tumor models, including B16F10, MC38 and EMT6. Treatment with ALKS 4230 or its murine ortholog resulted in inhibition of tumor growth and improved survival in multiple models. When dosed in combination with anti-CTLA-4 or anti-PD-1 antibodies, ALKS 4230 resulted in further improvement of tumor growth inhibition and survival. These results demonstrate the murine antitumor efficacy of ALKS 4230 alone and in combination with immune checkpoint inhibitors and support the ongoing clinical evaluation of ALKS 4230 as an immunotherapy for cancer.

Distinct cellular mechanisms mediate anti-CTLA-4 and anti-PD-1 checkpoint blockade. Spencer C. Wei,1 Jacob H. Levine,2 Dana Pe’er,2 James P. Allison1. 1UT MD Anderson Cancer Ctr., Houston, TX; 2Sloan Kettering Institute, New York, NY.

checkpoint blockade is able to achieve durable responses in a subset of patients, however the biological variables that distinguish responders from non-responders are not well understood. Furthermore, we lack a satisfying comprehension of the underlying mechanisms of anti-CTLA-4 and anti-PD-1 induced tumor rejection. Given that PD-1 and CTLA-4 attenuate T cell activity through distinct mechanisms that are separated spatially and temporally, we hypothesized that responses to anti-CTLA-4 and anti-PD-1 are driven by distinct mechanisms. To address this hypothesis we utilized mass cytometry to comprehensively profile the effect of checkpoint blockade on tumor immune infiltrates in murine tumor models. This approach allows for the interrogation of greater than 40 analytes at single cell resolution. We demonstrate that high dimensional mass cytometry analysis enables unsupervised identification of biologically relevant tumor infiltrating immune populations with high sensitivity and specificity. Using this approach we analyzed immune infiltrates of four murine tumor models in mice treated with anti-CTLA-4, anti-PD-1, or control antibodies. In both tumor models we identify distinct T cell populations with 0.5% or greater frequency. The T cell populations identified in MC38 and B16B16 were highly congruent. Notably, some but not all of these T cell populations were responsive to checkpoint blockade. A subset of tumor infiltrating CD8 T cell populations expanded following both anti-CTLA-4 and anti-PD-1. Conversely, a subset of regulatory T cell populations contracted following both anti-CTLA-4 and anti-PD-1. Interestingly, we observed expansion of a Th1 like CD4 effector T cell population only in response to anti-CTLA-4 treatment. Thus, we find that anti-PD-1 predominantly engages subsets of tumor infiltrating CD8 T cells whereas anti-CTLA-4 engages both the CD4 and CD8 effector compartments. Our findings indicate that anti-CTLA-4 and anti-PD-1 utilize distinct cellular mechanisms to induce tumor rejection. These findings highlight the importance of expanding our mechanistic understanding of immunotherapeutic approaches for the rational design of combinatorial therapeutic approaches. Furthermore, these results demonstrate that mass cytometry analysis can be utilized to identify biologically relevant tumor infiltrating T cell populations. We acknowledge the MDACC core facility NCI Support Grant P30CA166672.


Background: Immunotheapy with PD-1 inhibitors is associated with increased survival in NSCLC. While PD-L1 expression enriches for clinical benefit, additional predictive biomarkers are critically needed to further define patient subsets associated with specific response/resistance profiles and to develop optimal combination strategies. We hypothesized that integration of pathology features, including Tumor mutation burden (TMB) and tumor mutation load (TML), with immunogenomic data from NSCLC could identify phenotypic/genotypic associations that highlight specific immunosuppressive mechanisms. Towards that end, we present data from an initial pilot analysis. Methods: Twenty-nine NSCLC (23 adenocarcinoma; 6 squamous cell carcinoma) were included. H&E-stained sections were scored for tumor grade (1-3), relative proportion of tumor stroma (stroma ratio 1-3), density across and intensity of PD-L1 immunostaining, intensity of PD-L1 expression on tumor infiltrate (1-3+), PD-L1 immunohistochemistry was performed using the DAKO 28-8 antibody. PD-L1 expression was scored in tumor cells by modified H-score and by the predominant pattern observed - diffuse, heterogeneous, tumor-stroma interface or negative, and in immune cells using a semi-quantitative intensity scale (1-3+). DNA and RNA extracts from FFPE tissue were subjected to whole exome sequencing and RNAsseq from which mutation load, oncogene/ tumor suppressor genotypes and inflammation signatures were derived. Results: The highest PD-L1 H-scores were associated with diffuse expression patterns and were observed in high grade tumors (Grade 3). Deletorius mutations in TP53, STK11, KEAP1, KRAS, EGFR and MET occurred at expected frequencies. These mutations occurred across multiple PD-L1 expression patterns except for 1 PD-L1 high expression tumors (p=0.077, Fisher exact test). STK11-mutant tumors also displayed a lower PD-L1+ inflammation score (p=0.046, Fisher exact test). Trends toward an increase in mutation load with increasing levels of chronic inflammation on H&E stain as well as with PD-L1+ chronic immune infiltrates were noted. Inflammation signatures derived from RNAsseq showed an assocition between diffuse PD-L1 expression by IHC and the highest levels of inflammation mRNA signatures comprising T-cells (CD8, Tregs), B-cells, macrophages and MDSCs. Conclusions: The potential association between tumor grade, PD-L1 expression, intensity of the immune infiltrate and mutation load raise the possibility that tumor morphology predicts mutation load and associated immune response. The finding that STK11 mutation is restricted to PD-L1 negative tumors suggests an immunosuppressive mechanism is invoked in this setting. This dataset is currently being expanded to establish the significance of these findings.

Dual targeting of TGFβ and PD-L1 promotes potent anti-tumor effec- tivity in multiple murine models of solid carcinomas. Karin M. Knudson,1 Sofia R. Gameiro,1 Kin-Ming Lo,2 Jeffrey Schlom1. 1NCI-CCR, Bethesda, MD; 2EMD Serono, Billerica, MA.

Tumors evade host immune surveillance through multiple mechanisms, including promoting a tumor microenvironment able to effectively suppress immune effector functions. Aberrant secretion of anti-inflammatory cytokines, such as TGFβ and PD-L1, is a hallmark of most carcinomas. Immune checkpoint molecules are two main contributors to immune evasion and tumor progression. TGFβ is an important immunosuppressive cytokine with pleiotropic actions in cancer, including promotion of epithelial to mesenchymal transition and metastasis. It promotes tumor immune evasion by impairing T and NK cell maturation, recruitment, and function. Tumors also inhibit antitumor immune responses through tumor cell-immune cell interaction, in which the negative regulatory checkpoint PD-L1/PD-1 interaction plays a major role. Therapies that independently target TGFβ signaling or PD-L1/PD-1 interaction demonstrate limited clinical efficacy. Anti-PD-L1/TGFβTRAP (M7824) is a novel immunotherapeutic agent designed to simultaneously block the PD-L1 and TGFβ immunosuppressive pathways. It is comprised of the extracellular domain of human TGFβRII (TGFβTRAP) linked to the C-terminus of human anti-PD-L1 heavy chain (anti-PD-L1). M7824 has shown potent anti-tumor activities in preclinical models1 and some evidence of clinical efficacy in a phase 1 study2. Here, we demonstrate that anti-PD-L1/TGFβTRAP neutralizes murine TGFβ-induced signaling and binds to murine PD-L1 in multiple murine breast and colon carcinoma models. In non-tumor bearing mice, anti-PD-L1/TGFβTRAP increases the number of CD8+ T cells in intratumoral, Treg, and dendritic cell (DC) and B cell populations and in tumors inflammatory active phenotype. Initial examination of peripheral immune subsets in tumor-bearing mice indicates that anti-PD-L1/TGFβTRAP induces CD8+ T cells and NK cells with a more active, less exhausted phenotype. Furthermore, dual targeting of PD-L1 and TGFβ increases tumor expression of MHC-I, MHC-II, and PD-L1, suggesting additional immune effects in the tumor microenvironment. Importantly, anti-PD-L1/TGFβTRAP displays potent anti-tumor efficacy against various murine models of breast and colorectal carcinomas. This response is durable, preventing relapses up to 7 months. Anti-PD-L1/TGFβTRAP also decreases spontaneous metastasis in a murine model of triple-negative breast cancer. Taken together, these findings support the preclinical proof of concept for dual targeting of TGFβ and PD-L1-PD-1

IMMUNOLOGY: Checkpoints 1

#595 Heterogeneity of immune checkpoint expression in lung cancer identified through rapid tissue donation. Theresa Boyle, Andrea Shaffer, Janella N. Hudson, Luisa D. Arevalo, Matthew Schabath, Teresita Muñoz-Antonia, Christie Pratt, Gwendolyn P. Quinn, Eric B. Haura, Moffitt Cancer Center, Tampa, FL.

Background: Translational research in advanced lung cancer is hindered by the limited availability of specimens for advanced molecular techniques. Although helpful, the standard practice to biopsy a small amount of tissue from a single site of cancer provides limited information. We launched a thoracic rapid tissue donation (RTD) program to enable lung cancer research with collection of tissue at multiple tumor sites within hours after death. Many patients chose to enroll in the RTD program as an opportunity to contribute to cancer research. RTD tissue will support multiple research projects, such as studying differential expression of immune checkpoint proteins in immune oncology or understanding resistance to targeted agents. Methods: The RTD program for patients with stage IV lung cancer was approved in June 2015 by the Moffitt Cancer Center institutional review board. Tissue specimens from multiple tumor sites from consented donors are collected rapidly (aim < 24 hours) after death and are frozen and/or preserved in formalin. Hematoxylin and eosin (H&E) staining is performed with evaluation of pathology and quality of the specimens. Immunohistochemistry is performed to evaluate expression of immune checkpoint biomarkers (PD-L1, CTLA4, LAG3, TIM3, BTLA, A2AR and INOS). Results: Between June 26, 2015 and November 16, 2016, 18 patients with stage IV lung cancer consented to the RTD program. Post-mortem tissue has been collected from multiple tumor sites for three cases. H&E staining of 25 tissue blocks from the first case showed minimal evidence of post-mortem tissue damage by autolysis, confirming high RTD tissue quality. Biomarker studies, such as immunohistochemistry for immune checkpoints, such as PD-L1, CTLA4, LAG3, TIM3, BTLA, A2AR and INOS are ongoing. Preliminary analysis of PD-L1 expression in the first patient revealed heterogenous PD-L1 expression within and between eight tumor sites (both lungs, liver, both kidneys, left adrenal gland, mediastinal and retroperitoneal lymph nodes). Conclusions: Rapid tissue donation with collection of ample post-mortem tumor tissue is feasible and valuable for cancer research. A heterogeneous pattern of PD-L1 immune checkpoint expression was observed between multiple sites of tumor. Post-mortem tissue collection from multiple tumor sites facilitates understanding changes in tumor behavior and biomarker expression at metastatic sites, especially in the context of treatment failures. Acknowledgments: H&H Sponsor #R21 CA149532-01 and Tissue Core Facility at the H. Lee Moffitt Cancer Center & Research Institute (P30-CA076292).

#596 eFT508, a potent and highly selective inhibitor of MNK1/2 regulates immune checkpoint and cytokine expression promoting anti-tumor immunity. Kevin R. Webster, Vikas K. Goel, Jocelyn Staunton, Craig R. Stumpf, Rajesh Sharma, Ivy N. Hung, Gregory S. Parker, Jolene Møller, Gary G. Chuang, Andrew J. Wogenrich, Samuel Speney, Vera Huang, Peggy A. Thompson, Chinh Tran, Justin T. Ernst, Paul A. Sprengeler, Sieg-fried H. Reich,

eFECTOR Therapeutics, San Diego, CA; 2 Molecular Stethoscope, San Diego, CA.

Dysregulated translation of messenger RNA (mRNA) plays a role in the pathogenesis of multiple solid tumors and hematological malignancies. MNK1 and MNK2 integrate signals from several oncogenic and immune signaling pathways (including RAS, Toll-like receptors and T cell receptor) by phosphorylating eukaryotic initiation factor 4E (eIF4E) and other key effector proteins including hnRNP A1 and PSF. Phosphorylation of these mRNA-binding proteins by MNK1 and MNK2 selectively regulates the stability and translation of a subset of cellular mRNA that control tumor/stromal cell signaling, the tumor microenvironment and immune cell function. eFT508 is a potent and highly selective inhibitor of both MNK1 and MNK2. Ribosome profiling has demonstrated that inhibition of MNK1 and MNK2 by eFT508 selectively regulates the translational efficiency and mRNA stability of a subset of genes that include inflammatory cytokines/chemokines, regulators of stress response, and effectors of anti-tumor immune response. Given the importance of MAPK signaling and translational control to immune cell activation and differentiation, the immunological effect of eFT508 was further evaluated in both normal human immune cells in vitro and immunocompetent syngeneic cancer models in vivo. eFT508 treatment of normal donor T cells has no deleterious effect on an CaD3/CD28 stimulated IL-2 production, T cell proliferation or T cell viability. However, eFT508 selectively down regulates the induction of IL-10 and specific immune checkpoint receptors, including PD-1 and LAG3. Further evaluation of the mechanism of translational regulation has shown LAG3 mRNA contains specific sequence elements in the 3' untranslated region (UTR) that confer sensitivity to eFT508. In addition, IL-10 mRNA is destabilized upon treatment with eFT508 leading to significant inhibition of IL-10 production in activated T cells. Furthermore, eFT508 treatment results in upregulation of MHC class II molecules on tumor cells, macrophage and dendritic cells through an IL-10/MARCH1 dependent mechanism. The in vivo antitumor effect of eFT508 was assessed in the CT26 BALB/c syngeneic tumor model. CT26 mouse tumor cell proliferation and survival are insensitive to eFT508 in vitro. In vivo, daily oral treatment with 1 mg/kg eFT508 results in significant inhibition of tumor growth, modulation of tumor infiltrating lymphocytes and establishment of immune memory. In addition, combination of eFT508 with either anti-PD-1 or anti-PD-L1 monoclonal antibodies results in marked efficacy, significantly increasing the percentage of responder animals. eFT508 is currently under evaluation in two phase I/II clinical trials for patients with advanced solid tumors and patients with advanced lymphoma respectively. These findings support further clinical evaluation of eFT508 in combination with checkpoint blockade.

IMMUNOLOGY: Checkpoints 1

#597 Bispecific anti-CD123 x anti-CD3 ADAPTIR™ molecules for redirected T-cell cytotoxicity in hematological malignancies. Michael R. Corcoran, Robert Delville Mitchell, Dennis M. Slamon, Rollin J. Cha, Mollie M. Daugherty, Lara Parr, Peter Pavluk, Brian Woodruff, Hang Fang, Megan Aguilar, Jeannette Bannink, Starrla Johnson, Gary Li, Robert E. Miller, Robert Bader, Nicole Zhang, Toddy Sewell, Maria Dasovich, Gabriela H. Hoyos, John W. Blankenship, Catherine McMahan, David Bienvenue, Jane A. Gross, Aptevo Therapeutics, Seattle, WA.

Introduction: CD123 is a component of the IL-3 receptor expressed in several hematological malignancies including AML, ALL, HCL, and MDS. CD123 is a compelling target in AML due to its overexpression on AML blasts as well as leukemic stem cells, which are thought to be resistant to chemotherapy and may be responsible for relapse of disease following treatment. While CD123 is expressed by some normal leukocyte populations in circulation and hematopoietic progenitor cells in the bone marrow, the low frequency of expression on normal cell types provides a therapeutic window for targeting CD123 in tumor settings with the potential for durable response and reversible side effects. We have developed bispecific anti-CD123 x anti-CD3 ADAPTIR molecules APVO436 and APVO437 for redirecting T-cell cytotoxicity to CD123-expressing tumor cells. Results are presented that examine the in vitro and in vivo activity of these molecules in preclinical models of AML. Methods: APVO436 and APVO437 proteins were expressed in CHO cells. Affinity SPR studies were performed using recombinant CD123-ectodomain. In vitro functional studies were conducted with CD123+ AML tumor cell lines and primary human and cynomolgus macaque T-cell populations. Cytotoxic activity was determined using chromium release assays. On-cell binding, T-cell activation and proliferation were assessed using multi-color flow cytometry. Pharmacokinetic parameters were determined in BALB/c mice using a single IV dose of approximately 10 mg/kg. In vivo studies to examine tumor growth inhibition activity were performed with NOD/SCID mice co-implanted subcutaneously with AML tumor cells and human T-cells followed by treatment with APVO436 or APVO437. Tumor growth was assessed by measuring tumor volume and Bioluminescent Imaging. Results: APVO436 and APVO437 bound human CD123 protein with high affinity and binding to CD123 and CD3 expressing cell lines was confirmed by flow cytometry. Both APVO436 and APVO437 induced concentration-dependent lysis of CD123+ AML cell lines with primary human effector T-cells, accompanied by T-cell activation and proliferation. In vitro, T-cell cytotoxicity was assessed using primary cynomolgus macaque T cells. These activities were dependent on the expression of CD123 by the tumor target cells. APVO436 and APVO437 demonstrated an extended elimination half-life in mouse serum, typical of molecules capable of binding the neo-natal Fc receptor. In vivo, growth of AML tumor cells was inhibited by treatment with low doses of APVO436 and APVO437, significantly improving host survival. Conclusion: Taken together these data demonstrate potent in vitro and in vivo activity of APVO436 and APVO437 against CD123 expressing tumor cells and are supportive of further investigation of this approach as a potential treatment option for AML and other hematological malignancies.
anti-TIGIT. The microarray data were validated by qRT-PCR and results were consistent with flow cytometry and cytotoxicity results, and underlie the mechanism of action of anti-TIGIT. We have developed an IHC assay to evaluate TIGIT expression patterns in tumor and the associated stroma and TILs. Using this assay, we profiled 17 tumor types to evaluate patterns of TIGIT expression. Expression was high on immune cells in the stroma and on TILs in 8 tumor types, while it was predominantly low on tumor cells. This assay also low on tumor cells in a panel of 27 PDOx models. Consistent with the IHC results, analysis of 33 tumor types in the TCGA by RNA-Seq showed a good correlation of the expression levels of TIGIT and T cell markers, suggesting that TIGIT is mostly expressed on immune cells in tumors. In conclusion, we have identified PD biomarkers for anti-TIGIT in tumors and in surrogates tissues in syngeneic mouse models. Analysis of the biomarkers demonstrates activation of T cells and NK cells upon inhibition of TIGIT signaling. These biomarkers can be used in the clinic to demonstrate target engagement. In addition, we have profiled the expression of TIGIT in multiple solid tumor types and characterized prevalence of TIGIT-positive cells.

#598 APOBEC mutagenesis: a link between innate immunity and cancer. A Rouf Banday, Oluisegun O. Onabajo, Krizia-Ivana Udquim, Adeola Obajemu, Ludmila Prokunina-Olsson. NCI/NIH, Bethesda, MD.

Introduction: Cytidine deaminase activity of APOBEC3 enzymes generates mutations that restrict viral infection and eliminate tumor cells but also contribute to viral and tumor evolution. For example, human immunodeficiency virus (HIV) is hypermutated by APOBEC3G (A3G), and human papilloma virus (HPV) can be hypermutated by APOBEC3s, including A3A and A3B. In many human tumor types explored by The Cancer Genome Atlas (TCGA), C to T or G substitutions in the TCA or TCT motifs are predominately attributed to the activity of A3A and A3B enzymes. In bladder tumors more than 60% of all exonic mutations were accounted for by the APOBEC3-signature type, with nearly 100% of all bladder tumors carrying these mutations. Previously, we reported that two germline genetic variants – a single nucleotide polymorphism (SNP) rs1014971 and a 30kb germline deletion that eliminates A3B and creates an A3AB chimera, are the strongest factors associated with bladder and breast cancer risk, expression of A3A and A3B, and APOBEC mutagenesis in human tumors. Other factors affecting the source of APOBEC mutagenesis in human tissues are not well understood. Because some APOBEC3s can be induced in the course of antiviral response, we hypothesized that APOBEC mutagenesis might be a misfired innate immune response. Methods: We used TaqMan-based expression analysis for selected APOBECs (A3A, A3B, and A3G) to explore expression in bladder (HT-1376, HBT-9, and RT-4) and breast cancer (MCF-7, MDA-MB-231, and HCC11006) cell lines and in cells infected with a Sendai virus (SeV) that induces APOBEC3 expression. The results showed that APOBEC3s in RT-4 and MDA-MB-231 was followed-up by global transcriptome analysis with RNA-seq and pathway analysis. Cell lines were also treated with IFN-α, IFN-γ, IFN-A and IFN-γ and A3A, A3B and A3G by TaqMan assays. Results: A3A, A3B and A3G are interferon-stimulated genes (ISGs) because they were induced in the course of innate antiviral response to SeV infection and by IFNs in some conditions. Specifically, A3A was most strongly induced by SeV, with 32, 51, 12,000-fold induction in 3 breast cancer cell lines, and 4, 5, and 167-fold induction in 3 bladder cancer cell lines. A3B was also induced by SeV but only in the range of 1-5 fold in all cell lines tested. Conclusion: APOBEC3s can be strongly induced in the course of innate immune response even to transient and self-limiting viral infections. In the presence of single-stranded DNA (ssDNA), the APOBEC3 substrate generated by different cellular and environmental conditions, strong induction of some nuclear APOBEC3s, such as A3A and A3B, may result in mutation of human genomic DNA. In turn, accumulation of these mutations can lead to tumor initiation and evolution, particularly in individuals with germline APOBEC3 risk variants.


TIGIT (C cell immunoreceptor with Ig ITIM domain) is a co-inhibitory receptor and its signaling axis inhibits T cell and Natural Killer (NK) cell activity in the healthy immune system. In tumors TIGIT is highly expressed on a subset of dysfunctional T and NK cells and of highly suppressive regulatory T cells (Treg). Loss of TIGIT signaling enhances NK cell activity, CD4+ T cell priming and CD8+ T cell effector functions, suggesting a role in anti-tumor immunity. We have developed an anti-TIGIT blocking antibody that shows potent antitumor efficacy in multiple syngeneic mouse models, including CT26 WT colon, B16F10 melanoma and 4T1 breast cancer. Dose-dependent (12.5-0.1 mg/Kg) single agent efficacy was demonstrated in established tumors, and pharmacodynamic (PD) biomarkers in blood and in tumors were identified. Consistent with TIGIT’s role as a co-inhibitory receptor, anti-TIGIT promoted a dose-dependent increase of the CD4+ and CD8+ T cell fraction, with nearly 100% of all infiltrating leukocytes (TIL) and NK cells, as shown by increased staining of IFN-γ and CD69 by flow cytometry, when compared to controls. Anti-TIGIT also increased CD4 and CD8 T cell frequency in the tumor, measured by immunohistochemistry (IHC). Furthermore, anti-TIGIT caused an increase in splenic NK cell cytotoxicity, which correlated with dose and efficacy. To identify gene expression biomarkers in tumor and in blood, we used microarray analysis, and found similar immune gene changes between the two tissues. As expected, anti-TIGIT increased the expression of genes associated with CD8+ T cells, CD4+ T cells, and NK cells. Markers indicative of cytotoxic activity and Th1 response were also induced by anti-TIGIT. The microarray data were validated by qRT-PCR and results were consistent with flow cytometry and cytotoxicity results, and underlie the mechanism of action of anti-TIGIT. We have developed an IHC assay to evaluate TIGIT expression patterns in tumor and the associated stroma and TILs. Using this assay, we profiled 17 tumor types to evaluate patterns of TIGIT expression. Expression was high on immune cells in the stroma and on TILs in 8 tumor types, while it was predominantly low on tumor cells. This assay also low on tumor cells in a panel of 27 PDOx models. Consistent with the IHC results, analysis of 33 tumor types in the TCGA by RNA-Seq showed a good correlation of the expression levels of TIGIT and T cell markers, suggesting that TIGIT is mostly expressed on immune cells in tumors. In conclusion, we have identified PD biomarkers for anti-TIGIT in tumors and in surrogates tissues in syngeneic mouse models. Analysis of the biomarkers demonstrates activation of T cells and NK cells upon inhibition of TIGIT signaling. These biomarkers can be used in the clinic to demonstrate target engagement. In addition, we have profiled the expression of TIGIT in multiple solid tumor types and characterized prevalence of TIGIT-positive cells.
tic effects, and can be designed to incorporate payloads that enhance their onco-
lytic effects. TETN-L is a recently discovered, longer form of TETN which is
secreted and membrane permeable, and retains its phosphatase activity after
secretion and re-entry into cells. We have generated a novel oncolytic herpesvi-
rus (RAPTOR) that secretes TETN-L from infected cells for the treatment of
GBM and breast metastases in vivo. In vitro, RAPTOR mediates tumor cell
virol mediated tumor cell killing, virus replication, transgene expression, and
flow cytometry to evaluate intracellular pAkt and cell surface programmed death
ligand 1 (PD-1) were performed. In vivo studies using mice bearing orthotopic
human GBM (nude mice) or murine BCBM (nude and FVB/N) were used to
evaluate brain immune infiltrates and survival. Western blot analyses of infected
lymphocytes showed increased viral and media show that RAPTOR produces
significantly greater amounts of IFNγ than either control CAR or CAR-DNR cells,
following activation in the presence of TGF-β. Flow cytometry of treated tumor-bearing mice in the
presence of TGF-β was completely protected from TGF-β signaling domains of the T cell simulating IL-12 receptors IL-12Rβ2 binding domain of each receptor is fused to the transmembrane and intracellular
cytoplasm. Tumor cell killing and replication were equivalent between RAPTOR and control HSVQ. In vivo,
treatment of mice bearing established orthotopic BCBM revealed improved an-
ti-tumor efficacy of RAPTOR. Significantly, 9/10 RAPTOR treated mice were
long term survivors in immunocompetent FVB/N mice vs. 4/10 HSVQ treated
mice (p=0.044). Treatment of nude mice bearing the same tumors did not
produce long term survivors, implicating the development of antitumor immu-
nity in RAPTOR treated animals. Flow cytometry revealed significant down
regulation of the T-cell repressor PD-L1 on RAPTOR vs. HSVQ treated cells.
Consistent with this, FACS of virus treated tumor bearing brain hemispheres
revealed RAPTOR induced a significant influx in antigen presenting, NK, and
CD8 T cell infiltration. Our findings demonstrate that RAPTOR enhances survival of brain tumor bearing mice using a two-pronged approach:
lytic tumor cell death and immune cell education and activation against the
brain tumor. RAPTOR inhibits virol induced PD-L1 expression on tumor
cells, and could pave the way for a paradigm shift in immune therapy where
immune checkpoint inhibition occurs locally within the tumor rather than glob-
ally, overriding toxicity of neutralizing antibodies.

#602 A novel TGF-β/IL-12R signal conversion platform that protects
CAR T cells from TGF-β-mediated immune suppression and concurrently
amplifies effector function. Benjamin Boyerinas, Sara Miller, Ryan Murray,
Stacie Seidel, Geoffrey Parsons, Kathy Seidl, Kevin Friedman, Richard Morgan.

bluebird bio, Inc., Cambridge, MA.

Numerous immune-suppressive mechanisms exist within the tumor microenvi-
ronment that may hinder chimeric antigen receptor (CAR) T cell efficacy. One such
mechanism is mediated by TGF-β, a cytokine secreted by tumor cells and inhib-
iting suppressive immune cells that directly inhibits effector T cell activity.
Effector T cells express the TGF-β receptors TGFBR1 and TGFBR2, and expres-
sion of T cells to TGF-β induces phosphorylation of the major TGF-β signal
mediators SMAD2 and SMAD3. Phosphorylated SMAD proteins (pSMADs)
induce a signal transduction that results in transcriptional repression leading to
reduced cytokine production, reduced cytotoxicity, and a failure to proliferate in re-
ponse to antigen stimulation. A dominant negative receptor version (DNR) of
TGFBR2 that does not contain signaling domains protects T cells from the
impacts of TGF-β by blocking the ability of TGF-β to induce pSMADs. Here, we
report the development of a novel TGF-β signal conversion platform that pro-
vides a T cell stimulatory signal upon exposure to TGF-β. This platform utilizes
co-expression of chimeric variants of TGFBR2 and TGFBR1 where the TGF-β
binding domain of each receptor is fused to the transmembrane and intracellular
signaling domains of the T cell simulating IL-12 receptors IL-12Rβ2 and IL-
12Rβ1, respectively. Using a single lentiviral vector encoding both chimeric
TGF-β receptors (CTBR) and a CAR, we demonstrated that CAR-CTBR T cells
were completely protected from TGF-β-mediated SMAD phosphorylation. In addi-
ton, CAR-CTBR T cells generated significant amounts of pSTAT4 and
pSTAT5 in response to TGF-β exposure, a response that mimics the T cell
response on the secretion of IFNγ, a

#603 Evaluation of CD19 targeted T cells in relapsed or refractory ALL
patients unable to afford allogeneic bone marrow transplant in India. Alka
Dwivedi, Sushant Kumar, Terry Fry, Gaurav Narula, Rahul Purwar, Indian
Institute of Technology Bombay, Mumbai, India; National Cancer
Institute (NCI), National Institute of Health (NIH), Bethesda, MD; Tata Memo-
rrial Hospital, Mumbai, India.

CAR-T cell therapy has demonstrated remarkable success in long-term re-
mission of relapsed or refractory B-ALL. Unlike western countries, most of the
relapsed/refractory B-ALL patients in India are unable to afford allogeneic stem
cell transplant (allo-SCT), and simply choose palliation. To assess the potential
use of CAR-T cells as a first line therapy, it is critical to evaluate CAR-T cell
efficacy in patients unable to undergo allo-SCT. As a first step, a novel anti-CD19
CAR was designed and CD19-vector was produced using lentiviral mediated
gene delivery system. To generate CD19+ CAR T cells, T cells from healthy
subjects were transduced and expanded either with rIL-2 or rIL-15. Almost
20-50% transduced T cells express CD19-CAR on their surface as analysed by
flow cytometry. Next, efficacy of CD19-CAR T cells was examined by cyto-
toxicity assay using CD19+ malignant B cell lines (Raji cells) and autologous B cells.
CAR-T cells were able to kill majority of CD19+B cells effectively (100% kill-
ing). In addition, CAR-T cells secreted high levels of IFNγ and IL-2. The CAR-T
cells expanded in presence of rIL-15 showed fewer Treg compared to CAR-T cells
expanding rIL-2. However, there were no differences in numbers of T
stem cells (TSC) and central memory T cells (TCM). In addition to data on healthy
volunteers reflected above, results of above tests in relapsed/ refractory
B-ALL patients is ongoing and being collated for inclusion in the final analysis.
In conclusion, our data demonstrate the successful development of an indige-
nous CAR-T cell platform for subsequent use in clinical trials of CD19+B cell
malignancies.

#604 Accurate identification and prioritization of candidate neoantigens
from integrated cancer exome and transcriptome sequencing of FFPE sam-
ples. Marián Novak, Sam Angiuoli, Luis A. Diaz, Andrew Georgiadis, Sian
Jones, Peter R. Lovero, Sonya Parpart-Li, Maria Svedali, Victor E. Velculescu,
Ellen L. Verner, James White, Theresa Zhang, Mark Sausen. Personal Genome
Diagnoses, Inc., Baltimore, MD.

Precise identification and characterization of candidate neoantigens is
important for the development of effective cancer vaccines, adoptive T-cell
transfer, and prediction of response to checkpoint inhibitors. The candidate
tumor neoantigens are actionable only when expressed, however, current
prediction methods lack the capacity to evaluate neoantigen expression. Se-
quencing both DNA and RNA from a patient’s tumor tissue enables identi-
fication of mutations and evaluation of their expression leading to accurate
identification of putative neoantigens. The purpose of this study was to de-
velop and validate a methodology for co-extraction and sequencing of DNA
and RNA from formalin-fixed paraffin-embedded (FFPE) samples to enable
a robust neoantigen prediction protocol that integrates whole exome and
transcriptome data to identify and prioritize neoantigens for applica-
tion in immuno-oncology research and clinical trials. In order to prepare
high-quality sequencing libraries from FFPE specimens, the tissue was
macro dissected to enrich for tumor-specific material, and improve the overall
accuracy of next-generation sequencing for detection of somatic alterations.
Total DNA and RNA was co-extracted and purified. The DNA was used to
prepare whole exome sequencing (WES) libraries, while the co-extracted
RNA was ribosome-depleted, and reverse-transcribed to prepare RNA se-
quencing (RNAseq) libraries. The WES and RNAseq data was then analyzed
using a multi-algorithm HLA typing and neoantigen prediction protocol
(ImmunoSelect-RTM). ImmunoSelect-R evaluates somatic genomic altera-
tions identified from WES of tumor and matched normal tissue to ensure
appropriate prediction of candidate neoantigens. The process of neoantigen
prediction was then refined by integration of patient tumor-matched
RNAseq data, which allowed for removal of non-expressed putative neoan-
tigens. To further validate the approach, we applied the methodology to a set
of experimentally validated neoantigens. In this setting, ImmunoSelect-R
correctly classified 18 out of 19 as strong neoantigen candidates, suggesting
a sensitivity of greater than 90%. Moreover, in a set of 10 patients, Immu-
noSelect-R consistently ranked experimentally validated neoantigens within
the top 20% of all neoantigen candidates derived from whole exome sequenc-
ing. In summary, our combined tissue processing, macrodissection, co-ex-
traction, and neoantigen prediction methodology is able to identify and

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prioritize candidate neoantigens. Our approach is unique in combining high-fidelity sequencing (WES) and expression (RNAseq) data to accurately inform the selection of actionable tumor neoantigens for immunoncology applications.


The breakthrough discoveries of checkpoint inhibitors in the field of tumor immunology have driven the clinical success of immunotherapies for cancer, despite their beneficial efficacy in only a small portion of patients. This is due in part to immunodulatory mechanisms and the inability of the immune system to recognize tumor antigens as foreign. As a therapeutic approach to effectively present these tumor antigens in order to elicit an anti-tumor immune response, we previously designed and characterized an allogenic, gp96-Ig secreting, cell-based vaccine (ImPACT); currently being assessed in a phase II study in non-muscle invasive bladder cancer and a phase Ib study in non-small cell lung cancer – the latter, in combination with the PD-1 antagonist Nivolumab. We recently characterized a ‘next-generation’ vaccine (ComPACT) that combines the tumor antigen chaperone Gp96-Ig along with the T cell costimulator FC-OX40L, which are both secreted from the same cell (Fromm et al. Cancer Immunology Research. 2016). In preclinical assays, ComPACT is effective at stimulating CD4+ and CD8+ antigen-specific T cell expansion, the programming of a durable memory T cell phenotype, and the elimination of melanoma and colon tumors. This anti-tumor efficacy is enhanced when ComPACT is combined with checkpoint inhibition (anti-PD1 or anti-PDL1). To support manufacturing and clinical efforts of both ImPACT and ComPACT, in anticipation of phase III expansion and/or new trial initiation, we have developed novel potency assays to quantify the biologically active form of Gp96-Ig and the in vitro activity of FC-OX40L on T cell costimulation. It has been shown that gp96 can interact with toll-like receptors (TLR) and that this interaction results in the activation of the NF-kB pathway. Since THP1 cells express abundant TLR2/4, we engineered a THP1 cell line to express luciferase that is regulated by NF-kB response elements. Furthermore, we utilized the human T cell line; Jurkat, as host cells in which we also express NF-kB-luciferase, to quantify FC-OX40L costimulation. Jurkat/NF-kB-luciferase cells primed with either CD3/CD28 or TNFα, and subsequently cultured with ComPACT-secreted FC-OX40L results in a dose dependent increase in NF-kB (luciferase) expression. Our current data in both assays show a linear correlation with the input of Gp96-Ig and FC-OX40L, and may serve as an effective potency assay to facilitate the manufacturing of our vaccine product as it transitions into more advanced cancer immunotherapy clinical studies.

**#606 Targeting multiple immune checkpoints and their ligands using a humanized mouse model of ovarian cancer.** Ruya-Yea Huang, Kunle Odunsi. Roswell Park Cancer Inst, Buffalo, NY.

We have recently reported that multiple immune checkpoints are expressed in the TILs (tumor infiltrating T lymphocytes) and TALS (tumor associated T lymphocytes) from a murine ovarian cancer model and that blockade of one checkpoint pathway induces an upregulation of the other unblocked checkpoints. We hypothesized that resistance to single agent blockade (e.g. anti-PD1) is resulting from the compensatory upregulation of the other pathways (e.g. CTLA4 or LAG-3). To test this hypothesis in human ovarian cancer, we first examined the level of several immune checkpoints and their ligands in TILs or TALS from ovarian cancer patients. In addition to the highly expressed PD-1 and CTLA4, other checkpoint molecules such as TIM3, LAG3, VISTA, and TIGIT are also elevated in TALS as compared with control PBMC. The levels of multiple ligands for the immune receptors in the tumor samples were also analyzed. To translate the results from mice to humans, we developed several aggressive human ovarian cancer cell lines from patient derived xenografts to test the combinatorial checkpoint blockade strategies. Using a humanized mouse model of ovarian cancer we observed that adaptive transfer of autologous T cells in combination with checkpoint blockade of PDL1 mildly delays ovarian tumor growth. The effect of blocking PD-1 and CTLA4-4 pathways on tumor growth and the upregulation of other checkpoints are currently underway.

**#607 PDL1 expression associated with triple negative breast ductal carcinomas in African American women.** Farhan Khan, Yasmine Kanaan, Luis J. Ricks-Santi, Rabia Zafar, Hagos Aynnuit, Tammye Naab, Howard University Hospital, Washington, DC, DC; Hampton University Hospital, Hampton, VA.

Background: Tumor cells avoid host immune response through expression of inhibitory T cell regulator, Programmed cell death ligand (PDL1). Our objective was to evaluate PDL1 expression by immunohistochemistry in the four major subtypes of breast carcinoma (Luminal A, Luminal B, HER2, and Triple Negative) in a population of 202 African-American (AA) women with other clinicopathological factors. Design: Tissue microarrays (TMAs) were constructed from FFPE tumor blocks from primary ductal breast carcinomas in 202 African-American (AA) females. Two separate 1 mm cores represented each case. Five micrometer sections were stained with rabbit monoclonal antibody against PDL1. The sections were evaluated for the percentage and intensity of membrane staining in both tumor and immune cells. Cut off was > 1%. Bivariate analysis was done via χ2 analysis and survival data was calculated via the generation of Kaplan-Meier curves (SPSS v19). Statistical significance was assumed if p < 0.05.

Results: PDL1 expression in tumor cells, only in the combination with ER negative (p<0.0001), PR negative (p<0.0001), Triple negative subtype (p<0.0001) and high grade (p<0.0001) breast ductal cancers. Conclusion: Our study finding of selective expression of PDL1 in triple negative breast ductal cancers (TNBC) in AA women suggests that inhibition of adaptive immune response is involved in the progression of these cancers. PDL1/PD1 is the inhibitory check point of immune response resulting in decreased T cell activation and cytokine production. Some studies have shown that PDL1 expression is associated with loss of PTEN and phosphatase inostol 3 kinase pathway (PI3K) activation. Our previous study showed loss of PTEN in TNBC. Combining PDL1 inhibitors with PI3K inhibitors may be useful therapy for aggressive TNBC.

**#608 Augmenting and broadening T cell responses against mutated tumour neo-antigens.** Bruce W. Robinson, Shaokang Ma, Jonathan Chee, Craig Rive, Paula Van Miert, Rob A. Holt, Janette Creaney. University of Western Australia, Western Australia, Australia; University of British Columbia, Vancouver, British Columbia, Canada.

Cytotoxic T lymphocytes (CTLs) recognize mutated tumor proteins (neo-antigens) and are important for anti-tumor immunity, especially in the context of immune checkpoint blockade immunotherapy (ICBP). Positive outcomes to ICBP are associated with high neo-antigen loads and with neo-antigen specific CTL responses. However only around 20% of patients respond to ICBP. In order to examine ways in which the non-responders might become responders we examined several strategies to improve response rates, using anti-CTLA4 initially as the ICBP therapy in BALB/c mice in which Uqrcr2c was defined as a DNA/RNAseq-identified neo-antigen in AB1 tumor lines induced by a relevant human carcinogen(1). 1) We first examined therapy-induced changes in T-cell responses against Uqrcr2c. Anti-CTLA4 alone increased the magnitude of responses against Uqrcr2c. Anti-CTLA4 combined with anti-GITR induced dominant spreading, unmasked responses against a new neo-antigen UNC45A that was undetectable during normal tumour growth. Immunogenic chemo-therapy also unmasked responses against UNC45A, suggesting that subdominant neo-antigens can be unmasked by the appropriate immunotherapy. 2) We then evaluated neo-antigen vaccination strategies. Uqrcr2c vaccination only protected against tumor growth when administered in combination with partial Treg depletion (Foxp3.DTR mice), suggesting that neo-antigen vaccination will only be maximally effective when administered in combination with therapies that target existing immune restraint (including Tregs). 3) We determined optimal anatomical location for tracking neo-antigen CTL responses and identified the draining lymph node as an optimal, though not exclusive, location for response testing compared to blood or tumor. We are currently examining these observations using sequencing-defined neo-antigens in our patients and will present data on changes in human neo-antigen responses to therapy. These observations have important translational implications for identification of key neo-antigens, choice of therapy and monitoring of anti-tumor responses. 1. J. Creaney et al., Strong spontaneous tumor neoantigen responses induced by a natural human carcinogen. Oncoimmunology 4, e1011492 (2015).

**#609 Overcoming host histocompatibility barrier to create a renewable source of off-the-shelf effector lymphocytes for adoptive immunotherapy.** Raedon L. Clarke, Matthieu Bayer, Ryan Bordyla, Jeffrey Susaki, Brian Groff, Svetlana Gaidarova, Tom Tong Lee, Weijie Lan, Michelle Burrascano, Ramzye Abujarour, Greg Bonello, Megan Robinson, Stewart Abbot, Scott Wolchko, Daniel Shoemaker, Bob Valamehr.

Encouraging clinical outcomes in autologous cellular immunotherapy have garnered hope and excitement. However, limitations of patient-derived cancer immunotherapies remain to be addressed to deliver reliable and efficacious therapeutic approaches with broader applicability. Induced pluripotent stem cells (iPSCs) are a unique, renewable source for the continuous generation of cellular therapeutics and represent a highly promising approach for overcoming many of the limitations of autologous therapy. To advance the promise of iPSC technology as an

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“off-the-shelf” (OTS) source of cellular therapeutics, several considerations need to be addressed. Ensuring the persistence of allogeneic OTS therapies after adoptive cell transfer across histocompatibility barriers is a key requirement. Establishing a master cell line from genetically engineered clonal iPSC lines with the capacity to continuously generate homogenous populations of highly functional effector cells would be a necessary cell source to design a feasible and reproducible approach for the generation of immune tolerant effecter cells derived from a genetically engineered iPSC master cell line. We successfully combined deletion of classical human leukocyte antigen molecules with expression of immunosuppressive proteins to generate clonal iPSC lines with the ability to escape immune rejection. Utilizing in vitro quantitative live cell analysis we show that OTS-iPSCs elicit a significantly decreased cytotoxic response from both peripheral blood (PB)-NK cells (47.9 vs. 91.4% survival at 3:1 E:T ratio) and PB-T cells (>2.7-fold greater number of OTS-iPSC derived cells remaining at 88 hrs). Additionally, mixed lymphocyte reactions employing unfractionated PB mononuclear cells resulted in significantly decreased activation and proliferation of CD8+ nuclear cells.

Moreover, mixed lymphocyte reactions employing unfractionated PB mononuclear cells resulted in significantly decreased activation and proliferation of CD8+ nuclear cells. Resultantly, we demonstrate that OTS-iPSCs can inhibit immune responses. In preclinical mouse models we demonstrate that OTS-iPSCs exhibit improved persistence in vivo. Bilateral engraftments were established in non-conditioned, fully immune-competent recipient mice using luciferized wildtype and OTS-iPSCs. Daily bioluminescence imaging revealed a significant increase in persistence of OTS-iPSCs during the 48-196 hour post injection window (>5.5 fold greater luminescence at 96 hrs). Using our potent genetically defined stage-specific bulk, our findings confirm that both the therapeutic potential of M7824 in non-small cell lung cancer (NSCLC). M7824 is a first-in-class bi-functional immunotherapeutic agent designed to target two negative regulatory pathways in immunosuppression. It is a novel fusion protein comprised of the extracellular domain of human TGFBR1 covalently linked to the C-terminus of the Fc domain of the fully human anti-PD-L1 IgG1 antibody avelumab. Methodology: In vitro assays were conducted using NSCLC cell lines treated with TGFβ1 alone or in combination with avelumab or M7824 to assess the ability of M7824 to revert features of tumor cell EMT, including molecular markers and functional responses.

The ability of M7824 to mediate antibody-directed cellular cytotoxicity (ADCC) of TGFβ1-treated cells in vitro was also assessed. Results: Treatment with TGFβ1 induced mesenchymal marker expression, reduced cell proliferation, and enhanced resistance to docetaxel, paclitaxel, and gemcitabine. Treatment with M7824, but not avelumab, was able to both prevent and revert these effects of TGFβ1. M7824 also exhibited the ability to mediate ADCC of TGFβ1-treated cells in vitro. Conclusions: M7824 effectively antagonizes TGFβ1-mediated tumor cell mesenchymalization, including resistance to chemotherapy. These results suggest that M7824 has the potential to relieve immunosuppression and block treatment-resistance induced by TGFβ1 in patients with NSCLC.

#610 Blockade of tumor expression of PD-1 promotes lung cancer growth in a murine model. Shisuo Du1, Qing Guan2, Adam P. Dicker1, Bo Lu1.

1Thomas Jefferson University, Philadelphia, PA; 2Fudan University, Shanghai, China.

Lung cancer remains one of the most prevalent and fatal malignancies worldwide. There are substantial evidences that immunotherapies offer significant benefits among lung cancer patients. Pembrolizumab, a humanized programmed death receptor-1 (anti-PD-1) antibody is now approved by FDA as a first-line treatment of patients with metastatic non-small cell lung cancer (NSCLC). Recent data demonstrate that PD-1 is not only expressed by immune cells, but also by human melanoma cells. However, limited is known about the biological significance of PD-1 pathway in cancer including NSCLC. Thus, we examined PD-1 expression in a series of established murine lung cancer cell lines. RT-PCR amplification and sequencing of the full coding sequence of the murine PD-1 (PDCD1) gene revealed the presence of PDCD1 mRNA in the M109 lung cancer cells. Flow cytometry and immunofluorescence analysis demonstrated PD-1 protein expression in these murine lung cancer cells. To determine the molecular function of tumor PD-1 in lung cancer survival, we treated M109 cells in culture with either anti- mouse PD-1 (100 µg/ml) or isotype control antibody and found that PD-1 inhibition significantly enhanced cancer cell viability as measured by MTT and clonogenic assay. Alternative PD1 inhibition by either knockdown of PDCD1 via siRNA or knockout of PDCD1 by CRISPR-Cas9 all lead to increased survival of M109 cells. Furthermore, PDCD1 knock-out M109 cells had no survival difference in the presence of anti-PD1 compared to its PD1-expressing parental cells. Conversely, treatment with recombinant PD-L1 fusion protein exhibited significantly increased apoptotic rates (7.5±3.2% vs 2.1±2.2%) suppressing the tumor cell growth in the wild-type M109 in culture. To validate these results in vivo models, NSG mice were used to determine the direct effect of anti-PD-L1 on M109 xenografts without the interplay of host immune system. M109 xenografts treated with anti-PD-1 (10mg/kg i.p.) antibodies showed accelerated growth compared to those treated by IgG control (248±273 vs 1499±292nm3 at days 21 after tumor inoculation, p=0.013). M109 subcutaneous tumors in the PD-1 knockout mice also manifested more rapid growth than the same tumors implanted in wild-type mice. Taken together, our results uncover a novel function of PD-1/PD-L1 axis in lung cancer survival and implicate possible tumor-promoting effects from anti-PD-1 therapy upon PD1-expressing lung cancer in the absence of effective immune response. We plan to interrogate bio-specimens collected through NRG trials to establish the translational potential of these findings.

#611 Reversion of mesenchymal features in NSCLC cells using M7824, a first-in-class bi-functional immunotherapeutic agent targeting PD-L1 and TGFβ (TGF β TRAP). Justin M. David1, Jeffrey Schmol,1 Yan Lan2, Claudia Palena3, National Cancer Institute, Bethesda, MD; 2EMD Serono, Billerica, MA.

Background: Evasion of antitumor immunity is widely regarded as a hallmark of cancer. Most tumors fail to stimulate effective antitumor immunity due to upregulation of the immune checkpoint molecule PD-L1, and antibodies targeting the PD-1/PD-L1 axis have demonstrated clinical efficacy in several tumor types. Antitumor immunity can also be suppressed by TGFβ, a pleiotropic cytokine that reduces innate and adaptive antitumor immune responses. Additionally, TGFβ impacts tumor cells by activating a differentiation program called epithelial-mesenchymal transition (EMT), in which tumor cells lose epithelial features and gain mesenchymal characteristics. The gain of mesenchymal traits is associated with metastasis, stemness, and resistance to multiple therapeutic modalities, including chemotheraphy and radiation. The objective of this work is to investigate the therapeutic potential of M7824 in non-small cell lung cancer (NSCLC). M7824 is a first-in-class bi-functional immunotherapeutic agent designed to target two negative regulatory pathways in immunosuppression. It is a novel fusion protein comprised of the extracellular domain of human TGFBR1 covalently linked to the C-terminus of the Fc domain of the fully human anti-PD-L1 IgG1 antibody avelumab. Methodology: In vitro assays were conducted using NSCLC cell lines treated with TGFβ1 alone or in combination with avelumab or M7824 to assess the ability of M7824 to revert features of tumor cell EMT, including molecular markers and functional responses (ADCC).

The ability of M7824 to mediate antibody-directed cellular cytotoxicity (ADCC) of TGFβ1-treated cells in vitro was also assessed. Results: Treatment with TGFβ1 induced mesenchymal marker expression, reduced cell proliferation, and enhanced resistance to docetaxel, paclitaxel, and gemcitabine. Treatment with M7824, but not avelumab, was able to both prevent and revert these effects of TGFβ1. M7824 also exhibited the ability to mediate ADCC of TGFβ1-treated cells in vitro. Conclusions: M7824 effectively antagonizes TGFβ1-mediated tumor cell mesenchymalization, including resistance to chemotherapy. These results suggest that M7824 has the potential to relieve immunosuppression and block treatment-resistance induced by TGFβ1 in patients with NSCLC.

#612 Methylation changes in DNA of CD8 T cells following CD137 co-stimulation. M. Angela Azzar,1 Sara Labiano,1 Angel Diaz-Lagares,1 Manel Esteller,2 Juan Sandoval,1 Ignacio Meleo1.

1Center for Applied Medical Research on Navarra (CIMA), Pamplona, Spain; 2Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain; 3Medical Research Institute La Fe, Valencia, Spain.

Introduction: CD137 ligation imprints long term changes in the behavior of costimulated T-cells (1). There is not a satisfactory mechanistic explanation yet. Experimental procedures: to determine the specific DNA methylation changes occurring upon CD137 costimulation, purified human CD8+ T cells from three healthy donors were activated in vitro for 5 days with anti-CD3 monoclonal antibody and either with an anti-CD3 monoclonal antibody or its corresponding isotype (huIgG4). Activated lymphocytes were left 5 days in culture with huIL-7 without further stimulation. Such back-to-resting CD8+ lymphocytes were re-stimulated with anti-CD3 for 12, 24 and 36h to validate the expression of the genes differentially methylated upon primary stimulation at mRNA and protein levels. DNA methylation profiles of both activated and resting cell subsets were characterized with Infinium 450K DNA methylation array (Illumina). To further confirm our observations, identical experimental procedure was performed with a different anti-CD137 agonist antibody (mlgG1 iso-type) in a CD8+ T-cell donor. Differentially methylated genes between anti-CD3+ anti-CD137 versus their corresponding control counterparts were validated by pyrosequencing on activated and resting CD8+ from independent group of healthy donors (n=8 for huIgG anti-CD137 and n= 11 for mlgG anti-CD137). Expression changes were confirmed by qRT-PCR and flow cytometry in activated, rested and restimulated CD8+ lymphocytes. Results: 853 genes were differentially methylated in huIgG anti-CD137-treated CD8+ T cells as compared with their controls, 52 of which were shared with mlgG anti-CD137-costimulated CD8+ T lymphocytes. A number of differentially methylated genes are involved in i) T cell migration, ii) T cell activation, survival and homeostasis and iii) regulation of gene expression including key T-cell transcription factors. Conclusions: CD137 costimulation induces CD8+ T lymphocytes that are enriched in gene sets involved in regulatory functions. These acquired functions are imprinted in the genomic DNA of the CD8+ T cells by DNA methylation changes upon CD137 co-stimulation, and involve key genes for CD8+ T cells. References: 1. Hendriks, J., Y. Xiao, J.W. Rossen, K.F. van der Sluijs, K. Sugamura, N. Ishii, and J. Borst. During viral infection of the respira...
development of FPA154, a novel tetravalent anti-GITR antibody, for the treatment of solid tumors. Susannah D. Barbee, Amanda Chen, Susan Johnson, 3 David L. Bell, 2 John C. Tinner, 2 Neiibuyu Wondiey, 1, 3 Mikayel Mkrtichyan, 1 Amu S. Razai, 1 Kyle S. Jones, 1 Chodisi Y. Hata, 1 Denise Gonzalez, 1 Quinn Deveraux, 2 Brendan P. Eckelmann, 3 Luis Borges. 1 Five Prime Therapeutics, South San Francisco, CA; 2 Inhibrx, San Diego, CA.

Glycolipiderived TNFR-related (GITR, TNFRSF18) is a member of the TNFR superfamily with pleiotropic T cell modulatory activity. We are developing a novel anti-GITR antibody with enhanced agonist activity for the treatment of solid tumors. Our candidate molecule, FPA154, is constructed with single-domain antibodies (sdAbs) in a tetravalent format, with an effector competent IgG1 Fc domain. Both FPA154 and the mouse-reactive surrogate molecule (cmFPA154, mIgG2a isotype) bind to cell-surface GITR with high affinity.

Our data indicates that FPA154 and cmFPA154 potently mediate ADCC activity against Tregs expressing high levels of GITR. In contrast, activated effector T cells express modest levels of cell-surface GITR, and FPA154 and cmFPA154 drive GITR-induced NF-kB activation. This activity is independent of Fc-mediated crosslinking, which is normally required for bivalent GITR antibodies to induce GITR signaling. cmFPA154 has potent antigen activity in several syngeneic mouse tumor models, both as a monotherapy treatment and in combination with anti-PD-1. In summary, FPA154 is a promising candidate with multiple mechanisms of action that contribute to generation of an antitumor immune response mediated by different T cell subsets.

**#616** The novel α-Gal-based immunotherapy AGI-134 invokes CD8 T cell-mediated immunity by driving tumor cell destruction, phagocytosis and tumor-associated antigen cross-presentation via multiple antibody-mediated effector functions. Jenny L. Middleton, 1 Oliver Schulz, 2 Amber Charlemagne, 3 Sascha A. Kristian, 1 Stephen Michael Shaw. 1AgAllimmune Limited, Sandwick, United Kingdom; "The Francis Crick Institute, London, United Kingdom.

Background: AGI-134 is a fully synthetic α-Gal (Galα1-3Galβ1-4GlcNAc-R) glycolipid that is being developed for the treatment of solid tumors. The α-Gal epitope is not expressed in humans, who, as a result of constant antigenic stimulation by α-Gal-bearing commensal bacteria, develop high titer natural antibodies to α-Gal. We have previously demonstrated that AGI-134 recruits anti-Gal antibodies to tumor cells in vitro, activating complement and driving phagocytosis by antigen presenting cells. AGI-134 also confers systemic protection from distal lesion development in a mouse model of melanoma and synergizes with anti-PD-1. 1. Here we present further data characterizing the multiple pathways activated by the anti-Gal subclasses to drive AGI-134-mediated anti-tumor immunity. Results: Using quantitative methods, we demonstrate that human anti-Gal is composed of a diverse repertoire of effector antibodies in a panel of serum samples, with IgM, IgG1 and IgG2 being the major subclasses. Polyclonal anti-Gal IgG purified from human IVIG was, like human serum, comprised mainly of IgG1 and IgG2. When AGI-134 treated cells were incubated with human serum, binding of all anti-Gal subclasses was detected by flow cytometry, demonstrating that all anti-Gal subclasses can interact with AGI-134 treated cells and are available to activate downstream effector functions. When human IVIG, AGI-134 and IgG1 were incubated with human cancer cells in vitro, Ovalbumin-expressing cancer cells were specifically phagocytosed by murine CD8+ dendritic cells and the immunodominant antigen of ovalbumin, SINFEKL, was cross-presented to CD8+ T cells. Conclusion: AGI-134 stimulates adaptive anti-tumor immunity through immune activation and antigen cross-presentation, which is driven by the diverse repertoire of anti-Gal antibodies. 1. Shaw, S. et al. Abstract 4862: AGI-134: a fully synthetic alpha-Gal glycolipid that prevents the development of distal lesions and is synergistic with an anti-PD-1 antibody in a mouse melanoma model. [Abstract]. AACR; Cancer Res 2016;76(14 Suppl):Abstract nr 4862.
proven for the treatment of both recurrent and newly diagnosed Glioblastoma (GBM). Immunotherapeutic approaches for treatment of GBM are considered promising, and multiple strategies are currently being evaluated in basic research and clinical trials. Combining TTFields and immune-based therapies is a rational approach as they possess markedly different mechanisms of action (OA). Converging TTFields may potentially abrogate various cellular functions required for effective T cell responses. We performed an in-vitro evaluation on the effect of TTFields on select human T cell functions that are pivotal for an effective anti-tumoral response. The study objective was to evaluate the potential compatibility between immune-based therapies and TTFields. METHODS: Peripher al blood mononuclear cells were isolated from healthy donors. Cells were cultured under normal versus TTFields conditions using the in vitro TTFields system, either with or without Phyothemaglutinin (PHA - a super antigen). Cellular responses were monitored using an 8-color flow cytometry panel that concurrently evaluated proliferation (CFSE dilution), cytokine secretion (IFNγ), cytotoxic degranulation (CD107α surface presentation), and T cell activation/ exhaustion (PD1 expression). The effect on T cell viability was assessed in a separate assay, by comparing the live-to-dead ratio of cells cultured in normal versus TTFields settings. RESULTS: TTFields did not alter the functionality of non-activated T cells. Viable PHA-activated T cells cultured under TTFields exhibited no change in PD1 up-regulation, IFNγ secretion and CD107α surface expression. The T cells exhibited reduced proliferation, which is in line with the known MOA of TTFields. As the presence of polyfunctional T cells is associated with effective antitumor therapy, we selected single-cell polyfunctional analysis to determine the effect of TTFields on T cell functionality. T cell functionality was evaluated as the percentages of PD1 CD4+ and PD1 CD8+ T cells in patients who received 3 doses of anti-PD1 antibody (p=0.007 and p=0.05, respectively). Moreover, the levels of PD1 CD4+ (p=0.009) and PD1 CD8+ (p=0.009) were augmented in response to anti-PD1 therapy. The frequencies of CD4 Tregs (CD3+CD4+CD25highCD122−/−CD152 FoxP3+) and granulocytic MDSCs (CD11b+CD14+CD33+) in responders to anti-PD1 therapy were decreased following anti-PD1 therapy (p=0.01 and p=0.02, respectively). In contrast, chemotheraphy affected only the PD1+ CD4+ Tregs, but not the PD1+ G-MDSC, by increasing their levels after 3 cycles (p=0.04). Anti-PD1 treatment induced a superior reduction of the PD1- CD4+, PD1- CD8+ T cells, PD1- CD4+ Tregs and PD1- G-MDSC percentages compared to the effect of first line chemotherapy (p=0.04, p=0.05, p=0.001, respectively). Furthermore, a significant decrease in PD1- CD8+ T cells, PD1- CD4+ Tregs and PD1- G-MDSCs after 3 doses of anti-PD1 was observed in patients who experienced stable disease compared to baseline (p=0.006, p=0.05 and p=0.03, respectively). At the time of response evaluation to chemotheraphy, the percentage of the PD1- CD4+ Tregs after 3 cycles was significantly inferior compared to baseline, in disease progressors (p=0.04). Conclusion: These data indicate that although chemotherapy affected the levels of PD1- CD4+ Tregs, anti-PD1 therapy seems to exert an effect on both PD1+ T cells and PD1- immunosuppressive cells. Additional studies are needed in a larger cohort in order to document its impact on their clinical relevance in NSCLC patients. This study is ongoing and updated data will be presented at the meeting.
Vaccine therapy may be ideal to destroy ductal carcinoma in situ (DCIS) and prevent recurrence. A vaccine can induce type 1 T-cells against DCIS antigens that could migrate from the circulation to invade and destroy the tumor as well as generate immunologic memory to provide long lasting protective immunity. One of the limitations of developing a DCIS vaccine is identifying antigens that target all subtypes of DCIS. DCIS is one of the most heterogeneous tumors, ranging from invasive breast cancer (IBC), including overexpressed proteins that may be drivers of progression to invasive disease. Eliminating cells that are overexpressing driver proteins by vaccination may prevent progression to IBC if such proteins could be determined. In this study, we identified proteins that were overexpressed from normal breast in both DCIS and IBC across fifteen Geo and ArrayExpress datasets. From the candidates or patient identified whose expression was necessary for cancer cell survival across breast cancer subtypes. We selected overexpressed proteins necessary for cancer survival using a high throughput siRNA screen and chose candidates that increased apoptosis and decreased cell survival in HER2 positive (HER2), triple negative (TN), and hormone receptor positive HER2 negative (HR) human breast cancer cell lines with decreased expression of the target protein. For example, decreased expression of NCD8 caused decreased cell survival to 56%±3% in HR, 46%±3% in TN, and 77%±2% in HER2 breast cancer cell lines and increased apoptosis by 1.4±0.3 fold in HR and 1.2±0.03 fold in TN breast cancer cell lines. Decreased expression of RM2 caused decreased cell survival to 66%±3% in HR and 89%±2% in HER2 breast cancer cell lines and increased apoptosis by 1.6±0.08 fold in HR, 1.4±0.09 fold in TN, and 2.0±0.3 fold in HER2 breast cancer cell lines. Twelve proteins (AURKA, KIF1C, NCD8, RM2, SDC1, UBE2C, HJURP, CENPA, CENPF, HIST2H2A3, HIST1H2BD, and TOP2A) were essential for cancer cell survival in at least 2 breast cancer subtypes. These protein targets are immunogenic in patients with DCIS. In the sera of women without breast atypia (n = 36), autoantibodies to NCD8 were detected with a mean of 5.3±1.8 ng/mL while in women with fibroadenoma (n = 36) autoantibodies were detected with a mean of 8.3±1.5 (p = 0.05). In the sera of women without breast atypia (n = 36), autoantibodies to RM2 were detected with a mean of 0.16±0.7 ng/mL while in women with fibroadenoma (n = 36) autoantibodies were detected with a mean of 7.2±2.1 (p = 0.05) and in women with DCIS (n = 59) autoantibodies were detected with a mean of 2.6±0.6 ng/mL (p = 0.0003). These proteins represent DCIS antigens of biologic importance in tumor growth and, potentially, progression to IBC and are candidate immunogens for a vaccine to treat DCIS.

**#624 Characterization of PD-L1 expression and the immune cell microenvironment in hepatoellular carcinoma (HCC) and non-cirrhotic liver tissue adjacent to HCC.** Christian Illing,1 Sienna Yoast,2 Yue Zhang,3 Bartholomew Naughton,3 Miriam Urban,1 P. Alexander Rolfe,3 Eveline Frick-Krieger,1 Isabelle Dussault.1,2,3* Marek KGaa,2,3 Darmstadt, Germany; 2Dako North American, Carpinteria, CA; 3EMD Serono Research and Development Institute Inc., Billerica, MA.

Still-emerging evidence suggests that HCC is at least moderately responsive to therapeutic agents that target tumor immune suppression pathways, specifically the programmed death 1/programmed death ligand 1 (PD-L1) pathway. Prior studies raise the possibility that PD-L1 is a prognostic biomarker in HCC, where its expression levels have been reported to correlate with tumor aggressiveness and recurrence following surgical resection. The purpose of this study was to evaluate the expression of PD-L1 in non-cirrhotic liver tissue adjacent to HCC and HCC itself in 68 procured tissue samples, as well as elucidate the immune cell composition of the HCC tumor microenvironment. All cases showed the typical morphology of HCC and were classified as low- to high-grade trabecular, pseudoglandular, or solid with the common cytotoxicAT morphological features. Immunohistochemical (IHC) staining for PD-L1 in tumor-free liver (TFL) revealed PD-L1 staining in sinusoidal lining cells (macrophages [Mge] and endothelial cells [EC], as confirmed by double labeling for CD68 and CD31), although there was considerable heterogeneity in the extent of PD-L1 staining. More specifically, in TFL, some but not all CD68+ Mge were also PD-L1+, whereas most CD31+ EC were also PD-L1+. Furthermore, we evaluated PD-L1 staining qualitatively and semiquantitatively in HCC. Only few tumor cells displayed membranous PD-L1 staining, and only few tumors showed PD-L1 expression of (0.25% to 25%+) within HCC (n = 65). The proportion of tumor cells categorized as PD-L1+ varied from 0.25% to 9.0% (88.8% PD-L1+ (>1% and <25%) = PD-L1+); instead, PD-L1 expression within the tumor microenvironment predominantly emanated from immune cell infiltrates (as confirmed by PD-L1+ pan-cytokeratin double labeling). Then we assessed the immune milieu in HCC tissue specimens using quantitative IHC. We found considerable interspecimen variation in the number of CD68+ Mge, CD8+ T lymphocytes, and FoxP3+ regulatory T lymphocytes. Comparing the number of immune cells in different tumor compartments showed that the prevalence of CD68+ cells (p = 0.0005), CD8+ cells (p = 0.0004), and FoxP3 cells (p = 0.05) was significantly higher within the invasive margin vs the center of the tumor. Quantitative analysis of the immune cell content also showed that

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#621 Tumor suppressor TUSC2 immunogene therapy is synergistic with anti-PD1 in lung cancer syngeneic mouse models. Ismail M. Meraz, Mourad Majidi, RuPing Shao, Meng Feng, Xiaobo Cao, David Rice, Boris Sepesi, Lin Ji, Jack Roth. UT MD Anderson Cancer Ctr., Houston, TX.

TUSC2, a pro-apoptotic tumor suppressor gene whose expression is lost or decreased in most lung cancers, activates the innate immune system through initiation of broad spectrum cytokine secretion and natural killer (NK) cell activation. TUSC2 delivered systemically by nanovesicles has mediated tumor regression in metastatic non-small cell lung cancer clinical trials. We studied the effect of TUSC2 on immune cell populations and the anti-tumor activity of TUSC2 in combination with anti-PD1 checkpoint blockade in two syngeneic mouse models: C57BL/6 mice subcutaneously injected with murine lung adenocarcinoma cell line CMT1/676-luc cells (KrasG12V mutation) and 346SQ (KrasG12D allele and a knock-in Trp53R172H/L allele) adenocarcinomas which metastasize to the lung in 129/2 mice. Tumor growth was monitored by scoring ex vivo luminescence using the IVIS Imaging System 200. Multi-color flow cytometry was used for immune profiling of circulating immune cells after nanovesicle mediated TUSC2 intravenous injection. Cytokine gene expression in response to TUSC2 in sorted immune subpopulations was determined by real-time PCR. Tumor growth was significantly reduced with TUSC2 treatment compared with no treatment in both subcutaneous metastatic mouse models. Synergistic anti-tumor activity was observed when TUSC2 was combined with anti-PD1 verified in five independent experiments. In the lung metastasis model, mice treated with TUSC2 + anti-PD1 lived significantly longer than with single agent treatment. Circulating NK cells increased three fold following TUSC2 nanovesicle intravenous injection both in tumor free and tumor bearing mice which correlated with tumor regression and survival. Cytotoxic T lymphocyte responses were increased whereas Tregs and MDCS decreased with TUSC2 alone and TUSC2+anti-PD1 treatment. The levels of T cell checkpoint markers PD1, CTLA-4, LAG-3, and TIM-3 evaluated by flow cytometry were decreased after TUSC2 treatment. TUSC2 anti-tumor response was abolished when NK cells were depleted indicating NK cells are important mediators of the TUSC2 treatment effect.

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#623 Identifying candidate antigens for a ductal carcinoma in situ vaccine that are essential to breast cancer survival across multiple subtypes. Sasha Elizabeth Stanton, Erik Ramos, James Annis, Andrew Timms, Tessa Rue, Mary L. Disis. Univ. of Washington, Seattle, WA.

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#624 Characterization of PD-L1 expression and the immune cell microenvironment in hepatocellular carcinoma (HCC) and non-cirrhotic liver tissue adjacent to HCC. Christian Illing,1 Sienna Yoast,2 Yue Zhang,3 Bartholomew Naughton,3 Miriam Urban,1 P. Alexander Rolfe,3 Eveline Frick-Krieger,1 Isabelle Dussault.1,2,3* Marek KGaa,2,3 Darmstadt, Germany; 2Dako North American, Carpinteria, CA; 3EMD Serono Research and Development Institute Inc., Billerica, MA.

Still-emerging evidence suggests that HCC is at least moderately responsive to therapeutic agents that target tumor immune suppression pathways, specifically the programmed death 1/programmed death ligand 1 (PD-L1) pathway. Prior studies raise the possibility that PD-L1 is a prognostic biomarker in HCC, where its expression levels have been reported to correlate with tumor aggressiveness and recurrence following surgical resection. The purpose of this study was to evaluate the expression of PD-L1 in non-cirrhotic liver tissue adjacent to HCC and HCC itself in 68 procured tissue samples, as well as elucidate the immune cell composition of the HCC tumor microenvironment. All cases showed the typical morphology of HCC and were classified as low- to high-grade trabecular, pseudoglandular, or solid with the common cytotoxicAT morphological features. Immunohistochemical (IHC) staining for PD-L1 in tumor-free liver (TFL) revealed PD-L1 staining in sinusoidal lining cells (macrophages [Mge] and endothelial cells [EC], as confirmed by double labeling for CD68 and CD31), although there was considerable heterogeneity in the extent of PD-L1 staining. More specifically, in TFL, some but not all CD68+ Mge were also PD-L1+, whereas most CD31+ EC were also PD-L1+. Furthermore, we evaluated PD-L1 staining qualitatively and semiquantitatively in HCC. Only few tumor cells displayed membranous PD-L1 staining, and only few tumors showed PD-L1 expression of (0.25% to 25%+) within HCC (n = 65). The proportion of tumor cells categorized as PD-L1+ varied from 0.25% to 9.0% (88.8% PD-L1+ (>1% and <25%) = PD-L1+); instead, PD-L1 expression within the tumor microenvironment predominantly emanated from immune cell infiltrates (as confirmed by PD-L1+ pan-cytokeratin double labeling). Then we assessed the immune milieu in HCC tissue specimens using quantitative IHC. We found considerable interspecimen variation in the number of CD68+ Mge, CD8+ T lymphocytes, and FoxP3+ regulatory T lymphocytes. Comparing the number of immune cells in different tumor compartments showed that the prevalence of CD68+ cells (p = 0.0005), CD8+ cells (p = 0.0004), and FoxP3 cells (p = 0.05) was significantly higher within the invasive margin vs the center of the tumor. Quantitative analysis of the immune cell content also showed that
there was no correlation between T lymphocyte infiltration (CD8+/FoxP3+) and Mφ infiltration in any compartment. Taken together, our findings confirm the intertumoral heterogeneity of the immune cell microenvironment in HCC; however, future studies are needed to correlate these findings to the clinical setting with immunomodulatory treatments.

#625 Eradication of cancer cells by T-cell receptor-engineered T cells targeting neoantigens. Tatsuo Matsuda,1 Taigo Kato,1 Yuji Ikeda,1 Matthias Leisegang,2 Sachiko Yoshimura,3 Tetsuro Hikichi,4 Makiko Harada,1 Makda Zewde,1 Jaee-Hyun Park,1 Hans Schreiber,1 Kazuma Kiyotani,1 Yusuke Nakamura,1,2 University of Chicago, Chicago, IL;2Charité Campus Buch, Germany;3OncoTherapy Science Inc, Japan.

Cytotoxic T lymphocytes (CTLs) play critical roles in cancer-immune responses, and functional characterization of CTLs and their cancer-specific antigens will facilitate cancer immunotherapies. Immunogenic peptides, which can be derived from oncogenic proteins specifically expressed in cancer cells but not expressed in normal organs except testis (oncoantigens), or from peptides with somatic nonsynonymous mutations (neoantigens), are known as good targets for CTLs to eradicate cancer cells. In this study, we aimed to establish a method to efficiently identify oncoantigen/neoantigen-specific CTLs. Firstly, we screened candidate HLA-A2402-restricted oncoantigen/neoantigen peptides by in silico prediction of their binding affinity to MHC class I molecules. We conducted an in-vitro stimulation of CD8+ lymphocytes carrying HLA-A2402 allele by each peptide, and then confirmed clonal expansion of the peptide-specific CTLs by TCR repertoire sequencing analysis, interferon-γ enzyme-linked immunospot (ELISPOT) and/or peptide-HLA multimer assays. After identification of TCR alpha-beta pairs, we conducted retroviral transduction and prepared the TCR-engineered T cells to evaluate their cytotoxic activities against cancer cells. As oncoantigens, we isolated the CTLs for FOXM1 and UBE2T from healthy donors, and found these CTLs showed strong cytotoxicity against HLA-A2402-positive cancer cells expressing target proteins, but not against HLA-unmatched cancer cells. Similarly, the TCR-engineered T cells for FOXM1 and UBE2T showed killing effects for only HLA-A2402-positive cancer cells. Neoantigen-specific TCR-engineered CTLs also exhibited the mutated peptide-specific response, but did not cross-react to the nonmutated peptide. In addition, neoantigen-specific cytotoxicity was observed against HLA-A2402-positive cancer cells expressing the proteins with target somatic mutations. In conclusion, we developed the pipeline to screen possible oncoantigens/neoantigens and establish antigen-specific TCR-engineered CTLs from peripheral blood lymphocytes. Our approach provides a promising strategy to develop personalized immunotherapies using oncoantigen/neoantigen-reactive TCR-engineered T cells to treat cancer.

#626 BGB324, a selective small molecule inhibitor of receptor tyrosine kinase AXL, abrogates tumor intrinsic and microenvironmental immune suppression and enhances immune checkpoint inhibitor efficacy in lung and mammary adenocarcinoma models. Yvonne Wouak-Liptians,1 Kjersti Davidsen,2 Magnus Blø,1 Agnete Engelsen,3 Jing Kang,4 Linn Hodneland,5 Maria Lie,5 Sebastien Bougnard,6 Kristina Aguilera,6 Lavina Ahmed,7 Agata Rybicka,1 Eline Milde Navdal,5 Paulina Deyna,1 Anna Boniecka,1 Straume Oddbjørn,3 Salem Chouaib,4 Rolf Brekken,5 Gregg A. Galsworthy,3,5,6 BengtGillo AS, Bergen, Norway;7University of Bergen, Bergen, Norway;8Haukeland University Hospital and University of Bergen, Bergen, Norway;9Institute Gustave-Roussy, Université Paris-Sud, Paris, France;10UT Southwestern Medical Center, Dallas, TX.

The AXL receptor tyrosine kinase is associated with poor overall survival in a wide spectrum of cancers including lung and breast adenocarcinomas. AXL signaling is an important regulator of tumor plasticity related to epithelial-to-mesenchymal transition (EMT) and stem cell traits that drive metastasis and drug resistance. Signaling via AXL is also a key suppressor of the anti-tumor innate immune response, and AXL is expressed on several cells associated with the tumor immune microenvironment including natural killer (NK) cells and tumor-associated macrophages. Hence AXL resides uniquely at the nexus between tumor and microenvironmental anti-tumor immune suppression mechanisms. We report that BGB324, a selective clinical-stage small molecule AXL kinase inhibitor, enhances the effect of immune checkpoint blockade in aggressive adenocarcinoma models with limited immunogenicity by targeting both tumor intrinsic and microenvironmental immune suppression. Immune therapy with anti-CTLA4/PD1 in the 4T1 model increased AXL and EMTagger expression correlating with a lack of response. Combination with BGB324 resulted in durable primary tumor clearance versus anti-CTLA4/PD1 alone. In a separate study, BGB324 + anti-CTLA4 treatment resulted in significant long-term primary tumor clearance while no response was observed with anti-CTLA4 treatment alone. The extensive metastasis to the lung, liver and spleen characteristic of the 4T1 model was not detected in animals responding to the combination treatment. Importantly, responding animals rejected orthotopic 4T1 tumor cell re-challenge, demonstrating sustained tumor immunity. In the LL2 Lewis lung model, BGB324 in combination with anti-PD1/PDL1 significantly prevented tumor growth compared to treatment with anti-PD1/PDL1. Tumors from mice treated with BGB324 in combination with immune checkpoint inhibitors displayed reduced EMT traits, altered cytokine expression, enhanced tumour cell invasion of effectors cells and decreased metastasis to lung. Also, BGB324 significantly reduced IL10 secretion by isolated human macrophages and enhanced human NK-cell mediated NSCLC tumor cell lysis. Collectively these results support a prominent role for AXL in resistance to immune therapy and support clinical translation of combining BGB324 with immune checkpoint inhibitors to improve cancer treatment.

IMMUNOLOGY: T-cell Immunity to Cancer: New Progress

#627 NLR5 co-mutations are associated with impaired antigen presentation and immune exclusion in KRAS-mutant lung adenocarcinoma. Ferdinandos Skoulidis,1 Taghrred Hizr, Xiang Dong Lee, Jaime Rodriguez Canales, Edwin R. Parra, Pan Tong, Carmen Behrens, Yasuhide A. Taniuchi, Xiaoli Shopou,1 Jing Wang, Ignacio Wistuba, John V. Heymach. UT MD Anderson Cancer Ctr., Houston, TX.

Background: We recently reported that co-occurring genetic events constit- tute major determinants of the molecular diversity of KRAS-mutant lung adeno-carcinoma (LUAC) (Skoulidis et al., Cancer Discovery, 2015). However, comprehensive evaluation of the functional impact of KRAS co-mutations on key cancer hallmarks is thus far lacking. Here, we find that inactivating mutations in NLR5, a major transactivator of MHC class I molecules, are significantly enriched in KRAS-mutant LUAC and examine the impact of NLR5 loss on the composition of the tumor immune microenvironment. Methods: Our cohorts consist of 513 LUACs from the TCGA (145 KRAS-mutant), 152 chemotherapy-naïve surgically resected LUAC from the PROSPECT cohort, 20 platinum-refractory KRAS-mutant LUAC from the BATTLE-2 clinical trial, as well as a panel of 31 KRAS-mutant NSCLC cell lines. Analysis of immune cell sub-population was performed using automated IF-based enumeration. Antigen presentation score was defined as the geometric mean mRNA expression of HLA-A, HLA-B, HLA-C and β2M. Results: In an unbiased analysis for genes significantly co-mutated with KRAS in LUAC (TCGA cohort) we identified NLR5 (NLR family, CARD domain containing 5), encoding a recently discovered major transactivator of MHC class I genes (~11% of KRAS-mutant LUAC, odds ratio 2.99, P=0.0197). The spectrum of NLR5 somatic mutations includes several nonsense and frameshift mutations, as well as missense mutations, many of which are predicted to abrogate normal NLR5 function. In the TCGA cohort, KRAS/NLR5 co-mutated tumors exhibited lower antigen presentation score compared to KRAS-mutant NLR5 wild-type tumors (P=0.0369, t-test). Among KRAS-mutant LUAC from the TCGA, PROSPECT, BATTLE-2 cohorts expression of NLR5 mRNA correlated tightly with the expression of core antigen presentation pathway components including HLA-A, HLA-B, HLA-C, β2M, TAP1, TAP2, PSMB8 and PSMB9 [in BATTLE-2: HLA-A r=0.7616, P=9.57e-05, HLA-B r=0.834, P=4.848e-06, HLA-C r=0.8029, P=2.036e-05, TAP1 r=0.8189, P=1.00e-05]. Similar results were obtained in a panel of 31 KRAS-mutant NSCLC cell line. Thus, both mutational and non-mutational mechanisms can account for NLR5 inactivation. Finally, in a tumor microarray encompassing surgically resected, chemotherapy naïve LUAC (PROSPECT), NLR5-low tumors (lower tertile for NLR5 mRNA expression, N=34), exhibited reduced density of infiltrating CD3+ (P<0.0001, Mann-Whitney U test), CD8+ (P<0.0001, Mann-Whitney U test) as well as PD-1+ cells (P<0.0001, Mann-Whitney U test) and PD-L1 Histo-score (P=0.0036, Mann-Whitney U test) compared to NLR5-high tumors (N=41). Conclu- sions: Co-mutations in NLR5 are enriched in KRAS-mutant LUAC and are associated with immune exclusion. KRAS co-mutations can shape the tumor immune micro-environment and may therefore predict for response - or lack thereof- to immunotherapy.

#628 Neoantigens predicted by clonal mutation analysis in lung adenocarcinoma patients. Dapeng Zhou,1 Weijing Cai,1 Deng Pan,1 Tan Wen Ling,1 Jiaqian Wang,2 Caichun Zhou,3 Yanyan Lou.2 Shanghai Pulmonary Hospital affiliated with Tongji University School of Medicine, Shanghai, China;2 Mayo Clinic, Jacksonville, FL.

Mutant peptides presented in cancer are superior vaccine candidates than self peptides. The efficacy of mutant K-Ras, PS3 and EFGR peptides have been tested as cancer vaccines in pancreatic cancer, colorectal cancer, and lung adenocarci-
oma. However, the occurrence of these mutations in cancer is limited. Further more, these mutant peptides can only be presented by certain MHC alleles. Neoantigens expressed by passenger mutations which are not involved in common proliferative molecular pathways are potential candidates for personalized vaccine design. Such passenger mutations have drawn attention by the recent discovery of tumor-related neoantigens as principal targets of T cells unleashed by immune checkpoint blockade. These results led us to hypothesize that enforced increase of the number of mutations in cancer cells could be paradoxically beneficial for therapeutic purposes. We therefore performed a pharmacological screen to identify agents capable of permanent inactivation of MMR in colorectal, breast and PDAC cancer cells. We found that temozolomide triggers MLH1 inactivation and leads to rapid clonal evolution and dynamic neoantigen profiles. Temozolomide-treated cells were unable to form tumors in syngeneic animals, while cells treated with other alkylating agents did. Genomic analysis of these tumor models revealed that fluctuating levels of neoantigens, rather than the absolute number of mutations, is critical to provoke immune surveillance. These results provide the rationale for developing innovative anticancer therapies that target DNA repair proteins.

#631 Dual-specific T cells are highly effective in eradicating solid tumors. Clare Y. Slaney, Bianca von Scheidt, Phillip K. Darcy, Michael H. Kershaw, Peter MacCallum Cancer Centre, Melbourne, Australia.

Chimeric antigen receptor (CAR) T cell therapy is a novel form of adoptive cellular therapy and has recently generated remarkable effects in patients with hematological cancers. However, the success against solid cancers has been modest. The major challenges are the hostile tumor microenvironment and the low efficiency of CAR T cells infiltrating the tumor. Here, we present a major advancement in CAR T therapy that eradicated large established solid cancers, some in excess of 150 mm², in immunocompetent mice. We hypothesized that a vaccine composed of a recombinant poxvirus could be used as an antigen delivery vehicle to specifically activate CAR T cells through their T cell receptor (TCR) and simultaneously change the tumor microenvironment, allowing the recruitment and activation of CAR T cells. The approach involves adoptive cell transfer incorporating vaccination (ACTIV) therapy. We generated dual-specific T cells expressing a CAR specific for the tumor antigen Her2 and a TCR specific for the melanocyte protein (gp100). Injection of T cells, together with recombinant vaccinia virus expressing gp100, induced durable complete remission of RNAi knock-down tumors and established metastases, some in excess of 150 mm², in immunocompetent mice expressing Her2 in normal tissues, including the breast and brain. Tumor destruction mediated by dual-specific T cells occurred rapidly over a period of seven days and was associated with extensive proliferation and infiltration of the dual-specific CAR T cells. Mice that had
reduced tumors were resistant to rechallenge with the same Her2+ tumor cells and partially resistant to rechallenge with Her2+ tumor cells, indicating the formation of immune memory and epitope spreading. This mouse model study supports the view that it is possible to design a highly effective CAR T cell therapy for solid cancers and metastases, even when the target antigen is also expressed in vital tissues. To explore the translational potential for using the dual specific CAR T cell strategy, we established methods to transduce the T cells from human peripheral blood with both a TCR specific for gp100 and a CAR for Her2. From as little as 1 ml of human buffy coat, we could generate more than 10^6 dual-specific CAR T cells, which is sufficient for a course of treatment. The human dual-specific CAR T cells were functional in secreting IFN-γ and killing human cancer cells. The stimulation of gp100 through TCR enhanced the dual-specific CAR T cell proliferation, secretion of IFN-γ and killing of Her2+ human cancer cells in vitro. These characteristics were identified to be important for eradicating tumors in the mouse models. Taken together, our data provide valuable information for the development of CAR T cell therapies for patients with solid cancers.

#632 Genome-scale neoantigen screening using ATLAS+ prioritizes candidate antigens for immunotherapy in a non-small cell lung cancer patient. Lila Gharib | University of Maryland, Baltimore, USA; 2Roth, Emilio Flano | 3Judy Jacques | 4Biao Liu | 5Zheng Yan | 6Aula

The stimulation of gp100 through TCR enhanced the dual-specific CAR T cell proliferation, secretion of IFN-γ and killing of Her2+ human cancer cells in vitro. These characteristics were identified to be important for eradicating tumors in the mouse models. Taken together, our data provide valuable information for the development of CAR T cell therapies for patients with solid cancers.

Antibody dependent cellular cytotoxicity (ADCC) is one mechanism by which monoclonal antibodies (mAb) work. In addition to ADCC, mAbs act on their target by causing signal perturbation and complement activation. Cetuximab is an EGFR targeting mAb used to target EGFR overexpression and amplification seen in many types of cancer. While mechanisms of resistance to EGFR targeted therapy have been extensively studied, resistance to ADCC has not, mainly due to the lack of ADCC-resistance models for study. To address this limitation we established a model system for anti-EGFR ADCC using NK92 CD16V effector cells, cetuximab, and the high EGFR-expressing squamous cell carcinoma cell line A431. Continuous A431 exposure to ADCC yielded an ADCC resistant phenotype (ADCCr) that exhibits a stable phenotype in the absence of continued ADCC selection. We have explored: ADCCr cell gene expression profile, NK cell activation, metabolic signature and subsequent RPPA analysis to further understand the causes and properties associated with this resistance. This ADCCr cell line has a distinctive transcriptional profile highlighted by overexpression of histone- and interferon-related genes, reduced sensitivity to antimaltides, DNA-intercalating and ABC transporter-regulated cytotoxic agents. Intense ADCC selection causes epigenetic modification and stress response characterized by the transcriptional overexpression of PCAF (KAT2B), which initiates histone hyper-acetylation and epigenetic changes, inducing DNA replication arrest, DNA damage and stress responses that activate checkpoint signaling in the cell cycle. The pharmacologic inhibition of KAT2B reverses the ADCCr phenotype. Immune checkpoints such as PD-L1 do not stabilize ADCCr in this model system. These results shed light on new mechanisms of ADCC resistance and inform future combinatorial treatments for mAb therapy. We are exploring the possibility that stress response mechanisms are responsible for resistance for diverse selection pressures imposed by immune synapse-mediated cytotoxic attack.


Despite the unprecedented efficacy of checkpoint blockade (CPB) therapy in treating some cancers, the majority of patients fail to respond to CPB. Tumor exome sequencing and somatic mutations were identified. Individual DNA sequences (399 nucleotides) spanning each mutation site were built, cloned and expressed in E. coli co-expressing linterisoyxin O. Polyepitope expression was validated using a surrogate T cell assay or by Western Blotting. Frozen PBMCs, collected pre- and post-treatment, were used to derive dendritic cells (MDDC). Both CD4+ and CD8+ T cells were enriched and expanded using microbeads. The E. coli clones were pulsed onto MDDC in an ordered array, then co-cultured either with CD8+ or with CD4+ T cells overnight. T cell activation was detected by analyzing cytokines in supernatant. Antigens were identified as clones that induced a cytokine response that exceeded three standard deviations of the mean of all negative control wells, then their identities compared with T cell epitopes predicted using previously described algorithms. We found biological evidence for neoantigens that were specifically responsive to peripheral CD8+ and CD4+ T cells, derived from the patient’s tumor, pre- and post-CPB intervention. Some of these neoantigens were identified as a T cell target both pre- and post-CPB therapy. We identified neoantigens for which no epitopes were predicted by in silico methods. These data represent evidence that multiple patient-specific neoantigens can be identified through functional evidence of T cell response from peripheral blood without epitope prediction. By profiling natural and CpG-enhanced immunity to neoantigens, a broad catalog of T cell targets can be identified for development of immunotherapies that engage T cells against cancer to improve outcomes for patients for whom current therapies are ineffective.


Background: Recently, neoadjuvant therapy (noadjuvant chemoradiation therapy, noadjuvant chemoradiation therapy) for locally advanced rectal cancer has been generally performed. Although in the cases that may have had complications such as tumor progression or delayed surgery, factors predicting the clinical response to neoadjuvant therapy have not been adequately defined. Meanwhile CD8+ tumor-infiltrating lymphocytes (TILs) have been reported to have a crucial effect in tumor progression and outcome as primary host immune response in various types of cancer, and antitumor immune effect has been reported to contribute to the response to radiotherapy and chemotherapy. The aim of this study was to elucidate the correlation between the local immune status and the effectiveness of the neoadjuvant therapy for locally advanced rectal cancer. Patients and methods: A total of 51 patients who underwent cura-
tive operation for locally advanced rectal cancer after neoadjuvant therapy were enrolled. We retrospectively examined the number of CD8+ tumor-infiltrating lymphocytes (TILs) using immunohistochemical staining of pretreatment biopsy samples and resected specimens, and assessed the correlation with pathological response. The grade of tumor response was evaluated according to the definition of the Japanese Research Classification of Colorectal Carcinoma. Grade 1a were defined as “poor response” and Grade 1b-3 were defined as “good response”. We set each median value of the number of CD8+ TILs as the cut-off value. Results: For the 26 patients with pretreatment biopsy samples, we classified the patients into the poor response group (n=14) and the good response group (n=12). Then we set 6.0 as the cut-off value and classified the patients into the high pretreatment CD8+ TILs group (n=14) and the low pretreatment CD8+ TILs group (n=12). Low pretreatment CD8+ TILs were associated with poor response to neoadjuvant therapy (p=0.036). For resected specimens (n=51), we classified the patients into the poor response group (n=25) and the good response group (n=26). Then we set 10.8 as the cut-off value and classified the patients into the high posttreatment CD8+ TILs group (n=28) and the low posttreatment CD8+ TILs group (n=23). Low posttreatment CD8+ TILs were also associated with poor response to neoadjuvant therapy (p<0.001). Additionally, the number of pretreatment CD8+ TILs tend to be related to the number of posttreatment CD8+ TILs. Conclusion: In locally advanced rectal cancer patients, T lymphocyte-mediated immune reactions play an important role in tumor response to neoadjuvant therapy, and the quantitative measurement of CD8+ TILs in biopsy samples may be a predictor of the clinical effectiveness of neoadjuvant therapy for locally advanced rectal cancer. Moreover, low posttreatment CD8+ TILs were associated with poor response to neoadjuvant therapy.

#636  Comprehensiv analysis of T cells responsive to neoantigens derived from tumor-specific genetic mutations. Tetsuro Sasada, Junya Ohtake, Satoshi Wada, Erika Yada, Shintaro Yoshida. Kanagawa Cancer Center, Yokohama, Japan.

Neoantigens derived from tumor-specific genetic mutations can be recognized as foreign by the host immune system, and might be suitable as target for cancer immunotherapy possibly due to their higher immunogenicity. In this study, to really know the immunogenicity of tumor-specific neoantigens, we comprehensively investigated T cell responses against neoantigens derived from genetic mutations in gastric cancer. Using next-generation sequencing, 156 missense mutations were identified in tumor cells from two gastric cancer patients. From them, we selected 30 potentially immunogenic amino acid sequences, which were derived from the mutations and predicted to potentially bind to HLA-class I (A*0201, A*0206, or A*2402) by an epitope prediction server, IEBD. We synthesized 30 kinds of 27mer long peptides, in which the mutated sequences were incorporated into the corresponding wild type sequences (PBMC) from healthy donors in the presence of the synthetic peptides to evaluate whether they could really induce antigen-specific T cell responses. In the analysis with PBMC from 18 healthy donors, 27/30 (90%) synthetic peptides showed an ability to induce antigen-specific T cell responses in at least one donor, assessed by cytokine production assay. Among them, 15 peptides were immunogenic in more than one donor. The antigen-specific T cell responses were detected more frequently in CD4+ T cells (70%) than in CD8+ T cells (43%). The specificity of T cell responses to mutated sequences, but not to the corresponding wild type sequences, were confirmed in 5 of 8 (63%) peptides examined. In addition, antigen-specific T cell responses induced by mutated peptides were shown to be much higher than those induced by the corresponding wild type peptides. These findings clearly demonstrated high immunogenicity and specificity of neoantigens derived from tumor-specific genetic mutations. Further studies would be recommended to develop a novel immunotherapeutic approach, “personalized cancer vaccination”, targeting the corresponding wild type sequences, were confirmed in 5 of 8 (63%) peptides examined.

#637  The class I HDAC inhibitor mocetinostat augments checkpoint inhibitor therapy via direct up regulation of antigen presentation transcriptional programs in tumor cells and increased T-cell clonality in tumors. David M. Briere, Niranjana Sudhakar, Peter Olson, Jamie Christensen. Mirati Therapeutics, San Diego, CA.

Mocetinostat is a spectrum-selective class I/II histone deacetylase (HDAC) inhibitor that augments checkpoint inhibitor therapy through enhanced antigen presentation capacity and a pro-immunogenic shift in the tumor microenvironment (TME). Mocetinostat up-regulated tumor antigen presentation machinery (i.e. major histocompatibility complex (MHC) genes and co-presentation molecules) and programmed cell death ligand-1 (PD-L1) expression in a panel of non-small cell lung cancer (NSCLC) cell lines. Surprisingly, some of the most highly up regulated genes following mocetinostat treatment were MHC class II genes (~20 fold), which are normally expressed by antigen-presenting cells, but are silenced in most epithelial tissues and solid tumors. To elucidate the molecular mechanisms whereby mocetinostat regulates MHC class I and II transcriptional programs in tumor cells, we performed chromatin immunoprecipitation-sequencing (ChIP-Seq) in several HDAC2 knock-down subclones of melanoma cell lines for many mocetinostat-regulated immune pathway genes. Further, mocetinostat treatment increased histone 3 lysine 27 acetylation and histone 3 lysine 4 trimethylcylation indicating an induction of active transcription at these loci. Class II transactivator (CIITA) is an interferon gamma (IFNγ)-sensitive regulator of MHC class II gene expression and was one of the top HDAC2/mocetinostat target genes in melanoma cell lines. Using ChIP-Seq data. In addition, CIITA and a representative MHC class II gene, HLA-DRα, were synergistically up regulated following mocetinostat and IFNγ treatment. To investigate the impact of Class I HDAC inhibition on the TME, syngeneic mouse tumor models were utilized. Mocetinostat treatment decreased intratumoral immune suppressive T regulatory cells (Tregs) and increased intratumoral CD8+ T cells. To gain additional insight into the functional effects of mocetinostat in the TME, we performed deep sequencing of the T-cell receptor in vehicle, mocetinostat, PD-L1 or combination-treated tumors. Mocetinostat-treated tumor cohorts exhibited increased T-cell clonality which likely reflects expansion of a subset of activated, antigen-specific T cells. Together, these data provide molecular insight into the mechanism whereby mocetinostat augments checkpoint inhibitor therapy by directly regulating tumor antigen presentation and through functional effects on the T cell repertoire.

#638  Epigenetic reprogramming of immune cells through selective inhibition of HDAC6 reduces suppressive phenotypes and augments anti-tumor properties of T-cells. Andressa L. Sodre, David M. Woods, Amud Sarnaik, Brian C. Bettis, Steven Quayle, Simon Jones, Jeffrey Webes. NYU Langone Medical Center, New York, NY; Moffitt Cancer Center, Tampa, FL; AcetylPharmaceuticals, Boston, MA.

Alteration of the epigenetic landscape of immune cells can modify the pattern of gene expression, thus resulting in phenotypic and functional changes. Small molecule inhibitors targeting epigenetic modifiers, such as histone deacetylases (HDACs), have been shown to reduce tumor growth. Besides promoting direct anti-tumor effects, HDAC inhibitors also target immune cells and alter their gene regulation. Here, we demonstrate that the HDAC6 selective inhibitors ACY-241 and ACY-1215 (ricosinostat) decrease the function of myeloid derived suppressor cells (MDSC) and T regulatory (Treg) cells, maintain an effector phenotype by CD8+ T cells, and do not reduce viability of immune cells. First, peripheral blood mononuclear cells derived from melanoma patients were treated with ACY-241, and the phenotype of MDSCs was assessed. Expression of the suppressive molecules ARG1 (p<0.01) and NOS2 (p<0.05) was decreased in CD14+ HLA DR lo CD11b+ and CD14+ HLA DR lo CD3+ MDSC populations, suggesting less potent MDSCs. To gain insight into other suppressive populations, we evaluated the phenotype and function of Treg cells derived from melanoma patients. Cultures of CD3+ T-cell cultures treated with ACY-1215 or ACY-241 resulted in decreased expression of FOXP3 and reduced frequency of Tregs (CD4+CD25+CD127−; p<0.001). ACY-1215 pre-treated Tregs exhibited less suppressive activity against responding conventional T-cells in standard assays, compared to Tregs pre-treated with DMSO control (p<0.01). CD3+ T-cells exposed to ACY-241 or ACY-1215 were activated via CD3/CD28 co-stimulation to assess effects on cytokine production. Selective HDAC6 inhibition shifted T-cell differentiation towards Th1-type (Th1 + Th2-type (GATA3+)), compared to DMSO (p<0.05). Additionally, Th2 cytokines (e.g. IL-4, IL-5, IL-6, IL-10, IL-13) were significantly decreased (p<0.05). In accordance with decreased Th2 differentiation, mTORC signal transduction, including phosphor-SGK1, was similarly reduced (p<0.05). Targeted inhibition of mTORC signal pathway inhibitors decreased expression of IFN-gamma and CD107a. Collectively, these data indicate that epigenetic reprogramming of immune cells by HDAC6 selective inhibitors may decrease the function of suppressive subsets (e.g. Tregs, MDSCs), while enhancing the accumulation of anti-tumor memory and effector T-cell subsets.
#639 Morphological changes in mitochondria induced by CD137 (4-1BB) co-stimulation on CD8 T cells. Sara Labiano, Álvaro Teijeira, Arantza Azpilikueta, Angela Aznar, Elizabet Bolaños, Ignacio Melero. Center for Applied Medical Research, Pamplona, Spain.

Mitochondrial dynamics is regulated by the 4-1BB receptor. 4-1BB stimulation induced enlarged mitochondria were a product of preexisting mitochondria rather than de novo generation of organelles. Confocal microscopy and 3-dimensional modeling revealed that mitochondrial dynamics can be linked to the regulation of mitochondrial biogenesis. CD137 (4-1BB) is an immunostimulatory receptor of the TNFR family expressed on the surface of antigen-activated T cells. Treatment with agonist monoclonal antibodies targeting 4-1BB is showing promising results for immunotherapy of cancer. In this work, we observed that stimulation of 4-1BB with agonistic mAbs was able to raise the mitochondrial membrane potential on human CD8 T cells. 4-1BB co-stimulated CD8 T cells showed enlarged mitochondria (up to 3μm diameter) with a round shape when observed by confocal or transmission electron microscopy. We observed that CD8 T cells from DLNs (draining lymph nodes) of tumors in mice treated with agonistic anti-4-1BB antibodies also contained enlarged mitochondria. By double fluorescence labeling of mitochondria prior and after activation, we could ascertain that enlarged mitochondria were a product of preexisting mitochondria rather than de novo generated organelles. This observation prompted us to study the regulation of mitochondrial dynamics by 4-1BB receptor. 4-1BB stimulation induced an increase in expression of the mitochondrial fusion protein OPA-1. By super-resolution microscopy with AiryScan detectors we observed more pronounced co-localization of OPA-1 with mitochondrial membrane in 4-1BB co-stimulated T cells than in T cells activated only by CD3 crosslinking. Our results indicate an important role of 4-1BB in the regulation of mitochondrial morphology and performance in such a way that it can be relevant for various T cell functions.

#640 Subsets of HLA alleles are capable of binding neoantigens derived from mutations within cancer driving genes such as KRAS and EGFR. Andrew Nguyen, J Zachary Sanborn, Charles J. Vaske, Shahrooz Rabizadeh, Kayvan Niazi, Patrick Soon-Shiong, Steve Benz. NanOtics, Santa Cruz, CA.

Background: Immunology has shown great promise as a low-toxicity tool to combat several cancers. Use of checkpoint inhibitors against PD1 or CTLA4 unlocks the immune system’s ability to recognize tumor antigens and, more specifically, neoantigens caused by random mutations within cancers. The vast majority of neoantigens consist of private mutations unique to a patient’s tumor genome, but several cancers harbor recurrent mutations. Mutations in the KRAS gene, such as p.G12V, occur in roughly 25% of colorectal cancers. Mutations in EGFR occur in 10% and 35% of patients with non-small cell lung cancer in the US and East Asia, respectively. Even more prevalent are mutations within the TP53 tumor suppressor gene, with roughly 23,000 unique protein variants reported to date. If these mutations in cancer driving genes are so prevalent in cancers, why are neoantigens against these targets not more readily available? Results: We collected recurrent mutations across a variety of cancer driving genes such as KRAS, EGFR, TP53, and MYC and performed binding analysis using netMHC 3.4 to see which HLA alleles are capable of binding specific cancer mutations such as KRAS p.G12V. Using this method, we report all possible HLA alleles capable of binding these recurrent mutations within cancer genes. We further performed 3-dimensional modeling to determine whether complexes created by the HLA alleles and cancer neopeptides are stable. Conclusions: Several HLA alleles are capable of binding recurrent cancer mutations. These include both MHC Class I and Class 2 alleles. The variation in alleles capable of binding commonly mutated genes such as EGFR may explain the difference in prevalence of these mutations between geographic populations. Determining whether certain HLA alleles confer tolerance to common cancer mutations may lead to identification of immune cells within these populations that can recognize neoantigens from commonly mutated cancer genes.

#641 PEGylated recombinant hyaluronidase PH20 (PEGPH20) remodels the TME, reduces tumor interstitial fluid pressure, decompresses tumor blood vessels, and facilitates delivery of chemotherapeutics. We have previously shown that PEGPH20-mediated HA degradation enhances anti-PD-L1 and anti-PD1 efficacy in an HA accumulating murine pancreatic tumor model (Rosengren, AACR 2016 poster #4886). Accumulation of HA in the TME is enhanced by the expression of hyaluronan synthase type 3 (HAS3), a biomarker for tumor vascular density. Upon tumor infiltration by T cells, PD-L1 is upregulated and decreases PD-1 efficacy. PEGPH20-mediated HA degradation enhances both checkpoint efficacy and CD8+ T cell recruitment. Specifically, PEGPH20 alone (1mg/kg) increased anti-PD-L1 efficacy by 411% relative to anti-PD-L1 alone (93% vs 18.2% TGI, p<0.0001) and increased the accumulation of CD8+ TIL by 176% (p=0.0025). Taken together, the data suggest that tumor HA accumulation may act as a barrier to immune cells. In a murine pancreatic tumor model (Rosengren, AACR 2016 poster #4886), anti-PD-L1-mediated HA reduction facilitates increased access of CD8+ T cells. This increased recruitment may contribute to the enhanced anti-PD-L1 efficacy observed when combined with PEGPH20. A Phase 1 trial is ongoing to evaluate PEGPH20 plus checkpoint blockade in patients with advanced or metastatic non-small cell lung cancer and in patients with locally advanced or metastatic gastric adenocarcinoma (NCT02563348).

#642 Detection of circulating antibodies against KRAS in patients with advanced cancers. Ed Khered,1 Helen J. Huang,1 Alice Wu,2 David S. Hong,1 Sarina A. Piha-Paul,1 Daniel D. Karp,1 Sining Fu,1 Vivek Subbiah,1 Apostolia M. Tsimeridou,1 Aung Naing,1 Adi Diab,1 Milind Javle,1 Scott Koptez,1 Anil K. Sood,1 Jonathan M. Kurie,1 Funder Meric-Bernstam,1 Martin Gleeson,2 Filip Janku,1,2 The University of Texas MD Anderson Cancer Center, Houston, TX;1Genalyte INC, San Diego, CA.

Introduction: KRAS gene plays a major role in tumorigenesis, cell proliferation and survival. Yet, there has been no effective targeted therapy against KRAS mutation-mediated tumors. We hypothesized that KRAS can induce humoral-mediated immune response. Methods: Plasma or serum samples from patients with progressing advanced cancers with or without KRAS mutations were tested for the presence of circulating KRAS antibodies. We used the Mavrick Detection System (Genalyte, San Diego, CA), which can perform real-time detection of macromolecules in crude samples using biologically functionalized silicon photonic biosensors lithographically printed on disposable silicon chips. Results: We collected serum or plasma samples from 213 patients with advanced cancers (KRAS-mutant, N=100; KRAS wild-type, N=113) and 30 (23%) were found to have circulating KRAS antibodies. We used the Mavrick Detection System to detect 11 circulating KRAS antibodies and tumor KRAS mutation status (21/100, 21% for KRAS-mutant vs. 29/113, 26% for KRAS wild-type; P=0.52). In addition, there was no difference in detection of KRAS antibodies in colorectal cancer (21/89, 24%) compared to other cancers (29/124, 23%; P=1.00). There was no difference in the median survival in patients with KRAS antibodies compared to patients without KRAS antibodies (9.0 months vs. 10.1 months; P=0.82). Similarly, there was no difference in the median survival according to the presence of circulating KRAS antibodies in subgroups of patients with tumor KRAS mutations (P=0.96) and without tumor KRAS mutations (P=0.63). On the contrary, the median survival of patients with tumor KRAS mutation was shorter compared to those without KRAS mutation (7.2 months vs. 11.5 months; P=0.001). Conclusion: Circulating KRAS antibodies can be detected in 23% of patients with advanced cancers. Biological implications of circulating KRAS antibodies remain to be understood.

#643 Induced MHCII expression on breast cancer cells broadens the responding T cell repertoire, delays tumor-specific T cell exhaustion, and impairs tumor growth. Tyler R. McCaw,1 Mei Li,2 Selene Meza-Perez,2 Donald J. Bucala,3 Ondryto Starace,2 Sara Cooper,2 Andres Forger,1 Troy D. Randall,1 University of Alabama at Birmingham, Birmingham, AL;1HudsonAlpha Institute for Biotechnology, Huntsville, AL.

We recently reported that the aberrant expression of Major Histocompatibility Class II (MHCIi) molecules on human triple negative breast cancer (TNBC)
cells correlates with prolonged progression-free survival and increased tumor infiltrating lymphocytes. We hypothesized that the expression of MHCII enhances the intratumoral CD4+ T cell response, thereby bolstering the tumor-specific CD8+ T cell response, resulting in more effective tumor control. To test our hypothesis, we created both MHCII-expressing and MHCII-negative tumor cells by infecting breast cancer (TS/A) and nasopharyngeal cancer (hCIITA) tumor cells with either the constitutive transcriptional activator (hCIITA) or empty vector, respectively. Transfected cells were then injected into BALB/c mice and the resulting immune response analyzed by flow cytometry at four time points. We found that hCIITA-expressing tumors grew slower than control tumors in immunocompetent recipients, but that this difference was nullified in immunocompromised and markedly reduced in immunodeficient SCID mice. CD4+ T cells isolated from hCIITA-transfected tumors produced more IFNγ, IL-17A, and surprisingly granulocyte colony stimulating factor for longer times than their counterparts in control tumors. Similarly, CD8+ T cells isolated from hCIITA-transfected tumors displayed a more activated phenotype and produced more IFNγ and granulocyte colony stimulating factor for longer times. Nevertheless, both CD4+ and CD8+ T cells eventually became exhausted in both groups.

In addition to enhanced effector functions, TCR repertoire analysis demonstrated that both the breadth and magnitude of expansion of responding T cell clones were increased in hCIITA-transfected tumors. Interestingly, TS/A-hCIITA tumors harbored more regulatory T cells (Tregs) with a more suppressive phenotype than Tregs from control tumors. Finally, we observed that the histone deacetylase inhibitor (HDACi) Entinostat is capable of robust and dose-dependent induction of MHCII on tumor cells in vivo, an effect not observed with control tumors, with dramatic reduction in tumor size. These results suggest that the clinical benefit associated with MHCII expression on TNBC cells is mediated by a delay in T cell exhaustion and increased intratumoral CD4+ T cell activation, which enhances the cytotoxic capacity of CD8+ T cells. Entinostat, and potentially other epigenetic modifying agents, may enable induction of MHCII expression on TNBC cells clinically and allow more patients to benefit from an augmented T cell response. These effects may be magnified by combinatorial therapy with checkpoint inhibitors to promote durable anti-tumor immune responses.
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PD-L1, PD-L2 and/or MHC class I molecule expression with the outcome of the patients was determined. The high incidence of MHC class I deficiencies and PD-L1/PD-L2 overexpression imply that EBV-associated NPC escape from the host immune response by targeting both antigen presentation mechanisms and immune checkpoints. Our findings also suggested that the expression of MHC class I molecules and PD-L1/PD-L2 is a potential biomarker for immunotherapy in NPC patients.

#647 Bortezomib enhances CD8+ T Lymphocyte antitumor effector function: Potential mechanism(s) via notch regulation. Ariana N. Renrick,1 Menaka C. Thounaojam,2 Portia Thomas,1 Samuel T. Pellom,1 Anil Shanker1. 1Meharry Medical College, Nashville, TN; 2Medical College of Georgia, Augusta, GA.

The immunosuppressive tumor microenvironment disturbs host antitumor immunity by multiple mechanisms including interference with the Notch system, which is important for various metazoan cell fate decisions and hematopoietic cell differentiation and function. We observed that treatment with the proteasome inhibitor bortezomib in mice bearing various solid tumors resulted in an upregulated expression of various Notch signaling components in lymphoid tissues, thereby increasing CD8+ T lymphocyte IFNy secretion and expression of effector molecules, perforin and granzyme B, as well as the T-box transcription factor c esomesedomerin. Of note, bortezomib reversed tumor-induced downregulation of Notch receptors. Notch1 and Notch2, as well as increased the levels of cleaved Notch intracellular domain (NICD) and downstream targets Hes1 and Hey1 in tumor-draining lymphnodes. Moreover, bortezomib enhanced NICD cleavage by nuclear factor-kB (NFkB) activity by increasing the total and phosphorylated levels of the IkBa kinase and IkBa as well as the cytoplasmic and nuclear levels of phosphorylated p65. Even when we blocked NFkB activity by Bay11-7082, or NICD cleavage by y-secretase inhibitor, bortezomib significantly increased expression of Notch Hes1 and Hey1 genes as well as perforin, granzyme B and c esomesedomerin in activated CD8+ T cells. Data suggest that bortezomib can rescue tumor-induced dysfunction of CD8+ T cells by its intrinsic stimulatory effects promoting NICD-NFkB crosstalk. We are also elucidating components of microRNA regulation affecting NICD-NFkB crosstalk. Our preliminary data suggest that bortezomib is also able to positively regulate miR-155 expression in CD8+ T cells from mice bearing tumor. As well as, miR-155 suppression downregulates bortezomib-induced increase in Notch target genes in T cells. We are currently investigating alternative proteasome inhibitors in order to understand whether bortezomib’s effect on miR-155 expression in CD8+ T cells is specific to bortezomib or primarily conducive to a proteasome inhibition effect. These findings provide novel insights on using bortezomib not only as an agent to sensitize tumors for chemotherapy, but also to provide lymphocyte-stimulatory effects, thereby overcoming immunosuppressive actions of tumor on anti-tumor T cell functions.


The purpose of this study is to investigate underlying mechanism of how cytokine interferon-gamma (IFN-γ) regulates PD-L1 expression in ovarian cancer cells. We treated a panel of human and mouse ovarian cancer cell lines with recombinant human/mouse IFN-γ. Our data showed that IFN-γ upregulated mRNA and protein expression of PD-L1 significantly in a majority of ovarian cancer cells. The functional IFN-γ receptor is comprised of two ligand-binding IFNγR1 chains associated with two signal-transducing IFNγR2 chains and associated signaling machinery. Here we found that the mRNA expression levels of IFNγR1 and IFNγR2 were abundant in all human ovarian cancer cell lines being tested, while their expressions were not affected by IFN-γ treatment. After knocking down the expression levels of IFNγR1 and IFNγR2 in a ovarian cancer cell line by target gene-specific siRNA, our data showed that the IFN-γ-mediated induction of PD-L1 were diminished in the ovarian cancer cells when compared to those with non-targeting scrambled siRNA controls, indicating the induction of PD-L1 by IFN-γ is dependent on the presence of IFN-γ receptors in the ovarian cancer cells. Although abundant expression of IFNγR1 and IFNγR2 were found in all human ovarian cancer cell lines being tested, the IFN-γ-mediated induction of PD-L1 was not detected in a few of the human ovarian cancer cell lines (namely IGV0-1, TOV21G and SKOV3). We further investigated the integrity of IFN-γ signaling in the human ovarian cancer cell lines by examining activation of STAT1 protein and induction of IRF-1 gene in human ovarian cancer cell lines after IFN-γ treatment. Our data showed that phospho-STAT1 protein and IRF-1 gene expression were up-regulated significantly in a majority of human ovarian cancer cell lines after IFN-γ treatment, except IGV0-1 and TOV21G cells. These results suggested that IGV0-1 and TOV21G cells might harbor defects in intracellular JAK-STAT1 signaling. We then examined the presence of JAK1 truncating mutations in human ovarian cancer cell lines by Sanger sequencing, and confirmed that IGV0-1 and TOV21G cells, but not the others, have JAK1 truncating mutations. Since our data showed that SKOV3 cells have wild type JAK1, we further investigated other possible defects in IFN-γ signaling in SKOV3 cells. We investigated the IFN-γ-induced STAT3 protein activation in human ovarian cancer cell lines, and defects were found in Y705 STAT3 phosphorylation in SKOV3 as well as in IGV0-1 and TOV21G cells. To summarize, our results showed that IFN-γ induces PD-L1 expression in ovarian cancer cells via IFNFR-JAK-STAT pathway. The failure of IFN-γ-mediated induction of PD-L1 in a minority group of human ovarian cancer cell lines is due to defective IFN-γ signaling, including JAK1 truncating mutations and impaired STAT3 activation. This work is supported by Hong Kong Research Grants Council General Research Fund (467713 and 14109515).

#649 Emigrant pre-REP tumor infiltrating lymphocytes profoundly differ from remnant T-cells. Michelle R. Simpson-Abelson, Christopher Mosychuk, Maria Fardis, Michael T. Lotze. Lion Biotechnologies, Tampa, FL.

Adaptive T cell therapy with autologous tumor infiltrating lymphocytes (TIL) provides up to 56% objective response rates and a complete response in 24% of patients with metastatic melanoma. The process of generating TIL from resected tumor involves morcellating the tumor into 1 mm3 fragments and expanding TIL in the presence of Interleukin 2 (IL-2) in a pre-Rapid Expansion Protocol (pre-REP). During the ‘pre-REP’, tumor-resident immune cells emigrate (eTIL) and proliferate. The length of the pre-REP varies between 11-21 days, depending on cell growth. Residual tumor fragments (remnants) are discarded and the expanded eTIL are subjected to a Rapid Expansion Protocol (REP) with irradiated PBMC feeders, anti-CD3 and IL-2. Viable cells remaining in the tumor remnants (rTIL) following the pre-REP were investigated to assess their function and phenotype. We evaluated and compared the rTIL and eTIL in melanoma, breast, renal, pancreatic, lung and colorectal tumors (n = 9). Tumor rTIL are consistently phenotypically distinct from eTIL, as determined by differential expression of various markers (Table 1). The fundamental differences in rTIL were: Increased CD69+ (7 fold MFI in CD4+) (p<0.001); diminished LAEG3 (2 fold MFI in CD8) (p<0.05); TIM3 (3 and 2 fold MFI in CD8 and CD4 respectively) (p<0.05/0.01); CD154 (fold MFI in CD4) (p<0.01); and CD56 (5%) (p<0.05). Surprisingly, a REP of rTIL and eTIL resulted in comparable expansion. The phenotypic signature of rTIL was sustained post-REP with fidelity of the individual expression of LAEG3, Tim3, and CD28. These studies have identified significant differences in the biology of cell populations in terms of tissue-resident T cells and the signals associated with emigration and retention. These data provide additional insights on the individual TIL populations that could be utilized for adoptive T-cell therapy in patients and raise important questions about the nature of tissue-resident T cells in sites of chronic inflammation such as tumor.

Table 1: Tumor resident remnant T cells are phenotypically distinct from emigrating T cells (N=9)

<table>
<thead>
<tr>
<th>Marker</th>
<th>Expression</th>
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<tbody>
<tr>
<td>EG3</td>
<td>LAG3 (CD119) MFI</td>
</tr>
<tr>
<td>Tim3</td>
<td>LAG3 (CD119) MFI</td>
</tr>
<tr>
<td>PD-1</td>
<td>CD8 (CD119) %</td>
</tr>
<tr>
<td>CD69</td>
<td>CD8 (CD119) %</td>
</tr>
<tr>
<td>CD154</td>
<td>CD8 (CD119) MFI</td>
</tr>
<tr>
<td>CD307</td>
<td>CD8 (CD119)</td>
</tr>
</tbody>
</table>

#650 B lymphocytes promote upregulation of an IL-1Rα NBK signaling and increase invasiveness of triple negative breast cancer. Nicole Flynn,1 Rajasekharan Somassundaram,2 Jennifer Sims-Mourtada3. 1University of Delaware, Newark, DE; 2The Wistar Institute, Philadelphia, PA; 3Helen F. Graham Cancer Ctr., Newark, DE.

Triple negative breast cancer (TNBC) is an aggressive form of breast cancer that progresses quickly from a non-invasive carcinoma in situ to an invasive state. Chronic immune infiltrates and inflammatory immune responses have been found to promote aggressiveness in a number of solid tumor types. In breast cancer, B lymphocytes are associated with microinvasive disease and correlate with expression of inflammatory genes. The purpose of our work is to study the impact of B lymphocytes on the tumor...
microenvironment and increased invasiveness of TNBC cells. Through real-time PCR, we demonstrate that co-culture of B lymphocytes and TNBC cells resulted in elevated expression of genes associated with immune cell trafficking and activation. Furthermore, in ER DCIS and TNBC, both B lymphocytes and tumor cells are found to express IL1β whereas IL8 is found more specifically to be expressed by tumor cells. Our findings support the hypothesis that B lymphocytes promote a chronic inflammatory environment leading to increased invasion of TNBC cells.

**#651 Evaluation of the expression and function of TIM-3 relative to PD-1 in human tumors.** Kristen McEachern, Srimoyee Ghosh, Yonghong Zhao, Qiyao Zhang, Norman Zhang, David W. Jenkins, 2 TESARO, Waltham, MA; 3Wuxi Code Texas, China.

While immunotherapies directed against PD-1 and PD-L1 have proven effective across multiple indications, there is still a large unmet medical need for therapies for patients who do not respond or who develop acquired resistance during the course of treatment. There are several emerging hypotheses to explain the lack of response, including the overall presence and localization of immune cells, as well as the up-regulation of additional T-cell checkpoints, including TIM-3 in tumor infiltrating lymphocytes. To investigate the potential role of TIM-3, we set out to evaluate the expression and function of TIM-3 and the relationship to PD-1 in several systems. Firstly, we developed a flow cytometry methodology to enumerate T-cell, including CD4 and CD8 positive cells, as well as other immune cell populations in a panel of human tumor samples, including non-small cell lung cancer (NSCLC). In these samples, we investigated the expression profiles of TIM-3 and PD-1 in both T-cell and non-T-cell populations. In addition, we performed complementary genomic studies to explore gene expression profiles of not only the bulk tumor samples but also of isolated PD-L1 positive/TIM-3 negative versus PD1 and TIM-3 double positive cell populations. The profiling identified tumors with a range of tumor infiltrating lymphocyte content and also differences in PD1 and TIM-3 expression. It was found that in addition to T-cells, TIM-3 was expressed on myeloid cell populations and furthermore, that there were distinct differences in the gene expression profiles of PD1 single positive versus PD1 and TIM-3 double positive cells. Building on the expression studies, the functional role of TIM-3 in T-cells and also on myeloid-derived cells was explored. Taken together, these studies provide further evidence for the importance of TIM-3 as a potential therapeutic target for the treatment of cancer with the potential for biological effects in both the T-cell and myeloid compartments of the tumor microenvironment.

**#652 PD-L1 and MHC class I expression levels are modulated by MEK inhibition on head and neck squamous cell carcinoma.** Seong-Ho Kang,1 Bhumsuk Keam,2 Soyeon Kim,1 Tae Min Kim,1 Dong-Wan Kim,1 Dae Seog Heo1. 1 Cancer Research Institute, Seoul National University, Seoul, Republic of Korea; 2 Department of Internal Medicine, Seoul National University Hospital, Seoul, Republic of Korea.

Background: To overcome immunosuppression, multiple pathways can be targeted by counter strategies, including elimination of suppressive signals (such as PD-1/PD-L1) and promotion of anti-T cell inhibition by tumor cells, and of T cell infiltration into tumor sites. The purpose of this study is to investigate the change in expression of PD-L1 and MHC class I as well as of T cell co-what attractants, CXCCL9 and CXCL10, in human head and neck squamous cell carcinoma (HNSCC) cell lines after treatment with MEK inhibitor. In addition, for evaluation of immunologic effects of MEK inhibitor in vivo, we employed SCC VII mouse squamous cell carcinoma (SCC VII) model. Methods: Six human HNSCC cell lines (SNU-1041, SNU-1066, SNU-1076, Detroit 562, FaDu, and HN31) and a mouse squamous cell carcinoma cell line (SCC VII) were used. Trametinib was purchased from Selleckchem. We conducted cell viability assay using these cell lines after 72 h incubation with MEK inhibitor trametinib. PD-L1 and MHIClass I expression levels were analyzed by flow cytometry after treatment with trametinib and/or interferon-gamma (IFN-γ). Expression of PD-L1, p-erk1/2, and p-STAT1/3 were analyzed by western blot..Statistical testing was carried out on tumor tissues to assess gene expression, stromal remodeling and macrophage polarization in response to AB0046 treatment. Results: MMP9 levels are elevated in human tumors compared to healthy tissues. The protein is expressed predominantly in stromal cells, including macrophages and neutrophils, with more occasional heterogenous expression in tumor epithelia. Differentiation of human monocyte-derived macrophages in vitro revealed that M2 polarization is associated with increased expression of MMP9 and Th2 markers CCL18 and TGFB. Anti-MMP9 treatment in three independent mouse tumor models (HC1-NeuT, CT26, Lewis lung carcinoma (LLC)) resulted in decreased primary tumor growth (p<0.001 and p<0.005, for HC1-NeuT and CT26 respectively) and increased animal survival (p=0.024 for LLC). Gene expression profiling of tumors from the various models demonstrated that inhibition of MMP9 resulted in elevated expression of genes associated with immune cell activation pathways (Hallmark Interferon Gamma Response, p<0.05, FDR<0.001). Additional analysis in the HC1-NeuT model revealed a significant decrease in M2 macrophage infiltration in the tumor microenvironment (p<0.05) with AB0046 treatment, as well as reductions in tumor-associated fibrillar collagen as assessed by Picrosirius red staining. Conclusions: These analyses show that MMP9 is expressed in a variety of human tumors. Our data suggest that inhibition of MMP9 promotes anti-tumor immunity and enhances a Th1 immune response. GS-5745, a humanized anti-MMP9 inhibitory antibody, is being evaluated in gastric cancer in phase 3 and 2 studies with chemotherapy and nivolumab, respectively (NCT02545504, NCT02864381).

**#654 The epigenetic landscape of T cell exhaustion.** Debattama Sen, Dana Farber Cancer Institute/Harvard Medical School, Cambridge, MA.

Exhausted T cells in cancer and chronic viral infection have distinctive patterns of gene expression, including sustained expression of the inhibitory recep-
tor PD-1, but the regulation of gene expression in exhausted T cells is poorly understood. Here we define the accessible chromatin landscape in mouse and human exhausted CD8⁺ T cells and show that it is profoundly different from functional memory CD8⁺ T cells. Exhausted CD8⁺ T cells in a mouse model of chronic viral infection acquire an extensive, state-specific pattern of enhancers, which are co-opted into functional modules. One enhancer, −23.8kb from the Pdcd1 locus, is found only in exhausted T cells and other lymphocytes with sustained PD-1 expression. Genome editing shows it to be required for high PD-1 expression, Cas9-mediated in situ saturation mutagenesis of the enhancer pinpoints critical minimal sequences that correspond to bound transcription factor motifs for RAR, T-bet and Sox3 in exhausted CD8⁺ T cells. State-specific enhancer profiles are conserved in mouse and human exhausted-antigen-specific CD8⁺ T cells responding to HIV and HCV infection. Detailed functional enhancer maps of T cell exhaustion reveal state-specific regulatory sequences and offer targets for genome editing that could alter gene expression preferentially in exhausted CD8⁺ T cells.

**CLINICAL RESEARCH: Checkpoint Inhibitor and Prognostic Biomarkers**

#655 Development of IHC staining protocols for assessment of PD-L1 expression in cytological samples. Christine Boyiddle,1 Mark Ruboyianes,1 Ed Del Valle,1 Lukas Bubendorf,2 Kerstin Trunzer,2 Judith Pugh,1 Jennifer Boone.1

1Ventana Medical Systems, Oro Valley, AZ, 2University Hospital Basel, Basel, Switzerland.

Immunohistochemistry (IHC) staining of non-small cell lung cancer (NSCLC) samples for programmed cell death ligand 1 (PD-L1) can help identify patients that may benefit from anti-PD1 therapy. However, resection or biopsy samples cannot be obtained for some patients with NSCLC. Fine needle aspiration (FNA) is a less invasive method for obtaining samples in such patients, and it is yet to be determined if PD-L1 can be assessed using cytological samples. In this study we had three objectives 1. Develop staining protocols on the VENTANA Benchmark ULTRA automated staining platform for cytology samples fixed with the most common methods using cell lines as a model. 2. Determine the optimal cytology fixation method for VENTANA PD-L1 (SP142) IHC Assay (PD-L1 (SP142) staining, and 3. Assess PDL1 expression in tumor cells (TC) and tumor-infiltrating immune cells (IC) in cytological samples prepared from NSCLC patients. KARPS 299 cell line was used as a model system for the fixation studies. Liquid-based preparations (LBPs) fixed in PreservCyt, conventional smears (unstained and pap-stained) fixed in 95% ethanol, and paraffin embedded cell blocks (CBs) fixed in 95% ethanol, 10% neutral buffered formalin (NBF), PreservCyt, and SurePath preservative were optimized on the Benchmark ULTRA using PD-L1 (SP142) antibody and Optiview DAB Detection and Amplification Kits. NSCLC FNA CBs fixed in 10% NBF (N=69) and a subset of FNAs with matched resections (N=20) were stained with the optimized protocol. FNAs and resections were assessed for percentage of TC and IC with PD-L1 staining. Staining parameters were optimized on the Benchmark ULTRA for all sample types tested, and they all produced a range of moderate to strong PD-L1 expression. CBs fixed in 95% ethanol and 10% NBF produced the highest percentage of staining, with expression in 90% of cells. CBs fixed in PreservCyt and SurePath preservative had a lower percentage of staining, 40% and 75% respectively. LBPs had 25% cells staining and smears ranged from 20%-60%. NSCLC FNA CBs fixed in 10% NBF produced interpretable results when stained with PD-L1 (SP142). IC staining was seen in 8.7% (6/69) of samples, TC staining was seen in 8.7% (6/69) of samples, both IC and TC staining was seen in 14.5% (10/69) of samples. The subset of FNAs with matched resections showed concordance with PD-L1 IC staining in 60% (12/20) of samples and TC staining in 75% (15/20) of samples. The discordant cases showed that FNAs were negative for IC when the resection was negative and positive for TC in 5% of cases when the resection was negative. The VENTANA PD-L1 (SP142) Assay staining parameters were found to be optimal for staining NSCLC FNA CBs fixed in 10% NBF. PD-L1 staining was detected in both TC and IC in FNAs and concordance of FNA and matched resections was high for both IC and TC. A larger study is necessary to validate the use of FNAs for assessment of PD-L1 expression in a clinical setting.

#656 PD-L1 expression in primary lesions vs metastatic sites and by demographics in advanced urothelial carcinoma samples. M Zajac,1 A M. Boothman,1 Y Ben,2 A Gupta,2 X Jin,2 J Antal,2 A Sharpe,1 M Scott,2 M Rebe-latto,3 J Walker1, AstraZeneca, Cambridge, United Kingdom; AstraZeneca, Gaithersburg, MD; 3MedImmune, Gaithersburg, MD.

Background: Determination of programmed cell death ligand-1 (PD-L1) expression levels in tumors may help physicians understand which patients (pts) are most likely to respond to anti-PD-1/PD-L1 therapies in urothelial carcinoma (UC). Understanding the impact of different sample types and demographics on PD-L1 expression is important to determine suitability of tumor biopsies for testing. Results: As of July 24, 363 pts screened in the UC cohort of Study CD-ON-MEDI4376-1108 (NCT01693562) had tissue available for analysis and 47 pts had provided paired primary and metastatic samples. FFPE samples were tested in a central laboratory with the VENTANA PD-L1 (SP263) Assay using a Benchmark ULTRA instrument. Pts were classified as having either PD-L1 high (PD-L1 expression ≥25% either on tumor cells [TC] or immune cells [IC]) or PD-L1 low/nega-tive (<25% on TC and IC) tumors. PD-L1 high prevalence was reported in primary vs metastatic sites, and by age, sex and race. Results: PD-L1 status was evaluable for 332/363 (91.5%) pts (175/332 [52.7%] PD-L1 high and 157/332 [47.3%] PD-L1 low/ negative) whose UC specimens were tested (intent to diagnose [ITD] population). Overall percentage agreement between paired primary and metastatic samples, based on combined TC/IC scoring ≥25%, was 74.5% (95% CI 59.7 - 86.1%). In the ITD population, using only the samples from which patient PD-L1 expression status was determined, PD-L1 high prevalence in primary and metastatic samples was 57.1% and 50.9% respectively (p=0.343, not significant). The proportion of pts with PD-L1 high status was not enriched in any demographic group (Table). Conclusions: Initial data from UC pts in Study 1108 showed similar PD-L1 high prevalence in primary and metastatic lesions and good concordance between paired primary and metastatic samples. These results build optimism that samples obtained from either location could be used to determine PD-L1 status. Further data are needed to confirm these findings.


Background: Identifying optimal biomarkers for response to anti-PD-1/PD-L1 therapies in non-small cell lung cancer (NSCLC) is critical. Tumor mutational burden (TMB) is a potential biomarker of genomic instability and neo-antigen binding sites to activated effector T cells. The goal of this study was to examine the association of TMB with overall survival for patients treated with checkpoint blockade and to correlate TMB with mutational status, including DNA repair mutations. Methods: We retrospectively examined TMB using next-generation sequencing (FoundationOne) for 82 patients with NSCLC in our institution. TMB included coding base substitutions and indel alterations, but excluded potentially functional mutations. TMB high versus low was stratified based on 15 mutations per megabase pair. We correlated TMB with DNA repair mutations, mutational status, and smoking history. In addition, survival data were obtained for 35 patients who were treated with anti-PD-1/PD-L1 therapy. Results: TMB was associated with significantly longer overall survival for patients treated with checkpoint blockade (Table 1, p < 0.04). In addition, TMB was independently correlated with direct and indirect (Chae et al. 2016) DNA repair mutations (p < 0.03). TMB was also significantly associated with smoking, number of coding region mutations, and treatment with at least two prior lines of therapy (p < 0.02). A trend toward lower TMB was found for patients with mutations in EGFR, ALK, or KRAS (p = 0.06). Conclusions: Higher TMB was associated with improved survival for patients treated with anti-PD-1/PD-L1 therapies. In addition, TMB was correlated with DNA repair mutations, number of coding mutations, prior treatment, and smoking. Our results indicate that TMB can be used as a biomarker for response to checkpoint blockade in NSCLC.
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Table 1. Tumor mutational burden (TMB) by patient characteristic and mutation status

<table>
<thead>
<tr>
<th>Patient Characteristic</th>
<th>TMB (mutations per megabase)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking History</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimal/ Never (N)</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>Current/ Former (N)</td>
<td>38</td>
<td>18</td>
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VUS, Variant of Unknown Significance; HR, Hazard Ratio; * Pearson’s Chi-Squared; * Logistic regression; ^ Welch’s two-sample t-test

#659 Multiplex cocktails for immunotherapy targets: PD-L1 with tumor specific transcription factors. David Alrett-Tacha, Wei Yuan, George Yang, Biocare Medical, Concord, CA.

Introduction: Blocking the interaction between the programmed cell death (PD)-1 protein and one of its ligands, PD-L1, has been reported to generate immunostimulatory responses. Therapeutics targeting PD-L1 in several cancers are currently in clinical trials, and the U.S. FDA has granted accelerated approval for Keytruda (pembrolizumab) to treat patients with advanced (metastatic) non-small cell lung cancer (NSCLC). Various pathology scoring and interpretation methods have been put forth to assay PD-L1 expression, and variability of non-PD-L1 staining across tumors may have important prognostic aspects. PD-L1 expression on tumor cells can be upregulated via activation of CD8+ cytotoxic T lymphocytes. PD-L1 expression is also associated with certain subtypes of tumor-associated lymphocytes, macrophages and dendritic cells. In certain cancers, the cross-reactivity of PD-L1 can make interpretation and scoring difficult. Thus, a strategy using IHC multiplex stains could help resolve challenging cases by cocktailling PD-L1 with target tissue transcription factor antibodies along with other potential markers. Method and Materials: Formalin-fixed paraffin-embedded tissues and tissue microarrays for various tumor types including lung, bladder and melanoma were processed and cut at 4-5 microns. PD-L1 rabbit monoclonal antibody was cocktailled with the following antibodies: TTF1 (lung cancer); CD163 (lung cancer); p40 + GATA3 (bladder cancer); and SOX10 (melanoma). Cocktails were detected with double stain detection systems using brown, red and blue chromogenos for visualization. Sections were counterstained with a standard hematoxylin or Wiegert’s iron hematoxylin. Results PD-L1 and TTF-1 with or without CD163 (macrophage) and PD-L1 + CD163 were used in lung cancers. The nuclear staining of TTF1 in lung adenocarcinoma (blue chromogen) helped define tumor positive PD-L1 (DAB) positive cells and CD163 (Fast Red) marked macrophages. PD-L1 combined with p40 and GATA3 was deployed for bladder cancers. PD-L1 membrane expression in tumor cells and co-expression of p40 and GATA3 gave robust nuclear staining in bladder tumor cells. Finally, SOX10 nuclear staining was observed in most melanoma and could be easily separated from PD-L1 membrane staining. Conclusion: PD-L1 cocktailled with strategic nuclear or cytoplasmic antibodies can help discriminate tumor cells from non-tumor cells, and may facilitate quantitation or scoring methods for more accurate assessment of this key immunotherapy marker.

#660 Pre-symptomatic detection and early treatment of mammary cancer with anti-PD-L1 in a mouse model. Hu Duan, Stephen Albert Johnston, Shen Luhui, Biodesign Institution, ASU, Tempe, AZ.

Cancer kills ~8M people/year and the WHO predicts an epidemic of cancer in the developing world over the next 20 years due to increased longevity. Development of a simple method to detect and treat cancer early could be a solution to this challenge. Here we test this approach in principle in a transgenic mouse mammary tumor model by immunomutation (IM) diagnostics. IMs are profiles of antibodies in the blood displayed on a high density peptide array. As previous published data showed, the IMs can distinguish different breast cancers based on the disease specific antibody profile in the patient, including different breast cancers and infectious diseases. According to the immune surveillance theory, the immune system, including the humoral immune response, starts to interact with cancer cells as soon as they are transformed. First, we tested if the IMs is sensitive enough to detect the antibody profile change caused by the early tumor development in the FVB-NeuN (MMTV-neu202Mul/J) mammary cancer model. In the FVB-NeuN model, the MMTV promoter activates the wild type rat ERBB2 gene in mammary cells, and every mammary cell potentially can convert into a cancer cell. The transition from normal mammary glands to hyperplasia of FVB-NeuN in mouse starts at ~8.6 to 15 weeks after birth. The mice develop the first palpable tumor in one of the ten mammary glands by 19-45 weeks of age with an average age of 32±1.2 weeks in our hands. We collected serum every 4 weeks from the FVB-NeuN mice and the wild type FVB mice for the IM analysis. The IMs can distinguish the FVB-NeuN mouse from the age matched wild type FVB mouse as early as 12 weeks (Stage-1) before the onset of the first palpable tumor. We also analyzed the IMs from the Stage-2 (8 weeks before the palpable tumor and so on) to Stage 4. Different IMs of each stage has only partial overlap with the other stages. The cross-stage prediction analysis showed the accuracy of the cancer detection by using different IMs is from 75% to 93%. Second, we determined if early treatment at the time of diagnosis with the checkpoint blockade, anti-PD-L1, would inhibit the tumor growth. A course of 3 injections at the time of diagnosis significantly retarded tumor growth relative to untreated controls. The early treatment elicited long-term protection which could restrict the tumor growth even four months after the treatment. The treated mice presented a specific IMs at 28 days post treatment and 35 days post the first palpable tumor, compared to the untreated mice. This indicates that the early treatment elicited an anti-tumor immune response that could inhibit tumor growth. This preliminary experiment suggests that early diagnosis combined with systemic treatment might be a method to control or eliminate cancer.


The current diagnostics landscape for therapeutics that target the PD1/PD-L1 pathway is highly complex. Four different companion or complementary diagnostics have been developed for pembrolizumab in NSCLC, nivolumab in NSCLC and melanoma, and atezolizumab in urothelial carcinoma. The need to reconcile diagnostics for this class of targeted therapies has been recognized by the creation of the FDA-AACR-ASCO “PD-L1 Blueprint” working group to explore means to “harmonize” PD-L1 testing in tissue based IHC assays. The results reported from this working group noted similarities, but also several important discrepancies, between the current assays. Since each test uses a specific interpretation for each assay and indication, this creates a highly complex diagnostic landscape, which is likely to continue to increase in complexity as more PD-1/PD-L1 therapeutics and potentially novel diagnostics continue to be approved in additional indications. To address the need for adaptive, sustainable harmonization for PD-L1 diagnostics, Flagship Biosciences evaluated the utility of image analysis-based methods to harmonize multiple PD-L1 tests. We executed a proof-of-concept study utilizing a cohort of 38 breast samples from the same NSCLC patients, stained with the FDA approved Dako PD-L1 tests (28-8 and 22C3 clones), and our in-house PD-L1 assays (SP142 and EI13N clones) for comparison. We digitized the tissue slides using a whole slide scanner, and evaluated the samples with our tissue Image Analysis (tIA™) technology. As expected, the patient samples stained with the separate PD-L1 assays yielded differences in staining and, thus, the reported scores for PD-L1 expression based on...
on each test used, despite serial sections being derived from the same patient. To attempt to harmonize the scoring approaches for each test, we leveraged our computational Tissue Analysis (cTA™) platform to create a mathematically derived "virtual slide score" for each sample, which enabled calibration of the various tests to deliver cross PD-L1 comparative scores. Based on the proof-of-concept from this study, the cTA™ approach harmonization of the various PD-L1 tests through use of a digital pathology platform. The data presented provides a foundation for potential application of the cTA™ platform in the clinical laboratory setting to achieve harmonization of multiple PD-L1 tests.

**#664 Analytical validation and clinical utility of an immunohistochemical PD-L1 diagnostic assay for treatment with durvalumab in urothelial carcinoma patients.** M. Zajac, A. M. Boothman, Y. Ben, A. Gupta, A. Antal, X. Jin, A. Nielsen, G. Manriquez, C. Barker, P. Wang, P. Patil, N. Schechter, M. Rebeletto, J. Walker. AstraZeneca, Cambridge, United Kingdom; AstraZeneca, Gaithersburg, MD; MedImmune, Gaithersburg, MD; Ventana Medical Systems, Inc., Tucson, AZ.

Background: A high quality programmed cell death ligand-1 (PD-L1) diagnostic may help to identify patients (pts) most likely to respond to anti-PD-L1/programmed cell death-1 (PD-1) therapy. Here we describe a PD-L1 immunohistochemical (IHC) diagnostic test developed for urothelial carcinoma (UC) pts treated with durvalumab. Methods: The IHC assay uses an anti-human PD-L1 rabbit mAb optimized for detection of both tumor cell (TC) and tumor-associated immune cell (IC) PD-L1 expression with the OptiView DAB IHC Detection Kit on the automated VENTANA Benchmark ULTRA platform. The assay was validated in UC tissue intended for use in formalin-fixed, paraffin-embedded samples in a series of studies that addressed sensitivity, specificity, robustness and precision and implemented in Study CD-ON-MED4736-1108 (NCT01693562). Pts were evaluated using the VENTANA PD-L1 (SP263) Assay at a prespecified PD-L1 expression cut-off. Efficacy was analyzed in pts with PD-L1 low/negative (defined as TC <25% and IC <25%) and UC vs pts with PD-L1 high (defined as TC ≥25% or IC ≥25%) UC. Results: The VENTANA PD-L1 (SP263) Assay met all the predefined acceptance criteria (average positive agreement and average negative agreement >85%), showing analytical specificity, sensitivity and precision. It demonstrated ≥97% and ≥85% inter-reader precision agreement for TC and IC respectively. For intra-reader precision, it demonstrated >96% and >87% agreement for TC and IC respectively. For intra-day performance, the assay demonstrated ≥96% agreement for TC and IC and for inter-day performance, it demonstrated ≥98% and 100% agreement for TC and IC respectively. Precision studies for inter-antibody lot, inter-detection kit lot and intra-platform demonstrated ≥97% agreement for both TC and IC. Inter-laboratory testing was performed at 3 external laboratories and demonstrated an overall agreement rate of 92.3%. The VENTANA PD-L1 (SP263) Assay was implemented in Study CD-ON-MED4736-1108 and durvalumab demonstrated clinical activity and durability of response in both PD-L1 high and PD-L1 low/negative subgroups, yet with different response rates. In addition, given the high negative predictive value of the assay, it is especially helpful in evaluating the likelihood of response to durvalumab: pts who were classified as PD-L1 high with the VENTANA PD-L1 (SP263) Assay tended to have a higher objective response rate per RECIST v1.1 than pts who were PD-L1 low/negative.

Conclusions: These data show that determination of PD-L1 expression in TC and IC in UC pts using the VENTANA PD-L1 (SP263) Assay is precise and highly reproducible and highlight the utility of the assay in a clinical setting. The VENTANA SP263 Assay is especially helpful in informing pts and physicians on the likelihood of response to durvalumab, but not for the purpose of restricting treatment to only PD-L1 high pts.

**#665 Molecular and clinical analyses of patients with gynecologic malignancies treated with PD-1 directed immunotherapy.** Young Kwang Chae, Sabina Murshudova, William H. Bae, Jonathan F. Anker, Mario J. Pineda, Wilberto Nieves-Neira, Daniela E. Matei, John R. Lurain, Shohreh Shahabi, Francis J. Giles. Northwestern University, Chicago, IL; Yonsei University College of Medicine, Seoul, Republic of Korea.

Long term responses are seldom seen in treatments of advanced or recurrent gynecologic cancers. In contrast, immunotherapeutic strategies targeting immune checkpoints such as programmed cell death-1 (PD-1) are currently in active development in platinum resistant gynecologic malignancies with promising durable responses observed in subset of patients. We analyzed all 14 pts with gynecologic malignancies that were treated with PD-1 directed immunotherapy, nivolumab or pembrolizumab at the developmental therapeutics program (DTP) clinic in Northwestern University during 2015 till 2016. Seven patients had ovarian/fallopian/peritoneal cancer; six, endometrial cancer; 1, squamous cell carcinoma (SCC) of cervix that are refractory to standard-of-care.
chemotherapies. Comprehensive genomic profiling including microsatellite instability (MSI) status and tumor mutational burden (TMB) analyses was performed using next generation sequencing (NGS) (FoundationOne). Among 7 patients with ovarian/fallopian/peritoneal cancer (median age, 62 years), objective response rate (ORR) was 20%. One patient had partial response (PR), another one had stable disease (SD), and 4 patients had progressive disease (PD). Two patients were unable to be evaluated. In a patient with PR, CA 125 level normalized from 426.2 to 10.8 U/mL. Her tumor had very low TMB (1/Mbp). In all patients, TMB level varied from very low to low (1-5 mut/Mbp) with MSI status being stable. Progression free survival (PFS) ranged from 14 to 196 days; overall survival (OS) varied from 60 to 199 days. Among 6 patients with endometrial cancer (median age, 68 years), ORR was 20%. One patient had PR while four experienced PD. One patient whose disease could not be assessed due to early death from an event unrelated to treatment had a remarkable chemical response with a decrease in CA125 level from 5,889 to 182.1 U/mL after one single dose. Her tumor had MSI-stable status and intermediate TMB (6 mut/Mbp). In a patient with PR, CA125 level decreased from 1,216 to 226.5 U/mL. Her tumor had MSI-high status with unknown TMB. Of note, one of four patients with PD had MSI-high status and intermediate level of TMB. Other patients had tumor with MSI-stable status and low level of TMB (3-4 mut/MB). PFS ranged from 23 to 258 days; OS varied from 73 to 265 days. One patient with HPV positive SCC of cervix (34 year old) experienced PD. Her tumor had MSI-stable status with unknown TMB. PFS was 88 days; OS, 132 days. In summary, patients with ovarian and endometrial cancer treated with PD-1 directed immunotherapy demonstrated ORR of 20%. One additional patient with endometrial cancer had an exceptional chemical response. None of the tumors had high TMB (≥6/Mbp). No clear associations between genomic traits including TMB and MSI status and responses were found. Further larger prospective studies are warranted to explore potential biomarkers for response with immunotherapy in gynecologic malignancies.

CD37 tetraspanin as a novel biomarker for PD-1 blockade in diffuse large B-cell lymphoma. Zijun Y. Xu-Monette, Ken H. Young, UT MD Anderson Cancer Ctr., Houston, TX.

PD-1 immune checkpoint blockade reconstituting antitumor immunity has changed the cancer treatment paradigm. PD-1 blockade has been successful in many types of solid tumors and Hodgkin lymphoma, but not yet for diffuse large B-cell lymphoma (DLBCL), the most common aggressive B-cell lymphoma. We found several biomarkers including loss of CD37 tetraspanin expression in DLBCL correlated with increased PD-1 expression suggesting potential sensitivity of these DLBCL subsets to PD-1 blockade. CD37 (TSPAN26) is a member of the tetraspanin superfamily, widely expressed on normal and malignant mature B-cells and downregulated in plasma cells. It has been documented that CD37 plays important roles in T-cell-B-cell interactions, B-cell humoral response triggered by B-cell receptor cross-linking, and a balance between immune responses and tolerance. Interestingly, in a large cohort of DLBCL patients, we found that loss of CD37 expression in DLBCL predicts strikingly decreased overall and progression-free survival rates, and that PDCD1 gene expression was upregulated in CD37-negative activated B-cell-like (ABC) DLBCL by gene expression profiling, whereas the costimulatory molecule ICOSLG was upregulated in GCB-like cell-like (GCB) DLBCL. Using quantitative real-time multiplex technology, we further measured PD-1 and PD-1 ligand expression at the protein level on lymphoma cells and immune cells in the tumor microenvironment, and found that PD-1 protein levels were increased on both cytotoxic and helper T-cells infiltrating in CD37-negative DLBCL either of ABC or GCB subtype. These novel discoveries suggest that CD37 is important for sustained an-titumor adaptive immunity, that immune dysregulation plays an important role for poor clinical outcomes in DLBCL, and that CD37-negative DLBCL may be sensitive to PD-1 blockade. In summary, loss of CD37 tetraspanin was found to correlate with PD-1 overexpression in DLBCL clinical samples, and CD37 may serve as a novel biomarker for anti-PD-1 immunotherapy clinical trials in DLBCL.

Immunoprofiling circulating blood as a means to early detection of solid tumors. Amit Kumar, 1 Dimitry Gabrilovich, 2 Frank J. Rauscher, 2 George Dominguez, 2 Cyrus Shoelvar, 1 John Roop, 1 Anthony Campisi, 1 Alexander Polo 2. 1Itus Corporation, Los Angeles, CA; 2Wistar Institute, Philadelphia, PA.

Our goal was to evaluate whether profiling Myeloid Derived Suppressor Cells (MDSCs) in circulation correlated with the existence and stage of multiple biopsy-verified solid tumor types and to evaluate whether such analyses could enable early detection. The tumor micro-environment (TME) is replete with numerous immune cells, and the type and concentration of such cells can provide prognostic information. The link between high concentrations of MDSCs and poor prognosis is most likely due to the immuno-suppressive effects of such cells. Some fraction of these cells spill into the blood stream. We utilized flow cytometry to phenotypically quantify subsets of MDSCs and other leukocytes in the circulation of biopsy-verified cancer patients, as well as in age and sex matched healthy donors. Our results indicate a marked increase in MDSC levels in the circulation of tumor patients relative to healthy donors. We have analyzed patients presenting over a dozen solid tumor types (lung, breast, ovarian, colon, melanoma, liver, thyroid, pancreatic, uterine, osteosarcoma, appendiceal, jeio-myosarcoma, liposarcoma, and vulvar) and found notable differences in the immune-profiles of circulating blood in these patients. It appears that the immune response, as measured by our flow cytometry technique, is general for most tumor types. We will present correlations and inter-relations between different cell types and evidence of tumors. While our studies to date have been performed in an unblinded manner, we will present performance data (specificity and sensitivity) for our approach.

Evaluation of α-1-acid glycoprotein as a predictive biomarker for severe neutropenia induced by docetaxel in esophageal carcino ma patients. Yusuke Sasaki, 1 Ken Kato, 1 Hidekazu Hirano, 1 Hirokazu Shoji, 1 Yoshihata Honma, 1 Satoru Iwasa, 1 Atsuo Takashima, 1 Tetsuya Hanaguchi, 2 Kengo Nagashima, 3 Narikazu Boku 2. National Cancer Center Hospital, Hakodate Central General Hospital, Tokyo, Japan; 3National Cancer Center Hospital, Tokyo, Japan; 4Chiba University Graduate School of Medicine, Chiba, Japan.

Background: Serum α-1-acid glycoprotein (AAG) level is an independent predictor of response and a prognostic factor of survival in patients with non-small cell lung cancer treated with docetaxel chemotherapy. However, whether AAG is associated with the outcomes of esophageal cancer patients treated with docetaxel remains unclear. Methods: Between August 2009 and April 2014, pre-treatment serum samples were obtained from patients with clinical stage II/III esophageal cancer. Individuals were subsequently treated with neoadjuvant DCF or CF followed by surgery. DCF consisted of docetaxel and cisplatin on day 1, and continuous infusion of 5-fluorouracil on days 1-5, with this regimen repeated every three weeks for up to three cycles. CF consisted of cisplatin and 5-fluorouracil. Patients were divided into groups based on the median value of baseline AAG levels. Response to chemotherapy, survival, and the most severe grade of neutropenia were compared between the high and low AAG groups at each regimen. Results: A total of 129 patients were enrolled (44 received neo DCF and 85 neo CF). The median serum AAG level was 95 g/L (range 57-225 g/L) and 95 g/L (range 46-197 g/L) in the patients with neo DCF and CF, respectively. For all patients, overall survival (OS) was significantly shorter in the high AAG group (AAG ≥ 95 g/L) than the low AAG group (AAG < 95 g/L) (HR 2.18, p=0.03). Both in the neo DCF and neo CF patients, there was a similar trend in OS between individuals with high and low AAG levels. Multivariate analysis demonstrated that AAG levels (OR of AAG ≥ 95 g/L in both neo DCF and neo CF) was an independent prognostic factor. However, the relationship between response to chemotherapy and AAG level were similar both in DCF and CF patients. In addition, grade 4 neutropenia was observed in 50% of patients with low AAG level and 23% with high AAG (p = 0.06) in DCF patients. A correlation analysis revealed a statistically significant correlation between serum AAG level and nadir absolute neutrophil count (r = 0.53) and performance status in patients with high AAG. However, in CF patients, there was no difference in the frequency of severe neutropenia between individuals with high and low AAG level, and there was no correlation between AAG and nadir neutrophil count. Conclusions: Serum AAG level may be a prognostic marker for survival in stage II/III esophageal cancer patients treated with neoadjuvant chemotherapy. In addition, low serum AAG level is a potential predictive biomarker of docetaxel-induced severe neutropenia.

Quantification of estrogen (ER) and progesterin receptor (PR) as well as HER2/neu proteins and gene expression improves discrimination of clinical behavior by triple positive breast carcinomas. Zohair R. Hameed, Michael W. Daniels, D. Alan Kerr, James L. Wittliff. University of Louisville, Louisville, KY.

IHC analyses of ER, PR and HER2 proteins are used as clinical biomarkers for breast cancer management. However, IHC provides semi-quantitative results sometimes complicated by variation in methods and interpretation. Our goal was to ascertain clinical use of these three analytes to predict breast cancer recurrence when analyses for gene expression and protein products were quantified. Procedures: We examined ER and PR protein in 1059 de-identified carci-noma biopsies previously quantified by radio-ligand binding and/or enzyme immunoassay (EIA) using FDA approved reagents and HER2 oncoprotein determined by EIA to assess relationships between biomarker profiles and disease-free (DFS) and overall survival (OS) for 123 patients. Our comprehensive, IRB-approved Database also contained de-identified Microarray results obtained.
Gallbladder carcinomas involve multiple cascades of oncogenes like EGFR and Kras. Activation of Kras downstream pathways predict the sensitivity to anti EGFR treatment. For 2012, the first time it was established that GBC and cholangiocarcinomas have their unique somatic genomic landscape and thus be studied independently. This study evaluates whether EGFR is a prognostic factor in GBC and identifies the frequency of codon 12 and codon 13 Kras mutations, along with its association with subject survival. Seventy two GBC curative resections from North India were immunostained with anti EGFR monoclonal antibody and assessed for codon 12 and 13 KRAS mutation by real time PCR. Strong EGFR expression was observed in 25.4 percent cases. About 28/72; G12C, 17/72; G12R, 10/72; G12D and 18/72; G13D cases harboured point mutations in Kras gene, which was significantly higher as compared to control tissues. Multivariate analysis revealed that EGFR expression (p = 0.01; HR = 3.14; 95percent CI = 1.28-7.71) and codon-13 mutation (p = 0.001; HR = 38.34; 95percent CI = 4.90-300.01) had a significant impact on survival and were independent prognostic factors in GBC. Our study suggests that EGFR expression and codon 13 mutations are independent prognostic factors in the selected GBC cohort and showed a high frequency of Kras codon 12 mutations as well.

#670 Integrative genomic analysis of alterations driving anti-androgen treatment resistance in vitro. Dong Shen,1 Brendan Hodkinson,2 Deborah Ricci,1 Karin Verstraeten,2 Michael Schaffer,1 Michael Gormley,1 Shibu Thomas1,1 Janssen Research & Development, Spring house, PA; 2Janssen R&D BE, Beerse, Belgium.

Prostate cancer is one of the most frequently diagnosed cancers in the world. It is the second most common type of cancer and the fifth leading cause of cancer death in American men. Enzalutamide (MDV) and apalutamide (ARN-509) are recently the acquired F876L mutation of the androgen receptor (AR) was treated with prednisone in metastatic castration-resistant prostate cancer. Al- acetate (ABI) is an androgen biosynthesis inhibitor which is used in combina- It is the second most common type of cancer and the fifth leading cause of cancer death in American men. Enzalutamide (MDV) and apalutamide (ARN-509) are prostate cancer cells resistant to enzalut- amide and apalutamide. To systematically identify and understand the mecha- canisms of resistance, we evaluated 22RV1 and LNCaP prostate cancer cells which were resistant to the treatment of enzalutamide (MDV), apalutamide (ARN-509), and abiraterone acetate (ABI). We isolated the DNA and RNA from both resistant cells and their parent cell line. We did RNAseq for gene expression analysis, whole exome sequencing to identify the DNA alterations, and array CGH for copy number change analysis. We confirmed the acquired F876L mutation of the AR identified in LNCaP cells resistant to apalutamide. We identified the novel G644R and R630Q mutations of AR, R213* mutation of TP53, N372H mutation of BRCA1, and E626I mutation of ATM in LNCaP cells which were resistant to the treatment of enzalutamide and apalutamide. For copy number alterations, we identified acquired copy number gain on chromosome 3, 8, and 18 on 22RV1 resistant cells, and acquired copy number loss on LNCaP resistant cells. Besides acquired DNA alterations, we identified 16 up-regulated and 24 down-regulated genes which are common in both 22RV1 and LNCaP cells resistant to enzalutamide and apalutamide. Through pathway enrichment analysis, we identified the Wnt/B-catenin, cAMP signaling, and TREM1 signaling pathways were up-regulated in 22RV1 and LNCaP cells resistant to enzalut- amide and apalutamide. Detection of acquired genetic alterations in cells resis- tant to anti-androgen treatment provides us with important insights into the mechanisms of their resistance. Further testing of clinical samples collected at disease progression after treatment with these agents is required to validate these findings.

#671 Prognostic importance of EGFR expression and Kras gene mutations in gallbladder carcinoma. Anjali Singh,1 Abul Kalam Najmi,1 Pramod Mishra,2 Majid Talikoti,1 Jamia Hamdard, New Delhi, India; 2MAMC, Delhi University, New Delhi, India.

Multivariate analysis of prognostic factors

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#672 Baseline serum albumin as a prognostic factor in patients with stage I and II colon adenocarcinoma. Luis F. Onate-Ocana, Instituto Nacional de Cancerología, Mexico City, Mexico.

Background: Patients with stage I or II colon carcinoma (CC) have a significa- nt risk of recurrence after adequately performed curative resection. Adjuvant chemotherapy has not improved recurrence or survival in such patients. In this study, a prognostic model in patients with stage I or II CC is presented. Methods: Consecutive cases with CC treated at a cancer center in Mexico City from January 2008 to December 2014, with diagnosis of adenocarcinoma by colonoscopy and biopsy were included in this cohort. Patients were treated according to standard guidelines. Follow-up continued until June 2016; the Kaplan-Meier method and the Cox model were used to analyze the association of prognostic factors and overall survival (OS). Results: 1,259 cases of CC were treated; only 496 patients have stage I and II CC and were included in the database: 234 women (47.2%) and 266 males (52.8%) and their mean age was 59.9 years (SD 14.9); 96, 339, 31 and 30 cases were TNM stages I, IIa, IIb, and IIIc, respectively. Multivariate analysis of prognostic factors is depicted below. Predicted survival functions using this model, defined 4 risk groups that showed better prognostic value than simple TNM stages I-IIc. Conclusion: A new, simple, cheap and widely available prognostic factor in patients with CC: Albumin and other clinical factors add significant prognostic information to TNM classification. Feasibly, it can be used to develop multivariate prognostic models with clinical impact defining the use of adjuvant chemotherapy. The use of histopathological and immunohistochemical markers could improve the prognostic information of this model.

#673 Exploration of tissue morphologies in breast cancer samples using unsupervised machine learning. Riku Turkki,1 Dmitrii Bychkov,1 Nina Linder,2 Jorma Isola,3 Heikki Joensuu,4 Johan Lundin,5 1Institute for Molecular Medicine Finland, Helsinki, Finland; 2Translational Cancer Biology Research Program, Helsinki, Finland.

We applied a machine learning approach for exploration of tissue morpholog- y in hematoxylin and eosin (H&E) stained breast cancer tissue microarray.
(TMA) samples. We then investigated whether the morphological categories produced were associated with clinically relevant molecular biomarkers and 10-year overall survival. The data set comprised digitized (0.22 μm/pixel) and H&E stained TMA spots from tumor samples of 490 women who were diagnosed with primary breast cancer within a Finnish breast cancer database (Fin-Proc) collected in 1973 and 1992. In order to quantitatively describe the morphologies of the TMA spots, we divided the tissue images into rectangular sub-images (224x224 pixels), and extracted features with a pre-trained convolutional neural network. We then clustered the sub-images (n = 147,266) with a non-linear data embedding algorithm that creates a two-dimensional mapping of the tissue morphologies. Lastly, we defined a quantitative profile for each tumor, describing the morphologies within the tissue spot image by dividing the two-dimensional map of morphologies into 128 separate clusters with k-nearest neighbor clustering. Visual inspection of the two-dimensional embedding of tissue spot images verified that the morphologies clustered coherently, i.e. similar looking sub-images formed distinct clusters in the map. Interestingly, some morphological patterns were strongly associated with tumor estrogen receptor content, progestosterone receptor content, human epidermal growth factor receptor 2 status, and the proliferation marker Ki-67 status (p <0.0001 for each comparison). In exploratory analyses we identified one morphological category that was associated with a favorable 10-year overall survival with a risk ratio of 0.68 (CI95% 0.53-0.89, p = 0.002, power = 0.87). Our work demonstrates that unsupervised machine learning can be applied to explore and better understand the role of morphological patterns in breast cancer. Method: We quantitatively assess the morphology of cancer tissue may complement molecular biomarkers and potentially reveal novel prognostic and predictive factors.

**#674 HPV16 was the preponderant type of HPV infection in cutaneous squamous cell carcinoma.**

Gang Shi,1 Anne C. Fischer,2 Jonathan M. Zelnik,3 Patti E. Gravitt,4 Max Fischer,4 Inbal B. Sander,1 Jonathan D. Cuda,1 Janis M. Taube,1 Mohammed Lilo,1 W P Andrew Lee,1 Anthony P. Tufaro1.

1Johns Hopkins University School of Medicine, Baltimore, MD; 2Florida Atlantic University, Boca Raton, FL; 3Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 4University of Maryland School of Medicine, Baltimore, MD.

Purpose: Human papillomavirus (HPV) has been well established as a causative factor in mucosal oropharyngeal cancer and anogenital surface malignancies. However, its etiological role in cutaneous squamous cell carcinoma (cSCC) is still debated. The aim of this study is to identify the presence of HPV types in cSCC and identify their associations with cSCC. Methods: 77 cSCC blocks from 54 patients underwent DNA isolation and a broad spectrum of HPV PCR tests to detect the presence of HPV genotypes by the use of a universal primer pair GP5+/GP6+. The resultant PCR products were cloned to T Vectors and subsequently underwent DNA sequencing and Nucleotide BLAST search to identify the HPV types in cSCC. To ensure the accuracy of the HPV PCR test, 16 blocks from 10 head and neck SCC (oropharyngeal) patients were also included in the HPV presence and type analyses. Cutaneous SCC patients' demographics, pathology and clinical parameters were compared and analyzed statistically in conjunction with the HPV test results. Results: HPV DNA was found in 37 of 77 (48.1%) blocks representing 32 of 54 (59.3%) cSCC patients. HPV16 was the predominant type of infecting HPV in cSCC and accounted for 35 of 37 (91.5%) HPV positive and 31 of 37 (84.2%) HPV positive patients and blocks. Other HPV genotypes found included HPV18, 57, 10, 2, accounting for 4 of 32 (12.5%), 2 of 32 (6.25%), 2 of 32 (6.25%), 2 of 32 (6.25%) of the HPV positive patients and 4 of 37 (10.81%), 2 of 37 (5.41%), 2 of 37 (5.41%) of the HPV positive blocks, respectively. HPV infection was significantly associated with the cSCC tumor size in this cohort of patients. Tumors with a size of ≤20mm were more frequently associated with HPV. Moreover, the average tumor size of the HPV positive group was also significantly smaller than that of the HPV negative group. Conclusions: HPV infection is very commonly found in cSCC patients and HPV16 is the major type of HPV involved in cSCC oncogenesis, accounting for >80% of HPV infections. HPV16 is a contributory factor in such appears to be contributing to a smaller size of cSCC, which may improve the prognostic classification of patients to provide a new insight into tumorigenesis.

**#675 The interaction between hedgehog and EMT pathway in non-small cell lung cancer and its prognostic implication.**

Ho Jung An,1 Jae-Tae Jung,1 Young Il Soa,2 Chang Suk Kang1.1 St. Vincent’s Hospital, The Catholic University of Korea, Suwon, Republic of Korea; 2Yeouido St. Mary’s Hospital, The Catholic University of Korea, Seoul, Republic of Korea.

Introduction: The Hedgehog (Hh) signaling plays essential role in transcription regulation and embryonic development. In many cancers including lung cancer, it is related to carcinogenesis and poor prognosis. This signaling also mediates epithelial to mesenchymal transition (EMT) by paracrine mechanism in various diseases. We aim to study the interaction between Hedgehog and EMT pathway and its clinical implication in non-small cell lung cancer (NSCLC). Method: Total 271 cases of NSCLC who received surgical resection in Yeouido St. Mary’s Hospital between 1997 and 2011 were included. For immunohistochemical analysis of Hh and EMT related proteins, 4um sections were cut and Hh and EMT pathway related proteins were immunostained (Shh, Patched (Ptc), Smoothened (Smoo), Glialoma-associated oncogene (Gli-1) and Suppressor of fused (Sufu), and EMT associated molecules including E-cadherin and vimentin were analyzed. Staining intensity (IS) was estimated as follows (0 no staining), + (1 weak), + (2 distinct), 3 (strong). Intensity score (IS) ≥1 was defined as positive. Medical record was retrospectively reviewed. The study was approved by the institutional review board the hospital. Results: The median age was 63 (18-84) years, and 65.3% were male. Squamous cell carcinoma was diagnosed in 113 (41.7%), adenocarcinoma in 153 (56.5%), and mixed type in 153 (56.5%). Stage 1 and 2 were 46.9% and 30.6%, respectively, and 8 cases had distant metastasis. The Hh proteins showed positive correlation each other. For EMT, the expressions of Hh protein was significantly correlated with tumoral and stromal positivity of vimentin, and inversely with positivity of E-cadherin (P = 0.002 and P = 0.028, respectively). In survival analysis, expressions of Shh and Gli1 were independent predictor of disease free survival and cancer-specific survival (P = 0.007 and P = 0.025, respectively). Conclusion: Expression of Hh protein is associated with epithelial-mesenchymal transition and associated with poor prognosis in NSCLC.

**#676 Nuclear expression of insulin receptor (InsR) adds prognostic information in primary breast cancer patients with high BMI.**

Sofie Björn,1 Anja H. Rosendahl,1 Maria Simonsson,2 Andrea Markkula, Karin Jirström, Signe Borgquist,1 Carsten Rose,1 Christian Ingvar,1 Helena Jernström,1 Lund University, Lund, Sweden.

Aim and Introduction: The aim of this study was to elucidate the prognostic importance of nuclear expression of the insulin receptor (nucInsR) in primary breast cancer. The nucInsR is important for the metabolism, for the insulin-like growth factor (IGF) signaling network and is implicated in breast cancer. Insulin and InsR are interlinked with obesity and body mass index (BMI), which are also associated with breast cancer. The nucInsR is present both in the membrane and cytoplasm and can additionally translocate to the nucleus. The role of nucInsR is not well understood and the prognostic importance is unclear. Methods: The tumor-specific expression of nucInsR was evaluated by immunohistochemistry on invasive breast cancer tissue microarrays from 984 primary breast cancer patients included in a population-based cohort in Sweden. Patients were followed for up to 11 years, the median follow-up for patients still at risk was five years. nucInsR in relation to prognosis was analyzed using Cox regression, adjusted for age, invasive tumor size, axillary lymph node involvement, histological grade, estrogen receptor (ER) status, BMI, tumor storage time, and treatment. Results: Status of nucInsR was available for 900 patients (91.5%). nucInsRstatus was present in 214 patients (23.8%) and was associated with lower age (P = 0.004), and lower waist-to-hip ratio (P = 0.006), but not with BMI, ER or PR status. During follow-up, 107 patients had a first breast cancer event. The risk of first breast cancer event was significantly lower in patients with nucInsR<sup>+</sup> status compared to nucInsR<sup>-</sup> patients (HR = 0.58, 95% CI 0.35-0.96) and not significantly different in patients with BMI >30 kg/m<sup>2</sup>. Conclusions: These results indicate that nucInsR status may add prognostic information in primary breast cancer depending on BMI.

**#677 Progralin/PGP88 tumor tissue expression further stratifies survival outcomes of patients classified by their Nottingham Prognostic Index.**

Ginette Serrero,1 Douglas Hawkins,2 Olga Ioffe,3 Pablo Bejarano,4 Binbin Yue1.1 AEG Pharmaceuticals, Inc., Columbia, MD; 2University of Minnesota, Minneapolis, MN; 3University of Maryland Greenbaum Comprehensive Cancer Center, Baltimore, MD; 4Cleveland Clinic Florida, Weston, FL.

Background: The Nottingham Prognostic Index (NPI) combines nodal status, tumor size and histological grade and is used to provide predictive value information on survival for patients with primary breast cancer. Attempts to improve the performance of NPI have been carried out by inclusion of other factors, such as biomarker expression as well as addition of other structural features such as vascular invasion. In the present study, we investigated whether expression of the survival factor PGP88 (progralin), known to be overexpressed in breast cancer, would improve NPI’s predictive value. GPP88/Progralin is an 88 kDa autocrine growth and survival factor that is overexpressed in estrogen receptor

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positive (ER+), invasive ductal carcinoma (IDC) in association with resistance to anti-estrogens and aromatase inhibitor while the cells remain ER+. Previous training and validation pathological studies totaling 600 cases of ER+ IDC had demonstrated that GP88/Progranulin tissue expression was an independent prognostic marker of recurrence and mortality for early stage ER+ IDC. Methods: We processed 11 normal and 8 tumorous lung resection specimen. For all the kinomes extracted, we developed a corrective approach. We applied resection specimen. For all the kinomes extracted, we developed a corrective approach. The new biomarkers are essential for improving the survival and prognosis of hepatocellular carcinoma (HCC) patients. Alpha-fetoprotein (AFP) is the most widely used biomarker. But the low sensitivity and specificity limits its clinical application. Recent study validated the diagnostic capability of osteopontin (OPN) and dickkopf-1 (DKK1) and assessed the combination of AFP, DKK1, and OPN as a panel for the diagnosis of HCC. Based on these previous studies, we hypothesized that combination of OPN and DKK1 can be used as a marker for prognosis of patient to HCC after hepatectomy. From January 2006 to December 2008, patients undergoing hepatectomy for hepatocellular carcinoma were screened for samples that had been stored in the Bank of tumor. To target the selected patient, it was confirmed that the remaining tissue specimen is stored after diagnosis. Serum of the patients was used to investigate the OPN and DKK1 by ELISA. In the paraffin block were prepared unstained slide and OPN and DKK1 level checked by IHC. It examined the correlation between prognosis and biomarkers through statistical analysis. AFP, OPN (serum level) and DKK1 (serum level) were an independent prognostic factor for overall survival (OS) in HCC after hepatectomy (n = 60, P = 0.0204, 0.0167 and 0.0455 respectively). New biomarkers combinations based on the AFP existing biomarker were showing a falling curve of the overall survival (OS) and disease-free survival (DFS) in Kaplan Meier curve. In conclusion, combination of OPN, DKK1 and AFP as a biomarker could support the correct diagnosis for HCC after hepatectomy.

### #680 Prognostic impact of OPN and DKK1 in patient of hepatocellular carcinoma after hepatectomy, Yun Sung Seo, Hye Rim Byeon, In Hae Park, Seung Duk Lee. National Cancer Center, Goyang-si, Gyeonggi-do, Republic of Korea.

The new biomarkers are essential for improving the survival and prognosis of hepatocellular carcinoma (HCC) patients. Alpha-fetoprotein (AFP) is the most widely used biomarker. But the low sensitivity and specificity limits its clinical application. Recent study validated the diagnostic capability of osteopontin (OPN) and dickkopf-1 (DKK1) and assessed the combination of AFP, DKK1, and OPN as a panel for the diagnosis of HCC. Based on these previous studies, we hypothesized that combination of OPN and DKK1 can be used as a marker for prognosis of patient to HCC after hepatectomy. From January 2006 to December 2008, patients undergoing hepatectomy for hepatocellular carcinoma were screened for samples that had been stored in the Bank of tumor. To target the selected patient, it was confirmed that the remaining tissue specimen is stored after diagnosis. Serum of the patients was used to investigate the OPN and DKK1 by ELISA. In the paraffin block were prepared unstained slide and OPN and DKK1 level checked by IHC. It examined the correlation between prognosis and biomarkers through statistical analysis. AFP, OPN (serum level) and DKK1 (serum level) were an independent prognostic factor for overall survival (OS) in HCC after hepatectomy (n = 60, P = 0.0204, 0.0167 and 0.0455 respectively). New biomarkers combinations based on the AFP existing biomarker were showing a falling curve of the overall survival (OS) and disease-free survival (DFS) in Kaplan Meier curve. In conclusion, combination of OPN, DKK1 and AFP as a biomarker could support the correct diagnosis for HCC after hepatectomy.

### #681 Methylylomics profiling reveals epigenetically silenced HSYST2 sensitize ILS signaling and confers poor prognosis of ovarian cancer, Rui-Lan Huang,2 Hisang-Ju Chan,2 Lin-Yu Chen,2 Yaw-Wen Hsu,2 Tai-Kuang Chao,2 Po-Hsuan Su,2 Yu-Chun Weng,1 Chio-Chung Yuan,1 Hung-Cheng Lai1.1 Taipei Medical University-Shuang Ho Hospital, New Taipei, Taiwan;2 Taiwan International Graduate Program Molecular and Cell Biology, Ph.D. Program, Academia Sinica, New Taipei, Taiwan;3 Graduate Institute of Life Sciences, National Defense Medical Center, Taipei, Taiwan;4 Tri-Services General Hospital, National Defense Medical Center, Taipei, Taiwan.

The attempts of target therapy in ovarian cancer therapy mostly fail. There is need of biomarkers for better patient stratification in clinical trials. Epigenetics is a driving force for cancer development and may serve as a molecular marker of the disease. The present study was to analyze the clinical relevance of methylation phenotypes and figure out the mechanism in ovarian cancer. We integrated the analysis using public dataset of epigenomics and transcriptomics, and our hospital-based methylylomics database and tissue arrays of patients diagnosed with epithelial ovarian cancers (EOC). Bioinformatics results were verified and validated in cell lines and clinical samples. From 25 genes, we identified even genes, ADRA1A, CD248, HSYST2, NFEH, IGSF21, POU4F2, and TWIST1, of which hypermethylation was significantly correlated to the poor overall survival (OS). The association of NFEH and HSYST2 hypermethylation and poor 5-year OS was validated in our independent dataset and TCGA database. The NFEH/HSYST2 hypermethylated signature was an independent risk factor for 5-year OS (adjusted Hazard ratio = 2.93, 95% confidence interval 1.25 to 6.85, P = 0.013). In vitro experiment re-expressing HSYST2 repressed migration and invasion, and suppressed IL-6 signaling pathways. The combination of hypermethylation of HSYST2 and high expression of IL-6 confers the worse outcome. Our data suggested that methylation of NFEH/HSYST2 constituted a poor prognostic signature of ovarian cancer. The siluation status of proteoglycan modified by HSYST2 implicates an intrinsic sensitivity of ovarian cancer cells to IL-6, which shed a new light on the application of HS3ST2 as a biomarker for personalized medicine of targeting IL-6.

### #682 Novel Mesenchyme Homeobox2-target transcription axes are involved in cancer-drug resistance, overall survival and therapy prognosis in lung cancer patients: a functional epigenome wide study, Federico Avila Moreno,1 Leodl Armas Lopez,2 Patricia Piña-Sánchez,2 Oscar Arrieta,2 Enrique Gúzman de Alba,3 April Marcela Herrera-Solórito,1 Blanca Ortiz-Quintero,1 Patricia Santillán-Doherty,2 David C Christiani,1 Joaquin Zúñiga,1 Universidad Nacional Autónoma de México (UNAM), Mexico City, Mexico;2 Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico;3 Instituto Nacional de Cancerología (INCAN), Mexico City, Mexico;4 Instituto Nacional de Enfermedades Respiratorias (INER) Ismael Cosío Villegas., Mexico City, Mexico;5 Harvard School of Public Health, Boston, MA.

In recent years, the development of targeted therapy for cancer has been a major focus of research and clinical practice. One of the major challenges in cancer treatment is the development of drug resistance, which can lead to treatment failure and poor patient outcomes. In this study, we aimed to explore the role of a novel transcription axis, consisting of the transcription factor Mesenchyme Homeobox2 (Mhx2) and its target transcription factor, in the development of drug resistance in lung cancer. We used a functional epigenome-wide approach to identify changes in DNA methylation, histone modifications, and non-coding RNA expression that were associated with drug resistance in lung cancer cell lines. Our results showed that changes in the expression of Mhx2 and its target genes were strongly associated with drug resistance, providing a potential mechanism for the development of drug resistance in lung cancer. These findings suggest that targeting the Mhx2-Moreno and Intoc molecule, which could serve as a potential therapeutic target to overcome drug resistance in lung cancer.
CLINICAL RESEARCH: Checkpoint Inhibitor and Prognostic Biomarkers

#683 Quantification of rare PD-L1 expressing leukocytes and CTCs in peripheral blood of cancer patients. Adam Jendrisak, Jiyoun Byun, Mahipal Suraneni, David Lu, Rachel Krupa, Sarah Orr, Ryon Graf, Yipeng Wang, Mark Landers, Ryan Dittamore. Epic Sciences, San Diego, CA.

Background: Expression of PD-L1 on tumor tissue is associated with improved response to PD-L1 checkpoint inhibitors. Additionally, expression of PD-L1 on infiltrating T cells is associated with immune exhaustion. Currently, PD-L1 analysis is performed on surgically removed or biopsied samples, usually taken long before clinical decision points. Animals are monitored for survival and tumor growth. The immune biomarkers in CTCs as well as leucocytes will allow real time assessment of liquid biopsy based platform that is capable of simultaneously measuring immune biomarkers in CTCs as well as leukocytes will allow real time assessment of response to immune checkpoints inhibitors.

Lung cancer remains the most progressive malignant disease strongly resistant to oncological therapies including platinum-derived cancer drugs and Epidermal Growth Factor Receptor (EGFR)-Tyrosine Kinases Inhibitors (TKIs). Homeobox-related gene (HOX) transcription factors as Mesenchyme HOX-2 (MEOX2) have previously been associated with cancer-drug resistance, progression and metastasis in lung cancer patients. However, transcriptional mechanisms epigenetically modulated have not totally been elucidated in lung cancer therapy resistance. Here an epigenomic strategy was conducted to identify a novel MEOX2 gene sequence-promoter targets profile, associated or involved in therapy resistance mechanisms in human lung cancer. For that chromatin from human non-small cell lung carcinomas (NSCLC), MEOX2 versus RNA Pol II immunoprecipitation and hybridization assays using genomic tiling-arrays and bioinformatics analyses were performed, which set of quantitative and functional assays with clinical-outcome validation predictions analyses, were assessed. Stringent bioinformatics results identified a common profile of 13 gene promoter sequences, which included in others Sonic Hedgehog-GLI-1, ALDH1A2, MMP24, RUFY3 and ZEB1 (FDR<0.1) in NSCLC patients with different clinical outcome data. GLI-1 promoter sequences upstream -2,192 to -109 quantitatively validated, were occupied by MEOX2 and RNA Pol II in both NSCLC cell lines and NSCLC patients, as well as significantly enriched with the histone activation marks H3K27Ac and H3K4me3, in addition, confirmed by the ENCODE database bioinformatics analyses. Furthermore, a set of genetic silencing functional assays validated a novel transcriptional MEOX2-GLI1 axis in a cisplatinum dose-dependent manner, involved in cellular migration, invasion, and proliferation capacity. Finally, MEOX2-GLI1 axis expression was clinically validated and analyzed using Kaplan-Maier survival analyses on an independent cohort of 90 NSCLC patients, identifying a significant MEOX2-dependent GI1-1 overexpression statistically associated with clinical poorer overall survival prognosis and treatment response into cisplatinum-based first-line therapy and/or second-line EGFR-TKIs target therapy protocols. In conclusion, a chromatin-immunoprecipitation and epigenome-wide analysis based on the MEOX2-transcriptional observation study, identified novel MEOX2-transcriptional gene promoter axes involved in embryonic development, oxidoreductase activity, matrix metalloprotease, cellular polarity, epithelial-mesenchyme phenotype, cancer cell migration and invasion pathways. Promoting cisplatinum-based resistance mechanisms and EGFR-TKIs based therapy response prognosis in human lung cancer.

#684 Integrative proteomics and transcriptomics to define the cell surface landscape of neuroblastoma. Amber K. Weiner,1 Simone Sidoli,1 Tina Glisovic-Aplenc,2 John M. Maris,2 Benjamin A. Garcia,1 Sharon J. Diskin2. 1Univ. of Pennsylvania School of Medicine, Philadelphia, PA; 2Children's Hospital of Philadelphia, Philadelphia, PA.

BACKGROUND. Neuroblastoma (NB) is an embryonal tumor of the sympathetic nervous system that accounts for 12% of childhood cancer deaths. Despite intense multimodal therapy, survival probability for high-risk NB patients remains below 50% and relapsed NB is essentially incurable. To date, the cell surface landscape of NB at diagnosis and relapse is currently unknown. An unbiased survey of these proteins and their isoforms will greatly facilitate the identification of candidate immunotherapeutic targets for preclinical validation. METHODS. To identify proteins on the cell surface of NB, we performed plasma membrane protein extraction utilizing an optimized sucrose density gradient methodology followed by nano-liquid chromatography coupled to mass spectrometry (nLC-MS/MS) in NB cell lines. Proteomic data is integrated with RNA-sequencing data to assess the correlation between these data types, determine differential expression between NB (n=2242) and normal tissues (n=7889), and investigate NB-specific isoforms. In parallel, we have developed a software tool to aid in proteomic data analysis and interpretation. RE-

CLINICAL RESEARCH: Childhood Cancer Clinical Translational Research

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SULTS. To date, we have applied our proteomic approach to nine NB cell lines stratified by MYCN amplification. This methodology has yielded on average 66% (range: 60-68%) membrane protein enrichment with high reproducibility between biological replicates (80%; range: 78-84%). This has allowed us to confirm known cell surface proteins that are currently being developed as immunotherapeutic targets in neuroblastoma (e.g., GPC2 in NB-M1). We are also using this approach to investigate the mass spectrometry based proteomics data, where we identified 4826 membrane proteins, with RNA-sequencing data to evaluate proteins expressed uniquely in neuroblastoma and those that correlate with MYCN amplification. We are further determining how RNA and protein levels correlate on the cell surface and will utilize these data to detect NB-specific isoforms. In parallel, we have initiated a clinical trial at Baycrest Hospital in Toronto, an efficacious tool for applying to clinical trials whose cellular responses match clinically relevant differences between patient subsets, such as MYCN status, survival hazard ratio, or histopathological assessment. To evaluate the method, we used onTARGET prediction to identify compounds and gene targets relevant for clinical outcomes in the NIH-TARGET (n=249) and R2-AMC (n=98) neuroblastoma cohorts. The algorithm confirmed existing targets and made interesting predictions. Of the top-ranking compounds associated with a survival outcome, agents targeting the mTOR/P38 axis are highly prevalent. The algorithm correctly predicts targeting of the MYC pathway, and predicts possible modulators of MYC signaling, such as TRRAP, AU-RKA and BMP2. In addition to nominating agents with a favorable outcome, the algorithm can also flag compounds that might worsen prognosis, further facilitating prioritization of targets for evaluation. In summary, onTARGET may guide target selection for neuroblastoma studies and can be adapted for other cancers as well. The many targets that were identified immediately suggest an opportunity for continued evaluation in cells and in vivo models. This work has been initiated in collaboration with researchers at Lund University.

One of the main bottlenecks of anticancer drug development is the assessment of the in vivo relevance of emerging therapies. Previously, drugs that suppress the in vivo progression of neuroblastoma have been hard to identify. To address this problem, we propose a new computational technique, onTARGET, that enables researchers to select, with good accuracy, compounds that are likely to show meaningful activity in cell lines derived from neuroblastomas and patient tumors and potentially other cancers.

The in vivo activity of compounds that are selected by onTARGET can be validated through a panel of in vivo models, including human xenografts, both 11q-deleted and MYCN-driven transgenic nude mice, and both induced and primary patient-derived xenografts (PDXs) and patient tumors are ongoing to define the cell surface landscape in both diagnostic and relapsed NB.

### #686 GPC2 is an oncogenic and immunotherapeutic target in high-risk neuroblastoma

**Katherine S. Tajsic,1,2 Dustin M. English,1,2 Marcin K. Chojnowski,1,2 Robert C. Luo,1,2 Jessica A. Hightower,1,2 Michael L. Dyer,1,2 Maria F. Gotuzzo,1,2 Jennifer A. Monahan,1,2 Bryant B. Backer,1,2 Casey J. Kugler,1,2 Andrew J. Court,1,2 Michael E. Rock,1,2 Genie A. Sutcliffe,3 and Jeffrey I. Benkovic,1,2**

High-risk neuroblastoma (HR-NB) is the most common extracranial solid tumor in children and is characterized by aggressive behavior, limited response to conventional therapies, and high mortality rates. One of the main bottlenecks in the drug development process is the assessment of the in vivo relevance of emerging therapies. Previously, drugs that suppress the in vivo progression of Neuroblastoma have been hard to identify. To address this problem, we propose a new computational technique, onTARGET, that enables researchers to select, with good accuracy, compounds that are likely to show meaningful activity in cell lines derived from neuroblastomas and patient tumors and potentially other cancers. The algorithm correctly predicts targeting of the MYC pathway, and predicts possible modulators of MYC signaling, such as TRRAP, AU-RKA and BMP2. In addition to nominating agents with a favorable outcome, the algorithm can also flag compounds that might worsen prognosis, further facilitating prioritization of targets for evaluation. In summary, onTARGET may guide target selection for neuroblastoma studies and can be adapted for other cancers as well. The many targets that were identified immediately suggest an opportunity for continued evaluation in cells and in vivo models. This work has been initiated in collaboration with researchers at Lund University.

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### #687 Targeting tumor-promoting neuroblastoma microenvironment: inhibition of tumor development and progression by targeting mPGES-1 expression by cancer associated fibroblasts

**Anna Kock,1 Per-Johan Jakobsson,1 Per Kogner1,2,3,4,5,6, and Christian Lindahl7**

The tumor microenvironment is a network of cells, cell-cell and cell-matrix interactions that provide cues for tumor proliferation, survival, and invasion. In neuroblastoma, the tumor microenvironment plays an essential role in disease progression and treatment resistance. Early targeting of mPGES-1 may inhibit CAF infiltration and tumor growth. In this study, we investigated the role of mPGES-1 in the tumor microenvironment of human neuroblastomas and developed a computational strategy that enables researchers to select, with good accuracy, compounds that are likely to show meaningful activity in cell lines derived from neuroblastomas and patient tumors and potentially other cancers.

One of the main bottlenecks of anticancer drug development is the assessment of the in vivo relevance of emerging therapies. Previously, drugs that suppress the in vivo progression of neuroblastoma have been hard to identify. To address this problem, we propose a new computational technique, onTARGET, that enables researchers to select, with good accuracy, compounds that are likely to show meaningful activity in cell lines derived from neuroblastomas and patient tumors and potentially other cancers. The algorithm correctly predicts targeting of the MYC pathway, and predicts possible modulators of MYC signaling, such as TRRAP, AU-RKA and BMP2. In addition to nominating agents with a favorable outcome, the algorithm can also flag compounds that might worsen prognosis, further facilitating prioritization of targets for evaluation. In summary, onTARGET may guide target selection for neuroblastoma studies and can be adapted for other cancers as well. The many targets that were identified immediately suggest an opportunity for continued evaluation in cells and in vivo models. This work has been initiated in collaboration with researchers at Lund University.

### #686 onTARGET: a new computational strategy to predict in vivo relevant targets against neuroblastoma

**Elin Almstedt, Ingrid Lönstedt, Cecilia Krona, Sven Nelander. Uppsala University, Uppsala, Sweden.**

One of the main bottlenecks of anticancer drug development is the assessment of the in vivo relevance of emerging therapies. Previously, drugs that suppress the in vivo progression of neuroblastoma have been hard to identify. To address this problem, we propose a new computational technique, onTARGET, that enables researchers to select, with good accuracy, compounds that are likely to show meaningful activity in cell lines derived from neuroblastomas and patient tumors and potentially other cancers. The algorithm correctly predicts targeting of the MYC pathway, and predicts possible modulators of MYC signaling, such as TRRAP, AU-RKA and BMP2. In addition to nominating agents with a favorable outcome, the algorithm can also flag compounds that might worsen prognosis, further facilitating prioritization of targets for evaluation. In summary, onTARGET may guide target selection for neuroblastoma studies and can be adapted for other cancers as well. The many targets that were identified immediately suggest an opportunity for continued evaluation in cells and in vivo models. This work has been initiated in collaboration with researchers at Lund University.

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BACKGROUND: The alpha particle emitting radiotherapeutic [211At]-MABG theoretically has superior radiobiological properties for anti-tumor efficacy compared to the currently used agent ([131I]MIBG). Specifically, [211At]-MIBG does not target microscopic deposits due to the long path length of the beta particles, while alpha particles have both a short path length and higher linear energy transfer that can create clustered DNA double strand breaks. Here we sought to define the anti-tumor activity of [211At]-MABG in preclinical models of human neuroblastoma (NB). METHODS: [211At]-MABG was synthesized using a bismuth target via the 209Bi(2n,2n)211At reaction and used for solid-phase radiosynthesis of [211At]-MABG (Ultraspace, Progenics, NY). We determined NET (SLG6A2) mRNA and protein expression in 35 NB cell lines and created isogenic pairs by overexpression of NET in 5 NB cell models. We performed uptake, cytotoxicity, and biodistribution studies using these models with [131I]MIBG and [211At]-MABG and extrapolated human dosimetry. Additionally, dose escalation studies with [211At]-MABG (n=10 at each dose, range 10-100 uCi) were performed to determine toxicity in SCID mice. Therapeutic in vivo trials were conducted with NET transfected xenografts and patient derived xenograft (PDX) models injected intravenously with escalating doses of [211At]-MABG and [131I]MIBG or vehicle (n=10 at each dose, range 10-100 uCi) and will be reported.


Introduction: The 5 year mortality rate for stage 4 metastatic neuroblastoma (NBL) is ~50%; however, the stage 4S subset of NBL is typically classified as low-risk with tumors exhibiting spontaneous regression. To date, the underlying genetic mechanisms influencing this distinct phenotype remain unknown. Method: To identify genetic determinants specific to the 4S phenotype, we performed genome-wide association study (GWAS) analyses. Blood DNA samples from 6,195 NBL patients and 11,384 non-NBL controls were genotyped using Illumina SNP arrays. European ancestry subjects were selected for inclusion in a discovery [stage 4S (n=148), stage 4 (n=996), non-NBL controls (n=9,923)] or replication cohort [stage 4S (n=76), stage 4 (n=608), non-NBL controls (n=1,461)]. Following imputation using 1KG Phase 3, we conducted case:case comparisons including 4S vs. 4 and stage 4S vs. 1, followed by comparisons of NBL cases in each group to matched non-NBL controls. Variants uniquely associated with 4S NBL were defined as those meeting the following criteria: (1) stage 4S vs. 4 meta P<5.0 x 10^-6 and P<0.005 in the discovery cohort (2) 4S vs. non-NBL control P<0.05 and (3) either not significant (P>0.05) or odds ratio in the opposite direction in 4 vs. non-NBL controls. Results: 4S-specific variants that met our criteria spanned multiple regions with several harboring genes important to neuronal function including 9q34 (SARDH/DBH; rs2502746), 1p32.2 (KCNAH; rs12112252), 1p34 (KCNS4; chr 1:41317634), 7q34 (CNOT3; rs79403134) and 2q24.1 (KCNJ3; rs78829625). At the 9q34 locus, rs2502746 showed further evidence as an expression quantitative trait locus (eQTL) for Dopamine Beta-Hydroxylase Antisense RNA1 (DHAS-AS1 P=1.9 x 10^-6 sun-exposed skin) and Sarcosine Dehydrogenase SARDH expression (P=2.5 x 10^-6 whole blood). Other 4S-specific loci observed include 3q27.3 (rs113207181), 8p23.1 (rs12548388), and 15q12 (rs62189512). Notably, none of the previously identified NBL susceptibility loci were found to be associated with 4S NBL in the 4S vs. non-NBL control comparison, suggesting a unique genetic basis for this enigmatic subset. Discussion: Although a unifying mechanism underlying 4S NBL has yet to be identified, our results suggest a potential role for ion-channel and neuronal function genes. Ongoing efforts include investigating the role these genes and associated pathways play in NBL, including an integrative genomic, epigenomic and transcriptome analysis of stage 4 and 4S NBL.

**#689** Immune checkpoint targeting to improve immunotherapy in neuroblastoma. Myrna L. Ortiz-Ruiz, H. Lee Moffitt Cancer Ctr. & Res. Inst., Tampa, FL.

Neuroblastoma (NB) is the most common extracranial solid tumor in children. Current treatments account for only the at most 40% of survival rate in high-risk NB patients. Hence, there is a need to develop new strategies that disrupt immunosuppressive signals in the tumor microenvironment in order to achieve robust anti-tumor immune responses. Co-inhibitory signals through receptors such as PD-1 can lead to the inactivation of T cells. On the other hand, co-stimulatory signals through receptors such as 41BB can enhance T cell activity against tumors. These receptors can serve as tools to develop strategies to optimize and enhance T cell anti-tumor functions. In this study, we used 9464D NB tumor mouse model to evaluate antibody treatment as a possible immunostrategy. We further examine the infiltration and function of T cells after targeting checkpoints in 9464D NB tumor bearing mice. Blockade of various receptors in mice bearing 9464D tumor showed that anti-41BB delayed tumor growth without MYCN amplification. MYCN is a regulator of ornithine decarboxylase (ODC), the rate-limiting enzyme of polyamine biosynthesis. Inhibition of this pathway in MYCN-amplified NB tumors has been shown to be a target for treatment. Alpha-difluoromethylornithine (DFMO) inhibits ODC and is currently being used in a Phase II clinical trial for NB. BET inhibitors JQ1 and OTX-115 have been shown to be effective against MYCN-amplified cancers; it is hypothesized that they downregulate MYCN as well as cancer stem cell (CSC) signaling. We propose that inhibiting MYCN with a BET inhibitor, coupled with inhibition of ODC with DFMO, will result in enhanced inhibition of NB growth and tumor-initiating properties. Methods: Single and combination drug treatments were conducted on BE(2)-C, SMS-KCN, CHLA90, and one patient-derived cell line. CellTiter-Glo Luminescent Cell Viability Assay was used to determine cell viability in 96-well plates. IC50 values were calculated with a four-parameter variable-slope dose response curve using GraphPad Prism v.5 software. Drug combination studies were conducted in MYCN amplified tumors BE(2)-C and SMS-KCN using ray designs to evaluate for synergism. Incucyte ZOOM Live-Cell Imaging system was used for kinetic monitoring of cytotoxicity and apoptosis in NB cells. Western blots measured protein levels of apoptosis markers (cleaved caspase 3 and cleaved PARP) and CSC markers (Nanog, Sox2, NF-kB, CXCXR4, Lin28B, and MYCN). Neurosphere assays were used to evaluate tumor initiation via sphere formation. Results: Cell viability of MYCN amplified NB showed an IC50 range of necheckpoint targeting. Also we want to test adoptive transfer of T cells in tumor bearing mice after BMT. Further we want to measure the infiltration of other immune cells, such as myeloid cells and Tregs, in the tumor environment and possible target immune suppressive populations to see the effects in tumor growth.
After T cell receptor (TCR) identification, retrovirally TCR transduced CD8+ generated by priming with A*02:01 overexpressed and required for proliferation in ES. We isolated HLA-A*02:01eral and in Ewing sarcoma (ES) in particular. ES is a highly malignant bone releasing IGFs from IGFBPs. The insulin/IGF-axis is involved in cancer in gen-

Shivaprasad,1 Baskar Subramanian,1 David Azorsa,2 Zhongyu Zhu,3 Dimiter

FGFR4 in rhabdomyosarcoma and other cancers.

2Institute of Pathology of the LMU, Munich, Germany; 3Technische Universität

A*02:01- healthy donors directed against PAPPA, generated by priming with A*02:01 TCR peptide restricted T cells from A*02:01+ healthy donors directed against PAPPA, generated by priming with A*02:01 TCR peptide-loaded dendritic cells. After T cell receptor (TCR) identification, retrovirally TCR transduced CD8+ T cells were assessed for their in vitro specificity and in vivo efficacy in human ES bearing Rag2-/- mice. Engraftment in mice and tumor infiltration of TCR transgenic T cells in the mice was evaluated. The TCR transgenic T cell clone PAPPA-2G6 demonstrated specific reactive towards HLA-A*02:01+ PAPPA+ ES cell lines. We furthermore detected circulating TCR transgenic T cells in the blood in Rag2-/- mice and in vivo engraftment of the in bone marrow. Tumor growth in mice with xenografted ES was significantly reduced after treatment with PAPPA-2G6 TCR transgenic T cells compared to control treatment, and tumors from treated mice revealed tumor infiltrating PAPPA-2G6 TCR transgenic T cells. In summary, we demonstrate that PAPPA is a promising target for TCR based immunotherapy of ES. We demonstrate that TCR transgenic T cells recognize this target, home to the tumor, and causes tumor regres-

Pappalysin-1 is a suitable target for T cell receptor transgenic T cells to kill Ewing sarcoma in vivo and in vitro. Uwe Thiel,1 Andreas Kirschner, Melanie Thie1, Thomas GP Grünewald,2 Rebeca Alba Rubio,2 Günther Rich-

ter,3 Thomas Kirchner, Dirk Busch,2 Poul Sorensen,4 Stefan Burdach1. #692 Pappalysin-1 is a suitable target for T cell receptor transgenic T cells to kill Ewing sarcoma in vivo and in vitro.

#694 BK-216: a novel, first-in-class, small molecule inhibitor of EWS-FLI1 in early
development, for the treatment of Ewing Sarcoma. Sarvana P. Selvanathan,1 Eric Moseley,1 Garrett T. Graham,1 Katti Jessen,2 Brian Lan-

nutt,2 Aykut Uren,1 Jeffrey A. Toretsky3,3*Georgeotown University, Lombardi Comp. Cancer Center, Washington, DC; 3Oncertal Therapeutics, Inc., San Di-

go, CA.

One of the most significant challenges in creating more potent, less toxic treatments for patients to identify new cancer therapeutic targets that distin-

TK-216 displays anti-tumor activity in a number of ES xenograft

models and characterization of YK-4-279, an enantiomer-specific inhibitor of EWS-FLI1, which has been demonstrated to induce apoptosis, inhibit EWS-FLI1 trans-

scription, block RNA helicase A co-immunoprecipitation with EWS-FLI1, and result in alternative splicing to mimic EWS-FLI1 knockout. Continuous eff-

orts in structure-guided medicinal chemistry has yielded TK-216, an analog of YK-4-279 inhibitor of EWS-FLI1, which is 3-4 fold more potent with excellent drug-like properties. TK-216 potently inhibits the proliferation of ES cells. In-

duces apoptosis in a dose-dependent manner as measured by caspase-3 activity in multiple ES cell lines with different translocation variants. The effects of TK-

216 on alternative splicing (AS) were further validated using genes including ARID1A, CLK1, CASP3, PPP1R3B and RUNX2. The splicing pattern was similar between TK-216 and YK-4-279. In addition to the in vitro activity of TK-216, we show that TK-216 displays anti-tumor activity in a number of ES xenograft models. In summary, TK-216, a novel, first-in-class therapeutic which directly inhibits EWS-FLI1, offers a promising approach for the treatment of Ewing Sarcoma and is currently in Phase 1 clinical trials in patients with relapsed or refractory Ewing Sarcoma (clinicaltrials.gov - NCT02657005).

#695 Targeted nanoparticle therapy for poor prognosis Ewing’s sarcoma. Hyung-Gyo Kang,1 Jon Nagy,2 Julia Samulak,3 Timothy Triche1. USChil-

dren’s Hospital Los Angeles, Los Angeles, CA; 2Nano Valient Pharmaceuticals, Bozeman, MT.

Targeted nanoparticles have shown the potential to deliver anticancer drugs to cancer cells selectively and thus to overcome unexpected cytotoxicity and limited efficacy of chemotheraphy caused by non-selective delivery to normal cells. The optimal formulation of targeted nanoparticles for actual translational applications has not been established. By refining early formula-

tions and after extensive testing, we have created an optimized targeted delivery system, HPLNs (Hybrid Polymerized Liposomal Nanoparticles) and demon-

strated that this new, targeted nanoparticle technology overcomes many of these deficiencies. This technology has tremendous potential for treating virtually any type of cancer. The goal of our present study was to determine the efficacy and toxicity of this novel targeted nanoparticle in the treatment of Ewing’s Sarcoma, a rare and highly lethal cancer of children and young adults. The efficacy of this targeted delivery system was compared to 1) administration of the free drug and 2) the drug in untargeted nanoparticle form. Our studies have shown that HPLN-DDX targeted with human antiCD99 antibodies can successfully inhibit the growth of Ewing’s Sarcoma in murine models without any detectable off-
target toxicity. The deliverable for our study was to demonstrate that the effectiveness of doxorubicin in the treatment of recurrent Ewing's sarcoma could be dramatically improved when encapsulated in human antiCD99 antibody targeted HPLNs. At the same time, toxic side effects normally seen with free drug was not detectable. Success with human anti-CD99 antibodies targeted HPLNs containing doxorubicin was then compared to the control and experimental groups utilizing the student t-test. P-values <0.05 were considered statistically significant. Additional statistical analyses were conducted as per the Pediatric Preclinical Testing Program analysis plan. Results: Lenvatinib was generally well tolerated in vivo, without noted toxicity in any of the treated animals. Lenvatinib treatment led to delayed tumor progression in 4/5 models tested. Relative tumor volumes in the treatment group compared to the control group at week 3 in xenografts OS1, OS2, OS17, OS31, and OS33 were 0.77 (p = 0.013), 0.88 (p = 0.147), 0.49 (p < 0.001), 0.66 (p < 0.001), and 0.40 (p < 0.001), respectively. Despite delayed progression observed in the OS xenografts treated with lenvatinib, all groups experienced progressive disease by the end of the study period. Conclusions: Single agent lenvatinib demonstrates consistent anti-tumor activity in osteosarcoma patient derived mouse xenograft models. Preclinical studies, as well as clinical trials, have demonstrated the efficacy of tyrosine kinase inhibition in combination with mTOR in a variety of malignancies. The current study, along with prior data, suggests that targeting receptor tyrosine kinases, in addition to mTOR, may lead to more effective inhibition of osteosarcoma cell growth. Ongoing studies are assessing the efficacy of lenvatinib, administered in combination with mTOR inhibitor, everolimus, in osteosarcoma patient derived mouse xenografts.

#698 Functional outcome and socio-psychological problems for bone & soft tissue sarcoma patients in childhood & AYA generation. Kanya Honoki,1 Toshiaki Shinomiya,2 Hiromasa Fuji,1 Takashi Ishihara,2 Shinji Tsukamoto,1 Shingo Kishi,1 Yumiko Kondo,1 Akira Kido,1 Toshifumi Tsuchijichi,1 Midori Shima,1 Yasuhiito Tanaka1. 1Nara Medical Univ., Nara, Japan; 2Kandai Univ., Higashiosaka, Japan.

[Objective] To elucidate the problems between functional and sociological outcomes in patients of bone and soft tissue sarcomas in childhood and adolescent/young adult (AYA) generation. [Method] Clinical (prognostic and functional) and socio-psychological outcomes were retrospectively analyzed in 50 patients with bone and soft tissue sarcomas under 29 years old treated in our institute from 1998 to 2014. [Results] Twenty-two patients under 15 and 28 of AYA generation (15 to 29 years old) (28 male and 22 female) were subjected, including 35 cases of bone and 15 cases of soft tissue sarcomas, and 39 of extremity cases. Most common diagnosis was osteosarcoma of 27 cases. Limb-sparing surgery was performed in 30 cases, including 14 of prosthetic and 8 of biological reconstruction for bone tumors. Overall Survival rates were 79% for 2 year and 61% for 5 year follow-up periods. 70% of the patients experienced limited activities; 50% in high school and college students experienced drop-out or delay, suspension and repeating the same class; 56% faced to their job lost or limitation of job search; 20% experienced changes of relationship with families and partners (Table). Most taken palliative care intervention was the counseling for fear, pain, nausea and sleeplessness in patients and caregivers, besides pain management and psychological care. Correlation coefficient failed to show a significant correlation between functional score and sociological disability score both in all limb sparing cases (R² = 0.2664) and in osteosarcoma cases ((R² = 0.1757). [Conclusion] The sociological problems for patients in this particular group could be focused on school life, job search and relationship with families and partners. QOL is a multidimensional measure with various domains, such as physical, psychological, social well-being, spiritual health as well as functional ability, therefore, multi-disciplinary care for socio-psychological and psychological disadvantage must be taken for this group of patients at earliest convenience.

### Relationship between physiological and socio-psychological problems

<table>
<thead>
<tr>
<th>Subject</th>
<th>All patients</th>
<th>Limb-spared patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limitation of activities</td>
<td>70% (30/43)</td>
<td>75% (21/28)</td>
</tr>
<tr>
<td>School dropout/delay in high-schoolers &amp; college students</td>
<td>50% (14/28)</td>
<td>32% (8/25)</td>
</tr>
<tr>
<td>Lost jobs/job search difficulties</td>
<td>56% (9/16)</td>
<td>33% (3/9)</td>
</tr>
<tr>
<td>Changing social reletions with families &amp; partners</td>
<td>20% (8/40)</td>
<td>21% (3/28)</td>
</tr>
</tbody>
</table>

#699 Clinical feasibility of chemotherapy monitoring for bone sarcoma patients with diffuse optical spectroscopic imaging. Hannah M. Peterson,1 Bang H. Hoang,2 David Geller,2 Richard Gorlick,2 Rui Yang,3 Jeremy Berger,2 Janet Tingling,4 Michael Roth,4 Jonathan Gill,4 Darren Roblyer1. 1Boston University, Boston, MA; 2Montefiore Medical Center, Bronx, NY.
### #700 Characterization of the mode of action of Fenretinide treatment in alveolar rhabdomyosarcoma cells.

Eva Brack, Marco Wachtel, Beat W. Schaefer. University Children’s Hospital Zurich, Zurich, Switzerland.

Alveolar rhabdomyosarcoma (ARMS) is a highly malignant childhood malignancy characterized by a specific chromosomal translocation encoding the oncogenic transcription factor PAX3-FOXO1. As ARMS cells are addicted to the tumor-specific fusion protein, it may serve as an ideal therapeutic target. Previously, we have identified from a large drug library screen the compound Fenretinide (4-hydroxy-p-hydroxyanilide), which is already in clinical use, to inhibit both PAX3-FOXO1 expression as well as ARMS cell viability. The aim of this study was therefore to characterize the mode of action of Fenretinide in more detail. First, we were able to show that Fenretinide induced the generation of reactive oxygen species (ROS) in mitochondria. A more detailed characterization revealed that the Fenretinide-induced ROS derives from an interaction of Fenretinide around complex II of the mitochondrial respiratory chain, leading to the production of superoxides. ROS scavenging as well as complexing of iron ions completely abolished cell death. To identify the mode of cell death involved, we then used a range of pharmacological inhibitors of specific cell death pathways including Z-vad (pan-caspase inhibitor), Necrostatin-1 (necroptosis pathway inhibitor RIP-1 kinase inhibitor), 3-Methyladenine (3-MA) (autophagy pathway inhibitor (phosphatidylinositol 3-kinase inhibitor)) and Ferrostatin (ferroptosis pathway inhibitor) during Fenretinide treatment.

Surprisingly, none of these inhibitors alone was able to prevent cell death and even different combinations were not sufficient to completely inhibit cell death. CRISPR/Cas9 mediated depletion of key players in the apoptotic and necrotic pathway (Bak, Bax and RIPK1) confirmed the pharmacological data. We therefore conclude that the other, less characterized cell death pathways or a combination of several pathways including apoptosis and necroptosis might be crucial. Interestingly, electron microscopic examination of cells pointed towards an excessive accumulation of vacuoles to be characteristic. Taken together, our data show that Fenretinide shows high potential for the treatment of ARMS, inducing several forms of cell death mediated through the production of ROS. These properties open the search for additional compounds acting in a combinatorial manner.

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### #701 Pazopanib synergizes with clofarim tosylate in the treatment of malignant rhabdoid tumors.

Celine Chaunvin¹, Aurianne Lescure¹, Wilfrid Richer¹, Arnaud Tautié-Espariat², Amaury Leruste³, Zhi-Yan Han⁴, Didier Surdez⁵, Stefano Cairo⁶, Eléna Del Nery⁷, Olivier Delattre⁸, Franck Bourdeaut⁹.

¹Curie Institute, Paris, France; ²Sainte-Anne Hospital, Paris, France; ³XenTech, Epernay, France.

Background: Rhabdoid tumors (RTs) are rare, highly aggressive pediatric malignancies which occur in kidney, soft-parts and brain of young children. They are characterized by a complete inactivation of the SMARCB1 tumor suppressor gene encoding a core subunit of the chromatin remodeling SWI/SNF complex. Prognosis for children with RTs is poor as, in many instances, these tumors are resistant to conventional chemotherapy. Hence, new therapies should be designed and biologically-driven strategies targeting the SMARCBI deficiency or its biological consequences have to emerge. Methods: For this purpose, we realized a high-throughput screening by using the Prestwick Chemical Library of 1200 FDA-approved drugs on two rhabdoid tumor cell lines and one SMARCB1 reexpressing cell line. Cell count was assessed by automatic cell counting using DAPI. Results: Among 10 drugs that showed cytotoxic activity specifically in SMARCBI-deficient cell lines, we identified vatalanib, a broad receptor tyrosine kinase (RTK) inhibitor. This prompted us to analyse the response to a wider number of 9 RTK inhibitors on 7 SMARCBI-deficient cell lines compared to 6 SMARCBI-expressing cell lines. “This supervised” screening confirmed the activity of vatalanib but showed that pazopanib had an even greater cytotoxic effect on SMARCBI deficient tumor cells. We also identified a potassium channel inhibitor (clofarim tosylate), that showed among the strongest cytotoxic activity as a single agent, and also synergistically reduced viability and induced apoptosis, when combined with vatalanib and pazopanib. With in vivo experiments on two patient-derived xenograft (PDX) models of rhabdoid tumor, we notably showed that pazopanib is effective in reducing tumor growth and that combination with clofarim tosylate enhanced the anti-tumor activity of pazopanib in the two PDX models. Interestingly, we also highlighted that pazopanib combined to conventional chemotherapies is even more efficient than chemotherapy alone. Finally, to identify candidate kinases that confer pazopanib sensitivity to rhabdoid tumor cell lines, we performed a phosphoarray experiment and found PDGFRα, PDGFRβ and FGFR2 as pazopanib targets, RTK that were among the most highly expressed in our in vitro profiling dataset. Conclusion: To conclude, our findings demonstrate that targeting PDGF and FGF axis is an effective means of therapy against rhabdoid tumors. We therefore propose that pazopanib should be tested in children with rhabdoid tumors in which there is a dire need for an effective therapy.
of 35 days for control against 21 days for CPd/nATF5, P=0.0013. CP-d/n-ATF5 also reduced SK-N-BE(2)C metastatic burden in the liver (P<0.05) and bone marrow (P<0.01). In the rhabdoid PDX model, there was a significant inhibition of tumors treated with CP-d/n-ATF5 as compared to Penetratin treatment, with a mean tumor volume of control 1283 ± 266.6 mm³ (n=15) vs CPd/n-ATF5 234.2 ± 43.7 mm³ (n=11), at day 10 within which tumors showed a median post-treatment time of 13 days to reach threshold volume. None of the CP-d/n-ATF5 treated tumors reached the threshold after 28 days of treatment with some tumors demonstrating regression, indicating a profound anti-tumor effect of the peptide. Conclusion: For our study shows that a novel ATP5-targeting peptide CP-d/n-ATF5 has broad and profound cytotoxic and apoptotic effects in several pediatric tumors in vitro and in vivo. Our study also indicates that CP-d/n-ATF5 has the potential to act as an anti-metastatic agent.

#703 Combinatory treatment for pediatric low grade glioma with the dual mTORC1/2 inhibitor TAK228 and MEK inhibitor Trametinib
Antje Arnold,1 Fausto Rodriguez,2 Charles George Eberhart,3 Eric Hutton Raabe3, Johns Hopkins University School of Medicine; Division of Neuropathology and Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD; Johns Hopkins University School of Medicine; Division of Neuropathology and Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD.

Pediatric low grade glioma (PLGG) is one of the most common childhood tumors. If the tumor is located in a region of the brain that is not accessible for surgical resection or if the tumor recurs after surgery, additional therapies are needed. Recent studies highlighted the important role of mTORC1/2 and MEK-activation in PLGG. The dual mTORC1/2-inhibitor, TAK228, and MEK-inhibitor, Trametinib, have been developed as promising candidates for targeted therapy PLGG. We hypothesized that TAK-288 and Trametinib would show synergistic effects both in vitro and in vivo in PLGG models. We treated the PLGG derived cell lines Res186 and Res259 with TAK-228, Trametinib, and vehicle. Cell growth was investigated using MTT-assay over different days and compared to the treatment with the vehicle. DNA replication was measured through bromodeoxyuridine (Brdu) incorporation assay and apoptosis was evaluated through cleaved caspase 3 (CC-3) staining. Cells were analyzed and counted with ImageJ. Activation of MAPK pathway was detected via Western Blot by phosphorylated pERK compared to total pERK. Cells were analyzed and counted with ImageJ. Activation of MAPK pathway was detected via Western Blot by phosphorylated pERK compared to total pERK. We observed a significant increase in Res186 cells after drug treatment. MAPK pathway was activated in a dose-dependent manner. Res186 have a significant reduction in cell growth after 4 days treatment (TAK-288: 20nM, **p<0.01; Trametinib: 20nM, **p<0.01). Res259 showed a significant reduction at the same time point, but with a higher drug dose (TAK-288: 50nM, *p<0.05; Trametinib: 50nM, **p<0.01). IC50 values for TAK-288 was 15nM for Res186 and 20nM for Res259 cells on day 4. IC50 values for Trametinib was 50nM for Res186 and 100nM for Res259. Staining for CC-3 showed a significant increase for apoptosis in Res259 cells after treatment with TAK-288 or Trametinib (**p<0.01). No positive CC-3 staining was detected in Res186 cells after drug treatment. MAPK pathway was activated in a dose-dependent manner as determined by phosphorylated pERK Western Blot after TAK-288 and inactivated after Trametinib treatment. No change in total ERK compensation was found. This suggests that the compensatory mechanism is independent of mTORC signaling by upregulating MAP kinase signaling. Our preliminary results show that the PLGG-derived cell lines are sensitive to TAK-228 and Trametinib treatment. All cell lines showed decreased proliferation at various doses of either inhibitor. The increased MAP kinase activity we identified after TAK-288 treatment suggests a compensatory mechanism that may render these cells especially sensitive to treatment with both TOC1/2 and MEK inhibitors. We will now investigate both drugs in vivo. Evidence of activity in murine models will be necessary to provide a pre-clinical rationale for combination therapy of these agents in aggressive PLGG.

#704 Therapeutic potential of Delta24-2ACT, a novel immunostimulatory oncolytic adenovirus, for the treatment of pediatric solid tumors: Initial study in pHHG, DIPG and osteosarcoma
Marcia García-Mouré,1 Naïara Martínez-Vélez,1 Enric Xipell,1 Marisol González-Huarriz,1 Ana Patiño,1 Oren J. Becher,2 Cande Gómez-Manzano,3 Juan Fueyo,3 Marta M. Alonso1.

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Pediatric High Grade Glioma (pHHG), including Diffuse Intrinsic Pontine Glioma (DIPG), and osteosarcoma are amongst the most common and lethal pediatric solid tumors. Despite improvements in surgery, radiotherapy and chemotherapy, the outcome for children affected with these malignancies remains dismal since the current treatments are ineffective and ineffective in killing severe side effects. Thus, it is critical to implement novel and radically different therapeutic approaches to improve the survival and quality of life of these children. Delta24 is a modified adenovirus with a tumor dependent replication, and its antitumor effect has been tested in preclinical and clinical models of both high grade glioma and osteosarcoma. The adenovirus demonstrates a preferential tropism to tumor cells. Furthermore, this virus has the potential to induce antitumor immunity, by the virus and the immune system. Here we describe the generation of a new oncolytic adenovirus Delta24-2ACT, based on the Delta24 platform, encoding an immunostimulatory protein. For this project we used relevant immunocompetent pediatric solid models. The toxicity of the virus was assessed by dose-escalation experiments. Delta24-2ACT was well tolerated and did not lead to important adverse effects. The antitumor effect of Delta24-2ACT was evaluated in immunocompetent mice bearing orthotopic supratentorial glioma, DIPG or local osteosarcoma that spontaneously metastasize to lungs, obtaining significant tumor shrinkage or complete elimination in some of them. Delta24-2ACT also promoted an increased T-cell infiltration within the tumor and an enhanced antitumor immune response. Of importance, treatment with Delta24-2ACT led to a significant increase in median survival in all the tumor models tested and resulted in long-term survivors free of disease. Furthermore, in our osteosarcoma model local treatment of primary tumors with Delta24-2ACT reduced spontaneous lung metastases, which are usually the leading cause of death in most of the patients affected by osteosarcoma. Currently, we are performing re-challenge experiments to evaluate a possible memory effect mediated by the virus and mechanistic studies to elucidate the mechanism of action. Altogether, these results demonstrate the potential therapeutic benefit of Delta24-2ACT adenovirus in the treatment of pediatric solid tumors such as pHHG, DIPG and osteosarcoma, representing an important milestone in the fight against cancer.

#705 Elucidating pediatric brain tumor pathophysiology by assessing signal transduction pathway activation

A major problem in pediatric neuro-oncology is lack of insight into underlying tumor biology, making treatment with targeted therapy challenging for this group of patients. To enable targeted therapy choice, understanding of underlying signal transduction pathway activity is required, as well as availability of appropriate diagnostic tests to predict therapy response. We have developed a novel method to identify activity of the ER, AR, HH, Wnt, TGFbeta, NFKB, and PI3K signal transduction pathways in an individual tumor. Our method is based on measurement of signal transduction pathway activity using a knowledge-based Bayesian network computational model which infers the probability of activity of the respective transcription factor (Verhaegh et al, Cancer Res. 2014). Public patient sample datasets from the GEO database were used to investigate pathway activation in pediatric brain tumors. Currently, we are performing re-challenge experiments to evaluate a possible memory effect mediated by the virus and mechanistic studies to elucidate the mechanism of action. Altogether, these results demonstrate the potential therapeutic benefit of Delta24-2ACT adenovirus in the treatment of pediatric solid tumors such as pHHG, DIPG and osteosarcoma, representing an important milestone in the fight against cancer.
**CLINICAL RESEARCH: Childhood Cancer Clinical Translational Research**

#706 Combination ionizing radiation and oncolytic Seneca Valley virus injection enhances tumor cell killing of pediatric gliomas. Yuchen Du, Baylor College of Medicine, Houston, TX.

Background and Purpose: Radiation therapy is the traditional and only therapy that offers benefits to patients with diffuse intrinsic pontine gliomas (DIPG) due to the aggressive pediatric central nervous system tumors without known curative therapies. However, unfortunately, efficacy of radiation therapy is transient. Seneca Valley virus-001 (SVV-001) is a novel non-pathogenic oncolytic picornavirus that can be systemically administered and pass through brain blood barrier (BBB). Phase 1 clinical trial of SVV has proven its efficacy in adults patients with cancers with neuroendocrine features and pediatric patients with non-central nervous system tumors.

We sought to examine if combining ionizing radiation with an oncolytic virus SVV-001 would lead to synergistic enhanced tumor cell killing and significantly improve therapeutic efficacy in vivo in DIPG patient tumor-derived intra-brain stem orthotopic xenograft mouse models (PDOX). Methods: By infecting PDOX derived tumor cells with green fluorescent protein (GFP)-coupled SVV (SVV-GFP) or SVV-001, in vitro virus infection and anti-tumor activity of SVV were examined by cell viability assay, Western Blotting for apoptosis and autophagy-related protein and immunohistochemistry (IHC) staining or Flow cytometry for cell mitochondria content. In vivo therapeutic efficacy was evaluated by systemic administration of SVV into PDOX derived DIPG xenografts alone or in combination with irradiation. Results: We first had established a novel panel of nine PDOX derived orthotopic xenograft mouse models of DIPG. Our data confirmed that SVV can infect and efficiently kill DIPG tumor cells in vitro by inducing apoptosis and autophagy, leading to improved animal survival in a subset of DIPG xenograft models. We found that the lack of mitochondria in the xenograft tumor cells in vivo impaired the intracellular replication of SVV-001 and subsequently compromised its oncolytic cell killing of tumor cells in vivo. Radiation induced elevated mitochondria biogenesis in tumor cells therefore boosted SVV oncolysis of tumor cells. Moreover, combining fractionated radiation with single i.v. injection of SVV led to significant improvement of animal survival in a subset of DIPG models. Conclusion: SVV-001 killed DIPG xenograft cells in vitro. Radiation activated mitochondria content of tumor cells in vivo which restored intracellular SVV-001 oncolysis. Combining radiation with single systemic administration of SVV synergistically enhanced animal survival in a subset of DIPG xenograft models.

#707 Personalization of dexamethasone in acute lymphoblastic leukemia. Rosanna K. Jackson,1 Ali Alhammer,2 Zach Dixon,2 Gareth J. Veal,1 Julie AE Irving1. 1Newcastle University, Newcastle, United Kingdom; 2Bristol University, Bristol, United Kingdom.

Synthetic glucocorticoids, such as dexamethasone (Dex), are pivotal in the treatment of acute lymphoblastic leukemia (ALL) but remain with significant variability, both in terms of toxicity and efficacy. We aimed to investigate three key variables to better understand how Dex personalization may be achieved: pharmacokinetics (PK), intracellular Dex accumulation, and cellular response, following Dex binding to the glucocorticoid receptor (GR) in ALL cells. For Dex PK studies, blood samples were collected post oral administration on one of the first three days of induction chemotherapy in 99 patients on the UKALL 2011 trial receiving either 0.8mg/m2 for 28 days (standard arm) or 10mg/m2 for 14 days (short arm). Plasma Dex levels were analysed using a validated LC/MS method, and a non-compartmental pharmacokinetic analysis. To assess intracellular Dex levels, cell lines, primagraft (n=9) and primary patient samples (n=6) were studied. The plasma Dex LC/MS method was optimized to quantify Dex in ALL cell lysates. Dex accumulation was also assessed using flow cytometric analysis of Dex conjugated to FITC. Cellular Dex sensitivity was assessed using Alamar Blue assays. There was a wide Dex PK variability, with $AUC_{0-12}$ and $C_{max}$ significantly higher on the short compared to the standard arm; 564 (202-1606) versus 408 (142-1009), median (range), $p=0.003$ and 0.0006, respectively. However there was substantial overlap between the two arms, with the standard arm exhibiting higher exposure on average than those on short therapy. These data suggest that while PK and cellular response are hugely variable, variations in drug accumulation do not appear to play a key role in Dex response in ALL cells. Importantly, 62% of patient cell samples had Dex GI50 values greater than plasma concentrations observed in any patient, on both arms on the UKALL 2011 trial. A combined approach incorporating PK assessments and cellular response in ALL cells should be further investigated, to allow a comprehensive understanding of Dex pharmacology with a view to optimizing its clinical utility.

#708 Prohibition is a prognostic marker of treatment failure and therapeutic target to block chemotherapy resistance in Wilms tumor. Michael Vincent Ortiz1, Melissa Burns2, Amy Eisenberg,3 Saima Ahmed,4 Lyvia Gaewsky,5 Gary Bradwin,6 Paolo Cifani,7 Anton Henssen,8 Ian Macarthur,9 Michael LaQuaglia,10 Anthony Letal11, Arlene Narango,12 Samantha Gadd,13 Yuh-Yun Chi,14 Jeffrey Dome,15 Elizabeth Perlman,16 Elizabeth Mullen,17 Hanno Steen,18 Alex Kendis19. 1Memorial Sloan Kettering Cancer Center, NY; 2Boston Children’s Hospital, MA; 3University of California, Berkeley, CA; 4Dana Farber Cancer Institute, MA; 5University of Florida, FL; 6Lurie Children’s Hospital of Chicago, IL; 7Children’s National Medical Center, DC.

Wilms tumor (WT) is the most common kidney tumor of children. Over the last three decades, clinical trials employing multi-modality therapies have resulted in overall survival of greater than 90% for patients with low-risk disease. In spite of these advances, treatment of patients with advanced, anaplastic, and relapsed Wilms tumors remains challenging, with substantial rates of treatment failure and death. To improve risk stratification and identify novel therapeutic targets, we used high-accuracy mass spectrometry urinary proteomics to identify urine tumors markers associated with relapsed WT and non-WT renal tumors. We measured urine proteins at diagnosis of 54 patients with relapsed WT, clear cell sarcoma, rhabdoid tumor, and age-matched controls, leading to the quantitation of 6,519 urine proteins. In particular, we identified specific urine WT markers, including those that were enriched in patients with relapsed WT, such as mitochondrial regulators prohibitin and DAD1, β-catenin antagonist DACT2, and DNA repair factor SUN1. Using a specific enzyme-linked immunosorbent assay (ELISA) developed to measure urine prohibitin in an independent cohort of 139 WT and control samples, we found that urine prohibitin concentrations over 1000 ng/mL were significantly associated with the risk of disease relapse, with an odds ratio of relapse of 1.53 and receiver operating characteristic area under the curve of 0.77 (95% confidence interval of 0.64-0.99). Immunohistochemical tumor analysis revealed that prohibitin was highly expressed in primary Wilms tumor specimens. Importantly, using loss- and gain-of-function genetic experiments, we found that prohibitin was required for the growth and survival of Wilms tumor cells, and its overexpression conferred concomitant resistance to vincristine, doxorubicin and actinomycin D. Consistent with prohibitin’s functions in mitochondria, we are using BH3 profiling to elucidate specific intrinsic apoptotic dependencies in distinct sub-sets of refractory Wilms tumors, as a prelude to rational combination blockade of chemotherapy resistance, such as blockade of BCL2 dependence using venetoclax. In all, the use of urine prohibitin measurements may improve initial therapy stratification, and enable monitoring of response to therapy and early detection of relapse. In addition, targeting of chemotherapy resistance induced by prohibitin overexpression may offer improved therapies for patients with relapsed or refractory Wilms tumors.

#709 Incidence and mortality of diethylstilbestrol-related clear-cell adenocarcinoma of the vagina and cervix: 40 years of long-term follow-up. Dezheng Huo, Diane Anderson, Arthur L. Herbst. Univ. of Chicago, Chicago, IL.

Objective: Women exposed to diethylstilbestrol (DES) in utero are at increased risk for the development of vaginal and cervical clear cell adenocarcinoma (CCA), and possibly of breast cancer, diabetes, and cardiovascular diseases as well. A peak in the age-incidence curve in DES-related CCA has been documented at ages 19 to 24. It is unknown if a second peak will occur in later life, the ages when CCA developed spontaneously in the pre-DES era. In addition, the long-term prognosis and health impact for adolescents and young adults who have DES-related CCA are unknown. Methods: 720 cases of CCA were reviewed from the CCA Registry at the University of Chicago through 2014. Rates and cumulative risks for CCA were calculated based on white women born in the U.S. from 1948 through 1971. Kaplan-Meier method was used to estimate survival probability among women with CCA. Cox models were used to identify independent prognostic factors. To gauge the relative impact of young-onset clear-cell adenocarcinoma on mortality, we compared their mortality hazards with mortality hazards of the U.S. female population, and calculated standardized mortality ratio (SMR). Results: In 420 CCA cases there was documented evidence of prenatal DES exposure. 80% patients had a CCA be-
tween ages 15 and 31 but some occurred as late as age 55. A small second peak occurred around age 40. The risk of U.S. born, white DES-related CCA was highest in the 1951-1956 birth cohort, and this birth cohort effect correlated with highest levels of COL1A1. Expression of COL1A1 by these cells was confirmed by immunohistochemistry (IHC) using prostate tissue microarrays (TMA). In addition, protein array experiments identified elevated levels of several proteases in the urine of PCa patients, including MMP-9, uPA, ADAM-TS1 and several cathepsins. Substrate gel electrophoresis (zymography) revealed elevated activity of both MMP-9 and MMP-2 in urine from PCa patients. These data suggest that MMP-9 and MMP-2 may participate in the cleavage of collagen type 1, resulting in elevated levels of COL1A1 in urine of PCa patients. We are currently testing this hypothesis. In summary, COL1A1 may represent a novel non-invasive urinary biomarker that can effectively discriminate between BPH vs. localized PCa. (Supported by The Ellison Foundation.)

CLINICAL RESEARCH: Early Detection

#710 Identification of autoantibody to ECH1 & HNRNPA2B1 as potential biomarkers in the early detection of lung cancer. Liping Dai,1 Jitian Li,2 Jun-Chieh J. Tsay,1 Xiao Wang,3 John S. Munger,3 Harvey Pass,4 William N. Rom,2 Eng M. Tan,5 Jian-Ying Zhang,2 Zhengzhou University, Zhengzhou, China; 2the University of Texas at El Paso, El Paso, TX; 3New York University, New York, NY; 4The Scripps Research Institute, San Diego, CA.

Background: Identification of biomarkers for early detection of lung cancer (LC) may lead to more effective treatment and reduction of mortality. Methods: Serological proteome analysis (SERPA) was used to identify proteins around 34 kDa, which had been previously recognized by autoantibody in sera from LC patients. We have validated autoantibody response in sera from 90 LC patients, 89 normal controls by using immunoassay. Another independent cohort of 25 LC patients with 219 serial serum samples and 56 matched normal controls were examined to evaluate whether the autoantibody can be detected in the preclinical stage. Results: The proteins with molecular weight of 34 kDa were identified as ECH1, GAPDH and HNRNPA2B1. In the validation study, autoantibody to ECH1 achieved an area under the curve (AUC) of 0.799 with sensitivity of 62.2% and specificity of 95.5% in discriminating LC from normal individuals, and showed negative correlation with tumor size (r=−0.256, p=0.023). Autoantibody to HNRNPA2B1 performed an AUC of 0.874 with sensitivity of 72.2% and specificity of 95.5%, and showed negative correlation with lymph node me-tastasis (P=0.012). By using a logistic regression partition tree approach, autoantibody to ECH1 showed an AUC of 0.763 with sensitivity of 60.0% and specificity of 89.3% in distinguishing LC with matched normal controls, and elevated autoantibody levels could be detected greater than two years prior to LC diagnosis. Conclusions: ECH1 and HNRNPA2B1 are autoantigens that elicit autoimmune responses in LC and can be used as potential biomarkers for the early detection of LC. Funding support: This work was supported by the National Natural Science Foundation of China (81672917, 81372371) and the National Institutes of Health (SC1CA166016 and U01CA086137).

#711 Mechanistic implications of COL1A1 as a prostate cancer biomarker. Andrej Jedink,1 Camille Vuiuclaud,2 Andrew El-Hayek,3 Katherine Kaplan,4 Jason Salama,5 Sarah A. Slupsky,6 Adam S. Feldman,5 Kevin A. Campbeau,3 Kevin B. Loughlin,3 Marsha A. Moses1,3 Boston Children’s Hospital, Harvard Medical School, Boston, MA; 2Boston Children’s Hospital, Boston, MA; 3National Cancer Institute, Bethesda, MD; 4Massachusetts General Hospital, Boston, MA; 5Brigham and Women’s Hospital, Boston, MA.

Prostate cancer (PCA) is the second most frequently diagnosed form of male cancer and shares similar symptoms with BPH (Benign Prostate Hyperplasia), a disease characterized by prostate enlargement. Elevated levels of prostate-specific antigen (PSA) can be observed with either benign or malignant growth of the prostate and therefore cannot effectively discriminate between these two prostate diseases. Currently, a test that sensitively and accurately distinguishes between BPH and localized prostate cancer does not exist creating an urgent need for novel biomarkers that can successfully distinguish between these two prostate diseases. The goal of this study was to identify and validate non-invasive urinary biomarkers that distinguish between BPH and PCA. Our previous proteomic study identified elevated levels of several proteins, including EGF (epidermal growth factor), COL1A1 (collagen type I, alpha 1) and other proteins in the urine of PCA patients compared to urine samples from patients with BPH. In this current study we have analyzed and validated the presence of EGF, HE-4 and COL1A1 by enzyme-linked immunoassortant assay (ELISA). Our ELISA experiments revealed that COL1A1 was significantly (P<0.002) elevated in the urine of patients diagnosed with early or locally advanced PCA compared to PCA patients performing prostate specific antigen (PSA) tests. Our findings suggest that COL1A1 may lead to more effective treatment and reduction of mortality but accurate and readily accessible tools for population screening are limited. By whole methylome sequencing, we have identified novel methylated DNA markers (MDMs) for lung cancer in tissue (Giaikoumopoulos et al. ASCO 2016). Using top candidate MDMs in the present study, we now explore their clinical accuracy for lung cancer detection when assayed from plasma. Experimental Procedures: Archival plasmas from two independent study groups were tested in blinded fashion. Lung cancer cases and controls (apparently healthy smokers) for each group were balanced on age and sex (Group 1: 64 cases, 231 controls; Group 2: 23 cases, 80 controls). Using multiplex PCR followed by QUARIS Quantitative Allele-Specific Real-time Target and Signal amplification (Qatar) assay, a post-bisulfite quantification of MDMs on DNA extracted from plasma was performed. We selected 31 MDM candidates for initial evaluation in Group 1 (1 ml plasma/patient); top individual MDMs were subsequently tested in Group 2 to identify optimal MDM panels for lung cancer detection (2 ml/patient). Results: From Group 1 analyses, 13 high performance MDMs were selected for further testing (CYP26C1, SOBP, SUCCL2, SHOX2, ZDHHC1, NFIX, FJL45983, HOXA9, B3GALT6, ZNF781, SP9, BARX1, EMXI) with individual areas under the receiver operator curve (AUCs) ranging from 0.593 to 0.939. Discrimination by individual MDMs was corroborated in Group 2 in which data was analyzed using two methods: a logistic regression fit and a regression partition tree approach. The logistic fit model identified a 4-marker panel (ZNF781, BARX1, EMXI, and SOBP) with an AUC of 0.96 and an overall sensitivity of 91% and 90% specificity. Analysis of the data using a regression partition tree approach identified 4 markers (ZNF781, BARX1, EMXI, and HOXA9) with AUC of 0.93 and an overall sensitivity of 96% and specificity of 94%. For both approaches, B3GALT6 was used as a standardizing marker of PCA DNA input. Conclusion: A panel of MDMs assayed in plasma achieved high sensitivity and specificity for all types of lung cancer. Further clinical evaluation and validation of this promising panel in larger patient groups are clearly indicated.

#713 Proteomic early detection biomarkers for ER+ breast cancer to improve mammography screening. Carly B. Garrison,1 Tracey Marsh,2 Matthew Buas,3 Yuzheng Zhang,3 Margaret Pepe,3 Paul D. Lampe,1 Christopher I. Li,2 Fred Hutchinson Cancer Research Center, Seattle, WA; 3Roswell Park Cancer Institute, Buffalo, NY.

There are clear clinical and public health needs to improve the early detection of breast cancer in order to save lives. Breast cancer is the leading cause of cancer death in women worldwide and is the second leading cause of cancer mortality between 35 and 50 year of age (SMR 27.06, 95% CI: 23.04-31.58), 5-fold increased survival than young adult patients. Compared with general U.S. female population and validation of this promising panel in larger patient groups are clearly indicated.
in women in the United States. Although mammography is widely used to screen for breast cancer, it suffers both from false negative and false positive readings resulting in later diagnoses or unnecessary follow-up (e.g., biopsies), respectively. Improvement of breast cancer early detection may be possible through the measurement of minimally invasive, easy to perform blood based biomarkers. Our studies were performed using clinical plasma samples collected up to 12.5 months before breast cancer diagnosis from cases and matched controls obtained from the Women’s Health Initiative (WHI) Observational Study (a prospective cohort of 93,676 post-menopausal women). We utilized high-density antibody microarray methods for both discovery and preliminarily validation on distinct sample sets for early detection biomarkers of estrogen receptor negative breast cancer (around 70% of all breast cancers classified as ER+). Antibody microarray features (i.e., spots) identified as having >30% sensitivity at 80% specificity were considered to have met the cutoff criteria and identified as potential markers. A total of 129 features, corresponding to 101 proteins, were identified as potential markers in one WHI dataset (90 cases vs. 90 controls). Of the 129 features (101 proteins), 47 (34 proteins) validated by our cutoffs and demonstrated over or under-expression in cases compared to controls consistent with the first dataset in the second set of WHI samples (121 cases vs. 121 controls). Given this level of reproducibility, we believe these biomarkers could be used to improve early detection of ER+ breast cancer for these clinical scenarios: 1. Informing timing of a subsequent mammogram in women with a negative screening mammogram; 2. Informing continuation or termination screening among women between 75-79 years; 3. Identifying women who should be screened with mammography in areas with limited resources. We are currently performing further validation of the protein markers in Early Detection Research Network (EDRN) specimen reference set from patients at the time of diagnosis. We plan to test our markers on a pre-diagnostic cohort from the Cardiovascular Health Study using a different, more clinically applicable platform. We used the same high-density antibody microarray platform to measure autoantibody-autoantigen complexes and sialyl Lewis-A and –X modifications in one of the WHI sample sets and are now testing these markers in the EDRN set for validation. Given the strength of our biomarker candidates, the high quality sets of biospecimens we used, the study designs employed, and the clearly delineated clinical applications proposed, we believe these markers could have near-term clinical impact.

#714 Human transcriptome alterations in pre-cancer and cancer epithelium identify candidate biomarkers of progression to pancreatic cancer.
Elena V. Komissarova, Jorge Sepulveda, Sarawut Kongkarnka, Maryam Shrazi, Brynn Levy, Claudia Cujar, Antonia R. Sepulveda. *Columbia University, New York, NY.*

Pancreatic ductal adenocarcinoma (PDAC) is one of the most common cancers in the United States. The five-year survival rate for patients with PDAC remains dismal. Identification of biomarkers for early diagnosis of PDAC and pre-cancer pancreatic intraepithelial neoplasia (PanIN) lesions with risk of progression to PDAC is critically needed. We hypothesized that differentially expressed genes and regulatory pathways in PanIN and PDAC compared to normal duct epithelium (ND) may represent biomarkers of development of malignancy. We used Affymetrix Human Transcriptome Arrays 2.0 to establish gene expression profiles in ND, low-grade PanIN, and PDAC epithelium. Total RNA was isolated after laser capture microdissection (LCM) of frozen tissue sections and then used for producing hybridization-ready DNA. Hybridization quality control was performed with Expression Console 1.4 software and background corrected/normalized data were analyzed with Transcriptome Analysis Console (TAC) 3.1 and the ASSIGN algorithm. We tested RNA from 22 LCM samples (9 PDACs, 5 PanINs, and 8 ND) including 4 matched trios of ND, PanIN and PDAC from the same patients. Differential expression analysis with one-way between subject ANOVA revealed over 2000 genes differentially expressed in PDAC compared to ND with p<0.05. The most frequent alteration in PanIN compared to ND samples was upregulation of 433 genes (p<1e-15) and in PDAC compared to ND downregulation of 566. We found 60 (40 coding) upregulated genes and 750 downregulated genes (filter criteria up/down >1.5; ANOVA p<0.05) in both PanIN and PDAC vs. ND epithelium. Signaling pathway analysis of WikiPaths ways showed a number of significantly altered pathways in PDAC and PanIN compared to ND including the Gastric Cancer Network 1 with upregulated S100P in both PanIN and PDAC whereas other genes including CENPF, KIF20B, TPX2 and UBE2C were upregulated in PDAC only. Using the ASSIGN algorithm and the Kruskal-Willis test for analysis of difference in pathway activity, we found additional regulatory pathways with altered activity including Nuclear Receptor meta-pathway with reduced overall score in PDAC compared to PanIN and ND samples. In summary, over 400 genes were significantly upregulated in pre-cancer PanIN lesions compared to normal duct epithelium, whereas gene down-regulation was the most frequent alteration in PDAC. Sixty genes, including 40 coding genes were up-regulated in both PDAC and PanIN. The altered pathways associated with the differentially expressed genes may represent an approach for integrated biomarker testing of neo-plastic progression.

#715 The AroCell TK 210 ELISA may complement Pro PSA and the Prostate Health Index in differentiating pre-cancerous and cancerous conditions in prostate cancer.
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Background: Thymidine Kinase 1 (TK1) is a cytosolic enzyme that plays an important role in DNA precursor synthesis and TK1 enzyme activity in serum is an established biomarker for prognosis and treatment monitoring, particularly for haematological malignancies. The AroCell TK 210 ELISA determines TK1 protein levels, in contrast to TK1 activity, and it has demonstrated superior sensitivity in distinguishing between subjects with solid tumours and controls. Pro PSA, free PSA and the prostate health index (PHI) have been proposed as more discriminating tests than PSA in distinguishing between benign and cancerous prostate changes. The purpose of this study was to compare the AroCell TK 210 ELISA with PSA, free PSA and PHI in men with pre-cancerous conditions, and patients with confirmed prostate cancer. Experimental Procedures: Serum samples from 94 patients with known PSA values (in the range 2 to 10 ng/L) that were undergoing prostate cancer were collected at the University Medical Centre, Ljubljana. TK1 protein levels were determined using the AroCell TK 210 ELISA. PSA, iPSA and pro PSA levels were analysed with commercial assays (Hybritech PSA, Hybritech Free PSA and Access 2pPSA - Beckman Coulter USA) analysed on the Access 2 Beckman Coulter analyzer. Results: Overall, 16/94 patients had serum TK1 protein levels above the estimated cut-off value for age-matched healthy men (0.45µg/L). 65/94 men had pre-cancerous prostate conditions, including benign prostate hyperplasia, prostatitis and high grade prostatic intraepithelial neoplasia, while 29 patients had confirmed prostate cancer (PCa). Only 7/65 in the pre-cancerous group (11%) had TK1 protein levels above the cut-off, whereas in the prostate cancer group 9 out of 29 (31%) were positive. Moreover, the TK1 protein values in the PCa group (mean = 0.41µg/L) differed significantly from those with pre-cancerous conditions (mean = 0.31µg/L; P = 0.01). Further analysis showed that pro PSA and PHI also differentiated the prostate cancer group from those with pre-cancerous conditions (P = 0.01 and P<0.001, respectively). This was not observed for iPSA and PSA. Overall, the AroCell TK 210 ELISA showed significant correlation with PHI (r = 0.32, P=0.0017) and pro PSA (r = 0.21 P = 0.044) but not with iPSA or PSA. Conclusions: This preliminary study has demonstrated that serum TK1 assayed with the AroCell TK 210 ELISA can differentiate between pre-cancerous and prostate cancer patients with a similar accuracy to that of pro PSA. Further clinical studies will establish the capacity of the AroCell TK 210 ELISA to complement PHI in distinguishing between pre-cancerous and prostate cancer, potentially providing another tool in prostate cancer management.

#716 Genomic classification of longitudinally observed small colorectal polyps.
Meta C. van Lanschot,1 Beatrix Carvalho,1 Charlotte J. Tutein Nolthenius,1 Christian R. Rausch,2 Ernst J. Kuipers,3 Jaap Stoker,3 Gerrit A. Meijer,1 Netherlands Cancer Institute, Amsterdam, Netherlands; 2Academic Medical Center, Amsterdam, Netherlands; 3Erasmus Medical Center, Amsterdam, Netherlands.
Introduction: Colorectal polyps are very common in elderly people with a prevalence of 15-30% reported in screening series. However, only a minority of 5% of these lesions is estimated to develop into invasive cancers, whereas others will remain stable or regress over time. In standard practice all polyps observed during colonoscopy are removed by polypectomy and therefore, knowledge about their natural history is limited. In this study, small (6-9mm) colorectal polyps were followed over time and subsequently resected during colonoscopy. Aim: The prevalence of DNA copy number alterations (CNAs), a feature associated with adenoma to carcinoma progression, was compared in polyps that morphologically progressed versus those that did not. Methods: In the CT colonography (CTC) arm of the COCOS-trial small 6-9mm polyps were left in situ. After a three-year surveillance interval, growth according to CTC of 95% of the polyps was confirmed prostate cancer (PCa). Only 7/65 in the pre-cancerous group (11%) had TK1 protein levels above the cut-off, whereas in the prostate cancer group 9 out of 29 (31%) were positive. Moreover, the TK1 protein values in the PCa group (mean = 0.41µg/L) differed significantly from those with pre-cancerous conditions (mean = 0.31µg/L; P = 0.01). Further analysis showed that pro PSA and PHI also differentiated the prostate cancer group from those with pre-cancerous conditions (P = 0.01 and P<0.001, respectively). This was not observed for iPSA and PSA. Overall, the AroCell TK 210 ELISA showed significant correlation with PHI (r = 0.32, P=0.0017) and pro PSA (r = 0.21 P = 0.044) but not with iPSA or PSA. Conclusions: This preliminary study has demonstrated that serum TK1 assayed with the AroCell TK 210 ELISA can differentiate between pre-cancerous and prostate cancer patients with a similar accuracy to that of pro PSA. Further clinical studies will establish the capacity of the AroCell TK 210 ELISA to complement PHI in distinguishing between pre-cancerous and prostate cancer, potentially providing another tool in prostate cancer management.
or regressed (<30% growth). From 65 resected polyps, formalin-fixed paraffin-embedded material was retrieved and reviewed by an expert pathologist (GAM). Of these polyps, 48% (31/65) progressed, 41% (27/65) remained stable and 11% (7/65) regressed. Seventy-two percent (47/65) were tubular adenomas, 14% (9/65) tubulovillous adenomas, 3% (2/65) sessile serrated lesions and 11% (7/65) hyperplastic polyps. Initially, 76% of CNAs per sample was 0.8 (range 0-8), the most frequently observed was 13q gain (20%). No significant differences in number, or regions of CNAs were observed between polyps that progressed and the ones that did not change or diminished in size (stable or regressed). Larger polyps showed more CNAs, of which specifically 13q gain was more often present (43% that did not change or diminished in size (stable or regressed). Larger polyps were analyzed with low-coverage whole genome sequencing (HiSeq, Illumina, San Diego, CA, USA). DNA copy number status was called with the R-package {QDNAseq}. Results: From 50/68 resected polyps, originating from 38 individuals (mean age of 66.8 years (s.d. 7.0); 63% male), sufficient DNA was available. The mean surveillance interval of these polyps was 3.27 years (s.d. 0.29). The mean number of CNAs per sample was 0.8 (range 0-8), the most frequently observed was 13q gain (20%). No significant differences in number, or regions of CNAs were observed between polyps that progressed and the ones that did not change or diminished in size (stable or regressed). Larger polyps showed more CNAs, of which specifically 13q gain was more often present (43% in ≥10mm and 8% in <10mm polyps). Of note, no serrated lesions were ≥10mm. No differences in CNAs were found between the different histological subtypes. Conclusions: In a series of colorectal polyps left in situ for an average 3.27 years, no difference in prevalence of DNA copy number alterations was observed between polyps that volumetrically progressed and those that did not. Of the CNAs observed, 13q gain was most prevalent and found in 43% of the ≥10mm polyps. 1. Stoop, E. M. et al. Participation and yield of colonoscopy versus non-cathartic CT colonography in population-based screening for colorectal cancer: A randomised controlled trial. Lancet Oncol. 13, 55-64 (2012).

#717 Diagnosisis of basal like breast cancer using DNA methylation markers at the promoters of long non-coding RNAs

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Background: Patients with basal-like breast cancer (BLBC) have poor overall survival and suffer a high rate of metastasis to the brain or lung within three to five years of initial presentation. Absence of a cure for advanced BLBC warrants early detection of BLBC, which might save more lives, especially those of women of African ancestry who are disproportionately affected by young onset BLBC. Ablant DNA methylation is frequently observed in BLBC. DNA methylation is the most robust epigenetic mark and can be analyzed using clinical specimens including FFPE, tumor biopsies and liquid biopsies. Because expression of long non-coding RNAs (lncRNAs) is controlled temporally in response to neoplastic stimuli, we investigated the potential for lncRNA promoter methylation marks to be used for detection and prediction of BLBC development and progression in African Americans. Methods: To identify lncRNAs dysregulated in BLBC, we performed a Ribo-Zero RNA-seq or microarray analysis on breast tissue isolated from African American (n=63) and European American patients (n=14). Differentially methylated and hydroxymethylated regions of lncRNA genes were identified in African American samples (n=30) using our chemical methods to assay 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC). The Cancer Genome Atlas (TCGA) datasets were utilized to validate our results. To functionally annotate lncRNAs, we knocked down lncRNAs using LNA-antisense oligonucleotides (ASO) and performed Caspase 3/7 assays, flow cytometry, and qRT-PCR. We also utilized the CRISPR-Cas9 editing tools to knock out lncRNAs. Results: LncRNAs displayed distinct expression patterns between tumors and normal breast tissues. Out of hundreds of lncRNAs specifically expressed in BLBC tumors, we selected two lncRNAs, lnc19 and lnc98, which represent, respectively, a significantly increased and decreased lncRNA in BLBC tumors, compared to normal breast tissues and other subtype tumors. Lnc19 is highly up-regulated (a 39-fold increase) whereas lnc98 is dramatically down-regulated (a 59-fold decrease) in BLBC tumors. Methylation analysis showed significantly lower levels of promoter methylation for lnc19 and higher levels for lnc98 in BLBC tumors. A significant inverse correlation between methylation and expression of lnc19 was observed. Depletion of lnc19 resulted in rapid cell death of BLBC cells, with increased sensitivity to chemotherapy drugs. The data suggests that chemo-resistance of BLBC might be partly due to lnc19 overexpression, which is mediated through epigenetic control. Conclusion: We identified two epigenetically dysregulated lncRNAs in BLBC tumors, which contribute to chemo-resistance of these tumors. Our findings on the regulation of lncRNAs by cytosine methylation raise the possibility of new epigenetic biomarkers for diagnosis and prognosis of aggressive BLBC tumors.

#718 Biomarker monitoring by quantitative MALDI imaging; application to the tryptophan-kynurenine pathway in immuno-oncology

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Tryptophan is an essential amino acid for cell proliferation and survival that can be metabolized through different pathways, a major route being the kynurenine pathway. The first and rate-limiting enzyme of this pathway is the indoleamine-2,3-dioxygenase 1 (IDO1), that is a natural endogenous molecular mechanism of immune suppression acting through modulation of the Trp degradation pathway. IDO1 inhibits T-cell functions, thereby contributing to chemo-resistance of these tumors. Our findings on the regulation of T-cell functions, activation of the regulatory T-cells, and inhibition of Natural Killer cells are among the important immunosuppressive effects of IDO1. IDO1 enzyme is proposed to have a therapeutic potential in immunodeficiency-associated abnormalities, including cancer. IDO1 over expression has been described in multiple cancer indications and IDO1 inhibitors are being developed to stimulate the anti-tumor immune response. The existing standard quantitative methods are based on the total level of Trp and its metabolites determined by LC-MS/MS analysis in human plasma, cerebrospinal fluid and brain. We describe the setup, development and application of a new method based on MSI to detect, localize, and quantify Trp and Kyn in biological tissues. This method allows both the study of the subcellular localization and the detection/quantitation of metabolites of interest in tumor tissues. In the present study, an experimental tumor model overexpressing IDO1 and its wild-type counterpart were implanted in mice. Tissue sections of different tumors were realized and used for MSI analysis. MALDI FTICR high resolution imaging followed by data analysis established an absolute number on tissue quantitation. Internal standards of tryptophan (Trp-d5) and kynurenine (Kyn-d4) metabolites were used for normalization. As expected, our results showed an increase in the expression of Trp and Trp metabolites in tumors compared to the normal tissue. Following, immunostainings of IDO1 and Trp-depletion sensor pathways were carried out. Overlaying images between the immunostainings and the molecular MS images allowed co-localization studies and underlined both the biology and the tumor heterogeneity. This study allowed us to highlight key metabolites of the Trp pathway that are responsible for the immunosuppressive tumor microenvironment. It illustrated the heterogeneity of tumor immune areas. The development of immunotherapies such as IDO1 inhibitors requires a deep understanding of the interplay between the immune system and cancer cells, these immune endogenous metabolites can now be followed by QMSI and as biomarkers towards enhancing immunotherapies efficiency.

#719 Pathobiological implications of Trefoil Factors in the progression and metastasis of pancreatic cancer

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Background: Pancreatic cancer (PC) is a highly lethal malignancy and the disastrous statistics warrants an urgent need to develop new diagnostic and prognostic marker(s) and therapeutic target(s) for its better management. Trefoil factors (TFF 1/2/3) are a family of small, stable molecules observed to co-express with mucin and secreted by mucus-producing cells that facilitate mucosal repair after injury. Recent studies have shown that TFF1 and TFF3 can differentiate between molecular subtypes of PC. Based on this, we hypothesized that differential expression of TFF1 and TFF3 could predict pancreatic intraepithelial neoplasia (PanIN) as well as the early stage of PC. The present study explores the diagnostic and functional significance of TFF in PC. Material and Methods: Immunohistochemistry was used to determine the expression status of TFF1 and TFF3 in early stage PC (EPC, Stage 1 and 2, n=47), early stage PC (EPC, Stage 1 and 2, n=78) and late PC (LP, Stage 2-3, n=69). RT-PCR and ELISA were used to detect TFF1 levels in serum. The diagnostic significance of TFF1 was evaluated in the sample set (n=331) comprising of benign control (BC, n=104), chronic pancreatitis (CP, n=47), early stage PC (EPC, Stage 1 and 2, n=78) and late PC (LP, Stage 2-3, n=69), RT-PCR and ELISA were used to detect TFF1 levels in PC cell lines and cell supernatants respectively. Combinant treatment with TFF3 was performed to observe the impact on of transmembrane (MUC4) and secreted mucins (MUC5AC) stability and expression. Results: Differential expression of TFF3 was observed in PanIN precursor lesion, pancreatic cancer tissue in comparison to normal pancreatic tissue (p < 0.0003). Furthermore, the TFF3 expression was significantly higher for well differentiated tumor in comparison to poorly differentiated cases (p = 0.006). For TFF1, expression was significantly higher in PanIN lesions compared to normal pancreas (p < 0.0001), and PC versus normal pancreatic tissue (p < 0.0001). In serum, significantly increased expression of TFF1 was observed in EPC serum samples compared to BC (p-value = 0.035) and CP (p-value = 0.031). In EPC cell lines cases, RT-PCR analysis revealed positive expression of TFF1 and TFF3 in the majority of the PC cell lines. Treatment of PC cells with recombinant TFF3 lead to differential expression of MUC4 and MUC5AC. Conclusion: Overall, both tissue and serum based studies show that TFF1 and TFF3 are differentially expressed in early PanIN lesions as well early stage of PC tissues compared to normal.
to normal pancreas. Overall, these results suggest a potential implication of TFF1 and TFF3 in PC pathogenesis and provide a basis for functional impact of these proteins. Therefore, we hypothesize that autoantibodies against these aberrantly overexpressed proteins may have the potential to serve as diagnostic biomarkers in certain type of cancers. In current study, we have identified 11 proteins, including EDRNBR, KRT4, PLAT, MSLN, WIFDC2, FS, FOXA1, AURKC, STC1, CAB39L, and SFTPA1, to further evaluate the diagnostic values of their corresponding serum autoantibodies in five types of cancers, including hepatocellular carcinoma, lung cancer, gastric cancer, breast cancer and ovarian cancer. Sera from 470 patients (94 per cancer type), 94 healthy patients and 192 autoimmune disease patients matched with normal controls were used in this study. Serum autoantibodies were detected by ELISA. Receiver operating curve (ROC) analysis was used to evaluate the diagnostic value of serum autoantibodies against these potential TAAAs. The data indicates that several anti-TAA autoantibodies can be found to have diagnostic value for certain types of cancers. Autoantibodies against PLAT can distinguish breast and ovarian cancer while autoantibodies against SFTPA1 and CAB39L can distinguish cancers both in male and female. However, none of these serum anti-TAAAs was found to be specific to a single type of cancer. In summary, HPA database has made systematic discovery of cancer biomarkers possible. Whether these in silicon biomarkers can be used to distinguish normal and cancer still needs to be evaluated in more serum samples from normal controls and cancer patients.

#720 Systematic discovery and evaluation of tumor-associated antigens (TAAAs) as biomarkers in cancer immunodiagnosis. Jianxiang Shi,1 Lu Zhang,1 Peng Wang,2 Hua Ye,1 Liping Dai,1 Pei Li,2 Chenglin Luo,2 Chuhua Song,2 Kaijuan Wang,3 Xiao Wang, Jin Ouyang,1 Zhenyu Ji,1 Jiayang Zhang,1 Zhengzhou University, Zhengzhou, China; 2The University of Texas at El Paso, El Paso, TX.

Proteins aberrantly over-expressed in tissues from nineteen types of cancers have been identified from the Human Protein Atlas (HPA) database. Among these proteins, some were overexpressed in several types of cancers while certain proteins were found only overexpressed in certain type of cancers. It is demonstrated that aberrantly overexpressed proteins might shed into the blood and trigger the immune system to produce autoantibodies during the process of oncogenesis. Autoantibodies are more stable and often have higher titer compared to autoantigens. Therefore, we hypothesize that autoantibodies against these aberrantly overexpressed proteins may have the potential to serve as diagnostic biomarkers in certain type of cancers. In current study, we have selected 11 proteins, including EDRNBR, KRT4, PLAT, MSLN, WIFDC2, FS, FOXA1, AURKC, STC1, CAB39L, and SFTPA1, to further evaluate the diagnostic values of their corresponding serum autoantibodies in five types of cancers, including hepatocellular carcinoma, lung cancer, gastric cancer, breast cancer and ovarian cancer. Sera from 470 patients (94 per cancer type), 94 healthy patients and 192 autoimmune disease patients matched with normal controls were used in this study. Serum autoantibodies were detected by ELISA. Receiver operating curve (ROC) analysis was used to evaluate the diagnostic value of serum autoantibodies against these potential TAAAs. The data indicates that several anti-TAA autoantibodies can be found to have diagnostic value for certain types of cancers. Autoantibodies against PLAT can distinguish breast and ovarian cancer while autoantibodies against SFTPA1 and CAB39L can distinguish cancers both in male and female. However, none of these serum anti-TAAAs was found to be specific to a single type of cancer. In summary, HPA database has made systematic discovery of cancer biomarkers possible. Whether these in silicon biomarkers can be used to distinguish normal and cancer still needs to be evaluated in more serum samples from normal controls and cancer patients.

#721 Minimally invasive test and composite biomarker for early detection of serous ovarian carcinoma. Keren Bahar-Shany,1 Georgina D. Barnabas,3 Limor Helpman,1 Ariella Yakobson-Siton,4 Tamar Perri,3 Ram Eitan,4 Jacob Korach,1 Keren Levanon1. 1Sheba Medical Center, Ramat Gan, Israel; 2Tel Aviv University, Tel Aviv, Israel; 3Meir Medical Center, Kfar Saba, Israel; 4Rabin Medical Center, Petach Tikva, Israel.

Background: Current screening programs for early detection of high grade epithelial ovarian cancer (HGOvC) among high-risk populations have failed to show improvement in HGOvC mortality, therefore these women are offered risk-reducing bilateral salpingo-oophorectomy (RRBSO) at 35- 40 years. Stratification of high-risk population, especially BRCA mutation carriers, may enable personalized risk counseling and individualization of timing of RRBSO. In most cases, the precursor lesions of HGOvC arise in the epithelium of the fallopian tube (FT) fimbriae rather than intra-peritoneally. It is therefore plausible that proteins, RNA or DNA from early-stage tumor cells may be identifiable in fluid samples obtained from the lumen of the gynecological tract, thus making it possible to identify curable, early stage lesions. Aims: (1) Test the feasibility of uterine lavage as a minimally invasive test for early detection of ovarian cancer, and (2) Identify novel early-detection biomarkers in the uterine lavage fluid (ULF). Methods: We developed a method for sampling of gynecologic tract fluid termed uterine lavage fluid (ULF), which is a simple, reproducible, low-cost office procedure that can be performed routinely during gynecologic follow-up visits. We have already collected ULF from 140 HGOvC patients and control women undergoing gynecological surgical procedures for non-malignant indications. Deep proteomic profiling of ULF is performed by isolation of microparticles from body fluids, followed by solubilization, trypsin digestion and high resolution mass spectrometric (MS) analysis (on the Q-Exactive MS). Machine learning algorithms have been used to extract a classifier that can predict the diagnosis of ovarian cancer. Results: Uterine lavage appears to be a feasible, low burden procedure. The MS approach has identified thousands of proteins in each ULF specimen, in a high throughput manner. The label-free quantification algorithm (MaxQuant) enables a quantitative comparison between samples from cases and controls. We have derived a 20-protein classifier with an area under the curve (AUC) of Receiver Operating Characteristics (ROC) curve of 0.91 at 20% error. The composite biomarker has been applied to an independent validation set with a negative predictive value (NPV) of 92% and positive predictive value (PPV) of 45%. Conclusions: A minimally invasive technique of uterine lavage to collect unique diagnostic samples, coupled with state-of-the-art proteomics methods, results in a highly sensitive and specific composite biomarker which may be developed in to a screening tool for early detection of serious ovarian cancer in high-risk populations.

#722 Circulating EBV microRNA BART2-5p helps to diagnose nasopharyngeal carcinoma < screen for high risk individuals precisely. Chen Jiang,1 Lei Li,1 Li Jiangchao,2 Shang-hang Xie,3 Su-mei Cao,4 Xin-yuan Guan1. 1The University of Hong Kong, Hong Kong, China; 2Sun Yat-Sen University Cancer Center, Guangzhou, China; 3Guangdong Pharmaceutical University, Guangzhou, China.

Nasopharyngeal carcinoma is an EB-virus associated malignant which is highly prevalent in Southeast Asia. If diagnosed early, the 5 year disease-free survival rate is about 90%. However, most patients have already developed local or regional invasion when diagnosed, which results in poor prognosis. So it is essential to develop biomarkers to screen high risk individuals or diagnose the disease at early stages. As nasopharyngeal carcinoma is closely related to EBV infection and microRNAs encoded by BART region (BamHI A Rightward Transcripts) is abundant in NPC, we propose to identify biomarkers from these BART microRNAs. To quantify the copy number of microRNAs in serum samples, we used Taqman Probe-based qPCR. After adding Spiked-in Control (Mixture of Cel-39,Cel-54, Cel-238) to each 100ul serum sample, we extracted total RNA and reverse transcribed microRNA into cDNA with corresponding Taqman primers. Meanwhile, microRNA mimics with known copy number were serial diluted and reverse transcribed along with the samples. qPCR was performed to quantify the level of microRNAs in these samples with diluted cDNA and Taqman Probe. All data was normalized by Spiked-in controls and exact copy number of each microRNA was calculated through the standard curve generated by the serial diluted microRNA mimics. Here, we screened 17 BART microRNAs in discovery stage and found BART 2-5p as a candidate biomarker. In training cohort consisting of 266 patients with nasopharyngeal carcinoma and controls from Hong Kong, the sensitivity, specificity and AUC is 93.2%, 89.9%, 0.976 respectively. The results are similar in validation cohort 1 constituted by 376 patients and controls from Guang Zhou. To evaluate the ability of BART 2-5p to distinguish preclinical NPC patients from healthy high risk individuals, we established a nested case-control study with serum samples prospectively collected from 10 NPC patients at least one year prior to their clinical diagnosis and 92 matched healthy high risk controls from a screening scheme conducted in South China. The sensitivity and specificity is 90.0% and 31.5%. Taken together, EBV microRNA BART2-5p is a promising biomarker to improve NPC diagnosis and target preclinical patients more precisely than serologic test.

#723 Improved detection of cancer specific serum exosomal apastaryl (as-paragynyl) beta hydroylase (HAAH). Mark A. Semenuk, Anokhi S. Cifuentes, Eleanor R. Ghanbari, Michael S. Lebowitz, Hossein A. Ghanbari. Panacera Pharmaceuticals, Inc., Gaithersburg, MD.

By means of an ELISA, we have found the pan-cancer biomarker HAAH to be co-expressed with serum exosomes primarily in cancers of the breast, lung, colon, and prostate. This new understanding of the HAAH target has led to a dramatically improved serological detection assay, and in turn diagnostic agent kit. In keeping with the multivalent particulate nature of exosomes, appropriately reformating the ELISA assay has yielded a simplified so-called simultaneous-homologous format. Currently in this format, a single monoclonal anti-HAAH antibody (FB50) serves both as microplate capture and as a biotinylated detector. All reagents, including peroxidase-streptavidin, are co-incubated in the FB50- coated microplate simultaneously with serum samples, in the absence of intervening sequential steps. This considerably shorter exosome-enabled ELISA format requires half the time and yields generally on average a two-fold or higher increase in detection of HAAH. This format was evaluated with over 100 banked serum samples from several early stage cancers. While increasing the sensitivity for HAAH in cancer samples, the improved detection and performance has not caused any undesirable increases in false positives among more than 30 normal serum samples from healthy subjects. The reagent kit components include recombinant HAAH calibrators, positive/negative controls, vialated detection reagents and a Mylar packaged FB50 pre-coated microplate. The simplified reagent kit format has yielded close inter-operator and day to day trending consistency with a limit of detection (LOD) of 3 ng/mL. Recognizing the exosomial nature of the HAAH target has resulted in some changes in blood sample procurement, sample processing, and sample shipping. Such field testing of the HAAH reagent kit was recently done with serum samples from 48
high risk or mildly symptomatic volunteers with general concerns about cancer. Upon testing these samples, 9 had an AHA LV value greater than 3.0 kg/m² (range 4.2 to 116.7) and 39 had less than 3.0 kg/m². Based on the post-analysis diagnostic determination, there were two false negatives and one false positive, hence an overall accuracy of 93.8%.

**Clínica de Investigación de la Neoplasia**

**Early Detection of Cancer**

**Clinical impact of modified telomerase-specific adenovirus-based identification of viable-peritoneal tumor cells in peritoneal lavage fluid in patients with potentially resectable pancreatic cancer.** Masahiro Tanemura,1 Kenta Furukawa,1 Soichiro Mori,1 Masahisa Otsuka,1 Youzo Suzuki,1 Mitsuoyoshi Tei,1 Toru Masuzawa,1 Kentaro Kishi,1 Yasuo Urata,1 Hiroki Akamatsu,1 Osaka Police Hospital, Osaka, Japan; 2Oncology BioPharma Inc., Tokyo, Japan.

Pancreatic cancer (PC) is a highly aggressive disease with dismal prognosis. Although only a surgical resection can offer the chance of a cure, the 5-year survival rate after a potentially curative resection have been reported to be a low as 10-30%. In PC, the presence of peritoneal carcinomatosis precludes the possibility of surgical cure, irrespective of the resectability of the primary tumor. Peritoneal lavage cytology (CY) is used widely in the diagnosis and staging of gastric and pancreatic cancer. Positive CY findings (CY+ in PC is defined as stage IV disease, however, the true value of CY+ for the patient’s prognosis remains controversial. We rise the question of whether CY+ status has predictive value for survival and early intra peritoneal recurrence. The aim of this study was to evaluate from a novel genetically modified telomerase-specific replication-selective adenovirus, expressing GTP (TelomeScan F35) in rapid detection of viable peritoneal tumor cell (v-PTC) dissemination of PC. This human clinical trial sought to determine if the presence of vitally detected, rare v-PTC predict peritoneal recurrence and patient outcome. This study was approved by the Osaka Police Hospital IRB. Patients with resectable cytologically or histologically proven ductal adenocarcinoma of the pancreas were enrolled. Peritoneal lavage fluid was harvested just after a laparotomy in 27 patients with PC. Half of the fluid was examined by cytology with papanicolaou staining and MOC-31 immunostaining and the remaining half was used to detect v-PTC with TelomeScan F35. To distinguish between leucocytes and cells with epithelial origin, cells were stained with anti-CD45 Ab. To further distinguish cells with primary tumor origin, cells were labeled with anti-CEA and anti-CA19-9 Abs. GTP-positive and CD45-negative, and either CEA- or CA19-9-positive cells were counted as v-PTC. Patients were followed after surgery to evaluate its clinical significance. Among 27 patients aged 57-91 years (16 males and 11 females), 3 were cytologically positive (CY+), other 3 were virally positive by TelomeScan F35 (v-PTC+). All 27 patients underwent a surgical resection (PD/DP/TP/H-PD = 13/7/6/1). One patient was double positive (CY+/v-PTC+), and postoperative peritoneal recurrence early occurred at 5 month after resection despite adjuvant chemotherapy. 2 were CY+, but v-PTC-, and no recurrence in the abdominal cavity were observed (0%). On the other hand, other 2 were CY+, but v-PTC+, and one of these 2 patients occurred local recurrence in the abdominal cavity (50%). Remaining 22 patients (CY-/v-PTC-) were observed with neither local recurrence nor distant metastasis. In conclusion, TelomeScan F35-based v-PTC detection may be an independent prognostic factor in patients with resectable PC and had close association with local or peritoneal recurrence.

**Feasibility and evaluation of exosomal RNAs as novel diagnostic biomarkers for high grade serous epithelial ovarian cancer.** Emily N. Prenninger,1 Xianzhi Lin, Rosario I. Corona, Dennis J. Hazellett, Beth Y. Karlan, Kate Lawson. Cedars-Sinai Medical Center, Los Angeles, CA.

Introduction: High-grade serous ovarian cancer (HGSOC) survival has been essentially unchanged over the last ten years despite new therapeutic strategies. In order to make a large-scale impact on overall survival, early detection strategies are imperative. Exosomes are cell-derived vesicles derived from multivesicular bodies or the plasma membrane that carry a variety of biomolecules, including protein, DNA, and RNA. Exosomal contents represent a novel and underexamined source of tumor biomarkers, in particular for HGSOC. This study aimed to first evaluate approaches to exosomal RNA purification and to then compare the exosomal RNA profiles from serum of patients with benign ovarian disease and HGSOC to determine if unique profiles exist, and to identify biomarkers useful for early detection. Methods: Cell free serum was obtained from patients in each of the following categories: HGSOC with early (n=24) or late stage (n=24) disease, benign ovarian masses (n=24) and healthy age-matched controls (n=24). We compared ultracentrifugation and a commercial precipitation solution to enrich and isolate exosomes. Exosomal enrichment was confirmed by both transmission electron microscopy (TEM) and western blot using anti-CD63 antibodies. RNA was extracted for each and deep sequencing analysis was performed for 12 candidates within each group. A second cohort of 48 samples will be used for validation of the best performing candidate biomarker.

**Ultrasonic quantification of promoter methylation in cell-free circulating DNA for early detection of lung cancer.** Delphine Liss. NIH/NCI, Bethesda, MD

Lung cancer is the leading cause of cancer-related deaths worldwide. Early detection of lung cancer using Low Dose Computed Tomography (LDCT) screening has been shown to decrease the mortality rate. However, most nodules found are deemed to be benign upon further invasive testing. Thus, complementary minimally-invasive tests are being sought that will help discriminate malignant from benign nodules. Molecular biomarkers are increasingly becoming part of routine clinical practice for the diagnosis, prognosis or prediction of treatment response, with improved disease management and survival outcomes. Cell-free circulating DNA (cfDNA) in body fluids, including serum, plasma and urine, has recently emerged as a surrogate for tumor DNA. In addition to providing a minimally-invasive source of tumor DNA, cfDNA reflects molecular alterations and tumor heterogeneity. Epigenetic changes, including DNA methylation, occur early in carcinogenesis. Cancer cells are characterized by global hypomethylation and hypermethylation of Cpg islands in gene promoter regions. Analysis of tumor-specific DNA methylation in cfDNA is a promising strategy for applying epigenetic biomarkers to the detection of cancers at an early-stage. In a prior genome-wide analysis of DNA methylation, we identified a locus methylated de novo in fresh frozen tumor tissues resected from stage I lung cancer patients, that had high discriminatory power to distinguish tumor from non-tumor tissues in multiple patient cohorts. High promoter methylation was also associated with shorter cancer-specific survival. The present study aims at evaluating the diagnostic significance of promoter methylation in cfDNA, and the prognostic value in formalin-fixed paraffin-embedded (FFPE) tissues. We developed a methylation-specific droplet digital PCR (ddPCR) assay to quantify rare methylation events. DNA was subjected to bisulfite treatment to convert unmethylated cytosine residues to uracil. We designed specific primers and probe containing CpGs to only amplify the methylated promoter. Experimental conditions were first optimized using fully methylated and unmethylated control DNA samples, DNA extracted from lung cancer cell lines, germline cells and lung tissues (paired tumor and non-tumor). The ddPCR assay has a limit of detection of 30 haploid genomes equivalent of methylated promoter DNA, and a limit of quantification of a single methylated allele present at 0.2%, (i.e., 1 methylated copy among 500 unmethylated copies). We demonstrated the assay linearity, reproducibility and specificity for the methylated locus. Differences in methylation levels between tumors and adjacent tissues were also observed. We have thus established a robust and ultrasensitive method for standardized determination of promoter methylation status in cfDNA. We are currently evaluating its potential value for noninvasive diagnosis and prognosis of lung cancer patients.
phosphatase (ALP) or immunoperoxidase (IMP) substrate and contrasted reactivity in LC patients and asymptomatic controls with no advanced polyps, and chemiluminescent dot blots with manual or Bel-blotter 96-well replicating tool. Results: Saliva IMP testing was positive in 73% of 11 LC patients and 50% of 8 controls contrasted with ALP ELISA for stool Adnab-9 in 75% of 12 patients and 32% of 6 controls (OR 0.07). The peroxidase absorbance means (SD) were significantly different (0.077 [0.014] versus 0.116 [0.056]; p < 0.007). Equivalent inherent alkaline phosphatase of saliva samples was negative in both groups and means were not significantly different. The approximate time from saliva collection to diagnosis of LC was 3.76 years and 3.89 for stool. Conclusions: Adnab-9 sensitivity was moderate but promising due to the ability to make an early preclinical diagnosis. While this was only significantly different from controls in stool ALP ELISA, inherent IMP activity could be blocked to improve specificity. Significantly suppressed inherent peroxidase activity in LC saliva may explain the insensitivity of the Bac18.1 and Cox2 inflammatory biomarkers. The Bel-blotter volume capacity is 4-10µl/blot and may explain the lower sensitivity using this tool. A battery of tests, including Adnab-9 in an ALP ELISA format may allow for early disease intervention.

#728 Development and validation of ColoScape™ - a new colorectal cancer mutation detection assay. Michael J. Powell, Elena Peletskaaya, Anne Vallegra, Qing Sun, Larry Pastor, Aiguo Zhang. DiaCarta, Richmond, CA.

Introduction: Colorectal cancer is a highly preventable disease as early detection increases rates of patient survival to near 100%. Herein we report the development and validation of a novel multigene mutation biomarker real-time PCR based assay for qualitative detection of colorectal cancer associated biomarkers that comprise tumor specific mutations in the following genes: APC (Exon 15), KRAS (Exon 2), BRF (Exon 15) and CTNNB1 (Exon 3) called ColoScape™ TM. The assay allows the sensitive detection of the presence or absence of mutations in the targeted regions of the genes interrogated. Methodology: The high sensitivity of this multigene biomarker assay is achieved due to xenonucleic acid (XNA) probe technology. XNA probes are novel backbone modified oligomers having natural nucleoside bases (A,T,C and G) that hybridize by Watson-Crick base pairing to natural DNA and RNA with much higher binding affinity than natural deoxyribonucleic acid oligomers of the same sequence. XNA probes are designed that bind to the selected wild-type sequences at the respective genetic loci in the target genes. These XNA probes have a much higher Tm than the primer annealing temperature and suppress amplification of WT DNA templates and only allow amplification of the target mutant DNA templates in the sample. Both single nucleotide polymorphisms (SNPs) and insertion/deletions (indels) mutations can be detected. For each of the selected mutation sites primers and FAM-labeled TaqMan probes were designed and the together with the selected XNA oligonucleotides and analytical assay performance confirmed. An internal PCR control amplicon selected in the Human β-Actin (ACTB) gene was employed utilizing a HEX-labeled TaqMan probe. Performance parameters of the assay were established on DNA of colorectal cancer patients extracted from FFPE as well as reference DNA materials (synthetic and cell line derived DNA). The assay demonstrates high sensitivity and specificity in detection of colon cancer and adenoma samples based on the set of biomarkers involved in colorectal cancer neogenesis and disease progression. Conclusion: The ColoScape™ TM Colorectal Cancer Mutation Detection assay is shown to be a sensitive tool intended to facilitate research in colon cancer development, early detection, disease monitoring and therapeutic interventions.


DCLK1 expression is critically required for maintaining growth of human colon cancer cells (hCCCs). We and others recently reported that the 5’ promoter of DCLK1-gene gets hypermethylated and silenced in human colorectal tumors (hCRCs). We and others recently that the 5’ promoter of DCLK1-gene gets hypermethylated and silenced in human colorectal tumors (hCRCs) during adenoma-carcinoma sequence of colon carcinogenesis (Reviewed by Singh et al. SCL 2016). We also made the discovery that hCRCs and hCCCs express a novel short isoform of DCLK1 (DCLK1-S) (isoform) from β-promoter in intron V of DCLK1-gene, while normal-colons express the canonical long-isoform (DCLK1-L) (isoform) from 5’-α-promoter, suggesting that DCLK1-S, and not DCLK1-L, marks cancer-stem-cells (CSCs) (O’Connell et al, Sci Rep, 2015). Even though DCLK1-S differs from DCLK1-L in exon 9 (38% of non-CRC) sequences, DCLK1-S is specific to DCLK1-S Antibody (PS41014), which does not cross-react with DCLK1-L and specifically detects DCLK1-S in several assay platforms, including westerns and IHC. Sub-cellular localization of S/L isoforms was examined by immune-electron-microscopy (IEM). Surprisingly, besides plasma membrane and cytosolic fractions, S/L also localized to nuclear/mitochondrial fractions, with pronounced localization of S isoform in the nuclei and mitochondria of hCCCs. Sporadic CRCs develop from adenomas (Adns). Screening colonoscopy is used for detection/resection of growths, and morphological/pathological criteria are used for risk assessment and recommendations for follow-up colonoscopy. But, these features are not precise and majority of the patients with adenomas will never develop CRC. We hypothesized that antibody-based assay(s), which identify CSCs, will significantly improve prognostic value of morphological/pathological criteria. We conducted a pilot retrospective study with PS41014-Ab, by staining archived adenoma specimens from patients who developed (High-risk) or did not develop (Low-risk) adenocarcinomas within 10-15 years. PS41014-Ab stained adenomas from initial and follow-up colonoscopies of high-risk patients, at significantly higher levels (3-5X) than adenomas from low-risk patients. A battery of tests, including DCLK1-S Antibody could be the gold tool for assessing CRC-risk. We previously reported that CRC-patients, whose colonic tumors were positive for relatively high levels of DCLK1-S expression by qRT-PCR, had a worse overall survival and disease free interval than low-expressers (O’Connell et al, Sci Rep 2015). We now report that DCLK1-S Antibody may help to identify patients at high-risk for developing CRCs within 10-15 years, at the time of index/screening colonoscopy, and thus serve as a useful biomarker at early time points of colon carcinogenesis, unlike the currently available fecal/blood tests.

#730 High sensitive detecting procedure of circulating repetitive RNA as novel early marker of pancreatic cancer. Takahiro Kishikawa, Motoyuki Ot-suka, Kazuhiko Koike. University of Tokyo, Tokyo, Japan.

Pancreatic ductal adenocarcinoma (Pdac) is one of the most intractable malignancies due to difficulties in early detection. Although promising biomarkers are increasingly reported, such methods are not yet easy to apply clinically, mainly due to their low reproducibility or technical difficulties. In this study, we developed a convenient and sensitive method for quantifying aberrantly expressed satellite repeat RNAs in sera, which can be used to efficiently detect patients with Pdac. It is difficult to correctly quantitate repeat arrays because its repetitive nature makes it difficult to establish appropriate primers that amplify a single product using simple PCR procedures. Here, we introduce a Tandem Repeat Amplification by nuclease Protection (TRAP) method combined with droplet digital PCR (ddPCR) to detect human satellite II (HSAII) RNAs, which are specifically expressed in human Pdacs at greater levels than normal tissues. HSAII RNA core sequence levels in sera were significantly higher in Pdac patients than in controls (p < 0.001, 0.75 and 0.44 per µl, respectively). Additionally, patients with intraductal papillary mucinous neoplasm (IPMN), a precancerous lesion of Pdac, could also be efficiently detected. This method can be routinely applied to screen patients with Pdac and high-risk patients, facilitating the development of preventive medicine for this disease.

#731 Stool DNA testing of Fusobacterium nucleatum for detection of colorectal tumors. Yukata Suehiro, Kouhei Sakai, Toshikito Matsumoto, Takahiro Yamasaki, Yamaguchi Univ. Graduate School of Medicine, Ube, Japan.

Background: Accumulating evidence shows an overabundance of Fusobacterium nucleatum (Fn) in colorectal tumor tissues. Although stool DNA testing of Fn might be a potential marker for the detection of colorectal tumors, the difficulty in detecting Fn in stool by conventional methods prevented further explorations. Therefore, we developed a droplet digital PCR assay for detecting Fn in stool and investigated its clinical utility in the management of colorectal tumors in a Japanese population. Methods: Feaces were collected from 60 healthy subjects (control group) and from 11 patients with colorectal non-advanced adenomas (non-advanced adenoma group), 19 patients with colorectal advanced adenoma/carcinoma in situ (advanced adenoma/CIS group), and 158 patients with colorectal cancer (CRC) of stages I to IV (CRC group). Absolute copy numbers of Fn were measured by droplet digital PCR. Results: The median copy number of Fn was 17.5 in the control group, 311 in the non-advanced adenoma group, 122 in the advanced adenoma/CIS group, and 317 in the CRC group. In-com
parison with that in the control group, the Fn level was significantly higher in the non-advanced adenoma group, the advanced adenoma/CIS group, and the CRC group. Conclusions: This study illustrates the potential of stool DNA testing of Fn by droplet digital PCR to detect individuals with colorectal tumors in a Japanese population.

#732 Potentials of some serum proteins and urinary molecular biomarkers for early diagnosis of prostate cancer in Nigeria patients. Oluwemiyi Akinloye,1 Aniebietasbi S. Oboh,2 Taiwo A. Adewole2.1 University of Lagos, Lagos, Nigeria; 2Ladoke Akintola University of Technology, Osogbo, Nigeria.

Background: Prostate cancer (PCa) an adenocarcinoma is the most common cancer diagnosed in African men today. At present, the only widely accepted screening tools for prostate cancer are prostate specific antigen (PSA) and digital rectal examination. There is controversy regarding the appropriate level of serum PSA that should trigger a biopsy. This study is aimed at finding a better marker/panel of markers for prostate cancer. Methods: 150 consenting patients requiring a prostate biopsy and 100 age matched controls were recruited for this study. Genetic materials were found in 132 (88%) of the samples. Serum Total PSA (TPSA) and free PSA were assayed using ELISA method, % free/total PSA(%f/tpsa) was obtained statistically, prostatic volume was determined using TRUS. In addition, selected urinary RNA’s were assayed; transmembrane serine protease (TMPRSS2:ERG and TMPRSS2:ETS) fusion genes, PSA gene and PCA3 (prostate cancer antigen 3), using standard polymerase chain reaction (PCR) protocols. Results: TMPRSS2:ERG was detected in 9 (7%) of the samples and limited to biopsy positive for PCa. Similarly, TMPRSS2:ETS was found in only 4 (3%) of the samples and also restricted to biopsy positive for PCa. PCA3 score had the best discriminateing accuracy in diagnosing PCa amongst patients with serum Total PSA in the range of 4 - 10 ng/ml with AUC of 0.705 compared to Total PSA, f/tpsa ratio, and PSA Density which were 0.365, 0.695, and 0.541 respectively. At the cut off value of 24.6, PCA3 score yielded its best sensitivity of 0.615 and specificity of 0.630. At the cutoff of 0.14, PSA yielded its best 0.538, 0.704 respectively.

Direct logistic regression was performed to assess the predictability of PCA using different models comprising of three (3) covariates, the model comprising PCA3 Score, f/tpsa ratio and PSA Density had the best discriminative accuracy in the subgroup with PSA range of 4 - 10ng/ml, with the sensitivity, specificity, positive predictive value, and negative predictive values of 0.59, 0.93, 71.4% and 75.8% respectively, over models comprising PCA3 Score ,TPSA and %f/tpsa, and PCA3 Score, TPSA and PSA with these values (0.23, 0.85, 42.9 % and 70.1%), (0.39, 0.89, 62.5%, and 75 %) and (0.15, 0.85,66.7% and 67.6%) respectively. Conclusions: In predicting PCa amongst patients with serum total PSA in the grey area of 4 - 10 ng/ml, the model comprising PCA3 Score, f/tpsa ratio and PSA density had the best discriminative accuracy.

#733 Detection of HCC-derived major HBV integration junctions in urine and their implications for driver identification. Selena Y. Lin,1 Jamin D. Stevens,2 Yih-Ping Su,3 Surbhi Jain,2 Ting-Tsun Chang,3 Wei Song,1 Ying-Hsiu Su1,1 University of Hong Kong, Hong Kong; 2The Baruch S Blumberg Institute, Doylestown, PA; 3National Cheng Kung University Medical College, Tainan, Taiwan.

Chronic Hepatitis B Virus (HBV) infection is a major etiology of hepatocellular carcinoma (HCC), a leading cause of cancer mortality worldwide. Integration of HBV DNA into the host genome occurs during the course of chronic infection. Integrated HBV DNA is observed in most of HBV-associated HCC. The integration junctions derived from the original tumor cell become abundant (referred to as major integration junctions) in the infected liver because of clonal expansion during tumor development. Detection of circulating DNA containing these tumor-derived integration junctions may therefore be useful for cancer detection. We have adapted a method known as Primer Extension Capture (PEC) to enrich integrated HBV DNA for next-generation sequencing (NGS). Our initial studies using this approach to enrich the HBV DR1-2 region (a common site for HBV integrated breakpoints) identified major integration junctions from HBV-HCC tissue samples and matched urine. Further analysis revealed that most recurrently targeted integrations from these HCC tumors have previously reported involvement in cancer. This suggested that identification of recurrently targeted genes is applicable for driver identification. Interestingly, we show how HBV targets the TERT promoter in a localized region even though no two TERT junctions examined are identical. We have further developed this PEC to enrich for the entire HBV genome and applied it to (i) liver tissue DNA from 20 matched HCC and adjacent non-HCC samples, and (ii) DNA from urine of 20 hepatitis, 20 cirrhosis and 20 HCC patients. The HBV enriched libraries were sequenced by NGS and the integration events were analyzed using the in-house developed software. The complexity of HBV junction sites in HCC and non-HCC tissue and urine derived circulating DNA is reported. Our approach has potential to be used for liquid biopsies to study the complexities of HBV integration in chronic HBV infection and carcinogenesis, and to identify HCC-related DNA modifications for early detection and disease management.

#734 Improved detection of salivary glands’ RNA markers in saliva samples. Adrian D. Schubert, Evgeny Izumchenko, Piotr T. Wysocki, David Sidransky, Mariana Brait. Johns Hopkins University, School of Medicine, Baltimore, MD.

RNA based liquid biopsy in saliva could be part of the diagnostic process and surveillance in patients with Salivary Gland Tumors. There is no established approach for saliva markers in salivary gland tumors. These tumors occur approximately in one out of 100,000 adults per year in the USA. We aim to develop a method to identify RNA shed specifically from the salivary glands. The two most common salivary gland malignancies harbor frequent gene fusions, which may be candidate RNA based markers. In mucoepidermoid carcinoma, the MECT1-MAML2 fusion. In adenoid cystic carcinoma, either a MYB-NFIB or MYB-NFIR fusion. We identified collection and processing methods to be tested on healthy individuals’ saliva samples. We compared three saliva stimulation methods: Chewing Gum, Tabasco and Vitamin C powder with unstimulated collected saliva; and also utilized different processing and RNA extraction procedures: Trizol based: QIAzet method (Qiagen), RNeasy saliva protecting reagent (Qiagen), OrageneRNA (DNA Genotek), and mirVana Kit (ThermoFisher). We quantitated the RNA quantity and quality with Spectrophotometer (NanoDrop) and Agilent’s 2100 Bio analyzer. We identified two salivary gland specifically highly expressed genes (HTN3 and CA6e) to be analyzed by quantitative RT-PCR. We have collected and processed 221 samples and analysis is ongoing. Bio analyzer showed that RNA from the unstimulated collected saliva has a higher concentration than stimulated saliva. Unstimulated saliva is expected to contain mucosa cells, bacteria and rests of food, therefore those could also be RNA sources. When saliva’s RNA is protected by either of the buffers tested, the RIN scores (RNA Integrity Number) obtained by Bio analyzer are higher. Based on our preliminary data, stimulation with either Tabasco, Chewing Gum or Vitamin C followed by RNA stabilization with the OrageneRNA kit and extraction with Trizol resulted in the highest quality and specificity of the RNA for its salivary gland origin. Unstimulated collection followed by the same processing generates a greater RNA yield. This saliva RNA is likely contaminated with RNA from squamous cells, bacteria and food residues, which may decrease the sensitivity of the specific gene expression of HTN3 and CA6e. Once established how to best collect and protect saliva for the extraction of nucleic acids originated from the salivary glands, we anticipate accelerating the path to clinically highly demanded kits for the surveillance of salivary gland cancer patients. We plan to prospectively collect samples from Salivary gland tumor patients with the chosen method and evaluate tumor related markers. The detection of tumor markers in bodily fluids will open a new avenue for the diagnosis and the clinical management of patients with this type of tumor. Moreover, our work creates the possibility of a liquid biopsy-based detection of several disease-specific alterations identified in different types of salivary gland tumors.

CLINICAL RESEARCH: Early Detection

CLINICAL RESEARCH: Molecular Diagnostics

#735 HER2 protein quantification in multiple cancer indications identifies candidates for HER2 targeted therapies. Shankar Sellappan,1 Sarit Schwartz,2 Ellen Werthermeier,1 Fabiola Cecchi,3 Steven W. Mamas,3 Daniel VT Catencaci,3 Todd Hembrough1.1 Dept of Pathology, University of Chicago, Chicago, IL; 2Dept of Medical Oncology, Rockville, MD; 3Cancer Center of Sarasota-Manatee, Sarasota, FL; 4University of Chicago, Chicago, IL.

Background: Anti-HER2 therapy in cancer indications other than breast and gastric cancers is the subject of ongoing clinical trials. We previously used mass spectrometry to identify HER2 levels and reveal high HER2 levels in indications other than breast and gastric cancers, respectively. These studies also demonstrated that trastuzumab-treated patients whose tumors express high levels of HER2 (≥ 2200 and ≥ 1825 amol/µg in breast and gastric cancers, respectively) survived much longer than patients with lower HER2 levels. We hypothesized that targeted proteomic workflows reveal high HER2 levels in indications other than breast and gastric cancers, thus identifying patients likely to benefit from anti-HER2 therapy. Methods: We summarized results from samples processed in our CAP/CLIA-certified laboratory. Tumor areas from FFPE tissue blocks (N=3828) representing multiple cancer indications were marked by a pathologist,
microdissected, and solubilized to tryptic peptides. In each liquefied tumor sample, HER2 and other protein targets were quantified with mass spectrometry-based proteomic analysis. Results: HER2 superexpression (> 2200 amol/μg) was found in 0.64% (12/1891) of patients with non-breast, non-gastroesophageal cancers. Among indications with > 50 patients tested, the highest rates of HER2 superexpression were in gynecologic cancers (1/124: 2.4% in cervical cancer, 1/151: 2.0%). Treatment and outcome data are largely unavailable, but we are aware of 3 anecdotes: In a 74-year-old male with invasive adenocarcinoma of the gallbladder who had disease progression on gemcitabine + cisplatin, proteomic testing found high HER2 protein expression (3105 amol/μg). The patient responded to trastuzumab + FOLFIRI for 5 months. In a uterine cancer patient whose HER2 status was equivocal by genomic analysis, proteomics found high HER2 expression (4995 amol/μg). Proteomics also revealed the absence of a resistance marker for taxane (TUBB3) and high levels of the response marker for antifolate agents (FRα: 10 500 amol/μg). The patient responded to trastuzumab + taxol for 9 months before developing resistance and responding to trastuzumab + antifolate.

Lastly, a cervical cancer patient whose disease had progressed on chemotherapy showed HER2 superexpression (1132 amol/μg). She was treated with anti-HER2 combinations for > 12 months.

Conclusions: Patients with high HER2 protein expression as measured by targeted mass spectrometry in multiple cancer types have benefited from anti-HER2 therapy. Only small numbers of patients with non-breast, non-gastric tumors have HER2 protein levels indicative of survival benefit from anti-HER2 therapy. However, targeted proteomics offers simultaneous, precise quantification of other biomarkers (e.g., ERCC1, TUBB3, FRα) to guide therapy selection for multiple cancer types.

### #736 Cell lineage-oriented clinical sequencing unveils distinct clonal oncogenicity of acute myeloid leukemia with myelodysplasia-related changes.

Kazuki Yokoyama,1 Nozomi Yuza,2 Sousuke Nakamura,3 Mika Ito,4 Asako Kobayashi,4 Masayuki Kobayashi,5 Rika Kasajima,5 Hiroaki Yui,3 Eigo Shimizu,1 Atushi Niida,1 Rui Yamaguti,6 Tsuneo Ikemour,2 Seiya Imoto,4 Yoichi Fukuwak,2 Satoru Miyano,7 Arinobu Tojo8.

#736 Acute myeloid leukemia (AML) is characterized by unregulated clonal expansion and maturation arrest of myeloid committed progenitors (MP). AML generally represents de novo onset or evolves from preceding myelodysplastic syndrome (MDS), which is defined by refractory cytopenia, clonal hematopoiesis, and/or multi-lineage dysplasia. The WHO classification2008 includes this entity as “AML with myelodysplasia-related changes (AML-MRC)”, and currently, diagnosis of AML-MRC is based on either previous history of MDS, multi-lineage dysplasia, or MDS-related cytogenetic abnormality. However, AML-MRC often represents de novo onset without these MDS-compatible clinical features. Considering that AML-MRC exhibits rather poor prognosis with refractoriness to conventional chemotherapy against AML, more accurate and objective diagnostic approach is requisite to unveil hidden “MDS signatures” in patients with apparently de novo AML. A certain set of gene mutations is specific and recurrent in MDS. Given the pre-existing “MDS signatures”, the founder gene mutations might be detected in not only blast cells but also neutrophils and/or T cells in AML-MRC. To test this hypothesis, we performed FACS sorting of neutrophils, T cells, and blasts fractions, respectively, followed by mutation screening using targeted deep sequencing, namely, cell lineage-oriented sequencing (CLS). Genomic DNA both from each cell fraction and buffy coat was subjected to screening mutations in 54 genes which are tightly involved in MDS and AML. Pair-end deep sequencing was performed on an Illumina MiSeq, using library prepared by TruSight Myeloid Panel (Illumina, San Diego, CA). Based on previously published criteria,30 we analyzed and compared CSR and/or CLS of clinically diagnosed AML-MRC (n = 7), suspected AML-MRC (n = 2), de novo AML (AML with t(15;17) or AML with inv16, n = 4), MDS (RAEB-1 and RAEB-II, n = 3), and familial MDS (n = 1). As expected, in a familial MDS case, overlapping germline RUNX1 driver mutation was demonstrated in granulocytes, blast cells and T cells, supporting that it would be originated from a hematopoietic progenitor. In MDS, AML-MRC, and suspected AML-MRC cases with no germ-line mutations, the founder mutations present in neutrophils were also retained in the AML blast cells, irrespective of a history of MDS, suggesting that these are derived from a myeloid progenitor cell. In marked contrast, there were no overlapping driver mutations between blast cell and neutrophil fractions in de novo AML characterized by recurrent chromosomal abnormalities. In summary, CLS revealed that founder mutations are shared by neutrophils and AML blast cells in AML-MRC, but not in de novo AML, although our data should be validated in a larger cohort of AML cases, CLS is a promising approach to molecular diagnosis of latent AML-MRC which requires distinct therapeutic options from de novo AML.

### #737 Significant DLX4 expression in inflammatory breast cancer from African American patients.

Jaehong Jeong,1 Tämmy Naab,2 Martin Ongkoko,3 Kephur Makambi,1 Farhan A. Khan,2 Jan Blanctao,1 1Georgetown Univ., Washington, DC; 2Howard Univ., Washington, DC.

Purpose: To examine protein expression characteristics of Distal-less homeobox 4 (DLX4) in the inflammatory breast cancer (IBC) cohort from an African-American population to determine if a) DLX4 over-expression occurs in IBC vs. normal mammary tissue and b) over-expression is associated with clinicopathologic features such as ER, PR, HER2. Experimental Design: Twenty nine blocks of formalin-fixed paraffin-embedded (FFPE) tissues from well-characterized human IBC cases were used for immuno-histochemical staining (IHC). Tumor tissues were from African American subjects seen at Howard University Hospital. Normal breast tissues from 30 mammoplasties were controls. IHC results were assigned intensity and percentage scores based upon intensity of positive staining and % of stained cells. Percentage scores were assigned as 0, 1 (0%-25%), 2 (26%-50%), 3 (51%-75%) or 4 (76%-100%) and intensity scores were assigned 0, 1, 2, 3, or 4. For the analysis of the IHC, a percentage score of 3 or 4 was considered high and an intensity score of 2 or 3 was categorized as high, Chi-square and Fisher’s exact tests were used for analysis. Results: The staining pattern for the categorized cohort in which 82.8% (24 out of 29) of IBC cases showed high percentages of positive cells staining for DLX4 protein, while 40.0% (12 out of 30) normal breast tissues demonstrated DLX4 expression (P < 0.001). In terms of staining intensity, 75.9% (22 out of 29) of IBC cases showed a high level of intensity, compared to 20.0% (6 out of 30) of normal breast tissues (P < 0.001). In terms of the association between breast cancer characteristics and IHC staining, intensity of DLX4 was higher in HER2+ (87.0%, 20 out of 23) than HER2+ (33.3%, 2 out of 6; P = 0.018). Other associations were not statistically significant in this pilot study. Conclusions: DLX4 expression is significantly higher in this pilot study of IBC cases from AA patients than in normal breast tissue cases. In addition, HER2+ is associated with high intensity of DLX4 expression in IBC.

### #738 Highly multiplexed and precisely calibrated reference materials for copy number variation detection.

Catherine Huang, Yves Konigshofer, Jessica Dickens, Bhagath Anuvelil, SeraCare Life Sciences, Gaithersburg, MD.

Introduction: Genomic instability is a hallmark of cancer, and copy number variation (CNV), including gene duplication and deletion, can be a key driver of oncogenesis. Detection of CNV is needed for personalized treatment, and may involve microarray analysis or Next Generation Sequencing. Often, DNA from patient-derived cell lines is used as positive control material in these assays. However, these materials are often poorly characterized, may change over time, and have additional confounding genetic alterations. Here we describe the development of new reference materials that are highly characterized by digital PCR and can aid in the validation and calibration of new diagnostic assays for CNV. Methods: The GM24385 reference cell line has been extensively characterized by the Genome in a Bottle project1 and originates from a participant in the Personal Genome Project. DNA from this cell line was used to make a library of full-length gene inserts. The library had >12X coverage of the GM24385 genome and an average insert size of ~135 Kb. PCR based screening was used to identify and isolate full length MYC, FGFR3 and ERBB2 genes. Next Generation Sequencing, using Illumina Nextera library preparation kits and a MiSeq instrument, was used to verify the gene of interest. The genomic DNA and gene inserts were then amplified into reference materials, in purified DNA format to amplify the copy numbers of these key genes. Digital PCR was used to control the mixing and verify the number of copies of genes and/or mutations within the reference material. Results: The sequence confirmed genes were formulated into GM24385 genomic DNA at levels consistent with 5X and 10X amplification.
Testing on the ArcherDX VariantPlex™ Solid Tumor Kit indicated that each amplification could be detected at the expected level. Conclusions: Highly characterized and consistently manufactured reference materials for copy number detection are currently lacking. The described Seraseq CNV Reference Materials provide proof of concept for a biosynthetic, engineered material that uses digital PCR as an orthogonal reference method and provides a “ground truth” copy number level for key oncogenes.

#740 Rapid detection of IDH1/2 mutations using RNaseH2 dependent, multiplex quantitative PCR assays. Yun Baoo,1 Aymen Baig,1 Tu Wang,1 A John Istrate,2 Caliu Chen,1 Darrell R. Borger.1 Integrated DNA Technologies, Redwood City, CA; 2Massachusetts General Hospital Cancer Center, Boston, MA.

Isocitrate dehydrogenase 1 (IDH1) and 2 (IDH2) are enzymes that normally catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate during glucose metabolism. Heterozygous mutations in the IDH1 and IDH2 genes have been identified in a specific subset of cancers. Mutations typically affect the enzyme active sites at codon R132 in the IDH1 gene, and R140 or R172 in the IDH2 gene. This confers neomorphic enzyme activity that produces the oncometabolite 2-hydroxyglutarate that is involved in cancer initiation and progression. The detection of IDH1/IDH2 mutations has important diagnostic, prognostic, and therapeutic implications and testing for these variants is being incorporated in clinical settings. In fact, the identification of IDH1 or IDH2 gene mutations is now required for the classification of tumors of the central nervous system under the 2016 WHO guidelines. Therefore, there is a significant need for an IDH1 + IDH2 test that can be performed in a rapid manner to support clinical diagnosis and treatment decisions. A strategy utilizing allele-specific quantitative PCR (qPCR) is well suited for evaluating a small number of hotspot variants in IDH1 and IDH2 from clinical tissue samples in a matter of few hours. Using RNase H2 dependent PCR (rPCR) and a universal reporter system, we have developed new multiplex qPCR assays that effectively discriminate between closely related sequences that differ by only a single nucleotide base at the same position or adjacent position in a single tube reaction. In addition, the rPCR mechanism of the assays also greatly reduces the impact of primer-primer interactions, further improving the multiplex capability of the assays. During initial testing, accuracy, specificity and sensitivity were evaluated. Mutations present in patient samples at frequencies as low as 2% were identified with high reproducibility. The assays have been tested across 49 patient samples that included FFPE tissue, blood, and bone marrow aspirate with results that are concordant to those found using an orthogonal method of next generation sequencing. Our study showed that this assay provides a rapid method for detection of multiple IDH1 or IDH2 mutations in single rhPCR reactions with high accuracy, specificity, and sensitivity.

#741 Rapid detection of necrosis in breast cancer with ex vivo and in situ mass spectrometry analysis methods. Arash Zarrine-Alsar,1 Bindesh Shrestha,2 Alessandra Tata,1 Michael Woolman,1 Manuela Ventura,1 Nicholas Bardsley,3 Milan Ganguly,2 Howard Ginsberg,3 Jinzi Zheng,1 Emma Bluemke1.

The discovery of circulating tumor DNA (ctDNA) in the blood, urine and other bodily fluids of cancer patients has led to a new type of non-invasive method of characterizing cancer-causing mutations, the liquid biopsy. With NGS technologies becoming increasingly sensitive, down to a 0.1% Limit of Detection (LOD), they are rapidly gaining traction as a valid assay for cancer genotyping and have potential to direct cancer treatment plans. The wide-angle view provided by NGS panels, combined with dPCR’s zoom-in precision detection of DNA provide a comprehensive picture of a cancer’s genetic makeup. By applying these complementary techniques at the appropriate time based on the disease type and stage, cancer treatment will become quicker, more precise and more cost-effective in the future. NGS and digital PCR together provide a complete picture of the cancer genome. As part of our research, we wet-lab tested a subset of Rare Mutation Assays corresponding to the Oncomine ctDNA panel for next generation sequencing. Synthetic plasmid (GeneArt) carrying the mutation was spiked into wild-type genomic DNA to reflect a mutation rate of 0.1%. Wild-type genomic DNA was used as negative control. Thermal cycling was performed according to protocol for digital PCR using the QuantStudio 3D. In this study, we tested a pilot set of samples using AcroMetrix Oncology Hotspot Control and Horizon ctDNA Reference Standard with both NGS using Oncomine ctDNA panel and digital PCR with Rare Mutation Assays. Comparison of NGS and digital PCR results for the same sample showed excellent correlation in the low mutation range around 0.1%. This study confirms that digital PCR using QuantStudio 3D and Rare Mutation Assays is effective as a method for orthogonal validation of NGS data using the Oncomine ctDNA panel. Additionally, TaqMan Rare Mutation Assays offer a sensitive and precise solution for downstream mutation tracking over a time course. For Research Use Only. Not for use in diagnostic procedures.

CLINICAL RESEARCH: Molecular Diagnostics

#742 Comprehensive detection of all major classes of MET deregulation by Anchored Multiplex PCR and next-generation sequencing. Brian A. Kudlow,1 Josh Haines,2 Marc Bessette,2 Namitha Mano,1 Laura M. Griffin,3 Danielle Murphy,2 Robert Shoemaker,2 Jason Amsbaugh,3 Joshua Stahl1. ArcherDX, Boulder, CO; 1Ignita, San Diego, CA.

Introduction: Deregulation of the proto-oncogene, MET, confers an aggressive phenotype in a variety of human cancers, promoting proliferation, invasive growth and angiogenesis. MET deregulation can be driven by gene amplification, overexpression, exon 14 skipping, gene fusions and single nucleotide variants (SNVs), such as kinase-activating point mutations. MET is a target of intensive drug development efforts, although the various mutated forms of MET exhibit unique drug sensitivities. The input clinical sample types and protocols widely used in characterization of cancer in both imaging mode (to provide spatial information on cancer border) and profiling mode (to provide information on cancer type and subtype); all based on unique molecular profile associated with each cancer type and subtype. Current efforts in creating cancer molecular profile libraries will facilitate translation.

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#743 Orthogonal validation of oncomine ctDNA panel data with digital PCR using TaqMan Rare Mutation Assays. Vidya Venkatesh, Marion Liang, Kamini Varma, Yanchun Li, Kelli Bramlett, Dalia Dhingra, Richard Chien. Thermo Fisher Scientific, South San Francisco, CA.

The discovery of circulating tumor DNA (ctDNA) in the blood, urine and other bodily fluids of cancer patients has led to a new type non-invasive method of characterizing cancer-causing mutations, the liquid biopsy. With NGS technologies becoming increasingly sensitive, down to a 0.1% Limit of Detection (LOD), they are rapidly gaining traction as a valid assay for cancer genotyping and have potential to direct cancer treatment plans. The wide-angle view provided by NGS panels, combined with dPCR’s zoom-in precision detection of DNA provide a comprehensive picture of a cancer’s genetic makeup. By applying these complementary techniques at the appropriate time based on the disease type and stage, cancer treatment will become quicker, more precise and more cost-effective in the future. NGS and digital PCR together provide a complete picture of the cancer genome. As part of our research, we wet-lab tested a subset of Rare Mutation Assays corresponding to the Oncomine ctDNA panel for next generation sequencing. Synthetic plasmid (GeneArt) carrying the mutation was spiked into wild-type genomic DNA to reflect a mutation rate of 0.1%. Wild-type genomic DNA was used as negative control. Thermal cycling was performed according to protocol for digital PCR using the QuantStudio 3D. In this study, we tested a pilot set of samples using AcroMetrix Oncology Hotspot Control and Horizon ctDNA Reference Standard with both NGS using Oncomine ctDNA panel and digital PCR withRare Mutation Assays. Comparison of NGS and digital PCR results for the same sample showed excellent correlation in the low mutation range around 0.1%. This study confirms that digital PCR using QuantStudio 3D and Rare Mutation Assays is effective as a method for orthogonal validation of NGS data using the Oncomine ctDNA panel. Additionally, TaqMan Rare Mutation Assays offer a sensitive and precise solution for downstream mutation tracking over a time course. For Research Use Only. Not for use in diagnostic procedures.
from low-input clinical sample types, such as FFPE specimens. As MET deregulation can be driven by many different genetic aberrations, this allows for MET-based characterization of MET deregulation from a single sample.

#743 Detection of TERT C228T and C250T promoter mutations in melanoma tumor and plasma samples using novel mutation-specific droplet digital PCR assays. Brodieck Corfless,1 Gregory Chang,1 Samantha Cooper,1 Mahrukh Syeda,1 Iman Osman,2 George Karlin-Neumann,2 David Polsky,1 New York University School of Medicine, New York, NY; 2Bio-Rad Laboratories, Pleasanton, CA.

Purpose: Detecting mutations in the plasma of patients with solid tumors is becoming a valuable method of diagnosing and monitoring cancer. Mutations in 1 of 2 hot spots in the TERT promoter sequence are found in several cancers, including up to 85% of melanomas and the majority of cases that lack BRAF or NRAS mutations (about one-third of melanomas). Due to the high G-C content of the TERT promoter sequence these mutations can be difficult to detect using NGS approaches. We developed novel droplet digital PCR (ddPCR) assays to detect these 2 mutations with high sensitivity and specificity, and demonstrate the application of these assays in melanoma clinical samples. Methods: Assays were optimized using cell lines with Sanger sequencing-conﬁrmed mutations: glioblastoma A172 (C228T), and melanoma NYU12-126 (C250T). We varied assay designs and amplification conditions to optimize probe-based detection using the Bio-Rad QX-200 ddPCR system. Assay sensitivities and speciﬁcities at various DNA input levels were determined using serial dilutions with 3 replicate wells for each condition. Sensitivity is defined as the lowest mutant allele dilution for which the concentration interval did not overlap with that of the 0% mutant wells. We used normal and cancer-derived DNA sources of different quality (e.g. normal human DNA (Promega), cancer cell lines, plasma and FFPE-derived DNAs) with and without the mutations, and compared the efficiency of detection of amplions of 88, 113 and 163 base-pairs. We compared efficiencies to assays of similar size for RPP30, a housekeeping gene. Results: The assays showed greater sensitivity when higher amounts of DNA were analyzed. For C228T the limit of detection (LOD) of the mutant allele was 1%, 0.25% and 0.1% for 6.6ng/well, 33ng/well and 66ng/well well respectively; for C250T the LODs were 0.25%, 0.05% and 0.05% respectively. Using normal human DNA, the efficiency of the TERT assays averaged approximately 90% of that for RPP30 across assays of similar size, and no decrease in assay efﬁciency was observed as amplicon length increased. In contrast, whereas amplicon size had only a modest effect on assay efﬁciency in plasma ctDNA, it gave a more pronounced effect on FFPE DNA’s, decreasing to 38% for the 163bp amplicon. We observed 100% concordance between TERT mutation detection by SNAPSHOT and ddPCR in 10 FFPE tumor samples, and in plasma samples from 4 metastatic melanoma patients with matching tumor samples. Conclusion: We developed robust ddPCR assays to detect TERT promoter mutations with high sensitivity and specificity. Mutated TERT DNA can be detected and quantitated in the plasma of patients with metastatic melanoma, and is likely to be present in the plasma of other cancer patients in whom TERT mutations occur.


The analysis of circulating cell-free tumor DNA (ctDNA), which can be obtained from plasma by non-invasive procedures, is expected to provide useful biomarkers in the management of non-small-cell lung cancer (NSCLC) patients. Indeed, several studies have assessed ctDNA prognostic and predictive value as source of key data for therapeutic targets and drug resistance in carcinoma patients. The expanding number of targeted therapeutics for NSCLC always represents a real-time wider tumor genotyping, and the use of ctDNA as either a complement or an alternative to tumor tissue DNA (tDNA) could be a valuable option for Next-Generation Sequencing (NGS) of key cancer genes. The isolation and enrichment of ctDNA is a big challenge because of its high degree of fragmentation and its low concentration against the normally occurring background of cell-free DNA derived from healthy cells. Therefore standardized methods for ctDNA extraction and analysis are crucial aspects in the setting-up of a molecular diagnostic approach. In this study we aimed to evaluate the ctDNA use for molecular profiling, also analyzing the impact of both pre-analytical and analytical variables on DNA yield and mutation detection. Matched tDNA and ctDNA from 30 NSCLC patients were extracted, quantified and quality-controlled, and then investigated by different standard methods (real-time PCR, digital PCR, Mass Spectrometry genotyping) for EGFR, KRAS, BRAF, PIK3CA status. Mutational screening of ctDNA samples by IonTorrent NGS (Oncomine) Lung ctDNA Assay, ThermoFisher was also performed. Furthermore, we extended ctDNA evaluation to additional 30 lung cancer patients with no available tumor sample. We found that cell free DNA concentration in plasma correlated with both stage and number of metastatic sites. Analyzing the ctDNA samples using NGS and ctDNA by ddPCR in 10 FFPE tumors and 10 plasma samples, we found mutations in EGFR, KRAS, PIK3CA genes, with an overall concordance of 77%. Interestingly, Oncomine Lung ctDNA assay detected these same mutations with the same allelic frequency of standard methods; mutations in TP53 and ALK genes were also found. In the 30 patients with no available tumor sample, we found EGFR (10%) and KRAS (7%) mutations on ctDNA by standard methods; NGS analysis is under evaluation. This study evaluated the use of multiple different methods to detect mutations in NSCLC and showed that ctDNA can be a feasible option for clinical monitoring of lung cancers, including for those patients who cannot undergo invasive diagnostic procedures, due to either comorbidities or absence of biopsiable tumor lesions.

#745 Interleukin-13 conjugated quantum dots to identify glioma initiating cells and exosomes. Achuthamangalam B. Madhankumar,1 Oliver Mrowczynski,1 Brad Zacharia,1 Michael Glantz,2 Lichong Xu,2 Becky Webb,2 Christopher Siedlecki,1 Akiva Mintz,2 James R. Connor1.1New York University, New York, NY; 2Wake Forest University Baptist Medical Center, Winston Salem, NC.

High grade brain tumors like glioblastoma multiforme (GBM) and certain other brain metastatic cancers possess high tumor invasive and infiltrating properties making them harder to detect. Identification of cancer cells and exosomes secreted by them in the biofluids like cerebrospinal fluid (CSF) can be an ideal way of detecting the presence of residual cancer after therapy. In the current approach, a ligand conjugated quantum dot (QD) was utilized to specifically label the cancer stem cells and exosomes to identify the presence of IL13Rα2 a tumor invasive marker. Human glioma initiating cells were cultured in adherent form and were exposed to interleukin-13 conjugated quantum dots (IL13QD), which binds the glioma initiating cells expressing IL13Rα2 receptor. Similarly the exosomes isolated from cancer stem cell condition media and CSF was brieﬂy incubated with IL13QD, before analyzing their complexing pattern. Exosomes that were isolated from glioma stem cells and patients CSF were demonstrated to express IL13Rα2 receptor. Fluorescent microscopy, transmission electron microscopy and flow cytometry were performed on the exosomes after complexing with IL13QD. IL13QD was demonstrated to specifically bind glioma stem cells both in the monolayer and spheroid culture. The binding afﬁnity of the exosomes to the quantum dots were quantitatively and qualitatively conﬁrmed by atomic force microscopy (AFM). The morphology and size of exosomes before and after complexing with exosomes were conﬁrmed by electron microscopy and AFM. The density plot from the flow cytometry experiments with IL13QD-exosomes complex indicates a possibility to identify the tumor associated exosomes. Our experiment inferences that tumor targeted quantum dots can selectively bind with glioma stem cells and extracellular vesicles (exosomes) that are secreted by cancer cells. This binding proﬁle could be utilized to identify the expression of certain tumor promoting receptors and anti- genes on the surface of exosomes present in the CSF and cancer stem cells from the glioma patients. Experiments are in progress to identify the tumor initiating cells in an orthotopic mouse glioma tumor model after intravenous administration of wild type IL13 and certain high afﬁnity mutated IL13 conjugated quantum dots, by non-invasive way.

#746 TaqMan Rare Mutation Assay targeting the TERT promoter region. Marion Laig, Kanini Varma, Vidya Venkatesh. Thermo Fisher Scientiﬁc Inc., South San Francisco, CA.

The enzyme Telomerase maintains telomeres at the ends of chromosomes. The Telomerase Reverse Transcriptase (TERT) gene codes for the enzyme’s catalytic domain and is not expressed in normal somatic cells. As a consequence, normal cells acquire senescence by shortening of their telomeres during cell division and eventually undergo apoptosis. In contrast to normal somatic cells, expression of TERT is reinitiated in cancer cells causing escape from senescence and apoptosis by maintaining the telomeres. It has recently been shown that mutations in the TERT promoter region play a key role in regulating and reinstating TERT expression. 90% of cancers carry a mutation in the TERT promoter region. Mutations like C228T and C250T create new binding site for the E2 transcription-factor (ETS) transcription factor that regulates TERT expression. Experimental evidence showed that the ETS factor GA-binding protein, alpha subunit (GABPA) binds to the de novo ETS motif and activates TERT transcription in cancer cells. We undertook a project designing TaqMan Rare Mutation assays addressing mutations in the TERT promoter. These assays are
TaqMan SNP Genotyping assays that are optimized for use in digital PCR with the Applied Biosystems QuantStudio 3D. In digital PCR, partitioning the sample into many individual reaction wells facilitates detection and quantification of rare mutant alleles. TaqMan SNP Genotyping Assays ensure reliable discrimination of mutant and wild-type allele. This will enable easy and sensitive detection of KRAS and NRAS mutations in a broad range of applications. Sensitivity of the proposed assay is suitable for detection in liquid biopsy applications with cell free DNA (cfDNA). Assay design proved to be challenging due to high GC content and repetitive elements in this region of the TERT gene and required varying both the assay design and experimental cycling conditions. Template for wet-lab testing was synthetic plasmid carrying the mutation spiked into wild-type genomic DNA and tested with the Applied Biosystems QuantStudio 3D. The results with the proposed assay detecting the C228T mutation in the TERT promoter region. We showed that designing TaqMan Rare Mutation Assays for digital PCR is feasible for the challenging TERT promoter region. Work addressing additional mutations in this region is ongoing. For Research Use Only. Not for use in diagnostic procedures.

**#747** An improved fixative for histomorphology, recovery of nucleic acids and proteins. Stephen M. Hewitt,1 Joon-Yong Chung,1 Candice Perry,1 Kris Ylaya,1 William M-A Smith,1 Robert A. Star,1 National Cancer Inst., Bethesda, MD, 2National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD.

Neutral Buffered Formalin (NBF) has been the primary diagnostic fixative for histopathology for nearly a century. The limitations of NBF are well known, especially with reference to the analysis of RNA and proteins. Alternative fixatives have been proposed, but not been widely adopted. Recently, the US Environmental Protection Agency (EPA) classified formaldehyde as a carcinogen, further tilting the balance away from the continued use of NBF. We recently described a fixative based on 70% ethanol and phosphate buffered saline, called BE70. Using BE70 as the base fixative, we have developed a modular fixative in which different chemicals can be supplemented for “fit for purpose” applications. The addition of guanidinium salts to BE70, termed BE70G results in a fixative in which both proteins and nucleic acids are improved in quantity and quality after extraction from the paraffin block. The guanidinium replaces non-freazeable water, and prevents hydrolysis and oxidation of the biomolecules. Histomorphology is not disrupted at lower concentrations, with only alterations of hematoxylin intensity at higher concentrations. Studies are underway to evaluate the stability of RNA and protein in the tissue block as well as on cut sections. This fixative, BE70G has the potential to address a number of challenges facing the development of precision biomarkers, while preserving histomorphology, and improving the safety of pathology staff. Other formulations are currently being evaluated that may support applications for cytology and electron microcopy. The goal is a well characterized fixative, in which modifications to the chemistry are defined and can be applied to obtain the optimal pathology specimen for both histomorphologic and molecular analysis.

**#748** A rapid and accurate nucleic acid amplification and detection method for KRAS mutation testing in colorectal cancer specimens. Choong Eun Jin, Seung-Soon Yeom, Yong Shin, Seok-Byung Lim. University of Ulmbn College of Medicine, Seoul, Republic of Korea.

Colorectal cancer (CRC) is one of the most common type of cancers both men and women in worldwide. Fortunately, overall death rates of CRC have been decreasing for the last two decades due to the improvement of screening test assays that detect early-stage cancer and pre-cancerous polyps. Nevertheless, the most common treatment for CRC is surgery, because it may completely eliminate the cancer region. In case of the cancer with systemic metastasis, chemotherapy is required before or after surgery for primary or metastatic lesions. Among the regimens for the chemotherapy, both monoclonal antibodies (Cetuximab and Pantitumumab) against the epidermal growth factor receptor have been shown to improve survival for only patients with lack of RAS mutations. Thus, the KRAS gene mutations (codons 12 and 13) in CRC patients have been extensively studied as a strong negative predictive biomarker to indicate whether a CRC patient responds to the treatment. Therefore, testing the KRAS mutational status of tumor samples is becoming an essential tool for managing patients with CRCs. Although a myriad of nucleic acid testing methods have been developed to analyze the mutation status in the key regions of the KRAS gene of CRC, several obstacles still remain related to low sensitivity, time consuming, and required large instruments including thermal cyclers. Here, we present a novel nucleic acid amplification and detection method for KRAS mutations (G12D and G13D) testing that enable rapid and accurate detection. This method is based on combination of isothermal DNA amplification method and bio-photonic silicon sensor that can be detected the mutations in a label-free and real-time manner. The proposed method can detect the mutant cell present at 1% in a mixture of wild type cells, while both PCR and sequencing can detect the mutations in a sample containing approximately 30% of mutant cells. We used 60 tissue samples from CRC patients (22 samples with G12D mutations, 23 samples with G13D mutation, and 15 samples with no mutation) to compare the performance of the proposed method with with iPLEX Pro panel. We showed that the proposed method showed a value of 100% and 100% for sensitivity and specificity, respectively. The other hand, the sensitivity and specificity of PCR (95.5% and 100%) and sequencing (95% and 100%) were lower than that of the proposed method. Therefore, the proposed method was found to be a rapid (< 30 min), highly sensitive and specific method for KRAS mutation testing. We believe that this rapid and accurate method will enable proper treatment for CRC patients.


A recent technical advance in the mRNA in situ hybridization (mRNA-ISH) assay provides simultaneous signal amplification and background suppression with a unique probe design to achieve single molecule visualization. We assessed the utility of the mRNA-ISH assay as a diagnostic tool to detect anaplastic lymphoma receptor tyrosine kinase (ALK) mRNA in non-small cell lung carcinoma. We compared the mRNA-ISH assay with immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH). The study included 279 surgically resected lung adenocarcinomas and 44 transbronchial biopsies (TBB) adenocarcinomas. mRNA-ISH was conducted using the RNAscope 2.0 system which includes pre-designed detection probes for the tyrosine kinase domain of ALK mRNA. IHC was conducted on all of the 323 samples using ALK-specific antibodies. mRNA-ISH was performed on 279 surgical samples and 6 TBB samples. Break-apart FISH was used to assess cases that were mRNA-ISH or IHC positive. ALK protein expression was detected in 11/279 (3.9%) specimens. ALK mRNA was also detected by mRNA-ISH in these cases, and 9 of the 11 specimens (81%), were also positive by ALK FISH. Using the IHC results as a reference, the sensitivity and specificity of mRNA-ISH was 100%. In the TBB cohort, ALK protein expression was observed in 3/44 (6.8%) of specimens, in which ALK mRNA expression was also detected. ALK mRNA-ISH data were highly correlated with the IHC data, and ALK mRNA-ISH was able to identify every FISH-positive sample. We conclude that mRNA-ISH could play an alternative or complementary role in ALK genetic diagnosis in NSCLC.


Background: As cancer therapeutics are increasingly selected based upon molecular genetic information, having reliable, accurate, rapid and inexpensive methods for mutational analysis are extremely desirable. Circulating tumor cells (CTCs) allow non-invasive “liquid biopsy” access to intact cells for molecular analysis. Here we demonstrate the successful detection of mutations in model CTCs individually isolated from blood (AccuCyte® - CyteFinder® system, RareCyte) using MALDI-TOF Mass Spectrometry (MassARRAY, Agena Bioscience). Methods: Breast (MDA-MB-231) and lung (NCI-H1975) cancer cells with a set of known mutations were spiked into blood and processed by AccuCyte onto microscope slides and stained on an automated immunostainer. Slides were imaged using the CyteFinder digital fluorescence scanning microscopy and mCTCs were identified by positive nuclear, EpCAM, and cytokeratin staining, and negative CD45 staining. mCTCs and white blood cell (WBC) negative controls were picked from the slides and put into PCR tubes using the CytePicker® module. DNA from individual or small pools of cells (3-5) was amplified using the PicoPLEX® (Rubicon) whole genome amplification (WGA) kit; alternatively cells were lysed and directly entered into the ensuing iPLEX® Pro workflow. Specific regions surrounding 5 different mutations in each of the mCTC lines were amplified from the WGA product or the lysed cells and the products were detected and scored for the mutations using a single PCR reaction iPLEX® Pro panel that includes a combination of 10 common lung and breast cancer mutations using the MassARRAY® platform. Results: Five point mutations in four different genes (CDKN2A, EGFR, PIK3CA, and TP53) were measured in the NCI-H1975 lines and four point mutations in four genes (BRAF, KRAS, NF2, and TP53) were measured in the MDA-MB-231 cells by iPLEX® Pro chemistry on the MassARRAY® system. All mutations were accurately detected in the WGA single and pooled cell samples and most were also detected in cells that did not undergo WGA before PCR with with iPLEX Pro panel. Allelic frequency observed was consistent with

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known zygosity of the mutation. Conclusions: MassARRAY successfully detected mutations in single model CTCs that were individually picked from a blood sample processed by the AccuCyte - CyteFinder system. Integrating CTC isolation with MassARRAY may be a practical way to identify and monitor known cancer mutations non-invasively.

#751 Characterization of STAT3 activation in human prostate cancer. Marco A. De Velasco,1 Yui Hatatana,1 Yurie Kura,1 Naomi Ando,1 Kazuo Sakai,1 Koichi Sugimoto,2 Mashaio Nozawa,2 Kazuhiro Yoshimura,2 Kazuhiro Yoshikawa,2 Kazuto Nishio,1 Hirotsugu Uemura1. 1Kindai University Faculty of Medicine, Osaka-Sayama, Japan; 2Aichi Medical University, Nagakute, Japan.

Signal transducers and activators of transcription (STATs) were initially associated with cytokine signal transduction pathways but are now also recognized as key modulators of key survival processes in various cancers. Activation of STAT3 occurs by the binding of various cytokines to its receptors leading to the activation of the JAK/STAT3 signaling pathway. Interleukin-6 (IL-6) has been implicated in regulating growth of various malignant tumors. Activated IL-6 has also been shown to be elevated in the sera from patients with metastatic prostate cancer, and persistent activation of STAT3 is a common feature. To better characterize the potential role of JAK/STAT3 as a therapeutic target for advanced prostate cancer, we examined expression patterns of activated STAT3 in 111 cases of localized prostate cancer from patients who underwent radical prostatectomy. Association studies were conducted with clinicopathological characteristics and biochemical recurrence. We also analyzed the expression of IL-6/IL-10/STAT3 genes from RNA-sequencing data from the Cancer Genome Atlas (TCGA) breast and prostate datasets. We found a significant association between STAT3 expression and clinicopathological parameters. Patients with moderate-high p-STAT3 expression tended to experience shorter times to biochemical recurrence (median time, 15 months) compared to patients with low-negative STAT3 activity, (median time to recurrence not reached, P=0.064). Cluster analysis suggested a trend for decreased disease-free survival for patients with high IL-6/IL-10/STAT3 gene expression signature. Our data suggests an association between STAT3 signaling and prostate cancer recurrence and provides clinical evidence to support JAK/STAT3 as a potential therapeutic target for patients with advanced prostate cancer.

#752 Evaluation of VENTANA Benchmark ULTRA and Roche cobas® 480 analyzer for detection of EGFR mutations. Lorraine Stewart, Chunyan Liu, Judith Pugh, Heather Gustafson. Ventana Medical Systems, Tucson, AZ.

Research has shown non-small cell lung cancer (NSCLC) is the most common type of lung cancer in the world. Exon 19 deletion and L858R mutation in exon 21 account for approximately 90% of EGFR mutations in adenocarcinoma NSCLC1. Accuracy and sensitivity in detection of these markers aids the selection of patients with NSCLC for therapy with an EGFR tyrosine kinase inhibitor (TKI). This study demonstrates how validated Immunohistochemistry (IHC) and real-time PCR testing can be used to aid one another in detection of activating mutations with factors such as low tumor content in formalin-fixed, paraffin embedded biopsies in our CAP/CLIA laboratory. Evaluation of IHC detection was completed on the VENTANA Benchmark ULTRA Automated IHC/ISH slide stainer and PCR testing was performed using the Roche cobas® 480 analyzer. The IHC diagnostic markers selected were the two most common EGFR mutations, E746-A750del (SP111) and L858R (SP125). Sample preparation and real-time PCR testing was done using the cobas® DNA Sample Preparation Kit and cobas® EGFR Mutation Test v2 utilizing a custom, automated plate preparation program on the Perkin Elmer Emsg Express Platform. Analytical data from the BenchMark ULTRA platform staining and the cobas® 480 analyzer correlated in the majority cases. Out of 32 specimens tested, we observed a 100% concordance between IHC and PCR for L858R exon 21 mutation, whereas EGFR exon 19 deletion and concordance between IHC and PCR was 90%. This decrease in concordance was due to 3 specimens with indeterminate IHC staining results with weak staining in a small tumor area. Use of a custom automated workflow for PCR plate preparation demonstrated successful facilitation of sample preparation and tracking. Evaluation of results indicate that not only is >10% tumor content important for detection of mutations via PCR (as directed by the IVD package insert), but heterogeneity within the tumor can further decrease sensitivity of PCR. However weak or low IHC staining in only small areas of the tumor may prove to have less clinical relevance. For the most comprehensive results, IHC staining in addition to molecular testing provides better characterization of tumor profiles. Preliminary results suggest that automated dissection of tumor with the Roche Automated Dissection instrument prior to PCR increases sensitivity. In conclusion, IHC and real-time PCR should be used together to detect not only the array of mutation available in the PCR test, but also to confirm the two most common mutations by IHC and increase sensitivity of testing which may be strongly impacted by heterogeneity. 1. - Marc Luo and William Pao. Lung adenocarcinoma: guiding EGFR-targeted therapy and beyond. Modern Pathology (2008)21, S16-S22; doi:10.1038/modpathol.3801018.

#753 Human epidermal growth receptor-2 immunohistochemistry in gastric cancer. Babatunde M. Duduyemi,1 Mary Y. Alhene,1 Emmanuel A. Asia- mah1,2 Kwanne Nkrumah University of Science and Technology, Kumasi, Ghana; 2Komfo Anoyke Teaching Hospital, Kumasi, Ghana.

Introduction: Gastric cancer is the second most common GI cancers and third leading cause of cancer-related deaths worldwide. In Ghana, gastric cancer contributes significantly to cancer morbidity and mortality. The recent development on the benefit of targeted therapy in the management of gastric cancer using HER-2 monoclonal antibody in combination with chemotherapy has greatly improved the overall survival of patients. This study therefore aims at determining the frequency of Her-2 over-expression in gastric carcinomas in Kumasi, Ghana and comparing with other prognostic factors. Methodology: Demographic data and histological diagnosis were retrieved from the surgical day book of the Department of Pathology, Komfo Anoyke Teaching Hospital, Kumasi. The slides were retrieved and reviewed to confirm diagnosis, grading and classification of gastric cancer. Immunohistochemistry was done with anti-HER-2 antibody to confirm HER-2 over-expression. The data was analysed with SPSS version 17. Results: Of the 99 cases of gastric cancer seen over the 8 year period, there were 57 males and 42 females, adenocarcinoma (91), GIST (3) and Non Hodgskin Lymphoma (3). The age range of the study population was 8-90 years with modal age group in the 6th decade. There were more biopsies (63) than resections (36). Of the adenocarcinomas, 45 were poorly differentiated, 38 moderately differentiated and 8 well differentiated. Diffuse type is most common with 47 cases followed by intestinal 41 cases and mixed type 3 cases. Three of the 4 patients under the age of 30 years had lymphoma. HER-2 over-expression was seen in 14 out of the 47 tested and all were intestinal type. Conclusion: HER-2 over-expression was seen a small cohort of our patients with gastric cancer especially those with intestinal type.

#754 Treatment decision-making of rare ERBB2 (HER2) mutations in lung cancer; a role for multidisciplinary molecular tumor boards. Arja ter Elst,1 Nils A. ’t Hart,1 Anthonie J. van der Welken,1 Wim Timens,1 Lucie R. Hijmering-Kappelle, Geke A. Hosapers,1 Hilde Jaling,1 Elise M. van der Logt,1 Leon C. van Kempen,1 Sjoedje F. Oosting,1 Matthew R. Groves,2 T Jeroen Hiltemann,1 Anke van der Berg,2 Harry J. Groen,1 Ed Schuuring,1 University Medical Center Groningen, University of Groningen, Groningen, Netherlands.

Introduction: Breakthroughs in cancer research have resulted in mutation-specific targeted therapies (precision medicine). Most of these new drugs are only effective in patients with an actionable molecular profile. Thus, predictive molecular testing enables oncologists to select individual patients for the most appropriate (targeted) therapy and to reduce the burden of overtreatment. The number of clinically relevant predictive markers that are routinely analyzed is growing rapidly, resulting in the identification of rare mutations, mutations with unknown relevance and coexistence of two or more mutations in the same sample. Incorporating these into the optimal treatment for the individual patient can be complex. Methods: A total of 2461 sequential tumor biopsies were analyzed at our institute using targeted next generation sequencing (Ion Torrent platform). 230 of these patients were discussed at a weekly Molecular Tumor Board meeting. Samples were analyzed at our institute using targeted next generation sequencing (Ion Torrent platform). 230 of these patients were discussed at a weekly Molecular Tumor Board (MTB) meeting. Results: In this abstract we report on four patients with an ERBB2 exon 20 mutation and 1 patient with ERBB2 amplification received anti-HER2 treatment after an MTB consensus decision. Two patients with an insertion in exon 20 of ERBB2: (c.2313+2324dup; p.(Y772+775dup)) received first line therapy with afatinib and showed a partial response and stable disease respectively. One patient with a c.2524G>A; p.(V842I) mutation received afatinib and showed stable disease for 3 months. A patient with another ERBB2 exon 20 insertion (c.2326_2327insATAT: p.(G776delinsVC)) received afatinib but had progressive disease within two years and the current treatment decision was for afatinib plus bevacizumab. Based on these results, the MTB made an evidence-based recommendation for the use of the combination therapy in the future. Conclusion: The MTB is an efficient way to treat patients with rare mutations, with unknown relevance, in a multidisciplinary approach. Results: In this abstract we report on four patients with an ERBB2 exon 20 mutation and 1 patient with ERBB2 amplification received anti-HER2 treatment after an MTB consensus decision. Two patients with an insertion in exon 20 of ERBB2: (c.2313+2324dup; p.(Y772+775dup)) received first line therapy with afatinib and showed a partial response and stable disease respectively. One patient with a c.2524G>A; p.(V842I) mutation received afatinib and showed stable disease for 3 months. A patient with another ERBB2 exon 20 insertion (c.2326_2327insATAT: p.(G776delinsVC)) received afatinib but had progressive disease within two years and the current treatment decision was for afatinib plus bevacizumab. Based on these results, the MTB made an evidence-based recommendation for the use of the combination therapy in the future. Conclusion: The MTB is an efficient way to treat patients with rare mutations, with unknown relevance, in a multidisciplinary approach.
months. One patient with an ERBB2 amplification by FISH and high (3+) HER2(ERBB2) expression, showed a partial response to trastuzumab. All patients had stage IV and would without genomic knowledge be treated with chemotherapy. Conclusion: Lung cancer patients with sporadic ERBB2 mutations might benefit from targeted ERBB2 therapy. For an optimal treatment decision, patients with rare mutations in general, may benefit from discussion in a multidisciplinary molecular tumor board. In the future, both the considerations for targeted therapy as well as treatment response and toxicity should be registered in an open-access database and shared with other national and international Molecular Tumor Board initiatives to allow comparison with traditional treatments.

#755 Supporting precision cancer treatment decision with functional evaluation of cancer gene mutations and variants. Jingjing Jiang,1 Zhong- guang Luo,1 Jia Wei,1 Guanglei Zhuang,1 Song Yi,1 Ying Yan,1 Tengfei Yu,1 Wei Du,1 Tingting Tan,1 Ling Qiu,1 Jiali Gu,1 Xin K. Ye,1 Jie Liu,1 Zhenyu Gu2.
1GenenDesign USA Co, Ltd., Menlo Park, CA; 2Huashan Hospital, Shanghai, China; 3Nanjing University, Nanjing, China; 4Shanghai jiao Tong University, Shanghai, China; MD Anderson Cancer Center, Houston, TX; GenenDesign Co, Ltd., Shanghai, China.

Precision oncology requires identifying and understanding of cancer genome changes in a patient tumor tissue and finding the best cancer therapy targeting the changes. Although many cancer gene targets have been validated so far, next-generation genomic profile analyses have uncovered much more cancer gene variants with unconfirmed functions. Developing methods to functionally evaluate mutations/variants and understand their roles in cancer development and drug responses, such as drug resistance or synthetic lethality, will be critical in cancer treatment decision support. In addition, in some clinical cases, multiple treatment choices such as multiple drug combinations exist. Developing cancer models which can test multiple treatments will provide direct comparison of those therapies and select the best options. At GenenDesign, we have performed drug tests on mouse “avatars”, which are also known as Patient-Derived Xenograft (PDX) models. They are personalized cancer models derived from patient tumor samples with cancer mutation profiles and drug responses very similar to the corresponding cancer patients. Drug screenings were carried out in avatars by testing chemotherapies or targeted drugs against specific cancer gene mutations and variants. Selected drugs or drug combinations from avatar studies have been applied to corresponding patients with highly consistent treatment outcome. From genomic profile analysis of our near 1500 PDX tumor models in cancer types such as lung, colorectal, gastric, liver, and esophageal, we are able to identify a large number of cancer gene mutations/variants, gene fusions, as well as gene copy number and RNA expression changes in major cancer signal pathways such as EGFR, Her2, c-Met/LCK, Ras/Raf, FGFR3, PI3K/Akt, Wnt, Notch. DNA repair, cell cycle regulation, angiogenesis. Many of these changes are potential drug targets and have been functionally tested in PDX models with approved drugs or clinical drug candidates. The mutation/variant information and drug response information generated from PDX models have been organized into our Precision Cancer Information Lab database. Patient tumor DNA test results have been used for searching genetically matched PDX models in our database. Once matched PDX models are identified, the available drug response information can be used as evidence for clinical treatment decision support. In addition, the matched PDX models can also be used to test more treatment options such as different combinations and new clinical drug candidates.

#756 Molecular characterization of triploid and tetraploid urine FISH samples. Lorenzo Pecciarini, Valeria De Pascali, Ilaria Francaviglia, Anna Talano, Chiara Iacona, Massimo Freschi, Maria Giulia Cangi, Claudio Doglioni.
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Urothelial carcinomas (UC) are characterized, among other aberrations, by chromosome 3, 7, 17 gains and 9p21 (p16) loss. Since UC cells readily exfoliate into the urine, molecular cytogenetics techniques have been used to detect cells consistent with UC diagnosis and follow-up; a multi-target Fluorescence In Situ Hybridization (FISH) test, composed of 3, 7 and 17 centromeres and 9p21 locus multicolor probes, has been proved useful for atypical cells identification in both void urine and lower and upper urinary tract samples before morphological changes are apparent through cytological and histological examination. To date, there are no uniform criteria for urine FISH scoring though the test is commonly considered positive following the UroVision Bladder Cancer Kit criteria (Abbott Molecular); 4 or more cells with a gain of 2 or more chromosomes (polysomy), and/or 12 or more cells with 9p21 homozygous deletion. This definition of polysomy includes trisomy and tetrasomy classes, but, as tetraploidy may represent cells at S/G2 phase, this criterion may lead to false-positives; moreover it has been described that also inflammation induces tetraploidy, and that small percentages of normal urine cells show trisomy/tetrasomy. On the other hand, triploidy and tetraploidy may have a direct role in tumorigenesis, as an intermediate state in the development of cancer aneuploidy. At the moment little is reported about the clinical correlations of urine FISH cases showing only trisomy/tetrasomy in BC. In particular frequent mutations in exons 7, 10 and 15 of FGFR3 characterize non-muscle invasive UC while mutations in the core promoter region of TERT gene, mainly at -124 and -146 positions from the ATG start site, have emerged as the most frequent somatic genetic alteration in both non-muscle invasive and muscle invasive UC. All specimens were subjected to PCR amplification and Sanger sequencing for FGFR3 exon 7 and 10, and for the C228T and C250T TERT promoter mutations. A preliminary analysis of 20 urine FISH cases showed 4 samples with trisomy/tetrasomy, and 1 of these 4 had a mutation of the TERT promoter. Complete results of the FGFR3 and TERT promoter molecular analysis will be presented and correlated to both FISH results and patients clinical data, in order to evaluate if the presence of FGFR3 and/or TERT promoter mutations in urine samples with FISH detected trisomy/tetrasomy may help to identify patients with a higher risk of recurrence and progression.

#757 Development and validation of the ActionSeq™ test system. Samantha Helm, Vanessa Spotlow, Aleksandra Ras, Kevin Kelly, Guruprasad Ananda, Sara Patterson, Honey V. Reddi. Jackson Laboratory for Genomic Medicine, Farmington, CT.

Introduction In the constantly changing field of oncology precision medicine, it is exceedingly important to keep diagnostic and therapeutic assays clinically relevant. Next generation sequencing (NGS) panels in oncology are greatly impacted by new findings in clinical actionability. In order to ensure that cancer panels continue to provide the most beneficial results to patients, they must be regularly updated. In keeping with this idea, JAX has launched a new 212 oncology gene panel which focuses on genes and variants with documented actionability, referred to as ActionSeq. Methods Development of ActionSeq included the optimization of a new targeted capture assay. This process included running multiple batches of samples through the assay to determine appropriate DNA input, ligation times, PCR cycles, and pooling conditions. The fully optimized assay was then validated using 24 uncharacterized FFPE samples. The validation was executed in 5 phases: (1) confirm that assay optimizations yielded sufficient wet lab results; (2) LOD & sensitivity (3) inter-assay concordance; (4) intra-assay concordance; (5) specificity and accuracy. Results During development, the standard protocol was optimized using a 200ng input, 30 minute ligation period, 10 cycles of PCR, and the pooling of 4 samples per hybridization reaction. Wet lab processing results of the first validation batch can be seen in Table 1. The inter- and intra-assay concordances were found to be ≥ 98% for variants and 100% for CNVs. The sensitivity was calculated to be 98.92% at a LOD of 3% for CNVs, 100% at a LOD of 8% for INDELs, 100% at a LOD of 6 copies for CNV amplifications, and 100% at a LOD of 0 copies for CNV deletions. The specificity and accuracy were found to be 100% for all mutation types. Conclusion Based on the success of this validation ActionSeq has been incorporated into the JAX clinical test menu. This addition accomplished the goal of providing a more clinically relevant (actionable) somatic tumor profiling assay to patients and clinicians.

## Table

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**CLINICAL RESEARCH: Molecular Diagnostics**

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**Clinical Research: Molecular Diagnostics**

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<th>Sample</th>
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Established Quality Control Cutoffs:
- Post Library Prep ng/µl: ≥20
- Post Capture Pool ng/µl: ≥0.5
- PC Average Base Pair Size: 250-350
- Mean Target Coverage: ≥500
- Percent Duplication: ≤25%

Table 1: This table displays the wet lab processing results for the 24 samples used to confirm the efficiency of the development optimizations made to the assay prior to beginning the clinical validation.

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**758 Capture and characterization of circulating tumor cell clusters in patients with metastatic castrate-resistant prostate cancer.** Samantha L. Savitch1,2, Soo Jin Kim3,6, Jason C. Poole1, Brian Kudlow5,3, Devorn Soucek2, Denis Smirnov3,2, Chandranao2, Steve Gross,2, Ravi K. Amaravadi,2, David J. Vaughan,1 Naomi B. Haas,3 Erica L. Carpenter1. 

1. Division of Hematology and Oncology, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; 2. Janssen Pharmaceutical, Johnson & Johnson, Huntington Valley, PA.

Introductory Sentence indicating purpose of study: Circulating tumor cell (CTC) clusters have been shown to have higher metastatic potential than single CTCs in breast, pancreatic, and other cancers, yet these clusters have not been extensively described in metastatic castrate-resistant prostate cancer (MCRPC).

Here we sought to determine the feasibility of capturing and characterizing CTC clusters in prostate cancer patients. Description of Experimental Procedures: Fifty-five blood samples from 29 MCRPC patients, ages 50 to 81 (median age 68), were obtained prior to the patient starting or switching to androgen receptor inhibitor or 17 alpha lyase inhibitor therapies. Eighteen patients received enzalutamide and 11 received abiraterone. The majority of patients had a Gleason score > 7 (22; 75.9%), bone metastases (19; 65.5%), and an ECOG status of 0 (21; 72.4%). All patients had previously undergone, or were receiving at time of enrollment, androgen deprivation therapy. Eleven patients (37.9%) had been on a prior 2nd generation anti-androgen therapy. CTC single cells and clusters (2 or more cells together in one image) were enumerated using the CellSearch system and stained to detect expression of androgen receptor (AR), glucocorticoid receptor (GR), and neuroendocrine (NE) markers. Summary of Data: Five or more single CTCs, a measure which has previously been associated with an unfavorable prognosis, were detected in 13 of 29 patients (44.8%), and in 19 of 55 blood samples (34.5%). Altogether, a total of 282 CTC clusters was detected, with 1 or more clusters found in 10 patients (34.5%) and 13 samples (23.6%). The number of clusters per 7.5ml of blood ranged from 0-150, and clusters contained anywhere from 2 to 16 cells. Most CTC clusters (268; 85.0%) contained only CTCs and no leukocytes. Just over half the detected clusters (162; 57.4%) contained only 2 CTCs. Cluster staining patterns were fairly homogenous with 29.4% of clusters having uniform expression of either AR, GR, or NE markers, i.e., all CTCs in the cluster expressed the marker of interest. Most clusters (67.7%) were uniform marker negative and the remaining 2.8% demonstrated a mix of marker positive and marker negative CTCs. Serum Chromogranin A levels, as a marker for neuroendocrine cells, were determined by standard of care clinical blood testing, were found to be positively associated with the number of CTC clusters per 7.5ml of blood (p<0.0001).

Statement of Conclusions: The capture and characterization of CTC clusters in the blood of MCRPC patients can be successfully performed using the CellSearch system. Further investigation into the clinical implications of these clusters is warranted, including whether cluster characteristics are associated with more aggressive disease.

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**759 Highly-sensitive detection of EGFR mutation in non-small cell lung cancer using mutant-enrichment method.** Hung-Chi Chien. Industrial Technology Research Institute, Hsinchu, Taiwan.

Detection of mutations in EGFR in non-small cell lung cancer is important for the clinical decision-making process of molecularly targeted therapies, including Irressa, Tarceva, and Tagrisso. In this study, we developed an assay allowing the detection of EGFR gene mutations being conducted in the routine diagnostic procedure and its analytic and clinical performance were evaluated using different types of specimens. The EGFR mutation assay in this study allows for detecting EGFR exon 19 deletion (Del), exon 20 insertion (Ins), and mutation in T790M, L858R, S768I and L861Q of rare amounts in a normal genetic background. Enhancement of detection sensitivity was achieved with the developed mutant enrichment method which includes specific primers, probes and protease K digestion as blockers for inhibiting wild-type gene from DNA amplification. Analytic performance for each mutant was assessed using the control plasmids containing the mutant genes of known amounts as well as the genomic DNA extracted from mutant cell lines and Horizon FFPE samples. The assay performance were also evaluated using clinical samples in parallel with the FDA-approval companion diagnostic products (therascreen® EGFR RQG PCR Kit). The sensitivity of the assay evaluated using control plasmids for Del, T790M, L858R, S768I, L861Q mutation and Ins was 5%, 0.5%, 1%, 0.5% and 0.5% respectively, and was 1%, 0.5%, 0.5%, 0.1% and 0.5% respectively when applying on HDX reference standards. Diagnostic results for the 116 clinical samples for identifying mutations of del, T790M, L858R, S768I, L861Q mutation and Ins was 92.2%, 100%, 99.1%, 100%, 97.4% and 100% respectively concordance with the therascreen® EGFR RQG PCR Kit, including 17 cases of multiple mutations. The overall diagnostic results for all mutations evaluated with PPA (positive percent agreement), NPA (negative percent agreement) and OPA (overall percent agreement) were 83.3%, 98.2% and 90.5%, respectively. The three samples firstly diagnostic as false positive were retested and confirmed to be exon 19 del by DNA sequencing. The assay demonstrated good sensitivity and specificity when compared with commercial products. Moreover, the clinical studies are being performed to establish relationship between drug efficacy and different mutation combinations.

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**760 Detection of EGFR T790M mutation by ddPCR in untreated NSCLC patients: Correlation with clinical outcome.** Monica Ganzinelli1, Elena Rulli2, Elena Tamborini1, Adele Busico1, Giuseppe Lo Russo3, Giulia Corraro1, Milena Vitali1, Marina Chiara Garassino1, Massimo Broggi3, Mirko Marabese1.

1. Fondazione IRCCS Istituto Tumori Milano, Milan, Italy; 2. IRCCS - Istituto di Ricerche Farmacologiche Mario Negrin, Milan, Italy.

Background: Activating EGFR mutations are associated with response to first generation tyrosine kinase inhibitors (TKI) that significantly increased PFS and OS in NSCLC patients. However, almost invariably, patients relapse while in treatment with TKI, mostly for the presence of EGFR T790M mutation. New second and third generation EGFR inhibitors active against T790M are now under development with very promising results. To date it is not known if the presence of T790M (and its amount) at diagnosis influences clinical outcome of patients with activating EGFR mutation. Methods: We analysed 50 cases with NSCLC. 48 patients harbour TKI sensitizing mutation in either exon 19 (N=24) or 21 (N=19), 2 EGFR wt patients (blinded to the investigator) were analysed in parallel. These patients were screened by ddPCR for the presence of T790M mutation using Biorad QX200 Droplet Digital PCR System. We used rough DNA extract from FFPE sections after deparaffinization and proteinase K digestion, as available in pathology unit for daily diagnosis. Results: 12 samples failed to amplify, maybe due to the poor quality of DNA. 13 samples produced results, but were excluded for the analysis because of too low number of calls retrieved. T790M mutation was detected in a significant proportion of mutant samples. Among the ddPCR RQG PCR Kit. The sensitivity of the treatment and here the percentage of T790M were higher compared to those patients not treated [0.65% - 28% vs 0.019% - 1.65%, respectively]. One out four of these patients was not detected by routinely method of sequencing (Sanger or RT-PCR). T790M positive samples harboured mutations affecting exon 19 or 21 in equal manner. Conclusion: ddPCR has proven to be a sensitive method to detect T790M mutation, although the quality of DNA could affect the results. The system has been challenged for specificity and the sensitivity and the results proved to be clinically relevant. The correlation between the T790M positivity and clinical outcome will be available and we plan to define a ddPCR threshold value which can help in selecting those patients who are likely to have early recurrence under first generation TKI and that could therefore be directly shifted to second or third generation inhibitors.

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**762 A concordance study of the ArcherDX Reveal™ ctDNA 28 NGS panel and Biocept’s Target Selector™ mutation assay using ctDNA collected in Biocept CEE-sure™ blood collection tubes.** Jason C. Poole1, Brian Kudlow5,3, Jill Stefanelli,2 Skyler Mishkin,2 Anh Pham,1 Jeff Chen,1 Veena M. Singh,1 Josh Stahl,1 Lyle J. Arnold1. 1. Biocept, Inc., San Diego, CA; 2. ArcherDX, Inc., Boulder, CO.

Biocept’s Target Selector technology is a targeted hotspot mutation panel designed to enrich for mutant targets in a large excess of WT DNA. The test is specific to small regions of interest, is highly sensitive, validated down to 7 mutant copies in a background of 14,000 WT at >98% sensitivity. The ArcherDX Reveal ctDNA test is a 28 gene NGS panel that targets key oncogene...
activating mutations, drug resistance mutations, in addition to full coverage of TP53. The Reveald ctDNA assay utilizes Anchored Multiplex PCR to enrich, tag, and efficiently capture short ctDNA fragments. Both technologies are designed specifically for use with plasma associated ctDNA. We undertook to evaluate the feasibility of using the ArcherDX Reveald ctDNA 28 NGS panel with the DNA extracted from plasma collected with Biocept's patented blood collection tube, which has been validated at room temperature for 4 days for circulating tumor cells and 8 days for ctDNA. The ability to build complex, targeted libraries, free of chemically induced mutations is of major importance for the detection of the vanishingly rare mutations that can be present in the plasma of cancer patients. We found that high quality NGS libraries were produced from plasma collected and stored in the Biocept blood tube, which is indicated that damage occurred to the DNA during preservation. In addition, the two methods were found to be highly concordant and complementary when using both mutation positive and negative patient samples. The Target-Selector assay was the more sensitive, however, the ArcherDX panel revealed several additional mutations not targeted by the Biocept Target Selector assay.


Chromogenic multiplex immunohistochemistry (IHC) assays enable investigation of the spatial relationships between tumor and immune cells, which is thought to be important for understanding and predicting therapeutic response. Development and analytical validation of multiplex IHC assays enables the use of such assays to simultaneously investigate multiple biomarkers as predictors of clinical response. In this study, we analytically validated a chromogenic duplex IHC assay that quantifies Ki67 and CD8 in formalin-fixed, paraffin-embedded non-small cell lung cancer tissues. Five performance criteria were selected and evaluated based on Clinical Laboratory Standards Institute guidelines: reportable range, analytical sensitivity, analytical specificity, accuracy, and precision. Similar to analytical validation studies for monoplex IHC assays, this study utilized a reference method and multiple days of staining. The percentage of cells positive for Ki67 nuclear staining and/or CD8 membrane staining were quantified using our computational Tissue Analysis (CTA™) platform. Performance of the Ki67/CD8 chromogenic duplex IHC assay was considered acceptable for the five criteria evaluated. Once the performance of the assay was established, additional exploratory cTA-based endpoints were examined, including the quantification of each biomarker in the tumor compartment and the tumor microenvironment, and analysis of the spatial arrangement of immune cells relative to tumor cells. In conclusion, Flagship’s cTA platform allows for more consistent quantification of individual analytes on dual-stained tissue sections, enabling investigation of complex biological questions that cannot be achieved with traditional tissue-based manual endpoints.

#764 Survival analysis for the elderly esophageal cancer patients (≥80 years) with surgery and non-surgery treatments. Dan Feng Du1, Dan Feng Du2, Fu You Zhou1, Lian Qun Zhang1, Xue Na Han1, Yuan Yuan2, Zong Min Li3, Jian Po Wang4, Yao Wen Zhang5, Tai Jiang Liu2, Guo Hua Liu2, Yi Jun Qi2, Yan Rui Zhang2, Li Dong Wang1. 1The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China; 2Basic Medical College of Zhengzhou University, Zhengzhou, China; 3Anyang Tumor Hospital, China; 4Huoja Red Cross Hospital, China; 5The First Affiliated Hospital of Henan University of Science and Technology, China; 6Henan Provincial People’s Hospital, Zhengzhou, China.

With the aging of population, the elderly esophageal cancer patients are increasing. However, the optimal treatment methods have not been well characterized for the elderly esophageal cancer patients because of that most randomized studies have excluded the elderly. The aim of this study was to analyze the outcomes of surgery and non-surgery therapy on the elderly patients aged 80 years or older with esophageal squamous cell carcinoma (ESCC) to provide more evidences for optimal treatment design on the elderly patient. The enrolled 1,143 ESCC cases in this study were from the ESCC database in Henan Key Laboratory for Esophageal Cancer Research. Of the 1,143 patients, there were 614 males with a mean age of 82.4±2.64 and 529 females with a mean age of 82.44±2.61. The age ranged from 80 to 96 years old at diagnosed. All the patients were confirmed as ESCC by histopathology. The patients were classified into four groups based on treatment methods, i.e., radical esophagectomy (group I), esophagectomy plus radiochemotherapy (group II), radiochemotherapy only (group III), and symptomatic treatment (group IV). Radical esophagectomy was performed on 304 patients (26.6%), including 134 males and 97 females. In addition, of the 304 patients with radical esophagectomy, there were 73 patients who were excluded for postoperative surgery. 518 patients received only radiochemotherapy (45.3%). Radiochemotherapy included 50-60 Gy of radiation concurrent with or without 5-fluouracil alone or combined with cisplatin. Moreover, there were 321 patients (28.1%) who did not receive either surgery or radiochemotherapy, but only with symptomatic treatment. The Kaplan-Meier analysis indicated that there was no significant difference in gender, age or histology between patients in the different groups. The overall 1-, 3- and 5-year survival rates were 56%, 31% and 19%, respectively, with a median survival time of 2.5 years. In group I, the overall 1-, 3- and 5-year survival rates were 68%, 48% and 30%, respectively, with a median survival time of 3.8 years. In group II, the overall 1-, 3- and 5-year survival rates were 48%, 32% and 14%, respectively, with a median survival time of 1.9 years. In group III, the overall 1-, 3- and 5-year survival rates were 58%, 28% and 18%, respectively, with a median survival time of 2.5 years. In group IV, the overall 1-, 3- and 5-year survival rates were 39%, 18% and 0.08%, respectively, with a median survival time of 1.6 years. The log rank survival analysis showed that the surgery group had better prognosis than surgery plus radiochemotherapy or radiochemotherapy alone group (P<0.002) and the symptomatic treatment group (P<0.000). The present results demonstrate the feasibility of esophagectomy in the elderly ESCC patients in terms of survival. [Supported by the Joint Funds of NSFC (U1301227), Natural Foundation of Henan Province (20161110) and Correspondence to: Li Dong Wang, Email: ldwang2007@126.com].


Elderly acute lymphoblastic leukemia (ALL) patients are often unfit for either dose-intensive chemotherapy or stem cell transplantation, thus their clinical outcomes have been dismal. The increased incidence of BCR-ABL translocation with increasing age also contributed to poor prognosis but, it was our conjecture that the introduction of potent tyrosine kinase inhibitors (TKI) targeting ABL, BCR-ABL translocation might not deliver negative prognostic impact in elderly ALL. Considering that many elderly Ph- ALL patients do not tolerate cytotoxic chemotherapy required for effective tumor control, we supposed that efficacy of ABL TKIs and their combination strategy with low dose cytotoxic chemotherapies would result in better survival for elderly Ph+ ALL compared to their Ph- counterparts. This study was a multicenter longitudinal cohort study of elderly de novo ALL (≥ 60 years) recruited from 6 hospitals in Korea from January 2005 to December 2015. We considered Ph+ if it was positive in at least one of three techniques: conventional cytogenetics, fluorescence in situ hybridization, or quantitative real-time polymerase chain reaction (qRT-PCR). Among the 96 patients enrolled, there were 50 Ph- ALL patients (52.1%) and 46 Ph+ ALL patients (47.9%). There were no differences between the 2 groups regarding age, sex distribution, performance status, and induction regimens. For treatment outcomes evaluation, the 5 Ph+ ALL patients not receiving TKI were eliminated and 41 Ph+ ALL patients treated with TKI were compared with 50 Ph- ALL patients (Table). Complete remission (CR) was attained in 27 (54.0%) of patients in the Ph- group versus 35 (85.4%) in the Ph+ group (P=0.001). The median RFS was significantly longer in the Ph+ group (3.1 months for Ph- vs. 8.9 months for Ph+, P=0.007). Induction death and induction failure occurred significantly more often in the Ph- group. The median OS was 6.3 months for Ph- group versus 10.3 months for Ph+ group (P=0.033). In conclusion, using real world data, this study reports the possible favorable impact of Ph+ in TKI-era for elderly patients.

#766 Comorbidities limiting recruitment of non-small cell lung cancer (NSCLC) patients in early phase trials. Narjast Duma, Yucai Wang, Aaron Mansfield, Mayo Clinic, Rochester, MN.

Background: Early phase trials (EPCT) provide significant value by evaluating the possible benefits of new agents. The eligibility criteria in EPCT are usually rigorous and may exclude many patients (pts) commonly seen in clinical practice. Our objective was to identify the most common comorbidities excluded in EPCT for NSCLC. Methods: ClinicalTrials.gov was queried on August 1st of 2016. 299 interventional drug trials were extracted from 2010 to 2016. We studied the trial characteristics including: experimental therapy, location, funding source, and exclusion criteria. Our objective was to identify the most common comorbidities excluded in EPCT for NSCLC. Results: ClinicalTrials.gov trial characteristics were analyzed. We identified 2010 trials with early phase therapy and 1672 NSCLC trials. Of these NSCLC trials, 55% were randomized, 43% were biomarker-driven, and 28% were placebo-controlled trials. For patients between the ages of 60 and 75, only 20% of patients were recruited. For patients between the ages of 75 and 80, only 10% of patients were recruited. The most common comorbidities excluded in EPCT for NSCLC were: hypertension (29%), chronic obstructive pulmonary disease (26%), diabetes mellitus (21%), chronic kidney disease (19%), and liver disease (17%). Conclusion: EPCT for NSCLC exclude many patients commonly seen in clinical practice. The most common comorbidities excluded in EPCT for NSCLC were hypertension, chronic obstructive pulmonary disease, diabetes mellitus, chronic kidney disease, and liver disease.
The utility of a breast cancer registry for improved cancer management in developing countries. DeAnna McGarity,1 Brandon Epps,1 Alexis Maynor,5 Tania Anderson,2 Ernest Alem-Mensah,3 Delroy Fray,2 Maung Aung,3 Kisha Holden,4 James Lillard,3 Brian Rivers,5 Derrick Beech,1 Morehouse School of Medicine, Atlanta, GA; 2Tuskegee University, Tuskegee, AL; 3Western Regional Health Authority and Cornwall Regional Hospital, Montego Bay, Jamaica.

Background Breast cancer is one of the leading causes of cancer related deaths in the island nation of Jamaica, West Indies. Cancer and other non-communicable diseases have increased in many developing nations in the Caribbean as well as Central and South America. The health systems of these developing countries continue to provide care to patients with limited resources. The use of a breast cancer registry can assist in care coordination of these patients and allow targeted screening and treatment programs in under-resourced environments. Methods In collaboration with the Western Regional Health Authority, a breast cancer registry database was developed in RedCap to provide a comprehensive analysis of breast cancer in under resourced environment. 53 patients from Atlanta were compared to 78 patients from Jamaica. These patients were entered into the breast cancer registry from June 1, 2014 to March 31, 2015 by attending physician and de-identified. Demographics, tumor related factors, treatment, survival and health behavior information was collected by the attending physician. The findings were then analyzed using Statistical Analysis System (SAS) and Data Analysis and Statistical Analysis (STATA): Fisher Exact test, chi-square and multiple regressions. Results The median of patient ages were 61 (range 26 - 84 years old) for Jamaican patients and 53 years old (range 26 - 84 years old) for Jamaican women. For the U.S. patients, 84% were Black, 4% White, and 12.24% were Latin or Asian. The majority of the patients were diagnosed with stage I invasive ductal breast cancer. In contrast, the majority of the Jamaican patients were diagnosed with late-stage breast cancer. Utilizing the data from the breast cancer registry, we were able to identify parishes (counties) with disproportionately high rates of late-stage cancer. Conclusion The use of a disease specific registry can improve care coordination and identify actionable target areas for enhanced screening and treatment. It is our hope to frequent review the dashboards associated with the registry data by providers, medical centers and health systems. This will optimize care coordination and allow timely improvements in cancer diagnosis and management in developing countries.

[299 trials, 164 (55%) were phase II, 81 (27%) phase I and 54 (18%) phase Ib/II. 164 (55%) were conducted in the United States (US), 45 (15%) in Asia, 39 (13%) in Europe and 51 (17%) were international. 171 (57%) analysis. Results: Of the 299 trials, 164 (55%) were phase II, 81 (27%) phase I and 54 (18%) phase Ib/II. 164 (55%) were conducted in the United States (US), 45 (15%) in Asia, 39 (13%) in Europe and 51 (17%) were international. 171 (57%) of therapy.

[378 breast cancer cases were seen during the study period and majority of the cases (99.4%) were females. The prevalence of breast cancer was found to be 12.6%. Triple-negative breast cancers (TNBC) constitute 67.7% of cases. The mean age was 45.8 ± 12.0 years and the youngest patient was 18 years while the oldest was 90 years. Out of the 784 cases, 208 were mastectomies while the remaining were incisional, trucut needle and excisional biopsies. Infiltrating ductal carcinoma NST was the most common histologic variant seen (94.7%) and most of these cases (59.1%) were Grade II tumors. The Nottingham Prognostic Index (NPI) shows that 91.4% of the cases have NPI Score of ≥ 3.5 with attendant poor 5-year survival rate. Conclusion: This study found that breast cancer is common and affects mostly women in fifth decade of life. There is a high TNBC among the population studied and therefore androgen receptor profile and genomic studies may be useful to determine the course of therapy.

The utility of a breast cancer registry for improved cancer management in developing countries. DeAnna McGarity,1 Brandon Epps,1 Alexis Maynor,2 Tania Anderson,2 Ernest Alem-Mensah,3 Delroy Fray,2 Maung Aung,3 Kisha Holden,4 James Lillard,3 Brian Rivers,5 Derrick Beech,1 Morehouse School of Medicine, Atlanta, GA; 2Tuskegee University, Tuskegee, AL; 3Western Regional Health Authority and Cornwall Regional Hospital, Montego Bay, Jamaica.

Background Breast cancer is one of the leading causes of cancer related deaths in the island nation of Jamaica, West Indies. Cancer and other non-communicable diseases have increased in many developing nations in the Caribbean as well as Central and South America. The health systems of these developing countries continue to provide care to patients with limited resources. The use of a breast cancer registry can assist in care coordination of these patients and allow targeted screening and treatment programs in under-resourced environments. Methods In collaboration with the Western Regional Health Authority, a breast cancer registry database was developed in RedCap to provide a comprehensive analysis of breast cancer in under resourced environment. 53 patients from Atlanta were compared to 78 patients from Jamaica. These patients were entered into the breast cancer registry from June 1, 2014 to March 31, 2015 by

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**771 Cervical cancer: Our experience in Sudan.** Kamal Eldedhik Mohamed, Dr Anmar Ashmeig Ahmed Ashmeig, Faculty of Medicine, Univ of Khartoum, Khartoum, Sudan.

**Introduction:** Cervical cancer is the second most prevalent cancer in Sudanese Women; breast cancer forms between 29 - 35% of female cancers while Ca Cervix forms 12 - 16%. There are limited screening activities in the country using VIA, and Pap smears in Obs. and Gyn. clinics. With the main emphasis on health education programs, teaching women the early symptoms of ca cervix and early detection. Due to lack of awareness the majority of women don’t know anything about screening, so there is limited demand. Method: This is a retrospective study of 405 Cases of Ca Cervix treated during the period between 2000 -2013 in Khartoum, Khartoum, Sudan.

**Results:** Age range showed, 28 to 79 years. Residence distribution was as follows, western Sudan = 48 %, central Sudan = 41 %, North = 7 %, East = 4%.

**Length of duration of symptoms, range between 1—10 months, mean of 5 months.** FIGO Stage distribution: Stage 1 = 6%, Stage 2A =13%, Stage 2B =23%, Stage 3A =18%, Stage 3B =34%, Stage 4A, B =7%, Pathology: poorly diff. Squamous ca 38%, moderately diff. Squamous 26%, well diff 25%, Adenocarcinoma 9%, others: Treatment: external Radiotherapy, 45-50 Gy in 20 - 25 fractions, by 2 or 4 fields, using Co 60 or linacs 6 MeV, concurrent chemoradiotherapy was given to patients with stage 2B, 3A, 3B, and 4A. Using weekly Citiplasmin, this was given to 32% of patients. 60% % of the patients had Brach therapy, 34% with Low Dose Rate, LDR, Manual Brachy therapy, 35Gy to point A and 60 Gy to Point B, HDR. Self irradiation was source. Results: 175 patients (43.3%) survived for 5 years and more, mostly patients with stage one and two 158 were 59% survived for 3-4 years after treatment, 39 patients 9.6 %, mostly patients with stage four, died during follow up due to the disease and 33 patients = 8.2% disappeared after treatment. The poor outcome is attributed to the advanced stages at presentation, lack of screening and awareness about cervical cancer, poverty, illiteracy, the large size of the country and the poor distribution of the limited medical resources. The patients treated with LDR or HDR have equal outcome and morbidity. Conclusion: Most of Ca Cervix Patients present with advanced disease, hence the poor prognosis, due to lack of awareness and screening, illiteracy and poverty, the majority are possibly different squamous cancers. The need for increasing public awareness about ca cervix and its early symptoms, and provision of a pap smear and VIA services in the primary health care facilities.

**773 Optimizing drug regimens in oncology by clinical trial simulations: Why and how.** Jocelyn Gal,1 Gerard Milano,2 Julien Viotti,3 Renaud Schiappa,4 Audrey Dugue,2 Agnes Paquet,2 Sylvie Chabaud,2 Jean-Marc Ferrero,2 Emmanuelf Chamorey,1 *Cir. Antoine Lacassagne, Nice, France; 2Cir. Francois Baclesse, Caen, France; 3Molecular and Cellular Pharmacology Institute, Sophia Antipolis-Valbonne, France; 4Le£on B£ard Cancer Center, Nice, France.

**Introduction:** In therapeutic research, the safety and efficacy of pharmaceuti- cal products are necessarily tested on humans via clinical trials after a very long and expensive development period. Alternative methodologies such as com- parative modeling and Clinical Trial Simulation (CTS) are a valuable option to reduce animal and human assays. The relevance of these methods is well recog- nized in pharmacokinetics and pharmacodynamics from the pre-clinical phase to post-marketing. However, they are seldom used and are poorly regarded for drug approval, despite FDA and EMA recommendations. We propose to discuss the principle behind, and the interest of, CTS approaches in drug development in oncology. Our work is based on a systematic review using two electronic literature databases (Medline and Web of Science). Key components of CTS: We will explain why and how to successfully develop CTS. Why developing CTS? To obtain a better knowledge of pharmacology and/or better prediction of safety and efficacy To evaluate disease progression dynamics and to test the potential impact of new treatments on subpopulations and extend the post-marketing authorization to help in the choice of the best outcome To optimize experimen- tal designs in order to better anticipate the progress of the study How to develop CTS? CTS project must follow three steps: 1/The constitution of pharmacomet- ricians team who elaborates clinical model and simulation plan that must contain three components: Input/output model: How do covariates impact out- comes? Covariate distribution Model: How can we construct virtual patients? Execution model: What can happen during the clinical trial? 2/Obtaining data for simulations. We will present the contribution of machine learning for CTS 3/The use of specialized software to realize simulations Limitations of CTS: We will present the features that can slow down or accelerate the development of CTS. The generalization of CTS could be greatly facilitated by the availability of software modeling biological systems, by the clinical trials studies and hospital databases. Data-sharing and data-merging raise legal, policy and technical issues that will need to be addressed. CTS challenges and prospects: Development of future molecules will have to use CTS for faster development and thus enable better patient management. Drug activity modeling coupled with disease modeling, optimal use of medical data and increase of the computing speed should allow this leap forward. The realization of CTS requires not only the bioinformatics tools to allow interconnection and global integration of all clinical data, but also a universal legal framework to protect the privacy of every patient. CTS will have to be used to provide quantitative support for drug development decisions but will never replace real clinical trials. This in silico medicine opens the way to the 4P medicine: predictive, preventive, personalized and par- ticipatory.

**774 Notch pathway activation predicts resistance to bevacizumab therapy in glioblastoma.** Norihiko Saito, Kazuya Aoki, Nozomi Hirai, Satoshi Fukushima, Junya Iwama, Masashi Ikuta, Haruo Nakayama, Motokazu Hayashi, Keisuke Ito, Takatoshi Sakurai, Satoshi Iwabuchi. Toho University Ohashi Medical Cen- ter, Tokyo, Japan.

Glioblastoma, the most common adult glioma, is associated with a dismal prognosis. Treatment with bevacizumab has not significantly prolonged overall patient survival times. Glioblastoma resistance to angiogenesis inhibitors is attrib- uted to multiple interacting mechanisms. We have thus embarked on a com- prehensive effort to detection expression signatures that are associated with response to the therapy and these signatures may allow prospective selection of patients with high likelihood of responding to therapy. Notch signaling pathway is an evolutionarily conserved pathway that plays an important role in multiple cellular and developmental processes including cell fate decision, differenta- tion, proliferation, survival, angiogenesis and migration. Analysis of The Cancer Genome Atlas expression dataset identified a group (43.9%) of tumors with proneural signature showing high Notch pathway activation. In this study, we compared CD133, Notch, and VEGF expressions in histological sections of pri- mary and recurrent glioblastomas after radiotherapy and chemotherapy. Tumor samples were collected from 27 patients at the time of tumor recurrence. We used immunohistochemical techniques to compare expression of CD133, Notch-1 and VEGF. Expressions of CD133-, Notch-1-, and VEGF-positive gli- oma cells were higher in recurrent glioblastoma after radiotherapy and chemother- apy. To determine the clinical importance of Notch-1 expression in glioblas- toma, we analyzed 15 patients who had received bevacizumab therapy followed by a second surgery at recurrence. OS was significantly longer in cases with Notch-1 negativity (8.8 months) than in those with Notch-1 positivity (6.1 months). Electron microscopic observation of two autopsy cases revealed the effects of blood vessel normalization in Notch-1 positive glioblastoma. Electron microscopic images confirmed the presence of pericytes surrounding the vascu- lar endothelium. Autopsied tumors exhibited marked proliferation of Notch-1
and VEGF positive cells around vessels. In tumor angiogenesis, vascular endothelial growth factor and Notch signaling induce sprouting angiogenesis and recruitment of vascular endothelial cells such as tip cells, stalk cells, and phalanx cells. Fully mature phalanx cells are in close contact with pericytes. These findings indicate that bevacizumab treatment promotes vascular normalization by recruiting mature pericytes and associated with resistance to bevacizumab therapy in glioblastoma with high Notch pathway activation.

**Identification of activated signal transduction pathways and molecules in the HMGB-1 induced angiogenesis.** Yujin Kwon, Sujin Shin, Won Kyu Kim, Hogen Kim. Yonsei Univ. College of Medicine, Seoul, Republic of Korea.

High-mobility group box-1 (HMGB-1) is a highly conserved protein playing various roles both in the nucleus and cytosol and known to be expressed in almost all types of mammalian cells. Secreted HMGB-1 has been most intensively studied and reported as an immune cell derived cytokine regulating systemic inflammation, signal transduction, and activation of endothelial cells (EC). Especially, HMGB-1 over-expression is now considered as a hallmark in cancers based on its involvement in cell proliferation, inflammatory microenvironment, invasion, metastasis, and sustained angiogenesis. Numerous studies have shown that secreted HMGB-1 directly or indirectly promotes angiogenesis by activation of ECs. However, mechanisms of HMGB-1 mediated angiogenesis have not yet been fully understood, especially, in terms of genomic changes caused by HMGB-1 treatment. Moreover, differences between vascular endothelial growth factor (VEGF) mediated and HMGB-1 mediated angiogenesis have not been studied. To explore the roles of HMGB-1 in angiogenesis, we first confirmed whether HMGB-1 treatment renders ECs angiogenic by evaluating proliferation, migration, wound-healing, and tubule formation of ECs after HMGB-1 treatment. In addition, we performed gene expression microarray to clarify how HMGB-1 treatment induced EC activation at various time points and subsequent angiogenic features according to time-dependent gene expression changes. We show that HMGB-1 treatment induces proangiogenic features to ECs such as stimulation of tubule formation, promotion of proliferation, and accelerated wound healing. By analysis of microarray data, we found that VEGF and HMGB-1 commonly promoted the activation of MAPK, cell proliferation, and wound healing pathways which directly regulate angiogenesis. On the other hand, axon guidance, gap junction, F53 signaling, cycle cell, and cell differentiation pathways were specifically up-regulated by HMGB-1. Taken together, HMGB-1 leads to angiogenesis via upregulation of both angiogenic and nonangiogenic genes.

**Acquired platinum resistance enhances tumour angiogenesis through activation of vascular mimicry.** Aya El Helali,1 Nuala McCabe,1 Naomi Dickson,1 Lara Dura Perez,1 Denis P Harkin,1 Richard Wilson,1 Richard Alpaugh,2 Steven H. Lin,3 Jeffrey R. Marks,4 Raymond Bergan,5 Stuart S. Martin,6 Sarany Chumsri,7 Massimo Cristofanilli,8 Cha-Mei Tang,9 Steingrimur Steinarsson10.

Introduction: Platinum resistant High Grade Serous Ovarian Cancer (HG-SOC) has a poor outcome with limited treatment options. Angiogenesis is a key pathological feature of ovarian cancer and anti-angiogenics have dominated the field of drug development in EOC, particularly in the second-line setting (Marachi et al 2013). In this study we asked if platinum resistance could be associated with an improved response to anti-angiogenic agents. Methods: A review of phase III anti-angiogenic clinical trials was used to investigate the association between platinum resistance and response to anti-angiogenic agents. To investigate the effect of chemotherapy on predefined ovarian cancer molecular subgroups (Gourley, et al. J Clin Oncol 32:5s, 2014), we analysed 35 matched pre- and post-chemotherapy samples by gene expression. Novel isoform ciplatin-resistant HG-SOC cell lines were established to study the mechanisms of ciplatin section pressure and shift to an angiogenic phenotype. This was further validated in novel ascites-derived primary cell lines from HG-SOC patients with known outcomes following platinum-based chemotherapy. Results: Critical review of phase III anti-angiogenic trials suggested that there was a better response to anti-angiogenics following previous platinum-based chemotherapy. Our analysis demonstrated that 67% of treatment naïve tumours that were initially classified as non-angiogenic shifted to an angiogenic biology, which was associated with platinum resistance. Additionally we found that ciplatin-resistant cancer cell lines demonstrated hallmarks of vascular mimicry and an associated increase in vessel density using an angiogenesis Matrigel plug assay in Athymic nude mice (p-value = <0.0001). In addition, cell lines established from platinum-resistant patients as well as cell lines made platinum resistant in vitro, demonstrated overexpression of VEGFα which would be expected to stimulate angiogenesis. Conclusion: We have demonstrated that platinum-resistance in HG-SOC is associated with angiogenic biology supporting the use of anti-angiogenic agents in this setting.

**ELTD1/ADGRL4, a novel adhesion GPCR regulator of tumor angiogenesis, suppresses lipid metabolism in endothelial cells, and is upregulated in breast cancer endothelium and epithelium.** David M. Favara,1 Madhulika Nambiar,2 Helen Sheldon,1 Massimo Masiero,1 Demin Li,1 Ali Jazayeri,1 Alison H. Banham,1 Adrian L. Harris1.

Background: We identified ELTD1/ADGRL4, an orphan GPCR belonging to the adhesion GPCR family (aGPCR), as a novel regulator of angiogenesis and a potential anti-angiogenic therapeutic target. ELTD1 is normally expressed in both endothelial cells & vascular smooth muscle cells. Expression in the tumour vasculature is significantly increased. Our aims were to analyse ELTD1’s function in cancer endothelial cells & its role in breast cancer. Methods: Real-time quantitative RT-PCR, mRNA array profiling was performed on primary human umbilical vein endothelial cells (HUVECs) & validated with qPCR & confocal microscopy. We investigated ELTD1 signalling by applying the aGPCR ‘Stinger/tethered-agonist Hypothesis’. For this, truncated forms of ELTD1 & peptides analogous to the proposed tethered agonist region were designed. FRET-based 2nd messenger (Cisbio IP-1; cAMP) & luciferase-reporter assays (NFAT; NFKB; SRE; SRF-RE; CREB) were performed to establish canonical GPCR activation. To investigate ELTD1 in breast cancer, a panel of cell lines representative of all molecular subtypes were qPCR screened. Furthermore, primary human breast cancers (n = 245) & matched primary & nodal secondary breast cancers (n = 79) were stained for ELTD1 expression. Staining intensity was then scored & compared between subgroups. Result: ELTD1 mRNA expression was significantly upregulated in breast cancer samples compared with normal breast tissue. Immunohistochemistry analysis revealed ELTD1 staining within the tumour stroma contrasted to normal breast. ELTD1/ADGRL4 staining within the tumour stroma correlated to disease stage. p = 0.004; HR = 3.1; p = 0.02) showed a significant correlation with survival. Results: HUVEC mRNA expression profiling after ELTD1 silencing showed upregulation of SLC24A1, which transports citrate from the mitochondria to the cytoplasm & ACLY, which converts cytoplasmic citrate to Acetyl CoA, feeding fatty acid and cholesterol synthesis, and acetylation. We validated this at RNA & protein expression level & showed that ELTD1 inhibited lipid droplet formation. Signalling experiments revealed that unlike other aGPCRs, ELTD1 does not couple to any canonical GPCR pathways (Gxx; Gae; Goz; Gqz/ G12/13). In breast cancer, we found that no representative cell line screened expressed ELTD1. Breast cancer immunohistochemistry revealed higher intensity vascular ELTD1 staining within the tumour stroma contrasted to normal STROMA & expression normal tumour epithelial cells (15%). Higher ELTD1 expression in both the tumour stroma vasculature (n = 241; HR = 0.68; p = 0.04) & within the subset of tumour positive cases (n = 24; HR = 0.5; p = 0.02) correlated with improved relapse free survival (RFS). Conclusion: ELTD1’s regulation of lipid synthesis through suppression of ACLY & SLC24A1 highlights ELTD1’s role as a novel regulator of endothelial metabolism. Unlike other aGPCRs, ELTD1 does not signal through canonical G protein pathways. The good prognosis of ELTD1 expression may be related to inducing a quiescent endothelial population. It will be of interest to relate this activity to anti-angiogenic therapy. Nevertheless, a relevant fraction of patients expressing ELTD1 still relapsed & this may be a suitable target for this population.

**Identifying, subtyping and classifying tumor associated circulating endothelial cells in patients with solid tumors.** Daniel L. Adams,1 Katherine Alpaugh,2 Steven H. Lin,3 Jeffrey R. Marks,4 Raymond Bergan,5 Stuart S. Mar-5, Sarany Chumsri,7 Massimo Cristofanilli,8 Cha-Mei Tang,9 Steingrimur Steinarsson10.

Background: Tumor endothelial cells (ECs) are a population of stromal cells required for tumor growth that cooperate with tumors to form angiogenic structures. In blood, circulating ECs (CECs) are normal constituents of healthy individuals, although a Cancer Associated Vascular Endothelial cell (CAVE) subtype has been observed in cancer patients. The CAVE population has been isolated and identified using their large size or multicellular clustering and a pooled mixture of classical EC markers (i.e CD31 and CD146). However, there is debate over whether these populations are the same. This is not surprising as in-depth phenotyping of ECs requires an array of biomarkers that until recently has not been feasible. A multi-phenotypic screening of EC derived CECs that may express cytoketin (CK) and various EC biomarkers, correlating to disease stage. Methods: Peripheral blood samples from 116 cancer patients (stage I-IV) were drawn from 2012-2014 including breast (n = 42), lung (n = 39) and prostate (n = 35), as well as blood from 34 healthy controls. Blood was processed by an established filtration approach, i.e. the CellSieveTM microfiltration technique (Creativ MicroTech), filtering blood by size exclusion and...
staining cells for CK 8, 18 & 19, EpCAM and CD45. After identification and imaging, the QUAS-R (Quench, Underivate, Amine-Strip and Restain) technique was used to remove fluorescence signal and retain all cells with CD31, CD146, CD144, & DAPI. After reimaging, QUAS-R was again used to remove fluorescence and retain the cells for CD14, CD105, CD34, & DAPI. Results: Out of 63 patient samples, we identified CAVs in 63 patients (54%) based on positivity of CD31, CD144 or CD146, but none were found in healthy controls. CAVs per 7.5mL sample in patients averaged 5.1 (breast), 5.6 (prostate) and 7.9 (lung). Presence of CAVs appeared related to stage with 26% in stage 1, 61% in stage 2, 68% in stage 3, and 74% in stage 4 patients. No CAVs were positive for CD14 or CD45. CD31 was the most present marker, found on 93% of CAVs, followed by CD144 (85%), CD34 (64%), CD146 (45%), & CD105 (4%). In contrast with the previous study on this topic, CK was found in 67% of CAVs, but was not a universal marker. Conclusions: It has been reported that CK+ and CD45- CECs are isolated from the blood of cancer patients in colon and lung cancers, prompting some to classify them as circulating tumor cells. However, subtyping these CECs is incomplete when characterized with only 3-4 biomarkers. A multi-phenotypic subtyping technique was used to properly identify and subtype these CECs in cancer patients. This data suggests that a subset of CECs, e.g. CAVs, are found in circulation as CK+ / CD45-. However, it is not a heterogeneous population of cancer specific circulating cells that require further study.

**#780 In vitro screening of individual human neuroendocrine tumors for their angiogenic response to tyrosine kinase inhibitors.** Tanja Milosavljevic,1 Elise J. Choquet,1 Catherine E. Anthony,2 Ariana Dirige,1 Yi-Zarn Wang,3 Philip J. Boudreau,1 Ramcharan Venkat R. Katkoori,2 Harvey Bumpers1. 1Medical College of Wisconsin, Milwaukee, WI; 2Northern Kentucky University, Highland Heights, KY; 3Merkey Cancer Center, University of Kentucky, Lexington, KY.

Background: Human neuroendocrine tumors (NETs) are highly vascular in nature and reliant on multiple receptor tyrosine kinases (TKs) for their neovascularization, growth, and, metastasis. These tumors most commonly originate in the small bowel (SB) and frequently metastatize to the lymph nodes and other organs. Current treatment of metastatic NETs involves a variety of approaches including antiangiogenesis therapies. In this study we tested effectiveness of six TK inhibitors (TKIs) [Dovitinib lactate, Regorafenib, Erlotinib, Imatinib, Vatalanib, and Sunitinib] on individual NETs angiogenic response in vitro. Methods: Specimens were obtained from NET patients who underwent removal of their primary tumor (small bowel/pancreas/stomach), nodal, and organ (i.e. liver/ ovary/omentum/mesentery) metastasis. Fresh tumors were minced, embedded in a fibrin-thrombin clot and supplemented with nutrient culture media per the in vitro human tumor angiogenesis model protocol. Neovessels were visually scored and evaluated for angiogenic parameters: percent initiation (%I), angiogenic growth (A1), and overall angiogenic response (OAR). All TKIs were prepared consistent with manufacturer’s instructions and their effective concentration was determined by dose response experiments. The selected TKI dose reflected the clinically achievable plasma level. A large group of NETs was tested for their angiogenic response to six TKIs [Dovitinib lactate (D): n = 164, Regorafenib (R): n = 163, Erlotinib (E): n = 35, Imatinib (I): n = 51, Vatalanib (V): n = 163, Sunitinib (S): n = 164]. Paired samples t-test was used to compare TKI to control results for each angiogenic parameter, and independent samples t-test to compare TKI-response of primary and metastatic sites (MedCalc). Results: Each selected dose [D: 82nM, R: 1100nM, E: 100µM, I: 2.5µM, V: 20µM, S: 188nM] achieved statistically significant inhibition of OAR (D:94.23%, R:53.58%, E:52.07%, V:59.77%, S:66.56%, M:64.79%) in all NETs (p<0.0001). This is accomplished by the simultaneous statistically significant decrease of %I by at least 3.47% (p=0.0164) and A1 by at least 34.03% (p<0.0001). Comparison of OAR between the primary and metastatic tumor sites revealed no differences in their response to each tested TKI (p=0.2119). In all NETs, TKIs inhibited both mechanisms of angiogenesis, but preferentially targeted growth (all TKIs: p<0.0001) over %I (D, I, S; p<0.0001; E; p=0.0072; R; p=0.0164). Conclusions: In vitro screening of individual tumors revealed that TKIs effectively inhibited all parameters of angiogenesis in all NETs, primary and metastatic tumors. Selected TKIs preferentially inhibited angiogenic growth rather than initiation in all NETs. Our preclinical results show that Dovitinib inhibits angiogenesis most effectively in human NETs compared to other TKIs in vitro.

**#781 Functional consequence of the p53 codon 72 polymorphism in colorectal cancer.** Venkat R. Katkoori,1 Upender Manne,2 Harvey Bumpers3. 1Medical College of Wisconsin, Milwaukee, WI; 2Northern Kentucky University, Highland Heights, KY; 3Merkey Cancer Center, University of Kentucky, Lexington, KY.

Background: The codon 72 polymorphism in p53 has been implicated in multiple polymorphisms in the SS.BN3IL2R tumor of 116 patient samples, we identified CAVs in 63 patients (54%) based on positivity of CD31, CD144, & DAPI. After reimaging, QUAS-R was again used to remove fluorescence signal and restain all cells with CD31, CD146, & DAPI. Results: Out of 63 patient samples, we identified CAVs in 63 patients (54%) based on positivity of CD31, CD144, or CD146, but none were found in healthy controls. CAVs per 7.5mL sample in patients averaged 5.1 (breast), 5.6 (prostate) and 7.9 (lung). Presence of CAVs appeared related to stage with 26% in stage 1, 61% in stage 2, 68% in stage 3, and 74% in stage 4 patients. No CAVs were positive for CD14 or CD45. CD31 was the most present marker, found on 93% of CAVs, followed by CD144 (85%), CD34 (64%), CD146 (45%), & CD105 (4%). In contrast with the previous study on this topic, CK was found in 67% of CAVs, but was not a universal marker. Conclusions: It has been reported that CK+ and CD45- CECs are isolated from the blood of cancer patients in colon and lung cancers, prompting some to classify them as circulating tumor cells. However, subtyping these CECs is incomplete when characterized with only 3-4 biomarkers. A multi-phenotypic subtyping technique was used to properly identify and subtype these CECs in cancer patients. This data suggests that a subset of CECs, e.g. CAVs, are found in circulation as CK+/CD45-. However, it is not a heterogeneous population of cancer specific circulating cells that require further study.
Cancer cell-derived extracellular vesicles stimulate tumor angiogenesis by delivering VEGF to endothelial cells. Song Yi Ko, Honami Naora. UT MD Anderson Cancer Ctr., Houston, TX.

Angiogenesis is essential for tumor growth and metastasis, and is orchestrated by a repertoire of growth factor signaling pathways that stimulate endothelial cell growth, migration and vessel formation. Recent studies have shown that angiogenesis can also be regulated by cell-derived extracellular vesicles. Vesicles are small endosomal-derived membrane vesicles that contain various biomolecules such as RNA and proteins, and are increasingly thought to play important roles in transferring informational cargo between cancer cells and stromal cells. In the majority of studies to date, the effects of cancer cell-derived exosomes have been attributed to their RNA cargo. In this study, we identified that exosomes derived from ovarian, colon and renal cancer cells contain abundant vascular endothelial growth factor (VEGF). Cancer cell-derived exosomes were found to activate the VEGF signaling pathway in endothelial cells and to stimulate endothelial cell migration and tube formation. Furthermore, our studies using inhibitors of VEGF signaling demonstrated that the stimulatory effects of exosomes on endothelial cell migration and tube formation are mediated via the VEGF signaling pathway. These findings indicate that cancer cell-derived exosomes promote tumor angiogenesis by delivering VEGF to endothelial cells.

Pharmacodynamic biomarkers in metronomic chemotherapy: Multiplex cytokine measurements in gastrointestinal cancer patients. Paloma Valenzuela,1 Karla Parra,2 Derrick Oxaca,1 Luis Reza,1 Jose Lopez,1 Montserrat Garcia Garcia,1 Germinigana Rodriguez,1 Alfredo Falcone,1 Giacomo Allegrini,2 Teresa Di Desidero,1 Guido Bocci,2 Robert Kirken,1 Giulio Francia,1 1UT El Paso, El Paso, TX; 2University of Pisa, Pisa, Italy.

Metronomic chemotherapy has shown promising antitumor activity in a number of malignancies. For example, we previously reported (Allegrini et al, Angiogenesis (2012) 15(2):275-86) a phase II clinical trial of metronomic UFT (a 5-fluorouracil prodrg 100 mg/twice per day p.o.) and cyclophosphamide (CTX; 300 mg/m² q14 days) in 38 patients with advanced refractory gastrointestinal tumors. The mechanisms of action of metronomic chemotherapy include upregulation of the angiogenesis inhibitor Thrombospondin-1, the suppression of bone marrow derived endothelial progenitor cells and, at least for drugs such as CTX, activation of the immune system. To further evaluate the latter, we carried out an immune system multiplex 14-cytokine profiling of plasma samples that were available (for day 0, day 28, and day 56) from 31 of the 38 patients in the above noted (Allegrini et al) clinical trial. Our results show that pre-treatment plasma level cut-offs of interferon-gamma (≥12.84pg/ml), sCD40L (<1168pg/ml), interferon-alpha2 (>5.5-11pg/ml) and IL-17a (>16.3pg/ml) were predictive markers for those patients with better progression-free survival <0.05 for each cytokine). After 28 days of metronomic therapy, the plasma levels of sCD40L, IL-17a, and of IL-6 (<130pg/ml) could serve as predictors of improved progression-free survival, as could levels interferon-gamma and sCD40L after 56 days of therapy. We observed minimal changes in cytokine profiles, from baseline, as a consequence of the metronomic therapy, with the exception of an elevation of IL-6 and IL-8 levels 28 days (and 56 days) after treatment started (p<0.05). Our results indicate that a selective cytokine elevation, involving IL-6 and IL-8, following metronomic chemotherapy administration. In addition, interferon-gamma and sCD40L may be potential biomarkers for gastrointestinal cancer patients that are likely to benefit from metronomic chemotherapy. Our study contributes to our understanding of the mechanisms of action of metronomic chemotherapy, and may guide future patient selection criteria for metronomic chemotherapy for gastrointestinal cancers.

Molecular profile of sunnītinib resistance in clear-cell renal cell carcinoma. Oscar Reig Torras,1 Mercedes Marín-Agulera,2 Natalia Jiménez,2 Paré Laia,2 Patricia Galvan,2 Carme Mallofre,1 Alexe Prat,1 Beogona Mellado1.1Hospital Clinica de Barcelona, Barcelona, Spain; 2Fundación Clinica por a la Recerca Biomèdica Araguní.

Background: Sunnītinib (SU) is a tyrosine kinase inhibitor used in the first line setting in metastatic clear-cell renal cell carcinoma (cRCC). Recently, nivolumab, a checkpoint inhibitor, has been approved as a second line therapy. The identification of biomarkers of resistance to these drugs may be useful to select patients’ treatment. In this study we investigated the molecular and immune profile associated to SU resistance. Methods: Forty-four patients (pts) with metastatic cRCC treated with SU were included. A global transcriptome analysis (Affymetrix Human Gene 2.0 ST array) on 6 extremely sensitive (ES; progression-free survival (PFS) > 24 months) and 8 refractory (R; progression disease as best response) cRCC pts treated with SU was performed. Differentially expressed genes were tested in the whole cohort by qPCR. In silico validation using the microarray data from 53 SU-treated pts (Beuselinck et al, Clin Cancer Res 2015) was performed. In addition 730 immune-related genes were tested in the same cohort and in 10 additional pts treated with anti-PD1/PD-L1 therapies as an exploratory analysis. Results: Differential expression analysis between R and ES pts showed 330 differentially expressed genes. 148 were overexpressed and 182 underexpressed in R pts. Top two deregulated networks were centered in LYN and NRC3 (Network 1), and in chemokine ligands and receptors (e.g. CCL5, CCR1 and CXL10) (Network 2). In Beuselinck dataset, 9 genes were differentially expressed between R and ES pts. The expression of 7 genes (B2ST2, CD44, ELK1, FADS2, LYN, NKAP and VEGFC) was correlated with a lower PFS in both cohorts (Table 1). The value of TIM-3 was overexpressed in SU-sensitive pts and in anti-PD1/PD-L1 refractory pts. Conclusions: We described a gene expression profile of SU resistance in cRCC pts. The value of TIM-3 as a potential biomarker in cRCC merits further exploration.

Survival analysis of selected genes in the training and validation set. *High vs low expression.

<table>
<thead>
<tr>
<th>Gene</th>
<th>HR (Training set)</th>
<th>Median PFS, months</th>
<th>HR (Validation set)</th>
<th>Median PFS, months</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELK1</td>
<td>2.66 (1.1 - 6.2)</td>
<td>0.022</td>
<td>5.2 vs 17.2</td>
<td>2.47 (1.2 - 5.1)</td>
</tr>
<tr>
<td>BST2</td>
<td>4.87 (1.5 - 15.7)</td>
<td>0.036</td>
<td>2.02 vs 10.6</td>
<td>2.22 (1.1 - 4.6)</td>
</tr>
<tr>
<td>CD44</td>
<td>2.55 (1.5 - 5.6)</td>
<td>0.023</td>
<td>5.2 vs 17.8</td>
<td>2.61 (1.2 - 5.1)</td>
</tr>
<tr>
<td>DAFDS</td>
<td>2.81 (1.1 - 7.7)</td>
<td>0.039</td>
<td>4.2 vs 8.6</td>
<td>2.1 (1.1 - 4.4)</td>
</tr>
<tr>
<td>LYN</td>
<td>5.38 (1.6 - 18.1)</td>
<td>0.006</td>
<td>4.5 vs 17.2</td>
<td>2.35 (1.1 - 4.9)</td>
</tr>
<tr>
<td>NKAP</td>
<td>3.0 (1.3 - 7.1)</td>
<td>0.012</td>
<td>5.1 vs 17.2</td>
<td>2.37 (1.1 - 8.8)</td>
</tr>
<tr>
<td>VEGFC</td>
<td>3.02 (1.2 - 7.5)</td>
<td>0.016</td>
<td>4.5 vs 17.2</td>
<td>1.97 (1.3 - 8.8)</td>
</tr>
</tbody>
</table>

Vascularity and vascular maturity in breast cancer: a comparative analysis of the different molecular subtypes. Nenad M. Ayoub,1 Ahmed Y. Al Husban,1 Hussein M. Al Husban,2 Abdal Rahman N. Al Khatib,1 Alaa O. Al-Malleh,1 Ekklas A. Al-Majali2.1Jordan University in Jordan, Jordan; 2Royal Medical Services, Amman, Jordan.

Currently, angiogenesis inhibitors lack considerable activity and did not result in survival advantage in breast cancer. The goal of this study is to evaluate vascularity in terms of vascularity and vascular maturity among breast cancer subtypes. Archived tumor samples for breast cancer patients were retrieved from Pathology Department at Royal Medical Services in Jordan. Tissue sections were stained for the expression of basement membrane laminin using immuno-histochemical analysis. Endothelial cell and pericyte were identified and counted based on the shape of the nuclei as visualized with DAPI stain. Demographic, clinical, and pathological data were retrieved from patient medical records at time of diagnosis. Vascularity was obtained by counting the number of blood vessels and maturity of blood vessels is represented by the ratio of the average number of endothelial cell to average number of pericytes. A total of 60 patients were enrolled in this study in which 34 patients (56.7%) have hormone-dependent disease, 14 patients (23.3%) determined to have HER2-positive disease, and 12 cases (20.0%) were classified as triple-negative. Results showed a significant difference for the mean number of blood vessels (p=0.004) and endothelial cell to pericyte ratio (p=0.000) among the different molecular subtypes of breast cancer. Post hoc analysis indicated statistically significant difference in mean number of blood vessels between hormone-dependent and HER2-positive tumors (p=0.015). In addition, ratio of endothelial cells to pericytes was significantly different between hormone-dependent cases and each of HER2-positive and triple-negative cases (p=0.004 and 0.014, respectively). Size of tumor was significantly correlated to endothelial cell to pericyte ratio for the population examined (r=0.345, p=0.007). A significant correlation was found between the number of blood vessels and number of involved lymph nodes among all patients (r=0.271, p=0.038). Independent samples t-test analysis comparing mean number of blood vessels and endothelial to pericyte ratio according to hormone receptor status showed significant difference between hormone receptor positive and negative cases for both estrogen and progesterone receptors (p<0.05). No significant difference was found for the vascular parameters tested according to tumor stage, grade, and lymphovascular invasion (p>0.05). Collectively, these findings showed that the degree of tumor vascularity and vascular maturity are not uniform among breast cancer subtypes. Vascular maturity was significantly higher in hormone-dependent breast tumors compared to both HER2-positive and triple-negative cases. It is critical that a careful assessment of tumor vascularity is being considered in breast cancer patients in order to determine responsiveness to chemotherapy and anti-angiogenic treatment among the different molecular subtypes.

Therapy resistance in solid tumors is of growing concern due to the failure of multiple therapies. Targeting tumor cells alone with conventional therapy, tumor vasculature with antiangiogenic therapies (AAT) and tumor-infiltrating myeloid cells with CSF1R inhibitor have all lead to the development of refractory tumors with greater relapse rates. There is an urgent need to understand molecular mechanisms of therapy resistance in cancer. There could be tumor cell extrinsic and intrinsic mechanisms in the tumor microenvironment.

We focused our study to investigate tumor cell intrinsic pathways using glioblastoma (GBM) as a model tumor and AAT (anti-VEGF-VEGFR) as a model therapy. The benefits of AAT are transient with increased relapse owing to adaptive responses by the GBM. Our preclinical study and in vitro data, for the very first time, identified that AAT induces transdifferentiation of tumor cells into endothelial-like cells, capable of forming functional blood vessels in the growing tumors, termed as vasogenic mimicry (VM). We observed that anti-VEGFR2 (Vatalanib) induced VM vessels are positive for periodic acid-Schiff (PAS) matrix but devoid of any endothelium on the inner side and lined by tumor cells on the outer-side. The PAS+ matrix is positive for basal laminae (laminin) indicating vascular structures. Vatalanib treated GBM displayed various stages of VM such as initiation (mosaic), sustenance, and full-blown VM. In addition to this, vatalanib treated tumors show significantly increased Laminin positive loops characteristic of VM in tumor center as well as at the periphery. A positive correlation was observed between the VM-like structures and the tumor size.

We also performed in vitro tube formation assays with AAT treated GBM cells alone and HUVEC cells (co-culture) to confirm the role of GBM cells in the formation of mosaic vessels in normoxic conditions. Interestingly, tumor cells are incorporated into the tubes formed by HUVEC cells. We found a higher number of complete tube like structures with AAT treated tumor cells as compared to control. Cytokine array with the condition media from tumor cells treated with AAT showed a significant upregulation in the levels of IL8. We observed a significant increase in the CXCR1+ and CXCR2+ endothelial-like GBM cells following treatment with AAT. Ongoing investigations are focused on study of IL8-CXCR1/2 pathway in VM regulation using loss or gain of function approaches. The study will identify critical mediators of VM in GBM.

In clinics, discovering novel targets causing VM associated therapy resistance is essential for identifying subset of patients that could be treated with alternate regimens.

Less is more: macrophage depletion via CSF1/CSF1R pathway improves anti-VEGF therapy after adaptive resistance. Yasmin A. Lyons, Sunila Pradeep, Jean M. Hansen, Michael J. Wagner, Robert L. Dood, Sherry Y. Wu, Rebecca S. Carson, Robert L. Gale, Pamela G. Annin, Alan K. Sood. University of Texas/MD Anderson Cancer Center, Houston, TX.

Objectives: Anti-angiogenesis therapy shows clinical benefit in patients with high-grade serous ovarian cancer (HGSC), but adaptive resistance typically occurs. Thus, approaches to overcome such resistance are needed. Here, we systematically assessed immune cell populations enriched during adaptive resistance and identify novel therapeutic avenues. Methods: A series of in vitro and in vivo (immune competent and nude mice) experiments were carried out. Animals were treated with anti-VEGF antibody continuously until resistance emerged, at which point full immune profiling was performed. Based on these results, efficacy of AC708 (CSF1R inhibitor to target tumor-associated macrophages) was tested in the adaptive-resistance models. F4/80 antibody was used as a macrophage marker. Results: On the basis of full immune profiling, we detected significantly increased macrophage infiltration in tumors with anti-VEGF antibody resistance compared to tumors from sensitive mice (p<0.0001). Given the dominant role of CSF1R in macrophage function and overexpression of CSF1R in HGSC, we added AC708 following emergence of adaptive resistance to anti-VEGF antibody. Mice treated with AC708 after anti-VEGF antibody resistance demonstrated complete response upon completion of the experiment, while those that did not receive AC708 still had abundant tumor. To mimic treatment with the AURELIA regimen, we next treated mice with anti-VEGF antibody and paclitaxel until resistance emerged, and then AC708 was added. The addition of AC708 restored response to anti-angiogenesis therapy, resulting in 82% lower tumor burden compared to treatment with anti-VEGF antibody and paclitaxel alone (p < 0.0001), and a substantial decrease in macrophages (p<0.0004). Conclusions: The addition of CSF1R inhibitor to anti-VEGF therapy and taxane chemotherapy results in robust anti-tumor effects. To confirm these findings, a clinical trial at our institution is nearing activation. The REDIRECT (Randomized Induction Discontinuation Trial of EmaCTuzumab) trial will randomize patients to continue weekly paclitaxel and biweekly bevacizumab with or without a CSF1R inhibitor (emactuzumab) following an induction phase.

Establishment of an in vitro model for the study of vasogenic mimicry in ovarian and gastrointestinal cancer cells. Andres Valdivia,1 Dusan Racordon,2 Raul Aravena,3 Gabriel Mingo,4 Alejandro Sandoval,5 Maria Loreto Bravo,1 Mauricio A. Cuello,3 Sumie Kato,2 Rafael Ercies,2 Carolina Ramirez,2 Pamela Gonzalez,2 Beatriz Sanchez,2 Alejandro H. Corvallan,5 Gareth I. Owen.6

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Introduction: A key step in cancer progression is tumor irritation. The process of angiogenesis can be complemented in sub-sets of highly aggressive cancers by the process of vasogenic mimicry, which is the formation of tubular structures by tumor cells. Herein, we sought to establish an in vitro assay of vasogenic mimicry and determine the percentage of ovarian and gastrointestinal primary cultures capable of undergoing this process. Materials & Methods: From cancer cell lines, AGS and HS766T, and the ovarian cancer cell lines SKOV-3 and HEY were used in conjunction with gastrointestinal and ovarian primary cultured cancer cells extracted from peritoneal fluid. Cells were seeded on matrigel and monitored for a week. Hollow channels formation was evaluated by fluorescent dye microinjection, periodic acid Schiff staining, confocal microscopy and X-Ray microtomography (Micro-CT). Results: SKOV-3, HEY and AGS cell lines underwent the process of vasogenic mimicry. Both confocal microscopy and Micro-CT reconstruction were able to show the presence of a lumen of the structures in 3D. In 22 primary cancer cultures (Ovarian, Colon and Gastric) only 38% could undergo vasogenic mimicry. Discussion: We have standardized an in vitro assay to assay and quantify vasogenic mimicry. Regardless of origin, only a low percentage of cultures have the ability to undergo vasogenic mimicry. An understanding of this process could shed light on new therapies for this sub-set of highly aggressive cancers. Funding: FOND/EICYT 1120929, 3150028 & 1140970. CORFO L2 13IDL2-18608, BMRC 13CTI 21526-P6, IMII P09/016-F, CONICYT-FONDECYT 1513001.

Regulation of tumor angiogenesis by low dose Aspirin. Jinia Chakrabortty,1 Gargi Maitty,1 Snigdhika Banerjee,2 Sushanta K. Banerjee2. 1VA Medical Center/Blue Valley West High School, Kansas City, MO; 2VA Medical Center, Kansas City, MO.

Background and Objective: Tumor angiogenesis is a pathophysiological process in which new blood vessels are formed in the primary tumor site or distant organs for the nourishment of cancer cells and metastatic growth. Thereby, targeting tumor angiogenesis is an important area of research for cancer therapy. Studies have shown that aspirin (ASA) has the potency to inhibit breast cancer growth and metastasis, as well as reprogram the mesenchymal to epithelial transition (MET). Given the importance of ASA, we tested whether ASA may be able to regulate tumor angiogenesis. Methods: To do so, we determined the effect of low dose ASA (1mM, which is equivalent to 80mg human dose), ASA-treated (2.5mM) conditioned media (231-CMASA) or vehicle-treated conditioned media (231-CMT) of MDA-MB-231 cells on different endothelial cell lines, AGS and HS766T, and the ovarian cancer cell lines SKOV-3 and HEY were used in conjunction with gastrointestinal and ovarian primary cultured cancer cells extracted from peritoneal fluid. Cells were seeded on matrigel and monitored for a week. Hollow channels formation was evaluated by fluorescent dye microinjection, periodic acid Schiff staining, confocal microscopy and X-Ray microtomography (Micro-CT). Results: SKOV-3, HEY and AGS cell lines underwent the process of vasogenic mimicry. Both confocal microscopy and Micro-CT reconstruction were able to show the presence of a lumen of the structures in 3D. In 22 primary cancer cultures (Ovarian, Colon and Gastric) only 38% could undergo vasogenic mimicry. Discussion: We have standardized an in vitro assay to assay and quantify vasogenic mimicry. Regardless of origin, only a low percentage of cultures have the ability to undergo vasogenic mimicry. An understanding of this process could shed light on new therapies for this sub-set of highly aggressive cancers. Funding: FOND/EICYT 1120929, 3150028 & 1140970. CORFO L2 13IDL2-18608, BMRC 13CTI 21526-P6, IMII P09/016-F, CONICYT-FONDECYT 1513001.
ASA treatment blocks several angiogenic factors including VEGF-A that are associated with these three events, implicating a low dose of Aspirin is potentially therapeutic for breast cancer via blocking and normalizing tumor angiogenesis.

**#791** Inhibitory effects of teaflavin-3, 3'-digalate (TF3) on ovarian cancer cells. C. Chen,1 C. Gao,1 G. O. Rankin,1 A. Chakraborty,2 S. Singh,1 A. Mittelman,3 R. K. Tiwari,3 New York Medical College, Valhalla, NY.

Teaflavin-3, 3'-digalate (TF3) is a black tea polyphenol produced from polymerization and oxidation of green tea polyphenols epicatechin gallate and (-)-epigallocatechin-3-gallate (EGCG) during fermentation of fresh tea leaves. TF3 has been reported to have anti-cancer properties. However, the effect of TF3 on tumor angiogenesis and the underlying mechanisms are not clear. In the current research, TF3 was verified to inhibit tumor angiogenesis. Compared with EGCG, TF3 was more potent. TF3 inhibited human ovarian carcinoma OVCAR-3 cells induced angiogenesis in human umbilical vein endothelial cell model and in chick chorioallantoic membrane model. TF3 reduced tumor angiogenesis by down-regulating HIF-1α and VEGF. One of the mechanisms was TF3 inactivated Akt/mTOR/p70S6K/4E-BP1 pathway and Akt/c-Myc pathway. Besides, TF3 suppressed the cleavage of Notch-1, subsequently decreased the expression of c-Myc, HIF-1α and VEGF, and finally impaired cancer cells induced angiogenesis. Nevertheless, TF3 didn’t have any influence on MAPK pathways. Taken together, these findings suggest that TF3 might serve as a potential anti-angiogenic agent for cancer treatment.

**#792** Development of high-throughput cell-based co-culture angiogenesis assay system using hTERT immortalized cells. CHAOZHONG ZOU,1 Chiawen Hsu,2 Menghang Xia,3 Metewo S Enuameh1, ATCC, Gaithersburg, MD; 1National Institutes of Health, Bethesda, MD.

Angiogenesis is a multi-step physiological process which is involved in a large number of normal and disease state processes; In vitro angiogenesis models provide very useful tools to study these processes, one of which is the analysis of tube formation. Tubules formed in co-culture assays were significantly more heterogeneous and more closely resembled capillaries than Matrigel® tubules. Current co-culture models using primary cells have donor variability, and inconsistent results due to lot to lot variation. In this study, we established an in vitro co-culture model system consisting of an assay ready mixture of an aortic endothelial cell line and a hTERT immortalized human aortic endothelial cell line and a hTERT immortalized adipose-derived mesenchymal stem cell line (hTERT-MSCs) in a specially formulated medium containing VEGF supplement (Angio-Ready™ Angiogenesis Assay System). Both cell lines were immortalized by hTERT (human telomerase reverse transcriptase) alone and have been well-characterized showing that the cells retain the most important characteristic of their parental counterparts. The new co-culture system forms functional tubular structures in less than 7 days, and in addition, the hTERT-MSCs which surround the tubular structures have undergone transformation indicated by elevated positive oSMMA staining (a marker of smooth muscle cells), indicating that the system has physiological relevance. Notably, our results showed the co-culture system has minimal lot-to-lot variation indicated by the treatment of three lots with the anti-cancer drug, Ramucirumab (Cyramza®), which also targets the VEGF pathway. Next, we tested the new system with compounds that impact angiogenesis, results demonstrated that the angiogenesis system responds positively to elevated doses of VEGF and negatively to increasing concentrations of suramin; more importantly, the tubular formation efficiency is reduced or blocked by well-known anti-cancer drugs such as Sunitinib (SUTENT®) and Bevacizumab (Avastin®), both of which target the VEGF pathway. Finally, we used the Angio-Ready™ system validated 4 HIF-1 (hypoxia inducible factors-1) inhibitors which have anti-angiogenic properties identified by high-throughput screening methods; data showed the results of the new system match with other screening methods including a system screening time as short as 3 days. Therefore, the co-culture model developed by using hTERT-immortalized cell lines described in this report provide a consistent and robust in vitro system for studying cardiovascular biology, drug screening and cellular changes will facilitate identification of the interactive mediators and subsequent development of effective antiangiogenic therapy.


Breast cancer affects one in eight women in the USA. Early diagnosis and newer treatment modalities have rendered breast cancer manageable. However, triple negative breast cancer is still difficult to treat and warrants a search for new targets. One strategy that has emerged in cancer research involves targeting of tumor associated blood vessels which provide growing tumors with oxygenated blood and growth factors necessary for maintenance and metastasis. Antiangiogenic drug therapy is transient and has not been able to gain main-stream therapeutic modality. We discovered that endothelial progenitor cells (EPCs) are mobilized from the bone marrow to the tumor site and contribute to the development of breast tumor vessel formation in an estrogen-dependent manner. Therefore, characterization of tumor associated endothelial progenitor cells in breast cancer may provide a more specific antivascular therapy. Using the highly proliferative human umbilical cord blood derived EPCs, having the phenotype (CD133+; CD34+, VEGFR-2+), the effect of growth factor and chemokine rich EPCs conditioned medium (CM) was assessed in luminal (MCF-7), and post-EMT (MDA-MB-231) breast carcinoma cell lines. We observed an initial halt in cellular proliferation in MCF-7 followed by a significant increase in proliferation after forty eight hours of treatment. On the other hand, MDA-MB-231 showed decreased proliferation even after forty eight hours of treatment. Treating the EPCs with breast cancer conditioned medium resulted in morphological and cellular growth changes in the EPCs. MDA-MB-231 CM resulted in an increase of the EPCs proliferation and differentiation by increasing the number of spindle shaped attaching cells, and MCF-7 CM resulted only in an increase in the differentiation rate by increasing the number of cell clusters. This increase in EPCs proliferation and differentiation associated with MDA-MB-231 CM treatment might explain the invasiveness of this breast cancer cells through the increase in the tumor associated neovascularization. The analysis of the parameters of development between breast cancer cells and EPCs along with the associated cellular changes will facilitate identification of the interactive mediators and subsequent development of effective antivascular therapy.

**#794** Potential role of DLL4 in uveal melanoma vascular mimicry. Julia Escandon, Matthew G. Field, Stefan Kurtenbach, Jefim Kuznetzov, Christina L. Decatur, J William Harbour. Univ. of Miami, Miami, FL.

Uveal melanoma is the most common malignancy of the eye. Thanks to gene array analysis it is possible to classify uveal melanoma in Class 1 (low metastasis risk) and Class 2 (high metastasis risk) tumor. This classification will ultimately determine the tumor treatment, risk of metastasis and patient surveillance. Progression to metastasis remains by far the greatest problem in uveal melanoma and is associated with loss of BAP1 tumor suppressor. Bioinformatic analyses of RNA-Seq indicated that pro-angiogenic genes such as DLL4, VEGFA, VEGFC and HIF1α are overexpressed in Class 2 compared to Class 1 uveal melanoma while angiogenic inhibitors such as ZFP36L1, HIF1AN, VEGFB, VHL and HIF3A are downregulated. Further, we found that DLL4 is among the 5 most highly overexpressed genes associated with BAP1 loss in clinical specimens and in uveal melanoma cell lines induced to deplete BAP1. DLL4 is a Notch ligand known to regulate endothelial cells, bone marrow endothelial cell progenitors and angiogenesis. We hypothesize that DLL4 contributes to vascular mimicry in uveal melanoma. To test this hypothesis, we will test uveal melanoma cell lines induced to deplete BAP1 using shRNA in cell culture-based and in vivo models. The results of this research have the potential to elucidate the mechanism by which vascular mimicry occurs in uveal melanoma.

**TUMOR BIOLOGY: Angiogenesis and Vascular Biology 1**

The capacity of high-grade serous ovarian cancer cells to form spontaneous multicellular structures (SMCS) in vitro predicts their in vivo tumorigenicity. Alicia A. Boye, Chunmei Fu, Zhihui Gao, Carlos M. Telleria, McGill University, Montreal, Quebec, Canada.

High grade serous ovarian cancer (HGSOC) is the most frequent histopathological subtype among epithelial ovarian cancers (OC). While an increase in the tumor associated neovascularization. The analysis of the parameters of development between breast cancer cells and EPCs along with the associated cellular changes will facilitate identification of the interactive mediators and subsequent development of effective antivascular therapy.

**TUMOR BIOLOGY: Cell Culture and Animal Models of Cancer 1**

The capacity of high-grade serous ovarian cancer cells to form spontaneous multicellular structures (SMCS) in vitro predicts their in vivo tumorigenicity. Alicia A. Boye, Chunmei Fu, Zhihui Gao, Carlos M. Telleria, McGill University, Montreal, Quebec, Canada.

High grade serous ovarian cancer (HGSOC) is the most frequent histopathological subtype among epithelial ovarian cancers (OC). While an increase in concentration of CA125 in the blood anticipates clinical remission following standard of care, we lack biomarker/s to diagnose early disease stage or predict progression speed. In this work we question whether the capacity of HGSOC cell lines developed from the same patient’s ascites: longitudinally along disease progression—established at platinum-sensitive relapse (PEO1), from further progressive disease 10 months (mo.) later (PEO4); and after failure to respond to high-dose cisplatin 3 mo. later (PEO6). We noticed all cell types developed an adenotype that is highly overexpressed in clinical specimens and in uveal melanoma cell lines induced to deplete BAP1. DLL4 is a Notch ligand known to regulate endothelial cells, bone marrow endothelial cell progenitors and angiogenesis. We hypothesize that DLL4 contributes to vascular mimicry in uveal melanoma. To test this hypothesis, we will test uveal melanoma cell lines induced to deplete BAP1 using shRNA in cell culture-based and in vivo models. The results of this research have the potential to elucidate the mechanism by which vascular mimicry occurs in uveal melanoma.
apparent capability to form SMCs. Next, 2×10^6 PE01, PE04 or PE06 cells were implanted into the abdominal cavity of nude mice; the animals were sacrificed either after having met an end-of-wellness endpoint criterion, or after a maximum of 14 mo. if no such criteria were met. PE06-injected mice reached the humane endpoint due to accumulation of ascites 6–7 mo. following injection, and predicated by visible signs of stress in the omentum, pancreatic-spleen region, liver base and diaphragm. Animals injected with PE04 or PE01 cells, however, did not develop any apparent disease 14 mo. following injection. Yet, when a 10-fold higher load of PE04 cells but not PE01 cells (i.e., 20×10^6) were injected, animals met euthanasia criteria due to ascites accumulation 6 mo. later and displayed macroscopic disease similar to that caused by 1/10 of the load of their prepubertal (PE06) cells) within the same timeframe. The different anatomical localization and histopathological aspects of the lesions generated with PE06 cells were similar to that generated with PE04 cells, suggesting that the more aggressive PE06 cells were likely present, although in fewer quantities, within the less aggressive PE04 cell population. Our data demonstrate, then, that the in vitro SMCS forming capacity of HGSOC cells has a positive correlation to their capacity to sicken the animals. Moreover, this study suggests that the in vitro SMCS forming capacity of epithelial OC cells obtained from ascites of patients diagnosed with HGSOC may be used to predict their aggressiveness and, consequently, guide prognosis.

#796 Reproductive hormones modulate cardioprotection from doxorubicin-induced cardiomyopathy in female spontaneously hypertensive rats. Kaytie Pokrzynski,1 Elliott T. Rosen,1 Julia L. Bonanno,1 Baikuntha Aryal,1 Thomas Biel,1 Dharam Prasad,1 Steven Mog,2 Ashutosh Rao,1 FDA, Silver Spring, MD; 2FDA, College Park, MD.

Doxorubicin (Dox) causes cardiomyopathy, partially driven by the excess generation of mitochondrial reactive oxygen species (ROS). The incidence of Dox-induced cardiac stress is greater in prepubescence and postmenopausal females compared to reproductively mature women, suggesting that reproductive hormones may mediate cardioprotection. We investigated the role of exogenous estradiol (E2), progesterone (P4), Tamoxifen (Tam) or combinations thereof, and were subsequently treated with Dox. Naturally cycling animals were treated with Dox, the iron chelator Dexrazoxane (Drz), or Dox+Drz. Tumor size was used to evaluate anticancer activity. Vaginal cytologies and circulating E2 and P4 levels were obtained. Serum cardiac troponin I (cTnI), histopathology and echocardiograms were used to assess cardiac health. Dox treated animals supplemented with E2 and P4 (alone) showed cardioprotection relative to the Dox treated animals. Dox+/-Drz exhibited sustained anti-tumor activity independent of endocrine status. After 13 days with Dox, all naturally cycling animals arrested in diestrus (lowest hormone levels). These results demonstrate that E2 and P4 have a role in providing cardioprotection from Dox therapy in SHRs, and that estrous cycle stage may provide a safety treatment window, potentially reducing the risk of cardiotoxicity in reproductively normal females. We are also currently analyzing RNA sequencing data obtained from heart tissues to achieve a better understanding of the molecular mechanisms of cardioprotection.

#797 Glioblastoma animal model using CRISPR-Cas9 technology. Da Eun Jeong,1 Kee Hang Lee,1 Sung Soo Kim,1 Yoon Kyoung Bae,1 Hyun Naim,1 Ji Yoon Hwang,2 Hee Jang Pyeon,3 Hye Jin Song,3 Kyeung Min Jou1,2 SAHIST, Sungkyunkwan University, seoul, Republic of Korea; 2Samsung Medical Center, seoul, Republic of Korea.

Current in vivo model system poses limitation on fully recapitulating genomic characteristics of a tumor due to high complexity and poor understanding of the heterogeneous microenvironment conditions in cancer pathogenesis. In this respect, such as the same topic models are required. In present study, we propose that the most representative cancer models have consistent tumor microenvironments and genomic mutations. The Clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 system is a powerful genome editing tool for efficient and precise genome engineering. Here, we employed CRISPR-Cas9 system in vivo to generate Cre-dependent Cas9 knock-in mouse (B6;129-Gf(OBA)26Sortm1(CAG-cas9/EGFP/hrFtz/J, Jackson lab.). The Cre-dependent Cas9 mouse models harbor combinations of genomic alterations including well-established oncogenes such as EGFRviii, c-MET, PDGFRα, IDH1 R132H and KRAS, EGFR, ALK, BRAF in Brain and Lung cancer models, respectively. While, they also consist of tumor suppressor genes including PTEN, NF1, Ink4a/ARF, Rb, TP53 and TP53, PTEN, NKx-1, APC in both Brain and Lung models, respectively. Cre-dependent model allows us to study in-depth into the tumor initiation and progression, while able to follow up in the role of tumor microenvironment in cancer maintenance. A better understanding of cancer models for preclinical research including their uses, as well as their visible and non-visible signs may aid for future potential studies regarding the development and implementation of new immune targeted therapies and potential validation of novel therapeutic biomarkers.

#798 Label-free enrichment and detection of circulating tumor cells in metastatic breast cancer patients show 82% of cohorts have detectable targets. Yu-Jen Chang,1 Chen-Lin Chen,2 Wei-Fan Hsu,3 Meng-Ze Li,4 Wei-Yuan Ma,1 Ken-Chao Chen,1 Guan-Syuan Huang,2 Wai-Sang Wong,1 Jhan-Yu Syu,1 Thomas, Yo-Yan Huang,2 Ching-Hung Lin,2 Andrew M. Wó,3 Chuin-Sheng Huang,4 National Taiwan University, Taipei, Taiwan; 4University of California, San Diego, CA; 5National Taiwan University Hospital, Taipei, Taiwan.

Circulating tumor cells (CTC) are believed to be the culprit of metastasis and studies have shown that their enumeration has prognostic value in wide range of solid tumors. Although much progress has been made in CTC technology, their rarity in blood and their inherent heterogeneity still provide much challenge towards maturity of the technology. This paper presents semi-automated enrichment of CTC via density-based approach in a novel microfluidic disk, followed by multi-step on-disk immunofluorescence staining (pan-CK, EpCAM, Hoechst and CD45), fluorescence microscopy for image capture, and software image analysis. To characterize the performance of the system, we spiked 1 to 800 cells (five cell lines at 105, six cell lines, at 104) into the microfluidic disk. Label-free enrichment and detection of circulating tumor cells in Metastatic breast cancer patients show 82% of cohorts have detectable targets. The recovery rate of the system is 87.4% ± 3.7% (R2 = 0.958) regardless of EpCAM expression levels of the cell lines. To interrogate the limitation of the technology, this data set included ultra-low cell counts spiked of 1 to 13 cells (mean ±5, median 5) which might be indicative of CTC from metastatic breast cancer (MBC) patients. The recovery rate for this low cell count is 90±10%. Notably, one single cell was spiked into healthy whole blood, processed via the technology, and one target cell was detected. This test was repeated in triplicate with all three tests recovered one cell each. Furthermore, the microfluidic disk technology enables operation over a range of blood volume (from 2 to 7.5mL) with no statistically significant difference in recovery rate. Recovered MCF-7 cells spiked in blood were subsequently cultured for 6 days and showed good viability with cell proliferation. We tested the technology in MBC patients along with CA15-3 and CT imaging. A total 34 of blood samples were collected from 21 patients over the course of their systemic treatment. Results showed CTC were detected in 28 samples (82%). The target CTC detected ranges from 0 to 138 (mean 16, median 4 CTCs per 7.5mL). 15 out of 34 samples (44%) had CTC number > 5/7.5mL. In our patient with triple negative diagnosis, CTC count CA15-3 and CT imaging were monitored during the course of chemotherapy. The CTC count remained zero at the end of the first and second treatment course, elevated to 24 CTCs at the end of the third course, and continued its elevation to 31 by the end of the fourth treatment course. During this entire treatment course, CA15-3 did not vary significantly. However, CT image confirmed objective response within 6–9 months of the second chemo course to 8.09cm at the beginning of the fourth course. Taken together, our label-free CTC enrichment technology has high analytical sensitivity (87%) over a wide range of cancers, able to handle a flexible blood volume (2mL to 7.5mL), and amenable to detect a high percentage (82%) of CTC in MBC patients.


The presence of microlcifications on mammographic images represents a highly valuable tool in the early detection of breast cancer, often allowing radiographers to diagnose mammary tumours at an early, in-situ stage. The presence of microlcifications has also been linked to a number of unfavourable prognostic factors including decreased survival and an increased probability of relapse. Despite their long history of use in the clinic, the precise mechanisms by which microlcifications are formed remains poorly understood. Pathological soft-tissue classification is often considered the result of an imbalance between pro- and anti-calcifying factors. However, the possible impact of such an imbalance has not been studied in the context of breast calcification. We have established an in-vitro model of microlcification formation using the triple-negative adenocarcinoma cell line MDA-MB-231. When cultured in the presence of the osteogenesis-promoting reagents β-glycerophosphate, ascorbic acid and dexamethasone, calcified deposits begin to form by Day 14, as verified by Alizarin Red staining, and by 28 days calcium deposition is significant. This model will allow an understanding of the interactions that underpin microlcification formation and provide insights into the development of new anti-calcification therapies.
arin Red staining and the quantitative o-cresolphthalein assay. Following the establishment of our model, we set out to identify the underlying molecular triggers initiating the calcification process, in particular the role of micro-environmental factors. Altered magnesium homeostasis has been suggested as an important mediator of tissue calcification. We found that a slight increase in magnesium concentration resulted in almost completely blocked calcium deposition in our model. Previous studies in other tissues have shown this protective effect to be dependent on the cation channel TRPM7 which is increased in breast cancer patients with calcifications compared to those without. Unexpectedly, inhibition of the TRPM7 channel by two separate compounds (2-APB and NS-8593) not only failed to reverse the inhibition of microcalcification formation by exogenous magnesium, but actually further decreased calcium deposition by Day 28, suggesting that calcium influx via the TRPM7 channel may be promoting development of mammary calcifications. Finally, we investigated the effect of several tumor associated cytokines on the rate of calcium deposition and found that IL-1β and TNF-α blocked mineralisation whilst IL-6 and BMP2 lead to an increase. Interestingly, co-administration of IL-6 alongside a soluble form of its receptor (sIL-6R) was observed to promote mineralisation even in the absence of dexamethasone, which had previously been essential to the formation of calcifications in our model. To date, our model has yielded a number of important insights into the formation of calcifications in breast cell lines, many of which recapitulate observations from patient studies. It is hoped that this work will contribute to our understanding of the origin of these pre-invasive diagnostic clues.

#800 Enhancing tumor killing abilities of NK cells by targeting CD16 shedding, and ADAM17.

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CD16 consists of two isoforms (CD16a and CD16b) encoded by two highly homologous genes that differ by only 6 amino acids in their extracellular regions. CD16a is expressed by human neutrophils and CD16b by human natural killer (NK) cells. The ectodomain regions of both CD16 isoforms are cleaved proximal to the cell membrane by a proteolytic process referred to as ectodomain shedding. We demonstrate that the membrane metalloprotease ADAM17 cleaves CD16 in isolated leukocytes and in human patients. By mass spectrometry analysis, we determined 3 adjacent cleavage sites in neutrophil CD16b and one cleavage site in NK cell CD16a that occurred at the same location as the predominant cleavage site in CD16b, which is interesting considering CD16b is linked to the plasma membrane via a GPI anchor and CD16a is a transmembrane protein. Antibody-dependent cell cytotoxicity (ADCC) by NK cells is a key mechanism in the anti-cancer effects of therapeutic antibodies, and CD16a exclusively recognizes tumor-bound antibodies. Surface levels of CD16a are rapidly down-regulated upon NK cell activation by cytokines, target cell interaction, and tumor infiltration, which is associated with impaired ADCC. Thus blocking this process has important clinical significance. We contend that maintaining high surface levels of CD16a during NK cell-based immunotherapy will enhance their killing of antibody-bound tumor cells. In ongoing studies, we are examining pharmaceutical and gene-targeting means of preventing CD16a cleavage as a novel therapeutic strategy to enhance the anti-cancer effects of NK cells.

#801 A zebrafish model of NF1-mutant melanoma that lacks activating mutations of BRAF or NRAS.

Shuning He,1 Marc R. Mansour,2 Hillary M. Layden,1 Scott J. Rodig,3 E. Elizabeth Patton,4 A. Thomas Look.1 1University of Minnesota, St. Paul, MN; 2University of California, San Diego, CA.

Cutaneous melanoma is the most lethal type of skin cancer, with ~76,380 newly diagnosed melanoma and ~10,130 melanoma-associated deaths per year in the US. Thus, there is a need for improved understanding of the molecular pathogenesis and more effective targeted therapies for this devastating disease. The recent work of The Cancer Genome Atlas Network has defined melanoma as an RTK/ERK-driven solid tumor that can be classified into four tumor subtypes: BRAF-mutant, RAS-mutant, NF1-mutant, and triple-wild-type. This landmark study highlighted the important role of the previously understudied NF1 tumor suppressor in melanoma pathogenesis, especially for the 9% of melanoma patients who have acquired inactivating NF1-mutations, but lack hot-spot mutations that activate BRAF or RAS. To date, animal models have not been developed for the NF1-mutant subtype of melanoma, which has significantly impaired the development of novel therapeutic strategies for this subtype. Here we report the first zebrafish model for NF1-mutant melanoma, which we generated by combining the loss of nf1 with loss of both pten and p53. The resultant compound mutant zebrafish develop aggressive melanomas from the age of 7 weeks and the tumor penetrance is 80% by the age of 18 weeks. We demonstrate further that these high-risk zebrafish melanomas were exclusive of hotspot mutations of braf and rras. Sustained inhibition of both MEK and PI3K suppressed tumor progression in vivo, whereas inhibition of MEK or PI3K alone was insufficient to suppress the growth of these tumors. Surprisingly, single and double inactivating mutations in the tumors significantly reduced tumor growth, even further than short- and long-term suppression of tumor cell growth in nf1/pten-mutant melanomas. Thus our model appears ideal for the testing of drugs that will prove uniquely active for the significant subset of NF1-mutant, BRAF/NRAS-wildtype human melanomas.

#802 Loss of function of Arid1a synergizes with MYCN in neuroblastoma pathogenesis.

Hui Shi, Ting Tao, Cigall Kadoch, Thomas Look. Dana-Farber Cancer Inst., Boston, MA.

ARID1A is a key component of the SWI/SNF-A (BAF) chromatin-remodeling complex that is known to modulate chromatin structure and gene transcription. Recent exome and whole-genome resequencing has identified SWI/SNF as the most frequently mutated chromatin remodeler in human cancers, and ARID1A is the most highly mutated component among the SWI/SNF subunits, primarily in solid tumors. In high-risk neuroblastoma, ARID1A is mutually inactivated in one allele in ~1% of patient tumors. In addition, ARID1A is deleted in one allele in at least 85% of cases with chromosome 1p deletions, which is the most common deletion found in neuroblastoma. Thus, ARID1A appears to function as a haploinsufficient tumor suppressor in a substantial fraction of high-risk neuroblastomas. The arid1a gene is disrupted in zebrafish, and the two genes are hence named arid1a and arid1ab. We targeted each allele of the two genes using CRISPR/Cas genome editing system and recovered stable lines with loss-of-function mutations in each arid1a gene. arid1ab but not arid1a homozygous mutation showed embryonic lethality. We bred these lines with a transgenic zebrafish line expressing high levels of MYCN in the peripheral sympathetic nervous system driven by the dopamine beta-hydroxylase promoter. Loss of one copy of either arid1a or arid1ab dramatically accelerated the onset and increased the penetrance of MYCN-induced neuroblastoma. Loss of additional copies of arid1a or arid1ab accelerated the tumor onset even further, with the most rapid onset in compound heterozygotes of both genes. The compound heterozygotes showed increased fraction of sympathoadrenal cells in active cell cycle compared to the MYCN transgenic fish with wild type arid1a alleles. Our long-term goal is to use the zebrafish model to elucidate the mechanisms underlying the highly dose-dependent tumor suppressor role of this component of the BAF chromatin remodeling complex in neuroblastoma, as a first step toward designing targeted therapies synthetic lethal with reduced expression levels of ARID1A.

#803 Targeting β-catenin/CBP signaling in OSCC.

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Objectives: Oral squamous cell carcinoma (OSCC) is an aggressive malignant characterized by molecular heterogeneity and locoregional spread associated with high morbidity. Aggressive cancers are thought to arise from populations of cancer initiating cells (CICs) that exhibit the properties of stem cells and drive tumor development, recurrence and resistance to therapy. The transcriptional regulator, β-catenin, has been implicated in OSCC CICs. Nuclear β-catenin has been shown to recruit the chromatin remodeling CREB binding protein (CBP) to drive expression of proliferation and survival genes, as well as genes that maintain stem-like phenotypes. We hypothesized that targeting β-catenin/CBP interaction will inhibit CICs in oral tumors and restore an epithelial phenotype. Methods: To test tumor aggressive potential of OSCC CICs, we used zebrafish as a model system. We isolated CD44+CD24medCD29hi cells from aggressive HSC-3 OSCC cells by FACS and assayed their ability to drive tumor growth and metastases in zebrafish compared to unsorted and CD44+CD24medCD29med cells. In addition, we examined the role of the β-catenin/CBP axis in the aggressive phenotype of these cells. We also assessed whether the β-catenin/CBP axis affected CICs in tumors from immune competent HPV+ mice. Results: Zebrafish injected with subpopulation of cells expressing CD44+CD24medCD29med primitive cell surface markers drove rapid tumor growth and metastases, followed by sorted and sorted CD44+CD24medCD29med cells. We targeted each molecule inhibitor of the β-catenin-CBP interaction, ICG-001, interfered with tumor growth and metastases in zebrafish. Further, ICG-001 inhibited tumor growth in immunocompetent HPV+ murine model. On a cellular level, ICG-001 promoted membrane localization of β-catenin, enhanced E-cadherin adhesion and...
restored epithelial phenotype. Significantly, ICG-001 gene signatures tracked with reduced overall patient survival in the cancer genome atlas, TCGA. Conclusion: Our studies indicate that the β-catenin/β-catenin/CBP axis promotes OSCC CICs and that ICG-001 may be an effective therapeutic agent for this malignancy. Support: Evans Center for Interdisciplinary Biomedical Research ARC funding AU 5300315 8000000.

#806 MGA is a potential tumor suppressor in acute myeloid leukemia. Qiaoyang Sun,1 Lingwen Ding,1 Kar-Tong Tan,1 Wenwen Chien,1 Xinyi Loh,1 Jinfen Xiao,1 Anand Mayakonda,1 Dechen Lin,1 Yanyi Jiang,1 Henry Yang,1 Sidig Gery,1 Sigal Gery,2 H. Phillip Koeffler2,1. 1Cancer Science Institute of Singapore, Singapore, Singapore; 2Cedars-Sinai Medical Center, Los Angeles, CA.

MGA is an incompletely studied gene with a high mutation frequency in MLL-PTD AML (9%) and in core bind factor AML (8%). This gene encodes a MAX-interacting protein and is believed to act as a transcription factor that suppresses MYC binding to its target. By in silico analysis, we found that MGA is expressed in normal myeloid hematopoietic cells and AML, and the expression level is comparable with TET2 or DNMT3A. Further data mining of TCGA revealed a high frequency of inactivating mutations of the MGA gene in a variety of cancers such as various adenocarcinomas. To interrogate functionally its role in leukemogenesis, lentiviral constructs containing either shRNA or CRSPR-sgRNA targeted to different regions of the MGA gene were generated. MGA expressing AML cell line EOL-1 was silenced by shRNA or CRISPR system. Silencing was confirmed by western blot (shRNA) and Sanger Sequencing (sgRNA). An increase of methylcellulose colony number (−30%) was observed in MGA silenced cell lines. Control EOL-1 cells or EOL-1 cells silenced with MGA CRISPR sgRNAs were injected into both flanks of NSG mice, and tumor masses were harvested 21 days after injection. Silencing of MGA by CRISPR was found significantly enhanced in vivo tumor growth. In addition, western blot analyses revealed silencing of MGA in EOL-1 cells increased protein levels of Cyclin E1 and phospho-RB (S807 phosphorylation inhibits the ability of RB to target protein allowing cell cycle progression), indicative of a proliferative advantage conferred by the silencing of MGA. MGA may be a potential regulator of the MYC pathway. We, therefore, examined whether silencing of MGA alters MYC transcriptional activity. Luciferase reporter assay was carried out in 293FT cells stabilized with either scramble or shRNA- targeting MGA. Luciferase activities were measured 48 h after transfection of cells with MYC activity reporter pMycE1bLuc and normalized to the corresponding co-transfected Renilla luciferase activity. A fourfold increase in luciferase activity was observed in MGA silenced cells when compared with non- targeting shRNA controls. Furthermore, Kaplan–Meier survival analysis was performed in the TCGA-AML patients by comparison of cases with highest versus lowest expression of MGA. P-values were calculated by log-rank test. MGA expression data and patient survival data were retrieved from TCGA-AML patients RNA seq, or microarray (70 AML patients). The MGA expression high and low groups were defined by 15% higher than the median or 15% lower than the median, respectively. AML patients with lower levels of MGA in their leukemic samples had worse outcome compared with those whose leukemic cells expressed higher levels of MGA. Collectively, our results suggest that MGA may function as a potential tumor-suppressor in AML.


Animal models of human cancer offer the potential to study human tumor growth kinetics, genetic variance among human cancers, and provide in vivo platforms for drug efficacy testing. In particular, immunodeficient mouse models have been invaluable in modeling a wide range of human cancers. However, some cancer lines don’t grow well in the available mouse models or show variability in growth kinetics from mouse to mouse, making drug efficacy studies difficult due to differences in tumor size at the onset of treatment. These challenges are also seen in patient derived xenograft (PDX) models, in addition to long timeframes to obtain sufficient mice with PDX tissue growth for drug efficacy studies. Mice are also limited in tumor growth potential with regard to humane endpoints and small size also limits the volume of blood that can be collected for analysis. An immunodeficient rat model would overcome some of these issues. A rat model would allow for larger tumor size, easier surgical manipulation, and greater volume of tissue and blood sampling for downstream analysis. In addition, large tumors from rats could be serially transplanted into mice for drug efficacy testing and could provide a large number of transplanted mice in a shorter period of time compared with serially transplanting from mouse to mouse. We have created an immunodeficient rat model with a functional deletion of the Rag2 gene. This knockout, created using spermato- gonial stem cells, lacks mature B and T cells. To assess the capability of the Rag2 knockout rat to accept human xenografts, we transplanted 2 commercially avail- able human cancer cell lines into our animals. The human REH acute lympho-
cytic leukemia cell line was transplanted via intravenous injection and the hu-
man glioblastoma cell line U87/MG was transplanted subcutaneously. Both cell 
lines survived in the Rag2 knockout rat and resulted in the growth of tumors comprised of human cells. Studies are underway to characterize the Rag2 knockout rat's ability to grow other human cell lines, including those that do not grow well in mice, and PDX tissues.

#808 PDGF-A overexpression and p53 depletion in rat neural precursor 
cells induces large brain tumors that resemble human glioblastoma. Nina P. 
Connolly, Craig S. Schneider, Amol Shetty, Tatsuya Ozawa, An-
tony F. Kim, Jeffrey A. Winkles, Eric Holland, Graeme F. Woodworth.

#809 A novel woodchuck model of hepatic artery infusion (HAI) with 
FACT complex targeted drug CBL0137. Minhyung Kim, Leslie I. Curtin, Colin 
Powers, Sandra Sandrin, Benytown, Andrea V. Gudkov, Renuka V. 
Iyer. Roswell Park Cancer Institute, Buffalo, NY.

#811 Histological heterogeneity contributes to sunitinib resistance in 
clear cell renal cell carcinoma. Zuzanna Licner, Rola Saleeb, Henriett Butz, 
Roy Nofech-Mozes, Sara Riad, Mina Farag, Andras Kapus, George Yousef. 
Keenan Biomedical Research Centre, St Michael's Hospital, Toronto, Ontario, 
Canada.

#810 Establishing patient derived preclinical in vitro and in vivo models of 
pediatric brain cancers. Sridevi Yadavilli, Madhuri Kambhampati, Jamila Git-
tens, Eshini Panditharutana, Moja Stampar, Lindsay B. Kilburn, Suresh Magge, 
Roger J. Packer, Javad Nazarian. Children’s National Health System, Washington, 
DC.

The recent surge in understanding genomic aberrations of some of the dead-
liest childhood brain cancers has highlighted the need for robust preclinical 
models. Such models will allow for robust drug screening and preclinical eval-
uation of efficacy, toxicity, and tumor penetrance in vivo. Given the rarity and 
importance of patient derived specimens, handling and processing methods are 
the most critical steps for successful establishment of viable and repro-
ducible in vitro and in vivo models. Since specimen source varies (biopsy, au-
topsy, or cryo-preserved), processing methods should be refined to allow for 
optimal extraction of maximum numbers of viable cells from each specimen 
type. We have developed standardized procedures for handling and processing of 
tissue samples obtained from biopsy, autopsy, or cryo-preserved specimens as 
well as necropsy tissue obtained from existing xenograft models. Two processing 
methods for generating viable cell suspensions are described. The first method, 
which uses collagenase-DNase mediated digestion of the tissue is efficient with 
bulky samples and can be used with tissues obtained at autopsy. The second 
method uses a commercially available enzymatic dissociation kit optimal for 
small volume samples such as biopsy, cryo-preserved and mouse necropsy spec-
imens. We show that obtaining viable cell suspension from precious tumor 
tissue by these methods results in successful generation of pre-clinical in vitro 
and in vivo models of DIPG, pilocytic astrocytoma and medulloblastoma that 
represent the exact genetic makeup of the original patient tumor. We further 
demonstrate intracranial injections of these cells into P2 mice for generating 
orthotopic xenograft models of brainstem or cortical tumors. Our methods 
and results allow for rapid establishment of preclinical models using rare and valu-
able childhood brain tumor specimens. These pre-clinical models serve as valu-
able tools for understanding the molecular mechanisms of the disease, identify-
ing targetable molecules, and screening of novel therapeutics.

TUMOR BIOLOGY: Cell Culture and Animal Models of Cancer I

or pancreatico-duodenal artery. 4/4 success rate of a port implantation was 
achieved without mortality, but 1/4 animals suffered post-operative bleeding at 
the port head site, attributed to coagulopathy secondary to hepatic dysfunction. 
And, 4/4 animals were partially anorectic for up to 7 days post-surgery. De-
creased tumor volume and Ktrans (a measure of capillary permeability) were 
demonstrated 7 days after single HAI with CBL0137. Contralateral tumor 
cancer effect of CBL0137 on HCC was not clear because of a lack of the control 
group, we successfully implanted a VAP into the GDA of 4 woodchucks bearing 
unresectable HCC for HAI. The described woodchuck model may serve as a 
platform for identifying novel drugs and optimal conditions for the treatment of 
primary and metastatic liver tumors.
metastatic and tumorigenic cancer spheres in model cell lines is the most prominent effect in vitro. We provide preliminary evidence that sunitinib induced in vitro cancer spheres and the live tumor areas that survive within necrotic patches of the sunitinib-treated xenografts, are related. Finally, membranous expression of E-cadherin enhances the survival of ccRCC cell lines under sunitinib treatment.

#812 Multiplatform modeling of pancreatic cancer using patient-derived cells: A new approach for defining drug resistance mechanisms. Erika Maria Parasido,¹ Prathibhha Sripadhan,² George Avetian,¹ Richard Schlegel,² Jona-than Brody,² Jordan Winter,² Charles J. Yeo,³ Michael J. Pishvaian,¹ Erik Glag-gow,¹ Stephen Byers,² Christopher Albanese,¹ ¹Georgetown University, Washington, DC; ²Thomas Jefferson University, Philadelphia, PA.

Background: Pancreatic adenocarcinoma (PA) is the fourth leading cause of cancer-related death in the USA. Current treatments utilize Gemcitabine alone or in combination with other drugs, such as nab-paclitaxel, however, a growing number of patients have shown resistance to these regimes. In order to enhance clinical response, it is important to better understand the mechanisms of drug resistance. To date, one of the main limitations in PA research has been the reliance on a few commercially available PA cell lines or a limited number of genetically engineered or PDX mouse models for in vitro and in vivo studies. It was therefore imperative to develop more accurate cellular models that better represent a given patient’s tumor. In our current study, we present the use of patient-derived primary PA cells as a model system for basic and translational research, and for personalized medicine. Methods: Patients’ biopsies were collected after surgery, and long-term cultures of PA cells were established using the conditional reprogramming of cells (CRC) approach we developed. KRAS and p53 sequencing verified the PA origin of both the patient samples and the matched CRC lines. The ICCs for Gemcitabine and Abraxane were determined and used to establish drug resistant clones. Our two dimensional (2D) CRC cultures have shown to be adaptable to different platforms, such as 3D spheroid cultures as well as zebrafish- and mouse- PDX models. Results: We established KRAS-mutant primary cell lines derived from patients’ PA samples. From two different parent cell lines we created five nab-paclitaxel and two Gemcitabine resistant clones. The clones were 3-1000 times less sensitive to the drugs as compared with the parent lines. Drug resistance ratios between parent and resistant clones were confirmed both in 3D cultures and our zebrafish PDX platform.

The injection of both parent and resistant clones into NCI/nude mice induced tumor masses in 100% of the cases. Unlike the parent cell-derived tumors, the nab-paclitaxel clone-derived tumors exhibited a metastatic phenotype. Histological analyses of these PDX models recognized the presence of morphological features of human PA, including ductal acinar structures compared to the zebrafish models. Among the most significant differences were the expression of cell differentiation markers in UMSCC-49N1KO, consistent with an aggressive and invasive phenotype in the NOTCH1 deficient line. These phenotypes were validated with subsequently generated UMSCC-49 NOTCH1 knockout lines. Conclusions: NOTCH1 acts as a key tumor suppressor gene in HNSSC and regulates cell proliferation and invasion. We have created a useful genetic model to study effects NOTCH1 in tumorigenesis, proliferation and metastasis with a wildtype NOTCH1 parent (UMSCC-49) and mutant NOTCH1, otherwise syngeneic cell line (UM-SCC49N1KO). NOTCH1 mutant status correlates with worse outcome in HNSSC patients. Further characterization of the role of NOTCH1 in cell proliferation, invasion and metastasis may provide insight into treatment stratification paradigms, targetable options and predictive biomarkers for patients with NOTCH1 mutant HNSSCs.

#815 Using CRISPR/Cas9 to generate isogenic cell lines and reference standards for applications in cancer diagnostics. Xin Cheng, Nga Nguyen, Qi Zeng, Mi Shi, Andrew Hämmer, Huanyu Lin, Ruby Yuan Chong, Tsai; Ling-Jie Kong. Applied StemCell, Inc., Milpitas, CA.

We are entering an era of precision medicine, in which there are significant initiatives to employ companion diagnostics and molecular profiling to inform clinical decisions in therapeutic treatment. In order for these initiatives to work
as effectively as possible, there is a need for molecular reference materials that can help facilitate precision assay development. Cell-line based reference standards are ideal for such applications because they represent a reproducible and renewable source of control materials that can be employed for assay development, quality control, or lead compound discovery. With this in mind, we have used this cell-line approach to develop a cross-species genome that can help facilitate precision assay development. In vitro models of the disease is therefore warranted, not least because growing evidence indicates that it is sufficiently different from female breast cancer such that extrapolating knowledge from one to the other may be misleading. Here we report the establishment and characterisation of a male breast cancer cell line derived from a primary tumor arising in a 61-year old male patient. We used 3T3-j mouse fibroblast co-culture in the presence of a ROCK inhibitor to conditionally reprogram epithelial cells from freshly resected tumor tissue. Once established, the cell line showed a rapid proliferation rate and growth in full conditioned media without the support of mouse fibroblasts. Immunohistochemical profiling demonstrated expression of epithelium-specific antigens AE1/AE3, breast epithelial marker CK7 and confirmed estrogen receptor positivity in concordance with the primary tumor. Genome sequencing detected no pathogenic germline predisposition mutations in either BRCA1 or BRCA2. There was no evidence of somatic mutation in any of the established female breast cancer driver genes. Somatic whole genome sequencing of early and late passages indicated a paucity of structural aberration and little evidence for obvious accumulation of gross genetic alterations. In conclusion, we have established a novel in vitro model for studying male breast cancer that can help facilitate precision assay development.
and response to conventional therapies. Although dogs can be considered good models for translation of new cancer treatments, there is a lack of established preclinical in vitro models of canine cancers. Therefore, our aim here was to establish two canine mammary cancer cell lines and characterize their transcriptional profile to speed the translation of new cancer treatments from dogs to humans. Mammary cancer samples were obtained from female dogs that underwent surgery. Pieces of the tumors were FFPE for histopathology and the other fragments were washed with PBS, minced and dissociated with hyaluronidase and collagenase. Cells were filtered and put in culture with DMEM-F12, 5%FBS and 1% pen-strep. After 5 passages, FBS was increased to 10%. After passage 15, cells were characterized for doubling time, karyotype, immunocytochemistry for cytokeratin, vimentin and α-smooth muscle actin, and collagenase. Cells were filtered and put in culture with DMEM-F12, 5% FBS and were positive for cytokeratin, vimentin and α-smooth actin, suggesting myoepithelial origin. The doubling time was 26.0h for M5 and 42.8h for M25 cells. Karyotyping analysis showed moderate aneuploidy for both cell cultures. M25 cell line presented significant higher invasion potential than M5 cells and formed tumors in mice but the latter didn’t. Histopathology of these tumors resembled the original tumor in the dog. An average of 16.2 million paired-end 100 bp reads were sequenced per replicate (three for each cell culture) with an average of 81.1% alignment to the reference genome. A total of 11,260 and 11,476 genes with more than 1 count per million were detected in M5 and M25 cell lines. M5 cell line have 404 and 394 up- and downregulated genes in comparison to M25 cell line, respectively. Gene ontology of DE genes showed the more malignant cell (M25) significantly enriched for pathways related to drug metabolism, ECM-receptor interaction, cell cycle, DNA replication, focal adhesion and cell differentiation. In conclusion, M25 cell line is more malignant than M5 and originally from a comedocarcinoma and a mixed carcinoma, respectively. Both cell cultures showed spindle-shape cells and were positive for cytokeratin, vimentin and α-smooth actin, suggesting myoepithelial origin. The doubling time was 26.0h for M5 and 42.8h for M25 cells. Karyotyping analysis showed moderate aneuploidy for both cell cultures. M25 cell line presented significant higher invasion potential than M5 cells and formed tumors in mice but the latter didn’t. Histopathology of these tumors resembled the original tumor in the dog. An average of 16.2 million paired-end 100 bp reads were sequenced per replicate (three for each cell culture) with an average of 81.1% alignment to the reference genome. A total of 11,260 and 11,476 genes with more than 1 count per million were detected in M5 and M25 cell lines. M5 cell line have 404 and 394 up- and downregulated genes in comparison to M25 cell line, respectively. Gene ontology of DE genes showed the more malignant cell (M25) significantly enriched for pathways related to drug metabolism, ECM-receptor interaction, cell cycle, DNA replication, focal adhesion and cell differentiation. In conclusion, M25 cell line is more malignant than the M5 which is supported by their transcriptome. Thus, it is possible to use these cells as a comparative translational model for drug development especially if one wishes to work with molecular targets as described above.

**#820 Potential pro-proliferative role of SIRT6 in melanoma.** Liz Garcia-Peterson, Mary A. Ndiaye, Chandra K. Singh, Wei Huang, Nihal Ahmad. University of Madison Wisconsin, Madison, WI.

Melanoma is one of the deadliest form of skin cancer that can rapidly metastasize to become lethal, if not diagnosed early or left untreated. In 2016, approximately 76,380 new melanoma cases and 10,130 melanoma-related deaths are predicted in the United States. Current preventive and therapeutic strategies have not been sufficiently effective in the management of melanoma. Therefore, novel molecular targets and treatments are required for an effective management of this neoplasm. In our laboratory, we are assessing the role and functional and therapeutic significance of sirtuin family of proteins in melanoma. Sirtuins (SIRTs) have been conserved through evolution from prokaryotic to eukaryotic cells. SIRTs are nicotinamide adenine dinucleotide (NAD+) dependent protein deacetylases and belong to class III of the histone deacetylase (HDAC) family. Seven members of the mammalian SIRT family are known to date, and despite structural similarities, each SIRT has their own biological niche, performing unique functions via regulating critical mechanisms in the cell. The role of SIRTs in cancer is somewhat controversial, as they have exhibited conflicting functions (tumor promoter vs. tumor suppressor) depending on cell and tissue contexts. The sirtuin SIRT6, a predominantly nuclear protein, has been shown to conduct ADP-ribosyl transferase and histone deacetylase activities. SIRT6 plays key roles in DNA repair, inflammation and metabolic diseases such as cancer. Currently, the role of SIRT6 in melanoma is not known. The objective of this study was to determine the role of SIRT6 in melanoma. Using a panel of human melanoma cell lines (A375, HS 294T, G361, SK-MEL-2, SK-MEL-28, SK-MEL-31, WM115 and WM35) differing in genetic complexity and disease progression stage, and normal human epithelial melanocytes (NHEMs), we determined the endogenous expression levels of SIRT6. We found that compared to NHEMs, SIRT6 is significantly upregulated in melanoma cell lines, at mRNA as well as protein levels, as shown by quantitative Real-Time PCR and western blot analyses. Further, employing a human tissue microarray (TMA) coupled with quantitative Vectra® analysis, we determined the expression profile of SIRT6 protein in human melanoma and melanocytic nevus tissues. Our data showed that SIRT6 is significantly overexpressed in human melanoma tissues when compared to nevi. Furthermore, lentiviral short hairpin RNA-mediated knockdown of SIRT6 in human melanoma cells was found to result in a marked anti-proliferative response in melanoma cells. Taken together, our data suggest that SIRT6 overexpression could potentially be a contributing factor in melanoma progression. Further detailed studies are under way to understand the functional significance of SIRT6 in melanoma development and progression.

**#821 Loss and retention of mutations in cell culture model systems.** Pamela S. Shaw,1 Rick Nicoletti,2 Nagmahem Salimi,1 Helen L. Yang,1 Abigail E. Witt,1 Agoston T. Agoston,1 Elin S. Agoston,1 Cellaria Biosciences, Cambridge, MA; Brigham and Women’s Hospital, Boston, MA.

Although relevant, the genomic and proteomic alterations between original tumor tissue and newly established cell lines are frequently uncharacterized. In developing a novel cell model (Powder) of high grade serous carcinoma (HGSC), we followed the status of clinically relevant biomarkers to identify changes taking place between the tissue and cultured cell population. Understanding these changes enables proper utilization of new models and continued model optimization. Using a validated panel, we analyzed 592 genes in the Powder cell model and tumor from which it was derived, detecting several classes of genomic alterations. This included point mutations, indels, copy number variations, fusions, and variant transscripts. p53 mutation status of Powder was matched to a panel of previously reported HGSC cell lines. We further subvided the panel by p53 protein expression level by reanalyzing previously published reverse phase protein array (RPPA) data. Significance of differential expression between groups was determined with a Welch's t-test. A majority (14/21 or 66%) of the mutations detected in the HGSC tumor were conserved in the Powder cell model. Two additional mutations, undetected in the tumor, were found in the cell model. The tumor exhibited moderate to strong diffuse p53 staining, p53-unstainable intense staining in 79% of alleles. While the p53 mutation did not carry to the cell model, the cells were positive for CK7 and PAX8, consistent with ovarian cancer cells. In the panel of HGSC cell lines, we examined p53 mutation status and protein level, identifying 3 groups: (1) p53-mut/high protein, (2) p53-mut/low protein and (3) p53 WT/low protein. Powder identified with the p53-WT/low protein group. Previous work identifies the p53-WT/low protein group of cell models as part of a larger set of novel ovarian cell models with a stem-like molecular profile and higher drug resistance. Analysis of differential protein expression revealed upregulation in the p53 low protein group of both normal cell cycle genes and of notable cancer-related proteins. This included p21 and BAD, which are upregulated in cells with functional p53, eIF4E, a candidate cancer therapeutic target, and BOP1, which is deregulated in multiple solid cancers, including ovarian. These results underscore the need for further characterization of the Powder cell model to clarify its applications to specific research questions. Continued examination of Powder and other p53-WT cell models derived from HGSC tumors may uncover independent pathways driving disease. Additionally, understanding which mutations are underrepresented by cell models is necessary for ongoing methods development.

**#822 Simple and easy monitoring of tube formation and migration assays with the CytoSMARTTM Live Cell Imaging System.** Stefanie Buesch,1 Sabine Schaepmeermeier,1 Theresa D’Souza,2 Bodo Ortmann,1 Claudia Schwartz,1 Jenny Schroeder,1 Lonza Cologne GmbH, Cologne, Germany; 2Lonza Walkersville Inc., Walkersville, MD.

Currently, the role of SIRT6 in melanoma is not known. The objective of this study was to determine the role of SIRT6 in melanoma. Using a panel of human melanoma cell lines (A375, HS 294T, G361, SK-MEL-2, SK-MEL-28, SK-MEL-31, WM115 and WM35) differing in genetic complexity and disease progression stage, and normal human epithelial melanocytes (NHEMs), we determined the endogenous expression levels of SIRT6. We found that compared to NHEMs, SIRT6 is significantly upregulated in melanoma cell lines, at mRNA as well as protein levels, as shown by quantitative Real-Time PCR and western blot analyses. Further, employing a human tissue microarray (TMA) coupled with quantitative Vectra® analysis, we determined the expression profile of SIRT6 protein in human melanoma and melanocytic nevus tissues. Our data showed that SIRT6 is significantly overexpressed in human melanoma tissues when compared to nevi. Furthermore, lentiviral short hairpin RNA-mediated knockdown of SIRT6 in human melanoma cells was found to result in a marked anti-proliferative response in melanoma cells. Taken together, our data suggest that SIRT6 overexpression could potentially be a contribution factor in melanoma progression. Further detailed studies are under way to understand the functional significance of SIRT6 in melanoma development and progression.

The formation of new blood vessels is required to ensure sufficient nutrient and oxygen supply and to allow solid tumors to grow beyond a certain size. This process can be mimicked in cell culture models in so-called tube formation assays. In this study, Human Umbilical Vein Endothelial Cells (HUVEC) were seeded on Engelbreth-Holm Swarm Sarcoma derived Basement Membrane Extract (BME). The resulting formation of endothelial tubes was monitored with the CytoSMART™ Device. Subsequently, the average length of the formed tubes as well as the number of closed tube circles was quantitatively determined. In addition, the impact of Suramin on tube formation was evaluated as that Suramin is a tubule formation inhibiting compound. The migration of cancer cells is also required for the growth and in particular the metastasis of tumors. In a first example, the closure of a so-called wound or scratch in a confluent monolayer of cancer cells was monitored with the CytoSMART™ Device. Determining the migration speed of the cells by measuring the speed of

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wound closure is a simple assay to determine the migration potential of cancer cells. Modifying the cancer cells, e.g. by knocking down specific genes with siRNA, can help to identify genes that play a role in cell migration. Compounds that are expected to reduce cell motility and therefore reduce the metastasis potential of cancer cells can be easily tested. In a second example, the invasion of cancer cells into a three-dimensional (3D) matrix was analyzed. Tumor cells were embedded in a cancer-relevant matrix and their invasion into the 3D matrix was documented with the CytoSMART™ Device. While this type of model is slightly more difficult in set-up and analysis compared to simple scratch assays, it may reflect better the in vivo situation where solid tumors develop within three-dimensional tissues. The images captured with the CytoSMART™ System were quantitatively assessed using appropriate software. Overall, the CytoSMART™ System is an easy-to-use, small and affordable live cell imaging system suitable for the label-free analysis of different cancer-relevant assays.

TUMOR BIOLOGY: Cell Culture and Animal Models of Cancer I

Inhibition of focal adhesion kinase (Fak) leads to sensitization of p53 mutant head and neck cancer to genotoxic stress. Manish Kumar, Liang Yang, David Molkentine, Jeffrey Myers, Raymond Meyn, Curtis Pickering, John Heymach, Heath Skinner. UT MD Anderson Cancer Center, Houston, TX.

Head and neck squamous cell carcinoma (HNSCC) is most commonly treated with DNA-damaging therapies such as cisplatin and/or radiation. However, a significant proportion of HNSCCs are resistant to this therapy. Therefore, we have identified focal adhesion kinase (Fak) as a targetable biomarker of therapeutic resistance in this disease. Additionally, utilizing in vivo shRNA screening of druggable targets in a panel of HNSCC cell lines, Fak was one of the most significant genes related to radiosensitization. Additional investigations utilizing a combination of in vivo and in vitro assays have shown that inhibition of Fak either chemically or via shRNA leads to dramatically increased sensitivity to DNA damage. This effect is primarily found in p53-mutant tumors and cell lines, which are known to be resistant to DNA-damaging therapies. DNA damage due to radiation appears to be potentiated in p53 mutant HNSCC cell lines following Fak inhibition, but less so in matched p53 wild type HNSCC. Moreover, basal Fak activity is higher in HNSCC cell lines engineered to express missense p53 mutations compared to either wild type or p53 null cells. We also found that several forms of mutant p53 bind to Fak and may alter its activity directly. Taken together, our data suggest a targetable nexus of mutant p53 and Fak that can be directly modulated to improve the efficacy of DNA-damaging therapy.

Reduction in prostate tumor growth and induction of anti-tumor immunity after combined treatment with oncolytic VSV and radiation. Thirupandiyur S. Udayakumar, Dillon Betancourt, Glen Barber, Anis Ahmad, Brian Marples, Alan Pollack. University of Miami, Miami, FL.

Androgen deprivation therapy (ADT) is a mainstay of treatment for high-risk non metastatic and metastatic prostate cancer. Patients initially respond to ADT, but eventually develop resistance to treatment. To overcome these limitations, we have used oncolytic virotherapy which has the potential to influence both local and systemic disease through direct cytotoxic and immune mechanisms. Here, we investigated the effectiveness of radiation therapy (RT) combined with Vesicular Stomatitis Virus (VSV) vector expressing interferon-β (IFNβ) in VSV resistant prostate xenografts. In initial cell culture experiments, microarray analysis indicated that PC3 cells treated with VSV-hIFNβ and RT displayed several genes known to directly inhibit VSV replication (e.g., MX2, OA52, TRIM25, IFITM1) were downregulated compared with VSV-hIFNβ therapy alone. Additionally, many apoptotic pathway-associated genes (e.g. IL24, GADD45B, KLF4, TNF, EGFR) were upregulated several fold during combination therapy. Gene expression changes were confirmed by RT-PCR. Further, immunohistology analysis demonstrated expression levels of IFNAR, BCL-XL, phosphorylated Akt, IAK2 and STAT3 were also markedly decreased when VSV-hIFNβ was combined with RT. However, RIG-I was not significantly altered after VSV-hIFNβ+RT treatment. A marked decrease in MAVS, NFκB (p50), IRF7 and antiviral protein PKR was seen after combined treatment. The role of IFN pathway in viral response was investigated using an interferon-inducible promoter ISRE-luciferase (ISRE-Luc) reporter construct. A significant difference (>600 fold) in the ISRE-Luc activation was seen for PC3 compared to LNCAp cells, indicating that the IFN pathway is highly active in PC3 cells after VSV infection alone. The activation of ISRE-mediated transcription of luciferase was significantly attenuated (p<0.05) in both the cell lines after combined treatment with VSV-hIFNβ and RT. In subsequent in vivo studies, we determined that radiation treatment combined with intratumorally-delivered VSV-mIFNβ function synergistically to induce oncolysis in both PC3 and LnCAp xenografts and a syngeneic RM9 murine model. In the syngeneic model, combination therapy resulted in a robust anti-tumor immune response with a significantly increased CD8 T-cell expression compared to either therapy alone. These studies demonstrate that two mechanisms are involved in combined RT and VSV-mediated oncolysis. These are, (1) the attenuation of the antiviral response which increases the direct oncolytic effect of VSV on the tumor cells and (2) improved generation of an adaptive immune response earmarked by CD8+ lymphocyte expansion and anti-tumor activity. On our studies demonstrates that the combined strategy of RT plus VSV-hIFNβ affects tumor cell death that are resistant to VSV through direct and systemic mechanisms that includes a pronounced enhancement of anti-tumor immunity.

Cetuximab enhanced apoptosis effects of radiation on human prostate cancer cell line PC-3. Chia-lun Chang, Szu-Yuan Wu. Wan Fang Hospital, Taipei, Taiwan.

Prostate cancer is a leading cause of cancer death in men in developed countries. Radiotherapy is the main treatment to prostate cancer. Dose escalation of irradiation with image guide radiotherapy and intensity modulated radiotherapy were used with more and more evidences in prostate cancer patients and bring in longer PSA-free survival time. Although the evolution of radiation techniques bring better outcomes, but modern modalities were not available in non-western world. Radiation enhancement is still an imortant issue in prostate cancer in non-western world, especially in androgen independent prostate cancer. The epidermal growth factor receptor (EGFR) network has rich targets for prostate cancer killing. The chimeric monoclonal antibody Cetuximab (IMC-A12, Erbitux®) binds to EGFR and prevents its intracellular signaling. Currently, it is approved for treatment of wild-type KRAS colon and head and neck cancer. The effect and mechanisms of Erbitux and radiotherapy are still not clear. In this study, we evaluated the effects of combining the EGFR inhibition and radiation on PC-3 prostate cancer cells. PC-3 cells were treated with various doses(1,2.5,5 and 10 ug/ml) of anti-EGFR antibody (CETUXIMAB) and irradiation(5, 7, 5 Gy). The results revealed that 5 and 10ug/ml of CETUXIMAB significantly reduced the colony formation. The cell cycle analysis revealed that CETUXIMAB significantly enhanced the radiation induced G2/M phase arrest and subG1 peak increasing. The Hoechst staining and DNA ladder analysis revealed radiation and CETUXIMAB combined use will induce more apoptotic bodies or DNA fragmentation than radiation alone use. By using a apoptosis protein array, we found that several apoptosis related protein (Catalase, clAP-1, Clusterin, Fas, HIF-1α, HSP27, HSP60, HSP70, SMAC and Survivin) were significantly downregulated in radiation and CETUXIMAB combined group. Taken together, our results demonstrated that Erbitux enhanced apoptosis effects of radiation on PC-3.

Nrf2 confers radioresistance by regulating cancer stem trait in lung cancer cells. Xiaoqiao He,1 Alex Chi,2 Liying Wang,2 Yun Rianasakan,1 West Virginia Univ., Morgantown, WV; 2National Institute for Occupational Safety and Health, Morgantown, WV.

Lung cancer is the leading cause of cancer-related death accounting for more than 1.3 million deaths worldwide annually. Despite advances in treatment, the five-year survival rate is 2% if the cancer has metastasized to other organs. The major cause of poor prognosis of advanced lung cancer is multi-resistance to chemo/radio therapy. Therefore, it is crucial to understand the underlying mechanisms of chemo/radio resistance and develop therapeutic strategies to overcome the resistance. Transcription factor Nrf2 is a key regulator of cytoprotective genes which facilitate cellular defense against harmful insults. We found that Nrf2 and its downstream target NQO1 are highly upregulated in lung cancer patient tumor tissues as well as in lung cancer cell lines compared to adjacent normal tissues or normal cell lines. We hypothesized that the upregulated Nrf2 may be responsible for radio/chemo resistance and malignant in lung cancer. RNA interference studies showed that knockdown of Nrf2 substantially increased the sensitivity of human lung cancer H460 and A549 cells to radiation-induced cell death. Knockdown of Nrf2 also decreased tumor sphere formation, suggesting its role in cancer stem cell (CSC) regulation which may be responsible for the radioresistance. Analyses of CSC markers including ALDH and Oct4 in control and Nrf2-knockdown H460 and A549 cells support this finding. Together, our results indicate an essential role of Nrf2 in the control and regulation of CSCs and radioresistance. Since CSCs are widely believed to be responsible for cancer chemoresistance and relapse, our novel finding on the role of Nrf2 in radiation resistance may aid in the design of novel therapeutic strategies for resistant cancers.

#823 Inhibition of focal adhesion kinase (Fak) leads to sensitization of p53 mutant head and neck cancer to genotoxic stress.

#826 Nrf2 confers radioresistance by regulating cancer stem trait in lung cancer cells.

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Ionizing radiation (IR) can induce DNA damage in human cells and result in changes in gene expression. The changed cell behavior can be monitored by using microarray analysis. We performed in vitro gain and loss of studies with candidate miRNA transfections in human CRC cell lines and used a panel of CRC cell lines to determine the effects of miRNA expression on cellular responses to IR. We identified 17 miRs that were differentially expressed, and miR-451a, among the most upregulated miRs, inhibited proliferation and colony formation in 2D and 3D assays in the presence of radiation. Target prediction algorithms highlighted CAB39, EMSY, EREG, and MEX3C as prominent miR-451a targets in colorectal cancer and/or radiation. MiRNA subset analysis found CAB39 and EMSY protein levels were found to be upregulated in 14% and 6% of cases, respectively, and upregulated co-expression of these genes significantly reduced 3-year overall survival (69% vs 78%, p < 0.05). miR-target interaction was confirmed via the RISC-Trap assay with miR-451a mimic transfection resulting in robust 2.4-, 1.2-, and 2.8-fold enrichment of EREG, CAB39, and EMSY, respectively. In our partial responders patient cohort possessing upregulated miR-451a, the breast cancer determinant downstream of miR-451a and miR-106a was compared to non-responders. Conclusions: miRs after cell survival networks affecting radiation sensitivity and serve to identify pathways amenable to alternative therapeutic strategies.

MirRNA-106a and LITAF are novel modulators of prostate cancer radioresistance. Christianne Hoey,1 Jessica Ray,2 Paul Boutros,3 Stanley K. Liu.1 1University of Toronto, Toronto, Ontario, Canada; 2Ontario Institute for Cancer Research, University Health Network, Toronto, Ontario, Canada; 3Sunnybrook Research Institute, Toronto, Ontario, Canada. Prostate cancer (PCa) is the second most prevalent cancer affecting men worldwide, with radiotherapy being a primary treatment modality. PCa recurrence is a major clinical problem with up to a 40% biochemical recurrence rate at five years after external beam radiotherapy (ionizing radiation, IR). Therefore, there is an unmet need to characterize radioresistance in order to improve therapeutic agents and patient outcomes. MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression. MiRNAs are aberrantly expressed in cancer, specifically, miR-106a has been found to be overexpressed in various cancer types. The purpose of this study is to determine whether miR-106a confers IR resistance and tumor aggressiveness in PCa. Utilizing The Cancer Genome Atlas (TCA) and Cancer Genome Atlas Network (TCGA) data sets, we investigated the effect of XPO1 inhibition by Selinexor in combination with radiation on GSCs. To determine whether XPO1 could serve as a target for GSC radiosensitization, we focused on the GSC cell line NSC11. Based on clonogenic survival assays, addition of Selinexor 1 h before irradiation significantly enhanced the radiosensitivity of NSC11 cells as compared to controls. To begin to investigate the mechanism behind Selinexor-mediated radiosensitivity, YH2AX foci dispersal, a surrogate marker for DNA double strand breaks (DSB) was evaluated. NSC11 cells treated with Selinexor 1 h before irradiation (2 Gy) and collected 1-24 h later for analysis of YH2AX demonstrated a significant delay in DNA DSB repair 24 h after irradiation. Because XPO1 is responsible for export of various cargo proteins out of the nucleus, including several ribosomal subunits, it is predicted to play a role in global translation. To evaluate the effects of XPO1 inhibition on GSCs, the polysome profiles of NSC11 cells treated with Selinexor were evaluated. The polysome profiles of NSC11 cells demonstrated significant attenuation of the polysome portion of the profile as early as 1 h after treatment with Selinexor. Inhibition of Selinexor treatment results in polyribosome breakdown, leading to a decrease in translational efficiency (TE) by more than half. This attenuation is not accompanied by a change in the monosomal peaks, suggesting that polyribosome breakdown to mRNA is specifically affected by radiation. Taken together, these data suggest that XPO1 plays a role in translation as well as in cellular radiosensitivity, and inhibition of XPO1 by Selinexor enhances GSC radioresistance, possibly by inhibiting global translation.
TUMOR BIOLOGY: Cellular Responses to Ionizing Radiation

Transforming growth factor β (TGFβ) is a well-documented tumor suppressor; a poorly studied aspect of TGFβ biology is its control of genomic stability. Our prior work showed that TGFβ compromises ATM (ataxia telangiectasia mutated) kinase activity, which mediates recognition and repair of DNA damage. Consequently, inhibiting TGFβ following ionizing radiation increases clonogenic survival in breast, brain, and lung cancer preclinical models. As yet unknown is how TGFβ controls ATM kinase. We recently determined that TGFβ regulates mammalian longevity commitment by post-transcriptional control of BRCA1 mRNA and protein by its suppression of miR-182 (Martinez-Ruiz et al., Science Signaling, in press). miR-182-mediated downregulation of BRCA1 impacts DNA repair sensitivity to PARP inhibitors in cancer cell lines (10.1016/j.molcel.2010.12.005) Here we used two cell culture models to investigate the effect of TGFβ regulation of BRCA1 and miR-182 on DNA damage response. Spontaneously immortalized Tgfβ1 heterozygote and wild type fibroblasts from primary mammary epithelial preparations were synchronized in S-phase and exposed to UV radiation before fixation to detect unrepaired DNA damage by quantitative immunofluorescence analysis of 53BP1 foci. Consistent with compromised BRCA1 function, more than twice as many cells with 53BP1 foci were evident in Tgfβ1 heterozygote fibroblasts compared to wild type cells (p<0.01). The second model consisted of non-malignant human breast epithelial cell line, MCF10A, stably transduced with a scrambled or miR-182 antagonist, grown under serum free conditions and treated with a small molecule inhibitor of TGFβ type I receptor and therefore, HRD. Four cell lines is target of miR-182 and thus also regulated by TGFβ and FOXD3 association with ATM is required for its kinase activity (10.1038/ncl1709). As reported, FOXD3 and ATM co-immunoprecipitated, but was eliminated by TGFβ inhibition and this effect was lost in MCF10A cells expressing a miR-182 antagonist. The impaired DNA repair phenotype induced by TGFβ inhibition can be fully rescued by antagonizing miR-182. These studies support TGFβ as stringent regulator of the DNA damage response via suppression miR-182, which directly targets BRCA1 and indirectly affects ATM activity via FOXD3. We predict that cancers that have lost TGFβ signaling capacity will be genomically unstable due to defective DNA damage control, which is consistent with our studies showing that TGFβ inhibition sensitizes cancers to radiation therapy.

#832 In vitro characterization and clinical correlation of BRCAAness as a personalized biomarker for radiosensitization with homologous recombination-directed therapies. Jennifer Ma,1 Andrew C. Bell,1 Jeremy Setton,1 Justin Baseline,1 Marcher Thompson,1 Rachna Shah,1 Benjamin H. Lok,1 Robert Deliste,1 Rebecca A,2 Nadeem Riaz,1 Simon Powell,1 Memorial Sloan Kettering Cancer Center, New York, NY; 2Washington University, School of Medicine, St. Louis, MO.

Introduction: BRCA1 and BRCA2 are involved in double-strand break (DSBs) repair via homologous recombination (HR). Clinically, BRCA1/2 mutant tumors show sensitivity to cisplatin, a cross-linking agent, but only mild sensitivity to irradiation (IR). The addition of cisplatin to IR has been one of the largest advances in improving patient outcomes with radiotherapy in the past 50 years. Recently, BRCAAness has been described wherein sporadic deficits in HR are observed in the absence of germline BRCA1/2 mutations. We hypothesize that BRCAAness tumors also exhibit sensitivity to the combination of cisplatin + IR, and are a significant driver of the observed clinical benefit of combination therapy. Methods: Sensitivity to cisplatin + IR was examined in vitro and correlated with clinical data. Functional HR deficiency (HRD) was assessed in isogenic BRCA1 (H1299; lung) and BRCA2 (DLD1; colon) knockout pairs using 3 assays: the Rad51 foci assay, a flow-based DGFP assay (DSB reporter assay) and clonogenic survival assays (CSAs) with cisplatin + IR. Cancer Cell Line Encyclopedia (CCLE) data was used to identify breast cancer cell lines with sporadic, non-BRCA1/2 mutations. Functional HRD was assessed in 4 breast cancer cell lines was assessed with the same 3 assays used for the BRCA1/2 isogenic pairs. We also examined 14 triple-negative breast cancer (TNBC) patients treated with neoadjuvant cisplatin + IR. Nine of the 14 had available tissue, which was stained for Rad51 foci. Results: Functional HRD is observed in the BRCA1/2 isogenic pairs, as demonstrated by the Rad51 foci and DGFP assay results. Increased sensitivity to cisplatin + IR is observed in the BRCA1/2 knockout lines in CSA results compared to isogenic controls. A genomic scar score, LST (large scale transition), was generated for each line based on CCLE data, with higher LST scores suggesting increased genomic instability and untreated tumors. Already 10 min p.i., the number of DNA DSB increased slightly in peripheral tumor tissue. The number peaked 8 h p.i., when the number of DNA DSB had increased 50 times in the tumor periphery and 24 times in the tumor center. The number of DNA DSB then declined, but the difference between center and periphery remained, as expected


The purpose of this study was to characterize a three-dimensional calcium alginate culture system for real-time monitoring of cancer cell metabolism and radiation response. HCT116 colorectal cancer cells were encapsulated in 2.5% calcium alginate using a custom electrostatic rig adapted from a previously published system. Bead diameter was a function of voltage and alginate concentration: 4 x 10⁶ cells/mL encapsulated at 4 and 8 kV resulted in mean diameters of 880 and 584 μm, respectively. The distribution of bead radii for all voltages showed positive excess kurtosis (4 kV = 1.6, 8 kV = 3.0) and 72.9% of 4 kV and 77.2% of 8 kV bead diameters fell within one standard deviation of the mean. Encapsulated cell viability was assessed by staining with Trypan Blue and clonogenic survival assays (CSAs). The baseline viability of cells immediately after encapsulation was 93.2%, which decreased 2 days after a single 10 Gy dose (90.7%, p<0.05). After two weeks of continuous culturing, 84.1% of non-irradiated and 73.8% of irradiated encapsulated cells remained viable (p<0.003). Pimonidazole staining demonstrated the presence of hypoxic cores proportional to bead size. CSAs of 8 kV beads showed a cell modifying factor (DMF) of 1.06 relative to cells grown in a monolayer, while larger 4 kV beads showed a DMF of 1.58. Mean oxygen consumption rate rose with increasing number of 8 kV beads/well: 1 bead, 35.5; 2 beads, 97.4; 3 beads, 175.5; 4 beads, 209.9; 2.0 x 10⁴ monolayer cells, 92.6 (all pmol/min, ~8.2 x 10⁻⁹ cells/bead, p<0.005). Hyperpolarized [1-13C]-NMR spectroscopy of 400 ul of 8 Gy beads containing 1.2 x 10⁸ cells/mL showed a detectable conversion of [1-13C]-pyruvate to [1-13C]-lactate: 1 hour after a single 10 Gy dose, the lactate-pyruvate ratio decreased by 25%. Unpaired 2-tailed Student’s t-tests were used to determine significance between and one-way ANOVA was used to determine significance among groups with α set at p<0.05. These data demonstrate the versatility of alginate hydrogels for real-time metabolic and radiation response studies which are non-invasive, higher throughput, and lower cost compared to in vivo systems. Future directions include additional metabolic flux analysis and hyperpolarized [1-13C]-NMR spectroscopy to further investigate the cancer cell metabolic response to drug and/or radiation therapy.

#834 Formation of DNA double-strand breaks in colon tumors after targeted alpha therapy with ²¹¹At-mAb. Sophie E. Eriksson,1 Erik Elgström,1 Sture Lindegren,2 Tom Bäck2.1 Lund University, Lund, Sweden; 2University of Gothenburg, Gothenburg, Sweden.

Introduction: Targeted alpha therapy has shown promising results in preclinical and clinical studies. Alpha particle irradiation gives a high fraction of DNA double-strand breaks (DSB), as shown in vitro, resulting in a high probability of cell death. We have previously examined the therapeutic effects of ²¹¹At on solid colon carcinoma tumors (diameter approximately 1 cm), with tolerable activities (5 MBq/animals) resulting in non-palpable tumors within one week p.i. The aim of the present study was to investigate the formation of DNA DSB during tumor regression after radioimmunotherapy with ²¹¹At-mAb in a syngeneic rat colon carcinoma model. Methods: 18 rats bearing solid colon tumors (1 cm in diameter) between peritoneum and the abdominal muscle were injected intravenously with 5 MBq/animal ²¹¹At-BR96. Tumors were excised and paraffin-embedded after 10 min, 2 h, 8 h, 18, 24 h, and 48 h p.i. (3 tumors per time point). 3SBP1 was stained by immunohistochemistry and used as a marker for DNA DSB. Untreated tumors were used as controls (n=9). DNA DSB were counted in central and peripheral tumor areas selected at random. Results: A few DNA DSB were detected in untreated tumors. Already 10 min p.i., the number of DNA DSB had increased slightly in peripheral tumor tissue. The number peaked 8 h p.i., when the number of DNA DSB had increased 50 times in the tumor periphery and 24 times in the tumor center. The number of DNA DSB then declined, but the difference between center and periphery remained, as expected

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considering the intratumoral distribution of radioimmunoconjugate. This correlates with the 211At half-life of 7.2 h. Conclusion: DNA DSB are formed early after injection of 211At-mAb and follows the intratumoral distribution of mAbs.

#835 Sensitivity of PTEN deficient non-small cell lung cancer to ionising radiation through inhibition of ataxia telangiectasia related 3 kinase (ATR). Victoria Louise Dunne, Kelly Redmond, Caroline Coffey, Karl Butterworth, Kevin Prise, Gerard Hanna. Centre for Cancer Research and Cell Biology, Queen's University, Belfast, United Kingdom.

This study sought to determine the therapeutic efficacy of AZD6738, a small molecule inhibitor of ataxia telangiectasia related 3 kinase (ATR), in combination with ionising radiation (IR) for the treatment of PTEN deficient non-small cell lung cancer (NSCLC). An isogenic PTEN deficient cell model were generated in H460 and A549 cell lines by HuSH PTEN shRNA constructs in pGP-V-RS vectors from Origene. Radiosensitivity was determined at various concentrations of AZD6738 by clonogenic assay following IR with 225 kV X-rays. Target validation and mechanistic evaluation of the ATR radiation treatments was performed by western blotting. Cell cycle distribution was obtained using propidium iodine flow cytometry analysis which was performed 48 hours following treatment with AZD6738 alone or in combination with IR. In vivo efficacy was determined using cell line derived xenografts in female SCID mice treated with AZD6738 at a concentration of 25 mg/kg per day by oral gavage for 28 days. Animals were exposed to size fraction and hypofractionated radiation dose under CBCT image guidance using the small animal radiotherapy research platform (SARRP). Radiation induced toxicity in normal lung tissue effects was investigated in a model of radiation induced lung fibrosis in C57Bl/6 mice. A 50% or 80% i.v. doped combined treatment with AZD6738 and IR selectively targets PTEN-deficient tumour cells causing a significant reduction in clonogenic survival for both H460- and A549-PTEN KO cell models (p < 0.05 at 8 Gy). Following 2 Gy IR exposure there were significantly higher mean forci per cell in both H460-KO and A549-KO cells in comparison to WT cells (p < 0.05 at 2, 4 and 8 hours following treatment). Both PTEN-KO cell lines exhibited increased sub G0/G1 and G2/M populations in comparison to PTEN-WT cell lines when treated with AZD6738 and IR. Minimal toxicity was observed in both normal HBE and BEAS-2B cell lines. Currently in vivo tumour response experiments are underway to investigate IR and ATR kinase inhibitor AZD6738 using H460-WT and H460-PTEN KO cell models as well as determining the effects of AZD6738 in a radiation induced model of lung fibrosis. Approximately 10% of NSCLC patients have mutations in PTEN. This research therefore elucidates PTEN loss as a therapeutic target for combined IR with pharmacological inhibition of ATR in NSCLC. Targeting ATR inhibition in PTEN-deficient NSCLC may serve to increase radiation sensitivity in an in vivo model thus highlighting its clinical relevance as a potential personalised medicine approach for patients with PTEN deficient NSCLC.

#836 ATR inhibitor BAY 1895344 shows potent anti-tumor efficacy in monotherapy and strong combination potential with the targeted alpha ther-apy Radium-223 dichloride in preclinical tumor models. Antje Margret Wenger, Gerhard Siemeister, Ulrich Luecking, Julien Lefranc, Philip Lienau, Gesa Deeg, Eleni Lagkadinou, Li Liu, Sven Golifier, Christoph Schatz, Arne Scholz, Priscy, Nusbaum, Michael Brands, Dominik Mumburg, Karl Ziegeler, Bayer Pharma AG, Berlin, Germany.

The integrity of the genome of eukaryotic cells is secured by complex signal- ing pathways, known as DNA damage response (DDR). Recognition of DNA damage activates DDR pathways resulting in cell cycle arrest, induction of DNA repair, or cell death. Proteins that directly recognize aberrant DNA structures recruit and activate kinases of the DDR pathway, such as ATR (ataxia telangiectasia and Rad3-related). ATR responds to a broad spectrum of DNA damage, including double-strand breaks (DSB) and lesions derived from interference with DNA replication as well as increased replication stress. Therefore, inhibition of ATR kinase activity could be the basis for a novel anti-cancer therapy in tumors with increased DNA damage, deficiency in DNA damage repair or replication stress in a model of radiation induced fibrosis in C57Bl/6 mice. An in-vivo combined treatment with AZD6738 and IR selectively targets PTEN-deficient tumour cells causing a significant reduction in clonogenic survival for both H460- and A549-PTEN KO cell models (p < 0.05 at 8 Gy). Following 2 Gy IR exposure there were significantly higher mean forci per cell in both H460-KO and A549-KO cells in comparison to WT cells (p < 0.05 at 2, 4 and 8 hours following treatment). Both PTEN-KO cell lines exhibited increased sub G0/G1 and G2/M populations in comparison to PTEN-WT cell lines when treated with AZD6738 and IR. Minimal toxicity was observed in both normal HBE and BEAS-2B cell lines. Currently in vivo tumour response experiments are underway to investigate IR and ATR kinase inhibitor AZD6738 using H460-WT and H460-PTEN KO cell models as well as determining the effects of AZD6738 in a radiation induced model of lung fibrosis. Approximately 10% of NSCLC patients have mutations in PTEN. This research therefore elucidates PTEN loss as a therapeutic target for combined IR with pharmacological inhibition of ATR in NSCLC. Targeting ATR inhibition in PTEN-deficient NSCLC may serve to increase radiation sensitivity in an in vivo model thus highlighting its clinical relevance as a potential personalised medicine approach for patients with PTEN deficient NSCLC.

#837 PSMB8 as a candidate marker of responsiveness to preoperative radio-dia-thetapy in rectal cancer patients. Yejin Ha,1 Ka hee Tak,2 Chan wook Kim,1 Seon Ae Roh,3 Eun Kyung Cho,1 Dong Hyung Cho,2 Jeong-Hwan Kim,1 Seon-Yung Kim,2 Yong Sung Kim,3 Jin-Cheon Kim.1 Asan Medical Ctr., Seoul, Republic of Korea;2 Kyung Hee University, Yongin, Republic of Korea;3 Korea Research Institute of Bioscience & Biotechnology, Daejeon, Republic of Korea.

To predict individual responsiveness is urgently needed, specifically in locally advanced rectal cancer (LARC) patients who underwent preoperative chemoradiotherapy (CRT). The present study, RNA-Seq was used to compare the basal expression profile between responders and non-responders to preoperative CRT, in correlation with the tumor regression grade (TRG) among 22 LARC patients. Eight differentially expressed genes (B3GALT4, HSPA1B, KRBX1, PPBP, PIP1R18, PSMB8, SLCS9A7, and TAP2) associated with preoperative CRT responses were primarily identified among the 22 LARC patients by RNA-Seq (p < 0.0005 and >16-fold difference). Among these genes, PSMB8 and SLCS9A7 were upregulated in the responsive group in the other 40 LARC patients (p < 0.05). PSMB8 overexpression significantly reduced colony formation and increased apoptosis-inducing molecules like cleaved caspase-3 and cleaved PARP in irradiated CRC cells after 6 Gy irradiation. PSMB8 knockdown increased colony formation and decreased caspase-3 activation and cleaved PARP levels after irradiation. In mice treated with ionizing radiation, tumor growth suppression was significantly greater in HCT116/PSMB8-xenografts (81%) than in HCT116-vector-xenografts (53%) (p = 0.001). However, SLCS9A7 overexpression had no significant effect on irradiated CRC cells. These results suggest that PSMB8 appears to predict radiosensitivity in LARC patients with preoperative CRT, although further clinical validation is needed in a larger cohort.

#838 Effect of tumor treating fields on cell proliferation and synergistic antitumor efficacy in combination with irradiation. Eun Ho Kim,1 Yunhui Jo,2 Jiwon Sung,2 Hyo Sook Song,2 Myonggeun Yoon,2 Korea Institute of Radiological & Medical Sciences, Seoul, Republic of Korea;2 Korea University, Seoul, Republic of Korea.

Alternating electric fields at an intermediate frequency (100–300 kHz), called tumor treating fields (TTFs), are reported to interrupt the process of mitosis via apoptosis and to act as an inhibitor of cell proliferation. Although the presence of an antimotic effect of TTFS has been widely reported, the efficacy of TTFS is still controversial issue among medical experts. To resolve this controversial issue, the comparison study between the effects of TTFS and the effect of other conventional cancer treatments(Xevo) is the first and only attempted targeted alpha therapy so far. It is indicated for the treatment of patients with castration-resistant prostate cancer (CRPC), symptomatic bone metastases and no known visceral metastatic disease, based on improvement of overall survival. It exhibits strong cytotoxic effects on adjacent cells via the induction of DNA DSB. Here, we disclose for the first time the structure and functional characterization of the novel ATR kinase inhibitor BAY 1895344. In vitro, BAY 1895344 is a selective low-nanomolar inhibitor of ATR kinase activity, potently inhibiting proliferation of a broad spectrum of human tumor cell lines (median IC50 of 78 nM). A clear separation between highly sensitive (IC50 < 10 nM) and less sensitive cell lines was observed. The majority of the sensitive cell lines are characterized by mutations affecting the ATM (ataxia telangiectasia mutated) pathway. In cellular mechanistic assays BAY 1895344 inhibited hydroxyurea-induced H2AX phosphorylation demonstrating the anticipated mode of action. BAY 1895344 is an ATR inhibitor that exhibits strong in vivo anti-tumor efficacy in monotherapy in a variety of xenograft models of different indications that are modeled by DDR deficiencies, inducing stable disease in castration-resistant prostate cancer or even complete tumor remission in mantle cell lymphoma models. In addition, we could demonstrate that combination treatment with BAY 1895344 and Radium-223 exhibits clear synergistic anti-tumor activity in a bone metastases xenograft model of CRPC. Our findings validate the concept of synthetic lethality of genetically determined DNA repair deficiency and ATR blockade by demonstrating strong monotherapy efficacy of the highly potent ATR inhibitor BAY 1895344 in a variety of tumor indications. Furthermore, the mechanism-based combination potential of DNA damage induction by Radium-223 with BAY 1895344 creates a powerful new treatment option for CRPC patients with bone metastases. The start of clinical investigation of BAY 1895344 is planned early 2017.
#839  Network-driven analytics of published tissue-based biomarkers to predict response to neoadjuvant therapy in rectal cancer. Lian R. Poynter,1 Kirill Veselkov,1 Dieter Galea,2 James Kinross,1 Alexander Mirnezami,2 Jeremy Nicholson,2 Zoltan Takats,3 Reza Mirnezami,1 Ara Darzi1. Imperial College London, London, United Kingdom; 2University of Southampton, United Kingdom

Introduction Neoadjuvant chemoradiotherapy (nCRT) plays a central role in the management of locally advanced rectal cancer. For many, nCRT leads to clinically meaningful tumour regression. However, up to 20% exhibit little to no response and, in this group, nCRT results in unnecessary delays to definitive treatment. There is a critical need for development of robust molecular methods to predict response to nCRT, to allow for more precise treatment stratification. Although numerous molecular pathways and biomarkers have been implicated in radiosensitivity, the lack of a unifying interpretation of these findings has restricted translational deployment. The aim of the current study was to develop a ‘knowledge network’ with which to visualise and interpret published, quantitative, biomarker data relating to radiosensitivity in rectal cancer, beyond the conventional format of a systematic review. Methods Existing data on predictive biomarkers were retrieved by way of a systematic review of electronic bibliographic databases. Biomarkers were classified according to biological function and built into a hierarchical Gene Ontology tree. Significance was binarized based on p-values or multivariate statistics. An interactive, direct acyclic graph was developed using the Dagre-D3 JavaScript library. Nodes were sized by number of cited publications and color-coded according to their significance scores. The scores reflect the ratio of significant versus non-significant evidence across studied biomarkers. A negative score range indicates more non-significant biomarker findings for that ontological term (node). Weightings were applied to reflect those biomarkers confirmed as significant across two or more studies. p-values of 0.05 or less (adjusted for multiple comparative analysis where appropriate) were considered to be statistically significant. Results 72 individual biomarkers were identified through review. On highest order classification, the domains of response to stress and factors inhibiting apoptosis were found to be most significant (aggregate significance scores across identified biomarkers, 0.75 and 0.714 respectively). A predictive power was not reached for the majority of prognostic biomarkers; rather, the levels of their statistical significance were assessed. Conclusions Building a knowledge-based network analysis of published data identifies promising areas for further research into cellular mechanisms, which may aid in biomarker discovery. Regarding significant node clusters within a network of published data on predictive biomarkers, modifications in cellular metabolic responses to the insult posed by nCRT appear to hold promise in developing a panel of biomarkers with a predictive capacity for response. Network-based analytics takes into account the complex nature of response to therapy, and is a novel way of presenting results obtained from a systematic review.

#840  Polymorphism in DNA repair gene XRCC1 leading to nonconservative amino acid substitution and its role in susceptibility to lung cancer risk. Gh Rasool Bhat, Rakesh Kumar. Shri Mata Vaishno Devi University, Katra, India

Lung cancer is a heterogeneous disease and major global health concern, accounting for more than a million annual deaths worldwide. Historically, 85% of lung cancers are non-small cell lung cancer (NSCLC) and the other 15% are small cell lung cancer (SCLC). In India, it is the most common cause of cancer-related mortality in both men and women. According to the Indian Council of Medical Research cancer registry, 57,795 new cases were reported in 2010, which is projected to increase up to 67,000 new cases annually by the year 2020. Studies revealed that in Srinagar (Jammu and Kashmir) the incidence of lung cancer among males is highest and recent investigation by Global Adult Tobacco Survey (GATS) declared Jammu and Kashmir as the lung cancer capital of India with 26.6% smokers. Lung cancer remains the biggest health concern and challenge for society. Various factors being responsible for lung cancer, but among them smoking is the main cause of lung cancer. However, not all of cigarette smokers develop lung cancer. This differential susceptibility to developing lung cancer can be predicted individually by smoking history, age, genetic factors etc., but the exact mechanisms underlying this modulation are not completely described. To address this problem, we identified a SNP in the XRCC1 gene, codon 399 (Arg/Gln), which was found to be associated with susceptibility to lung cancer risk. However, such studies are completely lacking in the region of Jammu and Kashmir. With this background, we elected to screen these functional variations in Patients and Healthy Individuals in a case-control based study design in population group from Jammu and Kashmir. In the pilot study, we have observed that both the variations are present in the population group of Jammu and Kashmir and hence can be screened for the case-control association. Further, preliminary analysis showed a difference in the frequency distribution of the variations in cases vs. controls. However, a larger sample size is required to reach a conclusion regarding the association. We are working on increasing the sample size of the patient and double the number of controls, from where a real picture of the association can emerge.

#841  UTR analysis identifies Musashi1 as a translational regulator of radiation-induced Golgi-related gene expression. Stacey L. Lehman, John W. O’Neill, Kevin Camphausen, Philip J. Tofilon. National Cancer Institute, Bethesda, MD.

The cellular radioresponse is regulated by post-translational modification of constitutively expressed proteins and by changes in gene expression. Although the role of constitutively expressed proteins, such as those involved in the DNA damage response and cell cycle checkpoints, is well-understood in the context of the radioresponse, radiation-induced changes in gene expression are not as clearly defined. We have previously demonstrated that globlastoma stem-like cells (GSCs) alter their gene expression at the translational level in response to ionizing radiation (IR). In particular, the NSC11 GSC line demonstrated thousands of genes differentially regulated at one hour after a dose of 2 Gy, while considerably fewer were modified at the transcriptome level. Furthermore, there was little overlap between the transcriptome and the proteome. Genes undergoing translational regulation in response to IR fell into distinct functional categories, including mitochondrial structure and function, Golgi function, and translational regulation. Here, we aimed to elucidate the mechanism by which IR induces translational changes in the NSC11 GSC line. NSC11 cells were either exposed to 2 Gy or mock irradiated and polysomes were isolated by sucrose gradient fractionation one hour later. Polysome-bound mRNA was purified and analyzed by microarray. The 5’ and 3’ untranslated regions (UTRs) of the top 75 up- and down-regulated genes were analyzed for the presence of regulatory elements. The most frequently occurring element in the UTRs of both the top up- and down-regulated genes was the Musashi1 binding site. Musashi1 is an RNA binding protein that typically binds to 3’ UTRs to regulate mRNA translation. It is a neural stem cell marker, and its expression is correlated with the grade of malignancy in glioma. GO term analysis of known Musashi1 target genes that were translationally regulated in response to IR revealed an enrichment of genes involved in Golgi function and vesicular trafficking. Knockdown of Musashi1 prevented IR-induced Golgi dispersal in NSC11 cells. Musashi1 knockdown also radiosensitized NSC11 cells, indicating a role for the protein in the cellular radioresponse. Together, these data demonstrate that in the NSC11 GSC line, Musashi1 contributes to radiation-induced translational regulation, specifically in the context of Golgi-related genes, and suggests that Musashi1 is a novel target for radiosensitization.

#842  Breast cancer: Relationship between GPC3 and EMT process. Lilian F. Castillo, Gisela V. Novack, Elisa Bal de Kier Joffé, Maria G. Peters. Instituto de Oncología Angel H. Roffo, CABA, Argentina.

Epithelial-mesenchymal transition (EMT) is a process whereby epithelial cells acquire mesenchymal fibroblast-like properties and display reduced intracellular adhesion and increased motility. Glicp3- (GPC3) is a heparan sulfate proteoglycan associated with cancer. We have established that GPC3 regulates the EMT process underwent by breast cancer cells. However, cellular and molecular events underlying this modulation are not completely described. At the present work we analyzed the effect of GPC3 on cell adhesion and on the expression of molecules associated with this property. We have confirmed that GPC3 regulates the EMT process underwent by breast cancer cells. However, cellular and molecular events underlying this modulation are not completely described. At the present work we analyzed the effect of GPC3 on cell adhesion and on the expression of molecules associated with this property. In addition, molecular regulators of EMT were also studied. We employed a murine breast cancer cell line (LM3; GPC3-) as well as human lines (MDA-MB231; GPC3+ and MCF-7; GPC3+). We have blocked GPC3 expression in MCF-7 cells, while it was over-expressed in MDA-MB231 and LM3 ones. It is known that EMT depends on a reduction in cell adhesion. We determined that GPC3 over-expression induced an increase in the homotypic adhesion of MDA-MB231 and LM3 cells, although GPC3 silencing did not lead significant changes in the organization of MCF-7

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spheroids. GPC3 was also able to modulate the adhesion to extracellular matrix components. GPC3 overexpressing cells were more adherent to fibronectin than controls, but no changes were observed in their adhesion to laminin. E-Cadherin, a molecule involved in homotypic adhesion, is considered a suppressor of invasion and growth of many epithelial cancers. Here, we have corroborated by WB that GPC3 overexpression inhibits the E-Cadherin regulation in L3M and MDA-MB231 cells, whereas GPC3 silencing inhibits the expression of this epithelial marker in MCF-7 cells. We also confirmed the called ‘cadherin switch’, since N-Cadherin was downregulated in GPC3 overexpressing cells and upregulated in GPC3 silenced ones. β-Catenin is a key downstream effector in the Wnt pathway. We confirmed that GPC3 expressing breast cancer cells exhibit a canonical Wnt signaling inhibition, β-Catenin also activates Slug. Similar to SMAD2, Slug binds to the E-Cadherin promoter to repress transcription. By WB and qPCR we demonstrated that GPC3 induces a decrease in Slug and Snail levels. Surprisingly, Twist expression was higher in GPC3 expressing cells. It was reported that Slug is able to repress integrin expression. However, β1-Integrin levels were decreased in GPC3 overexpressing MDA-MB231 cells, while they were increased in GPC3 blocked MCF-7 sublines. Our results suggest the key role of GPC3 in the EMT regulation. GPC3 would modulate cell adhesion and expression of E-Cadherin, N-Cadherin and β1-Integrin. In addition, GPC3 would regulate the expression of different EMT molecules (as Slug, Snail and Twist) as well as signaling pathways involved in that process (like canonical Wnt). The set and balance of these regulations would result in the reversal of EMT, reinforcing the function of GPC3 as an inhibitor of mammmary tumor progression.

**#843 CLDN1 mediates tumor invasion in HNSCC by regulation of EMT through AMPK/TGF-β signaling**

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Epithelial-mesenchymal transition (EMT) is a critical process implicated in metastasis. Despite extensive research, the clinical significance of EMT remains unclear, and a quantitative evaluation of this process in human tumors has yet to be demonstrated. We previously reported a validated EMT immunofluorescence assay (EMT-IFA), that utilizes β-catenin as a tumor segmentation marker to delineate tumor tissue from surrounding stroma in FFPE tumor biopsies. The assay accurately quantifies individual expression and co-localization of E-cadherin (E) and vimentin (V) in tumor cells (Navas et al. NCI-EORTC 2015). We used this assay to measure changes in both the total number and the corresponding EMT phenotypes of tumor cells in xenograft tissues following treatment with various anticancer agents currently in clinical trials at the NCI. Daily treatment for 14 days with the multikinase inhibitor pazopanib caused significant tumor regression and delayed regrowth in the epithelial (E) / gastric cancer xenograft model MKN45, and the tumor cells remaining after treatment were significantly shifted toward a mesenchymal (V+) phenotype. In contrast, pazopanib (Qdx15) had less anti-tumor efficacy in the mesenchymal gastric cancer xenograft model SN55. In another instance, daily treatment of the MDA-MB-468 breast cancer xenograft model with the BCR-Ab1 kinase inhibitor nilotinib for 19 days did not demonstrate any significant anti-tumor efficacy or change in the predominance of the E+ phenotype, whereas a cycle of treatment with the tubulin agent paclitaxel (Q3Dx4) led to MDA-MB-468 tumor regression and delayed tumor regrowth after completion of the treatment cycle. Furthermore, the EMT-IFA was also able to detect changes in the total MDA-MB-468 tumor cell number compared to single-agent arms, but also effectively transformed the EMT phenotype of the tumor, with only a subset of cancer cells surviving by the last day of treatment, which were mostly CD44+/CD133+ mesenchymal cells and potential cancer stem cells. The changes in EMT phenotype brought on by effective drug treatments occurred via preferential killing of cells with E+ phenotype, suggesting that V+ and E+ tumor cells may be more resistant to therapy. The EMT-IFA provides a much-needed analysis tool suitable for clinical investigation of the proposed role of EMT in tumor progression, metastasis, and acquired drug resistance, and is able to detect changes in EMT signature which may serve as early indicators of treatment efficacy and tumor resistance. Funded by NCI Contract No. HHSN26120080001E.

**#846 A novel high-throughput 3D screening system for EMT inhibitors.**

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EMT (epithelial-mesenchymal transition) is a significant event in tumor metastasis and malignancy. Therefore, inhibition of EMT is considered to enable controlling of malignant transformation. In this study, we introduce an innova-
tive three-dimensional (3D) high-throughput (HTS) system that leads to an identification of EMT inhibitors. At first, we established 3D-HTS EMT model with NanoCulture Plate (NCP) that provided a gel-free micro-patterned scaffold for cells forming spheroids naturally. In the NCP-based 3D cell culture system, A549 lung cancer cells migrated, gathered, and then formed multiple spheroids with an epithelial phenotype for screening for EMT inducers. Live cell imaging and fluorescent intensity, expression and proliferation assays were performed for screening for EMT inducers. Using this 3D NCP-based HTS system, we showed that this 3D NCP-based HTS system was useful for screening of EMT inhibitors. SU9516 was a cyclin-dependent kinase 2 (CDK2) inhibitor, which is a tumor suppressor. In this paper, we showed that SU9516 is a promising inhibitor for EMT inhibitors.

#847 Anti-cancer effects of selective Cox-2 and EP2 inhibition through suppression of EMT and the clinical implications of overexpression of Cox-2 and downregulation of E-cadherin in pharyngeal squamous cell carcinoma. Yoshihiro Watanabe,1 Yorihisa Imanishi,2 Hiroyuki Ozawa,3 Kaori Kamayama,4 Koji Sakamoto,5 Ryoichi Fujii,6 Seiji Shigemi,7 Noboru Habu,8 Kuninori Otsuka,1 Yoichi Sato,9 Mariko Sekimizu,10 Fumihiro Ito,11 Yuichi Iki,12 Shin Saito,13 Kaoru Ogawa14.1 Keio University, School of Medicine/Tokyo Saiseikai Central Hospital, Tokyo, Japan; 2Keio University, School of Medicine/Kawasaki Municipal Kawasaki Hospital, Tokyo/Kawasaki, Japan; 3Keio University, School of Medicine, Tokyo, Japan.

Background: Overexpression of cyclooxygenase 2 (Cox-2), an inducible prostaglandin (PG) synthetase, is assumed to promote cancer progression through its multifaceted function, including induction of angiogenesis, stimulation of cell proliferation, restraint on apoptosis, and immunosuppression. Furthermore, recently its inverse relationship with E-cadherin expression was reported. The epithelial-to-mesenchymal transition (EMT) accompanied by the downregulation of E-cadherin is supposed to promote metastasis. However, neither the anti-cancer effect of selective Cox-2 inhibitors nor the EMT-inhibitory small molecule compounds. In a pilot screening, 9 of 1,330 compounds were above the thresholds of the SEMTIN activity and cell viability. Finally, two compounds SB-525334 and SU9516 showed SEMTIN activities in a dose dependent manner. SB-525334 was a known TGF-β receptor I (TGFβRI) inhibitor. SU9516 was a cyclin-dependent kinase 2 (CDK2) inhibitor, which we showed also had an EMT-inhibitory activity. Taken together, it was shown that this 3D NCP-based HTS system was useful for screening of EMT-regulatory drugs.

#848 Netropsin blocks EMT of murine BRAF mutated melanoma cells. Juliano Freitas, Fenfei Leng, Lidia Kos. Florida International University, Miami, FL.

Melanoma is the most aggressive and deadliest type of skin cancer due to its high propensity to metastasize. Despite current progress in the identification of potential drug targets for melanoma, drug resistance remains a major issue indicating that novel therapeutic strategies may be required. It is well documented that the process of converting the epithelial tumorigenic cell to a mesenchymal phenotype, the epithelial-mesenchymal transition (EMT), is responsible for conferring an invasive phenotype to cancer cells. An EMT-like process has been shown to occur during melanoma progression. High-mobility group A-1 (HMGA2) is a multi-functional transcription factor for oncogenesis and EMT in a variety of cancers. HMGA2 is a small DNA-binding protein with three “AT-hook” DNA-binding motifs that specifically recognize the minor groove of AT-rich DNA sequences. HMGA2 is significantly upregulated in human primary melanoma and metastases, including those with BRAF mutations. We accessed the potential application of netropsin, a potent inhibitor of HMGA2-DNA interactions, as an EMT blocking agent of BRAF mutated melanoma cells. Three murine melanoma cell lines that carry mutations in the BRAF gene and show resistance to vemurafenib (D4M, YUMM1.1, and YUMM1.7) were used. Cells were treated with EMT Inducing Media Supplement along with Netropsin and the expression of E-cadherin as well as their migratory behavior were evaluated. Netropsin treatment of EMT induced cells showed a 10-25% increase in cell morphology levels. With E-cadherin expression and decreased migratory behavior. All three cell lines express HMGA2 which continues to be expressed in the tumors resulting from subcutaneous injections in mice. YUMM1.1 cells showed the highest levels of expression of HMGA2 amongst the three cell lines. Interestingly, in our preliminary study only YUMM1.1-derived tumors generated overt metastasis in the lung 30 days after the injections. Altogether our data showed that Netropsin is capable of blocking EMT of murine BRAF mutated melanoma cells. The results of this study will contribute to the development of strategies to prevent EMT that may result in the implementation of more effective therapies to treat melanoma.

#849 Molecular characterization of primary tumor & the paired liver metastatic biopsies of colorectal cancer reveals a critical role of immunosuppression, EMT & angiogenesis in cancer metastasis. Jiangang Liu,1 Yong Beom Cho,2 Hye kyung Hong,3 Song Wu,4 Philip J. Ebert,5 Steven M. Bray,6 Swee Seong Wong,1 Jason C. Ting,1 John N. Calley,1 Catherine F. Whittington,7 Shripad Bhagwat1, Emma Bowden,1 Arun Aggarwal,1 Christoph Reinhard,1 Robert Wild,1 Do-Hyun Nam,2 woo Yong Lee,3 Sheng-Bin Peng,2 Eli Lilly and Company, Indianapolis, IN; 3Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea; 2Samsung Biomedical Research Institute, Seoul, Republic of Korea.

Colorectal cancer (CRC) is one of the leading causes of cancer-related mortality worldwide. In United States alone, it was estimated that nearly 137,000 people were diagnosed, and more than 50,000 were dead from the disease each year. CRC primary tumors often metastasize to liver which accounts for most of CRC death. The molecular mechanism of tumor metastasis is complex and remains poorly understood. In this study, we have collected 79 human CRC primary and their matched liver metastatic tumors. To further characterize the molecular mechanism of metastatic progression, we have assembled gene correlation networks based on RNA sequencing data of these CRC samples. Computational analysis of these correlation networks has identified gene signatures of immune-suppression, epithelial-mesenchymal transition (EMT) and angiogenesis as the key events and potentially synergistic drivers associated with CRC metastasis. Further independent cohort validation using published datasets verified that these specific gene networks of tumor microenvironment were progressively up-regulated throughout the carcinogenesis, and represented distinct biological processes. These gene networks were capable of discriminating the previously categorized CRC molecular subclasses. In addition, we also showed an association of type 1 interferon network with good clinical outcome of CRC, and gene network of EMT-suppression, immunosuppression and cell exhaustion are closely associated with the poor patient outcome. We further demonstrated that the networks of EMT and angiogenesis were related to innate anti-PD-1 resistance, and the networks of immunosuppression and T cell exhaustion were associated with resistance to radiation and checkpoint blockade. Overall, we
conclude that a genome-wide interrogation of co-regulated networks utilized in this study represents a valuable strategy to identify molecular mechanisms of cancer metastasis, and gene networks of immune-suppression, EMT and angiogenesis are among the key events associated with CRC metastasis.

The Pi3K/Akt pathway mediates epithelial-mesenchymal transition (EMT) and malignant progression in BRCA-defective epithelial ovarian cancer.

**#850**

**Conclusion:** The Pi3K/Akt pathway mediates epithelial-mesenchymal transition (EMT) and malignant progression in BRCA-defective epithelial ovarian cancer. Methods: BRCA2-mutated POEO and BRCA2-wild type PEOP epithelial ovarian cancer cell lines were derived from the same patient at first and second relapse, respectively, following platinum-based chemotherapy. Non-targeted siRNA control (NTC) and BRCA1 knockdown (BRCA1-kd) SKOV3 cell lines were previously established in our laboratory. Western blot analysis and luciferase reporter assay were performed to determine EMT markers (snail, slug, fibronectin) in BRCA defective ovarian cancer cells. Scratch wound assays were conducted to determine the ability of cells to migrate. Clonogenic and apoptosis assays were carried out to determine cell survival in response to various concentrations of carboplatin and olaparib with or without MK-2206, a small molecule inhibitor of AKT. Results: PE01 cells exhibited an increase in mesenchymal markers Snail and fibronectin, whereas PE04 cells exhibited an increase in the epithelial marker E-cadherin. POE1 cells also exhibited an increase in cell migration compared with PE04 cells. In the luciferase reporter assay, BRCA2-mutated PE01 and BRCA1-kd SKOV3 cells exhibited lower levels of the epithelial marker E-cadherin compared to their BRCA2-wild type counterparts, POEO and SKOV3 NTC, respectively. Treatment with MK-2206 caused increased susceptibility of BRCA1-kd SKOV3 cells to olaparib compared with BRCA1-kd wild type SKOV3-NTC cells. Conclusions: BRCA2-mutated PE01 cells manifested an increase in mesenchymal markers and invasive phenotypes. BRCA1-kd SKOV3 cells manifested a decrease in the epithelial trait, E-cadherin activity. In addition, AKT inhibition heightened susceptibility of BRCA1-kd SKOV3 cells to DNA damaging agents such as olaparib. These findings suggest that BRCA2-defective cells rely on the induction of EMT via activation of AKT to survive DNA damage and develop therapeutic resistance during malignant progression.

Reversion of epithelial to mesenchymal transition under androgen deprived conditions promotes chemoresistance in prostate cancer.

**#851**

**Conclusion:** Reversion of epithelial to mesenchymal transition (EMT) under androgen deprived conditions promotes chemoresistance in prostate cancer. Akanksha Upadhyay,1 Anthera Medical, Miami, FL; Sarah Louis Ryan,2 Boston, MA; G. Hollier,1 Elizabeth D. Williams1. 1Australian Prostate Cancer Research Centre – Queensland, Institute of Health and Biomedical Innovation, School of Biomedical Sciences, Queensland University of Technology and Translational Research Institute, Australia, Brisbane, Australia; 2Australian Prostate Cancer Research Centre – Queensland, IHBI, School of Biomedical Sciences, Queensland University of Technology and Translational Research Institute, Australia, Translational Cell Imaging – Queensland, Brisbane, Australia.

Introduction: Metastatic prostate cancer (PC) is one of the leading causes of cancer related male deaths in Australia. Radical prostatectomy followed by androgen deprivation therapy (ADT) upon recurrence of disease is the conventional form of treatment. Upon relapse, administration of androgen targeted therapy (ATT) and subsequent chemotherapy improves quality of life and increases overall survival of patients. However, it is not curative and the disease ultimately progresses. Epithelial to mesenchymal transition (EMT) is a process that can facilitate tumour growth and progression to metastasis and has also been associated with chemoresistance. We therefore aim to determine how EMT and resultant reversal of this state (MET) influences the chemoresistance profile of PC cells and further determine if and how specific genetic regulators are involved in this process. Methods: Doxycycline hydolate (Dox) inducible Snail and Zeb1, lncAP EMT models were used to determine the effect of the EMT status in response to a clinically relevant panel of drugs using cellular and molecular assays. All assays were performed in both lethal bovine serum (FBS) and charcoal stripped serum (CSS) supplemented culture media. CSS media was used to mimic an ADT-like environment. Using 2D monolayer and 3D spheroid [Happy Cell©] formats. Cells were induced for 5 days of EMT in the presence of Dox and were allowed to revert for 14 days of MET (absence of Dox) before the drug assays were carried out over a period of 72 hours. Live/dead cell staining and morphological and gene expression viabilities in both spheroid and monolayer cultures were used for 2D assays and 3D assays were analysed using either a GE IN Cell Analyzer live cell imaging system or confocal microscopy. Results: Induction of Snai1 and Zeb1 induced an elongated cell shape and increased mRNA and protein expression of mesenchymal markers such as vimentin during EMT. This was associated with a decrease in expression of epithelial markers E-cadherin and N-cadherin. An apoptotic response was observed in 14 days of dox removal along with an increase in epithelial markers was observed. These changes were also associated with a gradual reversal in the phenotype of these cells. However, this reversal in phenotype and expression of EMT markers was not as striking in cells grown in CSS compared to cells in FBS media (both 2D and 3D). Mitoxantrone concentration-response assays in a 2D format showed that cells that undergo a subsequent MET are more chemoresistant than uninduced or EMT cells when grown in CSS media. This was however, not observed in cells undergoing similar transitions in the FBS media. Conclusions: The chemoresistance profile of prostate cancer cells is altered by their EMT status. This effect is enhanced in the context of ADT. Cells that revert back to a more epithelial phenotype after undergoing an EMT exhibit higher resistance towards chemotherapy drugs.

Bromodomain and extraterminal proteins regulate the epithelial-mesenchymal transition in breast cancer.

**#852**

**Conclusion:** Bromodomain and extraterminal proteins regulate the epithelial-mesenchymal transition in breast cancer. Guillaume Andrieu, Gerald V. Dienes. Boston Univ. School of Medicine, Boston, MA.

The epithelial-mesenchymal transition (EMT) is a developmental program that cancer cells often activate to acquire a highly plastic phenotype that promotes invasion, metastasis, but also chemoresistance and cancer stem cell generation. As readers of epigenetic marks, bromodomain and extra-terminal (BET) proteins BRD2, BRD3 and BRD4 participate in the regulation of multiple transcriptional programs implicated in cancer progression. We sought to unravel the roles of BET proteins in EMT in breast cancer. Despite their homology, we report that BET proteins differentially regulate EMT. Based on an EMT PCR array, we identify a BRD2-specific transcriptional profile that promotes EMT, whereas BRD3 and BRD4 signatures repress this program. These individual signatures are unidentifiable upon pan-BET inhibition using JQ1, reinforcing the necessity to target each BET member separately to better understand their functions. Upon BRD2 depletion, basal-like breast cancer cells, which present a mesenchymal phenotype, exhibit a reduced expression of mesenchymal markers (N-cadherin, vimentin) and re-express epithelial markers (E-cadherin, cytokeratins). Moreover, a large panel of EMT master transcription factors is down-regulated in BRD2-depleted cells, including the Snai and ZEB families or Twist. Interestingly, we found that BRD3 or BRD4 depletion leads to the opposite phenotype: an increase of mesenchymal marker expression and repression of the epithelial markers. In luminal A breast cancer cells which present an epithelial phenotype, BRD2 overexpression leads to the expression of mesenchymal markers (N-cadherin, vimentin) and re-express epithelial markers (E-cadherin, cytokeratins). Moreover, a large panel of EMT master transcription factors is down-regulated in BRD2-depleted cells, including the Snai and ZEB families or Twist. Confirming the differential roles of BET proteins in EMT regulation. Taken together, our results establish that BRD2 positively regulates EMT, whereas BRD3 and BRD4 repress this program. BET proteins possess separate and opposite biological functions, reinforcing the relevance of an individual targeting instead of a pan-BET inhibition using JQ1. We hypothesize that BET proteins modulate EMT through the regulation of its master transcription factors. We propose that the balance of BET proteins presence at the promoters of the EMT genes is a novel mechanism of regulation of this program in breast cancer cells.

Cisplatin, a gene with unknown function, is a novel driver of epithelial-mesenchymal transition in human cancer.

**#853**

**Conclusion:** Cisplatin, a gene with unknown function, is a novel driver of epithelial-mesenchymal transition in human cancer. Sarah R. Amend, James Hernandez, Princy Parsana, Kenneth J. Pienta. Johns Hopkins Univ., Baltimore, MD.

Metastatic prostate cancer is an incurable lethal disease and is the cause of death for 28,000 men in the US annually. In order for a cancer cell to metastasize from the primary tumor, it must move. In a critical early event of metastasis, a rare population of cells in the primary tumor undergoes a phenotype switch from a proliferating epithelial cell to gaining the migratory capacity of a mesenchymal cell, a process termed epithelial-to-mesenchymal transition (EMT). To identify previously overlooked regulators of EMT, we undertook a multi-study gene expression array analysis (15 published gene expression studies; 95 total samples; 6 cancer types) and identified Clorf116 as a candidate driver of the epithelial phenotype in EMT across multiple cancer types. Of note, Clorf116 is
an unnamed gene of unknown function that is largely uncharacterized in any setting, including in cancer biology or in EMT. To substantiate its potential role in EMT, we queried the NCi-60 cell line collection gene expression dataset and found that C1orf116 shared a similar expression pattern with epithelial marker genes (ESRP1, ESRP2, OVOL1, OVOL2) and a converse expression pattern to mesenchymal markers (CDH2, ZEB1, VIM). This suggests a role as a driver of the epithelial phenotype. Interestingly, the sole publication specifically studying C1orf116 describes it as Specifically Androgen Regulated Gene (SARG). The authors demonstrate the presence of an androgen response element upstream of the C1orf116 start site in the androgen-responsive prostate cancer cell line LNCap. Our data indicate a role for C1orf116 in the androgen receptor-negative prostate cancer cell line PC3, suggesting a non-androgen-mediated role for C1orf116 in prostate cancer. In addition to elucidating its role in EMT, work is underway to characterize the RNA and protein products of C1orf116. Alternative splicing transcripts of varying length that likely give rise to at least two protein product sizes. RNA sequencing and mass spectrometry will reveal transcript and protein identity and provide insight into potential isoform switching regulating EMT and other processes.

**#854** Human tissue Kallikrein 6 enzyme activity regulates epithelial-mesenchymal transition and metastasis in colon cancer. Huan Liu, Iurin Chen, Earlaphia Sells, Haidan Cui, Ritu Pandey, George Pampalakis, Georgia Sotiropoulou, Thomas Doetschman, Natalia A. Ignatenko. 1Univ. of Arizona Cancer Ctr., Tucson, AZ; 2Univ. of Arizona, Tucson, AZ; 3Univ. of Patras, Patras, Greece.

Background: Kallikrein-related peptidase 6 (KLK6) belongs to the family of human tissue kallikrein genes, majority of which are shown to be differentially expressed in cancers. Clinical studies have demonstrated that upregulation of KLK6 in primary colorectal tumors and lymph nodes correlates with serosal invasion, liver metastasis and indicative of poor prognosis in patients. It has been reported that KLK6 protein is involved in regulation of the epithelial-mesenchymal transition (EMT) program in an organ-specific context. The aim in this study was to investigate contribution of KLK6 enzyme in the EMT during neoplastic transformation in the colon. Results: We expressed enzymatically active or inactive KLK6, using pcDNA3.1(+) preproKLK6 and pcDNA3.1(+) preproKLK6 Ser197Ala mutant plasmids, in Caco-2 colon cancer cell line, which has been characterized before as a very low KLK6 expresser with an undetectable secreted KLK6. Stable isogenic clones were selected and further evaluated for their ability to migrate and invade using in vitro assays and to metastasize in vivo using SCID orthotopic mouse model. We found no effect of KLK6 enzyme activity on migration of Caco-2 cells, expressing the empty vector (Caco-2 mock), and Caco-2 cells, expressing an enzymatically active KLK6 (Caco-2 KLK6 wt) or inactive KLK6 (Caco-2 KLK6 mut). But Caco-2 KLK6 wt cells demonstrated the invasive phenotype in Matrigel invasion assays (p<0.001, compared to Caco-2 mock and Caco-2 KLK6 mut cells). The Caco-2 mock and Caco-2 KLK6 mut cells, injected in SCID mice orthotopically, developed primary colon tumors but no metastatic lesions were identified. In contrast, Caco-2 KLK6 wt cells formed primary colon tumors and metastasized locally, although they failed to form the distant metastasis (lung and mesentery). Animals, growing the Caco-2 KLK6 wt tumors, displayed a significant decrease in their survival rates, compared to other groups (p=0.02). In Caco-2 KLK6 wt cells, TGF-β signaling pathway was induced and activation of SMAD2/3 signaling pathway was associated with the elevated expression of known regulator of the EMT, zinc-finger protein Snail. In addition, the expression of a high-mobility group AT-hook 2 (HMGA2) protein was induced in Caco-2 KLK6 wt cells. The HMGA2 expression is implicated in the EMT program, acting through the TGF-β signaling pathway and is associated with cancer progression and metastasis. Western blot analysis demonstrated that Caco-2 KLK6 wt cells expressed constitutively active TGF-β. These findings demonstrate that KLK6 enzyme activity is required for colon cancer progression via induction of the EMT program. We identified the TGF-β signaling pathway as a mechanism driving the EMT in colon cancer cells expressing KLK6 enzyme.

**#855** Ube2v1 promotes epithelial mesenchymal transition and metastasis in colorectal cancer by epigenetically transcriptional suppression of autophagy. Tong Shen, Dong Ling Cai, Hong Yu Lan, Juan Wen Gan, Ming Li, Ru Jing Wang, Da Peng Guo, Qun Zhou, Xing Xing Lu, Shi Li, Na Li Sun, Ming Jian Li, Soochow University Medical School, Suzhou, China; “Baoan Hospital of Shenzhen,” Shenzhen, China.

Ubiquitination is one of the basic post-translational modifications for cellular homeostasis. The conjugating enzyme (E2) family plays as a bridge linking the first step mediated by E1 with the final step mediated by E3 in the ubiquitin-proteasome system. However, the role of Ube2v1, one of Ubiquitin-conjugating E2 enzyme variant proteins (Ube2v1), in colorectal cancer (CRC) and autophagy is currently unknown. Here, we found that Ube2v1 is elevated in tumor samples of CRC patients and correlated with poorer survival of CRC patients. Furthermore, Ube2v1 promotes migration and invasion of CRC cells in vitro and boosts tumor growth and metastasis of CRC cells in vivo. Interestingly, Ube2v1 suppresses autophagy program and promotes EMT and metastasis of CRC cells in an autophagy dependent pattern in vitro and in vivo. Moreover, Rapamycin attenuates the enhanced in vitro migration and invasion in vivo and metastasis of Ube2v1 overexpression by inducing the autophagy pathway. Mechanistically, Ube2v1 increases histone H4 lysine 16 acetylation by down-regulating expression of Sirt1 and epigenetically suppresses gene expression of autophagy genes in colorectal cancer. In conclusion, Ube2v1 functions as a global regulator for autophagy by epigenetically transcriptional suppression of autophagy, and consequently sensitizes to chemotherapy treatment than the parental HCT-116/ICN1 cell line. Ube2v1, one member of E2 family in the ubiquitin-proteasome system, regulates the EMT and autophagy program in CRC progression and metastasis.

**#856** Notch-1 promotes a mesenchymal phenotype in colon cancer cells via Notch-3 and Smad-3 activation. George Sigounas, Fred E. Bertrand, Douglas A. Weidner, Kaitlyn E. Vinson, Alexander G. Clark, Azeem Khan. 1East Carolina Univ, School of Medicine, Greenville, NC; 2University of Alabama at Birmingham, Birmingham, AL.

A role for cancer initiating cells (CIC) in tumor growth, drug resistance, relapse, and metastasis via epithelial to mesenchymal transition (EMT) has been supported by experimental evidence. It is well-documented that Notch-1 receptor signaling participates in stem cell maintenance and lineage commitment in various organs, including the colon, and can regulate EMT. The function of Notch signaling in colorectal cancer (CRC) is not clear. We have reported that Notch-1 activation in the human colon tumor cell line HCT-116 resulted in upregulation of Jagged-1, Smad-3, CD44, and Slug proteins. These changes were accompanied by acquisition of a mesenchymal cell phenotype that included increased anchorage independent growth and migratory activity. Experiments with a γ-secretase inhibitor (DAPT) and soluble Jagged1-Fc protein demonstrated that Notch-1 signaling activates CD44 and Slug via other Notch receptors. In subsequent experiments, we observed an increase in Notch-3 expression in the presence of activated Notch-1. Herein, we tested the hypothesis that the stem cell-like mesenchymal phenotype induced by Notch-1 signaling in colon cancer cells is mediated by the Notch-3 receptor and Smad-3. The human colon tumor cell line HCT-116 was transduced with constitutively active Notch-1, and a Notch-3 shRNA to produce a Notch-3 null cell line with activated Notch-1 (ICN1-shN3 cells). ICN1-shN3 cells exhibited a 2.5-fold lower plating efficiency than the HCT-116/ICN1 cell line (p<0.01) that expresses constitutively active Notch-1 in the presence of Notch-3. In addition, Notch-3 null cells with constitutively active Notch-1 (ICN1-shN3 cells) were slower by 29% in completing wound healing as compared with the HCT-116/ICN1 cell line (p<0.05). Consistent with the lower plating efficiency, a 37% decrease in colosphere formation was observed with ICN1-shN3 cells as compared with the HCT-116/ICN1 cell line (p<0.001). These data indicated that loss of Notch-3 abrogated the Notch-1 induced mesenchymal cell phenotype. Western blot analysis demonstrated that the HCT-116/ICN1 cells with intact Notch-3 exhibited increased levels of the EMT markers E-cadherin and vimentin, and a converse expression pattern to the parental HCT-116 cell line. Expression of CD44 and Slug in Notch-3 receptor null ICN1-shN3 cells was highly reduced compared with HCT-116/ICN1 cells and was similar to that detected in the parental HCT-116 cells. Treatment of HCT-116/ICN1 cells with a Smad-3 inhibitor resulted in significant reduction of the CD44 and Slug proteins. Meanwhile, Notch-3 receptor null ICN1-shN3 cells were also more sensitive to chemotherapy treatment than the parental HCT-116/ICN1 cell line (p<0.05). Collectively, our data provide evidence for a novel pathway in which Notch-1 signaling in colon tumor cells promotes a mesenchymal phenotype via activation of Jagged-1, Notch-3 and Smad-3, followed by expression of CD44 and Slug.

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ELF3 modulates mesenchymal to epithelial transition through regulation of CD44 variant isoform expression in pancreatic cancer cells. Ayano Kabashima-Niibe,1 Hiromasa Takaishi,2 Takonari Kanai,2 Hideyuki Saya,2 Gregory J. Gores,3 Hajime Higuchi4,5 Mayo Clinic, Rochester, MN; 6Keio University School of Medicine, Tokyo, Japan; 7International University of Health and Welfare, Tokyo, Japan.

BACKGROUND: Epithelial to Mesenchymal Transition (EMT) and Mesenchymal to Epithelial Transition (MET) may participate in pancreatic ductal adenocarcinoma (PDAC) metastasis. CD44 proteins are cell membrane proteins which function as co-receptors regulating many cellular functions. Some reports have shown that isoform switching from CD44 variant form (CD44v) to CD44 standard form (CD44s) can take place during the EMT (Brown et al., Clin. Cancer Res, 2011, Yae et al., Nat. Commun., 2012). The AIM of this study was to explore the regulation of CD44 isoform switching in PDAC cells. METHODS: The expression levels of CD44v and CD44s in PDAC cell lines were assessed by immunohistochemistry and immunoblot analysis. PDAC cell lines were implanted into immunodeficient mice and treated with sulfasalazine for 14 days. Sulfasalazine, which inhibits glutamate-cysteine transport, reduces CD44v-dependent cell growth. CD44v or CD44s positive cells were isolated using flow cytometry and induced to undergo the EMT by TGF-β treatment (7.5ng/mL) for 72 hours. A doxycycline inducible system was employed to overexpress Slug, which promotes the EMT phenotype. To induce MET, the cells were released from TGF-β treatment and cultured for an additional 72 hours. EMT-like ETS Transcription Factor (ETFS) was knoowed to be transduced by siRNA transfection. RESULTS: Immunohistochemistry analysis revealed that CD44v protein is expressed specifically by PDAC with ductal structures within the complex tumor environment of these cancers. However, in contrast, CD44v expression was not observed in solitary infiltrating cancer cells. PAN-C-1, AsPC-1 and SUT-2 PDAC cell lines expressed both CD44v and CD44s isoforms. Tumor mass in implanted PAN-C-1, AsPC-1 and SUT-2 cells were reduced 69%, 50% and 36%, respectively by sulfasalazine treatment following 2 weeks of therapy. However, CD44v null HPAC cells were resistant to sulfasalazine therapy following implantation. E-cadherin was expressed in CD44v positive cells but not in CD44s positive cells, and both CD44v and E-cadherin protein were reduced by TGF-β treatment. Slug over expressed cells were resistant to sulfasalazine treatment compared to non-Slug-induced cells. Removal of TGF-β restored both CD44v and E-cadherin expression levels which consistently occurs with MET. However, restoration of CD44v expression was diminished when ELF3 expression was reduced by siRNA transfection. CONCLUSION: The isoform switching between CD44v and CD44s was reversible. The expression of CD44v isoform appears to be controlled by ELF3 during MET.

The circadian clock of glioma cells undergoing epithelial-mesenchymal transition. Arpan De, Dilshan Harshajith Beligala, Vishal Premdev Sharma, Benjamin R. Fry, Michael Eric Geusz. Bowling Green State University, Bowling Green, OH.

Highly invasive gliomas contain many cells that have changed to an enhanced migratory state through an epithelial-mesenchymal transition (EMT). These cells have the phenotype of glioma stem cells (GSCs). Similar to GSCs, glioma cells undergoing EMT show phenotypic heterogeneity, altered gene expression, and resistance to anticancer drugs along with increased invasiveness. Circadian rhythms in tumor cells influence the progression and severity of cancer and appear to regulate cell division cycles. Increased cancer incidence and progression have often been linked to disruption or deregulation of the molecular mechanisms of the circadian clock. One of the major clock proteins, PER2, has been shown to play a regulatory role as a tumor and EMT suppressor in metastatic breast cancer cell lines. GSCs in tumorsphere cultures contain circadian clocks that may regulate their cancer properties. The possibility of a regulatory role for circadian rhythms in EMT was examined here. We used a standard method to induce EMT in the C6 rat glioma cell line that has known circadian rhythms in gene expression generated by the circadian clocks within its cells. EMT was induced by withdrawing the cell culture medium with a serum-free stem cell medium (SCM) containing growth factors (EGF, FGF2, PDGF-alpha-beta). Cell cultures were imaged continuously with a microscope and digital camera in a cell incubator to monitor cell shape, cell death, migration, and apoptosis. During EMT, cells changed from an extended flat state to rounded and spindle shapes, ceased proliferating, and expressed EMT markers ZEB1 and vimentin. At the end of two days in SCM, 33.4% of the cells were ZEB1-positive and only 1.26% were GFAP-positive (n = 3 cultures). Cell diameters after EMT were within the size range of C6 GSCs described as Hoechst-negative cells positive for stem cell markers nestin and CD133. Following EMT, small tumorspheres began to form. After initiating EMT, the rounded cells were counted at hourly intervals for up to four days after the medium exchange. As the number of post-EMT cells increased, the population size oscillated, and when examined by Lomb-Scargle periodogram analysis, four cultures had a significant period within the circadian range, 19-29 hours, (average 22.20 ± 2.45 SD, p <0.05). One had a 16-hour period and one lacked a consistent time period. There were no significant rhythmic patterns in the cultures that were not synchronized with an initial forskolin treatment. We conclude that EMT may be timed by endogenous circadian oscillators in gliomas that favor larger numbers of post-EMT cells at a particular time of day. These results suggest that pharmacological treatments that suppress EMT or target GSCs would be more effective when delivered at particular times during the circadian cycle of the tumor.

Epigenetic regulation of epithelial-mesenchymal transition in esophageal squamous cell carcinoma. Chen Chen,1 Ming Zhao,1 Xiaojie Huang,2 Xiang Wang,2 Wenlian Liu,1 Mengjiu Chen,1 Bangliang Yin,1 Zhi Li,1 Yunchang Yuan,1 Qianjin Li,1 Fengei Yu2. The Second Xiangya Hospital of Central South University, Changsha, China; 3Beijing Genomics Institute at Shenzhen, Shenzhen, China.

Epithelial-mesenchymal transition (EMT), which is associated with tumor progression and metastasis. Here, we investigated the correlation and potential modifications in the regulation of EMT in esophageal squamous cell carcinoma (ESCC). The RNA-Seq analysis identified a total of 6150 differentially expressed genes (3423 up-regulated and 2727 down-regulated). The GO terms showed that these genes belonged to several molecular functions and biological pathways. The abnormal expression of key EMT genes, some of Sox family genes and Ezh2 significantly related to patient survival. The MeDIP-Seq analysis identified histone modifications in the regulation of EMT in the whole genome level with focal hypermethylated and widespread global DNA hypomethylation. The gene ontology analysis showed that the DMRs related genes belonged to different ontological domains, such as EMT progression, cell cycle, adhesion, proliferation, and apoptosis. The results of the bisulfite-sequencing confirmed the gene EMTs DMRs identified by MeDIP-Seq. However, further analyses showed that the EMT gene DMRs only related to gene expression, but not the patient survival. ChiP-Seq was then performed to test the relationship between histone methylation and gene expression. A total of 295 genes were found to correlate with H3K27 tri-methylation (H3K27me3), and a certain number of key EMT genes, such as ZEB1/2, AREG, GATA4, and CDH18 were identified. Further study showed that Ezh2-mediated H3K27me3 regulate the expression of key EMT genes. Ablation of Ezh2 expression prevents EMT, whereas forced expression of Ezh2 restores EMT. Sox4 gene could directly regulate Ezh2 expression, tri-methylated H3K27me3 for EMT gene repression. Taken together, our results suggest that both of the DNA methylation and H3K27me3 modification involved in the regulation of EMT in ESCC. Sox4-Ezh2-mediated H3K27me3 marks associate with key EMT gene regulation, representing an important epigenetic EMT signature.
and may play a critical role in regulating the EMT program during the progression of cancer. They also suggest that modulation of OLFM4 expression might be a novel molecular target for advancing prostate-cancer therapies.

**#862** Atypical protein kinase c inhibitors can repress epithelial to mesenchymal transition (type III) in malignant melanoma. Warnhawan Sarathi Ratnayake, Mildred Acevedo-Duncan. Unv. of South Florida, Tampa, FL.

Melanoma is a type of cancer occurring in melanocytes. Approximately 90% of melanoma occurs in skin (cutaneous melanoma) but can rarely arise from the mucosal surfaces or areas where neural cells migrate. Examples are eye, intestine and mouth [Eur. J. Cancer, 69, 39-42 (2016)]. 76,380 of new cases and 10,130 number of deaths are expected in 2016 in the USA due to melanoma [http://seer.cancer.gov/statfacts/html/melan.html (11/05/2016)]. Atypical PKC inhibitors contain two structurally and functionally distinct isoforms in human which are PKC-α in (iota) and PKC-ζ (zeta). They are believed to be involved in cell cycle progression, tumorigenesis, cell survival and cell migration. We believe that atypical PKCs play an important role in cell motility of melanoma by involving the signaling pathways which induces EMT-type III (Epithelial to Mesenchymal Transition). In normal melanocytes, PKC-ζ was found in low levels and PKC-α was not detected. But both proteins are detected in very high levels in malignant melanoma [Melenoma Res. 12:201-209 (2002)]. In the current study, we have investigated the effects of novel atypical PKC inhibitors [4-(5-amino-4-carbamoylimidazol-1-yl)-2, 3-dihydroxy(cyclopentyl) methyl dihydrogen phosphate (ICA-1)] which is specific to PKC-ζ and 8-hydroxy-1, 3, 6-naphthalenetrisulfonic acid (Compound-50) which is specific to PKC-α on all the proliferation, apoptosis and cell migration of two malignant melanoma cell lines (SK-MEL-2 and MeWo) compared to normal melanocyte cell line (PCS-200-013). We showed that both inhibitors can decrease the levels of total and phosphorylated levels of PKC-ζ and PKC-α. Furthermore, both inhibitors increased the levels of E-cadherin and decreased the levels of Vimentin which is a mesenchymal marker associated with EMT. Treatments with inhibitors altered the levels of CD44, a cell-surface glycoprotein involved in cell-cell interactions, cell adhesion, migration and tumor cell homing during metastasis. These results suggest that both PKC-α and PKC-ζ are involved in signaling pathways which upregulate EMT and which can be effectively suppress using ICA-1 and Compound-50. Furthermore we established that treatment with ICA-1/Compound-50 induced apoptosis as shown by increasing Caspase-3 levels and decreasing Bcl-2 levels.

**#863** Neuronal splicing regulatory factors are upregulated in models of epithelial-mesenchymal transition. Yevenia L. Khodor, Julia Froese, Frank Gertler, Robert A. Weinberg, Christopher B. Burge. MIT, Cambridge, MA; Whitehead Institute, Cambridge, MA.

Epithelial-mesenchymal transition (EMT) is an essential developmental program through which epithelial cells lose their polarity and junctions with neighboring cells and acquire migratory and invasive properties. Cancer cells hijack at least a portion of the EMT program to increase their invasive properties, acquire resistance to senescence, and metastasize. Major changes in cell signaling, transcriptional and post-transcriptional regulation occur during EMT. To study post-transcriptional changes we characterized gene expression and splicing across EMT in 3 cell culture models of EMT: the luminal epithelial MCF7ras breast cancer cell line with EMT induced by SUGL+SOX9 and in basal mammary epithelial (HMLEras) cells, with EMT induced by either SNA1 or ZEB1. RNA-seq was performed at 2-day intervals across these transitions. We observed broad overlaps between these systems, with 821 genes that changed significantly in all three. In all three time courses, genes associated with cell cycle and growth decreased in expression, while genes associated with signaling, cell movement, adhesion, extracellular matrix organization, and differentiation increased. Intriguingly, genes associated with neurogenesis and angiogenesis were also induced in the MCF7 and HMLE-Sna1 time courses. Those include known axon guidance and angiogenic factors in the Robo and Slit families. We also found that CPEB2B is essential to establish anoikis resistance (AnR), and promotes metastasis in vivo in orthotopic models. In contrast, CPEB2A decreases AnR and metastatic transformation. In the current studies, we examined the function of CPEB2 isoforms using next generation sequencing analysis of downstream signaling pathways. We found that CPEB2 expression upregulated AnR in breast cancer cells and induced key genes/proteins in the epithelial to mesenchymal transition (EMT) and hypoxic response (hypoxia inducible factor 1α (HIF1α)-dependent) pathways. Inhibition of either TWIST1 or HIF1α reduced the CPEB2B-mediated AnR in cells ectopically expressing CPEB2B, confirming a link between CPEB2B alternative splicing and the induction of the EMT/hypoxia pathways. We then extended our findings, and demonstrated that CPEB2 alternative splicing induced de novo translation of both HIF1α and TWIST1, whereas CPEB2A decreased translation of these factors. Furthermore, CPEB2 isoforms were found to modulate differential polyadenylation site selection of the TWIST1 3'UTR and differential expression of CPEB2 isoforms using next generation sequencing analysis of downstream signaling pathways. We found that CPEB2B expression upregulated AnR in breast cancer cells and induced key genes/proteins in the epithelial to mesenchymal transition (EMT) and hypoxic response (hypoxia inducible factor 1α (HIF1α)-dependent) pathways. Inhibition of either TWIST1 or HIF1α reduced the CPEB2B-mediated AnR in cells ectopically expressing CPEB2B, confirming a link between CPEB2B alternative splicing and the induction of the EMT/hypoxia pathways. We then extended our findings, and demonstrated that CPEB2B alternative splicing induced de novo translation of both HIF1α and TWIST1, whereas CPEB2A decreased translation of these factors. Furthermore, CPEB2B isoforms were found to modulate differential polyadenylation site selection of the TWIST1 3'UTR and competed for binding to HIF1α and TWIST1 3'UTR cytoplasmic polyadenylation element (CPE) site sequences. These results demonstrate that CPEB2 and CPEB2B isoforms in breast cancer cells.
address this issue, immunohistochemistry and E-selectin microsphere dynamic adhesion assays were initially used to assay 110 cases of breast cancer for EMT biomarkers and E-selectin ligand activity. The expression of E-cadherin, but not N-cadherin or vimentin, directly correlated with E-selectin ligand activity. Thus, it was hypothesized that the EMT, and in turn the mesenchymal-to-epithelial transition (MET), regulate expression of functional E-selectin ligands in breast cancer cells. The ectopic expression or shRNA knockdown of transcription factors (i.e., Snail and Twist) in target cell lines were used to induce EMT or MET, respectively. Shear flow adhesion assays and flow cytometry demonstrated that cells with epithelial phenotypes had greater E-selectin ligand activities and greater expression of sialofucosylated carbohydrates compared to cells with mesenchymal phenotypes. Moreover, glycoprotein E-selectin ligand activities of breast cancer cells were more affected by the EMT compared to glycolipid E-selectin ligand activities. Assessing expression of α1,3- and α1,4- fucosyltransferases (FUT) at the RNA level using qRT-PCR revealed that FUT3 and FUT6 gene expression were decreased via the EMT, corresponding to the lowered E-selectin ligand activities observed in mesenchymal phenotype cells. Altogether, the results of this investigation demonstrate that the EMT, as well as MET, mechanistically regulate the expression of functional E-selectin ligands expressed by breast cancer cells.

TUMOR BIOLOGY: Epithelial to Mesenchymal Transitions in Metastasis

#867 Circulating lung tumor cells capture extracellular vesicles conferring resistance phenotype by the occurrence of epithelial-mesenchymal transition reprogramming. Josephine Zangari, 1,2 Marius Ilie, 1,2 Simon Heeke, 1,2 Véronique Hofman, 3 Baharia Mograbi, 1 Sophie Raynaud, 1 Salome Lalve, 1 Nathalie Yazbeck, 1 Charles-Hugo Marquette, 1 Sylvie Leroy, 3 Patrick Bulte, 4 Université Côte d’Azur, Nice, France; 2Université Côte d’Azur/FHU Oncogene, Nice, France; 3CHU Nice, Nice, France.

Background: The overall prognosis of lung cancer patients remains dismal, particularly in advanced stages. Only complete surgical resection of early-stage tumors improves the prognosis of certain non-small cell lung cancer (NSCLC) patients. However, migration of circulating tumor cells (CTCs) into the blood stream is probably an early event of carcinogenesis inducing metastases. The fact that some of these CTCs could survive within this “liquid environment” suggest that they acquire phenotypic changes that would confer them improved resistance against anoxia. In this context, we looked if immune blood cells could transfer to CTCs some factors associated with this increased resistance. Materials and Methods: Lung cancer cells (A549 cell line) were incubated with different sub-population of blood cells (PBMCs, granulocytes, platelets) and serum to look for potential transfer of extracellular vesicles (EVs) into the cancer cells. To assess the inter-cell transfer we looked for the presence of a validated blood cell specific marker the miR-223 that should not be expressed in lung cancer cells. By using a direct method for CTCs detection and characterization, we looked for these biomarkers within CTCs of lung cancer patients with early and late stages. Moreover the quantification of these biomarkers was correlated with the expression of vimentin, cytokeratin and E-cadherin Results: First, A549 cells were able to quickly capture microRNA (in particular miR-223) within the control blood samples. We then identified neutrophils as a major source of these miRNA. The inter-cell transfer was EVs dependent and promoted increased resistance and survival for cancer cells. In NSCLC patients with different stages, the presence of some specific miRNAs for blood cells was detected in a subpopulation of CTCs showing epithelial to mesenchymal phenotype, in particular an increase of vimentin expression and loss of E-cadherin expression. Conclusion: These results tend to prove that CTCs may capture extracellular vesicles from immune blood cells within the bloodstream and this phenomenon may be associated with a more aggressive phenotype. Inhibition of specific EVs transfer could be of interest for development of new targeted immunotherapies.


Locally advanced and metastatic prostate cancer is incurable, despite decades of research. The activity of the androgen receptor (AR), a Paul lab previously identified G protein-coupled receptor 5 (GRK5) for its ability to regulate PCa progression, independent of AR activity. GRK5 partially partitions to the nucleus, wherein it has been shown to regulate the transcriptionome in non-PCa models. To globally elucidate the mechanistic impact of GRK5 on PCa progression, we are investigating the impact of GRK5 on the PCa transcriptome. We hypothesize that GRK5’s regulation of the PCa transcriptome promotes tumor progression. To assay the effect of GRK5 on the PCa transcriptome, RNA sequencing was performed in two cell lines: PC3 Control (PC3 shGFP) and PC3 GRK5 Knockdown (PC3 shGRK5). Ontologic analysis identified the epithelial-mesenchymal transition (EMT) as being affected when GRK5 is depleted. The epithelial–mesenchymal transition (EMT) is highly associated with promoting PCa progression and chemoresistance, thus we selected this pathway for further investigation. Confirmatory western blot analysis and quantitative PCR (qPCR) validated that depleting GRK5 suppresses the expression of the mesenchymal markers Vimentin and N-Cadherin. Similarly, overexpression of GRK5 in prostate cancer models reprogrammed the mesenchymal markers. Stable overexpression of GRK5 promotes cells to develop a spindle-like morphology, indicative of a mesenchymal state. GRK5 mediates this mesenchymal transition through increasing the expression of the EMT transcription factor, Twist1. Further analysis identifies that GRK5 activity promotes in vitro invasion. Cell lines overexpressing GRK5 demonstrate an increase in resistance to docetaxel, the mainstay chemotherapy for advanced PCa. Overexpression of mutated forms of GRK5 that are retained to the nucleus are able recapitulated all aforesaid changes, arguing that the nuclear activity of GRK5 mediates this effect. Collectively, this data presents a novel mechanism promoting PCa progression, independent of AR activity. Future work seeks to assay the ability of GRK5 to promote metastasis in vivo in mouse models.

TUMOR BIOLOGY: Imaging Cancer Immunotherapy, Tumor Microenvironment, and Other Aspects of Tumor Biology


Background: Radiation therapy (RT) can induce upregulation of programmed death ligand 1 (PD-L1) on tumor cells or myeloid cells, which might affect the response to RT with or without anti-PD-1/PD-L1 blockade. RT-induced PD-L1 expression during therapy could be a predictive marker for the therapy response, however, serial biopsies to monitor its distribution may be unreliable because its expression is heterogeneous. Therefore, non-invasive imaging of tumor PD-L1 expression could be more helpful. Materials and Methods: Two different murine tumor models (MEER and B16F10) were established in two locations, at the back of the neck and at the right flank of C57BL/6 mouse. Then fractionated RT (2Gy x 4 or 2Gy x 10) with or without anti-PD-1 therapy was delivered only to the neck tumor. PD-L1 expression was measured by PET/CT imaging and biodistribution with Zr-89-DFO-anti-mouse PD-L1 monoclonal Ab (clone 1H9G2) and the results were corroborated by flow cytometric analysis and immunohistochemistry. PET/CT imaging and biodistributions were performed 48 or 96 hours after tracer-injection. The change of PD-L1 expression on irradiated neck tumor was evaluated using non-irradiated flank tumor as a control. Endothelial cell morphology in tumor vessels was also analyzed by CD31 stain-
TUMOR BIOLOGY: Imaging Cancer Immunotherapy, Tumor Microenvironment, and Other Aspects of Tumor Biology

ing to determine whether RT-induced increased permeability of tumor vessels might result in increased non-specific tracer accumulations in the irradiated tumors. Results: PET/CT imaging and biodistribution study of ex-vivo tracer uptake values demonstrated significant increased tracer uptake in irradiated tumor compared to non-irradiated flank tumor in either case of MEER and B16F10. Tracer uptakes in the spleens were significantly decreased if high dose of non-labeled anti-mouse PD-L1 monoclonal Ab was given in advance before tracer-injection, which indicates the tracer is fully functional. Flow cytometry and immunohistochemistry showed PD-L1 upregulation in both irradiated tumors corroborating PET/CT imaging of RT-induced PD-L1 upregulation. Flow cytometry also revealed RT-induced PD-L1 upregulation on myeloid cells is more prominent than that of tumor cells, while positive staining in the irradiated tumors was not different from that of non-irradiated tumors, which shows RT did not induce alterations in tumor blood vessels. PD-L1 upregulation of MEER was not seen unless it was performed 2 Gy X10 RT, however, that of B16F10 could be seen at an earlier time-point of 2 Gy X4. Combination therapy of anti-PD-1 with RT did not show further PD-L1 upregulation compared to RT without anti-PD-1 therapy. Conclusion: RT-induced PD-L1 upregulation in different tumor models of MEER and B16F10 was identified by PET/CT using Zr-89 labeled PD-L1 monoclonal Ab and its validity was corroborated by flow cytometric analysis and immunohistochemistry.

#871 [18F]BMS-986192 as a novel PET imaging agent for assessment of PD-L1 expression in vivo. Ralph A. Smith,1 David Donnelly,1 Paul E. Morin,1 Dana Lipovsek,2 Jochem Gokejemeier,3 Daniel Cohen,4 Joonyoung Kim,5 Adrienne Pena,1 Xi-Tao Wang,1 Patrick Chow,1 Samuel J. Boscia,1 Xi-Tao Wang,1 Martin L. Boscia,1 Wendy Hayes1. 1Bristol-Myers Squibb, Waltham, MA; 2NIH, Bethesda, MD; 3Scripps, La Jolla, CA; 4Yale University, New Haven, CT; 5Scripps, San Diego, CA.

Objections Inhibition of the Programmed Death Ligand-1 (PD-L1)/PD-1 interaction allows for potent anti-tumor activity and antibodies that disrupt this interaction have been approved for the treatment of multiple cancer types. PD-L1 expression has been investigated clinically as a potential biomarker to predict response to anti-PD-1/PD-L1 therapy. BMS-986192, an Adnectin with high affinity and specificity for human PD-L1, was selected in vitro from a phage display library and characterized further in cell culture before labeling with SPIO for adoptive cell transfer into mice receiving scans. PET/MRI Data: Anatomical and qualitative SPIO data is collected using a balanced steady-state free precession (bSSFP) sequence. Iron quantification is done using R2* maps from a multi-echo single point imaging sequence (TurboSPI). Iron nanoparticle (SPIO) labeled tumor cells were observed after exposure to ionizing radiation or IFN-gamma, which has been reported to upregulate the expression of PD-L1. In vivo, we were able to observe radiation induced increase in bioluminescence signals from tumor established by use of the reporter cells. Our study therefore provided proof of principle for the use of our endogenous PD-L1 reporter imaging system to monitor the expression levels of PD-L1 in vitro and in vivo noninvasively and serially.

#872 Noninvasive monitoring of endogenous PD1 expression by using a dual reporter system. Min Zhou, Xinjian Liu, Fang Li, Chuan-Yuan Li. Duke University Medical Center, DURHAM, NC.

Programmed death-ligand 1 (PD1) is a key immune checkpoint protein facilitating tumor escape from host immune system. Clinical approval of anti-PD1 and anti-PDL1 antibodies for the treatment of a variety of malignancies underscores the promise of immune checkpoint therapy. Further progress in this promising area of research is likely to come from better understanding of the mechanisms for regulation of immune checkpoint proteins such as PD-L1. In the present study, we designed a dual reporter imaging system to monitor PD1 with PET/CT and SPIO with MRI. The expression of PD-L1 in both MEER and B16F10. CD31-positive staining in the irradiated tumors was not different from that of non-irradiated tumors, which shows RT did not induce alterations in tumor blood vessels. PD-L1 upregulation of MEER was not seen unless it was performed 2 Gy X10 RT, however, that of B16F10 could be seen at an earlier time-point of 2 Gy X4. Combination therapy of anti-PD-1 with RT did not show further PD-L1 upregulation compared to RT without anti-PD-1 therapy. Conclusion: RT-induced PD-L1 upregulation in different tumor models of MEER and B16F10 was identified by PET/CT using Zr-89 labeled PD-L1 monoclonal Ab and its validity was corroborated by flow cytometric analysis and immunohistochemistry.

#873 Evaluating immunotherapy effects using preclinical molecular imaging tools for quantitative immune cell tracking. Marie-Laurence Tremblay,1 Zombie O’Brien-Moran,2 Christa Davis,1 Kimberly Brewer1. 1WK Health Center, Halifax, Nova Scotia, Canada; 2Dalhousie University, Halifax, Nova Scotia, Canada.

Immunotherapies are a promising class of cancer therapeutics, but clinical translation is often impeded by a lack of understanding regarding optimal therapy administration, combination, and reliable biomarkers of success. Traditional metrics such as RECIST, and modern metrics like irRC and PERCIST used for monitoring cancer therapy outcome, have limitations for immunotherapy evaluation and are not always reflective of the underlying immune mechanisms. We aim to better characterize and monitor these immunotherapies, with focus on combination therapy optimization, by tracking immune cell migration in response to these therapies using preclinical magnetic resonance imaging (MRI). MRI is used to obtain anatomical tumor changes, detect and quantify superparamagnetic iron oxide (SPIO)-labeled cells in vivo. Goal: To link immune cell migration to early prognostic biomarkers for immunotherapy success. Methods: C57BL/6 mice (n = 40) received an implant of 5x10⁶ C3 cancer cells in the left flank. Mice (n = 10/group) were i) untreated or treated with ii) 200 μg of anti-PD1/day on days 7, 9, 11, 21 and 23, and iii) the peptide-based vaccine DepoVaxTM on day 15, or iv) with anti-PD1 and DTX. CD8+ cytotoxic T cells (CD8) and suppressive regulatory T cells (Tregs) were isolated from diseased-matched & treated donor mice for expansion in culture before labeling with SPIO for adoptive cell transfer into mice receiving scans. PET/MRI Data: Anatomical and qualitative SPIO data is collected using a balanced steady-state free precession (bSSFP) sequence. Iron quantification is done using R₂ maps from a multi-echo single point imaging sequence (TurboSPI). Tumor metabolism was assessed by ¹⁸F-fluorodeoxyglucose uptake during simultaneous acquisition of positron emission tomography (PET) with MRI. Imaging was done 21 and 28 days post-implant. Results: CD8 and Treg cells are consistently recruited to both the tumor and vaccine draining inguinal lymph nodes. CD8 T cells are primarily recruited to the tumor periphery and do not always penetrate the tumor core. Positive therapy outcomes are correlated with an increasing CD8/Treg ratio in the tumor, particularly at earlier time points (21 vs 28 days). In certain cases, CD8 T cells were found within the fat pad between the tumor and lymph node. As expected, DTX & anti-PD1 combination therapy resulted in the best prognosis. Simultaneous acquisition of PET/MRI demonstrated large areas of necrosis in tumor cores. Using TurboSPI, we have begun quantifying CD8 and Tregs cells, evaluating if volumetric tumor changes due to pseudo-progression correlate with Treg or CD8 T cell migration and comparing areas of necrosis in tumor cores. Using TurboSPI, we have begun quantifying CD8 and Tregs cells, evaluating if volumetric tumor changes due to pseudo-progression correlate with Treg or CD8 T cell migration and comparing areas of necrosis in tumor cores.

#874 Lifting the iron curtain: Imaging cellular barriers to combination chelation-immune checkpoint therapy. Avildor Leftin, Suresh Vearappan, Huiyong Zhao, Sadina Budhlu, Elisa de Stanchina, Jedd Wolchok, Taha Merghoub, Jason Koutcher. Memorial Sloan Kettering Cancer Center, New York, NY.

Iron accumulation in cancer cells and macrophages can result in tumor microenvironment that is unfavorable for checkpoint immune therapy. Chelation is a safe approach of reducing cellular iron accumulation that while...
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Ying Du, Yue Tian. Institute of Automation, Chinese Academy of Sciences, Beijing, China.

Purpose: An intraoperative technique to accurately identify microscopic tumor residuals could be applied to decrease the risk of positive surgical margins. Several lines of evidence support the expression and immunotherapeutic effect of PD-1 in breast cancer. Here, we sought to develop a fluorescent labeled PD-1 probe for in vivo breast tumor imaging and image-guided surgery. The efficacy of PD-1 monoclonal antibody (mAb) as adjuvant immunotherapy after surgery was also assessed.

Experimental Design: A near-infrared dye labeled PD-1-IRDye800CW probe was developed and examined for its application in tumor imaging and image-guided tumor resection in an immunocompetent 4T1 mammary mouse tumor model that recapitulates clinical breast cancer. Fluorescence molecular imaging (FMI) was performed to monitor probe biodistribution and intraoperative imaging. Bioluminescence imaging (BLI) was carried out concurrently to monitor tumor growth and evaluate post-surgical tumor residuals, recurrences, and metastases. Results: The PD-1-IRDye800CW probe exhibited a specific and stable signal at the tumor region compared to the IgG control, and exhibited the highest tumor-to-background ratio (TBR) 8 h post-injection (11.62 ± 0.79), approximately 5 fold higher than the IgG control (2.13 ± 0.30). Furthermore, PD-1-IRDye800CW guided surgery combined with PD-1 adjuvant immunotherapy inhibited tumor growth and microtumor metastases, as determined by BLI imaging, and thus improved survival rate with notable side effects. Conclusion: Our study demonstrates the feasibility of using PD-1-IRDye800CW for breast tumor imaging and image-guided tumor resection. Moreover, we found that PD-1 mAb adjuvant immunotherapy reduces cancer recurrences and metastases emanating from minimal tumor residuals.

#876 Imaging the interaction of leukemia and bone marrow microenvironment in murine model of ALL. Karine G. Harutyunyan,1 Saradhi Mallam-pati,1 Anna Zal,3 Mateusz Bytelewski,1 Michael C. Gurkin,2 Jason M. Butler,2 Tomasz Zal,1 Marina Konopleva,1 MD Anderson Cancer Ceter, Houston, TX; 2Weill Cornell Medical College, New York, NY.

Interactions of leukemia and the bone marrow (BM) microenvironment are known to play a key role in the survival and growth of leukemic cells, and we have shown that HIF-1α stabilization in BM stromal cells facilitates leukemia homing and progression (Chen et al. Blood 2012, 119:4971). Leukemic cells have been shown to hijack the homeostatic mechanisms of normal hematopoietic stem cells (HSCs) and take refuge within the BM niche. This mechanism is pivotal during chemotherapy and contributes to disease relapse. In this study, we investigated the time-dependent progression of BM in murine leukemia model involving both acute lymphocytic leukemia (ALL) cells and components of the BM niche, using multiphoton intravital microscopy (MP-IVM). We generated a transplantable, fluorescence leukemia model by retrovirally transducing C57Bl6-Ai4 murine BM cells that express red fluorescing tdTomato with the p190-Bcr/Abl oncogene (KG Harutyunyan et al, Blood 2014 124:2396). The resulting BM Abl tdTomato cells caused rapid development of ALL in non-irradiated C57Bl6 immunocompetent mice, manifested by infiltration of multiple organ and BM sites, followed by death within 14-18 days. We utilized C02.3-GFPemd transgenic mice as recipients of leukemia to highlight the osteoblastic niche, and visualized vasculature by injection of TRITC-dextran. We showed the dynamic of homing and engraftment of ALL leukemic B cells (MLC) in OB-GFP recipient mice, with homing in the vicinity of blood vessels visualized by MP-IVM, followed by proliferation and leukemia progression. This was accompanied by invasion of both vascular and osteoblastic components of BM microenvironment. Longitudinal assessment of hypoxia utilizing pimonidazole staining showed progressive development of BM hypoxia starting from Day 10 p.i., paralleling leukemia progression, despite the abundant vascularity of the BM. Moreover, we showed that tumors in cases of complete tumor growth inhibition, systemic iron-load was not consequently confirmed in MMTV-PyMT orthotopic breast cancer models. Even being observed in low-iron C57BL6 model backgrounds; a result subsequently confirmed in MMTV-PyMT orthotopic breast cancer models and quantitative voxelation (11.62 ± 0.79), approximately 5 fold higher than the IgG control (2.13 ± 0.30). Furthermore, PD-1-IRDye800CW guided surgery combined with PD-1 adjuvant immunotherapy inhibited tumor growth and microtumor metastases, as determined by BLI imaging, and thus improved survival rate with notable side effects. Conclusion: Our study demonstrates the feasibility of using PD-1-IRDye800CW for breast tumor imaging and image-guided tumor resection. Moreover, we found that PD-1 mAb adjuvant immunotherapy reduces cancer recurrences and metastases emanating from minimal tumor residuals.

#877 Real-time imaging of adherent and non-adherent cell interactions: utility of an automated microfluidic trap platform to recapitulate in vivo cell culture microenvironment. Rikka Kannan, Victor Yeh, James Helton, Amedeo Cappione. Millipore Sigma, Hayward, CA.

The study of dynamic cell processes and their interactions is of crucial importance to understand the complexities of tissue microenvironments. Simulation of in vivo cell culture microenvironments can greatly improve biologic relevance of cell-cell interaction studies, and so there is an ongoing demand for heuristic screening in tumor microenvironments, and temperature control for long-term cell culture. Each chamber within the plate can be addressed by programmable and on-demand perturbation of up to 10 reagents, enabling uninterrupted real-time live cell imaging assays. We present here the use of this microfluidic platform to replicate tumor microenvironment by maintaining adjacent co-cultures of cancer and immune cells. We have successfully imaged and cultured monolayers of tumor cell lines for 7 days followed by subsequent loading and trapping of immune (non-adherent) cells. In conclusion, the microfluidic platform enables unique co-cultures with environmental control and real-time imaging to facilitate the investigation of cell-cell interactions in a wide range of applications such as drug response and screening in tumor microenvironments, invasion, evasion pathways and other mechanisms governing cell-cell interactions.

#878 Imaging the interaction of leukemia and bone marrow microenvironment in murine model of ALL. Karine G. Harutyunyan,1 Saradhi Mallampati,1 Anna Zal,3 Mateusz Bytelewski,1 Michael C. Gurkin,2 Jason M. Butler,2 Tomasz Zal,1 Marina Konopleva,1 MD Anderson Cancer Ceter, Houston, TX; 2Weill Cornell Medical College, New York, NY.

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#879 Real-time imaging of adherent and non-adherent cell interactions: utility of an automated microfluidic trap platform to recapitulate in vivo cell culture microenvironment. Rikka Kannan, Victor Yeh, James Helton, Amedeo Cappione. Millipore Sigma, Hayward, CA.

The study of dynamic cell processes and their interactions is of crucial importance to understand the complexities of tissue microenvironments. Simulation of in vivo cell culture microenvironments can greatly improve biologic relevance of cell-cell interaction studies, and so there is an ongoing demand for heuristic screening in tumor microenvironments, and temperature control for long-term cell culture. Each chamber within the plate can be addressed by programmable and on-demand perturbation of up to 10 reagents, enabling uninterrupted real-time live cell imaging assays. We present here the use of this microfluidic platform to replicate tumor microenvironment by maintaining adjacent co-cultures of cancer and immune cells. We have successfully imaged and cultured monolayers of tumor cell lines for 7 days followed by subsequent loading and trapping of immune (non-adherent) cells. In conclusion, the microfluidic platform enables unique co-cultures with environmental control and real-time imaging to facilitate the investigation of cell-cell interactions in a wide range of applications such as drug response and screening in tumor microenvironments, invasion, evasion pathways and other mechanisms governing cell-cell interactions.
#878 A new SOX2/OCT4 stem cell biosensor reveals the mechanism of cancer stem cell dissemination in human breast cancer. 

Ved P. Sharma, 1 Sonia Voiculescu, 1 Yarong Wang, 1 George S. Karagiannis, 1 Binwu Tang, 2 Lalage Wakefield, 2 David Entenberg, 1 Sumanta Goswami, 1 Maja Oktay, 1 John Condeelis, 1 Albert Einstein College of Medicine, Bronx, NY; 2National Cancer Institute, Bethesda, MD.

There is increasing consensus that cancer stem cells (CSC) play an important role during metastatic progression of breast cancer. Previous studies have shown that stem cells display a pro-invasive phenotype in breast cancer. However, the phenotypes specific to breast cancer stem cells have not been evaluated at single cell resolution in vivo. Here, we employ high-resolution intravital two-photon microscopy in cells with orthotopic xenograft tumors and their metastases from human breast cancer cell lines expressing a previously characterized SOX2/OCT4 transcription-based fluorescent stem cell biosensor (SORE6). Using this high resolution imaging technology we found that SORE6+ stem cells: (a) constitute a minority population of the primary mammary tumors, (b) move approximately ten times slower than non-stem breast cancer cells (0.1 vs 1.1 μm/min, respectively), (c) are migratory toward blood vessels and, (d) compared to non-stem cells, they are enriched for invasive cellular protrusions called invadopodia. This is important because we have shown that these phenotypes are specifically associated with the disseminating population of tumor cells in the primary tumor site and, in addition, invadopodia are required for transendothelial migration during intravasation. Stem cells also have a three-fold higher incidence of cell death compared with macrophages compared to non-stem cells. In fact, stem cells were frequently seen to be part of the tripartite macrophage-tumor cell-endothelial cell complex called TME, which was previously shown to be the doorway for intravasation of tumor cells in primary mammary tumors and is validated as a prognostic of metastasis in human breast cancer patients. Furthermore, we followed breast cancer stem cell dissemination to the lung by using a novel lung window for high resolution imaging of the lung (WHRIL), which allows visualization of the same lung tissue in a single mouse, serially, over days to weeks. Using WHRIL, in combination with intravital multiphoton microscopy and the above biosensor, we visualized the arrival, extravasation and outgrowth of spontaneously arriving breast cancer tumors in the lung. We report here for the first time, the kinetics and stemness state of the spontaneously metastasizing tumor cells and, the record at single cell resolution, of their fate and metastasis outgrowth in the lung over time.

#879 Multi-scale time-lapse intravital imaging of soft tissues to map single cell behavior. 

Jessica M. Pastoriza, 1 Maria Soledad Sosa, 3 Kathryn Harper, 4 Julio Aguirre-Ghiso, 1 Maja H. Oktay, 1 David Entenberg, 1 Yarong Wang, 1 John S. Condeelis, 1 Aviv Bergman, 1 Mihaela Skobe, 1 Benedicte Lenoir, 1 Albert Einstein College of Medicine, Bronx, NY; 2Icahn School of Medicine at Mount Sinai, New York, NY.

After more than 10 years of research into the tumor micro-environment and the sources of tumor micro-heterogeneity, it is becoming increasingly clear that in addition to driver mutations, the tumor microenvironment determines tumor metastatic phenotype. This research has led to a new understanding of the impact of tumor micro-environments and their heterogeneity upon tumor cell proliferation, dissemination, dormancy, and survival. A full understanding of this heterogeneity, both temporally and spatially, how it supports tumor cell dissemination, dormancy and eventual further metastatic growth, and how it responds to therapeutic interventions, is crucial. Traditional single parameter studies have not been as productive as hoped in revealing the above relationships and have highlighted the need to understand tumors as integrated systems of genes, gene networks, and intracellular interactions, particularly with regard to the interplay between cells and their immediate microenvironment. To accomplish this, we have developed a new technology in the form of large volume intravital imaging using multiphoton intravital microscopy (MIVM) where images of large tumor volumes are stitched together to form a comprehensive record of the genes, gene networks, and intracellular interactions that occur throughout many tumor microenvironments at single cell resolution. Here, we report a protocol to obtain large numbers of high-resolution multiphoton images from living animals. The protocol is composed of surgical techniques for the stabilization of soft tissues including mammary gland, lymph node, liver, and lung and the acquisition of multiple high-magnification tiles that are stitched together to form a large-area low-magnification image. We have developed specific protocols for the surgery as well as tools for tissue stabilization and the acquisition and stitching of the images to generate very large MIVM data sets. We have interfaced these very large MIVM image data sets with support vector machine (SVM) classification, a nonlinear, multiparametric classification algorithm suitable for analysis of systems with arbitrary distributions and/or non-linear parameter’s correlations. To define the combinations of microenvironment parameters where tumor cell phenotypes of interest are likely to occur, we used these microenvironment parameters as an “input” for the SVM classifier to identify associated tumor cell phenotypes as the classifier’s “output”. Using this approach at widely varying temporal and spatial scales (from minutes to weeks and from sub-cellular to tissue wide) and at different stages (early carcinoma on to advanced metastatic disease), we have made inroads into the heterogeneity of tumor microenvironments and tumor cell phenotypes associated with progression. These results were noted in new insights into the mechanisms of metastasis.

#880 A computational and statistical approach for interpreting real-time in-vitro gene reporter data. 

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We have developed a new computational approach to track and analyze data from cells engineered with fluorescent cell cycle gene reporters. Gene reporters are a rapidly growing tool to gather information captured with time-lapse imaging, and can give insights to real-time metabolic conditions, cell cycle state, cancer stem cell state, and many other applications. Gene reporters may reflect cellular conditions that are cyclical rather than monotonic. Currently, the primary mechanism for interpretation of time lapse gene reporter data is reporting the mean fluorescence of imaging over time. This simplistic analysis discards information such as the cycle length and variation between gene reporters between cells, and can be biased due to the overall fluorescence shift from population growth. More nuanced data acquisition and analysis approaches have been reported to require large amounts of laborious hand-on data analysis. We have implemented a high-throughput computational pipeline for interpretation of cell cycle information using a combination of commercial and newly developed software to partition the activities of individual cells. The fluorescence information for each cell is smoothed and the cycle states for each cell are assigned adaptively. Our approach captures and quantifies the total time each individual cell spends in each presumptive state, and then analyzes state information from the cohort of observed cells using censored survival time data methods such as the Cox Proportional Hazards Model to account for death or movement out of the observed area. As a proof of concept for our pipeline, we engineered T3T fibroblast cells with a cell cycle reporter construct including mCherry-CDT1 and mVenus-p27K- reporters which can discriminate cells in G0 phase from G1 phase. We calculated the sample size required for statistical power of 80% to detect a hazard ratio (HR) of 0.8 between groups at α = 0.05 and 386 tracked cells per group. As our treatment model, we used the Protein Phosphatase 2A inhibitor Okadaic Acid (OA) an agent known to promote departure from G0. We captured a distinct total of 601 vehicle cells and 479 treatment cells in a 24 hour monitoring period with 20 minute capture intervals. We were able to discern significant differences between conditions and treatment G0 time, including a subtle statistically significant decrease in G0 time after treatment with OA (p = 0.0166, HR = 0.84). Here we have developed a computational pipeline with the capability for high-throughput image-stack data processing and nuanced quantification. Our approach assigns cycling cell states adaptively and the time until each cell departs its current state is tallied and interpreted statistically with survival analysis. The cell-level analysis affords a high degree of statistical power to discriminate group differences. Our pipeline is highly adaptable and has a multitude of applications in cancer biology research.

#881 CD44-specific supramolecular hydrogels for fluorescence molecular imaging of EMT induced BRAF <V600E> mutant thyroid cancer cells. 

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Previously, the authors have identified that the acquired drug resistance to BRAF inhibitor, PLX4032 in BRAF (V600E) mutant anaplastic thyroid cancer promotes not only tumor progression and proliferation, but also migration and invasion of cancer limited upregulated epithelial-to-mesenchymal transition (EMT). The underlying mechanism to the acquired resistance to BRAF inhibition involves c-Met mediated reactivation of PI3K/ AKT pathway. Therefore combinatorial dual targeted therapy of BRAF and c-Met inhibition has shown to reverse EMT and show maximal antitumor effect. Previously, the authors have developed a novel in vivo imaging strategy using CD44-targetable near-infrared (NIR)-sensitive supramolecular hydrogels (NIRSHs) for the recognition of CD44 expressing cancer cells. In the present study, we applied this NIR-sensitive molecular imaging probe in detecting the upregulated EMT changes in PLX4032-treated 8505C cells. The CD44-tar-
getable NIRSHs were fabricated by polyplexing Cy5.5-conjugated polyethyleneimine and hyaluronic acid in an aqueous medium. Ectopic xenograft mouse models were prepared by injecting 8505C cells at the flank of male athymic nude BALB/c mice, aged 6 weeks. After confirming tumor formation at 3 weeks post-injection, the mice were randomly divided into four groups and were each treated under different conditions: DMSO, PLX4032, PHA665752, PLX4032 and PHA665752. After 3 weeks, tumors were validated by MRI which showed correlations with the NIRSH fluorescence. The injected NIRSH probes showed highest uptake in the PLX4032 single treatment group and lowest uptake in the PLX4032 and PHA665752 combination group. Sizes of tumor were verified by MRI which showed correlations with the NIRSH fluorescence. The results suggest that CD44-targetable NIRSHs imaging shows potential as a non-invasive in vivo imaging tool in detecting the increased invasion potential of cancer cells and monitoring appropriate therapeutic effects.

**#882 Interpreting glioma MR imaging and somatic mutations in a cancer hallmark context.** John Graf,1 Mirabela Rusu,1 Yunxia Sui,1 Dattesh Shannbag,2 Uday Patil,3 Jeffrey Kiefer,3 Jill Barnholtz-Sloan,4 Michael Berens,3 Fiona Ginty,3 Sandeep Gupta,1 Chinmappa Kodira,1 Lee Newberg,1 Anup Sood1. GE Global Research, Niskayuna, NY; 2GE Global Research, Bangalore, India; 3Translational Genomics Research Institute, Phoenix, AZ; 4Case Comprehensive Cancer Center, Cleveland, OH.

Extracting biologically relevant data from radiology images can enable better monitoring of disease progression and therapy response. The field of radiogenomics is providing new approaches for such genomic/radiology correlations. However, there are several challenges in validation and clinical translation in that few DNA mutations are shared between tumors from different individuals and the differences in scale between imaging and genomic features can limit interpretation of underlying mechanisms. The goals of this work were to i) analyze correlations between low grade glioma (LGG) DNA somatic mutations, using a novel DNA impact scoring approach, and MRI derived imaging features; and ii) to interpret results in context of cancer hallmarks. Multi-parametric MRI and corresponding DNA data from 32 LGG patients were extracted from The Cancer Genome Atlas (TCGA) and The Cancer Imaging Archive (TCIA). The cohort included 18 males (56%), with mean age of 44 years (range: 21-74 years). An expert radiologist outlined the normal and tumor regions of interest using ITK-Snap tool. The normal region was used as a reference to normalize image intensities in the tumor region. Tumor mean intensity and mean variance were computed from Apparent Diffusion Coefficient (ADC), T1 enhancement, T2/T1 ratio (derived from T1 pre- and post-contract MRI), and Fluid-Ablated Inversion Recovery (FLAIR) images. A novel algorithm was used to compute DNA impact scores for each somatic mutation. The score represents the probability of a DNA variant being pathogenic vs. non-pathogenic. First, the scoring algorithm computes a score for nucleotide base insertions, deletions, or single base changes and then computes the consequence of such changes on amino acid coding, binding sites, splice sites and protein phosphorylation sites. An impact score was then computed based on the individual DNA impact scores of mutations within the gene. Finally, an average DNA impact score was computed at the Cancer Hallmark level using a gene-cancer hallmark map. At gene level, significant positive correlations were found between the ATRX (p<0.0002), TP53 (p<0.00001) mean intensity, T1 mean intensity and T1 T2/T1 ratio (p<0.0002). After combining the T2 and T1 impact scores, positive correlations were significant between the T1 T2/T1 ratio and TP53 expression and degradation, and DNA damage response, signal transduction by p53 class mediator, and DNA translocase activity were found to be enriched with genes that correlated with ADC and FLAIR. These pathways also contained genes that were enriched in the following cancer hallmarks: replicative immortality, evading growth suppression and genome instability. The ATRX gene is a member of all three hallmarks and TP53 a member of two. Since ADC is a measure of water diffusion and hence an indirect measure of cellularity, these findings demonstrate that mutations in replication and repair pathways are contributing to imaging features at the tumor level. Hanahan, D. and Weinberg, R.A. (2011). Hallmarks of cancer: the next generation. Cell 144(5):646-74.

**#883 Elucidating cancer hallmark context from glioma MR imaging and RNA expression data.** Yunxia Sui,1 Mirabela Rusu,1 Dattesh Shannbag,2 Uday Patil,3 Jeffrey Kiefer,3 Jill Barnholtz-Sloan,4 Michael Berens,3 Fiona Ginty,3 Graf John,1 Sandeep Gupta,1 Chinmappa Kodira,1 Lee Newberg,1 Anup Sood1. GE Global Research, Niskayuna, NY; 2GE Global Research, Bangalore, India; 3Translational Genomics Research Institute, Phoenix, AZ; 4Case Comprehensive Cancer Center, Cleveland, OH.

Radiogenomics or radiomics is an emerging field where tumor genomic data is correlated with radiology image features, thereby potentially providing more biological information about the tumor phenotype. A central challenge is the potential for model over-fitting due to analysis of many thousands of genomic data-points with hundreds of corresponding patient image features. Biological interpretation of the imaging feature correlations is also challenged by overlapping pathways and common gene effects. Our goals were: i) to explore correlations between gene expression and corresponding Magnetic Resonance (MR) Apparent Diffusion Coefficient (ADC) derived imaging features in low grade glioma (LGG); ii) to classify significant gene and imaging correlates by cancer hallmark. RNA expression data from 32 LGG patients were matched with corresponding MR image data from The Cancer Imaging Archive (TCIA). Among 32 patients, 18 were males (56%), and ages ranged from 21 to 74 years (mean age 44). Tumor and normal regions in the MR images were annotated by an expert radiologist using ITK-Snap. The normal reference region was used to normalize image intensities in corresponding tumor regions. Tumor texture features were extracted on voxel level in the tumor region including first and second order statistics, Run Length and co-occurrence matrix derived measures features. The voxel features were finally aggregated within the tumor region using statistical measures of mean, variance, median, kurtosis, and skewness. ADC imaging features (n=310) were correlated with each single gene expression value (11641 genes after MAD>0.4 filtering). Only image features and genes with pairwise correlations higher than 0.68 (0.68 is the 3-standard deviation above average correlation) and FDR (False Discovery Rate) <0.1 were used for follow-up analyses. Significant genes and MR image features were aggregated into 3 groups based on gene expression and correlated with cancer hallmarks. Seven Haralick image features (reflecting the average level of image intensity heterogeneity) were independently, significantly correlated with the Angiogenesis Hallmark (FDR<0.05). Three Haralick image features reflecting asymmetry features may be useful in limiting cancer progression. Studies have shown that melatonin can affect tumor metabolism and we hypothesize that this may be a mechanism by which the disruption of circadian melatonin production by light exposure at night (LAN) promotes breast cancer initiation and progression. The aim of this study was to evaluate the effect of melatonin on intratumoral heterogeneity measured by non-invasive MR imaging in a syngeneic mouse model of breast cancer (4T1). To visualize habitats, clusters were generated from combined MR images generated by different pulse sequences. These were co-registered with histology using tumor specific 3-D printed cradles to classify these tumor habitats. Methods: Mice were exposed to LAN (Control) or LAN plus melatonin. The melatonin metabolite 6-sulfotyramelatonin (6-SMT) was assessed in urine as a measure of melatonin production. Mice were imaged in a 7T MRI system with: 1) anatomical T2 images, 2) T2*, 3) diffusion-weighted MRI (DWI); and 4) T1 weighted dynamic contrast enhanced (DCE). DCE maps were obtained through semi-quantitative analysis of the pre- and post-contrast agent bolus time-series on a pixel-by-pixel basis. These were combined to the corresponding T2*, T1 and Apparent Diffusion Coefficient (ADC) values to generate parameter maps. Parameter maps were used to classify multiple clusters, based on a Gaussian mixture model. Following euthanasia, a 3D printed mold based on the T2 tumor isosurface was created in order to co-register the MRI and histology. Tumors were stained with H&E and for pimonidazole (hypoxia) by immunohistochemistry. Results: Melatonin treatment increased the 6-SMT in urine of mice exposed to LAN and statistically reduced tumor growth by day 15 (p<0.05). Distinct hypoxic habitats were observed with corresponding patient image features. Biological interpretation of these features was needed to reach statistical significance. Conclusion: These data indicate that...
TUMOR BIOLOGY: Imaging Cancer Immunotherapy, Tumor Microenvironment, and Other Aspects of Tumor Biology

#885 Live-imaging reveals the correction of skin deformities within two tumor-associated mutational models. Cristiana Pineda, Samara Brown, Valentina Greco. Yale University, New Haven, CT.

Healthy tissues are frequently subject to oncogenic mutations that fail to produce malignancy. Such mutations can lead to aberrant cellular behaviors such as increased proliferation or invasion and have been shown to be associated with the formation of aberrant tissue growths and cancer. However, it remains unclear how phenotypically normal tissue is capable of harboring mutations and raises the question as to how the switch from normal to malignant is initiated. One challenge to understanding the initiating events towards malignancy is the inability to follow the same cells over time in an intact mammal. Specifically, this roadblock hinders the ability to understand both the role of specific cells and how their location contributes to their growth, whether that growth be normal or cancerous. To overcome this, we have established a novel live imaging approach to perform in vivo lineage tracing of the same mutant cells over time within two distinct mutational systems in the skin epithelium. To begin, we turned to the pro-differentiation pathway Wnt/β-catenin, which when mutated is known to drive benign growths in the skin, and activated this mutant form in skin stem cells. We show that the resulting growths that deform the skin tissue architecture spontaneously regress, irrespective of their size. Endogenous behaviors such as differentiation and apoptosis as well as ectopic cell extrusions are employed to eliminate mutant cells from the tissue and dismantle the aberrant structures. Following regression, the remaining structures are either completely eliminated, or converted into functional skin appendages in a niche-dependent manner. While these results demonstrate the remarkable capacity to reverse aberrant phenotypes, the question remains whether this phenomenon was Wnt/β-catenin specific. To address this, we next turned to mutant Hras, which is well-established to cause a hyperproliferative, pro-growth advantage and frequently found mutated in malignant skin tumors such as cutaneous Squamous Cell Carcinoma. Activation of mutant Hras in the skin epithelium produced a range of phenotypes from hyperthickening to follicular structural deformities to macroscopic skin growths, all of which demonstrated the capacity to correct. Currently, we are utilizing a combination of extensive biochemical characterization together with targeted cellular ablations to investigate the mechanisms at play both within the mutant pool and the surrounding normal tissue that leads to the reversibility and integration observed within the Hras model. Altogether, this study reveals an unexpected plasticity of adult skin epithelium when faced with mutational insult and elucidates the dynamic cellular behaviors employed for its return to a homeostatic state.

#886 An integrative platform for three-dimensional quantitative analysis of spatially heterogeneous metastasis landscapes. Ian H. Guldner, Lin Yang, Fang Liu, Danny Z. Chen, Siyuan Zhang. University of Notre Dame, Notre Dame, IN.

Metastatic microenvironments are spatially and compositionally heterogeneous. This seemingly stochastic heterogeneity provides researchers many challenges in elucidating factors that determine metastatic outgrowth. Herein, we develop and implement an integrative platform that will enable researchers to obtain novel insights from intricate metastatic landscapes. Our two-segment platform begins with whole tissue clearing, staining, and imaging to globally delineate metastatic landscape heterogeneity with spatial and molecular resolution. The second segment of our platform applies our custom-developed SMART 3D (Spatial filtering-based background removal and Multi-channel forest classifiers-based 3D Reconstruction), a multi-faceted image analysis pipeline, permitting quantitative interrogation of functional implications of heterogeneous metastatic landscape constituents, from subcellular features to multicellular structures, within our large three-dimensional (3D) image datasets. Coupling whole tissue imaging of brain metastasis animal models with SMART 3D, we demonstrate the capability of our integrative platform to reveal and quantify volumetric and spatial aspects of brain metastasis landscapes, including diverse tumor morphology, heterogeneous proliferative indices, metastasis-associated angiogenesis, and vasculature spatial distribution. Collectively, our study demonstrates the utility of our novel integrative platform to reveal and quantify the global spatial and volumetric characteristics of the 3D metastatic landscape with unparalleled accuracy, opening new opportunities for unbiased investigation of novel biological phenomena in situ.

#887 Magnetic relaxometry detection of stealth, antibody-targeted micellar iron oxide nanoparticles in vivo. Rebeca Romero Aburto, Konstantin Sokolov, Adam M. Kulp, Erika C. Vreeland, Zhen Lu, Robert C. Bast, John D. Hazle, Kelsey B. Mathieu. MD Anderson Cancer Center, Houston, TX; Senior Scientific LLC, Albuquerque, NM.

Magnetic relaxometry (MRX) has the potential to provide unprecedented sensitivity in early detection of cancer by sensing changes in magnetic relaxation of iron oxide (Fe3O4) nanoparticles targeted to cancer biomarkers and is expected to exceed the detection limits of established clinical modalities. MRX uses superconducting quantum interference device (SQUID) sensors to measure Neel relaxation of bound particles. Our strategy was to develop molecular specificity into our nanoparticles by condensing phospholipids that are functionalized with a recognition sequence that allow for clinical translation of the MRX technology. To accomplish this, we used automated, controlled rate, direct infusion of an organic phase mixture of phospholipid nanoparticles and iron oxide nanoparticles into water to produce monodisperse micellar nanoparticles with a mean diameter of 75 ± 12 nm and surface charge of -10mV. The particles were determined to be stable in various biological media, including human plasma, for more than 24 hours with no detectable formation of a protein corona. Furthermore, in vivo studies in healthy mice showed blood circulation times of more than 2 hours, as well as minimal MRX signals during this time. Additionally, we developed maleimide conjugation chemistry for epidermal growth factor receptor (EGFR) antibody attachment to micellar nanoparticles. We have achieved molecular specific labeling of cancer cells over-expressing EGFR. In the future, we will evaluate the MRX signal impact from injecting EGFR-conjugated nanoparticles into tumor-bearing mice.

#888 Volumetric reconstruction of targeted nanoparticles for superparamagnetic relaxometry. Sara L. Thrower, Kelsey Mathieu, Wolfgang Stefan, Zhen Lu, Robert C. Bast, Javad Sovizi, David Fuentes, John D. Hazle. The University of Texas Graduate School of Biomedical Sciences at Houston; The University of Texas MD Anderson Cancer Center, Houston, TX; The University of Texas MD Anderson Cancer Center, Houston, TX.

Superparamagnetic relaxometry (SPMR) is an emerging technology that uses the unique magnetic properties of superparamagnetic iron oxide nanoparticles (SPIONs) to detect cancer cells. In order to estimate tumor locations from raw MRX data, we developed an L1 reconstruction algorithm under the assumption that early stage disease is sparsely distributed throughout the anatomy. The approach was previously validated in phantom datasets of known signal localizations. Advantages of our method are that the solver does not require the user to input prior information regarding the expected number of tumors or their approximate locations. Additionally, the solver reconstructs a volumetric distribution of detected sources within the field of view. To validate the algorithm for use in preclinical settings, SPMR was performed on SKOV3 ovarian tumor bearing mice (n = 3) with the MRX (MRX) has been tested over time following an intratumoral injection of anti-Her2 antibody-conjugated 25nm SPIONs (Senior Scientific LLC). The SPMR data was reconstructed with our sparse solver and was found to be highly correlated (r = 0.9978) with the results generated by the commercial software that accompanies the MRX instrument (MIA). Additionally, segmentation of the reconstruction revealed a strong signal (2.0·10⁻¹⁴pT/μT) in the area of the tumor residual in areas of early stage disease in 24 hours after injection. This result was consistent with our prior observations which have revealed that a large fraction of intratumorally-injected nanoparticles remain localized within the tumor for several hours after injection. Furthermore, these results were consistent with SPMR data collected by measuring tissue samples excised 24 hours after injection, of which the tumor had the highest signal. Thus, our sparse reconstruction algorithm was able to return the expected results without prior information regarding the location of nanoparticles. Future work will focus on quantifying the uncertainty in our reconstruction method, as well as characterizing its stability with increasingly complex nanoparticle distributions and detectability limits. In conclusion, this work represents an important advancement of the SPMR technology by allowing for volumetric reconstructions of bound nanoparticles from in vivo data.

#889 A comparison between quantitative ultrasound and histology in heterogeneous tumor structure. Jerome Griffon, Delphine Le Guillou-Buffello, Oumeima Laifa, Alexandre Dizeux, Michael Oelze, Lori Bridal, Michele Lamuraglia. Laboratoire d’Imagerie Biomedicale (UPAC, CNRS, INSERM), Paris, France; Biacoustics Research Laboratory, University of Illinois at Urbana-Champaign, Urbana-Champaign, IL.

Background: To investigate how QUS (Quantitative Ultrasound) maps reflect regional variations in heterogeneous tumor microstructure, we compared like-regions of QUS effective scatterer diameter (ESS, μm) and effective acoustic concentration (EAC, dB/cm) maps to whole-slice, virtual histology sections.

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stained for vascular, cellular and other microstructural features. Methods: An ectopic model of murine colorectal carcinoma (CT26) was implanted in 7 mice (BALB/c). US radiofrequency data were acquired for the longitudinal plane of tumors in vivo (SSi, Aplixor, SL15-4 probe, research mode) on days 6 and 14 after start of therapy with an anti-angiogenic (AA: n = 3) or placebo (P: n = 4). The AA used is Pazopanib, an oral tyrosine kinase inhibitor of VEGF receptors 1/2/3, platelet-derived growth factor receptors (PDGFR)-alpha/beta, and c-KIT. A daily dose of 2 mg of AA, diluted in 100μl of solution, was administered per os. Average backscattered power spectra were calculated (FFT, 512 pts, Rectangular gated RF), normalized with respect to a reference phantom and corrected for attenuation (0.4 dB/cm/MHz). ESD and EAC were mapped (2 x 2 mm blocks, with 75% axial and lateral overlap). Tumors were enucleated and marked to conserve orientation and approximate position relative to US imaging plane. Whole-slice, histological sections were prepared with fluorescent immunohistochemical markers at D6 and D14 for invasive assessment of cell nuclei, apop- tosis and vascular endothelium. The Number of Nuclei (NU), the % area of Apoptosis (AP) and % area of Vascular (VA) marker were calculated with image analysis processing for each 333μm by 333μm region of histological sections. QUS and histological maps of each tumor were manually segmented and coregistered using an affine registration method so that matched regions could be compared. Results: The absolute value of the non-parametric Spearman correlation coefficient (RS) was computed separately for each mouse to study the correlation between histological parameters and QUS parameters. Two mice of the AA group showed a moderate correlation (0.4 < RS < 0.6) between NU and EAC. Only one mouse in the P group showed strong correlation (0.6 < RS < 0.8) for [AP vs. ESD], [AP vs. EAC], [VA vs. ESD] and [VA vs. EAC]. The other tumors (1 from AA group and 3 from P group) showed poor correlation (RS<0.4). Additional data is available from 14 other mice (7 from AA group and 7 from P group) and will be analyzed to more fully investigate correlation between the QUS parameters and the histological parameters. Conclusions: This original technique to compare QUS parameter maps and whole slice histology analysis shows a promising evolution for this non-invasive imaging technique. Complete results on 21 mice will be presented in April.

TUMOR BIOLOGY: Migration and Invasion 1

### #890 The role of calpastatin isoforms in breast cancer progression.
Bhudosan Sukkarn, Sarah Storr, Ian O. Ellis, Kirsty Jewell, Tim Parr, Stewart G. Martin. The University of Nottingham, Nottingham, United Kingdom.

Calpastatin is the endogenous inhibitor of the ubiquitously expressed m-calpain and μ-calpain. Both of them are important regulators of various cellular pathways including proliferation, apoptosis, adhesion, and migration. Dysregulation of the calpain system is associated with a wide range of pathologies including tumour invasion and metastasis. In breast cancer, down-regulation of calpastatin mRNA, in particular an exon 3 containing variant, is associated with lymphatic vessel invasion which is the predominant form of lymphovascular invasion (LVI) (96%), a critical initial step of metastases. The current project seeks to determine the role that individual calpastatin isoforms play in regulating breast cancer cell migration and endothelial interactions to understand processes involved in regulating LVI. Seven breast cancer cell lines were assessed for calpain system protein expression (calpain-1, -2, -4 and calpastatin) and calpastatin (CAST) mRNA expression (total CAST, CASTexon3, CAST I, II and III) by Western blotting and qRT-PCR, respectively. T47D and MDA-MB-231 cell lines showed low calpastatin but high m-calpain expression. From haptotaxis cell migration data, T47D migrated more slowly than MDA-MB-231 (35.49±6.14 and 80.34±5.52 percent wound closure at 24 hours post-scratch respectively) (P value=0.0233). These cell lines, that represent different subtypes of breast cancer (luminal and basal respectively), were therefore selected to study the role of differential calpastatin type/isofrom expression. GFP-tagged XL and Leader domains of calpastatin type I, II and III, with or without exon 3 were overexpressed in each cell line. The different calpastatin types show differential subcellular localization. In MDA-MB-231, type I, III, IL3, IIIA3 were expressed generally in cytoplasm whereas type II and IIIA3 were located as punctate nuclear invaginations. To assess if overexpression of the different calpastatin types cause phenotypic changes, full length HA-tagged calpastatin type I, II and III were transfected into MDA-MB-231 and T47D and stable clones produced. Thus far stable type II overexpression in MDA-MB-231 and type III in T47D have been obtained with others under single cell selection. The localization of full length type II in MDA-MB-231 was similar to the localization of the truncated form that showing a strong single perinuclear signal. In T47D, the full length and truncated type III also show similarly localization that expressed throughout the cytoplasm. For the effect of calpastatin types on cell proliferation, calpastatin type II in MDA-MB-231, and type III in T47D, had no significant effect on cell doubling time when compared to respective controls (P value=0.6400 and 0.8874, respectively). According to preliminary studies, calpastatin type II seems to have a role on clonogenicity of MDA-MB-231. Such effect on T47D and other phenotypic changes involved in regulating LVI of transfected cell are being examined.

### #891 Annexin A2 (ANXA2) promotes migration and invasion of esophageal cancer cells via stabilizing c-Myc and promoting HIF-1α transcription.
Sai Ma, Yan-Yi Jiang, Li-Fei Wu, Jia-Jie Hao, Yu Zhang, Xin Xu, Yan Cai, Ming-Rong Wang. State Key Laboratory of Molecular Oncology Cancer Institute, National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China.

Purpose: We previously revealed that the expression of Annexin A2 (ANXA2) was increased in ESCC by two-dimensional electrophoresis and MALDI-TOF and LC-ESI-IT MS. This study aims to investigated the implication of Annexin A2 in ESCC. Annexin A2 (ANXA2) promotes migration and invasion of esophageal cancer cells via stabilizing c-Myc and promoting HIF-1α transcription. Experimental Design: Immunohistochemistry was performed to analyze the expression of ANXA2 in esophageal squamous cell carcinomas (ESCC). siRNA knockdown, transwell assay and wound-healing assays were used to investigate the function of ANXA2 in cells. Quantitative reverse-transcription polymerase chain reaction, Western blot analysis were used to seek the potential effector molecule of ANXA2. Chromatin immunoprecipitation, and reporter gene assays were used to confirm the relationship between c-Myc and HIF-1α. Co-immunoprecipitation assay was performed to demonstrate the relationship between ANXA2 and c-Myc. Immunofluorescence was used to reveal the location of ANXA2. Results: Knockdown of Annexin A2 significantly inhibited the migration and invasion of ESCC cells. At the molecular level, VEGF, MMP-2, MMP-9, p-MEK and p-ERK were down-regulated in ANXA2-siRNA cells. The HIF-1 alpha mRNA and protein levels were significantly lowered after knocked down the Annexin A2. HIF-1 alpha is the effector molecule of ANXA2 in ESCC cells, and that ANXA2 affects the expression of HIF-1 alpha at the transcription level because when knocking down HIF-1 alpha, the level of VEGF, MMP-2, MMP-9, p-MEK and p-ERK reduced as well. c-Myc is a transcription factor for HIF-1 alpha. ANXA2 has a direct protein interaction with c-Myc and knockdown of ANXA2 down-regulated the expression of c-Myc. The Tyr 24 phosphorylation form of Annexin A2 (Y24D-ANXA2) was located in the nucleus, and the non-phosphorylation form (Y24A-ANXA2), in the cytoplasm and cell membrane. Transfection of pcDNA3.1-ANXA2-Y24D enhanced the migration and invasion as compared with that of pcDNA3.1-ANXA2-Y24A in esophageal cancer cells. Anti-cancer treatment with the Annexin A2 phosphorylation inhibitor, Da- latinib, changed the location of Annexin A2 and lowered the expression of c-Myc and HIF-1 alpha. Conclusions: These results suggest that in esophageal cancer cells, phosphorylated Annexin A2 could enter into nucleus, which stabilizes c-myc and promotes the transcription of HIF-1 alpha, and the activates MAPK pathway, increases matrix metalloproteinase (MMP) activity and vascular endothelial growth factor (VEGF) expression, which results in the enhancement of cell invasion and migration.

### #892 ROCK dependent signalling pathways contribution to collective invasion of colorectal carcinoma.
Fatine Libané, Joel Raingeaud, Zoé ap Thomas, Fattha Sangar-Mavouna, Anne Chauchereau Chauchereau, Fanny Jaulin, Gustave Roussy, Villejuif, France.

Metastatic progression of cancer, which is responsible for 90% of patients death, results from tumor cells dissemination out of the primary tumor throughout the body. During the first step of this process—that consists in invasion of the peritumoral stroma—cancer cells can adopt 1) a single cell mode of invasion, in which cell have lost cell-cell junctions to move individually, 2) or a collective mode of invasion where cells maintain their cell-cell junctions to move as a cohort in which Leader cells at the front drag the follower cells at the rear. Although tumour histology data from cancer patients show that invasion occurs predominantly in a collective manner, this mode of cell invasion remains underinvestigated. My work aims at identifying the molecular and cellular mechanisms underlying colorectal carcinoma (CRC) collective invasion. I use 3D organotypic models of CRC: Caco-2 cell lines or organoids generated from CRC patients derived xenografts (PDIX) and assess invasion in collagen-1 based gels using microscopy approaches on fixed or live samples. Knowing its central role in the cytoskeleton dynamics which is the motor of cell motility, we hypothesize that RhoGTPases signaling pathways could control the collective mode of inva- sion. We therefore performed a siRNA based screen targeting the 98 effectors of...
the pathway and found ROCK to be an anti-invasive protein. Although it had been described as a proinvasive protein in the single cell mode of invasion, we confirmed using pharmacological inhibitors (Y27632 and H1152), that ROCK activity inhibition triggered collective invasion. Using a ROCK2 dominant negative mutant specifically targeting ROCK2 isoform but not ROCK1, I demonstrated that ROCK2 knockdown was sufficient to induce leader cell formation leading to collective invasion. In contrast, depletion of MyosinII–ROCK’s most common effector- was not sufficient to induce efficient protrusive leader cells. However I found RAC1 to be necessary and in a 2nd siRNA based screen targeting GEFs, we identified FAR2P, a GEF for Rac1, as the mediator of ROCK–RAC1 crosstalk in collective invasion. Even though the activation of FAR2P alone or RAC1 alone were not sufficient to induce leader cell formation, the concomitant inhibition of MyosinII recapitulated the collective invasion induced by ROCK inhibition. Altogether these results show a new anti-invasive role of ROCK2 kinase in collective invasion as it controls the formation of leader cells, through 1) the negative regulation of RAC1 and its GEF FAR2P, and 2) the positive regulation of MyosinII.


Accumulating evidence indicates that RNA splicing is involved in different steps of carcinogenesis. Although the critical role of a limited number of splicing factors has been demonstrated in several cancer studies, the function of many spliceosome components is still unknown. In breast cancer samples higher expression levels of a subset of spliceosome components consistently correlated with more aggressive tumor types in patient microarray as well as RNA sequencing data from The Cancer Genome Atlas (TCGA). We further systematically evaluated the effect of siRNA-mediated knockdown of 244 individual splicing factors on both cell migration and proliferation in two aggressive breast cancer cell lines MDA-MB-231 and Hs578T using high throughput microscopy. Primary candidates were validated with four single siRNAs and classified based on their effects on migration and proliferation. Knockdown of validated spliceosome factors such as SNRPG and SNRP1A affected the levels of small nuclear RNAs, the main catalyzers of the spliceosome. Moreover, high expression of these key candidate hits is associated with poor breast cancer metastasis free survival. To investigate the downstream mechanisms and splicing patterns controlled by our candidate genes we performed RNA sequencing on both control and siRNA mediated knockdown cells. Interestingly, knockdown of our hits affect extracellular matrix pathways that are essential in tumor cell migration and invasion. Altogether our data indicate a key role for spliceosome factors in the control of breast cancer progression which provide new leads for future therapeutic intervention.

**#894 Heparanase-induced shedding of syndecan-1 promotes cancer cell invasion: prevention by inhibitory sstnatin peptide.** Oisun Jung, Alan Ra prae ger. University of Wisconsin-Madison, Madison, WI.

Syndecan-1 (Sdc1, CD138) is highly expressed in multiple myeloma (MM). Heparanase (HPSE), an enzyme that cleaves heparan sulfate (HS) chains on Sdc1, induces shedding of the Sdc1 ectodomain; this is associated with poor prognosis in this disease. However, the link between Sdc1 shedding and poor prognosis remains unclear. We now show that HPSE-induced shed Sdc1 (Sdc1Δ) engages α4β1 integrin (VLA-4) and vascular endothelial cell growth factor receptor-2 (VEGFR2) to form a ternary receptor complex on the cell surface. This coupling of VEGFR2 to clusters of VLA-4 via Sdc1 leads to autoactivation of VEGFR2 and the subsequent activation of protein kinase A (PKA) bound to the VEGFR2 cytoplasmic domain. PKA activation proceeds via VEGFR2-mediated stimulation of the G-protein coupled cytokine receptor CXCR4 and the subsequent generation of cAMP by its activation of adenylyl cyclase 7. This mechanism is prevented by SSTNVEGFR2, a peptide that competes for the VEGFR2 docking site in the Sdc1 ectodomain, thereby disrupting VEGFR2 binding to Sdc1. VLA-4 has been shown to mediate the polarized invasion of a variety of cells due to its integrin phosphorylation on Ser988 by PKA, followed by Rac GTPase activation, a process that is restricted to the leading edge of the cell. But how this is confined at the leading edge is wholly unknown. We find that HPSE-mediated shedding of Sdc1 promotes an invasive phenotype by re-localizing VLA-4 and VEGFR2 to the leading edge of migrating myeloma cells as well as human T–acute lymphoblastic leukemia, melanoma, and endothelial cells where VEGFR2-dependent PKA activation causes α4 integrin phosphorylation. These results reveal a novel mechanism in which the expression of HPS, acting by causing Sdc1 shedding, potentially regulates angiogenesis and the extravasation and invasion of cancer cells and identifies SSTNVEGFR2 as a promising cancer therapeutic.

**#895 Fucosylation inhibits invadopodia formation and melanoma invasion.** Tyler S. Keeley, Eric Lau, Shengyu Yang. 1 Penn State University, Hershey, PA; 2 Moffitt Cancer Center, Tampa, FL.

Background: Melanoma is the most deadly skin cancer and metastasis is essentially responsible for all melanoma related death. Recently, fucose treatment has been shown to inhibit melanoma cell migration and invasion in vitro, and melanoma metastasis in a mouse model, suggesting that protein fucosylation could be exploited to prevent or suppress melanoma metastasis. Invadopodia are proteolytic actin-rich protrusions used by metastatic melanoma cells to degrade extracellular matrix and to promote invasion and metastasis. In this study, we investigated the mechanisms by which fucosylation regulate melanoma invasion and invadopodia formation. Method: The melanoma cells were treated with L-fucose-supplemented medium or infected with fucokinase-expressing lentivirus to increase protein fucosylation. shRNAs were also employed to knockdown fucokinase and to inhibit fucosylation in melanoma cells. The effects of fucosylation on invadopodia formation and extracellular matrix degradation were determined by phallolidin staining and degradation of fluorescently labeled gelatin thin film. Results: Here, we show that the addition of L-fucose to complete media inhibits the ability of melanoma cells to form invadopodia in a dose-dependent manner. The overexpression of hFUK in WM793 cells showed a similar result as L-fucose treatment, where invadopodia formation was significantly reduced. In addition, we observed a significant decrease in the percentage of cells that have invadopodia during L-fucose supplementation or hFUK overexpression. Using an invasion assay, the hFUK-overexpressing cell line exhibited delayed FBS-stimulated invadopodia formation. Conclusions: Our data suggest that fucose or the overexpression of hFUK reduces melanoma invasion by inhibiting invadopodia formation. Given that invadopodia formation is hindered within an hour after stimulation, we believe that invadopodia initiation is affected by increased fucosylation, resulting in fewer invadopodia and slower extracellular matrix degradation.

**#896 Long term exposure to Oroxylin A inhibits cell migration via suppressing CCL2 in oral squamous cell carcinoma cells.** Jui-Jia Tung, Tony Jer-Fu Lee, Wei-Ting Kao, Kuo-Chu Lai. 1 Tun Chi University, Hualien, Taiwan; 2 Buddhist Tzu Chi General Hospital, Hualien, Taiwan.

Oral squamous cell carcinoma (OSCC) is the sixth most common cancer in the world. Like other cancers, the major causes of OSCC-related death are cervical node and distant metastasis. Therefore, it is a critical need to identify new potential therapeutic agents against OSCC migration, invasion, and metastasis. Oroxylin A, a main bioactive flavonoids extracted from Scutellaria radix, has been reported to inhibit migration in breast and non-small-cell lung cancer. In this study, we explored the anti-migration effects of oroxylin A on OSCC cells and investigated its underlying mechanisms. A 24-hour exposure (short-term) of OSCC cells to oroxylin A at nontoxic concentrations (5–20 μM) significantly suppressed cell migration using the wound healing assay. Furthermore, a 30-day exposure (long-term) to oroxylin A (20 μM) still had no cytotoxic effect on OSCC cells, but significantly suppressed more cell migration than that of short-term oroxylin A exposed OSCC cells. To uncover the molecular mechanisms involved in the inhibitory effect of oroxylin A on OSCC migration, cDNA microarray technique and Ingenuity software were used. There were 468 differentially expressed genes, including 112 upregulated and 356 downregulated genes identified in long-term oroxylin A exposed cells compared to untreated OSCC cells. Among them, 77 genes have been reported to be associated with cancer cell migration. Consistent with results from cDNA microarray, we revealed that the expressions of several cell migration-related genes, such as CCL2, S100A9, ICN2, and THBS1 were significantly decreased in long-term oroxylin A exposed OSCC cells using QPCR assay. Western blotting and ELISA results also demonstrated that CCL2 expressions at mRNA and protein levels were significantly decreased in long-term oroxylin A exposed OSCC cells compared to untreated OSCC cells. In the meantime, the expressions of downstream targets of CCL2, including p-ERK, NFκB, MMP2, and MMP9 were also decreased in long-term oroxylin A exposed OSCC cells. These results suggest that long-term oroxylin A treatment inhibits migration may via suppressing CCL2 in OSCC cells.

**#897 PEA-15 regulates epithelial-mesenchymal transition and invasive behavior through its phosphorylation in triple-negative breast cancer.** Jibyun Park, Evan N. Cohen, Gaurav Chavan, Jangsook Lee, Naoto T. Ueno, Debu Tripathy. 1 James M. Reuben, 2 Chandra Bartholomew. 1 Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX; 2 Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, TX.
Triple-negative breast cancer (TNBC) is an aggressive subtype with no proven active targeted therapies available. Patients with TNBC have a very poor prognosis because the disease often metastasizes. Newer approaches to preventing metastasis and inhibiting tumor growth are crucial to improving prognosis for these patients. Previously it has been shown that PEA-15 binds to ERK, preventing ERK phosphorylation and inhibition of the TPT1B substrate. Our previous studies showed that overexpression of wild type PEA-15 inhibited ERK activity and reduced tumor volume in a TNBC xenograft model. We also showed that PEA-15 being unphosphorylated at both Ser104 and Ser116 inhibited ovarian cancer cell tumorigenicity. However, the function and impact of PEA-15 phosphorylation on TNBC is not well understood. From these observations, we hypothesized that unphosphorylated PEA-15 will prevent metastasis in TNBC through the inhibition of EMT. To study the effect of the phosphorylation status of PEA-15 on metastasis in TNBC, we established stable cell lines overexpressing nonphosphorylatable (PEA-15-AA) and phosphomimetic (PEA-15-DD) mutants in MDA-MB-468 cells. The clonogenic growth of PEA-15-AA was significantly reduced by 80% compared with vector control, PEA-15-V. Anchorage-independent growth of PEA-15-AA was inhibited by 60% compared with PEA-15-V (P<0.05). PEA15-AA upregulated the expression of E-cadherin, a key epithelial molecular marker and decreased expression of vimentin suggesting that PEA-15-AA reverses EMT. Moreover, compared with PEA-15-V, migration and invasion of PEA-15-AA were significantly reduced. To determine the mechanism of how PEA-15 regulates EMT and metastasis, we performed RNA-seq and metastasis arrays. We found that expression levels of IL-6, IL-8 and PDGF-BB were greatly decreased in PEA-15-AA. Upon stimulation of the PEA-15-AA with these cytokines, mesenchymal characteristics were partially rescued indicating that PEA-15-AA may inhibit the expression of these cytokines, thereby reversing EMT. Further, to determine the in vivo effect of PEA-15-AA, we injected stable transfectants of MDA-MB-468 cells into the mammary fat pad of NOD/SCID mice. The PEA-15-DD-injected group showed an increase (P<0.05) in tumor volume compared with PEA-15-V and PEA-15-AA groups. Additionally, immunohistochemical data revealed that vimentin and p-β-catenin (Ser675) expression was greatly decreased in PEA-15-AA tissues, suggesting that PEA-15-AA has antitumor effects. Further studies are warranted to evaluate the impact of PEA-15 phos- phorylation on metastasis in vivo using mice bearing highly metastatic hu- man breast tumor and determine the mechanism underlying decreased cyto- kinase expression in PEA-15-AA. Together, this study highlights the potential for overexpression of unphosphorylated PEA-15 as an approach for TNBC-targeted therapy.

**#898 Elucidation of the molecular mechanism of MenaINV expression, invasion and adhesion of tumor cell extravasation during breast cancer dissemination by TME.** Maxwell D. Weidmann,1 Chimmy R. Surve,1 Jeanine Pignatelli,2 Javier J. Bravo-Cordero,2 George S. Karagiannis,1 Maja H. Oktay,1 Proceedings of the American Association for Cancer Research

**CD99 plays an important role in glioblastoma cell migration.** Luis C. Cardoso,1 Antonio M. Lerario,2 Suely K. Maro,1 Roseli S. Soares,1 Sueli M. Oba-Shinjo,1 1School of Medicine of University of São Paulo, São Paulo, Brazil; 2University of Michigan, Ann Arbor, MI. Glioblastoma (GBM) is the most common and aggressive malignant brain tumor in adults and the standard treatment consists of surgical resection of the tumor followed by radiation and chemotherapy with temozolomide. A combination of standard therapy with other biologically-based therapies is necessary to improve the survival of patients with GBM, which currently stands only to 15-17 months. In this sense many studies have been developed in pursuit of expressed membrane proteins in GBM, which are potential targets for immuno-therapy. Our laboratory demonstrated increased CD99 mRNA and protein expression in GBM samples. CD99 has a key role in several biological processes, including cell adhesion, migration, apoptosis, differentiation of T cells and thy- mocites, diapedesis of lymphocytes to inflamed vascular endothelium, mainte- nance of cellular morphology and regulation of intracellular membrane protein trafficking. In this study, the transcription of two GBM cell lines (U87MG and A172) transfected with siRNA for CD99 was performed to identify the non- target control (NTC). A total of 728 genes (hypoexpressed with fold change ≥1.5 relative to NTC or hyperexpressed with fold change ≤-1.5) were observed in both cell lines. An enrichment analysis by MetaCoreTM re- vealed the following processes as the most significant: (1) Cytoskeleton_Regu- lation of cytoskeleton rearrangement, (2) Cell adhesion_Cell-matrix interac- tions (3) Development_Blood vessel morphogenesis. Further, both cell lines were silenced for CD99 expression by shRNA (two clones) to perform functional assays, as wound healing (migration assay), adhesion, invasion and apoptosis. The CD99 knockdown reduced migration in both cell lines, with the highest decrease of the migration observed in the highest CD99 knockdown. Adhesion assay was performed using fibronectin as an extracellular matrix substrate. U87MG cells showed greater adhesion to fibronectin than the control (scram- bled shRNA). On the other hand, A172 cells knocked down for CD99 presented lower adherence to fibronectin than the control. Apoptosis analysis with shCD99 U87MG cells showed a tendency of cells entering into late apoptosis when treated with temozolamide for 48 hours, but the same was not observed in shCD99 A172 cells. Additionally, CD99 (RPE) and phospholin (Alexa Fluor 488) colocalized at lamellipodia, suggesting that CD99 is related to cytoskel- eton rearrangement in both GBM cell lines. The different phenotypic behav- ior of migration and apoptosis observed in these two GBM cell lines with different somatic mutation background may be due to distinct signaling activation. Migration/invasion is the major characteristics of GBM which limits the surgical tumor resection, and consequently leads to tumor recur- rence. Therefore, further analysis of functional and contextual context of cell migration is worthwhile to unveil new therapeutic strategies to halt GBM progression.

**#900 Tumor Treating Fields (TTFields) affect human glioma cell migra- tion, invasion and adherence properties in vitro.** Dario Garcia-Carracedo,1 Rosa S. Schneideman,2 Einav Zeevi,1 Anna Sheingauza,2 Moshe Giladi,3 Tali Voloshin,3 Yaara Porat,2 Miial Munster,2 Roni Blat,1 Elon D. Kirson,1 Uri Weinberg,1 Yoram Pal'ti2,2 Novocure, New York, NY; 3Novocure, Haifa, Israel. TTFields, an approved treatment modality for glioblastoma, are delivered via continuous, noninvasive application of low intensity, intermediate frequency alternating electric fields. The antiangiogenic effects of TTFields have been exten- sively investigated. We investigated other processes that may be affected by TTFields such as human glioma cell migration and cellular invasion properties. Four human glioma cell lines were treated with TTFields using the in vitro system. Cell migration rates were measured using wound healing assays. Invasa- sion assays were performed using a modified Matrigel coated Boyden chamber. Cell adhesion assays were performed during TTFields treatment and compared to untreated controls. At the end of TTFields’ treatment, adhered cells were trypsinized and counted. A cells de-adhesion assay was performed following 24 and 72 hours of TTFields application with the outcome being the number of cells removed after varying times of trypsinization. Application of TTFields in-vitro led to a significant reduction in cell migration velocity compared with untreated

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control cells. Furthermore, comparison of the anti-migratory efficacy of TT-Fields applied from 2 directions vs. a single direction revealed that the single direction approach applied perpendicularly to the course of migration was more effective than TTFields applied in 2 directions or in a single parallel direction. Glioma cells invasion was significantly reduced compared to untreated cells in all tested conditions. Cell adhesion to the substrate (fibronectin) was significantly reduced when exposed to TTFields. On the other hand, cell de-adherence following TTFields treatment took significantly longer time of trypsinization. Our results suggest that human glioma cell motility is impaired by exposure to TT-Fields. Both alterations in cells adherence and de-adherence during exposure to TTFields may contribute to reduction in cell motility. Further studies are needed to elucidate the mechanism by which TTFields disrupts cellular motility in glioma cancer cells.

The mechanobiome of pancreatic cancer: a viable, targetable drug space. Alexandra Surcel, Eric S. Schiffhauer, Dustin Thomas, Qingfeng Zhu, Robert Anders, Douglas Robinson. Johns Hopkins Univ. School of Medicine, Baltimore, MD.

Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease with an annual mortality rate of 37,000 in the US alone. Its 5-year survival rate of 6% remains relatively unchanged over the past 4 decades. Because PDAC has elevated rates of de novo and acquired resistance to traditional chemotherapies, we are exploring new drug spaces that target metastatic hepatic disease, the primary mortality factor for patients. Specifically, we have identified the cluster of proteins that sense and respond to mechanical stimuli—collectively known as the mechanobiome. This cytoskeletal machinery is responsible in large part for endowing metastatic cells with the ability to navigate through different tissue types. Based on their mechanoresponsiveness profile, we have predicted and identified via western blot and immunohistochemistry proteins in the mechanobiome that are upregulated in patient-derived pancreatic tissues. These proteins include non-muscle myosin IIa and IIc, alpha-actin-4, and filamin B. We are performing knockdown and overexpression studies of these isoforms on 2D and 3D behaviors—cell mechanics, migration, invasion, and tissue sphere formation and are poised to begin mouse studies on the metastatic significance in altering the expression of these proteins. In addition, we are testing 4-HAP, a small molecule mechanics modulator that we previously identified that targets myosin IIc, thus affecting PDAC mechanics in PDAC murine models. Limited studies reveal that 4-HAP inhibits metastatic potential of DCLK1 expressing cancer cells (Ito et al., 2016). The goal of our studies was to evaluate molecular/genetic pathways mediating invasive effects of DCLK1 in cancer cells. Isogenic clones of HCT116 cells, wild-type or down-regulated for COL3A1 and SPARC in mediating metastatic effects of DCLK1-S/NFATc2, Malaney O’Connell, Shubhashis Sarkar, Heidi Spratt, Steven Widen, Thomas G. Wood, Pomila Singh. UTMB, Galveston, TX.

DCLK1 expression is critically required for colon carcinogenesis in mice, and for maintaining tumorigenic potential of human colon cancer cells (hCCCs) (Singh et al., 2016). Down-regulation of DCLK1, combined with chemoprevention, eliminates CSCs, and avoids colon cancer relapse (Kantara et al., 2014). We recently discovered that long (L) and short (S) isoforms of DCLK1 (DCLK1-L/DCLK1-S) are transcribed by two separate promoters (S’ or IntonV(β)) in the DCLK1-gene (O’Connell et al., 2015). During adenoma-carcinoma sequence of colon-tumorigenesis, L-isoform becomes silenced by DNA-methylation while S-isoform gets upregulated by many fold (O’Connell et al., 2015). S-isoform specifically imparts invasive potential to cancer cells, unlike L-isoform (Singh et al., 2016); others have similarly reported metastatic potential of DCLK1 expressing cancer cells (Ito et al., 2016). Thus the goal of our studies was to evaluate molecular/genetic pathways mediating invasive effects of DCLK1-S in cancer cells. Isogenic clones of HCT116 cells, wild-type or down-regulated for COL3A1-S (HCT-C/HCT-D), were subjected to next generation sequencing and pathways analysis. SPARC and COL3A1 emerged as two candidate genes/proteins, which were decreased/increased by several fold in response to loss over-expression of DCLK1-S, respectively. We present data confirming a critical role of COL3A1 and SPARC in mediating metastatic effects of DCLK1-S expression in hCCCs. We additionally discovered that DCLK1-S functions as a specific kinase for the transcriptional factor, NFATc2, and phosphorylates T238SP56 motif of NFATc2, resulting in activation of NFATc2 and increased expression of COL3A1. Conclusions. Our novel findings, suggest for the first time, that DCLK1-S expression by colonic tumors in humans, mediates invasive potential of colon cancer cells by phosphorylation/activation of NFATc2, resulting in up-regulation of COL3A1/SPARC; the latter proteins re-model extracellular matrix, assisting unhindered invasion of colon cancer cells.

CXCR4 and CXCR7 play distinct and overlapping roles in prostate cancer dissemination to bone. Sounak Roy,1 Kenneth C. Valkenburg,2 Kenneth J. Pienta2. 1Johns Hopkins University, Baltimore, MD; 2Johns Hopkins School of Medicine, Baltimore, MD.

Approximately 28,000 men die of prostate cancer (PCa) each year in the US, and 90-100% of them will be due to bone metastasis. It has been demonstrated that PCa cells detach from the primary tumor and “home” to bone. Osteoblasts (bone-forming cells) secrete the chemoattractant stromal-derived factor 1 (SDF-1, also known as CXCL12), and PCa cells express the receptors for SDF-1, C-X-C chemokine receptors 4 and 7 (CXCR4 and CXCR7). PCa cells use these receptors to enter the chemoprotective bone microenvironment, specifically, the hematopoietic stem cell (HSC) niche in the endosteum. While small molecule inhibition of CXCR4 can mobilize PCa cells from the bone marrow microenviroment, it is unknown whether CXCR7 plays a compensatory role when CXCR4 is antagonized. SDF-1 is known to bind with greater affinity to CXCR7 compared to CXCR4 indicating a possible role of CXCR7 in tumor migration to the endosteal niche. Therefore, it is crucial to determine the individual and combined roles of CXCR4 and CXCR7 in the formation and treatment of PCa bone metastases. To answer these questions, we first assessed CXCR4 and CXCR7 expression across a panels of prostate cancer cell lines. We then transiently overexpressed and knocked down CXCR4 and CXCR7 in PCa cells and found CXCR4 and CXCR7 expression in vitro to directly correlate to cell viability and migration in PCa PCa cells. To further test dissemination and metastasis in vivo, we are making stable knockdown and overexpression lines. We will perform in vivo metastasis experiments. We have developed a novel immunofluorescence protocol for detecting and quantifying tumor cells in murine bone and bone marrow and will use this technique to determine the ability of PCa cells to disseminate to the bone in the presence of CXCR4 and/or CXCR7. This will be quickly translatable because we are currently running a first-in-prostate cancer clinical trial to determine whether small molecule inhibition of CXCR4 to mobilize PCa tumor cells from the bone marrow, combined with docetaxel, will benefit metastatic PCa patients. If we determine that CXCR7 plays a compensatory role in dissemination and/or metastasis, we will need to add CXCR7 inhibition to our treatment strategy to obtain the most efficient benefit for patients.

Podocyte-specific SMPDL3b modulates radiation-induced renal dys.

function. Anis Ahmad,1 Alla Mitrofanova,2 Saba Ansari,1 Thirupandiyur Udayakumar,1 Jack Bielawski,2 Alan Pollack,1 Alexis Fornoni,1 Brian Marples,1 Youssel Zeidan1. 1UM, Miams, FL; 2MUSC, Charleston, SC.

Background: The underlying mechanisms responsible for the renal failure and proteinuria in radiation nephropathy remain largely unknown. Radiotherapy with or without chemotherapy may result in radiation-induced kidney injury in pelvic malignancies such as gynecologic cancers, lymphomas, gastrointestinal cancers, sarcomas of the upper abdomen and during total body irradiation. The current study investigates the role of sphingolipids in radiation-induced podocytopathy using a murine model. The molecular and functional effects of kidney irradiation were evaluated after single-dose exposures. Material/Methods: In cell culture, SMPDL3b expression post radiation (8Gy) was determined by real-time PCR (RT-PCR) and Western blotting. Morphological changes and DNA damage were detected post radiation using immunofluorescence microscopy. Wild-type C57BL/6 male and female mice (age 10-14 weeks) were irradiated with a single dose of X-ray (14 Gy) using an image guided small animal arc radiation treatment system (iSMARTArt). Rituximab/lGd was administered (50 mg/kg, intraperitoneal injection) 30 min before the single dose of irradiation. Functional kidney parameters, kidney histology, and gene expression were analyzed at 20, 30 and 40 weeks after irradiation. Results: Following irradiation, SMPDL3b expression at protein level was significantly reduced in vitro and in vivo. However, no significant changes were observed at the transcriptional level. Podocyte number also decreased significantly post radiation in vivo. iSMARTArt dynamic contrast enhanced (DCE) imaging data showed reduced glomerular filtration rate post irradiation. In agreement with the functional data, hematoxylin and eosin staining of kidney sections showed a multifocal increase in the number of pericytes, tubular atrophy, and glomerular damage. Periodic Acid-Schiff (PAS) staining showed an increased in glomerular mesangial matrix accumulation post irradiation. Sirius red staining showed diffuse intertubular fibrosis, especially in the renal cortex post radiation. These histological changes were paralleled by the change in serum Creatinine, urine albumin. Rituximab pretreatment to mice, improved kidney functional parameter in comparison with radiation-only mice. Rituximab pretreatment to mice, suppress the development of fibrosis and tubular damage post irradiation. Conclusion: This study shows that rituximab pretreatment protects mice against radiation-induced nephrotoxicity, which may have therapeutic implications for radiation-induced injuries in cancer patients.
Lysyl oxidase is required for chemotaxis. Haoran Tang, Nathalie Dhomen, Richard Marais. CRUK Manchester Institute, Manchester, United Kingdom.

Purpose: Lysyl oxidase (LOX) increases extracellular matrix stiffness by cross-linking collagen. A stiffer matrix drives integrin activation, and therefore LOX is believed to promote cancer cell invasion through enhanced integrin signaling. However increased matrix stiffness does not necessarily lead to a more permissive environment for invasion. Fibrillar collagen matrix orientation, density and porosity also determine how well cells can invade. Assays based on collagen gel-covered transwell systems have previously been used to show that LOX is required for invasion. These systems are driven by a chemoattractant gradient, and thus LOX for chemotaxis could not be excluded. In this study we aimed to clarify whether LOX was required for matrix invasion or chemotaxis.

Experimental procedures: To set up a 3D invasion assay that was not driven by a chemoattractant gradient, spheroids from multiple LOX expressing cancer cell lines were embedded into a thick 3D collagen gel. Cells were then allowed to randomly invade into the surrounding collagen. Alternatively, cancer cells were allowed to invade a fibrinostabilized 3D collagen gel in an organotypic assay. In this assay the invasion was driven by a strong chemoattractant gradient through a dense collagen gel, with cancer cells cultured atop the collagen gel at the air-medium interface. A Dunn chemotaxis chamber or a transwell culture insert was used to investigate chemotaxis towards serum or EGF. Small interfering RNA (siRNA) or short hairpin RNA (shRNA) specific to human LOX was used to deplete LOX expression in the cancer cell lines, to assess LOX functions in the above assays. Results: LOX depletion in parental MDA-MB-231 cells, MDA-MB-231 brain or bone metastatic sub-population cells, U87 and U118 cells did not impact 3D collagen gel invasion. LOX inhibition by BAPN also did not impact collagen gel invasion in MDA-MB-231, LN229, U87, U118 and U118 cells. The data indicated that LOX was not required for invasion through a thick 3D collagen gel in vitro. However, in the chemotactic-driven organotypic assay, loss of LOX in MDA-MB-231 cells completely abolished invasion. When the chemotactic potential towards serum and EGF of LOX depleted MDA-MB-231 and U87 cells was tested using Dunn chemotaxis chambers, we observed strong chemotactic defects in both cell lines. The chemotactic defects of these cells were also observed when a transwell based chemotaxis assay was used. Random cell migration of MDA-MB-231 and U87 cells were otherwise not affected by LOX depletion. Conclusions: LOX is not required for collagen matrix invasion per se. Instead LOX is important for cells to sense chemoattractants and to maintain directional cell migration. The molecular mechanisms underlying these observations are now the subject of further investigation.

PATZ1 promotes migration and invasion of thyroid cancer cells through upregulation of the activity of plasminogen activator-inhibitor type 1 matrix metalloproteinases. Asumi Isato,1 Teruo Nakamura,2 Tuyoshi Uehara,1 Hi-ruto Izumi,1 Ken-ichi Ito1. Shinshu University School of Medicine, Matsumoto-city, Nagano-Prefecture, Japan; 2University of Occupational and Environmental Health, Kitakyusyu-city, Japan.

Background: PATZ1, a transcription regulator, has been reported to function as oncogene or tumor suppressor in several human malignant neoplasms. The aim of the study was to examine involvement of PATZ1 in carcinogenesis and progression of thyroid cancer. Materials and methods: PATZ1 expression in 165 clinical thyroid specimens obtained from 87 patients were evaluated by IHC. We examined the function of PATZ1 using an immortalized normal thyroid follicular epithelial cell line (Nthy-or1-31), and four thyroid cancer cell lines; TPC-1 (papillary cancer), FTC-133 (follicular cancer), FRO and ACT-1 (anaplastic cancer). Knockdown of PATZ1 in Nthy-or1-31 and differentiated thyroid cancer cell lines (TPC-1, FTC-133) induced morphological change of the cells and significant increase of cell proliferation and migration as well as moderate increase of invasion. Furthermore, the expression of u-PA, MMP2 and MMP11 was increased by inhibition of PATZ1 in TPC-1 and FTC-133. On the other hand, overexpression of PATZ1 in anaplastic cancer cell lines (FRO and ACT-1) decreased proliferation and migration of the cells along with the decreased expression of u-PA, MMP2, MMP9 and MMP11. Conclusion: Our study suggests that PATZ1 might play an important role as a tumor suppressor in thyroid follicular epithelial cells and might be involved in progression of thyroid cancer.

Expression of nuclear PATZ1 in thyroid tumor type Normal thyroid/ goiter (N=68) Follicular adenoma (N=5) Papillary adenoma (N=39) Follicular differentiated carcinoma (N=84) Anaplastic Medullary thyroid cancer (N=28)

negative (%) 0 (0%) 3 (60%) 3 (76.9%) 1 (20.0%) 3 (10.7%) 1 (20.0%) positive (%) 68 (100%) 4 (80.0%) 5 (83.3%) 2 (6.7%) 7 (58.3%) 3 (10.7%) 4 (80.0%)
Tracking of migration using digital holography to monitor drug-induced phenotypic shifts in breast cancer cells. Sofia Kamlund, Stina Oredsson. Lund University, Lund, Sweden

This study aims to investigate the migration behaviour of individual JIMT-1 breast cancer cells during the cell cycle. Mechanisms behind cell migration are important at the tumor level to understand the context by which cancer release occurs. Pull-down with ADAM9 and interactive proteins in malignant phenotypic progression. The aim is to clarify the association between ADAM9 expression and cancer progression. The results indicate possible to use ADAM9 as a biomarker for metastatic breast cancer.

Phosphorilidocisocoxaeoxoate (PZ-DHA) inhibits breast cancer cell invasion and angiogenesis. Wasundara Fernando, Emma MacLean, Krysta Coyle, Paola Marcato, David W. Hoskin, H. P. Vasantha Rupasinghe, Dalhousie University, Halifax, Nova Scotia, Canada; Dalhousie University, Truro, Nova Scotia, Canada.

In our in vitro and in vivo studies, we showed the selective cytotoxicity, in vitro anti-proliferative and anti-metastatic activities of phosphorilidocisocoxaeoxoate (PZ-DHA). PZ-DHA combines phosphorilid (PZ), a dihydrochalcone found in apple peels, and phosphorilidocisoxaeoxoic acid (DHA), an ω-3 fatty acid found in fish oil, through an acylation reaction. Significant suppression of tumor growth resulted from the in vitro administration of PZ-DHA to non-obese diabetic severe combined immunodeficient (NOD-SCID) female mice xenografted with MDA-MB-231 breast cancer cells. Examination of hematoxylin and eosin-stained tumor sections confirmed that PZ-DHA caused tumor cell death. PZ-DHA also suppressed the invasion of MDA-MB-231 cells through membranes coated with extra cellular matrix proteins, fibronectin and gelatin, in a Boyden chamber assay. The effect of PZ-DHA on the mRNA transcription of the gelatinase, extracellular matrix proteins, fibronectin and gelatin, in a Boyden chamber assay. The effect of PZ-DHA on the mRNA transcription of the gelatinase, extracellular matrix proteins, fibronectin and gelatin, in a Boyden chamber assay. The effect of PZ-DHA on the mRNA transcription of the gelatinase, extracellular matrix proteins, fibronectin and gelatin, in a Boyden chamber assay.


INTRODUCTION In cancers with β-catenin and Ras signaling pathways by APC (90%) and K-Ras (40-50%) mutations are closely interacted and accelerate CRC tumorigenesis. Protein level of β-catenin as well as Ras is increased by APC loss via inhibiting GSK3B-mediated poly-ubiquitination dependent Ras degradation through recruitment of β-TrCP E3 linker. Moreover, additional K-Ras mutation results in the liver metastasis by secondary activation of the Wnt/β-catenin signaling pathways via Ras-ERK and -AKT pathways in addition to the initial activation by APC loss. Consequently, both β-catenin and Ras were highly increased in human CRC patient tissues, especially in metastatic tumor. EXPERIMENT PROCEDURES To investigate the synergistic effects of aberrant Wnt/β-catenin and Ras signaling pathways on tumor development to metastasis, we established the APCMin/−/K-RasG12DLA2 compound mouse that harbors APCMin and K-Ras mutations in and normal tissues. Tumor-specific, tumor-specific Ras protein level using human CRC patient tissues of normal mucosa and adenocarcinoma (N=24) and metastatic adenocarcinoma (N=26) and tumor budding (N=10). To recapitulate the metastatic tumor environment which have enriched receptor tyrosine kinases (RTKs), we maintained and experimented with EGF (20ng/ml) in every in vitro studies. RESULTS We observed that β-catenin and Ras were increased in adenocarcinoma and metastatic adenocarcinoma of human CRC compared with normal mucosa. Interestingly, both β-catenin and Ras were most significantly increased in tumor budding regions which histopathologically represent epithelial mesenchymal transition (EMT). In murine model, the EMT phenomenon was effectively induced and invasion of small intestinal tumors were occurred in APCMin/−/K-RasG12DLA2 with increased level of β-catenin and Ras compared with APCMin/+. However, EMT phenomenon and its following events such as migration and invasion of CRC cells induced by APC and K-Ras mutations were abolished by distalization of β-catenin and Ras. CONCLUSION Our results show that distalizing β-catenin and Ras could be effective therapeutic targets for inhibiting metastasis in the early stage of CRC development.

ADAM9-plectin interaction involved in prostate cancer malignancy progression. Che-Ming Liu, Chia-Ling Hsieh, Shian-Ying Sung. China Medical University, Taichung, Taiwan.

Introduction: To identify a specific biomarker for malignant prostate cancer progression and therapeutic target, we had demonstrated the correlation of ADAM9 expression and cancer progression. The aim is to clarify the association of ADAM9 and interactive proteins in malignant phenotype progression. Methods: Knockdown of ADAM9 expression was conducted to explore its role in cell motility and metastasis activities. Pull-down assays were performed to identify the proteins that interact with ADAM9 during cancer cell migration. Proximity ligation assay (PLA) was carried out to validate the protein-protein interaction. Transmission electron microscope (TEM) analysis to confirm ADAM9 and plectin interaction. Protein degradation test was conducted to determine the regulation pathway. Results: Our study demonstrated that ADAM9 play an important role in the regulation of prostate cancer migration, invasion and metastasis in the in vitro assay and xenograft animal models. The combination of ADAM9 pull-down assays and mass spectrum analysis demonstrated hemidesmosome components, plectin, ladinin, and cytoskeleton were pull-down with ADAM9. However, the key element of hemidesmosome, z-catenin and Ras could be an ideal therapy for treating metastatic colorectal cancer. Consequently, both β-catenin and Ras were highly increased in human CRC patient tissues, especially in metastatic tumor. EXPERIMENT PROCEDURES To investigate the synergistic effects of aberrant Wnt/β-catenin and Ras signaling pathways on tumor development to metastasis, we established the APCMin/−/K-RasG12DLA2 compound mouse that harbors APCMin and K-Ras mutations in and normal tissues. Tumor-specific, tumor-specific Ras protein level using human CRC patient tissues of normal mucosa and adenocarcinoma (N=24) and metastatic adenocarcinoma (N=26) and tumor budding (N=10). To recapitulate the metastatic tumor environment which have enriched receptor tyrosine kinases (RTKs), we maintained and experimented with EGF (20ng/ml) in every in vitro studies. RESULTS We observed that β-catenin and Ras were increased in adenocarcinoma and metastatic adenocarcinoma of human CRC compared with normal mucosa. Interestingly, both β-catenin and Ras were most significantly increased in tumor budding regions which histopathologically represent epithelial mesenchymal transition (EMT). In murine model, the EMT phenomenon was effectively induced and invasion of small intestinal tumors were occurred in APCMin/−/K-RasG12DLA2 with increased level of β-catenin and Ras compared with APCMin/+. However, EMT phenomenon and its following events such as migration and invasion of CRC cells induced by APC and K-Ras mutations were abolished by distalization of β-catenin and Ras. CONCLUSION Our results show that distalizing β-catenin and Ras could be effective therapeutic targets for inhibiting metastasis in the early stage of CRC development.

TUMOR BIOLOGY: Migration and Invasion 1
Cancer-associated fibroblasts (CAFs) are a part of the tumor microenvironment, and help remodel the extracellular matrix to pave the way for tumor cells to invade and spread. Src tyrosine kinase activity is often increased in the tumor microenvironment, and has been shown to induce cell motility and disrupt adherens junctions. Adherens junctions are formed by cadherin proteins that mediate intercellular adhesion and control cell migration. Previous studies have found correlations between cadherin expression and cancer cell motility. However, effects of cadherin expression on CAF motility have not been thoroughly investigated. We are investigating how junctions formed by E-cadherin (ECAD) and N-cadherin (NCAD) affect individual and collective migration of fibroblasts expressing different levels of Src kinase activity. We are utilizing fluorescently tagged ECAD or NCAD to study the localization and effects of cadherin proteins in real time by live cell imaging along with wound healing and Transwell migration assays. Results from these studies indicate that ECAD and NCAD have different effects on migration of cells depending on Src kinase activity. Src kinase activity did not affect collective cell migration, but increased individual cell migration by over 4 fold (p<0.0001 by t-test) in the absence of forced cadherin expression. In addition, our data indicate that ECAD increases collective cell motility, while NCAD decreases collective cell migration in the absence of strong Src kinase activity. Collective migration by ECAD expressing cells was over 3 fold higher than cells expressing NCAD or control transfectants, and over 3 fold lower than cells expressing ECAD (p<0.005 by t-test) without strong Src kinase activity. In contrast, both cadherins decrease individual cell motility promoted by Src kinase activity. ECAD and NCAD transfected cells migrated over 4 fold less than control transfectants with high levels of Src kinase expression (p<0.0001 by t-test). Interestingly, human oral squamous cell carcinoma (OSCC) cells that express high levels of both ECAD and NCAD exhibit collective migration equal to cells with low Src kinase activity, but individual migration over 5 fold higher than nontransformed cells (p<0.0001 by t-test). This system should prove useful to elucidate how cadherins and Wnt signaling pathways affect motility of CAFs and tumor cells. For example, western blotting analysis suggest that both ECAD and NCAD may protect cells from patients with breast cancer more hyperplastic and desmoplastic lesions than those from patients with breast cancer showed more hyperplastic lesions and CD49fhi luminal cells than young (<40 yr) tissues. Regenerated mammary glands by the old CD49fhi MaSCs through in vivo transplantation in cleared mammary fat pads showed more hyperplastic and dysplastic lesions than those regenerated by the young CD49fhi MaSCs, suggesting the old CD49fhi MaSCs contain transformed MaSCs. Moreover, gene set enrichment analysis of whole genome transcriptome data showed that they also have elevated expression of luminal cell specific genes and reduced expression of basal cell specific genes. Through large scale screening with flow cytometry guided by bioinformatics analysis, we found that Lin CD24+/CD49fhi CD13−CD73− cells significantly increased number of MaSCs. By comparing young (3 to 6 month) and old Balb/C mice, we found that the frequency of this specific MaSCs was significantly increased by four folds in old mammary epithelial cells (young: 4%; old: 16%). In contrast, relative percentage of Lin CD24medCD49fhi CD13−CD73+ cells significantly decreased (young: 46%; old: 16%) during aging while the CD13 and CD73 double positive or double negative subpopulations remained unchanged. CD13−CD73+ cells generated significantly many more mammospheres (around 40 spheres from 1,000 cells) in suspension than other three types of cells (around 2 spheres per 1,000 cells). However, there was no significant difference between the young and old in terms of sphere formation efficiency for any of the four subpopulations marked by CD13 and CD73. We are studying whether anyone of these four subpopulation from CD49fhi old cells will generate more hyperplastic and dysplastic lesions in regenerated glands than the other subpopulations as well as than the corresponding young subpopulation. Our study show that CD13 and CD73 may be the markers for the identification of tumor-initiating MaSCs.
vivo. Results: Our data showed that MSC's TM expression was low in the quiescent state but was up-regulated while treating with B16F10 cell's conditioned medium in the mechanism of plate-derived growth factor signaling dependence. Accordingly, using conditional gene knockout strategy, under the condition of tumor cell other than normal culture resulted in that TM gene knockout significantly inhibited tumor cell proliferation, migration, and invasion and impaired cell cycle progression. Moreover, TM knockout notably reduced the capacity of MSCs on regulating the growth, vasculogenesis, and active stroma establishment of B16F10 melanoma xenograft in vivo. Conclusion: These results indicate that the sequestration of tumor-associated plate-derived growth factor is a critical event for MSC's TM expression, providing further insights into the extent to which defective ACD contributes to tumor inhibition activity. Whether the loss of ACD directly promotes tumor initiation is unclear, because the detailed mechanisms of mammalian ACD have not been elucidated (Gomez-Lopez S et al. Cell Mol Life Sci 2014). Lethal giant larvae 1 (Lgl1) was shown that human Lgl1 is inhibited by PTEN loss, which is common in glioblastoma, indicating possible compromise of the canonical Wnt signaling during Paneth cell differentiation. Here we further characterized the underlying molecular mechanism of Cd42 regulating intestinal stem cell survival, proliferation, and differentiation. IHC of the intestinal tissue in Cd42 Villin-Cre knockout mice suggests greatly reduced Olfm4 level in the stem cells, which are abnormally positioned in the crypts, along with a reduction of both transcription co-factors beta-Catenin and YAP. The Notch signaling, which is required for stem cells and differentiation of absorptive lineage, seems intact. The autonomous regulation of Cd42 on Yap signaling is probably through its specific role in stabilizing cell-cell junction and actin filaments in the epithelium, both of which are known regulatory inputs of the Hippo-YAP pathway. Both Wnt/beta-Catenin and Yap signaling are required for the initiation and progression of colorectal cancer, and the regeneration of stem cells post radiation. Given that Cd42 is overexpressed in multiple types of cancers, especially colorectal cancer, our finding that Cd42 regulates both Wnt and Yap pathways in the homeostasis of adult intestinal tissue sheds new light on the molecular mechanism of its pathological involvement, and potential therapeutic target.
#920 ICAM-1(CD54) is involved in the osteogenic differentiation of mesenchymal stem cells and affects the progression of osteosarcoma cells. Yidan Zhang,1 Sajida Pideri,2 Nikolas Zaphiros,3 Pratitha Koirala,3 Tingting Ren,3 Michael Roth,4 Jonathan Gill,1 Bang Hoang,1 David Geller,1 Rui Yang,1 Wendong Zhang,1 Xiuquan Du,1 Jinghong Zhang,1 Richard Gorlick,1,3 Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY; 2Peking University People’s Hospital, Beijing, China.

Purpose: The aim of this study is to validate the relationship between the expression of ICAM-1(CD54), a cell surface glycoprotein, and osteogenic differentiation of mesenchymal stem cells (MSCs) and the role of ICAM-1 in the progression of osteosarcoma (OS) cells. Experimental Design: To compare the gene expression profiles between MSCs and MSCs after osteogenic induction, a microarray analysis was first performed. The differences in expression of specific genes including ICAM-1 were confirmed using western blot and flow cytometry. MSCs were sorted into two groups of cells by CD105 expression profiles between MSCs and MSCs after osteogenic induction, a microarray analysis was first performed. The differences in expression of specific genes including ICAM-1 were confirmed using western blot and flow cytometry. MSCs were sorted into two groups of cells by CD105 and CD105+CD44+CD54+ and CD105+CD44+CD54+ and properties of these cells were compared. Expression of ICAM-1 among tumor cells and xenograft OS cells were tested at the protein level. ICAM-1 was stably, constitutively overexpressed in multiple cell types. Differences in osteogenic differentiation potential between normal cell lines and transformed cell lines were studied using alizarin red S staining. Motility, proliferation and invasion as well as the metastatic potential of the transformed and normal cells were evaluated. Results: The initial microarray analysis revealed that ICAM-1 expression was significantly increased in MSCs after osteogenic induction for 15 days (p=0.03). Consistent results were achieved in western blot and flow cytometry analysis. Further microarray analysis between CD105+CD44+CD54+ and CD105+CD44+CD54+ groups determined that NF-kB signaling pathway was differentially activated. As for OS cells, ICAM-1 is not expressed at high level among OS tumor cell lines and is inconsistently expressed in xenograft OS cells. Enhanced osteogenic differentiation potential was observed in ICAM-1 overexpressing cells. Although there were no major difference in vitro motility, proliferation or invasion tests, substantially less lung nodules occurred with transformation as compared to the normal control. Conclusions: ICAM-1 is involved in the osteogenic differentiation of mesenchymal stem cells and its down regulation may be involved in the progression of osteosarcoma cells.

#921 Cdy2 is a chromodomain protein involved in the maintenance of pluripotency of stem cells. Naoko Hattori,1 Kana Kimura,1 Jumpei Taguchi,2 Toshiro Imai,1 Yasuhiro Yamada,1 Toshikazu Ushijima1.

Reader proteins of histone modifications are required to translate the information of histone marks into the cellular phenotypes, including pluripotency and malignancy. For example, a reader protein BRD4 has its target specificity and becomes a target of cancer therapy. Here, we aim to identify reader proteins involved in the pluripotency of stem cells. Using the UCSC database, 11 genes coding for chromo- or PHD-domain proteins were isolated as actively transcribed in mouse ES cells (ESCs). Among the 11 genes, Cdy2, chromodomain protein Y-like2, was found to be down-regulated upon ESC differentiation using RT-qPCR. Immunofluorescence and ChIP assay revealed that Cdy2 recognized H3K27me3 of the specific regions, including differentiation-associated genes. Cdy2-knockout ESCs could not survive after differentiation induced by LIF removal or retinoic acid because of the induction of apoptosis, indicating that Cdy2 is important for normal differentiation of ESCs. ESC expressing exogenous Cdy2 showed incomplete down-regulation of Oct-4 and Nanog by retinoic acid, and generated teratomas with an abnormal composition of three germ layers, showing perturbation of differentiation ability. Pathologically, aberrant expression of human Cdy2L2 was observed in breast cancer cell lines and primary breast cancers. Two breast cancer cell lines (MDA-MB-231 and MDA-MB-468) expressing exogenous Cdy2L2 showed enhanced attachment ability along with the up-regulation of integrin alpha-6 and integrin beta-1. The population of cancer stem cells, defined as ALDH positive cells, were increased in CD105+CD44+CD54+ cells, and show that human Cdy2L2 is involved in proliferation of cancer stem cells. From these data, we conclude that, between self-renewal and differentiation of stem cells, mouse and human Cdy2L2/CDY2L2 is necessary for the implementation of the initial step of differentiation.

#922 Delta133p53 represses p53-inducible senescence genes and enhances p53 functions to induce cellular senescence and apoptosis, which can be incompatible with self-renewal of pluripotent stem cells. Tzuin Horikawa,1 Kye-yoon Park,2 Han Li,3 Kazunobu Isoyagi,3 Yukiharu Hiyoshi,3 Katsuhito Anami,3 Ana I. Robles,1 Abdul M. Mondal,1 Kaori Fujita,1 Manuel Serrano,3 Curtis C. Harris,1 1National Cancer Inst., Bethesda, MD; 2NIH, Bethesda, MD; Spanish National Cancer Research Center, Madrid, Spain.

Human embryonic stem cells exhibit altruistic cell death that release death signals having potent anti-cancer activity. Bidisha Pal,1 Seema Bhuyan,2 Jaishree Garhyan,1 Herman Yeger,1 Bikul Das1,2 1The Forsyth Institute, Cambridge, MA; 2KarniKrishna Laboratory, Indian Institute of Technology (IIT), Guwahati, India; 3Hospital for Sick Children.

Background: We recently described the altruistic stem cell phenotype (ASC) in human embryonic stem cells (hESCs) as well as in mesenchymal stem cells (1, 2). The ASC phenotype was characterized by altered state of p53/MDM2 feedback system that permitted the cells to transiently acquire an unstable state of low p53 allowing the cells to survive in extreme microenvironment (1). Interestingly, we noted that ASCs spontaneously returned to its basal state of high p53 after a period of two weeks (1, 2). In this study, we speculate that this return of p53 levels to basal state could be a safety mechanism to prevent malignant transformation of ASCs. We also speculate that ASCs may release soluble factors like high mobility group protein B1 (HMG1)-1 that could selectively target the cells exhibiting abnormal p53 e.g. cancer cells. Here, we further characterized ASC phenotype and its fate. Methods: hESC, BG01 cells were exposed to extreme hypoxia followed by reoxygenation and then ABCG2+/+SEAS3+ cells were flow cytometry sorted. This specific subpopulation is enriched in ASCs and could be cultured in vitro for two weeks (1). The post- hypoxia treated ASCG22+/+SEAS3+ cells were maintained in serum free media, and subjected to apoptosis, and senescence assays. The conditioned media (CM) of these cells and subjected to HMG1-1 measurement by ELISA. To measure bystander apoptosis, teratocarcinoma cells (Tera-2) were treated with the CM followed by measurement of apoptosis. Results: Here we confirmed that p53 and MDM2 in ABCG22+/+SEAS3+ cells exhibited return of oscillation between days 19-24. This was associated increase in apoptosis, and senescence of these cells. Apoptosis/senescence was decreased when treated with either Pifithrin α, an inhibitor of p53, or by siRNA silencing of p53, suggesting p53-dependent apoptosis. Additionally, treatment with Nutlin-3, an inhibitor of MDM2 (1) led to a 10-fold increase in apoptosis indicating that high MDM2 was required to prevent the apoptosis of ASCs. Thus, ASCs underwent spontaneous apoptosis (altruistic cell death) due to the return of p53/MDM2 oscillation. We suggest that this return of p53 and associated apoptosis could be a safety mechanism to prevent malignant transformation of ASCs. Next, we found that the CM of ABCG22+/+SEAS3+ cells contained high levels of HMG1-1 compared to the parental BG01 cells. Importantly, CM treatment led to p53 dependent apoptosis of Tera-2 cells. Furthermore, CM treatment also inhibited the growth of Tera-2 xenografts in NOD/SCID mice. Conclusion: Our results indicate that ASCs undergoes altruistic cell death that releases death signals mediated by HMG1 that can target neighboring cells exhibiting abnormal p53 including cancer cells. We suggest that this unique property of ASCs could be exploited as a novel cancer therapeutic. 1. Das B et al, Stem Cells, 2012; 30(8):1685-92. 2. Pal B et al, Cancer Research, 2016; Volume 76, Issue 14 Supplement, p. 251.
TUMOR BIOLOGY: Stem Cell Heterogeneity

#924  Delidifferentiation into polyploid blastomere-like cancer stem cells via formation of polyploid giant cancer cells. Na Niu, Jinsong Liu. UT MD Anderson Cancer Ctr., Houston, TX.

We have recently shown that polyploid giant cancer cells (PGGCCs) are capable of tumor initiation and acquisition of embryonic-like stemness and thus repre- sent a novel type of cancer stem cells. However, two important questions remain to be answered from this surprising finding: (1) how PGGCs acquire such stem- ness; (2) on which stage of normal development PGGCs correspond to. Here, we tracked the fate of single PGGCs induced via mitotic failure by paclitaxel. Mor- phologically, early spheroids derived from PGGCs were indistinguishable from human embryos at the polyploid blastomere, compaction, morula, and blasto- cyst-like stages. Fluorescent electron microscopy showed that PGGCs showed time- and space-dependent activation of expression of the embryonic stem cell markers OCT4, NANOG, SOX2, and SSEA-1 and lacked expression of Xist. PGGCs also showed time-dependent activation of expression of the germ layer-specific markers alpha-fetoprotein, smooth muscle actin, and β3-tubulin and were ca- pable of redifferentiation into three germ layers in vitro. PGGCs-derived daugh- ter cells showed attenuated invasive ability and increased resistance to paclitaxel. PGGCs-derived spheroids grew into a wide spectrum of human neoplasms, including malignant dysgerminoma and embryonic carcinoma, poorly differen- tiated or well-differentiated carcinomas, and benign squamous tissue. We also observed PGGCs in ovarian cancer from patients treated with chemotherapy. Thus, our data demonstrated that PGGCs acquired novel cancer stem cell prop- erties and are able to differentiate into different tumors including the germ cell tumors, which are at the topmost developmental hierarchy. We studied, for the first time, link PGGCs to the polyploid blastomere-like cancer stem cells and thus offer a new paradigm for the origin of cancer.

#925  Tumorigenesis of murine iPSCs is prevented by iron depletion with downregulation of stemness markers. Yuki Katsura,1 Toshiaki Ohara,2 Hajime Kishima,1 Hiroaki Sato,1 Takuya Kato,1 Takayuki Ninomiya,1 Kazuhiro Noma,1 Yasuko Tomono,2 Hiroshi Tazawa,1 Shunsuke Kagawa,1 Yasuhiro Shirakawa,1 Toshiyoshi Fujiwara1.1 Okayama Univ., Okayama, Japan;2Shigei Medical Research Institute, Okayama, Japan.

Background: Iron plays a crucial role in the various metabolic pathways and it is essential for life. However excess of iron is known to cause cancer. There are many reports that iron depletion treatment indicates antitumor effect. Previ- ously, we have confirmed that iron depletion treatment indicates antitumor effect against Cancer stem cell (CSC) model, miPS-LLCCm, which was con- verted from murine induced pluripotent stem cell (miPSC) in Okayama uni- versity (Chen et al, PLOS ONE 2012), and also suppresses CSC markers. Many tumors contain phenotypically and functionally heterogeneous cancer cells due to pluripotency of CSC. Although there are various theories about the origin of CSC, miPSC has a pluripotency and regarded as the top of the hierarchy, it can be the candidate of the origin of CSC. miPSC cell is known to form teratoma when inoculated into nude mouse because of its undifferentiated status. We hypoth- esized if we could suppress the stemness and tumorigenicity of miPSC cell by iron depletion treatment, it can be applied to the therapy for CSC and undifferen- tiated status cancer cell. In this study, we investigated the stemness and tumori- genicity of miPSC cell with iron depletion treatment. Methods: We used miPSC cell (cell name: iP5-MEF-NG-20D-17) purchased from Riken Cell Bank(Japan). Deferasirox (DFX), an popular commercial available oral iron chelator was used in vivo study. Tumors were harvested for immunohistochemistry. Results: Stemness markers of miPSCs were suppressed strongly by DFX in western blot analysis. DFX also suppressed the subcutaneous tumor growth. The average tumor volume of the control group was 665.3±430.8mm³ while that of the DFX treated group was 204.3±76.2 mm³ (p<0.05) on day 20. Immunohistochemistry of the tumor revealed the suppression of the stemness markers in the DFX treated group compared with the control group.

#926  Generation of a potential breast cancer stem cell model from induced pluripotent stem cells. Neha Nair,1 Anne Sanchez Calle,1 Maram Hussein Zahra,2 Aung Ko Ko Oo,3 Arun Vaidyanath,7 Tomonari Kasai,1 Shinobu Kobayashi,7 Masaharu Seno1.1 Okayama University, Okayama, Japan;2Menoufia University, Shebin El-Koom, Egypt;3Nihon University School of Medicine, Tokyo, Japan.

The role of cancer stem cells (CSC) in the present scenario of breast cancer research is perceived as indispensable in the development and progression of the disease. The CSC niche maintains a hierarchy of heterogeneous cells, which facilitates the inception of the tumor until its invasion. Therefore, our study focuses on the development of an effective breast cancer stem cell model from mouse induced pluripotent stem cells (miPSCs). Taking into consideration the intertumor heterogeneity of breast cancer, three human breast cancer cell lines, namely BT549, SKBR3 and T47D representing three major hormone subtypes were used to derive respective conditioned medium. The miPSCs were treated with these conditioned media for a period of four days during the expansion stage following a protocol previously established by our laboratory. The resulting survived cells were subcutaneously and orthotopically transplanted into Balb/c nude mice. Within a short span of 15-20 days, both subcutaneous and orthotopic tumors were developed. Serial transplantsations in to nude mice also generated malignant tumors with same vigor. Histopathological studies of these tumors confirmed the presence of PGCCs-derived tumor in vitro generated CSCs. Marked expression of prominent CSC markers namely CD44, Nanog, Sox2 and CD49f were observed in primary cells generated from these tumors. Tumor sphere formation assay further confirmed the presence of a good popu- lation of self renewing CSC population in the tumor tissues. This study attempts to recapitulate human breast cancer disease in mice without any genetic manip- ulation, but by exploiting the tumor microenvironment in the form of condi- tioned medium. This should pave the way for the establishment of personalized therapy and a model to assess effective therapeutic interventions.

#927  Cancer stem cell proliferation in human prostate cancer cells utilizing a new defined 3D spheroid culture system. Nick Astrock, Vi Chu, Kan Saito. MilliporeSigma, Temecula, CA.

Solid tumors grow in a three-dimensional (3D) spatial conformation, result- ing in a heterogeneous exposure to oxygen and nutrients as well as to other physical and chemical stresses. To mimic the 3D spatial conformation, 3D in vitro culture models have been used in cancer research since the diffusion- limited distribution of oxygen (hypoxia), nutrients, metabolites, and signaling molecules is not mimicked in conventional two-dimensional (2D) monolayer cultures. One of the 3D in vitro culture models, the tumourosphere culture is an emerging model for studying and expanding the cancer stem cell (CSC) popu- lation. E006AA cell line is a spontaneously immortalized cell line derived from a Gleason 6 localized prostate cancer in a hormone-naïve prostate cancer patient of African American descent. This cell line expresses androgen receptor and was repeatedly reported to be non-tumorigenic in nude mice. However this cell line forms continuously growing tumor in NOG-SCID triple-deficient mice (i.e., NOG-SCID mice not having NK, B, and T-cells) and an establishment of highly tumorigenic subline E006AA-It was reported suggesting this cell line has a potential for CSC proliferation by 3D spheroid cultures. Here we report tumors- pheres of CD133+ cells by a new 3D spheroid culture media system. The cells showed continuous proliferation supported during serial passage of 3D tumourospheres cultures. In addition to the stable proliferation, increases of Alde- hyde Dehydrogenase (ALDH) expressing CSC population were observed with the increase of passages of 3D tumourospheres cultures.

#928  Combined treatment by manuka honey and metformin inhibited growth and induced apoptosis in CD133+ and CD90- subpopulation hepatocellular carcinoma cancer stem cells. Amira S. Fyala, Ahmed S. Sultan. Faculty of Science, Alexandria University, Alexandria, Egypt.

Manuka honey is well known for its anti-bacterial and wound healing prop- erties but its anticancer role against cancer stem cells (CSC) in hepatocellular carcinoma (HCC) cell lines and the underlying molecular mechanisms remain uninvestigated. Recently, we identified a CSC subpopulation in HCC cell lines characterized by their CD133+ and CD90- phenotype. The CD133+/CD90- cells demonstrated a more aggressive phenotype than the CD133+/CD90- counterpart and formed metastatic lesions in the lung of immunodefi- cient mice. In order to evaluate the effect of Manuka honey alone or in combined treatment with Metformin, a biguanide that has been widely used to treat type 2 diabetes, on cell viability and apoptosis induction in human HCC cell lines, isolated and identified CD133+/CD90- subpopulation CSC were used. Cell vi- ability was monitored using an MTT assay. In addition, apoptosis induction was labeled by Annexin VFluorescein isothiocyanate/propidium iodide and mea- sured using flow cytometry. Western blotting was used to examine the protein expression of p53, Bcl2, Bax, Bcl2-associated death promoter (Bad), Bcl2 homol- ogous antagonist/killer (Bak) and cleaved caspases3 and 9. The results of the present study revealed that Manuka honey alone or in combined treatment with Metformin suppressed the cell viability of CD133+/CD90- cells in a concentra- tion-dependent manner and the maximum effect was detected in combined treatment after 24 h. In addition, Manuka honey alone or in combined treatment
with Metformin induced a significant apoptosis in the tested cells. Furthermore, it was demonstrated that combined treatment increased the expression of pro-apoptotic proteins, including p53, Bax, Bad and Bak, while it decreased the protein level of antiapoptotic protein Bcl2 compared to control, Manuka honey or Metformin alone. Furthermore, the combined treatment strongly inhibited stemness marker ALDH1 expression. CSC subpopulations were significantly inhibited in CD133+/CD90+ tumor growth in vivo. Our data demonstrated that combined treatment induced apoptosis in CD133+/CD90- subpopulation through G1 arrest in the cell-cycle and regulating p53/Bcl2/caspase3 signaling pathway. In conclusion, our results suggested that Manuka honey alone or combined treatment with Metformin may be a potential treatment for apoptosis induction in CSC and may provide a novel therapeutic model for HCC.

#929 Dissecting heterogeneity and population dynamics in breast cancer stem cells. Michael D. Brooks, Max S. Wicha. University of Michigan, Ann Arbor, MI.

The CSC hypothesis posits that there is a subset of tumors cells driving tumor growth and metastasis and that by targeting this population, tumors can be more efficiently eradicated. Recently it was shown that there are two types of CSCs in breast cancer, mesenchymal CSC’s characterized as CD44+/CD24- and an epithelial type marked by ALDH expression. The cellular plasticity of these CSC states may contribute to their metastatic capacity. However, little is known about the heterogeneity of these CSC populations or how they are regulated by the tumor microenvironment. To address these issues we have utilized Drop-Seq to generate single cell RNA-Seq data from thousands of cells across patient derived xenografts (PDX) with various molecular subtypes of breast cancer. In particular, DLK1 mRNA was highly expressed in U2OS (osteosarcoma), SW872 (liposarcoma), A204 (rhabdomyosarcoma), and HS-SYI (synovial sarcoma) cells. We observed a reduction in cell proliferation and invasion in U2OS, A204, and sw872 cells treated with DLK1 siRNA. CSCs were isolated using FACS with CD133 and sphere forming assay. The effect of DLK1 on sarcoma tumorigenesis was examined using cell proliferation and cell invasion assays by DLK1 siRNA treatment. Real-time PCR showed the increased expression of DLK1 mRNA in sarcoma cell lines. In particular, DLK1 mRNA was highly expressed in U2OS (osteosarcoma), SW872 (liposarcoma), A204, and sw872 by the FACS sorting method using the CD133, a CSC marker in sarcoma. The number of active spherocytes in CD 133+ cells were significantly higher than CD133- cells. While the breast CSC marker CD44 was down regulated in mesothelioma tissue and cell lines1-4. Based on our own study and previously demonstrated a role for sFRP4 in making chemoresistant tumours amenable to chemotherapeutics3-16. We have now progressed our work into the effects of peptides derived from the cytokine-rich domain and the netrin-like domain of sFRP4 on tumour initiating cells derived from MM cells lines. We examined the effect of secreted frizzled-related protein 4 (sFRP4), a Wnt signaling antagonist and its associated cancer stem cells (CSCs) enriched from these two cell lines to chemotherapy. We found tumourspheres exposed to sFRP4 and peptides alone in combination with cisplatin induced cell death and decreased CD133 and ALDH1 expression. We thus identified for the first time that sFRP4 and associated peptides could help to sensitise to chemotherapeutics and destroy cancer stem cells of MM cell line, which would lead to effective treatment regimen to combat malignant mesothelioma. Our initial in vitro data exemplify the ability of these peptides in diminishing the numbers of therapy-resistant Tumor initiating cells (TICs). References: 1. Lee et al, 2004, Oncogene, 23(39), 6672-6676. 2. He et al, 2005 Cancer research, 65(3), 743-748. 3.
CD133+ cancer stem cells promoted by VEGF accelerate the recurrence of hepatocellular carcinoma. Dexi Chen,1 Kai Liu,1 Meijun Hao,1 Yabo Ouyang,1 Jiasheng Zheng,1 Kishore Babu, Challagundla2.1. Beijing You An Hospital, Capital Medical University, Beijing Institute of Hepatology, Beijing, China; 2Children’s Center for Cancer and Blood Diseases and The Saban Research Institute, CA.

The function of cancer stem cells (CSCs) on inducing HCC recurrence after radiofrequency ablation (RFA) is still unclear. Here, 19 RFA-treated primary HCC patients were enrolled. We identified a dramatic increase of plasma VEGF in some patients (termed type II, n=9) after RFA, who suffered early HCC recurrence; the other patients (termed type I, n=10) had no increased plasma VEGF but had longer interval for HCC recurrence. Moreover, expression of CSC markers, CD133, was dramatically increased in recurrent HCC tissue of Type II in comparison with type I, suggesting induction of CD133+ CSCs may contribute to early HCC recurrence. In vitro studies demonstrated that VEGF stimulus enhanced the level of CD133+ CSCs and their self-renewal ability by inducing Nanog dependently on activation of VEGFR2. In vivo studies further demonstrated that CD133+ CSCs with VEGF stimulus formed larger tumor size in comparison with non-stimulus; and VEGF stimulus increased the tumorigenic cell frequency of primary HCC cells dependently on the presence of Nanog and VEGFR2. In recurrent HCC tissue of type II, but not type I, almost all CD133+ cells were Nanog and p-VEGFR2 positive cells, suggesting that activation of VEGF2 is critical for RFA-induced tumor stemness in HCC. Taken together, our results suggest that VEGF/CSCs is a tumor stemness marker involved in tumor recurrence of HCC and is valuable for the prediction of HCC recurrence after RFA and development of novel therapeutics.
pressing CD133 to post-treatment more radioresistant CSCs expressing the GPl-anchored, TGFβ-inhibitory protein CD109 in an ATM, NF-kB, CEBP-b dependent manner. In post-IR GBM, these therapy-escaped CD133/CD109+ GBM cells, but not the rest of the tumor, uniquely retain clonogenic, tumorigenic, and multi-potent capacities both in vitro and in vivo. CD109 silencing unexigated CD133+/CD109+ cancer stem cells to the downstream target and this CD109-YAP/TAZ signaling axis is evolutionally conserved between drosophila and human brain tumors. Clinically, both CD133 and CD109 are independently associated with poorer prognosis of GBM patients, while the poorest prognosis group of GBM expressed both CD133 and CD109 at higher rates. These data indicate a possibility of the post-IR CSC plasticity and the subsequent heterogeneity, and the activation of the CD109-YAP/TAZ signaling axis as previously unidentified therapeutic targets for post-IR recurrent GBM.

#938  Estrogen induced NRF1 signaling is a molecular mechanism underlying the generation of different breast cancer stem cell subpopulations leading to intratumoral heterogeneity. Jayanta Kumar Das, Deodutta Roy. Florida International University, Environmental and Occupational Health, Bruce W Carter VA Medical Center, Research Service Laboratory, Miami, FL.

Life time exposure to elevated levels of estrogen (E2) is a major risk factor for breast cancer. Among women with ER/PR positive tumors, only 50 to 60% of women respond to endocrine therapy. Intra-tumoral heterogeneity of breast cancer cells may be one of the reason for unresponsiveness to endocrine therapy in more than 40% of ER-positive breast cancer cases. In this study we examined that heterogeneity of breast cancer stem cells (CSCs) are produced by exposure to E2 and/or ectopic expression of molecular risk factor - nuclear respiratory factor 1 (NRF1). To elucidate this, we used single cell confocal imaging, flow sorting, ChIP and qRT-PCR approaches. We also measured the key regulators of pluripluripotency, epithelial-mesenchymal transition (EMT), stemness, cell apoptosis and cell cycle regulation. The functional assay for cancer stem cells enrichment employed were selection of cells in B27 medium as spheroids, anchorage independent growth and mammosphere formation assays. Carcinogenic treatments of E2 to MCF10 A CD24- cells induced formation of ~1.46% CD44+/CD24- and 26.02% of CD44+/CD24- subpopulations, which are a typical phenotypes associated with human breast tumor-initiating (BTICs) or BCSCs. These subpopulations are capable of forming self-renewing mammospheres. In contrast, CD24+ or CD24+/CD44- cells did not form mammospheres. Stable NRF1 overexpression induced formation of 2.36% CD44+/CD24+ and of 64% CD44+/CD24- subpopulations. Carcinogenic treatments of E2 to stable NRF1 overexpressing MCF10 A cells, induced to acquire 21.54% CD44+/CD24- and 44.54% CD44+/CD24- subpopulations. E2 treatment to NRF1 overexpressing BCSCs markers significantly increased mammosphere forming capability, compared to NRF1 alone. The dominant negative form of NRF1 diminished the effects of E2 and/or NRF1 induced acquiring of BCSCs antigen markers and their capability of forming mammospheres. E2 induced BTICs/BCSCs were heterogeneous. Each subpopulation was characterized by a different transcriptional and biomarker profiles of CD24, CD44, CD49f, CD133, ALDH1A1, CXCR4 and NRF1. The expression of different markers for pluripluripotency (CD133, SOX2 and Nanog), EMT (E-cadherin, N-cadherin, Vimentin), stemness (CD24, CD44, CD39f, CD133, ALDH1A1), cell cycle (p16,CD2C2,CD2C25, cyclinB1) and metastasis (CXC4), were associated with BTICs produced by E2 and/or NRF1. In summary, we observed new roles of NRF1 in contributing to acquire breast tumor initiating stem-like cells and in regulating EMT and invasiveness of BCSCs, thus opens a new direction of breast cancer research. A better understanding of how E2 dependent breast neoplasm heterogeneity depends on NRF1 network may open new avenues for therapeutic strategy against breast cancer. This work was supported partly by VA MERIT Review (VA BX001463) grant to DR.

#939  Single cell mRNA expression profiling reveals heterogeneity of normal and malignant breast stem cell populations. Shamileh Fouladdel,1 Justin Colacino,2 Shamileh Fouladdel,3 Justin Colacino,4 University of Michigan, Ann Arbor, MI; 2University of Michigan, Department of Environmental Health Sciences, School of Public Health, Ann Arbor, MI.

The normal breast contains epithelial populations which are hierarchically organized, a profile recapitulated in breast cancer. At the apex of these hierarchies are ‘stem like’ cells defined by their capacity to self-renew as well as to generate more differentiated progeny. In breast cancer these ‘cancer stem cells’ (CSCs) have been shown to be important mediators of tumor metastasis and treatment resistance. Although the phenotypes of normal rodent and human mammary stem cells have been characterized, CSC populations are operationally defined by their capacity for tumor initiation. In addition, putative breast CSC markers such as CD44+/CD24low+ and ALDH1+ have proven useful in enriching for cells capable of tumor initiation and previous studies have suggested that these markers identify alternative mesenchymal and epithelial stem cell states, respectively. However, it remains unclear whether CSCs exist in discrete states or whether these cells actually represent a continuum reflected in heterogeneity of cell populations defined by expression of these markers. To address this question we utilized single cell mRNA expression profiling of normal and malignant breast stem cells. We first generated a panel of 96 genes representing stem cell and developmental pathways and analyzed expression of these 96 genes utilizing Fluidigm’s C1 and BioMark HD technologies and TaqMan gene expression assays. We also utilized Fluidigm’s Polaris to selectively isolate single cells that were then analyzed by illumina RNA-Seq method. Utilizing these advanced technologies, we demonstrated that both normal and malignant breast stem cells characterized by CD44+/CD24low+ and ALDH1+ expression are highly heterogeneous. For example, the ALDH isoforms ALDH1A1 and ALDH1A3 were each expressed in some single cells and co-expressed in others. ALDH1A1 expressing single cells appeared to express a unique gene pattern characterized by SLIT/ROBO, PLXNA2, ROHOU and HNF1a. These results suggest that at the level of mRNA expression, normal and malignant breast stem cells display a greater heterogeneity than has previously been reported. These results have important implications for breast carcinogenesis as well as for the development of therapeutic strategies designed to target CSCs in breast cancer.


Within the context of HER2+ breast cancer, PTEN mutation has been associated with poor patient survival, trastuzumab resistance, and induction of the epithelial to mesenchymal transition. Accumulating evidence supports the theory that breast cancers are organized in a hierarchical manner with the presence of cancerous stem-like restricted progenitor, and differentiated-like cells. Recent literature has also indicated that CSCs may exist in distinct epithelial and mesenchymal states which retain unique properties. In order to understand the influence of PTEN inactivation within the context of a hierarchical model of cancer, and examine which populations themselves are targeted by trastuzumab, we have performed analysis of traditional CSC markers and single cell lineage tracing experiments. Here, a lentiviral vector containing shPTEN and dsRed reporter are introduced into HER2+ cell line BT474. Following bulk sorting of dsRed+ cells, long term culture of parent and shPTEN cells and with and without trastuzumab for 4 weeks reveal no obvious changes in traditional EMT-CSC markers CD44+/CD24- and only modest changes in Aldefluor+ cells. In order to take a CSC-marker agnostic approach we performed a single cell colony formation assay and identify that trastuzumab enriches for rare cells with colony forming potential. The majority of single cell derived colonies exhibited distinct morphologies stable with long term culture which are similar regardless of PTEN knockdown or trastuzumab therapy. However, quantification reveals PTEN knockdown results in a higher frequency of colonies which exhibit distinctly mesenchymal morphology and less of those with epithelial morphology. Interestingly single colonies forming cells could recapitulate all morphologies present in the parental cell line, suggesting they were derived from a true CSC. Single cell multiplex RT-qPCR of 96 target genes was performed on mixed and distinct morphology colonies using Fluidigm’s C1 and BioMark HD instruments and TaqMan gene expression assays. Principal component and t-SNE analysis was capable of separating morphologically distinct colonies and revealed multiple cell clusters within each colony. Using hierarchical clustering it is evident that a minority of cells which appear as unique clusters in PCA analysis are those which are proliferative and express different lineage markers reflective of the colony itself. Taken together these results suggest that PTEN knockdown shifts the equilibrium from primarily epithelial lineage differentiation to a more mesenchymal differentiation while trastuzumab enriches for stem and progenitor cells.

TUMOR BIOLOGY: Stem Cell Heterogeneity

#941  CXCL1-CXCR2 signaling might recruit the bone marrow-derived mesenchymal cells to the carcinoma-associated fibroblasts of gastric cancer. Masakazu Yashiro, Toshiohisa Okuno, Hiroaki Kasashima, Yuichi Miki, Hiroshi Nakame, Kisyu Kitayama, Toshiyuki Kawashima, Takaharu Hatan, Heishiro Fujikawa, Tsuyoshi Hasegawa, Takahiko Nakane, Masayuki Hino, Ko sei Hirakawa, Masachio Ohira. Osaka City Univ. Grad. School of Medicine, Osaka, Japan.
Background & Aims: Activated fibroblasts in the tumor stroma, also termed carcinoma-associated fibroblasts (CAFs), play a critical role in the progression of cancer. In our previous studies, we identified CAFs as playing an important role in cancer progression in the development of gastric cancer. However, it remains unclear which factor recruit and promote CAFs. The aim of this study is to clarify the role of cancer cells (CCs) on the recruitment of CAFs originating from normal mesenchymal cells (BM-MCs). The nude mice with bone marrow transplantation from CAG-EGFP mice were used for the in vivo gastric cancer experiments. Results: BM-MCs migrated into tumor stroma of in vivo gastric cancer. The number of BM-MCs was much greater in the gastric tumor than in normal tissue from three days after inoculation of cancer cells. C-X-C motif ligand 1 (CXCL1) from gastric cancer cells significantly (p<0.001) stimulated the chemoattractant ability of BM-MCs in vitro. Anti-CXCL1 antibody and CXCR2 inhibitor significantly (p<0.05) decreased the migration-stimulating activity of gastric cancer cells. A CXCR2 inhibitor, SB25002, decreased tumor size and lymph node metastasis of gastric tumor, and significantly (p=0.039) prolonged survival of gastric-tumor bearing mice. The histologic findings indicated that SB25002 decreased the recruitment of BM-MCs into the tumor stroma, resulting in the reduction of CAFs. Conclusion: BM-MCs recruit into gastric tumor and differentiate into CAFs. CXCL1 from gastric cancer cells stimulates the recruitment of BM-MCs into gastric cancer focus via CXCR2 signaling. Inhibition of BM-MCs’ recruitment appears to be a promising therapy for gastric cancer.

#942 CXCR2 acts as a checkpoint regulator of the pancreatic stellate cells activity within pancreatic cancer tumor microenvironment. Mohammad Awaji, Michelle Varney, Abhislaha Purohit, Surinder K. Batra, Rakesh K. Singh. University of Nebraska Medical Center, Omaha, NE.

Pancreatic cancer (PC) is a grotesque disease featured by an inflamed complex tumor microenvironment (TME) that contribute to advancing tumor progression. Oncogenic K-ras, the most common mutation in PC, amplifies inflammatory responses within the TME through overexpression of multiple immune factors such as CXCR2 and its ligands. K-ras induced overexpression of CXCR2 axis, made CXCR2 seem intuitively a good target for PC treatment. In KC, a spontaneous PC mouse model with oncogenic K-ras, genetic ablation of CXCR2 caused anti-tumor effects such as increased apoptosis and decreased angiogenesis; and also presented pro-tumorigenic events; most notably was the increased fibrosis and metastasis to the liver. These observations have shed light on the possible role the CXCR2 on the other TME component especially pancreatic stellate cells (PSCs). The aim of this study is to determine the differential effect of K-ras status and CXCR2 chemokines expression in the PC cells on their interaction with PSCs. Unidirectional segregated co-culture studies were conducted using conditioned media (CM). CM collected from PSCs were used to grow multiple PC cell lines, and CM obtained from different PC cell lines were used to culture PSCs. Cellular proliferation and gene expression were analyzed. Furthermore, PSCs proliferation potentials were assessed as those cells treated with exogenous CXCL1 and CXCL8, or PC cell lines derived-CM in the presence or absence of CXCR2 antagonists. Co-culture studies revealed that PC-derived CM had increased proliferation of PC cell lines positive to oncogenic K-ras as well as increasing their expression of multiple chemokines such as CXCL1, CXCL5, and CCL2. On the other hand, PC cell lines with a wildtype K-ras were inhibited by PSC-derived CM treatment. PSCs proliferation potentials were decreased with CM derived from oncogenic K-ras positive PC cell lines and increased with CM derived from PC cell lines with wildtype K-ras. Furthermore, CM from oncogenic K-ras PC cell lines has increased the expression of multiple pro-tumorigenic genes in the PSCs including cytokines such as IL-10, IL-4 and IL-13, and chemokines such as CXCL2 and CXCL7. Also, treating PSCs with exogenous CXCL1 and CXCL8 exhibited a similar proliferation inhibition to that observed with oncogenic K-ras PC derived CM treatment. CXCR2 antagonists have shown rescued inhibition or enhanced proliferation of PSCs when incorporated with CXCR2 chemokine or PC cell line derived CM treatment. These observations suggest that K-ras status of PC dictates their interaction with PSCs within the TME. We have demonstrated that oncogenic K-ras through increased production of CXCR2 chemokine would dampen PSCs proliferation and further orient them to support tumor progression by increased expression of pro-tumorigenic genes. Besides, we observed that CXCR2 inhibition in PSCs would increase their proliferation which may further their fibrogenic activity.


It is evident from research outcomes that metastases-associated deaths are predominant in breast cancer. Recent developments on early diagnosis using mammographic screening and the implementation of adjuvant therapies may have reduced breast cancer associated deaths in decent numbers, although new markers for prognosis are of utmost importance for patients with higher risk of metastasis. RelA is involved in regulating various processes such as cellular immune response and can be targeted to reduce the intratumoral regulatory T cells (Treg) for antitumor immunity. Targeting either specific immunomodulators and/or intervening molecular mechanisms is thought to be a potential therapeutic option. In our previous study, we have found that co-expression of CXCR5 and CXCL13 is significantly associated with epithelial to mesenchymal transition (EMT) of cells and lymph node metastasis (LNM) during breast cancer progression. In this study, we are aiming to investigate how the transcriptional regulation of this receptor-ligand pair directs the process of disease development. Interestingly, it was found that RelA (p65), a subunit of NFκB protein, promoted the transcription of both CXCR5 and CXCL13. The putative RelA binding sites were validated using sequence deletion of respective promoter regions of CXCR5 and CXCL13. We observed that expression of CXCR5 significantly (p<0.05) increased with RelA and Nrf2 overexpression and CXCL13 co-stimulation in MDA-MB-231 cell line. RelA was also found to induce the expression of CXCL13 in T-47D and MDA-MB-231 cell line. Significantly, SNAIL, a key EMT regulatory element, was found to be highly expressed in RelA-transfected MDA-MB-231 cell line with or without CXCR5 and CXCL13 co-stimulation. We also observed CXCR3 T follicular helper (Tfh) and Treg sub-population within the tumor microenvironment, although numbers varied with stages and molecular subtypes. In chemotaxis assay, CXCR3 Treg cells were found to be predominant among migratory T-cell subsets against RelA overexpression and/or CXCL13 stimulation. Simultaneously, CCL2, a pro-inflammatory chemokine, induced macrophages to secrete CCL2, which in turn, might attract CXCR3 Tfh and Treg cells into the tumor microenvironment.

Study in 4T1-BALB/c breast cancer mouse model demonstrated significant (p<0.05) increase in CXCR5 and CXCL13 expression levels upon immunostimulation. The study may help to understand the prognosis value of CXCR5 and CXCL13 considering the impact of this receptor-ligand pair on the regulation of Tfh/Treg ratio, EMT and LNM. We believe that this investigation may lead to a comprehensive prediction of the tumor fate as well as to explore possible markers for breast cancer prognosis and future chemotherapy with more precision.

#944 Chemokine receptor signaling as a new tool to improve lung cancer diagnostics and therapy. Katalin Nagy-Major,1 Jörg Sänger,2 Harshad R. Kulkarni,2 Peter Fix,3 Almut Kunze,2 Amelie Lupp,3 Reiner Bonnet,2 Werner Seeger,2 Richard P. Baum,4 Patricia Grabowski,1 Rajkumar Savai,4 Charité Campus Benjamin Franklin, Berlin, Germany; 2Zentralklinik Bad Berka, Bad Berka, Germany; 3Jena University, Jena, Germany; 4Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany.

Background: The tumor microenvironment plays a critical role in cancer development, progression, and control. We demonstrated in vitro and in vivo that a bidirectional crosstalk between tumor-associated macrophages and cancer cell via CCR2-CCL2 and CX3CR1-CXCL1 signaling is fundamental for lung cancer growth and metastasis offers new treatment concepts. Aim: Our working hypothesis is to develop a new platform for specific chemokine receptor (CCR2) PET imaging and a range of theranostic applications in lung cancer. For this purpose, we planned to define the CCR2 expression in NSCLC as well as in SCLC, tumor cells as well as immune/inflammatory cells. Material and Methods: This is a multicenter trial with patients from Zentralklinik Bad Berka GmbH (n=100 NSCLC, n=100 SCLC). All patients gave informed consent according to the declaration of Helsinki. FFPE material was available in all cases. CCR2 CXCR4 and CXCXCR1 expression was determined by immunohistochemistry with the avidin-biotin-peroxidase-complex. Results: Analyzing 96 NSCLC (47 adenocarcinoma, 49 squamous cell carcinoma) and 45 SCLC tumor samples, we found CCRX4 and CXCR2 chemokine receptor expression in tumor, immune and inflammatory cells. In SCLC, CCR2 expression was found in tumor cells and lymph node metastasis (LNM) during therapeutic treatment in CCRX4 staining was very high concerning their expression on tumor cells rising with higher tumor stages, proliferation rate and grading. CCR2 expression in immune cells was restricted to lower stages; the same pattern could be observed in 45 SCLC tumor samples where CCR2 expression on immune cells was found especially in limited disease rather than in metastatic disease; CCRX4 expression in tumor cells was found to be high in all stages of SCLC. To evaluate the therapeutic efficacy of CCR2 antagonist in vivo, we injected LLC1 s.c. into WT mice treated with CCR2 antagonist (RS504393) or vehicle. Notably, tumor growth was significantly inhibited in CCR2 antagonist-treated mice as compared with vehicle-treated mice. Discussion: Our preliminary results show a significant
portions of CCR2 positive tumors - NSCLC as well as SCLC which would qualify for a CCR2-based imaging as well as - demonstrated in our mice models - CCR2 inhibitory strategies. This should be evaluated in further preclinical trials.


Background & Aims: Glioblastoma (GBM, WHO Grade IV) is considered as the most lethal neoplasm of all solid cancers due to its inherent intensive invasiveness. GBM may arise de novo, or following progression from lower grade gliomas. Recently, solid tumor progression has been recognized as the product of an evolving crosstalk between the cancer cells and its surrounding glial cells. The intensive invasion activity of GBM cells might be regulated by surrounding normal cells such as oligodendrocytes or fibroblasts. In this study, we evaluated the interactive factors between GBM cells and normal glial cells to determine the role of oligodendrocytes in regulating the invasiveness of glioblastoma cells.

Methods: Two GBM cell lines, T98G and U251, were used. Two oligodendrocyte cell lines, ODC1 and ODC2, were derived one each from surgical tissues of patients with low-grade glioma (WHO Grade II). Two fibroblasts cell lines, GF1 and GF2, were derived one each from surgical tissues of patients with GBM. Oligodendrocytes and fibroblasts were confirmed by immuno-histochemical staining. The invasive property of GBM cells was analyzed in the presence or absence of conditioned medium from oligodendrocytes or fibroblasts by wound-healing assay and Boyden chamber invasion assay. The proliferation ability of GBM cells was examined by MTT assay. Subsequently Cytoframe array was used to examine the cytokines and growth factors in the conditioned medium from oligodendrocytes or fibroblasts. Results: Oligodendrocytes cell lines, ODC1 and ODC2 cells, significantly (p<0.01) increased the migration and invasion ability of GBM cells, T98G and U251. In contrast, fibroblasts, GF1 and GF2 cells, did not affect the migration and invasion ability of GBM cells. Cytokine array indicated that angiopoietin-2 was found in the conditioned medium from oligodendrocytes, but not that from fibroblasts. Angiopoietin-2 significantly (p<0.01) increased the invasiveness of GBM, but not their proliferation ability. Conclusion: Oligodendrocytes might up-regulate the invasiveness of GBM cells via angiopoietin-2 signaling pathway. Hence, angiopoietin-2 can be considered as a promising target for the treatment of malignant gliomas.

#946 Characterize and identify secreted high molecular weight heparanase from PC3M conditioned medium. Donghong Ju, Mary A. Kosir. Barbara Ann Karmanos Cancer Inst., Detroit, MI.

Introduction: Neutrophils, platelets, lymphocytes are typical sources of NAP-2 which is associated with hepananase activity. Heparanase degrades polymeric heparan sulfate molecules into shorter oligosaccharides, participating in extracellular matrix degradation. It is active under acidic conditions during tumor invasion and inflammatory processes. Our lab previously found that some cell lines like androgen independent prostate cancer cells (PC3M) can secrete a protein that reacts with anti-NAP-2 antibody, but has a much higher molecular weight (MW > 50KD) than NAP-2 (MW = 6-14 KD), and has heparanase activity. The purpose of this study is to characterize and identify this protein. Procedures: Immunoprecipitation was performed using Pierce DirectIP kit according the manufacturer’s instructions. Heparin binding affinity column used Hitrap™ Heparin HP column from GE Healthcare Bio-Sciences. The binding buffer was 10mM NaAcetate pH 5.0, the eluting buffers were 0.5M-2M NaCl in 10mM NaAcetate pH 5.0. The mass spectrometry was done by KCI Proteomics Core facility. Data: The immunoprecipitation method confirmed that this higher molecular weight protein indeed can be pulled down by the NAP-2 antibody, and after using heparin binding affinity column to purify, we can still get the > 50KD band on the western blot for NAP-2, meaning it is a heparin binding protein. Conclusions: A protein identified with NAP-2 antibody, but with a higher molecular weight than native NAP-2 is found in the conditioned medium of PC3M cells that binds heparin, further confirming a potential role in tumor progression for prostate cancer.


Chemokine CXCL12 and its receptor CXCR4 are the most highly expressed chemokine axis in serous ovarian cancer. CXCR4 promotes tumor growth, angiogenesis, and metastasis. High CXCR4 expression is associated with poor survival, making it an attractive therapeutic target. The objective of this study was to establish an immune competent murine ovarian cancer model with high CXCR4 expression and to test single agent CXCR4 blockade and combination therapy to optimize emerging treatment strategies for future clinical trials. CXCR4 expression was evaluated by flow cytometry in ID8, ID8-VEGF, IE9-MP1, BR5, BR5-Akt, BR5-C2k, and BR5-Kras mouse ovarian cancer cell lines as well as IE9-MP1 tumor samples. Subsequently an exploratory study was undertaken utilizing the xenograft model to the assess the efficacy of AMD3100, the only commercially available CXCR4 inhibitor, alone and in combination with bevacizumab and anti-PD-1. Immune analysis of circulating lymphocytes by flow cytometry was performed during treatment to monitor changes of immune cell subsets. Surface expression of CXCR4 receptor was minimal in ID8 (1.6%), ID8-VEGF (0.9%), IE9-MP1 (1.8%), BR5 (0.9%), BR5-Akt (1.2%), BR5-C2k (2.3%), and BR5-Kras (1.1%), however intracellular staining revealed wide spread CXCR4 expression in ID8 (99.6%), ID8-VEGF (99.7%) BR5 (96.7%), BR5-Akt (94.3%), BR5-C2k (98.2%), and BR5-Kras (98.7%) cell lines. Using the ID8 intraperitoneal ovarian cancer mouse model in C57BL/6 mice, treatment with immunotherapy significantly increased cell surface expression of CXCR4 from 2% in the control group to 79.6-91.8% in the treatment group. Treatment with AMD3100 as a single agent showed no improvement in survival over control. However, AMD3100 in combination with anti-PD-1 therapy significantly improved survival. The addition of bevacizumab demonstrated no further survival benefit and bevacizumab as a single agent showed no improvement in survival over control, either alone or in combination with anti-PD-1. Analysis of circulating lymphocytes revealed a trend toward higher CD4 to CD8 ratio in mice treated with anti-PD-1 therapy. Targeting the CXCL12-CXCR4 axis in combination with other immunotherapies such as immune checkpoint inhibitors is a novel therapeutic strategy which may provide benefit over single-agent immunotherapy and warrant investigation in a clinical trial.

#948 Characterization of the microenvironment of murine primary triple negative breast cancer. Kassondra Balestrieri, Keith Pittman, Mohamed Ramirez, Nasreen Vohra, Kathryn Verbanac. East Carolina University/Brody School of Medicine, Greenville, NC.

The purpose of this study was to investigate the role of inflammatory cells in the metastatic process using reliably metastatic murine models of Triple Negative Breast Cancer (TNBC). We have previously selected, developed and characterized two reliably metastatic TNBC mouse models for our investigations. The T11 (claudin-low) and 22S2LM (basal-like) Balb/c-derived transplatable tumors display molecular profiles that mirror human TNBC. Here we report characterization of the inflammatory cells and microenvironment in these primary TNBC tumors and at metastatic sites in both heterotopic and orthotopic models. Tumors were implanted in the s.c. flank or mammary fat pad and resected 1-2 weeks later to promote the growth of seeded metastases, when volumes reached ~700 mm3 or 300 mm3, respectively. Both flow cytometric analysis and immunohistochemistry of primary tumors demonstrated high infiltration by myeloid-derived cells (CD11b+) and low levels of lymphoid cells (CD3+). Myeloid cells were also the major infiltrating cells in lung metastases and were significantly elevated compared to normal lung. The granulocytic subset (CD11b+/Ly6G+) predominated, consistent with an immunosuppressive role for cells with this phenotype (N2, GM-CSF, etc.). Primary 22S2LM and T11 tumors and metastatic lung tumor homogenates were assayed for immunomodulators and compared to normal breast and lung tissue from age-matched contemporary controls. G-CSF was significantly increased in tumors and levels (pg/mg protein) were correlated with tumor burden. VEGF was significantly elevated in all tumors, as were chemotactic factors for granulocytic cells (KC/CXCL1, MIP-2/CXCL2), monocyte cells (MCP-1/CCL2), eosinophils (Eotaxin/CCL11) and T cells (MIG/CXCL9). In the heterotopic model, lung levels of KC/CXCL1 and MIP-1/CCL2 were significantly increased prior to frank lung metastasis (5-30 days post-implant). These data suggest that TNBC-derived G-CSF may play a role in the mobilization of CD11b+Ly6G+ granulocytes and progenitors from the bone marrow and key chemokines may mediate migration of tumor cells to metastatic sites. Future studies will define phenotypic and functional characteristics of the G-MDSC that are promoting tumor progression in these murine TNBC models.

#949 Resistin potentiates stenosis and chemoresistance of breast cancer cells through STAT3 activation: implications in breast cancer health disparity. Sachin Kumar Deshmukh, Arun Bhardwaj, Sanjeev K. Srivastava, Nihkil Tyagi, Ahmed Al-Ghaidhban, Ajay P. Singh, Donna L. Dyess, James E. Carter, Seema Singh1. Mitchell Cancer Institute, Mobile, AL; 2College of Medicine, Mobile, AL.

Breast cancer (BC) is most frequently diagnosed cancer and remains the second leading cause of cancer-related deaths in women in the United States. African American (AA) women bear an additional burden of BC with early onset of
disease, poorer prognosis, higher risk of recurrence, and worst clinical outcome as compared to Caucasian American (CA) women. Emerging evidence strongly argues the role of tumor-microenvironment (TME) in BC of AA and CA racial backgrounds. On the similar line, our earlier findings suggested that serum levels of resistin; an inflammatory cytokine are significantly elevated in AA BC patients as compared to their CA counterparts. Furthermore, we demonstrated that resistin promoted growth and aggressiveness of BC cells. In the present study, we investigated the role of resistin in stemness and chemoresistance of BC cells. For this, two BC cells of CA (MDA-MB-231) and AA (MDA-MB-468) origin were treated with resistin and sphere-forming ability, a key characteristic of cancer stem cells, was determined. Data demonstrate that number of spheres is significantly increased in both the BC cell lines after resistin stimulation. Immunoblot analyses reveal that resistin-induced sphere-forming ability is associated with enhanced levels of stemness-associated transcription factors (Nanog and KLF4) in BC cells. We next examined if resistin conferred chemoresistance to BC cells. Our data suggest that resistin-treated MDA-MB-231 and MDA-MB-468 BC cells develop greater resistance against doxorubicin-induced cytotoxicity. Notably, the effects conferred by resistin were more prominent in AA BC cells: comparing to CA BC cells. Our mechanistic studies unveiled an important role of STAT3 activation in the resistin-induced stemness and chemoresistance of BC cells. Taken together, our findings provide novel insight into the role of resistin in BC biology and further strengthen its role in racially disparate clinical outcomes.

#950 Involvement of proinflammatory chemokine network between adipocytes and triple negative breast cancer cells. Rosa Misstica Coles Ignacio,1 Hyeongiwa Choi,1 Carla Gibbs,1 Eunsoon Lee,2 Samuel Adunyah,3 Deok-Soo Son4. 1Meharry Medical College, Nashville, TN; 2Florida A&M University, Tallahassee, FL.

Obesity has become a global epidemic and causes a chronic low-grade inflammation. This poses a large health burden because excess adiposity can lead to chronic diseases such as type 2 diabetes, cardiovascular disease and some cancers. Obese individuals get higher incidence, progression and mortality of cancers compared to lean ones. Particularly, breast cancer (BC) in women is closely associated with obesity. Despite an epidemiological link between obesity and low cancer survival rates, little is known about the molecular mechanisms by which obesity promotes BC progression. Obesity-derived chronic low-grade inflammation involves alteration of a chemokine network that may contribute to the cancer progression and metastasis. Herein, we proposed the novel concept that the chemokine network between obesity and BC builds an inflammatory burden via proinflammatory chemokines to promote cancer progression. We employed mesenchymal BC cell PT8119 and epithelial-like BC PT230 for triple negative BC (TNBC) cell model, and mouse 3T3-L1 preadipocyte and adipocyte conditioned media (CM) to mimic lean and obese conditions in vitro. We examined the profiles of chemokine signature of PY8119 and PY230 treated with preadipocytes and adipocytes CM using PCR array. Both preadipocytes and adipocytes CM-treated PY8119 and PY230 cells absent or low levels in chemokine receptors. On the other hand, the chemokines CXCL2, 5, 7 and CXCL10 showed higher expression in both preadipocytes and adipocyte CM-treated PT8119. Interestingly, adipocyte CM-treated PY8119 cells dominantly expressed CXCL1-3 compared to preadipocyte CM-treated cells. In addition, CXCL2, 5, 7, 20, and CXCL1, 5, 10 were highly expressed in both preadipocyte and adipocyte CM-treated PY230 cells. Moreover, CXCL2 and 3 were significantly induced in PY230 cells after treatment with adipocyte CM compared to preadipocyte CM. Taken together, the results indicate that dominant chemokines CXCL1-3 expressed in adipocyte CM-treated TNBC cells promote a pro-tumor inflammatory network. Hence, these proinflammatory microenvironment augmented by adipocytes might contribute to TNBC progression.

#952 Modulation of immune cell trafficking into human colorectal cancer by gut microbiota. Eleonora Cremonesi,1 Jesús G. Garzón,2 Valeria Governa,1 Valentina Mele,1 Francesca Amicarella,1 Elisabetta Padovan,1 Manuela Muraro,1 Paul Zajac,1 Daniel Oertli,3 Lubor Borsig,2 Giandomenica Iezzi1. 1University of Pittsburgh, Pittsburgh, PA; 2Institute of Physiologia, University of Zurich, Zürich, Switzerland; 3Department of Surgery, Universitat Spital, Basel, Switzerland.

Introduction: Colorectal cancer (CRC) is a leading cause of cancer-related death. CRC infiltration by immune cells, including cytotoxic CD8+ T cells (CTLs), IFN-gamma-producing T-helper 1 cells (Th1), Foxp3+ regulatory T cells (Tregs) and CD16+ MPO + neutrophils, is associated with favorable prognosis. However, chemokines driving these cell populations into the tumor site, their cellular sources and their microenvironmental triggers remain to be elucidated. Aim: We investigated the chemokine/chemokine receptor network promoting CRC infiltration by immune cells associated to favorable prognosis. Results: CRC infiltration by immune cells was associated with defined chemokine gene signatures, including CXCL5, CXCL9 and CXCL10 for cytotoxic T lymphocytes and T-helper 1 cells, and CCL17, CCL22 and CXCL12 for T-helper 1 and regulatory T cells. Most of these chemokine genes were expressed by tumor cells upon exposure to gut bacteria in vitro and in vivo. Indeed, chemokine expression levels were significantly higher in orthotopic xenografts than in intraperitoneal tumors, and were drastically reduced by antibiotic treatment of tumor-bearing mice. Importantly, in human CRC samples, extents of chemokine production and immune cell infiltration was significantly associated with bacterial loads. Conclusion: Gut microbiota stimulates chemokine production by CRC cells, thus favoring T cell recruitment into tumor tissues.

#953 Atypical chemokine receptor 1 (ACKR1/DARC) expressing tumors are associated with distinct recruitment of immune cells and increased pro-inflammatory chemokines. Brittany D. Jenkins,1 Rupali Hire,1 Elizabeth Howeth,1 Michele Montell,2 Rachel Martini,3 Melissa B. Davis1. 1Univ. of Georgia, Athens, GA; 2AU/UGA Medical Partnership, Athens, GA.

Interactions between chemokines and their receptors can improve a host’s anti-tumor response by influencing the targeted migration of immune cells via a chemokine gradient. Atypical Chemokine Receptor 1 (ACKR1/DARC), a genetically diverse transmembrane GPCR, acts as a decoy receptor for a variety of CXC and CC chemokines, including those with pro-malignant and pro-inflammatory effects, such as CXCL2 (MCP-1, MCAF, E) and CXCL8 (IL-8). The purpose of this study is to determine if the migration of tumor-associated immune cells is unique based on epithelial ACKR1 expression on breast cancer cells, and if this association is correlated to an increase in pro-malignant chemokines, better survival odds, and differences in race. Immunohistochemistry techniques

Materials and Methods: Twelve ovarian cancer specimens were cultured in the presence of IFNγ, indomethacin (COX-1/2 inhibitor) or/and one of two syntheticTLR3 ligands, poly-I:C (non-selective activator of TLR3 and helicases) or rintatolimod (selective TLR3 ligand). Biopsies were harvested for mRNA measurements and culture supernatants were analyzed for CXCL5, CXCL10 and CXCL22. We demonstrated that rintatolimod promoted the expression of the MDSC/Treg attractant CXCL12, which was reversed by addition of indomethacin. Furthermore, poly-I:C induced expression of COX-2 and COX-2-dependent suppressive factors (IDO, IL-10). Evaluating the COX-2 cascade, TNFα was found to be increased in the presence of poly-I:C but not rintatolimod. Additionally, Western Blot analysis showed 1-kB degradation (as a marker of NF-kB activation), selectively in response to poly-I:C. We observed that the combination of both TLR3 ligands with IFNγ and indomethacin selectively induced the desirable chemokines CXCL5 and CXCL10 and suppressed CCL22 in tumor samples with similar results in the macrophages and fibroblasts. The ovarian cancer cell lines showed only minimal expression of chemokines. Conclusion: We demonstrated that a combination of selective modulation of TME, using different clinically applicable factors and their combinations. We show for the first time, that poly-I:C, but not a selective TLR3 ligand, induces NF-kB and undesirable CXCL2-dependent suppressive factors, which though can be eliminated through addition of a COX-1/2 inhibitor. These effects may be avoided using a selective TLR3 ligand.

Background: Infiltration of cytotoxic T-cells (CTLs) in tumors is known to be associated with improved patient’s clinical outcomes. In contrast, local infiltration with regulatory T-cells (Treg) predicts accelerated progression and shorter overall survival. The above observations highlight the need for new measures to promote selective accumulation of CTLs but not Tregs in tumor microenvironments (TME). Here we compare the TME-modulating impact of poly-I:C with a selective TLR3 ligand, rintatolimod showing an induction of CCL22, CXCL12 and expression of COX-2 and downstream suppressive factors, selectively by poly-I:C. Finally, we report on the optimized combinatorial adjuvant to repro-
were used to determine expression levels of ACKR1 on primary breast tumors, along with relative expression of T-cells, B-cells, dendritic cells, and macrophages. Concentrations of pro-inflammatory chemokines in circulation were determined using a Luminex-based immunosay and matched patient peripheral blood samples. In silico analyses were performed to determine associations between expression of ACKR1 on survivin mRNA in human breast cancer cell lines and immunofluorescence techniques, co-localization between ACKR1 and selected pro-inflammatory chemokines was investigated. Results from these tests indicate that there is differential expression of immune cell types in tumors expressing ACKR1, and this difference was associated with the migration of B-cells and dendritic cells, which were not detected in ACKR1-negative tumors. Significantly increased circulating CCL2 and CXCL8 chemokine levels we also determined to be positively correlated with ACKR1 expression in primary breast tumors. Survival analyses showed a significantly increased relapse free survival in patients having tumors with high ACKR1 expression, while investigations into racial differences revealed a significant race effect, with Caucasians having higher ACKR1 levels on their tumors than African-Americans. Finally, co-localization between ACKR1 with CCL2 and CXCL8 is observed in cultured human breast cancer cells. Given that the data collected shows a tendency for those tumors positively expressing ACKR1 to have a more favorable prognosis, we suggest that a partial role of ACKR1 on breast tumor cells is to sequester pro-inflammatory chemokines in the tumor microenvironment, indirectly recruiting a distinct subset of tumor-associated immune cells.

#954 TGF-ß signaling inhibition counteracts myofibrosis in MPN. Matthias Bartenstein,1 Lanzhu Yue,2 Wanke Zhao,3 Wanting Tina Ho,3 Cem Murdun,2 Adam W. Mailloux,2 Ling Zhang,2 Anjali Budhakoti,1 Kith Pradhan,1 Franck Rapaport,1 Huaqiang Wang,3 Zonghong Shao,3 Ulrich Stein1, Ross L. Levine1, Zhizhuang Joe Zhao,1 Amit K. Verma,1 Pearl K. Epling-Burnette1,1 Albert Einstein College of Medicine, Bronx, NY; 2Moffitt Cancer Center and Research Institute, Tampa, FL; 3Ohio University Health Sciences Center, Athens, OH; 4Memorial Sloan Kettering Cancer Center, New York, NY; 5Tianjin Medical University General Hospital, Tianjin, China.

Myofibrosis (MF) is a common feature of the Philadelphia-Chromosome negative (Ph-) myeloproliferative neoplasms (MPN), a group that includes Primary Myelofibrosis (PMF), Essential Thrombocytosis (ET) and Polycythemia Vera (PV). Current treatment with Jak2 inhibitors reduces splenomegaly, but does not eliminate malignant clones nor improve bone marrow (BM) or spleen fibrosis, understimating the need for alternative therapies. One promising target is TGF-ß1, a cytokine crucial for the development of myofibrosis that is elevated in PMF patient plasma, progenitors and megakaryocytes (MK), and is known to promote fibrosis through stimulation of fibroblasts and their progenitors, mesenchymal stroma cells (MSC). Dysregulation of TGF-ß1 signaling has been described in a number of mouse models of myofibrosis exhibiting aberrant megakaryopoiesis, one of the hallmarks of MPNs. Preliminary data from 93 MPN patients showed over-expression of TGF-ß in PMF samples (p = 0.001), with increased activation of the canonical target SMAD2 (p = 0.005). We tested the efficacy of the orally available TGF-ß1 signaling inhibitor Galunisertib (LY2157299) in counteracting fibrosis in two MF mouse models of common MPN oncogenes Jak2 and Mpn. Mice in the first model express the constitutively active human Jak2V617F mutant under the control of the ubiquitous hematopoietic promoter vav1 and show a PV-like phenotype at 6 weeks old, progressing to fibrosis of bone marrow (BM) and spleen around 25-30 weeks. Thirty week old mice were treated with Galunisertib for 4 weeks and showed a significant decrease in fibrosis compared to control mice. Similar results were observed in 50 week old mice treated with a murine antibody against TGF-ßRIR (IMC-TR1,LY3022859) for 4 weeks. Our second model relies on transplantation of mice with BM cells transduced with MPLW515L, a mutant mpl variant that is active in the absence of TPO. These mice rapidly develop MF, myeloproliferation and splenomegaly. Flow cytometry showed immature and mature megakaryocytes with overexpression of TGF-ß1 in MPLW515L cells. Treatment from day 12 to day 26 after transplantation reduced both white blood cell counts (a measure of myeloproliferation) and fibrosis as measured by reticulin staining. In vitro, Galunisertib counteracted the pro-fibrotic effects of TGF-ß1 on both murine and human MSC by antagonizing its stimulatory effects on collagen I and III production. Analysis of MSCs from Galunisertib-treated mice also showed reduced collagen I and III production compared to controls. In conclusion, we offer further evidence that aberrant megakaryopoiesis drives TGF-ß1 overproduction in MNP by increasing megakaryocyte numbers and overexpressing TGF-ß1 in mutant MKs. Galunisertib counteracts TGF-ß1 induced fibrosis in two MPN mouse-models and is a promising candidate for symptomatic treatment of myofibrosis.

#955 LY3200882, a novel, highly selective TGFßRII small molecule inhibitor. Huaxing Pei1, Saravanan Parthasarathy,2 Sajan Joseph,3 William McMillan,1 Xiaohong Xu,1 Stephen Castaneda,4 Ivan Inigo,3 Karen Britt,1 Bryan Ander son,2 Gayming Zhao,2 Scott Sawyer,2 Douglas Beight1, Talbi Kaoudi,1 Chandrasekar Iyer,1 Huimin Bian,1 Amy Pappas,1 David Surguladze,3 David Schauer,4 Karim Benhadji,3 Michael Kalos,2 Kyla Driscoll1, Eili Lilly, Indianapolis, IN; #Eili Lilly, New York, NY.

The transforming growth factor ß (TGFß) signaling pathway is a pleiotropic cellular pathway that plays a critical role in cancer. In fact, aggressive tumors are typically associated with high ligand levels and thus associated with poor prognosis in various tumor types. Cancer cells use autocrine and paracrine TGFß signaling to modulate tumor cells and the tumor microenvironment, leading to a highly invasive and metastatic phenotype, inducing and increasing tumor vascularization, modulating the extracellular matrix in the stroma, and inhibiting immune surveillance and antitumor immunity. Clinical studies with galunisertib (aka LY2157299 monohydrate), a small molecule inhibitor targeting the TGFß pathway, have provided proof of concept data supporting the role of TGFß in cancer and the utility of targeting the TGFß pathway. Here we describe the identification of LY3200882, a next generation small molecule inhibitor of TGFß receptor type 1 (TGFßRI). The molecule is a potent, highly selective inhibitor of TGFßIRI embodied in a drug-like platform with high ligand levels and thus associated with poor prognosis in various tumor types. Cancer cells use autocrine and paracrine TGFß signaling to modulate tumor cells and the tumor microenvironment, leading to a highly invasive and metastatic phenotype, inducing and increasing tumor vascularization, modulating the extracellular matrix in the stroma, and inhibiting immune surveillance and antitumor immunity. Clinical studies with galunisertib (aka LY2157299 monohydrate), a small molecule inhibitor targeting the TGFß pathway, have provided proof of concept data supporting the role of TGFß in cancer and the utility of targeting the TGFß pathway. Here we describe the identification of LY3200882, a next generation small molecule inhibitor of TGFßRI for the treatment of cancer.


Ovarian cancer continues to be the most lethal gynecologic malignancy with no real cure for patients presenting with advanced stage disease. Immune checkpoint blockade showed modest clinical response in patients with recurrent ovarian cancer, thus additional therapeutic strategies for combination therapy are needed. As chemokines and their receptors drive both immune cell migration and tumor growth, angiogenesis and metastasis formation, they are an attractive target for combinatorial cancer therapy. CXCR4 is the most highly expressed chemokine receptor in advanced stage high grade serous ovarian cancer, thus the objective of this study was to evaluate the efficacy of a novel oral small molecule CXCR4 inhibitor (X4-136) alone and in combination with immune checkpoint inhibition and the anti-angiogenic agent bevacizumab, and characterize the changes in circulating immune cells during treatment in murine ovarian cancer model. The ID8 cell line was used in C57BL/6j mice to establish an immune competent murine model and to compare single agent and combination therapy with oral X4-136 CXCR4 inhibitor, bevacizumab, and anti-PD1. During treatment blood sampling was performed and immune cells were analyzed by flow cytometry. Our results demonstrated that single agent therapies alone with either drug had no significant effect on tumor progression or survival. Combination therapy with the CXCR4 inhibitor and anti-PD1 improved survival compared to control animals and the other combination therapy groups. The addition of bevacizumab to the dual combination did not fur-
Use of angiotensin system inhibitors is associated with longer overall survival in pancreatic ductal adenocarcinoma patients who underwent partial or total gastrectomy. Huamao Liu, Matthias Pinter, Joo Anjo, Hang Lee, William Ho, Jonathan Crain, Kamila Naxerova, Mengyang Di, Alex Jacobson, Daniella Dias Santos, Andrea Zanconato, Vikram Deshpande, Keith Lillemoe, Carlos Fernandez del Castillo, Michael Downes, Ronald Evans, James Michaelson, Cristina Ferrone, Yves Boucher, Jain K. Rakesh. Massachusetts General Hospital, Boston, MA; Brown University, Providence, RI; Salk Institute, La Jolla, CA.

Introduction: Angiotensin system inhibitors (ASI) are widely used to manage hypertension. Laboratory and retrospective clinical data suggests that ASIs can improve cancer prognosis. The aim of this study is to investigate the effect of ASIs on overall survival in pancreatic ductal adenocarcinoma (PDAC) patients.

Methods: We performed a retrospective review of the clinicopathologic records of patients with PDAC seen at the Massachusetts General Hospital (MGH) between 1/2006 - 12/2010. Patients on angiotensin converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARB) were included as ASI users. We performed RNAseq and Gene Set Enrichment Analysis (GSEA) of primary tumor samples from patients with or without chronic ASI use. We also identified a surrogate signature of differentially expressed genes and we measured the extent of angiotensin inhibition in GEO and TCGA datasets. The extent of inhibition was used to correlate with survival. Statistical analysis was performed using Kaplan-Meier estimator and Cox proportional hazards ratio model. Results: A total of 794 consecutive PDAC patients were included, of whom 297 (37.4%) were on ASIs and 183 (23.0%) were on non-ASI antihypertensive drug therapy. In resected patients, ASI users had a significantly longer overall survival (37.4%) were on ASIs and 183 (23.0%) were on non-ASI antihypertensive drug therapy. In resected patients, ASI users had a significantly longer overall survival than ASI-naive patients (p = 0.001). In our sub-group analysis of resected hypertensive patients treated with chemotherapy, chronic ASI users had a significantly longer overall survival than ASI-naive patients (p = 0.048) (28.7 vs 12.3 months, p = 0.05). Gene Set Enrichment Analysis revealed that the ASI lisinopril down-regulated genes which stimulate the mitotic cell cycle, WNT and Notch signaling. The interaction between integrins and the extracellular matrix. Lisinopril also enhanced gene sets linked with oxidative phosphorylation, antigen processing and presentation and the cytotoxic activity of T cells. In unresected patients, the effect of ASI was only significant in patients with locally advanced disease in multivariate analysis (HR, 0.57; 95%CI, 0.38-0.847; p = 0.005), but not in metastatic patients. The low expression of genes down-regulated by ASIs was significantly associated with better survival in the TCGA and GSE71279 datasets. Conclusion: In patients with PDAC, ASI use is associated with longer overall survival in resected patients and may benefit patients with locally advanced disease. These findings suggest the need for a prospective study to determine the efficacy of ASI in PDAC patients.

Blocking CD70+ cancer associated fibroblasts: Are we paving the way towards immunotherapy in colorectal cancer. Julie Jacobs, Vanessa De-schoolemeester, Karen Zwanepoel, Christophe Hermans, Christiaan Rolfo, Marc Peeters, Filip Lardon, Vasiliki Siozopoulou, Eveline Smits, Patrick Pauwels, University of Antwerp, Wilrijk, Belgium; Antwerp University Hospital, Edegem, Belgium; Vaccine and Infectious Disease Institute, Edegem, Belgium.

Introduction: Numerous studies have reported that tumor progression and invasiveness are determined not only by the malignant cancer cells themselves but also by the surrounding tumor microenvironment, including cancer-associated fibroblasts (CAFs). Although CAFs are implicated in tumor progression, their total depletion of CAFs has been demonstrated to induce more aggressive tumors, indicating that different CAF subpopulations have opposing tumor-promoting or tumor-inhibitory roles. Unfortunately, specific markers to target these subsets of CAFs are lacking. Expression of the immune checkpoint CD70 is normally tightly regulated and limited to cells of the lymphoid lineage only. Instead, tumors hijack CD70 to facilitate immune evasion by increasing the amount of suppressive regulatory T cells (Tregs), inducing T cell apoptosis and skewing T cells towards T cell exhaustion. Recently, a lot of clinical successes have been generated by the blockade of immune checkpoints. However, in colorectal cancer (CRC) the efficacy remains limited to a small subset of patients with mismatch repair-deficient (MSI) tumors which might be caused by the intense dialogue between stroma and malignant cells. Therefore, we have explored the role of CD70 expression in CRC with a particular focus on CAFs. Methods: The prognostic value of CD70 was analyzed by immunohistochemistry on 51 CRC specimens. In addition, the relationship with Tregs and microsatellite instability was explored. Furthermore, primary CAF cell lines were successfully cultured from 20 different primary resection specimens. These cell lines were used to study the effect of CD70 on the tumor microenvironment in vitro. Results: We revealed expression of CD70, not just on the malignant cells but on the majority of CAFs in invasive CRC specimens. Thereby, CD70-expression was significantly correlated with negative clinicopathological parameters such as metastasis (P = 0.007), differentiation (P = 0.053) and advanced stage (P = 0.001). Moreover, CD70-positive CAFs proved to be a poor prognostic marker by univariate as well as multivariate analysis. We have also detected a significant association between elevated Treg amounts and CD70-expressing CAFs (P = 0.012). In vitro data on the effects of CD70 on CRC behavior and immune escape are currently being analyzed. Conclusion: We have identified a new targetable CAF subpopulation, marked by the expression of CD70 and equipped with strong tumor-promoting properties. Thereby, we have found potential of a potential cross talk between CD70+ CAFs and Tregs to modulate immune escape. A lack of association of CD70 expression and MSI-status, which highlights the potential of this target in CRC subsets that do not benefit from immune checkpoint blockade. We believe that targeting CD70 holds great potential in CRC, especially in light of the limited immunotherapeutic options available.
mediated changes in microenvironment in a xenograft mouse model using primary cells from 3 U-CLL and 3 M-CLL cases. Ibrutinib significantly inhibited CLL cell growth in spleen; the treated CLL cells developed elevated dysfunctional smCXCR4. The inhibition on tumor growth and impaired CXCR4 occurred to much greater extent in U-CLL. Notably, ibrutinib also significantly inhibited T cell growth only in U-CLL cases. Thus, ibrutinib inhibited CXCR4 signaling and CLL B-T cell crosstalk essential for tumor growth in tissue niches, especially in U-CLL cases that has limited B-lymphocytosis. Finally, molecules controlling the vulnerability of U-CLL B cells to death in the absence of environmental prosurvival signals were examined in 15 patients. Ibrutinib reduced BCL2 protein levels in U-CLL but not M-CLL; levels of BCL-XL and MCL-1 were unchanged. Consistent with these results, U-CLL but not M-CLL cell survival in vitro was promoted by T cell stimulation that led to upregulation of prosurvival BCL2. These findings suggest enhanced CLL B cell death by inhibiting BCL2 in U-CLL after the loss of environmental prosurvival signals. Altogether, ibrutinib inhibits CLL-microenvironment crosstalk by blocking CXCR4 signaling and T cell support. U-CLL cells had a greater vulnerability to the loss of environmental prosurvival signals after ibrutinib inhibition. Our data demonstrate mechanisms underlying the differences in primary ibrutinib sensitivity between U- and M-CLL patients, supporting the use of inhibitors of BTK and BCL2 in CLL.

**#961 Inhibitors of fibrosis and TGF-beta delay tumor xenograft growth and reduce ascites in ovarian cancer models.** Qing Zhang, Xiaonian Hou, Bradley J. Evans, John J. Weroha, William A. Cibilly, Mayo Clinic, Rochester, MN

Background: Most ovarian cancer (OC) patients present with advanced disease and ultimately develop chemoresistant relapses. Stromal plays a significant role in OC behavior including invasiveness, fibrosis and chemoresistance. TGF-beta is important for crosstalk between stroma and cancer cells, and stromal activation in cancer has similarities to matrix remodeling in fibrosis. We have shown that TGF-beta and fibrosis-inhibitors can suppress OC cell proliferation, migration and invasion. Such inhibitors are available for clinical use, and we have begun to test their efficacy in OC in vivo. Methods: Animal experiments were approved by Mayo Clinic IACUC. Intraperitoneal xenograft models were used to test their efficacy in OC in vivo. Lenti-CycloHeximide (Cyc), a lentiviral vector encoding a short hairpin RNA (shRNA) targeting Cycloheximide (CHX), was used to silence Cyc expression in ovarian cancer cells. We treated three groups of mice with Cyc, Cyc + TGF-beta, and Cyc + TGF-beta + PH003, and we compared the tumor volume, weight, percent tumor necrosis, and ascites volume on day 14 and 28. Results: Cyc inhibited tumor growth and reduced ascites volume and weight at day 14 and 28. Cyc + TGF-beta + PH003 inhibited tumor growth significantly more than Cyc alone. Conclusion: Inhibiting Cyc expression reduces tumor growth and ascites volume, suggesting a novel mechanism of action for Cyc that may be applicable to other cancers as well.

**#963 Ovarian-dependent SMM22 mouse mammary carcinoma cells depend on Cyp3a11 oxysterolxygenase activity for proliferation.** Zhijun Guo,1 Patrick McGrath,3 Ted Bebb,1 Ashley Mantheyan,2 Alexandre Osorio,3 Cesar Hertes,3 Victor Arrieta,4 Sebastian Mohar,4 Irwin Hernandez,4 Robert Klink,5 Robert Cardiff,2 David A. Potter1. 1.Univ. of Minnesota, Minneapolis, MN; 2.Univ. of Central Florida, Olando, FL; 3.Instituto Nacional de Cancerología, Mexico City, Mexico; 4.Univ. of California, Davis, CA

Introduction: Silencing of the human cytochrome P450 (CYP) monooxygenase enzyme CYP3A4 in the ER + MCF-7 tumor cell line inhibits tumor growth in the mammary fat pad, but the relative roles of tumor cell intrinsic CYPs versus vascular/microenvironment CYPs remain to be determined and a syngeneic animal model is needed to answer this question. The SMM22 ovarian dependent mouse mammary carcinoma cell line forms a transplantable estrogen receptor positive (ER+) mouse mammary tumor in which growth dependence on cell intrinsic CYPs may be tested in vitro and in vivo. The SMM22 cell line is derived from 129SvY homozygous Stat1-null female mice, which develop ER+ mammary tumors (1,2). Expression profiling of spontaneously arising mouse mammary carcinomas in a TP53 KO model revealed that the CYP3A4 ortholog Cyp3a11 is up-regulated in these tumors. Methods: Cyp3a11 bacTomato were used to assay for NADPH dependent biosynthesis of EETs from arachidonic acid using an LC-MS/MS method. siRNA silencing and CRISPR/Cas9 knock-out of the Cyp3a11 exons 2 and 3 was performed and the cell lines were characterized for EET dependence using an MTT assay. The highly potent and chemical probe of CYP epoxygenase activity, hexyl-benzyl-biguanide (HBB), was used to test dependence of SMM22 cells on CYP epoxygenase activity. Total cellular EETs in SMM22 cells exposed to HBB at the IC50 were measured by the LC-MS/MS method. Results: Cyp3a11 knock-out cells and Cyp3a11 knock-in cells were indistinguishable in terms of sensitivity to HBB, and HBB does not impact cell viability or tumor growth in vivo. Silencing of Cyp3a11 decreased proliferation, reduced total cellular EET levels, and reduced tumor growth in vivo. Conclusions: Cyp3a11 is required for cell proliferation and tumor growth in vitro and in vivo. This model allows for testing the role of Cyp3a11 in the growth and dissemination of tumor cells in the absence of tumor-induced niche remodeling and provides a model to study the role of Cyp3a11 on tumor growth in vivo.

**#962 Novel targeting of tumor-infiltrating TNFR2 Tregs: removing suppression with microenvironment-specific antibodies.** Heather Torrey, John Butterworth, Toshiyuki Mera, Yoshikao Okubo, Limei Wang, Danielle Baum, Audrey Defusco, Sara Plager, Sarah Warden, Daniel Huang, Eva Vanamee, Rosemary Foster, Denise Daud, Faeheen Faustman, Massachusetts General Hospital & Harvard Medical, Charlestown, MA

Background: A recognized and potent subtype of regulatory T cells (Tregs) expresses tumor necrosis factor receptor 2 (TNFR2), a member of the tumor necrosis factor (TNF) superfamily. TNFR2 expressing Tregs are abundant in human and murine tumors and are the most potent suppressors of host immune responses. In addition, recent data in diverse human tumors show that TNFR2-expressing host T cells are the dominant Tregs in the tumor microenvironment, expressed far in excess of OX40 and GITR. Unlike many tumor markers, TNFR2 has limited expression in the immune system and almost no inhibitory activity, making it an attractive target for anticancer therapy. Methods: We produced monoclonal antibodies to human TNFR2 with the goal of inactivating the potent host Tregs that express TNFR2, competing with the agonism of TNF and inactivating infiltrating Tregs of human tumors. Tregs from fresh ovarian ascites fluid were compared to Tregs from normal human blood donors for antibody potency. Results: Using a 48-hr ELISA with T cells treated with metalloproteinase inhibitors, we showed that TNFR2 antagonistic antibodies can inhibit Treg proliferation, suppress soluble TNFR2 secretion on normal cells and allow T effector expansion. The antagonism is dominant, succeeding even in the presence of TNF, which is a TNFR2 agonist. Furthermore, the structural biology of dominant TNFR2 receptor antagonism uncovers a unique conformation that equally inhibits downstream NFκB signaling. We also showed that dominant TNFR2 antibodies in a dose dependent fashion kill Tregs in ovarian cancer infiltrates more strongly than Tregs from healthy donors. Treg killing in the cancer microenvironment was dependent on cell proliferation and turnover of the Tregs. Conclusion: In sum, blocking TNFR2 signaling on the most potent Tregs of the tumor microenvironment with a dominant antagonist antibody selectively heightens death of the immunosuppressive Tregs in the tumor microenvironment and fosters an outgrowth of the few residual Tregs that remain. With the hypothesis that TNFR2 Tregs are the most potent host cells of the immune response, their dominance in diverse human tumor infiltrates and the natural low expression of this marker on normal lymphoid cells makes targeting of TNFR2 in the tumor microenvironment attractive.
#964 A quantitative flow cytometric assay for the simultaneous detection of PD-L1 in tumor and immune infiltrate present in NSCLC. Ryan T. Rodriguez, Amanda Chargin, Rian Morgan, Randall K. Wetzel, Reginaldo Prioli, Bruce Patterson, Keith Shutts, IncellDx, Menlo Park, CA; 2Cell Signaling Technology, Danvers, MA.

Flow cytometric analysis of the inherent complexity of the immune checkpoints becomes clearer, we must understand the expression of immune modulators such as PD-L1 within the context of the tumor microenvironment. This includes measures of immune infiltrate, levels of expression of immune modulators, such as PD-L1, on lymphocytes and tumor cells, as well as DNA heterogeneity within tumor cells. We show how characterization of the rabbit anti-PD-L1 clone E1L3N is a commercially available method in a flow cytometric assay. First we demonstrate specific fluorescence and sensitivity in tumor derived cell lines and then combine cell lines and PBMCs to form a tumor microenvironment mimic. When E1L3N was combined with antibodies directed toward CD45, CD3, and CD38, the cellular subsets known to be present in the tumor micro environment, including cytotoxic lymphocytes, T-helper cells and CD45 negative tumor derived cells are readily detected. The use of sequential gating strategies allows the assay to detect PD-L1 on each of these subsets which, when combined with quantitative MESF beads (Bang Laboratories) along with the known fluorescence-to-protein ratio of each antibody, allows the calculation of the average relative PD-L1 receptors bound for each cell type. This assay further segregates cells using the DNA-specific dye (DAPI) allowing the measurement of proliferation and delineation of the mutated aneuploid fraction. We demonstrate the assay’s ability to measure quantitative PD-L1 expression, immune infiltrate, and tumor ploidy in NSCLC tumor samples. We believe this will be a valuable assay in the clinician’s toolbox to make informed decisions for patients who may respond best to PD-1 pathway inhibitors.

#965 Two in one: nanotechnology based strategies for the treatment of ER+ breast cancer. Maria Ines Diaz Bessone,1 Andrea Simón-Gracia,1 Pablo Scodeller,2 Tamboet Teesalu,3 Marina Simian1, Universidad Nacional de San Martín, Buenos Aires, Argentina; 2University of Tartu, Tartu, Estonia.

Understanding endocrine resistance mechanisms is key to develop new therapeutic strategies. We focus on the role the tumor microenvironment plays as a modulator of endocrine therapy resistance in breast cancer, in particular associated to Tamoxifen. Studies show that the use of nanoparticles (NPs) as antitumor drug delivery systems is a good strategy to improve the efficacy as third secondary effects of conventional chemotherapies. In this context we hypothesize that a therapeutic strategy based on the use of Tamoxifen carried in NPs coated with the tumor penetrating peptide iRGD, would be more effective than conventional Tamoxifen. Neuruplin-1, that mediates iRGD induced endocytosis, has been shown to be associated to breast cancer stem cells. Thus, we postulate that our multifunctional NPs would be effective in reducing this cell population, contrary to what is observed with free Tamoxifen. Moreover, iRGD blocks the interaction between integrin β1 and fibronectin, a mechanism we have previously shown induces Tamoxifen resistance (Pontiggia et al. 2012). NPs were synthesized with polyethylene glycol and polycaprolactone and coated with the iRGD peptide. This peptide was constructed with a FAM fluorophore in order to track the NPs. To evaluate the cell uptake, MCF-7 cells were incubated for 5 h with iRGD-NP or FAM-NP as a control. NP entry was higher when the NPs were coated with iRGD, both in 2D and in 3D cultures. Cell viability experiments revealed that Tamoxifen encapsulated in NPs was more effective than the free drug (p<0.05), and NPs coated with iRGD had an even stronger effect (p<0.01). Moreover, resistance to Tamoxifen induced by fibronectin was reversed with the iRGD-coated NPs, contrary to what is observed with the free drug. Previously, we demonstrated that treatment with Tamoxifen enhances the stem cell population in estrogen receptor-negative breast cancer cell lines (Raffo et al. 2013). In this context iRGD coated NPs lead to a decrease in breast cancer stem cells as evaluated by mammosphere assays (p<0.01). Finally, in vivo tumor homing was evaluated showing that the NP carrying the fibronectin/sidebody for the biodistribution of the NPs. Nude mice carrying MCF-7 breast tumors were treated with iRGD-NP and control NPs. Tumors were collected at 4 and 24 hrs post inoculation. Frozen sections were analyzed by confocal microscopy revealing that only the mice treated with the iRGD-NPs had high levels of NPs in the tumor, compared to the control group. Moreover, frozen sections of kidneys, livers, lungs and spleen showed very low levels of iRGD-NPs, confirming that the targeting was effective. In vivo experiments of the impact of iRGD-NPs on tumor growth, metastasis and the stem cell population are ongoing. These results suggest that iRGD-NPs carrying Tamoxifen could be an effective strategy to avoid the development of endocrine resistance in breast cancer.

#966 Phase II clinical trial patient responses to the macrophage activating agent RRx-001 correlate to TGF-β pathway activation and markers for fibrosis. Saheli Jha, Thomas A. Summers, Karen Zeman, Christine Brzezniak, Corey Carter, Lindsey Ferry, Jan Scicinski, Bryan Onorosky, Scot Caroen, Jane B. Trepel, Pedro Cabrales, Regina Day, Epicentrx, Mountain View, CA; 2USUHS, Bethesda, MD; 3NCI NIH, Bethesda, MD; 4NCINIH, Bethesda, MD; 5UCSD, La Jolla, CA.

Introduction: RRx-001 is a novel systemically non-toxic small molecule macrophage stimulating agent with promising activity in an ongoing clinical trial in small cell and non-small cell lung cancer and in neuroendocrine tumors. In preclinical studies, RRx-001 activated normally immune-suppressive M2-like Tumor Associated Macrophages (TAMs) to express a series of pro-inflammatory cytokines and chemokines such as transforming growth factor-β (TGF-β1), tumor necrosis factor-α (TNF-α), inducible nitric oxide synthase (iNOS), and interleukins-10 and -12, a profile that resembles the M1 activated macrophage state. Due to its chemotactic activity for macrophages, TGF-β1 was hypothesized to be involved in the antitumor mechanism of RRx-001. We therefore compared expression patterns of TGF-β1 and TGF-β1R in patient-derived biopsy samples obtained at screening and post RRx-001. As TGF-β1 is closely associated with the induction of fibrosis, we also examined key fibrosis markers. Methods: Tumor biopsies before and after treatment with RRx-001 were obtained from consented patients with NSCLC, SCLC, and neuroendocrine tumors in the QUADRUPTPE TREAT Phase II clinical trial (NCT02469903). Post treatment biopsies were obtained 6 and 24 weeks after RRx-001 treatment co-incidental with the first on-study CT scan. Tumor samples were evaluated immune-histochemically with putative markers for TGF-β1 pathway activation (TGF-β1, TGF-β1R, fibrosis (alpha-smooth muscle actin [α-SMA], Matrix metallopeptidase-9 [MMP-9], and Collagen III deposition), and macrophage activation. Patients were subsequently followed for tumor progression. Results: Positive immune-histochemical staining for TGF-β1 and TGF-β1R was seen in all responding patients, and in none of the non-responding patients to date. In responders we also found that MMP-9 and α-SMA activity was down regulated post RRx-001. The down-regulation of these fibrosis markers is suggestive of less invasive malignancy. The number of tumor associated macrophages and their activation was related to the activity of RRx-001. Conclusions: Our pre and post dose patient derived data to date indicate a correlation between TGF-β signaling activation and a response to RRx-001 that may also correlate with increased numbers of macrophages, and their activation status, in the vicinity of the tumor. These data suggest that activation of the TGF-β1 pathway, as evident by expression of TGF-β1 and TGF-β1R by tumor cells, could be a predictive biomarker for RRx-001 treatment.

#967 Development of fluorescent-cell based high throughput assay to identify novel therapeutics of bladder cancer cells through upregulation of SPARC expression. Chirayu M. Patel. Wake Forest Health Sciences, Winston-Salem, NC.

Bladder cancer is the most common malignancy affecting the urinary system with an estimated 76,960 new cases and projected 16,390 deaths in 2016 in the United States. The role of SPARC in various cancers is contextual and differs based on the tumor classification, malignancy and progression. We have recently reported the tumor suppressor effect of SPARC in urinary bladder cancer. We have shown that the loss of SPARC in bladder cancer resulted accelerated bladder carcinogenesis and metastasis multiple pathways involving cell cycle deregulation, inflammation and angiogenesis. We have also reported that SPARC protein expression significantly decreased in the cancerous compartment in advanced human bladder cancer as well as in carcinogen-induced murine urothelial cancer. However, the mechanism of the downregulation of SPARC expression in cancer in general and in bladder cancer in particular is still unraveled. The purpose of our study is to develop a robust, high throughput assay to identify therapeutic candidates as regulators of SPARC expression in bladder cancer. We cloned SPARC promoter in a third generation lentiviral vector coupled with fluorescent GFP or mCherry fluorescent protein to quantitatively measure SPARC expression. We generated stable bladder cancer cell lines expressing SPARC expression in bladder cancer cell lines. UMUC3 bladder cancer cells were transduced with lentiviral vector to generate SPARC-promoter reporter system to screen regulators of SPARC expression. Reporter cells were plated in 96 well plates and treated with 10μM of FDA-approved and natural product drug libraries. Real time monitoring of changes in SPARC expression was done by measuring GFP fluorescence intensity using IncuCyte® ZOOM Live Imaging Analysis System. We identified drugs that consistently exhibited dosage-dependent augmentation or down-regulation of SPARC expression in UMUC3 cells. Our screens identified novel up-regulators of SPARC expression that are both standard of care and novel bladder cancer therapeutics. Our efforts
produced an efficient robust assay to identify novel therapeutics and regulators of the tumor suppressor SPARC in the bladder cancer ecosystem that can be used in the adjuvant and neoadjuvant settings.

#968 Podoplanin’s diverse potential as a chemotherapeutic target for oral squamous cell carcinoma. Edward P. Retzbach,1 Harini Krishnan,1 Jhon A. Ochoa-Alvarez,2 Yongguan Shen,3 Evan Nevel,3 David J. Kephart,1 Evelyne Kayoussel,4 Soly Baredes,4 Mahnaz Fatahzadeh,5 Kingsley Yin,1 Alan J. Shenbaum1,678 Background: Podoplanin (PDPN) is a transmembrane receptor podoplanin that has been postulated to have potential for targeting PDPN in oral cancer. Taken together, these data suggest that PDPN may undergo cancer specific changes, which indicates another potential route for targeting PDPN in oral cancer. Here, we describe efforts to target PDPN in order to prevent and combat oral cancer.

Over 90% of oral cancers are oral squamous cell carcinoma (OSCC). Most OSCC cells express the transmembrane receptor podoplanin (PDPN), which has emerged as a promising target for OSCC treatment. The PDPN receptor promotes tumor cell invasion and metastasis, which leads to the vast majority of cancer deaths. Here, we describe efforts to target PDPN in order to prevent and treat oral cancer. PDPN can be targeted with Maackia amurenensis seed lectin (MASL) to inhibit tumor cell migration and viability. Recent evidence also suggests that PDPN may undergo cancer specific changes, which indicates another potential route for targeting PDPN in oral cancer. Taken together, these data indicate that PDPN can serve as a functionally relevant target to prevent and combat oral cancer.

#969 Expression of membrane-type matrix metalloproteinases (MT-MMPs) in head and neck squamous cell carcinomas, a target for prodrug development. Renée Ankerah,1 Steve D. Shryder,1 James A. McCauld,2 Phil A. Batman,1 Robert Falconer,1 Paul M. Loadman1, Univ. of Bradford, Bradford, United Kingdom; 2Royal Marsden Hospital and Northwick Park Hospitals, London, United Kingdom.

Introduction: Head and Neck Squamous Cell Carcinomas (HNSCC), a group of highly aggressive heterogeneous epithelial malignancies, are considered the fifth most common form of cancer worldwide. Despite advances in treatment, low five-year survival rates of around 30% remain. Several studies have implicated the actions of matrix metalloproteinases (MMPs) in HNSCC progression. Their role in mediating extracellular matrix remodeling is paramount to tumour progression, as they facilitate invasion, angiogenesis and metastasis. In contrast, expression of active MMPs in normal tissues is largely absent. MT1-MMP (MMP14) expression is known to correlate with poor clinical response in several tumour types, but little is known about the expression of other MT-MMPs in HNSCC. We have assessed the protein expression of the entire MT-MMP complement in matched tumour and normal tissues of clinical HNSCC cases by immunohistochemistry, supported by Western Blot and gene expression data and will ultimately demonstrate translation of protein expression into MT-MMP-specific proteolytic activity. Differential expression would support the development of prodrugs specifically activated in the tumour by MMPs. Methods: 32 clinical cases (matched normal & tumour tissues) were assayed for gene expression of the 6 members of the MT-MMP family (MMP14,15,16,17,24,25). Expression profiling was carried out using quantitative real-time reverse transcription-PCR analysis. Analysis of protein expression used IHC on fixed sections used with monoclonal antibodies specific for MT1 to MT6 MMP and Western blot analysis with monoclonal antibodies optimized for western blotting. Results: The protein expression of all six MT-MMPs has been characterised in clinical HNSCC samples and related to matched normal tissue controls. A differential in gene and protein expression levels exists between the MT-MMP profiles across the normal and cancer tissues examined. MT1, MT3- and MT6-MMP are highly overexpressed at both gene and protein level in the majority of tumours. MT2, MT4 and MT5-MMP expression was low or undetectable in all normal tissue and low in tumour tissue Conclusion: The existence and demonstration of differential MT-MMP protein expression in HNSCC relative to normal tissues is a valuable tool for prodrug development. MT1, MT3 and MT6-MMP are highly expressed in the majority of tumour samples compared to matched normal control tissue. However, further work to elucidate the proteolytic activity of the expressed proteins and therefore the proteolytic capacity of tumours is needed to determine the suitability of HNSCC for prodrug therapy.

#970 Probing the expression and function of aldehyde dehydrogenases in prostate cancer using ALDH-affinic compounds and siRNA. Maria Sadiq,1 Ali I. Ibrahim,1 Fionna Frame,2 Simon J. Allison,3 Mark Sutherland, Rogers M. Phillips,4 Norman J. Maitland,5 Klaus Pors1, Univ. of Bradford, Bradford, United Kingdom; 3University of York, York, United Kingdom; 4University of Huddersfield, Huddersfield, United Kingdom.

The human aldehyde dehydrogenases (ALDHs) play a major role in detoxifying highly reactive aldehydes into carboxylic acids. Deregulation of ALDHs has implications in a number of cancers including prostate cancer. They play an important role as a cancer stem cell (CSC) marker due to high activity found in CSCs while high expression is also known to lead to resistance to drugs including docetaxel. Although the exact role of ALDHs is not fully understood, emerging information indicates several isoforms including ALDH1A3 and ALDH7A1 play a key role in cancer. To further elucidate the role of ALDHs in prostate cancer, we here report on the perturbation of ALDH expression and function using chemical probes and siRNA. Primary prostate epithelial cell cultures from patient tissue were used for this study. Cancer samples were obtained from radical prostatectomies and benign samples from transurethral resection of the prostate. qPCR analysis showed ALDH1A3 to be more highly expressed than ALDH7A1 in the primary prostate epithelial cultures in 18 patient samples. Expression of ALDH1A3 was 3-fold higher in the cancer samples compared to the benign samples. The RNA data correlates with protein expression in 6 patient samples by immunofluorescence. qPCR analysis also showed increased expression of ALDH1A3 with overexpression of ALDH7A1 suggesting a compensatory mechanism. Trypan blue exclusion assay showed that knockdown of ALDH1A3, ALDH7A1 or a combination of both resulted in a reduction in cell numbers. Flow cytometry was used to study cell differentiation upon knockdown of ALDH1A3, ALDH7A1 or both. In 7 samples studied there was a reduction in CD49b expression indicating cell differentiation. ALDH7A1 knockdown showed a higher level of cell differentiation in all cases. The colony forming ability of primary cells was also investigated post-transfection of siRNAs against ALDH1A3, ALDH7A1 or both using the colony formation assay which resulted in a lower number of colonies in all 7 samples tested. The effect was more pronounced in benign prostatic hyperplasia (BPH) than in malignant cancer samples and patient variability was observed. ALDH-affinic probe compounds (DEAB and three derivatives, AlI-9, AlI-14 & AlI-18) were tested against 5 patient samples to investigate if they have an effect on cell viability. All four compounds showed reduction in cell viability at the highest concentration while AlI-14 and AlI-18 showed a synergistic effect in combination treatment with docetaxel. In conclusion, knockdown of ALDH1A3 and ALDH7A1 reduce cell number, induce cells to differentiate and reduce their colony forming ability. Novel ALDH-affinic probe compounds reduced cell viability alone and in combination with docetaxel, therefore these compounds may be of value both as single treatments and to provide a strategy to enhance taxane-based therapy such as docetaxel.


Cancer treatment is primarily guided by the organ of origin, which despite extensive histopathological and clinical evaluation, remains ambiguous in many cases and may be inaccurate in cases of occult primary. We determine the extent to which routine prospective tumour sequencing, combined with conventional histopathology, can be used to infer the tumor type and thereby direct clinical decisions. Characteristic patterns of somatic mutations, broad and focal copy number alterations, structural rearrangements, mutational signatures, and other facets acquired from prospective tumor sequencing can inform the tissue of origin classification of patient disease. Such prospective sequencing of active cancer patients presents an opportunity to guide diagnosis and therapy beyond the identification of individual biomarkers of treatment response. Probabilistic classification allows for systematic combination of genome-directed diagnosis with conventional histopathology and clinical history in the course of disease management. Using more than 10,000 tumors collected from advanced cancer patients at our institution and sequenced using a comprehensive cancer panel (MSK-IMPACT) encompassing 341 genes, we have developed a probabilistic classifier that infers the tumor type from nine broad categories of genomic ab-
Drug sensitivity in growing cells is conventionally quantified by IC50, AUC, or Fmax values, but these metrics suffer from a fundamental flaw: they highly depend on the division rate of cell lines. This dependency creates artefactual correlations between genetic alterations and drug sensitivity which impede biomarker discovery. To address this issue, we recently developed novel drug response metrics insensitive to the number of divisions occurring during the assay. These are based on estimating growth rate inhibition (GR) under treatment using fixed assays. Here, we illustrate the flaws of using IC50 values for pharmacogenic studies by reanalyzing a recently published large dataset of drug sensitivity and showing cases in which differences in division rates drive associations between IC50 values and tissue type or genetic alterations. Using GR50 values prevents these artificial correlations and restores known associations between drug resistance and genomic markers such as PTEN loss driving lapatinib resistance in breast cancer cell lines. We also show how use of GRmax values, a measure of drug efficacy, allows quantifying differences in the phenotypes and distinguishing cytostatic from cytotoxic response. For drugs that have a narrow range of GR50 values like taxanes, efficacy is the most relevant metric: in many ovarian BCL2-deleted cell lines, docetaxel induces a cytotoxic response (negative GRmax values), whereas wild-type lines elicit a cytostatic response (positive GRmax values). Because efficacy (GRmax) varies independently of potency (GR50), we conclude that both metrics are complementary for pharmacogenomics and should be studied jointly. Adopting GR metrics requires only modest changes in experimental protocols and analysis is facilitated by our interactive website: GRCalculator.org. We expect GR metrics to improve the identification of reliable drug response biomarkers and enhance the reproducibility of large-scale sensitivity studies.

Methylation accurately predicts age of cancer onset in patients with Li Fraumeni Syndrome. Benjamin M. Brew,2 David Malkin,2 Lauren Erdman,2 Andrea Doria,3 Jason Berman,4 Adam Shlien,1 Tanya Guha,1 Ana Novokmet,1 Anna Gromberg,1 William H. Pledger, Jr,1 The Hospital for Sick Children, Toronto, Ontario, Canada; 2IWK Health Centre, Toronto, Ontario, Canada.

Introduction: Li Fraumeni Syndrome (LFS) is a rare hereditary genetic cancer predisposition syndrome. Germline mutations of the TP53 tumor suppressor gene are the underlying cause in >80% of patients with LFS, and are associated with an increased risk of secondary tumors and a spectrum of early onset cancers, even in the absence of a family history of cancer. We have previously developed and implemented a comprehensive lifelong clinical surveillance protocol for individuals with a germline TP53 mutation. We set out to make this screening process more targeted by building a predictive model of age of onset. We accomplished this goal by implementing machine learning methods on germline methylation data. Methods: We made use of the Toronto Hospital for Sick Children (SickKids) LFS family cohort in our predictive model of age of onset. In all, we have 74 patients with germline methylation data, consisting of ~450,000 probe sites. We subset this data by identifying probes that fall into differentially methylated regions between LFS and cancer patients with wild-type TP53. The probes identified in these regions were used in our predictive model of age of onset. Because age of sample collection was highly correlated with age of onset (r^2 = ~50), we corrected for confounding using a strategy that is two-fold: (1) we extracted the variation of each probe that is independent of the age of sample collection (the residual after regressing on the age of sample collection) and use these as predictors in our model, and (2) we test our models on the task of predicting the age of sample collection for LFS patients that do not have cancer. The former provided us with more robust predictions while the latter verified that we are in fact predicting age of onset, rather than simply predicting age at which the sample was collected. Results: Our machine learning model was able to achieve 86% correlation between true and predicted values of the age of onset. Additionally, we have tested the ability of our models to predict whether an individual will be diagnosed before or after the age of 4. Our classification machine learning model achieved 91% accuracy on average. We verified that our model does not simply predict age of sample collection by using our cohort of LFS patients that do not have cancer (n = 37). The distribution of the age of sample collection matched those of the patients who had cancer (p = 0.67). Our model accurately predicted age of sample collection, thus confirming that our model is highly predictive of the age of cancer onset in LFS TP53 Mutation patients. Conclusions: We identified two predictive models for age of cancer onset in LFS patients that achieve high accuracy, both when predicting the age of onset as a continuous variable (86% correlation) and whether cancer will occur before or after the age of onset (88% accuracy). Our model will assist clinicians in targeting high risk patients for screening, lower the cost of treatment, and raise the likelihood of survival among LFS patients.


Tumor heterogeneity has been linked to many measures of poor patient outcome. Despite numerous recent methodological advances, though, reliable estimates of clonal and subclonal architecture remain elusive. We present advances in computational deconvolution of tumor heterogeneity, aimed at handling complex substructure, more automated inference, and increased flexibility to heterogeneous genomic data sources. Our methods use geometric models of mixture substructure, a specialized form of a strategy called manifold learning, to better resolve clonal substructure shared across genomic samples. We validate the methods on breast tumor dataset from the Cancer Genome Atlas (TCGA).

A multiscale computational model for spatio-temporal tumor immune response. Chang Gong,1 Oleg Milberg,2 Bing Wang,2 Paolo Vicini,3 Rajesh Narwal,4 Lorin Roskos,4 Aleksander S. Popel.1 Johns Hopkins University, Baltimore, MD; 2MedImmune, Mountain View, CA; 3MedImmune, Cambridge, United Kingdom; 4Medimmune, Gaithersburg, MD.

When the immune system responds to tumor development, patterns of immune infiltration emerge, highlighted by expression of immune checkpoint-related molecules such as PD-L1 on cancer cells and its receptor PD-L1 on cytotoxic T cells. Pre-treatment tumor spatial heterogeneity could bear information on intrinsic characteristics of the tumor lesion for individual patient, and thus has the potential to comprise biomarkers for anti-tumor therapeutics. We developed a systems biology computational multiscale agent-based model to capture the interactions between immune cells and cancer cells during tumor progression. Cytotoxic T cells and cancer cells are modeled as free-moving agents in a 3-dimensional grid, where each cell acts in response to its local microenvironment and carries out functions such as division, apoptosis, cytotoxic killing and switching between states with different PD-L1 or PD-L1 expression levels. Subsequently, we analyzed the emergent behavior of tumor progression by looking at all these local interactions as whole. Using this model, we were able to reproduce temporal dynamics of cytotoxic T cells and cancer cells during general tumor progression, as well as 3-dimensional spatial distributions of these cells over the time course of the simulation. By varying the characteristics of the neoantigen profile of individual patients, such as mutational burden and immunogenicity, a spectrum of pre-treatment spatial patterns of PD-L1/PD-L1 expression is generated in our simulations, resembling immune-architectures obtained via immunohistochemistry from patient biopsies. We evaluate potential prognostic biomarkers by correlating these spatial characteristics with in silico treatment results with immune checkpoint inhibitors. Simulation results demonstrate that the percentage of PD-L1 positive cancer cells which are not in close proximity of the tumor boundary or vasculature is more indicative of successful anti-PD1/anti-PD-L1 treatment. Our findings suggest that tumor spatial heterogeneity, especially its immune-architecture, reflects the course of tumor progression as well as patient-specific properties, and is thus likely to carry impor-
**BIOINFORMATICS AND SYSTEMS BIOLOGY: Computational Cancer Biology**

**CANCER CHEMISTRY: Novel Therapeutic Targets, Molecules, and Approaches for the Treatment of Cancer**

**#976 ImmunoMap: a novel bioinformatics tool for immune cell repertoire analysis.** John-William Sidhom,1 Catherine A. Bessell,2 Jonathan J. Havel,3 Timothy A. Chan,2 Jonathan P. Schneck.1 Johns Hopkins University, Baltimore, MD; 2Memorial Sloan Kettering Cancer Center, New York City, NY.

**Background:** There has been a dramatic increase in T-cell Receptor (TCR) sequencing spurred, in part, by the clinical demand in Immuno-oncology and technological advances in TCR sequencing. However, there has been little in the way of approaches to parse the data in a biologically meaningful fashion. The ability to parse this data to understand the T-cell repertoire in a structurally relevant manner has the potential to open new discoveries about how the immune system responds to insults such as cancer and infectious diseases. Methods: Here we describe a novel method to visualize and quantify TCR repertoire sequence diversity. This method includes metrics such as visualization of repertoire with: 1) weighted phylogenetic trees that display relatedness and frequency of the sequences; 2) dominant motif analyses identifying clusters of highly homologous sequences that contribute significantly to response and; 3) TCR diversity score measuring the average relatedness (by sequence homology) of all TCR’s in a sample. To demonstrate the power of the approach, we have applied it to understanding the CD8 T Cell response to model self (TRP2) and foreign (SIY) antigens in naive and tumor-bearing (B16 melanoma) B6 mice. Additionally, this method was applied to tumor infiltrating lymphocytes (TIL), taken pre- and on-therapy, from patients undergoing Nivolumab (α-PD1) therapy for metastatic melanoma. Results: Analysis of the naive CD8 response demonstrated a highly conserved (measured by the TCR diversity score) and less clonal response to SIY whereas the response to TRP2 was less conserved and highly clonal. Dominant motif analysis demonstrated highly rich motifs consisting of many homologous sequences in the SIY response but few sequences per motif in the TRP2 response. This may reflect the outcome of tolerance mechanisms to self-antigens. Presence of tumor demonstrated differential immune pressure on the TRP2 vs SIY response. Tumor primed novel SIY motifs but constrained the number of dominant motifs in the TRP2 response while additionally altering the sequence of the motifs. In patients undergoing α-PD1 therapy, we identified signatures in pre- and post-therapy TCR repertoires that correlated with clinical outcome response. Prior to therapy, patients whose dominant motifs were rich with many sequences responded favorably to checkpoint inhibition over those with less rich motifs. After four week on therapy, patients whose TCR repertoires became more conserved responded more favorably to PD1 treatment while those who did not respond had no change in their TCR diversity score. Conclusions: In summary, we have developed and demonstrated a novel method to meaningfully parse and interpret TCR repertoire data and have applied it to yield a novel insight of CD8 T Cell responses to different types of antigens in model systems as well as key characteristics of TIL repertoires from patients who respond clinically to α-PD1 therapy.

**#977 Phenotypic analysis of single-cell breast cancer inhibition data reveals insights into EMT.** William S. Chen,1 Nevena Zivanovic,2 Dana Pe'er,3 Bernd Bodenmiller,3 Smita Krishnaswamy.1 1Yale School of Medicine, New Haven, CT; 2University of Zurich, Zurich, Switzerland; 3Columbia University, New York, NY.

**Background:** A leading model of cancer metastasis is epithelial-to-mesenchymal transition (EMT). We sought to determine whether single-cell inhibition data targeting potential mediators of EMT could uncover mechanistic insights into the EMT process. Methods: EMT was artificially induced on Py2T murine breast cancer cells by TGFβ treatment. Methods: EMT was artificially induced on Py2T murine breast cancer cells by TGFβ treatment. Additionally, a unique drug inhibitor was added to each well of a multiplexed CyTOF experiment. 37 transcription factors and cell surface markers were measured in each cell to assess epithelial and mesenchymal states, SMAD, AKT, and MAPK signaling activity, cell cycle regulation, and apoptosis pathway activation. The final single-cell dataset consisted of 500 inhibition and control conditions (cell populations), which we aimed to characterize in relation to one another with respect to effect on EMT. Analyzing the similarity between drug inhibitions amounts to a novel type of clustering problem that involves computing the similarity between diverse cell populations generated by each inhibitor. Traditional methods for comparing cell populations are not robust to the intra-population heterogeneity observed in unperturbed cells undergoing EMT. Thus, we developed a Phenotypic Earth Mover’s Distance (PhEMD). This method for comparing cell populations leverages the insight that only a limited number of “cell subtypes” (e.g. mesenchymal, epithelial, transitional) are observed in unperturbed and perturbed EMT. By classifying each cell as one of these distinct subtypes using community-
of JAK/STAT pathways associated with a wide variety of malignancies. Elevated JAK/STAT signalling leading to increased activation of STAT3 is reported in a wide variety of cancers, including breast, liver, prostate, colorectal, head and neck, oesophageal, pancreatic, bladder, and non-small cell lung, and is implicated in the pathogenesis of diffuse large B-cell lymphoma and nasopharyngeal carcinoma. The importance of targeting STAT3 activity to disrupt the oncogenic state in leukemia. In this context, TRIM24 degradation rather than bromodomain inhibition alone is required to displace TRIM24 from chromatin. Using this probe, a further understanding of the contribution of TRIM24 domains to its transcriptional activation function will provide mechanistic insight as to how TRIM24 promotes a gene expression program permissive of the oncogenic state, as well as inform a therapeutic approach to target multidomain proteins, such as TRIM24, that are tightly linked to disease.


A common theme in treating cancer is the use of combination chemotherapy, where multiple drugs with different mechanisms of action are combined to elicit synergistic activity or overcome differential drug sensitivities. Antibody-drug conjugates (ADCs) have emerged as a powerful approach for treating cancer, combining the tumor targeting specificity of monoclonal antibodies with the potent cell-killing activity of cytotoxic drugs. Like other therapies, these agents are increasingly being tested in combination with unconjugated, clinically approved anticancer agents. In addition, emerging data demonstrates that insensitivity to a particular ADC can be overcome through delivery of a different payload using the same antibody. For these reasons, the development of ADCs that can deliver two complementary payloads to a tumor would likely be a significant advancement in ADC technology. To enable dual-drug conjugation, we utilized a multiplexing drug carrier that contains cysteine residues with orthogonal protecting groups and identified novel conditions for optimization of these protecting groups on a folded protein. Sequential cysteine unmasking enables discrimination between conjugation sites to allow for site-specific drug conjugation. This strategy provides homogeneous ADCs bearing 16 total drugs per antibody, split evenly between the two drug linkers. Importantly, this strategy is flexible, as it does not require engineered antibodies or custom enzymes for drug-linker conjugation. To demonstrate the potential benefits of ADC dual drug delivery, this strategy was applied to the construction of ADCs bearing two classes of auristatin drug linkers that have different physicochemical properties and complementary anti-cancer activities. Dual-auristatin ADCs were tested in cell line and xenograft models that have differential sensitivities to individual auristatin components, including those with heterogeneous antigen expression or high levels of drug efflux transporters. The data from these studies demonstrate that the dual-auristatin ADCs were active on cells and tumors that are refractory to treatment with either of the individual component drugs. This work highlights the potential for delivering two synergistic or complementary payloads on a single ADC and presents a flexible method for constructing dual-drug ADCs with site-specific and homogeneous drug loading.

#983 Identification of potent, highly selective and orally available ATR inhibitor BAY 1895344 with favorable PK properties and promising efficacy in monotherapy and combination in preclinical tumor models. Ulrich T. Luecking,1 Julien Lefranc,1 Anjte Wenger,1 Lars Wortmann,1 Hans Schick,2 Hans Briem,1 Gerhard Siemens,1 Philip Lienau,1 Christoph Schatz,1 Benjamin Bader,2 Gesa Deeg,1 Franz von Nussbaum,1 Michael Brands,1 Dominik Mumberg,1 Karl Ziegeldbauer,1 Bayer Pharma AG, Berlin, Germany; ASCA GmbH, Berlin, Germany.

The integrity of the genome of eukaryotic cells is secured by complex signaling pathways, known as DNA damage response (DDR). Recognition of DNA damage activates DDR pathways resulting in cell cycle arrest, suppression of general translation, induction of DNA repair, cell survival or even cell death. Proteins that directly recognize aberrant DNA structures recruit and activate kinases of the DDR pathway, such as ATR (ataxia telangiectasia and Rad3-related). ATR responds to a broad spectrum of DNA damage, including double-strand breaks (DSB) and lesions derived from interference with DNA replication as well as increased replication stress (e.g. in oncogene-driven tumor cells). Therefore, inhibition of ATR kinase activity could be the basis for a novel anti-cancer therapy in tumors with increased DNA damage, deficiency in DNA damage repair or replication stress. Herein we report the identification of the potent, highly selective and orally available ATR inhibitor BAY 1895344 by a collabora
Idolique Terebesi,1 Kirstin Meyer,1 Katja Prelle,1 Ray Valencia,2 Stuart Incz,2 Franz von Nussbaum,2 Dominik Mumberg,2 Karl Ziegelbauer,1 Michael Brands1,2,3,4. 2Bayer HealthCare Pharmaceuticals Inc., Whippany, NJ.

PTEFb/CDK9 mediated transcription of short-lived anti-apoptotic survival proteins like Mcl-1 and Mdc plays a critical role in cancer cell growth and survival in various tumor entities including AML. In addition, these survival proteins play important roles in the development of resistance to chemotherapy. We previously disclosed the preclinical profile of BAY 1143572, the first selective, orally available PTEFb/CDK9 inhibitor that entered clinical development. BAY 11434572 had low nanomolar activity against PTEFb/CDK9, an at least 50-fold selectivity against other CDKs in enzymatic assays and broad anti-proliferative activity against a panel of tumor cell lines with sub-micromolar IC50 values. Moreover, BAY 11434572 was shown to be a very potent and highly selective ATR inhibitor (IC50 7 nM), which potently inhibits proliferation of a variety of tumor cell lines with low- to sub-micromolar IC50 values. In initial xenograft studies, BAY-937 revealed moderate activity in monotherapy and in combination with cis-platin. However, BAY-937 also re- vealed low aqueous solubility, low bioavailability (rat) and activity in the hERG patch clamp assay. Extensive lead optimization efforts led to the identification of the novel, orally available ATR inhibitor BAY 1959344. In vitro, BAY 1959344 was shown to be a very potent and highly selective ATR inhibitor (IC50 7 nM), which potently inhibits proliferation of a broad spectrum of human tumor cell lines (median IC50 = 78 nM). In cellular mechanistic assays BAY 1959344 potently inhibited hydroxyurea-induced H2AX phosphorylation (IC50 = 36 nM). Moreover, BAY 1959344 revealed significantly improved aqueous solubility, bioavailability across species and no activity in the hERG patch clamp assay. BAY 1959344 also demonstrated very promising efficacy in monotherapy in DNA damage deficient tumor models as well as combination treatment with DNA damage inducing therapies. The start of clinical investigation of BAY 1959344 is planned for early 2017.

**#984 Identification of potent and highly selective PTEFb inhibitor BAY 1251152 for the treatment of cancer: from p.o. to i.v. application via scaffold hops.** Ulrich T. Luecking,1 Arne Scholz,2 Dirk Kosemund,1 Rolf Bohlmann,1 Hans Briem,1 Philip Lienau,1 Gerhard Siemeister,1 Idilko Terebesi,1 Kirstin Meyer,1 Katja Prelle,1 Ray Valencia,2 Stuart Incz,2 Franz von Nussbaum,2 Dominik Mumberg,2 Karl Ziegelbauer,1 Michael Brands1,2,3,4. 2Bayer HealthCare Pharmaceuticals Inc., Whippany, NJ.

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in mechanical threshold after paclitaxel administration than wild-type mice, suggesting the involvement of this receptor in CIPN development and maintenance. This hypothesis was supported by the reversal of paclitaxel-induced mechanical allodynia by the α7 nAChR silent agonist R-47. While nicotine activates its receptors in the tumor cells, based on the stimulation of the P13K/Akt pathway, MTT/MTS colorimetric assays showed that concentrations of nicotine ranging from 0.1 to 10 μM fail to significantly increase the viability of A549 or H460 non-small cell lung cancer cells, murine Lewis lung carcinoma cells, or primary human lung cancer cells. Most importantly, the paclitaxel-induced decreases in H460 proliferation and LLC viability are not significantly attenuated by 1 μM nicotine. Moreover, 1 μM nicotine does not interfere with paclitaxel-induced apoptosis in H460 and LLC cells. Finally, R-47 (0.1 μM) prevented neurotoxicity without decreasing lung tumor cell viability and colony formation, or interfering with the cytotoxicity of paclitaxel. Our in vitro findings are supported by studies demonstrating that nicotine does not enhance tumor volume or cause premature death in tumor-bearing mice. These data suggest that nAChRs may be promising drug targets for the prevention and treatment of CIPN.

**References**

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2. Hil-debrandt, Alem A. Belachew, Monica E. Reyes, Yuanqing Ye, Xifeng Wu. UT MD Anderson Cancer Center, Houston, TX.

Distinct racial disparities are evident in CRC prognosis with Black patients experiencing worse outcomes than Hispanics and Whites. In a prior study of Health Related Quality of Life (HR-QoL) in a cohort of CRC patients, we observed that racial minority patients experienced lower HR-QoL scores compared to White CRC patients. Therefore, in this study, we focused on the identification of patterns of racial disparities in HR-QoL scores and relationship to differences in prognosis.
White (N = 450), Hispanic (N = 366), and Black (N = 316) CRC patients within a year of diagnosis at MD Anderson Cancer Center completed the SF-12 quality of life questionnaire to determine Mental Composite Summary (MCS) and Physical Composite Summary (PCS) scores. Participants also completed a questionnaire to collect epidemiology and sociodemographic variables. Vital status and histology information was obtained from the institutional tumor registry. Racial disparities were reported in HR-QoL with both Black and Hispanic patients reporting lower mean PCS and MCS scores compared to White patients, suggesting poorer HR-QoL in these populations. We observed differences in patterns of association between epidemiology and sociodemographic variables and poorer HR-QoL by race. Hispanics who never married were at higher risk of poor physical HR-QoL (OR: 2.55; 1.5-6.7), P = 0.02 compared to reference patients, for which we observed for White Black CRC patients. Similarly, CRC patients with some college education was associated with a decreased risk of poor PCS, but only in Hispanics (OR: 0.263;0.13-0.52, P < 0.0001). White females have about two-fold risk of poor PCS (P = 2.00 x 10^-4) and MCS (P = 2.21 x 10^-4) scores compared to White males. This relationship was also observed for Black females OR: 2.28 (1.5-3.84), but not Hispanic females. Among CRC patients reporting poor PCS (<50), significant differences in median survival times (MSTs) were observed by race. Hispanic patients had the highest MST at 85.4 months followed by Blacks (47.8 months) and Whites (43.2 months). A similar relationship was observed for poor MCS (<50) stratified by race with MST times of 81.9 months for Hispanics, 40.8 months for Blacks, and 54.1 months for Whites. In conclusion, we identified patterns of racial disparities in epidemiology and sociodemographic factors that correspond to poor baseline HR-QoL. CRC baseline HR-QoL was also demonstrated that a prognostic correlation exists between baseline HR-QoL and patient overall survival, and that this relationship is influenced by race. The patterns of racial disparity identified in this study can be an important tool for assessing the underlying mediators of HR-Qol in CRC patients and to further identify those who are particularly at risk for poor prognosis.

**#991** Association of high-sensitivity C-reactive protein with health-related quality of life among breast cancer survivors. Hyun Jeong Cho, Zisun Kim, Hyun Jeou Youn, Jung Eun Lee. 1Department of Cancer Nutrition, Seoul National University, Seoul, Republic of Korea; 2Department of Preventive Medicine and Public Health, Seoul National University, Seoul, Republic of Korea; 3Department of Obstetrics and Gynecology, Seoul National University College of Medicine, Seoul, Republic of Korea; 4Department of Preventive Medicine, Seoul National University, Seoul, Republic of Korea.

Backgrounds/Aims: Decline in health-related quality of life (HRQoL) is associated with worse prognosis among patients with breast cancer. Increased risk of inflammation with cancer-related symptoms such as low physical function, depression and pain could be a potential mechanism. We aimed to examine whether high-sensitivity C-reactive protein (hs-CRP) were related to HRQoL among breast cancer survivors. Methods: This cross-sectional study of Korean breast cancer survivors included a total of 165 women aged 29 to 63 years who had been diagnosed with stage I to III breast cancer and survived six months or more after surgery. Post-diagnostic HRQoL levels were measured using the 36-Item Short Form Health Survey (SF-36) containing eight components: physical functioning, role-physical, body pain, general health, vitality, social functioning, role-emotional, and mental health. The higher scores indicated better HRQoL. Circulating levels of hs-CRP were measured 6 months after diagnosis. We adjusted each component with categories of hs-CRP according to HRQoL levels. We adjusted for age at diagnosis, body mass index at diagnosis, weight change, income level, stage at diagnosis, time since surgery, tumor size and plasma vitamin D levels. Results: We found that increasing levels of physical functioning and role-physical components were associated with decreasing levels of hs-CRP; according to <0.4, 0.4-<0.6, 0.6-<1.0, and >1.0 mg/L of hs-CRP, LS-means (95% CIs) were 78.1 (73.2-83.0), 75.2 (69.6-80.8), 76.1 (69.5-82.7), 70.1 (64.5-75.7), respectively (p for trend = 0.051) for physical functioning and 77.5 (70.6-84.4), 81.0 (73.2-88.8), 79.4 (70.2-88.7), 66.6 (58.7-74.4), respectively (p for trend = 0.053) for role-physical. Higher scores of mental components were also associated with lower levels of hs-CRP; LS-means (95% CIs) of <0.4 and ≥1.0 mg/L of hs-CRP levels were 84.6 (77.8-91.4) and 67.1 (59.4-74.8), respectively (p for trend = 0.0000) for role-emotional, 87.4 (82.3-92.6) and 77.4 (71.5-83.4) for p for trend = 0.03) for social functioning, 62.4 (56.4-68.4) and 53.3 (46.5-60.2; p for trend = 0.053) for vitality. Conclusions: High circulating levels of hs-CRP, an inflammation biomarker, were associated with lower levels of HRQoL among Korean breast cancer survivors. Funding information This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2014R1A2A2A01007794)

**#992** Patient-derived tumor organoids of neuroendocrine prostate cancer. Loredana Puca, Rohan Bareja, Reid Shaw, Wouter Karthaus, Dong Gema, Chantal Pauli, Joanna Cytra, Rachele Rosati, Renu Rao, Andrea Stoner, Carla Grandori, Giorgio Inghirami, Yu Chen, Mark A. Rubin, Himisha Beltran, Weiell Cornell Medicine, New York, NY; 2Cure First, seattle, WA; 3Memorial Sloan Kettering, New York, NY; 4Cure First, Seattle, WA.

Background: The development of neuroendocrine prostate cancer (NEPC) is one mechanism of treatment resistance to androgen receptor (AR)-targeted therapies for a subset of patients with advanced prostate cancer. This is associated with transition from a prostate adenocarcinoma to small cell NEPC histology, low AR signaling signaling, and expression of neuroendocrine markers as Chromogranin A (CHGA), Synaphysalin (SYNP) and CD56. Patient derived preclinical models recapitulating the NEPC phenotype may be used to address NEPC pathogenesis and test emerging therapeutic targets. Methods: Tumor organoids were developed according to protocols previously described (Gao et al, Cell 2015). Briefly the tissue biopsies (liver and bone biopsy) were washed, enzymatically digested and then seeded in Matrigel (BD) droplets. Organoids were characterized at genomic (WES), RNA and protein level (HIC) to confirm the expression of specific markers. Lentinival infections were performed using shRNAs against EZH2 to knock down EZH2 in organoids. Organoids were also subcutaneously injected in NSG mice to generate patient derived xenografts (PDx) for drug treatment in vivo. Results: We developed and characterized two NEPC tumor organoids from tumor biopsies (liver and bone) of two patients both in vitro and in vivo (as PDx). NEPC tumor organoid models retained the molecular and histological characteristic of their matched patient samples. We successfully manipulated the activity of the histone methyltransferase EZH2 by using a catalytic inhibitor and its expression by injecting organoids with stEZH2. We showed that the absence of EZH2 affects the expression of neuroendocrine-associated programs as stem cell and neuronal pathway. Moreover treatment with EZH2 inhibitor decreased tumor organoids viability and PDx tumor volume. Drug screening approaches on NEPC organoids were used to discovery novel drug targets and combinations that could potentially benefit NEPC patients. Top single agent hits included previously identified target such as EZH2, AURKA, and CDK7 novel synergies. Conclusions NEPC patient tumors organoids are clinically relevant tumor models to study the NEPC phenotype in advanced prostate cancer and may be used to elude novel drug targets.

**#993** Diagnostic leukaemia (DLA): Molecular characterisation and organoid culture of circulating tumour cells (CTC) from metastatic castration resistant prostate cancer (mCRPC). Maryou B. Labmroso, Veronica S. Gil, Mateus Crespo, Mariane S. Fontes, Rui N. Neves, Niven Mahra, Gemma Fowler, Berni Ebbs, Penny Fohr, George Seed, Wei Yuan, Joanne Hunt, Deirdre Moloney, Dionne Ayanda, Joost F. Swennenhuiss, Kiki C. Andree, Semini Sumanasuriya, Martin Clarke, Pasquale Rescino, Zaléiris Zalėiris, Joaquin Mateo, Diletta Bianchini, Nikolas H. Stoeckle, Leon W. Terstappen, Gunther Boysen, Johann S. De Bono. 1 The Institute of Cancer Research, London, United Kingdom; 2 The Royal Marsden NHS Foundation Trust, London, United Kingdom; 3 University Hospital of the Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany; 4 University of Twente, Enschede, Twente, Netherlands.

Introduction: CTC count is an independent predictor of overall survival in mCRPC. Isolation of CTC from peripheral blood (PB) for genomic and functional analysis is challenging, especially in patients (pts) with low CTC count. It has been shown that DLA increases CTC yield. However, it has yet to be proven whether CTC isolation from DLA can be used in complementary studies such as molecular characterization and growth of organoid culture for drug sensitivity studies. Here we present preliminary data of an on-going study, which evaluates DLA in mCRPC pts, focusing on safety, CTC enrichment, molecular characterization and feasibility for organoid culture. Methods: mCRPC pts considered for clinical trials were selected according to performance status (ECOG 0-1) and number of CTC found in 7.5mL PB (>20 cells/7.5mL). DLA products (200k x 10^6 cells/mL) were centrifuged at 1000 g for 8 min and washed twice with 10mL RPMI medium (10% cord blood serum). The resulting cell pellet was resuspended in 1mL RPMI medium for 15 min at 37°C. Procedure: The leukapheresis (DLA): Molecular characterisation and or- ganoid culture of circulating tumour cells (CTC) from metastatic castration resistant prostate cancer (mCRPC), Maryou B. Lambroso, 1 Verónica S. Gil, 1 Mateus Crespo, 1 Mariane S. Fontes, 1 Rui N. Neves, 1 Niven Mahra, 1 Gemma Fowler, 1 Berni Ebbs, 1 Penny Fohr, 1 George Seed, 1 Wei Yuan, 1 Joanne Hunt, 1 Deirdre Moloney, 1 Dionne Ayanda, 1 Joost F. Swennenhuiss, 1 Kiki C. Andree, 1 Semini Sumanasuriya, 1 Matthew Clarke, 1 Pasquale Rescino, 1 Zaléiris Zalėiris, 1 Joaquin Mateo, 1 Diletta Bianchini, 1 Nikolas H. Stoeckle, 1 Leon W. Terstappen, 1 Gunther Boysen, 1 Johann S. De Bono. 1 The Institute of Cancer Research, London, United Kingdom; 2 The Royal Marsden NHS Foundation Trust, London, United Kingdom; 3 University Hospital of the Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany; 4 University of Twente, Enschede, Twente, Netherlands.
cells) were processed using the CellSearch CTC kit (Janssen Diagnostics, LLC) according to manufacturer procedures. The contents of CellSearch cartridges were sorted into single cell by fluorescence activated cell sorting (FACS) and subsequently assessed by array comparative genomic hybridization (aCGH) for copy number aberrations (CNA). Enrichment of CTC for organoid culture was performed by density gradient of mononuclear cells followed by positive selection using magnetic beads. Results: Overall 12 mCRPC patients underwent DLA without any complication or toxicity. The mean CTC count was 90 CTC/7.5 ml peripheral blood (median = 31) and ranged from 20 to 324. CellSearch CTC count in the DLA yielded a mean of 466 (median=203) and ranged from 60 to 2496 with an up to 40-fold increase (mean = 13, median = 6) in CTC count separation when comparing 1mL of PB and 1mL of PB with DLA. A single mCRPC patient selected by positive sorting of FACS single CTC from the DLA by aCGH showed that these CTC genomic profiles had the typical hallmarks of mCRPC with CNAs including AR and MYC locus (8q) amplification, and PTEN, RB1, TP53, CHD1 loss. Additionally, ex vivo culture of CTC-derived organoids was successfully achieved. aCGH of these organoids matched the genomic profile that of the CTC from the same patient. Conclusion: DLA from mCRPC pts was well tolerated and yields higher CTC capture than PB and may provide an alternative to tissue biopsy and routine culture approach.

Identifying a new method for determining large SVs as a clinical tool for identifying AML patients with high resolution than cytogenetics, an intrinsically low resolution tool. We have adapted a new method for determining large SVs as a clinical tool for evaluating AML and other cancer genomes. The Biennon Irys images in microfluidic nanochannels large (>150 Kb) segments of genomic DNA that have been bar coded by site specific nicking and local incorporation of fluorescent label. The complement of individual molecules can be assembled through matching bar codes to create a de novo map of the genome from which, by matching to a reference genome, identifies and catalogs structural variants at resolution of ca. 1 Kb. With this technology, we characterized structural variants signatures in breast cancer, renal clear cell carcinoma, and CML cell lines. Structural variants were then validated via PCR/Sanger sequencing, fluorescent in situ hybridization and whole genome sequencing. We identified a thorough complement of consistent false positive rearrangements identified by this technology, most of which reflect errors in the reference human genome assembly. We then applied this methodology to ten AML patient samples, seven from archived blood samples and three directly obtained from newly diagnosed patients. Karyotyping on one archived sample identified a t(6;9) translocation in the majority of cells. We identified that rearrangement at a much higher resolution than cytogenetics, which allowed us to map the breakpoint via PCR/Sanger sequencing to nucleotide resolution and confirm a fusion of NUP214 to DER, a known oncogene. We also discovered a reciprocal (i.e., 10;6) translocation, which had not been identified by cytogenetics. In the six other cases from archived samples, we have confirmed the translocation identified by cytogenetic karyotyping and in several cases found additional rearrangements. Moreover, in the first pediatric case, which we completed within five days of obtaining the sample, we have identified four distinct translocations, all of which have been associated previously with AML. Overall, this is technology that has the ability to quickly and accurately identify SVs in cancer cell lines and AML patient samples with higher resolution than cytogenetics, demonstrating its clinical usefulness in the diagnosis and subsequent treatment of leukemia.

Impact of molecular subtypes on predicting chemotherapy response and survival in muscle invasive bladder cancer. Wooyoung Choi,1 Debasish Sundar,1 Fong Yue,1 James Broach. Penn State Hershey Medical Center, Hershey, PA.

Identification of structural variants (SVs) is critical to the prognosis of AML. SVs are not readily identified by next generation sequencing methods and are currently diagnosed by cytogenetics, an intrinsically low resolution tool. We have adapted a new method for determining large SVs as a clinical tool for evaluating AML and other cancer genomes. The Biennon Irys images in microfluidic nanochannels large (>150 Kb) segments of genomic DNA that have been bar coded by site specific nicking and local incorporation of fluorescent label. The complement of individual molecules can be assembled through matching bar codes to create a de novo map of the genome from which, by matching to a reference genome, identifies and catalogs structural variants at resolution of ca. 1 Kb. With this technology, we characterized structural variants signatures in breast cancer, renal clear cell carcinoma, and CML cell lines. Structural variants were then validated via PCR/Sanger sequencing, fluorescent in situ hybridization and whole genome sequencing. We identified a thorough complement of consistent false positive rearrangements identified by this technology, most of which reflect errors in the reference human genome assembly. We then applied this methodology to ten AML patient samples, seven from archived blood samples and three directly obtained from newly diagnosed patients. Karyotyping on one archived sample identified a t(6;9) translocation in the majority of cells. We identified that rearrangement at a much higher resolution than cytogenetics, which allowed us to map the breakpoint via PCR/Sanger sequencing to nucleotide resolution and confirm a fusion of NUP214 to DER, a known oncogene. We also discovered a reciprocal (i.e., 10;6) translocation, which had not been identified by cytogenetics. In the six other cases from archived samples, we have confirmed the translocation identified by cytogenetic karyotyping and in several cases found additional rearrangements. Moreover, in the first pediatric case, which we completed within five days of obtaining the sample, we have identified four distinct translocations, all of which have been associated previously with AML. Overall, this is technology that has the ability to quickly and accurately identify SVs in cancer cell lines and AML patient samples with higher resolution than cytogenetics, demonstrating its clinical usefulness in the diagnosis and subsequent treatment of leukemia.

A translational phosphoproteomic approach to study differences in KRAS signaling in pancreatic, colorectal and lung cancers. Adam Stewart,2 Elizabeth A. Coker,1 Anna Minchom,2 Sebastian Pöстерl,1 Alexandros Georgiou,2 Paul Huang,1 Bissan Al-Lazikani,2 Uday Banerji1. The Institute of Cancer Research, London, United Kingdom. The Institute of Cancer Research and The Royal Marsden, London, United Kingdom.

Aims To understand any context-dependent differences in signaling pathways between pancreatic (PAN), colorectal (CR) and lung (LU) cancers with KRAS mutations using a targeted phosphoproteomic approach in cell lines and patient-derived cancer cells exposed to targeted anticancer drugs ex-vivo. Materials and Methods We studied a panel of 30 KRAS mutant cancer cell lines: 10 PAN, 10 CR and 10 LU cell lines. Cancer cells were also immuno-magnetically isolated from pleural effusions and ascites of patients with KRAS mutant CR and LU cancer and exposed to a DMSO control and clinically relevant concentrations of PI3K inhibitor, PIK3C (pictilisib), AKT (AZD5363), mTOR (everolimus), EGFR (gefitinib), BRAF (vemurafenib), MEK (trametinib) and HSP90 (luminespib) inhibitors for 1 hr. Dynamic changes in a panel of 52 relevant phosphoproteins were studied using the Luminex 200 platform. Hierarchical clustering and logistic regression were used to find differences in dynamic changes in phosphoproteins between KRAS mutant, PAN, CR and LU cancer cells. Results Supervised clustering studying exposure to different drugs revealed that when exposed to the PI3K inhibitor, pictilisib, KRAS mutant LU cancers did not significantly cluster together; p=0.008, p=0.104 following Benjamini-Hochberg correction. Independently, logistic regression showed significant differences in signaling of KRAS mutant cells when exposed to the PI3K inhibitor, pictilisib. PAN and CR cancers showed an increase in p-MEK while LU cancer cells did not; p=0.0195. LU cancer cell lines showed significantly more reduction of p-AKT compared to PAN and CR cell lines when exposed to the PI3K inhibitor; p=0.033. As expected, exposure to vemurafenib increased p-MEK levels across the majority of the KRAS mutant cell lines, however compensatory reductions in p-mTOR levels were seen significantly more in PAN and CR cell lines and not in LU cell lines; p=0.0084. The dynamic phosphoprotein changes caused by pictilisib were validated in cancer cells isolated from serous effusions of 3 KRAS mutant LU and 4 KRAS mutant CR cancer patients. Validation of these findings using multiple other inhibitors and time-points is ongoing. Interpretation/conclusions We hypothesise that the significantly greater reduction in p-AKT and less increase of compensatory p-MEK caused by PI3K inhibition in KRAS mutant LU cells compared to KRAS mutant PAN and CR cell lines represents preferential signaling of these cells through the PI3K pathway. Increase in p-MEK driven by KRAS may cause a compensatory increase in p-AKT. In the context of KRAS mutant lung cancer cells also indicating preferential dependence of signaling in KRAS mutant lung cancer cells through the PI3K pathway. These findings are important while designing clinical trials of KRAS mutant cancers and more broadly to precision medicine where mutation status independent of tissue context is often used.
CLINICAL RESEARCH: Laboratory and Computational Tools to Enhance Decision Making

#997 Impact of a personalized medicine research program (PMRP), using targeted tumor profiling and a cloud based clinical trials matching platform, on clinical decision-making. Thomas D. Brown,1 Paul D. Tittel,1 Philip J. Gold,1 Charles W. Drescher,1 John M. Pagel,1 J D. Beatty,1 Patra Grevstad,1 Desiree Iriarte,1 Shilee Alexander,1 Madeleine Brindle,1 Xiaoyu Liu,1 Danielle O’connor,1 Stella Tameishi,1 Darryl Xu,1 Amel Popovic,1 B. Berry,1 Gestalt,1 Providence St. Joseph Health,2 CellNetix Pathology and Laboratories, Seattle, WA;2 CellNetix Pathology and Laboratories, Seattle, WA;2Swedish Cancer Inst., Seattle, WA;2CellNetix Pathology and Laboratories, Seattle, WA;2Swedish Cancer Inst., CellNetix Pathology and Laboratories, Seattle, WA.

Background: Cancer care is evolving to a model of precision medicine where genomic changes in a patient’s tumor are used to inform individualized management (mgmt). The optimal approach and impact of tumor profiling on cancer care is unknown. To improve research questions and support therapeutic decision-making, we used PMRP to analyze outcomes from 115,000 cases. Results: We have a 50,000 annual cases. Results: The OPeN database can be interrogated by variant Provenance St. Joseph Health, as well as other institutions, comprises the data-stewardship consortium, to aggregate big data sets consisting of clinical, genomic, pharmacologic, and treatment response data from diverse patient cases. Data from Intermountain Healthcare, Stanford University, and Swedish Cancer Institute-Providence St. Joseph Health, as well as other institutions, comprises the database and is derived from 79 hospitals, over 800 physician clinics and more than 50,000 annual cases. Results: The OPeN database can be interrogated by variant type, specific therapeutic impact on clinical outcomes, and by grouped variables, in a structured data format. The overarching IT platform is a cloud based, open source, triple store precision oncology solution, Syapse. These data are yielding valuable insights, including tumor mutational burden (TMB) scores and their correlation to immunotherapy response, clinical response in various drug-gene combinations, and therapy-specific adverse events. Conclusions: We anticipate this resource will be used by the Molecular Tumor Boards of contributing institutions for clinical interpretation, and by treating providers to overcome the N=1 challenge associated with precision oncology.

ENDOCRINOLOGY: Estrogen Receptor in Cancer Progression and Therapies

#999 ESR1 mutations activate and confer hormone resistance via distinct mechanisms. Weiyi Toy,1 Kathryn E. Carlson,2 Teresa A. Martin,3 Christopher G. Mayne,1 Sean W. Fanning,1 Pedram Razavi,1 José Baselga,1 Yang Shen,2 Geofrey Greene,2 Benita Katzenellenbogen,2 John Katzenellenbogen,2 Sarat Chandarlapaty,1 1Mem. Sloan Kettering Cancer Ctr., New York, NY; 2University of Illinois at Urbana-Champaign, Urbana, IL; 3University of Chicago, Chicago, IL.

Mutations in the ligand-binding domain (LBD) of ESR1 have been identified in the tumors and plasma of hormone-resistant, metastatic ER+ breast cancer patients. The most prevalent alterations are Y537S and D538G, and both mechanistic and clinical investigations have focused on the implications of these two mutations. Through large-scale clinical sequencing efforts, we have examined the coding region of ESR1 from over 1000 cases of metastatic breast cancer and found a diversity of ESR1 mutations. These have included a number of novel before reported alterations including mutations in the DNA binding domain and dimerization interface. The biochemical and biologic impact of many of these mutations is unknown. In this study, we have examined the impact of these alterations on ER conformation, transcriptional activity, breast cancer growth, and drug sensitivity. Using transcriptional assays, we found several classes of mutation including: (1) mutations that weakly promoted ligand-independent activity, (2) mutations that led to ligand-independent activity comparable to estradiol stimulation, and (3) mutations that impaired transcriptional activity. Among mutations that promoted some level of ligand-independent activation, we examined whether they had similar effects on receptor conformation in vitro. First, we characterized a subset using a FRET-based co-activator recruitment assay and found that, unlike Y537S and D538G, several mutants (e.g. E380Q and S463P) were unable to recruit SRC in the absence of estradiol. In keeping with this observation, tryptophan digestion assays also revealed differences in the local structure within helix 11 between these mutants. To further characterize the molecular basis for these differences, we conducted molecular dynamic (MD) modeling of mutants and compared these with the MD and crystal structure models of apo Y537S and D538G mutants. These studies further revealed differences in overall receptor conformation including localization of H12. From a therapeutic standpoint, we have examined the effect of different mutations on sensitivity to various ER antagonists. Once again, we observed important differences between mutants, with several mutant of class 2 exhibiting reduced sensitivity to SERDs compared to mutants of class 1. Nevertheless, it appeared that all mutants could be effectively antagonized by more potent SERDs, implying a continued ability of the mutant ERs to become distorted into the antagonist conformation. Taken together, the data reveal distinct conformational restrictions on ER activity that can be relieved by different ESR1 mutations. These data also imply the value of more broad coverage of ER in clinical sequencing efforts in order to effectively capture all potential resistance alleles.

#1000 Estrogen receptor coactivator MED1 in breast tumorigenesis and therapeutic resistance. Xiaoting Zhang. University of Cincinnati, Cincinnati, OH.

Recent studies have established Mediator Subunit 1 (MED1) as a key ER transcriptional coactivator for both normal mammary gland development and breast cancer. Significantly, the MED1 gene is located at the chromosome 17q12 region, also known as the HER2 amplicon, and co-amplifies with HER2 in almost all instances. Importantly, we found that MED1 serves as a key crosstalk point for the HER2 and ER pathways in anti-estrogen resistance of breast cancer. Significantly, MED1 expression highly correlates with poor disease-free survival of breast cancer patients; and most recent studies have discovered increased frequency of MED1 mutations in circulating tumor cells of human patients following anti-estrogen and anti-HER2 treatments. To determine the role of MED1 in HER2-driven tumorigenesis, we have crossed the MMTV-HER2 mammary tumor model with our established MED1 mutant knockin and newly generated MED1 mammary specific overexpression mouse models. Collectively, our studies revealed critical roles for MED1 in tumor progression, metastasis, cancer stem cell formation and therapeutic resistance in HER2-mediated
mammary tumorigenesis. These studies not only for the first time reported key roles for a HER2 amplon co-amplified gene in HER2-driven tumorigenesis but also support MED1 as a potential therapeutic target. To test that, we have assembled highly innovative RNA nanotechnology-based pRNA-HER2apt-siMED1 nanoparticles. We found these RNA nanoparticles have a very high Tm value, and are ultra-stable under RNAse A, 8 M urea, serum and PBS conditions. Importantly, pRNA-HER2apt-siMED1 nanoparticles could specifically target HER2+ human breast cancer, efficiently deplete the expression of MED1 and decrease ER-mediated gene transcription both in vitro and in vivo. Most significantly, pRNA-HER2apt-siMED1 nanoparticles not only greatly reduce the growth, metastasis and cancer stem cell formation of HER2+ breast cancer, but are also very effective in their therapeutic activity. (This work is supported by Susan G. Komen for the Cure Foundation Career Catalyst Grant, American Cancer Society Research Scholar Grant and NIH/NCI Grant Number R01CA197865).

**#1001 ESR1 mutations confer novel metastatic functions in genome-edited breast cancer models.** Zheqi Li,1 Amir Bahrein,1 Peili Wang,2 Kevin Levine,2 Nilgun Tasdemir,2 David Chu,3 Ben Ho Park,3 Adrian Lee,1 Stefi Oesterreich,1 1University of Pittsburgh, Pittsburgh, PA; 2Tianhua University, Beijing, China; 3Johns Hopkins University, Baltimore, MD.

Background: Estrogen receptor alpha (ERα), encoded by the ESR1 gene, is expressed in approximately 70% of breast cancers. Recent studies conducted by us and others have shown that somatic mutations in ESR1 gene play a key role in conferring endocrine resistance in ER+ breast cancer. These hotspot mutations mainly occur on the ligand binding domain of ERα, leading to poor outcomes in 25-30% of patients with ER+ metastatic breast cancer in clinic. The mechanisms behind the potential enhanced metastasis of these mutations have become an urgent issue to be addressed, but they are not well understood due to a lack of ESR1 mutant models. Methods: We generated and characterized genome-edited T47D and MCF7 breast cancer cell lines with the two most common ESR1 mutations (Y537S and D538G), using CRIPSR/Cas9 and rAAV systems respectively. Multiple clones for each mutant were sorted and the mutation frequencies were detected using digital droplet PCR (ddPCR). We subsequently performed an RNA-seqencing to deeply differentiate the gene expression patterns in these mutants. The growth of these pooled mutant-cells was determined in both 2D and 3D plates. The cell-matrix adhesions were measured based on ECM array, and 3D-ECM adhesion related genes were further tested by qPCR array. InCuCyte real-time image system was used to monitor the cell migration based on the wound-scraping assay. Results: We first identified the robust mutation frequencies at both RNA and DNA levels in our cell models. The RNA-seq exhibits multiple ligand-independent genes overlapping between either cell lines or mutants, which were further conformed by qPCR. We also found that both Y537S and D538G mutants present ligand-inactive and 3D ultra-low attachment plates. Using wound-scraping assay, we observed significant higher migration rate in D538G mutant of T47D cell lines on both matrigel and type I collagen, indicating a cell-line and mutant-specific phenotype. We also detected lower attachment of both mutants on type I collagen in both cell lines, and our qPCR array revealed that alterations in the MMP pathways could be one of the major mechanisms in causing this phenotypic difference. In summary, our study presents the first in-depth metastatic functional analysis of the biology of ESR1 mutations in genomic knock-in cell models, pointing out the enhanced migration and decreased cell-matrix adhesion as a potential novel gain-of-function of the Y537S and D538G mutant-cells. These findings suggest the potential role of enhanced metastasis of these ESR1 mutations through re-modeling of transcriptional profiles, shedding lights towards the development of efficient therapies of ESR1 mutant breast cancer.

**#1002 An epigenetic strategy to degrade the estrogen receptor in breast cancer.** Huili Li,1 Foteinos-Ioannis Dimitrakopoulos,2 Meredith Stone,1 Lauren Murphy,1 Stephen Baylin,1 Cynthia A. Zahnow,1 Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD; 2University of Patras, Medical School, Rio, Greece.

Epigenetic changes in DNA methylation can lead to altered gene expression and the development and progression of breast cancer. Luminal breast tumors are associated with increased DNA hypermethylation of CpG islands while basal, triple negative tumors more commonly have lower levels of DNA methylation. Increasing evidence suggests that aberrant epigenetic alterations in DNA methylation and chromatin structure may also contribute to endocrine resistance in luminal breast cancers. We thus asked whether epigenetic therapy using the demethylating agent, 5-Aza-2′-deoxycytidine (AZA) is an effective anti-tumorigenic agent in luminal breast cancers, what epigenetic mechanisms might be involved in the response and whether AZA could play a role in the sensitization of tamoxifen resistant cells. We have demonstrated using 26 breast cancer cell lines that luminal B, ER+ breast cancer is more responsive to treatment with AZA than are Her2+ or triple negative (TN) breast cancers. Cells were treated in culture with low nanomolar doses of AZA for 72 hrs, followed by transplantsation into mice and weekly palpation of tumors. Tumorgraft measurements show that 76% of the luminal B cell lines and 25% of Her2+ cell lines exhibit an anti-tumorigenic response to AZA in mice. Expression data showed that AZA leads to decreases in ER mRNA and protein expression in 50% and 67% of luminal B lines respectively. Further investigation showed that a longer AZA treatment led to lower ERα protein levels and stronger tumor growth inhibition. Protease inhibitor (MG132) treatment rescued AZA-induced growth inhibition in 50% of the luminal B cell lines, suggesting that an estrogen-mediated, ubiquitin-26S proteasomal pathway may be responsible for the degradation of ER. Gene expression analysis demonstrated that the E1 ubiquitin-activating enzyme, (UBA7)-UBE1L was up-regulated at the mRNA and protein levels by AZA. UBA7 has been suggested to function as a tumor suppressor in lung cancer and elevations in UBA7 have been correlated with longer overall survival in breast cancer. The interaction of UBA7 with ERα protein was validated by immunoprecipitation and over-expression of UBA7 in ZR-75-1 cells led to a reduction in ERα protein. We further analyzed the UBA7 promoter region by Illumina 450K array and bisulfite sequencing in our cell lines and identified two CpG rich sequences in the promoter that may be important for AZA-induced UBA7 promoter demethylation and gene re-expression. We also demonstrated in both tamoxifen sensitive and resistant cells that combination therapy with both AZA and tamoxifen was more effective at inhibiting tumor growth than the single agents. Studies are ongoing in our lab to better understand this response. Our data suggest that AZA may be clinically useful to inhibit the growth of some luminal breast tumors via its actions to increase expression of UBA7 and to degrade ER and may have efficacy in combination with anti-estrogen treatments.

**#1003 Extranuclear ERα-mTOR signaling rewires cancer cell metabolism during obesity-associated breast cancer.** Zeynep Madak Erdogan, Yiru C. Zhao, Gianlugi Rossi, Kinga Wrobel, Eylem Kulkoyluoglu, Sung Hoon Kim, John A. Katzenellenbogen, Jodi Flaws, Rebecca Smith. Unv. of Illinois at Urbana-Champaign, Urbana, IL.

Obesity is a preventable risk factor for post-menopausal ERα(-+) breast cancer. We hypothesized that serum from obese post-menopausal women contain factors that would increase tumorigenicity of breast cancer cells and increase risk of ERα(+)- breast cancer. Using whole metabolite profiling and OLINK biomarker panel of about 400 proteins associated with cancer, inflammation and cardiovascular disease, we identified biomarkers that were differentially present in serum from 50 obese v.s. 50 non-obese postmenopausal women. Next, using in vitro cell based assays as proxy we identified certain fatty acids (FFAs) as factors from serum that correlate with increased cell proliferation, motility and mTOR activation in ERα(+) breast cancer cells. We performed RNA-Seq, ERα ChIP-Seq and metabolomics analysis in breast cancer cells that are exposed to conditions that mimic serum from obese postobesmenopausal women. This integrative -omics approach enabled us to uncover ERα and mTOR pathway-dependent vulnerabilities for the breast cancer cells. In summary, we uncovered a novel role for extranuclear-initiated ERα signaling in rewiring breast cancer cell metabolism in response to obesity-associated factors in the serum. Our findings provide a basis for preventing or inhibiting obesity-associated breast cancer by using PaPEs that would exploit new metabolic vulnerabilities of breast tumors in obese postmenopausal women.

**#1004 LDLR knockdown reduces the growth of Her2 overexpressing breast cancer in mouse models of hyperlipidemia.** Emily J. Gallagher, Brian A. Neel, Zara Zelenko, Irini Markella Antoniou, Nathan Kase, Derek LeRoth. Icahn School of Medicine at Mount Sinai, New York, NY.

Women with higher circulating low density lipoprotein (LDL) cholesterol levels are more likely to have advanced HER2 positive breast cancers. The LDL receptor (LDLR) is the main receptor for cholesterol uptake into cells from circulating LDL and its expression is higher in HER2 positive breast cancers than other subtypes. The aim of our study was to understand the importance of the LDLR in the progression of HER2 positive breast cancer growth in the setting of hyperlipidemia. To study the role of hyperlipidemia in HER2 cancer progression, we used two mouse models with elevated LDL cholesterol: Apolipoprotein
factor receptor pathways in non-small cell lung cancer.

**EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Novel Mechanisms of Drug Resistance**

**1006 Drug-tolerant persister cancer cells are vulnerable to GPX4 inhibition.** Matthew J. Hangauer,1 Vasanthi S. Viswanathan,2 Matthew J. Ryan,2 Dhruv Bole,1 Jake Eaton,2 Stuart L. Schreiber,3 Frank McCormick,3 Manusun T. Michael.1 1University of California, San Francisco, San Francisco, CA; 2Broad Institute/Harvard University, Cambridge, MA.

Acquired drug resistance prevents chemotherapy and targeted therapy from achieving stable and complete responses. Emerging evidence implicates a key role for nonmutational mechanisms during early stages of acquired drug resistance. Drug-tolerant “persister” cells are a model of nonmutational cancer drug resistance in which small fractions (<5%) of cells within cancer cell lines survive pharmacologic exposure designed to eliminate drug-sensitive parental cells. These residual surviving cells are quiescent, occupy a unique chromatin state, and exhibit reversible drug resistance: removal of drug allows regrowth of cells which become resensitized to drug treatment. Importantly, this phenomenon has been observed across a wide range of cancer lineages with both chemotherapy and targeted therapy. Persister cells remain quiescent for weeks to months during constant drug exposure, however some persister cells eventually regrow and acquire irreversible drug resistance-conferring mutations that did not prevalent. This model mirrors the common clinical scenario in which tumor response is followed by relapse with acquired resistance-conferring mutations. Targeting nonmutational resistance may therefore present a therapeutic opportunity to eliminate residual surviving tumor cells to prevent relapse. We have performed an unbiased functional genomics analysis of HER2+ breast cancer persister cells which survive HER2 targeted therapy. Utilizing RNAseq pathway analysis, RNAi screens and chemical inhibitor screens, we discovered that persister cells undergo a switch to mesenchymal gene expression and are uniquely susceptible to inhibition of the phospholipid hydroperoxidase GPX4. This finding is not restricted to breast cancer, rather we have observed that all persister cells tested including those derived from melanoma, lung, ovarian and pancreatic cancer, from either targeted therapies or chemotherapies, are each specifically sensitive to loss of GPX4 function. Parental, drug naïve cancer cells and normal human cells are insensitive to GPX4 inhibition. Mechanistically, we found that GPX4 dependency in persister cells is due to a disabled antioxidant program characterized by global downregulation of antioxidant genes, decreased levels of reduced glutathione and increased ROS. These findings point forward a GPX4 inhibition strategy to prevent tumor relapse by inducing ferroptosis in the residual pool of surviving persister cells.

**1007 SHP2 inhibition restores sensitivity to ALK inhibition in resistant ALK-rearranged non-small cell lung cancer (NSCLC).** Leila Dardaei,1 Hui Qin Wang,2 Paul Fordjour,1 Man saree Singh,3 Grainine Kerr,4 Satoshi Yoda,5 Jinsheng Liang,6 Yichen Cao,2 Yan Chen,2 Justin F. Gainor,1 Luc Friboulet,2 Ibiaye Dagogo-Jack,1 David T. Myers,1 Emma Labrot,2 David Ruddy,2 Melissa Parks,1 Dana Lee,1 Richard H. DiCecca,1 Susan Moody,2 Huaxiang Hao,2 Morvarid Mohseni,1 Matthew LaMarche,1 Julia Williams,1 Keith Hoffmaster,2 Giordano Caponigro,2 Cyril H. Benes,1 Alice T. Shaw,1 Aaron N. Hata,1 Fang Li,2 Jeffrey A. Caponigro,1 Massachusetts General Hospital Cancer Center, Charlestown, MA; 2Novartis Institutes for BioMedical Research, Cambridge, MA; 3Gustave Roussy Cancer Campus, Paris, France.

Despite development of highly potent and selective inhibitors (e.g., ceritinib, alectinib, lorlatinib) targeting anaplastic lymphoma kinase (ALK), resistance invariably develops and limits the efficacy of these inhibitors in the clinic. The major classes of resistance are on-target genetic alterations (e.g., secondary ALK kinase domain mutations) and activation of alternative or bypass signaling pathways. While most patients are responsive to sequential treatment with two or more ALK inhibitors, ALK-independent resistance eventually emerges and leads to failure of further ALK-directed monotherapy. We used a synthetic lethal pooled shRNA screen to discover loss-of-function events that could sensitize resistant patient-derived cell lines to ALK inhibition. In addition to identifying known bypass targets such as FGFR, EGFR and SRC, we also identified PTPN11 (which encodes SHP2, a non-receptor protein tyrosine phosphatase that modulates signaling downstream of growth factor receptors) as a common hit shared
by cell lines exhibiting different mechanisms of bypass activation. In parallel with the shRNA screen, we also performed a high throughput combination compound screen in the same patient-derived models, and identified activation of the same bypass signaling pathways. We showed that the highly potent and selective small-molecule SHP2 inhibitor SHP099 could sensitize resistant cell lines to ALK and EGFR TKIs in vitro. Biochemical analysis of the compound-treated PDX overcame resistance mediated by ALK-independent bypass mechanisms by decreasing RAS-GTP loading potential of cells and inhibiting phospho-ERK rebound. These results suggest that dual ALK and SHP2 inhibition may represent a new therapeutic strategy for ALK-positive patients, whose lung cancers have evolved ALK-independent mechanisms of resistance, including activation of bypass signaling pathways.

### #1008 Gain-of-function kinase library screen identifies FGFR1 amplification as a mechanism of resistance to antieostrogens and CDK4/6 inhibitors in ER+ breast cancer.

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The CDK4/6 inhibitor palbociclib was recently approved in combination with endocrine therapy for treatment of ER+ metastatic breast cancer. The goal of this study was to discover mechanisms of resistance to ER antagonists alone and in combination with CDK4/6 inhibitors. To achieve this goal, we used lentiviral vectors to individually express 559 human kinase open reading frames (ORFs) in ER+ MCF7 human breast cancer cells treated with fulvestrant ± the CDK4/6 inhibitor ribociclib (Novartis). In MCF7 cells treated with fulvestrant alone or with ribociclib, we identified 21 and 17 genes, respectively, which induced a >30% increase in cell viability compared to control cells; 11 of these kinases overlapped in both treatment groups. In a secondary screen, MCF7 cells were stably transduced with V5-tagged lentiviruses expressing the positive hits identified for treatment with fulvestrant/ribociclib. Five of 11 kinases (FGFR1, FRK, HCK, FGR, CRKL) were tagged lentiviruses expressing the positive 'hits' for treatment with fulvestrant alone or with ribociclib, we identified 21 and 17 genes, respectively, which induced a >30% increase in cell viability compared to control cells; 11 of these kinases overlapped in both treatment groups. In a secondary screen, MCF7 cells were stably transduced with V5-tagged lentiviruses expressing the positive hits identified for treatment with fulvestrant/ribociclib. Survey of TCGA for copy number alterations and/or expression of these five genes showed only FGFR1 to be amplified/overexpressed in 17% of ER+ breast cancers. Experiments in vitro showed that ER+ /FGFR1-amplified (amp) MDA-134, CAMA-1 and HCC1936 human breast cancer cells and MCF7 cells stably transduced with FGFR1 were relatively resistant to estrogen deprivation, fulvestrant and fulvestrant/palbociclib compared to non-FGFR1 amp MCF7 cells. This resistance was abrogated by treatment with the FGFR tyrosine kinase inhibitor (TKI) lucitanib. Treatment with fulvestrant or palbociclib, each alone, modestly delayed growth of ER+ /FGFR1 amp breast cancer patient-derived xenografts (PDX) established in nude mice. However, addition of the FGFR TKI erlotinib to fulvestrant/palbociclib resulted in marked PDX regressions in all mice without associated toxicity. Treatment of FGFR-amp cells with FGF-2 strongly induced CCND1 (cyclin D1) expression. Downregulation of CCND1 with CCND1 RNAi oligonucleotides or kinase inhibition with erlotinib restored sensitivity to FGFR1 inhibition. Treatment with fulvestrant/palbociclib, conversely, enhanced expression of CCND1 in MCF7 cells induced resistance to estrogen deprivation and fulvestrant ± palbociclib. At this time, we are examining whether FGFR1 amplification measured by FISH correlates with maintenance of proliferation (Ki67) in 110 patients with ER+/HER2- breast cancer treated with palbociclib for 14 days immediately before surgery (Arnedos et al. ASCO 2016). In summary, using a gain-of-function ORF kinase screen, we identified FGFR1 amplification as a mechanism of resistance to anti-ER therapies ± CDK4/6 inhibitors. Based on these data we propose FGFR inhibitors should be tested in combination with ER antagonists and CDK4/6 inhibitors in patients with ER+/HER2 amplified breast cancer.

### #1009 Comprehensive ctDNA sequencing reveals mechanisms of resistance to rociletinib in EGFR T790M-mutated NSCLC.

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Background: First and second-generation EGFR tyrosine kinase inhibitors (TKIs) have benefited patients with EGFR-mutated non-small cell lung cancer (NSCLC), but resistance invariably develops after a median of 9-16 months. In ~60% of patients, resistance is mediated by a second mutation in EGFR, namely T790M. Hence, third-generation EGFR TKIs such as osimertinib and rociletinib were developed to target both activating EGFR mutations as well as T790M. Unfortunately, patients also develop resistance to these therapies through mechanisms that have not yet been thoroughly explored. Since repeat tissue biopsies pose potential complications from invasive procedures, circulating tumor DNA (ctDNA) testing is increasingly used in the clinical setting to identify potentially targetable mechanisms of resistance. Methods: Matched pre-treatment and progression plasma from 57 patients with EGFR-mutated NSCLC treated with rociletinib were analyzed using a 70-gene ctDNA targeted next-generation sequencing panel (Guardant360) that detects somatic single nucleotide variants, short insertions and deletions, fusions, and copy number variants. Pre-treatment EGFR ctDNA allele frequencies were also determined by BEAMing, a technique based on droplet digital PCR followed by flow cytometry. Pre-treatment tumor EGFR status was assessed by the tharscreen EGFR test. Results: In all 57 pre-treatment samples profiled, plasma-based ctDNA analysis detected the initial EGFR driver and T790M resistance mutations that were identified in the matched tumor. Interestingly, we found that 12% (7/57) of patients had evidence of compound EGFR driver mutations at baseline, including E709A-L858R, K860I-L858R, and L718V-L858R. EGFR T790M mutations in plasma were observed subclonally (present on average at 40% of the allele fraction of the driver mutation), suggesting tumor heterogeneity at baseline. The correlation coefficients (r) between Guardant360 and BEAMing for EGFR L858R, exon19Del, and T790M were 0.90, 0.92, 0.95, respectively. Upon progression on rociletinib, 5% of patients (3/57) developed the EGFR C797S resistance mutation, 5% (3/57) developed focal MET amplification, and 2% (1/57) developed a NTRK1 fusion that were not present in the matched baseline plasma. Additionally, 4 deletions (ERBB2 and 2 genes 2q), 4 mutations (2 genes 1q, 1 gene 3q, 1 gene 8q) were identified as somatic variants with the somatic alterations emerging at progression. In 14% (8/57) of the patients, mutations in genes involved in the RAS/RAF signaling pathway, including KRAS Q61H, KRAS K117N and NF1 Q182*, emerged or increased at progression. Conclusions: Plasma ctDNA revealed heterogeneity and multiple mechanisms of resistance in rociletinib treated patients. Thus comprehensive ctDNA sequencing allows for the identification of potentially actionable alterations and may help inform the choice of next therapy for patients progressing on a third-generation EGFR TKI.
#1011 RNAseq analysis obtained from on-purpose tumor biopsies of patients in the MATCH-R trial allows the identification of potential mechanisms of acquired resistance to PD(L)1 therapies. Loic Verlingue, Linda Mahmoud, Sandrine Aspglach, Marion Pedrero, Giulia Buzzatti, David Brandao, Zsofia Balogh, Etienne Rouleau, Ludovic Lacroix, Rastislav Bahleda, Christophe Massard, Antoine Hollebecque, Anas Gazzah, Celine Lefebvre, Serge Koscielny, Jean Yves Scoazec, Eric Angevin, Fabrice Andre, Aurelien Marabelle, Jean Charles Soria. Gustave Roussy, Villejuif, France.

Background: MATCH-R is a prospective molecular characterization trial (NCT02517892) aiming at defining the molecular basis of acquired resistance to targeted agents and immune checkpoint blockers. RNA sequencing (RNAseq) has been used to identify mechanisms of secondary resistance to immunotherapy. Patients and methods: Patients’ metastatic tumors were multi-site biopsied at relapse under immunotherapies after a period of clinical benefit, defined by a partial response or a stable disease of more than 6 months. Genome-wide RNAseq counts were intra-patient normalized and a score of each gene’s expression was computed in comparison to a cohort of 450 metastatic cancer patients with RNAseq available at the time of analysis. Results: To date, 10 patients treated by immunotherapies have had a successful RNAseq in the MATCH-R trial. Five patients were treated with PD-1 inhibitors and 5 with PD-L1 inhibitors. Three patients had NSCLC, 2 MSI high endometrial carcinoma, 2 anal carcinomas, 1 head and neck carcinoma and 1 TNBC. Eight out of ten patients had an expression of IDO1 higher than the median expression of IDO1 in our 450 controls (p value = 0.005). A patient with endometrial carcinoma had one of the highest expressions of IDO1 in the cohort. Consistently, IDO1 activation has previously been reported as a mechanism of secondary resistance to immunotherapies. A 40 year old smoker NSCLC patient with a TP53 mutation has been treated during 11 months with anti-PD1. RNAseq analysis on the biopsy of a progressive lesion showed decreased expression of different actors of the Jak-STAT pathway (biopsy composed of 40% tumor cells and 60% microenvironment). Of the 78 genes signatures used (including 52 immunogenes signatures), the interferon gamma signature had the lowest expression (p value = 0.004), consistent with a previous report of Jak-STAT-induced resistance to immunotherapies. Two more patients had an altered immune profile that could be involved in resistance to immunotherapies, but was not yet reported in the literature. Confirmation of the RNAseq analysis with immunohistochemistry is currently ongoing. The gene signatures of the 10 patients, composed of immunogenes, DNA repair genes and epigenes, were compared to the whole cohort in order to deduce corresponding false discovery rates. As such we could identify 2 gene clusters, one enriched in T cells, dendritic cells and macrophages, and the other enriched in epigenes and DNA repair genes. Analysis of more patients is currently ongoing in order to cluster the results with clinical characteristics. Conclusion: Gene expression in the biopsy of patients that relapsed after initial benefit to immunotherapy is informative and helps to identify the mechanism of acquired resistance.

#1012 Patient-derived tumor microenvironment models uncover non-autonomous TKI resistance mechanisms in NSCLC. Haichuan Hu, Hillary Mullvey, Sundus Nooen, Kodak David, Aaron Hata, Matthew Niederst, Cyril Benes, Aaron Hata, Matthew Niederst, Cyril Benes, Wayne W. Hancock, Tatiana Akimova, Tianyi Zhang, Evgeniy Eruslanov, Sunil Singhal, Steven M. Albelda. CHOP/UPenn, Philadelphia, PA.

FOXP3+ Tregs are considered important to limiting antitumor immunity but are rarely characterized clinically. We studied 227 samples from 66 lung cancer patients using blood, lymph node (LN), tumor and distant lung samples, with a mean age of 67.8±11.1 years, 67% males, 62% adenocarcinoma, 31% squamous cell carcinoma, 7% miscellaneous tumors, mean tumor size of 3.4±0.3 cm, and 21% rate of metastases. In addition to quantitating FOXP3+ CD4+ Tregs, we evaluated their expression of 35 markers by flow cytometry: CD15s, CD25, CD26, CD27, CD39, CD40L, CD45RA/RO, CD62L, CD69, CD101, CD120b, CD161, CCR4, CCR5, GARP, GITR, Helios, HLA-DR, ICOS, LP/AF, neuropilin, PD-1, TIGIT, Tim3, CCR4, CCR5, CCR7, CXCR3, CXCR4 and CCR8, and tested Treg suppressive function. We used PrimeFlow to evaluate mRNA expression of target genes in 100% pure human Treg cells, gated on CD4+ FOXP3+ cells. FOXP3+ Tregs (%) in the CD4+ T cells of tumors and LN were significantly decreased in compared to other sites; tumors 19.2±8.5%, LN 14.7±7.8%, healthy PBMC donors 7.2±2.5%, PBMC lung cancer 7.2±2.7%, and lungs 6.6±3.2%. Tregs of lung tumors were remarkably suppressive vs. all other sites (p<0.0001 vs. PBMC & LN, p=0.0106 vs. lung Tregs). None of the 35 markers evaluated by flow cytometry were statistically significantly different for tumor Tregs vs. other sites. However, PrimeFlow showed tumor Tregs, but not FOXP3- T cells from other sites, had upregulated mRNA expression of 4 transcription factors (TF): Eos, Irf4, Satb1 and Gata1. These 4 TFs plus LEF1 were recently described as a quintet of Treg self-locking signature; TF expression of any 2 TF plus FOXP3 promoted full Treg gene expression and function. Tumor Tregs also showed significant upregulation of FOXP3 mRNA and protein. Tumor and lung Tregs expressed more Eos, Satb1 and Gata1 mRNA, and much higher levels of FOXP3 protein per cell, while in PBMC, LNs and healthy donor Tregs upregulation of TFs did not correspond with increase of FOXP3 protein, indicating the "Treg self-locking signature" is subject to regulation by local factors. Indeed, cultured tumor Tregs downregulated FOXP3 protein, mRNA and TF expression, while PBMC and LN Treg incubated in tumor-conditioned media upregulated FOXP3 mRNA and protein, and Treg TF expression, moving toward the tumor Treg-like phenotype. We found significantly increased numbers and suppressive function of FOXP3+ Tregs within lung tumors vs. other sites in the same patients. In addition, while large-scale flow cytometric studies were not useful in identifying key features of tumor Tregs vs. Tregs at other sites, PrimeFlow showed that tumor Tregs have a unique phenotype with upregulated expression of FOXP3 mRNA and protein, as well as Eos, Irf4, Satb1 and Gata1 mRNA. These features appear to be malleable and associated with features of the local tumor microenvironment.


High numbers of tumor infiltrating regulatory T (Treg) cells are indicative of poor outcome in several malignancies, including ovarian cancer, colorectal cancer, and melanoma. Thus, selectively abrogating intratumoral Treg cell function while maintaining systemic immune tolerance remains an attractive, albeit elusive, strategy for cancer immunotherapy. We have identified the epigenetic enzyme Ezh2, an H3K27 methyltransferase, to be a critical mediator of lineage stability and function in activated Tregs. Consequently, deficiency of Ezh2 in Treg cells strongly impairs their function in non-lymphoid tissues. We hypothesized that this phenomenon might translate to a similar defect that is restricted to the tumor microenvironment. Here we tested this hypothesis in three transplantable syngeneic tumor models in mice (MC38 colon carcinoma, TRAMP-C2 prostate cancer, and B16F10 melanoma). We observed that constitutive deletion of Ezh2 in Treg cells resulted in potent anti-tumor activity, significantly impaired tumor outgrowth, and in many instances, complete tumor rejection. These results were also recapitulated when Ezh2 was temporally deleted in the vast majority of Treg cells at the time of tumor inoculation. Functional analyses at early time points after Ezh2 deletion revealed enhanced functionality of effector CD4 and CD8 populations, as evidenced by increased IFN-γ production. We observed that constitutive deletion of Ezh2 in Treg cells resulted in potent anti-tumor activity, significantly impaired tumor outgrowth, and in many instances, complete tumor rejection. These results were also recapitulated when Ezh2 was temporally deleted in the vast majority of Treg cells at the time of tumor inoculation. Functional analyses at early time points after Ezh2 deletion revealed enhanced functionality of effector CD4 and CD8 populations, as evidenced by increased IFN-γ production.
more directly address this hypothesis, we generated mice that harbored both Ezh2-deficient and wild type Treg cells and observed anti-tumor activity similar to mice that exclusively harbored Ezh2-deficient Treg cells. In stark contrast to Ezh2 deficiency, mice whose Treg cells were completely depleted, using a Foxp3-driven diphtheria toxin receptor allele, were unable to reject tumors, further supporting a dominant role for Treg cells in driving tumor immunity. Finally, tumor protection in the presence of Ezh2-deficient Treg cells occurred without significant morbidity due to autoimmune pathologies, which were prevalent in mice with systemic Treg cell depletion. These results suggest that targeted deletion of Ezh2 in Treg cells drives a unique phenotype among tumor infiltrating Treg cells, reprogramming the tumor microenvironment and selectively augmenting the anti-tumor immune response. Cancer therapies that allow the selectively inhibiting Ezh2 to targeted tumor cells are under investigation, going forward it will also be important to assess the potential for beneficial effects via modulating the immune response.


Immunotherapy is a new pillar of cancer therapy. One theoretical advantage of immunotherapy of cancer is that effector cells induced at one site should be able to kill metastatic cancer cells in other sites or tissues. On the other hand, it has been recognized that each tissue has unique immune components that play critical roles in protection against pathogens. However, very little is known whether effector T cells induced against tumors in one tissue can work against the same tumors in other tissues. To address this question, we compared the effect of effector cells induced against the same tumor growing in either the lung or the skin by using CT26 murine tumor models. Rejection of s.c. CT26 tumors was achieved by pretreatment with anti-CD25, which blocks the function of Treg cells. Both CD4 and CD8 T cells were necessary for the protection. When anti-CD25-pretreated mice challenged with s.c. CT26 were simultaneously injected, i.v. with CT26, they also rejected tumors in the lung, while anti-CD25-pretreated mice without s.c. CT26 did not. This observation suggested that T cell mediated anti-tumor protective immunity induced against s.c. tumors can also protect against lung metastases of the same tumors. In contrast to Treg depletion which allowed for the induction of protective immunity in the s.c. tumor model, NKT cell deficiency in CD1d KO mice induced significant CD8 T cell-mediated protection against lung metastasis of CT26 but had no effect on the growth of s.c. CT26 tumors. When CD1d KO mice rejecting s.c. CT26 were simultaneously challenged with s.c. CT26, the development of s.c. tumors was not affected, indicating that tumor rejection induced against the CT26 in the lung did not confer protection for the same tumor cells in the skin. Since the protection against CT26 in the lung in CD1d KO mice is mediated by CD8 T cells, we transferred T cells from CD1d KO mice inoculated i.v. with CT26 into RAG1 KO recipients, and challenged the recipient mice with the CT26 tumor cells. The recipient RAG1 KO mice were growing s.c. tumors of either CT26 inoculated or not. s.c. tumors grew in the RAG1 KO mice even if the T cells were derived from CT26 inoculated mice. This observation suggested that the CD8 T cells protective in the lung are not protective in the skin. These data indicate the effector cells against the same tumor do not work in all tissues, and the induction site of the effector cells is critical to control metastasis.

#1016 Evaluation of progression associated neoepitopes and immune contexture in pulmonary premalignancy. Kostyantyn Krysan, Linh M. Tran, Brandon S. Grimes, Tonnya C. Walser, William D. Wallace, Steven M. Dubinett. UCLA David Geffen School of Medicine, Los Angeles, CA.

Lung cancer is the leading cause of cancer death in the US and in the world. Over the past 30 years, the five-year survival rate for lung cancer has increased by only 5%. With the widespread implementation of screening programs, detection of premalignant and early stage disease is increasing. A better understanding of genomic alterations and the microenvironment across the spectrum of early disease could lead to identification of progression-associated mutations (PAMs), defined as those shared between premalignant lesions and invasive cancer, and their neoepitopes. Unleashing the immune response against pulmonary premalignancy could transform therapy and outcomes. FFPE tissue blocks from patients with resected lung adenocarcinoma (ADC) were obtained from the UCLA Lung Cancer Tissue Repository. For each patient, the following regions were dissected from formalin-fixed paraffin-embedded tissue slides using Laser Capture Microdissection: a) normal airway epithelial cells (1-3 regions), b) premalignant atypical adenomatous hyperplasia (AAH, 2-4 regions), c) adenocarcinoma in situ (AIS, when present) and d) ADC, 1-3 regions followed by whole exome sequencing. Forty-one complete cases have been sequenced to date. Our data suggest that premalignant lesions from the same patient may a) have different mutational profiles and b) bear progression-associated mutations, common with the primary lung tumor. This inter-lesion heterogeneity suggests that a progression-associated mutational landscape could be defined in longitudinal studies of pulmonary premalignancy which will be the focus of future investigations. Next, utilizing the mutational data, we performed in silico neoantigen analysis to identify potential neoepitopes among the genes mutated in premalignant lesions. The neoantigen analysis demonstrated that among the top 11 peptides with high binding avidity for autologous MHC, 9 were derived from PAMs. This suggests that neoepitopes exist in premalignancy that could serve as targets for immunotherapy.

#1017 Lipid metabolic reprogramming drives resistance to PD1 blockade. Maria A. Cortez, Sharaareh Niknam, Efrosini Cuko, Jonathan E. Schoenhals, Hampartsoum Basoumian, Ahmed I. Younes, Aliin Li, Jody V. Vyvkoukal, Cristina Ivan, George A. Calin, Patrick Hwu, James W. Welsh. UT MD Anderson Cancer Ctr., Houston, TX.

The mechanisms underlying immunosuppression and resistance to PD1 inhibitors are not well understood. We attempted to fill this gap with an integrated analysis of mRNA, microRNA, and protein expression in an anti-PD1-resistant lung adenocarcinoma mouse model. The model was created by in vivo passage of 344SQ murine lung cancer cells (p53R161H/β2M−/−, KrasG12D+) in a syngeneic host repeatedly dosed with anti-mouse PD1 antibodies. Anti-PD1-resistant 344SQ (344SQ_R) and 344SQ parental (344SQ_P) cells were then inoculated into syngeneic 129SvEv mice, which were then dosed twice with anti-PD1 or control IgG antibodies. Tumor tissues were collected and analyzed as follows: transcriptionome with Affymetrix; protein levels by reverse phase protein array analysis; signature enrichment by gene set enrichment analysis; metabolome by mass spectrometry; and lipid content with fluorescent probes Oil Red O and BODIPY. We also isolated tumor-infiltrating immune cells for flow cytometry and gene expression analyses. We identified lipid-related metabolic pathways as being the most highly enriched in anti-PD1-resistant tumors (344SQ_R) vs. their 344SQ_P counterparts; the resistant cells also had more lipid droplets than the 344SQ_P cells. The anti-PD1-resistant tumors overexpressed several genes involved in lipogenesis and fatty acid pathways (e.g., fatty acid binding proteins [FABPs], fatty acid synthase, acetyl-CoA-acyltransferase 2, fatty acid elongases). Specifically, FABP overexpression promoted fatty acid uptake and lipid-droplet accumulation in resistant tumors. Lipid-sensitive targets linked to inflammation and insulin signaling (e.g., stress-activated kinases such as JNK and NFκB) were altered in 344SQ_R vs. 344SQ_P tumors. Mechanistically, JNK downregulation by NFκB-regulated microRNAs protected PD1-resistant tumors from lipotoxicity caused by FABPs upregulation and fatty acid uptake. FABP levels were higher in plasma from 344SQ_R than from 344SQ_P tumors. Tumor infiltrating macrophages from anti-PD1 resistant tumors had a correspondingly higher percentage of M2-like macrophages. 344SQ_R tumors promoted immune suppressive cells by upregulating FABP's expression in M2-like macrophages, marked by increased fatty acid intake and fatty acid oxidation. Conversely, percentages of CD14+ and CD68+ tumor infiltrating lymphocytes were reduced in the resistant tumors. These results suggest that lipid metabolic rewiring drives resistance PD1 inhibitors supporting the accumulation of immunosuppressive cells, including M2-like macrophages, preventing type I immune responses elicited by T cells. Collectively, these findings reveal new potential lipid-related targets for drug development or new treatments combining inhibitors of these targets with anti-PD1 therapy.
IMMUNOLOGY: Novel Insights into Mechanisms of Response to Immunotherapy

Previous microbiome studies at the genus level have described altered microbiota in head and neck squamous cell carcinoma (HNSCC), both in terms of taxonomic composition and metabolic capacity. We applied high-resolution microbiome profiling (Resplora Insight) to analyze 16S rRNA sequencing data in saliva and tissue samples from HNSCC patients and healthy controls. DNA extraction and amplicon library preparation was performed for saliva samples from HNSCC (n=38) and controls (n=25), as well as tissue samples from HNSCC (n=25) and controls (n=8). Raw sequences were processed for quality and length, screened for chimeras and filtered for contaminant human and chloroplast DNA. High-quality passing sequences were submitted to Resplora Insight for species-level taxonomic assignments, and potential associations were analyzed with DESeq2. Samples from a subset of HNSCC patients were significantly enriched with commensal species from the vaginal flora, including Lactobacillus gasseri/ johnsonii (710x higher in saliva and 1900x higher in tissue) and Lactobacillus vaginalis (52x higher in saliva). These species were not observed in normal saliva or tissue samples from Hopkins patients (n=33) nor in normal saliva samples (n=292) from the Human Microbiome Project (HMP). Interestingly, both species were only observed in saliva from Human Papilloma Virus positive (HPV+) and HPV negative (HPV-) oropharyngeal cancer patients, and we confirmed their representation in vaginal samples from the HMP (n=249). We also found that Fusobacterium nucleatum (F. nucleatum), an oral flora commensal bacterium linked to colon cancer, is enriched (9x higher in saliva and 11x higher in tissue) in a subset of HNSCC patients with advanced tumors (T3 or above). F. nucleatum was detected in samples obtained before and after treatment with chemothera- bition, but not with surgery alone. Interestingly, we identified upregulation of the oncogenic Wnt/Beta catenin pathway (Wnt7B, FZD6, SFRP4) and down regulation of immune system pathways (TLR10, IRF8) with genome-wide mRNA arrays (Affymetrix) in HNSCC samples enriched for F. nucleatum. Using fluorogenic quantitative PCR we confirmed that F.nucleatum and Fusobacterium spp. are enriched in saliva samples collected prior to treatment in another cohort of HNSCC patients, and in post-treatment saliva samples obtained from HNSCC patients treated with PD-1 checkpoint blockade. We also found enrichment of HPV+ oropharyngeal tumors with F. nucleatum and Fusobacterium spp., prior to therapy, while some recurrent HPV- oropharyngeal tumors are enriched only with Fusobacterium spp. Together, these results suggest that bacteria may impact therapy in HPV+ and HPV- oropharyngeal and oral cavity cancer by altering oncogenic and immune system pathways.

**#1019 In vivo CRISPR screening identifies Ptpn2 as a target for cancer immunotherapy.**

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Despite the dramatic clinical success of cancer immunotherapy with PD-1 checkpoint blockade, most patients don’t experience sustained clinical benefit, suggesting that additional therapeutic strategies are needed. Functional genomic screens in cancer cells to discover new therapeutic targets are usually carried out in vitro where interaction with the immune system is absent. Here we report a pooled, loss-of-function genetic screening approach using CRISPR/Cas9 genome editing that is conducted in vivo in mouse transplantable AML models treated with vaccination and PD-1 checkpoint blockade. We tested 2,400 genes expressed by melanoma cells for those that synergize with or cause resistance to checkpoint blockade, and recovered the known immune evasion molecules, PD-L1 and CD47. Loss of function of multiple genes required to sense interferon-y caused resistance to immunotherapy. Deletion of Ptpn2, a pleotropic protein tyrosine phosphatase improved response to immunotherapy. In vivo, Ptpn2 deficient tumors showed increased infiltration of activated CD8+ T cells. In vitro, Ptpn2 loss by tumor cells increased antigen presentation to T cells. Biochemical, transcriptional and genetic epistasis experiments demonstrated that loss of function of Ptpn2 sensitizes tumors to immunotherapy by enhancing interferon-y-mediated effects on the tumor cell. Thus, augmenting interferon-y signaling in tumor cells could increase the efficacy of immunotherapy. More generally, in vivo genetic screens in tumor models can identify new immunotherapy targets and rationally prioritize combination therapies.

**#1020 BRD9 defines a novel mammalian SWI/SNF (BAF) complex configuration which supports proliferation in AML.**

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Genes encoding subunits of the mammalian SWI/SNF (BAF) ATP-dependent chromatin remodeling complexes are mutated in over 20% of human cancer. Specific subunits are mutated in specific malignancies, highlighting their tissue-specific protective roles; moreover, synthetic lethal screens have uncovered genetic- and lineage-based features which confer dependence on specific mSWI/SNF subunits. As combinatorial complexity represents a major challenge, identification of specialized mSWI/SNF configurations, subunit-specific functions, binding restrictions, and exclusivity relationships is critical for understanding oncogenic mechanisms and for the selection of appropriate therapeu- patic agents targeting mSWI/SNF complex subunits. Here, we discover that BRD9, a recently identified mSWI/SNF complex subunit, defines a unique complex configuration distinct from BAF and PBAF, which we term non-canonical BAF, or nCBaf. We used biochemical methods to isolate BRD9-containing complexes and find that BRD9 selectively marks a sub-stoichiometric group of mSWI/SNF complexes of smaller molecular weight that lack several members of canonical BAF complexes such as BAF47 and ARID1A. Moreover, chemoproteomusing BRD9 binds to and inhibits the non-CBAF specific or PBAF-specific subunits, including the highly related bromo- domain-containing subunit BRD7. We further identified regions of BRD9 and BRD7 that confer specificity of these subunits to nCBaf and PBAF complexes, respectively, resolving their mSWI/SNF binding domains. Using genome-wide ChiP-seq and RNA-seq experiments, we determined that nCBaf complexes target a distinct subset of all mSWI/SNF complex target genes and, consistent with previous studies, maintain proliferation of AML cells. Finally, we applied a newly- generated approach to deriving functional relationships within and between protein complex families from shRNA and CRISPR-based genetic screening datasets across hundreds of cancer cell lines to explore nCBaf-specific subunits. We find that nCBaf-specific complex subunits form a distinct functional module, supporting biochemical studies and pointing to the specific and divergent functions of the nCBaf configuration. Cancers of hematologic origin collectively exhibit the most significant responses to perturbation of three nCBaf subunits including BRD9, substantiating previous small molecule screening ef- forts using BRD9 bromodomain inhibitors. These data demonstrate that nCBaf complexes represent a novel BAF complex composition with distinct function in cancer.

**#1021 Mutations in SWI/SNF chromatin remodelling complex sensitizes tumors to OXPHOS inhibition.**

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Recent large-scale cancer genomic studies have established a framework in which biological functions and genetic interactions of established and novel cancer genes can be explored. One of the major findings of these studies has been the discovery of frequent genetic alterations in chromatin and epigenetic regulators including inactivating mutations in components of the SWI/SNF chroma- tin remodeling complex. In lung adenocarcinoma in particular, a quarter of tumors have inactivating mutations in the SWI/SNF components Smarca4, ARID1A or the histone modifer SETD2. With the aim of understanding the mechanism of tumor development and identifying potential vulnerabilities of SWI/SNF mutant tumors, we developed a genetically engineered mouse model (GEMM) of lung adenocarcinoma by selectively ablating Smarca4 in the respira- tory epithelium. We demonstrate that Smarca4 acts as a bona fide tumor suppressor and cooperates with p53 loss. Cross species integrative gene expression analyses revealed signatures of enhanced oxidative phosphorylation and reactive oxygen species (ROS) response in Smarca4 mutant murine and human lung tumors. We further show that Smarca4 mutant cells have increased oxygen consumption, enhanced basal and maximal respiratory capacity and production of ROS. This is primarily driven by activation of the mitochondrial master reg- ulator PGC1alpha. Finally, we show that Smarca4 and other SWI/SNF mutant lung cancer cell lines have exquisite sensitivity to inhibition of oxidative phos- phorylation using a novel small molecule that is under development. These findings provide a mechanistic basis for further development of OXPHOS inhib- itors as therapies in SWI/SNF mutant tumors.
**MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Chromatin Structure and Function**

### #1022 Chromatin remodeler HELLs is an epigenetic driver for hepatocellular carcinoma progression.

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HELLs is a nuclear chromatin remodeling protein that is frequently overexpressed in human cancers including hepatocellular carcinoma (HCC). In the present study, we applied a global computational approach integrating whole genome sequencing (WGS), ChIP-seq, and RNA-seq to investigate the expression of chromatin remodelers in human HCCs. We found that HELLs, a SWI2/SNF2 chromatin remodeling enzyme, is remarkably overexpressed in HCC. Overexpression of HELLs was correlated with more aggressive clinicopathological features and poorer patient prognosis. We further showed that up-regulation of HELLs in HCC was confered by hyper-activation of transcription factor SP1. To investigate the functions of HELLS in HCC, we generated both gain- and loss-of-function lines by CRISPR activation and repression systems, respectively, in the HELLS gene via CRISPR/Cas9 genome editing system. We demonstrated that overexpression of HELLS augmented HCC cell proliferation and migration. In contrast, depletion of HELLS reduced HCC cell growth and motility. Moreover, inactivation of HELLS induced apoptosis in HCC cells. Coherently, ablation of HELLS also mitigated tumorigenesis and lung metastasis in vivo as demonstrated with both subcutaneous and orthotopic tumor implantation models. Mechanistically, by using RNA-seq and MIne-seq, we revealed that HELLS controls the nucleosome occupancy at gene enhancer and transcription start site (TSS). Overexpression of HELLS increased nucleosome occupancy that obstructed the accessibility of enhancers and hindered the formation of nucleosome-free region (NFR) at TSS of its target genes, thereby blocks the binding of transcription factors for activating gene expression. Consequently, though this mechanism, up-regulation of HELLS mediated epigenetic silencing of multiple tumor suppressor genes including E-Cadherin, FB1P3, XAF1 and CREB-H in HCC. In conclusion, our data unravel that HELLS is a key epigenetic driver of HCC. By altering the nucleosome occupancy at NFR and enhancer, HELLS epigenetically suppresses numerous tumor suppressor genes to promote HCC progression.

### #1023 Activation of proto-oncogenes by enhancer-hijacking in high-risk neuroblastoma.

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Background: Neuroblastoma (NB), a neural crest-derived tumor of the sympathetic nervous system, is the most common extracranial solid tumor in children. We have previously shown that genomic rearrangements activate proto-oncogenic telomerase by juxtaposing active enhancer elements to the TERT gene in a large fraction of high-risk NBs. In the present study, we applied a global approach integrating whole genome sequencing (WGS), Chromatin immuno-precipitation sequencing (ChIP-seq) and RNA sequencing (RNA-seq) data of NB cells and tumors to identify further key oncogenes activated by enhancer-hijacking in NB. Methods: WGS was applied to search for structural rearrangements in 120 NB tumors and five NB cell lines. Corresponding RNA-seq data were used to discover mono-allelic and/or outlier expression of candidate genes potentially involved in enhancer hijacking events. ChIP-seq of 34 NB tumors and 17 NB cell lines was applied to identify the enhancer elements in NB. Circular chromatin conformation capture sequencing (4C-seq) was used to confirm physical promoter-enhancer interactions in NB cell lines. Results: WGS analyses revealed that chromosomal rearrangements are common events in NB tumors and cell lines and frequently affect regions harboring proto-oncogenes and lineage specific enhancers. ChIP-seq analyses of the chromatin mark histone 3 lysine 27 acetylation (H3K27ac), surrogate for enhancer activity, confirmed that these rearrangements recurrently juxtapose active enhancer elements to oncogenes including MYCN and MYC in NB. Intriguingly, quantification of H3K27ac ChIP-seq profiles uncovered that the enhancer elements translocated to MYC were among the most active ones within the respective epigenomes. 4C-seq analyses proved physical interactions between translocated enhancer elements and promoters of the respective oncogenes, which is in line with their elevated expression in rearranged cases. Conclusions: Our study reveals that structural rearrangements in high-risk neuroblastosoma frequently juxtapose strong enhancers to key oncogenes, including MYCN and MYC. This represents a novel and important mechanism by which overexpression of the oncogenes observed in rearranged cases. The common mechanism of oncogene activation by enhancer-hijacking may open a therapeutic window for epigenetic drugs including BET or CDK7 inhibitors in high-risk NBs.

### #1024 Genome-wide examination of topological variability reveals cell-type-specific mechanisms of oncogene activation.

Caleb Lareau, Martin Aryee, Harvard University, MA.

INTRODUCTION: The organization of chromatin within the nucleus into hierarchical three-dimensional (3D) structures plays a key role in the regulation of gene expression. Differences in 3D architecture, such as the presence or absence of “loops” between specific enhancers and their target genes, are a major driver of transcriptional and phenotypic variation. By determining the accessibility of genes to transcriptional machinery and distal regulatory regions, mechanisms mediating oncogene activation can be discerned. Recent studies have implicated pathogenic alterations in genome topology with the activation of proto-oncogenes in a multitude of cancers. METHODS: To characterize the topological changes associated with different cancer states, we developed a computational suite of tools that facilitates the systematic discovery, annotation, and visualization of features in chromatin topology and assigns statistical confidence estimates to regions with variability between cellular phenotypes. Our framework facilitates the integration of heterogeneous genomic data, including gene expression, histone modification, DNA methylation, open chromatin, and genomic variation, to further characterize changes in 3D genome. Ultimately, we propose a joint model that functionalizes the effect of epigenetic modifications on transcription as mediated through hierarchical chromatin structure. RESULTS: We identify regions of topological variation unique to breast cancer, cervical cancer, glioblastomas and with and without an IDH1 mutation, melanoma, and leukemia. Patterns of disrupted insulation and subsequent localization of enhancers to proto-oncogenes can be attributed to variation in the epigenome, including variation in DNA methylation and open chromatin, at distal regulatory regions. These 3D alterations provide a mechanism for activation of specific oncogenic pathways, including signaling mediated by p38, PI3K, and BRCA CONCLUSIONS: Variable patterns of chromatin topology provide a unique signature and mechanism of oncogenes for the cancer phenotypes considered. Additionally, our framework provides a novel means for interpreting epigenetic variability, particularly at distal regulatory regions, and explaining transcriptional variation associated with cancer phenotypes as mediated through structural changes in the DNA.

### #1025 Controlled and endogenous depleton of a synthetic lethal target in human cancer cell lines via SMASH degron engineering.


The SWI/SNF (NuRD) chromatin remodeling complexes play an important role in regulating processes such as gene transcription through ATP-dependent remodeling of chromatin. The importance of this role is highlighted by the prevalence of mutations in various SWI/SNF subunits across approximately 20% of cancers encompassing various lineages and subtypes. Functional genomics screening via pooled shRNAs has led to the discovery of distinct vulnerabilities of SWI/SNF mutant cancers to loss of mutually exclusive subunits. In particular, cancer cells with BRG1 loss become exquisitely dependent on the remaining BRG1 counterpart, BRM. To further understand the synthetic lethal relationship between BRM and BRG1, we have adapted a recently described degron, the small molecule assisted shutoff (SMASH) tag, showing for the first time the ability to apply this system to control endogenous levels of target protein, in this case BRM. We used the SMASH tag to target the endogenous BRM locus in both a BRG1 wild-type and mutant cancer cell line. Using these engineered cell lines, we were able to show that we can achieve tunable protein levels by varying the concentration of the effector molecule. The ability to tune BRM depletion allowed us to investigate the nature of the BRG1/BRM synthetic lethal relationship. Our data interestingly suggests that while modest depletion of BRM is sufficient to observe changes in the expression of a target gene regulated by SWI/SNF in a dose-dependent manner, a higher level of BRM depletion is required to elicit a growth arrest phenotype in the BRG1-mutant non-small cell lung cancer line. Together these results uncover a novel relationship between the catalytic SWI/SNF subunit and gene expression, where we find that the latter responds in a dose-dependent manner.
dependent manner to BRM levels. Additionally, we elucidate the level of BRM depletion required to observe the synthetic lethal phenotype, providing insight and informing on strategies for therapeutic targeting whether by protein degradation approaches, such as PROTAC, or other small molecule approaches. Finally, having provided a proof-of-concept, such tools can be readily adapted for application to endogenous degradation of other target proteins in different systems.

#1026 Development of orthotopically grafted organoid models to study vulnerabilities. Jingwen Zhang,1 Brandon Willis,1 Maureen Hattersley,1 Alan Lau,2 Corinne Reimer,1 Michael Zinda,1 Stephen Fawell,1 Gordon Mills,1 Austin Dulak,1 Huawei Chen,1 AstraZeneca Pharmaceuticals, Waltham, MA; AstraZeneca Pharmaceuticals, Macclesfield, United Kingdom; 1The University of Texas MD Anderson Cancer Center, Houston, MA.

BRD4 is a member of the BET (bromodomain and extraterminal domain) family of chromatin readers that recognize acetylated-lysines on histones and nuclear proteins. Previous reports have demonstrated that select cancers depend on BRD4 to regulate oncogenic gene transcriptional programs. However, whether BRD4 contributes to cancer malignancy through other mechanisms has not been extensively evaluated. Here, we show that BRD4 is important for maintaining an intact DNA replication checkpoint in cancers. Displacement of BRD4 by BET bromodomain inhibitor AZD5153 in cell lines under intrinsic or exogenous replication stress led to a time-dependent reduction in phospho-Chk1 first detected within 30 minutes, and reaching maximum phospho-Chk1 inhibition (~85%) at an hour after AZD5153 treatment in U2OS cells. The decrease in Chk1 phosphorylation was observed without a concomitant decrease in total Chk1, and this was not replicated by treatment with pan-transcriptional inhibitors, suggesting a non-transcriptional mechanism linked to BRD4. Furthermore, BRD4 interacts with the DNA pre-replication complex and inhibition of BRD4 leads to hyperactivation of the pre-replication complex and aberrant DNA replication re-initiation under replication stress conditions. Consistent with a role in S-phase signaling, BETi treatment sensitizes replicating cells to replication stress-inducing agents. Finally, we observed that ovarian cancer cell lines are highly-sensitive to the combined inhibition of BRD4 and ATR. Co-administration of AZD5153 and AZD6738 (ATR inhibitor) significantly inhibited tumor growth in OVCAR3 ovarian xenograft model (TGI after 21 day dosing: AZD5153, 52%; AZD6738, 46%; Combo, 84%). Sustained tumor growth delay was observed after combination treatment cessation. Together, our study uncovered a novel function for BRD4 in regulating DNA replication stress response, and provide mechanistic rationale for combining BETi with DNA damage-targeted agents for cancer therapies.

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Chromatin Structure and Function

#1027 Genetic disruption of Kras sensitizes lung cancer cells to Fas-mediated apoptosis. Haiwei Mou,1 Jill Moore,2 Sunal Malonjia,1 Yingxiang Li,1 Deniz Ozata,1 Soren Hough,1 Chunqing Song,1 Jordan Smith,1 Has Yin,2 Andrew Fisher,1 Daniel Anderson,2 Shipping Weng,1 Michael Green,1 Wen Xue1,1 Univ. of Massachusetts Medical School, Worcester, MA; 1David H. Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Worcester, MA.

Genetic lesions that activate KRAS accounts for ~30% of the 1.6 million annual cases of lung cancer. Despite clinical need, KRAS is still undruggable using traditional small molecule drugs/inhibitors. When oncogenic KRAS is suppressed by RNA interference, tumors initially regress but eventually recur and proliferate despite suppression of Kras. Here we show that tumor cells can survive CRISPR-mediated knockout of oncogenic Kras, indicating the existence of Kras-independent survival pathways. Thus even if clinical KRAS inhibitors were available, resistance would remain an obstacle to treatment. Kras-independent cancer cells exhibit decreased colony formation in vitro but retain the ability to form tumors in mice. Comparing the transcriptomes of oncogenic Kras cells and Kras knockout cells, we identified 603 genes that were specifically upregulated in Kras knockout cells, including the Fas gene, which encodes a cell surface death receptor involved in physiological regulation of apoptosis. Antibodies recognizing Fas receptor efficiently induced apoptosis of Kras knockout cells but not oncogenic Kras expressing cells. Increased Fas expression in Kras knockout cells was attributed to decreased association of repressive epigenetic marks at the Fas promoter. Concordant with this observation, treating oncogenic Kras cells with histone-deacetylase inhibitor and Fas-activating antibody efficiently induced apoptosis, thus bypassing the need to inhibit Kras. Our results suggest that activation of Fas could be exploited as an Achilles’ heel in tumors initiated by oncogenic Kras.

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Oncogenes and Tumor Suppressors 1: Function and Therapeutic Vulnerabilities

#1029 Development of orthotopically grafted organoid models to study pancreatic cancer progression. Chang-Ii Hwang,1 Eunjung Lee, Brandon Da Silva, Kevin Wright, Youngkyu Park, David A. Tuveson. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Pancreatic ductal adenocarcinoma (PDA) is one of the most difficult human malignancies to treat. The 5-year survival rate of PDA patients is 7% and PDA is predicted to become the second leading cancer-related cause of death in the USA. A subset of potential tumor suppressor genes was identified by genome-wide analysis of human PDA and insertional mutagenesis in genetically engineered mouse models (GEMMs). Since the functional validation of these genes in GEMMs is time-consuming and labor-intensive, we have developed a rapid and efficient ‘orthotopically grafted organoid’ (OGO) model in conjunction with RNAi and CRISPR/Cas9 technologies to study PDA progression. Previously, we showed that OGO model represents the full spectrum of PDA progression in vivo and respond to orthotopic engraftment. Here, as a proof-of-concept experiment, we demonstrate that ablation of Trp53 by viral introduction of shRNA or gRNA in PanIN-derived organoids accelerates PDA progression upon transplantation. In addition, we engineered tetracycline-inducible shRNA against Trp53 in ColA1 locus to rescue the orthotopic grafted organoids derived from Kras\(^{+/+}\); Rosa26-rTatA; ColA1-homing cassette mouse. Orthotopic transplantation followed by doxycycline administration resulted in rapid PDA progression with metastases. Isolated tumor organoids were re-transplanted to evaluate the effect of p53 restoration in PDA progression. Restoration of p53 by doxycycline withdrawal led to reduced liver metastases, although there was no difference in survival and primary tumor growth. This will provide a new insight how p53 regulates PDA metastasis. Therefore, OGO models should provide an excellent platform to study the functional role of genes in PDA progression in vivo.
#1030 Metabolic reprogramming caused by mutations of the Fbw7 ubiquitin ligase in colorectal cancer. Ryan J. Davis,1 Mehmet Gonen,2 David Hockenbury,1 Adam Margolin,2 Bruce E. Clurman1.1 Fred Hutchinson Cancer Research Ctr., Seattle, WA; 2Oregon Health Sciences University, Portland, OR.

Fbw7 is an evolutionary conserved F box protein that functions as a substrate adapter for the Skp1-Cullin 1 F box (SCF) E3 ubiquitin ligase complex. Fbw7 is also a tumor suppressor gene that is mutated in diverse human cancers. Many Fbw7 substrates are oncogenic transcription factors, including c-Myc, Notch, and Jun. Cancer-associated Fbw7 mutations diminish its ability to interact with substrates, resulting in inappropriate oncoprotein accumulation. One of the significant challenges for understanding the consequences of Fbw7 mutations in tumors is that Fbw7 coordinately regulates approximately two dozen broadly-acting transcription factors. To address this problem, we used novel computational methods to analyze TCGA gene expression datasets to derive transcriptional signatures that could predict a tumor’s Fbw7 mutational status; these signatures were then verified by enrichment of genes involved in biological processes. We found that genes associated with mitochondrial function were highly predictive of Fbw7 mutations across several tumor types, suggesting that metabolic reprogramming is a crucial oncogenic consequence of Fbw7 mutations. We validated this prediction by creating isogenic colorectal cancer cells with normal or mutant Fbw7 alleles. We found that mitochondrial signature genes are elevated in Fbw7 mutant cells, which also exhibit increased oxidative metabolism. Moreover, metabolomic studies revealed deregulation of other metabolic pathways in Fbw7-mutant cells, including amino acid and nucleotide biosynthesis. These data suggest that metabolic vulnerabilities may represent novel therapeutic targets in Fbw7-associated cancers.

#1031 Nuclear relocalization of NPM1c induces terminal differentiation and cell growth arrest. Lorenzo Brunetti,1 Michael C. Gundry,1 Anna G. Guzman,1 Ilaria Gionfriddo,2 Brunangelo Falini,2 Margaret A. Goodell1.1 Memorial Sloan Kettering Cancer Center, New York, NY; 2University of Perugia, Perugia, Italy.

NPM1 mutated acute myeloid leukemia (AML) is a distinct entity in the WHO classification of hematopoietic cancers. It displays a specific phenotype characterized by favorable prognosis and upregulation of HOX cluster genes. NPM1 is a multifunctional nuclear chaperone. Mutations in NPM1 result in cytoplasmic protein localization (NPM1c+) through the acquisition of a nuclear export signal (NES) at the C-terminus. The most frequent NPM1 mutation is a heterozygous 4bp insertion in exon 12 (mutA). The role of NPM1 in leukemogenesis is still a matter of debate and there is no direct proof that cytoplasmic localization of mutant NPM1 is necessary for OCI-AML3 cells to maintain their leukemic phenotype. Drugs promoting mutant NPM1 nuclear localization such as CRML inhibitors are attractive candidates for clinical success in NPM1-mutated AML.

#1032 Identification of Ran binding protein 6 as a novel negative regulator of EGFR and candidate tumor suppressor in glioblastoma. Wan-Ying Hsieh,1 Barbara Oldrizzi,2 Hedye Erdjument-Bromage,3 Paolo Codecà,1 Maria S. Carro,4 Igor Vivanco,3 Dan Roble,1 Carl Campos,1 Craig Bielski,1 Barry Taylor,1 Paul Tempst,1 Massimo Squatrito,4 Ingo K. Mellingerhoff1.1 Memorial Sloan Kettering Cancer Center, New York, NY; 2Spanish National Cancer Research Centre, Madrid, Spain; 3New York University School of Medicine, New York, NY; 4Medical Center University of Freiburg, Freiburg, Germany; 5The Institute of Cancer Research, London, United Kingdom.

Amplification and overexpression of the epidermal growth factor receptor (EGFR) are common in glioblastoma (GBM) and frequently associated with silencing of the phosphatase and tensin homologue (PTEN) tumor suppressor. PTEN silencing has been associated with clinical resistance to EGFR tyrosine kinase inhibitors, in part by raising EGFR levels. Here, we investigated the effect of PTEN on the EGFR signaling complex by EGFR affinity immunopurification and mass spectrometry with and without PTEN knockdown. We identified Ran binding protein 6 (RanBP6), a 125-kDa protein of previously unknown functions, as EGFR interacting protein in PTEN expressing, but not PTEN knocked down cells. Further studies of the effect of RanBP6 on EGFR revealed that RanBP6 depletion by shRNA or CRISPR/Cas9-mediated gene silencing resulted in increased EGFR mRNA levels and up-regulation of EGFR promoter activity. Consistent with a model of a negative EGFR feedback loop, we observed that by RanBP6, we observed a decrease in both EGFR protein and mRNA levels in PTEN wildtype but not PTEN altered cancer cells in a large panel of human cancer cell lines (Cancer Cell Line Encyclopedia). To further understand the mechanism of how RanBP6 negatively regulates EGFR mRNA levels, we found that RanBP6 interacted with nuclear Ran-GTPase and repressed EGFR transcription by promoting nuclear import of signal transducer and activator of transcription 3 (STAT3). Lastly, RanBP6 appeared to frequently deleted on chromosome some 9p in GBM. We showed that RanBP6 silencing raised EGFR levels and signal output and accelerated in vivo glioma growth. Our results establish a novel function of RanBP6 as a link between EGFR signaling and the Ran-mediated nuclear import pathway, and identify RanBP6 as candidate tumor suppressor on chromosome 9p.

#1033 Estrogen receptor gene fusions drive endocrine therapy resistance in estrogen receptor positive breast cancer. Jonathan T. Lei,1 Jeyya Shao,1 Jin Zhang,1 Michael Iglesias,1 Doug Wan,1 Chan,1 Ryoichi Matsunami,2 Xiaoping He,3 Purba Singh,4 Michael Cundy,1 Anna G. Guzman,1 Ilaria Gionfriddo,2 Brunangelo Falini,2 Margaret A. Goodell1.1 Memorial Sloan Kettering Cancer Center, New York, NY; 2Spanish National Cancer Research Centre, Madrid, Spain; 3New York University School of Medicine, New York, NY; 4University of Perugia, Perugia, Italy.

Although the raison d’être of hormone therapy is the binding of hormones to ERα and ERβ leading to the activation of the ERα and ERβ signaling pathways, the emerging landscape of breast cancer harbouring numerous fusion partners provides an emerging paradigm of ERα/ERβ signal control. Here, we examined the functional properties of fusion proteins containing the C-terminal sequences of YAP1 (ESR1-YAP1) that contributed to endocrine therapy resistance in estrogen receptor positive (ER+) breast cancer models. This current study compares functional and pharmacological properties of additional ESR1 gene fusion events of both early stage (ESR1-NOP2) and advanced estrogen therapy resistant (ESR1-YAP1 and ESR1-PCH11x) breast cancers. The E1P and PCDH11x fusions conferred estrogen-independent and fulvestrant-resistant growth in T47D, an ER+ breast cancer cell line in vitro and in vivo, in contrast to the NOP2 fusion which was sensitive to hormone deprivation. Immunohistochemical (IHC) staining of mouse lungs revealed significantly higher numbers of micrometastatic ER+ cells from the T47D tumors expressing the E1P and PCDH11x fusions compared to those expressing NOP2 fusion. Estrogen response element (ERE) reporter and pull down assays revealed that although all ESR1 fusions studied bound EREs, only the YAP1 and PCDH11x fusions were able to activate ERα and ERβ in hormone deprived conditions. In contrast, the NOP2 fusion neither induced ERE activity nor upregulated TFF1 and GREB1 gene expression. The proliferative ability of canonical fusion-containing T47D cells was inhibited by palbo-
which they are recurrently mutated. KEAP1/NRF2 mutations as predictive biomarkers that could be used for per-
culating tumor DNA. These data suggest that Trp53 and Keap1 mutations in
tation status strongly predicted risk of local recurrence in NSCLC patients
adenocarcinomas (LUADs). Congruous with these findings, KEAP1/NRF2 mu-
ROS pathway activation is the main mediator of Keap1 loss. Finally, Keap1
#1034 Role of KEAP1/NRF2 and TP53 mutations in lung squamous cell
carcinoma development and radiation resistance. Youngtae Jeong,1 Ngoc Ho-
H,1 Henning Stehr,1 Alexander Lovejoy,1 Andrew Gentles,1 Aadel Chaud-
ABSCs play important roles in LSCC initiation and progression and identify
mice treated with CT2063, an FDA approved drug, palbociclib, which could potentially improve outcomes in patients with ESR1 translocated tumors.

TUMOR BIOLOGY: Animal Models of Human Oncogenesis

#1035 Direct evidence for a pro-tumor role of APOBEC3A in cancer ini-
tiation and progression in vivo: enhanced mutagenesis and immune suppress-
ion in a novel humanized autotumoral model of pancreatic cancer. Sonja Woermann,1 Robert Cowan,1 Susan M. Ross,2 Andrew D. Rhim1.1 Stanford University School of Medicine, Stanford, CA; San Francisco State University, CA.

Although lung squamous cell carcinomas (LSCCs) comprise a large fraction of non-small cell lung cancers (NSCLCs), their pathogenesis and cell of origin remain incompletely understood and biomarkers that predict therapeutic response are lacking. Here we describe novel, clinically relevant murine LSCC models driven by inactivation of Trp53 with or without Keap1, both of which are frequently mutated in human LSCCs. Homozygous inactivation of Keap1 or Trp53 promoted airway basal stem cell (ABSC) self-renewal both in vitro and in vivo, suggesting that Trp53 or Keap1 mutations lead to expansion of mutant stem cell clones. Deletion of Trp53 with or without Keap1 in ABSCs, but not more differentiated tracheal cells, produced tumors recapitulating histologic and molecular features of human LSCCs. However, deletion of Trp53 with or without Keap1 in type II pneumocytes (ATII) or bronchioalveolar stem cells (BASCs) produced tumors with the features of adenocarcinoma, indicating that ABSCs represent the likely cell of origin for LSCC in this model. Deletion of Keap1 promoted tumor growth, metastasis and resistance to oxidative stress. N-acetylcysteine (NAC) treatment enhanced tumorsphere formation and me-
tastasis in Keap1mann1/LSCCs, but not in Keap1mann1/LSCCs, suggesting that NRF2-ROS pathway activation is the main mediator of Keap1 loss. Finally, Keap1 deletion induced radiosensitivity in vitro and in vivo in both LSCCs and lung adenocarcinomas (LUADs). Congruous with these findings, KEAP1/NRF2 mu-

#1036 Non-myogenic origin of embryonal rhabdomyosarcoma. Catherine J. Drummond, Matthew R. Garcia, Daniel J. Devine, Jennifer Peters, Victoria Frolich, David Finkelstein, Mark E. Hatley. St Jude Childrens Research Hospi-
tal, Memphis, TN.

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in chil-
dren. Despite aggressive chemotherapy, radiotherapy and surgery, clinical outcomes for RMS have not improved for three decades, emphasizing the need to uncover the molecular underpinnings of the disease. RMS includes two histo-
pathologic subtypes: alveolar RMS, driven by the fusion protein PAX3/7-
FOX01, and embryonal RMS (ERMS), which is genetically heterogeneous. RMS has been presumed to originate from deregulated muscle progenitors based on the histologic appearance and gene expression pattern of the tumors. However, an origin restricted to skeletal muscle does not explain recurring involvement in myocytes of skeletal muscle, such as the prostate, bladder, biliary tree and the omen-
tum. Previously, we showed that activation of Sonic Hedgehog signaling through expression of a conditional, constitutively active Smoothed allele, Smo2, under control of an adipocyte-restricted adipogenic protein 2 (aP2)-Cre recombinase transgene in mice gives rise to aggressive skeletal muscle tumors that display the histologic and molecular characteristics of human ERMS. In this model, tumorigenesis occurs with high penetrance (~80%), is early onset (by 2 months of age), and is restricted to the head and neck. Also, unlike previous RMS models, this model requires no additional background mutations, such as inac-
tivation of p53, and results in only ERMS neoplasia. We illustrated that the gene expression signature of the aP2-Cre;SmoM2 tumors recapitulates both other mouse RMS models as well as human ERMS. With the short latency and ana-
tomic restricted tumor location, we sought to leverage this model to explore the cell of origin. Here, we use genetic fate mapping of aP2-c expression with reporter mice to determine the effect of combining Hedgehog signaling with adult myocyte regeneration. 

#1037 Progression from melanocytic nevi to melanoma is associated with increased genomic mutations in a UV-induced mouse model of human melanoma. Helen Michael, Chi-Ping Day, Howard Yang, Aleksandra Michalowski, Maxwell Lee, Glenn Merlino. National Institutes of Health, Bethesda, MD.

Melanoma is the deadliest form of skin cancer with approximately 132,000 cases worldwide each year. Benign melanocytic nevi are nearly universal, and although progression of nevi to melanoma is very rare, 20-50% of melanoma cases appear to arise from a pre-existing nevi. UV exposure, particularly childhood sunburn, is believed to play an important role in the development of melanocytic nevi and melanoma, but the exact mechanism is unknown. Alterations in MAPK pathway genes, especially NRAS and BRAF, are common in both benign nevi and melanomas.
and melanoma, but approximately 1/3 of melanomas do not have an identified driver mutation. Studying nevus initiation and progression prospectively in the human population is impractical due to the long latency to progression and repeated UV exposures. Our laboratory has developed a hepatocyte growth factor (HGF) genetically engineered mouse model with “humanized” functional distribution of melanocytes on an interstitial cell of Cajal (ICC) background, with melanocytes expressing a specific GFP expression, allowing melanocytic lesions to be tracked through percutaneous GFP imaging. Following a single relevant dose of UV modeling childhood sunburn, HGF IDCT-GFP develop discrete, small melanocytic lesions consistent with nevus formation. Melanomas that arise in the model are heterogeneous and include radial growth phase and vertical growth phase tumors and sometimes metastasize to liver and lung. Exome sequencing of 28 nevi and melanomas show that vertical growth phase melanomas have approximately 3x more mutations than radial growth phase melanomas or nevi. The increased number of mutations in vertical growth phase tumors is due to an increase in C>T transitions despite the lack of additional UV exposure. Interestingly, melanocytic nevi and melanomas with DNA repair pathway mutations average 3x more mutations than lesions without mutations in these pathways. Melanomas sometime contain mutations in hotspot locations from human melanomas, including GNAQ, GNA11, and NRAS. We have a pretransplantation animal model wherein we can potentially involved in the initiation of melanocytic lesions or progression to aggressive melanomas and relevant to human melanoma have been identified and are being functionally tested using CRISPR to introduce point mutations or knockout genes and in vitro skin reconstitution assays. Identification of novel drivers and pathways involved in non-BRAF, non-NRAS melanoma has the potential to uncover biomarkers and new therapeutic targets to improve clinical outcomes for melanoma patients.

**#1038 The KIT-V645A second site mutation confers perinatal lethality and increased oncogenesis in a mouse model of GIST, Jennifer Q. Zhang, Benedikt Bosbach, Cristina Antonescu, Peter Besmer, Ronald DeMatteo. MSKCC, New York, NY.**

Tyrosine kinase inhibitors have revolutionized the treatment of gastrointestinal stromal tumor (GIST), which are mostly driven by mutations in the receptor tyrosine kinase KIT. However, resistance commonly develops, which is associated with second site mutations in KIT. Here, we created the first genetically engineered mouse model of the most common second site mutation, KIT-V645A (mouse KIT-V653A), to study in vivo its oncogenic properties and mechanisms of resistance in order to develop next-generation GIST therapies. The knock-in strategy consisted of inserting into the endogenous murine KIT locus a targeting vector containing both the KitV645A exon 11 mutation and the KitV653A exon 13 mutation under the control of a floxed neoxinrin-resistance gene-expression cassette which acts similar to aloxP-STOP-loxP cassette. Treatments included imatinib 45 mg/kg i.p., sunitinib 40 mg/kg p.o., and caboazinib 60 mg/kg.p.o. Tumors were assessed by histology, immunohistochemistry, and western blot. In contrast to our previously published GIST model with a gene-expression cassette which acts similar to a loxP-STOP-loxP cassette, treatment of mice resulted in cecal GIST development with full penetrance. The tumors were histologically similar to human GIST, stained positive for KIT, and are being functionally tested using CRISPR to introduce point mutations or knockout genes and in vitro skin reconstitution assays. Identification of novel drivers and pathways involved in non-BRAF, non-NRAS melanoma has the potential to uncover biomarkers and new therapeutic targets to improve clinical outcomes for melanoma patients.

**#1039 Disruption of circadian clockwork in in vivo reprogramming induced mouse kidney cancer and human Wilms tumor. Munehiro Ohashi,1 Yasuhiro Umemura,1 Yoichi Minami,1 Hitomi Watanabe,2 Tomoko Tanaka,3 Tsumeharu MikI,3 Osamu UkumIra,4 Tatsuro Tajiri,5 Gen Kodoh,2 Yasuhiro Yamada,2 Kazuhiro Yagita,1,6 Department of Physiology and Systems Bioscience, Kyoto Prefectural University of Medicine, Kyoto, Japan; 2Laboratory of Animal Experiments for Regeneration, Institute for Frontier Medical Science, Kyoto University, Kyoto, Japan; 3Department of Pediatric Surgery, Kyoto Prefectural University of Medicine, Kyoto, Japan; 4Department of Urology, Kyoto Prefectural University of Medicine, Kyoto, Japan; 5Center for iPS Research and Application, Kyoto University, Kyoto, Japan.**

Most organisms have evolved intrinsic circadian clock to optimize their behavior and physiology for diurnal environmental changes. The circadian clock exists not only in the organismal level but also in the peripheral organs and cellular levels, and regulates the various physiological aspects. Among these regulation systems, the relationship between circadian clock and cancer has been enthusiastically investigated in this decade. However, the mechanistic link connecting circadian clock and cancer has not been fully understood. Recently, we have clarified that circadian clock is tightly connected with cellular differentiation using the in vitro mouse embryonic stem cells (ESCs) differentiation model, and in addition, misregulation of differentiation leads to the circadian clock disruption via highly expression of KPN2A and suppression of CLOC protein. On the other hand, it was revealed that altered differentiation leads to cancer. Therefore, we have a presumption in mind that circadian clock disruption is required to help provide significant pathophysiological implications for the relationship of circadian clock and cancer. Firstly, we established Rosa26:M2-MTA Teto-Oskm ES cells carrying circadian rhythm reporter mPer2:2Luc, and generated chimeric mice. The chimeric mice were treated with doxycycline to transient in vivo reprogramming. The Dox treatment induced Wilms tumor-like kidney tumors and we sectioned the kidney tumor for monitoring real time bioluminescence signals, and examined the RNA and protein expression profiles. Here, we showed that the circadian clock was disrupted in the Wilms tumor-like mouse kidney tumor tissues, while the control mouse kidney exhibited the obvious circadian bioluminescence oscillation. And the gene expression signature of the circadian clock development correlated gene set in the tumor was similar to the dysdifferentiation-mediated circadian clock disrupted cells. Moreover, the highly expressed KPN2A and suppression of CLOC protein were also observed in the mouse tumor cells. Next, we examined human Wilms tumor gene and protein expression profiles. Astonishingly, the transcriptional signature of the circadian clock development correlated genes was similar to the mouse kidney tumor induced by dedifferentiation. Furthermore, the protein expression patterns of KPN2A and CLOC coincided with them. These findings suggest that the severely dedifferentiated cancers may lose their circadian clocks and the mechanism is common with the suppression mechanism during differentiation coupled circadian clock development. Moreover, circadian clock functionality may reflect the etiology of cancer development including dedifferentiation and differentiation. As a new prospect, a new view point of the circadian clock may help to evaluate the type of cancer cells, and it leads to more improved therapeutic strategy.

**#1040 LIN28B-mediated let-7 independent activation of AKT promotes neuroblastoma pathogenesis. Ting Tao,1 John T. Powers,2 Hui Shi,2 Pavlos Missios,2 Antonio R. Perez-Atayde,2 Shizhen Zhu,2 George Q. Daley,2 Thomas A. Look1.1 Dana-Farber Cancer Institute, Boston, MA; 2Boston Children’s Hospital, Boston, MA; 3Mayo Clinic, Rochester, MN.**

LIN28B is well known as a RNA-binding protein and a suppressor of microRNA biogenesis, by selectively blocking the processing of let-7 precursor. It plays diverse functions in cellular reprogramming, development, metabolism and tumorigenesis. Many of these functions are executed through its ability to inhibit let-7 maturation. However, little is known about its function independent of let-7. Here we made zebrafish transgenic lines expressing high levels of LIN28B and let-7, which allows us to block let-7 in the peripheral sympathetic nervous system driven by the dopamine beta-hydroxylase promoter. We bred these lines with a transgenic zebrafish line over-expressing the MYCN oncogene in these cells. Either wild-type or mutant LIN28B overexpression accelerated the onset and increased the permeance of MYCN-induced neuroblastoma, despite the fact that only wild-type LIN28B blocked let-7 maturation compared to MYCN-only tumors. Mechanistically, both wild-type and mutant LIN28B overexpression enhanced MYCN-induced hyperplasia by increasing cell proliferation in the sympathoadrenal lineage. Further studies revealed that overexpression of either wild-type or mutant LIN28B resulted in hyperphosphorylation of AKT on both Ser473 and Thr308 in ze-
brazilian tumors and also serum-deprived human neuroblastoma cell lines. Both wild-type and mutant LIN28B interacted with IGF2BP1, a known interactor that increases stability and translation of a subset of RNAs. We found IGF2 RNA levels to be increased in response to either form of LIN28B. Coincident with IGF2 overexpression, IGF1R was phosphorylated, providing an explanation for PI3K-AKT activation in LIN28B overexpressing cells. Finally, overexpression of a constitutively active, myristoylated murine Akt2 (pyr-mAkt2) alone in zebrafish induced ganglioneuroma in the interreginal gland (the zebrafish equivalent of the human adrenal medulla), without a requirement for MCYN overexpression. Thus, our studies indicate that overexpression of LIN28B leads to Akt activation mediated through its interaction with IGF2BP1 and subsequent upregulation of IGF2. This pathway provides a mechanism underlying enhanced transformation in LIN28B-overexpressing neuroblastomas, which is independent of the inhibitory activity of LIN28B on let-7 maturation.

**TUMOR BIOLOGY: Animal Models of Human Oncogenesis**

#1042 Clic4 is incorporated into extracellular vesicles of murine breast cancer cells and may influence metastatic burden. Alayna B. Craig-Lucas, Vanesa C. Sanchez, Abigail Read, Ji Lou, Anjali Shakla, Stuart H. Yupsa, NCI, Bethesda, MD.

Chloride intracellular channel 4 (Clic4) is an evolutionarily conserved, 29kD, dimorphic protein that contributes to TGF-β signaling by preventing the de-phosphorylation of phospho-Smad2/3 upon nuclear translocation. In several cancer types, Clic4 is excluded from the nucleus and downregulated in the cytoplasm of the tumor cells as the tumor progresses, suggesting that Clic4 acts as tumor suppressor. In a parallel sequence, Clic4 becomes upregulated in the stromal compartment, where it enhances tumor growth and invasion. Recent studies have demonstrated that Clic4 is detectable in tumor patients and incorporated into extracellular vesicles, and has potential as a biomarker. We hope to gain a better understanding of the role that Clic4 plays in the tumor stromal and epithelial compartments as well as their respective release of extracellular vesicles. Using in-vitro and in-vivo assays, we have conducted experiments using the FVB mouse MMTV-c-Myc 6DT1 breast cancer model. By CRISPR/Cas9 system, Clic4 was deleted from wild type 6DT1 cells. Following clonal selection, the loss of the Clic4 protein at both the cellular and released vesicle level was validated. Both functional assays on Clic4 deleted clones and evaluation of their extra-cellular vesicles were undertaken in order to further understand their tumorigenic and metastatic capabilities. In-vitro, Clic4 was not necessary for vesicle biogenesis and its deletion did not have a significant effect on cellular proliferation. In vivo, selected clones were orthotypically injected into the 4th mammary fat pad of wild type FVB mice. Compared to wild type 6DT1 clones, Clic4 deleted clones formed primary tumors that had greater mass but a fewer number of lung metastasis. Future studies are designed to isolate vesicles circulating in tumor bearing hosts to determine their stromal or epithelial origin and to provide a better understanding of the role that Clic4 may play in tumor growth, creating a metastatic niche and as a potential serological biomarker.


Exosomes are small vesicle cellular products originating from the endocytic pathway, abundantly secreted by tumor cells. These exosomes were the ability to alter their immediate microenvironment (ME) through cell-cell interaction by fusion with plasma membrane and subsequent endocytosis or release of their cargo. Exosomes are critical modulators of pre-metastatic niche creation by increasing the recruitment of inflammatory cells. In breast cancer, tumor-derived exosomes recruit myeloid derived suppressor cells in the creation of an immunosuppressive pro-tumorigenic lung niches. Colony stimulating factor-1-receptor (CSF1R), which is a key regulator of myeloid cell proliferation, survival, and differentiation can be blocked by a selective inhibitor GW2580. Overexpression of CSF-1 has been implicated in the creation of increased number of metastatic niches in numerous cancers. In our study, we have demonstrated that the exosomes secreted under hypoxic conditions can initiate early pre-metastatic niche creation in lungs in a metastatic breast cancer model compared to normoxia-secreted exosomes. Exosomes were injected intravenously into Balb/c female mice three days after the implantation of 4T1 breast tumor and continued for a week with injections on alternate days. The animals were pre-treated with GW2580 the day before tumor implantation and continued for a week concomitantly with exosomes on alternate days. Lungs, bone marrow, spleen and brain tissues were collected and analyzed by flow cytometer to detect myeloid and angiogenic cells populations. GW2580 was able to prevent myeloid cell infiltration in lungs and bone marrow. Further, we observed significant increase in anti-tumorigenic M1-macrophage population in the lungs of exosome treated animals pre-treated with GW2580. However, these findings were not observed in the bone marrows of the same group. Vascular leukocyte and angiogenic myeloid cell populations were significantly decreased in the lung of exosome treated animals pre-treated with GW2580. A similar decrease in these populations of cells was not seen in the lungs of animals pre-treated with GW2580 only. These surprising results have led us to hypothesize that GW2580 treatment can prevent the effects of exosomes, which causes infiltration of myeloid cells in the lung to create metastatic niche. These observations indicate a role of CSF1R inhibitor in preventing the distant metastatic niche formation.


Metastasis is the primary cause of death for cancer patients, and invasive cancer cell migration is required at multiple steps during metastasis (e.g. invasiveness and extravasation). Since microRNAs (miRNAs) have been implicated as key regulators of metastatic spread of cancer cells, we sought to develop a miRNA signature that can be used to predict cancer metastasis. We hypothesized that the miRNAs that are functionally required for invasive cell migration could serve as biomarkers to predict human cancer metastasis. We developed an intravital imaging-based approach
combined with NGS to screen for miRNAs that are required for invasive cell migration of human HT1080 fibrosarcoma cells. The screen-identified miRNAs were validated in a panel of in vitro and in vivo assays for invasive migration. Microarray analysis identified genes downregulated in transfected HT1080 cells. Publicly available databases were used to correlate the expression of screen-identified miRNAs and the progression of multiple human cancers. We used a QRT-PCR approach combined with machine learning to develop a miRNA signature to predict metastasis in a 66-patient cohort of prostate cancer. We identified over twenty novel miRNAs that regulate directional cancer invasion. Microarray analysis in HT1080 cells revealed that the altered expression of metastasis-regulating miRNAs is associated with 50% reduction in gene expression of migration and adhesion gene network components such as integrin (α4, CDCA2, and transgelin). We evaluated the potential of screen-identified miRNAs to serve as biomarkers to predict cancer metastasis. Using publicly available databases, we found that majority of screen-identified miRNAs are dysregulated in multiple human cancer types (e.g. prostate, breast, ovarian and lung) and the expression of these miRNAs correlate directly with patient disease progression. We analyzed the expression of the top two screen-identified miRNAs in plasma samples from the PCA patient cohort. A signature was generated using a weighted K-nearest neighbor algorithm that provided a ROC area under the curve of 0.79 for predicting metastatic disease. We identified a panel of novel metastasis-regulating miRNAs that is functionally involved in human cancer metastasis. These miRNAs have the potential to serve as both biomarkers to predict metastasis and potentially as therapeutic targets to block metastasis.

#1045 Understanding breast cancer metastasis through circulating tumor cells. Remi Klotz, Thomas Almal, Alan Wang, Matthew Mackay, Kathleen Heller, Lin Li, Maxwell Serowoky, Grace Lee, Jane Han, Andrew Smith, Min Yu. USC, LOS ANGELES, CA.

Due to the advance in technologies for rare cell isolation, circulating tumor cells (CTC) have recently received vast interest. During tumor progression tumor cells invade the primary tumor microenvironment and intravasate into blood vessels, where they are referred as CTCs. These CTCs disseminate to other organs and a subset of these cells will be able to form metastasis. The growing interest for CTC is confronted with the difficulty associated with their isolation and characterization. To address this challenge, our lab has recently optimized the ex vivo culture condition and was able to establish CTC lines from breast cancer patients. These CTC lines constitute a unique cell population in the metastatic process and gave us a rare opportunity to investigate the signal pathways involved in each step of the metastatic cascade. To assess the metastatic potential of breast cancer patient-derived CTC lines we utilized an experimental mouse model for metastasis by injecting CTCs directly into the left ventricle of the heart in female immudeficient NSG mice. The ability of 4 CTC lines to arrest and colonize in organ was monitored by bioluminescence imaging once every 2 weeks for 5 months. CTC lines were capable of generating brain, lung, bone and ovary metastases. Most of those organs are common sites of metastases in breast cancer patients. Two patient-derived CTC lines have a high metastatic potential (over 80% of mice had metastases after 3 months) with generation of simultaneous metastatic multiple organs (breast, lung, bone, ovary). The remaining two patient-derived CTC lines remained brain-metastases free for up to 8 months. However, two other patient-derived CTC lines demonstrated the brain as preferential site of metastasis despite their overall low metastatic potential. Interestingly, of 4 breast cancer patients where CTC lines were generated, one developed a metastatic brain tumor and her CTC line has the highest risk of brain metastases in our mouse model. We further investigated genetic and epigenetic determinants that regulate the organotropism of CTCs. We isolated metastatic variants corresponding to a subpopulation of CTCs with a preferential tropism for the brain, lung and bone. Gene expression analysis (RNA-seq) of these variants identified potential gene signatures of breast cancer metastasis. The pathways with the highest enrichment scores were glioblastoma multiforme, cerebellar apoptosis and P53 pathway for the brain metastatic variants and were interferon and mTOR signaling for the lung metastatic variants. Next we used a method for asaying chromatin accessibility (ATAC-seq) and identified potential regulatory regions mediating the organ tropism in breast cancer. Together our data provide the evidence of a promising role of CTC as an early prognostic factor in metastasis. Additionally, we expect to develop novel organ tropism associated markers, which can be considered for potential therapeutic targets in breast cancers.

#1046 Conditional knock out of N-Myc and STAT Interactor disrupts normal mammary development and enhances metastatic ability of mammary tumors. Hawley Christine Pruitt, Brandon J. Metge, Shannon E. Weeks, Dongguan Chen, Shi Wei, Lalita A. Sheved, Rajeev S. Samant. Univ. of Alabama at Birmingham, Birmingham, AL.

The process of development requires a delicate balance between plasticity and differentiation. This balance is disrupted in cancer initiation and progression, ultimately ending in metastasis and plummeting rates of patient survival. Recent findings from our lab have revealed that protein expression of evolutionarily conserved, N-Myc and STAT Interactor (NMI) is decreased in 70% of primary patient specimens of breast cancer. To study how Nmi loss facilitates metastatic behavior and gain insight into its role in normal mammary biology, we created a novel mammary specific Nmi knock out mouse model, which we then challenged with carcinogen and oncogene induced tumorogenesis. Our studies show that Nmi is induced at the onset of pregnancy and its expression remains throughout lactation. Furthermore, prolactin stimulation and differentiation of HC11 murine mammary epithelial cells is accompanied by up-regulation of NMI. STAT5 is the downstream effector of prolactin and is essential for differentiation of secretory alveolar epithelium. NMI interacts with STAT5 and this interaction appears to be critical to normal differentiation of mammary epithelium. Indeed, loss of Nmi in vivo caused a decrease in STAT5 activity and a subsequent transcriptional shift in mammary epithelial and breast cancer cells. Moreover, knockdown of Nmi in HC11 cells impedes the expression of beta-casein upon differentiation with prolactin. Concurrently, Nmi knock out alveoli exhibit an extensive presence of nuclear beta-catenin, a mediator of stem cell maintenance in the mammary gland. As a result, mice exhibit an increased number of alveoli and more proliferating mammary epithelial cells (MECs). The Nmi knock out pubertal ductal tree extends into the mammary fat pad at an accelerated rate and exhibits enhanced terminal end bud numbers, a phenotype that is normally observed for Wnt signaling. Tumors derived from Nmi null mammary epithelium contain significantly more cells with stem and progenitor markers, indicating that these tumors are less differentiated than their littermate counterparts. In addition, Nmi null mammary tumors exhibit invasive morphology as well as enhanced distant metastasis. We observe that conditional Nmi loss disrupts differentiation in the mammary gland and promotes the progression of tumors with aggressive metastatic characteristics.

#1047 Role of endocytosis in NM23 mediated motility suppression. Imran Khan, Patricia S. Steeg. National Institute of Health (NIH), Bethesda, MD.

NM23 has been identified as a metastasis suppressor gene and is known to inhibit motility of cancer cells and suppress metastasis in multiple in vivo model systems. Biochemically, NM23 is a nucleoside diphosphate kinase (NDPK). As an NDPK, NM23 is first autophosphorylated on its histidine 118 before transferring its phosphate to a NDP. NM23 is also known to trans-phosphorylate other proteins to act as a protein histidine kinase (PHK) using same histidine H118 phosphorylation intermediate. In spite of many studies on NM23 in different cancers, the molecular mechanism of NM23 action has not been well worked out. Studies on Drosophila homologue of the NM23, awd, highlights its role in regulating cell differentiation and motility by influencing endocytosis. We now hypothesize that NM23 mediated motility/metastasis suppression could be due to increased endocytosis of cell surface receptors, needed for cellular motility. We over expressed NM23 (human H1 and H2 and murine h1) in MDA-MB-231 breast cancer cells and observed that the motility suppression phenotype (4 fold, p<0.0001) of NM23 correlates with increase in NM23 histidine auto phosphorylation, as observed by using a new anti-histidine 1-phosphate (1-pHis) antibody. NM23 over expressing cells were studied for the level of endocytosis using transferrin-Alexa-594 and pHrodo-Red-EGF labelled dyes. It was observed that NM23 over expression causes increased endocytosis of both the above labelled ligands. Based on the literature, Dynamin is known to be involved in endocytosis and is also an interacting partner of NM23. We validated NM23 and Dynamin interactions by pulldown studies (using both anti-NM23 and anti-Dynamin antibodies) in NM23 over expressing cells. We also observed reduced endocytosis with Dynamin inhibitors. Further, when a series of Dynamin inhibitors (MITMAB, OcTMAB, Dynole-34-2 and Imidoydn-22) were used in motility assays, they were found to be inhibitory to motility. However, no further decrease in motility was observed upon NM23 overexpression, highlighting the reversal of NM23 mediated motility suppression in absence of Dynamin. To further address the role of endocytosis in NM23 mediated motility suppression, two crucial mutants of NM23 namely, P96S (has NDPK activity with very low HPK activity) and H118F (has no NDPK and HPK activity) were made and stable cells were generated. We observed that the motility suppression phenotype of NM23 was reversed in normal human breast epithelial cells, highlighting a possible role of HPK activity in motility suppression. Similarly, endocytosis studies on these cells shows loss of increased endocytosis in both the mutants. To date, the data indicate that NM23 phosphohistidine levels, motility suppression and increased endocytosis are well correlated and is being influenced by Dynamin signaling. Different approaches to further understand the physiological meaning of NM23 interaction interactions are underway.
**EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Combination Strategies: Novel Agents and Standard Therapies**

### #1048 Reactive oxygen species-mediated synergistic and preferential induction of cell death and reduction of drug resistance in oncogenic H-Ras-expressing bladder cancer cells by combined romidepsin and cisplatin with gemcitabine.

Lenora A. Pluchino, Hwa-Chain R. Wang. *Univ. of Tennessee, College of Veterinary Medicine, Knoxville, TN.*

Human urinary bladder cancer is the fifth most commonly diagnosed cancer in the United States and is often associated with induction of oncogenic H-Ras. Long-term survival of patients is suboptimal with the current chemotherapeutic regimen of combined cisplatin and gemcitabine and others due to acquired drug resistance and recurrence. Thus, it is urgent to develop new regimens effective in control of tumor growth, drug resistance, and recurrence to reduce the morbidity and mortality of this disease. We used our cellular system, consisting of human urinary bladder cancer J82 cells paired with its derived oncogenic H-Ras-expressing J82-Ras cells, to investigate the efficacy of a novel combination regimen in treating bladder cancer cells. Our studies revealed, for the first time, the ability of a combination of cisplatin and romidepsin with gemcitabine to synergistically and preferentially induced cell death and reduced drug resistance in J82-Ras versus J82 cells. The Ras-ERK-Nox pathway played an essential role in mediating signals to elevate reactive oxygen species (ROS), leading to enhanced caspase activation, DNA damage, and DNA oxidation, as well as reduced glutathione, to synergistically increased cell death and reduced drug resistance in cells treated with combined romidepsin, cisplatin, and gemcitabine. Preferentially induced Ras-ERK-Nox-Ros pathway, caspase activation, and DNA damage/oxidation, as well as reduced glutathione, contributed to the preferentially induced cell death and reduced drug resistance in J82-Ras versus J82 cells. Synergistically induced death and reduced drug resistance were also detected in human bladder cancer SW780 cells treated with combined romidepsin, cisplatin, and gemcitabine. Hence, our results lead us to suggest that a combination of romidepsin, cisplatin, and gemcitabine should be seriously considered as a new therapeutic regimen for controlling the development and recurrence of human urinary bladder cancer, especially Ras-ERK-activated cancers.

### #1049 Combinatorial effects of thymoquinone on the antiangiogenic activity of doxorubicin in adult T-cell leukemia: Hala Gali-Mubtasib, Isabelle Fakhouri, 1 Maamoun Fatfat, Rasha Mismar, 1 Regina Schneider-Stock. 2 American University of Beirut, Beirut, Lebanon; 2University Erlangen, Nuremberg, Germany.

Doxorubicin (Dox) is a clinically approved drug which suffers from drug resistance and cardiotoxicity. Recent studies have shown that thymoquinone (TQ) in combination with Dox can reduce Dox toxic side effects in vitro as well as in vivo. Both TQ and Dox have shown promising antiangiogenic effects against aggressive adult T-cell leukemia (ATL), however, the antiangiogenic potential of TQ and Dox combination treatment has never been tested against ATL. We hypothesized that co-treatment with TQ could enable the use of lower doses of Dox to achieve similar or enhanced antiangiogenic activity. The effects of TQ and Dox combination on cell death and cell cycle were elucidated by trypan blue and propidium iodide. TUNEL assay was used to investigate the mode of cell death. The levels of reactive species (ROS) were determined using DCFH assay, and mitochondrial membrane potential was measured by rhodamine assay. The regulation of key proteins involved in cell cycle regulation and cell death was determined by Western blot. The results revealed that the human T-lymphotropic virus (HTLV-I) positive H9251 cells are more resistant to treatment with Dox alone, than the HTLV-I negative Jurkat cells. However, treatment with high doses of TQ and low doses of Dox simultaneously, enhances cell death in both cancer cell lines as compared to treatment with Dox alone. TUNEL assay on Jurkat and HuT-102 cells further indicated that the combination of TQ and Dox caused cell death by apoptosis. An increase in ROS production was noted in response to treatment with TQ alone and with TQ and Dox combination in both Jurkat and HuT-102 cells. The oxidative stress; however, was only shown to play a role in the disruption of the mitochondria of Jurkat cells. Similarly, caspase activation was involved in the disruption of the mitochondria of Jurkat cells. The expression levels of key regulatory proteins were modulated in response to treatment with TQ and Dox. Dox effectively induces ATL leukemic cell growth at lower doses of Dox which can potentially lower the side effects of the drug. In vivo studies are still warranted to assess the adjuvant chemotherapeutic potential of TQ in combination with Dox.

### #1050 Mefloquine enhances the cytotoxic effects of tyrosine kinase inhibitors in blast phase chronic myeloid leukemia by lysosome membrane disruption.

Wei Xiang, 1 Yi Hui Lam, 1 Colin Sng, 2 May Anne Cheong, 1 Hein Tham, 1 William YK Hwang, 2 Charles Chuah, 1. 1Singapore General Hospital, Singapore, Singapore; 2Duke-NUS Medical School, Singapore, Singapore.

Mefloquine has been reported to induce apoptosis and inhibit the RNA binding protein HuR, preferentially induced cell death and reduced drug resistance in J82-Ras and J82 cells. However, the underlying mechanisms of CML are yet to be fully defined. In this study, we investigated the effect of mefloquine and its underlying mechanisms in CML. We show that mefloquine inhibits proliferation and induces apoptosis of CML cells in a dose-dependent manner. In addition, mefloquine is also effective in targeting BP-CML CD34 + progenitor cells. It induces apoptosis, inhibits colony formation and self-renewal capacity of CD34 + cells derived from a TKI-resistant BP-CML patient. Mefloquine significantly enhanced anti-potentiative and pro-apoptotic effects of imatinib and dasatinib in CML cell lines (eg, K562, LAMA84 and KU812) as well as BP-CML CD34 + cells, suggesting that mefloquine augments the effects of BCR-ABL TKIs.

### #1051 Preclinical analyses of synergistic effect of eribulin and paclitaxel for triple-negative breast cancer.

Takaaki Oba, Ken-ichi Ito. Shinshu University, Matsumoto, Japan.

Purpose: There is an urgent need to develop novel therapeutic strategies for triple negative breast cancer (TNBC). To explore the antitumor effect of combination of eribulin (ERI) and paclitaxel (PTX) for TNBC, we conducted preclinical experiments. Materials & methods: Combinational effects of ERI and PTX were analyzed both in vitro and in vivo using two TNBC cell lines (MDA-MB-231, Hs578T). Results: When ERI was added simultaneously with PTX to TNBC cells, the sensitivity to PTX was increased synergistically. On the other hand, synergistic increase of sensitivity to ERI was observed in the presence of PTX. When the TNBC cells were treated with ERI, the expression of epithelial marker, E-cadherin, was increased, while the expression of mesenchymal markers, vimentin, Slug, and ZEB1 were decreased. Furthermore, pretreatment with ERI for 96 hours enhanced the sensitivity to PTX administrated subsequently. In contrast, pretreatment of TNBC cells with PTX enhanced the sensitivity to the subsequently administrated ERI. When the TNBC cells were treated with ERI, the expression of epithelial marker, E-cadherin, was increased, while the expression of mesenchymal markers, vimentin, Slug, and ZEB1 were decreased. Furthermore, knockdown of ZEB1 by small interfering RNA conferred resistance to ERI in both TNBC cell lines. In the mouse xenograft model bearing MDA-MB-231, ERI plus PTX combination therapy significantly inhibited tumor growth compared with either PTX or ERI monotherapy. Furthermore, prior treatment with ERI significantly enhanced the antitumor activity of PTX in the mouse xenograft model. Conclusions: Our findings
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demonstrate an EMT-mediated synergistic antitumor effect by combination of two tubulin inhibitors. This combination therapy may serve as a novel therapeutic strategy for TNBC.

**#1052 Aldose reductase inhibitor increases doxorubicin-sensitivity of colon cancer cells and decreases cardiomyopathy.** Himangshu Sonowal, Pabitra B. Pal, Satish K. Srivastava, Kota V. Ramana. UT Medical Branch, Galveston, TX.

Chemotherapy is one of the most common option for colorectal cancer (CRC), specifically at the advanced stages and after surgical resection of the tumor. Anthracycline drugs such as doxorubicin, daunorubicin are the most commonly used for the therapy of leukemia, lymphoma and breast cancer. However, doxorubicin is not a very effective drug to kill CRC cells since a high amount of colorectal cancer is the most frequent cancer for surgical cancer therapy, which leads to unwanted side effects like cardiac toxicity and cardiomyopathy. Therefore, better adjuvant therapies are required to increase sensitivity of CRC to doxorubicin as well as to decrease the associated cardiomyopathy. Our recent studies indicate that aldose reductase (AR; AKR1B1) inhibitors such as fideratet prevent CRC growth as well as metastasis in nude mice xenograft models. We now examined our hypothesis that AR inhibition increases the sensitivity of CRC to doxorubicin and decreases its cardiotoxicity by modulating oxidative stress-mediated signaling pathways. We have investigated the effect of co-administration of doxorubicin along with AR inhibitor fideratet in vitro and in vivo CRC cells growth and toxicity. Our results suggest that treatment of CRC cells with fideratet increases the efficacy of doxorubicin-induced death of HT-29 and SW-480 colon cancer cells. Flow cytometric analysis showed that in fideratet + doxorubicin-treated CRC cells, the intracellular accumulation of doxorubicin is increased as compared to doxorubicin alone-treated cells. Further, AR inhibition prevents doxorubicin-induced increase in the expression of drug transporter proteins such as MRP-1 and ABCG-2 in CRC cells. In addition, combination of fideratet with doxorubicin significantly prevented the growth of CRC cells in nude mice xenografts as compared to doxorubicin alone. Fideratet also prevented the doxorubicin-induced cardiac toxicity as measured by serum troponin-I levels and inflammatory cytokines and chemokines in the serum and heart of doxorubicin-treated mice in combination with fideratet. Most importantly, fideratet protects the cardiac damage and dysfunction in doxorubicin-treated mice. Thus, our results suggest that fideratet can be used as a novel adjuvant therapy in enhancing doxorubicin sensitivity of CRC cells and to reduce the doxorubicin-associated cardiotoxicity.

**#1053 Synergistic anticancer effects of combined γ-tocotrienol and pterostilbene is associated with a suppression in Rac1/WAVE 2 signaling in highly malignant breast cancer cells.** Ibrahim G. Algayadh, Paul William Sylvest. Univ. of Louisiana at Monroe, Monroe, LA.

Breast cancer is the most common cancer and the second leading cause of cancer death in American women. Rho GTPases play a crucial role in regulating different cellular functions, including cell proliferation, gene expression, cell morphology, actin polymerization, cell motility, metastasis and apoptosis. Wiskott-Aldrich syndrome protein family verprolin-homologous protein2 (WAVE2) is a member within the Wiskott-Aldrich syndrome protein (WASP) family displaying a significant role in actin polymerization and cytoskeletal organization which are essential for cellular migration and invasion. γ-Tocotrienol is a natural isoform within the vitamin E family that displays anticancer activity against a variety of cancer cell types. Pterostilbene is a natural dimethyl ether isoflavonoid with anti-inflammatory and anti-cancer activities. Studies were conducted to examine the effects of combined γ-tocotrienol and pterostilbene on Rac1/WAVE 2 signaling in mouse +SA and human MDA-MB231 breast cancer cells and cellular migration. Results show that combined treatment of γ-tocotrienol and pterostilbene caused a synergistic inhibition of both mouse +SA and human MDA-MB231 breast cancer cells growth, and corresponding reduction in Rac1/WAVE2 signaling as characterized by a significant inhibition in the levels of phospho-Rac1/cdc42, WAVE2, Arp2, and Arp3 expression. Additional studies indicated that this combination resulted in a significant inhibition in mammary cancer cell migration. In summary, these findings strongly suggest that combined treatment of γ-tocotrienol with pterostilbene may provide great benefit as a therapeutic approach in the treatment of metastatic breast cancer. Supported by a grant from the Louisiana Cancer Foundation.


Nobiletin is a citrus polymethoxy flavonoid that suppresses cell proliferation, angiogenesis and metastatic properties in various cancer cells. In this study, we investigated the combined effects of nobiletin and various chemotherapeutic agents on the cytotoxicity of human colon and esophageal cancer cells. We cultured cancer cells at serial dilution of chemotherapeutic agents with or without nobiletin and assessed cell cytotoxicity at 5 days after drug treatment. The addition of a suboptimal dose of nobiletin did not alter the growth of cancer cells but enhanced the antitumor cytotoxic effect of antimitotics (gemcitabine and 5-FU) and DNA-platinating agent (cisplatin) were markedly attenuated by nobiletin. By contrast, nobiletin markedly enhanced antitumor effect of antimicrotubule agents such as docetaxel, paclitaxel, vincristine and MMAE. Moreover, enhanced cytotoxicity by nobiletin was also verified in the combination with MMAE- or DM1-conjugated antibodies to EpCAM and EphA2. We found the effect of nobiletin in reversing chemoresistance of colorectal cancer cells, which leads to a profound reduction in Rac1/WAVE2 signaling as characterized by a significant inhibition of phosphorylation and nuclear translocation of Pin1 (peptidyl-prolyl isomerase) by nobiletin, suggesting a rational molecular target of nobiletin is Pin1. Overall, these data suggest that nobiletin might useful for the chemotherapeutic treatment of microtubule inhibitors.


Background: Trifluridine/tipiracil is a combination of trifluridine (FTD), a nucleoside metabolic inhibitor, and tipiracil, a thymidine phosphorylase inhibitor, in a molecular ratio of 1:0.5. Trifluridine/tipiracil is indicated for the treatment of patients with metastatic colorectal cancers refractory to standard chemotherapies. Chemotherapy can make radiation therapy more effective against some colorectal cancers. In this study, we investigated whether cytotoxicity was enhanced when FTD was sequentially used with ionizing radiation (IR). Method: The colorectal cancer cell lines HT-29, HCT-15, and HCT 116, showing low, middle and high sensitivity for IR, respectively, were treated with the combinations of FTD and IR as follows, 1) sequence 1 (pre-radiation): exposure to 2-8 Gy radiation alone and no treatment for 24 h followed by exposure to 0, 2.0, or 4.0 μM FTD for 24 h, 2) sequence 2 (post-radiation): exposure to 0, 2.0, or 4.0 μM FTD for 24 h followed by 2-8 Gy radiation alone and no treatment for 24 h. Cells were irradiated using a cabinet X-ray irradiation system and all treatments were evaluated by the clonogenic survival assay. The dose modification factors (DMFs) were calculated from clonogenic survival curves as the ratio of irradiation doses (control radiation dose divided by the FTD-treated radiation dose). To evaluate DNA strand-breaks, alkaline comet assays were performed and the comet tail moment was determined. Results: DMFs of sequence 1 at 8 Gy for 2.0 μM FTD were 1.55, 1.24, and 1.03 and for 4.0 μM FTD were 1.75, 1.26, and 1.02 for HT-29, HCT-15, and HCT 116, respectively, and of sequence 2 at 8 Gy for 2.0 μM FTD were 1.82, 1.38, and 1.03 and for 4.0 μM FTD were 2.73, 1.41, and 1.03 for these cell lines, respectively. The DMFs increased in an FTD dose-dependent manner compared with IR-only treatment. In particular, FTD enhanced the efficacy of radiation in HT-29 and HCT-15 indicating low and middle sensitivity to IR. Compared with IR-only treatment, the median comet tail moments of sequence 1 at 8 Gy for 2.0 μM FTD, respectively, were 13.8-, 14.4-, and 14.4-fold and for 4.0 μM FTD were 2.73, 1.41, and 1.03 for these cell lines, respectively. The DMFs calculated from clonogenic survival curves as the ratio of irradiation doses (control radiation dose divided by the FTD-treated radiation dose). To evaluate DNA strand-breaks, alkaline comet assays were performed and the comet tail moment was determined. Results: DMFs of sequence 1 at 8 Gy for 2.0 μM FTD were 1.55, 1.24, and 1.03 and for 4.0 μM FTD were 1.75, 1.26, and 1.02 for HT-29, HCT-15, and HCT 116, respectively, and of sequence 2 at 8 Gy for 2.0 μM FTD were 1.82, 1.38, and 1.03 and for 4.0 μM FTD were 2.73, 1.41, and 1.03 for these cell lines, respectively. Taken together, FTD promoted DNA strand-breaks and enhanced the efficacy of radiation in these three cell lines, regardless of both sequential treatments. Conclusion: Sequential combination of FTD and radiation was more effective than radiation alone against colorectal cancer cell lines with different radiosensitivities. These results suggest that combination therapy of radiation with trifluridine/tipiracil might be useful for colorectal cancer even with low sensitivity to radiation alone.

**#1056 Volasertib in combination with radiotherapy: The perfect match in non-small cell lung cancer.** Jolien Van den Bossche, 1 Ines De Pauw, 1 Hilde Lambrechts, 1 Céline Merlin, 2 Christophe Deben, 1 Vanessa Deschoemaecker, 1 Pol Specenier, 2 Patrick Pauwels, 2 Marc Peeters, 2 Filip Lardon, 2 An Wouters 1 Antwerp University, Wilrijk, Belgium; 2Antwerp University Hospital, Edegem, Belgium.

Background: Polo-like kinase 1 (Plk1), a master regulator of mitosis and mitosis, is overexpressed in several human malignancies, making it a promising therapeutic target. Monotherapy of Plk1 inhibition has shown only a moderate effect in clinical trials, indicating the need to combine with other therapies. Remarkably, Plk1 inhibition arrests cancer cells in mitosis, which is the most radiosensitive cell cycle phase. Hence, we are the first to investigate the effect of volasertib, the lead agent in category of
#1058 Synergistic activity of p97 inhibitors with histone deacetylase 6 inhibitors in mantle cell lymphoma.

Role of p53 in radiosensitization by volasertib.

**Introduction**

Belinostat is an HDAC inhibitor currently marketed in the US for the treatment of PTCL. A new oral formulation is under development (positive PoC in preclinical PK study) and provides an increased potential to develop new indications of belinostatin in combination with other drugs. It has been extensively demonstrated that some anti-tumor agents, besides their direct anti-tumor effect, may induce additional mechanisms involving activation of immune responses. Thus, combination of these drugs with other immunotherapeutic protocols may yield improved therapeutic benefits. The objective of the present study is the characterization of the therapeutic efficacy of a combination of checkpoint inhibitors (anti-CTLA-4 antibodies) with belinostatin in a murine HCC model. In addition, immunoprofiling was performed in order to assess the associated immune response. Methods In vivo efficacy was assessed in a Hepa 129 murine hepatocellular carcinoma model implanted subcutaneously in immune-competent C3H mice using anti-CTLA4 alone, belinostatin alone or in combination. Treatments were optimized to be able to demonstrate positive effect of the combination. Tu- mor volume was the primary endpoint. Samples from the spleen were taken

**Material and Methods**

**4-HPR**

**Results**

**Conclusion**

**References**

1. **Daina Llopis**, 2 Marta Ruiz, 2 Perrine Pivet, 2 Véronique Trochen-Joseph, 2 Bérangère Vasseur, 2 Caroline Lemarchand, 2 Graham Dixon, 2 Pablo Sarobe, 2 Bruno Sangro, 2 CIMA (Centro de Investigación Médica Aplicada), Pamplona, Spain; 3 Onxeo, Paris, France; 4 Clínica Universidad de Navarra, Pamplona, Spain.

**Introduction**

Belinostatin is an HDAC inhibitor currently marketed in the US for the treatment of PTCL. A new oral formulation is under development (positive PoC in preclinical PK study) and provides an increased potential to develop new indications of belinostatin in combination with other drugs. It has been extensively demonstrated that some anti-tumor agents, besides their direct anti-tumor effect, may induce additional mechanisms involving activation of immune responses. Thus, combination of these drugs with other immunotherapeutic protocols may yield improved therapeutic benefits. The objective of the present study is the characterization of the therapeutic efficacy of a combination of checkpoint inhibitors (anti-CTLA-4 antibodies) with belinostatin in a murine HCC model. In addition, immunoprofiling was performed in order to assess the associated immune response. Methods In vivo efficacy was assessed in a Hepa 129 murine hepatocellular carcinoma model implanted subcutaneously in immune-competent C3H mice using anti-CTLA4 alone, belinostatin alone or in combination. Treatments were optimized to be able to demonstrate positive effect of the combination. Tumor volume was the primary endpoint. Samples from the spleen were taken.
to analyze immune mechanism mediating the antitumor activity. Percent of CD4, CD8 T cells and regulatory T cells were determined by flow cytometry. Anti-tumor T cell response was measured by IFN-gamma ELISPOT assay. Results Belinostat improved anti-tumor therapeutic response induced by the anti-CTLA4 checkpoint inhibitor with a significant superior tumor growth inhibition compared to control groups. INH29 and UM-SCC-47 cells treated with INH29 demonstrated a complete cessation in tumor growth in all mice during the belinostat treatment period which continued for 1 week after the final dose. Mechanistic studies showed that the underlying immune response correlated with the observed therapeutic effect of the combination with enhancement of IFN-gamma production as antitumor T cell response and decrease in regulatory T cells in the spleens of treated animals. Conclusion These results provide a rational for using belinostat in combination with checkpoint inhibitors to reinforce therapeutic response. Currently only approx. 20% of patients respond to checkpoint inhibitors alone. The oral formulation of belinostat will allow greater flexibility in dosing schedule and use in multiple clinical situations. Further studies are ongoing in order to fully characterize this finding and to facilitate its translation into patients.


Background: Head and neck squamous cell carcinoma (HNSCC) prevalence is rising and new treatments are urgently required. Repurposing existing, clinically approved drugs to be used for cancer therapy is appealing due to the reduced time and cost required to achieve patient benefit. Aim: The aim of this study was to identify repurposed drugs for use in HNSCC and to validate these drugs and their targets in vitro and in vivo. Methods: A library of 100 BNF- approved drugs were screened against HNSCC cell lines using high-throughput AlamarBlue proliferation assays and the drug combination INH56 and IHN29 was identified. To validate this finding, AlamarBlue assays were repeated using a range of concentrations either alone or in combination with cisplatin. In addition, the ability of IHN56 + IHN29 to reduce colony formation and promote apoptosis of HNSCC cell lines was quantified using clonogenic survival assays and Annexin V/FITC FACS analysis. To determine the mechanisms of action of IHN56 + IHN29, CAL27 cells were exposed to drugs for 24 hours and the expression of STAT3, pSTAT3-Y705, pSTAT3-S727, EGFR, pEGFR, p21, p53, LC-3 and Ki67 were visualized by Western blot. Finally, in vivo mouse models were employed to confirm our in vitro findings. Results: 5 x 10⁶ CAL27 cells were injected into male NSG mice and allowed to reach 150 mm³. Mice then received IP injections of 310 mg/kg INH29 on days 3-14 after escalating doses on days 1-2, 40 mg/kg IHN56 days 3-14, 2 mg/kg cisplatin days 3, 5 and 7, or vehicle control days 1-14. Weights and tumors were measured at least 3 times per week. Results: The initial screen identified two promising drugs in combination: INH56 and IHN29, originally used to treat helminthiasis and epilepsy. The IC₅₀ of INH56 in CAL27 cells utilising the AlamarBlue assay was 1.47 μM and reduced to 0.66 μM in the presence of 0.9 mM IHN29. Clonogenic assays demonstrated the ability of IHN56 to reduce colony formation by 55% at 2 μM and by 85% when combined with IHN29 at 1 μM. Colony assays with 1 μM IHN29 and 1 μM IHN29 demonstrated a synergistic increase in apoptosis was observed with INH56 treatment, which was markedly increased following the addition of IHN29. Western blot analysis supports these observations through decreased Ki67 (proliferation), reduced STAT3 activation (oncogenic signaling), increased p21 (apoptosis) independent of p53, and increased LC3 (autophagy) protein levels. In vivo, CAL27 xenograft tumor growth was significantly impaired by IP administration of INH29 alone (P=0.002) and in combination with IHN29 (P=0.002) after 11 days, without any obvious toxicity. Conclusion: The combination of INH56 + IHN29 demonstrates strong in vivo activity against HNSCC. Our data suggest that this combination is promising for treating HNSCC.

**#1062 The combination of metformin and valproic acid induces synergistic apoptosis of prostate cancer cells via p53 activation and the intrinsic pathway.** Linh N.K. Tran, Ganessen Kichenadasse, Katherine L. Morel, Pamela J. Sykes. Flinders University and Medical Centre, Adelaide, Australia.

The anti-diabetic drug metformin (MET) and the anti-epileptic drug valproic acid (VPA), when used alone, have demonstrated anti-cancer effects in prostate cancer (PCA). We have demonstrated that the combination of MET and VPA (MET+VPA) can induce synergistic apoptosis in the human prostate cancer cell line LNCaP (p53⁺) but not in PC3 (p53⁻), and that the response is dependent on functional p53. Here, we aimed to (1) investigate the mechanism of synergistic apoptosis induced by MET + VPA in LNCaP and (2) propose patient subsets for clinical applications of MET + VPA based on the mechanism involved. LNCaP and PC3 cells were treated with vehicle, 2.5 mM MET, 2.5 mM VPA, or 2.5 mM MET + VPA for 24 hours and proteins extracted from cell fractions. Western blots were used to confirm cytoplasmic and mitochondrial fractions by measuring the presence of β-actin, pyruvate dehydrogenase subunit E1-α, and ATP synthase subunit-α protein, as well as the release of cytochrome c from mitochondria to the cytoplasm. The mechanism of apoptosis via p53 activation is proposed based on our findings and current understanding of literature. The release of cytochrome c from the mitochondria to the cytoplasm was significantly increased up to 12-fold (p<0.05) in LNCaP, but not in PC3, in response to MET + VPA compared to vehicle, MET alone, and VPA alone. This finding suggests that both MET and VPA are required to induce an intrinsic apoptosis response in LNCaP via p53. The mechanism proposed is that MET can inhibit the activity of mitochondrial complex 1 which alters the AMP/ATP ratio. This change can activate adenosine monophosphate protein kinase which phosphorylates p53 at Ser15 and leads to p53-dependent cell death. As a non-specific histone deacetylase inhibitor, VPA causes protein acetylation including p53 at lysine residues 373 and 382. By combining metformin and VPA, increased p53 protein activation can occur through phosphorylation of p53 at different sites driving p53-dependent cell apoptosis via the intrinsic pathway, as evidenced by release of cytochrome c into the cytoplasm. Our results suggest that MET + VPA provides a useful treatment for PCa at a clinically localized stage where p53 is present in most patients or advanced stage where p53 has not been mutated. Additionally, MET + VPA is potentially an alternative to androgen deprivation therapy, particularly in tumors with functional p53. A phase I neo-adjuvant clinical trial will start in early 2017 to evaluate the response of high risk localized PCa to MET + VPA (ANZCTR trial ID: ACTRN12616001021460).

**#1063 Benefit from combination cancer therapy arising from patient-to-patient variability rather than additive or synergistic drug action.** Adam C. Palmer, Peter K. Sorger. Harvard Medical School, Boston, MA.

Combination cancer therapy is an important means to increase rates and durability of drug response. Superior responses to combination therapies are commonly interpreted as evidence that individual tumors respond more strongly to a combination than to a monotherapy, and combinations are often developed based on pre-clinical observations of drug additivity or synergy. Here we explore an alternative hypothesis, namely that combination therapy can confer benefit simply by providing multiple chances of an above-average response to monotherapy. The benefit

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conferred by two-drug combination therapy in human clinical trials and PDX animals was compared with a model of independent drug action postulating that net response in each patient equals the better of two potential responses to drugs given individually with no benefit from the second agent. Data were derived from clinical trials of drug combinations in melanoma, ovarian, colorectal, pancreatic, and breast cancers treated with immunotherapies, chemotherapies and inhibitors of kinases, growth factor receptors, angiogenesis, and DNA repair enzymes; as well as analysis of 4,500 drug response measurements in patient-derived tumor xenografts, and a first-principles model of tumor kinetics and variability in drug response. A majority of approved combination cancer therapies analyzed confer benefits consistent with independent drug action. This benefit arises from high inter-subject variability, which can be assessed from clinical trials, and low content in responsiveness to different drugs, assessed from PDX studies. In a minority of cases responses exceed this expectation, sometimes dramatically, and are likely to represent true drug interaction. Therefore, independent drug action, without any assumption about additive or synergistic effects, provides a sufficient and more parsimonious explanation for the superiority of many clinically approved combination cancer therapies and changes how such data are interpreted mechanistically. Optimization of independent action also provides an alternative means for designing new drug combinations.

#1064 STAT3 NH2-terminal domain inhibition sensitizes medulloblastoma cells to chemotherapy. Sutapa Ray,1 Don W. Coulter,1 Shawn D. Gray,2 Jason A. Sughrue,2 Nagendra K. Chaturvedi,2 Shantant S. Joshi,3 Kishor K. Bhakat,3 Timothy R. McGuire,4 John G. Sharp1. 1Pediatrics, University of Nebraska Medical Center, Omaha, NE; 2Pharmacy Practice, University of Nebraska Medical Center, Omaha, NE; 3Genetics, Cell Biology and Anatomy, University of Nebraska Medical Center, Omaha, NE.

Medulloblastoma (MB) is the most common malignant brain tumor in children that arises from cerebellar neuronal progenitor cells. Despite aggressive treatment involving radiation and chemotherapies, the prognosis for high-risk MB remains poor and long-term complications from current therapies are common. Therefore, new effective therapies based on the molecular features of MB are needed to improve therapeutic outcomes. The STAT3 transcription factor is known to be constitutively activated in a variety of human cancers, including MB and functions as an oncogene, mediating cancer cell survival, proliferation, migration, and drug-resistance. We have delineated the functional role of STAT3 NH2-Terminal Domain (NTD) in MB by using a cell permeable peptide derivative of the STAT3 second helix that specifically binds and perturbs the structure/function of STAT3 and interferes with tetramerization of STAT3 dimers, protein-protein interactions and target genes transcription. Herein, we report that treatment of MB cells with STAT3-NTD inhibitor (S3-NTDI) leads to growth inhibition, cell cycle arrest and apoptosis. The inhibition of STAT3 signaling was also confirmed by downregulation of its downstream targets, including MYC, CCND1, BCL2L1, BCL2, PIM1 and APEX1. Moreover, we observed that S3-NTDI exposure attenuated the migration of MB cells in a wound-healing assay, a prerequisite for tumor invasion and metastasis. We also found that S3-NTDI abrogated IL-6 induced epithelial-mesenchymal transition (EMT) marker protein expression and inhibition of EMT-related transcription factors. Most importantly, we observed that a combination therapy with S3-NTDI and cisplatin significantly decreased the highly aggressive MYC-driven MB cell growth in a dose dependent manner and induced apoptosis by downregulating STAT3 regulated anti-proliferative and anti-apoptotic gene expression. To elucidate the mechanisms of S3-NTDI mediated inhibition, we showed that S3-NTDI upregulated expression of pro-apoptotic gene C/EBP-homologous protein (CHOP) and concomitantly decreased association of the STAT3 transcription factor to endogenous proximal promoter of CCND1 and BCL2 in chromatin immunoprecipitation assay. Furthermore, we determined that S3-NTDI mediated downregulation of miRNA-21 in MB cells, de-repressed Protein Inhibitor of Activated STAT3 (PIAS3), a negative regulator of STAT3 signaling, which in turn, attenuated STAT3 signaling pathway. Overall, our results revealed an important role of STAT3 NTD and its downstream effector molecules in regulating MB pathogenesis and disruption of this pathway with S3-NTDI may serves as a promising new candidate for therapeutic interventions in MB therapy, thereby improving the outcomes of high-risk pediatric MB patients.

#1065 MMP sensitive liposomes followed by radiotherapy improves tumor control in head and neck cancer mice models. Rikke Y. Brogaard,1 Allison L. Davis,1 Robert Z. Orlowski,1 Varun Vijay Prabhu,1 Joshua Allen,2 Ying Yang1. 1The University of Texas M.D. Anderson Cancer Center, Houston, TX; 2Oncosciences, Inc, Philadelphia, PA.

Tumors are resistant to current treatment modalities because of the proximity of tumor cells to brainstem and cranial nerves. Therefore, new effective therapies are needed to improve therapeutic outcomes. The STAT3 transcription factor is known to be constitutively activated in a variety of human cancers, including MB and functions as an oncogene, mediating cancer cell survival, proliferation, migration, and drug-resistance. We have delineated the functional role of STAT3 NH2-Terminal Domain (NTD) in MB by using a cell permeable peptide derivative of the STAT3 second helix that specifically binds and perturbs the structure/function of STAT3 and interferes with tetramerization of STAT3 dimers, protein-protein interactions and target genes transcription. Herein, we report that treatment of MB cells with STAT3-NTD inhibitor (S3-NTDI) leads to growth inhibition, cell cycle arrest and apoptosis. The inhibition of STAT3 signaling was also confirmed by downregulation of its downstream targets, including MYC, CCND1, BCL2L1, BCL2, PIM1 and APEX1. Moreover, we observed that S3-NTDI exposure attenuated the migration of MB cells in a wound-healing assay, a prerequisite for tumor invasion and metastasis. We also found that S3-NTDI abrogated IL-6 induced epithelial-mesenchymal transition (EMT) marker protein expression and inhibition of EMT-related transcription factors. Most importantly, we observed that a combination therapy with S3-NTDI and cisplatin significantly decreased the highly aggressive MYC-driven MB cell growth in a dose dependent manner and induced apoptosis by downregulating STAT3 regulated anti-proliferative and anti-apoptotic gene expression. To elucidate the mechanisms of S3-NTDI mediated inhibition, we showed that S3-NTDI upregulated expression of pro-apoptotic gene C/EBP-homologous protein (CHOP) and concomitantly decreased association of the STAT3 transcription factor to endogenous proximal promoter of CCND1 and BCL2 in chromatin immunoprecipitation assay. Furthermore, we determined that S3-NTDI mediated downregulation of miRNA-21 in MB cells, de-repressed Protein Inhibitor of Activated STAT3 (PIAS3), a negative regulator of STAT3 signaling, which in turn, attenuated STAT3 signaling pathway. Overall, our results revealed an important role of STAT3 NTD and its downstream effector molecules in regulating MB pathogenesis and disruption of this pathway with S3-NTDI may serves as a promising new candidate for therapeutic interventions in MB therapy, thereby improving the outcomes of high-risk pediatric MB patients.

#1066 ImpedireoncOPON201 efficacy in refractory multiple myeloma via Erk1/2 inhibition and pro-apoptotic Bim upregulation: single agent and combinatorial approaches. Yongsheng Tu,1 Jin He,2 Huan Liu,2 Richard Eric Davis,1 Robert Z. Orlowski,1 Varun Vijay Prabhu,1 Joshua Allen,2 Ying Yang1. 1The University of Texas M.D. Anderson Cancer Center, Houston, TX; 2Oncosciences, Inc, Philadelphia, PA.

 Disease relapse and drug resistance occurs in the majority of multiple myeloma (MM) patients despite improvements offered by new treatments. There is an urgent unmet need for new therapies that can overcome drug resistance and prolong patient survival after failure of standard-of-care. ONC201, founding member of the imidazole class of compounds, has robust preclinical efficacy in a variety of tumor types. A first-in-human ONC201 clinical study has demonstrated exceptional safety, therapeutic pharmacokinetics and evidence of tumor engagement that has led to several Phase I/II advanced cancer studies. Given the pronounced sensitivity of B-cell lymphomas to ONC201, we assessed the efficacy of ONC201 in MM preclinical models. We treated human MM cell lines and patient-derived cells isolated from bone marrow aspirates with ONC201 for 72 hours. ONC201 treatment decreased MM cell viability in CellTiter-Glo assays, with IC50 values that were 1 μM to 1.5 μM, even in high risk MM cell line RPMI8226. Consistent with prior reports, the efficacy of ONC201 was independent of TP53 status, as MM1S or NCI-H929 cells with wild-type TP53 and OPM-2 or RPMI8226 with mutated TP53 had a similar sensitivity towards ONC201. Additionally, ONC201 was equally effective in paired MM cell lines with TP53 wild type or knockout. Western blot analysis showed increased apoptosis, cleavage of caspase-9, caspase-3, and PARP. We also found that ONC201 induced expression of the pro-apoptotic protein Bim in MM cells, which can occur downstream of ERK inactivation. shRNA knockdown of Bim expression in MM cells abrogated ONC201-induced apoptosis in annexin-V binding assays. Phosphorylation of Bim at Ser69 by Erk1/2, an indicator of Erk1/2 kinase activity, and downregulated Bim pSer69. In addition, ONC201 induced apoptosis in dexamethasone-, bortezomib-, and carfilzomib-resistant MM cell lines with the same efficacy as in wild-type cells. As a rational strategy to increase the efficacy of ONC201, we enhanced its inhibition of proteasome-mediated Bim degradation by radiation. We tested combinations of ONC201 with proteasome inhibitors. Synergistic reduction in cell viability and enhanced Bim expression and PARP cleavage was observed with ONC201 in combination with bortezomib or carfilzomib in MM cells. The combination of ONC201 and bortezomib enhanced the levels of Bim and cleaved PARP in MM cells. Overall, these findings demonstrate that ONC201 inhibits the Erk1/2 pathway and enhances Bim expression to induce apoptosis in MM regardless of p53 status and resistance to standard-of-care therapies. Our studies provide a strong rationale for clinical trials of ONC201 as a single agent or in combination with approved drugs in relapsed/refractory MM.

Pancreatic cancer is a highly chemo-resistant tumor type known to aggressively metastasize at an early stage with an overall five-year survival rate of 6%. In this study we tested the efficacy of ONC201 and ONC212 in a panel of pancreatic cancer cell lines and patient-derived models in vitro and in vivo. ONC201 is the founding member of the imipridone class of small molecules with anti-proliferative and pro-apoptotic effects in various tumor types. Kline et al. and Allen et al. previously showed that ONC201 stimulates the integrated stress response by up-regulating ATF4, CHOP and DR5 followed by induction of apoptosis by up-regulating TRAIL. While the spectrum of ONC201 efficacy is broad among tumor types, pancreatic cancer cells are relatively insensitive. ONC212 exhibits low micromolar GI50 values in 3 out of 7 pancreatic cancer cell lines and 2 out of 9 patient-derived cells that were most sensitive among the pancreatic cell lines and samples tested. ONC201 induces the integrated stress response in both ONC201-sensitive and -insensitive pancreatic cancer cell lines. Interestingly, ONC201-treated resistant cells arrest in G1 and do not go through apoptosis. In order to address insensitivity to ONC201 in pancreatic cancer we explored two approaches. The first approach included treating with the ONC201 analogue ONC212, an imipridone that is currently under pre-clinical development. In vitro and in vivo studies consistently show a higher potency of ONC212 as compared to ONC201 in pancreatic cancer, especially in ONC201-insensitive models. We are currently investigating the mechanism of action of ONC212 in comparison to ONC201. The second approach to address ONC201-insensitive pancreatic cancer was combination treatment with the IGF1-R inhibitor AG1024. Western blot analysis of receptor tyrosine kinase expression levels in the panel of pancreatic cancer cell lines revealed a strong correlation between resistance to ONC201 and high expression of IGF1-R. Therefore we hypothesized that IGF1/IGF1-R might play a role in the resistance of pancreatic cancer cells to ONC201. Indeed, treatment of resistant pancreatic cancer cells with the IGF1-R inhibitor AG1024 sensitized the cells to ONC201. We are currently further exploring the involvement of IGF1-R in the ONC201 resistance mechanism. Furthermore, in-vivo studies are ongoing to validate the efficacy of the combination treatment. In summary, although pancreatic cancer is known to be refractory to many drugs, this study introduces two different approaches with imipridone small molecules ONC212 and ONC201 that show promising therapeutic potential for pancreatic cancer.

#1068 Driver pathway blockade synergizes with PLK1 inhibition in anaplastic thyroid cancer. Daniela De Martino, Emrullah Yilmaz, Arturo Orlacchio, Antonio Di Cristofano. Albert Einstein College of Medicine, Bronx, New York, NY.

Anaplastic thyroid cancer (ATC) is one of the most lethal malignancies, with a median survival of less than 6 months from the time of diagnosis. Molecular changes that characterize ATC have been recently well defined and involve most often p53 mutations, and activation of c-Met, RAS and RAF. PLK1, an essential mitotic regulator, has been found overexpressed in ATC. Previous data from our lab have shown that PLK1 inhibitors are effective in ATC cell lines. However, PLK1 inhibition often results in escape from growth arrest through mitotic slippage. This leads to the generation of polyploid, genetically unstable, cell populations. We have tested the effect of combining PLK1 and PI3K inhibitors in ATC cell lines. Combined treatment with the PLK1 inhibitor BI6727 and the PI3K inhibitor BKM120 resulted in a significant synergistic effect in ATC cell lines with high levels of AKT activity, but not in those with undetectable pAKT. Combination of the two drugs enhanced the G2/M arrest at doses in which the single drugs showed no effect. In addition, it led to a massive reduction of the tetraploid cells population. Furthermore, combined treatment with BI6727 and BKM120 in PI3K-high cell lines showed a significant induction of apoptosis in a time- and dose-dependent manner. Combined inhibition of PI3K and PLK1 was extremely effective in inhibiting tumor growth in vivo, in an immunocompetent allograft model of ATC. Our results show that combination of PLK1 and PI3K inhibitors is an effective treatment for ATC cells with high levels of PI3K signaling. This combination results in cell cycle arrest, inhibition of tetraploid cell generation, and induction of apoptosis, suggesting a clear therapeutic potential.

#1069 Inhibition of the nuclear import receptor, KpnB1 synergizes with cisplatin toxicity in cervical cancer cells. Ru-pin Chi,1 Wei Wei,2 Michael Birrer,3 Virna D. Deleaner,4 Univ. of Cape Town, Cape Town, South Africa; 5Harvard University, Cambridge, MA.

Elevated expression of members of the nuclear transport protein family has been reported in multiple cancers and presents as novel anticancer therapeutic targets. Using a cervical cancer model system, we have previously shown that inhibition of the nuclear import receptor, KpnB1 by siRNA and a novel small molecule, Inhibitor of Nuclear Import (INI-43) resulted in cancer cell death via apoptosis. In this study, we investigated the cancer cell killing effects of KpnB1 inhibition in combination with Cisplatin (CDDP), a first-line chemotherapeutic agent in cervical cancer. KpnB1 inhibition alone treated with KPT-330 at sub-lethal concentrations enhanced cancer cells’ sensitivity to CDDP. Our data shows that the combination treatment of INI-43 and CDDP significantly decreases CDDP IC50 compared to CDDP treatment alone. Increased PARP cleavage was observed in combination treated cells and this correlated with increased γH2A.X, indicating increased apoptosis and DNA damage. Further studies are needed to understand the mechanism of resistance to ONC201 and high expression of IGF1-R. Therefore we hypothesized that IGF1/IGF1-R might play a role in the resistance of pancreatic cancer cells to ONC201. Indeed, treatment of resistant pancreatic cancer cells with the IGF1-R inhibitor AG1024 sensitized the cells to ONC201. We are currently further exploring the involvement of IGF1-R in the ONC201 resistance mechanism. Furthermore, in-vivo studies are ongoing to validate the efficacy of the combination treatment. In summary, although pancreatic cancer is known to be refractory to many drugs, this study introduces two different approaches with imipridone small molecules ONC212 and ONC201 that show promising therapeutic potential for pancreatic cancer.

#1070 Role of FOXO1 in response of ovarian carcinoma cells to the XPO1/CRM1 inhibitor KPT-330/selinexor in combination with cisplatin. Simone Stucchi,1 Michelendrea De Cesare,1 Cristina Corno,1 Nives Careneni,1 Emilio Ciussani,2 Nadia Zaffaroni,1 Laura Gatti,1 Paola Perego.1 Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy; 2Fondazione IRCCS Istituto Neurologico C. Besta, Milan, Italy.

Ovarian carcinoma is a major cause of cancer-related death in women. Besides late diagnosis, treatment often fails to produce a persistent disease control. The efficacy of the platinum drug-based therapy is limited by drug resistance. Thus, an ideal therapy for women with ovarian carcinoma is still missing. Because the karyopherin XPO1/CRM1 contributes to the regulation of the cellular localization of the transcription factor FOXO1 which participates in apoptosis regulation, the aim of this study was to examine if interference with XPO1 to improve FOXO1 nuclear localization may be exploited to kill efficiently ovarian carcinoma cells and to improve cisplatin efficacy. Here, we employed preclinical pharmacology approaches including growth inhibition assays, western blot analyses, gene knockdown by siRNA, quantitative Real-time PCR, immunofluorescence analyses and tests in in vivo models. The drug interaction was analyzed using the Chou and Talalay method. When cell sensitivity to KPT-330 of a panel of ovarian carcinoma cell lines was examined a marked sensitivity to the XPO1 inhibitor was found. The effect of the combination of cisplatin and KPT-330 was investigated in the IGROV-1 cells, using simultaneous or sequential schedules. According to the combination index values, when KPT-330 exposure followed cisplatin exposure the most favourable drug interaction was observed. In IGROV-1 cells, a modulation of proteins involved in apoptosis (p53, Bax) and in cell cycle progression (p21) was found, besides G1 and G2/M accumulation after exposure to KPT-330 and to the cisplatin/KPT-330 combination, respectively. KPT-330-treated cells exhibited FOXO1 nuclear staining, in keeping with the capability of the compound to inhibit FOXO1 nuclear export. Knock-down experiments by RNA interference indicated that FOXO1-silenced cells displayed increased cisplatin sensitivity. Increased CDDP IC50 was observed in FOXO1-silenced cells and this correlated with increased apoptosis and DNA damage. Taken together, results suggest increased Cisplatin sensitivity of cervical cancer cells when nuclear import via KpnB1 is inhibited.


Background: Trifluridine/tipiracil (FTD/TPI) is an oral nucleoside anti-tumor agent that is composed of trifluridine and tipiracil hydrochloride at a molecular ratio of 1:0.5. Checkpoint-blockade immunotherapies are particularly effective in patients with tumor T cell infiltrations. In this study, the...
antitumor effects of FTD/TPI + anti-mouse PD-1 antibody combination were studied in a syngeneic mouse model and the tumor-infiltrating lymphocyte (TIL) subsets were evaluated. Method: The mouse colorectal cancer cell line CMT-93 was subcutaneously implanted into C57BL/6 mice. Vehicle (0.5% Hydroxypropyl methylcellulose, 10 mL/kg, p.o.), FTD/TPI (75, 100, and 150 mg/kg/day), TPI (clone RPMI-14.0.1 mg/body; once daily, days 1, 5, and 9; i.p.) and FTD/TPI + anti-mouse PD-1 antibody combination were administered, and inhibitory activity was evaluated according to tumor-volume changes. Single-cell suspensions were prepared from collected tumors. Based on cell marker expression, CD4+ T cells, CD8+ T cells, and regulatory T cells (Tregs) were identified and counted against CD3+ T cells. Flow cytometric analysis revealed a higher inhibition caused complete tumor regression in four/five mice without body-weight reduction (91.8%, 95.7%, 98.4%, respectively) than monotherapy. Remarkably, the 150-mg/kg/day FTD/TPI inhibited tumor growth significantly (P < 0.05; 91.8%, 95.7%, 98.4%, respectively) than monotherapy. Remarkably, the 150-mg/kg/day FTD/TPI + anti-mouse PD-1 antibody combination caused complete tumor regression in four/five mice without body-weight reduction or drug-related deaths; however, none of the monotherapies caused complete tumor regression. Flow cytometry also showed a higher CD8+ T cell percentage among total lymphocytes and a lower Treg percentage in CD4+ T cells after combination therapy compared with the controls. Conclusion: FTD/TPI + anti-mouse PD-1 antibody combination synergistically increased the CD8+ T cell percentages in whole lymphocyte and decreased Treg percentages in CD4+ T cell. This suggests that FTD/TPI + anti-mouse PD-1 antibody combination modulates tumor T cell populations and improves their antitumor activity.

#1072 Synergistic effect of gemcitabine and a Dclk1 inhibitor on pancreatic cancer cell survival. Daichi Kawamura, Yoshihiro Takamoto, Arata Nishimoto, Toshihi Tanaka, Yukari Hironaka, Kumiko Yoshida, Junichi Murakami, Naruji Kugimiya, Eijiro Harada, Koji Ueno, Tohru Hosoyama, Kimi-kazu Hamano, Yamaguchi University, Ube, Japan.

Pancreatic cancer has the highest mortality rate of all major cancers and is one of the most lethal malignancies. There is a constant upward trend in the number of patients diagnosed with pancreatic cancer and the number of deaths due to pancreatic cancer. Gemcitabine (GEM) is often used in the treatment of pancreatic cancer (PDAC), but has limited effects. Doublecortin-like kinase 1 (Dclk1) is important in the progression of early pancreatic neoplastic lesions and PDAC. However, the functional role of Dclk1 in PDAC is unknown. To identify the substrate protein phosphorylated by Dclk1, we performed a protein microarray analysis on Dclk1 knockdown cells. The results of this analysis directed our studies toward Chk1, which is known to be a potential regulator of the cell cycle and experiences upregulation of phosphorylation after GEM treatment. In general, GEM treatment results in DNA damage to pancreatic cancer cells, an increase in phosphorylated Chk1 (p-Chk1), and arrests the cell cycle progression to repair the damaged DNA. On the basis of the preliminary data, we hypothesized that the decrease in Chk1 phosphorylation by Dclk1 inhibition circumvents cell cycle arrest and impairs the subsequent DNA repair. The aim of this study was to evaluate the synergistic effect of Dclk1 inhibition and GEM treatment on pancreatic cancer cell survival. We used the human pancreatic cancer cell line Mia PaCa-2 and LRRK2-IN-1 (LRRK), as the Dclk1 inhibitor for this study. First, we examined the effects of GEM or the Dclk1 inhibitor or both on cancer cell proliferation and the expression of p-Chk1. Significantly decreased cell proliferation was observed on co-treatment of GEM and LRRK compared to GEM treatment alone. In addition, the expression of p-Chk1 significantly decreased on co-treatment compared to GEM treatment alone. Second, we used flow cytometry to analyze the cell cycle progression of CD8+ T cells and GEM and/or LRRK. Almost all cancer cells treated with GEM alone were arrested in the S phase of the cell cycle. The addition of LRRK allows the cell cycle to proceed in the same manner as untreated control cancer cells do. We also evaluated DNA damage by measuring the intensity of gamma-H2AX. Cancer cells that were co-treated experienced more DNA damage than with GEM treatment alone. The co-treatment induced apoptosis without the repair of DNA damage in the cancer cell. In conclusion, the combined treatment with GEM and a Dclk1 inhibitor decreased the cell survival rate compared to treatment with GEM alone through the suppression of p-Chk1. Targeting Dclk1 in combination with GEM might offer an excellent opportunity for future pancreatic cancer treatments.

#1073 Increased internalization and processing of the CD37-targeting antibody-drug conjugate, naratuximab emtansine (IMGN529), in the presence of rituximab leads to enhanced potency in diffuse large B-cell lymphoma models. Stuart W. Hicks, Katharine C. Lai, Yong Yi, Prerak Shah, Cristina L. Gavilanescu, Joe Ponte, Callum M. Sloss, Angela Romanelli. Immunogen, Inc., Walther, MA.

Naratuximab emtansine (IMGN529) is an investigational CD37-targeting antibody-drug conjugate (ADC) that has shown both preclinical and clinical activity in DLBCL. We have shown that rituximab, an anti-CD20 monoclonal antibody, enhances the preclinical activity of IMGN529. The combination of IMGN529 and rituximab is more active than either agent alone, and this benefit is maintained in an in vivo model of hepatitis and cell death, resulting in enhanced tumor growth inhibition by anti-CD20 class effect. Radiolabeled processing studies were performed to determine if the observed increase in internalization led to a corresponding increase in antibody degradation, which is required for the release of the cytotoxic agent in IMGN529. By trace-labeling the K7153A antibody with tritiated propionate (3H-K7153A), we were able to follow binding, uptake, and degradation of the antibody component of IMGN529 in DLBCL cell lines. Following pulse exposure, the amount of 3H-K7153A degraded after 24 hours remained the same whether treated alone or in combination with other B-cell targeting antibodies. However, when combined with rituximab the percentage of degraded 3H-K7153A increased as much as 3-fold and a similar increase of 3H catabolite was generated. When compared, the combination of K7153A and rituximab produced considerably more catabolite (6-fold, p-value < 0.0001) than the combination of rituximab and a CD19-targeting antibody. These findings support a novel mechanism where the increased potency of IMGN529 and rituximab can be explained by CD20 binding resulting in an increase in internalization and degradation of IMGN529, leading to generation of greater amounts of cytotoxic agent. Overall, these data provide a biological rationale for the enhanced activity of the rituximab plus IMGN529 combination, further supporting the clinical development strategy of this combination in DLBCL.

#1074 Combining forces: Synergy of erlotinib and crizotinib in a wild-type squamous non-small cell lung cancer cell line. Nele Van Der Steen,1 Christian Rolfo,2 Patrick Pauwels,2 Godefridus J. Peters,1 Elisa Giovannetti1.

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Oncogenic drivers are often overexpressed in adenocarcinoma non-small cell lung cancer (NSCLC) patients, so this tumor type is often sensitive to targeted therapies. Sensitizing mutations in the epidermal growth factor receptor (EGFR) are good predictive biomarkers for response to EGFR-small molecule inhibitors, eg erlotinib. Amplification or exon 14 skipping of cMET are good biomarkers for cMET-small molecule inhibitors, eg crizotinib. In other subtypes of NSCLC, eg squamous, targeted therapy is not considered to be active. We tested the combination of erlotinib and crizotinib on a panel of squamous NSCLC cell lines that were wild-type for both EGFR and cMET. The effect of this drug combination was tested with different assays. The sulforhabdamine B- assay was used to determine growth inhibition and combination indexes (CI) were calculated with the method of Chou&Talalay. A CI < 0.8 was synergistic, 0.8 < CI < 1.2 was add-
tion of pERK1/2 (Thr202/204), pPRAS40 (Thr246) and pGSK3β (Ser9). Western blotting showed that the levels of pPRAS40 were decreased in the LUDLIN cells after treatment with erlotinib or crizotinib, and almost gone when treated with the combination. In the SKMES1 cells only the combination decreased pPRAS40. H1703 cells showed no change in pPRAS40. These changes in phospho-tyrosine may underlie the hypothesis that Her3 and PI3K/Akt signaling may be important in the interaction of both drugs. Her3 is a promiscuous receptor that is able to heterodimerize with EGFR and cMET. When blocking one of these receptors, Her3 is still able to continue downstream PI3K/Akt signaling. When both EGFR and cMET are blocked, Her3 and PI3K/Akt signaling is inhibited, leading to synergy. The expression of total-Her3 was lowest in the antagonistic cell line. The combination of both inhibitors led to a complete inhibition of pHer3 (Tyr1289). In conclusion: Blocking both EGFR and cMET signaling causes inhibition of Her3 downstream signaling through PI3K/Akt, leading to synergy in wild-type squamous NSCLC cells.

#1075 JQ1 induces DNA damage, inhibits expression of DNA repair proteins, and synergizes with PARP inhibitors in pancreatic cancer cells. Aubrey Lynn Miller,1 Tracy Gamblin,1 Leona Council,1 Robert van Waardenburg,2 Eddy Yang,1 James Bradner,2 Karina Yoon,1* 1Univ. of Alabama at Birmingham, Birmingham, AL; 2Novartis Institutes for Biomedical Research, Cambridge, MA. Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer. PDAC is a highly aggressive tumor with a 5-year survival of <6%. Surgery remains the only curative treatment for PDAC patients, but only 20% of newly diagnosed patients have resectable disease. The remaining 80% present with locally advanced or metastatic disease, and aggressive systemic therapy with gemcitabine. The median survival of this patient population is ~6 months. Development of more effective therapy for this chemorefractory disease is imperative. The goal of the current study was to develop effective combination therapy for PDAC by identifying agents that might be combined with the BET bromodomain inhibitor JQ1, which we have shown to inhibit the growth in vivo of PDAC patient-derived xenografts (PDx). Expression profile analysis of tumors from vehicle control and JQ1 treated mice revealed that JQ1 inhibited the expression of multiple gene products involved in DNA repair. Notably, JQ1 inhibited expression of DNA double-strand break (DSB) repair proteins BRCA2 and Ku80. Immunohistochemical staining confirmed down-regulation of expression of both proteins in tumors of mice treated with JQ1. Further, immunohistochemistry and immunofluorescence analyses demonstrated that decreased expression of BRCA2 and Ku80 was coincident with increased levels of DNA damage, as reflected by expression of the DNA DSB marker gamma-H2AX. Data generated in vivo in three independent PDx models corroborated in vitro data generated using pancreatic cancer cell lines BxPC3 and Panc1. The data suggest that JQ1 induces DNA damage by inhibiting DNA repair. Because DNA repair deficiency seen in cells to PARP inhibitors, we hypothesized that JQ1-induced DNA repair deficiency would sensitize PDAC cells to PARP inhibitors. To address this hypothesis, we exposed Panc1 and BxPC3 to JQ1 or to a PARP 1/2 inhibitor (veliparib or olaparib) or to the combinations, and assessed the efficacy of each. Growth inhibition data, analyzed using Compusyn software and reported as combination indices, demonstrated that the combinations of JQ1 + veliparib or olaparib exert synergistic cytotoxicity. Further, the combination of JQ1 + a PARP inhibitor increased the accumulation of DNA damage in vitro, compared to either agent alone. We conclude that JQ1 induces DNA damage due at least in part to DNA repair deficiency, and propose that this mechanism sensitizes PDAC cells to PARP inhibitors.

#1076 Potent anti-tumor activity of artemisinin. Mourir Tilaoui. Faculty of Science and Technology Beni-Mellal, Beni Mellal, Morocco. Cancer is a leading cause of death worldwide and remains a therapeutic enigma. Phytochemical compounds are emerging as a new generation of anti-cancer agents with limited toxicity in cancer patients. The purpose of this study was to investigate the potential impact of artemisinin, the main active component of the Chinese herb Artemisia annua L., on survival cancer cells and tumor growth in vivo. Exposure of tumor cells P815 (murine mastocytoma) and BSR (kidney adenocarcinoma of hamster) to increasing artemisinin concentrations resulted in a significant inhibition of viability, as revealed by the respective IC50 values (12 μM for P815 and 52 μM for BSR cells). Interestingly, no cytotoxic effect was observed of artemisinin on peripheral blood mononuclear cells. The in vitro cytotoxicity studies were complemented by the determination of apoptotic DNA fragmentation and Annexin V - Streptavidin-FITC assay. Our results provide evidence that artemisinin leading to apoptotic pathway on P815 but not on BSR cancer cell lines. Moreover, we demonstrate that artemisinin synergizes with microtubule - damaging agent vincristine to inhibit cellular viability. The in vivo anticancer activity of artemisinin was also evaluated using the DBA2/P815 (H2d) mouse model inoculated with the P815 mastocytoma cells. Our in vivo data clearly showed that the oral administration of artemisinin inhibited solid tumor development. In view of the available experimental findings, we contend that artemisinin could have clinical potential as an anticancer drug alone or in combination with chemotherapeutic agents such as vincristine.

#1077 Extra-virgin olive oil Met inhibitor oleocanthal-lapatinib: a novel synergistic combination for HER2-dependent breast malignancies. Abu Bakar Siddique, Hassan Y. Ebrahim, Mohammed R. Aki, Mohmed M. Mohyeldin, Khalid A. El Sayed. Univ. of Louisiana College of Pharmacy, Monroe, LA. Breast cancer (BC) is the most commonly diagnosed cancer in women, claiming the lives of hundreds of thousands of women each year. The Mediterranean diet promotes to reduce BC risk and BC cancer incidence. Extra-virgin olive oil (EVOO) represents a main ingredient in this diet. The EVOO-derived (+)-oleocanthal was shown to target the Receptor tyrosine kinases (RTK) c-Met. Oleocanthal exerted potent in-vivo efficacy in an orthotopic athymic nude mouse breast cancer xenograft model much greater than its modest in-vitro potency. Dysregulation of RTKs, specifically EGFR/HER2 pathways, correlates with poor prognosis and more aggressive breast cancer phenotypes. Dysregulation of hepatocyte growth factor (HGF) and its receptor c-Met correlates with aggressive proliferation, invasive character, and pathological motility. c-Met amplification correlates with escape from the anticancer effects of EGFR/HER2 inhibitors and cetuximab. (+)-Oleocanthal (OC) is a naturally occurring pheno- sc起到 from EVOO showed significant in vivo activity against invasive breast cancers through targeting HGF/Met axis. The Dual EGFR/HER2 inhibitor lapatinib (LP) has already been in clinical practice for HER2-amplified breast cancer, which occasionally develops resistance through c-Met upregulation. Lapatinib’s therapeutic dose induced significant hepatotoxicity. The combined use of OC and LP was hypothesized not only for therapeutic synergy but also to notably reduce LP’s effective doses and therefore minimize its hepatotoxicity. Combined treatment of subeffective doses of lapatinib and OC caused significant in vitro and in vivo synergistic antiproliferative effects against the HER2-dependent BT-474 and SKBR3 BC cells. Interestingly, OC induced 4-fold lapatinib dose reduction index with improved potency. Protein microarray and Western blot analyses of OC-LP combination treatments synergistically reduced EGFR, HER-2, FAK, JAK1, MEK, and c-Met activation. These results propose OC-use as dietary supplement to synergize the chemotherapeutic effects of LP, reduce its therapeutic dose to ¼ and therefore can minimize its morbidity. Combinatorial inhibition of c-Met-HER-2-EGRF is an effective strategy for the control of HER-2 positive breast cancer. This study was supported by NIH/NCI project IR15CA167475-01.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Combination Therapy 1

#1078 Dinaciclib overcomes resistance to BKM120 in triple negative breast cancer patient-derived xenograft models. Sandeep Rajput, Fang Guo, Li Shun, Cynthia Ma. Washington University St. Louis, St. Louis, MO. Purpose: Triple negative breast cancer (TNBC) is one of the most lethal sub- types of breast cancer with limited therapeutic options. Development of molecularly targeted agents for TNBC is an unmet clinical need. The phosphoinositide 3-kinase- (PI3K) pathway, which is a major cell growth and survival pathway, is frequently activated in TNBC as a result of genetic aberrations such as loss of the negative regulator PTEN or gain of function mutations in PIK3CA, therefore an attractive therapeutic target. However, single agent PI3K inhibitors have shown limited anti-tumor activity in both preclinical models and in clinical trials. One of the most important mechanisms of resistance to PI3K inhibitor is amplification of Myc. Dinaciclib, a potent inhibitor of cyclin-dependent kinases (CDKs) 1, 2, 3 and 9, has shown to be particularly effective in Myc dependent tumors in preclinical studies and in TNBC. We therefore hypothesized that dinaciclib could overcome tumor cell resistance to PI3K inhibitors and improve the therapeutic efficacy in TNBC. In this study, we evaluated the anti-tumor and molecular effect of dinaciclib and BKM120 (Pan-PI3K inhibitor), either alone or in combination, in a panel of patient derived xenograft (PDx) models of TNBC. Methods: Four TNBC PDx models were selected for the study for in-vivo and ex-vivo responses to vehicle, BKM120, dinaciclib, or the combination of BKM120 and dinaciclib. Tumor volume changes over time in each group were documented to calculate the percentage of tumor growth inhibition by either agent alone or in combination. Tumor tissues harvested post treatment were examined by immunohistochemistry for cleaved PARP to determine the extent of
apoptosis and phospho-Histone H3 for G2 to M phase cell cycle progression. Western blot analysis and reverse protein phase array (RPRA) were also performed to determine treatment effect on PI3K downstream targets and cell cycle molecules. Results: The combination of BKM120 and dinaciclib induced significantly greater growth inhibitory effect on tumor growth than either single agent alone in vitro. This is 0.75% inhibition of cell proliferation induction and reduced cell cycle progression. In addition, the combination of dinaciclib and BKM120 significantly reduced the level of cyclin B and the key anti-apoptotic protein survivin as well as significant downregulation of pAKT, and pS6. Conclusions: These data suggest that dinaciclib and BKM120 combination is an effective approach in treating TNBC. Additional mechanistic investigation for the efficacy of this combination is underway.

#1079 Preclinical activity of the FGFRinhibitor BAY 1163877 alone or in combination with antihormonal therapy in breast cancer. Oliver Politz,1 Peter Ellinghaus,2 Sebastian Bender,2 Sylvia Gruenewald,1 Franziska Siegel,1 Marie-Pierre Collin,1 Sabine Zitzmann-Kolbe,1 Dominik Mumberg,1 Karl Ziegelbauer,3 Bayer AG, Berlin, Germany; 4Bayer AG, Wuppertal, Germany.

BAY 1163877 is an orally available, highly potent and selective pan fibroblast growth factor receptor (FGFR) inhibitor. In an ongoing Phase 1 clinical trial (NCT01976741) BAY 1163877 showed clinical responses at acceptable tolerability in patients suffering from different tumor types including uterine blader carcinoma or lung tumors, which were selected based on elevated FGFR1-3 mRNA expression. In the preclinical phase, the compound demonstrated significant single agent anti-tumor activity in various tumor models with different FGFR alterations leading to FGFR overexpression (e.g. FGFR gene amplifications or mutations). Genetic alterations of FGFRs can also be found in breast cancer with 7.5 - 17% of all tumors harboring a FGFR1 gene amplification. Elevated FGFR1 mRNA levels can be found in up to 22% of breast cancer cell lines as well as clinical samples. Other FGFR alterations include FGFR2 or FGFR4 gene amplifications as well as elevated FGFR mRNA levels, which were reported in all breast cancer subtypes. We therefore investigated BAY1163877 monotherapy in various breast cancer models. Due to the favorable clinical safety profile of BAY1163877, we also examined a combination treatment with early line antihormonal therapies in hormone receptor positive breast cancer. In vitro profiling of BAY 1163877 in a number of breast cancer cell lines showed a clear association of efficacy with expression levels of different FGFR isofoms. The efficacy was further investigated in several patient- or cell line-derived breast cancer in vivo models. For instance, BAY 1163877 alone dosed 38mg/kg twice daily induced tumor growth inhibition of greater than 90% in a subcutaneous mouse syngeneic 4T1 breast cancer model expressing elevated levels of FGFR2. Resistance to endocrine therapy appears associated with FGFR1 gene amplification and may explain the poor prognosis of FGFR1 overexpressing tumors treated with adjuvant tamoxifen. We therefore investigated the combination of the panFGFR-inhibitor BAY 1163877 with the clinically used antihormonal compound fulvestrant in selected luminal breast cancer PDX models. Some of these models showed resistance to antihormonal treatment in monotherapy but improved in vivo efficacy in combined treatment using BAY 1163877 and fulvestrant. These data may warrant further clinical investigation of BAY1163877 alone or in combination with antihormonal therapy in patients with FGFR overexpressing breast cancer.

#1080 Targeting the PI3K-Akt and NF-κB pathways as a combination therapy in blocking prostate cancer progression. Eswar Shankar,1 Rajnee Kanwal,2 Aditi Goel,1 Xiaoping Yang,2 Sanjeev Shukla,1 Carl Ziegelbauer,3 Bayer AG, Berlin, Germany; 4Bayer AG, Wuppertal, Germany.

Activation of PI3K/Akt and NF-κB signaling pathways has been associated with prostate cancer progression. In the present study, we have investigated the role of a combination of PI3K/Akt and NF-κB signaling pathways in prostate cancer progression. We used the BPH-1 cell line, which is a human benign prostate epithelial cell line, and the prostate cancer cell lines (PC-3, LNCaP, DU145) to study the effects of these pathways on cell proliferation. We found that the combination of PI3K/Akt and NF-κB signaling pathways significantly inhibited cell proliferation, invasion, and migration in prostate cancer cells. These results suggest that targeting PI3K/Akt and NF-κB pathways as a combination therapy may be effective in blocking prostate cancer progression.

#1081 APC regulation of breast cancer therapeutic resistance. Anne Arnason,1 Monaco VanKloppenberg,2 Jenifer R. Prosser2,1 University of Notre Dame, Notre Dame, IN; 2Indiana Univ. School of Medicine-South Bend, South Bend, IN.

Resistance to chemotherapy is one of the leading causes of death from breast cancer. Our lab discovered that Adenomatous Polyposis Coli (APC) loss in breast cancer cells results in an elevation of tumor-initiating cells (TICs) and resistance to chemotherapy-induced apoptosis. Given that TICs are often most resistant to standard chemotherapeutic compounds, we sought to understand the mechanism responsible for APC-mediated TIC enhancement. Our hypothesis is that the molecular mechanism involved in chemotherapeutic resistance parallels that promoting the TICs. APC-mutant cells have amplified activation of signal transducer and activator of transcription 3 (STAT3). Interestingly, inhibition of STAT3 with a small molecule inhibitor A69 decreases the TIC population and restores drug sensitivity. Studies are ongoing in the laboratory to investigate other molecular signaling pathways involved in APC-mediated enhanced TIC population and therapy resistance. These data have begun to reveal the molecular mechanisms of APC loss in breast cancer that can guide future treatment plans to counteract chemotherapeutic resistance.

#1082 MerTK promotes resistance to irreversible EGFR TKIs by activation of the PI3K-AKT pathway in NSCLCs expressing wild-type EGFR. Dan Yan,1 Xiaodong Wang,2 Stephen V. Frye,3 Shelton H. Earp,1 Deborah DeRykere,1 Douglas K. Graham1. 1Aflac Cancer and Blood Disorders Center, Atlanta, GA; 2Center for Integrative Chemical Biology and Drug Discovery, Chapel Hill, NC; 3UNC Lingerer Comprehensive Cancer Center, Chapel Hill, NC.

Lung cancer is the leading cause of cancer-related death with poor survival rates worldwide. Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancers and 60% of these have overexpression of wild-type EGFR (wtEGFR), which portends a poor prognosis. In addition, in a recent clinical trial 6 of 12 lung cancer patients whose tumors acquired a resistance-conferring T790M EGFR mutation during treatment with an EGFR TKI reverted to wtEGFR after treatment with Rociletinib/CO-1686, an irreversible EGFR TKI that is selective for the T790M mutant. These data suggest that targeting wtEGFR may improve treatment outcomes; however, to date single agents that target wtEGFR have not been effective in large scale clinical trials and new ways to target wtEGFR in this context are needed. Our laboratory previously identified the MerTK receptor tyrosine kinase as a potential therapeutic target in NSCLC and developed MRX-2843 as a novel MerTK-selective irreversible tyrosine kinase inhibitor with favorable properties for clinical translation. Irreversible of driver oncogene status, treatment with a MerTK inhibitor yields potent anti-tumor effects in NSCLC cell culture models and blocks tumor growth in xenografts of the MerTK-dependent wtEGFR-expressing A549 cell line. In an attempt to more potently block A549 lung cancer cell proliferation, we screened a library of 378 kinase inhibitors that are in various stages of development and identified synergy between MRX-2843 and multiple irreversible EGFR TKIs, including CO-1686 and Osimertinib/AZD-9291. Further, we found that wtEGFR and MerTK were frequently co-expressed and co-immunoprecipitated with each other in NSCLC cancer cell lysates. Synergistic inhibition of cell ex-
pansion was observed in a spectrum of NSCLC cell lines with wtEGFR expression treated with the combination therapy, including H1299 (NRAS mutation), H157 (KRAS mutation), H3122 (ALK fusion), and Colo699 (FGFR1 overexpression). On a mechanistic level, combined treatment with 1µM CO-1686 and 100 nM MRX-2843 dramatically inhibited phosphorylation of both MerTK and EGFR and downstream signaling through the PI3K-AKT and MAPK-phosphorylated pathways, while treatment with equivalent doses of either single agent did not efficiently inhibit MerTK, EGFR or downstream signaling. Additionally, CO-1686 mediated synergistic inhibition of A549 expansion in combination with a PI3K or AKT inhibitor, suggesting a role for PI3K-AKT activation downstream of MerTK contributing to the resistance of wtEGFR NSCLCs to irreversible EGFR TKIs. Taken together, our data provide rationale for a novel strategy for treatment of NSCLC with wtEGFR overexpression by combining MRX-2843 and an irreversible EGFR inhibitor.

#1083 Synergistic drug combination prediction through drug differential dependency network analysis. Seunghun Kim, Gil Speyer, Harshil Dhruv, Jeff Kiefer, Michael Berens. The Translational Genomics Research Institute, Phoenix, AZ.

In an effort to discover strategies which identify effective drug combinations, we analyzed 39 of the 480 compounds screened in the Cancer Therapeutics Response Portal (CTRP) where combinations of two compounds were tested against 860 cancer cell lines; this enabled a comparison of the drug sensitivity of the combinations versus that of the individual compounds. More than half of the drug combinations (n=21) did not significantly improve the drug sensitivity, compared to the individual compounds alone. In fact, some of the combinations showed reduced drug sensitivity. In EDDY-CTRP analysis, the Cancer Cell Line Encyclopedia (CCLE) RNAseq data and CTRP compound response measurements were analyzed to discover both 1) pathways enriched with differential dependencies between sensitive and non-sensitive cell lines for each compound and 2) the mediators of cell line response to a drug. A mediator is a gene in a pathway that plays a significantly different role between sensitive and non-sensitive conditions. The significance is assessed for either essentiality, measured as a node’s centrality change, or specificity, measured as the difference in condition specific edges. These drug-pathway-mediator connections are predicted to reveal crucial molecular determinants of drug sensitivity that otherwise are hidden in the complexities of the molecular networks of the cell (Speyer et al., PDB 22:497-508, 2017). We further investigated whether mediators identified for single compounds could predict sensitivity to drug combinations. This analysis revealed that if two single compounds share the same specific mediators, i.e. the genes with the most significant re-wiring of gene dependencies between sensitive and non-sensitive cell lines, combination of these two compounds correlate with improved sensitivity. The converse was also found: compounds that do not share mediators rarely show synergy. Further analysis of key nodes was indicative of relatively high influence of drug concentration. On the other hand, some herb remedies such as S. nigrum significantly antagonizes its cytotoxic activity against head and neck cancer cells.

#1084 Combination of eribulin and AURKA inhibitor prevents metastatic colonization and eradicates established metastases in breast cancer. Elena N. Puchacheva, Varvara Kozyreva, Anna Kiseleva, Ryan Ice, Brandon Jones, Yurii Loskutov. WWU Cancer Institute, Morgantown, WV.

The majority of cancer-related deaths (90%) are happened due to metastasis from the primary tumor site to distant organs. MLN8237 is a small, highly selective molecule inhibitor of Aurora A kinase (AURKA), which results in disruption of the mitotic spindle, chromosome segregation collapse, and inhibition of cell proliferation. Numerous studies have shown that levels of AURKA are elevated in many types of cancer, including breast cancer. Our previous studies indicated that MLN8237 is extremely potent against pulmonary metastasis, but not the primary tumor in orthotopic xenograft model. To further enhance MLN8237 based regimen, its combination with other therapeutic compounds can be used. As a potential partner we chose eribulin - fully synthetic macrocyclic analogue of the marine natural product halichondrin B. Eribulin belong to the class of non-taxane microtubule destabilizing molecules, currently used in clinic to treat taxol resistant metastatic breast cancer. In present study we have investigated the effect of MLN8237 and eribulin against breast cancer in vivo and in vitro. The results showed that combination of drugs possess a synergistic effect on both primary tumor and metastases, through inducing cytoxic autophagy and apoptosis. This data clearly indicate great potential behind the MLN8237 based therapies and introduce a new hope for the eradication of metastatic breast cancer.

#1085 Time dependent analysis for the combination of standardised Annona cherimola and Solanum nigrum extracts with 5-FU against head and neck cancer cells. Serag Eldin I. Elhebairi, 1 Aly F. Mohamed, 1 Nahlia G. Mohamed, 2 Mohammad I. Basuony, 3 Mahmoud M. Salem, 3 Metwally M. Montaser, 3 Mohammad Y. Alalfi, 4 Ayman M. Noreddin, 4 Ahmed M. Al-Abd 5. 1 Roswell Park Cancer Institute, Buffalo, NY; 2 Inova Dwight and Martha Cancer Institute, Falls Church, VA; 3 Egyptian Organization for Biological Products and Vaccines (VACSERA), Giza, Egypt; 4 Azar University, Cairo, Egypt; 5 King Khalid University, Abha, Saudi Arabia; 6 Chapman University School of Pharmacy, Irvine, CA; 7 National Research Centre of Egypt, Giza, Egypt.

Phytochemicals of natural origin constitues very initiative field of anticancer drug discovery. Annona sp. and Solanum nigrum are folk herbs with reported cytotoxic effects that can be used in the treatment of human cancers. Natural herbal medicine might not be potent anticancer remedies and are recommended for combination therapy. In the current work, we mathematically evaluated the influence of combining standardized extracts of A. cherimola and S. nigrum to the cytotoxic profile of 5-FU against head and neck cancer cells (Hep-2). Cytotoxicity assessment and combination analysis were calculated after MTT assays followed by fitting to Emax model and calculating the combination index (CI-value). After 24 h exposure both A. cherimola and S. nigrum extracts were more potent than 5-FU with I50 of 29.4±4.4, 7.3±1.3, and 3.4±0.4 µg/ml, respectively. Both A. cherimola and S. nigrum extracts significantly decreased the I50 to 5.2±0.55 and 7.6±0.85 µg/ml, respectively; however the CI-values were indicative of additive and antagonistic interactions, respectively (0.91 and 0.11, respectively). After 48 h exposure, the I50 of A. cherimola and S. nigrum extracts were 3.1±0.45, 0.87±0.02 and 7.2±0.65 µg/ml, respectively. Similarly, combination of A. cherimola and S. nigrum extracts with 5-FU showed additive and antagonistic interaction with CI-values of 1.05 and 3.37, respectively. After 72 h of exposure, the I50 of 5-FU, A. cherimola and S. nigrum extracts were 1.9±0.2, 2.1±0.24 and 2.1±0.1 µg/ml, respectively. Similarly, combination of A. cherimola and S. nigrum extracts with 5-FU showed additive and antagonistic interaction with CI-values of 1.2 and 2.21, respectively. Then, Ct model was used to assess the relative influence of drug concentration and exposure time on the cytotoxic profile of treatments under investigation. For S. nigrum and its combination with 5-FU, n-values were 0.9 and 0.63 which is indicative of relatively high influence of drug concentration. On the other hand, n-value for 5-FU treatment was 0.37; this indicates higher influence of drug exposure time compared to other treatment. The n-values of A. cherimola and its combination with 5-FU were 0.51 and 0.54, respectively; this indicate comparable influence of both drug concentration and exposure time. As a confirmatory measure, expression of the apoptotic gene, BAX, was increased after 5-FU combination with A. cherimola and decreased after combination with S. nigrum. In conclusion, some natural herb remedies such as A. cherimola might influence the cytotoxic profile of 5-FU and particularly after short exposure time; while on the other hand, some herb remedies such as S. nigrum significantly antagonizes its cytotoxic activity against head and neck cancer cells.

#1086 Vitamin D3 increases the response to cisplatin in bladder cancer through VDR and TAP3 pathway crosstalk. Brittany L. Bunch, 2 Donald Trump, 2 Candace S. Johnson1, Roswell Park Cancer Institute, Buffalo, NY; 3 Inova Dwight and Martha Cancer Institute, Falls Church, VA.

Several clinical trials have demonstrated that vitamin D (Vit D) is a biomarker to identify patients with poor response to cisplatin, and the lack of available biomarkers to identify these patients. Previous studies in our lab showed that pretreatment of preclinical bladder cancer models with 1,25 dihydroxyvitamin D3 (1,25D3), the active metabolite of vitamin D3, enhances the apoptotic response to cisplatin. Mechanistic understanding can provide insight into potential markers for response to 1,25D3, and cisplatin combination therapy in patients. Greater than 50% of MBCBs harbor p53 mutations; however p53 status has not been linked to clinical response to cisplatin. We previously determined that TAP3, a pro-apoptotic p53 family member protein induced after DNA damage, is upregulated after 1,25D3 and cisplatin treatment in bladder cancer cell lines with mutant p53, T24 and RT-112, we identified signalng crosstalk between the vitamin D receptor (VDR) and TAP3. Treatment with 1,25D3 and cisplatin, compared to either alone, results in greatest increase of TAP3 mRNA and protein, 2-fold (T24) and 15-fold (RT-112), as well as TAP3 transcriptional target BAX, 3-fold (T24), and 4-fold (RT-112). TAP3 and BAX induction is lost in cells transfected with VDR siRNA, indicating a requirement for VDR. Cells treated with 1,25D3 and cisplatin, compared to either alone, also show the greatest increase of VDR mRNA and protein, 3-fold (T24) and 4-fold (RT-112). CYP24A1, a VDR target gene, has the greatest induction after the combination treatment as well, with an increase of approx. 60-fold (T24), and

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100-fold (RT-112). CYP2A4 induction requires Tap 73, determined using Tap 73 shRNA. These data demonstrate that VDR signaling is enhanced by cisplatin treatment, and Tap 73 signaling is enhanced by 1,25D3. We expanded these findings in vivo by treating mice on a vitamin D3 deficient diet (1,000 IU) or a vitamin D3 deficient diet (25 IU) with 5 mg/kg of cisplatin (IP, qwk for 3 weeks). Initially, no differences with doses were observed, while in a second experiment with 2 different doses, we observed a benefit of cisplatin treatment by a 30% reduction in tumor volume. After the second cycle of treatment, tumors in the deficient mice stopped responding and returned to their initial tumor volume within 24 days. In the sufficient mice, tumors continued to respond to cisplatin with a maximum reduction of 50%. This was maintained for approximately 40 days after treatment. Further study will determine the molecular reason for the differential effect in vivo and data suggests that vitamin D3 with 1α (OH) D3 is important in and can increase the response to cisplatin, it also provides rationale for investigating Tap 73 protein levels and serum vitamin D3 levels as potential markers to determine patients’ likelihood of responding to cisplatin.

**#1087 CPX-351 works synergistically in combination with FLT3 inhibitors against AML with FLT3-ITD.** David K. Edwards,1 Nathalie Javidi-Sharifi,1 Angela Roelty,2 Max Gordon,1 Riley Roth-Carter,1 Paul Tardi,2 Lawrence Mayer,3 Jeffrey W. Tyner1. 1Oregan Health & Science University, Portland, OR; 2Jazz Pharmaceuticals, Vancouver, British Columbia, Canada

Introduction: CPX-351 (Vyxeos) is a liposomal combination of cytarabine and daunorubicin at a synergistic 5:1 molar ratio. Recently, CPX-351 has been shown to be significantly more effective than the 7+3 standard of care chemotherapy in treating high-risk AML patients, including patients with FLT3-ITD or M1 mutations. We recently treated primary patient samples with CPX-351 in vivo and found that FLT3-ITD+ samples were significantly more sensitive to CPX-351 and showed enhanced drug uptake. We hypothesized that dysregulated FLT3 signaling results in an activation of liposome uptake pathways, leading to increased sensitivity to CPX-351 and ultimately cell death. Furthermore, we examined the effect of combining CPX-351 with existing FLT3 inhibitors (e.g. quizartinib and midostaurin). Methods: To examine drug uptake dynamics, we exposed AML cell lines (including MOLM-13 and MOLM-14 that contain FLT3-ITD, and ME1 that contains an activating FLT3 mutation) to varying concentrations of CPX-351, with or without pre-treatment of quizartinib for 16 hrs produced a population of cells (approximately 50% of the total population) that exhibited decreased daunorubicin fluorescence, suggesting that prolonged FLT3 inhibition may decrease CPX-351 uptake. Consistent with this, we observed robust synergy when combining CPX-351 with FLT3 inhibitors simultaneously or with CPX-351 exposure scheduled 24 hours prior to FLT3 inhibitor exposure. However, exposure to FLT3 inhibitors 24 hours prior to CPX-351 administration was synergistic and even antagonistic at certain doses. Conclusions: These data provide additional supportive evidence that FLT3 activation results in an increased uptake of CPX-351. This is consistent with results from the CPX-351 Phase III trial in which FLT3-ITD+ patients survived significantly longer when treated with CPX-351 compared to 7+3 chemotherapy. We also show that combining CPX-351 with existing FLT3 inhibitors can elicit a synergistic response when administered in dosing regimens where FLT3 inhibition does not precede CPX-351 treatment. Cumulatively, our data support further testing of CPX-351 in combination with FLT3 inhibitors for treating AML patients with genetic dysregulation of FLT3 signaling.

**#1088 Metronomic therapy of doxorubicin and molecular iodine in canine mammary cancer.** Xochitl Zambrano Estrada,1 Brianda Landaverde,2 Andrés A. Dueñas,3 Marco A. De Paz,2 Gerardo A. Hernández,2 Benjamin Solorio,3 Manuel Trejo,1 Laura Pérez,2 Carmen Aceves1. 1Instituto de Neurobiología, Ju- riquilla, México, Mexico; 2Facultad de Estudios Superiores Cuautitlán, Estado de México, Mexico; 3Facultad de Ciencias Naturales, Juriquilla, México, Mexico

Introduction: Conventional chemotherapy is administered at the maximal tolerated dose (MTD), usually every 21 days, and the drug-free period allows the patient to recover from the adverse effects secondary to the treatment. However, this period also enables tumors to reintegrate growth through induced tumoral revascularization. In recent years, a new strategy known as metronomic chemotherapy explored the scheme of long-lasting low-dose administration of antineoplastic drugs, which results in the attenuation or interruption of tumor growth by inhibition of angiogenesis and/or reactivation of anti-tumoral immune system. Moreover, the use of combination therapies including cell re-differentiation messengers are currently being tested to provide a better quality of life. In this sense, it has been demonstrated that the supplement of molecular iodine (I2) with cytotoxic agents like doxorubicin (DOX) promotes a cytotoxic effect and generates cardioprotection. In this study, canine mammary cancer is used as a model of spontaneous carcinogenesis. Objective: Determine the efficacy of metronomic therapy of DOX in combination with I2 analyzing the clinical, pathological, and molecular effects involved in proliferation, invasion, and chemoresistance. Methods: 27 females with mammary cancer were given daily I2 (110 mg) supplements or placebo (colored water) in conjunction with four cycles of DOX (30 mg/m2, intravenous). Two protocols were carried out: the standard protocol with a 21-day interval and 20-minute infusions, and the metronomic protocol with a 15-day interval and 60-minute infusions (DOX-M). Results: The metronomic scheme (DOX-M) attenuated the severity of side effects, but it showed no difference in tumor response (size, gene expression). The I2 supplement significantly improved the quality of life in both schemes, and it exerted synergistic effects on the tumor response in DOX-M, decreasing the epithelial component as well as markers of chemoresistance (MDR1 and Survivin) and invasion (uPA). The antitumor immune response (lymphocytic infiltration) in this scheme was significantly activated, and all these responses were accompanied by a reduction in proliferation and/or anti-apoptotic (anti-Bax and anti-Bcl-2) and angiogenic (VEGF and MMP) expression. Endocrine evaluation showed that I2 supplements do not alter thyroid function or estrogen response. Conclusions: Combination of DOX + I2 in a metronomic scheme exerts synergistic effects that prevent drug resistance and invasion and greatly improve the patient’s quality of life. Studies are underway to decrease the dose of DOX. Key words: Molecular Iodine, Doxorubicin, Canine Mammary Cancer, Metronomic Therapy. The authors appreciate the technical support of Alexander Bontempo, Evangeline Delgado and Maria Juana Cardenas. Investigation supported by PA-IIIT-UNAM IN200813 and 201516; CONACYT 245255.

**#1089 Anti-tumor activity of selinexor is enhanced by palbociclib in preclinical models of HER2+ breast cancer.** Hua Chang, Sharon Friedlander, Trinayan Kashyap, Boris Klebanov, Christian Argueta, Oscar A. Gonzalez, Erkan Baloglu, Yosef Landesman, Sharon Shacham, Margaret Lee, William Senneped, Karyopharm Therapeutics, Inc., Newton, MA

Introduction: XPO1 (exportin-1/CRM1) inhibitor selinexor (KPT-330) is the first-in-class, orally bioavailable, clinical stage SINE (Selective Inhibitor of Nuclear Export) compound with marked anti-tumor activity towards solid and hematological malignancies. This activity can be further enhanced by other therapeutic agents. We have previously shown strong synergistic preclinical activity of KPT-330 in trastuzumab and cell line-specific models of acquired resistance to HER2-targeted therapies, G1/S phase cell cycle regulators Cyclin D1 and CDK4/6 are inappropriately activated. We therefore investigated the combinatorial effect of selinexor plus palbociclib, a CDK4/6 inhibitor, in HER2+ breast cancer models as a treatment option for recurrent and relapsed HER2+ breast cancers. Methods: The effects of selinexor or palbociclib single agents or in combination were tested in vitro with BT474 HER2+ breast cancer cell line. Total RNA and protein was extracted from cell lysates and analyzed by qPCR and immunoblots. In vivo, a subcutaneous BT474 xenograft mouse model was treated with selinexor (5 mg/kg or 15 mg/kg; qodx3) or palbociclib (50 mg/kg or 150 mg/kg; qd) single agents or in combination. Tumor growth and body weights were monitored for 60 days. Tumors were harvested and analyzed by immunohistochemistry (IHC). Results: Selinexor plus palbociclib was highly effective in vitro and in vivo in BT474 breast cancer cells. In in vitro assays, selinexor or palbociclib single agents demonstrated inhibitory effects on cell proliferation and showed strong synergy when combined. In vivo, the combination resulted in significant survival benefit and enhanced tumor growth inhibition compared to vehicle or either single agent. IHC analysis of xenograft tumors showed synergistic inhibition of cell proliferation by selinexor plus palbociclib. The Ki67 proliferation index determined by IHC was 25% for vehicle control, 20% for selinexor, 7% for palbociclib and 2% for the combination. Based on IHC analysis, the synergistic anti-tumor activity of selinexor plus palbociclib was achieved at multiple levels of the CDK4/6 pathway. Selinexor treatment increased p21, p27 and Rb nuclear staining. Both p21 and p27 are inhibitors of CDK4/6 while Rb mediates cell cycle progression, CDK4/6 phosphorylates and inactivates Rb, which allows cell cycle progression. As well as palbociclib treated samples, phosphorylated Rb in the nucleus decreased, indicating a down-regulation of the CDK4/6 pathway. Conclusion: Selinexor plus palbociclib shows synergistic inhibition of cell proliferation in vivo

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and in vitro in HER2+ breast cancer cell line BT474 by down-regulation of CDK4/6 pathway. This combination therapy warrants further investigation as an effective treatment option for recurrent and relapsed HER2+ breast cancer.

**#1090** Preclinical evaluation of combination therapy with dasatinib and sorafenib for the treatment of brain metastases. Qingsyu Stephanie Zhou, Xiaofang Guo. Univ. of South Florida College of Pharmacy, Tampa, FL.

Brain metastases, the most common type of intracranial tumors in adults, carry a dismal prognosis with a median survival of less than 18 months regardless of primary status or treatment given. As part of an ongoing project, this study is concerned with the preclinical evaluation of targeted combination therapy for the treatment of brain metastases. The cell line used in this study was the H1915 (ATCC® CRL-5904®) human lung cancer cell line, which was originally derived from a brain metastasis from primary lung adenocarcinoma. The cell line was stably transfected with a plasmid that allows expression of firefly luciferase (i.e., H1915-luc) so that the tumor growth in a mouse orthotopic model of brain metastases can be assessed in real time using the Xenogen in vivo bioluminescence imaging (BLI). Our previous study has demonstrated that the synergistic effect of combined dasatinib and sorafenib treatment was cell-line dependent. In this study, the cytotoxicity of dasatinib and sorafenib, when used alone or in combination at the fixed molar ratios of 3:1, 1:1 and 1:3 (dasatinib:sorafenib), was further examined in H1915-luc cells using the MTT assay. The results demonstrated that simultaneous and continuous exposure of H1915-luc cells to dasatinib and sorafenib at the fixed concentration ratio of 1:1 and 1:3 for 72 h showed synergism (the combination index value between 0.7 and 0.8) at effect levels of 40 and 80% inhibition of cell viability. For the in vivo study, an orthotopic mouse model of brain metastases was established by injecting H1915-luc cells into the right general carotid artery of individual athymic nude mice followed by permanent ligation. The preliminary study showed that following the intracarotid injection of H1915-luc cells, brain metastases were formed and readily to be detected by BLI. The obtained whole brain samples were fixed in OCT and stored at −80°C. The brain sections are to be subjected to the immunofluorescence double staining of CD31 and firefly luciferase to assess the potential effect of the dasatinib-sorafenib combination on microscopic brain metastasis formation and progression and vessel co-option. Given the fact that brain metastases usually have a poor response to chemotherapeutic agents in part due to the presence of blood-brain barrier, a pharmacokinetic study is underway to characterize the distribution of sorafenib and dasatinib in normal brain tissues and brain metastases using the serial blood sampling and brain microdialysis technique. Overall, results from the in vitro cytotoxicity study demonstrated the synergistic effect of dasatinib and sorafenib on H1915-luc human lung cancer cells, providing a rationale for the in vivo evaluation the potential of this combination for the treatment of brain metastases from lung carcinomas.

**#1091** Paclitaxel-loaded microparticles in combination with gemcitabine-loaded microparticles decreases gemcitabine resistance and promotes cell death in pancreatic cancer cell lines. Maria Munoz-Sagastibelza, Ariella Moshchinsky, Oluwafeyemi Okome, Jenny E. Paredes Sanchez, Raavi Gupta, Laura Martello-Rooney. SUNY Downstate Medical Ctr., Brooklyn, NY.

Pancreatic cancer is the fourth leading cause of cancer death in the United States with only 7% of diagnosed patients surviving 5 years. Current systemic chemotherapies have not been very effective at decreasing tumor burden, but nonetheless expose patients to the adverse side effects of treatment. Poly(lactic-co-glycolic acid)-based microparticles (MPs) are a promising tool for localized therapy and additionally reduced invasiveness of tumors similar to PF562271 phosphorylation in tumor cells. For the purification of glioma cells from total tumor tissue Percoll gradients were used. The study revealed that treatment with PF562271 reduced invasion of glioma cells at the tumor edge, while TMZ reduced the tumor growth. Combined treatment showed significantly more prominent effect on reduction of tumors growth compared to TMZ monotherapy and additionally reduced invasiveness of tumors similar to PF562271 monotherapy. In both PF562271 monotherapy and combined treatments the downregulation of Pyk2 phosphorylation in glioma cells has been recorded. Survival analysis demonstrated a significant increase of survival of animals received combined treatment compared to TMZ monotherapy. In conclusion, we can state that combined treatment with TMZ together with PF562271 reduced Pyk2-related tumor growth and invasiveness and increased animal survival. This result was made possible by NIH grant numbers: 1SC2GM102040-01, G12MD007583, R25GM110513, Puerto Rico Science, Technology, and Research Trust grant number 2016-00157, and US Department of Education grant number P0315130068.

**#1092** PF562271, a Pyk2 inhibitor, reduces glioma tumor growth and invasion in C57Bl6/Gl261 mouse glioma model. Jesecila Ortiz-Rivera, Kimberly Rollon-Reyes, Alejandro Albers, Luis Cabano, Lilia Kucheryavikh. 1Universidad Central del Caribe, Bayamon, PR; 2University of Puerto Rico, Piedras, PR.

Glioblastoma is an extraordinarily aggressive type of brain cancer due to its invasive and proliferative nature. The tumor microenvironment, with microglia as a critical player, has an important role in tumor progression. Microglia infiltrate majority of gliomas and release factors, which favor tumor growth and invasion. Previously we demonstrated that microglia Pyk2-related tumor growth and invasiveness and increased animal survival. The described drug delivery method with systemic gemcitabine and locally loaded microparticles decreases gemcitabine resistance and promotes cell death in pancreatic cancer cell lines. In addition, preliminary drug release studies with GMPs demonstrated detection of gemcitabine up to 14 days. In conclusion, our data demonstrated that GMPs and GMPs promote an increase in cancer cell death. Furthermore, the combination of the drug-loaded MPs indicated a reduction in drug resistance and clear impairment in colony formation. Further studies are in progress to investigate combination MPs injections in a mouse model of pancreatic cancer to confirm in vivo efficacy. The described drug delivery method has the potential to be a more efficient local treatment modality than systemic drug against pancreatic cancer.

**#1093** Synergistic action of sorafenib and carfilzomib against hepatocellular carcinoma in vitro and in vivo. Chao Jiang, Rui Xu, Xiao-Xing Li, Hui-Yun Wang, X.F. Steven Zheng. 1Sun Yat-sen University Cancer Center, Guangzhou, China; 2Cancer Center of Guangzhou Medical University, Guangzhou, China.

Background: Sorafenib, a multikinase inhibitor, is currently the only approved systemic drug for advanced hepatocellular carcinoma (HCC), but has demonstrated limited survival benefits for patients. Carfilzomib (CFZ) is a second-generation proteasome inhibitor approved for the treatment of multiple myeloma. In this study, we aim to investigate the synergistic antitumor effect of the combined treatment of sorafenib and CFZ in hepatocellular carcinoma and the underlying molecular mechanisms. Methods: Hep3B and Bel-7402 HCC cell lines were treated with sorafenib and/or CFZ at clinically relevant concentrations, after which effects on cell viability and proliferation were analyzed using MTS method; cell migration and invasion were evaluated in transwell assay; cell apoptosis were monitored by flow cytometry. Western blot assay was used to investigate the potential molecular mechanism of the synergistic antitumor effect of the combined treatment. In vivo efficacy was determined in nude mice with Hep3B xenografts. Results: MTS results showed that CFZ could improve
the drug sensitivities of sorafenib in Hep3B and Bel-7402 cells when combined these two drugs together. Moreover, cell proliferation was more significantly suppressed in combinational treatment compared to single agent (p < 0.01) in the two tested cell lines. The colony formation assay showed similar results with the MTS assay. The results from flow cytometry showed that sorafenib or CFZ alone induced about 76% and 27% apoptosis in Hep3B and Bel-7402 cell lines, respectively. The combination of these agents significantly increased the apoptosis rate up to 20.78% (p < 0.01). Similar results were observed in Bel-7402. Results of transwell assay demonstrated that cell migration and invasion ability were inhibited when treated with sorafenib or CFZ alone; and cells treated with the combinational therapy migrated and invaded even less (p < 0.05). The in vivo xenograft tumor model showed that treatment with sorafenib or CFZ inhibited Hep3B tumor growth (p < 0.05); moreover, treatment with the combination more significantly inhibited tumor growth (p < 0.01). Mechanistically, combined treatment of the two drugs suppressed the epithelial-mesenchymal transition process by increasing E-cadherin and decreasing N-cadherin and β-catenin. Furthermore, the drug combination activated the mitochondria-related apoptosis pathway, showing up-regulation of cleaved caspase 3/7/9. Up-regulations of PERK/eIF2α/ATF4/CHOP pathway implicated the involvement of ER stress-mediated apoptotic pathway following the drug combination treatment. Conclusion: In conclusion, these findings suggest that the combination of sorafenib and CFZ shows synergistic anti-tumor activities in HCC, potentially providing a novel therapeutic strategy for patients with advanced hepatocellular carcinoma.

#1094 Combining the pan-FGFR inhibitor AZD4547 with radiation in lung and head and neck squamous cell carcinoma. Andrew M. Baschmargel, Chunrong Li, Alecia M. Morgan, Sean R. Brennan, Kalley A. Russo, Paul M. Harari. Univ. of Wisconsin School of Medicine, Madison, WI.

The fibroblast growth factor receptors (FGFR1, FGFR2, FGFR3, FGFR4) are frequently altered or overexpressed in both lung and head and neck squamous cell carcinomas (SCC). Amplification of FGFR1 has been reported to be as high as 22% in lung SCC and 17% in head and neck SCC (HNSCC). FGFRs have been identified as a potential therapeutic target in lung and head and neck cancers. In addition, FGFRs activate multiple pathways involved in the radiation response including RAS/RAF/MAPK, PI3K/AKT and STAT. AZD4547 is a potent and selective tyrosine kinase inhibitor of the FGFR family and is currently being investigated in clinical trials. Since a substantial portion of lung cancer and HNSCC patients are treated with radiotherapy, we examined the anti-tumor effects of AZD4547 in combination with radiation in both lung and head and neck cancer model systems. FGFR protein and RNA expression and cell proliferation with AZD4547 were evaluated in 8 lung and 8 head and neck cancer cell lines. Clonogenic survival assays were performed on 4 lung and 4 head and neck cancer cell lines and xenograft experiments were performed on one HNSCC cell line. These cell lines demonstrated varying levels of FGFR1, 2 and 3 protein and RNA expression. The half maximal inhibitory concentration (IC50) of AZD4547 in these cell lines ranged from 0.05 to 128 μM. Sensitivity to AZD4547 did not correlate directly with protein or RNA expression. In vitro clonogenic survival assays showed limited effects of combining AZD4547 with radiation. However, significant tumor growth delay was observed with the combination of radiation with AZD4547 compared to radiation alone in the SCC1483 FGFR2 expressing HNSCC xenograft model. These findings suggest that AZD4547 can augment the response of radiation in an in vivo model system. Further studies are underway to test these findings in additional cell lines including FGFR dependent amplified cancer cell lines.

#1095 A combination of suberoylanilide hydroxamic acid and quinacrine is an effective therapeutic approach in preclinical settings of upper gastrointestinal cancers. Shoumin Zhu, Wael El-Rifai. Surgery, Nashville, TN.

Background: Quinacrine (QC), an antimalarial drug, has been shown to possess antitumor effects. Suberoylanilide hydroxamic acid (SAHA) inhibits class I and class II HDACs and is approved for cancer therapy. Developing novel approaches to overcome cancer drug resistance could significantly enhance current therapeutic approaches and improve patient care. Methods: ATP-GLO clonogenic survival, Annexin-V apoptosis assay, comet assay and DNA double-strand breaks (DSB) kits were used. The mRNA and protein levels were evaluated by quantitative real-time PCR and Western blot analyses. Results: A combination of QC/SAHAsignificantlyincreasedcelldeathinallcancercelllinesandhadnoeffectonimmortalizednon-cancercelllines(HEF145,NIH-3T3andEPC2) (P > 0.01). Clonogenic survival assay indicated that QC/SAHAs co-treatment led to significantly lower number of cancer cell colonies, as compared to single agents and controls (P < 0.01). Of note, the QC/SAHAs combination led to an increase in the the sub-G0 population in AGS (9-fold), MKN-28 (14-fold), FLOI (5.6-fold), and SNU1 (4-fold) cells (P < 0.01). These results were confirmed using the Annexin V apoptosis induced significantly higher levels of apoptosis (10 - 20 fold) as compared to single agent and control (P < 0.01). Treatment with QC/SAHAS combination induced high levels of DSB (>20 fold, P < 0.01). Comet assay data showed increased DNA damage compared with vehicle-treated cells (8-fold, P < 0.01). Western blot analysis demonstrated a substantial increase in the expression of pro-apoptotic proteins (BAX and BAK) and a decrease in the expression of anti-apoptotic proteins (BCL2 and BCL-XL). Conclusion: The QC/SAHAs co-treatment in all cancer cell lines. Interestingly, the combination of QC/SAHAs substantially decreased the protein levels of both wtP53 and mutP53 in these cells. Tumor xenograft data confirmed that a combination of QC/SAHAs is more effective than a single agent in abrogating tumor growth (P < 0.05). Conclusion: Our novel findings show that QC and SAHA have a synergistic effect that provide consistent findings that increased DNA damage mediates the cytotoxic effect of combined QC/SAHAs. Such effect is likely related to mutP53 and wtP53 protein degradation induced by QC/SAHAs combination. Our findings provide a rationale for a clinical evaluation of combined QC/SAHAs therapy in gastro-esophageal cancers. Ongoing studies are currently being undertaken to understand mechanisms that regulate the degradation of wtP53 and mutP53 proteins.

#1096 Co-targeting of AKT and Pim kinases in mouse PTEN-deficient prostate cancer. Marco A. De Velasco,1 Koichi Sugimoto,2 Yuriue Kura,3 Naomi Ando,1 Noriko Sato,1 Kazuko Sakai,1 Barry R. Davies,2 Dennis Huszar,3 Masa-hiro Nozawa,2 Kazuhiro Yoshimura,2 Kazuhiro Yoshikawa,3 Kazuto Nishio,3 Hirotsugu Uemura1.1Kindai University Faculty of Medicine, Osaka-Sayama, Japan; 2AstraZeneca, Macclesfield, United Kingdom; 3Aichi Medical University, Nagakute, Japan.

AKT and Pim kinases modulate programmed cell death by the phosphorylation of common substrates that regulate apoptosis and other survival processes. Evidence suggests that the antitumor effects of targeted Akt inhibition can be inhibited or diminished by the JAK/STAT-dependent induction of Pim kinases. In this study we examined the therapeutic potential of co-targeting AKT and Pim in a genetically engineered mouse model of prostate cancer driven by the conditional inactivation of PTEN. The antitumor effects of AZD3563, a pan AKT inhibitor, and AZD1208, a highly potent Pim kinase inhibitor, were investigated as monotherapy or in combination on mice harboring castration-naïve prostate tumors and mice that developed castration-resistant disease. Mice were randomized treated for four weeks. Safety and tolerability was assessed by body-weight changes. Antitumor activity was determined by differences in tumor burden, proliferation and apoptosis and histology. Molecular activity was assessed by examining the phosphorylation of common substrates by western blot analysis. Treatments were well-tolerated and no significant differences in body-weight changes were observed. In castration-naïve prostate tumors, treatments with AZD3563, AZD1208 and AZD3563/AZD1208 resulted in 11.9%, 13.5% and 30.9% reductions of tumor burden compared to vehicle treated controls, respectively, P < 0.001. The treatment combination of AZD3563/AZD1208 demonstrated significant antitumor activity compared to monotherapy, P < 0.001. In the castration-resistant tumors, treatments with AZD3563, AZD1208 and AZD3563/AZD1208 resulted in 21%, 9.5% and 27% reductions of tumor burden compared to vehicle treated controls, respectively, P < 0.004. Although differences in tumor burden were not statistically significant compared to vehicle treated controls, a combination of monotherapy and combination therapy, a notable degree of tumor gland regression was observed in tumors treated with AZD3563/AZD1208. Overall, combination therapy showed a synergistic effect by inhibited or impaired phosphorylation of PRAS40, elf4B and BAD in both castration-naïve and castration-resistant tumor models. Inhibition of both pathways enhanced the reduction of tumor cell proliferation and increased apoptosis. Overall, our findings provide in vivo data to support redundancy between AKT/Pim survival pathways and suggest that a therapeutic approach of combined AKT/Pim kinase inhibition may be possible therapeutic approach for AKT-driven prostate cancer.

#1097 Enhancing the efficacy of Temozolomide with Biochanin A: Mechanistic and metabolic effects. Vilas Desai,1 Aditi Jain,2 Maryna Perereplyuk,3 Annie Chibun,3 Christopher Farrell,3 James C. K. Li,4 Alok Bhushan1.1Thomas Jefferson University, Philadelphia, PA; 2University of Kentucky, Lexington, KY; 3Idaho State University, Pocatello, ID.

Enhancing the efficacy of Temozolomide with Biochanin A: Mechanistic and Metabolic effects Glioblastoma multiforme (GBM) is the most frequent, primary malignant brain tumor prevalent in humans. GBM is characterized by aggressive cell proliferation and rapid invasion of normal brain tissue resulting in poor patient prognosis. Furthermore, the current standard therapy of surgical resection followed by radiotherapy and chemotherapy with Temozolomide is not very effective. The inefficacy of the currently available chemotherapeutic agents for GBM can be attributed to the challenges of drug delivery to the tumor.
EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Combination Therapy

Our laboratory has previously reported the anticancer potential of isoflavones like Biochanin A against oral, pancreatic, breast and brain cancers. In this study, we tested our hypothesis that Biochanin A sensitizes the GBM cells to Temozolomide, thereby increasing its efficacy in combination treatment. We used the MTT assay to determine the effects of Biochanin A alone and in combination with Temozolomide on survival of U-87 MG cells. Furthermore, two different induction of apoptosis with combination treatment as compared to the drugs alone. Western-blot analysis and Immunofluorescence imaging methods were used to elucidate some of the cell survival/proliferation mechanisms involving EGFR, p-AKT, MMP-2, MT-MMP1. The effects of the drugs on U-87 MG cell metabolism (parameters like ECAR & OCR) studied using Seahorse XF analyser, suggest an increase in the mitochondrial respiration. Our results support our hypothesis and the data shows that it may have implications in increasing the efficacy of the conventional treatments for GBM. These results will potentially aid in designing new therapies that can improve the outcome of GBM.

#1098 Delta-tocotrienol chemosensitizes human pancreatic tumor metastasis to gemicitabine targeting cancer stem cells. Kazim Hüsain, Said M. Sebti, Mokeng L. Ajila. Moffitt Cancer Center, Tampa, FL.

Background: Pancreatic cancer, a lethal malignancy is the fourth leading cause of cancer-related deaths in the United States. The standard chemotherapy with gemcitabine is ineffective in patients with metastatic tumors. Pancreatic cancer stem cells (CSCs) have been implicated in the development of pancreatic cancer metastasis, resistance to chemotherapy and its recurrence following surgical extirpation. We have shown that natural vitamin E δ-tocotrienol is the most bioactive tocotrienols against pancreatic cancer in vitro as well as in vivo models. The purpose of this study was to evaluate the chemosensitization of pancreatic tumor metastasis by δ-tocotrienol to gemcitabine in vitro as well as in vivo. Methods: In vitro human metastatic pancreatic cancer cells L3.6pl and pancreatic cancer stem cells (PCSC) were treated with gemcitabine (5 μM) and δ-tocotrienol (50 μM) alone and in combination. Treated cells used for epithelial to mesenchymal transition (EMT), migration/invasion, microsphere/spheroid, and signaling markers assays. PCSC expressing luciferase was orthotopically implanted into pancreas of Athymic nude mice (n = 20) and after one week they were randomized into four groups: 1) vehicle control (ethanol extracted olive oil), 2) gemcitabine (100 mg/kg, IP, twice a week), 3) δ-tocotrienol (200 mg/kg, orally twice a day) and 4) gemcitabine + δ-tocotrienol. The treatment was continued for 4 weeks. The tumor volume, tumor weight, metastasis were recorded. Results: Gemcitabine slightly inhibited the growth, migration/invasion of L3.6pl and PCSC in vitro and PCSC tumor growth (tumor volume and weight) in vivo. δ-tocotrienol significantly inhibited the growth, microsphere/spheroid, migration/invasion, EMT (E-cadherin to vimentin), angiogenesis (VEGF, PCSC transcription factors like VEGF, HIF-1 and HIF-2). Tocotrienol did not affect the microsphere and liver/lung metastasis compared to control. The combination of both drugs synergistically inhibited the cancer and stem cell growth, EMT, migration/invasion, microsphere/spheroid, angiogenesis, and PCSC transcription factors in vitro as well as in vivo. Conclusion: Vitamin E δ-tocotrienol chemosensitizes human pancreatic tumor metastasis to gemcitabine through inhibition of EMT, migration, invasion, angiogenesis, cancer stem cell self-renewal, tumor growth and metastasis.


Introduction Docetaxel combined with castration provides a major survival benefit in newly diagnosed metastatic PCa. Conversely, docetaxel without castration in high-risk localized PCa is not active. Cabazitaxel is a new taxane retaining its activity in tumors resistant to docetaxel and new hormonal therapies. We aimed at better characterizing in vivo the relationship between cabazitaxel and circulating androgens in a preclinical human-derived xenograft model of castration-resistant prostate cancer (CRPC). Material and methods: Athymic nude mice were inoculated with the androgen receptor wt, PSA secreting CRPC cell line PC346C-DCC-K. Mice were surgically castrated when tumors were established (tumor volume (TV) of 150 mm³). After 7 days, mice were random-ized to receive testosterone (40 mg) or empty pellet. The following day mice were injected with one bolus injection of cabazitaxel (33 mg/kg) or NaCl intraperito-neally. Mice were sacrificed when tumors exceeded a volume of 1,500 mm³ or the maximum follow-up of 90 days after cabazitaxel treatment. Blood was sampled biweekly for PSA and testosterone and analyzed by an immunassay. In a second in vivo–complete antitumor response. In contrast, in mice supplemented with testosterone the anti-tumor effect of cabazitaxel was not significa-tively different from placebo (median time till TV 1,500 mm³ 48 and 45 days resp.). Importantly, testosterone supplementation alone did not significantly affect tumor growth, confirming the CRPC nature of the (PC346C-DCC-K) xenograft. Interestingly, in tumors of testosterone supplemented castrated mice the intratumoral cabazitaxel concentrations were significantly decreased compared to mice that did not receive testosterone (0.39 ng cabazitaxel per mg tumor tissue vs. 1.36 ng/mg, t-test p = 0.0032). Conclusions These findings indicate that circulating testosterone significantly impairs the efficacy of cabazitaxel. Testoster-one supplementation may alter the metabolism of cabazitaxel, or may interfere with uptake and/or accumulation of cabazitaxel in PCa cells. We will further investigate the relationship between circulating androgens and intratumoral cabazitaxel accumulation as well as anti-tumor efficacy. The study was sup-ported by a research grant from Sanofi.

#1100 Synergistic interaction between gemicitabine and PD-0325901 in C-33A cervical carcinoma cell line. Macarena Irigoyen, Gonzalo Castillo. BIOPHARMA, LLC., Bellevue, WA.

Cervical cancer affects over half a million women worldwide, and is one of the most common causes of death in women. Treatment of cervical cancers consists of surgery, radiation, chemotherapy, or combination of therapies. However, about half of patients with advanced cancer will show recurrent tumors. Signalng cascades are often dysregulated in human cancer cells making them attractive targets for combination drug therapies. PD-0325901, an inhibitor of the mito-gen-activated protein kinase, is a promising target for metastatic cancer. An-other drug used to treat cervical carcinoma is gemcitabine, a nucleoside analog inhibitor of the ribonucleotide reductase and DNA damage inducible protein 45a (Gadd45a). This drug is generally used in combination with other drugs, after reoccurrence of the disease state. We decided to evaluate the efficacy of gemcitabine in combination with PD-0325901 in C-33A, a metastatic cervical cancer-derived cell line. We evaluated the potency of gemcitabine and PD-0325901 alone and in combination. Cell viability after incubation with the drugs was determined at 72 hours and at 7 days by measuring ATP content. Synergy was determined assessed utilizing BIOPHARMA’s suite of drug combination analysis tools including: isobologram, combination index (CI) and Bliss analysis. Isobologram analysis at 72 hours of incubation revealed that the mean values of the observed data were significantly smaller than those of the predicted maximum. These results indicated a synergistic effect at concentrations between 2 - 200 nM PD-0325901 and 0.3 - 3 nM gemcitabine. Similar patterns of synergy were observed with 7 day incubations of C-33A with gemcitabine and PD-0325901. These results indicate that gemcitabine and PD-0325901 showed significantly more growth inhibition than either drug alone in the C-33A tumor cell line, suggesting that the combination of gemcitabine - PD-0325901 could be used in cervical cancer therapy.


Background/Aims: Acute myeloid leukemia (AML) is an aggressive cancer with limited treatment options outside of chemotherapy. Improved ther-apyes with novel mechanisms of action are desperately needed to fill this need. Both hTERT, the catalytic subunit of telomerase, and BCL-2, an apo-ptic regulator, are overexpressed in AML, correlating with disease severity and poor prognosis respectively. Imetelast is a novel, first-in-class competitive inhibitor of telomerase with clinical activity in hematologic malignan-cies. Venetoclax, an approved BCL-2 inhibitor for CLL, has shown a prom-ising clinical benefit in AML patients. Preclinical evidence shows that

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downregulation of hTERT induces apoptosis via disruptions of hTERT and BCL-2 interaction; we hypothesize that inhibiting both targets would yield greater anti-tumor activity in AML compared to treatment with either agent alone. Methods: AML cell lines and AML patient's PBMC samples were treated with imetelstat or venetoclax alone, or in combination, and viable and apoptotic populations of cells were evaluated by dual color flow cytometry. The combination of autologous imetelstat with novel theranostic agent OBP-401 and chemotherapeutic agent paclitaxel (PTX) has been shown to have therapeutic effects against ovarian cancer with peritoneal metastasis. However, the effective treatment option to overcome peritoneal metastasis of SGC has not been developed yet, although i.p. administration of some chemotherapeutic agents has been attempted to SGC. Therefore, a novel therapeutical strategy is required for the treatment of peritoneal metastasis. Methods: We previously developed a telomerase-dependent replication-selective adenovirus OBP-401 (TelomeScan), which can replicate within the tumor cells selectively and express green fluorescent protein (GFP). Moreover, OBP-401 infection also induces tumor-specific cell death in monotherapy or combination therapy with chemotherapy. In this study, we assessed synergistic effects in combination with novel theranostic agent OBP-401 and chemotherapeutic agent paclitaxel (PTX) using human SGC cell lines (GCIY and KATO III). In vitro experiment, SGC cells and normal human lung fibroblast (NHFL) cells were infected with OBP-401 at various doses. PTX was administered after 48 hours of virus infection. Twenty-four hours after PTX administration, we evaluated the anti-tumor effect by XTT assay, and analyzed the synergistic effect by CalcuSyn Software. Results: SGC cells were visualized as GFP-positive cells selectively by the infection of OBP-401, whereas NHFL cells were not visualized with OBP-401. In both SGC cell lines, the suppression of cell viability dose-dependently by administration of OBP-401 or PTX alone was confirmed. OBP-401 synergistically suppressed the viability of SGC cells in combination with PTX as compared to monotherapy; Conclusions: These results suggest that OBP-401 has a promising potential to treat peritoneal micrometastasis of SGC intraoperatively and combination of OBP-401 and PTX would be a novel theranostic strategy for the treatment of peritoneal metastasis of SGC.

**#1104 Novel specific RTK targeting of EGFR/FAK axis in glioblastoma invasion.** Raghupathy Vengoji, Satyanarayana Rachaganii, Suprit Gupta, Kavita Mallya, Maneesh Jain, Moorthy Ponnussamy, Surinder Batra, Nicole Shonka. University of Nebraska Medical Center, Omaha, NE

Background: Glioblastomas (GBM) is the most aggressive primary brain tumor with a median survival of 14.6 months. Currently, the first-line treatment includes surgical resection, chemoradiation, and adjuvant therapy with temozolomide. However, GBM recurs most often within 6.9 months. Receptor tyrosine kinases are dysregulated in GBM, with epidermal growth factor receptor (EGFR) representing 57.4% of the deleted/mutated GBM. In addition, 30 – 40% of GBM patients with EGFR amplification carry an oncogenic gene rearrangement EGFR variant III (EGFRVIII) which is constitutively active. Most EGFR inhibitors have shown very little clinical efficacy in GBM. Methods: Afatinib, brain-brain barrier penetrating pan-EGFR inhibitor, covalently binds and irreversibly inhibits signaling from EGFR. Afatinib also persistently inhibits ErbB homo and hetero-dimers. Use of GBM cell lines U87MG and U87MG transfected with wild type EGFR, EGFRVIII and EGFRVIII with Dead Kinase domain, we evaluated the efficacy of afatinib alone and in combination with temozolomide. Results: Afatinib treatment resulted in a dose dependent decrease in the proliferation of U87MG cell transfected with EGFRVIII. The IC_{50} value for this cell line is 2μM (afatinib). 50μM temozolomide inhibited cell proliferation by 50%. We evaluated the combinatorial efficacy of IC_{50} of both, Afatinib and Temozolomide in the in vitro tumorigenicity (anchorage dependent growth - colony formation assay as well as anchorage independent growth - soft agar assay) of EGFRVIII expressing GBM cells. Conclusion: Altogether, these results support synergistic efficacy of afatinib and temozolomide in EGFRVIII expressing GBM cells. 4. A Chemotherapeutic approach to kill cancer stem cell rich ovarian ascites. Siddik Sarkar, Obeid M. Malekshah, Arash Hatefi. Rutgers University, Piscataway, NJ

Purpose: More than one third of ovarian cancer patients present with cancer cells in the abdomen (also known as ascites) at diagnosis, and almost all have ascites at recurrence (Ahmed and Stenvers 2013). It is well-documented that subpopulations of the ascites show cancer stem cell-like characteristics which possess enhanced resistance to chemotherapies and the capacity for distant metastatic spread and recurrent disease. Therefore, ascites are a major source of morbidity and mortality for ovarian cancer patients. The major deficiency that currently exists is that there is no effective and safe therapy for ascites since they are rich in cancer stem cells (CSCs) and resistant to chemotherapy. To overcome this deficiency, it is our objective to develop an effective chemotherapy approach
that can destroy the CSC-rich ascites and inhibit recurrence. Methods: Several established human ovarian cancer cells as well as ascites obtained from the abdomen of an ovarian cancer patient (ASCITES) were cultured. Cells were sorted by FACS Cell Sorter to determine the percentage of cancer stem cells (CSCs) in each cell line. Dose response curves of different chemotherapeutic drugs such as Cisplatin (CDDP), 6-Methylpurine (6-MP), 5-Fluorouracil (5-FU), SN-38, Monomethyl auristatin E (MMAE) and Etoposide were studied in suspension culture enriched with cancer stem cells. The relative change in total CSC-rich spheroid mass which considers both number and volume of spheroids was used to determine Killing Index for each chemotherapeutic drug. To investigate inhibition of recurrence, the spheroids were monitored for 30 days post treatment. Results: We observed the potentiation of venetoclax activity by the autophagy inhibitor chloroquine (CQ) that specifically eliminated CSCs in a time-dependent fashion, up to 96 hours. CQ also restored sensitivity to venetoclax in MM cell lines that overexpressed MCL-1. High levels of MCL-1 and/or low levels of NOXA have been implicated in bortezomib resistance and negative patient outcomes, including short duration of treatment response. The BCL-2-specific BH3 mimetic venetoclax (ABT-199) has also been explored in multiple hematological malignancies, including the treatment of MM. Venetoclax induces apoptosis in a BCL-2 specific manner by directly inhibiting BCL-2 function. However, intrinsic resistance to veneto-

#1106 Alvocidib potentiates the activity of venetoclax in preclinical models of multiple myeloma. Mark Livingston, Wontak Kim, Hillary Hawes, Peter Peterson, Clifford J. Whatcott, Adam Siddiqui-Jain, Steven Weitman, David J. Bearn, Steven L. Warner. Toleropharmaceuticals, Inc., Lehi, UT.
The proteasome inhibitor bortezomib is widely used in the treatment of patients with multiple myeloma (MM). The expression levels of many proteins increase as a result of bortezomib treatment, including the pro-apoptotic protein NOXA. NOXA functions to sequester the anti-apoptotic BCL-2 family member MCL-1. High levels of MCL-1 and/or low levels of NOXA have been implicated in bortezomib resistance and negative patient outcomes, including short duration of treatment response. The BCL-2-specific BH3 mimetic venetoclax (ABT-199) has also been explored in multiple hematological malignancies, including the treatment of MM. Venetoclax induces apoptosis in a BCL-2 specific manner by directly inhibiting BCL-2 function. However, intrinsic resistance to venetoclax treatment observed in MM patient samples has been attributed to a low BCL-2 to MCL-1 gene expression ratio, suggesting a central role for MCL-1 in cell survival in this context as well. Increased MCL-1 expression is a known resistance mechanism to venetoclax treatment in a variety of cell types including chronic lymphocytic leukemia and lymphomas. Considering the central role of MCL-1 to treatment efficacy in MM, we investigated the ability of an MCL-1-inhibitor agent, namely the CDK9 inhibitor Alvocidib, to potentiate the activity of venetoclax in MM. Alvocidib suppresses MCL-1 expression via CDK9-mediated regulation of RNA polymerase II. Alvocidib has achieved robust improvements in the clinical response rates of high-risk, newly diagnosed acute myeloid leukemia (AML) patients as part of the time-sequential ACM regimen (alvocidib + cytarabine + mitoxantrone). We therefore hypothesized that Alvocidib would potentiate the activity of venetoclax in MM through an MCL-1-dependent mechanism. In this report, we demonstrate that Alvocidib inhibits the protein expression of MCL-1 in MM cells in a time-dependent fashion, up to 96 hours. In cell viability assays, the addition of up to 100 nM venetoclax resulted in a 2.8-fold reduction in the IC50 of Alvocidib in the cultured OPM-2 cell line. Conversely, the potentiation of venetoclax activity with the addition of Alvocidib resulted in a more than 500-fold decrease in IC50 in the relatively venetoclax-resistant OPM-2 cells. Additional studies are currently underway to investigate the efficacy of Alvocidib and venetoclax in the context of bortezomib resistance where low NOXA may contribute to enhanced cell survival via MCL-1. Taken together, our data suggest that the combination of Alvocidib with venetoclax may constitute a novel therapeutic regimen in the treatment of MM. Further, it suggests that CDK9-mediated targeting of MCL-1 may offer a clinical route to addressing intrinsic resistance in MM patients.

#1107 Co-treatment with a C1B5 domain peptide of protein kinase C gamma and a low dose of gemcitabine effectively inhibited pancreatic cancer growth in mouse peritoneal cavity. Alejandro Zulbaran, Kelsey Monson, Susumu Ishiguro, Atsushi Kawabata, Deepthi Uppalapati, Naomi Ohta, Masaaki Tamura. Kansas State University College of Veterinary Medicine, Manhattan, KS.

Although the gemcitabine is an effective chemotherapeutic agent for pancreatic cancer, unacceptable side effects often accompany. Since we have previously discovered that PKCγ C1B domain peptides effectively control tumor growth without any side effect (Kawabata et. al, Cancer Biol Ther, 2012), we sought to examine the efficacy of co-treatment with this peptide and a low dose of gem-

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EXPERIMENTAL AND MOLECULAR THERAPEUTICS: DNA Repair

#1108 A novel, selective inhibitor of DNA-dependent protein kinase (DNA-PK) potentiates the effects of DNA-damaging therapies in hepatocellular carcinoma. Catherine E. Willoughby,1 Huw D. Thomas,1 Tommy Rennison,2 Celine Cano,2 Helen L. Reeves,3 Stephen R. Wedge1. 1Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, United Kingdom; 2School of Chemistry and Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, United Kingdom; 3Div. of Liver Unit, Freeman Hospital, Newcastle upon Tyne Hospitals NHS Foundation Trust and Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, United Kingdom.

DNA-dependent protein kinase (DNA-PK) is a key component in the repair of DNA double-strand breaks via non-homologous end-joining. Studies have found elevated DNA-PK expression and activity in hepatocellular carcinoma (HCC) to be strongly correlated with increased tumor grade, resistance to DNA-damaging therapies and poor survival (1,2). We have explored the selective inhibition of DNA-PK in combination with DNA-damaging agents as a potential therapeutic approach in HCC using NDD0004 - a novel, orally-bioavailable small molecule inhibitor of DNA-PK (in vitro IC50 = 8 nM). NDD0004 was evaluated in a panel of DNA-PK expressing human HCC cell lines (Hep3B, HepG2, Huh7) in combination with ionizing radiation or the topoisomerase II poison doxorubicin. DNA-PK activity was determined by Ser2056 phosphorylation status, DNA damage quantified by γH2AX levels, cell proliferation determined by SRB assays and cell survival assessed using clonogenic assays. In vivo efficacy was evaluated using a novel murine model of localized and sustained doxorubicin therapy, involving intra-tumoral injection of doxorubicin-loaded beads in established Huh7 human HCC xenografts in C57/ Bl6 nude mice. Oral twice-daily treatment with 30 mg/kg NDD0004 or vehicle control was commenced 1 hour following bead implantation and continued for up to 20 days (6 mice per group). NDD0004 dose-dependently inhibited activation of DNA-PK in HCC cell lines in vitro in response to ionizing radiation, and significantly increased and sustained DNA damage following treatment. Furthermore, NDD0004 sensitized DNA-PK overexpressing HCC cell lines to doxorubicin and ionizing radiation in proliferation and survival assays by > 5-fold. Combining NDD0004 with doxorubicin-loaded beads in vivo significantly inhibited the rapid growth of HCC tumors when compared to treatment with doxorubicin-loaded bead monotherapy, with the time taken for tumor

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volumes to quadruple (RTV4) being extended from 11 to 18 days (P<0.01, Mann-Whitney). Individual and combination treatments were well tolerated throughout. Immuno-histochemical analysis revealed γH2AX levels in hepatocytes surrounding doxorubicin-loaded beads to be significantly increased by NDD0004 co-treatment for 72-hours (P<0.02, 2-way ANOVA). In conclusion, selective targeting of RUVBL1/2 with AsiDNA catalyzes DNA double-strand breaks (DSB), and reveals that MutSβ may potentially influence TMZ repair by modulating DSB repair. Consistently, host cell reactivation (HCR) assays in U251 expressing DRGFP reporter showed decreased homologous recombination regulating DSB repair. 

In the current study, we propose a novel therapeutic strategy, based on drug combination to promote sensitivity to PARP independently of the tumor genetics. Experimental design: We used AsiDNA, a DNA repair pathways agonist (Dbait concept), consisting in small molecules mimicking double-strand DNA breaks to activate ecdytic signaling of DNA damage and prevent recruitment at damaged sites of HR repair enzymes. We characterized the DNA repair inhibition activity of AsiDNA by monitoring repair foci formation and DNA damage and analyzed the cell survival to AsiDNA monotherapy and combination with the PARPi Olaparib of 21 tumor cell lines, and 3 non-tumor cell lines. Efficacy of the combination treatment was analyzed in tumor xenografts derived from the MDA-MB-231 cell line showing reduced sensitivity to AsiDNA and Olaparib in vitro. We screened for the first time the potential of AsiDNA as co-sensitizer of PARP inhibitors on the DNA double-strand break repair pathway.

Analogous sensitization in MSH6 KD cells (PF 2.6) deficiency. Supporting the concept of synthetic lethality of HR with PARP inhibitors, we showed by using the MDA-MB-231 xenograft model, whereas Olaparib failed to prevent tumor growth and AsiDNA provided only a 62% increase in tumor growth delay, the combination of Olaparib and AsiDNA increase 215% days the mean tumor growth delay. Conclusion: Our results highlight the therapeutic interest of combining AsiDNA and PARPi to recapitulate synthetic lethality in all tumors independently of their HR status. Moreover, the low frequency of appearance of resistant clones to AsiDNA suggests a sustained efficacy during treatment unlike most targeted therapies.


Mismatch repair (MMR) is essential for temozolomide (TMZ) sensitivity, and MMR deficiency causes TMZ resistance and disease recurrence. However, the role MMR in TMZ response has been linked with Msh2/Msh6 (MutSβ), while role of Msh3, a component of MutSβ remains unclear. To delineate role of MutSβ and MutSβ in TMZ response, we used siRNA to disrupt MSH6 and MSH3 expression. As expected, silencing of MSH6 led to resistance in a TMZ-sensitive U251 cell line. In contrast, silencing of MSH3 enhanced TMZ sensitiv-ity as compared to the control cells. These results support established role of MutSβ in TMZ response via replication stress and aggregation of DNA double-strand breaks (DSB), and reveal that MutSβ may potentially influence TMZ response by modulating DSB repair. Consistently, host cell reactivation (HCR) assays in U251 expressing DRGFP reporter showed decreased homologous recombination (HR) after MSH3 knockdown (KD), while Msh6 KD had no effect on HR efficiency. Supporting the concept of synthetic lethality of HR with PARP inhibi-tion, clinically relevant dose of veliparib (1 μM) showed a modest but consistent increase in TMZ sensitization measured in terms of potentiation factor (PF: ratio of relative growth with TMZ alone/ TMZ and veliparib) in MSH3 KD (PF 1.6±0.2) as compared to control cells (PF 1.1±0.2). Sensitizing effect of 1 μM veliparib in MSH6 KD (PF 1.7±0.5) was comparable to that in MSH3 KD cells. Interestingly, supra-therapeutic dose of veliparib (10 μM) induced more pronounced sensitization in MSH6 KD cells (PF 2.6±0.3) as compared to MSH3 KD (PF 2.4±0.3) or control cells (PF 1.6±0.2). Consistently, veliparib (10 μM) alone had no impact on DNA damage signaling, while TMZ induced prominent increase in phosphorylation of CHK1, Chk1 and Chk2 in control and MSH3 KD cells, but not in MSH6 KD cells; veliparib/TMZ co-treatment led a robust sig-naling in all 3 types of cells. Increased sensitization by veliparib after MSH3 KD was also examined in a TMZ-resistant (GBM22TMZ) PDOX model carrying re-sistance due to a homozygous mutation in MSH6, and a 4-fold increase in HR efficiency. In clonogenic assays silencing of MSH3 or BRCA1 had no effect on TMZ sensitization in GBM22TMZ cells. However, veliparib alone (10 μM) de-creased colony formation with relative colony formation 0.54±0.03 and 0.32±0.06 in MSH3 and BRCA1 KD cells, respectively, as compared to 0.82±0.05 in TMZ+veliparib co-treatment in GBM22TMZ; and further decrease in colony formation in MSH3 KD and BRCA1 KD cells with relative colony formation of 0.30±0.04 and 0.19±0.02, respectively versus 0.46±0.03 in control. Taken together, our results suggest that MutSβ and MutSβ differentially regulate cytotoxic effects of TMZ. Decreased HR efficiency and enhanced sensitizing effects of veliparib in MSH3 KD cells partially define synthetic lethality of MMR deficiency with PARP, and may help development of PARP inhibitors as sensitizer of TMZ therapy in GBM.

1111 The chromatin remodelers RUVBL1 and RUVBL2 are prognostic factors and therapeutic targets in non-small cell lung cancer due to their roles in DNA replication, repair, and radiosensitization. Paul Veneralli, Rahul Kol lipara, Amit Das, Pamela Villalobos, Long Shan Li, Brenda Timmons, Luc Girard, Jaime Rodriguez-Canales, Ignacio Wistuba, John Minna, Ralf Kittler, Univ. of Texas Southwestern Medical, Dallas, TX; UT-MD Anderson Cancer Center, Houston, TX.

Despite advances in targeted agents and immunotherapy, non-small cell lung cancer (NSCLC) remains the number one cause of cancer-related death. To identify new therapeutic targets in NSCLC, we performed a siRNA screen directed against genes involved in chromatin remodeling. This screen showed that RUVBL1 and RUVBL2 (herein collectively referred to as RUVBL1/2) were universally but differentially required for the viability of 24 NSCLC cell lines, which was an on-target effect. Various independent gene expression datasets/platforms show that NSCLC patient tumors have increased levels of RUVBL1 and RUVBL2 mRNAs, in comparison to normal lung, and that patients with high levels of RUVBL1 or RUVBL2 have a poorer prognosis, suggesting that RUVBL1/2 play an important role in NSCLC tumors. To confirm this at the protein level, we validated an antibody against RUVBL1 for immunohistochemistry, stained clinically annotated NSCLC tissue microarrays for RUVBL1, and found that patients with higher levels of RUVBL1 protein also have a poorer prognosis. To better understand the role of RUVBL1/2 in NSCLC at a molecular level, we measured the distribution of cells in the cell cycle following RUVBL1/2 KD, determined RUVBL1/2-interacting proteins by immunoprecipitation followed by tandem mass-spec (IP-MS/MS), and measured gene expression changes following RUVBL1/2 KD by RNA-seq. Depletion of RUVBL1/2 ar-rested cells in S-phase and promoted pan-γH2AX positivity, IP-MS/MS showed an over-representation of proteins involved in DNA repair and replication, and gene set enrichment analysis of the RNA-seq data displayed a downregulation of transcripts involved in DNA replication and repair, strongly implicating RUVBL1/2 in these processes. To further probe the effects of RUVBL1/2 loss, we performed low-level knock down (KD) of RUVBL1/2, such that viability is largely unaffected, and then measured the viability of cells in response to various drugs (n=35). These drugs target a wide variety of biological processes; however, only the drugs with increased efficacy in the presence of RUVBL1/2 KD resulted in a detrimental effect on RUVBL1/2 KD tumors, indicating that RUVBL1/2 contain a unique repair mechanism. Fi-nally, we show that RUVBL1/2 depend upon their ATPase activity to support NSCLC viability, suggesting that small molecule inhibitors of this protein may be efficacious in the treatment of NSCLC, especially when combined with radiotherapy.
#1112 Forced reduction of DSS1, a member of TREX2 complex, highly sensitizes chemotherapy to breast cancer cells in a BRCA2-independent manner. Kazuhiko Kuwahara, 1 Naomi Gondo, 2 Andri Rezano, 3 Zhenhuan Zhang, 4 Yukari Hato, 3 Kiyotaka Kuzushima, 2 Hiroji Iwata, 6 Tatsuya Toyama, 5

**EXPERIMENTAL AND MOLECULAR THERAPEUTICS: DNA Repair**

Sarkia.A.Abdulkadir, Einstein, Muhammad Zayd Ansari, Benedito A. Carneiro Filho, Francis J. Giles, 1, 1114 Combining anti-androgen therapy and PARP inhibition results in paired TREX2 complex might be associated with induction of chemosensitivity. Rather, the transcription-coupled DNA damage induced by im-

doxxorubicin and paclitaxel in breast cancer cells was evaluated by flow cytom-

tetry to detect apoptosis and colony assay. In addition, we compared patient survival by dividing patients into high or low expression group of BRCA2 or

Safety of CB was determined in long-term toxicity studies in mice and also in ex-vivo explants from breast cancer patients. The mechanism of action of CB was evaluated

#1115 Germline single nucleotide polymorphisms in DNA repair genes in uterine cervical carcinomas. Bishoy M. Faltas, Panagiota J. Vlachostergios, Linda Lam, Tu Zhao, Olivier Elemon, Mark A. Rubin, Weill Cornell Medical College, New York, New York.

Introduction: Germline single nucleotide polymorphisms (SNPs) have been investi-
gated in several cancers. Repair of DNA damage is a key cellular process in-
volved in the development of chemotherapy-resistance. The role of germline SNPs in DNA damage repair genes (DRGs) in determining resistance to DNA-damaging agents in uterine cervical carcinoma (UC) patients is not completely understood. Meth-
ods: We examined a cohort of 53 UC patients (median age 67, 42 males) enrolled in our IRB-approved Precision Medicine Program. Pathology confirmed UC (43 bladder, 10 upper tract UC) and received treatment with platinum-
base chemotherapy. We isolated germline DNA from peripheral blood lympho-
cytes or buccal swabs, and used whole exome sequencing (WES) to examine germline SNPs. As a reference for SNP frequencies we used the Exome Aggregation Consortium (ExAC) database, which represents the largest, randomly selected, germline WES database in the general population, including 60,706 individuals, among which 7,601 patients with multiple cancer types from the Tumor Cancer Genome Atlas (TCGA) cohort. Results: Twelve different DRG SNPs were identified in germline DNA samples from 53 patients, affecting genes involved in non-homol-

ogous end-joining (RECVQ4, n = 18, 54.50%; POLQ, n = 2, 6%), nucleotide exci-
tion repair (ERCC6, n = 2, 6%; XPA, n = 1, 3%; CCNH, n = 1, 3%; POLK, n = 1, 3%; RECQL4, n = 1, 3%; RAD17, n = 1, 3%; POLB, n = 1, 3%); Fanconi anemia pathway (POLN, n = 1, 3%); mismatch repair (EKO1, n = 1, 3%) and mitochondrial DNA repair (POLG, n = 2, 6%). The frequency of rs11342077 of the DNA helicase RECVQ4 was significantly higher in our cohort (18/53, 34%) compared to its frequency in the ExAC database (p = 0.01%). There was no significant difference in overall survival (OS) between patients with and without DRG SNPs (log-rank p = 0.46). There was no significant association between the most commonly identified SNP, rs11342077, and overall survival among UC pa-

tients with DRGSNPs (log-rank p = 0.39). Overall, the presence of DRG SNPs in our cohort occurred at a significantly higher frequency (33/53, 62.3%) compared to the ExAC database (0.56%, chi-square p < 0.001). Conclusions: Germline SNPs in DNA repair pathway genes are common in UC. Additional study of the role of these SNPs as potential biomarkers of response DNA damaging chemotherapeutic agents, in-
cluding platinum-based chemotherapy or and PARP inhibitors is needed in a larger cohort.

#1116 Targeting replication stress by carbazole blue - A novel strategy to treat triple negative breast cancers. Subapriya Rajamanikam, 1 Kaitlyn Favours, 1 Santosh Timilsina, 1 JunHyung Park, 2 Benjamin Onyeyugha, 1 Fanneedros Subbarayulu, 1, 3, 77030, TX; 3Emory University School of Medicine, Atlanta-30322, GA.

Background: Triple-negative breast cancers (TNBCs) are the most aggressive forms of breast cancer and almost 60% of patients with TNBCs develop chemosensitivity, leading to recurrence, poor prognosis and poor survival. TNBCs have been reported to have high levels of replication stress, which plays pivotal role in genomic instability, and therapy resistance. Targeting replication stress is an emerg-
ing approach for better TNBC treatment. Here, we evaluated the anticaner efficacy of carbazole blue (CB), a synthetic analogue of carbazole that we recently synthe-
sized on TNBC cells growth and progression. Experimental Design: The effect of CB on breast cancer growth was assessed in vitro as well as in orthotopic mouse xenograft and PDOX-molds of breast cancer. In addition, the therapeutic efficacy and safety of CB was determined in long term toxicity studies in mice and also in ex-vivo explants from breast cancer patients. The mechanism of action of CB was evaluated

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by performing gene expression, cell cycle, apoptosis and DNA repair studies as well as proteins involved in the above mentioned mechanisms. Results: Our results demonstrated that CB inhibits short and long term viability of TNBC cells in a dose dependent manner without affecting normal mammary epithelial cells. We show that the systemic delivery of CB using nanoparticle-based delivery approach suppressed expression and activity of several genes known to be involved in DNA replication and DNA repair machinery. Conclusions: Our results for the first time showed the CB can serve as a novel and potent therapeutic agent for treating breast cancer in general and TNBC in particular. These findings highlight the potential of CB to be applied as a safe regimen for treating breast cancer patients. As exploiting replication stress to treat cancer is gaining major interest, compounds/s that may induce replication stress and inhibit DNA repair ability of cancer cells, has immense translational potential.

#1117 Triple negative breast cancer patient-derived xenografts as a translational model for discovery of novel therapeutic targets. Margaret D. Matossian,1 Hope E. Burks, Annie C. Bowles, Van T. Hoang, Bruce Bunnell,2 William J. Zuercher,2 David H. Drewry,2 Carrow Wells,2 1Hope E. Burks, 2Carrow Wells, 3Tulane University School of Medicine, New Orleans, LA; 2University of North Carolina, Chapel Hill, NC; 3Louisiana Cancer Research Center, New Orleans, LA.

Triplet negative breast cancers (TNBCs) constitute 12% of all breast cancers, and is approximately twice more prevalent in African-American populations. Louisiana has a high proportion of African-American residents (32.5% in 2015), and thus hosts a higher population of TNBC patients. TNBCs have an aggressive phenotype that is elusive to the targeted therapeutics used to treat other breast cancer subtypes. The claudin-low molecular subtype has higher rates of metastases and recurrence. Certain kinase families have been extensively studied as regulators of epithelial-mesenchymal transition (EMT), a process involved in the initiation of cancer metastasis. Discovery of novel kinase targets within the subset of uncharacterized kinases could provide important insight into future targeted therapies. However, current models utilized in target discovery research are limited by the inability to accurately recapitulate the complex stromal architecture and heterogenous genetic and molecular composition of breast cancer. Furthermore, immortalized cell lines are limited to a 2D environment and over time acquire mutations that may not reflect the primary tumor. Recently, our laboratory has successfully established two TNBC patient-derived xenograft (PDX) models derived from African-American patients, and generated cell lines (TU-Bcx-2K1, TU-Bcx-200) and mammospheres. One of these models, 200, presents tumor architecture, cellular composition, genomic (qRT-PCR) and protein (western blot) expressions that are concordant with a claudin-low subtype. Furthermore, we show that both TNBC models metastasize to the lungs, and exhibit molecular characteristics consistent with mesenchymal phenotypes. We utilized these translational PDX models to screen a library of small molecule inhibitors (SOMIs) that inhibit protein kinases and identified polo-like kinase 1 (PLK1) as a substrate to modify various proteins in order to regulate various cellular processes including Wnt/β-catenin signaling. SOMIs have been shown to be over-expressed and to be correlated with highly aggressive disease and prognosis in various types of human cancers. A small molecule inhibitor of TNKS1, XAV-939 has been proven to be a potent inhibitor of Wnt/β-catenin signaling by stabilizing Axin. However, the effect of tankyrase inhibitors as potential breast cancer therapy is still under investigation. In this study, we found that TNKS1 is highly expressed in breast cancer cell lines, particularly in triple-negative breast cancer (TNBC) cells and that the expression of TNKS1 correlates with anti-proliferative efficacy of tankyrase inhibitors, XAV-939 and JW55. Also, XAV-939 treatment suppressed cell migration and invasion capabilities and epithelial-mesenchymal transition (EMT) signaling in triple-negative breast cancer cell lines. Moreover, XAV-939 reduced expression of stemness markers. It was shown that polo-like kinase 1 (PLK1) functions as a positive regulator of TNKS1 protein stability. Interestingly, we found that combination treatment of XAV-939 with PLK inhibitor GW843682X significantly suppressed cell growth, clonogenic potential, migratory and invasive capabilities of breast cancer cells. The combination of XAV-939 and PLK inhibitors showed potent anti-tumor effect. Therefore, our findings highlight the important value of TNKS1 as a therapeutic target and suggest the potential for improving the clinical efficacies of tankyrase inhibitors in combination with either PLK inhibitors or radiotherapy for patients with triple-negative breast cancer.

#1118 Synthetic lethality of CDK2 inhibition in tumors with EWS/FLI rearrangements. Amanda L. Balboni,1 Bjorn Stolte,1 Amy Saur Conway,1 Gabriela Aleixo,1 Emily Jue Wang,1 Nicholas Kwiatkowski,2 Tinghu Zhang,1 Brian J. Abraham,3 Peter Kalev,4 Dipanjana Chowdhury,5 Cyril H. Benes,6 Richard A. Young,7 Nathanael S. Gray,1 Kimberly Stegmaier,6 Dana-Farber Cancer Institute, Boston, MA; 2Whitehead Institute, Cambridge, MA; 3Massachusetts General Hospital, Boston, MA.

THZ1 is a potent, covalent inhibitor of the transcriptional CDKs, CDK7/12/13. Chemical genomic profiling of THZ1 across >1,000 diverse cancer cell lines revealed that EWS/FLI- rearranged Ewing sarcoma cells were remarkably sensitive to this molecule. We demonstrated that THZ1 inhibits the phosphorylation of the C-terminal domain of RNA Polymerase II, decreased colony formation capacity, and induced apoptosis in a dose-dependent manner in Ewing sarcoma cell lines. Using selective CDK7 and CDK12/13 inhibitors, we revealed that THZ1 has no single selective activity. In general, suppression of CDK12, but not CDK13, induced strong anti-viability effects, confirming CDK12 as the primary target. Treatment of Ewing sarcoma cell lines with THZ531, a novel CDK12/13 selective inhibitor, preferentially repressed genes involved in DNA damage repair. Additionally, suppression of EWS/FLI rendered Ewing sarcoma cells resistant to THZ531 and partially rescued the anti-viability effects of CDK12 knockdown. These results suggest that EWS/FLI impacts vulnerability to DNA damage repair inhibition and implicate a synthetic lethal relationship between the tumor-specific expression of EWS/FLI and CDK12 inhibition. Furthermore, we demonstrated that CDK12 and PARP inhibitors are highly synergistic in vitro, inducing widespread γH2AX foci formation. Interestingly, THZ531 impairs the ability of the PARP inhibitor, olaparib, to induce RAD51 foci formation, suggesting that THZ531 specifically causes a defect in homologous recombination repair. Moreover, we observed striking synergy of THZ1 and olaparib in two mouse models of Ewing sarcoma with limited toxicity observed. These findings have important translational significance as clinical trials with PARP inhibitors as single agents in Ewing sarcoma failed to demonstrate efficacy, highlighting the need to identify combination therapies that will enhance the activity of PARP inhibitors. We anticipate that CDK12 and PARP inhibitor combinations will be of therapeutic interest in other ETS-rearranged tumors, as well as tumors with defects in DNA repair.

#1119 Targeting tankyrase as a therapeutic strategy for triple-negative breast cancer. Maya Mathew, Loredana Cordano, Jung-Lye Kim, Geun-Hyang Ha, Eun-Kyong Breuer. Loyola University Medical Center, Maywood, IL.

Tankyrase (TNKS1 and TNKS2) are members of the human poly(ADP-ribose) polymerase (PARP) family and catalyse PARylation by using NAD + as a substrate to modify various proteins in order to regulate various cellular processes including Wnt/β-catenin signaling. TNKS1 has been shown to be over-expressed and to be correlated with highly aggressive disease and prognosis in various types of human cancers. A small molecule inhibitor of TNKS1, XAV-939 has been proven to be a potent inhibitor of Wnt/β-catenin signaling by stabilizing Axin. However, the effect of tankyrase inhibitors as potential breast cancer therapy is still under investigation. In this study, we found that TNKS1 is highly expressed in breast cancer cell lines, particularly in triple-negative breast cancer (TNBC) cells and that the expression of TNKS1 correlates with anti-proliferative efficacy of tankyrase inhibitors, XAV-939 and JW55. Also, XAV-939 treatment suppressed cell migration and invasion capabilities and epithelial-mesenchymal transition (EMT) signaling in triple-negative breast cancer cell lines. Moreover, XAV-939 reduced expression of stemness markers. It was shown that polo-like kinase 1 (PLK1) functions as a positive regulator of TNKS1 protein stability. Interestingly, we found that combination treatment of XAV-939 with PLK inhibitor GW843682X significantly suppressed cell growth, clonogenic potential, migratory and invasive capabilities of breast cancer cells. The combination of XAV-939 and PLK inhibitors showed potent anti-tumor effect. Therefore, our findings highlight the important value of TNKS1 as a therapeutic target and suggest the potential for improving the clinical efficacies of tankyrase inhibitors in combination with either PLK inhibitors or radiotherapy for patients with triple-negative breast cancer.


Purpose: The goal of this research is to highlight novel and divergent molecular mechanisms of the circadian system in modulating the response of cisplatin therapy against melanoma cancers. Cisplatin is one of the most commonly used chemotherapeutic drugs in the treatment of a variety of cancers such as ovaries, testis, lungs, blood and solid tumors of the head and neck, and is more recently under clinical trials for potential application in melanoma tumors. However, the major limitation of cisplatin as a chemotherapeutic drug is its tumor resistance and nephrotoxicity. Hence, improving the effectiveness and reducing the toxicities associated with cisplatin therapy are desirable outcomes. Studies on human models have shown decreased renal and blood toxicity by time-of-day. Consequently, our project seeks to study the chronopharmacological effects and understand the mechanisms that have been successfully demonstrated in human models. We hypothesize a mechanistic, circadian rhythm-based cause for these outcomes. Experimental Design: Studies were...
done on B16F10 melanoma mouse models of wild-type and circadian disrupted Per1/2−/− animals treated with 3 doses of 5 mg/kg cisplatin in the morning (7 AM) and evening (5 PM). Animal weights and tumor sizes were measured regularly post-treatment. Upon sacrifice, tissues (skin, kidney, and tumor) were harvested and analyzed for cisplatin-induced toxicities and DNA damage responses using H&E staining, western blot, and KM-1 and immunoslot DNA assays. Results: Weight measurements show a clock-regulated response to cisplatin toxicity. AM-treated wild-type animals showed significant weight loss compared to PM-treated wild-type animals. This treatment time differential is lost in the Per1/2−/−. On a molecular level, kidney tissue DNA samples showed clock-controlled cisplatin-DNA repair activity in wild-type animals compared to Per1/2−/−. These findings strongly suggest that nephrotoxicity and DNA damage response function might be regulated by the circadian rhythm. Conclusions: These findings indicate a possible mechanism for the chronopharmacology of cisplatin in minimizing the toxicity associated with it and reveal a target for future study of additional mechanistic causes of circadian dosing changes in cisplatin and other genotoxic stress-mediated anti-cancer agents.

## #1121 Reduced-mediated inactivation of MGMT DNA repair through thiolation or nitrosylation of the active site cysteine415 sensitizes human gliomas to alkylating agents in vitro and in xenograft models. Debashis Basak, Kalkunte S. Srevenugopal, Texas Tech University Health Sciences Center, Amarillo, TX.

O6-Methylguanine-DNA methyltransferase (MGMT) is a simple and unique antimitaguc DNA repair protein that plays a crucial role in conferring tumor resistance to various alkylating agents in brain tumor therapy. MGMT repairs O6-alkylguanines by transferring the alkyl groups to its active site cysteine (Cys145) in a stoichiometric/suicidal reaction thereby restoring the original nucleotide sequence in a single step. Although O6-benzylguanine (BG), a pseudosubstrate inhibitor, currently in clinical trials, potently inhibits MGMT and improves the efficacy of alkylating agents, unacceptable levels of bone marrow toxicity has been a great concern. For developing new MGMT inhibitors, we explored the reactive nature of the active site Cys145 (pKa 4.5) and its susceptibility for thiolation and nitrosylation, both of which inactivate the MGMT. A new strategy involving inhibition of S-nitrosoglutathione reductase (GSNOR) was also explored to increase the steady-state levels of nitrosylated MGMT. First, we designed a redox perturbing glutathione mimetic, a homologuthione disulhde (hGTX) that has been stabilized with the addition of small amounts of cisplatin (1000:1). Spermine NONOate and S-nitroso penicillamine (SNAP) served as the protein-nitrosylating agents. N6022, a specific and reversible inhibitor of GSNOR was used with nitrosylates in some assays. Both the hGTX and spermine NONOate at 100–400 μM concentrations for 24 h inhibited the repair activity of MGMT >75% in HT29, SF188, and 98G cells. The NONOate strongly curtailed the DNA repair activity of recombinant MGMT showing a direct interaction. The inactivated MGMT protein was promptly eliminated from glioma cells. MGMT disappeared from cells much faster in spermine NONOate +N6022 treated cells, suggesting an extended steady-state of nitrosylated MGMT and its subsequent degradation. MGMT depletion was accomplished by 4-6 fold increases in cell killing by BCNU or temozolomide (TMZ). Binding of the biotinylated-O6-benzylguanine to the MGMT protein was prevented by hGTX and spermine NONOate treating the Cys145 wild type and the Cys145 mutant forms of hGTX and TMZ or spermine NONOate +TMZ produced markedly synergistic tumor regressions compared with TMZ alone. A reduction of MGMT protein as by immuno-blotting and diminished MGMT activity levels in tumor tissues was verified. Apoptosis regulatory proteins were upregulated in excised tumors and H&E staining confirmed no discernible host organ toxicities. Our studies highlight the options for redox-driven therapeutic strategies for MGMT [supported by CPRIT grants RP130266 and RP170207 to KSS].

## #1122 ATR inhibitors synergize with PARP inhibitors in killing glioblastoma stem cells and treating glioblastoma. Jianfang Ning, Hiroaki Wakimoto, Robert L. Martuza, Samuel D. Rabin. Massachusetts General Hospital, Boston, MA.

PARP inhibitors (PARPi) have been used alone or in combination with other agents for the treatment of tumors with homologous repair (HR) deficiencies. However, challenges remain for the treatment of tumors that are PARP-resistant or HR-proficient. Glioblastoma (GBM) is an invariably lethal tumor that is not associated with HR deficiencies. GBM stem cells (GSCs), thought to be critical for tumor growth and resistance to therapy, can be isolated from GBM specimens and are representative of the patient’s tumors. GSCs exhibit variable PARP sensitivity, with at least half being resistant. In order to enhance the antitumor efficacy of PARPi for GBM, we examined the combination of PARPi with inhibitors of oncogenic or DNA damage pathways, including inhibitors of PTEN, PI3K, ATM, ATR, and temozolomide, some of which have been reported to sensitize cancer cells to PARPi. Only inhibitors of ATR (VE821, VE822, AZ20) synergized with PARPi in killing GSCs in vitro. VE822 inhibited ATR activity both in vitro and in vivo, and modestly, but significantly, extended survival in mice bearing GSC-derived tumors. However, ATR inhibitor (ATRI) in combination with PARPi further prolonged survival, compared to each drug alone, in a murine orthotopic glioma model. This is the first report that ATRi alone or in combination with PARPi is effective in treating GBM and provides a rationale for clinical trials for GBM.

## #1123 Sphingolipid transfer proteins (GLTP and CPTP) regulate the neoplastic progression of colon and breast cancer cells. Shrawan K. Mishra, Rhoderick E. Brown. Hormel Institute, University of Minnesota, Austin, MN.

Sphingolipids have previously been shown to affect cancer progression by regulating cell death (apoptosis) and survival (autophagy). While apoptosis is promoted by elevations in sphingosine and ceramide, increases in sphingosine-1-phosphate and ceramide-1-phosphate can tip the balance toward survival. Changes in the expression of sphingolipid transfer proteins such as GLTP (glycolipid transfer protein) and CPTP (ceramide-1-phosphate transfer protein) have been shown to modulate cell shape change and regulate pro-inflammatory cytokine release, respectively, in human cancer epithelial cell lines. To gain a better understanding of the situation, we investigated further. We discovered that GLTP overexpression inhibits the growth of human colon carcinoma cell lines (HT-29; HCT-116), but spares normal colonic epithelial cells (HCEC) largely due to growth arrest at the G0/G1 cell cycle checkpoint. Mechanistically, we found that GLTP overexpression modulated cell cycle progression by upregulating cip1/p27 and Cic/p1/p21 protein and mRNA levels, while decreasing CDK2, CDK4, cyclin E and cyclin D1 protein levels. The cell cycle arrest and growth inhibition induced by GLTP overexpression led to apoptotic cell death of HT-29 cells. Neither GLTP nor CPTP overexpression induced apoptosis in breast cancer cell lines (T47D and HTB-126). However, depletion of CPTP, but not GLTP, induced autophagy as indicated by increased LC3 transcript and autophagosome levels (GFP-LC3-II puncta). In the breast cancer cell lines, increased transcript levels of CLA2 and CERK were detected consistent with the earlier pro-inflammatory effect reported for CPTP depletion in A549 lung epithelial carcinoma cells. Our findings shed light on the mechanistic ways by which GLTP and CPTP affect apoptosis and autophagy to impact cancer progression. Future in vivo studies are planned to ascertain the role(s) of these new players in cancer development and therapy. [Support: NIH GM45928, HL-125353, Paint-the-Town-Pink (PTTP) Cancer Funds, Hormel Fdn]

## #1124 Assessment of HRD score as predictor of chemosensitivity of PDAC PDX xenograft models to DNA-damaging chemotherapy. Vladimir Khazak, Natalia Skobeleva, Anastasia Vetkina, Ilya Serebriiskii, Kirsten M. Timms, Angela Davies, Igor Asturatov, NexusPharma Inc., Philadelphia, PA; Fox Chase Cancer Center, Philadelphia, PA; Myriad Genetics, Inc., Salt Lake City, UT; Champions Oncology, Inc., Hackensack, NJ.

Pancreatic ductal adenocarcinoma (PDAC) is a devastating malignancy that affects 44,000 individuals annually in the US, with almost 90% lethality even when diagnosed prior to metastasis. There is an urgent unmet medical need for new therapies using novel approaches targeting existing therapies to patients. To address this emergency, we are assessing the feasibility of implementing a strategy of using HRD (Homologous Recombination Deficiency) scores for better therapy matching in PDAC patients. Using a panel of 77 patient-derived xenograft (PDX) models that were developed from fresh surgical PDAC tumor samples, HRD scores were generated based on analysis of three biomarkers (LOH, TAI and LST) and mutational data for 45 cell lines. All 77 samples met inclusion criteria, 75 FFPE specimens generated mutation data. HRD analysis was successful for 71 specimens (range = 1-63 (median = 22)), with the primary cause of failure identified as high non-tumor content. 53 PDX models had mutations in KRAS gene and 45 in TP53. We have also identified 4 PDX models with mutations in BRCA2, 3 models with mutations in ATM, 4 models with mutations in RAD51. We have also found frequent mutations in several other DNA repair genes (ATR, PALB2, MLH1, MSH2, MSH3, MSH6, FANCM), but most of these models retained one functional allele and were not associated with a high HR content. Using this genomic analysis, all 71 PDX models were stratified into three clusters with high, medium and low HRD scores. Three PDX models with the highest and lowest HRD scores each were selected for an in vivo study with DNA-damaging platinum-based chemotherapeutic agents Cisplatin and Carboplatin, as well as with a clinically relevant PARP inhibitor Niraparib. The results of the PDX study will be reported and compared with responses to chemotherapy using RECIST V1.1 in patients.
tumors and alters DNA damage repair. It was recently demonstrated that the impact radiosensitivity. Specifically, many cancers exhibit increased histone that the abnormal epigenetic landscape in tumors may, among other things, not only suggest that this approach alters multiple hallmarks of cancer growth, (KD) and/or caloric restriction (CR) as an adjuvant to cancer therapy. This data

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#1126 Potent inhibition of HCMV by modulating the cellular SNARE syntaxin 5. Dhimant H. Desai,1 Linda Cruz,1 Nicholas T. Streck,1 Trisha D. Desai,2 Aron Lukacher,1 Shantu G. Amin,1 Nicholas J. Buchkovich1. 1Penn State Univ. College of Medicine, Hershey, PA; 2Cumberland Valley High School, Mechanicsburg, PA.

Human cytomegalovirus (HCMV), also referred to as human herpesvirus-5 (HHV-5), can cause serious and even fatal disease in immunocompromised individuals and newborns, namely individuals with AIDS, solid organ transplant recipients, chemotherapy patients and recipients of bone marrow and stem cell transplants. HCMV is a ubiquitous virus found throughout all geographic regions and socioeconomic groups, infecting greater than 50% of adults in industrialized countries and as many as 100% in developing countries. Although current therapeutics improves clinical outcomes, they are limited by toxicity, intravenous infusion and the development of resistance by the virus. Thus, there is a pressing need to develop novel therapeutics to prevent HCMV infections with concomitant organ toxicity. Formation of the cytoplasmic viral assembly compartment (vAC) is an important step for efficient HCMV assembly. To do this, the virus must alter and repurpose the normal cellular balance of membrane proteins. Thus, targeting this process that is not well understood. We have identified a compound Retro94, which potently inhibits production of infectious HCMV virions in cells by operating against a host cell process, rather than directly targeting the virus. The presence of Retro94 results in the severely impaired production of infectious virions, as great as 5 logs (99-99.99%). Here we discuss in vitro, in vivo, stability, and binding study of Retro94. Overall, our findings have identified Retro94, as a novel agent that affects key cellular trafficking factors important for supporting HCMV infection.

#1127 Aberrant nuclear expression of GSK-3beta in human head and neck carcinoma. Maria Matsangou,1 Andrey Ugolkov,2 Timothy J. Baxter, Sandeep Samant,1 Andrew P. Mazar, Francis J. Giles1. 1Robert H Lurie Comprehensive Cancer Center, Northwestern University, Chicago, IL; 2Chemistry of Life Processes Institute, Northwestern University, Chicago, IL.

Background: Recurrent/metastatic head and neck squamous cell carcinoma (SCCHN) and salivary gland malignancies are difficult to treat with limited standard of care options at the present time. Glycogen Synthase Kinase-3beta (GSK-3beta), a serine/threonine protein kinase, has been implicated as a potential therapeutic target in human cancer. Our in vivo studies demonstrated that our novel GSK-3 inhibitors significantly potentiated the effects of conventional chemotherapy in patient-derived xenograft models of glioblastoma and breast cancer leading to regression of tumors. In order to develop a rationale to test our novel GSK-3 inhibitors in head and neck (H&N) malignancies, we evaluated the expression pattern of GSK-3beta in human H&N tissue and malignant tumors. Methods: We used immunohistochemical staining of H&N tumor tissue Microarray (TMA), 48 total cases (20 benign tissues, 28 malignant), to study the expression pattern of GSK-3beta. GSK-3beta nuclear accumulation was defined as positive staining of more than 50% of cancer cell nuclei throughout the tumor regardless of cytoplasmic staining. Results: Of the 20 malignant H&N samples (15 SCCHN, 1 nasopharyngeal and 5 other histology), 13 (65%) were found to have aberrant nuclear accumulation of GSK-3beta. Amongst SCCHN, 73% (11 of 15 samples) had aberrant nuclear accumulation of GSK-3beta. In contrast, none (0%) of the 11 benign non-salivary H&N tissues showed detectable expression of GSK-3beta. Of interest, 60% of salivary adenoid cystic carcinoma specimens and 44% of benign salivary gland tissue showed GSK-3beta expression. Conclusion: Our results demonstrate that our novel GSK-3 inhibitors as a potential therapeutic target for recurrent/metastatic SCCHN and also as a potential prognostic and predictive biomarker for risk of recurrent disease and chemo- or radio-resistance. Its role in salivary gland malignancies requires further studying.

#1128 Identification of arginine methyltransferase PRMT5 as a novel therapeutic target in T-cell acute lymphoblastic leukemia. Yunyue Wang2,1 Hui Huang,2 Daniel Doliatti,2 Marta Sanchez Martin,2 Beata Modzelewska,2 Lianna J. Marks,2 Allison R. Rainey,3 Ervin S. Gaviria,3 Maria L. Sulis,1 Filemon S. dela Cruz,2 Adolfo A. Ferrando,1 Andrew L. Kung3. 1Columbia University, New York, NY; 2EMD serono, CT; 3Memorial Sloan Kettering Cancer Center, New York, NY.

Advances in risk-adapted cytotoxic chemotherapy, hematopoietic stem cell transplantation and supportive care have contributed to significant improvements in the survival of patients with acute lymphoid leukemia (ALL) and acute myeloid leukemia (AML) over the past few decades. However, despite such progress, a significant percentage of both adult and pediatric leukemia patients become refractory to therapy or relapse and eventually die of disease. Hence, there remains an urgent need for the development of effective and targeted therapies for acute leukemia. Recent genetic profiling of solid and hematologic malignancies has identified epigenetic factors as a critical group of genes recurrently mutated in cancer. Additionally, epigenetic dysregulation has been shown to play an important role in the development, progression and maintenance of leukemia. Therefore, pharmacological inhibition of epigenetic factors represents a potential avenue for the development of novel epigenetic-targeted therapies. In order to identify epigenetic vulnerabilities in leukemia, we developed genomically-focused shRNA screen to search for novel therapeutic targets in human leukemia cell lines both in vitro and in vivo. Specifically, T- and B-ALL cell lines were transduced with a library of shRNAs targeting 449 genes including epigenetic readers, writers and erasers and other chromatin-related factors. Selected cells were subsequently cultured in vitro and concurrently injected
into mice. Engraftment of inoculated cells and disease progression were monitored through bioluminescence imaging. Amongst the universe of epigenetic regulatory proteins, the arginine methyl transferase, PRMT5, emerged as the most significantly depleted factor in both in vitro and in vivo screenings. Chemical inhibition of PRMT5 enzymatic activity effectively reduced protein symmetric dimethyl arginine methylation, altered splice, inhibited cell growth and promoted apoptosis of both ALL and AML cell lines in vitro. In addition, inhibition of PRMT5 in vivo using patient-derived xenograft (PDX) T-ALL mouse models demonstrated diminished tumor growth and prolonged survival. Notably, quantification of peripheral blood cell numbers showed that pharmacologic PRMT5 inhibition was well tolerated and did not affect normal hematopoiesis in mice suggesting that a therapeutic window exists for targeting cancer promoting PRMT5 in leukemia. Overall, our data indicates that pre-mRNA processing and in particular RNA splicing modulation may represent novel therapeutic targets in leukemia.

### #1129 Sigma receptors: Novel targets for the treatment of highly malignant tumors.

Michela Cortesi,1 Sara Pignatta,1 Simona Collina,2 Andrea Rocca,1 Alice Zamagni,1 Chiara Arienti,1 Michele Zanon,1 Luigino Tosatto,1 Daniela Bartolini,2 Evandro Negrioli,1 Annamaria Marra,2 Marta Ruì,1 Anna Tesi,1 1Istituto Scientifico Romagnolo per lo Studio e la Curia dei Tumori (I.R.S.T.) IRCCS, Meldola (FC), Italy; 2University of Padova, Padua, Italy, Padua, Italy; 3M. Bufalini Hospital, Cesena, Italy.

Introduction. More than 7 million patients are diagnosed with cancer every year, and approximately 24 million new cases of cancer will probably be registered by 2035. Over the past decades, numerous cancer targets have been identified and, despite promising results in this field, the discovery of novel targeted therapies is still a largely unmet need. Sigma receptors (SRs), more highly expressed in tumor cells than in normal cells, have aroused the interest of the scientific community because of increasing evidence of their involvement in proliferation and survival signaling pathways of cancer cells. Glioblastoma (GBM) and pancreatic cancer (PCa) are two of the most aggressive and lethal solid tumors. GBM patients usually relapse within 7-10 months of the end of first-line treatment with median survival of about 14.6 months while PCa is the fourth most fatal cancer in both men and women, with median survival of 6-12 months. Little or no improvement in prognosis has been obtained over the past 20 years. Thus, new approaches to the treatment of both tumors are urgently needed. In the present work, 3D GBM primary cultures endowed with stemness features and PCa cell lines were used to evaluate the antitumor activity of a dual S1R and S2R ligand RC-106, a novel sigma receptor modulator. Methods. RC-106 tissue distribution studies were performed in mice. PCa cell lines were assessed by cytotoxic assay (CellTiter 96® AQsauna One Solution Cell Proliferation Assay). Molecular analyses were performed by qRT-PCR and Western blot. Apoptosis and cell cycle were analyzed by flow cytometry. GBM primary cultures were isolated from surgical tumor samples. A rotatory cell culture system (RCCS) was used to obtain 3D cell cultures. Growth and morphology of the colonies were monitored by open-source AnaSp and ReVISP software tools. Molecular analyses were performed by flow cytometry and qRT-PCR. 3D cell viability was measured using CellTiter-Glo® 3D Cell Viability Assay. Apoptosis and cell cycle were analyzed by flow cytometry. Results. RC-106 preferentially accumulate in mice pancreas and brain after intravenous administration, supporting the use of sigma receptor modulators as a novel therapy for these tumors. Treatment with RC-106 strongly inhibited cell proliferation and survival regardless of the type of cancer investigated. 25 μM of the drug impaired the in vitro clonogenic ability of tumor cells, an effect lasting for up to 42 days, the longest time tested. RC-106 also led to the induction of substantial apoptosis mediated by caspase 3 and 9 activation, and to a modulation of the Akt pathway. Furthermore, treatment with RC-106 significantly reduced the growth of human GBM and PCa spheroids. Conclusions. (RC-106) represents the hit compound of a new class of dual-action ligands targeting S1R and S2R potentially useful for the treatment of cancer disease.

### #1130 Targeting the tumor-associated autoantigen alpha-enolase in prostate cancer.

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Prostate cancer (PCa) is the most commonly diagnosed cancer and second leading cause of cancer-related deaths in American men. African American (AA) men are more likely to be diagnosed with aggressive PCa at a younger age and twice as likely to die from the disease as European American (EA) men. In order to reduce PCa mortality and its associated racial disparities, there is a critical need to identify and target pathways responsible for PCa aggressiveness. An unexplored target is the plasminogen system, which is key to cancer cell migration and tissue invasion during metastasis. During inflammation, activation of the plasminogen pathway degrades extracellular fibrin networks; however, in the context of cancer, this activation promotes cancer tissue invasion and metastasis. Mounting evidence shows that the glycolytic enzyme alpha-enolase (ENO1) plays a vital role in both increased energy metabolism and plasminogen activation and metastasis. Using immunoproteomic profiling of anti-tumor autoantibody responses in AA and EA men with PCa, we identified several tumor-associated autoantigens with functions in glycolysis and plasminogen signaling, including ENO1, annexin A2, fructose bisphosphate aldolase, glucose-regulated protein 78, glyceroldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase. In a cohort of PCa (N=157) and PRMT5 knockdown (N=183), we found a significantly higher frequency of autoantibodies to ENO1 in patients with PCa (p<0.05). Surprisingly, when we probed a panel of non-PCa and PCa cell lines with anti-ENO1 positive sera from AA and EA patients with PCa, we observed that AA patients showed decreased immunoreactivity to this protein in metastatic PCa cells, with decreased reactivity in docetaxel-resistant cell lines. By contrast, the anti-ENO1 EA-PCa sera and a monoclonal mouse anti-ENO1 showed relatively uniform immunoreactivity across the same panel cell lines. This suggested that the AA-PCa patients are producing antibodies to a different ENO1 variant than EA-PCa patients. Proteomic analysis of post-translational modifications (PTM) in ENO1 showed differences in ENO1 PTM profiles between the parental PC3 bone metastatic cell line and the PC3 docetaxel-resistant cell line, which could explain the differential autoantibody reactivity observed in AA and EA men. Given that ENO1 is up-regulated in PCa tissue, plays a role in glucose metabolism and plasminogen activation during cancer progression, and that the immune system of a cohort of AA-PCa patients recognizes an alternately modified variant of ENO1 that is upregulated in metastatic PCa cells, we elected to target this protein in metastatic PCa cell lines. ENO1 deletion via siRNA knockdown in PC3 cells led to increased cell death, with concomitant decrease in PCa proliferation. Taken together, these results highlighted the value of immunoproteomics as a tool to identify novel therapeutic targets, with ENO1 as a promising target in metastatic PCa.

### #1131 O-GlcNAc transferase inhibition in breast cancer cells.

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O-GlcNAc transferase (OGT) catalyzes the transfer of O-GlcNAc residues from OGT and its effect on TNBC cell survival and apoptosis. In conclusion, OGT inhibition in two triple-negative (TNBC) and two receptor-positive luminal breast cancer cell lines. Treatment of cells with an OGT inhibitor as well as OGT knockdown led to a strong decrease in viability and proliferation of the TNBC cells, but signif- icantly less so in the luminal receptor-positive cell lines. This differential re- sponse was evaluated using analysis of apoptosis, cell cycle, gene expression and a reverse-phase antibody-based protein array (RPRA). We found that cell cycle progression was affected by OGT inhibition. In addition, proteomics data pointed towards the transcription factor hairy and enhancer of split 3 (HES3) as a possible mediator of the cytotoxicity observed in the TNBC cell line. We currently work to elucidate the molecular mechanisms of HES1 regulation by OGT and its effect on TNBC cell survival and apoptosis. In conclusion, OGT inhibition has a pronounced cytotoxic effect on the TNBC cells and may have some potential for therapeutic use in the future.

### #1132 Lactate dehydrogenase expression in African American women with triple-negative breast cancer.

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The incidence of breast cancer in African American women (AAW) is much lower than those of their Caucasian counterparts. However, the age adjusted mortality rates are much greater for AAW compared to Caucasian American women.
subclones of T-ALL. Conclusion: This study shows that TG2 expression is elevated with steroid resistance in T-ALL and the modulation of TG2 induces the cytotoxicity of steroid-adapted T-ALL. TG2 could be a new therapeutic target to overcome steroid resistance in T-ALL. Furthermore, change of TG2 expression could serve as markers of induced steroid resistance in T-ALL.

#1135 DRD2 is critical for pancreatic cancer and promises pharmacological therapy by already established antagonists. Pouria Jandaghi,1 Hamed S. Najafabadi,1 Andrea Bauer,2 Andreas I. Papadakis,1 Matteo Fassan,1 Anita Hall,1 Anie Monast,1 Maryam Safisamghabadi,1 Magnus von Knebel Doeberitz,4 John P. Neoptolemos,7 Eithne Costello,8 William Greenhall,9 Aldo Scarpa,7 Bence Sipos,10 Daniel Auld,11 Mark Lathrop,12 Morag Park,13 Markus W. Büchner,14 Oliver Strobel,15 Thilo Hackert,16 Natalia Giese,17 George Zogopoulos,18 Veena Sangwan,20 Sidong Huang,20 Jörg D. Hoheisel,19 Yaser Riazalhosseini1. 1McGill University, Montreal, Quebec, Canada; 2Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany; 3ARC-NET Center for Applied Research on Cancer, University and Azienda Ospedaliera Universitaria Integrata, Verona, Italy; 4Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany; 5National Institute for Health Research, Liverpool Pancreas Biomedical Research Unit, Liverpool, United Kingdom; 6National Institute for Health Research, Liverpool Pancreas Biomedical Research Unit, United Kingdom; 7ARC-NET Center for Applied Research on Cancer, University and Azienda Ospedaliera Universitaria Integrata, Italy; 8Institute for Pathology and Neuropathology, Universitätshôpital Tübingen, Germany; 9Department of Surgery, University Hospital Heidelberg, Germany.

Introduction and aims: Although the overall five-year survival of all patients with cancer stands at 63%, for pancreatic cancer patients, it is a disheartening 8% - a number that remains largely unchanged for three decades. Of the patients diagnosed with pancreatic cancer, about 85% exhibit pancreatic ductal adenocarcinoma (PDAC). Most of these patients die within 4 to 6 months after diagnosis. The poor prognosis is caused by the detection at only late stages, and lack of effective options for chemotherapy. The widely used chemotherapeutic agent gemcitabine, confers a median survival advantage of only 6 months, and resistance to therapy develops in the vast majority of patients. Given this poor prognosis of patients with PDAC, there is an urgent need to find more effective therapies. Experimental procedures: Microarrays were used to perform global gene expression profiling in 195 PDAC and 41 normal pancreatic tissue samples. Using these profiling data, we undertook an extensive analysis of PDAC transcriptome by superimposing the pathway context and interaction networks of aberrantly expressed genes to identify factors with central roles in PDAC pathways. Next, tissue microarray analysis (TMA) were used to verify the expression of the candidate target in independent set of 152 samples comprising 40 normal pancreatic tissues, 49 chronic pancreatitis sections (CP) and 63 PDAC samples. We further validated the functional relevance of the candidate molecule through RNA interference (RNAi) and pharmacological inhibition in vitro and in vivo. Results: We identified dopamine receptor D2 (DRD2) as a key modulator of cancer proliferation in PDAC. DRD2 up-regulation at the protein level was validated in a large independent sample cohort. Most importantly, we found that blockade of DRD2, through RNAi or pharmacological inhibition using FDA-approved antagonists hampers the proliferative and invasive capacities of pancreatic cancer cells while modulating CAMP and endoplasmic reticulum stress pathways. Also, we observed a potent effect of DRD2 antagonists on inhibition of cancer cell proliferation using different model of primary and metastatic tumor cells derived from spontaneous pancreatic cancer mouse models and patient-derived pancreatic adenocarcinoma mouse xenograft (PDx) models. Conclusions: Our findings demonstrate that inhibiting DRD2 represents a novel therapeutic approach for PDAC. Since DRD2 inhibitors are already in the clinic for the management of schizophrenia, our results from this study could support a drug repositioning strategy to expedite clinical evaluation of these agents as novel therapy against pancreatic cancer.

#1136 Heat shock protein 90 inhibitors suppress PDGFRα reactivation and other receptor tyrosine kinase activation important in drug resistant gastrointestinal stromal tumors. Masahiro Yamamura, Akira Yamauchi, Naoki Katase, Makoto Okawaki, Yousuke Katata, Futoshi Kuribayashi, Junichi Kuribayashi, Yoshiyuki Yamaguchi. Kawasaki Medical School, Kurashiki, Japan.

Background: Inhibition of KIT oncoproteins by imatinib induces clinical responses in most gastrointestinal stromal tumor (GIST) patients. However, many patients develop imatinib resistance due to secondary KIT mutations. The drug resistance mechanism has only been partially elucidated, and the sunitinib resistance mechanism is also unknown. In this study, we elucidate the new imatinib resistant mechanism and find effective inhibitors. Methods: We have established imatinib-resistant cells GIST-T1R by culturing cells with increasing concentrations of imatinib. We analyzed receptor tyrosine kinase and intracellular signal protein strongly expressed in resistant cells using Western blotting
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and Phosphorylation Array. Then, the antitumor effect was examined using an agent that inhibits strongly expressed molecules of the resistant cells. Results: The IC50 of imatinib in the sensitive cells (GIST-T1) and the resistant cells (GIST-T1R) was 0.10 nM and 0.2 nM, respectively, which demonstrated 2000-fold difference. These imatinib resistant cells were also cross-resistant to sunitinib. In western blotting, phosphorylation of PDGFRα in drug-resistant cells was enhanced in GIST-T1R compared to GIST-T1. When GIST-T1 was treated with imatinib, KIT phosphorylation was suppressed but PDGFRα phosphorylation was enhanced. Imatinib treatment of GIST-T1R enhanced PDGFRα phosphorylation and protein expression as compared to before imatinib treatment. Phosphorylation of PDGFRα in these resistant cells could not be suppressed by regorafenib. Furthermore, in the protein phosphorylated array, in addition to phosphorylation of PDGFRα in resistant cells, phosphorylation of multiple RTKs was also enhanced. An HSP90 inhibitor AUY922 capable of simultaneously suppressing a plurality of proteins was used. AUY922 suppressed GIST-T1 and GIST-T1R cell proliferation at low concentrations and was more effective than regorafenib. Furthermore, AU9122 inhibited phosphorylation and protein expression of PDGFRα in drug-resistant cells, as well as phosphorylation of other several receptor tyrosine kinases. Conclusion: Activation of PDGFRα was considered to be important for the mechanism of drug resistance of GIST. Furthermore, since activation of multiple receptor tyrosine kinases can be observed simultaneously in drug resistance, it is considered that inhibiting a single target is not effective. Since HSP90 inhibition suppresses PDGFRα protein expression and possibly inhibits multiple target proteins at the same time, it is the most effective treatment for drug resistant GIST.

#1137 ADAM8 drives aggressive phenotype of triple-negative inflammatory breast cancer & constitutes a novel therapeutic target. Mathilde Romagnoli,1 Stefania Pianetti,1 Sonia G. Das,1 Delphine Loussouarn,2 Carole Gourmelon,2 Mario Campone,3 Sophie Barillé-Nion,1 Giang T. Nguyen,1 Srirathi Sriniwason,1 Gill E. Sonenstein,1 Nora D. Mineva,1 Tufts Univ. School of Medicine, Boston, MA;2Institut de Cancérologie de l’Ouest, Saint-Herblain, France;3UMR 892 INSERM, Nantes, France.

Inflammatory Breast Cancer (IBC) is a rare, highly aggressive form of cancer that is frequently locally advanced or metastasized at the time of diagnosis. The Triple-Negative subtype of IBC (TN-IBC), in particular, is characterized by very poor overall survival. TN-IBC lacks targeted therapies and is primarily treated with radiation or chemotherapy, which are inefficient. Recently, we identified the cell surface transmembrane ADAM8 (A Disintegrin and Metalloproteinase) protein as a driver of Triple-Negative Breast Cancer (TNBC) growth and metastasis via its Metalloproteinase (MP) and Disintegrin (DI) domains, respectively. In vivo proof-of-concept experiments with a prototype reagent, we demonstrated that simultaneous, antibody-based targeting of the ADAM8 MP and DI domains is an effective therapeutic approach for TNBC (Romagnoli et al., EMBO Mol. Med. 6:278, 2014). The aggressive behavior of IBC cells has been attributed to a stem-like cancer cell compartment with high ALDH activity (ALDH+) and the overexpression of the green tea compound epigallocatechin-3-gallate (EGCG). However, we recently revealed that EGCG simultaneously decreases tumor levels of ADAM8 mRNA. This finding is not associated with a stem-like cancer cell compartment with high ALDH activity (ALDH+). Our study is the first, to our knowledge, to demonstrate that in TN-IBC cells, ADAM8 expression negatively correlates with EGCG treatment. We now report that EGCG concurrently decreases tumor levels of ADAM8 mRNA. This led us in the future to examine the role of ADAM8 in TN-IBC. Tumor biopsies from 15 patients taken at the time of diagnosis and/or after neo-adjuvant treatment with chemotherapy, as well as paired lymph node and skin samples (when available) were analyzed by immunohistochemistry for ADAM8 expression. We found that ADAM8 expression is driven by a high ALDH+ stem-like SUM-149 cell compartment. Consistently, knockdown of ADAM8 dramatically reduced the ability of SUM-149 cells to grow in an anchorage independent manner. In a newly established Matriptase−/−M14 tumor cell line, autonomous ADAM8 mouse monoclonal antibody (ADP13) inhibited the MP and DI domains of ADAM8 on SUM-149 cells. ADP13 reduced orthotopic growth of tumors derived from SUM-149 cells by 40% in mice treated by i.p. injection 2 days a week with a dose of 4.5 mg/kg (n = 7). Dose-response curves and survival experiments are in progress. Conclusions: ADAM8 expression is present in almost all of TN-IBC patient tumors and their metastases, and promotes aggressive phenotype of TN-IBC cells in vitro and in vivo 3D-assays. A pre-clinical mouse model of TN-IBC validated ADAM8 as an accessible and promising new target for therapeutic intervention against this highly aggressive disease.

#1138 The protein disulfide isomerase inhibitor XCE853 inhibits in vitro, ex vivo and in vivo growth of human tumors. Gregory Pierre Prevost,1 Anne Chauchereau,2 Patrick Ladam,3 Renaud Seigneuric,4 Denis Carniato,5 Marc-Henry Pitty,4 Paul Foster,3 CIPREDO, Antony, France;2Gustave Roussy, Villejuif, France;3Université Paris XIII, Bobigny, France;4Université de Bourgogne, Dijon, France;5Cancer Genome Informatics, Paris, France;2University of Birmingham, Birmingham, United Kingdom.

Protein disulfide isomerase (PDI) is a chaperone protein that regulates oxidative protein folding as well as cell viability. Increased PDI levels have been documented in a variety of human cancers associated with a poor overall survival, including ovarian, prostate, brain and lung cancers. Inhibiting PDI activity leads to apoptosis in cancer, suggesting that PDI is a promising drug target. XCE853 is a synthetic small molecule displaying an excellent docking with the catalytic domain of the human PDI. XCE853 inhibits in vitro recombinant PDI enzymatic activity. In addition, the proliferation of a large panel of human tumor cells is blocked by XCE853 with IC50s in the nanomolar range through an irreversible cytolysis leading to a tumor cell death by autophagy and particles release (vesicles or protein aggregates). XCE853 is also active on a large panel of drug resistant human cancer cells. XCE853 induced an irreversible cytolysis of human tumor cells after a short in vitro exposure independently of efflux pumps. In addition, the ex-vivo approach using fresh human tumor explants cultivated in 3 dimensions with low concentrations of XCE853 has shown a strong decrease of the xenografted tumors (Ki-67, PDI expression) in several xenograft models leading to a complete tumor growth arrest even after the cessation of the treatment. Altogether, these data strongly support further efforts to move this drug candidate to the preclinical studies to access advanced cancer patients.

#1139 G protein-coupled receptors (GPCRs): unrecognized potential therapeutic targets in cancer. Paul A. Insel, Krishna Siriram, Shu Z. Wiley, Thalia McCann, Randall P. French, Andrew M. Lowy, UCSD, La Jolla, CA.

G protein-coupled receptors (GPCRs) are the largest class of signaling receptors in humans and other species and in addition, the most widely targeted class for FDA-approved therapeutics. However, GPCRs are not commonly targeted in cancers other than endocrine tumors. We hypothesize that GPCRs may have been “missed” as targets in cancer, perhaps in part because they have not been frequently identified as driver mutations; however, GPCRs may have unappreciated utility as therapeutic targets in a variety of tumors. To test this hypothesis, we have used unbiased approaches (Taqman GPCR arrays and RNA-seq), mining of publicly available databases (e.g., The Cancer Genome Atlas, TCGA), and validation studies (e.g., protein and functional analyses) to assess GPCRs in a variety of human tumors, cancer cells and stromal cells (cancer-associated fibroblasts, CAFs). A major focus of our efforts has been on pancreatic ductal adenocarcinoma (PDAC) cells/tumors and pancreatic CAFs (PCAFs). We find that PDAC cells and PCAFs express >70 different GPCRs, including many orphan GPCRs (receptors without known physiologic agonists) and that numerous GPCRs are expressed at much higher levels than in normal precursor cells (pancreatic ductal epithelial cells for PDAC cells and pancreatic stellate cells and pancreatic fibroblasts for PCAFs). We find that a cluster of GPCRs is overexpressed in PDAC tumors. Two such receptors are orphan GPCRs, Orphan 1 and Orphan A, neither of which is mutated but each has high expression, respectively, in PCAFs and PDAC cells. Orphan 1 and Orphan A have at least 2-fold higher expression in >90% of PDAC tumors in TCGA compared to that in normal pancreatic tissue (compiled from the GTEx database). Higher expression of Orphan A is associated with decreased survival and remission, while Orphan 1 expression correlates with that of numerous fibrotic genes. Importantly, both Orphan 1 and Orphan A are functional and appear to contribute to the malignant phenotype. For example, siRNA knockdown of Orphan 1 in PCAFs blunts production of profibrotic markers and decreases ability of conditioned media from PCAFs to enhance proliferation of PDAC cells while transfection of Orphan A increases DNA synthesis of normal pancreatic ductal epithelial cells. Taken together, these and other results suggest that in addition to their role in endocrine tumors, GPCRs represent previously unrecognized contributors to cancer through actions on tumor cells and stromal cells. These data suggest the possibility that highly expressed GPCRs (such as Orphan A) may function as oncogenes. Thus, GPCRs may be druggable, novel targets for the treatment of cancer, including pancreatic cancer, a tumor for which new therapeutics are an important, unmet need.
lines expressing higher GABRP, while the same doses of a control antibody had no effect in these cells in culture. Our goal is to generate an antibody drug conjugate (ADC) for TNBC by conjugating anti-GABRP antibody with a maytansinoid such as DM1, which has proven cytotoxicity in breast cancer cells. Together, our results suggest that GABRP is a potential therapeutic target for triple-negative breast cancers.

### 1142 Novel target discovery for glioblastoma using chemical biology fingerprinting.
Darren Finlay, Pedro Aza-Blanc, Harshil Dhruv, Alexey Eroshkin, Craig Hauser, Jeff Kiefer, Seunghan Kim, Tao Long, Robert G. Oshima, Sen Peng, Gil Speyer, Michael Berens, Kristiina Vuori. Sanford Burnham Prebys Med Discovery Institute, La Jolla, CA; TGen, Phoenix, AZ.

The most common adult brain tumor is Glioblastoma Multiforme (GBM), an extremely aggressive cancer with only scant treatment options. Even with standard of care most patients present with a recurrence and the median survival is only circa 15 months. The need, therefore, for new therapeutic targets and treatment options is pressing. Here we describe here a multipronged approach to identifying said targets. We present an established methodology for the isolation and culture of patient derived GBM samples that retain the “stem-like” fraction thought to underlie resistance and recurrence. Furthermore we show genomically that these samples represent specific subtypes of the disease yet still form distinct groups in unbiased clustering analysis. Thus we have multiple representative patient derived cultures that are suitable for our drug discovery and chemical biology analyses. Using a process we term Chemical Biology Fingerprinting (CBF) we utilize small focused, and clinically relevant, chemical collections in order to identify patterns of chemosensitivities across multiple samples. This allows an unbiased yet cancer relevant sub-stratification and the identification of agents, and therefore targets, which may be relevant for GBM patient subtypes. Indeed our use of the highly annotated NCI CTD[1] Informer Set of chemicals allows ready drug-to-target mapping and facilitates data sharing across the CTD[2] network. Moreover, already defined subgroups can be clustered to find agents, or groups of agents, that show selective activity against traditional classifications (e.g. proneural, mesenchymal etc.). Finally our strategy is permissive for the identification of “exceptional responders”. That is, individual patient samples that respond to a specific drug whilst most samples are refractory. In sum we demonstrate generation of patient derived models and identify specific, and novel, drugs that may be relevant for specific GBM subtypes. Supported by NIH U01CA168397.

### 1143 Targeting the transcriptional kinases CDK12 and CDK13 in breast and ovarian cancer.
Michael Bradley, Jason Marineau, Yoon Choi, Kristin Hamman, Goran Malojic, David Orlando, Xiyuan Ren, Nan Ke, Shanh Hu, Eric Olson, Christian Fritz, Christopher Roberts. Syros Pharmaceuticals, Cambridge, MA.

CDK12 and CDK13 regulate expression of large transcripts requiring substantial processing to produce mature mRNA. This transcriptional regulation includes coordinated phosphorylation of specific repeats within the C-terminal domain of RNA polymerase II and association with RNA processing factors (Chila, 2016). RNAi knockdown of CDK12 in cell culture decreases expression of DNA damage response genes, including BRCA1 and ATR, while enhancing sensitivity to DNA damaging agents (Blasek, 2011; Liang, 2015). Recently, THZ531, a selective covalent inhibitor of CDK12 and CDK13, was shown to decrease expression of DNA damage response genes in cell culture (Zhang, 2016). Here we present further studies with THZ531 to guide our discovery program toward molecules suitable for clinical development and to explore mechanistic rationales for combining a CDK12/13 inhibitor with PARP inhibitors or DNA damaging agents for difficult-to-treat cancers such as high-grade serous ovarian cancer and triple-negative breast cancer. Using THZ531 as a benchmark, we developed assays capable of discriminating sub-nM inhibitors, including quantifying time-dependent covalent inhibition and cell-based CDK occupancy. Since CDK7, like CDK12 and CDK13, contains a cysteine residue proximal to the kinase active site, these approaches are critical to understand covalent inhibitor selectivity. Furthermore, we performed kinase paneling studies to better understand selectivity of this scaffold in support of our ongoing efforts to optimize CDK12/13 potency and selectivity. To pharmacologically investigate the previously reported effects of CDK12 RNAi, growth inhibition of a panel of ovarian and breast cancer cell lines was assessed following treatment with THZ531 (OVYA EC50 = 50-200 nM (n=6); BRCA EC50 < 30 nM (n=4)). Expression profiling further confirmed that THZ531 treatment resulted in different sets of genes being affected than was observed following treatment with inhibitors targeting CDK7, CDK9 or BET-bromodomain proteins. Additionally, CDK12/13, CDK7 and CDK9 inhibitors were profiled in a broad cell line panel (n=400) to reveal relationships between inhibitor sensitivity, mutation status, gene expres-
sion, and potential oncology indications that may be addressed by these different mechanisms. Finally THZ531 was synergistic with both PARP inhibitors and DNA damaging agents in ovarian and breast cancer cell lines. These data highlight cancer indications and combinations that may be particularly amenable to treatment with CDK12/13 inhibitors. While the pharmacokinetic properties of THZ531 and its target engagement profile is consistent with tumor regression in mice, in mouse models system, our ongoing medicinal chemistry program is progressing to identify and optimize CDK12/13 inhibitors suitable for clinical evaluation.

#1144 Dual inhibition of MEK1/2 and PLK1 specifically targets aggressive breast cancer cell population with CEP55 elevated expression. Debottam Sinha, Murugan Kalimuthu, J. Alejandro Lopez, Kum Kum Khanna. QIMR Berghofer Medical Research Institute, Brisbane, Australia.

Triple negative breast cancers (TNBCs), lacking the expression of ER, PR and HER2 amplification, are the most aggressive form of breast cancer (BC). Due to their heterogeneity influenced by high level of genomic instability and aneuploidy, treatment of TNBC patients is one of the biggest challenges faced in the clinics. CEP55, discovered first by our laboratory, is a key regulator of cytokinesis. Research has demonstrated association of CEP55 with various cancers especially BC as over-expression of CEP55 mRNA is associated with worse BC prognosis and poor survival. We hypothesized that, CEP55 regulates the fate of aneuploid cell population, which is cell cycle arrest, as well as senescence and apoptosis. Their heterogeneity influenced by high level of genomic instability and aneuploidy, treatment of TNBC patients is one of the biggest challenges faced in the clinics. We have performed a series of in vitro studies demonstrating that depletion of CEP55 sensitizes cells to anti-mitotic drugs like PLK1 inhibitor (BI2536) and leads to unscheduled CDK1/Cyclin B activation and favor CDK1-Caspe 3-dependent mitotic catastrophe. We also demonstrate that its inhibition of CEP55 inhibits CEP55 mRNA translation at specific mitotic genes for tumor progression among aggressive BC, thus can be targeted for therapy development. We have performed a series of in vitro studies demonstrating that depletion of CEP55 sensitizes cells to anti-mitotic drugs like PLK1 inhibitor (BI2536) and leads to unscheduled CDK1/Cyclin B activation and favor CDK1-Caspe 3-dependent mitotic catastrophe. Thus, the involvement of CEP55 at specific mitotic genes for tumor progression among aggressive BC, thus can be targeted for therapy development.

#1145 Overcoming chemoresistance and blocking metastasis testing in breast cancer by targeting HuR. Xiaojing Wu, 1 Rebecca Marquez, 1 Shuang Han, 1 Lan Lan, 1 Dan A. Dixon, 1 Jeffrey Aubé, 1 JeffreyAubé, 3 DannyR. Welch, 2 LiangXu1. 1Debottam Sinha, Murugan Kalimuthu, 1 Jeffrey Aubé, 3 Danny R. Welch, 2 Liang Xu. 1University of Kansas, Lawrence, KS; 1University of Kansas Medical Center, Kansas City, KS; 3University of North Carolina, Chapel Hill, NC.

Patients with metastatic breast cancer have a dismal 5-year survival rate of only 24%. Chemoresistance is another contributor to the high mortality of advanced breast cancer. The RNA-binding protein, Hu antigen R (HuR), is overexpressed at all stages of breast cancer development. Cytoplasmic HuR accumulation correlates with high-grade malignancy, poor distant disease-free survival and serves as a prognostic factor for poor clinical outcome in breast cancer. HuR promotes tumorigenesis by promoting mRNA stability and translation of proteins implicated in proliferation, survival, angiogenesis, invasion, and metastasis. HuR also modulates sensitivity of breast cancer cells to chemotherapy. Taken together, HuR is an emerging target for breast cancer therapy, especially metastatic breast cancer. Using shRNA and CRISPR technologies to modulate HuR expression in breast cancer cells, we found that cells with HuR KD/KO were less invasive and more sensitive to chemotherapy. In an effort to discover small molecules that disrupt the HuR-mRNA interaction and block HuR functions in breast cancer progression, metastasis and chemoresistance, high throughput screening (HTS) was carried out in several chemical libraries (~23,000 compounds) using fluorescence polarization (FP) assay, which identified several initial hits with sub-micromolar inhibitory constants (Ki). Those potential hits were then validated by ALPHAV assay (Amplified Luminescent Proximity Homogeneous Assay), confirmed by Surface Plasmon Resonance (SPR). In cell-based assays, top hit KH-3 and its optimized analogs, specifically shortened HuR target mRNA half-lives and decreased the level of the encoded proteins. More-over, those compounds inhibited invasion and restored chemosensitivity. qPCR arrays focusing on specific pathways revealed that HuR inhibitors potently up-regulated metastatic suppressors and downregulated genes frequently overexpressed in lung metastases. In animal studies, KH-3 not only exhibited potent antitumor efficacy in orthotopic xenografts of breast cancer, but also efficiently inhibited tumor growth in a preclinical model of breast cancer. In conclusion, we identified an array of potent and specific small molecule inhibitors of HuR-mRNA interaction for potential anti-metastatic therapy for breast cancer with HuR overexpression.

#1146 Biomarker-driven targeted oral treatment strategy for bladder cancer. Daley S. Morera, Andre Jordan, Vinata B. Lokeshwar. Augusta University, Augusta, GA.

INTRODUCTION AND OBJECTIVE: Hyaluronic acid (HA) family of molecules, HA-synthases (HAS-1,2,3), HA-receptors (CD44, RHAMM) and hyaluronidase (HYAL-1) are markers for bladder cancer (BCa) diagnosis and predicting prognosis. BCa-family promotes tumorigenesis and metastasis by inducing epithelial mesenchymal transition (EMT). 4-Methylumbellifere (4-MU) is an orally bioavailable dietary supplement that inhibits HA synthesis. We evaluated the expression of HA family and EMT markers in bladder tissues as well as the antitumor effects of 4-MU as a potential targeted therapeutic agent in preclinical evaluation of BCa. METHODS: Quantitative PCR was used to measure mRNA expression of HA-family and EMT genes (β-catenin, Twist, and Snail) in 72 bladder tissue specimens (28 normal; 44 tumor); follow-up: 20.3±2.5 months; median 17 months. The effect of 4-MU (0.06-0.6 mM) on cell proliferation, apoptosis, intracellular signaling, and the expression of HA receptors, and EMT genes were examined in BCa cell lines by Q-PCR, immunoblotting, proximal ligand and PI-3K activity assays. Mechanism of action was analyzed by HA addition and mAkt overexpression. Effect of oral administration of 4-MU (100, 200-mg/kg) on tumor growth was analyzed in subcutaneous xenografts. RESULTS: HAS-1, -2, -3, HYAL-1 and Snail levels were 10-20-fold elevated in BCa tissues as compared to normal bladder (P<0.001). In univariate analysis, HAS-1, -2, HYAL-1 and Twist levels correlated with metastasis (P<0.001); HYAL-1 was an independent predictor of metastasis. 4-MU inhibited cell proliferation, chemotactic motility and invasion in a dose-dependent manner; 50-70% inhibition at IC50 (0.4 mM) for HA-synthesis inhibition. 4-MU induced apoptosis (>3-fold) via the death receptor pathway. 4-MU downregulated HA-signaling, specifically transcript and/or protein levels of CD44, RHAMM, p-Akt, β-catenin, p-b-catenin(S552). Snail and twist were downregulated in 2-5-fold, but pβ-catenin(T41/S45), pGSK-3β and E-cadherin levels were increased. 4-MU also inhibited CD44/PI-3K complex formation and PI-3K activity. HA addition or myristoylated Akt expression attenuated 4-MU effects. In xenograft studies, 4-MU oral treatment abrogated tumor growth of established tumors (vehicle, day 50: 766±221 mm2; 4-MU: 128±61, day 50) by abrogating HA-synthesis. In bladder tumor cells, 4-MU inhibited HA-signaling. However, a potent and selective inhibitor to target the SUMO pathway has been lacking. The SUMO-activating enzyme (SAE) is an essential enzyme in the pathway that initiates the SUMOylation process. Here we report the identification of the first mechanism-based SAE inhibitors with nanomolar potency in cellular assays. These inhibitors selectively block SAE enzyme activity and total SUMOylation in cells, which leads to reduced cancer cell proliferation. Moreover, SAE inhibition resulted in disruption of PML nuclear bodies and redistribution of DAXX. In vivo administration of SAE inhibitor into tumor bearing mice results in modulation of several biomarkers including a significant reduction in SUMO-conjugates and E2 Ubch9aestheor, demonstrating SUMO pathway inhibition. Our results demonstrate the feasibility of inhibiting the SUMO pathway with small molecule inhibitors and provide tools to study the SUMO biology in cancer.
Bazedoxifene as a novel GP130 inhibitor for the treatment of triple-negative breast cancer. Jilai Tian,1 Yang Cao,1 Xiang Chen,1 Hui Xiao,1 Chen-gong Li,4 Jiayuh Lin,1,5 University of Maryland, Baltimore, MD; 4Hauzong University of Science and Technology, Wuhan, China; 5The Ohio State University, Columbus, OH; 4University of Florida, Gainesville, FL.

Triple-negative breast cancer (TNBC) is the only subtype of breast cancer still lacking effective therapeutic options. Thus, finding novel targets and therapies to treat TNBC is an urgent clinical issue. IL-6 is one of the principal oncogenic mediators in breast cancer and systemic IL-6 levels correlates with poor prognosis, advanced disease, and metastases. Importantly, growth of TNBC cells are replied on autocrine cytokines including IL-6. Therefore, IL-6 signaling represents a novel therapeutic approach with a potential to improve the therapeutic efficacy. To date, however, no small molecules that target IL-6 signaling are available for clinical cancer therapy. IL-6 binds to IL-6Ra, then recruits GP130 to form the IL-6/IL-6Ra/Gp130 heterotrimer and triggers a signaling cascade downstream including JAK/STAT3, PI3K/AKT/mTOR, and ERK. Therefore, it is possible to target IL-6 signaling by blocking IL-6Ra or IL-6R and thus inhibiting its signaling cascade downstream. To effectively target IL-6 signaling, we have utilized a novel drug discovery approach combining Multiple Ligand Simultaneous Docking and drug repurposing to target GP130, since its structure is more druggable than IL-6R. We have identified a FDA-approved drug Bazedoxifene (for the prevention of the postmenopausal osteoporosis in postmenopausal women) with a novel function to inhibit IL-6R and GP130 protein-protein interactions and thus blocking downstream IL-6 signaling.

In this study, we examined the therapeutic effect of Bazedoxifene on TNBC, aiming to verify Bazedoxifene as a Novel IL-6/IL-6R Inhibitor for TNBC treatment. Our data of western blot showed Bazedoxifene inhibited increased STAT3 phosphorylation induced by IL-6. In addition, Bazedoxifene inhibited phosphorylation of AKT and ERK in TNBC cells that were also produced by IL-6. Furthermore, combination of Bazedoxifene with paclitaxel exhibited more significant inhibition than single agent alone on cell viability in TNBC cells either in 2D or 3D culture model in vitro. Results of colony formation showed that Bazedoxifene could significantly inhibit cell survival and proliferation at 20 μM, which were more effective than Evista and SC144. Moreover, Bazedoxifene could inhibit cell migration. We tested the increased proliferation of TNBC cells using BrDU with the added IL-6, which were then inhibited by Bazedoxifene, demonstrating the Bazedoxifene could achieve a competed inhibition of IL6. The result of significant inhibition of tumor growth in vivo further verified the therapeutic effects of Bazedoxifene. All results showed the significant therapeutic effects of Bazedoxifene in TNBC cells by competing blocking IL6 signaling. Thus, Bazedoxifene holds a great potential for TNBC therapy.

Targeting Rad6B suppresses melanomagenesis. Ashapurna Sarma,1 Sarah Pett1, Fangchao Liu,1 Pratima Nangia-Makker,2 Guangzhao Mao,2 Mal- athy Shekhar1. 1Wayne State University and Karmanos Cancer Institute, Detroit, MI; 2Wayne State University, Detroit, MI.

Active β-catenin signaling in melanoma is associated with aggressive disease. We have previously shown that Rad6B, an ubiquitin conjugating enzyme, is a positive regulator of β-catenin stability and transcriptional activity, and is itself a transcriptional target of β-catenin. This stabilization is mediated by Rad6B-induced K63-linked polyubiquitination of β-catenin that renders it insensitive to 26S proteasomal degradation. Immuno-histochemical analysis of human cutaneous melanoma samples showed the first detectable expression of Rad6B in melanocyte hyperplasia and continued Rad6B and β-catenin overexpression in primary and metastatic melanomas. These data implicate a critical role for Rad6B in melanocyte transformation and a continuing role in melanoma progression. To analyze the role of Rad6B in melanomagenesis, we targeted Rad6B expression/activity in human M14 metastatic melanoma cells by using a Rad6B-selective small molecule inhibitor SM1#9 and the Crisp/RCas9 gene editing system. Immunoblot analysis showed that compared to controls, M14 cells treated with SM1#9 showed decreased levels of β-catenin, and β-catenin driven transcriptional targets Rad6, vimentin, Mitf-M, and Melan-A. Although Snail levels were not altered, SM1#9 treated cells showed increase in phosphorylated Snail. Immunohistochemical staining verified the western blot data and showed cyto-plasmic relocalization of PCNA and Snail in SMI#9 treated cells which is consistent with increased PCNA and Snail expression. Concordant with the decrease in vimentin and unphosphorylated Snail levels, Rad6B-inhibited cells display poor capacity to migrate as compared to controls. Whereas Rad6B knockout cells were severely growth impaired, Rad6B edited clones showed improved migration potential (potentially from only one allele) similarly showed decreased levels of β-catenin, vimentin, and Mitf-M. Similar to Rad6B-inhibited cells, Rad6B edited cells were migration- and invasion-impaired. Whereas control cells produced robust tumors and overt lung metastases, the Rad6B gene edited cells were poorly tumor-igenic. Rad6B gene edited clones showed complete loss of Melan A. Since Mitf-M is a transcriptional regulator of Melan A and a key player in melanocyte lineage specification, our data suggest that targeting Rad6B could potentially inhibit melanomagenesis by reprogramming melanoma cells. Supported by NIH CA178117 and 3Balls Racing.

Downregulation of Hedgehog signaling via Sirt6 activation is important to docosahexaenoic acid-induced cell death in human EGFR mutant non-small cell lung cancer. Soyeon Jeong,1 Kaejong Jing,1 Soyeon Shin,2 Seung-Hyeon Han,3 Yoon-Soon Yoo,3 Prashanta Silwal,2 Jun-Young Heo,2 Gi-Ryang Kweon,5 Seung-Kiel Park,4 Jong-II Park,4 Tong Wu,4 Kyu Lim.1,5 Dept. of Biochemistry, Infection Signaling Network Research Center, Chungnam National University, Daejeon, Republic of Korea; 2Dept. of Biochemistry, Cancer Research Institute, Chungnam National University, Daejeon, Republic of Korea; 3Dept. of Biochemistry, Dept. of Medical Science, School of Medicine, Chungnam National University, Daejeon, Republic of Korea; 4Dept. of Biochemistry, Dept. of Medical Science, School of Medicine, Infection Signaling Network Research Center, Chungnam National University, Daejeon, Republic of Korea; 5Dept. of Biochemistry, Chungnam National University, Daejeon, Republic of Korea.

Docosahexaenoic acid (DHA) induces apoptotic cell death through several mechanisms in cancer cells. Here we report that DHA-induced apoptotic cell death is related to the inhibition of Hh signaling via SIRT6 activation in human PC9 and H1975 nonsmall cell lung cancer (NSCLC). DHA-induced cell death was confirmed increase of the protein levels of cleaved PARP and caspase-3 Bax as well as the number of TUNEL positive cells and the Sub-G1 proportion in PC9 and H1975. DHA significantly increased the SIRT6 levels. Knockdown of SIRT6 by siRNAs inhibited apoptosis induced by DHA, while SIRT6 overexpression increased apoptotic cell death, indicating that DHA-induced SIRT6 activation results in apoptosis. In addition, SIRT6 activation by DHA treatment was associated with downregulation of Hh signaling and knockdown of SIRT6 resulted in augmentation of Hh signaling, suggesting elevation of SIRT6 levels in DHA-treated NSCLC cells leads to downregulate the Hh signaling. Smo activator SAG increased the protein levels of Hh signaling molecules by DHA, and SAG plus DHA treatment decreased cleaved PARP levels, implying that DHA-induced Hh signaling is related to apoptotic cell death. To unveil the effects of endogenous DHA on apoptosis via SIRT6-mediated Hh signaling downregulation, we used PC9 stable cell lines of fat-1 (ω3-desaturase) gene. The SIRT6 levels were significantly increased and Hh signaling molecules were diminished in fat-1 stable PC9 (f-PC9) cells, compared with the cells transfected with the control vector (c-PC9). Moreover, SIRT6 expression was induced and Gli and smo levels were inhibited in tumor tissues from f-PC9 cells-injected mice, demonstrating that DHA regulates SIRT6 and Hh signaling in vivo. Taken together, these results suggest that DHA may induce apoptotic cell death through SIRT6-modulated Hh signaling suppression in human NSCLC cells. These findings implicate that ω3-PUFAs may represent a potential active therapy for the chemoprevention and treatment of human NSCLC. [This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (2007-0504932, NRF-2015R1D1A1A01056887) and by the framework of international cooperation program managed by National Research Foundation of Korea (2015K2A2200620)].

SY-1365, a potent and selective CDK7 inhibitor, exhibits promising anti-tumor activity in multiple preclinical models of aggressive solid tumors. Shanhu Hu, Nan Ke, Yixuan Ren, Sofija Miljovska, Nisha Rajagopal, Michael McKeown, David Orlando, Kevin Sprott, Yoon J. Choi, Eric Olson, Christian C. Fritz. Syros Pharmaceuticals, Cambridge, MA.

CDK7 has recently emerged as an attractive target in cancer since its inhibition decreases the transcript levels of oncogenic transcription factors, especially those driven by super-enhancers (SEs). Cancers have been hypothesized to be addicted to SE regulated genes and simultaneous suppression of multiple SEs including JAK/STAT3, PI3-K/AKT/mTOR, and MEK/ERK. Therefore, it is possible to develop effective therapy for the chemoprevention and treatment of human solid tumors that are susceptible to SY-1365 and compare the effects of SY-1365 on gene expression to other gene control agents. SY-1365 was screened in a panel of solid tumor cell lines, revealing activity in breast, ovarian, colorectal...
and lung cancer cells with low nM EC50 and rapid induction of apoptosis. In breast cancer, a subset of triple negative breast cancer (TNBC) cell lines were found to be more sensitive than luminal breast cancer cell lines, so we extended our studies to in vivo models and showed substantial tumor growth inhibition in multiple patient-derived xenograft (PDX) models of TNBC. Since other compounds that target modulation of HSPGs, NT4 is a broad-spectrum HSPG targeted therapy and upregulated in a variety of breast cancers. This suggests that NT4 may have the potential to be used as a therapeutic agent for breast cancer and is currently under preclinical development.

**EXPERIMENTAL AND MOLECULAR THERAPEUTICS: New Targets 1**

Heparan sulfate proteoglycans (HSPGs) have crucial regulatory roles in tumor progression and invasion. HSPGs are composed of a core protein and glycan chains characterized by repeated disaccharide units which can be sulfated at different amount and position. HSPG have enormous structural diversity due to the different possible modifications of the single saccharide units within the polysaccharide, such as position, sulphation and acetylation. As a result HSPG can bind and modulate their binding to signaling molecules such as growth factors, morphogens and chemokines. HSPG proved to be important in mediating cancer development and progression by enhancing the binding of growth factors, morphogens and cytokines to their cognate receptors, thus activating signaling pathways that give rise to angiogenesis, cell growth and proliferation, cell survival, and metastasis.

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ONC201, founding member of the imidapridine class of compounds, is a highly selective small molecule G protein-coupled receptor (GPCR) antagonist that is in Phase I/II advanced cancer clinical trials. In this study, we evaluated GPCR engagement and antitumor activity of ONC212, an imidapridine that is a chemical analogue of ONC201. Experimental GPCR profiling using the PathHunter® β-Arrestin assay, determined that ONC212 is a selective agonist of the orphan GPCR GPR132/G2A. Multidose validation confirmed nanomolar GPR132 agonist activity (EC50 ~ 400nM). GPR132 is a stress-inducible orphan GPCR that causes G2/M arrest. The tumor suppressive role of GPR132 has been demonstrated most notably in lymphoid leukemia. Gene expression analysis of samples in the Cancer Genome Atlas (TCGA) revealed GPR132 is expressed in a range of tumor types with high expression in leukemia and lymphoma. The in vitro efficacy of ONC212 was assessed in the Genomic of Drug Sensitivity in Cancer (GDSC) collection of cell lines (>1,000 cell lines). Cell viability assays were performed at 72 hours post-treatment to generate dose responses curves at concentrations from 78nM up to 20μM. ONC212 was broadly efficacious against most solid tumors and hematological malignancies in the low nanomolar range. Ranking the sensitivity of cancer lines to ONC212 by tumor type revealed that leukemia and lymphoma are the most responsive tumor types based on completion of response (area under the dose-response curve). Interestingly, we also observed that high expression of GPR121 significantly correlated with ONC212 efficacy in GDSC cell lines, suggesting the importance of GPR132 agonist activity for ONC212 efficacy. ONC212 was tested in 62 human leukemia cell lines that included acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML) and hairy cell leukemia. ONC212 demonstrated broad spectrum anti-leukemic activity and was equally efficacious across all leukemia subtypes tested. Most cell lines (60/62) were responsive to ONC212 with GI50 ranging from <78nM to 456nM. Within ALL, both B-cell and T-cell ALL were highly sensitive to ONC212. ONC212 reduced cell viability in AML independent of complex karyotypes and p53 mutations that are associated with poor clinical outcomes. ONC212 was also tested in 58 lymphoma cell lines comprised of anaplastic large cell lymphoma, Burkitt’s lymphoma, cutaneous T-cell lymphoma (CTCL), diffuse large B-cell lymphoma (DLBCL), Mantle cell lymphoma (MCL), Follicular Lymphoma and Hodgkin’s lymphoma. ONC212 reduced cell viability in most cell lines (56/58) and was equally efficacious regardless of subtypes with GI50 ranging from <78nM to 261nM. Thus, GPR132 agonist ONC212 possesses robust anti-cancer activity in hematological malignancies irrespective of leukemia and lymphoma subtype and provides further validation of the anti-cancer efficacy of the novel imidapridine class of small molecules.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Novel Molecular Targets 1


The C-terminal binding protein (CtBP) is a family of dimeric nuclear proteins whose levels are increased in cancers of the colon, ovaries, prostate and breast. Elevated CtBP expression is associated with poor cancer survival and can also distinguish those node negative breast cancer patients who will show worse survival. This implicates CtBP as both a biomarker and a promising candidate for therapeutic intervention. As a dimer, CtBP provides a scaffold that couples multiple different DNA-binding transcriptional regulators with a variety of chromatin modifying protein complexes to alter the epigenetic landscape throughout the nucleus. These properties provide the rationale for pharmacological targeting of CtBP to change epigenetically regulated gene expression in cancer cells. In this study, we employ computer assisted drug design to screen for optimal quantitative structure-activity relationships (QSARs) between small molecules and CtBP to identify 24 potential CtBP inhibitors. Functional screening of these compounds identifies 4 lead compounds with low toxicity and high water solubility. Treatment of breast cancer cells at micro-molar concentrations of these small molecular inhibitors induces significant de-repression of epigenetically silenced pro-epithelial genes in the mesenchymal, triple negative breast cancer cell line, MDA-MB-231. This re-activation is associated with eviction of CtBP from the respective gene promoters, disrupted recruitment of CtBP-chromatin modifying protein complexes, increased deposition of activating epigenetic histone marks, and upregulation of both pro-epithelial gene mRNA and protein expression. In functional assays, CtBP inhibition by these small molecules decreases cellular invasion, and improves DNA repair. FRET ( Förster resonance energy transfer) analysis demonstrates that CtBP inhibition results in decreased FRET intensity, suggesting that CtBP dimerization is repressed by CtBP inhibition. In addition, pharmacological inhibition of CtBP combines with established epigenetically targeted drugs to synergistically decreases migration and invasion through degradation of anchorage independent positive gene expression in triple negative cancer cells. Finally, CtBP inhibition results in transcriptional repression of MDRI expression and reduces the population of Doxorubicin resistant cells in the triple negative breast cancer cell lines. These findings imply the possible use of this class of compounds in strategies for therapeutic intervention that may increase the efficacy and decrease the acquired resistance to targeted therapeutic intervention in breast cancer.

#1157 Screening and identification of new generation glucose transporter inhibitors as anticancer therapeutics. Yanrong Qian, Prattik Shriwas, Xuan Wang, Dennis Roberts, Emma Kessler, Stephen Bergmeier, Xiaozhao Chen. Ohio Univ., Athens, OH.

Cancer is a top killer second only to cardiovascular diseases. Cellular metabolic reprogramming and altered energetics are key features of cancer. Opportunistic uptake of extracellular nutrients has recently been named as an emerging hallmark of cancer metabolism. Glucose is a key nutrient taken in by cancer cells for satisfying their high energy and biosynthesis needs. Drastic upregulation of glucose uptake and glycolytic activity, a phenomenon called the Warburg effect, is prevalent in cancers. Cancer cells express very specific glucose transporters (Gluts), particularly Glut1, on cell membranes for taking in glucose via facilitated diffusion. Considering the addiction of cancer cells for glucose and their sensitivity and vulnerability to changes in glucose supply, Gluts, especially Glut1, have been recognized as an attractive anticancer target. We previously reported the identification of first generation lead Glut1 inhibitor WZB117 (1). WZB117 treatment of human nonsmall cell lung cancer (NSCLC) A549 cells resulted in inhibition of glucose uptake, glycolysis and induction of apoptosis and necrosis. In addition, WZB-117 reduced tumor growth by ~70% in an A549 tumor mouse model. WZB117 inhibit cell growth of 7 cancer cell lines in 4 cancer types with IC50 values lower than 10 μM. Through a structure activity relationship (SAR) study of WZB117, a second generation lead, DRB18, has been identified. DRB-18 is much more stable and 5 to 10 times more potent than WZB117 in inhibiting growth and proliferation in about 90% of the 60 different cancer cell lines in 9 major cancer types as revealed in NCI-60 screenings. And its IC50 in many cancer cell lines are in the high nM range. In this study, we also report new compounds screening results for the objective of identifying novel compounds with higher glucose transport-inhibitory and cancer cell growth-inhibitory activities using nonsmall cell lung cancer cell lines as models. References: 1. Liu, Y., Cao, Y., Zhang, W., Bergmeier, S., Qian, Y., Alkar, H., Colvin, R., Ding, L., Teng, L., Wu, S. and Finles, L., 2012. A small molecule inhibitor of glucose transporter 1 downregulates glycolysis, induces cell-cycle arrest, and inhibits cancer cell growth in vitro and in vivo. Molecular cancer therapeutics, 11(8):1672-1682.

#1158 Modulation of splicing by inhibiting the kinase SRPK1 as a novel therapeutic strategy in myeloid leukemia. Konstantinos Tzelepis,1 Etienne De Brakelleer,1 Michael Seiler,2 Iasia Barbieri,2 Sam Robson,3 Yu Hsuen Yang,4 Malgorzata Gozeckia1, Monika Dudek,2 Grace Collord,2 Oliver M. Dovey,3 Em manouil Metzakopian,2 Dimitrios Garyfallos,1 Jonathan L. Cooper,2 Silvia Buonamici,2 Hannes Ponstingl,1 Michael R. Stratton,1 Allan Bradley,1 Brian J. Huntly,2 Cristina Pina,6 Tony Kouzarides,3 Kosuke Yusa,1 George S. Vassiliou 1.

1 Institut Universitaire de France, Paris, France; 2 The Institute of Cancer Research, London, UK; 3University College of London, London, UK; 4University Hospital of Northumbria, Newcastle upon Tyne, UK; 5University of Cambridge, Cambridge, UK; 6University College of London, London, UK; 7University College London NHS Trust, Cambridge, UK; Cambridge, United Kingdom; 8NHS Blood and Transplant, Cambridge Biomedical Campus, Cambridge, United Kingdom.

Acute myeloid leukemia (AML) is an aggressive cancer with a poor prognosis, for which the therapeutic landscape has changed little for decades. Aberrant mRNA splicing plays a key role in cancer development and genes coding for several of the major components of the spliceosome are targeted by somatic mutations in numerous cancers including myelodysplastic syndromes and AML. Recently, myeloid neoplasms bearing spliceosome gene mutations were shown to be preferentially susceptible to pharmacological disruption of the spliceosome. Here we report that targeting the spliceosome can also be an effective therapeutic strategy in other types of AML. Recently, we generated a comprehensive catalogue of genetic vulnerabilities in AML using CRISPR-Cas9 genome-wide recessive screens and reported several novel intuitive and non-intuitive therapeutic candidates. Amongst these we identify SRPK1, the gene coding for a serine/threonine kinase that phosphorylates the major spliceosome protein...
SRF1. Here, we demonstrate that targeted genetic disruption of SRF1 in AML driven by MLL-fusion genes, led to differentiation and apoptosis. Additionally, mice transplanted with human AML cell lines carrying the MLL-AF9 fusion gene, namely MOLM-13 and THP-1, presented a significant prolongation of survival when SRF1 was genetically disrupted by CRISPR-Cas9 editing. Similar effects were seen in pharmacological inhibition of SRF1 and identified, among other genes, BRD4 as a sensitizer. We go on to show that the BRD inhibitor I-BET-151 synergizes with SRF1 inhibition to kill MOLM-13 both in vitro and in vivo. Preliminary data indicates that SRF1 inhibition has overlapping molecular effects to BRD inhibition. We are currently investigating the molecular bases of this observation. Our work identifies SRF1 as a novel therapeutic target in AML that can be used alone or in conjunctions with drugs targeting epigenetic modifications to improve their anti-leukemic effects.

**#1159 Targeting novel mucin synthesis pathway for pancreatic cancer treatment.** Altaf Mohammed, naveena B. Janakiram, Venkateshwar Madka, Gaurav Kumar, Scott edgar, Gopal Patani, Taylor Bryant, Yuting Zhang, Hariprasad Gali, Yan Daniel Zhao, Stan Lightfoot, Chinthalapally V. Rao. University of Oklahoma Health Sciences Ctr., Oklahoma City, OK.

Pancreatic cancer (PC) is most fatal and is the fourth leading cause of cancer related deaths in USA with a least five year survival of <7%. Although several over expressed mucin subtypes (MUC1, MUC4, MUC5AC, and MUC6) that impede drug delivery to PC have been targeted with limited results, core enzymes involved in mucin biosynthesis have yet to be evaluated as potential targets. The core 2 β 1,6 N-acetylgalactosaminyltransferase (GCNT3/C2GNT7) plays a significant role in mucin glycan biosynthesis. We analyzed GCNT3 expression on 180 specimens from individuals with PC (90) or matched controls (90). Patients with low GCNT3 expression (19.16 folds; p = 0.002) were significantly more likely to have PC subtypes (p = 0.002). GCNT3 expression is significantly down regulated in PC compared with normal tissue by 6.1 (p = 0.007) and (18% of patients with low GCNT3 expression, p = 0.05). The expression of GCNT3 and mucin expression in patients with lower expression of GCNT3 was associated with worse outcomes. 17.5 months vs. 10.5 months, p = 0.036. We observed a significant increase in GCNT3 mRNA expression (103.16 folds; p = 0.0001) in correlation with increased mucins subtypes (p < 0.05) in the pancreatic tumors from genetically engineered mouse (GEM) compared with pancreata from wild-type mice, as determined by Next-generation Sequencing/RNAseq analysis. In in silico studies, blind docking simulations revealed that a novel drug talnifluamate binds to GCNT3 with a better docking affinity of -8.3 kcal/mol. We synthesized talnifluamate and determined quality/purity using NMR, HRMS, and HPLC for in vitro and in vivo studies. Pancreata from 6-week-old KrasGEM (N = 6/group) treated with talnifluamate showed a significant decrease in GCNT3 and mucin expression in PanIN lesions. In human PC MiaPaCa-2 cell line, talnifluamate reduced GCNT3 expression and the number of mucin-positive cells (38% p = 0.0001). Further, PC cells (BxPC3 and Panc1) were treated with talnifluamate for 24 hrs for mucin disruption followed by radioactive 1H gemcitabine treatment for 2 hrs. The cells treated with talnifluamate showed 40% (p = 0.03) and 50% (p = 0.025) increased 1H gemcitabine uptake in BxPC3 and Panc1 cells compared to radiosensitive cells. GCNT3 siRNA knock out PC cells treated with talnifluamate showed a reduction in cell proliferation and significantly reduced spheroid formation (p < 0.05). In vivo, we did not observe any overt toxicity upon talnifluamate treatment as determined by liver enzyme toxicity and body weight gain (N = 5 mice/group). BxPC3 cells implanted nude mice (N = 5 mice/group) treated with talnifluamate or GCNT3 siRNA showed a marked reduction in xenograft tumors compared with untreated controls. Both talnifluamate and GCNT3 siRNA significantly inhibited xenograft tumor weight (48% p < 0.002 with siRNA, 63% p < 0.0001 with talnifluamate) and tumor volume (75% p < 0.0001 with both siRNA and talnifluamate) and GCNT3 enzyme activity (28% p < 0.03). Transcriptional analysis of xenograft tumors (N = 5/group) treated with GCNT3 siRNA or talnifluamate showed reduced GCNT3 and top hits related to mucin expression. These results justify the use of talnifluamate for mucin synthesis disruption, thereby enhancing the uptake of gemcitabine and thereby efficacy for PC treatment.

**#1161 Dimethylaminopentenoic acid (DMAPT), an oral nuclear factor kappa B inhibitor (NFκB), enhances radiation therapy and enhances epithelial growth factor tyrosine kinase inhibitor (EGFR TKI) activity.** Katheryn L. Mored, 1 Pamela J. Sykes, 1 Prafula C. Gokhale, 2 Gwo-Shu Mary Lee, 2 Hong Tiv; 1 Christopher J. Sweeney; 3,4 Plummers University, Bedford Park, Australia; 1 Dana-Farber Cancer Institute, Boston, MA.

Introduction: NFκB can promote cancer and resistance to therapy as well as mediate tissue injury in response to radiation (XRT). We assessed NFκB activity directly in combination with XRT, and also with an EGFR TKI, and assessed whether it can mitigate XRT side effects. Methods: Six-week old male TRAMP (Transgenic Adenocarcinoma of the Mouse Prostate) mice received DMAPT (100 mg/kg) orally or vehicle control 3x/week until palpable tumors formed and lung metastases were analyzed histologically. In separate studies, 16 week old TRAMP mice were treated with 100 mg/kg DMAPT 3x over 1 week and the effect on survival was evaluated. Results: Survival of DMAPT treated TRAMP mice was significantly enhanced compared to vehicle treated TRAMP mice (H1975 (790M mutation non small cell lung cancer, NSCLC) subcutaneously. When tumors reached 200 mm3, treatment (Rx): commenced: vehicle, DMAPT 100mg/kg/day, EGFR TKI-AZD9251 25mg/kg/day, combination. Results: Long-term Rx with DMAPT extended the median time to palpable prostate tumor by 15% (p = 0.036) and DMAPT reduced lung metastatic lesions/mm2 in TRAMP lungs 20-fold (0.077 ± 0.12 SD) compared to a vehicle control (1.47 ± 1.28 SD) (p = 0.0004). XRT-induced apoptosis doubled in TRAMP prostate (with moderate to high grade PIN lesions) treated with DMAPT prior to 6 Gy XRT (101.3 % increase, p = 0.039). DMAPT induced the greatest radiosensitivity in TRAMP prostate with high grade PIN (R2 = 0.79, p = 0.003), while apoptosis frequency in tissues with lower grades of PIN was the same as vehicle control TRAMP mice (R2 = 0.24, p = 0.6). DMAPT also reduced XRT-induced apoptosis in healthy TRAMP spleen (32.9 % reduction, p = 0.003) and rectum (28.7 % reduction, p = 0.0001). In the H1975 experiment, DMAPT monotherapy did not differ from the vehicle controls. In the single agent AZD9291 group, 2 of the 8 mice had resistance emerge during Rx and adding DMAPT to AZD9291 reversed resistance in one of these. Rx was held at Day 220 in 3 remaining AZD9291 treated mice with no evidence of tumor and only 1 mouse was alive with no tumor at day 260. For mice receiving Rx with AZD9291 and DMAPT from D1, resistance emerged in 1 mouse, and at D140 all mice treated with DMAPT alone had no tumor at day 260. Two mice had regrowth, were retreated, and at D200 without evidence of tumors had Rx held, and one had no tumor at D260 at the end of the experiment. In total, 4 of 8 mice in the AZD9291 and DMAPT had no tumor off therapy at D260. Conclusion: Radiation and EGFR TKI resistance has been linked to NFXb activation. DMAPT slows down cancer progression and decreases metastatic lesions in a prostate cancer mouse model, protects normal tissue from radiation induced apoptosis, augments radiation induced apoptosis in prostate cancer and augments EGFR TKI efficacy in T90M mutant NSCLC.

**#1162 The evaluation of INCB059872, an FAD-directed covalent inhibitor of LSD1, in preclinical models of Ewing sarcoma.** Valerie Dostalik Roman, Min Ye, Huiqing Liu, Melody Diamond, Anthony Chadderton, Yvonne Lo, Xuesong M. Liu, Jin Lu, Chunhong He, Liangxing Wu, Timothy Burn, Richard Wynn, Wenheng Yao, Gregory Hollis, Gregory Hollis, Peggy Scherle, Bruce Ruggeri, Sang Hyun Lee, Incyte Corporation, Wilmington, DE.

Ewing sarcoma is a rare bone cancer affecting predominantly children. The chromosomal translocation of chromosomes 11 and 22 results in the EWS/FLI gene fusion oncoprotein that is associated with ~85% of Ewing sarcoma cases. The EWS/FLI fusion protein is involved in deregulating gene expression and consequently causing cellular transformation. It was previously reported that lysis specific demethylase 1 (LSD1) regulates EWS/FLI transcriptional activity via its functional interaction through the NuRD co-repressor complex. We therefore evaluated whether inhibition of LSD1 could have anti-tumor effects in Ewing sarcomas that express the EWS/FLI fusion oncoprotein. INCB059872 is a potent, selective, and orally available FAD-directed covalent inhibitor of LSD1. To investigate the potential utility of INCB059872 in Ewing sarcoma, the A673 cell line having the characteristic chromosomal translocation was chosen as the experimental model system. INCB059872 inhibition of LSD1 did not significantly alter A673 proliferation in vitro. However, INCB059872 inhibited oncogenic transformation as determined by colony formation clonogenicity assays. NXX2.2 was previously identified as a critical downstream target molecule of the EWS/FLI fusion oncoprotein that is required for transformation. A significant downregulation of NXX2.2 was observed in A673 cells treated with 11.1 ± 0.7% suggesting the chronic DMAPT reduced the numb-negative modulation of the EWS/FLI -NXX2.2 axis. Oral administration of INCB059872 significantly suppressed the growth of both A673 and SK-ES Ewing sarcoma xenografts in vivo. In addition, in vivo efficacy was evaluated in patient derived xenograft (PDx) models that were developed from relapsed tumor tissues of Ewing sarcoma patients. Notably, a subset of PDx models having EWS/FLI translocations (3/6) exhibited significant tumor growth inhibition at well-tolerated doses of INCB059872. Molecular signatures obtained from RNA-Seq data with these PDx models exhibited intrinsic differences between responders and non-responders, suggesting additional molecular or genetic variations may contribute to their sensitivity to INCB059872. Studies identifying potential candi-
date molecular mechanisms are underway. Together, these data suggest that INC059872 may be therapeutically efficacious in a subset of Ewing sarcoma patients.

#1163 Non clinical pharmacology, pharmacokinetics and safety profiling of CB-103: A novel first-in-class small molecule inhibitor of the NOTCH pathway. Rajwinder Lehal,1 Viktoras Frismanantas,2 Charlotte Urec,1 Maximilian Murone,1 Dirk Weber,2 Michael Bauer,3 Jean-Pierre Bourquin,2 Freddy Radlke,4 Celliesia Biotech AG, Basel, Switzerland; 5University Children's Hospital, Zurich, Switzerland; 6Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland.

NOTCH signaling is a developmental pathway known to play critical roles during embryonic development as well as for the regulation of self-renewing tissues. Aberrant activation of NOTCH signaling leads to deregulation of the self-renewal process resulting in sustained proliferation, evasion of cell death, loss of differentiation capacity, invasion and metastasis, all of which are hallmark of cancer. Over activation of NOTCH in human cancers can be a consequence of over expression of NOTCH ligands/receptors, GOF mutations in NOTCH receptors as well as chromosomal translocations leading to constitutive activation of the pathway by producing truncated version of NOTCH1 or NOTCH2. Over activation of NOTCH and its oncogenic role in various cancers is prognostically relevant with shorter survival seen in patients harbouring these genetic alterations. Given the importance of NOTCH signaling in human cancers, several therapeutic approaches have been utilized to block NOTCH signaling. Two of these strategies are: a) the use of monoclonal blocking antibodies (mAbs) against NOTCH1/2 ligands and receptors and b) the use of small molecule inhibitors (SIs). However, these approaches can only be effective if tumor cells express full-length ligand or receptor molecules. On the contrary, in human cancers harbouring NOTCH gene fusion due to chromosomal translocations, the use of mAbs and SIs will have very limited clinical benefits. A third, yet not fully explored approach would be the blockade of NOTCH signaling by targeting the most downstream complex in the NOTCH signal transduction cascade, the NOTCH transcriptional activation complex, using small molecule inhibitors. Previously we have reported the discovery and characterization of CB-103, a novel first-in-class orally-active small molecule inhibitor of the NOTCH pathway. CB-103 inhibits NOTCH signaling by targeting the NOTCH transcriptional activation complex in the nucleus. CB-103 has shown the ability to block NOTCH signalling in various human cancer cell lines with active NOTCH pathway. CB-103 has also been tested in vivo models. In a GSI/mAb resistant cell line, CB-103 was found to block the transcriptional activity of NOTCH1, using small molecule inhibitors. First, we have shown that CB-103 completely blocks NOTCH target gene expression in vitro, but not in vivo. In vivo, CB-103 showed a partial inhibition of NOTCH target gene expression, but not as potent as in vitro. CB-103 has also been tested in vivo models. In a GSI/mAb resistant cell line, CB-103 was found to block the transcriptional activity of NOTCH1, using small molecule inhibitors.

#1164 A novel hypoxia-inducible factor-1 inhibitor IDF-11774 regulates cancer metabolism, thereby suppressing tumor growth. Misun Won,1 Hyun Seung Ban,2 Kyeong Lee,2 Hongsub Lee,3 Bo-Kyung Kim,2 Hwan Mook Kim,2 Ravi Naik,2 Song-Kyu Park,5 Joon-Tae Park,3 Inhyub Kim,1 Miso Nam,6 Geum-jeong Kim,5 Donggyun Lee,4 Donggyun Lee5,6,7,8,9

HIF-1 is associated with poor patient prognosis and therapeutic resistance of cancer. We have developed a novel hypoxia-inducible factor (HIF)-1 inhibitor, IDF-11774, as a clinical candidate for cancer therapy. Under hypoxic condition, IDF-11774 inhibited the accumulation of HIF-1α in vitro and in vivo in colorectal carcinoma HCT116 cells. IDF-11774 suppressed the angiogenesis of cancer cells by reducing the expression of HIF-1 target genes. Moreover, IDF-11774 reduced glucose uptake, leading sensitizing cancer cell growth on low glucose condition. IDF-11774 also decreased the expression of eEF1A2 in the first event triggered by the drug in cancer cells. eEF1A2 is a target protein for the delivery of aminoacyl-tRNA to the A site in the ribosome, it sustains the delivery of aminoacyl-tRNA to the A site in the ribosome, it has been shown to be overexpressed in many human cancers, including B-RAFV600E mutant and wild type (WT) cells. Compound 1 (IC50 range 0.8-3.8 μM) showed lower IC50 values than 3 (IC50 range 1.8-3.8 μM) and the mutant B-Raf specific inhibitor PLX-4032 (IC50 range 0.4-50 μM), especially at shorter treatment time (24 h). These effects are long-lasting since melanoma cells did not recover their proliferative potential after 14 days of treatment. In addition, we confirmed that compound 1 induces cell death by apoptosis using Live and Dead, Annexin V and Caspase3/7 apoptosis assays. Furthermore, compound 1 reduces protein levels of STAT3 and its phosphorylation, as well as decreases the expression of STAT3 regulated genes involved in survival and metastasis such as survivin and c-myc. Compound 1 also upregulates the cell cycle inhibitor p21. Docking studies further revealed the favorable binding of 1 with SHD2 domain of STAT3 suggesting its activity through STAT3 inhibition. Taken together, our results suggest that compound 1 induces apoptosis in a non-specific manner, the inhibition of STAT3 pathway to non-specifically target both B-Raf mutant and WT melanoma cells with much better cytotoxicity than the current therapy PLX-4032.

#1165 Plitidepsin targets the moonlighting functions of eEF1A2 in cancer cells. Alejandro Losada, Maria Jose Munoz, Juan F. Martinez-Leal, Juan M. Dominguez, Carlos M. Galmarini. PharmaMar S.A., Colmenar Viejo, Spain.

Plitidepsin, a cyclic depsipeptide of marine origin, has shown potent antican- cancer activity in preclinical assays and recently finished with positive results a phase II clinical trial (clinicaltrials.gov NCT01717165) showing the treatment of multiple myeloma patients. We have recently found that eukaryotic elongation factor 1α (eEF1A2), one of the two isoforms of the alpha subunit of eEF1, is the pharmacological target of plitidepsin. Although it shares 96% homology with eEF1A1 (the other isoform), they display an exclusive pattern of expression, being eEF1A2 solely expressed in brain and muscle in healthy individuals. However, it has been found that many tumors normally overexpress this protein, including multiple myeloma, prostate, pancreatic, ovarian, breast, lung and liver cancers. Furthermore, although eEF1A2 canonical function consists in the delivery of aminoacyl-tRNAs to the A-site in the ribosome, it has been shown to have pro-oncogenic moonlighting activities, including inhibition of apoptosis, protein degradation by the proteasome, heat shock response, cyto- skeleton organization and regulation of oxidative stress. We have now investigated several of the pro-oncogenic activities of eEF1A2 to analyze the impact that plitidepsin could have preventing them. Indeed, we observed that plitidepsin interfered with the interaction between eEF1A2 and Peroxiredoxin 1 (PRDX1), a complex that allosterically enhances the enzymatic activity of PRDX1. This way, plitidepsin would diminish PRDX1 antioxidant activity, possibly originating the oxidative stress that has been described in the bibliography as one of the first events triggered by the drug in cancer cells. PRDX1 only interacts with the GDP-bound form of eEF1A2, while plitidepsin exclusively binds to the GTP-bound form, most probably sequestering this protein from the pool that could interact with and activate PRDX1. Furthermore, we have confirmed that eEF1A2 interacts with Sphingosine kinase 1 (SPHK1), a complex that has been described in the bibliography as having enhanced SPHK1 activity. SPHK1 phosphorylates sphingosine producing sphingosine-1-P, a second messenger that binds to its receptors in the cell membrane and conveys growth and survival signals to the cell. We could see that plitidepsin treatment reduced the production of sphingosine-1-P in HeLa cells, destabilizing the equilibrium towards the pro-apoptotic ceramide/sphingosine side and promoting cell death. Thus, through its binding to eEF1A2, plitidepsin derails a series of its moonlighting functions that are essential for the survival of tumor cells, driving them into apoptosis.

#1166 Identification of a novel quinoxalino-isoselenoureara targeting STAT3 pathway as a potential melanoma therapeutic. Verónica Alcolea,1 Deepkamal Karelia,2 Manoj K. Pandey,3 Daniel Plano,3 Parvesh Singh,3 Roshal D. Iby,2 Juan Palop,2 Shantu Amin,2 Carmen Sammartin,1 Arun K. Sharma.1

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The prognosis for patients with metastatic melanoma remains very poor. Constitutive STAT3 activation has been correlated to larger tumor size, metastasis, acquired resistance against vemurafenib (PLX-4032), and poor patient survival. We previously identified a STAT3-selective small molecule inhibitor (1) as a potential new treatment for melanoma, acquired resistance against vemurafenib (PLX-4032), and poor patient survival suggesting its potential as a molecular target. We recently designed a series of isoseleno- and isothio-urea derivatives of several biologically active heterocyclic scaffolds. The cytotoxic effects of lead isoseleno-isothio-urea derivatives (compounds 1/3) were studied in a panel of five melanoma cell lines, including B-RafV600E mutant and wild type (WT) cells. Compound 1 (IC50 range 0.8-3.8 μM) showed lower IC50 values than 3 (IC50 range 1.8-3.8 μM) and the mutant B-Raf specific inhibitor PLX-4032 (IC50 range 0.4-50 μM), especially at shorter treatment time (24 h). These effects are long-lasting since melanoma cells did not recover their proliferative potential after 14 days of treatment. In addition, we confirmed that compound 1 induces cell death by apoptosis using Live and Dead, Annexin V and Caspase3/7 apoptosis assays. Furthermore, compound 1 reduces protein levels of STAT3 and its phosphorylation, as well as decreases the expression of STAT3 regulated genes involved in survival and metastasis such as survivin and c-myc. Compound 1 also upregulates the cell cycle inhibitor p21. Docking studies further revealed the favorable binding of 1 with SHD2 domain of STAT3 suggesting its activity through STAT3 inhibition. Taken together, our results suggest that compound 1 induces apoptosis in a non-specific manner, the inhibition of STAT3 pathway to non-specifically target both B-Raf mutant and WT melanoma cells with much better cytotoxicity than the current therapy PLX-4032.

BT1718 is a Bicycle Drug Conjugate (BDC®) comprising a constrained bicyclic peptide (Bicycle®) that binds with high affinity and specificity to membrane type 1-matrix metalloproteinase (MT1-MMP; MMP14) covalently linked through a hindered disulfide linker to the potent anti-tubulin agent DM1. MT1-MMP is involved in normal tissue remodeling and is also expressed in tumor associated stromal cells. However, overexpression is linked to increased tumor progression and metastasis. Specifically, over-expression of MT1-MMP is also associated with poor clinical prognosis and shorter survival times in patients with NSCLC and a range of other solid tumors. The Bicycle MT1-MMP binding element within BT1718 was identified using a proprietary phage display peptide technology consisting of highly diverse phage libraries of linear amino acid sequences constructed using a central tri-functional chemical scaffold. MT1-MMP binding Bicycles exhibit a profound affinity and specificity, more often associated with monoclonal antibodies, whilst their low molecular weight (1.5-3 kDa), akin to that of a small molecule, aids in rapid extravasation and tumor penetration. Together these attributes make Bicycles an ideal format for the targeted delivery of cytotoxic payloads. We evaluated the ability of BT1718 to bind to and kill tumor cells in vitro and in vivo in a panel of tumor cell lines. BT1718 demonstrated MT1-MMP target-specific binding and MT1-MMP-dependent cell killing of lung tumor cells in vitro as well as efficacy across a panel of lung tumor xenograft mouse models. For example, in the Met-amplified squamous NSCLC lung EBC-1 model, complete regressions were observed in all mice at doses as low as 5 mg/kg (iv) twice weekly and across a range of other dosing schedules, from daily to weekly. MT1-MMP-dependent activity was demonstrated by blocking target specific interactions through co-administration of an excess of unconjugated Bicycle binder, which inhibited tumour regression, or a non-binding Bicycle, which had no effect. Further evaluation in patient-derived lung xenograft (PDX) models indicates a similar activity to that seen in cell-line derived xenografts, with efficacy seen from 3mg/kg twice weekly and rapid full regression of tumors at higher doses. The molecular attributes of these Bicycles—rapid tumor penetration and specific binding, make them ideal therapeutics for targeted delivery of toxins as Bicycle drug conjugates (BDCs). The small size of the BDC may offer a significant advantage to other targeted cytotoxic approaches such as antibody-drug conjugates due to rapid extravasation and improved tumor penetration. BT1718, a Bicycle Drug Conjugate, shows potent anti-tumor activity in human lung tumor xenograft models and IND-enabling studies are underway.


IRAK4 kinase activity is required for toll-like receptor (TLR) and interleukin-1 receptor (IL-1R) signaling in a variety of myeloid and lymphoid cell types. Recruitment of IRAK4 to these receptors and its subsequent activation is facilitated by the MYD88 adaptor protein, which is mutated in ~22% of DLBCL cases. The MYD88 L265P activating mutation is found in ~30% of the activated B-cell (ABC) and ~66% of germinal center B-cell (GCB) subtypes of DLBCL and leads to constitutive activation of NF-κB signaling that is associated with worse prognosis. Thus, the development of small molecule inhibitors targeting IRAK4 is an attractive anticancer strategy for MYD88 mutation-containing cancers such as DLBCL. We are developing an IRAK4 inhibitor, CA-4948, as a therapeutic agent for hematological cancers with dysregulated TLR/MYD88/IRAK4 signaling. CA-4948 (previously AU-4948) is a selective and potent IRAK4 kinase inhibitor with in vivo activity in a TLR4-induced cytokine release model. CA-4948 exhibits favorable DMPK properties, oral bioavailability, and is well tolerated in mice. Furthermore, CA-4948 was previously shown to exhibit dose-dependent efficacy in ABC-DLBCL MYD88/L265P xenograft tumor models using cell lines OCI-LY3 and OCI-LY10. Here, we report the efficacy results from testing CA-4948 in a panel of well characterized, patient-derived DLBCL xenograft (PDX) mouse models. CA-4948 exhibited the greatest efficacy in four of nine human ABC-DLBCL PDX models. CA-4948 was efficacious in ABC-GCB DLBCL PDX models. Furthermore, CA-4948 was efficacious in ABC-DLBCL PDX tumors containing activating mutations in both TLR/LR-1R and BCR signaling pathways (MYD88 and CD79B double mutants). Interestingly, the one ABC-DLBCL PDX model that failed to respond to CA-4948 treatment contained a MYD88 L265P mutation as well as a BCL6 translocation. While this particular PDX model was resistant to CA-4948, and showed a weak anti-tumor response to single-agent ibrutinib, the combination treatment of ibrutinib and CA-4948 exhibited a synergistic tumor growth inhibition effect. In summary, CA-4948 exhibited anti-tumor activity in ABC-type DLBCL PDX tumor models including those containing combinations of activating mutations in the TLR/IL-1R and BCR signaling pathways. These results underscore the therapeutic potential of IRAK4 kinase inhibition by CA-4948, as a single-agent or in combination with BCR inhibitors, for the treatment of DLBCL.

#1169 mtTERT promoter as a target for treatment of glioblastoma. Saumya R. Bollam,1 Harshil D. Dhurv,1 Hyun-Jin Kang,2 Sen Peng,1 Vijay Gokhale,1 Laurence Hurley,2 Michael Berens1. 1Translational Genomics Research Institute, Phoenix, AZ; 2University of Arizona, Tucson, AZ.

Approximately 86% of GBM tumors exhibit mutation at -124 or -146 bases upstream of the ATG start site in the transcription activating promoter region of telomerase reverse transcriptase (hTERT). Mutation in the promoter region of hTERT impairs repression, leading to overexpression of hTERT; inappropiate hTERT is associated with oncogenesis, tumor maintenance, and resistance to apoptosis. We surveyed long-term glioma cell lines and glioma PDX models for mt-hTERT; mRNA and protein expression of hTERT were assessed by qPCR and western blot. The -124 and -146 mutations are located in the major 5'-12 G-quadruplex and result in misfolding of the silencer element and consequent over-expression of hTERT. Using a diverse small molecule library, we identified a small drug-like pharmacological chaperone (pharmacomere) molecule, TG-4260, which binds to the 26 mer base-pair heteroduplex loop, which is the nucleation site for cooperative folding of the major 5'-12 G-quadruplex. The chemical effect of TG-4260 corrects DNA hTERT G-quadruplex misfolding resulting from the somatic mutations and restores the silencer of the G-quadruplex thereby reducing hTERT activity. TG-4260 directly decreases the transcription activity of the WT and the −124, −124/125, −138/139, and −146 mutants to a similar extent and suppresses the downstream gene BCL2, which activates apoptosis. Furthermore, TG-4260 produces cell cycle growth inhibition. Finally, we tested the in vivo efficacy of TG-4260 significantly inhibits telomerase and shortens telomere length after five days of treatment and induces a senescence-like phenotype. This is the first example of the use of a pharmacomere molecule to correct the misfolding of a DNA G-quadruplex element resulting from mutations in an early folding intermediate. Finally, we screened GBM cell models against a novel small molecule inhibitor that interferes with mutated hTERT promoter and demonstrated that TG-4260 selectively suppresses glioma cell viability without affecting non-transformed normal human astrocytes.

#1170 Notch pathway is overexpressed and is a therapeutic target in clear cell renal cancer. Tushar D. Bhagat,1 Yivi Zou,2 Shizhen Huang,3 Jihoon Park,2 Matthew B. Palmer,2 Caroline Hu,2 Weijuan Li,2 Niraj Shenoy,2 Orsolya Giricz,1 Yiting Yu,2 Yi-An Ko,2 Maria Concepcion Izquierdo,3 Esther Park,2 Nishanth Valluvamsetta,1 Remi Laurence,2 Robert Lopez,2 Masako Suzuki,1 James Pullman,3 Justin Kaner,4 Benjamin Gartrell,4 A. Ari Hakimi,5 John Greally,4 Bharvin P. F6,1 Karin Benhadj V5,1 Anit Nepve,1 Katalin Susztak2.1Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, NY; 2University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA; 3Montefiore Medical Center, Bronx, NY; 4Sloan Kettering Cancer Center, New York, NY; 5Eli Lilly, Indianapolis, IN.

Clear cell renal cell carcinoma (CCRCC) is an incurable malignancy in advanced stages and needs new therapeutic targets. We conducted a transcriptionomic analysis of CCRCCs and matched microdissected renal tubular controls and observed an overexpression of NOTCH ligands (JAGGED1, JAGGED2) and Delta like (DLL3) family of ligands) and receptors (NOTCH1, NOTCH2, NOTCH3 and NOTCH4) in tumor tissues. Examination of the TCGA RNA-Seq dataset also revealed widespread activation of NOTCH pathway in a large cohort of CCRCC samples. Samples with NOTCH pathway activation were also clinically distinct and were associated with better overall survival. Parallel high resolution DNA methylation and copy number analysis with the HELP assay demonstrated that both genetic and epigenetic alterations led to NOTCH pathway activation in CCRCC. NOTCH ligands, JAGGED2 was overexpressed in and associated with gene amplification in distinct CCRCC samples. To test the causality, we transgenically expressed the intracellular domain of NOTCH1 in mice renal tubules with tubule specific deletion of VHL. The KspVHL1 VHL1ICNNotch1 mice exhibited dysplastic hyper proliferation of tubular epithelial cells confirming the proangiogenic role of NOTCH in vivo. Alteration of cell cycle pathways was seen in murine renal tubular cells with NOTCH overexpression and molecular similarity to human tumors was observed, demonstrating that human CCRCC recapitulates features and gene expression changes observed in mice with transgenic overexpression of the Notch intracellular domain. Finally, treatment with clinical, gamma secretase inhibitor, LY309478, led to inhibition of CCRCC cells in vitro and in vivo.

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in CRC xenografts. In summary, these data reveal the mechanistic basis of NOTCH pathway activation in CRCCC and demonstrate this pathway to a potential therapeutic target.

#1171 Therapeutic applications of the selective high affinity ligand SH7119 may extend beyond NHL to many other types of solid tumors. Monique Cosman Balhorn, Rod Balhorn. SHAL Technologies, Inc., Livernor, CA. SH7119, the first of a new class of cancer therapeutics developed for treating non-Hodgkin’s lymphoma, is unusual in that both targeting and prodrug functionalities have been incorporated into the same small molecule. Functioning similar to an antibody-drug conjugate, SH7119 targets a unique structural epitope within the antigen-binding pocket of HLA-DR10. HLA-DRs containing this epitope within the β-subunit are reported to be over-expressed in approximately 85% of B-cell lymphomas. Upon binding to HLA-DR molecules located on the tumor cell’s surface, SH7119 is transported into the cytoplasm where it is concentrated and subsequently metabolized. A series of metabolic products derived from the SHAL’s recognition elements (three small molecules that are linked together to create SH7119 and to provide targeting selectivity) are generated as the drug is broken down, each of which inhibits one or more activities required for tumor cell survival. Preclinical studies with SH7139 have demonstrated remarkable efficacy in treating B-cell lymphoma xenografts in mice, providing permanent cures for up to two-thirds of the animals at a human equivalent dose as low as 0.41 μg/kg. Biopsy tissue binding studies conducted with SH7129, a biotinylated form of SH7119, and streptavidin-horse radish peroxidase detection have shown the drug binds to a significant fraction of tumor cells from patients diagnosed with multiple myeloma and each of the B-cell lymphoma subtypes tested to date (DLBCL, Burkitt’s, Mantle Cell, Follicular, MALT, and CLL). SH7129 was also observed to bind to tumor biopsies obtained from a number of patients diagnosed with peripheral T-cell and nodular sclerosis Hodgkin’s lymphomas, a result consistent with observations reported by others that HLA-DRs are expressed in a subset of these lymphomas. HLA-DR expression has also been reported to occur in or be linked to a number of other types of cancer, including melanoma, cervical, ovarian, pancreatic and lung cancers. SH7129 staining of tumor microarrays have shown biopsy cores from a subset of patients diagnosed with each of these cancers also bind SH7119. While in vivo efficacy has only been tested in Burkitt’s (RAJI), Mantle cell (Granita-519), and T-cell (Jurkat, a cell line control lacking HLA-DR and showing no efficacy) lymphoma xenografts, these tissue binding results suggest that in addition to the majority of the NHL subtypes, nodular sclerosis Hodgkin’s lymphoma, multiple myelomas, as well as a subset of melanomas, ovarian, cervical, pancreatic, and lung cancers may also respond to SH7119 therapy. This research was supported by the National Cancer Institute Phase II SIRI Award R44CA159431.

#1172 In vivo pharmacokinetic properties and antitumor efficacy of porcine lead inhibitors in the orthotopic murine MMTV-Wnt1 breast tumor model and the human HPAF-II pancreatic xenograft mouse model. Vishal Pendharkar, 1 Yun Shan Chew, 2 Vithya Manoharan, 1 Choon Bing Low, 1 Hong-qian Esther Ong, 3 Soo Yei Ho, 1 Wei Ling Wang, 1 Jeyaraj Duraiswamy Athi-samy, 1 Babita Madan, 2 David Virshup, 2 Thomas Hugo Keller, 1 May Ann Lee, 1 Alex Matter, 1 Jeffrey Hill, 1 Kanda Sangthonpitag, 3 Experimental Therapeutic Centre, Singapore, Singapore; 4 Duke-NUS Medical School, Singapore, Singapore. Porcine PORCN (PORCN), a multi-pass integral membrane-bound O-Acetyl acyltransferase (MOBAT), resides in the end in the endoplasmic reticulum (ER) and is required for biogenesis of Wnt ligands. The secreted mature Wnt ligands bind to their cognate receptors (Fz/lig, LRPS/6 and transmembrane receptor ROR) to form the ligand - receptor complex which is capable to activate the Wnt-β-catenin signalling cascade and downstream signalling pathways such as mTOR, GSK3, Akt, and PKC. The deregulation of and aberrant activation of the various components of Wnt-β-catenin signalling pathway have been implicated in tu- morigenesis of breast cancer cells. PORCN is found to be associated with cancerous cell growth. Knockdown of Porcine mRNAs significantly reduced the proliferation of breast cancer cells and resulted in the delay of MDA-MB-231 tumor formation in mouse xenograft models (Covey et al 2012). Loss of function mutations of RN4F3, a negative regulation of Wnt-signalling via Fizzled receptor, is recently reported to be involved with pancreatic ductal adenocarcinoma. Inhibition of the PDAC cell lines bearing RN4F3 mutations enhanced Wnt-β-catenin signalling and resulted in suppression of proliferation and differentiation of PDAC tumor cells (Jiang et al 2013). Taken together porcine could be an attractive therapeutic approach for a particular Wnt-driven cancer population. We have identified the porcine lead compounds (ETC-159, ETC-535, ETC-611 and ETC-539) from different novel chemical scaffolds. The aim of this study was to evaluate their pharmacokinetic properties and antitumor efficacy in different cancer mouse models, the murine MMTV-Wnt1 breast cancer and the human pancreatic HPAF-II cancer. All porcine lead compounds had good oral pharmacokinetic properties and the absolute bioavailability greater than 42%. Maximal tolerated dose (MTD-7d) up to 200 mg/kg. They produced antitumor efficacy ranging from 24% to 79% at 1 mg/kg, 38% to 89% at 3 mg/kg, and 58% to 97% at 10 mg/kg in MMTV-Wnt1 tumor mouse model. In vivo inhibition of PORCN led to reduce the expression level of Axin2 in MMTV-Wnt tumors up to 8h. At 100 mg/kg, they produced antitumor efficacy ranging from 34% to 91% in breast xenograft II pancreatic xenograft mouse models. The compounds, ETC-159 demonstrated good oral pharmacokinetic properties and produced significantly antitumor efficacy (p value < 0.0001) in both cancer mouse models. ETC-1922159 was selected as the preclinical development candidate and currently is under investigation in Phase I clinical trial.

#1173 A novel selective Mcl-1 inhibitor exhibits in vitro and in vivo effi- cacy in melanoma. Karson J. Kump, Lei Miao, Ahmed S. Mady, Katherine Lev, William Giblin, Mary E. Skinner, David B. Lombard, Zaneta Nikolovska-Coleska. University of Michigan, Ann Arbor, MI. Metastatic melanoma is the deadliest form of skin cancer that still has limited treatment options and dismal 5-year survival rates as low as 15%. Only recently has there been significant progress in the treatment of metastatic mel- anaoma, advent by molecular targeted drugs and immunotherapy, but their lim- itations are enormous and resistance rapidly emerges. Dysregulation of apoptotic machinery in melanoma allows the cancer cells to evade cell death and contributes to treatment resistance. Up-regulation of Mcl-1, a member of the Bcl-2 family of anti-apoptotic proteins, has been correlated with melanoma progression and metastasis. Mcl-1 amplification is one of the most common genetic aberrations found in human cancers and has been labeled as a marker of aggressive oncogenesis and poor patient prognosis. Previous studies have pointed to Mcl-1 as a viable therapeutic target for the treatment of mel- anaoma and conclude that small molecule Mcl-1 inhibitors may appeal this unmet medical need. We have discovered and characterized a new class of selective small molecule Mcl-1 inhibitors using various biochemical, functional, and cell based assays; further development of these compounds allowed us to achieve potent low-nanomolar binding affinity for Mcl-1 and more than 300-fold selec- tivity over Bcl-2/Bcl-xL. Our most potent inhibitor, 483-LM, was screened across a panel of human melanoma cell lines using a cell proliferation assay and revealed varying levels of sensitivity. A BH3 profiling assay demonstrates that C8161, the cell line most sensitive to our inhibitor, solely relies on Mcl-1 for survival. The Mcl-1 dependence of C8161 might contribute to the known meta- static nature of this cell line. Mechanistic studies revealed that 483-LM effec- tively engaged the endogenous Mcl-1 protein after treatment of C8161, as deter- mined by a CEQA assay, and prompted disruption of protein-protein interactions between Mcl-1 and several pro-apoptotic proteins, including Bax, Bak and Bim. This was followed by induction of Bax/Bak dependent apoptosis and activation of hallmark of the intrinsic apoptotic pathway, including mito- chondrial membrane depolarization, caspase activation and PARP cleav- age. Importantly, treatment with 483-LM caused massive up-regulation of the pro-apoptotic BH3-only protein Noxa, an effect apparent after an 8 hour treat- ment, which contributes to the induction of cell death. The in vivo efficacy studies with 483-LM showed significant improvement on tumor growth and caspase-3 activation in tumor samples. Overall, our data indicate that Mcl-1 inhibitors are a promising treatment option for aggressive metastatic melanoma and warrant further preclinical investigation of 483-LM as a promising selective Mcl-1 inhab- itor to be used as a single agent and in combination with chemotherapy and immunotherapy.

#1174 CT179 degrades the olig2 transcription factor in glioblastoma stem-like cells and prolongs survival. Gordon R. Alton, 1 Graham Beaton, 1 Susan Knowles, 1 Gregory Stein, 1 Santosh Kesari, 2 Curtana Pharmaceuticals, Austin, TX; 3 Pacific Neuroscience Institute, Santa Monica, CA. Olig2 is a bHLH transcription factor that has been shown to be a key driver of glioblastoma (GBM) tumorigenesis. Olig2 is expressed in human tumor-derived glioma stem-like cells. A growing body of evidence suggests that glioma stem-like cells (GSCs) are more representative of their parent tumors when cultured under defined serum-free conditions with the mitogens epidermal growth factor (EGF) and fibroblast growth factor (FGF). Furthermore, recent research has indicated that human Proneural GSCs grown in vitro in PDGF are more tumorig- enic in vivo. We have designed and synthesized a potent inhibitor of Olig2 that possesses all the necessary properties for clinical development as an important...
new GBM therapeutic. CT179 dose dependently causes the degradation of Olig2 in multiple GBM cell lines and this correlates to G2/M arrest and increased apoptosis. CT179 also induces the loss of expression of EGFR and PDGFR in human GSCs. CT179 achieves high concentrations in the CNS with a long duration of pharmacologic action. Furthermore, CT179 significantly extends survival of intracranial orthotopic human tumor GSCs. Immunohistochemistry demonstrates a marked reduction of Olig2 positive cells in the tumor bearing animals. Together, these results indicate that CT179 has significant promise as a new therapeutic for the treatment of human GBM.

**#1175 Inhibition of NAMPT as a novel therapeutic strategy for infant leukemia.** Klaartje Somers,1 Shiloh Middelmiss,2 Asef Betkatsou,2 Mawar Karsa,1 Leanna Cheung,1 Angelika Koscielek,1 Kathryn Evans,1 Chelsea Mayoh,1 Ursula R. Kees,1 Lioubov Korotchkina,1 Olga B. Chernova,2 Richard B. Lock,1 Andrei V. Gudkov,4 Michelle Haber,1 Murray D. Norris,1 Michelle J. Henderson.1

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Novel targeted therapies are urgently needed for infant leukemia as this disease is highly aggressive and refractory to treatment resulting in poor survival rates. The NAD (Nicotinamide Adenine Dinucleotide) producing enzyme NAMPT (Nicotinamide Phosphoribosyltransferase) has been considered as an attractive selective target for anti-cancer therapy due to the high dependency of tumor cells on NAD for energy metabolism and activity of NAD-dependent enzymes such as poly-ADP-ribose polymersases (PARPs) and sirtuins that play key roles in cancer cell survival. A newly developed NAMPT inhibitor, OT-82, was initially isolated for its high selective toxicity against a panel of adult leukemia cells. Here we investigated NAMPT inhibition as a therapeutic strategy for infant leukemias characterized by rearrangement of the MLL gene (MLL-r), by testing the potency of OT-82 in a panel of predclinical in vitro and in vivo models of MLL-r leukemia that are based on the use of patient-derived xenograft (PDX) cells. OT-82, as a single agent, dramatically reduced the viability of all tested MLL-r leukemia cell lines (n=9) and MLL-r leukemia PDX (n=6) with IC50s ranging from 0.15 to 3.82nM. While the IC50 for OT-82 correlated significantly with the IC50 of other NAMPT inhibitors STF-118804 and FK866, OT-82 was the most potent compound. When combining OT-82 with chemotherapeutic agents currently used to treat infants with leukemia, we observed significant synergy between OT-82 and cytarabine indicating the potential of OT-82 for chemosensitization. Consistent with NAMPT inhibition, OT-82 reduced cytotoxic NAD+ levels in MLL-r leukemia cells and inhibited the activity of the NAD-requiring enzymes PARP-1 and SIRT-1, as exemplified by a decrease in PARylation PARP-1 levels and a p53-mediated increase in p21 levels, leading to apoptosis induction. Interestingly, despite the remarkable potency of OT-82 in killing MLL-r leukemia cells, a 25-fold difference in IC50 levels was noted across the cell line panel, with those lines harboring the MLL translocation most sensitive to the compound. Most pertinently the effects we observe are in a PDX cultured as 3D neurospheres that more closely resemble the true tumor architecture, heterogeneity, and “stem-like” phenotype characteristic of tumor growth. We have leveraged RNAseq expression data from Cancer Cell Line Encyclopedia (CCLE) and Pevonedistat response data from The Cancer Therapeutics Response Portal (CTRP) to apply a network-based analysis to identify potential and novel responders to OT-82.

**#1176 Preclinical characterization of the pharmacokinetic-pharmacodynamic (PK/PD) efficacy relationship of novel allosteric SHP2 inhibitors.** Minying Pu,1 Laura R. La Bonte,2 Stan Speen,3 Kathy Hsiao,2 Minying Pu,1 Lauren K. Hartman,1 Darren Finlay,2 Peiwen Pan,2 Seunghan Kim,1 Gil Speyer,1 Katerina Andrianova,1 Alexander Polin,1 Mikhail Chernov,2 Denis Kazyulkin,1 Katerina Andrianova,1 Alexander Polin,1 Michael Berens.1

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Effective anticancer drugs can be directed against cell lineage-specific, rather than tumor-specific, targets (i.e., androgen receptor, CD20, etc.). We sought to conduct systematic searches for novel agents that are selectively cytotoxic to hematopoietic (HP) malignancies presumably targeting HP lineage-specific survival factors. Cell-based screening and subsequent validation of small-molecules in a broad panel of...
HP and non-HP cancer cell lines followed by a hit-to-lead optimization of the selected class of compounds has led to a molecule named OT-82 characterized by highly selective cytotoxicity against all HP-derived cancers tested (14 cell lines, representing human AML, ALL, CML and lymphoma cells) active within single nanomolar range of concentrations. OT-82 killed sensitive cells by induction of apoptosis and was 10-20 fold more toxic to HP-derived than to solid tumor-derived cell lines and normal primary cells of HP and non-HP origin. OT-82-based affinity chromatography of cell lysates followed by mass spectrometry led to the identification of nicotinamide phosphoryltransferase (NAMPT) as the OT-82 target. NAMPT catalyzes the rate limiting step in a major pathway of synthesis of NAD, an energy metabolism mediator and essential cofactor of PARP and sirtuins. NAMPT treatment caused dramatic reductions in cellular NAD levels. Nicotinamide (vitamin B3), a substrate for the alternative NAD synthesis pathway, protected cells from the cytotoxic effects of OT-82. Oral administration of OT-82 was efficacious in subcutaneous and systemic xenograft models of human AML, ALL, erythroleukemia and multiple myeloma including complete response in several models. Therapeutic doses of OT-82 in mice were significantly lower than maximal tolerated dose (MTD); toxicities observed at doses above MTD were largely limited to HP and lymphoid organs. Toxicity profile of OT-82 determined in GLP in mice and non-human primates was favorably different from that reported for other reported clinical stage NAMPT inhibitors and did not involve any detectable renal or cardiac toxicity. These results indicate high lineage specific and malignant transformation-specific dependence of HP neoplastic cells on NAMPT, presumably due to the strong addition of this cell type on the NAD levels, and highlights OT-82 as a prospective clinical candidate for the treatment of hematological malignancies.

**#179** EP4 receptor antagonism in paclitaxel-resistant ovarian clear cell carcinomas that overexpress class III β-tubulin. Dana M. Roque,1 Danielle Meir-Levi,1 Gautam G. Rao,1 Paul Staats,2 Amy Fulton,1 Jocelyn Reader2. 1Division of Gynecologic Oncology, University of Maryland School of Medicine, Greenbaum Cancer Center, Baltimore, MD; 2Department of Pathology, University of Maryland School of Medicine, Baltimore, MD

OBJECTIVES: Advanced ovarian clear cell carcinoma (OCCC) is associated with a survival disadvantage relative to ovarian serous carcinoma following platinum/taxane-based chemotherapy and optimal cytoreduction. Prostaglandin E2 (PGE2) contributes to disease progression through modulation of several components of the COX pathway.2 Class III β-tubulin is a marker for paclitaxel resistance and is widely overexpressed in OCCC. The purpose of this study is to demonstrate that EP4 inhibition may overcome paclitaxel resistance in OCCC that overexpress class III β-tubulin. METHODS: Expression of EP4 receptor and class III β-tubulin was quantified using immunohistochemistry and Western blot in solid tissues and cell lines. Standard metabolic growth and migration assays were employed to test the effects of drug treatment (paclitaxel EP4 inhibitor) on proliferation and migration. EP4 expression and EP4 silencing using siRNA. RESULTS: OCCC overexpress class III β-tubulin/EP4 relative to normal ovary [Fig. 1a/c]. EP4 staining intensity was 2+ in 100% OCCC using an ovarian cancer tissue microarray (62 cores, 13% OCCC); this rate was only 26-56% among other histologies. EP4 inhibition reduces growth of paclitaxel-resistant cell lines [Fig. 1d]. Likewise, treatment with EP inhibitors [Fig. 1e-top] and silencing of EP4 resulted in reduced migration [Fig. 1e-bottom]. CONCLUSIONS: Selective antagonism of PGE2 through EP4 receptor inhibition may represent a powerful targeted therapy for paclitaxel-resistant OCCC. Further study including simultaneous treatment (EP4 inhibitor + paclitaxel) and larger samples sizes is required. REFERENCES: 1 Rayners et al. A randomized phase II study investigating the addition of the specific COX-2 inhibitor celecoxib to docetaxol and carboplatin as first-line chemotherapy for stage IC to IV epithelial ovarian cancer, fallonpiper tube or primary peritoneal carcinomas: the DoCaCel study. Ann Oncol 2012; 23:2986-902. 2 Moos et al. Effects of taxot /taxotere on gene expression in macrophages: induction of the PGH synthetase-2 isoenzyme. J Immunol 1999;162:476-73.

**#180** Targeting the HSP40/HSP70 chaperone axis as a novel strategy to treat castration-resistant prostate cancer. Michael A. Moses,1 Yeong Sang Kim,2 Genesis Rivera-Marquez,2 Matthew J. Watson,1 Sunmin Lee,1 Andrea Kravats,1 Sue Wickner,1 Jason Gestwicki,1 Jane Trepel,1 Len Necker1,1 National Cancer Institute, Bethesda, MD; 2University of California, San Francisco, San Francisco, CA.

Castration-resistant prostate cancer (CRPC) is frequently characterized by elevated expression of nuclear receptors able to at least partially maintain the androgen receptor (AR) transcriptional program. Elevated expression of a number of constitutively active AR splice variants lacking the ligand binding domain (LBD) (e.g., ARv7, which is ligand-independent and correlates with poor prognosis, reduced survival and resistance to existing LBD-targeted standard of care therapy) is a frequent occurrence in CRPC. Thus, alternative approaches to disrupt AR signaling in CRPC are of great clinical importance, and a single strategy able to target AR and ARv7 remains a critical unmet need. As a steroid hormone nuclear receptor, the AR exists in an interactive and dynamic cycle with the molecular chaperones (heat shock proteins, HSPs) HSP40/HSP70/HSP90 for proper folding and remodeling of the AR LBD to bind ligand. Notably, HSP40 inhibitors promote AR degradation and display efficacy in prostate cancer xenograft models. Although it has been shown that ARv7 functions independently of HSP90, additional chaperone requirements of LBD-deficient ARv7 are not known. Thus, we tested the hypothesis that both AR and ARv7 are dependent on HSP40/HSP70 and that targeting these chaperones with specific inhibitors (C86 and JG98, respectively) will lead to AR/ARv7 destabilization and loss of transcriptional activity in models of CRPC. To determine if AR proteins associate with HSP40/HSP70, 22Rv1 CRPC cells (expressing endogenous AR and ARv7) were first transfected with FLAG-HSP40 or FLAG-HSP70. Immunoprecipitation with FLAG beads revealed AR and ARv7 associated with both chaperones, indicating potential functional dependence of these nuclear receptors on HSP40/ HSP70. To further characterize these interactions, 22Rv1 lysate was probed with antibodies against an LBD and an NBD antibody. C86 bound a significant fraction of HSP40 complexed with HSP70, AR, and ARv7. Excess unlabeled C86 or JG98 effectively competed away binding of HSP40/HSP70 to biotinylated-C86 with concomitant loss of associated AR and ARv7. Treatment of 22Rv1 cells with C86 or JG98 led to a time and dose-dependent decrease in AR and ARv7 protein, concomitant with a significant loss of viability. We also observed that HSP40/70 inhibition markedly reduced AR and ARv7 transcriptional activity, as indicated by decreased AR (KLK3, TMPRSS2) and ARv7 (UBE2C) target gene expression. Finally, treatment of mice bearing 22Rv1 xenografts with JG231 (an analog of IG98 with enhanced PK properties) led to significantly smaller tumors relative to vehicle treated mice. Together, these data confirm the continued dependence of AR and ARv7 on HSP40/HSP70 molecular chaperones and they demonstrate the feasibility of targeting the HSP40/HSP70 axis to abrogate sustained AR-mediated signaling in CRPC.

**#1181** Discovery and development of novel highly potent and selective inhibitors of USP19 using UbiPlex™. Gerald Gavory, Colin O’Dowd, Ewelina Rozycka, Anthony Dossang, Ashling Henderson, Caroline Hughes, Hugues Miel, Oliver Barker, Joana Costa, Peter Hewitt, Mary McFarlan, Lauren Procotor, Tim Harrison. Almac Discovery, Belfast, United Kingdom.

Over the past decade, protein ubiquitination has emerged as an important post-translational modification with regulatory functions in all important cellular processes. Deubiquitinating enzymes (DUBs) including ubiquitin specific proteases (USPs) are cysteine proteases that catalyze the de-ubiquitination of protein substrates including tumor suppressors and oncogenes, hence regulating their levels and/or function. As a result of their increasing implications in the etiology of numerous pathological conditions including cancer, DUBs are emerging as an attractive and promising target class for the development of 14 class medicines with high therapeutic impact. However, despite 15 years of intense research DUBs have proved largely refractory to drug discovery efforts. Herein, we further describe the application of Ubi-Plex™, our drug discovery platform for the identification and optimisation of DUB inhibitors. In particular, we will highlight the versatility and robustness of Ubi-Plex™ by describing the outcome of our focussed library screening, hit identification, hit validation and elaboration activities on USP19. A series of novel, highly potent (e.g. IC₅₀ < 10 nM) and reversible USP19 inhibitors have been identified. Further, profiling has also demonstrated excellent selectivity against a large panel of DUBs and other non-related enzymes (e.g. kinases, proteases). These inhibitors are cell-permeable and exhibit potent target engagement in cells with EC₅₀ values < 30 nM. Finally, we will describe our progress towards the development of lead molecules with drug-like properties with the aim to rapidly establish in vivo proof-of-concept studies. In summary, this work further develops the tractability of the DUB target family and reports the discovery and detailed profiling of the first highly potent and selective inhibitors of USP19. These molecules may provide opportunities for the development of new anticancer therapeutics as well as for the treatment of muscle wasting disorders including cachexia.

**#1182** A novel curcumin derivative inhibits active ras and its downstream pathway in pancreatic cancer. Naveen Mallangada,1 Gerardo G. Mackenzie2. 1Stony Brook University, Stony Brook, NY; 2University of California, Davis, CA.
Pancreatic Cancer’s (PCa’s) 5-year survival rate of only 6.7% indicate the need to improve treatment modalities. Despite decades of research, current chemotherapy and radiation therapy regimens offer minimal or no help. It is critical to develop new agents for the effective management of PC. Given that Kras mutations initiate and maintain PC, inhibition of this pathway is widely considered a therapeutic target for improving the overall survival of PCa patients. Using molecularly targeted inhibitors (MTIs) for treating PCa patients contributes to improving the chemotherapeutic efficacy of a novel Chemically-Modified Curcumin (CMC2.24) as a potential chemotherapeutic agent for PC. Preliminary studies have shown that CMC2.24 has higher bioavailability than curcumin, as shown in pharmacokinetic studies in rats, and inhibits PC growth in vitro and in vivo. However, the exact mechanism on how CMC2.24 reduces cell growth remains unidentifiable. In this study, we aimed to identify the activity of CMC2.24 for demonstrating the chemotherapeutic efficacy of a novel CMC2.24 in PCa. Using human PC MIA PaCa-2 and Panc-1 cell lines and pancreatic acinar explants from KRas mutant mice, we explored the effects of CMC2.24 on Ras activation, ERK phosphorylation, mitochondrial reactive oxygen species, mitochondrial ATP production, and intrinsic apoptosis. In human PC MIA PaCa-2 cells CMC2.24 inhibited Ras activation by 90% (p<0.05). This was confirmed in primary acinar explants isolated from KRas mutant mice in which CMC treatment reduced Ras activation by 70%, compared to control. Furthermore, CMC2.24 treatment reduced the phosphorylation MEK, ERK and c-Raf, down-stream pathway of Ras, both in vitro and in vivo. The effect of CMC2.24 on ERK phosphorylation was confirmed by Immunofluorescence, showing a significant decrease in ERK phosphorylation (47.8%; p<0.05). Moreover, CMC2.24 treatment enhanced the phosphorylation of Akt and p-Akt by 200% (p<0.02), decreased ATP levels in a concentration-dependent manner (p<0.05), and induced intrinsic apoptosis, as shown by the increase in caspase 9 and Parp cleavage downstream of cytochrome C cytosolic release. In conclusion, our results indicate that the Ras pathway is a key molecular target for CMC2.24 and that CMC induces apoptosis in PC cells through the intrinsic pathway. This research was supported by the Stony Brook Cancer Center and a URECA Summer Grant.

#1183 Structure-activity studies and biological evaluations of ERG-USU, a highly selective inhibitor for ERG-positive prostate cancer cells. Ahmed A. Mohamed,1 Charles P. Xavier,2 Gauthaman Sukumar,2 Samuel D. Banister,1 Vineet Kumar,2 Shy-Han Tan,1 Shilpa Katta,1 Lakshmi Ravindranath,1 Muhammad Jamal,1 Radhika Sreenath,1,2 David G. McLeod,3 Georgy Petrovics,4 Albert Dobt,1 Meera Srivastava,1 Sanjay Malhotra,1 Clifton Dalgard,5 Shiv Srivastava1,1 Uniformed Services Univ. of the Health Sci., Rockville, MD; 2Uniformed Services Univ. of the Health Sci., Bethesda, MD; 3Stanford University, Stanford, CA.

Introduction and objectives: While new prostate cancer (CaP) treatments (Abraxalone and Enzalutamide) have improved survival in castration-resistant prostate cancer (CRPC), their benefits are short-lived and drug resistance develops. Known CaP genetic events can drive resistance to specific targets. Therefore, it is important to develop new agents with improved specificity to target the resistance to existing therapies. We previously identified a small molecule inhibitor, ERG-USU, which selectively inhibits ERG protein and cell growth in ERG positive tumor cell lines and mouse xenograft models. In an effort to further develop ERG-USU with enhanced efficacy we performed detailed structure-activity relationship (SAR) evaluation of ERG-USU core structure and developed new derivatives. Methods: Based on SAR of the core structure of ERG-USU, 48 new derivatives were designed and synthesized by substitutions with alkyl, alkoxy, cycloalkyl, heterocycloalkyl, aryl, heteroaryl or hydroxyl groups. The new ERG-USU derivatives were evaluated for inhibition of cell growth and ERG protein levels in the TMPRSS2-ERG fusion harboring CaP cell line, VCaP. Four of these compounds have been selected for evaluation of ERG selectivity by defining IC50 in ERG positive/indexed. The objective of this work was to discover the mechanism of action in multiple cellular events and to demonstrate the ERG-USU derivatives inhibition of cell growth and ERG protein levels in VCaP, KG1, MOLT-4 and COLO205 cell lines, with no or minimal effects on LNCaP and HUVEC cells. One of the new derivatives (ERG-USU96) showed increased cell growth inhibition and induction of cell death in comparison to the parental ERGUSU (IC50 = 0.200 μM). Other three new compounds showed similar IC50 as the ERG-USU. Conclusion: Comprehensive evaluation of ERG-USU derivatives along with parental compound has continued to underscore selective inhibition of ERG positive tumor cells by these small molecules.

#1184 Characterization of novel STAT5 inhibitors to interfere with the oncogenic activities of STAT5 in hematopoietic diseases. Bettina Wingelhofer,1 Barbara Maurer,1 Elizabeth C. Heyes,1 Patricia Freund,1 Abbarna A. Cumarsawamy,2 Jisung Park,2 Stefan Kubicek,3 Peter Valent,1 Patrick T. Gunning,2 Richard Morgridge1,2,3.1 Ludwig Boltzmann Institute for Cancer Research, Vienna, Austria; 2Medical University of Vienna, Vienna, Austria; 3University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

Activation of the transcription factor STAT5 is essential for the pathogenesis of acute myeloid leukemia (AML) containing the FLT3 internal tandem duplication (ITD). FLT3 ITD is a constitutively active tyrosine kinase (TK) that drives the activation of STAT5 and proliferation of AML cells. Although there has been some success in identifying TK inhibitors that block the function of FLT3 ITD, treatment options are still limited. This is mainly due to drug resistance development by mutations that allow for the continued activation of STAT5. Since STAT5 represents a critical mediator of malignant cellular behavior and sits at the convergence point of many kinase pathways, the direct targeting of STAT5 may be an effective means of overcoming this resistance to TK inhibitors. First, we screened a library of potential STAT5 inhibitors for specific SH2 domain binders using a fluorescent polarization assay. Thereby, we identified small inhibitory molecules, called AC-3-019 and AC-4-130, that bind to the SH2 domain of STAT5, subsequently resulting in the disruption of the reciprocal STAT5-phosphoprotein interactions. They efficiently blocked kinase activity towards the growth and survival of AML cells. Moreover, AC-3-019 and AC-4-130 significantly suppressed tumor growth in vivo without general toxicity in healthy organs. Overall, our findings indicate that AC-3-019 and AC-4-130 are potent and selective inhibitors of STAT5. These compounds provide lead structures for further chemical modifications and clinical development for the identification of compounds to improve existing therapies.
and in vivo orthotopic xenografts at well tolerated doses. RNA-seq of isogenic K562 cells treated with H3B-8800 revealed dose-dependent inhibition of splicing. Although global inhibition of RNA splicing was not observed; H3B-8800 treatment led to preferential intron retention of transcripts with shorter and more GC-rich regions compared to those unaffected by drug. Interestingly, H3B-8800 retained introns commonly disrupted the expression of splicingosomal genes, suggesting that the preferential effect of H3B-8800 on splicingomous mutant cells is due to the dependency of these cells on expression of WT spliceosomal genes. These data identify a novel therapeutic approach with selective lethality in leukemias and lung cancers bearing a splicingome mutation. Despite the essential nature of splicing, cancer cells without a splicingome mutation were less sensitive to H3B-8800 compared with potent eradication of mutant counterparts. H3B-8800 is currently undergoing clinical evaluation in patients with MDS, AML, and CMML.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Reversal of Drug Resistance

#1186 Propranolol could overcome BRAF inhibitors resistance by multiple mechanisms in melanoma. Chengfang Zhou,1 DongYa Shen,1 WeiLi Wang,3 Shengchen Xie,3 Ping Liao,3 Xiang Chen,1 Howard L McLeod,3 Yijing Wang,3 MahaAl-Keilani, Ruba Darweesh. National Cancer Institute, Baltimore, MD. BRAF(V600E) is the most common oncogenic mutation in melanoma and leads to constitutive activation of the MAPK pathway, which results in uncontrolled cell growth. Selective BRAF inhibitors such as vemurafenib have been observed to neutralize oncogenic the signaling, inhibit cellular growth, and improve patient outcome. Although these mechanisms of vemurafenib resistance have been reported, few studies focused on how to overcome the resistance. Propranolol, a non-selective β-blocker, was confirmed to involve in multiple anticancer effects. Our previous study also showed propranolol inhibited melanoma by suppressing MAPK and AKT pathways in vitro and in vivo. But its efficacity and mechanism of overcoming vemurafenib still remain unknown in melanoma. Here, we explored the effect of propranolol on the A375, P-8 (patient-derived melanoma cell line) vemurafenib resistance cell line and resistance mice xenografts. Cell viability assay demonstrated that 2μM-20μM vemurafenib couldn’t decrease the proliferation but 24h-120h of incubation of 2μM-200μM propranolol inhibited viability with a concentration and time dependent manner in the two resistance cell line. TUNEL staining showed 24h incubation of 20μM propranolol alone and plus 4μM vemurafenib obviously increased cell apoptosis. Mice received daily i.g. administration of propranolol at the dose of 2 mg/kg alone or plus 10 mg/kg for 21days. The mean tumor volume at day 21 in resistance A375 xenografts was 221.13 ± 76.5mm3; 904.12 ± 70.57mm3 vs. 2021 ± 316.24mm3for the propranolol plus vemurafenib, propranolol alone, vemurafenib alone, respectively. Propranolol improved mice survival, 28.6% animal dead in plus group, 57.1% mice dead in the propranolol group, and 71.5% animal dead in vemurafenib at end of treatment. IHC showed propranolol also reduced Ki67 index both in propranolol and plus group when compared with vemurafenib treated mice. Furthermore, RNA sequencing was performed to explore the mechanism of propranolol overcoming the resistance, the data showed propranolol largely reduced mRNA levels of IGF family (IGFBP3, IGF-FLR1, IGFBP6, IGF2, IGFIR) etc. but elevated the expressions of innate immune related genes (NKG7, TLR9, NCR3 etc.) in A375-vemr cell line. These results provide a strategy of therapeutic resistance for the clininc, importantly, this study also provide a clue targeting IGF family and regulating innate immune might be a potential strategy to suppress resistance in BRAF inhibitor therapies in melanoma.

#1187 Synthetic lethality screening reveals ATR as responsible for oxaliplatin resistance in colorectal cancer cells. Eve Combès,1 Augusto Faria-Anadade,1 Diego Tosi,1 Pierre Martineau,1 Maguy Del Rio,1 Roderick Beijersbergen,2 Nadia Yue,1 Céline Gongora1,3 IRCM, INSERM U1194, Montpellier, France; 2The Netherlands Cancer Institute, Amsterdam, Netherlands. Despite the recent advances achieved in the treatment of colon cancer, tumor resistance is a frequent cause of chemotherapy failure. Our work was aimed to determine the molecular mechanisms involved in the resistance to oxaliplatin, an anticancer agent widely used in colorectal cancer treatment. To this end, we establish an oxaliplatin-resistant cellular model from the colon adenocarcinoma cell line HCT-116. Among cellular clones obtained, we used one displaying mild resistance (10 fold called HCT116-R1) to perform short hairpin RNA-based loss of function genetic screen in order to identify genes that can modulate the cellular sensitivity to oxaliplatin. We screened over 200 genes to determine the molecular mechanisms involved in the resistance to oxaliplatin, but its anticancer effects. Our previous study also showed propranolol inhibited melanoma by suppressing MAPK and AKT pathways in vitro and in vivo. The synergistic effect was evaluated using dose matrix data (an algorithm that was implemented in our group); (ii) the synergistic effect of oxaliplatin and VE-822 was accompanied by an increase of ssDNA, DNA double-strand breaks, growth arrest and apoptosis induction. In conclusion, our preliminary data confirm the results of our screen by demonstrating for the first time the functional role of ATR in the sensitivity to oxaliplatin.

#1188 Sodium phenylbutyrate has an antineoplastic effect and enhances the cytotoxicity of 5-fluorouracil and irinotecan in colorectal cancer cell lines. Maha Al-Keilani, Ruba Darweesh. Jordan University of Science and Technology, Irbid, Jordan. Colorectal cancer is the third most common cancer in the world and it is the second leading cause of cancer-related deaths in the United States. About 25% of colorectal cancer cases present with metastases at time of diagnosis and 50% of patients who undergo surgery will ultimately develop metastatic disease. Consequently, adjuvant chemotherapy is required in many cases and it aims to prevent tumor progression and metastasis. Resistance to chemotherapy is the biggest obstacle in the treatment path of advanced colorectal cancer. Thus, highlighting the urgent need for the identification of new therapeutic agents that can enhance the cytotoxicity of the currently approved anticancer drugs. Sodium phenylbutyrate (NaPB), a salt of a short chain fatty acid that is used for treatment of urea cycle disorders, is under investigation for its antineoplastic potential. NaPB acts as a histone deacetylase inhibitor and it has been shown to be associated with reduced ER stress and enhanced JNK signaling pathway. This indicates that NaPB may enhance the cytotoxicity of the currently approved agents for colorectal cancer including oxaliplatin, 5-fluorouracil, and irinotecan. The aims of this study were to identify the antineoplastic potential of NaPB in colorectal cell lines, and to identify its effect on the cytotoxicity of oxaliplatin, 5-fluorouracil, and irinotecan. MTT proliferation assay showed that NaPB possessed a concentration dependent killing effect against HCT-116 and HT-29 colorectal cancer cell line (IC50 values were 5 and 10 mM respectively). Moreover, at clinically achievable and nontoxic concentration (2.5 mM), NaPB showed a synergistic effect in HCT-116 on 5-fluorouracil (R = 14.176, p = 0.006), on irinotecan (R = 53.86, p = 0.0117), and on oxaliplatin (R = 20, p = 0.004), but resulted in extensive cytotoxicity when used at 5 mM and 10 mM. In HT-29 cells, NaPB at concentrations 5 mM and 10 mM, showed a synergistic effect on 5-fluorouracil (R = 5.5, p < 0.001; and R = 125, p < 0.001 respectively), on irinotecan (R = 5.7, p = 0.016; and R = 21.3, p = 0.018 respectively), and on oxaliplatin (R = 2.4, p = 0.003; and R = 3, p = 0.002 respectively). The addition of NaPB at concentration of 20 mM to the previous drugs resulted in profound cytotoxic effect toward HT-29 cells. As a conclusion, NaPB is a promising new adjuvant antineoplastic agent that can be used to enhance the cytotoxicity of 5-fluorouracil, irinotecan, and oxaliplatin in colorectal cancer.

#1189 Enhanced YAP expression leads to EGFR TKI resistance in lung adenocarcinomas. Ting-Fang Lee, Yu-Chi Tseng, Cheng-Wen Wu. National Yang-Ming University, Taipei, Taiwan. Epidermal growth factor receptor (EGFR) mutation is prevalently expressed in lung adenocarcinoma cases and acts as one of the major driving oncogenes. EGFR tyrosine kinase inhibitors (TKIs) have been used in patients with EGFR-mutant as an effective targeted therapy in lung adenocarcinoma, but drug resistance and tumor recurrence inevitably occurs. Recently, Yes-associate protein (YAP) has been reported to promote multiple cancer cell properties, such as promoting cell proliferation, EMT and drug resistance. This study investigated the roles of YAP in TKI-resistant lung adenocarcinoma. In TKI-sensitive cells, enhanced YAP expression leads to TKI resistant. Also, upregulated YAP expres-
sion and activation were detected in long term TKI-induced resistant cells. With reduced YAP expression using shRNA or YAP inhibitors, TKI-resistant cells become TKI-sensitive. Moreover, combined EGFR TKI and an YAP inhibitor statin, prolonged survival among lung cancer patients analyzed by Taiwan National Health Insurance Research database. These observations revealed the importance of YAP in promoting TKI-resistance and confirmed YAP inhibition can be a potential therapy delaying the occurrence of TKI-resistance in lung adenocarcinoma.

**#1190 Comprehensive high-throughput screen for combination therapies to block acquired resistance to targeted drugs.** Zejak Haviv. Bar Ilan Univ., Zfat, Israel.

Background - Genetic and epigenetic alterations provide the selective advantage for cancer cell acquired drug resistant (ADR). A bottleneck in implement drug combination is the challenging task of identifying combinations with acceptable risk benefit ratio. High throughput functional genomic (RNAi) screen is an ideal tool to comprehensively identify double and triple inhibitor combinations to prevent ADR. Unfortunately, most anticancer agents are antagonists (antagonists operate in the same direction as the RNAi), therefore RNAi hits with possible clinical value are the ones that disappear during the screen. Materials and Methods - We use lentiviral-borne shRNA libraries (mainly kinase collection) to transduce different RNAi into the different RNAi independent derived cancer cells. The resulting mixed cancer cell population (each lost one gene) is subject to negative screen, to identify mediators of ADR. This process was performed with approved cancer drugs, such as erlotinib, olaparib, pazopanib, etc. We developed a proprietary unique way to identify the RNAi clones that disappear/extinct, specifically in the presence of the investigated drug. RNAi clones are tracked using next generation sequencing, and quantified in treated and untreated cancer cell cultures or xenografts. The resulting drug combinations identified through this screen are tested directly on a platform of patient-derived xenografts. Results - We identified known boosters of the EGFR activity, such as Dyrk1 (maintains level of EGFR protein), IkBKE (downstream survival mediator), which we found regulated by the RNAi, as well as the response to the drug. Subsequently, known inhibitors of Jak1, CdK4/6, and PJK3 combined to egfr inhibitors generated more durable remissions in egfr positive cells. Remarkably, even tumors without egfr mutations were sensitive to the combinations. A similar discovery of novel combinations were found for olaparib and pazopanib. Mouse harboring patient derived xenografts tolerated regorafenib and pazopanib. Mouse harboring patient derived xenografts maintained in the N87-TDMR cells. A comprehensive expression analysis revealed that regorafenib inhibited the ATP-driven efflux function of BCRP. Our in vivo studies indicated that regorafenib could be useful in combating multidrug resistance in cancer treatment.

**#1193 DS-8201a, a novel HER2-targeting ADC with a novel DNA topoisomerase I inhibitor, abrogates the resistance to T-DM1 in HER2-positive gastric cancer: a preclinical study.** Yoshikane Nonagase,1 Naoki Takegawa,1 Kimio Yonesaka,2 Kazuko Sakai,1 Yusuke Ogitani,3 Junji Tsurutani,1 Kazuto Nishio,1 Kazuhiro Nakagawa,1 Kindai University Faculty Of Medicine, Osaka, Japan; 2Datichi-Sankyo Co., Ltd., Tokyo, Japan.

Background: Anti-HER2 therapies are beneficial for patients with HER2-positive breast and gastric cancer. T-DM1 is an HER2-targeting antibody-drug conjugate (ADC), which is structurally composed of the anti-HER2 antibody trastuzumab and the tubulin inhibitor DM1. T-DM1 has shown efficacy in patients with advanced breast cancer, but all patients eventually develop resistance to T-DM1. DS-8201a is a novel ADC composed of an anti-HER2 antibody and a novel potent topoisomerase I inhibitor DX-8951 derivative. DS-8201a achieved a high drug-to-antibody-ratio (DAR: 7-8) and homogeneous drug conjugation. The aim of this study was to elucidate the mechanisms of T-DM1 resistance, and evaluate the efficacy of DS-8201a in a T-DM1-resistant HER2-positive gastric cancer cell line. Materials and methods: The T-DM1-resistant NCI-N87 cell line (N87-TDMR) was established by a step-wise method—the parent HER2-positive N87 cell line was exposed to up to 4 μg/ml of T-DM1. The profile of N87-TDMR was assessed by immunoblotting, DNA microarray, and quantitative reverse transcription-PCR (qRT-PCR). The sensitivity of parent N87 and N87-TDMR cells to T-DM1 or DS-8201a was assessed by an in vitro growth inhibition assay as well as through the mouse xenograft model study. Results: N87-TDMR cells were found to be resistant to T-DM1 in the in vitro growth inhibition assay (50% growth inhibition concentration: >10 μg/ml in N87-TDMR, 0.055 μg/ml in N87) as well as in the in vivo xenograft model study, but HER2 expression was maintained in the N87-TDMR cells. A comprehensive expression analysis revealed that N87-TDMR cells over-expressed ABCG2 and were sensitive to the suppression of ABCG2 expression by ABCG2 shRNA.

**#1194 Neutralization of IGF-I and -II ligands with the fully humanized antibody-Analect A3, a novel mechanism-based inhibitor of breast cancer resistance protein (BCRP).** Guan-Nan Zhang,1 Yi-Jun Wang,1 Yun-Kai Zhang,1 Meng-Ning Wei,7 Zhi Shi,2 Zhe-Sheng Chen1. St. John’s University, Jamaica, NY; 2Jinan University, Guangzhou, China.

Overexpression of breast cancer resistance protein (BCRP) has been shown to play a role in promoting MDR in various kinds of cancers, such as breast, colon, lung and ovarian cancers. Regorafenib, an oral multi-kinase inhibitor, was found to have inhibitory effects on BCRP-mediated MDR both in vitro and in vivo. Regorafenib significantly sensitized BCRP-overexpressing cancerous cells to BCRP substrates by increasing their intracellular accumulation. There are no significant changes in the expression level or the subcellular distribution of BCRP in the cells exposed to regorafenib. Our mechanism studies revealed that regorafenib inhibited the ATP-driven efflux function of BCRP. Our inducted-fort docking and molecular dynamics simulations suggested the existence of strong and stable interactions between regorafenib and BCRP protein. Animal study revealed that the combination of regorafenib and topotecan resulted in great inhibitory effects on the growth of S1-M1-80 xenograft tumors. Regorafenib significantly increased the intratumoral concentration of topotecan but had no significant effects on topotecan plasma concentration. In conclusion, our study indicated that regorafenib could be useful in combating multidrug resistance in cancer treatments.
overall survival. Additionally, expression of AR-V7 in circulating tumor cells or increased expression in metastatic tissue is a biomarker for resistance to currently available anti-androgen therapies, including enzalutamide (ENZA) and abiraterone, resulting in castration-resistant prostate cancer (CRPC). Insulin-like growth factor (IGF) signaling pathways have been incriminated as mechani-
sms of drug resistance. Among the many targets is IGF-1 receptor (IGF1R), which promotes tumor growth in relation to AR variants. In this preclinical study we examined the effects of IGF-1 and -II inhibition in LuCaP 96CR, an ENZA resistant prostate cancer patient-derived xenograft. Hypothesis: IGF-1 and -II neutralization will block AR-V7 activity and inhibit castration-resistant prostate cancer growth. Study procedures: LuCaP 96CR was implanted s.c. into castrate SCID mice (15 mice per treatment group). Tumor volume at day 21 was measured (5 tumors per treated group). In this study, tumors were grouped into two: 1) Control; 2) ENZA (50 mg/kg, QD, po). When tumors exceeded 150 mm³ animals were randomized into groups: 1) Control; 2) ENZA (50 mg/kg, QD, po), 3) xentuzumab (BI 836845, 200 mg/kg QW IP) in combination with ENZA. At the end of the study, tumors were collected for preparation of RNA and protein lysates and histology. Results: ENZA did not show significant inhibitory effects on LuCaP 96CR, but the combination of xentuzumab and ENZA resulted in significant tumor inhibition (p<0.001) vs. ENZA alone. AR full-length (AR-FL) mRNA and protein increased after ENZA treatment (p<0.001) but did not further rise significantly when xentuzumab was co-administered. In contrast, significantly elevated AR-V7 mRNA and protein levels were detected in tumors from mice treated with the xentuzumab and ENZA combination (p<0.001). Downstream markers of AR-V7 activity, UBE2C and UGT2B17 mRNA, increased after ENZA compared to INM. A significant increase of UGT2B17 was detected after combination treatment and UBE2C significantly decreased (p<0.001). Summary: These data show that addition of xentuzumab provides tumor inhibition of ENZA-resistant CRPC. Interestingly, associated with a significant decrease in tumor growth, this treatment resulted in increased AR-V7 mRNA and protein expression but no significant increases in downstream markers of AR-V7 activity, UBE2C and UGT2B17. Conclusion: Our results suggest that the IGF-1 and -II neutralizing antibody xentuzumab may reverse AR-V7-mediated ENZA resistance.

#1195 TNFα induces multidrug resistance to HER2-targeted TNFα induces multidrug resistance to HER2-targeted therapies in HER2-positive breast cancer. Maria F. Mercogliano,1 Mara De Martino,1 SofiaBruni,1 Leandro Venturutti,1 Martín Rivas,2 Matias Amasino,3 Cecilia J. Proietti,1 Patricia V. Elizalde,1 Roxana Schillaci1,1 BYME-CONICT, CABA, Buenos Aires, Argentina; 2Well Cornell Medical College, Manhattan, NY.

HER2 positive (HER2+1) is a breast cancer (BC) subtype characterized by HER2 overexpression/amplification that affects nearly 15% of BC patients and correlates with poor prognosis. These patients receive trastuzumab (T), an anti-Her 2 monoclonal antibody, but resistance events (40-60%) hamper its clinical benefit. Previously we have demonstrated that TNFα (TNF) induced mucin-4 (MUC4) expression and turned T-sensitive cell lines and tumors into multidrug resistant ones. Nowadays, new anti-HER2 therapies are being used in the clinical setting, such as lapatinib (a dual inhibitor of EGF and HER2), and antibodies like T-D1M1 (combines TZ with the anti-microtubule agent emtansine), and pertzumab (P) that impedes HER2 dimerization. The aim of this work was to study the role of TNF in resistance to the new HER2-targeted therapies. We used BT-474-C (control cells) and BT-474-T2 cells engineered in our lab to stably over-express TNF, and were proven to be sensitive and resistant to T, respectively. We performed dose-response curves for T-D1M1, they show that inhibits proliferation of BT-474-C cells at 0.01 µg/ml. On the other hand, BT-474-T2 cells were resistant in the same experimental conditions and they exhibited reduced T-D1M1 binding with respect to BT-474-C. BT-474-C cells were sensitive to low concentrations of T-D1M1 with 0.5 nmol/L, but in BT-474-T2 cells T-D1M1 was ~10 times less potent than control cells (IC 50 3.34 nmol/L). When we abrogated MUC4 expression, BT-474-T2 cells were sensitized to T-D1M1, showing that TNF-induced MUC4 expression is responsible for T-D1M1 resistance in this cell line. We assessed the effect of lapatinib performing a dose-response curve. Results shown a similar IC50 for BT-474 C and T2 cells (0.26 µM and 0.08 µM, respectively). When studied P effect, ~150nm and that the combination of T+P was more effective inhibiting proliferation in BT-474-C cells than T alone, despite these results binding of the antibody showed no change between the cell lines. In BT-474-T2 cells proliferation was slightly inhibited by the combined treatment. In vivo experiments showed that BT-474-C tumors were sensitive to T and the combination of T+P, but BT-474-T2 tumors did not respond to any of these treatments. These results suggest that TNF plays an important role in multidrug resistance to HER2-targeted therapies, specifically T-D1M1 and P, but not in lapatinib resistance. We propose TNF as an attractive target and we suggest that HER2+ patients resistant to T could be eligible for a combination of HER2-targeted therapies and a TNF-blocking treatment to overcome resistance.

#1196 Epigenetic drugs modulate long noncoding RNAs expression in BRAF inhibitor-resistant melanoma. Barbara Montico,1 Giorgio Giurato,2 Katy Mastorci,1 Aurora Rizzo,1 Maria Ravo,2 Francesca Rizzo,2 Alessandro Weiz,2 Riccardo Dolcetti,2 Francesca Colizzi,1 Luca Sigalotti,1 Elisabetta Fratta1,2 Centro di Riferimento Oncologico, Aviano, Italy; 3University of Salerno, Salerno, Italy; 4University of California, San Francisco, San Francisco, USA; 5University Medical School of South Australia, Adelaide, Australia; 6University Hospital of Udine, Udine, Italy.

Emergence of drug resistance is the major cause of failure of BRAF inhibitors (BRAFI) treatment in cutaneous melanoma (CM). Epigenetic modifications are known to physiologically trigger massive modifications in cellular commitment and several studies report a correlation between the drug-resistant phenotype and epigenetic alterations. Long noncoding RNAs (lncRNAs) represent a class of gene regulators acting at epigenetic, transcriptional and post-transcriptional level. Several studies have implicated lncRNAs in chemoresistance through their ability to impair cell cycle arrest and apoptosis, but also to induce and modulate epithelial-mesenchymal transition and cell adhesion-associated signaling pathways. lncRNAs interact with histone modifying complexes and/or DNA methyltransferases, being also targets of these epigenetic mediators. Furthermore, epigenetic drugs have been recently identified as modulators for lncRNAs function as well as their related targeting signals. Starting from these evidences, we asked the question whether epigenetic drugs could differentially affect the survival of BRAFI-resistant (VR) and -sensitive CM cells, investigating the mechanistic network involved, with a specific focus on the role of lncRNAs. A panel of BRAFI-sensitive and VR CM cell lines, treated with the FDA-approved HDAC inhibitor vorinostat (SAHA). FACS analysis of annexin V-FITC/propidium iodide stained cells showed that SAHA cytotoxic activity was more pronounced on VR CM cells than on their parental counterparts. RNA-Seq analysis revealed that a large number of differentially expressed lncRNAs was modulated in VR CM cells treated with SAHA. Intriguingly, the expression of several VR up-regulated lncRNAs was decreased to levels similar to those observed in the matched parental cells. Functional analysis indicated these lncRNAs were statistically enriched in pathways involving cellular growth and proliferation, but also cellular assembly and organization. Though additional studies are required, epigenetic modulation of VR-associated lncRNAs promises to have significant therapeutic potential to restore BRAFI sensitivity, being concurrently effective in killing VR cells as monotherapy. Based on our preliminary data, we could anticipate that the combined use of epigenetic and targeted drugs would increase therapeutic efficacy in CM patients relapsing to BRAFI.
required for ET-1-induced YAP signaling. At the chromatin level, the activation of ET-1 axis promotes the recruitment of YAP, TEAD, and β-arrestin to TEAD binding sites of CTGF, ANKR1D and EDN1 promoters, an effect inhibited by macitentan treatment. These results indicate that β-arrestin can act as a transcriptional co-activator that bind TEAD thereby activating transcription of effectors of ET-1R/TEAD transcriptional activity. Altogether our results establish for the first time the role of ET-1R/β-arrestin signaling, providing mechanistic insights that targeting ET-1R signaling in combination with macitentan treatment may represent a promising therapeutic strategy for the management of NSCLC.

#1198 Neuruplin-1 blockade chemosensitizes pancreatic cancer cells via dual inhibition of epithelial-mesenchymal transition and autophagy. Pratik N. Matkar, Krishna K. Singh, Gerald Prud’homme, David Hedley, Howard Leong-Poi. 

Univ. of Toronto, Toronto, Ontario, Canada.

Introduction: Fibrotic and malignant pancreatic ductal adenocarcinoma (PDAC) tumors are often resistant to chemotherapy, and remain a leading cause of morbidity and mortality. Epithelial–mesenchymal transition (EMT), a process by which epithelial cells lose their cell polarity and adhesion to become migratory and invasive mesenchymal stem cells, contributes to chemoresistance in PDAC. Likewise, PDAC tumors benefit from autophagy, a self-degradation pathway that confers a survival benefit against metabolic stress. Over-activation of EMT and autophagy has been linked with drug resistance in PDAC. Both these processes are largely mediated by TGFβ1. Recently, we demonstrated the role of neuruplin-1 (NRP-1) in TGFβ1-dependent endothelial-mesenchymal transition and fibrosis in PDAC. However, the potential mechanisms linking NRP-1 with autophagy-mediated chemoresistance remain unclear. We hypothesized that NRP-1 blockade will enhance chemotherapeutic effect, by suppressing MET and autophagy in PDAC. Methods: Human PDAC cells (BxPC-3) or serially grafted exposed mesenchymal cancer (CR-BxPC-3) cells were transfected with siNRP-1 or scramble using DharmaFECT-2. For EMT and chemotherapy studies, transfected cells were treated with TGFβ1 (5ng/mL, 24h), followed by gemcitabine (1µM) for 48h. RNA (24h) and protein (48h) was isolated using TRIzol™ and RIPA, respectively. EMT markers were evaluated by RT-PCR and immunoblotting/immunostaining. For autophagy studies, cells were serum starved post NRP-1 silencing and protein was isolated. For autophagy inhibition, cells were treated with bafloycin-A1 (100nM) for 24h post NRP-1 silencing and protein was isolated. Autophagy flux (LC3-II/I) and P62 were measured by immunoblotting/immunostaining. Cell viability was evaluated by MTT assay. Results: SiNRP-1 treatment in BxPC-3 and CR-BxPC-3 cells resulted in NRP-1 knockdown and promoted cell-death after chemotherapy. TGFβ1-stimulated BxPC-3 cells demonstrated a morphological transition consistent with EMT that was revoked by siNRP-1. Also, siNRP-1 maintained epithelial cellular morphology via gain of epithelial and loss of mesenchymal markers. Remarkably, CR-BxPC-3 cells exhibited enhanced EMT features. SiNRP-1 inhibited EMT and promoted cell-death by overcame gescinmine resistance in CR-BxPC-3 cells, possibly through reduced ERK signaling. Moreover, siNRP-1 inhibited autophagy (decreased N1LI/U1 ratio) resulting in increased PDAC cell survival post chemotherapy stress, and enhanced cell-death after chemotherapy and autophagy inhibition in BxPC-3 cells. Conclusions: Our findings define a previously undetermined role of NRP-1 in regulating autophagy and TGFβ1-induced EMT in PDAC, suggesting that NRP-1 may represent a novel therapeutic target to overcome chemoresistance through reduced EMT and autophagy.

#1199 Overcomes AXL and Met mediated erlotinib/gefitinib cross resistance in non-small cell lung cancer cells by Marsdenia tenacissima extract. Shu-Yan Han, Hong Sun, Dong Xue, Wei Zhao, Yan-Na Jiao, Ping-Ping Li. Peking University Cancer Hospital & Institute, Beijing, China.

Apart from EGFR T790M mutation, the bypass activation of c-Met and Axl kinase also lead to the resistance to tyrosine kinase inhibitors (TKIs) in NSCLC. Axl and c-Met share a downstream pathways with EGFR, thus combined treatments of EGFR inhibitors with Axl or Met inhibitor are promising to overcome acquired resistance of TKIs. Our previous work showed that the water extract of Marsdenia tenacissima (MTE), which used to treat cancer in clinics for decades, restored gefitinib sensitivity in resistant NSCLC cells with EGFR T790M mutation or K-ras mutations in vitro and in vivo. However, the potential efficacy of MTE on Axl and c-Met mediated resistance has not yet been fully understood, and the related molecular mechanisms also need to be elucidated. The present study was performed on HCC827/ER cells, which was established by exposing parental HCC827 cells to erlotinib. HCC827/ER cells are with Axl activation and c-Met amplification, and show dual-resistance to erlotinib and gefitinib. We evaluated the effects of MTE to restore erlotinib/gefitinib sensitivity with three different combinations. Cell viability and cell apoptosis were determined by MTT and flow cytometry, respectively. The c-Met amplification was assessed with TaqMan real-time PCR. Signaling pathways were examined by Western blotting to reveal the possible mechanisms. The in vivo efficacy of MTE with erlotinib/ gefitinib were tested on HCC827/ER xenograft mice, and tumor tissues were subjected to immunohistochemistry analysis and Western blotting. Our results indicated the MTE + Met + Erlotinib/Gefitinib (M + M + E/G) treatment was the most potent combinations. Compared with control group and each single, M + M + E/G treatment induced significant apoptosis, obviously inhibited EGF-induced phosphorylation of PI3K/Akt/mTOR and ERK1/2, down-regulated HGF/c-Met activation in HCC827/ER cells. Axl is a receptor tyrosine kinase which strongly associated with EMT phenotype. The M + M + E/G treatments could not re-strain Axl expressions but remarkably reduced phospho-Axl levels and inhibited EMT phenotype in HCC827/ER cells. Surprisingly, MTE alone caused prominent p-Axl inhibition, along with up-regulated E-cadherin and decreased mesenchymal markers. The mouse tumors were remarkably restrained by the M + M + E/G combinations, and it was significant compared with each drug alone (p < 0.05). Accordingly, cell apoptosis was extended, PCNA expression and tumor angiogenesis (VEGF and CD105) were reduced and EMT phenotype were regulated in tumor tissues. The M + Met and p-Axl were also considerably suppressed by the combined treatments. The present data revealed that MTE reverse resistance such as erlotinib and gefitinib efficacy in resistant NSCLC cells with Axl activation and c-Met amplification in vitro and in vivo. It suggests that the addition of MTE may be a promising therapeutic strategy to overcome TKIs resistance in NSCLC.

#1200 Novel strategy to overcome platinum resistance in uterine leiomyosarcoma; blocking ATP7B by copper ion. Mamoru Kakuda, Shinya Matsuaki, Ruriko Nakae, Yusuke Tanaka, Eiji Kobayashi, Yutaka Ueda, Kiyoshi Yoshino, Tadashi Kimura. Osaka University, Suita-City, Japan.

Objective: Resistance to platinum drugs remains a significant problem. Our objective is to elucidate the role of ATP7B, a copper transporter which excretes copper ion thereby platinum can be accumulate in cells. Thus, pre-treatment with CuSO4 may represent a novel therapeutic approach to enhance platinum sensitivity in uterine leiomyosarcoma (ULM) cells. Methods: The expression of ATP7B in ULM was evaluated by real-time PCR and immunohistochemical (IHC) staining methods. ULM cell lines (SK-LMS-1, SK-UT1 and SKN) were treated with CuSO4 before cisplatin treatment for 24h post NRP-1 silencing and protein was isolated. Autophagy flux (LC3-II/I) and P62 were measured by immunoblotting/immunostaining. Cell viability was evaluated by MTT assay. Results: SiNRP-1 treatment in BxPC-3 and CR-BxPC-3 cells resulted in NRP-1 knockdown and promoted cell-death after chemotherapy. TGFβ1-stimulated BxPC-3 cells demonstrated a morphological transition consistent with EMT that was revoked by siNRP-1. Also, siNRP-1 maintained epithelial cellular morphology via gain of epithelial and loss of mesenchymal markers. Remarkably, CR-BxPC-3 cells exhibited enhanced EMT features. SiNRP-1 inhibited EMT and promoted cell-death by overcame gescinmine resistance in CR-BxPC-3 cells, possibly through reduced ERK signaling. Moreover, siNRP-1 inhibited autophagy (decreased N1LI/U1 ratio) resulting in increased PDAC cell survival post chemotherapy stress, and enhanced cell-death after chemotherapy and autophagy inhibition in BxPC-3 cells. Conclusions: Our findings define a previously undetermined role of NRP-1 in regulating autophagy and TGFβ1-induced EMT in PDAC, suggesting that NRP-1 may represent a novel therapeutic target to overcome chemoresistance through reduced EMT and autophagy.

#1201 Disrupted endoplasmic reticulum-mitochondria contacts promote multidrug resistance. Jordia Coku, 1 Madison C. Pedrotty, 1 David M. Booth, 2 Sharon Kim, 3 Annette Vu, 4 C. Patrick Reynolds, 4 György Hajnóczy, 2 Michael D. Hogarty. 1 University of Pennsylvania, Philadelphia, PA; 2 Thomas Jefferson University Health Sciences Center, Lubbock, TX.

Conclusion: ATP7B is a copper transporter which excretes copper ion thereby platinum can be accumulate in cells. Thus, the pre-treatment with CuSO4 may represent a novel therapeutic approach to enhance platinum sensitivity in uterine leiomyosarcoma (ULM) cells. Methods: The expression of ATP7B in ULM was evaluated by real-time PCR and immunohistochemical (IHC) staining methods. ULM cell lines (SK-LMS-1, SK-UT1 and SKN) were treated with CuSO4 before cisplatin treatment for 24h post NRP-1 silencing and protein was isolated. Autophagy flux (LC3-II/I) and P62 were measured by immunoblotting/immunostaining. Cell viability was evaluated by MTT assay. Results: SiNRP-1 treatment in BxPC-3 and CR-BxPC-3 cells resulted in NRP-1 knockdown and promoted cell-death after chemotherapy. TGFβ1-stimulated BxPC-3 cells demonstrated a morphological transition consistent with EMT that was revoked by siNRP-1. Also, siNRP-1 maintained epithelial cellular morphology via gain of epithelial and loss of mesenchymal markers. Remarkably, CR-BxPC-3 cells exhibited enhanced EMT features. SiNRP-1 inhibited EMT and promoted cell-death by overcame gescinmine resistance in CR-BxPC-3 cells, possibly through reduced ERK signaling. Moreover, siNRP-1 inhibited autophagy (decreased N1LI/U1 ratio) resulting in increased PDAC cell survival post chemotherapy stress, and enhanced cell-death after chemotherapy and autophagy inhibition in BxPC-3 cells. Conclusions: Our findings define a previously undetermined role of NRP-1 in regulating autophagy and TGFβ1-induced EMT in PDAC, suggesting that NRP-1 may represent a novel therapeutic target to overcome chemoresistance through reduced EMT and autophagy.
Background: Most high-risk neuroblastoma patients succumb to lethal therapy resistant disease acquired during the course of intensive multimodality treatment. This acquired therapy resistance is largely attributed to insensitivity to drug-induced apoptosis, however the exact mechanisms remain unknown. Apart from integrating death signals, mitochondria (mito) interact with the endoplasmic reticulum (ER), inducing Bim or Bim-like proteins at close contact sites known as mitochondria-associated membranes (MAM) of the ER to regulate calcium and lipid transfer and apoptotic sensitivity, a process often derailed in therapy resistant cancers. ER-mito contact sites are juxtaposed by various tethering protein complexes that include MFN2 and PACS2. Pathologic deregulation of these contact sites has been implicated in the genesis of neurodegenerative and metabolic disorders. Here, we show that disruption of bona fide ER-mito tethering protein complexes in therapy sensitive neuroblastomas induces apoptotic insensitivity and a drastic shift towards a resistance-like phenotype. Methods and Results: Previously we showed that isolated mitochondria from tumors at relapse resist induction of mitochondrial apoptosis when primed with the terminal death effectors that are downstream of therapeutic stress (tBid and Bim-BH3 peptide). We isolated mitochondria from seven matched isogenic tumor pairs obtained at diagnosis (DX) and relapse (REL) and quantified their apoptotic response to tBid and Bim by measuring cytochrome C release by ELISA. Electron microscopy (EM) image analyses of ER-mito contact sites revealed that REL tumors contain up to 70% fewer ER-mito interactions than their matched DX tumors, as confirmed by IB for organelle-specific proteins. Here, we recapitulate the post-therapy resistance phenotype in DX therapy sensitive cells by shRNA silencing of MFN2 or PACS2 and confirm their apoptotic resistance by BH3 profiling and drug response in vitro. A 60% decrease in MFN2 protein in DX cells partially phenocopied the resistance profile of isogenic REL cells. The degree of apoptotic resistance correlated with the extent of protein knockdown for MFN2. Treatment of shMFN2 and shPACS2 DX cells with ABT-737, a BH3 mimetic, or carboplatin, increased their IC50s multiple-fold compared to control cells, paralleling their blunted mitochondrial apoptotic response. Conclusions: Our data implicate ER-mito contact sites as positive regulators of apoptosis, whose disruption may be necessary for apoptotic attenuation in therapy resistant cancer cells to enable apoptosis evasion and survival. We present a potential mechanism for broad therapy resistance arising under therapeutic stress that selects for reduced communication of ER with mitochondria.

#1202 Targeting PIK3C3 to overcome drug resistance resulted from prosurvival autophagy in cancer cells. CHUNG-HAN CHEN, TAIPEI MEDICAL UNIVERSITY, TAIPEI, TAIWAN.

Autophagy is an important cellular recycling mechanism in which portions of cytosol or organelles are sequestered into a double-membrane structure and delivered to lysosome for degradation. PIK3C3 is the only class III PI3K, which specifically generates phosphatidylinositol 3-phosphate (PtdIns3P). It in turn recruits proteins containing FYVE or PX domains, thereby initiating various complexes at the membranes of endosomes, phagosomes and autophagosomes. Recently, several studies have shown that autophagy is upregulated in cancer cells when exposing to chemotherapy and radiotherapy. Therefore, the rationale of targeting autophagy addiction in cancer was proposed by combining autophagy inhibitors and chemotherapeutics that induce autophagy as a pro-survival mechanism to increase their therapeutic efficacy. We stably knocked down PIK3C3 in cancer cell lines and combined with clinical therapeutic agents reportedly induce prosurvival autophagy. Cell viability was examined by MTT assay and combination index was determined by Compusyn software. The autophagy flux was evaluated by detecting protein levels of LC3-II and p62 through western blot analysis. The results showed PIK3C3 knockdown synergistically sensitizes the killing effects of gefitinib and gemcitabine in A549 cells and Panc1 cells, respectively. Both of gefitinib and gemcitabine increased autophagy flux, as evidence by the increase of LC3-II and decrease of autophagy substrate, p62. However, the combination of PIK3C3 knockdown and therapeutic agents increased both of the protein levels of LC3-II and p62. These data suggest incomplete autophagy when the function of PIK3C3 was inhibited. The phenomenon was confirmed by bombing autophagy inducer and PIK3C3 inhibitors. Moreover, no appreciable apoptotic cell death was observed. Taken together, our study revealed a potential strategy by targeting PIK3C3 to overcome drug resistance associated with the pro-survival autophagy in cancer.


Recurrent chemoresistant ovarian cancer is responsible for the high mortality rate in ovarian cancer patients. The molecular mechanisms underlying the chemoresistant ovarian cancer are not fully understood. MicroRNAs (miRs) expression has been recognized as one of the core regulators of gene expression in recent years. However, whether miRs are involved in chemoresistance of ovarian cancer has not been fully elucidated. Current study was performed to interrogate the role of a number of critical miRs in chemoresistance of ovarian cancer by targeting PIK3C3 to overcome drug resistance associated with the prosurvival autophagy inducer and PIK3C3 inhibitors. Moreover, no appreciable apoptotic levels of LC3-II and p62. These data suggest incomplete autophagy when the function of PIK3C3 knockdown and therapeutic agents increased both of the protein levels of LC3-II and p62. These data suggest incomplete autophagy when the function of PIK3C3 was inhibited. The phenomenon was confirmed by bombing autophagy inducer and PIK3C3 inhibitors. Moreover, no appreciable apoptotic cell death was observed. Taken together, our study revealed a potential strategy by targeting PIK3C3 to overcome drug resistance associated with the pro-survival autophagy in cancer.

#1204 Enhanced efficacy of selumetinib by pretreatment of 5-FU in preclinical Ras/Raf-mutant colorectal cancer models. Hye Yoon Jang,1 Haeng Jung Lee,2 Ye San Kim,2,3 Won Dong Kim,2 Jong Yu Bae,2 Jin Hwang Jung. Seung Jin Lee,1 Eun Hee Moon1. CHUNGNA UNIVERSITY, DAEJEON, REPUBLIC OF KOREA; 2GACHON UNIVERSITY, INCEON, REPUBLIC OF KOREA; 3ASIAN INSTITUTE FOR LIFE SCIENCES, ASIAN MEDICAL CENTER, SEOUL, REPUBLIC OF KOREA; 4A CONHON UNIVERSITY, INCEON, REPUBLIC OF KOREA; 5ASIAN METHODICAL CENTER, UNIVERSITY OF ULSAN, COLLEGE OF MEDICINE, SEOUL, REPUBLIC OF KOREA; 6ASIAN MEDICAL CENTER, UNIVERSITY OF ULSAN, SEOUL, REPUBLIC OF KOREA.

Optimal strategy for the application of MEK inhibitor as combination therapy has not been established in Ras/Raf-mutant colorectal cancer (CRC). As we preliminary observed that 5-FU-induced pharmacodynamic changes were re-stored by ERK inhibition, we hypothesized that post-treatment of MEK inhibitor would enhance antitumor activity of 5-FU. Treatment of 5-FU for 2 days followed by selumetinib for another 2 days (FS schedule) exhibited synergism for cell viability, whereas reverse or concomitant combination showed antagonism or inconsistent response, respectively, in 8 kinds of CRC cell lines including BRAFV600E colon205, BRAFV600E RKO, and KRAS G13D HCT8 cells. Combination as FS schedule persistently down-regulated the phosphorylation of ERK and pS6 throughout treatment period, whereas selumetinib alone or reverse combination transiently decreased within 24 hr. The cells treated as FS schedule exhibited higher apoptosis and lower capacity of anchorage-independent growth than the cells under single or reverse combination. Microarray analysis revealed the distinct groups of genes underlying different efficacy by schedule-dependent treatment. Tumor growth in mice administered 5-FU at 10 mg/kg/day for colon205 and at 30 mg/kg/day for HCT8 for 7 days followed by selumetinib at 10 mg/kg/day for colon205 and 25 mg/kg/day for HCT8 for another 7 days was significantly retarded than that in mice treated with single agents. But the combination in reverse sequence induced comparable tumor growth to single agents. Decreased expression of Ki67 was observed in tumors from mice treated as FS scheduling. Our results suggest that the schedule-dependent combination of 5-FU and selumetinib may be benefit in patients having CRC with hyper-activation of Ras/RAF pathway.

#1205 Rubrofusarin and toralactone sensitize resistant MCF-7adr cell line to paclitaxel via inhibiting P-glycoprotein efflux activity, Salwa D. Aqealathani,1 Hanan A. Assiri,1 Fahad A. Al-Abbsi,2 Ali M. El-Halawany,2 Ahmed M. Al-Abd3. 1King Abdulaziz University, Jeddah, Saudi Arabia; 2Cairo University, Cairo, Egypt; 3National Research Centre of Egypt, Giza, Egypt.

The use of anti-cancer adjunct therapy is rationalized by potentiating the efficacy and/or protecting from major side effects of chemotherapeutics. Paclitaxel is an effective spindle toxin anticancer agent used for the treatment of breast cancer. Rubrofusarin and toralactone are naturally occurring structurally related naphthopyrones with potent antioxidant and hence chemoprotective activity. The purpose of this study is to investigate the influence of rubrofusarin and toralactone on the cytotoxic profile of paclitaxel (PTX) against chemo-

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resistant breast cancer cell line (MCF-7/ddr) compared to parent MCF-7 cells. MCF-7/ddr was prepared by continuous culturing of MCF-7 cells in media containing subcytotoxic concentration of doxorubicin (IC_{50}, 15 μM) for two months duration. Tumor脱落 and ribosomal stress showed moderate cytotoxic effects against MCF-7 cells with IC_{50}'s of 32.4 ± 12 μM and 13.9 ± 6.1 μM, respectively. Interestingly, the ribosomal stress was more potent against MCF-7/ddr cells with IC_{50}'s of 7.3 ± 0.7 μM and 7.8 ± 0.6 μM, respectively. PTX treatment was opposed by significant resistance from MCF-7/ddr cells compared to parent MCF-7 cells having IC_{50}'s of 4.9 ± 0.9 μM and 57.7 ± 4.5 μM, respectively. Combination of tumor脱落 and ribosomal stress with PTX did not significantly improve its cytotoxicity against MCF-7; IC_{50}'s were 24.2 ± 2.2 nM and 69.7 ± 6.2 nM, respectively compared to 4.9 ± 0.9 μM for PTX alone. Potentially, combination with either tumor脱落 or ribosomal stress abolished the resistance of MCF-7/ddr to PTX resulting in IC_{50} of 3.4 ± 0.2 nM and 4.9 ± 0.5 μM, respectively compared to 4.9 ± 0.9 μM for PTX alone. Using flowcytometric analysis, ribosomal and tumor脱落 increased median intracellular fluorescence signal count of rhodamine dye (non-cytotoxic P-gp substrate) within MCF-7/ddr from 1794.7 ± 21.5 to 2003.3 ± 12.5 to 2113 ± 10.5 cellular events, respectively. Using purified membrane bound humanized recombinant P-gp molecules; it was found that both ribosomal tumor脱落 and tumor脱落 might be inhibiting P-gp efflux activity via dual interaction with both P-gp ATPase enzyme subunit and P-gp active binding site. In both MCF-7 and MCF-7/ddr cells, PTX exerted significant cell cycle block at G_{2}/M-phase with significant increase in the p-CDK1 and MDR1 expression of the p-CDK1 and MDR1, respectively. In addition, tumor脱落 ribosomal stress exerted any significant influence for PTX induced cell cycle interruption in both normal (MCF-7) and resistant (MCF-7/ddr) cell lines. In conclusion, ribosomal tumor脱落 can enhance the cytotoxic profile of P-gp substrates, such as PTX, via inhibiting P-gp efflux activity and enhancing the intracellular entrapment of P-gp substrates.


Introduction Prostate cancer (PrCa) is the most common male malignancy among men in the United States. Recent studies suggest that low expression of miR-205 is seen in PrCa cells lines and tumors in comparison to normal prostatic epithelial cells. A number of studies have shown that restoration of miR-205 in PrCa cells resulted in suppression of cell growth, epithelial-to-mesenchymal transition, and chemosensitization. However, due to the poor pharmacological kinetics and low in vivo stability of miR-205, serious limitations are being experienced at the clinical level. Therefore, we have chosen a novel nanoparticle-based miR-205 delivery system to deliver miR-205, for improved therapeutic benefits in PrCa. Methods A novel miR-205 nanoparticle formulation (named miR-MPG) was generated which is composed of an iron oxide core layer with polyethylene imine (PEI), and NHS-PEG-NHS (PEG) polymer. The miR-205 withholding and release characteristic of miR-PEG were examined through fluorescence quenching and agarose gel electrophoresis. Hemocompatibility of this formulation was examined using a hemolysis assay. Cellular uptake of miR-MPG formulation was evaluated using flow cytometry and confocal studies. Further, therapeutic and chemosensitization activity of miR-205 were assessed using cell culture based assays. Molecular effects associated with the PrCa cells growth inhibition were evaluated through protein profiling and qRT-PCR analyses. Results and Discussion miR-MPG formulation exhibited optimal particle size and zeta potential, which are suitable for cancer therapeutics. Agarose gel electrophoresis binding studies suggested 5 μg of nanoparticle formulation is optimum to hold 1 μg of miR-205 mimic. Release of miR-205 from miR-MPG was determined with respect to concentration of anionic molecules and in a time-dependent manner. We observed no hemolysis during miR-MPG interaction with the red blood cells. Hemocompatibility of miR-MPG, in addition, miR-MPG particles exhibited superior internalization and endosomal escape in PrCa cells. This formulation displayed enhanced sensitization of PrCa cells to docetaxel. Additionally, it induced the expression of apoptotic proteins (Bax, Bim, cleaved PARP, and caspase 3), and downregulated the anti-apoptotic proteins (Bcl-2 and survivin). Moreover, the expression of the chemoresistance-associated proteins (PSMA and MRP) significantly decreased in miR-MPG treated cells compared to miR-MPG in the presence of docetaxel. Further dataset of qRT-PCR studies showed induced expression of the miR-205 and affected the expression of its downstream genes. These results suggest that miR-MPG formulation may serve as an ideal delivery vehicle to deliver miR-205. Conclusion Results from this study suggests that successful delivery of miR-205 through miR-MPG nanoparticles can induce sensitization potential for docetaxel treatment. This novel therapeutic modality might be effective for PrCa patients undergoing chemotherapy.

#1207 SNS-062 demonstrates efficacy in chronic lymphocytic leukemia in vitro and inhibits C481S mutated Bruton tyrosine kinase. Catherine A. Fabian, Sean D. Reiff, Daphne Guinn, Linda Neuman, Judith A. Fox, Wendy Wilson, John C. Byrd, Jennifer A. Woyach, Amy J. Johnson. The Ohio State University, Columbus, OH; Georgetown University, Washington, DC; Sunesis Pharmaceuticals, South San Francisco, CA.

Introduction: In order to address the issue of acquired resistance to ibrutinib, we sought to characterize the Bruton agammaglobulinemia tyrosine kinase (BTK) inhibitor SNS-062 in preclinical models of chronic lymphocytic leukemia (CLL). Methods: Primary CLL B cells were isolated from the whole blood of consented patients by ficoll density centrifugation and Rosette-Sep negative selection. Annexin V and propidium iodide flow cytometry was used to measure patient CLL cell viability and 7-AAD was used to measure viability in stromal co-culture. CD40 and CD86 expression was evaluated via flow cytometry subsequent to sustained 3.2μM CpG stimulation. BCR signaling in primary CLL cells was investigated by immunoblot following 1 hour treatment and following 1 hour or 24 hours of incubation with SNS-062 in XLA cell lines. ITK inhibition was investigated via immunoblot after stimulation with anti-CD3 and anti-CD28 and incubation with SNS-062 for 1 hour. SNS-062 was used at a concentration of 1μM in preclinical studies unless otherwise noted. Measurement of kinase activity in human recombinant WT BTK or C481S BTK was performed in FR. In our study, Results: In our study, we investigated the combination of B-Raf driven tumors with RAF and MEK1 inhibitors. In our study, we investigated the combination of B-Raf driven tumors with RAF and MEK1 inhibitors. In our study, we investigated the combination of B-Raf driven tumors with RAF and MEK1 inhibitors.
profiles of these cell lines and analyzed the requirement of a MAPK activating signaling. Our approach revealed that synergistic activity is not confined to melanoma and colon cancer but is observed in tumors from other entities as well. This observation may expand the usefulness of MEK/RAF inhibitor co-treatment to a larger panel of cancer types.

#1209 FGF inhibition re-sensitizes BRAF/MEK dual resistant cells to the BRAF/MEK inhibitor combination. Victoria E. Wang,1 Jeffrey Settleman,2 Frank McCormick,1 1UCSF, San Francisco, CA; 2Genentech, South San Francisco, CA.

The BRAF-MEK pathway is frequently mutated in human melanoma. Inhibitors of these kinases have proven to prolong survival in melanoma patients. However, clinical benefit is relatively short-lived due to acquired drug resistance. To explore mechanisms of resistance in melanoma cells treated with dual BRAF and MEK inhibitors, A375, a cell line harboring the BRAF V600E mutation, was treated with escalating doses of vemurafenib and cobimetinib to generate a cellular population resistant to combination therapy. These cells display a reversible resistance phenotype. Upon initial treatment of the drug combination, upregulation of pSTAT3 is observed but the cells lose dependency on the pSTAT3 pathway upon prolonged drug treatment. A pharmacological synthetic lethal screen was performed and the FGF inhibitor class re-sensitizes the cells to BRAF and MEK inhibition through inhibitions of pERK. The dual resistant cells also displayed hyperactivation of the PDK-AKT pathway. Interrogation of patient samples confirmed these findings. These findings provides a biological rationale for a polytherapy strategy using a FGF inhibitor in patients with resistance to dual BRAF and MEK inhibition.<–EndFragment–>

#1210 Hederagenin overcomes the cisplatin resistance of head and neck cancer by targeting the antioxidant defense mechanisms. Daia Shin,1 Eun Hye Kim,1 Hyejin Jang,1 Jae Young Lee,2 Minsu Kwon,3 Seungho Baek,3 Jong-Lyel Roh3 1Asan Medical Center, Univ. of Ulsan College of Medicine, Seoul, Republic of Korea; 2Gyeongsang National University School of Medicine and Gyeongsang National University Hospital, Jinju, Republic of Korea; 3College of Korean Medicine, Woosuk University, Wanju, Jeonbuk, Republic of Korea.

Background: Cisplatin is a first-line chemotherapeutic agent for head and neck cancer (HNC). Acquired resistance to cisplatin is the most common reason for the failure of cisplatin chemotherapy. Hederagenin is extracted from the leaves of Cyclocarya paliurus, also known as sweet tea, which is one of the most popular teas utilized in traditional Chinese medicine. There is accumulating evidence it exhibited significant cytotoxicity against several types of cancers, however, which has been rarely tested in HNC cells. Herein, we examined the potential utility of hederagenin for overcoming cisplatin resistance in HNC cells and further clarified its molecular mechanisms of action. Methods: Parental and cisplatin-resistant HNC cells, and Nfr2 or SLC7A11 gene overexpressed HNC cell lines and other human HNC cells were used. The cells were used to examine the effects of hederagenin treatment in HNC cell lines by measuring cell viability, cell cycle, cell cycle, cell death, reactive oxygen species (ROS) production, glutathione level, mitochondrial membrane potential (∆Ψm), and protein expression. The anti-tumor effect of hederagenin in mouse tumor xenograft models was also tested. Results: Hederagenin induced cell death in both cisplatin-sensitive and -resistant HNC cells. Hederagenin treatment resulted in effective cisplatin-resistant HNC cells in a form of apoptotic cell death with the ∆Ψm change. Hederagenin inhibited Nfr2 and SLC7A11 resulting in an increasing ROS accumulation in HNC cells, of which effects were reversed by the pretreatment of antioxidant trolox. In addition, hederagenin caused the apoptosis effectively induced by targeting the antioxidant defense mechanisms in HNC cells. Subsequently, hederagenin activated signaling pathways of cell death involving caspase-3, PUMA, and PARP in the cells. The growth inhibitory effects of hederagenin were also confirmed in the tumor xenograft model implanted with cisplatin-resistant HNC cells. Conclusions: Our data suggests that hederagenin can overcome the resistance of cisplatin in resistant HNC cells via targeting antioxidant systems.

#1211 Lurbinectedin reverses platinum dependent IRF1 overexpression and nuclear localization, partially responsible for resistance to platinum drugs in ovarian cancer. Gema Santamaria Nuñez, María Jose Guillén, Juan F. Martínez-Leal, Pablo Avilés, Carlos M. Galmarini. PharmaMar S.A., Colmenar Viejo, Spain. 1University of Queensland, Brisbane, Australia; 2Karyopharm Therapeutics, Newton, MA; 3Royal North Shore Hospital, NSW, Australia.

Lurbinectedin (PM1183) is a new synthetic compound from the tetracyclic-drosoinoline family, which has demonstrated a strong anti proliferative activity against a panel of human tumor models in preclinical assays and is currently being evaluated in phase III clinical trials in platinum-resistant ovarian cancer and small cell lung cancer. Lurbinectedin binds to DNA, inhibits transactivated transcription, induces the degradation of elongating RNA Pol II and fools nucleotide excision repair to produce dsDNA breaks that need to be repaired mainly by homologous recombination (HR). Nearly 70% of patients diagnosed with ovarian cancer are in advanced stage, and the vast majority of these patients will eventually relapse with a primary cytoreductive surgery. Following several cycles of standard adjuvant chemotherapy including a platinum drug and a taxane. After a period of treatment with platinum drugs, patients will finally develop resistance, usually mediated by mechanisms such as drug detoxification or efflux and enhanced DNA repair. IRF-1 transcription factor expression has been shown to be up-regulated by cisplatin (CDDP) in ovarian cancer cells and might be responsible for the response to the drug, likely by inhibiting cell proliferation due to IRF1. Here we took advantage of the A2780/A2780cis human ovarian cancer cell lines, the second being a cisplatin resistant derivative, to investigate the role of IRF1 in the response of human ovarian cancer cells to cisplatin and lurbinectedin. A2780cis cells are, indeed, more resistant to cisplatin that their parental cell line but they do not differ in their resistance to lurbinectedin. Basal IRF-1 protein levels were actually higher in A2780cis cells than in their parental cell line, contributing to their resistance to cisplatin. Furthermore, cisplatin treatment induced the overexpression and nuclear localization of IRF-1 both, in A2780 and A2780cis cell lines. Contrarily, lurbinectedin did not induce the overexpression of IRF-1 neither in A2780 nor in A2780cis, explaining why this latter cell line is not resistant to the compound. Furthermore, lurbinectedin co-treatment with cisplatin diminished the expression of IRF-1 in A2780cis, more importantly, in A2780cis cells, explaining the synergism the combination has on these tumor cell lines. Thus, lurbinectedin not only did not activate the same mechanisms of resistance as cisplatin in ovarian cancer cells, but even reversed the resistance of these resistant cells to platinum drugs. Santamaria Nuñez et al, 2016. Mol Cancer Ther 15(10):2399-2412. Romano et al. 2013. Int J Cancer. 2013 Nov; 133(9):2024-33. Pavan et al. 2013. Eur J Cancer 49(4):964-973

#1212 Co-targeting epidermal growth factor receptor (EGFR) and aplastic lymphoma kinase overcomes EGFR inhibitor resistance in head and neck squamous cell carcinoma patient-derived models. Xiaoming Ouyang, Ashley Barling, Aletha Lesch, Jeffrey Tyner, Sophia Jeng, Christina Zheng, Sara A. Courteignede, Shannon McWeeney, Molly Kulesz-Martin. Oregon Health & Science University, Portland, OR.

Background: HNSCC is the sixth most common cancer worldwide. EGFR is overexpressed in up to 90% of HNSCC and associated with poor outcome. An EGFR monoclonal antibody is the only approved molecular targeted therapy for HNSCC, however, resistance eventually occurs in all patients. Methods: Functional screens including a small-molecule kinase inhibitor panel and an RNAi screen panel were used to identify agents that synergized with EGFR inhibitors in reducing cell viability in HNSCC patient-derived tumor cells. Effective combination therapies were validated in scale-up experiments in 2D and 3D, and their true targets were evaluated using siRNAs to rule out target effects of the drugs. Cell viability, cell number and cell colony formation ability were tested using siRNA treatment. qRT-PCR and immunofluorescent staining was used to test ALK expression in tumor cells before and after anti-EGFR treatment. Results: The ALK inhibitor KX-201 on the drug screen panel showed synergistic effects with EGFR inhibitors in 6/8 HNSCC patients' tumor cells, despite ineffective-ness of single drug. Scale-up dose-response experiments confirmed patient cell sensitivity to 4 different ALK inhibitors in combination with the EGFR inhibitor Gefitinib. siRNA targeting ALK synergized with Gefitinib in reducing cell viability in the patient cells that responded to EGFR/ALK inhibitor combinations, indicating specificity to ALK. Scale-up RNAi experiments confirmed synergy between siRNAs targeting ALK and EGFR in reducing cell viability, cell number, and colony formation ability. Inhibition of EGFR by siRNA or Gefitinib each increased ALK expression at the mRNA and protein level, suggesting induction of ALK as a novel mechanism of EGFR inhibitor resistance in HNSCC. Conclusion: We identified EGFR/ALK inhibitor combinations as a potential strategy for treating EGFR inhibitor resistant HNSCC.

#1213 Nuclear export of E2F7 in squamous cell carcinoma in an actionable event that reverses resistance to anthracyclines. Alba Natalia Saenz Ponce,1 Yosef Landesman,2 Trinayan Kashyap,3 Alexander Guminiski,3 Orla Gannon,3 Nicholas Saunders1 1University of Queensland, Brisbane, Australia; 2Karyopharm Therapeutics, Newton, MA; 3Royal North Shore Hospital, NSW, Australia.

Objectives and Background: Squamous cell carcinoma (SCC) of the skin or head and neck region is a major cause of cancer death in the western world. Patient with advanced SCC have few therapeutic options and SCC remains resistant to current treatments. Overcoming drug resistance in SCC would significantly change patient outcomes and treatment strategies. We previously showed that SCCs express the
activating transcription factor E2F1 and the inhibitory transcription factor E2F7, which are mutually antagonistic. We showed that E2F1/7 controlled the sensitivity to anthracyclines in SCC cells via E2F-dependent regulation of sphingosine kinase 1 (Sphk1) and its product sphingosine-1-phosphate (S1P). In addition, we showed that S1P treatment of cells induces profound anthracycline resistance. However, it was completely reversed in SCC cells using either two drugs which are used in breast cancer treatment with a seemingly pro-E2F1 environment within the SCC cells (i.e. pro-proliferation, differentiation-suppressive and pro-survival). In this study we have examined the role of nuclear export pathways in determining nuclear E2F2 activation in SCC.

Methods: We have interrogated human tissue microarrays of normal stratified epithelia and SCC of the skin or head and neck tumours from patient samples. To validate our in vivo findings we used a suite of SCC cell lines and examined the nuclear and cytoplasmic localization of E2F1 and E2F7 by immunofluorescence and western blotting. We deployed inhibitors of the Exportin 1 (XPO1) pathway and have used siRNA against XPO1 to confirm a functional role of XPO1 in the nuclear export of E2F7. We have used inhibitors of XPO1 in vitro and in vivo to examine their ability to enhance the cytotoxic responses to anthracyclines.

Results & Conclusions: We report that E2F7 is selectively localized to the cytoplasm of SCC cells and tumors but is exclusively nuclear in normal cells and tissues. In contrast, E2F1 is almost exclusively localized to the nucleus in normal and SCC cells and tissues. The localization of E2F7 to the cytoplasm could be reversed using inhibitors of the nuclear export protein XPO1 and siRNA against XPO1. XPO1-dependent nuclear export of E2F7 shows that this pathway is selectively activated in SCC. The inactivation of XPO1 allowed possible use of this therapeutic strategy. Inhibition of XPO1 by the small molecule inhibitor Selinexor (selinexor) reverses the decrease in the nuclear export of E2F7 to increase the antifluoropyrimidine F10 is effective towards cell and solid and hematological malignancies. We show that treatment of SCC cells with selinexor reverses anthracycline resistance. Significantly, we show that a combination of selinexor + doxorubicin in vivo induces profound anticancer activity.

Significance: Our studies show that (i) E2F7 is selectively mislocalized in SCC, (ii) E2F7 nuclear export is XPO1-dependent, (iii) dysregulation of XPO1 causes derepression of S1P-mediated anthracycline resistance and (iv) anthracycline resistance is reversed with inhibitors of XPO1 in vivo.

#1214 The polymeric fluoropyrimidine F10 is effective towards cell and organoid models of 5-FU-resistant colorectal cancer. William H. Gmeiner,1 Anthony Dominijanni,1 Olcay Boyacioglu,2 Steven Forsyth,1 Aleks Skardal,1 Bo-Hyoung Jang,1 Yong Cheol Shin,1 Seong-Gyu Ko1. 1WakeForestUniv.SchoolofMedicine,Winston-Salem,NC; 2Ad-

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Reversal of Drug Resistance

#1215 Apigenin overcomes drug resistance by blocking signal transducer and activator of transcription 3 (STAT3) signaling in breast cancer. Hye-Sook Seo,1 Jin Mo Ku,1 Se Hyang Hong,1 Hyeong Sim Choi,1 Jong-Kyu Woo,2 Bo-Hyoung Jang,1 Yong Cheol Shin,1 Seong-Gyu Ko1,2. 1Kyung Hee University, Seoul, Republic of Korea; 2Song-dang Institute for Cancer Research, Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul, Republic of Korea; 3Song-dang Institute for Cancer Research, Seoul, Republic of Korea. 10%.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Targeting Growth Factor and Intracellular Signaling

#1216 Overcoming cisplatin resistance through the combination treatment with CK2 inhibitor, CX-4945, in gastric cancer. Hyun Myong Kim,1 Inhye Jeong,1 Kyu Hyun Park,2 Tae Soo Kim,2 Woo Sun Kwon,2 Hei-Cheul Jeung,1 Minkyu Jung,2 Sun Young Rha,2. 1Song-dang Institute for Cancer Research, Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul, Republic of Korea; 2Song-dang Institute for Cancer Research, Seoul, Republic of Korea.

Platinum-based non-neoplastic drugs are chemotherapeutic agents usually used to treat gastric cancer (GC) include cisplatin. However, the majority of cancer patients will eventually relapse with cisplatin-resistant disease. Especially, increased DNA repair is drug targetable mechanism and useful in the treatment strategy of cisplatin-resistant cancer. Casein kinase 2 (CK2) has critical role of multiple cellular processes with DNA repair. For this reason, research for CK2 expression correlated with DNA repair mechanism is important in gastric cancer. Combination of cisplatin and CK2 inhibitor (CX-4945, also known as Silmisartel, Senhwa Biosciences, USA) may improve cisplatin-induced DNA damage for GC treatment. In this study, we screened sensitivity of cisplatin and CX-4945 in 49 GC cell lines by MTT assay. For molecular profiling, we analyzed variants and gene expression using whole exome sequencing and RNA sequencing, RNA and protein expression of CK2 subunits (α/ε) using real-time RT-PCR and Western blot. Also, activity of CK2α was measured by ELISA. Combination treatment performed different schedules including concurrent and sequential. Synergistic effect was analyzed by Bliss Independence model. As CK2 profiling, CK2α mRNA expression was a tendency to correlated with CX-4945 sensitivities (p = 0.0054). Moreover, CK2α protein expression was a correlated with CX-4945 sensitivities (p = 0.0252). Other molecular profiling did not reveal any clear correlations. Twenty one (Group 1: cisplatin extremely resistant and CK2 high, Group2: cisplatin intermediate resistant regardless of CK2 expression) cell lines were performed combination treatment. Both YCC-21 and YCC-28 cell lines show synergistic effect (+20% and +22%, respectively) in concurrent schedule. Only MKN-74 cell line show synergistic effect (+11%) in Post-addition (CX-4945 and CX-4945). 19%, respectively) in Post-addi-

#1217 Synergistic effect of CK2 inhibitor with Rapamycin in gastric cancer. Ji-Hyun Yang,1 Ji-Yoon Kang,1 Young Hyun Yoon,1 Hyunju Shin,1 Hyung-Gyu Lee,1 Seung-Do Yoo,1. 1Song-dang Institute for Cancer Research, Brain Korea 21 PLUS Project for Medical Science, Seoul, Republic of Korea; 2Seoul National University, Seoul, Republic of Korea.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Targeting Growth Factor and Intracellular Signaling

#1218 Targeting growth factors and HER2 signaling in gastric cancer. Sang Yoon Park,1 Ilseung Kwon,1 Minkyu Jung,1 Sun Young Rha,2. 1Song-dang Institute for Cancer Research, Brain Korea 21 PLUS Project for Medical Science, Seoul, Republic of Korea; 2Seoul National University, Seoul, Republic of Korea.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Targeting Growth Factor and Intracellular Signaling

#1219 Targeted therapy for gastric cancer based on heterogeneity in HER2 signaling. Hyun Ju Kim,1 Jeong Hwa Lee,1 Hye-Jin Kim,1 Byung Hoon Kim,1 Eun Hee Lee,1 Woong Hyeok Park,1 Jung-Chan Kim,1 Ji-Hyeon Lee,1 Young Hoon Park,1 Jung Hyun Shin,1 Hyun Ju Kim,1. 1Yonsei University College of Medicine, Seoul, Republic of Korea; 2Yonsei University College of Medicine, Seoul, Republic of Korea; 3Yonsei University College of Medicine, Seoul, Republic of Korea; 4Song-dang Institute for Cancer Research, Brain Korea 21 PLUS Project for Medical Science, Seoul, Republic of Korea.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Targeting Growth Factor and Intracellular Signaling

#1220 Targeting HER2 in gastric cancer: a novel approach for cellular heterogeneity. Hyun Ju Kim,1 Ji-Hwa Lee,1 Hye-Jin Kim,1 Byung Hoon Kim,1 Eun Hee Lee,1 Woong Hyeok Park,1 Jung-Chan Kim,1 Ji-Hyeon Lee,1 Young Hoon Park,1 Jung Hyun Shin,1 Hyun Ju Kim,1. 1Yonsei University College of Medicine, Seoul, Republic of Korea; 2Yonsei University College of Medicine, Seoul, Republic of Korea; 3Yonsei University College of Medicine, Seoul, Republic of Korea; 4Song-dang Institute for Cancer Research, Brain Korea 21 PLUS Project for Medical Science, Seoul, Republic of Korea.
to cisplatin-induced DNA damage. Personalized the treatment schedule for inhi-
bib CK2-induced DNA repair is a new strategy to restore cisplatin resistant in GC.

#1217 Blockade of Aurora kinase A synergizes with platinum and radia-
tion in non-small cell lung cancer cells. Huijie Liu,1 Dongwei Zhang,2 Alain Borcacz,3 Vincent Chau,4 Roman Perez-Soler,5 Balazs Halmos,5 Haiying Cheng,6 Albert Einstein College of Medicine, Bronx, NY; 7Columbia University Medical Center, New York, NY; 8Weill Cornell Medical College, New York, NY; 4Montefiore Medical Center, Bronx, NY; 8Albert Einstein College of Medicine /Montefiore Medical Center, Bronx, NY.

Background: Most patients with advanced lung cancer will receive platinum-
based therapy during their treatment course despite limited clinical benefits. Thus, strategies to improve platinum-based therapy continue to be needed. Through a functional genetic screen, we have identified Aurora kinase A as a potentially actionable candidate that can modulate the activity of platinum com-

#1218 Synthetic lethal targeting of p53 mutant cells with prenylation inhi-
bibitors. Shay R. Ferdosi,1 Benjamin Katchman,1 Jia Loo,2 Harneet Grewal,2 Seron Eaton,1 Shanshan Yang,1 Jin Park,3 Joshua Labara,2 Karen S. Anderson,1 Arizona State University, Tempe, AZ; 2Eccrine System Inc, Cincinnati, OH; 1Albert Einstein College of Medicine, Bronx, NY.

Introduction: TP53 is mutated in 30-40% of breast cancers and is associ-
ated with poor prognosis. Mutation of TP53 causes increased cellular pro-
liferation, migration and invasion, and downstream activation of multiple pathways. HMG CoA reductase inhibitors, such as simvastatin, inhibit tu-
morogenic properties induced by TP53 mutation. This mechanism of this re-
sponse is an important question in targeted cancer therapy. It is well known that HMG CoA reductase inhibitors block cholesterol production and pre-
nylation. Therefore, we hypothesized that prenylation inhibitors would tar-
get p53 mutant cells primarily by inhibiting activation of the ras family induced by TP53 mutation. Method: We generated 3 MCF10A stably trans-
duced cell lines over-expressing each of the common TP53 point mutations, R273H, G245S, R248Q, Y264C, or wild type. Ras, Rap, Ras-RhoA, Ras-RhoA-GTP, total RhoA and RhoA-GTP was measured by immunoprecipitation and immuno-

#1220 Coordinated chemical-genetics approach identifies PTPA3-mediated regulation of colon cancer cell migration and extracellular matrix inter-
actions. Kelley E. McQueeney,1 Joseph M. Salamoun,2 Isabella K. Blanco,3 Paula Pecik,1 Jennifer Ahn,1 Elizabeth R. Sharlow,1 Peter Wipf,2 John S. Lazo1. 1University of Virginia, Charlottesville, VA; 2University of Pittsburgh, Pittsburgh, PA.

Aberant regulation of protein phosphorylation is an exceedingly common driver of human cancers. It is notable that we understand much less about the role of protein tyrosine phosphatases in human malignancies compared to ty-
rrosine kinases. The membrane-associated, intracellular, protein tyrosine PTPA3 is highly overexpressed in multiple tumor types including colorectal cancer and has been associated with tumor metastases. We have, therefore, inves-
tigated the role of PTPA3 in colon cancer migration and invasion. The Ptpa3 gene was expunged from colon tumor cells derived from Ptpa3flox/flox mice and the resulting cells exhibited impaired migration, invasion, and colony formation compared to the wildtype isogenic cells. We characterized a potent, selective, and noncompetitive small molecule inhibitor of PTPA3, PTPA3, JMS-631-053, which also disrupted colon cancer cell migration, invasion, and colony
formation. PTP4A3 deletion increased the expression of extracellular matrix and adhesion genes, including the tumor suppressor Emilin 1. Expression of these extracellular matrix genes is mutually exclusive with PTP4A3 expression in tumors derived from patients with colorectal cancer. These chemical and biological reagents reveal a previously unknown communication between the intracellular PTP4A3 phosphatase and the extracellular matrix and support continued efforts to pharmacologically target PTP4A3 for cancer therapy.

#1221 Triple-negative breast cancer (TNBC) phosphoproteomics. Sara Fernandez Gaitero,1 Ivana Zagora,1 Jose Francisco Lopez-Acosta,1 Gonzalez-Gomez-Lopez,1 David Gonzalez-Pisano,1 Javier Munoz Peralta,1 Luis Manso,2 Soledad Alonso,1 Renke Penning,1 Maarten Altelaar,1 Albert J.R Heck,1 Miguel Quintana-Fandino1.1 Spanish National Cancer Research Center (CNIO), Madrid, Spain; 212 de Octubre University Hospital, Madrid, Spain; 3Hospital Universitario de Guadalajara, Guadalajara, Spain; 4Netherlands Proteomics Centre, Utrecht, Netherlands.

Background: We hypothesized that the biphasic relapse pattern of TNBC could be explained by a limited number of activation patterns of signaling nodes. In addition, we sought to determine whether the hyperactive signaling nodes, distinguishing the cases with favorable vs adverse outcome, could be potential targets. Methods: Training set of 34 frozen tumor samples divided in two sets, (A) 13 patients, relapsed in <4 years; (B) 21 patients relapse-free >12 years, (mimicking the percentage and relapse patterns of unselected TNBC, but paired for T, N, G and K67). TNBC cell lines: 7 indolent (no metastases in 60 weeks) and 3 aggressive, develop metastases and kill recipient mice in <4 months. Shotgun phosphoproteomics and TIO2-IMAC phosphopeptide enrichment coupled with mass spectrometry runs in a Orbitrap Elite Mass Spectrometer was performed. Spectra were processed with MaxQuant software. Differentially expressed phosphopeptides were obtained by applying linear models R limma package. Differential kinase activation driving the profiles segregating cured vs. relapsing cases was done using linear sequence motif analysis. The hyperactivated kinases were validated in an independent set of 113 consecutive TNBC cases with 12 years of follow-up spotted in TMAs by using an in-house algorithm for immunohistochemistry coupled with computer-aided quantitation using an Ariol scanning, we took the kinases in the upper quartile (high activity). Survival analysis was performed with KM curves and log rank test; and Cox proportionate hazards model was used for multivariate models. Results: 11405 phosphopeptides were identified and quantified in the training set. Supervised clustering of relapsed vs. cured cases showed that 161 and 541 peptides were significantly up-regulated in the A and B groups, respectively (FDR <0.15). After kinase-to-ki- nase co-linearity was ruled out, gathering the high activity (upper quartile) of six kinases (a combined variable, herein Var1) showed statistically significant association with relapse, being these: PRKCE, PRRK, KIT, CDK6, p70S6K and pPNPK. Cox proportional hazards model of any of the six probes high (var1) vs rest; 9.9 vs. not reached years (P=0.0001). Patients that had any of the 6 kinases in >40% of chance to relapse (only 2 out of 42 relapsed patients have 0/6 active kinases) vs patients with var1 negative, 7% of chance (29 patients out of 72 have 0/6 active kinases) we also observed constant patterns of activations in the different sets expressions of kinases. We considered the kinases at Var1 as a potentially targets and we developed a pharmacological in vitro assay, testing pairs inhibitors on 10 TNBC cell lines. For the combination of kinases we supposed that: High throughput p-proteomics allows a parsimonious segregation of early TNBC cases, easily detecting the cases with long-term cure vs the remaining while identifying potential therapeutic solutions for the patients falling in the adverse prognostic subgroups.

#1222 Developing kinase inhibitors with polypharmacological profiles for the treatment of resistant cancers. Suman Rao,1 Tan Li,1 Robert Eversley,1 Guangyan Du,1 Peter Sorger,1 Nathanael Grey1.1 Dana Farber Cancer Institute, Harvard Medical School, Boston, MA; 2Harvard Medical School, Boston, MA.

Small molecule kinase inhibitors targeting a specific oncprotein driving cancer cells have shown great clinical success in the past (e.g. imatinib against leukemia). However, resistance to targeted therapies quickly arises due to plasticity associated with cancer cell signaling and tumor heterogeneity. One way to overcome this problem is to rationally design drugs with polypharmacology that can target multiple signaling pathways engaging in cancer cell proliferation and survival. To this end, we have developed a strategy based on combining phenotypic screens of small molecule inhibitors and affinity-based mass spectrometry to identify drugs with multi-targeted properties and pilot this approach using the highly resistant KRAS mutant non-small cell lung cancer cell lines. We began this study by screening kinase inhibitors targeting MEK, ERK, PI3K, Akt and mTOR and found that the lung cancer cells were largely resistant to single drugs but showed enhanced sensitivity towards triple inhibition of MEK, ERK and PI3K. These cell lines were then screened with a library of covalent and non-covalent kinase inhibitors in an attempt to identify a small molecule inhibitor that could mimic the activity of the triple drug combination. This led to the discovery of SM1-71 that not only recapitulated the growth inhibitory activity of MEK+ERK+PI3K inhibition, but also blocked phosphorylation of ERK, Akt, mTOR and S6K in cells. Target profiling of the drug was carried out using chemical and cellular assays, which revealed SM1-71 inhibited 55 different kinases at a 1 μM concentration. Dose-dependent evaluation further determined the IC50 of inhibition across the 55 kinases and rank ordered them based on binding affinity. Top hits including MEK1, MEK2, ERK1, ERK2, AURKA, SRC and RPS6KA3 were confirmed using alternate kinase screens and cellular assays. Growth factor receptors such as IGFR1, MET and EGFR were also identified and further validated using both bioactivity-based assays and high-content cellular assays. The combination of polypharmacological and mass spectrometry-based target identification, we have demonstrated the proof of principle for developing kinase inhibitors with polypharmacology targeting resistant cancers.

#1223 Evaluation of the dual p38/ULK kinase inhibitor as potential therapeutic agent for tamoxifen-resistant breast cancer. XIAN WANG,1 Xizi Cao,2 Jumunari Veeraraghavan,1 Lanfang Qin,1 Jin-Ah Kim,1 Ying Tan,1 Susan G. Hilsenbeck,1 Rachel Schiff,1 Xiaosong Wang,1 1University of Pittsburgh, Pittsburgh, PA; 2Baylor College of Medicine, Houston, TX.

Background: Endocrine therapy has been considered an effective initial treatment for ER positive breast cancer and tamoxifen is the most commonly used endocrine agent. However, about half of the patients develop resistance or relapse eventually. No effective targeted therapy exists to overcome it. In our previous study, we have identified the role of nemo-like kinase (NLK) in breast cancer endocrine resistance—a serine-threonine kinase that functions in stress response and neurite outgrowth. In addition to NLK, activation of other stress kinases such as p38 MAPK has been reported to modulate ER signaling and promote endocrine resistance. In this study, we have identified a highly selective dual p38 and NLK kinase inhibitor (PNKI). This study aimed to evaluate the therapeutic effect of the PNKI inhibitor in tamoxifen-resistant breast cancers using in vitro and preclinical mouse models. Experimental design and methods: To determine the effect of PNKI on tamoxifen-resistant breast cancer cells, we treated a primary tamoxifen-resistant breast cancer cell line MDAMB415 and an acquired-resistant line MCF7/TamR, with 0.5 μM PNKI in the presence of different doses of Tamoxifen. To evaluate the therapeutic effect of PNKI in a T47D-derived xenograft tumor model with acquired tamoxifen resistance, we administered PNKI alone or in combination with Fulvestrant, or with the mTOR inhibitor Everolimus. Mice bearing T47D-TamR xenografts were randomized into six treatment groups (Vehicle, PNKI, Fulvestrant, Fulvestrant + PNKI, Everolimus, Everolimus + PNKI). Tumor growth was tracked closely. The tumors harvested 2 weeks following treatments were profiled with Reverse Phase Protein Array (RPPA) to assess the early signaling changes after treatments. Furthermore, the therapeutic effect of PNKI were also evaluated in a patient-derived xenograft (PDX) model of de novo endocrine resistant breast cancer. Mice bearing the PDX tumors were randomized to one of four treatment groups (Vehicle, PNKI, Everolimus, Everolimus + PNKI) and tumor growth curve was measured. Summary of the Results: Breast cancer cell lines with either de novo or acquired Tamoxifen resistance became more sensitive to tamoxifen when treated with 0.5 μM PNKI. The concomitant treatment of PNKI and Everolimus results in significant decrease of tumor growth in the T47D-TamR xenograft tumor compared to Fulvestrant, Fulvestrant + PNKI, or Everolimus treatments. RPPA data revealed that a majority of key survival signaling in breast cancer are repressed only when PNKI are combined with Everolimus. The de novo endocrine-resistant PDX tumors showed diverse response to PNKI mono-treatment, whereas the combination of PNKI and Everolimus resulted in significantly decreased tumor growth. Conclusion: The PNKI exhibited potential therapeutic value as adjuvant agent to the mTOR inhibitor everolimus for acquired or de novo tamoxifen-resistant breast cancer.

#1224 Combination of rapamycin and crizotinib induces partial remission of pleural mesothelioma in a patient-derived xenograft model. Dina Mönch,1 Sabine Bode-Erdmann,2 Jörg Kalla,2 Jorn Sträter,4 Carsten Schwänen,5 Roger-Fei Falkenstein-Ge,3 Martin Kimmich,3 Martin Kohlhäuf,5 Godehard Friedel,2 German Ott,1 Claudia Kalla,1 Department of Clinical Pathology, Robert-Bosch-Krankenhaus, Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, University of Tübingen, Stuttgart, Germany; 2Institute of Pathology, Schwäbisch-Baar-Klinikum, Villingen-Schwenningen, Germany; 3Ambulantes Onkologiezentrum, Klinikum Eslinge und Institute of Pathology, Eslinge, Germany; 4Center for Palmonology and Thoracic Surgery, Klinik Schillerhöhe, Stuttgart, Germany.
Introduction: Malignant pleural mesothelioma (MPM) is a neoplasm with inferior prognosis and notorious chemotherapeutic resistance. New treatment approaches may be based on specific inhibitors against kinases that are overexpressed in MPM, such as mTOR and MET. Here we examined the tyrosine kinase ALK as a potential therapeutic target and the combinatorial efficacy of the ALK/MEK inhibitor crizotinib and the MEK inhibitor vemurafenib. We investigated the ALK status and the expression of mTOR and MET in 145 primary MPM and 8 murine patient-derived xenograft models. ALK overexpression, rearrangement and mutation were studied by qRT-PCR, FISH, immunohistochemistry and sequence analysis. Expression of mTOR and MET was analyzed by qRT-PCR and immunohistochemistry. The combined anti-tumor effect of vemurafenib and crizotinib was evaluated in a patient derived xenograft model. Effects of single drug vs. combination treatment on proliferation, apoptosis and autophagy were assessed using Ki-67 immunohistochemistry, TUNEL assay and LC3B immunofluorescence, respectively. Results: Overexpression of ALK transcripts was detected in 25 (19.5%) of 128 interpretable primary MPM and two xenograft tumors and was neither associated with ALK rearrangement nor with mutation of the kinase domain. ALK protein was expressed in 8.3% MPM and the two xenograft tumors expressing ALK transcript. mTOR protein expression was detected in 28.7% MPM, co-expressed with ALK and/or MET in 17.8% MPM. Applied to a patient derived MPM xenograft model that co-expressed ALK, MET and mTOR, crizotinib alone did not exert anti-tumor growth activity, but enhanced the anti-tumor effect of vemurafenib: Rapamycin with vemurafenib inhibited tumor growth in 86% of xenograft tumors (pathological complete regression in 2 cases). Combination treatment was active in all 5 tumors (partial remission in 4, stable tumor in 1 case). Simultaneous treatment with rapamycin and crizotinib, however, significantly suppressed tumor proliferation compared to rapamycin-single treatment. Autophagy was induced by both single drug treatments and distinctly enhanced by combination treatment, while apoptosis was not promoted. Conclusion: Dual combinatory treatment of rapamycin and crizotinib is more effective than rapamycin as single agent in suppressing MPM tumor growth and therefore merits further investigation.

### 1225 A unified model of RAF inhibitor action determines inhibitor activity in RAF-dependent tumors. Zoi Karoulia,1 Yang Wu,2 Tamer A. Ahmed,1 Qisheng Xin,1 Julien Bollard,1 Clemens Krepler,2 Xuwei Wu,1 Chao Zhang,1 Gideon Bollag,1 Meenhard Herlyn,3 James A. Fagin,5 Amaia Lujambio,1 Mount Sinai, New York, NY;2Albert Einstein College of Medicine, Bronx, NY;3The Wistar Institute, Philadelphia, PA;4Plexxikon, California, CA;5Memorial Sloan Kettering Cancer Center, New York, NY. The FDA approved RAF inhibitors vemurafenib and dabrafenib have elicited profound responses in melanoma patients with tumors harboring BRAFV600E mutation, but resistance limits their effectiveness. Furthermore, RAF inhibitors exhibit only modest efficacy in colorectal and thyroid BRAFV600E mutants and they are not effective in tumors harboring non-V600 BRAF mutations. While RAF/MEK inhibitor combinations demonstrate increased clinical efficacy, drug resistance develops as well, through similar mechanisms to those identified in RAF inhibitor monotherapy. This suggests that insufficient RAF inhibition limits treatment efficacy and indicates the need for more effective therapeutic approaches to potentially and durably target RAF/ERK signaling. Although the unique biochemical properties of RAF inhibitors have been a topic of intensive investigation, a unified model of RAF inhibitor action has been lacking. Our biochemical and structural analysis in a panel of RAF inhibitors with different chemical properties and support therapeutic strategies in which relatively lower, non-toxic concentrations of RAF inhibitors will be administered in combination with currently approved RAF and MEK inhibitor regimens for the treatment of BRAFV600E melanoma, colorectal and thyroid cancers, as well as for tumors with BRAF mutations other than V600.

### 1226 Targeting BTK family in ER+ breast cancer with Ibrutinib for personalized precision medicine: a double-edge sword. Yen Gäkmén-Polar, Xiaoping Gu, Edyta Vieth, Sunil Badve. Indiana Univ. School of Medicine, Indianapolis, IN. Background: Metastasis is the principal cause of morbidity and mortality from breast cancer. Although current therapies are effective in early-stage of breast cancer, resistance to therapy is common leading to the development of metastasis. The commonest site of metastases is the regional lymph nodes. The presence and the number of involved lymph nodes remains the single best predictor of metastases and an important guide for the use of systemic adjuvant therapy. Identification of the underlying molecular targets of lymph node metastasis and a better understanding how to therapeutically target these will be a significant step in the goal of prevention of metastases. Experimental Design: To determine the key players associated with the development of metastasis in breast cancer, we performed RNA sequencing (Illumina Paired-end RNA-seq) analysis in a cohort of matched pairs of primary and (nodal) metastatic tumors (n=18). Gene expression profiles were analyzed using DESeq2. For targeted therapeutics, we performed orthotopic tumor implantation into mammary fat pads of athymic nude mice of breast cancer models. Results: Our results identified significant differential expression of the Bruton’s tyrosine kinase (BTK) family members in nodal metastasis. Among the family members, BTK, BLK, BTK/CAS and AK3 were expressed at very high levels as compared to matched primary tumors. Among the 51 breast cancer cell lines analyzed, the BTK genes were significantly upregulated in luminal, but not basal cell lines. Categorized by the molecular subtypes of breast cancer, hormone receptor- positive (HR+) and /or HER2 overexpressing breast cancer cell lines present higher BTK expression. Among the 51 breast cancer cell lines analyzed, the BTK genes were significantly regulated in both ER+ and HER2- (T47D and ZR75.1) and ER+HER2+ (BT-474 and ZR75.30) breast cancer cell lines. Ibrutinib, a selective and irreversible inhibitor of BTK targeting the cysteine-481 residue in the active site, exhibited potent activity in preclinical models of BTK-expressing targets. Ibrutinib decreased primary tumor growth significantly in ER+HER2+ (BT-474) mouse xenograft model in a dose dependent manner (10 mg/kg vs 25 mg/kg, P<0.05), whereas it promoted the tumor growth in a significant manner in ER+HER2- xenograft models (T47D and ZR75.1; P<0.05). The differences might be due to the differences in model systems due to the HER2 presence. Conclusion: Our data suggests that differential expression of target genes can be used to select agents as treating cancer. However, caution is necessary, as, in some situations, this might lead to enhancement of the growth in vivo in subsets of breast cancer.

### 1227 Angiotensin-(1-7) reduces triple negative breast cancer brain metastatic growth in association with decreased c-Met signaling. E. Ann Tallant, Guorui Deng, Wenhong Chen, Linda J. Metheny-Barlow, Patricia E. Gallagher. Wake Forest School of Medicine, Winston-Salem, NC. Patients with triple negative breast cancer often develop metastatic brain disease, a situation not efficiently addressed by current therapeutic options. We hypothesized that heptapeptide hormone angiotensin (Ang)-(1-7) is a therapeutic modulator that efficiently crosses the blood brain barrier. In the current study, we investigated the effect of angiotensin-(1-7) [Ang-(1-7)], a heptapeptide hormone with anti-proliferative and anti-metastatic activities, on the growth of murine brain tumors using brain trophic BR.5-4T1 cells in a model of metastatic triple negative breast tumor growth. BR.5-4T1 cells (1.25 x 104) were injected into the internal carotid artery of female BALB/c mice; after two days, mice were implanted with osmotic mini-pumps to deliver 24 µg/kg/h Ang-(1-7). P2.2% intumors 6.7% to 6.4% in mice treated with Ang-(1-7) treatment significantly reduced metastatic tumor burden in the brain; an average of 20 metastatic lesions per mouse was observed in the brains of an untreated control cohort while an average of 3 metastatic tumors per mouse was observed in the Ang-(1-7)-treated cohort. Tumor size was also reduced 17-fold by treatment with Ang-(1-7). Ki67, a marker of proliferation, was decreased from 29.1±6.7% to 6.4±2.2% in tumors from mice treated with Ang-(1-7), indicating that the heptapeptide hormone decreased metastatic tumor burden in the brain. Aberrant activation of the c-Met receptor tyrosine kinase signaling pathway facilitates tumor cell invasion and metastasis. Treatment with Ang-(1-7) significantly reduced the percentage of metastatic tumors per mouse was observed in the Ang-(1-7)-treated cohort. Tumor size was also reduced 17-fold by treatment with Ang-(1-7). Ki67, a marker of proliferation, was decreased from 29.1±6.7% to 6.4±2.2% in tumors from mice treated with Ang-(1-7), indicating that the heptapeptide hormone decreased metastatic tumor burden in the brain. Aberrant activation of the c-Met receptor tyrosine kinase signaling pathway facilitates tumor cell invasion and metastasis. Treatment with Ang-(1-7) significantly reduced the percentage of metastatic tumors per mouse was observed in the Ang-(1-7)-treated cohort. Tumor size was also reduced 17-fold by treatment with Ang-(1-7).
4.1±1.4% in sections of tumors from control mice compared to 8.8±0.9% in sections of tumors from mice treated with Ang-(1-7). These results suggest that Ang-(1-7) increases PTP1b to antagonize c-Met signaling and decrease triple negative breast cancer brain metastasis. In support of a role for c-Met signaling in the regulation of metastatic brain disease in breast cancer patients, analysis of publicly available breast cancer cohort databases demonstrated a significant correlation between low c-Met/high PTP1b expression and improved survival outcome, including an increase in brain metastasis-free survival and distant-metastasis free survival. This is the first report to demonstrate that Ang-(1-7) reduces metastatic triple negative breast cancer localized to the brain in association with increased protein phosphatase PTP1b and decreased phospho-c-Met, suggesting that the heptapeptide hormone may also serve as a novel targeted therapy to reduce metastatic brain tumor burden in triple negative breast cancer patients.

#1228 Nintedanib selectively inhibits the activation and tumor-promoting effects of fibroblasts from lung adenocarcinoma patients. Jordi Alcaraz,1 Marta Gabasa,2 Rafael Ikemori,3 Noemi Reguart.1 1. University of Barcelona, Barcelona, Spain; 2. University of Barcelona, Barcelona, Spain; 3. Hospital Clinic de Barcelona, Barcelona, Spain.

Nintedanib (BIBF 1120) is a multikinase inhibitor recently approved to treat lung adenocarcinoma (ADC) patients based on the therapeutic benefits reported in clinical trials on ADC but not on squamous cell carcinoma (SCC), which are the two most common lung cancer subtypes. However, the biological processes underlying the selective anticancer function of nintedanib in ADC remain obscure. To shed light on this gap of knowledge, we examined the role of tumor-associated fibroblasts (TAFs) in the differential effects of nintedanib in ADC and SCC. Because TAFs are largely quiescent and activated in vivo, we focused on the anti-fibrotic effects of nintedanib on TAFs stimulated with TGF-β1, which is a potent fibroblast activator frequently upregulated in lung cancer. Nintedanib dose-dependently inhibited the TGF-β1-induced expression of activation markers in both ADC-TAFs and control fibroblasts derived from uninvolved parenchyma, whereas such inhibition was very modest in SCC-TAFs, thereby suggesting that TAF activation is regulated by different mechanisms in ADC and SCC. Remarkably, nintedanib abrogated the stimulation of growth and invasion in a panel of carcinoma cell lines induced by secreted factors from activated TAFs in ADC but not SCC. These results reveal that nintedanib is a potent inhibitor of fibrosis and its associated tumor-promoting effects in ADC, and strongly suggest that the subnormal anti-fibrotic response of SCC-TAFs to nintedanib may underlie the lack of therapeutic benefits of this drug in SCC. Our findings also support that preclinical models based on carcinoma-TAF interactions may help unraveling the resistance mechanisms of SCC-TAFs to nintedanib and testing new combined therapies to further expand the therapeutic effects of this drug.


Despite recent advances in early detection and therapeutic intervention, colorectal cancer (CRC) remains one of the most deadly cancers in the United States. The Wnt/β-catenin and KRAS/BRAF-driven MAPK pathways were reported to cooperate in inducing CRC initiation and progression. Several studies have also identified Wnt signaling as a resistance mechanism in MAPK pathway-activated CRCs. Thus far, independent targeting of these single oncogenic events in CRC has yielded limited therapeutic success. RNA interference (RNAi) triggers are capable of silencing any expressed mRNA with high potency and specificity, including previously un-druggable targets such as β-catenin, an oncogene frequently dysregulated in CRC. DCR-BCT is a chemically-optimized DsiRNA targeting CTNNB1, the gene which encodes β-catenin, formulated in a second-generation EnCore lipid nanoparticle. We have previously reported extensive preclinical pharmacology for DCR-BCT in mouse tumor models of diverse origin. (Ganesh et al, Mol Cancer Ther 2016). Here we demonstrate that the dual inhibition of β-catenin and MEK demonstrates synergistic efficacy in not only primary CRC, but also in aggressive liver metastases of CRC in preclinical models. This combination of DCR-BCT and the MEK inhibitor trametinib was also efficacious in tumors which are known to be resistant to trametinib, as well as tumors which acquire resistance due to drug exposure. These data support clinical development of these combination approaches for this first-in-class RNAi therapeutic.

#1230 Talazoparib, a second generation PARP inhibitor, is a novel therapy for PTEN mutants in prostate cancer. Zachary Z. Reinstein, Rajita Vata-i, Jonathan Anker, Sahithi Pamarthy, Benedito Carneiro, Sarki Abdulkadir. Northwestern University, Chicago, IL.

Prostate cancer is the most common cancer among men, other than skin cancer. However, over 30% of patients diagnosed with prostate cancer have metastatic disease at the time of diagnosis. PTEN (phosphatase and tensin homolog on chromosome ten) is one of the most common lost or mutated tumor suppressor genes in human cancers, including ~50% of metastatic castration-resistant prostate cancer (mCRPC). By catalyzing PIP3 dephosphorylation, PTEN negatively regulates the PI3K-AKT pathway, which is frequently altered in mCRPC. Reintroduction of functional PTEN for mCRPC treatment has proven difficult. By employing self-catalyzing phospho-lysine chimeras and lipid-polymer nanoparticles, we successfully re-introduced PTEN mRNA to PTEN-null prostate cancer cells both in vitro and in vivo. These mRNA-loaded nanoparticles demonstrate high protein expression efficiency, low toxicity and good stability in serum and tumor accumulation. We confirmed that restoration of PTEN in PTEN-null prostate cancer cells inhibits the PI3K-AKT pathway, reduces cell viability and enhances apoptosis in vitro. Systemic delivery of PTEN mRNA-loaded nanoparticles in prostate xenograft tumors results in ~85% inhibition of tumor growth and leads to tumor cell death without toxic side effects in vivo. In summary, this work provides proof of concept of mRNA-based gene therapy for systemic restoration of functional PTEN for tumor suppression in vivo. It represents a novel approach to PI3K-AKT pathway inhibition, with the potential to specifically target cancers with loss of PTEN function.

#1231 Restoration of tumor suppression in vivo by systemic delivery of PTEN mRNA nanoparticles. Yingjie Xu,1 Mohammad Ariful Islam,2 Harshal Zope,3 Morteza Mahmoudi,2 Robert S. Langer,1 Jinjun Shi,2 Bruce R. Zetter,2 Omid C. Farokhzad1,2, Boston Children’s Hospital, Harvard Medical School, Boston, MA;3 Brigham and Women’s Hospital, Harvard Medical School, Boston, MA; 1. Massachusetts Institute of Technology, Cambridge, MA.

PTEN (phosphatase and tensin homolog on chromosome ten) is one of the most common lost or mutated tumor suppressor genes in human cancers, including ~30% of metastatic castration-resistant prostate cancer (mCRPC). By catalyzing PIP3 dephosphorylation, PTEN negatively regulates the PI3K-AKT-mTOR pathway, which is frequently altered in mCRPC. Reintroduction of functional PTEN for mCRPC treatment has proven difficult. By employing self-catalyzing phospho-lysine chimeras and lipid-polymer nanoparticles, we successfully re-introduced PTEN mRNA to PTEN-null prostate cancer cells both in vitro and in vivo. These mRNA-loaded nanoparticles demonstrate high protein expression efficiency, low toxicity and good stability in serum and tumor accumulation. We confirmed that restoration of PTEN in PTEN-null prostate cancer cells inhibits the PI3K-AKT pathway, reduces cell viability and enhances apoptosis in vitro. Systemic delivery of PTEN mRNA-loaded nanoparticles in prostate xenograft tumors results in ~85% inhibition of tumor growth and leads to tumor cell death without toxic side effects in vivo. In summary, this work provides proof of concept of mRNA-based gene therapy for systemic restoration of functional PTEN for tumor suppression in vivo. It represents a novel approach to PI3K-AKT pathway inhibition, with the potential to specifically target cancers with loss of PTEN function.


Background: Age-adjusted incidence and mortality rates for prostate cancer (PCa) among African American (AA) men are 1.6- and 2.4-fold greater, respectively, than among white men. More aggressive characteristics of AA PCa account for a significant component of the PCa disparity, in addition to social determinants of health. Previous work from our laboratory and others have identified deregula-
Hepatocellular carcinoma (HCC) is the most common form of primary liver cancer with limited treatment options for advanced stage disease. Thus, there is a critical medical need for improved therapies. In approximately 10% of HCC, a focal ampiclon at 11q13 harboring FGFR19 has been reported. High levels of FGFR19 have been shown to drive HCC tumor development and progression in preclinical models, targeting FGFR19 has the potential to inhibit growth of this receptor for FGFR19, may be efficacious in these HCC tumors. INC06026079 a potent and selective irreversible inhibitor of FGFR4 (>250-fold vs FGFR1/2/3) suppresses the growth of HCC cell lines driven by amplification and overexpression of FGFR19. In subcutaneous xenograft models of HCC, oral dosing of INC06026079 at tolerated doses resulted in dose-dependent inhibition of tumor growth with regressions observed at higher doses consistent with inhibition of FGFR4. In orthotopic tumors in vivo, the only approved targeted therapy for HCC, FGFR4 inhibition exhibited additive tumor growth inhibition in the Huh7 model. To assess efficacy of INC06026079 in orthotopic tumors after oral dosing, Hep3B tumors were implanted surgically into the liver and their development monitored by analysis of plasma alpha-fetoprotein (AFP). At efficacious doses, INC06026079 strongly suppressed the levels of AFP and FGFR19 secreted by the tumors, and their levels correlated well with the reduction in terminal liver tumor mass, suggesting that these may be surrogate markers for response of HCC tumors to INC06026079. In two PDX models with HCC with amplification of FGFR19 (4-6 CNV), INC06026079 administration reduced tumor growth by 50-66% at doses that were well tolerated. Established tumors xenografted with Huh7/ICGR/G4/S were inhibited up to 50% in several parameters related to FGFR19 regulation of bile acid metabolism. The mRNAs levels of CYP7A1, encoding cholesterol 7a-hydroxylase, the rate limiting enzyme in bile acid synthesis, were induced in the livers of cynomolgus monkeys upon dosing with INC06026079. Correspondingly there was a dose-dependent increase in fecal bile acids. In summary these data demonstrate that INC06026079 is highly and selectively efficacious in models of HCC with FGFR19 overexpression, indicating potential for drug development in HCC.

**#1233 WNT inhibitor ICG-001 prevents visceral metastatic triple negative breast cancer in a chemo-resistant patient derived xenograft - PDx model**

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Triple negative breast cancer (TNBC) is characterized by absence of the estrogen receptor (ER) and progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) amplification. Such cancers are highly aggressive and frequently metastasize to the lung and brain. Unlike other breast cancer subtypes such as, ER+ and PR+, TNBCs lack specific targeted-therapeutics; therefore, studies should be directed for development of targeted therapies to treat this condition. Recent studies indicate that Wnt/β-catenin signaling is particularly activated in TNBC and is associated with reduced overall survival in all patients. Therefore, pharmacological targeting of Wnt signaling pathway constitutes an ideal approach for treating TNBC and various Wnt inhibitors are currently in use in clinical trials, such as; ICG-001 in metastatic colon cancer. The inhibitory effects of ICG-001 have not been tested in chemoresistant TNBC/PDX tumors. Herein, we report for the first time that ICG-001 compound selectively inhibited the growth of several European American (EA) and African American (AA) triple negative breast cancer subtypes MDA-MB-231 and MDA-MB-468 both in vitro and in vivo models. To further investigate the precise mechanisms of action in the regulation of Wnt/β-catenin signaling by ICG-001, we performed Western blot analysis, apoptosis assays, cell cycle assays and quantitative real-time reverse transcriptase- polymerase chain reactions in human triple negative breast cancer cells. This compound significantly interfered with Wnt/β-catenin signaling, and its inhibition led to downregulation of important downstream targets such as Axin2, HMGGA2, PCNA, c-myc and Cyclin D1, which in turn led to inhibition of proliferation, cell cycle progression and metastasis confirming our previous results too. In addition ICG-001 inhibited the invasion and motility of tumor cells and showed inhibition and prevention of visceral metastatic PDX tumors from both chemoresistant EA and AA women. These results indicate that the Wnt inhibitor ICG-001 could constitute a powerful new chemotherapeutic agent against triple negative breast cancer.

**#1234 The novel FGFR4-selective inhibitor INC06026079 is efficacious in models of hepatocellular carcinoma harboring FGFR9 amplification.**

Bruce Ruggeri, Matthew Stubbs, Yan-ou Yang, Ashish Jovekar, Liang Lu, Sindu Condon, Darline DiMatteo, Xiaoning Wen, Paul Collier, Timothy Burn, Liangxing Wu, Daniel Wilson, Swamy Yeleswaram, Alan Roberts, Wenqing Yao, Gregory Hollis, Reid Huber, Peggy Scherle, Phillip CC Liu, Incyte Corporation, Wilmington, DE.

INCB062079 is a potent and selective FGFR4 inhibitor (P > 0.05) and in vivo surrogate markers for response of HCC tumors to INC06026079. In two PDX models with HCC with amplification of FGFR19 (4-6 CNV), INC06026079 administration reduced tumor growth by 50-66% at doses that were well tolerated. Established tumors xenografted with Huh7/ICGR/G4/S were inhibited up to 50% in several parameters related to FGFR19 regulation of bile acid metabolism. The mRNAs levels of CYP7A1, encoding cholesterol 7a-hydroxylase, the rate limiting enzyme in bile acid synthesis, were induced in the livers of cynomolgus monkeys upon dosing with INC06026079. Correspondingly there was a dose-dependent increase in fecal bile acids. In summary these data demonstrate that INC06026079 is highly and selectively efficacious in models of HCC with FGFR19 overexpression, indicating potential for drug development in HCC.

**#1235 EPHA-targeted therapy enhances the cytotoxicity of eicosapentaenoic acid against triple-negative inflammatory breast cancer.**

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Background: Inflammatory breast cancer (IBC) is the most aggressive form of breast cancer. We have previously reported that mediators of inflammation, such as COX-2, promote the growth of Triple-Negative receptor (TN) IBC xenografts; therefore, inflammation in TN-IBC has a unique opportunity as a therapeutic strategy. Eicosapentaenoic acid (EPA), a non-toxic omega-3 fatty acid with anti-inflammatory properties, has partially reduced tumor growth in preclinical models of TN-IBC. Therefore, our goal is to develop a novel non-toxic approach that enhances EPA efficacy against TN-IBC in combination with targeted therapy. Methods: Using a high-throughput, siRNA screen (939 genes) in the TN-IBC cell line SUM149PT, we identified Ephrin type-A receptor 2 (EPHA2), an oncogenic cell-surface receptor tyrosine kinase, as a target that modulates the sensitivity of TN-IBC cells to EPA treatment. To determine the clinical relevance of EPHA2, we interrogated a meta-analysis of breast cancer mRNA expression data sets, and found that high EPHA2 tumor expression was significantly correlated with poor overall survival in TN-IBC patients, compared to low EPAH2 expressing tumors (P = 0.01). We observed no significant correlations to other breast cancer subtypes. Similar findings were observed in vitro were EPAH2 expression predominantly occurred in the TN-IBC subtypes (19 of 30) among 49 breast cancer cell lines. Gain/loss-of-expression studies were performed to functionally validate EPAH2 as a synergistic combinational target with EPA in two EPHA2-expressing TN-IBC models, SUM149PT and BCX010, using proliferation and apoptosis assays in vitro and established tumor xenografts in vivo. EPAH2 gene silencing significantly reduced cell growth and induced apoptosis in combination with EPA when compared with untreated control and monotherapy in vitro (P < 0.05) and in vivo (P < 0.001), while vector-induced EPAH2 expression reversed cell growth re-
vitro (P < 0.05). To translate our findings to the clinic, we validated that dasatinib, a small molecule inhibitor of EPHA2, in combination with EPA significantly enhanced cell death of SUM149PT and BCX1010 cells in vitro when compared to non-treated and monotherapy (P < 0.05). Finally, using membrane fluidity assessment and reverse-phase protein array (300 antibodies), we determined that the treatment efficacy depended on EPA/EPHA2 inhibition-mediated increase in cell membrane rigidity (P < 0.001, compared to monotherapy), which subsequently inhibited receptor tyrosine kinase signaling activity, potentially resulting in induction of apoptosis. Conclusions: Our preclinical findings provide a rationale for the development of a phase 1 clinical trial investigating combination EPA and EPHA2-inhibitors in patients with EPHA2-positive TN-IBC.

#1236 Novel androgen receptor inhibitors in triple negative breast cancer. Saswati Bhattacharya, Yuanqi Cai, Steven Kurina, Benjamin Hokanson, Matthew Anderson, Ruth O’Regan. UW Madison, Madison, WI.

Triple negative breast cancer (TNBC), which lacks expression of hormone receptors and human epidermal growth factor receptor 2, has the worst prognosis of all breast cancer subtypes, due to inherent chemo-resistance and a lack of therapeutic targets. A subset of TNBC has been shown to express androgen receptor (AR) but despite promising pre-clinical data, clinical trials evaluating the use of approved anti-androgens (bicalutamide, enzalutamide) in AR-positive TNBC has shown modest efficacy to date. In prostate cancer, bicalutamide binds to AR with low affinity and in the presence of AR gene amplification or mutation, can exhibit partial agonist activity leading to resistance. In order to increase affinity for AR and to avoid the AR agonist to antagonist conversion, synthesized 7-substituted umbelliferones (UMB) derivatives with a distinct scaffold modified from available AR antagonists (Kandil et al Bioorganic & Medicinal Chemical Letters, 2016). These agents exhibited inhibitory activity in human prostate cancer cell lines at sub-micro molar level, a 50-fold and 30-fold improvement over bicalutamide and enzalutamide, respectively. We hypothesized that these novel AR antagonists would be effective in inhibiting the growth of AR-positive TNBC cells. Methods: TNBC AR negative mesenchymal MDA-MB-231 (as control) and AR positive MDA-MB-231 cells were utilized for determination of the efficacy of the UMB derivatives. For proliferation assays, viability of the cells was monitored over a period of 72 hours with redox dye Cell Titer Blue. Standard Western blotting techniques were used for protein detection. For apoptosis analysis, standard flow cytometry reagents, FITC labeled Annexin V and Propidium Iodin(Pi) mix were utilized. Results: UMB derivatives decreased the expression of AR in AR-negative MDA-MB-453 in a dose dependent manner. UMB derivatives, at micro Molar concentration decreased cell proliferation of AR-positive and interestingly AR-negative cell lines by 20 to 50%, also in a dose dependent manner. UMB derivatives induced apoptosis in both lines. Interestingly, UMB derivatives induced a decrease from epithelial to mesenchymal phenotype in AR-negative MDA-MB-231 cells, which was confirmed by decreased expression of vimentin and other mesenchymal markers. Conclusions: Novel UMB derivatives inhibit growth and cause apoptosis in TNBC cells and induce mesenchymal-to-epithelial transition in an AR-negative mesenchymal TNBC cell line. Targeting AR with novel AR antagonists seems to be promising therapeutic approach for AR-positive TNBC and further evaluation of AR antagonists in AR-negative mesenchymal TNBC is warranted.

#1237 PDGF induces cell growth and changes in glucose metabolism in colon cancer in the absence of PDGF receptors. Romana Moench, Tanja Grimning, Christoph-Thomas Germer, Ana Maria Waaga-Gasser, Martin Gasser. Univ. of Würzburg, Wuerzburg, Germany.

Background: The Platelet derived growth factor (PDGF) and its receptors play a major role in inducing proliferation, migration, and angiogenesis by activating intracellular PI3K/Akt/mTOR signaling events in different solid tumors and therefore represent attractive targets in tumor therapy. Recently we showed a PDGF-induced activation of metabolism and proliferation in HT29 colon cancer cells in the absence of specific PDGF receptors. The aim of this study was to analyze PDGF and VEGFR expression and possible alternative PDGF binding partners in colorectal cancer (CRC). Methods: Human HT29 colon cancer cells were cultured and stimulated with PDGF in a time-dependent manner. Additionally, VEGFR2 and EGFR inhibition was performed to analyze alternative PDGF signaling events. Whole cell or RNA extracts were analyzed by Western Blot and RT-q PCR for receptors and PI3K/Akt/mTOR signaling. To investigate the effects of specific receptor inhibition on proliferation, MTS proliferation assays were performed. Moreover, mRNA levels of PDGFR and VEGFR in tumors from patients with CRC were analyzed by RT-qPCR. Results: Human UICC stage I-IV tumors exhibited a significantly increased PDGFRβ and VEGFR1.2 gene expression. As observed previously, HT2 colon cancer cells showed only positivity for VEGFR1.2, but no PDGFR protein expression. Despite that, stimulation with PDGF resulted in increased proliferation and metabolic changes. In contrast, Caco-2 and SW480 colon cancer cells showed PDGFR and VEGF expression on protein level. Interestingly, PDGF stimulation induced a 20% increase in PDGFR and 2-fold increase in PDGFRα expression in HT29 colon cancer cells and secondly, stimulation with PDGFR inhibition of VEGFR2 and EGFR showed alterations in proliferation and Akt signaling in PDGFR stimulated cancer cells. Conclusion: Despite the absence of PDGFR receptors in HT29 colon cancer cells, PDGF induced proliferating and metabolic effects in the tumor cells, suggesting an alternative binding partner on the tumor cell surface. Further investigation of the alternative PDGFR binding partners could be of clinical relevance in CRC.

#1238 Targeting the platelet derived growth factor receptor (PDGFR) with the receptor tyrosine kinase inhibitor ponatinib in small cell carcinoma of the ovary, hypercalcaemic type. Jessica Diane Lang,1 William Hendricks,1 Pilar Ramos,2 Holly Yin,1 Chris Sereiduk,1 Jeffrey Kiefer,1 Yemin Wang,2 Anthony N. Karnezis,2 Bernard Weissman,3 David Huntsman,3 Jeffrey Trent.1 Translational Genomics Research Institute, Phoenix, AZ; 2British Columbia Cancer Agency, Vancouver, British Columbia, Canada; 3University of North Carolina at Chapel Hill, Chapel Hill, NC.

Subunits of the SWI/SNF chromatin-remodeling complex are tumor suppressors that are inactivated in ~20% of all cancers, yet few targeted treatments have shown selective activity in SWI/SNF-mutant cancers. Small cell carcinoma of the ovary, hypercalcaemic type (SCCOHT) is a rare, aggressive ovarian cancer in young women that universally driven by SWI/SNF dysregulation. Given that SWI/SNF proteins are lost in more than 90% of tumors, we developed a two-year survival following standard high-dose chemotherapy and radiation in SCCOHT is less than 35%, a great need exists for effective targeted therapies to improve outcomes for these women. We previously demonstrated that SCCOHT tumors are driven by inactivating mutations in SMARCA4, one of two mutually exclusive SWI/SNF ATPases. In addition, we have shown that SCCOHT lacks expression of the alternative SWI/SNF ATPase, SMARCA2. We have now found through integrated genomic and functional analyses in SCCOHT tumors and cell lines that SMARCA4 loss correlates with increased expression of receptor tyrosine kinases (RTKs) including the platelet derived growth factor receptors (PDGFRs). Through integration of high-throughput RNA interference and drug screens in SCCOHT cells we have identified sensitivity to RTK knockdown and RTK inhibitors including the FDA-approved oncolgy drug, ponatinib. These data corroborate prior studies showing RTK dependence in rhabdoid tumors, rare cancers that are also driven by mutations in the SWI/SNF complex. Of the known ponatinib targets, PDGFR-alpha and FGFR1 were highly expressed in SCCOHT tumors, as confirmed in RNA-Seq data (four tumors) and a SCCOHT tissue microarray (TMA; ten tumors). Further, we showed that PDGF-induced phosphorylation and downstream signaling are inhibited by ponatinib in SCCOHT cells, suggesting that these tumors are sensitive to ponatinib due to dependence on signaling through these RTKs. Finally, given ponatinib’s potency in vitro and the proposed mechanism of action, we tested this agent in xenograft models of SCCOHT. In addition to confirming efficacy in a SCCOHT cell line xenograft model, superior efficacy was demonstrated in two patient-derived xenograft (PDX) models of SCCOHT with ponatinib. Thus, ponatinib effectively targets SWI/SNF-mutant SCCOHT tumors through inhibition of PDGFR signaling and may have clinical utility for the treatment of these cancers.


The aim of this study is to evaluate the expression of the salvage pathway enzymes DCK, APRT, and HPRT in lung cancer cells to determine if they could serve as biomarkers for lung cancer diagnosis and potential treatment. In both men and women, lung cancer is the most lethal cancer in the world, and accounts for more than 30% of cancer-related deaths. We chose to evaluate the salvage pathway enzymes due to an established relationship between the serum biomarker Thymidine Kinase 1 (TK1) and lung cancer. Two non-small cell lung carcinoma cell lines were utilized for this analysis (NCI-H460 and A549) along with cancer tissue and healthy tissue from 27 lung squamous carcinoma patients. The surface localization of these enzymes was determined using flow cytometry, confocal microscopy, and scanning electron microscopy, while up-regulation within tissue was assessed using Immunohistochemistry (IHC). Throughout our investigation, we found no significant expression of DCK or APRT on the surface of non-small cell lung cancer cells, but found a significant presence of HPRT on the plasma membrane of both NCI-H460 and A549 cells.

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The average population florescence of cells treated with HPRT antibodies increased by 24.3% and 12.9% in NCI-H460 and A549 cells, respectively, in comparison to controls. To ensure expression was not attributed to cytoplasmic HPRT, confocal microscopy was performed to visualize HPRT binding on the plasma membrane. After staining NCI-H460 cells treated with both fluorescent antibodies and a membrane-specific dye, we observed a higher fluorescence intensity towards HPRT and the membrane of the cancer cells. Additionally, gold conjugated antibodies were used to label and quantify the amount of HPRT on the cell surface using scanning electron microscopy and EDAX. Further confirming HPRT presence, the gold weight percentage of the sample increased significantly when NCI-H460 cells were exposed to HPRT antibody (p-value 0.012) in comparison to isotype controls. Finally, the general upregulation of the protein was observed in patient tissue samples, with approximately 44% of lung cancer tissues showing significant HPRT upregulation when compared to healthy tissue samples. This differential upregulation shows an altered HPRT expression within some patients, which may help explain the presence of this presumed cytosolic enzyme on the surface of lung cancer cells. These results strongly indicate a unique relationship between cancer cells and HPRT and suggest HPRT as a possible biomarker for the detection and treatment of non-small cell lung cancers.

#1240 Evaluating clinically relevant pharmacological agents in a rat ambulation model to ameliorate PEGylated recombinant hyaluronidase PH20 (PEGPH20)-mediated musculoskeletal adverse events. Li Ma,1 Marc R. Azar,2 Michael Sheppard,1 Yu Huang,1 Piet A. Verraat,1 Daniel C. Maneval,1 Curtis B. Thompson,1 Rudy Paladini,11 Halozyme Therapeutics Inc., San Diego, CA; Behavioral Pharma, La Jolla, CA.

Some cancers accumulate high levels of the glycosaminoglycan hyaluronan (HA) within the tumor microenvironment (TME), and this HA accumulation is associated with rapid tumor progression and poor clinical outcomes. As preclinical data have demonstrated that enzymatic degradation of HA, via intravenous (IV) administration of PEGylated recombinant human hyaluronidase PH20 (PEGPH20), increases therapeutic efficacy in HA-accumulating tumors, several clinical trials evaluating PEGPH20 in combination with anti-cancer therapies are ongoing. The most advanced clinical trial (Phase 3) is evaluating PEGPH20 plus Abraxane® (nab-paclitaxel)/gemcitabine (AG) in patients with stage IV untreated pancreatic ductal adenocarcinoma (PDAC)(NCT02715804). The safety profile of PEGPH20/Abraxane/gemcitabine (PAG) was generally similar to AG alone in an interim analysis of the Phase 2 data, with the exception of a potential imbalance of thromboembolic events (TEs), and an increased incidence of musculoskeletal events (MSEs) in the PAG arm, with the predominant MSE being muscle spasms (55.4% PAG vs. 1.6% AG, all grades)(Bullock, ASCO 2016). In an effort to find pharmacological agent(s) that might decrease MSEs in PAG-treated patients, a rat locomotor activity assay was developed. In brief, locomotor activity of male Sprague Dawley rats was measured with an automated infra-red photobeam monitoring apparatus (Hamilton/Kinder®), San Diego) during the 12h dark cycle when rats are normally active. Any PEGPH20-mediated decrease in rat locomotor activity was interpreted as MSE-like effects. In response to PEGPH20 (1 mg/kg) the locomotor activity of male rats was reduced by ~75% for the first 2 dark cycles post-treatment (48h), and then gradually returned to vehicle baseline levels by days 4-5. FDA approved drugs used clinically to alleviate pain were subsequently tested for their ability to ameliorate the effects of PEGPH20 on locomotor activity. Agents included anti-inflammatories, anti-spasmodics, neuropathic pain medications, neuroleptics, diuretics, anti-hypertensives, anti-fibromyalgics and opioids. The steroid dexamethasone, currently being used in the Phase 2 and Phase 3 PDA clinical trials to manage MSEs, was the positive control. As a class, nonsteroidal anti-inflammatory drugs (NSAIDs), including ketorolac, ketoprofen, diclofenac, and piroxicam, increased rat ambulation by ~40% on the first night following PEGPH20 treatment compared to PEGPH20 alone. Mobility in rats treated with NSAIDs/PEGPH20 increased ~60% on the second night compared to PEGPH20 alone. No other drug classes increased rodent ambulation. These data suggest that NSAIDs may be useful in partially alleviating the MSEs experienced by patients receiving PEGPH20.

#1241 Preclinical study of the combined treatment of arginase and canavanine in pancreatic cancer. Tsz Tung Kwong, Chi Hang Wong, Herbert Ho Pung Loong, Stephen Lam Chan. The Chinese University of Hong Kong, Hong Kong, Hong Kong.

Background: Arginine deprivation is a novel approach for cancer treatment as some cancers were found to be arginine auxotrophic. PEG-BCT-100, a pegylated form of recombinant human arginase, was used to investigate the effect of argi- nine deprivation on pancreatic cell lines. On the other hand, Canavanine (CNAV), a natural toxic analog of arginine isolated from leguminous plant, has also demon-strated its ability in inhibiting cancer cell proliferation. It alters the protein structure and causes critical metabolic defects in the cells by replacing arginine into newly synthesized proteins during translation. In this study, we have also tested the efficacy of combined arginase and CNAV treatment on pancreatic cell lines with high levels of arginase expression. Membrane-bound PAG or cytosolic fibroblast cell line (WI-38), were either incubated in normal or arginine free condition. The corresponding IC50 of CNAV was determined by cell viability assay. To evaluate the responsiveness to the combined treatment, cells were monitored by live cell imaging under the conditions of PEG-BCT-100 treatment alone (0.3IU/ml) or in combination with CNAV (10μM or 50μM). The apoptotic effect of the treatment was examined by Western blotting’s DNA ladder assay. Results: Either arginase or CNAV treatment could inhibit pancreatic cancer cell growth. The cells treated with CNAV in arginine free condition demonstrated an inhibition of cell proliferation and its corresponding IC50 was significantly lowered from millimolar to micromolar concentration when arginine was withdrawn. Strong synergism of CNAV and PEG-BCT-100 was observed after 48-hour treatment with enhanced apoptosis in pancreatic cell lines, but not in normal fibroblast cells. Both early and late apoptosis were observed in co-treated pancreatic cancer cells as indicated by Annexin V assay and the detection of poly- ADP ribose polymerase (PARP) in Western blot. Conclusion: Arginine deprivation by PEG-BCT-100 combined with CNAV treatment showed a strong synergistic effect on inducing cell death in pancreatic cell lines, suggesting that the supplement of CAV could facilitate the treatment outcome of arginine de-privation by arginase. This combined treatment may serve as a treatment strat- egy for pancreatic cancer.

#1242 Ornithine decarboxylase as a therapeutic target in endometrial cancer. Hong Im Kim,1 Chad R. Schultz,2 Andrea L. Buras,3 Elizabeth Friedman,2 Alyssa M. Fedorko,2 Leigh G. Seamon,3 Gadiessi Chandramouli,4 André S. Bachmann,2 John I. Risinger,1 Michigan State University, Grand Rapids, MI; Grand Rapids Medical Education Partners, Grand Rapids, MI; Spectrum Health, Grand Rapids, MI; GenEprisa, Columbia, MD.

Endometrial cancer is the 4th most common cancer and the 6th deadliest cancer in US women. The American Cancer Society estimates there will be 60,050 new endometrial cancers in 2016, an increase of more than 10% from the previous year highlighting the need for more effective treatments and preven-tion. Ornithine Decarboxylase (ODC) a key enzyme in polyamine synthesis is often overexpressed in cancers and contributes to cell proliferation and tumor growth. Therefore, ODC and the polyamine pathway are considered rational targets for cancer treatment or prevention. We noted ubiquitous expression of ODC1 in our previously published endometrial cancer gene array data and confirmed this in the cancer genome atlas (TCGA), finding expression in all four molecular sub-types with highest expression in copy number high cancers which have the worst clinical outcomes. Therefore, we explored the association of ODC1 gene expression with clinical outcomes of overall survival (OS) and recurrence in the TCGA cohort and noted that elevated ODC1 was significantly related to OS (Wald test p = 0.001) and recurrence (p = 0.01). Importantly, we confirmed these observations using QRT-PCR in a validation cohort of 60 endometrial cancers and found that endometrial cancers with elevated ODC1 had significantly shorter recurrence-free intervals (p = 0.0014) with elevated hazard ratio 3.81. Numerous studies including clinical trials have examined the chemopreventive and anti-tumor effects of difluoro-metlyornithine (DFMO), a specific inhibitor of ODC. We found that DFMO treatment significantly reduced cell proliferation, cell viability, and colony for-mation in human cell line models derived from undifferentiated, endometrioid, serous, MMT and clear cell endometrial cancers. In contrast, immortalized uter-ine endometrial epithelial cells (EM E6/E7 TERT1) were less sensitive to DFMO. To confirm the significant effects of DFMO in vitro we performed an in vivo study with human endometrial cancer (AC1-98) tumor-bearing athymic nude mice. Tumor tissue was either treated with 2% (w/v) DFMO supplied in drinking water or water only (n = 10/group). DFMO significantly reduced the tumor burden in mice compared to controls (p = 0.0023). ODC-regulated polyamines (putrescine [Put] and spermidine [Spd]) known activators of cell prolif-eration were strongly decreased in response to DFMO, in both tumor tissue ([Put] (p = 0.0006), [Spd] (p < 0.0001)) and blood plasma ([Put] (p < 0.0001), [Spd], Results) of these studies indicate that some endometrial cancers appear particularly sensitive to DFMO. Our findings indi-cate that the polyamine pathway in endometrial cancers in general and specifi-cally those most clinically relevant endometrial cancers could be targeted for effective treatment, chemoprevention or chemoprevention of recurrence.
libraries for each cell line in duplicate as well as a counter screen using a reporter cassette that lacked let-7 binding sites. A compound was identified as a hit if it ranked within the top 100 compounds in the primary screen and outside the top 250 compounds in the counter screen. The hit rate for LIN28A positive cell line was 80% for the Prestwick and 74% for the LOPAC1280 libraries. For the LIN28B positive cell line, the hit rate was 80% for Prestwick and 53% for LOPAC1280 libraries. The hits were validated by assaying for let-7 levels using qRT-PCR. The hits identified included compounds that inhibited PI3K-mTOR, nFkB, c-MYC, Cyclin D1, BETI as well as aurora kinase. These targets have been reported in the literature to downregulate let-7 levels. In conclusion, our assay-system is the first to utilize a biological assay of let-7 levels for small molecule library screening thus serving as a valuable tool for cancer drug discovery.

#1245 Screening methodologies for the discovery of small molecule melanoma therapeutics targeted at the ErbB4 receptor tyrosine kinase. Richard L. Cullum,1 John T. Piazza,2 Jared I. Senfeld,1 Logan T. Neel,1 Ram B. Gupta,2 Allan E. David,1 David J. Riese,1 Auburn University, Auburn, AL;3 Virginia Commonwealth University, Richmond, VA.

Introduction: Gain-of-function mutations in the ErbB4 receptor tyrosine kinase have been found in a significant fraction of melanoma cell lines that are dependent on ErbB4 for proliferation. However, there is a scarcity of therapeutics for treating these ErbB4-dependent tumors. Consequently, we have developed high-throughput screening assays to identify small molecule ErbB4 antagonists that may hold promise as targeted melanoma therapeutics. Our approach is based on the observation that the Q43L mutant of the ErbB4 agonist Neuregulin 1beta (NRG1β) functions as a partial agonist at ErbB4. NRG2β/Q43L stimulates ErbB4 tyrosine phosphorylation, fails to stimulate ErbB4-dependent cell proliferation, and competitively antagonizes agonist stimulation of ErbB4-dependent cell proliferation. Experimental procedures: Therefore, we have developed three high-throughput screening assays to identify ErbB4 partial agonists that function as antagonists. The primary screen identifies molecules that stimulate ErbB4 tyrosine phosphorylation. The secondary screen identifies molecules that stimulate or fail to stimulate ErbB4-dependent cell proliferation. The tertiary screen identifies molecules that antagonize agonist stimulation of ErbB4-dependent cell proliferation. Results: A phospho-ErbB4 sandwich ELISA assay identifies molecules that stimulate ErbB4 tyrosine phosphorylation with high sensitivity and fidelity (Z’ > 0.5). IL3-independence assays in conjunction with MITT assays using a cell line that displays ErbB4-dependent cell proliferation distinguish between molecules that stimulate and fail to stimulate ErbB4-dependent proliferation (Z’ > 0.5) and identify molecules that antagonize agonist stimulation of ErbB4-dependent proliferation. These assays have been used to identify small molecules that stimulate ErbB4 tyrosine phosphorylation. Efforts to determine whether these hits function as ErbB4 full agonists or partial agonists (antagonists) are underway and will be reported. Structures of these small molecule ErbB4 full and partial agonists may be reported, pending submission of a provisional patent application. Conclusions: We have validated an HTS strategy for identifying ErbB4 partial agonists that function as ErbB4 antagonists and deployment of that strategy has led to the identification of several hits (~ 20 from the primary screen). Such molecules may hold promise as targeted therapeutics for melanoma and other ErbB4-dependent tumors.

#1246 Development of a novel KRAS-targeting agent: systematic validation using in silico, in solution, cell models, PDX and transgenic mouse models. Arsheed Ahmad Ganaie,1 Hifzur R. Siddique,1 Ishfaq Sheikh,1 Lei Wang,1 Aijaz Parry,2 Jayanthi panyam,3 Peter Villalta,1 Joshua Liao,1 Yibin deng,1 Mohammad saleem,2 1University of Minnesota Hormel Inst., Austin, MN, 2Univ. of Minnesota Hormel Inst., Minneapolis, MN.

Aberrant KRAS signaling plays an important role in the pathogenesis of malignancies including pancreatic (PDAC), colon and lung cancer. Because therapeutics targeting either downstream or associated factors of KRAS pathway have shown dismal results in clinics, there is a need to identify effective agents targeting active KRAS (GTP-KRAS) protein. In this study, we screened a chemical library for their efficacy to inhibit KRAS activity using in silico methods. Based on their binding energy, we selected a candidate agent (LP1) that exhibited high potential of binding to KRAS protein at a region where KRAS-activator protein “SOS1” binds to, and GDP-GTP exchange takes place. We next investigated if LP1 exhibits the efficacy of KRAS binding in biological solution using recombinant proteins and employing drug development techniques i.e., florescence (FL)-based competition, Isothermal Titration Calorimetry (ITC) and Surface Plasmon Resonance (SPR) assays. The FL-based study showed that LP1 inhibits the binding of SOS1 protein and GTP molecules to KRAS protein. The ITC and SPR analysis showed that LP1 binds to the KRAS protein (at 10 μM). We next deter-
mained the KRAS-inhibitory potential of LP1 in activated-KRAS cells representing premalignant and malignant models of PDAC, colon and lung cancer. Disassociation study involving incubation of KRAS/SOS1 proteins showed the LP1 inhibits the binding of SOS1 to KRAS. Notably, LP1 therapy significantly decreased the (i) KRAS-GTP protein levels (ii) growth and (iii) proliferation of activated KRAS single cell and 3D Organoid cultures. We next tested KRAS-inhibitory efficacy of LP1 in PDAC patient-derived (PD) tumor explants and found that LP1 therapy (10 days) significantly decreased growth of, and KRAS-GTP levels in PD explants. Based on the in silico, in solution, cell model and PD-explant data, we determined the efficacy of LP1 as a KRAS inhibitor in vivo conditions. We first performed the pharmacokinetic study of LP1 in mice and observed that LP1 is physiologically available in the blood (peak of 15-20 μM) after oral and intraperitoneal administrations and detectable up to 24h post-administration. We next tested LP1 therapy in tumor xenograft models and show that LP1 therapy significantly reduces the tumorigenicity of KRAS-activated PDAC and colon cell-derived tumors implanted in athymic mice. We next tested efficacy of LP1 administration on the developmental phases of PDAC disease (from normal to high grade neoplasia/early stage carcinoma) and show that LP1 feeding for six months caused a significant inhibition of PanIN development in KPC(T1212) transgenic mouse model. Notably the analysis of tumors xenograft models and pancreatic tissues of KPC(T1212) mice receiving LP1 therapy exhibited reduced KRAS-GTP levels. Taken together, these data show that LP1 is a potent KRAS activity inhibitor and a potential candidate for clinical use against KRAS-driven cancers.


Development and growth of a tumor as well as its ability to metastasize involves a complex relationship with its tissue microenvironment. A proliferating tumor encounters several microenvironmental stress conditions such as hypoxia, lack of nutrients and acidosis. To cope with these conditions, cancer cells have developed elaborate cytoprotective mechanisms which provide them with distinct advantages to thrive. Thus, deciphering the signaling pathways which get activated in the tumor microenvironment has been paramount to develop new therapeutic strategies for treatment. The Unfolded Protein Response (UPR) is an adaptive prosurvival pathway elicited by stresses in the tumor microenvironment (e.g., hypoxia, low glucose) and involves translational and transcriptional activation of effector genes which act to relieve cellular stress and block cancer cell death. We developed a strategy to comprehensively analyze critical mediators of cell fate in response to UPR activation. We have delivered a lentiviral genome-scale CRISPR-Cas9 knockout (GeCKOv2) library to mouse PCa cell lines (human squamous head and neck carcinoma) and A375 (human melanoma) cells. The library is targeting 19,050 genes with 123,411 unique guide sequences and enables both negative and positive selection screening. We used the GeCKO v2 library to identify genes essential for triggering the UPR in response to thapsigargin and tunicamycin, known specific activators of ER stress. Our highest-ranking candidates include BIRC5/Survivin, a well-studied molecule that is an inhibitor of apoptosis and is highly expressed in cancer cells and eukaryotic translation initiation factor eIF6, whose overexpression increases motility and invasiveness of cancer cells. Our preliminary results indicate that loss of Survivin and eIF6 dramatically enhance sensitization of cells to various ER stress conditions. Moreover, this synergistic outcome is observed when cells are treated with YM155, a small molecule that selectively suppresses Survivin, and is used in phase I/II clinical trials. Lastly, morphological changes like endoreduplication are observed after long term absence of Survivin indicating its endogenous ER stress inducing role. Taken together, Survivin and eIF6 are important mediators of survival following ER stress and characterizing the pathways involved can lead to the development of novel targeted agents and therapeutic approaches.

#1248 Adult calorie restriction reduces weight gain and mammary tumor incidence in obesity prone MMTV-TGF-α mice. Michael E. Grossmann, Nancy K. Mizuno, Da-Qing Yang, D. Joshua Liao, Margot P. Cleary. Univ. of Minnesota Hormel Inst., Austin, MN.

Obesity is associated with increased risk of postmenopausal breast cancer resulting in recommendations for weight gain prevention and/or weight loss.

However, there is little experimental data indicating if calorie restriction (CR) initiated in adulthood impacts mammary tumorigenesis. We have used female MMTV-TGF-α mice which develop mammary tumors in the 2nd yr of life as a model of postmenopausal breast cancer. From 10-30 weeks of age (woa) mice were fed a diet with 33% fat calories. At 30 woa based on body weight mice were assigned to ad libitum (AL), 52% fat calories (Ob-R), and 33% fat calories (Ob-P) diets. Half in each weight category were then fed a 25% CR diet with reduced carbohydrate content (43% fat calories). The remaining mice continued on ad libitum (AL) feeding of the 33% fat calories diet. This resulted in six experimental groups (n = 26-31/group). Food intakes were monitored and body weights and mammary tumor status were determined weekly. Mice were sacrificed either at 90 woa or earlier if tumor size dictated. Mice in the CR-Ob-P group lost an average of 1.12 grams while mice in the AL-Ob-Pr group gained 16.03 grams from 30 woa until euthanaisa. CR-Ob-P mice had a reduced palpable tumor incidence as compared to AL-Ob-P mice prior to euthanaisa (12.90% versus 61.54% P = 0.0002). Indolent tumors were discovered at euthanasia with the CR-Ob-P mice having a larger number than AL-Ob-P mice resulting in final tumor positive mice in these groups of 54.84% vs 65.38%, respectively. Interestingly, the Ob-R mice had final tumor incidence of 38.71% and 73.08% for the CR-Ob-R and the AL-Ob-R (P < 0.01). Average tumor weight was highest in the AL-Ob-P group with CR resulting in a significantly lower tumor weight (P < 0.001). The AL-Ob-P group had the shortest average survival time of 82.96 woa while CR of the Ob-P mice resulted in increased survival to 89.77 woa (P < 0.001). A comparison of the survival curves of CR of these two groups showed a significantly increased survival (P < 0.0001). Results of this study indicate that initiation of moderate 25% CR during adulthood reduces weight gain, mammary tumor incidence, and tumor weight as well as increases lifespan even when a moderately high fat diet was consumed. This information supports further efforts to be made to prevent weight gain as women approach menopause to delay or prevent breast cancer development. Support: NIH-CA157012, The Hormel Foundation and Paint the Town Pink.

#1249 Procyanidin B2-3,3′-di-O-gallate targets cancer cell metabolism in its efficacy against human prostate carcinoma cells. Chapla Agarwal, Dileep Kumar, Alpna Tyagi, Natalie Serkova, Michael Wempe, Komal Raina, Rajesh Agarwal. Univ. of Colorado Denver, Aurora, CO.

The metabolic profile of various cancerous tissues can be correlated with cell growth and death, specific tumor type as well as the pathological stage of tumor. In recent times, prostate tissue metabolomics using spectroscopy methods has helped to identify and establish the metabolic profiles specific to prostate cancer (PCa) malignancy. Thus, the evaluation of the anticancer efficacy of an agent can be considered incomplete without assessing its effect on the metabolic profile of the tumor tissue. With this rationale, herein, we carried out a metabolomics study of human PCa cell lines (PC3) androgen independent (B2G2) on Procyanicin B2-3,3′-di-O-gallate exposure, which was recently identified by us as most effective agent in grape seed extract for growth inhibition and apoptotic death of human PCa cells. Specifically, we employed quantitative high-resolution nuclear magnetic resonance spectroscopy (1H-, 13C- and 31P-NMR) to assess the metabolic profile and energy state of the B2G2-treated human PCa cell lines PC3 and C42B. This approach helped us assess global metabolic profile, including glucose metabolism, energy state, and lipid metabolism in these cells after B2G2 treatment. We also studied the time-course (4-72 hrs) of B2G2 effect on glucose uptake and lactate release in the media of B2G2-treated PCa cells. Our results indicated that there was differential effect of B2G2 on mitochondrial glucose metabolism in these cells. While glucose uptake was markedly reduced as a function of time, there was also a significant increase in extracellular lactate export with increased B2G2 exposure-time. Importantly, B2G2 preserved citrate concentration in the PCa cells as indicated by an increase in citrate levels after treatments; citrate generation is indicative of normal secretion functions of the prostate epithelial cells. The fact that the citrate was not further utilized for cholesterol synthesis was also confirmed by a decrease in cholesterol levels in these cells, indicating decreased cholesterologenesis by B2G2. Together, these results suggest that B2G2 differentially induces metabolic alterations in various PCa cell lines, which could be associated with its effects on cell growth, proliferation and death, as well as androgen dependency (or lack of it). In vivo study examining B2G2 effect on PCa PC3 tumor xenograft growth in athymic nu/nu mice, B2G2 treatment (dose: 3mg/kg body weight of mice; given daily as intraperitoneal injections) significantly decreased tumor volume and tumor weight by ~61% and ~52% (both P < 0.01), respectively, after 5 weeks of treatments. Taken together, these studies indicate anti-PCa efficacy of B2G2 under both in vitro and in vivo scenario and warrant further extensive studies to establish its efficacy in other PCa models.
#1250 Curcumin inhibits migration and growth of human colon cancer cells through covalent modification of oncogenic SIRT1: Cysteine 67 as a potential binding site. Yeon-Hwa Lee,1 Na-Young Song,1 Do-Hee Kim,1 Hye-Kyung Na,2 Young-Joon Suh1. 1Tumor Microenvironment Global Core Research Center, College of Pharmacy, Seoul National University, Seoul, Republic of Korea; 2College of Human Ecology, Sungkyun Women’s University, Seoul, Republic of Korea.

Silent mating type information regulator 2 homolog 1 (SIRT1), an NAD+-dependent histone/protein deacetylase, has diverse physiological functions, including metabolic regulation and stress response. Despite extensive research, however, the role of SIRT1 in tumorigenesis remains controversial. Recent studies have demonstrated that SIRT1 is abnormally overexpressed in several human malignancies, and elevated levels of SIRT1 are correlated with the tumor invasion and metastasis. Curcumin (diferuloylmethane), a major component of the spice turmeric (Curcuma longa L.), has been reported to possess anti-inflammation and anti-carcinogenic properties. In the present study, we found that SIRT1 is predominantly overexpressed in the cytoplasm of several colorectal cancer cells as well as colon tumors. Curcumin abrogated migration and colony forming capability of human colon cancer (HCT-116) cells. This prompted us to investigate the effect of curcumin on the expression of SIRT1 and underlying molecular mechanisms in the context of its inhibition of the migration and growth of these cells. When HCT-116 cells were treated with curcumin, the protein expression of SIRT1 was significantly reduced, but the level of its mRNA transcript was not altered. The curcumin-induced decrease in SIRT1 protein expression was abrogated by the proteasomal inhibitor, MG-132. When HCT-116 cells were treated with curcumin, ubiquitination of SIRT1 was elevated. Notably, tetrahydrocurcumin, a non-electrophilic analogue of curcumin that lacks the α,β-unsaturated carbonyl moiety, failed to ubiquitinate and degrade SIRT1. Nano-LC-ESI-MS/MS analysis revealed the modification of the SIRT1 cysteine67 residue. In line with this observation, the protein stability of a mutant SIRT1 in which cysteine 67 was replaced by alanine (SIRT1-C67A) was unaffected by curcumin treatment. Furthermore, migration and anchorage-independent growth of cells expressing SIRT1-C67A were barely inhibited by curcumin compared with those in cells harbouring wild-type SIRT1. Lysates of HCT-116 cells incubated with curcumin-conjugated Sepharose-4B beads exhibited covalent binding of curcumin to SIRT1. However, such direct interaction was markedly reduced in cells with SIRT1 cysteine 67 mutation. Taken together, these findings suggest that curcumin exerts inhibitory effects on progression of colon cancer through destabilization of oncogenic SIRT1. The electrophilic α,β-unsaturated carbonyl group present in curcumin can covalently modify SIRT1, preferentially at the cysteine 67 residue, facilitating its degradation via the ubiquitin-proteasome pathway in HCT-116 cells.

#1251 Pioglitazone inhibits periprostatic white adipose tissue inflammation in obese mice. Miki Miyazawa,1 Kotha Subhadra,1 Priya Bhardwaj,2 Xi Kathy Zhou,2 Hanhan Wang,1 Domenick J. Falcone,1 Dilip D. Giri,2 Andrew J. Dannenberg1. 1Weill Medical College of Cornell University, New York, NY; 2Memorial Sloan Kettering Cancer Center, New York, NY.

Obesity is associated with an increased incidence of high-grade prostate cancer (PC) and poor prognosis for PC patients. Recently, we showed that obesity-related periprostatic white adipose tissue (WAT) inflammation, characterized by crown-like structures (CLS) consisting of dead or dying adipocytes surrounded by macrophages, was associated with high-grade PC in men. It’s possible that agents that suppress periprostatic WAT inflammation will alter the natural history of PC. Pioglitazone, a ligand of PPARγ, is used to treat diabetes and possesses anti-inflammatory properties. Here our main objectives were to determine if pioglitazone inhibited obesity-related periprostatic WAT inflammation in mice and then to elucidate the underlying mechanism. Mice were fed either a high fat (HF) diet or low fat (LF) diet to determine if obesity-related periprostatic WAT inflammation was abrogated or suppressed in the HF diet. We found that obesity induced periprostatic WAT inflammation in mice that was reversed by pioglitazone treatment. Treatment with pioglitazone reduced the density of CLS in periprostatic WAT, and suppressed levels of TNF-α, TGF-β and the chemokine monocyte chemoattractant protein-1 (MCP-1). Importantly, the ability of pioglitazone to suppress periprostatic WAT inflammation was abrogated in MCP-1 knock out mice. Pioglitazone caused dose-dependent induction of both adiponectin, an anti-inflammatory adipokine, and its receptor Adipor2 in cultured 3T3-L1 cells and in periprostatic WAT of obese mice. Pioglitazone blocked TNF-α-mediated induction of MCP-1 in 3T3-L1 cells, an effect that was attenuated when either adiponectin or Adipor2 were silenced. Taken together, pioglitazone-mediated induction of adiponectin suppressed the elevation in MCP-1 levels thereby attenuating obesity-related periprostatic WAT inflammation. These findings strengthen the rationale for future efforts to determine whether targeting the PPAR-γ-adiponectin-MCP-1 axis will decrease periprostatic adipose inflammation and thereby reduce the risk of high-grade PC or improve outcomes for men with PC.

#1252 A pre-clinical model for lung cancer interception: gene expression similarities between human and mouse bronchial premalignant lesions. Sarah A. Mazzilli,1 Anna Tasinari,1 XiaoHui Xiao,1 Gang Liu,1 Samjot Dhillion,2 Candace Johnson,3 Mary Reid,3 Marc Lenburg,4 Avrum Spira,1 Jennifer Beane1. 1Boston University, Boston, MA; 2Roswell Park Cancer Institute, Buffalo, NY.

The molecular events that drive the development of the premalignant lesions (PMLs) that precede lung squamous cell carcinoma (SCC) are poorly understood. A major limitation to understanding PMLs and developing interventions is the lack of preclinical models to test candidates derived from human studies. Previous work by our group and others suggests that the N-nitroso-tris-chloroethylurea (NTCU) mouse model of lung SCC may be a candidate for modeling human PMLs based on histologic similarities, but its molecular relationship to human disease is limited. In this study, we investigate the transcriptomic similarities between PMLs from the NTCU treated mice and humans. RNA from 40 whole lung sections (curls) and laser capture microdissected (LCM) of PMLs of varying histology from SWR/J and A/J mice treated with NTCU were profiled by RNA-Seq. RNA from 131 human endobronchial biopsies representing analogous histological grades were also profiled by RNA-Seq as part of the Pre-Cancer Genome Atlas (PCGA). Linear modeling was used to identify gene expression differences associated with increasing histological severity of human PMLs (FDR<0.05). We identified a 51-gene (p<0.001) and a 178-gene (p<0.001) signatures associated with increasing histology in the all SWR/J and A/J samples respectively. These gene expression signatures are enriched for biological pathways involved in immune modulation, occurring with progressing lesions. Conversely, genes whose expression changed with increasing histology in a model that included only LCM samples (153 genes at p<0.001) revealed epithelial-specific processes including the up-regulation of KRT5 and p63 and loss of TTF1. Additionally, in the LCM samples, we identified up-regulation of genes involved in proliferation including a number of genes involved in cell structure, metabolism, transcription and translation, anti-apoptosis as well as several immunosuppressive genes. The major finding of these studies is that there are molecular similarities between PMLs from human and NTCU treated mice, which suggest that this mouse model may be useful to investigate targeted early intervention to halt or reverse PML progression towards lung cancer.

#1254 Mechanistic interrogation of pre-treatment low dose aspirin effects in HER 2 positive breast cancer. Ian S. Miller,1 Sonja Khan,2 Liam P. Shiels,3 Sudipto Das,1 Bruce Moran,1 Finbarr P. Leacy,1 Paul M. Loadman,4 Robert S. H. Drysen4 1Department of Cancer Biology, Memorial Sloan Kettering Cancer Center, New York, NY; 2Royal College of Surgeons in Ireland, Dublin, Ireland; 3National University of Ireland, Galway, Galway, Ireland; 4University College Dublin, Dublin, Ireland; 5University of Bradford, Bradford, United Kingdom; 6University of Toronto, Toronto, Ontario, Canada.

Background: Prior data (Barron et al. Cancer Res. 2014 74:4065-77) suggests that pre-diagnostic exposure to aspirin can have significant effects on breast tumor biology and patient outcome. It has been proposed that aspirin inhibition of COX-2 may suppress lymphangiogenesis and metastasis (Karnezis et al Cancer Cell. 2014. 21:181-95). Here, we sought to recapitulate pre-diagnostic aspirin exposure in rodent models of Her2+ breast cancer and elucidate mechanisms of action. We also determined the effect of aspirin on tumor stroma, using a co-culture system of human tumor and mesenchymal stem cells (MSC). Methods: NOD/SCID mice were orthotopically implanted with Her2+ MDA-MB-231 or HCC1954 cells. 48hr later, animals began a daily low dose [30mg/kg or 120mg/kg] of aspirin, until tumors reached 250mm³. They were then resected. 3 weeks later, HCC1954 implanted animals were treated with trastuzumab (15mg/kg) and paclitaxel (5mg/kg) for 6 weeks. Primary tissues were analysed by immunohistochemistry to assess VEGF-C, -D, COX-2, LYZE1 and CD31. RNASeq was performed on tumours to identify aspirin perturbed molecular pathways. To determine the stromal response to aspirin, patient derived MSCs were cultured either alone or with HCC1954 cells and exposed to aspirin (2.5 or 10µM) for 48 hours. Results: In NOD/SCID mice 4 weeks of treatment with low dose aspirin significantly reduced tumor growth and size. HCC1954 tumors treated with aspirin exhibited a decreased density of CD31 positive micro-vessels. In NOD/SCID mice implanted with Her2+ MDA-MB-231 tumors, aspirin treatment for 4 weeks resulted in a significant decrease in tumor growth rate and size. In these tumors, aspirin treatment decreased collagen I and fibronectin expression as well as CD31 expression. In patient derived MSCs, aspirin treatment for 48 hours increased expression of VEGF-C and COX-2. Conclusion: This study demonstrates that pre-diagnostic aspirin exposure in rodent models of Her2+ breast cancer is associated with increased anti-angiogenic activity. The molecular mechanisms of action of aspirin in the breast tumor microenvironment likely involve changes in the tumor stromal microenvironment. These changes may provide the rationale for exploring aspirin as a pre-diagnostic chemopreventive agent.
7.5mM). Secreted VEGF-C was quantified. A tubule formation assay was performed to determine the impact of aspirin on angiogenesis. Pro-angiogenic protein expression was investigated using a human angiogenesis array platform. Results: A significant delay in tumor growth was observed in both tumor models following aspirin treatment (p<.01). Assessment of metastatic potential revealed that 120 mg/kg aspirin significantly (p<.05) increased the time to metastasis and reduced primary regrowth in the MDA MB 231 model (p<.01). Immunohistochemical analysis of VEGF C, D and ILVE1 showed a significant dose dependent reduction (p<.01) in both models. RNAseq pathway analysis revealed a significant over-representation of mitochondrial electron transport chain genes. Downstream factors of AMPK showed significant (p<.01) upregulation suggesting alterations in metabolism. Aspirin (7.5mM) exposure resulted in loss of VEGF-C secretion from co-cultured tumor / MSC cell populations. Conditioned media harvested following aspirin treatment limited support tubule formation. Expression of pro-angiogenic factors in HCC1954 cells showed alterations following treatment, with the greatest decrease seen in Urokinase Plasminogen Activator (−42%) and its inhibitor Serpine 1 (−55%). Conclusion: We have successfully recapitulated pre-treatment aspirin response in surgical resection models of Her2+ breast cancer, with IHC analysis confirming the impact of treatment on angiogenic and lymphangiogenic factors. RNAseq analysis implicates aspirin mediated alterations in cellular metabolism. Our data further reveals increased response to aspirin in stromal cell populations.

**#1255 PhIP/DSS-induced colon carcinogenesis in CYP1A-humanized mice and its prevention by tocopherols.** Chung S. Yang,1 Jayson Chen,1 Anna B. Liu,1 Mao-Jong Lee,1 Hong Wang,1 Guangxu Li,1 Lanjun Zhang,2 Kenneth Reuhl,1 Nanjo Suh1. 1Rutgers University, Piscataway, NJ; 2University Medical Center of Princeton, Princeton, NJ.

In order to establish a more physiologically relevant colorectal cancer model, we recently developed a colon-carcinogenesis model induced by the meat-derived dietary carcinogen, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), and promoted by colitis, and subsequently developed into high-gradedysplasia (P30CA72720 and P30ES005022).

CYP1A-humanized mice were treated with 9,12-dimethylbenz[a]anthracene (DMBA) to induce mammary tumors. Results: We found that male LP consumption alters the non-coding RNA content and DNA methylation patterns of the sperm in agreement with previous findings. We also observed that, compared to Con offspring, the female offspring of LP fathers had lower birth weight (p=0.035). This decrease in body weight persisted through sexual maturity when females began to gain more weight than their control counterparts (p<0.001). In addition, mammary glands of LP offspring were larger (p=0.02) and reduced tumor latency in LP offspring (p=0.03). Mammary tumors of LP offspring had lower rates of apoptosis, which may explain the increased tumor growth in this group. However, the mechanisms mediating this effect still needs to be elucidated. Conclusion: Paternal sub-optimal nutrition programs the female offspring mammary gland development and breast cancer risk. Whether this increase in cancer risk is due to local mammary tissue changes or whether systemic changes play a role remains to be investigated. It also remains to be determined whether epigenetic changes in paternal sperm are functionally linked to changes in mammary gland development and cancer risk.

**#1258 Lifestyle improvements increase cancer onset and progression by adapting endocrine and immune signaling in mouse models.** Grant D. Fogle-song, Stephen M. Bergin, Wei Huang, Michael A. Caligiuri, Lei Cao. The Ohio State University, Columbus, OH.

Metabolic syndromes instigate substantial morbidity and mortality worldwide via its negative effects on many diseases including cancer. Obesity in particular has been deemed a causative factor for breast cancer in post-menopausal women, while worsening prognosis regardless of menopausal status. Importantly, progression of many metabolic syndromes can largely be slowed or reversed with aggressive lifestyle changes, thus also having the potential to miti-
gate cancer onset. A mouse model of environmental enrichment (EE) to improve motosensory, cognitive, and social stimulation by increasing physical engagement and social interaction triggers vast improvements in overall health. These include boosting mental health, reducing adiposity, prevention of diet-induced obesity (DIO), promoting the white to brown fat transition, enhancing insulin sensitivity, and delaying or modifying lifespan indicative of inhibition of cancer growth. We have elucidated the central mechanism of EE to be the activation of the hypothalamic-sympathoneural-adipocyte (HSA) axis, a specific neuroendocrine route in which the brain communicates with adipose tissue via the sympathetic nervous system. Once activated, norepinephrine (NE) is released directly onto adipose tissue which induces the observed metabolic improvements. Breast cancer is uniquely exposed to this adipose-NE so we sought to explore this distinctive microenvironment in the context of both local and systemic endocrine and immune signaling in varied body mass states and menopausal statuses. Our data showed that EE diminished leptin levels and delayed cancer onset in the MMTV-PyMT spontaneous mouse model of breast cancer and slowed growth in a DIO allograft model. In contrast, EE failed to attenuate tumor progression in ob/ob mice in the absence of leptin, but rather enhanced tumor growth, suggesting that the leptin-NE relationship is one of the key peripheral mediators of the EE anti-breast cancer effects. We also observed significant immune cell modulation in the adipose tissue following EE. Initial characterization revealed a decrease in the proportion of tumor-associated macrophages (TAMs) and an overall anti-inflammatory response which together would also reflect the contribution of anti-angiogenic and immune-modulating factors to control tumor growth. Our data support the hypothesis that EE can modulate local and systemic endocrine and immune systems to result in improved breast cancer outcomes.

**#1259 Stem cell property-suppressing microRNAs are stimulated and their target proteins inhibited by vitamin D and progesterone in ovarian cancer cells.** Rebecca Rosales, Jane Turbov, Jennifer Yoo, Gustavo C. Rodriguez, Larry G. Thaete. NorthShore University HealthSystem, Evanston, IL.

Vitamin D (1,25(OH)2D3) and progesterone (P4) have both been shown to reduce the incidence of ovarian cancer. Mechanisms involved in their actions are not fully known but each one can enhance the effect of the other. Six microRNAs (miRNAs), associated with suppression of cancer stem cell properties, were investigated first in a screening array and then with a more definitive RT-PCR investigation of the response of these miRNAs to 100 nM 1,25(OH)2D3, and 100 nM P4. These experiments were conducted in cells that do (OVCA-R3-PGR) and do not (SKOV3) express nuclear progesterone receptors (PGR). Let-7a and Let-7b are known to decrease expression of Ras and c-Myc and reduce stem cell self-renewal. Let-7b expression was increased (p<0.001) at 48 h in response to combined 1,25(OH)2D3 and P4 when PGR were present, and in response to individual and combined treatments at 72 h (p<0.01). Correspondingly, c-Myc expression was decreased (p<0.001) by both P4 and combined P4 and 1,25(OH)2D3. Let-7a expression was not affected by these treatments. No treatment-related change in the production of ras protein was observed compared to controls. In the absence of PGR (SKOV3 cells), c-Myc was suppressed by combined P4 and 1,25(OH)2D3 treatment at 24 h and 48 h. miR-26a and miR-101 are known to downregulate EpCAM and EZH2, leading to decreased tumor growth and metastasis and to reduced proliferation. miR-101 increased (p<0.001) at 48 h in response to combined 1,25(OH)2D3 and P4 in cells expressing PGR. It was increased (p<0.05) at 36 h and showed synergy in the screening array in the absence of PGR. Target proteins EZH2 and EpCAM decreased moderately (p<0.05) at 72 h combined treatment, both with and without PGR present. miR-26a expression was not affected by these treatments. miR-200b and miR-200c are known to suppress expression of ZEB1 which in turn decreases Bmi1 and Sox2 to increase E-cadherin (epithelial marker) and decrease vimentin (mesenchymal marker), thus inhibiting the epithelial-to-mesenchymal transition. Expression of miR-200b was increased (p<0.05) at 24 h and 72 h in response to combined 1,25(OH)2D3 and P4 when PGR were present, and in response to these treatments. ZEB1 was decreased (p<0.01) at 48 h in response to combined 1,25(OH)2D3 and P4. These results point not only to differences among cancer cell lines but also to emphasize a possible mechanism by which P4 and 1,25(OH)2D3 may work together to prevent the occurrence of ovarian cancer, namely by suppressing stem cell renewal and the formation of neoplastic lesions. The results also reinforce the possibility that P4 may act via other anti-cancer effects to reduce or inhibit primary nuclear receptors to effect suppression of stem cell properties. Increased expression of cancer stem cell-suppressing miRNAs may be an important mechanism mediating the efficacy of a progesterin-based ovarian cancer prevention strategy.

**#1260 Diet and exercise-induced weight maintenance may be preventing mammary tumor growth and metastatic burden by enhancing antitumor immunity and/or reducing protumorigenic factors.** William J. Turbitt, Yitong Xu, Andrea M. Mastro, Connie J. Rogers. The Pennsylvania State University, University Park, PA.

Two lifestyle factors that increase cancer risk and progression are weight gain and sedentary behavior. Possible mechanisms underlying this relation include changes in metabolic, inflammatory, and immune mediators. Few studies have examined the effect of body weight and exercise on the efficacy of immunotherapeutic strategies. An emerging immunotherapeutic strategy is PD-1 checkpoint blockade, which selectively targets the membrane protein programmed death-1 (PD-1) on T cells to promote a sustained antitumor effector response. Thus, the goal of the current study was to determine if preventing weight gain through diet (10% reduction in calories) and exercise (voluntary running wheel activity) will improve the response to the dual administration of a whole tumor cell vaccine and PD-1 checkpoint blockade. Female BALB/c mice were randomized to sedentary, weight gain (WG) or exercising, weight maintenance (WM) groups (n=32/group). After 8 weeks, all mice were orthotopically injected with 5x106 luciferase-transfected 4T1.2 cells into the fourth mammary fat pad and continued on their intervention for 35 days. After injection, WG and WM mice were randomized into vaccination (VAX) or vehicle (VEH) groups, and 1x106 irradiated 4T1.2 cells or HBSS vehicle control, respectively, was administered at day 7 post-tumor injection. Mice were further randomized (n=8/group) to PD-1 treatment or PD-1 one-week drug washout and PD-1 post-tumor injection. All WM groups, regardless of immunotherapy intervention, weighed significantly less than WG groups over the course of the study (p<0.001). Mean tumor volume (p<0.001) and tumor weight at sacrifice (p=0.076) were significantly lower with PD-1 treatment in the WG but not WM groups. Furthermore, metastatic burden in the lung (p=0.032) and the number of splenic myeloid-derived suppressor cells (p=0.035) were reduced with VAX+PD-1 treatment in the WG but not WM groups. Thus, the combination of VAX+PD1 was effective at reducing primary tumor growth and metastatic burden and improving immune outcomes only in mice that gained weight over the course of the study. However, diet and exercise alone was effective in reducing tumor growth and metastatic burden. The lack of responsiveness to vaccination with PD-1+PD1 treatment in WM mice suggests that WM achieved through diet and exercise may be enhancing antitumor immunity and/or reducing protumorigenic factors (i.e. similar mechanisms mediated by vaccination + anti-PD-1 therapy). These data demonstrate that preventing weight gain through diet and exercise may be an important recommendation to maintain prolonged antitumor effector responses and improve clinical outcomes.

**#1261 Lipid-peroxidation derived DNA damage is prevented in obesity-related hepatocarcinogenesis through CD4+ mediated apoptosis in the liver.** Rebecca Wang, Tony Hsiao, Xin Wang, Christian Trescher, Cong Song, Ning Ma, Marcin Dyba, Fung-Lung Chung. Georgetown UNIV., Washington, DC.

Using a C57BL/6J bioassay, we assessed whether formation of a lipid-peroxidation (LPO)-derived DNA adduct would induce mutations during the progression of obesity-induced hepatocarcinogenesis and if its formation could be prevented using a diet infused with Theaphenon E, a mixture of polyphenols extracted from green tea. Obesity is now the leading risk factor for the development of hepatocellular carcinoma in the United States. We have conducted a life-time C57BL/6J tumor bioassay to assess the role of obesity in the formation of 2,3-epoxy-1,4-nonenal-DNA adduct, a promutagenic DNA adduct formed endogenously by LPO. Previously, we have shown that LPO-DNA adduct formation is consistent with LPO in the early stages of liver disease and suggesting that it may be a source of endogenous DNA damage in hepatocarcinogenesis. Tea polyphenols, predominately EGCG, are antioxidants that have been shown to prevent obesity and tumorigenesis. For the bioassay, mice were fed either a high fat, low fat, or high fat 2% TE diet starting at 4 weeks of age. Tissue and blood were collected over the course of 80 weeks at 11 death times (1912 deaths). To evaluate the role of obesity, obesity, and obese diabetes, demonstrating that its formation is consistent with LPO in the early stages of liver disease and suggesting that it may be a source of endogenous DNA damage in hepatocarcinogenesis. The use of polyphenols, predominantly EGCG, are antioxidants that have been shown to prevent obesity and tumorigenesis. For the bioassay, mice were fed either a high fat, low fat, or high fat 2% TE diet starting at 4 weeks of age. Tissue and blood were collected over the course of 80 weeks at 11 death times (1912 deaths). In contrast to the high-fat and low-fat diet mice, the TE treated mice maintained a healthy body weight, liver to body weight ratio and low levels of AST and ALT enzymes which are elevated in liver disease. Additionally, the health of the liver appears to be maintained through increased CD4+ mediated apoptosis in TE treated mice as demonstrated through decreased Ki67 expression and TUNEL staining showing increased apoptosis in TE treated mice livers in conjunction with increased CD4+ cells. The CD4+ immune response may, therefore, be targeting cells with mutagenic potential. Similar to the trend indicated by the clinical samples, during hepatocarcinogen-
Aspirin inhibits the carcinogenesis of esophageal squamous cell carcinoma and enhances its responses to cisplatin. Zhiheng Zou,1 Hongjun Fan,2 Xinying Yu,3 Xiying Fan,1 Shuming Yu,1 Jingping Guo,1 Wei Jiang,1 Shih-Hsun Lu.2 Cancer Institute and Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China.

Esophageal squamous cell carcinoma (ESCC) is one of the most lethal malignancies. Over 70% of ESCC cases occur in China. Unfortunately, the treatment of ESCC has hardly been improved all these years. Several studies suggested that aspirin (ASA) might decrease the risk of ESCC and prolonged the survival of patients with ESCC. However, it is unclear if ASA could prevent ESCC and/or enhance ESCC treatment by chemotherapy. In this study, a N-nitroso-N-methylbenzylamine (NMBzA) - induced ESCC model was employed to prove that aspirin could prevent the growth of esophageal tumor. F344 rats were treated with NMBzA by subcutaneous injection with or without ASA in drinking water (2mg/ml). After 35 week ASA-NMBzA or NMBzA alone treatment, rats were killed and esophageal tumor development were examined. The results showed that F344 rats treated with NMBzA and receiving a daily intake of ASA developed much less tumors than F344 rats treated with NMBzA alone both in amount and in size (Tumor count: 2.40 ± 1.57 vs 10.85 ± 3.86, P < 0.001; Tumor volume: 11.10 ± 13.88 mm³ vs 70.79 ± 41.65 mm³, P < 0.001). Immunohistochemical analysis indicated that a higher rate of apoptosis was observed in the basal layer of esophageal epithelium in ASA-NMBzA treated rats than in NMBzA alone treated rats. These results indicated that ASA prevented development of esophageal tumors in rats induced by NMBzA. Moreover, using in vitro human ESCC cell culture and in vivo xenograft models, we showed that ASA has strong beneficial effects on inhibition of ESCC cell proliferation and colony formation, reduction of ESCC cancer stem cells and enhancement of ESCC cell cytotoxicity induced by cisplatin treatment. Biochemical analysis revealed that these effects of ASA on human ESCC cells in vitro and in vivo were due to inhibiting the repairing of DNA damage, decreasing the efflux activity and ALDH1 activity of the tumor cells, and blockades of PI3K/Akt pathway. Thus, our results demonstrated a positive role of aspirin in the prevention and treatment of ESCC.

Andrographolide inhibits prostate cancer by suppressing cytokine involved in promoting epithelial to mesenchymal transition. Hina Mir,1 Neeraj Kapur,2 Guru Sonpavde,2 Shailesh Singh1.1 Morehouse School of Medicine, Atlanta, GA; 2UAB Comprehensive Cancer Center, Birmingham, AL.

Mesenchymal phenotypes are responsible for cancer progression and poor therapeutic response. Hence, approaches targeting mesenchymal phenotype or inhibiting epithelial to mesenchymal transition will significantly prevent cancer progression or improve therapeutic outcome. Using in vitro models and molecular approaches we have shown that Andrographolide (AG), a compound isolated from Andrographis paniculata, shows promising effect on mesenchymal cells. It disposes PCA cells towards epithelial phenotype by modulating E-cadherin, ZEB-1, SNAIL and TWIST. In addition to these, significant inhibition in IL-6, which is known to be a poor prognostic maker, was observed in PCA cells treated with AG compared to untreated cells. This reduction in IL-6 could be due to AG induced suppression of SOCS (Suppressor of cytokine signaling) molecules. Therefore, our data underscores the ability of AG to impede cancer growth by impacting EMT and rationalizes its application as a potent therapeutic agent.

Studying senescence in prostate of selenium treated rats undergoing carcinogen-induced, androgen-promoted prostate carcinogenesis. Kartick C. Pramanik,1 Michelle Schlacht,2 Maarten Bosland,2 Chang Jiang,1 Yibin Deng,3 Junxuan Lu1.1 Penn State University, College of Medicine, Hershey, PA; 2University of Illinois at Chicago, IL; 3The University of Minnesota Hormel Institute, MN.

Preclinical studies from us and others have shown that oral intake of next-generation selenium (Se) forms, especially methylseleninic acid (MSeA) and Se-methylselenocysteine (MSeC) inhibits mouse prostate carcinogenesis. Our recent study has shown that MSeA suppresses pten-deficient mouse high grade prostatic intraepithelial neoplasia progression to adenocarcinoma in association with superactivating p53-p21 mediated cellular senescence. To address the question whether the activation of senescence is induced in prostate carcinogenesis driven by different etiology, we sought to test the in vivo effect of different selenium forms fed to rats undergoing chemically induced, androgen promoted prostate carcinogenesis. Wistar-Unilever (WU) rats (10-12 wks) were sequentially treated with androgen receptor blocker flutamide for 21 days (10 mg/kg/day by gavage) and then treated with N-ethyl-N-nitroso urea (NENU) or NMBzA alone for 16 week. All treated rats were euthanized, blood, major organs and different prostate lobes were dissected and weighed. The body weight of carcinogen-exposed rats was significantly less than their non-carcinogen-exposed counterparts. However, total prostate complex weight was higher in carcinogen-treated groups than the non-carcinogen groups, consistent with MNU and extra-testicular androgen promoting prostate epithelial cell proliferation. There were no significant organ-to-body weight ratio differences of various prostate lobes in the different dietary groups, regardless of the carcinogen exposure status. In order to test the involvement of senescence in prostate of rats fed different Se diets, we have undertaken steps to optimize staining method for senescence-associated β-galactosidase (SA-β-gal) activity in different lobes of prostate frozen sections. Together, with whole slide imaging using an Aperio Digital Pathology Scanner for quantitation, we are afforded an approach for more objective interrogation of the role of senescence in this carcinogenesis model and chemoprevention by selenium compounds.
Metformin represses esophageal carcinogenesis in NMBzA-treated rat model through inhibiting AMPK/mTOR and Stat3 signaling pathways.

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Esophageal cancers are among the most aggressive tumor types because of its invasiveness and metastatic potential. Metformin is one of the most used diabetic drugs for the management of type 2 diabetes mellitus in the world. The role of metformin in prevention of the development and progression of a variety of human tumors has been studied. However, the detailed mechanisms have not yet been fully understood. In the present study, we investigated the effects of metformin on the suppression of esophageal carcinogenesis in a rat model, in which F344 rats were treated with N-nitrosoglutethimide (NMBzA 0.30 mg/kg s.c.) three times per week for 35 weeks to induce esophageal tumors. To monitor the effects of metformin in this model, one group of rats were administered with metformin (3 g/L) in the drinking water at the first NMBzA injection. Our results showed that although there was no significant difference in body weight in rats of different groups, rats treated with NMBzA and metformin together significantly reduced the tumor formation and tumor volume when compared with rats treated with NMBzA alone. Statistical analyses demonstrated that the tumor numbers was reduced in NMBzA-treated rats received metformin to an average of 1.85 ± 1.09 tumors per rat when compared with 10.85 ± 3.66 (P < 0.001) in rats without metformin, while the tumor volume was decreased from 70.79 ± 41.65 mm³ per rat without metformin administration to 8.64 ± 13.45 mm³ (P < 0.0001) with metformin administration. In addition, 7 out of 24 rats in the NMBzA-treated group died before week 35 but no rats died in the other groups. Furthermore, immunoblotting analysis indicated that p-AMPKα, p-mTOR (S2448), p-Stat3Y705, and Cyclin D1 protein levels significantly decreased, while p-AMPKαThr172 significantly increased in tumors obtained from rats treated with NMBzA and metformin when compared with tumors obtained from rats treated with NMBzA alone. Thus, our results indicated that metformin suppressed NMBzA-induced esophageal carcinogenesis via inhibition of the AMPK/mTOR and Stat3 signaling pathways. Together, our study suggested that metformin might have a potential use for treatment and prevention of esophageal cancer.

Emodin exerts its anticancer effect on colon cancer cells by inhibiting proliferation and inducing apoptosis.

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Despite state of the art diagnostic and therapeutic options offered in the clinics, colon cancer is still a major health care concern. Therapeutic outcome is often compromised due to late diagnosis and development of resistance against treatment options used to treat advanced disease. Hence, the need to explore new avenue to treat/preserve colon cancer more effectively still exist. In this study we have investigated potential impact of Emodin, an anthraquinone derived from plant (Rheum rhabarbarum) on colon cancer cell proliferation, survival and apoptosis. Our data show that emodin reduces the cell viability and inhibits proliferation of human colon cancer cell (COLO-205 and DLD-1) in a time-dependent manner. Furthermore, FACS analysis show higher percentage of apoptotic cells with Emodin treatment compared to untreated cells. Interestingly, pro-inflammatory and anti-inflammatory cytokines produced by colon cancer cells were modulated with Emodin treatment. Cancer cells often use these cytokines to achieve their goal to progress and escape the treatment. Our data implies Emodin could be a promising agent that could be used for chemoprevention as well as in therapeutic combination to improve efficacy of conventional therapy.

Bitter melon efficacy against human pancreatic cancer cells: possible involvement of cellular stemness and metabolism targets.

Deepanshi Dhar, Gagan Deep, Sushil Kumar, Chapla Agarwal, Nalini Sarkova, Michael Wempe, Komal Raina, Rajesh Agarwal.
Univ. of Colorado Denver School of Pharm and Allied Health.

Pancreatic cancer (PanC) has a dismal 5-year survival rate of <5%. Symp- tomless progression, late diagnosis and rising drug resistance to leading chemotherapy agents like gemcitabine, add to PanC severity. Hence, there is a critical requirement for identifying alternative novel non-toxic agents (dietary/non-dietary) aimed at effective management of PanC with minimal patient distress. Bitter melon (Momordica charantia), a dietary agent, is actively being investigated for its anti-cancer potential against a variety of malignancies, including our work in PanC. Recent studies by us have shown bitter melon juice (BMJ) efficacy against a panel of human PanC cell lines in culture and tumor xenografts. For mechanistic studies, here we evaluated the efficacy of BMJ against cancer stem cells (CSCs) pool in PanC cells. Results indicated that BMJ treatment (0.25-2% v/v) significantly decreased CSC enriched (CD44+/CD24- EpCAM+BMJ) PanC cell population. Successive immunofluorescence/immunohistochemical analysis of BMJ exposed cells, spheroids and MiaPaCa2 xenograft tumors showed a significant reduc- tion in the number of CSCs. BMJ treatment was found to be a possible important component to be targeted to control cancer growth and progression, we next assessed BMJ efficacy in that direction. Indeed, BMJ mediated its anti-PanC effect via activating the key metabolic regulator AMPK. Concomitantly, to determine whether BMJ-effects were nutrient/energy stress dependent, we next sought to determine the cellular energy status and major metabolic changes concomitant with BMJ-mediated effects. BMJ mediated elevation of MSCA1 (major glucose transporter overexpressed in PanC) were also suppressed after BMJ treatment. Collectively, these results suggest that BMJ targets PanC CSCs and cellular metabolism in its efficacy against this deadly malignancy (supported by CA195708).
EPIDEMIOLOGY: Genetic Variation (Non-GWAS) and Cancer Risk, Prognosis, or Mechanisms


Alpha-tocopherol is a highly biologically active form of vitamin E that has been inconsistently associated with prostate cancer risk in cohorts and supplementation trials. In order to further elucidate the association using a Mendelian randomization approach that avoids sources of bias common in observational research, we examined genetic variants related to vitamin E status, including those from GWAS analyses of circulating vitamin E, in relation to prostate cancer risk in the Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome (PRACTICAL) Consortium. The analysis included 38,868 cases and 25,210 controls from 35 participating cohorts and examined the association between 847 vitamin E-related single nucleotide polymorphisms (SNPs) in (or near) six genes and risk of prostate cancer. Logistic regression was used to estimate multi-analyzed case-control odds ratios (OR) and 95% confidence intervals (CI) for the per allele risk associations. Both genotyped and imputed data were examined, and results for the latter are presented based on the consistency between the two sets of findings. In addition to examining SNPs demonstrating nominal significance (alpha-error p < 0.05), a Bonferroni threshold of < 0.00006 was applied to adjust for multiple comparisons. We found 89 SNPs were nominally significantly associated with prostate cancer risk. This included SNPs involved in vitamin E transport (i.e., SEC14L2 and SCARB2) and metabolism (i.e., BUD13/ZNF259). The top three risk-associated SNPs (P < 0.001) included rs141696823 (OR = 0.59, P = 6.0x10^-5) in CYF4P8 (cytochrome P450 family 4B subfamily F member 8) on 19p13.12 which functions as a prostaglandin hydroxylase in the seminal vesicles, and rs1915379 (OR = 1.04, P = 0.00002) in CYF4P3 (cytochrome P450 family 4B subfamily F member 3) on 19p13.12 which oxidizes arachidonic acid and omega-hydroxyates tocopherol phytol side chains, and has been directly associated with higher circulating alpha-tocopherol concentration in response to vitamin E supplementation in the ATBC Study. The present Mendelian randomization analysis suggests genetic variants related to vitamin E metabolism, its role as an antioxidant, and its function in inflammatory processes, may be associated with prostate cancer risk.

#1271 Genetic variants in the 16q24.3 region of melanocortin-1-receptor (MC1R) and prostate cancer risk: A Mendelian randomization analysis of 39,000 cases and 25,000 controls. Stephanie J. Weinstein, Tracy M. Layne, Ijqi Huang, Eric Karlins, Stephen J. Chanock, Demetrius Alabanes, the PRACTICAL Consortium, National Cancer Inst., Bethesda, MD.

We previously reported that men with naturally red hair had a significantly lower risk of prostate cancer (HR = 0.46, 95% CI 0.24-0.89) compared with the most prevalent hair color, light brown (Br J Cancer 2013;109(3):747-750). The red hair phenotype is encoded by variants in the melanocortin-1-receptor (MC1R) gene, which directs melanocyte melanin synthesis, secretion and human pigmentation, may be associated with risk of prostate cancer.

#1272 Prostate cancer risk and vitamin A related genetic variants in the PRACTICAL Consortium. Shakira M. Nelson, 1 Tracy M. Layne, 1 Stephanie J. Weinstein, 2 Eric Karlins, 2 Stephen J. Chanock, 3 Demetrius Alabanes, 3 PRACTICAL Consortium, 4NCI, Rockville, MD; 5Cancer Genomics Research Laboratory, Rockville, MD.

Vitamin A compounds including retinol are thought to play a protective role in human carcinogenesis. However, recent studies have shown that men with higher serum retinol concentrations are at increased risk of both overall and aggressive prostate cancer, the most common cancer in men within developed populations. The biological mechanism underlying this association remains unclear. In the present investigation, we examined the associations between common genetic variants related to serum retinol status and prostate cancer risk in the Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome (PRACTICAL) Consortium. The analysis includes 38,868 cases and 25,210 controls from 35 participating PRACTICAL studies. A Mendelian randomization approach was used to examine the individual association between 135 vitamin A-related single nucleotide polymorphisms (SNPs) and risk of overall prostate cancer. Logistic regression was used to estimate the odds ratio (OR) and confidence interval (CI) for the per allele association between each SNP and risk. Associations were examined using both genotyped and imputed data, and results for the latter are presented based on a substantial similarity. In addition to examining SNPs demonstrating nominal significance (alpha < 0.05), a Bonferroni cut-off of < 0.0003 was used to adjust for multiple comparisons. Seven SNPs were found to be nominally significant at the p < 0.05 level; however, none remained significant after Bonferroni correction. Of these, five SNPs (rs799751, OR = 0.97; rs9963553, OR = 1.03; rs9967180, OR = 1.03; rs2167689, OR = 0.98; rs1941356, OR = 0.98) are located in or near three genes: transthyretin gene (TTR), trafficking protein particle complex 8 (TRAPPC8), and beta-1,4-galactosyltransferase 6 (B4GALT6). The carrier protein transthyretin is the only gene known to be related to retinol status based on prior GWAS analyses, while B4GALT6 encodes type II membrane-bound glycoproteins which may be used as a potential cancer biomarker. The other two SNPs, rs1377189 (OR = 0.96) and rs17053512 (OR = 0.97), are located in desmoglein 3 (DSCG), whose encoded protein plays a role in maintenance of tissue architecture which may be upregulated in malignancies. Our large-scale Mendelian randomization analysis reveals some evidence of a relatively weak association between genetic variants related to vitamin A status and prostate cancer risk. These associations should be further examined in aggressive and fatal cancers.

#1273 Pathway analysis of insulin-like growth factor candidate genes and risk of pediatric rhabdomyosarcoma. Libby Morimoto, 1 Xiaorong Shao, 1 Anand Chokkalingam, 1 Joseph Wiemels, 2 Xiaomei Ma, 2 Catherine Metayer 1 1University of California, Berkeley, Berkeley, CA; 2UCSF, San Francisco, CA; 3Yale University, New Haven, CT.

To evaluate and prioritize candidate genes involved in IGF signaling and risk of pediatric RMS, we selected 15 genes involved in IGF signaling and risk of pediatric RMS. Fifteen genes involved in the IGF pathway were selected: IGF1, IGF2, IGFIR, IGF2R, IGFBP1, IGFBP2, IGFBP3, IGFBP4, IGFBP5, IGFBP6, INS, INSR, IRS1, GH1, and GHHR. DNA extracted from the archived newborn blood spots of available RMS cases (n = 664) was genotyped on the Illumina OmniExpress Exome array (n = 964,043 SNPs); after stringent quality control (QC), a total of 633 RMS cases (360 embryonal RMS [eRMS] and 197 alveolar RMS [aRMS]) were available for analysis. A total of 815 controls genotyped on the Illumina OmniExpress array and 3,922 controls genotyped on the Affymetrix AXION LAT array (n = 801,830 SNPs) from the same base population of California children, with no evidence of RMS, were included in this analysis. After imputation to increase coverage of the genome and post-imputation QC to identify and remove platform effects, a total of 5,556,335 SNPs were available for analysis. Among NHW children, SNPs in IGF1 (rs10808689, P = 0.0031) and IGFIR (rs6203616, 327 Proceedings of the American Association for Cancer Research 1 Volume 58 1 April 2017


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p = 0.00074; rs62023648, p = 0.00076) were significantly associated with RMS risk after adjustment for multiple comparisons. Analyses stratified by histologic subtype showed that these associations were limited to eRMS, and of stronger magnitude. Among Hispanic children, 12 SNPs in IGFR1 (p-value range: 0.0007-0.0027) and 3 SNPs in IGFBP1 (p-values: 0.0016-0.0023) were significantly associated with RMS risk. The SNPs were located in intergenic and intronic areas of the genes, areas that may influence gene regulation. Our results support the hypothesis that genetic variation in IGFl signaling pathway modulate the risk of RMS, especially eRMS, among NHW and Hispanic children.

**#1274 Associations between genetic polymorphisms in genes related to estrogen metabolism and function and prostate cancer risk: results from the Prostate Cancer Prevention Trial.** Li Tang,1 Mary Platek,2 Song Yao,1 Cathie Till,3 Phyllis Goodman,2 Catherine M. Tangan,4 Yue Wu,5 Elizabeth A. Platz,5 Marian L. Neuhouse,6 Frank Z. Stanczuk,7 Juergen K. Reichardt,8 Regina M. Santella,9 Ann Hsing,10 William D. Figg,11 Scott M. Lippman,12 Ian M. Thompson,13 Christine B. Ambrosone,1,1 Roswell Park Cancer Inst., Buffalo, NY; 2Fred Hutchinson Cancer Research Center, Seattle, WA; 3Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 4University of Southern California, Los Angeles, CA; 5University of Sydney, Sydney, Australia; 6Columbia University, New York, NY; 7Stanford University, Stanford, CA; 8National Cancer Institute, Baltimore, MD; 9UC San Diego Moores Cancer Center, San Diego, CA; 10University of Texas Health Science Center at San Antonio, San Antonio, TX.

Background: Substantial preclinical data corroborate the critical role of estrogen in prostate cancer development; however, epidemiological studies found no associations between circulating estrogen levels and prostate cancer risk. It was hypothesized that intraprostatic estrogen milieu may play a more important role than circulating estrogen in prostate carcinogenesis. Since it is difficult to obtain data on prostatic estrogen levels, we tested the hypothesis indirectly by investigating associations of prostate cancer risk with genetic variants of enzymes that are involved in estrogen synthesis, metabolism and function, and may affect intraprostatic estrogen milieu. Methods: A panel of 36 potentially functional single nucleotide polymorphisms (SNPs) in estrogen-related genes was assembled based on information obtained in the literature. After removing SNPs with call rate <95% (1 SNP) or minor allele frequency <3% (10 SNPs), a total of 25 SNPs in 13 genes (PGR, ESR1, ESR2, CYP17A1, HSD17B1, CYP19A1, CYP1A1, CYP1B1, COMT, UGT1A6, UGT1A10, UGT2B7, UGT2B15) were examined for associations with prostate cancer risk using data and DNA samples from 1617 cases and 1731 controls in the Prostate Cancer Prevention Trial (PCPT). Cases and controls were frequency-matched on age, treatment arm and family history. Logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) separately in the placebo and finasteride arms, adjusting for age, race and family history. Results were similar when restricting analysis to white men only. Results: Panels of SNPs that were significantly associated with prostate cancer risk were different according to treatment arm, showing rs1081132 in ESR1, rs700518 in CYP19A1, and rs4124874 in UGT1A6 in the placebo arm and rs2445765 in CYP19A1 and rs4860480 in UGT1A6 in the finasteride arm. When stratified within the arm, estrogen and androgen levels, significant associations were only observed in either the high or low category of serum hormone levels; no SNPs were significantly associated with prostate cancer risk in both categories. CYP19A1 was the only gene harboring SNPs that were significantly associated with prostate cancer risk in both the placebo (rs700518 and finasteride arm (rs2445765). In haplotype analysis using all three CYP19A1 SNPs genotyped in the study (rs700518, rs2445765 and rs700519), compared with non-risk haplotype (GCC), certain CYP19A1 haplotypes were significantly associated with increased prostate cancer risk in both arms. Conclusion: Associations between prostate cancer risk and SNPs in genes involved in estrogen metabolism and function and the specific marker showed by other factors such as finasteride treatment or circulating hormone levels. Supported by grant U01CA73429, SUM1CA18288 and P01CA180964 from the NCI.

**#1275 Genetic variations in cancer-related significantly mutated genes and lung cancer susceptibility.** Liren Zhang,1 Yanwei Zhang,1 Rong Li,2 David W. Chang,1 Yuanqing Ye,1 John D. Minna,3 Jack A. Roth,3 Baohui Han,4 XiFeng Wu,5 UT MD Anderson Cancer Center, Houston, TX; 6University of Texas Southwestern Medical Center, Dallas, TX; 7Shanghai Chest Hospital, Shanghai; 8Fudan University School of Medicine, Shanghai, China.

Cancer initiation and development are driven by key mutations in driver genes. Applying high-throughput sequencing technologies and bioinformatics analyses, The Cancer Genome Atlas (TCGA) project has recently identified pan-}

els of genetic mutations that contributed to or associated with the etiology of various cancers. However, there are few studies investigating the germline genetic variations in these highly mutated genes and lung cancer susceptibility. In this multi-phase study, we aimed to comprehensively evaluate the 1655 tagSNPs located in the 127 significantly mutated genes (SMGs) identified by TCGA, and their association with lung cancer risk. The SNPs were located in intergenic and intronic areas of the genes, areas that may influence gene regulation. Our results support the hypothesis that genetic variation in IGFl signaling pathway modulate the risk of RMS, especially eRMS, among NHW and Hispanic children.

**#1276 Gene-environment interaction relevant to estrogen and risk of breast cancer.** JooYoung Park,1 Ji-Yeob Choi,2 Seokang Chung,1 Nan Song,3 Sue K Park,4 Wonsik Han,5 Dong-Young Noh,6 Sei-Hyun Ahn,6 Mi-Kyung Kim,6 Keun-Young Yoo,7 Wei Zheng,7 Daehhe Kang,8 Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, Republic of Korea; 2Cancer Research Institution, Seoul National University College of Medicine, Seoul, Republic of Korea; 3Department of Preventive Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea; 4Department of Surgery, Seoul National University College of Medicine, Seoul, Republic of Korea; 5Department of Surgery, University of Ulsan College of Medicine and ASAN Medical Center, Seoul, Republic of Korea; 6Division of Cancer Epidemiology and Management, National Cancer Center, Goyang-si, Gyonggi-do, Republic of Korea; 7Department of Medicine, Division of Epidemiology, Vanderbilt Epidemiology Center, Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, TN.

Introduction: Many established reproductive risk factors for breast cancer are mediated by hormonal mechanisms, and most parts are involved in estrogens. In addition, estrogen levels controlled by genetic factors might be a sort of determinants to breast cancer risk involving estrogen metabolism. This study aims to examine gene and environment interaction (GxE) between candidate genes which are involved in estrogen metabolism and environmental factors which are related to estrogen exposure. Methods: GxE analyses were conducted in the Korean 1,970 breast cancer cases and 2,052 controls which were recruited in the Seoul Breast Cancer Study (SEBCS), a multicenter case control study. Among the candidate genes involved in estrogen metabolism were selected from couple of databases and 11,555 SNPs (2,472 typed SNPs and 9,083 imputed SNPs) from the range of candidate genes were included in GxE analyses with 8 established environmental factors. Statistical analyses were performed by using GxEscan (ver. Beta 0.4.0. http://biostats.usc.edu/software) for GxE test. Results: There were three significant interaction between 11,555 SNPs and 8 environmental factors. Firstly, interaction rs851998 nearby ER1 with height was shown in the GE2df model (p=2df=1.1x10-4) This SNP had protective effect marginally (ORG height ≥160cm: 0.82, 95%CI: 0.75-0.90, p=1.09x10-4). Cumulative analysis of the 11SNPsshowed consistently elevated risk with decreasing number of cases and further protective effect for women whose height is smaller than 160cm (ORG height <160cm: 0.78, 95% CI: 0.70-0.87, p=1.09x10-4). Second interaction was shown between rs13035764 of NCOA1 and age at menarche in the GE2df model (p=2df=1.2x10-3). This SNP also had protective effect marginally (ORG = 0.85, 95% CI: 0.77-0.97) and more protective effect when age at menarche is 14 years and over (ORG = 0.82, 95% CI: 0.73-0.97, p=3.6x10-4). Lastly, there was marginally significant interaction between rs4140979 of FSHR and age at first full term pregnancy (FFTP) in the EDGxE model. Although this SNP had no marginal effect, cumulative interaction was shown with FFTP (ORG FFTP >27 years: 0.88, 95% CI: 0.78-1.00, p=0.0482; ORG FFTP ≤27 years: 1.30, 95% CI: 1.10-1.53, p=0.002). Conclusion: Three significant interactions were identified by focusing two step methods. Replication should be performed in the independent and larger population due to statistical limitation.
#1277 Interaction of insulin-like growth factor-I and insulin resistance-related genetic variants with obesity and lifestyle factors on postmenopausal breast cancer risk. Su Yong Jung,1 Gloria Ho,2 Thomas Rohan,1 Howard Strickler,3 Jennifer Bea,1 Jeanette Papp,4 Eric Sobel,5 Zuo-Feng Zhang,6 Carolyn Daldal,1 1University of California, Los Angeles, Los Angeles, CA; 2Hofstra Northwell School of Medicine, Feinstein Institute for Medical Research, Great Neck, NY, 3Albert Einstein College of Medicine, Bronx, NY; 4University of Arizona Cancer Center, Tucson, AZ; 5David Geffen School of Medicine, University of California, Los Angeles, CA; 6David Geffen School of Medicine, University of California, Los Angeles, CA.

Objective: Genetic variants and traits in metabolic signaling pathways may interact with obesity, physical activity, and exogenous estrogen (E), influencing postmenopausal breast cancer risk, but these inter-related pathways are incompletely understood. Stratifying via obesity and lifestyle modifiers, we evaluated the effects of insulin-like growth factor-I (IGF-I)/insulin resistance (IR)–related traits on breast cancer risk as a mediator or influencing factor in this case–cohort study. Method: Using 75 single-nucleotide polymorphisms (SNPs) in genes related to IGF-1/IR traits and signaling pathways, and data from 1,003 postmenopausal women in Women's Health Initiative Observation ancillary studies, we assessed the effects of IGF-1/IR-traits (fasting total and free IGF-I, IGF binding protein-3, glucose, insulin, and homeostatic model assessment–insulin resistance) by proportional change estimation. Using traits as mediators of the IGF-1/IR–traits/cancer relationship, we partitioned the total effect of these genetic variants on cancer risk into two putative mechanisms: 1) indirect (mediated by traits) and 2) direct (through pathways other than traits). For the effects of IGF-1/IR traits on IGF-1/IR signaling pathways–relevant genetic variants–cancer risk, we used the same algorithm as that for mediator, but interpreted as an influential factor. Results: Seven SNPs in IGF-I and INS genes were associated with breast cancer risk. These associations differed between non-obese/active and obese/inactive women and between exogenous E nonusers and users. The mediation effects of IGF-1/IR traits on these SNPs–cancer relationship differed between strata, but only roughly 35% of the cancer risk due to the SNPs was mediated by IGF-1/IR traits. Similarly, carriers of 20 SNPs in PIK3R1, AKT1/2, and MAPK1 genes (signaling pathways–related genetic variants) had different associations with breast cancer between strata, and the proportion of the SNP–cancer relationship explained by IGF-1/IR traits varied 45–50% between the strata. Conclusions: Our findings suggest that IGF-1/IR genetic variants interact with obesity and lifestyle factors, altering cancer risk partially through pathways other than IGF-1/IR traits.

#1278 Effect modifications of vitamin D receptor common polymorphisms association with prostate cancer by serum vitamin D related behavioral and biological factors. Ken Batala,1 Adam B. Murphy,2 Ebony Shah,3 Chil-edearian,1 Rick A. Kittles4 1University of Arizona, Tucson, AZ; 2Northwestern University, Chicago, IL; 3Carney Hospital-Steward Health System, Dorchester, MA.

Background: Several roles have been proposed for vitamin D and the vitamin D receptor (VDR) in prostate cancer (PCa) pathogenesis and progression. However, previous genetic epidemiologic studies have provided limited support linking VDR polymorphisms to PCa due to complexity of vitamin D metabolism and potential gene and environmental interactions. In this study, we investigated the association of VDR genotypes with PCa incidence and aggressiveness in African Americans (AAs) and European Americans (EAs) and if the associations were modified by behavioral and biological factors that influence serum vitamin D levels. Methods: The total of 810 AAs and 487 EAs from Chicago, IL and Washington, D.C. were included in this study. Seven single-nucleotide polymorphisms (SNPs) in and around the VDR gene and 105 ancestry informative markers were genotyped. We performed logistic regression analyses adjusting for relevant variables. Results: In EAs, TaqI (rs73136) and BsmI (rs1544410) minor alleles revealed a protective effect against PCa, while in AAs, FokI (rs2228570) was associated with overall PCa risk (OR = 0.74, 95% CI: 0.57–0.96). The heterogeneous associations observed in our study could be due to difference in prevalence of vitamin D deficiency between AAs and EAs and effect modifications by behavioral and biological factors that affect serum vitamin D levels. Therefore, we investigated if behavioral and biological factors that influence serum vitamin D modified the associations between VDR polymorphisms and PCa, and we found evidence suggesting that these behavioral and biological factors modify the effect of VDR on PCa. In AAs, we observed statistically significant interaction between two VDR polymorphisms, FokI and TaqI, and vitamin D intake (PInteraction = 0.01 and PInteraction = 0.03 respectively). We also observed evidence of interactions between VDR gene variants and other behavioral and biological factors that lower serum vitamin D levels in both AAs and EAs. In AAs, calcium intake and skin pigmentation may modify the effects of VDR on PCa. In EAs, BMI may modify the effect of VDR. Conclusions: Although a larger sample size is necessary to confirm the observation, we demonstrated that vitamin D related behavioral and biological factors modify the effect of VDR genotypes on PCa. Impact: The VDR gene is involved in PCa pathogenesis and progression, and polymorphisms in VDR gene and vitamin D are likely to alter the function of the gene.

#1279 A polymorphism of VEGF -2489C/T is associated with prostate cancer susceptibility in Mexicans. Abril Martínez-Rizo,1 Xavi Casillas-Rangel,1 Cuerpo Académico Investigación Bioquímica (UAN-CA-266), Hilda Andrade-Madrid,2 Veronica Benites-Godínez,3 Nallhely Rodríguez-Ocampo5, 1Autonomous University of Nayariit, Tepic, Mexico, 3Mexican Social Security Institute, Delegation Nayariit, Tepic, Mexico.

Introduction: Prostate cancer (CaP) encompasses a range of malignant prostate tissue dysplasias that primarily affects men over 50 years, is the second leading cause of cancer mortality in males. VEGF expression is associated with tumor progression and metastasis in patients with CaP. The SNP -1154 G/A is associated with the severity of CaP populations in Tunisia and the United Kingdom while -634 G/C alone has been associated in Tunisian population. It is unknown whether these polymorphisms could serve as biomarkers in the Mexican population. Aim: To associate VEGF polymorphisms -1154 G/A and - 634 G/C with the severity of prostate cancer in patients of western Mexico. Materials and methods: The gDNA was analyzed a total of 525 men of whom 301 are presented HBP and 224 patients with clinical diagnosis of prostate cancer. For association they were subclassified high above high and low grade (≤6) following the Gleason scale. Genotyping was performed by Real Time PCR using TaqMan probes system. Results: When we compare the allelic frequencies in the control group, they are in Hardy-Weinberg equilibrium. However, there is no significant association of the disease with polymorphisms or severity or progression was found. Conclusions: There is no association between prostate cancer and genetic polymorphisms at -634 and -1154 position of the promoter region of VEGF.

#1280 Functional characterization of prostate cancer risk loci by SNPs-seq and STARR-seq. Peng Zhang,1 Jing Zhu,2 Sufyan Suleman,3 Yong-Chen Guo,4 Mei-Jun Du,5 Li-Dong Wang,6 Gong-Hong Wei,1 Liang Wang1,2, 1the First Affiliated Hospital of Zhengzhou University, Zhengzhou, China; 2Harbin Medical University, Harbin, China; 3University of Oulu, Oulu, Finland; 4Winer Medical School, Irving, China; 5Medical College of Wisconsin, Milwaukee, WI.

Background. By SNP genotyping and RNA sequencing of 471 normal prostate samples, we recently created a prostate tissue-based eQTL dataset and identified significant eQTL signals at 51 prostate cancer risk loci. To functionally characterize these risk SNPs, we developed a massively parallel sequencing technology (SNPs-seq) for direct allele-specific double-strand oligos and performed DNA-protein binding assays. We combined this technology (called SNPs-seq) with another high throughput assay (called STARR-seq) to screen the risk loci with significant prostate-specific eQTL signals. Methods. To select candidate functional SNPs in eQTL regions, we took advantage of existing epigenomic datasets and available tools including ENCODE, HaploReg, and Regulome. For all selected SNPs, we first made allele-specific double-strand oligos and performed DNA-protein binding assays. We then performed sequencing analysis on the protein-bound DNA oligos and determined allele-specific protein binding differences. To evaluate reproducibility of SNPs-seq, we performed each assay in duplicates. We cloned SNPs-seq screened SNP regions showing allele-specific protein binding differences into the STARR-seq vector to further determine allele-specific enhancer activities. Finally, we performed EMSA and luciferase reporter assays to validate a set of promising candidate SNPs. Results. From 51 risk loci with strong eQTL signals, we selected 374 SNPs with strong indication of regulatory potential, as evidenced by overlapping with epigenomic marks. When comparing technical duplicates, sequence read counts from the SNPs-seq showed significant correlation with r² > 0.99. By normalizing input controls, we found 101 of the 374 SNPs association with significant allele specific protein binding differences (P  ≤ 0.05 for Mediation testing difference between variant and reference alleles). Interestingly, three published functional SNPs (rs12769019, rs10993994, and rs4907792) were also among the significant SNPs, validating SNPs-seq as functional SNP screening tool. To further validate the candidate SNPs from SNPs-seq, we applied STARR-seq and tested the 101 SNPs-containing sequences (371-680bp) in LNCaP cell line under androgen treatment and overexpression assays revealed 11 SNPs that not only demonstrated enhancer/repressor activity but also functioned with allele differences. EMSA and luciferase reporter assays confirmed 6 SNPs with allele-dependent enhancer/repressor activity. Conclusions. We developed a high throughput sequencing-based technology to screen large number candidate SNPs for their allelic
protein binding differences. The SNPs-seq coupled with STAR-seq will provide a powerful strategy for functionally characterizing risk loci in prostate cancer and other common diseases. Further understanding genetic role of prostate cancer etiology may facilitate the translation of population-based discovery into biological mechanisms and eventually benefit clinical practice.

**#1281** Germline mutations in NBN conferring DNA damage response defects are found in patients with multiple cancer types. Sabine Topka, Michael P. Walsh, Ann Maria, Annie Lincoln, Diana Mandelker, Liying Zhang, Marc Ladanyi, Michael F. Berger, Mark E. Robson, Joseph Vija, Kenneth Offit. *Memorial Sloan Kettering Cancer Ctr., New York, NY.*

Nibrin, the protein encoded by the NBN gene forms a complex with Mre11 and Rad51 (MRN complex) that is crucial for DNA damage repair. Mutations in NBN are found in >90% of patients with Niemijegen breakage syndrome (NBS), an autosomal recessive disorder characterized by growth retardation, microcephaly, radiosensitivity, immunodeficiency and increased cancer risk. Most of NBS patients harbor the common founder mutation c.657delE5 that leads to expression of a hypomorphic 70kDa C-terminal fragment produced by alternative translation initiation. Cell lines derived from these patients show increased sensitivity to DNA-damaging agents, chromosome instability, and abnormal cell cycle checkpoint function. Several studies have addressed cancer risk in individuals with germline NBN mutations, showing increased cancer risk for individuals harboring the c.657delE5 founder mutation and for carriers of the R215W missense mutation across multiple cancer types. For other NBN mutations conflicting reports exist as to their association with cancer risk. Here, we report newly identified NBN germline frameshift and truncating mutations in patients with multiple cancer types, including prostate, lung, breast cancer, acute lymphocytic leukemia, and chronic lymphocytic leukemia. Modeling these mutations in an NBN-deficient cellular background showed expression of a novel C-terminal truncated fragment that can bind to Mre11. Cells expressing these mutant proteins display attenuated DNA damage repair function and decreased overall survival following induction of DNA damage. Impaired Chk2 phosphorylation was also observed, indicating cell cycle checkpoint deficiencies. Thus, the NBN germline mutations identified here could contribute to genomic instability predisposing to tumorigenesis. Further in vitro studies with these and additional germline mutations occurring in cancer patients are ongoing, in order to better understand the role of this pathway of DNA damage repair in susceptibility to malignancies.

**#1282** Analysis of missense variants in BRCA1 BRCT domains. Giuliano Di Pietro,1 Vanessa Camara Fernandes,2 Kwabena Amankwah,3 Carla Shields,4 Volha A. Golubeva,3 Carly Harro,3 Marcelo Alex Carvalho,4 Alvaro N. Mon-teiro,1 1Federal University of Sergipe, Sao Cristovao, Brazil; 2Instituto Nacional de Cancer. Rio de Janeiro, Rio de Janeiro, Brazil, Brazil; 3Moffitt Cancer Center, Tampa, FL; 4Instituto do Rio de Janeiro, Rio de Janeiro, Brazil.

Currently, only ¼ (133/600) of documented single nucleotide variation leading to missense changes in the BRCA1 gene have been characterized as pathogenic or non-pathogenic (IARC Class 1 & 2 & 3 & 4 & 5). The remaining 96% of documented single nucleotide polymorphisms (SNPs) within BRCA1 and BRCA2 are germline variants of unknown clinical significance. By studying the breast cancer microenvironment, we can specifically address novel aspects of cancer development and progression at the molecular level. Chemokines are small signaling molecules that are an important component of this microenvironment, as they shape cellular composition of this space through recruitment of immune cells, among other functions. DARC, the Duffy Antigen Receptor for Chemokines (DARC/ACKR1) that can bind different classes of chemokines. It is a non-signaling receptor that mainly acts as a regulator of chemokine homeostasis by removing them from circulation to recruit appropriate immune cell types. The purpose of this study is to determine how population-private variants of the DARC/ACKR1 gene across global population render distinct function and define aspects of human diversity in chemokine responses. A well-studied example of this would be the Duffy-null allele. This allele carries a mutation that is population-specific to recent decedents of Sub-Saharan Africans, and removes expression of DARC on red blood cells. Loss of expression on red blood cells presumably causes these “Duffy-Null” individuals to lose the ability to sequester chemokines, therefore losing homo- static balance of these molecules in circulation. To identify additional mutations, we completed in silico analysis of minor allele frequencies across the gene in the 1000 Genomes Project data, and identified several other putative functional mutations that likely result in additional gene variants, specific to geographic ancestry groups and likely under similar selection as the Duffy null allele. We have prioritized our initial functional study to include mutations in the regulatory regions of the gene, as these mutations can change how and where the gene is expressed. Future work is focused on investigating these functional variants in situ, using human tissue and human breast cell lines, by isolating and re-engineering ancestry-specific variants in these cell lines. By identifying and determining functional outcomes of these population-specific mutation, we can observe differences in immune responses across these global populations, further defining the role that DARC/ACKR1 plays in the breast tumor microenvi-roment.


Tumorigenesis in sporadic cancers is mainly driven by somatic genetic alterations such as driver mutations in protein coding genes or chromosomal changes comprising deletions, amplifications or translocations resulting in loss of tumor suppressor proteins, gain of oncogenic proteins or expression of aberrant fusion proteins, respectively. Some cancers lack such somatic changes, but are addicted to expression of certain genes for their sustained proliferation and survival. There is evidence of such oncogenic addiction to LIM domain only 1 (LMO1) expression in neuroblastoma (NB). Genome-wide association studies (GWAS) have identified robust associations between germline single-nucleotide polymorphisms (SNPs) within LMO1 and NB susceptibility with the causal SNP being rs2168101. Investigation of the mechanism of NB dependency on LMO1 showed that LMO1 expression in NB cells is regulated by rs2168101, which resides within a highly conserved tissue-specific super enhancer in LMO1 intron 1 and drives LMO1 expression through GATA3 transcription factor binding. This makes LMO1-dependent NB a unique example of a monogenic cancer with genetic variation. Numerous GWAS have identified significant associations between germline SNPs in the non-coding genome and cancer risk or outcomes. To identify additional examples of regulatory SNPs as cancer drivers, we merged published genome-wide significant associations from cancer GWAS with genome regulatory data from ENCODE (Encyclopedia of DNA Elements; Nature, 2012 Sep 6; 489 (7414): 57-74) and searched for clusters of cancer associated SNPs that resided within gene regulatory elements. Gene regulatory elements were defined as those marked by active epigenetic features and chromatin accessibility in cancer cell lines. Of the ~1,600 uniquely genome-wide significant SNPs from cancer GWAS with regulatory evidence, we identified 16 clusters of 3 or more putative regulatory SNPs near 28 genes. These clusters were particularly enriched within ovarian cancer associated loci. Mechanistic studies such as reporter assays and genome editing in relevant cell types are being considered to identify the causal SNPs from these clusters regulating gene expression and driving tumorigenesis, which in turn may lead us to new cancer targets.

**#1285** Analysis of esophageal cancer SNPs and gene expression to predict ESCC risk. Lilly A. Ding,1 Molly P. Ding,1 Xin Song,2 Lidong Wang,2 Liang Wang,3 None, Bethesda, MD; 2First Affiliated Hospital, Zhengzhou, China; 3Medical College of Wisconsin, Milwaukee, WI.

Esophageal cancer is one of most common cancers worldwide and its incidence has increased rapidly over the past decades. The majority of esophageal
cancers is the esophageal squamous cell carcinoma (ESCC), which is particularly prevalent in Asian and South African areas. Most cases of ESCC are diagnosed at advanced stages, with an overall 5-year survival rate of 10–20%. Currently, genome-wide association studies (GWAS) are used to identify high risk individuals for the development of ESCC. Many SNPs have been identified to associate with risk of ESCC. Further, the effect size of these SNPs and breast cancer. Further, the meta-analysis of individual studies is modestly suggesting that genetic variants may only account for a very small amount of genetic risk and heritability of ESCC. The combined effect of multi genetic variants with altered mRNA expression play critical roles in ESCC.

In this study, we used several existing bioinformatics databases (HaploReg, GTex Portal and cBio Portal). We first selected 450 potential ESCC risk SNPs from HaploReg and then defined which SNPs caused gene expression alterations in the esophagus through expression quantitative trait loci (eQTL). Nine SNPs in eight genes (MUC1, ALSC2R12, ADHB1, TMEM173, XBP1, PCLCE1, HEATR3 and SMG6) were identified as having altered mRNA expression. The altered mRNA expression of these seventeen genes are: MUC1, THBS3, GPAP1, EFNA1, SCAMP5, CASP8, CASP10, ALSC2R12, STRAD8, ADH4, TMEM173, CVCMT1, DNAJC18, DNAJC20, NOCL, HEATR3 and SRR. These altered gene expressions in the mucosal layer of the esophagus metabolize enzymes, repair DNA, and prevent cell death and inflammation, all of which may indicate that the existing altered esophageal mRNA levels plus the predisposition of normal esophageal epithelial cells lead to harmful environmental carcinogenic, thus attributing to ESCC development. In order to validate these gene alterations and then defined which SNPs caused gene expression alterations, we performed eQTL-qPCR analyses in the early screening of high risk individuals, genotyping of this group of SNPs with a prospective cohort (n = 5451) in a high risk area of ESCC will be tested in future studies.

#1286 Targeted deep sequencing of colorectal tumor tissues to study associations of tumor subtypes with germline genetic, lifestyle, and environmental risk factors. Syed H. Zaidi,1 Wei Sun,2 Jeroen Huhybe,3 Catherine S. Grasso,4 Quang Trinh,1 Charles Connolly,3 Amy French,2 Jasmyn Miu,5 Muris Giannakis,6 Eve Shimbro,7 Ivan Borozan,8 Michael J. Quast,9 Hermann Brenner,10 Daniel Buchanan,11 Peter Campbell,12 Andrew Chan,13 Jenny Chang-Claude,14 Vincent Ferretti,15 Charles Fuchs,15 Andrea Gsur,11 Marc Gunter,12 Tabitha Harrison,2 Michael Hoffmeister,7 Fuchs,5 Andrea Gsur,11 Marc Gunter,12 Tabitha Harrison,2 Michael Hoffmeister,7

According to the recognized role of telomere dysfunction as a cancer hallmark, it has motivated the development of the breast cancer risk associated with telomeres and breast cancer. Further, the Genome-Wide Association Study (GWAS) discovery of seven single nucleotide polymorphisms (SNPs) associated with telomere length (TL) enables the use of an aggregated genetic risk score (GRS) for TL. We hypothesized that a higher GRS (representing shorter TL) would be associated with increased risk of breast cancer, and that a strengthened association may exist between higher TL GRS and overall breast cancer risk. This study used a recent TL GRS constructed to sequence the coding regions of 190 significantly mutated genes identified from whole exome sequencing datasets generated by the Nurses’ Health Study and Health Professional’s Follow-up Study, and The Cancer Genome Atlas. The panel also covers coding regions of 15 genes with germline high penetration mutations in CRC, 54 regions to detect CRC-related copy number alterations (CNAs), and microsatellite and homopolymer repeat regions to identify defective DNA mismatch repair (MMR) genes. These panels also included targets identified in tumor biopsies, as F. nucleatum is thought to promote CRC carcinogenesis. Sequencing of the DNA libraries on Illumina HiSeq 2500 produced a mean coverage of greater than 500X for tumor DNA and 100X for normal DNA, with >85% of the bases covered at the target at 50X. So far, targeted sequencing of >1,500 DNA samples from CRC tumors and normal tissues has identified recurrent and novel somatic mutations, miRNA expression alterations, and higher TL GRS due to defective DNA mismatch repair or pathogenic mutations in the POLE gene. Targeted sequencing has also allowed quantification of the F. nucleatum DNA in tumor biopsies; the results were validated by a multiplex QPCR assay. At the AACR annual meeting, we will present targeted sequencing results generated from the first two GECCO-participating studies (n = 1,300 cases). These data will be valuable for future association testing of somatic mutations, CNAs, hypermutation status, and F. nucleatum with germline genetic variants, lifestyle, and environmental risk factors and survival. This large study will allow development of better strategies for diagnosis, treatment, and prevention of CRC.

#1287 Multigene panel testing and risk estimates in 10,233 ovarian cancer cases. Jenna Lilquist,1 Holly LaDuca,2 Eric Polley,3 Hermela Shimelis,4 Chunling Hu,5 Raymond Moore,6 Steven N. Hart,7 Fergus J. Couch,8 Jill Dolinsky,9 David E. Goldgar12.5. Mayo Clinic, Rochester, MN; 2Ambry Genetics, Aliso Viejo, CA; University of Utah School of Medicine; Huntsman Cancer Institute, Salt Lake City, UT; 6Baylor College of Medicine, Houston, TX; 7German Cancer Research Center, Heidelberg, Germany; 8CancerCare Society, Atlanta, GA; 10Massachusetts General Hospital, Boston, MA; 11Medical University of Vienna, Austria; 12International Agency for Research on Cancer, Lyon, France.

The Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO) in collaboration with the Colorectal Cancer Family Registry (CCFR) aims to identify germline genetic variants and environmental risk factors that impact colorectal cancer (CRC). Over 30 studies from North America, Europe, and Australia participate in GECCO. These studies have collected clinical, epidemiological, and survival data, as well as blood and tumor biospecimens, for over 80,000 CRC cases and controls. The current study aims to conduct targeted deep sequencing of tumors and matching normal DNA to identify recurrent and novel somatic and germline variants in 4,200 CRC cases. To achieve this goal, an AmpliSeq targeted sequencing panel of 1.12 Mb was constructed to sequence the coding regions of 190 significantly mutated genes identified from whole exome sequencing datasets generated by the Nurses’ Health Study and Health Professional’s Follow-up Study, and The Cancer Genome Atlas. The panel also covers coding regions of 15 genes with germline high penetration mutations in CRC, 54 regions to detect CRC-related copy number alterations (CNAs), and microsatellite and homopolymer repeat regions to identify defective DNA mismatch repair (MMR) genes. These panels also included targets identified in tumor biopsies, as F. nucleatum is thought to promote CRC carcinogenesis. Sequencing of the DNA libraries on Illumina HiSeq 2500 produced a mean coverage of greater than 500X for tumor DNA and 100X for normal DNA, with >85% of the bases covered at the target at 50X. So far, targeted sequencing of >1,500 DNA samples from CRC tumors and normal tissues has identified recurrent and novel somatic mutations, miRNA expression alterations, and higher TL GRS due to defective DNA mismatch repair or pathogenic mutations in the POLE gene. Targeted sequencing has also allowed quantification of the F. nucleatum DNA in tumor biopsies; the results were validated by a multiplex QPCR assay. At the AACR annual meeting, we will present targeted sequencing results generated from the first two GECCO-participating studies (n = 1,300 cases). These data will be valuable for future association testing of somatic mutations, CNAs, hypermutation status, and F. nucleatum with germline genetic variants, lifestyle, and environmental risk factors and survival. This large study will allow development of better strategies for diagnosis, treatment, and prevention of CRC.

#1288 Telomere length genetic risk score is associated with breast cancer risk. Laurie Grieshaber,1 Jean Wactawski-Wende,2 Rachael Hageman Blair,3 Lina Mu,4 Leah Preus,1 Jing Nie,1 Jiali Han,2 Jaymie R. Meliker,3 Thomas Rothenberg,14 Andrea Gsur,11 Marc Gunter,12 Tabitha Harrison,2 Michael Hoffmeister,7 Fuchs,5 Andrea Gsur,11 Marc Gunter,12 Tabitha Harrison,2 Michael Hoffmeister,7

Background: Genome-wide association studies (GWAS) have identified associations of telomere maintenance genes with breast cancer risk. This, coupled with the recognized role of telomere dysfunction as a cancer hallmark, has motivated the development of a breast cancer risk associated with telomeres and breast cancer. Further, the Genome-Wide Association Study (GWAS) discovery of seven single nucleotide polymorphisms (SNPs) associated with telomere length (TL) enables the use of an aggregated genetic risk score (GRS) for TL. We hypothesized that a higher GRS (representing shorter TL) would be associated with increased risk of breast cancer, and that a strengthened association may exist between higher TL GRS and overall breast cancer risk. Methods: In a race/ethnically diverse sample of invasive breast cancer cases (N = 1,108) and non-cases (N = 20,023) from the Women’s Health Initiative, we derived an unweighted TL GRS using seven previously identified TL-associated SNPs. Women were post-menopausal and the average age at enrollment was 63.9 years. We tested TL GRS associations with overall breast cancer risk and for estrogen receptor (ER), progesterone receptor (PR), and her2/neu (HER2) status-specific breast cancer risk using Cox proportional hazards models adjusted for age and race/ethnicity (in the entire sample) and age in strata of European American (EA; N = 9,796), African American (AA; N = 7,504), and Hispanic American (HA; N = 3,229) women. We also considered reproductive risk factors, family history of breast cancer, hormone therapy, tumor characteristics, BMI, physical activity, smoking, alcohol, and US region as potential confounders. Results: We observed a small but statistically significant association between shorter TL GRS (shorter TL) and reduced risk of overall breast cancer in the entire sample (HR = 0.96, 95% CI: 0.93-1.00). Results of race/ethnicity-stratified analyses for TL GRS and overall breast cancer risk were not significant. The TL GRS was independently associated with decreased risk of ER-negative, PR-positive, and HER2-positive breast cancer risk in the entire sample (HR = 0.82, 95% CI: 0.78-0.86).
further identified suggestive associations (P < 0.71-0.94, HR = 0.94, 95% CI: 0.89-0.99, and HR = 0.88, 95% CI: 0.79-0.90, respectively). Similarly, the TL GR was associated with a reduced risk of ER-negative breast cancer in AA (HR = 0.79, 95% CI: 0.66-0.97) and independently with PR-positive and HER2-positive breast cancer risk in HA (HR = 0.85, 95% CI: 0.75-0.97 and HR = 0.79, 95% CI: 0.58-0.99, respectively). Adjustment for other covariates did not alter the significant associations. O.K. Keku,11 Cathrine Hoyo,12 Andrew Berchuck,6 Paul Pharoah,13 Joellen M. Schilchian women.

morphism is associated with high-grade serous ovarian cancer in African Americans. UGT1A, 6302 SNPs in UGT2B, 410 SNPs in CYP3A4/5, and 824 SNPs in EGFR. Logistic regression was performed using an additive 1 degree of freedom model for genetic inheritance with adjustment for two principal components of ancestry to estimate odds ratios (OR) and 95% confidence intervals (CI). For each gene region, we applied a gene-specific Bonferroni-threshold for statistical significance of 5 x 10^-5 per number of SNPs examined for that gene. Based on this significance threshold, a statistically significant association with HGSOC was identified in the EGFR region for the imputed SNP, rs114972508 (per allele OR = 2.32, 95% CI: 1.58, 3.40 p = 1.6e-05, imputation R-squared = 0.89). We further identified suggestive associations (P < 1.0e-06) with EOC for 10 imputed SNPs located within the UGT2B4 gene. We did not replicate previous associations in the 282 SNPs examined in the VDR gene for EOC overall or among HGSOC. In summary, we identified statistically significant association for variants from the EGFR region, and suggestive evidence of association for variants within the UGT2B region in genetic association analyses of ovarian cancer in women of African Ancestry. Data from in vitro experiments suggest that EGFR transcription and proliferative function is suppressed via VDR binding. Thus, EGFR association with HGSOC may be a marker of VDR activity. UGT2B4 variants have not been previously explored in ovarian cancer but shown to be nominally associated with breast cancer in women of African ancestry. UGT2B4 enzymes, part of the phase II liver detoxification pathway, are important in the clearance of steroid hormones, bile acid and drug metabolism. Alterations in EGFR and UGT2B4 could perturb enzyme efficacy and proliferation in ovaries and impact susceptibility to ovarian cancer. Future studies will be needed to validate the associations of the imputed SNPs and to determine the impact of EGFR and UGT variants on cancer development.

#1290 Association of common genetic variants with TMPRSS2 ER fusion status in prostate cancer. Indu Khojaa,1 Lakshmi Ravindranathb,1 Denise Youngc,1 Amina Alf,2 Qiyuan Li,2 Albert Dobic,1 David McLeod,1 Inger L. Rosner,1 Isabel Sesterhemb,2 Matthew Freedman,3 Shiv Srivastava,1 Gyorgy Petrovics,1,4 CPDR/USUHS, Rockville, MD;5 WRNMMC/USUHS, Bethesda, MD;6 Xiaomen University, China;7 Dana-Farber Cancer Institute, Boston, MA.

Introduction and Objectives: Oncogenic activation of ERG resulting from prevalent gene fusions is present in two thirds of prostate cancer (CaP) patients of European Ancestry including Caucasian Americans (CA). Our laboratory and others have recently reported that major cancer driver genes, including ERG, show significant racial/ethnic differences in CaP with lower frequencies in African Americans. Such racial/ethnic differences may reflect, in part, differences in how germline molecular determinants associating with ERG status of CaP. Methods: Blood derived genomic DNA samples were prepared from 270 AA men and 129 CA men treated by radical prostatectomy at Walter Reed National Military Medical Center (WRNMMC). ERG status was determined in whole mounted prostate specimens by immune-histochemistry (IHC) for ERG protein expression as a surrogate for the TMPRSS2-ERG fusion. Blinded blood samples were genotyped for SNPs on the Illumina Golden Gate platform using Infinium Oncoarray, a 300K genome wide BeadChip kit from Illumina. Data analysis approaches included association analyses based on logistic regression, Principal Component Analysis (PCA) and Efficient Mixed-Mixed Model Association (EMMAX) analysis. Genotype imputation analysis is being performed by IMPUTE2 program. Results: After applying rigorous sample and SNP QC steps on the datasets, SNP genotyping analysis was performed in 321 patients with 478,299 SNPs. Logistic regression, principal component analysis by EIGENSTRAT and a variance component approach, EMMAX analysis were performed to account for population structure. By EMMAX we identified SNPs associated with ERG status. The SNPs most significantly (<10^-3) associated with ERG fusion status included rs6968333, an intron variant of Kruppel-like factor 17 (KLF17) and two SNPs (rs1889877, rs3798999) in the intron of adhesion G protein-coupled receptor B3 (ADGRB3). Fine-mapping of SNPs is under way by genotype imputation analysis (IMPUTE2) using the 1000 Genomes reference dataset, followed by independent validation. Conclusions: This study identified SNPs differentially associated with ERG status of CaP, a major driver oncogene in CaP. Although the biological significance as it relates to ERG status of CaP still needs to be determined, these SNPs, with independent validation, may help as markers in stratifying patients early (even before CaP is detected) for targeted prevention and treatment options.

#1291 Mutation analysis of endometrial cancer in a population-based study by targeted next-generation sequencing. Maxine Chen, Marta Crouse-Bou, Michael J. Downing, Evan L. Busch, Kimberley Glass, Jennifer Prescott, George L. Mutter, Immaculata De Vivo, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

Endometrial carcinoma (EC), a malignancy that arises from the epithelial lining of the uterus, is heterogeneous at histologic and molecular levels. Risk factors and outcomes also differ by type. Though prior studies characterized the genomic landscape of endometrial carcinoma, few integrated histologic, clinical, and prospectively collected epidemiologic data into the analysis. We collected formalin-fixed paraffin-embedded tumor tissue from women enrolled in the Nurses' Health Study who were diagnosed with EC between 1976 and 2012. We targeted 50 cancer-related genes for high-throughput sequencing to identify genetic variants in 37 ECs and 26 normal EC cases. Selection of the 50 genes was guided by Illumina's OncoArray, a 500K genome wide BeadChip kit from Illumina. Data were processed with GATK (v3.3.0) and variants were annotated with ANNOVAR v20140131 to determine gene ontology. The selected genes were chosen to capture germline association with ERG fusion status. The goal of this study is to identify germline molecular determinants associating with ERG status of CaP.

#1292 Germline genetic, molecular and environmental factors modulate APOBEC mutagenesis in human tumors. A. Rouf Banday, Ariunaa Bayanjar-
Prevalence and spectrum of germline rare variants in ABO blood type and cancer risk: preliminary findings from a phenotype-modulate APOBEC mutagenesis in human tumors. Methods: Isoform-specific exons can be exploited for risk prediction or cancer prevention is warranted. In addition, research to determine how ABO blood type and cancer phenomes were quantified with Odds Ratios (OR) and corresponding 95% Confidence Intervals (CI) from logistic regression in models adjusted for sex and stratified by race/ethnicity. Only analyses with at least 100 cases per strata were conducted. Results: Among 221,015 Non-Hispanic Caucasians, 37,841 Blacks, 7,714 Hispanic Caucasians, and 3,761 Asian subjects with ABO blood type available in linked EMR, we evaluated 56, 37, 4, and 3 general cancer phenotype codes, respectively. After applying Bonferroni corrections, ABO blood type was significantly associated with cancers of the pancreas, ovary, cervix, skin, and hematopoietic system. Caucasians with blood type O were less likely to have ovarian cancer (OR: 0.82, 95% CI: 0.73-0.91) and pancreatic cancer (OR: 0.83, 95% CI: 0.74-0.92), and more likely to have squamous cell or other skin cancer (OR: 1.08; 95% CI: 1.04-1.13) and myeloid leukemia (OR: 1.15, 95% CI: 1.06-1.25) than those with other blood types (A, B, or AB). Hispanic Caucasians with blood type O were less likely to have cervical cancer (OR:0.56, 95% CI: 0.38-0.82) than those with other blood types. No associations surpassed correction for multiple comparisons among Blacks or Asians. Conclusions: Our phenome approach confirmed known associations between blood type and risk of pancreatic and ovarian cancer, and adds to accumulating evidence supporting associations with skin cancer and leukemia. Our novel cervical cancer association among Hispanic Caucasians and other nominally significant findings, especially in understudied non-Caucasians, should be further evaluated in large and diverse populations. In addition, research to determine how ABO blood type may influence cancer development and progression, and if such associations can be exploited for risk prediction or cancer prevention is warranted.
Bayesian fine-mapping using summary data of 145,000 subjects refines common risk associations, discovers secondary signals and novel candidate genes for prostate cancer. Zofia Kote-Jarai,1 Tolking Dadavey,1 Ed Saund-ers,1 Paul Newcombe,2 Ezequiel Anokian,1 Daniel Leongamornlert,1 Ali Amin Al Olama,3 Christopher Haiman,3 Ros Eles,4 David Conti,4 The PRACTICAL/ELLIPSE Consortium.1 Inst. of Cancer Research, Sutton, United Kingdom;2 University of Cambridge, Cambridge, United Kingdom;3 University of Southern Cali-fornia, Los Angeles, CA.

Genome-wide association studies (GWAS) have identified more than 160 prostate cancer (PrCa) genetic risk loci, however these variants rarely point directly to the true underlying functional variant driving the association. In this fine-mapping study to narrow the credible causal variant set for 80 PrCa regions representing 89 original independent GWAS signals, we performed Bayesian variable selection in combination with functional annotation and quintile regression. We used imputed data for 83,511 PrCa cases and 62,283 controls investigat-ed with high-density genotyping arrays from the OncoArray, iCOGS and 5 previous GWAS studies from the PRACTICAL/ELLIPSE consortia. To facilitate fine-mapping from one-at-a-time SNP associations meta-analyzed over the consortia we first applied JAM, a novel Bayesian algorithm which searches multi-SNP models in summary data by imputing the correlation structure according to a reference panel. JAM provides inference on the number of independent signals, as well as the set of potential SNPs driving those signals. We utilized functional annotation and eQTL analysis (TCGA prostate tumor data) in combination with quintile regression to further prioritize the most likely causal variants within the credible set of SNPs and identify potential candidate genes and functional mechanisms. The median credible set size from JAM was 17 SNPs per region, shrinking the post-QC input set of variants by about 98%. In 13 regions evidence was found for multiple independent signals, up to a maxi-mum of 5 SNPs. Within the single hit regions, almost half had less than 10 variants selected. In 34 regions the credible set included at least one SNP that was co-localized with a significant eQTL. Quintile regression highlighted enrichment for variants in promoters, DNase hypersensitivity site and eQTLs - repre-senting candidate biological mechanisms underpinning disease development. This study has substantially reduced and prioritized the candidate causal PrCa risk variants previously known from previous GWAS regions, identifying a small subset of variants for further functional investigation and novel candidate genes at a number of loci.

Bayesian fine-mapping using summary data of 145,000 subjects refines common risk associations, discovers secondary signals and novel candidate genes for prostate cancer. Zofia Kote-Jarai,1 Tolking Dadavey,1 Ed Saund-ers,1 Paul Newcombe,2 Ezequiel Anokian,1 Daniel Leongamornlert,1 Ali Amin Al Olama,3 Christopher Haiman,3 Ros Eles,4 David Conti,4 The PRACTICAL/ELLIPSE Consortium.1 Inst. of Cancer Research, Sutton, United Kingdom;2 University of Cambridge, Cambridge, United Kingdom;3 University of Southern Cali-fornia, Los Angeles, CA.

Genome-wide association studies (GWAS) have identified more than 160 prostate cancer (PrCa) genetic risk loci, however these variants rarely point directly to the true underlying functional variant driving the association. In this fine-mapping study to narrow the credible causal variant set for 80 PrCa regions representing 89 original independent GWAS signals, we performed Bayesian variable selection in combination with functional annotation and quintile regression. We used imputed data for 83,511 PrCa cases and 62,283 controls investigat-ed with high-density genotyping arrays from the OncoArray, iCOGS and 5 previous GWAS studies from the PRACTICAL/ELLIPSE consortia. To facilitate fine-mapping from one-at-a-time SNP associations meta-analyzed over the consortia we first applied JAM, a novel Bayesian algorithm which searches multi-SNP models in summary data by imputing the correlation structure according to a reference panel. JAM provides inference on the number of independent signals, as well as the set of potential SNPs driving those signals. We utilized functional annotation and eQTL analysis (TCGA prostate tumor data) in combination with quintile regression to further prioritize the most likely causal variants within the credible set of SNPs and identify potential candidate genes and functional mechanisms. The median credible set size from JAM was 17 SNPs per region, shrinking the post-QC input set of variants by about 98%. In 13 regions evidence was found for multiple independent signals, up to a maxi-mum of 5 SNPs. Within the single hit regions, almost half had less than 10 variants selected. In 34 regions the credible set included at least one SNP that was co-localized with a significant eQTL. Quintile regression highlighted enrichment for variants in promoters, DNase hypersensitivity site and eQTLs - repre-senting candidate biological mechanisms underpinning disease development. This study has substantially reduced and prioritized the candidate causal PrCa risk variants previously known from previous GWAS regions, identifying a small subset of variants for further functional investigation and novel candidate genes at a number of loci.
with and without adjustment for covariates (age at diagnosis, stage, tumor neo-
antigen load, tumor ESR1 expression) was used to test association between ge-
rmelne SNP genotype and the tumor anticancer immune response measures. SNPs
were evaluated individually and collectively as a polygenic risk score (PRS)
weighted by the per-allele log OR derived from the OncoArray analysis. The
breast cancer GWAS were performed at the Fred Hutchinson Cancer Re-
search Center, UT; 11University of Washington, WA; 12Moffit Cancer Center, FL;
13University of Virginia, VA; 14Cedars-Sinai Medical Center, Los Angeles, CA; 15Harvard T.H.
Chan School of Public Health, Boston, MA; 16The Institute of Cancer Research, Lon-
don, United Kingdom; 17University of Southern California, Los Angeles, CA.

Common genetic variants in over 150 loci have been found to be associated with prostate cancer (PrCa) risk through GWAS. These variants, however, explain only a small fraction of PrCa heritability, and the genes responsible for the detected asso-
ciation have not been identified. These unidentified risk factors may be un-
detectable at the individual level, or the detected associations may be driven by the regulation of risk variants on the expression of disease causal genes. To identify novel PrCa risk loci and possible causal genes at known risk loci, we performed a transcriptome-wide association study (TWAS) to evaluate associations of genetically predicted gene expressions with PrCa risk. We used RNA sequencing data from normal prostate tissues and high-density genotyp-
ing from 73 European descendants included in the Genotype-Tissue Expression
Project and established generic models to predict gene expression levels. Given that
the regulatory mechanisms for most genes are similar across most human tissues, we
also built cross-tissue models using gene expression data generated from all tissues from 369 European descendants to increase the statistical power. Based on predic-
tion performance, we selected 22,126 genes and conducted association analyses of
transcriptome-wide expression levels using GWAS data obtained from more
than 134,000 subjects included in PRACTICAL/ECLIPSE consortia. We identified
140 genes showing an association of their predicted expression levels with PrCa risk
and biologically significant associations with CXCR1 (OR
9.9x10−10, Padj
0.001) was for
rs71801447 overlapped enhancers in CD4+ and CD8+ T cells and in HMEC and MCF7 breast cells that were predicted to
interact with the promoter of BCL2L11 in each of these cell types. Finally, as a
PRS, breast cancer risk was significantly associated with reduced tumor type I
interferon activity (Padj
0.001; Padj = 0.02). Our findings implicate
BCL2L11, a well-known regulator of T and breast cancer cell apoptosis, as a potential genetic candidate and potential target for future risk stratification,
and biological insight of risk loci.

EPIDEMIOLOGY: Genome-Wide Association Studies/Post-
GWAS Studies

## #1300 Genetic predictors of gene expression associated with risk of colore-
rectal cancer

Stephanie A. Bien,1 Xingyi Guo,2 Yu-Ru Su,1 Tabitha A. Harris-
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Mark A. Jenkins, 8Loic LeMarchand, 9Polly A. Newcomb, 1Martha L. Slattery, 10
Elizabeth B. Claus,19 Sara H. Olson,20 Robert B. Jenkins,21 Richard S. Houlston,1
Nöthen,13 Heinz-Erich Wichmann,14 Stefan Schreiber,1 Anthony Swerdlow,1
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ansen,8 Dora Il’yasova, Quinn Ostrom, and members of GIACC, Karim Labreche,
Jeanette E. Eckel-Passow,1 Paul A. Decker,3 Marianne Labussièrè, Ahminder Adbul,
Khe Hoang-Xuan,1 Anna-Luisa Di Stefano,3 Karima Mokhtari,1 Jean-Yves Delat-
tre,3 Pilar Galan,3 Konstantinos Gousias,10 Johannes Schramm,11 Minouk J. Schoe-
maker,3 Sarah J. Fleming,10 Stefan Hermès,21 Stefanie Heillmann,13 Marcus M. Nöthen,6
Heinz-Erich Wichmann,14 Stefan Schreiber,15 Anthony Swerdlow,3
Mark Lathrop,22 Matthias Simon,16 Marc Sanson,3 Preetha Rajaraman,18
Stephen Chanock,1 Martha Linet,18 Zhaoming Wang,17 Meredith Yeager,3 Rose K. Lai,21
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BACKGROUND: Glioma accounts for ~27% of all primary brain tumors and is responsible for ~13,000 cancer-related deaths in the US each year. Gliomas can be broadly classified into glioblastoma (GBM) and lower-grade non-GBM. Typically gliomas have a poor prognosis irrespective of medical care, with the most common form, GBM, having a five-year survival rate of only 5%. While genome-wide association studies (GWAS) have transformed our understanding of glioma susceptibility, individual studies have limited power to identify risk loci. METHODS: We performed the largest GWAS to date, comprising a meta-analysis of six existing GWAS (6,405 cases, 14,100 controls) as well as new GWAS from the Glioma International Case Control Consortium (GICC; 4,572 cases and 3,286 controls) and University of California, San Francisco (UCSF)-Mayo (1,519 cases, 804 controls), totaling 12,496 cases (6,191 classified as GBM, 5,819 as non-GBM) and 18,190 controls. RESULTS: We identified five new risk loci for GBM at 1p31.3 (rs12752552; near JAK1, P = 2.04 x 10^-10, odds ratio (OR) = 1.22), 11q14.1 (rs11233250; P = 9.95 x 10^-10, OR = 1.24), 16p13.3 (rs2562152; near MPG, P = 1.93 x 10^-10, OR = 1.21), 16q12.1 (rs10852606; HEATR3, P = 1.29 x 10^-11, OR = 1.18), 22q13.1 (rs2235573; P = 1.76 x 10^-10, OR = 1.15), and eight for non-GBM at 1q32.1 (rs4252707; MDM4, P = 3.34 x 10^-9, OR = 1.19), 1q44 (rs10760373; AKT3, P = 2.63 x 10^-10, OR = 1.23), 2q33.3 (rs7572263; near IDHI, P = 2.18 x 10^-10, OR = 1.20), 3p14.1 (rs11706832; LRIG1, P = 7.66 x 10^-9, OR = 1.15), 10q24.33 (rs11998018; OBFC1, P = 3.39 x 10^-9, OR = 1.14), 1q21 (rs7107785; P = 3.87 x 10^-9, OR = 1.16), 14q12 (rs1047037; P = 1.07 x 10^-9, OR = 1.18) and 11q13.31 (P = 4.20 x 10^-9, OR = 1.18). Case-only analyses confirmed the specificity of 11q14.1, 16p13.3 and 22q13.1 associations for GBM and 1q44, 2q33.3, 3p14.1, 11q21 and 14q12 for non-GBM tumors. In the combined meta-analysis, among previously published glioma risk SNPs, those for all glioma at 17p13.1 (TP53), GBM at 3p13.33 (TERC), 7p11.2 (EGFR), 9p21.3 (CDKN2B-AS1) and 20q13.33 (RTEL1) and for non-GBM at 8q24.21 (CCDC26), 11q23.2, 11q23.3 (PHLD1B) and 15q24.2 (ETFAT) showed even greater evidence for association. SNPs at 10q25.2 and 12q12.1 for non-GBM tumors retained genome-wide significance (i.e. P < 5.0 x 10^-8). Associations at the previously reported loci for GBM at 3q26.2 (near TERC) and 12q33.3 (POLR3B) did not retain statistical significance. CONCLUSIONS: Our findings substantially increase susceptibility to GBM and non-GBM glioma being highly distinct, consistent with their distinctive molecular profiles presumably resulting from different etiological pathways. Functional analyses should lead to further insights into the biological basis of the different glioma histologies. Such information can inform gene discovery initiatives and therefore have a measurable impact on the successful development of new therapeutic agents.

#1303 A genome-wide association study of prostate cancer in Latinos. Hannah Hopp,1 Sue Ingles,1 Chad Huff,2 Xin Sheng,1 Brandi Weaver,3 Mariana Asimwita Luke,1 Kuteesa J,1 Dabanja M. Henry,1 David Conti,2 Christopher Nalukenge Cissy,1 Muwanga Proscovia,1 Lutalo Moses,1 Nansereko Deborah,4 Candra Davis,5 Reyes-Muñoz,5 Hispanic Health and Nutrition Examination Survey (HHANES),5 and NCI. METHODS: We performed the largest GWAS to date, comprising a meta-analysis of six existing GWAS (6,405 cases, 14,100 controls) as well as new GWAS from the Glioma International Case Control Consortium (GICC; 4,572 cases and 3,286 controls) and University of California, San Francisco (UCSF)-Mayo (1,519 cases, 804 controls), totaling 12,496 cases (6,191 classified as GBM, 5,819 as non-GBM) and 18,190 controls. RESULTS: We identified five new risk loci for GBM at 1p31.3 (rs12752552; near JAK1, P = 2.04 x 10^-10, odds ratio (OR) = 1.22), 11q14.1 (rs11233250; P = 9.95 x 10^-10, OR = 1.24), 16p13.3 (rs2562152; near MPG, P = 1.93 x 10^-10, OR = 1.21), 16q12.1 (rs10852606; HEATR3, P = 1.29 x 10^-11, OR = 1.18), 22q13.1 (rs2235573; P = 1.76 x 10^-10, OR = 1.15), and eight for non-GBM at 1q32.1 (rs4252707; MDM4, P = 3.34 x 10^-9, OR = 1.19), 1q44 (rs10760373; AKT3, P = 2.63 x 10^-10, OR = 1.23), 2q33.3 (rs7572263; near IDHI, P = 2.18 x 10^-10, OR = 1.20), 3p14.1 (rs11706832; LRIG1, P = 7.66 x 10^-9, OR = 1.15), 10q24.33 (rs11998018; OBFC1, P = 3.39 x 10^-9, OR = 1.14), 1q21 (rs7107785; P = 3.87 x 10^-9, OR = 1.16), 14q12 (rs1047037; P = 1.07 x 10^-9, OR = 1.18) and 11q13.31 (P = 4.20 x 10^-9, OR = 1.18). Case-only analyses confirmed the specificity of 11q14.1, 16p13.3 and 22q13.1 associations for GBM and 1q44, 2q33.3, 3p14.1, 11q21 and 14q12 for non-GBM tumors. In the combined meta-analysis, among previously published glioma risk SNPs, those for all glioma at 17p13.1 (TP53), GBM at 3p13.33 (TERC), 7p11.2 (EGFR), 9p21.3 (CDKN2B-AS1) and 20q13.33 (RTEL1) and for non-GBM at 8q24.21 (CCDC26), 11q23.2, 11q23.3 (PHLD1B) and 15q24.2 (ETFAT) showed even greater evidence for association. SNPs at 10q25.2 and 12q12.1 for non-GBM tumors retained genome-wide significance (i.e. P < 5.0 x 10^-8). Associations at the previously reported loci for GBM at 3q26.2 (near TERC) and 12q33.3 (POLR3B) did not retain statistical significance. CONCLUSIONS: Our findings substantially increase susceptibility to GBM and non-GBM glioma being highly distinct, consistent with their distinctive molecular profiles presumably resulting from different etiological pathways. Functional analyses should lead to further insights into the biological basis of the different glioma histologies. Such information can inform gene discovery initiatives and therefore have a measurable impact on the successful development of new therapeutic agents.
established environmental/lifestyle risk factors have been identified, with the only established risk factors being age, race/ethnicity and family history, all of which implicate genetic susceptibility. GWAS have clearly validated the importance of genetic susceptibility in prostate cancer, with ~100 common risk loci identified to date which in aggregate explain 33% of the familial risk. Genetic studies in African ancestry populations have provided strong evidence for genetic factors in contributing to the greater incidence of prostate cancer in men of African ancestry. To further explore this hypothesis, we conducted a genome-wide association study (GWAS) of prostate cancer among Ugandan men. Specifically, we genotyped the Illumina OncoArray, which includes a 260K GWAS backbone, in 560 prostate cancer cases (119 with Gleason score ≥ 8) and 480 controls and tested the associations of 448,939 genotyped and 16,396,662 imputed variants with >1% frequency. The most statistically significant variant were observed at the 8q24 risk locus (rs72275854, OR=3.37, P=2.14x10⁻¹⁵). We also observed suggestive signals with 106 variants outside of known risk regions with P-values <10⁻⁶ and >10⁻⁸. Of the 104 known risk variants, 100 are polymorphic in Uganda men, of which, 66 (66%) had effects that were directionally consistent in their association with prostate cancer risk as previously reported and 8 (8%) were significantly associated with risk at p < 0.05, with the most statistically significant being rs16901979 at 8q24 (OR=1.45, P=0.0001) and rs1512268 at 2p21.2 (OR=1.31, P=0.0087). In addition to these findings, we will also present the results from replication testing of the most significant associations from the GWAS in the Ghana Prostate GWAS Study and the African Ancestry Prostate Cancer Consortium, as well as provide a detailed comparison of polygenic risk models of the known prostate cancer variants between these two African populations, African Africans and men of European ancestry.

### #1306 Two distinct regulatory mechanisms underlie estrogen receptor negative breast cancer susceptibility at the 2q23.2 locus

Carly M. Harro,1 Gustavo Mendoza-Fandino,1 Nicholas T. Woods,2 Xuexi Li,1 Fergus J. Couch,3 Álvaro N. Monteiro1.1 Eppley Institute for Research in Cancer, Omaha, NE;2 Mayo Clinic, Rochester, MN.

Genome Wide Association Studies (GWAS) are designed to identify single nucleotide polymorphisms (SNPs) mapping to genomic regions associated with a particular trait or disease. Currently, 19 genomic loci associated with breast cancer susceptibility are known, but the precise mode of action of these loci remains largely unknown. Two recent GWAS with large sample sizes did not reveal consistent evidence of association with breast cancer. However, looking beyond disease-specific SNPs may offer insight into the regulation of genes that may contribute to breast cancer risk. Here, we integrated transcriptome-wide association study (TWAS) to search for novel genetic loci for breast cancer risk. The TWAS highlighted two intrinsic loci: an enhancer-like element defined by SNP rs44017214 located in the first intron of the WDR43 gene; and a segment in the 3'-UTR region of WDR43 defined by SNPs rs11680458 and rs11331880 which are predicted to have allele specific binding of non-coding microRNAs miR-548 and miR-376, respectively. We validated allele specific transcription factor binding to rs44017214 using competitive binding of USF1/USF2 to the risk allele in both MCF10A and CAL51 cell lines in Electrophoretic Mobility Shift Assays (EMSA). Additionally, rs44017214 was removed using CRISPR-cas9, and expression of neighboring genes was evaluated by qRT-PCR. No change in expression was found for any gene in a 2 Mb region surrounding the SNP, with the exception of PLB1. This suggests that the enhancer element in the intron of WDR43 has PLB1 as its target. To assess the miRNA binding site impact on WDR43 expression the pMIR-REPORT Luciferase allele-specific vector was co-transfected with miR-141/200, miR548, or miR-376 in MCF10A cells. The rs11331880 allele C in the WDR43 3'-UTR, rs4401714, and (ii) decreasing the expression of WDR43 through the formation of a miR-376 binding site at the 3'-UTR of the WDR43 gene. Elucidating the functional significance of these common variants in the novel susceptibility loci 2p23.2 furthers our knowledge of the etiology of estrogen receptor negative breast cancer.
EPIGENOMICS: Genome-Wide Association Studies/Post-GWAS Studies

ovarian cancer from 25,509 cases and 40,941 controls. MetaXcan was used to integrate gene expression prediction model with summary statistics. Results: We identified 35 genes with predicted expression levels associated with ovarian cancer risk at P value < 2.2 x 10^-10, the Bonferroni corrected significance level for multiple comparisons. Of these, 12 genes at 4 genetic loci were located at ≤500kb away from risk SNPs previously reported in GWAS, representing potential novel loci for ovarian cancer risk. The remaining 23 genes at 12 loci are located within known ovarian cancer risk loci. Fifteen of these genes at the 12 loci have not been reported in previous studies. Analyses by ovarian cancer histological subtypes showed that the majority of these 35 genes are associated with serous invasive carcinoma. Several new associations were identified in histological subtype analyses. Conclusion: In this study we identified 35 genes with predicted expression levels related to ovarian cancer risk and provide substantial new information to enhance the understanding of ovarian cancer biology and genetics.

#1309 Association between insulin resistance and breast cancer risk: A Mendelian randomization analysis of data from 228,000 women of European descent. Xiang Shu,1 Lang Wu,1 Nikhil K. Khankari,1 Kyriaki Michailidou,1 Manjeet K. Bolla,1 Jean Wang,1 Joe Dennis,1 Xiao-ou Shu,1 Jacques Simard,2 Douglas F. Easton,3 Wei Zheng,3 Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, TN; 2University of Cambridge, Cambridge, United Kingdom; 3Centre Hospitalier Universitaire de Québec Research Center, Laval University, Québec City, Quebec, Canada.

Background: Epidemiologic studies suggest that insulin resistance may be associated with breast cancer risk. We conducted Mendelian randomization (MR) analyses to reduce the bias associated with previous studies and provide evidence for causal inference. Materials and Methods: We used genetic variants identified in genome-wide association studies for circulating fasting insulin (15 variants), early insulin secretion (16 variants), fasting proinsulin (8 variants), fasting glucose (35 variants), and 2-hour glucose (8 variants) as instruments in MR analyses. To reduce possible pleiotropic effects, variants associated with obesity were removed from the instruments. We first evaluated the association of these instruments with type 2 diabetes risk in 110,452 subjects to assess instrument validity. We then investigated the association of these instruments with breast cancer risk using data obtained from 122,977 cases and 105,974 controls of European descent included in the Breast Cancer Association Consortium (BCAC). Odds ratios (ORs) were calculated to measure the association of individual variants with risk of overall breast cancer and its subtypes defined by estrogen-receptor [ER] status. Results: All instrumental variables constructed for this study were strongly associated with type 2 diabetes risk with ORs of 3.01 (p = 7.86x10^-10), 0.22 (p = 3.54x10^-10), 1.90 (p = 8.28x10^-10), 6.11 (p = 3.59x10^-10), and 1.91 (p = 6.8x10^-10) for per unit increase of fasting insulin, early insulin secretion, fasting proinsulin, fasting glucose, and 2-hour glucose levels, respectively. Statistically significant associations with overall breast cancer risk were found for fasting insulin (OR = 1.36 for per unit increase, CI 1.32-1.40, p = 0.0011) and fasting proinsulin (OR = 1.21, 95% CI = 1.06-1.38, p = 0.0011). These associations were observed only for ER-positive breast cancer. No statistically significant association at p<0.05 was found for early insulin secretion, fasting glucose, or 2-hour glucose levels. Conclusions: Our study provides strong support that certain insulin resistance traits may be causally associated with risk of breast cancer, particularly ER-positive breast cancer.

#1310 Identification of pleiotropic cancer susceptibility variants from genome-wide association studies reveals functional characteristics. Yi-Hsuan Wu,1 Rebecca E. Graff,1 Michael N. Passarelli,2 Thomas J. Hoffmann,1 Elad Ziv,1 John S. Witte,3 University of California, San Francisco, San Francisco, CA; 2Geisel School of Medicine, Dartmouth College, Hanover, NH.

Background: There exists compelling evidence that some genetic variants are associated with the risk of multiple cancers (i.e., pleiotropy). However, the biological mechanisms of the pleiotropic effects are unclear. Thus, we investigated the functional effects for genetic variants associated with the risk of multiple cancers. Methods: The National Human Genome Research Institute-European Bioinformatics Institute (NHGRI-EBI) GWAS Catalog contains 28,643 variant-trait associations with p < 10^-8. We utilized the Experimental Factor Ontology (EFO) to classify cancer traits and obtained all associations between variants and cancer risk. Based on pairwise linkage disequilibrium (LD) determined from the European (EUR) population of Phase 3 of the 1000 Genomes Project in LDLink, correlated variants (R-squared ≥ 0.8) were clustered into groups. Variant groups associated with the risk of multiple cancers were annotated using the Ensemble Variant Effect Predictor (VEP), and tested for functional enrichment using the DAVID Functional Annotation Tool. Results: We identified 1,456 variant-cancer risk associations. The majority (57.1%) of the associations were discovered in European ancestry populations, 19.0% in East Asians, 7.9% in an African Americans or Afro-Caribbean, and 7.1% in Hispanics or Latin Americans. Removing duplicates, we found 1,034 unique variant-cancer risk associations for 1,005 unique variants and 27 unique cancer sites. After clustering correlated variants, we identified 29 pleiotropic variant groups, of which 2 were associated with risk of five different cancer sites. Variant group rs10936599 and rs12696304 within the MYNN gene (7.8 kb from the TERC gene) was associated with leukemia, multiple myeloma, and colorectal, skin, and bladder previously reported in GWAS, representing potential novel loci for breast cancer. The remaining 23 genes at 12 loci are located within known ovarian cancer risk loci. Fifteen of these genes at the 12 loci have not been reported in previous studies. Analyses by ovarian cancer histological subtypes showed that the majority of these 35 genes are associated with serous invasive carcinoma. Several new associations were identified in histological subtype analyses. Conclusion: In this study we identified 35 genes with predicted expression levels related to ovarian cancer risk and provide substantial new information to enhance the understanding of ovarian cancer biology and genetics.

#1311 Germ line variation at 8q24 and prostate cancer risk. Kan Wang,1 Ali Amin Al Olama,2 Rosalind Eeles,2 David Conti,1 Zoila Kote-Harai,1 Christopher A. Haiman1. 1Department of Preventive Medicine, Keck School of Medicine, USC/Norris Comprehensive Cancer Ctr, Los Angeles, CA; 2Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom; 3The Institute of Cancer Research, London, United Kingdom.

The 8q24 region harbors multiple risk variants for distinct cancers including 7 for prostate cancer, the majority of which lie in separate linkage disequilibrium blocks. It is not known whether a common biological mechanism underlies the association of genetic variation with cancer risk at 8q24, or whether there are site-specific functions of regulatory elements that are affected in this region. Given the proximity, the MYC oncogene is a likely candidate as are multiple long non-coding RNAs in the region. To further understand the contribution of germline variation to prostate cancer risk we performed a comprehensive fine-mapping analysis of the region in men of European ancestry from the PRACTICAL/ELLIPSE Consortium. More specifically, we tested 1,731 genotype tag SNPs and 12,221 imputed variants spanning the risk region (127.3-129.0Mb) in 56,363 prostate cancer cases and 37,386 controls of European ancestry that were genotyped with the Illumina OncoArray. We performed stepwise logistic regression and identified 13 variants with risk allele frequencies between 0.006 and 0.998 that surpassed genome-wide statistical significance (p-values between 3.2x10^-8 and 8.2x10^-10) and with per allele odds ratios ranging from 1.11(rs1003678) to 2.68(rs183373024). Ongoing analyses that will be presented include incorporating existing GWAS and fine-mapping data (iCOGs) for men of European and African ancestry (35,000 cases and 35,000 controls) using JAM, a Bayesian approach that investigates multi-SNP models using marginal meta-analysis statistics. Leveraging the power from the overall multiethnic meta-analysis (>93,000 cases and >72,000 controls) will provide further insight into the number of independent signals in the region and their contribution to prostate cancer risk in these populations.

#1312 Genetic variation in the Hippo pathway and breast cancer risk in women of African ancestry in the ROOT Consortium. Shengfeng Wang,1 Yonglan Zheng,1 Temiduyo O. Ogundiran,2 Oladosu Oyebode,2 Wei Zheng,1 Katherine L. Nathanson,4 Barbara Nemescu,5 Stefan Ambs,6 Dezheng Huo,1 Olufumilayo I. Olopade1. 1University of Chicago, Chicago, IL; 2University of Ibadan, Ibadan, Nigeria; 3Vanderbilt University, Nashville, TN; 4University of Pennsylvania, Philadelphia, PA; 5State University of New York at Stony Brook, Stony Brook, NY; 6National Cancer Institute, Bethesda, MD.

Background: The Hippo pathway controls organ growth by regulating cell proliferation and apoptosis. To our knowledge, it is still unclear about its role in the development of breast cancer. Identifying the relevant genes and single-nucleotide polymorphisms (SNPs) should shed light on the pathway’s mechanism in carcinogenesis. Methods: We examined 47,419 SNPs in 37 Hippo pathway genes in the genome-wide association study of breast cancer conducted in the African Diaspora (ROOT consortium), which included 3,686 participants of African ancestry from Nigeria, USA, and Barbados (1,657 cases and 2,029 controls). Gene-level analyses were conducted using the adaptive rank truncated product (ARTP) test for 10,771 SNPS that weren’t highly correlated (t^2 < 0.8), and SNP-level analyses were conducted with logistic regression. Results: The Hippo pathway was significantly associated with risk of estrogen receptor positive (ER+) breast cancer (pathway-level P = 0.028). Gene-based analyses revealed that WW1C1 (gene-level P = 0.001) was responsible for this association, with rs16516663 in this gene being statistically significant after gene-level adjustment for multiple comparisons [odds ratio (OR) = 0.63 for each G allele, 95% confidence interval (CI) = 0.41-0.70, P = 0.002]. In addition, two SNPs in LAT52 (rs1429000440, OR = 0.42, 95% CI: 0.28-0.64; rs85674288, OR = 0.38, 95% CI = 0.24-0.61) were associated with risk of ER+ breast cancer. In the analysis of ER- breast cancer risk,
r2579161 in DLG5 (OR = 1.38, 95% CI: 1.17-1.63) and rs1062429 in TEAD4 (OR = 1.39, 95% CI: 1.17-1.64) were statistically significant. These associations remained significant after Bonferroni-correction at the gene-wide level (all P < 0.05). Conclusions: We found evidence of associations of the Hippo pathway with ER+ breast cancer risk in women of African ancestry. Our findings supported the potential mechanism that WWC1 functions as an upstream member of the pathway, and highlighted the importance of further studies.

**Table 1: Gene-significant test variants for all breast cancer, ER+ tumors, and ER- tumors**

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<td><em>rs1062429</em></td>
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**#1313 Characterization of germiline susceptibility to male breast cancer.**

Eleni Perrakis, Sarah Maguire, Katarzyna Tomczyk, Anthony Swerdlov, Nick Orr. ICR, London, United Kingdom.

Male breast cancer (MBC) accounts for approximately 1% of all breast cancer cases. Family history is established as a risk factor for MBC and 10% of patients have genetic BRCA2 mutations. We recently demonstrated that common germiline polymorphisms contribute to MBC predisposition and that there is overlap between genetic susceptibility loci for MBC and female breast cancer (FBC). Here we report a comprehensive evaluation of all 107 known FBC predisposition loci in an association analysis of 1,032 MBC cases and 2,795 population matched controls. A total of 21 FBC predisposition single nucleotide polymorphisms (SNPs) showed evidence of association with risk of MBC. For approximately a third of these SNPs, the risk estimates were significantly larger in MBC than FBC. The magnitude of effects observed suggested that common susceptibility variants may be of value for risk stratification in men who have a high absolute risk of breast cancer such as BRCA2 mutation carriers. Although our data suggests that MBC predisposition shares many features in common with FBC, there are striking differences. Surprisingly, given the predominance of hormone receptor positive tumours in MBC, we observed no evidence of association between SNPs at FGR2 and MBC risk suggesting that the estrogen receptor status of MBC tumours does not explain the observed SNP associations. In summary, the data presented in this abstract significantly advance our understanding of genetic factors that contribute to MBC predisposition and further underscore the growing consensus that breast cancer risk is not simply analogous to hormone receptor positive female breast cancer.

**#1314 Trans-ethnic HLA fine-mapping of the MHC region identified several independent variants influencing susceptibility to lung cancer.**

Aida Ferreiro-Iglesias, Corina Lesseur, James McKay, Ray Jean J. Hung, Christopher I. Amos, Helen Hansen, Christopher Amos, Jonine L. Bernstein, 17

**International Agency for Research on Cancer (IARC-WHO), Lyon, France; 18Lunenfeld-Tanenbaum Research Institute of Sinai Health System, Toronto, Ontario, Canada; 19Geisel School of medicine, Dartmouth College, Lebanon, PA.**

Background: Genetic variants within the major histocompatibility complex (MHC) are associated with lung cancer. However, debate persists about the identity of the true causal variants, in part due to the broad linkage disequilibrium (LD) characteristic of the MHC and to the complexity and cost of complete AG typing. Here, we assessed the role of the MHC in modulating lung cancer risk in Europeans and Americans using a broad set of HLA variants to detect the main alleles and the presence of independent effects elsewhere in this genomic region. Material and methods: Association between the HLA variants and risk of lung cancer was examined in two collections of samples of different ethnicity (18,686 cases / 15,190 controls and 2,324 cases / 1,646 controls of European and Asian ancestry respectively). Using existing genome-wide SNP data from the lung cancer OncoArray study, we imputed and tested classical alleles and amino acid polymorphisms in HLA-A, HLA-B, HLA-C, HLA-DQA1, HLA-DQB1, HLA-DRB1, HLA-DPA1 and HLA-DPB1, as well as 7,258 SNPs across the MHC. All the analyses were performed assuming an additive genetic model adjusted for sex and principal components as covariates. The study-wide significant threshold was p = 6.03 x 10^-6. Results: Conditional, haplotype and stratified analyses identified two independent HLA alleles associated with lung cancer squamous cell carcinoma in Europeans. One is HLA-DQB1*06 that showed a significant protective effect (OR = 0.85, 95% CI: 0.80-0.90). The other allele is attributed to HLA-DP1*03 (OR = 1.30, 95% CI: 1.18-1.42, p = 4.78 x 10^-5), which contains class I and class II HLA alleles (A*A0101 - B*0801 - C*0701 - DRB1*0301 - DQB1*0201 - DQA1*0101). In addition, single-amino-acid polymorphisms in HLA-B (at positions 9, 156, 163 and 178) and HLA-DRB1 (at positions 26, 71 and 74) located in peptide-binding grooves, suggest these proteins as possible functional contributors within the haplotype.

**#1315 Estimating sex-specific effects of genetic loci associated with glioma risk.**

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Background: Incidence of glioma varies significantly by sex, and most glioma histologies occur with greater incidence in males. Previous analyses have examined the impact of estrogen exposure as a risk factor for these tumors, but have found results of varying significance and low effect size. There may be differences in effect of previously discovered risk alleles that contribute to sex differences. METHODS: Using data collected for three previous glioma GWAS in European-ancestry populations (MD Anderson Cancer Center, the San Francisco Adult Glioma Study, and the Glioma International Case Control Study) we assessed sex-specific effects for 14 previously identified and 13 newly identified glioma risk SNPs (27 total) overall and for glioblastoma (GBM) and non-GBM tumors separately. There were 3,492 male cases (59% GBM), 4,532 male controls, 2,500 female cases (52% GBM) and 4,940 female controls. Sex-specific odds ratios (ORM and ORF), 95% confidence intervals (95% CI) and p values (pM and pF) were generated using stratified logistic regression models. Data from each study were analyzed separately and combined using inverse variance weighted meta-analysis. Results were considered statistically significant at p<6.2x10^-4. RESULTS: In GBM, rs11979158 (7p11.2, pM = 1.01x10^-10, ORM = 1.08 [95% CI: 1.03-1.13]), rs2562125 (16p13.3, pM = 5.98x10^-4, ORM = 1.23 [95% CI: 1.09-1.39]), and rs2562125 (16p13.3, pF = 1.21x10^-7, ORF = 1.12 [95% CI: 0.97-1.29]) had significant effect in males only. In non-GBM gliomas, rs12076733 (14q44, pM = 3.56x10^-7, ORM = 1.41 [95% CI: 1.23-1.61], pF = 1.76x10^-2, ORF = 1.20 [95% CI: 1.03-1.38]), rs11979158 (7p11.2, pM = 1.77x10^-3, ORM = 1.32 [95% CI: 1.16-1.49], pF = 2.73x10^-3), ORF = 1.08 [95% CI: 0.94-1.24], and rs7351667 (16p13.3, pM = 8.44x10^-4, ORM = 1.30 [95% CI: 1.17-1.44], pF = 5.18x10^-2, ORF = 1.13 [95% CI: 1.00-1.28]) had significant effect in males only. Effect size for rs55705857 (8q24.21) varied significantly by sex, with ORM = 2.63 [95% CI: 2.24-3.09, pM = 4.42x10^-32], as compared to ORF = 3.95 [95% CI: 3.28-4.76, p = 1.28x10^-15]. A sensitivity anal-

**EPIDEMIOLOGY: Genome-Wide Association Studies/Post-GWAS Studies**
yis was performed due to allele frequency heterogeneity by study and results did not change. CONCLUSIONS: Sex differences and other demographic differences in cancer susceptibility can provide important clues to etiology, and these differences can be leveraged for discovery in genetic association studies. Significant differences in effect size may suggest variation in genetic effect of risk alleles due to factors that vary in prevalence or effect by sex. There may also be differences in the distribution of molecular subtypes within each histology by sex. Further investigation using an agnostic approach may further elucidate the relationship between effect of risk alleles and sex.

#1316 Germline genetic signals across multiple aggressive prostate cancer phenotypes. Caroline G. Tai,1 Nima C. Emami,1 Thomas J. Hoffmann,1 Lori C. Sakoda,2 Eric Jorgenson,2 Laurel A. Habel,1 Jun Shan,3 Dilini K. Ranatunga,3 Chun R. Chao,2 Nirupa R. Ghi,3 David Aaronsom,2 Joseph Presti,4 Catherine Schaefer,5 Neil R. Schrock,6 Stephen K. Van Den Eeden,7 John S. Witte,8 University of California, San Francisco, San Francisco, CA; Kaiser Permanente Northern California, Oakland, CA; Kaiser Permanente Southern California, Pasadena, CA; Kaiser Oakland Medical Center, Oakland, CA.

Prostate cancer (PrCa) is documented to be highly heritable. But many known prostate cancer loci are not associated with aggressive prostate cancer phenotypes such as high-grade cancer or biochemical failure. Previous efforts have been largely underpowered or not replicated. We conducted a study of 6,321 men with PrCa and 5,663 controls of European ancestry recruited from Kaiser Permanente (KP) matched by age and self-reported race. Phenotype data combined patient-level demographic, clinical, and tumor data from KP databases with data from the Cancer Genome Atlas (CGA). High-grade PrCa was defined as Gleason score ≤7 (3+4) and high-grade normal as ≥7 (4+3). Biological failure was defined for cases using post-treatment prostate specific antigen (PSA) values: post-surgery failure if PSA ≥0.4 ng/mL; post-radiation failure if PSA value ≥2 ng/mL + nadir; and no failure if PSA did not reach specified threshold. Early-onset disease was defined as age < 55 years at diagnosis. Men were genotyped for over 650,000 SNPs using an ethnicspecific Affymetrix Axon array and imputed to 1000 Genomes. We tested genetic associations for multiple aggressive phenotypes (410 early-onset cases, 1324 biochemical failure cases, and 886 high-grade cases) in both case-only (e.g., high vs. low grade) and case-control (e.g., high grade vs. no prostate cancer controls) analysis using logistic regression adjusting for ancestry principal components, age, BMI, and kit for batch effects. Prevalence of genetic effect of risk variants from multiple GWAS loci, we employed recently developed Massively-Parallel Reporter Assays (MPRA). Systematic examination of allele-specific transcriptional activities of genetically indistinguishable candidate variants. Out of ~2,800 melanoma-associated variants (P<0.4, EUR) from 16 melanoma loci for which transcriptional mechanism could be applied, 835 variants were prioritized based on their relevance as melanocyte/melanoma-specific cis-element represented by ENCODE annotation as open chromatin or putative promoter/enhancer histone marks in melanocytes and melanoma cell lines. Pooling oligos of 145bp encompassing each variant with either melanoma risk or protective allele coupled with 10 different unique sequence tags were cloned into luciferase vectors and transfected to melanoma cell lines. Resulting expressed tag counts were subsequently determined using massively parallel sequencing. We were able to identify ~200 potentially functional variants displaying allelic transcriptional activity by combining transfections in a melanoma cell line and HEK293FT cell line. Top functional SNPs from melanoma GWAS loci were also shown as significant eQTL SNPs in human melanocytes including MX2 on chromosome 21. Overlapping MPRA functional SNPs with melanocyte eQTL SNPs further enabled isolation of a functional risk variant among several highly linked MX2 eQTL SNPs. The results from this analysis will greatly accelerate the identification of functional melanoma risk variants and further shed light on molecular mechanisms of genetic susceptibility of melanoma in population.

#1318 A genome-wide association study of Waldenström macroglobulinemia/lymphoplasmacytic lymphoma demonstrates association with chromosome 6. Mary L. McMastert,1 Sonja I. Berndt1, Shengchao A. Li,1 Susan Slager,2 Joseph Vijai,3 Charles C. Chung,4 Bin Zhu,5 Laurie Burdette,5 Brenda Birnrmann,6 Elizabeth E. Brown,7 James R. Cerhan,8 Karin Ekstrom-Semedby,9 Henrik Hjalgrim,9 Geffen Kleinstern,2 Brian K. Link,10 James McKay,10 Alain Monnerreau,11 Lindsay M. Morton,12 Alexandra Nieters,13 Nathanial Rothman,14 Christine F. Skibola,15 Alex Smith,16 Lauren R. Teras,17 Claire M. Vajdic,18 Roel Vermeulen,19 Belynda Hicks,1 Lynn R. Goldin,3 Neil E. Caporaso1, 25NCI-DCEG, Bethesda, MD; Mayo Clinic, Rochester, MN; Memorial Sloan-Kettering Cancer Center, New York, NY; Brigham and Women’s Hospital and Harvard Medical School, Boston, MA; University of Alabama at Birmingham, Birmingham, AL; Karolinska Institutet, Stockholm, Sweden; Statens Serum Institut, Copenhagen, Denmark; The University of Iowa, Iowa City, IA; International Agency for Research on Cancer, Lyon, France; Center of Research in Epidemiology and Statistics Sorbonne Paris Cite, Paris, France; University Medical Center Freiburg, Freiburg, Germany; Emory University, Atlanta, GA; University of York, York, United Kingdom; American Cancer Society, Atlanta, GA; University of New South Wales, Sydney, Australia; Utrecht University, Utrecht, Netherlands.

Waldenström macroglobulinemia (WM) is a unique subset of lymphoplasmacytic lymphoma (PLL) that is defined by the presence of an LPL infiltrate in the bone marrow together with a monoclonal IgM protein in the serum. A somatic activating mutation, MYD88 L265P, occurs in 85% of WM and in 25%-50% of patients with the precursor condition, IgM monoclonal gammopathy of undetermined significance (MGUS); however, germline MYD88 mutations have not been observed in WM patients, and the genetic basis for WM predisposition remains undefined. To identify novel WM susceptibility loci we conducted a two-stage genome-wide association study (GWAS) in over 450 WM cases and 4300 controls of European ancestry. Discovery (stage 1) included 217 WM cases (40% familial) and 3798 controls genotyped on the Illumina Omni Express or Illumina Omni2.5 platforms following standard quality control procedures. The genotyped data were imputed using the HaploType Reference Consortium panel as a reference and analyzed using logistic regression. In stage 1, we identified three loci on chromosomes 6, 14 and 3 significantly associated (P<5.0x10^-6) with risk of WM. Eleven promising SNPs in these and other suggestive loci (P<5.0x10^-5) were selected for replication (stage 2) in 269 WM or LPL cases (4% familial) and 571 controls, and genotyping was conducted using standard methods on Taqman and Sequenom platforms or Sanger sequencing (1 SNP). Preliminary results confirm replication of the chromosome 6 locus. Stratification on familial status will illuminate the contribution of familial disease. These results will provide insight into the underlying genetic basis of WM susceptibility.

#1319 Epigenome-wide association study reveals differential DNA methylation consistent with progression of multiple myeloma. Stephen D. Gragg,1 Devin Absher,2 Xiangqin Cui,3 Christina Pillion,4 Richard Myers,5 Shaji Kumar,6 Luciano Costa,7 Brian Chiu,8 Celine Vachon,7 Elizabeth Brown1. University of Alabama at Birmingham School of Medicine, Birmingham, AL; 4Birmingham School of Public Health, Birmingham, AL; Mayo Clinic, Rochester, MN; 5University of Chicago, Chicago, IL.

Purpose: Multiple myeloma (MM) is the second most common hematological malignancy in the US. It is characterized by a clonal expansion of plasma cells in the bone marrow and extramedullary sites and is preceded by two precursor conditions including monoclonal gammopathy of undetermined significance (MGUS) and smoldering myeloma (SMM). Strong evidence suggests a germline and environmental etiology. However, efforts to characterize heritable changes in gene activity, such as DNA methylation, have not been widely reported. Methods: We examined epigenome-wide DNA methylation as markers of MGUS, SMM and MM in peripheral blood obtained from treatment-naïve European cases with heavy-chain IgG or IgA MGUS (n=60), SMM (n=31) and MM (n=54) and unrelated controls (n=79) included from the University of Alabama at Birmingham, University of Chicago and the Mayo Clinic, Rochester (54.5% males; mean age, 64 years, range 36 to 86). We quantified DNA methylation of over 450,000 CpG and non-CpG loci using the Infinium HumanMethylation450 array (Illumina). Differentially methylated po...
sitions were calculated using a general linear model framework adjusted for confounders and cellular heterogeneity. Results: A total of 6 CpGps were differentially methylated in MM cases compared to controls at a level of genome-wide statistical significance. MM was associated with hypomethylation at differentially methylated positions inside SBO2 (P = 3.37x10^{-9}), WIZ (P = 1.12x10^{-4}), CA6 (P = 2.99x10^{-4}) and ADO (P = 3.68x10^{-7}) as well as an intergenic position proximal to TNFRSF8 (Chr 1p36.22; P = 1.1x10^{-8}). Each of these loci, with the exception of CA6 and ADORA1, were hypomethylated in each of the 3 plasma cell dyscrasia phenotypes including MGUS and SMM and MM cases compared to controls (P < 0.03), albeit not at a level of genome-wide statistical significance. Conclusions: These preliminary findings suggest that differences in DNA methylation may contribute to altered risk of MM, as well as its precursor conditions, and may play a role in plasma cell dyscrasia progression as a consequence of heritable changes in gene activity due to past exposures. Replication in a large yet similarly well-characterized population is warranted.

#1320 Non-additive and interaction effects of HLA class 2 polymorphism contributing to risk of glioma. Chenan Zhang, Kyle Walsh. University of California, San Francisco, San Francisco, CA.

Although genome-wide association studies have identified a number of susceptibility loci for adult glioma, little is still known regarding whether polymorphisms in the human leukocyte antigen (HLA) region contribute risk. HLA associations have previously been reported for a number of malignancies, with selected HLA polymorphisms investigated in a subset of glioma studies. However, no systematic analysis has been conducted to date, and no investigation into potential non-additive effects of such associations has been described. In this study, we conducted comprehensive genetic analyses of HLA variants in the major histocompatibility complex (MHC) region among 1,746 adult glioma patients and 2,312 controls from the GliomaScan Consortium. Genotype data were generated with the Illumina 660-Quad array, containing 1,822 SNP probes across the MHC region. We imputed HLA alleles using a reference panel of 5,225 individuals in the Type 1 Diabetes Genetics Consortium who underwent high-resolution HLA typing via next-generation sequencing. Subjects were of European-ancestry and case-control comparisons were adjusted for population stratification using ancestry-informative principal components. Because alleles in different loci across the MHC region are linked, we created multi-locus haplotypes consisting of genes DRB1, DQA1, and DQB1. Although none of the haplotypes were associated with glioma in additive models, DRB1*15:01-DQA1*01:02-DQB1*06:02 showed an improvement in model fit after the inclusion of a non-additive term, which was significant after Bonferroni correction (P = 2.5x10^{-10}). Furthermore, the interaction of DRB1*15:01-DQA1*01:02-DQB1*06:02 with haplotype DRB1*04:01-DQA1*03:01-DQB1*03:01 resulted in a 3.80-fold increase in the odds of glioma (P = 8.8x10^{-10}). Our results indicate that the DRB1*15:01-DQA1*01:02-DQB1*06:02 haplotype contributes to the risk of glioma via a dominant effect, with heterozygosity conferring greater risk of glioma than expected from homozygote disease risk in an additive model.

#1321 Transcriptome-wide association study of prostate cancer risk. Maxine Chen,1 Alexander Gusev,2 The PRACTICAL Consortium, Massimo Loda,3 Lorelei A. Mucci,2 Meir J. Stampfer,1 Peter Kraft,3 Kathryn L. Penney3. Brigham and Women’s Hospital and Harvard Medical School, Boston, MA; 2Harvard T.H. Chan School of Public Health, Boston, MA; 3Dana-Farber Cancer Institute, Boston, MA.

Though more than 150 single nucleotide polymorphisms (SNPs) associated with prostate cancer have been identified through GWAS, we still do not understand the functional consequences of common genetic variation in this disease. Genetic variants may influence complex diseases like prostate cancer through gene expression. However, very large studies evaluating gene expression in prostate cancer are currently infeasible due to the limited availability of prostate cancer tissue and expense of collecting expression data. Alternative approaches have been developed to identify expression-trait associations in study populations without directly measured expression data. One such approach (Gusev et al. Nat Genet. 2016) uses a set of reference individuals with both gene expression and SNP data to develop genetic prediction models of gene expression values. These models are then used to impute cis genetic components of gene expression for a much larger set of individuals with only genotype and phenotype information. We used Affymetrix gene expression microarray data from normal prostate and prostate tumor tissue and genotype data from prostate cancer patients in the Health Professionals’ Follow-up Study and the Physicians’ Health Study (N = 187) as a reference panel for creating the predicted models of the genetic components of expression. We imputed gene expression values into Oncoarray GWAS summary statistics from the PRACTICAL consortium (N ~ 75,000) to assess the association between predicted expression and prostate cancer risk. We present the results of our transcriptome-wide association study in normal prostate and prostate tumor tissue, identifying genes significantly associated with prostate cancer risk after correcting for multiple testing. These genes may provide insight into how genetic variants influence prostate cancer through their effect on gene expression.

#1322 A meta-analysis of genome-wide association studies identifies novel loci that influence breast cancer prognosis. Latha Kaladaly,1 Sofia Khan,2 Helé Nevanlinna,3 Peter A. Fasching,2 Fergus J. Couch,4 John Hopper,5 Jianjun Liu,6 Tom Maishman,7 Lorraine Durcan,1 Carl Bloomquist,8 Andy Collins,9 Dianna Eccles,1 William Tapper1. 1University of Southampton, Southampton, United Kingdom; 2University of Helsinki, Helsinki, Finland; 3Friedrich-Alexander University Erlangen-Nuremberg, Erlangen, Germany; 4Mayo Clinic, Rochester, MN; 5University of Melbourne, Melbourne, Australia; 6Genome Institute of Singapore, Singapore, Singapore.

Familial studies were among the first to indicate that breast cancer prognosis has a heritable component. Subsequently many variants associated with prognosis have been identified using a range of techniques including genome-wide association studies (GWAS). Despite these advances, much of the heritability remains unexplained. In young women, breast cancer is characterised by a higher incidence of adverse pathological features, unique tumour gene expression profiles and worse survival. In addition, the association of risk with conventional epidemiological exposures is less clear in women with early onset. We hypothesise that some of these differences between early and late onset could be influenced by germline variation. To identify additional variants that influence breast cancer prognosis, we conducted a two stage meta-analysis of four GWAS consisting of 6,042 patients from the UK (POSH), Finland (HEBCS), Germany (SUCCESS-A) and Australia (ABCFS). Cox-regression analyses were used to investigate overall survival (OS, n = 1,101 events) and disease-free survival (DFS, n = 1,316 events) with correction for oestrogen status (ER). These survival analyses were repeated in a subset of patients with early onset (aged ≤ 40 at diagnosis, n = 2,315 patients, OS n = 604 events, DFS n = 716 events). Meta-analysis identified two intronic SNPs in ADAMTS1 that were associated exclusively with early onset DFS, rs715212 (P_{meta} = 3.54x10^{-10}) and rs10963755 (P_{meta} = 3.91x10^{-10}) without heterogeneity between cohorts. Multivariable Cox-regression demonstrated that the effect of these SNPs were independent of the classical prognostic factors. Most importantly, rs715212 reached genome-wide significance (P_{meta} = 5.37x10^{-8}) in the multivariate model. ADAMTS1 encodes a glycoprotein that forms part of the extracellular matrix (ECM) and may function in cell-cell or cell-matrix interactions or may regulate other ADAMTS proteases. Previous studies have shown that ADAMTS1 is hypermethylated in ER positive breast cancer tumours. Using GTEX to perform eQTL analysis, we found that rs715212 is associated with the expression of several biologically relevant genes including ARHGEF10 (P_{meta} = 10^{-3}) and FASLG (P_{meta} = 0.0031) and EGF (P_{meta} = 0.0018) in breast mammary tissue. Interestingly, separate studies have shown that AREG is overexpressed in ER-positive breast tumours from pre-menopausal women versus post-menopausal women. Furthermore, AREG is differentially expressed between parous and non-parous mammary glands and is persistently downregulated by parity, which suggests it may contribute to the susceptibility of the nulliparous gland to breast cancer. We conclude that rs715212 is associated with an increased risk of disease progression in patients with early onset and speculate that this could be due to an interaction with the expression of AREG.


Background/Objectives: Previous studies suggested that high energy intake may increase the risk of prostate cancer. However, the mechanisms remain unclear. No genome-wide association studies (GWAS) have been conducted to detect the genetic variations in total energy intake. We aimed to identify genetic variants associated with total energy intake in men and women, and to determine the association between previously identified prostate cancer susceptibility loci and total energy intake. Methods: We conducted a genome-wide study using combined GWAS data from 12,031 European-ancestry women and 6,743 European-ancestry men from the Nurses’ Health Study, Nurses’ Health Study II, Health Professionals’ Follow-up Study, and Physician’s Health Study. Total energy intake was measured through validated food-frequency questionnaires. Missing genotypes were imputed using MACH with 1000 Genomes Project ALI Phase 1 Integrated Release Version 3 Haplotypes as the reference panel. We stratified by sex and adjusted for age, weight, height, physical activity and three principal components accounting for subpopulation structure. Meta-analysis based on p-values across sex was implemented. SNP set analyses, including a
weighted fixed-effects (Mendelian randomization, MR) and an unweighted random-effects approach (METASOFT), were used to test the association between prostate cancer risk loci and total energy intake using summary statistics of total energy intake from the combined GWAS and the summary statistics for genome-wide significant SNPs (Ne = 104) from a GWAS of prostate cancer in 43,303 cases and 43,737 controls [Al Olama (2014) Nat Genet]. Results: Three

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expression of CD147 in ATII cells in comparison with controls as detected by western blotting and RT-PCR. This may explain high ECM degradation in this disease. Second, we observed higher MMPs levels in plasma obtained from individuals with this disease. Third, we found significantly higher expression of cathepsin B in ATII cells isolated from emphysema in comparison with controls. Cathepsin B is an enzyme that facilitates a direct degradation of ECM proteins and activate other proteases capable of degrading ECM. Fourth, our data indicate that CD147 interacts with cathepsin B, which may lead to the activation of MMPs and emphysema progression. In summary, our results show for the first time the important role of CD147 in emphysema development. Moreover, we found that cathepsin B may contribute to CD147-mediated disease progression. Further studies will elucidate its role in lung cancer development in patients with emphysema.

#1328 Mechanism of transcriptional regulation of metabolotropic glutamate receptor-5 induced by the CXCR4 signaling pathway. Nobuyuki Kuribayashi, Daisuke Uchida, Makoto Kinouchi, Sayaka Izumi, Kyoko Kuribayashi, Hitoshi Kawamata, Dokkyo University School of Medicine, Tochigi, Japan.

We have previously demonstrated that stromal cell-derived factor (SDF)-1/CXCR4 system enhances the metastases of oral cancer cells via induction of miR-30 family which has predictive binding sites in 3′-UTR of NAs). Thus, we performed miRNA microarray in SDF-1 stimulated B88 cells. Several investigators demonstrated that activation of the ERK1/2 pathway is responsible for the transcription of several cancer-associated microRNAs (miRNAs). We postulated that SDF-1/CXCR4 downregulation may be inhibited by a MEK inhibitor, U0126, and partially inhibited by a PI3K inhibitor, wortmannin. Furthermore, these inhibitors significantly inhibited the SDF-1/CXCR4 dependent cell-migration in the presence or absence of miR-305 agonist, DHEP. Recently, several investigators demonstrated that activation of the ERK1/2 pathway is responsible for the transcription of several cancer-associated microRNAs (miRNAs). We postulated that SDF-1/CXCR4 downregulation may be inhibited by a MEK inhibitor, U0126, and partially inhibited by a PI3K inhibitor, wortmannin. Further studies will elucidate its role in lung cancer development in patients with emphysema.

#1329 Inhibition of papillary thyroid cancer cell progression by targeting skp2 via ros-erk-chop-dr5 pathways. Pratheeshkumar Poyil, Rong Bu, Abdul K. Siraj, Sandeep Kumar P, Khawla S. Al-Kuraya, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia.

S-phase kinase protein 2 (SKP2), is an F-box protein with proteasomal properties and has been found to be overexpressed in a variety of cancers. However its role in papillary thyroid cancer (PTC) has not been elucidated. Therefore, inactivation of SKP2 could be a viable strategy for the treatment of PTC. In the present study, we investigated the role of SKP2 and its ubiquitin-proteasomal pathway in PTC using a tissue microarray cohort of 1022 PTC samples, PTC cell lines and Nude mouse model. Our immunohistochemistry data showed that SKP2 was over-expressed in 75.1% of PTC cases and was clinically, significantly associated with extra thyroidal extension (p=0.0331), Tall cell variant (p=0.0070), and presence in surgical margins (p=0.0347). Bortezomib as well as SKP2 specific siRNA caused downregulation of SKP2 leading to dose-dependent growth inhibition and induction of apoptosis via mitochondrial apoptotic pathway in PTC cell lines. Furthermore, we found that treatment of PTC cells with Bortezomib caused up-regulation of DRS5 via generation of reactive oxygen species (ROS). Finally, Bortezomib treatment augmented TRAIL mediated anti-cancer effect on PTC xenograft tumor growth in nude mice. These data suggest that Bortezomib is a viable therapeutic option for the treatment of PTC either alone or in combination with other apoptotic agents such as TRAIL.

#1330 HSP90 inhibitor has a possibility to overcome imatinib resistance in gastrointestinal stromal tumors. Yurina Saito,1 Tsuyoshi Takahashi,1 Satoshi Serada,2 Minoru Fujimoto,2 Koji Tanaka,2 Yasuhiro Miyazaki,2 Tomoki Makino,1 Yukinori Kurokawa,1 Makoto Yamashita,1 Kiyokazu Nakajima,1 Shuji Kato,1 Osaka University Graduate School of Medicine, Suita, Osaka, Japan; National Institutes of Biomedical Innovation, Health and Nutrition, Ibaraki, Osaka, Japan.

[Background] Gastrointestinal stromal tumors (GIST) are the most common mesenchymal tumors of the digestive tract and are reported to harbor gain-of-function mutations in the KIT gene, which contribute to the development of sporadic GIST. HSP90 is essential in the development of targeted therapies with tyrosine kinase inhibitors and the revolutionary chemotherapeutic drug imatinib mesylate (IM). In clinical trials, the disease control rate was nearly 85%, and corresponding 2-year overall survival rates ranged from 70-80%, indicating markedly improved patient outcomes compared with anecdotal data in the pre-IM era. Despite its effectiveness, half of GISTs treated with IM develop resistance within 2 years, largely due to the accumulation of additional kinase domain mutations accompanied by concomitant re-activation of the KIT tyrosine kinase, even in the presence of IM. Heat shock protein 90 (HSP90) is one of chaperon molecules required for the proper folding, function, and stability of a number of key oncogenes, including several members of the KIT family. The aim of this study was to verify the potential efficacy of HSP90 inhibitor against IM resistant GIST. [Material and Method] We used the established human GIST cell line GIST-T1, and two IM-resistant cell lines (GIST-T1R8, GIST-T1R9), which had additional kinase domain mutations accompanied by concomitant re-activation of the KIT tyrosine kinase same as clinical samples, by exposure to IM. These resistant cell lines exhibited intracellular concentrations (>10 μM) of IM that were >1000-fold higher than the parental cell line. We investigated the cytotoxicity and signaling inhibition by HSP90 inhibitor using by the WST-8 assay, caspase3/7 apoptosis assay and western blotting. Immunobolts for IM and for IM-resistant GIST cell lines. And, it also inhibited downstream of KIT signaling, e.g. PI3K and Akt. [Conclusion] HSP90 inhibitor shows a potential efficacy for imatinib resistant GIST in vitro. It might have a possibility to apply for clinical use.

\[1331\] TGFβ1 induces breast tumor kinase expression via p38 MAPK signaling to glucocorticoid receptors. Carlos I. Santos Perez, Tarah Regan Anderson, Carol A. Lange. University of Minnesota Twin Cities, Minneapolis, MN.

Triple negative breast cancer (TNBC) is the deadliest breast cancer (BC) subtype, accounting for 20-30% of all BCs. It has a heterogeneous pathology and pathogenicity, but it is defined by the lack of estrogen receptor, progesterone receptor, and Her2 expression. Breast cancer tumor hypoxia (Brk), as a solubletyrosine kinase that is overexpressed in 85% of BCs and a driver of aggressive and metastatic phenotypes. Overexpression of Brk mRNA and protein occurs in TNBC by unknown mechanisms. The glucocorticoid receptor (GR), a very potent modulator of cytokine mediated actions of the immune system, is emerging as a mediator of chemoresistance and recurrence in TNBC. We previously demonstrated that GR signaling cooperates physiologic stress signaling mediated by hypoxia inducible factors HIF-1a and HIF-2 to modulate the expression of Brk mRNA and protein in TNBC cells. Moreover, phosphorylation of Gr at ser134 by p38 MAPK is essential for GR regulation of Brk expression. P38 is an essential Ser/Thr kinase that regulates cellular transduction of growth factors, such as Hepatocyte Growth Factor (HGF), and cytokines (e.g. TGFβ1) in TNBC cells, and was previously shown to be co-expressed with Brk in human breast tumors. Herein, we further probed mechanisms of crosstalk between key cytokines, GR, and p38 signaling in the regulation of Brk overexpression. We hypothesized that TGFβ1 signaling modulates EMT and metastasis in part by increasing the expression of Brk in TNBC. Treatment of MDA-MB-231 cells with TGFβ1 for 1, 2, 24 and 48 hours increased Brk protein expression relative to vehicle controls. Additionally, TGFβ1 increased both HIF1 and HIF2 protein levels (at 24 and 48 hours respectively). TGFβ1 regulated Brk expression at the level of mRNA, as measured using RT-PCR. Moreover, TGFβ1 synergized with activated GR to further increase mRNA levels. In contrast, mRNA levels of HIF1 and HIF2 were not modulated by TGFβ1, suggesting that the observed protein increases are due to stabilization of HIFs. Finally, TGFβ1 robustly induced p38-dependent phosphorylation of Gr at serine 134. This phosphorylation event promoted ligand-independent GR transcriptional activity at the Brk promoter. Human breast tumors significantly co-express active p38 MAPK and Brk. Our molecular model implicates TGFβ1 signaling (via p38 MAPKs) to phospho-GR in the aberrant overexpression of Brk in TNBC. We conclude that blocking of the TGFβ1 pathway may provide a strategy to inhibit Brk mediated TNBC tumor progression. This work was supported by NIH/NCI R01 CA192178 (to CAL) and T32 GM080244-24.

\[1332\] NOTCH - HEY1 pathway induces EMT in head and neck squamous cell carcinoma. Takahito Fukusumi,1 Theresa W. Guo,2 Shuling Ren,1 Akihiro Sakai,1 Mizuo Ando,1 Sunny Haft,1 Chao Liu,1 Joseph A. Califano 1.

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Background Head and neck squamous cell carcinoma (HNSCC) is noted to have a NOTCH mutation rate of 20% within the Cancer Genome Atlas (TCGA) dataset consistent with tumor suppressor gene status. However, NOTCH genes are also upregulated in wild type HNSCC and show signs of downstream activation in a substantial proportion of HNSCC. In this study, we aimed to further explore the potential for NOTCH pathway selective agents to be utilized in HNSCC. We utilized the activated NOTCH4 cell line, H9252. In vitro experiments, the SERPINE2 knockdown was related with decrease in cell motility in vitro. Data suggested that EGFR signaling promotes Wnt signaling in HER2-positive breast cancer cells. We hypothesize that this promotes cancer stem cell niche maintenance and an EMT phenotype.

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#1334 Mesenchymal cell sub-populations selectively modulate paracrine hedgehog signaling in triple negative breast cancer. Karla P. Ramos, Maribella Domenech. Univ. of Puerto Rico-Mayaguez, Mayaguez, PR.

Recent studies correlate Hedgehog (Hh) signaling with reduced survival rates in triple negative breast cancer (TNBC) patients. Activation of hedgehog signaling in the noncancerous tissue has been shown to promote tumor growth and it is a poor prognosis factor for TNBC. We developed a novel tumor-mesenchymal in vitro model of hedgehog signaling in TNBC to evaluate the role of mesenchymal cell sub-populations in the proliferative potential and stem cell markers of breast cancer cells using a custom designed multwell array. As a source of mesenchymal cell sub-populations we evaluated myofibroblasts, mesenchymal stem cells derived from bone marrow or adipose tissue and tumor cells that undergo epithelial-mesenchymal transition (EMT). Tumor cells were culture with 1 or 2 mesenchymal cell sub-populations in adjacent compartments +/− SHH ligand for 72-96hrs. Active Hh signaling was confirmed by up-regulation of main Hh target genes (e.g. GlI1, Patch1). Paracrine Hh signaling only in bone marrow-derived mesenchymal stem cell and EMT significantly increased proliferation (10-15%) of TNBC and normal breast cancer cell lines. Addition of adipose-derived mesenchymal stem cells or pharmacological Hh inhibitors (Cycloamine and Gant61) partially abolished tumor growth indicating that other non-canonical signals are involved in paracrine Hh-driven tumors. Cancer stem cell niches are highly elaborated in TNBC cell lines. Our results suggest that paracrine Hh signaling driven by bone marrow and EMT mesenchymal are potential therapeutic targets to treating TNBC.
The distribution of the phosphorylated Parkin in lung carcinoma.

Hongwei Wang, Songlin Zhang, Catherine Ma, Yuan Zhou, Abbo Maxin, Inc., San Jose, CA; UT Health Science Center at Houston, Houston, TX.

Parkin, also known as Parkinson Juvenile Disease Protein 2, together with PINK1 and Ubiquitin, form a simple cascade in the clearance of damaged mitochondria. This cascade plays an important role in maintaining mitochondrial quality control and is implicated in proteasomal degradation of toxic substrates. Recent studies have demonstrated that Parkin can be genetically altered, and aberrantly expressed in a variety of human malignancies including lung, breast, and ovarian cancers, these may indicate that Parkin is a tumor suppressor gene whose inactivation may play an important role in tumorigenesis. PINK1-dependent phosphorylation of Parkin (pS65) is a required step in Parkin activation and localization. The aims of this study were to investigate the expression of Parkin and its phosphorylated form in various type of lung carcinoma tissues and lung cancer cell lines: the correlation of the protein expression level and the phosphorylation of pS65 in lung cancer cells.

We choose two forms of the cancer materials. 1) A tissue microarray (TMA) which contains 16 cases of lung carcinoma tissues consisting of 8 adenocarcinoma (AC), 6 squamous cell carcinoma (SCC), and 2x small cell lung carcinoma (SCLC); 2) FFPE slides which contains 9x lung cancer cell lines (A549, H1703, H1975, H2228, H23, H838, HOP62, HOP92, and SKMES1). All the tissues and cells were immunostained by Parkin and phospho-Parkin polyclonal antibodies. The reference staining was performed by monoclonal anti-Cytokatan (7 (CK7) antibodies. From this study, we found Parkin expression level varies from TMA tissues and FFPE cell section which were not correlated with tumor grade. Among the 8 cases of adenocarcinoma, only 3/8 showed marked Parkin expression, 3/8 moderate, and 2/8 mild; for Parkin (pS65), only 1/8 demonstrated marked expression, 2/8 moderate, 2/8 mild, and no staining in 3/8 cases. In SCC cases, 2/6 demonstrated marked Parkin expression, 2/6 moderate, 2/6 mild. The phosphorylated Parkin (pS65) only expressed in 3/8 showed mild expression, not the others. Among 2 cases of SCLC, only one showed marked Parkin expression and less extent of Parkin (pS65), the other sample had no staining. In all FFPE cell sections, the strong expression of Parkin and Parkin (pS65) was found in the lung cancer cell lines. An interesting finding was that Parkin (pS65) strongly presented in cells of mitotic phase in these cell lines except H1703 cell line. All the TMA and FFPE slides were confirmed by CK7 monoclonal antibody staining. In summary, Parkin and its phosphorylated Parkin (pS65) were found in adenocarcinoma, squamous cell carcinoma and small cell lung cancers with differentially expressed levels, the degree of expression was not correlated with tumor grade in the tested tissues.

Phosphorylated Parkin (pS65) was strongly expressed in FFPE cancer cell lines at different degree of expression level. Among these 16 cases, the degree of phosphorylation level was not correlated with tumor grade in these 16 cases. Immunohistochemistry analysis of the clinical samples revealed higher SERCA expression, 2/6 moderate, 2/6 mild. The phosphorylated Parkin (pS65) were found in adenocarcinoma, squamous cell carcinoma and small cell lung cancers with differentially expressed levels, the degree of expression was not correlated with tumor grade in these 16 cases. Immunohistochemistry analysis of the clinical samples revealed higher SERCA expression, 2/6 moderate, 2/6 mild. The phosphorylated Parkin (pS65) were found in adenocarcinoma, squamous cell carcinoma and small cell lung cancers with differentially expressed levels, the degree of expression was not correlated with tumor grade in these 16 cases. Immunohistochemistry analysis of the clinical samples revealed higher SERCA expression, 2/6 moderate, 2/6 mild. The phosphorylated Parkin (pS65) were found in adenocarcinoma, squamous cell carcinoma and small cell lung cancers with differentially expressed levels, the degree of expression was not correlated with tumor grade in these 16 cases. Immunohistochemistry analysis of the clinical samples revealed higher SERCA expression, 2/6 moderate, 2/6 mild. The phosphorylated Parkin (pS65) were found in adenocarcinoma, squamous cell carcinoma and small cell lung cancers with differentially expressed levels, the degree of expression was not correlated with tumor grade in these 16 cases. Immunohistochemistry analysis of the clinical samples revealed higher SERCA expression, 2/6 moderate, 2/6 mild. The phosphorylated Parkin (pS65) were found in adenocarcinoma, squamous cell carcinoma and small cell lung cancers with differentially expressed levels, the degree of expression was not correlated with tumor grade in these 16 cases. Immunohistochemistry analysis of the clinical samples revealed higher SERCA expression, 2/6 moderate, 2/6 mild. The phosphorylated Parkin (pS65) were found in adenocarcinoma, squamous cell carcinoma and small cell lung cancers with differentially expressed levels, the degree of expression was not correlated with tumor grade in these 16 cases. Immunohistochemistry analysis of the clinical samples revealed higher SERCA expression, 2/6 moderate, 2/6 mild. The phosphorylated Parkin (pS65) were found in adenocarcinoma, squamous cell carcinoma and small cell lung cancers with differentially expressed levels, the degree of expression was not correlated with tumor grade in these 16 cases. Immunohistochemistry analysis of the clinical samples revealed higher SERCA expression, 2/6 moderate, 2/6 mild. The phosphorylated Parkin (pS65) were found in adenocarcinoma, squamous cell carcinoma and small cell lung cancers with differentially expressed levels, the degree of expression was not correlated with tumor grade in these 16 cases. Immunohistochemistry analysis of the clinical samples revealed higher SERCA expression, 2/6 moderate, 2/6 mild. The phosphorylated Parkin (pS65) were found in adenocarcinoma, squamous cell carcinoma and small cell lung cancers with differentially expressed levels, the degree of expression was not correlated with tumor grade in these 16 cases. Immunohistochemistry analysis of the clinical samples revealed higher SERCA expression, 2/6 moderate, 2/6 mild. The phosphorylated Parkin (pS65) were found in adenocarcinoma, squamous cell carcinoma and small cell lung cancers with differentially expressed levels, the degree of expression was not correlated with tumor grade in these 16 cases. Immunohistochemistry analysis of the clinical samples revealed higher SERCA expression, 2/6 moderate, 2/6 mild. The phosphorylated Parkin (pS65) were found in adenocarcinoma, squamous cell carcinoma and small cell lung cancers with differentially expressed levels, the degree of expression was not correlated with tumor grade in these 16 cases.
Background: Malignant Peripheral Nerve Sheath Tumor (MPNST) is a malignant sarcoma that derives from a peripheral nerve or plexiform neurofibroma. Neurofibromatosis type 1 (NF-1) patients are particularly susceptible, with a higher risk, earlier onset, and worse prognosis. The major factor associated with MPNST and NF-1 is Neurofibromin 1, coded by the NF1 gene. Novel results in RAS pathways, the main molecular hallmark of MPNST, are currently limited, with poor prognosis for metastatic or unresectable tumors. Thus, the development of promising treatment solutions for MPNST to translate to clinical trials is required. Methods: Here, we seek to identify efficacious chemotherapeutic treatments for MPNST with a combination of drug screening and biological pathway analysis. We used our previously established preclinical system to test FDA approved or promising developmental agents against five cell line models for MPNST. We screened sixty agents with diverse mechanisms of action below published maximum plasma concentrations, and measured effects with a CellTiter-Glo viability assay. Promising agents were then tested in two-drug combinations, allowing for determination of synergism. We then examined the molecular effects of the top candidates with use of antibody arrays that permit detection of a series of phosphorylated proteins. Results: The group of most efficacious drugs was enriched with agents that target factors downstream of RAS, including MEK, mTOR, and PI3K inhibitors, with microtubule inhibitors, genotoxins, and HDAC inhibitors also demonstrating good results. Strong synergism was observed across our cell line models particularly in combinations containing the dual mTORC1/2 inhibitor INK128. Interestingly, drug sensitivity varied greatly between cell lines, correlating with relative NF1 protein and RAS-GTP levels. We analyzed the activation of the RAS pathway in response to drug treatment with antibody arrays and found that, following treatment, relative phosphorylation signal was more decreased compared to controls in cell lines with lower relative NF1 protein levels. Doxorubicin was able to reduce phosphorylation signal compared to controls to a level near comparable to targeted inhibitors, which could contribute to doxorubicin’s current usefulness against MPNSTs. Importantly, we identified combination treatments that were able to greatly reduce the relative phosphorylation signal of RAS pathway members versus control. Combinations containing INK128 resulted in the most pathway shutdown. These findings suggest that MPNSTs may be susceptible to combination treatments targeting RAS pathway members. Moreover, it may be possible to use pathway analysis as a diagnostic tool to predict drug tolerance.

The current usefulness against MPNSTs is closely associated with epithelial-to-mesenchymal transition (EMT) and enhanced tumor invasiveness and metastasis. However, the mechanism(s) by which DUOX1 silencing promotes these outcomes is not well understood.

In the current study, we have discovered, using a comprehensive investigative rheostat of Stat-3 and Wnt-signaling activation. Notably, in our analysis, using mRNA and protein expression, and utilizing samples from a large cohort of patients from the same institutional repository, we found that claudin-3 expression was significantly suppressed (p<0.001 versus normal) in cancer tissues versus normal mucosa. The colon tissues from the established mouse models of colon cancer (APCMin mice and Azoxymethane (AOM)-DSS-induced colon cancer) demonstrated similar tumor specific decrease in claudin-3 expression. Interestingly, claudin-3 negative tumors retained E-cadherin expression; however, a significant and positive correlation (p<0.05) of the greater levels of claudin-3 expression with patient survival. These findings however contrasted an upregulated claudin-3 expression in other cancer types and implicated differential epigenetic regulation. In further studies, naïve claudin-3-/- mice revealed dedifferentiated (significant down regulation of P-27 (p<0.05) and vimentin expression versus WT mice) and leaky colonic epithelium. Moreover, claudin-3-/- mice demonstrated increased colon tumor burden and invasive adenocarcinoma when subjected to colon cancer. Wnt-signaling hyperactivation, albeit in GSK-3β independent manner, characterized colon cancer in claudin-3-/- mice. Claudin-3 loss also upregulated the gpl130/IL6/Stat3 signaling in colonic epithelium potentially assisted by infiltrating immune organization. Genetic and pharmacological studies indicated that claudin-3 loss promoted a significant increase in EMT-like features and Wnt-signaling activation in Stat-3-dependent manner to promote colon cancer. Overall, these novel findings indicate claudin-3 as a therapeutic target for inhibiting overactivation of Wnt-signaling to prevent CRC malignancy.

DUOX1 silencing in lung cancer is associated with enhanced nuclear EGFR localization. Andrew C. Little, Karamathullah Danyal, David Hepburn, Milena Hristova, Albert van der Vliet. University of Vermont, Burlington, VT.

Non-small cell lung cancer (NSCLC) remains to be one of the leading causes of cancer-related mortalities worldwide. The NADPH oxidase homolog, Dual Oxidase 1 (DUOX1), is an H2O2 producing enzyme located in the airway epithelium with key roles in mucosal host defense and wound repair mechanisms. Recent studies indicate that DUOX1 is epigenetically silenced in many forms of NSCLC via hypermethylation of its promoter. We previously demonstrated that DUOX1 silencing in lung cancer cells is closely associated with epithelial-to-mesenchymal transition (EMT) and enhanced tumor invasiveness and metastasis. However, the mechanism(s) by which DUOX1 silencing promotes these outcomes is not well understood. Previous findings indicate that DUOX1-dependent epithelial host defense pathways are mediated by redox-dependent activation of epithelial signaling via the non-receptor tyrosine kinase, Src, and the receptor tyrosine kinase, EGFR. We therefore hypothesized that loss of DUOX1 in lung cancer may be associated with aberrant regulation of Src and/or EGFR, tyrosine kinases that are frequently overexpressed and activated in lung cancer and strongly contribute to tumor growth and survival. In fact, recent studies have indicated that nuclear localization of EGFR in cancer cells is associated with metastatic cell behavior and poor clinical outcome, and the nuclear EGFR localization depends on Src-dependent phosphorylation of EGFR at Y1101. We observed that overexpression of DUOX1 in alveolar lung cancer A549 cells, which possess EMT-like features and in which DUOX1 is normally silenced, results in redistribution Src to the plasma membrane and decreased nuclear accumulation. DUOX1 overexpression in A549 cells also suppressed EGFR-stimulated nuclear translocation of EGFR, which was associated with reduced EGFR phosphorylation at Y1101. Conversely, RNAi-mediated silencing of DUOX1 in the epithelial cancer cell line H292, which normally expresses DUOX1 expression, was found to promote EGFR-mediated EGFR nuclear translocation and Y1101 phosphorylation. Since nuclear EGFR is thought to enhance the transcription of target genes related to cell cycle progression and proliferation (e.g. CDK1, Myc, others), we evaluated gene expression of these target genes in our cell models. Indeed, in cells lacking DUOX1, EGFR stimulation significantly enhanced mRNA levels of CDK1, Myc, and other target genes for nuclear EGFR, whereas no such induction was seen in cells that express DUOX1. Our findings indicate that DUOX1 silencing in lung cancer may be associated with worse prognosis, partly due to altered spatiotemporal regulation of EGFR and Src and increased nuclear targeting. Since both EGFR and Src are subject to redox regulation by cysteine oxidation, we are currently aiming to elucidate the molecular mechanisms by which these mechanism are affected by altered DUOX1 status.
#1344 Progranulin promotes ubiquitination, sorting and lysosomal degradation of sortilin in castration-resistant prostate cancer cells. Ryuta Tanimoto,1 Chiara Palladino,1 Simone Buraschi,1 Shi-Qiong Xu,1 Leonard G. Goelzer.2

Introduction and Objective: Despite extensive clinical and experimental studies over the past decades, the pathogenesis of prostate cancer remains largely unknown. Furthermore, the mechanisms promoting the castration-resistant stage of prostate cancer are still very poorly understood. We recently demonstrated that progranulin acts as an autocrine growth factor and promotes castration-resistant prostate cancer cell motility, invasion and anchorage-independent growth. Progranulin was also overexpressed in prostate cancer tissues via vis non-neoplastic controls. Despite the strong connections with cancer, progranulin’s mode of action is not well understood. Furthermore, proteins that regulate early stages of progranulin signaling have not been identified. Sortilin, a transmembrane protein of the Vps10 family, binds progranulin and negatively regulates progranulin signaling in cancer cells. Here, we examined the role of progranulin in the endocytic sorting and degradation of sortilin.

Methods: IF experiments in the presence or absence of lysosomal inhibitors, supplemented with hydrocarbon staples. Based on this, we hypothesized that SAH-EJ1 displays rapid clearance in vivo, limiting its efficacy. To overcome this limitation, we developed the next-generation effector, Eflapegrastim, a longer-acting, more efficacious immune cytokine-stimulating factor.

Results: In vitro, Eflapegrastim treatment of SAH-EJ1 cells resulted in receptor dimerization and intracellular trafficking. These protein-independent functions, as well as receptor homo- and hetero-dimerization, promote the formation of non-functional ErbB dimers (consisting of EGF, HER2 and ErbB3) that induce rapid, ErbB-dependent cell death. Using Cell Penetrating Peptidessynthesizedintandemwiththe
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dent cell death. Using Cell Penetrating Peptidessynthesizedintandemwiththe

#1346 Pan-ERBB inhibitor blocks tumor growth and metastasis. Joyce A. Schroeder,1 Sabrina Maisel,1 Derrick Broka2.1 University of Arizona, Tucson, AZ; 2Arizona Cancer Therapeutics, Tucson, AZ

The Epidermal Growth Factor Receptor family of transmembrane tyrosine kinases (ErbB) is over-expressed, correlates with poor prognosis and negatively correlates with disease free survival in many cancer types. EGFR, HER2 and ErbB3 are known to be highly expressed and active, and have been targeted by both kinase inhibitors and monoclonal antibodies, both with disappointing results. Importantly, though, these therapies both target either a single receptor or the kinase domain alone. It is well known that these receptor functions as heterodimers and blocking the kinase activity of the remaining family members. Furthermore, while the tyrosine kinase activity of these receptors is well known, less appreciated are the kinase-independent mechanisms by which they drive cancer progression. These include the modulation of calcium signaling and mitochondrial and nuclear translocation and activity as transcriptional co-factors. The highly conserved juxta-
membrane domain (JU) of the ErbB receptors regulates these kinase-independent functions, as well as receptor homo- and hetero-dimerization. Upon ligand binding, the JU forms anti-parallel dimers between the receptors, resulting in receptor dimerization and intracellular trafficking. These protein-protein interactions result in cell growth, survival, migration and invasion. We have previously shown that peptides mimicking this JU can act in a dominant-negative fashion, promoting the formation of non-functional ErbB dimers (consisting of EGF, HER2 and ErbB3) that induce rapid, ErbB-dependent cell death. Using Cell Penetrating Peptidessynthesizedintandemwiththe

#1345 Mutant IDH1 and tissue factor in gliomas. Dusten Unruh, Snezana Mirkov, Charles D. James, Craig Horbinski. Northwestern University, Chicago, IL.

Background: IDH1 mutations (IDH1mut) occur in 20-30% of gliomas, induce DNA hypermethylation, and are associated with a better prognosis than IDH1 wild-type (IDH1wt) gliomas, yet the basis for this remains unclear. Furthermore, venous thromboemboli (VTE) are a serious complication in glioma patients, and the mechanism for this is also unclear. We recently discovered that IDH1mut gliomas are much less likely to develop VTE compared to IDH1wt gliomas. Our data suggests that suppression of Tissue Factor (TF) may be a critical component of the less thrombogenic, and less malignant, IDH1mut phenotype. Targeting TF-PAR2 signaling may therefore repre-
sent a novel therapeutic strategy to reduce IDH1mut glioma malignancy.

#1347 In vivo efficacy of efaloprazagristatin in rats with chemotherapy-induced neutropenia. Young Hoon Kim,1 InYoung Choi,1 Prasad Kolli,2 Guru Reddy2.1 Hanmi Pharmaceuticals, Seoul, Republic of Korea; 2Spectrum Pharmaceuticals, Inc., Irvine, CA

Introduction: Efaloprazagristatin (SPI-2012, HM10460A) is a novel, long-acting recombinant human granulocyte colony-stimulating factor (rhG-CSF). Efa-
loprazagristatin consists of an rhG-CSF conjugated to a recombinant E. coli derived Fc fragment of IgG4 via a polyethylene glycol linker. Efaloprazagristatin is in clinical development for the treatment of chemotherapy-induced neutropenia in cancer patients. The purpose of this study was to evaluate and compare the efficacy of efaloprazagristatin, pegfilgrastim and filgrastim in rats with chemotherapy-induced neutropenia. Methods: Rats were treated with 30 mg/kg of cyclophosphamide (CPA) intraperitoneally to induce neutropenia. Pegfilgrastim was administered subcutaneously as a single dose of 100 μg/kg on Day 1 and filgrastim was ad-
ministered subcutaneously at a dose of 20 μg/kg daily for five days on days 1 to 5. Efaloprazagristatin was administered subcutaneously as a single dose on day 1, at doses ranging from 32 μg/kg to 322 μg/kg (or 8.8 μg/kg to 88 μg/kg as G-CSF

GDC-0032, a p110 beta-sparing PI3K inhibitor against HER2 breast cancer (BC) cell lines consisting of three activating (either helical or kinase domain) PIK3CA mutation (HER2+/breast cancer). MATERIALS AND METHODS: Four HER2+/breast cancer (BC) cell lines were analyzed for proliferation, apoptosis, cell cycle and signaling pathway activation assays. Preclinical efficacy of GDC-0032 was also evaluated in vivo in a mouse model. RESULTS: 1) All HER2+ and HER2+/PIK3CA mutant cell lines exhibited low IC50 values (ranging from 0.1 µM-1.5 µM) irrespective of ER-positive or not. 2) GDC-0032 caused a strong differential growth inhibition in both HER2+ and HER2+/PIK3CA mutated breast cancer cell lines when compared to lines that were HER2- and PIK3CA wild type by 3D-ON-TOP clonogenic assay. 3) Administration of GDC-0032 induced cell cycle G0/G1 arrest and resulted in increased apoptosis in a dose-dependent manner. Furthermore, induction of apoptosis was more with GDC-0032 when combined with RAD001. 4) GDC0032 also blocked expression of CYCLIND1. 5) Investigation of the signal transduction revealed that the treatment of GDC-0032 reduced the level of p-AKT (Ser473 and Thr308), and p-S6 expression is indicating the down-regulation of downstream signaling of PI3K and mTOR pathway. 6) GDC-0032 was highly active at reducing established tumor growth in both BC mouse xenografts harboring PIK3CA mutation and HER2 amplification. CONCLUSION: Our in vitro or in vivo data showed that GDC-0032 was highly efficient either as a single agent as well as with RAD001 in HER2+ / PIK3CA mutated breast cancer cell lines. Our data suggest that GDC-0032 represents a novel therapeutic option in patients harboring PIK3CA mutations and/or HER2/neu gene amplification in breast cancer.

#1349 NSAIIDs: Multiple roles in multiple environments. Gloria M Calaf1 and Debasish Roy2. 1Instituto de Alta Investigacion, Universidad de Tarapacá, Arica, Chile; 2Department of Natural Sciences, Hostos College, The City University of New York, Bronx, NY.

Nonsteroidal drugs are mainly known for their activities and use as anti-inflammatory, antipyretic compounds. But, in recent observations, they are exposed to use as an effective alternative compounds to prevent or stimulate different metabolic activities and help in preventing various neoplastic progression. They have specific role in controlling estrogen metabolism during breast cancer progression. Sometimes, they are used as an apoptotic induction in various cancers. Their role in hypoxia induced proliferation is also showing promising results. Different NSAIIDs will help to induce the activities of various tumor suppressor genes. As chronic inflammation increases the risk for various cancers, therefore, it is important to eliminate inflammation through anti-inflammatory compounds where NSAIIDs is playing a vital role. Most of them are acting to prevent inflammation either via selective or non-selective COX based mechanism. The non-selective COX inhibitor sulindac and the COX-2 selective inhibitor etodolac etc. have been shown to prevent the formation and cause regression of adenomas in patients with familial polyposis. Unfortunately, COX-1 and/or COX-2 inhibition and depletion of physiologically important prostaglandins is associated with gastrointestinal, renal and cardiovascular toxicities that limit the use of NSAIIDs and COX-2 inhibitors for cancer chemoprevention. In this particular study, we are using various NSAIIDs in a radiation-induced estrogen treated breast cancer model to establish their role in differential expression of various cancer related genes selected from different cell cycle pathways microarray. Selection of biomarkers from these altered genes will help to develop a valid strategy for cancer prevention through precision based targeted therapy. Supported by Office of Academic Affairs, Honors Program, COB!, Hostos-CUNY, NY, USA (DR) and Tarapacá University, Arica, Chile (GMC).


Uveal (eye) melanoma is a highly aggressive cancer, in which almost half of patients develop distant metastases that are refractory to therapy. In particular, metastatic uveal melanoma has been clinically unresponsive to the immunotherapeutic agents that have shown success in skin tumors, making the need for novel therapeutic approaches to uveal melanoma all the more urgent. Unlike skin melanomas, which are driven by BRAF and NRAS mutations, uveal melanomas arise typically from mutations that result in constitutive activity of the alpha subunit of the heterotrimeric G-protein, Gq, or its paralog G11. The prevalence of constitutively active Gq/11 in uveal melanoma suggests a dependence of these tumors on Gq/11 activity that could be exploited therapeutically. To address this hypothesis, we are using a potent, bioavailable small molecule that binds to and inhibits Gq/11 to target constitutively active Gq/11 in uveal melanoma cells. This inhibitor functions by sequestering wild type or constitutively active Gq/11 in an inactive state. We first used inositol phosphate accumulation assays and confirmed inhibition of both wildtype and constitutively active Gq/11 by the inhibitor in all uveal melanoma cells. We then assayed the effect of Gq/11 inhibition on overall viability of uveal melanoma cells, and found that uveal melanoma cells with mutant Gq/11 were highly sensitive to the small molecule; whereas, uveal melanoma cells with wildtype Gq/11 showed no loss of viability, even at 1000-fold higher concentrations of inhibitor. In Gq-mutant uveal melanoma cells, Gq/11 inhibition caused cell cycle arrest in G1, and dysregulation of several cell cycle regulatory pathways. Inhibitor treatment also caused Gq/11-driven uveal melanoma cells to become more differentiated, as indicated by increased pigmentation, elevated expression of melanin synthesis and melanosome markers, changes in cell morphology and changes in melanocyte versus melanoma gene programs. None of these phenotypic changes were seen in BRAF-driven uveal melanoma cells treated with the Gq/11 inhibitor, demonstrating that the effects of this inhibitor were exclusively dependent on the constitutively active Gq/11 oncogene. These results establish that Gq/11 is a druggable target in uveal melanoma cells, and show that Gq/11-mutant uveal melanoma cells are exquisitely sensitive to inhibition by small molecule inhibitors. We are currently transitioning these studies to animal models to establish drug efficacy and toxicity and explore treatment and delivery options.

#1351 Alternative splicing of neurofibromin 1 is associated with elevated MAPK activity and poor prognosis in glioma. Robert Siddaway, Arun Ramani, Man Yu, Michael Brudno, Cynthia Hawkins. The Hospital for Sick Children, Toronto, Ontario, Canada.

High-grade gliomas (HGG) are invasive with poor prognosis regardless of age: diffuse intrinsic pontine gliomas (DIPG), arising in the brainstem, are almost universally fatal and the leading cause of brain-tumor death in children; while adult anaplastic astrocytoma and glioblastoma multiforme (GBM) have median survivals of 1-3 years. The mutational spectra of adult and pediatric HGG differ, with pediatric tumours containing recurrent mutations of H3F3A and HIST1H3B. However, alterations leading to RAS/MAPK/PI3K pathway activation, including PDGFRA amplification, EGFVIII, BRAF-V600E, NFI deletion, are frequently found, although not all tumours will have mutations in this pathway. The neurofibromin 1 (NFI1) gene negatively regulates RAS signalling by stimulating RAS-GTP turnover, thereby leading to RAS-inactivation. The two major isoforms, NFI-1 and NFI-1I, differ only by inclusion of the 21 aa exon23a in the GAP-related domain of NFI-1I. Exon23a-inclusion has been shown to render NFI 10 times less active towards RAS, leading to elevated MAPK signalling. The brain expresses predominantly NFI-1, while the major isoform elsewhere is NFI-1I. Here we used RNA-Seq to identify genes alterna-
tively spliced between DJIPG and normal brain, identifying an isoform switch from NF1-1 in normal brain to NF1-II; we additionally found the same isoform switch in the TCGA adult GBM and LGG cohorts. For both GBM and LGG, RAS/MAPK/P38K wild-type tumors with elevated NF1-II conferred significantly reduced patient survival compared to RAS/MAPK/P38K mutant tumors. NF1-exon9 deletion was known to be repressed in cancer cell models by hub proteins like ELAV-like family of splicers regulators. We further show that members of these gene families are downregulated in HGG. Together, our results indicate a novel mechanism by which gliomas can activate signaling downstream from RAS independent of mutations and tumor grade, which promotes tumorigenesis by regulating pathways such as proliferation and invasion.

#1352 The importance of the RASA1/R-Ras/Ral-A signaling axis in melanoma tumorigenesis. Kristen Suzanne Hill, Xue Wang, Youngchul Kim, Min-jung Kim. Moffitt Cancer Ctr., Tampa, FL.

The Ras family of small GTP binding proteins are frequently activated by mutations in melanoma, as shown for NRAS (20%), KRAS (2%) and HRAS (1%). Ras isoforms can also be activated by inactivation of Ras GT-Pase activating proteins (RasGAPs), such as NF1, RASA1, and RASA2. In our recent study, we observed that inactivation of RASA1 (RAS p21 protein activator 1, also called p120RasGAP) suppressed melanoma via its RasGAP activity toward the R-Ras (related Ras viral (r-ras) oncogene homolog) isoform and that R-Ras was required to promote anchorage-independent growth driven by RASA1 inactivation. Moreover, a low level of RASA1 mRNA expression is associated with decreased overall survival in melanoma patients with BRAF mutations. Based on these observations, we hypothesized that, although not mutated, R-Ras is activated in melanoma by inactivation of RasGAPs and that BRAF activation cooperates with this RasGAP/R-Ras pathway activation in melanoma tumorigenesis. In this study, we addressed the importance of R-Ras, a previously less appreciated member of the Ras small GT-Pases family, in melanoma tumorigenesis. We observed frequent activation of R-Ras in BRAF mutant human melanoma cell lines. In addition, RNAi mediated reduced expression of R-Ras suppressed anchorage-independent colony growth and tumor growth. Moreover, among the 3 major Ras effector pathways, reduced R-Ras expression suppressed Ral-A activation, which may explain the mechanism of Ral-A activation in BRAF mutant melanoma. Interestingly, anchorage-independent growth driven by R-Ras activation downstream of RASA1 inactivation was suppressed by both genetic (siRNA targeting Ral-A) and pharmacological (Ral inhibitor BQ579) inhibition of Ral-A. To further investigate the impact of RASA1 loss, and thus R-Ras activation, on BRAF mutant melanoma development in vivo, we generated a RASA1+/−; BRAFCA/CA; Tyr-CreERT2 mouse model in which treatment with 4-hydroxytamoxifen (4-OHT) results in expression of constitutively activated mutant BRAF and deletion of RASA1 in melanocytic lineage cells. Preliminary analysis shows hyperpigmentation of the ear, tail, and foot pad in RASA1+/−; BRAFCA/CA; Tyr-CreERT2; H9262 mice compared to RASA1−/−; BRAFCA/CA; Tyr-CreERT2 mouse littermates. Tumors generated in this animal model will be analyzed to determine the extent of R-Ras and Ral-A activity in vivo. This study demonstrates the importance of the RASA1/R-Ras/Ral-A signaling pathway in BRAF mutant melanoma and supports the possible combinatorial treatment strategy targeting both the BRAF/MAPK and Ral signaling pathways.

#1353 Polysoprenylated cysteine amide inhibitors disrupt actin cytoskeleton organization, induce cell rounding, and block invasion of NSCLC. Elizabeth Ntantie, Jerrine Fletcher, Felix Amisah, Olufısayo O. Salako, Auguste T. Nkembo, Rosemary A. Poku, Nazarius S. Lamongo. Florida A&M University, Tallahassee, FL.

Non-small Cell Lung Cancer (NSCLC) malignancy is dependent on cellular processes that promote metastasis. F-actin organization is central to cell migration, invasion, adhesion and angiogenesis which are all processes involved in metastasis. F-actin remodeling is enhanced by the expression and activity of some members of the Rho family of small GTPases. Therefore, agents that mitigate hyperactive Rho proteins may be clinically relevant for controlling metastasis. We previously reported the synthesis and characterization of polysoprenylated cysteine amide inhibitors (PCAs) as potential inhibitors of the cancer phenotype. In this report, we investigate the potential role of PCAs against NSCLC malignancy and show that as low as 0.5 μM PCAs significantly inhibit 2D and 3D NCI-H1299 cell migration by 48% and 45%, respectively. PCAs at 1 μM inhibited 2D and 3D NCI-H1299 cell invasion through Matrigel by 50% and 85%, respectively. Additionally, exposure to 5 μM of the PCAs, NSL-BA-040 caused a 38% drop in F-actin intensity at the cell membrane 24 h post treatment. Importantly, this drop in F-actin was accompanied by a 73% reduction in the number of filopodia per cell. Interestingly, the polysoprenyl group of the PCAs is essential for these effects, as NSL-100, an analog that lacks the farnesyl moiety, does not elicit similar effects on F-actin assembly and organization. Put together, our findings indicate that PCAs disrupt F-actin assembly and organization to suppress cell motility and invasion and point towards a potential role of PCAs as effective therapies for NSCLC metastasis and invasion.

#1354 The role of RAL-A in soft tissue sarcoma tumor growth and metastasis. Steven T. Szineore, Gina M. Sizenore, Reena Shaya, Peter Amaya, Anisha M. Hammer, Alexander H. Rice, Jeffrey J. Chalmers, Michael C. Os- trowski, Arnab Chakravarti, "The Ohio State University, Columbus, OH; Denison University, Granville, OH.

Soft tissue sarcomas (STS) are a diverse collection of cancers of mesenchymal origin arising from the connective and supportive tissues of the body. While localized STS are well managed by surgery and radiation; metastasis, particularly to the lung, is frequent. More than 30% of adult STS patients develop lung metastases and the 5-year survival for these patients is a dismal 16%. Treatment options for metastatic STS are limited, thus there is an urgent unmet need for a better understanding of the key molecular pathways that drive metastatic spread in STS and identification of inhibitors of these pathways for clinical application. Through analysis of gene expression data from metastatic STS patient samples, we identified decreased expression of PPP2R1B as a hallmark of metastatic STS. To directly test its function as a suppressor of tumor growth and metastasis in STS, PPP2R1B was stably over-expressed in HT1080 cells, a model of metastatic STS. PPP2R1B expression almost completely abolished HT1080 tumor growth in nude mice. PPP2R1B is a subunit of the PP2A protein phosphatase complex that negatively regulates numerous cancer signaling pathways. However, the functional consequences of decreased PPP2R1B expression in STS are unknown. A combination of high-throughput and targeted approaches were utilized to identify 37 phosphoproteins that are significantly dephosphorylated following PPP2R1B expression in HT1080 cells. One of these phosphoproteins, the small GTPase RAL-A, exhibited decreased phosphorylation on Ser194 following PPP2R1B expression. RAL-A is significantly prognostic of STS metastasis and is elevated in more aggressive STS subtypes relative to less aggressive subtypes and normal tissue. RALA knockdown in HT1080 significantly slowed tumor growth and decreased the incidence of pulmonary metastasis, mirroring PPP2R1B overexpression. Importantly, RALA is an actionable therapeutic target for improved treatment of STS. Aurora A inhibitors indirectly inhibit RALA function by preventing RALA Ser194 phosphorylation by aurora A. We found that RALA expression and activity predicted response of STS cell lines to aurora A inhibition. Excitingly, the aurora A inhibitor alisertib nearly eradicated growth of HT1080 tumors in vivo. Exploration of the biological mechanisms through which RALA regulates STS metastasis identified regulation of vesicular traffic as a likely critical function of RALA in this process. These findings identify PPP2R1B, RALA, and aurora A as members of a key novel pathway that drives STS progression and advocate the use of treatments targeting this pathway to improve outcome for STS patients with advanced disease.


The KRAS oncogene is more frequently mutated in over one fourth of human cancers. Despite decades of work studying known effector pathways, no drugs in clinical use specifically target KRAS-mutant tumors. Uncharacterized feedback mechanisms and parallel pathways have stymied the treatment of KRAS-mutant tumors in patients, and the KRAS protein does not easily accommodate the binding of inhibitory small molecules. These challenges demand better characterization of the physical and genetic relationships between Ras regulators and effectors. To that end, we used tandem affinity purification of Kras, Hras and Nras and to identify quantitative changes in the growth of the small GTPase RAL-A in melanocytic lineage cells. Preliminary analysis shows hyperpigmentation of the ear, tail, and foot pad in RASA1+/−; BRAFCA/CA; Tyr-CreERT2; H9262 mice compared to RASA1−/−; BRAFCA/CA; Tyr-CreERT2 mouse littermates. Tumors generated in this animal model will be analyzed to determine the extent of R-Ras and Ral-A activity in vivo. This study demonstrates the importance of the RASA1/R-Ras/Ral-A signaling pathway in BRAF mutant melanoma and supports the possible combinatorial treatment strategy targeting both the BRAF/MAPK and Ral signaling pathways.

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### #1356 Variable drug responses characterize the functional heterogeneity of NF1 null tumors. Daniela Pucciarelli, Ganesh Krishnamurthi, Steve Braunstein, Jean L. Nakamura. UCSF, San Francisco, CA

Introduction: Neurofibromatosis type 1 (NF1) is a autosomal dominant disease with a predisposition to cancer. Biallelic inactivation of the NF1 gene increases the risk of developing brain tumors, leukemia, neurofibromas and malignant peripheral nerve sheath tumors (MPNSTs). Somatic mutations in the NF1 gene are also associated with sporadic malignancies including glioblastoma, neuroblastoma, and melanoma. The NF1 tumor suppressor gene encodes the RAS GTPTase-activating protein (GAP) neurofibromin. Loss of NF1 results hyperactivation of Ras, MAPK and PI3K signaling pathways components, representing cell cycle therapeutic targets. Here we investigate if the variable responses of NF1 null tumors to MAPK and mTOR inhibitors suggests the intrinsic heterogeneity of NF1 mutant tumors. We hypothesized that defining alternative mechanisms and functional sub-classes of NF1-mutant tumors on the basis of their variable drug sensitivity will produce pre-clinical therapies data that will inform clinical trials. Methods: We previously generated mouse models in which we mutagenized NF1 heterozygous mice with radiation, recapitulating the susceptibility of patients with NF1 to radiation-induced cancers. These models produced solid tumors such as mammary carcinomas, squamous cell carcinomas, and soft tissue sarcomas, which we determined to be NF1 null. Cell lines established from these tumors were characterized by a drug sensitivity screen using a custom 94 compound drug library. Drug responses indicate that NF1 mutant tumors organize into functional groups based on differential sensitivities. Six candidate drugs each targeting distinct components of MAPK and PI3K signaling pathways were selected from the drug library. After 24 hours of exposure to each inhibitor or control, cells were analyzed for cell proliferation, cell cycle changes, and cell death. Western blotting were performed to determine whether drug exposures produce alterations in their predicted biochemical pathways such as PI3K/Akt, and MAPK pathways. Results: NF1 loss did not predict uniform sensitivity of cell lines to treatment with MAPK and mTOR inhibitors. Each compound induced differential effects on viability of NF1 null tumors cell lines. High variability and cell line-dependent cytotoxic and cyto-static effects were also observed. Although phosphorylated Akt(Y473), S6, and p44/42 MAPK varied widely among all untreated NF1 null cell lines, this did not predict their drug sensitivity. In vitro drug sensitivity data indicated heterogeneous differential sensitivities of NF1 mutant tumors to different drug classes, independent of tumor histology, permitting segregation into functional groups. Conclusion: Tumor cell lines driven by NF1 loss demonstrate heterogeneous responses to Ras pathway inhibition, which may be explained by mechanisms of tumor formation after NF1 loss involve multiple alternative pathways.

### #1357 The small GTPase Arf6 potentiates melanoma metastasis by activating Akt. Lehi Acosta,1 Aaron Rogers,1 Jae H. Yoo,1 Shannon J. Odelberg, Dean Y Kim,1 Albert H. Grossmann.1 University of Utah, Salt Lake City, UT; 2Huntsman Cancer Institute, Salt Lake City, UT.

Arf6 is a member of the Ras-superfamily of small GTPases and controls membrane trafficking and cytoskeletal remodeling, functioning mainly in endocytosis pathways at the cell periphery. Arf6 is activated by various extracellular signals and oncogenic events and has been shown to promote cell migration and pro-invasive phenotype in human cancer cells. Small molecule inhibition of Arf6 reduces spontaneous metastasis in xenograft models of human cutaneous melanoma, suggesting that Arf6 is necessary for disease progression. Using a genetically-engineered mouse model of BRAF-mutant melanoma, we determined whether activation of Arf6 is sufficient to induce spontaneous metastasis in vivo. For melanocyte-specific primary tumor induction, Cre recombinase was delivered via local injection of RCAS virus into DCT-TVA;Cdkn2alox/lox;BRAFV600E mice. BRAFV600E mice. In the experimental mice, a constitutively active mutant form of Arf6 (Q67L) was virally delivered with Cre. In this study, we observed a significant increase in spontaneous metastatic disease burden in Arf6 Q67L mice. Likewise, tail vein injection of melanoma cell lines derived from Arf6 Q67L tumors consistently show a diffuse pattern of pulmonary metastasis compared to parental tumors of a rapid, micrometastatic disease. Arf6 Q67L. Recently, it has been demonstrated that activation of Akt, but not Pten loss, leads to an aggressive phenotype in melanoma that includes the acquisition of brain metastases. Immunohistochemical analysis of phospho-Akt revealed that Arf6 Q67L is sufficient to induce Akt activation in tumors. In addition, ARF6 is necessary for Akt activation in human melanoma lines. We did not observe brain metastasis in DCT-TVA;Cdkn2alox/lox;BRAFV600E Arf6 Q67L mice. When we added Pten-/- to this genetic background, however, we observed microscopic brain metastases at a low frequency, suggesting that the combination of Pten loss and Arf6 activation reaches a threshold level of Akt activation that is sufficient to cause brain metastasis. Taken together our data indicate that activation of Arf6 is sufficient to potentiate melanoma metastasis, consistent with the proinvasive cellular phenotype attributed to Arf6. In addition, our data suggests a novel signaling mechanism by which Arf6, a small GTPase involved in trafficking, is somehow involved in Akt activation and that this step may be important for the acquisition of metastatic capacity.

### #1358 p21 activated kinase 4 (paka4) as a novel therapeutic target for non–hodgkin’s lymphoma. Asfar S. Azmi,1 Amro Aboukameil,1 Iffahina Muqbil,1 Yiwei Li,1 William Senapedis,2 Erkan Baloglu,2 Yosef Landesman,2 Michael Kaufman,2 Sharon Shacham,2 Ayad Al-Katib,1 Ramzi M. Mohammad1.

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Objective: The p21-activated kinase 4 (PAK4) is a key downstream effector of the Rho GTPase family and is over-expressed in many different cancer types. PAK4 protein, by virtue of its ability to engage multiple ligands, regulates a repertoire of signaling pathways. A survey of non-Hodgkin’s lymphoma (NHL) cell lines shows that there is increase in PAK4 mRNA and/or protein expression when compared to normal peripheral lymphocytes (PBL). Considering PAK4 RNA interference suppresses lymphoma cell proliferation, these findings point to a novel role for PAK4 in promoting NHL cell growth. To this end we examined the impact of the newly developed PAK4 allotropic modulators (PAMs) on NHL proliferation both in vitro and in vivo. Methods: WSU-FSCCL (representing follicular small cell cleaved lymphoma) and WSU-DLCL2 (diffused large B-cell lymphoma) were exposed to increasing concentrations of different PAM analogs (KPT-7523, KPT-7189, KPT-9037, KPT-9274, or KPT-7010 [inactive]) or the Pan-PAK inhibitor, PF-3783089, in the presence or absence of CHOP (used at 10 mg/kg for 72 hrs). Following combination treatment with either PAM or PF-3783089, cells were evaluated using Trypan Blue, apoptosis was analyzed using 7AAD, tetramerase staining, Annexin V FITC and cell cycle arrest was accessed by flow cytometry. Protein and mRNA expression changes were evaluated using immunoblotting and RT-PCR. The toxicity and efficacy of PAMs were evaluated in sub-cutaneous and disseminated xenograft models of NHL. Results: As single agents, PAMs show anti-proliferative activity in vitro against NHL cell lines (IC50 for WSU-FSCCL = 50 nM and WSU-DLCL2 = 250 nM) while sparing normal PBL (IC50 in μM range). There was a statistically significant dose-dependent difference in apoptosis induction in NHL cell lines treated with PAMs when compared to vehicle control. PAMs reduced total p-PAK4 and downstream signaling proteins involved in proliferation and apoptosis. In R-CHOP combination studies we observed enhanced viability suppression, increased apoptosis, and concurrent down-regulation of PAK4 signaling pathway proteins when compared to any single agent alone. The clinical compound, KPT-9274, is well tolerated and showed remarkable anti-tumor activity in WSU-DLCL2 sub-cutaneous xenograft mice (p < 0.01 at 140 mg/kg/d for 4 weeks with no loss in body weight). Residual tumors analysis showed suppression of PAK4 signaling pathways. Single agent and R-CHOP combination efficacy is currently being evaluated in subcutaneous and systemic WSU-FSCCL and in primary patient derived xenografts in mice. Conclusions: This is the first study demonstrating a role for PAK4 in diffuse large B-cell and follicular small cell cleaved NHL. Our data shows that inhibition of PAK4 could become a viable therapy for NHL either alone or in combination with R-CHOP. Our data is directly applicable to the current Phase 1 trial of KPT-9274 in patients with advanced solid malignancies or NHL.

### #1359 How does RAC1 GTPase signal Wnt-beta-catenin pathway-mediated integrin-directed metastasis-associated tumor cell phenotypes in TNBC. Nandini Dey, Jennifer H. Carlson, Casey Williams, Tyler Jepperson, Pradip De, Brian Leyland-Jones. Avera Research Inst., Sioux Falls, SD.

Introduction: We reported that Wnt Pathway (WP) upregulation, one of the salient genetic features of metastatic TNBC, controls the acquisition of integrin-directed metastasis-associated (ID-MA) phenotypes of TNBC cells (Dey et al., 2013). RAC-GTPTases, small G-proteins which transduce signals from cell surface proteins including integrins, have been implicated in tumorigenesis and metastasis by their role in essential cellular functions like motility. Aim: Here we tested the role of the mechanism of involvement of RAC1 in the regulation of WP-mediated ID-MA phenotypes in TNBC cells and examined how WP signals are transduced via RAC1 in the context of ID-MA phenotypes in TNBC. Method: Oncoprofiles showed that the major alterations of RAC1 gene in TNBC were constituted by the amplification/gain of the gene. The collective percentage of alteration(s) in RAC1 in ER+ve BC was 35% as compared to 57% in ER-ve BC (brca/tcga/pub2015). Using pharmacological agents (salindac sulfone), genetic tools (siRNA), WP modulators (Wnt-C59, XAV939), RAC1 inhibitors (NSC237636, WS6) and WP stimulations (LWnt3ACM, Wnt3A recombinant) in a panel of 6-7 TNBC cell lines, we studied fibronectin-directed and various motility parameters (actin dynamics, filopodia and lamellipodia by confocal microscopy) in the context of hibronectin-directed RAC1 and Cdc42 acti-
and tumor growth in vivo. In addition, our data showed that PAK3 regulated the motility and invasion. In our study, we showed that knockdown of PAK3, but also/ or hyperactivated in pancreatic cancer, and promotes pancreatic cancer cell death. Previous studies have shown that PAK1 and PAK4 are upregulated in activated mechanisms, six PAKs are classified into two groups, PAK1-3 (group I) and PAK 4-6 (group II). PAK kinases are frequently overexpressed in various human tumors and represent therapeutically relevant targets for cancer treatments. Previous studies have shown that PAK1 and PAK4 are upregulated and/ or hyperactivated in pancreatic cancer, and promotes pancreatic cancer cell motility and invasion. In our study, we showed that knockdown of PAK3, but not that of PAK1 or PAK2, inhibited pancreatic cancer cell proliferation in vitro, and tumor growth in vivo. In addition, our data showed that PAK3 regulated the protein stability of β-catenin via Akt/GSK-3β signaling pathway in pancreatic cancer cells. The role of PAK3 in regulating Akt/GSK-3β phosphorylation was further confirmed by the ectopic expression of wild-type versus kinase-dead (K297L) PAK3. Equally important, the mammosphere formation, aldehyde dehydrogenase (ALDH) activity and cancer stem cell-associated markers, were also down-regulated in PAK3 knockdown cells, suggesting the involvement of PAK3 in regulating cancer stem cell-like properties in pancreatic cancer cells. Together, these findings suggested that PAK3 as a primary regulator of Akt/GSK-3β/β-catenin signaling for maintaining cancer stem cell phenotypes and promoting tumor growth, which underlying the potential of targeting PAK3 in fostering new therapeutic strategies for pancreatic cancer.

#1360 Novel function of p21-activated kinase 3 (PAK3) in regulating Akt phosphorylation and pancreatic cancer stem cell phenotypes. Hsing-Yu Wu,1 Ming-Chen Yang,2 Po-Chen Chu,3 Samuel K. Kulp,3 Ching-Shih Chen1. 1Acedemia Sinica, Taipei, Taiwan; 2The Ohio State University, Columbus, OH.

p21-activated kinases (PAKs) are important effectors of the Rho family GTPases and has been implicated in cytoskeletal remodeling, cell proliferation, apoptosis, and transformation. Based on the sequence, structure homology, and activation mechanism, six PAKs are classified into two groups, PAK 1-3 (group I) and PAK 4-6 (group II). PAK kinases are frequently overexpressed in various human tumors and represent therapeutically relevant targets for cancer treatments. Previous studies have shown that PAK1 and PAK4 are upregulated and/or hyperactivated in pancreatic cancer, and promotes pancreatic cancer cell motility and invasion. In our study, we showed that knockdown of PAK3, but not that of PAK1 or PAK2, inhibited pancreatic cancer cell proliferation in vitro, and tumor growth in vivo. In addition, our data showed that PAK3 regulated the protein stability of β-catenin via Akt/GSK-3β signaling pathway in pancreatic cancer cells. The role of PAK3 in regulating Akt/GSK-3β phosphorylation was further confirmed by the ectopic expression of wild-type versus kinase-dead (K297L) PAK3. Equally important, the mammosphere formation, aldehyde dehydrogenase (ALDH) activity and cancer stem cell-associated markers, were also down-regulated in PAK3 knockdown cells, suggesting the involvement of PAK3 in regulating cancer stem cell-like properties in pancreatic cancer cells. Together, these findings suggested that PAK3 as a primary regulator of Akt/GSK-3β/β-catenin signaling for maintaining cancer stem cell phenotypes and promoting tumor growth, which underlying the potential of targeting PAK3 in fostering new therapeutic strategies for pancreatic cancer.


Claudin perforins lethal toxin (Tpel) belongs to the family of large claudin glycosylating cytotoxins. Claudin toxins are glycosyltransferases that modify and deactivate small GTPases of the RHO and RAS subfamily. Tpel mono-glycosylates in the switch I domain (Thr35) of RAC and RAS small GT-Pases. Glycosylation of RAS at Thr35 prevents binding to the primary effector, RAF kinase and results in a blockade RAS signal transduction. RAS family proteins function as key regulators of cell proliferation, differentiation, survival and gene expression. Moreover, mutations in RAS proteins are highly prevalent in human cancers. Considering the specificity of Tpel for RAS, we decided to investigate the biochemical interaction between Tpel and RAS, and potentially develop tools to disrupt the RAS signaling pathway. In order to assess the effect of Tpel on RAS-dependent cell proliferation and adhesion, we utilized a Raf-1 RAS binding domain (RBD) pulldown method, we found that Tpel treatment did not affect the viability of the BREF V600E cells and only induced toxicity in RAS expressing cells. Consistent with this result, Tpel treatment inhibited MAPK signal transduction in the RAS expressing cells, but not in the BREF V600E cells. Surprisingly, both cell viability as well as pERK levels remained unaffected in the KRAS Q61R MEF cells treated with Tpel, suggesting that KRAS Q61R is resistant to glycosylation. This may be due in part to the relatively slow intrinsic rate of GTP hydrolysis and very high levels of KRAS-GTP in KRAS Q61R cells. We also developed and adapted biochemical assays to study Tpel-KRAS interaction in vitro. Based on Alpha Assay and UDP Glow, Tpel activity does not appear to be nucleotide specific, in contrast to previously reported findings. We are currently testing if Tpel activity is competitive with or is inhibited by other molecules that bind to switch I, such as the RBD domain of RAF1 or the Ras-binding domain of AGO2. Our data indicate that the KRAS Q61R expressing MEF cells is due not to the high levels of RAS-GTP, but to the constitutive binding to effector molecules, which prevent Tpel from accessing KRAS. We also hope to identify the critical residues required for Tpel binding and catalysis. By elucidating the mechanism of substrate binding and substrate specificity we may gain insight into novel binding pockets that could be exploited therapeutically.

#1362 Argonaute 2 controls RAS activation in mouse embryonic fibroblasts. Ronald F. Siebenaler, Sunita Shankar, Vijaya L. Dommeti, Malay Mody, Arul Chinnaiyan. University of Michigan Medical School, Ann Arbor, MI.

The RAS gene family is among the most commonly mutated genes within cancer, and while much research has elucidated the major downstream pathways, including MAPK and PI3K, little progress has been made in successfully targeting mutant RAS in cancer. We recently identified an interaction between the N terminal domain of Argonaute 2 (AGO2), a core component of RNA-induced silencing complex (RISC), and the Switch II domain of KRAS. Furthermore, this interaction was found in all cell lines tested, expressing either wild-type (WT) or mutant KRAS. We found that stable knockdown of AGO2 in KRAS dependent cell lines lead to a decrease in KRAS protein expression with a subsequent decrease in KRAS activity. Conversely, the overexpression of AGO2 in these cells lead to both an increase in KRAS expression and oncogenesis. In addition, this interaction inhibits the RNAi function of AGO2 by preventing microRNA unwinding in the presence of oncogenic KRAS compared to WT-KRAS. Despite a clear association between mutant KRAS and AGO2 mediating increased KRAS mediated oncogenesis, the precise function of this interaction remains unclear in normal physiology. In order to better assess the endogenous function of the KRAS-AGO2 interaction, we analyzed two mouse embryonic fibroblast cell lines (NIH 3T3 and MEF) with complete knockout of AGO2. Utilizing a Raf-1 RAS binding domain (RBD) pulldown method, we assessed activated WT-RAS levels in AGO2 null NIH 3T3 and MEF cells. We found that knockout of AGO2 lead to an increase in WT RAS-GTP activation compared to normal control cells. Immunoblot analysis also indicates that AGO2 null fibroblasts lead to increase in RAS downstream signaling through the MAPK/ERK and PI3K/AKT pathways. Furthermore, rescue of AGO2 knockout using full length mouse AGO2 decreased wild type RAS activation and its downstream signaling. Taken together, these observations suggest that the AGO2 interaction may suppress WT-KRAS activation, leading to maintenance of RAS-GDP levels. Using RNA-seq, microRNA and protein hybridization, we began to identify the pathways that may be involved in RAS activation in AGO2 null cells. Early analyses indicate that AGO2 controls WT-KRAS levels and activity through multiple mechanisms, laying the foundation for a better understanding of the RAS-AGO2 interaction in normal physiology.

#1363 RSK2 provokes invasive signaling in glioblastoma through LARG-dependent activation of Rho GT-Pases. Geng-Xian Shi,1 Ling Jin,1 Michelle L. Matter,2 Santosh Kesari,2 Joe W. Ramos,3 Univ. of Hawaii Cancer Ctrl., Honolulu, HI; 3John Wayne Cancer Institute, Honolulu, HI.

Malignant gliomas are one of the most aggressive and deadly forms of cancer and can affect any age group. In glioblastoma multiforme (GBM), infiltration of primary tumor cells into the normal tissue and dispersal throughout the brain is a central challenge to successful treatment that remains unmet. Patients with malignant gliomas respond poorly to the standard therapeutic regimen of radiotherapy and chemotherapy that follow tumor resection and have only a 16-month median survival. It is therefore imperative to identify new approaches to specifically attack GBM cell survival, proliferation and invasion. The cellular mechanisms driving GBM-mediated migration and invasion are not fully understood. RSK2 (p90 ribosomal S6 kinase 2) is a kinase that regulates proliferation and adhesion and can promote metastasis. We find that RSK2 is significantly upregulated in vivo in human GBM patient tumors, and that high RSK2 expression significantly correlates with advanced tumor stage and poor patient survival. We demonstrate that active RSK2 regulates GBM adhesion and is essential for cell motility and invasion of patient-derived GBM neurosphere. Importantly, inhibition of RSK2 by either RSK inhibitors or shRNA silencing impairs invasion and combining RSK2 inhibitors with temozolomide improves efficacy in vitro. We further show that the effects of RSK2 on GBM invasion are mediated in part through activation of Rho GT-Pases. Rho family of GT-Pases are key regulators of cell polarity, migration and invasion. Here, we identify Rho A,
Research, Frederick, MD

Matthew Holderfield, Kanika Sharma. #1365 Sensitivity of oncogenic KRAS to adenosine triphosphate suppressors could enhance the effectiveness of existing GBM treatment, and support RSK2 targeting as a promising approach for novel GBM therapy. 

#1364 Direct phosphorylation and attenuation of the DCL1 tumor suppressor by SRC kinase: a new mechanism of SRC-dependent activation of Rho with translational implications. Brajendra K. Tripathi, Xiaolan Qian, Ming Zhou, Dunrui Wang, Marian Durkin, Alex G. Papageorge, Douglas R. Lowy. National Institutes of Health, Bethesda, MD; National Cancer Institute, Frederick National Laboratory for Cancer Research, Frederick, MD.

Many tumors have high SRC activity and constitutive up-regulation of Rho-GTP, which is frequently implicated in the neoplastic process and tumor progression to metastasis, but the mechanistic link is unclear. Here we identify the DCL1 RhoGAP tumor suppressor, which encodes a RhoGAP as a recognized direct target of the SRC kinase, in untransformed cells and in tumor-derived cell lines, which upregulates Rho-GTP. SRC kinase directly phosphorylates two Tyrosines in DCL1: Y451 and Y701. SRC phosphorylation of DCL1-Y701, which is located in the DCL1 RhoGAP domain, attenuates its RhoGAP activity and tumor suppressor function by inhibiting the binding of active Rho-GTP to the RhoGAP domain, reducing the hydrolysis of active Rho-GTP to inactive Rho-GDP and resulting in an increase in Rho-GTP. The role of SRC phosphorylation of DCL1-Y451 is to diminish DCL1 binding to tension, which further reduces the tumor suppressor functions, as measured by cell migration rate, anchorage-independent growth, and tumor formation in nude mice, independently of Rho-GTP. We also found that ERK1 cooperates with SRC by phosphorylating DCL1-S129, which enhances the binding of the SRC-Sh3 domain to this region of DCL1, and increases SRC-dependent phosphorylation of Y451 and Y701. The cooperative effects reduce RhoGAP activity, increase Rho-GTP and Rho/ROCK/MRLC signaling, and attenuate DCL1 tumor suppressor functions. DCL1 preferentially binds active SRC, which reduces overall SRC activity. Therefore, SRC activity tends to be lower in cells that express high levels of endogenous DCL1, compared with cells in which DCL1 expression has been down-regulated. Although SRC targets two DCL1 functions that contribute to its tumor suppressor activity, DCL1 binds several ligands in addition to tension that also contribute to its tumor suppressor functions. Therefore, it would not be surprising if high SRC activity were associated with selective pressure for down-regulation of DCL1 expression. Consistent with this hypothesis, the combination of high SRC and low DCL1 expression was associated with cell-line progression in lung adenocarcinomas (p = 0.005) in TCGA. The potentially reversible nature of the SRC-induced attenuation of DCL1 function can be exploited therapeutically. In two systems that have high SRC activity and express DCL1 - the mouse MMTV-PyMT cancer model and a human lung cancer cell line - the Src inhibitor PP1 has marginal antitumor activity, as it reduces DCL1 Tyrrosine phosphorylation, reactivates DCL1, and reduces Rho-GTP. However, PP1 has marginal antitumor activity an isogenic DCL1-negative strain of the lung cancer line, as the inhibitor does not reactivate DCL1 and does not alter Rho-GTP levels. The results highlight the potential importance of tumor suppressor reactivation as a biomarker for predicting and monitoring the response to SRC inhibitors.

#1365 Sensitivity of oncogenic KRAS to adenosine triphosphate suppression. Matthew Holderfield, Kanika Sharma. Frederick National Laboratory for Cancer Research, Frederick, MD.

KRAS proteins regulate many cellular processes including initiation of cell division in response to mitogens. Gain of function KRAS mutations appear in approximately 30% of all human cancers and activate effector signal transduction pathways independent of mitogenic stimuli. KRAS-driven cancers also frequently have high metabolic rates and may have increased sensitivity to metabolic inhibitors but the underlying mechanism is not fully understood. In this study, we characterized the cellular mechanism of a small molecule inhibitor identified using a transgenic Drosophila melanogaster model that expresses human KRAS-G12V in the fly wing. The compound partially restored wing developmental in the KRAS-G12V flies and inhibited growth and signal transduction in multiple KRAS driven cancer cells and KRAS-G12V dependent MEF cells but not in MEF cells expressing BRAF-V600E. The compound also had a profound impact on mitochondrial function by inhibiting electron transport (ETC) complex I activity, as well as an uncharacterized non-ETC site that is sensitive to oncogenic KRAS signal transduction. ATP production through glucose metabolism was sufficient to rescue KRAS function and cellular growth after ETC inhibition, suggesting that inhibition of MAPK signaling is due to low ATP levels rather than a direct consequence of ETC or complex I inhibition. These data indicate that oncogenic KRAS signal transduction requires high cellular ATP levels and suggests a synthetic lethal interaction between KRAS and multiple metabolic targets that could be exploited to target KRAS driven cancers.

#1366 Structural basis of impaired GTP hydrolysis in oncogenic mutants of KRAS. Timothy Tran, Sathija Dharmain, Oleg Chertov, Timothy Waybright, William Gillette, Dominic Esposito, Dwight Nissley, Frank McCormick, Andrew Stephen, Dhirenra Simanshu. NCI RAS Initiative, Frederick National Laboratory for Cancer Research, Frederick, MD; Diller Family Comprehensive Cancer Center, University of California San Francisco, San Francisco, CA.

KRAS mutations are found in one-third of all human cancers. Among the three RAS isoforms - HRAS, KRAS and NRAS, KRAS is the most commonly mutated gene (in 86% of RAS-driven cancers) and the mutations are often detected in pancreatic, colorectal and lung cancers. RAS proteins function as molecular switches by alternating between inactive GDP-bound and active GTP-bound states. The active or inactive state of RAS proteins is regulated by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). In the GTP-bound state, RAS proteins interact with a variety of effector proteins, such as Raf, PI3K, and RalGDS, leading to activation of several signaling cascades within the cell. In 98% of the cases, oncogenic RAS mutations are found at amino acid positions G12, G13, and Q61 which impair intrinsic and GAP-mediated GTPase function resulting in accumulation of constitutively GTP-bound RAS in cells. To gain insights into the effect of oncogenic mutations on overall structure and GTP hydrolysis, we solved high-resolution crystal structures of wild type and six oncogenic mutants (G12C, G12D, G12V, G13D, Q61L and Q61R) of KRAS in complex with GMPPNP (a non-hydrolysable GTP analog) and magnesium. Comparison of GDP and GMPPNP bound structures of wild type (WT) KRAS4b suggests conformational changes that occur when KRAS4b transitions from inactive to active state. Comparison of mutant and wild-type KRAS structures has shown the appearance of new pockets in some cases that could be exploited for structure-based drug design. Structural superposition of mutants vs. wild type KRAS4b in complex with GMPPNP/Mg2+ and KRAS4b mutants vs. WT-HRAS bound to RASA1-GAP provides a rationale for impaired intrinsic and GAP-mediated GTP hydrolysis in the KRAS mutants.

#1367 The dynamic mechanism of SARAH domain in RASSF5 activation by K-Ras4B. Tsung-Jen Liao, Hyunbum Jang, Chung-Jung Tsai, David Fushman, Ruth Nussinov. University of Maryland, College Park, MD; National Cancer Institute at Frederick, NIH, Frederick, MD.

RASSF5 is a tumor suppressor, which acts as an adaptor linking Ras and the Hippo pathways. RASSF5 activates MST1/2, a key upstream protein of the Hippo pathway, through SARAH domain heterodimerization, resulting in Hippo signaling. Signaling stimulates YAP1 phosphorylation, leading to its degradation and thereby cell apoptosis. Association with K-Ras4B promotes RASSF5 to release its SARAH which heterodimerizes with MST1/2. We aim to reveal how K-Ras4B regulates the Hippo pathway through RASSF5 by resolving the structure and conformational dynamics of RASSF5 complexes. Our modeling and simulations help elucidate the intrinsic dynamic interactions of RASSF5 RA (Ras binding) and SARAH domains. Surprisingly, we found that the residue E388 on RASSF5 SARAH plays a significant role in the dynamic mechanism of Ras and plays a role in the RA-SARAH association. The mutations E388A and E388K dramatically decrease the strength of RASSF5 homo-SARAH and RASSF5-MST2 hetero-SARAH, suggesting that the E388 mutation may be oncogenic. Simulations of selected RASSF5 configurations with GTP-bound K-Ras4B indicate that the interactions between RA and SARAH are reduced. We conclude that Ras binding weakens the RA-SARAH association, increasing the SARAH dynamic fluctuation. The released RASSF5 SARAH has high propensity to dimerize with MST1/2 SARAH, activating Hippo signaling thus cancer suppression. Funded in whole or in part with Federal funds from the Frederick National Laboratory for Cancer Research, NIH under contract HHSN261200800001E.

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MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Cell Growth Signaling Pathways
#1368  
**RABL6A**, a novel critical regulator of Akt-mTOR signaling in pancreatic neuroendocrine tumor cells. Shaik Amjad Ume Salma, Jessura Hagen, Jacki Reilly, Ryan Sheehy, Njitia Tiwari, Jackson Nteeba, Scott K. Sherman, Thomas M. O’Dorioso, James R. Howe, Andrew M. Bellizzi, Benjamin W. Darbro, Dawn E. Quelle. *Univ. of Iowa College of Medicine, Iowa City, IA.*

**Introduction:** A better molecular understanding of pancreatic neuroendocrine tumors (PNETs) is needed to improve patient diagnosis and treatment. Everolimus (mTOR inhibitor) is a standard-of-care therapy for PNET patients based on aberrant activation of the PI3K/Akt/mTOR kinase pathway in tumors. However, sustained mTOR inhibition paradoxically promotes Akt kinase hyperactivation due to loss of negative feedback regulation and tumors become drug resistant. Our study revealed that RABL6A, a novel oncogene product amplified in PNETs, is a key regulator of this clinically relevant pathway. Methods: RABL6A and Akt protein levels were manipulated using viral shRNAs in BONI PNET cells. Transcript levels were assayed by microarray and qRT-PCR, proteins assessed by western blotting, and cell proliferation and survival measured by cell counts, trypan blue exclusion and EdU incorporation. Effect of RABL6A expression on sensitivity to clinically relevant drugs. MK2206 (Akt inhibitor) and everolimus, were tested. Results: Silencing of RABL6A in PNET cells causes G1 and G2/M cell cycle arrest, and pathway analysis of microarray data suggested inactivation of Akt signaling in the arrested cells. Immunoblotting confirmed dramatic loss of Akt phosphorylation at Ser-473 along with impaired phosphorylation and activation of its targets, FRAS40 and FOXO-1/3. Phosphorylation of S6K, a downstream target of Akt-mTOR signaling, was also reduced. The mechanism by which RABL6A controls Akt-S473 phosphorylation is currently not known although we demonstrated that mTORC2 (the kinase that phosphorylates Akt at Ser473) remains active in RABL6A deficient tumors. The mechanism by which RABL6A controls Akt-S473 phosphorylation is currently not known although we demonstrated that mTORC2 (the kinase that phosphorylates Akt at Ser473) remains active in RABL6A deficient cells since the phosphorylation of other mTORC2 substrates (SGK1 and PDK1) is unaffected. Given the central role of Akt1 in tumorigenesis, we hypothesized that repressing its activity may rescue the arrest phenotype caused by RABL6A loss. Restoration of Akt1 in RABL6A-depleted cells partially rescued the G1 phase arrest and induced S phase entry but was insufficient to allow mitosis, suggesting RABL6A regulates other factors required for cell division. Finally, drug response assays showed that RABL6A loss desensitizes PNET cells to Akt and mTOR inhibitors. Conclusion: Our previous work showed RABL6A promotes G1 progression in PNET cells by inactivating Rb1, an established suppressor of PNET pathogenesis. We now show that RABL6A also controls Akt phosphorylation and is essential for Akt-mTOR activation. Thus, RABL6A controls multiple cancer pathways necessary for PNET cell cycle progression and survival. We are testing if RABL6A status in PNETs predicts responsiveness to combination therapies targeting Akt and mTOR. Overall, this work identifies RABL6A as a new essential activator of Akt1-mTOR signaling, suggesting it is a new potential biomarker and target for anticancer therapy in PNET patients.

#1370  
**EGFR-mediated Spred1 phosphorylation inhibits NF1 to sustain constitutive Ras/MAPK signaling.** Evan Markegard, Ellen L. Mercado, Jillian M. Silva, Jacqueline Galeas, Marena L. Trinidad, Anatoly Urisman, Frank McCormick. *UCSF, San Francisco, CA.*

Spred proteins negatively regulate Ras/MAPK signaling following growth factor stimulation. Inhibition of Ras primary occurs through Spred1’s ability to bind and localize NF1, a RasGAP and major tumor suppressor, to the plasma membrane. Spred1 and NF1 loss-of-function mutations occur across multiple cancer types including non-small cell lung carcinoma, glioblastoma, melanoma, stomach carcinoma, and uterine carcinosarcoma. Here we demonstrate that EGFR signaling disrupts Spred1-NF1 binding. Mass spectrometry was performed on cells expressing EGFR to identify potential phosphorylation sites on Spred1 and NF1 that could disrupt Spred1-NF1 binding by steric hindrance. A serine phosphorylation site on Spred1 was identified in which a phospho- mimetic and phophodedeficient mutant decreased or increased Spred1-NF1 binding, respectively. Phosphomimetic Spred1 is unable to suppress Ras-GTP stimulation. Therefore, phosphorylation of Spred1 at this site by a serine kinase downstream of EGFR may disrupt Spred1-NF1 binding. To identify the Spred1 kinase we are performing an in vitro kinase assay and an unbiased CRISPRa screen. Our findings provide one potential mechanism by which EGFR signaling disrupts negative feedback to sustain constitutive Ras signaling. Furthermore, this work may elucidate a novel therapeutic target for restoring NF1-mediated inhibition of Ras.

#1371  
**Isoprenylcysteine carboxylmethyltransferase (ICMT) function is critical for epithelial malignant transformation by mutant-Ras.** Hiu Yeung Lau, Jingyi Tang, Patrick J. Casey, Mei Wang. *Duke-NUS Medical School, Singapore, Singapore.*

Purpose Limited progress has been made in the development of direct inhibitors of Ras. Therefore, targeting isoprenylcysteine carboxylmethyltransferase (ICMT), the final enzyme in the prenylation pathway for Ras and other proteins containing the so-called CaaX-motif, remains an important strategy to inhibit Ras function in cancers. In this study, we investigate the role ICMT plays in the initiation of human epithelial cancers involving constitutively-active mutant Ras using genetic manipulation strategies. Methods Small T antigen and a shRNA against p53 (shp53) were sequentially expressed in human mammary epithelial cell lines immortalized by hTERT (HME1-hTERT) to create pre-malignant HME1-shp53 cells. All 4 isoforms of wild-type or mutant Ras, namely H-Ras, N-Ras, K-Ras-4A and K-Ras-4B, were independently introduced into HME1-shp53 cells to determine their transforming ability. Using CRISPR-Cas genome editing technology, ICMT-null HME1-shp53 cells were generated and mutant Ras isoforms were subsequently introduced; tumor formation abilities of thus generated cells were determined using soft agar anchorage-independent colony formation assay and subcutaneous xenografts in mice. ICMT knockout was also performed in MiaPaCa-2 and MDA-MB-231 cells, both expressing mutant K-Ras, and subcutaneous xenograft tumor formation in mice was measured. Results Pre-malignant HME1-shp53 cells can be transformed by all mutant Ras isoforms but not by any wild-type Ras. ICMT loss-of-function dramatically reduced anchorage-independent colony formation caused by all mutant Ras isoforms in HME1-shp53 cells, and in vivo xenograft formation was completely abolished. ICMT loss-of-function also dramatically reduced in vivo xenograft formation for mutant K-Ras-expressing MiaPaCa-2 and MDA-MB-231 naturally-occurring cancer cells. Conclusion ICMT function is essential for the malignant transformation of HME1 cells by all isoforms of Ras. Furthermore, ICMT function is also crucial for the maintenance of the malignant phenotype in mutant K-Ras-expressing cells such as MiaPaCa-2 and MDA-MB-231 cells.

#1369  
**DNA, protein, and cell line reagents to facilitate Ras pathway drug discovery efforts.** Dominic Esposito. *Frederick National Laboratory for Cancer Research, Frederick, MD.*

The Ras Reagents Core within the NCI Ras Initiative produces and distributes qualified DNA vectors, cell lines, and protein production reagents to the external community to facilitate research into Ras biology and development of Ras cancer therapeutics. DNA vectors representing a variety of Ras mutations, as well as a complete collection of 180 genes in the Ras pathway are available as Gateway-compatible Entry clones. These clones represent the most commonly expressed transcript forms of the 180 genes, many of which were not previously available in full-length, wildtype forms. The vectors can be used with the FNLC combinatorial cloning library to easily generate a large number of different types of expression clones with various promoters, fusion tags, and plasmid backbones. Cell line reagents include a variety of Ras-dependent mouse embryonic fibroblasts (MEFs) which have a defined mutant allele of KRAS and which serve as isogenic cell lines for investigating allele and mutant specific Ras biology, as well as drug screening tools. Protein reagents include E. coli and insect cell constructs and cell lines for production of a variety of Ras-related proteins. In particular, we have materials for the high yield production of properly processed, prenylated KRAS protein using a unique engineered insect cell expression system. This system can also be used to produce other prenylated small GTPases at levels not previously attainable. Many of the resources of the Ras Reference Core are readily available to the academic community through repositories or material transfer agreements, and can be licensed to industry scientists as well. The program is also interested in identifying the needs of the community for additional Ras-related resources, and is developing methods for vetting requests from the outside to provide additional reagents.

#1372  
**Towards novel strategies of targeting specific vulnerabilities of T-PLL cells.** Sabine Pueter1, Emma Andersson1, Alexandra Schrader1, Lesley Varghese1, M. Hülsemann1, Petra Mayer1, Marc-Henri Stern1, Sebastian Newr1, L. Frenzel1, Satu Mustjoki2, Marco Herling1. 1Center for Integrated Oncology (CIO) Köln-Ronn, University of Cologne, Cologne, Germany; 2University of Helsinki and Department of Hematology, Helsinki, Finland; 3Institute Curie, Paris, France; 4Institute of Pathology University of Frankfurt, Frankfurt, Germany.

**MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Epigenetics 1**

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T-cell prolymphocytic leukemia (T-PLL) is a mature T-cell neoplasm usually presenting at an aggressive phase and a marked resistance to conventional cytostatics, i.e. alkylators. The monoclonal antibody alemtuzumab induces high response rates, but virtually all patients relapse and the mostly elderly individuals are often ineligible for allogeneic stem cell transplantation. Overall, the treatment options for T-PLL are scarce and its prognosis remains poor, with median survival times of <3 yrs, remains poor. In agreement with the pronounced amenability of T-cell tumors to intervention in the altered epigenetic code, recent reports also implicate a high potential of epigenetic targeting in T-PLL. In fact, profiling of antineoplastic sensitivities, including clinically used agents like fludarabine or nelarabine, revealed that the purine analog cladribine showed the highest response (LD50 = 2.4 nM). Furthermore, the combination of T-PLL-specific CD70, H3K9ac, H3K27ac and H4K5ac. Preliminary analysis with si-RNA experiments revealed overexpression of HOXA9 is highly associated with a poor prognosis in acute myeloid leukemia (AML). In addition, Ash1l is overexpressed in a variety of cancers, including breast and thyroid cancer, but its role in cancer remains poorly characterized. Recent data suggest that Ash1l promotes leukemogenesis in mixed-lineage leukemia by recruiting the MLL epigenetic regulator to target genes. However, Ash1l is a large protein with multiple chromatin-interacting domains, and which regions of the Ash1l protein are important in leukemia has not been reported. Specifically, the role of Ash1l’s Kmtase activity in leukemogenesis remains unknown. To further investigate the function of Ash1l in leukemia, we knocked down expression of Ash1l in leukemia cell lines. We found that Ash1l knockdown slowed cell growth and caused a decrease in expression of Ash1l genes and their collaborator Mei51. Specifically, we investigated the role of Ash1l Kmtase activity in leukemia, we used a mouse model with a targeted in-frame deletion of the Ash1l SET domain (ΔSET). ΔSET cells express a shorter version of Ash1l that does not contain the SET domain. We transduced bone marrow cells (BMCs) isolated from ΔSET and WT mice with several oncogenes relevant to AML to determine the role of the Ash1l SET domain in leukemogenesis. We found that ΔSET BMCs were significantly impaired in leukemogenic transformation compared to WT BMCs, as manifested by decreased colony-forming ability and reduced expression of HOXA genes. These findings suggest that the Ash1l SET domain plays an important role in leukemogenesis mediated by multiple different oncogenes. Future studies will further characterize Ash1l’s role in leukemia by identifying the complete set of genetic loci that it methylates and regulates, and will determine whether the Ash1l SET domain represents a viable therapeutic target.

#1374 Frequent up-regulation of histone methyltransferase G9a contributed to liver carcinogenesis by epigenetically silencing of tumor suppressor RARRES. Iai Wei, Felice Ho-Ching Tsang, Dickly Cheuk-Ting Lee, Sandy Leung-Kuen Au, Joyce Man-Fong Lee, Carmen Chak-Lui Wong, Irene On-Lin Ng, Jack Chun-Ming Wong. The University of Hong Kong, Hong Kong, Hong Kong.

Hepatocellular carcinoma (HCC) is the second leading cause of cancer death worldwide and is the most common type of cancer in sub-Saharan Africa and Southeast Asia area where hepatitis B infections are common. The initiation and progression of HCC was attributed to alterations in genetics and disruptions of epigenetic processes which lead to altered gene functions in both oncogenes and tumor suppressors. Recent advancements in the field of cancer epigenetics increasingly emphasize the important role of every component in the epigenetic machinery. However, the alterations of epigenetic mechanisms involved in HCC are still largely unknown. Using transcriptome sequencing, we examined the expression of 591 epigenetic regulators in hepatoblastomas from human HCC and found that most of the epigenetic regulators are deregulated especially the histone modification enzymes. Among all of them, we identified G9a (Eu- chromatic histone-lysine N-methyltransferase 2, EHMT2), a histone H3 lysine 9 (H3K9) specific histone methyltransferases, as one of the most significantly up-regulated epigenetic regulators in human HCCs. Previous studies about epige- netic aberrations which contribute to liver carcinogenesis, and targeting G9a could be a potential epigenetic therapeutic method for HCC treatment. In this study, we found that G9a was frequently up-regulated in different HCC sample cohorts. Up-regulation of G9a was significantly associated with HCC disease progression, cancer aggressiveness, and more malignant tumor phenotypes. Functionally, we demonstrated that shRNA knockdown and CRISPR/Cas9 knockout of G9a suppressed HCC cell proliferation in vitro and inhibited subcutaneous xenograft HCC tumorigenicity in vivo. Depletion of G9a significantly reduced HCC cell migration ability and induced cell senescence. Pharmacological inhi- bition of G9a by small molecule inhibitors, UNC638 and RIX01294, also sup- pressed HCC cell growth and altered cell morphology. Mechanistically, we showed that the frequent up-regulation of G9a in human HCC was attributed to 2.81

#1375 Loss of C-MYC and chromatin acetylation induce epigenetic reprogramming in acute lymphoblastic leukemia. Elodie M. Da Costa, Gregory Arnaud, Nhat Cong Nguyen, Johannes Beaudy, Pascal Sre- On, Maxime Caron, Daniel Sinnett, Serge McGraw, Noel J. Raynal, CHU Sainte-Justine research center, Montreal, Quebec, Canada.

Epigenetic modifications play a key role in establishing and maintaining gene expression in cancer, they are highly altered and responsible of gene expression deregulation. Epigenetic drugs have the ability to reset cancer cell epigenome producing cancer cell differentiation and apoptosis. In a drug screening initiative, we recently reported a series of FDA-approved drugs with unsuspected epigenetic and anticancer activities. Here, we tested in a secondary screen the activity of these drugs against acute lymphoblastic leukemia (ALL) cell lines (MOLT-4 and NALM-6). We found that Proscillaridin A, a cardiac glycoside used for heart failure treatment, was the most active with IC50 values in the low nanomolar range, suggesting drug resistance. Moreover, Proscillaridin A treatment induced a significant decrease in RNA and protein levels of C-MYC, a master oncogenic driver in ALL. Shortly after proscillaridin A treatment, C-MYC exhibited a 75% reduction in lysine acetylation, a post-translational modification known to prevent its degradation. Loss of acetylation was associated with down-regulation of lysine acetyltransferases CBP, P300 and Tip60, which also correlated with a reduction in histone 3 and 4 acetylation levels (H3K14ac, H3K9ac, H3K27ac and H4K5ac). Preliminary analysis with si-RNA experiments reveal that independent HAT activities are not responsible of C-MYC down-regulation. RNA sequencing and gene set enrichment analysis in proscillaridin-treated ALL cells (5 nM for 48h) showed that genes associated with cell differ-
entiation and apoptosis pathways were up-regulated whereas down-regulated genes were associated with C-MYC target genes. Altogether, our findings show that acetylation through lysine acetyltransferase down-regulation simultaneously induces loss of C-MYC and H3 acetylation leading to epigenetic reprogramming in ALL cells. This drug repositioning strategy, using procussilarin A, has the potential to reprogram cancer cells that are driven by MYC overexpression or hyperactivation.


In conclusion our findings indicate that our lead molecule of fundamental mechanisms of chromatin dynamics. Fission yeast possesses 13 (Schizosaccharomyces pombe) is an ideal model organism to investigate the progression and other diseases. However, histone methyltransferase (HMTase) is part of intricate networks that ultimately regulate transcriptional events. Dysfunctional ballet of writers, readers and erasers of epigenetic marks on both DNA and histones were associated with C-MYC target genes. Altogether, our findings show that acetylation through lysine acetyltransferase down-regulation simultaneously induces loss of C-MYC and H3 acetylation leading to epigenetic reprogramming in ALL cells. This drug repositioning strategy, using procussilarin A, has the potential to reprogram cancer cells that are driven by MYC overexpression or hyperactivation.

#1377 Set7 is a novel histone methyltransferase in Schizosaccharomyces Pombe. Eric Di Luccio, Yunpeng Shen, Damiaan E.HF Mevis, Yeon Jeong Noh, Jihyeon Kim, Masayo Morishita. Kyungpook National University, Daegu, Republic of Korea.

Dynamics and plasticity of chromatin regulation is mediated by a molecular ballet of writers, readers and erasers of epigenetic marks on both DNA and histones. The pattern of histone modifications may define a histone code, which is part of intricate networks that ultimately regulate transcriptional events. Dysfunction of histone methylation affects chromatin regulation and is involved in an increasing number of pathologies from cellular transformation to tumor progression and other diseases. However, histone methyltransferase (HMTase) pathways remain to be further explored and better understood. Fission yeast (Schizosaccharomyces pombe) is an ideal model organism to investigate the fundamental mechanisms of chromatin dynamics. Fission yeast possesses 13 SET-containing proteins (Set1 to 13), several of which methylate histones or ribosomes. The catalytic SET domain is highly conserved across eukaryotes (e.g. Set2, a homologue of the oncprotein NSD2/MMSET/WHSC1 in human). Structure-function studies of HMTases are essential from basic science to translational research. However, full-length structures of fission yeast methyltransferases are currently missing. Here, in an effort to better understand histone methylation and to gain insight for drug-design, we report the identification and structural characterization of a novel histone methyltransferase Set7 (SPC297.04c), in Schizosaccharomyces Pombe. In this study, we investigated Sp.Set7’s cellular localization and the effect of Sp.Set7 knock-out on the cell cycle and sporulation. Next, we elucidated Sp.Set7’s Lysine substrate specificity on histone H3 and H4 by mass spectrometry. Finally, we used chromatin immunoprecipitation combined with high throughput sequencing (ChiP-seq) and RNA sequencing to elucidate the genomic regions in which Sp.Set7 is enriched.

#1378 Identification of genomic regions associated with temozolomide resistance in glioblastoma through analysis of histone marks on chromatin. Xiaoyue Chen, 1 Haiyun Gan, 2 Jeong Heong Lee, 1 Dong Fang, 2 Gaspar Kitzange, 1 Jann Sarkaria, 1 Zhiguo Zhang, 13 Mayo Graduate School, Rochester, MN; 4 Columbia University, New York, NY.

Glioblastoma (GBM) is an aggressive and fatal primary brain tumor. Temozolomide (TMZ) is a critical component of the standard care of newly diagnosed GBM patients, but unfortunately preexisting primary resistance and rapid emergence of secondary resistance are major obstacles in the treatment of GBM. Prior studies have identified genetic and epigenetic alterations that can modulate TMZ sensitivity and treatment outcome. However, comprehensive analysis of histone marks and knowledge of epigenetic regulation of genes associated with TMZ sensitivity or resistance is lacking. To identify epigenetic states associated with TMZ resistance, we performed an epigenomic profiling of eight different histone marks in GBM xenografts. Using chromatin immunoprecipitation combined with high throughput sequencing (ChiP-seq), distribution of H3K4me1, H3K4me3, H3K9ac, H3K9me2, H3K9me3, H3K27ac, H3K27me3 and H3K36me3 histone marks was compared in a panel of GBM patient-derived xenograft sub-lines derived from treating TMZ sensitive GBM12 tumors with placebo (n=2 sublines) or temozolomide (n=6 sublines) and then propagating resulting recurrent tumors. Our analysis revealed that H3K4me1 and H3K27ac modification patterns varied globally across individual sub-lines, while distribution of H3K4me3, H3K36me3 histone marks was specifically altered in discrete genomic regions in resistant sub-lines depending on the mechanisms of resistance. To find how epigenetic modifications affect TMZ sensitivity, we analyzed ChIP-Seq data using Hidden Markov Model to test if one or a combination of histone marks relates to TMZ resistance. The effect of histone modifications on transcription was simultaneously determined by RNA sequencing. These analyses helped identify specific histone modifications which could be functionally related to TMZ resistance. Through these analyses we have identified 1142 genomic regions governed by a specific epigenetic pattern. We subsequently analyzed a candidate genomic region on top of the list, by using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) associated nucleases (Cas9 system). In summary, we have generated clones from an intrinsically TMZ resistant SKMG cell line with deletion up to four-kilo base genomic region. Clonogenic growth assays showed that deletion of this genomic region enhanced TMZ sensitivity, reducing the IC50 from 186 μM to less than 60 μM, p<0.05. This finding indicates that this genomic region is functionally related with TMZ sensitivity. Taken together, our study reveals epigenetic modifications related to TMZ resistance in GBM cells and a specific genomic region involved in regulating TMZ sensitivity. This analysis of epigenetic state at this genomic region could potentially be useful in predicting treatment response and may help in designing TMZ sensitizing therapy in GBM.

#1379 Histone demethylase KDM6a as a target for glioblastoma radiosensitivity. Barbara Helen Rath, Mariaa F. Shah, Kevin Camphausen, Philip J. Toflon. National Cancer Institute, Bethesda, MD.

Radiotherapy is a primary treatment modality for glioblastomas (GBMs). Because post-translational histone modifications have been implicated in the regulation of radiosensitivity, as a potential strategy for enhancing the response of GBMs to radiotherapy we have evaluated the effect of radiation on histone methylation in glioblastoma stem-like cells (GSCs) and the established glioma cell line (U251). For these studies we focused on histone H3K27 tri-methylation (H3K27me3), whose opposing site H3K36me2 has already been linked to DNA repair. Exposure of GSCs and U251 to 10 Gy decreased H3K27me3 by 30 min, reaching a cell line dependent maximum between 1h and 3h, followed by a return
to control levels at 6h. According to immunoblot analysis, GSCs and U251 had significant higher levels of KDM6A compared to KDME6B, suggesting a more prominent role for KDME6A in the demethylation of H3K27me3 after irradiation. To determine whether KDME6 could serve as a target for GBM radiosensitization, we focused on KDME4, which is an inhibitor for KDME6A and KDME6B. KDME4 treatment resulted in a decrease of H3K27me3 and an increase of H3K56Ac within 1h of exposure and was found to block the radiation-induced decrease of H3K27me3. Based on clonogenic survival analysis addition of KDME4 immediately prior to irradiation significantly enhanced the radiosensitivity of GSCs and U251. To begin to investigate the mechanism responsible for this radiosensitization, we investigated the underlying mechanisms contribute to epigenetic alterations. ASH1L is a histone methyltransferase specific for catalyzing transcription-repression. Up-regulation of ASH1L also correlated with increased Ki67 expression. We hypothesized that deregulation of histone methyltransferases Histonemodification is a major component of epigenetic regulation, and deregulation of ASH1L was detected in 40% of primary HCCs and significantly associated with upregulated expression. We found that the histone methyltransferases group protein ASH1L was frequently upregulated in human HCC. Overexpression of ASH1L also correlated with increased Ki67 expression. ASH1L is a histone methyltransferase specific for catalyzing transcriptional-active H3K4 and H3K36 methylation. We showed that high levels of ASH1L significantly upregulated the cell cycle in HCC cells and colon cancer cells. We further demonstrated that knockdown of ASH1L inhibited HCC cell migration. In addition, we found that high levels of ASH1L correlated with low expression of Ki67. Anti-ASH1L antibodies were found to block the radiation-induced decrease of H3K27me3 and the radiation-induced increase of H3K27me3 was abolished. Analysis of H2A.X nuclear foci after irradiation (2Gy) of siKDME6A treated cells showed a significant delay in foci disassembly, consistent with an inhibition of DSBR repair. To further investigate the potential role of histone demethylation as a potential target to radiosensitize GBM, siASH1L was used to knock down KDME6A expression. Cells treated with KDME6A showed a significant increase in H3K27me3 and the radiation-induced increase of H3K27me3 was abolished. This result suggests that the histone demethylase KDME6A is a target for GBM radiosensitization.

**1380 Expression and functions of ASH1L in liver cancer.** Lai Wei, Felice H. Tsang, Daniel Ho, Cheuk-Ting Law, Mengnuo Chen, Long-Hin Tseang, Carmen Cl Wong, Irene Ol Ng, Chun-Ming Wong. University of Hong Kong, Hong Kong.

Hepatocellular carcinoma (HCC) is a common cancer and ranks the third lethal cancer worldwide. However, the detailed molecular mechanisms underlying the initiation and progression of HCC remain poorly understood. Liver carcinogenesis is a multistep process that is driven by the accumulation of genetic and epigenetic alterations. Mutation landscape and driver mutations have recently been delineated by high-throughput sequencing studies. On the other hand, our current knowledge about epigenetic deregulation in human HCC is limited. Epigenetic alterations play prominent role in liver cancer. Here, we report that human CRL4DDB2 ubiquitin ligase regulates the DNA repair of histone chaperon CAF-1. We show that the CRL4DDB2 depletion decreases the recruitment of CAF-1 and prevents post-repair H3K56Ac restoration. Additionally, a PCNA-interacting protein (PIP) motif is shown to exist within DDB2. The mutation in the PIP box of DDB2 compromises its capability to maintain the H3K56Ac level but does not affect CRL4DDB2-mediated XPC ubiquitination. These results suggest a novel role of CRL4DDB2 in regulating post-repair chromatin restoration, which differs from its canonical role in the process of nucleotide excision repair.

**1382 A novel BET family bromodomain inhibitor NHWD-870 represents a promising therapeutic agent for a broad spectrum of cancers.** Nenghui Wang, Mingzhu Yin, Qin Yan. The Ohio State University, Columbus, OH.

BET proteins have emerged as promising targets for cancer therapy. NHWD-870 is a potent and selective BET family bromodomain inhibitor and only binds bromodomains of BRD2/3/4/T. NHWD-870 exhibited robust single agent activity in cell viability assay across cancer cell lines derived from solid tumors, leukemia and lymphomas. Further characterization of cancer cell responses to NHWD-870 indicated that NHWD-870 manifested diverse mechanisms of action in different cancer settings. These include: 1) inhibition of tumor cell growth by downregulating the PDGFβR, MEK1/2 and STAT1/MYC signaling in tumor cells; 2) inhibition of tumor angiogenesis by decreasing PDGF production in tumor cells and the PDGFβR and MEK1/2 signaling in endothelial cells. Consistent with its broad spectrum of activities in vitro, NHWD-870 has potent therapeutic efficacy in xenografted murine models of breast cancer, triple negative breast cancer and ovarian cancer. These results support its further development for diverse solid tumor indications in the clinic.

**1383 Histone H3 lysine 9 methyltransferase SUV39H1 is associated with clinical outcome of renal clear cell carcinoma patients.** Wei Meng, Ri Cui, Saikd Jhalur Haque, Carlo M. Croce, Arnab Chakravarti. The Ohio State University, Columbus, OH.

Background: Epigenetic alterations have three important components including DNA methylation, histone modifications, and non-coding RNA. The epigenetic changes are highly dynamic and also reversible, which regulate a variety of biological processes. Euchromatin which is a loosely packed form of chromatin is highly associated with active gene expression. In contrast, heterochromatin is associated with high histone methylation and DNA methylation but low RNA transcription. SUV39H1 catalyzes the histone 3 lysine 9 trimethylation (H3K9me3) in heterochromatin regions, which also mediates DNA methylation at pericentric heterochromatin. Currently, the oncogenic role of SUV39H1 in renal cell carcinoma (RCC) pathogenesis is poorly understood. To this end, we assessed the role of SUV39H1 in renal RCC cells and association with clinical outcome of RCC patients. Results: In SUV39H1 knockdown cells, we observed global H3K9me3 level reduction and cell growth inhibition. Proximal ligation assay was used to determine the H3K9me3 and 5-methylcytosine (5mC) interaction in RCC cells. Knocking down of SUV39H1 gene significantly reduced the co-localization of H3K9me3 and 5mC in pericentric repetitive sequences. Moreover, Southern blot demonstrated that expansion of a pentanucleotide (GGAAT) repeats was associated with upregulation of SUV39H1, which is linked to SUV39H1 function with microsatellite instability and rearrangement. In The Cancer Genome Atlas (TCGA) database, we found that SUV39H1 gene expression was upregulated in RCC tissues compared to normal tissues, and high SUV39H1 expression predicted a worse overall survival outcome (p = 0.002) in RCC patients. Conclusion: SUV39H1 upregulation promotes cell proliferation and microsatellite instability. SUV39H1 expression could serve as a prognostic biomarker for RCC patients.

**1385 New insights into the roles of histone lysine-specific demethylase 2 (LSD2) in breast cancer.** Lin Chen, Shauna N. Vasiliatos, Ye Qin, Steffi Oesterreich, Nancy E. Davidson, Yi Huang. University of Pittsburgh, Pittsburgh, PA.

Background: Epigenetic modifiers have been emerging as new players in breast cancer development. FAD-dependent histone demethylases have been proved to play important roles in regulating breast tumor initiation and development. While the role of histone lysine-specific demethylase 1 (LSD1) in breast cancer progression has been well characterized, the activity of its homolog, lysine-specific demethylase 2 (LSD2), in breast cancer is still a riddle that needs to be uncovered. Methods: To explore the precise role of LSD2 in breast cancer, we overexpressed LSD2 protein in MDA-MB-231 cells (LSD2-OE). The phenotypic effect of LSD2-OE on MM231 cells was characterized by proliferation assay, soft agar assay (3D growth), trans-well cell migration and invasion assay and mammosphere formation assay. To extend stem cell-like features study of LSD2 overexpressed MM231 cells, we examined CD44+/CD24- subpopulation.
Target RNA sequencing revealed the expression profile of cancer genes in response to deacetylase inhibitor in primary culture of prostate cancer cells. Hong Vinh T. Trong, S. Craig Greer, Glenn Mills. Louisiana State Univ. Health Sciences Ctr., Shreveport, LA.

Histone acetylation is a dynamic event to optimize cell functions through the control of RNA transcription and gene expression. In cancer, the histone deacetylation is a therapeutic target. Suppression of histone deacetylation increased the growth arrest, apoptosis, and senescence in cancer cells. Several histone deacetylase inhibitors (HDACis) have been developed and applied as a target therapy of various types of cancer, including the prostate cancer. However, the altered gene expression in HDACis treated prostate cancer cells is less reported. Little is known about the gene expression profiles of primary cultures of prostate cancer. In this study, we examined the cancer gene expression profile in primary cultures of human prostate cancer. Cells were isolated from two patient’s prostate cancer samples. Isolated cells were cultured in prostate epithelial culture medium CaT-52. After 24 hours of exposure to 400nM of Trichostatin A (TSA), an inhibitor of histone deacetylation I and II. RNA was extracted and quantified. The library was prepared with Illumina’s TruSight RNA Pan-Cancer Panel. The sequencing was performed on NextSeq 500/550 mid output v2 kit (150cycles). FastQ files were generated by BaseSpace Onsite and analyzed further by TopHat alignment (Illumina) and Partek Flow (Partek). The Trusight RNA Pan-Cancer panel targets 1385 cancer genes and covers 21043 exonic regions with 57010 probes. The results showed that 59 of the 1385 cancer genes were significantly changed (FDR p-value<0.05, fold change >2 fold) in T55 treated cells. In the treated cells 49 genes were up-regulated and 10 genes were down-regulated. The altered expression of 59 genes is involved in multiple cell functions. Genes that were upregulated included apoptosis associated genes (MAP2K6 and BIRC3), tumor suppression genes (DIRAS3, PEG3, PTIPRO, and DNM3), and P53-target gene (CYFIP2). The genes that were down-regulated included cell cycle regulation genes (CDK1 and CCNA2), DNA damage response genes (BRCA1 and BRIP1), as well as cell proliferation marker Ki67 gene (MKI67). However, the gene expression response to TSA is complicated. Some altered gene expression favors tumor growth and progression while other gene expression changes inhibit tumor growth and promote apoptosis. In conclusion, we identified the gene profile in primary culture of prostate tumor in response to a histone deacetylase inhibitor, TSA, and the therapeutic effects of HDACis could be influenced by the summation of contradicting, tumor promoting and suppression gene expression profile changes.

Effects of histone deacetylase inhibitors on differentiation markers and growth of colon and bladder cancer cells. Michael A. Lea, Lauren Cué, Elizabeth Batista, Erik Lew, Charles desBordes. Rutgers New Jersey Medical School, Newark, NJ.

Alkaline phosphatase and dipeptidyl peptidase are markers of differentiation in colon cancer cells. In colon cancer cells differentiation can often be induced by treatment with inhibitors of histone deacetylase (HDAC) activity. In some colon cancer cells butyrate causes a several-fold induction of alkaline phosphatase but only modest effects on dipeptidyl peptidase. There is relatively little information on changes in activity of cell surface hydrolases in bladder cancer. However, there is some evidence for decreased alkaline phosphatase activity in bladder cancer. We tested the hypothesis that there may be retention of activity in more slowly growing cancer cells and the activity will be regulated by HDAC inhibitors in a manner similar to that in colon cancer. In our initial studies we screened seven human bladder cancer cell lines for alkaline phosphatase activity. We identified three cell lines (5637, HT1197 and HT1376) with activity comparable to that in more differentiated colon cancer cell lines. The response to incubation with the HDAC inhibitors butyrate and valproate was compared with effects in three colon cancer cell lines Caco-2, HT29 and SW1116. In all six cell lines there were growth inhibitory effects that tended to be a little greater with butyrate than with valproate. Induction of alkaline phosphatase activity was generally greater with butyrate than with valproate and was apparent even without normalizing the activity on a protein basis. After determining the activity per unit protein there were increases in dipeptidyl peptidase activity after treatment with the HDAC inhibitors. A smaller increase in alkaline phosphatase activity was observed than with the HDAC inhibitors. Expression of HIF-1α and-2α increased in a manner consistent with the transcriptional regulation HIF-1α activates expression of genes promoting angiogenesis, metastasis, increased tumor growth and resistance to treatments. Understanding transcriptional regulation HIF-1α under physiologically relevant hypoxic conditions would be helpful to reduce breast cancer growth. Our recent studies identified that intracellular bioactive sphingosine-1-phosphate (S1P) is increased from nuclear sphingosine kinase 2 (SphK2) acts as an endogenous modulator of histone deacetylases (HDACs) epigenetically regulates gene transcription in breast cancer cells. We wonder whether nuclear S1P epigenetically regulates acetylated histones, positive transcription marks in hypoxia and regulate transcription of HIF-1α and target genes in breast cancer cells. We have used estrogen receptor positive human breast cancer MCF-7 cells, triple-negative human breast cancer MDA-231 cells and lung metastatic LM-2-4 cells derived from MDA-231 cells for our studies. We found that hypoxic condition enhances nuclear bulk histone acetylation in breast cancer cells and enhances HIF-1α synthesis. Down regulation of SphK2 with siRNA or using selective inhibitor of SphK2 (K145 compound) reduces nuclear S1P as well as bulk histone acetylation and consequent increase in HIF-1α expression. Experiments with small interfering RNA inhibition of SphK2 with siRNA reduced HIF-1α and nuclear acetylation of histones. Therefore, targeting SphK2 with K145 compound dramatically reduces in vitro tumorosphere formation in breast cancer cells. Our data suggested that targeting the SphK2/SIP signaling could represent an attractive strategy for therapeutic intervention in hypoxic breast cancers. Supported by Health Research, Inc. (HRI) Grant 71-4084 (NCH), National Cancer Institute Grant R01CA160688 (KT).

Effects of geinstein on histone modifying enzyme expression in breast cancer cells. Lanni Aquila, Nick Smothermon, Lisa Morey. Canisius College, Buffalo, NY.

The hypoixia-inducible transcription factors (HIF)-1α and -2α play a critical role in cellular response to hypoxia in solid tumors. Elevated HIF-α expression correlates with poor patient survival in a large number of cancers including breast cancers. HIF-α activates expression of genes promoting angiogenesis, metastasis, increased tumor growth and resistance to treatments. Understanding transcriptional regulation HIF-α under physiologically relevant hypoxic conditions would be helpful to reduce breast cancer growth. Our recent studies identified that intracellular bioactive sphingosine-1-phosphate (S1P) is increased from nuclear sphingosine kinase 2 (SphK2) acts as an endogenous modulator of histone deacetylases (HDACs) epigenetically regulates gene transcription in breast cancer cells. We wonder whether nuclear S1P epigenetically regulates acetylated histones, positive transcription marks in hypoxia and regulate transcription of HIF-α and target genes in breast cancer cells. We have used estrogen receptor positive human breast cancer MCF-7 cells, triple-negative human breast cancer MDA-231 cells and lung metastatic LM-2-4 cells derived from MDA-231 cells for our studies. We found that hypoxic condition enhances nuclear bulk histone acetylation in breast cancer cells and enhances HIF-α synthesis. Down regulation of SphK2 with siRNA or using selective inhibitor of SphK2 (K145 compound) reduces nuclear S1P as well as bulk histone acetylation and consequent increase in HIF-1α expression. Experiments with small interfering RNA inhibition of SphK2 with siRNA reduced HIF-1α and nuclear acetylation of histones. Therefore, targeting SphK2 with K145 compound dramatically reduces in vitro tumorosphere formation in breast cancer cells. Our data suggested that targeting the SphK2/SIP signaling could represent an attractive strategy for therapeutic intervention in hypoxic breast cancers. Supported by Health Research, Inc. (HRI) Grant 71-4084 (NCH), National Cancer Institute Grant R01CA160688 (KT).
RNA was extracted, followed by an RT-PCR, and amplification of the genes of interest via PCR. Changes in gene expression were quantitated using ImageJ. Overall, it was concluded that the exposure to genistein impacts the gene expression of both SET8 and SIRT1 within human prostate cancer cells.

#1390 Deregulation of RNA N6-adenosine methylation contribute to liver carcinogenesis. Mengnuo Chen, Larry L. Wei, Cheuk Ting Law, Felice HC Tsang, Iris MI Xu, Joyce LF Lee, Carmen Wong CL Wong, Irene DL Ng, Chun-Ming Wong. The University of Hong Kong, Hong Kong, Hong Kong.

Primary liver cancer, particularly the most common type hepatocellular carcinoma (HCC), is the third leading cause of cancer death worldwide. Case is even worse in Asia due to extremely high prevalence of HBV/HCV infection, which enhances the risk of HCC. However, the exact mechanism of how HCC develops is still not clearly defined. Previous studies have shown strong evidence for epigenetic alterations in human carcinogenesis, which mainly focus on abnormal DNA methylation, histone modifications, and chromatin remodeling. Recent studies suggest that diverse chemical modifications on RNAs, also known as "epi-transcriptome", constitute another layer of gene expression regulation. N-6-methyladenosine (m6A) is the most abundant modification on eukaryotic mRNA, which has been found to functionally influence mRNA stability, alternative splicing, and translation efficiency. However, the roles of m6A deregulation in human carcinogenesis remain unclear. Through whole-transcriptome sequencing, we identified that METTL3, the major component of m6A methyltransferase, was significantly up-regulated in human HCC. Up-regulation of METTL3 negatively associated with poor patient overall and disease-free survival, which have been linked to the non-cleaved N-glycosidically bound Kras and its potential to mediate pancreatic cancer initiation. Congruently, pharmacological inhibition of G9a using BRD4770 displays an inhibitory effect on Kras-induced cell proliferation. Combined, these data provide evidence for a key role of the me3K9H3-G9a pathway as a mediator of the oncogenic Kras response and defines a novel point of potential therapeutic intervention for PDAC.

#1392 Development of a novel mouse model for intrahepatic cholangiocarcinoma. Rebecca Marcus,1 Wai Chin Foo,2 Anirban Maitra,1 Sonal Gupta1.

1Sheikh Ahmed Bin Zayed Al Nahyan Center for Pancreatic Cancer Research, The University of Texas MD Anderson Cancer Center, Houston, TX; 2The University of Texas MD Anderson Cancer Center, Houston, TX.

Cholangiocarcinoma (CCA) is a poorly understood cancer of the biliary epithelium and the second most common type of liver cancer. Surgery currently offers the only potential for cure; however, most patients present with advanced disease and are therefore unreatestable. The Cancer Genome Atlas (TCGA) analysis and other recent genomic studies have revealed discrete epigenetic perturbations amongst CCA tissues originating from different anatomic sites. Intrahepatic cholangiocarcinoma (ICC) arises from the intrahepatic bile ducts, and a subset of ICCs harbor activating mutations in the cholangiocyte regulatory factor BRCA-associated protein 1 (BAP1). Loss of this protein may be associated with global epigenomic and transcriptomic alterations that ultimately contribute to tumor progression and metastasis dissemination. While elucidating the molecular pathways of ICC may identify potential targeted therapies and improve early detection, inquiry into this disease has been hampered by a lack of genetically faithfull animal models. We developed a genetically engineered mouse model (GEMM) of ICC that incorporates an activating mutation in BAP1 combined with Kras activation. An Albumin-Cre promoter was used to induce hepatoblast-specific mutations. Mutant Kras cooperates with loss of BAP1 and results in lethal hepatic transformation and dose-dependent survival. Kras activation alone results in extended disease latency and survival > 50 weeks. Loss of BAP1 alone or heterozygous loss of BAP1 combined with mutant Kras shortens disease latency, with mice surviving 39 weeks on average. A significant reduction in survival is seen with homozygous loss of BAP1 and Kras activation (KrasBAP1−/−). These mice survive on average 23 weeks (p = 0.0045). Histopathologic evaluation of KrasBAP1−/− mice demonstrates focal biliary precursor lesions, frank ICC, and hepatocellular carcinoma (HCC). Mice with heterozygous deletion of BAP1 and Krash activation loss of BAP1 alone, or Krash activation alone develop HCC only. Given the bipotential nature of hepatoblasts, the ICC phenotype of our GEMM may be enhanced by inducing biliary tree-specific mutations. Adenoviral Cre enzyme (Ad-Cre) is used to achieve such combinatorial specificity, and a novel surgery was developed whereby retrograde biliary tree administration of this enzyme is performed. Surgery utilizing GFP-tagged hepatoblast-associated transgenes confirm administration targeted to the biliary tree. Retrograde biliary tree injection of Ad-Cre into ROSA26mT/mG mice demonstrates Cre recombinase expression within cholangiocytes, thereby establishing proof-of-principle. Ad-Cre injection in Kras BAP1−/− mice to induce cholangiocyte-specific BAP1 deletion and Kras activation is ongoing.

#1393 Chromatin remodelers as potential new targets for therapy of pediatric sarcoma. Joana G. Marques,1 Berkley Gryder,2 Marco Wachtel,3 Iaved Khan,2 Beat Schaeffer.1 University Children’s Hospital, Zurich, Switzerland; 2National Institutes of Health, Bethesda, MD.

Fusion-positive rhabdomyosarcoma (FP-RMS) is a pediatric malignancy driven by the fusion transcription factor PAX3-FOXO1, which generates an aberrant gene expression signature leading to cell transformation. Since FP-RMS cells are highly addicted to the fusion protein, it is in focus as target for alternative therapies. Nevertheless, PAX3-FOXO1, as a transcription factor, does not contain structural cavities and has a low druggability. We therefore hypothesize that we can affect this aggressive subtype of RMS by targeting the co-regulators that collaborate with the fusion protein in regulating transcription. Recently, we have identified the NuRD (Nucleosome Remodeling and Deacetylase) complex as a potential partner of PAX3-FOXO1 in gene expression modulation. The NuRD complex is unique among chromatin remodeling complexes due to its dual enzymatic activity (histone deacetylation through HDAC1/2 and nucleosome positioning by CHD4 - chromodomains-DNA-bind-
ing protein 4), offering new possible therapeutic targets. Silencing of two core members of NuRD, CHD4 and RBBP4, led to a drastic decrease in FP-RMS cell viability. Additionally, CHD4 depletion caused a complete regression of mouse tumor xenografts, but it did not affect proliferation of myoblasts, fibroblasts or fusion negative RMS cells, despite the fact that these cells also carry high CHD4 expression and are highly sensitive to the fusion protein. We investigated these huge discrepancies and learnt that it affects the expression of approximately 50% of PAX3-FOXO1 target genes with most of these genes being upregulated, suggesting an activating role for CHD4 in these cases. Consistent with a positive effect of CHD4 on gene expression, Chip-seq experiments with FP-RMS cell lines demonstrated that NuRD promotes promoter and enhancer regions of highly expressed genes and co-localizes with the fusion protein at regulatory regions of a subset of its target genes. Next, we studied the influence of this nucleosome remodeler on the chromatin status by DNase hypersensitivity assays and determined that the presence of a DNase signal at PAX3-FOXO1 binding sites is concordant with the presence of CHD4. Hence, we suggest a scenario where CHD4 plays an essential role on FP-RMS tumorigenesis by allowing chromatin to acquire an open architecture that enables PAX3-FOXO1 mediated gene expression. In summary, our data propose that CHD4 has a crucial role as a co-regulator of PAX3-FOXO1 driven gene expression. To our knowledge, CHD4 is the first identified chromatin remodeler associated with PAX3-FOXO1 transcriptional activity, thus highlighting the relevance of epigenetic regulation in FP-RMS tumor development and opening chromatin remodelling as a possible new field of action against this tumor, which is driving ongoing work aimed at finding first-in-class small molecules to inhibit CHD4 function.

#1394 Bioorthogonal Profiling of Protein Methylation (BPPM) identified MCM5 as a new substrate for SETD8 in DNA replication. Fabio Pittella Silva, Gil Blum, Chamara Senevirathne, Luo Minkui. Memorial Sloan Kettering Cancer Center, New York, NY.

SETDb is the only member of the SET domain containing methyltransferase family, which catalyzes mono-methylation of K20 on histone H4 (H4K20me1). Lysine residues of non-histone proteins such as p53 and proliferating cell nuclear antigen (PCNA) are also mono-methylated, while lysine residues in Numb were found to be dimethylated. As a consequence, SETDb methylation activity is implicated in several fundamental cellular processes such as transcriptional regulation and heterochromatin formation as well as processes that ensure genomic stability including DNA replication and the DNA damage response. Although it has been suggested that SETDb is involved in DNA replication as a positive regulator of origin licensing through H4K20 methylation and by supporting Okazaki fragment processing through PCNA methylation, to date, there is no evidence whether other key protein in the replication fork is directly modified by SETDb. To address this question, we used Bioorthogonal Profiling of Protein Methylation (BPPM) with engineered enzyme and synthetic SAM analogues to profile new substrates for SETDb. We genetically engineered SETDb and identified mutants amenable to accommodate non-native SAM analogues containing a terminal alkyne moiety for click chemistry. The engineered SETDb can transfer this distinct chemical moiety into target proteins for subsequent pulldown and identification of the modified substrates. Among the new substrates identified by BPPM, MCM5 was the most abundant, which was further validated by immunoblotting. We hypothesized that MCM5 is a new substrate for SETDb that is mono-methylated by SETDb. To test this hypothesis, we performed western blotting with anti-MCM5 antibody and the presence of MCM5 was confirmed. We also performed Co-IP experiments and confirmed the interaction between SETDb and MCM5. We also performed CHIP-seq experiments with HeLa cell line and confirmed the enrichment of MCM5 at the promoters of genes that are involved in DNA replication. These results suggest that MCM5 is a new substrate for SETDb in DNA replication.

#1395 Deciphering inosinomine in glioblastoma versus normal cortex. Angela Gallo,1 Alessandro Silvestris,2 Valeriana Cesaroni,1 Valentina Tassinarini,1 Nicolò Mangraviti,1 Ernesto Picardi,2 Graziano Pesole.1 Ospedale Pediatrico Bambino Gesù, Roma, Italy; 2Università di Bari, Bari, Italy.

Cancer is driven by alterations of the genomic information, which carries mutations in key genes providing selective advantage for clonal multiplication of cancer cells. However, mutations within DNA are not the only source for cell alteration. RNA molecules are targets of a series of post-transcriptional modifications, such as splicing and RNA editing, that can affect sequence, structure and stability. The most common type of RNA editing in humans converts Adenosine in RNA targets into Inosinome (A-to-I) and is catalyzed by two adenosine deaminases that act on dsRNA (ADARs) family of enzymes (ADAR and ADAR1B). Inosines are subsequently interpreted as guanosines by several cellular proteins and could ultimately lead to a genomic mutations (A-to-IG). At present, it has been estimated that over 4 millions editing sites exist in our transcriptome in- tendency, and RNA editing mediated by ADARs at RNA level are necessary for our survival and their levels is highly regulated in different tissues and during development. Considering the importance of ADAR activity in our cells we believe that if ADAR are not well regulated they may contribute to cancer on set and/or progression. The advent of high-throughput RNA sequenc- ing has enabled identification of RNA editing sites and global analyses of cancer RNA editing. ADARs mediated RNA editing dynamically contributes to genetic alterations in cancer, including high-grade gliomas. Gli- oblastoma (GBM) is one of the most common and aggressive primary brain tumor in humans and despite advances in understanding the molecular mechan- isms underlying these tumors, current treatments are ineffective. In order to elucidate the glioma-specific RNA editing signature, we analyzed 146 RNA-Seq from primary glioblastomas from the TCGA dataset compared to 132 normal brain cortex RNA-Seq from the GTEx database. To our knowledge, A-to-I editing events has been detected using a collection of more than 4 million annotated edited substrates and the REDTools suite of python scripts with stringent filter by. means of the Cuifffquant/Cuifffdiff tools, we have also compared global transcriptome profiles and ADAR genes expression pat- terns at RNA and protein level across GBM samples and we found that the general down regulation of editing events at both recoding and non -recoding (Alus) sites that correlate with a down expression of ADARB1 enzyme. No differences were observed with ADAR expression. Overall, we observed a strong editing landscape perturbation in glioblastoma that could be important for identifying the most effective target genes for possible therapeutic intervention.

#1396 Role of epigenetic reader and alternative mRNA splicing variant MBDB2_v2 expression in triple-negative breast cancer. Emily A. Garcia,1 Bin Sao,1 Cristina Mitrea,2 Gregory Dyson,1 Julie Boerner,1 Lisa Polin,1 Stan R. Terlecky,2 Alicia Bollig-Fischer.1 Wayne State University, Detroit, MI; 2Seton Hall University, South Orange, NJ.

Background: Among all breast cancer patients, those diagnosed with the triple-negative breast cancer (TNBC) subtype have the worst prognosis, in part attributable to the fact that TNBC lacks targets for effective molecularly-targeted therapies. The concept that EGFR inhibitor drugs could be used as a targeted treatment against TNBC has been put forth because roughly 50% of TNBC express high levels of EGFR. However clinical trials targeting EGFR did not significantly improve patient outcomes. Our recent work offers a potential ex- planation as to why EGFR inhibitors failed and supports an innovative therapeu- tic approach combining an EGFR tyrosine kinase inhibitor with the novel antioxidant biologic, CAT-SKL. Our data indicate that inhibition of cancer stem cell-like cells depends on antioxidant-induced downregulation of an alternative mRNA splice variant of the methyl-CpG binding domain 2 gene, MBDB2_v2. Objective and Methods: The purpose of the present study was to evaluate the importance of MBDB2_v2 in TNBC and better understand how CAT-SKL regu- lates its expression. Using TNBC cell lines we investigated the effects of MBDB2_v2 expression on tumor initiation capacity. Furthermore we studied MBDB2_v2 mRNA levels in an in vivo tumor xenograph mouse model of TNBC and in human TNBC samples using an existing database. Then by RNA-seq analysis we assessed how CAT-SKL regulates MBDB2_v2 expression in TNBC cells through differential gene expression. Results: The results support that MBDB2_v2 expression is regulated by redox signaling in TNBC cells, which is tightly linked to TNBC cell tumor initiation capacity. Furthermore, MBDB2_v2 was upregulated in tumors harvested from overweight mice, which also displayed increased tumor take rate, suggesting that a pro-inflammatory tumor microenvironment could play a role in promoting MBDB2_v2 expression. In addition, RNA-seq analysis identified an overrepresentation of mRNA splicing factors with a repression down regulation regulated by CAT-SKL treatment of TNBC cells. From this gene set two splicing co-factors are highlighted: SRRM1 and CPSF3. These co-factors are critical for splicing factor function, 3’ end formation and polyadenylation; and both have been linked via protein-protein interaction to a relevant exonic splicing enhancer site in exon 3 of the MBDB2_v2 transcript. Finally, according to the Kaplan Meier Plotter database high MBDB2_v2 levels in BC were associated with shorter relapse free survival. Conclusion: Our investi- gation sheds light on the relevance of MBDB2_v2 expression in TNBC, and how a select antioxidant regulates its expression. The application of which holds promise of a novel, targeted therapeutic modality for TNBC, the subtype with the worst prognosis and highest need for treatment options.
#1397 Antisense oligo nucleotide of Annexin A4 improved platinum resistance in ovarian clear cell cancer. Reisa Kakukuri,1 Satoshi Nakagawa,2 Tadashi Iwamiya,1 Eiji Kobayashi,1 Shinya Matsuzaki,1 Yutaka Ueda,1 Kiyoshi Yoshino,1 Yuva Kashara,1 Satoshi Obioka,1 Tadashi Kimura,1 Satoshi Serada,1 Tetsuo Naka,1 Minoru Fujimoto1. 1Osaka University, OSAKA, Japan; 2Sakai city medical center, OSAKA, Japan; 3National institute of Biomedical Innovation, OSAKA, Japan.

Introduction: Ovarian cancer in Japan are classified as clear cell carcinoma (CCC) more than 20 %, this percentage is higher than in Europe and United States. Besides, it is well known that CCC of ovary is highly resistant to cancer chemotherapy including carboplatin and paclitaxel treatment. We reported that Annexin A4 expression was observed in CCC tissues by using quantitative real-time polymerase chain reaction. Annexin A4 expression was also observed by immunohistochemical analysis. Elevated Annexin A4 level has been detected in various epithelial cancer cell lines and have reported associating with chemoresistance to platinum-based cancer drugs. To overcome the platinum chemoresistance, we thought antisense oligonucleotides (ASOs) to be a good therapeutic option in a way of highly specific therapy for improving chemoresistance by suppressing the expression of Annexin A4 in cancer cells. Methods: We generated ASO targeting Annexin A4 with 2’, 4’-bridged nucleic acid. And we analyzed suppression of Annexin A4 in A549-transfected RMG-1 cell line (CCC) in vitro using real time PCR and western blotting. In 16 types of ASOs targeting Annexin A4, 2 ASOs were eligible. Cells were seeded in 96-well plates (2,000 cells per well). Next day, cells were transfected with ASOs using lipofectamine 2000 and were exposed to various concentrations (0-100 μM) for 72 hr. Then, drug concentrations resulting in a 50 % inhibition of cell growth (IC50 values) were calculated. Intracellular platinum accumulation in Annexin A4 overexpressing cells was analyzed. To assess the improvement of platinum resistance in vivo, we used ICR nu/nu mice xenografted subcutaneously with RMG-1 cells. Intraperitoneal injection of cisplatin 3mg/kg after intratumoral administration of ASO 1mg/kg each twice a week were given to xenograft mice. Results: By real time PCR analysis, among strong 16 types of ASOs targeting Annexin A4, 2 ASOs showed strong knockdown efficiency (about 80 % knockdown) compared to negative control ASOs. Western blotting analysis showing knockdown of Annexin A4 expression was observed in Annexin A4 ASO transfected cells compared to no treatment or control ASOs in vitro. ASO-transfected RMG-1 cells was less resistant to cisplatin (IC50 = 3.3±M) compared with control cells (IC50 = 5.2±M). Same result were obtained with carboplatin. Platinum resistance was significantly improved in treated with Annexin A4 ASO and cisplatin compared to control ASO and cisplatin treated group in vivo. Conclusion: By transfection of ASOs targeting Annexin A4, platinum resistance have improved in vivo and in vitro, Annexin A4 have associated with efflux of platinum anti-tumor drug. In conclusion, antisense oligonucleotides for Annexin A4 will be a therapeutic option for ovarian clear cell carcinoma with chemoresistance to platinum anti-tumor drug.

#1400 Using tumor spheroids to evaluate the efficacy of EZH2 inhibitors in clear cell renal cell carcinoma (ccRCC). Darmood Wei, Youfeng Yang, Christopher J. Ricketts, Carole Sourbier, Laura S. Schmidt, William M. Linehan. NCI, Bethesda, MD.

Enhancer of zeste homolog 2 (EZH2) is a key component of the polycomb repressive complex 2 (PRC2). EZH2 is frequently overexpressed in a wide variety of human malignancies including non-Hodgkin lymphoma, gastric cancer, pancreatic cancer, and lung cancer. Thus it has potential to become a therapeutic target. Characterization of EZH2 as a therapeutic target in clear cell renal cell carcinoma (ccRCC) has not been fully explored. ccRCC has been defined by mutation of the von Hippel-Lindau (VHL) tumor suppressor gene in combination with chromosome 3p loss. Recent sequencing efforts have revealed that several chromatin remodeling genes encoded on chromosome 3p are often mutated, of which PBRM1 is the most frequent (41%). The PBRM1 gene codes for the BAF180 protein, a SWI/SNF chromatin remodeling complex subunit. Loss of BAF180 in ccRCC may disrupt the PBAF variant of the SWI/SNF complex. The SWI/SNF complex remodels the chromatin landscape by either sliding or evicting the nucleosomes from the chromatin. This chromatin remodeling modulates the accessibility to promoter regions by transcriptional machinery. It is through this mechanism that the SWI/SNF complex can regulate a range of cellular processes. It has been demonstrated that the SWI/SNF complex can act antagonistically to the PRC2 complex by evicting PRC2 complex from the promoters of tumor suppressors. Disruption of the SWI/SNF complex would impede the eviction of the PRC2 complex, similarly observed in SNF5-deficient malignant rhabdoid tumors. Therefore, we hypothesize that PBRM1 inactivation disrupts specific SWI/SNF complexes allowing EZH2 to bind and repress target tumor suppressor genes. Thus inhibition of EZH2 in ccRCC may present as a targeted therapeutic option in tumors with PBRM1 mutations. We have investigated EZH2 in ccRCC cell lines with PBRM1 mutations and observed that these cell lines have overexpression of EZH2 in comparison to RPE1 (renal cortex proximal tuibule epithelium cell line). We examined the effects of two EZH2 inhibitors (GSK126 and EPZ6438) on ccRCC tumor spheroids. Our preliminary data suggests EZH2 inhibition results in reduced growth of PBRM1 mutant cell lines grown as tumor spheroids.


Genetic and environmental factors cooperate to assure precise genome-wide epigenetic regulation of the mammary epithelia cell transcriptome. Our interest in regulatory epigenetic mechanisms that, by determining mammary epithelial cell decisions, are pivotal to deter the onset and progression of breast cancer, let us identify a regulatory epigenetic mechanism of mammary morphogenesis that links a physiological regulatory lipid of environmental origin (all-trans retinoic acid, RA) with sphingolipids capable of determining either cell death (ceramide, CER) or cell life (sphingosine 1 phosphate, S1P). Whenever RA, for different reasons, fails to perform the epigenetic transcriptional control of neutral sphingolipids...
gomyelinase 2 (nSmase2/SM3DP3), involved in the synthesis of pro-apoptotic CER, SIP fosters both pro-proliferative and pro-invasive activity. Apparently, in the absence of epigenetic control of CER production by RA, SIP contributes to determine RA tumorigenic action. This study provides the rationale for combination therapeutic approaches with epigenetic drugs and inhibitors of either sphingosine kinase, the enzyme involved in SIP synthesis, or SIP receptors. This study was supported by the NCI R01 CA127614 grant (NS).

#1402 NME3 links mitochondrial fusion to DNA repair in nuclear genome. Zee-Fen Chang. National Taiwan University, Taipei, Taiwan.

In mammals, there are 10 isoforms of NME (NDP Kinase), which catalyze the reversible reaction of NTPs formation from NDP and ATP. By RNA interference, NME3 was found critical for DNA repair. Data from analysis of the steady-state levels of nRTPs and dNTPs suggest that NME3 does not play a critical role in determining the total pools of these nucleotides. However, its mitochondrial localization and catalytic function are necessary for its function in DNA repair. Super-resolution microscopy analysis indicates the localization of NME3 at mitochondrial outer membrane. We further used APEX-mediated biotinylation to identify proteins interacting with NME3. Among them, Mfn1 is validated to associate with mitochondrial NME3 in vitro and in vivo. We provide evidence that NME3 is required for DNA damage-induced mitochondria fusion via GTP-mediated fusion of Mfn1. The lack of this process increased nucleotide oxidation and sustained DNA damage in nuclear genome. These data highlight the function of NME3 in mitochondrial fusion, which is required for the maintenance of genome integrity.

#1403 PCNA-dependent cleavage and degradation of SDE2 regulates response to replication stress. Ukhyun Yo1, Winson Cai, Jingming Wang, Yoojin Kwon, Alan D. D’Andrea, Hyungjin Kim. Stony Brook University, Stony Brook, NY, Dana-Farber Cancer Institute, Boston, MA.

Maintaining genetic integrity during DNA replication is essential for cellular survival and for preventing tumorigenesis. Proliferating cell nuclear antigen (PCNA) functions as a processivity factor for DNA replication, and posttranslational modification of PCNA plays a key role in coordinating DNA repair against replication-blocking lesions by providing a platform to recruit factors required for DNA repair and cell cycle control. Here, we identify human SDE2 as a new genome surveillance factor regulated by PCNA interaction. SDE2 contains an N-terminal ubiquitin-like (UBL) fold, which is cleaved at a diglycine motif via a PCNA-interacting peptide (PIP) box and deubiquitinating enzyme activity. The cleaved SDE2 is required for negatively regulating ultraviolet damage-inducible PCNA monoubiquitination and counteracting replication stress. The cleaved SDE2 products need to be degraded by the CRL4<sup>C372</sup> ubiquitin E3 ligase in a cell cycle- and DNA damage-dependent manner, and failure to degrade SDE2 impairs S phase progression and cellular survival. Collectively, this study uncovers a new role for CRL4<sup>C372</sup> in protecting genome integrity against replication stress via regulated proteolysis of PCNA-associated SDE2 and provides insights into how an integrated UBL domain within linear polypeptide sequence controls protein stability and function. Knowledge on such mechanism will be useful to identify novel cancer therapeutic interventions exploiting deregulated ubiquitin signaling and SDE2 activities in cancer.

#1404 Regulation of 53BP1 by the structural nuclear protein NuMA. Naik Salvador Moreno. Wake Forest Baptist Medical Center, Winston Salem, NC.

Cells repair DNA double-strand breaks (DSB) through two major pathways: homologous recombination (HR) and non-homologous end joining (NHEJ). The choice between these two pathways is critical to cell survival and is altered in cancers. 53BP1 is a protein with an important role in this choice: it promotes NHEJ by blocking CIP-dependent DNA resection. Here, we present a new negative regulation mechanism of 53BP1 that relies on the structural protein NuMA. This protein participates in mitotic spindle assembly, influences chromatin organization during the interphase and modulates the chromatin response to DNA damage. We identified NuMA in a proteomics analysis of a 53BP1-interacting proteins. The interaction was confirmed by reciprocal immunoprecipitation and with a FRET assay. In response to DSB, we measured greater than 50% decreased 53BP1-NuMA binding, which suggests that NuMA may restrain 53BP1 diffusion in the absence of DNA damage. Fluorescence correlation spectroscopy (FCS) measurements in cells expressing GFP-tagged 53BP1 support this hypothesis. Moreover, NuMA overexpression inhibited 53BP1 recruitment at DNA damage sites in laser microirradiation assays, whereas NuMA silencing had the opposite effect. 53BP1 plays an essential role in B cell class switch recombination and survival of PARP inhibitors in A1-null cells. NuMA overexpression prevented immunoglobulin switching, and over-wrote cancer cell sensitivity to PARP inhibitors to the same extent as 53BP1 loss of function. Our results help understand how 53BP1 is controlled, and shed light on the mechanisms regulating PARP1 sensitivity.

#1405 A novel chemotherapeutic agent to treat tumors with DNA mismatch repair deficiencies. Kyungjae Myung, Yongliang Zhang, Young-Un Park. Institute for Basic Science, Ulsan, Republic of Korea; NIH, MD.

Impairing the division of cancer cells with genotoxic small molecules has been a primary goal to develop chemotherapeutic agents. However, DNA mismatch repair (MMR)-deficient cancer cells are less susceptible to conventional chemotherapeutic agents. Here we have identified baicalein as a small molecule that selectively kills MutS<sup>-</sup>-deficient cancer cells. Baicalein binds preferentially to mismatched DNA and induces a DNA damage response in a mismatch repair-dependent manner. In MutS<sup>-</sup>-proficient cells, baicalein binds to MutSα to dissemble from DNA and induce cell cycle arrest and subsequent cell death. In contrast, continued replication in the presence of baicalein in MutS<sup>-</sup>-deficient cells results in a high number of DNA double-strand breaks and ultimately leads to apoptosis. Consistently, baicalein specifically shrinks MutS<sup>-</sup>-deficient xenograft tumors and inhibits the growth of AOM-DSS-induced colon tumors in colon-specific MSH2 knockout mice. Collectively, baicalein offers the potential of an improved treatment option for patients with tumors with a DNA MMR deficiency.

#1406 Towards an RNA expression-based signature for oncogene-induced replication stress. Sergi Guerrero, Rudolf Fehrmann, Marcel ATM van Vugt. University Medical Center Groningen, Groningen, Netherlands.

Introduction Certain tumor subgroups, including triple negative breast cancers (TNBCs), are characterized by high levels of genomic instability (GI). These tumors are typically very aggressive and difficult to treat. Increasingly, we realize that GI is fueled by replication stress (RS). RS can be induced by expression of oncogenes, including CCNE1, MYC and CDC25A, which perturb initiation, elongation and/or termination phases of DNA replication. RS poses a threat to cellular viability, and tumor cells with high levels of RS will therefore increasingly depend for their survival on DNA damage checkpoint and repair pathways. Possibly, this increased dependence creates therapeutic opportunities. Unfortunately, it is currently difficult to determine levels of RS in tumors, a requirement to efficiently screen for new treatments. The aim of this work is to develop an RNA expression-based signature for oncogene-induced replication-stress. Material & Methods A panel of cell lines (RPE-1, RPE-1-P5<sup>+/+</sup>, MDA-MB-231, BT-549 and HCC-1806) was engineered to doxycycline-inducibly express a set of oncogenes (CDC25A, CCNE1 or MYC). Single DNA fiber replication analysis in combination with immunoblot analysis of RS markers was used to validate the cell line models. MTI assays were performed to assess sensitivity to ATR and Weel inhibitors. RNA-sequencing was used to uncover expression changes upon RS induction. Results Treatment with doxycycline resulted in high expression levels of CDC25A, CYCLINE or MYC. Expression was homogeneous but present in the far majority of RPE-1 cells (40% CDC25A, 88% CCNE1 and 93% MYC). DNA fiber analysis was subsequently used to study the alteration of DNA replication dynamics. Severe reduction on ongoing DNA synthesis velocity was shown in engineered cells (RPE-1 and MDA-MB-231) under doxycycline treatment, confirming prominent RS. In line with elevated levels of RS, oncogene induction resulted in elevated levels of ATR activity, as judged by phospho-CHK1 and phospho-RPA levels. Importantly, oncogene-expression enhanced sensitivity to Weel and ATR checkpoint inhibitors. Subsequently, RNA samples were harvested under the presence and absence of doxycycline. In parallel to oncogene-expression, hydroxyurea, a chemical agent known to increase stalled replication forks was applied as a DNA damage control. RNA-sequencing analysis will be performed to identify differentially expressed genes upon oncogene expression. Commonly identified genes over multiple cell lines and shared between the different oncogenes will be used to build a RNA expression-based signature. Conclusively, our results indicate that oncogene expression provokes RS in multiple cell line models and induces impaired replication fork stability and sensitivity to cell cycle checkpoint inhibitors. These models are therefore valid to develop an RNA expression-based qualifier in order to facilitate patient selection towards novel therapeutics.
Translational regulation of RPA2 via IRES by UNR and eIF3a.

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Purpose: Translation is a critical step in the process of genetic information expression. Internal ribosome entry site (IRES) element is a RNA sequence with a complex structure, which plays an important role in cap-independent translation regulation. The activation of RPA2 IRES element can cause its abnormal expression and finally effects DNA repair pathway. We conducted series of assays to explore the mechanism of translation initiation regulation of RPA2 via IRES by UNR and eIF3a Methods: Biotin pull down assay was taken to investigate the physical interaction between RPA2 IRES and UNR. UNR was knocked down and overexpression in H1299, A549 and SK-MES cell lines. Western blot and real-time PCR were used to detect protein level and mRNA level respectively. For interaction of eIF3a with UNR, CO-IP assay and co-localization assay were conducted. And GST pull down assay was carried out to further identify their exact binding domains. RPA2 IRES-interacting domains of UNR and eIF3a were explored with EMSA assay. Results: UNR protein could directly bind to RPA2 IRES as well as to eIF3a, and UNR regulated the protein expression of RPA2 in H1299, A549 and SK-MES cells, while the mRNA level of RPA2 remained no change. UNR interacted with the first domain of eIF3a and with RPA2 IRES via its own first domain. However, we have not found a clue for the direct interaction of eIF3a with RPA2 IRES yet. Conclusion: UNR cooperated with eIF3a to regulate the RPA2 IRES activity and hence to regulate the RPA2 protein expression. This might be a regulation mechanism of cellular internal ribosomal entry site affecting translation initiation, the rate-limiting step of translation.

LIM protein Ajuba participates in the ATR response by direct interaction with RPA70.

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DNA damage response (DDR) pathways are essential for genome stability and cell survival. Specifically, the ATR kinase is activated by DNA replication stress or UV irradiation. An early event in this activation is the phosphorylation and local recruitment of RPA, a single strand DNA binding protein. Our lab has shown that the LIM protein Ajuba associates with RPA, an OB fold-containing protein, and this interaction prevents an unscheduled ATR response. We hypothesize that Ajuba represses the ATR pathway through direct interaction with RPA, thereby preventing activation of ATR. To test this hypothesis, we employed E. coli and in vitro translation system for the production of recombinant proteins. Our data show that Ajuba directly associates with the large subunit of RPA, RPA70. The domains involved in the interaction were the C-terminal OB folds of RPA70, and the region between the PreLIM and LIM domains in Ajuba. We speculate that this interaction mediates the association of Ajuba with the RPA complex in cells. Understanding the implication of the Ajuba and RPA interaction will allow us to gain insights on the regulation of DDR, which is involved in early events of cellular transformation and tumorigenesis in human cells.

Analysis of 16,172 patient-derived tumor samples indicate TXX2 as being essential for survival of genomically instable cancer cells.

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The purpose of this study was to evaluate the clinical significance of TXX2 expression in a large cohort of patient-derived tumor samples. The expression of TXX2 was measured in 16,172 patient-derived tumor samples using 3 independent technologies: FqMRNA, a genome-wide association analysis, and RNA sequencing. The results showed that TXX2 expression was significantly higher in genomically instable tumors, suggesting that TXX2 might play a role in genomic instability.

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To investigate the clinical significance of TXX2 expression, we analyzed the expression of TXX2 in a large cohort of patient-derived tumor samples using 3 independent technologies: FqMRNA, a genome-wide association analysis, and RNA sequencing. The results showed that TXX2 expression was significantly higher in genomically instable tumors, suggesting that TXX2 might play a role in genomic instability.

Differential mismatch repair-dependent damage responses in sub-populations of cells from human intestinal organoids.

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Hereditary mutations in DNA mismatch repair (MMR) genes cause the cancer predisposition syndrome called Lynch syndrome. Patients predominantly develop colorectal cancer and some extracolonic cancers at a young age. The MMR pathway repairs mismatches created during DNA replication, but also can induce apoptosis and cell cycle arrest in response to certain DNA damaging agents as a protective mechanism to eliminate cells at risk of accumulating mutations. Our understanding of this MMR-directed DNA damage response has come primarily from studies in human cancer cell lines. We wished to examine the role of the MMR-dependent damage response in a model that may be more relevant to the human intestine. Therefore, we have created human intestinal organoids (HIOs) in vitro either by directing the differentiation of human embryonic stem cells (hESCs) or through culturing of adult intestinal tissue samples. We have used these HIOs to specifically study the MMR-dependent damage response to DNA alkylation damage. We have found that whereas hESCs undergo rapid apoptosis in response to alkylation damage, the differentiated HIOs or those created from adult tissues undergo not only apoptosis, but also senescence and accelerated differentiation following damage. Using CRISPR-Cas9 gene editing in hESCs, we also created MMR-deficient HIOs that failed to respond to the drug indicating that these responses are MMR-dependent. We determined that the cells undergoing apoptosis following DNA damage are most likely the intestinal stem cells suggesting that different cell types in the intestine have different MMR-dependent responses to damage. Together these results
suggest that loss of MMR function in the intestinal crypts may provide a selective advantage particularly in an environment conducive with DNA damage that promotes tumorigenesis.

**#1412** High EGFR somatic mutation frequency targeting the TK domain in colorectal cancer patients with microsatellite instability (MSI). Safoora Dehimi,1 Michael Slifker,2 Eric A. Ross,2 Wafik S. El-Deiry1. 1Department of Hematology/Oncology and Molecular Therapeutics Program, Fox Chase Cancer Ctr., Philadelphia, PA; 2Department of Biostatistics, Fox Chase Cancer Ctr., Philadelphia, PA.

CRCs arise through genetic changes that impact various driver genes and in some tumors increased mutation rate in microsatellite unstable tumors. The hypermutable phenotype associated with microsatellite instability (MSI) results from loss of the mismatch repair system (MMR) activity. MSI is detected in 15% of all CRCs, and such tumors have a better prognosis and different chemotherapeutic outcome patterns, including high clinical benefit from immune checkpoint therapy or relative resistance to 5-FU as compared to microsatellite stable (MSS) tumors. The epidermal growth factor receptor (EGFR) signaling pathway plays an essential role in carcinogenesis of CRCs and is known to be overexpressed in MSI CRCs while highly mutated in Lung cancer. We analyzed the mutation frequency of deregulated pathways including EGFR and its downstream genes KRAS and BRAF in CRCs. We found a significantly elevated EGFR mutation frequency in CRC MSI-H subtype (45.5% vs. 6.5% in MSS CRCs, p<0.0000001). Although KRAS and NRAS are mutated with high frequency in both MSI-H and MSS groups, BRAF corresponding to RTK-RAS pathways is more altered in MSI-H than MSS CRCs (32.67% vs 13.10%; p=0.001), consistent with the known association between MSI-H CRCs and BRAF mutations. We hypothesized that there might be a mutation pattern in EGFR in CRC subtypes that provide a rationale for EGFR-targeted therapy for a subtype of CRC. Of 1104 profiled CRCs in the COSMIC v73 database, somatic EGFR mutations were mapped for 101 MSI-H versus 916 MSS CRCs. EGFR mutations mapped on the protein structure revealed that mutations are mainly targeting tyrosine kinase (TK) domain while the extracellular domain remained mostly wild-type with potential for targeting by anti-EGFR monoclonal antibodies. It is known that downstream genes such as KRAS as well as expression levels of EGFR ligands can affect the sensitivity of CRCs to anti-EGFR therapy. We didn’t detect any difference in mRNA expression level between MSI-H and MSS group but our analysis is indicative of the presence of G12D mutations in a high proportion of KRAS-mutated MSS CRCs, therefore resistance to anti-EGFR antibodies such as cetuximab would be expected. However, EGFR tyrosine kinase inhibitors such as erlotinib and gefitinib could be considered for further testing in patients with MSI-H tumors that have a lower frequency of G12D KRAS mutations and may have mutant EGFR.

**#1413** Replication obstacles formed within common fragile sites under replication stress are targeted by the global genomic nucleotide excision repair pathway. Martın Mistrik,1 Lucie Beresová,1 Eva Vesela,1 Ivo Chamrad,1 Jiri Voller,1 Masayuki Yamada,1 Tomas Furst,1 Rene Lenobel,2 Katarina Chroma,1 Martin Mistrik,1 Lucie Beresová,1 Eva Vesela,1 Ivo Chamrad,1 Jiri Voller,1 Masayuki Yamada,1 Tomas Furst,1 Rene Lenobel,2 Katarina Chroma,1 Jan Gursky,1 Jiri Bartek1. 1Palacky University in Olomouc, Faculty of Medicine and Dentistry, Olomouc, Czech Republic; 2Palacky University in Olomouc, Faculty of Science, Olomouc, Czech Republic.

CFSs are genomic loci present in higher vertebrates that are particularly sensitive to various forms of replication stress and suffer from increased breakage and rearrangements in tumors. They have rather enigmatic evolutionary role and employ various DNA repair and checkpoint mechanisms promoting their stability. We used an original approach for identification of CFSs’ associated factors based on DNA probe designed to match the high flexibility island sequences typically present in some of the highly expressed CFS’s. This probe was used as affinity bait for fishing specifically enriched proteins which were further identified using SILAC and quantitative mass spectrometry. Among already known CFS’s stabilizers we identified also hits so far not implicated in CFS’s maintenance. Interestingly, most of these novel hits are components of the known CFS’s stabilizers we identified using SILAC and quantitative mass spectrometry. Among already used as affinity bait for fishing specifically enriched proteins which were further investigated in vitro and in vivo for their potential to enhance sensitivity of tumor cells to chemotherapy.

**#1414** Chromatin remodeling histone chaperone FACT complex modulates AP site damage repair in chromatin and sensitizes cancer cells to chemotherapeutic drugs. Heyu Song, Shrabasti Roychoudhury, Dan Feng, Kishor Bhakat. University of Nebraska Medical Center, Omaha, NE.

Recognition and repair of DNA lesions in the genome are critical for maintaining genome stability and the progression of the cell cycle; DNA replication defects lead to cancer development. The most frequently formed DNA lesion in the genome is the apurinic/apyrimidinic (AP) site which is mutagenic and blocks transcription and replication. The primary enzyme to repair AP sites in mammalian cells is the AP endonuclease (APE1), which often overexpresses in diverse cancer types and its overexpression is associated with patients’ resistance to chemotherapeutic treatments. The involvement of AP endonuclease (APE1) through the BER pathway is extensively investigated in vitro. However, how AP site is recognized by APE1 in the context of highly complex nucleosome structure in chromatin is unknown. Because DNA is packaged tightly in nucleosome, the ability of repair proteins to access sites of DNA damage and facilitates repair of the damage requires chromatin remodeling activities. Here, we show that APE1 interacts with chromatin remodeling histone chaperone complex, FACT (facilitates of chromatin transcription) via its N-terminal domain in cells. By immunoprecipitation of endogenous APE1 from chromatin fraction and separation of protein bands in SDS-PAGE followed by MALDI-TOF-TOF analysis, we have identified both subunits (SPT16 & SSRP1) of FACT as the prominent APE1 interacting partners. Subsequently, we confirmed the interaction of APE1 with FACT complex through a co-immunoprecipitation assay analysis and SPT16 and SSRP1 interact with APE1 in the nucleus and in chromatin. Interestingly, we found rapid eviction of histones with concomitant degradation of FACT complex upon induction of DNA damages. Downregulation of FACT complex abrogates the nucleosome eviction, the recruitment of repair protein APE1 in chromatin and the repair of AP sites, demonstrating the functional importance of nucleosomes disruption in BER pathway and identifying chromatin remodeling protein required for the process. Notably, knock-down of FACT showed increased sensitivity of cancer cells to many chemotherapeutic drugs. We also found that FACT down-regulated cells have much higher accumulation of AP site damages in the genome compared to control cells. Our study revealed a key role of nucleosome remodeling complex FACT in DNA damage repair in BER pathway. This study also suggests that histone chaperone complex could be a potential target for enhancing sensitivity of tumor cells to chemotherapy.

**#1415** SAN1: a novel senataxin associated nucleaserequired for the repair of interstrand crosslinks. Alex Andrews, Ian Macara. Vanderbilt University, Nashville, TN.

The DNA damage response (DDR) is a set of complex signaling pathways capable of sensing DNA damage, and activating a large number of enzymes involved in the remodeling and repair of the genome. Mutations in the genes involved in the DDR lead to the development of many cancers. One particularly dangerous type of DNA damage that can occur is an interstrand crosslink (ICL). ICLs can lead to the development of double strand breaks through the blockage of DNA replication and transcription. Although ICLs can arise endogenously from molecules such as aldehydes, most commonly they are induced from chemotherapeutic drugs such as Cisplatin and Mitomycin C (MMC). These drugs are commonly used in the treatment of breast and ovarian cancers. A better understanding of which proteins are involved in the repair of ICLs is critical for understanding resistance, toxicity, and response in patients treated with ICL inducing agents. The repair of ICLs requires the coordination of several DNA repair pathways including the Fanconi Anemia pathway, homologous recombination (HR), and nucleotide excision repair (NER).

The Fanconi Anemia pathway is essential for the repair of these lesions as it is responsible for the recognition of the ICL lesion, as well as the recruitment of several nucleases responsible for unhooking and removal the cross-linked nucleotides. Recently, we identified an uncharacterized 5’ nuclease that interacts with the RNA/DNA helicase Senataxin, which we have named senataxin-associated nuclease 1 (SAN1). Senataxin has been shown to act on R loops, RNA/DNA hybrids that are a source of endogenous DNA damage. Deletion of the SAN1 gene in HeLa cells or in mouse embryonic fibroblasts leads to the sensitization of cells to Cisplatin and Mitomycin C (MMC), but not to ionizing radiation that induces double strand breaks. Importantly, the defect in ICL repair can be restored using WT SAN1 but not with a mutant that is catalytically inactive. Treatment of SAN1-/- HeLa cells with MMC also leads to radial chromosome formation, a characteristic of cells deficient in ICL repair. Additionally, SAN1 partially colocalizes with FancD2 MMC induced foci, and treatment with MMC results in increased DNA damage and R loops in SAN1-/- cells. In conclusion, this study highlights the discovery of a novel nuclease involved in the repair ICLs, a process critical for understanding resistance and response to chemother-
apies such as Cisplatin and MMC. Future work is aimed at determining the specific step of ICL repair that SAN1 participates in, how and when during repair SAN1 is recruited to ICL sites, and which other DNA repair proteins SAN1 functions with.

#1416 Development of small molecule inhibitors for cancer therapy by targeting RPA and XPA nucleotide excision repair proteins. Navnath S. Gavande,1 Pamela S. VanderVare-Carozza,2 Tyler L. Vernon,1 Katherine Paweleczak,3 John J. Turchi1. 1Indiana University School of Medicine, Indianapolis, Indiana, IN; 2NERx Biosciences Inc, Indianapolis, IN.

Targeting DNA repair and the DNA damage response for cancer therapy has recently gained increasing attention with inhibitors of the PARP enzyme showing a therapeutic efficacy in various cancer models. However, the clinical benefit of DNA repair inhibitors has been limited due to the problem of collateral damage to cells with homologous recombination deficiency. Here, we show that targeting RPA and XPA with small molecule inhibitors potently ablates DNA repair and synthetic lethality of cancer cells with homologous recombination deficiency.

#1417 Expression of BARD1 induces genetic instability and is associated with cervical carcinogenesis. Maxim Pilyugin,1 Pierre-Alain André1, Nicole Concin,2 Gerda Hofstetter,2 Irmgard Irminger-Finger1. 1Rutgers University, New Brunswick, NJ; 2University Hospital Innsbruck, Innsbruck, Austria.

Cervical cancer is always linked to infection with the human papillomavirus (HPV), which causes hyper-proliferation of cervical epithelial cells and neoplasia and cancer. The viral oncoprotein interaction with host tumor suppressor proteins partially explains the uncontrolled proliferation, which ultimately progresses to cervical cancer. However, little is known about the specific factors that provide or inhibit the malignant transformation. Since a portion of HPV infections never evolves into cancer, the identification of discriminatory factors would be an important step towards the development of prognostic and therapeutic tools. Based on this notion, we investigated whether an isoform of BARD1, BARD1α, which we found frequently expressed in cancer cells, plays a role in the malignant transformation of cervical epithelial cells. Using immuno-histochemistry staining on tissue sections and RT-PCR we found that BARD1α is expressed in proliferating cervical cancer cells, while normal BARD1 is repressed. BARD1 might be a driver of carcinogenesis and might be a critical event in the progression from hyper-proliferating cervical epithelium after HPV infection to cervical cancer. Together, our results show that an isoform of BARD1, BARD1α, is increasingly expressed in precancerous stages of cervical cancer and the specific inhibition of BARD1α may represent a novel approach for therapeutic interventions to prevent cervical carcinogenesis after HPV infection.

#1418 G9a methyltransferase plays a role in ATM-dependent DNA damage response. Lizahira Rodriguez-Colon,1 Yasueva Ginjula,2 Atul Kulkarni,1 Safia Ansari,1 Shridar Ganesan1. 1Rutgers Cancer Institute of New Jersey, New Brunswick, NJ; 2Rutgers University, New Brunswick, NJ.

Induction of DNA damage leads to a choreographed set of local chromatin changes that ensures an efficient recruitment of DNA repair factors. One prin-

#1419 Role of a novel Senataxin-associated nuclease in DNA repair. Heather McCartney, Ian Macara. Vanderbilt University, Nashville, TN.

Yeast two-hybrid analysis has identified a novel, uncharacterized Senataxin-interacting protein with 5′ exonuclease activity, and homology to the FEN1 nuclease domain. Senataxin is an RNA/DNA helicase that functions in resolving R loop structures which occur as a result of normal replication and transcription, but can also persist resulting in genomic instability. We found that SAN1 (Senati-


Metnase, a human SET-transposase fusion protein contains two functional domains: a SET domain and transposase domain. Transposase domain exhibit strand transfer and end joining activity while set domain is responsible for histone lysine methyltransferase activity (KMT). Metnase also increases the efficiency of double strand break repair by non-homologous end joining (NHEJ); however, its role in the excision of clustered DNA damage remains to be inves-
tigated. We hypothesize that 5′-3′ endonuclease activity of metnase could possibly substitute for APE1 in BER and serve as an alternative initiator of BER in tumor cells lacking APE1. In the present study, we examined in vitro endonuclease activity of Metnase using abasic site containing artificial DNA, and de-
scribe its activity in BER. Its unique 5′ endonuclease activity was selective only for abasic lesions, but other base modifications such as 8-oxoguanine, uracil, 5-hydroxy-methylcytosine could not be cleaved. Metnase increases lesion removal and reduces clustered abasic lesions from DNA, and allows completion of short-patch or long-patch BER. APE1 has difficulty cleaving 5′ of clustered abasic lesions. This endonuclease that promotes BER in clustered oxidative DNA damage is not known. Metnase also cleaves multiple abasic lesions (clustered DNA damage) and facilitates the repair of DNA if the multiple DNA damages are 3 or more nucleotides apart on the opposing strands. However, repair of multiple DNA damages remains inefficient if these are in close proximity of each other (<3 nt apart on the opposing strands) and results in DNA double strand break (DSB). These results suggest that Metnase can promote BER of oxidative nucleotides, where APE1 is unable to function.
#1421 Evaluation of DDR-targeting strategy using ATR inhibitor in biliary tract cancer. Ah Rong Nam,1 Ji Eun Park,1 Ju Hee Bang,1 Mei Hua Jin,1 Do Youn Oh,2 Yung Jue Bang2. 1Cancer Research Institute, Seoul National University College of Medicine, Seoul, Republic of Korea; 2Department of Internal Medicine, Seoul National University Hospital, Seoul, Republic of Korea.

Background: The DNA damage response (DDR) is a multicomplex network of signaling pathways involved in DNA damage repair, cell cycle checkpoints and apoptosis. The ataxia telangiectasia and Rad3-related (ATR) protein kinase is a key enzyme in the DDR that activates checkpoint kinase 1 (Chk1), resulting in cell cycle arrest. Tumor types with loss of ATM function and/or high replication stress are expected to be more susceptible to DDR targeting. In biliary tract cancer (BTC), DDR related pathway, which includes RAP1, MSH6, BRCAP1, ATM, MLIH1, MSH2, is altered in about 20% of cases. TP53 module is observed in 33.9% of BTC cases (Nat Genet 2015). The purpose of this study is to test DDR targeting strategy using ATR inhibitor in biliary tract cancer. Methods: Using 9 kinds of BTC cells, MTT assay and colony formation assay were done for determining growth inhibitory effect of AZD6738, an ATR inhibitor. Cell cycle analysis was done by FACS Calibur flow cytometer and the methods described by Chou and Talalay were used to determine whether a synergistic effect existed between AZD6738 and cytotoxic chemotherapeutic agents (cisplatin, 5-FU, gemcitabine). The alkaline comet assay was done to measure of DNA damage in individual cells. Tumor xenograft model was used for in vivo test of AZD6738. Results: Among 9 BTC cells, SNU478 and SNU869 were most sensitive to AZD6738 which showed low expression of BTM and ATM and/or p53. AZD6738 blocked ATR-mediated Chk1 phosphorylation and increased rH2AX, a marker of DNA damage, in sensitive cells. AZD6738 significantly increased apoptosis (cleavage of PARP and caspase-7) and G2/M arrest, increased level of p21, and presence of DNA damage, in sensitive cells. AZD6738 and cytotoxic chemotherapeutic agents demonstrated synergistic effects in colony formation assay, cell cycle analysis and comet assay. In xenograft model of SNU478, AZD6738 monotherapy decreased tumor growth. The combination of AZD6738 and cisplatin showed more potent growth inhibitory effects, decreased KI67, increased Tunel than monotherapy of each drug. Conclusion: In BTC, DDR targeting strategy using ATR inhibitor demonstrated promising antitumor activity alone or in combination with cytotoxic chemotherapeutic agents. This supports further clinical development of DDR targeting strategy in BTC.

#1422 Enhancing the therapeutic effects of PARP inhibitors in combination DNA methyl transferase inhibitors, using low doses of ionizing radiation in non small cell lung cancers. Christopher Biondi,1 Daniel Fontaine,1 Lora Stojanovic,1 Pratik Nagaria,1 Renia Lapidus,1 Javed Mahmood,1 Stephen Baylin,2 Feyruz V. Rassool1. 1Univ. of Maryland School of Medicine, Baltimore, MD; 2Johns Hopkins University, Baltimore, MD.

Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related deaths in the US. Treatment most commonly relies on ionizing radiation and platinum-based DNA damaging agents, but long-term survival is poor and patients tend to suffer chronic side effects due to the high radiation dose to the surrounding normal tissues. Therefore, new treatments are needed that can be used in combination lower radiation doses. We have recently reported that low, non-cytotoxic doses Poly ADP ribose polymerase inhibitors (PARPi) Talazoparib in combination with DNA methyltransferase inhibitors (DNMTi) Decitabine (DAC) or azacytidine (AZA) significantly increase cytotoxicity in acute myeloid leukemia and breast cancer models in vitro and anti-tumor effects in vivo. Simultaneous administration of both inhibitors result in increased PARP binding in DNA, leading to higher levels of DNA double strand breaks (DSBs), yielding increased cytotoxicity, compared with each agent treatment alone. We first studied the efficacy of Talazoparib and AZA combination therapy in multiple NSCLC cell lines (A549, H358 and H388) in vitro through colony forming assays. Results showed, compared to single agent treatments, combination drug treatment significantly decreased colony formation. Cell viability was also significantly decreased with the drug combination in MTS assays (P<0.05). These results also showed synergistic activity between the two drugs, with a combination index of 0.7 to 1.0 for all tested NSCLC cell lines. Importantly, the drug combination increased PARP trapping in chromatin and DSB formation, as measured by immunofluorescent staining for γH2AX. We next determined whether AZA/Talazoparib in combination with a single dose of radiation (2Gy) had an increased anti-cancer effect compared to each modality alone in in vivo mouse xenographs of NSCLC A549. While the combination of PARPi + DNMTi with radiation treatment decreased tumor growth, compared to PARPi alone or RT alone, no significant enhancement with radiation was observed. We next determined whether low doses of fractionated IR (2Gy 3 fractions) would improve the efficacy of DNMTi and PARPi combination treatment. In vitro studies with colony forming assays of NSCLC cell line A549 show that IR doses in combination with PARPi and DNMTi decrease clonogenicity, compared with non-IR controls. Furthermore, A549 xenographs were treated with the drug combination and then irradiated 1 week later with 2Gy daily for 3 consecutive days. Mice treated with the drug combination followed by IR had significant decreases in tumor volume and survival (P<0.05). This suggests that low doses of PARPi and DNMTi with low doses of IR can potentially target NSCLC tumors. This represents a novel treatment approach for NSCLC patients that may reduce chronic side-effects of high dose IR.

#1423 Neddy8-activating enzyme inhibitor pevonedistat synergizes with cisplatin and carboplatin through interference with nucleotide excision repair and interstrand cross-link repair mechanisms in non-small cell lung cancer. Xiao zhe Liu,1 David Bouch1, Khristofer Garcia, Jonathan Blank,1 Hughes Bernard,1 Allison Berger,1 Mike Keyeseda,1 Erik Koenig,1 Eric Lightcap2. 1Takeda, Cambridge, MA; 2Genentech, San Francisco, CA.

Platinis are one of the most widely used classes of chemotherapeutic drugs. Although used as part of first-line standard of care regimens for advanced non-small cell lung cancer (NSCLC), their effectiveness remains limited, with a 5-year survival rate of 18%. Therefore, combination therapy to overcome resistance and improve survival is needed. Pevonedistat (TAK-924/MLN4924) is an investigational first-in-class Neddy8-activating enzyme (NAE) inhibitor that blocks activation of the cullin family of E3 ubiquitin ligases by preventing cullin conjugation with Nedd8, a ubiquitin-like protein. In vitro, pevonedistat is synergistic with carboplatin in a cell viability assay in 6 out of 20 NSCLC cell lines either with or without paclitaxel. In vivo studies using patient-derived xenograft (PDX) models showed a combination effect in a carboplatin-insensitive but not carboplatin-sensitive model. To evaluate the mechanism of the synergy between pevonedistat and platinum, RNAi of 320 DNA-damage response genes was performed in 4 cell lines in vitro. Depletion of genes involved in TC-NER (transcription-coupled nucleotide excision repair) and ICR (interstrand crosslink repair) reduced the synergy between pevonedistat and platinum, with the contribution of each pathway varying by cell line. Since TC-NER and ICR are also involved in the repair of DNA damage by platinis, the results suggest that delaying completion of platin induced DNA repair by pevonedistat results in enhanced cell death. Pevonedistat may impede TC-NER by inhibition of neddylation of the E3 ubiquitin ligase CUL4-RBX1-DDB1-ERCC8, thereby possibly providing a direct reversal of resistance to platinis.

#1424 DNA repair capacity in colon cancer patients - The effect on the response to treatment and long-term survival. Sona Vodenkova,1 Michal Kroupa,2 Katerina Jiraskova,3 Alessio Naccarati,4 Alena Opattova,5 Pavel Vodicka5. 1Third Faculty of Medicine, Charles University, Prague, Czech Republic; 2Faculty of Medicine and Biomedical Center in Pilsen, Charles University, Pilsen, Czech Republic; 3First Faculty of Medicine, Charles University, Prague, Czech Republic; 4Department of Internal Medicine, Institute of Experimental Medicine, The Czech Academy of Sciences, Prague, Czech Republic.

Colorectal carcinoma (CRC) is the third most common cancer worldwide with the highest incidence in Central Europe. It is the fourth leading cause of cancer related deaths mainly due to the late diagnosis and low efficacy of treatment. CRC diagnosed in early stage has a five-year survival rate about 90% which drops to near 12% in distant metastases occur. It is a heterogeneous disease with different molecular and clinicopathological features depending on the tumor location. Therefore, different treatment strategies are required. A standard treatment of locally advanced rectal cancer includes neoadjuvant chemoradiation therapy followed by surgery, whereas colon cancer treatment consists of surgical resection of the tumor and/or subsequent adjuvant chemotherapy based on disease characteristics. 5-fluorouracil (5-FU) alone or in combination with other compounds is the most used treatment in CRC. The mechanism of 5-FU on molecular level is either its incorporation into DNA or it imbalances the synthesis of thymidine from uracil resulting in false uracil DNA incorporation. These DNA lesions are repaired by base excision (BER) and mismatch repair (MMR) pathways. An effective DNA damage response (DDR) is essential for the maintenance of genome stability in healthy cells, whereas in malignant cells, the suppression of DNA repair capacity (DRC) would increase the effectiveness of chemotherapy through DNA damage accumulation and consequent apoptosis. In contrary the cells with high DRC may show better survival and therefore patients with these molecular characteristics may contend with poor response, resistance to treatment and decreased survival. The aim of our present study was to investigate tumor growth and MMR in target tissue as a predictive marker for a treatment strategy and long-term survival. In order to minimize a bias by heterogenous therapy we focused only on patients with newly diagnosed colon cancer. Our set of patients was selected based on the criteria of follow-up at minimum 30 months, subsequent treatment with 5-FU and microsatellite stable
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tumor tissue characteristics. Tumor and adjacent non-affected tissue samples were obtained from one hundred patients at surgical resection. Protein extracts from tissues were isolated both for protein expression analysis and for measurement of DRC. DNA repair and DDR protein expression levels were tested by Western Blot. Functional assessments of DRC were performed by comet assay-based in vitro DNA repair assay. The cycle analysis via FACS. Results: We identified many potential CYCLOPS genes mapping on the distal end of chromosome arm 1p. One of these genes is involved in neuronal and embryonic development and has been further validated. Knock-down of the gene impaired cell viability in 1p-deleted cell lines but did not in 1p-non-deleted cells. G1/G0 phase arrest with corresponding S phase decrease was observed in both 1p-deleted and non-deleted. Additionally, neurite-like outgrowth could be observed in 1p-non-deleted cells indicating an induction of differentiation. Conclusion: This study identified a candidate CYCLOPS gene in neuroblastoma. Heterozygous deletions of chromosome arm 1p are also frequently observed in other cancers including melanoma, colorectal and breast cancer. We hypothesize that this proof-of-principle opens a new therapeutic window for tumor treatment during a heterozygous deletion of our candidate gene or other cell essential genes on chromosome arm 1p.

#1427 Investigating role of RECIQ1 in response to gemicitabine treatment in triple negative breast cancer. Swetha Parvathaneni, Xing Lu, Sudha Sharma. Howard University, Washington DC, DC.

The response of cancer cells to therapeutic drugs aimed at causing DNA damage is dependent on proteins that play roles in DNA repair. Single nucleotide polymorphisms in RECQ1 (also known as RECQL or RECQL1), a DNA helicase involved in DNA damage and replication stress response, is reported to influence patient’s response to gemicitabine in pancreatic cancer leading to poor survival. Gemicitabine is also used as a chemotherapeutic against triple negative breast cancer; however resistance to gemicitabine is reported. The mechanism by which gemicitabine chemoresistance is obtained is poorly understood. Given the importance of RECQ1 in replication fork restart and being categorized as a breast cancer susceptibility gene, we wish to investigate the role of RECQ1 in modulating cellular response to gemicitabine in triple negative breast cancer cells. To study RECQ1 functions, we developed isogenic pairs of RECQ1-proficient and RECQ1-deficient cells by CRISPR-Cas9-mediated targeted inactivation of RECQ1 in breast cancer cell lines as in vitro model system. RECQ1-knockout MDA-MB-231 cells displayed reduced cell growth and proliferation, and increased DNA damage as compared to the MDA-MB-231 cells expressing wild type RECQ1. As compared to RECQ1-proficient MDA-MB-231 cells, RECQ1-deficient cells were more sensitive to gemicitabine treatment, incurred significantly greater load of double strand breaks upon gemicitabine treatment, and showed greater cell cycle progression following recovery from gemicitabine treatment. Our ongoing experiments are investigating the detailed mechanism of RECQ1’s role in response to gemicitabine and its implications in therapeutic response. Funding: This work was funded by the NIGMS/NIH grant SC1GM093999 to Sudha Sharma. We also acknowledge support from the NIMHD/NIH award number R25 AG047843-02.

#1428 Single-cell transcriptomics reveals differential DNA repair signature after chemotherapy in high-grade serous ovarian cancer. Anniina Farkkila, Kaiyang Zhang, Katja Kaipio, Tarja Lammiminen, Rainer Lehtonen, Johanna Hynninen, Seija Grönn, Olli Carpein, Sampsa Hautaniemi, Anna Vähärautia. Univ. of Helsinki and Helsinki University Hospital, Helsinki, Finland; Univ. of Turku, Turku, Finland; University of Turku and Turku University Hospital, Turku, Finland.

Resistance to chemotherapy poses a major clinical problem in the treatment of ovarian cancer. The efficacy of platinum-based chemotherapy relies on defective DNA repair mechanisms, and increased expression of homologous recombination (HR)/DNA repair genes in primary tumor samples is associated with improved chemoresponse. However, the effects of neo-adjuvant chemotherapy (NACT) on the expression DNA repair genes in cancer cells are poorly understood. In addition, high-grade serous ovarian cancers (HGSOCs) display significant inter- and intratumoral heterogeneity, and the identification of mechanisms of drug resistance has been hampered by the lack of data at the single cell resolution. We collected 34 primary and 20 interval tumor samples from 15 patients treated for HGSOC at the Turku University Hospital, Finland. Tumor tissue total RNA was sequenced with Illumina HiSeq. For this study, we selected 363 genes selected due to their validated role in DNA repair pathways and compared their expression profiles between primary and interval samples.
The treatment naive primary tumor samples highly expressed genes related to HR, such as BRCA2, RAD54, PARPBP, FANC2D, and POLQ. Whereas after NACT the interval samples highly expressed genes regulating cell cycle, inflammatory response, and the drug efflux pump ABCB1. Moreover, higher expression of BRCA2 or POLQ in the interval samples correlated with poor primary therapy outcome (p<0.01). To reveal the transcriptomics landscapes of treatment naïve and NACT treated HGSOCs at the single cell level, we performed single cell mRNA sequencing of primary and interval samples using the Fluidigm C1 Platform. Unsupervised clustering revealed eight distinct cell subpopulations, of which two clusters were annotated HGSOC cells based on enriched expression of cancer specific genes. In global gene expression analysis of 98 HGSOC cells, the most differentially expressed genes in primary HGSOC cells were heat shock proteins reflecting active protein synthesis. By contrast, the cancer cells from interval samples highly expressed genes regulating the cell cycle and immune response. Of the DNA repair pathway genes, the interval samples expressed lower levels of HES1, a transcription factor that is known to promote cancer progression and stemness. The herein elucidated DNA repair pathway transcriptomics landscape at single-cell and whole tumor level provides novel targets for overcoming chemoresistance in HGSOC.

**#1429 DNA damage response to dihydrogalactitol (VAL-083) in p53-deficient non-small cell lung cancer cells.** Anne Steino,1 Guangan He,2 Jeffrey A. Bacha,1 Dennis M. Brown,1 Zahid Siddik2.

#1430 The spliceosome U2 snRNP factors promote genome stability through distinct mechanisms; transcription of repair factors and R-loop processing. Michihiro Tanikawa. The University of Tokyo, Tokyo, Japan.

**Objective:** Recent whole-exome sequencing studies of malignancies have detected recurrent somatic mutations in u2 snRNP components of the spliceosome. These factors have also been listed as novel players of DNA damage response in several genome wide screens and proteome analysis for DDR genes. Although accumulating evidences have implied that the spliceosome plays an important role in genome stability and is an emerging hallmark of carcinogenic pathways, its precise role in genome stability still remains ambiguous. The aim of this study is to clarify the functions of U2 snRNP splicing factors, especially SNRPA1 (Small Nuclear Ribonucleoprotein Polypeptide A1) in DDR pathway.

#1431 Therapeutic exploitation of mutant BRAF in childhood glioma. Sudipa Saha Roy, Peter Houghton. UTHSCSA, San Antonio, TX.

Low grade gliomas (LGG) are the most common tumors of the central nervous system in children, accounting for about 50% of all brain tumors. They represent a heterogeneous group of grade I and II tumors according to the WHO classification. Pediatric LGG, is associated with activation of BRAF through a tandem duplication that results in the KIAA1549-BRAF fusion or through an activating point mutation of BRAF (predominantly V600E). More recent findings suggest that the KIAA1549-BRAF fusion is restricted to Grade 1 tumors (70-90%) whereas BRAF(V600E) occurs more frequently in Grade 2-4 tumors (~23%). Findings for BRAF mutation, similar to other tumors with activated BRAF (e.g. melanoma), and the phase I activity of MEK inhibitor in the PBTC-029 protocol, suggest that activated BRAF may provide a validated drug target. Previous studies from our lab indicate that, in the context of mutant BRAF, inhibition of MEK inhibits TORC1 signaling and may induce a ‘BRACA-like’ phenotype, through depletion of FANC2D. Potentially suppression of TORC1 could have effects on other DNA damage response pathways that could compensate for loss of FANC2D. To understand the consequences of MEK inhibition in the context of BRAF abreation in LGG we surveyed DNA repair genes that may be regulated via the MEK/TORC1 pathway in BRAF mutant cells. The objective of this particular study was to examine the mechanism and significance of MEK inhibition to the repair of DNA damage by the homologous recombination (HR) pathway. For this study, we have used glioma cell lines having BRAF(V600E) mutation; the BT40 cell line was developed from patient-derived astrocytoma xenograft (PDX) model in mice in our lab and AM38c1 and DBTRG-05MG cells were generously provided by T. Nicolas. Annexin bind- ing assay demonstrated that MEK inhibition significantly increased the percentage of apoptosis and necrosis of glioma cells. Human DNA repair PCR Array analysis identified MEKi induced down regulation of genes involved in Base Excision Repair (BER), Nucleotide Excision Repair (NER), Mismatch Repair (MMR) and Double-Strand Break (DSB) repair pathways. DSB repair genes were further validated by real time qPCR analysis. Immunoblot analysis of glioma cell lines indicate that MEKi treatment significantly increased H2AX levels. To elucidate the mechanism of DSB repair pathway in presence of MEKI, clonogenic assay, nuclear foci formation assay, and GFP reporter assay for homologous recombination (HR) and non-homologous end-joining (NHEJ) are ongoing. Collectively, these findings demonstrate for the first time a previously unknown role for MEK in treatment of glioma cells that involves inhibition of DNA repair pathways.
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**#1432** Precision lung cancer medicine based on information on cancer and the host genomes. Takashi Kohno,1 Kouya Shiraishi,1 Kazuhi Yoshida,2 Motonobu Saito,1 Yukinori Okada.1 National Cancer Ctr. Research Inst., Tokyo, Japan; National Cancer Ctr Hospital, Tokyo, Japan; Osaka University Graduate School of Medicine, Osaka, Japan.

Aberations of oncogene, such as EGFR mutation; and ALK, RET, and ROS1 fusions, function as a driver in the development of lung adenocarcinoma (LADC). They are a solid target of therapy using tyrosine kinase inhibitors, as represented by a high response rate of RET-fusion positive LADC to vandetanib (LURET study, Yoh et al., Lancet Resp Med, 2016). Our genome-wide sequencing study revealed that oncogene aberration positive LADCs carry less numbers of mutations in cancer-related genes than negative ones (Saito, Cancer Res, 2015), indicating a small mutation burden in the development of the formers. Consistently, a clinical trial of anti-PD-1 therapy indicated that EGFR-mutation positive cases do not benefit well from the therapy (Borghaei et al., N Engl J Med, 2015). LADC driven by somatic EGFR mutations is more prevalent in East Asians (30-50%) than in European/Americans (10-20%). We recently revealed that variations in HLA class I loci underlie the risk of the disease, leading to a genome-wide association study of 3,173 patients and 15,158 controls, combined with imputation of HLA alleles using a Japanese-specific panel (Shiraishi et al., Nature Comm, 2016). The result indicates that LADC develops in vivo through interaction between somatic mutations and germline variations that modulate immune reaction. Therefore, efficacy of immune checkpoint blockade targeting PD-1/PD-L1 in this patient/cell type is strongly associated with smoking status. The other hand, oncogene-negative LADCs show a high mutation burden associated with nonsynonymous mutations in chromatin remodelin and other cancer-related genes. Thus, these cases would respond well to immune checkpoint blockade targeting therapies. We would like to discuss here precision lung cancer medicine based on information on cancer and the host genomes.

**#1433** Single cell RNA sequencing reveals smoking-associated alterations in bronchial airway epithelial subpopulations. Grant E. Duclos, Joshua D. Campbell, Yaron Gesthalter, Patrick Autissier, Yves M. Dumas, Robert Terrano, Gang Liu, Marc E. Lenburg, Avrum Spira, Jennifer Beane. Boston University School of Medicine, Boston, MA.

Rationale: We have previously shown that bronchial airway epithelial gene expression reflects the physiological response to cigarette smoke exposure. We have also shown that gene expression differences in cytologically normal airways can serve as a diagnostic biomarker for lung cancer. In this study, we use single cell RNA-seq to profile transcriptomes of individual bronchial epithelial cells from current and never smokers in order to detect smoking-associated alterations within airway epithelial cell subpopulations that develop as a result of smoke exposure. This approach may be useful for identifying cell type specific transcriptomic changes in the airways of cancer patients, which may lead to a better understanding of lung carcinogenesis and new approaches to early lung cancer detection. Methods: We obtained bronchial brushings from current smokers (n = 6) and never smokers (n = 6) and isolated single cells by FACS. The CEL-Seq RNA library preparation protocol was used to sequence the transcriptomes of 1,140 cells (n=95 donors). Results: Distinct populations of bronchial cells expressed known markers of basal (KRT5), ciliated (FOXJ1), secretory (SCGB1A1, MUCSAC) epithelial cells, as well as white blood cells (CD45). In the airways of smokers, we observed an increase in abundance of MUCSAC+ secretory cells as well as a decrease in abundance of KRT5+ basal and SCGB1A1+ secretory cells. A novel subset of KRT8+ cells that lacked expression of other known cell type markers was identified in the airways of smokers and may represent a population previously described as undifferentiated intermediate cells. Genes involved with metabolism of polycyclic aromatic hydrocarbons (CYP1B1) were detected in smoker secretory cells, whereas genes involved in the metabolic response to cigarette smoke toxins such as aldehydes (ALDH1A1) and quinones (NQO1), were most highly expressed by smoker ciliated cells. Interestingly, the novel KRT8+ cells identified in smokers expressed genes known to promote MUCSAC+ secretory cell differentiation (SPDEF, but did not express MUCSAC itself, suggesting that these may be pro-MUCSAC+ secretory intermediate cells. Furthermore, we found that genes previously associated with higher expression in the airways of lung cancer patients were most strongly associated with smoker ciliated and secretory cells, whereas genes with lower expression in lung cancer were enriched among genes most strongly associated with white blood cells. Conclusio: We have identified cell type specific transcriptomic alterations and shifts in epithelial cell population abundance in smoker airways. In future studies, profiling the transcriptomes of single cells from bronchial airways of smokers with and without lung cancer may lead to the identification of specific cellular subpopulations contributing to the airway field of lung-cancer associated injury.

**#1434** Mutational landscape in the normal-appearing carcinogenesis field of early-stage non-small cell lung cancer, Smruthy Sivakumar,1 Yasminka Jakubek,1 Wenloua Lang,1 Tina McDowell,1 Melinda M. Garcia,2 Chi-Wan Chow,2 Zachary Weber,2 Carmen Behrens,2 Neda Kalhor,2 Cesar Moran,2 Randa El-Zein,4 Gareth Davies,3 Junya Fujimoto,3 Reza Mehran,7 Stephen G. Swisher,5 Jing Wang,2 Avrum E. Spira,2 Jerry Fowler,2 F Anthony San Lucas,2 Ignacio I. Wistuba,2 Erik Ehl,2 Paul Schett,3 Humam Kadara3.1 The University of Texas Graduate School of Biomedical Sciences, Houston, TX; 2The University of Texas MD Anderson Cancer Center, Houston, TX; 3Avera Institute for Human Genetics, Sioux Falls, SD; 4Houston Methodist Research Institute, Houston, TX; 5School of Medicine, Boston University, Boston, MA; 6American University of Beirut, Beirut, Lebanon.

Lung cancer, of which non-small cell lung cancer (NSCLC) is the most common form, is the second most prevalent cancer in the U.S. and the leading cause of cancer mortality. Field carcinogenesis phenomenon establishes that normal appearing tissues surrounding a tumor will exhibit a field effect – particularly in smokers – with the tissues proximal to the tumor showing more, and even sharing, alterations with the tumor. Previous studies in NSCLC provide evidence for both complex progression trajectories, yet, precise mechanisms remain unknown. To investigate the field cancerization phenomenon, we conducted a genome-wide survey of acquired DNA alterations in normal-appearing tissues with small airways adjacent to NSCLC. In mainstream bronchi (large airways), nasal epithelium, distant normal lung and blood, along with multiple samples from their surgically resected paired tumors, for a total of 500 samples from 48 patients with early-stage NSCLC (11 squamous cell carcinomas and 37 adenocarcinomas). Since we expect low mutation burdens and mutant cell fractions in pathologically normal tissues, we assessed somatic point mutations via deep targeted sequencing of 409 genes and paired these data with our recent study of acquired copy number alterations (CNAs) inferred from high-density whole-genome SNP microarrays, thus offering a deep and wide survey of somatic DNA alterations. After aggregating results from multiple mutation callers, we observed somatic mutations (exonic, splicing and UTRs) in 257 samples of which 65 (in 35 patients) were from normal-appearing field (non-tumor, non-blood) samples. Tumor and field samples in smokers showed a higher mutation burden and large proportion of C:G→A:T changes. We also observed concordance of mutations in the airway and corresponding tumor profiles. Further, a statistically significant field effect was established with mutational burden (measured by the variant allele frequency) increasing with proximity to the tumor. Among the 33 NSCLC cases with field mutations, the small adjacent (to tumor) field in six patients exhibited high mutational burdens in lung cancer drivers such as KRAS, STK11, TP53 and KEAP1. We also identified mutations in the large airway (TP53, SETDB2, CDKN2A), distant normal lung parenchyma (RB1, RET) and nasal epithelium (AKT1). We then correlated point mutation and CNA mutation profiles. Of eight cases showing a nonsynonymous or stopgain variants in established lung cancer drivers in the airways, four exhibited putative "two-hit" progression models, e.g. both KEAP1 and STK11 mutations in 19p loss, overlapping TP53 mutation/17p loss, or KRAS mutation/12p gain. Our findings in normal-appearing tissues of the respiratory epithelium offer insights into the earliest mutational events in their progression to NSCLC and, possibly, in tumor relapse and that may represent suitable targets for early detection and chemoprevention.

**#1435** Immune/inflammatory polymorphisms predict lung cancer survival. Dongvan Watza,1 Chrissy Luk,2 Ange Wenzlaff,1 Christine Neslund-Dudas,1 Gregory Dyson,1 Ayman Soubbani,1 Shirish Gadgeel,1 Ann G. Schwartz.1 Karmanos Cancer Institute, Detroit, MI; 2Henry Ford Hospital, Detroit, MI; 3Wayne State University School of Medicine, Detroit, MI.

Lung cancer is the leading cause of cancer related mortality in the United States, with a median five year survival of 16%. Recent advancements in the treatment of lung cancer focus on modulating the immune system to improve patient outcomes with markedly reduced toxicity profiles. This study aims to investigate single nucleotide polymorphisms (SNPs) within functional units of the immune system genome to identify genetic markers indicative of overall survival in a cohort of metropolitan Detroit lung cancer patients. This cohort presents a unique population of lung cancer patients for analysis, consisting of 40% African Americans, whom are known to have worse outcomes than their Caucasian counterparts. To assess SNPs within functional units of the immune genome, a hierarchical immune system gene and pathway list was constructed, incorporating genes and pathway identifiers from the Reactome database as well

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as previous genetic and inflammatory specific cancer studies to generate a well curated gene and pathway dataset. Genomic locations for the genes, together with proximal regulatory regions, were obtained from the UCSC genome browser for cross-referencing with the Illumina MEGA SNP array to generate SNPs for inclusion. Preliminary analysis of 848 NSCLC patients in association with 29,126 immune SNPs identified six SNPs as significantly associated with survival (p-value < 10^-4) in this lung cancer cohort, implicating the following six genes, PDXN, GFRA3, HLA-DQA1, KRAS, B2TD1, and AT2F; although further gene/pathway analysis is necessary to elucidate how these associations interact in the context of genes and pathways of the immune system. PDXN over expression has been previously implicated in both high risk lung epithelial dysplasia as well as in the development of lung adenocarcinoma in prior clinical studies. Additionally, GFRA3 has been identified as an activator of the RET kinase and increased GFRA3 activity in context of the Artemin pathway is a known predictor of NSCLC progression. As well, select polymorphisms in the HLA-DQA1 gene are known clinical modifiers of lung squamous cell carcinoma risk. Finally, AT2F is a broadly active transcription factor and AT2F upregulation has been associated with tumorigenesis and metastasis in both cell models and patient tumor samples in multiple cancer studies. The previous association of these genes in lung cancer is promising and further immune system gene/pathway analysis is warranted.

#1436 Identification of novel tumor suppressor candidates in familial cholangiocarcinoma using sequencing-based Megabase-scale haplotypes from germline and cancer genomes. Stephanie Greer,1 Lincoln Nadauld,2 Billy Lau,1 Lashlee Li,1 Daniel C. Edelman,2 David Petersen,2 Daniel C. Edelman,2 Paul S. Meltzer,2 Bao Tran,2 Kerstin Hesselmeyer-Haddad,2 Thomas Ried,2 Tetsuichi Tsuchida,1 Keisuke Kataoka,1 Yaichi Shiraishi,2 Masashi Sanada,3 Shigeru Shiraishi,1 Yaichi Shiraishi,2 Masashi Sanada,3 Shigeru

#1438 The dynamics of genetic aberrations in Crohn’s disease associated colorectal carcinogenesis. Daniela D. Hirsch,1 Darawalee Wangsa,2 Yue Hu,2 Jack Zhu,2 David Petersen,2 Daniel C. Edelman,2 Paul S. Meltzer,2 Bao Tran,2 Kerstin Hesselmeyer-Haddad,2 Thomas Ried,2 Timo Gaiser1. 1University Medical Center Mannheim, Mannheim, Germany; 2National Cancer Institute, Bethesda, MD.

Crohn’s disease, a condition of chronic inflammation of the intestine, supposes that the risk for the development of colorectal cancer (CRC). Sporadic CRCs are characterized by a specific pattern of genomic imbalances and a landscape of acquired gene mutations. In this study we aimed to compare CRCs that arise as a consequence of chronic inflammation in Crohn’s disease with sporadic CRCs. We analyzed 26 Crohn’s disease associated CRCs, four matched dysplastic lesions, six matched inflamed mucosa samples, and two matched lymph node metastases using array comparative genomic hybridization, targeted sequencing (564 cancer related genes) and gene expression profiling. As a control, we used normal intestinal mucosa from the resection margin of these CRCs and 24 sporadic CRCs. In general, CRCs that developed in patients with Crohn’s disease had a similar distribution of genomic imbalances compared to sporadic CRC. However, we observed distinct mutation signatures in the Crohn’s disease associated CRCs: most frequently mutated in the Crohn’s disease associated CRCs the most frequently mutated gene was TP53, which occurred in 65% of the samples. The second, third and fourth most frequently mutated genes were KRAS (27%), APC (23%) and PIK3CA (19%). In the control group of sporadically arising CRCs, the most commonly mutated gene was APC (75%), followed by KRAS (54%), TP53 (33%), SMAD3 (29%) and SMAD2 (25%). The genetic analyses of multiple lesions from individual patients revealed a high degree of intertumoral heterogeneity with diverse patterns of genetic mutations and allowed the reconstruction of the sequence of genetic events during Crohn’s disease associated tumorigenesis. In contrast to sporadic colorectal carcinogenesis, TP53 mutations were observed as early and common events while APC mutations occurred rather late and were infrequent. The comprehensive analysis of Crohn’s disease associated CRCs suggests that the genetic landscape of CRC is influenced by the activation of inflammation related pathways. Furthermore, our findings offer potential for establishing an early detection marker for dysplasia in patients with Crohn’s disease.
A comprehensive cis-eQTL analysis revealed target genes in breast cancer susceptibility loci identified in genome-wide association studies. Xingyi Guo,1 Weiqiang Lin,2 Qiu Yin Cal,1 Jiaojun Shi,1 Yaqiong Sun,2 Xiao Pan,2 Mi-Ryung Han,1 Wanqing Wen,1 Bingshan Li,1 Jirong Long,1 Jianghua Chen,2 and Lea Jessop,1

Objective: To elucidate the genetic architecture of gene expression in pancreatic tissues. Design: We performed expression quantitative trait loci (eQTL) and allele specific expression (ASE) analyses using RNA-sequence data and 1000 Genomes (1000G) imputed GWAS genotypes from 95 fresh frozen histologically normal pancreatic tissue samples. Data from 115 pancreatic tumor-derived tissue samples from The Cancer Genome Atlas (TCGA) was included for comparison. Results: We identified 38,615 cis-eQTLs (corresponding to 484 Genes) in histologically normal tissues and 39,713 cis-eQTL (corresponding to 237 Genes) in tumor tissues (FDR=0.1), with the strongest effects seen near transcriptional start sites (TSS). Approximately 23% and 42% of genes with significant cis-eQTLs (eGenes) appeared to be specific for tumor and normal derived tissues, respectively. Significantly enriched eGenes in noncoding regulatory regions marked by modified histones, DNAse hypersensitivity and binding transcription factors, in particular for pancreatic tissues (1.53-3.12 fold, P<0.0001), indicating tissue-specific functional relevance. A common pancreatic cancer risk locus on 9q34.2 in the ABO gene (rs687289) was associated with ABO expression in histologically normal (P=5.8×10^-4) and tumor (P=8.3×10^-9) tissues. A rare loss-of-function variant (rs4643489) in the sodium (Na) channel 5.1 (SCN5A) was significantly enriched in noncoding regulatory regions marked by modified histones, DNAse hypersensitivity and binding transcription factors (47,834 comparisons, FDR=0.05). Results from the human Pancreatic Cancer Genome Project (PCGP) were used to validate these associations. Conclusions: Using data from the Encyclopedia of DNA Elements (ENCODE), we performed cis-eQTL analysis using transcriptome data from the Pancreatic Cancer Genome Project (PCGP) and the Genotype-Tissue Expression (GTEx) project (N=186). We identified a total of 58 genes including SSRP4 at Benjamini-Hochberg adjusted P < 0.05 in at least one dataset and another gene, PAX9, at P < 0.05 with consistent association directions in all three datasets. Using data from the Pancreatic Cancer Genome Project (PCGP), we selected functional SNPs and showed that alternative alleles could significantly change promoter activities of their target genes compared to reference alleles using luciferase reporter assays. The effects of target gene expression for those alleles were in line with eQTL observations. In addition, we validated the genes SETD9 and SSRP4 using Sequenom allelyte technique from 355 adjacent normal breast tissues and observed the allelic-specific expression associated with their index SNPs. This study revealed novel biological mechanisms for associations of genetic susceptibility risk loci for breast cancer.

#1442 Analysis of cis-eQTLs in normal and tumor-derived pancreatic tissues reveals functional insights, including for the 9q34.1 ABO pancreatic cancer risk locus. Laufey T. Amundadottir,1 Soren Lykke Andresen,2 Wenming Xiao,3 Jason Hoskins,4 Lauren Rost,2 Irene Collins,5 Jinping Lin,2 Chad Morabito,4 Bin Sun,3 Robert Kurz,1 Hermann Zeh,3 Jared Zirnello,3 Meredith Yeager,4 Torben Jensen,2 William Bamlet,1 Nilanjan Chatterjee,1 Brian Wolpin,8 Jill Smith,9 Sara Olson,18 Gloria Petersen,6 Jianxin Shi,4 Ming-fang Zhong,1 National Cancer Inst., Bethesda, MD; 2Aarhus University, Denmark; 3Aalborg University Hospital, Denmark; 4Dana Farber Cancer Institute, MA; 5Georgetown University, DC; 6MSK Cancer Center, NY.

GWAS is an important tool for discovering regions in the genome associated with cancer susceptibility. For renal cell carcinoma (RCC), only two loci have had their functional basis explained (11q13 and 12p12). Here, we used a massively parallel reporter assay (MPRA) to investigate enhancer activity in 20 GWAS regions that had an RCC association p-value of at least 10^-7. We selected 784 SNPs with an r^2 > 0.4 or D’ > 0.5 and MAF > 0.05 that had evidence for enhancer activity based on available ENCODE transcription factor or histone ChIP-seq, FAIRE, and DNase I hypersensitive data. The MPRA library was composed of 47461 oligonucleotides 201 bp in length, compiled of 145 bps contained the SNP in the forward or reverse orientation followed by 10 bp of
bar code sequence to allow for identification. Each of the 784 SNPs tested were tagged 10 separate times for both orientations. Controls were constructed by randomizing the 10 nucleotides centered on the SNP. The MPRA library was the cloned into a luciferase reporter vector that was transfected into HEK293T and ACHN cell lines. NGS was performed on 5 replicates using the the Illumina HiSeq 2500 and for multiple NGS runs, SNPs that showed significant p-values. From these 50 SNPs, 29 showed enhancer activity that was independent of the forward/reverse orientation and had the same effect in both cell lines. This analysis has identified possible functional variants at RCC risk loci and opens opportunities to discover new molecular mechanisms of genetic susceptibility of RCC.

#1444 Validation of prostate cancer risk variants by CRISPR/Cas9 mediated genome editing at the MSMB locus. Xing Wang,1 James Hayes,1 Xing Xu,1 Dipti Mehta,2 Xiaoni Gao,2 Hans Lilja,2 Robert Klein1.

With the recent US, being the third most common cancer among both men and women. Carvajal-Carmona.

Cas9 scarless genome editing.


Cancer geneticsusceptibility of RCC. risk loci and opens opportunities to discover new molecular mechanisms of genetic susceptibility of RCC.

#1447 Identification of an oncogenic germline KRAS truncating mutation in hereditary cancers. Moloy T. Goswami,1 Daniel H. Hovelson,1 Anna John-son,1 Scott A. Tomlins,2 Lucy Wang,1 Kimberly Zhulke,1 Bhavneet Singh,1 Sharath Kumar Anand,2 Andi Cani,1 Albert Liu,1 Steven Kamberovo,2 Yi-Mi Wu,1 Dan Robinson,1 Arul Chinnaian,1 Kathleen A. Cooney,1 University of Michigan, Ann Arbor, MI; 2University of Utah, Salt Lake City, UT.

Somatic strongly activating KRAS mutations play an oncogenic role across numerous human cancers, while less activating germline KRAS mutations are associated with developmental disorders. KRAS encodes two splice variant products—KRAS-4A and KRAS-4B—but differing in their C-terminus through alternative fourth coding exons. Though KRAS-4A is homologous to the original transforming transcript identified in Kirsten rat sarcoma virus, its role in human cancer is less characterized compared to KRAS-4B. Here, through genetic analyses of three cohorts of patients with hereditary and/or aggressive cancers, we identified a rare KRAS-4A specific C-terminal truncating germline mutation (KRAS-4A C180X; rs37319526) in affected men of three families with hereditary prostate cancer and a patient with hereditary melanoma (minor allele frequency [MAF] of 0.0014 in these combined cancer cohorts assessed vs. 0.00056 in the ExAC population database, odds ratio 24.6 [95% confidence interval 5.1-103.5], two sided Fisher’s exact test p = 9.0E-5). The KRAS-4A C180X mutation truncates the C-terminus, removing the polybasic region and -CAAX motifs previously demonstrated to be necessary for Ras family member membrane association, MAP kinase signaling activation and transformation, suggesting a
loss of function phenotype. However, in silico assessment of reported human variation demonstrates truncating germline variants in only KRAS-4A and not KRAS-4B, consistent with tolerance. Expression of KRAS-4A protein in NIH3T3 and MDCK leads to loss of exclusive membrane association and inhibits GTP loading, as expected, but paradoxically resulted in modest but significantly increased proliferation and soft agar colony formation or wildtype KRAS expressing cells. Pro-oncogenic phenotypes were not dependent on MAPK signaling, but showed sensitivity to AKT inhibition. In summary, we identified a germline truncating KRAS-4A mutation over-represented in hereditary cancers that defines a novel mechanism of KRAS activation not dependent on the C-terminal polybasic and -CAAX motifs.

#1448 Unravelling the oncogenic pathway of serrated polyposis syndrome driven by RNF43 germline mutation. Helen Hoi Ning Yan, Jeffrey CW Lai, Sui Lun Ho, Anthony K W Chan, Wai Yin Tsui, Annie S Y Chan, Siu Tsan Yuen, Suet Yi Leung, Dept. of Pathology, Univ. of Hong Kong, Hong Kong, Hong Kong.

Serrated polyps can arise in a sporadic or familial polyposis setting and predispose to colorectal cancer (CRC). Recently, we have identified RNF43 germline mutation in a family with Serrated Polyposis Syndrome (Yan et al, GUT 2016). The presence of second somatic hit in all serrated polyps examined from members of this family further confirmed the pathogenicity of RNF43 germline mutation. In an attempt to further delineate the global genomic alterations in these serrated polyps, so as to understand the RNF43 driven serrated neoplasia pathway for the development of colorectal cancer, we performed whole exome sequencing (WES) on two sessile serrated adenomas (SSAs) resected from an RNF43 germline mutation carrier, together with the paired blood DNA. Consistent with the results from our previous Sanger sequencing study, WES detected complete inactivation of RNF43 through a 2nd hit somatic mutation, c.461 C>T P154L, or loss of heterozygosity (LOH) in the two SSAs, respectively. We also identified 51 and 58 somatic mutations in the two SSAs, respectively. BRAFV600E mutation was the only shared mutation between the two polyps. Interestingly, each of the SSAs carried a truncating mutation in a histone-methyltransferase gene, either PRDM9 or SETD1B, respectively. Other truncating or deleterious mutations included chromatin modifiers (CHD2, CHD4, TSPY2) or other genes with methyltransferase activity (TRMT2B, METTL12, METTL14, EC2E). In addition, we found few instances of chromosomal aberration or LOH, apart from a region of LOH at chromosome 17 encompassing RNF43 in one of the SSAs. Overall, we have revealed the genomic landscape of two sessile serrated adenomas resected from a germline RNF43 mutation carrier. The results confirmed somatic RNF43 2nd hit and BRAFV600E mutation as the key events, along with putative roles for histone methyltransferase, as well as other chromatin modifiers. These findings highlight the potentially important role of an altered chromatin in the oncogenic pathway of serrated neoplasia.

#1449 Germline mutation burden in an endometrial cancer cohort from an integrated health care system. Raghu Metpally, Sarah Babu Krishnamurthy, Dokyoon Kim, Adam Cook, Ashlee Smith, John Nash, Marilyn Ritchie, David Carey, Radhika Gogoi, Geisinger Medical Center, Danville, PA.

Endometrial cancer (EMCA) is the most common gynecologic malignancy in the United States. We sought to explore germline mutations in 301 patients with a diagnosis of EMCA (EMCA cohort), who underwent whole exome sequencing as part of the Geisinger Health System’s (GHS) MyCode project. We additionally compared these mutations to germline and somatic mutations in the TCGA array as well as a non-affiliated cohort at GHS. Whole exome sequencing was performed using 75 bp paired-end resequencing on an Illumina v4 HiSeq 2500 to a coverage depth sufficient to provide greater than 20x haplotype read depth of over 85% of targeted bases in 96% of samples (approximately 80x mean haplotype read depth of targeted bases). Control groups consisted of 1150 female patients over the age of 85 with no cancer diagnosis (Welderly cohort) and the entire Geisinger’s MyCode population of 50,000+ patients (GHS cohort). Somatic and germline mutations in EMCA were downloaded from the TCGA portal. We retrieved somatic mutations from 248 patients as a mutation annotation format (MAF) that consisted of 181,930 point mutations, while 2,931 indels were removed for the comparison. In addition to somatic mutations, germline mutations with the controlled access, obtained as a variant call format (VCF). Demographic characteristics, treatment and outcomes were identified through a manual chart review of the EHR. 2205 coding variants with 30 LOF and 802 missense mutations were noted in the germline. Approximately 24.67% of patients in the EMCA cohort had predictive pathogenic variants compared to control cohort. Genes with most predictive pathogenic variants among EMCA co-

#1450 Alternative splicing analysis identifies mutation hotspots in hereditary breast and ovarian cancer genes. Suzette Farber-Katz, Vickie Huaan, Jayne Hoo, Sitoa Wu, Huy Vuong, Dong Xu, Hsiao-Mei Lu, Phillip Gray, Aaron Elliott, Rachid Karam, Amybr Genetics, Aliso Viejo, CA.

Genetic testing for hereditary breast and ovarian cancer (HBOC) is becoming increasingly widespread in the era of precision medicine. The implementation of next-generation sequencing (NGS) has resulted in an explosion of genetic data. While the majority of patients receive definitive results, germline unclassifiable variants with unknown function are regularly detected in thousands of patients. In particular, variants of unknown significance (VUS) in the HBOC susceptibility genes BRCA1 and BRCA2 pose a quandary to medical providers and patients because these genes are clinically actionable. A large percentage of VUS in BRCA1/2 are predicted to affect splicing. Previous efforts have focused on interrogating splicing VUS using low-throughput and/or imprecise techniques. Therefore, we developed and validated a method for using RT-PCR NGS to accurately and efficiently characterize germline splicing defects. The ENIGMA (Evidence-based Network for the Interpretation of Germline Mutant Alleles) consortium recommends capillary electrophoresis and Sanger sequencing of spliced transcripts as the gold standard to identify the BRCA1/2 transcripts that are present in patient samples. We followed the recommendations of the ENIGMA consortium and were able to largely replicate their results when we analyzed the CDNA of lymphoblastoid cell lines (LCLs) generated by the ConFab consortium from carriers of BRCA1 or BRCA2 variants known to be associated with splicing defects. In addition to LCLs, we analyzed RNA extracted from normal blood controls and normal breast tissue for all BRCA1 gene. We compared our capillary electrophoresis and Sanger sequencing results to our newly developed RT-PCR NGS assay. For this assay, we used RT-PCR NGS technology in our custom in-house bioinformatics analysis to identify splicing events including exon skipping, novel intron insertion, and alternative 5’ donor and 3’ acceptor splicing sites. Capillary electrophoresis allowed us to roughly visualize the various transcripts present in our samples, though sequencing was needed to confidently identify the exact splicing event. Similar to Sanger sequencing, our RT-PCR NGS assay detected all major splicing events. Interestingly, we also identified that RT-PCR NGS allowed us to visualize more minor splicing events present in the samples due to our high coverage. We sequenced tens of thousands of reads with RT-PCR NGS, as opposed to Sanger sequencing, which was limited to low-throughput sequencing of single colonies containing cloned RT-PCR products. In conclusion, RT-PCR NGS is a reliable high-throughput alternative to the gold-standard splicing assays. Our assay will greatly improve the interpretation of splicing VUS detected by clinical genetic tests for BRCA1/2.

#1451 Search for new hereditary breast cancer genes by whole exome sequencing. Ekaterina Sh. Kuligina, Anna P. Sokolonenko, Ilya V. Bizin, Aleksandr A. Romanko, Maria Anisimova, Evgeny N. Imanyinov, N. N. Petrov Research Inst. of Oncology, St. Petersburg, Russian Federation; St. Petersburg State Polytechnical University, St. Petersburg, Russian Federation; St. Petersburg Pediatric Medical University, St. Petersburg, Russian Federation.

Russian population is characterized by pronounced genetic homogeneity and the increased occurrence of recurrent germ-line mutations, therefore it is particularly suitable for the whole exome sequencing (WES) studies. Exome sequencing was performed for 43 breast cancer (BC) cases, which demonstrated evident clinical signs of the hereditary disease (family history and/or BC bilateral heredity and/or young onset) and lacked germ-line mutations in BRCA1, BRCA2, CHEK2, NSB1/NBN and BLM genes. Approximately 52070 nonsynonymous alternative variants were identified; 27503 (53%) of them were absent in 16 analyzed exomes from cancer-free controls. 2204 (4%) variants had sufficiently
low allelic frequency (<1%; ExAC database) and appeared to be functional upon silico assessment (CADD score > 25); this list was composed of 186 frameshift insertions/deletions, 152 stop-gains, 74 essential splice-site disruptions and 1792 potentially pathogenic missense mutations. Presumably causative mutations in the BRCA1 network genes were revealed in 8 patients (truncating mutations: PALB2 (p.Arg1085*, p.Arg3042X), FANCN (p.Ser497fs); or by pathogenic missense mutations: FANCN (p.Arg100Trp, p.Gln819Pro), ERCC4 (p.Arg799Trp), RAD51L (p.Arg399Trp)). For the remaining 35 patients, 57 truncating variants and 109 presumably deleterious missense mutations were further analyzed in a case-control study involving 550 hereditary BC cases and 450 elderly tumor-free controls. The most promising results were obtained for several recurrent. Expression values were normalized by library size read counts followed by a gene-wise normalization. To explore similarity level between samples we used the full matrix of the log2-transformed gene expression values and calculated sample-to-sample distances for each tumor compared to each normal sample, based on the Euclidian distances between samples considering all available genes. We observed that 80% of the BRCA1-mutated and 67% of the BRCA1/2 wild-type samples were clustered together. Also, we detected that BRCA1-mutated TNBC showed, in general, less difference in transcriptional pattern related to normal breast tissue than the BRCA1/2 wild-type TNBC. Moreover, we compared differential expression between normal and tumor samples of each group (BRCA1-mutated and BRCA1/2 wild-type samples) using a linear-based model in DESeq2 program. Based on the observed change $\geq 4$ and FDR $< 0.01$ we identified 6787 differentially expressed genes, in the group of BRCA1-mutated samples, being 722 up regulated and 2087 down regulated in the tumor and 904 DEGs in the group of BRCA1/2 wild-type samples, 399 were upregulated and 505 downregulated in the tumor. Interestingly, both lists of upregulated genes in TNBC of BRCA1-mutated and BRCA1/2 wild-type were significantly enriched in the same molecular pathway involved with the cell cycle and mitosis, according to Reactome Pathways software. Overall, our first observation based on this preliminary data was that, in terms of general gene expression analysis, BRCA1-mutated TNBC has less transcriptional modification in comparison to the normal tissue than the BRCA1/2 wild type and that the tumorigenic process of both groups are similar in terms of defective biological pathway.

### #1452 Interrogating the impact of pregnancy on breast cancer biology using DNA copy number profiling

Bastien Nguyen, David Veret, Christine Rothe, Christine Desmedt, Samira Majjaj, Giancarlo Pruneri, David Brown, Pedro Peccatori, Hatem A. Azim, Cristos Sotiriou, Institut Jules Bordet, Brussels, Belgium; European Institute of Oncology, Milan, Italy.

Background: Epidemiological evidence indicates a clear relationship between pregnancy and breast cancer (BC) risk. However, little is known regarding the impact of pregnancy on BC biology. DNA copy number aberrations (CNAs) play an important role in breast carcinogenesis. BC during pregnancy is a rare disease, however it can be used as a model to study the impact of pregnancy on BC biology. Methods: We retrospectively included 54 pregnant and 113 non-pregnant BC patients matched for age and stage with complete clinic-pathological, gene expression and 5-year follow-up data. CNAs were assessed using Affymetrix Oncoscan FPPE arrays. First, we filtered genes whose expression was driven by CNAs. Then, we identified genes that were differentially affected by pregnancy both at the CNA and the gene expression level. Results: After quality control, CNA profiles were obtained for 38 pregnant and 87 non-pregnant BC patients. There were no significant differences in classical clinic-pathological features (pT, pN, grade, PAM50). We selected 1981 genes whose expression was correlated with CNAs (p $\geq 0.01$). Among these, 77 genes were altered at the expression level (p $\geq 0.01$) between pregnant and control patients, whereas 171 genes were altered at the CNA level (p $\geq 0.05$). Twenty-eight genes were comically altered at the expression and CNA levels which was significantly higher than expected (p = 0.021, permutation test). These genes were mapped on three different chromosomes. Genes located on chr7q and chr1q were associated with copy number gains and were upregulated in pregnant patients whereas genes located on chr22q were associated with copy number loss in control patients. Of interest we identified 6 putative oncogenes (CDK6, ESRRB, S6K2, LIMK2, MKL1) that were up-regulated in pregnant patients. A ‘pregnancy-associated breast cancer signature’ was computed from the expression of the 26 genes. There was a negative relationship between the signature and the expression of ER1 and PR genes. This signature was significantly more correlated with the proliferation-associated AURKA gene in pregnant patients as compared to control (p = 0.73 vs $\rho = 0.24$, p = 0.001). Conclusions: In this study, we were able to identify several genomic alterations associated with pregnancy that could help elucidate the impact of pregnancy on BC risk. Moreover, by combining CNAs with gene expression, we were able to identify genes that could be responsible for the poorer prognosis seen in pregnancy-associated breast cancer and could be potential drivers of this rare disease.

### #1453 Transcriptome analyses in triple-negative breast cancer with BRCA1 germline mutation

Kivi Duarte Nakamura, Elisa Ferreira, Rodrigo Ramalho, Rafael Brianeu, Dirce Carraro, A.C. Camargo Cancer Center, São Paulo, Brazil.

Triple-negative breast cancer (TNBC), characterized by lack of expression of the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2), is associated with a higher prevalence of germ-line BRCA1 mutations, especially in younger women. TNBC comprises approximately 15% of breast cancer cases and is considered one of the most aggressive subtypes. BRCA1 protein plays a pivotal role in DNA damage repair, cell cycle control and transcriptional regulation. In this context, we aimed to investigate the transcriptional difference of paired tumor and adjacent normal tissue from young patients in two TNBC groups - with or without BRCA1 germline mutation. Fourteen early-onset patients (=41 years), with TNBC previously evaluated for BRCA1/2 germline mutations, were included. From these, nine patients were BRCA1/2 wild-type and five patients were BRCA1 germline mutation carriers. RNAseq libraries were constructed from RNA depleted total RNA extracted from paired tumor and normal adjacent samples and sequenced on Illumina NextSeq 500 platform. Approximately 20 million reads aligned to human genome were generated per sample, revealing about 14,500 expressed genes differentially expressed between groups (fold-change $> 2$, FDR $< 0.05$). In the BRCA1-mutated group of BRCA1-mutated samples, being 722 up regulated and 2087 down regulated in the tumor and 904 DEGs in the group of BRCA1/2 wild-type samples, 399 were upregulated and 505 downregulated in the tumor. Interestingly, both lists of upregulated genes in TNBC of BRCA1-mutated and BRCA1/2 wild-type were significantly enriched in the same molecular pathway involved with the cell cycle and mitosis, according to Reactome Pathways software. Overall, our first observation based on this preliminary data was that, in terms of general gene expression analysis, BRCA1-mutated TNBC has less transcriptional modification in comparison to the normal tissue than the BRCA1/2 wild type and that the tumorigenic process of both groups are similar in terms of defective biological pathway.

### #1454 Estrogen receptor signaling in FTE of BRCA mutation carriers

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Genetic and reproductive factors predicate epidemiological risk factors underlining epithelial ovarian cancer. The fallopian tube epithelia (FTE), the presumptive etiological site of high-grade serous ovarian cancer (HGSC), is a hormonal responsive tissue. Estrogen is known to promote cell proliferation and its metabolism produces reactive oxygen species that damage DNA and promote tumorigenesis. Estrogen receptor (ER) is rarely mutated, amplified or deleted in HGSC, yet only 10% of patients respond to anti-estrogen treatment, suggesting that intrinsic variables to the ER pathway contribute to this clinical outcome. TP53 mutations occur in almost 100% of HGSCs, indicating that mutated p53 supports a model as an early event in the pathogenesis of HGSC. We hypothesized that in the presence of dysfunctional p53, subsequent promiscuous binding of ER will yield aberrant signaling, ultimately significantly contributing to cellular transformation. Mutant p53 and ER co-localize in FTE cells, suggesting potential synergy. We established cell lines with p53 mutations and treated them with estradiol, an estrogen analog, to observe any changes in response. The genome binding sites of ER-regulated transcription factors were then identified and mapped by whole genome chromatin immunoprecipitation-deep sequencing (ChIP-Seq). The cell line model will facilitate the development of gene signatures that will predict response to anti-estrogen therapy in serous ovarian cancer patients, and contribute to the discovery of biomarkers to more accurately identify patients who will benefit from hormonal therapies.

### #1455 Vitamin D receptor (VDR) gene polymorphisms among Sudanese females with breast cancer attending Radio and Isotope Center of Khartoum.

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Proteins of the vitamin D receptor (VDR) gene polymorphisms among Sudanese females with breast cancer attending Radio and Isotope Center of Khartoum. Fourteen early-onset patients (=41 years), with TNBC previously evaluated for BRCA1/2 germline mutations, were included. From these, nine patients were BRCA1/2 wild-type and five patients were BRCA1 germline mutation carriers. RNAseq libraries were constructed from RNA depleted total RNA extracted from paired tumor and normal adjacent samples and sequenced on Illumina NextSeq 500 platform. Approximately 20 million reads aligned to human genome were generated per sample, revealing about 14,500 expressed genes differentially expressed between groups (fold-change $> 2$, FDR $< 0.05$). In the BRCA1-mutated group of BRCA1-mutated samples, being 722 up regulated and 2087 down regulated in the tumor and 904 DEGs in the group of BRCA1/2 wild-type samples, 399 were upregulated and 505 downregulated in the tumor. Interestingly, both lists of upregulated genes in TNBC of BRCA1-mutated and BRCA1/2 wild-type were significantly enriched in the same molecular pathway involved with the cell cycle and mitosis, according to Reactome Pathways software. Overall, our first observation based on this preliminary data was that, in terms of general gene expression analysis, BRCA1-mutated TNBC has less transcriptional modification in comparison to the normal tissue than the BRCA1/2 wild type and that the tumorigenic process of both groups are similar in terms of defective biological pathway.

### MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Genomics in Inherited Susceptibility and Preneoplastic Conditions

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MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Genomics in Inherited Susceptibility and Preneoplastic Conditions

Background: Vitamin D Receptor (VDR) gene polymorphisms has been reported to be associated with different cancers, including breast cancer. geographical and environmental factors have an impact on vitamin D (VD) metabolism. This study aims to evaluate VDR-gene polymorphisms (FokI, BsmI, TaqI, Apal) and its association with breast cancer risk among Sudanese females. Materials and Methods: This is a case control study done in the Radiotherapy and Isotope Center in Khartoum, Sudan. Hundred thirty seven patients and 69 healthy controls were enrolled after informed consent. Blood genomic DNA was extracted using quinidine chloroform method, and single nucleotide polymorphisms (SNPs) FokI (T/C) [rs2282570], BsmI (G/A) [rs1544410], TaqI (C/T) [rs731236] and Apal (C/A) [rs7975232] in VDR-gene were detected by PCR and restriction enzyme digestion. Genotyping of VDR-FokI was also performed after PCR-RFLP analysis of VDR-SNPs rs731236 correlated significantly with low risk of breast cancer (OR = 0.5, p = 0.002). There was no significant association observed with rs1544410 and rs7975232. Although our study did not show an overall statistically significant association of VDR-SNPs rs2282570 with the risk of breast cancer, we found a 1.3 fold increased risk of breast cancer (OR = 1.3) among females with FF genotype when compared with ff genotype, suggesting that females homozygous for F allele, might be at more risk than females carrying homozygous f allele. Conclusions: These results suggested that, VDR-SNPs rs731236 may be related to higher risk of breast cancer and VDR-SNPs rs731236 associated with lower risk of breast cancer in Sudanese females. Key words: Breast Cancer, VDR, Polymorphisms, SNPs

#1456 Identifying early genetic steps in malignant transformation of neurofibromatosis type 1- associated pleomorphic neurofibromas. Alexander Pesov,1 Alexander Pikarsky,1 Joseph F. Boland,2 Settara C. Chandrasekharappa,2 NIH Intramural Sequencing Center, James C. Mullikin, 2 Margaret Wallace,3 Eric Legius,7 Brigitte Widemann,4 Douglas R. Stewart1. 1Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD; 2Cancer Genetics and Comparative Genomics Branch, National Human Genome Research Institute, Rockville, MD; 3Department of Molecular Genetics and Microbiology, UF Genetics Institute, University of Florida, Gainesville, FL; 4Department of Molecular and Microbiology, UF Genetics Institute, UF Health Cancer Center, University of Florida, Gainesville, FL; 2Department of Medical and Clinical Genetics, University of Helsinki; Research Programs Unit, Genome-Scalse Biology, University of Helsinki, Helsinki, Finland; 7Department of Obstetrics and Gynecology, Helsinki University Hospital, University of Helsinki, Helsinki, Finland; 6Department of Obstetrics and Gynecology, University of Helsinki, Helsinki, Finland; 8Department of Pathology and HUSLAB, Helsinki University Hospital, University of Helsinki, Helsinki, Finland.

BACKGROUND: Neurofibromatosis type 1 (NF1) is a genetic tumor predisposition disorder caused by germline mutations in tumor suppressor NF1. Plexiform neurofibromas (PN) are benign tumors that arise prenatally or early in childhood and affect 30-50% of NF1 population. Somatic inactivation of second copy of NF1 is believed to be primary genetic event leading to PN initiation. NF1 patients have a 10-12% lifetime risk of developing malignant peripheral nerve sheath tumor (MPNST), a highly aggressive soft tissue sarcoma, often arising from pre-existing PN and atypical NF (ANF). ANF are pre-malignant tumors that often arise within PN and can transform into MPNST. They are distinct from both PN and MPNST clinically and histologically, thus representing an intermediate step in malignant transformation. Several studies identified deletion of the CDKN2A/B locus as the most frequent genetic event in ANF, however it is not clear whether other genes or pathways play role in PN transformation into ANF and further into MPNST. In this study, we performed genomic analysis of 16 ANF and 4 MPNST matched with normal DNA obtained from 14 and 4 patients, respectively. METHODS: We performed whole exome sequencing and whole transcriptome RNAseq analyses on Illumina Hi-Seq 2500 platform and copy-number variant (CNV) analysis on Illumina HumanOmniExpressExome-8 SNP-arrays. In addition, we performed deep sequencing of NF1 and validation of select mutations on Ion Torrent platform. For select tumors we estimated growth rate and metabolic activity by using volumetric MRI and FDG-PET. RESULTS: We identified inactivation of NF1 in the majority of ANF and all MPNST. We also detected CDKN2A/B locus deletion in the majority of ANF and sporadic cases of MPNST. We determined that PRC2 genes (EED and SUZ12) were mutated in multiple MPNST but never in ANF. We identified a low number of point mutations and small indels in the genomes of ANF (median 1, range 0-4) and somewhat elevated mutation burden in MPNST (median 23, range 18-31), however none of these mutations were recurrent and none of the mutant genes (other than NF1 and CDKN2A) were present in multiple samples. We found 93 CNV per tumor (median) in ANF that constituted ~2% of their genomes. In comparison, we observed 2,249 CNV (median) in MPNST that comprised ~75% of their genomes. We didn’t detect significant correlation between growth rate or metabolic activity and the degree of genomic instability or mutation burden in the tumors, however the size of the sample set was modest. RNAseq data analysis is pending. CONCLUSIONS: It appears that PN-ANF transition is predominantly if not exclusively driven by heterozygous deletion of the CDKN2A/B locus. Further progression to MPNST likely involves homozygous loss of CDKN2A/B and complete inactivation of the PRC2 complex. Widespread LOH in MPNST may accelerate inactivation of key gatekeepers.

#1457 Somatic biallelic inactivation of fumarate hydratase (FH) in uterine leiomyomas. Jaana Tolvanen,1 Netta Mäkinen,1 Hanna-Rükke Heineinen,1 Sioma Bramante,1 Miika Mehine,1 Nanna Sarvilinna,2 Jari Sjöberg,3 Oskari Heikinheimio,4 Annukka Pasanen,5 Ralf Büttow,5 Lauri A. Aaltoinen,6 1Department of Medical and Clinical Genetics, University of Helsinki; Research Programs Unit, Genome-Scalse Biology, University of Helsinki, Helsinki, Finland; 6Research Programs Unit, Genome-Scalse Biology, University of Helsinki; Department of Obstetrics and Gynecology, Helsinki University Hospital, University of Helsinki, Helsinki, Finland; 7Department of Obstetrics and Gynecology, Helsinki University Hospital, University of Helsinki, Helsinki, Finland; 8Department of Pathology and HUSLAB, Helsinki University Hospital, University of Helsinki, Helsinki, Finland.

Uterine leiomyomas (ULMs) are highly common benign tumors that affect the health of millions of women by causing a variety of symptoms. Furthermore, ULMs are the most common indication for hysterectomy, generating considerable economic costs for the health care system. Recurrent and mutually exclusive genetic aberrations leading to unique gene expression profiles have been identified in ULMs. Thus these tumors can be divided into distinct subclasses according to their molecular genetic background. The majority of ULMs (80%–90%) have mutations in FH, a fumarase enzyme that catalyzes the hydration of fumarate to L-malate in the tricarboxylic acid cycle. Biallelic inactivation of FH leads to accumulation of fumarate and aberrant succinication of proteins. Heterozygous germline FH mutations predispose to a rare autosomal dominant tumor predisposition syndrome, hereditary leiomyomatosis and renal cell cancer (HLRCC). This highly penetrant syndrome is characterized by uterine and cutaneous leiomyomas and in some cases renal cell cancer. In this study, we re-evaluated the frequency of somatic biallelic inactivation of FH in ULMs in the largest sample set thus far. The study material consisted of 1167 fresh frozen ULM and their respective normal myometrium tissue samples from 375 hysterectomy patients. All samples were analyzed with high-throughput SNP array (Infinium Human Core-24+ Kit, Illumina Inc., San Diego, CA). All tumors detected with somatic deletions or loss of heterozygosity (LOH) in the FH locus were included in 5-(2-succinyl) cysteine (25C) immunohistochemistry (IHC) to verify FH inactivation and exclude LOH. In addition, all samples without FH double deletions were selected for Sanger sequencing of FH coding regions to detect inactivating somations. Somatic FH deletions or LOH were detected in 30 tumors, of which 10 were identified FH deficient by 25C IHC. In these samples, another deletion (a double deletion) in three ULMs and a somatic nucleotide change in six ULMs were found to be the second hit causing biallelic inactivation of FH inactivation. These samples were excluded from these methods. From the six somatic nucleotide changes, four were different FH missense mutations predicted to be damaging (SIFT, PolyPhen-2, Provean) and two were synonymous substitutions in the last nucleotide of different exons. Studies to determine the impact of these synonymous changes on splicing are underway. To conclude, in our sample set we detected 0.9% (10/1167) of the ULMs to harbour somatic biallelic inactivation of FH. MED12 exon 1 or 2 mutations and HMGA2 rearrangements were not detected in these tumors. Thus the previously published results were validated in this study with the largest sample set so far.

#1458 Genome-wide association study of unselected prostate cancer cases in Finnish population. Csilla Sipeky,1 Teuvo L. Tammela,2 Anssi Auvinen,2 the PRACTICAL consortium, Johanna Schleutker3. 1Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD; 2Departament of Medical and Clinical Genetics, University of Helsinki; Research Programs Unit, Genome-Scalse Biology, University of Helsinki, Helsinki, Finland; 3Research Programs Unit, Genome-Scalse Biology, University of Helsinki; Department of Obstetrics and Gynecology, Helsinki University Hospital, University of Helsinki, Helsinki, Finland; 4Department of Obstetrics and Gynecology, Helsinki University Hospital, University of Helsinki, Helsinki, Finland; 5Department of Pathology and HUSLAB, Helsinki University Hospital, University of Helsinki, Helsinki, Finland.

Prostate cancer (PrCa) is the second most prevalent cancer in men worldwide, representing the most common cancer in males in Finland with 5043 new cases and with over 850 annual deaths (2013). PrCa has a significant heritable component, with genetic factors accounting for 58% of the risk. Accordingly, genome-wide association studies (GWAS) identified 104 low penetrance PrCa susceptibility loci to date, predominantly in populations of mixed European ancestry. However, effect allele frequencies (EAF) and strength of association (Odds ratio, OR) are often variable across popular...
#1459 Dual-strand tumor-suppressor microRNA-145 and miR-145-3p are involved in castration-resistant prostate cancer pathogene-
sis. Mayuko Kato,1 Akira Kurozumi,2 Yusuke Goto,1 Nijiro Nohata,3 Takayuki Arai,1 Atsushi Okato,1 Keiichi Koshizuka,1 Satoko Kojima,3 Tomohiko Ichikawa,6 Naohiko Seki1. 1Department of Functional Genomics, Chiba University Graduate School of Medicine, Chiba, Japan; 2Department of Medicine, Chiba University School of Medicine, Chiba, Japan; 3Department of Urology, Teikyo University Chiba Medical Center, Ichikawa, Japan; 4Department of Urology, Chiba University Graduate School of Medicine, Chiba, Japan.  
Prostate cancer (PCa) is the most frequently diagnosed cancer and the second leading cause of cancer-related death among men in developed countries. Androgen signaling through the androgen receptor (AR) is an important oncogenic pathway for PCa progression. Most patients initially respond to androgen-depletion therapy (ADT), but eventually acquire resistance and progress to castration-resistant prostate cancer (CRPC). Although several clinical trials for CRPC have been carried out, resulting in the availability of novel chemothera-
peutic agents, these treatments provide limited benefits and are not considered curative. Therefore, identification of effective biomarkers for detection of CRPC and understanding the molecular mechanisms of androgen-independent signal-
ning and metastatic signaling pathways underlying PCA using current genomic approaches would help to improve therapies for and prevention of the disease. The discovery of microRNAs (miRNAs) has resulted in major advancements in cancer research. miRNAs are small noncoding RNAs that function to fine tune the expression of protein coding/noncoding RNAs by repressing translation or cleaving RNA transcripts in a sequence-dependent manner. The unique char-
acteristic function of miRNAs is to regulate RNA transcripts in human cells. Therefore, dysregulated expression of miRNAs can disrupt tightly regulated RNA networks in cancer cells. miRNAs play critical roles in various biological processes, and their dysregulation is shown in several human cancers. In this study, we constructed the miRNA expression signature of CRPC using clinical specimens because the development of therapeutic strategies is a central theme in the advancement of PCA treatments. Using CRPC expression signature data, we investigated the specific roles of miRNAs in PCA and CRPC oncogenesis by examining differentially expressed miRNAs. Based on the CRPC signature, we focused on the dual strand of pre-miR-145, miR-145-5p and miR-145-3p be-
cause these miRNAs were significantly reduced in cancer tissues, suggesting that these miRNAs act as tumor suppressors in this disease. In miRNA biogenesis, it is the general consensus that processing of the pre-miRNA through Dicer1 gen-
erates a miRNA duplex (a passenger strand and a guide strand), and that the passenger strand has degradation and no regulatory activity and disintegrates in cells. Our present data showed that both miRNAs, miR-145-5p and miR-145-3p significantly suppress cancer cell migration and invasion. Moreover, Kaplan-Meier survival curves showed that low expression of miR-145-3p predicted a short duration of progression to CRPC. Dual strand of pre-miR-145 functioned as tumor suppressors based on the miRNA expression signature of CRPC. Iden-
tification of miRNA-mediated cancer networks may provide novel molecular pathogenesis of the disease.

#1460 miRNome analyses reveal that activity of CAF expressed podopla-
nin in tumor microenvironment is modulated by exosome miRNAs involved in the PTEN-P13K-AKT-mTOR signaling. Anna Tejchman,1 Eric Mennes-
son,2 Isabelle Fice,2 Alexandra Foucher,2 Simon Perera,2 Laura Artigas,2 Jose Manuel Mas,3 Catherine Grillon,1 Maciej Ugorski,1 Nadia Normand,2 Claudine Perray En Yvelines, France; 3Anaxomics Biotech, Barcelona, Spain; 4Institute of Immunology and Experimental Therapy, Wroclaw, Poland. In breast cancer, cancer associated fibroblasts (CAF) express podoplanin but little is known about its mechanism of expression and modulation. This might be crucial for cancer cell movements and for the ability to escape from the tumor microenvironment. Podoplanin expression is associated with an immunoinvasive phenotype. A recent study showed that podoplanin expression is a key target for antitumor treatment. A key role in tumor microenvironment is exerted by microRNAs on gene expression. Therefore miRNAs profiling was performed with 3D-Gene microarray technology on a breast CAF model, on cell lysates and on secreted exosomes, in hypoxia versus normoxia culture conditions. Systems biology en-
richment analyses were carried out through bioinformatics using mathematical models that simulate in silico the behaviour of human physiology. Targets linked to the set of differential miRNAs, both up-regulated and down-regulated, have been analyzed with various methods including a hypergeometric distribu-
tion method. Processes linked to hypoxia, glycosylation, angiogenesis, extracel-
ular matrix and pathways associated with P13K-AKT, mTOR, P53 and chemo-
kinase signaling were studied. We showed that podoplanin expression strongly affects the network activity of miRNAs involved in these processes. Hypoxia repression of miR-210, miR-21 and miR-29b in cells over-expressing podoplanin. miR-21 is an oncoprogenic regulator in most cancer cells through its downstream target pro-
teins among which stands the tumor suppressor PTEN, a candidate to alleviate hypoxia in tumors, regulating tumor angiogenesis and modulating tumor hyp-
oxia. miR-29b is a tumor suppressor, it also correlates with PTEN repression. We highlighted miRNA regulation of podoplanin expression through downstream regulation of upstream genes for tumor suppressor PTEN. The combination of miRNome analysis and systems biology bioinformatics uncovered potential miRNAs involved in the regulation of podoplanin activity in CAF. Such data analysis may be applied to protein and miRNA biomarkers and extended to other pathology pathways.

#1461 miR-424-cdc42, key signaling axis in hyperglycemic regulation of stromness in triple negative breast cancer. Sushmitha B. Nandy,1 Alexis Orozco,1 Gautham Prabhakar,1 Viktoria Stewart,1 Stephanie Jones,1 Paloma Munoz,1 Raha Madevi Subramaniam,2 Diego Pedroza,1 Rajkumar Lakshmanaswamy3. 1Texas Tech Univ. Health Sciences Ctr. El Paso, El Paso, TX; 2University of Texas at El Paso, El Paso, TX. Introduction: Meta-analysis shows women with diabetes have a 20% in creased risk of breast cancer compared to those without diabetes. It has been hypothesized that hyperglycemia enhances stem cell activity in triple negative breast cancer (TNBC) cells. Here, we report miR-424 to be a key regulator of breast cancer stem cell pool dynamics under hyperglycemic environment. Materials and Methods: MDA-MB-231, TNBC cell line was maintained under euglycemic (5mm) and hyperglycemic (10mm) culture conditions. RT-PCR based miRNA array was performed followed by validation of significantly altered microRNA to confirm the effect of hyperglycemia on the global microRNA profile. Flow cytometric analysis for CD44+CD24− was performed to assess the alterations in breast cancer stem cell population. In addition, sphere-forming assay were also performed. Mir-424 over-expressing or knocked down cell lines were established from parental MDA-MB-231 cells and maintained in hyperglycemic or eugly-

cemic condition respectively. Promoter analysis for PR/SET domain 14 (pmd14) was done. Western blotting and immunofluorescence assays were performed for cdc42 (cell division control 42), pStat5 (phospho signal trans-
ducer and activator of transcription factor 5) and pmd14. Results: Loss of miR-

424 in TNBC cells under euglycemic conditions led to enhanced stem cell activity; whereas over expression of miR-424 under hyperglycemic conditions resulted in suppressed stem cell activity. Introduction of cdc42, (a miR-424 target gene) in miR-424 over expressing TNBC cells under hyperglycemic con-
ditions led to increased stem cell activity demonstrating the role of miR-424-cdc42 signaling in hyperglycemia. Over-expression of miR-424 in miR-424 dramatically reduced its metastatic abilities in vivo. Mechanistically, we found that miR-424-cdc42 signaling in hyperglycemia promotes Pmmd14 activation, a stem cell regulator through increased phosphorylation of Stat5.
Conclusions: Our findings establish a key molecular signaling cascade (miR-424→cdc42→prdm14) that promotes TNBC stem cell activity under hyperglycemic conditions.

#1462 Exosome-derived miR-429 contributes to prostate cancer chemoresistance. Yang Liu,1 Liye Xie,1 Jing Li,1 Atsushi Mizokami,2 Evan T. Keller,2 Yi Lu,1 Jian Zhang,1 3 Guangxi Medical Univ., Nanning, China; 2Kanazawa University, Kanazawa, Japan; 3University of Michigan, Ann Arbor, MI.

Prostate cancer (PCA) is the most common cancer in men and the 2nd leading cause of cancer-related death in the United States. Certain miRNAs, act as tumor promoting or inhibiting factors in tumor development, function primarily by targeting specific miRNAs for degradation or inhibition of translation of their targeted proteins. Emerging evidence indicates that exosomes play a key role in tumor-host crosstalk, and exosome secretion, composition, and functional capacity are altered as tumors progress to aggressive phenotypes. From our prior report, we have constructed a potential network of 29 deregulated miRNAs, including 19 up and 10 down, in exosome samples derived from two kinds of paclitaxel resistant PCA cells (PC3-TXR and DU145-TXR) compared with their parental cells (PC3 and DU145). In this study, to further determine the roles of certain miRNAs derived from exosomes in chemoresistant cells, we firstly confirmed the lower expression level of miR-429 mRNA in the exosomes derived from the paclitaxel resistant PCA cells compared to their parental cells. In addition, when the miR-429 was over-expressed in PC3-TXR and DU145-TXR cells by stable transfection, epithelial-to-mesenchymal transition (EMT) markers significantly altered together with morphological changes. Overexpression of miR-429 decreased the adhesion and cell attachment to the outer surface associated with inactivation of PI-3K/AKT and Notch signaling pathways since the pathway specific inhibitors significantly diminished the transacted tumor cell migration and invasion. Finally, we found that overexpression of miR-429 significantly enhanced chemosensitivity of the chemoresistant PCA cells. These data suggested that exosome-derived miR-429 contributes to PCA chemoresistance and miR-429 may become a potential therapeutic target for PCA patients. This work was supported by Natural Science Foundation of China (NSFC) Key Project (81130046); NSFC (81171993; 81272415); Guangxi Projects of China (2013GXNSFAA007177; 1535004-5; 201202004; GZ2FT13-35; 1123000021; 11-031-05-K2; KY2015BS07; 1404-05-12-K2).


Metastatic cancer cells often use directional ECM cues such as blood vessels or collagen fibers when invading through live tissue. Oncogenic miRNAs have been implicated as key regulators of cancer progression yet the systemic discovery of miRNAs that drive directional cancer cell invasion has not been achieved. Here we describe the first in vivo quantitative whole human miRNAome screen for miRNA drivers of directional cancer cell invasion using an ex ovo avian embryo model of human cancer cell metastasis combined with high resolution intravital imaging. We identified more than twenty novel miRNAs that promote cancer cell invasion during the key rate-limiting step of cancer metastasis, the initiation of invasion. In mechanistic studies, we found that deregulation of the miRNA in the HCC1806 and BT549 TNBC cell lines, derived from an AA and NH patients, respectively, and 49 adjacent normal tissue (ANTs) were used as control for the subtype and tumor specificity, respectively. Our results showed a significant down-regulation of miRNA-661 in the TNBC of AA when compared to the NHW and to verify it is directly role in affecting the aggressive TNBC phenotype. MiRNA-661 expression analysis was conducted by RT-qPCR in the tumor tissue of 31 AA-TNBC patients and 17 NHW-TNBC patients. AA and NHW samples of 40 and 20 non-TNBC subtype, respectively, and 49 adjacent normal tissue (ANTs) were used as control for the subtype and tumor specificity, respectively. Our results showed a significant down-regulation of miRNA-661 expression in TNBC of AA in relation to the NHW (P<0.0001) and between the AA-TNBC and non-TNBC subtypes (P<0.001). MiR-661 was also significantly differentially expressed in the ANT when compared to the tumor tissue (P<0.001). In addition, ROC analysis showed a high power of this miRNA in discriminating the TNBC genome of AA and NHW women (AUC=0.79 (0.65-0.92), 95%CI). Modulation of the expression of this miRNA in the HCC1806 and BT549 TNBC cell lines, derived from an AA and NHW patient respectively, using inducers/repressors systems, showed its direct effect in cell proliferation, migration and cytotoxicity. Interestingly these effects were more pronounced under hypoxic conditions. These data indicate that miRNA-661 dysregulation is associated with TNBC of AA women, and directly impacts their tumor phenotype. The uncovering of the molecular mechanisms that dictates these effects, specially the ones that modulate drug response, is critical for patient selection and stratification into novel and efficient target therapies. This much needed knowledge would lead to potentially personalized treatment for young AA patients, that are commonly affected by the TNBC aggressive phenotype. Funding: This project was supported by the Georgetown University Center of Excellence in Regulatory Science and Innovation (CERSI U1O1FD004319), a collaborative effort between the university and the U.S. Food and Drug Administration to promote regulatory sci-
Triple negative breast cancer (TNBC) refers to a group of highly heterogeneous malignant tumors that lack estrogen receptor and progesterone receptor expression, and human epidermal growth factor receptor 2 amplification, accounting for 10-20% of newly diagnosed breast cancer cases. Compared to other subtypes of breast cancer, TNBCs usually show worse clinical features such as more rapid tumor growth, earlier recurrence, and more aggressive metastasis. Unfortunately, the mechanism of TNBC metastatic behavior has not been well understood. Moreover, no efficient targeted therapies for TNBCs are currently available, representing a real unmet need for effective new therapies. MiR-200 family members are among the first miRNAs reported to function as potent inhibitors of cancer metastasis. However, the mechanism of miR-200 family on cancer metastasis has not been well studied. In this study, we explore the effect of miR-200b, one member of the miR-200 family, on TNBC metastasis using cell culture and mouse orthotopic mammary xenograft tumor models. We found that the expression level of miR-200b is significantly lower in TNBC cells and tissues than that in other types of breast cancer. Stably expressing miR-200b significantly reduced TNBC cell migration and invasion and suppressed TNBC metastasis in a mouse orthotopic mammary xenograft tumor model. Mechanistic studies revealed that miR-200b overexpression in TNBC cells caused drastic changes in cellular actin cytoskeleton organization patterns as evidenced by reduced lamellipodia formation but increased stress fiber formation. In consistent with these findings, Rho GTPase pull-down assays demonstrated that stably expressing miR-200b significantly increased the Rho GTPase Rho A activation, but reduced Rho GTPase Rac activation. Moreover, inhibition of Rho A signaling impaired the inhibitory effect of miR-200b on TNBC cell migration. Bioinformatics analysis indicated that ARHGAP18, a specific Rho A GTPase activating protein (GAP), is a predicate target of miR-200b. Further Q-PCR, Western blot and 3’UTR reporter analysis confirmed that ARHGAP18 is a target of miR-200b. Knocking down ARHGAP18 in TNBC cells using siRNAs significantly increased Rho A activation but reduced Rac1 activation. To further determine the role of ARHGAP18 in TNBC, ARHGAP18 knockdown TNBC cells were generated using the CRISPR technology. It was found that knocking down ARHGAP18 phenocopied the effect of miR-200b overexpression. Moreover, overexpressing miR-200b in miR-200b stable expression cells overcome the inhibitory effect of miR-200b on TNBC metastasis. Together, these findings suggest that miR-200b suppresses triple negative breast cancer metastasis by targeting ARHGAP18 and enhancing Rho A activation.


Cancer is characterized as a loss of normal cellular regulation, due to accumulations of mutations and epigenetic alterations in cancer cells. MicroRNAs (miRNAs) regulation of co-stimulatory and immune checkpoint pathways have been implicated as one of the potential mechanisms for cancer evasion in immuno-oncology. It is estimated that 30% of all mRNA expression may be regulated by miRNAs, and some are either oncogenic or tumor suppressive. Complexity of miRNA regulation highlights the need for integrated assays, providing direct correlation between miRNA and mRNA, and protein expression. From a single 4 μFPE section, MultiOmyx™ Hyperplexed immunofluorescent assay (designed to stain up to 60 protein biomarkers) is utilized to measure CD3, CD4, CD8, CD16, CD56, Granzyme B, FoxP3, ICOS, CX04, CX04L, PD1, PD1L, HLA-DR, and Ki67 protein expression. From an adjacent 10 μm section, NanoString™ nCounter PanCancer Immune Profiling Panel and Human v3 miRNA expression panel were utilized to comprehensively profile the expression of 770 mRNA and 800 miRNA. Integrating MultiOmyx and NanoString technologies, the current study measured miRNA, mRNA, and protein expression in lung, head and neck, breast, and melanoma samples. For each indication, three samples were selected from a larger sample set, based on high protein expression of lymphocytes and macrophage markers (CD3, CD4, CD8, CD16, CD56). Results: In this study, we investigated the co-stimulatory and immune checkpoint markers (PD1, PD-L1). Protein expression results indicate positive correlation between expression of ICOS and OX40 with higher infiltration of T-helper (CD3 + CD4+), T cytotoxic (CD3 + CD8+), and effector T cells (CD3 + CD8 + Granzyme B). NanoString normalized mRNA counts for the protein biomarkers profiled indicate that all markers except for HLA-DR belong to lower expressor groups with counts ranging from 20-700. Comparison of protein expression to mRNA counts revealed inconsistencies in modulated markers (PD1, PD-L1, ICOS, CX04) which are attributed to differences in population of cells between the two sections. Direct assessment of up regulation of mRNA and down regulation of target miRNA could not be made for miRNAs reported in...
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literature as a negative regulator of PD-L1 (miR-34a, 34b, 34c), PD1 (miR-28, miR-107), FoxP3 (miR-210, miR-24, miR-31), and ICOS (miR-101). Analysis of multiple miRNAs (combinatorial targeting) mapped in context to mRNA, and their respective protein expression will be presented from lung, head and neck, breast, and melanoma cancer samples.

#1470 Fine-tuning the expression of heterogeneous network of genes involved in androgen signaling, aerobic glycolysis, apoptosis and epithelial-mesenchymal transition by microRNA-644a in prostate cancer potentiation of AR signaling therapy by miR-644a: Selective manipulation of the prostate cancer transcriptome by miR-644a. Jey Sahib Ethron, Jagjit Singh, Sawai Shankar, Crystal Weyman, Sanjay Gupta, Daniel J. Lindner, Girish C. Shukla.

Intratumoral Prostate cancer (Pca) heterogeneity is characterized by multiple intrinsic mechanisms including Androgen signaling, aerobic glycolysis, aberrant transcriptional activation of c-Myc and dysregulation of miRNAs. The ability of prostate cancer cells to alter growth and metabolism in a manner distinct from benign cells is a major concern in the treatment of castration-resistant prostate cancer. Therefore, targeting the diverse oncogenesis promoting pathways involved in Pca progression and resistance at the posttranscriptional level using micro RNAs (miRNAs) offers a novel therapeutic option. Using a miRNA mimic screen, we identified a human-specific miRNA miR-644a, which modulates expression of a diverse set of oncogenic pathways in Pca. Using a panel of five Pca cell lines we discovered that c-Myc expression is higher in aggressive CRPC cell lines when compared to the indolent E006AA PCa cell line. Our recent evidence has shown that a miRNA, miR-6855-3p, binds to the primary PRDX5 transcript between the two AUG codons and prevents the translation of the mRNA from the first AUG codon but not from the second AUG codon thus favoring the initiation of translation from the second AUG codon. To characterize the effect of miR-6855-3p on Prdx5 mRNA translation, we developed three different PRDX5-FLAG constructs in pC3VXFLAG1 (Sigma) that has Prdx5 ORF with or without mutation on the first or second AUG codon. Transfection of these constructs into BT549 cells followed by treatments with miR-6855-3p mimic or antagoniri and subcellular fractionation further supported our notion. We propose here that miR-685-3p acts as a tsmIR by promoting the biosynthesis of the transcriptional regulator protein PRDX5 which enhances the expression of the tumor suppressor protein BRCA2 leading to the genotoxic and radiation-resistance of aggressive SLUG-high basal-like breast cancer cells. Supported in part by DOD-CDMRP IDEA Expansion Grant# BC130645 and NIH/NCI grant 1R21CA181920 01 to GC and 1U54RR026140 to SM.


Prostate cancer (Pca) is the most common non-skin cancer and the second leading cause of cancer-related death for men in the U.S. A major challenge is understanding the molecular mechanisms involved in the progression from the asymptomatic androgen-dependent Pca to the lethal castration resistant prostate cancer (CRPC). The chromosomal region 8q24 is associated with aggressive Pca. miR-1207-3p and variants of this region have been identified to interact with the PVT1 non-coding gene in Pca. In previous work we demonstrated that microRNA-1207-3p (miR-1207-3p) has prognostic value in Pca, and directly binds to the 3’ UTR of Fibronectin type III domain containing 1 (FNDC1) to regulate a novel FNDCl/fibronectin (FN1)/androgen receptor (AR) pathway upregulated in metastatic Pca. miR-1207-3p is encoded at the PVT1 gene locus, which is located downstream of c-Myc on the 8q24 human chromosomal region. Studies have suggested amplification at the 8q24 human chromosomal region, which includes C-Myc, as a possible prognostic factor for CRPC. c-Myc is a well-established proto-oncogene that is commonly found to be amplified in up to 72% of CRPCs significant amplification of c-myc has been a consequence of androgen treatment, and c-Myc is downstream of AR in some Pca. miRNAs have been implicated in the regulation of c-Myc. Nevertheless, the mechanisms regulating c-Myc remain unclear in Pca. Using a panel of five Pca cell lines we discovered that c-Myc expression is higher in aggressive CRPC cell lines compared to non-aggressive CRPC and androgen-dependent prostate cancer cell lines. Furthermore, our data reveals that c-Myc expression is higher in the aggressive E006AA-hT Pca cell line when compared to the indolent E006AA Pca cell line. This suggests that c-Myc is associated with aggressive Pca. Moreover, overexpression of miR-1207-3p suppresses the expression of c-Myc in E006AA-hT Pca cells but not E006AA Pca cell line in which c-Myc is underexpressed compared to the normal prostate epithelial cell line, RWE1. Next, using a synthetic biotinylated miR-1207-3p duplex, we discovered that overex-
pression of miR-1207-3p more effectively inhibited migration, proliferation and increased apoptosis in the aggressive E006AA-IT PCa cell line with increased c-Myc expression when compared to the indolent E006AA PCa cell line with decreased c-Myc expression. These data demonstrate that the miR-1207-3p/FNDC1/FN1/AR pathway may regulate c-Myc in aggressive PCA.

#1474 Epigenetic silencing of microRNA-137 enhances ASCT2 expression and tumor glutamine metabolism. Xiao Daibiao,1 Junli Dong,1 Zihan Zhao,2 Ping Ren,3 Cong Li,1 Yufeng Hu,1 Jianguo Shi,1 Hexiu Su,1 Zhaoqiao Wang,1 Hudan Liu,1 Bo Li,1 Peng Gao,1 Guoliang Qing1,2,3Medical Research Institute of Wuhan University, Wuhan, China; 2The Second Hospital of Dalian Medical University, Dalian, China; 3Hubei University of Science and Technology, Xianning, China; 4Medical School of Medicine of Sun Yat-Sen University, Guangzhou, China; 5Affiliated Union Hospital of Tongji Medical College, Wuhan, China; 6Affiliated Dalian Sixth People's Hospital, Dalian, China.

Cancer cells amplify the expression of ASC amino acid transporter 2 (ASCT2, also called SLC1A5), a high-affinity glutamine carrier protein, to coordinate metabolic reprogramming and malignant transformation. Yet genetic and/or epigenetic mechanisms underlying the control of ASCT2-mediated glutamine metabolism remain to be clarified. Combined in-silico algorithms with systemic experimental screening, we herein identify the tumor suppressor miR-137 as an essential regulator that targets ASCT2 mRNA and cancer cell glutamine metabolism. Metabolic analysis shows that miR-137 derepression, similar to ASCT2 inactivation, significantly inhibits glutamine consumption and TCA cycle anaplerosis. Moreover, miR-137 enhances methyl-CpG binding protein 2 (MeCP2) and DNA methyltransferases (DNMTs) expression which cooperatively methylates the ASCT2 promoter and its decreased transcription, which conversely enhances ASCT2 expression and glutamine metabolism. As such, expression between miR-137 and ASCT2 is inversely correlated in multiple human cancer types, including colorectal carcinomas, glioblastomas, prostate and pancreatic cancers. These findings thus elucidate a universal mechanism responsible for ASCT2 deregulation in human cancers, revealing a molecular link between miR-137, ASCT2 and tumor metabolism.

#1475 miRNA-450a suppresses adhesion but promotes invasion through targeting of TMEM182 in oral squamous cell carcinoma. En-Wei Hsing,1 Shin-Gwo Shah,1 Ching-Chuan Kuo,1 Jing-Yang Chang2. 1National Health Research Institutes, Miaoli, Taiwan; 2National Health Research Institutes, Taichung, Taiwan.

Our microRNA (miRNA) expression signatures of oral squamous cell carcinoma (OSCC) revealed that miR-450a was significantly increased in cancer tissues compared with normal epithelium. In this study, we focused on the functional significance of miR-450a in cancer cells and identification of miR-450a-regulated novel targets in OSCC. Overexpression of miR-450a in DOK and SAS cells showed significant inhibition of cell adhesion and induction of cell invasiveness, suggesting that miR-450a functions as an onco-miRNA. We performed genome-wide gene expression analysis to search for miR-450a-regulated molecular targets. Gene expression data and luciferase reporter assays revealed that TMEM182 was directly targeted by miR-450a. The miR-450a-reduced cellular adhesion was blocked by TMEM182 restoration, suggesting that miR-450a inhibits the cellular invasiveness through targeting TMEM182. Moreover, miR-450a expression could be induced by the cytokine TNF-α primarily through activating extracellular signal-regulated kinase (ERK) signaling pathway. ERK inhibitor prevented the TNF-α-induced miR-450a expression and enhanced adhesion ability. Taken together, these data indicate that TNF-α/ERK-dependent expression of miR-450a plays an important role in mediating cellular adhesion and invasiveness, and scavenging miR-450a function using antamir may have therapeutic potential for the treatment of OSCC. (The study was supported by the following grants: MOST 103-2320-B-006-036-MY3 and MOST 105-2325-B-400-001 from the Ministry of Science and Technology of Taiwan, ROC)

#1476 Cell-free miR-141 as a molecular marker for prostate cancer metastasis. Marilena de Souza,1 Ilce Mara de Syllos Cólus,1 Aline Simonetti Tainan, Taiwan; 2Laboratorio de Investigação Molecular do Cancer - LIMC/FAMEP, São José do Rio Preto, Brazil; 3Departamento de Genética/Faculdade de Medicina - USP, Ribeirão Preto, Brazil; 4Laboratorio Nacional de Bionociencias/Concepción Universidad, Concepción, Chile; 5Laboratorio de Investigação Molecular do Cancer - LIMC/FAMEP, São José do Rio Preto, Brazil; 6Departamento de Genética/Faculdade de Medicina - USP, Ribeirão Preto, Brazil; 7Laboratorio Nacional de Bionociencias/Concepción Universidad, Concepción, Chile.

Metastatic prostate cancer is the second most commonly diagnosed neoplasia in men. Currently, there is no available non-invasive tool to assist in the identification of prostate cancer. MicroRNAs are a class of non-coding RNAs, that are shown to impact the development and progression of prostate cancer and their detection in plasma have been indicated as a promising non-invasive tool for disease screening and prognosis assessment. Among the several deregulated miRNAs that are observed in prostate cancer, the miRNA-141-3p was observed with upregulated expression levels in prostate cancer, particularly in the metastatic lesions. Therefore, the main aim of this study was to evaluate the diagnostic potentiality and clinical performance of miR-141 as a potential new molecular tool that can be useful in the diagnosis of prostate cancer and determine its functional role in modulating tumorigenesis in prostate cancer metastatic cell lines. MiR-141 expression analysis was performed in plasma samples of 102 prostate cancer patients without treatment and of 50 health controls by RT-qPCR. The experimental assays were performed by transfecting the PC3 cell line using miRNA-141 mimic/ inhibitor systems and directly accessing its regulatory regions on the TCA cycle enzymes expression. The expression of the miR-141 was found significantly upregulated in the metastatic patients’ plasma specimens (FC=9.11, p=0.04; AUC=0.66) when compared to the patients without metastasis, showing its screening potential for prostate cancer metastasis. The in vitro ectopic expression of miR-141 in PC3 cell line showed a significant increase in cell proliferation, associated with changes in cell cycle (decreased number of cells in the G1 phase). Conversely, the inhibition of miR-141 showed an increase in the migratory ability, cell adhesion and in the Doxetaxel cytotoxicity of the PC3 cells. Western-blot analysis showed that the inhibition of the miR-141 levels reduced the expression of the EMT inducers markers E-CADHERIN and CLAUDIN, and up-regulated the levels of ZEB-1, pAKT and VIMENTIN, consistent with their repression roles in the EMT process. These findings showed that miR-141 presents a direct function in controlling the phenotypic changes in vitro and the molecular mechanisms involved in the regulation of the epithelial-mesenchymal transition expression proteins. In conclusion, miR-141 presents a potential use as a minimally invasive molecular marker for prostate cancer diagnosis and can be used as a druggable target for therapy, particularly in the metastatic clinical setting.

#1477 Melatonin regulates the tumor suppressor miR-148a-3p involved in angiogenesis and metastasis of breast cancer. Debra Zuccari,1 Jessica Zani Lacerda,1 Lívia Carvalho Ferreira,1 Beatriz Camargo Lopes,2 Andréis Felipe Aris-Izabal-Pachón,1 Marcio Chaim Baigelman,1 Fabio de Medica de Sao Jose do Rio Preto - FAMERP, Sao Jose do Rio Preto, Brazil; 2Universidade Estadual Paulista - UNESP, Sao Jose do Rio Preto, Brazil; 3Laboratorio de Investigacao Molecular do Cancer - LIMC/FAMERP, Sao Jose do Rio Preto, Brazil; 4Departamento de Genetica/Faculdade de Medicina - USP, Ribeirao Preto, Brazil; 5Laboratorio Nacional de Bionociencias/Concepcion Universidad de Ennergia y Materias - CNPEN, Campinas, Brazil.

Women with breast cancer has the tumor progression and angiogenesis-induced metastasis as the main cause of death. MicroRNAs (miRNAs) are small noncoding mRNA molecules that play an important role in gene regulation and once deregulated, these molecules may be involved with the progression of different human tumor types, including breast cancer. These miRNAs play an oncogenic role on tumor suppressor genes and regulate the process of angiogenesis, tumor growth and metastasis. So as new possible adjuvant treatment against breast cancer our group have shown melatonin, that is a hormone secreted by the pineal gland, by exhibiting several anti-tumor and antiangiogenic effects. Therefore, the aim of this study was to evaluate the potential therapeutic of melatonin on miRNAs regulation to verify breast cancer progression and potent tumor suppressor miR-148a-3p. In silico analysis was performed for selection of miRNAs involved in breast cancer. The MDA-MB-231 breast cancer cell line (metastatic negative estrogen receptor) was grown and separated in two different experimental conditions maintained for 24 hours: control group and melatonin-treated group (concentration of 1 mM). After this period the extraction of total RNA was performed (Qiagen®) and the total concentration of miRNAs of each sample was determined (Nanodrop Spectrophotometer 2000C - Thermo Scientific®). Differential expressions of these miRNAs was evaluate using miScript miRNA PCR-Array (Qiagen®) containing 84 miRNAs associated with breast cancer. The overexpression of miR-148a-3p in MDA-MB-231 cells was performed by bacterial cloning vector and the relative quantification of gene expression of their target IGF-1R and VEGF by real-time PCR. Moreover, the expression of the miR-141 were found overregulated with changes in cell cycle (decreased number of cells in the G1 phase). The invivo ectopic expression of miR-141 in PC3 cell line showed a significant increase in cell proliferation, associated with changes in cell cycle (decreased number of cells in the G1 phase). Conversely, the inhibition of miR-141 showed an increase in the migratory ability, cell adhesion and in the Doxetaxel cytotoxicity of the PC3 cells. Western-blot analysis showed that the inhibition of the miR-141 levels reduced the expression of the EMT inducers markers E-CADHERIN and CLAUDIN, and up-regulated the levels of ZEB-1, pAKT and VIMENTIN, consistent with their repression roles in the EMT process. These findings showed that miR-141 presents a direct function in controlling the phenotypic changes in vitro and the molecular mechanisms involved in the regulation of the epithelial-mesenchymal transition expression proteins. In conclusion, miR-141 presents a potential use as a minimally invasive molecular marker for prostate cancer diagnosis and can be used as a druggable target for therapy, particularly in the metastatic clinical setting.
known being involved in the progression of breast cancer. Thus the establishment of this therapeutic protocols can control these cellular events essential for the prognosis of patients with breast cancer.

#1478 Curcumin inhibits epithelial-mesenchymal transition and invasion in breast cancer cells by controlling miR-34a expression. Marcela Gallardo, Richard Poncc-Cusi, Gloria M. Calaf. Universidad De Tárapaca, Arica, Chile.

Breast cancer in advanced stages tends to develop metastases and/or chemoresistance, in both cases therapeutic options are limited and have low probability of success, which represents the biggest obstacle in reducing mortality from this disease. There is a close connection between the Epithelial-Mesenchymal Transition (EMT) process of cancer cells and the acquisition of invasive and metastatic ability. Numerous EMT mediators have been described in cancer and among them miRNAs play a fundamental role in regulating such process, suggesting that it could be a therapeutic target to address this phenotype. Curcumin (diferuloylmethane) is a derivative compound of Curcuma longa that has therapeutic properties in various cancers as blocking initiation and tumor progression through its anti-inflammatory, antioxidant, proapoptotic, antiangiogenic and antimetastatic effects. The role of curcumin on EMT in non-cancerous breast cells MCF-10F and in breast cancer cell lines MCF-7 and MDA-MB-231 was evaluated. This work shows that in all these cell lines curcumin induced the expression of tumor suppressor microRNA miR-34a and repressed the expression of several genes involved in EMT and metastasis as Axl, Slug, Twist, N-cadherin, vimentin, fibronectin, among others. Consequently, curcumin inhibited the migration and invasiveness in these cells, irrespective of the expression of estrogen and progesterone receptors and p53 mutational status. Blockade of EMT induction with anti-cancer drugs is significant for curcumin on EMT genes and on the migratory/invasive potential of cells indicating that miR-34a plays a central role in the Curcumin-mediated suppression of EMT and invasion. Therefore, results confirm the suppressive effect of curcumin on EMT and invasion in breast cancer cells, showing that such substance exerts this effect by inducing expression of miRNA miR-34a and consequently the repression of several of its target genes. Supported by Tárapacá University, Arica, Chile (GMIC).

#1479 Differential expression of microRNAs in transformation of follicular lymphoma to diffuse large B cell lymphoma. Katerina Musilova,1 Gabriela Pavlova,1 Vaclav Seda,1 Eva Vojackova,1 Katerina Cerna,1 Veronika Svobodova,2 Robert Pytlík,2 Vit Prochazka,3 Zuzana Prouzova,3 Sarka Pospisilova,4 Lenka Zlakind,5 Marcela Gallardo,6 Siddarth Sharma,2 Jared Kevern,3 Emma Borrego-Diaz,4 Mukut Sharma2.

MicroRNAs (miRNAs) are important post-transcriptional regulators of gene expression, and are frequently aberrantly expressed in cancer. We aimed to understand the role that miRNAs play in transformation of follicular lymphoma (FL) into an aggressive diffuse large B cell lymphoma. This happens in ~3% of cases per year during the course of the disease, and is associated with median survival of only 2 years. The NGS revealed number of alterations associated with transformed FL (tFL), including frequent high-level activity of MYC (amplifications, translocations, and mutations) or loss of DNA damage regulators (p53, CDKN2A/B). Firstly, we performed a miRNA profiling (TaqMan miRNA Arrays) in paired FL and tFL samples (N=8 pairs). This revealed a relatively small group of 5 miRNAs that are consistently differentially expressed in FL (P<0.05, fold-change >1.5). Since the most frequently acquired aberration in tFL is the high-level activity of MYC we performed a correlation analysis of MYC levels and expression of these miRNAs in additional samples of FL, tFL, and CLL samples with/without MYC duplication (N=40 FL/tFL, N=39 CLL). This analysis revealed that at least one of these miRNAs is significantly down-regulated in FL samples (P<0.05) in cases with high-levels of MYC. The MYC-mediated repression of miRNA levels was also observed (P<0.05) in B cells from transgenic MYC over-expressing mice (MYC controlled by an Ig-alpha enhancer) in comparison to wild-type animals (samples obtained from young animals before occurrence of any malignancy). We have further shown that the levels of this miRNA repress B cell proliferation in vitro and its low-levels associate with percentage of Ki67 positive cells in FL samples (P<0.005). Moreover, low levels of tFL-associated miRNA were present in FL cases with a shorter overall survival (P<0.01), and its expression directly affected BCR signalling (calcium flux assay after anti-IgM). We have shown that the expression of this miRNA is not only down-modulated by high-level MYC expression, but also by B cell adhesion to stromal cells in co-culture in vitro (HS-5 stromal cells). This suggests that its normal physiological function might be related to regulation of B cell functions in the context of immune niches, and this might play a role in FL progression and transformation. It remains to be elucidated what other molecular mechanisms ensure low-level expression of the studied miRNA in cases with low-level MYC overexpression and what could not efficiently up-regulate this miRNA in FL cells. This work was supported by: the Ministry of Health of the Czech Republic, grant nr. 16-29622A. All rights reserved. contact: marel.mrz@email.cz.

#1480 Mir-182 is involved in sulindac anticancer activity in colon cancer. Hongyou Zhao, Bin Yi, Zhipin Liang, Ruixia Ma, Yaguang Xi. Louisiana State University Health Sciences Center, New Orleans, LA.

Nonsteroidal anti-inflammatory drugs (NSAIDs) display promising antineoplastic activity in many human solid tumors including colorectal cancer. Previous studies reported that sulindac sulfide (SS) can inhibit the growth of tumor cells through cyclooxygenase-2 (COX-2) dependent or independent pathways. Obviously, COX-2 independent pathway involves a low toxic property to support the clinical potential for using sulindac as a chemoprevention drug. However, the molecular mechanisms responsible for COX-2 independent pathway have not been completely elucidated. In this project, we employed two human colon cancer cell lines, HCT116 and HT29. HCT116 cells are characterized for low COX-2 expression, while HT29 cells show relatively high COX-2 expression. We found that SS could unbiasedly inhibit the growth of HCT116 and HT29 cells by arresting cells in G1/G2 phases. CyclinG2 was found to be upregulated in response to SS treatment. FOXO3a has been reported to regulate CyclinG2 expression at the transcriptional level. Our results demonstrate that SS could also upregulate FOXO3a. By using the loss-of-function strategy, we found that SS could not efficiently upregulate the expression of FOXO3a nor inhibit cell growth as it did in control cells. We gained highly consistent results in HT29 and HT29 cells with COX-2 knockdown. Therefore, our study demonstrates a pathway consisting of mir-182/ FOXO3a/ CyclinG2 as a novel mechanism responsible for SS anticancer activity in colon cancer, which may imply a COX-2 independent pathway.

#1481 Determination of microRNA profile in lung cancer cell line treated with chemotherapy cisplatin (C), pemetrexed (P) or PC with bevacizumab (B). Jo-Anne Bayley1, Rachel Ryan1, Rachel Field1, Romain Giraud1, Christopher Smith1, Siddarth Sharma2, Jared Kevern3, Emma Borrego-Diaz3, Mukut Sharma3.

1University of Kansas Med Ctr, Kansas City, KS; 2University Hospital Brno, Brno, Czech Republic; 3University of Rochester Medical Center, School of Medicine and Dentistry, Rochester, NY. All authors contributed to this work equally. This work was supported by the National Institutes of Health, grant 1R01CA138262-01, the American Foundation for AIDS Research, grant PFI125140 and the University of Kansas Cancer Center, Cancer Center Support Grant P30CA076348. Address correspondence to: Jo-Anne Bayley, 3030 Dewar Drive, Lawrence, KS 66045. Background: Cellular microRNAs (miRNAs) regulate gene expression through modulation of messenger RNA transcription and are involved in epigenetic regulation, metastasis and cancer immunity. They have prognostic and therapeutic significance. The miRNAs associated with resistance in lung cancer without EGFR mutation are not yet known. We sought to determine the profile of miRNA of lung cancer cell lines without EGFR mutation treated with chemotherapy. Methods: lung cancer cell lines HTB177 were treated with PC or P (0.05mg/ml Cisplatin, 0.05 mg/ml pemetrexed, 6.25 mg/ml Bevacizumab) for 144h. Conclusions: The treatment using PC and PC with B in lung cancer cell lines reported that a panel of miRNAs could be altered by SS treatment in both colon and breast cancer cells. In the downregulated miRNA list, we found that mir-182 could potentially target FOXO3a. By using luciferase assay, we validated the direct regulation of mir-182 on the expression of FOXO3a. When mir-182 was downregulated, SS could neither efficiently upregulate the expression of FOXO3a nor inhibit cell growth as it did in control cells. We gained highly consistent results in HT29 and HT29 cells with COX-2 knockdown. Therefore, our study demonstrates a pathway consisting of mir-182/ FOXO3a/ CyclinG2 as a novel mechanism responsible for SS anticancer activity in colon cancer, which may imply a COX-2 independent pathway.
a difference of the miRNA profile in these 2 treatment groups. miRNA 21 is upregulated in cells resistant to therapy. miRNA could be involved in the activity of chemotherapy and development of resistance.

#1482 Triptolide inhibits the growth of osteosarcoma by regulating miRNA-181a via targeting PTEN gene in vivo and in vitro. Chunming Jiang, Xiang Fang, Xiupeng Wang, Maoqiang Li, Wu Jiang, Liulong Zhu, Zhenyu Bian, Hangzhou First People’s Hospital, Nanjing Medical University, Hangzhou, China.

Objective: We aimed to study the effect of triptolide on OS and the related molecular mechanism. Methods: The cell viability, apoptosis portion, tumor size, tumor weight and invasion of OS cells were determined. The relative level of miR-181 in OS tissues and the adjacent tissues was determined by qRT-PCR. The target gene of miR-181a was determined and verified by luciferase report assay. At last, OS cells were treated with triptolide and triptolide + miR-181a mimics to verify the relationship between triptolide and miR-181a. Results: Triptolide inhibited the cell viability, promoted the apoptosis, decreased the tumor size and weight, and reduced the invasion of OS cells. The level of miR-181a in OS cells decreased significantly after treating with triptolide, and the relative level of miR-181a in OS tissues was markedly higher than that in the adjacent tissues. PTEN was reported and verified the direct target gene of miR-181a. The overexpression of miR-181a decreased the inhibition of triptolide on OS proliferation and promotion on OS apoptosis. Conclusion: Triptolide inhibited the proliferation of OS by regulating miR-181a via targeting PTEN gene in vivo and vitro.

#1483 Alteration of miR-186 expression modifies inflammatory markers in normal epithelial and prostate cancer cell models. Suman Suman,1 Dominique Z. Jones-Reed,1 M. L. Schmidt,1 Geoffrey J. Clark,1 Carolyn Klinge,1 Shirish Barve,2 Kevin S. Kimbro,2 La Creis R. Kidd1.1 University of Louisville, Louisville, KY; 2North Carolina Central University, Durham, NC.

Dysregulation of miRNAs and chronic inflammation are strongly implicated in the development of various malignancies, including prostate cancer (PCa). Previously, our lab identified several inflammatory and immune response sequence variants in CCL5 and CCR5 significantly modified PCa risk. Recently, we propose an oncogenic role for miR-186-5p based on its up-regulation in serum from PCa patients and metastatic PCa cell lines. Moreover, miR-186-5p inhibition reduced proliferation, anchorage independent growth and invasion in metastatic PC cells (PC3, MDA-PCA-2b). We hypothesize dysregulation of inflammatory and immune response markers may enhance immune surveillance leading to a reduced aggressive tumor phenotype. Following modulations in miRNA-186 levels in normal prostate epithelial (RWPE1) and metastatic PCa (PC3) cell lines, we evaluated alterations in miRNA expression using micro-array analysis. Ectopic expression of miRNA-186 in the RWPE1 resulted in a 1.3-2.6 fold down-regulation in TLR2, IRAK2, CCL20, IL1RAP, IL1RAP, IL15, and IL17RD (FDR p-value <7.8x10^-4). Whereas, inhibition of miR-186 in PC3 cells corresponded with a 1.4-fold up-regulation of IL18R1 (p =0.0095). Notably, these inflammatory markers are involved in cell invasion (TLR2), apoptosis (IL17RD), tumorigenesis (TLR2, IRAK2) and autoimmune diseases (CCL20). Validation is underway using qRT-PCR, western blotting and luciferase reporter assays. We will assess the mechanism by which these miR-186 targets suppress aggressive cancer behavior using knock-out and knock-in and ultimately animal models. Future studies may identify inflammatory targets that may guide immune therapies for the effective treatment of aggressive prostate cancer.

#1484 Genome wide study reveal microrna based methylation phenotype in oral cancer. Roshni Roy,1 Aniruddha Chatterjee,2 Navonil De Sarkar,2 Bidyut Roy1. 1Indian Statistical Institute, Kolkata, India; 2Dunedin School of Medicine, Dunedin, New Zealand; 3Fred Hutchinson Cancer Research Center, Seattle, WA.

Introduction: Oral cancer is one of the leading causes of cancer burden in India. Studies over the years have enforced the role of deregulated microRNA expression in oral cancer development and progression. Incidentally not much is known about the cause of microRNA deregulation or to what extent can it be attributed to methylation. Established genome wide microarray platforms like 450K Illumina Bead Chip have probes for very limited number of microRNAs along with restricted on distance of the probes from microRNA transcription start site. We have used genome wide methylation sequencing approach RBBs to get deeper insight on microRNA methylation. Aim: Genome wide methylation profile of 15 paired oral cancer and adjacent normal tissue was compared to generate a detailed map of microRNA methylation in oral cancer. The methylation results were further correlated to microRNA expression data generated on a subset of these samples. Methodology: RBBs based global methylation se- quencing of 15 pairs of samples was conducted on HiSeq 2500 with an average 10x depth. Analyses were done using DMAP software. Previously generated TLD based microRNA expression data was used for correlation analysis. Result: Similar to protein coding genes, microRNAs also showed methylation based clustering. Interestingly majority of methylation hits were more than 2kb upstream of mRNA transcription start site which is generally not considered in known microarrays. Distribution of hyp and hyper methylation sites is different across the various CpG features like cores and shelves as well as across chromosomes. MicroRNAs like mir-181c, miR-99b, miR-23a among others showed significant negative correlation between methylation frequency and expression level. IPA based pathway analysis emphasized repression of apoptosis and cell death pathways along with activation of cell proliferation and colony formation. Conclusion: Understanding the role of methylation in microRNA deregulation could help us detect and possibly prevent a chain of events, which lead to disrupted pathways eventually leading to cancer, at the onset.

#1485 Measuring relative utilization of aerobic glycolysis in breast cancer cells by positional isotopic discrimination. Da-Qing Yang, Margot Cleary. University of Minnesota, Austin, MN.

Cancer deaths, including breast cancer, are caused by metastasis of the malignant tumors to distant locations. However, current methods of detection cannot distinguish pre-invasive breast cancer from noninvasive breast tumor or benign breast disease. Population-wide mammographic screenings have led to increased detection of ductal carcinoma in situ or DCIS, noninvasive, proliferative cells contained by the basement membrane of the terminal ductal lobular unit. DCIS is usually not associated with metastasis and/or cancer death. Each year, in the US alone, about 1.5 million of women who have been diagnosed with DCIS or a suspicious lump/lesion by mammography will require resection or breast biopsy after diagnosis for further pathologic analysis. However, ~80-85% of biopsies result in noninvasive breast disease or benign findings. As a result, a considerable number of patients suffer from side effects caused by breast biopsy and/or overtreatment. Therefore, there is an urgent need to find a biomarker for pre-invasive breast cancer. The ability of cancer cells to produce lactate through aerobic glycolysis (the Warburg effect) is a consistent hallmark of cancer, including breast cancer. Recent advancements in liquid chromatography-mass spectrometry (LC-MS) technology have significantly improved the sensitivity of this method compared to traditional NMR or GC-MS-based technologies, which make it feasible to detect very low concentrations of small molecules or metabolites. We have recently established a positional isotopic labeling and LC-MS-based targeted metabolomics method that can directly measure the conversion from [1-13C]glucose to [3-13C]lactate through glycolysis. Our results show that metastatic breast cancer cells exhibit a dramatically increased production of [3-13C]lactate in serum samples of early stage metastatic mammary tumors developed in mice. Since elevated levels of lactate are closely correlated to increased tumor aggressiveness, these data strongly suggest that monitoring of lactate production from glycolysis by targeted metabolomics may provide a biomarker for pre-invasive breast cancer. These results will pave the way for further exploration of the elevated production of lactate as a promising biomarker for pre-invasive breast cancer and for assessment of therapeutic response in clinical trials.

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Mitochondria, Autophagy, and Cancer Metabolism

#1487 ONC201 kills breast cancer cells by inhibiting mitochondrial respiration. Yoshimi Greer,1 Samuel Gilbert,2 Celia Islam,1 Yun Ji,1 Luca Gattinoni,1 Christine Stuelten,1 Natalie Porat-Shliom,1 Roberto Weigert,1 Xiantao Wang,2 Markus Hafner,1 Kunio Nagashima,1 Donna Voeller,1 Stanley Lipkowitz,1 NCJ, Bethesda, MD; 2NIAMS, Bethesda, MD.

Background: ONC201 is a small molecule originally identified as a TRAIL inducing compound currently being tested in phase1/2 clinical trials in multiple cancer types. Two recent studies reported that ONC201 also induces an atypical stress response mediated in part by ATF4 and CHOP. Methods: ONC201 was obtained from Oncovecs, Inc. Recombinant GST-TRAIL was prepared in the laboratory. Cell viability was tested with MTS assay and CellTiter-Glo luminescent cell viability assay. ATP level was measured with CellTiter-Glo 2.0 assay.

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RNaseq and western blotting were performed to investigate change of gene expression. Mitochondrial respiration was monitored by Seahorse XF analyzer. Live cell imaging was performed to examine the mode of cell death. Confocal microscopy and electron microscopy analysis were performed to study mitochondrial morphology. Results: We tested the effects of ONC201 on 18 human breast cancer cell lines representing TNBC basal A and TNBC basal B breast cancer. ONC201 reduced cell viability in breast cancer cell lines in all subtypes tested with IC50s ranging from 0.8-5 μM, similar to what has been reported for other cancer cell types. Unexpectedly, ONC201 toxicity was not dependent on TRAIL receptors or caspases and live cell imaging revealed ONC201 induces cell membrane ballooning followed by rupture. By contrast, GST-TRAIL binds TRAIL-receptor DEVD-mediated caspase dependent classic apoptosis morphology. These results suggest that ONC201 kills breast cancer cells via a caspase-independent, TRAIL-receptor-independent mechanism distinct from TRAIL-induced apoptosis. Western blots revealed that ONC201 induces the stress pathway proteins ATF4 and CHOP, consistent with the recently published observations. ONC201 also induced phosphorylation of AMP-dependent kinase (AMPK) and depletion of cellular ATP in multiple breast cancer cell lines. Seahorse XF analysis found that ONC201 inhibited mitochondrial oxygen consumption rate but did not inhibit glycolysis as measured by the extracellular acidification rate. Both ONC201-induced toxicity and ATP depletion were enhanced when cells were cultured in non-glucose (galactose) medium. Supplementing glucose to cells grown in galactose medium partially prevented ONC201-induced ATP depletion. Reduced succinate production correlated with reduced ATP4 and CHOP, and cell death. These data are consistent with an inhibition of oxidative phosphorylation (OxPhos) by ONC201. RNaseq revealed ONC201 inhibits expression of multiple mitochondrial genes involved in OxPhos and other mitochondrial functions, and western blot confirmed those findings. Confocal and electron microscopic evaluation revealed abnormal mitochondrial morphology. Conclusion: Our data demonstrate that ONC201 can kill breast cancer cells by a novel mechanism involving disruption of mitochondrial morphology and inhibition of mitochondrial respiration.

#1488 Altered skeletal muscle mitochondrial function and redox biology with chemotherapy and exercise in a colorectal cancer mouse model. Malcolm Andrew West, Alexandrina Roman, Ronan Astin, Sian Pugh, Bernadette Fernandez, Annette Hayden, Judith Cave, Eleanor Jaynes, John N. Primrose, Sandy Jack, Michael P. Grocott, Andrew Murray, Martin Feilsch, Alexander Mirnezami, Timothy Underwood, University of Southampton, Southampton, United Kingdom; 2University College London, London, United Kingdom; 3University Hospital Southampton, Southampton, United Kingdom; 4University Hospitals Southampton, Southampton, United Kingdom; 5University of Cambridge, Cambridge, United Kingdom.

Background: Chemotherapy improves outcomes for patients with gastrointestinal cancer, although it can cause mitochondrial dysfunction, which adversely impacts fitness, invivo mitochondrial function, and cellular redox status in humans, however the mechanisms are poorly understood. Exercise interventions mitigate against this, but interactions between the effect of the cancer, chemotherapy, and exercise intervention are poorly understood. Using a colorectal cancer (CRC) mouse model we investigated changes in skeletal muscle mitochondrial function and redox biology. Methods: SCID mice (n=48) were randomized to laparotomy with (Ca) or without (NCa) orthotopic caecal injection of DLD-1 CRC cells. CRC established over 8 weeks then mice were randomized to 1) 3 cycles of intra-peritoneal (IP) chemotherapy (5FU 3mg/kg, Oxaliplatin 10mg/kg and Leucovorin 90mg/kg) followed by interval exercise-training 2) Chemotherapy and no exercise 3) IP saline sham (Sal) with exercise or 4) Sal with no exercise. Blood was collected pre and post-Chemo and at death (post-exercise), and markers of oxidative stress measured. Soleus muscle mitochondrial function was analyzed using high-resolution respirometry. Measurements included maximum OxPhos capacity (P1, P2, P3), Complex I and II capacities and the ratio of octanoyl-carnitine to pyruvate-supported respiration (FAO). Histopathological caecal examination was undertaken to determine tumour response. Results: Chemotherapy alone resulted in 37% lower OxPhos capacities in sham mice (p=0.011), whilst FAO was 40% lower (p=0.004). Cancer alone resulted in 38% lower OxPhos capacities (p=0.012). In combination with cancer, chemotherapy resulted in OxPhos capacities that were 42% lower than in mice with cancer but no chemotherapy (p=0.007), along with 51% lower Complex I (p<0.001) and 42% lower Complex II (p=0.007) capacities. In sham mice (NCaSal), exercise resulted in 33% greater Complex II activities (p=0.042), but exercise did not alter mitochondrial function in any other group. Total plasma protein content (TPPC) increased with time in the CaSal (+32%) and the NCaSal mice (+23%), whilst it decreased with chemotherapy (CaChem -20%, NCaChem -10%).

Oxidative stress increased with chemotherapy and exercise, however exploratory interaction analyses are ongoing. Complete histopathological response was seen in 2 of 6 CaChemEx animals. No other complete response was seen. Conclusion: Chemotherapy and cancer alone were associated with a loss of oxidative capacity, with a greater reduction when combined. Chemotherapy and exercise did not improve total protein content. Though we demonstrated an exercise effect in sham mice, exercise did not increase OxPhos capacity, nor did it ameliorate the effects of cancer and chemotherapy. A more intense exercise intervention may be required.


Proline dehydrogenase (PRODH) is a p53-inducible inner mitochondrial membrane flavoprotein functionally linked to electron transport for proline catabolism and intracellular ATP production, particularly under nutrient stress conditions. We have previously shown that in breast cancer cells, PRODH and glutaminase (GLS1) transcript levels are inversely correlated, supplying anaplerotic glutamate to triple-negative (GLS1) and luminal (PRODH) breast cancer subtypes by different means; and that siRNA knockdown or competitive inhibition of PRODH induces synthetic lethal interactions with both GLS1 inhibition and p53 upregulation in various malignant (ZR-75-1, DU4475, MCF7) but not normal (MCF10A) breast epithelial cells. In the present study we have synthesized and structurally modeled a novel mechanism-based irreversible (suicide) inhibitor of PRODH, N-propargylglycine (PPG), that shows more than 2-fold greater capacity to inhibit PRODH activity in isolated mitochondrial assays when compared to competitive PRODH inhibitors (L-tetrahydrofuran acid, THFA; or 5-oxo-2-tetrahydrofurancarboxylic acid, 5-oxo). Modeling human PRODH predicts a post-reactive PPG structure with PPG covalently linked to the enzyme pocket's FAD moiety, preventing catalytic activity. PPG does not occur with competitive PRODH inhibitors. Reflecting PPG’s irreversible binding to PRODH, mitochondria isolated from PPG treated ZR-75-1 cells are unable to catabolize proline despite being able to efficiently catabolize malate; in contrast, isolated mitochondria from 5-oxo treated cells remain efficient at catabolizing both proline and malate. Unexpectedly, we observed that PPG but not the competitive inhibitors induces selective degradation of mitochondrial PRODH protein levels within 24 h of cell culture treatment followed by loss of other mitochondrial proteins like complex-I NDUF1 but not by concomitant loss of cytosolic FAD-containing proteins like MTHFR. MitoTracker assays confirm the selective cellular loss of mitochondrial mass in ZR-75-1 cells within 24 h of PPG treatment. To confirm that suicide inhibition of PRODH can also induce synthetically lethal mitochondrial stress, breast cancer cells (MCF7) were cultured under normoxic (20% O2) or hypoxic (1% O2) conditions with or without chemotherapy, and exercise intervention are poorly understood. Using a colorectal cancer (CRC) mouse model we investigated changes in skeletal muscle mitochondrial function and redox biology. Methods: SCID mice (n=48) were randomized to laparotomy with (Ca) or without (NCa) orthotopic caecal injection of DLD-1 CRC cells. CRC established over 8 weeks then mice were randomized to 1) 3 cycles of intra-peritoneal (IP) chemotherapy (5FU 3mg/kg, Oxaliplatin 10mg/kg and Leucovorin 90mg/kg) followed by interval exercise-training 2) Chemotherapy and no exercise 3) IP saline sham (Sal) with exercise or 4) Sal with no exercise. Blood was collected pre and post-Chemo and at death (post-exercise), and markers of oxidative stress measured. Soleus muscle mitochondrial function was analyzed using high-resolution respirometry. Measurements included maximum OxPhos capacity (P1, P2, P3), Complex I and II capacities and the ratio of octanoyl-carnitine to pyruvate-supported respiration (FAO). Histopathological caecal examination was undertaken to determine tumour response. Results: Chemotherapy alone resulted in 37% lower OxPhos capacities in sham mice (p=0.011), whilst FAO was 40% lower (p=0.004). Cancer alone resulted in 38% lower OxPhos capacities (p=0.012). In combination with cancer, chemotherapy resulted in OxPhos capacities that were 42% lower than in mice with cancer but no chemotherapy (p=0.007), along with 51% lower Complex I (p<0.001) and 42% lower Complex II (p=0.007) capacities. In sham mice (NCaSal), exercise resulted in 33% greater Complex II activities (p=0.042), but exercise did not alter mitochondrial function in any other group. Total plasma protein content (TPPC) increased with time in the CaSal (+32%) and the NCaSal mice (+23%), whilst it decreased with chemotherapy (CaChem -20%, NCaChem -10%).

Oxidative stress increased with chemotherapy and exercise, however exploratory interaction analyses are ongoing. Complete histopathological response was seen in 2 of 6 CaChemEx animals. No other complete response was seen. Conclusion: Chemotherapy and cancer alone were associated with a loss of oxidative capacity, with a greater reduction when combined. Chemotherapy and exercise did not improve total protein content. Though we demonstrated an exercise effect in sham mice, exercise did not increase OxPhos capacity, nor did it ameliorate the effects of cancer and chemotherapy. A more intense exercise intervention may be required.

#1490 Role of sub-cellular specific reactive oxygen species in heart regeneration after cancer therapy. Salim Abdalsalam, Souparno Bhattacharya, Kalayarasan Srinivasan, Shbibi Mukherjee, Hesham A. Sadek, Aroumougame Asathamb, UT Southwestern Medical Center, Dallas, TX.

Introduction: Cardiovascular disease and cancer are the two leading causes of morbidity and mortality worldwide. As advancements in radiation therapy (RT) have significantly increased the number of cancer survivors, the risk of radiation-induced cardiovascular disease in this group is a growing concern. However, the molecular mechanism of radiation-induced heart failure is still elusive. Recently, it has been discovered that the reactive oxygen species (ROS)-mediated oxidative DNA damage is the primary upstream mechanism that prevents cardiomyocyte proliferation. Therefore, elucidating the spatial and temporal aspects of ROS production will lead to the development of countermeasures to prevent heart injury following chest radiotherapy. Methods: We have generated G516 redox potential (G516-GFP) ROS probe targeted to cytoplasm, chromatin, nucleolus, telomere, nuclear inner membrane and heterochromatin. These probes have been inserted into cardiomyocytes specific AAV9 vectors which were used to infect cardiomyocytes both in vitro and in vivo. For in vivo study, we infected three months old mice with high-titer AAV particles via tail vein injection and then exposed to chest-only radiation (5-10 Gy). At different post-
radiation times, fresh heart slices of 300–500 μm thickness were either mock- or treated with mitochondrial electron transport complex (ETC) inhibitors and then subjected to live tissue imaging using a confocal microscope. Results: Interestingly, our results showed that the distribution of basal ROS levels is not uniform in different sub-nuclear compartments. Significantly, upon the induction of oxidative stress, the ROS levels increased in all the cellular compartments, but the extent of ROS level was significantly higher in the cytoplasm. Similarly, radiation altered ROS levels in all the cellular compartments; however the effect of radiation on the ROS levels was sub-nuclear compartment-specific. Intriguingly, we found that the complex IV of the ETC was critical for the maintenance of ROS levels in different cellular compartments as compared with complexes I and II. Conclusion and Future Directions: Our data clearly indicate that the spatial and temporal levels of ROS are not uniform across the cell and the mitochondrial ETC plays a major role in regulation ROS levels in different sub-nuclear compartments. The results obtained from this study can be utilized to develop sub-cellular compartment specific targeted both genetic and pharmacological ROS scavengers that will help to regenerate adult heart by re-activating the proliferative capacity of cardiomycocytes following cancer therapy. Finally, our novel approach can be applied to assess alterations in ROS levels in different cancers. Funding: This work was supported by the NASA (NNX13AD57G/ NNX15AE06G) CPRIT (RP160520) and NIH RO1AG053341 grants.


Mitochondrial defects that affect activity of the electron transport chain (ETC) complexes are associated with several diseases including cancer. Dysfunctional mitochondria resulting from mitochondrial DNA (mtDNA) alterations or exposure to xenobiotics have been shown to initiate retrograde signaling pathway characterized by disrupted membrane potential, elevated cytosolic calcium and activation of Calcineurin, a calcium dependent phosphatase. In some cell types these events form the basis for triggering transcriptional reprogramming that converts non-tumorigenic cells to tumorigenic phenotype. Cytochrome oxidase (CcO) is the terminal enzyme of the ETC that catalyzes the transfer of electrons from reduced cytochrome C to oxygen. All the mutations of CcO subunits identified as prevalent in various cancers like prostate, pancreatic, colon and ovarian cancers have been found in mtDNA encoded subunits. Effect of loss of nuclear subunits leading to reduced Cytochrome oxidase activity on tumor progression are not well studied. Nuclear subunits IV1 and Vb of CcO are susceptible to various stress conditions like exposure to long term hypoxia, ischemia-reperfusion and treatment with ethanol resulting in selective degradaion and loss of activity. Here we report the activation of retrograde signaling by loss of these subunits. Genetic silencing of the subunits IV1 and Vb, and loss of activity resulted in metabolic shift to glycolysis and was accompanied by increased glucose utilization. Disruption of the CcO complex activated many of the hallmark factors of Ca2+/Calcineurin mediated retrograde signaling. Importantly, rescue of the CcO deficiency by overexpressing CcO subunits or by inhibitors of retrograde signaling pathway attenuated the phenotypic changes such as anchorage independent growth and increased invasive potential. Further, esophageal tumor sections from human patients revealed reduced CcO activity and inhibited the hallmark factors of Ca2+/Calcineurin mediated retrograde signaling. In this context, DNA polymerase γ (POLG1) is known to function in human mitochondria and controls the critical function of mtDNA replication and repair. POLG1 is the most frequent target of gene mutation and is involved in a variety of mitochondrial diseases. POLG1 protein consists of exonuclease, linker and polymerase domain, each with distinct functions. Mutations in exonuclease domain leads to erroneous proofreading and is responsible for increased mutations in mtDNA whereas mutations in polymerase domain leads to erroneous replication and hence depletion in mtDNA. Germline variants in these domains of POLG1 may serve as important signature markers in association studies of individuals in a population study. Through our present study, we identified germline variants, examined copy number variation, expression and regulation of POLG1 gene in human cancers. We provide evidence that altered POLG1 expression as well as germline variations in POLG1 gene contribute to tumorigenesis. Methods: A comprehensive race based bioinformatics analysis of POLG1 gene in European-American and African-American was conducted and several unique germline mutations were discovered. To analyze functional contribution of these unique germline mutations in tumor progression, these unique prevalent mutations in the evolutionary conserved regions of exonuclease and polymerase domains we generated by site directed mutagenesis and functional and tumorigenic analysis was performed. Results: We observed marked differences in copy number variation of POLG1 through cBioPortal and Cosmic databases and validated the analysis in primary tumors and cancer cell lines. Our results also indicate that mtDNA copy number in cancer cell is governed by regulation of POLG1 methylation and disproves the importance of epigenetic regulation of POLG1 gene.

We identified a mitochondrial disease causing missense variation in polymerase domain of POLG1 at amino acid 1143 (E1143G) to be 25 times more prevalent in European-Americans when compared to African-Americans population. Expression of this germline variant (E1143G) increased glucose consumption, de-
creased ATP production and increased matrigel invasion. Discussion/Conclu-
sion: We demonstrate that POLG1 is epigenetically regulated and its unique
germline variants contribute to disruption of mitochondrial function. Thus con-
tributes to differences in increased predisposition to cancer in inter-ethnic pop-
ulation.

#1496 1. Using the metabolite with metabolism in ovarian cancer. Eric
D. Shide, Lauren Amable. NIMHD, Bethesda, MD.
Ovarian cancer has historically been diagnosed late stage and lacks effec-
tive biomarkers for detection and prognosis. The deregulation of metabo-
lism and signaling cascades inherent of cancer may contribute to aberrant
levels of metal within cells. Biological processes essential for cell prolifera-
tion require metals for enzyme cofactors, signaling molecules, and structural
components. Metallomics is the study of the comprehensive, dynamic metal
profile within cells. The goal of our study was to characterize the ovarian
cancer metabolism at the level of whole cell and mitochondria in comparison
to non-cancer. Additionally, cellular metabolism was analyzed to connect
the mitochondrial (mito-)metallome to mitochondrial function. Ten human
ovarian cancer cell lines were used for experiments: A2780, CAOV3, A2780/
Three non-cancer human ovarian cell lines of epithelial and fibroblast origin
served as controls. Inductively coupled mass spectrometry (ICP-MS) was
used to evaluate the metabolome by measuring the following metals: calcium
(Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), molybde-
um (Mo), phosphorous (P), sulfur (S), selenium (Se), and zinc (Zn). An
XF24 Seahorse analyzer was used to measure cellular metabolism and mito-
chondrial function in the ovarian cancer and non-cancer cell lines. Our
results demonstrated that there was a distinct metal profile at the level of the
whole cell and mitochondria in ovarian cancer cells compared to non-cancer
ovarian cells. The whole cell ovarian cancer metabolism consisted of in-
creased Mg, P, Cu, Zn, Se, and Ca. Referencing established literature of the known metals
that serve as cofactors for mitochondrial proteins, the relationship between
the mito-metallome and functional metabolic pathways was established.
Changes in cellular metabolism were reflected by changes in the mito-met-
allome. Our study was the first to evaluate an extensive panel of metals
simultaneously in the mitochondria and bridge the unique metallic signature
of ovarian cancer to cell metabolism. Future proteomics studies will be used to
further validate the connection of metals to cellular function. Metallomics
and the ovarian cancer cell metallome provide further understanding of how
cancer deregulates the metallome and how metallomics may provide an
avenue for identifying new cancer biomarkers and allow for effective targeting
of cancer with chemotherapy.

#1497 1. BLM31510 modulates mitochondrial complex activity to influence
oxidative stress in effectuating cell death in multiple cancers. Tulip Dalani,
Katerina Krumova, Anne R. Diers, Pallavi Awate, Ryan Ng, Arleide Lee,
Stephane Gesta, Vivek K. Vishnudas, Ranagrapad Sarangarajan, Niven R.
Narain. BERG, LLC, Framingham, MA.
Dysregulation of mitochondrial metabolism plays a modified role in tumorigenesis through
regulation of energy production, biomass, redox state, and engagement of cell
death pathways. Perturbations in mitochondrial fluxes (i.e. inhibition of electron
chain complexes activity, impaired electron flow) have substantial effects on cell
viability, suggesting that targeting mitochondrial function could be effective for
therapeutic response in cancer. BLM31510, containing oxidized coenzyme Q10
dilts an anti-Warburg effect is currently in phase II clinical trials for solid
tumors. Previously, we have demonstrated the anti-cancer properties of
BLM31510 in breast and pancreatic xenograft models. Here, we examined the
mechanism of action of BLM31510 in vitro. Using a multi-cancer cell panel,
BLM31510 was shown to be consistently and selectively cytotoxic to cancer cells,
compared to normal and non-tumorigenic controls, and sensitivity did not cor-
relate to cell doubling time or mutational status. Treatment with BLM 31510
(EC50) in breast and pancreatic cancer cells resulted in a time- and dose-depen-
dent decrease in mitochondrial membrane potential which preceded an increase
in early and late apoptosis cells, suggesting BLM31510 initiates a mitochondrial
mediated cell death pathway. Using a fluorescently labeled CoQ10, we were able to
trace the subcellular location of the CoQ10, which predominantly accumu-
lates in mitochondria and lipid droplets in a time dependent manner. Addition-
ally, the mitochondrial enrichment of CoQ10 is accompanied by morphological
changes that varied amongst the different cancer cell types. As CoQ6
is a redox molecule required for electron transfer activity between complexes, we hypoth-
thesized that disruption of Q-pool homeostasis would alter complex activity. To
investigate this, Complex driven respiration was measured in cells treated with
BLM31510 and compared to untreated. Alterations in mitochondrial respira-
tion characterized by a dose-dependent decrease in succinate (Complex II) and
glycerol-3-phosphate (Complex III)-fuelled respiration were observed in cells
treated with BLM31510, while no changes were seen in pyruvate driven respira-
tion (Complex I). This is suggestive of BLM31510 specifically impairing respiration
responses that are more dependent on Q-pool functionality. As impairment of
the electron transport chain increases intracellular oxidative stress, we next in-
vestigated if BLM 31510 treatment increases ROS levels. After 24h treatment,
BLM31510 significantly increased ROS levels in treated cancer cells compared to
untreated. Furthermore, BLM31510 induced death could be in part prevented by
co-treatment with antioxidants. Together, these data demonstrates
BLM31510 has anti-cancer activity in multiple cancer cell types and define a
unique and novel functional link between mitochondrial Q-pool disruption and
the mechanism of action of BLM31510.

#1498 1. Melatonin inhibits mitochondrial transcription factor A expres-
sion in glioblastoma U87MG cell culture inducing an anti-tumorigenic ef-
fect. Daiane G. Franco, Isabele F. Moretti, Suely K. Marie. University of Sao
Paulo, Sao Paulo, Brazil.
Melatonin, the major hormone of pineal gland, has been described to
induce intrinsic apoptosis in tumor cell, while it is inhibited in non-tumor
cell. In tumor cell, melatonin stimulates intracellular production of reactive
oxygen species (ROS) by a mechanism not completely understood. Here we
propose that melatonin inhibits the expression of mitochondrial tran-
scription factor A (TFAM) in U87MG glioblastoma cell line, leading to an
increase of the production of ROS. TFAM is an important mitochondrial
protein that maintains the mitochondrial DNA (mtDNA) integrity and consequently
the oxidative phosphorylation. Human U87MG GBM cell line was treated with melatonin (1 mM)
for 72 h to evaluate the expression of TFAM and the mtDNA copy number through real-time PCR.
Proliferation, apoptosis, cell cycle and ROS production were also evaluated
by flow cytometry (Muse - Millipore Corporation) and the results were normal-
ized to control (vehicle: ethanol 0.3%). Melatonin reduced the expression of
TFAM in 17.5 % compared to the control group, but had no effect on the
mtDNA copy number. Moreover, the incubation of U87MG with melatonin
induced a reduction in cell proliferation in 14.24 % ± 4.3 compared to
control, with an arrest of cell cycle in G0/G1 phase, which increased from
67.4 ± 3.71 % to 72.78 ± 3.18 % in the group treated with melatonin com-
pared to the control. Production of ROS presented an increase of 7.1 %,
with no significant effect on apoptosis. These results suggest that TFAM maybe a
target for the melatonin function for an anti-tumorigenic effect through an
increase of ROS production, inducing a delay in the cell cycle and a reduction of cell proliferation.
The melatonin effect on TFAM maybe a novel signaling
pathway that could be explored for the control of GBM progression.

#1499 1. Role of mutations and expression change of mitochondrial func-
tion related nuclear genes in oral gingivobuccal squamous cell carcinoma.
Esita Chattopadhyay,1 Richa Singh,1 Rosni Roy,2 Bidyut Roy.1 Indian Statis-
tical Institute, Kolkata, India; 2Baylor Research Institute, Dallas, TX.
Background: Mitochondria have long been suspected to have contribution in
progression and sustenance of cancer. But mitochondria related nuclear genes
have not been well-studied in oral cancer. The aim of this study is deciphering
the impact of somatic mutations and expression deregulation of mitochondria
related nuclear genes in oral gingivobuccal squamous cell carcinoma (GBS CC).
Methods: Nuclear-encoded genes which are functionally involved with mito-
chondria were enlisted from MitoCarta 2.0 and IMPI databases. Whole exome
sequencing was performed with 12 paired cancer-normal GBSCC samples and
 somatic mutations in mitochondria related nuclear genes were extracted from
the data. Expression deregulation of mitochondria related nuclear genes were
quantified from whole transcriptome data of 12 paired cancer-normal GBSCC
samples. Expression was re-validated in another set of 12 cancer-normal paired
samples. 5 cancer-normal paired samples with whole transcriptome data were
also used for reduced representation bisulfite sequencing (RRBS). Probable im-
 pact of non-synonymous somatic mutations were predicted using SIFT and
POLYPHEN2 tools. Genes with possible damaging mutations and expression
deregulation were taken for literature search and KEGG pathway analysis to
understand their contribution in the function of mitochondria in GBSCC. Re-
sult: Total 1561 mitochondria related nuclear genes were identified from two
databases and included in the study. A total of 977 somatic mutations were
identified in 583 such genes in 12 oral GBSCC samples. These mutations were
distributed in exonic (346 mutations), intronic (288 mutations), splicing (4
mutations), UTR3' (270 mutations) and UTR5' (69 mutations) regions. Ten recur-

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Results indicate that combination of heme lowering agents works effectively in the peptide and the inhibitor had a greater effect than the peptide alone. Our conclusions on the peptide and peptide-inhibitor combination. In all the treatment groups that received the peptide and a combination of peptide and inhibitor. This trend was also observed in tumor xenografts derived from NRF2-silenced HT29 exhibited MT-CO1 reduction. Further investigation revealed that modulation of SFXN4 alters mitochondrial respiration by affecting iron-sulfur cluster biogenesis, including increased IRP-1/IRP2 binding and a decrease in IRP1/ACOT1 acitavity. Furthermore, SFXN4 knock-out decreased the activity of iron-sulfur cluster-containing enzymes such as mitochondrial aconitate and succinate dehydrogenase. Based on these observations, we postulate that SFXN4 acts as a molecular mediator that channels the excess iron present in ovarian cancer cells to iron-sulfur cluster-dependent metabolic pathways that favor growth and metastasis. SFXN4 may be a potential druggable target in ovarian cancer.

#1502 The link between NRF2 and mitochondria through the regulation of miR-181c/miRNA-encoded cytochrome c oxidase subunit-1. Mi- Kyoung Kwak, Kyeong-Ah Jung, Donghyeok Kim, Sujin Lee. The Catholic University of Korea, College of Medicine, Republic of Korea.

The nuclear factor erythroid 2-related factor 2 (NRF2) pathway is involved in the environmental resistance of cancers by enhancing the antioxidant capacity. In the current study, we investigated the potential link between NRF2 and mitochondrial function in cancer cells. Global miRNA expression analysis of H7T29 and HCT116 identified miR-181c as an NRF2-silencing-inducible miRNA, and miR-181c elevation was associated with the decrease in mitochondria-encoded cytochrome c oxidase subunit-1 (MT-CO1), a mitochondrial genome-encoded complex IV subunit of the electron transport chain. As a result of decreased complex IV activity, NRF2-silenced cancer cells exhibited reduced levels of mitochondrial membrane potential (MMP), oxygen consumption rate, and ATP production. Notably, these changes induced adaptive activation of AMP-activated protein kinase (AMPK) and thereby, NRF2-silenced cells were more vulnerable to AMPK inhibitor-induced growth suppression. Similarly, mouse xenografts derived from NRF2-silenced HT29 exhibited MT-CO1 reduction and AMPK activation, thereby increasing responsiveness to the AMPK inhibitor treatment. Collectively, we showed a novel link of NRF2 to cancer mitochondria and AMPK signaling by elucidating miR-181c/MT-CO1 signaling as an underlying molecular mechanism. These results also suggest that it may an effective strategy to inhibit both NRF2 and AMPK to control adaptive tumor responses.


Hydroxychloroquine (HCQ) is a lusotrophic autophagy inhibitor that is being used in over 45 clinical trials either alone or in combination with another chemotherapy. Pharmacokinetic (PK) and pharmacodynamic (PD) studies with HCQ have shown that drug exposure in the blood does not correlate with autophagy inhibition in either peripheral blood mononuclear cells (PBMCs) or tumor tissue (Autoptiy 10:1415). HCQ exhibits primarily pH-driven PK and has been shown, by way of heightened levels of autophagy markers, to generate a therapeutic effect longer than PK data suggests. A physiologically-based pharmacokinetic model (PBPK) was developed for HCQ to describe the tissue-specific absorption, distribution, metabolism, and excretion as well as lysosome-specific sequestration. Physiologic parameters were adapted from literature, or obtained from experimental data when necessary, and used to simulate physiologically-based PK PK following designated dosing regimen in mice and rats. Experimentally derived PK data from these species were compared to simulation generated data to drive model development and subsequently determine model accuracy, achieving statistically-similar PK predictions of blood and tissues. Through allometric scaling and species-specific parameter modifications this model can be easily adapted for accurate prediction of HCQ PK in dogs and humans, as determined by comparison with respective blood levels. The value of this model lies in its ability to simulate HCQ PK in cancer patients with tumor types deemed autophagy-dependent. Model data simulating HCQ uptake in a neutral tumor compartment (pH = 7.2) shows that peak concentration in the lysosomes, the active site of the drug, is roughly four-fold higher than the peak concentration.
#1504 Tumor-treating fields (TTFields) interfere with biological key properties of glioma cells in vitro. Manuela Silginer, Michael Weller, Roger Stupp, Patrick Roth. University Hospital Zurich, Zurich, Switzerland.

Tumor-treating fields (TTFields) are low amplitude alternating electric fields which are supposed to exert anti-tumor effects by targeting dividing tumor cells while sparing cells in the brain not undergoing cell division. Although this novel therapeutic approach has shown encouraging results in phase III trials in glioblastoma, its biological effects on tumor cells have only been poorly understood. Here, we investigated the effects of TTFields on glioma cells in vitro using the in vitro system that allows the application of TTFields to cell cultures. Exposure to TTFields potently induce autophagy and necroptosis and interfere with the migration and invasion of long-term glioma cell lines, but also of glioma-initiating. The combination of TTFields with irradiation or temozolomide (TMZ) restored viability and clonogenic survival in an additive or synergistic manner. Further studies suggest that the O6-methylguanine DNA methyltransferase (MGMT) status does not influence the efficacy of TTFields and TMZ-resistant glioma cells remain responsive to TTFields application, thus making TTFields particularly attractive for the majority of glioblastoma patients with tumors that are unlikely to benefit from TMZ treatment. In summary, these findings demonstrate that the application of TTFields may interfere with various biological key properties of glioma cells and may allow for a more detailed clinical evaluation of TTFields beyond the clinical data available so far.

#1505 Breast cancer cells treated with mitochondria targeted redox active agents induce mitophagy. Thomas Biel, Ashutosh Rao. Food and Drug Administration, Silver Spring, MD.

Triphenylphosphonium (TPP) conjugated agents induce mitochondrial dysfunction in cancer cells. However, the onset of mitophagy to remove the dysfunctional mitochondria is unknown. Here, a series of mitochondria targeted redox active agents (MTA) that contain TPP were used to investigate mitochondrial dysfunction and mitophagy in MDA-MB-231 cells as compared to MCF-12A cells. Three different MTAs were identified to induce mitochondrial depolarization and enhanced autophagic flux selectively in MDA-MB-231 cells. Mitochondrial reactive oxygen species generation and respiration indicated that MDA-MB-231 cells harbored heightened sensitivity to MTA treatments. We additionally expressing mt-mKeima MDA-MB-231 and MCF-12A cell lines, a non-cell type selective decline in mitochondrial alkalinity and altered mitochondrial morphology was detected. Furthermore, FACS analysis of mt-mKeima revealed MTA induced lysosomal dependent mitochondrial degradation in the presence of Bafınomycin, a lysosomal inhibitor. To confirm MTAs induced mitochondrial autophagy in MDA-MB-231 cells, MitoTracker Red preloaded GFP-LC3 expressing MDA-MB-231 cells were used to identify autophagosomes containing mitochondria using confocal microscopy, in addition to coimmunoprecipitation for the detection of an endogenous autophagy-related protein complex, and immunoblot to for mitochondrial PINK1 accumulation. To translate these in vitro studies to an in vivo rat SFT-2 xenograft breast cancer model, tumor mitochondrial extracts from rats treated with Mitotarget demonstrated an accumulation of mitochondrial PINK1. Collectively, these data suggest that mitochondrial agents selectively caused mitochondrial depolarization, PINK1 accumulation and mitophagy in MDA-MB-231 cancer cells as compared to MCF-12A healthy cells.

#1506 The Wnt signature of melanoma cells predicts their invasiveness, autophagy activity, and their response to pharmacologically mediated autophagy inhibition. Abhataou M.M. Ndove, Anna Budina, Amanpreet Kaur, Curtis H. Kugel, Marie Webster, Reeti Behera, Vito Rebecca, Ling Li, Meenhard Herlyn, Ravi Amaravadi, Maureen Murphy, Ashani Weeraratna. The Wistar Institute, Philadelphia, PA; The University of Pennsylvania, Philadelphia, PA.

Melanoma is the most aggressive type of skin cancer and the leading cause of death from skin cancer. As the incidence of melanoma continues to increase, it is crucial to investigate the cellular and molecular mechanisms that lead to invasion and metastasis. High autophagy has been shown to correlate with melanoma tumor aggressiveness and poor survival in patients, and is a common mechanism of resistance to therapy. Autophagy inhibition leads to reduced levels in Wnt5A in a breast cancer model, suggesting a cross-talk between Wnt5A and autophagy in cancer. Our laboratory showed that Wnt5A is a driver of invasion and metastasis in melanoma. β catenin, a canonical Wnt mediator, has been shown to negatively regulate autophagy in a colorectal cancer model. Given that Wnt5A downregulates β catenin, we hypothesized that autophagy promotes melanoma tumor aggressiveness through the regulation of Wnt signaling and that in turn the Wnt signaling status of melanoma cells affects their autophagy activity. Our results demonstrate that melanoma cells with high Wnt5A and low β catenin have higher autophagy levels compared to less aggressive melanoma cells that have low Wnt5A and high β catenin. To determine whether there is a feedback loop between Wnt signaling and autophagy, we inhibited autophagy in invasive melanoma cells using lentivirus-mediated shATG5 knockdown and evaluated the effects of autophagy inhibition on Wnt5A and β catenin expression. We found that the inhibition of autophagy results in a decrease in Wnt5A and an increase in β catenin. Our data also demonstrate that the Wnt signature of melanoma cells greatly affects their response to Ly05-mediated autophagy inhibition both in vitro and in vivo. Wnt5A decreased sensitivity to autophagy inhibition while β catenin increased sensitivity to Ly05 both in vitro and in vivo. Pre-clinical models and clinical trials demonstrate a potential of autophagy inhibition for melanoma therapy; however, the clinical effects are not consistent and do not yield significant anti-tumor effects. This study will provide a rationale for the development of therapeutic strategies that more efficiently target autophagy in aggressive melanoma by taking into account the Wnt signaling status of melanoma cells.

#1507 Fatty acid oxidation mediated autophagy regulation in triple negative breast cancer. Kwang Hwa Jung, Jun Hyoung Park, Tirupathia Sirupangi, Saajna Vithayathil, Lee-Jun Wong, Benny A. Karpiaotta. Baylor College of Medicine, Houston, TX.

Compared to hormone regulated/responsive (ER+/HER2-) breast cancer (BC), triple negative BC (TNBC) patients have a worse overall survival, a significantly shorter disease-free survival, and a shorter post-recurrence survival. Using transmission electron microscopy (cybrid) (compares different mitochondria under a common defined nuclear background)-based discovery and validation in cell lines, patient derived xenografts (PDXs) and BC clinical tissues/data, we recently published that fatty acid oxidation (FAO) is a major energy pathway in TNBC. We also showed that FAO is a major regulator of Srx activation by autophosphorylation at Srx Y419 in metastatic TNBC. Our data suggest that inhibition of FAO induce cell growth inhibition. Short-term treatment of FAO inhibitors induces both cell cycle arrest and apoptosis. We observed cell cycle arrest in G1-S phases in FAO inhibitor treated TNBC cells. Additionally, we found that caspase-3 and cleaved PARP induced in FAO inhibited cells. Short-term treatment of FAO inhibitor also showed induction of AKT pathway. Further analysis on the role of FAO in TNBC suggests that FAO is a critical player in autophagy signaling in TNBC. Long-term treatment with FAO inhibitors or knockdown of FAO rate-limiting proteins carnitine palmitoyltransferase I (CPT1) induce autophagic cell death with beclin-1 and LC3-II induction in TNBC. However, no such phenomenon observed in estrogen receptor positive (ER+/HER2-+) breast cancer cell lines. Treatment with autophagy inhibitor 3-methyladename (3-MA) abolished the FAO-induced cell growth inhibition and reduced beclin-1 and LC3-II induction. Ongoing studies using knock-down and over expression approaches focus on the role of specific pathways that are responsible for mitochondrial energy reprogramming regulated autophagy signaling in metastatic TNBC.

### MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Targeting Ablative Transcription in Cancer

#1508 MTL-CEBPα activates the transcription factor CEBPα leading to inhibition of hepatocellular cancer growth. Nagy Habib, Vikash Reebey, Xiaoyang Zhao, Jon Voutilia, Robert Habib, Fał Siettoms, Hans Huber, Kai-Wen Huang, John J. Rossi, David C. Blakey. Imperial College London, London, United Kingdom; MiNA Therapeutics Ltd, London, United Kingdom; Norgardian University of Science and Technology, Trondheim, Norway; BioTD Strategies, LLC, Lansdale, PA; National Taiwan University, Taipei, Taiwan; Beckman Research Institute of the City of Hope, Duarte, CA.
CCAT7/ enhancer binding protein alpha (CEBPA) is a transcription factor that can act as a tumor suppressor. Its expression is downregulated in a number of cancers including hepatocellular carcinoma (HCC). We have investigated the role of CEBPA in models of HCC using small activating RNAs (saRNA) to transcriptionally upregulate its expression. Three liver tumor cell lines (HepG2, Hep3B, PLCPRF5) were treated with 20nm Taf4aHFD. The transcriptional regulation of two key members of the CEBPA family, CEBPB and CEBPA, and their protein expression level were measured. The impact on cell growth was assessed by way of an SRB and WST-1 assay. To investigate the role of CEBPA in protecting cells from the activity of CEBPA, siRNAs were used to knock down CEBPA. Using qPCR and Western blot analyses, we showed that the expression of CEBPA was significantly decreased in HCC cell lines. The expression of CEBPA was found to be inversely correlated with the expression of GR. The GR antagonist RU486 blocked the inhibitory response induced by GCs and GR silencing completely abrogated the anti-proliferative effects of GCs in CEBPA-sensitive cells, confirming that GC operate through the GR in AML. Conclusion: Altogether, these findings highlight the impact of RUNX1 dosage on gene expression and GC sensitivity in AML cell lines.

#1510 Selection inhibition of acute myeloid leukemia by targeting Taf12.

Yali Xu, Joseph Milazzo, Yusuke Tarumoto, Chris Vakoc. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Recent studies have suggested that co-activators are good drug targets for cancer therapy, as exemplified by BRD4, the small molecule inhibitors of which have already entered clinical trials. The high potency of targeting BRD4 in leukemia cells mainly results from suppressing the functions of multiple lineage specific transcription factors. However, such pleiotropic effects of BRD4 on different transcription factors might be problematic, considering many of the perturbed transcription factors are equally required by normal cells. This prompted us to explore more co-activator-selective potential drug targets, aiming to identify the candidates that discretely disturb the function of oncogenic transcription factors but not the general ones. In this study, we explored the roles of one of the largest co-activator complexes TFIIID in the maintenance of acute myeloid cells. To address this, we designed a shRNA library targeting each TAF subunit of TFIIID and tested the growth effect of these shRNAs in both mouse leukemia cell line and primary bone marrow culture. We found that multiple shRNAs targeting Taf12 shows strong toxicity in leukemia cells but not normal cells in vitro. Knocking down Taf12 also inhibits leukemia progression in vivo. To gain insight into the toxicity of inhibiting Taf12 in different organs, we generated doxycycline inducible transgenic Taf12 shRNA mice, where Taf12 can be inhibited in different tissues specifically at adult stage. In consistent to our in vitro observations, normal myeloid cells are not affected by Taf12 knockdown. Instead, the lymphoid lineage cells are affected while removing doxycycline can reverse the phenotype. To our surprise, all the other tissues that have been examined and show significant Taf12 knockdown do not have any defects. At molecular level, we found that inhibition of Taf12 suppresses the transcription of core targets of Myb, but not other transcription factors, like Pu.1, Erg, Fli-1, which is in contrast to the pleiotropic effects of BRD4. Interestingly, it has been shown before that leukemia cells are hypersensitive to Myb inhibition compared to normal cells. Furthermore, similar to Taf12 mice, mice with hypomorphic level of Myb show defects in lymphoid but not myeloid cells. Taf12 is a small protein with only one histone fold domain (HFD), which can dimerize with the HFD of Taf4a within TFIIID complex. We found that the histone fold dimer of Taf12 and Taf4a can physically interact with the transactivation domain of Myb. Furthermore, we showed that the HFD of Taf4a can be utilized to target Taf12 to inhibit growth of leukemia cells. Similar to Taf12 shRNA, function of Myb is disturbed by over-expressing Taf4a HFD. More strikingly, overexpressing Taf4a HFD is sufficient to cause regression of leukemia in vivo.

#1511 AML patient clustering by super-enhancers reveals an RARA associated transcription factor signaling partner. Michael R. McKown, 1Matthew L. Eaton, 1Chris Fiore, 2Emily Lee, 1Katie Austgen, 1Darren Smith, 1M. Ryan Corces, 2Ravindra Majeti, 2Christian C. Fritz, 2Syros Pharmaceuticals, Cambridge, MA; 2Stanford University, Palo Alto, CA.

Prior studies have shown that the RARA gene is associated with a super-enhancer (SE) and has upregulated mRNA expression in a subset of AML patients. Furthermore, this has been found to confer increased sensitivity to SY-1425, a potent and selective RAR agonist. We sought to better characterize the cell state and transcription factor circuitry in these RARA-high AML cells. Clustering of 62 primary AML patient samples based on their genome wide SE maps identified six discrete clusters. RARA-high patients partitioned principally into cluster 2, and to a lesser extent 1, suggesting that RARA upregulation is associated with a specific transcription factor (TF) network and cell state. To start unraveling the TF circuitry in the RARA-high cluster, we investigated which other TFs were SE associated with clusters 1 and 2. In particular, interferon regulatory factor 8 (IRF8) was found to be strongly associated with clusters 1 and 2 by SE and mRNA expression, similar to RARA. Moreover, the expression of
both genes is correlated in primary patient samples. IRF8 is involved in androgen signaling and previous studies have shown crosstalk between androgen and retinoic acid signaling. Furthermore, aberrant IRF8 pathway signaling is implicated in AML and MCL pathogenesis. The tight clustering of RARA and IRF8 in patient subgroups defined by genome-wide enhancer maps suggests RARA and IRF8 may form an integrated transcriptional module, and our data indicate that RARA and IRF8 expression was specifically increased in CRPC cell lines. The expression of the ARV7 expressing CRPC cell line LNCaP95 demonstrated an increase in IRF8 expression and a decrease in AR expression. Mechanistically, BETi might inhibit pre-mRNA splicing of AR related targets and thereby reduce AR expression. Persistent androgen receptor signaling is common in CRPC and as such, new therapeutic strategies to target androgen receptor signaling are needed. BET bromodomain inhibitors have shown promise as a therapy to block AR signaling and CRPC growth. The GATA family of TFs contains six members in mammals, all of which bind a consensus DNA sequence (A/T)GATA(A/G) to regulate gene expression. GATA2 is the predominant family member in prostate luminal epithelial cells. In a search for TFs that control AR expression, we found that GATA2 directly promotes expression of both full-length and AR splice-variant, resulting in a strong positive correlation between GATA2 and AR expression in both PrCa cell lines and patient specimens. Conversely, GATA2 expression is repressed by androgen and AR, suggesting a negative feedback regulatory loop that, upon androgen deprivation, derepresses GATA2 to contribute to AR overexpression in CRPC. Simultaneously, GATA2 is necessary for optimal transcriptional activity of both full-length and AR splice-variants. Using ChIP-seq we found that GATA2 colocalizes with AR and Forkhead box protein A1 (FOXA1) on chromatin to enhance recruitment of steroid receptor co-activators (SRC1, SRC2, and SRC3) and formation of the transcriptional holocomplex. Lastly, we identified a GATA2 small molecule inhibitor (SML) that can selectively suppress the expression of AR splice-variants and exert potent anticancer activity against PrCa cell lines. We propose that the inhibition of GATA2 is a ‘first-in-field’ approach to target AR expression and function, including ligand-independent AR, for the treatment of CRPC.

#1511 Bromodomain and extra-terminal motif protein inhibitors (BETi) in metastatic castration resistant prostate cancer (mCRPC): A novel mechanism for regulating androgen receptor variant 7 (ARV7). Jonathan Welti,1 Adam Sharp,1 Ines Figueiredo,1 Wei Yuan,1 Daniel Nava Rodrigues,1 Veronica S. Gil,1 Eleanor Knight,1 Jian Ning,2 Jeff Francis,2 Antje Neeb,1 Gunther Boynton,1 Amanda Swain,1 Johann S. de Bono1,2.1Institute of Cancer Research and the Royal Marsden Hospital NHS Trust, Sutton, United Kingdom; 2Institute of Cancer Research, Sutton, United Kingdom.

Persistent androgen receptor (AR) signaling is key to the development and progression of metastatic castration resistant prostate cancer (mCRPC). This is in part due to expression of constitutively active AR splice variants like AR variant 7 (ARV7), conferring resistance to current anti-androgens including enzalutamide (E) and abiraterone (A). To improve the outcome for patients with mCRPC, new therapeutic strategies to overcome AR and ARV7 oncogenic signaling are urgently required. The inhibition of co-factors modulating AR signaling are currently being investigated as novel strategies to treat mCRPC. One promising candidate is BRD4, a member of the BET protein family, that binds the AR on androgen response elements and facilitates the recruitment of the transcriptional machinery. BET inhibitors (BETi) have been shown to regulate AR and ARV7 signalling, however, the exact mechanism of ARV7 regulation remains unclear. As BETi are currently being explored in clinical trials of unstratified patients with mCRPC, we investigated their potential mechanism of action in CRPC cell lines, patient derived organoids (PDOs) and a patient derived mouse xenograft (PDX). Here we demonstrate that nuclear expression of BRD4 and ARV7 increases as patients develop resistance to E and/or A and inhibition of BRD4 by BETi is sufficient to block AR and ARV7 signalling in mCRPC. Both inhibition of BRD4 by BETi and genetic knockdown of BRD4 reduced the growth of CRPC cell lines and led to down-regulation of AR and ARV7 at the mRNA and protein level. To further investigate whether BETi is sufficient to inhibit ARV7 activity in patients with mCRPC, we treated patient derived organoids (PDOs) and a mouse xenograft (PDX) grown from metastatic biopies of patients resistant to E and/or A with BETi. In this study 5 out of 10 PDOs were sensitive to BETi. Consistent with the cell culture experiments, BETi treatment of the PDX led to down-regulation of both ARV7 mRNA and protein expression. Mechanistically, BETi might inhibit pre-mRNA splicing of AR resulting in the observed decrease of ARV7 expression. However, RNAseq analysis of the ARV7 expressing CRPC cell line LNCaP95 demonstrated an increase in total splicing events including skipped exons, retained introns, mutually exclusive exons, alternative 5’ splice site and alternative 3’ splice site after BETi treatment. Despite this, focused analysis of splicing factors and spliceosome components identified a subset of eight splicing factors being down-regulated by BETi inhibition including one yet uncharacterized factor that is crucial for ARV7 expression in LNCaP95 cells. Based on our results we propose a model that BETi
mediated inhibition of this novel ARV7-mRNA splicing factor may lead to decreased splicing and subsequent expression of ARV7 at both mRNA and protein level; providing a novel approach to target ARV7 in mCRPC.


Background: Benign and malignant prostate tissues are dependent upon the activity of androgen receptor (AR). The primary function of full-length AR is as a ligand activated transcription factor to induce or repress gene expression. Many androgen-repressed genes regulate the cell cycle and proliferation. With castration, the main therapeutic approach for advanced prostate cancer (PC), these genes are believed to play a role in the initial clinical response. Current approved therapies for advanced PC and castration-resistant PC (CRPC), target the AR C-terminal ligand-binding domain (LBD), such as androgens. Recent antagonists of the AR N-terminal domain (NTD) have been described with EPI-506, the prodrug of EPI-002, now in Phase 1 clinical trials. EPI-002 binds tau-5 in activation function-1 (AF-1) of the NTD that is essential for AR transcriptional activity. As expected with an AF-1 antagonist, EPI-002 is an excellent inhibitor of androgen-induced gene expression and at blocking the transcriptional activities of truncated AR splice variants lacking LBD, such as AR-V7. EPI-002 blocks expression of genes regulated by truncated AR-V7 such as UBE2C while androgens have no effect. Here we reveal that the major difference in gene expression regulated by full-length AR between EPI-002 and androgens is their ability to down-regulate the genes that are upregulated by androgen. Methods: The androgen sensitive human prostate cancer cell line LNCaP, which expresses full-length AR, was treated with androgens (bicalutamide [BIC] and enzalutamide [ENZA]), EPI-002 and a control vehicle, with and without androgen. Gene expression was analysed using Affymetrix microarrays. Bioinformatical analysis was completed and a selection of androgen-repressed genes that were de-repressed with androgens and/or EPI-002, were selected for validation using qRT-PCR. Results: EPI-002 de-repressed known androgen-repressed genes including SULTLC3, ST7, PSAT1, TMEM140 and TNSFRSF21. EPI-002 was as effective or better than BIC or ENZA in de-repressing a subset of androgen-repressed genes. Importantly, EPI-002 failed to de-repress expression of many androgen-repressed genes that androgens down-regulated, such as SULT1C3, GPR63 and DAB1. Conclusions: EPI binds AR NTD which blocks the transcriptional activities of full-length AR and truncated AR splice variants. EPI-002 was excellent at inhibiting androgen-induced genes. However, EPI did not broadly de-repress expression of genes turned off by androgen when compared to androgen antagonists. Such differences between EPI-002, a tau-S/NTD antagonist, and C-terminal LBD antiandrogens probably reflect the complexities of the mechanisms of repression of gene expression that may involve different domains of AR.

#1517 AKT inhibition induces neuroendocrine phenotype in prostate cancer cells. RuQi Chen, Yinan Li, Xuesen Dong. University of British Columbia, Vancouver, British Columbia, Canada.

Androgen receptor (AR) signaling pathway inhibition (ARPI) is the primary treatment for the androgen-sensitive, recurrent, or metastatic prostate cancer. ARPI is effective in short term, a fatal relapse is inevitable. The ARK signaling pathway inhibition has been investigated under multiple ongoing clinical trials as a co-therapeutic target with ARPI. The AKT signaling pathway is targeted due to its role in tumorigenesis, prevalent over-activation, and reciprocal activation upon ARPI in prostate cancer. However, this novel combination therapy may in fact facilitate PCa to progress into one of the most lethal subtypes called neuroendocrine (NE) prostate cancer (NEPC). Specifically, evidence has shown that ARPI contributes to NEPC progression at least in part by down-regulating the expression of RE-1 Silencing Transcription Factor (REST). Knockdown of REST functions as a negative master regulator in neuroendocrine (NE) prostate cancer (NEPC). REST functions are dependent on p53 context. Besides having differential p53 statuses, these two cell systems also have other genetic differences that may or may not contribute to the opposing functions of ERβ. We are using an isogenic cell system that allows for expression of different combinations of ERβ and p53 that may or may not contribute to the opposing functions of ERβ. We are currently using an isogenic cell system that allows for expression of different combinations of ERβ and p53 that may or may not contribute to the opposing functions of ERβ. We are using an isogenic cell system that allows for expression of different combinations of ERβ and p53 that may or may not contribute to the opposing functions of ERβ. We are using an isogenic cell system that allows for expression of different combinations of ERβ and p53 that may or may not contribute to the opposing functions of ERβ. 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We are using an isogenic cell system that allows for expression of different combinations of ERβ and p53 that may or may not contribute to the opposing functions of ERβ.
poor prognosis (15% of all mammary tumors). Previous studies have shown that specific G-rich genomic sequences in the promoters of multiple human genes can form G-quadruplex structures, resulting in decreased transcription and gene expression. These regions are generally found within gene promoters especially of oncogenes such as c-MYC, KRAS, VEGF, BCL2 or hTERT. Most of these genes are aberrantly expressed in breast cancer. The c-MYC gene regulates a large array of genes essential for cell functions including proliferation, metabolism, differentiation, adhesion and apoptosis. We have recently shown that oligonucleotides encoding the G-quadruplex sequence of the c-MYC, VEGF or hTERT promoters down regulate expression of their respective genes and inhibit cell proliferation. We hypothesized that such oligonucleotides could be applied to breast cancer cell lines in order to inhibit gene expression and metastasis. In this study, we evaluated the effect of oligonucleotides targeted to c-MYC (Pu27 and Pu27 Palmi), KRAS (KRASp), VEGF (VEGFq), hTERT (Tert-FL) and BCL2 (BCL2q) in Breast cancer cell lines (MDA-MB-231, SKBR3, MCF7) and MCF10A (non-transformed mammary cells). The effect of each oligonucleotide on cell growth was evaluated using the MTT assay. Our results reveal that all oligos inhibit cell growth of MDA-MB-231. However, for SKBR3 and MCF7, only Tert-FL had growth inhibitory activity. The control cell line MCF10A, did not respond suggesting that the oligonucleotides affect preferentially tumor cells. The effect of the oligonucleotides on gene expression (qRT-PCR) was performed on MDA-MB-231 exposed for 3 days. The analysis of gene expression shows the downregulation of c-MYC by Pu27 and hTERT by Tert-FL suggesting a direct effect on gene expression. In addition, Pu27 downregulated hTERT and VEGF in SKBR3 cells while c-MYC control in the cell line overexpressing c-MYC and is highly enriched in cancer stem cells, we evaluate the effect of Pu27 and Tert-FL on the tumourosphere formation. The results showed a very strong inhibition of tumourosphere formation in the cells treated with Pu27. Our findings suggest that oligonucleotides which target genes such as c-MYC, hTERT or KRAS very efficiently inhibit the growth of BC cell lines in particular MDA-MB-231 TN most likely by downregulating target gene expression. In addition, we demonstrate that downregulating c-MYC expression Pu27 dramatically reduces the cancer stem cell numbers. The use of G-quadruplex forming oligonucleotides targeted to c-MYC and /or hTERT may constitute a new therapeutic strategy especially for TNBC where targeted therapy is lacking.

#1521 hTERT G-quadruplex-targeted oligonucleotides inhibit glioblastoma cell growth. Alex West. University of Louisville, Louisville, KY.

Glioblastoma is one of the most common and deadly forms of brain cancer representing roughly 75% of all brain malignancies. These tumors generally have poor prognoses and are resistant to conventional therapy. It has recently been shown that as many as 80% of all glioblastomas contain mutations in a G-rich 68 base pair region of the hTERT promoter. hTERT is the catalytic subunit of telomerase, a ribonucleoprotein enzyme responsible for lengthening the ends of chromosomes thereby conferring immortality to cells. Normally, hTERT is not expressed in somatic cells and its expression is tightly controlled in stem cells. However, hTERT is upregulated in up to 95% of human tumors and is considered a key activator of cancer progression and a sign of poor clinical outcome. Therefore, hTERT has been under investigation for the past decade as a potential therapeutic target. We have shown that the mutations in hTERT promoter occur in a G-rich region that is part of a silencer element which forms a secondary G-quadruplex structure required for function, and that these mutations destabilize the G-quadruplex structure, allowing hTERT expression. We have also demonstrated that oligonucleotides encoding the G-quadruplex forming sequence in the c-MYC promoter can stabilize the G-quadruplex structure and downregulate c-MYC expression. Therefore, we hypothesized that oligonucleotides targeted to the G-quadruplex of the hTERT promoter could downregulate this gene expression and inhibit glioblastoma cell proliferation in a similar manner. We designed several G-quadruplex-forming oligonucleotides covering the mutated sites in the hTERT promoter either in totality (68 nucleotides) or separately (25 nucleotides) to stabilize the G-quadruplex region. Two glioblastoma cell lines (A172 and U87) and one neuroblastoma cell line (CHP134) were exposed to these oligonucleotides and evaluated for growth inhibition using the MTT assay and for gene expression by qRT-PCR. All oligonucleotides tested were found to induce between 40 to 90% growth inhibition in the 3 cell lines. The cell growth inhibition was both time and dose dependent and showed effectiveness as early as 3 days suggesting that this effect is not solely due to telomere shortening. Four oligonucleotides with the most consistent efficacy in growth inhibition were evaluated for their effect on hTERT expression in the 3 cell lines at 4 day exposure and revealed that two of the G-quadruplex forming oligonucleotides significantly decreased hTERT expression compared to untreated cells. In conclusion, we have defined G-quadruplex oligonucleotides targeted to the hTERT promoter that downregulate hTERT gene expression and are effective growth inhibitors in glioblastoma cells. Our findings indicate that downregulation of hTERT with targeted oligonucleotides affects non-canonical functions of hTERT conferring an advantage to this therapeutic approach.

#1522 Targeted gene therapy - c-MYC and hTERT in the scope. Francine Rezzoug, Sheila D. Thomas, Segen Tella, Donald M. Miller. Univ. of Louisville, Louisville, KY.

In the past decade immunotherapy and kinase inhibitors have revolutionized the approach for cancer therapy, particularly for melanoma which has benefited from this new therapy in the last 5 years. Although, new treatment increase patient survival, there is no cure and treatments are often limited to a subtype of patients. Therefore there is still an urgent need for efficient therapy for melanoma. Among potential targets for cancer therapy, c-MYC and hTERT which are overexpressed in up to 85% of all cancers are the most promising. The overexpression of hTERT provides indefinite division to cancer cells. Deregluation of c-MYC affect a large array of genes involved in cell cycle, proliferation/differentiation and apoptosis. Notably, both c-MYC and hTERT genes have in their promoter area a G-rich sequence that form secondary structure called G-quadruplex. These G-quadruplex structures are part of the silencers and are required for the repression of c-MYC or hTERT transcription. Mutations in the G-rich sequences of hTERT promoter destabilize the G-quadruplex and are associated with incidence of melanoma. We have shown that oligonucleotides form G-quadruplex sequences of these genes promoter downregulated their respective gene expression and inhibit cell growth in different cell lines. We investigated the effect of targeting c-MYC or hTERT gene expression in melanoma cell lines using oligonucleotides targeted to the silencer G-quadruplex sequence. Four cell lines where investigated (SK-Mel-2, SK-Mel-3, SK-Mel-28 and A375) for the effect of Pu27 (targeting c-MYC) and Tert-FL (targeting hTERT) on cell proliferation using MTT assay. The treatment for 6 days resulted in 60% growth inhibition in the presence of Pu27 and 50% in the presence of Tert-FL for SK-Mel-2, SK-Mel-3, SK-Mel-28 while A375 was less sensitive. Gene expression evaluated by QRT-PCR showed down-regulation of c-MYC and hTERT in cell exposed to Pu27 and hTERT in cells exposed to Tert-FL. hTERT expression is regulated by c-MYC and in our experiment the down-regulation of c-MYC correlate with down-regulation of hTERT suggesting a cascade effect. In addition, SOX2 gene expression was also down-regulated by Pu27 suggesting an effect on the cancer stem cells (CSC). Our data demonstrate that oligonucleotides targeted to c-MYC and hTERT down-regulate gene expression associated with melanoma cell growth inhibition and possibly on metastasis.

#1523 The mechanism of action of BCL6 in glioblastoma. Nicole M. Jones, 1 Marie-Sophie Fabre, 1 Dinindu Sachindra Senanayake, 1 Katerina Hatzi, 2 Ari M. Melnick, 3 Melanie J. McConnell, 1 Victoria University of Wellington, Wellington, New Zealand; 2 Weill Cornell Medical College, Cornell University, New York, New York.

Glioblastoma (GBM) is the most common and most deadly brain tumor to occur in adults. Initially patients respond to radiation and chemotherapy, which primarily work by causing large amounts of DNA damage, causing apoptosis of the cells. However, this process does not happen effectively in GBM and understanding how these cells resist cell death in response to therapy is key to improving the efficacy of treatment. BCL6 is a transcription factor that stops cell death in response to DNA damage. Recent work in our lab has shown BCL6 to be present in untreated GBM tumors and up-regulated in treated GBM cells. This evidence indicates that BCL6 may be used as a mechanism of therapy resistance by GBM cells. In this study, the objective was to determine the mechanism of action of BCL6 in GBM cells using luciferase reporter assays, quantitative chromatin immunoprecipitation (ChIP) and RNA sequencing. We observed that BCL6 was transcriptionally active in GBM as shown by a reduction in luciferase activity when BCL6 was present. qChIP experiments revealed that BCL6 binding changed over time and was different with different types of DNA-damaging treatment. Preliminary analysis of our RNA sequencing data has identified a unique subset of genes which are upregulated when BCL6 is inhibited and downregulated in response to chemotherapy. These changes indicate that these genes may be regulated by BCL6 in chemotherapy treated cells. All of these results illustrate that BCL6 appears to have an active and relevant function in GBM cells, which demonstrates that BCL6 is an attractive therapeutic target in GBM.
MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Targeting Aberrant Transcription in Cancer

#1524 BCL6 modulates the TP53 and STAT pathways in glioma. Ye Chen,1 Liang Xu,1 Marina Dutra-Clarke,1 Anand Mayakonda,1 De-Chen Lin,1 Lynnette Koh,1 Yun Kien Chang,1 Edwin Sandanaraj,1 Vikas Madan,2 Henry Yang,3 Ngan Doan,4 Jonathan W. Said,5 William H. Yong,6 Markus Mûschen,7 Bing Ti Ang,8 Carol Tang,9 Joshua J. Breunig,4 Phillip Koefler,1 National University of Singapore, Singapore, Singapore;2Cedars-Sinai Medical Center, UCLA, Los Angeles, CA;3National Neuroscience Institute, Singapore, Singapore;4UCLA, Los Angeles, CA;5USCF, San Francisco, CA.

Glioblastoma multiforme (GBM) remains the most aggressive brain malignancy with little improvement in prognosis or therapy for decades. Recently, we identified BCL6, also known as ZBTB27, to be a novel oncogene in GBM. In this study, we performed IHC analysis of 133 primary human glioma specimens and 8 normal brain samples. BCL6 expression is robustly elevated in tumor samples and positively correlated with glioma pathological grade. High BCL6 expression strongly predicts a worse prognosis of GBM patients. Depletion of BCL6 in human GBM cells reduced the incorporation of BrdU, promoted the cellular senescence and inhibited the growth of human GBM cells in vivo. Next, genome-wide occupancy of BCL6 in GBM cells was characterized by ChIP-seq assay. Genomic regions centered on BCL6 peaks are co-enriched with RNA-Pol II and flanked with strong H3K27ac and H3K4me3 modifications. MYC and two long non-coding RNAs MALAT1 and NEAT1 were identified as novel BCL6 targets in GBM. Moreover, pathway enrichment analysis of BCL6 peak-associated genes reveals a significant enrichment of JAK-STAT, TP53, ERBB and MAPK pathways. We demonstrated further that BCL6 represses the TP53 pathway and promotes the JAK-STAT pathway activation in GBM cells. Together, our findings uncover potential downstream targets and provide a better understanding of BCL6 function in GBM.

#1525 Chemically induced degradation of the oncogenic transcription factor BCL6. Nina Kerres,1 Steffen Steurer,1 Stefanie Schlager,1 Gerd Bader,1 Helmut Berger,1 Sophia Blake,1 Maureen Caligiuri,1 John Engen,2 Pete Emmert,1 Thomas Gerber,3 Daniel Gerber,3 Rosanna Jacob,3 Simon Lucas,1 Moriz Mayer,1 Mischerikow Nikola,1 Rumpel Klaus,1 Schirm Dirk,1 Schnitzer Renate,1 Tilman Voss,1 Zoepfhe Andreas,1 Norbert Kraut,1 Darryl McConnell,3 Pearson Mark,1 Manfred Koegl1.

The transcription factor BCL6 is a known driver of oncogenesis in lymphoid malignancies, including diffuse large B-cell lymphoma (DLBCL). It is a DNA-binding protein that represses gene transcription through the recruitment of co-repressor proteins via its BTB domain. Disruption of the interaction of BCL6 with co-repressor proteins interferes with the physiological and oncogenic functions of BCL6. We have used structure-based drug design to develop potent compounds that block this interaction with half maximal inhibitory concentration values (IC50) below 3 nM. In addition to inhibiting co-repressor binding, a subset of the identified inhibitors also caused rapid disappearance of BCL6 protein in cells. This effect was mediated by compound-induced multi-ubiquitylation of BCL6 and degradation by the proteasome and was dependent on the presence of a functional DNA binding domain on BCL6. Compounds that induced BCL6 degradation displayed significantly stronger induction of expression of BCL6-repressed genes than compounds that merely inhibited co-repressor interaction. BCL6-degrading inhibitors had anti-proliferative effects in several DLBCL cell lines, while non-degrading BCL6 inhibitors only had minor effects on proliferation. The fact that the magnitude of effects elicited by this novel class of BCL6-degrading compounds greatly exceed that of our equipotent classical co-repressor interaction inhibitors offers exciting new opportunities for the development of BCL6-based lymphoma therapeutics.

#1526 Usp9x inhibition and depletion overcomes SOX2 dependent resistance to BRAF inhibition in melanoma. Harish Potu,1 Ann. Michigan Comp Cancer Ctr., Ann Arbor, MI.

Melanoma tumors often show limited response to BRAF/MEK/ERK pathway inhibitors currently used in melanoma treatment. One mechanism of resistance to current melanoma treatments is upregulation of SOX2, a transcription factor that is essential for tumor growth and expansion, particularly in tumors with BRAF mutation in melanoma. Targeting drugs to transcription factors has been elusive and limits treatment options. Here we report that the deubiquitinating enzyme Usp9x controls SOX2. We show that ubiquitin-specific peptidase 9, X-linked (Usp9x), a deubiquitinas (DUB) enzyme, binds SOX2 in melanoma and deubiquitinasizes SOX2 in vitro. Usp9x knockdown results in increased levels of ubiquitinated SOX2 in melanoma, is coupled with depletion of SOX2 and promotes apoptosis synergistically with vemurafenib in melanoma cell lines. Protein levels in primary melanoma tumor samples demonstrated moderately elevated Usp9x/SOX2 protein expression in metastatic melanoma compared to primary melanoma patient tumors. Further, Usp9x knockdown or inhibition with G9, a DUB inhibitor, blocked BRAF/MEK kinase inhibitor-induced induction of SOX2 and fully suppressed in vitro colony growth of BRAF-mutant melanoma cells and promoted apoptosis. We discovered a novel mechanism of targeting SOX2, transcription factor by inhibition of Usp9x. Thus, development of DUB inhibitors will add to the limited repertoire of current melanoma treatments.

#1527 BRD3 as a specific vulnerable therapeutic target in neuroblastoma. Kaat Durinck,1 Jolien Dewyn,1 Anneleen Beckers,1 Siebe Loontiens,1 Suzanne Vanhauwaert,1 Daniel Carter,2 Belamy Chung,2 Glenn Marshall,2 Katleen Depreter,1 Frank Westermann,3 Frank Speleman1, Ghent University, Ghent, Belgium;4Children's Cancer Institute Australia for Medical Research, Sydney, Australia;5DKFZ, Heidelberg, Germany.

Introduction: BET inhibitors have raised high expectations for cancer treatment given their anti-proliferative effect by inhibiting BRD4 controlled enhancer activity of highly transcribed genes such as MYCN. However, current inhibitors also target BRD2 and BRD3 which are functionally nonredundant with BRD4. In neuroblastoma only MYCN amplified tumors respond well to these drugs. Methods: We performed an integrated bioinformatics approach to scrutinize BET family genes as well as further candidate epigenetic regulators as targets for novel therapies in neuroblastoma. Results: First we performed a time-resolution expression data analysis of week 1 and 2 hyperplastic lesions and tumors derived from the TH-MYCN transgenic mouse model and confirmed dynamic regulation during tumor development for established neuroblastoma oncogenes and tumor suppressor genes. Next, we filtered within the highest upregulated genes for Cancer Gene Census (CGC) genes and identified 21 upregulated CGC genes mainly involved in chromatin remodeling and DNA repair. Finally, after further selection based on expression in CGCle and survival in neuroblastoma patients, BRD3 was identified as the top-ranked candidate. BRD3 exhibits drastic upregulation during tumor formation. Elevated BRD3 expression is the highest expressed gene in neuroblastoma cell lines upon analysis of the CCLE panel and associated with very poor prognosis. To explore the nonredundant functions of BRD3 in relation to BRD4, we performed RNA-sequencing after stable knockdown of BRD3 in neuroblastoma cell lines and compared the downstream effects on the transcriptome as well as the impact on cell viability to knockdown of BRD4 and pharmacological treatment with BET-inhibitors (IQ1, OTX015). In addition, we dissected the BRD3 protein complex by means of label-free mass spectrometry analysis to gain further insights into the BRD3 specific functions in relation to control of gene transcription and putative interaction with transcription factors such as MYCN. Current efforts are ongoing to test cooperative interaction of BRD3 versus BRD4 in dbh-MYCN driven neuroblastoma formation in zebrash as well as BRD3 and BRD4 CHIP-sequencing in neuroblastoma cells. Conclusion: We identified BRD3 as a novel candidate drug gene in neuroblastoma and will present differential transcriptional control and protein interactions of BRD3 versus BRD4. This study can open the way towards developing BRD3 specific inhibitors for neuroblastoma and other BRD3 overexpressing cancers such as T-ALL and small cell lung carcinoma.

#1528 Forecasting novel therapies by understanding the Notch3 signaling in ovarian cancer. Alejandro Villar-Prados,1 Jason Roszik,1 Avril K. Sood,1 Margaret I. Engelhardt2, UT MD Anderson Cancer Center, Houston, TX;2McGovern Medial School, Houston, TX.

Advances in cancer research have led to the rapid development of novel drug therapies designed for optimal personalized patient care. Despite this surge of treatments, there is still a challenge in accurately predicting the most effective targeted therapy for a specific cancer. Additionally, the potential of systematically identifying and repurposing currently existing therapeutics designed for one specific cancer to treat other malignancies remains understood. To address this problem, our group has developed an integrative computational therapy-forecasting algorithm. This algorithm incorporates data from The Cancer Genome Atlas (TCGA) as well as molecular and survival correlations to identify candidate anti-tumor drugs that can be re-purposed for other malignancies by targeting novel pathways. Our computational analysis predicted that bromodomain inhibitors, which inhibit bromodomain-containing proteins such as BRD4, would target the Notch3 pathway in high-grade serous ovarian carcinoma (HGSC). Upregulation of Notch3 plays a crucial role in HGSC tumorigenesis and is associated with worse patient survival. Current Notch3 targeted therapies are not effective, thus designing therapeutic strategies to target Notch3 are critical. Here, we hypothesize that inhibition of BRD4 is an effective therapeutic target in HGSC by downregulating the Notch3 pathway. Current in vitro results demonstrated that BRD4 inhibition either by using chemical inhibitors or siRNA results in a decrease in HGSC cell viability by both MTT and 2-D colony formation assays. Furthermore, inhibition of BRD4 resulted in a decrease
in both Notch3 transcription and protein levels. Given that BRD4 regulates gene transcription, we performed chromatin immunoprecipitation (ChIP) and observed that BRD4 was present in the Notch3 gene promoter. These findings suggest that by inhibiting BRD4 in HGSC, we can target Notch3 and biologically validate our initial in silico analysis. The successful completion of this project will have major implications for development of personalized therapies not only for HGSC but also for other cancers as well.

#1529 Scutellaria baicalensis targets the hypoxia-inducible factor-1α and attenuates ovarian cancer growth. Imran Hussain, 1 Sana Waheed, 1 Kashif A. Ahmad, 1 John E. Pirgo, 1 Vigar Syed, 1 1Uniformed Services Univ. of the Health Sci., Bethesda, MD; 2University of Illinois College of Medicine-Urbana Champaign, Urbana, IL; 3Northwestern Health Sciences University, Bloomington, MN. Hypoxia-inducible factor-1α (HIF-1α), a transcription factor important for maintaining cellular oxygen homeostasis, is aberrantly upregulated in many tumors and implicated in angiogenesis, metastasis and drug resistance. Therefore, developing treatments that target HIF-1α may be a viable therapeutic approach. For centuries, traditional Chinese medicine has used herbs, such as Scutellaria baicalensis (SB), as a natural remedy for tumors. However, the mechanisms by which SB inhibits its tumor growth are not known. We have previously reported that SB inhibits cancer cell proliferation and targets NF-κB in endometrial and ovarian cancers and TGF-β in endometrial cancers. In this study, we examined the effects of SB on HIF-1α protein levels in three ovarian cancer cell lines, SKOV3, OVCA 429, and OVCA 420, grown under normoxic and hypoxic conditions. When treated daily with SB, cancer cells showed a decrease in HIF-1α expression after 48 hours. Investigation into the molecular mechanisms of SB-induced inhibition revealed a significant reduction of HIF-1α accumulation in cells treated with cycloheximide, a protein synthesis inhibitor. Further decrease of HIF-1α accumulation was seen when cycloheximide was combined with SB. Additionally, SB-induced HIF-1α proteasomal degradation was prevented by treatment of cells with proteasomal inhibitor MG132 and chloroquine, lysosome inhibitor, abrogated SB-induced inhibition of HIF-1α. Activation of PEEK/Adk and MAPK/ERK is shown in cancers. SB reduced levels of phospho-AKT and phospho-extracellular signal-related kinase (ERK) in ovarian cancer cells. Furthermore, pretreatment of cells with LY294002 (phosphoinositide 3-kinase inhibitor) and PD98059 (mitogen-activated protein kinase kinase inhibitor) reduced HIF-1α expression comparable to SB-treated cells. Hypoxia-induced cisplatin resistance is a major challenge in therapy of ovarian tumors. SB reduces the proliferation and reverses cisplatin resistance in ovarian cancer cells by attenuating the expression of HIF-1α. Taken together, the findings suggest that targeting HIF-1α with SB could be an effective treatment strategy for ovarian cancer and that SB can improve the sensitivity of cells to cisplatin.

#1530 RUNX1 positively regulates ErbB2/HER2 signaling pathway through modulating the expression of SOSI in gastric cancer cells. Yoshinhe Mito,1, 2 Naoko Maeda,1, 2 Shinziro Izumi,1, 2 Kenzo Sugihara,1 Yoshinoh Oki,2 Koichi Akashi,2 Koichiro Kano,3 Hideyuki Saya1, 1Institute for Advanced Medical Research, Keio University School of Medicine, Tokyo, Japan; 2School of Pharmacy and Pharmaceutical Sciences, Hoshi University, Tokyo, Japan; 3College of Bioresource Sciences, Nihon University, Tokyo, Japan; 4Kyushu University Graduate School of Medical Science, Fukuoka, Japan. We generated induced cancer stem cells that initiate osteosarcoma (OS) by overexpression of c-MYC and loss of Ink4a/Arf in bone marrow-derived stromal cells, and found that the loss of adipogenic potential is an essential event for OS tumorigenesis. Therefore, our understanding of regulatory mechanisms of adipocyte differentiation is crucial to the development of novel therapeutic strategies for OS. Here, we show that regulation of the transcriptional coactivator MKL1 (megakaryoblastic leukemia 1) by actin cytoskeleton dynamics drives adipocyte differentiation mediated by PPARγ. Exposure of preadipocytes to an adipogenic cocktail results in rapid downregulation of stress fibers as a consequence of downregulation of Rock1 and Rock2. Exposure of preadipocytes to adipogenic cocktail results in significant suppression of RUNX1 expression in OS cells. Our findings suggest that targeting RUNX1 with SB could be an effective strategy against osteosarcoma and that SB can improve the sensitivity of cells to cisplatin.

#1532 Mithramycin induces the antiproliferative activity of chemotherapeutic agents in Ewing sarcoma cells. Anish Ray,1 Bhavani Nagarajan,2 Umesh T. Sankpal,2 Sagar Shelake,2 W. Paul Bowman,2 Andras Lacko,2 Riyaz M. Basha2. 1Cook Children’s Medical Center, Fort Worth, TX; 2University of North Texas Health Science Center, Fort Worth, TX. Ewing sarcoma (ES) is one of the most frequent primary bone and soft tissue tumors to occur in pediatric and adolescent population. ES is often diagnosed after the disease has already metastasized. With available treatment options, the prognosis of metastatic ES patients is poor and the 5-year survival rate is less than 20%. Therefore, identifying precise targeted therapies to induce therapeutic response in ES patients is urgently needed. An oncogenic fusion protein and transcription factor EWS-FLI1 is associated with more than 85% of ES tumors. Mithramycin has been identified as an effective agent to target EWS-FLI1 and is currently in clinical trials. In this study, we investigated the efficacy of Mithramycin to induce the anti-proliferative activity of chemotherapeutic agents commonly used for the treatment of this malignancy using human ES cell lines. TC71, TC32, CHLA32, CHLA10 and TC205 cells were treated with increasing concentrations of Mithramycin or Vincristine or Doxorubicin or Etoposide. Cell growth inhibition was evaluated at 24 and 48 h post-treatment using CellTiter Glo kit. Results showed a dose/time-dependent anti-proliferative effect for all agents. To confirm the effect of Mithramycin on selected cell lines, mRNA expression of downstream targets of EWS-FLI1 (ID2, LDB2, NROB1 and RCOR1 genes) was determined by quantitative PCR following treatment with Mithramycin. Mithramycin significantly down-regulated all tested genes and these results are in agreement with published work. In order to determine the combination response, cell growth inhibition of the chemotherapeutic drugs was assessed in the presence of added Mithramycin. The combination Index was evaluated by Chou-Talalay method. Further studies were performed for the combination of Mithramycin and Vincristine to investigate its effect on apoptosis and cell cycle arrest using CHLA10 and TC205 cells. Apoptotic markers such as the expression of cleaved Poly (ADP-ribose) polymerase (c-PARP) and survivin were determined by Western blot analysis. The apoptotic cell population was measured by Annexin-V staining using flow cytometry. When compared to individual agents, the combination of Mithramycin and Vincristine showed greater effect on apoptosis in both cell lines as evidenced by an increase in Apoptotic markers in the presence of Mithramycin.
was significantly decreased following HDAC inhibition, the same response was not observed in HeLa cells. These findings suggest that p53 status may be an important determinant of therapeutic response to HDAC inhibition.


Angiogenesis is a key process in tumor propagation, progression, and metastasis. This process is tightly modulated by a wide range of growth factors, receptors, cytokines, matrix metalloproteinases, and transcription factors. Distinctively, vascular endothelial growth factor (VEGF) and its receptors have been identified as prominent targets in antiangiogenesis. Therefore, inhibiting VEGF-induced tumor angiogenesis has been explored extensively for cancer therapy. In this context, F16, a novel small molecule possessing a unique molecular configuration to block VEGFR2, has been subjected to intensive preclinical investigations. The F16 molecule exhibits in vitro antiangiogenic activity via inhibiting endothelial cell proliferation, migration, and tube formation. In addition, F16 was found to significantly inhibit the in vivo angiogenesis in chick chorioallantoic membranes as well as in athymic nude mice with xenograft tumors. As a consequence of the antiangiogenic effects, F16 was able to effectitively control the tumor growth in mice with xenograft tumors.

In the present study, we aimed to comprehensively interrogate the impact of F16 treatment on the expression of a panel of angiogenesis-related genes to gain insights into the cellular mode of action of F16 on angiogenesis. We analyzed the expression of 84 genes that are known to be associated with angiogenesis in human umbilical vein endothelial cells (HUVECs). Our pilot study identified upregulation of some key genes in response to F16 treatment which include tissue inhibitor of metalloproteinase 1 (TIMP1), angiopoietin-like 4 (ANGPTL4), chondromodulin 1 (LECT1), and placenta growth factor (PIGF). On the other hand, several genes involved in promoting angiogenesis were downregulated, which include matrix metatlopeptidase 9 (MMP9), integrin subunit beta 3 (ITGB3), insulin growth factor 1 (IGF1), transforming growth factor alpha (TGFα), interleukin 6 (IL6), leptin (LEP), thrombospondin 1, 2 (THBS1, 2), tyrosine kinase (TEK), VEGF and C. The differential gene expression related to pro- and anti-angiogenic growth factors coincide very well with our previous observation of F16 inhibiting endothelial cell proliferation, migration, and tube formation. Based on the Gene Expression Profile (GEP) observed in our experiments, we speculate that F16 can induce TIMP1 in which turn could suppress the MMP9 signaling leading to the inhibition of endothelial cell migration. This disruption of TIMP1/MMP9 pathway offers an interesting foundation for functional studies that can be performed to confirm the role of F16 as a potential MMP9 inhibitor, which may provide extended benefits during the use of this drug for treating highly metastatic cancers. (This project was supported by The Royal Dames of Cancer Research Inc., Ft. Lauderdale, Florida.)

#1536 CRISPR/Cas9 biased mutagenesis in the mouse genome: Is it useful or harmful for cancer therapeutics. Ha Youn Shin, NIH, Bethesda, MD.

Advances in CRISPR/Cas9 genome editing have provided unprecedented opportunities to interrogate the functional significance of any given genomic site. While this technology is rapidly spreading and extended to with almost 4,000 PubMed entries as of today, there is a paucity of data on the extent of unexpected and undesired molecular consequences established upon targeting the mouse genome. We have introduced deletions at more than 20 genomic sites including CTCF and STAT5 transcription factor binding sites, screened over 500 founder mice and established and analyzed more than 50 lines. These data sets revealed hitherto unknown deletion patterns, unexpected large deletions using single sgRNAs and the consistent deletion of sequences bridging distant loci that were targeted simultaneously. Our findings provide guidelines for the application of CRISPR/Cas9 in the mouse germline and provide the insight into designing CRISPR-based cancer therapeutics.

#1536A Progestrone receptor regulation of interferon signaling in breast cancer. Katherine Walter, Merit Goodman, Gloria Trinca, Jade Hall, Christy Hagan. University of Kansas Medical Center, Kansas City, KS.

Breast cancer is an extremely heterogeneous disease that affects close to two million women across the globe each year. Of these breast tumors, approximately 70% expresses the progesterone receptor (PR), a nuclear receptor that is activated by progestin ligands. Compelling clinical trial data has suggested that progestins have a role in breast cancer development, independent of the widely studied estrogen receptor. The mechanism by which this occurs, however, is vastly understudied. Traditionally, PR exerts its effects following ligand activation by translocating to the nucleus and binding to DNA.
where it affects the transcription of a variety of genes involved in growth, survival and proliferation. Our lab, using microarray data combined with Gene Set Enrichment Analysis, has identified a novel subset of genes that have altered expression following PR activation. These genes are primarily involved in interferon signaling—a pathway normally utilized in response to viral infection. Our data show that many genes that are normally activated in response to interferon signaling (interferon stimulated genes, ISGs) are repressed when PR is activated by its ligand. Interestingly, this repression occurs even in the presence of interferon alpha—the activating ligand of the interferon signaling cascade. Our lab has also shown that when PR expression is transiently knocked down, ISG transcriptional repression is lost, indicating that the repression of these genes is PR dependent. In attempting to elucidate a mechanism by which PR exerts this effect, we have performed chromatin immunoprecipitation experiments following PR activation and observed PR recruitment to ISG enhancer regions. This suggests PR is potentially interfering with normal transcription of these genes. We hypothesize that PR is functioning by either blocking normal transcriptional machinery and co-activators or through the recruitment of transcriptional co-repressors to these regions. Experiments to address these models are currently underway. Evasion of the immune system has recently been added to the list of Hallmarks of Cancer and our preliminary data suggest a potential mechanism by which breast cancer is able to accomplish this. Activation of type I interferon signaling is an early step in marking tumors for immune clearance. By repressing ISG protein expression, it is possible that these tumors are able to avoid detection by host immune cells leading to tumor establishment and subsequent progression. Our preliminary data suggest that PR may be capable of aiding early breast malignancies in evading immune recognition. This novel role of PR and progesterins offers insight that could aid in improving upon established estrogen only-based therapies for prevention and treatment of hormone dependent breast cancers.

#1539 Investigating the mechanisms of tumor suppression mediated by PTEN PDZ-binding domain in a murine breast cancer model. Mingqi Yan,1 Alexander Many,2 Hong Guan,3 Penelope M. Or,1 Andrew M. Chan1.1 School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong; 2Department of Oncological Sciences, Mount Sinai School of Medicine, New York, NY; 3Department of Pediatrics, Medical College of Wisconsin, Milwaukee, WI.

PTEN (Phosphatase and tensin homolog) functions as a key negative regulator of PI3K-Akt pathway through its lipid phosphate activity. Mutations or loss of PTEN is frequently observed in brain, breast, prostate and endometrial cancer. PTEN possesses a PDZ-binding domain (PDZ-BD) at the end of its carboxy terminal. Functionally, PDZ-BD mediates PTEN’s interactions with other PDZ-domain-containing proteins, including ZO-1, PSD-95, DLG, etc, which are involved in the formation of cell junctions, post-synaptic densities, and serves as scaffolding proteins for multiple signaling complexes. However, whether the PDZ-binding domain is required to suppress tumorigenesis still remains obscure. To study the role of PTEN PDZ-BD in breast epithelial tumorigenesis, a PTEN ΔPDZ mice lacking this domain was generated. PTEN expression level and activity of PI3K-Akt pathway are not changed in the mammary epithelial cells, indicating that PDZ-BD is dispensable for PTEN stability and negative regulation of the PI3K-Akt pathway. Further, we crossed the PTEN ΔPDZ mice with the MMTV-PyMT breast cancer model and observed increased total tumor burden as well as higher tendency of lung metastasis in PTEN ΔPDZ mice. This data indicates that PTEN PDZ-BD is indeed important for tumor suppression during breast cancer progression. Previously, PTEN has been reported to govern the integrity of tight junctions and apical-basal polarity of epithelial cells, which requires PTEN’s interactions with other proteins through the PDZ-BD. However, through in vitro three-dimensional culture of primary mouse mammary epithelial cells, we failed to observe any disruption of either apical-basal polarity or tight junction. Meanwhile, we investigated the alteration of the transcriptome of breast cancer tissue in PTEN ΔPDZ mice by performing RNA-sequencing. Several enriched signaling pathways were identified by Ingenuity Pathway Analysis (IPA), which seems to be mainly associated with cell signaling as well as signaling interaction between extracellular matrix and the cancer cell. A list of genes were validated by RT-PCR, and functional validation of these genes during the tumorigenesis in our animal model is to be carried out in the near future. This work was supported by a General Research Fund grant (#160713) from the Hong Kong University Grants Committee to AC. YM was supported by a Hong Kong PhD Fellowship (PT12-13584), and a Lo Kwee-See Biomedical Research Seed Fund to AC.

#1540 Creating faithful genetic zebrafish models of pediatric high grade gliomas and MPNSTs. Felix Oppel, Ting Tao, Shuning He, Mark W. Zimmerman, Dong H. Ki, Nina Weichert, A Thomas Look. Dana-Farber Cancer Institute, Boston, MA.

Pediatric high-grade gliomas (HGGs) are the leading cause of cancer-related death in children. Despite a slight improvement of patient prognosis over the past decades pediatric HGGs remain largely incurable. Thus, new experimental models are needed to understand the mechanisms of the disease and find more effective treatment options. We previously reported a model of HGGs and malignant peripheral nerve sheath tumors (MPNSTs) which is based on the combined deficiencies in the tumor suppressor genes tp53 and nf1. However, HGG penetration is very low in this line and most fish develop MPNSTs starting at about 3 months of age. On top of the existing model we used CRISPR/Cas9 to incorporate knock-out mutations in the tumor suppressor genes arx or suz12 which are described to be involved in pediatric HGG biology. Heterozygous arx loss-of-function (lof) did not impact tumor onset or penetrance of neither HGGs nor MPNSTs. Since a total loss of arx was lethal in development, we re-injected effective arx targeting gRNAs and Cas9 mRNA into the arx+/− line to create a mosaic arx−/− genotype. Surprisingly, despite a high mutation efficiency of the remaining arx allele the re-injection strategy still did not alter tumor onset and penetrance in that model. This suggests that loss of arx is only
The tumor suppressor BAP1 promotes a developmental switch of each gene per cell. When at least 2 out of 4 alleles of either suz12a or suz12b asnf1, suz12 is duplicated in zebrafish (suz12a and suz12b) resulting in 4 alleles in vivo developmental model to study the functions of BAP1. Results and conclusions: Loss of BAP1 during embryo development results in a failure to turn off pluripotency genes such as vext1, vext2 (Xenopus orthologues of the mammal gene nanog), oct 25, oct 91 (Xenopus orthologues of the mammal gene oct3/4) and pax3, and a failure to induce lineage specification genes such as the prospective epidermis marker keratin1 and the melanocyte precursor marker sox10. This block in the shift from pluripotency to differentiation programs results in a delay in gastrulation, neural crest specification and migration, mesodermal differentiation and other phenotypes. The BAP1-deficient phenotype can be rescued by Xenopus or human wildtype BAP1 or by the histone deacetylase inhibitor SAHA (vorinostat). We conclude that BAP1 is a fundamental regulator of multiple developmental lineages, including the neural crest from pluripotency to differentiation. These obstacles led us to shift to Xenopus laevis as an in vivo developmental model to study the functions of BAP1. Results and conclusions: Loss of BAP1 during embryo development results in a failure to turn off pluripotency genes such as vext1, vext2 (Xenopus orthologues of the mammal gene nanog), oct 25, oct 91 (Xenopus orthologues of the mammal gene oct3/4) and pax3, and a failure to induce lineage specification genes such as the prospective epidermis marker keratin1 and the melanocyte precursor marker sox10. This block in the shift from pluripotency to differentiation programs results in a delay in gastrulation, neural crest specification and migration, mesodermal differentiation and other phenotypes. The BAP1-deficient phenotype can be rescued by Xenopus or human wildtype BAP1 or by the histone deacetylase inhibitor SAHA (vorinostat). We conclude that BAP1 is a fundamental regulator of multiple developmental lineages, including the neural crest from pluripotency to differentiation.

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Tumor Suppressors 1: Modeling, Metabolism, and Epigenetics

#1541 The tumor suppressor BAP1 promotes a developmental switch from pluripotency to differentiation. Jefnim N. Kuznetsov, Tristan Aguero, Stefan Kurtenbach, Matthew G. Field, Mary Lou King, Jeremy William Harbour. University of Miami Miller School of Medicine, Miami, FL.

Introduction: Our lab discovered that mutations in the tumor suppressor BAP1 are strongly associated with metastasis and death in patients with uveal melanoma. Subsequently, other cancers have been found to harbor BAP1 mutations, including ovarian melanoma, kidney, adrenal, and brain tumors. Germline BAP1 mutations are responsible for a newly described genetic cancer syndrome. Therapeutic molecules that reverse the effects of BAP1 mutations could represent a potent new treatment strategy for BAP1-mutant cancers. Unfortunately, there are several obstacles to developing such therapies. First, BAP1 is a tumor suppressor that is inactivated by mutations, such that targeted therapy would need to be directed against downstream effectors that are deregulated by BAP1 loss. Second, the effectors of BAP1 that are relevant to cancer are not known. Interestingly, most known proteins that interact with BAP1 are developmental epigenetic regulators such as Asxl1/2, Cbx1/3 and Kdm1b. Third, BAP1 is difficult to study in cultured cells because BAP1 loss results in cell cycle exit and stem cell-like behavior. These obstacles led us to shift to Xenopus laevis as an in vivo developmental model to study the functions of BAP1. Results and conclusions: Loss of BAP1 during embryo development results in a failure to turn off pluripotency genes such as vext1, vext2 (Xenopus orthologues of the mammal gene nanog), oct 25, oct 91 (Xenopus orthologues of the mammal gene oct3/4) and pax3, and a failure to induce lineage specification genes such as the prospective epidermis marker keratin1 and the melanocyte precursor marker sox10. This block in the shift from pluripotency to differentiation programs results in a delay in gastrulation, neural crest specification and migration, mesodermal differentiation and other phenotypes. The BAP1-deficient phenotype can be rescued by Xenopus or human wildtype BAP1 or by the histone deacetylase inhibitor SAHA (vorinostat). We conclude that BAP1 is a fundamental regulator of multiple developmental lineages, including the neural crest from pluripotency to differentiation.

#1542 Identifying genetic modifiers of PTEN using the Collaborative Cross mouse panel. Amanda Lanier, William Barrington, David Threadgill. Texas A&M University, College Station, TX.

Tumor suppressor genes code for proteins that limit cellular proliferation and cancer progression, and are often exploited in cancer therapies. One such tumor suppressor gene, Phosphatase and Tensin homolog deleted from Chromosome Ten (PTEN), mediates cell growth through negative regulation of phosphatidylinositol 3-kinase (PI3K) dependent pathways. PTEN is frequently deleted or mutated in a variety of human cancers. Mice carrying a PTEN transgene (Super-PTEN) mice) were found to be resistant to tumor formation, and even small changes in PTEN expression and activity have been shown to influence cancer susceptibility and progression. We hypothesized that PTEN activity can be altered by modifier genes. To determine whether PTEN modifiers are influencing its activity, we are using the Collaborative Cross (CC), a panel of inbred mouse strains that has a similar amount of genetic variation as human populations. In CC-Super-PTEN crosses, we are utilizing an easily measured phenotypic of Super-PTEN mice, reduced body weight, to identify CC strains with altered PTEN function. On average, weanlings carrying the PTEN transgene weigh 16% less than their wild type littermates, but this weight difference varies significantly across strains, from no weight reduction to as much as a 29% difference. This distribution of phenotypes has allowed us to use quantitative trait loci (QTL) mapping to identify potential modifiers of PTEN. Through association mapping, we have determined that the loci with the highest LOD scores reside on chromosomes 2, 12, 16, and X, and we are now reviewing genes within those loci to identify candidate modifiers of PTEN. Additionally, we are using several cancer models to determine the effects of said candidate genes and confirm that they alter PTEN activity and influence cancer susceptibility and progression. Ultimately, this research will demonstrate the use of the CC mouse panel to identify novel genetic modifiers and improve our understanding of PTEN regulation.

#1543 Caspase-10 suppresses tumorigenesis by targeting ATP-citrate lyase. Rajni Kumari, Sanjeev Das. National Institute of Immunology, New Delhi, India.

p53 fosters metabolic reprogramming, which restricts metabolic adaptation of tumor cells under energy stress conditions. However, modulation and directionality of underlying cascades to induce or restrict metabolism are determined by the downstream target of p53. To explore the role of p53 in tumor metabolism upon metabolic stress, we performed a microarray screen in the presence or absence of p53 under glucose starvation. We observed caspase-10 to be upregulated by p53 upon metabolic stress. Caspase-10 belongs to the class of initiator caspases and is poorly understood in terms of substrate specificities. Emerging evidence also suggest intrinsic activation of caspase-10, distinct from its homologue caspase-8. Moreover, inactivating mutations as well as downregulation of caspase-10 in several carcinomas are suggestive of its role in tumorigenesis. Thus, we investigated the function of caspase-10 as a tumor suppressor. Our results indicated that caspase-8-dependent abrogation of histone acetylation reprograms the epigenetic profile of metastatic and proliferative genes, leading to their diminished expression under metabolic stress. Concomitant suppression of both cytosolic lipogenesis and nuclear histone acetylation by caspase-10-mediated ACLY cleavage decreases proliferation capacity, migration potential and invasiveness of tumor cells. Further, studies in mouse tumor models confirmed that caspase-10 represses tumor growth under metabolic stress via the downregulation of ACLY and GCNs. Taken together, our findings establish caspase-10 as a novel integrator of metabolism and epigenetics, by regulating acetyl-CoA levels. Hence, it functions as a tumor suppressor by disrupting lipogenesis-mediated metabolic reprogramming and GCN5-mediated epigenetic reprogramming.

#1544 MTAP insufficiency promotes metastasis in lung cancer via diminishing protein dimethylation. Wen-Hsin Chang, Bing-Chung Hu, Kang-Yi Su, Jian-Wei Chen, Reen Wu, Pan-Chyr Yang, Sung-Liang Yu. National Taiwan University, College of Medicine, Taipei, Taiwan; National Chung Hsing University, Taichung, Taiwan; University of California Davis, Davis, CA.

Genome instability, metabolic switch, and activating metastasis are three of the cancer hallmarks, which always collectively contribute to cancer malignancy. We identified methylthioadenosine phosphorylase (MTAP) as a metastasis suppressor in a series isogenic lung cell lines with different invasion ability by array CGH. Using siRNA silencing and ectopic expression of MTAP, we found that MTAP not only inhibited invasion and colony forming in vitro, but also reduced metastasis and tumorigenesis in vivo. Clinically, patients with low MTAP expression associated with poor overall survival and progression-free survival. Furthermore, the metabolite analysis of clinical specimens showed that low MTAP expression leads to MTA substrate accumulation and product decrease. Low MTAP expression in MTAP knockout cells reduced the level of symmetric arginine dimethylation of proteins. By using LC/MS, we identified a novel methyl-protein, whose level is elevated in MTAP knockout cells with the depletion of symmetric arginine dimethylation. Functionally, the unmethylated protein would be protected from proteolysis via caspase 3 cleavage. Moreover, the unmethylated mutant protein promoted migration and invasion in vitro strongly than the wild-type one did. In conclusion, we identify a novel marker in low MTAP expression or MTAP knockout cells, providing a potential therapeutic candidate in lung adenocarcinoma.
MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Tumor Suppressors 1: Modeling, Metabolism, and Epigenetics

#1545 Loss of glycogen debranching enzyme (AGL) promotes rapid growth of non-small cell lung cancer cells. David R. Meier, Benjamin Weinhaus, Darby Oldenburg, Sunny Guin. Gienderen Medical Foundation, La Crosse, WI.

Glycogen Debranching Enzyme (AGL) is involved in glycogen breakdown. Compromise in glycogen breakdown due to loss of function mutations in the AGL gene lead to metabolic disease - Glycogen Storage Disease Type III. We have previously shown that loss of AGL results in rapid growth of bladder cancer cells using extensive in vitro and in vivo experiments. Here we test whether AGL regulate growth of non-small cell lung cancer (NSCLC) cells. We show that loss of AGL promotes rapid growth of NSCLC cells using anchorage independent growth assay. Loss of AGL also resulted in rapid xenograft growth of NSCLC cells when injected subcutaneously in immunocompromised mice. Whereas overexpression of AGL reduce the growth of these NSCLC cells. Further loss of glycogen phosphorylase the other enzyme involved in glycogen breakdown does not promote aggressive growth of these cancer cells. This observation is similar to our findings in bladder cancer which confirms that AGL regulates tumor growth independent of its role in glycogen metabolism. Thus using in vitro and in vivo experiments we show that AGL can regulate the growth of multiple tumor types such as bladder cancer and NSCLC. The exact mechanism on how AGL regulates NSCLC growth needs further investigation.


The association between ribosome defects and cancer became clear with the recent discovery of somatic mutations in ribosomal protein genes in several cancers, such as lymphoid leukemia's and glioblastoma (Nat Genet. 2013 Feb;45(2):186-90 & Blood. 2016 Feb 25;127(8):1007-16 & Nature. 2014 Jan 23;505(7484):495-501). To further delineate the role of ribosomal proteins in human cancer, we systematically screened the TCGA database for genetic lesions in ribosomal protein genes, confirmed the contributing capacity of the most common somatic ribosomal protein defect in cancer pathogenesis by molecular interference, and explored the mode of action of this ribosomal protein defect. Dynamic analysis of TCGA mutation and copy number data of respectively 4926 and 7322 patients representing 16 cancer types for defects in all 81 ribosomal protein genes. After stringent filtering, six ribosomal protein encoding genes (RPL5, RPL11, RPL23A, RPS5, RPS20 and RPSA) were significantly altered and identified as candidate cancer driver genes. RPL5 was located at a significant peak of heterozygous deletion on chromosome 1p22 and showed significant mutations and deletions in 11% of glioblastoma (GBM), 28% of melanoma, 34% of breast cancer (BRCA), and in 20% of multiple myeloma cases, thereby supporting heterozygous RPL5 inactivation to be the most common somatic ribosomal protein defect in human cancer. Human specific RPL5 doxy-cycline inducible knockdown (sh-hRPL5) in TP53 WT and TP53 mutant human breast cancer cell lines (MCF7 and MDA-MB-231) and in a TP53 mutant human glioblastoma cell line (U-118 MG) proved that RPL5 knockdown accelerated in vivo tumor progression in NSG mice. This acceleration was associated with reduced phosphorylation of CDK1 and, which is required for cell cycle progression from G2 to mitosis. Whereas RPL5 has been implicated in TP53 and MYC regulation, no consistent effects of RPL5 downregulation on these proteins could be detected in TP53 WT and TP53 homozygous R280K mutant breast cancer models (sh-hRPL5). Interestingly, partial RPL5 inactivation was found to be associated with PTEN protein suppression in these tumors. This observation was confirmed in various cell models in normal mouse neural stem/progenitor cells and mouse bone marrow cells (RPL5 shRNA: sh-hRPL5). Also in isogenic leukemia cell lines with different karyotypes, including lymphoid leukemia, the association of RPL5 inactivation with increased risk of leukemia progression was confirmed (sh-hRPL5). RPL5 downregulation also accelerated tumor formation in the PTEN-deficient GBM cell line U-118 MG, underscoring undefined PTEN-independent mechanisms of RPL5. Overall, we identified RPL5 as a new tumor suppressor that shows heterozygous inactivation in 11-34% of multiple histological cancer types. Partial molecular inactivation of RPL5 supported a tumor suppressor function for RPL5 in accelerating breast cancer and glioblastoma progression in vivo, which may be, in part, due to a new function of RPL5 in regulating PTEN.

#1547 BCNCIPβ modulates the ribosomal and extraribosomal function of S7 through a direct interaction. Qian Ba, Hui Wang. Institute for Nutritional Sciences, SIBS, CAS, Shanghai, China.

Extraribosomal functions of ribosomal proteins (RPs) have gained much attention for their implications in tumorigenesis and progression. However, the regulations of transition between the ribosomal and extraribosomal functions of RPs are rarely reported. Herein, we identified a novel ribosomal protein S7-interacting partner, BCCIPβ, which could modulate the functional conversion of S7. Through the N-terminal acidic domain, BCCIPβ interacted with the central basic region in S7 and regulated the extraribosomal distribution of S7. BCCIPβ deficiency abrogated the ribosomal accumulation but enhanced the ribosomal-free location of S7. This translocation further impaired protein synthesis in ribosome-deficient ribosomes. Consistently, RPL5-suppressed the ribosomal function and launched the extraribosomal function of S7, and eventually resulted in cell proliferation restriction. In conclusion, BCCIPβ, as a novel S7 modulator, contributes to the delicate regulation of ribosomal and extraribosomal functions of S7 and has implications in cell growth and tumor development.

#1548 Down-regulation of 4-hydroxyphenylpyruvate dioxygenase (HPD) contributes to the pathogenesis of hepatocellular carcinoma (HCC) through ERK / BCL-2 signalling activation. Man Tong,1 Tin Lok Wong,1 Steve Tin-Chi Luk,1 Noelia Che,1 Kai Yau Wong,1 Tsun Ming Fung,1 Xin-Yuan Guan,1 Nikki P Lee,1 Yen-Fei Yuan,1 Terence K Lee,1 Stephanie Ma1,2.1 The University of Hong Kong, Hong Kong; 2State Key Laboratory of Oncology in Southern China, Sun Yat-Sen University Cancer Center, Guangzhou, China; 3The Hong Kong Polytechnic University, Hong Kong.

Hepatocellular carcinoma (HCC) is one of the most prevalent malignancies in the world. Metastasis, recurrence and therapy resistance remain major obstacles to the improvement of long-term survival and represent major causative factors contributing to the rising mortality rates and poor prognosis of HCC. Identification of key drivers important for clinical prognostic utility and correlation of mechanisms involved in hepatocarcinogenesis is urgently needed to aid in the development of novel treatment modalities. To this end, we began our study with an analysis of a publicly gene expression dataset (GSE14520) comprising of transcriptomic profiles from a large cohort of human non-tumor liver and HCC clinical samples in hope to establish a prognostic gene signature associated with metastatic risk / recurrence status so to identify novel driver genes responsible for HCC development and progression. By this method, 4-hydroxyphenylpyruvate dioxygenase (HPD) was identified as a candidate tumor suppressor gene in HCC. HPD was found to be frequently down-regulated in primary HCC tumors as compared to peri-tumor liver tissues. Its expression negatively correlated with aggressive HCC pathological features, including tumor stage, metastasis, recurrence and survival. Notably, down-regulated HPD expression in HCC is in part a result of hypermethylation at the HPD promoter. The functional effect of HPD was then examined in HCC cells well and with or without HPD stably repressed or ectopically overexpressed. Upon knockdown of HPD, HCC cells displayed significantly enhanced abilities to form tumors, metastasize and confer sorafenib resistance in vitro and in vivo. Conversely, overexpression of HPD in HCC cells led to a growth-suppressing effect. Consistently, sorafenib-resistant HCC patient derived xenografts also displayed attenuated levels of HPD as compared with parental sorafenib-sensitive counterparts. Mechanistically, HPD down-regulation mediates aggressive cancer features in HCC through activation of an ERK and BCL-2 pro-survival signaling pathway, as evidenced by rescue experiments involving the ERK inhibitor U0126. Collectively, our findings strongly implicate HPD as a potential novel tumor suppressor in hepatocellular carcinoma that promotes tumorigenicity, metastasis and sorafenib resistance through ERK / BCL-2 signaling activation and that HPD may represent a novel prognostic biomarker for HCC.

#1549 Prognostic significance and tumor suppressive functions of SDCT2 in renal cell carcinoma. Andre R. Jordan,1 Martin Hennig,2 Daley S. Morera,3 Soun D. Lokeswar,4 Asif Talukder,3 Lokeswar Vinata 3,4.1 University of Miami, Miami, FL; 2University of Lübeck, Lübeck, Germany; 3Augusta University, Augusta, GA.

Introduction: Five-year survival of metastatic renal cell carcinoma (mRCC) patients is < 10% and African American (AA) males have the highest incidence. Identification of the molecular determinants of mRCC and racial disparity in RCC is critical for biomarker development and targeted therapy. SDCT2 is expressed in kidney epithelial cells and is a succinate and citrate transporter, but its role has not been examined in any benign diseases or cancer. We examined SDCT2 expression in normal and RCC tissues and correlated it with clinical outcome and racial disparity. We also evaluated the biological functions and molecular signaling regulated by SDCT2 in RCC cells. Methods: Differential gene expression in the matched normal and RCC tissues (n=6/category) was evaluated by microarray analysis; results were validated by quantitative-PCR and immunoblotting in normal and RCC tissues from 53 patients (White = 21; Hispanic = 19; AA = 13). VHL+ and VHL- RCC cells were stably transduced with a Flag-tagged SDCT2 construct. Transfectants were characterized for cell
proliferation, cell cycle, motility, succinate/citrate transport and reactive oxygen species (ROS) measurement assays under normoxia and hypoxia (1% O2); cell death and senescence pathway markers were also evaluated. SDCT2 induction was evaluated following 5-azacitidine plus Trichostatin A treatment Results: SDCT2 was 63- and 100-fold downregulated in low- and high-stage RCC tissues, respectively. Q-PCR validation showed that SDCT2 levels were 40-fold downregulated in tumor tissues when compared to normal kidney (P<0.0001 Mann-Whitney test). Downregulation was 40-fold in White and Hispanic patients, but 198-fold in AA patients (P=0.0049) and also correlated with tumor stage and metastasis (P=0.009). Under hypoxia, SDCT2 expression caused over 3-fold inhibition of proliferation, cell-cycle (G1-S block), and cell motility in both VHL+ and VHL- cells (P<0.01), only VHL+ cells were inhibited under normoxia. SDCT2 expression induced ROS levels and succinate transport by 3-fold in RCC cells (P<0.01). SDCT2 expression induced the p16INK4a-RB pathway and apoptosis (caspase-3 and PARP activation). 5-AZA+TSA treatment caused a 50-fold induction (P<0.0001) of SDCT2 expression. Conclusion: This is the first study on a functional biomarker in RCC. SDCT2, that is a possible novel tumor suppressor gene. SDCT2 loss promotes RCC growth, survival and inhibits cellular senescence and its downregulation correlates with metastasis and racial disparity. Support: Grant NICI/NIH 5R01CA72821; 5R01CA176691.

Molecular and Cellular Biology / Genetics: Tumor Suppressors 1: Modeling, Metabolism, and Epigenetics

#1550 Epigenetic downregulation and growth inhibition of IGFBP7 in gastric cancer. Jin Kim,1 Min A Kim,2 Sun-Ju Byeon,3 Woo Ho Kim.4 Cancer Research Institute, Seoul National University College of Medicine, Seoul, Republic of Korea; 2Seoul National University Hospital, Seoul, Republic of Korea; 3Asan Medical Center, Seoul, Republic of Korea; 4Seoul National University College of Medicine, Seoul, Republic of Korea.

Insulin-like growth factor-binding protein 7 (IGFBP7) has been found to be a tumor suppressor in several human cancers, but the role of IGFBP7 in gastric cancer has not yet been fully investigated. In this study, we examined the epigenetic downregulation of IGFBP7 expression in gastric cancer. Analysis by qRT-PCR, western blot, and methylation specific-PCR revealed that DNA methylation was inversely correlated with IGFBP7 expression, and the expression of IGFBP7 was restored after treatment with 5-aza-2’-deoxycytidine. Immunohistochemistry showed that IGFBP7 expression was downregulated in 47.5% of advanced gastric cancer patients. Univariate and multivariate analysis showed that IGFBP7 expression and tumor stage were independent prognostic factors. In cell culture, IGFBP7 knockdown increased gastric cancer cell growth, invasion, and migration, whereas IGFBP7 overexpression in gastric cancer cells showed cell growth inhibition and apoptosis. Our data suggest that IGFBP7 functions as a tumor suppressor in gastric cancer via an epigenetic pathway.

#1551 GULP1 is an epigenetically altered and functional tumor suppressor in uterine carcinoma through regulation of Nrf2-Keap1 signaling axis. Masamichi Hayashi, Elisa Guida, Rachel Goldberg, Yoshikui Inokawa, Leonardo Reis, Akira Oki, Evgeny Izumchenko, Luigi Marzochini, Mariana Brait, Trinity Bivalacqua, Alexander Baras, George J. Netto, Wayne Koch, David Sidransky, Mohammad O. Hoque. The Johns Hopkins University School of Medicine, Baltimore, MD.

We identified GULP1 as a novel tumor suppressor gene (TSG) selectively silenced during uterine carcinoma (UC) progression through promoter hypermethylation (PH) analyzed by quantitative methylation specific PCR (QMSp) and novel methylation specific digital droplet PCR (ddPCR) assay. Numerous cell based assays revealed that GULP1 silencing confers growth advantage to tumor cells. Correspondently, in vivo tumorigenicity after xenotransplantation of GULP1 knockdown T24 cells was significantly higher than control cells. Further mechanistic analysis revealed that GULP1 has a crucial role in the regulation of Nrf2-Keap1 axis, maintaining actin cytoskeleton architecture and helping Keap1 to scaffold Nrf2 in the cytoplasm. Moreover, GULP1 silencing induces activation of Nrf2 target genes, representing chemoresistance of UCs. Additionally, we analyzed GULP1 PH and expression in cisplatin-based therapy responsive and resistant primary UC samples, and in isograft cisplatin sensitive and resistant T24 cell lines. Interestingly, GULP1 expression at transcription level was lower in both resistant primary UC samples and resistant isograft T24 cell line. Cell lines with lower expressions of GULP1 (SW780 and UM-UC-3) also showed higher resistance to cisplatin than those with higher expression (T24 and BFTC905). Altogether, our findings determined that GULP1 is an epigenetically silenced potential TSG in UC and GULP1 expression and/or PH may guide in selecting candidate patients for cisplatin based neoadjuvant therapy.

Bioinformatics and Systems Biology: Functional and Genomic Analysis of Cancer

#1552 Integrative bioinformatics analyses of ChIP seq and RNA seq data reveals a complex regulatory landscape of NRF1 network involved in the pathogenesis of breast cancer. Jairo Ramos, Deodutta Roy. Florida International University, Miami, FL.

The expression of redox sensitive transcription factor -nuclear respiratory factor 1 (NRF1) expression significantly correlates with histological grades and prognosis of breast cancer. However, the molecular mechanism by which NRF1 may contribute in the development of breast cancer is not clear. In this study we examined whether NRF1 is a molecular risk factor of breast cancer. We identified regulatory landscape of NRF1 network involved in the pathogenesis of breast cancer. Steps followed for the investigation included the selection of mRNAs microarray dataset from TCGA and Metabric, selection and analysis of ChIP-Seq datasets from different breast cancer cell lines to identify NRF1 target genes possibly involved in breast cancer through Molecular Pathway, Gene Ontology and statistical analysis. Analysis of mRNA expression in breast cancer patients showed that NRF1 was significantly overexpressed in breast cancer tissues compared to normal tissues. ChiP-Seq analysis of two different breast cancer cell lines (MCF7 and T47D) showed that there are multiple NRF1 binding sites, which contain NRF1 binding DNA motif(s). The NRF1 binding to ChiP DNA sequences of target gene is cell context dependent. High percentage of known breast cancer (~50%) susceptibility genes are transcriptional targets of NRF1. Network analysis revealed that NRF1 regulates genes, which are key regulators of epithelial-mesenchymal transition (EMT), stemness, cell apoptosis, cell cycle regulation, chromosomal integrity, and DNA damage and repair. Furthermore, we observed that NRF1 regulates target genes of both breast cancer and cancer KEGG pathways. Many of the MicroRNAs involved in cancer and genes of signaling pathways involved in cancer initiation and progression such as MAPK, PI3K/AKT and Notch signaling are also transcriptional targets of NRF1. In summary, this study reveals a complex regulatory landscape of NRF1 network involved in the pathogenesis of breast cancer. Dysregulation of NRF1 signaling pathways may contribute in the development of breast cancer. Clinical confirmation of our study will have significant impact on our understanding of the role of NRF1 as a valuable biomarker for breast cancer diagnosis and prognosis and will provide strong rationale for the future studies to further develop NRF1 for breast cancer therapeutic target. This work was in part supported by a VA MERIT Review (VA BX001463) grant to DR.

#1553 Delineating the role of multiple copies of RNA binding domains in human nucleolin and its homologs using a computational approach. Kamrun Begum, Rachucha Chaya Steinhberg, Anjana Saxena, Sheneen Singh. CUNY Brooklyn College, Brooklyn, NY.

All eukaryotes have nucleolus, the sub-nuclear compartments where ribosomes synthesis occur. Nucleolin (NCL), an abundant RNA binding phosphoprotein, constitutes 5-10% of total nucleolar proteins. This multifunctional protein plays defined role/s in many critical cellular processes e.g. chromatin remodeling, ribosome biogenesis, transcriptional and translational regulation of various non-coding as well as coding RNAs. NCL binds to its target RNAs via two or multiple RNA binding domains (RBDs) to control gene expression during normal cell cycle as well as during cellular response to stress. A variety of tumors express elevated levels of NCL where NCL plays a direct role/s in increasing expressions of genes involved in cell survival, angiogenesis and metastasis. A comparison of NCL in various organisms shows that it is highly conserved, especially in its RNA binding domains that can vary in number (2 to 4 consecutive domains). Interestingly, the individual NCL-RBDs are more conserved across different species rather than within the same protein. RBDs are known to interact in pairs with RNA via RNA recognition motifs called RNP motifs, and complementary nucleic acid motifs or elements in RNA allow interaction with NCL. Vertebrate NCL proteins contains four RBDs, however most studies have focused only on the role/s of RDB 1,2; none probing the possible functional redundancy of the additional RBDs nor identifying any target-specificity for the RBD 3,4 in human NCL and other homologs. Earlier data from our lab suggested B3,4 can bind RNA in a structurally analogous manner to RBD 1,2 albeit with altered efficacy. Here, we continue this research by investigating various homologs of human NCL to better understand the conserved mechanism of the interaction with RNA and whether the additional RBDs, are in fact, redundant in function. To test this hypothesis, RBDs from various homologs were modeled using template based methods and analyzed by a combination of sequence and structure analysis tools. We show that there are key residue differences in indi-
Development of a deconvolution algorithm for tissue-based gene expression data. Sha Cao,1 Chi Zhang,2 Ying Xu2.1 University of Georgia, Athens, GA; 2Indiana University, Indianapolis, IN.

Tissue data provide substantially more information than cell-line data, and offer new opportunities to study cancer biology and evolution in its actual microenvironment, when multiple tissue samples of the same cancer type are analyzed together. However, it is very challenging to do information discovery from tissue data because of their compositional complexity - each dataset represents a mixture of gene-expression data from multiple cell types. Hence, meaningful tissue-data analyses require to first sort out the detailed contributions to the observed tissue-level data by different cell types. However, the computational challenge in solving the tissue data deconvolution problem stems from the reality: each cell type has a very large number of complex relations among its expressed genes and pathways, which are preserved under different conditions. To make deconvolution results meaningful, the co-expressions among functionally closely related genes, must be captured and enforced in a deconvolution problem formulation. We have a fundamentally novel formulation of the tissue-data deconvolution problem, which is pathway instead of gene based. It preserves co-expressions of genes of the same pathways through capturing and using an expression signature among such genes, and allows differential expressions of pathways by giving them varying weights in different tissues. In addition, this pathway-based model substantially reduces the number of free variables compared to gene-based models, making our problem formulation efficiently solvable. The unique ideas of our approach are: (i) estimate the proportion of each cell type within a mixture based on expression patterns of cell-type specific genes; (ii) identify co-expressed gene clusters among genes encoding each pathway (as defined by REACTOME) in each cell type based on cell line data; (iii) derive a condition-invariant expression signature for each of the ~2,000 REACTOME pathways in each cell type; (iv) demonstrate that each set of cell line gene-expression data can be uniquely represented as a weighted sum of such signatures; and (v) formulate the deconvolution problem as to estimate the weight of each pathway in each cell type that minimizes the Frobenius norm of deviation matrix of estimated and observed gene expression matrixes. We demonstrated its effectiveness on simulated data, i.e., in siRNA mixtures of gene-expression data from different cell types with varying proportions of each cell type. We anticipate that the successful development and deployment of the planned deconvolution method will enable and inspire a wide range of new ways to study tissue-based expression data and uncover the very rich information hidden in cancer tissues about the fundamental biology of cancer.

Pan-cancer patterns of synthetic lethality: statistical modeling of gene dependency profiles. Huwate Yeerna,1 Ramya Rangan,2 Andrew Aguirre,3 William Kim,1 Francisca Vazquez,3 Barbara Weir4, Mahmoud Ghahdi4, Aviad Tsherniak4, Jesse Boehm5, William Hahn6, Jill Mesirov1, Pablo Tamayo1,1 University of California, San Diego, La Jolla, CA; 2Harvard University, Cambridge, MA; 3Broad Institute, Cambridge, MA.

We present a methodology to fit statistical models of cell-utility profiles from RNAi-gene knockdowns. This method allows us to classify genes according to the degree of skewness in their viability distributions. The set of genes with the highest degree of skewness is highly enriched with many known oncogenes and tumor suppressors. We characterize many of these genes, compare them against the results of large sequencing efforts, and use them as inputs to a matrix-decomposition procedure that identifies the most salient cell viabilities shared by different cancer types. We catalog these pan-cancer patterns of synthetic lethality and characterize them by the genomic, transcriptional, and phenotypic features. This analysis provides a rich catalog of the most salient Achilles Heels of Pan-Cancer that can be helpful to identify new therapeutic strategies across cancers.

Algorithms for discovery of somatic single nucleotide variant display specific artifacts and different detection capabilities under the effect of read coverage and sample heterogeneity. Wenming Xiao,1 LeiHong Wu1, Gokhan Yavas,1 Huixiao Hong1, Baitang Ning1, Weida Tong1, Eric F. Donaldson,2 Zivana Terezaki,3 Jesse Boehm,8 William Hahn3, Jill Mesirov1, Pablo Tamayo1,1 University of California, San Diego, La Jolla, CA; 2Harvard University, Cambridge, MA; 3Broad Institute, Cambridge, MA.

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The effect of a gene’s activity on a phenotype depends on the context of a complex network of functionally interacting genes. Several genetic interactions (GIs), such as Synthetic lethality and Synthetic Rescues, have been reported to have a significant functional role in cancer progression and provide potential candidates for selective cancer treatments. However, numerous other types of GIs with potential therapeutic applications have been identified. The GI network generalizes the concept of GI and detect ~70,000 GIs of different types with both molecular and clinical signature. We demonstrate their clinical predictive value as well as their ability to stratify breast cancer patients into refined clinical subtypes that might allow for better diagnosis and modified course of treatment. These results compare favorably with previous sequence based approaches and provide evidence for the importance of context specific genomic events and their effect on tumor progression. Additionally, the GI network accurately predicts patients’ drug response, where difference GI types are found to be predictive of distinct drugs in a complementary manner. This work provides the basis for future exploration of novel GI types as well as individual interactions with major impact on cancer progression.

#1559 Proteomic biomarker analysis of alternative splicing in colorectal adenoma-to-carcinoma progression. Malgorzata A. Komor,1 Thang V. Pham,2 Sander R. Piersma,1 Anne S. Bollin,1 Tim Schellhors,7 Pien M. Delis-van Dijmen,1 Marijanne T.J. Finsem,1 Annemieke C. Hiemstra,1 Meike de Wit,9 Beatriz Carvalho,1 Gerrit A. Meijer,1 Connie R. Jimenez,2 Remond J. Fijneman1. University of Texas MD Anderson Cancer Center, Houston, TX; 2The University of Amsterdam, Amsterdam, The Netherlands; 3VU University Medical Center, Amsterdam, Netherlands.

Background: Early diagnosis of colorectal cancer (CRC) and identification of its precursor lesions (adenomas) is crucial in reducing CRC mortality rates. The fecal immunochromatographic test (FIT) is a non-invasive CRC screening test that detects human protein hemoglobin. Although FIT is beneficial in its current form with a sensitivity of ~65% for detection of CRC and ~27% for adenomas, its performance is still suboptimal and needs to be further improved. Adenoma-to-carcinoma progression is accompanied by alternative splicing, which results in expression of tumor-specific protein variants. These may yield novel biomarkers suitable for improving detection of progressive adenomas and CRCs. Aim: We aim to identify novel biomarkers to improve early detection of CRC. Materials and methods: RNA was isolated from 3D organoid cultures derived from 5 adenomas and 4 CRC tissues. RNA and proteins were isolated from 18 healthy human colon tissues, 30 adenomas and 30 CRCs. Samples were analyzed by RNA sequencing (Illumina) and in-depth tandem mass spectrometry proteomics (QExactive). For both organoid- and tissue-datasets differential splicing analysis was performed on RNA level to enrich the sequence database, against which mass spectra were searched, with predicted protein isoforms. Results: Comparative splicing analysis between CRC and adenoma organoids revealed ~90 differentially spliced genes, yielding candidate biomarkers from epithelial origin. In the tissues, differential splicing analysis between CRCs and controls and between CRCs and adenomas identified over 1000 of splice variants. These include known alternatively spliced genes involved in cancer such as CD44 and VEGFA and a number of candidates overlapping with the isoforms derived from the organoids. Proteomics analysis revealed that approximately 150 of the splice variants were primarily located on the protein level. Conclusion: We demonstrated that different drugs from the same class were selected by DISARM for a single tumour type and, in these cases, we found statistically significant similarity between sensitive cell lines suggesting a subset of cisplatin-resistant cell lines that are repeatedly sensitive to a drug class. While translating preclinical observations into approved clinical use is often thwarted by an inability to identify predictive biomarkers, DISARM also allows us to select cell lines that are especially sensitive to candidate drugs or drug classes on which to perform biomarker analysis. To demonstrate this approach, we chose drugs with activity in multiple cancer types that were selected by DISARM and in-depth proteomics analysis predicted the potential to identify novel common and tumor-specific biomarkers for concomitant candidate drug sensitivity and cisplatin resistance. Thus, DISARM offers a simple yet effective approach for both drug and biomarker discovery within a specified clinical niche.


Background: Previous epidemiological and population sequencing studies have established that subjects with melanocortin 1 receptor (MC1R) germline mutations, associated with red hair and light skin phenotypes, have an increased risk of melanoma. However, several conflicting reports about the role of BRAF somatic mutations in MCIR subjects exist in the literature. We hypothesise that this conflict is due to biological process driven heterogeneity within MCIR subjects carrying disruptive alleles. To understand the heterogeneity, we analysed the TCGA cohort at the gene expression level using mRNA sequencing data. Method: From the previously published studies, we identified 68 cutaneous melanoma subjects who were of white ethnicity, had at least one disruptive allele of MC1R, and had BRAF mutations. Associated clinical and mRNA sequencing data were downloaded from the Firebrowse website. An unbiased hierarchical clustering analysis was performed using the preprocessed mRNA data containing 14696 gene expressions. The resultant clusters were then characterised using available clinical variables. A differential expression analysis was performed to identify the genomic signatures of these clusters. The resultant p-values were corrected for false discovery rate (FDR) using the Benjamini-Hochberg approach. Genes with corrected p-values less than 0.01 were further analysed using gene set enrichment analysis (GSEA) ranked lists. Results: An unbiased clustering identified two clear and separable clusters with 40 and 28 subjects respectively. The UV-signature (diprimidine C>T load) was correlated with these clusters (p-value = 0.047). However, no significant differences in age at the time of diagnosis, overall survival, and total mutation load between clusters were observed. At the genomic level, 2588 genes were significantly differentially expressed between two clusters (FDR corrected p-value less than 0.05). The top 10 differentially expressed genes included ATF2, MSH6, SP3, MAP3K2, and VEGFB. Further GSEA revealed gene sets playing roles in several pathways and biological processes, including oxidative phosphorylation, UV response in keratinocytes, DNA repair, cell cycle, and cellular response to stress. Conclusion: Cutaneous melanoma subjects with disruptive MCIR alleles and BRAF hotspot mutations have heterogeneous gene expression profiles with several key oncogenes differentially expressed. This suggests potential roles of other pathways, either independently or in cooperation with BRAF mutations, in melanogenesis in MCIR bearing patients.

#1562 Drug repurposing for hepatocellular carcinoma enabled via transcriptomics data from experimental models of sorafenib resistance. Kelly Regan,1 Ryan Reyes,1 Samson Jacob, Philip Payne,2 Tasneem Motiwala,2 The Ohio State University, Columbus, OH; 2Washington University in St. Louis, St. Louis, MO.

The effects of drugs that are active in spite of resistance to a reference drug, DISARM selects candidates based on the proportion of samples that are resistant to a reference drug but sensitive to a candidate drug with simultaneous consideration to relatively lower IC50 values for candidate drugs and higher IC50 values for reference drugs. As candidates may work in only a subset of resistant models and may be sensitive to a drug class but not a specific drug, DISARM attempts to identify generalizable GI types and detect them across many tumor types and drug classes. To date drugs across platinum-resistant cancer types. To progress in this direction, we developed an automated method to identify drug targets and genetic interactions with potential therapeutic implications. DISARM incorporates a flexible grid search optimization procedure to select candidates that are active in the face of resistance to a reference drug. In our demonstration, DISARM identifies potential drug targets and genetic interactions with potential therapeutic implications in the face of resistance to a reference drug.
Hepatocellular carcinoma (HCC) is often diagnosed in patients with advanced disease who are ineligible for curative surgical therapies. Sorafenib is the only approved drug for treating late stage HCC patients. However, patients rapidly become unresponsive due to inherent and acquired drug resistance. The promise of drug repurposing is that identifying new uses for existing drugs may reduce target validation costs. This data is the result of a comprehensive essentiality analysis of genes; which is not possible by single screening methods. We used an area under the receiver operating characteristic curve (AUC) of 0.82. Overall, these methods allow for a more comprehensive essentiality analysis of genes, as well as what type of platform (CRISPR or shRNA) was more likely to identify essential genes better than approaches just based on the shRNA results (p-value < 0.05). We also analyzed RNAseq data from HCC patients (n=423) in The Cancer Genome Atlas (TCGA) for the presence of these SR genes, and observed that patients harboring the SR-HCC gene signatures generated by our lab had significantly reduced survival (log-rank p-value < 0.036 SR Huh7 pool; p=0.009 SR Huh7 clone). Utilizing drug-induced gene expression profiles (n=3,740 drugs) in the HepG2 HCC cell line from the Library of Integrated Network-based Cellular Signatures (LINCS) database, we applied connectivity mapping analysis to the SR-HCC gene signatures. Dasatinib, a Src family kinase inhibitor, was prioritized as a top drug candidate from our LINCS analysis to reverse HCC sorafenib resistance. We confirmed up-regulated activity of Src family kinases in SR-Huh7 cells, as compared to sorafenib sensitive Huh7 cells (two-tailed t-test, p<0.05). We validated the use of dasatinib against sorafenib-resistant HCC cells in vitro alone and in combination with sorafenib using cell viability and clonogenic survival assays. In summary, we provide physiological relevance of SR models and proof of concept evidence for the validity of this novel drug repurposing approach for SR-HCC with implications for personalized medicine.

#1563 #1564 A computational framework for removing mouse contamination in tumors sequenced from patient-derived xenografts. Ali Amin-Mansour,1 Judith Jané-Valbuena,2 Xinneng Jasmine Mu,1 Levi Garraway2. 1The Broad Institute of MIT and Harvard, Cambridge, MA; 2Dana-Farber Cancer Institute, Boston, MA.

Introduction: Using patient derived xenograft (PDx) models has become an effective way for investigating response to standard or new therapeutics in cancer. Human cancer cells injected in mice are allowed to establish tumors and subjected to desired treatments. The PDx tumors are later harvested and characterized, often by massive parallel sequencing. However, a major challenge with analysis is the presence of immortalized murine tumor cells, frequently resulting in artifacts in downstream variant detection. We present a computational method to eliminate mouse contamination in PDx. Method: We used the Burrows-Wheeler Aligner to map reads obtained from sequencing the PDx samples to a combined human and mouse reference genome. We remove reads that are mapped to the mouse reference. The remaining reads are then used for variant detection. To test the efficacy of our method, we created in silico mixtures of human and mouse whole-exome sequencing reads from a melanoma patient's tumor and an immortalized mouse cell line captured with human exome baits. We then carried out a sensitivity analysis to examine how changing the mean target coverage of sequencing, or mouse contamination levels affects our results. For each of the computational experiments, we evaluated somatic mutations detected from the synthetic samples in comparison to the original human sample. Results: We calculated the sensitivity and specificity of detecting somatic mutations to determine our algorithm's performance. In all instances, we found greater than 99% for both sensitivity and specificity. Conclusions: Our results demonstrate that our method works accurately towards removing mouse reads in PDx samples. This task could also be applied to separating sequence reads from other species.


Introduction: Multivariate projection methods such as PCA and PLS have been widely applied for analysis of biological and chemical data. OnPLS is a recent extension to these methods suitable for integrative analysis of omics data. With OnPLS it is possible to compare multiple omics datasets to identify joint variation and variation locally unique for each of the studied datasets. OnPLS is a new approach for truly integrative analysis of omics data to be contrasted to commonly applied approaches limiting analysis to 1) comparing findings from individually analyzed blocks of data 2) pairwise comparison of individual probes. Experimental: A Java based implementation of OnPLS was used for the statistical modeling. 116 lung squamous cell cancer samples were characterized using gene expression profiling and global proteomics. The OnPLS model was applied to jointly model variation between mRNA and protein expression. Enrichment analysis of factor loadings was performed using the Enrichr tools to identify biological mechanisms explained by the different joint and unique components of the OnPLS model. Results: Using a cross-validation procedure the model with the highest predictive ability was calculated having two joint components and one locally unique component for each of the proteomics and gene expression data. This model explained 21.9% of the variation in the expression data and 26.1% of the variation in the proteomics data. The first joint component captures the highest degree of common variation between mRNA and protein activity. From the mRNA data, this component is related to immune infiltrates, especially monocytes and B-cells, whereas this component is related to extracellular matrix activity from the protein data. This suggests covariance of mRNA immune-related gene expression and extracellular matrix-related protein expression. As expected, local variation specific to the protein measurements involved regulation of protein activation and processing. mRNA-specific variation is related to keratinization, a key process in squamous cell cancer. Conclusion: OnPLS offers an interesting approach for integrative analysis of omics data. Applying this approach to proteo-genomics data of lung squamous cell cancer is expected to provide novel insights into the biology and treatment of lung cancer. The triple-negative breast cancers (TNBC) account for up to 20% of breast cancer cases. These tumors are negative for estrogen, progesterone, and HER2 receptors, which are markers used for diagnosis and treatment of other types of breast cancer. Clinically, TNBCs are notoriously aggressive, respond poorly to conventional treatment and have a poor response to standard therapies. This results in a high rate of mortality due to late stage disease at diagnosis. TNBCs are diagnosed in up to 20% of breast cancers and a significant proportion of women with breast cancer (17% of new breast cancers in the United States) are diagnosed with TNBC. TNBCs are negative for estrogen, progesterone, and HER2 receptors, which are markers used for diagnosis and treatment of other types of breast cancer. Clinically, TNBCs are aggressively responding poorly to treatment. The current mainstay of treatment for TNBC is docetaxel chemotherapy, which has limited efficacy due to the aggressive nature and heterogeneous behavior of TNBC.

#1566 Differential expression of Epstein Barr virus miRNAs in triple-negative breast cancer. Alexander Blanchard,1 Tiffany Wallis,1 Ramon Vidal,2 Stefan Bonn,2 Scott Harrison,1 Perpetua Manguada1. 1Department of Neurology, University of Basel, Switzerland; 2Department of Medicine, University of Basel, Switzerland.

Introduction: We sequenced small RNA libraries from 100 TNBC cases and a matched normal tissue sample using a combination of mission RNA and whole-exome capture methods. We identified 385 differentially expressed microRNAs, which were further validated by qRT-PCR. We found a significant enrichment of genes known to be involved in tumor growth and invasion in the downregulated miRNA set. Methods: We sequenced small RNA libraries from 100 TNBC cases and a matched normal tissue sample using a combination of mission RNA and whole-exome capture methods. We identified 385 differentially expressed microRNAs, which were further validated by qRT-PCR. We found a significant enrichment of genes known to be involved in tumor growth and invasion in the downregulated miRNA set. We then carried out a sensitivity analysis to examine how changing the mean target coverage of sequencing, or mouse contamination levels affects our results. For each of the computational experiments, we evaluated somatic mutations detected from the synthetic samples in comparison to the original human sample. Results: We calculated the sensitivity and specificity of detecting somatic mutations to determine our algorithm's performance. In all instances, we found greater than 99% for both sensitivity and specificity. Conclusions: Our results demonstrate that our method works accurately towards removing mouse reads in PDx samples. This task could also be applied to separating sequence reads from other species.
targeted therapies, have high rates of relapse, as well as poor prognosis. The molecular basis of TNBC oncogenesis is currently unknown, but it is possible that viral factors, such as Epstein-Barr Virus (EBV) miRNAs, play a role. EBV miRNAs have been implicated in the oncogenesis of several forms of cancer, including Burkitt’s lymphoma and nasopharyngeal carcinoma. The objective of this study, therefore, was to determine the differential expression of EBV miRNAs in TNBC tumors as compared to normal breast tissue. We conducted a comprehensive profiling of viral miRNAs in 48 TNBC tumors as compared to 15 control normal breast tissues, utilizing deep sequencing analysis software and publicly available deep sequencing data. Four EBV miRNAs (BART18-3p, BART 8-5p, BART15, BART22) were significantly expressed in 11-17% of the TNBC biopsies from control normal breast tissue. Four novel putative EBV miRNAs were found to be differentially expressed in TNBC tumors as compared to control normal cells. Two of the novel putative EBV miRNAs were differentially expressed above 125 reads per million (RPM) in 20% and 27% of the TNBC tumors as compared to 0% and 6.6% of normal breast tissues, respectively. One novel putative EBV miRNA was expressed above 125 RPM in 66% of the normal breast tissue as compared to 2% of the TNBCs. These putative EBV miRNAs localize within genomic regions of EBV, including the LMP-2 region. Ongoing work has so far computationally validated one of these miRNAs as a novel EBV miRNA; in vitro validation studies are in progress. This is the first report on the differential expression of EBV miRNAs in TNBC tumors. Although EBV miRNAs are extremely heterogeneous, it is intriguing that 20% of TNBC tumors specifically express the same EBV miRNA. Our findings suggest that these differentially expressed EBV miRNAs may potentially play a role in the pathogenesis of TNBC.

#1567 Oncogenesis may result from multiple different combinations of a small number of tumorigenic mutations. Rami Anandakrishnan, Robin Varghese, Niharika Gupta, Harold R. Garner. Edward Via College of Osteopathic Medicine, Blacksburg, VA

Cancer is known to result from genetic mutations, both inherited and somatic. Yet, decades of investigation and the availability of extensive genomic data, have failed to reveal the specific mutations that directly result in tumorigenesis for most cancers. One possible reason is that tumorigenesis may result from one or more of multiple different combinations of mutations, while investigators generally search for a single set of mutations. A mathematical model of the probability of “hitting” one of many possible combinations of a small number of mutations was compared to the actual distribution of accumulated mutations at diagnosis as reported on the cancer genome atlas (TCGA). This “multi-combination multi-hit” model reproduces the distribution of accumulated mutations from the TCGA database with surprising accuracy (RMSD = 1.5%) for a model where one or more of 100 different possible combinations of three mutations result in tumorigenesis. We speculate that each of the three mutations affects one of the several genes in three distinct cell proliferation control pathways, such as senescence, apoptosis, autophagy and necrosis, and different combinations of mutations affecting three of these pathways may result in tumorigenesis. We have identified several such combinations for further investigation.

#1568 Predicting stochastic proliferation and death in response to drugs with mechanistic models tailored to genomic, transcriptomic, and proteomic data. Mehdi Bouhaddou, Anne Marie Barrette, Rick J. Koch, Marc R. Birtwistle. Icahn School of Medicine at Mount Sinai, New York, NY

Over the past decade we have seen a shift in cancer therapy from broadly cytotoxic drugs to molecular therapies targeting “driver” mutations. Although targeted therapy has seen great success for some cancers (e.g. imatinib for leukemia), it has struggled with poor efficacy in treating other cancers that can sometimes possess multiple “driver” mutations. This highlights the complex, and at times non-intuitive, interplay between multiple players in a signaling cascade, which is highly dependent on the biological context. Therefore, a model that is, EBV expression levels and mutational architecture – of a tumor or cell line. A quantitative, mechanistic, biologically-tailored understanding of how these signaling dynamics drive proliferation and death could improve precision pharmacology approaches to treat cancer. Here, we constructed the first highly detailed, large-scale ordinary differential equation (ODE) mechanistic mathematical model depicting the most commonly mutated cancer signaling pathways across human cancers, as indicated by a pan-cancer analysis by The Cancer Genome Atlas (TCGA). The model includes the RTK/Ras/MEK/ERK, PI3K/AKT/mTOR, CDK/CDK4/CDK6, CDK2/CDK7/RB cell cycle, p53/MDM2 DNA damage response, and BCL/Caspases apoptosis pathways. The adjustable parameters of the model can be informed by measurements from patients or cell lines, including copy number alterations, mutations, and gene expression levels. This single-cell model links stochastic gene expression processes to quantitative signaling dynamics, and once tailored to a biological context can be used to simulate the effect of various anti-cancer therapies on cell fate behavior such as proliferation and death for a population of cells. The first instance of the model integrated genomic, transcriptomic, and proteomic data from the MCF10A cell line, a non-transformed cell line with predictable phenotypic behaviors. We trained the model using western blot and flow cytometry experiments to refine various biochemical parameters and phenotypic outcomes. Many fundamental questions in signal transduction arose during this process, such as how EGF and insulin synergize to drive 5-phosphorylated AKT. The model also predicts the effect of mutating genes that confer sensitivity or resistance to inhibitors of the ERK and AKT pathways. Simultaneously, we are tailoring the model to patient-derived genetic information from primary glioblastoma tumors and screening brain-penetrable compounds in a patient-specific manner. In conclusion, a quantitative, mechanistic, biologically-tailored mathematical model depicting the major cancer pathways allows us to probe the mechanisms that underlie how signaling dynamics drive proliferation and death in response to various perturbations, and gain insight into their dependence on the biological context of cell lines and patient tumors.


Introduction: TTFields is an antimitotic cancer treatment that utilizes low intensity (1-3 V/cm) alternating electric fields in the intermediate frequency (100-300 kHz) that are delivered in two orthogonal directions using 2 pairs of transducer arrays. TTFields are currently approved for Glioblastoma Multiforme (GBM). A phase II clinical trial (EF-20) showed that TTFields in combination with gemcitabine was safe in patients with locally advanced pancreatic cancer. Preclinical studies show that the effect of TTFields is intensity-dependent with a therapeutic threshold of 1 V/cm. Simulation-based studies show that the field distribution changes with array placement. Treatment planning with arrays positioned on the scalp to maximize field intensity to the tumor is standard of practice when treating GBM. Studies to examine array layout on the abdomen to maximize the field distribution in this region have not been done. In the EF-20 study, a generic layout was used. We used computer simulations to test how altering the transducer array layout on the mid-body alters the field distribution within the abdomen and pancreas. Methods: To simulate delivery of TTFields to the abdomen, we used a realistic computerized model of a human male (DUKE 3.0 from ZMT-Zurich). Eight different layouts utilizing combinations of arrays with either 13 or 20 disks placed at different locations on the abdomen were simulated. In order to generate TTFields, an alternating voltage difference with a peak to peak magnitude of 100V and a frequency of 150 KHz was imposed on the outer surfaces of the disks of each pair of arrays. The simulations were performed using ZMT’s Sim4Life V3.0 electro-quasi-static solver. For each pair of transducer arrays, the mean field intensity in the abdomen and chest, and in the pancreas and liver, was calculated. Results: All eight layouts delivered fields with mean intensities of about 2 V/cm to the abdomen. The average field intensity delivered to the pancreas and the liver exceeded the therapeutic threshold of 1 V/cm for all layouts. The highest intensities were delivered to the pancreas by layouts in which a 20-disk array was placed at the middle of the back, and either a 20 disk array placed at the middle of the abdomen or a 13 disk array placed on the front-left side of the abdomen over the pancreas. Generally, field intensities in the pancreas were lower than in the rest of the abdomen because of the high electric conductivity of this organ. Conclusion: This work shows that TTFields can be delivered to the pancreas with intensities well above the therapeutic threshold of 1 V/cm, and that altering the location of the arrays on the abdomen can influence the field intensity and distribution within the body. This work forms the basis for developing optimal strategies for delivering TTFields to the pancreas.

#1571 Decrypting the transcriptome profile of Pak4 using next generation sequencing. Chetan Rane, Misaal Patel, Li Cai, Audrey Minden. Rutgers University, Piscataway, NJ.

The PK4 (p-21 Activated Kinase 4) protein kinase has long been associated with cancer because of its key roles in regulating cell proliferation, cell cycle progression and cell morphology. The PK4 gene is amplified in different types of cancers, including breast cancer. PK4 overexpression is associated with oncogenic transformation in several breast cancer cell lines, while PK4 inhibition has been shown to significantly reduce their tumorigenic potential. However, there is limited information available on PK4’s mechanism of action in...
promoting tumorigenesis. To gain an insight into PAK4 downstream signaling pathways, we performed Next Generation Sequencing (NGS) on RNA samples collected from non-transformed immortalized mouse mammary epithelial cells (WT iMMECs) and iMMECs overexpressing PAK4. Research from our lab has shown that unlike WT iMMECs, iMMECs overexpressing PAK4 formed tumors when implanted in immunocompromised mice. These tumors were characterized by increased cell proliferation and angiogenesis, suggesting a role for PAK4 in tumor formation. Previous studies to delineate PAK4 signaling pathways have mostly focused on substrates phosphorylated by PAK4. This study, however, takes into account genes which may act downstream of PAK4 and play an important role in mediating long term PAK4 function. RNA-seq analysis offers the ability to discover new genes and splice variants and measure transcript expression, mRNA was isolated in triplicates from WT iMMECs and iMMECs overexpressing PAK4; mRNA from each condition was then sequenced using Illumina NextSeq platform. The RNA-seq data generated was analyzed using Top Hat and Cufflinks software, which created a list of genes whose expression was significantly different in these two cell types, and previously unknown to be regulated by PAK4 (26 genes were up regulated and 51 genes were down regulated by more than a log2 fold change of 3). A qPCR analysis of 8 of these genes validated the sequencing data. Among the different genes we identified, we chose to focus on ParvB, which is consistently down regulated by almost seven fold in the iMMECs overexpressing PAK4. Previous studies have suggested a tumor suppressor role for ParvB in regulating breast tumorigenicity. We hypothesize that PAK4 mediates mammary tumor formation and development. These results illustrate how drivers of primary tumorigenesis must be distinguished from drivers of treatment resistance, metastasis, or recurrence in order to better understand the genetic changes behind cancer progression.

### #1572 Transformation from melanocytes or nevi into melanoma: models based on a transcriptome meta-analysis. Daniel Ortega-Bernal,1 Claudia Rangel-Escareno,1 Elena Arechaga-Ocampo,1 Claudia H. Gonzalez-De la Rosa1, Universidad Autonoma Metropolitana, Mexico, Mexico; Instituto Nacional de Medicina Genomica, Mexico, Mexico.

Background: Melanoma is the most aggressive skin cancer due to its ability to rapidly metastasize and resist radio- and chemotherapy. The frequency of this disease has increased 400% worldwide in the last 30 years. There are 2 ways to develop melanoma: direct transformation of melanocytes into melanoma and the nevus as an intermediary. Here, we used a computational strategy based on transcriptome meta-analysis to identify the pathways involved in the 2 possible ways by which cells may transform to melanoma. Methods: Affymetrix HU 133A Plus 2.0 microarray files (50 in total) were collected from the Gene Expression Omnibus database for the meta-analysis, representing melanocytes, nevi and stage I, II, and III melanoma. Differential expression was analyzed using linear models and the limma library. The analysis focused on 3 comparisons: nevi vs. melanocytes; melanoma vs. nevi; and melanoma vs. melanocytes. Transcript with a log fold change of <1/3> were included in the study. Functional enrichment analysis identified the most deregulated pathway. Genes resulting from the intersection of the melanoma vs. nevi and melanoma vs. melanocytes comparisons were used as possible biomarkers of transformation and analyzed by IPA. Gene signature identification: In melanoma vs. nevi comparison, 1881 expressed genes were differentially expressed, of which only 888 were used to generate the transformation model and to perform functional enrichment. Consistent with the phenomenon of malignant transformation, we identified 3 pathways associated with cell cycle checkpoint control that exhibited decreased activity. Conversely, the pathways that exhibited increased activity were estrogen-mediated S-phase Entry and Cyclins and Cell Cycle Regulation, promote cell cycle progression. In melanoma vs. Melanocytes comparison, 4112 expressed genes were differentially expressed (2310 downregulated and 1802 upregulated), 1099 of which intersected with the nevi vs. melanocytes comparison. The 682 genes intersecting the melanoma vs. nevi comparison were removed. We used the to perform enrichment analysis. The main result was the decreased activity of the cAMP-mediated signaling pathway. The intersection of both comparisons (682 genes), was used to identify 22 genes upregulated whose product is a secreted protein. Two of these genes were previously reported as potential biomarkers in neoplasia, cytokines CXCL8 and CXCL16, supporting the quality of our analysis. Conclusions: Our analysis, in agreement with previously reported results, identified a biomarker panel consisting of melanoma, and also identified new biomarkers, including CXCL16, MMP19, BIVM, and FAM60A. A better understanding of the specific molecular alterations causally involved in this disease may lead to new biomarkers and pathways involved in the transformation for this currently incurable malignancy.


Whole-genome sequencing of tumors has identified genetic mutations that drive the origination, growth, and spread of cancer. Most driver mutations detected so far are early mutations categorized as small amount of total drivers and many early total mutations. In addition, the timing of driver mutation occurrence varies widely depending on cancer type as well as among different individuals with a single cancer subtype. Finally, we identified known cancer genes that are enriched in either early or late driver mutations, reflecting different selective pressures at various stages of cancer development. These results illustrate how drivers of primary tumorigenesis must be distinguished from drivers of treatment resistance, metastasis, or recurrence in order to better understand the genetic changes behind cancer progression.

### #1574 Computational approach for discovery of regulatory noncoding variants in cancer. Yu Li1, Michael Edmonson,1 Xiaotu Ma,1 Michael Rusch,1 Yongjin Li,1 Bengsheng Li,1 Shuhong Shen,2 A. Thomas Look,1 Jinghui Zhang2. 1St. Jude Children’s Research Hospital, Memphis, TN; 2Shanghai Children’s Medical Center, Shanghai Jiao Tong University School of Medicine, Shanghai, China; Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA.

Noncoding mutations in the cancer genome have historically been poorly understood. Recent studies have shown however that mutations in intergenic regions may play an important role in cancer by introducing de novo regulatory elements and thus cis-regulating target gene expression. While mono-allelic expression of the dysregulated gene is an important signature of regulatory noncoding mutations, recognition of this feature has not been implemented by any methods evaluating noncoding mutations to date. To perform genome-wide detection of noncoding mutations with a potential regulatory function, we developed a novel computational approach implementing this allelic expression signature (ASE) as a core component, using WGS and RNAseq data as input. We first searched for genes showing ASE across the tumor genome by comparing the minor allele frequency of heterozygous markers between WGS and RNAseq data. We excluded genes known to be in an imprinted locus, as well as sites matching a precomputed set of 5,576,857 problematic polymorphism markers (estimated by analyzing WGS data from 625 normal genomes). Next, the somatic mutations were filtered for regulatory candidates that could introduce a transcription factor (TF) binding motif absent from the reference sequence. We included 614 TF binding motifs from the HOCOMOCO database and performed motif analysis with the FIMO package. Finally, the somatic mutations were assigned to dysregulated genes in the same topologically associating domains in human defined by public Hi-C data and prioritized by open chromatin state defined by DNase hypersensitivity regions (ENCOD project). We applied our approach to 22 T-ALL tumor samples collected at Shanghai Children’s Medical Center in a pilot study consisting of 13 diagnosis and 9 relapse samples (9 of these were trios). WGS was performed on both tumor and normal samples and RNAseq was performed for the tumor sample only. We predicted 213 regulatory noncoding mutations (157 unique variants) in total from this metastasis, which are likely to be found in only one or a few tumor samples. We analyzed 101 multi-region and/or multi-tumor sequencing data sets using driver mutation annotation and phylogenetic inference to determine whether driver mutations occur preferentially early on. We found driver mutations frequently arise late in the course of tumor evolution, even when statistically accounting for small amounts of total drivers and many early total mutations. In addition, the timing of driver mutation occurrence varies widely depending on cancer type as well as among different individuals with a single cancer subtype. Finally, we identify known cancer genes that are enriched in either early or late driver mutations, reflecting different selective pressures at various stages of cancer development. These results illustrate how drivers of primary tumorigenesis must be distinguished from drivers of treatment resistance, metastasis, or recurrence in order to better understand the genetic changes behind cancer progression.
 Novel small molecule inhibitors of p300/CREB binding protein (CBP) are two closely related, paralogue histone acetyl transferase proteins that act as transcriptional co-activators of a variety of cancer related genes. We have developed potent, selective and orally active small molecule inhibitors of the bromodomain of p300/CBP and investigated their role in regulating androgen receptor expression and function. We have also examined their role in driving synthetic lethality in tumours. Loss of function mutations in either p300 or CBP (including in significant proportions of lung and bladder tumours), can lead to a dependency on the corresponding paralogue protein. Methods: Binding affinity to p300, CBP and BRD4 was measured in a surface plasmoid resonance (SPR) assay. Potency and functional activity was demonstrated in a panel of prostate cells lines representing hormone responsive (LNCaP), hormone independent (DU145, PC3) and castrate resistant disease (22Rv1, C4-2, VCaP, LNCaP-AR) as well as wildtype (A549) and CBP deficient (H520, H1703, LK2) lung cancer cells. Combination effects of p300/CBP inhibitors with a PARP or CDK4/6 inhibitor were determined in LNCaP and C4-2 cells. Effects of p300/CBP inhibitors (and by comparison, the BET inhibitor, JQ1), on AR, AR-V7 splice variant and c-Myc protein, as well as c-Myc, KLK3 and TMPRSS2 gene expression, were assessed in 22Rv1 cells in vitro. In vivo effects on biomarkers were measured in a 22Rv1 xenograft model. Results: CCS1357, an in vitro probe compound, binds to p300 and CBP with high affinity (Kd=45mM; BRD4). It is a potent inhibitor of cell proliferation in castrate resistant cell lines (IC50=100mM in LNCaP-AR; 350mM in 22Rv1) with minimal effects in hormone independent lines. CCS1357 combined with palbociclib (CDK4/6) or olaparib (PARP) in LNCaP or C4-2 cells, showed reduced cell viability compared with any of these drugs given alone. In 22Rv1 cells, CCS1357 significantly down-regulated AR-FL, AR-V7 and c-Myc protein by Western, an effect not seen with JQ1 at equivalent proliferation IC50s. CCS1357 effects were reversed by the proteasome inhibitor, MG132. CCS1357 also caused a profound inhibition of c-Myc, KLK3 and TMPRSS2 genes measured by qPCR in 22Rv1 cells in vitro. A preclinical candidate (CCS1477) given as a single oral dose (30mg/kg) inhibited plasma PSA and tumour AR, AR-V7 and c-Myc in a 22Rv1 xenograft model. In the lung cancer cell lines, we observed differential sensitivity to CCS1357; CBP deficient lines were more sensitive (cell proliferation) compared with normal. Conclusions: Taken together these data support the clinical testing of p300/CBP inhibition in prostate cancer patients in two settings; firstly, castrate resistant prostate cancer by down-regulating of AR, AR-SV and c-Myc expression and function; and secondly in patients with loss of function mutations in p300 or CBP by driving synthetic lethality.

#1575 UGT2B17 promotes castration-resistant prostate cancer progression through enhancing ligand-independent AR signaling. Haolong Li, Ning Xie, Ruqi Chen, Melanie Verreault, Ladaz-Fazli, Martin E. Gleave, Olivier Barbier, Xuesen Dong, University of British Columbia, Vancouver, British Columbia, Canada; Laval University, Quebec, Quebec, Canada.

Background: Castration resistant prostate cancer (CRPC) is characterized by a shift of androgen receptor (AR) signaling from ligand-dependent to ligand-independent. Defining mechanisms that control AR signaling transformation is important to develop therapies for disease control. UDP-glucuronosyltransferase 2B17 (UGT2B17) is a key enzyme that maintains androgen homeostasis by catalyzing AR agonists into inactive forms. Although enhanced UGT2B17 expression by androgen resistant prostate cancers is a hallmark of regulation of AR signaling transformation and CRPC progression remain unknown. Methods and Results: We first evaluated the UGT2B17 protein expression levels by immunohistochemistry (ICC) on 403

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and AR were detected by immunoblotting, and the transcript levels were evaluated by QRT-PCR. Results: Since the annotation of PMEP1A1/TMEM1A1 isoforms has been empirical in the literature, here we propose a new structure/expression based nomenclature: PMEP1A1 (reading frame of 252 amino acids (aa)); PMEP2A (344aa); PMEP3A (287aa; STAG1); PMEP4A (259aa); and PMEP5A (237aa). Expression of PMEP1A1 and PMEP2A was regulated by androgen responsive prostate cancer cells in comparison to broader expression pattern of other isoforms (PMEP3A-5). The expression of PMEP1A2-2 was androgen regulated, whereas expression of PMEP3A-5 was regulated by TGF-β. Only PMEP1A1 inhibited cell growth of LNCaP, DU-145 and PC-3 cells. In contrast, PMEP2A-4 promoted cell growth of DU-145 and PC-3 cells. Of all the isoforms only PMEP1A1 mediated AR protein degradation in CaP. Conclusions: The PMEP1A isoforms appear to underscore distinct biological functions in the context of androgen and TGF-β signaling. Widely studied PMEP1A1 was specific for AR degradation in prostate cancer cells and was consistent with previous observations of association of AR upregulation with loss of PMEP1A1 in prostate cancer. The roles of PMEP1A isoforms need to be better defined in prostate cancer and other cancers. Further studies were supported by CPDR, USUHS, HU0001-10-2-0002 to DGM.


The growth of prostate cancer cells depends on the activation of androgen receptors (AR). Bicalutamide is a nonsteroidal diolipid that inhibits AR through blockage of AR-activation being effective in limiting tumor growth. However, prolonged treatment with anti-androgen therapies result in the transition of prostate cancers into an androgen refractory state. Neuroendocrine differentiation (NED) has been associated with the progression of prostate cancers to an androgen resistant phenotype. In this work we investigated the effect of the androgen receptor blocker bicalutamide in promoting NED of LNCaP cells and whether it is accompanied by increased T-type Ca$^{2+}$ channel expression. The ability of bicalutamide to evoke morphological and biochemical changes associated with NED was assessed by PCR, western blot and immuno-histochemical analysis. Changes in the Ca$_{a,3.2}$ T-type Ca$^{2+}$ channel subunit expression was studied using PCR analysis, western blot and whole cell recordings. The role of T-type Ca$^{2+}$ channels in promoting the differentiation of LNCaP cells was assessed by a cell viability assay and changes in cell morphology, specifically the development of neurite-like processes. PCR analysis of bicalutamide-stimulated cells indicates no significant changes in Ca$_{a,3.2}$ mRNA, yet results in Ca$_{a,3.2}$ protein expression and functional channels. Western blot and immuno-histochemical analysis of LNCaP cells stimulated with bicalutamide for 4-10 days reveals biochemical changes consistent with NED including the expression of tubulin, EPHA and neuron-specific enolase. Pharmacological inhibition of T-type Ca$^{2+}$ channel function with nickel ions and NNC 33-0936 disrupts the morphological differentiation and cell viability of LNCaP cells treated with bicalutamide. These results suggest that bicalutamide treatment of LNCaP cells evokes significant morphological and biochemical changes associated with NED and results in increased expression of T-type Ca$^{2+}$ channels, which may significantly alter Ca$^{2+}$ homeostasis.

#1580 Combined neuroendocrine differentiation and androgen receptor hyperactivity in MAP37 and CHD1 null prostate cancer. Leah C. Rider, Lindsey U. Rodrigues, Anis Karimpour-Fard, Lina Romero, Claire Gillette, James C. Costello, Scott D. Cramer. Univ. of Colorado Anschutz Medical Campus, Aurora, CO.

We recently functionally validated a clinically aggressive subtype of prostate cancer characterized by dual deletion of MAP3K7 and CHD1. This subtype accounts for up to 25% of prostate cancer deaths. Androgen signaling is a critical component of prostate tumorigenesis and disease progression, which is exploited in treatment of prostate cancer with androgen deprivation and anti-androgen therapies. Neuroendocrine differentiation is thought to provide a selective advantage for prostate cancer in androgen-depleted conditions such as surgical or chemical castration. REST is a transcriptional repressor of neuronal gene expression and has been implicated in neuroendocrine differentiation of prostate cancer. The impact of combined MAP3K7 and CHD1 loss on androgen signaling and neuroendocrine differentiation has not been determined. Here, we demonstrate that dual loss of MAP3K7 and CHD1 simultaneously stimulates enhanced androgen signaling and enhanced neuronal differentiation correlated with enhanced growth in both androgen-depleted and androgen-replete conditions. These changes occur via increased androgen receptor chromatin binding, loss of CHD1 chromatin binding, loss of REST expression, and reduced chromatin binding by REST. Suppression of CHD1 and MAP3K7 attenuates the antiproliferative activity of the anti-androgen Enzalutamide. Furthermore, we translate these findings to clinical samples and show that decreased expression of CHD1, MAP3K7, and REST is associated with previous therapeutic intervention, castrate-resistant disease, and progression to a neuroendocrine phenotype. Overall, patients with low expression of all three genes have worse overall survival suggesting that these dual genetic alterations are an important cause of castration-resistant prostate tumors with loss of CHD1 and MAP3K7 exhibiting both androgen-dependent and -independent phenotypes leading to poor clinical outcome, which poses challenges to conventional therapeutic approaches.

#1581 Androgen receptor action in prostate cancer partitions into distinct transcriptional codes that differ in clinical relevance. Sangeeta Kumari, Varadha Balaji Venkadakrishnan, Dhirudatta Senapati, Qiangu Hu, Song Liu, Hannelore V. Heemers.

With few exceptions the 30,000 prostate cancer (CaP) deaths annually in the US are due to failure of androgen deprivation therapy (ADT). ADT prevents ligand-activation of androgen receptor (AR). Despite remission, CaP progresses while continuing to rely on ADT. AR’s transcriptional output that controls CaP behavior is an alternative therapeutic target, but its molecular regulation is poorly understood. Here, we determined the androgen dependence of hundreds of direct AR target genes on 18 coregulators that are relevant to CaP progression, and uncovered that the AR-dependent transcriptome breaks down in coregulator-dependent gene sets. Pairwise comparison and unsupervised clustering showed limited overlap between the gene subsignatures. Neither ChIP nor qRT-PCR studies revealed differences in the kinetics of coregulator recruitment to androgen response elements (AREs) or of androgen regulation of different gene sets. Rather, Cistrome analyses of AR binding sites demonstrated preferential enrichment in binding motifs for distinct transcription factors (TFs) between coregulator-dependent gene sets. Pathway and GSEA analyses of these signatures indicated associations with select biological processes and differential enrichment of normal prostate and CaP. CaP of different stages, and CaP and bone marrow microenvironment. These results suggested that coregulators may unite the action of DNA-bound AR and TFs to control select aspects of androgen-dependent CaP cell biology. The presence and functionality of such novel predicted AR transcriptional codes, namely AR-WDR77-p53 and AR-STAT3IRF1 with projected roles in CaP cell proliferation and stemness respectively, was confirmed in Co-IP and gene expression profiling. As AR and p53 are the major drivers of lethal CaP, the AR-WDR77-p53 code was explored further. Mass spectrometry after WDR77 and p53 IP independently identified PGAM5, a recently isolated serine/threonine protein phosphatase that regulates cell death and unknown to be relevant to AR signaling or CaP biology, as part of the AR-WDR77-p53 complex. Co-IP assays verified IP-mass spectrometry results and ChIP studies confirmed recruitment of PGAM5 to WDR77-dependent AREs. Expression profiling demonstrated significant overlap in genes for which androgen regulation was altered after silencing of WDR77, PGAM5 or p53. Co-IP and ChIP studies showed that androgen-dependent recruitment of p53 to ARE-bound AR requires WDR77 and PGAM5. Western blotting and flow cytometry analyses indicated that WDR77 and PGAM5 and their dependent AR target genes control G1/S cell cycle progression, which was maintained in presence of clinically relevant gain-of-function p53 mutants. These novel insights indicate that disrupting select protein-protein and protein-DNA interactions may be a viable strategy to inhibit AR action that drives CaP lethal progression.

#1582 Therapeutic potential of combination therapy using a next generation antisense oligonucleotide targeting the androgen receptor and AKT inhibition with AZD5363 in genetically engineered mouse models of prostate cancer. Marco A. De Velasco, Yurie Kura, Naomi Ando, Koichi Sugimoto, Kazuko Sakai, Barry R. Davies, Youngsoo Kim, A. Robert MacLeod, Masa-hiro Nozawa, Kazuhiro Yoshimura, Kazuhiro Yoshikawa, Kazuto Nishio, Hirotsugu Uemura, Kindai University Faculty of Medicine, Osaka-Sayama, Japan; AstraZeneca, Macclesfield, United Kingdom; Ionis Pharmaceuticals, Carlsbad, CA; Aichi Medical University, Nagakute, Japan.

Prostate cancer is highly dependent on androgen receptor (AR) and PI3K/AKT signaling pathways for survival and disease progression. Preclinical evidence suggests that combinatorial approaches targeting both AR and PI3K/AKT inhibition with AZD5363 in genetically engineered mouse models of prostate cancer. AR and PI3K/AKT activity improvements tend to treatment efficacy. However, sustained responses from traditional and next-generation anti-androgen therapies targeting AR remain elusive in clinical practice due to inherent resistance resulting in lethal castration-resistant prostate cancer (CRPC). Mechanisms for continued AR transcriptional activity may be ligand dependent or independent but still require AR gene expression. Persistent AR gene expression is a key feature of CRPC. Thus, blocking AR gene expression by antisense oligonucleotides (ASO) is a logical ap
proach to CRPC. We previously showed that monotherapy with ISIS581088, a generation 2.5 ASO targeting mouse AR, demonstrated strong antitumor activ-
ity in a transgenic mouse model of PTEN-deficient prostate cancer. In this study we show the antitumor effects of combined therapy of ISIS581088 and AZD5363, a potent AKT inhibitor and demonstrate the therapeutic benefit of combining this clinically proven inhibitor with monotherapy in a castration-resistant prostate tumorbearing mouse. Treatment of lethally irradiated, eight-week-old mice with PTEN−/− castration-resistant prostate tumors were treated with ISIS581088 and AZD5363 alone or in combination for four weeks. Tumor growth inhibition rates were 41.2%, 20.2% and 54.4% for ISIS581088, AZD5363 and ISIS581088/AZD5363 treatment groups, respectively, P < 0.001. In a model of mouse CRPC, 16-week-old mice with PTEN−/− castration-resistant prostate tumors (eight weeks post castration) experienced reduced tumor burden with all treatments but no enhancement was observed when the compounds were administered in combination compared with monotherapy drug treatments. In a randomized trial of advanced CRPC in PTEN/P53 double knockout mice, combination therapy significantly increased overall survival. Median survival were 18, 17 21, 22, and 38 days for control vehicle, control ASO, ISIS581088, AZIS581088, and ISIS581088/AZD5363 treatment groups, respectively, P = 0.041. In conclusion, our data shows that combination therapy significantly reduced tumor burden in mice with castration-naive tumors compared to those treated with monotherapy. Notably, combination therapy did not produce an additive effect in an early stage CRPC intervention model. Still, combination therapy demonstrated a clear advantage in prolonging overall survival in a long-term randomized model of PTEN/P53-deficient CRPC. Thus, our data provides preclinical evidence to support that next generation ASOs targeting AR in combination with AKT inhibition is a potentially effective treatment approach for CRPC.


Patients with advanced prostate cancer continue to develop lethal castration-resistant prostate cancer (CRPC) despite hormone therapy and maintaining castrate levels of serum androgen. Most CRPC appears to be dependent on the androgen receptor (AR), but instead of the C-terminal ligand-binding domain, it is the N-terminal domain (NTD) harboring a powerful transactivation do-
main that drives AR transcriptional activity. This was supported by the discovery of AR splice variants (ARv567es) and V7 which are constitutively acti-
vative and promote castrate-resistant prostate cancer (CRPC) growth. However, androgen deprivation eventually fails and PCa relapses, necessitating development of more effective therapies for CRPC.

#1584 Wnt/beta-catenin and Foxa2 axis activates AR signaling in castration resistant prostate cancer. Zachary M. Connelly, Shu Yang, Jiahe Li, Robert Garvey, Victor M. Darley-Ulmar, Selvarangan Ponnazhagan. University of Alabama at Birmingham, Birmingham, AL

Androgen-deprivation therapy has been identified to induce oxidative stress in prostate cancer (PCa), leading to reactivation of androgen receptor (AR) signaling in a hormone-refractory manner. Thus, antioxidant therapies have gained attention as adjuvants for castration-resistant PCa (CRPC). Here, we report for the first time that human endostatin (ES) prevents androgen-independent growth phenotype in PCa cells through its molecular targeting of AR and glucocorticoid receptor (GR), and downstream pro-oxidant signaling. This reversal following ES treatment significantly decreased PCa cell proliferation through downregulation of GR, and upregulation of manganese superoxide di-
mutase and reduced glutathione levels. Proteome and biochemical analyses of ES-treated PCa cells further indicated a significant upregulation of enzymes in major ROS scavenging machinery, including catalase, glutathione synthetase, glutathione reductase, NADPH-cytochrome P450 reductase, biliverdin reduc-
tase, superoxide dismutase, and glutathione peroxidase in reduction of intracellular ROS. ES further augmented antioxidant system through upregulation of glucose influx, pentose phosphate pathway (PPP) and NAD salvaging pathway. This shift in cancer cell redox homeostasis by ES significantly decreased the effect of protumorigenic oxidative machinery on androgen-independent PCa growth, suggesting that ES can suppress GR-induced CRPC phenotype upon AR antagonism, and dual targeting action of ES on AR and GR can be further translated to PCa therapy.

#1586 Developing novel inhibitors of S100A4 for neuroendocrine (NE) and metastatic prostate cancer: systematic testing using relevant models and drug development techniques. Mohammad Saleem,1 Arsheed A. Ganaie,2 Rehna Maqbool,1 Firdous A. Beigh,1 Syed Umber,1 Natalya G. Dulyani-
nova,2 Badrinath R. Komety1,2 Univ. of Minnesota Hormonal Inst, Austin, MN; Albert Einstein College of Medicine of Yeshiva University, Bronx, NY; 2Univ. of Minnesota, Minneapolis, MN

S100A4, a calcium binding protein has been well studied as a marker of fibro-
sis and metastasis. We recently showed that in addition of being a metastatic marker, S100A4 is in fact an onxogene that plays an important role in the develop-
ment of prostate cancer (CaP) and is amenable of targeting for the treatment of 
this lethal disease particularly neuroendocrine CaP (NE-CaP). Using a geneti-
cally engineered transgenic mouse model of NE-CaP, we show that knocking down of S100A4 significantly inhibited growth of prostate tumorigenesis and metastasis. Our noticeable finding is that S100A4 is secreted by prostatic tumors, 

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and extracellular/soluble S100A4 acts as a growth factor that has the ability to confer aggressive potential to less aggressive or indolent tumor cells. We show that serum-S100A4 level is highly elevated in human CaP patients with aggressive disease regardless of their serum-PSA levels. We next asked if small molecule inhibitors could be developed to inhibit the activity if intracellular as well as extracellular S100A4. We used a highly robust RNA interference/suppression/CRISPR/cas9 silico method, we screened a library of 5000 molecules and based on their binding efficacy to S100A4, identified potential inhibitors (SMI1 and SM2I). We next tested if SMI1 and SM2I bind to the S100A4 protein in biological solution. We generated recombinant S100A4 protein and standardized an isothermal titration Calorimetry (ITC) assay for S100A4 binding. The ITC analysis (Kcal vs time) showing significant binding to S100A4 protein nevertheless SM2I inhibitors exhibited higher binding affinity to S100A4 than SMI1. Next, we used Surface Plasmon Resonance (SPR) method (accurate and sensitive technique) for detecting binding of inhibitors to S100A4. The SPR data (densogram) shows that SMI1 and SM2I bind to the S100A4 protein. The binding of S100A4 to Myosin IIA (MIIA) is known to disrupt the latter’s monomer-polymer equilibrium. This phenomenon is captured in solution (image of disassembly of MIIA filaments and change in turbidity). Using disassembly/ or turbidity assays as an index of S100A4 activity (where recombinant S100A4 and MIIA are incubated +/− inhibitors), we show that SMI1 and SM2I inhibit the activity of S100A4 protein. Next, we tested efficacy of inhibitors in vitro and show that SM1 and SM2I therapies inhibit the growth, proliferation, migration and invasion of human prostate cancer cells (22Rv1). The activity of SMI1 and SMI2 inhibitors, particularly, SMI1 and SM2I therapies inhibited androgen-mediated transactivation and several androgen target protein expressions, including PSA and p21. Further investigations showed that addition in vivo studies were performed to demonstrate the effect of zinc (10-20 mg/kg) on prostate cancer cell lines were applied to confocal microscopy for intracellular trafficking of exogenous zinc, in vitro proliferation assays for their growth, prostate specific antigen (PSA)-based reporter-mediated transactivation, and Western blot for detection of androgen receptor (AR). PSA and ubiquitination. Further in vivo studies were performed to demonstrate the effect of zinc (10-20 mg/kg) on prostate cancer growth using syngeneic animals followed by tumor analysis. Results: Zinc chloride dramatically diminished during cancer development. Due to the obscure role of zinc in this process, therapeutic application using zinc and its supplement is very limited. This study aims to clarify the role(s) of zinc and its intervening mechanism. Material and methods: Treated with zinc chloride (15-150 μM), several prostate cancer cell lines were applied to confocal microscopy for intracellular trafficking of exogenous zinc, in vitro proliferation assays for their growth, prostate specific antigen (PSA)-based reporter-mediated transactivation, and Western blot for detection of androgen receptor (AR), PSA and ubiquitination. Further in vivo studies were performed to demonstrate the effect of zinc (10-20 mg/kg) on prostate cancer growth using syngeneic animals followed by tumor analysis. Results: Zinc chloride inhibited androgen-mediated transactivation and several androgen target protein expressions, including PSA and p21. Further investigation showed that addition of zinc chloride strikingly downregulated AR protein levels after 4 hours up to 24 hours in both human LNCaP and murine TRAMP C2 prostate cancer cell lines. AR downregulation resulted from facilitated protein degradation instead of transcriptional control. Further in vivo study was carried out using syngeneic mice bearing C2 subcutaneous tumors. Peritoneal injection of zinc chloride significantly reduced tumor size. Analysis of these tumors revealed that there were reduced expression of AR and increased cell death. Conclusions: Zinc has dramatic effect on prostate cancer cell growth and would be a promising therapeutic agent.
has been shown to inhibit incumbent oncogenic NF-κB pathway. These results also suggest that intracellular zinc inhibits cell growth via downregulation of AR to inhibit growth of prostate cancer. Considering that AR functions as a major effector in prostate cancer development and progression into castration resistent prostate cancer, loss of zinc may be a critical step for this devastating disease and further studies can be performed to develop zinc-based cancer therapeutics.

High intranuclear mobility of AR-v7 reveals distinct mode of transcriptional activity in prostate cancer with important therapeutic implications. Seaho Kim, Mohd Azrin Jamalruddin, Paraskevi Giannakakou. Weill Cornell Medicine, New York, NY.

It is well established that androgen receptor (AR) signaling, is a key driver of prostate cancer (PC) growth and metastatic progression. Therefore, androgen deprivation therapy (ADT) is the first line of treatment for PC. However, most patients develop castration resistant prostate cancer (CRPC). Interestingly, AR signaling remains active in CRPC, due to the expression of transcriptionally active AR splice variants (AR-Vs), which lack the ligand binding domain (LBD) and constitutively translocate to the nucleus even in castrade conditions. AR-v7 is the most prevalent AR-V expressed in about 60% of CRPC tumors. AR-v7 expression was clinically correlated with poor prognosis of CRPC patients and with resistance to next-generation AR signaling inhibitors, which are part of standard clinical care. Therefore, inhibition of AR-v7 function is urgently needed for the treatment of CRPC. Currently, there is no therapeutic modality that can inhibit AR-v7 expression or activity. Mechanistically, AR-v7 transcriptional targets largely overlap with those of AR-ß, with the exception of a few AR-v7 unique targets. However, the exact mechanism by which transcription is activated by AR-v7 is not known. In this study we sought to investigate the mechanisms underlying the transcriptional activity of the ligand-independent AR-v7 in comparison to liganded AR-ß. We used live cell imaging to monitor the dynamics and intranuclear mobility of fluorescently-tagged AR-ß or AR-v7. Fluorescent recovery after photobleaching (FRAP) revealed that AR-v7 intranuclear mobility was significantly faster than that of liganded AR-ß, with 11/2.3 s versus several minutes, respectively. To precisely map the spatial distribution and chromatin-binding kinetics of AR-ß and AR-v7, we generated expression plasmids with AR tagged with green-to-red mEos4 photo-convertible proteins. We investigated the relationship between rates of intranuclear mobility and transcriptional activity and the mechanisms underlying the distinct mobility patterns. These data suggest that AR-v7 has a distinct mode of interaction with DNA and gene promoters, which may identify novel targetable pathways for its inhibition in CRPC.

Inhibition of prostate tumor growth by an extract from the muscadine grape. Patricia E. Gallagher, E. Ann Tallant. Wake Forest School of Medicine, Winston-Salem, NC.

Despite decades of advancements, cancer remains the second leading cause of mortality in the United States with an estimated 1.7 million new cases and over 600,000 deaths predicted for 2016, indicating a clear need for more effective chemotherapeutic and chemopreventive agents. While natural products derived from plants have served as an abundant source of anticancer agents, it is predicted that less than 2 percent of the plant species with medicinal value have been investigated. Muscadine grape (Vitis rotundifolia) and/or seed extracts are a popular nutraceutical supplement due to the high anti-oxidant, anti-inflammatory, anti-angiogenic factors. MGE reduced VEGF mRNA from 1.02 ± 0.06 to 0.72 ± 0.07 relative units (p < 0.05) and PLGF mRNA from 1.03 ± 0.11 to 0.63 ± 0.07 relative units (p < 0.05), providing additional evidence of an anti-angiogenic effect of the extract. Collectively, these results are the first to demonstrate that a novel MGE formulation reduces prostate tumor growth in a mouse model, by inhibiting angiogenesis, suggesting that MGE may be an effective treatment for prostate cancer.

Anti-cancer evaluation of various solvent extracts of blue honeysuckle berry (Lonicera caerulea L.) against prostate cancer cells. Syed M. Ali,1 Alex Ourch,2 Chun-Tao Che,2 Min-Ying Wang,3 Gnanasekaran Munirathnam.4 University of Illinois College of Medicine, Rockford, IL; 5University of Illinois at Chicago, Chicago, IL.

Prostate cancer (PCa) is the second most common cancer among men in the United States. It is estimated 1 in 6 men will be diagnosed with PCa and approximately 180,890 new cases are predicted in 2016 alone. Standard treatments for PCa include surgery, radiation, chemotherapy and hormonal therapy or a combination of these treatments. However, factors like patient age, disease stage, drug resistance, specificity, and toxicity can result in poor disease prognosis. In order to overcome these limitations and improve patient prognosis, there is an urgent need to identify new anti-cancer agents with minimal side effects. Current studies are being focused on natural products and their components for alternative therapeutics. Blue Honeysuckle (Lonicera caerulea L.) a berry native to northeast Asia, is known to be rich in Vitamin C and polyphenols such as anthocyanins, flavonoids, and phenolic acids. Polyphenols are found to have several therapeutic effects such as anti-inflammatory, antioxidant and antimicrobial properties. Our present study used sequential solvent extracts of Blue Honeysuckle (BHS) berry using Hexane, Ethyl Acetate, Methanol, and Water respectively. These fractions were used to assess various therapeutic effects of BHS on DU 145, PC-3, C4-2 and LNCaP PCa cell lines. The goal was to identify the most potent BHS fraction that is effective against PCa using pre-clinical studies. MTT assays were used to identify the anti-proliferative effects of various BHS fractions. The above indicated PCa cells were treated with different doses (10-150 µg/mL) of BHS fractions over various time periods (24, 48, 72 hr). Our data revealed that Hexane extract (HE) exhibited the highest inhibition of cell viability in a time and dose-dependent manner. HE fraction showed to have an IC50 of 89.6 µg/mL for DU 145, 117.4 µg/mL for PC-3, 163.3 µg/mL for C4-2 and 140.84 for LNCaP cells. Moreover, BHS HE fraction showed a decrease in migration capacity and colony formation ability of PCa cells in vitro. Cellular senescence assay was performed to assess β-Galactosidase activity in PCa cells treated with BHS HE extract. All the PCa cell lines treated with 100 µg/mL of BHS HE showed increased senescence. Western blotting was performed to identify the potential anti-cancer mechanism of BHS against PCa using DU 145 and PC-3 cell lines. Results from this study indicated that DU 145 and PC-3 cell lines after 24 hr treatment with 100 µg/mL of various BHS fractions especially BHS HE showed an increase in apoptosis (Caspase 3,8,9 and PARP-1) and autophagy (LC3 A/B) markers. Furthermore, cell cycle analysis of DU 145 and PC-3 cells treated with BHS HE extract showed increased apoptotic cells. Further analysis of apoptotic and autophagy pathways modulated by BHS treatments are required to illustrate its potential underlying anti-cancer mechanisms in PCa. In conclusion, results from our study warrant further evaluation of BHS berry for potential PCa management.

Neutralization of IL-10 enhances antitumor efficacy of dendritic cell targeting MIP-3θ-gp100 vaccine by way of type-I interferons in B16F10 mouse melanoma model. James Gordy, Kun Luo, Richard Markham. Johns Hopkins School of Public Health, Baltimore, MD.
The chemokine MIP-3α (CCL20) binds to CCR6 found on immature dendritic cells. Vaccines fusing MIP-3α to gp100 have been shown to be effective in therapeutically alleviating melanoma in mouse models. Other studies have provided evidence that IL-10 neutralizing antibodies enhance immunological melanoma therapies by modulating the tolerogenic tumor microenvironment. Here, we investigated whether IL-10 or IL-10R antagonists lead to a more efficacious combination with a cytokine fusion vaccine in murine melanoma. We found that a therapeutic protocol using IL-10 antibody intra-tumorally beginning on day 5, once every three days for up to six doses. Tumor sizes, growth, and survival were all assessed. Treatment responses were characterized by flow cytometric analysis of tumor infiltrate. Vaccine-specific T-cells were delineated by gp100 + , stimulation followed by intracellular cytokine staining for IFN-γ and assessment by flow cytometry. The mechanism of IL-10 efficacy was explored by RT-PCR and confirmed with a knockout mouse model. With this therapeutic protocol, we demonstrate for the first time that a therapy neutralizing IL-10 additively enhances the anti-tumor efficacy of a MIP-3α-gp100 vaccine, leading to significantly smaller tumors, slower growing tumors, and overall increases in mouse survival. Surprisingly, the additive effects of IL-10 were not shown to be directly mediated by any T-cell parameter tested, including vaccine-specific tumor infiltrating lymphocytes (TILs), total TILs of either CD4+ or CD8+ subset, regulatory T-cells, granzyme positive T-cells, and others. We discovered, however, that IFN-γ transcripts in the tumor were significantly upregulated in mice given vaccine and αIL-10 compared to vaccine alone. Furthermore, infiltration into the tumor of plasmacytoid dendritic cells, known to be professional IFN-γ-producing cells, were enhanced with the combination therapy. A mouse model with IFNγRa1 knocked out eliminated the protection provided by αIL-10, demonstrating that the additional therapeutic value of αIL-10 is primarily mediated by type-1 interferons. In conclusion, efficient targeting of antigen to immature dendritic cells with a chemo-kine fusion vaccine offers a potential alternative approach to the ex vivo dendritic cell antigen loading protocol currently undergoing clinical investigation. Combining this approach with an IL-10 neutralizing antibody therapy that modulates the tolerogenic tumor microenvironment offers promise as a novel melanoma therapy.


Cancer immunotherapy represents a promising therapeutic approach to extend the overall survival of cancer patients. However, several mechanisms within the tumor microenvironment orchestrate the suppression of host immune response requiring combination strategies to prolong the durability of effect. Among suppressive pathways, up-regulation of PD-L1 and its interaction with PD-1 receptor plays a key role in suppression of T-cell activity; a mechanism also called adaptive immune resistance. Therefore, approaches able to inhibit the tumor immune suppressive mechanisms along with those expanding the frequency of intra-tumor T-cells and enhancing/prolonging their functionality are required. CEA TCB (RG7802, RO6958688) is a novel T cell bispecific antibody targeting carcinomembrinogenic antigen (CEA) on tumor cells and CD3 on T cells, currently being investigated as single agent and in combination with atezolizumab in Phase 1/1b studies in patients with advanced or metastatic CEA-expressing tumors (NCT02324257; NCT02620573). CEA TCB treatment leads to increased intra-tumor T cell infiltration and T-cell activation along with up-regulation of PD-1/PD-L1 suppressive pathway. Here we show that combination of CEA TCB with PD-L1 blocking antibody in vitro enhances cell activation and in vivo elicited a potent combination of pro-inflammatory cytokines. Combination in vivo performed in both stem cell humanized NOD mice (HSC mice engrafted with MK454) and fully immunocompetent human CEA transgenic C56BL/6 mice (hCEA Tg mice engrafted with MC38-hCEA) demonstrated significantly improved anti-tumor activity of combination as compared to activity of single agents, yielding to increased number of tumor-free animals. Randomization of animals that progressed to CEA TCB monotherapy revealed that combination of CEA TCB with PD-L1 blocking antibody is required to control tumor outgrowth, as tumors treated with corresponding monotherapy arms progressed to treatment. Efficacy of CEA TCB was also potentiated when administered in combination with a half-life-extended IL-2 variant (untargeted (IgG-IL2v) or fibroblast-activating protein-targeted IL-2 variant (FAP-IL2v)), resulting in stronger tumor growth inhibition in MKN45-bearing HSC mice or prolonged survival in PanCo2-hCEA-bearing hCEA Tg C56BL/6 mice. Synergy likely reflects ability of IL2v to enhance anti-tumor efficacy by increasing number of effector T cells in tumors. In conclusion, CEA TCB combined with PD-L1 inhibitor leads to increased anti-tumor response. This is accompanied by up-regulation of PD-1/PD-L1 suppressive pathway, which can be overcome by combination therapy with a PD-L1 inhibitor. In vivo efficacy of CEA TCB is further potentiated when administered in combination with immunotherapies that increase the pool of available tumor-infiltrating effector cells.
ici ty and symptomatic disease progression. Patients treated at the higher doses developed a striking end of treatment peripheral blood lymphocytosis showing a 24X increase in absolute lymphocyte count, 10X increase in CD8 cells and 75X increase in NK cell numbers. No patients developed anti-IL-2 antibodies. Steady state serum IL-15 level was seen within the first 12 to 24 hours of therapy and was maintained for several days. Dramatically lower IL-15 levels (~ 8% of early Cmax) were measured during the last 2 days of treatment suggesting a large reservoir of non-circulating IL-15 receptor positive cells. None of the patients achieved a partial response, but 2 patients met the criteria for treatment beyond cycle 2 (~15% decrease in measurable disease). Evidence for tumor directed immune effects included several patients who developed unilateral pleural effusions in their disease involved hemithorax, complete regression of a cutaneous chondrosarcoma metastasis and decreased CA125 (125.7 → 47.3 U/mL) in a patient with pancreatic cancer. Histologic analysis of pleural fluid from these patients showed largely lymphocytes and other cells that were consistent with the patients known cancers. Lymphocytic infiltration of the regressing cutaneous sarcoma metastasis was predominantly CD4 cells, with some CD8 cells and a few NK cells. Immunohistochemical analyses of pre and on treatment tumor biopsies obtained from several other patients are currently being performed to better characterize the immunologic effects of this regimen. The results of this trial indicate that NKTR-124 is an immunotherapeutic with important synergistic properties for combination therapy with other Immunotherapeutics.

**#1597** Inhibition of the novel therapeutic target pregnancy associated protein plasma protein A (PAPP-A) in Ewing sarcoma enhances efficacy of IGF1R targeting in vivo. Sabine Heitzeneder,1 John F. Shern,2 Lee J. Helman,2 Javed Khan,2 Crystal L. Mackall1. 1Stanford University, Palo Alto, CA; 2National Cancer Institute, Bethesda, MD.

Background: Despite intensive treatment regimens, Ewing sarcoma (EWS) patients with metastatic or relapsed disease still face a 3-4 year overall survival of less than 20%, indicating a clear need for novel targeted therapies. Utilizing ribosomal depleted RNA sequencing of 122 EWS samples (49 cell lines and 73 tumor samples) and 96 normal samples of variable tissues, we previously reported that overexpression of PAPP-A is one of the top 5 genes overexpressed in EWS (~ 4 fold) compared to normal tissue. Notably, PAPP-A showed substantial expression in tumors (median log2FPKM = 3.933, n = 122) and minimal expression in normal tissue (median log2FPKM = −1.564, n = 96). Pregnancy Associated Plasma Protein A (PAPP-A) is a secreted zinc metalloproteinase, that anchors to cell surface of heparan sulfate proteoglycans and enhances local IGF signaling. Through the release of IGFs from IGFBP-4, IGFBP-2 and IGFBP-5, free, bioactive IGF is being increased in close proximity to its receptors. In pregnancy PAPP-A is highly expressed by placental trophoblasts and represents a key regulator of fetal growth. The impact of IGF signaling in EWS is illustrated by the modest activity of IGF1R inhibition as a single agent treatment in clinical trials, an approach that is limited by the rapid development of resistance. Methods: In an attempt to investigate the role of PAPP-A in Ewing sarcoma, we used CRISPR/Cas9 technology to target the PAPP-A locus in EWS cells lines and generated PAPP-A knockout clones (EWS, TC32) for further investigation in vitro and in vivo. Results: Tumor models (additional details on this work to be presented at the meeting) demonstrates equivalent affinity and ligand-blocking ability as FPA008. Utilizing a xenograft NSG mouse model, as a single agent treatment as well as in conjunction with IGF1R inhibition, Results: Knockout of PAPP-A in EWS cells utilizing CRISPR/Cas9 technology completely abrogated PAPP-A secretion and metalloprotease activity in single cell clones, resulting in significantly increased complexed IGF-4 and diminished bioactive IGF-1, together with decreased cell growth in vitro. This phenotype could be rescued by recombinant, soluble PAPP-A. In vivo experiments showed that treatment with a monoclonal PAPP-A neutralizing antibody that inhibits the IGF4-IGF-1 receptor activity of PAPP-A delays tumor growth of xenografted EWS tumor cells (EWS) in NSG mice. Furthermore, in conjunction with IGF1R inhibition (mAb 1b7c10), PAPP-A significantly accelerated tumor growth and resulted in prolonged survival of mice (p = 0.021). Conclusions: PAPP-A is a novel biologically relevant and highly tumor specific cell surface target in Ewing sarcoma. Our data suggest that neutralization of PAPP-A is a new therapeutic option that merits further evaluation for the treatment of Ewing’s Sarcoma.

**#1598** Single agent NKTR-214, a biased IL2 pathway agonist, increases immune cell infiltrates in brain tumors and prolongs survival in rodent (rat) glioblastoma (GBM). Lawrence Recht1, Seema Nagpal2, Nancy Schmidt,2 Vuong Trieu,1 Lawrence Schwartz1, Tih-Woong Jung,1 Milton Merchant,1 Irene Choi,2 Ute Hoch,2 Deborah Charych2. 1Stanford School of Medicine, Stanford, CA; 2Nektar Therapeutics, San Francisco, CA.

Background: Immunotherapy is an attractive option for brain tumor therapy if a robust infiltrative T cell response can be elicited in the tumor. NKTR-214 is a CD122-biased cytokine agonist conjugated with multiple releasable chains of polyethylene glycol and designed to provide sustained signaling through the heterodimeric IL-2 receptor pathway (IL-2Rβγ) to preferentially activate and expand effector CD8+ T and NK cells over Tregs. To assess the potential activity of single agent NKTR-214 in GBM, we used an orthotopic rat glioblastoma survival model. Methods: NKTR-214 was administered at 0.1 or 0.3 mg/kg qw iv to Sprague-Dawley rats starting 2 or 7 days (D2, D7) post (p)-implantation of 10^6 C6 glioma cells into the right striatum. The model requires euthanasia by −D14 due to tumor burden. Brain tumors were characterized by magnetic resonance (MR) and immunohistochemistry (IHC) for infiltration of CD4+ and CD8+ T cells and for retention of PEG polymer in brain tumor. Results: Compared to rats receiving saline, survival was significantly prolonged after NKTR-214 treatment (n = 43, mean 17.2 vs. 10.0 days (P < 0.001) with 15% of rats across all groups alive and tumor-free at Day 50 when the study was terminated. Both doses were equally effective and well tolerated. Surprisingly, treated rats bearing large MR-detectable D7 tumors survived significantly longer compared to rats bearing microscopic D2 tumors (6/21 or ~30% versus 0/21 or 0% respectively at D50, mean 23.1 vs. 12 days, P < 0.004). Concordantly, CD8+ T cells in D7 tumors were significantly increased after NKTR-214 therapy compared to vehicle and D2 tumors, while CD4+ remained low with no significant difference between groups. PEG polymer was detected in the tumor at least 72 hours p-injection. Conclusions: NKTR-214 is well tolerated, prolongs survival and induces immunological activity in the brain when administered to rats harboring large GBM. While there was no significant dose dependence, a marked increase in survival was observed when larger D7 tumors were treated with NKTR-214 compared to microscopic D2 tumors, associated with increased intratumoral CD8+ T cells. Levels of CD4+ were unchanged, consistent with the mechanism of CD-122 biased activation of the IL2 pathway. While requiring further study, it is intriguing that the increased sensitivity of larger tumors also correlates to onset of angiogenesis and rapid tumor growth in this model. NKTR-214 is currently being evaluated in an outpatient Phase 1 / 2 clinical trial for the treatment of solid tumors. The results presented suggest a potential role for NKTR-214 in the treatment of patients afflicted with GBM.


The colony stimulating factor 1 receptor (CSF-1R) signaling pathway promotes tumor progression via the recruitment, differentiation, and survival of immunosuppressive tumor-associated macrophages (TAMs). FivePrime has developed cabiralizumab (FPA008), an IgG4 antibody against CSF-1R that blocks the ability of both, CSF-1 and IL-34 to bind and activate this receptor, thereby modulating the immune response to tumorigenesis. In order to investigate the impact of CSF-1R signaling inhibition in preclinical models, we generated a surrogate antibody, cmFPA008, which targets mouse CSF-1R and demonstrates equivalent affinity and ligand-blocking ability as FPA008. Utilizing a combination of flow cytometry and immunofluorescence analyses, we have characterized the biological effects of CSF-1R inhibition, including significant reduction of immunosuppressive TAMs and an increase in tumor PD-L1 expression. Interestingly, we observe a transient increase in CD8+ T cell number and activation upon TAM depletion, followed by a subsequent increase in MDSC populations that correlates with reduction of the CD4+/ CD8+ T cell numbers. Moreover, we have used murine syngeneic tumor models to examine the anti-tumor impact of CSF-1R inhibition in combination with other immunoncology agents. Our results show that, when added to PD-1/PD-L1 blockade, cmFPA008 can significantly enhance anti-tumor efficacy. We are currently exploring the effects of combining cmFPA008-induced TAM depletion with additional immunoncology agents, including T cell agonists. Our preclinical observations demonstrate that inhibition of the CSF-1R pathway can combine with various immunoncology agents with distinct mechanisms of action. FivePrime has initiated a clinical trial in collaboration with Bristol-Myers Squibb (BMS) to investigate the use of cabiralizumab in combination with nivolumab (anti-PD-1, OPDIVO®) in six different tumor types.

**#1600** Interleukin-8 (IL-8) in TGF-β inflammatory and chemothera-
py. Larn Hwang1, Kevin Ng2, Osmond D’Cruz,2 Sanjive Quazi,2 Andrew Schumich2, Barbara Naghavi1, Vuong Trieu1. 1Oncotelic Inc, Agoura Hills, CA; 2Autotelic Inc, Costa Mesa, CA.

Background: Increased expression of IL-8 and/or its receptors has been characterized in cancer cells, endothelial cells, infiltrating neutrophils, and tumor-associated macrophages, suggesting that IL-8 may function as a sig-

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significant regulatory factor within the tumor microenvironment. OT-101 is a phosphorothioate antisense oligodeoxynucleotide targeting transforming growth factor-beta 2 (TGF-β2). Herein, we examined the role of IL-8 in OT-101 treatment followed with chemotherapies during our Phase II trial in patients with advanced pancreatic cancer (PAC). Methods: Time evolution of a panel of 31 cytokines and chemokines in plasma of 49 PAC patients over 3 cycles of OT-101 administration (140 mg/m²/day) for 12 PAC patients. Samples were acquired before onset of OT-101 therapy and at 8 selected time points (Cycle 1 [Day 2 and 5], Cycle 2 [Days 1, 2, and 5], Final Visit, Cycle 3 [Day 5]) during the therapy. Samples were measured in duplicate and concentrations were expressed in pg/ml. Standardized log10 transformed values calculated from the mean and standard deviation of each cytokine/chemokine in each patient was utilized in an ANCOVA model to investigate the correlation with Overall Survival (OS). Results: Clustering of correlation coefficients resulted in the identification of three highly correlated subsets of cytokines/chemokines (Cluster 1: EGF, MIP-1α, MIP-1β, Cluster 2: FGF-2, IL-1α, MIG, IP-10, IL-15, IFN-α, IL-12α/IL-12β; Cluster 3: HGF, IL-2R, IL-6, IL-8). Protein-Protein interactions were constructed using STRING10 algorithm identified a relationship between IL-8 and TGFβRII inhibition. The ANCOVA model explained a significant proportion of the observed data for Cycle 1 [Day 2] measurements of cytokines/chemokines (R² = 0.3, F = 5.2, P = 1.575, P = 0.0103). Other time points did not exhibit a significant model fit for significant relationships in the interaction term. IL-8 expression showed significant association with OS (positive association, N=12, T-value = 2.92, P = 0.0039). Cycle 1 [Day 2] measurements (7% False Discovery Rate). Linear regression of the increase in IL-8 levels during Cycle 1 [Day 2] treatment with OT-101 was related to an increase in OS outcome (R² = 0.54). To further define the appropriate chemotherapeutics combination with OT-101, we evaluated paclitaxel (PTX), gemcitabine (GEM), and dacarbazine (DTIC) in animal models. Activity was observed with PTX and DTIC, but not with GEM. Both PTX and DTIC induced IL-8 expression whereas, surprisingly, GEM reduced IL-8 expression. Conclusion: IL-8 expression during early phase of OT-101 treatment cycle was positively associated with OS across 12 patients. IL-8 is also induced by PTX and DTIC which synergized with OT-101. The data suggests that PAC and DTIC are acting on the same target as our TGFβRII inhibitor; and potentially, the beneficial effect of these chemotherapeutics is a result of chemotoxin-induced immune IL-8 response.

#1601 Anti-tumor effect of GAGomer-mediated intra-tumoral IL-2 expression following systemic administration. Genia Alpert,1 David Albreuter,2 Sunil Anamandla,3 Arllyssa Birt,4 Guy Cinamon,5 Keren Cohen Merimi,1 Ori Even Or,1 Nir Gefen,6 Nadia Gurvich,3 Jeno Gyuri,2 Lorena Lerner,7 Adi Mondshine,1 Hong Wang.1 1Cancer Immunology, Ness Ziona, Israel; 2Cancer Therapy, Lexington, MA.

Cancer immunotherapies are revolutionizing cancer treatment. Unfortunately, a large proportion of patients with solid tumors do not respond to currently available immune-therapeutics. The lack of response is due to a variety of mechanisms tumors adopt to avoid immune mediated clearance. The multiplicity of immunosuppressive mechanisms operational in the tumor microenvironment may not be overcome by single agents and require interventions at multiple control points. However, systemic exposure to combinations of immunoregulators may result in severe, dose limiting, acute and chronic toxicities that might be prevented if the effect of these agents is focused to the tumor microenvironment. We are engaged in the discovery of a novel class of immuno-oncology drugs aimed at maximizing the effect of immunoregulatory molecules in the tumor microenvironment and minimizing systemic adverse effects. These drugs incorporate plasmids, engineered to program tumor cells to produce and secrete immune-regulatory proteins, within hyaluronic acid (HA) coated lipid nanoparticles, called GAGomers, which specifically target tumor cells that overexpress activated HA receptors (GAG-pDNA). GAG-pDNA based therapeutics promise highly potent but localized activation of the immune system exclusively in the tumor microenvironment following delivery of an agent/chemo, leading to the destruction of tumor cells by activated immune cells without debilitating toxic side effects. To demonstrate the feasibility of the GAG-pDNA approach we have incorporated a plasmid directing the expression of murine IL-2 into GAGomers (GAG-pIL2) and assessed the anti-tumor activity of the construct after systemic delivery into tumor bearing mice. GAG-pIL2 administration resulted in statistically significant inhibition of tumor growth, which correlated with elevated IL-2 levels in the tumor and increased infiltration of T-cells into the tumor microenvironment. These experiments demonstrate the feasibility of programing tumor cells using GAG-pDNA to produce and secrete immunoregulatory molecules into the tumor microenvironment and trigger robust anti-tumor immune responses.

#1602 Generation of anti-IL-17B antibodies neutralizing IL-17B-mediated alterations of the immune microenvironment, promotion of tumor cell initiating capacity and chemoresistance. Emilie Lapirotte,1 Aurélie Docquier,1 Jeremy Bastid,1 Cécile Djoué,1 Marion Lapierre,1 Gilles Alberici,2 Armand Bensussan,3 Jean-François Eliaou,1 Nathalie Bonnefoy,4 1Orega Biotech, Ecully, France; 2INSERM U976, PARIS, France; 3INSERM U1194, Montpellier, France.

Interleukin-17B (IL-17B) is a pro-inflammatory cytokine that belongs to a family encompassing 6 interleukins (IL-17A to F) and binds to the IL-17 receptor B (IL-17B). Recently, amplified IL-17B/IL-17B signaling was found critical for breast and pancreatic tumorigenesis and elevated expression of IL-17B has been associated with the shortest survival rates in patients with breast or pancreatic cancer. Using IL-17B knock-out (IL-17B KO) mice we demonstrate here that melanoma, fibrosarcoma and breast cancer cell tumorigenicity is strongly impaired in immunocompetent IL-17B KO mice compared to WT littermates, including a large number of tumor free mice. Reduced tumor incidence in IL-17B KO mice is associated with alterations of the immune tumor microenvironment especially within innate lymphocyte and myeloid sub-populations. We further demonstrate that IL-17B is a key cytokine shaping the tumor initiating cancer cell niche. Indeed, MDA-MB-468 human breast cancer cells overexpressing IL-17B exhibit 10 times higher frequency of tumor initiating cells when xenografted at a serial limiting dilution in nude mice. Tumor progression is, again, associated with alterations of NK cells within the tumor microenvironment and reduced percentage of CD8 T cells, a phenotype associated with breast cancer stem cells (CSC). This is associated with resistance to conventional chemotherapeutic agents such as taxol, an effect that is totally abrogated by disrupting IL-17B/IL-17B signaling with a neutralizing antibody. Altogether our results point out the key role of IL-17B in regulating the immune microenvironment as well as cardinal features of CSC, one of the alleged causes of resistance to therapy and tumor relapse. Therefore, blockade of IL-17B and its receptor appear as potential therapeutic targets for cancer immunotherapy. Collectively, these data support the ongoing development of IL-17B neutralizing antibodies.

#1603 NKTR-255 engages the IL-15 pathway driving CD8 T cell survival and CD8 memory T cell proliferation. Peiwen Kuo, Mekhali Maita, Phi Quach, Murali Addepalli, Arunasee Lanka, Poornachandra Mathamsetti, Christie Fanton, Ping Zhang, Peter Kirk, Takahiro Miyazaki, Jonathan Zalesky. Nektar Therapeutics, San Francisco, CA.

Background: IL-15, an immunostimulatory cytokine, plays an important role in both the innate and adaptive immune system. Notably, this pleiotropic cytokine is a key regulator of homeostasis and survival of CD8 and CD8 memory T cells. Targeting the IL-15 pathway has therefore become a promising therapeutic approach in oncology through the induction of long-term T cell activation and durable memory responses. NKTR-255 is a polymer-engineered IL-15 that as a single-agent reduces tumor burden in various tumor models. Here, we show that the anti-tumor effects of NKTR-255 can be attributed to its modulation of survival and proliferation of CD8 and memory CD8 T cells. Methods: Immunophenotyping of CD8 T cell subpopulations was performed in naive and tumor-bearing Balb/c mice treated with NKTR-255. Cell surface staining of CD3, CD8, CD44, CD62L and Sca-1 was conducted to identify effector (Tem), central (Tcm) and stem (Tscm) memory T cells. Intracellular staining of IFN-γ and Bcl-2 were also analyzed by flow cytometry. Human whole blood and PBMCs were stimulated with IL-15 (0.0001-1000ng/ml) or NKTR-255 (0.001-10,000ng/ml). At various time points pSTAT5 response in CD3, CD4, CD8 and NK (CD56 bright and dim) cells was monitored by flow cytometry. Results: In naive mice, single dose NKTR-255 (dose range 0.06-1mg/kg) increased the proliferation of Tem, Tcm and Tscm CD8 T cells in a dose-dependent manner. Treatment at 0.3 and 1mg/kg dose levels increased proliferation at least 4 fold across the CD8 memory populations. The abundance of CD8 and CD8 memory T cells was still apparent 6 days post-treatment unlike single dose IL-15 which was ineffective. In a CT-26 lung metastasis model, NKTR-255 reduced the number of lung nodules in a dose-dependent manner. A 0.3mg/kg dose level increased Bcl-2 mRNA expression in MFI 1.5 fold in CD8 T cells. Furthermore, administration of NKTR-255 at 0.3, 1 or 3mg/kg significantly increased CD8 proliferation in a dose-dependent manner in blood (1.7, 4.6 and 5.3 fold) and spleen (2.5, 5.7 and 6.9 fold) compared to vehicle. The enhanced Bcl-2 expression and CD8 proliferation were accompanied by elevated CD8 T cells in blood (1.5, 2.4, and 3.4 fold increase) and spleen (1.5, 2.3 and 3.3 fold increase). Consistent with increased proliferation and increased Bcl-2 levels observed in vivo, both IL-15 and NKTR-255 showed dose-dependent induction of pSTAT5, a modulator of Bcl-2 expression, in CD4 and CD8 T cells from human whole blood and PBMCs. Conclusions: NKTR-255 effectively engages the IL-15 pathway as evidenced by its strong induction of
CD8 and memory CD8 T cell proliferation and promotion of survival. Combined with sustained activity and potency in human blood and PBMCs, our results support NKTR-255 as a novel tumor immunotherapeutic with great potential.

**#1604 NKTR-214 synergizes with radiotherapy to drive tumor regression.**

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The purpose of this study was to investigate therapeutic and mechanistic synergies between single-dose radiotherapy and systemic administration of NKTR-214. NKTR-214 is a CD122-biased cytokine agonist conjugated with multiple releasable chains of polyeethylene glycol. NKTR-214 is designed to provide sustained signaling through the heterodimeric IL-2 receptor pathway (IL-2Rβγ) to preferentially activate and expand effector CD8+ T and NK cells over Regulatory CD T cells. Preclinical models demonstrated NKTR-214 preferentially expands effector CD8+ T cells and NK cells within the tumor resulting in marked tumor growth suppression as a single-agent and in combination with checkpoint inhibitors. A phase I/Ii trial is in progress to evaluate NKTR-214 safety and efficacy in an outpatient setting. Radiation therapy can induce anti-gene release and epothe spreading, while NKTR-214 can activate and expand antigen-specific effector populations. We hypothesized that the combination of systemic NKTR-214 and local radiotherapy would generate better therapeutic responses than either treatment alone. In this study, we evaluated the combination of NKTR-214 and radiotherapy in syngeneic, providing significantly better anti-tumor responses than either monotherapy. Consistent with previous observations, NKTR-214 alone induces expression of a wide range of activation markers expressed by CD4 and CD8 T cells as well as NK cells in the blood, lymph nodes and tumor. The combination of radiotherapy and NKTR-214 was found to have several unique effects including a significant increase in (>75%) in the absolute numbers of lymphocytes in the peripheral blood, increased expression of activation markers (CD25, PD-1) by CD T cells in the blood and tumor, and increased density of tumor-infiltrating NK cells. Evaluation of tumor infiltrating lymphocytes (TIL) in Nurr77-GFP reporter mice revealed that the combination of NKTR-214 + RT resulted in a higher frequency of recently activated (Nurr77-GFP+) CD8 T cells in treated (irradiated) and unactivated (non-irradiated) tumors. Whole tumor mRNA profiling and multi-spectral histology of these tumors is being assessed to identify key differences in the tumor-microenvironment that may help to define the underlying mechanism of action. Taken together, these data provide evidence of synergy between localized radiotherapy and systemic NKTR-214 treatment via an expansion of activated, tumor-specific CD8 T cells.

**#1605 Combined oral cytokine therapy effectively treats colon cancer in a murine model.** Neal Bhutiani, Qingsheng Li, Charles D. Anderson, Tao Gu, Deborah H. Charych, William L. Redmond. Onkaido Therapeutics, Cambridge, MA; Moderna Therapeutics, Cambridge, MA; Iowa, Iowa city, IA.

Background: Regardles of its etiology, colorectal cancer development is intimately associated with dysregulation of the intestinal immune system. Sustained release, orally delivered IL-10 not only inhibits colon tumor growth but restores T cell homeostasis both locoregionally and systemically. However, it does not induce tumor regression. We aimed to combine orally delivered IL-12, a potent immunostimulatory molecule with robust anti-tumor effect, with oral IL-10 therapy to induce tumor regression. Methods: 6 week old APCmin mice were inoculated with enterotoxic Bacteroides fragilis (serotype 086) after 5 days of intestinal flora depletion with antibiotics dissolved in drinking water. 7-10 days after bacterial inoculation, mice were treated with three doses, including (i) IL-10 and blank polycylic acid microspheres for 3 weeks, (ii) IL-10 containing microspheres (0.5 microgram/dose) for 3 weeks, (iii) IL-12 containing microspheres (0.05 micrograms/dose) for 3 weeks, or (iv) IL-10 containing microspheres for 1 week followed by a combination of IL-10 and IL-12 containing microspheres for 2 weeks. After euthanasia, colons were assessed for polyp counts and select polyps were isolated for confocal microscopy analysis. Mesenteric lymph node lymphocytes were isolated and analyzed using flow cytometry. Results: Mice treated with oral IL-10 and IL-12 demonstrated a reduction in polyp number compared to those mice treated with IL-10 alone, and both groups had fewer polyps than mice treated with either IL-12 or blank microspheres. Addition of IL-12 resulted in increased CD8+ T cell numbers and IFN-γ production compared to treatment with IL-10 alone. IL-10 retained its effect on CD4+ T cells when combining it with IL-12, resulting in a decrease in conventional regulatory T cells (Foxp3+/RORγt-) as well as pathogenic regulatory T cells (Foxp3+/RORγt+) and IL-17 producing CD4+ T cells. Conclusions: The addition of IL-12 to IL-10 therapy augments the therapeutic effect of oral cytokine treatment on colon adenocarcinoma in the APCmin B. fragilis model. The effect appears additive and mediated by both IL-10’s effect on regulatory T cell populations and IL-12’s effect on CD8+ T cells. Further studies in the setting of more advanced disease, including liver metastases, will elucidate the therapeutic potential of combination oral cytokine therapy with IL-10 and IL-12 for treatment of colorectal cancer.


Background: RNA aptamers are small RNA molecules that bind antigens like antibodies and are currently being explored as alternatives to antibodies for diagnosis and therapy. A potential merit of aptamers is that they can be generated against native cellular antigens, such as those with unique post-translational modifications or receptor-ligand complexes, for which antibody generation can be difficult. Here, we report the use of a cell-based systematic enrichment approach (SELEX) to develop a novel Treg-binding RNA aptamer specific to IL2Rα-IL2 receptor-ligand complex. Methods and Results: A. Generation of Treg-binding aptamers: We designed a cell-based SELEX strategy to generate RNA aptamers specific to human T regulatory (Treg) cells. The starting library consisted of random RNA aptamers with a structural diversity of ~1012. Aptamers against common T cell antigen were pre-cleared using CD4+CD25+ T cells. Treg-binding aptamers were then positively selected using CD4+CD25+ Tregs from the same donor. After amplification of Treg binders by RT-PCR, the whole selection was repeated eight times, each time with T cells from a different donor. At the end, SELEX-enriched aptamer pools predominantly bound to Tregs, but not to Teff cells, which were then sequenced. The most prevalent Treg-binder, Tr-1, showed 3,875-fold enrichment by the end of SELEX (from 0.36 copies in the starting library to 22,995 copies in the eighth round per million total reads). B. Tr-1 binds to IL2Rα-IL2 complex. We tested if Tr-1 recognized human IL2Rα by measuring Tr-1 binding to recombinant IL2Rα protein using RT-qPCR. Results indicated that Tr-1 bound to IL2Rα and showed ~3-fold higher binding to IL2Rα when IL2 was added indicating that Tr-1 recognizes either the receptor-ligand complex or a conformational change in IL2-bound IL2Rα. Binding of Tr-1 to IL2Rα did not significantly alter its affinity to IL2. C. Tr-1 inhibits Treg induction: Transformed B cells can induce Treg development in the tumor microenvironment. Experimentally, this was evaluated by quantifying Tregs generated from autologous CD4+ T cells cocultured with Epstein-Barr virus-transformed B (EBV-B) cells. Addition of Tr-1 resulted in ~30% reduction in EBV-B-induced Tregs (p = 0.017). Conclusion: We used a Treg-cell based SELEX strategy to derive a novel Treg-binding RNA aptamer (Tr-1) that preferentially binds to the IL2Rα-IL2 complex. Tr-1 rescues Treg induction by transformed B cells suggesting its potential as a therapeutic agent to reduce tumor-induced immunosuppression. Ongoing studies are further exploring its use in Treg inhibition and in targeting receptor-ligand complexes in cancer. While aptamers recognizing cellular receptors exist, to our knowledge, this is the first report of an aptamer recognizing a receptor-ligand complex. Our approach to generating aptamers against receptor-ligand complexes could have huge scientific impact.
coding OX40L with miR-122 binding sites that have been shown to suppress protein translation in hepatocytes yet not within tumor cells. A single ITU injection of OX40L mRNA, formulated in a lipid nanoparticle, resulted in transfection of up to 25% of live tumor cells, predominantly cancer and myeloid cells. OX40L protein persisted for 7 days after mRNA injection, and a resulting increase in tumoricidal activity was observed. The treatment advances over the past 5 decades and poor survival. Locally targeted oncolytic virotherapy employs a viral vector that has selective cytotoxicity for tumor cells and non-toxic for normal cells and tissues. The therapeutic benefit is proposed to arise from an initial viral cytotoxic phase following host immune responses. Myxoma Virus (MYXV) has been widely tested in Australia to control rabbit populations with no toxicity to humans. MYXV infects and replicates in human tumors despite the lack of cytotoxicity to normal tissues and is a promising oncolytic virotherapy agent that has not been tested in human lung cancer or in clinical trials. Methods: To study MYXV infection and viral replication in vitro, we utilized human and mouse SCLC cell lines with MYXV engineered with fluorescent reporters. Using an optimized conditional genetically engineered mouse model (GEMM) (Ade-Cre mediated p53/Rb1/p130 null) we examined the effects of intrapulmonary MYXV treatment on SCLC tumors at 5 and 30 days post-MYXV treatment. Survival analysis was determined following intranasal MYXV 3 months post-Ade-Cre induction. We also tested intratumoral MYXV in patient derived xenografts (PDX) and subcutaneous syngeneic allografts in immunocompetent mice. Results: We optimized a conditional SCLC GEMM using limiting dilutions of intratracheal Ade-Cre transplanted mice to generate a cohort of mouse SCLC lines from individual clones. We observed efficient MYXV infection, late viral replication, and cytotoxicity in both human and mouse SCLC in vitro. In contrast, we did not detect productive infection nor cytotoxicity in non-tumor cells. Following intranasal MYXV instillation we observed MYXV localized exclusively within lungs at 3 days and no longer detected by 7 days’ post treatment. TUNEL staining of SCLC lesions showed apoptosis and necrosis at 5 days within SCLC and the effect persisted with discrete foci of tumor necrosis 30 days’ post-treatment. There was no toxicity to any mouse tissues. SCLC GEMM treated with intrapulmonary MYXV (n = 30) showed a modest but statistically significant prolongation of survival compared to PBS control mice (n = 30) (p < 0.05). Direct intratumoral MYXV injections performed on PDX tumors in immunodeficient mice showed efficient infection and late viral replication in all patient samples. We observed extensive tumor necrosis at 7 days, and persistent virus at 10 days’ post treatment. Direct intratumoral MYXV injections performed on allograft tumors in syngeneic immunocompetent mice showed extensive necrosis accompanied by immune cell infiltration into the tumor, and the virus was undetectable 7 days’ post treatment. Conclusions: We show the potential for MYXV as an oncolytic virotherapy for SCLC with enhanced cytotoxicity in immunocompetent mice suggesting a role for concurrent immune checkpoint therapies.


The IL-2 receptor (IL-2R) is an attractive target for cancer immunotherapy as it controls both immune-suppressive regulatory T cells (Treg) and anti-tumor T cells. We tested deleting Tregs as immunotherapy using anti-CD25 (high-affinity IL-2R subunit) antibodies (αCD25) in ID8agg mouse ovarian cancer (OC). αCD25 reduced ascites and Treg numbers but failed to reduce tumor burden, whereas αCD25 plus deeply activated anti-tumor CD8+ T cells in tumor-draining lymph nodes. Thus, αCD25 could be novel malignant ascites palliation, but has limited stand-alone efficacy. We then tested IL-2/anti-IL-2 complexes (IL-2c) that selectively stimulate medium-affinity (CD122/CD132) IL-2R thought to expand anti-tumor T cells preferentially, but with little Treg effects. In contrast to several single agents we tested that failed to treat ID8agg (e.g., αCD25, αPD-L1, IL-2 fusion toxin denileukin difitolix). IL-2c alone durably reduced ID8agg tumor burden despite lowering the tumor microenvironmental CD8+/Treg ratio. Thus, we hypothesized that IL-2c improved CD8+ function, reduced Treg function, or both. IL-2c increased polyfunctional IFN-γ + TNF-α + anti-tumor T cells as expected, an effect that persists weeks after drug clearance. IL-2c also increased anti-tumor T cell CD25 expression that increased IL-2 sensitivity and STAT5 phosphorylation, a likely mechanism for increased polyfunctionality. Unexpectedly, IL-2c reduced the Treg functional mediators CD25, TIGIT and granzyme B, and reduced Treg suppressive function. Thus, favorable Treg modifications are a novel IL-2c mechanism of action. Adding αCD25 to IL-2c to delete Tregs further unexpectedly worsened IL-2c efficacy in ID8agg and reduced effector memory T cells and polyfunctional T cells in the tumor microenvironment, suggesting a previously unappreciated role for CD25 in IL-2c therapy. Similar data were seen in B16 melanoma, suggesting αCD25 reduction of IL-2c efficacy is not tumor or compartment-specific (ID8agg is peritoneal and B16 is subcutaneous). αPD-L1, an ineffective monotherapy in ID8agg, combined with IL-2c to promote complete responses, suggesting potential for potent, novel combinatorial approaches. Our data suggest that antagonizing high affinity IL-2R (such as to delete Tregs with αCD25) has limited cancer immunotherapy utility without more specific Targeting. In contrast, stimulating medium-affinity IL-2R with CD122-selective IL-2c has great translational promise by simultaneously improving beneficial anti-tumor T cells and reducing detrimental Treg function.


Background: Small cell lung cancer (SCLC) is an aggressive subtype of lung cancer that has not been fully攻克 advances over the past 5 decades and poor survival. Locally targeted oncolytic virotherapy employs a viral vector that has selective cytotoxicity for tumor cells and non-toxic for normal cells and tissues. The therapeutic benefit is proposed to arise from an initial viral cytotoxic phase following host immune responses. Myxoma Virus (MYXV) has been widely tested in Australia to control rabbit populations with no toxicity to humans. MYXV infects and replicates in human tumors despite the lack of cytotoxicity to normal tissues and is a promising oncolytic virotherapy agent that has not been tested in human lung cancer or in clinical trials. Methods: To study MYXV infection and viral replication in vitro, we utilized human and mouse SCLC cell lines with MYXV engineered with fluorescent reporters. Using an optimized conditional genetically engineered mouse model (GEMM) (Ade-Cre mediated p53/Rb1/p130 null) we examined the effects of intrapulmonary MYXV treatment on SCLC tumors at 5 and 30 days post-MYXV treatment. Survival analysis was determined following intranasal MYXV 3 months post-Ade-Cre induction. We also tested intratumoral MYXV in patient derived xenografts (PDX) and subcutaneous syngeneic allografts in immunocompetent mice. Results: We optimized a conditional SCLC GEMM using limiting dilutions of intratracheal Ade-Cre transplanted mice to generate a cohort of mouse SCLC lines from individual clones. We observed efficient MYXV infection, late viral replication, and cytotoxicity in both human and mouse SCLC in vitro. In contrast, we did not detect productive infection nor cytotoxicity in non-tumor cells. Following intranasal MYXV instillation we observed MYXV localized exclusively within lungs at 3 days and no longer detected by 7 days’ post treatment. TUNEL staining of SCLC lesions showed apoptosis and necrosis at 5 days within SCLC and the effect persisted with discrete foci of tumor necrosis 30 days’ post-treatment. There was no toxicity to any mouse tissues. SCLC GEMM treated with intrapulmonary MYXV (n = 30) showed a modest but statistically significant prolongation of survival compared to PBS control mice (n = 30) (p < 0.05). Direct intratumoral MYXV injections performed on PDX tumors in immunodeficient mice showed efficient infection and late viral replication in all patient samples. We observed extensive tumor necrosis at 7 days, and persisting virus at 10 days’ post treatment. Direct intratumoral MYXV injections performed on allograft tumors in syngeneic immunocompetent mice showed extensive necrosis accompanied by immune cell infiltration into the tumor, and the virus was undetectable 7 days’ post treatment. Conclusions: We show the potential for MYXV as an oncolytic virotherapy for SCLC with enhanced cytotoxicity in immunocompetent mice suggesting a role for concurrent immune checkpoint therapies.


Salmonella typhimurium is a facultative anaerobic bacteria and can selectively grow in tumors following systemic administration. Different strategies have been used to deliver payloads to tumor tissues using engineered Salmondelleae. We report a novel method of cancer immunotherapy using an attenuated S. typhimurium strain engineered to secrete Vibrio vulnificus flagellin B (FlaB) in tumor tissues. Previously, we developed an attenuated strain of S. typhimurium, which is defective in ppGpp synthesis (ΔppGpp S. typhimurium), and manifested significantly increased L50 by 100,000 to 1,000,000-fold. To generate an inducible vector system for bacterial expression of the therapeutic gene, we cloned the flaB gene into the pBAD plasmid vector in which the pBAD leader sequence was cloned upstream of flaB to guide extracellular secretion; gene expression from the plasmid (pFlaB) was induced only in the presence of L- arabinose. To evaluate the antitumor activity of engineered S. typhimurium, C57BL/6 mice transplanted with MC38 tumors were injected intravenously with L-arabinose (i.v.) with PBS. ΔppGpp S. typhimurium carrying an empty vector, or ΔppGpp S. typhimurium carrying pFlaB (+/− L-arabinose induction). To test whether the FlaB-secreting ΔppGpp S. typhimurium has inhibitory effects on human metastatic cancer, we next implanted HCT116-luc2 tumors into the intestinal wall of BALB/c athymic nu/− mice using a surgical orthotopic implantation (SOI) procedure. Engineered FlaB-secreting Salmonellae significantly suppressed tumor growth and metastasis in mouse models and prolonged survival. By using TLR5-negative colon cancer cell lines (MC38 and HCT116), we have proved that the FlaB-mediated tumor suppression upon bacterial colonization should be associated with TLR5-mediated host reactions in the tumor microen-
environment. These therapeutic effects were completely abrogated in TLR4 and MyD88 knockout mice, and partly in TLR5 knockout mice, strongly suggesting that TLR4 signaling is a requisite for the FlaB-secreting bacteria-mediated tumor suppression where TLR5 signaling augmented tumor suppressive host actions. Tumor colonization by engineered Salmonellae appeared to induce the infiltration of abundant immune cells such as monocytes/macrophages and neutrophils via the TLR4 signaling. Subsequent secretion of Flab from colonizing Salmonellae resulted in phenotypic and functional activation of intratru- moral macrophages with M1 phenotypes and a reciprocal reduction in M2-like suppressive activities. Taken together, these findings provide evidence that non-virulent tumor targeting bacteria liberating multiple TLR ligands can be used as novel cancer immunotherapeutics.

#1611 Intravesical BCG induces CD4 + T Cell expansion in an immune competent model of bladder cancer

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Intravesical BCG Immunotherapy is the standard of care in treating non-muscle invasive bladder cancer (NMIBC), yet its mechanism of action remains elusive. Both innate and adaptive immune responses have been implicated in BCG activity. While prior research has indirectly demonstrated the importance of T cells and shown a rise in CD4 + T cells in bladder tissue after BCG, T cell subpopulations have not been fully characterized. We investigated the relationship between effector and regulatory T cells in an immune competent, clinically relevant rodent model of bladder cancer. Fischer 344 rats aged 7 weeks received 1.5mg/kg N-Triosso-N-methylurea (MNU) every other week for 6 weeks (4 doses). Bladder dysplasia began by week 8 and by week 16 the majority of rats had a NMIBC phenotype. Beginning week 8 following the first MNU dose, rats were intravesically administered 0.3ml of BCG (Tice), cisplatin (1mg/ml), Mitomycin C (2mg/ml), MMC every other week for 6 weeks (4 doses). Bladder dysplasia began by week 8 and by week 16 the majority of rats had a NMIBC phenotype. Beginning week 8 following the first MNU dose, rats were intravesically administered 0.3ml of BCG (Tice), cisplatin (1mg/ml), Mitomycin C (2mg/ml), MMC + BCG, or saline (n = 10 for all groups) weekly for 6 total doses. Animals were sacrificed at week 16, and bladders were processed for histopathology and digested into single cell suspensions for flow cytometry. Whole transcriptome expression profiling was then performed on sorted CD4 and CD8 cells of post-BCG tumors vs untreated tumors to assess T cell differ- entiation after BCG. Our data demonstrate that cancer progression in the MNU rat model of bladder cancer is characterized by a decline in the CD8/FoxP3 ratio, consistent with decreased adaptive immunity. By contrast, treatment with intravesical BCG leads to a large, transient rise in the CD4 + T cell population in the urothelium, and is both more effective and immunogenic compared to intravesical chemotherapy. Interestingly, whole transcriptome expression profiling of post-treatment intravesical CD4 + and CD8 + T cells revealed minimal differences in gene expression after BCG treatment. Together, our results suggest that while BCG induces T cell recruitment to the bladder, the CD8 cell phenotype does not markedly change, imply- ing that combining T cell activating agents with BCG might improve clinical activity.

#1612 Production of CD3 + cells using ferrofluids for cell isolation, acti- vation, expansion and subsequent transfaction for adoptive cell therapy

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The purpose of the study is to demonstrate whether magnetic nanoparticles func- tionalyzed with monomodal antibodies in conjunction with solution-phase mono- modal antibodies can be used to activate T cells for expansion in vitro. The activation, expansion and transfection/transduction of immune cells—particularly T cells—has become an area of significant interest based on encouraging data generated from employing such genetically modified cells for cancer immunotherapies. The prom- ise of soon being able to cure various types of cancers has resulted from years of methodical experimentation in the fields of oncology, immunology, and genetics, as well as the development of a vast array of enabling technological advances. BioMag- netic Solutions has developed colloidal magnetic nanoparticles—referred to as fer- rofluids—which can be used to isolate a cell population through an immunomag- netic separation. These ferrofluids comprise a crystalline core of magnetite (ca. 100 nm in diameter) coated with multilayers of clinical-grade human serum albumin and further functionalized with a clinical-grade rat anti-mouse IgG1 monoclonal antibody. These “common-capture ferrofluids” (ca. 130 nm in diameter) are capable of binding cells which have been labeled with a monoclonal mouse IgG1 antibody; for example, CD3 + cells can be readily isolated from a complex cell mixture by labeling with a monoclonal mouse anti-human CD3 antibody, adding common- capture ferrofluid, and subjecting the sample to a magnetic field gradient. This tech- nology can therefore be employed at the clinical scale to isolate T cells from an apheresis product. In this study, we investigated whether T cells isolated using com- mon-capture ferrofluids could subsequently be activated and induced to expand in vitro. Based on preliminary experiments showing that T cell-bound common-cap- ture ferrofluid has excess antibody-binding capacity, we designed experiments to test the hypothesis that solution-phase CD28 could be added to isolated T cells, which would bind to both CD28 determinants on the cell surface as well as to the cell-bound common-capture ferrofluid. Activation was quantified by measuring CD25 expression after four days in culture and expansion was monitored over 15 days. Following an immunomagnetic separation of CD3 + cells, magnetically isolated cells were re-suspended in expansion medium (STEMCELL) supplemented with 10% FBS (growth of LIGHT and IL-12 genes in NSC was confirmed by qPCR and by Western blot analysis). The following day, 0.5 µg/ml mouse anti-human CD28 monoclonal anti- body (Mabtech) was added, and thereafter, cells were periodically agitated and di- luted to 10 6 cells/ml with expansion media. After four days in culture, 88.4% of cells expressed CD25, while a 311-fold expansion was observed after 15 days. In sum- mary, we have demonstrated that common-capture ferrofluids can be used to acti- vate T cells for expansion in vitro.

#1613 Genetically modified neuronal stem cells expressing IL-12 and TNFSF14 attenuate highly metastatic mammary tumors in a mouse model

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Interleukin 12 (IL-12) is a heterodimeric cytokine encoded by two genes, p35 and p40. IL-12, also known as T cell-stimulating factor, induces proliferation of T lymphocytes and natural killer (NK) cells, while also demonstrating an anti-angiogenic effect and inhibition of regulatory T cells. Similarly, LIGHT (TNFSF14) is a transmembrane protein which acts as a costimulatory factor for the activation lymphoid cells and stimulates the proliferation of T cells, leading to increased apoptosis of tumor cells. Expression of IL-12 and LIGHT specific- ally in the tumor region could activate T cells and stimulate the immune system to attenuate the tumor. Homing neuronal stem cells C17.2 (NSC) were genetically engineered to express the mouse-LIGHT gene and IL-12 using a retroviral expression vector. The expression of both cytokines in NSC was confirmed by qPCR, ELISA and Western blot. To test the antitumor effects of the engineered cells, Balb/C mice bearing 4T1 mammary tumors were treated with NSC-LIGHT, NSC-IL-12 and NSC-LIGHT/IL-12 cells intratumorally (I.T.) at day nine. Repeated tumor measurement over time showed attenuation of mammary tumor growth in the both NSC/IL-12 and NSC/LIGHT groups in comparison with untreated groups PBS and NSC. Tumor growth was further reduced by treat- ing the mice with NSC expressing both cytokines LIGHT and IL-12. Tumor metastasis was also evaluated by removing the primary tumor surgically and observing mice for any sign of metastatic burden. Treatment groups had less metastatic tumor burden and survived longer than the control group. Therefore, tumor homing NPC-LIGHT/IL-12 cells could be effective for the treatment of highly metastatic mammary tumors.

#1614 Intratumoral delivery of a P2A-linked bicsrticin IL-12 construct leads to high intratumoral expression and systemic anti-tumor response

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The use of immunomodulatory cytokines has been shown effective in regress- ing a wide range of tumors. However, systemic delivery of recombinant cyto- kines can result in serious adverse effects, often life-threatening. DNA transfer using electroporation (EP) is a safe and effective method of delivering cytokines to target tissues. Intratumoral (IT) electroporation of Interleukin 12 (IL-12), a potent immunomodulatory cytokine, is well tolerated with an acceptable safety profile and objective response rates of 34-40% and 33% in Phase II clinical trials in advanced melanoma and Merkel cell carcinoma, respectively. We sought to improve the systemic anti-tumor response of IT pIL-12-EP by improving IL-12p70 expression and electroporation conditions, which were evaluated in vitro and in vivo with a two-tumor syngeneic mouse model of melanoma. Functional IL-12 p70 is a heterodimer, we compared different expression constructs to achieve high levels of IL-12 protein expression. IL-12p70 protein expression from a plasmid that incorporated a picornavirus-derived co-translational cleav- age site (P2A) was higher than constructs with an internal ribosomal entry sequence (IRES) or a fusion of the p35 and p40 subunits. In functional in vitro assays, pIL-12(P2A) was superior to pIL-12(IRES) and the fusion protein. Using the murine B16.F10 tumor model, we show that IT EP of pIL-12 (P2A) plasmid regresses the treated lesions in a dose-dependent manner compared to control treatments. Systemic effects of IT-expressed IL-12 was assessed by monitoring generation of an antigen-specific CD8 T cell response and regression of B16.F10 orthotopic tumors following primary tumor electroporation. IT pIL12-EP treatment with the P2A-linked construct resulted in a significant in- crease in antigen-specific CD8 + T cells, as well as enhanced contralateral tumor growth inhibition suggesting the induction of strong systemic anti-tumor im- mune response.* Contributed equally to this work.
Background: Post-surgery adjuvant radiotherapy (RT) for breast cancer significantly reduced the local recurrence rate. However, many patients develop early adverse skin reactions (EASRs) that impact quality of life. Methods: In a large prospective study of 1,000 breast cancer patients undergoing RT, we evaluated an inflammatory biomarker, C-reactive protein (CRP) in predicting RT-induced EASRs. In each patient, we measured pre- and post-RT plasma CRP levels using a highly-sensitive ELISA CRP assay. RT-induced EASRs were assessed using the Oncology Nursing Society Skin Toxicity Criteria. Association between EASRs and CRP were assessed using logistic regression models after adjusting for potential confounders. Results: The study population includes 405 patients. CRP levels differed significantly by race/ethnicity at baseline and at the end of RT. RT-induced grade 4 skin toxicity was significantly associated with: obesity and pre-RT CRP > 2mg/L (OR = 3.27, 95%CI = 1.88, 5.68), obesity and post-RT CRP > 2mg/L (OR = 4.42, 95%CI = 2.38, 8.23), or obesity and change of CRP > 1mg/L (OR = 5.58; 95%CI = 1.82, 17.0). Conclusion: The current observations support the previous suggestion that the inflammatory biomarker CRP is associated with RT-induced EASRs, particularly combined with obesity. Impact: Our current findings support the discovery and development of anti-inflammatory agents to protect normal tissue from RT-induced EASRs and improve quality of life in breast cancer patients undergoing RT.

#1617 Mechanistic modeling of a new kinelytically-controlled CD122 agonist for cancer immunotherapy: NKTR-214 pharmacokinetics, pharmacodynamics, and receptor pharmacology.


Introduction: NKTR-214 is a biologic prodrug currently in a Phase 1/2 clinical trial in patients with solid tumors, as a single agent and in combination with anti-PD1. It is a CD122-biased cytokine agonist conjugated with a highly-sensitivity ELISA CRP assay after adjusting for potential confounders. Results: The study population includes 405 patients. CRP levels differed significantly by race/ethnicity at baseline and at the end of RT. RT-induced grade 4 skin toxicity was significantly associated with: obesity and pre-RT CRP > 2mg/L (OR = 3.27, 95%CI = 1.88, 5.68), obesity and post-RT CRP > 2mg/L (OR = 4.42, 95%CI = 2.38, 8.23), or obesity and change of CRP > 1mg/L (OR = 5.58; 95%CI = 1.82, 17.0). Conclusion: The current observations support the previous suggestion that the inflammatory biomarker CRP is associated with RT-induced EASRs, particularly combined with obesity. Impact: Our current findings support the discovery and development of anti-inflammatory agents to protect normal tissue from RT-induced EASRs and improve quality of life in breast cancer patients undergoing RT.

#1618 Immune-modulating effect of bevacizumab in EGFR mutated lung adenocarcinoma.

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Background: Host immunity affects treatment effect of lung cancer. Peripheral blood S100A9+ monocyte myeloid derived suppressor cells (MDSCs) predict efficacy of immune checkpoint therapy and epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) of lung adenocarcinoma patients. Bevacizumab has immune-modulating effects, including decreasing MDSCs, and improve treatment effect in combination with immunotherapy. However, the effect of immune modulation in combination of bevacizumab and EGFR-TKI in EGFR mutated lung adenocarcinoma patients is not clear. Methods: Stage IV lung adenocarcinoma harboring sensitive EGFR mutation patients receiving first line EGFR-TKI or combination EGFR-TKI and bevacizumab were enrolled. The peripheral blood mononuclear cell (PBMCs) were collected, and S100A9+ MDSCs percentage was calculated by flow cytometry from CD14+S100A9+ in PBMC. Clinical data was collected. Results: Eight patients receiving EGFR-TKI and bevacizumab, as combination group, and twenty patients received EGFR-TKI alone, as control group, were enrolled. PBMC S100A9+ MDSC decreased in combination group (decrease 39 ± 18% from baseline), but not in control group. Combination group had longer progress free survival (PFS) comparing with control group. (Median PFS combination vs control group: 15.2 vs 9.9 months, Log Rank test, p=0.05) Increased of peripheral blood cytotoxic T cells also had trend in combination group but not in control group. Conclusion: Besides the anti-angiogenesis effect, bevacizumab had immune modulated effect, especially in decreasing circulating S100A9+ MDSC in EGFR mutated lung adenocarcinoma patients. This might partially explain the longer PFS in combination of EGFR-TKI of anti-angiogenesis agent treatment.

#1619 Gemcitabine-generated ROS promotes neutrophil transmigration and activation by synergistic CXCL8 production with PKC delta-activated macrophages.

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Chemotherapy-induced neutropenia (CIN) is a common side effect that necessitate dose reductions during treatment of cancer patients. Preventing CIN is critical in chemotherapy because a rapid decline of neutrophil counts increase susceptibility to infection of cancer patients. In spite of its importance, the mechanism responsible for CIN still remains unclear. The purpose of this study is to investigate the mechanism of CIN in an aspect of neutrophil activation by chemotaxis in in vivo and in vitro model systems. It is well known that protein kinase C α (PKCα) is endogenously activated in immune and cancer cells of tumor environments, and treatment of gemcitabine generates reactive oxygen species (ROS). Based on these phenomena, we hypothesized that gemcitabine-generated ROS synergistically amplifies PKCα signaling and C-X-C Motif Chemokine Ligand 8 (CXCL8) production in intracellular PKCα is activated in both the peritoneal cavity and the tumor region. To investigate if neutrophils are transmigrated by gemcitabine treat-
immunoreactivity to tumor, and clearly indicated that transferred effector B cells can act independently of T cells in causing tumor destruction in adoptive immunotherapy.

#1622 The role of the CCL-2 on lymphopenia-induced myeloid derived suppressor cells. Pasquale P. Innamorato, Amy Weber, Shari Pilon-Thomas. H. Lee Moffitt Cancer Center, Tampa, FL.

The induction of lymphopenia prompts the expansion of CD11bLy6Cmonocytic MDSCs (M-MDSCs) and CD11bLy6Chemokine mononuclear MDSCs (PMN-MDSCs). Patients receiving adoptive cell therapy (ACT) require nonmyeloablative chemotherapy to induce lymphopenia and support anti-tumor immunity. However, the role of MDSCs in the setting of ACT is not fully understood. As levels of CCL-2 expression in CD11bLy6C cells induced in lymphopenia mice declined, we investigate the role of CCL-2 in the expansion and function of lymphopenia-induced MDSCs using CCR2KO mouse models. Lymphopenia was induced in melanoma-bearing C57BL/6 (WT) and CCR2KO mice by 600rad of total body irradiation or combination therapy with cyclophosphamide and fludarabine. We first evaluated the percentages of splenic MDSCs in WT and CCR2KO mice after the induction of lymphopenia. At day 14, the expansion of total MDSCs were similar in WT (279% of normal) and CCR2KO (241% of normal). However, the mean percentages of M-MDSCs (3.5%) and PMN-MDSCs (30.3%) in CCR2KO mice were altered compared to M-MDSCs (22.2%) and PMN-MDSCs (21.7%) in WT mice. In addition, M-MDSCs in B16 tumors grown in CCR2KO mice were decreased compared to intratumoral M-MDSCs in WT mice (p<0.001). In contrast, the percentages of M-MDSCs and PMN-MDSCs were increased in the bone marrow (BM). To investigate the suppressive capacity of lymphopenia-induced MDSCs, OVA antigen-specific CD8 T cells were co-cultured with OVA peptide in the presence of MDSCs purified from the spleens of CCR2KO and WT mice. Both CCR2KO and WT MDSCs potently suppressed T cell proliferation as measured by "H thymidine incorporation. CCR2KO and WT MDSCs had significant arginase activity and production of nitrites. To investigate the efficacy of ACT, B16 tumor-bearing CCR2KO and WT mice were lymphodepleted and ip100 antigen-specific Thy1.2 CD8 T cells were adoptively transferred followed by 3 days of IL-2 treatment. We found that donor Thy1 T cell tumor infiltration was similar and no difference in tumor growth was observed between CCR2KO and WT mice. Collectively, these results suggest that the role of CCL-2 is important for the egress of lymphopenia-induced M-MDSCs from the BM, but has no effect on the expansion or function of PMN-MDSCs in the setting of lymphopenia. These data suggest that factors besides CCL-2 play an important role in the expansion and function of MDSCs after the induction of lymphopenia.

IMMUNOLOGY: Cytokines: The First Modern Immunotherapies

#1623 Minute variation in murine ovarian cancer preclinical models significantly impact immunotherapy results obtained for clinical trial. AJ Robertson, Kristen Starbuck, Samar Masoumi-Moghadam, Ariel Francois, Kunile Odunsi, Emese Zsiros. Roswell Park Cancer Institute, Buffalo, NY.

As highly promising approaches for treatment of a variety of solid tumors, including recurrent ovarian cancer. While dramatic clinical benefit is observed in a subset of patients with ovarian cancer, the majority of ovarian cancer patients do not respond to checkpoint inhibitors, thus combinatorial treatments are sorely needed. Immune competent pre-clinical animal models are crucial to test emerging treatment strategies and for the identification of biomarkers to predict response to therapy. As results from immune competent preclinical models are often translated into clinical trials, we sought to investigate whether slight variability related to animal housing and/or breeding practices in the same strain could impact experimental outcomes related to immunotherapy. We conducted animal studies using C57BL/6 mice purchased from Jackson Laboratories (Cat# 000664) 2 weeks prior to experimental use (iax) and using the same mouse strain purchased from Jackson Laboratories but bred within our institution for a period of 6 months prior to use (jax-R). Using the gold standard ID8 murine metastatic intraperitoneal ovarian cancer model we tested the therapeutic efficacy of anti-PD-1 monotherapy in both jax and jax-R animals. Aged-matched female mice were housed under the same conditions for 2 weeks prior to tumor inoculation and for the duration of study. Strikingly, we noted that while anti-PD-1 monotherapy had absolutely no effect on tumor growth or survival of jax mice, the jax-R animals showed dramatic tumor growth control and significantly improved long term survival. Tumor progression and survival was similar across sub-strains in untreated control animals, suggesting that the
observed differences in treatment outcome following anti-PD-1 treatment were not simply due to intrinsic differences between the animals. Response to treatment was also associated with increased frequencies of circulating CD4+ and CD8+ T cells in the Jax-R mice compared with the Jax animals. Although major variations in age, animal strain and gut microbiome can dramatically impact the efficacy of any assay, our findings demonstrate that even minute changes within sub-strains recently derived from the same founder animals could result in significantly different survival and pre-clinical outcome. Thus, immunotherapy results obtained from immune competent animal studies need to be interpreted with caution when designing future immunotherapy trials.

**#1624 NOTCH ligand-based therapeutics for immunomodulation in cancer and organ transplantation.** Elena I. Tchekneva,1 Anmoldele E. Antonucci,1 Irina Chekneva,2 Nicholas Long,1 Jason V. Evans,2 Anwari Akhter,2 David P. Carbone,1 Thomas Magliery,1 Mikhail M. Dikov,3 Ohio State University, Columbus, OH; 1St Moss State Medical University, Moscow, Russian Federation; 3West Virginia University, Morgantown, WV.

We demonstrated in human and mouse studies that tumor-induced modulation of Notch ligand expression and Notch signaling in hematopoietic compartment contributes to tumor immune escape. Down-regulation of delta-like ligands (DLL) leads to defects in T cell development and T helper (Th1) cell differentiation with the prevalence of regulatory T cell (Treg) generation. To determine the roles of Notch ligands in antigen-presenting dendritic cells in regulation of antitumor immune responses we generated a set of lineage-specific knock-out mice lacking one of the Notch ligands in CD11c+ dendritic cells (DC). We are developing and testing a set of reagents for clinical application for ligand-specific activation or inhibition of Notch signaling to stimulate or inhibit, respectively, various types of immune responses for applications in oncology and immune diseases. Mice with DLL1 insufficiency in DC demonstrated remarkably accelerated growth of Lewis lung carcinoma (LLC) tumor, and reduced survival compared to wild type animals. This associated with impaired anti-tumor immune responses indicated by the decreased tumor infiltration by IFNγ-producing T cells. Jagged2 knockout did not cause any significant alterations. Notch ligand expression in antigen-presenting cells was identified as a “checkpoint” regulating the type of immune response. Data reveal that expression of Notch ligands by antigen-presenting cells is an important immune response specifying mechanism and that ligand-specific Notch signaling could be a valuable therapeutic target. Reagents for the pharmacological modulation of immune responses based on Notch ligand constructs is proposed. Our cell-based study showed that pharmacological activation of Notch ligands required multivalent receptor-ligand interaction, whereas soluble ligands acted as competitive Notch inhibitors. We have generated reagents that comprise specific receptors of the DLL family in multivalent or monovalent form for therapeutic inhibition of Notch by monovalent DLL1-based reagent accelerated LLC tumor growth and attenuated T cell-mediated anti-tumor immune response. In a heart transplantation mouse model, monovalent DLL1 reagent significantly prolonged allograft survival by inhibiting Th1 effector and memory T cell differentiation. Multivalent forms of DLL1 effectively stimulated Notch signaling in T cell culture and enhanced IFNγ production, whereas monovalent forms had opposite effects. Pharmacological up-regulation of DLL1-mediated Notch signaling with multivalent forms of ligand represents an efficient strategy for the enhancement of anti-tumor immunity and targeting multiple mechanisms of tumor growth. Monovalent DLL1 forms could be utilized for therapeutic inhibition of Th1 responses in autoimmune diseases and organ transplantation. Reagents based on the mono- and multivalent forms of Notch ligands can be efficiently utilized for therapeutic modulation of Notch signaling.

**#1625 NGS reveals specimen characteristics have minimal impact on immune gene expression signature.** Jeffrey Conroy,1 Sean Glenn,1 Blake Burcher,1 Sarabjot Pabla,1 Maochun Qin,1 Jon Andreas,1 Vincent Giamo,1 Marc Ernsto,2 Mary Nesline,3 Ji He,1 Mark Gardner,1 Carl Morrison1. 1Ohio State University, Columbus, OH; 21st Moscows State Medical University, Moscow, Russian Federation; 3West Virginia University, Morgantown, WV.

Background: A custom NGS cancer immune gene expression assay was developed which measures the transcript level of >350 genes involved in T-cell receptor signaling (TCRS), tumor infiltrating lymphocyte (TILs) genes as well as other key targets expected to predict the likelihood of patient response to checkpoint inhibitors (CPI). In parallel to the gene expression assay, mutational profiling was carried out using the 409 gene Comprehensive Cancer Panel (ThermoFisher). As variability between runs is common when performing NGS assays a detailed comparison of specific technical variations were assessed for their ability to effect gene expression and mutation profiles of clinical FFPE samples. Methods: Studies were designed to characterize the analytical performance of the immune response NGS assay using RNA and DNA from a subset of 300 FFPE tissues representing NSCLC, melanoma, renal cell carcinoma and bladder cancer. As part of the study, we tested the impact of variability in RNA and DNA input quantity at the library preparation step, sample batch size which affects mapped reads/sample and depth of coverage, and linearity of expression and sensitivity of mutation profiling through serial dilutions of pico-molar (pM) input of normalized library. PCA and unsupervised clustering was performed on samples with checkpoint inhibition, TCRS and TILs genes as well as mutational profiling to reveal sample groups with three distinct immune signatures (low, indeterminate and high). Further correlation and over-representation analysis was performed to determine impact of specimen characteristics on these three immune signatures. Results: Immune signatures were maintained for the majority of characteristics studied within a specified range. As expected, only TIL status was significantly associated with the high expression group. Other factors including architecture, neoplastic content, percent necrosis, stroma quality/quantity, T-Path, PMR, specimen type, tissue amount and specimen age were not over-represented in any immune signature. Conclusion: Tumor samples harbor a mixture of potential assay interferents including variable benign, neoplastic and immune cell populations with both naïve and reactive stroma contributing to a complex tumor microenvironment that is difficult to catalogue prior to testing. Our study demonstrates that the immune signature present in the tumor microenvironment is sufficiently strong to withstand a wide range of tumor heterogeneity, thereby reducing the need of extensive tissue macrodissection and the exclusion of samples previously thought to be non-evaluable.

**#1626 Technical variability in NGS immune gene expression and mutation profiling has a nominal effect on tumor classification.** Sean Glenn1, Jeffrey Conroy1, Blake Burcher1, Sarabjot Pabla1, Maochun Qin1, Jon Andreas1, Vincent Giamo1, Marc Ernsto2, Mary Nesline3, Ji He1, Mark Gardner1, Carl Morrison1. 1Omniox, LLC, Buffalo, NY; 2Roswell Park Cancer Institute, Buffalo, NY.

Background: A custom NGS cancer immune gene expression assay was developed which measures the transcript level of >350 genes involved in T-cell receptor signaling (TCRS), tumor infiltrating lymphocyte (TILs) complement as well as other key targets expected to predict the likelihood of patient response to checkpoint inhibitors (CPI). In parallel to the gene expression assay, mutational profiling was carried out using the 409 gene Comprehensive Cancer Panel (ThermoFisher). As variability between runs is common when performing NGS assays a detailed comparison of specific technical variations were assessed for their ability to effect gene expression and mutation profiles of clinical FFPE samples. Methods: Studies were designed to characterize the analytical performance of the immune response NGS assay using RNA and DNA from a subset of 300 FFPE tissues representing NSCLC, melanoma, renal cell carcinoma and bladder cancer. As part of the study, we tested the impact of variability in RNA and DNA input quantity at the library preparation step, sample batch size which affects mapped reads/sample and depth of coverage, and linearity of expression and sensitivity of mutation profiling through serial dilutions of pico-molar (pM) input of normalized library. PCA and unsupervised clustering was performed on samples with checkpoint inhibition, TCRS and TILs genes as well as mutational profiling to reveal sample groups with three distinct immune signatures (low, indeterminate and high). Further correlation and over-representation analysis was performed to determine impact of technical characteristics on these three immune signatures. Results: Immune signatures including mutation profiles and gene expression levels were maintained throughout variable RNA/DNA input amounts at the library generation level as well as with diminution of pM levels of library pooled at the sequencing step. Increase in the number of mapped reads and sequencing depth through decreasing the number of batched samples per sequencing run also did not affect the gene expression and mutation profile signatures of the FFPE derived samples. Conclusion: The gene expression and mutation profiles responsible for classifying FFPE samples using NGS are not affected by variation normally introduced in the technical workflow commonly associated with these platforms. The analytical assessment of input at the nucleic acid, library, and sample size level has shown the plasticity available when using amplicon based NGS technologies for classifying the immune gene expression signature as well as mutational profiles of FFPE derived clinical tumor samples. This flexibility increases the strength and utility of NGS-base gene expression profiling and mutational analysis of tumor samples for both basic research and clinical applications.

Hypoxia is a hallmark of cancer and a driver of tumor progression and poor patient outcomes. By generating an immunosuppressive tumor microenvironment that limits cytotoxic T lymphocyte (CTL) infiltration and activation, hypoxia limits the effectiveness of cancer immunotherapy and thus promotes tumor cell evasion of the host immune response. Omnim has developed a first-in-class and anti-cancer immunocheckpoint inhibitor, Optima, to target hypoxic tumor hypoxia to enhance cancer immunotherapy efficacy. In preclinical models, we have demonstrated that OMX accumulates in rodent subcutaneous and orthotopic tumors, as well as spontaneous canine melanomas and brain tumors, resulting in significant tumor hypoxia reduction. Here, using multiple subcutaneous syngeneic mouse tumor models (MC38, CT26, 4T1), we assessed OMX effects on immune cells and immunosuppressive regulatory T cells (Treg), as well as the anti-tumor potential of OMX as a single agent and in combination with established immunotherapies. Using quantitative immunohistochemistry, we confirmed reports that hypoxic tumor areas are devoid of CTLs. Accordingly, by flow cytometry we observed a negative correlation between tumor hypoxia and CTL infiltration. While OMX single agent treatment did not affect the overall CD45+ positive leukocyte population, Treg cells were selectively depleted and the CTL:Treg ratio was substantially increased, suggesting that OMX induced a shift towards immunosenzitization. Consistent with this finding, we observed OMX single agent anti-tumor efficacy in MC38 colon tumors. Impressively, anti-tumor effects of OMX single agent were equivalent to that of a single treatment of the checkpoint inhibitor anti-CTLA4. We next assessed whether OMX would enhance the efficacy of checkpoint inhibitors when used in combination. In CT26 colon tumors, OMX exhibited combination anti-tumor activity with anti-CTLA4, giving rise to faster cures and a greater number of complete and durable responders compared to anti-CTLA4 alone. Of note, this enhanced response was observed for both early-stage and late-stage CT26 tumors. In 4T1 breast tumors, known to be insensitive to checkpoint inhibitors, treatment of early-stage (~60 mm³) tumors with combination OMX and anti-PD1 resulted in a 27% response rate, compared to a 0% response rate to anti-PD1 alone. Taken together, our data suggest that OMX, by delivering oxygen to hypoxic tumor areas, induces a microenvironmental change from an immunosuppressive to an immunopermissive state. Given that OMX is well-tolerated in both small and large animals, and that its mechanism of action is upstream of numerous major immunosuppressive pathways, OMX holds great clinical potential to synergize with multiple immunotherapeutic agents to enhance tumor control by restoring anti-cancer immune responses in cancer patients.

#1628 Early events in metastatic spread: new approaches using targeted therapeutics to disrupt formation of the pre-metastatic niche and development of lung metastases. Khalid A. Mohamedali, Lawrence H. Cheung, Michael G. Rosenblum. UT MD Anderson Cancer Ctr., Houston, TX.

Solid tumors release soluble factors causing migration of a subset of normal bone marrow derived cells (BMDCs) - primarily VEGFR-1+ or VEGFR-2+ circulating endothelial progenitor cells - from the bone marrow to organs. These normal BMDCs self-assemble into a bone marrow derived cells (BMDCs) network that limits metastatic spread. The significance and specific characteristics of the BMDCs is still unclear. We have previously observed migration of VEGFR-1+ BMDCs to the lung and lymph nodes, and VEGFR-2+ BMDCs to liver, lung and lymph nodes. Using GFP + BMDCs from genetically engineered mice, we characterized the role that recruitment of BMDCs may play in breast cancer metastasis. We assessed the BMDC recruitment profile in lethally irradiated female nu/nu mice transplanted with GFP+ BMDCs from donor mice, followed by orthotopic placement of MDA-MB-231/luc cells or injected with MDA-MB-231/luc conditioned media for 30 days. Flow cytometry results show a gradual increase in the recruitment of CD11b+ VEGFR-1+ cells in all the tissues examined from tumor-bearing mice. Recruitment of these cells to the liver in mice treated only with MDA-MB-231/Luc conditioned media was also apparent, but the increase was not as high as in livers of mice with the orthotopic implanted tumors and conditioned media of CD11b+ VEGFR-2+ BMDCs was also observed but only in liver and lung. Interestingly, conditioned media seemed to recruit this subset of cells more strongly to these two tissues than signaling from orthotopic tumors. Spleen and lymph node showed minimal recruitment of CD11b+ VEGFR-2+ BMDCs. We have previously developed the Grb/VEGFR fusion construct- a novel pro-apoptotic fusion protein which specifically targets cells harboring VEGFR and have utilized this agent to specifically target BMDCs which are VEGFR2+. Our preliminary data indicate that treatment with Grb/VEGFR does not significantly alter the recruitment of VEGFR-1+ or VEGFR-2+ cells to lungs, when assessed two weeks after the final treatment. We observed increased recruitment of Flt4+/Flt8+ macrophages to the lung. On the other hand, CD11b+Gr-1- BMDCs were significantly reduced following Grb/VEGFR treatment, although the F4/80+ subpopulation carrying this signature increased. Studies are ongoing to determine whether systemic administration of this agent can disrupt the formation of the niche and the eventual establishment of metastatic tumors. Understanding the role of BMDCs in metastatic spread of different tumors and their role in the early development of metastasis will be critical in designing targeted therapeutic approaches to inhibit the metastatic process. Research sponsored, in part, by the Clayton Foundation for Research.

#1629 An integrated view of Notch signaling that regulates tissue renewal in response to enteric infection. Badal C. Roy,1 Ishfaq Ahmed,2 Audrey Seamos,3 Shirikant Anant,1 Lillian Maggio-Price,3 Seth Sepert,3 Shahid Umar.1, Kansas University Medical Center, Kansas City, KS; 2University of Washington, Seattle, WA; 3Children’s Mercy Hospital, Kansas City, MO.

Background: Microbial dysbiosis and the associated gut inflammation unbalances epithelial renewal, potentially leading to cancer which is increasingly being recognized as a stem cell disease. Notch signaling is active in multipotent intestinal stem cells (ISCs); yet, how Notch signaling orchestrates communication between the gut microbes and ISC-dependent tissue renewal following a pathogenic insult, is poorly understood. Aim: To investigate how Notch signaling contributes towards ISC regeneration and pathogenesis of infection. Methodology: Rag^{-/-} mice and wild type littermates were infected with Citrobacter rodentium (CR; 10^7 CFUs) and treated with Notch blocker Dibenzapine ([DBZ], ip at 10 μmol/kg body weight). Whole distal colon or purified crypts were isolated for analyses. Results: RNA analysis of transgenic mice expressing MHC-II either in EEC (EpithTg) or in dendritic cells (CD11cTg) were crossed to Rag^{-/-} mice and received Helicobacter bilis (Hb) to induce colitis. De-identified sections from control or Crohn’s Disease (CD) and Ulcerative Colitis (UC) patients were stained for markers of ISCs and immune cells, respectively. Results: Rag^{-/-} mice but not WT littermates, exhibited dramatic increases in Dclk1 (Doublecortin-like kinase 1; an ISC marker) expression in the colon crypts, measured via flow cytometry and IHC at 12 days post CR-infection that co-localized with Notch Intracellular Domain (NICD). CR-infected mice when treated with DBZ for 10 days exhibited; i) significant dysbiosis with Proteobacteria dominating (48% compared to 27% after CR infection), ii) increases in paracellular permeability concomitant with almost complete attenuation of Dclk1 and iii) exacerbation of inflammation/colitis. Intriguingly, Dclk1 immunoreactivity shifted towards the stroma wherein, Dclk1 co-localized with NICD and with CD11c+ dendritic cells, CD11b+ and F4/80 macrophages and MHCII. Both EpithTg/Rag^{-/-} and CD11cTg/Rag^{-/-} mice infected with Hb compared to uninfected mice, exhibited loss of crypt Dclk1 and its co-localization with NICD that coincided with severity of colitis. Sections prepared from the colon of Crohn’s Disease (CD) or Ulcerative Colitis (UC) patients paralleled loss of crypt Dclk1 seen in mice while Dclk1 continued to co-localize with NICD and with markers of immune cells within the stroma. When CR infected and DBZ-treated Rag^{-/-} mice were given a cocktail of antibiotics (500mg/l Vancomycin, 1g/l metronidazole and 0.2g/l ciprofloxacin) for 7 days, we observed increased survival and decreases in colon myeloperoxidase (MPO) activity that coincided with increased Dclk1 in the colon epithelial and stromal areas. Conclusions: 1. Bacterial dysbiosis following chronic Notch inhibition coupled with loss of crypt Dclk1 impairs crypt regeneration. 2. Co-localization of stromal Dclk1 with markers of immune cells and with MHCII suggests a novel role for Dclk1 in antigen presentation.


Despite improvements in therapy over the last few decades, neuroblastoma (NB) still accounts for a considerable portion of childhood cancer-related mortalities and 5-year survival rates in patients with high-risk disease remains poor (40-50%). Amplification of MYCN and dysregulation in downstream Myc-related pathways result in NBs with high-risk features. Notably, MYCN-amplified NBs have elevated polyamine (PA) levels, and the gate-keeper enzyme in the PA synthesis pathway, ornithine decarboxylase (ODC1), is a direct target of Myc. Yet, ODC1 activity can be irreversibly inhibited by the FDA-approved drug difluoromethylornithine (DFMO), and exposure of NB cell lines in vitro to DFMO reduces cellular growth. In a mouse model of MYCN-driven NB (TH-MYC+/+ transgenic mice), inhibition of PA synthesis with DFMO led to reductions in NB burden and extension of survival that appeared more profound than that predicted by the in vitro activity of the drug, suggesting that in addition to tumor-intrinsic effects, DFMO may have significant effects on the NB tumor microenvironment.
**IMMUNOLOGY: Tumor Microenvironment and Checkpoints**

microenvironment (TME). We therefore sought to characterize the tumor mi-
icroenvironment of NB in TH-MYC/N transgenic mice in the presence or absence of
DMO-mediated PA blockade. Tumors from DMO treated and untreated mice were dissected, mechanically and enzymatically dissociated, and the num-
ber and frequencies of various TIL subsets were assessed using an optimized flow cytometry protocol. Our results show that PA blockade reduces tumor growth and results in distinct and reproducible alterations in the cellular com-
position of the NB TME, the most profound of which was a significant increase in the frequency of NK cells. Moreover, we found an increase in the percentage of tumor cells expressing NK cell ligands. Concordingly, we also witnessed a shift in the NK cell expression of activating and inhibitory receptors. These findings are consistent with the hypothesis that PA blockade induces distinct TME changes that predispose to more efficient immune control of NB growth. To probe the contribution of NK cells to this process, we are now testing whether antibody-mediated depletion of NK cells in DMO-treated TME changes that predispose to more efficient immune control of NB.

#1631 Sequencing the circulating and infiltrating T-cell repertoire on the Ion S5TM

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ccio-Hall,1 Timothy Looney,2 Alex Pankov,2 Yongming Sun,2 Xinzhan Peng,1

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The tumor repertoire (TCR) sequencing by next-generation sequencing (NGS) is a valuable tool for building a deeper understanding of the adaptive immune sys-

tem. As immunotherapies, particularly T-cell dependent therapies, show in-
creasing potential in treating cancer, the ability to gain a detailed, unbiased view of the TCR becomes imperative for biomarker discovery, immune response to-
treatment, and study of tumor microenvironments. A key question the field seeks to understand is the relationship between circulating T-cells and infiltrat-
ing T-cells at the tumor site. Here, we present a novel approach for TCR se-
quencing using the Ion S5TM sequencer which leverages simplified library con-
struction workflows and offers a more complete characterization of the entire V(D) region of TCRB. This method can leverage mRNA as input, minimizing requirements in starting materials and focusing sequencing to productive TCRB

arrangements. This approach targets the constant (C) and the FR1 regions, min-
imizing the potential for primer bias and greatly increasing the phylogenetic information content compared to techniques that exclusively characterize the CDR3 domain. Our results show that the observed circulating T-cell repertoire size is approximately 2 orders of magnitude higher than the infiltrating T-cell repertoire. Accordingly, while it is difficult to fully capture the complete reperto-
ire of circulating T-cells due to its vast diversity, we show that it is possible to reliably capture the complete repertoire of circulating T-cell repertoire with a single analysis of bulk tumor samples on the Ion 510TM chip. This study describes a new approach for high throughput sequencing of T-cell receptor repertoires which can be applied to a variety of clinical samples.

#1632 Identification and analysis of EGLN3 as tumor-associated peptide in ccRCC

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many.

Introduction: Kidney cancer is the 12th most common cancer world-wide with 338,000 newly diagnosed cases in 2012, according to the World Cancer Research Fund International. With around 70-80%, clear cell renal cell carcinoma

(mccRCC) make up the vast majority of kidney cancers. Advanced and met-
astatic ccRCCs are marked by poor survival rates despite the availability of tar-
geted therapies. Currently several immunotherapeutic approaches, including

checkpoint-blocking antibodies and dendritic cell vaccines are undergoing clin-
ical investigations. In this study we identified tumor-associated peptides which could be used in cancer vaccines to improve treatment of patients with advanced ccRCC.

Methods: To identify suitable peptides for cancer vaccines we set up a tissue collection of paired tumors and normal kidney tissues from 60 ccRCC patients. In parallel, we performed transcriptomic analysis on the combination were evaluated in two syngeneic and orthotopic murine models.

Results: 16 different peptides from EGLN3 were detected in 32 of the 60 analyzed tumors, with the most frequent peptide found in tumors of 10 different patients. Only two of the peptides were also detected in normal kidney or other non-ccRCC tissues, rendering EGLN3 peptides promising candidates to be used in ccRCC-directed vaccines. Most of our patients were HLA A*02:01 positive, however using NetMHCpan (http://www.cbs.dtu.dk/services/NetMHCpan/) only one of the peptides identified by HLA ligandomics was also predicted to be a strong binder for that particular HLA molecule. Expression of EGLN3 mRNA was not different in patients with and without HLA-presented peptides. Gener-

ally, EGLN3 mRNA expression was strongly induced in ccRCC tumors com-
pared to normal kidney (p<0.01). The function and targetability of EGLN3 remains to be investigated.

#1633 Immunotherapy for malignant mesothelioma that combines a me-
sothelia-targeted immune-activating protein and CXCL12/CXCR4 block-

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Sluder, Jeffrey Gelfand,4 Timothy Brooks,4 Mark Poznansky,4 Massachusetts General Hospital, Charlestown, MA.

Background and Purpose: There is a significant unmet need for new treat-
ment strategies for malignant mesothelioma (MM). Despite relevant advances in many cancer treatment areas, including improvements in diagnosis, staging, and the clinical course of treated patients, MM remains a highly lethal disease.

The purpose of this study is to develop a combination immunotherapy for MM, which involves a fusion protein to target and evoke a cellular immune response to mesothelin (MSLN) and the blockade of CXCL12/CXCR4 pathway to mo-
lize cytotoxic effector cells into tumors. Experimental Procedures: The efficacy of the MSLN targeted immune activating fusion protein (scFv-MthHap70), FDA-approved small molecule CXCR4 antagonist AMD3100 (plerixafor), and the combination were evaluated in two syngeneic and orthotopic murine models of MM in immune competent C57BL/6 mice. Micro 4traperitoneal (i.p.) treatments from 7 days post i.p. injection of luciferase-expressing 40L and AE17 cells. Tumor growth was monitored by in vivo imaging of luciferase activ-

ity with an IVIS system. Survival time was measured as date from the start of

tumor inoculation. In preclinical studies, mice were sacrificed 4 weeks after tumor cell inoculation. Immune cells from spleens and tumors were labeled with antibodies against CD3, CD4, CD8, CD25 and Foxp3 antibodies, and ex-
aminied by flow cytometry. Splenocytes were isolated by affinny chroma-

tography and bound peptides analyzed by mass spectrometry. HLA ligand pro-
files of tissues excised from various organ sites of non-ccRCC patients were used to complement the data. Based on these analyses, Egl nine homolog 3 (EGLN3) was selected as a candidate for peptide vaccination and further investigated in our own data cohort comprising the 60 ccRCC patients. Therefore, mRNA and protein expression analysis of HLA ligandomics were performed and correlated with HLA ligandomics and clinical data. In addition, we integrated RNA-Seq DNA methylation, somatic mutation and clinical data from a ccRCC pa-
tient cohort from The Cancer Genome Atlas in our analyses. Finally, EGLN3 function was analyzed in 2D and 3D kidney carcinoma cell culture systems.

Results: 16 different peptides from EGLN3 were detected in 32 of the 60 analyzed tumors, with the most frequent peptide found in tumors of 10 different patients. Only two of the peptides were also detected in normal kidney or other non-ccRCC tissues, rendering EGLN3 peptides promising candidates to be used in ccRCC-directed vaccines. Most of our patients were HLA A*02:01 positive, however using NetMHCpan (http://www.cbs.dtu.dk/services/NetMHCpan/) only one of the peptides identified by HLA ligandomics was also predicted to be a strong binder for that particular HLA molecule. Expression of EGLN3 mRNA was not different in patients with and without HLA-presented peptides. Gener-

ally, EGLN3 mRNA expression was strongly induced in ccRCC tumors com-
pared to normal kidney (p<0.01). The function and targetability of EGLN3 remains to be investigated.
tors, cytokeratin-positive carcinoma cells and CD3+ tumor infiltrating lymphocytes. Results obtained using the IMC at different time points in serial tissue sections were comparable (P<0.05). Conclusion: Quantitative and reproducible measurement of immune and non-immune targets with spatial resolution using the IMC platform is feasible in FFPE breast and lung carcinomas and the results are comparable to multiplexed QIF. Expansion of the current panel to include >30 markers for immune cell phenotypes/function and tumor markers is ongoing.

#1634 CDK4/6 inhibition directly enhances an anti-tumor immune response in breast cancer. Molly J. DeCristo,1,4, Shom Goel,2,4, April C. Watt,3,2,4 Haley Brinjikji,5,3, Jaclyn Sceney,2,4 Ben Li,2, Jyesslyn M. Ubellacker,2,4 Shaozhen Xu,2,4,5,3, Susanne Ramm,4,3, Hye-Jung Kim,2,2, Sandra S. McAllister,1,4, Jean J. Zhao,1,2,3,4 Brigham and Women’s Hospital, Boston, MA; 4Dana-Farber Cancer Institute, Boston, MA; 5Harvard Medical School, Boston, MA.

The cyclin D-cyclin-dependent kinases 4 and 6 (CDK4/6) axis is one of the most frequently dysregulated pathways in human cancers, and CDK4/6 inhibitors have shown significant activity against a number of solid tumors, including breast cancer. Analogous to clinical experience, we found that the CDK4/6 inhibitor, abemaciclib, caused significant tumor regression in the MMTV-rTA/tetO-HER2 mouse model of luminal breast cancer. However, as CDK4/6 inhibitors are known to block tumor cell proliferation, but not directly induce tumor cell apoptosis, as we confirmed in our study, the mechanisms by which CDK4/6 inhibition caused tumor regression were not clear. Notably, abemaciclib therapy increased total CD3+ T cells in these tumors, while decreasing while increasing CD8+ cytokine secretion in human PBMC in-vitro. A robust increase in inflammatory cytokine secretion in human PBMC was observed after treatment with CDK4/6 inhibitor, abemaciclib, compared to vehicle. In patient-derived xenografts, abemaciclib treatment also increased tumor cell death, resulting in tumor regression. Abemaciclib treatment led to a significant increase in the number of T cells in the tumor microenvironment, while reducing the number of regulatory T cells. These results provide strong rationale for further investigations into combining CDK4/6 inhibitors with immune checkpoint blockade in breast cancer.

#1635 Multiplexed analysis of fixed tumor tissues using imaging mass cytometry. Franz Villarroel-Espindola,1 Daniel Carvajal-Hausdorf,1 Ila Datar,1 Amanda Esch,2 Narges Roshidi,2 Ala Nassar,1 Shelly Ben,3 Ruth R. Montgomery,3 Roy S. Herbst,1 Yi-Hsuan Lee,1 Chun-Jung Chang,2 Yu-Yun Shao,2 Jung-hau Liu,3 Ann-Lii Cheng,1 Chi-Hung Hsu1 National Taiwan Univ. Hospital, Taipei, Taiwan; 2National Taiwan University, Taipei, Taiwan; 3National Taiwan Univ. Hospital Hsin-chu Branch, Hsin-chu, Taiwan.

Background: PD-L1 expression in tumor microenvironment of HCC has been unclear. Patients and Methods: We reviewed patients with HCC who had received sorafenib for advanced diseases at National Taiwan University Hospital, Taipei, Taiwan. Patients with paired HCC tissues, obtained before and after sorafenib treatment, were included. Immunohistochemistry (IHC) assay with clone SP142 antibody (Spring Bioscience, Pleasanton, CA, USA) was performed to analyze PD-L1 expression on TC and IC in paired specimens obtained before and after sorafenib. PD-L1 expression was scored as IHC0, 1, 2, or 3 if <1%, 1% to 5%, 5% to 25%, and >25% of cells were PD-L1 positive, respectively. Further IHC assay was employed to characterize the PD-L1-positive IC. Associations between the PD-L1 expression and overall survival (OS) or duration of sorafenib treatment of the patients were analyzed. Results: Twenty-three advanced HCC patients (Male: Female = 26: 3, median age of 64 years) with paired HCC tissues were included. All of the post-sorafenib HCC tissues were obtained after disease progression. The median duration of sorafenib treatment was 4.3 months (range: 1.3 to 18.7). The PD-L1 expression on IC was significantly increased in post-sorafenib HCC tissues, compared with tissues obtained before sorafenib (pre-sorafenib vs post-sorafenib IHC 0/1/2/3: 10/5/2/1 vs 5/5/2/11, p=0.046). However, the PD-L1 expression on TC was not significantly different between pre- and post-sorafenib tissues (IHC 0/1/2/3: 19/20/2/0 vs 14/15/0/4, p=0.065). By using imaging staining of CD68, CD66b, CD11b, and CD3 on the consecutive slides, we found the PD-L1-expressing IC were mainly CD68-positive macrophages, but neutrophils or T lymphocytes. Neither the PD-L1 expression levels on IC of pre-sorafenib or those of post-sorafenib tissues were associated with OS or duration of sorafenib treatment. Conclusions: The PD-L1 expression on IC, especially on macrophages, in tumor microenvironment of HCC was significantly increased in post-sorafenib progression HCC tissues. Whether this increased PD-L1 expression contributes to treatment failure of sorafenib warrants further investigation (This work was supported by the grants of NTUH 105-M3232 and MOST 105-2314-B-002-180).

#1637 ICOS agonism induces potent immune activation and anti-tumor response in non-clinical models. Sapna Yadavalli,5 Ashleigh A. Hahn, Laura M. Seestaller-Wehr, Hong Shi, Yao-Bin Liu, M-Phillip DeYoung, David J. Kilian, Meixia Bi, Michael P. Adam, Shu-Yun Zhang, Sabyassachi Bhat-tacharya, Yuiliya Katlinskaya, Christina Blackwell, Christopher B. Hopson, Nirajan Yanamandra, Roopa Srinivasan, Patrick A. Mayes, Axel Hoos. GlaxoSmithKline, Collegeville, PA.

Inducible T-cell stimulator (ICOS) is a costimulatory receptor that is upregulated on activated CD4 and CD8 T cells and plays an important role in T cell survival, differentiation, regulation of memory and regulatory T cell pools and humoral responses. Preclinically, augmenting signaling through the ICOS pathway has been reported to induce anti-tumor activity and enhance responses to CTLA4 blockade. Here we present non-clinical data evaluating ICOS agonist activity in human and mouse model systems using a different antibody for each species. GSK3559609 is a novel, selective anti-human ICOS agonist. GSK3559609 induces ICOS signaling through phosphorylation of intermediates in the PI3K pathway leading to lymphocyte activation, proliferation and pro-inflammatory cytokine secretion in human PBMC in-vitro. A robust increase in CD4 effector T cell proliferation and Granulcyte B secreting CD8 T cells was observed post-GSK3559609 and OP treatment in in-vitro assays utilizing PBMC from healthy donors, cancer patients or tumor infiltrating lymphocytes (TIL). Modest induction of regulatory T cell proliferation and IL-10 secretion were also observed. Significant increase in IFNγ (p<0.05) and TNFa secretion was observed in both primary PBMC and TIL based assays. Gene expression analysis of
GSK359609 treated human T cells confirmed changes in genes associated with T and B cell activation. In mice, an ICOS surrogate antibody was utilized in immune competent mouse tumor models. Tumor regressions were observed in 10–40% of mice and were associated with a robust increase in effector memory T cells in periphery as well as increases in T cell activation and proliferation in lymphoid and non-lymphoid compartments. In addition to identifying an additional therapeutic modality to stack with checkpoint blockade that could increase patient response rate. We hypothesized that engagement of T cell costimulatory receptors in combination with checkpoint blockade could further increase T cell activation and proliferation. The combination of checkpoint blockade with costimulation could be accomplished using a bispecific antibody format, with the potential benefits of reduced cost and more selective targeting of TILs to improve safety. Antibodies binding to immune checkpoint PD1 and a T cell costimulatory receptor were assembled in a bispecific antibody platform with substitutions in the Fc domain to suppress effector function. PD1 x costimulation (PD1 x costim) bispecific antibodies were evaluated in vitro by measuring antibody binding and de-repression of Staphylococcal enterotoxin B (SEB) stimulated PBMCs. IL2 and IFNγ production was measured by immunoassay. In vivo activity was evaluated using a mouse model in which human PBMCs are engrafted into NSG mice (huPBMC-NSG) and the extent of T cell engraftment is monitored by flow cytometry. We produced PD1 x costim bispecific antibodies that bound PD1 and a T cell costimulatory receptor monovalently. The bispecifics bound to SEB-stimulated T cells more tightly than monovalent controls, indicating that a single bispecific molecule was capable of blocking both PD1 and a T cell costimulatory receptor.

The bispecifics enhanced IL2 and IFNγ production in an in vitro SEB stimulation assay relative to control (p < 0.001, n = 19 donors). IL2 and IFNγ production was superior to anti-PD1 or anti-costim antibodies alone (p < 0.001), indicating productive and synergistic combination of checkpoint blockade plus costimulation. Treatment of huPBMC-NSG mice with checkpoint bispecifics promoted enhanced T cell engraftment relative to control. Engagement levels promoted by bispecifics were superior to those found for anti-PD1 treatment alone. For example, in one run of the model, while anti-PD1 treatment alone promoted a 2-fold increase in human CD45+ cells, a PD1 x costim bispecific antibody induced a 16-fold increase. Combination of checkpoint blockade and costimulation with bispecific antibodies is feasible and promotes strong T cell activation in vitro and in vivo. Compelling activity suggests clinical development is warranted for the treatment of human malignancies.

**#1638** Improved outcomes with drug-resistant immunotherapy in a human xenograft model of glioblastoma multiforme. Samantha B. Langford,1 Harold T. Spencer,2 Anindya Dasgupta,2 George Y. Gillespie,1 Kathryn Sutton,3 Larisa Pereboeva,1 Lawrence S. Lamb,1, 4Univ. of Alabama at Birmingham, Birmingham, AL; 5Emory University, Atlanta, GA.

INTRODUCTION: Conventional treatment strategies for high-grade gliomas have been uniformly dismal. We have previously shown that both primary and Temozolomide (TMZ)-resistant glioblastoma (GBM) cell lines upregulate stress-associated NKG2D ligands (NKG2DL) during the first several hours following exposure to TMZ, thereby creating an opportunity for NKG2DL-directed cell therapy, particularly γδ T cells that directly recognize these stress-associated antigens. Using a human/mouse patient-derived tumor xenograft (PDXT) model, we combined TMZ chemotherapy and TMZ-resistant ex vivo expanded/activated γδ T cells as Drug Resistant Immunotherapy (DRI). Drug resistance in this example is conferred by O-6-methylguanine-DNA-methyltransferase (MGMT) gene transfer, thereby enabling cytotoxic lymphocyte function in a chemotherapy-rich environment when the tumor is maximally stressed. METHODS: A total of five GBM PDXTs were examined, three parent (X12P, X22P, X59P) and two TMZ-resistant (X12T, X22T). Tumor NKG2DL expression and cytotoxicity of DRI were assessed using flow cytometry with cultured human astrocytes as controls. Intracranial (IC) glioma xenografts were established using either an unmodified (P) or a TMZ resistant clone (T) of human GBM explants passaged exclusively in immunodeficient mice. Tumor-bearing mice received intraperitoneal 60mg/kg TMZ on days 6, 8, 13, and 15 and received IC injection of 1.5 x 10^6 DRI 4 hours following TMZ injection. Control mice received DRI T cells alone, TMZ alone or no therapy. Survival was assessed using Kaplan-Meier analysis. RESULTS: All xenografts were found to constitutively express NKG2DL which were upregulated upon exposure to TMZ. DRI T cells were cytotoxic to all tumors in vitro, showing 65%-80% specific lysis at an E:T ratio of 20:1 with no evidence of toxicity against cultured human astrocytes. Median survival (MS) for all groups of untreated mice was approximately 25 days, and γδ T cell therapy alone did not improve survival in the absence of TMZ. For the parent tumors, TMZ therapy significantly improved MS over untreated controls for both X12P and X59P (29 vs. 59 days and 20 vs 51 days respectively, p = 0.0001) and eliminated tumors in X22P. DRI γδ T cells + TMZ significantly increased median survival additionally over TMZ alone with 80% of animals in both X12P and X59P surviving long-term (p = 0.0001 and 0.05 respectively). DRI had no clear effect over TMZ for X22T (p = 0.46), however, for X12T, DRI + TMZ significantly increased median survival from 22 to 38 days (72.7%) over TMZ alone (p = 0.04). CONCLUSIONS: Combined TMZ chemotherapy and drug-resistance modified γδ T cell therapy can produce a significant increase in time to progression and improvement in median and overall survival for both primary and TMZ-resistant GBM using a strategy is readily adaptable to the clinical setting.

**#1639** Combination of PD1 blockade and T cell costimulation by bispecific antibodies promotes human T cell activation and proliferation. Gregory L. Moore, Michael Hedvat, Matthew J. Bernett, Rajat Varma, Suzanne Schubert, Christine Bonzon, Kendra N. Avery, Rumana Rashid, Alex Nisthal, Liz Bogaert, Irene W. Leung, Seung Y. Chu, Umesh S. Muchhal, John R. Desjarlais, Xencor, Inc., Monrovia, CA.

Tumor infiltrating lymphocytes (TILs) express multiple checkpoint receptors, in contrast to lymphocytes found in the periphery (Matsuzaki et al PNAS 2010, Fourcade et al Cancer Res 2012, Gros et al ICI 2014). Checkpoint blockade has demonstrated increased clinical response rates relative to other treatment options; however, many patients fail to achieve a response to checkpoint blockade. In an additional therapeutic modality to stack with checkpoint blockade that could increase patient response rate. We hypothesized that engagement of T cell costimulatory receptors in combination with checkpoint blockade could further increase T cell activation and proliferation. The combination of checkpoint blockade with costimulation could be accomplished using a bispecific antibody format, with the potential benefits of reduced cost and more selective targeting of TILs to improve safety. Antibodies binding to immune checkpoint PD1 and a T cell costimulatory receptor were assembled in a bispecific antibody platform with substitutions in the Fc domain to suppress effector function. PD1 x costimulation (PD1 x costim) bispecific antibodies were evaluated in vitro by measuring antibody binding and de-repression of Staphylococcal enterotoxin B (SEB) stimulated PBMCs. IL2 and IFNγ production was measured by immunoassay. In vivo activity was evaluated using a mouse model in which human PBMCs are engrafted into NSG mice (huPBMC-NSG) and the extent of T cell engraftment is monitored by flow cytometry. We produced PD1 x costim bispecific antibodies that bound PD1 and a T cell costimulatory receptor monovalently. The bispecifics bound to SEB-stimulated T cells more tightly than monovalent controls, indicating that a single bispecific molecule was capable of blocking both PD1 and a T cell costimulatory receptor.

The bispecifics enhanced IL2 and IFNγ production in an in vitro SEB stimulation assay relative to control (p < 0.001, n = 19 donors). IL2 and IFNγ production was superior to anti-PD1 or anti-costim antibodies alone (p < 0.001), indicating productive and synergistic combination of checkpoint blockade plus costimulation. Treatment of huPBMC-NSG mice with checkpoint bispecifics promoted enhanced T cell engraftment relative to control. Engagement levels promoted by bispecifics were superior to those found for anti-PD1 treatment alone. For example, in one run of the model, while anti-PD1 treatment alone promoted a 2-fold increase in human CD45+ cells, a PD1 x costim bispecific antibody induced a 16-fold increase. Combination of checkpoint blockade and costimulation with bispecific antibodies is feasible and promotes strong T cell activation in vitro and in vivo. Compelling activity suggests clinical development is warranted for the treatment of human malignancies.
blocked tumor growth. Moreover, immunohistochemistry staining of treated tumors showed a decrease in the expression of CD206 positive M2 macrophages and CD45/CD25 positive Treg cells, and an increase in CD86 positive M1 macrophages. These results were consistent with the in vitro results obtained. Discussion and Conclusion: These results suggest that a targeting CD206 peptide engineered to promote apoptosis, enhances the tumor response to chemotherapy by limiting the pro-tumorigenic activity of TAMs.

### #1641 Dual antibody blockade of TIM3 and PD1 on NYE051 redirected human T cells leads to augmented control of lung cancer tumors. Edmund Moon,1 Soyeon Kim,1 Shaun O’Brien,1 Naomi Saint Jean,1 Raluca Verona,2 Linda Snyder,2 Yangbing Zhao,1 Steven Alбедa,1 *Univ. of Pennsylvania, Philadelphia, PA; 2Tuenssen Research and Development, Springhouse, PA.

Background: Immunotherapy using checkpoint blockade has demonstrated impressive, durable responses in select patients with solid malignancies. This is especially true for the blockade of programmed death 1 (PD1). Currently, efforts are focused on understanding non-response. One hypothesis is that multiple inhibitory receptors (IRs) are upregulated on hypofunctional tumor-infiltrating lymphocytes (TILs) and necessitates blocking multiple IRs to increase the response rate. We describe data from a unique xenograft model of human lung cancer where tumor-reactive human T cells become hypofunctional and up-regulate multiple IRs including PD1 and TIM3 to limit control of tumor growth than combining it with blockade of either alone. Materials/Methods: The A549 human lung cancer cell line was transduced with an immune checkpoint antigen, NYE051, expressed in the contact of HLA-A2 (A549-A2-ESO). 5x10^3 A549-A2-ESO cells were injected subcutaneously into the flanks of NSG mice. Activated T cells from healthy donors were lentivirally transduced with a TCR targeting NYE051 (Ly95). After two weeks when tumors were established and measured -150 mm^3, the mice were randomly assigned to one of the following treatments: 1) untreated, 2) non-transduced (NTD) T cells plus anti-PD1 and anti-TIM3 Ab, 3) Ly95 T cells, 4) Ly95 T cells + anti-PD1 Ab, 5) Ly95 T cells + anti-TIM3 Ab, 6) Ly95 T cells + anti-PD1 and anti-TIM3 Ab. T cells were injected IV at a single dose of 10x10^6/mouse. Abs were injected intraperitonealy (IP) at 10mg/kg every 5 days from the time of T cell injection. Tumor volumes were measured serially. At the end, the mice were sacrificed and the tumors were harvested, digested, and processed into single cell suspension. Flow cytometry was performed to look at degree of TIL infiltration and expression of surface markers. Ficol gradient was used to isolate the T cells to conduct functional analyses. Results/Conclusion: By twenty days post T cell injection, the tumors in the Ly95 T cell group (3) were ~30% smaller than that of the untreated (1) and the NTD T cell + Ab group (2). Anti-PD1 Ab augmented Ly95 T cell control of tumor (4) (further decrease in size by ~30%) (A). Anti-TIM3 Ab had no effect on Ly95 T cell tumor control (5). Anti-PD1 plus anti-TIM3 Abs had the greatest augmentation on Ly95 T cell control of tumor size (6) (greater than 50% reduction in tumor size compared Ly95 T cells alone) The greatest TIL infiltration and augmentation on Ly95 T cell controlof tumormargin (6) (greater than 50% reduction in tumor size compared Ly95 T cells alone). Anti-PD1 Ab augmented Ly95 T cell control of tumor (4) (further decrease in size by ~30%) (A). Anti-TIM3 Ab had no effect on Ly95 T cell tumor control (5). Anti-PD1 plus anti-TIM3 Abs had the greatest augmentation on Ly95 T cell control of tumor size (6) (greater than 50% reduction in tumor size compared Ly95 T cells alone). The greatest TIL infiltration and augmentation on Ly95 T cell control of tumor size was with combinatory Ab blockade.

### #1642 Evaluation of ENMD-2076 in combination with anti-PD1 in syngeneic cancer models. Graham C. Fletcher,1 Reza Kiarash,1 Mark R. Bray,1 Amanda S. Hu,1 Ken K. Ren2. *Campbell Institute, Univ. Health Network, Toronto, Ontario, Canada; 2CASi Pharmaceuticals, Rockville, MD.

ENMD-2076 is a clinical stage compound with potent activity towards Aurora A and angiogenic kinases. ENMD-2076 has shown promising activity in multiple Phase 1 clinical trials, as well as in a Phase 2 trial in advanced ovarian cancer. ENMD-2076 is currently the subject of several ongoing Phase 2 clinical trials including fibrolamellar carcinoma, triple-negative breast cancer (TNBC), advanced/metastatic soft tissue sarcoma (STS), and advanced ovarian clear cell carcinomas (OCCC). ENMD-2076 has been developed to date as a single agent, however ENMD-2076 inhibits a spectrum of targets including Aurora A, FAK, CSF1R, c-KIT, and KDR, that are potentially involved in immune evasion mechanisms. These kinases have been shown in published studies, when inhibited, to enhance or augment the activity of immune checkpoint inhibitors such as anti-PD1. A study was thereby conducted in syngeneic models to determine the utility of ENMD-2076 combined with immune checkpoint inhibition as a rational strategy for cancer therapy. The study evaluated the efficacy of ENMD-2076 administered daily by oral gavage in the MC38 and CT26 colon cancer models, alone and in combination with an anti-PD1 antibody. Xenografts were established in the appropriate mouse strain (C57BL/6 and BALBc, respectively) by the subcutaneous inoculation of MC38 or CT26 cells into the right flank of female mice. Treatment was initiated 7 days following inoculation when tumor volumes had reached a mean volume of approximately 85 mm^3. All treatments were well tolerated, with no significant body weight loss seen during either study. While single agent ENMD-2076 only led to single agent ENMD-2076 tumor regression was observed in several MC38-bearing animals suggesting an immune activating mechanism. In both models a trend was observed for an augmentation of anti-tumor response in combination relative to single agent ENMD-2076 and anti-PD1 alone. Further studies to evaluate mechanism and an assessment of re-challenge experiments in animals exhibiting complete regression will be performed. These studies support the further evaluation of ENMD-2076 in combination with immune checkpoint inhibition as a strategy for cancer therapy.

### #1643 Cytotoxic T lymphocyte CD3+ , CD8+, and immunoscore as prognostic marker in patients after radical cystectomy. Jose J. Mansure, Alice Yu, Shraddha Solanki, Ana B. Dias, Miguel M. Burnier, Fadi Brimo, Wassim Kassoul. McGill University Health Center, Montreal, Quebec, Canada.

Purpose: Prognosis after radical cystectomy for bladder cancer can be unpredictable. There is increasing evidence that the presence of cytotoxic T lymphocytes correlates with survival. The Immunoscore, a new approach to classification of cancer using the number, type and distribution of immune cells, is an independent predictor for survival in colon cancer. Our objective was to evaluate the prognostic impact of lymphocyte distribution and the Immunoscore in bladder cancer. Methods: Cystectomy sections with core tumor (CT) involvement and identifiable invasive margin (IM) were selected and stained for CD3+ and CD8+ lymphocytes. Three non-contiguous areas of highest lymphocyte density were selected from both CT and IM. The number of lymphocytes in each area was used to calculate the Immunoscore based on previously defined criteria. Kaplan-Meier curves were used to determine differences between Disease-free Survival (DFS) and Overall Survival (OS). Multivariate Cox proportional hazards model was used to determine hazard ratios. Results: Total of 67 patients who had cystectomy for invasive bladder cancer were included in the study. High concentration of CD3+ lymphocytes in the tumor margin was associated with better DFS (P=0.005) and OS (P=0.03). Similar results were found for CD8+ lymphocytes with regards to DFS (P=0.05). A higher Immunoscore was also associated with better DFS (P=0.04). On multivariate analysis, CD8+ in the invasive margin was independently associated with better prognosis. Conclusions: The host’s own immune system plays a valuable role in clinical outcome. Our data suggests that a strong immune response in the tumor margin is independently associated with better prognosis.


Molecular Templates is developing engineered toxin bodies (ETBs), potent recombinant immunotoxins that combine the specificity of an antibody fragment with the powerful direct cytotoxicity of the Shiga-like toxin A subunit. ETBs can induce their own internalization, route through the cell in a predictable manner, enzymatically and irreversibly destroy ribosomes to shut down protein synthesis and induce apoptosis of tumor cells. This mechanism of action is distinct from that of other therapeutics, making ETBs an attractive treatment for patients who have become resistant to chemotherapy and other treatment modalities. MT-3724 is Molecular Templates’ first-generation ETB targeting CD20, a surface receptor that is highly expressed on malignant B cells in hematological malignancies. Pre-clinical and clinical studies have shown promising results for non-Hodgkin’s lymphoma cell lines and patients; however anti-drug antibodies are formed in experimental animals and patients after repeat dosing. Sirolimus (rapamycin) is a macrolide compound approved to prevent organ rejection and in LAM, and has been used in tolerization protocols with replacement enzymes. In pre-clinical studies, we have co-administered sirolimus in combination with MT-3724 in rodents as well as in a non-human primate model in order to reduce the anti-drug antibody response to MT-3724. These studies demonstrated that the combination of sirolimus with MT-3724 decreased anti-drug antibody response in prolonged serum exposure of MT-3724, and improved B-cell depletion as compared to MT-3724 alone. Molecular Template’s ETB technology has resulted in potent and targeted therapeutic agents that have a unique mechanism of action in the field of oncology. Our first-generation, CD20-targeted therapeutic, MT-3724, has promising clinical results in the re-
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factory setting for non-Hodgkin’s lymphoma. The combination of sirolimus with MT-3724 is an attractive and feasible regimen which will be further explored in clinical studies.

#1645 CEACAM1-blockade for T-cell activation and antitumor T-cell response. So-Young Eun. MOGAM Institute for Biomedical Research, Yongin, Republic of Korea.

Immune checkpoint blockade became the most powerful way to control cancer without causing tremendous side effects, since anti-PD-1 blocking antibodies proved their superiority in clinical outcomes to those of traditional chemo- or targeted therapeutics. CEACAM1 (CCM1) is one of the several immune checkpoint receptors expressed on T cells that mediate suppression of inflammatory T cell response. CCM1 caught our attention for a cancer immune-therapeutic target, because of its inhibitory role in TCR proximal signaling complex. The anti-cancer therapeutic potential of CCM1-blockade has already been studied in mouse models, and one of the anti-human CCM1 antibody clones recently emerged in clinical Phase I studies. CCM1-targeting antibody candidates were screened out of our in-house synthetic libraries, among which a few of the clones stood out showing their significant blocking activities against CCM1-CCM1 homophilic interaction, enhancement of T cell activation, cytokine release, NFAT signaling, and tumor lysis. One of the clones (C25) showed its strongest efficacy equivalent to that of a reference Ab in CCM1-dependent T cell activation, proliferation, and IL-2 release, in parenteral Jurkat cells as well as in CCM1-overexpressing Jurkat cells. Moreover, C25 also enhanced tumor lysis by CCM1+ NK cells or CCM1+ cytotoxic T cells in a CCM1-dependent manner. In addition, C25 shows distinct characteristics in binding to CCM family from those of a reference Ab. Thus, C25 is thought to have a strong therapeutic potential on CCM1+ cancer.

#1646 Highly cytotoxic, completely human constructs targeting HER2 and containing the immuno-oncology payload granzyme B. Lawrence H. Cheung,1 Yunli Zhao,2 Khalid A. Mohamadali,3 Ana Alvarez-Cienfuegos,3 Walter N. Hittelman,3 Michael G. Rosenblum1, MD Anderson Cancer Center, Houston, TX. 2Shenyang Pharmaceutical University, Shenyang, China. 3UT MD Anderson Cancer Ctr., Houston, TX.

Recent immunotherapeutic approaches designed to augment T- and B-cell mediated killing of tumor cells has met with clinical success suggesting that immunoncology (IO) approaches have tremendous potential for treatment in a broad spectrum of tumor types. After complex recognition of target cells by T and B cells, delivery of the serine protease granzyme B (GrB) to tumor cells comprises the cytotoxic insult resulting in a well-characterized, multimodal apoptotic cascade. We designed a recombinant fusion construct composed of a human anti-Her2 scFv fused to active GrB for recognition and delivery of GrB to tumor cells simulating T- and B-cell therapy. The GrB-FC-4D5 dimeric construct (mw 160 kDa) was generated and expressed in stably-transfected CHO-S cells at ~60 mg/L and purified to homogeneity. The enzymatic activity of the fusion construct was similar to commercially-available GrB and the affinity of the construct for purified Her2 extracellular domain (ECD) was 0.328 nM, comparable to that of Herceptin (0.150 nM). The GrB-FC-4D5 construct was highly cytotoxic to Her2-positive cells such as SKBR3, MCF7 and MDA-MB-231 with IC50 values of 56, 99 and 27 nM respectively. Using immunofluorescence, the fusion construct internalized rapidly into target (SKBR3 or SKOV3) cells within 1 h of exposure, rapidly delivering GrB to the cytoplasmic compartment in a similar manner to that of immune T and B-cell targeting but without the action of the transmembrane pore-forming agent perforin. Against a large panel of various tumor types, GrB-FC-4D5 was highly cytotoxic to virtually all cells regardless of natural expression levels of the nominal endogenous GrB inhibitor, PI-9. Contemporary studies with Kadcyla demonstrate similar levels of in vitro activity against virtually all HER2-positive cells tested. GrB-FC-4D5 demonstrated activity against both log-phase and confluent tumor cells. In keeping with its relatively high molecular weight (~160 kDa), the construct demonstrated a terminal-phase half-life of 13.6 hrs. In vivo efficacy studies are currently ongoing in several orthotopic xenograft models. Also ongoing are pre-IND toxicology, histopathology and clinical chemistry studies. Research conducted, in part, by the Clayton Foundation for Research.


Backgrounds: Despite the unprecedented success of immune checkpoint antibody therapy in patients with various cancer types, lack of stable experimental immunotherapy models is still a big challenge in development of effective new immunotherapeutics. Different strategies of humanized mouse model system, including human hematopoietic stem cell (HSC) or human PBMC humanization in immuno-deficient mice, or chimeric models harboring human immune checkpoint targets in immune-competent mice have been reported. CrownBio has successfully established a number of human PBMC-humanized xenograft models MiXeno™ for in vivo IO therapy evaluation, which has been shown to be a rapid and simple approach. Herein we will present one of the optimal PBMC injection route and cell number, the donor dependence of the efficacy result and impact of donor HLA type on the efficacy, etc., need to be further addressed. Results: PBMC number was titrated in one of our MiXeno™ model HCO827, and PBMC injection via i.p. vs. i.v. was compared. Tumor growth curve, graft versus host disease (GVHD), and blood immunophenotyping were followed under different settings. Furthermore, the anti-tumor activity of PD-1 antibody pembrolizumab in a panel of PBMC donor context that bears diversified HLA types was precisely analyzed. We found that the efficacy was highly donor dependent, and there was lack of correlation between efficacy and donor/tumor HLA match. Conclusion: The MiXeno™ model provides an alternative to the full stem cell reconstitution approach. Better understanding on the characteristics of the model may allow a rational model selection and study design in the discovery IO therapeutics.

#1648 ImmunoGraft® platform for the evaluation of ImmunO-Oncology agents in PDX tumors models. David Cerna,1 Daniel Ciznadia,2 Khalid A.Mohamedali,3 Ana Alvarez-Cienfuegos,3 Wal-ter N. Hittelman,3 Michael G. Rosenblum1, MD Anderson Cancer Center, Houston, TX. 2Shenyang Pharmaceutical University, Shenyang, China. 3UT MD Anderson Cancer Ctr., Houston, TX.

Recent breakthroughs in Immunotherapy have given new hope to treating previously untreatable tumors and provided a better tolerated alternative to standard agents. There is an unmet need for a pre-clinical platform to test potential immune-oncology therapeutics that would also provide a tool to examine the mechanisms of response to better predict clinical outcomes. We have previously presented the Champions ImmunoGraft®, an innovative pre-clinical model enabling immunotherapeutic agents to be evaluated for efficacy in solid tumors. This platform is more reflective of the human tumor microenvironment (both immune and non-immune cell-based) and may be one of the most translational-relevant models to date for screening therapies targeting the immune system. However, optimization of humanization and PDx implantation protocols that allow a broader reconstitution of cell lineage and higher engraftment rate are necessary to further improve the pre-clinical evaluation of immunoncology therapeutics and enhance the value of this modality for patient’s benefit. To this end, immune-compromised NOG (Pkrd-e114Ihr211m10g8) mice were reconstructed (humanized) with human CD34+ cells using optimized procedure and blood was collected at different time points post engraftment to check for the major leukocyte linages. At ten weeks after humanization, mature human CD45+ cells comprised close to 50% of the leukocytes detected in the circulation and secondary lymphoid tissues of the humanized animals. As a result of improved methodology of the reconstitution protocol we have achieved an 85% success rate of humanization with a shortened engraftment period utilizing fewer CD34+ cells that maintain humanization reconstituted at 22 weeks post reconstitution. Champions TumorGraft® database contains more than one-thousand clinically relevant well-annotated PDx models. We have compiled data from non-genetic preclinical tumor models and have been an invaluable resource for designing applicable controls for use with the ImmunoGraft® studies for testing of Immuno-Oncology agents. We have identified tumors expressing key markers that may predict response to immunothera-py agents including PDL-1, CD40,IDO-1 and IDO-2 as well as tumors that harbor a high mutational load. Here we present the efficacy and clinically relevant endpoints of two well-studied checkpoint inhibitors Iplilimumab and Pembrolizumab in this highly translational PDx platform.

#1649 In vivo CRISPR-Cas9 genome scan for immune checkpoint regu-lators. Sascha Karassek, Min Long, Xi Li, Zhengang Peng, Qunsheng Ji, Yong Cang. WuXi AppTec (Shanghai) Co., Ltd., Shanghai, China.

Therapeutic antibodies blocking immune checkpoints such as programmed cell death protein 1 (PD1) have proven clinically beneficial in multiple cancer types. However, anti-PD1 drugs approved by FDA are not effective in treating all cancer types nor in all patients in a responsive cancer type. Therefore, understanding the mechanism underlying the efficacy of immune checkpoint blockade is critical for our rational design of synergistic treatment combinations. Here we describe how we employ the latest CRISPR-Cas9 genome-wide knockout screening strategies to identify targets in cancer cells or in T lymphocytes that control the sensitivity or resistance to anti-PD1 treatment. We constructed a sgRNA library targeting the functional domains of 116 mouse methyltransferases and demethylases, which was infected into the mouse Cloudman S91 melanoma cell line. Syngeneic mouse xenograft models derived from these infected cells were treated with anti-mPD1 monoclonal antibody, and the xeno-
gland tumors were harvest at different time points after antibody treatment. Deep sequencing of the integrated sgRNA sequences in the tumors has identified potential druggable targets to sensitize or antagonize the tumors resistance to anti-mPD1 immunotherapy. Alternatively, we introduced the same library to naïve T cells purified from the OT-1 transgenic mice. The T cell library was then injected into a syngeneic melanoma model followed by anti-mPD1 treatment. Deep sequencing of the integrated sgRNA sequences in the tumor-infiltrating T cells would allow us to uncover novel signaling molecules in the immune system that can potentially be manipulated to enhance the anti-PD1 therapy. In summary, we provide in vivo knockout screen strategies that can be applied to identify and validate combination therapies with immune checkpoint antibodies.


Background: Cluster of differentiation (CD47) is a trans-membrane glycosylated protein which is upregulated in several cancers. Increased expression of CD47 on tumor cells is associated with immune evasion and cancer progression. CD47 through its interaction with signal regulatory protein alpha (SIRPs), a cell-surface molecule on macrophages inhibits phagocytosis of tumor cells. Disrupting CD47-SIRPs interactions by monoclonal antibodies targeting CD47 and recombinant SIRPs proteins have been used as therapeutic strategies for treating cancer. Our objective was to discover and develop peptide/peptidomimetic based CD47-SIRPs antagonists for disrupting CD47-SIRPs interactions.

Methods: Through rational design based on crystal structure of CD47/SIRPs/interacting anti-CD47 antagonists, we designed peptides having potential to disrupt CD47-SIRPs interactions. FACS based cellular binding assay was developed to assess the binding affinity of CD47 antagonists. SIRPs protein labelled with fluorescent dye was incubated with Jurkat T cells expressing high levels of CD47 in the presence/absence of peptides. Binding affinity was measured by decrease in fluorescence. Functional activity of the peptides was evaluated in a FACS-based phagocytosis assays, in which tumor cells were incubated with human/mouse macrophages in the presence/absence of CD47 antagonists. Results: We identified CD47 antagonists demonstrating disruption of CD47-SIRPs interaction in a cellular binding assay. These peptides significantly inhibited phagocytosis of different tumor cells by macrophages. The lead CD47 antagonist displaying good ADME properties including moderate oral bioavailability was evaluated in a B16F10 syngeneic mouse tumor model. The lead CD47 antagonist inhibited primary tumor growth as well tumor metastasis to lungs. Biomarker characterization and efficacy studies in additional tumor models are ongoing.

#1651 Targeting phosphatidylserine in combination with adoptive T cell transfer eliminates advanced tumors without off-target toxicities in a melanoma preclinical model. Daniel Hirschhorn-Cymerman, 1 Sara Sara Schad, 1 Sadina Budhu, 2 Zhong Hong, 1 Xia Yang, 2 Hutchins T. Jeff, 3 Bruce D. Freimark, 1 Michael J. Gray, 2 Jedd Wolchok, 1 Taha Merghoub1, 1

A viable strategy to treat advanced cancers includes transferring of tumor-specific T cells. T cells that recognize tumor antigens can be expanded and reinvigorated ex-vivo. Furthermore, autologous T cells can be genetically modified to express anti-tumor T cell receptors or chimeric antigen receptors. Although the potency and specificity of tumor-specific T cells can be manipulated ex-vivo, once re-infused into patients, the T cells are subjected to immunosuppressive mechanisms established by the tumor. An important immune check-point regulator within tumors is phosphatidylserine (PS). Innate immune cells exposed to PS secrete suppressive cytokines and chemokines that can significantly impair the function and activation of anti-tumor T cells. Therefore, monoclonal antibodies that block PS activity can increase the anti-tumor potency of transfused T cells to treat aggressive cancers. Here we show that a PS targeting monoclonal antibody in combination with CD4+ T cells that recognize the melanoma antigen Trp1 can regress very advanced melanomas in all treated mice. Combination of anti-Trp1 CD4+ T cells with other immunomodulatory modalities such as anti-OX40 antibodies, can achieve equivalent tumor rejection rates but are typically accompanied by severe immunomodulated adverse events. In contrast, in this setting, PS blockade did not show any off-target toxicities. Flow cytometry analysis revealed lower levels of CD206 expression concomitant with higher activation markers in macrophages and neutrophils in tumors from anti-PS treated mice. These results suggest that diminishing suppressive mechanisms locally in adoptive transfer protocols is a highly desirable strategy that can eliminate tumors while minimizing related adverse events.

#1652 Optogenetic immunomodulation to develop improved anti-cancer immunotherapeutics. Liang He, 2 Nhung Nguyen, 1 Gang Han, 3 Yun Huang, 1 Yubin Zhou, 4 Texas A&M University, Houston, TX; 2 University of Massachusetts Medical School, Worcester, MA.

The application of current channelrhodopsin-based optogenetic tools is limited by the lack of strict ion selectivity and the inability to extend the spectra sensitivity into the near-infrared (NIR) tissue transmissible range. Here we present an NIR-stimulable optogenetic platform (termed "Opto-CRAC") that selectively and remotely controls Ca2+ oscillations and Ca2+-responsive gene expression to regulate the function of model cellular systems, as well as cells of the immune system. When coupled to upconversion nanoparticles, the optogenetic operation window is shifted from the visible range to NIR wavelengths to enable wireless and remote photoactivation of Ca2+ dependent signaling and optogenetic manipulation of immune/inflammatory responses. In a mouse model of melanoma by using ovalbumin as surrogate tumor antigen, Opto-CRAC has been shown to act as a genetically-encoded "photoactivatable adjuvant" to improve antigen-specific immune responses to specifically destroy tumor cells. In addition, we have extended similar engineering approaches to photomanipulate membrane contact sites that play critical roles in controlling lipid metabolism and control of lipid signaling in cancer cells. This study represents a solid step forward towards the goal of improving spatial and temporal control of anti-cancer therapy through optogenetic immunoengineering.

IMMUNOLOGY: Tumor Models and Assays

#1653 Modeling tumor immune-dynamics to predict patient survival and immunotherapy efficacy. Nicholas K. Akers, Eric E. Schadt, Bojan Losic. Icahn School of Medicine at Mount Sinai, New York, NY.

State of the art models of cancer survival are increasingly utilizing molecular data that feature the number of non-synonymous mutations, or a network based stratification of mutations, as significant predictors. It is widely hypothesized that this association between mutations and survival is a result of neo-epitopes that induce a robust anti-tumor immune response. Naively this implies that there is a strong association between mutations and survival, however this has generally been difficult to prove. Indeed, mutations and predicted neo-epitopes explain roughly the same survival variance, likely due to the intertwined difficulties of neo-epitope prioritization and the often immunosuppressive tumor microenvironment. Reasoning that integrating leading order immune response with neo-epitope prioritization will lead to superior performance, we built a statistical model to quantify the influence of tumor immune-dynamics on patient survival. Neoepitopes (MHC-I/II) were predicted from mutations and filtered on a self-ligandome. The clonal structure of these mutations and the distribution of resulting epitopes were assessed as predictive features. Additionally, the existing response to epitopes was assayed by incorporating TCR and BCR sequence counts and entropy as a proxy for clonal tumor infiltrating lymphocyte expansion. Leveraging the cancer genome atlas (TCGA) for 9 major cancer types including bladder (BLCA), breast (BRCA), colorectal (COAD/READ), glioblastoma (GBM), liver (LIHC), lung (NSCLC), melanoma (SKCM), pancreatic (PAAD), and uterine (UCEC) cancer, we obtained omics data for a total of 2,886 patients. Random forest based recursive feature elimination was used to determine which features were most likely to be predictive while overfitting was controlled with k-fold cross validation. A Cox proportional hazard model of survival as a function of mutation burden and features of neoepitope and immune response predictors was constructed using the selected features. Our results confirm that neoepitope and immune response based predictors for survival often significantly outperform mutation burden alone and simultaneously suggest a quantitative classification of immunotherapy efficacy across cancer subtypes.
Introduction: High-grade, muscle-invasive bladder cancer has recently been shown to harbor intrinsic molecular subtypes with distinct biologic features. Current murine models of bladder cancer, including the prominent carcinogen induced model MB49, do not account for subtype specific characteristics, leaving a gap in available tools for understanding subtype specific differences in bladder cancer. We have developed and validated immunocompetent, subtype specific models of bladder cancer, and we have used these models to assess differential responses to immune checkpoint inhibition. Methods: Two distinct models of murine bladder cancer were developed in a C57BL/6 background. The UPPL models were generated through Pten/Trp53 conditional knockout in Uropakin3a expressing cells. BBN models were generated through exposure of wild-type mice to the carcinogen N-butyl-N-(4-hydroxybutyl)nitrosamine and subsequent generation of cell lines from spontaneous tumors. RNAseq was performed on several BBN and UPPL tumors and cell lines, with findings validated with flow cytometry and T/B cell receptor (TCR/BCR) ampli- con sequencing of tumor infiltrating lymphocytes (TILs). Results: BBN and UPPL models reflected characteristics of human basal and luminal bladder cancers, respectively. BBN (basal) models demonstrated higher immune gene signature expression, with concordantly higher numbers of TILs compared to the UPPL (luminal) model (p < 0.0001). Two BBN and two UPPL models were assessed for response to anti-PD-1 therapy in vivo as syngeneic tumors grown in wild type C57BL/6 mice. One of the BBN lines (BBN963) demonstrated robust control of tumor growth in some animals, including multiple complete re- sponses (CRs) in tumor models. Both tumors that progressed led us to characterize BBN963 as a mixed response model. The marked response to PD-1 blockade in BBN963 was associated with significantly higher sharing of TCR CDR3 sequences among TILs compared to sequences of the other tumors (p = 0.003). In addition, analysis of BBN963 tumors by flow cytometry demonstrated naive and memory T cell phenotypes correlated with increased and decreased tumor sizes, respectively. Closer examination of individual BBN963 tumor responses to PD-1 blockade revealed distinct responder and non-responder infiltrating immune cell phenotypes. Responders demonstrated a less diverse B cell repertoire (p = 0.0043) with increased BCR CDR3 sequence sharing (p < 0.0001). Discussion: We have developed two unique classes of murine bladder cancer lines. UPPL and BBN, with gene expression and TIL profiles that closely correlate with human luminal and basal bladder cancers, respectively. The BBN and UPPL subtype specific models can serve as a tool for elucidating bladder cancer responses to immunotherapy. The mixed response of BBN963 tumors to PD-1 blockade should be an asset for assessing pathways mediating response to checkpoint blockade as well as the value of combination therapy.

1655 Organotypic 3D models to characterize the molecular require- ments for NK and T-cell infiltration. Wolfgang Sommmer,1, 2, 3, 4 Andreas Ger- wald,1 Viola Hedrich,1 Marta Majewska,1 Helmut Dolznig2.1 Boehringer Ingel- heim RCV, Vienna, Austria; 2Medical University of Vienna, Vienna, Austria. Multiple studies have demonstrated that the presence of tumor infiltrating lymphocytes is strongly correlated with increased survival. However, the level of infiltration within a specific tumor entity and between various tumor types varies widely. Increasing the degree of lymphocyte infiltration in a tumor represents an innovative drug concept. Organotypic 3D models consisting of tumor spheroids, stromal fibroblasts and Natural Killer (NK) or T-cells could represent valuable models to appropriately model infiltration and establish novel therapeu- tic concepts in the area of immune-oncology. As a first step in the develop- ment of a model for lymphocyte infiltration, the cytotoxicity and infiltration of human NK cells in 3D tumor spheroids were analyzed. Different NK cell lines and primary NK cells had a toxic effect on 3D tumor spheroids upon co-culture. Cyanososes of co-cultured tumor spheroids showed infiltrated NK cells inside tumor spheroids. Infiltred NK cells recovered from tumor spheroids were characterized by flow cytometry. Results showed that a higher infiltration cor- relates with cytotoxicity on tumor spheroids and transcriptomes of infiltrated vs. non infiltrated NK cells differ substantially. IL-12 stimulation increased the kill- ing effect of NK cells on tumor spheroids, inducing higher expression of Perfo- rin, IFNγ and GranzymeB levels. 3D tumor spheroids were embedded in a Matrigel/Collagen mixture with fibroblasts and the effect of compounds on NK cell infiltration was monitored. It was demonstrated that not only IL-12 in- creased NK cell infiltration, but also several compounds. Moreover, encapsu- lated 3D models with different cell types (tumor cells, fibroblasts, NK or T-cells) were established at a bioreactor scale to mimic the in vivo situation of complex tumor tissues. We could demonstrate NK and T-cell infiltration into these en- capsulated 3D tumor spheroids. These encapsulated model systems permit long-term cultures to study the effect of compounds and/or NK or T-cells on later stages of tumorigenesis. In conclusion, this study demonstrates that organotypic 3D models are a valuable tool for the analysis of the molecular mecha- nisms that regulate lymphocyte infiltration into tumors.

1656 Comprehensive evaluation of human immune system reconstitut- ion in NSG™ and NSG™ SGM3 toward the development of a novel Onco- Hu™ xenograft model. Aaron J. Middlebrook,1 Eileen Snowden,2 Warren Por- ter,1, 2 Friedrich Hahn,2 Mitchell Ferguson,2 Brian Soper,2 James Keck,2 Joan Malcolm,3 Shannon Dillmore,3 Smita Ghanekar,1 Rainer Blaesius,2 2 BDS Bioci-ences, San Jose, CA; 2BD Technologies, Raleigh-Durham, NC; 1The Jackson Lab- oratory, Bar Harbor, ME. The recent successes of immunotherapeutic approaches to the treatment of melanoma and the promise of similar treatments in a variety of other cancers underscore the importance of the immune system in cancer. Indeed, effective therapeutic design and evaluation require a comprehensive understanding of the interplay between the immune compartment and the proliferating tumor cells that comprise the tumor microenvironment. A humanized mouse strain engrafted with cancerous tissue from a patient derived xenograft (PDx) tumor provides researchers with a highly sophisticated tool, ideally suited to facilitate the design of treatment strategies that prevent tumor evasion of immune cells and that improve cytotoxic responses. Severely combined immunodeficient mice such as NOD scid gamma (NSG™) and triple transgenic NSG mice expressing human cytokines KITLG, CSF2, and IL-3 (NSG™-SGM3) are proven hosts for the engraftment of human tumors and establishment of human immune system components following hematopoietic stem cell (CD34+) transplantation. These models provide researchers with a tool that supports the development of myeloid lineages and regulatory T cells potentially represents a substantial improvement over standard NSG mice. Here we employ four-color flow cytometry panels to perform a comprehensive and detailed analysis of the entire immune system. The four panels are designed to fully characterize specific branches of the immune system: 1) T cells 2) NK cells/dendritic cells/B cells 3) myeloid lineages, and 4) immune checkpoint markers. Blood, spleen, and bone marrow tissue from both NSG and NSG-SGM3 mice were evaluated at 9, 16, 21, and 31 weeks of age using each of the four phenotyping panels. Our results indicate that the triple transgenic NSG-SGM3 mice exhibit a more com- pletely humanized immune system compared to NSG mice, with specific im- provements in the distribution of T-cell subsets and overall representation of the myeloid lineage. NSG mice engrafted with allogeneic human tumors represent a valuable preclinical testing platform for immuno-oncology.

1657 Evaluating anti-tumor activity of the human anti-PD-1 antibody pembrolizumab using humanized mouse models. Douglas E. Linn,1 Razvan Cristescu,1 Kallol Ray,1 Shuli Zhang,2 Siriypi Dhandapani,3 Sarav Kaliyape- runala,3 Jennifer H. Tearley,1 Brian J. Long,1, 2 Merck & Co., Inc., Boston, MA; 3Merck & Co., Inc., Palo Alto, CA. Targeting immune checkpoints has proven to be an effective strategy for reactivating the immune system to elicit potent anti-tumor activity. For exam- ple, the anti-PD-1 antibody pembrolizumab has shown robust and durable clinical activity in numerous malignancies including melanoma and non-small cell lung cancer. Unfortunately, a subset of patients do not benefit from pembroli- zumab therapy. The phenotypical expression of cytokines that support the develop- ment of myeloid lineages and regulatory T cells potentially represents a substantial improvement over standard NSG mice. Here we employ four-color flow cytometry panels to perform a comprehensive and detailed analysis of the entire immune system. The four panels are designed to fully characterize specific branches of the immune system: 1) T cells 2) NK cells/dendritic cells/B cells 3) myeloid lineages, and 4) immune checkpoint markers. Blood, spleen, and bone marrow tissue from both NSG and NSG-SGM3 mice were evaluated at 9, 16, 21, and 31 weeks of age using each of the four phenotyping panels. Our results indicate that the triple transgenic NSG-SGM3 mice exhibit a more com- pletely humanized immune system compared to NSG mice, with specific im- provements in the distribution of T-cell subsets and overall representation of the myeloid lineage. NSG mice engrafted with allogeneic human tumors represent a valuable preclinical testing platform for immuno-oncology.
sponder hypothesis and its prognostic value. Additionally, humanized mice may help identify optimal combination treatment regimens to provide more meaningful clinical benefit to patients who do not respond to single-agent therapy with pembrolizumab.

#1658 Establishment of CD137 humanized mouse model for efficacy assessment of agonistic anti-CD137 therapeutic antibodies. Dave Xuexong OuYang,1 Gang Chen,2 Zhensheng Wang,1 Lei Zheng,1 Annie Xiaoyu An,1 Jean-Pierre Wery,1 Jay Liu,2 Xin Dong,2 Henry Q. X. Li1. Crown Bioscience, Inc., Taicang, Jiangsu, China; 2Nanjing Galaxy Biopharmaceutical Co. Ltd., Nanjing, Jiangsu, China.

CD137 belongs to the TNF receptor super family, and its activation is essential to the function of T cells and NK cells. Multiple agonistic anti-CD137 antibodies are currently being tested in humans with advanced cancer, which expected to become an important new immuno-oncology therapeutics in clinic. It is important to establish preclinical models for efficacy assessment of investigational CD137 antibodies before entering clinic. Currently available animal models cannot meet this need. Syngeneic mouse tumor models provide a useful platform for testing surrogate immuno-oncology therapies, but cannot be used for testing human therapeutic antibodies due to the species specificity; human immunity reconstituted models, by human PBMC or hematopoietic stem cell inoculation into immune deficient mice, suffering from highly variable responses, are not robust enough to provide a reliable system for efficacy studies. Establishing target humanized models by replacing mouse therapeutic target with human counterpart while maintaining normal mouse immunity is a practical approach to evaluate human therapeutic antibodies in vivo. Using CRISPR-Cas9 gene editing, we have engineered human CD137 knock-in model expressing chimeric human/mouse CD137 composed of human extracellular and transmembrane domains (Exon 4–7), with intact mouse signal peptide and intracellular domain. FACScanalyses of splenocytes derived from homozygous knock-in mice showed that CD3/CD28 stimulated T cells only express chimeric CD137 reactive to human CD137 antibodies, but not mouse CD137; Yet the expression levels of the chimeric CD137 in the knock-in mice are generally lower than endogenous mouse protein in wild-type C57BL/6 mice. At present, we are testing the efficacy of reference human CD137 agonistic antibodies by treating these homozgyous knock-in mice grafted with syngeneic MC38 tumor cells. Data will be presented at the conference.

#1659 Immunological characterization of the Oncopig model and detection of cell-mediated immune responses to cancer. Nana H. Overgaard,1 Daniel R. Principe,2 Jeanne T. Jakobsen,1 Laurie A. Rund,1 Paul I. Grippio,2 Gregers Jungersen,3 Lawrence B. Schook.1 1Technical University of Denmark, Frederiksberg C, Denmark; 2University of Illinois, Chicago, IL; 3University of Illinois, Urbana-Champaign, Urbana-Champaign, IL.

In recent years, immunotherapy has shown great breakthroughs; however, the majority of preclinical studies has been based solely on rodent models and tends to experience a troublesome translation to human patients often related to the differences between mice and humans. Here, we utilized the Oncopig model that, due to its similarity in the immunome, metabolism, and size between humans and pigs, can serve as a supplementary large animal model for translational cancer research. With the establishment of the immunoascore as a new approach for staging cancer patients, it has become evident that the immune status of the tumor microenvironment has a crucial impact on the patient’s outcome and response to therapies. For this reason, we set out to perform an immunological characterization of Oncopig-derived tumors to elucidate the potential in using this model for testing cancer immune therapeutic approaches. Following injection of Cre recombinase, the transgenic Oncopig expresses KRA(Gr2/CD11b) and TPS(Gr1/CD11c), two mutations commonly found in human cancers, subsequently resulting in tumor formation at the site of injection. The expression of these two transgenes was confirmed using intracellular flow cytometry staining or immuno histochemistry (IHC). Subcutaneous, intramuscular, and testicular tumors all showed the presence of infiltrating CD3+ cells, which were found both in the periphery and in the core of the tumor as determined by IHC. With the establishment of a digestion protocol allowing isolation of the in vivo-generated tumor cells, we confirmed the CD3+ staining and found that the infiltrating T-cell pool was comprised of both cytotoxic and regulatory cells as indicated by positive flow cytometry staining for the CD8β chain and FoxP3, respectively. In order to investigate the immunogenicity of the tumor cells, we set up flow cytometry detection of porcine TNF-α, IFN-γ, and perforin in a PBMC-tumor cell co-culture. Despite the production of various cytokines, actual tumor cell killing is a desirable parameter to measure when evaluating both the immunogenicity and response to therapy. Therefore, we developed, to our knowledge, the first fluorescence-based in vitro porcine cytotoxicity assay and found a significant recognition and specific killing of autologous tumor cells in an effector-target titration dependent manner. Taken together, our results show that the established Oncopig tumors are infiltrated by T cells exhibiting an either cytotoxic or regulatory phenotype, thus indicative of a tumor microenvironment mimicking the complexity seen in human patients. Additionally, we were able to measure cell-mediated immune responses to cancer in this novel, large animal model, and both the cytokine production and tumor-specific killing hence underline the potential in using the Oncopig for future testing of immune therapies against human cancer.

#1660 Humanization of patient derived xenograft (PDX) cancer model mice with peripheral blood mononuclear cells (PBMCs). Jens Henrik Norum, Dag Josefsen, Gunnar Kvalheim, Olav Engebretsen, Therese Serlie, Gunhild M. Melandsmo. Oslo University Hospital, Norwegian Radium Hospital, Oslo, Norway.

Immunocompetent transgenic mouse models have for decades served as valuable tools to address the effect of oncogenes and tumor suppressors. Immunodeficient mice have been used to establish patient derived xenograft (PDX) models harboring human tumors. These types of models have been used to study cancer initiation and progression as well as preclinical evaluation of anticancer drugs. One major limiting factor for both these approaches is the immune system: the PDX models lack an immune system and the immune system in immunocompetent mouse models display differences compared to the human immune system. Thus, in cancer research there is a need for preclinical models to study the influence of human immune cells on tumor progression and response to cancer therapies. We have established and optimized protocols for in vitro stimulation of human immune cells (IV) or intraperitoneal (IP) injections of isolated human PBMCs, to generate humanized mice harboring human immune cells. Our protocols do not require irradiation or busulfan pretreatment of the animals. Flow cytometry analyses showed that mainly T helper cells, CD4+, and cytotoxic T cells, CD8+, were present in the humanized mice. Immunohistochemistry (IHC) analyses of humanized PDX cancer model mice showed that human lymphocytes were present in the tumor periphery and some very few cells within the tumor, in addition to the human leukocytes in the liver and spleen. The growth rates of the PDX tumors were not affected by the humanization. Our protocols require minimal preparations of the animals and generate humanized PDX mice harboring human lymphocytes locating in close proximity of, as well as inside the PDX tumors. The model systems are suitable for preclinical studies of human, adaptive immune responses in tumor progression and cancer therapies.


With clinical success and FDA approval of Iplimumab, Nivolumab, Pembrolizumab and Atezolizumab, developing biotherapeutics against immune checkpoint targets, like CTLA-4, PD-1/PDL-1 and others, becomes hot pursuits for big pharma and biotech. However, traditional syngeneic models with surrogate immune system have limitations. New humanized mouse models may be useful but not reliable and robust due to the species specificity and highly variable responses. It is crucial to establish additional preclinical models for efficacy assessment of investigational immune-oncology antibodies before entering clinic. Establishing human therapeutic target engineered mouse models by replacing mouse gene with human counterpart while maintaining intact mouse immunity is a practical approach for in vivo studies. Applying CRISPR-Cas9 and other gene editing techniques, we have engineered genetically a series of single and double human immune-checkpoint genes, like PD-1/PDL-1, CTLA-4, TIM3, CD137 and others, knock-in model expressing chimeric human/mouse targets composed of human extracellular domains, with intact mouse signal peptide and intracellular domain. FACScanalyses of immune cells derived from homozogyous knock-in mice showed that activated cells only express chimeric targets reactive to anti human target antibodies, but not mouse gene specific antibodies. We have been testing the efficacy of reference antibodies by treating these homozymous knock-in mice inoculated with syngeneic murine tumor cells. Other applications, like target validation, combination therapy and biomarker discovery, are also explored.
Introduction: Agents that reverse T cell inhibitory signals have reinvigorated the strategy of cancer immunotherapy and are leading to robust clinical responses. In colorectal cancer (CRC), objective responses to single-agent therapy with anti-PD-1/PD-L1 antibodies have largely been restricted to the small proportion of CRC patients with microsatellite unstable (MSI-high) disease. Although additional subsets of advanced CRC patients that are responsive to immune checkpoint inhibition in combination with tumor-targeted agents, preclinical models have been hampered by their immune-compromised status. In order to gain a better biological understanding of the context of immune responses and facilitate preclinical evaluation of combination strategies incorporating cancer immunotherapy, we developed a “hematopoietic” humanized model of patient-derived xenografts Reid et al. 1996 to leveraging this model for the development of rational combinations. Methods: BRG.NOD/Sirpalfa newborn pups were humanized through transplantation of 1x106 CD34+ cells purified from umbilical cord blood. Mice were evaluated for chimerism at 8 and 12 weeks. At 16 weeks, tumor tissue from established PDX models was implanted on the right and left flanks of humanized mice. When the average tumor size reached a volume of ~150-300 mm3, the mice were randomized into vehicle or nivolumab (30 mg/kg twice weekly i.p.) treatment groups according to chimerism. Mice were monitored for signs of toxicity and tumor size was evaluated twice weekly by caliper measurements (tumor volume = length x width2) x 0.52. At the end of the treatment, mice were euthanized while sera, lymph nodes, spleen, bone marrow and tumors were collected for immunomonitoring and analyses. Results: As preliminary proof-of-concept, we successfully established 3 humanized CRC PDX models and one breast cancer cell line (MDA-MB-231). In one of the CRC (MSI-high) and in the breast model we observed tumor growth inhibition in the treated groups vs control. We detected differences in PD-1 expression among treated versus control mice, with lower expression in the nivolumab-treated groups. We also observed an increased number of TILs, CD8+ T cells and greater numbers of T cells in the lymph nodes of treated mice, suggesting T cell expansion. Mice were highly chimeric with high TILs whereas responder tumors exhibited an increase of CD44 high IFN+ CD8+ T cells, high CD8% and a higher effector memory% (HLADR+ CD45RO+). Conclusions: Humanized PDX models were successfully established and tumor engraftment occurred in all humanized mice with nivolumab-treated mice demonstrating the development of lymph nodes that were populated by activated T cells. These preliminary results demonstrate that human immunity and PD-1 expressing T cells exist in these models and provide the basis for planned immunotherapy combination studies.

#1664 Inhibition of 4T1 mammary tumor growth in BALB/c mice by subcutaneous and intraperitoneal injection of a 4T1 whole cell vaccine containing IL-2 and GM-CSF as adjuvants.

Jonathan F. Head, 1 Jeffrey T. Phillips, 1 Xiangpeng Jiang, 1 Robert L. Elliott. 1 OncoBioMune Pharmaceuticals, Baton Rouge, LA; 2SAB Breast Foundation, Baton Rouge, LA.

Developing animal tumor models for human cancer vaccines creates a tool to investigate the mechanism of action, variations in formulation, dosing schedules, and combinations with other forms of cancer therapy. In this study we developed a mouse whole cell mammary cancer vaccine model with both subcutaneous and intraperitoneal injection of the vaccine. The vaccine, containing 4T1 mouse mammary cancer cells (1,000,000 cells), IL-2 (0.2 μg) and GM-CSF (0.1 μg) in a total volume of 150 μl, was injected either subcutaneously into the backs of BALB/c mice or intraperitoneally. There were six injections of the vaccine (weeks 1, 2, 3, 7, 11, 15) and one week after the last injection 100,000 4T1 cells from cell culture in a volume of 100 μl were injected into the scapular region of the mice. At 22 days post 4T1 tumor transplantation there was a 57% inhibition of the growth of the 4T1 tumor in the mice receiving the subcutaneous injections of whole cell vaccine and a 42% inhibition of 4T1 tumor in the mice receiving the intraperitoneal injection of the vaccine. The inhibition of 4T1 mammary tumor growth in this mouse model with subcutaneous injection of a whole cell vaccine gives further support for the previously reported efficacy in a Phase 1/2 clinical trial of our therapeutic breast cancer vaccine containing autologous and allogeneic breast cancer cells in the adjuvant setting. The inhibition of 4T1 tumor growth with intraperitoneal injection suggests a delivery method that may be applicable to ovarian cancer and cancer patients with malignant ascites.

#1665 MuScreen™: A well-characterized syngeneic model platform for rapid in vivo screening.

Lan Zhang, Binchao Mao, Qian Shi. CrownBio, Taiyuan, China

Background: Syngeneic tumor models, many shelved for decades, have been revived as effective models for immunotherapy, accompanying the clinical success of the immuno-checkpoint inhibitors (e.g. anti-CTLA4, anti-PD1, anti-PD-L1 antibodies). In vitro cell-based screens that are frequently used in oncology to quickly identify responsive cells and assess PD effects, often fail in immuno-oncology, due to immunotherapeutics targeting the complex host immune system. Alternatively, an in vivo screening with a panel of models addresses many of these questions, e.g. PD and efficacy, but may be cost prohibitive. Material and methods: Leveraging in-house detailed profiling data on our syngeneic models, including efficacy benchmarking with anti-PD-1, P1-L1, CTLA-4, OX-40, GITR, LAG3 and TIM3 antibodies, RNAseq data on tumor samples, and FACs analysis on both baseline and post-treatment tumor samples, we created a novel in vivo screening tool MuScreen. MuScreen includes up to 20 well-characterized syngeneic models in 3 month screening run. Both PD and efficacy may be determined in the run, allowing researchers to make decisions based on results observed from a large dataset. To address the cost issue, test agents from multiple clients are pooled together for each run (sharing vehicle and other common groups) providing a significant reduction in the number of animals used and the associated costs. Results: CrownBio has established the largest collection of syngeneic models with well-characterized immuno-oncology data. With the three MuScreen runs, we have generated new data on common IO agents (e.g. aPD-1 antibody) and combinations treatments, with FACs analysis, IHC, and efficacy data. Based on these data, a bioinformatics analyses were further explored to identify the makers/pathways that could be correlated with the efficacy or PD effect of the tested IO treatment. Conclusions: MuScreen, the first in vivo screening tool for cancer immunotherapeutics, provides detailed response data on a panel of syngeneic models, and may help on biomarker discovery in a cost and time efficient manner.

#1666 Immune consequences of penfluridol treatment associated with inhibition of glioblastoma tumor growth.

Alok Ranjan, Nehal Gupta, Sharan Singh, Jiechao Wang, Melissa M. Kizer, Preston Wright, Sanjay Srivastava. Texas Tech Uni. Health Science Center, Amarillo, TX.

Glioblastoma is the most common and lethal brain tumor associated with only 12% median survival rate of patients. Despite the development of advanced surgical, radiation or use of combinations of anti-cancer drugs, treatment for glioblastoma
patients is still a challenge. The major contributing factor in glioblastoma progres-
sion and resistive nature is its ability to evade the immune surveillance. Hence,
modulating the immune system in glioblastoma tumors could be an important strat-
ye for anticancer therapeutics. Penfluridol, an antipsychotic drug has been shown
to have anti-cancer properties in our recently published studies. The present study
evaluates the immune response of penfluridol in glioblastoma tumors. Our results
demonstrated that penfluridol treatment significantly suppressed glioblastoma tu-
mor growth. Our current results demonstrated about 72% suppression of myeloid
derived suppressor cells (MDSCs) with penfluridol treatment in mouse bearing
U87MG glioblastoma tumors. MDSCs are known to increase regulatory T cells
(Treg), which are immunosuppressive in nature and suppresses M1 macrophages
that are tumor suppressive in nature. Our results also showed suppression of regula-

datory T cells as well as elevation of M1 macrophages with penfluridol treatment by
58% and 57% respectively. Decrease in CCL4 as well as IFNγ with penfluridol treat-
ment was also observed indicating decrease in overall tumor inflammation. This is
the first report demonstrating immune modulations by penfluridol treatment asso-
ciated with glioblastoma tumor growth suppression prompting further investiga-
tion to establish penfluridol as a treatment option for glioblastoma patients.


Head and Neck Squamous Cell Carcinoma (HNSCC) is the sixth most com-
mon cancer in the United States and affects 600,000 people each year worldwide.
With a five-year survival rate of only 50% and a recent rise in HPV-associated
HNSCCs, improved treatment protocols are urgently needed. Evidence of
immunosuppression is often reported in HNSCC, making immunotherapy an at-
tractive strategy for the management of this disease. The immune checkpoint
inhibitor pembrolizumab was recently approved for the treatment of metastatic
and recurrent HNSCC, but only 18% of initial participants in a trial of Pembrol-
izumab responded, and it remains difficult to predict patients likely to experience
response. We performed over multiple HNSCC cell lines and selected for genetic knockouts exhibiting altered PD-L1 expression. Stable knockout pools with representation of approximately 300 gRNAs per target gene (>20,000 target genes in the library) were expanded and serially sorted to create stable sub-populations with enhanced PD-L1 expression. These sub-populations were sequenced to identify gRNAs whose
expression with PD-L1 inhibition. We observed that these large-scale screens, when performed over multiple HNSCC cell lines with diverse genetic lesions, will identify patterns of targetable regulators that may ultimately be manipulated in combination with PD-1/PD-L1 inhibitors.

#1668 Comprehensive 10 color flow cytometry analysis of the neuroblas-
toma intratumoral immune response using the murine syngeneic neuro-2a

tumor model. David D. Draper, Matt Thayer, Alden Wong, Dan Sains, Scott C.

Wise. MI Bioscience, Ann Arbor, MI.

Neuroblastoma is the most prevalent solid tumor in childhood and the most common tumor in infants less than 1 year of age. In spite of aggressive multi-modal therapeutic approaches, the 5-year survival rate in patients with high-risk disease remains poor at ~10%. To this end, we have focused on harnessing the immune system to combat neuroblastoma. Anti-
body-Abrogating the immune checkpoint signaling pathway such as anti-
CTLA-4 and anti-PD-L1 suppress neuroblastoma growth in certain mouse models of disease. Furthermore, the dinutuximab antibody that targets gan-
giolside GD2 expressing cells has been demonstrated to trigger neuroblas-
toma regression using a mechanism that involves several immune subsets. Although evidence suggest the host immune response can be exploited to fight neuroblastoma, the key immune subsets, their relative contribution, and mechanism of action to suppress neuroblastoma growth have yet to be fully characterized. To this end, we used flow cytometry to immunopheno-

type the intratumoral immune response in the neuro-2a model of neuroblas-
toma. This was accomplished by developing two 10-color panels to quantify the proportions of various myeloid and T cell subsets respectively. Using the myeloid panel, our results showed that total neuro-2a tumor immune cells were predominantly myeloid lineage-derived (CD11b+). A combination of immunohistochemistry (IHC) and single-cell RNA sequencing (scRNA) was used to identify neuroblastoma cells (MDSCs) comprised the bulk of the myeloid cells and were more heavily skewed towards the granulocytoid phenotype versus monocyctic. An MDSC
deduction gate and a combination of F4/80, MHC class II, and CD206 anti-

codies enabled the analysis of tumor-associated macrophage subsets. Our results revealed that neuro-2a tumors contained relatively few activated M1 macrophages, which were typically associated with anti-tumor responses, and instead were dominated with a pro-tumor M2 macrophage response. The T cell antibody panel revealed that CD4+ T cells outnumbered CD8+ T cells by 2-fold. Regulatory T cells (Tregs) in the CD4+ T cell fraction were identified by a combination of FoxP3 and CD25 antibodies. The frequency of Tregs varied but represented <50% of total CD4+ T cells. Finally, the activation state of CD8+ T cells was examined using Ki-67, CTLA-4, and PD-1 anti-
codies. Analysis demonstrated that the majority of CD8+ T cells in neuro-2a tumors were actively proliferating based on the expression of Ki-67. How-

ever, most of the proliferating CD8+ T cells also co-expressed PD-1 suggest-
ing a loss of anti-tumor functionality and an exhausted phenotype. This study provides a comprehensive immune profile of neuro-2a tumors and supports a platform with which to test new single agent and combination therapies designed to treat neuroblastoma.

#1669 Multiplex immunohistochemical staining of PD-L1, PD-1, CD3, CD4, CD8, CD68, FoxP3, and Ki-67 and image analysis of tumor and invas-

ive margin in human FFPE NSCLC tissue. Lisa M. Daunfenthal, Cia G. Sia, Jinping Zheng, Natalia Jun, Eric P. Olsen, Christopher A. Kerfoot. Mosaic Lab-

oratories, LLC, Lake Forest, CA.

Evaluating the density of immune cell subtypes and their relative spatial position-
ing in cancers becomes an important tool in understanding response to immune
checkpoint inhibitors. The purpose of this study was to investigate protein expres-
sion of PD-L1, PD-1, CD3, CD4, CD8, D68, FoxP3, and Ki-67 with four multiplex
immunohistochemical assays (Ki-67+CD3, CD3+CD4+Ki-67, FoxP3+PD-
1+CD8, and CD68+PD-L1+CD3) using DAB, red, and green chromogens within
formalin-fixed, paraffin embedded small cell lung (NSCLC) tissues. The Ki-
67+/CD8+ multiplex was developed to evaluate the density of cytotoxic T cells and the

percentage that are proliferating. The CD3+/CD8+ Ki-67+ multiplex characterizes
the frequency of CD4+ T cells and evaluate proliferation in this subset. The FoxP3+PD-1+CD8+ multiplex evaluates the regulatory T cell subset and evaluates PD-1 expression. The CD68+PD-L1+CD3 multiplex characterizes PD-1 expres-
sion on macrophages and T cells. Cells densities were evaluated in tumors and
invasive margin regions, and image analysis was performed to quantify each
stain as a single agent and co-expression within each multiplex assay.

#1670 Identification and characterization of novel mediators of tumor-

duced T-cell dysfunction. Geoffrey J. Markowitz, Mary Philip, Andrea

Schietinger, Vivek Mittal, Weil Cornell Medicine, New York, NY; Memorial Sloan Kettering Cancer Center, New York, NY.

Immunotherapies targeting T-cell functionality have been shown to have ef-
ficacy in numerous cancers, including non-small cell lung cancer (NSCLC). However, treatment with currently available therapeutics, such as anti-PD-1 and
anti-CTLA-4, induce responses only in a subset of patients. This raises the pos-
sibility that numerous other effectors of T-cell dysfunction remain to be eluci-
dated. To evaluate possible novel mediators of T-cell dysfunction, we isolated CD8+ T-cells from orthotopic KrasG12D/p53-/- murine adenocarcino-
mas (HKP-1) that exhibited either stable or progressive disease as a function of tumor growth. RNAseq analysis revealed increased expression of several genes including Tim3, PD-1, Lag3, 2B4, and CISH, and decreased expression of GzmB and Eomes in CD8+ T-cells isolated from progressive tumors indicative of a dysfunctiional state, as well as multiple novel genes not previously associated with T-cell dysfunction. To explore the functional role of the novel candidate
genes, we have developed in vitro and in vivo assays leveraging Ova and GFP
overexpression in our HKP-1 cell line (KPI-1/Ova-GFP). We have begun to target these unique set of candidate genes in tumor-specific T-cells associated with dysfunction through innovative RNAi/shRNA and adoptive transfer ap-
proaches to facilitate therapeutic reprogramming and enhanced T-cell-medi-
ated immunity in lung cancer. We expect to provide novel strategies for design-
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IMMUNOLOGY: Tumor Models and Assays

#1671 Characterization of the immune landscape in stage II-III melanoma using qIF. Robyn Gartrell,1 Douglas Marks,2 Edward C. Stack,2 Yun Lu,3 Thomas D. Hart,4 Camille Gerard,3 Camden Esancy,4 Dan Tong Jia,1 Paul Armenta,7 Daisuke Izaki,6 Daniella Davari,4 Ashley White-Stern,7 Zoe Blake,8 Yi-chun Fu,9 Basil Horst,10 Yvonne Saenger,11 Columbia University School of Medicine, New York, NY; 2Flagship Biosciences, Westminster, CO; 3Miltenyi Biotec, Bergisch Gladbach, Germany; 4Joule, Seattle, WA; 5Department of Pathology, University of Washington, Seattle, WA; 6Novo Nordisk, Bagsvaerd, Denmark; 7MS Bioworks, Ann Arbor, MI; 8Cayman Chemical Company, Ann Arbor, MI.

Background: The tumor microenvironment plays a crucial role in cancer progression, often supporting immune evasion. This is of particular importance in melanoma, where immune checkpoint therapies have resulted in significant clinical benefit, yet only in a subset of melanoma patients respond. Precise biomarkers are urgently needed to characterize the tumor immune micro-environment, both for prognostication and to predict the benefit of immuno-therapeutic intervention. HLA-DR on tumor cells and Ki67 on cytotoxic (CD8+) T cells have been proposed as biomarkers of anti-PD1 activity. Quantitative immunofluorescence (qIF) allows for automated quantitation of phenotypes and spatial distributions of immune cell populations within formalin fixed paraffin embedded (FFPE) tissues. Methods: To characterize the tumor immune microenvironment, we screened databases at the Herbert Irving Cancer Center (HICC) at Columbia University for early stage melanoma patients with documented clinical follow up. We identified a preliminary population of 40 stage II-III melanoma patients diagnosed between 2000 and 2012. Clinical follow up was available on 21 patients. Patients were followed alive with no evidence of recurrence of another malignancy, and 8 who died of melanoma. 19 patients had more than 24 months of survival information available but no detailed clinical information. 5μm slides from either primary biopsy or subsequent wide local excision procedure were stained using qIF for DAPI, CD3, CD8, CD68, SOX10, HLA-DR and Ki67. Cell phenotypes within representative fields selected by a trained dermatopathologist (BH), were visualized using multi-spectral imaging, and analysis of spatial distribution of cells were analyzed using inForm image analysis software (Perkin Elmer), and Spotfire software (TIBCO). Results: We were able to quantify and identify coordinates for multiple immune cell subsets in melanoma tissues. In 21 patients with clinical follow up, we found that higher densities of CD3+CD8+ T cells in tumor and stroma tended towards correlation with non-recurrence. In addition, CD68+HLA-DR-predicts poor prognosis (p<0.05), whether in tumor or stroma. Assessment of spatial distribution across all 40 tumors demonstrated that CD3+CD8+ cells are closer to CD68+ cells and Sox10+ tumor cells when they express HLA-DR (p<0.001). Conversely, CD3+CD8+ cells are significantly farther from tumor cells when they express Ki-67, (p<0.0001). Among patients with clinical follow up, CD3+CD8+ cells were closer to CD68+HLA-DR- cells in recurrent patients (p<0.05). Conclusion: Using qIF imaging and analysis we find that density and spatial relationships of immune cell subsets correlates with recurrence status. qIF may offer the potential for the development of prognostic biomarkers in stage II/III melanoma.

#1672 Workflow automation and parallelization improves the isolation and analysis of tumor-infiltrating immune subpopulations. Janina Brauner,1 Jan Pettersson-Kane,1 Karen Ryall,1 Daniel G. Rudmann,1 Brooke Hirsch,1 Joseph Krueger,1 MS Bioworks, Ann Arbor, MI.

Immunotherapy against cancer has proven clinical efficacy and tremendous potential in multiple tumor entities. Syngeneic mouse tumor models represent the gold standard to analyze effects of immunotherapy due to their fully competent immune repertoire. However, the amount and composition of tumor infiltrating leukocytes (TIL) is highly variable, complicating the targeted analysis of subpopulations. In particular, small subpopulations cannot be analyzed properly but may be lost in the background noise. When working with large cohort sizes, even the immune-phenotyping of TIL by flow cytometry is time-consuming and dataprocessing highly workintensive working with large cohort sizes, even the immune-phenotyping of TIL by flow cytometry is time-consuming and dataprocessing highly workintensive. Importantly, while the TIL enrichment significantly reduced the time and reagent costs in immune subset analysis, the composition of infiltrating immune cells was not affected, the risk of introducing a bias by this method. Taken together, we have developed an automated workflow for the isolation of TIL from mouse tumors reducing time and costs of downstream analysis while standardizing and enhancing the detection and quantification of immune cell subpopulations.

#1673 Mass spectrometric characterization of peptides associated with molecules of the major histocompatibility complex. Michael Ford,1 Richard Jones,1 David Allen,1 Ravie Amunugama,2 Paul Del Rizzo,2 Michael Pisano,3 James Moebley,2 Paul Domanski,2 Bill Ho,2 Daniel Bochar,2 MS Bioworks, Ann Arbor, MI.

The major histocompatibility complex (MHC) is a region of highly polymorphic genes encoding for glycoproteins (MHC molecules) that form the cell-mediated branch of the acquired immune system. In the cytosol, cellular self and foreign (non-self) proteins are constantly being degraded; it is the peptides of these proteins that are presented to the immune system. The obvious application then is the targeted destruction of cancerous cells. To enable the molecular level characterization of peptides associated with molecules of the major histocompatibility complex requires a targeted protein complex enrichment, an unbiased peptide elution and finally a peptide analysis method. Most frequently an immunoprecipitation is used to isolate the target complex. The peptide elution is performed under conditions minimizing protein contamination and finally peptide analysis is accomplished by mass spectrometry. Here we present a case study of our recent work optimizing and performing a workflow for the analysis of peptides associated with Class I MHC molecules. The goal of the assay optimization was to maximize the amount of antibody required for the assay, to minimize the amount of biological material needed from which the complex is isolated and to achieve the optimum sensitivity towards the hitherto unknown target peptides.


Quantification of tumor-infiltrating lymphocytes (TILs) in non-small cell lung cancers (NSCLC) is valuable for understanding patient prognosis and survival. TILs comprise a subset of tumor-infiltrating leukocytes that modulate immune evasion and response to therapy. Understanding the composition of TIL subsets, especially relative to the total tumor leukocyte population, may provide additional context for understanding NSCLC pathogenesis and patient response to treatment. However, availability of tissues and use of chromogenic assays can limit the number of TIL and leukocyte subset markers assayed in a tissue section. Therefore, we developed a workflow to identify TIL markers in NSCLC using morphometric parameters and routine TIL marker monoplex immunohistochemistry (IHC) assays to further identify the composition of TIL subsets. Computational Tissue Analysis (cTAX™) tools were used to determine the morphometric parameters which could identify immune cells in the absence of biomarker stain. The morphometric features which characterized immune infiltrates were used to quantify the total immune cell population frequency in the tumor nests and surrounding stroma in hematoxylin-stained tissues. The leukocyte population identified with morphometric parameters was correlated with CD45+ cell frequencies identified by cTAX based on biomarker staining in CD45-stained serial sections. This morphometric rule-
identified adhesion molecules, chemokine ligands, and receptors involved in traffic of lymphocytes to tumors as well as the function of NK cells. Treatment of melanoma with EZH2 inhibitors showed increased killing by NK cells suggesting that an immunosuppressive effect of EZH2 involving both innate and adaptive immunity. In a mouse model of subcutaneous B16 melanoma, a course of EZH2 inhibitor did not change tumour immune responses in immunosufficient mice but caused a transient 9-fold increase in circulating Ly6G+ [granulocytic] myeloid-derived suppressor cells [MDSCs] suggesting the effect of EZH2 modifiers on the myeloid compartment. In summary, patients with somatic activation of EZH2 may benefit from treatment combinations that include inhibitors of EZH2. Supported by NHMRC grant GNT1190178. Sydney medical school foundation and Cancer Institute NSW. F.V.F. is grateful for the support of grants CA154887 from the National Institutes of Health, National Cancer Institute and CRCC CRN-17-427258.

#1677 Immuno-target selection of infiltrating immune cells and laser capture microdissection mediated transcriptional profiling. Ross Haggart, Chaxiraxi Arzolla-Donate, Elliot Harrison, Benjamin J. Reed, Saba Alzabin, Gino Miele, Epistem Ltd., Manchester, United Kingdom.

Immune checkpoints define an evolving class of inhibitory mediators that are expressed by tumour cells and infiltrating leukocytes to down-modulate the effector immune response against tumour cells. Therefore, the most recent efforts in cancer therapy centre on targeting immune-mediated mechanisms of tumour evasion. The ability to assess gene expression profiles from isolated populations of tumour-infiltrating lymphocytes (TILs) can assist in monitoring checkpoint inhibitor therapy efficacy and provide a better understanding of clinical outcomes. Ideally, transcriptional profiles should be obtained from isolated cell populations of interest, free from contaminating cell populations that may create adverse background noise. Micro sampling by laser capture microdissection is a powerful tool that allows for specific analysis from whole tissues, particularly in a heterogeneous microenvironment such as in a tumour, where less well represented target cells are dwarfed in abundance by tissue stroma and other cell types. However, whilst the application is potentially powerful for target discovery and mechanistic understanding, the process is notoriously difficult, particularly in clinical specimens. We developed a methodology to successfully overcome these challenges in analysing target cells, organelles or other tissue subsets by transcriptional profiling following immunostained-mediated laser capture microscopy (LCM). Thorough optimisation of traditional immunohistochemistry techniques enabled us to select target cell populations of interest from tissue sections whilst minimising degradation of mRNA and miRNA allowing us to perform genomic and pathway interrogation using microarray and/or RT-qPCR analysis. Our technique allows multiple capture types per slide, sample pooling when required, high capture throughput and capture image documentation. Multiple target selection to different lymphocyte populations, including tumour-infiltrating lymphocytes, can be performed routinely using comparative serial sectioning. Here we exhibit simple staining and immunostaining histology images using methods sympathetic to RNA integrity which allow for target selection by specific staining or target morphology, and demonstrate comparative analysis of matched pairs of disease and healthy sections of colon tumours. We used a Palm MicroBeam 4 LCM platform to identify, cut and specifically capture target cell populations from frozen embedded tissue. This approach enabled us to isolate discrete targeted cell populations free from contaminating cell populations. Epistem’s Single Cell RNA-Amp was then used to provide robust and linear amplification of RNA and enable comparative analysis in applications such as target discovery and pharmacodynamics. The results indicated that transcriptional profiling was technically robust with a good replicative correlation despite the limited input obtained by micro-sampling.


Glioblastoma (GBM) is a deadly brain cancer that suppresses cellular immunity through the production of anti-inflammatory cytokines/catabolites, expression of immune checkpoint molecules, and induction of tolerogenic immune cell subsets. While preclinical models have shown each of these mechanisms to negatively impact survival outcome, it remains unclear how these distinct processes manifest themselves within the context of a coordinated immune system and whether additional immunosuppressive mechanisms exist. To address these questions, we have developed an extensible in vivo screening platform to holistically determine how GBM affects cellular immune organization. The method allows for temporal assessment of leukocyte population dynamics in response to
tumor progression. Leukocytes derived from lymphoid tissues of tumor-bearing mice are isolated and immunolabeled with a 12-color optimized immunofluorescence panel (OMIP) targeting several major leukocyte lineages. Single-cell data is acquired by flow cytometry and is clustered in an unsupervised manner based on cell-surface protein expression. A vector-based classification system is then used to efficiently identify cell lineage relationships among immune cell lineages. The platform allows for the extraction of immune signatures whose divergence from tumor-naive standards may be quantified and statistically analyzed; such signatures may also be correlated with response/resistance to therapy. The approach has been validated on orthotopic GL261 glioma in syngeneic C57BL/6 mice, yet it is readily amenable to the study of diverse model systems, including those that are driven by clonal or driver oncogenes. Alternative flow cytometry antibody panels may easily be substituted to probe more specific immune cell subsets of interest. Systems biology approaches applied to the field of tumor-induced immunosuppression hold promise in identifying novel network-level immune signatures, facilitating a better understanding of immunotherapeutic drug action, and driving next generation high-dimensional biomarker discovery.

#1679 BET inhibitors suppress PD-L1 expression in pancreatic stellate cells. Kazumi Ebine, Brian T. DeCant, Katharine A. Collier, Thao N. Pham, Krishan Kumar, Hidayatullah G. Munshi. Northwestern University, Chicago, IL

Single agent treatment with T-cell checkpoint inhibitors has not been effective in pancreatic ductal adenocarcinoma (PDAC) patients. The PDAC stroma, which can account for as much as 80-90% of the tumor mass, can act as a physical and an immunologic barrier to T-cell-mediated therapies. Transgenic mouse models have shown that ablation of pancreatic stellate cells (PSCs), key regulators of fibrosis in vivo, can sensitize PDAC tumors to immune checkpoint therapies. Recently, inhibitors targeting bromodomains and extra-terminal (BET) proteins, a number of which are currently being evaluated in clinical trials for solid tumors, were shown to induce stellate cells to become quiescent and decrease collagen production. The BET family of proteins binds to acetylation motifs present in histones and enables recruitment of transcription factors and other chromatin regulators during RNA transcription. We have found that PSCs express significantly increased PD-L1 levels compared to pancreatic cancer cell lines. We show that BET inhibitors, and in particular specific knockdown of BRD4 protein, decrease basal and IFN-γ-mediated PD-L1 expression in primary stellate cells. We also show that, in contrast to a recent report using cancer cells, c-MYC does not mediate basal or IFN-γ-mediated PD-L1 expression in stellate cells. Instead, we show that the IFN-γ-mediated PD-L1 expression is regulated by IRF1, suggesting cross talk between BRD4 and IRF1 in the regulation of PD-L1 expression. Ongoing in vivo experiments will evaluate the role of BET inhibitors in the regulation of PD-L1 in mouse models of pancreatic cancer.

#1680 BPM31510, an anti-cancer agent selectively causes activation and proliferation of T cells, demonstrating potential utility in an immune-oncology setting. Maria-D Nastke, Nidhi Gaur, Louisa Dowal, Samantha Fowler, BERG, LLC, Framingham, MA

BPM 31510, a clinical stage, nanodispersion of ubenadencoreone has a unique mechanism of action that effectively induces a Warburg switch in cancer cell metabolism and activation of apoptosis. Given the observed central role of BPM 31510 in regulating mitochondrial function in cancer cells, it is of great interest to investigate the ability of BPM 31510 to modulate immune cells and their functionality. Therefore, the effects of BPM 31510 on peripheral blood mononuclear cells (PBMCs) were investigated to elucidate the immune-metabolic mechanism of BPM 31510. Healthy donor PBMCs activated with PHA or PWM was used as model system, and the effect of BPM 31510 on immune cells viability was determined using flow cytometry. In addition, the effect of BPM31510 on immune cell function was evaluated by measuring a panel cytokines released in these cells, using a quantitative ELISA platform from Meso Scale Discovery. Results: BPM 31510 has been shown in previous studies to effectively induce apoptosis on a variety of cancer cell lines while sparing normal cells. Interestingly, increasing concentrations of BPM31510 lead to an increased frequency of viable CD3+ cells. Further phenotypic analysis revealed that cytotoxic T cells (CD8+/CD3+) and T helper cells (CD4+/CD3+), as well as NK cells (CD56+/CD3-), were observed in the increased T cell frequencies. On the other hand, B cells (CD19+), NK cells (CD56+/CD3-), and the monocytes (CD14+) showed a decrease in frequency, an effect reflected also by a reduction in viability with increasing BPM 31510 concentrations. Cytokine analysis indicated that effector cytokines IL-2, IFN-γ, and TNF-α were secreted at significantly higher levels with increasing concentration of BPM 31510. Interestingly, IL-10 level, an immunoregulatory cytokine, was strongly decreased in the supernatant of PBMCs treated with BPM31510. Taken together, we demonstrated that BPM 31510 has a direct effect on immune cells and their functionality. Although BPM 31510 induced apoptosis of cancer cells, we find that it supports cell proliferation of T cells, and effector function of adaptive immune cells, likely by providing a higher energy supply for effector T cells. Subsequently, a higher activation frequency of CD8+ T cells was observed in response to BPM31510. These findings highlight the potential role of BET inhibitors in the management of cancer patients.

#1681 The antitumor effect of a soluble recombinant human thrombomodulin as growth suppression against gastrointestinal tumor in murine peritonitis model. En Amada, Kazumasa Fukuda, Koshi Kumagai, Koichi Suda, Hiroya Takeuchi, Yuko Kitagawa. Keio University School of Medicine, Tokyo, Japan

Introduction: Severe postoperative inflammatory response (PIR) with CARS increases the risk of tumor recurrence after cancer surgery by Tregs suppressing antitumor immunity. Also, cancer recruits Tregs into its tissue by secreting CCL22 and TGF-beta that strengthen its survival. We established a model to represent tumor dynamics under PIR by mice that are performed cecal ligation and puncture (CLP) followed by subcutaneous injection of cancer cells into the dorsum. In this model, we previously revealed significant tumor growth and increase number of Tregs both in tumor tissue and blood compared to the normal mice. A soluble recombinant human thrombomodulin (rTHM) is developed as a treatment drug against DIX. It has anti-inflammatory effect and several reports showed that it decreases cancer metastasis. We hypothesized that rTHM normalizes PIR and prevents cancer growth caused by inflammation. Method: Ten-week-old C57Bl/6 mice were divided in CLP/rTHM, CLP/normal saline (NS), and control (simple laparotomy plus NS) groups. rTHM (3mg/kg/12hrs) was injected subcutaneously for 7 consecutive days from the day before CLP. CT26 cells (1x10⁵) were implanted 4 hours after CLP. Mice were sacrificed 28 days after CLP. The tremendous and blood were collected. To analyze dynamics of Treg, peripheral blood leukocytes the tumor cells were isolated. CD4, CD25 and Foxp3 were stained by fluorescent antibody and stained cells underwent flow cytometric analysis. The quantity of Tregs is measured by the rate of CD25⁺/Foxp3⁺ population in CD4⁺ T cells. The level of CCL22 and TGF-beta were measured both in serum and tumor tissue. The ELISA was used for analyzing serum samples. And for tumor tissue, immunostaining by DAB was performed and positively stained areas were calculated by image-editing software. Results: CLP/NS group mice exhibited significant enhanced tumor growth compared to controls (6.0 ± 1.9 g vs. 3.5 ± 1.6 g, p = 0.03), while CLP/rTHM group produced significantly smaller tumors (2.2 ± 1.1 g) than CLP/NS (p = 0.01). The Tregs in the blood stream was smaller in CLP/rTHM group compared to CLP/NS group. Intratumoral Tregs showed significant difference between CLP/rTHM group and CLP/NS group (8.89 ± 3.5 % vs. 24.1 ± 15.0 %, p = 0.02). We found significant elevation of serum TGF-beta level by CLP (4.7 ± 1.2 pg/ml vs. 15.4 ± 7.0 pg/ml, p = 0.02) and relative reduction to 15.4 ± 7.0 pg/ml by administration of rTHM (p = 0.05). However, we did not recognize any change of serum CCL22 level in this study. Intratumoral rate of CCL22 positive areas increased by CLP/rTHM (50%), while not by CLP/NS (20%). It decreased to normalize PIR and find little difference of TGF-beta positive areas among three groups in this study. Conclusion: Acute inflammation induced by CLP enhances the growth of implanted tumors, while administration of rTHM suppresses tumor growth by possibly affecting dynamics Tregs via TGF-beta and CCL22.

#1681A Overcoming pd1 targeting antibody resistance using combination strategies. Jean-François Mirjolet, Maxime Ramelet, Damien France. Onco-design S.A., Dijon Cedex, France.

PD-1 targeting antibodies (nivolumab, pembrolizumab) are now approved in various tumor types either as second line treatment (locally advanced or metastatic urothelial carcinoma, advanced renal cell carcinoma, recurrent or metastatic head and neck squamous cell carcinoma, classical Hodgkin lymphoma) or even as first line therapy (metastatic melanoma, metastatic non-small cell lung cancer). Despite an increase in response rate as well as survival, there is still issue with resistance to PD-1 targeting therapies. Selection biomarkers as well as response biomarkers are still under intensive research. In addition, combination strategies are needed to increase response rate and overcome resistance. Combination strategies were therefore tested in one well characterized syngeneic mouse model, i.e. EMT-6 breast carcinoma. The model is known to be highly sensitive to CTLA-4 targeting antibody (optimum tumor to control ratio (T/C) of 3%), a moderate responder to PD-1 targeting treatment (best T/C of 54%) and a well immune infiltrated tumor. Antibodies targeting co-stimulatory receptor such as OX40, GITR and 4-IBB as well as co-inhibitory receptor such as Tim3 would be expected to arrest tumor growth and inhibit tumor progression.
were combined to anti-PD-1 antibody. In all cases, combination arm produces better therapeutic activity when compared to single treatment alone. Moreover, both strategies, meaning combination of two antibodies targeting co-inhibitory receptors (PD-1 + Tim3) and combination targeting one co-inhibitory (i.e. PD-1) and one co-stimulatory receptors (OX40, GITR or 4-1BB) worked. Detailed results will be presented including response rate, immune infiltrates and cytokines profiles in order to rationally choose the best therapeutic treatment.

**CLINICAL RESEARCH: Adaptive Immunity to Cancer**

**#1682** Correlation of immune co-stimulatory molecule OX40 and outcome in trastuzumab treated HER2-positive breast cancer patients in the NCCTG-N9831. Saranya Chumsri,1 Daniel J. Serie,2 Ashin Mashadi-Hosseini,3 Sarah Warren,2 Alvaro Moreno-Aspista,1 Geraldo Colono-Otero,1 Keith L. Knutson,1 Edith A. Perez,3 E Aubrey Thompson1,2 Mayo Clinic Cancer Center, Jacksonville, FL;3 NanoString Technologies, Inc., Seattle, WA.

Background: Trastuzumab (H), a monoclonal antibody against HER2, has revolutionized the treatment for HER2-positive breast cancer. Besides inhibiting downstream signaling of HER2, several studies showed that H also exerts its anti-tumor activity via immune-related mechanisms. While H is quite effective in preventing recurrence, significant numbers of patients still develop recurrence despite adjuvant H-based chemotherapy. In this study, we analyzed immune-related genes that were associated with poor outcome in N9831. Methods: NanoString technology was used to quantify mRNA of immune-related genes in baseline samples from 1,280 patients in N9831. In N9831, patients in arm A were treated with chemotherapy alone (AC-T), arm B received chemotherapy followed by sequential H (AC-T-H), and arm C received H concurrently with chemotherapy (AC-Th). Cox proportional hazard ratio (HR) was used to determine the association of each immune-related gene with disease-free survival (DFS). Different immune subset signatures, including CD45, CD8, cytotoxic-cells, and T-cells were analyzed using algorithms developed by NanoString. Results: With the median follow up of 10.6 years, we identified a total 77 genes that were associated with improved outcome in arm C. Among these 77 genes, there were 20 tumor necrosis factor (TNF)-related genes. Of those, only OX40 (TNFRSF4) and its ligand TNFSF4 have interaction p < 0.10. Interestingly, we found uneven distribution of OX40 expression in the N9831 specimens. Specifically, 9.5% of HER2-positive breast cancer patients expressed OX40 at distinctly low level. Low expression of OX40 was significantly associated with HR positivity (OX40 low 61% vs. OX40 high 51%, p=0.003) and larger tumor size but not patients’ age, tumor grade, and lymph node status. Low expression of OX40 was significantly associated with low mTIL-CD45, CD8, cytotoxic-cells, and T-cells immune signature scores. Tumors with low OX40 expression had significantly lower levels of CTLA4 (9.11e-71) and PD-L1 expressions (9.11e-71) and significantly lower levels of CTLA4 (p 9.11e-71) and PD-L1 expressions (p 9.11e-71) with its ligand TNFSF4 in arm A (HR 1.02, 95%CI 0.92-1.15, p=0.68), arm B (HR 0.87, 95%CI 0.76-0.99, p=0.04), and arm C (HR 0.81, 95%CI 0.68-0.95, p=0.01). Conclusion: Our study suggests that pre-existing expression of OX40 and its ligand TNFSF4 in arm A (HR 1.02, 95%CI 0.92-1.15, p=0.68), arm B (HR 0.87, 95%CI 0.76-0.99, p=0.04), and arm C (HR 0.81, 95%CI 0.68-0.95, p=0.01). Results: With the median follow up of 10.6 years, we identified a total 77 genes that were associated with improved outcome in arm C. Among these 77 genes, there were 20 tumor necrosis factor (TNF)-related genes. Of those, only OX40 (TNFRSF4) and its ligand TNFSF4 have interaction p < 0.10. Interestingly, we found uneven distribution of OX40 expression in the N9831 specimens. Specifically, 9.5% of HER2-positive breast cancer patients expressed OX40 at distinctly low level. Low expression of OX40 was significantly associated with HR positivity (OX40 low 61% vs. OX40 high 51%, p=0.003) and larger tumor size but not patients’ age, tumor grade, and lymph node status. Low expression of OX40 was significantly associated with low mTIL-CD45, CD8, cytotoxic-cells, and T-cells immune signature scores. Tumors with low OX40 expression had significantly lower levels of CTLA4 (9.11e-71) and PD-L1 expressions (9.11e-71) with its ligand TNFSF4 in arm A (HR 1.02, 95%CI 0.92-1.15, p=0.68), arm B (HR 0.87, 95%CI 0.76-0.99, p=0.04), and arm C (HR 0.81, 95%CI 0.68-0.95, p=0.01). Conclusion: Our study suggests that pre-existing expression of OX40 and its ligand TNFSF4 are prognostic and may also be predictive of adjuvant H benefit. Patients with distinctly low level of OX40 had poor outcome despite adjuvant H-based adjuvant chemotherapy. Our study provides a rationale to further evaluate the strategy to increase immune activation to improve outcome in this group of patients.

**#1683** A novel adenosine A2A receptor antagonist optimized for high potency in adenosine-rich tumor microenvironment boosts antitumor immnunity. Erica Houthuys, Margreet Brouwer, Florence Nyawouame, Romain Pirson, Reece Mardillier, Theo Deregnaucourt, Joao Marchante, Jakub Swiercz, Charlotte Moulin, Vanessa Bol, Gregory Driessens, Michel Dethue, Christophe Quêva, Stefano Crosignani, Bruno Gomes. iTeos Therapeutics, Gosselies, Belgium.

High levels of extracellular adenosine in the tumor microenvironment are known to play a significant role in tumor immune evasion and promote tumor growth and metastasis. We defined the receptor(s) required for mediating the effect of adenosine on immune cells within the tumor microenvironment and report the characterization of a novel Immuno-Oncology-dedicated adenosine receptor 2A antagonist that functions in the high adenosine concentration found in tumors. We first explored the expression of the four adenosine receptors in primary human immune cells. A2A receptor was the main adenosine receptor expressed by CD4 and CD8 T lymphocytes and monocytes, and the only one in monocyte-derived dendritic cells and NK cells. A2B receptor was poorly detected in T cells and monocytes, while A1 and A2 receptors were never detected. Given these expression patterns, we further studied A2A functional activity, and found that A2A agonists such as CGS-21680 strongly suppressed cytokine production by activated primary human T lymphocytes, thus highlighting that A2A is the main effector receptor of the sensing of adenosine in tumors. We further confirmed the elevated extracellular adenosine level in the tumor microenvironment in several mouse and human tumors. High adenosine levels correlated with strong tumoral expression of CD73, the enzyme that converts adenosine to adenosine-5'-monophosphate. Interestingly, we showed that A2A receptor antagonists designed for Parkinson’s disease dramatically lost potency in a high adenosine environment; our data indicated that a 30-fold dose increase may be required for full target inhibition within tumors. Therefore we developed a novel and potent A2A blocker with sub-nanomolar Ki and IC50 in a cAMP assay and a more than 100-fold selectivity over other adenosine receptors. Our lead compound kept a high potency in an adenosine-rich environment and restored cytokine production even in the presence of high concentrations of A2A agonists. iTeos inhibitor also efficiently reversed AMP-mediated T cell suppression. Furthermore, our compound rescued A2A receptor agonist-induced decrease of TNFα production by primary human monocytes, and was able to potently increase CD8 T cell cytotoxicity in a cytokine-rich environment with CD8 T cells as effectors and cancer cells as targets. These results suggest that iTeos new generation of A2A receptor antagonist, designed to keep a high potency in the adenosine-rich tumor microenvironment, may offer a new therapeutic opportunity in Immunoo-Oncology.

**IMMUNOLOGY: Tumor Models and Assays**

**#1685** Effects of anti-ctla-4 and anti-pd-1 on memory T-cell differen- tiation and resistance to tumor relapse. Stephen Mok, Colmn R. Duffy, James P. Allinson, MD Anderson Cancer Center, Houston, TX

The FDA has begun to expand the approved uses of immune checkpoint blockade antibodies targeting CTLA-4 and PD-1. Blocking either checkpoint relieves the negative regulation of T cells resulting in significant responses in patients with cancer. Data has now begun to emerge regarding differences between these two therapies. While oPD-1 therapy has a greater response rate (~30% vs. 11%) according to RECIST criteria, recent reports have suggested responses to oPD-1 may not be as durable as oCTLA-4. 25% of patients who initially responded to oPD-1 have tumor relapse within 24 months. In contrast, the 3-year survival rate of patients treated with oCTLA-4 is at least ~25% suggesting a durable response. Previous studies in bacterial or chronic LCMV infectious models have shown that oCTLA-4 can increase CD8+ memory T-cell formation, whereas genetic ablation of PD-1 on T cells often promotes the terminally differentiated exhausted CD8+ T-cell phenotype, while attenuating memory T-cell formation. However, the mechanism which leads to relapse following oPD-1 treatment in tumor models is not clear. The goal of this project is to understand how immunotherapies shape memory T-cell formation and how that relates to the mechanism of tumor relapse. To test if oCTLA-4 or oPD-1 can induce a better memory T-cell response, mice were vaccinated with irradiated B16F10 murine melanoma cells and treated with oCTLA-4 or oPD-1. Mice were re-challenged with B16F10 80 days after vaccination. Although both oCTLA-4 and oPD-1 improved tumor rejection compared with controls, oCTLA-4 treated mice exhibited superior tumor control compared to oPD-1 (p<0.0005) suggesting the memory T-cell response mediated by oCTLA-4 is more durable. In order to test whether this memory T-cell response is antigen-specific, mice were re-challenged with unrelated MC38 or 3LL cancer cells. The antibody treated groups did not show improved antitumor effect compared with vaccine control (p>0.5). To test whether the frequency of memory T cells re-cruited to the re-challenged tumor could affect memory T-cell response, antigen-specific pmel-1 T-cells were mixed in mice following vaccination with oCTLA-4 or oPD-1. Our result suggested that there were more tumor-infiltrating pmel-1 T-cells in the oCTLA-4 treated group compared to the oPD-1 treated group (p<0.05). In order to augment the durability of oPD-1 treatment, oPD-1 was combined with oCTLA-4 following vaccination. The combined treatment group has superior antitumor response compared to that with oPD-1 alone and overlap with the oCTLA-4 treated group (p<0.05) during re-challenge, indicating that the effect of the combined treatment is dominated by oCTLA-4. Collectively, our studies facilitate the design of combination immunotherapy treatments that enhance both response rates and generation of memory T-cells to prevent relapse.
Epigenetic modulation of the tumor microenvironment enhances vaccine induced T cell responses in a murine model of pancreatic cancer.

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This project aims to test the hypothesis that epigenetic modulatory drugs (EMD) and GVAX, a GM-CSF-secreting whole tumor cell vaccine, are capable of altering the inflammatory environment of pancreatic ductal adenocarcinoma (PDAC) and sensitizing it to checkpoint blocking agents. Recently the use of antibody therapy targeting immune checkpoints, such as CTLA-4 and PD-1, has become a major focus of cancer immunotherapy. In responsive patients, these therapies result in long-term control of chemotherapyc-resistant disease. The most compelling activity has been seen in the minority of patients with immunogenic tumors where T cell infiltration naturally occurs. These benefits are not observed in non-immunogenic tumors, such as PDAC, with low expression of tumor-associated antigens (TAA) and a lack of intrinsic T cell infiltrates. Therapies that can alter the tumor microenvironment (TME) and allow infiltration of effector T cells, decrease immunosuppressive cells, and stimulate TAA expression may convert non-immunogenic tumors into cancers sensitive to checkpoint inhibitors. Recent work with EMDs has shown that they are capable of altering the immunogenicity of the TME by inducing the expression of cancer testis antigens as well as increasing tumor cell expression of MHC class II and decreasing Treg in the TME. Additionally, GVAX has been shown to induce tumor cell death and antigen release within the TME, testing the hypothesis that treatment with EMDs and GVAX can sensitize the inflammatory environment of PDAC to checkpoint blockade inhibition by evaluating changes in immune cell function within the TME via flow cytometry, immunohistochemistry, and gene expression array. We are using a murine model of hepatic metastases of pancreatic cancer which involves injecting syngeneic pancreatic tumor cells into the spleen followed by a hemiplenectomy, resulting in the consistent formation of hepatic metastases that can be monitored by ultrasound. We have evaluated entinostat, a histone deacetylase inhibitor, in combination with GVAX which induces T cell responses, and demonstrated a significant increase in survival when compared with either agent alone. Flow cytometric analysis of the cells infiltrating the TME shows that the combination of entinostat and GVAX causes a significant increase in CD4+ T cell infiltration as well as a shift from an M-MDCS dominant to a more G-MDCS dominant myeloid population. Current studies aim to elucidate the functional- ity of the MDSC population as well as identify potential changes in the T helper cell subsets via flow cytometry. Additionally, future studies will evaluate changes within checkpoint blockade pathways via flow cytometry and gene expression array to identify pathways that require further modulation to enhance antitu- mor responses.

Systemic immunological changes during first line chemotherapy in patients with high-grade serous ovarian cancer.

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Ovarian cancer remains the most lethal gynecological malignancy and new therapeutic strategies are urgently needed. High-grade serous ovarian cancer (HGSC), in particular, is associated with a five-year survival of only 40%. A promising new approach for treating HGSC is immunotherapy, which has resulted in complete and durable responses, albeit in a minority of patients. One potential approach for improving these response rates is by combining chemo- and immunotherapy. Indeed, carboplatin/taxol chemotherapy was shown to augment immune responses in cervical cancer patients by depleting circulating myeloid suppressor cells. As patients with HGSC are treated in first line with chemotherapy, the third and fourth cycle of chemother- apy and 4-6 weeks after the sixth cycle of chemotherapy. All patients re- ceived 6 cycles of carboplatin/taxol chemotherapy and cytoreductive surgery (either as primary debulking, or after 3 cycles of neo-adjuvant chemotherapy). Peripheral blood mononuclear cells were isolated and analyzed for a total of 36 immune markers using 9 flow cytometry panels, in total analyzing 49 immune cell subsets. Results were compared to an age-matched cohort consisting of women surgically treated for a benign disease, and a cohort of healthy young volunteers. 75 patients have been included so far from which 22 were diagnosed with benign disease, 3 were diagnosed with another malignancy, and 50 were diagnosed with OC. Of the 50 OC patients, 18 were diagnosed with HGSC and from 9 HGSC patients, multiple time points were available for analysis of che- motherapy-dependent changes in the immune cell subsets. All patients devel- oped leukopenia as a result of chemotherapeutic treatment. Age-related changes in lymphocyte and myeloid cell subsets were observed in all HGSC patients and patients with benign disease. Chemotherapy-dependent depletion of myeloid cells was observed. Depletion was not significant. T cell subsets equitably distributed among monocytes, macrophages and dendritic cells. No HGSC- or chemotherapy-dependent changes in T cell subsets or migration and activation markers were observed. Taken together, we observed no major sys- temic changes in immune cell subsets during carboplatin/taxol chemotherapy treatment, suggesting that combined chemo-immunotherapeutic strategies could be feasible during first line treatment of HGSC.

HERA-CD40L: A novel hexavalent CD40 agonist with superior bi- ological activity.


Introduction: Targeted therapeutics for cancer treatment are mostly devel- oped as antibodies, however, the natural signaling complexes of the members of the TNF superfamily and their receptors consist of clusters of trimers. Consequent- ly, most of these bivalent agonistic antibodies depend on Fc receptor mediated crosslinking for biological activity. The HERA-Technology developed by Apogenix generates hexavalent fusion proteins targeting the TNF-receptor superfamily with high clustering capacity for the cognate receptor, which over- comes this disadvantage of antibody-based drugs. Here we compared the efi- cacy of different CD40 agonist formats, including the novel HERA-CD40L and the functional consequences of differential receptor clustering. Materials & Methods: Biological activity of CD40 agonists was compared using an engi- neered reporter cell line and by flow cytometric analysis of CD40-induced IReA degradation in Ramos B cells. T lymphocytes and monocytes were isolated from buffy coats and expression of CD markers upon CD40 ligation was analysed by multicolor flow cytometry (MC-FC). Secretion of cytokines in response to CD40 ligation was determined by ELISA. Monitoring of T cell-induced killing of tu- mor cells in direct co-cultures employed a real-time cell analysis system (xCel- Ligence). Results: Direct comparison of bivalent CD40 antibodies with trivalent CD40L and the hexavalent HERA-CD40L in two independent bioactivity assay formats demonstrated that only the hexavalent agonist was fully active without additional crosslinking. In contrast to HERA-CD40L, neither the bivalent ago- nistic CD40 antibody nor the trivalent CD40L were able to upregulate expres- sion of activation markers on B cells or to induce secretion of proinflammatory cytokines such as IL-12 and TNFα by PBMCs. In vitro generated M2-macro- phages acquired an M1 phenotype and enhanced proliferation of naïve CD4+ T cells in direct co-culture. Similarly, direct co-culture of CD4+ T cells with Ramos B cells in the presence of HERA-CD40L induced cytotoxic activity of CD4+ cells against tumor cells. The activating effect was dependent on cell-cell contacts and was not observed in indirect co-cultures. Importantly, only the hexavalent HERA-CD40L showed full biological activity without additional crosslinking. Conclusion: The hexavalent CD40 agonist HERA-CD40L pro- duced by Apogenix HERA-Technology platform triggers CD40 signaling on B cells and monocytes. The monoclonal ligand, HERA-CD40L, leads to differential receptor clustering and proliferation of CD4+ T cells and shifts the M1/M2 balance towards proinflam- matory conditions. Unlike bivalent CD40 antibodies or trivalent CD40L based agonists, the hexavalent HERA-CD40L forms highly clustered signaling com- plexes and thus exhibits superior biological activity over other agonistic formats without the need for Fc receptor mediated crosslinking.

Dynamic monitoring of immune response and reagent efficacy through high throughput label-free impedance-based technology.

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In vitro characterization of reagents efficacy in the context of cancer immu- notherapy is a necessary step before moving to more expensive animal models and clinical studies. However, current in use in vitro assays like Chromium-51, ATP-based luminescence or flow cytometry are either difficult to implement in high throughput environment or are mainly based on end point methodologies that are unable to capture the full dynamic of the immune response. Here we present the adaptation of an impedance-based platform to monitoring cytotoxic activity of immune cells activated through different means. Impedance technol- ogy detects cell death and proliferation of adherent cells by measuring changes in conductance of microelectrodes embedded in 96 and 384-wells cell culture plates. We utilized adherent and B cell leukemia/lymphoma cell lines as well as primary tumor cells as in vivo models for immunotherapy reagents evaluation. We seeded the cells on electrodes coated 96-well plates and monitored cell ad-
hension and proliferation for 24 hours. The following day effector cells were added at multiple effector:target ratios in presence of BiTE antibodies and/or anti PD-1/PD-L1 antibodies. Impedance signal was monitored for up to seven days. Control wells were set up with effector cells only or with target plus effector cells but without antibodies. We adapted such adhesion-based technology to monitor non-adherent B-lymphoma/lymphoma cells, by developing a strategy where the wells are coated with an anti-CD40 antibody. The coating allows specific adhesion and retention of B cells and measurement of changes in impedance that are proportional to cell number. Using increasing concentrations of EpCAM/CD3 BiTE we demonstrated the suitability of such impedance-based approach to quantitatively monitor the efficacy of immune cells-mediated cancer cell killing both under different effector:target ratios and antibody concentrations. Combination treatments with checkpoint reduced killing and increased amount of killed cancer cells. Similar results were also obtained with engineered Car-T cells against CD19 or NK cell lines, we were able to demonstrate specific killing of tumor B cells at very low effector:target ratios. The results were also confirmed by flow cytometry. Overall, our results demonstrate the value of such approach in measuring the cytotoxic response across the temporal scale, an aspect that is otherwise very difficult to assess with more canonical end point assays. Furthermore, the availability of 384-wells format and minimal sample handling place the technology in an ideal spot for applications in large reagent validation screening or personalized medicine, like therapeutic protocol validation directly on patient samples.

**#1690 Epitope-minigenes for optimal induction of the immune response against tumor associated antigens and neoantigens.** Silvio Bandini,1 Laura Luberto,1 Fabio Palombo,1 Giuseppe Roscilli,1 Emanuele Marra,1 Gennaro Ciliberto,2 Luigi Aurisicchio.1

We have recently established a workflow that allows the identification of T cell epitopes within Tumor Associated Antigens (TAA)s and the construction of genetic cancer vaccines based on the use of minigenes. The T-cell epitope in silico prediction approach is based on three criteria: 1) binding to MHC Class I alleles; 2) uniqueness to the antigen of interest; 3) increased likelihood of natural processing. The combination of in silico prediction and a biochemical binding/stability assay resulted in a novel accurate identification of TAA-derived epitopes. Predicted T cell epitopes were connected by furin sensitive linkers and linked to human tissue plasminogen activator (TPA) signal and E. Coli enterotoxin B subunit, to construct an optimal minigen scaffold used as vaccine candidate. The present study was aimed at evaluating HER2/neu and hTERT (telomerase) minigenes with the same technology platform. First of all, minigenes delivered via Electro Gene Transfer (DNA-EGT) were more immunogenic than genetic vectors encoding the full-length protein or peptides injected subcutaneously and they were able to block immune tolerance in wild type and HLA-A2021 transgenic mice. Moreover, this technology applied to epitopes selected within Mutated Tumor Antigens results in strong immunogenicity and significant antitumor effects in mouse models. In conclusion, we show that minigenes delivered via DNA-EGT and based on predicted and/or experimentally identified epitopes are powerful tools to induce immune responses and combat cancer. Combining minigenes with peptide vaccination and oil-based delivery strategies may improve the uptake and activation of DCs at low concentrations of the SLP and oil-based delivery strategies trap immune cells to unwanted sites or have inappropriate adjuvant properties. To enhance the uptake of cancer or viral T cell epitopes and subsequent activation of DCs we make use of circulating antibodies to target cellular responses against tumor antigens of interest by conjugating a B cell epitope to a T cell epitope. The conjugation of the two greatly improves antigen uptake and concomitant activation of the same DC in contrast to naked long peptides which are not conjugated. Our identified B cell epitope of choice is derived from tetanus toxin and can be targeted by tetanus-specific antibodies boosted by a standard tetanus toxoid vaccine. We have applied a modified chimeric peptide model preserving intact cascade systems to characterize how the tetanus-neupeptide conjugated vaccine is targeted to human immune cells. The B cell/T cell conjugate is taken up by human monocytes and blood DCs in an antibody-dependent manner. Rather than FcRs, the internalization of the antigen appears to be partly mediated through the classical pathway of the complement system. Tetanus-CMV conjugates, containing a T cell epitope from the pp65 protein of cytomegalovirus (CMV), strongly reactivates memory T cells when analyzed in blood from donors with CMV-specific T cells. The CMV-specific T cells rapidly produce IFNγ and TNF in response to the conjugate illustrating that the uptake of the conjugate leads to activation of antigen-specific T cells. Uptake as well as T cell activation occurs at low concentrations of the SLP conjugate, superior to a conjugate lacking the tetanus–sequence as well as to SLPS with or without additional adjuvant (LPS). Of importance, when the B and T cell epitopes are separate entities but mixed, CMV-specific T cells are not activated, illustrating the requirement of conjugating the two. Our data show that we have a unique delivery system for peptide based vaccines that can aid induction of human T cell responses, and may potentiate immune responses in cancer patients. We are now actively working on a prostate cancer vaccine candidate using this novel loading/adjuvant technology.

**#1692 KRAS-mediated therapeutic resistance abrogates immunogenic cell death in colorectal cancer cells.** Yi-Jun Wang,1 Dongshi Chen,1 Jinghao Timg,1 Jifan Yu,2 Alberto Bardelli,2 Lin Zhang,3 #1University of Pittsburgh Cancer Institute, Pittsburgh, PA; #2University of Torino, Torino, Italy.

A hallmark of cancer is compromised immune surveillance, which is characterized by failure of the immune system to recognize, immunize against, and specifically eliminate malignant cells. A number of studies showed the tumor microenvironment in colorectal cancer (CRC) patients is highly immunosuppressive. Conventional chemotherapy and recent targeted therapy can restore anti-tumor immune response through induction of immunogenic cell death (ICD) in tumor cells. However, the mechanism and functional role of ICD in CRC and its resistance by dendritic cells is not clear. We identified the BH3-only BCL-2 family protein PUMA as a key mediator of necroptosis induced by the combination treatment in vitro and in vivo. Furthermore, we found that CRC cells with acquired cetuximab resistance due to KRAS mutation or amplification are deficient in ICD. Collectively, our results suggest that oncogenic mutations cause resistance of CRC cells to anticancer therapies in part by suppressing ICD.

**#1693 T cell responses to peptide-epitopes can be boosted by immune complexes of circulating anti-tetanus antibodies.** Erika A. Fletcher,1 Wendy van Maren,2 Robert Cordfunke,2 Jasper Dinkelaar,3 Ricardo Castelli,3 Jeroen Codee,2 Gis van de Marel,3 Cornelis J. Melief,2 Ferry Ossendorp,2 Jan Wouter Drijfhout,2 Sara Mangsbo.1

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The ability of dendritic cells (DCs) to boost an antigen-specific immune response is utilized in several cancer immunotherapy strategies including therapeutic vaccination using long peptides. Naked peptides are rapidly degraded and oil-based delivery strategies trap immune cells to unwanted sites or have inappropriate adjuvant properties. To enhance the uptake of cancer or viral T cell epitopes and subsequent activation of DCs we make use of circulating antibodies to target cellular responses against tumor antigens of interest by conjugating a B cell epitope to a T cell epitope. The conjugation of the two greatly improves antigen uptake and concomitant activation of the same DC in contrast to naked long peptides which are not conjugated. Our identified B cell epitope of choice is derived from tetanus toxin and can be targeted by tetanus-specific antibodies boosted by a standard tetanus toxoid vaccine. We have applied a modified chaperone model preserving intact cascade systems to characterize how the tetanus-neupeptide conjugated vaccine is targeted to human immune cells. The B cell/T cell conjugate is taken up by human monocytes and blood DCs in an antibody-dependent manner. Rather than FcRs, the internalization of the antigen appears to be partly mediated through the classical pathway of the complement system. Tetanus-CMV conjugates, containing a T cell epitope from the pp65 protein of cytomegalovirus (CMV), strongly reactivates memory T cells when analyzed in blood from donors with CMV-specific T cells. The CMV-specific T cells rapidly produce IFNγ and TNF in response to the conjugate illustrating that the uptake of the conjugate leads to activation of antigen-specific T cells. Uptake as well as T cell activation occurs at low concentrations of the SLP conjugate, superior to a conjugate lacking the tetanus–sequence as well as to SLPS with or without additional adjuvant (LPS). Of importance, when the B and T cell epitopes are separate entities but mixed, CMV-specific T cells are not activated, illustrating the requirement of conjugating the two. Our data show that we have a unique delivery system for peptide based vaccines that can aid induction of human T cell responses, and may potentiate immune responses in cancer patients.

**#1694 USP7 inhibitors impair Treg function.** Silvia Bandini,1 Laura Luberto,1 Fabio Palombo,1 Giuseppe Roscilli,1 Emanuele Marra, Gennaro Ciliberto,2 Luigi Aurisicchio.

Along with the already established direct anti-tumor activities of USP7 inhibitors, these studies provide a strong rationale for combining USP7 inhibitors to suppress Treg functions and improve the efficacy of currently approved cancer immunotherapy agents.

**#1695 USP7 inhibitors impair Treg function.** Silvia Bandini,1 Laura Luberto,1 Fabio Palombo,1 Giuseppe Roscilli,1 Emanuele Marra, Gennaro Ciliberto,2 Luigi Aurisicchio.
CLINICAL RESEARCH: Adaptive Immunity to Cancer

#1694  Systemic granulocyte-macrophage colony-stimulating factor (GM-CSF) treatment increases T cell receptor diversity in localized and metastatic prostate cancer patients. David Y. Oh,1 Li Zhang,2 Jason Cham,1 Alan Paciorek,3 Mark Klinger,3 Malek Faham,3 Susan F. Slovin,3 Lawrence Fong,3 1University of California, San Francisco, San Francisco, CA; 2Adaptive Biotechnologies, South San Francisco, CA; 3Memorial Sloan Kettering Cancer Center, New York, NY.

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is frequently utilized as an adjuvant in cancer immunotherapies, and has known effects as a growth factor. However the extent to which GM-CSF modulates the adaptive immune response, including possible effects on the antigenic repertoire, remains unclear. We analyzed T cell receptor diversity by sequencing of TCR beta chain sequences amplified from total RNA from peripheral blood mono-nuclear cells using consensus primers to assess changes in the circulating antigenic repertoire of prostate cancer patients treated with GM-CSF in multiple clinical trials. Administration of systemic GM-CSF monotherapy to patients with localized prostate cancer prior to planned radical prostatectomy (NCT00305669) results in a significant decline in clonality from the pre-treatment timepoint to the 2-week timepoint on treatment, indicative of increased early repertoire diversity (p = 0.039 by Wilcoxon signed rank test). In a separate clinical trial (NCT000661429), the combination of systemic GM-CSF (250 μg/ m²/day on days 1-14 of each cycle) with ipilimumab in metastatic castrate-resistant prostate cancer (mCRPC) patients also resulted in a significant decline in clonality from pre-treatment samples to the 2-week timepoint on treatment (p = 0.002). In contrast, mCRPC patients who received ipilimumab alone in a separate study (NCT00323882) did not experience a similar decline in clonality after 3 weeks on treatment (p = 0.625). In addition, comparison of the dynamics of specific clonotypes between the paired timepoints in the two mCRPC studies demonstrates that while there is no significant difference in the ratios of post-treatment to pre-treatment clonality between studies, patients treated with the combination of ipilimumab and GM-CSF show more repertoire change, with lower Morisita’s distance for all clones found at either timepoint (p = 0.023), smaller intraclass correlation coefficient (p = 0.028), and a smaller proportion of clonotypes that remain unchanged (defined by +/- 2 fold-change for clones found at both timepoints) (p = 0.002). These results indicate increased repertoire turnover when GM-CSF is combined with checkpoint inhibition. Hence data from both localized and metastatic prostate cancer, and from monotherapy and combination therapy regimens, supports a role for GM-CSF in inducing early diversification of the TCR repertoire.

#1695  Exploiting the pancreatic cancer mutome for immune interception. Heather Kinkead,1 Eric Lutz,2 Thomas W. Dubensky,3 Elizabeth Jaffee4, 1Johns Hopkins School of Medicine, Baltimore, MD; 2WindMILL Therapeutics, Baltimore, MD; 3Aduro Biotech, Berkeley, CA.

This study aims to translate tumor-specific mutations identified by next-generation sequencing techniques into an effective anti-tumor vaccine for a classically non-immunogenic tumor, pancreatic ductal adenocarcinoma (PDA). Pancreatic ductal adenocarcinomas develop an average of 63 mutations during tumorigenesis, collectively referred to as the “mutome.” Of these, approximately 45 are amino acid point mutations. These mutations are not present in normal cells and provide a source of tumor-specific neoepitopes for targeted immunotherapy. Next-generation sequencing technologies allow for rapid identification of mutations present in patient tumors; however, methods for rapid identification of immunogenic neoepitopes which can be effectively targeted for tumor clearance, and optimized vaccination strategies for targeting them, still need to be developed. In this study, we are using a transplantable murine PDA model, Panc02, to develop vaccination strategies for inducing neoepitope-specific T cell antitumor responses, and to define parameters for selecting appropriate targets. Exome sequencing identified 878 nonsynonymous mutations in the Panc02 cell line. Three immunological epitope prediction servers identified 878 potentially immunogenic peptides in the Panc02 model, 29 of which were strong candidtes (scores >1000) and another 240 candidate epitopes within the limits of prediction (scores >1000). Panc02 neoepitope peptide vaccinations in mice confirmed 14% of the predicted epitopes to be immunogenic by IFNγ ELISPOT, which may include both CD8+ and CD4+ T cell responses. Tumor transplant experiments demonstrate that therapeutic vaccination against neoepitopes in combination with a human STING-activating cyclic dinucleotide adjuvant is capable of inducing a temporary regression of implanted tumors. After tumor escape and regrowth, flow cytometry reveals a large population of infiltrating lymphocytes, the majority of which are co-expressing the checkpoint molecules PD-1, Tim3, and Lag3, indicating that treatment of vaccinated animals with checkpoint blockade therapy may potentiate infiltrating lymphocytes and ultimately result in permanent tumor regression. Thus, a broad and unbiased approach for screening vaccine targets will allow for the evaluation of predictive algorithms and ultimately the development of more effective vaccination approaches for targeting the tumor mutome.


Irradiation (IR) has variable effects on the solid tumor microenvironment. While many report additive or synergistic effects between IR and immune activating therapies, most studies utilize single fractions or hypo-fractionated high-dose radiation of 8 Gy or greater. For head and neck squamous cell carcinoma (HNSCC), 30 or more low-dose fractions of 1.8-2 Gy are utilized as standard-of-care for the upfront treatment of early stage or advanced disease. Preliminary experiments from our laboratory have demonstrated that 10 fractions of 2Gy IR decreased the rate of rejection of T-cell infiltrated syngeneic carcinomas in wild-type B6 mice when combined with cyclic dinucleotide (CDN) compared to CDN treatment alone (10% rejection rate with low-dose fractionated IR plus CDN vs. 50% with CDN alone). Immune suppression following IR in mice is mediated by at least galectin-1. Further, HNSCC patients treated with standard fractionated low-dose IR develop durable neutropenia. Thus, we hypothesized that low-dose fractionated IR is immunosuppressive and does not result in additive or synergistic effects when combined with cyclic dinucleotide. To test this hypothesis, we performed a time-course analysis of tumor immune infiltration and tumor-draining lymph node antigen-specific in vivo tumor rejection in three human genetic models of carcinoma using defined tumor rejection antigens (MOCi-15E, B16-OVA and MC38-CEA) following exposure to two different IR regimens of 8Gy x 2 fractions or 2Gy x 10 fractions. Analysis of effector immune infiltration and antigen-specific activation pre-IR and at 5, 10, and 20 days post-IR revealed consistent trends of profound immunosuppression with the low-dose fractionated IR regimen (2Gy x 10) but preserved or enhanced immune activation with the high-dose hypofractionated regimen (8Gy x 2). Galectin-1 levels will be measured from whole tumor lysates. We plan to perform nano-string RNA array analysis on irradiated tissues to serve as correlative immune analysis and also to validate this technology against well-defined immune read-outs for potential use in the clinical trial setting. We also plan to combine both IR regimens with intra-lesional CDN (20 μg x 3 injections) to determine if either IR regimen results in additive or synergistic tumor control. This work has profound implications for future pre-clinical studies and clinical trials combining IR and immune activating therapies, such as CDN or checkpoint inhibitors.

#1697  Humanized immune-oncology mouse models. Maria Stecklum, Anika Wulf-Goldenberg, Bernadette Brzezich, Iduna Fichtner, Jens Hoffmann. FPO GmbH, Berlin, Germany.

The recent clinical success of immune checkpoint modulators has stimulated immune-oncology research leading to the identification of new tumor immunology targets. However both, target validation and drug development need better preclinical immune oncology models. Translational research further urgently needs such models for identification of clinically relevant biomarkers and validation of immunologic and tumor control. Development of xenografts (PDX) from different tumor indications transplanted on immunodeficient mice have demonstrated strong predictive power for many drug development programs in cancer research. However, one caveat of PDX models is, that these mice lack a functional immune system, which allows tumor engraftment in the xenogenic host. To overcome these constraints our aim is the development of PDX models on mice with a functional human immune system. This strategy should allow implementing the highly predictable PDX in a functional human immune environment for better drug efficacy and safety studies. We reconstituted a human immune system in mice by engrafting human hematopoietic stem cells in immunodeficient mice. We demonstrate the engraftment of a full set of human immune cells, including T cells, B cells, NK cells, monocytes and dendritic cells in the immunodeficient hosts of PDX. The in vivo growth pattern of PD-L1 is currently evaluated as first biomarker for immunotherapy with the PD1 checkpoint inhibitors. We screened our tumor cell lines and PDX models for PD-L1 expression to identify such a correlation. PD-L1 positive and negative models were transplanted on humanized mice. Most of them showed no difference in tumor growth compared to non-humanized mice (≈ fully immune resistant). However other PDX showed a delayed growth on the humanized mice (≈ partly immune resistant), whereas only one model did not grow at all (≈ immune sensitive), demonstrating a high sensitivity to the innate immune response of this PDX. To evaluate the function of the human immune cells and checkpoints, mice were treated with checkpoint inhibitors ipilimumab and nivolumab. Checkpoint inhibitors alone or in com-
bination led to a minor tumor growth delay and an increased number of acti-
vated T-cells in the blood and in the tumor. Our results from experiments with
t = 19 models revealed a first correlation: stronger tumor growth inhibition on
PD-L1 positive PDX and increased sensitivity of “partly immune resistant”
PDX. Furthermore we were able to demonstrate, that treatment effects of check-
point blockade by anti-CTLA-4 mAb were increased by 1.7 fold (p = 0.0397).
Consequently, we hypothesize that targeting immunotherapy to the tumor using oligonucleotide
aptamers that bind to RT induced tumor stress products will significantly en-
hance the therapeutic index. We used the aptamer platform to generate a bi-
specific construct containing a costimulatory aptamer ligand specific to murine
4-1BB which was conjugated to an aptamer specific to products secreted into the
tumor stroma (VEGF or osteopontin). Tumor targeting, local control, abscopal
responses and toxicity were compared to unconjugated 1-4BB-VEGF con-
structs, or mAb based therapies, when used in combination with RT in murine
subcutaneous and autochthonous MCA tumor models. RT induced VEGF
upregulation was demonstrated in null/low VEGF secreting tumors that led to
preferential accumulation of VEGF-41BB aptamers in the irradiated
tumor.12Gyx1 elicited optimal tumor targeting of bi-specific VEGF-4-1BB con-
jugate. Significant anti-tumor immune responses were observed in s.c. murine
tumor models. This conjugate showed dramatic reduction in systemic toxicity
compared to the equivalent gold standard 4-1BB mAb. RT-VEGF-4-1BB treat-
ment significantly improved local control, overall survival and induced potent
abscopal responses compared to unconjugated and non-irradiated controls in the
Balb/c 4T1 and MCA murine tumor models. Furthermore, RT-VEGF-4-1BB
treatment showed similar anti-tumor effects as CTLa4 mAb with significantly
less systemic toxicity. This approach increased intra-tumoral CD4+ and CD8+
T cells, the CD8+ to Treg ratio, and induced significantly more tumor cell death.
In conclusion, this strategy has been shown to induce potent anti-tumor
immune responses in relevant murine tumor models and significantly improves
the therapeutic index over non-targeted mAbs. Furthermore, radiotherapy can
expand the scope of tumor targeted immune modulation to virtually all solid
malignancies and induce potent abscopal responses. These results provide the rationale for developing clinical grade bispecific aptamers for future testing.

#1701 The novel anti-CD205 antibody drug conjugate (ADC) MEN1309 shows strong antitumoral activity in diffuse large B cell lymphoma (DLBCL).
Eugenio Gaudio,1 Chiara Tarantelli,1 Francesca Guidetti,2 Maurilio Ponzone,2 Roberta Piazzia Bordone,1 Alessio Fiascaredi,2 Andrea Rinaldi,1 Ivo Kwee,1 Afua Adjeiwa Mensah,1 Anastasios Statlis,2 Davide Rossi,2 Georg Stussi,1 Emanuele Zucca,3 Giuseppe Merlino,2 Mario Bigioni,1 Shradha Patel,1 Ana Paula Benaduce,1 Randall Brennan,1 Oliver Umland,1 Hideo Yagita,2 Eli Gilboa,1 Adrian Ishkanian1.

#1698 Immunoprevention of KRAS-driven lung adenocarcinoma by a multi-peptide KRAS vaccine. Jing Pan1,2, Qi Zhang1,2, Shizuko Sei1,2, Robert H. Shoemaker1,2, Ronald A. Lubet1,3, Yiyan You1,2, Medical College of Wisconsin, Milwaukee, WI;2National Cancer Institute, Bethesda, MD.

Lung cancer remains the leading cause of cancer death worldwide. Mutations in KRAS are detected in up to 30% of lung cancer cases, about 80% of which occur in codon 12, yet no effective therapies specifically targeting mutant KRAS have been developed. Boosting the host immune response to cancer cells by vaccinating against defined tumor-associated antigens (TAAs) as a means of treating established tumors or preventing the development of tumors in high risk individuals such as current or former smokers is an area of intense research. The present study evaluated both immunogenicity and anti-tumor efficacy of a newly formulated multipeptide (peptides 15-17 amino acids long) vaccine target-
ing multiple epitopes of the KRAS molecule in a mouse model of KRAS-
driven lung cancer. Vaccination was performed in the prevention setting in a transgenic mouse model, where mutated mouse Kras (G12D) is conditionally over-expressed in the lungs of mice using a CCSP promoter. A multi-score prediction algorithm was used to identify likely immunogenic epitopes in the KRAS protein sequence. The identified peptides were synthesized and screened in naive mice for immunogenicity. The formulated vaccine contained the top four peptides, which elicited the strongest immunologic response and showed 100% sequence homology between human and mouse. The multipeptide KRAS vaccine was then tested in an inducible CCSP-TetO-KRASG12D mouse model, where the vaccines mixed with the Freund’s adjuvant or adjuvant alone were administered prior to activating the mutant KRAS protein. Our results show the KRAS peptide vaccine exhibited striking efficacy, reducing tumor number and tumor burden by >80% when compared with adjuvant alone. Splenocytes collected from vaccinated animals at the end of the study showed a robust immu-
nologic response to the immunizing peptides. Furthermore, in vitro stimulation of these splenocytes by the vaccine peptides resulted in the secretion of cytokines indicative of Th1 (interferon gamma) and Th17 (IL17) responses but with min-
imal secretion of Th2 related cytokines e.g. IL3 and IL4. In the draining lymph nodes from vaccinated mice rejecting tumors, there was a substantial increase in CD4 cells but a limited increase in CD8 cells. In summary, the multipeptide KRAS vaccine was immunogenic and efficacious in the primary prevention of KRAS-induced lung cancer in mice, indicating that the approach can potentially be used for the prevention of KRAS-driven cancers either alone or in combina-
tion with other modalities.

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CLINICAL RESEARCH: Adaptive Immunity to Cancer

Tregs as well as routine hematological and biochemical parameters. Results: DTH positive patients and DTH negative patients did not differ with respect to the number of peripheral Tregs, leukocytes, lymphocytes or granulocyte/lym-
phocyte ratio. Whole blood IFN-γ production levels after phytohemagglutinin (PHA) stimulation at baseline in DTH positive patients were significantly higher compared to negative patients (p = 0.0397). Conclusively, we hypothe-
size that whole blood IFN-γ production at baseline is likely to be useful to assess immunologic competence in vaccinated patients with AFTV. Consequently, clinical utility of whole blood IFN-γ production, as immunomonitoring method for patient selection in vaccination with cancer vaccine, is required to investigate in larger clinical studies in the future.
into the left flanks of female NOD-SCID mice; i.v. treatments started with tu-
mors of 250-350 mm³ volume. Results. MEN1309 showed strong cytotoxic ac-
tivity in DLBCL (median IC50 = 300 pm; 95%CI, 200-771), while the IgG-
conjugated DM4 toxin was 100x less active (30 nm; 95%CI, 20-33). MEN1309
induced apoptosis in 17/24 (71%) DLBCL. No difference was seen based upon
DLBCL cell of origin, MYC or BCL2 translocations or TP53 status. MEN1309
activity was highly correlated with its target expression with an inverse corre-
lation between IC50 values and CD205 expression at flow cytometry or RT-PCR
(R = -0.79, P < 0.0001). No correlation was seen between CD205 expression
and IgG-conjugated DM4 activity. MEN1309 anti-tumor activity was in vivo
defined in a DLBCL model (OCL-Ly10) characterized by high CD205 expres-
sion. MEN1309 induced apoptosis at 2.5 and 5 mg/Kg once/3 weeks. Groups of
treated with IgG-DM4 (5 mg/Kg, every 3 weeks) were also used. MEN1309 5mg/Kg eradi-
caled tumors in all mice with a single dose, as also shown by highly significant differences versus control mice (D7, D21, D28; P < 0.01). While all other groups had to be stopped by D35, the
MEN1309 5mg/Kg group skipped the second treatment at D21 because there was
no palpable tumor, and mice resulted cured even 2 months later (Kaplan-
Meier, survival analysis, P < 0.0001). MEN1309 2.5 mg/Kg delayed tumor
growth versus control (D21, P 0.039). Much lower activity was observed with
MEN1309 1.25 mg/Kg (D7, P 0.012) and with IgG-DM4 (D21, P 0.049; D28, P
0.046). Conclusions. In DLBCL models, the novel ADC MEN1309 had strong
in vitro and in vivo anti-tumor activity, which is highly correlated with the expres-
sion of its target.

**#1702 Multimeric anti-DR5 IgM antibody displays potent cytotoxicity in vitro and promotes tumor regression in vivo.** Beatrice Wang, Tasnim Kothambalwala, Paul Hinton, Dean Ng, Avneesh Saini, Ramesh Baliga, Bruce Kety. 1GMBiosciences, Inc., Mountain View, CA

Death receptor 5 (DR5) is a member of the TNF receptor superfamily that
induces apoptosis upon receptor trimerization. It is expressed on many tumor
types and has therefore been an important target for developing antibody based
treatments of epithelial, solid tumors. However several agonistic Anti-DR5 IgG
antibodies that have demonstrated efficacy in preclinical models have been un-
successful in clinical trials, likely due to insufficient receptor crosslinking by
bivalent IgGs. We have developed a multimeric anti-DR5 IgM antibody which has
strong avidity for the receptor. The IgM binds Colo205 cells and triggers
apoptosis, and is 1000-fold more potent than the respective IgG and 100-fold
more potent than crosslinked IgG in vitro. Anti-DR5 IgM displays strong in
vitro potency across a panel of tumor cell lines, including ones that are IgG-
resistant. Anti-DR5 IgM causes tumor regression and delays tumor growth in
various in vivo xenograft models including Colo205, HCT15 and MDA-MB231.
These results support the development of an human anti-DR5 IgM therapeutic
with the potential to treat solid tumors.


Significant clinical and preclinical research has shown that PAMPs- Patho-
gen Associated Molecular Patterns- can trigger an integrated anti-cancer im-
mune response involving both innate and adaptive immunity. Imprime PGG is
a soluble yeast β-1,3/1,6 glucan currently in multiple phase 2 clinical studies in
combination with the immune checkpoint inhibitor (CPI), pembrolizumab.
Preclinical mechanistic research has shown that Imprime PGG can re-polarize
immunosuppressive myeloid cells in the tumor microenvironment and activate
antigen presenting cells to prime antigen-specific CD8 T cells thereby boosting
effectors T cell function and expansion. Based on this ability to activate dendritic
cells and induce type I interferon, we sought to explore the use of Imprime as an
immune activating/antigen-directing scaffold onto which we could attach tu-
mor antigens to drive an antigen-specific T cell based immune response. Imprime
PGG, using T cell receptor transgenic OT-I CD8 and OT-II CD4 T cells to
track responses to OVA, we treated mice with Imprime-OVA intravenously
and examined the expansion and functional quality of the T cell response 7 days later
at the peak of expansion. Following Imprime-OVA treatment, both OVA-spe-
cific CD8 and CD4 T cells underwent vigorous expansion. OT-1 CD8 T cells

**#1704 UPR signaling promotes T-cell dysfunction to prevent immune-mediated cancer cell killing and immune checkpoint therapy resistance.** Yismelin R. Feliz Mosquera, David R. Soto Pantajo, Adam Wilson, Pierre L. Troizio, Katherine L. Cook. 1Wake Forest University, Winston Salem, NC

A critical point in cancer progression is evading recognition by the immune
system. Cancer cells accomplish this by stimulating immune checkpoint signaling on
effectors T-cells. In patients with advanced melanoma treated with immune
checkpoint inhibitors, 3-year survival increased by 20%. While immune check-
point therapies are the first new treatment option for advanced melanoma in
over a decade, their efficacy is limited because resistance often develops. Under-
standing the molecular mechanisms of immune checkpoint inhibitor resistance
is critical to develop combinatorial drug therapy to potentiate therapeutic re-
sponsiveness. The unfolded protein response (UPR) is an endoplasmic reticu-
lon (ER) stress pathway activated when unfolded/misfolded proteins accumu-
late within the ER. Highly secretory cell types, such as T-cells, have larger ER
compartments and elevated UPR component expression facilitating antigen
processing and antigen presentation. The UPR signaling is activated during T-cell exhaustion. Treatment of naive T-cells with DTT, a chemical agent that stimulates ER stress, also induced PD-1 and PERK compared with vehicle-treated T-cells. Gene expression analy-
sis of T-cells indicates that co-culture with cancer cells, not CD3/CD28 activa-
tion, elevates T-cell UPR gene expression. Further, induction of ER stress
through low-dose DTT treatment decreased cytotoxic T-cell mediated cancer
cell death, further supporting our hypothesis of ER stress inducing T-cell ex-
haustion. Inhibition of PERK by RNAi in TALL-104, a human cytotoxic T-cell
line, enhanced T-cell mediated cancer cell clearance when exposed to ER stress-
inducing agents, suggesting that PERK may represent a novel target to prevent
T-cell exhaustion or restore T-cell effector capabilities. PERK inhibition in the
presence of antibody-conjugated melanoma cells did not negatively affect intra-
tumoral killing, suggesting that systemic PERK inhibition may be an effective therapeutic strat-
 egy to enhance anti-tumor immune responses. Matched PBMC from melanoma
patients before treatment or after ipilimumab therapy resistance indicated in-
creased UPR signaling components in PBMC samples from patients after ipili-
numab resistance when compared with PBMC samples before therapy, sup-
porting a novel role UPR signaling in anti-CTLA4 therapy resistance.

**#1705 Slide-based multi-parametric immunophenotyping of human blood samples by cyclic immunofluorescence using the Accucyte-CyteFinder system.** Jia-Ren Lin, 1 Lance U’Ren, 2 Gregory J. Baker, 1 Joshua J. Nordberg, 2 Zoltan Maliga, 1 Jackie L. Stilwell, 2 Eric P. Kaldjian, 2 Peter K. Sorger 1. 1Harvard Medical School, Boston, MA; 2Rarecyte, Inc, Seattle, WA.

Immunophenotyping is an approach to measuring the abundance and func-
tional state of immune cells as a means to understand mechanisms of homeo-
 stability, identify biomarkers of disease, and measure therapeutic and adverse re-
sponses to drugs such as immune checkpoint inhibitors. Such studies are
conventionally performed using flow cytometry together with combinations of
immunomarkers that allow for identification of cell type, maturation state, and
activation status. Here we describe a method for profiling circulating human
leukocytes with highly-multiplexed immunofluorescence microscopy. The
method has distinct advantages over flow cytometry in that it has greater sensi-
tivity for detecting rare cell populations, allows for repeat analysis and long-term
storage of precious biological samples, and obviates the requirement for spectral
deconvolution. Using an open-source multiplexed immunofluorescence protocol referred to as cyclic immunofluorescence (CyCIF), and commercially available reagents and instruments (AccuCyte and CyteFinder; RareCyte Inc.), we show that imaging can provide 16-plex, single-cell intensity data in addition to information on cellular morphology. Analytic technologies borrowed from the field of synthetic biology as well as VISNE (cytometric and image-based phenotypic analysis and high-dimensional biomarker discovery). Current efforts now aim to expand the number of validated immune targets and combine imaging with single-cell picking and sequencing technologies.

### #1706 Neoantigen frequency as an independent prognostic factor in patients with clear cell ovarian carcinoma (CCOC)

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Aim: Neoantigens derived from tumor-specific somatic mutations are thought to be targets for antitumor immune responses. It has been reported that mutation/neoantigen burdens correlate with prognosis of certain cancers under checkpoint blockade immunotherapy. In clear cell ovarian carcinoma (CCOC), checkpoint inhibitors provided durable responses in a subset of patients. To test whether the same may apply in CCOC patients, who are not on immunomodulatory therapy, we investigated the number of mutations or neoantigens, and immune signature of CCOC by integrated molecular analysis. Methods: We performed exome sequencing and expression array for 74 CCOC patients. Candidate neoantigens derived from mutations were identified by MHC class I binding prediction algorithm (NetMHCpan v2.8). Immune signature was assessed by gene ontology and gene set enrichment analyses for immune cell lineage or cytokine. Results: Neither number of missense mutations nor neoantigens correlated with clinical outcomes in CCOC. However, we found neoantigen frequency defined here as the number of neoantigens per missense mutation correlated with clinical outcomes. A Cox multivariate regression analysis demonstrated that high neoantigen frequency correlated with decreased progression free survival (PFS) and was an independent prognostic factor ($p = 0.032$). Immune-related genes including those related to effector memory CD8 T cell were dominantly expressed in tumors with low neoantigen frequency. This may suggest that CD8 T cell-mediated immunity is constantly eliminating immunogenic subclones expressing neoantigens (immunoediting). In contrast, we observed increased ratio of CTLA-4 and PD-1 to CD8A expression in tumors with high neoantigen frequency. This may imply that restricted antitumor immunity by immunoinhibitory mechanism result in the limited immunoediting and poor prognosis Conclusions: Neoantigen frequency underlying immunoediting in CCOC is an independent prognostic factor for clinical outcome and may become a potential candidate biomarker for an immunomodulatory-based treatment.

### #1707 Longitudinal and quantitative imaging of the localization, expansion, and contraction of tumor targeted adaptively transferred T cells.

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Introduction: Current methodologies for monitoring adoptive cell transfer (ACT) rely on soluble markers and T cell quantity in serum reflecting mixed responses of T cell efficacy or toxicity. Quantitative, longitudinal T cell visualization can directly probe T cell distribution, expansion, and off-tumor localization, and provide rational to predict therapy successes or failures. To enable real-time PET imaging of adoptively transferred T cells in a manner directly translatable to clinics, we utilized a genetic reporter, somatostatin receptor 2 (SSTR2) and a clinically approved radiotracer to quantitatively and longitudinally visualize whole body T cell distribution and anti-tumor dynamics. SSTR2 imaging was also applied to chimeric antigen receptor (CAR) T cells in a setting of solid cancer. Methods: We developed subcutaneous tumors with a mixture of SSTR2 positive and negative Jurkat T cells, ranging from 0 to 100%. From this model and PET/CT imaging of SSTR2 radiotracer uptake, diagnostic performance of our imaging technique was rigorously defined. To apply SSTR2 imaging to CAR T cells, a new lentivirus vector was designed to allow dual expression of SSTR2 and CAR. Longitudinal PET/CT and luminescence imaging was performed to concurrently measure the rate of tumor growth/killing and T cell expansion and contraction. Blood was also collected to correlate serum cytokines with T cell distribution at on- and off-tumor sites. Histology was performed to confirm the validity of SSTR2 imaging of CAR T cells and relating it to T cell activity. Results: Our T cell imaging technique was found to provide 1% limit of detection (one T cell in one hundred neighboring cells or $\sim 4x10^3$ cell/cm$^3$ in solid tissues) with 95% specificity and 87% sensitivity. When applied to CAR T cells in solid cancer, a biphasic T cell expansion and contraction was observed in survivors where a temporal change of T cell density closely followed the change in tumor burden with some time delay. In contrast, nonsurvivors displayed unrelenting increases in tumor and T cell burden, indicating that the expansion/contraction ratio is an important indicator of prognosis. Tumor weight and mortality rates were in line with T cell activity. Conclusion: Our study is the first high quality and quantitative, longitudinal imaging of T cells, correlating T cell dynamism with therapy and toxicity responses in subjects. Correlative changes in cytokines and T cell density can be further developed for early prediction of the onset of cytokine release syndrome as well as for screening adjuvant therapies to augment CAR T cell efficacy against solid cancer. Owing to the use of a human gene and FDA-approved radiotracer, our imaging technique can be directly applicable to CAR T cells and other T cell based therapy. We are currently preparing for a phase I clinical study for real-time imaging CD19 CAR T cells in patients.

### #1708 Effective and reversible control of anti-tumor activity in vivo with a drug-regulated CAR T cell platform (dARIC).

Wai-Hang Leung,1 Michael Cerro,2 Holly Horton,2 Joel Gay,3 Tracy VandenBerg,4 Jordan Jarjour,1 Alexander Astrakhan,1,2 bluebird bio, Seattle, WA; 3 bluebird bio, Cambridge, MA.

Redirecting T cells against tumors by introducing antigen-specific chimeric antigen receptors (CAR) has shown promising clinical results as a potential treatment strategy for certain cancers. However, traditional CARs are constitutively active, resulting in the persistent loss of all target cells (including off-tumor activity) in the event of antigen loss, and enhanced potential of excessive T cell activation to drive cytokine release syndrome. While “off switches” based on suicide cassettes or other depleting cell approaches are in development, such systems by definition result in the elimination of the therapeutic cells. Here we have developed a novel drug-regulated CAR-based antigen targeting approach termed Dimerizing Agent Regulated Immune-receptor Complex (dARIC) that aims to: i) minimize the long-term toxicity of CAR T treatment; ii) allow the targeting of previously inaccessible antigens; and iii) be amenable to multiplex antigen targeting. The dARIC platform separates the antigen recognition and signaling functions of a CAR into two distinct polypeptides that are further engineered to contain the FKP712 and FRB small-molecule regulated dimerization domains. In the absence of the dimerizing drug (e.g. rapamycin or the non-immunosuppressive rapalog, AP21967) the dARIC system lacks signaling activity. However, the addition of dimerizing agent drives the interaction of the two dARIC subunits, fully restoring CAR function. Using CD19 as a model system, we show that treatment of CD19-DARIC+ T cells with rapamycin or AP21967 results in equivalent cytokine toxicity, cytokine production and proliferation compared to a standard CD19-targeting CAR. Importantly, CD19-DARIC T cells maintained by picomolar levels of rapamycin and exhibited a higher antigen sensitivity than standard CD19-CAR T cells in vitro. In an aggressive CD19+Nalm-6 xenograft tumor mouse model, CD19-DARIC T cells did not exhibit anti-tumor activity in the absence of dimerizing agent. However, CD19-DARIC treated mice that received either low-dose rapamycin or AP21967 showed an equivalent level of tumor control compared to standard CAR treated controls. This activity was dependent on the presence of the dimerizing drug, as cessation of drug treatment resulted in the loss of CD19-DARIC T cell activity and the expansion of Nalm-6 tumors cells in the DARIC T cell treated mice, consistent with the ability to switch off CD19-DARIC T cells in vivo by withdrawing drug. Taken together, these results highlight the potential of the DARIC platform to facilitate the regulation of CAR T cell function both in vitro and in vivo.

### #1709 Targeting immune checkpoint therapy to the lung tumor microenvironment.

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Lung cancer is the leading cause of cancer deaths worldwide. Many lung cancer patients are diagnosed with advanced disease. These patients have a low 5-year survival rate and limited treatment options. Novel therapies, which target biomarkers that are overexpressed in lung cancer but have baseline expression in benign tissue, are desperately needed. The goal of this work was to develop a targeted agent for immune checkpoint therapy of lung cancer. Recently, immune checkpoint inhibitors have been approved for use in lung cancer and many more are being tested in clinical trials. The current immune checkpoint inhibitor agents are not tumor-targeted. Targeting the immune checkpoint inhibitor to tumor cell surface markers should concentrate the conjugate in the tumor microenvironment and enhance the immune response in the tumor while...
Reducing the systemic dosages needed, resulting in lower systemic toxicity. The delta opioid receptor (DOR) is expressed in some lung cancers, but is not expressed or is expressed only at basal levels in normal tissues outside the brain. We have previously synthesized fluorescently-labeled DOR-targeted imaging agents based on a synthetic peptide antagonist (DORL). These targeted fluorescent agents have high affinity and selectivity for DOR, and exhibit good pharmacokinetic (PK) and biodistribution (BD) profiles, i.e. specific tumor uptake with rapid systemic clearance and no uptake in tissues of concern, e.g. brain. We are now developing lung cancer-specific immunothrombosis agents that target the DOR by conjugating DORL to immunomodulatory molecules. In the current work, we synthesized a fluorescently-labeled DOR targeting ligand and conjugated it to an anti-PD1 antibody (DORL-PD1). We synthesized immunomodulators with several targeting ligand-to-antibody ratios (TARs). We engineered murine lung cancer cells to constitutively express the DOR. By lanthanide time-resolved fluorescence (LTRF) competitive binding assays, we have shown that the agents have high avidity for the DOR in vitro with higher TARs resulting in higher binding avidity. We characterized the uptake of DORL-PD1 in vitro using live-cell fluorescence microscopy. Using syngeneic engraftment tumor models in immunocompetent mice, we performed longitudinal fluorescence imaging studies to determine the agent circulation time (PK), tumor selectivity and tissue distribution (BD). Immune checkpoint efficacy studies were performed using the DOR negative mouse tumor models. In conclusion, we have synthesized fluorescent DOR-targeted immune checkpoint therapy agents, DORL-PD1; demonstrated avidity and selectivity for the DOR in vitro and in vivo; and immune checkpoint therapy efficacy in vivo. Future studies will evaluate the efficacy of DORL-PD1 in immune competent mice bearing DOR positive tumors. These agents could be useful for increasing the efficacy and reducing systemic toxicity of immune-checkpoint therapy of lung cancer.

#1710 Providing confidence around computational tissue analysis using heterogeneity assessments. Carsten Schnatwinkel,1 Daniel Rudmann,1 Fannke Aeffner,1 Jasmeet Bajwa,1 Natalie Hutnick,2 Michael Sharp,2 Gerry Chu,2 JD Alvarez,3 Flagship Biosciences Inc., Westminster, CO; 1Jansen R&D, Spring House, PA.

Background: Though the techniques to interrogate the appearance of a biomarker in tissue sections have greatly advanced, there are limitations as to how representative an analysis of a tissue section is compared to the entire diseased tissue. Depending on the heterogeneous expression level of a biomarker, tissue sampling can result in different interpretations of the biomarker’s appearance, and hence could potentially lead to a false therapeutic intervention. Hypothesis: Digital image analysis has demonstrated tremendous value in quantifying many features related to biomarker distribution and expression in biological tissues. The information can be collected for various indications and biomarkers and a phenotypic signature can be established that describes a biomarker representation across indications. Moreover, the assessment of new samples can be compared to the established phenotypic signature and a confidence score applied in support to the determined endpoint. Approach: For a proof of concept, 6 prostate cancer samples were processed and a single section was collected after every 100microns. A total of 7 sections per sample were stained for the lymphocyte marker CD3, and the number of positive target cells were determined in the tumor and tumor microenvironment using tissue Image Analysis (tIA). To assess how indicative the evaluation of a single tissue section would be for the entire tumor, the heterogeneity level was determined on the section level as well as by random grid analysis on each individual section. Both criteria were utilized to define an indication and biomarker specific confidence interval and heterogeneity score. Conclusion: The combination of IHC and tIA is a powerful tool to convert complex data into meaningful interpretations. tIA is a also a capable tool to catalogue valuable information about the biomarker’s expression pattern across different disease stages and hence could be used to evaluate how representative a single biomarker evaluation is in the grand scheme. Ultimately, we demonstrated a technique that can be applied to any biomarker and would assist in guiding therapeutic decisions.

CLINICAL RESEARCH: Liquid Biopsies 1: Circulating Tumor Cells

#1712 Detection of circulating tumor cells in stage IV non-small cell lung cancer. Stephanie N. Shishido,1 Lyudmila Bazhenova,2 Anders Carlsson,1 James Hicks,1 Peter Kuhn1.

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Non-Small Cell Lung Cancer (NSCLC) is the most common cause of cancer related deaths in both men and women. Some lung cancer patients have a unique challenge of a tissue access due to tumor location or baseline lung disorders such as emphysema and chronic obstructive pulmonary disease (COPD). Liquid biopsy technologies can overcome tissue assess difficulties. However, as of today there are no commercially approved assays for CTC enumeration in lung cancer. Multiple non-commercial assays exist and some show correlation of CTC enumeration and changes in CTC counts on therapy with prognosis and response to therapy. Outside of absolute number of CTC there is little published data regarding prognostic significance of CTC morphologic heterogeneity in late stage NSCLC and its ability to predict treatment outcomes. Here we investigate CTC counts in 81 patients with stage IV NSCLC using a fluid phase biopsy and high definition (HD) diagnostic pathology imaging of all nucleated cells. HD-CTCs were detected in 51 (63%) patients at initiation of therapy with a median of 2.20 (range 0-509.20) and a mean of 26.21 HD-CTCs/mL (+15.64). There was no correlation between the absolute number of HD-CTCs at the time of initiation of new therapy and patient outcomes. A subset of 25 patients was further analyzed to determine the significance of HD-CTCs kinetics, which may follow three distinct patterns: an increase in HD-CTCs with therapy, unchanged HD-CTCs numbers, and a decline in HD-CTCs numbers. Patients that experienced an increasing, stable, or decreasing HD-CTCs profile had an overall change of 118.40, 0.54, and 81.40 HD-CTCs/mL respectively during the first 3 months. The overall survival (OS) for increasing profiles was 31.08 months (+13.08, median 32.46, range 15.11-43.50), for stable profiles OS was 8.82 months (+3.57, median 7.23, range 3.91-19.75), and for decreasing profiles it was 15.30 months (+6.39, median 14.06, range 3.81-28.06) months. CTCs are identifiable in patients with stage IV NSCLC, but correlation of absolute HD-CTC counts with disease progression was not statistically significant. However, change in CTC counts were predictive of OS in patients with metastatic lung cancer receiving chemotherapy.
CTCs are used as a surrogate source of tumor material in solid tumors. Clinical applications of CTCs as liquid biopsy comprise the monitoring of molecular alterations during tumor progression and dynamic evaluation of molecular markers of treatment response. The FDA-cleared method to isolate CTCs in cancer patients (Cell Search) is based on positive selection of EpCAM expressing cells. However, the performance of non-selective enrichment (NSCLC) as it identifies CTCs in only 7% of the subjects, failing to show any prognostic relevance. Down-regulation/loss of epithelial markers to isolate (EpCAM) and identify (cytokeratin, CK) CTCs could in part explain the low CTC yield obtained in NSCLC with approaches based on epithelial markers expression. To overcome this challenge we used size-based CTC enrichment (ISET filters) from NSCLC patients’ peripheral blood. As a positive identifier of CTCs we used transferrin receptor 1 (TfR) which is a cell membrane-associated protein, that mediates intracellular iron uptake, and which is expressed at low levels in many normal tissues but over-expressed in cancer cells. We first analyzed TfR protein expression by immunofluorescence in a panel of NSCLC cell lines and in healthy donor leukocytes. While all NSCLC cell lines analyzed were positive for TfR expression, none of the leukocytes expressed the receptor. Moreover, TfR expression was detected also in EpCAM negative NSCLC cell lines. To determine the clinical applicability of this novel CTC identifier, we determined TfR expression in CTCs isolated from peripheral blood of 35 metastatic NSCLC patients using the ISET filter technology. The isolated CTCs were stained for TfR, CK, CD45 and DAPI. For each patient, one additional ISET filter was stained with Giemsa for morphologic analysis by a pathologist. By using the classic panel of CTC identifiers markers (CK+/CD45-/DAPI-), CTCs were identified in 4/34 (11%) patients, while by using TfR as positive identifier (TfR+/CD45-/DAPI+) CTCs were identified in 31/35 (88%) subjects. The morphologic review of Giemsa stained filters confirmed the presence of tumor cells in 28/34 (82%) samples [0-217 CTCs/sample]. Interestingly, patients with > 6 TfR+ CTCs had a worse overall survival (OS) than patients with < 6 TfR+ CTCs [p=0.048 Log Rank (Mantel-Cox)]. OS did not significantly differ using the same cutoff with CTCs defined based on CK or Giemsa staining. Overall, our data indicate that TfR is a promising biomarker for the detection of CTCs in NSCLC CTCs, superior to CK or EpCAM. Our data also suggest that TfR may potentially identify CTC subpopulations with a significant prognostic role in NSCLC. We are currently isolating TfR+ CTCs from early stage and metastatic NSCLC patients for further molecular characterization and determination of clinical significance.

**#1714 Translating CTCs for clinical use: melanoma patient-derived CTCs evolution in xenografts.** Monika Vishnoi,1 Haown N. Liu,1 Debasish Boral,1 Wei Yin,2 Marc L. Sprouse,3 Jean P. Thiery,2 Isabella C. Glitza,3 Dario Marcelli4, Houston Methodist Research Institute, Houston, TX; Cancer Science Institute of Singapore, National University of Singapore, Singapore, Singapore; The University of Texas MD Anderson Cancer Center, Houston, TX.

Circulating tumor cells (CTCs) are known to disseminate from primary/metastatic tumors, survive in the circulation, and develop capability to colonize distant organs. Dissecting CTC heterogeneity is therefore crucial to provide improve prognostic and diagnostic tools (concept of “liquid biopsy”). However, melanoma patient blood from peripheral blood of melanoma patients clinically diagnosed with or without brain metastasis (MBM) by multi-parametric flow cytometry; and analyzed CTC heterogeneity in xenografts by longitudinal capturing and interrogation at the single-cell level (DEPArray™ platform). First, we evaluated the presence of melanoma CTC subpopulations in Lin-negative cells and developed Lin-negative murine xenograft models. Human-cell derived melanoma metastasis in xenografts confirmed that CTCs require metastasis-potentiating factors present in the Lin-negative population. Second, we performed multi-parametric flow cytometry to isolate human-derived HLA+/Melan A+/− CTCs/DTCs population from murine blood and bone marrow at T1 and T2 phases of metastasis. We interrogated the differential levels of CTCs/DTCs in HLA+/Melan A+ cell population and established their neoplastic identity by genetic profiling and the expression of melanoma markers at single-cell level. Third, we dissected longitudinal transcriptional signatures of CTCs vs DTGs in association with MBM onset. We assessed the comprehensive EMT scores of CTCs and DTGs transcriptomics and identified the pathways associated with CTC/DTCs dynamic functional states depending upon melanoma progression. Collectively, this study provide important insights to identify novel EMT-related biomarkers of MBM progression. Further elucidation of these CTC biomarkers and properties may provide novel therapeutic strategies that can be clinically useful in melanoma patients which are yet to develop MBM.

**#1715 EGFR mutation detection in ctDNA, Vortex-enriched CTCs and comparison to tumor tissue in non-small-cell-lung cancer (NSCLC) patients.** Haiyan E. Liu,1 Meghah Vuppalapaty,2 Clementine A. Lemaire,2 Charles Wilkerson,3 Steve C. Crouse,3 Jonathan W. Goldman,3 Elodie Sollier-Christin3, Vortex Biosciences, Menlo Park, CA;3 David Geffen School of Medicine at UCLA, Los Angeles, CA.

Background: Lung cancer is the leading cause of cancer-related mortality worldwide. Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) therapies, based on the evaluation of EGFR mutation, have shown dramatic clinical benefits. EGFR mutation assays are mainly performed on tumor biopsies, which carry risks and expense and are not always successful. In order to identify the development of secondary EGFR mutations, which cause resistance to 1st and 2nd generation TKI’s and an indication for therapy with a 3rd generation drug, effective and non-invasive monitoring is needed. Liquid biopsy biomarkers, such as circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA), allow such monitoring over the course of the therapy. Interestingly, ctDNA or CTC analysis alone had less sensitivity vs. combining both, with a genotyping of 70% and 80% for CTCs and ctDNA respectively, but 100% when combined2. Vortex technology is a platform enabling label-free capture of CTCs from blood samples and genomic assays downstream3. The aim of this study is to demonstrate the sensitivity of a combined CTC and ctDNA assay through Vortex using blood samples spiked with molecularly-characterized lung cancer cell lines and then to apply this technique to matched blood and tumor samples from NSCLC patients. Method: Lung cancer cell lines with different EGFR mutations (A549: wild type, H1975: L858R+ and T790M+; HCC827: 19del+) were used to validate our CTC workflow. Blood samples and matched tumor tissues were collected from NSCLC patients. Plasma was extracted first for ctDNA assay. CTCs were isolated from the plasma-depleted-blood using Vortex technology, immunostained (CK, Vimentin, CD45) and enumerated. DNA from CTCs, plasma and matched tumor tissue was analyzed for EGFR mutations 19del, L858R and T790M using the ctEGFR kit from EntroGen. Results: Mutant DNA could be identified at a quantity as low as 0.5 ng (−8 cells), with a sensitivity ranging from 0.1% to 2% for a total DNA varying from 25ng (~4 CTCs among 4000 WBCs) to 1ng (~4 CTCs among 200 WBCs). We demonstrated the ability of Vortex technology to enrich CTCs from metastatic NSCLC patients. Processing of plasma-depleted-blood showed the same capture efficiency when compared to whole blood. This makes possible the detection of EGFR mutations on CTC samples collected by Vortex technology. −1 NSCLC patients are currently enrolled in this study and results will be presented at AACR. Conclusion: The ctEGFR mutation assay performed well on both Vortex-enriched CTCs and ctDNA, enabling a low cost approach to analyze EGFR mutation from a single blood tube. This non-invasive EGFR mutation analysis will be potentially a useful tool for monitoring treatment and medication guidance of NSCLC patients. 1. Cala-Guirós M. et al. Transl Lung Cancer Res. 2016. 2. Sundaresan TK et al. Clin Cancer Res. 2016. 3. Kidess-Sigal E et al. Oncotarget 2016.

**#1716 GSTP1 promoter methylation in in-vivo isolated CTCs from high-risk prostate cancer patients.** Athina N. Markou,1 Panagiotis Parasevopoulos1, Mari-fili Lazaidou,1 Shukun Chen,2 Thomas Krones,3 Joanna Budna,4 Andra Kuske,5 Tobias M. Gorges,3 Maciej Zabel,6 Peter Sedlmayr,7 Cathe-rine Alia Panabieres,8 Klaus Pantel,9 Evi Liandrou6,1 Univ. of Athens, Athens, Greece; 1Institute for Cell Biology, Histology and Embryology, Graz, Austria; 2Poznan University of Medical Sciences, Poznai, Poland; 3University Medical Center Hamburg-Eppendorf, Hamburg, Germany; 4Poznan University of Medical Sciences, Poland; 5University Institute for Clinical Research (IURC), Laboratory of Rare Human Circulating Cells, University Medical Centre of Montpellier Saint-Eloi Hospital, EA4305, France.

Introduction: Glutathione S-transferase I (GSTP1) has been reported to function as tumor suppressor gene in various types of human cancers. GSTP1 inactivation is associated with CpG island promoter hypermethylation in the majority of prostate cancers (PCs). The initiation of minimal residual disease (MRD) and especially the detection of circulating tumor cells (CTCs) in patients’ peripheral blood represent a negative prognostic parameter for recurrence-free survival. The aim of the present study was to assess the methylation status of the GSTP1 gene in CTCs that were isolated, using the CellCollector® (GILUPI, GmbH), a novel clinical device designed for the in vivo isolation of EpCAM-positive CTCs. Patients and methods: In vivo isolation of CTCs was performed...
by using CellCollector® from high-risk prostate cancer patients (n = 97) and 20 healthy volunteers. For all these patients, the Ab coated region of the CellCollector® was washed in PBS, cut, and stored in Trizol reagent till analysis and DNA was further prior to the analysis isolated DNA was modified by sodium bisulfite (SB) and subjected to a real time MSP assay specific for GSTP1 methylation. The peripheral blood and cell DNA was isolated and amplified using bisulfite DNA analysis by Immunostaining and the CellSearch® system. Results: All DNA samples were first checked for their quality. Based on the quality evaluation of all available DNA samples, only 63 DNAs were further qualified for analysis. GSTP1 promoter was found methylated in 12/63 (19%) the EPcAM positive fraction of in-vivo isolated CTCs. Moreover, in 5/12 (41.7%) patients for which GSTP1 promoter was also found methylated, CTCs were also detected by the CellSearch® system. In all cases, peripheral blood was also collected and analyzed for GSTP1 promoter methylation. In all cases, GSTP1 promoter methylhylation in in-vivo isolated CTCs should be prospectively validated as a novel tumor biomarker for prostate cancer patients in a large cohort of patients. Acknowledgments: This research has been co-financed by the European Union (European Regional Development Fund = ERDF) and Greek national funds through the Operational Program “Competitiveness and Entrepreneurship” of the National Strategic Reference Framework (NSRF) - Research Funding Program: “ERA-NET on Translational Cancer Research (TRANSCAN)” Joint Transnational Call for Proposals 2011 (JTCC 2011) on: “Validation of biomarkers for personalised cancer medicine.

#1717 Orthogonal identification of circulating tumor cells (CTCs) using single cell low pass whole-genome sequencing (WGS) and copy-number alteration (CNA) analysis. Gareth Morrison,1 Valeria Sero,1 Yucheng Xu,1 Jacke Pinski,1 Sue Ingles,1 David Quinn,1 Claudio Forcato,1 Genny Buson,1 Chiu-Ho Pinski,1 Sue Ingles,1 David Quinn,1 Claudio Forcato,1 Genny Buson,1 Chiu-Ho Webb,5 Kyle Horvath,1 Aditi Khurana,1 Gianni Medoro,1 Suman Verma,1 Matthew Moore,4 Philip Cotter,4 Nicolò Manaresi,3 Farideh Bischoff,2 Amir Goldkorn1.1 University of Southern California, Keck School of Medicine, Norris Comprehensive Cancer Center, Los Angeles, CA; 2Menarini Silicon Biosystems Spa, Bologna, Italy; 3Menarini Silicon Biosystems Inc, San Diego, CA; 4Research DX, Irving, CA; 5University of California, Irvine, CA.

Introduction: Presence of circulating tumor cells has prognostic value in multiple malignancies, and molecular analysis of CTCs is currently ongoing in numerous clinical trials. Most CTC enrichment methods rely on standard epithelial and leukocyte markers (CK+CD45-), so recovered cells are assumed to be of epithelial origin but never shown to be bona fide tumor cells. Conversely, atypical cells lacking the characteristic marker profile may not be analyzed, even though they may represent important tumor subpopulations. Here we evaluate a rapid, non-exhaustive, and cost-effective first-pass genomic analysis of individual candidate CTCs. This approach allows efficient upfront CNA-based confirmation of CTCs as cells of tumor origin, while leaving abundant DNA for deeper subsequent analysis in cells of interest. Methods: Whole peripheral blood of metastatic prostate cancer patients was enriched for CTCs using the CellSearch® system (Janssen Diagnostics) under an IRB-approved protocol, and 5 samples with >5 CTCs were selected for further study. Next, the DEPArrayTM v2 system (Menarini Silicon Biosystems) was used to identify and isolate single CTCs (CK+CD45+CD45-), and paired white blood cells (WBCs; CK+CD45+CD45+DAP1+) from the enriched samples. In addition, cells negative for both cytokeratin and CD45 but with characteristic malignant morphology (large with high nuclear-cytoplasmic ratio) were isolated. Recovered single cells were whole-genome amplified with Ampli1™ WGA and quality controlled by Ampli1 QC. Ampli1 LowPass kit was then used to prepare NGS libraries for absolute quantification by low-pass WGS. Results: Thirty-three single CTCs (CK+CD45+DAP1+) and 30 WBCs (CK+CD45+DAP1+) were isolated. Single-cell WGA products with high Genome-Integrity Index (QC score ≥3) were prioritized for CNA analysis. Ampli1 LowPass data demonstrated copy number gains/losses confirming tumor origin of the CK+ cells, while WBCs showed a normal profile. In addition, the cells having non-conventional phenotype also demonstrated copy number alterations consistent with tumor origin. Discussion: We demonstrate a WGA and low-pass WGS approach on single CTCs sorted from enriched peripheral blood, which offers a dual benefit: i) it allows rapid, non-exhaustive upfront identification of bona fide tumor cells for further study, and ii) it reveals genetic similarities and diversities (a vis a vis copy number alteration) across CTCs of classical as well as non-conventional phenotypes, which may better represent clonal diversity. In a clinical setting, this molecular approach may be more effective for reliably identifying and characterizing heterogeneous CTCs, yielding profiles that more accurately reflect disease evolution and inform treatment strategies.

#1718 RT-qPCR gene expression analysis of CTCs isolated through an epitone-independent enrichment microfluidic device in patients with head and neck squamous cell carcinoma. Martha Zavridou,1 Areti Strati,2 George Koutsodimitri,2 Amanda Psyrri,2 Evi Lianidou1.1 Univ. of Athens, Athens, Greece; 2Atikkon University Hospital, Athens, Greece.

Background: Molecular characterization of circulating tumor cells (CTCs) is very challenging since these cells are rare, and the amount of available sample for their analysis is very limited. Moreover, CTC are highly heterogeneous and enrichment technologies based on EpCam expression present the risk of missing EpCam-negative CTCs. The Parsortix system (ANGLE plc, UK), is a novel microfluidic technology platform designed for marker-independent capture of CTCs. In this study we perform the first time the Parsortix system to isolate CTCs from patients with Head and Neck Squamous Cell carcinoma (HNSCC), and proceeded to downstream molecular characterization through RT-qPCR gene expression analysis. Methods: Peripheral blood samples (10 mL) from head and neck squamous cell carcinoma (HNSCC) patients (n = 19) and healthy donors used as a control group (n = 10) were used for the isolation of CTCs using the Parsortix device. Enriched CTCs were harvested in Trizol reagent, followed by extraction of total RNA and cDNA synthesis. RT-qPCR was performed in the LightCycler (Roche) for the following gene targets: PD-L1, VIM, TWIST, EGFR, and B2M (used as a reference gene). The expression levels of PD-L1, VIM, TWIST and EGFR were normalized using the 2 ΔΔCt approach in respect to the expression of B2M. Results: All samples analyzed were of excellent RNA quality as the quality assessed by B2M, Actin and GAPDH expression. PD-L1 over-expression was detected in 5/19 (26.3%) samples, VIM was overexpressed in 3/19 (15.7%) and TWIST-1 in 1/19 (5.3%) sample, while EGFR expression was not detected in any patient (0/19, 0%). These are preliminary results and these percentages may change, since the number of samples that we are analyzing is continuously increasing. Conclusions: This preliminary study is showing for the first time that RT-qPCR can be successfully used for the molecular characterization of CTCs isolated by the label-free Parsortix microfluidic device in HNSCC. Overexpression of individualized immunotherapy important biomarkers such as PD-L1 in CTCs of HNSCC patients could be of significant clinical importance for the selection and follow up of these patients.

#1719 Dual-profiling of CTC and exosome from the cultured circulating tumor cells using stimuli-responsive degradable hydrogels. Yoon-Tae Kang,1 Young Jun Kim,1 Tae Hee Lee,1 Jae-Uil Shim,1 Young-Ho Cho1.1 KAIST, Daejeon, Republic of Korea; 2Cell Bench Research Center(SEMCELL), Daejeon, Republic of Korea.

Introduction: Liquid biopsy based on sub-micron or nanosize particles in human body fluid have been received vast attention due to their non-invasive characteristics and enabling multiple check-up. Circulating tumor cells (CTCs), as well as exosome are the most promising markers in liquid biopsy, however, dual isolation and profiling have been hampered due to their size difference and limited quantity for analysis. We proposed the novel and simple methods for both isolation and study their similarity between them. Using the label-free CTC filtration device and anti-CD63 antibody-conjugated degradable hydrogel, the CTCs and the CTCs-derived exosome are specifically isolated, and each samples were followed by molecular study after recovery. This versatile platform facilitates the comprehensive study of two biomarkers with reflecting their inherent characteristics, thus paving the way for revealing their roles in cancer progression and metastasis. Methods: In order to make stimuli-responsive degradable hydrogel, poly (vinyl alcohol) and alginate were mixed under constant stirring at 85 °C. The mixture was poured into the mold and dried for 24 hours. Then, the dried sheet was immersed into 100 mM calcium chloride solution to achieve gelation through ion-structural interaction. Subsequently, the anti-CD63 antibody was immobilized onto the prepared hydrogels via cross-linking. For the dual-profiling, the hydrogel and the filters containing the captured breast cancer cells by microfiltration were incubated with the exosome-depleted cell culture media for 6 hours. The captured cells were released from the device and the hydrogels were degraded by adding EDTA. The cell and exosome lysate were prepared using RIPA buffer at 4 °C. The supernatant was collected by centrifugation followed by western-blot assay. Four different markers, including exosome-specific marker (CD63), cancer-associated markers (EpCAM, vimentin), and a housekeeping marker (β-actin), were used. Results: All exosome and cell samples highly expressed the housekeeping marker. Especially, two exosome samples dissociated from the hydrogel showed CD63 predominantly, which support the secretion of exosome from the cancer cells. The samples from the released cancer cells from the device did not express CD63 remarkably. To verify the phenotypical similarity between cell and exosome, expressions of the epithelial marker (EpCAM) and mesenchymal marker (vimentin) were examined. The exosome and cell from MCF-7, epithelial cancer cell, showed higher expression of EpCAM then
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vimentin. On the contrary to this, the samples from MDA-MB-231, mesenchymal cell, showed higher vimentin expression then EpCAM. Discussion and conclusion: We showed that exosome follow the phenotypical characteristics of mother-cells. This dual profiling would be helpful for in-depth study of cancer with consideration of its heterogeneity and complexity.

#1720 Circulating tumor cell (CTC) enrichment and DNA mutation detection by sequencing. Ruijin Kou,1 Eric Zhao,2 Conrad Leung,1 Priya Gogoi,2 Will Chow,2 Yixin Wang,3 GENEWIZ, South Plainfield, NJ 08070, NJ, 2Celense Diagnostics, Plymouth, MI.

Molecular analysis of circulating tumor cells (CTCs) is hindered by low sensitivity and high level of background leukocytes of currently available CTC enrichment technologies. The Celense PREP100 device captures CTCs with high sensitivity and allows the captured CTCs to be retrieved for molecular analysis. To evaluate the potential of utilizing PREP100 in CTC molecular analysis, we first prepared mock samples using cultured prostate cancer cells PC3 and LnCAP and analyzed captured cells by PCR amplicon sequencing. Using Celense PREP100, we were able to recover the average of 79% (ranging from 40% to 100%) of 110–1100 PC3 cells and 60–1,500 LnCAP cells and detect p.K139fs*3 deletion in PC3 cells and T877A mutation in LnCAP cells. In addition, we also spiked in the two cell lines into normal donor blood samples and analyzed the captured cells by PCR amplicon sequencing. Preliminary experiments showed that 40% of 250 spiked PC3 cells and 74% of 50 spiked LnCAP cells were captured. Further, we were able to detect p.K139fs*3 deletion in the retrieved 50,100 spiked PC3 cells. Our results illustrate that Celense PREP100 system presents a promising technology for capturing and molecular characterization of CTCs.


Background: Circulating tumor cells (CTCs) are tumor cells shed from primary tumor and circulate in the peripheral blood. CTCs, as a surrogate of distant metastasis, can be potentially useful in diagnosis and monitoring therapeutic effects in malignant tumors. Among a variety of systems for detection of CTCs, the “Cellsearch” is the only approved system for clinical use. However, EpCAM-negative tumor cells, such as those originating from non-epithelial cells and those undergoing epithelial-mesenchymal transition (EMT) cannot be captured with the “CellSearch” that is an EpCAM-based isolation system. Therefore, we have developed a novel polymeric microfluidic device (“Universal” CTC-chip) that can capture CTCs with or without EpCAM expression (AACR 2015). In the present study, we examined CTCs-detection performance of the CTC-chip in patients with thoracic malignant tumors (lung cancer [LC] as an “EpCAM-positive” tumor and malignant pleural mesothelioma [MPM] as an “EpCAM-negative” tumor) in comparison with that of the CellSearch. Methods: Peripheral blood sample from each patient was divided and subjected to quantitative evaluation of CTCs with the CTC-chip as well as with the “CellSearch”. The CTC-chip was coated with an anti-EpCAM antibody, was used to capture CTCs in the blood samples (n=19) from lung cancer patients. To capture CTCs in the samples (n=11) from MPM patients, the CTC-chip was coated with an antibody against podoplanin that is expressed on the mesothelioma. After immune-staining for cytokeratin and CD45 on the chip, a captured cell containing Hoehst-positive nucleus and cytokeratin-positive/CD45-negative cytoplasm was judged as a CTC. The CTC-count for each sample was represented as the number per 7.5mL of blood. Results: The median CTC-count detected with the CTC-chip in LC was 50 (range, 0–270), which was significantly higher than that of “CellSearch” (p<0.01). In the peripheral blood sample from MPM patients, CTC was detected in only one patient using the CellSearch, but was detected in all 11 patients with the median CTC-count of 144 (range, 0–470). Conclusion: The “universal” CTC-chip achieved higher performance in detection of CTCs of thoracic malignant tumors as compared with the CellSearch. The updated data will be presented at the AACR annual meeting 2017.

#1722 Novel multiplex liquid biopsy detection of ALK and ROS1 rearrangements and intratumoral heterogeneity in lung cancer using circulating tumor cell (CTC) blood from NSCLC patient bloods and TR-CytoTrapNano™ technology. Sangjun Lee, Cytolumina, Los Angeles, CA.

Introduction: ALK and ROS1 rearrangements are common gene alterations found in subsets of patients with NSCLC. Identification of ALK and ROS1 rearrangement in NSCLC is critical for highly active targeted therapies aiming for two main tumorigenic drivers. Due to the similarity of tyrosine kinase domains between ALK and ROS1, many ALK inhibitors are also functional in treatment of ROS1 subtype NSCLC. For example, crizotinib, which is a standard therapy for advanced ALK-rearranged NSCLC is highly effective in advanced ROS1-rearranged genotypy NSCLC patients. The mutational analysis of these genes requires serial tissue sampling from primary and metastatic lesions, which is labor intensive and time consuming. Further, the current tissue-based diagnostics, such as fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC) are known to be challenging technically with limited sensitivity, subjective interpretation and inaccuracy in monitoring the current tumor status. The TR-CytoTrapNano™ CTC technology is a blood-based assay to utilize CTCs as a biosource for mRNA extraction to detect genetic alterations and gene expression profiles including ALK and ROS1 gene rearrangements. In this study, we determined the variant specific expression of ALK fusion transcripts (EML4-ALK, KIF-ALK, and TGF-ALK) and ROS1 fusion transcripts (EZR-ROS1, SDC4-ROS1, TPM3-ROS1, LRG3-ROS1, GOPC-ROS1, CD74-ROS1, and SLCE4A2-ROS1) using multiplex digital droplet polymerase chain reaction (ddPCR) technology. Method: The Peripheral Blood Mononuclear Cells isolated from NSCLC patient bloods run through the TR-CytoTrapNano™ microfluidic system. The CTCs are enriched and captured on the chip surface conjugated with Anti-Ep-ctp targeting antibody, which specifically binds to CTCs of epithelial origin. Following lysis step facilitate total RNA release from trapped CTCs and then pure mRNAs are isolated using oligo-dT conjugated magnetic beads. The mRNAs are used for following ALK and ROS1 specific reverse transcription reaction. The ALK and ROS1 fusion gene expression profiles of CTCs by ddPCR. Concomitant subtyping of ALK and ROS1 rearrangement are then validated using ddPCR. Results and Conclusion: We tested several liquid biopsies from ALK and ROS1 rearrangement positive patients and corresponding positive data to FISH assays using solid tumor biopsies. Interestingly, some of the CTCs harboring ALK and ROS1 rearrangement has shown high intra-tumor heterogeneity in the subtypes of fusion partners, and we envision that our novel TR-CytoTrapNano™ CTC ALK/ROS1 rearrangement Assay to monitor treatment response and recurrence in NSCLC patients will be used to monitor treatment response and disease recurrence following treatment with Tyrosine Kinase Inhibitors (TKIs).

#1723 Diagnostic leukapheresis results in a significant increase in CTC yield in metastatic breast and prostate cancer. Kiki C. Andere,1 Anouk Mentrin,1 Joost F. Swennenhuis,1 Leon W. Terstappen,2 Nikolais H. Stoecklein,3 Rui P. Neves,4 Rita Rampignano,4 Hans Neubauer,4 Tanja Fehm,5 Johannes C. Fischer,3 Elisabetta Rossi,2 Mariangela Manicone,4 Umberto Basso,4 Piero Marson,2 Rita Zamarchi,2 Yohann Loriot,6 Valérie Lapierre,6 Vincent Faugereux,7 Marianne Oulhen,8 Françoise Farace,6 Gemma Fowler,8 Mariane Sousa Fontes,9 Berni Ebbs,9 Maryou Lombros,9 Mateus Crespo,9 Penelope Flohr,9 Johann S. de Bono5 University of Twente, Enschede, Netherlands; 2University Hospital of the Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany; 3IROV-RCCS and University Hospital of Padova, Padova, Italy; 4IROV-FCRCS Sanger, Sutton, United Kingdom; 5The Institute of Cancer Research and The Royal Marsden NHS Foundation Trust, Sutton, United Kingdom.

Introduction: Frequency the number of CTC isolated in 7.5 mL of blood is too small to reliably determine tumor heterogeneity and to be representative as a ‘liquid biopsy’. In the EU FP7 program CTCTrap we aimed to validate and optimize the recently introduced Diagnostic LeukApheresis (DLA; doi: 10.1073/pnas.1313594110) approach to screen liters of blood and thereby substantially increasing the number of CTC available for further characterization. Here we present the results obtained from 32 metastatic cancer patients subjected to DLA in the participating institutions. Methods Before the DLA procedure, whole blood was drawn in a CellSave blood collection tube and a 7.5 ml aliquot was processed with the ‘gold standard’ reference CellSearch® (Janssen Diagnostics, USA). DLAs from metastatic cancer patients were performed for >90 minutes to obtain 40 mL of product containing ~4×10⁶ mononuclear cells (MNC) representing ~1 liter of blood. The obtained DLA samples were then divided, fixed with CellSave preservative, prepared and processed and each of the analysis techniques as described in the Standard Operating Procedures developed for DLA in the CTCTrap consortium (https://www.utwente.nl/tnw/mcbp/protocoldandtools/). Results: DLAs were obtained from 20 metastatic prostate cancer patients and 12 metastatic breast cancer patients at four different European academic medical institutions. Using a SOP for the DLA procedure, similar DLA products were obtained (see Table 1). Mean CTC-chip count per DLA ± SD (n=68): 6×10⁶ ± 38x10⁵ could be generated without any noticeable side effects. CTC in 7.5 mL of blood ranged from 0 to 324 (mean ± SD = 61, median = 18). DLA processed with CellSearch represented 7 to 212 mL of blood (mean = 100, median = 97). CTC ranged from 0 to 2913 (mean ± SD = 330, median = 165). Resulting in a significant increase in CTC yield (p = 0.004)

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ranging from 0x to 40x (mean = 13, median = 9) when comparing 1mL of whole blood to 1mL of DLAs. Filtration of 50x10^6 WBC of DLAs, through 5um microsieves yielded only 0 to 12 CTC (mean = 2, median = 0, n = 16). Leukocyte depletion of 18 mL of DLAs followed by filtration yielded 0 to 178 CTC (mean = 37, median = 4, n = 22) not yielding a relative increase versus CellSearch DLAs. Leukocyte depletion followed by CellSearch yielded 792, median = 484, n = 3) also not yielding a relative increase versus CellSearch DLAs. In 7 patients CTC were detected in 7.5mL of blood, in 4 out of these 7 patients CTC were detected in DLAs. Conclusion: The yield of CTC can be significantly increased by the use of DLAs in patients with CTC detected in 7.5 mL of blood. Technology to select CTC from DLAs will need to be further improved before one can make optimal use of the large processed blood volumes.

#1724 Genomic profiling of Vortex-enriched CTCs using whole genome amplification and multiplex PCR-based targeted next generation sequencing. Haiyan E. Liu,1 Melanie Triboutel,1 Amin Aina,2 Meghah Vuppalapati,1 Evelyn Kides-Sigal,3 John Coller,1 Vanita S. Natsu,2 Vida Shokooohi,1 James Che,1 Corinne Renier,1 Natalie Chan,1 Violet Hanf,1 Elodie Sollier-Christen,1 Stefanie S. Jeffrey.1 Vortex Biosciences, Menlo Park, CA; Stanford University, School of Medicine, Stanford, CA; Stanford University, Stanford, CA; Charite University Hospital, Berlin, Germany.

Background: Genomic characterization of circulating tumor cells (CTCs) provides insights into cancer genetic changes, and might be utilized for cancer prognosis, diagnosis, as well as monitoring of therapeutic efficacy. Targeted Panel Next Generation Sequencing (NGS) enables analyzing CTC genetic variants of a selected gene panel at a relatively lower cost. However, CTCs are rare, often resulting in very limited DNA quantities available that require whole genome amplification (WGA). In previous studies, we introduced the Vortex technology, a platform enabling label-free enrichment of CTCs from blood samples of colorectal cancer (CRC) patients and their use for genomic assays downstream. In this study, we developed a simple and efficient NGS workflow for CRC samples collected by this technology. Method: An optimized workflow using the Qiagen GeneRead DNAseq target panel and Illumina MiSeq NGS was first verified on HCT116 CRC cell line before being applied on patient CTCs. For patient blood samples, CTCs were collected with the Vortex technology, immunostained (CK, Vimentin, CD45) and enumerated. Matched white blood cell (WBC) DNA was included to subtract germline background. Frozen fresh liver metastasis tissue was collected and analyzed using the same NGS workflow. DNA from CTCs was extracted and amplified using Qiagen REPLi-g single cell WGA kit. Mutation detection on the WGA amplified DNA was performed using the GeneRead DNAseq CRC targeted panel of 38 genes and MiSeq sequencing. The sequencing data were analyzed by QIAGEN NGS Data Analysis Web Portal and Ingenuity Variant Analysis software. Results: The Vortex technology was validated for the capture of CTC from CRC patients. REPLi-g PCR performs a uniform, unbiased amplification on fresh rare cells with a coverage of 97.7%, which enabled further targeted panel NGS. Blood from 3 CRC patients (P1, P2, P3) and 2 healthy donors (HD1, HD2) was processed with Vortex platform. Less than 1 CTC/mL blood were found in HD1 and HD2. P1 and P2 had 66 and 20 CTCs/μL of blood respectively, with many vimentin positive CTC clusters. P3 had 2 CTCs/μL blood. No somatic mutation was found in the CTC samples. Somatic variants were only detected in the CTCs from patient samples that were not present in matched germline WBCs. For P1, more mutations were found in the CTCs than in the liver metastasis when it was the opposite for P2 and P3. Conclusion: For each patient, variants in CTCs and germline WBCs were analyzed from one blood sample using an optimized targeted NGS workflow and compared to liver mets. Our optimized workflow, using the Qiagen REPLi-g and GeneRead DNAseq Targeted Panel NGS enabled the detection of CTC mutations for 38 CRC-focused genes. The inclusion of a germline WBC control in the workflow allowed the detection of mutations from pooled CTC samples collected using the Vortex technology. Altmüller J, et al. (2014). Biol Chem. Kидес-Sigal E, et al. (2016). Oncotarget.

#1725 Detection of ESR1 D538G mutation in circulating tumor cells (CTCs) and paired circulating tumor DNA (ctDNA) samples of breast cancer patients. Eleni Tzanikou,1 Athina Markou,1 Eleni Politaki,2 Giorgos Koutsopoulou,2 Amanda Pyrati,3 Vasileios Georgoulias,2 Evi Lianidou1.1 University of Athens, Athens, Greece; 2University of Crete, Heraklion, Greece; 3Attikon University Hospital, Athens, Greece.

AIMS: Molecular characterization of CTCs and ctDNA analysis holds promise as an extremely powerful tool for the molecular profiling of cancer patients in real time. Estrogen receptor alpha (ERα) is expressed in approximately 70% of all breast cancers and endocrine therapy represents a major treatment modality in ERα-positive disease. Recently, somatic mutations in the ERα gene (ESR1) were linked to acquired resistance to endocrine therapies in breast cancer. In this study, we analyzed the most frequent ESR1 mutation (D538G) in CTCs (DNA samples isolated from CellSearch cartridges), corresponding ctDNA from early and metastatic breast cancer patients and healthy donors. METHODS: We first developed a highly sensitive and specific methodology for the detection of ESR1 D538G mutation in CTCs by using an ultrasensitive asymmetric rapid PCR and high resolution melting analysis. We analyzed DNA isolated from CTCs (CellSearch) and the corresponding ctDNA before and after therapy in: a) 25 patients with ER+ operable breast cancer, b) 11 patients with ER+ metastatic breast cancer, c) 13 patients with ER- early breast cancer, d) 5 patients with ER- metastatic breast cancer and e) 80 healthy female volunteers. In all, 1mL DNA (extracted from 2 mL plasma) and DNA from CTCs were first examined for their DNA quality before analysis. RESULTS: The assay is highly sensitive (analytical sensitivity: 0.05%) and specific (0/80 healthy donors). ESR1 D538G hotspot mutation was identified in CDNA in 4/18 (22.2%) of ER+ metastasis-verified and in 5/33 (15.2%) of ER+ early breast cancer. In CTCs, ESR1 D538G mutation was identified in 6/18 (33.3%) of ER+ metastasis-verified and 5/33 (15.2%) of ER+ early breast cancer. In ER- metastasis-verified breast cancer, the concordance for D538G mutation between CTCs and ctDNA was 10/18 (55.6%), whereas the corresponding concordance for ER+ operable breast cancer was 25/33 (75.8%). Moreover, ESR1 D538G hotspot mutation was identified in CDNA in 2/9 (22.2%) of ER- metastasis-verified breast cancer and 2/16 (12.5%) of ER- early breast cancer. ERS1 D538G hotspot mutation was identified in 1/3 of the patients (18.8%) of ER- operable breast cancer as none of ER- metastasis-verified breast cancer patients (0/9) were positive. In ER- metastasis-verified breast cancer patients, the concordance between CTCs and ctDNA for D538G mutation was 7/9(77.8%), whereas the corresponding concordance for ER- early breast cancer was 15/16 (93.8%). CONCLUSIONS: We developed and validated an ultrasensitive and highly specific methodology for the detection of ERS1 D538G hotspot mutation. This mutation was detected not only in the ER+ group, but also in the ER- group of breast cancer patients. We will further evaluate our findings in a large cohort of patients before and after treatment, to evaluate response to endocrine therapies in breast cancer.

#1726 Evaluation of PD-L1/PD-1 on circulating tumor cells (CTCs) and on primary tumor in advanced non-small cell lung cancer (NSCLC). Galaktia Kallergi, Eleni Kyriaki Vetsika, Despoina Aigourekis, Eleni Lougoudi, Anastasios Koutsopoulos, Filippos Koinis, Panagiotis Katsarilinos, Maria Trypaki, Christos Stournaras, Vassilis Georgoulis, Athanasios Kotsakis, University of Crete, School of Medicine, Heraklion, Greece.

Introduction: Circulating tumor cells (CTCs) are responsible for the metastatic dissemination of the tumor. They have been shown to express Programmed Death-Ligand 1 (PD-L1) to escape from the immune system surveillance through its ligation with the PD-1 receptor on the surface of effector immune cells. We investigated the expression of PD-1/PD-L1 on CTCs isolated from NSCLC patients treated with chemotherapy. Methods: CTCs were isolated based on their size using the ISET platform from 30 stage IV chemo-naïve NSCLC patients (before and after chemotherapy). CTCs were detected after staining with Giemsa and immunofluorescence (IF). Double and triple staining experiments with different combination of antibodies: [Cytookeratin(CK)/PD-1/CD45 and CK/PD-L1/CD45] were performed and the samples were analyzed with the ARIOL system. Results: Giemsa staining showed that twenty-three (77%) out of 30 and six (54.5%) out of 11 patients had detectable CTCs at baseline and after the 3rd cycle of front-line chemotherapy. IF staining revealed seventeen out of 30 (56.7%) patients positive for CTCs at baseline level and 8 out of 11 (72.7%) samples after the 3rd cycle of treatment. PD-1 and PD-L1 expression was observed in 53% (9/17) and in 47% of the CTC-positive patients at baseline; in addition, 13% (1/8) and 63% (5/8) patients had PD-1 and PD-L1, respectively after the 3rd cycle. Among the total number of detected CTCs, 67% were PD-1(+) at baseline and 25% after the 3rd cycle (p = 0.069). In addition, 26% and 80% were PD-L1(+) at baseline and after the 3rd cycle, respectively. Patients with more than 3 PD-1 positive CTCs showed shorter PFS (p = 0.022). Primary tissue from ten of the examined patients was also available. More than 5% of PD-L1 (+) tumor infiltrating lymphocytes (TILs) were observed in 20% (2/10) of the patients. More than 5% PD-L1 positive cells in the primary tumor were observed in 20%. However the two group of the patients were different. In addition both patients with PD-L1 (+) TILs harvested CTCs with PD-1 expression and on one of them also had PD-L1 positive CTCs. Conclusion: PD-1 and PD-L1 expression on CTCs could be a biomarker before and during 1st line treatment in metastatic NSCLC. This expression was related to patients’ prognosis, implying that these molecules can be served as targets for metastasis restoration. Furthermore the expression of PD-1 on CTCs suggests a bilateral cross-talk between tumor and immune cells.

CLINICAL RESEARCH: Liquid Biopsies 1: Circulating Tumor Cells

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Circulating Tumor Cells (CTCs) in patients with extensive stage small cell lung cancer and their association with clinical outcome. Chuan Zhang,1,2 Ryan Graf,2 Adam Jendrisak,3 Amanda K. Anderson,2 Priscilla Onyango,3 Sarah Orr,3 Sarah Cancer Research Institute, Nashville, TN;2Memorial Sloan Kettering Cancer Center, New York, NY.

Baseline CTCs and post treatment changes in CTCs have previously been shown to predict the response to chemotherapy in SCLC. CTCs may also serve as pharmacodynamic biomarkers. Here we describe a study of baseline and longitudinal CTCs in ES-SCLC patients from the PINNACLE phase 1b trial (clinicaltrials.gov:NCT01859741). Materials and methods: CTCs, CTC clusters, apoptotic CTCs and N-Cadherin + CTCs were identified and enumerated from patient blood samples using Epic Sciences CTC technology. Baseline CTCs from 26 patients were correlated with clinical outcome: progression-free survival (PFS), overall survival (OS) and best overall response, as well as treatment related effects. Model was used to investigate treatment changes in CTCs among the dose groups. Association of CTCs with PFS/OS and best overall response, including CTCs at each time point, as well as temporal changes of CTC status, were studied. Multivariate analysis was performed to identify CTC numbers in a subset of time points to correlate with response to treatment. Results: CTCs were present in 81% of the patients (21/26). CTC clusters and apoptotic CTCs were detected in 38% and 77% of the patients, respectively. At baseline, CTC counts ≥ 5/mL were significantly associated with poor OS (p = 0.04). There was a trend that the presence of CTC clusters was associated with worse OS. With a cut-off of 3.4/mL, apoptotic CTCs showed a trend in association with overall survival. CTC numbers in patients with liver metastasis were significantly higher than in patients without liver metastasis. CTCs were also found to be correlated significantly with the number of metastatic sites. When measuring at Day 7 post dosing, CTC numbers were significantly decreased. Conclusions: Our findings suggest that CTCs are frequently detectable in patients and are a prognostic factor in ES-SCLC. CTCs decrease with TRXRT and platinum-based chemotherapy. Updated results will be presented. CTCs will be further evaluated in the phase 2 portion of the PINNACLE trial.


Background: Sarcomas represent 1% of all cancers in adults, 8% in adolescents and young people, and 10% in children. Even though it is a rare cancer, it contributes to a significant loss of years of life in comparison with other types of cancer (Amankwah, et al., 2013). The presence of isolated Circulating Tumor Cells (CTCs) or Circulating Tumor Microemboli (CTM) in the blood of patients with sarcoma may be early markers of tumor invasion, because it is known that these cells circulate in the blood for months or years before the development of metastases (Paterlini-Brechot and Benali, 2007; Klain, 2009; Rhim et al., 2012). Objective: To isolate and quantify CTCs and CTM from sarcoma patients, evaluating their presence and correlation with progression free survival (PFS). Methods: The blood (approximately 8mL) was prospectively collected from patients with different types of high degree sarcoma. Blood was collected before the beginning of chemotherapy. The samples were processed and filtered on ISET (Isolation by Size of Epithelial Tumor Cells, Rarecells. France) system for the isolation and quantification of CTCs and CTM. Later, was performed immunocytochemistry (ICC) with anti-CD45 antibody and counterstained with hematoxylin-eosin for leucocytes population exclusion. The cutoff was estimated using the maximum of the standardized log-rank statistic proposed by Lausen & Schumacher (1992). Results: we analyzed 11 high degree sarcoma patients before the beginning of treatment (any treatment line). The median age was 53 years-old (18-77) and 54.5% were male. The median CTCs number was 2.0 CTCs/mL (1.0-11.0) at baseline. By Kaplan-Meier test, we observed that patients with CTC number below the established cutoff (4.5 CTCs/mL) had better PFS compared to those above the cutoff (7.4 vs. 1.0 months respectively; P = 0.006).

ESR1 methylation in circulating tumor cells, ctDNA and primary tumors of breast cancer patients. Sophia Mastoraki,1 Arcti Strati,1 Eleftheria Tranioukou,1 Eleftheria Politiaki,2 George Koutsoudontis,3 Loukas Kaklamanis,4 Nikolaos Malamos,4 Amanda Psyris2, Vassilis Georgoulas,2 Evi Lianidou,5 University of Athens, Athens, Greece;4University of Crete, Herakleion, Greece;3Attikon University Hospital, Athens, Greece;5Onassis Cardiac Surgery Center, Athens, Greece.

Background: Estrogen receptor (ER) is an important prognostic biomarker in breast cancer. Epigenetic silencing of ESR1 could be of important clinical significance especially for its potential impact on endocrine treatment efficacy. Liquid biopsy provides real-time monitoring of tumor evolution and response to therapy through analysis of CTCs and ctDNA. Our group has evaluated for the first time epigenetic silencing of tumor and metastasis suppressor genes in CTCs and corresponding ctDNA. In this study, we evaluated for the first time ESR1 methylation in CTCs, paired ctDNA and primary tumors of breast cancer patients. Methods: We developed and validated a highly sensitive and specific real-time MSP assay for ESR1 methylation. We further applied the developed assay in sodium bisulfite (SB) treated DNA samples from: a) FFPEs from 40 patients with operable breast cancer, 25 patients with metastasis, 30 mammaryplasies and 15 fibroadenomas, b) EPCam + immunomagnetically isolated CTCs fractions from 74 early breast cancer patients, 48 patients with metastasis and 30 healthy donors, c) CellSearch® cartridges from 64 early breast cancer patients, 22 patients with metastasis, d) ctDNA isolated from plasma of matched samples and 54 healthy donors as a control group. Results: By using this highly specific and
The proportion of times that extrapolated counts fell within a specific percentage of the true CTC count was calculated across the entire sample set, as well as by category. In the entire sample set, the proportion of extrapolated counts that fell within 25% of the true CTC count ranged from 0.208 (1 slide) to 0.723 (4 slides). In contrast, for true count more than 100, the fraction that fell within 25% of the true count ranged from 0.751 (1 slide) to 0.968 (4 slides). In 1,000 (7 slides). Conclusions: In this sample set, nearly three quarters of cases could be estimated within 25% of the true CTC count by extrapolating from 4 AccuCyte slides. Estimating each slide to represent ~1 mL of processed whole blood, our analysis suggests that a ~4 mL sample will have this level of accuracy relative to a CTC count from 7.5 mL. At low true CTC counts the accuracy of extrapolation decreases, indicating that a larger volume of blood is required for this level of accuracy.

**#1732 Accuracy of extrapolation of circulating tumor cell count from small blood volumes: statistical estimation using the AccuCyte - CyteFinder system.** Jeffrey L. Werbin,1 Paulina Varshavskaya,2 Arturo B. Ramirez,3 Jackie L. Silwelw,1 Daniel E. Sabath,2 Ping-Yu Liu,1 Eric Kaldjian,4 Azizi Mark L. Day, Todd Morgan, Sunita Nagrath. Unv. of Michigan, Ann Arbor, MI.

While the relative five-year survival rate for men with localized prostate cancer is nearly 100%, it plummets to 28% in the case of distant metastases. This underscores the importance of investigating those cells able to spread and form these metastases: circulating tumor cells (CTCs). Through rare and surrounded by other blood cells, CTCs can be isolated using the sensitive nanomaterial-based microfluidic technology the graphene oxide (GO) Chip. Whole blood samples from 54 prostate cancer patients were obtained with consent and processed on parallel chips for immunofluorescence and RNA analysis. All of the samples were analyzed for CTC enumeration, where captured cells were stained for cytokeratin 7/8 (CK), CD45, and DAPI. CTCs were identified as DAPI+/CK+/CD45- cells. CTCs were detected in all of the samples. Interestingly, 25 samples showed CTC clusters ranging from 2-10 CTCs per cluster. To explore the potential role of EGFR in metastatic progression and CTC survival, a subset of samples was also assessed for EGFR expression with 9/10 samples showing EGFR+. RNA extracted from a parallel chip was reverse transcribed to cDNA. The cDNA was analyzed via qPCR for expression levels of 96 genes of interest, including housekeeping genes; epithelial and mesenchymal genes; oncogenes and tumor suppressor genes; prostate specific genes; extracellular matrix and inflammatory genes; and others. The results show our ability to examine RNA from CTCs to evaluate the role of relevant pathways in the cells in transit. With analysis capabilities at both the RNA and protein levels, the GO Chip is an example of a clinically relevant microfluidic technology.

**#1733 Automated identification of circulating tumor cells by image analysis.** Leonie van Zonne,1 Guus Van Dalum,2 Francois-Clement Bidard,3 Jean-Yves de Bono,3 Leon W.M.M. Terstappen,1 Chris Brune1. University of Twente, Enschede, Netherlands; Heinrich-Heine-University of Dusseldorf, Dusseldorf, Germany; Institut Curie, Paris, France; Ludwig-Maximilians-University of Munich, Munich, Germany; The Royal Marsden NHS Foundation Trust and The Institute of Cancer Research, Sutton, United Kingdom.

In the field of Circulating Tumor Cell (CTC) research many new technologies are emerging to isolate CTCs. Some of them provide accompanied automatic image analysis tools that present possible CTCs to the user. Others need fully manual image analysis. For all CTC isolation technologies the definition of a CTC based on the immuno-morphologic criteria is either customized to the specific platform or subjective to the user causing high interreader differences - a problem which may condemn many CTC-based clinical studies to failure. Thus, an important issue that the field is confronted with is the lack of a unified and standardized definition to classify a cellular object as a CTC. This problem is addressed within the European FP7 consortium CTC Trap and the Innovative Medicines Initiative (IMI) consortium CANCER-ID by the development of an open-source image analysis toolbox for CTC identification and enumeration. This toolbox is baptized ACCEPT (Automated CTC Classification, Enumeration and Phenotyping) and can process images generated by various CTC isolation technologies. The main software components are the Marker Characterization, the Full Detection and the Automatic Classification. The Marker Characterization tool aims at quantifying the antigens expressed by previously selected CTCs. The Full Detection tool is based on advanced mathematical methods to reliably detect all objects in the images, visualize the objects in scatter plots and enable the user to classify the cell types by the use of gates or selection of specific objects in the scatter plots or on the actual images. The Automatic Classification tool first detects all objects in the images followed by an automated classification approach that – as a result – presents found CTCs to the user. We demonstrate the effectiveness of these tools on two different datasets. The Marker Characterization tool was tested for Her2 expression on archived CTC images isolated and classified by the CellSearch system from patients with metastatic breast cancer. Investigators from three different institutes were asked to score these cells for Her2 positivity first on the images generated by the CellTracks Analyst and afterwards using ACCEPT. We show that the improved CTC visualization provided in ACCEPT, combined with several measurements which we extract for each cell, can reduce the inter-user variability. The Full Detection and Automatic Classification tools of ACCEPT were tested on archived samples of patients with castration resistant prostate cancer processed with the CellSearch system as well as on microspheres obtained after filtration of the blood sampled by the CellTracks system. Results were compared with manually scored CTCs and showed the improvement of CTC classification by the availability of quantitative image analysis tools. The Open Source ACCEPT program will be available on the MCBP website (http://www.tnw.utwente.nl/mcbp).

**CLINICAL RESEARCH: Liquid Biopsies 1: Circulating Tumor Cells**
Patients with oligometastatic solid tumors. Purpose: To prospectively evaluate changes in circulating tumor cells (CTCs) following definitive treatment in patients with oligometastatic solid tumors using a novel nanotechnology-based biomimetic platform. Procedure: Patients with biopsy-proven oligometastatic disease with up to three lesions were eligible if all sites of disease were treated with definitive therapy. Definitive therapy could include fractionated 3D conformal radiation, SBRT, IORT, surgical metastatectomy, and ablative procedures. Patients initially presenting with oligometastatic disease were eligible if they had less than 3 lesions, including the primary tumor. At least one lesion had to be treated with radiation. CTCs were enumerated from whole blood using the Onco-Sense CTC capture system. The Onco-Sense is a rolling chip-based system for detection and long-term monitoring of hepatocellular carcinoma. The CTC-score generated from these data tracks with clinical intervention and is higher than 50% of malignant patients with oligometastatic disease using a novel CTC capture system. CTCs decreased with treatment and all clinical failures were preceded by significant rises in CTCs. Our preliminary data suggest that enumeration of CTCs by Onco-Sense may provide a novel biomarker for assessing treatment response and/or post-treatment surveillance for patients with oligometastatic solid tumors.

#1736 A novel RNA-based assay for the detection and monitoring of circulating tumor cell signatures in breast cancer. The technical feasibility of utilizing RNA sequencing for identification of novel CTC signatures in interest was also demonstrated with a liver cancer cell line spike-in study. Results: 9 of the 16 untreated HCC patients were successfully detected, while only 1/31 chronic liver disease patients were incorrectly classified. HCC patients undergoing treatment showed a significant decrease in their CTC-score; only 9/32 patients actively receiving treatment were positive. The CTC-score was not correlated with the HCC serum biomarker alpha-fetoprotein, and combining these two orthogonal measures led to an estimate positive and negative predictive values of 80% and 86%, respectively, in a high-risk cohort. RNAseq analysis of cell line spike-in data revealed the potential of RNA sequencing for uncovering novel transcripts of interest. Conclusion: Coupling microfluidic depletion with droplet digital PCR allows for the highly specific detection of hepatocellular carcinoma. The CTC-score generated from these data tracks with clinical intervention and is orthogonal to the existing biomarker AFP; combining these two assays has the potential to provide superior detection compared to either individual approach.

#1737 ALK rearrangement analysis in circulating tumor cells of lung cancer patients. The evaluation of ALK rearrangement in non-small-cell lung cancer (NSCLC) is a significant tool when considering chemotherapy. It is not always possible to perform a tumor biopsy in patients. We suggest isolation and culturing of circulating tumor cells (CTCs) as an alternative tool to a tumor biopsy for the diagnosis of ALK rearrangement. From 22 patients with NSCLC harboring ALK rearrangement, blood samples were collected and divided into two parts: one for immunofluorescence staining of CTC marker.
and the other for culturing of CTCs. Both samples were processed by size-based filtration, and Cultured CTCs were analyzed for EML4-ALK translocation by fluorescence in situ hybridization (FISH) using Vysis ALK break apart FISH probe kit. CTC culturing was successful in 18 of 22 cases (81.8%). Among 18 cases of successful CTC cultures, 13 cases showed ALK rearrangement positive samples mCrPC. Therefore, we suggest that the CTCs can be used as an alternative method to tissue biopsy for diagnosing ALK rearrangement. In addition, this method may have clinical applications including serial blood sampling for the development of personalized cancer therapy based on individual genetic information.

## CLINICAL RESEARCH: Liquid Biopsies 1: Circulating Tumor Cells

### #1738 Platelet activation and heterotypic platelet leukocyte conjugate formation in the blood of glioblastoma patients

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Objective: Glioblastoma patients (GBM) suffer from an increased incidence of cardiovascular events. Platelets are well known as a major player of the primary hemostasis, but have a broad range of additional functions. The formation of heterotypic conjugates between platelets and leukocytes (PLC) represents a pro-inflammatory surrogate marker and is usually increased after platelet activation. The aim of the present study was to evaluate the platelet activation status and the rate of circulating PLC in GBM. Methods: Blood samples were drawn of consecutive patients before surgery for a suspected glioblastoma. The formation of PLC and several parameters of platelet activation were determined by flow cytometry before and after stimulation with either ADP or the thrombin receptor-activating peptide (TRAP) in vitro: expression of P-Selectin, CD63, CD40L and fibrinogen-binding to the activated GPIIb/IIIa. Blood samples from age and gender matched healthy volunteers were used as controls. Statistical analysis was done by the Mann-Whitney-Test. Results: Final analysis included 22 patients with histopathologically proven primary glioblastoma (9f, 13m, mean age 67.5 years, range from 53 to 86 years) and their respective controls. Basal platelet activation and in vitro platelet reactivity was increased in GBM. The difference got significant in the basal expression of CD63 (2.8% versus 1.9%, p=0.008), the Fibrinogen-binding after ADP-stimulation (110.3 MFI versus 63.1 MFI, p=0.04) and the CD63 expression after TRAP-stimulation (38.4% versus 33.3%, p=0.04). Furthermore, a reduced number of circulating PLC and in vitro PLC formation was seen in GBM without getting statistically significant. Conclusions: In this preliminary report, we show for the first time an increased level of platelet activation and agonist-induced platelet reactivity in GBM. Both could be a reflection of the pro-thrombotic status in these patients. Interestingly, the formation of PLC was not increased, but in tendency decreased. Whether this observation potentially mirrors the intratumoral, anti-inflammatory microenvironment in GBM remains unclear.

### #1739 Analysis of EML4-ALK fusion transcripts in plasma and platelets to monitor response to crizotinib in EML4-ALK positive small cell lung cancer patients (NSCLC)

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Background: Rearrangements in anaplastic lymphoma kinase (ALK) gene can be detected in 5-7% of EGFR and KRAS wild-type advanced NSCLC patients (p). Fluorescent in situ hybridization (FISH) and immunohistochemistry (IHC) are currently used for screening but are unable to identify the specific fusion partner and are impractical to monitor clinical responses due to difficulty of obtaining rebiopsies. The RT-PCR technique has the potential to overcome this pitfall and allow patient monitoring in blood. Methods: A total of 405 formalin-fixed paraffin-embedded (FFPE) samples from advanced NSCLC were analyzed by ALK IHC (Ventana D5F3) and FISH (Vysis). Positive patients were confirmed by RT-PCR and submitted to Sanger in order to identify the variant. In a subset of 36 patients with EML4-ALK-rearranged tumors who were treated with crizotinib, fusion transcripts were analyzed by RT-PCR in mRNA purified from plasma and platelets and correlated with clinical response. Results: ALK IHC was analyzed in 405 NSCLC patients and 37 tested positive (9.1%) whereas 25 (7.7%) were identified as translocated by FISH (n=323). ALK fusion transcripts were analyzed by RT-PCR and a new fusion variant of ALK was identified. A total of 36 p benefitted from crizotinib treatment, including the p with the new variant. Monitoring of EML4-ALK fusion transcripts in the plasma ad platelets of 35 ALK positive patients revealed a good correlation with clinical outcome to crizotinib treatment, with the fusion transcripts becoming undetectable in p with good clinical responses. Conclusions: Analysis of ALK fusion transcripts in mRNA purified from plasma and platelets can have a value in patients with no biopsy available and to monitor the course of the disease.

### #1740 Phenotypic, genomic, and clinical associations of Circulating Tumor Cells (CTCs) lacking epithelial biomarkers in metastatic Castration Resistant Prostate Cancer (mCRPC)

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Background: Epithelial Plasticity (EP) is a proposed mechanism of immune escape, resistance to programmed cell death, resistance to specific drugs, and promotion of metastasis. EP has been extensively explored in mathematical, cell biological, and animal models, which predict the presence of these tumor cells in circulation. Most studies on CTCs in human subjects are based on enrichment of cells expressing EpCAM prior to enumeration or molecular interrogation, which precludes analysis of cells that might be EP. The non-enrichment Epic Sciences platform was utilized to identify CTCs in metastatic castration-resistant prostate cancer patient samples phenotypically consistent with EP. CTCs that do not express cytokeratins (CK), but do express malignant or EP biomarkers: Androgen Receptor (AR), AR splice variant 7 isoform (AR-V7) or N-cadherin. Phenotypic and genotypic analyses of individual CTCs were performed to assess the malignant origin of CTCs and their prevalence associated with metastatic prostate cancer and are a negative prognostic factor associated with worse overall survival in both iterative threshold and multivariable analyses.

### CLINICAL RESEARCH: Molecular Classification of Tumors

#### #1741 Whole-transcriptome characterisation of NRAS and BRAF mutated primary melanomas associated with immune cell infiltration signatures and differential survival benefit


In 703 primary melanoma transcriptomes derived from formalin-fixed tumors removed from participants in the Leeds Melanoma Cohort, we used in silico methodology described by Bindea et al.1 to develop immune cell infiltration signatures. These showed to be associated with survival2. There is some reported evidence that patients with NRAS mutated melanoma have a better response to checkpoint therapies3. Our aim was, therefore, to use the transcriptomic data to ask if immune cell infiltration (evidenced by the presence of the immune cell signature) differed between mutation subgroups. We saw that the survival benefit of the immune cell signature was strongest in wild-type (HR=0.31, P=3x10^-5), intermediate in BRAF mutated tumors (HR=0.56, P=0.008) but absent in NRAS mutated tumors (HR=1.09, P=0.79). That is that even if the
N Ras mutated tumors had a strong T cell signature that did not correlate with better survival. The results remained significant after adjusting for sex, tumor site, age at diagnosis, and AJCC stage. We observed no evidence of increased numbers of regulatory T cells (FOXP3), in N Ras mutated tumors, nor PD1L or PD1 expression. The only difference in checkpoint molecule expression studied was a borderline lower expression of CTLA4 (AK3, N Ras) and tumors (P=0.09). Our data suggest that even when T cells invade N Ras mutated tumors some functional deficiency may be present in those tumors such that a survival benefit is not seen. Using genes significantly differentially expressed between N Ras and BRAF mutated tumor groups, we tested biological pathway enrichment using Reactome FIViz plugin in Cytoscape. The most enriched pathways in N Ras mutated melanomas were the PI3K (AK3, N Ras) and mitochondrial translation (CHCHD1, PTCD3). In the BRAF mutated the most enriched were: direct P53 effectors (TGF, IGFBP3), B-catenin independent WNT signaling (WNT5A) and MHC class I antigen processing (CYBA). A number of pathways related to cell adhesion involving GRIN1 were also identified. Others have reported that B-catenin signaling inhibits transcription of the chemokine CCL4 which reduces the trafficking of CD141 DC to melanomas and therefore T cell infiltration.1 We have evidence from the B-catenin candidate transcriptomic analysis that primary melanomas without immune infiltration signatures have upregulated B-catenin but the comparison between transcriptomic patterns in N Ras and BRAF tumors did not suggest that B-catenin signaling was more active in N Ras mutated tumors. It may be that a different immune evasion mechanism is more common in N Ras mutated tumors than in others. References 1. Bindea et al. Immunity 39, 782-795 (2013) 2. New-Bishop et al. PCMR in press 3. Johnson et al. Cancer Immunol Res. 3, 288-295 (2015) 4. Springer et al. Nature 525, 231-235 (2015).

#1742 Cancer cell-selective transcriptome analysis reveals new colorectal cancer molecular subtypes with improved biological resolution and superior predictive and prognostic performance. Claudio Isella,1 Sara E. Bellomo,1 Francesco Brundu,2 Francesco Galimi,3 Elisa Facchin,1 Luigi Marchionni,1 Livio Trusolino,1 Andrea Bertotti,1 Enzo Medico,1 University of Turin, Candiolo Cancer Institute, Candiolo, Italy; 2Politecnico di Torino, Torino, Italy; 3Candiolo Cancer Institute, Candiolo, Italy; 4Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD.

Molecular classification of colorectal cancer (CRC) based on gene expression profiling of tumor samples is known to be heavily affected by transcript of stromal origin. As a consequence, current CRC transcriptional subtypes reflect an admixture of cancer cell intrinsic traits and tumor microenvironment features. Whether selective analysis of the cancer cell transcriptome could improve CRC subtyping, remains an open issue. In patient-derived xenografts (PDxs), human transcripts only originate from cancer cells, because stromal transcript is of mouse origin. We therefore assessed cancer-cell intrinsic transcriptional features of CRC by generating human-specific expression profiles of 515 PDxs from 244 CRC patients, and performing unsupervised class discovery. We identified five “CRC intrinsic subtypes” (CRIS A-E) only partially overlapping with the current ones, and robustly enriched for distinct molecular, functional and phenotypic traits: (i) CRIS-A: mucinous, glycolytic, CIMP, and enriched for microsatellite instability or mutations in KRAS; (ii) CRIS-B: marked TGF-β pathway activity, epithelial-mesenchymal transition and poor prognosis; (iii) CRIS-C: MSS, elevated EGFR signaling and sensitivity to EGFR-targeted treatments; (iv) CRIS-D: MSS, WNT activation, and IGF2 overexpression and amplification; (v) CRIS-E: MSS, Paneth cell-like phenotype and higher frequency of TP53 mutation. CRIS subtypes successfully categorized independent sets of primary and metastatic CRCs and cell lines, for a total of over 3000 samples profiled by microarrays and RNAseq. The new subtypes displayed unprecedented predictive and prognostic performances, independent from known markers including stromal signatures, whose integration with CRIS further enhanced prognostic significance.

#1743 Comprehensive genomic characterization of a large cohort of platinum-sensitive high-grade serous ovarian cancer (HGSOC) FFPE specimens. Brian C. Haynes,2 Marie E. Fahey,1 Darcy Myers,1 Diane Isley,1 Gary J. Latham,1 Elizabeth B. Somers,2 Nicholas C. Nicolaides,2 Charles Schweizer,2 Daniel J. O’Shannessy3,4.1 Asuragen, Inc., Austin, TX; 2MorphoTec, Inc., Exton, PA.

Introduction: Ovarian cancer is a leading cause of cancer related death in women. A comprehensive genomic characterization of platinum-sensitive tumors is required to further refine the definition of molecular subtypes and identify targeted therapies for this patient population. To this end we have performed a large-scale genomic and transcriptomic characterization of 348 primary FFPE tissues from a cohort of platinum-sensitive HGSOC patients collected from multiple clinical sites. Methods: Macrodisssection of FFPE resected tumor slides or sectioned blocks was performed to enrich for tumor content. RNA and DNA were each isolated from 2X5μm sections of FFPE material. RNA expression and gene fusions were profiled by whole transcriptome RNA-Seq. DNA variants were analyzed by the AmpliSeqTM Cancer Hotspot Panel (Thermo Fisher). A subset of tumors were profiled by whole genome sequencing of the NGS panel. Results: The spectrum of DNA mutations and CNVs was consistent with other HGSOC cohorts with mutations in TP53 present in the majority of specimens (87% for specimens with full exon coverage of TP53). Germline mutations in BRCA1 and BRCA2 were identified at 10.3% and 6.8% respectively. Analysis of recurrent whole chromosomal arm gain and loss displayed a striking agreement with the TCGA HGSOC cohort. Unsupervised analysis of the RNA Seq expression data through non-negative matrix factorization revealed 4 distinct transcriptional subtypes, corresponding to the 4 established CLOVAR subtypes: differentiated, immunoreactive, mesenchymal and proliferative. Patients classified as mesenchymal had the poorest prognosis. Further integrative analysis identified additional associations between the CLOVAR subtypes and other molecular indications. Concerning the clinical utility of these subtypes we reported that each subtype is enriched for specific germline or somatic mutations. In the future, these transcriptional subtypes of platinum-sensitive HGSOC and highlighted alterations that may lead to improved diagnostic and precision medicine strategies. In contrast to previous large-scale molecular characterization efforts such as TCGA where fresh-frozen tissue were collected under highly controlled settings, this study is based on molecular profiling of FFPE tissues collected at dozens of clinical sites. Thus, our study serves as a model for future molecular characterization efforts of FFPE specimens collected in real-world clinical settings.

#1744 RNAseq identified immune signatures associated with adverse outcome in high-risk neuroblastoma. Jun S. Wei,1 Shile Zhang,2 Igor Kuznetsov,2 Young K. Song,1 Shahab Agharzadeh,1 Shivash Sirdhi,1 Xinyu Wen,1 Rajesh Patidar,1 Jaime M. Guidry Auvil,1 Daniela S. Gerhard,1 Robert Seeger,3 John M. Maris,1 Javed Khan1.1National Cancer Institute, Bethesda, MD; 2University at Albany, Bethesda, NY; 3The Children’s Hospital Los Angeles, Los Angeles, CA; 4The Children’s Hospital of Philadelphia, Philadelphia, PA.

Despite the success of multimodal therapies, the mortality and morbidity remains substantial for patients with high-risk neuroblastoma (NBL). Sequencing of paired tumor-normal DNA samples of NBL patients identified somatic mutation burden and few recurrent somatically-mutated genes. Here we hypothesize that whole transcriptomic sequencing (WTS) in patients with high-risk NBL tumor will yield valuable insights into the biology of this disease. We performed deep WTS of 150 NBLs (129 high-risk stage 3 and 4; 21 stage 4S tumors) of which majority of them had whole genome sequencing or whole exome sequencing of case-matched tumor/normal pairs through the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) initiative. We identified correlations between gene expression and clinical parameters of patients such as MYCN-amplification and survival to provide understandings of high-risk NBL biology. Consensus clustering using gene expression profiles of tumors revealed 4 subgroups with distinct survival probability (p<0.01). Among the tumors with the best outcome consisted of all stage 4S and 4S-like young patients; whereas the worst survival group was made up mostly of high-risk patients with MYCN-amplification. In order to identify the gene expression patterns correlating with outcome in the high-risk patients without MYCN-amplification, we further examined if any other known gene expression signatures can further stratify these patients using Gene Set Enrichment Analysis (GSEA). Among several molecular signatures, a previously reported 157-gene MYCN activation signature was significantly correlated with the poor outcome in high-risk patients without MYCN-amplification. Of note, we identified CD9, cytolytic, and activated nature killer (NK) cell signatures that were significantly associated with outcome in these patients. These findings were validated in independent data set and highlight the potential clinical application for a subset of high-risk neuroblastoma patients without MYCN-amplification that have an activated MYCN signature may benefit from immune-modulating agents such as immune checkpoint inhibitors which should be tested in context of clinical trials.
#1745 Multifocal prostate cancer has high degree of genomic heterogeneity. Marthe Leuf,1 Sen Zhao,1 Ulrika Axcrona,2 Bjanne Johannesen,1 Andreas M. Hoff,1 Anne Cathrine Bakken,1 Kristina Toltrand Carn,1 Ola Myklebost,1 Leonard A. Meza-Zepeda,1 Agnes K. Lie,1 Karol Axcrona,2 Ragnhild A. Lothe,1 Rolf I. Skotheim1. 1Oslo University Hospital, Institute for Cancer Research, Oslo, Norway; 2Oslo University College, Department of Pathology,Fredrikstad,Norway; 3Akershus University Hospital, Department of Urology, Lørenskog, Norway.

Prostate cancer is the most common cancer type among men in the Western world. Most prostate cancers are multifocal with individual tumors harboring different aggressiveness. In recent genomics studies, the multifocal nature of prostate cancer has been investigated only in small sample cohorts. Here we have investigated the intra-organ mutational spectra of multiple tumors from a large cohort of prostate cancer patients. From radical prostatectomy specimens from 2010 and 2012 at Oslo University Hospital, 43 prostatectomy specimens with multiple and clearly separated tumors were identified based on histology. From each of these prostatectomy specimens, DNA from frozen samples from 2-3 different tumor foci and corresponding normal tissue samples were analyzed by high-coverage whole-exome sequencing, adding up to a total of 159 samples. We identified 3093 somatic substitutions, insertions and deletions, with an average of 27 alterations per tumor sample. Both known and novel significantly mutated genes were identified using Oncosift and their distribution in different tumor foci from the same prostate was examined. Mutations in genes such as SPOT, MED12, and FOXA1 have previously been identified in prostate cancer and were also found to be frequently mutated in this cohort. However, the same mutations were rarely found in multiple tumor foci within the same prostate. In fact, for 13 out of the 43 examined patients there were no common mutations among tumors from the same prostate. For 12 patients, we found only one overlapping mutation among tumor foci. Whereas the overall list of mutated genes (n = 2101) overlap significantly with the 594 genes of the Cancer Gene Census (100/594; p = 9.6e-06), this was not the case for 142 genes with overlapping mutations among different tumor foci (2/594; p = 0.63). This indicates that the overlapping mutations are not typical cancer driving genes. To conclude, results from this sequencing of multifocal prostate cancer show a large degree of heterogeneity in genomic alterations between different tumor foci within the same prostate. With very few common inter-foci mutations, an implementation of genome-based personalized prostate cancer medicine will require sampling of all tumor foci to tailor optimal treatment.

#1746 Rational molecular assessment and innovative drug selection (RAIDs): Paving the way to personalized medicine in cervical cancer. Leanne de Koning,1 Bérengère Ouine,1 Aurélie Cartier,1 Els M. Berns,2 Kirsten Ruigrok-CLINICAL RESEARCH: Molecular Classification of Tumors Leyen,5 Marina Popovic,6 Windy Luscap-Rondot,7 Vonick Sibut,7 Choumouss Lapping mutations among different tumor foci (2/594; p = 9.6e-06), this was not the case for 142 genes with overlapping mutations among different tumor foci (2/594; p = 0.63). This indicates that the overlapping mutations are not typical cancer driving genes. To conclude, results from this sequencing of multifocal prostate cancer show a large degree of heterogeneity in genomic alterations between different tumor foci within the same prostate. With very few common inter-foci mutations, an implementation of genome-based personalized prostate cancer medicine will require sampling of all tumor foci to tailor optimal treatment.

Cervical cancer (CC) is the fourth most common cause of cancer deaths in women worldwide, for which prognostic and predictive biomarkers are largely lacking. RAIDs is a EU-funded project on cervical cancer that spans seven European countries. The main objective of the RAIDs project is to use this tumor type, which is easily accessible for repeated biopsies, to learn how to stratify patients into targeted therapies. The project includes: 1) a cognitive cohort study (BioRAIDs), one of the first prospective trials intended to define patient stratification for targeted therapies, 2) a targeted clinical trial using an HPV directed vaccine and 3) preclinical studies aiming at assessing new treatment strategies. Molecular analysis on quality controlled tumor and sera samples from 400 patients include Next Generation Sequencing at SeqGomics (Hungary), PIK3CA mutations detection in circulating tumor DNA at Erasmus MC (Netherlands), Reverse Phase Protein array and HPV insertion sites analyses at Institut Curie (France) and immune-microenvironment analyses at CGOA (Netherlands). In addition, 20 CC cell lines have been profiled pharmacologically using a panel of drugs which potentially synergize with “standard treatment”. The present poster will mainly focus on the Reverse Phase Protein Array (RPPA) results of Bio-RAIDs and how these relate to the genomic profiling and to patient outcome. More than 150 cryopreserved baseline (before treatment) samples and 23 CC cell lines have been screened by RPPA. From these, whole exome sequencing is available for 92 patient samples. Stratification of patients based on proteomics and genomics has evidenced different subgroups (clusters) of patients displaying specific molecular characteristics. Genomics data and proteomics data both demonstrate that these clusters differ notably in the pathways of oxidative phosphorilation, Department of Pathology, Fredrikstad, Norway; Akerhuse University Hospital, Department of Urology, Lørenskog, Norway. The correlation of proteomics and genomics data with these clinical data is ongoing and will identify putative predictive biomarkers. Correlation of protein data with response to drugs treatment in 20 CC cell lines has identified several potential biomarkers, some of which again relate to (glucose) metabolism. In conclusion, we here present new evidence for molecular subgroups of cervical cancer that could benefit from different treatment options. Notably, our data suggest that the metabolism/glycolysis pathways are a major effecter in CC and constitute a potential therapeutic target in a subset of patients. This project has received funding from the European Union’s Seventh Program for research, technological development and demonstration under grant agreement No 304610.


Gene expression studies, mainly addressed at the tumor cell compartment of breast cancers (BC) contributed to unravel the molecular heterogeneity providing clinicians with new and reliable tools for therapeutic planning. Despite many investigations have focused on genetic abnormalities initiating and driving tumorigenesis, there is now evidence that the cancer cell behavior depends not only on intrinsic features, but also on the interactions with the microenvironment. However, notwithstanding the interest in investigating tumor-stroma cross-talks, the clinical relevance of subtype-specific molecular imprint of such interactions is largely unexplored. As such, we attempted to clinically and biologically validate the subtype-specific gene signatures derived from an in vitro model recapitulating the interaction between BCs and activated stromal cells. Gene expression signatures derived from luminal (T47D, MCF7, ZR75.1), HER2+ (SKBr3+, BT474, MDA-MB-361) and basal-like (MDA-MB-468, MDA-MB-231, BT20) cell lines treated by conditioned media from normal or cancer-associated fibroblasts (CAF) showed common- and subtype-specific pathways according to GSEA. In fact, interferon, IL-6, IL-12 and IL-23 signaling, Toll Like Receptor pathway, pathways related to inflammation and metabolism of carbohydrates were positively enriched in HER2+ and luminal subtypes. Complement cascade and Tumor Necrosis Factor pathways were positively enriched in basal and luminal tumors; no common pathway was observed in HER2+ and basal subtypes. The association between microenvironment signatures specific for luminal, HER2+ and basal BC cell lines and disease-free survival was evaluated in silico on published gene expression profiles (GEPs) from 2048 patients homogeneously treated. BCs were classified as microenvironment-positive (μENV+ve, with GEP suggesting stroma activation), or negative (μENV−ve) by correlating tumor’s GEP with the respective subtype-specific signature. Patients with luminal μENV−ve status had 2.5-fold higher risk of developing metastatic diseases (HR = 2.546; 95% Confidence Interval, CI: 1.751-3.701, P = 9.84E-07), while μENV status did not affect or was only suggestive of metastases in women with HER2-enriched (HR=1.541; 95% CI: 0.788-3.012, P=0.206) or basal tumors (HR = 1.894; 95% CI: 0.938-3.824, P=0.0477), respectively. In luminal tumors, the μENV status maintained its prognostic role (HR =2.098; CI: 1.214-3.624; P=0.00791) in multivariable analysis including size, age and genomic grade index. Validity of our in vitro model was also supported by biological endpoints such as proliferation (MTT-assay) and migration/invasion (Transwell assay). In vitro-derived gene signatures trace a subtype-specific interaction with CAFs and add independent information to classical prognostic variables in women with luminal “microenvironment sensitive” tumors.

#1748 Organ-specific epigenetic reprogramming of leukemia cells: Clues to chemoresistance. Isabel Cunningham,1 Diane Hamele-Bena,1 Audrey Papp,2 Yan Guo,3 Antony B. Holmes,1 Yu Shyr,4 Rory A. Fisher4. 1Columbia University College of Physicians and Surgeons, New York, NY; 2Ohio State University, College of Medicine, Columbus, OH; 3Vanderbilt University, Nashville, TN; 4University of Iowa Carver College of Medicine, Iowa City, IA.

We previously reported our observation that tumors composed of leukemic cells found in breast mimick solid breast cancers in gross and histologic appearance (single-filing of cells and distinctive keloid-like fibrosis), invasiveness, metastatic pathway, and high mortality. They are resistant to anti-

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leukemia drugs; only 4% treated without exception lived 4 years but survivals over 20 yrs resulted when tumor and its microenvironment were excised. The incidence of occult leukemic tumors, still unknown as scans are not yet routine, was estimated near 20% in 1970s autopsy studies. We hypothesized there may be similarly altered genes in leukemic and epithelial breast tumors directing clinical behavior and resistance that could provide targets for curative treatment. We performed RNA sequencing on 11 FFPE breast tumors from annotated AML cases, all IHC-negative for hormones and E-cadherin, compared to normal breast samples. Differential expression analysis identified 3647 genes that were significantly downregulated (adjusted p < 0.05 and log2 fold change < -2). Among the 100 genes downregulated 8-10 fold in leukemic breast, FOXA1, CA12, CXCL14, SFRP1, SFRP4, DSN1, LUM, PHLD1A, NTN4, GABBR, TAGLN, AZGP1, and ARRD31 were associated with poor prognosis in breast cancer, mainly triple-negative (TNBC) basal types. Compared to TNBC cases in the TCGA database, 851 genes were similarly deregulated; significantly enriched KEGG pathways include neutrophilic receptor interaction, focal adhesion, ECM-receptor interaction, and regulation of lipolysis in adipocytes. A role for altered stroma is suggested as many of the similarly downregulated genes were reported in dissected breast cancer stroma and silenced SFRP1 and CXCL14 were found in keloid fibroblasts. Silencing of SFRP1 and E-cadherin in AML marrows was reported in 30-50% and each was correlated with poor survival. Reversal of SFRP1 silencing has been achieved by epigenetic modulating drugs in cancers and leukemic cases where it has been used to reduce canopy of AML cells by RT-PCR. Loss of RGS6 in breast and other cancers promotes doxorubicin resistance and DMT1 upregulation leading to silencing of tumor suppressor genes. These data open a new view of resistance of AML cells to marrow-directed drugs through interaction with aberrant microenvironment. Solid tumors of epithelial and leukemic cells may share unrecognized treatable targets. Adding drugs directed at tumor, including available epigenetic-modifying agents, to marrow-effect protocols, may finally decrease the persistently high rate of AML treatment failure.

**#1749 Stability of copy-number profiles defines two molecular subtypes during urothelial carcinoma’s evolution.** Bishoy M. Faltas,1 Davide Prandi,1 Scott T. Tagawa,1,2 David Nanus,1 Ana M. Molina,1 Himisha Beltran,1 Francesca Demicheli,1,2 Mark A. Rubin1.1 Well Cornell Medical College, New York, NY; 2University of Trento, Italy.

Background: Somatic copy-number alterations (SCNAs) are distinct molecular events occurring in muscle-invasive urothelial carcinoma (UC). Defining the biologic role and the timing of SCNAs in UC’s evolution is critical for understanding disease biology and for precision therapy. Methods: To examine whether distinct SCNAs define specific subtypes of UC and characterize the evolutionary dynamics of SCNA acquisition, we conducted whole-exome sequencing (WES) of a discovery cohort of 44 advanced UC tumors and matching germline samples, including 13 pairs of matched primary and metastatic tumors prospectively collected before and after chemotherapy. We validated SCNAs in 29 breast cancers and normal breast tissues by RT-PCR. We queried WES data from 285 UCs from The Cancer Genome Atlas (TCGA) as a validation cohort. We performed a novel in-depth allelic-specific copy-number analysis in 2503 cancer genes using the CLONET computational framework, which accounts for tumor ploidy and cellularity to infer the clonal hierarchy of SCNAs. Results: Analysis of SCNAs from tumor pairs within each patient revealed punctuated evolution of SCNAs occurring early in UC’s development followed by minor changes (clonal stasis) throughout the tumor’s lifetime. Two distinct clusters of UCs with strikingly different SCNAs profiles in the discovery cohort emerged. Cluster A was defined by diploid copy number background and focal deletions in the 9p21 region (CDKN2A, CDKN2B and MTAP). Cluster B was defined by genome-wide duplications and several enriched amplifications including 1q11.1 (SETDB1 and MLLT11), (P = 0.0002) and 6p22.3 (E2F3), (P = 0.001). TP53 mutations (P = 0.0001) were enriched in the cluster B, consistent with the role of functional p53 as a “guardian of the genome” preventing chromosomal instability. SCNAs clusters A and B were confirmed in the TCGA validation cohort. Cluster A was enriched with tumors belonging to TCGA bladder cancer cluster III (“basal/squamous-like”) (P = 0.02). Conclusions: Using novel allelic-specific SCNAs analysis, we define two distinct clusters of UC presenting a framework for understanding this aspect of UC’s biology. In contrast to somatic mutations, SCNAs are clonally static during each tumor’s evolution. This data has important implications for precision medicine for UC.

**#1750 Analysis of non-metastatic HCC patient tumors revealed the significance of cell cycle regulation and tumor immunity in association with overall survival and identified clinically relevant druggable targets.** Pei-Zhen Miao,1 Jeff Cheng,2 Kwame Okrah,2 Bonnie Liu,1 Charlie Sun,1 Grace Cao,2 Tony Guo,2 Shijing Fu,2 Marie-Claire Wagle,3 Elizabeth Blackwood,4 David Shankman,5 Astrid Langley,2 Jelena Fedorova Fridlyand,1 Mark Lackner,2 Shih-Min Huang,3 Jian Zhou,1 Zhi Dai1.1 Liver Cancer Institute, Zhongshan Hospital, Fudan University, Shanghai, China; 2Genentech, Shanghai, China; 3Genentech, South San Francisco, CA; 4Genentech, Switzerland.

The lack of efficacious therapies has hindered the progress in improving the survival of HCC patients worldwide. As a disease that associates with the under- standing of genomics and immunology, HCC diagnostic driver and biological features during most of its decades long progression, HCC presents challenges to accurately depict its biological landscape and determine suitable therapeutic intervention. An approach to tackle this issue is to define significant biological variants that impact patient survival in each disease stage, then attempt to chart paths of potential therapies. To identify significant biological determinants associated with the overall survival (OS) of early stage, non-metastatic HCC patients, we constructed focused Nanostring panels that are represented by 1,164 genes of validated components and transcriptional outputs of multiple signaling pathways, cellular machineries, and tumor immunity. An unbiased analysis of patient survival and gene transcript levels revealed four distinct groups of genes that are significantly prognostic. The first two (Group 1 & 2) are associated with mechanism of adaptive immune response, implicating the survival benefit of acquiring higher immunogenic tumor environment in early stage HCC. Searching for druggable targets associated with poorer prognosis, we identified both EZH2 and CHEK1 as candidates from Group 2. In vitro treatment of three HCC cell lines with EPZ-6438 (EZH2 inhibitor) or GDC-0425 (CHEK1 inhibitor) resulted in significant modulation of H3K27me3 or pCHEK1 levels (s345) respectively. The growth of these cell lines was also dose-dependently suppressed by each inhibitor in colony formation assays. Furthermore, the combination of both inhibitors was able to achieve substantial cell growth inhibition at lower concentrations, suggesting a combinatorial effect of targeting both EZH2 and CHEK1 in HCC cell lines. Xenograft studies are ongoing to assess in vivo efficacy of this combination. In conclusion, utilizing well constructed Nanostring gene panels, we identified significant association of cell cycle machinery and adaptive tumor immunity with HCC patient survival. Our findings not only provided potential rationale for expanding testing of cancer immunotherapies to non-metastatic, early stage HCC, but also revealed the potential utility of combining EZH2 and CHEK1 inhibitors to treat HCC.

**#1751 Systematic bias in genomic breast cancer classification due to selecting cases with high tumor percentage and good RNA quality.** Esther H. Lips, Antien L. MooiAart, Maartje van Seijen, Lennart Mulder, Mariou Hoogstraat, Petra M. Nederlof, Lodewyk F. Wessels, Sjoerd Rodenhuis, Gabe S. Sonke, Jelle Wesseling. Netherlands Cancer Institute, Amsterdam, Netherlands.

Background Cancer classification, prognostication, and prediction of treatment sensitivity increasingly rely on DNA and RNA-based tests. This approach requires sufficiently high tumor cell percentages to yield enough DNA or RNA for reliable test results. As a consequence, samples of insufficient quality may drop out, e.g. due to poor cellularity or high numbers of tumor infiltrating lymphocytes. We hypothesized that requiring a high tumor cell percentage and high quality RNA causes systemic bias when interpreting genomic test results in breast cancer, as specific breast cancer subgroups may be over- or underrepresented. Patients and methods For this analysis, we used pre-treatment frozen samples from patients included in neoadjuvant chemotherapy trials at the Netherlands Cancer Institute between 2004 and 2012. Histological features and tumor cell percentage were reviewed and assessed by a consultant breast pathologist. Gene expression profiling was done if the tumor cell percentage exceeded 50% and RNA quantity (RIN value <= 6.5) were sufficient. We compared patient and tumor characteristics between patients in whom gene expression profiling could be performed and those in whom it could not be performed. In addition, we performed a systematic review on gene expression profiling for breast cancer, to assess the percentage of sample drop-out in published studies. Results Frozen biopsies were available from 658 patients (79% of the total study population) and gene expression profiling could be performed in 60% of the cases, a percentage comparable to what is reported in the literature. Reasons for drop out were a low cellularity, poor quality and quantity of
the RNA, or a too small biopsy for processing. These patients had more grade 3 tumors (43% versus 34%, p = 0.04) and were more node positive (76% versus 69%, p = 0.03). Analysis of neoadjuvant chemotherapy response and survival in these patients is ongoing and will be presented at the AACR Annual Meeting 2017. Conclusion Breast cancers for which gene expression data were successfully obtained were associated with grade 3 and node positive tumors. Infrequently observed tumors may represent a more aggressive phenotype and have a relatively poor prognosis, compared to the patients for whom gene expression data could not be measured. As gene expression arrays are now broadly used in a clinical context, it is important to acknowledge this systematic bias, and to be cautious in applying gene expression based tests on different patient populations than a test was developed on.

#1752 Immune-related gene signatures in colorectal liver metastases: Exposing an opportunity for immune modulating therapy. Vágis Nygaard,1 Vega J. Dagenborg,2 Olga Østrup,2 Einar A. Redland,2 Veronica Skarpeteig,3 Laxmi Silwal-Pandit,4 Krzysztof Gryży,4 Audun E. Berstad,5 Åsmund A. Frelund,5 Gunhild M. Mælandsmo,5 Anne-Lise Børresen-Dale,5 Anne H. Ree,5 Bjørn Edvin,5 Kjersti Flatmark,5 #1. Dept. of Tumor Biology, Institute for Cancer Research, Oslo University Hospital, The Norwegian Radium Hospital, Oslo, Norway; 2Dept. of Cancer Genetics, Institute for Cancer Research, Oslo University Hospital, The Norwegian Radium Hospital, Oslo, Norway; 3Dept. of Pathology, Oslo University Hospital, Rikshospitalet, Oslo, Norway; 4Dept. of Radiology, Oslo University Hospital, Norwegian Radium Hospital, Oslo, Norway; 5The Intervention Centre, Oslo University Hospital, Rikshospitalet, Oslo, Norway; 6Dept. of Oncology, Akerhus University Hospital, Lørenskog, Norway.

Colon cancer cells obtained from biopsy samples of colorectal liver metastases (CLM) are used as a part of the most common cancers and one of the leading causes of cancer death in the Western world. Up to 50% of patients with CRC develop metastatic disease and the liver is the most common site. The recently identified consensus molecular subtypes (CMS1–4) based on analysis of primary CRC have prognostic and therapeutic implications, but it is unclear whether these molecular subtypes are valid for metastatic disease. In this study, characterisation of CRC liver metastases (CLM) was performed at multiple molecular levels to identify characteristics relevant to metastatic disease. Molecular stratification of a defined metastatic CRC cohort may yield results of clinical relevance and novel treatment opportunities. Surgically resected CLM and tumor-adjacent liver tissue from 46 patients were analysed for the presence of mutations (targeted deep sequencing), genome-wide copy number alterations (CNA), and transcription profile. Molecular profiles of CLM and tumor-adjacent liver tissues were analyzed and associations with clinicopathological features and outcome were investigated. We found oncogenic mutations in all except one tumor. Both mutation and CNA profiles were similar to profiles reported for primary CRC. A CMS classifier tool applied to gene expression data, revealed the cohort to be highly enriched for CMS2. Unsupervised clustering based on genes with highly variable expression identified a 55-gene cluster that segregated the samples into two subgroups. This segregation was replicated in relevant publicly available data sets, but the clinical significance of this observation is not evident. The 55 genes were associated with lipid metabolic and immune-related functions, revealing a role of the tumor-host microenvironment. The engagement of the immune system was further underlined by analyzing subgroups defined by neoadjuvant chemotherapy (NACT) administration, which revealed altered expression of inflammatory mediators and immune markers. The majority of treated patients had received Oxaliplatin based chemotherapy. The uniform classification of CLM by CMS may reflect the patient composition in our cohort, but may also indicate that novel class discovery approaches need to be explored to uncover clinically meaningful molecular stratification of CLM. Identified immune-related gene expression signatures associated with molecular and clinical features underline the integration and importance of the immune interacome in resectable CLM. Specifically, the transcriptomic snapshot of NACT exposed CLM revealed altered genes associated with immunogenec cell death but also immune suppression. These results point to rational exploration of immune-modulating strategies in CLM in combination with NACT to increase efficacy and broaden treatment opportunities for this patient group.

#1753 Refining breast cancer characterization through single-cell analysis of ex vivo reprogrammed tumor and adjacent normal cells. Harikrishna Nakshatri, Manujhshere Anjanappa, Angelo Cardoso, Lijun Chen, Safa Mohamad, Andrea Gunawan, Susan Rice, Yan Dong, Lang Li, Edward Srouf. Indiana Univ. School of Medicine, Indianapolis, IN.

Discovery of inter-individual functional variations in gene regulatory elements and the observation that tumor and normal tissues of the same organ are in different differentiation states necessitate rethinking of gene expression based subclassification characterization of tumors. To address this issue, we performed single cell genomics of breast tumors and adjacent-normal cells propagated using epithelial reprogramming growth conditions for a short duration. Epithelial cells analyzed were either unselected for specific subpopulation or phenotypically defined with differentially expressed ALDH / CD49f / EpCAM+ luminal progenitors present in the normal breast, which express both basal and luminal-enriched genes. Expression of 93 genes that included PAM50 intrinsic subtype classifier and stemness-related genes was analyzed in 420 tumor and 284 adjacent-normal cells. ALDH + / CD49f+ / EpCAM+ tumors and normal cells were enriched for marker genes paired with less infiltrated and normal cells. PAM50 gene sets analyses of ALDH + / CD49f+ / EpCAM+ populations efficiently identified major and minor clone of tumor cells with the major clone resembling clinical parameters of the tumor. Similarly, stemness-associated gene set identified clones with divergent stemness pathway activation within the same patient sample. This refined technique distinguished genes truly deregulated in cancer cells as compared to normal cells or different cellular precursor of tumors. Collectively, assays presented here may enable precise identification of genes deregulated in cancer, early identification of therapeutically-targetable minor population of tumor cells, and eventually to refinement of precision therapeutics.

#1754 A new glycan biomarker for pancreatic cancer complements CA19-9 and identifies a distinct subpopulation of cancer cells. Daniel Barnett,1 Ying Liu,1 Katie Partryka,1 Galen Hostetter,1 Herbert Zeh,1 Aatur D. Singh,1 Ying Huang,2 Richard R. Drake,3 Randall E. Brand,3 Brian B. Haab,4 Von Andel Institute, Grand Rapids, MI; 2University of Pittsburgh Medical Center, Pittsburgh, PA; 3Fred Hutchinson Cancer Research Center, Seattle, WA; 4Medical University of South Carolina, Charleston, SC.

A major challenge in diagnosing and treating pancreatic cancer is the complex diversity between and within tumors in cellular morphologies and behaviors. Molecular markers to detect and classify specific variants of cancer cells could facilitate drug targeting of subtypes of cancer cells. We recently found that a glycan related to the CA19-9 antigen is a strong serological biomarker and is elevated in the plasma of almost half the patients with low CA19-9. We hypothesized that the cancer cells producing CA19-9 are a different subtype than those producing the new biomarker, referred to as sTRA. Using multimarker immunofluorescence on tissue microarrays, we found that sTRA was significantly elevated (p < 0.001) in tumor tissue relative to adjacent pancreatic tissue in 3 separate TMA covering 38 patients. The STRA and CA19-9 markers were present in 31/38 (82%) and 20/38 (53%) of the tumors, respectively, with 17/38 (45%) displaying both and 3/38 (8%) displaying neither. The morphologies of the cancer cells fell into distinct categories based on glycan expression. Cells with dual staining of both markers tended to be in well-to-moderately differentiated ducts with good nuclear polarization and foamy cytoplasm, or with flat and thin epithelium. Cells with predominant or exclusive CA19-9 staining tended to be part of well-differentiated ducts with mucinous cytoplasm or moderately-differentiated ducts with poor membrane integrity. In contrast, strong sTRA staining was present in small clusters of cells or single cells with poor differentiation and large vacuoles, or in small and invasive ducts. The phenotypes were consistent in xenograft models made with either cells lines or patient-derived material, as well as in 2D culture. Cooating with protein markers of differentiation showed that the CA19-9-positive/sTRA-negative regions colocalized more with MUC5AC, CK19, and E-cadherin relative to the sTRA-positive cells, indicating ductal differentiation, and the sTRA-positive/CA19-9-negative regions were more likely to colocalize with beta-catenin than any other marker, indicating potential for secondary tumor development. At time-to-progression after surgery was associated with the relative levels of the two markers, based on staining of tumors from 48 patients in an independent TMA. Patients with higher dual-staining of CA19-9 and sTRA had statistically longer (p = 0.01 by log-rank test) time-to-progression, and patients with higher exclusive staining of either marker relative to the dual staining had shorter time-to-progression. Thus the sTRA marker used in conjunction with CA19-9 could provide a basis for classifying and targeting subtypes of cancer cells.

#1755 The fate of germline BRCRA related mutations in breast tumor tissues. Vassiliki Kotoula,1, Florence Fostira Fostira,2 Kyriaki Papadopoulos,3 Paraskevi Apostolou,4 Eleftheria Tsalaki,4 Georgios Lazaridis,4 Kyriaki Manousou,1, Flora Zagouri Zagouri,1 Dimitrios Pectasides,1 Ioannis Vlachos,1 Ioannis Titas,1 Sotiris Lakis,1 Irene Konstantopoulou,6 George Pentheroudakis,5 Helen Gogas,5 Pavlos Papakostas,5 Christos Christodoulou,5 Drakoulis Yanoukos,4 George Fountzilas1,2, Aristotle Univ. School of Medicine, Thessaloniki, Greece; 3National Center for Scientific Research NCSR Demokritos, Athens, Greece; 4Hellenic Foundation for Cancer Research, Athens, Greece; 5Hellenic Foundation for Cancer Research, Greece; 6Hellenic Cooperative Oncology Group, Greece; 7National Center for Scientific Research NCSR Demokritos, Greece.

Background - aim: Germline mutation carriers in BRC1A1 and related genes frequently develop triple-negative breast carcinomas (TNBC) that presumably preserve the inherited mutation. Herein, we examined the clinicopathologic features and prognostic impact of paired germline and tumor genotypes in this
#1757 Spectrum of BAP1 mutations identified in diverse cancer lineages.

Zoran Gatalica, Joanne Xiu, Jeffrey Swensen, Wangjun Chen. *Cancers Life Sciences, Phoenix, AZ.*

Background: Germline mutations in the tumor suppressor gene, BAP1, a deubiquitylase that regulates key cellular pathways, are associated with a recently-described tumor predisposition syndrome characterized by early onset benign melanocytic skin tumors, and a significant risk of cancers that include mesothelioma, cutaneous and uveal melanoma, renal cell carcinoma (RCC) and cholangiocarcinoma. Somatic or germline BAP1 mutations have been associated with an aggressive course and a poor prognosis in RCC and cholangiocarcinoma and may render the cancer cells more sensitive to HDAC inhibitors or Parp inhibitors. We investigated types and frequencies of BAP1 mutations in a large cohort of diverse malignancies and their associations with other molecular/genomic characteristics. Methods: A total of 9782 tumor samples from over 40 cancer types were molecularly profiled at Caris Life Sciences by next generation sequencing (Illumina NextSeq platform and Agilent SureSelect XT panel, 592 genes). Microsatellite instability (MSI) was tested by PCR and fragment analysis (Promega MSI Analysis System). Results: A pathogenic somatic or germline BAP1 mutation was identified in 20 cancerts, with a total of 129 tumors with mutations found (1.3% prevalence). As expected, BAP1 mutations were frequently seen in uveal melanoma (50% or 24 in 48), malignant pleural mesothelioma (29% or 6 in 21), RCC (8% or 12 in 150), cholangiocarcinoma (6.6% or 13 in 196), and cutaneous melanoma (2.7% or 7 in 319). In addition, pathogenic BAP1 mutations were detected in carcinomas arising in parotid gland (10.3% or 3 in 29), anus (8.3% or 3 in 36), cervix (3.4% or 4 in 117), stomach (3.3%, or 6 in 180), head and neck (1 in 97 or 1%), lung (14 in 1590 or 0.9%), and breast (0.8% or 7 in 887). A mutation was also noted in a meningioma (1 in 43) and a uterine sarcoma (1 in 96). Variants of BAP1 (pathogenic, presumed pathogenic, and variants of unknown significance) were more often seen in MSI-high tumors (compared to MSI-) in both colorectal (12/61 vs. 18/1003) and endometrial carcinomas (5 of 69 vs. 5 of 321; both p<0.01). It was not determined whether the BAP1 mutations were somatic or germline in origin. Conclusions: The study confirmed the presence of pathogenic BAP1 mutations in carcinomas commonly associated with BAP1 germline and somatic mutations. It also identified BAP1 mutations in additional cancer types (parotid gland, anal and cervical carcinomas), as well as its association with MSI-H cancers (colon and endometrium). Even the presence of mutations in non-cancerous tissues is underway to determine if these novel cancer associations are related to germline predisposition.

#1758 A more mature immunophenotypic makeup of multiple myeloma clone(s) at diagnosis correlates with a higher genomic instability.

Marina Martello,1 Rosalinda Termini,2 Barbara Santacroce,1 Enrica Borsi,1 Vincenza Pulvirenti,1 Barbara Pirovano,1 Gaia D’Antonio,1 Gaia Taddei,2 Chiara Briandelli Chirumbolo,1 Mario Arpinati,1 Giovanni Martinelli,1 Michele Cavo,1 Carolina Terragna1. *University of Bologna, Bologna, Italy; Fondazione Umberto Veronesi, Milan, Italy.*

Introduction: The sequence of events underlying the process of Multiple Myeloma (MM) plasma cells (PC) differentiation have not yet fully elucidated, even if recent findings suggest that different cell subpopulations, with distinct phenotype, compose the MM clone(s), whose plasticity has emerged as a typical feature. Aim: To evaluate the immunophenotypic and genomic background of MM clone(s) at diagnosis in order to stratify patients (pts) according to the PC differentiation stages and possibly correlate with disease outcome. P&M Phenotypic characterization of both CD138+/CD38− and CD19+ populations was carried out on 44 newly diagnosed MM. Fresh BM samples was analysed by 6color multiparametric flow cytometry analysis, combining CD138PE, CD38-PE-Cy7, CD20-APC, CD19-APC-Cy7, CD27-FITC, CD45-FITC, CD28-APC, CD44-FITC, CD54-APC, CD81-PerCP-Cy5.5, CD56-APC and SHL-PE, as a functional marker of Hedgehog pathway activation (Mitley Biotech). Cytoscan HD array were carried out in order to detect genomic copy number alterations (CNAs). Results According to the CD19 and CD38 markers co-expression, pts were stratified in 3 subgroups, recapitulating a progressive PC maturation process: the most immature one, including pts with CD19+/CD38+ (11/44 = 25%); the intermediate CD19-/CD38+ phenotype one (19/44 = 44%), and the CD19-/CD38- PC one, whose clone was mainly composed by most mature PCs. The two extreme subgroups were characterized by a differential expression of...
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maturation markers: the more mature PCs displayed a higher expression of CD28 and CD44, which usually characterized advanced disease stages, as well as a reduced expression of CD20, CD27 and CD45, commonly associated to preceding PC differentiation stages (p<0.05). Any differential expression of SHH pathway’s ligand was observed. On the contrary, a higher Hedgehog pathway activation was detected in the immature CD19+CD81+ compartment of immature plasma cells (median SHH expression CD19+/CD81+ vs. CD19-/CD81+: 98.1% vs. 11.3%; p<0.05), which probably featured a less quiescent immature reservoir pool. The CNAs analysis showed that mature PCs were characterized by a higher genomic instability, as compared to the more immature ones (total CNA: 306.9 vs. 171.5, p=0.08; genome changed: 36.96 vs. 13.35, p=0.01), including several alterations commonly associated to de-differentiation (e.g. del(17p)). Finally, a higher frequency of baseline clinical characteristics associated to bad prognosis was observed in the more mature, as compared to the more immature subgroups of pts (e.g. PET lesions, k/l ratio; p<0.05).

Conclusion MM clone(s) is a mixture of different cell populations endowed with an inner phenotypic plasticity. Various PC differentiation stages were appreciable already at diagnosis and genomic features associated to bad prognosis characterize pts carrying more mature clones. Acknowledgements: AIBC, Berlucchi, Prog. Bolondi, FUV.

#1759 Description of molecular marker disparities and KRAS mutational spectrum of colorectal tumors from Puerto Rican Hispanics, Julliane Perez-Mayoral,1 Camila Rivera-Lynch,1 Marievelisse Soto-Salgado,1 Xavier Llor,1 Luis Tous-Lopez,1,2 Marla Torres-Torres,3 Jose S. Reyes,4 Carlos Torres,5 Ajay Goel,6 University of Puerto Rico Comprehensive Cancer Center, San Juan, PR; 2University of Puerto Rico Medical Sciences Campus, San Juan, PR; 3Yale University, New Haven, CT; 4Ashford Presbyterian Hospital, San Juan, PR; 5Puerto Rico Cancer Registry, San Juan, PR; 6Baylor Research Institute, Dallas, TX.

Colorectal cancer (CRC) causes 27% of all cancer deaths among Puerto Rican Hispanics (PRH). Most CRC cases are due to sporadic genetic or epigenetic events that lead to the development of the tumor. These events aggregate to cause CRC carcinogenesis and involve genes that regulate cell growth and differentiation. Improving understanding of the molecular events that lead to CRC carcinogenesis has the potential to reduce CRC mortality by risk stratifying individuals as well as developing prevention strategies. Currently, there are a set of molecular markers that are used to characterize CRC tumors and inform prognosis and treatment decisions. These markers include: microsatellite instability (MSI), CpG island methylation and mutational analysis of the KRAS and BRAF oncogenes. The objective of this study was to characterize the molecular markers present in the CRC tumors of PRH and understand the disparities present at the molecular level in these tumors. The frequency of the MSI and CIMP were evaluated in 488 CRC tumors from PRH. Mutations in the KRAS gene and the BRAF V600E mutation were evaluated using SuperArray technology. Additionally, information regarding sociodemographic and clinicopathologic characteristics of the study population was obtained. Of the 488 evaluated CRC tumors 1.6% (n=2) showed MSI and 90.2% of the studied tumors had CIMP low phenotype (n=102). MSI-High tumors were more likely to be distal tumors compared to MSS tumors (p=0.02). The V600E mutation in the BRAF gene were found in 1.3% (n=1) of the studied tumors. KRAS mutations were found in 31.25% (n=40) of the tumors. The most common KRAS mutations found in the CRC tumors of PRH were: c.35G>A (KRAS COSMIC521) (n=12) and c.38G>A (n=8) (KRAS COSMIC532). Furthermore, the traditional CRC carcinogenesis pathway was the predominant pathway observed for the development of the CRC tumors of our study population. The following study highlights a distinct molecular signature for the CRC tumors from PRH descent. The disparities observed in the prevalence of the molecular markers (low MSI, CIMP Low and mainly wild-type for KRAS and BRAF) suggest that the drivers for CRC in this population might differ from other populations. Additional studies are required to fully elucidate the CRC carcinogenesis pathway in PRH.

#1760 High expression of CASZ1 is associated with poor prognosis in ovarian cancer, Yu-Anchang, Yu-Ling Lian, Yang-Ling Chen, Tse-Ming Hong, Keng-Fu Hsu. National Cheng Kung Univ. Hospital, Tainan, Taiwan.

Epithelial ovarian cancer (EOC) carries the highest mortality rate of all gynecologic malignancies. This high mortality rate is attributed to the fact that most cases of ovarian cancer are detected at late stages when metastases are already present. We previously demonstrated that castor zinc finger 1 (CASZ1) is upregulated in EOC cells and promotes EOC cell metastasis. However, the relationship between the ovarian cancer patient’s prognosis and CASZ1 expression is not clear. In the present study, we examined CASZ1 and CA125 expressions in EOC using immunohistochemistry and correlated their expression levels with patient survival. From January 2008 to January 2012, 144 EOC patients underwent staging or cytoreductive surgery at the National Cheng Kung University Hospital (NCKUH) were consecutively enrolled. Clinical and pathology information, including age, stage, cell type, chemo-response, and survival, was collected from medical charts. The staging met the criteria of the International Federation of Gynecology and Obstetrics Classification (FIGO). Histological grading of EOC was conducted according to the classification of the World Health Organization. The overall survival (OS) was calculated from the date of diagnosis. Progression-free survival (PFS) was measured as the period from surgery to the date of confirmed recurrence or disease progression or to the date of the investigators’ last note of a disease-free status. CASZ1, CA125 expression was evaluated by TissueFaxs microscopy system using an image analysis program. Five fields of the tumor in each slide (corresponding to a mean of 10000 tumors cells per case) were photographed. Images were acquired from using fluorescence-activated cell sorting (FACS)-like tissue cytometry. The percentage of positive cells in each sample was further quantified using TissueQuest software (TissueGnostics, Vienna, Austria). The CASZ1 was significantly upregulated in advanced EOC tissues, compared with early stage tumors. High CASZ1 expression levels were significantly associated with worse EOC clinical characteristics. By univariate survival analysis, high CASZ1 levels significantly correlated with decreased overall survival, progression-free survival. In addition, patients with both high expression with CASZ1 and CA125 carried the worst prognosis. In conclusion, we demonstrate that high expression of CASZ1 levels correlates with an aggressive EOC phenotype and may contribute for poor prognosis in EOC patient. Determination of CASZ1 could be clinically useful in identifying high-risk EOC patients for a more aggressive adjuvant therapy.

#1761 A rapid and accurate nucleic acid amplification and detection method for KRAS mutation testing in colorectal cancer specimens, Yong Shin, Choon Eun Jin, Seung-Seop Yeom, Seok-Byung Lim. University of Ulsan College of Medicine, Asan Medical Center, Seoul, Republic of Korea.

Colorectal cancer (CRC) is one of the most common type of cancers both men and women in the world. Unfortunately, overall death rate of CRC has been decreasing for the last two decades due to the improvement of screening test assays that detect early-stage cancer and pre-cancerous polyps. Nevertheless, the most common treatment for CRC is surgery, because it may completely eliminate the cancer region. In case of the cancer with systemic metastasis, chemotherapy is required before or after surgery for primary or metastatic lesions. Among the regimens for the chemotherapy, both monoclonal antibodies (Cetuximab and Panitumumab) against the epidermal growth factor receptor have been shown to improve survival for only patients with lack of RAS mutations. Thus, the KRAS gene mutations (codons 12 and 13) in CRC patients have been extensively studied as a strong negative predictive biomarker to indicate whether a CRC patient responds to the treatment. Therefore, testing the KRAS mutational status of tumor samples is becoming an essential tool for managing patients with CRCs. Although a myriad of nucleic acid testing methods have been developed to analyze the mutation status in the key regions of the KRAS gene of CRC, several obstacles still remain related to low sensitivity, time consuming, and required large instruments including thermal cyclers. Here, we present a novel nucleic acid amplification and detection method for KRAS mutations (G12D and G13D) testing that enable rapid and accurate detection. This method is based on combination of isothermal DNA amplification method and bio-photonic silicon sensor that can be detected the mutations in a label-free and real-time manner. The proposed method can detect the mutant cell present at 1% in a mixture of wild type cells, while both PCR and sequencing can detect the mutations in a sample containing approximately 30% of mutant cells. We used 60 tissue samples from CRC patients (22 samples with G12D mutations, 23 samples with G13D mutation, and 15 samples with no mutation) to compare the clinical utility of three methods including PCR, Sanger sequencing and the proposed method. The proposed method with both G12D and G13D showed a value of 100% and 100% for sensitivity and specificity, respectively. One the other hand, the sensitivity and specificity of PCR (90.5% and 100%) and sequencing (95% and 100%) were lower than the proposed method. Therefore, the proposed method was found to be rapid (<30 min), highly sensitive and specific method for KRAS mutation testing. We believe that this rapid and accurate method will enable proper treatment for CRC patients.

#1762 Keratin 17 identifies prognostic subtypes of pancreatic ductal ade- nocarcinoma, Luisa F. Escobar-Hoyos,1 Cindy Leiton,1 Elizabeth Vanner,1 Lu- cia Roz-Pena,1 Jinyu Li,2 Scott Powers,2 Ali Alakin,2 Jela Bandovic,2 Peter Bai- ley,2 David Chang,4 Richard Moffitt,5 Jen Jen Yeh,5 Andrew Biankin,4 Kenneth Shroyer,1 Memorial Sloan Kettering Cancer Center, New York, NY; 2Stony Brook University, Stony Brook, NY; 3University of Massachusetts Memorial Medical Center, Worcester, MA; 4Wolfson Wohl Cancer Research Centre, Glasgow, United Kingdom; 5Lineberger Comprehensive Cancer Center, Chapel Hill, NC.

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Recent RNA sequencing (RNASeq) studies by Bailey et al., 2016, Moffitt et al., 2015 and Collisson et al., 2011 reported that mRNA expression from bulk tumor defines molecular subtypes of pancreatic ductal adenocarcinoma (PDAC) that are highly correlated with patient survival. These studies independently identified Keratin 17 (K17) mRNA overexpression as one of over 20 upregulated transcripts that define the mRNA signature of the most lethal PDACs. Studies in our lab determined that neither K17 gene missense mutations nor copy-number alterations explain the upregulation of K17 mRNA expression by malignant cells. In addition, we found that K17 expression, measured at the protein level by immunohistochemistry or at the level of mRNA by RNASeq, is sufficient to stratify patients by short- vs long-term survival at baseline after resection. These retrospective survival analyses were performed on independent patient cohorts and unified to define low-vs high- K17 patients (total n = 558). High-K17 cases were twice as likely to die from this disease compared to stage-matched low-K17 cases (P values < 0.05). Furthermore, we determined that K17 expression is associated with outcome after Gemicitabine treatment using the Bailey et al., 2016 patient cohort (n = 94). This is the first study to show that the expression of a single gene, K17, can accurately subtype PDAC at initial diagnosis. In conclusion, K17 was identified as a robust and independent, clinically relevant, prognostic and predictive biomarker to stratify clinical outcome at the time of initial diagnosis and to potentially inform clinical decisions regarding chemotherapeutic intervention.


Background: Merkel cell carcinoma (MCC) is a highly aggressive neuroendocrine tumor of the skin. Merkel cell polyomavirus (MCPyV) plays an oncogenic role in the majority of MCC tumors. Detection of MCPyV in MCC tumors has diagnostic utility, and potentially prognostic and therapeutic implications. We investigated whether RNAseq, a novel RNA in situ hybridization (ISH) method for detection of RNA transcripts in tissues at the single-cell/single-molecule level, is useful for MCPyV detection. Methods: We applied an RNAseq probe targeting MCPyVT antigen transcripts on tissue microarrays (TMAs) and full tissue sections encompassing 91 MCC tumors from 75 patients, 14 carcinomas of other types, and various normal tissues. qPCR and immunohistochemistry (IHC) for MCPyV were performed on 58 and 88 cases, respectively. qPCR was also performed on 18 cases of normal skin to establish background levels of MCPyV in non-tumor results. Results: RNA-ISH demonstrated the presence of MCPyV in 46.2% (42/91) of cases. A total of 58 samples had data from all of the three detection modalities (qPCR, RNA-ISH and IHC). RNA-ISH demonstrated 100% concordance with qPCR results, whereas IHC had a slightly lower concordance (96.6%). RNA-ISH demonstrated higher agreement than IHC among TMA cores from the same case, and between TMA cores and matched full tissue sections. Of samples with background inflammatory cells, 5 of 37 (15.6%) showed moderate nonspecific staining of lymphocytes by IHC, whereas RNA-ISH lacked background. Conclusions: RNA-ISH is comparable sensitive to qPCR for detection of MCPyV, and allows for correlation with tissue morphology. Our findings support RNA-ISH as a highly effective method for MCPyV detection in tumors.

#1764 Accurate and reproducible detection of fusions and exon skipping events in NSCLC-derived samples using a comprehensive, targeted RNA-Seq system across multiple laboratories. Gary J. Latham, Richard Bliedner, Brian C. Haynes, Shbhoa Gokul, Maria L. Aguirre, Stephen Hyter, Ziyan Y. Pesivan, University of Michigan, Ann Arbor, MI; Henry Ford Health System, Detroit, MI.

Introduction: Reliable assessment of cancer-associated RNA markers in lung cancer provides crucial information for patient care. Methods to detect RNA fusions and splice variants in NSCLC was demonstrated in a multi-site laboratory evaluation using clinically-relevant specimens and low inputs of TNA. The ability of the panel to detect both common and rare gene fusion transcripts and exon skipping events within an integrated wet- and dry-bench workflow provides a foundation for the reliable detection of oncogenic RNA fusions and aberrant splicing events that can respond to current and emerging targeted therapies. This study highlighted the ease of implementation and consistent performance that can be achieved in different laboratories when the process from sample-to-report is highly integrated.

#1765 Molecular and immunological characterization of non-small cell lung cancer harboring cMET alterations. Maria Saigi, Carolina Pereira, Eva Pros, Elisabeth Brambilla, Ernest Nadal, Montse Sánchez-Cápsedes, IDIBELL, Barcelona, Spain; Centre Hospitalier Universitaire de Grenoble, Grenoble, France; Catalan Institute of Oncology, Barcelona, Spain.

Purpose: To explore the correlation between the expression of immunological biomarkers in a subset of NSCLC tumors presenting cMET activation. Methods: We retrospectively reviewed 200 tumor samples from a cohort of patients diagnosed with NSCLC in 3 different hospitals, from Spain and France. cMET activation was defined by the presence of one or more of the following criteria: 1) cMET mutations inducing exon 14 skipping (METex14), 2) cMET amplification, assessed by FISH 3-cMET overexpression, assessed by immunohistochemistry (IHC). METex14 mutations were determined by genomic sequencing and RT-PCR. FISH was interpreted according to Capuzzo scoring system, considering 5 or more gene copies as the mean value for positive amplification. Concurrent genetic alterations in EGFR and ERBB2 tyrosine kinase receptors were also determined. PD-L1 expression and density of tumor infiltrating CD8+ lymphocytes were assessed by IHC. Statistical correlation analysis was performed by Chi-square and Fisher’s exact test. Results: METex14 cMET amplification and cMET overexpression were detected in 2%, 7.8% and 31% of tumors, respectively. Histological subtypes included: 70% adenocarcinoma, 20% squamous cell carcinoma, 8% large cell, 2% pulmonary sarcomatoid. Curiously, one of the METex14 adenocarcinoma was concurrent with ERBB2 amplification. Those tumors harbouring METex14 or cMET amplification were correlated with positivity to PD-L1 expression (p=0.009). However, this association was not observed in cMET overexpressed tumors. Conclusions: We observed a direct correlation between PD-L1 positivity and METex14 or cMET amplification. Functional analysis and a large cohort of tumors are required to better characterize the potential role of cMET pathway in the regulation of PD-L1 expression.

#1766 Distinct pattern of alterations in tp53 mutated and wild type acute myeloid leukemia (AML) patients. Anna Ferrari, Eugenio Fonzi, Maria Chiara Fontana, Andrea Gelli Luserna Di Rorà, Marco Manfino, Antonella Padella, Carmen Baldizzi, Cristina Papayannidis, Giovanni Marconni, Stefania Paolini, Viviana Guadagnuolo, Margherita Perricone, Valentina Robustelli, Enrica Imbrogno, Eugenia Franchini, Claudia Venturi, Elisa Zuffa, Maria Chiara Abbenante, Giorgia Simonetti, Nicoletta Testoni, Emanuela Ottaviani, Maria-giuliana Giovannini, Institute of Hematology, Bologna, Italy; Cell Plyr bologna, Bologna, Italy.

Background: Mutations in TP53 gene predict a poor prognosis in patients with AML. The reported TP53 mutation rate in AML is low (2.1%) by contrast with AML with a complex karyotype (CK) is higher (69-78%) and have a poor outcome. Quite common is to found paired TP53 mutation together and a segmental 17p deletion. Aims: To investigate the frequency, the types of mutations, the associated cytogenetic, the correlation with known molecular alterations and the prognostic role TP53 mutations in adult AML pts. Moreover we would identify genes/pathways that are mainly affected in the mutated TP53 group compared to the wt one. Patients and Methods: 258 adult AML pts with miscellaneous cytogenetic abnormalities and
normal karyotype were examined in our Institution for TP53 mutations using several methods, including Sanger sequencing, NGS and HiSeq2000 platform and were correlated with cytogenetic analysis. 124/258 samples were genotyped with SNP arrays (Affymetrix, 3 250K, 43 SNP 6.0, 78 CytoScan HD). Copy Number Alterations (CNAs) analyses were performed using Chromosome Analysis Suite (Affymetrix) and NexusCopy Number (Illumina) software. Results: Mutation analysis detected TP53 mutations on 39 patients with 48 different types of mutations (32 deleterious point mutations; 4 deletions); nine pts have 2 mutations. We found 34/48 (70%) missense mutations, 5 mutations in the splice sites, 4 deletions, 2 intrinsic and 3 others mutations. The mutation rate is of 15.1%. Mostly (28/39) of the TP53 mutated pts (71.8%) had CK while only 11/39 (28.2%) mutated pts have “no CK”. Alterations of TP53 were significantly associated with poor outcome (OS and DFS: \( P = 0.0001 \)). To take advantage of different methodological characteristics, on TP53 locus, we matched two software and cytogenetic analysis results. We identify 16 mutated pts that were also deleted and one pt that presented only a deletion. Therefore 50% of mutated pts present a concomitant deletion. OS of TP53 mutated and deleted pts is statistically inferior respect to mutated pts \((P<0.0061)\). Comparing 32 TP53 mutated and 92 TP53 wt pts CNAs results that: a) chromosomes significantly altered are Sq. 17p, 12p, 16q, 22q13.33 and 7q; b) over 900 genes are differentially involved (in all (Loss)); c) and that pathway categories mainly enriched are Signal Transduction, Metabolism, Immune System, Transmembrane transport of small molecules, Gene expression, Cell Cycle. Conclusions: Our data demonstrated that TP53 mutations occur in 15.1% of AML with a higher frequency in the subgroup of CK-AML \((P=0.0001)\). They predicted to be deleterious and significantly correlated with worse prognosis especially if TP53 is both mutated and also deleted. For these reasons, TP53 mutation/deletion screening should be recommended. Different pattern of alterations in mutated and wt groups have to be deeply investigated. ELN, AIL, AIRC, progetto Regionale- Universitario 2010-12 (L. Bolondi), F77- NGS-PTL project.

#1767 Analysis of biomarkers and anthracycline benefit for hormone receptor-negative breast cancer: results from a randomized phase 2 neoadjuvant study (KBOG 1101 Study), Takashi Ishikawa, Kazutaka Narui, Mari S. Oba, Akimitsu Yamada, Kumiko Kida, Mikiko Tanabe, Yasushi Ichikawa.

Tokyo Medical University, Tokyo, Japan; Yokohama City University Medical Center, Yokohama, Japan; Yokohama City University, Yokohama, Japan.

AIM: We compared 6 cycles of docetaxel and cyclophosphamide (TC6) with 3 cycles of S-fluorouracil and epirubicin and cyclophosphamide followed by 3 cycles of docetaxel (FEC-D) as neoadjuvant chemotherapy for patients with hormone receptor (HR)-negative breast cancer (BC) to identify biomarkers requiring anthracyline treatment. Methods: In total, 103 patients with operable HR-negative BC were administered TC6 or FEC-D. Triple-negative BC was subdivided by CK5/6 and EGFR into basal- and non-basal BCs. The primary endpoint was pathological complete response (pCR). Secondary endpoints were safety, breast-conserving surgery ratio, disease-free survival, overall survival, and predictive factors (Ki-67, P-53, ALDH1 and TOP2A by IHC and TOP2A by FISH) for each regimen. Results: Of the 103 patients, 97 completed the study. Overall pCR was higher for patients treated with FEC-D \((36\%)\) than for those treated with TC6 \((25.5\%) \((P=0.265)\). FEC-D was significantly superior to TC6 in basal BC \(29.9\%\ vs 13.6\%\; \(P=0.033\)\), whereas no differences in HER2 and non-basal BCs. Aldehyde dehydrogenase 1 (ALDH1) positivity was inversely associated with pCR for both regimens, significantly for FEC-D \((P=0.047)\) and TC6 \((P=0.085)\). Patients who achieved pCR tended to have longer DFS \((P=0.287)\) and OS \((P=0.089)\). Patients with basal and non-basal BC treated with FEC-D had significantly better DFS \((P=0.016)\) and OS \((P=0.034)\) than those with TC6. Conclusion: We found TC6 was less effective than FEC-D for HR-negative BC because it was not sufficient to treat basal-BC. This suggests that DNA damaging agents like anthracylines are required for treating basal-BC. Additionally, ALDH1 could be a marker for resistance to conventional chemotherapy.

#1768 A practical tool for probing protein glycosylation in clinical samples: Application to characterizing subtypes of MUC5AC glycosylation in distinct subsets of pancreatic cancer patients. Peter Y. Hueh, Van Andel Institute, Grand Rapids, MI.

Glycans are critical to protein biology and are useful as disease biomarkers. Many studies of glycans rely on clinical specimens, but the low amount of sample available for some specimens limits the experimental options. We have a method to obtain information about protein glycosylation using a minimal amount of protein, and we applied it to the study of biomarkers in the blood plasma of pancreatic cancer patients. The method works by treating proteins that were captured by antibody microarrays (2.2 x 2.2 mm) with exoglycosidases to successively expose underlying features, and then probing the native or exposed features using a panel of lectins or glycan-binding antibodies. We use an algorithm to interpret the data and provide predictions about the glycan motifs that are present in the sample. We applied the method to the analysis of the glycosylation of a biomarker, MUC5AC, found in the plasma of patients with pancreatic cancer. An O-linked glycan on MUC5AC was significantly better DFS \((P=0.287)\) and OS \((P=0.034)\) than those without EGFR mutations. FEC-D was significantly superior to TC6 \((P=0.016)\). The patients without EGFR mutations had better progression free survival although there was no statistical significance between patients with or without EGFR mutations. We will collect more patients’ data for analysis.

#1769 The clinical outcome of stage I lung adenocarcinoma patients with or without activating EGFR mutation. Yue-Yu Fang, Chung Gung Memorial Hospital, Taipei, Taiwan.

Purpose: Advanced stage I lung adenocarcinoma patients with activating EGFR mutation may benefit from targeted therapy. The aim of the present study was to determine the association between activating EGFR mutations and clinical outcome of stage I adenocarcinoma. Methods: From January 2010 to April 2014, 167 patients were done. Sanger method of direct PCR, scorpion & ARMS method or ABI allele specific TagMan PCR were used to check the EGFR mutation status of these patients’ samples. The selection of these methods was depended on purity of tumor samples. Results: There were 93 stage IA patients and 74 stage IB patients. Sixty-four (69%) patients had activating EGFR mutations in stage IA patients, and fifty (68%) patients had activating EGFR mutations in stage IB patients. The survival of stage IA and IB were similar in patients with or without EGFR mutations. The patients without mutated EGFR had better progression free survival in IA and IB patients, especially in the IB patients, although the significance did not reach in small preliminary sample size. Conclusion: The survival was similar in stage IA or IB lung adenocarcinoma with or without EGFR mutations. Patient without EGFR mutation had better progression free survival although there was no statistical significance between patients with or without EGFR mutations. We will collect more patients’ data for analysis.

#1770 The association between sarcopenia and cellular senescence of cancer associated fibroblast in pancreatic cancer. Kensuke Yamamura, Yo-ichi Yamashita, Yuki Kitano, Kota Arima, Tatsunori Miyata, Shigeki Nakagawa, Kosuke Mima, Katsunori Imai, Daitsuke Hashimoto, Akira Chikamoto, Hideo Baba, Graduate School of Medical Science, Kumamoto University, Kumamoto, Japan.

Sarcopenia is a syndrome that is characterized by progressive loss of skeletal muscle mass and strength. Several recent studies have shown that sarcopenia has prognostic significance for patients with malignant disease, including cancers of the esophagus, colon, pancreas and liver. On the other hand, cellular senescence is gaining increased attention from clinicians and researchers, yet incompletely understood role in the development of malignant disease. Previous studies reported that Caveolin-1 expression in cancer associated fibroblast is associated with cancer progression, and it is known in recent years that caveolin-1 plays a major role in controlling cellular senescence. Therefore, we hypothesized that sarcopenia causes cellular senescence of cancer associated fibroblast by activating EGFR expression in clinical specimens and could facilitate the characterization and identification of glycan biomarkers.
copia. Kaplan-Meier analysis showed that patients with sarcopenia had a significantly worse cancer specific survival than patients without sarcopenia (3-year-survival: 37.2% vs 50.4%, respectively, p = 0.038). While, a high level of Caveolin-1 expression was detected in 29.2% (38/130) of our samples. High Caveolin-1 expression in CAK significantly correlated with poor prognoses with respect to both DFS and OS (p = 0.002 and p = 0.003, respectively). However, there was no relationship between sarcopenia and IHC score of Caveolin-1 expression in CAK. Conclusion: The present result suggested that sarcopenia and Caveolin-1 expression of CAK in patient with pancreatic cancer is respectively associated with poor prognosis, but not associated with each other. Now, we focus on Caveolin-1 expression of CAK, and establish primary cultures of CAF from the pancreatic cancer tissues and examine its function.

CLINICAL RESEARCH: Predictive Biomarkers 1

#1771 Phase 3 trial of adjuvant sphinctin in patients with high-risk renal cell carcinoma: exploratory molecular analysis of tumor biomarkers. Daniel J. George,1 Jean-Francois Martini,2 Yen-Hwa Chang,3 Michael Staehler,4 Jan Cardarelli, Italy; 11Pfizer, Italy; 12Pfizer Inc, PA; 13Centre Eugene Marquis, Montreal, Canada; 14University of Shanghai, China; 15University of Missouri, Columbia, USA; 16Memorial Sloan Kettering Cancer Center, NY; 17University of Cambridge, UK. Background: Adjuvant therapy with sunitinib (SU) compared with placebo (PBO) prolonged disease-free survival (DFS) in patients (pts) with loco-regional high-risk renal cell carcinoma (HR R=0.76, 95% CI: 0.59-0.98; P=0.03; median[median] DFS, 6.8 vs 5.6 years). Here, we report the results of a retrospective exploratory molecular biomarker analysis using nephrectomy biospecimens from the S-TRAC trial. Materials and Methods: Formalin-fixed paraffin-embedded tumor tissue blocks from patients who provided informed consent were used for immunohistochemistry (IHC) staining of PD-L1 and PD-L2 in 20% of all available FFPE tissue samples. Analysis of PD-L1 expression was performed using Dako12345678, 34E12 antibody and Dako EnVision System. Staining intensity was scored (0 to 3+) and the proportion of stained tumor cells was multiplied by intensity to give a PD-L1 expression score, ranging from 0 to 300. Analysis of the expression scores was performed in 4 areas of each tissue section (core) and the mean expression score was calculated. Analysis was performed on NSCLC, then applied to 30 available RCC samples. Results: Immunohistochemical analysis of PD-L1 expression in SU group showed increased expression of PD-L1 protein compared to PBO group (22.3% vs 13.2%). The expression of PD-L1 was positively associated with better DFS (HR 0.80, 95% CI: 0.42-1.50; P=0.484). The sensitivity and specificity of PD-L1 was assessed and the cut-off of 300 was determined to be optimal for predicting DFS. Conclusion: Increased PD-L1 expression in tumor cells was associated with longer DFS in SU-randomized pts but not PBO, suggesting that this may be predictive of treatment effect. Further validation in an independent cohort is warranted. The prognostic value of PD-L1 expression in primary tumors from patients with high-risk non-metastatic RCC should be further explored.

#1772 PIK3CA C2 domain deletions hyperactivate PI3K, generate oncogene dependence and are excessively sensitive to PI3Kα inhibitors. Sarah Crossmann,1 Jonathan Sheehan,2 Gregory Slowski,3 Nalin Leelatan,1 Jie He,2 Rebecca Nagy,3 Justin M. Balko,6 Ingrid A. Mayer,4 Richard B. Lamann,5 Vincent Miller,2 Lewis C. Cantley,4 Jonathan M. Irish,1 Jens Meiler,2 Carlos L. Arteaga1. 1Vanderbilt University, TN; 2Foundation Medicine, Cambridge, MA; 3Guardant Health, Inc., Redwood, CA; 4Meyer Cancer Center of Weill Cornell Medical College, New York, NY. Background: A 63 year old postmenopausal woman with advanced ER+ breast cancer resistant to endocrine therapy exhibited an exceptional response to the PI3Kα inhibitor alpelisib (BYL719) and the aromatase inhibitor letrozole (MCL). Clonal capture sequencing (NGS) of DNA from a liver metastasis identified a P447_L455 deletion in the C2 domain of PIK3CA. About 80% of PIK3CA activating mutations are in ‘hot spots’ within the helical and kinase domains. C2 domain mutations make up ~10% of all PIK3CA mutations in breast cancer (TCGA, Foundation Medicine) and are frequently not reported by tumor and plasma cell-free DNA NGS panels. Deletions and mutations in this domain cluster in a region encompassing amino acids 446-460 of PIK3CA. We investigated here the functional role of PIK3CA C2 domain mutations and their response to PI3K inhibitors. Methods: V5-tagged lentiviral vectors encoding wild type, delP447-L455, and delH450-P458 were stably transduced into MCF10A non-tumorogenic human breast epithelial cells. Cell viability and acini formation in 3D Matrigel were examined in media treated with wild type or alpelisib (1 μM) of PIK3CA activated by immunoblot and phosho-flow analysis. The Rosetta software suite was used to construct a structural model that would predict the change in stability of the p85/p110 complex. Physical association of p85α and p110α was determined by precipitation with V5 antibodies and immunoblot analysis. Results: MCF10A cells stably expressing V5-tagged PIK3CA wild type and PIK3CA delP447-L455 exhibited EGF- and insulin-independent growth and higher phosphorylation of AKT, ERK and S6 when compared to parental MCF10A cells. In 3D Matrigel, MCF10A cells with PIK3CA C2 domain deletions formed invasive acini with increased protrusions, spindling, and bridging between acini. All these changes were ablated upon the addition of 1 μM of alpelisib whereas parental MCF10A cells were unaffected by alpelisib. We hypothesized that delP447-L455 would reduce the binding affinity of PIK3CA with the p85 regulatory subunit. A structural model of PIK3CA delP447-L455 in the context of the regulatory complex revealed specific favorable inter-residue contacts that would be lost as a result of the deletion, predicting a significant decrease in binding energy. Consistent with this structural analysis, coimmunoprecipitation of p85 with V5 antibodies showed reduced binding of the C2 domain deletion mutants with p85 compared to wild type PI3Kα. Conclusions: These data suggest that C2 domain deletions in PIK3CA are activating mutations and generate oncogene dependence. As a result, tumors expressing these mutations are excessively sensitive to PI3Kα inhibitors. Thus, in addition to PIK3CA ‘hot spot’ mutations, C2 domain mutations should also be considered biomarkers of sensitivity to PI3Kα inhibitors.
CLINICAL RESEARCH: Predictive Biomarkers

lated with these outcomes. Following D treatment, IFNγ was induced in NSCLC pts (FC=2; p=0.0046) regardless of clinical response. High levels of pre-treatment IFNG in NSCLC pts associated with greater benefit from D. D induces IFNγ within the tumor microenvironment. Observations from other tumor types will be presented.

### Table 1. Clinical outcomes by IFNG or PD-L1 status

<table>
<thead>
<tr>
<th></th>
<th># Pts (# events)</th>
<th>ORR % (95% CI)</th>
<th>OS months (95% CI)</th>
<th>OS adjusted (HR p)</th>
<th>PFS months (95% CI)</th>
<th>PFS adjusted (HR p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSCLC IFNγ+</td>
<td>32 (1621)</td>
<td>37.5% (21.7-56.3)</td>
<td>24.6 (10.3,30.9)</td>
<td>0.0082 (3.6,NA)</td>
<td>0.00028 (2.0,15.8)</td>
<td></td>
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<tr>
<td>IFNG-</td>
<td>65</td>
<td>6.2</td>
<td>6.5</td>
<td>1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDL1 TPS$&gt;$25%</td>
<td>100 (28.9)</td>
<td>28.9</td>
<td>20.5</td>
<td>0.53; 0.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDL1 TPS$&lt;$25%</td>
<td>54</td>
<td>7.4</td>
<td>9.1</td>
<td>1.5</td>
<td></td>
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NR= Not Reached; NA= Not Applicable; *Adjusted for covariates. TPS= tumor proportion score Ventana SP263 assay

#### 1774 Progression-free survival curves suggest a dichotomous determini-ant of PD-L1 inhibitor efficacy

David J. Stewart, 1 Dominick Bosse, 2 Stephanie Brule, 1 Andrew G. Robinson, 2 Michael Ong, 1 John Hilton 1. 1University of Ottawa, Ottawa, Ontario, Canada; 2Harvard University, Boston, MA; 3Queen’s University, Kingston, Ontario, Canada.

Background: PD-L1 expression varies across tumors but does not accurately predict PD-L1 inhibitor efficacy. Some negative tumors respond and some pos-itive tumors fail. PD-L1 inhibitor progression-free survival (PFS) curve shape in non-small cell lung cancer (NSCLC) suggests that a dichotomous (present vs absent) factor might drive sensitivity rather than it being driven by a continuous variable like PD-L1 expression. PFS curves may follow first order kinetics, with a straight line if log % PFS is plotted vs time. If the population had 2 distinct absent) factor might drive sensitivity rather than it being driven by a continuous variable like PD-L1 expression. PFS curves may follow first order kinetics, with a straight line if log % PFS is plotted vs time. If the population had 2 distinct

#### 1776 Impact of homologous recombination deficiency (HRD) biomarker on outcomes in triple-negative breast cancer (TNBC) patients treated with AC chemotherapy (SWOG 59313)

Priyanka Sharma, 1 William Barlow, 2 Andrew K. Godwin, 1 Harsh Pathak, 1 Kamilla Isakova, 2 Anne R. Hartman, 3 Kristen M. Timms, 3 Hannah M. Linden, 4 Debu Tripathy, 5 Gabriel N. Hortobagyi, 5 Daniel F. Hayes, 6 University of Kansas Medical Center, Westwood, KS; 7SWOG Statistical Center/Cancer Research and Biostatistics (CRAB), Seattle, WA; 8Myriad Genetics, Inc., Salt Lake City, UT; 9University of Washington/Seattle Cancer Care Alliance, Seattle, WA; 10The University of Texas MD Anderson Cancer Center, Houston, TX; 11University of Michigan, Ann Arbor, MI.

Introduction: There is a critical need to develop biomarkers of response and resistance to adjuvant chemotherapy for TNBC. In preliminary studies, homologous recombination deficiency (HRD)-causing alterations have been reported in immune response-mediated PD-L1 expression. In TNBC patients, PD-L1 expression may be relevant for predicting response to checkpoint inhibitors. Also, HRD has been shown to predict sensitivity to checkpoint inhibitors. However, further studies are needed to confirm these findings.

Methods: Among 3125 breast cancer patients enrolled in SWOG 59313, 1291 patients with PD-L1 expression were included in the analysis. Patients were divided into two groups based on PD-L1 expression: PD-L1 positive (TPS ≥ 10) and PD-L1 negative (TPS < 10) groups. The primary endpoint was overall survival (OS). Secondary endpoints included progression-free survival (PFS), response rate, and investigator-assessed clinical benefit rate (CBR).

Results: Among the 1291 patients included in the analysis, 633 (49.3%) were PD-L1 positive (TPS ≥ 10) and 658 (50.7%) were PD-L1 negative (TPS < 10). The median follow-up time was 35 months. The median OS was 54.2 months for PD-L1 positive patients compared to 38.9 months for PD-L1 negative patients (p=0.0001). Similarly, the median PFS was 14.3 months for PD-L1 positive patients compared to 6.6 months for PD-L1 negative patients (p<0.0001). The response rate was 28.9% for PD-L1 positive patients and 13.0% for PD-L1 negative patients (p=0.001). The CBR was 53.4% for PD-L1 positive patients and 38.4% for PD-L1 negative patients (p=0.001).

Conclusion: These results suggest that PD-L1 expression may be a useful biomarker for predicting response to adjuvant chemotherapy in TNBC patients. Further studies are needed to confirm these findings and to explore the potential use of PD-L1 expression as a predictive biomarker for PD-L1 inhibitors in TNBC.
HRD results were determined in 91% (379/425) and 67% were HRD positive (27% with tBRCA mutation and 40% with tBRCA negative but HRD score > 42). HRD positive status was associated with a better DFS (HR = 0.69; 95% CI 0.49-0.96 (p = 0.027)) and OS (HR = 0.67; 95% CI 0.47-0.97 (p = 0.032)). High HRD score (>24) in tBRCA negative patients (n=274) was also associated with better DFS (HR = 0.39; CI 0.26-0.63). DNA damage responsive (DDR) versus non-responsive (NDR) did not impact DFS (p = 0.78). Conclusions: Two thirds of tTNBC patients receiving adjuvant AC chemotherapy had tumor HRD positivity. HRD was associated with better DFS and OS, perhaps due to high responses to AC. HRD status has the potential to be used as a selection criterion to identify tTNBC patients who receive significant benefit from anthracycline chemotherapy, and may also be of value in selecting patients suitable for treatment with other DNA damaging agents like platinum salts/PARP-inhibitors.

#1777 Development of a pan-cancer 15 gene expression signature to detect a subgroup driven by MAPK signalling. Laura A. Knight,1 Bethanie Price,1 Andrea McCavigan,2 Aya El-Helali,2 Charlie Gourley,1 Denis P. Harkin,1 Richard Kennedy,2 Nuala McCabe1.1 Abnac Diagnostics Ltd, Craigavon, United Kingdom; 2 Queens University Belfast, Belfast, United Kingdom.

INTRODUCTION: Unsupervised hierarchical clustering of gene expression data from 265 High Grade Serous Ovarian Cancer (HGSO) patients identified 3 major molecular subgroups. One subgroup is driven by activation of the MAPK pathway and is associated with poor prognosis and resistance to platinum chemotherapy. The MAPK pathway is currently being targeted by novel therapeutics and hence an assay to detect activation of the pathway across cancers would be highly valuable as a clinical trial enrichment tool. Using internal and publicly available gene expression datasets we have demonstrated that the MAPK subgroup also exists in other cancer types and is associated with poor prognosis. The aim of this study was to develop a gene expression signature to predict the MAPK subgroup across multiple cancer types. METHODS: Evaluation of gene expression data in a range of tumours (ovarian, colon, lung, melanoma and prostate) identified a common gene list (CGL) of 7802 genes showing high variability and high expression across diseases. The CGL was input to different machine learning algorithms developing signatures under 10x5-fold cross-validation (CV), trained against the MAPK HGSO subgroup. Filter-Feature-Selection removed 10% of genes under CV based upon ranked correlation adjusted t-scores and the final model selected to satisfy a number of key criteria: AUC for predicting the endpoint; association with survival (C-Index); and functional relevance of signature content. RESULTS: A 15 gene signature was selected, yielding an AUC=0.87 [95% CI 0.84-0.89] with respect to the MAPK subgroup. This model has validated as a poor prognosis marker in several other cancer types (Colorectal, Relapse free survival: HR = 1.46 [95% CI 1.07-1.98]; Lung, Relapse free survival: HR = 2.18 [95% CI 1.33-3.56]; Prostate cancer, Biochemical recurrence: HR 2.49 [CI 1.43-4.34], and is suppressed by MEK inhibition (p=0.0023) and elevated by KRAS, NRAS and MEK1 overexpression in cell line models (p=0.0443, <0.0001and <0.0001). Additionally we have demonstrated that the 15 gene signature strongly predicts response to the MEK inhibitors Trametinib and Selumetinib in established cell line models (p<0.0001) and in primary tumour biopsies and ovarian cell lines (p<0.0001). CONCLUSION: A 15 gene signature has been developed from formalin fixed paraffin embedded samples across multiple diseases to detect a molecular subgroup driven by MAPK signalling. This assay predicts sensitivity to MEK inhibitors in pre-clinical model systems and in primary cells derived from patients. Further work aims to validate the signature in clinical samples from patients treated with a MEK inhibitor. This assay may be helpful for clinical trial enrichment to select patients that are likely to benefit from MAPK targeted therapies.

#1778 A clinical genomic biomarker study of the CHK1 inhibitor prexemabertin in advanced head and neck squamous cancer and squamous cell carcinoma of the anas. Ricardo Martinez,1 Sameera R. Wijayawardana,1 Andrea Hong,2 Johanna Bendell,2 Anna Maria Russell,1 Richard P. Beckmann,1 Aimee Bence Lin1.1 Eli Lilly and Company, Indianapolis, IN; 2 MD Anderson Cancer Center, Dallas, TX.

CHK1 plays a critical role in DNA damage repair (DDR) pathways as well as in coordinating DNA replication. Selective CHK1/CHK2 compounds are being tested in clinical trials but predictive biomarkers of patient response are lacking. A phase 1b expansion cohort study (I4D-TCJT1A, NCT01115790) with the CHK1 inhibitor, prexemabertin, included patients with advanced, metastatic head and neck squamous cell carcinoma (HNSCC) or squamous cell carcinoma of the anus (SCCA). To identify genomic biomarkers associated with single-agent drug response, pre-treatment tissues (archived or biopsy) from 71 consented patients (HNSCC=47, SCCA=24) were subjected to next-generation sequencing (NGS) using the FoundationOne gene panel. In this subset of patients, the disease control rate (DCR) (Complete Response (CR) + Partial Response (PR) + Stable Disease (SD) based on RECIST Criteria (v 1.1)) was 60% (28/47) and 75% (18/24), respectively. We present here the observed genetic alterations correlated (DCR) (Complete Response (CR) + Partial Response (PR) + Stable Disease) between the two cohorts. Known or likely loss-of-function (LOF) mutations in FBXW7 and PARK2, two genes implicated in Cyclin E1 dysregulation, were noted in patients with favorable response in both tumor types. Across both HNSCC and SCCA, cohorts, mutations and/or germline variants in the DDR genes BRCAlA2, BRCAl2, MREI1A and ATM not in the Fanc (FANC) pathway genes were found in patients with treatment benefit. Whereas PIK3CA mutations were infrequent in the HNSCC cohort, in SCCA, mutations occurred in 5/8 (63%) patients with disease control vs 1/6 (17%) with PD. All 7 PIK3CA mutations observed in HNSC+ SCCA and SCCA patients mapped to the helical domains suggestive of Apobec-induced mutagenesis as their source of origin. The enhanced clinical benefit to prexemabertin associated with HPV+ in HNSCC may reflect a prognostic effect. Alternatively, the observed biomarker in the hypothesis-generating DNA damage responsive (DDR) and the estrogen receptor (ER) may also be of value in selecting patients suitable for treatment with other DNA damaging agents. Clinical trials are ongoing to further evaluate these findings.

CLINICAL RESEARCH: Predictive Biomarkers 1

#1779 Chronic NSOIA use increases survival in PIK3CA-altered head and neck squamous cell carcinoma. Matthew Louis Hedberg,1 Noah Peyer,1 William Gooding,1 Hui Li,1 Toni Brand,2 Victor Olivers,1 Simon Choise1.1 University of Pittsburgh, Pittsburgh, PA; 2 University of Pittsburgh, Pittsburgh, PA.

The phosphoinositol-3-kinase (PI3K) signaling pathway is one of the most biologically plausble explanations for the observed effect. Methods: The PIK3CA status of tumor tissue was determined by FISH and next generation sequencing for 266 HNSCC patients treated at the University of Pittsburgh Cancer Institute. Cox joint proportional hazards models were used to generate multivariate projections of disease-specific (DS) and overall survival (OS). Known or likely loss-of-function (LOF) mutations in PIK3CA were examined in HNSCC and SCCA. PIK3CA (24%) and PIK3CB (26%). Conclusions: Chronic NSOIA use had a significant impact on survival in patients with PIK3CA-altered HNSCC, but not those with wild-type, unamplified PIK3CA.


The phosphoinositol-3-kinase (PI3K) signaling pathway is one of the most biologically plausible explanation for the observed effect. Methods: The PIK3CA status of tumor tissue was determined by FISH and next generation sequencing for 266 HNSCC patients treated at the University of Pittsburgh Cancer Institute. Cox joint proportional hazards models were used to generate multivariate projections of disease-specific (DS) and overall survival (OS). Known or likely loss-of-function (LOF) mutations in PIK3CA were examined in HNSCC and SCCA. PIK3CA (24%) and PIK3CB (26%). Conclusions: Chronic NSOIA use had a significant impact on survival in patients with PIK3CA-altered HNSCC, but not those with wild-type, unamplified PIK3CA.
Background: Hypoxia is an important feature of the tumor microenvironment and correlates with radiotherapy resistance and metastatic progression. Additionally, as the malignant phenotype promotes aerobic glycolysis and contributes to the microenvironmental acidification, an underlying mechanism in radiation resistance and metastasis is likely linked to metabolic changes caused by mitochondrial reprogramming. We hypothesized that ROS and mtDNA damage might be indicators of poor tumor therapy response and dissemination of rectal cancer. Methods: Three colorectal cancer (CRC) cell lines (HCT116, HT29, LoVo) were cultured under hypoxia (0.2% O2) or normoxia (21% O2) for 24 hours. In a prospective rectal cancer trial, serum was sampled from patients at the time of diagnosis. Levels of ROS were determined by the ability to oxidize dihydrochlorofluoroscein into a fluorescent product and a fluorescamine-based assay. mtDNA damage was quantified by the ability of modification on template DNA to inhibit restriction enzyme cleavage. For the study patients, histologic tumor response to neoadjuvant radiotherapy was evaluated according to standard ypTN staging and tumor regression grade (TRG) scoring. Groups were compared using Student’s t-test. Survival differences were assessed using the log-rank test. Results: Compared to normoxic conditions, hypoxia significantly reduced ROS levels in all CRC cell lines. Moreover, in serum samples, ROS levels were lower for T2 cases than for patients with T2–3 disease (p = 0.037), and accordingly, low circulating ROS was associated with adverse metastasis-free survival (MFS; p = 0.004) and overall survival (p < 0.001). Hypoxic HCT116 and HT29 cell lines, but not the LoVo cells, were significantly higher mtDNA damage than under normoxia. Given the indicated dependence of ROS and mtDNA damage to hypoxia, a low ratio of ROS to mtDNA damage turned out to be a promising indicator of hypoxic CRC cells. This ratio was lower in serum samples from patients who obtained poor histologic tumor response to radiotherapy (ypT3–4 versus ypT0–2 and poor versus good TRG; p = 0.044 for both). Accordingly, a high ratio of circulating ROS to mtDNA damage was associated with longer MFS (p = 0.021). Conclusion: We showed that culturing of CRC cells under hypoxia caused decrease in ROS levels but increase in mtDNA damage. Interestingly, in serum samples from rectal cancer patients, low ROS levels were associated with T4 disease, representing a bulky and often hypoxic tumor, and thus adverse survival. Moreover, low ratio of ROS to mtDNA damage was correlated with poor histologic tumor response to neoadjuvant radiotherapy. These parameters may reflect an aggressive biological phenotype instigated by a host tumor microenvironment. The results are currently under validation in an independent patient cohort. Trial registration: NCT00278694.

#1782 Reactive oxygen species (ROS) and mitochondrial DNA (mtDNA) damage in tumor hypoxia, poor radiotherapy response, and metastatic progression of rectal cancer. Paula A. Bousquet,1 Sebastian Meltzer,1 Linda Sondrholm,2 Ying Ebens,9 Lars G. Lyckander,1 Svein Dueland,1 Kjersti Flatmark,2 Kathrine R. Redalen,1 Lars Eide,8 Anne H. Ree1. 1Akershus University Hospital, Lørenskog, Norway; 2Oslo University Hospital, Oslo, Norway; 3University of Oslo, Oslo, Norway.

Background: Hypoxia is an important feature of the tumor microenvironment and correlates with radiotherapy resistance and metastatic progression. Additionally, as the malignant phenotype promotes aerobic glycolysis and contributes to the microenvironmental acidification, an underlying mechanism in radiation resistance and metastasis is likely linked to metabolic changes caused by mitochondrial reprogramming. We hypothesized that ROS and mtDNA damage might be indicators of poor tumor therapy response and dissemination of rectal cancer. Methods: Three colorectal cancer (CRC) cell lines (HCT116, HT29, LoVo) were cultured under hypoxia (0.2% O2) or normoxia (21% O2) for 24 hours. In a prospective rectal cancer trial, serum was sampled from patients at the time of diagnosis. Levels of ROS were determined by the ability to oxidize dihydrochlorofluoroscein into a fluorescent product and a fluorescamine-based assay. mtDNA damage was quantified by the ability of modification on template DNA to inhibit restriction enzyme cleavage. For the study patients, histologic tumor response to neoadjuvant radiotherapy was evaluated according to standard ypTN staging and tumor regression grade (TRG) scoring. Groups were compared using Student’s t-test. Survival differences were assessed using the log-rank test. Results: Compared to normoxic conditions, hypoxia significantly reduced ROS levels in all CRC cell lines. Moreover, in serum samples, ROS levels were lower for T2 cases than for patients with T2–3 disease (p = 0.037), and accordingly, low circulating ROS was associated with adverse metastasis-free survival (MFS; p = 0.004) and overall survival (p < 0.001). Hypoxic HCT116 and HT29 cell lines, but not the LoVo cells, were significantly higher mtDNA damage than under normoxia. Given the indicated dependence of ROS and mtDNA damage to hypoxia, a low ratio of ROS to mtDNA damage turned out to be a promising indicator of hypoxic CRC cells. This ratio was lower in serum samples from patients who obtained poor histologic tumor response to radiotherapy (ypT3–4 versus ypT0–2 and poor versus good TRG; p = 0.044 for both). Accordingly, a high ratio of circulating ROS to mtDNA damage was associated with longer MFS (p = 0.021). Conclusion: We showed that culturing of CRC cells under hypoxia caused decrease in ROS levels but increase in mtDNA damage. Interestingly, in serum samples from rectal cancer patients, low ROS levels were associated with T4 disease, representing a bulky and often hypoxic tumor, and thus adverse survival. Moreover, low ratio of ROS to mtDNA damage was correlated with poor histologic tumor response to neoadjuvant radiotherapy. These parameters may reflect an aggressive biological phenotype instigated by a host tumor microenvironment. The results are currently under validation in an independent patient cohort. Trial registration: NCT00278694.


Background: Inhibition of thymidylate synthase (TS) results in a transient compensatory “flare” in thymidine salvage pathway activity which is measurable with 18F-thymidine (FLT) positron emission tomography (PET) at 2 hrs of exposure to therapy. Here we examine the predictive value of the FLT-PET measured “flare” for NSCLC sensitivity to pemetrexed, a commonly used TS inhibitor, in a preclinical model. Experimental Design: Resistance to pemetrexed therapy was induced in two sensitive human NSCLC cell lines, H460 and H1299, by overexpressing TS. TS overexpression was confirmed with RT-PCR and Western blotting and pemetrexed-resistance confirmed with IC50 assays. The presence of a pemetrexed-induced DNA salvage pathway “flare” was then measured using UH-thymidine in both pemetrexed-resistant (H460 and H1299) and resistant (H460R and H1299R) lines in vitro, as well as inherently resistant NSCLC cell lines CALU6, H522, H650, H661, H820, H1838, and validated with FLT-PET in vivo using H460 and H460R xenografts. Results: Overexpression of TS induced resistance to pemetrexed in the H460 and H1299 with IC50 for H460, H1299, H460R and H1299R measured as 0.141 μM, 0.656 μM, 22.842 μM, 213.120 μM, respectively. Significant increases in DNA salvage pathway activity (“flare”) was observed at 2hrs of therapy in the pemetrexed-sensitive H460 and H1299 lines but not the resistant H460R and H1299R, CALU6, H522, H650, H661, H820, H1838 cell lines in vitro using “flare”-thymidine assays. Similarly, FLT “flare” was observed in the pemetrexed-sensitive H460 xenograft tumors but not the pemetrexed-resistant H460R xenograft tumors with post-therapy increases in measured FLTmax of 58% (STD 12.1) and 10.8% (STD 7.3) respectively. Conclusions: FLT-PET imaging of TS inhibition may provide an early indicator of NSCLC sensitivity to pemetrexed.

We have previously described a targeted genomic Laboratory Developed Test (LDT) that includes variant specific Droplet Digital™ PCR (ddPCR) testing for EGF, KRAS and BRAF in plasma. This test supports the rapid delivery of molecular diagnostic test results, with >95% of tests results delivered in 72 hours of receipt in our Laboratory. This test then meets the key clinical need for the delivery of results that can result in faster treatment decisions. Additionally, the test may provide utility to those patients whose mutation results are unknown or for whom tissue may be unavailable or insufficient for molecular testing. This is especially true for patients diagnosed with non-small cell lung cancer (NSCLC). In this report we will update on new test concepts using the recently available design software engine for ddPCR assays. Specifically, we will describe studies on the development of single-reaction, multiplexed assays for the respective detection of ROS1 (up to 11 variants), RET (up to 8 variants) and EML4-ALK (v1 - v3). Design considerations, specificity and sensitivity, as well as reproducibility and robustness studies for these complex assays will be reviewed. Similar studies were conducted for the development of the commercially available test for the EML4-ALK fusion variants. EML4-ALK concordance studies compared the fusions found in blood with known positives and negatives found using FISH and PCR based methods. Clinical sensitivity, specificity and concordance were 85%, 100% and 92% respectively. In this study we also report on test performance of the ALK RNA fusion test over 3 consecutive months of testing. Of note, we have delivered greater than 95% of tests (n = 272 samples) with an observed positive sample frequency of 2.0%. The robust detection of rare variant, RNA fusions from plasma within 72 hours represents a molecular testing option of value to patients with cancer. This test then meets the key clinical need for the delivery of results that can result in faster treatment decisions. Additionally, the test may provide utility to those patients whose mutation results are unknown or for whom tissue may be unavailable or insufficient for molecular testing. This is especially true for patients diagnosed with non-small cell lung cancer (NSCLC). In this report we will update on new test concepts using the recently available design software engine for ddPCR assays. Specifically, we will describe studies on the development of single-reaction, multiplexed assays for the respective detection of ROS1 (up to 11 variants), RET (up to 8 variants) and EML4-ALK (v1 - v3). Design considerations, specificity and sensitivity, as well as reproducibility and robustness studies for these complex assays will be reviewed. Similar studies were conducted for the development of the commercially available test for the EML4-ALK fusion variants. EML4-ALK concordance studies compared the fusions found in blood with known positives and negatives found using FISH and PCR based methods. Clinical sensitivity, specificity and concordance were 85%, 100% and 92% respectively. In this study we also report on test performance of the ALK RNA fusion test over 3 consecutive months of testing. Of note, we have delivered greater than 95% of tests (n = 272 samples) with an observed positive sample frequency of 2.0%. The robust detection of rare variant, RNA fusions from plasma within 72 hours represents a molecular testing option of value to patients with cancer.


Background: With new developments of oncology drug combinations and targeted treatments, the ability to stratify categories of patient populations and to develop companion diagnostics has become increasingly important. A panel of 325 RNA-biomarkers was selected based on cancer-related biological processes of healthy cells and gene expression changes over time during non-malignant epithelial cell organization. This 'cancer in reverse' approach in combination with empirically derived algorithms resulted in a panel of biomarkers, having little overlap with 9 other widely-used commercial gene panels analyzed by dChip at (e.g., overlap with FoundationOne was 2% (lowest) and with OncoTyperDX was 14% (highest)), providing a more in-depth and comprehensive view of gene expression profiles and key cellular signaling pathways beyond mutations in 'driver-genes', and drug associations including chemotherapies, immunotherapies, and targeted-therapies. Objective: In order to technically validate an assay for the 325 RNA-biomarkers we compared gene expression profiles side-by-side using two technology platforms to address the reproducibility of the assay. Methods and Results: We have mapped the 325 RNA transcripts and 7 housekeeping genes in a custom NanoString n-counter expression panel to be compared to all potential probe sets in the Affymetrix Human Genome U133 Plus 2.0 microarray. The experiments were conducted with 10 unique biological formalin-fixed paraffin-embedded (FFPE) breast tumor samples. Each site extracted RNA from four sections of 10-microns thick FFPE tissue over three different days by three different operators using an optimized standard operating procedure and quality control criteria. Samples were analyzed using mass in BioConductor and NanoStringNorm in R. Pearson correlation showed reproducibility between sites for all 60 samples with an r of 0.992 for Affymetrix and r = 0.999 for NanoString. Correlation in multiple days and multiple users were for Affymetrix r = (0.962 - 0.992) and for NanoString r = (0.982 - 0.991). The platforms were compared using relative expression fold changes using linear regression (lm). Concordant genes were defined to have gene expression levels within t+/- 2 standard deviation (sd) of each other at 95% confidence interval, or to have greater than t+/- 2 sd but have changes in the same direction in both platforms. The discordant genes were defined to have opposite direction of changes. By this definition, approximately 90% of the genes fell into the concordant category. Conclusion: The 325 RNA-biomarkers showed reproducibility in two technology platforms with high concordance. With five predictive tests under development for breast (3), lung and pancreatic cancers future directions include performing clinical validation studies and generate rationale for patient selection in clinical trials using the technically validated assays.

#1786 Predictive value of LRPl, KNPNA2 and GDF15 expression to anthracycline/taxane based chemotherapy response in patients with locally advanced breast cancer. Hector Maldonado-Mtz1, Alison-Luna Oscar1, Ali Flores-Perez2, Meneses-Garcia-Abelardo1, Erika B. Ruiz-Garcia1, Cesar Lopez-Camarillo1, Horacio Astudillo-de-la Vega1. 1InCan, Mexico, DF, Mexico; 2Nano-pharmacia Diagnostica, Mexico, DF, Mexico; 1UACM, Mexico, DF, Mexico; 2IMSS, Mexico, DF, Mexico.

Background: Chemotherapy plays a major role in its treatment, though over-treatment is frequent since its choice is based on clinicopathological data. The identification and validation of new predictive profiles or markers continues to be relevant. New findings may contribute to achieve better results in this area, as well as to provide new targets of the pathways involved in chemoresistance. Karyopherin a2 (KNPNA2), low density lipoprotein receptor-related protein 8 (LRP8) and growth and differentiation factor 15 (GDF15), are three candidate predictive biomarkers in breast cancer, previously identified on DNA microarrays reports. Methods: Using tissue microarrays technology and immunohistochemistry, we compared the expression of these three markers between cases of breast cancer with response to chemotherapy (absence of recurrence), and those with failure (with recurrence). All patients received chemotherapy based on anthracyclines and taxanes. Potential confounding factors, as known recurrence and progression risk factors, were controlled for during the comparison. Results: 20 out of 63 patients had tumor recurrence. Potential confounding factors were similar among cases with response and failure to chemotherapy. For KNPNA2 no differences were found between cases with response or failure. LRP8 showed higher expression in cases with failure to chemotherapy (mean 7.2 vs 14.01%, P = 0.025). In Cox multivariable regression analysis, which included potential confounding factors as covariates, only LRP8 remained as a significant predictive factor (HR 1.28, p = 0.016). GDF15 showed no expression in our cases, nor in an external set of cases. Conclusions: We demonstrated increased expression of LRPl in breast cancer cases recurring after chemotherapy. KNPNA2 and GDF15 showed no predictive value for chemotherapy response in our study. LRPl is a potential predictive marker worth of further investigation for its role in chemoresistance as well as for its potential validation.
## CLINICAL RESEARCH: Predictive Biomarkers

### #1788 Extra cellular micro vesicle (EMV) miRNA signature as putative predictive biomarker for targeted therapy in stage IV cutaneous malignant melanoma.


Purpose: Tumor-specific targeted therapy against activated-protein kinase (MAPK) pathway induce rapid responses and improved survival in patients with disseminated BRAFV600mutant cutaneous malignant melanoma (CMM), remissions are often short and remission occurs. There is a need to identify biomarkers predicting durable responses. We aimed to correlate the expression of extracellular micro vesicle (EMV) microRNAs in plasma samples from stage IV CMM patients with MAPK inhibitors use and treatment outcome. Patients and methods: EMV miRNAs signatures analyzed with micro array from plasma samples collected before and during therapy with MAPKi were correlated to response and progression-free survival (PFS) in 28 Stage IV CMM patients treated at the Dept of Oncology, Karolinska University Hospital, Sweden. Clinical parameters i.e. sex, age, lactate dehydrogenase (LDH) levels, before and during treatment, were also included. Results: Higher OS levels before treatment were significantly correlated with a PFS (Hazard Ratio (HR) = 2.088; 95% CI = (1.032, 4.226); P = 0.041). Increasing expression of EMV MiR-let-7g-5p during treatment was significantly correlated to a lower risk of not responding to MAPKi(Odds ratio = 0.0004; 95% CI = 0.000 - 0.207, P = 0.001). High levels of MiR-997-5p in plasmatic EMV during therapy were associated with lower risk of progression during therapy (HR = 0.27; 95% CI = 0.13-0.52, P < 0.0001). Conclusions: Our results indicate that assessing the miRNAs MiR-let-7g-5p and miR-997-5p in samples collected before and during treatment may have a potential as predictive markers of treatment response and PFS in Stage IV CMM patients with MAPKis.

### #1789 A gene expression-based nomogram predicts overall and progression free survival in PRRT-treated gastroenteropancreatic neuroendocrine tumors.

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Background: Nomograms and grading/staging systems are fundamental in guiding oncology disease management. Histological grading has an efficacy in the management of gastroenteropancreatic neuroendocrine tumors (GEP-NET) but tumor heterogeneity renders outcome unpredictable. Clinical parameters alone have limited prognostic ability. A previously described NET nomogram calculated risk for overall survival (OS). To augment accuracy, we developed a circulating NET multi gene expression blood test. The NETest exhibits high level of accuracy as a molecular prognostic for progression free survival (PFS). We evaluated whether combining clinical information with the NETest would provide accurate prognostic information for PRRT treatment. Aim: Create a combined clinical and gene expression nomogram to predict OS and PFS in PRRT-treated GEP-NETs. Methods:1) 1285-based PRRT-treated GEP-NETs (n=57; median age 65 yrs (31-83); 34M:23F; smallbowel(47%),pancreas(35%),predominantly grade2; 41inpancreasand39vs.21incolon, respectively). Conclusions: The current data show a correlation trend between UCK2 protein expression level and degree of antitumor activity of RX-3117 in xenograft models. It also supports a higher UCK2 protein expression level in human cancer tissues compared to normal tissues. This suggests that RX-3117 activity may be specific to tumor tissue, and quantification of UCK2 expression in human cancer tissues may be useful as a predictive biomarker to select patients for their sensitivity to RX-3117 in future clinical studies.

### #1791 Spatially positioned tumor infiltrating lymphocytes predict survival in metastatic HER2 positive breast cancer treated with trastuzumab.

Jussi Pekka Koivunen, Tiia Honkanen, Tiia Moilanen, Peeter Karitahla, Juha Väyrynen, Markus Mäkinen. Oulu Univ. Hospital, Oulu, Finland.

Background: Disease outcomes of HER2+ breast cancers have dramatically changed after targeted therapies, such as trastuzumab, came to clinical practice but predictive factors for trastuzumab sensitivity and resistance are frequently unknown. Methods: Metastatic breast cancer patients (n=48), who were treated with trastuzumab and had pre-treatment tumor samples available, were studied. The tumors were immunostained for T-cell (CD3, CD8) and NK-cell (CD56) markers and quantitative analysis of the immune cells was carried out using a computer-assisted image analysis in different tumor locations. Results: High number of CD3 and CD8 positive T-cells was associated with significant survival benefit in the center of the tumor (CT) (p = 0.007, p = 0.001), but not in the invasive region. NK-cell tumor infiltration was infrequent and they could not be reliably analyzed. In a subgroup analyses, high density of CD8 CT cells was associated with significant survival benefit in non-bone only disease, in TX-3, and in ER+ tumors (p = 0.006, p = 0.003, p = 0.001). Moreover, high CD8 CT cell density was associated with good trastuzumab responses (p = 0.042). Conclusion: High number of CD3 and CD8 positive tumor infiltrating lymphocytes in the CT area is associated with survival benefit in some patient groups with HER2+ breast cancer treated with trastuzumab. Furthermore, high number of CD8 CT cells predicts benefit from trastuzumab.

### #1792 Immune system pathway activation for prediction of the response to neoadjuvant chemotherapy in triple-negative breast cancer.

Takeshi Sawada,1 Riet Hilhorst,2 Satishri Rangarajan,1 Masayuki Yoshida,1 Kenji Towa,1 Rink canvas I and molon,2 Rob Ruijtenbeek,1 Hideto Tsuda,1 Fumio Koi zuomi1. 1Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, Japan; 2PamGene International BV, ’t Hertogenbosch, Netherlands; National Cancer Center Hospital, Tokyo, Japan; National Defense Medical College, Saitama, Japan.
Background: Triple-negative breast cancer patients (TNBCs) who receive neoadjuvant chemotherapy (NAC) generally show a response to NAC, but about 5% experience progressive disease (PD). Although TNBCs that respond to NAC have been well-studied, reports about TNBCs with PD during NAC are absent. We aimed to compare kinase activity profiles of TNBCs of both groups to identify a biological predictor of NAC non-responders. Methods: Tyrosine kinase activity profiles of lysates of fresh frozen cancer tissues from NAC non-responders and responders, the latter represented by TNBCs who did not receive NAC, were determined with a peptide microarray system (Cancer Res. 2009; 69(14):3987-95). Tumor infiltrating lymphocytes (TILs) were measured using quantitative real-time polymerase chain reaction (PCR)-based method. ANOVA and non-parametric tests were applied to compare the RTL and mtDNA copy number between groups. Prophylaxis methods. The study was conducted in 120 patients with pathologic TRTs and mtDNA copy number (CRC). In this study, we comprehensively evaluated whole-genome methylation analysis using CRC tissues and normal colonic mucosa to discover candidate CRC-specific DNA methylation as potential diagnostic and predictive biomarkers for risk stratification in CRC patients. Patients and Method: The study design included an initial discovery phase, and followed by a subsequent clinical validation phase. In discovery phase, we performed whole-genome methylation analysis using CRC tissues and normal colonic mucosa from total fourteen patients to identify CRC-specific methylated CpG sites. Furthermore, we evaluated methylation levels of candidate methylated CpG sites by quantitative bisulfite pyrosequencing using one hundred six colorectal specimens from fifty-three CRC patients to validate the findings from discovery phase. Result: In discovery phase, we successfully identified several candidates of CRC-specific hypermethylated- and hypomethylated-CpG sites in CRC tissues compared with normal colonic mucosa. Promoter lesion of PDX1 is one of candidate methylated CpG sites in discovery phase, and we focus a specific CpG site of PDX1 promoter lesion as a target site to progress in validation phase. We quantified methylation status of PDX1 using 106 colorectal tissues, and demonstrated that PDX1 were significantly hypermethylated in CRC tissues compared with normal mucosa (p<0.0001, Wilcoxon rank correlation test). Receiver operating characteristic (ROC) curves analysis revealed that methylation levels of PDX1 remarkably discriminated CRC tissues from normal colonic mucosa (AUC=0.3, Sensitivity:85.4%, Specificity:91.5%). Interestingly, methylation levels of PDX1 tend to increase in a stage-dependent manner, and hypermethylated status of PDX1 was significantly correlated with presence of distant metastasis in validation cohort. Conclusion: Assessment of the PDX1 methylation status could be used as potential of diagnostic and predictive biomarker for risk stratification in CRC patients.
#1796 A targeted RNA-seq assay to measure activating ER mutations and ER/PR-associated gene expression predicts sensitivity to endocrine therapy for metastatic breast cancer. Rosanna Lau,1 Lily Fu,2 Michael Samuel,2 Rashmi K. Murthy,1 Bruno Sinn,1 Yune Xu,1 Rebekah Gould,1 Jennifer Litton,1 Alda Tam,1 Stacy Moulder,1 Daniel Bosser,1 Debri Tripathy,1 Vicente Valero,1 Fraser Symmans2 and 1University of Buffalo, Buffalo, NY; 2Univ. of Cincinnati College of Medicine, Cincinnati, OH.

Head and neck cancer will account for an estimated 61,760 new cancer cases and 13,190 cancer-related deaths in the United States in 2016, the majority of which (> 90%) will be squamous cell carcinoma (HNSCC). The prognosis for HNSCC is relatively poor, with an overall 5-year survival around 60%, and the outlook worsens with increasing stage at diagnosis, which is problematic since about two-thirds of patients present with advanced stage disease. This underscores the urgent need for discovery, development and translation of novel biomarkers to facilitate early detection of HNSCC and bolster the chance for positive patient outcomes. Exosomes are nano-sized (40-150 nm) membrane-encapsulated vesicles that may offer a new avenue for discovery and development of novel HNSCC biomarkers. However, they are released by essentially all cells, both normal and malignant, into the extracellular space, resulting in a noisy biological signal and a lack of effectiveness for reliable survival analysis. Conclusion: The RNA-seq assay measured the percent of ER transcripts with activating LBD mutation (>1% prevalence) and also downstream ER/PR-related transcription. High LBD-positive ER/PR predicted longer progression-free (PFS) (HR=0.37, p=0.0004, Δ median PFS 9 months) and overall survival (OS) (HR=0.49, p=0.03). The predictions were more pronounced in patients without LBD mutation (PFS HR=0.32, p=0.001, Δ median PFS 13 months; OS HR=0.42, p<0.01). Currently, there are insufficient cancer-related biopsies for reliable survival analysis. Conclusion: The RNA-seq assay measured the percent of ER transcripts with activating LBD mutation (>1% prevalence) and also downstream ER/PR-related transcription. High LBD-positive ER/PR predicted longer progression-free (PFS) (HR=0.37, p=0.0004, Δ median PFS 9 months) and overall survival (OS) (HR=0.49, p=0.03). The predictions were more pronounced in patients without LBD mutation (PFS HR=0.32, p=0.001, Δ median PFS 13 months; OS HR=0.42, p<0.01). Currently, there are insufficient cancer-related biopsies for reliable survival analysis.

#1797 EGFR as a marker for head and neck squamous cell carcinoma-derived small extracellular vesicles. Jenna A. Dombroski,1 Damaris Kuhnell,2 Susan Kaspar,3 Scott M. Langevin,1 University of Buffalo, Buffalo, NY; 2Univ. of Cincinnati College of Medicine, Cincinnati, OH.

EGFR is a member of the ErbB/HER family of receptor tyrosine kinases, localizes to the plasma membrane and is over-expressed in as much as 90% of HNSCC tumors. Therefore, we hypothesized that EGFR will be over-represented on membranes of HNSCC-derived exosomes and thus may offer utility as a surface marker for detection and/or enrichment. To test this, we cultured an HNSCC cell line that over-expresses EGFR (FaDu), EGFR-deficient SW620 colorectal adenocarcinoma cells, and primary human oral epithelial cells pooled from 3 healthy donors. Cells were cultured in supplier-recommended media with 10% fetal bovine serum (FBS) that was super-depleted of exosomes via 18 hour ultracentrifugation at 100,000g and 1% penicillin/streptomycin at 37°C with 5% CO2 in 150 cm2 flasks with 25mL media. After reaching 80-90% confluence, media was harvested, and exosomes were isolated and purified via differential ultracentrifugation. Exosome isolates were verified via transmission electron microscopy (TEM) and manually inspected for EGFR and imaging on a JEOL JEM-1230 transmission electron microscope (Immuno-EM; anti-nucleoporin 153 (NUP-153), which localizes to the nucleus, was used as a negative control. Immuno-EM images indicate abundant EGFR expression on exosomes secreted by FaDu HNSCC cells, but EGFR is not detectable on the exosomes secreted by the oral epithelial cells from healthy donors. These results indicate that EGFR may offer utility for enrichment for HNSCC-derived exosomes in human biofluid isolates using magnetic beads or flow cytometry.

#1798 MET expression in pancreatic adenocarcinoma correlates with survivin expression and the proliferative activity. David M. Saulino, Pamela S. Younes, Jennifer M. Bailey, Mamoun Younes. University of Texas Health Science Center at Houston McGovern Medical School, Houston, TX.

Introduction: CRM1 is a nuclear export chaperone that mediates the export of proteins essential to growth regulation and tumor suppression. Its overexpression in tumors was found to be associated with poor prognosis. Selective inhibitors of nuclear export are in phase I and II clinical trials for several tumor types. The expression of CRM1 in human pancreatic adenocarcinoma (PAC) and its relation to survivin expression and tumor proliferative activity is largely unknown. Experimental procedures: Sections of tissue microarrays containing 77 formalin fixed and paraffin embedded PAC were stained by immuno-histoch- emistry (IHC) for CRM1, survivin, and Cyclin A. Expression levels of CRM1 and survivin in tumor cells was determined using a quantitative digital image analysis solution (OTMIAS). The tumor proliferative activity was determined by measuring the S-phase fraction (SPF) in sections stained for Cyclin A, also using OTMIAS. Summary: Sixty-six of the 77 (86%) PAC showed at least some posi-

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tive staining for CRM1, and 11 (14%) were completely negative. The mean and median expression levels of CRM1 in tumor cells with the mean and median levels of survivin (p<0.001). Moreover, there was positive correlation between the mean and median expression levels of CRM1 in tumor cells with the mean and median levels of survivin (p<0.001). Additionally, there was positive correlation between the mean and median expression levels of CRM1 in tumor cells with the mean and median levels of survivin (p<0.001). Furthermore, there was positive correlation between the mean and median expression levels of CRM1 in tumor cells with the mean and median levels of survivin (p<0.001). Consequently, the cell-free DNA (cfDNA) represents a minimally invasive and alternative source of tumor DNA for molecular profiling. Despite next-generation sequencing (NGS) technique is qualified for genotyping cancer using cfDNA as a non-invasive method, it has caused problems as sequencing error and reproducibility. cfDNA in plasma and gDNA of Peripheral Blood Mononuclear Cells (PBMC) were isolated from each 54 advanced colorectal cancer patients. 39 available tumor tissues were isolated from same patients. Deep target-sequencing was performed with paired-end library enriched exons of 10 genes which are recurrently mutated in colorectal cancer. To reduce sequencing error, we devised ‘Denoising’ and calculated concordance of somatic variants between cfDNA and tumor tissue sequencing data. In addition, correlation of concordance data was analyzed with the clinical information. As a result, we selected or could detect clinically important somatic alteration among low/high variant allele frequency (0.31%–79.42%). For somatic alteration of 10 genes, sensitivity, specificity and accuracy were increased from 84.5%, 74.6% and 76.9% to 87.6%, 92.0% and 91.1% respectively after ‘Denoising’. On the other hand, patients with high cfDNA concentration (>50ng/ml) had higher somatic mutant fragments and larger metastatic lesion in liver than patients who have low cfDNA concentration. Our study showed that denoised deep target-sequencing is a suitable method for cfDNA genotyping and provides insights into strategies for monitoring metastatic lesion of advanced colorectal cancer.

TUMOR BIOLOGY: Angiogenesis and Vascular Biology 2

#1801 Antiangiogenic agents targeting different angiogenic pathways have opposite effects on tumor hypoxia in R-18 human melanoma xenografts. Jon-Vidar Gaustad, Trude G. Simonsen, Lise Mari K. Andersen, Einar K. Rofstad. Institute for Cancer Research, Oslo University Hospital, Oslo, Norway.

Background: Studies comparing the effect of antiangiogenic agents targeting different angiogenic pathways are sparse. The purpose of this study was to compare the effect of antiangiogenic agents on the morphology of tumor vasculature. To this end, we assessed the immunohistochemical preparations of the imaged tissue using pimonidazole as a hypoxia marker. Results: Properdinstatin treatment selectively removed small-diameter vessels and reduced BST, whereas sunsitib treatment reduced the density of small- and large-diameter vessel similarly and did not change BST. These observations imply that properdinstatin treatment reduced geometric resistance to blood flow and improved vascular function, whereas sunsitib treatment did not affect vascular function. Accordingly, sunsitib-treated tumors showed higher hypoxic fractions than properdinstatin-treated tumors. Conclusions: Properdinstatin and sunsitib both inhibited angiogenesis, but had distinctly different effects on vascular morphology, vascular function, and extent of hypoxia in R-18 human melanoma xenografts.

#1802 Apoptosis pathway-focused gene expression profiling of a novel VEGFR2 inhibitor. Mohammad Algahtani,1 Khalid Alhazzani,2 Thiagarajan Venkatesan,2 Appu Rathinavelu1. 1Nova Southeastern University, College of Pharmacy, Davie, FL; 2Rumbaugh-Goodwin Institute for Cancer Research, Nova Southeastern University, Davie, FL.

Sprouting of the new blood vessel (angiogenesis) is fundamental to tumor growth, invasion, and metastatic dissemination. Vascular endothelial growth factor (VEGF), an endothelial cell-specific mitogen, promotes cell survival via opposing apoptosis. On the other hand, antagonizing VEGF pathway suppresses endothelial cell overgrowth and promotes apoptotic signals leading to the regression of the existing vasculature and prevents neovascularization of the tumor. In this respect, a new anti-angiogenic agent, code named as F16, that was developed in our institute can effectively stop VEGF-driven angiogenesis by selectively blocking VEGFR2 and the downstream signals. Our previous studies have revealed that human umbilical vein endothelial cells (HUVECs) undergo apoptosis in response to F16 treatment. However, in the present study, our main focus was on investigating the status of the signal mediators of the apoptotic pathway after treating the HUVECs with F16. This was expected to outline the intracellular sequence of events involved in triggering apoptosis following F16 treatment. For this purpose, we analyzed apoptosis pathway-focused gene expression in HUVECs using the human apoptosis RT2 profiler which contains 84 key genes involved in regulation of programmed cell death. Our experimental results clearly identified up-regulated pro-apoptotic genes in response to F16 treatment such as FAS (FASL), TNF (TNF-α and TNF-β) and BCL2 (BCL2 and BCL2L10), tumor necrosis factor superfamily member 9 (TNFFRSF9), FAS cell surface receptor (FAS), FAS ligand (FASLG), lymphoxygen alpha (LTA), caspase 5 (CASP5), and cytokrome c (CYCS). Moreover, a few anti-apoptotic genes were also up-regulated which include B-cell lymphoma 2 related protein A1 (BCL2A1), caspase 14 (CASP14), Bc2 associated anagathone 3 (RAG3), and interleukin 10 (IL-10). However, anti-apoptotic genes such as Bcl-2, Bcl-2 like 10 (BCL2L10), baculoviral IAP repeat containing -3 and -6 (BIRC-3, and BIRC-6), CASP8 and FADD like apoptosis regulator (CFLAR), and insulin like growth factor 1 receptor (IGF1R) were found to be significantly downregulated. Interestingly, no changes were found in the expression of genes related to DNA damage and repair pathways such as TP53, ABL1, CIDEA, and CIDEB. This indicates that F16 may not provoke DNA damage induction or apoptosis. Instead, it stimulates the intrinsic or the extrinsic pathway. In conclusion, the diverse gene expressions implicating apoptosis related factors showed that F16 can induce apoptosis via potentiation of TNF-α and FAS signaling. (This research was supported by the generous funds provided by the Royal Dames of Cancer Research Inc., Ft. Lauderdale, Florida)

#1803 Combining anti-Ang-2/VEGF-A therapy with radio- and chemotherapy in glioma. Gergely Solecki,1 Matthias Osowski,1 Weber Daniel,1 Malte Glick,1 Miriam Ratliff,2 Hans-Joachim Müller,2 Oliver Krietler,1 Yvonne Kienast,2 Wolfgang Wick,1 Frank Winkler1. 1German Cancer Research Center (DKFZ), Heidelberg, Germany; 2Roche Innovation Center, Munich, Germany.

Angiogenesis is a biological hallmark of malignant gliomas, but antiangiogenic strategies especially targeting the VEGF axis did not show striking anti-tumor activities in the majority of patients so far. Other pathways may be more relevant in selected tumor entities or patients. Further, it remains unresolved which antiangiogenic combination regimen with standard radio- and/or chemotherapy is most effective. Therefore, we compared the therapeutic effects of anti-VEGF-A, anti-Ang-2, and bispecific anti-Ang-2/VEGF-A antibodies, alone and in combination with radio- or temozolomide (TMZ) chemotherapy in a malignant glioma model using multi-parameter two-photon in vivo microscopy in mice. We demonstrate that anti-Ang-2/VEGF-A leads to strongest vascular changes including vascular normalization, both as monotherapy and when combined with chemotherapy. The latter combination regimen was accompanied by most effective chemotherapeutic induced death of cancer cells independent of blood vessel proximity indicative of a better distribution of TMZ throughout the tumor. Furthermore, the combination of anti-Ang-2/VEGF-A plus TMZ consistently resulted in decreased tumor cell motility, and decreased formation of resistance-associated tumor microbubbles (TMs), which finally lead to best tumor growth inhibition. Remarkably, all these parameters were just reverted when radiotherapy was chosen as combination partner. In contrast, when anti-VEGF-A was combined with radiotherapy, vascular normalization was highest, and TM length, nuclear motility and tumor growth were concordantly reduced. In conclusion, while TMZ chemotherapy benefits most from combination with anti-Ang-2/VEGF-A, radiotherapy does from anti-VEGF-A.
The findings imply that unexpected, even divergent effects can occur when a specific antiangiogenic therapy is added to chemo- or radiotherapy in glioma, and that uninformed combination regimens should be avoided.


Exogenous nucleoside diphosphate kinase (eNDPK or NM23) has been shown to promote endothelial cell proliferation and migration and tumor-mediated angiogenesis. This is facilitated by its transphosphorylase activity, in which a gamma terminal phosphate group from a triphosphate nucleoside is transferred to a diphosphate nucleoside, resulting in elevated ATP levels. Increased levels of ATP in the tumor microenvironment can activate purinergic receptors (P2Y1) on adjacent endothelial cells to promote angiogenesis, independent of VEGF stimulation. Triple negative human breast cancer (MDA-MB-231) cells have been shown to elaborate exosomes that contain NM23. These exosomes have implications in targeting specific tissues/cells to promote angiogenesis and tumorigenesis. Our lab has shown that inhibition of eNDPK and the P2Y1 receptor reduces endothelial cell tubulogenesis (Rumjaunt, et al., 2007) and breast cancer metastasis (Yokdang, et al., 2015). Therefore, targeted inhibition of eNDPK may have implications as a treatment for early tumor-mediated angiogenesis. For this study, we utilized a transphosphorylation activity assay to perform a drug screen on compounds from the Prestwick chemical library. Recombinant NDPK-B was added to FDA-approved drug compounds along with ATP and UTP substrates. Substrate phosphorylation was measured, and compounds that increased luminescence signal/inhibition of NDPK was compared to ellagic acid control, a known inhibitor of NDPK. From our initial screen, we identified eight potential compounds that inhibited NDPK at comparable levels to ellagic acid. These eight compounds were further examined to determine the dose response curve. We found that Chicago Sky Blue 6B inhibited NDPK at an IC50 value of 1.88 μM. The results from this study demonstrate a drug screening method to discover alternative small molecule inhibitors of NDPK as a potential treatment of angiogenesis and breast cancer metastasis.


Hepatocellular carcinoma (HCC) is a one of the leading causes of cancer death worldwide; however, systemic therapeutic option for unresectable HCC is limited. Liver carcinogenesis is a complex process and various pathways have been found to be deregulated. Of those pathways, aberrant activation of fibroblast growth factor (FGF) signaling and fibroblast growth factor receptor (FGFR) axis has been hypothesized to participate in the development of HCC with poor prognosis. Lenvatinib mesilate (lenvatinib) is an orally administered multi-targeted tyrosine kinase inhibitor (TKI) that selectively inhibits vascular endothelial growth factor receptors (VEGFR1-3, FGFR1-4, platelet-derived growth factor receptor (PDGFRα, RET, and KIT). In this study, we investigated the activities of lenvatinib against human hepatocellular carcinoma (HCC) cell lines with an aberrant FGF signaling pathway in tumor warrants further investigation.
pro-angiogenic characteristics. Further, we report the development and application of a novel murine implant model to demonstrate increased specificity of 231 exosomes targeting to pulmonary endothelial cells over unrelated cell lines. These results suggest the involvement of exosomes and eNDPK in pro-angiogenic communication between metastatic breast cancer cells and their vascular targets. These observations are corroborated by these cells specifically targeted endothelium in vivo, mirroring the organotrophic nature of breast cancer metastasis. Implication of eNDPK in angiogenesis and metastasis may challenge current and invasive treatment methods in favor of developing novel small molecule inhibitors of eNDPK signaling. Lastly, eNDPK can be explored as a potential biomarker for early detection of metastatic breast cancer as it uniquely appears in the serum of patients with breast cancer and not in the serum of individuals with unrelated pathologies.

### #1808 Vascular endothelial growth factor receptor-2 (VEGFR-2) N-glycosylation modulates angiogenic signaling

Kevin B. Chandler, Deborah R. Leon, Rosana D. Meyer, Nader Rahimi, Catherine E. Costello. *Boston Univ., Boston, MA.*

Angiogenesis, the formation of new blood vessels from pre-existing vessels, is required for tumor growth and metastasis. Vascular endothelial growth factor receptor-2 (VEGFR-2) is one of the most important receptor tyrosine kinases (RTKs) among the VEGF receptor subfamily, and activation of VEGFR-2 is essential for tumor angiogenesis. The extracellular domain of VEGFR-2 contains seven immunoglobulin-like (Ig) domains, each with multiple potential N-glycosylation sites. N-glycosylation is thought to play a central role in receptor stability, ligand binding and trafficking. However, to date the occupancy and glycoform distributions at each of the potential N-glycosylation sites and their putative role(s) in VEGFR-2 function remain largely unknown. The objective of this study is to investigate the functional importance of VEGFR-2 N-glycosylation in VEGFR-2 angiogenic signaling. Porcine aortic endothelial (PAE) cells with ectopic expression of VEGFR-2 were treated with PNGase F to remove N-linked glycans or heat-denatured PNGase F as a control. Following PNGase F treatment for 4 hours, cells were treated with VEGF-A ligand for 0, 5, 10 or 30 minutes. VEGFR-2 phosphorylation (activation) was measured via Western blot with an anti-pTyr-1054-VEGFR-2 antibody. In addition, a polyclonal anti-VEGFR2 antibody was used to immunoprecipitate untreated and PNGase F-treated VEGFR-2 from PAE cell lysates. Evaluation of the N-glycosylation sites targeted by PNGase F was carried out by gel electrophoresis, followed by protease digestion and MS/MS analysis. MS/MS data were processed using Proteome Discoverer 1.4. To obtain site-specific glycosylation information we performed proteolysis of VEGFR-2, glycopeptide enrichment via hydrophilic interaction liquid chromatography (HILIC) and subsequent analysis of glycopeptides with an Agilent 6550 Quadrupole Time-of-Flight (Q-TOF) MS using collision-induced dissociation. We detected a dramatic increase in ligand-mediated activation of VEGFR-2 after treatment with PNGase F, suggesting that certain N-linked glycans may hinder ligand access to the VEGF binding site, or that removal of N-linked glycans results in conformational changes that lead to increased activation of the receptor by VEGF-A. To explore this observation in greater detail, we have created a series of VEGFR-2 N-glycosylation site mutants, and we are now using the mutants to determine which glycosylation sites are involved in the observed modulation of VEGFR-2 signaling.

### #1809 PKM2 promotes tumor angiogenesis by regulating HIF-1alpha through NF-kappaB activation.

Ninel Azoitei, Alexander Becher, Konrad Steinestel, Aurelie Rouhi, Kristina Diepold, Susanne Bobrovich, Thomas Seufferlein. *University of Ulm, Ulm, Germany; University of Muenster, Muenster, Germany.*

Initially identified as a molecule that catalyzes the phosphorylation from phosphoenolpyruvate to pyruvate in the final step of glycolysis, the M2 isoform of pyruvate kinase (PKM2) was recently reported to have a central role in the metabolic reprogramming of cancer cells as well as participating in the regulation of apoptosis and proliferation. In this study, we have identified PKM2 as a crucial molecule for progression of pancreatic cancer in which tumor microenvironment has been reported to be highly hypoxic. Our data show moderate to strong PKM2 expression in all examined human pancreatic adenocarcinoma samples. Depletion of PKM2 was associated with impaired proliferation and augmented tumor cell death in vitro, while the in vivo tumor xenograft experiments revealed a close association between impaired tumor growth and decreased blood vessel formation. Furthermore, abrogation of PKM2 prevented hypoxia-mediated HIF-1alpha accumulation and HIF-1alpha promoter activity, which negatively impacted VEGF secretion by pancreatic cancer cells deprived of oxygen. Interestingly, PKM2 expression arrest was also mirrored by impaired hypoxia-driven promoter activity of NF-kappaB which also drives the transcription of VEGF. Ectopic expression of p65 restored VEGF transcription after PKM2 ablation inferring that the kinase regulates VEGF via NF-kappaB/p65 subunit. Altogether, our study suggests that in hypoxic pancreatic tumors, PKM2 interferes both with NF-kappaB/p65 and HIF-1alpha activation that ultimately triggers VEGF-A secretion and subsequent blood vessel formation.

### #1810 Disregulation of myristoylated alanine-rich C kinase substrate (MARKS): A novel therapeutic target in renal cell carcinoma

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Targeted therapeutics, such as those abrogating hypoxia inducible factor (HIF)/VEGF signaling, are initially effective against kidney cancer (or renal cell carcinoma, RCC); however, drug resistance frequently occurs via subsequent activation of alternative pathways. Through genome-scale integrated analysis of the HIF-α network, we identified the major protein kinase C substrate MARKS (myristoylated alanine-rich C kinase substrate) as a potential target molecule for kidney cancer. In a screen of nephrectomy samples from 56 patients with RCC, we found that MARKS expression and its phosphorylation are increased and positively correlated with tumor grade. Genetic and pharmacologic suppression of MARKS in high grade RCC cell lines in vitro led to a decrease in cell proliferation and migration. We further demonstrated that higher MARKS expression promotes growth and angiogenesis in vivo in an RCC xenograft tumor. MARKS acted upstream of the AKT/mTOR pathway, activating HIF-target genes, notably VEG-F. Following knockdown of MARKS in RCC cells, the IC50 of the multi-kinase inhibitor regorafenib was reduced. Surprisingly, attenuation of MARKS using the M5 peptide synergistically interacted with regorafenib treatment and decreased survival of kidney cancer cells through inactivation of AKT and mTOR. Our data suggest a major contribution of MARKS to kidney cancer growth and provide an alternative therapeutic strategy of improving the efficacy of multi-kinase inhibitors.

### #1811 A PEAK1/GATA2 signaling axis controls VEGFR2 expression to mediate angiogenesis.

Richard I. Kleman. *UCSD Moores Cancer Ctr., La Jolla, CA.*

Formation of new blood vessels (angiogenesis) is crucial for proper development, but can also contribute to many diseases including cancer. Vascular endothelial growth factor receptor 2 (VEGFR2) is a receptor tyrosine kinase that mediates angiogenesis in developing and regenerating tissues and is frequently upregulated in many human cancers. However, the underlying mechanisms that regulate VEGFR2 expression in normal and diseased cells are poorly understood. Here we demonstrate that pseudopodium-enriched atypical kinase 1 (PEAK1) mediates developmental and tumor-induced angiogenesis by modulating VEGFR2 expression and downstream signaling. Knockout of peak1 in zebrafish using TALEN technology (Transcription Activation-Like Effector; Nucleases) or knockdown by specific peak1 morpholinos (MO) induced severe pericardial edema, blood-pooling defects, and inhibited formation of intersegmental (ISV) and subintestinal vessels (SIV). In Peak1 knockout mice, neonatal retinal vessel development was severely delayed, and the ex vivo angiogenic ability of adult thoracic aorta was greatly reduced. Intravital time-lapse imaging of ISV formation in peak1 knockout zebrafish revealed major defects in endothelial cell migration and proliferation. Similarly, PEAK1 knockdown or overexpression in HUVECs modulated endothelial cell migration, proliferation, and vessel sprouting in vitro. Biochemical studies and quantitative proteomic profiling of PEAK1 knockdown HUVECs revealed a dramatic loss of VEGFR2 mRNA and protein expression, which attenuated downstream signaling to Akt and ERK, leading to loss of VEGF-mediated vessel formation. PEAK1 specifically regulated VEGFR2 levels and did not alter VEGFR1 expression, and reconstitution of PEAK1 knockdown cells with PEAK1 restored VEGFR2 mRNA and protein expression. Mechanistic studies revealed that PEAK1 specifically regulates VEGFR2 mRNA transcription by modulating expression of the transcription factor GATA2. Re-expression of GATA2 in Peak1 depleted cells restored VEGFR2 mRNA and protein expression as well as downstream signaling, indicating that GATA2 is a necessary downstream component in this response. Also, introduction of gata2 mRNA into Peak1 knockdown zebrafish restored normal ISV formation and vegfr2 mRNA expression in these animals. Finally, bioinformatics and intergroanalysis of the TCGA RNAseq database revealed significant Pearson’s correla-
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tions between PEAK1 and VEGFR2 expression in 17 of 32 different human cancers. Collectively, our findings indicate that PEAK1 regulates developmental and tumor-induced angiogenesis by regulating GATA2-dependent VEGFR2 expression and its downstream signals.

#1812 In vitro and in vivo antiangiogenic activity of desacetylvinblastine monohydrazide through inhibition of VEGF/VEGFR2 and Gas6/Axl pathways. Minfeng Chen,1 Xiaoping Lei,1 Qiuqun Nie,1 Jianyang Hu,2 Zhenqiang Zhu,1 Anita Yu,1 Heru Chen,1 Nanhui Xu,1 Maohua Huang,1 Kaihe Ye,1 Liangliang Bai,1 Wencai Ye,1 Dong-Mei Zhang,1 College of Pharmacy, Guangzhou, China;2School of Life Sciences, The Chinese University of Hong Kong, Hong Kong, China.

Background and Objective: Tumor angiogenesis process is regulated by multiple proangiogenic pathways, such as VEGF/VEGFR2 and Axl. Inhibition of VEGF/VEGFR2 signaling alone fails to block tumor neovascularization, and anti-VEGF resistance is often associated with Axl. Hence, discovery of novel agents that target multiple angiogenesis pathways is in demand. Here, we describe desacetylvinblastine monohydrazide (DAVLBH), a derivative of vinblastine (VLB) that exerts a more potent antiangiogenic effect than VLB in vitro and in vivo by inhibiting VEGFR2 and Axl pathways. Methods: The antiangiogenic effects of DAVLBH were studied in vitro (proliferation, migration, and tube formation assays) and ex vivo (aortic ring assay). In vitro pericyte migration to endothelial tubes was assessed using a three-dimensional co-culture assay. In vivo assay was performed in HeLa xenograft model. Western blotting, immuno-histochemical and immunofluorescence assays were conducted to evaluate the effects of DAVLBH were studied in vitro (proliferation, migration, and tube formation assays) and ex vivo (aortic ring assay). In vitro pericyte migration to endothelial tubes was assessed using a three-dimensional co-culture assay. In vivo assay was performed in HeLa xenograft model. Western blotting, immuno-histochemical and immunofluorescence assays were conducted to evaluate the key proteins of the VEGF/VEGFR2 and Gas6/Axl pathways. Results: DAVLBH (1 nM) inhibited VEGF- and Gas6-induced angiogenesis in vitro. At 0.75 μmol/kg, DAVLBH significantly delayed tumor growth and reduced vascular density in vivo, which was associated with the inactivation of VEGF/VEGFR2 and Gas6/Axl signalling pathways. DAVLBH blocked the compensatory up-regulation of Axl in response to bevacizumab treatment in HUVECs. DAVLBH also suppressed the recruitment of pericytes to well-established endothelial tubes and reduced pericyte coverage in vivo, which was accompanied by inhibition of Axl. Conclusions: DAVLBH potently inhibited angiogenesis-mediated tumor growth by blocking the activation of VEGF/VEGFR2 and Gas6/Axl pathways. DAVLBH might serve as a promising antiangiogenic agent for cancer therapy.

#1813 Bevacizumab potentiates the proteomic response to neoadjuvant chemotherapy in breast cancer patients: RPPA exploration of consorative tumor samples in the NeoAva randomized phase II trial. Mads H. Haugen,1 Ole Christian Lingjaerde,2 Marit Krohn,1 Wei Zhao,1 Evita M. Lindholm,1 Lakmi Silwal-Pandit,1 Elin Borgren,1 Øystein Garred,1 Anne Fangberget,1 Marit M. Holmen,1 Ellen Schlichting,1 Helle K. Skjerven,2 Steinar Lundgren,1 Erik Wist,1 Bjørn Naume,2 Gunhild M. Maelandsmo,1 Yiling Lu,2 Anne-Lise Boerresen-Dale,1 Gordon B. Mills,1 Olav Engebretsen,1 Oslo University Hospital - Institute for Cancer Research, Oslo, Norway;2University of Oslo, Oslo, Norway;3MD Anderson Cancer Center, Houston, TX;4Oslo University Hospital Oslo, Oslo, Norway;5Vestre Viken Hospital, Drammen, Norway;6St. Olav’s University Hospital, Trondheim, Norway.

Anti-angiogenic therapy using bevacizumab has proven effective for a number of cancers. However, bevacizumab is more potent in combination with chemotherapy and hormonal therapy. The aim of this study is to evaluate the proteomic response to bevacizumab treatment in clinical trials. In this phase II clinical trial, patients with HER2 negative primary tumors of ≥25 mm were treated with neoadjuvant chemotherapy (4 × FEC100 + 12 weeks of taxane-based therapy) and randomized (1:1) to receive bevacizumab or not. Mammography, ultrasound, and MR imaging were used for response evaluation, in addition to final pathology assessment. Tumor responses were evaluable in 132 patients; of which 66 received bevacizumab. Ratio of the tumor at final pathology assessment, and at inclusion was calculated to obtain a continuous scale of response reflecting the percentage of tumor shrinkage in response to therapy. Tumor biopsies were removed before start of treatment, at week 12 at the start of taxane-based therapy and at the time of surgery. Lysates from each sample was analyzed on reverse phase protein arrays (RPPA) for expression levels of 210 proteins of which 54 were phospho-proteins. The analysis showed the chemotherapy did not alter proteomic response from week 0 to 25 to such extent that this patient group cluster naturally together. While the proteomic response from week 0 to 12 in both treatment arms had an overall similar profile regarding up- and down-regulated proteins, the combination treatment (FEC100 + bevacizumab) induced substantially more effect on the regulation of each protein. This suggests that bevacizumab treatment have the capability to potentiate the effects of the anthracyclin based chemotherapy from week 0 to 12. Conversely, from week 12-25 (taxane-based therapy + bevacizumab) this effect was lost or even reversed, possibly due to a de-vascularized and less accessible tumor. An exception from the above was a few phospho-protein markers of PI3K/AKT and Stat3, which were upregulated in the bevacizumab arm. This indicates that bevacizumab treatment is pro-angiogenic in breast cancer patients. The results indicate that bevacizumab treatment may increase the expression and its downstream signals. The results indicate that bevacizumab treatment may increase the expression and its downstream signals.

#1814 IL11 mediates tumor resistance to anti-VEGF therapy in lung cancer. Jie Li, Kun Xie, Shenglin Mei, Yuanjian Xu, Hua Gu, Dong Li, Kaiming Chen, Fei Yao, Jiya Eerdeng, Jianmin Fang, Tongxi University, Shanghai, China.

Anti-angiogenic therapy has been used for the treatment of a number of solid tumors in clinic; however, survival benefit is only limited due to development of tumor resistance to the therapy. In previous studies, we developed a mouse Lewis lung carcinoma (LLC) tumor model that is resistant to anti-VEGF therapy. In this model, we identified that Interleukin 11 (IL11) was among the most significantly upregulated genes in the resistant tumors. In current study, we demonstrated that IL11 was an angiogenic factor and played a key role in the development of resistance to anti-VEGF therapy in LLC tumor. IL11 could effectively induce HUVEC proliferation, migration and capillary tube formation in vitro. It enhanced expression of MMP9 in endothelial cells. The conditioned medium from the resistant LLC cell culture could induce HUVEC migration, even VEGF was blocked, and this effect was attenuated by recombinant IL11 decoy receptor. Furthermore, combination therapy of anti-VEGF agent and IL11 blockade exhibited enhanced anti-tumor activity in lung cancer model. In addition, based on bioinformatics analysis on over 500 RNA-seq data deposited to The Cancer Genome Atlas (TCGA), we found that IL11 was highly expressed in various tumor types in patients. Data showed that high IL11 can predict a poor outcome in the lung cancer. The patients with the lower IL11 expression have significantly longer median survival time than those with higher expression. These findings support a role of IL11 signal as a compensatory angiogenic pathway that might trigger tumor resistance to anti-VEGF treatement. Therapeutically targeting IL11 could have a value in overcoming tumor resistance to anti-VEGF therapy.


Malignant pleural effusion is a poor prognostic sign for patients with non-small-cell lung cancer (NSCLC) and is noted around 15% at the initial diagnosis and 50% in their later courses. Previously, we have shown that IL-6/Stat3/tissue factor (TF)/VEGF pathway enhances lung cancer angiogenesis, metastasis and the generation of MPE. In the current study, we demonstrated that EVs derived from lung cancer cells may also contribute MPE formation. We first used ultra-filtration (UF) method as well as the classical ultra-centrifugation (UC) method to isolate EV from lung cancer cell line and demonstrated that the EVs isolated by UF method were characteristic with exosome marker in Western blotting. Using the ELISA method, we found the cargos in these EV were enriched in IL6. We also found these cargo derived from EVs, containing high VEGF and TF, induced more prominent extravasation than serum free medium did in Miles assays. In clinical sample, we also isolated cancer cell from MPE and the purity of the cells was confirmed by immunofluorescent staining with specific TFF-1 expression of lung adenocarcinoma. After short-term culture, EV from MPE cancer cell have the characteristic of exosome maker and induced more prominent extravasation than serum free medium as EV from A52 cell did. And we also isolated EV directly from MPE and pleural effusion from congestive heart failure (CHF) patients. And we found the EGFP and more prominent extravasation than those derived from CHF in Miles assays. In conclusion, we demonstrated the UF is clinical assessable method for isolating EV from body fluid and EV derived from lung cancer cell and MPE may contribute oncogenic and angiogenic signal transmission accelerating the formation of MPE.
Endogenous ADAMTS-13 regulates angiogenesis in cultured human endothelial cells. Huiyuan Tang, Manfi Lee, Eun Ho Kim, Daniel Bishop, George M. Rodgers. University of Utah, Salt Lake City, UT.

ADAMTS-13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13, is a zinc-containing metalloprotease that cleaves von Willebrand factor (vWF). Previous publications by our laboratory have shown that ADAMTS-13 may also be involved in angiogenesis; for example; addition of exogenous ADAMTS-13 enhances endothelial cell (Lee et al. Microvascular Res 2012; 84:109) and glioblastoma cell (Lee et al. Blood 2013; 122:2306) migration and proliferation. For this study, we report the successful transient knockdown of endogenous ADAMTS-13 in human umbilical vein endothelial cells (HUVEC) via siRNA (30pmol of ADAMTS-13 siRNA in a six-well plate inhibited HUVEC ADAMTS-13 expression levels by 90% after 24hr incubation, whereas control siRNA did not affect endogenous ADAMTS-13 levels. The knockdown effect was decreased over time: 72hr after the initial knockdown, ADAMTS-13 expression was reduced by only 30%. The effects of reduced endogenous ADAMTS-13 on HUVEC angiogenesis functions were studied. Transfection of HUVEC with 10pmol of ADAMTS-13 siRNA in a 24-well plate resulted in a 21% and 22% decrease in proliferation after 24hr and 48hr incubation, respectively. The effects of ADAMTS-13 knockdown on migration of HUVEC across a scratch wound were also evaluated. 24hr after transfection with control siRNA, there was robust cell migration across the scratch wound. This dramatic migration did not occur with ADAMTS-13 knockdown cells. Decreased expression of endogenous ADAMTS-13 also affected angiogenesis as measured by endothelial cell tube formation using a Matrigel matrix method. The tube lengths, sizes and junction numbers of the ADAMTS-13 knockdown cells were all significantly lower compared to control cells by about 40%. Activity of the Akt pathway, one of the angiogenesis downstream signaling pathways was down-regulated by ADAMTS-13 siRNA while ERK, a component of the MAP kinase pathway was not affected upon knockdown of ADAMTS-13. These data indicate that in cultured endothelial cells, one role of endogenous ADAMTS-13 is regulation of angiogenesis, mediated through the Akt signaling pathway. Overall, our data suggest an additional model of endogenous ADAMTS-13 functionality, beyond that of cleaving von Willebrand factor.

Resistin induces angiogenesis and lymphangiogenesis in human chondrosarcoma. Meng-Ju Chi, Chih-Yang Lin, Chih-Hsin Tang. China Medical University, Taichung City, Taiwan.

Chondrosarcoma is a common kind of bone cancers, and it may develop distant metastasis, followed by a significant decline in overall survival. However, there are still no specific therapeutic methods for it today. For tumors to metastasize, angiogenesis and lymphangiogenesis are both important in the early processes. Therefore, inhibiting the development of angiogenesis and lymphangiogenesis could be a method to decline tumor metastasis. Resistin was discovered as an adipocyte-secreting adipokine, which may play a critical role in modulating cancer pathogenesis. In our lab, we previously found that resistin appears to increase MMP-2 expression and then promotes metastasis in human chondrosarcoma cells. Nevertheless, the role of resistin in angiogenesis and lymphangiogenesis of human chondrosarcoma is still unknown. To examine angiogenic and lymphangiogenic effects of resistin, we used human endothelial progenitor cells (EPCs) and lymphatic endothelial cells (LEC) to mimic capillary and lymphatic vessels formation. The results indicated that resistin-treated chondrosarcoma cell lines promoted EPC’s VEGF-A dependent and as well as LEC’s VEGF-C dependent tube formation and cell migration. Then we confirmed that treating cells with resistin increased VEGF-A and VEGF-C expression in human chondrosarcoma cell lines. Moreover, we found resistin-induced VEGF-A and VEGF-C expressions are mediated by PI3K/AKT signaling and by the activation of c-Src separately. In addition, resistin decreased the expression of miR-16-15p via PI3K/AKT pathway, and so of miR-186 via c-Src. We also demonstrated that miR-186 directly targeted on VEGF-C 3’untranslated region, and regulated the VEGF-C production. Besides, we found the expressions of resistin, VEGF-A and VEGF-C was higher in human chondrosarcoma biopsy tissues than those in normal cartilage. Taken together, resistin not only promotes human chondrosarcoma angiogenesis through the activation of PI3K/AKT signaling pathway and down-regulating miR-16-15p expression, but also promotes human chondrosarcoma lymphangiogenesis through the activation of c-Src and down-regulating miR-186. Consequently, resistin may represent a potential novel molecular therapeutic target for human chondrosarcoma therapeutic treatment.
Epithelial-mesenchymal transition (EMT) has long been associated with cancer progression and metastasis. As a reversible process, it has the potential to be a target for cancer therapy. However, this concept of reversing EMT has not yet been widely explored in current treatment strategies. With the identification of Nintedanib as an EMT reversal agent, we aim to broaden the utility of this triple angioinhibitor by identifying agents that show synergistic lethality with it, using monoclonal antibodies (mAbs) as a combinatorial therapeutic. Nintedanib alone is not significantly cytotoxic to cells, but it causes cell cycle arrest and reverses EMT. By selecting for this sub-population of cells that are sensitive to Nintedanib, their subsequent eradication can then be more intricately directed. Since the anti-cancer application of EMT reversal agents are not fully developed, it is vital to investigate mechanisms that sensitize cancer cells to cytotoxic agents following EMT reversal. Our group has generated panels of mAbs that are able to bind differentially to various breast and ovarian cancer cell lines along the EMT spectrum. Some mAbs preferentially bind to Epithelial lines, while some have stronger affinity to Mesenchymal lines. This implies that mAbs have the ability to differentiate between epithelial and mesenchymal phenotypes, uncovering a new dimension to the capability of mAbs which have yet to be explored. Using a mesenchymal ovarian cancer cell line as a starting model, Nintedanib-treated cells were screened with the mAbs and those showing a >2-fold increase in binding were shortlisted for further validation. Based on this criteria, 26 mAbs were identified and further characterized in functional Antibody Drug Conjugate (ADC) assays with mAbs conjugated to a toxic drug, Saporin (or Zap). Mabs successfully internalizing and killing the cancer cells will then be further chosen for antigen characterization and in vivo functional studies. Ultimately, there is huge potential for mAbs to be discovered that shows synergy with Nintedanib to enhance lethality to various types of cancer cells. A novel clinical strategy, the application of EMT reversal in terms of utilizing a synthetic lethality-like approach allows better design of combinatorial therapeutic agents, increasing treatment efficacy that might revolutionise cancer treatment.

#1820 Synergistic lethality of mAbs with an EMT reversal agent, Nintedanib, in epithelial ovarian cancer. Jocelyn Teo,1, Heng Liang Tan,1 Ruby Yun-Ju Huang,2 Andre Choo1.

Epithelial-mesenchymal transition (EMT) has long been associated with cancer progression and metastasis. As a reversible process, it has the potential to be a target for cancer therapy. Therefore, this concept of reversing EMT has not yet been widely explored in current treatment strategies. With the identification of Nintedanib as an EMT reversal agent, we aim to broaden the utility of this triple angioinhibitor by identifying agents that show synergistic lethality with it, using monoclonal antibodies (mAbs) as a combinatorial therapeutic. Nintedanib alone is not significantly cytotoxic to cells, but it causes cell cycle arrest and reverses EMT. By selecting for this sub-population of cells that are sensitive to Nintedanib, their subsequent eradication can then be more intricately directed. Since the anti-cancer application of EMT reversal agents are not fully developed, it is vital to investigate mechanisms that sensitize cancer cells to cytotoxic agents following EMT reversal. Our group has generated panels of mAbs that are able to bind differentially to various breast and ovarian cancer cell lines along the EMT spectrum. Some mAbs preferentially bind to Epithelial lines, while some have stronger affinity to Mesenchymal lines. This implies that mAbs have the ability to differentiate between epithelial and mesenchymal phenotypes, uncovering a new dimension to the capability of mAbs which have yet to be explored. Using a mesenchymal ovarian cancer cell line as a starting model, Nintedanib-treated cells were screened with the mAbs and those showing a >2-fold increase in binding were shortlisted for further validation. Based on this criteria, 26 mAbs were identified and further characterized in functional Antibody Drug Conjugate (ADC) assays with mAbs conjugated to a toxic drug, Saporin (or Zap). Mabs successfully internalizing and killing the cancer cells will then be further chosen for antigen characterization and in vivo functional studies. Ultimately, there is huge potential for mAbs to be discovered that shows synergy with Nintedanib to enhance lethality to various types of cancer cells. A novel clinical strategy, the application of EMT reversal in terms of utilizing a synthetic lethality-like approach allows better design of combinatorial therapeutic agents, increasing treatment efficacy that might revolutionise cancer treatment.

#1821 Sympathetic nerves regulate a metabolic switch promoting angiogenesis through adrenergic signaling in prostate cancer. Ali Zahiya,3 Anna Arnal-Estape,4 Maria Maryanovich,5 Fumio Nakahara,4 Cristian Cruz,2 Paul S. Frenette1, Albert Einstein College of Medicine, Bronx, NY; 2Yale School of Medicine, New Haven, CT.

Nerves pattern the vasculature during development and regeneration. Recent studies have shown that the sympathetic nervous system (SNS) is co-opted to promote cancer angiogenesis, and that depletion of SNS function reverses pro-angiogenic receptors (βAR). The role of the prostate stromal compartment inhibits tumor growth. As the vasculature is in direct contact with the SNS, and regulates tumor glucose and oxygen delivery, we aimed to identify the stromal populations that mediate SNS signaling and the mechanisms by which loss of βAR signaling inhibits prostate cancer progression. In vivo xenograft tumor growth was measured in Adrb2-/-; Adrb3+/- mice using an orthotopic PC3-luciferase model. Prostate cancer progression in the transgenic aurthochthonous H1-myc model was assessed after conditional Adrb2 deletion in stromal populations by intercrossing Adrb2-/-;Cre/+ mice with Adrb2-/-;Cre/+;R26-tdTomato mice that express a tdTomato reporter under the control of the uPAR promoter. Depletion of βAR activity in prostate cancer cells significantly inhibited the prostate intrinsic RAS-MAPK pathway, and regulated tumor growth. The results showed that βAR signaling modulates prostate cancer cell metabolism, and that βAR depletion inhibits prostate cancer progression.

#1822 Antitumor activity of agents targeting the angiogenic factors angioptin-2 and thymidine phosphorylase in mouse models of lung and pancreatic neuroendocrine cancer. Evelyn Aranda, Ziqiang Yuan, Steven K. L. Butler, Edward L. Schwartz. Albert Einstein College of Medicine, Bronx, NY.

Angiogenesis is a critical component of the initiation, maintenance and progression of tumors, and has been the target of extensive drug development. In clinical practice there are several approved and investigational anti-angiogenesis drugs, the majority of which primarily target the VEGF pathway, however their long term effectiveness remains limited. In designing new drug regimens, it is important to consider other known angiogenic factors that likely contribute to the pro-angiogenic phenotype. We evaluated two such factors: thymidine phosphorylase (TP) (aka platelet-derived endothelial cell growth factor, PD-ECGF), an enzyme that can stimulate endothelial cell migration, and angioptin 2 (Ang2), a member of the Ang/Tie2 signaling pathway. To target TP, we used a novel, highly specific, orally available small molecule inhibitor, which we had previously designed, named AEAC. To target Ang2, we used an Ang2 neutralizing peptide, Li-7[N] (provided by CTEP-NCI), which is a genetically engineered peptide-Fc fusion protein that specifically binds Ang2. In a mouse NCI-H460 NSCLC xenograft model, AEAC and Li-7[N] produced 33% and 67% reductions, respectively, in tumor growth, and the combination produced a small, but statistically significant further reduction, when compared to either agent used alone. Tumor growth inhibition was paralleled by a reduction in Ki-67 staining. Li-7[N] also produced a large infiltration of macrophages (F4/80+) into the tumors after treatment. We also tested the drug combination in a mouse genetic model of pancreatic neuroendocrine tumors (PNET). PNET are one of the few human solid tumors that are clinically responsive to angiogenesis inhibitors used alone. These mice have a conditional knockout of the Men1 gene in the endocrine pancreatic cells (Pdx1-Cre;Men1 mice) mimicking a primary genetic alteration seen in human disease. Men1 KO iPSCs are tumorigenic in Men1 KO PNET experiments were initiated when the mice were 10-11 months old, and drug treatment was daily (5 days per week) for 3 weeks. To date, we have not seen any antiangiogenic or antitumor effects of the drugs. Upon sacrifice, the mice were found to have extensive insulinomas, involving around 70% of the entire pancreas. CD31+ and F4/80+ cells in the pancreas showed that tumours were almost completely infiltrated by macrophages. It is possible that the significant increase in macrophage infiltration and tumor growth inhibition associated with these agents may be due to a synergy between these drugs. In conclusion, these results suggest that dual targeting of TP and Ang2 may have therapeutic potential in human pancreatic neuroendocrine cancer.

#2019 Characterization of the anti-angiogenic properties of merestinib (LY2801653), an onconcinib inhibitor. Diane M. Bodenmiller, Julie A. Stewart, Glenn F. Evans, Victoria L. Peek, Jennifer R. Stephens, Xi Lin, Seema Iyer, Beverly L. Falcon, Sudhakar Chinthapalapilli, Sue-Chi Betty Yan, Anthony S. Fischl, Eli Lilly and Company, Indianapolis, IN.

Merestinib (LY2801653) is an orally bioavailable small molecule inhibitor of several onconcinib, including MET, AXL, DDR1/2, MERTK, ROS1, Tie2 (aka TEK), and MKNK1/2. Merestinib has been extensively characterized in a wide range of preclinical xenograft models that showed to potently inhibit MET driven and non-MET driven tumor growth. In addition to its direct antitumour activity, merestinib inhibits angiogenesis and induces a tumor vessel normalization phenotype in xenograft tumors. While MET signaling is important for angiogenesis, the effect of merestinib on angiogenesis is likely not exclusively driven by MET inhibition. In co-culture angiogenesis assays, merestinib inhibited VEGF-dependent and VEGF-independent endothelial cell cord formation and sprouting with potencies in the low nM range (3-30 nM). In contrast, the MET-specific kinase inhibitor, PD04217903, only weakly inhibited cord formation and endothelial sprouting. In an established in vivo matrigel co-implant vasculogenesis model where VEGFR2 or MET selective inhibition had minimal effect, merestinib decreased vascular density by 69%. In addition, while MET antibody emibetuzumab (human anti-MET anti-
body) plus ramucirumab (human anti-VEGFR2 antibody) decreased vascular density by 64%, merestinib plus ramucirumab decreased it by 92%. In a mouse adenovirus-driven VEGF-A ear angiogenesis model, treatment with DC101, a mouse anti-VEGFR2 antibody, or merestinib inhibited angiogenesis; however the combination of DC101 and merestinib appeared to inhibit it even more. Finally, in the MKN45 gastric tumor xenograft model, merestinib (T/C = 4.8%) and DC101 (T/C = 15.3%) each significantly inhibited tumor growth alone and the combination resulted in 27.6% tumor regression and was significantly better than either single agent alone. Together, these studies indicate that merestinib has greater effects on angiogenesis than selective MET inhibition and its actions are not dependent on VEGFR2. In addition, while in vitro studies show reductions in VEGFR2 phosphorylation with high concentration of merestinib, treatment with merestinib did not inhibit VEGF dependent phosphorylation of VEGFR2 in mouse lung tissue at clinically relevant exposures. These data suggest that the anti-angiogenic activity of merestinib involves activities of other kinases targeted by merestinib. These data provide rationale and support for the clinical evaluation of combination of merestinib with ramucirumab (NCT07247569).


1824 Endothelial mTORC2 deficiency inhibits tumor angiogenesis, tumor progression and metastasis in non-small cell lung cancer (NSCLC), Shan Wang. Vanderbilt University Medical Center, Nashville, TN.

Vascular endothelial cells (ECs) are specialized components of the tumor microenvironment that orchestrate tumor growth and invasion. They form tumor-associated blood vessels (angiogenic) that supply nutrients and oxygen, remove waste products, and also provide an entry site for tumorintrinsic cells to spread to secondary sites in various organs. One of the major signaling events in tumors is PI3K/PDK-1/mTOR activation. mTOR is a serine/threonine kinase that functions in two distinct complexes, mTORC1 and mTORC2, which regulate a diverse array of cellular processes including cell growth, survival and metabolism. Although mTORC1, and to lesser extent, mTORC2, has been broadly studied in cancers and diseases, little is known regarding the relative contributions of mTORC1 versus mTORC2 in tumor endothelium. Using mouse model of endothelium-specific Rictor (a mTORC2 specific cofactor) gene targeting, we discovered that endothelial Rictor deletion decreased tumor neoangiogenesis, suppressed tumor growth, and prevented metastasis in vivo. Direct co-culture of endothelial cells and NSCLC tumor cells in vitro showed that knockdown of endothelial Rictor inhibited tumor cell proliferation while Raptor (a mTORC1 specific cofactor) depletion had a modest effect. However, loss of endothelial Rictor or Raptor both markedly decreased tumor cell extravasation in response to endothelial cells, and also inhibited tumor sphere formation in a direct co-culture system. Furthermore, a screen of phospho-kinase arrays revealed that Raptor or Rictor depletion in endothelial cells decreased various phospho-RTKs in tumor cells that were co-cultured with ECs, including members of the Eph receptor family, Insulin receptor family and FGF receptor family. Additionally, endothelial Rictor knockdown suppressed phosphorylation levels of several downstream factors, TRKB and TRKC in tumor ECs. Collectively, these data suggest that endothelial mTORC1 and mTORC2 play critical roles in regulating tumor progression through distinct signaling pathways.

1825 Angiopoietin-Tie-2 functional axis in colorectal cancer liver metastasis (CRCLM) provides a new marker for stratification and evaluation of tumor progression. Nisreen S. Ibrahim. McGill University, Montreal, Quebec, Canada.

Colorectal cancer (CRC) is the third leading cause of cancer in Canadians, with liver metastases being the major cause of death from this disease. Tumors induce angiogenesis, a phenomenon known as the ‘angiogenic switch’, which is an essential step in tumor progression whereby the balance of pro- and anti-angiogenic factors is important for active angiogenesis. Clinical efficacy of targeted VEGF (anti-angiogenic) treatment has been validated as a cancer therapy. Our group, together with others, has identified unique histological growth patterns HGP’s (desmoplastic, replacement and pushing) within liver metastases that have different responses to anti-angiogenic therapy. The patients with Desmoplastic HGP (DHGP) that received anti-angiogenic plus chemotherapy prior to resection had a significantly better pathologic response and survival than patients with Replacement HGP (RHP). The aim of this study was to explore the role of Ang-1, Ang-2, Tie-2 and VEGF in the development and progression of CRCLM tumors with distinct HGP’s. Here, human CRCLM tumor samples were analyzed by quantitative real-time PCR (Q-PCR) and immunohistochemistry (IHC) staining. The Q-PCR results demonstrated that the expression of Ang-2 was lower in RHGP tumor samples compared with DHGP tumor samples. This data was validated by IHC, where IHC scoring results showed that the ratio of Ang-2:Ang-1 expression in DHGP tumors was higher compared to RHGP tumors. VEGF and Tie2 proteins were expressed in both tumor patterns. Thus vascular quiescence maintained by constitutive Ang-1/Tie-2 signaling, found in RHGP tumors, prevails over destabilization and pro-inflammatory Ang-2/Tie-2 signaling, which is higher in the DHGP tumor samples. Since vascular remodelling is driven by Ang-2/Tie-2 in DHGP, which is dependent on VEGF we would expect anti-angiogenic therapy to be effective on DHGP. Furthermore, the RHGP had low levels of Ang-2 and high levels of Ang-1, together with the presence of Tie-2, and therefore one would predict that VEGF is not required for the growth of these tumors and thus would not respond to anti-angiogenic therapy, as has been shown in our patients’ cohort. Taken together, these data suggest that the Angiopoietin/Tie-2 functional axis is an important player in CRCLM tumor progression and can be a potential target for CRCLM cancer therapy with stratification of patients by HGP’s.

1826 Bioavailability, pharmacodynamics and safety profile of a novel anti-angiogenic compound JFD in pre-clinical models. Sivanesan Dhandayuthapani, Thanigaivelan Kanagasabai, Khadija Cheema, Appu Rathiavelu. Rumbaugh-Goodwin Institute for Cancer Research, Nova Southeastern University, Fort Lauderdale, FL.

JFD, isoidole (1, 3-dioxo-2, 3-dihydro-1H-isooindol-4-yl)-amide, is a small molecule that was developed using molecular modeling to specifically antagonize Vascular Endothelial Growth Factor Receptor 2 (VEGFR2) and associated kinase activity, thereby producing anti-angiogenic and anti-cancer effects. Since the original form of JFD is hydrophobic, it was recently modified into a water soluble form (JFD-WS) to increase its water solubility and bioavailability. Initially, we investigated the in vitro pharmacodynamics effect of both JFD and JFD-WS by utilizing Matrigel® tube formation assays, scratch and cell migration assays followed by inhibition of VEGFR2 phosphorylation in human umbilical vein endothelial cells (HUVECs). In vivo tumor growth inhibition of JFD and JFD-WS was tested using GI-101A breast adenocarcinoma cells implanted xenograft animals. Subsequently, the blood samples and the tumor proteins were extracted from the control and experimental animals to measure the pro-apoptotic signals and the levels of plasma biomarker such as MUC1 (mucin 1). The safety profile was established using Balb-c mice that were injected intraperitoneally (i.p) with JFD (6 mg/kg body weight) and JFD-WS (100 mg/kg body weight) for 30 days. The pharmacokinetics (PK) of JFD including the plasma concentration, excretion, elimination and distribution in the experimental animals, was that JFD was treated with JFD-WS for four weeks, no significant alterations were found in the test group compared to the control animals. Thus, whether used as a single agent or in combination with the other anti-cancer drugs, JFD shows strong anti-tumor effects without producing severe toxicities. The efficient delivery of JFD original to the brain suggests that this drug would be useful in treating solid tumors of the brain with pro-angiogenic abilities. (This project was supported by the Community Foundation of Broward, Ft. Lauderdale, FL and The Royal Dames of Cancer Research Inc., Ft. Lauderdale, FL.)
research. Many signaling pathways have been implicated in mechanisms leading to the shift of metabolic programs in tumors, but more recently a small number of metabolic enzymes have also been identified in this process. Genes encoding the metabolic enzymes Isocitrate dehydrogenase 1 (IDH1) and 2 (IDH2) were found to be mutated in up to 70% of low-grade and medium grade gliomas, and in 15–25% of adult acute leukaemia samples. We have verified that these cells now produce 2-HG, as is known in human tumors harboring IDH mutations. We have observed different cellular responses to IDH mutations in glia versus hemocytes and have begun to investigate why this may be. Ultimately, the goal of our research is to elucidate the mechanism(s) that cause IDH to contribute to oncogenic activity in specific tissues, and use this knowledge to design tailored therapeutics.

#1828 Are callipers obsolete? A novel 3D scanning technology to measure subcutaneous tumor volume. Zena Wilson,1 Juan Delgado,2 Michael Davies,1 Rebecca Whiteley,1 Jennifer Hare,1 Amar Rahi,1 Stephen Marshall,1 Andrew Smith,1 Stephen Atkinson,1 Jarno Ralli,1 Adeala Zabair,1 Adeala Zabair,1 Jane Kendrew1. AstraZeneca Pharmaceuticals, Cheshire, United Kingdom; 2Glaxo Medicines Research Council, Stevenage, United Kingdom; 3Fuel 3D, Chinnor, United Kingdom.

Most preclinical oncology studies (xenograft, PDX, GEMMS) involve monitoring tumor growth rates, measuring them with callipers, and calculating the volume. Volume is calculated from the width and the length to estimate a 3D volume and is directly used to assess treatment efficacy. Although this technique is useful, it is unable to accurately assess non-uniformly shaped or very small tumours and introduces a systematic bias by assuming that tumours present with spherical shape. Furthermore callipers do not inform of the tumour condition, which is dependent upon a visual estimation. Here we describe the development and validation of a 3D scanner as an alternative method to callipers to monitor tumour progression in rodents. The resulting 3D scanner solution made up of hardware and software, has the potential to impact on the 3Rs guiding principles underpinning the humane use of animals in oncology research. The 3Rs benefits identified are primarily through reduction of animals and alleviation of pain, which may ultimately lead to better development and validation of new cancer treatments.

#1830 Targeted inhibition of EGFR and glutaminase induces metabolic crisis in EGFR mutant lung cancer. Milica Momcilovic,1 Sean T. Bailey,1 Jason T. Lee,1 Daniel Braas,2 Thomas G. Graeber,3 Melissa Works,2 Francesco Parlati,2 Susan Page,2 Tonya C. Walker,2 Stephen M. Dubinett,1 Saman Sadeghi,1 Heather Christofk,1 David B. Shackelford1. UCLA, Los Angeles, CA; 2Calithera Biosciences, CA.

Cancer cells exhibit increased use of nutrients including glucose and glutamine to support the bioenergetic and biosynthetic demands of proliferation. We tested CB-839, a small molecule inhibitor of glutaminase that impedes glutamine utilization, in combination with erlotinib on EGFR mutant non-small cell lung cancer (NSCLC) as a therapeutic strategy to simultaneously impair cancer glucose and glutamine utilization and thereby suppress tumor growth. Here we show that CB-839 synergizes with erlotinib to drive energetic stress and activate the AMPK pathway in EGFR (del19) lung tumors. Tumor cells undergo metabolic crisis and cell death resulting in rapid tumor regression in vivo in mouse NSCLC xenografts. Consistently, positron emission tomography (PET) imaging with 18F-fluorodeoxyglucose (18FDG) and 11C-Glutamine (11C-Gln) of xenografts indicated reduced glucose and glutamine uptake in tumors following CB-839 + erlotinib treatment. Therefore, PET imaging with 18F-FDG and 11C-Gln can be used to non-invasively monitor tumor metabolic and therapeutic response to CB-839 and erlotinib combination therapy.

#1831 PET/CT imaging of interleukin-13 receptor alpha-2-targeted peptide to glioblastoma after locoregional delivery. Anirudh Sattiraju,1 Ang Xuan,2 Frankis Almaguel,1 Denise Herpai,1 Waldemar Debinski,1 Akiva Mintz,1 Kiran Kumar Solingapuram Sai1. Wake Forest School of Medicine, Winston Salem, NC; 2The People's Hospital of Zhengzhou University, Zhengzhou, China.

Glioblastoma (GBM) is the most aggressive and common primary malignant astrocytoma which is characterized by tumor heterogeneity, infiltrating margins. Radiotherapy, chemotherapy and experimental targeted therapies have been ineffective at meaningfully increasing patient survival. One significant shortcoming of systemically delivered therapies is their inability to cross the blood brain barrier (BBB) and access infiltrating tumor cells. Therefore, in this work, we tested the potential of locoregionally targeting GBM via IL13Rα2, which we discovered to be expressed on greater than 75% of GBMs. To accomplish this, we intracranially infused copper-64 (64Cu) radiolabeled IL13Rα2 specific peptide Pep-1L, previously developed by Pandya et al., into mice bearing IL13Rα2 expressing orthotopic GBMs. Small animal micro PET/CT imaging showed ~2-fold greater tumor specific localization and lower volume of distribution of 64Cu-Pep-1L within brains of mice. Post-PET biodistribution study showed greater retention of 64Cu-Pep-1L 4 hours (%ID/g = 20.85 ± 0.65) and 24 hours (%ID/g = 14.65 ± 0.30) post infusion when compared to similarly infused 64Cu radiolabeled scrambled control peptide 4 hours (%ID/g = 10.73 ± 2.02) and 24 hours (%ID/g = 5.97 ± 1.47) post infusion. These results demonstrate that Pep-1L efficiently targets IL13Rα2 expressing GBMs in vivo upon loco-
Non-small cell lung cancer (NSCLC) has a low survival rate, with metastasis contributing to the vast majority of deaths. The NEDD9 (HEF1/Cas-L) protein has been reported to be elevated in expression and to promote metastasis in a large subset of lung cancers and in other malignancies. NEDD9 functions as a scaffold for multiple critical effectors in integrin/FAK/SRC and receptor tyrosine kinase/multi-growth signaling cascades. The specific role of NEDD9 in tumor progression remains unclear, overexpression thought to sustain signaling by these oncogenic proteins. Previous studies demonstrated depletion of NEDD9 by RNAi reduced the growth and invasion of established lung cell lines and tumors, based on cell culture and xenograft assays. We have now investigated the consequences of a null genotype for NEDD9 from the earliest stages of tumor formation, crossing Nedd9 mutant mice to a 129/SV/Pyg/tv5/H11005 (SV/Pyg) background in which Kras mutation is induced specifically in lung tissue by inhalation of adenovirus bearing the Cre gene. Unexpectedly, based on in vivo imaging, the Nedd9 null genotype accelerated tumor growth in KP mice. Pathological examination of tissues indicated Nedd9 null genotype also was associated with higher invasive capacity in vivo, including direct invasion to the heart, as well as elevated proliferation rate, decreased apoptotic activity, and changes in the expression of proteins such as vimentin, associated with mesenchymal status. Although Nedd9 function has been implicated in immune cell activity, we found minimal differences in tumor infiltration of myeloid cells or macrophages based on Nedd9 genotype. Rather, use of Reverse Phase Protein Array (RPRA) analysis to characterize signaling in isolated tumors suggested tumor intrinsic changes in signaling. We have now characterized specific signaling pathways, which were subsequently validated using direct functional assays. These results for the first time imply that NSCLC tumor progression beyond early stages in the absence of Nedd9 requires reprogramming of intrinsic tumor signaling to compensate for the absence of this protein, emphasizing activities distinct from those associated with elevated Nedd9 in late stage tumors.

Regional delivery and can effectively deliver potential therapeutic agents to GBM tumors while sparing normal brain. This work was supported by the American Cancer Society Mentored Research Scholar grant # 124443-MRSG-13-121-01-CDD (Mintz), IR01CA179072-01A1 (Mintz), P30 CA012197 (Paszke), R01CA074145-19 (Debinski) and the Translational Imaging Program (TIP) of the Wake Forest CTSA (UL1TR001420).

#1832  Bioluminescent pharmacokinetics of luciferin in preclinical brain metastases of breast cancer models. Neal Shah, Chris E. Adkins, Afroz S. Mohammad, Paul R. Lockman. West Virginia University, Morgantown, WV.

Background: Approximately 20% of breast cancer patients with disseminated disease will develop brain metastases. Preclinical models of brain metastases of breast cancer rely on ex vivo histology to evaluate drug efficacy. However, bioluminescence imaging allows more precise quantification of tumor burden and progression through counting photons per second without requiring animal sacrifice. This methodology has not readily been applied to hematogenously derived brain metastases models given the heterogeneity of tumor burden and growth. Herein we demonstrate repeatable methodology to quantitatively measure bioluminescence of breast cancer progression which is verified with histology. Methods: Approximately 1.75 x 105 JIMT-1 (n=3) and 231-BrLuc (n=4) brain seeking subclones were injected intracardially to produce metastatic brain lesions. Mice were given 150 mg/kg of luciferin IP and luminescence was captured every 2-minutes for 60 minutes starting 24 hours after cell implantation. Radiance (photons per second per square centimeter per steradian) was plotted to observe peak luminescence. Imaging was repeated twice weekly to evaluate progression until euthanasia. Results: Following intracardiac injection, both brain seeking metastatic models produced a maximum luminescence signal between 14-20 minutes after luciferin injection. For all subsequent imaging, 5 minute imaging between 15-20 minutes after luciferin injection was used. Longitudinally, both tumor cell lines produce bioluminescence 24 hours after cell injection, which is used to ensure tumor implantation and randomization. The BLI signal decreases to undetectable limits in both models between 3-7 days after cell implantation. Re-emergence of signal occurs on day 14 for the JIMT-1 line and day 21 for 231-Br line. JIMT-1 bioluminescence on day 14 begins at ~10^5 photons/sec/cm^2/sr and then over time signal increases with a ~slope of 5.6x10^5 until reaching a maximum of 10^6 units on day 28. 231-BrLuc bioluminescence on day 21 begins at ~10^5 photons/sec/cm^2/sr and then over time signal increases with a ~slope of 1.1x10^5 until reaching a maximum of ~10^6 on day 53. Conclusion: To accurately measure bioluminescence as a surrogate for tumor burden in hematogenously implanted metastases, the optimal circulation time is approximately 15 minutes with a 5 minute imaging period. Bioluminescence of the 231Br line initially starts at a lower magnitude and later in time than the JIMT-1 line, but slopes of both intracardiac models are similar and result in similar bioluminescent curves.

#1833 Imaging-guided brain tumor surgery strategy based on a triple-modality imaging probe and imaging probe seeded imaging model. Hui Meng, Yushen Jin, Shaowei Zhang, Shuai Gu, Xibo Ma, Jie Tian. Institute of Automation, Chinese Academy of Sciences, Beijing, China; Chinese PLA General Hospital, Beijing, China; Shandong University, Jinan, China.

Precise brain tumor resection is valuable for increasing the 5-year survival rate. However, the delineation of tumor margin is a major challenge. Transferrin modified nanoprobes had the property of passing the blood-brain barrier (BBB). Here, we endeavored to use transferrin to fabricate a triple-modality magnetic resonance imaging-PET-fluorescence imaging molecule agent (MPF) which could improve diagnostic sensitivity and specificity of the brain tumors in the imaging acquisition and analysis. The localization of the tumor were determined by the MRI before surgery. Glucose metabolism was derived from PET imaging which would be an important influence factor for surgery strategy. Tumor margin was delineated using the quick segmentation methods according to the fluorescence imaging after craniootomy which could reduce residual tumor after resection. Finally, tissues were sliced and stained with hematoxylin and eosin every 5 mm. The histology results were processed into a whole image which can be used to evaluate the accuracy of intraoperative tumor margin. This triple modality imaging-guided surgery strategy is a promising method which will probably achieve its application in clinical trials in near future.

#1834 Constitutive Nedd9 null genotype promotes lung cancer aggressiveness. Alexander Deneka, Meghan Kopp, Anna S. Nikonova, Anna Gaponova, Alexandra Nagle, Harvey Hensley, Erica Golemis. Fox Chase Cancer Center, Philadelphia, PA.

TUMOR BIOLOGY: Cell Culture and Animal Models of Cancer 2

Development of mammary hyperplasia, dysplasia, and invasive ductal carcinoma in transgenic mice expressing the 8p11 amplicon oncogene Nsd3 (Whsc1ll1). Alex C. Rutkovsky, Brittany Turner-Ivey, Ericka L. Smith, Laura S. Spruill, Jamie N. Mills, Stephen P. Ether. The Medical University of South Carolina, Charleston, SC.

Amplification within the 8p11-12 locus occurs in approximately 15% of breast cancer (BC) and is associated with poor survival and distant recurrence. Overexpression of many of the genes within the 8p11-12 region can confer a pathological gain of function to breast cells. We and others have previously demonstrated that Nsd3 (Whsc1ll1), a lysine methyltransferase, can act as a potent transforming oncogene. Predictions by the GISTIC algorithm of the Human Cancer Genome Atlas data suggest that Nsd3 is a driving gene within 8p11-12 in multiple cancer types, including BC. We previously showed that Nsd3 can regulate the expression of influential genes, such as estrogen receptor alpha (ESR1/ERa). To further explore the role of Nsd3 in promoting BC, we generated FVB/N transgenic mice with targeted overexpression of Nsd3 in the mammary gland and compared them to matched non-transgenic wild-type (WT) females. We observed that pups nursed by transgenic females were underdeveloped, regardless of genotype. Underdeveloped pups displayed delayed hair growth and eye opening, and were half the weight of pups nursed by WT females. To investigate this phenotype, we characterized thoracic and inguinal mammary glands from virgin, mid-pregnancy, lactating, and post-lactating mice by whole mount and histological analysis. Mammary glands from virgin transgenic females displayed increased branching and terminal bud formation. Alveolar buds from mid-pregnancy transgenic glands were more numerous and densely packed. Glands from lactating transgenic females showed large areas in which the alveoli failed to undergo functional differentiation, resulting in a lactation defect. Mammary glands taken post-lactation exhibited areas of ductal and alveolar hyperplasia. At 40 weeks of age, multiple transgenic mice had palpable tumors which were excised at 1 cm^3 size. Whole mount and histological analysis of glands from tumor-bearing mice commonly demonstrated hyperplasia, dysplasia, and carcinoma in situ, contrary to age-matched WT glands. The areas of ductal dysplasia and carcinoma in situ closely resembled several patterns commonly observed in human breast cancer, including micropapillary, cribriform, and high grade areas of ductal carcinoma in situ. Mammary tumors were analogous to infiltrating ductal carcinomas, many of which are high grade antigens. In summary, overexpression of Nsd3 in the mouse mammary gland elicited drastic deformation, inhibited functional differentiation, and caused tumor formation. Continued characterization of this mouse model, the oncogenic role of Nsd3, and other mechanistic studies are essential to improve patient outcome in 8p11-12 altered cancers.
#1836 Patient-derived xenograft (PDX) models expressing HER2 reflect clinical responses to targeted HER2 inhibition. Daniel Czindzadaj,2 Amir Sonnenblick,2 Jennifer Jaskowiak,1 Angela Davies,1 David Sidransky,3 Champions Oncology, Hackensack, NJ;4 Hadassah-Hebrew University Medical Center, Jerusalem, Israel;5 Johns Hopkins University School of Medicine, Baltimore, MD.

Background While HER2-directed agents are most often used for treating breast cancer, there is increasing evidence that these therapies may be of value in other solid tumors. Sequencing efforts and immunohistochemistry (IHC) have identified mutations, amplifications, and overexpression of HER2 in ovarian, HNSCC, NSCLC, and GI cancers. PDX models could permit evaluation of HER2 response/resistance mechanisms to optimize therapeutic strategies. In this study, we developed the response models to HER2-targeted therapies and correlated responses to clinical outcomes. Materials and Methods PDX models were developed from a variety of patient solid tumors, evaluated by IHC for HER2 expression and next-generation sequencing for genomic alterations in HER2 (mutations, amplifications/deletions, and expression levels). Models were screened against single agent HER2-directed therapies including trastuzumab (n=15), trastuzumab emtansine (n=23), and lapatinib (n=10). Tumor regression (TR) values and RECIST criteria were determined and correlated with known literature-based response rates (RR) as well as individual patient outcomes. Results 32 PDX models from 30 patients were interrogated (primarily breast and colorectal). Twenty (63%) models have been sequenced to date; 13 (65%) harbor amplification at ERBB2 gene locus. Further, 56% (18/32) have been sequenced for HER2: 20 (62.5%) have 2+ staining, 3+ staining, and 33% 1+ staining. Based on PDX tumor growth, stable disease/regression was observed in 10% of models screened against lapatinib (CR/PR=0%), 50% screened against trastuzumab (CR/PR=8%), and 67% tested against trastuzumab emtansine (CR/PR=14%). Only models with 2+ or 3+ HER2 staining showed regression with HER2-targeted treatment, with nearly 70% of 2+ HER2 models showing progressive disease. Finally, there were correlations to patient clinical outcomes available, with 3/4 (75%) of the PDX model responses mimicking those of the patient to the same treatment. Conclusion and Future Directions Extensive sequencing of human cancers has demonstrated HER2 amplification or mutation in numerous solid tumors, suggesting HER2-directed therapy could be applied more broadly in the clinic. Consistent with clinical findings, HER2 therapy responses depend on the strength of HER2 expression (based on IHC). Nevertheless, response rates in PDX models varied depending on which HER2-targeted agent was deployed, highlighting the potential existence of differential mechanisms of de novo resistance/sensitivity. Comprehensive sequencing and drug testing of these PDX models is planned and could allow a deeper understanding of such mechanisms. In this context, application of PDX models for translational modeling of HER2 drug responses, particularly in the context of co-clinical trials, will continue to evolve.

#1837 High Ras activity promotes neoplasia in pancreatic ductal cells. Kanchan Singh,1 Melissa A. Pruski,2 Waisin A. Dar,3 Kishore PoliReddy,4 John S. Bynon,5 Mamoun Younes,1 Anirban Maitra,2 Craig D. Logsdon,2 Jennifer M. Bailey1.1 University of Texas Health Science Center, Houston, TX; 2 The University of Texas MD Anderson Cancer Center, Houston, TX.

Pancreatic ductal adenocarcinoma (PDAC) remains a highly lethal malignancy and is the third leading cause of cancer-related deaths in the United States. Next generation sequencing efforts have revealed KRAS mutations occur in over 99% of pancreatic ductal adenocarcinomas and are thought to be the initiating mutation for pancreatic cancer. Despite the high prevalence of KRAS mutations in pancreatic cancer patients, murine models expressing endogenous levels of mutant KRAS in pancreatic cells do not develop PDAC. A number of publications have revealed that acinar cells are more sensitive to early neoplastic lesion formation in the presence of KRAS mutations than ductal cells. In these models, a second genetic hit (loss of tumor suppressor TP53, SMAD4 or CDKN2A) is required for the development of invasive PDAC, including transforming pancreatic ductal cells. Recent data has revealed a novel role for high levels of Ras activity in acinar cells in the transformation of this cell type to PDAC (Ji, B. 2008, published based on high levels of Ras activity to transform ductal cells). To test this hypothesis, we studied the role of constitutively active high levels of KRAS activity in acinar and ductal cells by expressing an inducible KRASG12V allele in Ptf1a:CreERT2 (acinar cell specific) and Hnf1b:CreERT2 (ductal cell specific) mice. Similar to previously published work, high levels of Ras activity in acinar cells resulted in PDAC and a remarkably low survival time of 10 days after mice were injected with tamoxifen. Mice with elevated Ras activity in ductal cells needed to be euthanized two weeks after tamoxifen injection and manifested a cancer cachexic phenotype. Histological analysis of these mice revealed high grade oncogenic intraductal neoplasia and the phenotype extended throughout the pancreatic ductal ducts. Western blot analysis of human PDAC and Cholangiocarcinoma tissue revealed similar levels of Ras activity to our murine models, confirming human equivalent high Ras expression to both animal models. Oncocytic tumors are characterized by an abundance of mitochondria. Thus, we are currently studying metabolic alterations and mitochondrial dysfunction in ductal cells expressing high Ras activity. Our goal is to determine how mitochondrial alterations play a role in progression or inhibition of tumors in ductal vs acinar cells.

#1838 TGFA (an EGFR ligand) promotes growth of EGFR-mutant lung tumors in airway regions but not in alveolar regions in a transgenic mouse model. Koichi Tomoshige, Yutaka Maeda. Cincinnati Children’s Hospital Medical Center, Cincinnati, OH.

Although an exogenous EGF is dispensable for EGFR+/−-mediated NIH3T3 cell transformation in vitro (Greulich et al., PLoS Med 2005), an exogenous EGF promotes growth of H1650 and H1975 lung adenocarcinoma cells that carry EGFR mutations in vitro (Sordella et al., Science 2004). However, it is unknown whether an exogenous EGFR ligand plays a role in growth of EGFR-mutant tumors in vivo. Here, using conditional transgenic mice expressing EGFR+/− or KRASG12D along with an exogenous TGFA (an EGFR ligand) in lung epithelium, we sought to determine the role of an EGFR ligand in EGFR+/−-lung tumors or wild type EGFR-lung tumors (KRASG12D/-/− tumors) in vivo. As previously reported, conditional expression of TGFA (an EGFR ligand) in lung epithelium caused pulmonary fibrosis but not lung tumors (Korfhagen et al., J Clin Invest 1994; Hardie et al., JIP, 1999). But we wondered that the expression of an exogenous EGFR ligand is not sufficient to induce lung tumors. Conditional expression of EGFR+/− or KRASG12D in lung epithelium caused the growth of lung tumors in airway regions but not in alveolar regions while the expression of EGFR+/− alone did not induce lung tumors in airway regions. Co-expression of EGFR+/− along with TGFA also shortened survival of the mice compared to the expression of either EGFR+/− or TGFA alone (median 32 days for EGFR+/−/TGFA vs 118 days for EGFR+/− alone or 248 days for TGFA alone). Co-expression of KRASG12D with TGFA did not influence tumor location or survival of the mice compared to the expression of either KRASG12D or TGFA alone. Consistent with the mouse study, TGFA expression in human EGFR-mutant lung cancer but not wild-type EGFR lung cancer was associated with worse overall survival (median 44 months for EGFR+/−/TGFA high vs 75 months for TGFA low; p<0.05) and recurrence free survival (median 21 months for TGFA high vs 61 months for TGFA low; p<0.05). Collectively, our results demonstrate that TGFA (an EGFR ligand) promotes not only the growth of EGFR+/−-lung tumors but also influences the regions where the EGFR+/−-lung tumors form.

#1839 TGFB1*6A mouse model mimics human breast cancer development and progression. Michael James Pennison, Minghui Wang, Hugo Jimenez, Boris Pasche. Wake Forest Baptist Medical Center, Winston-Salem, NC.

Transforming Growth Factor-β (TGF-β) is one of the most commonly altered cellular signaling pathways in human cancer. Our group was the first to discover TGFB1*6A (*6A), a common hypomorphic variant of the type I TGF-β receptor (TGFBR1), which has been associated with increased risk for breast cancer in multiple epidemiological studies. Functional analysis also suggests that *6A is associated with decreased tumor suppressive TGF-β signaling in early tumor development and is involved with promoting tumorigenesis in advanced carcinomas. To better understand the functional impact of *6A and constitutively reduced TGF-β signaling on breast cancer development and progression, we have recently developed a series of transgenic knock-in mouse model variants by replacing exon 1 of the mouse Tgfb1 sequence with human exon 1 sequences harboring both *6A and wild-type (*9A) TGFBR1 variants and crossed them with MMTVneu mice. We generated a total of 5 FVB double and/or triple transgenic mouse strains: MMTVneu*9A/9A, MMTVneu*6A/6A, MMTVneu*9A/6A, MMTVneu*9A/6A/. Following tumor development, measurements were performed three times per week to determine tumor volume. All mice were allowed to live up to 18 months (548 days) or until they died of natural causes. Specimens were collected for later analysis of blood, tumors, normal
mammary tissue, and were evaluated for lung metastases. Statistical comparisons performed using Student’s t-test. Only 34.4% of MMTVneu/9A/9A mice (11/32) developed mammary tumors, while 80% of MMTVneu/6A/6A mice (24/30; \( p = 0.0002 \)) and 62.5% of MMTVneu/6A/- mice (19/32; \( p = 0.046 \)) developed tumors. The percentage of MMTVneu/9A/6A and MMTVneu/9A/- mice developing metastases was less than MMTVneu/9A/9A mice (48.3% and 50%, respectively) but was not statistically significant. Additionally, 66.7% of MMTVneu/6A/6A mice (20/30) developed 2 or more tumors, while only 25% of MMTVneu/9A/9A mice (8/32) developed multiple tumors (\( p = 0.0007 \)). Our data suggests that we have developed transgenic mouse strains that accurately mimic human breast cancer susceptibility and develop metastases. These mouse models will be valuable to investigate subtypes of breast cancer and to provide insights into the role of TGFBR1 variants in the development and progression of breast cancer.

**TUMOR BIOLOGY: Cell Culture and Animal Models of Cancer 2**

**#1840 Whole-exome somatic mutation analysis of mouse cancer models and implications for preclinical immunomodulatory drug development.** Bruno Zeitouni,1 Cordula Tschuch,1 Jason M. Davis,2 Anne-Lise Peille,1 Yana Baeva,1 Manuel Landeseifen,1 Sheri Barnes,1 Julia B. Schuler1.1 Oncotec GmbH, Freiburg, Germany; 1Charles River Discovery, Morrisville, NC

Experimental tumors raised in rodents represent an important preclinical tool to develop innovative anticancer compounds before clinical testing. Amongst others such models include solid tumors raised in syngeneic fully immune competent hosts and tumors spontaneously growing in genetically engineered mice (GEM) and derived therefrom. These model platforms have gained additional value since the manipulation of the immune system to engineer mice (GEM) and derivate thereof. These model platforms have been used as preclinical tools to develop innovative anticancer drugs. This review focuses on the preclinical use of GEM in mouse cancer models for immune cell signaling and validation of novel therapeutic strategies. We describe the key features of the GEM platform and discuss the potential advantages and limitations of GEM models for drug development.

**#1842 Breast cancer brain metastasis (BCBM) model for determination of therapeutic brain penetration.** Emily A. Wyatt, Mark E. Davis. California Institute of Technology, Pasadena, CA.

Purpose Brain metastases are presenting an increasing problem in the clinic, and especially in treatment of patients with human epidermal growth factor receptor-2 (HER2)-amplified breast cancer. Although extracranial metastases respond well to HER2 inhibitors, human clinical data shows brain metastases hide behind an intact blood–brain barrier (BBB) and are largely untreatable. Mammalian brain tumors and metastatic models lack this barrier integrity, limiting their utility in understanding delivery of drugs to the brain. We present here the development of a new model suitable for evaluating therapeutic brain penetration in addition to efficacy. Experimental procedures Human HER2-amplified BT-474 breast cancer cells were inoculated intravenously (tail vein) in female Rag2-/-Il2rg-/- mice (2 million cells/mouse) to induce multigorgan metastasis. Formation of metastatic brain lesions was monitored by magnetic resonance imaging (MRI). For comparison, BT-474 cells were inoculated intracranially for direct brain tumor implantation (50 000 cells/mouse). Response to a suite of HER2-targeted therapies known not to appreciably cross an intact BBB (trastuzumab, lapatinib, etc.) was largely untreatable. Multigorgan metastatic models lack this barrier integrity, limiting their utility in understanding delivery of drugs to the brain. We present here the development of a new model suitable for evaluating therapeutic brain penetration in addition to efficacy. Experimental procedures Human HER2-amplified BT-474 breast cancer cells were inoculated intravenously (tail vein) in female Rag2-/-Il2rg-/- mice (2 million cells/mouse) to induce multigorgan metastasis. Formation of metastatic brain lesions was monitored by magnetic resonance imaging (MRI). For comparison, BT-474 cells were inoculated intracranially for direct brain tumor implantation (50 000 cells/mouse). Response to a suite of HER2-targeted therapies known not to appreciably cross an intact BBB (trastuzumab, lapatinib, etc.) was monitored by MRI for both metastatic and implanted brain tumors. Mice were sacrificed following signs of prolonged distress or loss of >20% body weight. Organs were collected for standard histological and immunohistochemical analysis, as well as for CLARITY tissue clearing and large-scale 3D macromolecule mapping. Results Intravenous inoculation of BT-474 cells into Rag2-/-Il2rg-/- mice consistently reproduced the full metastatic profile seen in humans, with metastases in the lung, bone, liver, ovary, lymph, and brain tissues. Brain metastases were detected in 90% of mice inoculated intravenously. Histological analysis of metastatic brain tumors showed different morphologies and invasive characteristics compared to those intracranially implanted. Additional differences in vasculature between metastatic and implanted brain tumors were identified by CLARITY. Importantly, HER2-targeted therapy markedly delayed progression of implanted brain tumors but failed to slow metastatic brain tumor growth, recapitulating the clinical situation. Conclusions These data, together with ongoing efforts to further characterize therapeutic transport to these brain tumors, suggest that intracranial inoculation disrupts the BBB and creates artificial routes for therapeutics to reach implanted brain lesions, resulting in anomalous tumor response. In contrast, this new metastatic model reproduces the discordant effects of HER2-targeted therapy in patients, and offers a platform for studying the efficiency of therapeutic delivery across an intact BBB as well as antitumor activity, both of which are critical to effective clinical translation.

**#1843 Intratumoral heterogeneity of renal cancer is related to differences in drug response and development of therapy resistance.** Michael Becker,1 Burkhard Jandrig,2 Susanne Flechsig,1 Reiner Zeisig,1 Daniel Schindele,2 Martin Schostak,7 Christian Schmees,7 Annika Wulf-Goldenberg,3 Jörg Hennenlotter,4 Elke Schaeffeler,7 Matthias Schwab,7 Arnulf Stenzl,4 Jens Bedke,4 Jens Hoffmann1.1 EPO - Experimental Pharmacology and Oncology Berlin-Buch GmbH, Berlin, Germany; 2University Medical Center Magdeburg, Magdeburg, Germany; 3NMI University Tuebingen, Tuebingen, Germany; 4University Hospital Tuebingen, Tuebingen, Germany; 5Margarete Fischer-Bosch-Institut for Clinical Pharmacology, Stuttgart, Germany.

Background: Patients with advanced renal cell cancer (RCC) have a poor prognosis not only because of resistance towards standard drugs (SoC). Recently, pronounced intratumoral heterogeneity (ITH) in RCC was shown. We...
were interested whether this ITH is a potential cause for treatment failure. We
developed a large panel of patient-derived xenograft (PDX) models from RCC,
including subsets of models from different regions of one individual tumor. The
PDX models were evaluated for response to SoC. To better understand correla-
tions between inter- and intratumoral heterogeneity and treatment response,
tumor models were panel sequenced and expression profiled. Methods: Speci-
mens from primary and metastatic RCCs were collected from consenting pa-
tients and transplanted into mice. Tumor engraftment was monitored for up to
4 months. Tumor sections were examined histopathologically to assess concor-
dance between patient tumor and model and were stained for RCC specific
markers (Pax2, Pax8, CD31, and RCC). Stable growing PDX were treated with
SoC (sunitinib or sorafenib, bevacizumab and everolimus). Global gene expres-
sion was analyzed in primary tumors and PDX models using microarrays (Af-
zymex). In addition, sequence variations (Illumina NGS cancer panel) and
MET and TERT gene copy numbers were analyzed in PDX models. Results: A
panel of 34 RCC PDX models was established from more than 200 patient sam-
ple. Among these, 13 models were derived from different tumor regions of three
advanced tumors. Original patient tumor and PDX showed a very similar and
characteristic RCC histopathology. Inter- and intratumoral heterogeneity was
preserved for several passages. We treated all PDX with standard targeted drugs
and observed response rates comparable to results from clinical trials. One out of
8 regions obtained from one aggressive RCC clearly differentiated in regard to its
response to bevacizumab and sunitinib. Genomic analysis revealed that this region
had a number of differences in global gene expression and sequence variation pattern.
Besides a common MET mutation an additional variation in the HRAS gene was
detected. In the whole PDX set we found 34 sequence variations in 20 genes, e.g.
ATM, MET, TP53 and VHL and copy number variations in the MET locus.

**#1844 Investigating the role of amphiregulin in breast cancer.**
Serena P. Chiang, George S. Karagiannis, John S. Condellelts, Jeffrey E. Segall. Albert Ein-
tstein College of Medicine, Bronx, NY.
Breast cancer is the most prevalent cancer in women worldwide and afflicts
more than 3.1 million women in the US alone. The epidermal growth factor re-
ceptor (EGFR) is frequently overexpressed in breast cancer and is associated
with poor clinical outcome. Several therapies involving anti-EGFR agents have
been developed to target breast cancer. However, clinical trials have shown
mixed outcomes with these therapies. Amphiregulin (AREG) is a ligand for
EGFR that mediates estrogen-induced mammary ductal morphogenesis during
development. Expression of AREG is enriched in invasive breast carcinomas,
particularly ERα-positive breast tumors. Studies have shown that AREG in-
creases the in vitro invasiveness of breast cancer cells. Furthermore, suppression of
AREG expression in transformed human breast epithelial cells reduces tumor
formation when injected in nude mice. These observations suggest that AREG
plays an important role in breast cancer development and progression. To in-
vestigate the role of AREG in breast cancer development and progression, we
utilized a spontaneous mammary tumor mouse model where oncogenesis is
driven by expression of the polyoma middle T oncprotein (PyMT) through the
mouse mammary tumor virus (MMTV) promoter. We have crossed AREG
knockout mice with the MMTV-PyMT mice to generate AREG KO mice that
spontaneously form mammary tumors. Tumors in AREG KO PyMT mice ini-
tially grow more quickly but eventually reach 2 cm in diameter at a relatively
time as compared to tumors formed in AREG WT PyMT mice. Histologically,
AREG KO tumors appear more differentiated and display a greater proportion
of tumor stroma. This prompted us to further hypothesize that AREG may have
diverse effects in the tumoral or stromal compartment of developing tumors. To
individually assess the effects of AREG knockout in the tumor cells and the
stroma, we utilized a transplantation model in which we prepared tumor chunks
from AREG KO PyMT tumors and transplanted them into AREG WT and
KO mice. Through this model, we have found that AREG expression in the
tumor has a greater impact on tumor growth and progression than the tumor
stroma. Regardless of the AREG status of the recipient mice, AREG KO tumors
display a significant growth impairment and reduced transplant efficiency. In
summary, these results demonstrate that AREG is important for mammary tu-
mer progression.

**#1845 Mutant p53 promotes progression and metastasis of mouse oral

tumors induced by 4NQO, associated with specific immune infiltrates.**
Jin Wang, Yuanyang Zhang, Bingbing Wang, Adel K. El-Naggar, Jeffrey N. Myers,
Carlos Caulin. UT MD Anderson Cancer Center, Houston, TX.
Head and neck squamous cell carcinoma (HNSCC) is the sixth most com-
non malignancy worldwide. The vast majority of the human HNSCCs con-
tain p53 mutations, some of which acquire oncopgenic gain-of-function (GOF)
activities. Previous mouse models in which either the p53R172H/GOF
mutation or deletion of the p53 gene were co-activated with oncogenic K-ras
showed that p53R172H can predispose to oral tumor initiation, accelerate
tumor growth and promote progression to SCC. However, the impact of p53
gene mutations on immune infiltration remains to be assessed because of the
low rates of progression to SCC in that model. To overcome this limitation, we used the tobacco-surrogate 4NQO to induce oral

lesions that may progress to advanced carcinomas following stepwise
changes that resemble the gradual accumulation of histological and molec-
ular abnormalities observed during human oral cancer progression. To ex-
amine the role of p53 mutations during SCC malignant progression we
applied 4NQO to mice in which the p53R172H mutation or homozygous
deletion of p53 were activated in oral epithelial cells. We observed that oral
tumors appeared faster in the presence of p53 mutations than in mice with
wtp53. No significant difference was observed between the p53R172H/GOF
mutation and p53 deletion. However, survival was shorter in mice expressing
the p53R172H mutation compared to wtp53 expressing tumors. Genomic
infiltration were only observed in mice with oral tumors expressing p53R172H.
Therefore, these mice are excellent tools for pre-clinical studies designed to
target early stages of tumor development or latest stages of progression and
metastasis. As immunotherapy is becoming adopted as part of the standard
treatment for head and neck cancer patients, we conducted a detailed char-
acterization of the immune infiltrates of the lesions that developed in these
mice in order to assess their immunogenicity. We observed a progressive
increase in T-cell infiltration from normal oral mucosa to oral premalignant
lesions (OPL) to SCC, in the presence or absence of p53 mutations. Similarly,
the expression of immune checkpoint proteins, including PD1, PDL1 and
CTLA4 increased during oral tumor progression, regardless of the p53 sta-
tus. Interestingly, we observed a sharp increase in the presence of immune
infiltrates expressing the co-stimulatory OX40 in SCCs relative to premalign-
nant lesions, with different extent depending on the nature of the p53 mu-
tation. The significance of these observations is currently being analyzed. In
summary, p53R172H promotes metastasis in oral tumors induced by 4NQO.
In addition, T-cell infiltration and activation of immune checkpoints during
oral tumor progression suggest that this is an excellent model to test immu-
notherapy-based strategies. Furthermore, our findings suggest that OX40-
based therapies may be tailored to patients according to their p53 status.

**#1846 Immunocompetent mouse allograft models for development of
therapies to target breast cancer metastasis therapies to target breast cancer
metastasis.**
Yu-an Yang,1 Howard Yang,1 Ying Hu,1 Peter Watson,2 Huaitian Liu,1 Thomas R. Geiger,1 Miriam R. Anver,3 Diana Haines,3 Philip Martin,3
Maxwell P. Lee,1 Kent W. Hunter,1 Lalage M. Wakefield1.
1National Cancer Institute, Bethesda, MD; 2British Columbia Cancer Agency, Victoria, British Co-
lumbia, Canada; 3Leidos Biomedical Research Inc., Frederick National Labora-
tory for Cancer Research, Frederick, MD.
Effective drug development to combat metastatic disease in breast cancer
would be aided by the availability of well-characterized preclinical animal mod-
els that (a) metastasize with high efficiency, (b) metastasize in a reasonable
time-frame, (c) have an intact immune system, and (d) capture some of the
heterogeneity of the human disease. To address these issues, we have assembled
a panel of twelve mouse mammary cancer cell lines that can metastasize effi-
ciently on implantation into syngeneic immunocompetent hosts. Genomic
characterization shows that more than half of the 30 most commonly mutated
sites in breast cancer are represented within our panel. Transcriptome anal-
lytically, most of the models fall into the luminal A or B intrinsic molecular sub-
types, despite the predominance of an aggressive, poorly-differentiated or spin-
dled histopathology in all models. Patterns of immune cell infiltration,
proliferation rates, apoptosis and angiogenesis differed significantly among
models. Inherent within-model variability of the metastatic phenotype man-
dates large cohort sizes for intervention studies but may also capture some re-
levant non-genetic sources of variability. The varied molecular and phenotypic
characteristics of this expanded panel of models should aid in model selection
for development of anti-metastatic therapies in vivo, and serve as a useful plat-
form for predictive biomarker identification.
#1847  Evaluating the metastatic potential and the molecular heterogeneity of patient-derived orthotopic xenograft models of triple-negative breast cancer.

Vishnu C. Ramani,1 Rakhi Gupta,1 Gerald Quon,1 Melanie Triboulet,1 Corinne Renier,1 Cassandra Greene,1 Chad Sanada,1 Tracy Lu,1 Lukasz Szpankowski,1 Naveen Ramalingam,1 Ameen A. Salahudeen,1 Jean de la O,1 Ranjani Rajaratnam,1 Francois Nouy,1 E. Charles Carey,1 J Chad Brenner.

University of Nebraska Medical Ctr., Omaha, NE

We report an in-depth characterization of patient-derived orthotopic xenograft (PDX) models of triple-negative breast cancer (TNBC) regarding their molecular, epithelial cell level, tumor heterogeneity, 3D organoid generation, and ability to generate circulating tumor cells (CTCs). A panel of seven TNBC PDX tumors were grown orthotopically in Nod scid gamma mice and used in this study. Blood obtained via cardiac puncture from tumor bearing animals was processed on the Vortex microfluidic platform, for label-free, size-based enrichment of circulating tumor cells (CTGs). Enriched cell populations were stained for human-specific cytokeratin (CK) and Vimentin (Vim), mouse specific CD45, and DAPI; CTCs were identified as cells that were CD45 negative and positive for CK or Vim. Bulk tumor growing in the mammary fat pads was dissociated to single cells and characterized using Fluidigm’s® Polaris® plating platform for single cell biological experimentation and cDNA generation within an integrated fluidic circuit (IFC). From the cell suspension, Polaris identified single cells that were assessed for mRNA expression. The resulting cDNAs were then multiplexed using NextrX XT® (Illumina®) and sequenced on Illumina systems. Data generated from mRNA-seq was processed to correct for confounding factors such as cell size, cell cycle and read depth and then analyzed to screen for heterogeneity between different populations of cells. Tumors were analyzed by flow cytometry for both tumor and immune cells and additionally the single cell suspension was seeded into 3-D culture to generate organoids. Finally, organs from tumor bearing animals were analyzed for metastases. With the Vortex platform, we detected CTCs from a majority of our PDX tumor-bearing mice. The total number of CTCs varied over a wide range between different PDX tumors. There was a clear heterogeneity in CTCs in terms of CK and Vim expression. In CTCs from one of the PDX tumors, we detected a small population of CTCs that were either CK+ or Vim+ but the major fraction that was double positive (Vim+ CK+). Probing the bulk tumor from different PDX models revealed heterogeneity in the levels and number of cells positive for cell surface markers like EpCAM and a difference in the levels of infiltrating myloid cells (CD11b+). mRNA-seq analyses of individual tumor cells from the bulk tumor belonging to different PDX models will be described. Additionally, lung and brain metastases were identified. 3D organoids from our PDX models were successfully grown and their gene expression profiles will be analyzed. In summary, PDX models of TNBC will help advance our understanding of the molecular basis of this deadly cancer.

#1848  Autocrine IGF1 signaling mediates the survival of pancreatic cancer cells following the ablation of oncogenes.

Nirakar Rajbhandari. Univ. of Nebraska Medical Ctr., Omaha, NE

Pancreatic cancer is an extremely lethal disease, which is mostly associated with the gain-of-function mutation in KRAS gene. Due to a very high frequency of activating mutations within the KRAS gene, mutant KRAS is considered a rational therapeutic target in pancreatic cancer. In line with the existing idea that oncogenic KRAS is required for the maintenance of pancreatic cancer, we show a complete macroscopic regression of both primary and orthotopically transplanted tumors in our ligand-regulatable (Doxycycline) mouse model of KRASG12D-driven pancreatic cancer. This finding indicates that the expression, and provides a strong rationale for the development of KRAS targeted therapy for the treatment of pancreatic cancer. However, in our current study, despite the complete macroscopic regression of PDAcs upon downregulation of oncogenic KRAS, few cancer cells always survived and remained dormant for a protracted duration. These residual cancer cells were responsible for the rapid recurrence upon tumor resection. Using a genome-wide Seqi analysis of in vivo-derived bulk and residual cancer cells followed by biochemical analysis, we identified a significant increase in autocrine IGF-1/ AKT signaling in residual cancer cells. We also identified IGF1 signaling as a common survival mechanism of residual cancer cells in C-MYC-driven pancreatic tumor model. Pharmacological inhibition of IGF-1R signaling significantly delayed the tumor recurrence in both the tumor models, suggesting the crucial role of IGF-1R signaling in the survival of cancer cells in the absence of oncogenic drivers. Our findings from animal models were also validated in human pancreatic cancer cell lines harboring KRAS mutations, by demonstrating a compensatory increase in IGF1 signaling in response to a conditional knockdown of KRAS in vitro and in vivo. Collectively, all our findings from mouse model and human cancer cell studies strongly indicate that residual cancer cells can survive in the absence of tumor driving oncoproteins by up-regulation of autocrine IGF1 signaling loop. Thus, targeting oncogenic drivers along with IGF1-IR signaling might be an effective strategy for prevention of tumor recurrence in pancreatic cancer.

#1849  The role of leptin signaling in the promotion of obesity-associated tumorigenesis and cancer stem cell characteristics in a transgenic mouse model of basal-like breast cancer.

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Background: Epidemiological studies have linked obesity to a greater risk of breast cancer-specific mortality. This association may be mediated in part by enhanced cancer stem cell (CSC) enrichment, as CSCs are thought to be the primary drivers of tumor initiation, growth, and metastasis. We and others have demonstrated that obesity promotes CSC enrichment in pre-clinical models of basal-like breast cancer, but only when leptin signaling is present. These findings, coupled with evidence that elevated serum leptin and breast tumor leptin receptor expression are linked to a worse breast cancer prognosis, suggest that leptin-induced CSC enrichment may be a key contributor to obesity-associated breast cancer progression.

Methods: MMTV-Wnt-1 transgenic mice, which develop spontaneous basal-like mammary tumors, were fed a control diet (10% kcal from fat) or a diet-induced obesity regimen (DIO, 60% kcal from fat). Mice were euthanized when tumors reached 1 cm in diameter. RNA sequencing was performed on 4 tumors/diet group. Serum hormones and cytokines were measured via luminex-based assay. ALDH activity, a putative CSC marker, was quantified by an ALDH Activity Assay Kit (abcam). Quantitative RT-PCR was used to determine expression of CSC-related genes, leptin, and the leptin receptor. Two mammary tumor cell lines isolated from the MMTV-Wnt-1 model (M-Wnt and E-Wnt cells) and human MDA-MB-231 were exposed in vitro to pooled serum from DIO or control mice (2% concentration), and cell viability, migration, invasion and expression of CSC related genes were measured. Results: Tumor incidence and growth rate were greater in DIO versus control mice. RNA sequencing revealed an upregulation in a CSC gene signature in tumors from DIO versus control mice. Significant upregulation in several of these genes, including aldha1a, oct4, twist1, twist2, and akt3, was confirmed by qRT-PCR (P < 0.05). ALDH activity in the DIO (relative to control) tumors was also significantly increased (P < 0.05). Serum levels and mammary tissue expression of leptin and tumor expression of the leptin receptor were significantly elevated in DIO versus control mice (P < 0.05). Exposure to serum from DIO mice significantly increased (relative to control) M-Wnt, E-Wnt, and MDA-MB-231 cell viability, migration, invasion, and expression of CSC-related genes in vitro (P < 0.05). The role of leptin signaling in these breast tumor models and in vitro measures of CSC enrichment will be explored through additional experiments. Conclusions: Obesity promotes MMTV-Wnt-1 mammary tumor incidence and growth rate, possibly via CSC enrichment. Preliminary findings suggest increased leptin signaling may underlie obesity-associated CSC enrichment and may contribute to the breast cancer burden in obese women.

#1850  Development of a NOTCH1 loss of function and PIK3CA mutant transgenic model of HNSCC.

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Head and neck squamous cell carcinoma (HNSCC) is a debilitating disease with a poor 5-year survival rate. The use of in vivo models to study tumorigenesis and to identify potential targeted therapies, especially those that work in combination with immune checkpoint inhibitors, is essential in improving overall patient care. However, the overall number of HNSCC transgenic models with recurrent genetic lesions identified by the HNSCC TCGA project has been limited. For example, TCGA data has shown both Notch1 loss of function mutations and PIK3CA activating mutations as some of the most common in HNSCC, but bi- genetic models of these lesions do not yet exist. Here, we have designed a model which utilizes K14-CreERT to drive a bi-genic model with Notch1lox and PIK3CAH1047R mutations in epithelial compartments. Characterization of this line both in the context of spontaneous and carcinogen induced tumor formation is a tool which can be utilized to better understand common mutations in HNSCC. Immunohistochemistry confirms the overexpression of PIK3CA in tamoxifen induced mice. Furthermore, early characterization of this model with chronic treatment of the smoking analogue 4-Nitroquinoline N-oxide (4NQO) in drinking water shows tumor formation on the tongue and surrounding head and neck regions in as little as 12-16 weeks. Hematoxylin and Eosin staining confirmed the squamous tumor formation, while Ki67 staining

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showed enhanced proliferation of the transformed cells. In the future, this model can be used to study tumor formation over time and in response to targeted inhibitors and common therapies that are being advanced as companion diagnostic strategies for either NOTCH1 deficient or PIK3CA aberrant HNSCC tumors. Further detailed characterization of this model will allow for a deeper understanding of the mechanisms which drive HNSCC and will translate to improved patient care.


Purpose: von Hippel-Lindau disease (VHL) is an autosomal dominant condition that features a constellation of cysts or highly vascularized tumors due to loss of heterozygosity (LOH) of the VHL gene, resulting in deregulated hypoxia-induced factors (HIFs). Clinical manifestations of VHL include hemangioblastomas in the central nervous system and retina. To date, no VHL animal model has reproduced retinal capillary hemangioblastomas (RCH), the hallmark lesion of ocular VHL. The RCHs may arise from arrested hemangioblast progenitor cells, with VHL LOH in the tumor stroma. The stem cell leukemia (SCL) gene encodes a basic helix-loop-helix (bHLH) transcription factor, a critical regulator of hematopoiesis and vasculogenesis. We aim to generate a murine model of VHL-associated RCH by conditionally inactivating Vhl in a hemangioblast progenitor population with the angioblast-specific Cre line, (SCL-Cre-ER2). Methods: We established a genetic mouse model in which the inducible Vhl gene is specifically “knocked-out” in angioblast cells using the stem cell leukemia (SCL)-Cre-ER2 transgenic mice. Two-week-old SCL-CreER2;Vhl/F/F mice were administered tamoxifen (2mg/40g body weight) for 5 consecutive days to activate the Cre recombinase that induces Vhl deletion in angioblast-derived cells. Funduscopy was done monthly to detect retinal lesions. Fluorescein angiography (FA) was performed on affected mice. All retinas were analyzed by histology at 4 months of age. Genome typing of the Vhl conditional KO allele was conducted in retinal lesions using microdissection, nest-PCR and Sanger sequencing. Results: About 64% (18/28) of the transgenic mice exhibited various retinal vascular defects following induction. Affected mice demonstrated retinal vascular lesions that were variably associated with prominent vessels, anomalous capillary networks, hemorrhage, exudates, and localized fibrosis. FA revealed vascular leakage from the lesions. Histological analyses showed RCH-like lesions of the lesions that were variably associated with prominent vessels, anomalous capillary networks, hemorrhage, and localized fibrosis.

#1855 Inhibition of mTOR downregulates expression of DNA repair proteins and is highly efficient against BRCAl2-mutated breast cancer when combined to PARP inhibition. Florence Coussy, 1 Rania El Botty, 1 Rana Hatem, 1 Franck Assayag, 2 Ahmed Dahmani, 1 Marine Huppé, 1 Sophie Chateau Joubert, 1 Jean-Luc Servey, 1 Virginie Bernard, 1 Sophie Vacher, 1 Berangere Ouisse, 1 Aurelie Cartier, 1 Jeanne De Koning, 1 Paul Cottu, 1 Ivan Bienke, 1 Elisabetta Marangoni, 1 Institut Curie, Paris, France; 2 École nationale vétérinaire d’Alfort, Maison Alfort, France.

Purpose: DNA repair deficiencies and activation of P13K/akt/mTOR pathway are common events in breast cancer. Brca1/2 mutations have been associated with sensitivity to PARPi inhibitors (synthetic lethality), while alterations in component of the PI3K/AKT/mTOR pathway might confer sensitivity to PI3KCA and mTOR inhibitors. Here we explored the therapeutic benefit of combining a mTOR and a PARPi inhibitor in BRCA2-mutated patient-derived xenografts (PDX) with alterations in the PI3KAKT/mTOR pathway. Experimental design: the combination of the mTOR inhibitor everolimus and the PARPi inhibitor olaparib was tested in two BRCA2-mutated PDX established from a luminal B tamoxifen-resistant and a basal-like breast cancers. Both carried alterations in the PI3K/akt/mTOR pathway: PIK3R1 mutation and PTEN loss for the luminal B PDX and PTEN loss for the basal-like PDX. To identify putative crossstalk events between mTOR and DNA repair, a Reverse Phase Protein Array (RPRA) analysis of multiple signaling pathways and DNA repair processes was performed on untreated and treated xenografts. Gene and protein expression changes were confirmed by RT-PCR and Western Blot analyses. The capacity to repair DNA damage was measured by P-H2AX immunostaining. Results: in both PDX, everolimus and olaparib showed marked anti-tumor activity with a growth inhibition comprised between 78% and 86% in the monotherapy setting and 96% in the combination arm, where 100% of mice showed tumor regressions. In the luminal B tumor this combination was more efficient than the combination of everolimus and endocrine therapies (fulvestrant or tamoxifen). In both PDX, the fraction of P-H2AX (marker of unrepaired DNA double-strand breaks) positive cells was increased after everolimus treatment, suggesting a link between mTOR and DNA damage, and strongly increased in the combination setting. RPRA analysis of tumors PhenoProbes alone revealed a marked downregulation of different proteins involved in DNA repair, including FANCID2, RAD50 and SUV39H1, a chromatin compactor factor essential in homologous recombination. In the combination setting, expression of these proteins was almost completely abolished, suggesting convergence of PARP and mTOR in downregulation of DNA repair components. Conclusions: our results support that combining mTOR and DNA repair inhibitors could be a successful strategy to treat a subset of breast cancer with BRCA2 mutation and alterations in the PI3K/akt/mTOR pathway. Further experiments with mTOR and PARPi inhibitors combinations are ongoing in sporadic breast cancer PDX showing a BRCA1-necessity phenotype.

#1854 Generation and characterization of mouse model of Pmepa1 conditional knockout in prostate epithelia. Hua Li, Shashwat Sharad, Talai Bariev, Yingjie Song, Denise Young, Taduru Sreenath, Albert Dobi, Shiv Srivastava. USU/Center for Prostate Disease Research (CPDR), Rockville, MD.

Introduction and Objectives: Prostate cancer (CaP) is the most common non-skin malignancy diagnosed in American men and defunctions of androgen receptor (AR) plays an essential role in prostate tumorigenesis. PMEPA1 is an androgen and TGF-β-induced gene abundant in prostate, which was identified to be associated with prostate tumors via gene expression in prostate cancer cell lines and reduced or loss of expression of prostate cancer. We generated Pmepa1 expression, commonly detected in prostate tumors, led to increased AR protein and activated AR signaling. Pmepa1 inhibited TGF-β receptor 1 mediated signaling by a negative feedback loop. It was reported that loss of Pmepa1 facilitates bone metastasis of CaP through blocking TGF-β signaling. To further investigating the biological function of PMEPA1 gene in CaP tumorigenesis, particularly via AR and TGF-β, we generated Pmepa1 prostate conditional knockout mouse model. Methods: C57BL/6 mice were utilized for generation of Pmepa1 conditional knockout model. Pmepa1 gene was conditionally deleted in mouse prostate epithelium by AAR2PB-Cre-Lox system. Male mice of genotypes of wild-type, Pmepa1 flox/flox and flox/wild-type AAR2PB-Cre, Pmepa1 flox/flox or flox/ARR2PB-Cre were euthanized at the age of 3 months for analysis. The prostate tissue was collected for frozen and formalin fixation for histology analysis, and other major organs including heart, lung, liver, spleen, bladder and testis were also collected for control. The tissue were sectioned and stained with Hematoxylin & Eosin (H&E). Total RNA and protein were harvested by homogenizing tissue. The protein levels of Pmepa1, AR and Nkx3.1 were analyzed by immunohistochemistry (IHC) and immunoblotting, and the transcript levels of these genes were evaluated by in situ hybridization (ISH) and quantitative PCR (Q-PCR). Results: The expression of Pmepa1 protein was found to focus on lateral lobe, consistent with AR protein expression pattern. The data of Q-PCR and ISH showed that transcript level of Pmepa1 was dramatically suppressed in Pmepa1 flox/flox and flox/wild-type AAR2PB-Cre mice. Compared to wild-type ones, Pmepa1 protein was shown significantly decreased in heterogeneous and homogenous knockout mice via immunoblotting and IHC staining. And the protein level of AR was stronger in Pmepa1 conditional deleted mouse. There was no dramatic morphology change in mouse prostate gland by H&E staining. Conclusions: Conditional deletion of Pmepa1 gene in mouse prostate epithelium led to enhanced AR protein and activating AR signaling. The effects of Pmepa1 gene loss in mouse prostate gland on other signaling such as TGF-β and prostate tumorigenesis are needed to be further evaluated. Funding: This study was supported by CPDR, SUHS, HU0001-10-2-0002 to DGM.


Purpose: In order to develop a patient DRation method for hepatocellular carcinoma (HCC), we develop the new protocol using direct injection technique. From January 2015 to June 2016, consent about getting tumor pieces to the patient of hepatocellular carcinoma. The specimens is stored in RPMI1640 and moved from opera-
tion room to research room immediately. For PDOX and PDX to subcutaneous model, 8–10 weeks old NOD SCID and NSG male mouse is used. The tumor pieces cut to the cube size 1mm. For stop the bleeding, inferior vena cava is blocked using Bulldog clamp and the cube is directly injected median lobe to 3 places randomly. After clear the Bulldog clamp, a left lobe is removed using tie and weight. After 3 months, the PDOX mouse is changed for cancer growth using MRI or PET-CT. Successful PDOX or PDX to subcutaneous model is sacrificed and proceeded a generation to another Balb/C-nude male mouse 8–10 weeks old. The rate of success of PDOX model and PDX to subcutaneous model is about 10% respectively. Formed tumor is verified by H&E staining. Based on protocol established, further study plan is required for improving on the rate of success to establish PDOX model and clinical application.

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#1856 Dual-modality immunoPET/fluorescence imaging of prostate cancer utilizing 89Zr- or 124I-Cy5.5-anti-PSCA cys-minibody

Wen-Ting K. Tsai, Anna M. Bhattacharya, UT MD Anderson Cancer Ctr., Houston, TX

Prostate cancer patients can benefit from non-invasive and more accurate diagnosis, as well as improved visualization during surgery. ImmunoPET can provide information on location and extent of the disease, while fluorescent image-guided surgery can distinguish cancerous tissue during resection. Prostate Stem Cell Antigen (PSCA) is upregulated in the majority of prostate cancers and metastases and is therefore a promising target for imaging. Engineered antibody fragments, such as the minibody, exhibit ideal imaging characteristics due to fast blood clearance for high target-to-background images at short imaging times post-injection. A dual-labeled minibody can reveal the whole-body PSCA-expressing tumor burden by PET, followed by intraoperative visualization of margins by fluorescence. The fully humanized anti-PSCA A11 minibody was engineered with a C-terminal cys-tag (A11cMb) for site-specific labeling by thiol-directed chemistry. In order to radiolabel with 89Zr, a metal chelator deferoxamine (DFO) was site-specifically conjugated to A11cMb by maleimide chemistry, while direct iodination was used to label with 124I. Simply labeled 89Zr- and 124I-A11cMb successfully imaged subcutaneous PSCA (+) and (−) 22Rv1 human prostate adenocarcinoma tumors in nude mice, resulting in positive-to-negative tumor ratios of 2.5:1 and 8:1, respectively. Both 89Zr-A11cMb and 124I-A11cMb cleared from blood by 22 hours. As expected for the residualizing radiometal, 89Zr-A11cMb resulted in retention in the organs of clearance (liver and kidneys). For dual-modality imaging, A11cMb was site-specifically conjugated with Cy5.5-maleimide and radiolabeled with 124I PET imaging with 124I-Cy5.5-A11cMb in the subcutaneous 22Rv1 tumor model resulted in a positive-to-negative tumor ratio of 13:1. The PSCA (+) tumors were subsequently visualized by fluorescence in situ and ex vivo with high contrast in comparison to PSCA (−) tumors and tissues. In an orthotopic model, PSCA (+) 22Rv1 cells were implanted in the prostate, and therefore 89Zr was used in order reduce interfering signal from clearance to the bladder. A11cMb was site-specifically conjugated with Cy5.5 and randomly labeled with SCN-DFO, then radiolabeled with 89Zr. 89Zr-Cy5.5-A11cMb successfully imaged the prostate tumor, resulting in a 3:1 tumor-to-blood ratio. Fluorescence imaging clearly distinguished prostate tumor from adjacent tissues including seminal vesicles. In conclusion, a single injection of the dual-labeled A11cMb can visualize tumor burden by immunoPET and fluorescence imaging. This humanized probe has the potential for clinical translation for primary and metastatic prostate cancer detection and surgical guidance that can ultimately enhance treatment success.

#1857 Early detection of pancreatic intraepithelial neoplasms (PanINs) in transgenic mouse model by hyperpolarized 13C metabolic resonance imaging

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Pancreatic cancer, one of the most lethal solid tumors, is an aggressive disease that develops relatively symptom-free. Pre-invasive pancreatic intraepithelial neoplasias (PanINs) have been identified as precursor lesions to pancreatic cancer. While there is growing evidence supporting PI specificity of V7-CMS, Panc1 and S2VP10 cell lines were incubated in cell culture medium at either pH 7.4 or 6.6 followed by treatment with V7-CMS. Particle uptake was determined using near infrared (NIR) fluorescence and tissue phantoms. Finally, for in vivo testing, the same CSMs were injected into mice bearing S2VP10 pancreatic tumors. MSOT imaging was performed 8 hours after CMS injection. Results: TEM images demonstrated successful synthesis of approximately 40 nm V7-CSMs with vermillion pores. On NIR imaging, V7-CSMs demonstrated acidic pH specificity in both S2VP10 and Panc 1 cells at pH 6.6, with particle localization and signal intensity 8-fold and 5-fold higher, respectively, than that observed at pH 7.4. In tissue phantoms, increased pH specificity was also observed in both S2VP10 and Panc 1 cells at pH 6.6. Particle location and signal intensity on MSOT was demonstrated successful synthesis of approximately 40 nm V7-CSMs with vermillform pores. On NIR imaging, V7-CSMs demonstrated acidic pH specificity in both S2VP10 and Panc 1 cells at pH 6.6, with particle localization and signal intensity 8-fold and 5-fold higher, respectively, than that observed at pH 7.4. In tissue phantoms, increased pH specificity was also observed in both S2VP10 and Panc 1 cells at pH 6.6. Particle location and signal intensity on MSOT was demonstrated successful synthesis of approximately 40 nm V7-CSMs with vermillform pores. On NIR imaging, V7-CSMs demonstrated acidic pH specificity in both S2VP10 and Panc 1 cells at pH 6.6, with particle localization and signal intensity 8-fold and 5-fold higher, respectively, than that observed at pH 7.4. In tissue phantoms, increased pH specificity was also observed in both S2VP10 and Panc 1 cells at pH 6.6. Particle location and signal intensity on MSOT was...
Background: Early diagnosis of Invasive lobular carcinomas (ILCs) is clinically challenging due to its histopathologic features making it difficult to detect using mammography. Because of the diffuse infiltration of ILC cells into the surrounding stroma, ILC is also associated with a higher incidence of positive resection margins after breast-conserving surgery. Therefore, there is a significant unmet clinical imaging need to present in early detection, clinical staging and possibly intraoperative imaging to assess surgical margins. We propose to detect mammographically occult ILC by the in vivo detecting of malignant signaling pathways activated in ILC. Heat shock protein 90 (Hsp90) protein is 1-3% of the total cellular protein in most cells and acts as a molecular chaperone for more than 200 reported client proteins. Recently, we developed a series of tethered Hsp90 inhibitors that specifically target a tumor-specific form of Hsp90 associated with poor outcomes. Using near infrared (nIR) probe-tethered Hsp90 inhibitors, we demonstrated that Hsp90 is actively re-internalized and can be used to image murine and human breast cancer in vitro and in vivo. Methods and Results: We tested the imaging efficacy of the nIR-tethered Hsp90 inhibitor, HS196, in ILC models in vitro and in vivo. An inactive structural analog HS199 was used as a control to monitor for non-specific (non-Hsp90 dependent) uptake. Two ILC cell lines, MDA-MB-134 VI and SUM44-PE, and one patient-derived ILC xenograft, HCl-013 EI, were tested. nIR signals of HS196 in vitro and in vivo were detected by Odyssey (LI-COR) and Pearl Trilogy (LI-COR). nIR imaging in vivo imaging was performed on two mice with HCl-013 EI xenograft in SCID-beige mice. After injection of 10 nmol compounds via tail vein, quick and stronger accumulation and longer retention of the HS196 in the ILC xenograft was observed, while HS199 showed significantly weaker accumulation and faster clearance (by 24 h after injection). Significant uptake of HS196 by HCl-013 EI tumors was confirmed by ex vivo imaging of tumors 24 h after compound injection. Histological and flow cytometry analysis of HCl-013 EI tumors showed strong nIR signals in tumor cells. Conclusions: The non-radioactive, nIR imaging strategy, using a novel nIR-Hsp90 inhibitor compound, HS196, was effective in the non-invasive imaging of ILC tumors. This finding suggests that new molecular imaging techniques, not dependent on micro calcifications or architectural distortion, may be a novel strategy to meet the needs of ILC patients to detect, clinically stage and assess margins during surgical resection. Ongoing pre-clinical models will be employed and first in human testing of this approach in planned.

#1860 Direct observation of colorectal cancers using field-emission scanning electron microscopy with a thin polymer membrane, the NanoSuit. Hisakazu Miura, Katsuhiko Sumita, Kinji Kamiya, Takanori Sakaguchi, Yasuharu Takaku, Issa Ohta, Takahiro Hariyama, Hiroyuki Konno. Hamamatsu Univ. School of Medicine, Hamamatsu, Japan.

Background: Field-emission scanning electron microscopy (FE-SEM) enables us to observe nano-sized objects with great depth of focus and high resolution. However, the observation of biological samples including colorectal tissues using an FE-SEM has been difficult because it requires to evacuate its inside to prevent electron scattering, therefore all organisms containing ca. 70% water are rapidly evaporated and consequently caused structural disruption and collapse. To overcome the limitations of the conventional SEM, equipment such as low-vacuum scanning electron microscopy and environmental scanning electron microscope were developed. However, they are not reliable enough to investigate living organisms or wet tissues with high resolution level. We have recently reported that a simple surface modification of a thin extra layer, coined the term ‘NanoSuit®’, can keep organisms alive in the high vacuum (10^-9 to 10^-7 Pa) of the SEM. We now modified the technique and developed a new solution, which enables FE-SEM observations of wet tissues. In this study, we utilized this technique to observe real images of colorectal cancers and their adjacent normal mucosa at high resolution. Materials and methods: Colorectal tissue samples and their adjacent normal mucosa were cut with a scalpel from specimens surgically resected. All patients enrolled in this study provided written informed consent. Observations were carried out with an FE-SEM (S-4800, Hitachi or JEM-7100F, JEOL, Japan) at acceleration voltage of 1.0 kV. The vacuum level of the observation chamber was 10^-3 Pa. The newly developed surface shield enhancer (SSE) solution was used to make NanoSuit® for wet tissue observation. To form the NanoSuit®, the specimens were dipped for 1 min into the SSE solution and blotted briefly thereafter put on dry filter paper to remove excess solution. Specimens were then introduced directly into an FE-SEM to form a NanoSuit® following irradiation of the electron beam. Results: Fine structures of intestinal crypt and villi were successfully observed the real mucosal surfaces and cancer lesions of colorectum, suggesting an invasive front of colorectal cancer. Conclusions: We successfully observed the real mucosal surfaces and cancer lesions of colorectum with high resolution by an FE-SEM using a newly developed vacuum-proof suit, the "NanoSuit®": this novel technique will enable us to investigate further physiopathology of GI tract including cancers.

#1861 LRHH-conjugated magnetite nanoparticles for breast cancer detection and contrast enhancement in MRI. Jingjie Hu1, John Obayemi2, Karen Malatesta3, Derek Adler2, Sean Wang2, Edward Yurkow2, Winston Soboyejo3.

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Breast cancer is one of the most frequently occurring types of cancer in women. Furthermore, at the time of initial diagnosis, some tumors may have metastasized. There is, therefore, a critical need for novel procedures for the early detection of breast tumors and their metastases. One of the specific targeting receptors for human breast cancer cells is Luteinizing Hormone Releasing Hormone (LHRH). Approximately 52% of human breast cancers express binding sites for LHRH. Within this context, LHRH-functionalized nanoparticles have been considered for potential applications in cancer detection and treatment. In theoretical studies of the entry of Luteinizing Hormone Releasing Hormone (LHRH)-conjugated Magnetite Nanoparticles (MNPs) into MDA-MB-231 breast cancer cells. The internalization of LHRH-conjugated MNPs into breast cells is studied using confocal fluorescence microscopy. The receptor-mediated entry of nanoparticles into breast cancer cells is also elucidated using the ligand's fluorescence and kinetic models. The trends in predicted nanoparticle entry times and the size range of the engulped nanoparticles are shown to be consistent with the experimental observations. The specific interaction between LHRH receptors and LHRH can be used to enhance the specific targeting of breast cancer cells. Furthermore, LHRH-MNPs demonstrated specific enhancement for in-vivo signal in magnetic resonance imaging (MRI) for breast tumors and their metastases in the body. Lastly, nanoparticle concentration in different organs is presented to show that the conjugated MNPs are accumulated mainly into the tumor sites and their metastasis. While unconjugated MNPs are shown to accumulate in the liver, kidney, spleen and heart. The current results show that LHRH-conjugated magnetite nanoparticles have the potential for the specific targeting and detection/treatment of breast cancer.

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Otali, Gerald Dryden, Nejat Egilmez, Lacey R. McNally. Tomography. Observed when SKOV3 cells were incubated with anti-HER2 NPs, which was unconjugated, PEG NPs regardless of cell number. The highest MRX signal was more, there was little to no MRX signal for all cell samples incubated with controls; p = 0.0004 by MSOT two days after inoculation. These findings were also associated with inflammatory changes observed on histologic analysis. Conclusions: MSOT represents a non-invasive diagnostic modality that effectively identifies colitis in a murine model. With improvements in depth of tissue penetration, MSOT may hold potential as a sensitive, accurate, non-invasive imaging tool in evaluation of both disease status and early detection of malignancy in patients with IBD.

Feasibility of magnetic relaxometry for early ovarian cancer detection: preliminary evaluation of sensitivity and specificity in cell culture and in mice. Kelsey Mathieu, Zhong La, Hailing Wang, Adam Kulp, John Hazle, Robert C. Bust. The University of Texas MD Anderson Cancer Center, Houston, TX; 2UAB, Birmingham, AL.

Introduction: Most ovarian cancers are diagnosed in a late, incurable stage, which has prompted efforts towards earlier detection and more effective screening strategies. To be considered effective, screening must provide sufficient sensitivity and specificity to impact patient mortality while minimizing false positives. Magnetic relaxometry (MRX), which detects binding of targeted iron oxide nanoparticles (NPs) to cancer cells, offers the promise of improved sensitivity and specificity over conventional early detection modalities. Methods: We investigated the sensitivity and specificity of MRX by scanning ovarian cancer cell samples containing 10^5, 10^6, and 10^7 cells incubated with a fixed amount (57 μg Fe₂O₃) of anti-HER2 antibody-conjugated, PEG-coated NPs or unconjugated, PEG-coated NPs (Senior Scientific LLC). To further evaluate specificity, we used cell lines with both high and low expression of HER2 (SKOV3 and HEY, respectively). To assess MRX under in vivo conditions, we subcutaneously injected 10^5, 10^6, and 10^7 anti-HER2 NP labeled SKOV3 cells into nude mice (n = 9) and immediately performed MRX scanning. Prior to performing this study, we verified successful antibody-NP conjugation through an ELISA assay, which confirmed the presence of anti-HER2 antibody in NP pellets. Additionally, we performed flow cytometry to confirm a high level of specific binding between SKOV3 cells and anti-HER2-conjugated NPs. Results: Our in vitro data revealed strong linearity between cell number and MRX signal for both SKOV3 and HEY cells incubated with anti-HER2 NPs (R² = 0.99 and 1, respectively). Furthermore, there was little to no MRX signal for all cell samples incubated with unconjugated, PEG NPs regardless of cell number. The highest MRX signal was observed when 10^7 SKOV3 cells were incubated with anti-HER2 NPs, which was 2.1 ± 0.3 and 15.7 ± 1.4 times higher than when 10^7 HEY cells were incubated with anti-HER2 NPs or when 10^6 SKOV3 cells were incubated with unconjugated PEG NPs, respectively; significantly higher MRX signals (relative to the controls; p < 0.01) were also noted for samples containing 10^5 SKOV3 cells incubated with anti-HER2 NPs. When scanning live mice injected with 10^7 anti-HER2 NP labeled SKOV3 cells, the MRX signal was significantly higher than the signal from the mice prior to the injection (p < 0.001). Additionally, MRX signal versus cell number in the injected mice was highly correlated (r = 0.99) with the MRX data from the corresponding cell sample scans. Conclusion: MRX is sufficiently sensitive to detect 1 million cells in culture or 10 million cells in mice with a high level of specificity. Sensitivity may be improved by using nanoparticles coated with antibodies against antigens that are overexpressed by a larger fraction of ovarian cancers and will be the focus of future work.


Superparamagnetic Relaxometry (SPMR) is combination technology for the early detection of cancer. Conceptually, PEGylated superparamagnetic iron oxide (Fe₂O₃) nanoparticles (NPs) coupled with a targeting monoclonal antibody are systemically administered and target solid tumors by both passive (EPR) and active (receptor targeting) mechanisms. Once bound to the target cells, the NPs are magnetized by a brief, low field magnetic pulse to create magnetic field patterns that are detectable by superconducting quantum interference device (SQUID) detectors. The pattern of decay, known as Néel relaxation, exhibits a difference in latency specific to bound nanoparticles and differs from the Brownian decay exhibited by free/unbound particles. As a proof of concept, we developed PEGylated NPs that are covalently bound to an anti-Her-2 monoclonal antibody. In vitro, the nanoparticles exhibit specific binding to the Her-2 negative BT-474 breast cancer cells, with little to no binding to Her-2 positive MCF-7 cells. Similar patterns of selective targeting were observed in vivo. To improve the specificity of SPMR signals in tumors we developed a novel system that selectively enhances the magnetic signal at tumor sites and reduces the contribution of similar signals at off target sites such as the liver and spleen. For this study, we created phantoms of varying strength to mimic tumor and non-specific signals. We observed that by creating non-homogenous excitation magnetic field patterns, we reduced the contribution of the non-specific signals by an order of magnitude, effectively increasing the signal to noise ratio of the tumor signal. These results are clinically relevant and support the use of SPMR in the detection of small tumors in cancer patients.
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#1867 Near infrared fluorescent imaging of brain tumor with IR780 dye incorporated phospholipid nanoparticles. Shiuling Li,1 Jennifer Johnson,1 Anderson Peck,2 Qian Xie2. 1Van Andel Research Institute, Grand Rapids, MI; 2East Tennessee State University, Johnson City, TN.

Background: Near-IR fluorescence (NIRF) imaging is becoming a promising approach for tumor detection and diagnosis. The intracranial clinic-guided oncological surgery. While heptamethine cyanine dye IR780 has excellent tumor targeting and imaging potential, its hydrophobic properties limit its clinical use. In this study, we developed nanoparticle formulations to facilitate the use of IR780 for the fluorescent imaging of malignant brain tumor. Methods: Self-assembled IR780-liposomes and IR780-phospholipid micelles were prepared. The intracellular accumulation of IR780-nanoparticles in glioma cells were determined using confocal microscopy. The in vivo brain tumor targeting and NIRF imaging capacity of IR780-nanoparticles were evaluated using U87MG glioma ectopic and orthotopic xenograft models and a spontaneous glioma mouse model driven by RAS/RTK activation. Results: The loading of IR780 into liposomes or phospholipid micelles was more stable. In tissue culture cells, IR780-nanoparticles prepared by either method accumulated in mitochondria, however, in animals the IR780-phospholipid micelles showed enhanced intra-tumoral accumulation in U87MG ectopic tumors. Moreover, IR780-phospholipid brain also showed phospholipid intra-cranial tumor accumulation and potent NIRF signal intensity in glioma orthotopic models at a real-time, non-invasive manner. Conclusion: The IR780-phospholipid micelles demonstrated tumor-specific NIRF imaging capacity in glioma preclinical mouse models, providing great potential for clinical imaging and image-guided surgery of brain tumors.

#1868 Molecular imaging of human esophageal cancer for early detection. Xibo Ma, Hui Meng, Jie Tian. Institute of Automation, Chinese Academy of Sciences, Beijing, China.

Esophageal cancer was the sixth common cancer for incidence and ranks fourth for death rate in China, and squamous cell carcinoma is the main histological type. It is usually in an advanced stage at the time of diagnosis, resulting in an overall 5-year survival rate of <15%. Early detection can lead to an improvement in patient outcomes but is limited by non-specific contrast mechanisms. As all we know, antibody and peptides establish a biological basis for image contrast, which could achieve sufficient target-to-background ratio for in vivo detection. In this paper, an EGF antibody (Cetuximab) conjugated with a NIR dye (NIRDye800) was used for early detection of esophageal cancer. We demonstrated our technology could identify esophageal cancer in small size (<1 mm) and high-grade dysplasia from low-grade dysplasia. And 4.2-fold greater fluorescence intensity for esophageal cancer compared with surrounding normal esophageal tissues. No toxicity was observed in our experiments. The results indicated that this targeted, antibody-based imaging agents have great potential in early detection of esophageal cancer and other epidermal cancer.

#1869 Improved image-guided surgical resection of glioblastoma with [18F]fluoroethyltyrosine Cerenkov luminescence imaging. David Y. Lewis,1 Richard Mair,1 Alan J. Wright,2 Kieren Allinson,2 Scott K. Lyons,2 Tom Booth,2 Robert Bielik,1 Dmitry Soloviev,1 Kevin M. Brindle 1, 1University of Cambridge, Cambridge, United Kingdom; 2Addenbrooke's Hospital, Cambridge, United Kingdom.

The ability of a surgeon to completely resect a tumor is directly related to outcome in high- and low-grade gliomas. However current intraoperative navigational tools are useful only in a subset of glioma patients and do not detect the full extent of disease. We modelled orthotopic brain tumors by stereotactically implanting human U87, rat F98 and C6 glioblastoma cells into the forebrains of rats. We show here that a new hybrid PET-Cerenkov luminescence imaging approach using O2- [18F]fluoroethyl-L-tyrosine (FET) can accurately delineate tumor margins pre- and intraoperatively. We demonstrate consistency in the successful localisation and quantification of tumor burden using PET and Cerenkov luminescence imaging. The Cerenkov signal in individual tumors was directly proportional to the signal detected in corresponding FET PET scans (ρ = 1.06 ± 0.01; R2 = 0.98; p < 0.0001 and subsequent autoradiography indicated equivalence between modalities. Cerenkov luminescence was better able to discriminate tumor from healthy brain tissue than the current ‘gold standard’ for intraoperative mapping. 5-ALA (5aminolevulinic acid), indicated by a greater area under the ROC curve in human xenograft (0.968 ± 0.003 vs. 0.893 ± 0.019; p = 0.003) and syngeneic rat glioma models (0.970 ± 0.010 vs. 0.774 ± 0.046; p = 0.006). The quantitative accuracy of Cerenkov luminescence enabled us to determine a threshold of 2.1 x 10^6 p1 sec-1 cm-2 sr-1 MBq-1 that separated U87 human glioblastoma and normal brain tissue, more precisely guiding tumor excision than 5-ALA. At the optimal threshold the specificity for detecting syngeneic rat and human xenograft gliomas was better with FET Cerenkov than 5ALA (91.1 ± 2.7 % vs. 67.2 ± 5.2 %, p = 0.007 and 91.2 ± 1.0 % vs. 67.2 ± 5.2 %, p = 0.0008, respectively). We confirmed these findings at higher spatial resolution on cryosections using FET autoradiography and 5ALA confocal microscopy, with FET demonstrating higher specificity and sensitivity for tumor detection than 5-ALA. FET PET-Cerenkov luminescence imaging has the potential for guiding resections in a much broader range of glioma patients than current approaches. Previous clinical experience with FET offers a facile route for clinical translation of this technology.

#1870 Fluorescence-guided soft tissue sarcoma surgery using a cetuximab-imaging probe conjugate. Nicole K. Behnke, Jason M. Warram, Andrew C. Prince. The University of Alabama at Birmingham, Birmingham, AL.

BACKGROUND: Soft tissue sarcomas (STS) are a heterogeneous group of solid malignancies whose treatment includes margin-negative resection. Large tumor size and anatomic constraints make margin assessment challenging. Fluorescence-guided surgical resection can help delineate intraoperative margins; preclinical studies demonstrate improved oncologic outcomes in other malignancies using cancer-specific imaging probes. Recent literature describes catespin activated probes selective for STS, but no studies using disease-specific chemotherapeutic agents conjugated to imaging probes. This novel strategy has potential to replace unnecessary healthy tissue resection and improve oncologic outcomes by reducing margin-positive resections. Epidermal growth factor receptor (EGFR) is overexpressed in multiple subtypes of STS and is a potential target for fluorescence-guided surgery. Recent studies show fluorescently labeled cetuximab, an FDA- approved, anti-EGFR antibody, to be safe and useful for margin assessment in other malignancies. Our aim was to evaluate its potential for STS, examining tumor-targeting specificity of two drug-probe conjugates, and comparing them to the described catespin-activated probes. We hypothesize the drug-probe conjugates would successfully target STS, with superior tumor specificity. We also aimed to determine the smallest tumor detectable by our conjugate. METHODS: Athymic nude mice with subcutaneous HT1080 fibrosarcoma tumors were injected with one of five probes: IRDye800CW fluorescent probe conjugated to either cetuximab (anti-EGFR) or DC101 (anti-VEGFR2), IRDye800CW conjugated to an isotype control (IGc), or a catespin-activated probe (Integrisense 750 and Prosense 750). Fluorescence imaging was performed daily with open- and closed-field systems. Tumor-to-background ratios (TBR), signal washout times and normalized signal averages were evaluated. On day 9, smallest resectable game evaluation was performed to assess sensitivity for detecting residual post-resection tumor.

RESULTS: At day 9 post-injection, the TBR of the cetuximab-IRDye800CW group (11.1) was significantly greater than Integrisense750 (6.88, p = 0.005), the IgG-IRDye800CW control (4.44, p = 0.0005), Prosense750 (2.35, p = 0.00009), and DC101-IRDye800CW (1.87, p = 0.0003). During in vivo imaging, cetuximab-IRDye800CW outperformed all other agents by several folds of contrast enhancement. The smallest resectable game evaluation demonstrated 1mm3 fragment detection using the cetuximab-IRDye800CW probe. CONCLUSIONS: This study demonstrates superiority of cetuximab-IRDye800CW for disease-specific imaging in a subcutaneous animal model of STS. The novel strategy of coupling improved margin-negative surgical resection with established chemotherapy has considerable translational significance and is an avenue for exploration with other drugs used to treat STS.

#1872 Fluorescence-guided surgery in the tumor microenvironment in a syngeneic mouse model of EL-4 lymphoma. Kosuke Hasegawa,1 Atsushi Suetugu,1 Miki Nakamura,2 Takuro Matsumoto,1 Takahiro Kunisada,1 Masahito Shinizu,1 Shigetoyo Saiji,1 Hisataka Moriwa,1 Michael Bouvet,2 Robert M. Hoffman2. 1Gifu Univ. Graduate School of Medicine, Gifu, Japan; 2University of California, San Diego, CA.

We report fluorescence-guided surgery (FGS) of the tumor microenvironment of the EL-4 RFP lymphoma cells growing subcutaneously in C57BL/6 GFP transgenic mice. Using a portable hand-held Dino-Lite digital imaging system, subcutaneous tumors were resected by FGS. The tumor surface contained both RFP cancer cells and GFP stromal cells. The tumor microenvironment was clearly visualized and resected with the Dino-Lite. Host stromal cells, including adipocyte-like cells and blood vessels with lymphocytes, were observed along the tumor by confocal microscopy in addition to cancer cells by color-coded confocal imaging. Stromal cells were within the tumor and that cancer cells also in-
vaded the TME. Color-coded FGS, which distinguished cancer and stroma, significantly prevented local recurrence, which bright-light surgery or single-color FGS could not.

#1873 Intraoperative assessment of breast tumor margins using multimodal photoacoustic tomography (MarginPAT). Kyle McElyea,1 George Sankey,2 Rui Li,3 Lu Lan,4 Ji-Xin Cheng,5 Linda K. Han,4 Pu Wang,1 Indiana University School of Medicine, Indianapolis, IN;2 Purdue University, West Lafayette, IN;3 Indiana University Health Simon Cancer Center, Indianapolis, IN;4 Virginia Tech, West Lafayette, IN.

Radical mastectomies are progressively becoming a surgery of the past. Women today are increasingly opting for lumpectomies, a less invasive treatment option. Clinical data has shown no difference in survival or clinical outcomes between the two surgical groups in early stages of breast cancer. There is, however, an undesired outcome yet to be adequately addressed. Lumpectomies have, in some cases, failed to remove marginal malignant tumor cells left as a product from surgery. Following surgery, tumor biopsies are analyzed for marginal tumor cells. Biopsies are cut in, fixed, processed in a tissue processor, embedded into a paraffin block, and stained with H&E. After processing, presence of marginal cancerous tissue is determined by a pathologist. This process takes 3-5 days, ultimately requiring the patient to undergo reoperation if a positive margin is discovered. Presently, the reoperation rate is 20-30%. A device capable of imaging removed tissue to determine remaining marginal tumor during surgery would greatly reduce the reoperation rate. Multiple intraoperative imaging tools existing or are emerging for breast tumor margin assessment. 0.7mm spatial resolution in FGS can be achieved in tumor vasculature at an additional curative dose compared with treatment with shells of radiation avoiding hypoxia (anti-boosts) proved tenets of radiation biology: a few surviving hypoxic clonogens cause radiation treatment failure. In response, we implemented rapid 3D printing Tungsten loaded, highly conformal plastic blocks to compare treating ~100% of hypoxic tumor voxels with hypoxia avoidance, treating similar volumes of well oxygenated tumor. Only then did we observe significant (p<0.01) tumor cure differences between hypoxic boost and anti-boosts. This is the first validation of the curative effectiveness of avoiding hypoxia based dose painting in mammalian tumors.

#1875 [18F]HX4 shows potential as a predictive biomarker for the radiosensitizing capacities of metformin in a NSCLC xenograft model. Sven De Bruycker,1 Christel Vangestel,1 Tim Van den Wyngaert,2 Steven Deleye,3 Dominique Vanderghinste,1 Steven Staels,1 Sigrid Stroobants1.2 University of Antwerp, Wilrijk, Belgium;3 Antwerp University Hospital, Edegem, Belgium.

Introduction Metformin (MET) may improve tumor oxygenation and thus radiotherapy (RT) response. However, appropriate imaging biomarkers for patient stratification, critically needed to be able to advance to clinical trials with MET as a radiosensitizer, are still lacking. In this proof-of-concept (POC) study, we first assessed the effect of acute MET administration on NSCLC xenograft tumor hypoxia with PET using the novel hypoxia PET tracer [18F]HX4. Second, we verified the influence of a single dose of MET prior to RT on tumor treatment outcome. Experimental procedures A549 tumor-bearing mice (n=9; animals were inoculated in both hind legs) underwent a [18F]HX4 PET/CT scan to determine baseline tumor hypoxia. The next day, mice were administered 100 mg/kg MET IV. [18F]HX4 was administered IV 30 min later, whereupon a second [18F]HX4 PET/CT scan was performed two days after this second scan, mice were randomized into three groups (n=3/group): a control group (1), a RT group (2), and a MET+RT group (3) with comparable tumor volumes (259±103; 218±81 and 247±121 mm3, resp.). Animals were administered 0.9% saline (groups 1-2) or 100 mg/kg MET (group 3) IV, followed by a single dose of 10 Gy 30 min after administration (groups 2-3). Control animals of group 1 underwent sham RT. During the entire study, tumor growth was monitored triweekly by caliper measurements. Calculation of the relative tumor volumes (RTV) was initiated when tumors reached a mean baseline volume of 100 mm3 with the formula RTV = Vtumor/Vbaseline. Tumor growth inhibition (TGI) was calculated for each tumor in both treatment groups with the formula TGI = 1-(RTVtreated/RTVcontrol). Results MET significantly altered tumor hypoxia, as the mean [18F]HX4 tumor-to-blood ratio (TBR) was reduced from 3.03±0.27 to 2.82±0.25 (p<0.004) after MET administration. The tempering influence of MET on tumor hypoxia improved RT response, as fifteen days after irradiation TGI was 63±7% in the MET+RT group as compared to only 36±5% in the RT group, however significance was not reached. To date, tumors are still being measured triweekly and this will be continued until a volume of 1500 mm3 is reached. Then, tumors will be sacrificed for histological validations on tumor tissue to assess differences in a.o. proliferation and hypoxia. Conclusions Using non-invasive imaging, we showed in A549 xenograft tumors that MET acts as a radiosensitizer possibly by decreasing tumor hypoxia. Our results imply that [18F]HX4 PET imaging holds potential as a predictive biomarker for the beneficial effect of MET in the treatment of NSCLC; however, full proof of this in a POC study did not allow us to draw conclusions. These promising findings will be validated in a larger follow-up study in order to validate [18F]HX4 PET as a predictive biomarker for MET and RT response.

#1875A Preclinical proof of concept for the first Nanocyclix TKI-PET radiotracer targeting activated EGFR positive lung tumors. Philippe Geneste,1 Cyril Berthelot,1 Olivier Raguen,2 Sylvie Chalon,2 Xavier Tison,3 Sophie Sorriere,2 Peggy Provent,1 Marc Hillairet de Boisferon,1 Johnny Verrouaille,2 Celine Mothes,4 Fabienne Gouraud,4 Louisa Barre,5 Denis Guilloteau,6 Petra Blom,7 Jan Hoflack,7 Oncodesign S.A., Dijon Cedex, France;4 INSEM, Tours, France;5 INSERM/ CERRP, Tours, France; Cyclopharma, Saint-Beauzire, France;6 CEA, LDM-TEP, Caen, France.

Background: [18F]HX4 is a positron emission tomography (PET) ligand for EGFR. Progesterone receptor (PR) is a known target of EGFR inhibitors. In vivo, we evaluated the efficacy of HX4 in a xenograft model of the tumor cell line H520. The results were compared with those of [18F]FDG. The biodistribution of both ligands was measured in nude mice after intravenous injection. Results: In the model of the H520 tumor cell line, the uptake of HX4 was higher than that of [18F]FDG, with a statistically significant difference in tumor uptake (p<0.001). The biodistribution of both ligands was similar in the liver, lungs, spleen, and muscles. Conclusion: [18F]HX4 is a promising PET ligand for the detection of EGFR-positive tumors. Future studies will be needed to evaluate the potential of this tracer for the diagnosis and staging of EGFR-positive tumors.
TKI. Methods: New compound ODS2004436 was characterized in vitro for its activity and metabolism. After radiolabeling with $[^{18}F]$, the compound was evaluated in vivo in three clinically relevant lung cancer cell lines (NCI-H441 – EGFR_wild-type (WT); NCI-H3255 – EGFR_L858R; NCI-H1975 – EGFR_L858R/T790M) xenografted in nude rats. Results: The biochemical and cellular profiles were comparable to gefitinib on EGFR_WT and activated EGFR_L858R. The cellular activity was in the nanomolar range ($5 \pm 2$ nM) on NCI-H2355 and weak on NCI-H441 (42 $\pm 27$ nM) suggesting that our compound might inhibit to a lesser extent non activated WT-EGFR. We observed a better biochemical inhibition (254 nM vs 1350 nM) and cellular activity on NCI-H1975, the EGFR double mutant (4.8 $\pm 0.7$ µM vs 18 $\pm 8.7$ µM) of ODS2004436 compared to gefitinib. These data showed that our compound is suitable to bind to in vivo activated EGFR. In vitro binding experiments showed that $[^{18}F]$,ODS2004436 specifically bound to patient tumor samples expressing EGFR. The in vivo experiments in rats showed that $[^{18}F]$,ODS2004436 was rapidly cleared from the blood. Nevertheless the tumor uptake is stable overtime (up to 180 min) with a mean tumor/muscle ratio of 6.5 $\pm 0.9$. The tumor uptake is stable overtime (upto 180 min) with a mean tumor/muscle ratio of 6.5 $\pm 0.9$. Moreover, we observed a good correlation between the radiotracer uptake in the tumors and eEGFR immunostaining, suggesting that $[^{18}F]$,ODS2004436 is a good biomarker of activated EGFR, regardless of the mutation. Conclusions: Based on this study, we proved in vivo that $[^{18}F]$,ODS2004436 binds selectively to activated EGFR, and is a good candidate to evaluate the EGFR activity in NCLS using PET imaging. Clinical evaluation of this novel radiotracer is ongoing (first in-man phase 0/I clinical trial NCT02847377).

TUMOR BIOLOGY: Imaging for Cancer Diagnosis and Image-Guided Therapy

#1876 Cell cycle-dependent front polarized cell migration requires Aurora kinase A. Tony Lok Heng Chou, Lixin Zhou, Jennifer Won, Pooja Mohan, Oksana Nemirovsky, Abbas Fotovati, Torsten Nielsen, Nelly Pante, Christopher Maxwell. University of British Columbia, Richmond, British Columbia, Canada.

PROBLEM: Metastasis accounts for 90% of deaths from carcinomas. BACK-GROUND: To initiate migration, epithelial cells must front-rear polarize and reorganize the microtubule cytoskeleton directed toward the lamellipodia or leading edge. During mitosis, centrosome maturation, duplication, spindle pole assembly and chromosome segregation require Aurora kinase A (AURKA), which is optimally active when complexed with TPX2. Accumulating evidences identify non-mitotic functions for AURKA, such as cell migration and ciliary reorganization. Overexpression of AURKA leads to tumorigenesis in breast cancer. Mechanisms that activate non-mitotic AURKA and promote cell migration as well as metastasis are yet to be uncovered.

RESULTS: In a large breast cancer tissue microarray (n = 3,175), the abundance of phosphorylated RHAMM (a substrate of AURKA) predicted overall and relapse-free survival in ER-negative, basal-subtype, and triple negative breast tumors. We studied the AURKA-TPX2-RHAMM signaling axis in mammary (MCF10A and MUMG-FUCCI) and HeLa cells. In scratch wound assays, migratory cells were found to have front-polarized centrosomes, which correlated with G2 cell cycle phase, phosphorylated AURKA and elevated microtubule nucleation at centrosomes. Small molecule inhibition of Aurora kinase activity impaired centrosome polarity and retarded the kinetics of cell migration. In parallel, silencing RHAMM altered TPX2 localization as indicated by immunofluorescence and immuno-EM, which located TPX2 to the nuclear envelope and nuclear pore basket. Nuclear import of recombinant TPX2 was attenuated in RHAMM silenced cells and nuclear envelope accumulation was elevated.

CONCLUSION: The AURKA-TPX2-RHAMM axis regulates centrosome polarity, microtubule nucleation and directional cell migration while phosphorylated RHAMM predicts survival in aggressive forms of breast cancer.

#1877 Coordination of Rac1 and Cdc42 by centrosomal microtubules in focal adhesion dynamics and directed cell migration. Jean-Cheng Kuo. National Yang-Ming University, Taipei, Taiwan.

Cancer metastasis causes the increase of mortality in most cancer patients, and abnormal cell migration contributes to this disease progression. To control cell migration, the dynamical control of a protein complex within focal adhesions has to be regulated appropriately. It is known that focal adhesions can be regulated dynamically by microtubules. To examine the effects of centrosomal microtubules and non-centrosomal microtubules in regulating focal adhesion dynamics and directed cell migration, we first generated centrosome-deficient cells. We find that centrosomal microtubules and non-centrosomal microtubules control focal adhesion dynamics through modulating focal adhesion composition, which dynamically control the activity of small GTPases Rac1 and Cdc42. Deficiency of centrosomal microtubules significantly disrupts the balance of Rac1 and Cdc42, thereby inhibiting directed cell migration. This study unveils the effects of centrosomal cellular adhesions, and indicates an important role for Rac1 and Cdc42 activation that controls actin polymerization, cell polarization and directed cell migration.

#1878 PDZ-RhoGEF functions as a critical signaling effector for TROY. Zonghui Ding, Alison Roos, Jean Kloss, Harshil Dhruv, Marc Symons, Nhan L. Tran, Joseph C. Loftus. Mayo Clinic Arizona, Scottsdale, AZ; Dr. Translational Genomics Research Institute, Phoenix, AZ; The Feinstein Institute for Medical Research at Northwell Health, Manhasset, NY.

Glioblastoma Multiforme (GBM) is the most common tumor of the CNS with a median survival of approximately 15 months. The highly aggressive invasion of malignant cells into the surrounding normal brain tissue renders complete surgical resection impossible, increases therapeutic resistance, and virtually assures tumor recurrence. We have previously demonstrated that TROY (TNFRSF19) has an important role in GBM invasion and resistance. TROY expression is correlated with glioma grade and inversely correlated with patient survival. Increased TROY expression stimulated cell migration/invasion in vitro and in vivo and confers resistance to both ionizing radiation- and temozolomide-induced apoptosis via activation of Akt and NF-kB. These data indicate that TROY stimulated migration/invasion is associated with signaling pathways that also increase survival and therapeutic resistance, however, the mechanistic basis of this signaling remains unclear. To investigate the mechanism by which TROY induces GBM cell invasion, we performed immunoprecipitation of the TROY receptor coupled with MALDI-TOF and MS/MS analysis and identified PDZ-RhoGEF as a binding partner for TROY. We validated the interaction of TROY with PDZ-RhoGEF by immunoprecipitation and western blotting, and demonstrated that PDZ-RhoGEF can exchange for both RhoA and RhoC in glioma cells and is implicated in GBM cell invasion, proliferation, and survival. In order to assess the specific role of PDZ-RhoGEF in TROY signaling, we examined the effects of PDZ-RhoGEF on TROY-mediated activation of NF-kB. While overexpression of TROY alone strongly stimulated NF-kB transcriptional activity in serum-free conditions, increased expression of PDZ-RhoGEF alone had no effect on NF-kB activity. Co-transfection of PDZ-RhoGEF with TROY synergistically promoted NF-kB activation. Mutation of the TRAF binding domain (TBD) in the cytoplasmic domain of TROY abolished its capacity to activate NF-kB, even in the presence of PDZ-RhoGEF, suggesting that the TBD is important for TROY induced NF-kB activation. Furthermore, knockdown of PDZ-RhoGEF by shRNA attenuated NF-kB activation in TROY expressing cells, blocked TROY mediated invasion of primary GBM xenograft cells and increased sensitivity to temozolomide. Collectively, these data indicate that PDZ-RhoGEF plays an important role in TROY signaling and provides insights into a potential node of vulnerability to limit GBM cell invasion and decrease therapeutic resistance.

#1879 Transcriptional regulation of KAI1 by p73 in colorectal cancer metastasis. Woo Kyun Bae, Kyung Hyun Yoo, Keunsoo Kang, Lothar Hennighausen, Ik-Joo Chung. CUNHL Jeonnam, Republic of Korea; Sookmyung Women’s University, Seoul, Republic of Korea; Dankook University, Cheonan, Republic of Korea; NIH, Bethesda, MD.

Background: p73 is a member of the p53 family transcription factors and known tumor suppressor. However, its transcriptional activity is complex owing to its multiple isoforms, and limited information is available regarding the transcriptional and functional regulation of the p73 gene in colorectal cancer. In this study, we examined the role of p73 in colorectal cancer. Methods: We established a TaP73 overexpressing cell line using the Tet-on inducible expression system and analyzed global RNA expression by RNA-sequencing (RNA-seq) experiments. We studied the effects of TaP73 expression on the expression, invasiveness, and EMT of colon cancer cells. We conducted experiments in animals with liver metastasis of colorectal cancer. To investigate the roles of p73 in colon cancer, we examined the samples from patients who had undergone surgical resection for colorectal cancer between 2009 and 2013. mRNA expression of TaP73 and KAI1 was analyzed in 108 colorectal cancer specimens and adjacent non-cancerous tissues by real-time RT-PCR. Results: RNA-seq showed the association between TaP73 and KAI1 expression. Migration and invasiveness of colon cancer cells were reduced by TaP73 expression, but induced by KAI1 knockdown. TaP73 regulates KAI1 expression in colon cancer cells. TaP73-induced decrease in invasive- ness and epithelial-mesenchymal transition (EMT) was abrogated by KAI1 expression.
knockdown in TAp73 overexpressing cells. Migration ability of TAp73 overexpressing cells was recovered by KA1 knockdown. Hepatic metastases were significantly reduced in mice injected with TAp73 overexpressing cells and increased in those injected with TAp73 overexpressing cells with KA1 knockdown. mRNA expression of TAp73 and KA1 was higher in colorectal cancer cells. Comparing non-cancer- and cancer-expressing cells, the difference between TAp73 and KA1 mRNA expression was detected in early stage primary colorectal cancer, but not in the advanced stage. Conclusions: This study suggests that p73 (TAp73) acts as a tumor suppressor in the progression of colorectal cancer and regulates the expression of KA1, which is a key target of p73 in colorectal cancer liver metastases.

#1880 Bulk lung cancer cell migration is more common than single cell migration and regulated by different genes. Helmut H. Popper, Sylvia Eidenhammer. Medical Univ. of Graz, Graz, Austria.

Introduction: There are two different forms how tumor cells migrate: single or small cell cluster movement as it is seen in small cell carcinoma, and movement by large clusters of organized cells as seen most often in adenocarcinoma or squamous cell carcinoma. This latter migration form is not understood.

Drosophila border cells forming wings migrate in large cell complexes similar to what is seen in the carcinomas and the genes identified in these cells might regulate the same process in carcinomas. Methods: 30 cases of pulmonary squamous cell carcinomas and adenocarcinomas were selected based on the identification of large tumor cell clusters visible within the stroma as well as in blood vessels. Immunohistochemistry was done for receptor of activated C kinase (Rack1), large tumor cell clusters visible within the stroma as well as in blood vessels. Drosophila border cells forming wings migrate in large cell complexes similar to movement by large clusters of organized cells as seen most often in adenocarcinoma or squamous cell carcinoma. In addition immunohistochemistry was also done for Src-kinase, Twist, Snail, and TGFβ1. Tyrosine kinase substrate 5 (Tks5), E-cadherin, SARI (suppressor of AP-1), and vimentin, all known to be associated with epithelial-to-mesenchymal transition (EMT) and formation of invadopodia. Results and Conclusion: Most well differentiated pulmonary carcinomas migrate in large cell clusters, for example acinar adenocarcinomas form nicely structured acini deep within the stroma and even within blood vessels. These carcinomas do not undergo classical EMT. Proteins expressed by the four genes (Rack1, brk, mad, and sax) associated with border cell movement in drosophila could also be identified in pulmonary carcinomas and might coordinate bulk cell movement. Other members of TGF-beta signaling cascade were identified. Inhibition of Src by Rack1 may be important for border cell migration and cluster cohesion maintenance. Proteins usually seen in single tumor cell migration, such as vimentin (a sign of EMT) could not be proven in the tumor cell clusters. Findings of this study show that similar mechanisms are working in pulmonary carcinomas and that bulk cell migration is probably another way of metastasis. Further investigation using cell culture system and genetically designed adenocarcinomas expressing these genes should prove our findings and contribute in understanding migration of carcinoma bulk complexes.

#1881 STIM1 and Orai control non-Hodgkin lymphoma cell migration. Simon Latour,1 Isabelle Mahouche,2 Floriane Cherrier,3 Jean-Philippe Merlio,2 Sandrine Poglio,4 Laurence Bresson Bepoldini.1 INSERM U1218 ACTION, Bordeaux, France.2 INSERM UMR1053 BaRITOn, Bordeaux, France.3 Medical Univ. of Graz, Graz, Austria.

Non-Hodgkin lymphoma is one of the most common cancer in United States representing 4% of all cancer. Diffuse large B cell lymphoma (DLBCL) is the most common and aggressive non-Hodgkin B lymphoma. One of the characteristic features of this disease is the dissemination of the cancer cells, through the lymphatic system in the secondary lymphoid organs and extranodal sites, leading to the death of patients. Chemokines such as the Stromal Derived Factor 1 (SDF1) control the spread and the homing of the cancer cells. It is known that SDF1 activates various signalling pathways involved in cell proliferation, transcription or migration. Moreover, SDF1 induces an increase in intracellular calcium concentration whose role is still unknown in B cells. Store-operated Ca2+ entry (SOCE) is a major Ca2+ influx pathway in this type of cells. By definition, SOCE is activated by Ca2+ release from the endoplasmic reticulum. Two genes are responsible for SOCE activity: Stromal interaction molecule 1 (STIM1), an ER Ca2+ sensor that detects store depletion and ORAI1, the pore-forming subunit of Ca2+ release-activated Ca2+ (CRAC) channel. Several studies performed on adherent cells showed that ORAI1/STIM1 proteins are involved in cancer cell migration. However, the molecular mechanisms involved in cell migration differ widely between adherent and non-adherent cells. We studied the role of both actors of calcium entry: ORAI and STIM1 in DLBCL pathiology and more precisely in basal and SDF1-induced of DLBCL cell migration. Using Tissue MicroArrays approach we revealed that both ORAI and STIM1 are under-expressed in DLBCL tumoral tissue compared to normal lymphoid tissue. Next, using calcium imaging experiments we confirmed that SDF-1 triggered Ca2+ responses in two DLBCL cell lines (SUDHL4 and HLY1) involving intracellular Ca2+ store mobilization and extracellular Ca2+ influx. Based on these observations, we investigated the role of ORAI1 and STIM1 on SDF1-induced Ca2+ influx using pharmacological and RNA interference approaches. The inhibition of ORAI1 by BTP2 as well as the under-expression of ORAI or STIM1 by shRNA, prevented Ca2+ influx induced by SDF1 suggesting the involvement of ORAI and STIM1 in this process. Regarding this results, we studied the role of ORAI1 and STIM1 on DLBCL cell migration in vitro and in vivo. Our results show that basal or SDF1-induced cell migration was significantly inhibited by under-expression of STIM1 or ORAI1 in SUDHL4 and HLY1 cell lines. These results suggest that STIM1 and ORAI1 play a key role in the DLBCL cell migration. The identification of STIM1 and ORAI proteins as key players in the migration of DLBCL cells might provide new therapeutic targets for the treatment of this pathology.

#1882 Tankyrase-binding protein TNKS1BP1 regulates cancer cell invasion. Tomokazu Ohishi,1 Haruka Yoshida,1 Masamichi Katori,1 Toshiro Migita,1 Yukiko Muramatsu,2 Mao Miyake,1 Yuichi Ishikawa,2 Akio Saito,2 Shun-ichiro Iemura,1 Tohru Natsume,4 Hiroyuki Seimiya1.

TNKS1BP1-depleted cells and enhanced cell invasion in a PARP activity dependent manner. Under approval by Institutional Review Board of Japanese Foundation for Cancer Research, we performed immunohistochemical analysis of clinical pancreatic cancer. We found that TNKS1BP1 protein expression is decreased in invasive regions of the tumors. Taken together, these observations suggest that the tankyrase-TNKS1BP1 axis represents a novel post-translational modulator of cell invasion and its aberration promotes cancer malignancy.

#1883 Fibillar 1D tumor microenvironment is the key driver associating high-speed tumor cell motility with nuclear shape in breast cancer. Ved P. Sharma,1 James Williams,2 Edison Leung,3 Joseph Sanders,2 Robert Eddy,1 James Castracane,1 John Condeelis2.

Unlike normal breast stroma, which contains curly collagen fibrils, aligned collagen fibers oriented perpendicular to blood vessels are seen in both human breast tumors and mouse models of breast cancer. These linear collagen fibers provide “highways” for tumor cells to migrate toward blood vessels in a directional migration mode known as tumor cell streaming. Streaming is characterized by tumor cell migration at high speed and directional persistence on 1D collagen fibers. Previous studies utilizing linear ECM substrates have shown that tumor cells adopt elongated morphology and display increased speeds on linear 1D substrates compared to their 2D motility. However, the relationship between 1D geometry of ECM fibers in breast tumor microenvironment and the underlying mechanotransduction mechanism regulating high-speed migration of tumor cells is not well understood. Here, we analyzed in vivo ECM architecture by SHG intravital imaging and found a narrow peak of fiber diameters falling in the range 2-3 μm. These fibers were composed of collagen I and fibronectin. Based on these findings, we developed a high fidelity in vitro nanofiber system to study the molecular mechanisms underlying tumor cell streaming migration. Breast
cancer cells plated on 2 μm ECM-coated fibers showed enhanced motility matching in vivo velocities averaging 1.2 μm/min. We varied 1D fiber diameter (0.7-20 μm) and found that tumor cells move the fastest with highest persistence on smaller fibers within a narrow range of diameters from 0.7-3 μm. High tumor cell speeds correlated with enhanced alignments of F-actin and focal adhesions along the fiber axis. Unexpectedly, we also observed nuclear deformation during carcinoma cell migration on narrow fibers in vitro, similar to nuclear deformation observed in vivo. This was a surprising finding because nuclear deformation in vivo was assumed to be caused by squeezing through ECM pores. Thus, we hypothesized that actomyosin forces not only regulate cell motility parameters, but also nuclear deformation independently of ECM pore size. To test this, we disrupted the transmission of cytoskeletal forces to the nucleus by knocking down LINC complex proteins - SUN1 and SUN2, and found increased nuclear elongation and cell motility parameters, through the upregulation of actin polymerization. These results indicate that in carcinoma cells, F-actin associated forces are shared between the leading edge (to maintain cell speed) and the nucleus (to dynamically regulate nuclear shape). LINC complex disruption releases F-actin forces acting on the nucleus to the cell front, leading to higher tumor cell motility speeds. In summary, our results provide new insights into the interplay between actomyosin contractility and the LINC complex in the regulation of nuclear shape and high-speed tumor cell motility during carcinoma cell metastasis.


Background: Breast cancer (BCa) is a genetically heterogeneous disease and many genes contributing to BCa risk remain to be identified. Genome-Wide Association Studies (GWAS) and subsequent fine-mapping studies (>50) have strongly implicated genetic alterations at the CCDC170/C6ORF97-ESR1 locus (6q25.1) as being associated with the risk of BCa. ESR1, encoding the estrogen receptor, might be a more obvious candidate for influencing risk. Surprisingly, our analysis using genome-wide differential allele-specific expression (DASE), an indicator for BCa susceptibility, suggested that the genetic alterations of CCDC170, but not ESR1, accounts for GWAS-associated BCa risk at this locus. CCDC170 is a coiled-coiled-domain-containing protein of poorly understood function. BCa-specific truncation and missense mutations in CCDC170 also have been detected, with the truncations being implicated in driving Luminal B subtype BCa. Together these findings demonstrate that the CCDC170 gene is involved in BCa, but the underlying molecular mechanisms for its role in tumorigenesis are unknown. Experimental designs and results: By using the approaches of confocal microscopy and cell imaging analysis, here we report for the first time that CCDC170 is associated with the Golgi apparatus and perinuclear microtubules (MTs), and support a role for CCDC170 in the Golgi-associated microtubule network. We have shown that overexpression of CCDC170 triggers Golgi reorganization and stabilizes Golgi-associated MTs, accompanied by dramatically increased acetylation of α-tubulin that is driven by the actin-filament transferase ATAT1. The Golgi-associated MT network has been proposed to regulate cell polarity and migration. In support of this concept, we have shown that CRISPR knockout increases, and overexpression of CCDC170 decreases, BCa directional cell migration in vitro. We also found that the BCa-specific truncations result in mislocalization of CCDC170 and/or diminished stability of Golgi-associated MTs. Lastly, we identified candidate CCDC170 functional binding partners (e.g. MAP4) that are consistent with their localization and proposed function. These partners may serve to mediate the acetylation and stabilization of MTs. Conclusions: Taken together, our findings demonstrate that CCDC170 plays an essential role in Golgi-associated MT organization and stabilization, and provide a mechanism for how perturbations in CCDC170 could alter Golgi-mediated cell polarity, and thereby drive BCa and other abnormalities. This work was partially supported by the Susan G. Komen for the Cure (KG100274), NCI (CA186853), and Eileen Stein Jacoby Fund.

#1885 VEGFA/NRP1 signal contributes to filopodia formation in breast cancer cells. Marina Kiso, Sunao Tanaka, Masakazu Toi, Fumiaki Sato. Kyoto Univ. Graduate School of Medicine, Kyoto, Japan.

Background and objective: VEGF and NRP1 are the ligand and receptor, respectively, for VEGFA/NRP1 signal. VEGF activates the ERK/mTOR pathway to support tumor progression and impaired migration. Exogenous VEGF addition to VEGFA-KO 231 cells recovered WT 231 cell like morphology and induced cell migration. Next, we generated soluble neuropilin-1 (sNRP1) overexpressed 231 cells. sNRP1 traps VEGF to function as an antagonist. sNRP1-231 cells exhibited small and rounded morphology and impaired cell migration similar to that of VEGFA-KO 231 cells. Knock down of NRP1 by using siRNA in 231 cells also caused impaired cell migration. We evaluated the cell morphology using scanning electron microscope. We identified filopodia formation in VEGFA-KO 231 cells compared to WT-231 cells. Exogenous rhVEGF addition to VEGFA-KO 231 cells recovered filopodia formation. Filopodia is a fingerlike protrusion that comes out from the cell periphery by an actin polymerization. Filopodia is required for cell development and functions such as cell migration. Our results indicate, because of the absence of VEGF/NRP1 signal, filopodia formation is abrogated. As a result of diminished filopodia formation, VEGFA-KO 231 cells showed small and rounded shape and reduced migration. Bevacizumab and NRP1 bind VEGF at amino acid motifs from exon 3-4 and exon 7 of VEGF, respectively. Thus, our findings suggested that VEGF could play a role in filopodia formation via NRP1 for 231 cells, and that sNRP1 could not block the VEGF/NRP1 signal. Discussion NRP1 is a receptor of VEGFA, Semaphorine and other growth factors, and expressed in a variety of cancer cells. Intracellular portion of NRP1 lacks kinase domain. Previous study showed that the VEGF stimulated to form a complex between NRP1 and GIPC1/Syt/RhoA, and promoted tumor cell proliferation independently from VEGFRs (Yoshida et al., 2002). The precise downstream regulator, regarding VEGF/NRP1 signaling, is unknown. In conclusion, inhibition of VEGF/NRP1 signaling caused rounded morphology with diminished filopodia formation, which impaired cell migration of 231 cells. These findings would provide profound insights regarding molecular mechanisms in resistance to bevacizumab treatment.

#1886 mGluR1 drives invasion of proneural subtype of glioblastoma cells. Alena Gladwin, Sen Peng, Jeff Kiefer, Seungchun Kim, Michael Berens, Harshil D. Dhruv. TGen (The Translational Genomics Research Institute), Phoenix, AZ.

A major cause for the therapeutic failure and subsequent morbidity and mortality of glioblastoma (GBM) is the aggressive invasion of malignant cells into the surrounding normal brain that effectively renders complete surgical resection impossible and virtually assures recurrent tumor growth. Multi-omic profiling of GBM led to their molecular sub-classification into two distinct molecular subtypes: proneural (PN) and mesenchymal (MES). However, very little is known about shared or distinct invasion processes of cells in these genomically different subtypes. Using Microarray gene expression profiles of microdissected paired stationary core and invasive rim samples from 19 patients, we demonstrated that invasive gene signature of MES subtypes differs from PN. Specifically, using three orthogonal but intersecting bioinformatic approaches, i.e. gene set variation analysis, causal network analysis, and LIFEGOL analysis; we discovered that genes differentially expressed between PN core and rim could, to a meaningful degree, be accounted for based on their annotation as “regulated by the transcription factor REST”. REST functions as a repressor of gene expression. Of the genes repressed by REST, mGluR1 (Metabotropic Glutamate Receptor 1) was significantly overexpressed at the rim of PN glioma cells as compared to the core. Finally, we also investigated the role of mGluR1 in glutamate induced glioma cell migration; our results show that glutamate stimulates migration of proneural-like glioma cells (A127) as compared to non-proneural-like glioma cell (T98G). mGluR1 activation by glutamate has shown to induce activation of Pyk2 and Src in astrocytes; knockout of mGluR1 is not embryonic lethal. In summary, our data demonstrate that glutamate induced glioma cell migration of PN subtype of GBM is dependent on mGluR1 and thus raises the prospect that therapeutic targeting of mGluR1 may be a novel approach to controlling the invasion of this deadly disease.

#1887 Untethering heterochromatin: how loss of HP1α enhances cell invasion by altering nuclear envelope integrity. Tracy K. Hale, Sarah Bond, David Wheeler. Massey University, Palmerston North, New Zealand.

Malignant cell invasion is accompanied by complex changes in nuclear shape and organisation. The onset of invasion correlates with the loss of HP1α in many solid tumours including those of the thyroid, kidney, colon and breast, while in cancer cell lines HP1α suppresses invasive potential. In normal cells HP1α maintains the condensation of transcriptionally silent heterochromatin and seques-
tters it at the inner periphery of the nuclear envelope; an event critical for the maintenance of nuclear stability and envelope rigidity. This role for HP1a leads us to hypothesise that the un-tethering of heterochromatin from the nuclear periphery that would occur after loss of HP1 causes a corresponding disruption to the nuclear periphery that is a weakness of these cells that could be exploited.

#1888 3D matrix confinement triggers vascular mimicry through a conserved migratory and transcriptional response. Daniel Ortiz, Brian Tsui, Tyler Goshia, Colleen Ricker, Hannah Carter, Stephanie I. Fraley. University of California, San Diego, La Jolla, CA.

An initial step in solid tumor metastasis involves the migration of tumor cells through extracellular matrices. Several cancer cell migration strategies exist in vivo, and the local properties of collagen fibrils are implicated in modulating migration behaviors. Yet, individual tumor cells also display heterogeneity in their intrinsic ability to migrate and metastasize. It remains unclear to what extent intrinsic and extrinsic heterogeneity contribute to the emergence of distinct migration phenotypes and whether certain migration phenotypes contribute more to metastasis than others. To study this, we generated 3D collagen matrices of varying densities and monitored single cancer cell migration in these matrices with time-lapse microscopy. We observed a collagen density threshold at 2.5mg/ml, above which 86% of MDA-MB-231 breast cancer cells transition from single mesenchymal migration to collective cell migration, with a 50% increase in persistence after cell division. After seven days, these collectively migrating cells created networks coated with basement membrane molecules resembling a clinical phenotype known as vascular mimicry (VM). The remaining 14% of cells migrated randomly and eventually formed spheroids. HT-1080 fibrosarcoma cells also responded similarly, migrating persistently and forming cellular networks. Next we sought to identify the physical feature of high-density collagen driving VM. Neither hypoxia or matrix stiffness was sufficient to induce VM. However, PtK2 PEG and matrix contraction triggered VM non-specifically. RNA sequencing revealed collectively migrating cells up-regulated a conserved transcriptional program significantly enriched for annotations of vascular development and motility regulation processes. This gene module predicted survival in human tumor transcriptome datasets. Our results suggest that the VM phenotype arises in a subpopulation of cells from a conserved transcriptional and migratory response to confinement in 3D collagen.

#1889 Formation and merger of H69 and H69AR small cell lung cancer (SCLC) tumor cell clusters (TCCs) in vitro. Nico O. Ruprecht,1 Martin Hungerbuehler,2 Philipp Kellmann,2 Nathalie Harder,2 Günter Schmidt,3 Carlos Goshia, Colleen Ricker, Hannah Carter, Stephanie I. Fraley. University of California, San Diego, La Jolla, CA.

A novel migration of tumor cell invasiveness, termed mutual cellular pervasion (MCP), was observed in vitro. It involved TCCs formed by SCLC cells. Suspension cultures of H69 and H69AR cells were grown in flavin-free RPMI 1640 medium with 10% fetal bovine serum as the only source of flavins. Experiments and subcultivations were performed under light with wavelengths above 520 nm. TCC morphology was studied by confocal microscopy and transmission and scanning electron microscopy. Interactions of TCCs were followed by video microscopy in phase contrast and fluorescence modes. Data were analyzed using the Definiens software suite. The interaction of H69AR TCCs with confluent monolayers of human umbilical vascular endothelial cells (HUVEC) was visualized. Freshly subcultivated, singularized tumor cells rapidly formed aggregates. After two days, solid TCCs prevailed whose surface cells were connected by desmosome-like junctions. Apoptosis but not necrosis became obvious in central parts of clusters with diameters above 100 to 150 μm. TCCs enlarged through both cell proliferation and serial cluster formation. In the course of TCC merger, four stages were distinguishable: (1) directed cluster movement leading to contact of clusters, (2) formation of a near circular, symmetric structure termed contact disc. It consists of planar surface areas to which both TCCs involved contribute equally. (3) Merger of TCCs took place in the contact disc, and was accompanied by active movement of adjacent cells. (4) Starting from the merger zone, major surface areas of newly formed clusters were mutually perverted by cells from both participating cell clusters. After numerous merger events, only low numbers of TCCs reaching sizes of up to 2 to 3 mm were present at subcultivation. No spontaneous bursting, shrinkage, degeneration, or decay of TCCs has been observed. However, when TCCs of H69AR cells were confronted with confluent layers of HUVEC, drastic changes occurred in both formations. Formation of intense oscillatory cellular movements were triggered on TCC surfaces, preceding the release of cell aggregates which moved away from TCCs to the HUVEC layer. At the same time, HUVEC were disconnected from each other and started moving either randomly or in an oscillatory fashion. Individual tumor cells attached to the much larger HUVEC and spread on their surface, resulting in full covering which secluded HUVEC from the medium. To our knowledge, neither formation nor merger of malignant TCCs in vitro have been described so far. TCC merger involves movements and remodellings of TCC surface resulting in the invasion of tumor cells into non-tumor tissue, pervasion does not lead to destruction but results in TCC growth. However, key features of tumor cell invasion in vivo, such as chemotaxis, spreading and TCC formation as well as disruption of endothelial layers, manifest under conditions in vitro, thus facilitating related experimental studies.

#1890 PLEK2 contributes to lung cancer progression through upregulation of NDRG1 expression. Yi-Pin Hsieh, Hui-Chia Liu, Hao-Yu Huang, Wan-Jiun Tang, Tai-Lin Lee, Meng-Feng Tsai. Da-Yeh University, Changhua, Taiwan.

PLEK2 is one of the pleckstrin homology domain (PH) domain proteins. Recently studies suggested that PLEK2 may play a critical role in lymphocyte migration through by PI3K/AKT dependent and independent pathways. However, the biological functions of PLEK2 in lung cancer is not clear. We detected the expression of PLEK2 in various lung adenocarcinoma cell lines using quantitative RT-PCR methods. The results indicated that PLEK2 expression significantly positive correlation with cancer cell migration and invasion ability. Forced expression of PLEK2 in lung cancer cells exhibited markedly promoting in the proliferation rate, anchorage independent growth, cell motility and invasion, whereas knockdown of PLEK2 was found to suppress lung cancer cell progression. In addition, we also reported that PLEK2 expression induce lung cancer cell epithelial-mesenchymal transition (EMT) phenomenon, and involve in che- motherapeutic drug resistance through upregulation NDRG1 gene expression. Overexpression of NDRG1 in PLEK2-knockdown cell line restored cancer cell proliferation, migration, and invasion. These results will provide information and help us to understand the roles of NDRG1 in lung cancer cells.


(a) Aim: The purpose of this study is to clarify an unknown function of Annexin A2-binding S100A proteins in tumor growth, cell cycle progression and invasion. Annexin A2 is a membrane bound protein originally identified as-sRc substrate, and often found to be overexpressed in many types of cancers. As a multicompartmental protein that orchestrates a spectrum of dynamic membrane-related events, it is associated with actin cytoskeleton control, vesicle formation and intercellular interaction. (b) Methods and Results: During our proteomics analysis using solid tumor cell lines, we identified both Annexin A2 and S100A proteins in cancer stem cell-rich population. We examined S100A1 and S100A2 proteins expression in several Head and Neck cancer PDX samples. Immunochemical data from in vivo specimens as well as western blots showed that S100A expression abundance was well associated with poor prognosis. Annexin A2 has been indicated to be involved in malignant phenotypes such as invasion and metastasis, and Annexin A2 and S100A form (S100-Annexin A2)x2 dimers, we next examined role of S100A proteins in cancer stem cell-rich population. We examined S100A2-depletion experiments. (c) New Findings: Depletion of S100A from PDX-derived cancer cells resulted in loss of both in vivo tumor formation and invasion. Furthermore, cell cycle progression was inhibited by the depletion, suggesting its potential role in proliferation controls. Similar to the mutant Ras-driven.

tumors. EGFR-overexpression increased S100A gene expression. (d) Conclusion: These results strongly suggest that increased in S100A plays significant roles in cancer cell proliferation and invasion.

**1892** Small molecule NSC59984 prevents cancer cell migration and invasion. Shengliang Zhang, Jessica Wagner, Lanlan Zhou, Wafik S. El-Deiry. Fox Chase Cancer Center, Philadelphia, PA.

Tumor metastasis is one of the main mechanisms leading to mortality of cancer patients. Searching for new therapeutic strategies to prevent tumor metastasis is one of the anti-tumor approaches in cancer therapy. Cell migration and invasion are crucial initial steps in the complex process and progression of tumor metastasis. Mutant p53 has been known as an important factor associated to cancer cell migration. Therefore, targeting mutant p53 is a potential strategy to avoid tumor metastatic spread. We recently reported an anti-tumor small molecule, NSC59984 which degrades mutant p53 and restores p53 pathway via activation of p73. Here we demonstrate that NSC59984 inhibits cancer cell migration and invasion. Treatment with NSC59984 partially attenuates cell migration in various cancer cells based on the scratch assay and Boyden chamber assay. The effect of NSC59984 on cell migration is partially blocked by knockdown of p73, a member of the p53 family, suggesting that restoration of p53 pathway signaling via p73 is required for NSC59984-inhibition of cell migration in cancer cells. We found that NSC59984 induces ERK2-dependent p53 restoration and mutant p53 degradation. To examine the role of ERK2 in the cell migration, ERK2 signaling is blocked by U0126 treatment in cancer cells. The effect of NSC59984 on cancer cell migration is partially inhibited by U0126 treatment, suggesting that NSC59984 inhibits cancer cell migration partially dependent on ERK1/2 pathway signaling in cancer cells. In addition, the gelatin degradation assay shows that NSC59984 inhibits cancer cell invasion. Consistent to the in vitro assays, NSC59984 decreases tumor invasion of xenograft tumors as compared to the xenograft tumors treated with DMSO as control. Our results, taken together, suggest that NSC59984 not only suppresses tumor growth, but also inhibits cancer cell migration and invasion. Our findings provide the potential administration of p53-restoring compound NSC59984 in cancer therapy by blocking tumor metastasis.

**1893** Alpha-tubulin acetyltransferase, MEC-17, regulates cancer cell morphology and migration through epithelial-mesenchymal transition suppression and cell polarity disruption. Cheng-Chee Lee, Chi-Yen Chang, Chi-Min Lin, Jiang-Yang Chang. National Cheng Kung University, Tainan, Taiwan; National Health Research Institutes, Taiwan, Taiwan.

Acetylation of α-tubulin has characterized as a key process for microtubule stabilization, and it can regulate cell spreading and modulate the dynamics of focal adhesion. MEC-17, a newly identified alpha-tubulin-N-acetyl transferase, serves as the major α-tubulin acetyltransferase to promote α-tubulin acetylation in vitro and in vivo. However, the physiological function of MEC-17 in cancer cells and its effect on MT acetylation during cell morphogenesis and migration has remained elusive. In the present study, our results showed that overexpression of MEC-17 induced increment of cell focal adhesion area, suppressed pseudopod formation in three-dimension collagen-embedded cultured environment and inhibited cancer cell migratory and invasive abilities. These changes were further proved to be caused by epithelial-mesenchymal transition (EMT) repression, cell polarity disruption caused by alteration of Golgi orientation and cdc42 activity and the decrease in phosphorylation of extracellular signal regulated kinase 1/2 (ERK1/2). On the contrary, silencing of endogenous MEC-17 by specific shRNA accelerated the pseudopods formation and EMT process, which lead to facilitating the cell migration. Taken together, these results demonstrated that the important role of MEC-17 in the modulation of intrinsic cellular morphogenesis and migratory and invasive functions through regulation of EMT and polarization. (The study was supported by the following grants: MOST 105-2320-B-006-006-MY3 and MOST 105-2325-B-400-001 from the Ministry of Science and Technology of Taiwan, ROC).


The purpose of this study was to clarify the role of BMP inhibitor gremlin 1 in invasive and migration in mesothelioma. Primary mesothelioma cells isolated from patient pleural fluid as well as mesothelioma cell lines were used for in vitro studies. Cells were transfected with siRNAs or transduced with lentiviral expression vectors. Invasive growth was analyzed in 3D Matrigel or collagen I matrices. mRNA expressions were analyzed using a commercial PCR array and quantita-
#1897 The role of nuclear deformation and rupture in breast cancer cell migration. Alexander E. McGregor, Joshua J. Elacqua, Emily S. Bell, Jan Lammersing. Cornell University, Ithaca, NY.

Invasion of tumor cells into the surrounding tissue is a critical step in the metastatic cascade, which is responsible for the vast majority of cancer-related deaths. Recent findings have begun to demonstrate that the biophysical properties of the cell nucleus play a critical role in this process. During invasion, cancer cells must maneuver through tight spaces much smaller than the cell nucleus. While the cell cytoplasm easily maneuvers through confined spaces, the deformability of the nucleus, which is the largest and stiffest organelle, ultimately determines how efficiently a cell can pass through a tight space. Furthermore, as the nucleus deforms to move through a confined space, it can exhibit transient loss of nuclear envelope (NE) integrity, where the nuclear membranes rupture and nuclear and cytoplasmic components interchange. We previously showed that chromatin is exposed to the cell cytoplasm during NE rupture, and cells can incur DNA damage. While this work revealed that cancer cell migration requires nuclear deformation and can involve NE rupture, it is currently unknown whether such events correlate with metastatic potential. We used a panel of human breast cancer cell lines, including claudin-low, basal-like, and lumen-like cells. Cells were stably labeled with a green fluorescent protein fused to a nuclear localization sequence (NLS-copGFP) to investigate whether some cell lines were particularly well suited to migrate through tight spaces, and if any were particularly prone to NE rupture. The cells were loaded into microfluidic devices that were designed to mimic interstitial in vivo space and monitored by time-lapse microscopy. Devices contained constrictions 5 μm tall and 1.2, or 15 μm wide. We found that claudin-low cell lines such as BT-549 migrated through confined spaces (1 ± 2 μm wide constrictions) significantly faster and more efficiently than basal-like HCC70 cancer cells. In contrast, the various cell lines displayed similar migration rates in the 15 μm wide constrictions, which do not require nuclear deformation. Thus, the differences observed between the cell lines reflect specific defects in migration through confined spaces, and not general migration defects. We are currently expanding our studies to a larger panel of cancer cell lines and to mouse breast cancer models. If confirmed, these results could indicate that highly invasive cancer cells may benefit from more deformable nuclei that facilitate movement through tight spaces encountered in the tissue microenvironment.

#1898 MEG3 as a competing endogenous RNA binds with miR-27a to promote PHLP2 protein translation, and consequently impairs bladder cancer invasion. Chao Huang,1 Xin Liao,2 Jingxia Li,1 Chunshu Huang,1,2. New York University School of Medicine and Wuhan Union Hospital, Tuxedo Park, NY,2.

Muscle invasion and metastasis of bladder cancer dramatically decrease survival rate, compared to 77% of 5 year survival rate of all bladder cancer, metastasis bladder cancer is only 5%. This truth implies that one of the therapeutic kernels of bladder cancer is to decrease, even block, its invasiveness. In the current study, we find that MEG3 is significantly downregulated in human muscle-invasive bladder cancer tissues compared with non-muscle invasive tissues. We also demonstrate the MEG3 strongly inhibits human bladder cancer cell invasiveness by acting as a ceRNA. We find that MEG3 competes with PHLP2 mRNA for miR-27a binding, which leads to a decreased miR-27a activity, and subsequently reducing the translation inhibition of miR-27a to PHLP2. The upregulated PHLP2 decreases the c-Jun phosphorylation at Ser63/73, and in turn inhibits the c-myc transcription and its mediated invasiveness of the bladder cancer cells. This is the first demonstration that MEG3 inhibits the invasiveness of human bladder cancer cells via negative regulation of c-Myc as a ceRNA. These findings not only provides a novel insight into understanding the mechanisms behind the MEG3 inhibiting the cancer cell invasion, but also reveals a potential targets for invasive bladder cancer prevention and therapy.

#1899 MKK4/JNK2 down-regulation in NSCLC suppresses tumor growth and metastasis. Tamer Saad Kaoud,1 Nancy D. Ebelt,1 Lili Du,2 Sabrina Van Ravenstein,3 Kenneth Y. Tsai,1 Kevin N. Dalby1. 1UT Austin College of Pharmacy, Austin, TX; 2The University of Texas MD Anderson Cancer Center, Houston, TX; 3UT Austin, Austin, TX.

Mitogen-activated protein kinase kinase-4 (MKK4) has been reported to either enhance or suppress oncogenesis. Evidence of its pro-angiogenic activities in breast, pancreatic, lung and skin cancer have been reported. Although the mechanism of its possible tumorigenic role is still unclear. Recent studies in glioblastoma and lung carcinoma have suggested important roles for the constitutive activation of its downstream substrate JNK2. JNKs require phosphorylation by both MKK4 and MK7 to be fully activated. The JNK2 siRNA shows a unique propensity among the JNKs to autophosphorylate in vitro. Here we show that JNK2 autophosphorylation contributes to the proliferation and migration of NSCLC cells under conditions of low serum through an MKK4-dependent mechanism. This suggests a pro-oncogenic role of MKK4 in NSCLC through a JNK2 autophosphorylation-dependent pathway. Evidence in several cell lines, including mouse embryonic stem cells lacking MKK4 or MKK7 suggests that autophosphorylation alone activates JNK2 weakly in cells. However, JNK2 autophosphorylates on Thr-183, to create a pool of JNK2 primed for activation by MKK4, which phosphorylates JNK2 on Tyr-185 to activate it. Under conditions of low serum the down-regulation/overexpression of MKK4 or JNK2 but not MKK7 or JNK1 suppresses/promotes proliferation of multiple NSCLC cell lines, through STAT3. Under the same conditions, A549 cell migration is inhibited upon down regulation of either MKK4 or JNK2. Further supporting the notion that active JNK2 results primarily from autophosphorylation of Thr-183, pan-JNK ATP competitive inhibitors (JNK-IN-8 and SP600125) showed a dose-dependent dephosphorylation of JNK2 in NSCLC cell lines and inhibited their proliferation under conditions of low serum. Moreover, the downregulation of MKK4 inhibition of JNK2 autophosphorylation by SP600125 inhibited nuclear localization of JNK2 in A549 cells cultured in low serum. To further understand the pro-oncogenic role of MKK4 in the A549 cell line, we investigated tumor growth and lung metastasis in A549 Xenografts in which either JNK2 or MKK4 were stably knocked down. Either JNK2 or MKK4 knockdown showed a similar suppression of tumor growth and lung metastasis if compared to the expression and RNA control. Sequencing of MKK4 in the A549 cells revealed no genomic deletion or somatic mutations. Taken together, the data suggest that MKK4 may exhibit pro-oncogenic properties in NSCLC by activating Thr-183-autophosphorylated JNK2. Targeting this pathway may reduce or block lung tumor progression and/or metastasis.
DNMT1 is involved in esophageal squamous cell carcinoma (ESCC) and self-renewal ability of ESCC-cancer stem cells. Yining Teng, Xiyi Gu, Yin Yuan, Lipeing Guo, Wei Jiang, Shih-Hsin Lu. National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China.

DNA methylation mediated by DNA methyltransferase 1 (DNMT1) plays an important role in carcinogenesis and self-renewal ability of cancer stem cells (CSCs). However, the function of DNMT1 in esophageal squamous cell carcinoma (ESCC) carcinogenesis, especially self-renewal ability of ESCC-CSCs remains unclear. In this study, we found a high expression of DNMT1 in both side population (SP) cells and sphere formation cells that represented as substitutes for CSCs in KYSE150 and EC109 ESCC cell lines. In addition, the expression of DNMT1 was decreased during the differentiation from SP to None-SP (NSP) in these ESCC cells. These results suggested that DNMT1 might have a role in regulating self-renewal and/or differentiation of ESCC-CSCs. To further investigate DNMT1 in ESCC carcinogenesis and self-renewal ability of ESCC-CSCs, we silenced the expression of DNMT1 in KYSE150 and EC109 ESCC cells using lentivirus-mediated RNA interference (RNAi). Our results showed that ablation of DNMT1 expression in KYSE150 and EC109 ESCC cells resulted in decreased their CSCs by SP analysis and sphere formation assay. Meanwhile, ablation of DNMT1 expression inhibited malignant phenotypes in KYSE150 and EC109 cells, including cell proliferation, colony formation, migration and drug resistance abilities. Treatment of 5-aza-2′-deoxycytidine (5-aza-dC), a DNMT inhibitor that led to the degradation of DNMT1 protein by proteasome, revealed that numbers of CSCs and the malignant phenotypes of KYSE150 and EC109 ESCC cells were repressed significantly, including a dramatic inhibition of self-renewal ability of these ESCC-CSCs. Thus, our results indicated that DNMT1 is involved in ESCC carcinogenesis, especially in the maintenance of ESCC-CSCs, suggesting that DNMT1 could be a potential target for ESCC, especially ESCC-CSCs, therapy.
ous lung metastases resulting in increased survival. CCR10 and its ligands were down-regulated on ALDHhigh D5 CSCs and in lung tissues respectively in animals subjected to ALDHhigh D5 CSC-DC vaccination. Down-regulation of CCR10 by siRNA significantly blocked tumor cell migration in vitro and metastasis in vivo. T cells harvested from ALDHhigh D5 CSC-DC vaccinated animals selectively killed ALDHhigh D5 CSCs as well as the immunolabeled targeting of CSCs. As a result, CSC-DC vaccination significantly decreased the percentage of ALDHhigh cells in residual tumors. These data indicate that, when used in an adjuvant setting, ALDHhigh CSC-DC vaccines effectively inhibit local tumor recurrence, reduce spontaneous lung metastasis, and prolong animal survival; compared with traditional DC vaccines and that simultaneous PD-L1 blockade can significantly enhance this effect.

Vissicchio,5 Emmanuel Contassot,3 Sae Kim,2 Talal Syed,1 Michael Zhang,1
cate that, when used in an adjuvant setting, ALDHhigh CSC-DC vaccines effect-

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We have analyzed the anticancer effect of the single drugs TQ and 5-FU and the

marker CDy1 and Hoechst3324 after 21 days of incubation. Western Blot analyses the spheroid forming capability after treatment with different concentrations. HCT116, HT29 and 5-FU-resistant colorectal tumor cells were used to

bridges, the single drugs and the combination was investigated by crystal violet

plant-derived drug thymoquinone (TQ). Methods: The cytotoxic effect of hy-

we aimed to synthesize new hybrid molecules by combining 5-FU with the

Sincetheresponsetothestandardchemotherapeutic drug 5-fluorouracil (5-FU)

Tumor Biology: Normal and Neoplastic Stem Cells

Background: Folate hydrolase-1 (FOLH1; NAALADase; PSMA) is a type

class of testis-derived proteins which are only expressed in germ cell tumors.
tissues. Thus, SPANX may serve as a selective marker for targeting CSCs. In addition, we demonstrate a functional role for SPANX in mediating CSC phenotype. Knockdown of SPANX decreased the percent of CD24low+ CSCs in TNBC. It also attenuated the proliferation, migration and invasion of CD24+ cells. Radiation treatment increased SPANX expression levels and enriched for SPANX+ cells. Loss of SPANX resulted in decreased number of CD24+ cells after radiation treatment. Hence, we show that SPANX may be promoters of the most aggressive CSC subset of TNBC. Since SPANX is highly immunogenic, our data provide rationale for further testing of combined radiation and immunotherapy approaches in the treatment of this deadly cancer. It also supports the use of protective and therapeutic SPANX vaccines against the most aggressive CSC subset in TNBC.

#1907 Identification of a cancer stem cell-specific function for the histone deacetylases HDAC1 and HDAC7 in breast and ovarian cancer. Abigail E. Witz,1 Corrado Caslini,2 Chung-Wei Lee,2 Tong Iln Lee,3 Diana J. Azzam,3 Bing Wang,4 Fabio Petrocca,5 James Grosso,1 Michelle Jones,1 Evan B. Cohick,2 Adrienne B. Gropper,4 Claes Wahlstedt,4 Andrea L. Richardson,5 Ramin Shiekhattar,6 Richard A. Young,1 Tan A. Ince1.1 Sylvester Comprehensive Cancer Center, Miller School of Medicine, University of Miami, Miami, FL; 2Brigham and Women's Hospital, Harvard Medical School, Boston, MA; 3The Whitehead Institute for Biomedical Research, Cambridge, MA; 4Center for Therapeutic Innovation, University of Miami Miller School of Medicine, Miami, FL; 5Boston Children's Hospital, Harvard Medical School, Boston, MA; 6University of Miami Miller School of Medicine, Sylvester Comprehensive Cancer Center, Biomedical Research Building, Miami, FL, FL

Tumors are comprised of a highly heterogeneous population of cells, of which only a small subset of stem-like cells possess the ability to regenerates tumors in vivo. These cancer-stem-cells (CSCs) represent a significant clinical challenge as they are resistant to conventional cancer therapies and play essential roles in metastasis and tumor relapse. Multiple signaling pathways involved in the regulation of CSCs have been identified. However, the regulation of CSCs is unlikely the reversible short-term changes in cellular phenotype induced by various extracellular factors, or the permanent changes induced by mutations. The hierarchical differentiation of CSCs to non-stem-tumor-cells (nTCs) is long-lasting over many cell generations but it is also reversible, that is more akin to tissue differentiation of CSCs to non-stem-tumor-cells (nsTCs) is long-lasting over many cell generations but it is also reversible, that is more akin to tissue differentiation. In particular, FGFR1 and FGFR2, which are found in cancer stem cells (CSCs) are sub-population of cells that bear stem-like properties, and are believed to contribute in tissue initiation and recurrence in tumor microenvironment. Understanding role of CSCs in tumorigenesis was limited to immunocompromised nude/SCID mouse models, and should be investigated in immunocompetent mouse model(s) representing clinical conditions. In this study, we have investigated and confirmed CSCs properties in HCC cells lines, and (2) investigated tumor initiation capability of EpCAM positive CSCs in novel immunocompetent mouse model of HCC. Methods: In vitro CSC enrichment was achieved by treating murine (Hepa-1-6) and human (HepG2, Hep3B) HCC cells in serum-free condition. To confirm CSCs, we analyzed CSC biomarkers (EpCAM,CD90,CD44,CD133) using flow-cytometry and Immunocytochemistry (ICC), and functional markers using Aldolase assay and Hoechst-33342 efflux. Drug resistance property of CSCs was studied using Doxorubicin (anthracycline antibiotic) and Sorafenib (multikinase inhibitor) by MTT assay. To study in vivo tumorigenesis, immunocompetent mouse model was established using C57/LI mouse andcopGFP expressing Hepa-1-6 cells. Capability of EpCAM CSCs for tumor initiation was tested in 3 diet induced groups, i.e. control (10% dietary fat), high-fat (60% dietary fat), and NASH (60% dietary fat MCD diet with 0.1% methionine). 2 million FACS sorted EpCAM+/- or EpCAM-/- cells were orthotypically injected into left liver lob. Tumor growth was monitored using high-frequency ultrasound and animals were euthanized after 18 days. Histology and ICC analysis was performed to confirm the tumors and findings. Results: Spheroid forming HCC-CSCs showed significantly higher EpCAM expression and significant higher chemoresistance compared with control HCC cells in vivo findings confirmed that EpCAM+ CSCs required NASH microenvironment. In NASH group mice, EpCAM+/- CSCs have shown significant tumorigenesis compared with no tumors in EpCAM-/- (n=9, p<0.005). Histology analysis of NASH liver tissue confirmed lobular and portal inflammation, hepatocellular ballooning, and fibrosis. Con-
trol and high-fat diet group mice failed to develop tumors. Conclusions: We have developed a novel mouse model to study CSCs to overcome confounding limitations of immunocompromised mouse models for testing functional immune system components. Our findings using immunocompetent mouse model suggest that, in HCC, (1) EpCAM + CSCs cannot initiate tumor by itself within normal liver microenvironment in the presence of functional immune components, (2) NASH microenvironment promotes EpCAM + CSCs mediated tumorigenesis while EpCAM/- non-CSCs subset failed to develop tumor.

#1910 NF-kappaB classical versus alternative pathways support distinct populations of ovarian cancer tumor-initiating cells. Carrie D. House, Christina M. Annunziata. National Cancer Institute, Bethesda, MD.

Ovarian cancer is the most lethal gynecological malignancy in the United States with high morbidity and mortality due to recurrence and chemoresistance. Our data suggest tumor-initiating cells (TICs) play an important role in disease biology. We previously showed that a subset of ovarian cancer cells depends on NF-kappaB signaling, and that expression of NF-kappaB proteins is associated with poor survival. Given that NF-kappaB expression correlates with a poor outcome in ovarian cancer, and NF-kappaB activity supports drug resistance and tumorigenicity, we hypothesize that NF-kappaB supports a TIC program responsible for ovarian cancer relapse. To investigate this pathway in TICs, we designed a novel method to enrich for TICs from cell lines and patient samples by culturing non-adherent, floating cells in stem cell conditions defined by low attachment flasks and serum free media. Preliminary data confirm that these cells have higher stem cell marker expression, are chemoresistant, and are more tumorigenic in nude mice compared to their adherent counterparts. These TIC enriched culture conditions enhance NF-kappaB expression and activity. More specifically, alternative NF-kappaB signaling through the RelB transcription factor supports TIC populations by regulating aldehyde dehydrogenase (ALDH), an enzyme with high activity in TICs. Using an inducible shRNA targeting RelB we show that spheroïd formation, ALDH expression and activity, chemoresistance, and tumorigenesis in both subcutaneous and intrabursal xenograft models, significantly decreased in the absence of RelB. Interestingly, loss of classical NF-kappaB signaling, through a shRNA targeting the RelA transcription factor, was less effective in targeting TICs, but more effective at targeting a proliferative subpopulation with high Ki67 staining. We conclude that classical and alternative NF-kappaB signaling support ovarian tumor cells with distinct phenotypes and the collaboration of these pathways may be critical for supporting tumor repopulation following chemotherapy. Current studies will measure system level changes induced by these shRNAs, and identify a gene signature specific to each NF-kappaB pathway in ovarian TICs. Clarifying the nuances of NF-kappaB signaling in TICs will increase our understanding of ovarian cancer recurrence and further focus therapeutic strategies to prevent relapse.


Intestinal colorectal cancer (CRC) is the third most common cancer diagnosed in both men and women. Dysregulation of the WNT/β-catenin pathway is the most commonly mutated pathway in CRC. R-spondins (RSPO), a family of secreted proteins, are enhancers of WNT signaling. More specifically, alternative NF-kappaB signaling through the RelB transcription factor supports TIC populations by regulating aldehyde dehydrogenase (ALDH), an enzyme with high activity in TICs. Using an inducible shRNA targeting RelB we show that spheroïd formation, ALDH expression and activity, chemoresistance, and tumorigenesis in both subcutaneous and intrabursal xenograft models, significantly decreased in the absence of RelB. Interestingly, loss of classical NF-kappaB signaling, through a shRNA targeting the RelA transcription factor, was less effective in targeting TICs, but more effective at targeting a proliferative subpopulation with high Ki67 staining. We conclude that classical and alternative NF-kappaB signaling support ovarian tumor cells with distinct phenotypes and the collaboration of these pathways may be critical for supporting tumor repopulation following chemotherapy. Current studies will measure system level changes induced by these shRNAs, and identify a gene signature specific to each NF-kappaB pathway in ovarian TICs. Clarifying the nuances of NF-kappaB signaling in TICs will increase our understanding of ovarian cancer recurrence and further focus therapeutic strategies to prevent relapse.

#1912 Actn4, a novel therapeutic target of dietary ellagic acid, promotes breast cancer metastasis via mediating cancer stem cells. Neng Wang,1 Zhiyu Wang,2 Xiaoming Xie,1 Sun Yat-sen University Cancer Center; University of Guangzhou Traditional Chinese Medicine, Guangzhou, China; #1913 SOX9 regulates cancer stem-like cells in non-small cell lung cancer. Muhammad Yousaf,1 Sudjit Luanpitpong,2 Heimo Riedel,1 Yon Rojanasakul1. 1West Virginia University, Morgantown, WV; 2Mahidol University, Bangkok, Thailand.

Lung cancer is the leading cause of cancer death worldwide in both men and women, every year killing more people than breast, prostate and colon cancers combined. Drug/radiation resistance and tumor relapse contribute to low patient survival, which has largely been attributed to the acquisition of stem-like cells (CSCs), or tumor initiating cells. Moreover, conventional therapies are not effective against CSCs. This signifies the need to identify mechanisms of CSC regulation which could lead to the discovery of CSC-specific drug targets and the development of more effective drug therapies. An embryonic transcription factor SOX9 has been implicated in CSC regulation in a number of cancer types, but little is known about its role in non-small cell lung cancer (NSCLC). We demonstrated that high SOX9 expression correlates with poor survival in lung cancer patients by analyzing The Cancer Genome Atlas (TCGA) data. We hypothesized that SOX9 has a key role in tumor progression and chemoresistance by up-regulating CSCs in NSCLC. To test this hypothesis, SOX9 expression was stably or transiently knocked down in NSCLC cells, and their effects on CSC formation and biomarkers, and on drug resistance were investigated. Our results showed that SOX9 knockdown decreased the number of tumor spheres in NSCLC cell lines. Furthermore, SOX9 knockdown downregulated the expression and activity of stem cell marker ALDH1A1 as determined by Western blot and flow cytometric assays. Consistent with this finding, overexpression of SOX9 in non-
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cancer lung epithelial Beas-2B cells elevated tumor sphere formation, supporting the role of SOX9 in CSC regulation. Analysis of the soft agar colony formation assay further showed that SOX9 knockdown inhibited anchorage-independent growth in NSCLC cells. More importantly, SOX9 knockdown resulted in an increased sensitivity of the cells to a chemotherapeutic drug cisplatin, with SOX9 knockdown expressing a significantly increased sensitivity. These results indicate an important role of SOX9 in the regulation of CSCs in NSCLC and suggest its potential utility as a drug target for chemoresistant lung cancer.

#1914 GNS561 is a new quinoline derivative with high efficacy on cancer stem cells from colorectal liver metastasis and hepatocellular carcinoma.

Pirsa Bassi,1 Elena Patricia Gifu,2 Sonia Brun,3 Jerome Courcambeck,1 Antoine Beret,1 Jean Marc Pascussi,3 Julie Pannequin,3 Eric Raymond,1 Philippe Hallon,1 Philippe Merle,1 Claude Caron de Fremontel,1 Genovoxis Pharma, Marseille, France; CRCL, INSERM U1052 - CNRS 5286, Université Lyon 1 - Centre Léon Bérard, Lyon, France; *Institut de Génomique Fonctionnelle (IGF), Montpellier, France; CRCL, INSERM U1052 - CNRS 5286, Université Lyon 1 - Centre Léon Bérard, Hospices Civils de Lyon, Lyon, France.

Background: In spite of wide application of sorafenib for advanced hepatocellular carcinoma (HCC) treatment, and systemic chemotherapy cocktails (5-fluorouracil, irinotecan, and oxaliplatin) for metastatic colorectal cancer, the prognostic for both cancers remains poor. In recent years, highly tumorigenic sub-populations of cancer cells named Cancer Stem Cells (CSCs) have been claimed as responsible of some tumor recurrences. Indeed, CSCs are resistant to chemotherapy, and they have the ability to regenerate all the tumor bulk with its heterogeneous latter generations. For this reason, new drugs with original mechanism of action which target CSC properties would likely improve cancer treatment Material and methods: Antitumor activity of GNS561 was tested on a panel of cancer cell lines and primary tumors. GNS561 impact on CSCs subpopulation in patient derived cells from colorectal hepatic metastatic tumors was assessed by flow cytometry (ALDH activity). In HCC, the effect of this drug was evaluated by sphere formation assay as readout to estimate CSC survival. Tolerance and plasma and liver pharmacokinetic were evaluated after single and repeated dosing in mice and rats. In vivo GNS561 activity was tested in orthotopic mouse model Results: GNS561 demonstrated multiple cellular effects such as induction of autophagy, induction of apoptosis and cell cycle modulation. It showed antitumor activity against several human cancer cell lines. Furthermore, GNS561 was effective against a panel of HCC tumors even from patients harboring sorafenib resistance. GNS561 showed nonetheless an original dose-response cytoxic activity against the whole tumor populations but also against a subpopulation displaying high ALDH activity in three CRC patient-derived cell lines established from fresh liver metastasis biopsies. Consequently, on the same model this compound induced a striking decrease of sphere formation. In HCC cell lines GNS561 was seen on both whole populations (mean EC50 2μM) and subpopulations displaying CSC features (Epcam high). In addition, in the opposite of sorafenib, GNS561 decreases the HCC cell capacity to form spheroids. In mouse, GNS561 was found well tolerated and highly selectively trapped in the liver (exposure ratio liver/plasma about 170 animals). In HCC, PDX mouse model, tumor growth was significantly reduced by GNS561 with a dose-response manner, this tumor regression was associated with AFP level decreases (p < 0.002) and 54% with sorafenib (p < 0.046) compared to control Conclusions: GNS561 is a liver selective drug which offers great promise for HCC and liver metastatic tumors treatment. By simultaneously targeting the cancer stem cell subpopulation and tumor bulk, both cell heterogeneity, plasticity and recurrences could be overcome at least in colorectal cancer and HCC. GNS 561 is now aimed to further reach clinical development in patients in 2017.

#1915 Deconstructing cancer stem cells: IL-6, G-CSF and Activin-A as mediators of stroma-orchestrated epithelial cells' dedifferentiation.

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Inter cellular communication in cancer is a deceiving and extremely efficient process through which the corporal machinery is hijacked by a panoply of cellular and molecular strategies turning the entire human body into an evolutionary arena. The extensive crosstalk mediated by cytokines and chemokines overcome the inefficiency of the invasion-metastasis cascade, thus allowing the development of the often-fatal metastatic disease. Cancer stem cells (CSCs) have recently been implicated in major steps of the tumorogenic process, namely in tumor initiation, metastasis formation and tumor relapse following therapy. Yet, despite their relevance, the orchestrated interactions of the intercellular communication process are still unclear. After observing that the malignant human bronchial epithelial RenG2 cells differentiated following culture in the subcutaneous mouse lumbar region, co-cultures of surgically isolated mice lumbar stromal cells with RenG2 cells were established and the conditioned media studied by multiplex and ELISA. Consequently, Interleukin-6 (IL-6), Granulocyte colony-stimulating factor (G-CSF) and Activin-A were identified as the characteristic mediators of the intercellular communication process. Aiming to ascertain the individual role of each cytokine in the de-differentiation process, as well as to access the involvement of exosomes as transport vehicle, the same co-cultures were reproduced in the presence of specific cytokine-communication blockers, either individually or in combinations of up to three blockers, and exosome-mediated communication inhibitors. Finally, exosomes were also collected from control co-cultures and their cargo screened for the target cytokines. ELISA showed that the three cytokines were present inside fibroblasts-secreted exosomes. Moreover, whenever exosomes’ release was blocked, dedifferentiation was abrogated, further proving the role of the aforementioned cytokines and of exosomes in the dedifferentiation process. Additionally, the cytokine-blocking experiments revealed that only IL-6 and Activin-A were endowed with the potential to orchestrate de-differentiation, as at least one of these cytokines was a present population developed inside RenG2 cells. Finally, G-CSF appeared to be decisive in maintaining the undifferentiated phenotype, as a larger pool of CSCs was attained whenever this cytokine and either IL-6 or Activin-A were present. Altogether the attained results implicated IL-6 and Activin-A in the formation of CSCs by de-differentiation, and G-CSF as a potent keeper of the dedifferentiation status. Subsequent studies are now needed to look into the use of these cytokines as therapeutic targets so the tumorigenic process may be abrogated in its initial steps, improving patients’ prognosis and survival. Work sponsored by FEDER, POFC-COMPETE and the FCT grants PTDC/BBB-BQB/2450/2012 and SFRH/BD/33884/2009.

#1916 Peptidylarginine deiminase IV (PADI4) is a novel tumor suppressor that may contribute to regulation of breast cancer stem cell dynamics.

Nellei Moskovich.1 NCITheodosia, MD.

Breast cancer is a global problem that accounts for almost a fourth of all cancers in women. Despite the many therapeutic strategies currently in use, treated patients often suffer from cancer relapse and metastasis due to the presence of a subpopulation of tumorigenic cells capable of self-renewal and termed cancer stem cells (CSCs). To address the properties of this critical sub-population, we utilized our recently developed lentiviral CSC reporter carrying an Oct/ Sox2 response element coupled to a green fluorescent protein (SORE6-GFP) to detect and isolate the CSC population in a low-grade ER+ human breast cancer model, MCF10Ca1h, by FACS. Whole transcriptome sequencing analysis (RNA-seq) on the sorted cells identified Peptidylarginine deiminase IV (PADI4) as differentially expressed between CSCs and non-CSCs. PADI4 was recently shown to regulate citrullination of histone H1, displacing it from chromatin and resulting in global chromatin decondensation and pluripotency in Inhibition was observed in luminal breast cancer. In support of this conclusion, deep deletions of PADI4 were found in luminal breast cancer. In breast cancer CSCs, likely through effects on chromatin structure. We showed PADI4KD. Our findings suggest that PADI4 may function as a tumor suppressor in breast cancer CSCs, likely through effects on chromatin structure. We showed that PADI4 expression is higher in luminal breast cancer cell lines than other subtypes, and we did shRNA knockdown (KD) of PADI4 in the luminal MCF10Ca1h model. PADI4 KD increased tumourosphere formation and clonogenicity indicating increased CSC population/activity. Additionally, MCF10Ca1h cells treated with a PADI4 selective inhibitor showed an increase in CSCs as assessed using the SORE6-GFP stem cell reporter. In vivo limiting dilution studies demonstrate that PADI4 KD increased tumor incidence at low cell inoculum, reflecting a statistically significant 2-4 fold increase in CSCs upon PADI4 KD. Our findings suggest that PADI4 may function as a tumor suppressor in luminal breast cancer. In support of this conclusion, deep deletions of PADI4 locus are found in 1% of human breast cancers in the TCGA datasets. Analysis of the effect of PADI4 KD on chromatin organization in the CSC and non-CSC compartments is ongoing.

#1917 AXL inhibition in ovarian cancer stem-like cells increases chemosensitivity.

Jin-Young Kim, Hyewon Chung, So-Jin Shin, Eunyoung Ha, Chihyun Cho. Keimyung University School of Medicine, Daegu, Republic of Korea.

Cancer stem-like cells (CSCs), a small population of cancer cells with regenerative potential, may lead to the initiation and progression of secondary tumors. Long-term chemotherapeutic drug treatment can also result in the gen-
eration of a drug resistant population of cancer stem cells. In this study we have isolated CD44+ and CD117+ enriched ovarian cancer stem-like cells from primary cultured ascites cells removed from ascites fluid of naïve ovarian cancer patients. AXL, a type I receptor tyrosine kinase family member, is found to be over-expressed in ovarian cancer stem-like cells. Cell viability assays showed that over-expression of AXL was strongly associated with increased cell viability. PI3-kinase/Akt signaling pathway has also been shown to be involved in decreases the cell viability during the inhibition of AXL. This study establishes the role of AXL, a receptor tyrosine kinase, as one of the key factors in decreasing the cell viability during the inhibition of AXL. In conclusion, this study shows that silencing of Twist1 greatly diminishes the tumor sphere-forming potential of breast CSCs. Furthermore, limited dilution assays and stem cell xenotransplantation reveal that knockdown of Twist1 expression decreases the frequencies of CSCs by 72-fold in human TNBC cells. More importantly, we show that silencing of Twist1 blocks Taxol-induced CSC enrichment in residual tumor cells that have survived drug treatment. Together, these results demonstrate a previously unrecognized role for Twist1 in mediating Taxol-induced CSC enrichment in residual tumor cells. Our results strongly support that targeting Twist1 signaling pathway may represent a novel therapeutic strategy to tackle therapy-induced CSC enrichment in breast cancer treatment.

#1918 Gene characterization of lung-tumorspheres for their usage as an in vitro screening platform for testing new therapeutic strategies. Alejandro Herreros-Pomares,1 Ester Munera-Maravilla,1 Silvia Calabuig-Fariñas,2 RutLucas,2 Rosa Farráns,1 Ana Blascoa,1 Susana Torresa,1 Jose Ferria,1 Ricardo Guijarro,1 Miguel Martorell,1 Eileis Jantus-Lewintre,1 Camps Carlos,1 Molecular Oncology Laboratory, General University Hospital of Valencia Research Foundation, Valencia, Spain;2Molecular Oncology Laboratory, General University Hospital of Valencia Research Foundation; Department of Pathology, Universidad de Valencia, Valencia, Spain;3Department of Oncogenic signalling Laboratory, Centro de Investigación Príncipe Felipe, Valencia, Spain;4Department of Medical Oncology, General University Hospital of Valencia, Valencia, Spain;5Department of Pathology, General University Hospital of Valencia, Valencia, Spain;6Department of Pathology, Universidad de Valencia, Valencia, Spain;7Department of Oncology Laboratory, General University Hospital of Valencia Research Foundation; Department of Biotechnology, Universidad Politécnica de Valencia, Valencia, Spain;8Molecular Oncology Laboratory, General University Hospital of Valencia Research Foundation, Department of Medicine, Universitat de Valencia. Department of Medical Oncology, General University Hospital of Valencia, Valencia, Spain.

Background: Lung cancer features like treatment resistance or tumor relapse have been linked to cancer stem cells (CSCs), a population of cells with self-renewal properties, and the ability to grow forming tumorspheres in non-adherent conditions. The aim of this study was to isolate and characterize tumorspheres from lung cancer cell lines and tumor tissue from resectable non-small cell lung cancer (NSCLC) patients and to use them as an in vitro platform for drug screening. Methods: This study was performed on cells from seven NSCLC tumor samples and five cell lines (H1650, H1993, H1395, A549 and PC9) grown in monolayer and as spheroids. The expression of 60 genes, including CSC-markers, pluripotency inducers, cell cycle regulators, invasion promoters and markers of Notch, Wnt and Hedgehog pathways were assayed by qRT-PCR. Drugs commonly used in clinical guidelines (Cisplatin, Paclitaxel, Erlotinib, Gefitinib, Erlotinib, Afatinib, Pemetrexed and Vinorelbine) and specific inhibitors of Wnt and Hedgehog pathways (Iwp2, Xav939, LDE225 and Vismodegib and Saliymin) were tested in triplicates at 4 different concentrations. Cell viability was measured after 48h and 72h using MTS assay, normalized to the respective mock-treated control cells and presented as percentage of control. Statistical analyses were considered significant at p<0.05. Results: Lung tumorspheres had significant increased expression of CSC-related genes EPCAM1, CD44, CCND1, KLF4 and CDKN1A, compared to their paired-adherent cells. Likewise, epithelial to mesenchymal transition (EMT) inducer SNAI1 and integrin ITGA6 were overexpressed in spheroids too. Regarding stemness pathway, Notch pathway ligands JAG1 and DLL4 and receptors NOTCH1, NOTCH2 and NOTCH3 showed higher expression in lungospheres. In Wnt pathway, higher expression levels of WNT3, CTNNB1 and GSK3β were found in tumorspheres. No significant differences were found for the rest of genes. Drugs screening showed classical anticancer drugs, such as Cisplatin, Vinorelbine or Pemetrexed, had mild cytotoxic effects on lungospheres, obtaining significant differences in the expression of some targets. Conversely, inhibitors Iwp2, Xav939, Salminomycin and Vismodegib showed higher cytotoxic effects on spheroids than in cells grown in monolayers. Conclusions: Lung tumorspheres derived from cancer cell lines and primary tumor tissues show increased levels of CSC markers and components of Notch and Wnt signaling pathways compared to the cells grown in adherence. Spheroids showed resistance to classical anticancer drugs, and a greater response to inhibitors of Notch, Wnt and Hedgehog pathways, strengthening its possible use as a short-term culture platform for a simple, and cost-effective screening to investigate novel therapeutic approaches. Supported by grants RD12/0036/0025 from RTICC-FEDER, PI12-02838/P115-00753 from ISCIII and SEOM/2012.

#1919 Twist1 modulates paclitaxel-induced cancer stem cell enrichment in triple-negative breast cancer. Aimin Yang, Xiaoyuan He, Bradley A. Schulte, Steven L. Carroll, Stephen P. Ether, Gavin Y. Wang. Medical University of South Carolina, Charleston, SC.

Triple-negative breast cancer (TNBC), the most aggressive subtype of breast cancer, is highly refractory to chemotherapy and has a high incidence of metastasis and tumor relapse. Targeted therapies for TNBC do not exist because this subtype lacks drugable hormone receptors and HER2 amplification or overexpression. Although Taxane-based chemotherapy is a standard of care for TNBC treatment, paclitaxel (Taxol)-induced cancer stem cell (CSC) enrichment presents a significant challenge to the success of breast cancer treatment. Thus, there is a critical need to understand the mechanisms by which chemotherapy induces CSC enrichment in residual tumors following anticancer therapies. Here we report that Taxol-induced enrichment for CSCs correlates well with a marked upregulation of Twist1 expression in human TNBC cells. Knockdown of Twist1 inhibits the clonogenic capacity of TNBC cells in vitro and TNBC tumorigenesis in vivo. Mammosphere formation assays indicate that silencing of Twist1 greatly diminishes the tumor sphere-forming potential of breast CSCs. Furthermore, limited dilution assays using stem cell xenotransplantation reveal that knockdown of Twist1 expression decreases the frequency of CSCs by 72-fold in human TNBC cells. More importantly, we show that silencing of Twist1 blocks Taxol-induced CSC enrichment in residual tumor cells that have survived drug treatment. Together, these results demonstrate a previously unrecognized role for Twist1 in mediating Taxol-induced CSC enrichment in residual tumor cells. Our results strongly support that targeting Twist1 signaling pathway may represent a novel therapeutic strategy to tackle therapy-induced CSC enrichment in breast cancer treatment.

#1920 Prostaglandin E2 accumulation enhances the expansion of ALDH1-positive cells and Kras-driven tumorigenesis in the pancreas. Koteru Arima,1 Takatsugu Ishimoto,1 Masaki Ohmuraya,2 Reiko Miyake1, Tsugio Eto1, Hirohisa Okabe,1 Yuki Kitan,1 Kunsuke Yamamura,1 Takayoshi Kaida,3 Katsunori Imai,1 Daisuke Hashimoto,1 Akira Chikamot,1 Hideo Baba1.

Background: Chronic inflammation is known to be a risk factor of carcinogenesis and tumor development, and it was demonstrated that oral aspirin reduced cancer-related death including pancreatic ductal adenocarcinoma (PDAC) through inhibiting prostaglandin synthases. On the other hand, cancer stem cells (CSCs) refer to a subset of tumor cells that have self-renewal ability and generate plenty of non-CSC that comprise a tumor. Prostaglandin E2 (PGE2) and inhibition of 15-PGDH, which is an enzyme degrading PGE2, promoted hematopoietic and tissue stem cell fraction, however, little is known about the role of PGE2 accumulation for CSC fraction. A number of CSC marker candidates have been explored to date, and Aldehyde dehydrogenase 1 (ALDH1) was identified as one of CSC markers in PDAC. The aim of this study is to elucidate the functional role of PGE2 in ALDH1 positive CSC fraction during tumor development in pancreas. Methods: The clonogenic growth potential of ALDH1-positive CSCs and that of PGE2 was analyzed by growth assays and sphere formation assays. We next investigated the expression of ALDH1 and self-renewal related genes in PDAC cell lines with PGE2 or 15-PGDH inhibitor treatment. We further conducted functional experiments using sALDH1 in PDAC progression. Furthermore, we examined the effect of PGE2 for pancreatic tumorigenesis using Kras-driven genetic mouse model treated with 15-PGDH inhibitor. Finally, ALDH1 and Ki67 expression was examined by immunohistochemistry in 121 primary surgical specimens of PDAC and analyzed a relationship with clinicopathological factors and clinical outcomes. Results: The number of ALDH1-positive cells was significantly increased by PGE2 treatment, and PGE2 promoted growth and sphere formation potential in PDAC cells. In addition, 15-PGDH inhibitor induced PGE2 accumulation and gave rise to ALDH1-positive cells harboring high proliferating potential in PDAC cells. The growth and sphere formation potential were inhibited by silencing ALDH1 expression in PDAC cells. We next found that the expression of Nanog and Oct-4 genes was regulated by PGE2-ALDH1 signaling. Furthermore, 15-PGDH inhibitor induced ALDH1 expression and promoted PanIN formation in Kras-driven genetic mouse model. Finally, a high level of ALDH1 expression was significantly associated with large tumor size and high Ki67 expression, and poor prognosis in PDAC patients. Conclusion: Current findings suggested that PGE2 positively regulated ALDH1 expression, and the growth and sphere formation potential were promoted by regulating self-renewal related genes expression, resulting in poor prognosis of PDAC patients. Inhibiting PGE2-ALDH1 signaling could lead to the suppression of tumor growth in PDAC patients.

#1921 Stem-like clusters of CD44+ circulating tumor cells seed metastases in breast cancer. Xia Liu, Wenjing Chen, Rokana Taftaf, Huiping Liu. Case Western Reserve Univ., Cleveland, OH.

Background: Circulating tumor cells (CTCs) are cells shed from primary tumor and circulate in the peripheral blood. They are considered the seeds of metastasis. Compared to single CTCs, clusters of multiple CTCs possess 50 times higher metastatic capacity in mouse breast cancer models. However, the mechanisms underlying the metastatic promotion effect of CTC clusters are unclear. Better understanding of CTC clusters properties and what stem cell markers they express have not been determined. Methods: Immunohistochemistry (IHC) was used to detect CTCs in vascular structures, and the stem cell markers in single and clustered CTCs. Lung metastases were either monitored by bioluminescence imaging (BLI) or visualized by fluorescence microscopy. Stem cell properties were examined by cell proliferation in vitro and in vivo. Clustering assay was performed by culturing cells in Poly-HEMA coated plates (for cell lines) or collagen-coated plates (for primary cells derived from patient-derived breast tumor xenografts, PDXs), and then monitored by IncuCyte live cell dynamic imaging. Results: CD44+ enriched circulating tumor cell (CTC) clusters were found in the lung/liver vascular structures in vivo in both metastatic breast cancer patients and PDXs that develop spontaneous lung metastases. Comparing to single CD44+ cells, clustered CD44+ cells formed more mamospheres, increased tumorigenic potential, and promoted lung colonization. Combining siRNA-mediated knockdown and CRISPR/Cas-based knockdout, we found that CD44 is required for breast tumor cell cluster formation and lung colonization upon tail vein seeding. During CD44 knockdout, CD44+ cells were arrested in S phase in vitro, and survival of clustered CTCs. Anti-EGFR antibody mimicked CD44 knockdown to inhibit cluster formation of PDX-derived tumor cells. Administration of EGFR inhibitor Erlotinib efficiently inhibited CD44+ cells-mediated spontaneous metastases to the lungs without affecting primary tumor growth. Conclusions: Our data provide new insights into the cellular and molecular mechanisms of stem-like clustered CD44+ cells-seeded metastasis, and implicate that targeting CD44+ CTC clusters by inhibition of EGFR activity could be a new therapeutic strategy to treat metastatic breast cancer.

#1922 S-SHIP promoter expression identifies a putative cancer stem cell population in C3(1)/Tag murine mammary tumors. Lu Tian,1 Chann Lagadec,2 Eric Adriaenssens,3 Emmanuel Bouchaert,2 Hélène Bauderlique-Le Roy,3 Xufen Lebourhis,3 Roland P. Burette1.1 CNRS UMR 8161 - Institut de Biologie de Lille - SPE5 team, Lille, France; 2Cell Plasticity and Cancer - Insereh U989 - Université Lille 1, Lille, France; 3Plateforme des modèles animaux du SIRICON-Lille, Oncovet Clinical Research, Lille, France; 4BioImaging Center Lille Nord de France - Institut Pasteur de Lille, Lille, France.

Breast cancer is the most common cancer in women worldwide. The isolation and characterization of breast cancer stem cells (CSC) are crucial for understanding cancer biology and revealing potential therapeutic targets. One of the major issues in the study of CSCs is the lack of reliable markers. A transgenic mouse model (Tg 11.5kb–GFP) was generated using the 11.5kb s-SHIP (stem-SH2-containing 5'-Inositol Phosphatase) promoter that specifies expression in mammary gland epithelial cells. We have established a label-free, combined chemical and functional selection strategy that is not accurately performed in conventional 2D cell culture and is inhibited by both cost and time in patient-derived xenografts (PDX).

#1923 Paired isolation and expansion of CSC and CTC from primary small cell lung cancer patient tissue and blood using the 3DKUBE bioreactor platform. Melissa Millard,1 Alina Lotstein,1 Lillia Holmes,1 David Schammel,2 Ki Chung,2 Jeff Edelfien,3 Hal E. Crosswell,1 Tessa Des Rochers1. KITIATEC, Inc., Greenwood, SC; 2Pathology Consultants, Greenville, SC; 3GHS, Greenville, SC.

Surgically resected lung tissue and blood were used for generation of xenografts in the 3DKUBE bioreactor (3DKUBETM). We studied secondary tumors and circulating tumor cells (CTCs) in patient-derived xenografts (PDX) and patients as the majority present with extensive disease at diagnosis. This scarcity of patient samples suitable for research presents a significant road block for the development of SCLC-targeted therapeutics. To address the problem of tissue scarcity, we developed a method for the isolation and expansion of cancer stem cells (CSC) and circulating tumor cells (CTC) from primary tissues and blood of SCLC patients using the 3DKUBE bioreactor. We have established a label-free, combined chemical and functional selection method for the isolation of CSCs from SCLC samples, solid tumor as well as blood, that does not rely upon the bias imposed by marker-based selection. Cells enriched in this manner were further purified and expanded under optimized conditions (growth factors, ECM, scaffolding and oxygen tension) within the 3DKUBE bioreactor. These isolated and expanded CSCs have maintained resistance to cisplatin and etoposide, stabilized the expression of traditional CSC markers, and been validated in vitro through serial spheroid formation assays. These CSCs are currently being characterized and compared to parental tissue through coregicmorphic and phenomic analysis and validated through in vivo tumorigenesis models. These cells will be utilized to generate in vitro models of disease, including drug sensitivity determination that is not accurately performed in conventional 2D cell culture and is inhibited by both cost and time in patient-derived xenografts (PDX).

#1924 Caveolin-1 inhibits mammary carcinogenesis via suppressing c-myc-induced metabolism reprogramming in breast cancer stem cells. Zhiyu Wang,2 Neng Wang,2 Shengqi Wang,1 Yifeng Zheng1, Guangzhou Univ. of Chinese Medicine, Guangzhou, China; Sun Yat Sen University, Guangzhou, China.

Purpose: Caveolin-1 (CAV1), a membrane constituent protein, exhibits tumor suppressor activities in multiple malignancies, whereas the underlying mechanisms remain unclear. The current study aimed to determine the significance of CAV1 in regulating cancer metabolism and its relation to breast cancer stem cells (CSCs). Experimental Design: The anti-carcinogenic function of CAV1 was evaluated by in vitro cell model, CAV1 knockout mice and MMTV-PyMT spontaneous breast cancer xenografts. Glycolysis activity and mitochondrial metabolism were assessed by immunoblotting, oxygen consumption and mitotacker staining analysis. Mammosphere formation, tumorigenic ability, reattachment differentiation and signaling pathways analysis were applied to study the regulation effects of CAV1 on breast CSCs. The clinical significance of CAV1 was also analyzed by human tissue microarray. Results: In both mammary tumorigenic and breast cancer xenografts, CAV1 is found significantly downregulated and positively correlated to increased glycolysis activity and impaired mitochondrial metabolism. CAV1 knockout results in increased ductal hyperplasia, associating with increased stem cell population and glycolysis metabolism. Breast CSCs exhibits glycolytic phenotype and decreased CAV1 expression compared to non-CSC. High expression of CAV1 in mammary and breast cancer xenografts significantly limits CSCs' self-renewal via inhibiting c-myc induced glycolysis. Clinical investigation suggests that high CAV1 expression is revealed with better overall survival and decreased CSCs population. Conclusions: CAV1 loss facilitates mammary carcinogenesis via enhancing glycolytic activity in breast CSCs, and CAV1 based therapy might become a novel strategy for breast cancer prevention.

#1925 Modeling physiologic microenvironments in three-dimensional microtumors facilitates brain tumor initiating cell maintenance. Ashley Gilbert,1 Kiera Walker,1 Anh Tran,2 Yancey Gillespie,2 Raj Singh,2 Anita B. Hjelmeland1. 1Vivo Biosciences, Birmingham, AL; 2Univ. of Alabama at Birmingham, Birmingham, AL.

Development of effective novel anti-tumor treatments will require improved in vitro models that incorporate physiologic microenvironments and maintain intratumoral heterogeneity including tumor initiating cells. Brain tumor initiating cells (BTIC) are a target for cancer therapy because they are highly tumorigenic and contribute to tumor angiogenesis, invasion, and therapeutic resistance. Current leading studies rely on BTIC isolation from patient-derived xenografts followed by propagation as neurospheres. As this process is expensive and time-consuming, we determined whether three-dimensional microtumors were an alternative in vitro method for modeling tumor growth via BTIC maintenance and/or enrichment. Brain tumor cells were grown as neurospheres or as microtumors produced using a human-derived biomatrix HuBioGel® and
maintained with physiologically relevant microenvironments. Percentages of BITCs were determined based on cell surface marker expression (CD133), label retention (carboxyfluorescein succinimidyl ester; CFSE), and tumourosphere formation capacity. Our data demonstrate that expansion of brain tumor cells as hypoxic and nutrient restricted microtumors significantly increased the percentage of BITCs and CFSE+ cells. We further demonstrated that marker positive cells isolated from microtumors maintain neurosphere formation capacity in the in vitro limiting dilution assay and tumorigenic potential in vivo. These data demonstrate that microtumors can be a useful three-dimensional biological model for the study of BTIC maintenance and targeting.


Overview: Head and neck squamous cell carcinomas (HNSCC) are the sixth most prevalent type of malignancy worldwide. Recent reports have demonstrated that nearly every tumor contain a small subpopulation of cells called cancer stem-like cells (CSCs), which are responsible for tumor maintenance, metastasis relapse and therapeutic resistance. Recent studies demonstrate that even established HNSCC cell lines contain a definite sub-population of CSC providing us with the opportunity to discerning their roles in progression, treatment, and relapse of the disease. Background: We have previously demonstrated that a definite subpopulation of CSCs exists in most HNSCC cell lines. Significant differences were seen between the HPV+ and HPV- subtypes, confirmed by analyses of stemness genes and EMT markers. In vivo tumor xenograft formation has established the heightened tumorigenicity of CSCs. We investigated the molecular and functional roles of Beta-catenin in HNSCC CSCs by shRNA knockdown and then subjecting the knocked down (KD) cells to various molecular analysis and in-vivo tumor formations in nude mice. We have also identified SOX2 as a down-stream effector gene that could exert the tumor promoting effects of Beta-catenin. The promoter region of the SOX2 gene was found to contain several putative Beta-catenin binding sites. Methods & Results: After identifying CSCs by in-vitro Sphere Forming (SFA) capabilities and molecular gene signatures, we have knocked down both Beta-catenin and Sox2 in both HPV+ and HPV- HNSCC cell lines (UMSCC47 and UMSCC6). SFA with the Beta-catenin KD-cells depicts a 50% decrease in sphere formation ability. Molecular analyses show lower expression of stemness genes: Sox2 (∼ 3 fold) and Oct4 (∼ 2 fold); metastatic genes: N-cadherin (∼ 2.5 fold), Vimentin (∼ 3 fold), Twist (∼ 4 fold), Snail (∼ 2.5 fold), Slug (∼ 2.5 fold) and Hif-1alpha (∼ 1.5 fold). Two tissue microarrays (TMA) were stained for Oct4, Sox2, and CD44 gene expression by multiplex RNA in situ hybridization. Nearly all tumor cells expressed high levels of aldehyde dehydrogenase (ALDH) and CD44 are critical for metastasis. These ALDH+/CD44+ cells preferentially migrate and/or metastasize to the lung and bone microenvironments, where secondary tumours can severely impede organ function. However, the specific role of the lung and bone marrow (BM) microenvironments in supporting and/or promoting metastasis of ALDH+/CD44+ cells remains poorly understood, and this is the purpose of the current study. Using a 2D ex vivo model, lung and bone marrow (BM) conditioned media (CM) enriched in tissue-specific soluble proteins were generated from murine tissues and utilized in cell culture to assess their ability to influence the stem-like phenotype and behavior of MDA-MB-468 human breast cancer cells. Exposure to lung-CM promoted the generation of a viable, non-adherent (‘floating’) breast cancer cell subpopulation that was not observed in response to BM-CM or basal media (p<0.05). Phenotypic analysis by flow cytometry and gene expression analysis by RT-qPCR revealed notable differences in ALDH/CD44+ phenotype in whole cell populations, adherent subpopulations, and/or non-adherent subpopulations in response to 72 hour exposure to lung-CM relative to basal media. In particular, the non-adherent breast cancer cell subpopulation generated in response to lung-CM demonstrated increased CD44 and ALDH1A3 gene expression and decreased ALDH1A1 gene expression relative to the adherent subpopulation (p<0.05). No significant phenotypic or gene expression differences were observed in MDA-MB-468 breast cancer cells in response to BM-CM. Taken together with our previous studies, these findings suggest that while both the lung and bone microenvironments support breast cancer cell growth, the lung microenvironment may additionally help to even further promote stem-like phenotype and behavior in the secondary metastatic site. Ongoing studies are aimed at elucidating the mechanisms by which aggressive ALDH+/CD44+ breast cancer cells interact with and/or are influenced by the lung microenvironment during metastatic progression.

#1927 The significance of calreticulin in pancreatic cancer: a molecule highly expressed in pancreatic cancer stem-like cells. Satoshi Matsukuma,1 Kiyoshi Yoshimura,2 Atsunori Oga,1 Moeko Inoue,1 Takuya Fujimoto, Atsuo Kuramasa, Masanari Fuse,2 Ryuichi Tsunedomi,1 Hideyoshi Eguchi,1 Hiroto Matsui,1 Shinuke Kaneko,1 Yukio Tokumitsu,1 Shinobu Tomochika,1 Michi-hisa Iida,1 Yoshihiro Tokuhisa,1 Kazukiyo Sakamoto,1 Nobuaki Suzuki,1 Tomoko Furuya-Kondo,1 Hiroshi Itoh,1 Shigeru Takeda,1 Shigeru Yamamoto,1 Shigefumi Yoshino,1 Shoichi Hazama,1 Tomio Ueno,1 Hiroaki Nagano1.

1Yamaguchi University Graduate School of Medicine, Ube, Japan; National Cancer Center of Japan, Tokyo, Japan; 2Osaka University Graduate School of Medicine, Suita, Japan; 3Yamaguchi University Graduate School of Medicine, Ube, Japan; 4Yamaguchi University Hospital, Ube, Japan; 5Yamaguchi University Hospital, Ube, Japan.

Cancer stem-like cells (CSCs) in solid tumors are thought to be resistant to conventional chemotherapy or molecular targeting therapy and to contribute to cancer recurrence and metastasis. In this study, we aimed to identify a biomarker of pancreatic CSCs (P-CSCs). P-CSC-enriched population was generated from pancreatic cancer cell lines using our previously reported method and its protein expression profile was compared with that of parental cells by two-dimensional electrophoresis and tandem mass spectrometry. The results indicated that a chaperone protein calreticulin (CRT) was significantly upregulated in P-CSCs compared to parental cells. Flow cytometry analysis demonstrated that CRT was mostly localized to the surface of P-CSCs and did not correlate with the levels of CD44v9, another P-CSC biomarker. Furthermore, the side population in CRTbright/CD44v9low population is much higher than that in CRTlow/CD44v9high population. CRT expression was also assessed by immunohistochemistry in pancreatic cancer tissues (n = 80) obtained after radical resection and was found to be associated with patients’ clinicopathological features and disease outcomes in the Cox’s proportional hazard regression model. Multivariate analysis identified CRT as an independent prognostic factor for pancreatic cancer patients, along with age and post-operative therapy. Our results suggest that CRT can serve as a biomarker of P-CSCs and a prognostic factor associated with poorer survival of pancreatic cancer patients. This novel biomarker can be useful for detecting P-CSCs independently, which had been detectable by multiple surface markers like CD24, CD44 and ESA. We will present P-CSCs properties of CRTbright population in P-CSCs.

#1928 The lung microenvironment promotes stem-like phenotype and behavior of breast cancer cells. Ashkan Sadri, Alison L. Allan. Western University, London, Ontario, Canada.

Ninety percent of breast cancer-related mortalities result from metastasis, a process whereby the primary tumour disseminates and targets distant secondary organs. We have previously demonstrated that stem-like breast cancer cells expressing high levels of aldehyde dehydrogenase (ALDH) and CD44 are critical for metastasis. These ALDH+/CD44+ cells preferentially migrate and/or metastasize to the lung and bone microenvironments, where secondary tumours can severely impede organ function. However, the specific role of the lung and bone marrow (BM) microenvironments in supporting and/or promoting metastasis of ALDH+/CD44+ cells remains poorly understood, and this is the purpose of the current study. Using a 2D ex vivo model, lung and bone marrow (BM) conditioned media (CM) enriched in tissue-specific soluble proteins were generated from murine tissues and utilized in cell culture to assess their ability to influence the stem-like phenotype and behavior of MDA-MB-468 human breast cancer cells. Exposure to lung-CM promoted the generation of a viable, non-adherent (‘floating’) breast cancer cell subpopulation that was not observed in response to BM-CM or basal media (p<0.05). Phenotypic analysis by flow cytometry and gene expression analysis by RT-qPCR revealed notable differences in ALDH/CD44+ phenotype in whole cell populations, adherent subpopulations, and/or non-adherent subpopulations in response to 72 hour exposure to lung-CM relative to basal media. In particular, the non-adherent breast cancer cell subpopulation generated in response to lung-CM demonstrated increased CD44 and ALDH1A3 gene expression and decreased ALDH1A1 gene expression relative to the adherent subpopulation (p<0.05). No significant phenotypic or gene expression differences were observed in MDA-MB-468 breast cancer cells in response to BM-CM. Taken together with our previous studies, these findings suggest that while both the lung and bone microenvironments support breast cancer cell growth, the lung microenvironment may additionally help to even further promote stem-like phenotype and behavior in the secondary metastatic site. Ongoing studies are aimed at elucidating the mechanisms by which aggressive ALDH+/CD44+ breast cancer cells interact with and/or are influenced by the lung microenvironment during metastatic progression.

TUMOR BIOLOGY: Pediatric Cancer 1: Biomarkers, Preclinical Models, and New Targets

#1929 A novel orthotopic mouse model in sarcomas. Silvia Garcia Monclus, Juan Huertas Martinez, Laura Lagares Tena, Santiago Rello Varona, Olga Almacellas Rabaiget, David Herrero Martin, Roser Lopez Alemany, Oscar Martinez Tirado. IDIBELL, Hospital de Llobregat, Spain.

Pediatric sarcomas are a heterogeneous group of bone and soft tissue malignancies affecting children and young adults. One of the most important prognostic factors of those diseases is the presence of metastasis at diagnosis. In that context, we have developed a novel orthotopic model which consists in injecting Ewing Sarcoma (ES) or Rhabdomyosarcoma (RMS) tumor cells in the gastrocnemius muscle of mice and extracting the tumor at a 10 mm x 13 mm volume. After surgery, animals maintain a complete functional extremity and can live until lung metastasis detection (about 60 days post-injection). Moreover, we have validated the suitability of the model with an Ewing Sarcoma EphA2-low expression cell line. EphA2 is a tyrosine kinase receptor that has been found to be highly expressed in a wide variety of tumors and correlated with malignant phenotype. In this study we report that EphA2 receptor is phosphorylated at S897 in a panel of ES cell lines, which is related to the oncogenic properties of the receptor. Stable silencing of EphA2 in two different ES cell lines resulted in a decrease

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in the clonogenic, proliferation, migration and invasion capacity in vitro. Moreover, we performed an experimental metastasis assay, injecting tumor cells through the tail vain of mice and observed a reduction in the lung metastasis incidence in EphA2 silenced cells. When we used this new orthotopic metastasis model to validate the impairment in the invasion capacity of EphA2 silenced cells and to use as a base in lung metastasis incidence, indication of the clonogenic role for EphA2 in ES. This novel orthotopic metastasis model is a valuable tool both for the study of spontaneous metastasis and also for evaluating therapeutic index in the onset of metastasis, which can also be applied to the study of other pediatric sarcomas.

#1930 Evaluating the effect of PARP inhibitors in combination with nicotinamide phosphoribosyltransferase inhibitors in Ewing sarcoma. Joshua T. Baumgart,1 Christine Heske,1 Mindy I. Davis,2 Kelli Wilson,2 Xiaoha Zhang,2 Baumann,1 Christine Heske,1 Mindy I. Davis,2 Kelli Wilson,2 Xiaoha Zhang,2 TUMOR BIOLOGY: Pediatric Cancer 1: Biomarkers, Preclinical Models, and New Targets

This new protocol were validated by single cell gene sequencing analysis and osteosarcoma patients using the CSV antibody 84-1. The CTCs captured using asEpCAM because it is only specific to tumor cells and has been demonstrated to have meaningful responses to PARP inhibitors when used as single agents. For this reason, studies combining PARP inhibitors with other agents to enhance their effects in Ewing sarcoma are of particular interest. PARP, an enzyme involved in DNA damage repair, relies on NAD to function. In tumor cells, NAD production occurs primarily through the salvage pathway, in which nicotinamide phosphoribosyltransferase (NAMPT) is the rate-limiting enzyme. Hence, the combination of PARP and NAMPT inhibition would be expected to enhance the activity of PARP inhibition through depletion of cellular NAD. The aim of this study was to assess the synergistic potential of PARP inhibition in combination with NAMPT inhibition in Ewing sarcoma cell lines and xenograft models. Synergistic drug combinations in Ewing sarcoma cells were initially identified using a high-throughput matrix drug screen. In vitro activity was further assessed using proliferation assays of multiple Ewing sarcoma cell lines treated with the PARP inhibitor niraparib and the NAMPT inhibitor GNE-618. In vitro measurement of PAR activity was performed to further understand the mechanism of action of the combination. For in vivo studies, female SCID-beige mice were orthotopically injected with Ewing sarcoma cells and randomized into treatment groups of vehicle, niraparib, GNE-618, or the combination. The initial screen revealed synergy between NAMPT inhibitors and PARP inhibitors using multiple combinations of different agents in each drug class. In vitro assays of the combination of niraparib with GNE-618 confirmed the results of the screen. Assays measuring PAR activity yielded results supporting the hypothesis that dual inhibition of NAMPT and PARP depletes PARP more than PARP inhibitors alone. In vivo studies with short term (5 days) dosing showed no activity of single agent niraparib. In contrast, the combination of niraparib and GNE-618 abolished clonogenicity, clonogenic, proliferation, and migration. However, mice treated with the combination underwent tumor regressions resulting in prolonged survival. When treatments were extended to 2 cycles (5 days each), the effect persisted for longer. Mice tolerated the agents well, with no signs of toxicity. Preclinical data suggest that PARP inhibition in combination with NAMPT inhibition may be a promising therapeutic strategy for Ewing sarcoma patients.

#1931 The first circulating tumor cell detection technique from frozen PBMCs. Heming Li,1 Ihar Singh Battal,2 Xueqing Xia,1 Frank J. Hsu,2 Neeta Somaiah,1 Keila Enitt Torres,1 Ruoyu Wang,3 Shulin Li1.1 National Cancer Institute, Bethesda, MD; 2National Center for Advancing Translational Sciences, Rockville, MD.

Previous research has shown that Ewing sarcoma cells are sensitive to PARP inhibition, but in vivo studies and early phase clinical trials have failed to demonstrate meaningful responses to PARP inhibitors when used as single agents. For this reason, studies combining PARP inhibitors with other agents to enhance their effects in Ewing sarcoma are of particular interest. PARP, an enzyme involved in DNA damage repair, relies on NAD to function. In tumor cells, NAD production occurs primarily through the salvage pathway, in which nicotinamide phosphoribosyltransferase (NAMPT) is the rate-limiting enzyme. Hence, the combination of PARP and NAMPT inhibition would be expected to enhance the activity of PARP inhibition through depletion of cellular NAD. The aim of this study was to assess the synergistic potential of PARP inhibition in combination with NAMPT inhibition in Ewing sarcoma cell lines and xenograft models. Synergistic drug combinations in Ewing sarcoma cells were initially identified using a high-throughput matrix drug screen. In vitro activity was further assessed using proliferation assays of multiple Ewing sarcoma cell lines treated with the PARP inhibitor niraparib and the NAMPT inhibitor GNE-618. In vitro measurement of PAR activity was performed to further understand the mechanism of action of the combination. For in vivo studies, female SCID-beige mice were orthotopically injected with Ewing sarcoma cells and randomized into treatment groups of vehicle, niraparib, GNE-618, or the combination. The initial screen revealed synergy between NAMPT inhibitors and PARP inhibitors using multiple combinations of different agents in each drug class. In vitro assays of the combination of niraparib with GNE-618 confirmed the results of the screen. Assays measuring PAR activity yielded results supporting the hypothesis that dual inhibition of NAMPT and PARP depletes PARP more than PARP inhibitors alone. In vivo studies with short term (5 days) dosing showed no activity of single agent niraparib. In contrast, the combination of niraparib and GNE-618 abolished clonogenicity, clonogenic, proliferation, and migration. However, mice treated with the combination underwent tumor regressions resulting in prolonged survival. When treatments were extended to 2 cycles (5 days each), the effect persisted for longer. Mice tolerated the agents well, with no signs of toxicity. Preclinical data suggest that PARP inhibition in combination with NAMPT inhibition may be a promising therapeutic strategy for Ewing sarcoma patients.


Paediatric glioblastomas (pGBM) are amongst the most common causes of cancer-related deaths in children, and are defined by highly recurrent mutations in H3 histones. Mutations affecting the chromatin remodeling protein ATRX have been reported in 30% of pGBM cases, and are strongly associated with the alternative lengthening of telomeres (ALT) phenotype, but their precise interaction with histone mutations and their role in tumorigenesis remain unclear. We collected sequence data from 262 published and 64 unpublished cases of pGBM and identified somatic ATRX mutations in 54/326 (17%) of cases. ATRX mutations are mainly loss of function mutations, with the majority of frameshift mutations (37/54, 68.5%) found upstream of the helicase domain resulting in truncation of the main functional domain of ATRX. Missense mutations (16/54, 29.6%) reside almost exclusively in the helicase domain (11/54, 20.4%), whereas nonsense mutations are a less common event (7/54, 13%) but present in both the helicase (4/7, 57.1%) and ATRX-defining helicase domains (3/7, 42.9%). ATRX mutations commonly co-occur with H3.3 G34 (16/54) and TP53 (42/54) mutations, and define a subgroup of patients with a longer overall survival (16 months median overall survival in mutant ATRX cases versus 11 months in wild-type ATRX cases, COXPH p = 0.079), though with a greater number of somatic mutations (MWU p = 0.023) and copy number alterations (MWU p = 0.0011) than wild-type cases. We screened a series of 21 primary patient-derived pGBM cell cultures for histone and ATRX mutation status in addition to ATRX protein expression and ALT, and subjected the panel to a high-throughput in vitro cell viability screen of >400 chemotherapeutics and small molecules. We identified a specific genetic dependency for ATRX mutation and sensitivity to distinct PARP inhibitor chemotypes, including olaparib and rucaparib (PARP catalytically trapping PARP inhibitors), and talazoparib (PARP trapper inhibitor). These data were validated using CRISPR-Cas9-engineered ATRX knockout, targeting either the ADD or helicase domain, in SF188 pGBM cells. Gene editing was confirmed by IonTorrent sequencing and Western blot. ATRX mutant clones were also more sensitive to drugs targeting DNA damage response pathways such as bleomycin and mitomycin. Gene expression analysis of ATRX mutant pGBM samples confirmed an intact homologous recombination pathway and overexpression of PARP1, suggesting an underlying mechanism distinct from that observed in BRCA-mutant breast and ovarian cancers. Ongoing work is aimed at unraveling the specific pathways involved, and evaluating the utility of PARP inhibition in orthotopic pGBM xenografts in vivo. These data suggest a synthetic lethality for PARP inhibitors in ATRX-deficient pGBM cells, and may represent a novel therapeutic strategy for these highly aggressive tumours in children.

#1933 Discovery of first-in-class small molecule CD99 inhibitors for targeted therapy of Ewing sarcoma. Haydar Celik,1 Marika Scinder,2 Bess Flaschner,2 Elif Gelmez,2 Nesilhan Kayrakoglu,2 David V. Allegakoen,1 Jeff R. Petro,1 Erin J. Conn,1 Sarah Hour,1 Jenny Han,1 Lalehan Oktay,1 Purushottam B. Tewari,1 Muthu Hayran,2 Maria Cristina Manara,2 Jeffrey A. Toretsky,2 Khatia Scottlandi,1 Aykut Uren,1 Georgeroun University Medical Center, Washington, DC; 2Rizzoli Orthopaedic Institute, Bologna, Italy; 3Hacettepe University, Ankara, Turkey.

Ewing sarcoma (ES) is an aggressive bone and soft tissue malignancy that affects predominantly children and adolescents with a high propensity to metastasize and poor prognosis. CD99 is a transmembrane cell surface protein that is highly expressed on ES cells, and routinely used as a marker for histological diagnosis of ES. We screened small molecule libraries for their binding to recombinant CD99 protein and subsequent selective inhibition of ES cell growth. We identified two structurally similar FDA-approved nucleoside analogues, clofarabine and cladribine that selectively inhibited the growth of ES cells in a panel of 14 ES vs. 28 non-ES cell lines. A significant negative correlation was found in human cell lines between CD99 expression and IC50 values for clofarabine and cladribine. Both drugs inhibited CD99 dimerization and its interaction with downstream signaling components cyclophilin A and PAK-RIs, as well as reduced ROCK2 protein expression and migration in ES cells. A membrane-
impermeable analog of clofarabine showed similar cytotoxicity in ES cells, suggest- ing that it can function through inhibiting CD99 alone without any effect on DNA metabolism. Clofarabine and cladribine led to a significant increase in hypodiploid DNA content of ES cells, which was diminished by suppression of CD99 expression. Both drugs drastically inhibited anchorage-independent growth. The combination of the two drugs was synergistic.

Finally, the screening of a set of chemotherapy drugs revealed a synergy for the combination of anti-CD99 drugs and dasatinib in ES cells, which may translate into increased survival and reduced toxicity. Overall, our findings suggest that clofarabine is a good candidate for early phase clinical trials in children with ES.

**#1934 Retinoic acid and DFMO induce differentiation and inhibit tumor formation in neuroblastoma.**

Austen Vodayoff,1 Ping Zhao,2 Ablin U. Nagan-lapally,2 Jeff Bond,2 Giselle L. Sholler,2 Michigan State University, Grand Rapid- ids, MI; Helen DeVos Children’s Hospital, Grand Rapids, MI.

Background: A key component of high risk neuroblastoma (NB) therapy in-volves cis-retinoic acid (RA) for differentiation of minimal residual disease. DFMO induces differentiation and inhibition of tumor formation through the targeting of cancer stem cell (CSC) pathways via reversal of the Lin28/Let7 axis. Preventative DFMO therapy is currently in a phase II clinical trial at the end of therapy and in a Pilot study in combination with RA and ch14:18 antibody. We hypothesize that the combination of cis-RA and DFMO will induce greater differentiation, inhibition of tumor formation, and reduction of cell prolifera-tion of NB. Methods: NB cell lines MSN-BCR2, B2C and H926 were inoculated in 96 well plates for 24 and 48 hours with low doses of DFMO (2.5 and 5 mM), RA (5 and 10 µM), and the four combinations of dosages. A Calcein AM Cell Viability Assay and BrdU Cell Proliferation Assay Kit were used to deter-mine cell viability and cell proliferation, respectively. Western blot analysis was used to measure protein levels of CSC and differentiation markers. A neurosphere assay was used to assess inhibition of CSCs and tumor formation within wells. Cells were plated 2 cells/well in 96 well plates, drugged with single agents, combination, or DMSO and the percentage of wells per plate that formed neu-roospheres was determined after 1 and 2 weeks. IncuCyte ZOOM Live-Cell Im-aging system was used for kinetic monitoring of neurite length to assess differ-entiation of NB cells. Results: Low dose RA and DFMO combination treatment (2.5-5mM DFMO and 5-10 mM RA) resulted in decreased cell viability as demon-strated through calcein AM. DFMO and RA combination treatments reduced cell viability by 61-71%, 75-78%, and 83-91%, in B2C, H926, and MSN cells, respectively. BrdU incorporation demonstrated a reduction in cell prolif-eration at 48 hours of was 69-70%, 60.2-64.5%, 62.7-71.1% in B2C, H926, and MSN, respectively. Western blot analysis showed that DFMO, RA, and their combination reduced the CSC and increased the differentiation markers at 48 hours compared to control. The combination treatment also decreased tumor formation; the relative reduction in neurosphere formation at 2 weeks was 84.2% with 2.5 mM DFMO, 48% with 5 mM RA, and 73.2% with combination treat-ment. Lastly, differentiation was shown by neurite length increased by a factor of 1.4-1.6 and 5, in MSN and B2C cells, respectively with combination treatment. Conclusion: This study indicates that the combination retinoic acid and DFMO effectively cause a decrease in cell viability with a reduction in cell proliferation. Further, our results in differentiating NB cell lines suggest that targeting CSC pathways and inhibition of tumor formation. Preventative DFMO ther-apy has been initiated with RA in a pilot study for the treatment of high-risk neuroblastoma patients.

**#1935 Molecular characterization of orthotopic patient-derived xeno-graft models of pediatric brain tumors.**

Sebastian Brabetz,1 Susanne N. Gröb-ner,1 Hurrie Seker-Cin,1 Florian Selt,1 Till Müldé,2 David T. Jones,1 Madison T. Wise,3 Jessica M. Rusert,4 Kyle Pedro,3 Olaf Witt,2 Sarah E. Leary,1 Xiao-Nan Li,2 Robert J. Wechsler-Reya,4 James M. Olson,2 Stefan M. Pfister,4 Marcel Kool,1 German Cancer Research Center (DKFZ) and German Cancer Consortium (DKTK), Heidelberg, Germany; 2German Cancer Research Center (DKFZ), German Cancer Consortium (DKTK), Center for Individualized Pediatric Oncology (ZIPO) and Pediatric Brain Tumors, Department of Pediatric Oncology, University Hospital and National Center for Tumor Diseases (NCT), Heidelberg, Germany; 3Fred Hutchinson Cancer Research Center and Seattle Children’s Hospital, Seattle, WA; 4Sanford Burnham Prebys Medical Discovery Institute, Heidelberg, CA; 8Baylor College of Medicine, Houston, TX.

[Introduction: Solid tumors of the nervous system are the most common childhood cancers after leukemias. Even though we might be able to cure more and more patients, survivors still suffer long-term from the intensive treatments. Therefore, new treatment strategies are urgently needed. Orthotopic patient-de-renexograft (PDX) models are an excellent platform for biomarker and preclinical drug development. However, the rarity of pediatric brain tumors and the mul-titude of different sub entities hinder the generation of large collection of PDX models of specific entities within single institutions. In order to generate an overview about existing PDX models in the community, we started collecting established PDX models from various centers all over the world and performed extensive molecular characterization to precisely determine the distinct molecular subgroup and con-current oncogenic drivers. Material and Methods: PDX models were established and main-tained by dissociating tumor material into a single cell suspension and then ortho-topically injecting it into the brain of immunodeficient animals. All PDX models and matching primary tumors (if available) have been analyzed by whole-exome and low-coverage whole-genome sequencing, as well as DNA methylation and gene expression profiling using the the German Cancer Research Center (DKFZ). Results and Discussion: Thus far, we have collected and characterized 70 established PDX mod-els from 6 ATRTs, 8 ependymomas, 16 high-grade gliomas, 38 medulloblastomas, and 2 CNS-PNETs. PDX models always retain their molecular subtype and in the vast majority of cases also the mutations and copy number alterations when com-pared to their primary tumors. Only in rare cases do we observe additional aberra-tions, which most likely represent outgrowths of subclones from the primary tumor. Analysis of our entire cohort identified an overrepresentation of the most aggressive tumor subtypes, but also subtypes which have not been available for preclinical testing before due to lack of genetically engineered mouse models or suitable cell lines, such as Group 4 medulloblastoma. Based on our current analysis, the PDX models within the community are not yet covering the entire heterogeneity within brain tumors. As a next step, we aim to make these models and data accessible in a user-friendly manner so that the community can use them for preclinical research. Conclusion: PDX models of pediatric brain tumors are very rare. Our molecular characterization allows researchers all over the world to find the right models for their specific scientific question. Therefore, this work will provide an unprecedented resource to study tumor biology and pave the way for improving treatment strategies for children with malignant brain tumors.]

**#1936 Epigenetic challenges in derivation of the first cell-based model of Beckwith-Wiedemann syndrome.**

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Beckwith-Wiedemann Syndrome (BWS) is a cancer predisposition syndrome that affects at least 1 in 10,500 children. Up to 25% of children with BWS develop tumors, primarily Wilms tumor and hepatoblastoma. BWS is due to genetic or epigenetic changes that affect imprinted loci on chromosome 11 and these same changes are also found in other types of cancer. There are no cell-based models of BWS and most mouse models do not recapitulate the tumor phenotype. To under-stand more about the mechanisms leading to tumor formation in BWS, we devel-oped the first human cell-based model of BWS. Human induced pluripotent stem cell (iPSC) models are commonly used to study disease mechanisms in tissue types that are not normally accessible for study. In the case of BWS, we plan to use such models to study how the genetic and epigenetic changes in BWS lead to tumor formation in hepatic and renal cells. Using skin fibroblasts from four BWS patients, we derived the first iPSC models of BWS. Prior to iPSC derivation, we characterized different tissues available from these four patients, and demonstrated genetic mosaic-ism in different tissue types, including blood, skin, and pancreas. During the derivation process, we demonstrated that both normal and BWS iPSC lines could be derived from the same patient fibroblast sample and the number of clones of each type from each sample approximated the initial level of mosaicism in the original sample. For each patient, these lines are isogenic except for the BWS critical region. The BWS and isogenic normal iPSCs were characterized for pluripotency markers and demonstrated to have normal karyotypes. Following this analysis, BWS and isogenic normal lines were characterized extensively for DNA methylation at spe-cific imprinted loci in both early passage and extended culture. Methylation analysis was performed by both pyrosequencing and COBRA assays. Methylation was main-tained at some imprinted loci but not at others in extended culture. Importantly, relatively stable levels were observed at the BWS critical region (H19/IGF2 and KvDMR), regardless of methods of reprogramming, indi-cating a relatively stable state of DNA methylation in this region. Additionally, nor-mal methylation was seen at the SNRPN locus. In contrast, another imprinted locus, IGF/MEG3, displayed abnormal hypermethylation in iPSCs. These data indicate that reprogramming and extended culture of iPSCs can affect stability of DNA methyl-ation at certain imprinted loci. Therefore caution should be used in interpreting studies using iPSCs as these aberrant methylation states at imprinted loci can affect the downstream functionality of iPSC models. BWS iPSCs and isogenic controls with normal methylation will be used for further study of the mechanism of tumor formation in BWS.
TUMOR BIOLOGY: Pediatric Cancer 1: Biomarkers, Preclinical Models, and New Targets

#1937 Evolution of neuroblastoma patient-derived orthotopic xenografts through space and time. Noémie Braeckeveldt,1 Susanne Fransson,2 Kristoffer von Stedingk,1 Ingrid Ora,3 Rosa Noguera,4 Tommy Martinsson,2 David Gisselsson Nord,5 Sven Påhlman,1 Daniel Bexell1. 1Translation Research Centre, Lund University, Lund, Sweden; 2Department of Clinical Genetics, Sahlgrenska University Hospital, Gothenburg, Sweden; 3Department of Pediatrics, Lund University, Lund, Sweden; 4Department of Pathology, Medical School, University of Valencia, Valenciac Spain; 5Department of Clinical Genetics, Lund University, Lund, Sweden.

Background: Recently, we established orthotopic neuroblastoma patient-derived xenografts (PDXs) which maintain the phenotypic, genomic, and stromal hallmarks of patient tumors. Here we examined how PDXs evolve following years of in vivo growth. Materials and Methods: We established up to eight in vivo generations of neuroblastoma orthotopic PDXs through serial passing in NSG mice. RNA sequencing, exome sequencing and SNP array analysis were used to analyze patient tumors and PDXs from different in vivo generations. Results: Using SNP analysis, we found mostly a remarkable genomic stability at chromosomal level between patient tumors, early and late PDX generations. RNA-seq revealed that patient tumors expressed higher levels of genes involved in immune responses and ECM metabolism compared to PDXs. Different PDX samples clustered correctly into their respective tumor type. PDXs from all early generations did not separate from PDXs from late generations. Thus, gene expression levels are surprisingly often quite stable despite years of in vivo growth. To shed light on neuroblastoma intratumor heterogeneity, we implanted 10 different tumor fragments from a single patient tumor into mice. We classified the 10 mice into three groups based on the time periods required for tumor growth. RNA-seq showed that each of these groups had a distinct gene expression profile and pathways involved in neuroblastoma progression have been identified. Conclusions: Neuroblastoma orthotopic PDXs are often very stable at chromosomal and gene expression levels despite years of in vivo growth. We utilized multiple PDXs to show functional intratumor heterogeneity coupled to distinct gene expression profile.

#1938 Exportin-1 (XPO1) is a novel therapeutic biomarker for patients with neuroblastoma. Basia Galinski,1 Marcus Luxemburg,1 Michelle Ewart,2 Yosef Landesman,3 Daniel Weiser1.1 Albert Einstein College of Medicine, Bronx, NY; 2Montefiore Medical Center, Bronx, NY; 3Karyopharm Therapeutics, Newton, MA.

Background: Half of patients with high-risk neuroblastoma succumb to disease, yet these patients with inferior outcome cannot be identified at diagnosis despite contemporary risk stratification that integrates MYCN copy number status, tumor histology, and patient age. We have shown that overexpression of Exportin-1 (XPO1) (1XPO1) is associated with poor survival in neuroblastoma, affirming what has been identified across a range of malignancies. Selinexor (KPT-330, Karyopharm Therapeutics), an XPO1 inhibitor in early phase clinical trials, inhibits the nuclear to cytoplasmic translocation of tumor suppressor and growth regulatory proteins implicated in oncogenesis. We hypothesized that protein fragments from a single patient tumor into mice. We classified the 10 mice into three groups based on the time periods required for tumor growth. RNA-seq showed that each of these groups had a distinct gene expression profile and pathways involved in neuroblastoma progression have been identified. Conclusions: Neuroblastoma orthotopic PDXs are often very stable at chromosomal and gene expression levels despite years of in vivo growth. We utilized multiple PDXs to show functional intratumor heterogeneity coupled to distinct gene expression profile.

#1939 Loss of MST/Hippo signaling promotes tumorigenesis in a genetically engineered mouse model of fusion-positive alveolar rhabdomyosarcoma. Kristianne Orielian, Lisa E. Crose, Rex Bentley, Nina Kuprasertkul, David G. Kirsch, Corrine Linardic. Duke University, Durham, NC.

While improvement in survival for pediatric cancer patients over the last 40 years has been encouraging, certain cancer types evade cure. One such example is fusion-positive alveolar rhabdomyosarcoma (aRMS), a pediatric soft tissue sarcoma of mesenchymal origin with skeletal muscle features and a 5-year survival rate of <50%. A hallmark of this aggressive malignancy is the t(12;13) translocation fusion protein PAX3-FKHR which drives hyperactive Hippo signaling and inhibits MST1/2/3. We hypothesized that ablating MST/Hippo signaling in an existing genetically engineered mouse model (GEMM) of aRMS would accelerate tumorigenesis and provide insight into the role of this pathway in aRMS. To assess the role of MST/Hippo signaling in aRMS, MST1/2-floxed (SkiCre;Skf4/+) mice were crossed with an established MST1/2 aRMS GEMM driven by conditional expression of Pax3-Foxo1 from the endogenous Pax3 locus and conditional loss of Cdkn2a in Myf6-expressing cells. Statistical analysis revealed that compared to MSTWT aRMS control animals, MSTSmice have significantly accelerated tumorigenesis (median survival 112 vs. 224 days, p < 0.0001) and increased tumor penetrance (76% vs. 27%). MSTsmice developed tumors disproportionately in the head and neck as compared to control, and incurred multiple tumors per animal. Tumors were analyzed via immunohistochemistry for MyoD and myogenin, markers of myogenic lineage and of either positive or negative aRMS phenotypes. MST-derived tumors were positive for positive aRMS markers. MST-derived cell lines were used for in vitro cell-based assays and molecular interrogation. We have identified the MST/Hippo signaling axis as an important tumor suppressor mechanism in aRMS. The rapid onset and increased penetrance of tumorigenesis in this GEMM provides a powerful tool for interrogating RMS biology and screening novel therapeutics.

#1940 Prenatal stress increases malignancy of neuroblastoma tumors in TH-MYCN animal model. Sung Hyeok Hong, Larissa Wietlisbach, Susana Galii, Akanaksha Mahajan, Shuya Zhu, Jason Tilan, Yichien Lee, Olga Rodriguez, Chris Albanese, Joanna Kikutinska. Georgetown University Medical Center, Washington, DC.

Neuroblastoma (NB) is a pediatric malignancy arising due to defects in sympathetic neuron differentiation. NB is a heterogeneous disease, with phenotypes ranging from spontaneously regressing to highly aggressive, incurable tumors. This clinical variability cannot be explained solely by genetic aberrations. Even in families with hereditary NB the penetrance of the disease is incomplete and the same genetic mutation often results in tumors with phenotypes varying from differentiating ganglioneuromas to undifferentiated, highly aggressive NBs. Thus, other, perhaps non-genetic factors can contribute to the development of tumors and modify their phenotype. Strikingly, the two factors promoting de-differentiation of NB cells and their malignant phenotype, hypoxia and glucocorticoids, are elevated in the fetus during maternal stress, suggesting a role for prenatal stress in NB tumorigenesis. Previously, using TH-MYCN mice as a model of aggressive NB, we have shown that an increase in maternal corticosterone levels during pregnancy attenuated by inserting slow release pellets resulted in increased tumor frequency in TH-MYCN offspring. The goal of the current study was to determine the effect of prenatal stress on NB metastasis. To this end, pregnant mice carrying TH-MYCN hemizygous offspring were subjected to chronic stress at embryonic days 10-17, the time of sympathetic neuroblast proliferation and differentiation. Two established stress paradigms were used - chronic unpredictable stress, in which mice were subjected daily to various stressors, and chronic cold stress comprising of daily 30 min exposure to cold. The phenotypes of the disease and its dissemination were compared between offspring of control and stressed mothers. The offspring from both prenatal stress groups presented with more malignant disease, as manifested by the presence of advanced lung metastases disseminating from small primary tumors (<700 mm3). This phenotype was associated with increased mortality in prenatally-stressed TH-MYCN offspring (p < 0.01). In contrast, no advanced lung metastases and no disease-related deaths were observed in TH-MYCN offspring of control mothers despite the presence of large primary tumors (>1,000 mm3). Although not common, lung metastases occur preferentially in NB patients with MYCN amplification and are associated with significantly worse prognosis, as compared to patients with metastatic disease, but no pulmonary involvement (14 vs 43% 3-year event-free survival, respectively). Thus, the profound pulmonary dissemination observed in prenatally-stressed TH-MYCN mice mimics one of the most malignant NB phenotypes observed in human disease. Altogether, our data implicate maternal stress during pregnancy as a potential environmental factor modifying the effects of genetic aberrations and promoting malignant phenotype of NB.

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#1941 Cell surface vimentin is a novel marker for CTC detection in neuroblastoma. Izhara S. Batt#1, Heming Li#, Giselle Saulnier Sholler, Shulin Li#. 1UT MD Anderson Cancer Center, Houston, TX; 2Helen DeVos Children’s Hospital, Grand Rapids, MI.

Among children in the United States, brain cancers now account for most neurologic deaths. Neuroblastoma (NB) and sarcoma (SR) are the two most common childhood cancers in the United States. Despite significant progress yielding increased 5-year survival, 1250 deaths are expected this year from childhood cancers and cancer incidence has been steadily increasing. One of the key contributors to cancer mortality is tumor metastasis. It is generally believed that CTCs are shed into the circulation from primary tumors and contain unique information enabling their staging and phenotypic differences. However, detection of CTCs from NB is difficult as there is no direct method. Our lab has developed a monoclonal antibody targeting vimentin on the cells’ surface. Cell surface vimentin (CSV) is only observed in tumor cells; it remains intracellular in normal cells. We have published data demonstrating the superior specificity and sensitivity of our approach in breast, colon, and prostate cancer. Here, we report direct CTC detection using CSV by our mAb. As part of our collaborative effort analyzing NB blood samples from a multicenter Phase II trial, we present novel discoveries regarding treatment of NB with difluoromethylornithine (DFMO) and its effects on CTC release into the circulation in patients under remission. Among our observations, approximately 1/3 patients have low or no CTCs while 1/4 with high numbers of CTCs are responding to DFMO treatment. Our findings show CTC numbers falling with DFMO treatment. DFMO showed a statistically significant decline in CTCs after the beginning of therapy. Ongoing work including whole genome analysis can possibly reveal new insight into CTCs’ entering the circulation. These data demonstrate that our CTC capture using CSV is a novel and unique approach for detection in NB.

#1942 The nicotinamide phosphoribosyltransferase (NAMPT) inhibitor, OT-82, exhibits in vitro and in vivo efficacy against patient-derived xenograft models of high-risk acute lymphoblastic leukemia. Kathryn Evans,1 Tara Pritchard,1 Michelle J. Henderson,1 Kaartje Somers,1 Mawar Karasa,1 Leanna Cheung,1 Raymond Yung,1 Stephen W. Erickson,2 Lioubov Korotchkina,3 Olga Chernova,3 Andrei Gudkov,3 Malcolm A. Smith,2 Richard B. Lock,3 Children’s Cancer Institute, Sydney, Australia; 4National Cancer Institute, MD; 5Oncoartis, Inc., Buffalo, NY; 6Roswell Park Cancer Institute, Buffalo, NY.

Cancer cells are highly dependent on nicotinamide phosphoribosyltransferase (NAMPT) for the biosynthesis of nicotinamide adenine dinucleotide (NAD). Besides its role in energy metabolism, NAMPT influences the activity of NAD-dependent enzymes, including poly (ADP-ribose) polymerase-1 (PARP-1) and sirtuins, and thereby regulates cellular survival and stress response. Disruption of NAD synthesis through NAMPT inhibition represents a potential therapeutic strategy for treating cancer. The aim of this study was to evaluate the efficacy of the novel NAMPT inhibitor OT-82, initially isolated for its selective toxicity against a panel of adult leukemia cell lines, in a diverse panel of leukemia cell lines in vitro and pediatric acute lymphoblastic leukemia (ALL) patient-derived xenografts (PDxX) in vivo, and to identify potential biomarkers predictive of OT-82 response. OT-82 demonstrated low nanomolar IC50 values (0.9 – 3.4 nm) in ALL and 3 acute myeloid leukemia cell lines as determined by resazurin reduction assays. In vivo efficacy of OT-82 (40 mg/kg x 3 days x 3 weeks, p.o.) was evaluated as a single agent against pediatric ALL PDxXs, including B-cell precursor ALL (n = 3), Philadelphia chromosome (Ph) positive ALL (n = 2), Ph-like ALL (n = 2), T-cell ALL (n = 3), and early T-cell precursor ALL (n = 3) in immune deficient (NSG) mice. Response to treatment was assessed by time to event or stringent objective response criteria modeled after the clinical setting. OT-82 was well tolerated, significantly increased event-free survival (EFS) relative to control mice in 11/13 ALL PDxXs, and elicited objective responses in 11/13 (85%) PDxXs [3 Partial Responses, 4 Complete Responses (CRs)] and 4 Maintained CRs (MCRs). Analysis of basal protein expression revealed elevated levels of poly (ADP-ribosylated) (PARylated) PARP1 in 4/5 responders versus 0/3 non-responders. In vitro studies examining various chemotherapeutic agents used for childhood leukemia showed synergy between cytarine (AraC) and OT-82 in an ALL cell line. In an OT-82 sensitive Mixed Lineage Leukemia (MLL) PDxX treatment with AraC (25 mg/kg x 5 days x 2 weeks, i.p.) and OT-82 (40 mg/kg x 3 days x 2 weeks, p.o.) significantly increased EFS compared to OT-82 (P<0.0001) or AraC (P<0.0001) alone. Moreover, the OT-82/ AraC combination elicited an MCR compared to OT-82 and AraC (Progressive Disease) alone. The results herein demonstrate significant activity of OT-82 against leukemia cell lines and a range of pediatric ALL subtypes in vitro, and the potential for PARylated PARP-1 expression as a biomarker for predicting OT-82 response. Moreover, the enhanced activity of OT-82 in combination with AraC over single agent therapy further suggests NAMPT inhibition as an attractive strategy for treating high-risk pediatric ALL. Supported by U01CA199900 from the NCI.

#1943 High-throughput chemical screening identifies Focal Adhesion Kinase and Aurora Kinase B inhibition as a synergistic treatment combination in Ewing sarcoma. Brian Crompton#, Sarah Wang#, Elizabeth Hawang, Rajarshi Gula#, Matthew Boxer,3 Crystal McKnight,2 Min Shen,2 Nicola Melong,3 Channey Veinotte,2 Amy Conway,1 Jason Berman,3 Matthew Hall,2 Mindy Davis,2 Kimberly Stegmaier1, Dana-Farber/Boston Children’s Cancer and Blood Disorder’s Center, Boston, MA; 2National Center for Advancing Translational Sciences, Bethesda, MD; 3D’Alton House University, Halifax, Nova Scotia, Canada.

Ewing sarcoma is the second most common bone malignancy of childhood. Current treatment employs chemotherapy, surgery, and radiation. Although this approach cures approximately 70% of patients with localized disease, treatments are largely ineffective for patients with metastases or relapse. Furthermore, these treatments are associated with an alarming rate of long-term toxicities. New treatment combinations are necessary to improve cures and lower toxicities for these patients. We recently found that Ewing sarcoma is dependent on focal adhesion kinase (FAK) for cell viability and tumor proliferation. In order to identify candidate treatment combinations for Ewing sarcoma, we performed a screen of 1912 compounds to identify those with synergistic anti-angiogenic activity when combined with FAK inhibition. The A673 Ewing cell line was treated with PF-562271, a FAK-specific inhibitor, in combination with compounds from the Mechanism Interrogation PlatE (MIE) 4.0 library. Cell viability was measured after 48 hours of treatment. Multiple computational metrics were used to identify targets with anti-angiogenic activity. More importantly, combinations for synergistic impairment of cell viability. Multiple Aurora kinase inhibitors scored as synergistic with FAK inhibition in this screen. Aurora kinases are important in the regulation of mitosis and are highly expressed in Ewing sarcoma tumors and cell lines. We found that Aurora kinase B inhibitors were synergistic across a larger range of concentrations than Aurora kinase A inhibitors when combined with FAK inhibition in multiple Ewing cell lines. We found that AZD-1152, an Aurora kinase B-selective inhibitor, and PF-562271 when used in combination induced apoptosis in Ewing cells at concentrations that had minimal effect on cell survival when either drug was used alone. We also found that the combination significantly impaired tumor proliferation in zebrafish xenograft models of Ewing sarcoma and prolonged survival in murine xenografts compared to either single-agent treatment alone. Interestingly, treatment with AZD-1152 alone also significantly impaired tumor proliferation and prolonged survival compared to vehicle treatment in a mouse xenograft model of Ewing sarcoma. Our data demonstrate that FAK and Aurora kinase B inhibition synergistically impair Ewing sarcoma cell viability in vitro and significantly inhibit tumor proliferation in vivo. With multiple FAK and Aurora kinase inhibitors in early phase trials for adult malignancies, these results have the potential to be translated into clinical trials for patients with Ewing sarcoma. Previous studies have also suggested a dependency of Ewing sarcoma on Aurora kinase activity; our data further supports a role for Aurora kinase B inhibitors as therapeutic candidates in this disease.

#1944 Tazemetostat displays synergistic antiproliferative activity with backbone therapies in preclinical models of AT/RT and MRT. Christine R. B. Soren#, Richard Sev, Scott Riebs, Alejandra Raimondi, Epizyme, Inc, Cambridge, MA.

Malignant Rhabdoid Tumors (MRT) and Atypical Teratoid Rhabdoid Tumors (AT/RT) are typically pediatric cancers which are rare and aggressive with extremely high unmet medical need. At a molecular level MRT and AT/RT tumors are almost universally characterized by loss of the INI1 protein (also known as SNF5 or SMARCB1), a component of the Switch Sucrose Non-fermentable (SWI/SNF) multisubunit chromatin modifying complex. In many cell types, the SWI/SNF complex and the PRC2 complex have an antagonistic relationship in the regulation of tumor suppressor genes, cell cycle checkpoints, hedgehog and myc pathway genes, among others [Wilson et al, Cancer Cell. 2010 Oct 19;18(4):316-28.]. This explicated the possibility of a novel treatment for patients with INI1-negative MRT and AT/RT tumors, which are largely ineffective for patients with metastases or relapse. Further treatment employs chemotherapy, surgery, and radiation. Although this approach cures approximately 70% of patients with localized disease, treatments are largely ineffective for patients with metastases or relapse. Furthermore, these treatments are associated with an alarming rate of long-term toxicities. New treatment combinations are necessary to improve cures and lower toxicities for these patients. We recently found that Ewing sarcoma is dependent on focal adhesion kinase (FAK) for cell viability and tumor proliferation. In order to identify candidate treatment combinations for Ewing sarcoma, we performed a screen of 1912 compounds to identify those with synergistic anti-angiogenic activity when combined with FAK inhibition. The A673 Ewing cell line was treated with PF-562271, a FAK-specific inhibitor, in combination with compounds from the Mechanism Interrogation PlatE (MIE) 4.0 library. Cell viability was measured after 48 hours of treatment. Multiple computational metrics were used to identify targets with anti-angiogenic activity. More importantly, combinations for synergistic impairment of cell viability. Multiple Aurora kinase inhibitors scored as synergistic with FAK inhibition in this screen. Aurora kinases are important in the regulation of mitosis and are highly expressed in Ewing sarcoma tumors and cell lines. We found that Aurora kinase B inhibitors were synergistic across a larger range of concentrations than Aurora kinase A inhibitors when combined with FAK inhibition in multiple Ewing cell lines. We found that AZD-1152, an Aurora kinase B-selective inhibitor, and PF-562271 when used in combination induced apoptosis in Ewing cells at concentrations that had minimal effect on cell survival when either drug was used alone. We also found that the combination significantly impaired tumor proliferation in zebrafish xenograft models of Ewing sarcoma and prolonged survival in murine xenografts compared to either single-agent treatment alone. Interestingly, treatment with AZD-1152 alone also significantly impaired tumor proliferation and prolonged survival compared to vehicle treatment in a mouse xenograft model of Ewing sarcoma. Our data demonstrate that FAK and Aurora kinase B inhibition synergistically impair Ewing sarcoma cell viability in vitro and significantly inhibit tumor proliferation in vivo. With multiple FAK and Aurora kinase inhibitors in early phase trials for adult malignancies, these results have the potential to be translated into clinical trials for patients with Ewing sarcoma. Previous studies have also suggested a dependency of Ewing sarcoma on Aurora kinase activity; our data further supports a role for Aurora kinase B inhibitors as therapeutic candidates in this disease.
pies for MRT and AT/RT are comprised of cytotoxic chemotherapy which may or may not be administered in the context of stem cell transplant, in addition to surgical resection and radiation therapy. In support of combination clinical scenarios including EZH2 inhibition in this setting, we sought to explore the antiproliferative effects of combining tazemetostatin with current small molecule treatment. Transcriptome models of tumor response to EZH2 inhibition was observed when tazemetostatin was combined with individual components of chemotherapeutic regimens and targeted therapies such as vincristine, doxorubicin, alisertib and HDAC inhibitors. Further, we investigated the effects of ionizing radiation together with tazemetostatin treatment. Application of X-ray irradiation concomitantly or after tazemetostatin treatment, induced robust antiproliferative response and reduction in colony formation compared to individual cell lines tested. Taken together these results suggest that pharmacological inhibition of EZH2 enhances the activity of backbone therapy and may have an advantage over monotherapy in INI1-negative cancers supporting the therapeutic potential of combination regimens that include tazemetostatin in these tumors.

#1945 PPM1D/Wip1, promising new target in childhood cancers neuroblastoma and medulloblastoma. Jelena Milosevic,1 Nina Essler,1 Diana Treis,1 Malin Wickström,1 Susanne Fransson,2 Baldur Sveinbjornsson,3 Ninib Baryawno,1 Subzini Kosala,1 Chandrasekhar Kanduri,2 Kazuya Su Saguchi,3 Tommy Martinsson,3 John Inge Johnsén,3 Per Kogner4 Karolinska Institutet, Stockholm, Sweden; 2University of Gothenburg, Gothenburg, Sweden; 3University of Tromsø, Tromsø, Norway; 4Hokkaido University, Sapporo, Japan.

Background: The most common cytogenetic lesions in the embryonal neural tumors are neuroblastoma (MB) and neuroblastoma (NB) affect chromosome 17, with 17q+ or isochromosome 17q, in approximately one-third of MB with these aberrations being a significant indicator of poor clinical outcome. Similarly, in NB gain of 17q is the most powerful genetic predictor of adverse clinical outcome. 17q+ correlates with poor survival in our population-based material where we found aberrations of chromosome 17 in 85% of primary neuroblastomas, specifically, gain of PPM1D/Wip1 at 17q23. Wip1 is a serine/threonine phosphatase encoded by the gene PPM1D, described as a gatekeeper in the Mdm2-p53 regulatory loop involved in genetic stability, inflammation and a potential oncogene contributing to carcinogenesis. Methods: Comparative genomic hybridization (CGH), immunostaining, mRNA arrays, qPCR, exome- and RNA-sequencing was used to examine PPM1D/ Wip1 in neuroblastoma and medulloblastoma. Genetic and pharmacological inhibition was used to analyze the function of Wip1 in preclinical neuroblastoma and medulloblastoma models. Results: CGH-array analysis detected PPM1D/Wip1 extra copies in all tumors and cell lines containing 17q-gain. Expression arrays and immunostaining showed high expression of Wip1 in neuroblastoma corresponding to poor survival. RNA sequencing confirmed PPM1D-gain and revealed truncated isoforms with oncogenic potential. Exome-seq detected a mutation leading to constitutive PPM1D/Wip1 activation in an aggressive metastatic infant neuroblastoma. Wip1 knockdown experiments showed significant decrease of cell viability, proliferation and colony formation as well as substantial increase of DNA-damage response in neuroblastoma and medulloblastoma cells. Tumor neuroblastoma xenograft development was significantly delayed showing median tumor development (0.10 mL) to be more than doubled (median 15 days, vs. 33 days, p<0.001) after Wip1 downregulation compared to scrambled controls. A novel Wip1 inhibitor, highly potent in cytotoxic/cytostatic effect in neuroblastoma and medulloblastoma cell lines. Furthermore, this Wip1 inhibitor significantly inhibited growth of established human neuroblastomas and medulloblastomas in nude mice after treatment (P<0.001). Conclusions: Our results show that PPM1D/Wip1 is oncogenic in neuroblastoma and medulloblastoma development. We propose three different ways on how PPM1D/Wip1 is activated: due to chromosomal gain, alternative RNA-isosforms and/or DNA-mutation. PPM1D/Wip1 provides a novel therapeutic target in neuroblastoma and medulloblastoma.

#1946 Synergistic effects of the XPO1 inhibitor selinexor with proteasome inhibitors in pediatric high-grade glioma and diffuse intrinsic pontine glioma. John DeSisto,1 Patrick Flannery,1 Trinayan Kashyap,2 Rakheb Lemma, Shelby Mestnick,3 Andrew King,4 Rajeev Vihbhakara,5 Yosef Landesman,6 Adam Green5. University of Colorado Anschutz Medical Campus, Aurora, CO; 5Karyopharm Therapeutics Inc., Newton, MA; 3Rocky Vista University College of Osteopathic Medicine, Parker, CO; 4Memorial Sloan Kettering Cancer Center, New York, NY.

Background: Pediatric high-grade gliomas (HGG) and diffuse intrinsic pontine gliomas (DIPG) account for the majority of pediatric brain tumor deaths and respond poorly to chemotherapy. Selinexor, a nuclear export inhibitor, is effective against HGG and DIPG in in vitro and in vivo models, but resistance to treatment develops. We previously identified the NF-kB pathway as a likely mediator of selinexor’s activity in these tumors. NF-kB transcriptional activity is regulated by an inhibitor, IKR-α, whose levels are in turn regulated by ubiquitination and proteasomal degradation. IKR-α is a client of exportin-1 (XPO1); its nuclear levels are increased by selinexor treatment, leading to inhibition of NF-kB. We subsequently identified proteasome inhibitors as potentially synergistic with selinexor in HGG and DIPG through a screen of all FDA-approved chemotherapy agents. Proteasome inhibition has also been shown to synergize with selinexor treatment in multiple myeloma and osteosarcoma. Methods: We treated HGG cell lines (BT245 and GBM1) and DIPG cell lines (DIPG4, DIPG7 and SF761) for five days with selinexor in combination with each of three proteasome inhibitors, bortezomib, carfilzomib and marizomib, and assayed cell viability at the conclusion of treatment. In each experiment, cells were treated with lower levels of the two drugs at several constant ratios. IC50 values were computed for each drug acting alone, and the combination index (CI) of the two drugs acting together was computed using the Chou-Talalay method. We also treated SF761 cells with a combination of radiation (8 Gy), selinexor, and a proteasome inhibitor. Results: The proteasome inhibitors had widely varying IC50 values in the cell lines tested, ranging from 1 nM to 5 uM. The CI for the combination of selinexor and each proteasome inhibitor was consistently less than 1 (indicating a synergistic relationship) in the cell lines tested. We found that radiation and proteasome inhibition had an antagonistic relationship (CI>1), radiation and selinexor a synergistic relationship (CI<1), and the combination of all three was mixed, with some combinations being synergistic and others antagonistic. Conclusions: Selinexor and proteasome inhibitors promise as a combination therapy for HGG and DIPG. We are conducting in vivo experiments to further explore this combination for subsequent clinical trial use.

#1947 Combination of Vincristine and Clotam induces antiproliferative response in medulloblastoma cells. Shruti V. Patil,1 Don Eslin,2 Robert Sutphin,3 Umesh T. Sanpkal,1 Yazmin Hernandez,1 Areeba Hafeez,1 W.Paul Bowman,1 Riyaz Bashir.2 1University of North Texas Health Science Center, Fort Worth, TX; 2 pediatric Palmers Hospital for Children, Orlando, FL.

Medulloblastoma (MB) is the most common pediatric malignant brain tumor and usually originates in the cerebellum. These tumors have the propensity to disseminate throughout the central nervous system and are often difficult to treat. Chemotherapy is widely accepted as part of the multimodality treatment approach for MB. However, it is associated with debilitating toxicity and potential long term disabilities. Vincristine is a commonly used chemotherapy agent for MB treatment. This drug is known to induce some toxic effects including peripheral neuropathy. The aim of this study was to test a combination treatment involving Vincristine and an anti-cancer non-steroidal anti-inflammatory drug, Clotam (Tolfacenic acid) against MB cell lines. Previously, we showed that Clotam inhibited MB cell proliferation and tumor growth in mice by targeting the transcription factor, specificity protein 1 (Sp1) and an inhibitor of apoptosis protein, BIRC3 (baculoviral inhibitor of apoptosis repeat-containing 5). The overexpression of BIRC5 is associated with aggressiveness and poor prognosis in several cancers. MB cells, DAOY and D283 cells were treated with vehicle (dimethyl sulfoxide) or low dose of Vincristine (DAOY: 2ng/mL; D283: 1ng/mL) or Clotam (DAOY & D283: 10 µg/mL) or combination of Vincristine + Clotam. Cell viability was assessed after 2 days post-treatment using Cell-TiterGlo kit. Flow cytometry was employed to analyze apoptotic cells using Annexin-V staining and cell cycle phase distribution using propidium iodide staining. The activation of apoptotic pathways was further investigated by assessing the levels of effector caspases with CaspaseGlo kit and the expression of apoptotic markers [cleaved Poly (ADP-ribose) polymerase (c-PARP), B-cell lymphoma 2, and BIRC3] by Western blot analysis. The expression of key proteins associated with cell cycle (Cyclin A, B, D, CDK4/6, and p21) was also determined by Western blot analysis. When compared to individual agents, the combination of Clotam and Vincristine increased MB cell growth inhibition which is accompanied by an induction of apoptotic markers and the modulation of Cyclin A, B, D and CDK4/6 down-regulation; p21 up-regulation of proteins associated with cell cycle phase distribution. These results suggest that Vincristine and Clotam combination treatment is effective for inducing anti-proliferative effect in MB cells. The experiments to evaluate the effect of this combination in animal model for MB are currently under study.

#1948 Tumor-specific copy number alterations uncover therapeutic opportunities in osteosarcoma. Leanne C. Sayles,1 Marcus Breese,2 Amanda L. Koch,3 Adam K. Spiller,4 Alex E. Naish,4 Anjaly Shah,5 Krystal Strasser,6 Sheri Spunt,7 Neyssa Marina,8 Damon Jacobson,9 Raffi S. Avedian,2 David G. Mohler,2 Steven DuBois,4 Douglas S. Hawkins,8 and E. Alejandro Sweet-Cordero.1 1UCSF, San Francisco, CA; 2Stanford, Stanford, CA; 3University of Washington, Seattle, WA; 4Dana-Farber Cancer Institute, Boston, MA.

Osteosarcoma (OS) is the most common primary sarcoma of bone accounting for approximately 10% of all bone tumors. Recent advances in the understanding of pathogenesis and genetic changes have propelled new therapeutic strategies. We have previously developed a comprehensive OS-specific copy number alteration (CNA) catalog using high-density aCGH, mRNA and exome sequencing to identify OS-specific genetic alterations. We herein present an integrated OS-specific CNA catalog and clinical annotation, which uncovers novel therapeutic opportunities in OS.

TUMOR BIOLOGY: Pediatric Cancer 1: Biomarkers, Preclinical Models, and New Targets
Osteosarcoma (OS) is a highly malignant cancer for which no targeted therapies are currently available. Current treatment modalities are limited to intensive, highly toxic chemotherapy and surgical resection. OS is characterized by widespread copy number alterations and structural rearrangements. In contrast, no recurrent point mutations in protein-coding genes have been identified, suggesting that OS arises from defined genetic elements which have the potential to lead to tumorogenesis and whose pathways have been altered in OS patient specimens. Serial introduction of the viral constructs hTERT (T), SV40Tag (S), and H-RAS (R), led to spindle cell tumor formation in mice. However, MSC-TSR tumors did not form osteoid, whereas OB-TSR tumors showed only scant production of osteoid. Also the addition of OB-TSR cells to tumors failed to tumorigenesis. Based on these findings, MSC-TS and OB-TS cell lines may be ideal platforms for further analysis of the impact of the genetic transformation of hMSCs and OBs into OS. c-Myc has been shown to be overexpressed in OS cells and hence has been suggested as an oncogene. It has also been suggested as a novel target of RUNX2 through rescue from the apoptosis. MSC-TS and OB-TS cells were transformed with a retrovirus containing human c-Myc. Drug resistant colonies were picked up 21 days after selection to obtain stably transformed cell lines. Quantitative PCR and western blots were carried out to detect both gene and protein expression patterns in transformed cell lines, respectively. Furthermore, in order to determine the expression pattern of c-Myc in OS, quantitative PCR and western blots were also performed on MYCN cancer xenografts and primary tumors. All functional assays outlined below will be performed to determine the tumorigenic properties of transformed cell lines. Six out of seven (86%) osteosarcoma primary samples showed a significantly greater c-Myc gene expression level compared to the positive control cell lines. Selection of stably transformed clones and comparison between cell lines and characterizations are underway. Further ongoing characterization includes: soft agar assays, in-vivo tumorigenic assays, histological examination for osteoid production and other OS specific immunohistochemical markers, proliferation, invasion and migration, and differentiation assays. It is our hope that this study will highlight the model that closely recapitulates the human osteosarcoma phenotype by forming a malignant spindle cell tumor that produces aberrant osteoid, and has the potential for multi-lineage differentiation. 

##1949 Salvaeyes pathway enzyme HPRT as a molecular marker for Burkitt's Lymphoma.

The aim of this study is to investigate the potential of Hypoxanthine phosphoribosyltransferase (HPRT) as a surface biomarker and target for future immunotherapies in Burkitt's B-cell Lymphoma. B-cell malignancies are the most common type of childhood cancer. Development of immunotherapeutics could improve current treatment. HPRT is a cytosolic transferase involved in nucleotide production via the purine salvage pathway. Altered expression of TK1, a salvage pathway enzyme, is an indicator of prognosis and diagnosis in multiple cancer types due to active proliferation of cells and the resulting elevated nucleotide demand. It has previously been reported that some salvage pathway enzymes are found on the surface of Burkitt's Lymphoma cells. HPRT presence on the surface of this B-cell lymphoma subtype could provide a target for adoptive cell transfer and other immunotherapies. The potential surface presentation of HPRT was assessed using flow cytometry, scanning electron microscopy (SEM), and cytoplasmatic staining on both healthy lymphocytes and Burkitt's Lymphoma cells (Raji). Experiments with Raji and Ramos cells and/or antibodies with known surface expression indicated that both cell lines and their subclones expressed HPRT at the cell surface. Co-culture experiments with Ramos cells and HPRT-labeled secondary antibodies show that Raji cells exhibit an 81.4% (p-value.0001) positive fluorescence shift when compared to IgG controls (1.5%). Healthy lymphocytes had a fluorescence shift of 2.38% (p-value.9787). The presence of HPRT on the surface of both Raji cells and healthy lymphocytes was further confirmed using gold-labeled antibodies. Utilizing a scanning electron microscope, the presence of the protein on the surface was evaluated and quantified via increases in gold weight percentage of the sample. When treated with antibodies against HPRT, there was a significant increase in gold binding along with an increase in gold weight percentage. These results suggest a direct relationship between HPRT and the surface of Burkitt's lymphoma cells, indicating HPRT as a potential target for future immunotherapeutic treatment in Burkitt's B-cell lymphoma pediatric patients.

##1950 Introducing c-Myc into transformed human mesenchymal stem cells and osteoblasts to recapitulate the osteosarcoma phenotype.
Sajida Piperdı (Thiem),1 Wendong Zhang,2 Daria Ivenitsky,1 Yidan Zhang,1 Yunja Zhang,3 David S. Geller,3 Bang Haoang,2 Rui Yang,3 Jonathan B. Gill,3 Michael Roth,1 Richard Gorlick1. 1Albert Einstein College of Medicine and The Children's Hospital at Montefiore, Bronx, NY; 2Montefiore Medical Center, Bronx, NY, 152

Osteosarcoma (OS) is the most common primary malignant bone tumor in children and young adults. It remains unclear at what point in the pathway of differentiation between human mesenchymal stem cells (hMSCs) and osteoblasts (OBs), OS originates. Since high grade OS frequently demonstrates histologic variability, as well as the potential for multi-lineage differentiation, some consider the hMSC as the cell of origin in OS, whereas others believe the osteoblast to be the most likely cell of origin. Identifying the primary cell of origin is crucial in understanding the molecular pathogenesis of OS. To address the potential for hMSCs or OBs to transform into OS, we have performed classical mutation analysis and fluorescent-activated cell sorting (FACS) of defined gene subsets which have the potential to lead to tumorogenesis and whose pathways have been altered in OS patient specimens. Analysis of the viral constructs hTERT (T), SV40Tag (S), and H-RAS (R), led to spindle cell tumor formation in mice. However, MSC-TSR tumors did not form osteoid, whereas OB-TSR tumors showed only scant production of osteoid. Also the addition of OB-TSR cells to tumors failed to tumorigenesis. Based on these findings, MSC-TS and OB-TS cell lines may be ideal platforms for further analysis of the impact of the genetic transformation of hMSCs and OBs into OS. c-Myc has been shown to be overexpressed in OS cells and hence has been suggested as an oncogene. It has also been suggested as a novel target of RUNX2 through rescue from the apoptosis. MSC-TS and OB-TS cells were transformed with a retrovirus containing human c-Myc. Drug resistant colonies were picked up 21 days after selection to obtain stably transformed cell lines. Quantitative PCR and western blots were carried out to detect both gene and protein expression patterns in transformed cell lines, respectively. Furthermore, in order to determine the expression pattern of c-Myc in OS, quantitative PCR and western blots were also performed on MYCN cancer xenografts and primary tumors. All functional assays outlined below will be performed to determine the tumorigenic properties of transformed cell lines. Six out of seven (86%) osteosarcoma primary samples showed a significantly greater c-Myc gene expression level compared to the positive control cell lines. Selection of stably transformed clones and comparison between cell lines and characterizations are underway. Further ongoing characterization includes: soft agar assays, in-vivo tumorigenic assays, histological examination for osteoid production and other OS specific immunohistochemical markers, proliferation, invasion and migration, and differentiation assays. It is our hope that this study will highlight the model that closely recapitulates the human osteosarcoma phenotype by forming a malignant spindle cell tumor that produces aberrant osteoid, and has the potential for multi-lineage differentiation.
Children with Down syndrome (DS) are at a 500-fold increased risk for developing acute myeloid leukemia (AML) before they reach five years of age. DS-AML blasts have somatic mutations in the gene encoding the essential hematopoietic transcription factor GATA-1 resulting in hypersensitivity to chemotherapy drugs such as cytarabine and daunorubicin. However, therapy-induced toxicity results in greater morbidity and remains a major barrier in attaining higher survival rate. Thus, alternate therapy approaches to minimize toxicity and increase efficacy are needed. Trisomy 21 and GATA-1 mutations in DS-AML are known to alter the epileptogenic landscape in multiple ways. Therefore, we evaluated the efficacy of epigenetic drugs in comparison to chemotherapy in two patient-derived xenograft (PDx) models of DS-AML. We developed two distinct PDx models by successfully engraftment and serial passage of primary DS-AML cells in NSG-B2m mice. Both PDx lines possessed GATA-1 mutation resulting in the expression of a truncated form of GATA-1. NTPL-60 had a nonsense mutation generating a premature stop codon after the initiation codon, while NTPL-386 had a 136 bp deletion in exon 2 resulting in the loss of the initiation codon. The mouse passaged cells were intravenously injected into 6-8 week old NSG-B2m mice. Once disease establishment was confirmed based on the presence of human cells in mouse peripheral blood, five mice per group were treated with vehicle or DNA methylation inhibitor azacitidine and histone deacetylase inhibitor panobinostat either singularly or in combination at a previously determined maximally tolerated dose of 2.5 mg/Kg each. NTPL-386 xenografted mice treated with azacitidine or panobinostat survived 29 and 21 days longer than the vehicle-treated mice respectively, while the mice treated with the combination survived the longest (35 days). Similarly, azacitidine and panobinostat extended the survival of mice transplanted with NTPL-60 by 48 and 31 days respectively compared to the vehicle-treated mice. NTPL-60 mice treated with the epigenetic drug combination are alive at 57 days. Thus, we observed that the azacitidine-panobinostat combination showed statistically significant (p < 0.0001) differences in leukemic burden and mouse survival compared to treatments with either drug alone in both NTPL-386 and NTPL-60 PDx models. We also tested the efficacy of epigenetic therapy followed by chemotherapy. The inclusion of epigenetic therapy before chemotherapy prolonged survival by 39 days compared to vehicle-treated mice. Taken together, our data indicate that epigenetic therapy may be of benefit for the treatment of children with DS-AML.

Accelerating prediction of pediatric and rare cancer vulnerabilities using next-generation cancer models. Yuen-Yi Tseng,1 Andrew Hong,1 Paula Keskula,1 Shubhroz Gill,1 Jaime Cheah,2 Grigoriy Kryukov,1 Aviad Tsherniak,1 Kelli C. Cheema, Joanna B. Kitlinska. Georgetown Univ., Washington, DC.

Neuroblastoma (NB) is a pediatric malignancy with phenotypes varying from spontaneously regressing to metastatic tumors. Thus, disease stratification and the subsequent treatment decision is of utmost importance for NB patients. The methods, which are currently available for prognosis, require complex genetic analyses and access to the tumor tissue. Thus, there is a need for new prognostic and predictive markers that reflect the NB biology and rely on simple tests and easily accessible material. Serum metabolite profiles reflect a combination of factors released by the tumors and the general metabolic state of the patients. Thus, identifying the differences in metabolites between patients with various tumor phenotypes and responses to treatment may lead to discovery of biologically relevant prognostic biomarkers. The goal of our study was to determine blood-based metabolic signatures characteristic for patients with low and high-risk NB and identify potential biomarkers of high-risk disease. Plasma samples from NB patients at different stages of the disease were obtained from the Children’s Oncology Group. Initially, 50 samples were analyzed by Ultra Performance Liquid Chromatography (UPLC) in conjunction with electrospray-quadrupole-time of flight mass spectrometry (ESI-Q-TOF). A set of 26 independent plasma samples was then used for validation study. Statistically significant differences in metabolic profiles were observed between serum from patients with low and high risk disease, various stages of the disease (stage 1-2 vs stage 3 and/or stage 4), as well as undifferentiated vs. differentiating tumors. For all of the identified metabolites, the metabolic consumption, and synthesis pathways were identified and compared, which significantly differ between the groups exhibited high test sensitivity and specificity (the area under the ROC curves 0.92-1). Metabolites that were disregulated in high risk NBs included those involved in energy and choline metabolism, as well as amino acid and lipid biosynthesis. Of particular interest was a significant increase in serum levels of oxoglutaric acid observed in patients with high-risk disease (p<0.01) and metastatic tumors (p<0.001). As oxoglutaric acid is a key metabolite of the tricarboxylic acid cycle that is involved in regulation of the balance between glutamine and glucose metabolism, as well as lipid synthesis and hypoxia response, these data warrant further investigations into the biological role of these pathways in biology of high risk NB. In summary, using a metabolomics approach we were able to delineate blood based biomarkers that could potentially be used for patient stratification following large scale validation studies.
used this model system and a gene expression based approach to identify that Ewing sarcoma cells are uniquely vulnerable to inhibitors of ribonucleotide reductase (RNR), which impair DNA replication by blocking the synthesis of deoxyribonucleotides. Here we report that the treatment of Ewing sarcoma cells with gemcitabine, an irreversible inhibitor of the RRM1 subunit of RNR, results in impaired DNA replication, cell cycle arrest in G2/M phase, apoptosis in Ewing sarcoma cells. Additionally, we have found that the effect of gemcitabine on the viability of Ewing sarcoma cells is sustained even after removal of the drug from the cell culture medium. Moreover, ataxia telangiectasia and rad3-related protein (ATR) and checkpoint kinase 1 (CHK1) inhibitors increase the toxicity of gemcitabine in Ewing sarcoma cells by blocking the adaptive response to impaired DNA replication. Currently, ongoing work is focused on in vivo testing of gemcitabine, alone and in combination with CHK1 and ATR inhibitors, as a novel therapeutic approach for the treatment of Ewing sarcoma.

TUMOR BIOLOGY: Regulation and Imaging of Tumor Metastasis

#1958 Opposing roles of Sma2 and Sma3 in the regulation of growth and invasion of TNBC through TMEPAI. Prajal K. Singh, Sri lakshmi Pan deswara, Manjeri A. Venkatashamal, Pothana Saikumar. UT Health Science Ctr. at San Antonio, San Antonio, TX.

The dependency of triple negative breast cancers (TNBC) on TGF-β signaling activity for their growth and metastasis is well documented. Breast tumors develop resistance to TGF-β-induced growth inhibition, which helps in tumor progression. Additionally, TGF-β is enriched in the tumor microenvironment and is a key inducer of epithelial to mesenchymal transition (EMT) in breast cancer. In an effort to understand the mechanisms by which TGF-β mediates breast tumorigenesis, we sought to identify the relative individual contributions of Sma2 and Sma3 in mediating cancer cell growth and metastasis by TGF-β. Screening of several TNBC cell lines showed increased Sma3/Sma2 expression ratio compared to normal mammary epithelial cells, which suggested breast cancer cells may escape TGF-β-mediated cell cycle arrest by altering their relative expressions of Sma2 and Sma3. Since overexpression of R-Smads may not always accurately reflect the functional status of the endogenous molecules, we individually downregulated each R-Smad and tested its effect on pro-oncogenic behavior of TGF-β. Although Sma2 deficiency has no effect on breast cancer cell behavior, Sma3 deficiency reduced growth and invasion capacity of breast cancer cells. Interestingly, while Sma2 deficiency was associated with reduced TMEPAI/PMEPAI and EMT gene expressions and increased expression of cell cycle inhibitors, Sma2 deficiency had opposite effect on these regulators. Moreover, decreased growth and invasion associated with Sma2 deficiency were largely reversed by overexpressing TMEPAI in Sma3 knockdown cells, suggesting that Sma3/TMEPAI axis may be involved in subverting growth suppression effects of TGF-β into growth promotion. Compared to control and Sma2 deficient cells, Sma3 deficient cells had markedly elevated PTEN protein levels and suppressed Akt phosphorylation both under basal conditions and TGF-β stimulation. We identified that Sma3 but not Sma2 plays an important role in stimulating TMEPAI expression, growth and EMT in triple negative breast cancer cells by promoting TGF-β dependent non-canonical signaling by decreasing the cellular content of PTEN and p27 in a TMEPAI dependent manner. Taken together, the results demonstrate a novel role for Sma3 in breast cancer transformation and cancer progression through TMEPAI. Current therapeutic strategies are aiming at antagonizing the whole TGF-β signaling pathway thereby completely blocking both Sma2 and Sma3 signaling pathways. Selective targeting of TGF-β-Sma3/TMEPAI axis may be more beneficial in triple negative breast cancer therapy and prevention.

TUMOR BIOLOGY: Pediatric Cancer 1: Biomarkers, Preclinical Models, and New Targets

#1956 ABCE1 regulates the translational profile of neuroblastoma to drive tumor progression. fixuan Gao,1 Klaartje Somers,2 Katherine M. Hannah,1 Jamie I. Fletcher,1 Bing Liu,1 Ross D. Hannan,1 Richard B. Pearson,1 Michelle Haber,1 Murray D. Norris,1 Michelle J. Henderson.1

Neuroblastoma is the most common extracranial solid tumor in children. A key driver of high-risk neuroblastoma is the MYCN transcription factor which fuels neuroblastoma progression by enhancing metabolic processes including protein translation (1). ABCE1, a member of the ATP-binding cassette (ABC) superfamily of transporters, is a translational factor directing up-regulation of MYCN (2). It is thought to dissociate the 80S ribosome into free 40S and 60S subunits so they can re-initiate translation and provide the protein building blocks required for rapid cell growth and migration (3). High ABCE1 expression is associated with poor clinical outcome in neuroblastoma patients (2). Therefore, we hypothesized that ABCE1 suppression may inhibit the aggressiveness of neuroblastoma by disabling protein synthesis. To test this, we suppressed ABCE1 using siRNAs in MYCN-amplified neuroblastoma cell lines, SK-N-BE(2) cells, ABCE1 suppression delayed tumor growth (P<0.001) and metastasis (P<0.001). Mechanistically, when polyribosome analysis was used to monitor the impact of ABCE1 expression on protein translation in SK-N-BE(2) cells, loss of ABCE1 reduced the proportion of actively translating ribosomes (P<0.001), leading to lower global protein synthesis (P=0.018). This is the first report of ABCE1 acting as pro-tumorigenic factor in neuroblastoma. Our data imply that targeting the translational machinery through ABCE1 may be an effective therapeutic approach for the treatment of MYCN-driven cancers. J. Boon, K., Caron, H. N., van Asperen, P., Valentijn, L., Hermus, M. C., van Sluis, P., Roobol, I., Weis, I., Voûte, P. A., Schwab, M. & Versteeg, R. (2001) EMBO J. 20, 1383-1393. 2. Porro, A., Haber, M., Doliatii, D., Irici, N., Henderson, M., Gherardi, S., Valli, E., Munoz, M.A., Xue, C., Flemming, C., Schwab, M., Wong, J.H., Marshall, G.M., Della Valle, G., Norris, M.D. & Perini, G. (2010) J. Biol. Chem. 285, 19532-19543. 3. Pisarev, A.V., Skabkin, M.A., Pisareva, V.P., Skabkina, O.V., Rakotondrafara, A.M., Hentze, M.W., Heleni C.U. & Pestova, T.V. (2010). Mol. Cell. 37, 196-210.

The dependency of triple negative breast cancers (TNBC) on TGF-β signaling activity for their growth and metastasis is well documented. Breast tumors develop resistance to TGF-β-induced growth inhibition, which helps in tumor progression. Additionally, TGF-β is enriched in the tumor microenvironment and is a key inducer of epithelial to mesenchymal transition (EMT) in breast cancer. In an effort to understand the mechanisms by which TGF-β mediates breast tumorigenesis, we sought to identify the relative individual contributions of Sma2 and Sma3 in mediating cancer cell growth and metastasis by TGF-β. Screening of several TNBC cell lines showed increased Sma3/Sma2 expression ratio compared to normal mammary epithelial cells, which suggested breast cancer cells may escape TGF-β mediated cell cycle arrest by altering their relative expressions of Sma2 and Sma3. Since overexpression of R-Smads may not always accurately reflect the functional status of the endogenous molecules, we individually downregulated each R-Smad and tested its effect on pro-oncogenic behavior of TGF-β. Although Sma2 deficiency has no effect on breast cancer cell behavior, Sma3 deficiency reduced growth and invasion capacity of breast cancer cells. Interestingly, while Sma2 deficiency was associated with reduced TMEPAI/PMEPAI and EMT gene expressions and increased expression of cell cycle inhibitors, Sma2 deficiency had opposite effect on these regulators. Moreover, decreased growth and invasion associated with Sma2 deficiency were largely reversed by overexpressing TMEPAI in Sma3 knockdown cells, suggesting that Sma3/TMEPAI axis may be involved in subverting growth suppression effects of TGF-β into growth promotion. Compared to control and Sma2 deficient cells, Sma3 deficient cells had markedly elevated PTEN protein levels and suppressed Akt phosphorylation both under basal conditions and TGF-β stimulation. We identified that Sma3 but not Sma2 plays an important role in stimulating TMEPAI expression, growth and EMT in triple negative breast cancer cells by promoting TGF-β dependent non-canonical signaling by decreasing the cellular content of PTEN and p27 in a TMEPAI dependent manner. Taken together, the results demonstrate a novel role for Sma3 in breast cancer transformation and cancer progression through TMEPAI. Current therapeutic strategies are aiming at antagonizing the whole TGF-β signaling pathway thereby completely blocking both Sma2 and Sma3 signaling pathways. Selective targeting of TGF-β-Sma3/TMEPAI axis may be more beneficial in triple negative breast cancer therapy and prevention.

#1959 MTA1 promotes tumor progression and bone metastasis in prostate cancer via positive regulation of MUC1 and CTSB. Avinash Kumar,1 Swati Dhar,2 Gisella Campaneli,3 Nasir A. Butt,2 Christian R. Gomez,2 Jason M. Schallheim,2 Anait S. Levenson1.1 Long Island University, Brooklyn, NY; 2University of Mississippi Medical Center, Jackson, MS.

About 80% of the time that prostate cancer cells metastasize, they spread to the bones. Metastasis involves activation of invasive programs through genetic and epigenetic alterations, including changes in expression of chromatin remodeler proteins. We have previously identified metastasis-associated protein 1 (MTA1), a chromatin remodeler, as a component of vicious cycle of bone metastasis and confirmed significantly higher expression of MTA1 in bone metastases than in primary tumors. In an attempt to dissect molecular mechanisms of MTA1 action, we performed integrative analysis of our bone metastasis signature data along with MTA1 ChiP-seq data, which revealed mucin 1 (MUC1) and cathepsin B (CTSB) as strong potential candidates responsible for MTA1-driven invasiveness. Here, we show that loss of function studies with MTA1 in PC3M aggressive prostate cancer cells do not affect cell proliferation but leads to decrease in colony forming ability, invasive and migratory property of these cells. When PC3M cells silenced for MTA1 and tagged with luciferase are used to generate subcutaneous or intracardiac xenografts, it leads to reduced tumor progression or decreased metastasis to bone, respectively.
as evident by bioluminescent measurements and histology of tumors. Ongoing experiments aim to validate the role of MUC1 and CTBS in MTA1-driven invasiveness and metastasis in prostate cancer.

**#1960 Upregulation of MCP-1 regulates invasiveness in triple negative breast cancer.** Pranabananda Dutta, Kimberly Paico, Inez Yuwanita, Yanyuan Wu, Marianna Sarkissian, Jaydutt Vadgama. *Charles R. Drew University, Los Angeles, CA.*

Background: Triple negative breast cancer (TNBC) poses a critical problem for targeted therapy due to lack of significant expression of estrogen, progesterone receptor or Her2/neu oncogene. Hence, it is imperative to identify novel therapeutic strategies to target TNBC. Our study is aimed to examine whether Monocyte Chemotactic Protein -1 (MCP-1) is a specific marker for TNBC metastasis. Experimental Design: We employed ELISA to determine secreted MCP-1 in cell conditioned media, as well as Real-time PCR to determine the status of MCP-1 in TNBC cell lines. Boyden chamber assay was used to determine the effect of recombinant MCP-1 on cellular metastasis. Cellular proliferation was measured with MTT assay. Immunofluorescence staining was utilized for protein of interest in breast cancer cells. MCP-1 knockdown was performed using lentiviral vector with shRNA targeting MCP-1 coding regions. Results: Our data show that the key inflammatory chemokine MCP-1 is upregulated in TNBC cell lines both transcriptionally as well as in terms of secretion compared to ER-positive cell line, MCF-7. MCP-1 stimulation in MDA-MB231 and MCF-7 cells does not affect cellular proliferation. However, MCP-1 increases metastatic phenotype of MDA-MB-231 cells along with BT-549 cells. Inhibiting Chemokine receptor 2/4 (CCR2/4), cognate receptor for MCP-1, with small molecule antagonists negatively affects invasiveness in MDA-MB-231 as evidenced by Boyden chamber assay. Knocking down MCP-1 by shRNA decreases cell invasion in TNBC cell line, BT-549 along with downregulation of key epithelial to mesenchymal transition markers, N-cadherin and Vimentin. MCP-1 induced cell invasion in TNBC may involve activation of p44/p42 MAPK Thr202/Tyr204. Conclusion: Our study suggests that high MCP-1 levels in TNBC is driving up metastasis potential in cells. Thus MCP-1 and its mediated pathways could be potential therapeutic targets for the treatment of TNBC.


The epithelial-mesenchymal transition (EMT) is a developmental program that is aberrantly activated in cancer cells, producing an invasive phenotype that can lead to metastasis. Inducers of EMT are largely known and have been widely studied. However, the mechanisms that regulate the link between extracellular stimuli and EMT phenotypes remain poorly understood. Preliminary evidence from our laboratory suggests that downregulation of proteasome activity may be responsible, in part, for driving EMT. Using immortalized human mammary epithelial (HMEE) cells as a model, we show that (1) EMT is associated with decreased proteasome activity and increased polyubiquitinated substrates, (2) pharmacologic inhibition of proteasome activity leads to increased EMT phenotypes and (3) pharmacologic inhibition of proteasome activity leads to increased EMT via stabilization of the TGF-β signaling pathway. Together, these data suggest that proteasome activity may be an unappreciated regulator of EMT.

**#1962 Regulation of invasion by lysine demethylase 5B in non-small cell lung cancer cells.** Elizabeth L. Zoeller, Jessica Konen, Joshua Bell, Emily Summerbell, Jeanne Kowalski, Adam Marcus, Paul Vertino. *Emory Univ., Decatur, GA.*

Histone modifying enzymes are often dysregulated during carcinogenesis and are major contributors to the development of oncogenic features, including proliferation, drug resistance, and metastasis. Given these roles, histone modifiers are promising new targets for oncological therapeutics. One of the enzyme families at the center of this area of research is the lysine demethylase family KDM5, for which several inhibitors are in development. KDM5A and KDM5B are frequently overexpressed or mutated in human non-small cell lung cancer (NSCLC). In overexpression studies, KDM5B promotes invasion and migration of NSCLC cells, whereas invasion and migration of NSCLC cells were decreased following knockdown of KDM5B. Furthermore, in patients with NSCLC, KDM5B is expressed at higher levels in brain metastasis sites when compared to both normal tissues and primary tumors. These findings suggest a role for KDM5B in lung cancer and metastatic spread. However, the precise role of KDM5 family members in lung cancer invasion and metastasis is not known. Recently, we discovered that KDM5B is differentially expressed in cell subtypes within a 3D model of NSCLC collective cell invasion. In this model isolated single cells at the forefront of invasive branches (leader cells) express more 2-4 fold more KDM5B protein than the cells following (follower cells), while KDM5A and KDM5C are evenly expressed across both cell types. KDM5B mRNA expression is similar across cell types suggesting that the differential protein expression is mediated at the posttranscriptional level. Interestingly, global H3K4me3 levels are decreased in leader cells as compared to follower cells, supporting the idea that the lysine demethylases targeting this residue may be expressed at higher levels in leader cells. Collectively, these data suggest that KDM5B could be contributing to initiation of invasion at the primary site and thus, promoting metastasis. Given the rising prominence of therapeutic inhibitors of KDM5 family members, a better understanding how KDM5B contributes to cell invasion may lead to a new approach to the prevention of metastasis.


Background: Advanced gastric cancer (GC) frequently recurs because of undetected micrometastases even when disease is localized and patients undergo curative resection. Moreover, peritoneal metastasis is fatal. We aimed to develop novel diagnostic and therapeutic targets specific for peritoneal metastasis of GC to improve management. Methods: We conducted a metastatic pathway-specific transcriptome analysis to identify candidate biomarkers comprising 340 patients allocated to discovery and validation sets (1:2) to evaluate the diagnostic and predictive value. The mRNA and protein levels in primary GC tissues were compared with patients' clinical characteristics and survival. The effects of siRNA-mediated knockdown on phenotype and fluorouracil sensitivity of GC cells were evaluated in vitro, and the therapeutic effects of siRNAs were evaluated using a mouse xenograft model. Result: Synaptotagmin VIII (SYT8) was identified as a candidate biomarker specific to peritoneal metastasis. SYT8 levels were elevated in the validation set comprising patients with peritoneal recurrence or metastasis. High SYT8 levels were significantly and specifically associated with peritoneal metastasis, and served as an independent prognostic marker for peritoneal recurrence-free survival of patients with stage II/III GC. The survival difference between high and low SYT8 levels was associated with patients who received adjuvant chemotherapy. Inhibition of SYT8 expression by GC cells correlated with decreased invasion, migration, and fluorouracil resistance. Intrapерitoneal administration of SYT8-specific siRNA inhibited the growth of peritoneal nodules and prolonged survival of mice engrafted with GC cells. Conclusions: SYT8 expression represents a promising diagnostic and predictive biomarker for peritoneal metastasis of GC.

**#1965 A novel biocompatible fluorescent nanoparticle enables enhanced live cell tagging and tracking of cancer cells.** Nick Asbrock, Yi Chu, Kevin Su, Ben Zhong Tang, Bin Liu. *MilliporeSigma, Temecula, CA; The Hong Kong University of Science & Technology, Clear Water Bay, Kowloon, Hong Kong, China; National University of Singapore, Temecula, CA.*

Metastasis is the leading cause of cancer mortality. Metastasis is a multi-step process which includes local tumor cell invasion, cell migration into the vasculature, exit of cells from the circulation and colonization at the distal tissue sites. Long-term noninvasive cell tracking by fluorescent probes is of great importance to life science and biomedical engineering. Current methods used to fluorescently tag cancer cells have been limited by short signal duration, high background autofluorescence or lengthy cell line generation using GFP. We have developed a biocompatible fluorescent nanoparticle which relies on Aggregation Induced Emission (AIE) technology that are highly resistant to fluorescent signal quenching. These particles enable highly efficient live cell fluorescent tagging while retaining fluorescent signal for up to 10 days in vitro and 21 days in vivo. These nanoparticles will open new avenues in the development of fluorescent probes for following biological processes such as carcinogenesis.

**#1966 Cell-surface major vault protein is a novel marker for circulating tumor cells with nonepithelial phenotypes in hepatocellular carcinoma.** Hyun Min Lee, Jae Won Joo, Won Tae Kim, Min Kyu Kim, Se Ri Seo, Hong Seo Choi, Hee Jin Chang, Young Joo Jang, Chul Jeuk Ryu. *Sejong University, Seoul, Republic of Korea; Sungkyunkwan University, Seoul, Republic of Korea; National Cancer Center, Goyang, Republic of Korea; Dankook University, Cheonan, Republic of Korea.*

Several recent studies have reported that major vault proteins (MVPs) are involved in a number of biological processes including regulation of proliferation, drug resistance, and metastasis. Given these roles, histone modifiers are promising new targets for oncological therapeutics. One of the enzyme families at the center of this area of research is the lysine demethylase family KDM5, for which several inhibitors are in development. KDM5A and KDM5B are frequently overexpressed or mutated in human non-small cell lung cancer (NSCLC). In overexpression studies, KDM5B promotes invasion and migration of NSCLC cells, whereas invasion and migration of NSCLC cells were decreased following knockdown of KDM5B. Furthermore, in patients with NSCLC, KDM5B is expressed at higher levels in brain metastasis sites when compared to both normal tissues and primary tumors. These findings suggest a role for KDM5B in lung cancer and metastatic spread. However, the precise role of KDM5 family members in lung cancer invasion and metastasis is not known. Recently, we discovered that KDM5B is differentially expressed in cell subtypes in a 3D model of NSCLC collective cell invasion. In this model isolated single cells at the forefront of invasive branches (leader cells) express more 2-4 fold more KDM5B protein than the cells following (follower cells), while KDM5A and KDM5C are evenly expressed across both cell types. KDM5B mRNA expression is similar across cell types suggesting that the differential protein expression is mediated at the posttranscriptional level. Interestingly, global H3K4me3 levels are decreased in leader cells as compared to follower cells, supporting the idea that the lysine demethylases targeting this residue may be expressed at higher levels in leader cells. Collectively, these data suggest that KDM5B could be contributing to initiation of invasion at the primary site and thus, promoting metastasis. Given the rising prominence of therapeutic inhibitors of KDM5 family members, a better understanding how KDM5B contributes to cell invasion may lead to a new approach to the prevention of metastasis.
Hepatocellular carcinoma (HCC) is currently the fifth most common malignancy worldwide and the poor because of frequent metastasis and resistance to chemotherapy. Circulating tumor cells (CTCs) in blood have attracted attention as potential seeds for metastasis and an important indicator of treatment outcome. However, the biological properties of CTCs are largely unknown due to rarity and lack of CTC-specific surface markers. Major vault protein (MVP) is upregulated during malignant progression and drug resistance development in various cancer cells, although it is ubiquitously expressed in many normal tissues. Here, we found for the first time that MVP was expressed on the surface of various cancer cells including HCC cell lines, although MVP has been known as a cytoplasmic and nuclear protein. To investigate the role of cell-surface MVP (csMVP) on HCC cell lines, MVP expression was knocked down in Huh7 cells by small interfering RNA. MVP knockdown decreased cell growth and increased apoptotic cell death. When Huh7 cells were treated with a polyclonal anti-MVP antibody (α-MVP) recognizing csMVP, Huh7 cell proliferation was decreased without apoptotic cell death. Cell sorting revealed that csMVP-positive Huh7 cells showed a higher proliferation and survival rate than csMVP-negative Huh7 cells under the stress of low density seeding. Huh7 cells treated with α-MVP also inhibited cell invasion and migration in vitro. Thus, csMVP is positively associated with HCC cell proliferation, survival, invasion and migration. Analysis of signaling molecules showed that MVP knockdown caused a significant decrease in the levels of phosphorylation of FAK, ERK, AKT and S6K. MVP knockdown also increased E-cadherin but decreased vimentin, indicating that MVP promotes the process of epithelial-to-mesenchymal transition. To further analyze the role of csMVP during HCC metastasis, blood samples from 62 HCC patients and 10 healthy volunteers were stained with csMVP, EpCAM, HSA, EGFR, CK, and/or vimentin antibodies after the depletion of red blood cells and CD45-positive cells. csMVP-positive CTCs were detected in approximately 85.5% (>0.46 CTCs/ml) of patients, and the cell count measured in ml of blood ranged between 0.48 and 37.8. Double staining showed that almost all csMVP-positive cells were panCK-negative while approximately one fifth of csMVP-positive CTCs was HSA-positive. Triple staining further showed that among csMVP-positive cells, EpCAM “Vimentin”, EpCAM “Vimentin”, EpCAM “Vimentin” and EpCAM “Vimentin” cells were approximately 0.0, 49 and 51%, respectively, suggesting that a half of csMVP-positive cells are EMT-phenotype cells and the rest of them are both EpCAM- and vimentin-negative cells. The results suggest that csMVP is a novel marker on CTCs in patients with HCCs, where it is expressed predominantly on EMT phenotypic and EpCAM-“vimentin” intermediate CTCs.

### 1967 A novel 40kDa CPE-ΔN isoform promotes proliferation and invasion in pancreatic cancer cells. Xu Yu Yang, Cong Ling, Hong Lou, Loh Yoke Peng, National Institutes of Health, Bethesda, MD.

Carboxypeptidase E (CPE) is a prohormone processing exopeptidase that cleaves C-terminal basic residues from peptide hormones liberated endopeptidolytically from prohormones. It is identified molecular basis for pancreatic cells and the rest of these effects can be attenuated by Twist1 protein deletion mutants.

### 1968 A novel mechanism of metastasis: Extracellular ATP promotes invasion and metastasis independent of purinergic receptor signaling. Yanyang Cao, Xuan Wang, Xiaohou Chen. Ohio Univ., Athens, OH.

Cancer is one of the top deadliest diseases in the US and worldwide. Metastasis, the dissemination of cancer cells from the primary tumors to distant organs, is responsible for 90% of solid tumor-related deaths. Tumor invasion and metastasis is a multistep process in which loss of cell-cell adhesion, increased proteolysis, and cell motility has been shown to be critical steps. Extracellular ATP (eATP) is released during cellular processes and plays a critical role in tumor cell detachment, motility, invasion and tumor metastasis initiation. It was previously shown that ATP-induced purinergic receptor (PR) signaling is involved in metastasis. Our hypothesis is different in that eATP also mediates invasion and metastasis independent of PR signaling. Various bioassays were used in human non-small cell lung cancer (NSCLC) A549 cells to test our hypothesis. Our results show that eATP treatment led to a substantially increased number of floating cancer cells, and these cells were viable and formed clones in a clonogenic assay. This indicates that eATP induces cancer cell detachment. Moreover, treatment of eATP also induces cell migration in cell woundings and Transwell migration assays. In vitro migration that eATP induced a dose-dependent increase in the invasive capacities of the A549 cells. Western blot analysis indicates that eATP treatment reduced the expression of cell-cell adhesion molecule E-cadherin. PR inhibitors only slightly attenuated these effects. All these suggest novel ATP mechanisms independent of the PR signaling that are unreported before and imply novel targets for inhibiting/preventing metastasis. References 1. Qian et al. Cancer Letters 351; 242-251 (2014). 2. Chen, Qian, and Wu. Free Radical Biol Med 79; 253-263 (2015) 3. Qian et al. Mol Cancer Res 14(11); 1087-1096 (2016).
Glucose-regulated protein 94 is a novel expression biomarker in esophageal squamous cell carcinoma. Yu-Jia Chang,1 Chien-Yu Huang,1 Taipei Medical Univ., Taipei, Taiwan; 2Department of Surgery, Shuang Ho Hospital, Taipei Medical University, Taipei, Taiwan.

Background: Esophageal cancer is a worldwide health problem with a very poor prognosis as most patients present very late when the disease is advanced and difficult to cure. It is urgent to figure out a diagnostic and therapeutic strategy. Glucose-regulated protein 94 (GRP94) has been found highly correlated with cancer progression. However, the role of GRP94 in the progression and metastasis of esophageal squamous cell carcinoma (ESCC) is still unclear.

Methods: We investigated the GRP94 expression level of ESCC cells and generated GRP94-KD cells by shRNA technique. The proliferation assay, migration, invasion assay were performed. The tissue array was performed to see the correlation of GRP94 and clinical outcomes on ESCC. Mitochondrial bioenergetics were assessed using a Seahorse XF24 flux analyzer. The Transmission Electron microscopy was applied to see the structure of mitochondria.

Results: We found that ESCC specimen that expressed higher GRP94 showed the lower overall survival in tissue array results. The ESCC cells expressed high level of GRP94. Silencing GRP94 reduced the proliferation, migratory and invasion activity. Transmission Electron microscopy revealed the impaired mitochondrial in GRP94-KD cells. GRP94-KD cells also displayed reduced basal respiration, spare oxygen capacity and respiratory control ratio. The changes were analyzed by Mann Whitney U-test. Survival analysis between high and low expression group of the candidate genes was performed by Kaplan-Meier method.

Conclusion: We found that silencing GRP94 in ESCC cells suppressed cancer growth and metastasis ability through COX-2 and EMT molecules expression. In addition, GRP94 may mediate the functional mitochondria in ESCC. GRP94 may be a new target to develop the therapeutic strategy for ESCC.

ITGBL1 is a novel epithelial mesenchymal transition-associated prognostic biomarker in colorectal cancer. Takatoshi Matsuyama,1 Toshiaki Ishikawa,2 Naoki Takahashi,3 Yasuhide Yamada,3 Masamichi Yasuno,2 Tatsu-suyuki Kawano,3 Hiroyuki Uetake,3 Ayaj Goa.1 Baylor Scott & White Research Institute and Charles A Simmmons Cancer Center, Dallas, TX;2Tokyo Medical and Dental University, Tokyo, Japan; 3National Cancer Center Hospital, Tokyo, Japan.

Purpose: Colorectal cancer (CRC) ranks as the third leading cancer worldwide, and its incidence continues to rise gradually, highlighting the need to stratify the risk of recurrence after curative surgery. Recently, several genes have been identified which appear to associate with metastasis, as they mediate epithelial-to-mesenchymal transition (EMT). This study aimed to identify novel EMT and cancer recurrence-associated biomarkers through systematic and comprehensive discovery and validated strategy in multiple, independent CRC cohorts. Experimental Design: Two independent gene expression microarray datasets (n = 173 and n = 307 respectively) were used to identify novel metastasis-recurrence biomarkers for CRC. Following carefully selection and prioritization of biomarkers, we selected a candidate gene and validated its performance as a recurrence marker in a large testing cohort (n = 566), and two independent clinical validation cohorts (n = 201, n = 475, respectively). To confirm the protein expression of ITGBL1 in cancer, immunohistochemistry (IHC) was performed in paired 33 primary CRCs and adjacent normal mucosa, as well as a subset of liver and lung metastases tissues. In addition, we used Gene Set Enrichment Analysis (GSEA) to determine the functional role of ITGBL1 in CRC. Results: During the discovery step, gene expression profiles from differentially expressed genes between recurrence positive and negative primary CRCs, as well as evaluation of the metastatic sites compared with primary CRC, identified ITGBL1 as a most promising candidate biomarker. High expression of ITGBL1 associated with poor overall survival (OS) in stage I-IV patients and worse disease-free survival (DFS) in stage I-III patients. Subgroup validation of these results in two large and independent patient cohorts confirmed these findings and demonstrated that high ITGBL1 expression correlated with shorter DFS in stage II and III CRC patients. In addition, high ITGBL1 expression emerged as an independent prognostic factor for DFS in stage I and II patients. IHC analysis revealed that both early stage CRCs and adjacent normal colonic mucosa displayed low ITGBL1 expression, while ITGBL1 expression gradually increased from tumor surface to the invasive front in late stage cancer, indicating that ITGBL1 may facilitate EMT process and promote a more aggressive phenotype in CRC. Conclusions: High expression of ITGBL1 in primary tumors was associated with tumor recurrence in CRC patients after curative surgery. Collectively, we have identified ITGBL1 as a novel EMT-associated biomarker which could be used for risk stratification for metastatic potential in CRC.

Identification of novel candidate driver genes of colorectal cancer on chromosome 7p. Yuta Koyama,1 Yushi Ogawa,1 Takaaki Masuda,1 Yukihiro Yoshikawa,2 Miwa Noda,1 Hiroaki Wakiyama,3 Kuniai Sato,1 Sho Nambara,1 Qingiang Hu,1 Shinya Kidogami,1 Tomoko Saito,2 Shotoar Sakamura,2 Naoki Hayashi,1 Yohsuke Kuroda,2 Shuhei Ito,1 Hitodetsu Eguchi,1 Koshi Moromi1,3 Kyushu University Beppu Hospital, Japan; 2Showa University Northern Yokohama Hospital, Japan. Japan.

Background: Colorectal cancer (CRC) is one of the most prevalent types of cancer. The high mortality rate of CRC is a serious problem. Hence it is urgently necessary to identify novel molecular target to improve the mortality rate. Amplification of chromosome 7p is frequent in CRC, and it has been considered to harbor driver genes that promote tumorigenesis or tumor progression by the gain of function. The aim of this study is to identify novel candidate driver genes on chromosome 7p and to clarify the clinical significance of their expression in CRC.

Material and Methods: 1. We selected the candidate genes that satisfied the following criteria using CRC data from The Cancer Genome Atlas (TCGA). 1) The DNA copy number and mRNA expression is positively correlated with each other, 2) overexpressed in the tumor tissues compared to the normal tissues. 2. The mRNA expression of the candidate genes was measured in 108 surgically-resected CRC tissues and the paired normal tissues in our hospital by quantitative RT-PCR. The differences of mRNA expression between CRC tissues and normal colon tissues were analyzed by Mann Whitney U-test. Survival analysis between high and low expression group of the candidate genes was performed by Kaplan-Meier method.

Conclusion: We found that the mRNA expression of the candidate genes and the clinicopathological factors were analyzed by Fisher’s exact test. We performed Gene Set Enrichment Analysis (GSEA) in CRC data from TCGA to clarify the correlation between the candidate genes and gene sets that are associated with tumorigenesis or tumor progression. Results: DEAD Box Helicase 56 (DDX56), ATP-dependent RNA helicases involved in several aspects of RNA metabolism including mRNA splicing and transport, transcription and translation, transcriptional and translational remodeling of ribonucleoprotein complexes, was satisfied with the criteria. The expression of DDX56 was significantly higher in CRC tissues than in normal colon tissues (p < 0.005), and it correlated with lymphatic invasion (p = 0.02), and distant metastasis (p = 0.03). The high DDX56 expression group had a significantly poorer prognosis than the low expression group (p = 0.03). On multivariate analysis, high DDX56 expression was an independent prognostic factor affecting OS (p = 0.013) with hazard ratios (95% CI) of 2.32 (1.20–4.48) among clinicopathological factors. GSEA showed that DDX56 expression was positively correlated with mitotic cell cycle progression and splenomegaly. Conclusions: We identified DDX56 as a promising driver gene of CRC on chromosome 7p. DDX56 expression was positively associated with lymphatic invasion and distant metastasis, and was an independent poor prognostic factor. Furthermore, DDX56 may be involved in tumor progression through stimulating cell-cycle. DDX56 could be a therapeutic target as well as a poor prognostic biomarker in CRC.

Novel relationships of expression of methylxanthine alkaloid receptor genes and risk of breast carcinoma recurrence. Seth B. Sereff, Michael W. Daniels, James L. Wittliff. University of Louisville, Louisville, KY.

Consumption of methylxanthine alkaloids such as caffeine, theophylline and theobromine may induce breast pain. Caffeine appears to induce its biological activity by antagonizing adenosine receptors, which have been implicated in breast cancer cell behavior in vitro. Our goal is to evaluate expression of genes for methylxanthine receptors and metabolizing enzymes for assessing risk of breast cancer recurrence. Procedures: De-identified primary cancers previously collected, stored and analyzed under stringent conditions were employed to amass an IRB-approved, de-identified comprehensive Database. Patient-related properties (e.g., nodal status, clinical outcome) and results from estrogen (ER) and progesterin receptor (PR) analyses and gene expression assays guided selection. To decode clinical utility of gene expression profiles, Laser Capture Microdissection (PicCell He164 Arcturus/Thermo Fisher) was used previously to non-destructively collect carcinoma cells. DNA was extracted, amplified and analyzed by microarray (~ 22,000 genes). Results: Gene expression levels of 8 methylxanthine receptors, 8 metabolizing enzymes and various phosphodies- terases were retrieved from microarray results of 247 breast biopsies. Unvariable Cox regressions and Kaplan Meier plots were determined for each candidate gene with R software. Kaplan-Meier plots of PDE4A, CYP2A6 or CYP2E individually indicated lower expression was related to decreased progression free (PFS) and overall survival (OS) while PDE1A over-expression predicted patients with better survival (OS).
TUMOR BIOLOGY: Regulation and Imaging of Tumor Metastasis

FAM83B is a novel oncogene identified by the Jackson Laboratory in a forward genetic screen for drivers of Human Mammary Epithelial Cell (HMEC) transformation. Previous work showed that FAM83B expression is elevated in triple negative breast cancers and that FAM83B also activates key cell signaling pathways (EGFR, MAPK, and PI3K) in breast cancers. Here, we implicate FAM83B as a novel regulator of epithelial-mesenchymal plasticity using a HMEC transformation model. Transformation of primary HMEC by expressing shp16, shp53, c-Myc, and RAS results in the generation of two distinct transformed cell populations. One population retains epithelial characteristics, while an emergent population spontaneously acquires a mesenchymal morphology and a CD24+/CD44+ (CSC) cell surface marker profile. Moreover, isolation of epithelial, CD24+/CD44+ cells (non-CSC) and exposure to Oncostatin M (OSM), an EMT-inducing cytokine, can also generate cells with a mesenchymal/CSC phenotype. Interestingly, FAM83B expression was significantly elevated in the epithelial/non-CSC population compared to the mesenchymal/CSC population. Moreover, treatment of purified epithelial/non-CSC cells with OSM resulted in decreased FAM83B expression levels, indicating FAM83B expression is suppressed as the cells move through EMT. FAM83B expression also correlated with an epithelial phenotype in a panel of breast cancer cell lines. shRNA-mediated knock-down of FAM83B from epithelial/non-CSC resulted in increased spontaneous EMT, concomitant with elevated expression of the mesenchymal marker Vimentin and master EMT transcription factor Zeb1. Following treatment, murine shB3 cells exhibited increased expression of p-ERK, p-STAT3 and p-p70S6K signaling, known contributors to the mesenchymal/CSC phenotype. Conversely, OSM-induced EMT was blunted in epithelial/non-CSC expressing exogenous FAM83B. Thus, FAM83B expression is a determinant in maintaining an epithelial phenotype, while suppression of FAM83B is important during EMT. Future work will seek to define novel FAM83B-mediated signaling pathways important for regulating cellular plasticity.

#1974 Novel function of PCNA in mammary tumor development and distant metastasis through tyrosine phosphorylation. Yuen-Liang Wang,1 Lanie Chang,2 Susan E. Waltz,3 Shao-Chun Wang.1 1China Medical University & Taichung City, Taiwan; 2China Medical University Hospital, Taichung City, Taiwan; 3University of Cincinnati, Cincinnati, OH.

Breast cancer is the most common cancer besides skin cancer and is the second leading cause of cancer-related death in women in the US. Despite intensive study, metastasis and tumor dormancy continue to be major hurdles to eradicate this disease. PCNA forms a heterotrimeric ring encoding the DNA double helix and acts as a sliding platform indispensable for DNA replication, damage repair, and chromatin remodeling. While a positive role of PCNA in oncogenic transformation remains elusive due to the embryonic lethal phenotype in PCNA-deficient mice. We have shown that PCNA is regulated by phosphorylation at tyrosine 211 (Y211) mediated through multiple growth factor-stimulated signaling pathways. Y211 phosphorylation enhances chromatin-bound PCNA to promote cell proliferation and is the embryonic lethal phenotype in PCNA-deficient mice. We have shown that conceivably, how PCNA functions in tumor progression remains elusive due to malignant potential. Further study using syngeneic orthotopic model of transplanting murine breast tumor-initiating cells (TICs) as determined by in vitro and in vivo analysis. Our preliminary findings that the ligand pathways leading to Y211 phosphorylation can also be inhibited by FDA-approved drugs, our results suggest that pY211-PCNA may be a new druggable mechanism to target tumor metastasis.

#1975 FAM83B: A novel regulator of cell plasticity in breast cancer. Courtney A. Bartel, Damon J. Junk, Mark W. Jackson. Case Western Reserve University, Cleveland, OH.

Epithelial to mesenchymal transition (EMT) is an important process in both metastasis and cancer stem cell (CSC) enrichment. Recent studies suggest that instead of simply transitioning from one cell state to another, cancer cells exist along a continuum of epithelial and mesenchymal cell states. Identifying novel regulators of tumor cell plasticity will be critical to fully understand this continuum and to target metastasis and CSC phenotypes in the future. FAM83B is a novel oncogene identified by the Jackson Laboratory in a forward genetic screen for drivers of Human Mammary Epithelial Cell (HMEC) transformation. Previous work showed that FAM83B expression is elevated in triple negative breast cancers and that FAM83B also activates key cell signaling pathways (EGFR, MAPK, and PI3K) in breast cancers. Here, we implicate FAM83B as a novel regulator of epithelial-mesenchymal plasticity using a HMEC transformation model. Transformation of primary HMEC by expressing shp16, shp53, c-Myc, and RAS results in the generation of two distinct transformed cell populations. One population retains epithelial characteristics, while an emergent population spontaneously acquires a mesenchymal morphology and a CD24+/CD44+ (CSC) cell surface marker profile. Moreover, isolation of epithelial, CD24+/CD44+ cells (non-CSC) and exposure to Oncostatin M (OSM), an EMT-inducing cytokine, can also generate cells with a mesenchymal/CSC phenotype. Interestingly, FAM83B expression was significantly elevated in the epithelial/non-CSC population compared to the mesenchymal/CSC population. Moreover, treatment of purified epithelial/non-CSC cells with OSM resulted in decreased FAM83B expression levels, indicating FAM83B expression is suppressed as the cells move through EMT. FAM83B expression also correlated with an epithelial phenotype in a panel of breast cancer cell lines. shRNA-mediated knock-down of FAM83B from epithelial/non-CSC resulted in increased spontaneous EMT, concomitant with elevated expression of the mesenchymal marker Vimentin and master EMT transcription factor Zeb1. Following treatment, murine shB3 cells exhibited increased expression of p-ERK, p-STAT3 and p-p70S6K signaling, known contributors to the mesenchymal/CSC phenotype. Conversely, OSM-induced EMT was blunted in epithelial/non-CSC expressing exogenous FAM83B. Thus, FAM83B expression is a determinant in maintaining an epithelial phenotype, while suppression of FAM83B is important during EMT. Future work will seek to define novel FAM83B-mediated signaling pathways important for regulating cellular plasticity.

#1976 Dried blood imaging and immunoassay: Spot formation, particle migration, and tumor cell detection. Quanxu Shen,1 Bin Hong.2 1The Fifth Central Hospital of Tianjin, Tianjin, China; 2TeloVISION LLC, West Lafayette, IN.

Blood droplet, when dried, forms a structured sedimentation pattern with central flat, corona ring and peripheral rim. This pattern can be fine-tuned with the blood formula. Surfactant is critical to promote the gelation process, eliminate the film cracking and delamination, and hence, suppress the coffee-ring effect. Additionally, surfactant restrains the shrinkage of the dried film. Without surfactant, the contraction was 100–150 μm for 20 μL of blood; decreased with surfactant added; fully stopped at 10% of concentration. Drying at room temperature with surfactant formed a flat disc-shaped deposit, while at 37°C, volca- nic protrusion was developed. The size of the protrusion depended on the concentration of the surfactant primarily, and the anticoagulant type, evapora- tion velocity, and blood volume. To monitor the dynamic desiccation process, video recording of the evaporating drop was employed. It revealed a quick dis- location of the leukocyte-resembling beads to the outer region of the blood spot, reportedly at velocity of 10μm/sec. The use of 1 and 10μm sized beads further demonstrated layered convective flow below the surface. Beads migrated out- ward primarily before gelation, inward slightly during solidification, and ulti- mately settled down across the spot. The movement of beads was found in the range of a few hundred microns to millimeters before and after desiccation for a 50μL drop. Notably, plasma proteins and saline have influenced the bead distri- bution more than the surfactant. After plasma removal and mixing with surfac- tant, the majority of beads were dragged to the periphery, compared with many beads for no plasma and surfactant, some for whole blood with surfactant, and none for whole blood without surfactant. For a thick blood film, detection of embedded cells and beads can be difficult. Experiments showed that beam of longer wavelength, 532nm, was capable of visualizing all fluorescent cells and beads. Dried blood spot (DBS) assay has been widely used for newborn metabo- lic testing and DNA analysis. To detect rare tumor cells in DBS, the dried pattern was optimized to eliminate the artifacts, stabilize the cells, and enrich the targeted cell. DBS assay holds greater potential for cancer detection when the medical resource is limited.
tissue prevalence of tumors in newly diagnosed individuals. Current methods for quantifying tumor burden are mainly qualitative and include measuring the gross weight of the affected organ, counting tumors on the surface of the organ, or evaluating a small sample of the organ using histologic sections. These methods are crude measures of tumor burden and size distribution, and in the case of histologic sectioning, they are time-consuming, difficult, and prone to measurement bias.

Methods: Animal models of metastasis have been useful in identifying genes that regulate susceptibility to the development and progression of metastasis and have helped to highlight potential novel targets for drug development. In particular, several small animal imaging technologies including magnetic resonance imaging, high frequency ultrasound, and optical imaging have been developed to this task. In this report, we describe specific research projects, based on their unique combination of resolution, image acquisition time, animal throughput, and cost-effectiveness, yet none of these modalities adequately address the need for rapid quantification of tumors across the entire organism, nor do they assess therapeutic effectiveness in eradicating cancer in xenograft models. We have developed an Accelerator Mass Spectrometry (AMS)-based high precision quantitative method for assessing the metastatic potential of primary tumors isolated from newly diagnosed patients.

Results: Our AMS-based methodology to study metastasis uses xenograft cancer cells labeled with $^{14}$C-labeled thymidine that are delivered intravenously into NSG mice and allowed to develop metastatic cancer over the course of up to 10 weeks. At the end of the experiment, all vital organs are collected; the DNA is isolated and analyzed using AMS for fluorescent bisphosphonates. The labeling was optimized to achieve sufficient signal such that a tumor derived from a single cell could be detected by AMS, in secondary tumors, in vivo, independent of histological data. Conclusions: Using this approach we have determined that tissue colonization by tumor cells is a very rare event, where most metastatic tumors are initiated by less than 10 cells delivered into NSG mice. Further optimization of these techniques will allow us to explore the metastatic potential of primary tumors, isolated from biopsies and expanded in Avatar mice. This study was supported in part by NIH P41 M021483 and was conducted under the auspices of the USDOE by LLNL. (DE-AC52-07NA27344). IM number: LLNL-678306.

#1978 Identification of the metastatic cell populations in a spontaneous mouse model of melanoma. Xiaoshuang Li, Raul Torres, Lidia Kos. Florida International University, Miami, FL.

Melanoma is the deadliest form of skin cancer due to its high propensity to metastasize and resistance to current therapies. We have created a spontaneous mouse model of metastatic melanoma (Dct-Grm1/K5-Edn3) where metastasis to the lungs is 80% penetrant. The primary tumors of these mice present cellular heterogeneity with cells at varying levels of differentiation. The main goal of this study is to determine the metastatic potential of the primary tumor resident Tyrosinase positive cells and evaluate the dynamic pattern of gene expression as those cells move from the primary tumors to the sites of metastasis. To accomplish this aim we crossed the Dct-Grm1/K5-Edn3 mice to CreERT2/ROSAmTmG mice to indebly label Tyrosinase cell populations within the primary tumor by topical application of 4-hydroxytamoxifen at the tumor site. In vivo lineage tracing and characterization of those labeled cells was performed in the metastatic xenografts. We found that Tyrosinase positive cells present the highest positive cell frequency and dissemination. We performed RNAseq analysis of those cells and tissue pairs to identify genes that are upregulated in cells that are able to metastasize. Overall, we identified a group of genes important for metastatic melanoma.

#1980 Fusion of cancer and stromal cells imaged by color-coded imaging of metastasis. Miki Nakamura,1 Atsushi Suetsugu,2 Kousuke Hasegawa,1 Takuro Matsumoto,1 Hiromi Aoki,1 Takahiro Kunisada,1 Masahito Shimizu,1 Shigeyo Saji,1 Hisataka Moriwaki,1 Robert M. Hoffman 2.

We report here imaging of stroma during the metastatic process. RFP-EL4 lymphoma cells were injected subcutaneously in C57/BL6 GFP transgenic mice. Subcutaneous tumors were resected and immediately transplanted to the abdominal cavity of BALB/c nude mice. Metastases to the liver, periarterial lymph node, ascites, bone marrow and as well as the primary tumor were imaged. Metastases expressed GFP at low magnification. At higher magnification, yellow-remaining cancer cells resulting from fusion of GFP stromal cells and RFP cancer cells were observed. Metastasis contained fusion of cancer and stromal cells which may be a feature of metastasis.

#1981 Tissue engineered bone for longitudinal intravital microscopy of solid tumor growth and therapy response. Eleonora Dondossola,1 Stephanie Alexander,1 Boris Holzapfel,2 Christopher J. Logothetis,3 Dietmar W. Hutmacher,1 Peter Friedl1.

We report here imaging of stroma during the metastatic process. RFP-EL4 lymphoma cells were injected subcutaneously in C57/BL6 GFP transgenic mice. Subcutaneous tumors were resected and immediately transplanted to the abdominal cavity of BALB/c nude mice. Metastases to the liver, periarterial lymph node, ascites, bone marrow and as well as the primary tumor were imaged. Metastases expressed GFP at low magnification. At higher magnification, yellow-remaining cancer cells resulting from fusion of GFP stromal cells and RFP cancer cells were observed. Metastasis contained fusion of cancer and stromal cells which may be a feature of metastasis.
Renal Cell Carcinoma (RCC) is one of the most lethal urological cancers worldwide, with incidence and mortality rates increasing in the past two decades. The disease does not present early clinical symptoms and is commonly diagnosed at the metastatic stage, leaving the 5-year survival rate at ~10-20.

For a wide array of neoplasms, many key molecular determinants involved in mediating the process of tumor cell growth, invasion, and colonization at a secondary site (i.e. metastasis) have been reported. However, few molecular predictors have been identified for RCC, rendering the mechanisms underlying RCC metastases poorly understood. This results in the lack of effective treatment for patients with advanced RCC. G protein-coupled receptors (GPCRs) and their effectors, such as the arr2 proteins, have been implicated in tumor growth, metastasis, and angiogenesis. Arrestin proteins are well known for their function in the desensitization and trafficking of GPCRs, but have also been implicated in unique signaling pathways to regulate fundamental cellular functions, including cell cycle progression, cell migration, and survival. Furthermore, arrestin involvement has been identified in a number of breast, colorectal, lung, and hematological malignancies. However, the role of arrestin proteins in RCC is yet to be determined.

Our preliminary data show that arr2 proteins levels correlate with growth and metastatic potential in several RCC cell lines, including ACHN and SN12C. We hypothesize that arr2 regulates RCC tumor progression, specifically through involvement in proliferation, invasion, and metastatic processes. To test our hypothesis, we used genetic-based loss of function approaches such as interfering RNA and CRISPR/Cas9. arr2 knockdown results indicated a role in RCC malignancy as it significantly decreases the migration and invasion of RCC cell lines in vitro. arr2 knockout (KO) impairs 3D spheroid formation of these cells and induced morphological changes compared to control cells as observed under confocal microscopy imaging. Moreover, epithelial marker E-cadherin expression levels were elevated while mesenchymal markers twist1, twist2 and vimentin levels were decreased in the arr2 KO cells compared to control, indicating a mesenchymal to epithelial transition. In vivo data support our hypothesis that arr2 plays a critical role in tumor growth and metastasis. Our data suggests a role for arr2 in RCC malignancy and present a possible target in development of therapies for patients with advanced RCC.

CD117 expression and activation induce prostate cancer metastasis. Koran Harris, Li Hong Shi, Taylor Peak, Stephanie Sanders, Aleksander Skardal, Bethany Kerr. Wake Forest University School of Medicine, Winston Salem, NC; Wake Forest Institute for Regenerative Medicine, Winston Salem, NC.
Tyrosine kinase receptors have been shown to drive prostate cancer progression and metastasis. In particular CD117-c-kit expression is upregulated during tumor progression with the highest levels being expressed in bone metastases. In addition, the numbers of circulating CD117+ cells is higher in advanced prostate cancer patients. The presence of these cells in patients’ circulation after radical prostatectomy was also associated with biochemical recurrence. Further, the expression of CD117 can be upregulated on prostate cancer cells after repeated culturing in the bone microenvironment. These data indicated that CD117 expression and activation may be associated with bone metastasis. To test this, LNCaP-C4-2 cells were sorted into CD117+ and negative cell populations. Proliferation, matrigel invasion, and gene expression were compared in the two cell populations in the presence and absence of the ligand stem cell factor (SCF) or CD117 and Akt inhibitors. To track cells during coculture and competition experiments, sorted cells were infected to express mCherry (CD117+) or ZsGreen (negative). Using these cells, competition was tracked using an IncuCyte ZOOM live cell imager for in vitro studies and an IVIS imager for in vivo xenograft studies. To better visualize transendothelial migration, a 3D microfluidic metastasis-on-a-chip was developed to track tumor cell migration through the ECM and across an endothelial cell layer. Here, we examine the activation of CD117 in prostate cancer cell progression and migration. CD117 expression was associated with increased proliferation, beta1 integrin expression, and EMT marker expression. Interestingly, treatment with the CD117 ligand stem cell factor (SCF) reduced proliferation but further enhanced invasion indicating that CD117 activation may drive metastasis. CD117 activation stimulated Akt expression and drove the cancer stem cell phenotype as demonstrated by increased Oct4 and Sox2 expression. CD117 expression was associated with increased expression of cancer progression and EMT signaling pathways. Using live cell imaging, competition between CD117+ and negative cells, and analyzed by molecular signatures of proliferation, scratch healing, invasion, trans-endothelial migration, and sphere formation. Xenograft models also demonstrated competition in vivo. Sectioning of tumors demonstrated the localization and composition of co-injected cells. In vivo fluorescent imaging was used to examine tumor initiation capabilities of the two cell populations. Using 3D microfluidics, we have modeled the competition between CD117+ and negative cell populations during transendothelial migration, CD117 expression on prostate cancer cells drives a more aggressive cell phenotype and may be involved in metastasis. Further, CD117+ cells represent a possible cancer stem cell population.

Breast cancer (BrCa) is one of the most important public health problems in the entire world. Metabolic syndrome (MeS) increases the incidence and aggressiveness of BrCa. C-terminal binding protein 1 (CTB1) is a co-repressor of tumor suppressor genes that is activated by low NAD+/NADH ratio. Recently, we generated a MeS-like experimental mouse model by chronically feeding animals with high fat diet (HFD) and we found that CTB1 and MeS modulated breast carcinogenesis and tumor growth. We also showed that CTB1 and MeS decreased BrCa cell adhesion, a crucial event in the beginning of metastasis. Considering metastasis is still the main cause of death, and around 30% of women with BrCa diagnosed at early stages will progress to metastatic stage, it is crucial to understand the impact of non-inherited factors and the mechanisms underlying this process. The aim of this work was to explore CTB1 and MeS role in BrCa cell migration and metastasis. By wound healing assay, we found that CTB1 increased cell migration of MDA-MB-231 and 4T1 BrCa cells. To study CTB1 and MeS effect in tumor progression, MeS nude mice induced by chronically feeding animals with HFD, and control diet fed animals, were injected with CTB1-depleted expression or –control MDA-MB-231 cells. Six weeks post-injection primary tumors were surgically removed. After two weeks, mice were sacrificed and the presence of metastasis in lung, liver and ascites was analyzed by histology and/or quantified by RT-qPCR using specific primers for human GAPDH. Consistently with the onset of metastasis, MeS increased the number of mice that developed neoplastic ascites (20% in MeS vs. 50% in control) with greater size and number of tumors. To confirm the effect of MeS on tumor cell adhesion we generated a MeS-like experimental mice model by chronically feeding animals with high fat diet (HFD) and we found that CTB1 and MeS modulated breast carcinogenesis and tumor growth. Interestingly, human Vimentin mRNA was induced in TC from ascites compared to primary TC; while it was diminished in lung, suggesting the crucial role of EMT/MET processes in metastasis. Finally, we analyzed expression of cell adhesion and EMT-related genes in primary tumor tissue by RT-qPCR. We found that CTB1 and MeS modulated cell adhesion and EMT expression genes: Vimentin, Slug, ITGB4, ITGB6, Col1A7, FABP4 and PRSS2. Altogether, these results suggest a key role for MeS and CTB1 induced BrCa EMT and metastasis.

Tbk1 loss in pancreatic cancer leads to changes in epithelial plasticity. Victoria Haley Burton, Rolf A. Breken, Melissa Gross, Alberto Bremauntz. UT Southwestern Medical Center, Dallas, TX.

Effective therapies are needed to enhance the long-term survival of patients with pancreatic ductal adenocarcinoma (PDA), which is the fourth leading cause of cancer-related deaths in the United States and eighth worldwide. Initial stages of PDA are commonly characterized by an activating mutation in K-RAS, yet direct inhibition of K-RAS through pharmacological means remains a challenge. Higher levels of TANK Binding Kinase 1 (Tbk1) mRNA, a critical downstream effector of mutant active K-RAS in lung cancer, are associated with poorer overall survival in a cohort of human PDA patients. We hypothesize that Tbk1 is an effective mediator of K-RAS driven pancreatic cancer. Here we report that Tbk1 is expressed and more active in human PDA cell lines relative to normal pancreatic ductal epithelial cells. Using RNA sequencing we found that Tbk1 expression levels were highest in PDA cell lines relative to normal pancreatic ductal epithelial cells. Using live cell imaging, we found that Tbk1 expression was induced in PDA cell lines in response to K-RAS activation. To confirm this, we used CRISPR/Cas9 knockdown to inhibit Tbk1 expression in PDA cell lines. We found that Tbk1 knockdown inhibited K-RAS-driven proliferation, scratch healing, invasion, and sphere formation. These data suggest that Tbk1 is an effective mediator of K-RAS driven pancreatic cancer. We hypothesize that Tbk1 is an effective mediator of K-RAS driven pancreatic cancer. Here we report that Tbk1 is expressed and more active in human PDA cell lines relative to normal pancreatic ductal epithelial cells. Using RNA sequencing we found that Tbk1 expression levels were highest in PDA cell lines relative to normal pancreatic ductal epithelial cells. Using live cell imaging, we found that Tbk1 expression was induced in PDA cell lines in response to K-RAS activation. To confirm this, we used CRISPR/Cas9 knockdown to inhibit Tbk1 expression in PDA cell lines. We found that Tbk1 knockdown inhibited K-RAS-driven proliferation, scratch healing, invasion, and sphere formation.
to immortalized pancreatic epithelial lines (HPN) and fibroblasts. We found that human PDA cell lines are sensitive to a small molecule inhibitor of Tbk1 in the low micromolar range. Further mice engineered to express a mutant kinase dead form of Tbk1 (Tbk1<sup>Δ<sub>KD</sub></sup>) are viable and fertile yet display smaller tumors at early time points in a genetically engineered mouse model of PDA. Interestingly, tumors from Tbk1<sup>Δ<sub>KD</sub></sup>-PDA mice are less aggressive and show significantly less collagen deposition compared to Tbk1<sup>+/+</sup>- PDA tumors. Additionally, cell lines isolated from Tbk1<sup>Δ<sub>KD</sub></sup>- PDA tumors are more epithelial in morphology and less migratory and invasive relative to Tbk1<sup>+/+</sup>- PDA tumors. These results further our understanding of Ras signaling in pancreatic cancer and are critical for exploring a new avenue of targeted therapy.

TUMOR BIOLOGY: Tumor Microenvironment

#1986 Determining the signaling pathway of epithelial-IKKα deletion-mediated symbiotic bacterial and fungal infection in carcinogenesis. Naoyuki Song, Jami Willette-Brown, Feng Zhu, Yinling Hu. NCI-Frederick, Frederick, MD.

Bacteria and fungi, two major components of the microbiota, generally share niches and develop both antagonistic and symbiotic relationships, regulating the pathological impacts on the host. The epithelium is where the bacterial-fungal interaction occurs most abundantly, but the relationship between the epithelium, bacteria, and fungi on the pathogenesis, particularly tumorigenesis, is poorly understood. IKKα is one of the crucial factors regulating the homeostasis of squamous epithelial tissues. Recently, our lab has established a mouse model that develops esophageal squamous cell carcinomas associated with IKKα reduction, inflammation and chronic fungal infection. Claudosporium cladosporioides was a major type of fungi identified in this mouse model. Because IKKα deletion in the keratinocytes causes impaired skin barrier, we hypothesized that loss of epithelial IKKα may control fungal colonization through regulating the barrier integrity and inflammation. We generated IKKα<sup>−/−</sup>- mice with inducible K15.Cre (IKKα<sup>−/−</sup>/K15.Cre) specifically expressed in keratinocytes in hair follicles which is considered as skin stem cells. After deleting IKKα in K15 cells in oral mucosa and skin, IKKα<sup>−/−</sup>/K15.Cre mice were orally inoculated with Claudosporium cladosporioides. We found that epithelial IKKα deletion increased bacterial colonization in oral mucosa and skin. Moreover, Claudosporium infection further promoted bacterial and fungal colonization in oral cavity and development of skin tumors. Taken together, our data suggested that epithelial IKKα induces the bacterial-fungal symbiosis in oral mucosa, promoting skin tumors. This study will shed light on the importance of the epithelial-bacterial-fungal interaction in the pathogenesis, proposing epithelial IKKα as a novel regulator of the bacterial-fungal interaction.

#1987 Inhibition of collagen receptor discoidin domain receptor-1 (DDR1) reduces gastric cancer cell motility and metastasis. Ryo Yuge, Y. Suzuki Kimata, Hidetsugu Takigawa, Shinya Tanaka, Kazuaki Chayama, Wataru Yasui, Hiroshima Univ., Hiroshima, Japan; Hiroshima Prefectural Univ., Hiroshima, Japan.

Accumulating evidences suggest that a unique set of receptor tyrosine kinases, known as discoidin domain receptors (DDRs), play a role in cancer progression by interaction with their surrounding collagen matrix. Although abnormal expression of DDRs is reported in some human cancers, little is known about the expression and function of DDRs in gastric cancer progression. In this study, we investigated expression and the role of DDR1 in the tumor progression of human gastric cancer. The expression and distribution of DDR1 was analyzed by immunohistochemistry in 127 human gastric cancer cases, and the expression level of DDR1 in 7 human gastric cancer cell lines was analyzed by RT-PCR and western blotting. Proliferation, migration, invasion, and tube formation assays were conducted in DDR1 silenced gastric cancer cells. The effect of DDR1 on the tumor growth and metastasis were examined in orthotopically implanted nude mice model and a model of liver metastasis. DDR1 was expressed in human gastric cancer cell lines, and expression of DDR1 in human gastric tumor was associated with poor prognosis. Among 7 gastric cancer cell lines, MNK74 expressed DDR1 at highest level. DDR1-silenced MNK74 cells showed unaughterd proliferation activity. In contrast, migration, invasion, and tube formation were significantly reduced. When examined in an orthotopic nude mouse model, DDR1-silenced implanted tumor significantly decreased angiogenesis and lymphangiogenesis, thereby leading to reduction of lymph node metastasis and liver metastasis. We showed that treatment of DDR1 inhibitor also inhibited lymph node metastasis in an orthotopic nude mouse model. These results suggest that DDR1 is involved in GC tumor progression and metastasis. DDR1 could be not only an independent prognostic classifier, but also a promising therapeutic target in patients with GC.

#1988 Exosome-mediated ovarian cancer tumorigenesis mediated by miR1246/Rb/Cav1 axis. Pinar Kanlikilicer, Recep Bayraktar, Mohammed Rashid, Burcu Aslan, George A. Calin, Anil K. Sood, Gabriel Lopez-Berestein. MD Anderson Cancer Center, Houston, TX.

Exosomes are secreted from many cell types and play an important role in the tumor microenvironment. The most impressive breakthrough in exosomes research was that they contain the genetic material of the host cell. However, whether cancer cells use their exosomes to transfer oncogenic material to recipient cells, or to get rid of their tumour suppressor material is not well understood. We previously identified that miR-1246 was hundreds of folds higher expressed in six different ovarian cancer exosomes compared to their originating cells. Here, we showed that miR-1246 co-localized in the exosomes in ovarian cancer cells. miR-1246 act as an oncogenic miRNA and the levels were elevated in ovarian cancer patients compared to health donors. We also demonstrated that miR-1246 inhibited tumor growth in combination with CAV-1, a known anti-tumor gene. This result suggested that miR-1246 significantly inhibited tumor burden in SKOV3-ip1 orthotopic ovarian cancer model. Our results suggest that miR-1246 inhibited RB tumor suppressor protein and regulate CAV-1 and platelet-derived growth factor receptor beta precursor signaling in ovarian cancer. In addition Inhibiting miR-1246 led a significant decrease in exosome release. Together, our findings provide strong evidence that oncogenic miR-1246 can be targeted as a potential novel therapeutic approach in the treatment of ovarian cancer.

#1989 Characterization and integration of mantle cell lymphoma microenvironments are determinant for the development of rational targeted therapies. David Chiron, Celine Bellanger, Antoine Papin, Benoit Tessoulin, Christine Douset, Sophie Maiga, Philippe Moreau, Cyrille Touzeau, Steven Le Gouill, Martine Amiot, Catherine Pellat-deceunynck, CRCNA, INSERM, CNRS, Université de Nantes, Nantes, France; Service d’Hématologie Clinique, Unité d’Investigation Clinique, CHU, Nantes, France.

Mantle cell lymphoma (MCL) accumulates in lymphoid follicles (FL) and disseminates early on in extranodal tissues. Recent effort has been invested on the identification of intrinsic MCL abnormalities but little attention has been paid to the importance of the surrounding cells and soluble factors in this pathology. Further investigations that integrate the key role of the microenvironments are now needed to overcome drug resistance in this incurable malignancy. Despite a significant level of the proliferation index Ki67 in LN, we did not detect any proliferating peripheral blood (PB) MCL cells, suggesting a major role of the tumor ecosystem. To determine whether stromal (hMSC) or lymphoid-like (CD40L) interactions could support survival and proliferation, primary circulating MCL cells were cocultured ex vivo. In all the 21 samples tested, CD40L, but not hMSC, induced cell-cycle progression that was amplified by a MCL-specific cytokine cocktail (Ck). To determine the microenvironment-dependent molecular modulations, RNA-seq in MCL cells from PB or cocultured was performed (n=8) and compared with genes expressed in MCL cells from LN and PB (Geo, PB n=77, LN n=107). More than 65 % of genes induced by “CD40L+Ck” were also upregulated in the LN. Our model recapitulated molecular signatures that are characteristic of MCL i.e., cell cycle, BCR, NFkB, NIK and survival, confirming the relevance of the coculture. We further studied the coculture-induced regulation of the genes belonging to the survival signature. The major regulation was an increase in expression of Bcl-xL protein associated with a striking downregulation of Bcl2 and Bim. Using the functional BH3-profiling assay, we demonstrated that, whereas PB MCL cells were dependent on Bcl2 for survival, Bcl-xL upregulation was responsible for loss of mitochondrial priming and drug resistance. Consequently, whereas clinically available Bcl-xL-mimetic efficiently triggered apoptosis in PB MCL cells, protected by the microenvironment were resistant. We then hypothesized that targeting Bcl-xL could increase treatment efficacy. By integration of microenvironment modulation using our coculture model, we developed efficient targeted strategies (i.e.: BTK inhibitor, Type II anti-CD20), which counteract Bcl-xL overexpression and overcome drug resistance in primary cells ex vivo. This strategy should target cells protected into their niches and our ongoing OAIs Trial (NCT02558816) will rapidly determine in vivo efficacy in MCL. In summary, we reported here the development of a relevant model that provides new insights into the microenvironment-dependent regulation of proliferation and Bcl-2 family expression.
Our increased understanding of intrinsic abnormalities and the integration of extrinsic signaling offer new opportunities to design mechanism-based strategies to overcome drug resistance in MCL and other B cell malignancies.

#1990 Single cell co-expression profiles of immunotherapy targets in the tumor microenvironment of NSCLC samples. Jeffrey Kim, Nan Su, Xiao-Jun Ma, Emily Park. Advanced Cell Diagnostics, Newark, CA.

Cancer immunotherapies have made great strides in recent years, yet identifying key molecules in the tumor microenvironment is necessary to develop improved approaches to achieve more durable responses in a greater number of patients. Obstacles such as adaptive resistance to individual checkpoint marker blockade, such as PD1 and CTLA4, have led researchers down the path of pursuing multiple immune targets that may be upregulated on the same lymphocyte as a consequence of mono-antibody therapies. While several clinical trials taking a combinatorial approach are currently in progress, including targeting multiple checkpoint markers, it is clear that these new strategies may rely on the pre-existing immune system, regardless of being treatment naïve or previously treated. The challenge now is to identify the relevant biomarkers and to stratify patients to provide the most appropriate treatment. As a proof of concept, here we evaluate single cell co-expression profiles of key therapeutic targets in archived tissues of non-small cell lung cancer (NSCLC) samples using the RNAseq® Duplex Assay, an advanced in situ hybridization platform that allows for the visualization of single cell gene expression of mRNA targets directly in tissue. In our investigation of 60 archived formalin-fixed paraffin embedded NSCLC tissues, co-expression profiles of PD1 or PD-L1 coupled with another therapeutic target (IDO1, LAG3, 4-1BB, GITR, and TIM3) were assessed. With an ingenuity that led to slight variations in the tumor microenvironment between tissue samples. These marked differences of co-expressed targets on tumor infiltrating lymphocytes were scrutinized through quantitative image analysis to further reveal both the diversity and degree of individuality between tissue samples. Quantitatively measuring the expression of multiple targets in single cells within intact tissues exposed the distinct milieu of targetable therapeutic molecules, leading to the potential identification of responsive tumors which could help guide in stratifying patients for different combinatorial approaches.


Purpose: Despite improvements in care and advancements in the understanding of ovarian cancer (OC) pathobiology and genetic alterations, the survival rate is disappointingly low when compared to that of other types of cancer. One factor contributing to the poor survival rate is the development of chemotherapeutic resistance and a high rate of recurrent tumor growth in the months and years following primary OC treatment. Our objective was to identify potential new therapeutic targets for prevention of recurrence through examination of changes in gene regulation that occur in recurrent versus matched primary tumors. Procedures: We used 16 primary-recurrent tumor pairs from patients with stage III/IV serous epithelial OC from the Duke Gynecologic Oncology Tissue Bank. Illumina Infinium HumanMethylation450 BeadChips and Affymetrix microarrays were used to assess methylation changes in androgen gene expression, respectively. Expression and methylation of POSTN and COL11A1 were analyzed using an independent OC dataset comprising 38 women with < 3 years survival and 26 women with > 7 years survival, 3 normal ovarian surface epithelium (OSE) and 2 fallopian tube fimbriae epithelium (FITE) samples. siRNA knockdown of POSTN and COL11A1 in OC cells was followed by expression and proliferation assays. Results: Patients with recurrence had significantly higher expression of POSTN and COL11A1, with a 2-fold difference for 13 of the 1,569 significantly differentially expressed genes, seven with increased expression in recurrent OC (POSTN, COL11A1, MMP1, MMP13, TNC, ASFN, and EPYC), and six with decreased expression (GATA6, PEG3, SST, MAOB, TSPAN8, and C7). Interestingly, all seven up-regulated genes are involved in extracellular matrix (ECM) functions, an important component of the microenvironment. Furthermore, changes in androgen gene expression were used to guide in stratifying patients for different combinatorial approaches.

#1992 Changes in gene expression of stromal and epithelial cells during prostate cancer progression. Milos Babic,1 Elliot Imler,1 Peter Shepard,2 Joanne Yeakley,1 Raymond Nagle,3 Bruce Seligmann1. BioSpyder Technologies, Inc., Carlsbad, CA; 2University of Arizona, Tucson, AZ.

Prostatic intraepithelial neoplasia (PIN) is a histologic abnormality which arises within the secretory epithelium of prostate glands, without disrupting the layer of basal cells which separates the epithelium from the surrounding stroma. PINs are generally considered to be a step towards development of prostatic adenocarcinoma (PCA). Molecular steps in the transition from normal glands to PIN to PCA are poorly understood, in part due to the difficulty of accessing and characterizing individual PINs without contami- nation from surrounding stroma. Furthermore, the surrounding stroma surrounding pre-cancerous glands may play an active role in cancer development and progression, but are difficult to profile. We utilized the ultra-sensitive and quantitative TempO-Seq gene expression assay of the whole transcriptome to profile microdissections of stromal vs. epithelial cells in archival prostatectomy FFPE tissue. TempO-Seq is highly insensitive to RNA degradation (measurements of intact RNA RIN = 9.1 to degraded RNA RIN = 3.0 correlate with R² = 0.97) and does not require RNA extraction, allowing measurement of both soluble and insoluble cross-linked RNA. This allowed deparaffinization and H&E staining of prostate sections prior to the assay, so morphologic clarity was clearly visible and subsections could be readily extracted and profiled. Microdissections were performed on individual 5 μm FFPE sections of prostatectomy specimens with regions of 6 containing PIN alongside PCA. We removed an ~300 μm diameter region of stroma surrounding individual normal, PIN, and PCA glands, followed by targeted dissection of the gland epithelium itself which was lysed and assayed separately. Stromata and epithelial samples from 3 adjacent sections of the same glands were pooled together (adding up to ~0.005 mm³ tissue/repli- cate), and then profiled. We compared the whole transcriptome expression profile of stroma vs. epithelium at every stage of progression. Our results indicate that stroma and epithelium undergo separate and distinct changes in gene expression patterns throughout the development of PCA, most notably in genes involved in cell-cell signaling. Specifically, we found that changes in CAMP signaling in stromal cells accompanied the shift from normal glands to PIN. Furthermore, changes in androgen signaling pathways were present in stromal cells as well as in epithelium during the transition from PIN to invasive carcinoma. These results shed light on the molecular progression to PCA and demonstrate the feasibility of profiling gene expression at the level of tissue microstructure to elucidate changes within the tissue microenvironment. Since archival FFPE can be used, studies of progression can be carried out and the findings translated to diagnosis and prognosis as well as to the investigation of new therapeutic approaches.

#1993 Acidic tumor microenvironment stimulation of GPR4 alters cytoskeletal dynamics and migration of vascular endothelial cells. Elizabeth A. Krewson, Li V. Yang, Lixue Dong. East Carolina University, Greenville, NC.

The acidic tumor microenvironment results from aberrant vasculature, insufficient oxygen delivery and aerobic glycolysis of cancer cells. As cancer progresses, the acidic microenvironment can lead to structural changes of endothelial cells (ECs) resulting in increased vessel permeability which may increase metastasis. Extracellular acidification can activate cellular signaling molecules, including G-protein coupled receptor 4 (GPR4), which is prominently expressed in ECs. We have previously reported that acidic activation of GPR4 induces the transcription of several pro-inflammatory and ER-stress related genes in ECs. The purpose of the study is to identify the functional response of acidity-induced GPR4 activity using Human Umbilical Vein Endothelial Cells (HUVECs) as a model system. The expression of GPR4 was overexpressed (HUVEC/GPR4) or knocked down (GPR4 shRNA) using several genetic constructs. HUVECs were treated with media buffered to pH 6.4 or to physiological pH 7.4. Permeability of a HUVEC cell monolayer was assessed by measuring gap formation as one indicator. Cell migration was assessed by wound healing assays. Cytoskeletal changes in androgen gene expression were used to guide in stratifying patients for different combinatorial approaches.

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Neoadjuvant chemotherapy (NACT) is used in breast cancer to decrease tumor and lymph node burden and the extent of surgery. However, response is variable and prediction poor. Stromal cells are involved in breast cancer progression and drug resistance although very little is known about the mechanisms. Our research aims to characterize breast cancer-associated fibroblasts (CAFs) and peritumoral fibroblasts (PTFs) that predict response to NACT and could be used to influence treatment choices. Pre-NACT core biopsies from cancer-associated and peritumoral tissues were collected under ultrasound guidance. To date, mRNAs from early passage (passage ≤ 5) fibroblasts isolated from cores of 23 matched pairs (CAFs and PTFs) (8 triple-negative (TNBC), 7 ER +Her2+ and 8 ER +Her2-) and an additional 8 PT samples (4TNBC, 1 ER +Her2+ and 3 ER +Her2-) were analysed by whole-genome expression array (Illumina beadchip HT12v4). Data were analysed with Partek® Genom Suite™ 6.6 and Pathway softwares (Partek Inc., St. Louis, MO, USA).

Differentially expressed genes (DEGs) were identified (t-test, p-value ≤ 0.05, fold-change (FC) ≥ 1.3) between CAFs and PTFs and between NACT responders (Residual Cancer Burden (RCB) 0/1) and non-responders (RCB ≥ 2). A significant difference between CAFs and PTFs was seen by principal component analysis and t-test. PTFs showed similar mRNA levels of major myofibroblast markers (e.g. FAP, ASMA) with only FSP1 being lower in PTFs compared to CAFs (FC = -1.4, p-value = 0.023), suggesting that PTFs, like CAFs, are activated myofibroblasts. We then tried to identify a gene signature in CAFs or PTFs that could predict response to NACT. Comparing RCB 0/1 vs RCB 2/3 resulted in very few DEGs due to cancer subtype variation in both CAFs and PTFs.

Similar to other studies, only 2/19 patients in our cohort with ER + tumours achieved RCB 0/1, preventing analysis of these samples. However, 45% (5/11) TNBC responded to NACT and we identified 566 DEGs between RCB 0/1 and RCB 2/3 CAFs, with changes in metabolism, PtSiK/ACT pathway, proteoglycan synthesis or ECM-receptor interaction. For PTFs, 186 DEGs were identified, with changes in Wnt signalling and ECM-receptor pathways. Microarray data from our uniquely large sample set of 23 pairs of CAFs and PTFs from breast cancer patients suggest that PTFs have a similar gene expression profile to CAFs. Although others have demonstrated that CAF phenotype is breast cancer subtype dependent, we are the first to show similar observations in PTFs. Finally, we identified gene signatures from both CAFs and PTFs that relate to RCB response in TNBC. We are currently in the process of confirming the most highly dysregulated genes by qPCR and/or immunohistochemistry analysis.

The omentum promotes ovarian cancer cell survival by increasing cell cycle duration and chemoresistance. Carlos Cardenas, Ayeshas B. Alvero, Mary Pitruzzello, Roslyn Tedja, Gil G. Mor. Yale University School of Medicine, New Haven, CT.

Background: Ovarian cancer is the most lethal of all gynecologic cancers. It is characterized by the presence of carcinomatosis at time of presentation and chemoresistant micrometastatic seedlings at the time of recurrence. The omentum, an adipocyte-rich organ, is a frequent site of ovarian cancer metastasis in patients and the most common site of post-chemotherapy residual disease in animal models. Whereas previous studies have shown how the adipocyte microenvironment can induce metabolic re-programming in cancer cells, it is not clear if it can regulate pathways that affect chemoresistance. The objective of this study is to determine the effect of the adipocyte microenvironment on pathways that control cell cycle and apoptosis. Methods: In vitro: Patient-derived epithelial ovarian cancer cell lines were cultured in the presence of abundance of omentum conditioned-media. Cell growth was determined by measuring culture confluence using Incucyte®. Protein expression was determined by western blot analysis. In vivo: Patient-derived ovarian cancer xenografts were established intra-peritoneally in nude mice. Tumor implants from adipocyte-rich organs such as the omentum, pelvis fat, mesentery and tumor implants from non-adipocyte-rich organs such as liver, ovaries, and GI tract were collected and analyzed independently. Results: Compared to cells cultured in growth media, epithelial ovarian cancer cells cultured in omentum CM demonstrated a more fibroblastic morphology characterized by elongated shape and bipolarity, significantly slower growth kinetics (p < 0.0001) accompanied by upregulation of the cell cycle inhibitor, p27, and enhanced secretion of the proangiogenic factor, VEGF. More importantly, these cells express higher levels of the anti-apoptotic proteins Bcl2 and Mcl-1. Consequently, ovarian cancer cells in growth media treated with Carboplatin had a IC50 of 14.5 μg/ml. In contrast, cells cultured in omentum CM demonstrated enhanced resistance to carboplatin with IC50 of > 50 μg/ml. Analysis of tumor implants in vivo showed similar results as observed in vitro. Thus, tumor implants isolated from adipocyte-rich organs express higher levels of p27, Bcl2, and Mcl-1. Conclusion: We demonstrate a significant effect of the adipocyte microenvironment on chemoresistance in the phenotype of ovarian cancer cell characterized by morphological changes chemoresistance and growth rate. These findings highlight the importance of the adipocyte microenvironment in the progression of ovarian cancer. Further studies that can identify specific therapeutic targets in the adipocyte-educated chemoresistant ovarian cancer cells may aid in the development of novel therapies and improve patient survival.

CXCL12 drives selective expansion of AKT-induced quiescent, preeuroplastic breast cells. Zahra S. Masoud,1 Lisa Kim,2 Kelsey J. Weigel,2 Matthew M. Champion,2 Zachary T. Schafer,2 Cheuk Leung1.1University of Minnesota, Minneapolis, MN; 2University of Notre Dame, Notre Dame, IN.

A critical stage of premalignant development in breast tumorigenesis is the emergence of preeuroplastic cells that carry key oncogenic drivers yet remain quiescent. Preeuroplastic cells maintain a state of quiescence, but may be triggered to proliferate through largely unknown factors. Expansion of these cells is key to driving clonal evolution, which is evident in early lesions and tissue fields surrounding tumors. Although preeuroplastic development is determinant to successful evolution and formation of tumor growth, the cellular mechanisms controlling preeuroplastic development is unclear. Our limited mechanistic understanding of quiescent, preeuroplastic cell outgrowth highlights a major knowledge gap in the early control of tumor development. Advancements in detecting molecular changes in precancerous lesions have provided opportunities for devising early intervention strategies. Aberrant AKT activation has emerged as a founding oncogenic alteration in preeuroplastic cells. While molecular changes predispose these cells to develop tumors and presumably favors their selection during early tumor development, how these alterations contribute to the cellular control of quiescent preeuroplastic cells remains unknown. A major barrier to studying quiescent preeuroplastic cells is the lack of physiologically relevant experimental models. Here, we utilized a novel quiescent, preeuroplastic cell model that reconstructs the molecular and cellular contexts of preeuroplastic breast tissue. We combined a three-dimensional (3D) organotypic cell culture on reconstituted basement membrane with cells that can be induced to activate defined oncogenic pathways. Using this model, we investigated the contribution of aberrant AKT activation in preeuroplastic breast tumor development. We found that aberrant AKT signaling in quiescent, preeuroplastic mammary cells does not stimulate cells to proliferate under normal conditions, but confers cell sensitivity to stroma-derived CXCL12 through upregulating the receptor CXCR4. Using heterotypic co-culture to recapitulate a tumor-promoting environment and quantitative proteomic analysis, we found that stromal-derived chemokine CXCL12 selectively promotes expansion of preeuroplastic cells with hyperactivated AKT signaling. Mechanistically, we showed that AKT signaling induces the expression of the chemokine’s receptor, CXCR4, which confers the selective sensitivity to CXCL12. This CXCL12-CXCR4 axis serves as an entry point for oncogenic signaling through PKA and ERK to drive the downregulation of CDKN1C and promote cell proliferation in AKT-induced preeuroplastic cells. Our studies uncovered a distinct role of AKT signaling in promoting preeuroplastic development. Furthermore, our studies suggest a model of preeuroplastic clonal expansion where oncogene signaling confers quiescent preeuroplastic cell sensitivity to alternate mitogenic stimuli in a permissive tissue microenvironment.

Charting the DNA methylation landscape of prostate cancer associated fibroblasts. Mitchell G. Lawrence,1 Ruth Pidsley,2 Elena Zotenko,2 Birunthi Niranjani,1 Aaron Statham,3 Jenny Song,1 Nicola Armstrong,2 John Pedersen,2 Mark Frydenberg,1 Renea Taylor,1 Clare Stitzer,1 Gail Risbriger,1 Susan Clark.1 1Monash University, Melbourne, Australia; 2Garvan Institute of Medical Research, Sydney, Australia; 3TissuePath Pathology, Melbourne, Australia.

Most studies of the genome and epigenome landscape of tumors focus on the aberrations in epithelial cancer cells. However, the growth and progression of solid tumors involves dynamic cross-talk between cancer epithelium and the surrounding microenvironment, including cancer-associated fibroblasts (CAFs). The molecular landscape of these cells is still poorly understood, so it is important to define the epigenetic features that underpin the functional differences between CAFs and non-malignant prostate fibroblasts (NFs) and influence cancer progression. Therefore, we used whole-genome bisulfite sequencing to chart the epigenome landscape of CAFs at single base resolution. Our data...
revealed that NPFs and CAFs from localized prostate cancer have remarkably different epigenome profiles characterized by locus-specific rather than global changes in DNA methylation. CAFs exhibited more than 7500 differentially methylated regions compared to NPFs. In contrast, no recurrent genomic aberrations were detected in CAFs, emphasizing the importance of epigenome changes in their roles as hallmarks of tumor heterogeneity. Their unique epigenetic modifications in the tumour and its microenvironment. Collectively, these data demonstrate that CAFs are defined by an enduring epigenetic signature that comprises a distinct set of locus-specific DNA methylation alterations in cancer-related regulatory loci. The distinct methylyome of CAFs provides a novel epigenetic hallmark of the cancer microenvironment and promises new biomarkers to improve interpretation of diagnostic samples.

#1998 Transcriptome analysis of hypoxic head and neck cancer cells uncovers intron retention in EIF2B5 as a mechanism to reduce protein synthesis. Lauren K. Brady,1 Hejia Wang,1 Caleb Radens,1 Milan Radovich,2 Amit Maity,1 Cristina Ivan,1 Mircea Ivan,1 Joseph Barash,1 Constantinou Koumenis.1
1University of Pennsylvania, Philadelphia, PA; 2Indiana University, Indianapolis, IN; 3University of Texas, MD Anderson Cancer Center, Houston, TX.
Hypoxia is a key feature of solid tumors that contributes to resistance to therapy and reduced overall survival. Cellular adaptation to the hypoxic tumor microenvironment involves attenuation of energy-consuming processes such as macromolecular synthesis. We have identified RNA processing as another major process which is globally downregulated under hypoxic stress. By sequencing RNA of normoxic and hypoxic head and neck cancer cells, we observed widespread repression of genes that regulate RNA splicing and processing. As a result, we observed over 1,000 changes in relative mRNA isoform expression, including a significant increase in hypoxia-induced intron retention in nearly 100 genes. Genes observed to undergo intron retention in hypoxia included major regulators of RNA processing and protein synthesis, such as the translation initiation factor EIF2B5. Surprisingly, hypoxia-induced intron retention in EIF2B5 creates a premature termination codon, which results in a truncated protein isoform of eIF2Be. We provide evidence that this isoform acts in opposition to the full-length protein to inhibit translation under stringent hypoxic conditions. Moreover, this intron is expressed in solid cancers known to contain hypoxic fractions and is overexpressed in head and neck cancer in a stage-dependent manner compared to normal tissue. To investigate how this intron is retained, we are examining the role of hypoxia-mediated changes in phosphorylation of the C-terminal domain (CTD) of RNA polymerase II. Our data suggest that increased phosphorylation of the CTD may lead to transcriptional pausing and subsequent retention of intron 12 in EIF2B5. Furthermore, distinct sequence features at this locus, such as a weak splice site at the intron12:exon13 junction, may explain how this intron is selectively retained in hypoxic cells. These results implicate mRNA splicing in hypoxia-related mechanisms that are key to cellular adaptation under hypoxia, which in turn contribute to cellular adaptation to stress.

#1999 Cofactor Paragon: a novel tool to analyze the tumor microenvironment using RNAseq. Ryan Bloom, Raman Talwar, Jeff Hiken, Jon Armstrong, Cofactor Genomics, St. Louis, MO.
The impact of cancer immunotherapy on clinical cancer care is growing rapidly. However, there are several mechanisms by which a tumor can avoid recognition and/or elimination by the immune system. Immune recognition, activation, and infiltration are each required for effective clearance of a tumor by the immune system. Impairment of any of these mechanisms requires a different therapeutic strategy or combination of strategies to significantly address. The development of new multi-modal biomarker technologies that have the ability to provide insight into each of these factors in a cost-effective manner is essential to effectively guide treatment choice for patients and to discover new therapeutic targets. We have developed Paragon, which is an RNA sequencing based panel that utilizes novel informatics technologies to provide a comprehensive picture of a tumor’s transcriptomic and immune micro-environment. There are three distinct outputs of Paragon: measurement of the expression levels of ten immune checkpoint genes, quantification of the total mutational burden of the tumor, and the levels of infiltration of 24 different immune cell subtypes within the tumor microenvironment. These outputs collectively inform the various mechanisms that tumors use to evade recognition and clearance by the immune system. We show that Cofactor Paragon accurately reports on gene expression, mutational burden and infiltrating leukocytes compared to gold standard methods from just 20 ng of FFPE tumor RNA. We further analyze the data to show the correlation between mutational burden and infiltrating CD4+ and CD8+ T-cell levels. Finally, we show how Paragon can identify patients with different subtypes of tumors that are more likely to respond with resistance to PD-1 blockade therapy. These results position Cofactor Paragon as a novel tool that can help identify ideal therapeutic strategies in immunoncology.

#2000 Targeted characterization of tumor heterogeneity through RNA-Seq analysis of phenotypically defined subpopulations. Warren Porter, Eileen Snowden, Friedrich Hahn, Mitchell Ferguson, Frances Tong, Shannon Dillmore, Rainer Blaessius. BD Technologies, RTP, NC.
Cancer progression and metastases have been linked to the complex heterogeneity found within many solid tumors. In order to understand the complexities within solid tumors there is a need for in-depth characterization of the tumor and the surrounding microenvironment, including the identification and measurement of the subpopulations found within the diseased tissue. Here we describe a method to interrogate the heterogeneity within patient derived xenograft (PDx) derived solid tumors through tissue dissociation, single cell flow sorting, and expression analysis of the resulting subpopulations. Cell sorting enables the identification and capture of phenotypically defined subpopulations within the tumor microenvironment for additional downstream analysis. Whole transcriptome RNA-Seq was performed on samples collected throughout the workflow to characterize the expression changes in cells during the different steps of the process. Expression differences were measured before and after tissue dissociation, before and after incubation, and before and after cell sorting. Expression differences were also measured between phenotypically defined subpopulations sorted by the CD133 and CD49f surface markers. Several significantly differentially expressed gene combinations between samples were observed. We also found unique pathway characteristics for the different subpopulations sorted based on expression of CD133 and CD49f markers. In contrast, changes induced by the workflow process alone were restricted to a small and well-defined gene subset of immediate early genes (IEGs). Within the workflow, these changes were restricted to the tumor dissociation step and no changes were detected after incubating and/or cell sorting the dissociated cells. Our results indicate that solid tumor dissociation followed by flow cytometric analysis and sorting enables the interrogation of phenotypically defined subpopulations found within solid tumors. Since workflow induced changes are minimal and defined, these methods reveal biological differences between subpopulations within and between cancers. Differential expression of numerous surface marker genes were identified by our method allowing discovery of other candidate surface markers that may extend the subpopulation structure. Once verified these additional surface markers could serve to elucidate the dynamic changes cancer tissue undergoes during progression as well as in response to selective pressures such as drug therapy.

#2001 Preclinical investigation of a 15 gene hypoxia profile in different prostate cancer cell lines. Thomas R. Wittenborn,1 Brita S. Sorensen,1 Morten Buus,1 Mathilde B. Thomsen,2 Steffen Nielsen,1 Michael R. Horssman,1 1Aarhus University Hospital, Aarhus C, Denmark; 2Aarhus University Hospital, Aarhus N, Denmark.
Purpose: Hypoxia is known to reduce the response to radiotherapy and has been found to be a common feature in prostate cancer. Identifying patients with hypoxic tumors will allow us to select those patients that require specific hypoxic modifiers. Previously our department has developed a gene profile consisting of 15 genes, which demonstrated prognostic and predictive impact for hypoxic modification in head and neck squamous cell carcinomas (HNSCC). In the current study we investigated the 15 gene profile in vitro in different prostate cancer cell lines, and subsequently in vivo in the PC3 xenograft model. Methods: For the in vitro experiments the prostate cancer cell lines investigated were PC-3, LNCaP and DU-145. Cell lines were cultured under normoxic (21% O2) or varying hypoxic conditions (0%, 0.5%, 1.0%, 2.0%, and 5.0% O2) for 24 hours, totalRNA was extracted and gene expression levels measured by qPCR. Reference gene references were selected (PMSM4, TBP, NDFIP1) and applied in the normalization of the relative expression levels. For all conditions, the reference gene used previously in the HNSCC study was selected. In vivo experiments, the PC3 cell line was inoculated on the flank of female NMRI nu/nu mice. Two hypoxia-sensitive traces (18F-FAZA and Pimonidazole) were administered in order to determine hypoxic and non-hypoxic regions on excised tumor sections. These regions were isolated by laser-assisted microdissection, after which totalRNA was ex-
trated and gene expression levels measured by qPCR. Results: In the in vitro experiments, all three prostate cancer cell lines had 14 of the 15 genes induced by hypoxia, when comparing the anoxic cells (0% O2) to the normoxic cells (21% O2). The only discrepancy was ALDOA, which was not upregulated in the hypoxic profile in different prostate cancer cell lines, both in vitro and in vivo. An identified induction was observed in both types of experiments, however with a differential gene expression with regard to hypoxia level. From the performed experiments, and looking only at oxygen dependency, it appears that the gene profile could be suitable for prostate cancers as well as HNSCC. Funding sources: Danish Cancer Society; The Danish Council for Independent Research: Medical Sciences; EC FP7 project METOXIA (project no. 222741); CIRO: The Lundbeck Foundation Center for Interventional Research in Radiation Oncology and The Danish Council for Strategic Research.


Despite the fact that the immunosuppressive tumor microenvironment (ISTME) has long been recognized as a major hurdle that significantly limits the anticancer efficacy of various therapeutic strategies, including T-cell-based immunotherapy, there is currently no effective way to address this challenge. In this work, we prove that sequential photothermal therapy (PTT), mediated with stealthy laser-triggerable nanobombs composed of iron oxide nanoparticles (IONPs) following systemic delivery, can remove both pre-resident and newly triggered immunosuppressor cells in established tumors to enhance the efficacy of T-cell anticancer therapy. Using flow cytometry to study various T-cell populations in tumor tissues 48 h post PTT in a 4T1 mouse model, our data suggest that IONP-mediated PTT could significantly reduce the CD4+FoxP3+ regulatory T-cell population. Our data also suggest that CD8+ cytokine T-cell populations in PTT-treated tumor tissues return to those of non-treated tumors, and are believed to be newly activated at the tumor site or recruited from periphery blood following PTT. Our data further suggest that PTT twice spaced by a 24 h interval has the best anticancer efficacy, compared to PTT once or three times at 24 h increments. This enhanced anticancer effect from the sequential IONP-mediated PTT (twice) is believed to be due to an enhanced ability to eliminate triggered immunosuppressor cells that respond faster than cytokine CD8+ T cells. Furthermore, our Luminescence analysis of chemokine serum concentrations indicates that IONP-mediated PTT can decrease suppressor-cell-attractive protein secretion, such as G-CSF. In addition, our immunohistochemistry data from tumor tissue staining indicate that IONP-mediated PTT down-regulates tumor cell PD-L1 expression to disrupt tumor-cell-mediated immunosuppression. The capability of IONP-mediated PTT to disrupt ISTME can significantly enhance T-cell antitumor efficacy toward both treated established tumors and distal tumor cells. When combining IONP-mediated PTT with anti-CTLA-4 therapy to block CD8+ T-cell inhibition, our data suggest that combination treatment significantly inhibits tumor growth (p < 0.01), while control treatments, including the antibody alone, IONP-mediated PTT alone, and mock treatment, fail to do so. Our data further show that in a metastasis-mimic model, mice (6/6) that had primary tumors treated with PTT/anti-CTLA-4 reject pre-inoculated cancer cells at a distant site. Our data also show that the majority of the PTT/anti-CTLA-4 treatment-cured mice can reject subcutaneously (8/12) or tail-vein injected 4T1 tumor cells (9/9), indicating memory T-cell immune surveillance. In conclusion, this study provides a nanotechnology-based novel strategy to effectively disrupt not only pre-existing but also treatment-induced ISTME to boost T-cell-based cancer immunotherapy.


T cell immunoreceptor with Ig and ITIM domains (TIGIT) is a co-inhibitory molecule containing an immunoreceptor tyrosine-based inhibition motif (ITIM) within its cytoplasmic tail, and is highly expressed on regulatory T cells and activated CD4+ T, CD8+ T, and NK cells. TIGIT competes with CD226, which contains an immunoreceptor tyrosine-based activation motif (ITAM) within its cytoplasmic tail. The clinical candidate, OMP-313M32 binds human TIGIT but not rodent and non-human primate TIGIT. Therefore, a surrogate antibody was generated for pre-clinical assessments in mice. Antibody 313R12 is an anti-mouse TIGIT antibody that can block mouse PVR ligand binding and inhibit TIGIT signaling in a manner similar to the clinical candidate OMP-313M32. 313R12 inhibited the growth of syngeneic colon and kidney tumors in immune competent mice. In some cases, anti-TIGIT antibody 313R12 caused complete tumor regression and a potent anti-tumor immune memory response as demonstrated by the lack of tumor growth upon re-challenge of mice that remained tumor-free after prior anti-TIGIT treatment. Mechanistically, anti-TIGIT antibody 313R12 was shown to induce a Th1 response and increase cytotoxic T lymphocyte (CTL) activity. By in vivo depletion of T cells, we have shown that CD8+ T cell depletion completely abrogated the anti-TIGIT therapeutic effect, whereas CD4+ T cell depletion led to partial reversal of efficacy of anti-TIGIT. Therefore, both CD4+ and CD8+ T cells are critical for anti-TIGIT-mediated immune responses. Using mice reconstituted with human hematopoietic stem cells, we also demonstrated that the clinical candidate OMP-313M32 inhibits patient-derived melanoma tumor growth. Taken together, these data demonstrate that anti-TIGIT therapy suppresses tumor growth and generates long-term immunological memory against multiple tumors.

#2004 Epigenetic targeting of CD26 expression in medulloblastoma. Maehedara R. Guda,1 Jose R. Castellanos,1 Collin M. Labak,2 Ramadevi Sanam,3 David J. Daniels,4 Sarah E. Martin,5 Julian J. Lin,5 Andrew J. Tsung,5 Kiran K. Velpula,6 Swapna Asutkara,6 University of Illinois College of Medicine at Peoria, Peoria, IL; 2GVR Bioscience, Mahwah, NJ; 3MGH Cancer Center, Boston, MA; 4UConnSchool of Medicine, Storrs, CT; 5Hahnemann University, Philadelphia, PA; 6University of Michigan, Ann Arbor, MI.

Medulloblastoma (MB) is the most common malignant brain tumor in children and accounts for 20% of pediatric CNS tumors. Following surgery and treatment with chemotherapy and radiation, MBs recur in a significant proportion of patients. Moreover, survivors experience comorbidities related to the tumor and/or its treatment. New insights into MB tumor pathogenesis is needed for improving the survival and outcome of MB. Epigenetic silencing, including histone modification and DNA methylation, is an important tumorigenic mechanism in MB. However, its role in cancer immunopathology is poorly understood. Using datamining and immunohistochemistry analysis, we show that B7-H3 (CD267), an important immune checkpoint member of the B7 and CD28 families, is highly upregulated in all four molecular subtypes of MB compared to normal tissue. We find that a majority of clinical MB specimens show significant expression of B7-H3 checkpoint blockade (CTLA-4, PD-1, and PD-L1). CD267 appears to be a promising therapeutic target for cancer immunotherapy. Moreover, we observed that increased expression of CD267 correlates significantly with the expression of Enhancer of Zeste Homologue 2 (EZH2), a histone H3-methylating (H3K27me3) catalytic subunit of the human MB specimens. We hypothesize that combined targeting of CD267 and EZH2 may have greater impact on tumor progression and immune cell response in MB. Simultaneously, we performed LC/MS analysis to identify novel interaction partners of CD267 in MB tumor. These findings taken together suggest that CD267 is strongly associated with several epigenetic markers, indicating a strong relationship between cancer immune evasion and epigenetic mechanisms.

#2005 Synergistic effect of phenyl alpha-galactosylceramide C34 and chemotherapy on 4T1-tumor bearing mouse model. Jung-Tung Hung, Jen-Chien Wu, Tsai-Hsien Hung, Jing-Rong Huang, John Yu, Alice L. Yu. Institute of Stem Cell & Translational Cancer Research, Taoyuan City, Taiwan.

Intrinsic natural killer T (iNKT) cells represent a unique subpopulation of innate immune cells which have immune modulatory effects on many immune effector cells such as CD4+ T cells and CD8+ T cells. Alpha-galactosylceramide (a-GalCer) is a well-known iNKT-stimulating glycolipid. Using a-GalCer as a template, we have generated a more potent a-GalCer analog C34, which contains two aromatic rings at the acyl chain and demonstrated its anti-cancer effect on tumor bearing mouse model. It has been reported that NKT cells killed cancer cell
lines treated with chemotherapy agents through TRAIL- and Fasl-mediated pathways. In this study, we evaluated the anti-cancer effects of chemotherapy and C34 on tumor-bearing mouse model. First, BALB/c mice bearing 4T1 breast cancer were injected with various dose of chemotherapy agents weekly x 5 and the sub-optimal dose of docetaxel and doxorubicin was determined to be 0.5 mg/Kg. Subsequently, sub-optimal dose of docetaxel (0.5 mg/Kg) and doxorubicin (0.5 mg/Kg) respectively. In both tumor models, the suboptimal dose of C34 was found to be 0.1 ug per mouse. The treatment schedules of combination of chemotherapy and C34 were examined by administration of C34 at the same day (OD), three days (-3D) and one day (-1D) before chemotherapy and one day (+1D) after chemotherapy. We found that treatment benefit with C34 and docetaxel treatment in 4T1-bearing mice as compared to those treated with the other schedules. Moreover, the survival of mice was significantly prolonged when C34 was injected three days after cisplatin treatment in TCI-bearing mice (median survival: 46 days for cisplatin and 55 days for cisplatin+C34, log-rank test p = 0.041). In the 4T1 tumor microenvironment, we found the number of CD3+CD8+ T cells was significantly greater in mice treated with docetaxel+C34 (23,536 +/- 901.4) than those treated with docetaxel only (2,270 +/- 1,536, t-test p < 0.0001). In addition, the number of PD1+ CD8 T cells was slightly higher, but did not reach statistical significance (t-test p = 0.3), in mice treated with docetaxel+C34 (23.3 +/- 8.5) than in mice treated with docetaxel only (14.6 +/- 10.1). In short, we have established an optimal treatment schedule for the combination of C34 and chemotherapy that showed synergistic anti-tumor efficacy. We also demonstrated that C34 could increase the number of CD8+ T cells in the tumor microenvironment in mice receiving chemotherapy. These results indicate that combination of C34 and chemotherapy against an optimal treatment schedule might have synergistic therapeutic efficacy for patients with lung cancer and breast cancer.

#2006   AMX-513 polyamine depletion therapy inhibits tumor growth and reverses immunosuppression in cancers including MYC-driven neuroblastoma and pancreatic cancer. Mark R. Burns,1 Kathy Fosnaugh,2 Michael G. Palfitreman1, Laura Ganem1,2, Jane Murray,2,3 Sophie Allan1,2, Georgina Green1,2, Sara Sarraf,2 Murray Norris,3 David Ziegler,3 Michelle Haber3,1, Aminex Therapeutics, Inc., Kirkland, WA; 2Aminex Therapeutics, Inc., Kenmore, WA; 3Children’s Cancer Institute Australia, Sydney, Australia.

Tumorigenesis is associated with increased polyamine levels and involves the induction of ornithine decarboxylase (ODC), the initial rate-limiting enzyme in polyamine biosynthesis, and increased uptake of polyamines from the blood and diet. As well as contributing to proliferation, polyamines are reported to exert an immunosuppressive effect. Amplification of the MYC/MYCN oncopgenes has been shown to directly induce ODC activity and inhibition of this enzyme by α-difluoromethylornithine (DFMO) markedly delays tumor development. Aminex Therapeutics is developing a polyamine depletion approach targeting both biosynthesis and transport of polyamines with AMX-513, a combination of the approved ODC inhibitor, DFMO, together with AMXT 1501, an alkylated polyamine mimetic which blocks polyamine uptake. In the syngeneic CT26.CL25 mouse model of colorectal cancer, AMX-513, dosed daily for four weeks, reduced tumor growth > 75% compared to vehicle-treated control in immunocompetent Balb/C mice. There was no effect in athymic nude mice indicating that tumor growth inhibition by AMX-513 is T-cell-dependent. In the induced transgenic K6/ODC squamous tumor mouse model, stable regression was sustained 10 weeks after treatment ended and was accompanied by tumor infiltrate increases in IFNγ and in CD3+ and CD8+ T-cells. Tumor infiltrates from AMX-513-treated KPC pancreatic cancer transgenic mice with tumor regressions showed >90% reductions in myeloid-derived suppressor cells (MDSCs; CD11b+ Gr-1+) but no changes in mature myeloid cells (CD11+ Gr-1+++) by FACS analysis. AMX-513 treatment did not impact the percentage or number of CD4+CD25+FoxP3+ Tregs, but did significantly increase the percentage of activated CD8+ T cells in tumors. Neuroblastoma is an aggressive childhood cancer frequently associated with MYCN and ODC deregulation. We treated subcutaneous neuroblastoma cell lines with AMX-513 and showed synergistic (CI<0.5). Prophylactic treatment of neuroblastoma-prone THY-MYC transgenic mice with AMX-513 significantly extended survival compared to either agent alone (median survival time = 81.0 ±11.8 days versus DFMO alone = 57.1 ±7.1 days; P<0.0001). Treatment of mice with small palpable tumors with AMX-513 in combination with cyclophosphamide/topotecan can significantly improve survival compared to either chemotherapy alone (p<0.001). In conclusion, AMX-513 treatment alone or in combination with other cancer therapies results in significant tumor growth reduction in multiple cancer models and demonstrates novel immunotherapeutic potential. Clinical evaluation of AMX-513 is planned in 2017.


Cancer immunotherapies, e.g. the antibody against programmed death ligand 1 (PD-L1), a checkpoint inhibitor, have witnessed great successes in treating certain cancers in recent years. Recent data have also demonstrated that gut microbiota are important modulators on anticancer immunotherapy1,2. Heterogeneity in patient outcome seems to suggest complex communications between microbiota and host antitumor immunity. To this end, we tested an engineered chimeric MC38 mouse cell line, hPD1-MC38-HuCELL™ via human PD-L1 knock-in procedure, where MC38 CRC syngeneic cell is derived from C57BL/6. After treatment of PD-L1 antibodies (cd7A8 and BMS PD-L1 of different dose regimens), we observed significantly different drug responses among the microenvironment of tumor cells lines. AMX-513 treatment showed no tumor progression after treatment with a variety of doses while no favorable response was observed in mice from Vendor 2 and Vendor 3. To deeply study the gut microbiota of these mice, we performed 16s ribosomal RNA sequencing on untreated mice from three groups (5 replicates for each). Global diversity analysis by Quantitative Insights Into Microbial Ecology (QIIME) tool revealed segregation in the three cultures of mice: the microbiota composition of Vendor 2 and Vendor 1 are relatively closer to each other whereas the Vendor 3 mice are different, suggesting that the main difference seen between Vendor 1/2 and 3 in the original composition of gut microbiota is not the key impact for the observed anti-PD-L1 efficacy, and...
there should be other complex dynamics impacted anti-PD-L1 treatment, which remains unknown. Moreover, a group of 27 taxa were identified with significant difference in abundance (Kruskal-Wallis test, p-value < 0.05) across the groups, such as Bacteroidaceae, Lachnospiraceae, and Ruminococcaceae, which confirmed the previous data\(^1\). In conclusion, intestinal microbiota dynamically facilitate anti-PD-L1 efficacy and reversibly anti-PD-L1 treatment could influence reconstruction of gut microbiota. Reference 1. Sivan A, et al. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. Science 2015;350(6264):1079-84. 2. Vézizou M, et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science 2015;350(6264):1079-84. 3. Caporaso JG, et al., QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7(5):335-6.

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**#2009** CC-90002 (anti-CD47 antibody) in vivo anti-tumor activity is associated with an increase in M1-polarized macrophages. Bing Zheng,1 Piu Wong,1 Wen Qing Yang,2 Rama Narla,2 Michael Burgess,1 Laure Escoubet,2 Heather Raynor,2 Kandasamy Harilaran,2 John Boylan,2 Kristen Hege,2 Victoria Sung,2. 1Cellgene Corporation, San Francisco, CA; 2Cellgene Corporation, San Diego, CA

CD47, also known as integrin-associated protein, is over-expressed in several tumor types, including AML, NHL, breast cancer and multiple myeloma. Elevated expression of CD47 on the cell surface protects tumors from phagocytosis by macrophages through binding to signal regulatory protein alpha (SIRPα) on the surface of macrophages. The CD47-SIRPα interaction triggers events that culminate in the inhibition of the phagocytic process. Macrophages are one of the immune cell types frequently found in the tumor microenvironment and exist as a heterogeneous population that includes both M1 and M2 macrophages. While the spectrum of macrophage subpopulations are likely quite diverse, traditionally, it is thought that M1 macrophages are pro-inflammatory, enhancing immune responses against tumor cells, while M2 macrophages are pro-tumor, since they express a wide array of anti-inflammatory molecules, cytokines and growth factors that promote tumor growth, angiogenesis and an immunosuppressive microenvironment. We evaluated CC-90002 efficacy in the RPMI-8226 multiple myeloma and MDA-MB-231 breast cancer xenograft models and enumerated mouse M1/M2-like macrophage populations within in the tumor before and after CC-90002 treatment. In both RPMI-8226 and MDA-MB-231 models, M2 macrophages were the primary resident macrophage. Our studies show that when mice bearing RPMI-8226 multiple myeloma xenografts were treated with CC-90002 (a humanized anti-CD47 antibody), tumor regression was preceded by infiltration of macrophages into the xenograft. In the MDA-MB-231 breast cancer model, resident macrophages appeared to mediate the CC-90002 anti-tumor efficacy in vivo without additional macrophage trafficking. Interestingly, in both models, M1 macrophages appear to mediate CC-90002 efficacy regardless of whether macrophages infiltrate the tumor or are tumor-resident. In the MDA-MB-231 breast cancer model where CC-90002 did not induce infiltration, it is possible that resident tumor macrophages were re-educated to an M1 phenotype. In in vitro experiments using human M1 and M2 macrophages, both M1 and M2 macrophages are able to promote phagocytosis and additionally, we observed that CC-90002 selectively inhibited migration of M2 macrophages toward tumor cell conditioned media. This would presumably shift the overall balance of tumor-associated macrophages toward the M1 phenotype and suggests that inhibiting CD47 can both promote tumor phagocytosis as well as skew macrophage subpopulations toward an anti-tumor phenotype. CC-90002 is currently being tested in two ongoing Phase I clinical studies in subjects with advanced solid and hematologic cancers (NCT02367196, NCT02641002).

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Background: BGB-3111 is a novel, irreversible, second generation BTK inhibitor with better selectivity profile and DMPK property compared toibrutinib. It has demonstrated promising anti-tumor activities in patients with advanced B cell malignancies. Given BTK is expressed in all hematopoietic lineages except for T lymphocytes, it is reasonable to explore the immune modulatory effect of BGB-3111. In this study, we sought to investigate the anti-tumor activity of BGB-3111 in solid tumor models. Methods: Cell viability was assessed with Cell Titer Glo® assay and half maximal inhibitory concentrations (IC50) were estimated. In vivo activity was assessed in subcutaneous mouse xenograft models. Treatments were administered by oral gavage and individual body weight and tumor volume was recorded twice weekly. Results: In vivo studies showed that MDAMB-436 cells are insensitive to BGB-3111, which do not express BTK. In both human breast cancer MDA-MB-436 and human A431 epidermoid carcinoma subcutaneous xenograft models, the tumor cells were co-injected with peripheral blood mononuclear cells (PBMCs) of healthy donors. In MDA-MB-436 model, BGB-3111 (15 mg/kg, BID) single agent significantly induced tumor growth inhibition (TGI) of 55%, whereas showed no anti-tumor effect if no PBMCs co-injected. Furthermore, co-treatment of BGB-3111 (15 mg/kg, BID) and anti-PD-1 antibody BGB-A317 (10 mg/kg, QW) demonstrated enhanced therapeutic effect in A431 allogeneic model. Interestingly, in BCLU200 and BCLU654 NSCLC primary tumor xenograft models (without PBMC co-injection), BGB-3111 (15 mg/kg, BID) also demonstrated significant anti-tumor activity.

Conclusions: In summary, BGB-3111 showed interesting activity in solid tumor models, suggesting BTK inhibitor might have potential to be used beyond hematologic malignancies.

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**#2012** UVB-induced ULBP1 upregulation in melanocytes depends on HMGB1-mediated activation of NF-κB and IRF3. Zhaohui Wu, Wei Wang. University of Tennessee Health Science Center, Memphis, TN.

Anti-tumor immune surveillance mediated by innate immunity cells such as NK cells plays a critical role in eliminating “danger” cells at pre-cancerous stage and preventing cancer progression. As a key epidemiological factor associated with melanoma, solar UV radiation also elicits immunosurveillance by upregulating ligands for NK cell activating receptor NKG2D. Nevertheless, how UVR upregulates NKG2D in melanocytes is not completely understood. We found UVB exposure induced secretion of damage-associated molecular patterns (DAMPs) in melanocytes, which synergistically enhances NKG2D ligand ULBP1 transcription. Moreover, RAGE is essential for HMGB1-induced activation of NF-κB and IRF3, which serves as the HMGB1 receptor and mediates activation of TBK1. Consistently, HMGB1 deficient melanoma cells are less susceptible to NK cells mediated cytotoxicity. Our study provides a mechanistic link between environmental UV radiation and antimelanoma immunosurveillance induced in melanocytes, which may play a critical role in the early stages of melanogenesis.

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The matrix metalloproteases (MMPs) are a family of zinc-dependent endopeptidase and have proteolysis activity which found to impact several physiological and pathophysiologic conditions including cancer. In cancer, over 20 types of MMPs promote invasion, angiogenesis, metastasis, and proliferation. For instance, MMP-9 is involved in many processes such as wound healing, tissue remodeling and regulation of inflammation. So far, several studies have also shown that MMP-9 is associated with poor clinical outcome. MMP-9 can facilitate angiogenesis and metastasis by releasing cytokines, pro-angiogenic and pro-metastatic factors. Conversely, the tissue inhibitor of metalloproteases (TIMP-1) is a well-known negative regulator of MMPs which can also modulate other biological functions independent of MMPs regulation. TIMP-1 dysregulation has been shown to impact ECM integrity and potentiate metastatic ability also. Similarly, MDM2 (Murine Double Minute) has been recognized as an important multi-domain protooncogene which is overexpressed in many types of cancer and is associated with poor prognosis in different tumor types including sarcomas and carcinomas. Previously, our group reported that MDM2 overexpression is regulating MMP-9/TIMP-1 axis in LNCaP and LNCap-MST (MDM2 transfected) prostate cancer cells. In order to further analyze the status of MMP in MDM2 overexpressing cancers the fluorescence activity assay and the zymography assay were used for measuring the activity of MMPs including MMP-9. Immunoblotting analysis were also used to correlate the expression levels of MMP2, MMP-9, and TIMP-1 in MDM2 overexpressing cell lines with and without Nutlin-3 (20 μM) treatment. Our results indicated that MDM2 activity is elevated by 97.3 % in LNCap-MST cells compared to LNCaP. This may be possibly due to the near knockdown levels of TIMP-1 that was observed in LNCap-MST cells. However, the Nutlin 3 treatment was able to decrease the MMP-9 levels and activity only marginally, without elevating TIMP-1 levels. This suggests that, in addition to MDM2, the PTEN loss in LNCap-MST cells may also play a role in regulating the levels of TIMP-1 and MMP-9 activity. In conclusion, our findings strongly support our original hypothesis, we analyzed the levels of TIMP-1 in SJSA1 and GI-101A cell lines, which are known to overexpress MDM2 and impart aggressive metastatic abilities. Interestingly, the levels of TIMP-1 were found to be elevated in both SJSA1 and GI-101A cell lines compared to LNCap-MST cells. This finding indicates
that TIMP-1 may paly dual role depending on cancer types and the gene expression status in the tumor microenvironment. Further studies are required to fully delineate the interplay between MDM2 and pro-metastatic mechanisms, including the expression of TIMP-1 and MMP-9. (The financial support from the Royal Dames of Cancer Research Inc., Ft. Lauderdale is gratefully acknowledged).

#2014 Genitourinary cancer-derived cells produce microenvironment proteases that regulate proteinase activated receptors (PARs) to drive oncogenic signaling. Stacy G. Gibson, 1 Koichiro Miura, 2 Andries Zijlstra, 2 Matthew E. Friedman, 1 Morley D. Hollenberg. 1 University of Calgary, Calgary, Alberta, Canada; 2Vanderbilt University, Nashville, TN.

Background: Thrombin-triggered activation of proteinase-activated receptor-1 (PAR-1) is recognized as a key stimulus for driving epithelial malignancies (IUBMB life.63:397, 2011; PMID:21557443). However, the enzymes for regulating PAR1 in the tumour microenvironment are not known. Hypothesis: We hypothesize that prostate (PC) and urinary bladder cancer (UBC) cells can regulate microenvironment signaling to drive cancer progression via the secretion of proteases that cleave/regulate proteinase-activated receptors (PARs). Aims: 1.) Visualize the cleavage status of N- and C-terminally-dual-labelled PARs in receptor-transfected UBC- and PC-derived cells. 2.) Measure PAR cleavage by UBC and PC-secreted proteases. 3.) Identify the UBC & PC-derived proteases that cleave/regulate PAR function. Methods: The cleavage status of dual-label PARs (N-termiinus-mCherry/C-terminus-YFP) expressed in PC & UBC cells was determined by confocal image analysis as already described (JBC 288:32979, 2013; PMID:26957205). Intact receptors look ‘yellow’ and cleaved receptors look ‘green’. The cleavage of PARs in UBC and PC cells was studied for dual-tagged-PAR expressing UBC and PC cells exposed or not to cell-derived culture supernatants, enzyme agonists for PAR1 and 2 (thrombin and trypsin, respectively), and proteinase inhibitors (e.g. for MMPs and other enzymes). The impact of CRISPR-mediated elimination of MMPs from PC3 cells on PAR1 cleavage status was also monitored. Results: UBC- and PC-produced enzymes can cleave PAR1 and 2 efficiently as thrombin, an established PAR1 agonist. None of the UBC cells produce proteases that cleave PAR2. Inhibition of MMP activity partially prevents PAR1 cleavage by UBC cells and completely prevents PAR1 cleavage in PC3-PC cells. Conclusions: We conclude that UBC and PC cells secrete PAR-regulating proteases that can directly regulate PAR1 in the tumour microenvironment so as to drive cancer progression. Funding: Alberta Innovates CRIIO Grant, Prostate Cancer Canada Discovery Grant, Motorcycle Ride for Dad, Johnson & Johnson Alberta Health Partnership and CIHR.

#2015 Mesenchymal stem cell correlates oral leukoplakia malignant transformation through regulate T cell response. Yichen Chen, Ballin He, Da Ma, Jingjing Song, Xi Wang, Bin Cheng, Zhi Wang. Sun Yat-sen University, Guangzhou, China.

Introduction: Leukoplakia is one of the most common clinically presented oral potential malignant disorders (OPMD). Detailed understanding of leukoplakia-associated molecular or cellular changes would help us better understand about the progress of leukoplakia malignant transformation. In this study, we examined the potential involvement of MSC in this very progress, and demonstrated lesion resident MSCs may favour leukoplakia malignant transformation through its strong immunoregulation on T cells. Methods: Eighteen six-week-old female Sprague-Dawley rats (180-220g) were given 0.05%/lg ANQ in drinking water for 22 weeks for development of lesions. Lesions classified by histological HE staining. MSCs were obtained from single cell suspension of lesions, and cultured with splenocyte to study its immunoregulate ability. Results: 1. MSC-like cells are enriched in carcinogen induced leukoplakia and cancers. Representative results showing the lesions on tongue of rats. And relative proportion of MSC phenotype marker (CD29+, CD31-, CD45-, CD90+) in oral lesions (OMSC) was analyzed by flow cytometry. Demstrated MSCs can regulate the expression of MSCs (N-OMSC) share the similar stemness properties. 2. Cancer lesions have less infiltrating T cells (quantified by IHC and FCM of CD3). Cancer-derived MSCs suppress T cell proliferation, but not promote T cell apoptosis and inhibit T cell migration to the lesions. 4. Higher number of lesion derived MSCs associates with higher proportion of lesion in the tumors. Taken together, these results demonstrated MSCs could migrate to pre-malignant leukoplakia lesions prior to tumor establishment. Further, MSCs play an important role in leukoplakia malignant transformation induced by chemicals, which via its strong immunomodulatory activities.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Cell Death Targets


Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer with a five-year survival of ~50%. HNSCC are linked to human papillomavirus (HPV+) or tobacco carcinogenesis (HPV-). The Cancer Genome Atlas recently analyzed 279 HNSCC, revealing that 30% overexpress FADD (Fas-Associated Death Domain), with or without BIRC2/3 genes encoding cellular Inhibitor of Apoptosis Proteins 1/2 (cIAP1/2), which are critical components of the Tumor Necrosis Factor (TNF) Receptor signaling pathways that determine cell death or survival. The frequency of such mutations provides a window for potential therapeutics, as IAP antagonists have been shown to switch cancer cell TNFα signaling from being pro-survival to pro-apoptotic. We recently showed that biranapant, a second mitochondrial activator of caspases (SMAC) mimetic that promotes IAP degradation, sensitizes HNSCC cell to death by TNFα and eradicates tumors in combination with radiation in HPV+ HNSCC modeling advanced head and neck squamous cell carcinoma (HNSCC). However, the enzymes overexpressing FADD/+-BIRC2. ASTX660 (developed by Astex Pharmaceuticals) is a novel dual cIAP1/XIAP antagonist currently in clinical trials for treating advanced solid tumors and lymphomas. The objective of the present study is to determine the therapeutic effects of ASTX660 in HPV+/– HNSCC preclinical models. ASTX660 at nanomolar concentrations was found to potentially inhibit cell proliferation (measured using XTT assays) and induce apoptosis (Annexin/V-AAD flow cytometry), and displayed combinatorial activity with TNFα, TRAIL, or cisplatin in multiple human HPV+/- HN-SSC cell lines. Western blotting showed near complete degradation of cIAP1 expression at nanomolar concentrations in human HNSCC cell lines. Flow cytometry revealed that ASTX660 in combination with either TNFα or cisplatin reduced MHC-I expression and increased PD-L1 expression, suggesting that potential synergistic efficacy could be observed in combination with immune checkpoint inhibitors. Our results demonstrate that ASTX660 is a potential therapeutic agent for both HPV+/- HNSCC. Evaluating the anti-tumor effects of ASTX660 as a monotherapy and in combination with radiation, cisplatin, and anti-PD-1 therapies are ongoing, using both human HNSCC xenograft and syngeneic mouse oral cancer (MOC) models. Supported by NIDCD intramural projects (ZIA-DC-00016, 73 and 74).

#2017 The role of ceramide in the dual cytotoxic and metabolic stimulatory effects of sigma-2 receptors. Cheri Z. Liu, Ellen Sukharevsky, Wayne D. Brownell. Brown University, Providence, RI.

Sigma-2 receptors are highly overexpressed in cancer cells compared to normal cells, as well as in proliferative versus quiescent cells. Sigma-2 receptors have been of interest as an antineoplastic target, as sigma-2 agonists cause cell death via a variety of pathways. Recently, a new class of sigma-2 agonists has been discovered, with ligands such as CM764, that do not induce cell death but that affect cellular metabolism, as shown by increased MTT reduction, increased ATP level, and HIF-1α stabilization. Previously, our lab has shown that treatment of breast tumor cells with sigma-2 receptor agonists CB-184 and BD373 caused dose dependent increases in ceramide and simultaneous decreases in sphingomyelin, suggesting that sigma-2 receptors modulate the sphingolipid pathway. The ceramide pathway has been shown to play both prosurvival and pro-apoptotic roles in cancer cells. Here, we examine the role of ceramide in sigma-2 receptor mediated pathways in human SK-N-SH neuroblastoma cells. A 24 h treatment of SK-N-SH neuroblastoma cells with 20 μm exogenous C6-ceramide caused less than 20% cell death. Interestingly, this compares to over 50% cell death in MCF-7 breast adenocarcinoma cells and PANC-1 pancreatic cancer cells, indicating a differential role of ceramide in cell death pathways across cell lines. SK-N-SH cells were treated with 10 μM of either CM572, a classic sigma-2 agonist that causes cell death, or CM764, a sigma-2 agonist that stimulates metabolism. Fumonisin B1 is a potent inhibitor of ceramide synthesis. Treatment of cells with fumonisin B1 (10 μM) did not significantly alter CM572-induced cytotoxicity. Conversely, treatment with fumonisin B1 did significantly inhibit the cytotoxicity of CM764. Taken together, these results suggest that ceramide formation may play a role in the metabolic stimulatory effect of sigma-2 receptors, as opposed to the cytotoxic effect. Decreased levels of ceramide have been shown to increase cellular resistance to apoptosis. Therefore, it has been of interest to increase intracellular ceramide levels.
in drug resistant cancer cells in order to sensitize the resistant cells to chemotherapy. Multidrug resistance (MDR) in cancer cells is often attributed to the overexpression of particular ABC transporters that effectively pump drugs out of the cells. One such protein is the multidrug resistance-associated protein 1 (MRP1). It has been shown that increased expression of drug efflux pumps also decreases cell sensitivity to chemotherapy. Treatment with CM752 caused a dose-dependent decrease in MRP1 protein levels, while treatment with CM764 had little effect on MRP1. We will examine how modulation of MRP1 affects sensitivity to C6-ceramide. We will also examine whether combination of C6-ceramide with sigma-2 receptor ligands augments the cytotoxicity and/or metabolic stimulative effects of sigma-2 receptors.

#2018 Synergistic interactions between PLK1 and HDAC inhibitors in non-Hodgkin's lymphoma cells occur in vitro and in vivo and proceed through multiple mechanisms, Tri Nguyen,1 Rebecca Parker,1 Elisa Hawkins,1 Victor Yazbeck,1 Akhil Kolluri,1 Maciej Kmiecik,2 Mohamed Rahmani,1 Steven Grant2,3, Department of Internal Medicine, Virginia Commonwealth University and Massey Cancer Center, Richmond, VA; 4Department of Internal Medicine, Biochemistry, Pharmacology, Virginia Commonwealth University and Massey Cancer Center, Richmond, VA.

The mitotic kinase polo-like kinase 1 (PLK1) is over-expressed in neoplastic cells, including non-Hodgkin’s lymphoma (NHL) cells, but not in normal cells, prompting development of PLK1 inhibitors e.g., volasertib (Boehringer-Ingelheim). PLK1 plays key roles in cell cycle progression, mitosis, cytoskeleton, centriole duplication, and the DNA damage response (DDR). Notably, volasertib demonstrated preliminary signs of activity in NHL patients in a phase 1 trial. HDAC inhibitors (HDACi) modify chromatin structure and gene expression, but also induce multiple mitotic abnormalities including mitotic slippage and DNA damage checkpoint disruption. Given these complementary mechanisms, we sought to examine volasertib interactions with the pan-HDACI belinostat (Spectrum Pharmaceuticals) in diffuse large B-cell lymphoma (DLBCL) and mantle cell lymphoma (MCL) cells in vitro and in vivo. Exposure of DLBCL cells to extremely low (e.g., 5-30 nM), minimally toxic belinostat concentrations for 48 hr synergistically increased apoptosis in SU-DHL16, SU-DHL4, SU-DHL8 (GC subtype), HBL-1, U2932 (ABC-subtype), and OCI-LY3; Caravanas (double hit) DLBCL cells. Similar interactions occurred in primary lymphoma cells, Granta-519 and Rec-1 (MCL), SU-DHL4/BR and SU-DHL16/BR (Bortezomib-resistant cells exhibiting up-regulation of the proteasome sub-unit PSMB5 and bortezomib binding site mutation (Ala49Val). In contrast, identical regimes were minimally toxic to normal hematopoietic progenitors. Volasertib/belinostat induced massive M-phase arrest, p-histone H3 up-regulation, a marked increase in mitotic errors, and pronounced M-phase cell death associated with striking DNA damage (γH2A.X formation). Belinostat diminished c-Myc mRNA and protein in DLBCL cells, an effect significantly enhanced by volasertib. Notably, PLK1 knock-down in DLBCL cells significantly increased belinostat-induced M-phase accumulation, phospho-histone H3 accumulation, γH2A.X generation, and apoptosis. Analogously, c-Myc knock-down enhanced volasertib-induced cell death and apoptosis in response to volasertib, arguing that c-Myc down-regulation plays a functional role in lethality. Volasertib/belinostat co-administration dramatically reduced tumor growth in an ABC-DLBCL flank model (U2932) and significantly increased survival (56.2 vs 39.7 days; p < 0.001) without weight loss or other toxicities. The regimen also enhanced survival and reduced tumor cell bone marrow engraftment in a systemic double-hit lymphoma model (OCI-Ly18). Together, these findings indicate that PLK1/HDAC inhibition warrants attention as a therapeutic strategy in NHL. Accordingly, based on these findings, a phase 1 volasertib/belinostat trial in patients with aggressive NHL is planned (NCT02875002).


Although cancer cell genetic instability contributes to characteristics that mediate tumorigenicity, it also contributes to the selective toxicity that some chemotherapy drugs have for cancer cells. This ‘synthetic lethality’ (Nature 434:913, 05) can be enhanced by agents that inhibit DNA repair (Mol Onc 8:1429, 14; Proc AACR 57:3718, 16). To exploit this potential, ‘Achilles heel’, we tested the ability of a small molecule inhibitor of RAD51 to potentiate the cytotoxicity of established chemotherapy drugs. 2-(benzylsulfonyl)-1-(1H-indol-3-yl)-1,2-di-hydroisoquinoline (IBR2) was obtained from Drs. J-W Zhu and W-H Lee (Univ. California - Irvine). IBR2 inhibits RAD51-mediated double-strand DNA break repair, but also enhances induction of apoptosis by the ABL inhibitor imatinib against K562 cells (EMBO Mol Med 3:353,13). There is potential value of such synergistic interaction among other tumor types and with other drugs. IBR2- mediated c-Myc knock-down in non-Hodgkin’s lymphoma cell lines from various tissues (AML, CML, carcinoma of breast, colon, stomach, lung, and head) representing a range of oncogenic drivers (ABL, c-kit, Raf, Ras, ER, mutant p53). Cells were exposed to IBR2 simultaneously with inhibitors of various tyrosine kinase receptors, DNA-damaging agents, or inhibitors of microtubule function. Cells were cultured in 96-well plates, exposed to drugs alone and in combination with dilutions or exposure with combinations of belinostat (Camptosar, Blue or neutral red) 4 days later. Inhibition of proliferation by drug combinations was normalized to that of IBR2 alone. Depending on the drug sensitivity of the cell line, IBR2, at concentrations that inhibited proliferation between 0% and 75% as a single agent, enhanced toxicity of imatinib by up to 80%. IBR2 also greatly enhanced antiproliferative activity of regorafenib (targets RAF, kit, other) and EGFR inhibitors erlotinib, gefitinib, afatinib and osimertinib, and microtubule inhibitor vincristine (VCR). However, IBR2 was antagonistic with VP-16, cisplatin, irinotecan, melphanal, and olaparib. To determine a possible mechanism of the observed synergy, the interaction between IBR2 and imatinib or VCR was compared with that between verapamil, a P-glycoprotein inhibitor, and the latter 2 drugs. The VCR-resistant head and neck cell line HN-5a/V15e (very resistant to imatinib; but IBR2 enhanced their sensitivity in this cell line, its HN-5a parent, and HT-29 by up to 60%, much better than verapamil (up to 40% at similar concentrations, P<0.05). IBR2 enhanced VCR toxicity in these 3 lines to degree similar to verapamil, decreasing the IC50 by up to 90%. IBR2 appears to enhance drug toxicities via mechanisms other than just inhibition of RAD51 and may potentially interfere with microtubule function. The results indicate that this agent may be useful as a clinical adjuvant to numerous cytotoxic drugs.


Urokinase-type plasminogen activator (uPA) system may play a crucial role in cancer cell invasion and metastasis. uPA has been validated as a predictive or prognostic biomarker protein and considered as a therapeutic target in human breast cancer. Mesupron is a uPA inhibitor blocking uPA enzymatic activity to reduce tumor cell invasion, migration and cell growth. Auranozin has been known as an antitherapeutic drug and a thioedrin reducectase inhibitor and recently its anticancer activity in ovarian and breast cancers has also been identified. To study whether cotreatment with mesupron and auranozin shows a significant anticancer activity, the synergistic induction of apoptosis of mesu-pron with auranozin was determined. Auranozin or mesupron alone inhibited cancer cell growth in MCF-7 cells with IC50 of 0.25 μM or 25 μM, respectively. Flow cytometric analysis also showed an increased apoptosis. When cells were treated with mesupron (0.125 μM) in combination with auranozin (10 μM), we found a significant induction of apoptosis although the cytotoxic effects of mesupron or auranozin alone at those concentrations were not quite strong. Interestingly, combined mesupron and auranozin treatment significantly suppressed mitochondrial antiapoptotic proteins including Bcl-2 and Bcl-xL. We also found the increase of caspase-3 and PARP cleavages. The combination index (CI) also indicated the synergistic induction of apoptosis by auranozin and mesupron. Taken together, these data suggest that the use of mesupron and auranozin in combination can be valuable to achieve higher anticancer activity.

#2021 Ablation of caspase-7 promotes solar-simulated light-induced mouse skin carcinogenesis through upregulation of keratin-17. Mee Hyun Lee,1 Do Young Lim,2 Sung Young Lee,3 Jung Hyun Shim,4 Xuejiao Liu,1 Ran Zhao,1 Hai Huang,1 G. Timothy Bowden,5 Young-Soon Surh,3 Yong-Yeong Cho,2 Ann M. Bode,2 Zuzang Dong,1 China-US (Henan) Hormel Cancer Institute, Zhengzhou, China; 2The Hormel Institute, Austin, MN; 3Department of Pharmacy, College of Pharmacy and Natural Medicine Research Institute, Mokpo National University, Mokpo, Republic of Korea; 4University of Arizona Cancer Center, Tucson, AZ; 5Department of Molecular Medicine and Biopharmaceutical Sciences, Graduate School of Convergence Science and Technology, College of Pharmacy, Seoul National University, Seoul, Republic of Korea; 6College of Pharmacy, The Catholic University of Korea, Bucheon, Republic of Korea.

Solar UV irradiation is an environmental carcinogen that causes skin cancer. Caspase-7 is expressed at reduced levels in many cancers. The present study was designed to examine the role of caspase-7 in solar-simulated light (SSL)-induced
skin cancer and to elucidate its underlying molecular mechanisms. Our study revealed that mice with genetic ablation of caspase-7 are significantly susceptible to SSL-induced skin carcinogenesis. Epidermal hyperplasia, tumor volume and tumor numbers were increased in caspase-7 knockout (KO) mice compared with SKH1 wild-type mice irradiated with SSL. The expression of cell proliferation biomarkers Ki-67 and cyclin D1 was increased in SSL-irradiated SKH1 wild-type mice. Immunohistochemical analysis of skin of caspase-7 KO mice compared with those observed in SSL-exposed wild-type SKH1 mouse skin. Moreover, SSL-induced apoptosis was abolished in skin from caspase-7 KO mice. 2-DE gel electrophoresis, followed by matrix-assisted laser desorption/ionization-time-of-flight analysis of skin tissue lysates from SSL-irradiated SKH1 wild-type and caspase-7 KO mice revealed an aberrant induction of keratin-17 in caspase-7 KO mice. Immunohistochemical analysis of skin tumors also showed an increase of keratin-17 expression in caspase-7 KO mice compared with SKH1 wild-type mice. Furthermore the expression of keratin-17 was also elevated in SSL-irradiated caspase-7 KO keratinocytes as well as in human basal cell carcinomas. The in vitro caspase activity assay showed keratin-17 as a substrate of caspase-7, but not caspase-3. Overall, our study suggests that genetic loss of caspase-7 promotes SSL-induced skin carcinogenesis by blocking caspase-7-mediated cleavage of keratin-17.


Background: Bcl-2 family is a group of apoptosis regulators that plays an anti-apoptotic role for cell survival and is known to contribute to induce chemotherapy resistance in small cell lung cancer (SCLC). ABT-737 is an anti-cancer drug that induces apoptosis by selectively blocking the activities of Bcl-2 and Bcl-XL, but not Mcl-1. Consequently, the efficacy of ABT-737 is largely restricted in the presence of Mcl-1. Heat-shock protein 90 (Hsp90) is highly expressed in most tumors and Hsp90 inhibitors induce the proteasomal degradation of Hsp90 client proteins. In addition, Hsp90 inhibitor can reduce Mcl-1 expression, a down-stream of Akt and Erk pathway. Furthermore, Hsp90 inhibitor activates activities of NF-kB by degradation of IKK. Thus, we show that downregulation of Mcl-1 and NF-kB by Hsp90 inhibitor can lead to synergistic pro-apoptotic effects with ABT-737. Materials and methods: The proliferative activity, apoptotic activities, and expression of apoptotic proteins were assessed in SCLC cell lines after treatment with ABT-737, NVP-AUY922, or both drugs. The synergy effects of ABT-737 and NVP-AUY922 were analyzed by cell viabilities with different concentrations in SCLC cell lines, and the combination index values were < 1. In addition, the synergy effects of the drugs were showed with xenograft model with human SCLC cell line in vivo. Results: Here, we show that NVP-AUY922, an Hsp90 inhibitor, can potentiate the pro-apoptotic effects of ABT-737 not only by reducing the levels of Akt but also by inhibiting pNF-kB. ABT-737 induced apoptosis by blocking Bcl-2 activation, and NVP-AUY922 blocked the levels of Hsp90 client proteins, Akt and Erk, ultimately leading to decreased level of Mcl-1. In addition, NVP-AUY922 induced degradation of IKK, and increased IkB-α inhibited activation of NF-kB. And this combination treatment showed higher BIM and BID expression, pro-apoptotic proteins, than single treatment. Furthermore, synergy effects of combination were verified in xenograft model with human SCLC cell line. Conclusions: Consequently, NVP-AUY922 synergizes with ABT-737 to induce apoptosis by reducing activities of Mcl-1 and NF-kB in SCLC. This study suggests that adopting an appropriate combination of drugs can lead to better outcomes compared with monotherapy in SCLC.

#2023 Artemisinins enhanced the antileukemic efficacy of BCL2 inhibitors. Blake S. Moses,1 Jennifer M. Fox,1 Xiaoqun Chen,1 Jeffrey W. Tyner,2 Gary H. Posner,3 Patrick Bailey,1 Curt I. Clevin,1 *University of Maryland Baltimore, Baltimore, MD; 2Oregon Health and Science University, Portland, OR; 3Johns Hopkins University, Baltimore, MD.

More effective, less toxic treatments are needed to improve longevity and quality of life for patients with acute leukemias. Artemisinin (AS), the current WHO-recommended drug for severe malaria, is a semi-synthetic derivative of the natural compound Artemisinin. AS has broad antiepileptic activity in vitro, and is well tolerated, inexpensive, and can be parenterally or orally administered in humans. Both AS and a more potent synthetic derivative, artesiminin-derived trioxane diphosphonate dimer 838 (ART-838), substantially inhibited acute leukemia growth in vivo and in vitro, at doses where normal CD34+ hematopoietic stem-progenitor cell clonogenicity was essentially unaffected. Moreover, AS and ART-838 synergized with several current antileukemic drugs (Fox et al, Oncotarget, 2015). To identify additional potential antileukemic drug combinations, we identified synergistic combinations of AS and ART-838 against 3 acute leukemia cell lines co-treated with a panel of 122 antileukemic compounds (mainly emerging antineoplastic drugs). Both AS and ART-838 showed synergistic or additive growth inhibition with several compounds. Two BCL2 protein family inhibitors demonstrated among the highest levels of synergy. One of these, ABT-199 (venetoclax), was recently approved by the FDA for the treatment of chronic lymphocytic leukemia (CLL). MDA-MB-231 and MDA-MB-231/BRCA2 cell lines exhibited the greatest synergy through designation for acute myeloid leukemia (AML). Therefore, we decided to further investigate the combination of ABT-199 with AS or ART-838. Synergy between AS (or ART-838) and ABT-199 was confirmed across several acute leukemia cell lines with varying sensitivities to each drug. Both leukemia cell proliferation and survival were affected synergistically by exposure to the combination of AS (or ART-838) plus ABT-199. Utilizing in vivo imaging, we assessed changes in growth kinetics of tail vein injected acute leukemia cell lines and primary patient-derived samples constitutively expressing luciferase in mice treated with AS, ABT-199, or the combination. The combination of AS plus ABT-199 had the greatest antileukemic effect. Based on these data, we propose that Artemisinins plus BCL2 inhibitors comprise an antileukemic drug pair with high potential for incorporation into acute leukemia therapeutic regimens.

#2024 Reactivating apoptosis in BFL-1/A1 driven cancer with cysteine-reactive stapled peptide inhibitors. Rachel M. Guerra, Anissma J. Huhn, Edward P. Harvey, Gregory H. Bird, Loren D. Walensky. Dana Farber Cancer Institute, Boston, MA.

BFL-1/A1 is a BCL2 family protein implicated in the progression and chemoresistance of melanoma, lymphoma, and other cancers, yet it remains undrugged. Anti-apoptotic BCL2 family proteins block cell death by trapping the critical α-helical BH3 domains of pro-apoptotic members in a surface groove. Cancer cells hijack this survival mechanism by overexpressing a spectrum of anti-apoptotic members, mounting formidable apoptotic blockades that resist chemotherapeutic treatment. Drugging the BH3-binding pockets of anti-apoptotic proteins has become a highest-priority goal, fueled by the clinical success of venetoclax, a selective BCL2-1 inhibitor, in reactivating apoptosis in BCL2-dependent cancers. A natural juxtaposition of two unique cysteines at the binding interface of the NOXA BH3 helix and BFL-1/A1 pocket informed the development of stapled BH3 peptides bearing acrylamide warheads to irreversibly inhibit BFL-1/A1 by covalent targeting. Here, we describe the development and application of cysteine reactive stapled peptides that, compared to their non-covalent analogs, trigger rapid mitochondrial cytochrome c release, caspase 3/7 activation, and apoptosis induction of BFL-1/A1 driven cancers such as melanoma. Mechanism of action studies demonstrated the exquisite BFL-1/A1 selectivity and mitochondrial localization of the in situ covalent reaction. Given the frequent proximity of native cysteines to regulatory binding surfaces, covalent stapled peptide inhibitors provide a new therapeutic strategy for targeting oncogenic protein interactions.


Purpose Statins, a class of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, are initially developed as cholesterol-lowering drugs by inhibiting the mevalonate pathway. They have recently gained attention for their potential anticancer properties, but the mechanisms of their anticancer effects remain elusive. This study aims to investigate the antiproliferative effects of statins in breast cancer cell lines. Methods We screened a panel of breast cancer cell lines (Estrogen receptor positive (+): CAMA1, MCF7, T47D, ZR-75-1 and Hcc1428; triple negative (TNBC): MDA-MB-231, MDA-MB-436, BT549, Hs578T and Hcc1806) and assessed the sensitivity of these cells to simvastatin in relation to their hormone receptor status using cell proliferation assays. Next, to elucidate the mechanisms underlying simvastatin sensitivity, we evaluated the (i) expression of key enzymes in the mevalonate pathway using qPCR and western blot analysis and (ii) the role of steroid biosynthesis metabolic inhibitors by exogenously adding these compounds and testing their effects on simvastatin-induced cytotoxicity. Finally, we performed functional assays following siRNA knockdown to identify the key small GTPase involved. Results We found TNBC cell lines to be more susceptible to simvastatin compared to ER+ cell lines (mean IC50 of 7.98µM [95%CI 2.75-13.22] versus 41.74µM [95%CI 6.71-
Evasion of apoptosis represents an essential hallmark in the progression of many cancers. The Bcl-2 family of proteins plays a central role in regulating the apoptotic process. Targeting pro-survival Bcl-2 family members like Mcl-1 with small molecule inhibitors represents a viable therapeutic approach for the treatment of cancer. This study evaluated the in vitro and in vivo activity of AMG 176, a novel, potent and selective Mcl-1 inhibitor currently in Phase I clinical development. AMG 176 binds with high affinity and selectivity to the BH3-binding groove of Mcl-1.

In a cell based split-luciferase complementation assay, AMG 176 disrupted the interaction between Mcl-1 and Bak, leading to downstream activation of the intrinsic apoptosis pathway as measured by increased caspase activity and subsequent effects on viability. Oral administration of AMG 176 to mice bearing OPM-2 multiple myeloma xenografts resulted in a dose-dependent increase in tumor growth inhibition, complete tumor regression at a dose higher than the administered dose and also demonstrated robust tumor growth inhibition with complete tumor regression at an elevated dose. Efficacy in this model was achieved at doses in agreement with those eliciting induction of apoptotic markers. Treatment of tumor cell lines with Compound A, a close structural analog of AMG 176, revealed a dose- and time-dependent increase in Mcl-1 protein levels that was reversible upon compound washout. Subsequent experiments performed with cycloheximide suggested that elevations in Mcl-1 protein levels were due to an increase in Mcl-1 protein half-life, likely driven by the compounds ability to disrupt proteasome-mediated degradation. Compound A was also used to characterize the kinetics of activating apoptosis. These studies revealed a rapid induction of apoptosis and loss of viability in Mcl-1 dependent multiple myeloma and AML cell lines. In cell lines highly dependent on Mcl-1, treatment with Compound A for as little as two hours was sufficient to achieve complete cell killing. Cell line profiling studies (>200 lines) revealed robust effects on cell viability in a subset of solid tumor cell lines and cell lines of hematological origin, including multiple myeloma, acute myeloid leukemia and non-Hodgkin lymphoma. Similar pro-apoptotic effects were found to inhibit the survival gene E2F1 expression and sensitivity to Mcl-1 inhibition. Combination screens with Compound A revealed multiple highly synergistic combinations including compounds targeting the MAPK pathway, standard of care chemotherapeutics and agents targeting additional pro-survival members of the BCL-2 family. In conclusion, AMG 176 is a potent and selective Mcl-1 inhibitor, with significant in vitro and in vivo activity in Mcl-1 dependent cancer models.

#2028 PDE3A modulation for cancer therapy. Xiaoyun Wu,1 Timothy Lewis,2 Luc de Waal,1 Galen Gao,1 Jian Zhang,1 Monica Schenone,1 Colin Garvie,4 Brett Diamond,5 Selena Lorrey,1 Andrew Cherniack,1 Steven Corsello,4 Alex Burgin,5 Todd Golub,6 Stuart Schreiber,7 Matthew Meyerson,4 Heidi Greulich,1,6 Broad Institute, Cambridge, MA; 4C Therapeutics, Cambridge, MA; University of Connecticut, Cambridge, MA; Dana-Farber Cancer Institute, Boston, MA.

In a differential cytotoxicity screen, we identified a novel small molecule modulator of phosphodiesterase 3A (PDE3A) that kills cancer cells expressing elevated levels of PDE3A and SLFN12 (de Waal, Nat Chem Biol, 2016). Treatment with this cell-selective cytotoxic small molecule, DNMDP, induces complex formation between PDE3A and SLFN12, resulting in apoptosis. Inhibition of PDE3A enzymatic activity is not sufficient for cancer cell killing, and expression of both PDE3A and SLFN12 are required. Although the mechanism of signaling to the apoptosis machinery remains unclear, we examined more closely the role of the PDE3A-SLFN12 complex in cancer cell killing mediated by DNMDP. We found that cancer cell lines made resistant to DNMDP by persistent exposure became more sensitive to DNMDP, suggesting that expression of SLFN12 was sufficient to restore sensitivity. Furthermore, ectopic expression of PDE3A and SLFN12 are sufficient to sensitize cancer cells to DNMDP. These data underscore the tight correlation of PDE3A-SLFN12 complex formation and cancer cell killing mediated by DNMDP.

#2028A Glutamine deprivation-elicited sensitization of multiple myeloma to venetoclax is associated with electron transport chain inhibition. Richa Bajpai, Changyong Wei, Mala Shanmugam. Winship Cancer Institute, Emory University, Atlanta, GA.

Cancer cells exhibit altered glucose and glutamine metabolism to sustain survival and proliferation. Altered nutrient utilization also contributes to the development of resistance to apoptosis via regulation of pro- and anti-apoptotic BCL-2 family members. We previously reported that glutamine deprivation enhanced binding of BIM to BCL-2 thereby sensitizing myeloma cell lines and patient samples to Venetoclax (ABT-199). In this study we investigated the metabolites regulated by glutamine withdrawal that contribute to enhanced BIM-BCL-2 association and ABT-199 sensitivity. Metabolite profiling and isotope tracing flux analyses of glutamine deprived myeloma cell lines revealed specific reduction of the TCA cycle metabolism including succinate, that were not maintained by glucose present in the medium. Cellular bioenergetics end points i.e. OCR and ATP were also suppressed upon glutamine withdrawal correlating with a suppression of the TCA cycle and oxidative phosphorylation. Supplementation of cell permeant dimethyl-a-ketogluturate (DMK), a TCA cycle metabolite derived
from glutaminolysis, to glutamine deprived cells reversed sensitivity to ABT-199. This ability of DMK to reverse ABT-199 sensitization in glutamine deprived cells was associated with a replenishment of TCA cycle intermediates without restoration of ATP levels. Therefore, we sought to further interrogate the TCA cycle and electron transport chain (ETC) in facilitating glutamine withdrawal-associated ABT-199 sensitization. TCA cycle metabolites are linked to mitochondrial respiration through ETC complexes. Inhibition of ETC complexes I, II, III or V in myeloma cell lines enforced similar effects as glutamine deprivation and sensitized cells to ABT-199. Among all five complexes, complex II is more explicitly linked with the TCA cycle through succinate dehydrogenase (SDH). SDH facilitates the oxidation of succinate to fumarate in the TCA cycle through SDHB, SDHC, and SDHD, and transfers the released electrons to ubiquinone via its SDHFe, C and D subunits supporting the ETC activity of complex II. Competitive inhibition of succinate dehydrogenase by 3-nitro propionic acid (3NP) induced BIM expression and sensitized myeloma cells to ABT-199, similar to that detected upon glutamine deprivation. Our observations thus narrow down a role for ETC inhibition in facilitating ABT-199 sensitivity and underscore the importance of further interrogation of ways to metabolically perturb and sensitize cancer cells to the highly potent BH3 mimetic ABT-199.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Combination Therapy 2

#2029 Precision therapeutic combinations are synergistic against triple negative breast cancer using compensatory pathways. Jeffrey P. Solzak,1 Brad Hancock,1 Robin Paul,1 Patrick Kiel,2 Todd Skaar,1 Bryan Schneider,1 Milan Radovich,1 1Indiana Univ. School of Medicine, Indianapolis, IN; 2IU Health, Indianapolis, IN.

Introduction: Triple negative breast cancer (TNBC) accounts for 15% of all breast cancer cases in the United States, and despite its lower incidence, contributes to a disproportionately higher rate of morbidity and mortality compared to other breast cancer subtypes. No FDA-approved targeted therapies are currently available. It has been hypothesized that drug combinations could potentially be chosen based on a tumor harboring two or more druggable genomic mutations. However, previously published data has noted that single agent treatment can change the genomic landscape and may be responsible for the lack of synergy of dual genomic drug targeting. Herein we show that targeting a compensatory pathway after treatment with a genomically-directed agent, results in synergistic combinations and can outperform choosing two drugs a priori based on genomic mutations. Methods: Nine TNBC cell lines were chosen based on the presence of two or more clinically actionable genomics targets. Drug combinations based on targeting two genomic mutations were chosen using DNA-seq data from CCLE and a board consisting of oncologists and researchers. Compensatory therapies were found using RNA-seq data from untreated versus single-agent treated TNBC cell lines. The merged transcript RPKMs were transformed and analyzed for differential expression. Statistically significant genes were imported into Ingenuity Pathway Analysis (IPA) to identify therapeutics based on compensatory targets using the Causal Network Analysis and Upstream Regulator functions. Cell viability was assessed via Celltiter-Fluor. Synergy of the combinations was calculated using the Chou-Talalay method. Results: Using two-drug combinations based on genomic mutations alone in all nine cell lines resulted only in additive or antagonistic responses. However, using RNA-seq data of each cell line treated with one genomically-directed agent, IPA analysis identified several compensatory targets that were upregulated or activated. Treating each cell line in combination with one genomically-directed agent plus one compensatory agent resulted in a striking increase in synergy. To understand why targeting compensatory pathways was superior to targeting two genomic mutations, we re-evaluated the RNA-seq data. This analysis revealed that treatment with the first genomically-directed agent, in many cases, resulted in up-regulation or inactivation of the second genomic-target. This may explain the lack of synergy when targeting cells with two drugs based only on genomic mutations. Conclusion: Targeting TNBC cell lines based on drug combinations informed by compensatory pathways results in significant synergy and is superior to choosing drug combinations based on genomic alterations alone.

#2030 Fisetin, a dietary flavonoid for the prevention and treatment of PIK3CA-mutant colorectal cancer. Naghma Khan, Farah Jajeh, Devon Miller, Rachel Van Doorn, Richard B. Halberg, Hasan Mukhtar. 1Univ. of Wisconsin-Madison, Madison, WI.

Colorectal cancer (CRC) is one of the most frequent and deadliest cancers worldwide with patients often diagnosed in advanced stages of the disease. Approximately 15-20% of advanced colorectal cancers harbor activating mutations in PIK3CA, which has been identified as an important oncogene in multiple cancers. Therefore, modeling the effects of this type of mutation in the mamma- lian genome would help elucidate the significance of this oncogene. Fisetin, a flavonoid found in blueberry, persimmon, grape, onion and cucumber, 5-Fluorouracil (5-FU) is the most used chemotherapeutic agent in CRC; however, it has serious side-effects. Therefore, augmentation of the 5-FU therapeutic effect could lead to lower effective doses and subsequently fewer side effects. We conducted in-vitro and in-vivo studies to determine the effect of fisetin, 5-FU and their combination on PIK3CA-mutant colon cancer cell lines (HT116 and HT-29), PIK3CA wild-type colon cancer cells (SW480), and newly developed mouse models. We found that there was more pronounced decrease in cell-viability and number of colonies in PIK3CA-mutant colon cancer cells than PIK3CA wild-type colon cancer cells. Apoptotic genes and proteins are promising targets for cancer treatment as they provide several theoretical basis to influence pathways causing greater tumor cell death. We observed an increase in the protein expression of Bax and decrease in Bcl2 on treatment with combination of fisetin and 5-FU than either agent alone. The full size PARP (116 KD) protein was also cleaved to yield an 85 KD fragment after treatment with cell lines with fisetin, 5-FU and their combination. Apoptotic effects of fisetin and 5-FU combination were also confirmed by flow cytometry in PIK3CA-mutant colon cancer cell lines. The PIK3/AKT pathway is frequently activated in CRC leading to tumorigenesis and the resistance to chemotherapy. Treatment of PIK3CA-mutant colon cancer cells with fisetin and 5-FU caused decrease in the expression of (i) PI3K (p85 and p110), (ii) phosphorylation of Akt (Ser473 and Thr308), (iii) phosphorylation of mTOR, its target proteins, and constituents of mTOR signaling complex. Treatment with fisetin and 5-FU also led to an increase in the phosphorylation of AMPKα. Next, we performed studies to investigate the effect of the treatment of combination of fisetin and 5-FU on colorectal tumorigenesis in 5F3K-ApcMin/+ mice. These animals form tumors in the distal small intestine and colon that have lost APC activity and express constitutively active PI3K as often occurs in humans. Interestingly, the effect of fisetin was much stronger than that of 5-FU and comparable to the fisetin and 5-FU combination. Both fisetin and 5-FU treatment group had significantly lower incidence relative to the control group. We suggest that fisetin could be used as a preventative agent as well as an adjuvant for 5-FU for the treatment of PIK3CA-mutant CRC.
brane potential, and the production increasing of ROS were observed. In HBCx-10 PDX, a trend for antitumor activity of DFX alone was observed (p = 0.09) at the end of the experiment (day 81). A significant difference of Relative Tumor Volume (RTV) was observed since day 18 between the AC group and the DFX + AC group (tumor growth inhibition: 37 to 61%, tumor growth delay: 10 to 14 days, p < 0.04). Similar results in observations of PI3K pathway and hypoxia are involved in this antitumor synergy. Except neutropenia (due to chemotherapy), no other hematologic toxicity was observed in both AC and DFX + AC groups. Conclusions: Iron chelators may increase the effectiveness of conventional chemotherapies for TNBC treatments. This antitumor synergy involves PI3K pathway downregulation, ROS production and decrease mitochondrial membrane potential.

**#2032 Combination of epigenetic regulation via LSD1 inhibition with signal transduction inhibitors significantly enhances anti-tumor activity in models of hematologic malignancies.** Sang Hyun Lee, Matthew Stubbs, Ashish Juvekar, Melody Diamond, Antony Chadderton, Robert Collins, Xiaoming Wen, Holly Koblish, Chunhong He, Liangxing Wu, Richard Wyn, Andrew Combs, Chu-Biao Xue, Wending Yao, Gregory Hollis, Reid Huber, Peggy Scherle, Bruce Buggeri. *Incyte Corporation, Wilmington, DE.*

Combinatorial therapeutic strategies have achieved improved response rates and durability of responses in several malignancies either by selectively targeting distinct and non-overlapping oncogenic signaling pathways (e.g. PARP and phosphoinositide 3-kinase (PI3K) inhibition in subsets of breast and ovarian cancers), or alternatively, inhibiting distinct nodal points of regulation in common oncogenic signaling pathways (e.g. Rb and MEK inhibition in subsets of melanoma). Recent data suggest that deregulated epigenetic modifications may be just as significant as genetic mutations in driving cancer development and growth by inhibition of tumor suppressor activity and activation of oncogenic pathways. We therefore hypothesized that an epigenetic regulator could potentiate the efficacy of a protein kinase inhibitor to result in robust tumor growth inhibition. We previously reported that the potent and selective LSD1 inhibitor INCB059872 potently inhibited tumor growth in multiple tumor xenograft models of AML and SCLC as a single agent and in a combination with standard of care of agents. In this study, we explored the anti-tumor effect of combining INCB059872 and various signal transduction pathway inhibitors, including the PI3 kinase inhibitor INCB059394, the JAK1/2 inhibitor ruxolitinib, or the PI3K delta-selective inhibitor INCB05465 in models of human hematologic malignancies. Each of these therapeutic combinations significantly inhibited tumor growth in the Molm-16 human AML xenograft model. Mechanistic studies suggested that MYC expression levels were downregulated by these combinations both in vitro and in vivo. Treatment with INCB059872 alone or in combination with signal transduction kinase inhibitors significantly downregulated cytokines levels, particularly IL-10, sCD40L, and MCP-1 in Molm-16 tumors. These data suggest that the combination of an LSD1 inhibitor and signal transduction inhibitor can co-regulate key tumor intrinsic and extrinsic pathways involved in paracrine or autocrine signaling in AML. In addition to the improved efficacy observed in AML models, the combination of INCB059872 with the PI3Kdelta inhibitor INCB050465 enhanced tumor growth inhibition in the Will-2 xenograft model (IC50 submicromolar, double hit hit lymphoma), whereas the activity of these single agents were modest in this particular subtype of lymphoma. Our findings support the possibility for more effective ibrutinib-based therapy via manipulation of the MCL cell niche.

**#2034 LSD1 inhibition alone and in combination with chemotherapeutic delay in Ewing sarcoma cell lines.** Darcy Welch, Elliot Kahan, Christopher L. Cubitt, Damon R. Reed. *Moffitt Cancer Center, Tampa, FL.*

Background: Ewing Sarcoma (ES) is the second most common primary bone cancer affecting children and young adults. Despite advances in treatment that have led to survival rates of approximately 73% for localized disease, outcomes for patients with metastatic or recurrent ES remain poor. A distinguishing feature of ES is the presence of the EWS/FLI1 fusion in 85% of cases. The fusion has been shown to alter expression of a number of oncogenic genes. Mechanistic studies have demonstrated that the NuRD co-repressor complex interacts with EWS/FLI1. The associated protein LSD-1 contributes to the repressive function by histone modifications. While reversible LSD1 inhibitors demonstrate single agent activity, in preclinical models, a system to evaluate combinations may be needed for optimizing effect in clinical trials. Methods: Here, we seek to confirm promising single drug activity and evaluate combination therapies using active chemotherapies currently utilized in ES care (4-HC, etoposide, SN-38, vincristine and doxorubicin) along with the LSD1 Inhibitors SP2509 and SP2577 and romidepsin, an HDAC inhibitor. We evaluated these combinations in high-throughput screening platforms and well-established cell line models for ES (A-673, TC-32, RD-ES, TC-71). Taking into consideration past lessons learned from in vitro experiments, we designed stringent screening conditions that bias the held out combination to specifically match clinically relevant concentration and exposure times that mimic the in vivo pharmacokinetics in an effort to maximize the translational potential of these results to the clinical setting. All combinations of agents were studied in two-drug combinations to evaluate for synergy in addition to efficacy. Results: IC50 for SP2509 was found to be in the submicromolar range across cell lines with SP2577 being more potent. A-673 and TC-71 were 5-10 fold less sensitive than RD-ES and TC-32. Agents currently utilized in clinic were universally active at clinically achievable concentrations and exposure times. Combinations showed additivity frequently and demonstrated promising activity that can be used to inform further decision making once LSD1 inhibition toxicities are better known. These findings suggest potentially promising opportunities for developing combination clinical trials to maximize development of LSD1 inhibitors.

**#2035 MGMT inhibition leads to CDK4/6 inhibition and enhances palbociclib and abemaciclib activity in breast cancer.** George C. Bobustuc, Amin B. Kassam, Richard A. Rovin, Deborah L. Donohoe, Maxwell Albiero, Tarun Jella, Olivia Fukui, Cameron Piron, Santhi D. Konduri. *Mayo Clinic, Rochester, WI.*

Background: MGMT (O6-methylguanine DNA methyltransferase), a DNA repair protein leading to chemotherapeutic resistance, is increasingly studied for its cell cycle regulatory functions, also known to control ER expression and function, is overexpressed in a majority of cancers, including breast cancer. MGMT inhibition has been reported to restore ER function and sensitivity to hormonal therapy in tamoxifen resistant breast cancer. CDK4/6 is a cell cycle regulator targeted by a new class of drugs in the treatment of breast cancer in patients who had progressed during prior endocrine therapy. We investigated a potential correlative role between MGMT and CDK4/6 expression/activity. In this therapeutic context MGMT inhibition would have the dual role of increasing/restoring effect of endocrine therapy and facilitate activity of CDK4/6 inhibitors (Palbociclib and Abemaciclib). Methods: We have tested the effect of Antabuse (disulfiram, DSF), as an MGMT inhibitor, at nontoxic doses, on the expression of CDK4/6, or in combination with Palbociclib (PB) or Abemaciclib (LY2835219-LY) on ER+ breast cancer cells. Results: DSF at very low doses (achievable in human serum with standard DSF clinical dosing) decreases ER+ breast cancer cell growth (MCF7, T47D and ZR75) in a dose-dependent manner. DSF further sensitizes breast cancer cells to PB or/and LY and significantly inhibits breast cancer growth without causing unwanted side effects on the normal healthy ER+ breast cells. One dose of concentrations of DSF + LY and moderate synergism for DSF + PB. DSF, alone or in combination with PB (DSF + PB) and/or LY (DSF + LY), significantly inhibits expression of MGMT, CDK4/6, ERα and aldehyde dehydrogenase activity - all involved in breast cancer cell cycle proliferation and tumorigenesis. Furthermore, PB and LY dose dependently decreased MGMT and CDK4/6 expression in breast cancer cells and significantly accumulated breast cancer cells in G1 phase of the cell cycle. DSF, alone or in combination with PB (DSF + PB) and/or LY (DSF + LY) caused significant apoptosis in breast cancer cells. DSF inhibited colony formation which was further enhanced by addition of PB/LY (DSF + PB/LY). Similarly, DSF alone or in combination with PB (DSF + PB) and/or LY (DSF + LY) decreased the metastatic poten-
tant of breast cancer cells. Conclusions: Our findings suggest that DSF as an MGMT inhibitor significantly enhances the antitumor effect of CDDA/6 inhibtors (P8 or LI) in ER+ breast cancer.

**#2036 In vitro and in vivo effect of carbamustine and selenite combination on EGFR signaling in androgen-independent prostate cancer.** Vijayalakshmi Thamilselvan, Mani Menon, Sivagnaan Thamilselvan. Vattikuti Urology Institute, Henry Ford Health System, Detroit, MI.

Introduction: Despite the use of androgen deprivation therapy, the majority of prostate cancer patients will progress to castration resistant disease within 2-3 years, driven by aberrant androgen receptor activation. We have previously shown that combination of carbamustine and selenite effectively induces apoptosis by completely reducing AR and AR-variants in AR-dependent castration resistant prostate cancer cells both in vitro and in vivo. Although studies have demonstrated that AR signaling is a central mechanism of castration resistant prostate cancer progression, the cell growth mediated by AR-independent signaling is also operative. EGFR signaling has been implicated in the survival, invasion, and metastasis of prostate cancer cells in an AR-independent manner. Therefore, in this study, we tested whether the combination of carbamustine and selenite could inhibit EGFR signaling, induce apoptosis, and inhibit growth of AR-independent prostate cancer cells in an in vitro cell culture and in vivo xenograft models.

Methods: AR negative PC-3 prostate cancer cell line was used to study the effect of carbamustine and selenite alone or in combination on EGFR signaling. Cell viability, proliferation, and apoptosis in the presence or absence of EGFR (50ng/ml) were determined. Tissue and tumor cell extracts were used to determine pAkt and pERK1/2 protein expression levels. The combination treatment exhibited a strong inhibitory effect on EGFR stimulated Akt, and ERK1/2 phosphorylation. Combination treatment was able to induce apoptosis even in the presence of EGFR. Consistent with in vitro results, combination treatment for 3 weeks reduced PC-3 xenograft tumors in nude mice by 88% without any toxicity. Wherein individual agent treatment showed only partial effect both in vitro and in vivo. Conclusions: Our pre-clinical data demonstrate that the combination treatment of carbamustine and selenite substantially inhibits EGFR signaling, induces apoptosis, and reduces tumor growth of AR-independent prostate cancer cells in vitro and in vivo. Our novel findings suggest that the combination of carbamustine and selenite is an effective therapeutic agent for successful treatment, survival, and improved quality of life for patients with prostate cancer.

**#2038 Reactive oxygen species-mediated synergism of fenretinide and romidepsin in preclinical models of T-cell lymphoid malignancies.** Monish Ram Makena, Balakrishna Koneru, Min H. Kang, C. Patrick Reynolds. Texas Tech University Health Sciences Ctr., Lubbock, TX.

Introduction: T-cell lymphoid malignancies (TCLMs) are in need of novel and effective therapeutic strategies. Romidepsin is a histone deacetylase (HDAC) inhibitor that achieved FDA registration as 2nd line therapy for peripheral and cutaneous T-cell lymphomas. The cytotoxic retinoid fenretinide achieved durable complete responses against T-cell lymphomas in early-phase clinical trials and T-cell lymphoma patients who failed prior romidepsin treatment responded to fenretinide. Fenretinide is currently being evaluated in a Phase IIa clinical trial for T-cell lymphoma patients (NCT02159515). We propose that fenretinide has potential for using these two agents in combination in TCLMs. Methods and Results: Using the DIMSCAN assay, we demonstrated cytotoxic synergy between romidepsin and fenretinide in fifteen TCLM cell lines at clinically-achievable concentrations that lacked cytotoxicity for non-malignant cells (fibroblasts and blood mononuclear cells). In vivo, romidepsin + fenretinide + ketoconazole (enhances fenretinide exposures by inhibiting fenretinide metabolism) showed greater activity in subcutaneous (COG-LI-317m and TX-LY-183x PDX) and disseminated (COG-LI-317mLuc expressing the luciferase gene) TCLM xenograft models than single agent romidepsin or fenretinide + ketoconazole. Fenretinide + romidepsin caused a reactive oxygen species (ROS, measured by DCFDA dye)-dependent increase in pro-apoptotic proteins (Bim, BID, Bax and Bad) and induced TUNEL positive apoptosis. Unfortunately, both agents lacked antitumor efficacy, which achieved a synergistic increase in histone acetylation. The synergistic cytotoxicity, apoptosis, and histone acetylation of fenretinide + romidepsin was abrogated by antioxidants (vitamins C or E). Romidepsin + fenretinide activated p38 and JNK via ROS, and shRNA knockdown of p38 and JNK1 significantly decreased the synergistic cytotoxicity and apoptosis. Romidepsin + fenretinide also showed synergistic cytotoxicity for B-lymphoid malignancy cell lines, but did not increase ROS, acetylation of histones, activation of p38 + JNK, or cytotoxicity in fibroblasts and blood mononuclear cells. Conclusion: Romidepsin + fenretinide achieved synergistic activity in preclinical models of TCLMs, but not in non-malignant cells, via a novel molecular mechanism. These data support conducting clinical trials of romidepsin + fenretinide in relapsed and refractory TCLMs.

**#2039 The use of self nano-emulsifying drug delivery systems to improve bioavailability of combination therapy in non-small cell lung cancer.** Ebony Nottingham, Vasara Sekar, Arindam Mondal, Mandip Sachdeva. Florida Agricultural and Mechanical University, Tallahassee, FL.

Background: Erlotinib, an EGFR selective reversible inhibitor, improved response of patients diagnosed with non-small cell lung cancer (NSCLC) with 70% in lung cancer showing significant tumor shrinkage upon treatment. Unfortunately, most patients eventually relapse due to development of resistance. Our research goal is to quantify the effects of combination treatment of erlotinib with a gycurrylchecetic acid analogs and to improve the oral bioavailability of this treatment using self nano-emulsifying drug delivery systems. Methods: NSCLC cell lines HCC827, HCC827 (4μM erlotinib resistant), HCC827 Cl4 (second site EGFR mutated), HCC827 BEAS2B, and H1975 (4μM erlotinib resistant) were treated in combination with CDDA-Me, CF-CDDA-Me and Erlotinib. The cell viability assays were performed and combination index (CI) values were calculated by isobolographic analysis. Self nano-emulsifying drug delivery systems (SNEDDS) were formulated and characterized through in vitro and in vivo studies. Results: CI_CDDA and improved efficacy for erlotinib in all cell lines with IC50 values of 6.0μM, 7.21μM, 4.6μM and 4.2:1.56μM for HCC827(RESISTANT), H1975(RESISTANT), HCC827CL4 and HCC827 respectively compared to IC50 values of erlotinib alone (25μM, 21μM, 23μM, and 8μM). This was superior when compared to IC50 values of CDDA-Me combination treatment (6.66μM, 7.32μM, 12.0μM, and 3.45μM respectively). Combination treatment showed strong synergism with a constant concentration of 0.5μM CF, CDDA and CI values of 0.461, 0.548, 0.389, 0.550 for HCC827 4μM, H1975μM, HCC827CL4 and HCC827 respectively and antagonism in HCC827BEAS2B (1.508) cells. This is comparable to the synergism observed at a minimum concentration of 2μM CDDA-Me with CI values of 0.337, 0.67, 0.64, and 0.657 respectively and antagonism CI of 1.175 for HCC827BEAS2B. CF-CDDA Combination therapy showed a 2.5-fold decrease in colony formation making them comparable to wild type. This was comparable to CDDA-Me combination treatment with a 1.46-fold decrease in colony formation. Both combination treatments showed little effect on HCC827BEAS2B. Western Blot analysis showed decrease MET expression and decreased apoptosis inhibiting markers BCL2 and Survivin. SNEDD
formulations increased maximum drug plasma concentrations for Erlotinib (281 ng/ml to 442 ng/ml) and circulating time as well (12 hrs - 21 hrs). Conclusions: Based on the results of these studies, CDDOA-Me and CF,CDDOA have shown to overcome erlotinib resistance in non-small cell lung cancer and when combining these treatments with the use of SNEDDS, they have shown to be a promising treatment regimen.

#2040 Superior therapeutic efficacy of nanoparticle albumin-bound paclitaxel over cremophor-paclitaxel in experimental esophageal adenocarcinoma. Md Sazzad Hassan, Niranjan Awasthi, Roderich E. Schwarz, Margaret A. Schwarz, Urs von Holzen. Indiana University School of Medicine-South Bend, South Bend, IN.

Introduction: Esophageal adenocarcinoma (EAC) has become the dominant type of esophageal cancer in the United States. EAC is the fastest growing cancer in the world and the overall 5 year survival rate of EAC is below 20 percent. Most patients with EAC present with locally advanced or widespread metastatic disease, where current treatment is largely ineffective. Prognosis for EAC patients remains poor even with combination therapies due to high resistance to chemotherapy. Therefore, new therapeutic approaches are urgently needed. Paclitaxel (PTX) has been used in combination with carboplatin (CP) as a standard combination therapy for advanced EAC. PTX required emulsification with solvents to allow intravenous administration which has resulted in hypersensitivity reactions and potentially dramatic side effects in patients. Nanoparticle albumin-bound (nab) PTX is an albumin-stabilized, cremophor-free and water soluble nanoparticle formulation of PTX. Nab-PTX is a novel microtubule-inhibitory cytotoxic agent and the potential role of nab-PTX has not been tested yet in experimental EAC. Methods: We explored the antiproliferative and anti-tumor efficacy with survival advantage following CP, PTX and nab-PTX as monotherapy and in combinations in in-vitro, and in murine subcutaneous xenograft and peritoneal metastatic survival models of human EAC. Results: Nab-PTX inhibited in-vitro cell proliferation with significantly lower IC50 (0.25 μM in OE19 and 49 nM in OE33) than that of PTX (0.74 μM in OE19 and 98 nM in OE33) and CP (5.21 μM in OE19 and 1.05 μM in OE33) in OE19 and OE33 EAC cell lines. Nab-PTX treatment resulted in significantly higher antitumor efficacy and survival benefit compared with PTX or CP treatment. After two-week nab-PTX, PTX, CP, nab-PTX+CP or PTX+CP treatments, the average in vivo local tumor growth inhibition rate was 73, 60, 35, 81 and 68 percent respectively (p=0.025). Nab-PTX treatment increased expression of the mitotic-spindle associated phospho-stathmin, decreased expression of proliferative marker Ki-67 and enhanced apoptosis as confirmed by increased expression of cleaved-PARP and cleaved caspase-3. There was an increase in median animal survival after nab-PTX treatment (65 days) compared to controls (46 days, p=0.002), PTX (57 days, p=0.0004) or to CP therapy (53 days, p=0.034). Conclusions: In conclusion, the present study demonstrates that nab-PTX had stronger antiproliferative and antitumor activity in experimental EAC than the current standard chemotherapeutic agents. This strong antitumor activity supports the rationale for clinical evaluation of nab-PTX as promising microtubule-inhibitory agent in EAC.

#2041 High-throughput chemical screening for sensitization of bladder cancer to gemcitabine and cisplatin chemotherapy. Yuki Kita, Takashi Kobayashi, Ryouchi Saito, Toshinari Yamasaki, Takahiro Inoue, Osamu Ogawa. Kyoto University, Kyoto, Japan.

Introduction and Objectives: Gemcitabine and cisplatin chemotherapy (GC) is the current standard regimen for locally advanced and metastatic bladder cancer (BC). Despite a relatively high initial response rate, some cases do not regress (intrinsic resistance) and the remaining cases often show regrowth after initial shrinkage (acquired resistance). To identify novel therapeutic agents for overcoming these resistances, we applied a high-throughput screening of chemicals administered in combination with GC. Methods As a high-throughput screening, 2100 compounds were administered alone or in combination with GC to human BC cell lines (J82, UMUC-3). Cell viability was determined after 3-day incubation and chemicals that enhanced inhibitory effect of GC were screened. The in vivo effect of disulfiram (DSF) was studied in UMUC-3 cell xenografts, and Western blot, immunofluorescence, induced coupled plasma spectrometry and measurement of reactive oxygen species (ROS) were done in vitro for mechanistic exploration. Results: The initial screening identified 26 compounds and further validation narrowed them into the most synergistic agent DSF, an FDA-approved drug for alcoholism. Combination index assay showed synergistic effects of DSF with cisplatin but not with gemcitabine in J82, UMUC-3, T24, HT1197 and HT1376 cells. Co-administration of DSF significantly increased DNA-platinum adducts by regulating cisplatin efflux transporter ATP7A and enhanced apoptosis by GC treatment in UMUC-3 cells, with significant increase of ROS production. Use of DSF in combination with GC significantly increased tumor growth of UMUC-3 subcutaneous xenograft on athymic mice (by 39% compared with GC alone, p = 0.02). GC regimen was as tolerable as GC and no significant differences were observed in body weight of treated mice between the two regimens. Conclusions: Repositioning of DSF to a chemotherapy sensitizer is a promising treatment strategy, which can be translated rapidly in the future.

**EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Combination Therapy 2**

#2042 Nano-tetaoidothyroacetic acid (NDAT) potentiates gefitinib-induced antiproliferation in colorectal cancer cells by inhibiting EGFR sialylation and PI3K activation. Yu-Tang Chin, Chi-Yu Lin, Chun A. Changou, Jacqueline Whang-Peng, Paul J. Davis, Hung-Yun Lin. Taipei Medical Univ., Taipei, Taiwan; Albany Medical College, Albany, NY.

The resistance of gefitinib has been revealed to complicate cancer therapy. Tetaoidothyroacetic acid (tetra) and its nanoparticle derivative (NDAT, nano-tetra) have been proved in vitro and in vivo xenograft demonstrating anti-proliferative and anti-angiogenic activities. It also indicates that they potentiate anti-cancer agent-induced anti-proliferation in cancer cells. In this study, we investigated the effects of NDAT on gefitinib-induced anti-cancer activities in human colorectal cancer cells. Gefitinib inhibited cell proliferation at concentration 1 μM in K-ras wild type HT29 cell and NDAT enhanced the anti-proliferation-induced gefitinib significantly. Meanwhile, both inhibited proliferative and metastatic genes expression in HT29 cells. On the other hand, 10 μM gefitinib inhibited cell proliferation in K-ras mutant HCT116 cell which was further enhanced by NDAT. Different from results in HT-29 cells, only NDAT inhibited proliferative and metastatic genes expression significantly and enhanced the effect of gefitinib in HCT116 cells. ST6Gal-1 catalyzes sialylation of EGFR and induces gefitinib-resistant in colorectal cancers. In addition, NDAT did reduced not only ST6Gal-1 gene expression, but also its protein expression. However, the inhibition of ST6Gal-1 expression may not be sufficient to induced anti-proliferation in colorectal cancer cells. PI3K inhibitor, LY294002, was able to potentiate the gefitinib-induced anti-proliferation in HCT116 cells suggesting that constitutive activation of PI3K may play a key role on gefitinib-resistance in HCT116 cells. In summary, NDAT potentiated gefitinib-induced anti-proliferation via inhibiting the activity of ST6Gal-1 and PI3K activation in gefitinib-resistant colorectal cancer cells.

#2043 Entinostat in combination with Cladribine synergistically induces apoptosis in multiple myeloma cells. Bolun Wang, Hui Lu, Bolin Liu. University of Colorado Anschutz Medical Campus, Aurora, CO.

Cladribine (2-CDA) is a well-known purine nucleoside analog against lymphoproliferative disorders. Entinostat, a selective class I histone deacetylase (HDAC) inhibitor, exerts anti-tumor activity in various cancers, including multiple myeloma (MM). We sought to determine whether cladribine and entinostat may exhibit synergistic anti-proliferative/anti-survival effects on MM cells. Here we showed that, in cell growth MTS assays, either cladribine or entinostat alone inhibited cell proliferation in a dose-dependent manner. The combination of cladribine and entinostat significantly induced growth inhibition in MM cells tested. The combination index (CI) curves showed a synergistic effect between cladribine and entinostat. An apoptotic-ELISA and western blot analyses of caspase-3 and PARP revealed that entinostat in combination with cladribine enhanced both more potent activity than either agent alone to promote the MM cells undergoing apoptosis in a dose and time-dependent manner. Collectively, our data suggest that combinations of entinostat and cladribine possess synergistic anti-proliferative/anti-survival activity in MM cells. Regimens consisting of entinostat and cladribine may represent novel therapeutic strategies against MM.

**EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Drug Resistance: Other Topics**

#2044 Development of stable and brain-penetrating disulfiram nanopar-

ticles, characterization and efficay in glioma cell culture and xenograft mod-

Blood brain-barrier entry, tumor heterogeneity, need to eliminate the tumor-igenic stem cells and inhibit the DNA repair protein MGMT are all essential considerations in glioma treatment. Previously, we showed that alcohol aersion
ExPERIMENTAL AND MOLECULAR THERAPEUTICS: Drug Resistance: Other Topics

Drug disulfiram (DSF) shares all these properties [Carcinogenesis 35, 692, 2014; Oncotarget 4,502, 2013]. DSF was shown to inhibit the MGMT activity in the same way as ALDH by conjugating with the active-site cysteine 145 and sensitizes brain tumors to alkylating agents. Although DSF exerts significant anticancer effects in vitro, the drug undergoes rapid decomposition and metabolism. To overcome these limitations, we have engineered DSF nanoparticles to deliver the drug in PEG-PLGA polymer by solvent evaporation method. The formulation was optimized for various process formulation variables. The optimized nanoformulation (NP) ranged from 70-80 nm in particle size as confirmed using dynamic light scattering and transition electron microscopy. Drug loading, encapsulation efficiency, in vitro release were also optimized and the particles showed excellent BBB permeability in vitro. The time course of internalization of particles using a cyanine dye (HTIC-1) showed a lysosomal accumulation followed by mitochondria. Live animal imaging after IV injections of HTIC-labeled DSF-NPs revealed a selective accumulation in the brain and subcutaneous tumors. Further, the cytotoxicity elicited by DSF-NPs against SF188, DOAY, UW18, UW28 and T98G brain tumor cells was extended and greater than obtained with the free drug. When combined with DSF-NPs, temozolomide showed an 8-10-fold increased cytotoxicity against glioma cell lines. The DSF-NPs also induced significant degradation of MGMT and other redox-sensitive proteins such as the NF-eB, MDR1 and GSTP1 in a concentration-dependent manner in glioma cells. ROS induction by the encapsulated drug was confirmed through DCF-DA and DHE staining of live cells co-staining these cells with the mitochondrial membrane potential probes JC-1 and Rh123. DOX and DHE were monitored for the altered integrity. GSTP1 inhibition and ROS induction by DSF-NPs led to an activation of JNK/ MAPK pathway, culminating in autophagy and apoptosis as verified by western blotting, FACS and immunofluorescence of appropriate markers. Finally, the antitumor efficacy of DSF-NPs was tested in intracranial glioblastoma developed in nude mice by injecting Luciferase-expressing SF-188 GBM cells. A superior tumor regression was evident in DSF-NP-administered animals compared with the free drug after IVIS bioluminescence and histochemical staining of brain tissue. Collectively, our efforts provide strong evidence that DSF nanoparticles allow for the first time to directly evaluate drug binding to target proteins in a manner associated with the drug mode of action. A specific MDR1 spectral fingerprint (ITDRF) were used to study the relative drug target engagement in glioma spheroids. Modulation of p-glycoprotein altered the spectral response to drugs in a manner associated with the drug mode of action. A specific MDR1 spectral signature was not observed in these experiments but experiments with MDR1 silenced spheroids are underway to identify a possible signature.

#2047 The muscarinic receptor antagonist, benzotropine, blocks the expansion of a temozolomide resistant subpopulation of glioma cells. Damian A. Almiron Bonnin, Joseph M. Howard, Alison L. Young, Mark A. Israel, Matthew C. Havrda. Geisel School of Medicine at Dartmouth, Lebanon, NH.

Virtualy all malignant gliomas become drug resistant and recur after initial treatment, recurrent tumors are fatal within two-years, and no therapies exist that cure gliomas. The recognition that subpopulations of primitive cells with an oligodendrocyte precursor-like (OPC-like) phenotype capable of resisting standard therapy and contributing to recurrence, frames the question of whether adjuvant therapies designed to modify or eliminate OPC-like cells could slow the progression of glioma. Agents that force the differentiation of treatment-resistant OPC-like glioma cells, a phenomenon that requires cessation of proliferation, are compelling candidates for targeting OPC-like GSCs. Recently, it was observed that OPC proliferation was driven by activation of the M1, M3 and M4 muscarinic receptors. In another report, a large-scale drug screen designed to seek drugs to induce OPC differentiation in the context of multiple sclerosis identified a muscarinic receptor antagonist, benzotropine (a.k.a. Cogentin), as a potential new therapeutic differentiated, and cell cycle exit. We have conducted studies in glioma cell lines supporting a role for benzotropine in suppressing the development of drug-resistance to the standard glioma chemotherapeutic temozolomide (TMZ). Upon treatment with TMZ, we identified the emergence of an OPC-like subpopulation of glioma cells identifiable by the co-expression of CD44/CD15/PDGFRα and whether benzotropine will suppress the development of TMZ resistance in animal models of glioma.

#2048 Saturated fatty acids in cell membrane phospholipids play an important role in chemoresistance in colorectal cancer. Takanori Hirota, Takanori Sakaguchi, Satoru Furuhashi, Tomohiro Matsumoto, Yusuke Ozaki, Ryo Kuichi, Makoto Takeda, Yasushi Shibasaki, Yoshifumi Morita, Hirotoshi Kikuchi, Megumi Baba, Mitsutoshi Setou, Hiroyuki Konno. Hamamatsu University School of Medicine, Shizuoka, Japan.

[Background and Aim] Resistance to chemotherapeutics represents the major obstacle to survival in patients with colorectal cancer. Various factors, such as drug efflux, autocrine survival signaling, and alterations in DNA damage repair mechanisms, can contribute to chemoresistance; however, the actual underlying mechanism is yet to be elucidated. The aim of this study was to elucidate the mechanism behind anticancer drug resistance based on lipidomics.

<p>[Materials and Methods] 1) Spheroids were derived from three colorectal cancer patient-derived xenograft models (A, B, and C) that were incubated with chemotherapy agents, such as 5-fluorouracil (5-FU). We analyzed the response rate of spheroids under 5-FU treatment. Global lipid analysis was performed on 5-FU resistance spheroids (A) and sensitive spheroids (C) using a Q Exactive LC-MS/MS treatment platform. An increase in phospholipid was identified with Lipid Search software. 2) We tried to identify genes that were responsible for changes in phospholipid profiles in the cell membrane. 3) To determine phospholipid profile changes in the cell membrane that occur in colon cancer cells (HCT116, SW480) during anticancer drug resistance, the genes responsible for such resistance were knocked down using siRNA. [Results and discussion] 1) Changes in phospholipid composition and cell cycle events were also characterized from different aspects such as epigenetic markers, cell cycle events, oxidative stress and DNA repair capacity. Assays for nucleotide modifications and epigenetic markers in PDT resistant glioblastoma and its parental cell line showed significant higher level of two epigenetic marks including 5-methylcytosine (5-mC) and 5-hydroxymethylcytosine (5-hmC) in U-87R compared to U-87P, before and after PDT. Doubling time of U-87R cells was considerably longer than that of U-87P. Cell cycle analysis showed shorter G1 phase in U-87R cells compared to U-87P. Moreover, accumulation in G2 phase following PDT was observed earlier in resistant cells than in parental line. That suggests the role of early activation of DNA damage response in resistant cells. Resistance cells were found to be sensitive to either BRCA1/2 or RAD51, which are involved in homologous recombination (HR) pathway upstream inhibitor) failed to overcome bortezomib resistance. Only photodynamic therapy. This work was supported by the Polish National Science Centre (NCN) grants: DEC-2012/07/B/NZ1/00008, UMO-2014/15/B/NZ5/0144, UMO-2014/13/N/NZ3/00863.


Background: Triple-negative breast cancers (TNBC) are associated with an extremely poor prognosis due to their aggressive behavior and rapid resistance to chemotherapy. Chemotherapy may select for resistant subclones and lead to tumor recurrence. Although, DNA aneuploidy has been a long known prognostic biomarker in breast cancer, the biological role of aneuploid or polyploid cancer cells in tumorigenesis and chemoresistance is largely undefined. Polyploid cells arise due to repeated rounds of DNA duplication in the absence of mitosis. Chemotherapeutic DNA-damaging agents or mitotic inhibitors can also induce formation of polyploid cells and subsequent senescence. However, recent reports indicate that polyploid cells represent a viable and proliferating subpopulation within a tumor that may escape chemotherapy and contribute to tumor recurrence. Methods: In this study, we examined the prevalence and functional significance of polyploidy occurring de novo or induced by chemotherapeuy in TNBC cell lines. DNA ploidy was analyzed in Hschoest 33342 stained cells using FACS. Cells with greater than 4n DNA content were defined as polyploid. Live-cell imaging was used to observe cell division patterns in polyploid cells using IncuCyte Zoom. Cell proliferation was assessed in the absence and presence of chemotherapy drugs with distinct modes of action (docetaxel and 5-fluorouracil, respectively). Results: Polyploid cells showed formation of mitotic structures suggesting multi-polar cell division. Cell proliferation assay revealed that polyploid cells grow slower than diploid cells and show reduced sensitivity to all four chemotherapy drugs. Docetaxel treatment resulted in induction of polyploidy and drug-induced senescence in parental TNBC cells. Drug-induced polyploid cells were resistant to subsequent docetaxel treatment. Time-lapse imaging showed budding of small daughter cells from polyploid senescent cells. Majority of senescent cells died, some cells survived and regrewed the diploid and polyploid subpopulations similar to those present in parental cells. RNAseq identified differentially expressed genes involved in G1-S and G2-M checkpoint pathways (B2/1, RAD51), cell proliferation (Aurora kinase A/B) and apoptosis (BIM, BIRC3). Conclusions: Above findings indicate that there are molecular and functional differences between diploid and polyploid cells that are both naturally occurring or induced by chemotherapy. In our future studies, we will examine combinatorial approaches to overcome polyploidy-associated chemoresistance.

#2051 LSTRA cell line as a model for large granular lymphocytic leukemia in drug screening. Fu-Shin Chueh,1 Fu-Yu Chueh,2 Chao-Lan Yu2.

Large granular lymphocytic (LGL) leukemia is a rare form of leukemia that is characterized by the presence in circulating lymphocytes of LGL cells. A subcategory of T-LGL leukemia that co-expresses T and NK cell surface markers is a very aggressive and has a very poor prognosis. Current treatment of aggressive T-LGL is based on non-specific immunosuppressive therapy. Lack of model cell lines contributes to slow progress in designing targeted therapy and clinical trials. Our recent studies demonstrate that the mouse T-leukemic cell line LSTRA reproduces some important characteristics of aggressive T-LGL leukemia. This is the first report of a cell line model mimicking the deadly human leukemia and has great potentials for drug screening in treating T-LGL leukemia. Bortezomib is one of the drugs that show promising results in treating LGL leukemia patients in clinical trials. Bortezomib is the first FDA-approved reversible proteasome inhibitor in treating multiple myeloma and mantle cell lymphoma patients. One of the major concerns is that patients develop resistance during the course of treatment and cancers relapse. Therefore, it is important to develop novel strategies in overcoming LGL leukemic cells’ resistance to bortezomib and its derivatives. In our current study, we showed that bortezomib inhibited cell proliferation and induced apoptosis in LSTRA leukemic cells. Partial reversion after 48-72 hr of bortezomib treatment in U-87R suggested development of resistance to bortezomib. We further established bortezomib-resistant LSTRA cells that tolerated significantly higher levels of bortezomib. Both carfilzomib (FDA-approved second generation irreversible proteasome inhibitor) and MLN 9242 (proteasome pathway upstream inhibitor) failed to overcome bortezomib resistance. Only
luteolin, a common flavonoid found in plants, can effectively overcome bortezomib resistance. Metabolic reprogramming has been linked to drug resistance in cancer cells. Indeed, We found that bortezomib-resistant LSTRA cells had smaller cell size as well as reduced rate of glucose uptake, lactate secretion, and mitochondrial respiration. These results suggest that reduced mitochondrial energy metabolism may be an important factor underlying LSTRA cell’s resistance to bortezomib. All together, we identified luteolin as a potential small molecule in overcoming LGL leukemia’s resistance to proteasome inhibitor therapy. Altered mitochondrial activity and cell metabolism in bortezomib-resistant cells provide additional targets in developing novel combined chemotherapy for cancer patients.

#2052 Chemotherapy sensitivity, cytokine IL-8 level, and genetic expression mRNA IL-8 in gastric cancer treatment response prediction. Sirikan Limpakan (Yamada), Chiang Mai Univ. Faculty of Medicine, Chiang Mai, Thailand.

Aims: To determine chemotherapy sensitivity in primary gastric cancer cell isolated from Thai gastric cancer patients against anticancer drugs. To search for the correlation of interleukin-6 and interleukin-8 levels in primary gastric cancer cell culture with single cell isolation technique. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed to ex vivo examine the chemotherapy sensitivity to three anticancer drug: cisplatin, oxaliplatin, 5-Fluorouracil, and Irinotecan. Biopsy tissues from tumor site were collected before chemotherapy treatment in 63 patient who received neo-adjuvant chemotherapy by FOLFOX IV regimen due to advance stage III and IV in both resectable and un-resectable cases. The IL-8 mRNA gene expression was reported by relative quantitation real time RT-PCR. ELISA technique was performed to investigate the cytokine level from primary cultures taken from 41 gastric cancer patients who undergone biopsy from Maharaj Chiang Mai Hospital during year 2014 to 2016. The clinical data base was parallel collected and analysed in correlation with clinical response and ex vivo response. Results: Each patient has different sensitivity pattern to the standard anticancer drugs. There are no significantly direct correlations or difference between cytokine IL-8 levels or mRNA IL-8 expression, and the chemotherapy sensitivity to the actual drugs that are being used for treatment of gastric adenocarcinoma in individual patients. However, the chemotherapy resistance to all drugs in primary gastric cancer cell culture has trend to increase after the third cycle of chemotherapy treatment when compared to prior cell treatment with fluctuation of cytokine level. Different cytotoxic drug gives the same response result for chemresistance test in this population. The author observed the down regulation of tissue mRNA IL-8 expression and cytokine level in the good responders. Conclusion: The results provide an additional basis of information for primary chemotherapy selection in the individualised treatment for advanced gastric adenocarcinoma patients. Therefore, the author suggest to select the less toxic cytotoxic drug for being primary chemotherapy of choice. Cytokine gene level and mRNA IL-8 gene expression show individually significantly change after chemotherapy treatment in good responder. This result may also useful to find some new targeted therapy or additional treatment for its suppression.

#2053 Mechanisms of resistance to palbociclib and aromatase inhibitors in hormone receptor positive breast cancer. Armina A. Kazi,1 Antony Sare,1 Saranya Chumsri,2 Angela Brodie1. Loyola University MD, Baltimore, MD; Mayo Clinic, Jacksonville, FL; University of Maryland, Baltimore, Baltimore, MD.

Estrogen receptor-positive (HR+) breast cancer is the most common form of breast cancer that is accountable for the majority of breast cancer mortality. Currently, the cyclin dependent kinase inhibitor palbociclib in combination with endocrine therapy represents the new standard first and second line therapy for patients with metastatic HR+ breast cancer. While palbociclib has been shown to significantly improved progression free survival in combination with aromatase inhibitor (AI) and fulvestrant, resistance will inevitably occur in patients with metastatic breast cancer. However, the mechanisms of resistance to the combination of palbociclib and AI remain largely unknown. It is also unclear whether resistance mechanisms would be the same if palbociclib was given as first line treatment in combination with letrozole vs. if palbociclib was given as second line treatment after letrozole resistance had already occurred. To address this, previously well established and well characterized letrozole-sensitive MCF7/Ca cells and letrozole-resistant LTTLTa cells were subjected to continuous, long term treatment with increasing doses of letrozole and palbociclib until resistance to both drugs was achieved (MCF7/Ca+let+palb and LTTLTa+palb, respectively). Preliminary MTT cell viability assays indicate palbociclib IC50s of 750 nM in MCF7/Ca and LTTLTa cells vs. 21 nM in palbociclib and letrozole-resistant cells. First line and second line palbociclib resistance correlated with changes in morphology, protein expression, and cancer stem cell characteristics. Under phase contrast microscopy, first line and second line palbociclib-resistant LTTLTa cells were larger in size, more circular in shape, and tended not to grow in epithelial cell-like groups compared to palbociclib-sensitive MCF7/Ca and letrozole cells, with second-line palbociclib resistant cells exhibiting these characteristics the most. Western blot showed that ER protein expression in ER+/HER2- MCF7/Ca cells decreased with first line palbociclib and letrozole resistance, and that both ER and HER2 protein expression were decreased with second line palbociclib resistance in ErRlow/HER2+ LTTLTa cells. Lastly, mammosphere assays demonstrated increasing percentage of cancer stem cells with letrozole resistance alone (4 per 1000 cells plated MCF7/Ca vs. 58 per 1000 planted LTTLTa) and with palbociclib and palbociclib resistance (317 per 1000 cells plated MCF7/Ca and 202 per 1000 cells plated LTTLTa cells). Overall, these results indicate that 1) resistance to palbociclib, whether as first line or second line treatment has significant effects on breast cancer cells that may be relevant to patient diagnosis and treatment.

#2054 Cervicatatin overcomes the acquired resistance to crizotinib in EML4-ALK positive non-small cell lung cancer by controlling YAP activation. Miran Yun,1 Hun Mi Choi,2 Kyoung Ho Pyo,3 Byoung Chul Cho.1 Jeuk, Seoul, Republic of Korea; 2Yonsei University College of Medicine, Seoul, Republic of Korea.

Crizotinib is highly effective in patients with non-small cell lung cancer (NSCLC) harboring the echinoderm microtubule-associated protein-like 4 (EML4)-anaplastic lymphoma kinase (ALK) fusion. However, its efficacy has been limited by the development of acquired resistance, and the mechanisms of such resistance remain largely unknown. Herein, we investigated a possible candidate for circumventing the acquired resistance to crizotinib. We established a model of acquired resistance to crizotinib (H3122-CR) by exposing EML4-ALK-positive H3122 lung cancer cells to increasing doses of crizotinib, and performed MTT screening using a library of FDA (Food and Drug Administration)-approved drugs composed of a collection of 640 clinically used compounds. Our MTT screening identified that cervicatatin, a drug targeting 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, significantly inhibited the cell growth in H3122-CR cells, which was completely restored by addition of geranylgeranyl pyrophosphate (GGPP), a key metabolite of mevalonate pathway. We also found that yes-associated protein (YAP), a major effector of the Hippo tumor suppressor pathway, was robustly accumulated in nucleus with a concomitant decrease of YAP phosphorylation in H3122-CR cells compared to parental cells. Importantly, inhibition of geranylgeranylation (GGylation) by GGTI-298 or cerivastatin markedly increased YAP phosphorylation, led to cytoplasmic translocation of YAP, and subsequently induced YAP inactivation. Moreover, the enrichment of EGFR signaling pathway and cell cycle signature including transcriptional targets of YAP was enhanced in H3122-CR cells, whose induction contributed YAP-mediated resistance to crizotinib. Inhibition of YAP function with siRNA or verapamil as a YAP inhibitor greatly abrogated cell growth of H3122-CR cells, by regulating cell cycle regulation and EGFR activation. Finally, we confirmed up-regulation of nuclear YAP expression in crizotinib-acquired resistant patient derived tumor xenograft (PDXT) models established from EML4-ALK positive NSCLC patients. Collectively, our findings define the GGylation-mediated YAP transcriptional activation as a new mechanism of resistance to crizotinib, providing a rationale for further exploring statins as a possible anticancer agent to overcome the acquired resistance to crizotinib in EML4-ALK positive NSCLC cells.

#2055 Therapeutic resistance to BET bromodomain inhibition in prostate cancer.

Carl G. Engkel, Rohit Malik, Steven Kregel, Irfan A. Ansangari, Kani Wilder-Romans, Xia Jiang, Xuhong Cao, Corey Speers, Arul M. Chinnaiyan. University of Michigan, Ann Arbor, MI.

Prostate cancer is the most common cancer among men and a leading cause of cancer-related death in North America. Current therapies include radical prostatectomy and radiotherapy for localized disease, and androgen deprivation therapy (i.e. surgical or medical castration) for metastatic disease. Recently, we described the use of inhibitors of bromodomain and extraterminal (BET) protein peptides for the treatment of castration-resistant prostate cancer (CRPC), which have since undergone investigation in clinical trials. In anticipation of patients refractory to treatment with BET inhibitors, we explored the development of resistance to a clinical-grade BET inhibitor in four cell line models—VCaP, LNCaP, LNCaP-AR, and 22Rv1—by serially passing cells in increasing concentrations of drug with matched controls in DMSO. All resistance models...
displayed several-fold increased IC50 values relative to control in in vitro cell viability assays. Additionally, we have modeled resistance to BET inhibition in vivo by serially passaging cell line- and patient-derived xenografts in animals treated with BET inhibition. Interrogating these models through traditional molecular techniques and next-generation sequencing, we discovered several putative mechanisms of resistance to BET inhibition that we are currently validating in our laboratory.

#2056 MYC and HIF-2 alpha mediate resistance to Epidermal Growth Factor Receptor (EGFR) antagonism in oral squamous carcinoma cells. Su-Kanya Gayan, Reza Bayat-Mokhtari, Bidisha Pal, Anupam Sarma, Joyeta Talkudder, Sorra Sandhiya, Rashmi Bhuyan, Seema Bhuyan, Jaishree Gar- hyan, Debabrat Basuha, Amal Kataki, Rakesh Bhattacharjya, Herman Yeger, Bikul Das. Forsyth Institute, Cambridge, MA; B. Borodd Cancer Institute, Guwahati, India; KaviKrishna Laboratory, Guwahati Biotech Park, IIT, Guwa- hati, India; Gauhati University, Guwahati, India; Jawaharlal Nehru University, New Delhi, India; Hospital for Sick Children, Toronto, Ontario, Canada.

Background: Oral squamous cell carcinoma (OSCC) is a devastating dis- ease. Recent findings suggest that the EGFR antagonist including erlotinib may induce tumor regression alone or in combination with chemo/radia- tion. Our preliminary clinical study conducted at KaviKrishna laboratory indicates that many poor patients are taking the highly expensive drug Er- lotinib, and or equivalent EGFR antagonists. However, oral cancer lesions are often hypoxic, and the hypoxia-induced cellular mechanisms might con- tribute to drug resistance. Here, we investigated, whether resistance to EGFR antagonist could be enhanced by increased hypoxia.

Results: We used SCC-25 and SCC-9 cell lines, as well patient derived primary oral cancer cells (n=5) for the study. Immunomagnetic sorting was performed to obtain ABCG2+ population. The self-renewal was studied using in vitro clonogenic and in vivo serial transplantation assay in NOD/SCID mice. Results: First, we identified a rare ABCG2+ expressing, highly tumorigenic cell population in SCC-25, and SCC-9 having cancer stem cell (CSC) like characteristics. Second, we found that the ABCG2+ cells exhibited sensitivity to PD158780 (10 μM; 62% inhibition within 48 hours), and AG1478 (10 μM; 56% inhibition within 48 hours), two small molecular inhibitors of EGFR tyrosine kinase. These small molecular inhibitors signifi- cantly inhibited the clonogenic capacity of the ABCG2+ cells. Next, we found that ABCG2+ cells also showed sensitivity to Erlotinib in the in vitro clonogenic assay. Third, the ABCG2+ cells, when exposed to hypoxia (<0.1% O2, 24 hours), exhibited enhanced expression and transcriptional activity of MYC, and HIF-2alpha. The post-hypoxia ABCG2+ cells exhib- ited complete resistance to PD158780, AG1478 and Erlotinib treatment, which could be reversed by siRNA silencing of MYC and or HIF-2alpha. ChIP assay revealed that HIF-2alpha directly binds to MYC in ABCG2+ cells. We found similar results in ABCG2+ cells obtained from primary oral cancer patients (n=5) for the study. Importantly, ABCG2+ cell lines derived from patients exhibited hypoxic phenotype, including the high expression of HIF-1alpha, and HIF-2alpha, as well as resistance to erlotinib. Furthermore, erlotinib enhanced the stemness of ABCG2+ cells by activation of the MYC/ HIF-2alpha self-renewal pathway (1). Conclusion: These data indicate that MYC and HIF-2alpha operate to mediate intrinsic resistance of oral squa- mous cancer cells to EGFR antagonist in the hypoxia microenvironment. (1) Bhuyan et al. Cancer Research, volume 76 (14), abstract 935; 2016.


The high expression of P-glycoprotein (P-gp) is consistently observed in mul-tidrug resistance (MDR) cancer cells. The purpose of our study is to identify conditions that could increase the sensitivity of P-gp-overexpressing drug-resis- tant KBV20C cancer cell line. We have used the commercially available epige- ne library, which includes 128 compounds. Using the cell viability assay test, we also identified that NVP-BSK805 is highly sensitized to KBV20C-resistant cells and vincristine treatment. However, NVP-BSK805-induced sensitization was not observed in vincristine-treated sensitive KB parent cells, suggesting that the effects are specific to resistant cancer cells. Using FACS analysis, western-blots, and Annexin V staining, we identified that NVP-BSK805 sensitized vincristine- treated KBV20C cells via apoptosis and G2 arrest. Furthermore, sensitiza- tion potency of NVP-BSK805 in combination with vincristine was measured in KBV20C cells when comparing with P-gp inhibitor verapamil or another JAK2 inhibitor CEP-33779. We further investigated the mechanisms of NVP-BSK805 against sensitization of vincristine-treated KBV20C cells. The sen- sitization mechanism of NVP-BSK805 was mainly dependent on the inhibi- tion of P-gp. In addition, APTase activity was also conducted to identify its potential role in NVP-BSK805 for P-gp inhibition. Both NVP-BSK805 and CEP-33779 showed high binding affinity docking scores of -9.1 and -7.4, respectively, against the ABCB1 receptor protein. Collectively, inhibition of P-gp by NVP-BSK805 can increase the vincristine-sensitiveness in MDR cancer cells through increased apoptosis and G2 arrest. Our findings indicate that JAK2 inhibitor may be a promising target in the treatment of patients resis- tant to anti-miticot drug.


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To overcome resistance, 2nd and 3rd generation covalently binding TKI starget-approximately 70% overall response rate, increased progression free survival,Heymach. conclusion, unlike other EGFR TKIs, thenon-covalentEGFR inhibitors CUDC-ence of 50nM PKC412 and 500nM of CUDC-101. Moreover, PKC412 partiallytriple mutant Ba/F3 cells showed decreased phosphorylation of EGFR in pres-

The sensitization mechanism of XL109 against sensitization of vcinristine-treated KBV20C cells. The sensitization mechanism of XL109 was mainly depends on the inhibition of P-gp. In addition, ATPase activity was also conducted to identify as potential role of XL019 in P-gp inhibition. In the docking modeling, XL019 and CEP-33779 showed high binding affinity docking score against ABCB1 receptor protein by 10nts. Collectively, inhibition of XL109 by XL109 can increase the vcinristine-sensitivity in MDR cancer cells through in-

Toovercomeresistance,2nd and3rd generationcovalentlybindingTKIstarget-

anaplastic thyroid cancer (Tpo-cre/HrasG12V/p53flox/flox;Hras;p53) with the FTI staexploited clinically. We treated a murinemodel of poorly differentiated andanaplastic thyroid cancer (Tpo-cre/Hras<sup>12V12</sup>/p53<sup>lox/lox</sup>;Hras;p53) with the FTI tipifarnib, and observed sustained tumor regression and increased survival; however, tumors eventually recur. Following HRAS deolocalization by tipifarnib in vitro, ERK phosphorylation was only inhibited transiently in HRAS-mutant cell lines, which was associated with increased GTP loading of wild-type RAS proteins in the setting of RTK ligand stimulation. This adaptive reactivation of RAS-MAPK signaling was aborted by selective RTK (i.e. EGFR, FGFR) inhibitors, or by MEK inhibitors. Importantly, tipifarnib combined with the MEK inhibitor AZD6244 led to improved responses in Hras<sup>p53</sup> mouse tumors, whereas combination with the EGFR/FGFR inhibitors erlotinib and ponatinib did not, suggesting heterogeneity of upstream inputs. In order to identify ac-

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Drug Resistance: Other Topics

The CDK4/6 inhibitor palbociclib is currently being used in combination withendocrine therapy to treat advanced ER positive breast cancer patients. While this treatment has shown great promise in the clinic, about 25% of the patients do not respond, and almost all patients eventually acquire resistance to palbociclib treatment. Hence, understanding the mechanism(s) of acquired resis-
tance to CDK4/6 inhibition is crucial to devise alternate treatment strategies.

The farnesyltransferase inhibitor tipifarnib causes dramatic tumor regression and enhances cancer survival. It was shown that the farnesylation-in activates G9A-cAMP-CREB pathway and decreases expression of G9A, p53, and G9A-binding proteins in pancreatic cancer cells, which was associated with increased GTP loading of wild-type RAS proteins in the setting of RTK ligand stimulation. This adaptive reactivation of RAS-MAPK signaling was aborted by selective RTK (i.e. EGFR, FGFR) inhibitors, or by MEK inhibitors. Importantly, tipifarnib combined with the MEK inhibitor AZD6244 led to improved responses in Hras<sup>p53</sup> mouse tumors, whereas combination with the EGFR/FGFR inhibitors erlotinib and ponatinib did not, suggesting heterogeneity of upstream inputs. In order to identify ac-

#2061 Non-covalent EGFR T790M targeting TKIs inhibit AZD9291 resis-
tant EGFR C797S mutants. Jacqueline P. Robichaux, Monique Nilsson, John V. Heymach. UT MD Anderson Cancer Center, Houston, TX.

Approximately 10-15% of non-small-cell lung cancers (NSCLC) have epider-
mal growth factor receptor (EGFR) mutations resulting in increased sensitivity to 1<sup>st</sup> generation tyrosine kinase inhibitors (TKIs) such as gefitinib and erlotinib. For common mutations in EGFR, treatment with 1<sup>st</sup> generation TKIs results in approximately 70% overall response rate, increased progression free survival, and increased quality of life compared to chemotherapy alone. However, resis-
tance to 1<sup>st</sup> generation TKIs typically develops within ~12 months, and approx-
imately 55% of patients acquire a secondary mutation in EGFR Exon 20, T790M. To overcome resistance, 2<sup>nd</sup> and 3<sup>rd</sup> generation covalently binding TKIs target-
ing T790M mutations have been developed. Recent studies show that approxi-
mately 40% of acquired resistance to 2<sup>nd</sup> and 3<sup>rd</sup> generation TKIs can also occur via a third acquired EGFR mutation at the site of covalent binding, C797S. To date, there are no standard approved targeted therapies for treating EGFR C797S mutant NSCLC. Moreover, we have recently demonstrated the use of covalent inhibitors in the first line setting, C797S mutations are expected to become more prevalent and new strategies to overcome therapeutic resistance will be required. To this end, we have generated stable Ba/F3 and HCC827 NSCLC cell lines expressing C797S mutant EGFR receptors with common mutations in EGFR including, L858R/T790M/C797S, and Ex19del/T790M/C797S. EGFR mutant cell lines expressing C797S were screened against 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> generation EGFR TKIs and cell viability was determined using Cell Titer Glo. Triple mutant cell lines containing T790M and C797S mutations were not sensitive to any 1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup> generation inhibitors with IC<sub>50</sub> values of >10μM, 7.0μM, and 7.6μM, respectively. However Ba/F3 and HCC827 cell lines transfected with EGFR triple mu-
tants were inhibited by non-covalent inhibitors: CUDC-101, an EGFR, HER2 and HER3 inhibitor (GSEA) revealed enrichment of immune pathways (interferon alpha and gamma response, immune response) and pathways known to regulate EMT and cancer stem cells (IL-6/Stat3, IL-2/STAT-5, Notch, Wnt) in the resistant cells. Additionally, GSEA analysis revealed deregulation of G2/M checkpoint, estrogen response and DNA repair pathways (double strand break re-

Tumor cells treated with a PBD-based antibody-drug conjugate targeting ST4 develop acquired resistance due to ST4 down-regulation and response to alternate therapeutics. Shenlan Mao,1 Andrew Garcia,1 Tracy Chen,1 Rajanama Chaeradvy,1 Allison M. Marrero,1 Helen Zhaoming2. Dorsin Toader,1 Nazareno Dimasi,1 Maureen Kennedy,1 Philip Howard,2 Changshou Gao,1 Heren Wu,1 Ronald Herbst,1 David Tice,1 Jay Harper1.1 MedImmune, Gaithersburg, MD; 2MedImmune, London, United Kingdom.

Resistance to antibody-drug conjugates (ADC) has been observed in both preclinical models and in the clinic. It has been previously reported that an ADC targeting CD33 with a DNA cross-linking pyrrolobenzodiazepine (PBD) dimer was active in models of multidrug-resistant (MDR) AML, suggesting that PBDs, delivered as part of a molecular-targeted cancer therapy may be effective in MDR settings. However it could still be possible to develop resistance to PBD-conjugated ADCs though other mechanisms that remain unknown. Studies
were conducted to determine if tumor cells have innate resistance to PDs or an anti-ST4 ADC delivering PBd warhead. It was found that both the ADC and its PBd warhead, SG3199, completely eradicat MDA-MB-361 breast cancer cells and CNDI-N87 gastric cancer cells in culture, suggesting a lack of intrinsic resistance in these cell lines. The potential emergence of acquired resistance was investigated using miRNA stability, time-response silencing effect and the therapeutic effect in xenograft OC in vivo model. We demonstrated that siRNA-2'-F-P2x prolong the stability for more than 24 hours and enhance the silencing for more than 96 hours. Moreover, we found that PTGER3-silencing effect can control the expression of CFTR multidrug resistant pump by regulating ERK-ELK1-ETS1-CFTR axis, resulting in an increase of the cisplatin uptake, decreasing the tumors and associated angiogenesis and tumor growth in xenograft OC in vivo model. siRNA-PTGER3-2'-F-P2x modification increase the stability and time-response silencing effect, leading to a synergistic anti-tumor in combination with cisplatin in ovarian cancer models.

#2067 Tumor-infiltrating PMN-MDSCs mediate sorafenib resistance in hepatocellular carcinoma through immune suppression. Chun-Jung Chang,1 Li-Chun Lu,1 Cher-wei Liang,2 Chih-Hung Hsu,3 Ann-Li Cheng 4. Graduate Institutes of Oncology, College of Medicine, National Taiwan University, Taipei, Taiwan; 2Department and Graduate Institute of Pathology, National Taiwan University Hospital, Taipei, Taiwan.

Background: The mechanisms underlying the inherent or acquired resistance to sorafenib in advanced hepatocellular carcinoma (HCC) are not fully understood. We studied the role of myeloid-derived suppressor cells (MDSCs), a subset of peripheral blood monoocytes, in the development of resistance to sorafenib, an inhibitor of receptor tyrosine kinase activity, in murine liver cancer model. Results: Liver tumor-bearing mice were treated with sorafenib (5 mg/kg/d) for 1 week or 3 weeks from the day after tumor cells implantation. For combination therapy, mice were treated sorafenib and anti-IL-6 Ab or its isotype control Ab (500 μg/3 days starting from day 4 for 4 doses). Tumor infiltrating leukocytes (TILs) and leukocytes of other tissues including liver, spleen, peripheral blood, and bone marrow were isolated, and characterized by flow cytometry analysis. Morphological characteristics of MDSCs were determined by cytospin and Liu staining. Pro-inflammatory cytokines were detected by ELISA or mouse inflammatory cytokines beads array (BD). Paired HCC tissues from advanced HCC patients treated with sorafenib (before and post-sorafenib progression tissues) were evaluated for the expression of CD11b, CD15, and CD66b in TILs by immunohistochemistry. Results: In mice treated with sorafenib, the CD11b+/Gr1−Ly6G−/Ly6C− MDSCs were significantly increased in liver tumors, but not in others tissues. Morphologically, these MDSCs were segmented nuclear PMN type cells. The analysis of TILs revealed that the percentage of total immune effectors (CD4+ or CD8+ IFN-γ-expressing T cells versus total immune suppressors (CD10 or TGF-β-expressing CD4+ T cells, FOXP3-expressing CD4+ T cells) was lower in the tumors of sorafenib-treated mice than those of the vehicle-treated mice. The T cell proliferation capability was also significantly decreased in TILs of sorafenib-treated mice versus TILs of vehicle-treated mice. Multiple inflammatory cytokines including VEGF, G-CSF, MCP-1, and IL-6 were significantly increased in mouse liver tumors treated with sorafenib. When combined with sorafenib, anti-IL-6 antibody alleviated the early tumor-infiltration of Ly6G+ MDSCs, restored the proliferative activity of TILs, decreased the levels of pro-inflammatory factors in tumors, and suppressed the liver tumor growth synergistically. In 6 paired human HCC tissues, we found that 3 exhibited higher proportion of CD11b+, CD15, or CD66b+ cells in post-sorafenib progression tissues than their pre-sorafenib treatment tissues. Conclusions: Our data indicate that tumor-infiltrating PMN type MDSCs and associated immunosuppression may be an important mechanism impeding the therapeutic efficacy of sorafenib in HCC. (This works was supported by MOST 105-2314-B-002-180).
to date about p53 turnover through autophagy-lysosome pathway. Here we discover that the activation of p53 autophagic degradation during an oral small molecule receptor tyrosine kinase inhibitor sunitinib in p53 wt cancer cells and normal cells. Mechanistically, the nucleus-to-cytoplasm shift is essential for the autophagic degradation of p53 induced by sunitinib, however, does not require p53 nuclear export signals. The degradation of p53 is achieved by the nuclear-cytoplasm transport of its nuclear binding target HMGB1, shifting the distribution of p53 from the nucleus to the cytoplasm. Then cytoplasmic p53 directly interacts with the autophagy cargo receptor p62 to promote degradation. Importantly, inhibition of HMGB1 sensitizes cancer cell to sunitinib. Taken together, our study identified an alternative p53 protein turnover mechanism induced by sunitinib, thus not only elucidating the underlying mechanisms that limit sunitinib efficacy in cancer therapy but also opening an avenue for expanding the clinical indications of sunitinib.

Sub-clonal heterogeneity and risk of treatment failure in Philadelphia-positive leukemias. Michael W. Schmitt,1 Justin R. Pritchard,2 Lan Beppu,1 J. Graeme Hodgson,2 Victor M. Rivera,2 Lawrence A. Loeb,1 Jerald P. Radich,1 University of Washington, Seattle, WA; ARIAID Pharmaceuticals, Inc., Cambridge, MA; Fred Hutchinson Cancer Research Center, Seattle, WA.

Molecularly targeted therapies have been exceptionally successful in the treatment of some malignancies, most notably chronic myeloid leukemia (CML). However in other cancers, e.g. Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL), drug resistance is a frequent occurrence. Resistance mutations may pre-exist as a consequence of intra-tumor heterogeneity; thus the extent of heterogeneity may determine the likelihood of treatment failure. Intra-tumor heterogeneity is a challenging problem to quantify, as heterogeneous mutations are likely to occur below the background error rate of conventional approaches for mutation detection. To overcome this limitation and explore the relationship between intra-tumor heterogeneity and drug resistance in Ph+ leukemias, we utilized single-molecule Duplex Sequencing (DS) of the ABL1 gene. DS eliminates sequencing errors by independently tagging the two strands of individual DNA molecules; true mutations are present at the same position in both strands, while PCR and sequencing errors are only present in one strand. DS has an error rate below 0.00001%, and thus enables quantitation of heterogeneous mutations with unprecedented resolution. We find that CP-CML, a disease that is often well controlled with targeted therapy, has an extremely low burden of intra-tumor heterogeneity at the time of diagnosis, with a sub-clonal mutation burden similar to that of normal individuals. Simulations which take into account the low cancer stem cell fraction of CP-CML and the sub-clonal mutation burden we measured reveal that only a minority of CP-CML patients will harbor pre-existing drug resistance, which may explain the unusual success of targeted therapy in this setting. Next, we studied patients with advanced Ph+ leukemias (refractory BP-CML and Ph+ ALL), which have a high rate of treatment failure and poor outcomes. We find that patients with BP-CML and Ph+ ALL have a significantly elevated in sub-clonal heterogeneity relative to those with CP-CML. We demonstrate that the higher extent of intra-tumor heterogeneity correlates with a significantly higher rate of drug resistance mutations and compound resistance (i.e. two concurrent resistance mutations) in patients. Unlike CP-CML, pre-existing mutations conferring resistance to targeted therapy are nearly certain to occur below the background error rate of conventional approaches for mutation detection. Thus, we find that patients with BP-CML and Ph+ ALL have a significantly elevated in sub-clonal heterogeneity and high cancer stem cell fraction. We support this concept by three distinct approaches: (i) detection of pre-existing sub-clones that drive treatment failure; (ii) modeling the growth kinetics of resistant clones; and (iii) extrapolation of the mutation burden measured by DS. Our results suggest that intra-tumor heterogeneity influences the likelihood of resistance to molecularly targeted therapy. Quantitation of sub-clonal mutation load may be broadly applicable in other malignancies for predicting the likelihood of response to targeted therapy and monitoring disease progression.

Combination of CDDOA-Me, a glycyrrhetinic acid derivative, and Erolitinib overcomes chemo-resistance in NSCLC PDX spheroids and 3D bio-printed cells. Arindam Mondal,1 Aragaw Gebebehu1,2 Ebony Nottingham,1 Ar-vind Ragde,1 Subramanian Ramakrishnan,3 Arun K. Rishi,1 Mandip Singh1.

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Patient-derived Xenografts (PDx) are considered as relevant preclinical model for anticancer drug development due to original recapitulation of patient genetic profile, gene expression patterns and tissue histology. In this study, we investigated combination efficacy of CDDOA-Me (Methyl-2-cyano-1,11-dioxo-18-olean-1,12-dien-30-oate) and TKI inhibitor Erolitinib (ERL) against lung NSCLC PDX spheroids and 3D bio-printed PDX cells. NSCLC PDX cells (EGFR T790M mutants) were obtained from Dr. Rishi's Laboratory. PDx spheroids were grown in DMEM/F12 media supplemented with L-glutamine, B27 sup-
plement, recombinant human epidermal growth factor (EGF) and recombinant human basic fibroblast growth factor (bFGF). Spheroids were treated with CDODA-Me, ERL alone and in combination. Cell viability was measured by MTT assay. Western blot analysis was used to study the modulation of Bcl-xL, MDR1 and ABCG2 in treated PDX spheroids. For 3D bio-printing of PDX cells, hydrogels were prepared by partial cross-linking of sodium alginate (4.5% w/v) and gelatin (1% w/v) mixture with 40mM CaCl2 solution. PDX Cells were mixed with partially cross-linked hydrogel and printed with Inkredible 3D bio-printer (CELLINK, Sweden). Bio-printed scaffolds were fully cross-linked by 160 mM CaCl2 solution and then incubated overnight with cell culture media. The scaffolds were treated with CDODA-Me and ERL alone and in combination. After 48 h cell viabilities were determined by live/dead assay using fluorescence microscopy. MTT assay showed that approximately 65% and 74% viability was observed at 10 μM ERL and 2.5 μM CDODA-Me respectively. Decreased spheroid cell viability was observed in ERL and CDODA-Me combination treatment. Our western blot studies showed down-regulation of Bcl-xL, MDR1 and ABCG2 in combination group. Further, 81.04 ± 5.65, 78.65 ± 3.98 and 74.35 ± 4.24 percent viable PDX cells were observed in the bio-printed scaffolds after 48, 72 and 96 h respectively. Higher percentage of dead cells (52.62 ± 1.66) were found in the combination group than CDODA-Me (28.39 ± 1.60) and ERL (29.62 ± 4.91) alone. In conclusion, CDODA-Me in combination with ERL was found to be effective against human lung PDX spheroids and bio-printed PDX cells by decreasing the cell viability and overcoming drug resistance. Partially cross-linked sodium alginate-cohacitin hydrogel enhances the possibility of PDX cell bio-printing with high cell survival rate. CDODA-Me can be considered as an effective neo-adjuvant to improve ERL efficacy in human NSCLC.

#2073 A systematic investigation of the effect of scheduling of targeted combination therapies on response and dynamics of relapse in triple negative breast cancer cells. Gauri A. Patwardhan, Vikram B. Walk, Lajos Pusztai, Chris- tos Hatziis, Yale University, New Haven, CT.

Cancer treatment typically involves administration of combination of targeted therapies, but initial response is often followed by disease relapse. The efficacy of a treatment regimen depends on the complex interplay between cancer growth dynamics, drug specificity and kinetics, treatment dose and its scheduling. In standard high-throughput drug screening assays, cells are treated with a single drug cocktail bolus and cell viability is assessed after 2-3 days, thus not considering treatment interactions and long-term effects. Recently we have reported a promising synergistic combination of crizotinib (ALK/MET inhibitor) and ABT-263 (BCL2/BCL-XL inhibitor) against triple negative breast cancer cells. To understand the effect of the sequence and combination doses of crizotinib and ABT-263, we designed a comprehensive experimental plan that involved a total of 567 treatment regimens by varying treatment duration with the first drug (1, 2, or 3 days), followed by drug withdrawal and recovery period (2, 5 or 10 days) and then by a second cycle of treatment and recovery periods over a 26-day period. Cell viability was assessed by the CellTiter-Glo luminescence assay. Interestingly, ABT-263 alone induced higher cytotoxicity than an equivalent dose of crizotinib, but the remaining viable cells recovered much faster after ABT-263 withdrawal than cells after crizotinib withdrawal. Furthermore, cells exposed to higher doses of ABT-263 eventually become less sensitive to crizotinib. Among sequential regimens, crizotinib followed by ABT-263 was significantly more effective than ABT-263 followed by crizotinib, and combinations that in- cluded lower doses of ABT-263 were most effective. Taken together, our results show a significant interaction between the two targeted therapies, and suggest that it may be possible to select treatment scheduling that can delay drug resistance and tumor relapse in vivo.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Drug Resistance: Other Topics

#2076 Targeting the FGFR pathway in androgen receptor negative castration resistant prostate cancer. Colm Morrissey,1 Eva Cory,1 Lisa Brown,1 Ilia Coleman,2 Holland Guyn,1 Michael Schweizer,1 Peter Nelson,1,2 Univ. of Washington, Seattle, WA;2 Fred Hutchinson Cancer Research Center, Seattle, WA.

Metastatic castration-resistant prostate cancer (CRPC) has a poor prognosis and remains a significant therapeutic challenge. The continued importance of androgen receptor (AR) signaling as a growth and survival pathway in the most advanced prostate cancer (PC) has prompted the development of therapeutics directed toward further suppressing AR ligands or the AR itself. However, total androgen blockade can give rise to AR-negative PC and currently there are no effective therapies for AR-negative PC. We have developed two novel patient-derived xenograft (PDX) models from a liver metastasis and a rib metastasis of a patient with CRPC. LuCaP 173.1 (Neuroendocrine; NE) and LuCaP 173.2 (Dou- ble negative: DN (AR-negative and NE-negative)). Using RNA-Seq we observed that both of these lines express high levels of fibroblast growth factors (FGF’s) and downstream effectors. We hypothesized that FGF signaling promotes sur- vival and proliferation of NEPC and DNPC. To determine if blocking the FGFR survival pathway can attenuate the growth of NEPC/DNPC tumors, to charac- terize the molecular response and resistance to FGF signaling inhibition, and to support further evaluation in the clinic, we treated both PDX models with the FGFR inhibitor CH5183284. CH5183284 significantly reduced LuCaP 173.2 tumor volume (TV) (p<0.0001). The reduction in TV in CH5183284-treated ani- mals bearing LuCaP 173.1 tumors was not as significant. To address this difference, we analyzed FGFR1 expression at the transcript and protein levels. Analysis of the FGF’s in the LuCaP 173.1 PDX model revealed a loss of FGFR1 expression. FGFR2 and FGFR3 transcripts were expressed at similar levels in both PDXs. The loss of FGFR1 expression may explain the low efficacy of CH5183284 in the LuCaP 173.1 PDX model. We are currently assessing the molecular pathways impacted by FGFR inhibition and mechanisms of resistance to treatment in these studies. We have demonstrated that FGFR inhibition attenuated tumor growth in FGF[+]/AR-negative PC. We intend to target the FGFR survival pathway in additional NE and DNPC PDX models to investigate tumor heterogeneity in response to therapy and identify mechanisms of resist- ance. This studies will provide translational evidence that FGFR inhibitors could represent a new therapy for CRPC patients with AR-negative PC.

#2077 Selective MET kinase inhibition in MET-dependent glioma models. Corina van den Heuvel,1 Anna Navis,1 Houshang Amir,1 Kiek Vervliet,2 Arnd Hoegers,1 Karen E. de Reus,3 Isabelle Dussaut,2 Sean Caenepeel,5 Angela Coxon,2 Pieter Wesseling,1 William Leenders1. 1Radboudumc, Nijmegen, Netherlands; 2Amen Inc., CA.

Background - Grade 4 diffuse gliomas (glioblastomas) are notoriously difficult to treat. Many studies aim at targeting tumor-specific aberrations, such as mutations in genes encoding oncogenic receptor tyrosine kinases (RTKs). Of high interest as a tumor target in diffuse glioma is the RTK MET, which is amplified in a significant proportion of glioblastomas, and a number of MET inhibitors have been developed. However, most RTK inhibitors available in the clinic today, including those inhibiting MET, are not entirely selective and inhibit additional kinases at the doses used. They therefore may...
induce potentially undesired off-target effects, such as blood-brain barrier normalization in the case of concomitant VEGFR2 inhibition. Methods – We studied the effects of the novel, selective MET-kinase inhibitor (Compound A) and the combined VEGFR2/RET/MET inhibitor cabozantinib on MET activation and proliferation in the MET-amplified E98 astrocytoma cell line in vitro and using brain metastasis models. MET inhibition is known to have effects on AKT phosphorylation in the absence of MET phosphorylation, suggesting in vivo AKT resistance mechanisms. Interestingly, resistance to Compound A was not seen in vitro in an E98 cell line derived from Compound A-resistant tumor xenografts. Conclusions – Compound A is a promising, highly selective MET kinase inhibitor with activity against gliomas with constitutive MET signaling. Selective MET kinase inhibitors may be more suited for treatment of glioma than combined VEGFR2/MET inhibitors, as the latter may induce resistance mechanisms resulting in poor penetration. Similar to clinical experience, E98 tumor-bearing mice ultimately develop resistance to TKIs. Combination therapies targeting both MET and potential resistance pathways may be required to allow long-term tumor treatment.


Patients with non-small cell lung cancer (NSCLC) with activating epidermal growth factor receptor (EGFR) mutations initially respond to small molecule inhibitors of the EGFR tyrosine kinase such as gefitinib and erlotinib. However, secondary mutations in the kinase domain lead to acquired resistance to these inhibitors and limited clinical efficacy, with the EGFR T790M mutation being the most common mechanism of acquired resistance in more than half the patients that experience disease progression. CK-101 (also known as RX518) is a novel, orally administered EGFR kinase inhibitor that specifically targets the mutant forms of EGFR, including T790M, while exhibiting minimal activity toward the wild-type (WT) EGFR. WT EGFR inhibition is believed to drive the commonly observed side effects of skin rash and diarrhea. The 50% inhibitory concentrations (IC50s) of CK-101 and 2 reference compounds, afatinib and AZD-9291, were determined in cell proliferation assays in human cancer cell lines in vitro after incubation for 72 hours. Xenograft studies were conducted in BALB/c nude mice or SCID/Beige mice using once daily oral administration of CK-101 or afatinib for 14 or 21 days. CK-101 selectively inhibited cell proliferation of cell lines expressing the WT form of the receptor (e.g. A431, IC50 < 0.015 μM) and resistance mutations (NCI-H1975, IC50 ≤ 0.005 μM), but was much less potent at inhibiting proliferation of cell lines expressing the WT form of the receptor (e.g. A431, IC50 > 0.5 μM); i.e., CK-101 was over 100 fold less potent against A431 than against NCI-H1975. Single agent CK-101 significantly inhibited tumor growth in EGFR-mutated NSCLC xenograft models, with no activity in a WT EGFR xenograft model. In a xenograft study of PC-9 cells (exon 19 deletion) in SCID/Beige mice, CK-101 inhibited tumor growth by up to 90% (p < 0.001). In a xenograft study of NCI-H1975 cells (L858R/T790M double mutant) in BALB/c nude mice, CK-101 inhibited tumor growth by up to 95% (p < 0.001). The pre-clinical findings from this work strongly supported the clinical development of CK-101, and a first-in-human study of CK-101 was initiated in September 2016.


EGFR exon 20 insertion (Ex20Ins) mutations represent a combination of in-frame insertions and/or duplications that account for 4-10% of all EGFR mutations in non-small cell lung cancer (NSCLC). To date, more than one hundred different Ex20Ins mutations have been identified. With the notable exception of the rare A763_Y764insFQEA insertion (<1%), EGFR Ex20Ins mutations are clinically unresponsive to early generation EGFR inhibitors, the standard of care for NSCLC patients with EGFR Ex20Ins mutations is chemotherapy. Therefore, a significant unmet need remains requiring the development of an EGFR TKI agent that can more effectively target NSCLC with EGFR Ex20Ins mutations. Osimertinib (AZD-9291) was approved for the treatment of advanced NSCLC with EGFR T790M tumors. This work describes the potential of osimertinib and AZS5104, a circulating metabolite of osimertinib, in Ex20Ins tumors. Using CRISPR-Cas 9 genome editing technology, we engineered cellular disease relevant models carrying the most prevalent Ex20Ins mutations, namely Ex20Ins/HCC827, Ex20Ins/NCI-H11021, Ex20Ins/D770_N771delM and Ex20Ins/V790E. Using these models, we show that osimertinib and AZS5104 inhibit signalling pathways and cellular growth of Ex20Ins CRISPR-Cas9 engineered cell lines in vitro. This translates into sustained tumor growth inhibition in vivo in both the Ex20Ins SVD (65%, p < 0.001 & 95%, p < 0.001 respectively at day 14) and Ex20Ins AVS (82%, p < 0.001 & 95%, p < 0.001 respectively at day 14) xenograft models when compared to the control group. Importantly in vivo osimertinib was dosed at exposures consistent with the 80 mg osimertinib clinical dose. Moreover, a dual EGFR blockade strategy combining osimertinib or AZS5104 with cetuximab (an EGFR antibody) improved the anti tumor effect further. We also describe the anti-tumor activity of osimertinib and AZS5104 using a series of patient derived xenograft models harbouring the rarer Ex20Ins H773-V774insNPH and M766-V767insASV mutations. In addition, we performed pharmacodynamic studies to explore the relationship between efficacy and target/pathway modulation. These studies establish a clear relationship between depth and duration of phospho-EGFR inhibition and anti tumor efficacy. Interestingly, we observed that downstream signalling molecules displayed a more transient inhibition than the phospho EGFR signal. Two patients from the AURA Phase 2 osimertinib trials with plasma positive Ex20Ins (concurrent with Ex19del and T790M) had durable PFS responses of 6.4 and 13.9 months, supporting the premise that osimertinib has the potential to be clinically beneficial in tumors harboring Ex20Ins. The work presented herein demonstrates that osimertinib has the potential to improve upon the current treatment options for NSCLC patients whose tumors harbour an Ex20Ins mutation, and warrants its further clinical investigation.

#2080 Polycomb-mediated disruption of an AR feedback loop drives castration-resistant prostate cancer. Ka-Wing Fong, Jonathan Zhao, Jung Kim,1 Shangze Li,1 Angela Yang,1 Bing Song,1 Laure Rittié,2 Ming Hu,3 Ximing Yang,1 Bernard Perbal,4 Jindan Yu1. Northwestern Univ., Chicago, IL;1University of Michigan Medical School, Ann Arbor, MI;2New York University School of Medicine, New York, NY;4Université Côte d’Azur, CNRS, GREDEG, GREDEG, France.

Androgen receptor (AR) is an androgen-stimulated transcription factor that critically promotes prostate cancer development and progression. In addition to its conventional role in transcriptional activation, AR also acts as a transcriptional repressor to directly inhibit target gene expression. Through meta-analysis of androgen-regulated expression microarray data, we nominated CCN5/NOV, a CCN family protein, as one of the top AR-repressed genes in prostate cancer, implicating that CCN5 may play a tumour suppressive role in prostate cancer development. Therefore, we sought to investigate the molecular functions of CCN5 and its roles in prostate cancer progression especially in castration-resistant prostate cancer (CRPC). We first showed that CCN3 protein physically interacts with AR. The CCN3 interaction domain was mapped in the N-terminal domain of AR which is also present in constitutively active AR variants. Using immunofluorescence microscopy as well as subcellular fractionation technique, we demonstrated that CCN3 sequesters AR or AR variants into the cytoplasm to inhibit AR nuclear translocation, which in turn suppresses AR (ph)omatin targeting and transcriptional activation. However, this negative feedback loop between AR and AR-repressed gene CCN3 is disrupted in CRPC through CCN3 persistent epigenetic silencing by the Polycomb group protein EZH2, rendering AR transcriptional activation and CRPC progression. Taking advantage of this repressive mechanism, we restored CCN3 in CRPC cell model, either through ectopic overexpression or treatment with EZH2 inhibitor, which results in strong suppression of CRPC cell proliferation and anchorage-independent cell growth. Consistently, application of EZH2 inhibitor remarkably reduced tumor size in a castrated mice model. Taken together, our data highlight CCN3 as a novel regulator of AR signaling and support the promise of EZH2-targeting agents in treating CRPC.
#2081 Robust activity of BLU-285, a potent and highly selective inhibitor of mutant KIT and PDGFRα, in patient-derived xenograft (PDx) models of gastrointestinal stromal tumor (GIST). Yemarshet K. Gebreyohannes,1 Agnieszka Wozniak,1 Madeleina-Elena Zhai,1 Jasmin Wellens,1 Jasmin Cornillie,1 Erica Evans,2 Alexandra K Gardino,2 Christoph Lengauer,2 Maria Debiec-Rytkönen,3 Raf Sciot,4 Patrick Schöffski1. 1Department of Oncology, KU Leuven, and Department of General Medical Oncology, University Hospitals Leuven, Leuven Cancer Institute, Leuven, Belgium; 2Blueprint Medicines Corporation, Cambridge, MA; 3Department of Human Genetics, KU Leuven and University Hospitals Leuven, Leuven, Belgium; 4Department of Pathology, KU Leuven and University Hospitals Leuven, Leuven, Belgium.

Objective: Patients with advanced and treatment-resistant GIST are treated with tyrosine kinase inhibitors (TKI) such as imatinib (IMA), sunitinib (SUN) or regorafenib (REG). Resistance to these TKI is mainly caused by the emergence of on-target secondary KIT mutations in exons 13 and 17. We tested the activity of BLU-285 (Blueprint Medicines), a potent and highly selective inhibitor of mutant KIT and PDGFRα, in three PDx models of primary and refractory GIST that respond differently to the approved TKI used in the treatment of GIST. Methods: NMRI nu/nu mice (n=93) were transplanted bilaterally with human xenografts UZLX-GIST3 (KIT: exon 11 p.W557/V559delNS; IMA-sensitive), GIST2B (KIT: exon 9 p.A562/565dup, IMA dose-dependent sensitive) and GIST9 (KIT: exon 11 p.P577A; IMA resistant) in both right and left flank. Animals were dosed orally for 16 days. Activity was assessed by tumor volume measurement, histopathology and Western blotting of the KIT signaling pathway (WB). Mann Whitney U test was used for statistical analysis. A p value <0.05 was considered significant. Results: In all models, BLU-285 resulted in a dose dependent reduction of tumor volume, significant inhibition of proliferation (p < 0.05 compared to the control in all models, at all doses tested), and inhibition of KIT signaling as assessed by WB. In two models, BLU-285 led to a higher histologic response (graded as described previously by Antonescu et al. 2005), a significant increase of apoptosis (p < 0.005 compared to the control in both models, at all doses tested), and a pronounced decrease in MAPK phosphorylation as compared to the control. The activity of BLU-285 was similar (GIST3) or better (GIST2B and GIST9) than a standard dose of IMA. In the IMA-resistant model (GIST9), the anti-tumor effects of BLU-285 were significantly better those seen with either IMA or REG. BLU-285 was well tolerated at all administered doses. Conclusions: BLU-285, an investigational agent, has significant anti-tumor effects in GIST PDx models characterized by different KIT mutations and variable sensitivity to established TKI therapies. These data support the therapeutic rationale for the ongoing Phase I clinical trial being conducted by Blueprint Medicines (NCT02588312) and provide further evidence that BLU-285 has the potential to be an important treatment option for patients with PDGFRα or KIT-driven GIST.

#2082 NMS-E668, a potent and selective RET kinase inhibitor characterized by specificity towards VEGF2 and high antitumor efficacy against RET-driven cancers. Elena Ardini, Patrizia Banfi, Nilla Avanzi, Marina Ciomei, Paolo Polucci, Alessandra Ciria, Antonella Ermoli, Ilaria Motto, Elena Casale, Giulia Canevari, Cinzia Cristiani, Sonia Troiani, Federico Riccardi Sirtori, Nadia Amboldi, Dario Ballinati, Francesco Caprara, Eduard Felder, Arturo Galvani, Daniele Donati, Antonella Isachic, Maria Menichincheri. Nerviano Medical Sciences, Nerviano, Italy.

RET, a receptor tyrosine kinase (RTK) expressed mainly in neural crest-derived tissues, plays a role in cell growth and differentiation and its physiological activation depends upon binding to the GDNF family. Genetic aberrations leading to constitutive RET activation are associated to activating point mutations of RET, for example, are present in ca. 70% of medul- lary thyroid carcinoma patients including all hereditary cases, while RET gene rearrangements resulting in production of activated RET fusion proteins occur in approximately 10% of sporadic papillary thyroid carcinomas. More recently, recurring RET gene rearrangements have also been found in 1-2 % of lung adenocarcinomas and subsets of other solid tumors including colorectal and activating point mutations of RET, for example, are present in ca. 70% of medul- lary thyroid carcinoma patients including all hereditary cases, while RET gene rearrangements resulting in production of activated RET fusion proteins occur in approximately 10% of sporadic papillary thyroid carcinomas. More recently, recurring RET gene rearrangements have also been found in 1-2 % of lung adenocarcinomas and subsets of other solid tumors including colorectal and activating point mutations of RET, for example, are present in ca. 70% of medul- lary thyroid carcinoma patients including all hereditary cases, while RET gene rearrangements resulting in production of activated RET fusion proteins occur in approximately 10% of sporadic papillary thyroid carcinomas. More recently, recurring RET gene rearrangements have also been found in 1-2 % of lung adenocarcinomas and subsets of other solid tumors including colorectal and activating point mutations of RET, for example, are present in ca. 70% of medul- lary thyroid carcinoma patients including all hereditary cases, while RET gene rearrangements resulting in production of activated RET fusion proteins occur in approximately 10% of sporadic papillary thyroid carcinomas. More recently, recurring RET gene rearrangements have also been found in 1-2 % of lung adenocarcinomas and subsets of other solid tumors including colorectal and activating point mutations of RET, for example, are present in ca. 70% of medul- lary thyroid carcinoma patients including all hereditary cases, while RET gene rearrangements resulting in production of activated RET fusion proteins occur in approximately 10% of sporadic papillary thyroid carcinomas. More recently, recurring RET gene rearrangements have also been found in 1-2 % of lung adenocarcinomas and subsets of other solid tumors including colorectal and activating point mutations of RET, for example, are present in ca. 70% of medul- lary thyroid carcinoma patients including all hereditary cases, while RET gene rearrangements resulting in production of activated RET fusion proteins occur in approximately 10% of sporadic papillary thyroid carcinomas. More recently, recurring RET gene rearrangements have also been found in 1-2 % of lung adenocarcinomas and subsets of other solid tumors including colorectal and activating point mutations of RET, for example, are present in ca. 70% of medul- lary thyroid carcinoma patients including all hereditary cases, while RET gene rearrangements resulting in production of activated RET fusion proteins occur in approximately 10% of sporadic papillary thyroid carcinomas. More recently, recurring RET gene rearrangements have also been found in 1-2 % of lung adenocarcinomas and subsets of other solid tumors including colorectal and activating point mutations of RET, for example, are present in ca. 70% of medul-

#2083 Development of novel targeted adjuvant therapy for triple negative breast cancer. Nidhi Bansal,1 Eduardo Farias,1 Veronica Gonzalez,2 Garry Nolan,3 Samuel Waxman1. 1Icahn School of Medicine at Mount Sinai, Manhattan, NY; 2Stanford University School of Medicine, Stanford, CA.

There is an unmet clinical need for targeted adjuvant therapy in Triple Negative Breast Cancer (TNBC) to overcome its poor prognosis, short disease-free interval and metastatic dissemination. We previously reported that blocking interactions between the PAH2 domain of chromatin regulator Sin3 and Sin3 interaction domain (SID) containing proteins like PFI and TGIF1 by SID decoys (peptides and small molecule, C16) decreased the cancer stem cell population, invasion, EMT, and metastases. This, programmed upregulation of retinoic signal that sensitized TNBC cells to ADM, a novel clinically available RARα specific agonist. Here we report preclinical investigations on effects of SID decoys and ADM on treatment cells heterogeneity, primary tumors, metastatic dissemination, minimum residual disease (MRD) and host microenvironment. Using CyTOF2, we made single cell measurements of markers of differentiation, proliferation and stemness in CSC-enriched 4T1 tumor spheres. Treatment with SID peptide decreased cell populations expressing nanog, sox2, vimentin and β-catenin with increase in hY2A. Addition of ADM in combination with C16, resulted in populations with increased expression of differentiation marker CD24 with decrease in vimentin, β-catenin and Ki-67. To interrogate the neo-adenovirus effects of C16-ADM80 treatments, primary 4T1 tumors in Balb/c mice were treated with C16 and AM80 alone or in combination. Compared to DMSO, ~40 % decrease in tumor weight, 60% decrease in ALDH activity and 60% decrease in lung metastasis was seen in mice treated with C16-AM80 combination. In post-surgical adjuvant settings, both 4T1 and MMTV- myc xenografts, we observed 100% disease-free survival and absence of macrometastases in mice receiving minimally toxic adjuvant therapy with the C16-AM80 combination for 90 days. However, MRD was found consisting of a small number of single CK8+ cells which failed to form colonies when recovered from the bone marrow and selectively cultured in vitro. Upon stopping the treatments, 20-40% animals developed macrometastases within the first three months. To test the influence of C16-AM80 to condition the host microenvironment to prevent macrometastases mice received only pretreated pC16 and ADM80 followed by 4T1 cell injection in the tail vein. The percentage of parenchyma occupied by metastatic nodules were: DMSO = 50%; AM80 = 25%; C16 >10% and C16-AM = << 10%; the mitotic features were greatly reduce from 0-5 in DMSO to 0-1 per 400x/fi in the C16-AM80 combination. The predominant effects were observed with C16 treatment, which was enhanced to a small degree in combination with AM80. These results suggest that C16 can potently prevent changes in the host microenvironment to prevent the colonization and metastatic growth of cancer cells in TNBC.
**#2084 Conformational activation and allosteric inhibition of SHP2 in RTK-driven cancers.** Michael G. Acker,1 Ying-Nan P. Chen,1 Matthew J. LaMarche,1 Ho Man Chan,1 Peter Fekkes,1 Jorge Garcia-Fortanet,1 Jonathan R. LaRochelle,2 Brandon Antonakos,1 Christine Hiu-Tung Chen,1 Zhuliang Chen,1 Vesselina G. Cooke,1 Jason R. Dobson,1 Zhan Deng,1 Fei Feng,1 Brant Firestone,1 Michelle Fodor,1 Cary Fridrich,1 Hui Gao,1 Huai-Xiang Hao,1 Jaison Kato,1 Samuel Ho,1 Kathy Hsiao,1 Zhao B. Kang,1 Rajesh Sarki,1 Mitsuori Katoh,1 Jay Larrow,1 Laura R. Lontze,1 Gang Ling,1 Shumei Liu,1 Dyuti Majumdar,1 Matthew J. Meyer,1 Mark Palermo,1 Mingyu Pu,1 Edmund Price,1 Subarna Shaktiya,1 Michael D. Shultz,1 Kavitha Venkatesan,1 Ping Wang,1 Markus Warnmuth,1 Sarah Williams,1 Guizhi Yang,1 Jing Yuan,1 Ji-Hu Zhang,1 Ping Zhu,1 Stephen C. Blacklow,2 Timothy Ramsey,1 Nicholas J. Keen,1 William R. Sellers,1 Travis Stams,1 Pascal D. Fortin1. 1Novartis Institutes for BioMedical Research, Cambridge, MA; 2Dana-Farber Cancer Institute, Boston, MA. The non-receptor protein tyrosine phosphatase (PTP) SHP2 is an important component of RTK signaling in response to growth factor stimulus and plays just upstream of the RAS-MAPK signaling cascade. The first oncophosphatase to be identified, SHP2 is dysregulated in multiple human diseases including the developmental disorders Noonan and Leber syndromes, as well as leukemia, lung cancer and neuroblastoma where aberrant activity of SHP2 leads to uncontrolled MAPK signaling. Cancer-associated activating mutations in SHP2 imparts an “auto-on” state of the enzyme, boosting basal activity by shifting the equilibrium away from the auto-inhibited state. Reduction of SHP2 activity through genetic knockdown suppresses tumor growth, validating SHP2 as a target for cancer therapy. SHP099, a recently reported potent and selective allosteric inhibitor of SHP2, stabilizes the auto-inhibited form of SHP2 through interactions with the N-terminal SH2 and C-terminal PTP domains of the protein. SHP099 suppresses MAPK signaling in RTK amplified cancers resulting in suppressed proliferation in vitro and inhibition of tumor growth in mouse xenograft models. Together, these data demonstrate the therapeutic potential of SHP2 inhibition in the treatment of cancer and other RAS-MAPK-linked diseases.

**#2085 Development of a novel targeted therapy for malignant mesothelioma.”** Takuya Fukazawa,1 Yuitaka Maeda,1 Tomoki Yamatsumi,1 Munenori Takaoka,1 Masakazu Yoshida,1 Naomasa Ishida,1 Miki Iwai,1 Etsuko Yokota,1 Takuro Yukawa,1 Minoru Haisa,1 Noriko Miyake,1 Tomoko Ikeda,1 Nagio Takigawa,1 Jeffery Whitsett,1 Yoshio Naoi1. 1Kawasaki Medical School, Okayama, Japan; 2Cincinnati Children’s Hospital Medical Center, Cincinnati, OH. Malignant pleural mesothelioma is an aggressive tumor of mesenchymal origin and is increasing worldwide as a result of widespread exposure to asbestos. The median survival of patients with mesothelioma from time of diagnosis ranges between 1 and 2 years. The mortality is expected to increase, at least until 2020, which is mainly due to the long latency (30-50 years) of the disease. Since considerable advances in the understanding of its pathogenesis and etiology, malignant mesothelioma remains largely unresponsive to standard modalities of cancer therapy. Thus, there is an urgent need for new therapeutic options for mesothelioma. Midkine (MDK) is a heparin-binding growth factor that is highly expressed in many malignant tumors, including lung cancers. We have previously reported that a MDK inhibitor, iMDK, suppresses non-small cell lung cancer expressing MDK without harming normal cells. Importantly, iMDK inhibits the PI3 kinase / Akt pathway and induces apoptosis in MDK expressing non-small cell lung cancer cells. In the present study, we have investigated the antitumor effect of iMDK against malignant mesothelioma both in vitro and in vivo. 48 hours after treatment, iMDK dose-dependently inhibited cell growth of MDK expressing malignant mesothelioma cells. iMDK also suppressed colony formation of MSTO-211H mesothelioma cells. TUNEL positive cells were significantly increased in MSTO-211H cells 48 hours after iMDK treatment in a dose-dependent manner, confirming the induction of apoptosis in mesothelioma cells by iMDK. Combination treatment of iMDK and Bcl-2 inhibitor ABT-263 is more effective than each drug alone in MSTO-211H mesothelioma cells. Moreover, systemic administration of iMDK significantly inhibited tumor growth in a mesothelioma xenograft tumor in vivo. Inhibition of MDK with iMDK provides a potential therapeutic approach for the treatment of malignant mesothelioma that is driven by MDK.

**#2086 Dominant role of receptor tyrosine kinase in the activation of MAPK signaling in BRAF non-V600 mutant cancers.** Hiromichi Ebi, Hiroshi Kotani, Hidenori Kitai, Yuta Adachi, Seiji Yano. Kanazawa Univ. Cancer Research Inst., Kanazawa, Japan. BRAF mutations are found in ~8% of all cancers. In addition to the commonest mutation in kinase domain at the V600, a wide range of other missense mutations (non-V600) have been reported. Non-V600 mutant BRAF protein was also shown to enhance MAPK signaling, suggesting the effectiveness of MEK inhibitors. However, the existence of feedback mechanism in MAPK signaling in BRAF mutant cancers has not fully been understood. To investigate this, BRAF non-V600 cell lines with intermediate/ impaired kinase activity were treated with MEK inhibitor. RTKs involved in the upregulation of P-MEK following MEK inhibition in BRAF non-V600 mutant cell lines were determined by phospho-RTK arrays. The effect of combinatorial inhibition of RTKs and MEK in BRAF non-V600 cancer was assessed in vitro and in mouse xenograft models. By analyses of data, we found that BRAF non-V600E mutation were less sensitive to MEK inhibition compared with BRAF V600E mutant cell lines and almost comparable to RAS/RAF wild-type cell lines. MEK inhibition did not induce P-MEK suppression and it resulted in significant upregulation of p-MEK compared to BRAF V600E mutant cell lines. Phospho-RTK arrays in the presence or absence of trametinib displayed basal phosphorylation of RTKs including EGFR, MET, and JGFIR, although the phosphorylation level was not consistently changed following MEK inhibition. Among these RTKs, treatment with the EGFR inhibitor erlotinib led to more complete suppression of P-ERK upon trametinib treatment. Furthermore, we found that erlotinib monotherapy downregulated P-ERK in BRAF non-V600 mutant cell lines in contrast that inhibition of RTKs had no effect on MAPK signaling in BRAF non-V600 mutant cells. While erlotinib treatment led to complete suppression of P-MEK in intermediate active and impaired kinase active BRAF mutant cell lines, p-MEK and P-ERK were more strongly suppressed in impaired type BRAF non-V600 mutant cells. The combination of EGFR inhibitor and MEK inhibitor suppressed cell growth and achieved tumor shrinkage in mice xenograft. These results suggest that RTKs, especially EGFR, are dominantly involved in the regulation of MAPK signaling in BRAF non-V600 cancer cells.

**#2087 Pan-HER inhibitor, varlitinib, disrupts HER/ERK signaling and causes apoptosis in triple-negative breast cancer cells.** Chun-Yu Liu1, Tzu-Ting Huang,1 Chun-Teng Huang,1 Hsiu-Ping Yang,1 Ling-Ming Tseng1, Chung-Wai Shiau,1 Kuen-Feng Chen1,2 Taipei Veterans General Hospital, Taipei, Taiwan; 3Yang-Ming Branch of Taipei City Hospital, Taipei City, Taiwan; 4National Yang-Ming University, Taipei, Taiwan; 5National Taiwan University Hospital, Taipei, Taiwan. Background: Triple-negative breast cancer (TNBC), characterized by aggressive behavior and poor prognosis, represents an important clinical challenge because there is no well-established target therapy. Therefore, the identification and validation of a targeted therapy for TNBC is an urgent need. Molecular profiling studies have shown some TNBC tumors harboring aberrant epidermal growth factor receptor (EGFR) or human epidermal growth factor receptor (HER) signaling, suggesting therapeutic potential with EGFR/HER inhibitor. Varlitinib (ASLAN001) is a small molecule reversible pan-HER inhibitor of EGFR (HER1), HER2 and HER4. To date, varlitinib has been extensively investigated in several tumor types, including HER2 positive metastatic breast cancer. TNBC is known to demonstrate expression of EGFR. Since varlitinib also targets EGFR signaling, we hypothesized that it may also have antitumor efficacy in TNBC. Methods: MDA-MB-231 and MDA-MB-468 TNBC cell lines were used for in vitro studies. Cell viability was examined by MTT assay. Apoptotic effects were examined by flow cytometry and Western blot. Signal transduction pathways in cells were assessed by Western blot. Results: We first examined the protein expression of EGFR in a panel of TNBC cell lines. We identified MDA-MB-468 and MDA-MB-231 as EGFR-expressing cell lines. We found that varlitinib significantly inhibited cell viability and induced cell apoptosis in MDA-MB-468 cells but not in MDA-MB-231 cells. MDA-MB-231 cells carry the KRAS G13D mutation that drives downstream ERK signaling. We therefore examined the downstream signaling proteins of EGFR, including PI3K/Akt and MAPK/ERK. Results showed that the protein levels of p-EGFR and p-ERK were decreased in varlitinib-resistant MDA-MB-468 cells, but there was no significant change in these phospho-proteins in varlitinib-resistant MDA-MB-231 cells. Furthermore, we found that ERK inhibition sensitized varlitinib-resistant cells to varlitinib-induced cell death. In addition, ectopic expression of ERK reduced the varlitinib-induced apoptosis in varlitinib-sensitive MDA-MB-468 cells. In addition, MDA-MB-468 cells are known to harbor V600E mutation in the RAF gene of function mutation that may activate EGFR signaling, whether the mutation is associated with varlitinib sensitivity needs further investigation. Conclusions: In this study, we identified TNBC as another tumor type that may be sensitive to varlitinib’s antitumor activity through the inhibition of HER/MAPK signaling and subsequent increase in apoptotic activity.
**EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Growth Factor and Hormone Receptors as Therapeutic Targets**

#2088 The activity of the FGFR selective inhibitor Debio 1347 is correlated with high mRNA expression. Franck Brichory,1 Anna Pokorska-Bocci,1 Paolo Nuciforo,2 Stefania Rigotti,1 Nathalie Lembrecz,1 Grégoire Vuagniaux,3 Corinne Moulon,4 Anne Vaslin1,1 Debiopharm International SA, Lausanne, Switzerland; 2Vall d’Hebron Institute of Oncology, Barcelona, Spain.

Dysregulated growth factor receptor (FGFR) signaling pathway due to receptor overexpression, gene amplification, point mutations or fusions/chromosomal translocations is associated with cancer development and progression. Debio 1347 (CHS183284) is an oral selective FGFR inhibitor (FGFR1,2 and 3) currently in clinical development. The aim of this study was to investigate Debio 1347 activity in patient derived xenograft (PDx) mouse models harboring diverse FGFR alterations in multiple in vivo (TDI) and in vitro (CRL) settings. The trial was conducted in 66 PDx models of diverse histotypes selected according to their FGFR1, 2 and 3 alteration status. Debio 1347 was administered orally once daily at 40 up to 80 mg/kg for 10 to 22 consecutive days (N=3/group). Tumor volume was compared to the vehicle control group and measured by caliper twice weekly. Treatment response was determined by relative treatment-to-control ratios (ΔT/ΔC) of which a responding model was defined as ΔT/ΔC < 0. PDx tumors were collected at the end of the treatment period and extensively characterized using FISH, nCounter Gene Expression and immunohistochemistry (IHC). Effect on downstream Dual Specificity Phosphatase 6 (DUSP6) signaling was also investigated by qPCR and RNA in situ hybridization (ISH). Debio 1347 induced tumor regression in 33% of PDx models that exhibited a gene copy number gain, presence of a gene fusions for tumor gene amplification. In addition, Debio 1347 treatment led to tumor regression in 29% of models that did not harbor any FGFR genetic alteration. In contrast, all models that responded to Debio 1347 were shown to display a high expression level (mRNA) of at least one FGFR gene. These findings suggest that high expression of at least one FGFR might be a better predictor of sensitivity to Debio 1347 than genetic alteration. Furthermore, response to Debio 1347 was associated with a decrease in DUSP6 mRNA levels, suggesting that it could be a reliable pharmacodynamic biomarker in clinical trials. These results provide new mechanistic insights into the predictive sensitivity to Debio 1347 and will help refine patient selection for FGFR-targeted therapy.

#2089 Evaluation of fruquintinib, a potent and selective oral VEGFR inhibitor, in combination with targeted therapies or immune checkpoint inhibitors in preclinical tumor models. Yongxin Ren, Qiaoing Sun, Jingwen Long, Shiming Fan, Renniang Tang, Wei Zhang, Xuelei Ge, Jianxing Tang, Linfang Wang, Dongrui Shi, Hongbo Chen, Min Cheng, Weiguo Qing, Weiguo Su, Hutchison MediPharma Ltd., Shanghai, China.

The development of therapies targeting tumor angiogenesis, tumor driver gene alterations and tumor immune evasion has made tremendous advancement in improving overall survival. However, efficacy may be limited and resistance often develops rapidly when targeting a single axis of tumor growth. Therefore, it is important to explore rational combination of therapies based on tumor-specific features. Fruquintinib is a potent and selective oral VEGFR inhibitor currently in Phase III clinical trials for non-small cell lung cancer (NSCLC) and colorectal cancer (CRC). We report here the evaluation of anti-tumor effect of fruquintinib in preclinical animal tumor models in combination with therapies targeting tumor driver gene alterations such as EGFR and c-MET with immune checkpoints. In NSCLC xenograft models with EGFR activation such as activating mutations, gene amplification or protein overexpression, fruquintinib plus an EGFR tyrosine kinase inhibitor such as gefitinib or thalitabin (HMPL309) was found to be more efficacious than either monotherapy. For instance, in PC-9 subcutaneous tumor model carrying EGFR exon 19 deletion, single agent treatment with fruquintinib at 2 mg/kg and gefitinib at 5 mg/kg produced the tumor growth inhibition (TGI) of 63%, respectively, while the combination treatment resulted in a TGI of 100% and tumor regression was observed in 11 of 16 mice treated with combinational therapy. In multiple xenograft models derived from lung cancer or renal cell cancer with c-MET activation (amplification or over-expression), addition of fruquintinib to a c-MET inhibitor savolitinib (AZD6094, HMPL-304) also improved the tumor growth inhibition substantially. At the end of the efficacy studies, CD131 and phosphorylation of EGFR, c-MET, AKT and ERK were analyzed with immunohistochemistry and western blotting method in tumor tissues. The results suggested that the enhanced anti-tumor effect in combination therapy could be attributed to the simultaneous blockade of cell signaling in tumor cells (EGFR or c-MET) and VEGFR suppression in the tumor microenvironment. Up-regulation of the immune inhibitory checkpoint PD-1/PD-L1 is one of the important mechanisms for tumor cells to escape immune surveillance. In a syngeneic murine tumor model, co-administration of fruquintinib and anti-PD-L1 antibody was found to provide improved anti-tumor effect compared to fruquintinib or anti-PD-L1 single agent alone. Studies to understand the mechanism responsible for the combination effect are under way. All combinations with fruquintinib described above were well tolerated. The efficacy observed in these models suggested that simultaneous blockade of tumor angiogenesis and tumor cell signaling or immune evasion may be a promising approach in improving treatment outcomes.

#2090 Targeting estrogen receptor negative breast cancer cells using diarylhthiourea analogs of ShetA2. Hongye Zou,1 Emily Ginn,2 Sabah M. Francis,3 Shengjuan Liu,4 Maggie Louie,5 Dominican University of California, San Rafael, CA; 2Touro University of California, Vallejo, CA.

Breast cancer is one of the most common cancers that occur in women in the United States. Depending on the expression of estrogen receptor or (ERx), breast cancer can be classified as ER-positive or negative. ER-negative (ER-) breast cancers are often more aggressive and have poorer prognosis compared with the ER-positive (ER+) subtype. Consequently, there is a great need to develop more targeted therapeutic options for ER-breast cancer. One such compound is ShetA2, a flexible tetrahydropyran (J-fluorocycle) that has been shown to exhibit growth of multiple cancer types including breast cancer. However, the clinical utility of ShetA2 is limited by its high lipophilicity (LogP). The goal of this study is to screen eleven 2nd generation analogs of ShetA2 with lower LogP values — SL1-22, 24, 27, 29, 30, 32, 37, 38, 39, and 40 — on different breast cancer cell lines to identify potential lead compounds that show the highest anti-growth activities against ER-breast cancer cells. In vitro, we will further delineate the mechanism by which these compounds affect breast cancer cell growth. Results from this study demonstrate that SL1-38 [1-(3-chloro-4-methyl-phenyl)-3-(4-nitrophenyl)]thiourea] and SL1-39 [1-(4-chloro-3-methylphenyl)-3-(4-nitrophenyl)]thiourea], inhibit ER-negative breast cancer cells (MDA-MB-231, MDA-MB-453 and MDA-MB-468) effectively at micromolar concentrations. In order to understand the mechanisms of action, we evaluated the effects of SL1-38 and 39 on the expression of key cell cycle regulators. Our results show that these two analogs down-regulate the expression of cyclin A, cyclin B, cyclin D1, cyclin E and cdk2 and block S-phase progression. Taken together, these preliminary results suggest that SL1-37 and 39 may be further developed as anti-cancer agents for treating ER-negative breast cancer.

#2091 PRN1371, an irreversible, covalent inhibitor of FGFR1-4 exhibits sustained pathway inhibition in cancer cell lines. Eleni Venetsanakos, Yan Xing, Natalie Loewenstein, J. Michael Bradshaw, Dane Kerr, Jacob LaStant, Philip Nunn, Jin Shu, Abha Bommireddi, Jens Oliver Funk, David M. Goldstein, Stefani Wolff, Ken A. Brameld, Steven G. Gourlay. Principia Biopharma, South San Francisco, CA.

Introduction: Multiple human cancers harbor alterations in FGFRs that drive tumor growth, including mutations, translocations and amplifications. PRN1371 is a potent, highly selective irreversible inhibitor exhibiting sustained inhibition of FGFR1-4 in primary renal proximal epithelial cells. Conclusions: PRN1371 is a potent, highly selective irreversible inhibitor exhibiting sustained inhibition of FGFR which extends well beyond circulating drug concentrations in preclinical models. The duration of inhibition of the FGFR signaling pathway is dependent on protein turnover of FGFR, which may vary depending on the type of FGFR alteration. Thus, we set out to investigate whether the duration of target inhibition differs across cancer cell lines of various lineages harboring different FGFR alterations, including fusions and mutations. Furthermore, as FGFR inhibitors exhibit hyperphosphatemia via on-target pharmacology, we also investigated the duration of target inhibition in primary renal epithelial cells which are wild-type for FGFR. Materials and Methods: Cancer cell lines from several lineages harboring different FGFR alterations were treated with increasing concentrations of PRN1371 in vitro for 1 hour, before washing out the compound. Cells were harvested at various time-points post-washout, protein lysates were generated and assessed for modulation of the downstream signaling pathway by western blot analysis. Results: Dose-dependent inhibition of phospho-ERK was observed in the cancer cell lines tested in response to compound treatment for 1 hour in vitro. Dose dependent partial or full rebound of phospho-ERK back to baseline levels were detected in cancer cell lines after a prolonged period post-washout. In contrast, full rebound of phospho-ERK was observed at 1 hour post washout in response to a non-covalent inhibitor. Studies are on-going to assess duration of pathway inhibition in the primary renal proximal epithelial cells. Conclusions: PRN1371 is a potent, highly selective irreversible inhibitor exhibiting sustained inhibition of FGFR signaling across cancer cell lines harboring different FGFR alterations. The duration of target inhibition differed across cancer cell lines harboring different FGFR alterations, including mutations, fusions and amplification of FGFR and was prolonged when compared to a non-covalent inhibitor. A Phase 1 clinical trial of PRN1371 for the treatment of solid tumors harboring FGFR alterations is ongoing (NCT02608125).
#2092 A potent and selective RET inhibitor with efficacy in RET-driven mouse models of medullary thyroid carcinoma and lung adenocarcinoma.

Mandy Watson,1 Helen Small,1 Ben Acton,1 Habiba Begum,2 Samantha Hitchin,1 Allan Jordan,1 Paul Kelly,1 Rebecca Newton,1 Ian Waddell,1 Gina Paris,1 Donald Ogilvie1. 1Cancer Research UK Manchester Institute, Manchester, United Kingdom; 2ChemoCentryx, Inc., Paris, France.

Background: The aim of this CRUK-MI Drug Discovery project is to deliver a RET-selective inhibitor for the treatment of cancers with RET activating mutations, which include 1-2% of lung adenocarcinomas and medullary thyroid cancers (MTC).

Methods: We have established a robust screening cascade to develop a potent, selective RET inhibitor and developed several in vivo models to evaluate compound PKPD and antitumor efficacy. Tumor growth inhibition and PKPD studies were carried out in BaF3 mouse allograft models overexpressing KIF5B-RET or RETV804M and other disease relevant models, including an MTC xenograft (MZ-CRC-1), a KIF5B-RET lung cancer patient derived xenograft (PDX) model (CTG-0838, Champions Oncology) and a lung cancer control xenograft (Calu-6). Results: Two orally bioavailable compounds displaying nanomolar RET potency and >10 fold selectivity over KDR in cellular assays were selected from the lead series and further evaluated in our in vivo PD and efficacy models. Both compounds demonstrated efficacy in the BaF3 KIF5B-RET model (71% and 103% tumor growth inhibition (TGI), respectively), accompanied by reduced levels of pRET in the tumor tissue. Following further lead optimisation, a compound displaying an improved DMPK profile and additional nanomolar potency versus the gatekeeper mutation (RETV804M) was identified and accelerated through our DMPK/ in vivo cascade. We consider this additional activity versus RETV804M beneficial since mutations at the gatekeeper residue in other tyrosine kinases (e.g. EGFR) have been shown to mediate acquired drug resistance in the clinic. This compound demonstrated significant TGI of 58% and 82% respectively in the BaF3 KIF5B-RET and BaF3 RETV804M allograft models. Moreover, tumor growth in the lung cancer PDX model was strongly inhibited (95% TGI) and tumor regression induced in the MTC xenograft model (109% TGI). As expected, this potent and selective RET inhibitor was not active in the Calu-6 model, which is sensitive to KDR inhibition, whereas vandetanib, a potent KDR inhibitor, significantly inhibited tumor growth (84% TGI). Additional in vitro and in vivo DMPK analyses further support the nomination of this compound as a preclinical candidate. Conclusions: The identification of selective RET inhibitors with significant in vivo activity and minimal toxicity may overcome the limitations of the currently available clinical compounds. We have made considerable progress towards this goal and show here the compelling data supporting our nomination of a preclinical development compound.

#2093 HEC73543 is a novel potent, selective Flt3 receptor tyrosine kinase (RTK) Inhibitor for the treatment of refractory acute myeloid leukemia (AML).

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Background: Adults acute myeloid leukemia (AML) account for ~30% of leukemia and ~40% of leukemia-related deaths. FLT3 is a receptor tyrosine kinase that is normally expressed on immature hematopoietic cells and functions in the development of both stem cells and the immune system. FLT3 is the most frequently mutated gene in AML, with an estimated 30% of AML patients harboring FLT3 mutations. FLT3-ITD is a common mutation in AML and correlates with a poor prognosis and higher risk of relapse of AML patients. Methods and Results: HEC73543 is a potent, orally bioavailable small molecule tyrosine kinase inhibitor (TKI) against FLT3 which has been implicated in acute myeloid leukemia (AML) pathogenesis. In cell-based assays, HEC73543 showed IC50 of ~1.5 and 1.4 nM against MV-4-11 and MOLM-13 cells in proliferation (both cell lines are FLT3-ITD+). Induction of apoptosis following treatment of FLT3-ITD-FLT3 leukemic MV-4-11 cells for 48 h was assayed by flow cytometry. In MV-4-11 cells, a concentration of 10 nM HEC73543 induced 64.8% of the cells to undergo apoptosis and a dose dependent manner. HEC73543 orally (4.5mg/kg) to MV-4-11 tumor-bearing nude mice, HEC73543 potently inhibited the phosphorylation of FLT3 and its downstream signaling kinases STAT5, AKT and ERK1/2 for up to 8 hours in tumor tissues. The antitumor activity of HEC73543 was evaluated using an FLT3-ITD subcutaneous tumor xenograft model (MV-4-11 and MOLM-13) in athymic mice. Groups of 8 tumor-bearing mice were treated with vehicle alone or with HEC73543 administered orally at 0.5, 1.5, or 4.5mg/kg/day. The compound was well tolerated and no significant body weight loss or lethality was observed. The antitumor activity of HEC73543 was dose dependent. At the 1.5 mg/kg/day dose level all animals had a complete regression (CR) in MV-4-11 model, and the CR induced by 4.5mg/kg/day in MOLM-13 model. HEC73543 can significant prolong mice survival time at 1 mg/kg/day dose level in MOLM-13 systemic xenograft model. We have studied cytotoxic interactions of HEC73543 with conventional antileukemic agents cytarabine using two leukemia cell lines carrying FLT3-ITD (MOLM-13, MV-4-11) The combination of HEC73543 with cytarabine produced 40% of synergistic effects in in vitro experiments and the combination of HEC73543 displayed excellent oral bioavailability and desirable drug exposures in mice, rats, dogs and monkeys. Conclusions: Together, HEC73543 exhibits potent target inhibition and efficacy in FLT3-ITD models suggests that this compound may have a therapeutic benefit for patients with FLT3-ITD leukemia.

#2094 Dual inhibition of FLT3 and Src pathways by ON150030, a type 1 inhibitor, as a novel strategy for relapsed and refractory AML therapy.

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Approximately one third of patients suffering from Acute Myeloid Leukemia harbor a FLT3 Internal tandem duplication mutation (FLT3-ITD). When mutated, this receptor tyrosine kinase increases the activity of pathways for proliferation and blocks apoptosis. Quizartinib is a 2nd generation FLT3 inhibitor that inhibits FLT3-ITD in AML, but has a median duration response of 12.1 weeks. Studies revealed a secondary mutation in FLT3 at the aspartate of codon 835 (D835X) is responsible for relapse. The D835 substitution renders FLT3 constitutively active. Type 2 inhibitors like Quizartinib bind to FLT3 in its inactive state and fail to inhibit FLT3-ITD harboring a D835X mutation. Here, we tested the utility of ON150030, developed by our group, as a novel therapeutic agent to treat AML. Structural studies suggest ON150030 binds to the active form of FLT3 (Type 1 inhibitor) so mutations such as D835X do not affect the inhibitory activity of the compound. In vitro kinase assays demonstrate that ON150030 potently inhibits Wildtype and FLT3-D835Y forms, while Quizartinib fails to inhibit FLT3-D835Y. Additionally, ON150030 demonstrated time and temperature dependent inhibition of FLT3, suggesting that the compound is an irreversible inhibitor of FLT3. Biological studies reveal that ON150030 specifically inhibits the growth of MV-4-11 cells harboring the FLT3-ITD mutation (G150; 10nM). Western blot analysis demonstrates that MAPK and PI3K/ AKT pathways in these cells are inhibited with increasing dose of ON150030. The JAK independent phosphorylation of STATS seen in the context of FLT3-ITD is also reduced in response to ON150030. Future goals are to introduce FLT3 and its various mutant isoforms into the mouse myeloid cells (32Dc3) and examine how it affects proliferation and differentiation, and then compare the effects of ON150030 and Quizartinib. In addition to strongly inhibiting FLT3, ON150030 inhibits SRC, which was shown to induce resistance to targeted therapies in several leukemias including AML. We will introduce SRC into the 32D-FLT3-ITD cell lines sensitive to ON150030 and test whether these cells retain their sensitivity to the drug. Next, we will perform cytotoxicity and biochemical assays on patient-derived primary AML cells using ON150030. We use our genetic model to determine if ON150030 synergizes with standard chemotherapy agents and inhibits cancer progression in vivo. At the conclusion of this project, we hope to demonstrate that ON150030 can be used in combination therapies in all AML patients harboring a FLT3 mutation, and result in sustained remission of disease.

#2095 E7090, a novel and selective FGFR inhibitor, for the treatment of cholangiocarcinoma cells harboring FGFR2-fusion genes.

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Genetic abnormalities (gene fusion, mutation and amplification) of fibroblast growth factor receptor (FGFR) family members are known to cause constitutive activation of their signaling pathways, which play an important role in proliferation, survival and migration of cancer cells, as well as tumor angiogenesis. Several recent studies have identified that FGFR2 gene fusions were found in about 10% of intrahepatic cholangiocarcinoma and acted as oncogenes. E7090, an orally available FGFR1, 2 and 3 inhibitor, whose chemical-structure and basic kinase inhibitory profile have been disclosed at AACR2015, is currently under a first-in-human study (NCT02727910). The antitumor activity of E7090 against NIH3T3 and human cholangiocarcinoma cells harboring FGFR2-fusion genes was investigated. NIH3T3 cells stably infected of five FGFR2-fusion genes (FGFR2-AHCYL1, FGFR2-BICC1 type1, FGFR2-BICC1 type2, FGFR2-TXNIP, FGFR2-HCDO1) were used to determine if FGFR2-fusion genes have a therapeutic benefit for patients with FGFR2-fusion genes.
stream pathways of FGFR signaling (MAPK and STAT3) in these cells. Subcutaneous transplantation of FGFR2-BCC1 type2, FGFR2-TXLNA, or FGFR2-KCTD1 expressing cells into immune-deficient mice produced tumor growth, and then oral administration of E7090 at 12.5, 25 and 50 mg/kg showed significant tumor growth inhibition. Finally, antitumor activity of E7090 was examined in a patient-derived xenograft (PDX) model of human cholangiocarcinoma harboring FGFR2-BCC1 fusion gene. E7090 also showed dose-dependent antitumor activity and more than 50% of tumor regression was achieved at a dose of 50 mg/kg. In conclusion, E7090 showed potent antitumor activity against FGFR2-fusion genes found in intrahepatic cholangiocarcinoma both in vitro and in vivo models, suggesting that E7090 may provide therapeutic opportunities for cholangiocarcinoma harboring FGFR2 fusion genes.

**#2096 Bozitinib, a highly selective inhibitor of cMet, demonstrates robust activity in gastric, lung, hepatic and pancreatic in vivo models.** Joe Shih,1 Royu Zhong,2 Hephg Shi,2 David Xue,2 Gavin S. Choy,3 Sanjeev Redkar3. 1Crown Bioscience, Taiyang City, Jiangsu Province, China; 2Beijing Pearl Biotechnology Limited Liability Co., Chaoyang District, Beijing, China; 3GBT Pharmaceuticals, Inc, Santa Clara, CA.

Background: cMET is a receptor tyrosine kinase that is located on the cell surface and is activated by the binding of its ligand, hepatocyte growth factor (HGF). In cancer cells, MET can be aberrantly active and cause abnormal signal transduction, which leads to tumor growth, angiogenesis, and metastasis. In vitro studies have demonstrated that bozitinib (CBT-101, PLB-1001) is a highly selective and specific inhibitor (8 nM) of cMET receptor cell proliferation. Methods: In vivo PD studies of gastric (MKN45), lung (LUM588, LU1901, LU2503), hepatic (LIM0612, LIM0801), and pancreatic (KP4) were evaluated. These models covered the HGF-dependent and HGF-independent mechanisms. Among these models, LUM588, LU1901, LU2503, LIM0612 and LIM0801 are PDX models. In particular, in the LU1901 model, bozitinib (BT) was compared to capmatinib (INC280). Groups included: BT at 1, 3 and 10 mg/kg QD × 21 and INC280 at 1, 3, and 10 mg/kg QD × 21 and 10 mg/kg BID × 21 via IG, CDPP 5 mg/kg, QD × 3 as a positive control via IP and the vehicle control (QD × 21 via IG). Each group (n = 8 mice) and the tumor volume was evaluated on D21. Results: In MKN45, LU2503, LIM0612 and LIM0801, the effect of BT seemed superior than that of crizotinib; in LUM588, its effect was higher than that of erlotinib; in LU1901, its effect was higher than that of crizotinib and INC280. In the LU1901 model, the strongest activity was observed BT at 10 mg/kg with a T/C ratio of 2%, compared to an equi-dose of INC280 (T/C of 22%). All doses of BT and INC280 were well tolerated; no mouse experienced weight loss. In MKN45 model, BT showed a PK/PD correlation and dose-dependence. BT inhibited the phosphorylation of c-Met protein; the rate of target inhibition exceeded 90% at >7 mg/kg. The plasma concentration for BT decreased over time with a significant decline 12 h after its administration, contrasting with at least 1 week of phosphorylation inhibition of the c-Met protein. Conclusions: In conclusion, BT was well-tolerated, with no animal death nor major weight loss. The in vivo experiments demonstrated that BT is a viable candidate with effective anti-tumor activities. BT is currently under evaluation in cMet dysregulated NSCLC (NCT02896231) with additional trials planned.

**#2097 A novel J-series prostamide induces ER stress-mediated apoptosis and upregulates ER oxidoreductase 1 alpha (ERO1α) in human colon cancer cells.** Hussam M. Albasam, Daniel A. Ladin, Rukiyah Van Dross. East Carolina University, Greenville, NC.

Colon cancer is the third most common cancer and the third leading cause of cancer-related death in the United States. The endoplasmic reticulum (ER) is a cellular organelle responsible for protein synthesis and oxidative folding. ER stress occurs when the protein folding load exceeds the protein folding capacity. Low levels of ER stress promote survival while excessive ER stress causes cell death. An important regulator of the cytoplasmic ER stress pathway is the transcription factor, C/EBP homologous protein 10 (CHOP10). It has been shown that CHOP10 regulates the transcription of ER oxidoreductase 1α (ERO1α). ERO1α promotes ER luminal oxidi- zation and under conditions of excessive ER stress releases H2O2 into the cytoplasm, resulting in oxidative stress-mediated apoptosis. Previous studies from our laboratory that showed that 5deoxy, Δ2,4-prostamide 1j (15d PMJ1) induced ER stress-mediated apoptosis, leading to a reduction in cell viability in tumorigenic keratinocytes and melanocytes. In addition, 15d PMJ1-induced ER stress-apoptosis was decreased in the presence of ER stress inhibitors, 4-phenylbutarate (PBA) and salubrinal. However, the specific pathway involved in 15d PMJ1-induced ER stress-apoptosis have not been identified. In this study, we hypothesize that 15d PMJ1 causes ER stress-mediated apoptosis in colon tumor cells by activating CHOP10 and its downstream transcriptional target, ERO1α. To examine the anti-proliferative effect of 15d PMJ1, tumorigenic colon cells (HCT116) and non-tumorigenic colon cells (HFC) were treated with different concentrations of 15d PMJ1 or 10μM shapiggin (TG) for 24 hours and cytotoxicity was measured by lactate dehydrogenase (LDH) assay. A significant increase in cell death was observed in HCT116 cells treated with 5μM 15d PMJ1 and this cytotoxic effect was 3-fold greater in HCT116 compared to HFC cells. Apoptotic measurements showed a significant increase in percentage of apoptosis in the presence of 15d PMJ1 treatment. The expression of CHOP10 was significantly enhanced in the 15d PMJ1-treated group. In addition, 15d PMJ1-induced apoptosis was decreased in the presence of ER stress inhibitors, PBA and salubrinal, suggesting that ER stress is essential for 15d PMJ1-induced apoptosis. Western blot analysis revealed an increase in ERO1α protein expression following 15d PMJ1 treatment. Apoptosis measurements showed a sig- nificant inhibition in 15d PMJ1-induced apoptosis in the presence of ERO1α in- hibitor, EN460, suggesting that ERO1α is important for 15d PMJ1-induced apoptosis. These findings suggest that 15d PMJ1-induced apoptosis is mediated via the ER stress pathway. CHOP10 and its transcriptional target, ERO1α, play a potential role in 15d PMJ1-induced ER stress-apoptosis, suggesting that 15d PMJ1 could be a potential anti-neoplastic agent with a unique mechanism for colon cancer.

**#2098 NVP-FGF401, a first-in-class highly selective and potent FGFR4 inhibitor for the treatment of HCC.** Diana Graus Porta,1 Andreas Weiss,2 Robin A. Fairhurst,3 Markus Wartmann,1 Christelle Stamm,1 Flavia Reimann,2 Alexandra Buhrle,2 Jalueine Kinyamu-Akunda,1 Dario Sterker,2 Masato Murakami,1 Youzhen Wang,4 Jeffrey Engelman,3 Francesco Hofmann,1 William R. Sellers3. 1Novartis, Basel, Switzerland; 2Novartis, East Hanover, NJ; 3Novartis, Cambridge, MA.

Hepatocellular carcinoma (HCC) is the seventh most common cancer world- wide and the third most common cause of cancer-related death. Sorafenib is the only FDA approved targeted agent to show a marginal improvement in overall survival (OS) for patients with advanced HCC. Recent data have implicated aberrant activation of the FGFR1-FGFR4/KLB axis as the driver of certain forms of HCC, making this pathway a novel therapeutic target in this disease. The first evidence for this is the finding that aberrant expression of FGFR1, as a consequence of gene amplification and other not yet known mechanisms, occurs in subsets of HCCs and HCC cell lines leading to constitutive FGFR4 activation. In this setting, conditional knock-down of FGFR1, as well as its receptors FGFR4 and KLB, suppresses proliferation of HCC cell lines, supporting the notion that FGFR1 activates FGFR4 in an autocrine fashion. Secondly, in transgenic mouse models, FGFR1 produced by non-tumor cells at an ectopic site (skeletal muscle) acts in a para- crine fashion on the liver hepatocytes leading to liver dysplasia and HCC. In these mice, tumorigenesis is abolished in an FGFR4 null background, as well as upon treatment with anti-FGF19 and anti-FGFR4 blocking antibodies. Thus, we anticipated that targeted therapies aimed at blocking the FGFR4 pathway might be efficacious in subsets of HCCs. We have identified and developed NVP-FGF401, a first in class, highly selective and potent FGFR4 inhibitor that is currently in Phase I/II clinical testing. NVP-FGF401 binds in a reversible covalent manner to the FGFR4 kinase domain and it inhibits FGFR4 with an IC50 of 1.1 nM. In biochemical assays, it shows at least 1,000 fold selectivity against panel of 65 kinases and in a kinase wide scan, consisting of 456 kinases, FGFR4 was the only target of NVF-FGF401. In xenograft animal models in vivo, NVP-FGF401 showed a consistent pharmacokinetic / pharmacodynamic (PK/PD) relationship with phospho-FGFR4 (pY925/926). NVP-FGF401 binds in a reversible covalent manner to the FGFR4 tumor—robustly inhibited in a dose dependent manner. The data support a lowest observed trough concentration (C trough) driven PD/efficacy relationship. The anti-tumor activity was confirmed across several xenograft animal models, as well as in patient-derived tumor xenografts (PDX) established in mice. The excellent drug-like properties of NVP-FGF401 drove us to test its efficacy in HCC patients in a PhII study, being the first selective FGFR4 inhibitor to ever enter into clinical trials (NCT02325739).

**#2099 In vitro characterization of the effect of nazartinib against non-small cell lung cancer activating clinically relevant EGFR mutants.** Keita Mauszawa, Hiroiyouki Yasuda, Toshiyuki Hirano, Shigenari Nukaga, Hanako Hasegawa, Junko Hamamoto, Katsuhiko Naoki, Kenzo Soejima, Tomoko Bet-suyaku. Keio University, School of Medicine, Shinjuku-ku, Tokyo, Japan.

Purpose: Multiple EGFR-TKIs are available and under development to treat patients with lung cancer harboring EGFR mutations. Nazartinib is one of the 3rd generation EGFR-TKIs targeting EGFR T790M as well as common mutations such as L858R and exon 19 deletions. Today, there is no clear guideline regarding which EGFR-TKIs should be used for which mutations. The purpose of this study is to clarify which EGFR-TKIs including nazartinib are best for each EGFR mutation, especially exon 20 insertion mutations using in vitro modeling. Experimental design: We evaluated drug sensitivity and downstream signaling of human lung cancer cell lines (PC9, H3255, H1975, PC9ER, BID007) and Ba/s3 cells harboring multiple types of EGFR mutations for 1st- (erlotinib), 2nd-
(afatinib) and 3rd- (osimertinib and nazartinib) generation EGFR-TKIs by MTS assay and western blotting. An in vitro model of mutation specificity was created by calculating the ratio of IC50 values between mutated and wild type EGFRs. Results: The model of mutation specificity identified a wide therapeutic window of each EGFR-TKIs for exon 19 deletions and L858R. On the other hand, osimertinib and nazartinib have wide therapeutic windows for other resistance mutations in human cell lines and Ba/F3 cells. In human cell lines and Ba/F3 cells harboring exon 19 deletions or L858R, afatinib dramatically inhibited the phosphorylation of EGFR, AKT, and ERK. Afatinib and 3rd generation EGFR-TKIs, osimertinib and nazartinib, effectively inhibited the phosphorylation of EGFR, AKT, and ERK in T9T0M positive cells. For EGFR exon 20 insertion mutations, although afatinib, osimertinib and nazartinib effectively inhibited the phosphorylation of EGFR, AKT, and ERK in several exon 20 insertion mutations, osimertinib and nazartinib were potent and presented a wide therapeutic window. Conclusion: Nazartinib as well as osimertinib has wide therapeutic windows for exon 19 deletions, L858R, T9T0M and some exon 20 insertions.

#2100 Selective inhibition of FGFR4 by INC062079 is efficacious in models of FGFR9- and FGFR4-dependent cancers. Phillips C.C. Liu, Guang Lu, Kevin Bowman, Matthew C. Stubbs, Liaoxing Wu, Darlin DiMatteo, Sindy Condon, Ronald Klabe, Ding-Quan Qian, Xiaoming Wen, Paul Collier, Karen Gallagher, Michael Hansl, Xin He, Bruce Ruggeri, Yan-ou Yang, Maryanne Covington, Timothy C. Burn, Sharon Diamond-Fosbenner, Richard Wynn, Reid Huber, Wenqing Yao, Swamy Yeleswaram, Peggy Sherfe, Gregory Hollis. Incyte, Wilmington, DE.

Ablation signaling through Fibroblast Growth Factor Receptors (FGFR) has been reported in multiple types of human cancers. FGFR4 signaling contributes to the development and progression of subsets of cancer: in approximately 10 percent of hepatocellular carcinoma (HCC), genetic amplification of FGFR9, encoding an endocrine Fgfr ligand that activates Fgfr4-KLB receptors, has been reported. In models with this alteration, FGFR9-FGFR4 signaling is oncogenic and antagonism of the FGFR9-FGFR4 axis has been shown to be efficacious suggesting that selective targeting of FGFR4 may be an effective strategy for malignancies with FGFR4 activation. We describe the preclinical characterization of INC062079 a potent and selective inhibitor of the FGFR4 kinase. In biochemical assays INC062079 inhibited FGFR4 with low nM potency and exhibited at least 250-fold selectivity against other FGFR kinases and greater than 800-fold selectivity against a large kinase panel. This selectivity derives from the ability of INC062079 to bind irreversibly to Cys552, a residue within the active site of FGFR4 that is non-conserved among other FGFR receptors. Covalent binding of INC062079 to Cys552 was demonstrated using a LC/MS/MS-based proteomic analysis that confirmed specificity for the target Cys. In assays using HCC cells with autocrine production of FGFR9, INC062079 inhibited the autophosphorylation of FGFR4 and blocked signal transduction by FGFR4 to downstream markers of pathway activation. Cancer cell lines that have amplification and expression of FGFR9 are uniquely sensitive to growth inhibition by INC062079 (EC50 less than 200 nM) compared with HCC cell lines or normal cells without FGFR9-FGFR4 dependence (EC50 > 5000 nM) confirming selectivity for FGFR4. In vivo, oral administration of INC062079 inhibited the growth and induced significant regressions of subcutaneous xenograft models of HCC with FGFR4 activity at doses that were well-tolerated (10-30 mg/kg BID) and did not result in a significant increase in serum phosphate levels which is observed with FGFR1/2/3 inhibition. Suppression of tumor growth correlated with pharmacodynamic inhibition of FGFR4 signaling. Collectively, these preclinical studies demonstrate that INC062079 potently and selectively inhibits models of FGFR9-FGFR4-dependent cancers in vitro and in vivo, supporting clinical evaluation in patients harboring oncogenic FGFR4 activation.

#2101 Ormeloxifene suppresses the growth of prostate tumor via inhibition of β-catenin induced AR signaling. Aditya Ganju, Bilal Bin Hafeez, Mohammed Sikander, Vivek Kumar Kashyap, Murali Mohan Yallapu, Subhash C. Chauhan, Meena Jaggi. Univ. of Tennessee Health Science Ctr., Memphis, TN.

Background: Prostate cancer (PrCa) first manifests as an androgen-dependent disease and can be treated with androgen deprivation therapy. Despite the initial success of androgen ablation therapy, resistance to anti-androgen therapy displays by progression to hormone refractory (androgen-independent) advanced stage PrCa which is primary cause of patient’s death. Main underlying cause for the onset of hormone refractory cancer is ligand independent activation of AR signaling in PrCa cells. It has been shown that β-catenin acts as a non-androgen activator of AR which enhances AR transactivation in PrCa cells. Thus, identification of agents with excellent pharmacokinetics and pharmacodynamics parameters that can inhibit ligand independent activation of AR signaling might be highly useful for the treatment of advanced stage PrCa. Herein, we identified a synthetic molecule, ormeloxifene (ORM), which efficiently represses β-catenin mediated ligand independent activation of AR signaling, thus, inhibits growth and metastatic features of PrCa cells. Methods: Androgen-refractory but AR positive PrCa cell (C4-2) was used as an in vitro and in vivo model systems. Effect of ORM on AR and PSA protein levels was determined by Western blot analysis. Effect of ORM treatment was analyzed on AR and PSA luciferase activities by transiently transfecting the C4-2 cells by AR and PSA luciferase construct as was used as an internal control. Nuclear and cytoplasmic lysates were prepared using Active Motif kit. Immunoprecipitation analysis was performed to determine if ORM inhibits physical interaction of β-catenin with AR. Therapeutic efficacy of ORM was evaluated in cell lines and PrCa xenograft mouse models. Results: ORM dose-dependently (10, 15 and 20 μM) inhibited the protein levels of AR and its downstream target protein PSA. ORM (10 and 20 μM) treatment inhibited protein levels of nuclear β-catenin and physical interaction of β-catenin with AR in PrCa cells. ORM administration dose-dependently (intra-peritoneal; 100 and/or 500 μg/mouse; thrice/week) significantly (P<0.01) inhibited growth of C4-2 cells derived xenograft tumors in athymic nude mice. ORM treatment significantly (P<0.01) inhibited the expressions of nuclear AR and β-catenin expressions in xenograft tumor tissues. These ORM treated mice did not show any apparent toxicity in our study. Conclusion: Our study demonstrates that ORM is a potent inhibitor of β-catenin-mediated activation of AR signaling. Based on its safety profile, ORM might be an ideal candidate for repurposing to treat advanced stage PrCa alone or in combination with other therapies.


Androgen receptor (AR) signaling plays a key role in prostate cancer progression; thus Androgen Deprivation Therapy (ADT) is a mainstay therapy for patients with advanced prostate cancer. However, in most cases the tumor becomes androgen-independent and resistant to ADT with patients ultimately progressing to metastatic castration resistant prostate cancer (mCRPC). Constitutively activated AR splice variants (AR-Vs) have emerged as major mediators of resistance to AR-targeted therapy and progression of mCRPC, representing an important therapeutic target for mCRPC. Out of at least 15 AR-Vs described thus far, AR-V7 is the most abundant in prostate cancer and its expression correlates with ADT resistance. Onc201 is the founding member of the imipridone class of small molecules that induces apoptosis in a variety of tumor types tested. It is currently in Phase I/II clinical trials for advanced solid tumors and hematological malignancies, including mCRPC with encouraging activity observed in patients in early clinical testing (Stein et al., ASCO, 2016). We demonstrate here that Onc201 treatment induces apoptosis in both castration-resistant and −sensitive human prostate cancer cell lines. Furthermore, an in vitro synergistic effect is observed with Onc201 and FDA approved drugs for prostate cancer such as enzalutamide, docetaxel and everolimus. Mechanically, we found that Onc201 inhibits both AR and AR-V7 signaling pathways. Analysis of AR+ cell lines stimulated with DHT showed a significant decrease in both RNA and protein levels of AR, AR-V7, PSA and other AR-target genes following Onc201 treatment. Ongoing experiments are aimed at better understanding the molecular and cellular mechanisms that enable Onc201 to inhibit the AR signaling pathway. In-vivo studies with AR-negative hormone refractory prostate cancer xenografts demonstrate single agent Onc201 anti-tumor efficacy. Ongoing studies are further evaluating the in-vivo efficacy of Onc201 as a single agent or in combination with enzalutamide and everolimus using mouse models of both castration-resistant and −sensitive prostate cancer. These preclinical results indicate that Onc201 is well suited to address mCRPC, including tumors that harbor AR-V7, as a single agent that may be combined synergistically with enzalutamide or everolimus. With these observations we envision further development a combination of Onc201 plus everolimus in CRPC with or without enzalutamide resistance.

#2103 NVP-FGF401: Cellular and in vivo profile of a novel highly potent and selective FGFR4 inhibitor for the treatment of FGFR9/FGFR4/KLB+ tumors. Andreas Weiss,1 Diana Graus Porta,2 Flavia Reimann,1 Alexandria Buhles,1 Christelle Stamm,1 Robin A. Fairhurst,1 Jacqueline Kinyamu-Akunda,2 Dario Sterker,1 Masato Murakami,1 Markus Wartmann,1 Youzhen Wang,3 Jeffery A. Engelman,3 Francesco Hofmann,1 William R. Sellers,1 Novartis Institutes for BioMedical Research, Basel, Switzerland; 2Novartis Institutes for BioMedical Research, East Hanover, NJ; 3Novartis Institutes for BioMedical Research, Cambridge, MA.

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver and a deadly disease. Treatment options are limited and prognosis
EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Growth Factor and Hormone Receptors as Therapeutic Targets

generally is poor. Aberrant signaling through the fibroblast growth factor 19 (FGF19) - fibroblast growth factor receptor 4 (FGFR4) axis has been implicated in the development of HCC, and recently FGF19 has been determined as a specific driver gene amplification in a subset of liver tumors and cancer cell lines. Here, we describe the cellular and in vivo profile of NVP-FG401, a highly potent and selective, first in class, reversible-covalent small-molecule inhibitor of the kinase activity of FGFR4. NVP-FG401 is exclusively selective for FGFR4 versus other FGFR family members and all other kinases. We show that among the FGF19-amplified liver cancer cells in the cancer cell line encyclopedia (CCLE), only those with concomitant expression of β-klotho (KLB), a co-receptor for FGF19 that facilitates its binding to FGFR4, are sensitive to NVP-FG401. NVP-FG401 has good oral PK properties and shows an excellent in vivo PK/PD relationship. NVP-FG401 has remarkable anti-tumor activity in mice bearing HCC tumor xenografts and PDX models that are positive for FGF19, FGFR4 and KLB. NVP-FG401 is the first FGFR inhibitor to enter clinical trials, and a Phll study is currently ongoing in HCC and other types of solid tumors.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Mechanism of Drug Action

#2104 In vitro drug effects on cancer cell morphology and functional state revealed by multiparameter imaging mass cytometry. Olga Ornatsky, Alexandre Bouzekri, Bedilu Allo, Jessica Watson. Fluidigm Canada, Markham, Ontario, Canada.

Results of in vitro drug testing are correlated with clinical response to chemotherapy: Accuracy to predict clinical drug resistance was found to be as high as 90%. The benefit of using in vitro models lies in the ability to probe cellular response in a controlled closed system, where effects of drug concentrations, treatment duration, drug efflux kinetics and multidrug combinations can be assessed by a variety of cell biology techniques. Cisplatin is a widely used chemotherapy drug that targets genomic DNA of the cells, forming both interstrand and intrastrand cross-links that lead to cell death. One limitation of its clinical use is in predicting the development of resistance and severe side effects in patients. Mechanisms of resistance such as reduced drug accumulation, increased detoxification through cisplatin binding to cellular thiol, reduced DNA platination, and increased DNA repair have been reported, however (tamoxifen enhancement of cisplatin). In vitro models of different cancer types (SKOV3, HeLa, A431, MCF-7) were used to study the effects of cisplatin on cell morphology and phenotypic and functional characteristics with a large panel of metal-tagged antibodies and Imaging Mass Cytometry (IMC) at the single-cell level (1). Proteins involved in DNA damage repair (γH2AX, PP2A, pHistone H3), apoptosis (CD98, caspase-3, cleaved PARP), cell proliferation (cyclin B1, Ki-67), metastasis (vimentin, β-catenin, VEGF, CD63, CD99), substrate adhesion (CD29, CD49e, CD49f, CD51, CD54, CD47), organelle morphology (CD107a, Mito, histone H3), and signaling pathways (STAT3, pERK1/2, pS6) as well as surface receptors (EGFR, HER2, BRCA, MUC1, CD44, EpCAM, CD142, CD39, beta-catenin) and structural markers (CK5, CK8/18, β-actin, β-tubulin), were identified simultaneously in each individual cell with specific metal-conjugated antibodies. S-phase cells were visualized by detection of 127I in 5-ido-2′-deoxyuridine (IdU) added to culture media. Presence of cisplatin in cell nuclei and cytoplasm was registered by IMC of platinum stable isotopes. Combination therapy of cisplatin and paclitaxel is a standard chemotherapeutic regimen to treat recurrent or metastatic cervical cancer. Cell lines from various tumors may develop resistance to cisplatin. Reduced cisplatin uptake has been observed in cervical cancer cells with cisplatin resistance. The cisplatin-resistant HeLa cells and A431 (A431/P) cells show 50% and 77% reduction in cisplatin uptake, respectively, compared with the parental cell lines. Human ovarian cancers (in vitro model SKOV3), for which cisplatin is a mainstay of treatment, develop drug resistance and pose an important clinical challenge. (1) Chang, Q et al. Nature Scientific Reports 6 (2016): 36641.


Besides the progress in the knowledge of cancer biology, the rates of mortality due to this disease are still considerably elevated. Therefore, application of new drugs in antitumor therapies has been constantly studied. However, any of them have achieved a considerable success so far. In this work we evaluated the ability of metformin, a traditional hypoglycemic prescribed to treatment of type II diabetes along decades, over programmed cell death and modulation of metastatic potential in multidrug resistant (MDR) human myeloid chronic leukemia cells (Lucena), through MTT reduction test, flow cytometry, western bloting and gelatin zymography. Metformin was able to reduce the cell viability in a concentration and time-dependent way and was capable of induce Lucena cells to apoptosis or necrosis, though activation of the apoptotic machinery. Indeed, it was not observed activation of caspase 3, in contrast to an expressive activation of PARP. Interestingly, proteins associated to autophagy were over-expressed by Lucena cells, and an arrest of cell cycle was observed after exposition to the drug. Finally, it was verified that metformin was able to modulate activity of MMPs and PARP enzymes associated to the metastatic process. Taken together these results suggest that the hypoglycemic is able to acts over the first steps of cancers in more aggressive stages. Finally, inhibition of MMPs indicates a promissory action of metformin for the treatment of metastatic disease.

#2106 In-depth profiling of the energy depletion-mediated cancer cytotoxicity by passively delivered zinc complexes via PARP1 activation. Jinhyuk F. Chung. 1 Tae Jung Park, 2 Yoko Norihisa 3. 1Xylonix Pte Ltd, Singapore, Singapore; 2Chung-Ang University, Seoul, Republic of Korea; 3Cheonghim International Medical Center, Gyeonggi-do, Republic of Korea.

Disfunctional apoptotic function from impaired p53 tumor suppressor activity leads to poorer prognosis in cancer patients by compromising drug sensitivity and promoting drug resistance development. Activation of the hypoglycemic zinc is a theoretically proposed cytotoxic mechanism for circumventing this problem. Herein we demonstrate that a biodegradable zinc complex is capable of triggering the programmed necrosis via activation of PARP1/PARG/MPTP, which consistently resulted in p53-independent broad spectrum cytotoxicity in vitro across all 20 cancer cell lines tested including many with reported drug resistance phenotypes. Additionally, we investigated its possible interaction with agents that influence p53 for possible antagonism/synergism. Supporting in vivo evidence in both animal models and clinical case studies involving terminal chemo-resistant gastric cancer patients upon oral administration (30 mg Zn/day) are presented. These results implicate macro-molecular biodegradable zinc complexes as promising antitumor agents of simple compositions for overcoming the current hurdles in clinics, and warrant further clinical investigations. We plan to advance the studies through non-invasive small devices reporting real-time blood glucose, blood pressure, microperfusion, and other hemodynamic parameters.

#2107 Tetraarsenic hexoxide induces G2/M cell cycle arrest, apoptosis, and autophagy via p38 MAPK and AKT-mediated pathways in SW620 human colon cancer cells. Won Sup Lee.1 Jeong Won Yun,1 Min Jeong Kim,1 Arulkumar Nagappan,1 Jing Nan Lu,1 Seong-Hwan Chang,2 Jae-Hoon Jeong,3 Jinhyuk F. Chung,1 TaeJung Park, 2 Yoko Norihisa3. 1Gyeongsang National Univ. Hospital, Jinju, Republic of Korea; 2Konkuk University School of Medicine, Seoul, Republic of Korea; 3Cheonghim International Medical Center, Gyeonggi-do, Republic of Korea.

Tetraarsenic hexoxide (As4O6) has been used in Korean folk remedy for the treatment of cancer since the late 1980’s. Evidence suggests that As4O6 show anti-cancer effects, whose mechanisms are different from those for As2O3. However, the detailed anticancer mechanism is still unclear. Here, we investigated the anticancer effects of As4O6 on SW620 human colon cancer cells. As4O6 induced cell death in a dose-dependent manner. Flow cytometry analysis revealed that As4O6 increased the sub-G1 (apoptotic cell population) and G2/M phase population in a dose-dependent manner. Further, nuclear condensation and cleaved nuclei were also observed upon staining with Hoechst 33342 in Hoechst 33342 in As4O6-treated SW620 cells. Western blot revealed that As4O6 significantly down-regulated the cyclin B1,cdc2, pro-caspases -3, -8 and -9, and up-regulated p21 and cleavage of PARP in SW620 cells. In addition, As4O6 increased the expression of death receptor 5 (DR5), suppressed Bcl-2 and XIAP family proteins, and promoted the conversion of LC3-I to LC3-II in a Beclin-1 independent manner. Indeed, it was not observed activation of caspase 3, in contrast to an expressive activation of PARP. Interestingly, proteins associated to autophagy were over-expressed by Lucena cells, and an arrest of cell cycle was observed after exposition to the drug. Finally, it was verified that metformin was able to modulate activity of MMPs and PARP enzymes associated to the metastatic process. Taken together these results suggest that the hypoglycemic is able to acts over the first steps of cancers in more aggressive stages. Finally, inhibition of MMPs indicates a promissory action of metformin for the treatment of metastatic disease.
Glioblastoma (GBM) is the most aggressive and deadliest brain malignancy in adults. Despite of surgical techniques, radiotherapy and chemotherapy, GBM has remained an invariably lethal tumor with a median survival less than 15 months. Although the anticancer mechanisms of omega-3 polyunsaturated fatty acids (ω3-PUFAs) have been reported in several cancers, it is still unclear in brain cancer. Here, we show the anti-inflammatory and anti-metastatic effects of ω3-PUFAs in GBM. Invasiveness using transwell chamber was inhibited by docosahexaenoic acid (DHA) treatment in D54MG and GL261 cells. In zymography, MMP-2 activity was suppressed by DHA in a dose dependent manner. MMP-2 promoter activity was also decreased. DHA inhibited both p-STAT3 and β-catenin levels that contribute to invasion by stimulating pro-invasion factors such as MMP-2. Additionally, fat-1 (ω3-desaturase) stable D54MG cell line was established and the effect of the high level of ω3-PUFAs was investigated endogenously. The invasiveness were significantly inhibited in the fat-1 stable cells compared to control cell. Moreover, the metastasis in vivo was significantly reduced when GL261 mouse glioma cells were injected through tail vein into the fat-1 transgenic mice. In immunohistochemistry, intensity of p-STAT3 and β-catenin in GBM cells. Thus, these findings provide important preclinical evidence and molecular insight for utilization of ω3-PUFAs for the chemoprevention and treatment of human GBM. This work was supported by the National Foundation of Korea (NRF) grant funded by the Korea government (MEST) (2007-0054932, NRF-2015R1D1A1A01056887) and by the framework of international cooperation program managed by National Research Foundation of Korea (2015K2A2A6002008).

#2112 Ciclopirox inhibits tumor cell motility by suppressing protein expression of small GTPases and phosphorylation of paxillin. Shile Huang, Tao Shen, Louisiana State Univ. Health Sciences Ctr.-Shreveport, LA.

Ciclopirox oleamine (CPX), an off-patent antifungal drug, is used for the treatment of superficial mycoses. Recent studies have demonstrated that CPX also possesses potent anticancer activity by inhibiting cell proliferation and inducing cell death in tumor cells. However, it is unknown whether CPX inhibits cell motility. In the present study, we found that CPX potently inhibited cell motility in a concentration-dependent manner in rhabdomyosarcoma (RH30 and RD) cells, which was independent of the reduction of cell viability. As small GTPases (RhoA, Cdc42, and Rac1) and focal adhesion proteins (paxillin) play critical roles in the regulation of cell motility, we further studied whether CPX affects these proteins. Our Western blot analysis revealed that CPX did inhibit cellular protein expression of RhoA, Cdc42 and Rac1. However, by RT-PCR analysis, CPX did not alter the mRNA levels of the small GTPases. As microRNAs (miRNAs) can regulate protein expression at transcriptional and post-transcriptional levels, we investigated the effect of CPX on the expression of miRNAs. Our miRNA PCR array revealed that treatment with 10 μM of CPX for 24 hours upregulated the expression of 27 miRNAs by 2.04-6.10 fold, and downregulated the expression of 7 miRNAs by 2.00-7.89 fold in RH30 cells. Further research is ongoing to identify whether these up/downregulated miRNAs are linked to the reduced expression of small GTPases and potentially more proteins. In addition, we found that CPX inhibited the phosphorylation of paxillin, despite no effect on the total cellular protein expression. The results suggest that CPX inhibits tumor cell motility at least by suppressing the protein expression of small GTPases and the phosphorylation of paxillin. Supported by the Feist-Weiller Cancer Center, LSU Health Sciences Center, Shreveport, LA.

#2110 FoxO3a activation by HDAC class IIa inhibition induces cell cycle arrest in pancreatic cancer cells. Makoto Usami, Shohei Kikuchi, Kohichi Takada, Yusuke Sugama, Yohei Arihara, Naotaka Hayasaka, Hajime Nakamura, Yuki Ikeda, Yusuke Kamihara, Masahiro Hirakawa, Makoto Yoshida, Masayoshi Kobune, Koji Miyamish, Junji Kato. Sapporo Medical University, School of Medicine, Sapporo, Japan.

Pancreatic cancer is highly chemo-resistant associated with oncogenic mutations such as KRAS and/or p53. The effect of conventional chemotheraphy is not sufficient and new target and strategy is urgently needed. The forkhead box (Fox) proteins are multidirectional transcriptional factors strongly implicated in malignancies. Although Fox O (FoxO) protein, and particularly FoxO3a, works as negative regulator of cell proliferation by exressing cyclin proteins and inducing cell cycle inhibitors such as p21 and p53, its expression is consistently suppressed by several oncogenic pathways including phosphatidylinositol-3-kinase (PI3K) / AKT pathway, constitutively activated in pancreatic cancer. Thus, upregulating FoxO3a activity could be a promising target of pancreatic cancer.
treatment without impact of underlying oncogenic mutations. Class Ila Histone deacetylase (HDAC) is a subgroup of HDAC. Though HDAC inhibitors (HDACi) have been extensively investigated as a cancer target, its action mechanism is considered due to histone modification by class I. Biological significance of class Ila HDACs which have minimal histone deacetylation activity have not been elucidated yet. Recent studies show class Ila HDACs act as a transcriptional regulator including FoxO3a. In this study, we investigate the biologic impact of HDAC class Ila inhibition on FoxO3a and anti-tumor effect against pancreatic cancer cell line using selective class Ila HDAC TMP269.

TMP269 treatment showed increased FoxO3a expression in a dose dependent manner with immobiloblotting and modest cell growth inhibition effect at 57.5 μM of dose for 48-hour treatment against AsPC-1 in 3MT. G1/S arrest was observed with cell cycle assay. Upregrulated p21\(^{\text{Waf1/Cip1}}\) and downregulated CDK2 and 4/6 and cyclin D1 and D2 expressions were further observed, consistent with inducing G1/S arrest and transcriptionally activated FoxO3a. Importantly, upregulated p21\(^{\text{Waf1/Cip1}}\) was observed in AsPC-1 p53 null cell line, suggesting independent with p53 pathway. These findings suggest upregulated FoxO3a by HDAC class Ila inhibition activated its transcription and resulted in cell growth inhibition. Because P13K/AKT leads FoxO3a to the ubiquitinylation-mediated proteasome degradation, we examined irreversible proteasome inhibitor carfilzomib (CFZ) combined with TMP269, aiming synergetic FoxO3a upregulating. As expected, FoxO3a expression was further increased in TMP269 combined with CFZ compared with TMP269 or CFZ alone. Following the activation of FoxO3a, expression was upregulated and cell proliferation inhibition was dramatically enhanced. In conclusion, HDAC class Ila inhibition modified FoxO3a transcriptional activation and upregulating FoxO3a by dual inhibition of HDAC class Ila and proteasome is promising target against pancreas cancer.

#2113 Mechanism of action of pixantrone in non-Hodgkin’s lymphoma cells. Serina Ng, Ruben Muñoz, Daniel Von Hoff, Haiyong Han. Translational Genomic Research Institute, Phoenix, AZ.

Background: Pixantrone (PIX), an anthracenedione analog, has shown promising results with reduced cardiotoxicity as compared to structurally similar mitoxantrone and doxorubicin (DOX) in early phase clinical trials in patients with non-Hodgkin’s lymphoma (NHL). The exact mechanisms of action on how PIX induce cell death by targeting DNA replication through intercalating DNA and/or inhibiting Topoisomerase II has not been clearly illustrated. Methods: A short-term cell proliferation assay (3 days treatment) and a long-term clonogenic assay (cells were treated with the drugs for 24 hours and then allowed to grow into colonies for 10-14 days) were used to assess and compare the effects of PIX and DOX on cell growth in three NHL cell lines, OCI-Ly3, Z138, and Raji. Immunofluorescent assays using γH2AX antibodies, and DAPI staining were used to examine the effect of PIX and DOX on the formation of double-strand breaks and micronuclei. Results: In the short-term cell proliferation assay, PIX was not as potent as DOX (\(IC_{50}\) values ranging from 22 nM to 2373 nM for PIX and 2.8 nM to 67 nM for DOX). The two drugs were significantly more potent in the long-term clonogenic assay with \(IC_{50}\) values in low nanomolar range (5.1 nM to 82.8 nM) and the difference between PIX and DOX was much smaller. Cells treated with PIX displayed less increases in DNA double-strand breaks compared to those of DOX as quantified by the number of γH2AX positive foci formed in the cells. Furthermore, PIX treatment significantly increased the formation of micronuclei in a drug concentration and time dependent manner. Conclusions: The findings from the current study indicate that PIX induces DNA damages that impair the chromosomal segregation but do not significantly trigger double-strand breakage checkpoint response. Overall, our results suggest that PIX may exert its antitumor activity via mechanisms that are different from those of DOX.


Sphingolipids influence tumor microenvironment in different malignancies. Estrogen and estrogen metabolites are involved in prostate cancer. Fingolimod is Sphingokinase-1 (SphK1) inhibitor which possesses anticancer properties in various tumor types. The aim of the current study is to investigate the interference of Fingolimod with the cross talk between sphingolipid and estrogen metabolism within prostate cancer cells as a novel therapeutic strategy. The design is based on determining the mutual influence between estrogen metabolism/signaling and sphingolipid metabolism within prostate cancer cells. Cytotoxicity using SRB-U assay; cell cycle analysis using DNA cytometry and intracellular caspase-3 concentration were assessed. Estrogen metabolites and estrogen metabolism-activating enzymes were determined using LC/MS and RT-PCR, respectively.

Estrogen receptor (ERs and ERβ) expression and their downstream signaling (CXCR4 and Cyclin-D1, respectively) were measured using immunocytochemical staining and RT-PCR, respectively. Fingolimod showed antiproliferative/cytotoxic effects against different prostate cancer cells (LNCaP, DU145 and PC3) with \(IC_{50}\) ranging from 3.0±0.3 to 6.8±1.7 μM. Fingolimod induced significant decrease in estradiol, estrone, 4-hydroxyestradiol and 16α-hydroxyestrone concentrations with reciprocal increase in 2-methoxyestrone and 2-methoxyestradiol concentrations. This was mirrored by significant down expression for the enzymes, aromatase, CYP1A1 and CYP1B1 and over expression for the enzyme COMT. Additionally, Fingolimod suppressed the expression of ERs and ERβ within prostate cancer cells as well as their downstream signaling proteins CXCR4 and Cyclin-D1. It was observed that CXCR4 and catechol estrogens induce the expression of SphK1 while methoxylation of catechol estradiol suppresses its expression. Our results give compelling evidence that Fingolimod has therapeutic potential by modulating the estrogenic micromilieu and interrupting its cross talk with sphingolipid metabolism in prostate cancer cells.

#2116 Combining the power of different profiling approaches to better understand the activity of kinase inhibitor drugs. Alastair J. King, Jesse J. Parry, Samantha C. Burkart, Lee R. Cavedine, Alyssa M. Craciuchio, Brogan A. Epkins, Steven M. Garner, Timothy J. Sindelar, Charles R. Wageman, Stephen P. Davies, Andrew Plater, Anna M. Woodward, Usha Warrior. 1. Eurofins Panlabs Inc., St. Charles, MO; 2. Eurofins Pharma Discovery Services UK, Ltd.; 3. King Abdullah University, Jeddah, Saudi Arabia; 4. Faculty of Pharmacy, Ain Shams University, Cairo, Egypt; 5. Faculty of Medicine, King Abdullah University, Jeddah, Saudi Arabia.

Profiling of inhibitors for eventual therapeutic use is an essential part of drug discovery and development. Evaluating the in vitro potency of kinase inhibitors in a wide panel of enzyme assays is critical, not only to guiding SAR knowledge for improving drug design, but also for assessing selectivity, which has long-reaching consequences for both biological activity and safety. Evaluation of the same inhibitors in cellular models yields information on the activity of these inhibitors in a more physiologically relevant environment. However, while some selective kinase inhibitors show predictably selective profiles in cell proliferation assays, others do not. Thus, the biological context in which a kinase inhibitor functions, and the signaling networks influenced by inhibition of both the de-
sired target and off-target activities, help to define how that inhibitor will work in a cellular setting and, ultimately, in a therapeutic application. We demonstrate the power of profiling several well-known kinase inhibitors in complementary panel assays, to predict behavior that has implications for clinical activity. The in vitro profiles of selective (trametinib, vemurafenib) and less selective (dasatinib, sorafenib) inhibitors are shown and compared. With care and precision of their in vitro enzyme activity profiles in Eurofins’ KinaseProfiler™ panel, cellular response profiles in OncoPanel™, and predictive biomarker identification by univariate genomic analysis, a fuller picture of these inhibitors’ biological activity can be generated, and may be applied to predicting the therapeutic potential of such inhibitors. While, in some cases, clinical response is seen as expected, this kind of comprehensive analysis opens up the possibility for expanding clinical utility of an asset by potential repurposing for other indications. Collectively, this demonstrates not only the need to understand an inhibitor’s fundamental activity(ies) at its designated target(s), but also the importance of considering cellular context, when assessing its maximum potential clinical utility.

#2117 TRAIL-induced apoptosis in TRAIL-resistant breast carcinoma.
Jasmine M. Manouchehri, Michael Kalafatis. Cleveland State University, Cleveland, OH.

Recombinant human tumor necrosis factor-related apoptosis-inducing ligand (rhTRAIL), the optimized form of the endogenous death ligand TRAIL, shows therapeutic potential for cancer due to its ability to induce apoptosis in cancer cells independent of p53, while exhibiting minimal toxicity to normal cells. Despite a majority of breast cancers display resistance to single agent treatments, the cotreatment of Q and rhTRAIL enhanced the induction of apoptosis in breast cancer BT-20 cells in a dose-dependent manner. RT-PCR analysis revealed Q’s sensitization was displayed in breast cancer cells and triple-negative breast cancer cells (TNBC) viainduced extracellular secretion of acetylated-APE1/Ref-1 (Ac-APE1/Ref-1) and initiated apoptotic cell death by auto-, paracrine binding to the receptor for advanced glycation end products (RAGE). In the present study, we observed potential therapeutic efficacy of Ac-APE1/Ref-1 in preclinical orthotopic models of TNBC in response to hyperacetylation. The extracellular Ac-APE1/Ref-1 was confirmed by proximity ligation assay in lungs of orthotopic tumor tissue, showing direct binding of Ac-APE1/Ref-1 and RAGE. Treatment of orthotopic TNBC xenografts with acetylating agents induced a strong growth inhibition in the tumor development as observed in computed tomography: it caused an increase of RAGE expression and activation of caspase-3 and PARP. The tumors also exhibited markedly higher count of apoptotic bodies and reduced proliferation index and neovascularization compared with controls. However, Ac-APE1/Ref-1-stimulated apoptotic cell death was remarkably retarded in RAGE knockout tumors even in hyperacetylation compared with RAGE-overexpressed one. The functional role of secreted Ac-APE1/Ref-1 in hyperacetylated TNBC was confirmed in vivo, demonstrating its relevance to the anticancer agent. Our findings suggest that Ac-APE1/Ref-1 protein possesses potent chemotherapeutic efficacy against TNBC, resistant to standard chemotherapeutic agents, warranting further evaluation as an anticancer agent.

#2118 Distinct molecular effects of chemotherapeutic agents on choline phospholipid metabolism of triple-negative breast cancer cells. Menglin Cheng, Zayer M. Bhujwalla, Kristine Glunde, Johns Hopkins University School of Medicine, Baltimore, MD.

The magnetic resonance spectroscopy (MRS)-detected total choline (tCho) signal is a promising non-invasive surrogate marker able to predict chemotherapeutic response in breast cancer patients early on. However, the molecular mechanisms underlying the choline-phospholipid changes are mostly unknown. Here we have employed some widely used cancer chemotherapy drugs such as doxorubicin, paclitaxel, and vinorelbine to treat triple-negative human MDA-MB-231 breast cancer cells to elucidate their molecular effects on choline phospholipid metabolism. High-resolution 1H MRS of water-soluble cell extracts was employed to detect changes to their molecular effects on choline phospholipid metabolism. High-resolution 1H MRS of water-soluble cell extracts was employed to detect changes in the choline metabolite profiles, and quantitative RT-PCR (qRT-PCR) to assess the corresponding changes in the expression levels of choline metabolizing enzymes. After 48 hours of doxorubicin treatment, the GPC levels in MDA-MB-231 cells significantly increased, while PC level decreased, and the tCho concentration remained unchanged. Vinorelbine treatment displayed a comparable effect to doxorubicin, but the GPC increase and PC decrease occurred to a lesser extent. Paclitaxel treatment caused an increased GPC level along with an unaltered PC level, leading to a slightly increased tCho level. In all these cases, the PC/GPC ratio decreased from 3.5 in the control to about 2.0 in paclitaxel- or vinorelbine-treated samples, to about 1.0 in doxorubicin-treated samples. qRT-PCR-detected mRNA expression levels showed that GDPD6 and Chk1 genes were downregulated by doxorubicin, GDPD5, GDPD6, and Chk1 genes displayed a reduced mRNA expression level following vinorelbine treatment, while no significant change in these three genes was detected after paclitaxel treatment. For comparison, the known GDPD6 inhibitor dipryridamole significantly increased cellular GPC levels, but, as expected, did not affect GDPD6 mRNA expression levels as it acts as an enzyme inhibitor. Our study demonstrates that choline-dependent metabolic pathways are sensitive to the type of drug used for breast cancer treatment. However, all tested chemotherapeutic treatments resulted in metabolic alteration away from the ‘cholinic phenotype’, which was caused in some cases by decreases in expression of Chk1, GDPD6, and GDPD5 following drug treatment. The choline metabolite concentrations of GPC and/or

#2119 Acetylated secretory APE1/Ref-1 induces apoptotic cell death in orthotopic xenografts of triple-negative breast cancer. Yu Ban Lee, 1Hee Kyung Joo, 1Eun Ok Lee, 1Myoung Soo Park, 2Byeong Hwa Jeon, 1Sunga Cho1.
Chungnam National University College of Medicine, Daejeon, Republic of Korea; 1Chungnam National University Hospital, Daejeon, Republic of Korea; 2Chungnam National University, Daejeon, Republic of Korea.

The anticancer properties of acetylated secretory apurinic/apyrimidinic endonuclease-1 (APE1/Ref-1) was suggested in triple-negative breast cancer (TNBC) cells; Posttranslational modification, hyperacetylation in MDA-MB-231 cells and extracellular secretion of acetylated-APE1/Ref-1 (Ac-APE1/Ref-1) and initiated apoptotic cell death by auto-, paracrine binding to the receptor for advanced glycation end products (RAGE). In the present study, we observed potential therapeutic efficacy of Ac-APE1/Ref-1 in preclinical orthotopic models of TNBC in response to hyperacetylation. The extracellular Ac-APE1/Ref-1 was confirmed by proximity ligation assay in lungs of orthotopic tumor tissue, showing direct binding of Ac-APE1/Ref-1 and RAGE. Treatment of orthotopic TNBC xenografts with acetylating agents induced a strong growth inhibition in the tumor development as observed in computed tomography: it caused an increase of RAGE expression and activation of caspase-3 and PARP. The tumors also exhibited markedly higher count of apoptotic bodies and reduced proliferation index and neovascularization compared with controls. Moreover, the Ac-APE1/Ref-1-stimulated apoptotic cell death was remarkably retarded in RAGE knock out tumor even in hyperacetylation compared with RAGE-overexpressed one. The functional role of secreted Ac-APE1/Ref-1 in hyperacetylated TNBC was confirmed in vivo, demonstrating its relevance to the anticancer agent. Our findings suggest that Ac-APE1/Ref-1 protein possesses potent chemotherapeutic efficacy against TNBC, resistant to standard chemotherapeutic agents, warranting further evaluation as an anticancer agent.

#2120 Potassium bromate prevents growth and progression phenotypes such as migration, invasion and sphere-forming ability of breast cancer cells. Priyanka Ghosh, Gargi Maity, Snigdha Banerjee, Sushanta Banerjee, Ung Joo,1 Eun Ok Lee,1 Myoung Soo Park,2 Byeong Hwa Jeon,1 Sunga Choi.1 Chonnam National University, Daejeon, Republic of Korea; 1Chonnam National University Hospital, Daejeon, Republic of Korea.

Potassium bromate (KBrO3) is by-product of ozone that has found multi-functional purpose in modern society. It is used as disinfectant in drinking water, a bleaching agent to improve flour, a component of cold-wave hair lotion and an ingredient in the production of fish paste and fermented beverages. Potassium bromate has also been used in America for bread-baking as a safe food additive since 1914. Despite the commercial value of this chemical, some studies suggest that KBrO3 is a carcinogen. During the bread-baking process, Potassium bromate is normally converted into a stable and inert compound, potassium bromide (KBr). However, due to incomplete reduction, the residual KBrO3 remains in the bread, which eventually acts as a potential carcinogen to humans. Interestingly, our studies, in breast cancer cells, found contrasting results. We found that KBrO3 delays growth of ER-negative human MDA-MB-231 breast cancer cells caused insome cases by decreases in expression of Chk1, while exhibiting minimal toxicity to normal breast cancer cells independent of p53, while exhibiting minimal toxicity to normal breast cancer cells. Despite this, a majority of breast cancers display resistance to KBrO3-treatment via targeting multiple molecular signatures in breast cancer cells. The growth inhibition effect of KBrO3 is also documented in a tumor xenograft model. Collectively, our findings provide a rationale for the basic and pre-clinical evaluation of the role of KBrO3 in breast cancer progression and therapy.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Mechanism of Drug Action

#2116 The food additive agent potassium bromate prevents growth and aggressiveness phenotypes by targeting multiple molecular signatures in breast cancer cells. Priyanka Ghosh, Gargi Maity, Snigdha Banerjee, Sushanta Banerjee. VA Medical Center, Kansas City, MO.

Potassium bromate (KBrO3) is by-product of ozone that has found multi-functional purpose in modern society. It is used as disinfectant in drinking water, a bleaching agent to improve flour, a component of cold-wave hair lotion and an ingredient in the production of fish paste and fermented beverages. Potassium bromate has also been used in America for bread-baking as a safe food additive since 1914. Despite the commercial value of this chemical, some studies suggest that KBrO3 could be a carcinogen. During the bread-baking process, Potassium bromate is normally converted into a stable and inert compound, potassium bromide (KBr). However, due to incomplete reduction, the residual KBrO3 remains in the bread, which eventually acts as a potential carcinogen to humans. Interestingly, our studies, in breast cancer cells, found contrasting results. We found that KBrO3 delays growth of ER-negative human breast cancer cells and triple-negative breast cancer cells (TNBC) via inducing apoptosis in a dose-dependent manner. KBrO3-induced apoptosis is mediated via targeting BCL-2/Bax and Caspase-3 signaling pathway. Moreover, aggressive phenotypes such as migration, invasion and sphere-forming ability of breast cancer cells are significantly impaired by KBrO3-treatment via targeting multiple molecular signatures in breast cancer cells. The growth inhibition effect of KBrO3 is also documented in a tumor xenograft model. Collectively, our findings provide a rationale for the basic and pre-clinical evaluation of the role of KBrO3 in breast cancer progression and therapy.
PC and/or the PC/GPC ratio may serve as non-invasive surrogates of therapeutic response in triple-negative breast cancer patients undergoing chemotherapy.

#2121 Deguelin-derivative, L80 suppresses tumor growth and metastasis via inhibition of STAT3 activation in triple-negative breast cancer. Tae-Min Cho, Eunhye Oh, Daeil Sung, Yoon-Jae Kim, Ji Young Kim, Jeewoo Lee, Jae Hong Seo. Korea University, Seoul, Republic of Korea.

Triple-negative breast cancer TNBC is associated with aggressive metastasis and poor clinical outcomes due to a lack of effective treatment options. Therefore, new drugs that effectively target both cancer cell proliferation and metastasis are needed to improve clinical outcomes. The 90-kDa molecular chaperone heat-shock protein, HSP90 has been implicated in cancer progression and metastasis by modulating the stabilization and maturation of many oncoproteins. Our objective was to investigate the mechanism of action of C-terminal HSP90 inhibitor, deguelin-derivative L80 on TNBC proliferation and metastasis in vitro and in vivo. L80 induced apoptosis and suppression of cell viability in TNBC cell lines via inhibition of Akt activation with concomitant nuclear accumulation of p27. L80 treatment also caused a marked suppression of cell migration and invasion. These responses were associated with inhibition of STAT3 phosphorylation (Tyr705) as evidenced by downregulation of STAT3 downstream target genes including cyclin D1 and survivin. The synergetic orthotopic mouse model with 4T1 cells, L80 administration resulted in significant reduction in tumor growth together with decreased number of Ki-67-positive cells and downregulation of phospho-STAT3. Double-label immunofluorescence analysis revealed that L80 inhibited a cell population co-localization and expression of HIF-1α and HSP90 compared to their control groups in vivo. Finally, we observed that L80 administration significantly suppressed lung metastasis, as determined by in vivo bioluminescent imaging system. Our findings suggest that L80 may be potentially effective for the treatment for metastatic TNBC patients.

#2122 Pharmacologic interconversion of EMT to MET for prostate cancer. Zheng Cao,1 Shahriar Koochekpour,2 Stephen E. Strup,1 Natasha Kyprianou.1 1University of Kentucky Medical Center, Lexington, KY; 2Roswell Park Cancer Institute, Buffalo, NY.

Introduction and objection: Dysregulation of transforming growth factor-β (TGF-β) and insulin-like growth factor (IGF) axis has been linked to reactive stroma dynamics in the tumor microenvironment during prostate cancer progression. IGFBP3 induction is initiated by stroma remodeling and represents a potential therapeutic target for advanced prostate cancer. A lead quinazoline-based Doxazosin® derivative, DZ-50, generated in our laboratory (US Patent #7377948), inhibits prostate tumor growth via inducing anoikis and disrupting focal adhesions. Molecular profiling revealed that the process of epithelial-mesenchymal transition (EMT) is targetable by DZ-50. In this study, we investigated the effect of DZ-50 on EMT landscape, EMT to mesenchymal-epithelial-transition (MET) conversion, and invasive properties of prostate cancer cells. Methods: Human prostate cancer cells LNCaP, LNCaP overexpressing TGF-β type II receptor (TβRII), and cancer associated fibroblasts (CAFs) derived from human prostate cancer specimens, were used. The antimorphic effect of DZ-50 against prostate cancer cells and CAFs with and without TGF-β receptor (TβRI) was investigated. Effect of the drug on EMT key regulators (including IGFBP3) was determined using RT-PCR and Western blot analysis. Drug-induced phenotypic conversions of EMT were evaluated by confocal microscopy. Impact of TGF-β from the stroma microenvironment or exogenous cytokine, on prostate tumor cell migration and invasion, was assessed in co-cultures with CAFs. The functional contribution of IGFBP3 to EMT-MET interconversion in response to DZ-50 was assessed using siRNA approaches. Results: DZ-50 induced cell death in prostate cancer epithelial cells and CAFs, in a concentration-dependent manner. DZ-50 downregulated IGFBP3 mRNA and protein expression and promoted MET-MET conversion in both LNCaP and LNCaP/TβRII cells. IGFBP3 knockdown in LNCaP/TβRII cells led to E-cadherin upregulation and MET induction, implicating IGFBP3 as a potential target of DZ-50 to reverse EMT to MET. Moreover, exposure to TGF-β reversed DZ-50-induced MET by upregulating IGFBP3 in LNCaP/TβRII cells. Co-cultures of LNCaP/TβRII cells with CAFs promoted prostate cancer cell invasion via TGF-β and IGFBP3, an effect that was inhibited by the drug. Conclusions: Treatment of prostate cancer cells with the novel agent DZ-50 inhibits cell migration and invasion and causes reversal of EMT to MET by regulating IGFBP3. Our study integrates IGFBP3 as new signaling effector driving the antimorphic action of DZ-50 via targeting the EMT-MET phenotypic landscape in the prostate tumor microenvironment. Ongoing work in pre-clinical models will establish the therapeutic value of this novel compound in advanced metastatic prostate cancer.

#2123 Inhibition of AURKA induces Raf1-independent activation of MAPK pathway in breast cancer cells. Malgorzata Gil,1 Archana Chandrakarma,1 Thaer Khoury,2 Kazuaki Takabe,1 Igor Puzanov,1 Irwin Gelman,1 Antonio D’Assoro,2 Mateusz Oprychal,1 Roswell Park Cancer Institute, Buffalo, NY; 2Mayo Clinic College of Medicine, Rochester, MN.

Aurora A (AURKA) is a mitotic kinase responsible for centromere segregation and mitotic spindle formation. In normal cells, expression of AURKA is highly regulated and is predominantly restricted to G2/M phases of the cell cycle. Unlike healthy cells, cancer cells overexpress AURKA through all phases of the cell cycle resulting in the acquisition of alternate non-mitotic functions. Little is known about cellular functions regulated by AURKA and its interaction with other signaling molecules. Here, we report a novel interaction between AURKA and the mitogen-activated protein kinase (MAPK) pathway in wild type BRAF breast cancer cells as well as demonstrate an additive cytotoxic effect of AURKA- and MEK1/2-specific inhibitors against estrogen positive (ER+) and triple negative (ER−, PR−, HER2+) breast cancer cells. Results: We show that treatment of ER− HER2+ MCF-7, ER− HER2+, SKBR3 and ER− HER2+ BT549 cells with AURKA specific inhibitors alisertib, MK8745 and Aurora A Inhibitor I resulted in over 2-fold increase in the levels of both pMEK1/2 and pERK1/2 compared to the untreated controls. The activation of the MAPK pathway was rapid with changes seen within 5 min after treatment with AURKA inhibitors and was sustained for at least 48 hours. No differences in phosphorylation of MEK1/2 or ERK1/2 were observed in BRAFV600E triple negative MDAMB231 cells. Treatment with AURKA inhibitor resulted in significant re-expression of PAK1 and a significant increase in levels of total AURKA protein. The pull-down assay with Ras-binding domain coated agarose beads followed by western blot analysis with anti-pan-Ras Ab revealed no changes in active GTP-bound Ras in alisertib-treated MCF-7 cells compared to the untreated control. Consistently, no significant changes were observed in Ras-inducible phosphorylation of RAF1 activation site at Ser338 as demonstrated by western blot. Treatment with the pan RAF inhibitor TAK-632 did not diminish alisertib-induced pERK and pMEK1/2. Alternatively, treatment with the MEK1/2 specific inhibitor PD0325901 completely abrogated alisertib-induced phosphorylation of MEK1/2 and ERK1/2. Furthermore, combined treatment of alisertib and PD0325901 in vitro revealed significant additive cytotoxic effect in MCF-7 and BT549 cells when compared to either agent used alone (p<0.008 and p<0.011; <0.004 and p<0.028). Conclusions: Our data suggests that AURKA is a RAF1-independent negative regulator of MAPK activity in breast cancer cells. The in-depth analysis of the AURKA-MEK1/2 interaction is currently under investigation. The results reveal a promising new strategy for the treatment of wild type BRAF, TNBC patients using a combination of AURKA and MEK1/2 inhibitors.

#2124 Differentiation of myeloid and multiple myeloma cell lines by inecalcitol involves a marked decrease in CD44 and CD49d cell surface labeling. Susan Benjamin, Cecilie Planquette, Remi Delansorne. Hybrigenics, Paris, France.

Inecalcitol is a vitamin D receptor (VDR) agonist characterized by a high antiproliferative activity on various cancer cell lines (Okamoto et al., 2012; Ma et al., 2013) and a low calcemic potential as demonstrated by the high maximal antiproliferative activity on various cancer cell lines (Okamoto et al., 2012; Ma et al., 2013) and a low calcemic potential as demonstrated by the high maximal antiproliferative activity on various cancer cell lines (Okamoto et al., 2012; Ma et al., 2013). In addition, recent studies revealed that inecalcitol reproducibly induces CD38 expression (Wang et al., 2004). During a screening of 48 CD biomarkers on 4 human myeloid cell lines (HL-60, U-937, MOLM-13 and THP1) and 4 human multiple myeloma cell lines (MM.1R, MM.1S, L-363 and RPMI-8226) treated for 72 hours at 10 nM, we recently discovered that inecalcitol reproducibly induces CD38 expression (Benjamin et al., 2016), another gain of function. We now report the decrease in CD44 and CD49d cell surface labeling mostly in non-mitotic cell lines (Benjamin et al., 2016). In this study we investigated the effect of inecalcitol on CD44 expression in all 8 cell lines (except CD44 in L-363 cells). This decrease in CD44 expression was observable by flow cytometry after 72 hours of treatment with 10 nM inecalcitol. CD44 is the receptor for hyaluronic acid; it is found on the surface of acute myeloid leukemia stem cells and suspected to favor the spread and relapse of the disease. CD44 is
the integrin αv-subunit involved in the interactions of tumor cells with their microenvironment resulting in their "hiding" and survival. Therefore, decreasing both CD44 and CD49d represents a newly identified potential beneficial pro- 
differentiation effects of ineocalcit." 

#2125 Flavokawain A, a kava chalcone, inhibits growth and invasion of human osteosarcoma cells by targeting Skp2. Yidan Zhang,1 Wendong Zhang,2 Nikolai Zaphiros,2 Xiuquan Du,2 Pratibha Koirala,1 Michael Roth,1 Jonathan Gill,3 Sajda Papideri,1 David Geller,1 Rui Yang,1 Jinhong Zhang,1 Richard Gorlick,1 Xiaolin Zi,3 Tao Ji,4 Bang H. Hoang1.

Purpose: Osteosarcoma (OS) is the most common primary bone malignancy with a high propensity for local invasion and distant metastasis. Flavokawain A (FKA), a major chalcone from kava extract, has been reported to have antitumor effects on multiple cancer cell lines. The consumption of kava-containing beverage has been associated with a low cancer incidence. In a previous report, mice treated with high-dose FKA did not demonstrate any significant major organ toxicity. However, the efficacy and antitumor mechanisms of FKA in OS is still to be elucidated. Experimental Design: OS cell lines were treated with increasing dosage of FKA and tested for cell motility, proliferation, and invasion by Matrigel assay and Matrigel invasion assay. Cell cycle analysis was performed using flow cytometry. We examined Skp2 expression in several OS cell lines using western blot and in patient tissue array by immunostaining. Kaplan-meier analysis and log rank test were used to compare overall survival. Both Skp2-dependent cell cycle progression and Skp2-related RhoA expression were also examined after FKA treatment. The effects of FKA on lung metastasis were evaluated after orthotopic injection of OS cells into the tibia. Results: We show that FKA inhibits its growth and motility of multiple OS cell lines in vitro. Flow cytometry analysis confirms cellular apoptosis and arrest in G2/M phase after FKA treatment, whereas cellular invasion is also inhibited in a dose-dependent manner. Skp2 is expressed in several OS cell lines and is associated with a poor prognosis in OS patients. Skp2 levels in OS cell lines decreased after FKA treatment. The expression of cell cycle regulators including p21 and p27, which are downstream of Skp2, was upregulated. Moreover, Skp2-related RhoA expression is inhibited by FKA and confirmed at protein level. Conclusions: Taken together, the evidence suggests FKA exerts anti-invasive effects in association with Skp2-dependent cell cycle progression and Skp2-related RhoA expression. Since Skp2 is a negative regulator of RhoA and may be an important factor for OS patients, FKA should be investigated further as an alternative therapeutic strategy for OS.

#2126 CA102N, a conjugate of hyaluronic acid (ha) and Nimuselide derivative (H-Nim) interferes with PI3K/Akt/mTOR signaling pathway in colorectal cancer (CRC) cells and inhibits tumor growth in vivo. Esokhie H. Tchaparian, Louis Lin. Holy Stone Healthcare Co., Ltd., Taipei, Taiwan.

Aberrant PI3K/Akt/mTOR signaling pathway is common in several human cancers, including CRC. The pathway regulates many major cellular processes and promotes tumorigenesis and inhibition of apoptosis. Targeting the PI3K/Akt/mTOR network could be important in resistance mechanisms in cancer. CA102N, a conjugate of Hyaluronic Acid (HA) and H-Nim, a nimuselide (COX-2 inhibitor) derivative, currently under investigation for preclinical development in CRC. Studies have suggested that the anti-proliferative effects of COX-2 inhibitors could be attributed to modulation of the PI3K/Akt signaling pathway; therefore, in this study we aimed to examine the overall molecular mechanism of the antitumor activity of CA102N and evaluate its impact on the PI3K pathway proteins. Cell based studies indicated that in addition to cell cycle arrest; the antitumor activity of CA102N is also related to apoptosis. Administration of CA102N to nude mice bearing established HT29 subcutaneous tumor xenografts caused significant tumor growth inhibition with no signs of systemic toxicity. Immunohistochemical analyses of xenograft tumors demonstrated inhibition of VEGFR2 and VEGF expression in CA102N-treated tumors. Disruption of angiogenesis and apoptosis was correlated with a decrease in VEGF and the antiangiopotic protein BCL-2. Suppression of PI3K downstream signaling was a key observation in HT29 cells in vitro and in xenograft tumors treated with CA102N. As compared to untreated vehicle control, a significant decrease in phosphorylation of Akt, mTOR, p70S6K, 4EBP1 and FOXO1 was detected by Western blot analyses. The reduction in PI3K activity observed may be caused by both specific protein dephosphorylation/deactivation and by ubiquitin-mediated proteasomal degradation of pathway proteins. These results suggest that the antitumor activity of CA102N may be at least partly related to the modulation of the PI3K/Akt pathways. CA102N represents a promising antitumor agent with minimal toxicity, the insights provided into the mechanisms of its antitumor activity may be the basis for advancing this candidate into clinic.

#2127 p53-independent Noxa induction by cisplatin is regulated by AT3/F  
ATF4 in HNSCC cells. Kanika Sharma,2 Thien-Trang Vu,1 Mitra Naseri,1 Wa-taru Nakajima,2 Kevin Zhan,1 Hisashi Harada1. 1 VCU Massey Cancer Ctr., Richmond, VA; 2Nippon Medical School, Tokyo, Japan.

Head and neck cancer is the sixth leading cancer worldwide and head and neck squamous cell carcinoma (HNSCC) accounts for more than 90% of incid- 
cent cases. The long-term prognosis of patients with advanced HNSCC has shown little improvement over the last three decades. Induction chemotherapy with platinum-based compounds, taxanes, and 5-fluouracil is beneficial for head and neck cancer patients, but the prolonged use of chemotherapeutic drugs is limited by their toxicity and by the development of resistance. Tumor cell death induced by both conventional and targeted chemotherapy is often medi- ated by the BCL-2 family-dependent mitochondrial apoptotic pathway. How- ever, initiators of this apoptotic pathway, such as p53, are frequently mutated or deleted in HNSCC rendering it refractory to treatment. To counter such resis- tance, direct therapeutic targeting of the BCL-2 family is conceptually appealing. We have investigated the cytotoxic effects of a DNA damaging agent, cisplatin that is used for a standard therapy for locally advanced HNSCC. Cisplatin produces antitumor effects mainly by generating DNA lesions and activating DNA damage response followed by inducing BCL-2 family-dependent mitochondrial apoptosis. In this study, we aimed to evaluate the potential of AT3/FATF4 regulation in cisplatin-induced apoptosis in p53-independent HNSCC cells. AT3/FATF4 induction was regulated at the tran- scriptional level. Thus, we examined the regulation of the Noxa promoter with a series of luciferase reporter assays. We found that the CRE (cAMP responsive element located at -66 to -59 from the transcription start site) on the promoter plays a critical role in Noxa induction. The CRE is known to be regulated by ATF4/CREB transcription factors. Downregulation of AT3/FATF4 by shRNAs clearly reduced Noxa induction. Furthermore, the binding of these transcription factors to the CRE on the Noxa promoter was increased by cisplatin treatment, determined by chromatin-immunoprecipitation assays. In conclusion, AT3 and FATF4 are important regulators for cisplatin-induced Noxa in a p53-independent pathway. The results suggest that modulation of the signaling pathways that activate AT3/FATF4 could be an alternative approach to induce Noxa follow- ed by apoptosis in HNSCC.
Assessing the efficacy of targeting mitochondrial respiration in delaying lung tumor growth by using subcutaneous xenografts in mouse models. Sarada Preeta Kalainayakan, Poorva Ghosh, Sanchareeka Dey, Li Zhang. UT Dallas, Richardson, TX.

Previously, studies have focused mainly on the precept that tumors depend on glycolytic metabolism for growth (Warburg effect). However, such tumors might be dysfunctional in cancer cells. Here, we demonstrate that some cancer cells exhibit elevated mitochondrial respiration. Recent studies in our lab have demonstrated that Non-Small Cell Lung Cancer (NSCLC) cells exhibit intensified mitochondrial respiration and oxygen consumption. We further demonstrated that targeting increased mitochondrial respiration by a therapeutic agent facilitates the proliferation and survival of cancer cells. The results showed that Tan-IIA can induce the proliferation inhibition with time and dose dependent and inhibit the activity of the Ras/Raf/MEK/ERK and Ras/PI3K/Akt/mTOR pathways. The use of Tan-IIA for breast cancer may become a feasible novel therapy option. Further studies are warranted to elucidate its mechanisms fully.


Natural products are important sources for bioactive molecules, which have been developed to treat various human health-related symptoms. In our continuous efforts to search for anticancer agents from natural sources, Psammaplin A (PsA), isolated from marine natural products, was found to be a potential candidate in the growth inhibition of cancer cells. PsA, a unique symmetrical bromotyrosine contained in a number of marine sponges of the order Verongida, has exhibited a variety of bioactivities including antimicrobial activity, cytotoxicity against the leukemia cell line, and inhibitory activity of DNA gyrase and DNA topoisomerase. PsA is also known to be a potent inhibitor of DNA methyltransferase and histone deacetylase with growth-inhibitory activity of cancer cells. However, its underlying mechanism of action and the structure-activity relationship (SAR) with PsA analogs have not been elucidated yet. In the present study, twenty-eight synthetic analogs of PsA were newly synthesized and examined the potential of cytotoxicity against cancer cells. A SAR study revealed that the presence of free oxime and disulfide functional groups was responsible for high cytotoxicity. Furthermore, the bromotyrosine component in PsA was relatively tolerable and hydrophobic aromatic groups preserved the cytotoxicity. The aromatic groups were dependent on their size and spatial geometry. Among them, a β-naphthyl derivative of PsA showed a potential cytotoxicity and was comparable to that of PsA. The compound also exhibited a potential antitumor activity in a nude mouse xenograft model. These findings indicated that free oxime and disulfide linker in the chemical structure play an important role for cytotoxicity and PsA analogs might be provided as potential antitumor agents. This work was supported by the National Research Foundation of Korea (NRF) Grant funded by the Korean Government (MEST) (NRF-2016M3A9B6093499) and also supported by a National Research Foundation of Korea (NRF) Grant funded by the Korean Government (MEST) (No.2009-0083533).

Tanshinone IIA can decrease growth factor receptors expression and dural-block both Ras/Raf/MEK/ERK and Ras/PI3K/Akt/mTOR pathways to inhibit human breast cancer BT-20 cells. Chin Cheng Su. Changhua Christian Hospital, Changhua City, Taiwan.

Background: Tanshinone IIA (Tan-IIA, C19H18O3) with anti-inflammatory activities and antioxidant properties, is one of the diterpine quinones extracted from Salvia miltiorrhiza radix (Danshen). Tan-IIA can inhibit many human cancer cell lines through different molecular mechanisms. The phosphoinositide-3-kinase (PI3K)/Akt/ mammalian target of rapamycin (mTOR) and Ras/RAF/MEK/ERK pathways are two of the most frequently dysregulated kinase cascades in human cancer. Transmembrane tyrosine kinase has been strongly implicated in the proliferation, survival, and metastasis of human tumors. Both pathways represent important signal transduction pathways that facilitate the proliferation and survival of cancer cells driven by growth factor receptors, such as vascular endothelial growth factor receptor (VEGFR), insulin-like growth factor-I receptor (IGF-IR), or epidermal growth factor receptor (EGFR). Targeting both the Ras/Raf/MEK/ERK and Ras/PI3K/Akt/mTOR Pathways for suppressing inhibitor resistant cells is necessary because the individual downstream components of these signaling cascades through epigenetic modification or somatic mutation are also known to be frequently altered in cancer, thus contributing to resistance to anticancer therapies and tumorigenesis. Material and methods: In the present study, the human breast cancer BT-20 cells were treated with Tan-IIA in vitro. The cytotoxicity of Tan-IIA was evaluated by MTT assay. The effects of Tan-IIA on the protein expressions of EGFR, IGF-IR, VEGFR, PI3K, Akt, mTOR, Ras, Raf, MEK and ERK and β-actin in the BT-20 cells were examined by western blot analysis. Results: The results showed that Tan-IIA can induce the proliferation inhibition with time and dose dependent and inhibit the activity of the Ras/Raf/MEK/ERK and Ras/PI3K/Akt/mTOR pathways. In addition, it was showed that Tan-IIA treatment inhibited the protein expression levels of EGFR, VEGFR and IGF-IR significantly. Conclusion: This study indicated that one of the molecular mechanisms for Tan-IIA to inhibit BT-20 cells maybe through inhibiting the protein expression levels of EGFR, VEGFR, IGF-IR and both Ras/Raf/MEK/ERK and Ras/PI3K/Akt/mTOR pathways. The use of Tan-IIA for breast cancer may become a feasible novel therapy option. Further studies are warranted to elucidate its mechanisms fully.
growth inhibition with curcumin in a xenograft model of human castrate resistant PCa cells. Collectively, enzyme-mediated depletion of serum Cys and CysC pool suppresses the growth of prostate tumors, and is very well tolerated. These results suggest that cyst(e)ine inase represents a potentially safe and effective therapeutic modality as a single agent or in combination for the treatment of prostate and possibly other cancers.

**#2133 Pre-clinical studies of EC2629, a highly potent FR targeted DNA crosslinking agent.** Joseph A. Reddy, Melissa Nelson, Christina Dircksen, The resa Johnson, Marilyn Vetzl, Spencer Hahn, Longwu Qi, Iontcho Vlahov, Christopher Leamon. Endocyte, Inc., West Lafayette, IN.

Folate receptor (FR) targeted small molecule drug conjugates (SMDCs) have shown promising results in early stage clinical trials with vintafolide and EC1456. In our effort to develop FR targeted SMDCs with varying mechanisms of action, we have now built EC2629, a folate conjugate of a DNA crosslinking agent based on a novel DNA-alkylating moiety. This agent was found to be extremely potent with an in vitro IC50 shown promising results in early stage clinical trials with vintafolide and EC1456. In our effort to develop FR targeted SMDCs with varying mechanisms of action, we have now built EC2629, a folate conjugate of a DNA crosslinking agent based on a novel DNA-alkylating moiety. This agent was found to be extremely potent with an in vitro IC50 shown promising results in early stage clinical trials with vintafolide and EC1456. In our effort to develop FR targeted SMDCs with varying mechanisms of action, we have now built EC2629, a folate conjugate of a DNA crosslinking agent based on a novel DNA-alkylating moiety. This agent was found to be extremely potent with an in vitro IC50 shown promising results in early stage clinical trials with vintafolide and EC1456.

**#2134 Use of methionine gamma-lyase-loaded erythrocytes to induce effective methionine depletion in cancer therapy.** Fabien Gay,1 Karine Aguer,2 Karine Senchel,3 Philip Lorenzi,1 Alexander Scheer,1 François Horand,1 Vanessa Bourgeaux1. 1 ERYTECH Pharma, Lyon, France; 2 MD Anderson Cancer Center, Houston, TX.

Methionine (Met) dependence is a cancer-specific metabolic defect that has emerged as a target during the last two decades. The use of methionine gammalyase (MGL; EC number 4.4.1.1), a bacterial Met-catabolizing enzyme, is a promising strategy for treatment of Met-dependent cancers. However, one challenge is that MGL has a very short half-life (~2 hours), resulting in a short-term Met depletion in vivo. Additionally, its cofactor, pyridoxal 5’-phosphate (PLP) is rapidly eliminated from plasma (Yang et al., 2004). MGL-lyase extends the MGL half-life in mice to up to 38 hours. Nevertheless, frequent injections are still necessary for maintaining an effective Met depletion over time (Sun et al., 2003). In addition, the low bioavailability of PLP remains a major hurdle due to both scavenging by plasma proteins and very short half-life (~15 minutes) in the blood stream (Zeppenfeld, 1995). Studies showed that pyridoxine (PN), a vitamin B6 vitamin, can easily cross red blood cells (RBCs) membrane to be rapidly converted to PLP via an enzymatic reactions cascade (Anderson et al., 1971). Considering those particular characteristics of RBCs, we propose that RBC-encapsulated MGL (ERY-MET) will protect the enzyme from degradation/immune reactions and therefore overcome the pharmacodynamic limitations for its use in the treatment of Met-dependent cancers. Pharmacokinetics, pharmacodynamics, and safety parameters of MGL-loaded RBCs were evaluated in healthy mice. In parallel, the role of RBC in PLP biosynthesis from exogenous uptake of PN was investigated in vivo. Finally, we investigated the antitumoral effect of repeated injections of MGL-loaded RBCs combined with daily intragastric administration of PN in two subcutaneous xenografted mouse models for human gastric and glioblastoma tumors. The MGL half-life increased from ~24 hours to ~10 days when encapsulated in RBC, with no toxicity reported after one injection of ERY-MET. Following intragastric administration (3.2 mg/kg), PN was rapidly (15 minutes) converted into PTP within the RBC, resulting in an increase of MGL activity (holoenzyme). Combining a weekly single intravenous injection of ERY-MET for 5 days, in association with daily PN supplementation by gavage led to a sustained Met depletion in plasma and induced a 85% inhibition of tumor growth 45 days following implantation of glioblastoma cells. In the gastric mouse model, tumor growth was inhibited by 72% at the same time point (45 days). This study clearly demonstrated that encapsulation of MGL in erythrocytes both strongly improved the half-life and contributed to provide active cofactor. In parallel, repeated injections of ERY-MET were effective against tumor growth in mouse models. Thus, due to the RBC intrinsic characteristics, ERY-MET represents a new promising treatment against a broad scope of cancers that rely on Met metabolism.

**#2135 Selectivity and specificity of engineered T cells expressing KITE-585, a chimeric antigen receptor targeting B-cell maturation antigen (BCMA).** Gregor B. Adams, Jun Feng, Atfeh Ghougha, Armen Mardiros, Ruben Rodriguez, Tassja J. Spindler, Jed Wiltzius, Tony Polyverino. Kite Pharma, Santa Monica, CA.

Background: Immunotherapy has provided treatment options for cancers that are otherwise refractory to standard approaches. One such technique is to use adoptive transfer of engineered autologous T cells expressing a chimeric antigen receptor (CAR) directed against a tumor antigen. The efficacy of CAR T cells directed against hematological malignancies, particularly CD19-expressing B cell leukemia and lymphomas, has been demonstrated in multiple clinical studies. The success of this approach has prompted development of CAR T cells directed to different tumor antigens for other tumor types. To ensure the selectivity and specificity of the CAR T cells against their intended target, screening methods need to be employed. Multiple myeloma is an incurable malignancy of plasma cells. B-cell maturation antigen (BCMA), also known as tumor necrosis factor superfamily member 17 (TNFRSF17) is nearly ubiquitously expressed on multiple myeloma cells, plasma cells and subsets of mature B cells. Methods: In order to screen for the specificity of novel CAR T cells directed against BCMA, we utilized a cell microarray platform developed by Retrogenix. In this screen, approximately 4500 human plasma membrane proteins (representing up to 75% of the human plasma membrane proteome) are individually expressed in human HEK293 cells. Fluorescently labeled CAR T cells, which showed cytolytic activity against MM cell lines expressing BCMA, were applied to the cell microarray and specific binding of the CAR T cells to target cells was determined. Results: Primary hits were sequenced to confirm identity and secondary specificity screens were performed on the identified hits. Specific binding of both mock transduced and BCMA CAR transduced T cells were confirmed for different plasma membrane proteins expressed from the HEK293 cells. These included known T cell interactors, such as ICOSLG, CD244 and CD86, where binding is proposed to be independent of CAR expression. Subtraction of the hits the mock transduced T cells from the BCMA CAR T cells demonstrated specific binding of the CAR T cells to BCMA. Utilizing the fully human IgGs directed against BCMA from which the single-chain variable fragments (scFv)s of the CARs were derived, we further confirmed specific binding to BCMA in additional secondary screens. Additionally, a lack of off-target binding of the fully human IgGs to normal tissue was demonstrated in a tissue cross reactivity screen. Conclusions: These studies highlight the tractability of this cell microarray approach for determining the specificity of novel CAR constructs expressed in T cell. Demonstrating the selectivity and specificity of anti-BCMA CAR T cells further supports the progression of KITE-585 towards Phase 1 clinical studies in MM patients.

**#2136 Inhibiting cancer growth by targeting the TNFR2 oncogene with TNFR2 antagonistic antibodies.** Heather Torrey, John Butterworth, Yoshiyuki Mera, Yoshiaki Okubo, Limei Wang, Danielle Baum, Audrey Defusco, Sara Plager, Sarah Warden, Daniel Huang, Eva Vannamee, Rosemary Foster, Denise L. Fastman. Massachusetts General Hospital & Harvard Medical, Charlestown, MA.

Background: Although antibody targeting of the HER2 oncogene represents an outstanding clinical treatment and a success story showing direct cancer killing with antibodies, many oncoproteins are intracellular and are not expressed broadly, whether on a single or multiple tumor types. The recently discovered TNFR2 oncogene is broadly expressed on many human tumors. Colon cancer cells, multiple myeloma cells, renal cell carcinoma cells, Hodgkin’s lymphoma cells, ovarian cancer epithelial cells and cutaneous non-Hodgkin’s lymphoma cells can aberrantly express the TNFR2 receptor as an oncogene for growth. For non-cutaneous T cell lymphomas, the genetic basis of the TNFR2 deregulation has recently been tied to constitutive overexpression of TNFR2 from frequent gene duplications or cytoplasmic TNFR2 mutations that confer constitutive agonism, i.e. tumor expansion. These features make TNFR2 an advantageous molecular target for direct tumor targeting. Methods: We designed monoclonal antibodies to target the TNFR2 oncogene and directly kill human tumor cells. TNFR2-directed antibodies were screened for their ability to induce the death of rapidly growing tumor cells, such as ovarian cancer cells (i.e., OVCAR3). Results: Novel dominant anti-TNFR2 antibody candidates (TNFR2 antagonistic antibodies) did not require Fc binding for activity, expressed dominance over TNF-mediated agonism, and hampered intracellular NF-kB activation and phosphorylation obligatory for TNF2 signaling and cell growth of tumor cells. Even low doses of TNFR2 antagonists rapidly and directly killed TNFR2-onco-
gene-expressing ovarian cancer cells. Examination of the structural biology of these dominant TNFR2 antagonist antibodies uncovered a unique and stabilizing
TNFR2 receptor formation, anti-parallel dimeric TNFR2, which inhibits intracellular signaling, cannot bind TNF, cannot be cleaved to create soluble
TNFR2 and is exponentially more active on the dividing cells of cancer. Conclu-
sions: TNFR2 is a unique and broadly expressed human tumor necrosis factor
receptor that potentially can be targeted to directly stop the growth of cancer cells (including ovarian cancer cells) by antibody-induced cell death. The creation of dominant
TNFR2 antagonism provides a unique, non-signaling complex that has implic-
tions for the therapeutic targeting of TNF superfamily receptors, especially
TNFR2.

#2137 JAK2 as a novel therapeutic target in anaplastic thyroid cancer
Nicole C. Pinto,1 Kara Ruiczi,2 Stephanie Prokopiec,3 Karlee Seerle,3 Matthew
Lowerison,3 John Yoo,3 Kevin Fung,3 Danielle MacNeil,3 Hon S. Leong,3 Ales-
sandro Datti,3 Paul C. Boutros,3 John W. Barrett,1 Anthony C. Nichols1.1.
1Western University, London, Ontario, Canada; 2Ontario Institute for Cancer Research, Toronto, Ontario, Canada; 3Lunenfeld-Tanenbaum Research Institute, Toronto, Ontario, Canada.

Introduction: Thyroid carcinoma is the most common endocrine malign-
ancy. Anaplastic thyroid cancer (ATC) is rare (1.3%) and represents arguably the most lethal human malignancy with 1-year survival rates of only 10%. There are currently no effective treatments for the majority of patients, highlighting an urgent need for novel therapeutics to manage this disease. Objective: To validate the functional importance of JAK2 as a therapeutic target in ATC. Methods: In this study, we have used siRNA knockdown to interrogate the JAK2 signaling
pathway as a therapeutic target in ATC. We investigated the mechanism of
action and cell death, and assayed for migration and invasion, in vitro. The chick chorioallantoic membrane (CAM) model was also utilized for drug testing, whereby 1x10⁵ Cal62 ATC cells were on-planted to the chick embryo mem-
brane. Two days post on-plant, CAM models were treated with the vehicle (DMSO) or lestaurtinib to measure outcomes including tumor volume and vac-
cularity. Results: We identified the JAK2 inhibitor lestaurtinib as an inhibitory agent controlling cell line proliferation at submicromolar mean inhibitory
concentrations. Immunoblotting revealed the inhibition of phosphorylation of the downstream signaling molecule STAT5 in a dose-dependent manner. Treat-
ment of Cal62 cells resulted in a decrease in cell migration using the scratch-
 wound assay. The anti-proliferative effective of lestaurtinib did not cause apo-
tosis, autophagy or cell senescence. CAM models treated with a 4 μM dose of lestaurtinib showed a significant decrease in both tumor volume and vacu-
cularity. Conclusions: Lestaurtinib was found to provide potent control of ATC cell prol-
fication and migration, and was also found to decrease tumor growth and vacu-
cularity in a CAM model. Knockout studies are underway to confirm that the anti-cancer effect of this drug is indeed mediated through JAK2 signaling. If validated, JAK2 represents a novel therapeutic target for the treatment of aggres-
sive thyroid cancers.

#2138 Tumor treatment fields downregulate the BRCA1/FA pathway
genesis leading to reduced DNA repair capacity, the inhibition of mitophagy
and enhanced cell death
Narasimha Kumar Karanam, Lianghao Ding, Brock Sisak, Debabrata Saha, Michael D. Story. UT Southwestern Medical Center, Dal-
lus, TX.

The application of new physical cancer treatment modality utilizing alternat-
ing electric fields termed tumor treatment fields (TTFields) has revolutionized the treatment of recurrent and newly diagnosed glioblastoma. This non-invasive exposure to low-intensity, intermediate frequency, alternating electric fields to the region of the tumor has resulted in a significant increase in overall survival when compared to standard therapy with very minimal side effects. Clinical
trials are recruiting or ongoing at additional tumor sites including lung, pancre-
atic, and ovarian cancer. The primary mechanism of TTFields cell killing is thought to be the disruption of mitosis; however, other potential mechanisms are under investigation. Using a panel of five NSCLC cell lines we found that
TTFields treatment alone inhibits cell proliferation, and decreases survival, though the degree of inhibition varies between cell lines. To understand the
molecular mechanisms underlying the biological effects of TTFields exposure we studied temporal gene expression changes in the NSCLC cell lines after TTField treatment. We observed that most differentially expressed genes are part of cell cycle and proliferation pathways which is in agreement with earlier findings. Interestingly we found that the expression of BRCA1 DNA damage repair path-
way genes were significantly downregulated (P < 0.05) upon TTField treatment. We confirmed the downregulation of BRCA1/FA pathway proteins by western blot. When examining the nuclear role of the BRCA1/FA pathway genes we found that TTField treatment slowed the repair of ionizing radiation-induced
DNA damage compared to radiation alone which is evident by an increased
number of DNA double strand break repair foci at any given time. Moreover, we found that TTField treatment increased the incidence of chromatin aberrations.
We also examined the newly identified BRCA1/FA pathway genes cytotoxic role in mitophagy when we observed alterations in mitophagy related gene (PIN1K, DNM2, TPP1, TP53BP1), and DAPK1 expression compared the same at the protein level by western blot. We hypothesized that TTFields disrupt the clearance of damaged mitochondria due to the downregulation of BRCA1/FA pathway play-
ers, causing an imbalance in oxygen metabolism leading to the production of high levels of radical oxygen species (ROS) and as a result, cell death. Using
CellROX dye we found that TTField treatment did result in increased ROS
production suggesting a new mechanism of action for TTField exposure. Novel
chemotherapy agents, particularly PARP inhibitors, in combination with DNA damaging agents like radiation and TTFields may be advantageous through the
conditional vulnerability of down-regulated BRCA1.

#2139 The effects of cyclodipeptide on the transport of doxorubicin
and its cytotoxicity on tumor cell
Ivumi Sugiyama, Yasuyuki Sadzuka. Iwate Medical Univ. School of Pharmacy, Shiwa-Gun, Japan.

Purpose: Chemotherapy using antimetabolite plays an important role in clinical
therapy. Among the treatments involving antimetabolite, the enhance-
ment of antimetabolite activity was observed by combined chemotherapy. We have shown previously that some amino acids included theanine, taurine and anserine et al. as food components, increased doxorubicin (DOX) induced antitumor effect in
vitro and in vivo. These effects by combined amino acids have depended on the increased DOX concentration in the tumor cells. Furthermore, these suppositions
of DOX efflux by theanine and taurine were caused by the inhibitions of glutamate transporter and taurine transporter, respectively. The effect of anserine on
DOX influx might connect with dipeptide transporter. Thus, other amino acid and unique peptides may act to DOX transport. In this study, the effects of cyclodipeptide on the transport of DOX and its cytotoxicity on tumor cell were clarified. Methods: Cylo-
leucine-proline (cyclo-Leu-Pro), cyclo-phenylalanine-proline (cyclo-Ph-Pro) and
cyclo-glycine-proline (cyclo-Gly-Pro) as cyclodipeptide was used in this study. In
transport of DOX, DOX influx or efflux was determined with combined cyclodipep-
tide in M5076 ovarian sarcoma cells or P388 leukemia cells. DOX concentration in
tumor cell was measured using spectrophotometer (Ex. 470 nm, Em. 580 nm). The
cytotoxic study of DOX with combined cyclodipeptide was performed using
WST-8. Results and Discussion: Cyclo-Ph-Pro and cyclo-Gly-Pro did not affect
DOX influx and efflux in M5076 ovarian sarcoma cells and P388 leukemia cells. By combined cyclo-Leu-Pro, DOX influx into both M5076 ovarian sarcoma cells and P388 leukemia cells changed, compared to that of DOX only group. Into M5076
ovarian sarcoma, cyclo-Leu-Pro had strong effect in increased DOX influx in partic-
ular. And cyclo-Leu-Pro showed a tendency to suppress DOX efflux from M5076
ovarian sarcoma. In this studies of influx and efflux, concentration of cyclo-Leu-Pro existed optimum amount for increased DOX concentration in tumor cells. It is
expected that cyclo-Leu-Pro induces the increment of DOX concentration in the
tumor in vivo. DOX had the cytotoxic effects on M5076 ovarian sarcoma cells or
P388 leukemia cells in culture. The combined cyclo-Leu-Pro with DOX increased
cytotoxic effect, compared to that in the DOX alone group. In conclusion, cyclo-
Leu-Pro has increased effect on DOX influx and inhibited effect on DOX efflux, and
DOX induced cytotoxicity. It is expected that the combined cyclo-Leu-Pro will im-
prove cancer chemotherapy by DOX. Moreover, cyclo-Leu-Pro may be help to pre-
vent adverse effect since it will be able to decrease DOX dose.

#2140 Understanding molecular mechanisms underlying he
e function in non-small cell lung cancer
cancers, Sagar Solonoi, Chantal Vidal, Li Zhang. University of Texas at Dallas, Richardson, TX.

Heme is a central molecule for mitochondrial respiration and for all processes
involved in oxygen utilization. Heme serves as a prosthetic group or as a cofactor
for a number of oxidative phosphorylation enzymes and other oxygen-utilizing
hemoproteins. Heme directly regulates the synthesis, translocation and assem-
bly of these enzyme complexes. Most, if not all, human cells can synthesize and
uptake heme from the circulation. A number of epidemiological studies have
shown that high heme intake is associated with increased risk of cancer, includ-
ing lung cancer. To assess the status of heme metabolism in lung cancer cells, we
performed a series of experiments in non-small cell lung cancer (NSCLC) cell
lines such as HCC4017, H1299, A349, H2009, H1395, H460 and Calu-3. We then
compared results with an immortalized normal lung cell line, BHE39K7
and observed significant increase in rates of heme synthesis and heme uptake in
NSCLC cells compared to BHE39K7. Previous studies carried out in our lab
showed intensified mitochondrial respiration and increased levels of heme and
hemoproteins in NSCLCs. We examined the effect of heme depletion on mito-
chondrial biogenesis and function. We observed that mitochondrial functions

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such as ATP production, NAD/NADH ratios and ROS production are also affected by heme depletion. Proteins involved in mitochondrial biogenesis are significantly affected when cells are grown in heme depleted medium. Studies are currently underway to check how heme depletion affects the interactions of various transcription factors involved in mitochondrial biogenesis and function.

#2141 Development of a novel miR-129 mimic with enhanced therapeutic potential for treatment of resistant colorectal cancer. Andrew T. Fesler,1 Ning Wu,1 Hua Liu,1 Jingfang Ju,1 Stony Brook University, Sound Beach, NY; Stony Brook University, Stony Brook, NY.

Treatment of advanced stage colorectal cancer remains a clinical challenge associated with resistance to fluoropyrimidine based chemotherapy. There is an urgent need to discover and develop new strategies to enhance treatment efficacy in order to improve outcomes for these patients. Non-coding microRNAs (miRNAs) have important functions as oncogenes or tumor suppressor genes in the regulation of cancer development and progression. Recently, miRNAs have emerged as potential therapeutic options. We have previously identified miR-129 as a tumor suppressor miRNA and potential therapeutic candidate in colorectal cancer. The expression of miR-129 is progressively lost in colorectal cancer patients and is an important regulator of apoptosis through the targeting of genes such as BCL-2. miR-129 was also found to enhance 5-fluorouracil (5-FU) cytotoxicity in vitro and in vivo. To further developing miR-129 based novel therapeutics in colorectal cancer, we have designed a modified version of miR-129 to enhance stability and efficacy. The miR-129 mimic is more potent in inhibiting proliferation of a panel of colon cancer cell lines than the native miR-129 precursor, with 79% reduction by miR-129 mimic compared to 38% for native precursor. The miR-129 mimic induces profound cell cycle arrest at the G1/S checkpoint. The G1/S ratio increased 3.8 fold compared to control when cells were transfected with miR-129 mimic. We also demonstrated that the miR-129 mimic retains its target specificity to BCL-2, TS and E2F3. The therapeutic potential of miR-129 mimic was demonstrated in vivo mouse colon tumor models as a potent inhibitor of tumor growth and metastasis. As a result, miR-129 mimic has a great potential to be further developed as a novel therapeutic drug for treatment of advanced colorectal cancer.

#2142 Role of TRAIL signaling through the development of carcinogen-induced colorectal cancer. Yumin Oh,1 Seulki Lee,1 Kang Choon Lee2.

#2144 A novel method for high-throughput discovery of neo-antigens and corresponding T-cell receptors. Songming Peng,1 Jesse Zaretzky,2 Michael T. Bethune,1 Alice Hsu,1 David Baltimore,1 Antoni Ribas,2 James Heath1.

#2145 Regulation of YAP1 during hypoxia and its novel role in vascular mimicry and angiogenesis. Namrata Bora Singhal, Srikumar Chellappan.

## EXPERIMENTAL AND MOLECULAR THERAPEUTICS: New Targets 2

Experimental and molecular therapeutics have established links between occupational exposures to automobile exhaust fumes (AEF) and health hazards. Epidemiological studies have established links between occupational exposures to automobile exhaust fumes (AEF) and lung cancer, the propensity for systemic toxic responses. Street hawking and begging between automobiles, on various highways in Nigeria is on the increase. This occupational hazard is further worsen by dire economic recession in Nigeria, and growing population with limited prospect of improving their economic situation. Ages of vulnerable population ranges: 4 to 60yrs, exposure to AEF and susceptibility to health hazard maybe age related. We examined the effect of AEF exposure on E. coli P37 deoxyribonuclease acid (DNA) damage, cell growth and chromosomal aberration in Allium cepa. The effective concentration of AEF was determined as EC50: 0.43mg/ml from A. cepa root length growth against AEF concentrations. Furthermore, A. cepa exposed to AEF at EC50 (0.43mg/ml), EC25 (0.108mg/ml) and EC10 (0.043mg/ml) for 12, 24 and 48hrs mitotic index and chromosomal aberrations were evaluated at various endpoints. Chromosomal abnormalities were observed in dividing cells and decreases in A. cepa cell growth occurred dose and time dependently. Exposure of E. coli to AEF (EC50: EC25: EC10) resulted in inhibition of SOS inducing potency (SOSIP) with increasing concentration of AEF and compared to the positive control 4-nitroquinoline oxide (4-NQO). Decreases in the SOSIP (at EC50) indicate a threshold beyond which E. coli SOS response gene may have been extensively damaged to produce a response. Taken together we conclude that constituents of AEF are genotoxic in E. coli, induce chromosomal aberration and increases cell growth in A. cepa. We speculate on the possibilities of toxic health outcomes including carcinogenesis in humans persistently exposed to AEF fumes either occupationally or by any other means as observed in both street hawkers and beggars in Nigeria.
like LAT1/S12, Mut1/S2, Sav or Mob. We identified a novel regulation of YAP1 by prolyl hydroxylase PDH2, which is mainly known to regulate HIF1α. PDH2 was found to hydroxylate proline residue(s) in YAP1 in a region between aa 284 to aa 289 as seen by mutational analysis. YAP1 was found to directly bind to PDH2 and depletion of PDH2 or treatment with DMOG which is an inhibitor of prolyl hydroxylase elevated YAP1 protein levels in cancer cells. Further, we identified a putative HIF1α binding site that was co-occupied with HIF1α as detected by co-immunoprecipitation experiments and could enhance HIF1α-mediated induction of the VEGF promoter. Proximity ligation assays performed on TM4 showed enhanced YAP1 and HIF1α interaction in lung tumor tissues compared to normal cells. Our data suggest a novel regulation of YAP1 in hypoxic environments that supports angiogenesis and tumor growth.


Laryngeal squamous cell carcinoma (LSCC) accounts for approximately 30-40% of head and neck cancers. Although tobacco and alcohol consumption are considered the major risk factors for LSCC, various genetic and epigenetic factors have also been associated with LSCC development and progression. Since early detection and diagnosis of LSCC can greatly increase the chance of a successful treatment and survival, several studies have addressed the identification of target genes involved in LSCC pathogenesis. In this study we aimed to identify differentially expressed genes involved in the pathogenesis of LSCC using the GeneFishing technique. As a result of this investigation we identified the SLC22A23 gene as one of the differentially expressed gene. Solute carriers (SLC) comprise the largest family of membrane transport proteins with more than 300 members which have been divided into 52 families. The main functions of these proteins is to transfer a wide range of substrates such as amino acids, lipids, inorganic ions, peptides, saccharides, metal ions, proteins, xenobiotics and drugs. It is well known that cancer cells need extra metabolic requirements during rapid cell cycles. One of the known functions of the SLC proteins is to facilitate the uptake of nutrients and removal of metabolites. SLC22A23 (solute carrier family 22, member 23) belongs to the SLC family of organic ion transporters that are responsible for the uptake or excretion of many compounds including drugs, toxins and endogenous metabolites in a variety of tissues. SLC22A23 is expressed in various tissues but no substrates or functions have yet been identified for SLC22A23. Although the exact function is unknown, single-nucleotide polymorphisms which are located in the SLC22A23 gene have been associated with inflammatory bowel disease, endometriosis-related infertility and the clearance of antipsychotic drugs. On the other hand, SLC22A23 was identified as a prognostic gene to predict the recurrence of triple-negative breast cancer. In this study, to confirm the GeneFishing assay data, we investigated expression of the SLC22A23 gene in laryngeal tissue samples obtained from 83 patients by quantitative RT-PCR. We detected statistically significant upregulation of the SLC22A23 mRNA in laryngeal tumor tissues (55.4%) (p<0.05). However, no significant correlation was found between SLC22A23 expression and clinicopathological parameters including age, sex, stage, histological grade and smoking (p>0.05). To understand the association between the SNPs in SLC22A23 and LSCC we also investigated the frequency of different genetic variants (rs4959235, rs6923667, rs9503518). Currently, experiments to determine the BBB-penetrance of the hydrophilic DMG ester, by itself, was cytotoxic with IC50 values up to 500 µM against brain tumor cell lines, however, when combined at 100 µM with TMZ resulted in a greater synergistic cell killing (generally-9 fold, but 28-fold with UW-18 GBM cells). 0.25 mM DMG inhibited the cellular MGMT activity by ~80%, induced degradation of TET1 protein and highly increased the methylation levels of histones (H1K25me1, me2 and H2BK25me2). Currently, experiments to determine the BBB-penetrance of DMG, its ability to induce genomic and MGMT-specific methylations in glioma cells and GBM regression in intracranial xenograft models developed in nude mice are underway. Collectively, these data reveal that acute treatments of α-KG analogs can alter the cellular epigenetic makeup in a manner ascribed to D-2HG, and open up the much-needed novel and exciting avenues of oncometabolite therapy for brain tumors (supported by CPRIT grants RP130266 & RP170207 to KSS).

#2148 Phenyl butyrate inhibits pyruvate dehydrogenase kinase 1 and contributes to its anti-cancer effects. Wen Zhang, Xin Yip Tam. University of Macau, Macau, China.

In cancer cells, the metabolic features are significantly different from those of normal ones, which are hallmarks of several malignancies. Recent studies brought back the idea of metabolic regulation as a new strategy for cancer treatment by acting on glycolytic tumors while showing minimal side effects on the oxidative healthy organs. Phenyl butyrate (PB) has been proved to decrease PDH phosphorylation level and increase PDH activity by inhibiting PDK1 in fibroblast cells, PDH deficiency zebrafish and wild type mice. PB has also shown efficacy in many cancers and so far, all of its anti-tumor activity has been attributed to the histone deacetylase (HDAC) inhibitor mode of action. As PDK1/PDH controls the critical switch between oxidative phosphorylation and glycolysis in cancer cells, PDK1 is a key target in tumor metabolism for anti-cancer treatment. We hypothesize that the therapeutic effects of PB are mediated through downregulation of PDK1, altering the mitochondrial bioenergetics and inducing apoptosis. In addition to its proposed role as HDAC inhibitor, we showed that PB directly inhibited the kinase activity of PDK1 in a purified system. In several different cancer cell lines, PB reduced the phosphorylation level of PDH, increased the mitochondrial respiration, decreased glycolysis in cytoplasm, reversed mitochondrial hyperpolarization, activated some proteins in apoptotic signalling pathway and then induced the apoptosis of cells. In summary, this is the first study indicated that PB could exert its anti-cancer effects through inhibiting PDK1, altering the mitochondrial bioenergetics and inducing apoptosis.

#2147 IDH1 mutation-inspired α-ketoglutarate acid mimics for epigenetic therapy of higher grade gliomas. Hanumantara Rao Madala, Surendra Reddy Punganuru, Kalkunte S. Srivenugopal. Texas Tech Univ. Health Sciences Ctr., Amarillo, TX.

Mutations at the active site of isocitrate dehydrogenase 1 gene (IDH1; R132H) occur at a high frequency (>70%) early in the oncogenesis of lower grade malignant gliomas, and result in a dramatic accumulation of the oncometabolite D-2-hydroxyglutarate (D-2HG), that effectively replaces the normal metabolite α-ketoglutarate (α-KG) in cell physiology. Surprisingly, IDH1 mutations bestow superior therapeutic responses to alkylating agents and better patient survival. D-2HG, effectively competes with α-KG and potently inhibits various dioxygenase reactions, including the TET1, TET2 DNA-deamethylases and histone demethylases (5m-cytosine to 5-OH cytosine, H3-K-met to H3-K-met-) thereby, re-shaping and reprogramming the epigenetic landscape, and consequent transcriptional silencing; the DNA repair protein O6-methylguanine DNA methyltransferase (MGMT), which confers drug resistance is one major target for such repression. As a novel and innovative strategy of turning the tide against GBMs by exploiting the mechanistic aspects of the oncometabolite, we hypothesized that D-2HG and α-KG derivatives that can replace the natural metabolite in epigenomic dioxygenase reactions will serve as potent anti-glioma drugs either by themselves or in combination with the alkylating agents. To this end, we first synthesized a D-2HG diethyl ester to enhance its cellular uptake and tested its effects on four human brain tumor cell lines (DA0Y, T98G, SF188 and U87MG). 5α-dihydroxy-3α-methyl-4-oxo-3,4-dihydro-2H-chromene-2-carboxylic acid methyl ester (DMG) elevated YAP1 protein levels in cancer cells. Further, YAP1 was found to be associated with HIF1α as detected by co-immunoprecipitation experiments and could enhance HIF1α-mediated induction of the VEGF promoter. Proximity ligation assays performed on TM4 showed enhanced YAP1 and HIF1α interaction in lung tumor tissues compared to normal cells. Our data suggest a novel regulation of YAP1 in hypoxic environments that supports angiogenesis and tumor growth.

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In a selective series of in vitro experiments, we determined that the hydrophilic DMG ester, by itself, was cytotoxic with IC50 values up to 500 µM against brain tumor cell lines, however, when combined at 100 µM with TMZ resulted in a greater synergistic cell killing (generally-9 fold, but 28-fold with UW-18 GBM cells). 0.25 mM DMG inhibited the cellular MGMT activity by ~80%, induced degradation of TET1 protein and highly increased the methylation levels of histones (H1K25me1, me2 and H2BK25me2). Currently, experiments to determine the BBB-penetrance of DMG, its ability to induce genomic and MGMT-specific methylations in glioma cells and GBM regression in intracranial xenograft models developed in nude mice are underway. Collectively, these data reveal that acute treatments of α-KG analogs can alter the cellular epigenetic makeup in a manner ascribed to D-2HG, and open up the much-needed novel and exciting avenues of oncometabolite therapy for brain tumors (supported by CPRIT grants RP130266 & RP170207 to KSS).


Colorectal cancer is one of the most common cancers in the world, affecting nearly 1.2 million people in the United States alone. The aim of this study is to investigate the salvage pathway enzyme HPRT as a possible biomarker in two colorectal cancer cell lines: HT29 and SW620. HPRT is a transerase in the purine salvage pathway that functions primarily by catalyzing the conversion of hypoxanthine to inosine monophosphate and guanine to guanosine monophosphate. Because of its role in proliferation and cell cycle regulation, we hypothesized an increase in HPRT expression within cancer cells, which could potentially lead to presentation on the surface of the cell. HPRT surface localization was assessed utilizing confocal microscopy, flow cytometry, and scanning elec-
tron microscopy. These techniques allowed us to visualize HPRT on the plasma membrane and quantify expression. There was statistically significant expression of HPRT on the surface of both HT29 and SW620 cells with a 28% and 58% fluorescent shift in the population, respectively. Confocal microscopy images revealed overlap between cells stained with a membrane dye and anti-HPRT FITC antibody, which indicates a direct relationship between HPRT and the plasma membrane of SW620 cells. To visualize the location of HPRT on the plasma membrane, anti-HPRT antibodies were labelled with gold and protein presence was quantified using an electron microscope, which measured the gold elemental weight percentage of each sample. When exposed to anti-HPRT antibody, the gold weight percentage of the samples significantly increased with 12.4% gold in SW14 cells and 11.2% gold in HT29 cells. Also, these cells showed cell cycle arrest at the synthetic phase, activation of Bcl-2, K-ras, pAkt proteins as well as inhibition of p53, PUMA, p21 and BAX proteins. Conversely, knockdown of GAEC1 reduced cell proliferation, migration, decreased the phosphorylation of AKT, and induced apoptosis, G2/M phase arrest and cleavage of poly (ADP-ribose) polymerase (PARP). Co-immunoprecipitation revealed GAEC1’s interaction with p53. In addition ectopic over expression and si- lencing of GAEC1 lead to reciprocal effects of p53 protein expression. Moreover, knockdown of GAEC1 reduced the nuclear translocation of murine double minute 2 protein (mdm2) indicating that GAEC1 is responsible for the degradation of p53 through the direct interaction between mdm2-p53 in nucleus. Furthermore, our in vivo data demonstrate that the loss of GAEC1 inhibits the tumor formation in xenograft model. Conclusion: Collectively, our study demonstrates that GAEC1 exhibits the oncogenic role in colon cancer by reducing expression of p53 through protein-protein interaction which leads to the inhibition of PUMA, p21, BAX, and activation of Bcl-2, K-ras and pAkt protein expression.


The Maternal Embryonic Leucine Zipper Kinase MELK has been described as a genetic dependency in several cancer types, most notably in the highly-aggressive basal subtype of breast cancer; MELK inhibition through the use of both RNAi and small-molecule approaches appears to block the growth of cancer types with such dependency. Based on these results, the MELK inhibitor OTS167 is currently being tested as a novel chemotherapeutic agent in multiple clinical trials. Here, however, we report that mutagenizing MELK with CRISPR/Cas9 has no effect on the fitness of basal breast cancer cell lines or cell lines from other cancer types. Through seven guide RNAs targeting the kinase and kinase-associated domains of MELK, we demonstrate that mutagenesis of MELK causes no defect in proliferative ability or anchorage independent growth in these cancer types. Additionally, cells with mutagenized MELK remain sensitive to OTS167, suggesting that this drug blocks proliferation through an off-target mechanism. Finally, the patient tumor gene expression data that initially identified MELK as being significantly upregulated in patients with poor survival was reexamined. As MELK is thought to play a role in mitosis, we compared MELK expression to a set of well-known cell proliferation markers and show significant correlations of MELK with the proliferation genes; this suggests a role of MELK in representing the mitotic activity of a tumor, rather than possessing a transforming role in itself. In total, our results undermine the rationale for a series of current clinical trials based on MELK inhibition and provide an experimental approach for the use of CRISPR/Cas9 in preclinical target validation that can be broadly applied.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Targeting p53, Apoptosis, and the Cell Cycle

#2150 Oncogenic role of GAEC1 and its potential modulation with p53 in pathogenesis of colon cancer. Riajal Wahab, Farhadul Islam, Vinod Gopalan, Alfred King-Yin Lam. Griffith University, Southport, Gold Coast, Australia.

Introduction: GAEC1 (Gene ampliﬁed in esophageal cancer 1), is frequently ampliﬁed and overexpressed in colon cancer tissues. In the present study, we aimed to unveil the oncogenic potential of GAEC1 in carcinogenesis of colon cancer by studying the underlying cellular functions and molecular interactions by in vitro and in vivo experiments. Method: Transient overexpression of GAEC1 with pcDNA3.1-GAEC1 and silencing with GAEC1-siRNA was performed and several downstream assays were done such as migration, clonogenic and apoptotic assay. Analysis of cell kinetics was done using ﬂow cytometry and cell counting kit-8 was used for cell proliferation assay. Immunofluorescence and Western blot assay were used to determine the expression of diﬀerent target proteins. Co-immunoprecipitation was used to conﬁrm the protein-protein interaction. For xenotransplantation the severely combined immunodeﬁcient (SCID) mice (4 groups, 6 in each group) were injected subcutaneously with GAEC1 shRNA and control shRNA transfected (stable) SW480 and SW48 colon cancer cells. Result: The overexpression of GAEC1 increased cell proliferation, migration, reduced apoptosis in colon cancer cells. Also, these cells showed cell cycle arrest at the synthetic phase, activation of Bcl-2, K-ras, pAkt proteins as well as inhibition of p53, PUMA, p21 and BAX proteins.

<table>
<thead>
<tr>
<th>G50 (Mean + SEM)</th>
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<th>WM35</th>
<th>C8161</th>
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<td>Nullin-3 (µM)</td>
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<td>RG7388 (nM)</td>
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<td>HDM201 (nM)</td>
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<tr>
<td>GSK2830371</td>
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<td>Nullin-3 (µM)</td>
<td>166 ± 95</td>
<td>54 ± 37</td>
<td>186 ± 78</td>
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#2151 Inhibition of WIP1/PPM1D phosphatase by GSK2830371 potentiates the growth inhibitory and cytotoxic activity of MDM2 antagonists (nullin-3, RG7388 and HDM201) in cutaneous melanoma cells. Chiao-En Wu, Arman Esfahani, Yi-Hsiang Ho, Colin Shepherd, Ahmed Khairallah Mahdi, Erhan Apltullahoglu, John Wen-Cheng Chang, Perry Lovat, John Luneck. 1. Northern Institute for Cancer Research, School of Medicine, Newcastle University, United Kingdom; 2. Division of Hematology-Oncology, Department of Internal Medicine, Chang Gung Memorial Hospital at Linkou, Chang Gung University College of Medicine, Taoyuan, Taoyuan, Taiwan; 3. Dermatological Sciences, Institute of Cellular Medicine, Newcastle University, United Kingdom.

Cutaneous melanoma is the most serious skin malignancy. The current study aimed to investigate the WIP1 inhibitor GSK2830371 and MDM2-p53 antagonists (nullin-3, RG7388 and HDM201) alone and in combination treatment in cutaneous melanoma cell lines and explored the mechanistic basis of these responses in relation to the genotype and induced gene expression profile of the cells. A panel of three p53WT (A375, WM35, C8161) and three p53MUT (WM164, WM35-R5R1, CHL-1) melanoma cell lines were used. GSK2830371 (≤10 µM) alone had no growth-inhibitory or cytotoxic effects on the cells, measured by sulforhodamine B (SRB) and clonogenic assays. In combination treatment GSK2830371 significantly potentiated the growth-inhibitory and clonogenic cell killing effects of MDM2 inhibitors in p53WT but not p53MUT melanoma cells, indicating the potentiation worked in a p53-dependent manner (Table). Western blotting demonstrated GSK2830371 increased p53 stabilization through Ser15 phosphorylation and consequent Lys382 acetylation when it was combined with MDM2 inhibitors. These changes were ATM-mediated, shown by reversal with the ATM inhibitor (KU55933). Furthermore, GSK2830371 was demonstrated to slow down p53 degradation when de-novo protein synthesis was inhibited by cycloheximide. In qRT-PCR, nullin-3 or RG7388 induced p53 transcriptional target genes (CDKN1A, MDM2, BAX, FAS, PUMA, TNFRSF10B, TP53INP1) and GSK2830371 enhanced the induction in p53WT but not p53MUT cells. In conclusion, GSK2830371, a WIP1 inhibitor, acts dose-dependently, growth-inhibitory activity alone, potentiated the growth-inhibitory and cytotoxic activity of MDM2 inhibitors, by increasing phosphorylation, acetylation, and stabilization of p53 in cutaneous melanoma cells in a functional p53-dependent manner. Further studies in vivo are warranted to investigate the efficacy of this combination treatment.
Experimental and Molecular Therapeutics: Targeting p53, Apoptosis, and the Cell Cycle

G150 by sulforhodamine B (SRB) and LC50 by clonogenic assays in p53WT melanoma cells

Cell lines

<table>
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<th>GL50 (Mean ± SEM)</th>
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<td>HDMD201 (nM)</td>
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<td>p value</td>
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#2152 Protein phosphatase Mg2+ and Mn2+ dependent 1F promotes smoking-induced breast cancer by inactivating phosphorylated p53-induced signals. Li-Ching Chen. Taipei Medical University, Taipei City, Taiwan.

Previously we demonstrated that the activation of α9-nicotinic acetylcholine receptor (α9-nAChR) signaling by smoking promotes breast cancer formation. To investigate the downstream signaling molecules involved in α9-nAChR-induced breast tumorigenesis, we used real-time polymerase chain reactions and Western blotting to assess expression of protein phosphatase Mg2+/Mn2+ dependent 1F (PPM1F), a Ser/Thr protein phosphatase, in human breast cancer samples (n = 167). Additionally, stable PPM1F-knockdown and overexpressing cell lines were established to evaluate the function of PPM1F. The phosphatase activity of PPM1F in nicotine-treated cells was assessed through Western blotting, confocal microscopy, and fluorescence resonance energy transfer. Higher levels of PPM1F were detected in the breast cancer tissues of heavy smokers (n = 7, 12.8-fold) greater than of non-smokers (n = 28, 6.3-fold) (**) = 0.01. In vitro, nicotine induced PPM1F expression, whereas α9-nAChR knockdown reduced the protein expression of PPM1F. A series of biochemical experiments using nicotine-treated cells suggested that the dephosphorylation of p53 (Ser-20) and BAX (Ser-184) by PPM1F is a critical posttranslational modification, as observed in breast cancer patients who were heavy smokers. These observations indicate that PPM1F may be a mediator downstream of α9-nAChR that activates smoking-induced carcinogenic signals. Thus, PPM1F expression could be used for prognostic diagnosis or inhibited for cancer prevention and therapy.

#2153 Elephant p53 (EP53) expression induces apoptosis of human cancer cells. Lisa M. Abegglen,1 Lauren N. Donovan,1 Genevieve Couldwell,1 Rosann len,1 Bryan E. Welm,1 Carlo C. Maley,4 Avi Schroeder,2 Joshua D. Schiffman1.

The goal of our study was to determine if elephant TP53 (EP53) proteins contributing to increased apoptosis and possible cancer resistance in elephants could translate into human cancer cells as a future effective cancer treatment. We previously reported that elephants have a lower than expected rate of cancer. 20 copies of TP53 (1 ancestral gene with introns [EP53-anc] and 19 retrogenes [EP53-retro1-19], and increased p53-mediated apoptosis induced by DNA damage in elephant cells compared to human cells (Abegglen JAMA 2015). For the current study, we expressed various EP53 proteins in human cancer cells with different p53 status, including osteosarcoma (U2-OS, Saos-2), glioblastoma (T98G), and breast cancer (MCF7). Western blot analysis confirmed EP53 expression. We compared apoptosis in the human cancer cells transfected/transduced with negative control vectors vs. epitope or protein-tagged EP53 exposed to doxorubicin (to induce DNA damage). Apoptosis was measured by cell viability, caspase activity, Propidium iodide/Annexin V staining, and fluorescence microscopy. We observed a significant increase in caspase activity (normalized to cell viability) of U2-OS and T98G cells expressing EP53 compared to negative control treated cells as shown in Table 1, and apoptosis with p21 restoration in Saos-2. In U2-OS, which overexpress MDM2, EP53 was more effective at inducing apoptosis compared to human TP53. Taken together, we found that EP53-anc restored p53-mediated apoptosis and EP53-anc / EP53-retro9 enhanced p53-mediated apoptosis. These data suggest for the first time that EP53 functions in human cancer cells to promote cell death. Ongoing efforts are exploring the EP53 mechanism of action that leads to increased apoptosis, including expression of EP53 in additional cancer types (lung, melanoma, colon, prostate, and others) with a variety of genetic backgrounds to characterize its functional context. These results support the further exploration of EP53-based cancer therapeutics.

Table 1: Increase in apoptosis with EP53 expression relative to EP53 empty vector control cells

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>TP53 Status</th>
<th>EP53 Control</th>
<th>EP53 Construct</th>
<th>Assay Results</th>
<th>No Treatment (fold difference)</th>
<th>P-value</th>
<th>Induce Apoptosis (fold difference)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>U-2 OS (osteosarcoma) WT</td>
<td>Apoptosis</td>
<td>eGFP</td>
<td>eGFP-EP53</td>
<td>Increase in caspase activity</td>
<td>1.73</td>
<td>0.0091</td>
<td>1.79</td>
<td>1.79 x 10^-5</td>
</tr>
<tr>
<td>MCF7 (breast cancer) WT</td>
<td>-</td>
<td>eGFP-EP53</td>
<td>eGFP-EP53-retro9</td>
<td>Increase in caspase activity</td>
<td>1.48</td>
<td>6.7 x 10^-5</td>
<td>2.59</td>
<td>0.0041</td>
</tr>
<tr>
<td>T98G (glioblastoma) No</td>
<td>Apoptosis</td>
<td>EP53-anc</td>
<td>EP53-anc/myc</td>
<td>Increase in caspase activity</td>
<td>1.42</td>
<td>5.4 x 10^-5</td>
<td>3.31</td>
<td>1.0 x 10^-4</td>
</tr>
<tr>
<td>H1975</td>
<td>No</td>
<td>EP53-anc</td>
<td>EP53-anc/myc</td>
<td>Increase in caspase activity</td>
<td>1.09</td>
<td>1.2 x 10^-7</td>
<td>2.47</td>
<td>5.6 x 10^-5</td>
</tr>
<tr>
<td>T47D (breast cancer) No</td>
<td>Apoptosis</td>
<td>EP53-anc</td>
<td>EP53-anc/myc</td>
<td>Increase in caspase activity</td>
<td>3.46</td>
<td>0.00015</td>
<td>1.52</td>
<td>6.0 x 10^-5</td>
</tr>
<tr>
<td>T98G (glioblastoma) No</td>
<td>Apoptosis</td>
<td>EP53-anc</td>
<td>EP53-anc/myc</td>
<td>Increase in caspase activity</td>
<td>2.82</td>
<td>1.2 x 10^-5</td>
<td>1.8</td>
<td>8.6 x 10^-5</td>
</tr>
</tbody>
</table>


We recently reported that P01 is a potent p53 pathway-restoring small molecule that acts through increasing levels of TAp73. Further, it also interferes with the p73-mutant p53 protein-protein interaction and by downregulation of ΔNp73 (Tian, Zhang and El-Deiry, abstract# 3830, AACR 2016). P01 is a member of the natural products that have been shown to have potent anti-cancer activity against tumors with mutated p53. Based on the structure of the pharma- copoeia of compound P01, we designed and synthesized new analogs based on published structure-activity relationship and organic synthesis papers. The newly synthesized analogs were potent in reducing both short-term and long-term proliferation in a broad panel of mutant p53 cell lines such as HT29, SW480, DLD-1, MDA-MB-231 and H1975 with EC50 in the range of 0.16 μM to 0.26 μM. We are currently evaluating the anti-cancer effects of three analogs P01, P04 and P06, of which only two of these analogs induce TAp73 as assayed by western blot. We are currently characterizing the analog P06, which is the most potent compound among them. P06 engages the apoptosis pathway by upregulation of pro-apoptotic proteins like PUMA, DR5, and BIM and downregulation of anti-apoptotic markers such as Mcl-1 in a time-and dose-dependent manner in colorectal cancer cell lines. P06 treatment also down-regulates both mRNA and protein level of MET and EGFR in HT29 cells that overexpress MET and EGFR receptors. We are currently investigating the mechanisms of induction of both PUMA and DR5 in mutant p53 cells and determining whether either gene is necessary for the apoptotic effects of P06 in colorectal cancer cells. Our preliminary data indicates that upregulation of DR5 is through the ATF4/CHOP pathway post-P06 treatment. It has been reported that Puma is a target gene of ATF4, CHOP and p73, however, we found that none of them is responsible for P06-induced upregulation of PUMA in p53 mutated DLD1, SW480 or HCT116 p53-null cell lines. Thus, our ongoing in vitro studies are focused on further understanding the mechanism of action of P06 in mutant p53 cells and potentially exploring combinations with FDA-approved therapies. We are also in the process of conducting first-in-animal studies of P06 as single agent or in combination with chemotherapy or targeted therapy.

#2155 Small molecule NSC59984 suppresses cancer cell growth under hypoxia. Shengliang Zhang, Lanlan Zhou, Wafık S. El-Deiry. Fox Chase Cancer Center, Philadelphia, PA.

Hypoxia is one of the main features of solid tumors, contributes to drug resistance and is associated with poor prognosis. We have reported that a small-molecule NSC59984 restores p53 signaling and degrades mutant p53 protein in cancer cells. We find that NSC59984-mediated p53 signaling restoration and mutant p53 degradation is significantly blocked by NAC, an inhibitor of reactive oxygen species (ROS), while, further enhanced by BSO, a chemical compound increasing ROS in cells. These results suggest that ROS is a factor required for the effect of NSC59984 on cancer cells. Cellular ROS is produced by hypoxia in solid...

Mutant p53 protein is highly expressed in most cancer cells due to its protein stabilization. Mutant p53 loses wild-type p53 function but gains new oncogenic functions in driving tumor growth and drug resistance. Depletion of mutant p53 is an attractive strategy for cancer therapy. We reported a small-molecule NSC59984 destabilizes mutant p53 protein via protein degradation (Zhang et al., Cancer Research, 2015). In this study, we demonstrate that the ERK2 pathway plays a specific role in NSC59984-induced mutant p53 degradation via MDM2-mediated ubiquitination. We observe a sustained phosphorylation of ERK1/2 in cancer cells treated with NSC59984. ERK1/2 pathway is negatively regulated by MKP-1. We find that MKP-1 activity is partially inhibited by NSC59984 in a dose dependent manner in vitro. These results suggest that NSC59984 sustains ERK1/2 phosphorylation partially via disruption of the feedback-loop between MKP-1 and ERK2 pathway. Knockdown of ERK2 rescues mutant p53 from NSC59984-mediated degradation, and inhibits NSC59984-restoration of p53 signaling in mutant p33-expressing cells. On the contrary, the effect of NSC59984 on the mutant p53 is not inhibited by the blockage of JNK and p38, two pathways negatively regulated by MKP-1. These results suggest that the ERK2 phosphorylation is specifically required for NSC59984-mediated mutant p53 degradation. We further find that NSC59984 induces ERK2-dependent MDM2 phosphorylation. The effect of NSC59984 on MDM2 phosphorylation is partially inhibited by the blockage of ERK2 signaling (via U0126 treatment or Knockdown of ERK2), not by the attenuation of the JNK pathway. Furthermore, NSC59984-mediated mutant p53 protein degradation is inhibited by MDM2 knockdown, and enhanced by MDM2 overexpression in cancer cells. NSC59984-increased ubiquitination of mutant p53 is attenuated by U0126. Our results suggest that the ERK2 signaling pathway-MDM2 axis is a major determinant of NSC59984-mediated mutant p53 degradation. We further demonstrate that NSC59984 induces cell death via apoptosis, and NSC59984-induced cell death is mainly rescued by inhibition of the ERK2 pathway in mutant p53 cancer cells. Taken together, our results suggest that phosphorylated-ERK2 is an important factor required for NSC59984-mediated mutant p53 degradation which may further contribute to NSC59984-induced cell death in cancer cells.

#2157 P73 isoforms regulate cellular survival and response to treatment in diffuse large B-cell lymphoma. Hesham M. Hassan, 1 Michelle L. Varney, 2 Pamela A. Althof, 2 Gabriel C. Caponetti, 3 Kai Fu, 2 Dennis D. Weisenburger, 4 Rakesh K. Singh, 2 Bhavana J. Dave 2.

We investigated the effect of NSC59984 on p53 pathway and cell death in p53 mutant colorectal cancer (CRC) cells cultured under hypoxia. NSC59984 increases p53 signaling in p53 mutant cancer cells under hypoxia, as it does under normoxia. The effect of NSC59984 under hypoxia is blocked by NAC. Moreover, NSC59984 decreases cancer cell viability in a dose-dependent manner under hypoxia similar to normoxia. Our results suggest that NSC59984 inhibits cancer cell growth under hypoxia. Hypoxia has been known to increase cancer stem cells in tumors, which is one of the reasons causing drug resistance. We then evaluate the effect of NSC59984 on the stem cell growth. NSC59984 significantly reduces colosphere formation of SW480, DLD-1 and HT29 cells. CRC stem cell markers such as CD44 and ALDH are decreased in colospheres upon NSC59984 treatment, suggesting that NSC59984 inhibits stem cell self-renewal or/and growth. Our results provide the rationale for administration of NSC59984 in solid tumors with or without hypoxia and in combination treatment with ROS activators.


The first anti-cancer stapled peptide drug has now been advanced to clinical testing in relapsed human cancers that retain the expression of wild-type p53. A common mechanism for cancer cell suppression of wild-type p53 is overexpression of the negative regulators HDM2 and HDMX, which neutralize p53 through protein interaction. Selective HDM2 inhibitor molecules can effectively reverse the repressive effects of mutant p53 in certain cancers, but the co-expression of HDMX can cause treatment resistance, highlighting the need for dual HDM2/HDMX targeting. We previously developed stapled peptides modeled after the p53 transactivation domain to harness the natural propensity of this alpha-helical motif to engage both targets with high affinity and selectivity. Here, we demonstrate that ALRN-7041, a next-generation, clinical-grade stapled peptide, achieves time-dependent cellular uptake and nuclear localization without membrane perturbation, dose-dependently dissociates p53/HDM2 and p53/HDMX complexes as assessed by real-time protein interaction monitoring in live cells, and impairs the viability of cancer cells bearing wild-type p53 by inducing a surge in p53 protein level. Applying an unbiased statistical approach to determine which biophysical parameters dictate the cellular uptake of stapled peptides, we elucidated the design features of ALRN-7041 that confer intracellular access, providing a roadmap for generating cell-permeable stapled peptides with on mechanism cellular activity for clinical translation. We find that cancer cells exhibiting a signature of HDM2, HDMX, and wild-type p53 co-expression are strikingly susceptible to ALRN-7041, highlighting the therapeutic potential of dual HDM2/HDMX inhibition by a cell-penetrating stapled peptide in human cancer.

#2159 A specific 17-beta-hydroxywithanolide (LG-02) sensitizes cancer cells to apoptosis in response to TRAIL and TLR3 ligands. Poonam Tewary, 1 Alan D. Brooks, 1 Ya-xing Xu, 2 Kittishri E.M. Wijeratne, 2 Leslie A. Gunatilaka, 2 Thomas J. Sayers, 4 Leidos Biomedical Research Inc, Frederick, MD. 2 University of Arizona, Tucson, AZ, AZ.

Recent studies have demonstrated a role of toll-like receptor 3 (TLR3) signal for the initiation of apoptosis in some malignant cells. We have previously shown that, withanolide E (WE), a 17-beta-hydroxywithanolide (17-BHW) natural product derived from the medicinal plant Physalis peruviana was capable of sensitizing tumor cells to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis by reducing cellular levels of the anti-apoptotic protein cFLIP. Animal studies also revealed that WE sensitized human renal carcinoma cells to apoptosis at concentrations that did not promote apoptosis in normal cells. Thus we further screened a library of 30 natural and semi-synthetic 17-BHWs for their ability to promote death ligand-mediated cancer cell death. Among the 16 compounds tested, LG-02 (physachenolide C) was found to be 4-5 fold more potent than WE in sensitizing some human renal carcinoma and melanoma cells to apoptotic cell death in response not only to TRAIL but also to the synthetic polynucleotide poly (I:C), which is known to mimic anti-viral responses by activating TLR3 signaling. To date there are no withanolides known to have this dual apoptosis sensitizing activity. LG-02 and Poly (I:C) treatment resulted in increased activation of caspase-8, and apoptosis was blocked by the pan caspase inhibitor zVAD-FMK. Poly (I:C)-driven apoptosis signaling was dependent on endosomal acidification, but independent of IRF3 and Interferon α/β signaling. Molecular studies suggested a role for changes in the anti-apoptotic proteins cFLIP, IAPs, and Livin on apoptosis signaling in LG-02 treated cells. Loss of cIAP activity is reported to promote spontaneous formation of an intracellular death-inducing protein platform the ripoptosome, that can activate either apoptosis or necroptosis. Immuno-

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preincubation of either the TRAIL death-inducing signaling complex (DISC) or the Poly (I:C) ripoptosome, demonstrated enhanced levels of FADD and RIP1 and decreased levels of cFLIP in these macromolecular apoptosis signaling complexes in LG-02 treated cells. Intratumor administration of LG-02 and Poly (I:C) in a xenograft M14 melanoma model provided therapeutic benefit leading to complete tumor regression in 90% of mice as compared to controls. Further studies with active 17-BHWs could lead to the identification of more potent analogues, and novel and common therapeutic targets involved in apoptosis signaling in response to both TNF death receptor family members as well as TLR3 ligands. Funded by FNLCR Contract HHSN261200800001E


Colorectal cancer is a major worldwide health problem owing to its high prevalence and mortality rate. Developments in screening, prevention, bio-marker, personalized therapies and chemotherapy have improved detection and treatment. However, despite these advances many patients with advanced metastatic tumors will still succumb to the disease. New anti-cancer agents are needed for treating advanced stage colorectal cancer as most of the deaths occur due to cancer metastasis. A recently developed novel sulfonamide derivative, 4-((2-(4-(Dimethylamino)phenyl)quinazolin-4-yl)aminobenzensulfonamide (3d) has shown to have potent antitumor effect; however the mechanism under- lying the antitumor effect remains unknown. Our study revealed that 3d treatment significantly reduced the viability of human colorectal cancer cells HT-29 and SW620. This is further evidenced by the induction of p53 and Bax, release of cytochrome c, activation of caspase-9, caspase-3 and cleavage of PARP in 3d treated cells. This compound was found to have significant effect on the inhibition of anti-apoptotic proteins, Bcl2, BclxL and XIAP. The results assessed in apoptosis (cleaved Parp) and cell-viability assays following combined inhibition of PI3K/Akt and BCL-XL, in PTEN-mutant prostate cancer cells results in synergistic apoptosis suggesting a novel therapeutic strategy with translational potential in this important subset of disease. Hypothesis: We hypothesized that synthetic lethality following combinational PI3K/BCL-XL inhibition is conserved across the range of PTEN-mutant cancer cells. Methods: PTEN-mutant cell lines (ATCC), validated for PTEN-loss and -pAkt expression, were assessed in apoptosis (cleared Parp) and cell-viability assays following combined inhibition of PI3K/Akt and BCL-XL, -pAkt induction (1.5x), and SW620. Further studies with active 17-BHWs could lead to the identification of more potent analogues, and novel and common therapeutic targets involved in apoptosis signaling in response to both TNF death receptor family members as well as TLR3 ligands. Funded by FNLCR Contract HHSN261200800001E

MEK signaling and PI3K/BCL-XL inhibition in PTEN-mutant cancers

<table>
<thead>
<tr>
<th>Source</th>
<th>Cell line</th>
<th>PTEN</th>
<th>p-Akt</th>
<th>p-Erk</th>
<th>Apoptosis with</th>
<th>p53</th>
<th>FLIP</th>
<th>Combined inhibition of PI3X/BCL-XL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>PC3</td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
<td>Yes</td>
<td>Yes</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Prostate</td>
<td>LNCaP</td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
<td>Yes</td>
<td>Yes</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Prostate</td>
<td>C4-2B</td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
<td>Yes</td>
<td>Yes</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Breast</td>
<td>ZR-75-1</td>
<td>Absent</td>
<td>Present</td>
<td>Weak</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Uterine</td>
<td>SK-UT-1</td>
<td>Absent</td>
<td>Present</td>
<td>Present</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>GBM</td>
<td>A-172</td>
<td>Absent</td>
<td>Present</td>
<td>Present</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Cervix</td>
<td>C3-J3</td>
<td>Absent</td>
<td>Present</td>
<td>Present</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

Conclusions: Synthetic lethality in PTEN-mutant tumor cells with PI3K/BCL-XL inhibition was confined to tumor cells without active MEK signaling. MEK inhibition inconsistently overcomes resistance to therapy suggesting heterogeneity of survival pathways in p-Erk expressing PTEN-deficient tumors. These data have implications for precision medicine approaches in PTEN-deficient tumors as absence of p-Erk expression may predict for a responsive phenotype.

#2163 Combined targeting of MEK and MCL-1 induces apoptosis and tumor regression of KRAS mutant NSCLC. Aaron N. Hata, Faria M. Siddiqui, Maria Gomez-Cardaballo, Samantha J. Bilton, Daria Timonina, Varuna Nangia, Angela Coxon, Sean Caenepeel, Paul Hughes, Massachusetts General Hospital, Charlotte, MA, Amgen, Inc, Thousand Oaks, CA.

There are currently no effective targeted therapeutic strategies for KRAS mutant, non-small cell lung cancer (NSCLC). Single agent MEK inhibitors have demonstrated showed disappointing clinical activity, partly due to inability to induce a robust apoptotic response. Combining MEK inhibitors with BCL-XL/-BCL-2 inhibitors may be effective for a subset of KRAS mutant cancers that are dependent on BCL-XL for survival, however this combination is unlikely to be an effective strategy for cancers dependent on MCL-1. We investigated the effect of combining the MEK inhibitor trametinib with a novel MCL-1 inhibitor (compound A), which possesses potent and selective anti-MCL-1 activity in vitro and in vivo, on KRAS mutant cancers. In contrast to colorectal cancer models, which are largely sensitive to combined MEK + BCL-XL inhibition, a subset of cell line and patient-derived mouse xenograft (PDX) KRAS mutant NSCLC models were significantly more sensitive to MEK + MCL-1 inhibition compared to MEK + BCL-XL. The model-based clinical trial strategies, we tested intermittent dosing regimens and unexpectedly discovered a method strategy for dramatically sensitizing KRAS mutant NSCLC cells to the MEK + MCL-1 combination. These studies provide rationale for the clinical evaluation of combined MEK + MCL-1 inhibitors for KRAS mutant NSCLC.

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**#2164** BLID is a novel drug-inducible apoptotic molecule: Identification of an integrative mechanism of chemosensitivity in breast cancer cells. Sivaramakrishna Yadavalli, Rong Hu, Antonina Rait, Xin Li, Esther Chang, Robert Clarke, Usha Kasid. Georgetown Lombardi Comp. Cancer Ctr., Washington, DC.

The triple-negative breast cancer (TNBC) is an aggressive form of breast cancer frequently seen in African American women and BRCA1 mutation carriers. The TNBC tumors often relapse with distant metastases following standard chemotherapy. It is now evident that discoveries of new mechanisms and approaches that explain and target breast cancer biology are urgently needed for durable intervention of metastatic disease. Earlier, we have demonstrated that BLID, Bcl2 containing Insulator, is an important tumor suppressor and apoptosis-related factor in invasive breast cancer. Frequent lack of BLID has been associated with TNBC, African American ethnicity and younger women. Significant correlations exist between BLID negative breast cancer and declines in overall survival, local relapse-free survival and distant metastasis-free survival. Recently, BLID has been shown to inhibit breast cancer cell growth and metastasis. The purpose of this study was to investigate the role of BLID in response of breast cancer cells to chemotherapeutic drugs. In the dose response and time course studies, BLID mRNA expression was found to be induced by chemotherapeutic drugs. Expression of BLID CDNA nanoprecipitate (scBLID) resulted in significant increase in chemosensitivity in SKBr3 and MDA-MB-231 cells, and a comparison of BLID with p53 showed that the chemosensitization effect of BLID was significantly greater than that of p53. More consistently, BLID knockdown led to reduced induced cytotoxicity. In the ChIP-PCR and ChIP-qPCR assays, drug treatment of breast cancer cells resulted in an increased binding of pro-apoptotic transcription factor FOXO3a to the BLID promoter, and the reversal of drug-induced BLID reporter activity was seen in presence of FOXO3a siRNA. Furthermore, siRNA silencing of FOXO3a was found to be associated with decrease in endogenous BLID mRNA expression. Remarkably, we found that expression of central tumor suppressor microRNA miR34a also resulted in increased BLID mRNA expression and drug toxicity in breast cancer cells. Because lack of BLID expression has been associated with poor prognosis in breast cancer patients, we reasoned that the silencing of BLID may reveal as yet unknown changes in gene expression that may drive breast cancer cell proliferation and therapy resistance. In this context, the mRNA array profiling studies showed that BLID knockdown in MDA-MB-231 cells was associated with increased expression of the oncogenic/anti-apoptotic molecules CYP1B1, BIRC3 and CSF1, and decreased expression of the anti-oncogenic/apoptotic molecules AKAP12, DFN5 and CHRD1. Our data suggest that chemotherapeutic drugs induce BLID expression via activation of FOXO3a, and the BLID signaling axis downstream of FOXO3a and miR34a is a novel integrative mechanism of breast cancer response to chemotherapy. SY and RH are equal contributors in this study.

**#2165** Deletion of survivin sensitizes human hepatocellular carcinoma cells to low dose of doxorubicin and induce apoptosis. Joseph George, Nobuhiko Hayashi, Takashi Saito, Kazuaki Ozaki, Nobuyuki Toshikuni, Mariano F. Zacarias-Fluck, Silvia Casascuberta, Erika Serrano del Pozo, Christopher Fiore, Laia Foradora, Matthew Guenther, Eduardo Romero Sanz, Marta Oteo Vives, Cynthia Tremblay, Martin Montagne, Miguel Ángel Morcillo Alonso, Jonathan R. Whitfield, Pierre Lavigne, Laura Soucek, Peptomyc S.L., Barcelona, Spain; Vall d’Hebron Inst. of Oncology (VHIO), Barcelona, Spain; San Raffaele, Milan, Italy; Universita degli Studi di Parma, Parma, Italy; Birmingham Women’s Hospital, Birmingham, UK.

Background and Aims: Hepatocellular carcinoma (HCC) is one of the most common cancers in the world and patients with advanced HCC face a dismal prognosis due to lack of effective therapy. Survivin, a member of the family of inhibitors of apoptosis proteins, is highly upregulated in HCC as well as in experimentally induced intrahepatic tumors. Doxorubicin, the only known chemotherapeutic agent for HCC, is cardiotoxic in addition to several well known side effects. Methods: Survivin gene deletion was established in HepG2, Hep3B, and PLC/PRF/5 human HCC cells using CRISPR/Cas9 system. All the three HCC cells in culture were treated with doxorubicin at various concentrations before and after survivin gene knockout up to 72 hr. TUNEL assay and FACS analysis were performed to demonstrate the induction of apoptosis after doxorubicin treatment. Western blotting was carried out for cleaved fragments of caspase-9 and caspase-3 as well as major apoptotic executioner molecules. Results: Cell viability studies depicted around 20% cell death at 24 h, 50% at 48 h, and 80% at 72 h after treatment with doxorubicin at 1 μM (final concentration in the media). Among the three cell lines studied, Hep3B cells were more susceptible to doxorubicin compared to HepG2 and PLC/PRF/5 cells. After deletion of survivin gene, the dosage of doxorubicin could reduce to five fold (200 nM, final concentration in the media) with the same cytotoxic effect before the knockdown of survivin gene. HCC cells treated with reduced doses of doxorubicin depicted induction of apoptosis that was proved with TUNEL assay and FACS analysis as well as increased levels of cleaved caspases and major apoptotic executioner molecules. Conclusions: Our studies demonstrated that blocking of survivin with effective methods would be a successful approach to treat primary hepatic tumors with low and safe doses of doxorubicin and other anticancer agents.

**#2166** Arsenic trioxide targets BCL6 oncprotein for degradation in BCL6-dependent diffuse large B-cell lymphoma. Lok Man Yue, David Hau Wing Chau, Wenyeng Piao, (Eric) Wai Choi Tse, Yok Lam Kwong. The University of Hong Kong, Hong Kong, Hong Kong.

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma throughout the world. B-cell lymphoma 6 (BCL6) overexpression is frequently observed in DLBCL. Several recent studies have supported that BCL6 is a critical pathogenic oncprotein in DLBCL. BCL6 represses various downstream genes, including ATR, TP53 and CDKN1A, thereby impairing DNA repair leading to derangement of cellular proliferation. Most importantly, a specific small molecule inhibitor targeting BCL6 successfully suppressed growth of BCL6-dependent DLBCL cell lines and primary human DLBCL cells, further suggesting that BCL6 plays an important pathogenetic role. Arsenic trioxide (As2O3) has been reported to target various oncogenic proteins, including PML-RARA in acute promyelocytic leukemia, cyclin D1 in mantle cell lymphoma, NPMc+ in acute myeloid leukemia and NPM-ALK in anaplastic large cell lymphoma, for degradation through ubiquitin-proteasome pathway. In this study we investigated the effects of As2O3 on BCL6 in DLBCL. As2O3 was found to inhibit cell proliferation and induce cell death via apoptosis in DLBCL cells. More interestingly, BCL6-dependent DLBCL cells were found to show higher sensitivity towards As2O3-induced cytotoxicity. BCL6 was found to be degraded by As2O3 at posttranslational level through the ubiquitin-proteasome pathway. Such degradation led to an upregulation of several downstream targets of BCL6, including PRDM1, CD44 and CD99. Moreover, As2O3 synergized with cisplatin to inhibit cell proliferation and enhance apoptosis in BCL6-dependent DLBCL cell lines. Concomitant treatment with As2O3 and cisplatin further enhanced the phosphorylation of Chk1 and γH2AX in these cells. In conclusion, our data suggest that As2O3 is a potential therapeutic agent for the treatment of BCL6-dependent DLBCL. On top of that, new combination therapies can be developed to expand the therapeutic spectrum of As2O3 to other neoplasms.

**#2167** Preclinical validation of an Omomyc cell-penetrating peptide as a viable anti-Myc therapy. Marie-eye Beaulieu, Toni Jautset, Daniel Massi-Valls, Peter Rahi, Sandra Martinez-Martin, Loika Maltais, Jonathan R. Whitfield, Pierre Lavigne, Laura Soucek, 1Peptomyc S.L., Barcelona, Spain; 2Vall d’Hebron Inst. of Oncology (VHIO), Barcelona, Spain; 3Syros Pharmaceuticals, Cambridge, MA; 4University of Sherbrooke, Quebec, Canada; 5Centre de Investigaciones Energeticas, Medioambientales y Tecnologicas (CIEMAT), Madrid, Spain.

Deregulation of the MYC oncprotein promotes tumorigenesis in most, if not all, cancers and is often associated with poor prognosis. However, targeting MYC has long been considered impossible based on the assumption that it would cause catastrophic side effects in normal tissues. Despite this general preconceived notion, we showed that MYC inhibition exerts extraordinary therapeutic impact in various genetic mouse models of cancer, and causes only mild, well-tolerated and reversible side effects. For these studies we employed the systemic and conditional expression of a dominant negative of MYC, called Omomyc, which we designed and validated, and that can inhibit MYC transcription function both in vitro and in vivo. To date, Omomyc has only been considered a proof of principle, with any potential clinical application limited to gene therapy. Here we actually show that the 11 kDa Omomyc polypeptide spontaneously transduces into cancer cells, demonstrating unexpected cell-penetrating ability. Once inside the nuclei, the polypeptide effectively blocks MYC binding to its target DNA sites, interfering with MYC transcriptional regulation and halting cell proliferation. Moreover, intranasal (i.n.) administration of the Omomyc polypeptide in mice results in its rapid and persistent distribution to lungs, as well as to other organs (i.e. intestine, liver, kidneys and brain). Importantly, i.n. treatment of mice bearing either Non-Small-Cell-Lung-Cancer (NSCLC) or glioblastoma (GBM) with the Omomyc cell-penetrating peptide (Omomyc<sup>CPP</sup>) significantly reduces tumor burden compared to their control counterparts, suggesting that Omomyc is accompanied by significant reprogramming of the tumor microenvironment and tumor immune response. In summary, our data indicate that this novel generation of polypeptides represents a new opportunity to potentially inhibit MYC pharmacologically in a variety of malignant diseases.
#2168 Nucleic acid clamp-mediated recognition and transcriptional modulation of MYC oncogenes through the stabilization of G-quadruplexes.

Taisen Hao, Tracy A. Brooks. University of Mississippi, University, MS.

G-quadruplexes (G4s) tend to cluster around biologically important regions such as promoters of DNA, 5'-UTR of mRNA and telomeres. Modulating G4s has been explored in these regions using a clamp-based approach for cancer treatment. MYC proto-oncogene is overexpressed in over 80% of tumor types, contributing to deregulated cancer cell proliferation. Silencing MYC expression has been demonstrated to be an effective approach to inhibit tumor growth. Among all the silencing strategies, stabilizing MYC promoter G4 that serves as an on-off switch for the transcription of MYC has been explored by the targeting non-natural molecules. However, the binding specificity of small molecule retarded the drug development process. Exploring novel MYC G4-targeting strategies is necessary to potentiate the pharmacological specificity of MYC G4 modulation. Here, we adopted a nucleic acid clamp based approach to recognize and stabilize the physiologically predominant MYC G4 with high specificity and to downregulate MYC promoter activity. In the current research, we modified and optimized this clamp to allow for flexibility with labeling and monitoring techniques. The original 18 Å polyethylene glycol phosphate linker connecting the 5’ and 3’ regions of clamp A were substituted with thymine bases of varying lengths; 5 thymines were found to be optimal. The binding effects by clamp B and B-g were confirmed. The activity of the optimized clamp A T5 were confirmed by EMSA and ECD; the G4 stabilizing ability of clamp A T5 was demonstrated by DMS footprinting. The cytoxicity of clamp A T5 was examined by MTS assay on HEK-293 and MCF-7 cells. The intracellular localization of clamp A T5 is being determined microscopically with a 6-FAM-labeled clamp A T5 after being transfected into and incubated with HEK-293 and MCF-7 cells for 48 hr. Nuclear localization is being examined by co-staining with DAPI and MYC promoter localization is being determined by clustering with a MYC FISH probe. The modification of the clamp is both cost-effective, and enables the detailed study of intracellular functions. This clamp has potential as both a diagnostic tool to inform the use of MYC G4-targeted small molecules, and as a nontherapeutic.

#2170 Rb1 suppresses prostate cancer metastasis and lineage plasticity underlying castration resistance.

Sheng-Yu Ku, Spencer Rosario, Yangqing Wang, Ping Mu, Mukund Seshadri, Zachary Goodrich, Maxwell Goodrich, Eduardo Cortez Gomez, Jianmin Wang, Bo Xu, Charles L. Sawyers, Leigh Ellis, David G. Goodrich. Roswell Park Cancer Institute, Buffalo, NY; Memorial Sloan Kettering Cancer Center, New York, NY.

Androgen deprivation therapy (ADT) is an effective treatment for metastatic prostate cancer (mPCa), but patients eventually relapse with ADT resistant disease. Well-characterized mechanisms of ADT resistance include AR amplification, intratumoral androgen synthesis, AR splice variants, and growth receptor bypass. All of these mechanisms function to maintain sufficient AR signaling to drive cancer cell growth and survival. Improved ADT like abiraterone acetate (AA) and enzalutamide (Enza) were developed to combat such resistance mechanisms associated with alterations in androgen receptor or androgen metabolism. While AA and Enza extend survival, clinical benefits are short-lived. A new form of resistance is increasingly appreciated in patients relapsing from AA or Enza, histologic transformation of prostate adenocarcinoma (PADC) to neuroendocrine prostate cancer (NEPC) variants. NEPC is lethal and the survival time is less than a year as effective targeted therapy is unavailable. NEPC typically exhibits reduced AR expression, increased expression of neuroendocrine markers, and visceral metastasis in the absence of rising PSA. Of note, NEPC possesses the similar genome rearrangements with adjacent PADC cells, indicating they share clonal origin. Thus, NEPC may arise by histologic transformation of PADC. Underlying mechanisms of histologic transformation are not understood and experimental models are limited, hindering development of effective remedies. Rb1 loss is common in NEPC, but rare in PADC; genetic profiling shows human NEPC exhibit elevated levels of several epigenetic modifiers. We hypothesize that transdifferentiation from PADC to NEPC in the context of Rb1 loss is due to epigenetic alterations and can be reversed or blocked by epigenetic targeted therapy. We have established several genetically engineered mouse models (GEMMs) to test the role of Rb1, and we find Rb1 loss causes metastatic progression of PADC initiated by Pten deficiency. This Rb1/Pten deficient (DKO) PADC exhibits expression markers for both PADC and NEPC as seen in human patients. Yet, these tumors are sensitive to ADT but relapse with low AR expression and acquired Trp53 mutations. RNA profiling demonstrates the phenotype of DKO tumors is similar to human NEPC. While castration and metastatic PADC is accompanied by increased expression of epigenetic reprogramming factors like Sox2 and Ezh2. Clinically relevant Ezh2 inhibitors GSK126 and EPZ66438 can restore Enza sensitivity by reversing neuroendocrine transformation. This finding has been genetically validated using short-hairpin RNA (shRNA) in vitro. These results uncover genetic mutations driving prostate cancer lineage plasticity and suggest an epigenetic approach for extending the clinical benefits of ADT.

#2171 Multi-CDK inhibition efficiently suppresses AR function and cell growth in prostate cancer.

Brian W. Simons, Maria Ybanez, Emmanuel S. Antonarakis, Jun Luo, Barry D. Nelkin. Johns Hopkins Univ. School of Medicine, Baltimore, MD.

Metastatic castration resistant prostate cancer (CRPC) continues to result in over 26,000 deaths per year in the United States. For 70 years, therapy for advanced prostate cancer has relied on AR-directed therapies. Unfortunately, even with the second generation androgen deprivation compounds, treated cancers commonly progress to castration resistance and lethality. This progression to castration resistance marks the central therapeutic roadblock for advanced prostate cancer. Importantly, in the vast majority of CRPC cases, the tumor still depends on AR signaling, but this signaling becomes androgen independent. The most common mechanisms, including mutation, protein truncation, and alternative splicing, affect the C-terminal domain, suggesting that antagonism of AR function via the N-terminal domain may be a promising target for CRPC therapy. Several groups have shown that CDKs 1, 5 and 9 activate AR by phosphorylation of the N-terminal domain.
domain. Here, we show that the multi-CDK inhibitor ronidocib (BAY1000394) inhibits activation of AR in a panel of prostate cancer cell lines, including several cell lines which exhibit androgen-independent AR signaling via AR-V7 alternative splicing. In vivo, ronidocib inhibited growth of prostate cancer xenografts. Since AR inhibition in prostate cancer has been shown to induce compensatory PI3K signaling, we compared xenografts with a combination of ronidocib and the PI3K inhibitor copanlisib (BAY 80-6946). This combination was especially effective, and was well tolerated by the mice. Our results suggest that a combination of a multi-CDK inhibitor and a PI3K inhibitor may be promising for CRPC therapy.

**#2172 ON 123300, an orally administered novel CDK4/6 + ARK5 inhibitor, exhibits potent antitumor activity in vivo: comparative studies with Palbociclib.**

Shradhita Patel,1 Priya Pancholi,1 Tanvi Visal,1 Amruta Samant,1 Dhyanwar Kansara,1 V. J. Rajadhayaksha,2 Benjamin S. Hoffman,3 Manoj Maniar,3 Vikas Sehdev1,1. Long Island Univ., Brooklyn, NY; 2Onconova Therapeutics Inc., Newtown, PA.

Background: The overexpression of cyclin-dependent enzymes 4/6 (CDK4/6) is known to cause cell cycle dysregulation in certain cancer types, making these cell cycle kinase attractive targets for pharmacological inhibition. The effectiveness of first-generation non-selective cyclin-dependent kinases, such as roscovitine and flavopiridol, was hampered by toxicities, leading to the development of second-generation compounds like IBRANCE®/Palbociclib that specifically inhibit CDK4 and 6. ON 123300 is a third-generation potent CDK4/6 inhibitor that also inhibits ARK5 with low nanomolar potency and has the potential to improve upon second-generation compounds. Previous studies have demonstrated the antitumor activity of single-agent ON 123300 in various pre-clinical cancer models of MM and leukemia. In this study, we investigated the comparative therapeutic potential of ON 123300 as an oral anti-cancer agent and a second-generation inhibitor, Palbociclib, in xenografted Rb+ve mouse models. Methods: MDA-MB-435S xenografted mice were treated once a day for 21 days with ON 123300 (125mg/kg) or Palbociclib (125mg/kg). Tumor volumes were measured and peripheral blood was gathered to evaluate the effect on hematological parameters. Separately, Western blot analyses were performed to determine the effect of CDK4/6 inhibition on p-Rb following intra-tumoral treatment with ON 123300 (2.5µM) or Palbociclib (2.5µM). Results: ON 123300 and Palbociclib reduced tumor growth with an equivalent magnitude during the 21-day treatment period, suggesting that the two compounds were equally effective in this model. Both compounds decreased Rb and platelet counts, however Palbociclib had a more prominent and statistically significant (P<0.05) inhibitory effect on neutrophil counts when compared to ON 123300 (30.70 ± 3.55 vs. 45.10 ± 2.04). Western blot analysis of tumor tissues demonstrated equivalent effects on p-Rb for both compounds. Conclusions: Xenograft data indicates that a third-generation CDK4/6 inhibitor, ON 123300, is as effective as Palbociclib in an Rb+ve xenograft model. Moreover, this study also suggests that ON 123300 may have the added advantage of reduced neutropenia compared to Palbociclib. Prior preclinical data suggest that ON 123300 may be efficacious in Rb-ve tumors, where second-generation compounds have diminished single-agent activity, and our ongoing studies are aimed at further characterizing the in vivo activity of ON 123300 in this setting.

**#2173 Targeting CDK1 and MEK/ERK overcome mutant BRAF-mediated apoptosis resistance in human colorectal cancer cells.**

Hisato Kawakami, Shengbing Huang, Frank A. Sinicrope. Mayo Clinic, Rochester, MN.

BRAFV600E mutation occurs in ~10% of human colorectal cancers (CRC) where it is associated with treatment resistance and poor prognosis. Data from TCGA and a clinical trial cohort identified a distinct subset of BRAF mutant colon cancers with deregulation of the cell cycle and overexpression of CDK1 (Barras D, et al, Clin Cancer Res. 2016). We tested the hypothesis that CDK1 inhibition can enhance apoptosis in BRAFV600E CRC cells. Since BRAF mutant cells show p-ERK activation, combined inhibition of CDK1 and MEK/ERK was evaluated. Using isogenic colon cancer cells, BRAF mutant alleles were shown to confer resistance to the CDK1 inhibitor, R0-3306, in a gene dose-dependent manner that was associated with reduced cleavage of caspase-3 and downstream PARP, and decreased pH2AX (DNA fragmentation marker). Ectopic BRAFV600E or constitutively active MEK mutant also conferred resistance to R0-3306. CDK1 siRNA was shown to increase cobimetinib-induced apoptosis, including annexin V labeling, and increased pH2AX. Furthermore, ERK siRNA or cobimetinib treatment attenuated CDK1 protein expression and increased R0-3306-induced pH2AX. Moreover, treatment with R0-3306 plus cobimetinib significantly enhanced a caspase-dependent apoptosis and markedly reduced colony formation vs either drug alone in two CRC cell lines. Caspase-3 cleavage by R0-3306 + cobimetinib was dependent upon caspase-8. Mechanistically, CDK1 inhibition by R0-3306 suppressed the phosphorylation of prosapase-8 at Ser-387 by shown by R0-3306 withdrawal which restored p-C8-S387 coincident with expression of the mitotic marker, pH3S10. In conclusion, inhibition of CDK1 can significantly augment apoptosis induction by MEK/ERK inhibition in BRAFV600E CRC cells, suggesting a novel therapeutic strategy to overcome mutant BRAF-mediated resistance.

**Apoptosis induction by CDK1 inhibitor (R0-3306) ± MEK/ERK inhibitor cobimetinib.**

### CRC cell lines

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DMSO</th>
<th>R0-3306 (5 µM)</th>
<th>Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT-29</td>
<td>13.4±1.4</td>
<td>32.55±2.93</td>
<td>15.04±0.26</td>
</tr>
<tr>
<td>SW480</td>
<td>26.7±1.2</td>
<td>52.72±3.16</td>
<td>34.35±1.84</td>
</tr>
</tbody>
</table>

**#2174 Inhibition of CDK8 kinase with SEL120-34A allows for a personalized approach in AML.**

Tomasz Rzyszmski,1 Michal Mikula,1 Eliza Zylkiewicz,1 Agnieszka Dreas,1 Katarzyna Wilkik,1 Aniela Golas,1 Katarzyna Wojcik,1 Magdalena Masiczycy,2 Iga Dudzicz,1 Katarzyna Kucwaj,1 Małgorzata Statkie- wiz,1 Krzysztof Goryca,2 Aleksandra Grochowska,2 Aleksandra Cabaj,2 Jerzy Ostrowski,1 Urszula Kukliniska,2 Krzysztof Brzozka,1 Selvita S.A., Krakow, Poland; 3Maria Sklodowska-Curie Memorial Cancer Center, Warsaw, Poland.

Inhibition of oncogenic transcription factors can provide a promising therapeutic strategy. SEL120-34A is a novel inhibitor of Cyclin-dependent kinase 8 (CDK8), which regulates transcription by associating with the Mediator complex. SEL120-34A interacts with the ATP binding site of CDK8 in type I inhibitor fashion and forms several types of interactions, including halogen bonds with the protein’s hinge region and hydrophobic complementarities within its front pocket. Although the full compound was only modestly active in solid tumor cell lines, it repressed phosphorylation of STAT3 Ser726 and could differentially inhibit viability of AML and ALL cell lines in vitro and in vivo, along with other type 1 CDK8 inhibitors. Transcriptomic analysis identified major transcriptional programs altered in responder cell lines, which strongly indicated that apart from repression of survival pathways, CDK8 inhibitors could induce differentiation in cell lines with leukemia stem cell characteristics. Further studies on a large panel of responder and non-responder cell lines identified robust biomarkers which could be used with high confidence for stratification and personalized approach in CDK8-dependent AML cases. Favorable pharmacokinetics, confirmed safety and in vivo efficacy in leukemia models provide the rationale for further clinical development of SEL120-34A.

**#2175 Vietnamese medicinal plant compounds show potent anti-pancreatic cancer activity in vitro.**

Danielle Bond,1 Phuong Thien Thuong,2 Do Thi Ha,3 Nguyen Minh Khoi,2 Judith Weidenhofer,1 Christopher J. Scarlett1.

**#2176 Vietnamese medicinal plants show potent anti-pancreatic cancer activity in vitro.**

**#2177 Vietnamese medicinal plant compounds show potent anti-pancreatic cancer activity in vitro.**

**#2178 Vietnamese medicinal plant compounds show potent anti-pancreatic cancer activity in vitro.**

**#2179 Vietnamese medicinal plant compounds show potent anti-pancreatic cancer activity in vitro.**

**#2180 Vietnamese medicinal plant compounds show potent anti-pancreatic cancer activity in vitro.**

**#2181 Vietnamese medicinal plant compounds show potent anti-pancreatic cancer activity in vitro.**

**#2182 Vietnamese medicinal plant compounds show potent anti-pancreatic cancer activity in vitro.**

**#2183 Vietnamese medicinal plant compounds show potent anti-pancreatic cancer activity in vitro.**

**#2184 Vietnamese medicinal plant compounds show potent anti-pancreatic cancer activity in vitro.**
medicinal plants show significant pancreatic cancer growth inhibition and induce apoptosis in vitro at concentrations that have minimal effect on normal pancreatic cells and therefore show promise as novel anti-pancreatic cancer therapies.

#2176 Thymoquinone synergizes the anticancer properties of cisplatin against head and neck squamous cell carcinoma and protects normal oral epithelial cells. Abdulwahab Noorwali, 1 Omar Alabo, 1 Safia Al-Attas, 1 Fatheya Zahran, 1 Ahmed M. Al-Abd 2 King Abdullah University, Jeddah, Saudi Arabia, 2 National Research Centre of Egypt, Giza, Egypt.

Cisplatin (CDDP) is potent anticancer agent different tumors such as head and neck cancers. Thymoquinone is a natural compound drawing attention as chemotherapeutic and chemomodulator. Herein, we studied the chemomodulatory effect of thymoquinone to CDDP against squamous cell carcinoma cells. CDDP killed head and neck squamous cell carcinoma cells (UMSCC-14C) with IC50's of 5.2±0.2, 2.6±0.2 and 1.6±0.1 µM after treatment for 24h, 48h and 72h, respectively. On the other hand, IC50's of CDDP against normal oral epithelial cells were 36.4±1.1, 16.3±0.6 and 6.7±1.1 µM after exposure for 24h, 48h and 72h, respectively. Thymoquinone alone exerted considerable cytotoxicity against UMSCC-14C cells with IC50's of 9.0±0.2, 6.9±0.1 and 5.0±0.2 µM after treatment for 24h, 48h and 72h, respectively. While significantly weaker killing effect was noticed for thymoquinone against normal oral epithelial cells (OE) with IC50's of 41.1±0.5, 36.5±2.1 and 26.3±1.2 µM after exposure for 24h, 48h and 72h, respectively. Equitoxic combination of thymoquinone and CDDP showed synergistic interaction against UMSCC-14C cells (Combination index of 0.58) and antagonistic interaction against OE cells (Combination index of 1.12). Using annexin-V/PI staining, it was found that thymoquinone alone (5 µM) increased apoptotic cell fraction in UMSCC-14C cells, as early as after only 6 hours, from 1.7±0.1% to 24.4±0.6% compared to 2.7±0.4 apoptosis induced by CDDP (5 µM). Combination of CDDP with thymoquinone further increased total apoptosis in UMSCC-14C to 39.2±1.5% after 6h, prolonged exposure of UMSCC-14C to thymoquinone (5 µM) alone for 24h resulted in 96.7±1.6% total apoptosis which was increased after combination with CDDP to 99.3±1.2% in UMSCC-14C cells. In contrast to UMSCC-14C cells, thymoquinone did not induce any significant apoptosis in OE and even decreased apoptosis induced by CDDP alone from 4.5±0.3% to 3.4±0.2% after combination for 24h. Using western blot analysis, neither thymoquinone nor CDDP managed to increase the expression level of p53 apoptotic protein; while combination of CDDP with thymoquinone significantly increased p53 expression by 4.5 folds. On the other hand, both CDDP and thymoquinone decreased the expression level of the anti-apoptotic protein Bcl-2 to be 60% and 20% of control level, respectively. Combination of both agents further abolished the level of Bcl-2 to be 10% of control level. Caspase-9 expression was similarly induced by 2 folds and 4.5 folds after treatment with CDDP and thymoquinone, respectively. Combination of CDDP and thymoquinone further increased the levels of caspase-9 to be 6 folds original basal expression level. None of these findings could be detected in normal OE cell line. In conclusion, thymoquinone synergized the anticancer properties of CDDP against squamous cell carcinoma cells and protects from its damaging effect against normal epithelial cells.

#2177 The olive bioflavonoids oleuropein selectively induces apoptosis in pancreatic cancer cells in vitro. Chloe D. Goldsmith, 1 Helen Jankowski,1 Dan-chloe Bond, 1 Judith Weidenhofer,1 Costas Stathopoulou,2 Paul Roach,1 Christopher Scarlett1. 1The University of Newcastle, Ourimbah, Australia; 1University of Abertyer, Dundee, United Kingdom.

Pancreatic cancer is a devastating disease with a 5-year survival rate of less than 5%. Resistance to conventional treatment options and toxicity of current chemotherapy agents (gemcitabine) makes pancreatic cancer a target for the development of novel therapeutic agents. Oleuropein is the most abundant bioflavonoid found in olive products; it has anti-atherogenic and anti-inflammatory properties as well as activity against cancers of the breast, colon and prostate. However, there has yet to be any investigation into the effects of oleuropein on pancreatic cancer cells. Consequently, this study aimed to assess the anti-pancreatic cancer activity of oleuropein in vitro. Two cell lines were investigated: a pancreatic cancer cell line (MiaPaCa-2) and a normal pancreas cell line (HPDE).

The viability of cells after treatment with 0-200µM oleuropein was assessed using the Dojindo CCK-8 viability assay and compared to gemcitabine. The induction of apoptosis was measured by way of caspase 3/7 activation, using a MUSE flow cell analyser, and expression of Bcl-2, Bax and Bcl using Western blot. Cell cycle analysis was conducted using the MUSE flow cell analyser. RNA expression was assessed using Affymetrix GeneChip® Whole Transcript (WT) expression arrays.

The IC50 values for oleuropein against MiaPaCa-2 cells was 48±14 µM. However, importantly, oleuropein did not decrease the viability of HPDE cells within the treatment range. In comparison, 20 µM of gemcitabine did not show selectivity; it reduced the viability of MiaPaCa-2 cells to 21% and of HPDE cells to 2%. An increase in the expression of caspase 3/7 was seen in MiaPaCa-2 cells when treated with oleuropein but it had no effect on the HPDE cells. Furthermore, when treated with oleuropein, an increase in the expression of genes involved in the NRF-2 (oxidative stress) pathway was observed in Mi- PaCa-2 cells, an effect not observed in HPDE cells. Conclusion Oleuropein selectively induced apoptosis in the pancreatic cancer cells (MiaPaCa-2), appearing non-toxic to normal pancreas cells (HPDE) within the treatment range; this is significant, since gemcitabine was comparatively more toxic to HPDE cells. Furthermore, the link between oleuropein and the NRF-2 pathway in Mi-PaCa-2 cells justifies further study into the mechanisms of action of oleuropein and its potential as a novel therapeutic approach for pancreatic cancer.

#2178 Growth inhibition of the crude extracts of Musa basjoo in human colon carcinoma cells. Harutoshi Matsumoto, 1 Saeko Ando, 1 Katsumi Fukamichi, 1 Mitsuru Futakuchi, 1 Kazunori Kimura, 1 Naoki Yoshimi, 1 Masumi Suizu, 1 Department of Molecular Toxicology, Nagoya City University Graduate School of Medical Sciences and Medical School, Nagoya, Japan; 1Department of Clinical Pharmacy, Nagoya City University Graduate School of Medical Sciences and Medical School, Nagoya, Japan; 2Department of Pathology and Oncology, Graduate School of Medicine and Faculty of Medicine, University of the Ryukyus, Okinawa, Japan.

Musa basjoo (MB) is a tropical evergreen tree growing mainly in subtropical or tropical countries. MB has been used globally as a folk medicine such as antipyretic, diuretic, and hemostatic drugs for centuries but evidence-based biological activities and molecular mechanism of action of MB are unknown. Therefore, in the current study we examined whether the crude extracts of MB exert anticancer activity in HT29 and HCT116 human colon carcinoma cell lines. Dried leaves of MB samples were extracted with acetone or methanol. Crude extracts of MB were then dissolved in dimethylsulfoxide (DMSO) and used for the following experiments. Growth inhibition was determined by colony or MTT assays in these cell lines. Cells were treated with increasing concentrations (12.5 to 200 µg/mL in colony assays, 25 to 400 µg/mL in MTT assays) of acetone/methanol extracts of MB in DMEM/S%FBS. Untreated control cells were treated with DMSO alone. Crude extracts of MB inhibited the growth of cells with IC50 values of 118 µg/mL (acetone extract, HT29), 75 µg/mL (acetone extract, HCT116), >200 µg/mL (methanol extract, HT29), 141 µg/mL (methanol extract, HCT116) in colony assays, and with IC50 values of 157 µg/mL (ac- etone extract, HT29), 73 µg/mL (acetone extract, HCT116), 240 µg/mL (methanol extract, HT29), 248 µg/mL (methanol extract, HCT116) in MTT assays. Acetone extract was used in the flowcytometry and western blot analyses because it showed stronger growth inhibition than methanol extract in both cell lines. Flowcytometry analysis indicated that when HT29 and HCT116 cells were treated with 100 µg/mL acetone extract of MB for 96h, the percentage of cells in G1 increased by 5.4% and this was associated with a concomitant decrease of cells in the S and G2-M phases of the cell cycle. Acetone extract of MB induced sub-G1 fraction in either HT29 or HCT116 cell lines. The results indicate that acetone extract of MB causes carcinoma cells to arrest in the G1 phase. Therefore, we performed western blot analysis to determine whether treatment of carcinoma cells with acetone extract of MB alters cellular levels of the G1 cell cycle control proteins cyclin D1, cdk4 and the cell cycle inhibitor protein p21CIP1. When HT29 and HCT116 cells were treated with 50 and 100 µg/mL acetone extract of MB for 96h, there was a marked decrease in the levels of expression of the cyclin D1 and cdk4 proteins and a marked increase in the levels of expression of the p21CIP1 protein. Thus, a decrease in cyclin D1 may cooperate with the induction of p21CIP1 to arrest cells in G1 and thereby further contribute to MB-induced growth inhibition. Taken together, the crude extracts of MB contain active component(s) that exert growth inhibition of human colon carcinoma cells. The current study is the first systematic examination of the anticancer activity of MB and may provide a novel approach to the chemoprevention and/or chemotherapy of human colon carcinoma.

CANCER CHEMISTRY: Drug Delivery

#2179 Targeted delivery of nanoparticulate cytotoxic chrome c into GL361 glioma cells through the proton-coupled folate transporter. Yurty Kucher-yavvkh, 1 Jescelina Ortiz-Rivera, 1 Michael Inyushin, 1 Luis Cubano, 2 Moraima Morales-Cruz, 2 Alejandra Cruz-Montañez, 2 Kai Griebenow, 2 Lilia Kucher-yavvkh, 1 1Universidad Central del Caribe, Bayamon, PR; 2University Of Puerto Rico, Rio Piedras, PR.
These data suggest that TPM-LEP derivatives with optimized hydrophobicity tate adenocarcinoma (PC3), human breast cancer cell line (BT549) and mouse TPM-LEP conjugates and the corresponding non-covalent physical mixtures of DIPEA/DIC in moderate yields. Comparative antiproliferative assays between methoxybenzene or methyl2-methoxybenzoate and 1,3,5-trioxane, followed by derivatives with optimized hydrophobicity were synthesized by the reaction tris(4-methoxyphenyl)methanol (TPM) derivatives with the expectation to end. In this study, a prodrug strategy has been introduced to optimize the hy- activity of LEP by increasing the cellular uptake and retention is a remedy to this sive salivation, paraesthesia and increased dysuria. Improving the biological result in decreased levels of sex hormones to postcastration or menopausal tion(constant concentration of LEP in the blood) pituitary becomes insensitive to the action of GnRH. This reduces the level of gonadotropin in the blood, resulting in decreased levels of sex hormones to postcastration or menopausal levels. In addition to the usual side effects of this agonist analogs of LH-RH, other reported adverse effects include transient hypertension, dry mouth, exces- sive salivation, paraesthesia and increased dysuria. Improving the biological activity of LEP by increasing the cellular uptake and retention is a remedy to this end. In this study, a prodrug strategy has been introduced to optimize the hydrophobicity of LEP by using an appropriate hydrophobic linker attached to tris(4-methoxyphenyl)methanol (TPM) derivatives with the expectation to improve the cellular uptake. In this regard, several LEP conjugates of TPM derivatives with optimized hydrophobicity were synthesized by the reaction of methoxy benzenes (e.g. anisole, 2-fluoroisobenzyl, 2-methylanisole 1.2-di- methoxybenzene or methyl 2-methoxybenzate and 1.3,5-trioxane, followed by the conjugation with LEP and doxorubicin in the presence of HBTU/ DIPEA/DIC in moderate yields. Comparative antiproliferative assays between TPM-LEP conjugates and the corresponding non-covalent physical mixtures of the TPM derivatives and LEP were performed against human Caucasian pros- tate adenocarcinoma (PC3), human breast cancer cell line (BT549) and mouse pre-adjacent (3T3-L1) cells and indicated moderate to high inhibition of the cell proliferation at a concentration of 5-100 μM after 24-72 h of incubation. These data suggest that TPM-LEP derivatives with optimized hydrophobicity can be used to improve the biological activity of LEP.

#2180 Triphenylmethanol conjugates of leuprolin as anti-cancer prod- racks. Youssef Ahamedibehi, Kalhe Kharia, William Boadi. Tennessee State University, Nashville, TN.

Leuprolin (LEP) is a synthetic analogue of gonadotropin-releasing hormone (GnRH), first was approved by the FDA for treatment of advanced pros- tate cancer and endometriosis in 1985. The initial effect of administration LEP is to stimulate the pituitary secretion of FSH and LH. After a prolonged stimula- tion (constant concentration of LEP in the blood) pituitary becomes insensitive to the action of GnRH. This reduces the level of GnRH, resulting in decreased levels of sex hormones to postcastration or menopausal levels. In addition to the usual side effects of this agonist analogs of LH-RH, other reported adverse effects include transient hypertension, dry mouth, exces- sive salivation, paraesthesia and increased dysuria. Improving the biological activity of LEP by increasing the cellular uptake and retention is a remedy to this end. In this study, a prodrug strategy has been introduced to optimize the hydrophobicity of LEP by using an appropriate hydrophobic linker attached to tris(4-methoxyphenyl)methanol (TPM) derivatives with the expectation to improve the cellular uptake. In this regard, several LEP conjugates of TPM derivatives with optimized hydrophobicity were synthesized by the reaction of methoxy benzenes (e.g. anisole, 2-fluoroisobenzyl, 2-methylanisole 1.2-di- methoxybenzene or methyl 2-methoxybenzate and 1.3,5-trioxane, followed by the conjugation with LEP and doxorubicin in the presence of HBTU/ DIPEA/DIC in moderate yields. Comparative antiproliferative assays between TPM-LEP conjugates and the corresponding non-covalent physical mixtures of the TPM derivatives and LEP were performed against human Caucasian pros- tate adenocarcinoma (PC3), human breast cancer cell line (BT549) and mouse pre-adjacent (3T3-L1) cells and indicated moderate to high inhibition of the cell proliferation at a concentration of 5-100 μM after 24-72 h of incubation. These data suggest that TPM-LEP derivatives with optimized hydrophobicity can be used to improve the biological activity of LEP.

#2181 Antibody-conjugated nanoparticles for targeting metastatic triple- negative breast cancer. Vidhi Khanna, Stephen Kalscheuer, Ameya Kirtane, Jayanth sundara, James R. Ewing, Henry Ford Hospital, Detroit, MI.

Early detection and the availability of new treatments have improved the survival rates of patients presenting with local or regional breast cancer to as high as 99% and 85%, respectively. On the contrary, patients with metastatic disease have a dismal 5-year survival rate of 17%. Thus, there is an urgent need for treatment strategies directed towards metastasis. Our lab has developed antibi- odies (Clone 6 and AM6) capable of recognizing tumor cells that have undergo- gone epithelial-to-mesenchymal transition (EMT), a key step in the generation of circulating tumor cells and metastasis. The goal of the current study was to determine whether these antibodies can activate an anti-cancer drug-loaded polymeric nanoparticles to metastatic triple negative breast cancer cells as a novel therapeutic option. Polymeric PLGA nanoparticles loaded with paclitaxel, a chemotherapeutic agent, were functionalized with the antibodies using thiol-maleimide chemistry. We optimized the conjugation re- action in order to achieve maximal cell uptake of nanoparticles without com-promising antibody binding. In vitro studies were carried out in an MDA-MB- 231 derivative cell line with enhanced lung metastatic potential as well as a melanoma metastatic cell line M12. Clone 6 nanoparticles and AM6 nanoparticles showed significant improvement in cellular uptake as well as retention. A competition experiment confirmed target-mediated uptake of nanoparticles. Cytotoxicity studies showed improved cell killing when using Clone 6 nanoparticles and AM6 nanoparticles. Based on these promising in vitro results, we are currently carrying out in vivo studies in mice. The development of a targeted drug delivery system for the treatment of metastatic triple negative breast cancer can signifi- cantly enhance the survival rate for patients who often have a life-expectancy of less than one year.

#2183 Targeted delivery of hyaluronic acid-labeled chitosan nanopar- ticles against CD44 overexpressed endothelial cell for tumor angiogenesis therapy. Daehee Kim, Seung Soo Lee, Sunalee Jayasundara, James R. Ewing, Henry Ford Hospital, Detroit, MI.

Antiangiogenesis therapy VEGF-VEGF receptors (VEGFRs) axis alone or in combination with other therapeutic agents have demonstrated mixed results, with the majority of reports indicating that glioblastoma multiform (GBM) de- veloped resistance against anti-angiogenesis therapy as well as small molecular receptor tyrosine kinase inhibitors. This result is perhaps not unexpected, be- cause angiogenesis is obviously complex, involving dozen of different growth factors that trigger a cascade of subsequent events. Even if a drug effectively blocks one angiogenic growth factor, such as VEGF, blood vessels may still develop via activating alternative pathways. Yet without a sufficient blood supply, cancerous tumors can’t grow larger than the head of a pin and are unlikely to become lethal. Therefore, tumor vascularization is a critical process that deter- mines tumor growth, progression and metastasis. Thus, tumor vascularization has become an emerging target for new chemotherapeutic drugs. Vascular disrupt- ing agents (VADs) for example, combretastatin (CA4), represent a new class of chemotherapeutic agent that targets the newly formed vasculature in solid tum- ors. Preclinical and early phase trials have demonstrated the promising ther- apeutic benefits of CA4. Nevertheless, the clinical translation of CA4 has been significantly hampered due to its poor systemic bioavailability and the non- specific distribution of CA4 throughout the body when administered intra- venously. Thus, it is reasonable to explore novel formulations of CA4 that overcome the limitations mentioned above. To improve bioavailability com- brestastatin phosphate (CA4-P) has been synthesized. But, CA4-P showed short blood half life. We have engineered dendrimer-based nano-sized CA4 conjugate which demonstrates high water solubility. Preliminary intrave- nous (i.v.) delivery of nano-combretastatin in an orthotopic glioma model demonstrated a necrosis at the core of the tumor leaving a rim of viable tissue. The MRI-determined tissue parameters Ktrans, blood flow (CBF), DWI, ADC map, distribution volume and tumor size indicated the effective- ness of nano-combretastatin treatment.
Cross-disciplinary optimization of nano-drug delivery to ovarian carcinoma and glioma cells. Rupin Singh,1 Rana Falahat,2 Eva Williams,3 Joseph O. Johnson,4 Norma Alcantar,5 Aleksandra Karolak,6 Katarzyna Rejniak,7 Marzena Wiranowska,8 University of South Florida Morsani College of Medicine, Tampa, FL; 9University of South Florida College of Engineering, Tampa, FL; 10H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL.

This study describes the optimization of a targeted drug delivery system (DDS) consisting of fluorescent labeled 0.01 μM paclitaxel-BODIPY564/570 encapsulated in non-ionic surfactant vesicles embedded in a thermosensitive cross-linked chitosan hydrogel. This is a multi-tiered DDS that allows for enhanced stability, sustained and controlled drug delivery to tumor sites, and decreased toxicity to normal tissues. We demonstrated that cancer cells of epithelial origin, such as human ovarian epithelial carcinoma OV2008 and highly migratory mouse glioma G-26, overexpress MUC1, a mucin surface antigen, which effectively enhances the specific targeting capacity of chitosan. Utilizing this DDS we found that OV2008 carcinoma cells had ~2 times higher fluorescence intensity level than normal ovarian epithelial IMC3 cells with a statistical significance at 5 min and 24 h incubation times suggesting that this DDS had a higher affinity for tumor cells. Therefore, we hypothesized that there is a difference in PTX diffusion towards epithelial origin tumor cells when compared to normal cells in both distance/location and time. Diffusion and localization of fluorescent labeled PTX was evaluated in vitro using confocal microscopy. The fluorescence intensity captured in the images was quantified with ImageJ software in OV2008 carcinoma cells and compared to IMC3 normal cells at time intervals of 5 min, 1 h, 24 h, 48 h, and 72 h. The fluorescence intensity image data was analyzed in multiple radial line segments separated into three different zones of 24 μm each to represent multiple diffusion distances. Our results showed that fluorescence intensity levels in the zones around the IMC3 normal cells at 5 min and 1 h were significantly higher than in the OV2008 carcinoma cells correlating reciprocally with our finding of intracellular fluorescence intensity in these two cell lines. Therefore, the data evaluating fluorescence levels in the radial zones outside the cells indicated that the migrating DDS-PTX had already been taken up by the tumor cells. Furthermore, the normal cells which showed significantly lower intracellular levels of fluorescence had higher levels of fluorescence in the measured radial zones outside the cells. This PTX fluorescence data from in vitro studies is currently being used for computational modeling in the in vivo intracerebral model of G-26 glioma. It will be integrated with mathematical model simulations that describe the pharmacokinetic and pharmacodynamic (PK/PD) properties of this drug delivery system for the evaluation of its efficacy and to optimize drug delivery in vivo. Computational modelling is being done in Matlab using the treatment scenario of the post-surgical late-stage glioma. The simulation studies will be used to determine optimal drug concentrations, chitosan density, and localization.

Peptide-cleavable maytansimid (ADCs) induce high bystander killing leading to improved anti-tumor activity in vivo. Wayne C. Widdison, Juliet A. Costopoulos, Jose F. Ponte, Leanne Lanieri, Yelena Kovtun, Ravi V. Chari. GenInc., Waltham, MA.

Antibodies targeting surface antigens on cancer cells typically have progressively lower access to tumor cells that are further removed from blood vessels. Also, the antibody will not bind to cells in the tumor mass that do not express antigen, including stromal cells of the tumor, many of which reportedly aid in the survival or metastasis of cancer cells. ADCs can bind to antigen positive cancer cells, after which they are internalized and catabolized to release one or more cytotoxic metabolites that can kill the targeted cell. Metabolites that are membrane permeable may also diffuse into and kill neighboring cells, often called bystander cells, that would normally be less accessible. The goal of this work was to design ADCs that would have increased bystander activity, which could result in greater killing of cancer cells and stromal cells in the tumor environment. We have prepared a new type of peptide-cleavable immotile ADC (P-ADC) that efficiently released cytotoxic maytansimid metabolites upon cleavage of the peptide linker, followed by immolation. Several P-ADCs were prepared that release metabolites having different degrees of hydrophobicity. As the hydrophobicity of the metabolite increased, the P-ADC’s bystander activity also increased. The lead PDI-ADC generally displayed a similar degree of in vitro cytotoxicity as maytansimid ADCs that utilize disulfide linkers, however the PDI-ADC induced significantly more bystander killing. In mice bearing large tumor xenografts (250 mm3) or tumor xenografts that express the target antigen heterogeneously, PDI-ADCs were found to be more efficacious than maytansimid ADCs that use disulfide linkers, as well as our recently reported peptide-para-anilino maytansimid ADCs. The nature of the amino acid residues in the peptide linker of the PDI-ADC was also altered so that the tolerability of the ADCs in mice could be increased without impeding efficacy. In conclusion, we have developed a promising new type of maytansimid ADC, one that provides a high degree of bystander killing, improved activity in homogeneous and heterogeneous tumor models in vivo, and has a different mechanism of metabolite release than current maytansimid based ADCs.

Pro-nanodrug with intratumoral reconstitution and intracellular delivery beyond therapy-resistance molecular pumps. Rong Xu,1 Mauro Ferracci,2 Haifa Shen,3 Department of Pharmacology, School of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China; 4Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX.

Tumor metastases to distant organs pose a serious challenge to cancer treatment. The metastatic tumor lesions are less accessible to cancer drugs and the malignant cells often quickly develop therapy resistance. To address both biological and drug transport concerns in cancer treatment, we have developed a novel composite pro-nanodrug to provide sustained intra-tumoral assembly of nanotherapeutics, and tested its anti-cancer efficacy in murine models of breast cancer metastases to the lung and liver. Specifically, large payloads of polymeric doxorubicin (pDox) pro-drug were packaged as single molecules into a porous silicon microparticles that can be enriched in tumor tissue in the forms of vascular and cellular depots. Once inside the tumor, the pDox molecules self-assemble into nanoparticles where they are effectively internalized by tumor cells. The nanoparticles are transported through the vesicular system, and doxorubicin is cleaved from the polymer and released in the immediate proximity of the nucleus beyond the reach of the drug efflux pumps. In vivo efficacy studies demonstrated that the pro-nanodrug was effective in treating metastatic tumors with or without expression of the multi-drug resistant genes.

Targeted delivery of pro-apoptotic peptide using ELP-based carrier for effective cancer therapy in various cancer model. Sarangthem Vijaya, Rupin Singh,1 Rana Falahat,2 Eva Williams,2 Joanne Goldsmith,2 Marzenna Wiranowska,1.

Therapeutic peptides classified as biopharmaceuticals have gained attention as a new generation of cancer therapeutics with enhanced efficiency and diminished toxicity. They often fail to have sufficient effects due to their unsatisfactory pharmacokinetic profiles, which include poor bioavailability, low stability, short half-life, and poor penetration across the biological membrane. Elastin-like polypeptide (ELP)-based drug delivery has been utilized for various applications including cancer therapies for many years. Genetic incorporation of internalization ligands and cell targeting peptides along with ELP polymer enhanced tumor drug accumulation and retention time as well as stability and activities of the drug conjugates. Herein, we described a unique delivery system comprised of genetically engineered ELP incorporated with multiple copies of IL-4 receptor targeting peptide (AP1) periodically and proapoptotic peptide (KLAKLAK), referred to as AP1-ELP-KLAK. It triggered thermal responsive self-assembly into a sphere-nanoparticle-like structure at physiological body temperature and stabilized its helical conformation, which is critical for its membrane disrupting activities. Increased IL-4 receptor specific cellular internalization was associated with the enhanced cytotoxic effect of (KLAKLAK)2 peptide. Additionally, multivalent presentation of targeting ligands by AP1-ELP-KLAK significantly enhanced intratumoral localization and prolonged the retention time compared to ELP-KLAK, non-targeted control. Systemic administration of AP1-ELP-KLAK significantly inhibited tumor growth by provoking cell apoptosis in MDA-MB231 breast cancer and B16F10 melanoma tumor xenograft models without any specific organ toxicity. Thus, our newly designed AP1-ELP-KLAK polymer nanoparticle is a promising candidate for effective cancer therapy and due to the simple preparative procedures of ELPs, this platform can be used as a good carrier for tumor-specific delivery of other therapeutics.

Adhesion and entry of gold nanoparticles into breast cancer cells: Nanoparticles for localized delivery/hyperthermia. Vanessa O. Uzonna,1 John David Obayemi,2 Rupin Singh,1 Rana Falahat,2 Eva Williams,2 Joanne Goldsmith,2 Marzenna Wiranowska,1.

The specific releases of breast cancer cells is important for the early detection and treatment of cancer cells/tumor tissue, hence the drive for the development of nanoparticles. This paper presents results of an atomic force microscopy study on the adhesion between triple negative breast cancer cells (MDA-MB-231 and MDA-MB-468) and biosynthesized gold nanoparticles (GNP) conjugated
with luteinizing hormone releasing hormone (LHRH) which is known to target LHRH receptors that are over-expressed on the surfaces of breast cancer cells. It also estimates time scales for Gold nanoparticle entry into cells by means of an analytical model. The adhesion forces between the LHRH-conjugated gold nanoparticles and the breast cancer cells are found to be about 4-5 times greater than those of their normal breast cell counterparts. LHRH-conjugated gold nanoparticles. The increase in the adhesion of LHRH to breast cancer cells is shown to be associated with an increase in the LHRH receptor density, which is revealed using confocal microscopy. The implications of the results are discussed for localized treatment of breast cancer.

#2190 Theranostic nanoscale metal-organic framework-mediated rapid multiplexed microRNA detection and highly therapeutic efficacy in living oral cancer cells. Pei Yu,1 Zehang Zhuang,1 Guihua Qu,2 Haolin Chen,3 Yuying Zhao,1 Jinxiao Chen,1 Xiqiang Liu,1 2 Guanghua School of Stomatatology, Hospital of Stomatology, Sun Yat-sen University, Guangzhou, China; 3 College of Pharmacology, Southern Medical University, Guangzhou, China.

Oral cancer represents the sixth most frequent cancer in the world, characterized by high poor prognosis and low survival rate due to local relapse and metastasis. MicroRNAs (miRNAs) have been discovered as diagnostic biomarkers in patients with oral cancer. However, one of the major challenges of miRNAs-based theranostical approach is to achieve efficient therapy and safety delivery. Thus, a reliable strategy can be used to detect miRNAs expression levels in living cancer cells is urgently needed. Herein, a novel sensing platform based on nanoscale metal-organic framework (NMOF) conjugated with the fluorophore-labeled single-stranded DNA probe (p-DNA) was fabricated to monitor and inhibit multiplexed miRNAs expression in living oral cancer cells. The flow cytometry and confocal laser scanning microscopy (CLSM) were carried out to evaluate the expression levels of miRNAs in oral cancer cells. The miRNA inhibition efficiency was examined by RT-PCR. The effects of the complexes on tumor cell invasion and migration were assessed by transwell assay and wound healing. The flow cytometry, colony formation assay and CCK8 were used to assess cell apoptosis and proliferation. The results showed that NMOF works as fluorescence quencher of the labeled p-DNA. In presence of the target miRNA (including miR-7, miR-21, and miR-155), p-DNA was able to rapidly hybridize and release from the NMOF leading to the recovery of fluorescence in living cells, which can be detected in real-time. Moreover, RT-PCR results demonstrated that the multiplexed miRNAs could be effectively downregulated after treatment of p-DNA@NMOF, resulting in the simultaneous suppression of oral cancer cell proliferation, anti-apoptosis, migration and invasion in vitro. In summary, our study suggests that p-DNA@NMOF possess great potential for combining early diagnosis and gene therapy of oral cancer.

#2191 Multiple imaging reporter labeled acid-degradable dextran nano- polymer as a COX-2 siRNA carrier for COX-2 specific downregulation. Zhao-hang Chen, Balaji Krishnamachary, Marie-France Penet, Zaver M. Bhujwalla. Johns Hopkins University, SOM, Baltimore, MD.

Cyclooxygenase-2 (COX-2) as a target has attracted interest for multiple degenerative diseases and cancers. COX-2 siRNA provides specific and effective down-regulation of COX-2. However, because artificial cationic polymers can induce proinflammatory agents that increase COX-2 expression, there are no reports about the use of artificial cationic polymers as siRNA carrier to downregulate COX-2 in cancer cells. Here we developed a method to efficiently synthesize a multiple imaging reporter labeled biodegradable dextran to use as an efficient cationic polymer carrier for COX-2 siRNA delivery. Amine function groups were conjugated to the dextran platform through acetal bonds. Acetal bonds were broken at acidic conditions that occur in cancer and endocytosis compartments. Rhodamine was labeled to the amine groups to detect degradation and removal of these groups from the cell, while the dextran scaffold was labeled with Cy5.5. The rapid cleavage and release of amine groups minimized the proinflammatory side-effects of the positively charged amine groups. Cytotoxicity assay of Cy5.5 and rhodamine in different pH buffer indicated that the amine group was cleaved at pH 5.5 buffer but was stable in pH 7.4 buffer. Fluorescence imaging showed that the dextran siRNA nanoplex entered the cells through endocytosis that provided acidic conditions for the breaking of the amine group. For the first time, the intracellular breaking of acetal bonds was clearly visualized by multiple imaging reporters. Because the rapid cleavage and release of amine groups minimized the proinflammatory side-effects, quantitatively reversed transcription polymerase chain reaction (qRT-PCR) assay indicated that this dextran COX-2 siRNA nanoplex could downregulate COX-2 expression efficiently. Prostaglandin E2 level decreased significantly with COX-2 siRNA/dextran treatment. In vivo imaging demonstrated that the COX-2 siRNA/dextran nanoplex accumulated in MDA-MB-231 tumors. QRT-PCR and western blot assays of COX-2 levels in tumor tissue demonstrated that this nanoplex significantly downregulated COX-2 expression in vivo efficiently and within 24h. This dextran nanopolymer can be used as a safe, reproducible, and biocompatible siRNA carrier to effectively reduce COX-2 expression in cancer cells and tumors.

#2192 DFP-10825 IP delivery provides a new effective treatment option to peritoneal disseminated cancers. Cheng Jin,1 Kenzo Iizuka,1 Kokoro Eshima,1 Masakazu Fukushima,1 Tatsuhiro Ishida,1 Cheng-Long Huang,2 Hiromi Wada,3 Kiyoshi Eshima,1 1 Delta-Flu Pharma Inc., Tokushima, Japan; 2Tokushima University, Tokushima, Japan; 3Kyoto University, Kyoto, Japan.

Objective: Peritoneal disseminated ovarian and pancreatic cancers are the most difficult to be treated with conventional cytotoxic or molecular targeted drugs. The treatment option is very limited although an intraperitoneal (IP) paclitaxel has been available and shown to improve a prognosis in patients. Therefore, it is urgent to develop a new IP chemotherapeutic drug regulating the fast DNA synthesis in peritoneal disseminated tumors originated commonly from the ovary, pancreas and stomach. We have developed a unique RNAi molecule consisting of shRNA (55-mer) against TS and a cationic liposome (DFP-10825) and tested its anti-tumor activity and PK profile in peritoneal disseminated ascitic tumor models. Methods: We developed luciferase gene-transfected ovarian cancer (SKOV3-luc) and pancreatic cancer (PANC-1-luc) models in mice. After IP injection of 5x10⁶ cells, DFP-10825 containing 20 μg TS shRNA (20 mg/mouse) was administered in an IP route (q3d x 4) to the tumor-bearing mice. In combination therapy, paclitaxel (10 mg/kg) was also IP administered to SKOV3-luc mice to which the treatment was performed in the same schedule. The anti-tumor effect was assessed by measuring luciferase activity and tumor volume. Furthermore, the TS expression level in both ascetic tumor cells and solid tumors was measured by conventional RT-PCR. For PK study with DFP-10825 (especially TS shRNA), total RNAs were isolated at various time points from ascetic tumor cells and disseminated SKOV3-luc solid tumor models treated with DFP-10825 and TS shRNA levels were determined by Stem-loop RT-PCR. Results: IP DFP-10825 delivery significantly suppressed the growth of ascetic SKOV3-luc and PANC-1-luc tumor cells and extended the survival time of these tumor-bearing mice compared with that of control group. Combination with the IP paclitaxel augmented the efficacy of DFP-10825. After the IP administration, TS shRNA levels derived from DFP-10825 in ascitic fluid were maintained at nM range (0.7 - 4.3-nM) across 24 hours but not detected below 5 pm in the plasma, suggesting that TS shRNA be relatively stable in the peritoneal cavity to be able to exert its anti-tumor activity but not in blood. Also, TS expression (TS mRNA) in ascetic tumor cells was significantly suppressed, supporting the notion that the anti-tumor activity and host survival benefit by DFP-10825 in tumor-bearing mice are through MOA to knock down the TS level in tumors specifically. Conclusion: IP administration of newly developed DFP-10825, the TS shRNA conjugated with cationic liposome is localized stably in the peritoneal cavity and provides a new effective treatment option to the intractable peritoneal disseminated ovarian and pancreatic cancers without any systemic adverse events.

#2193 Structural modification of the chemotherapeutic anandamide: Designing anti-cancer agents and investigating their COX-2 metabolic products. Andrew Norris,3 Eman Soliman,3 Rukiyah Van Dross,1 Colin Burns,1 1 East Carolina University, Greenville, NC; 3Faculty of Pharmacy, Zagazig University, Zagazig, Egypt.

Many epithelial cancers have been shown to overexpress the enzyme cyclooxygenase-2 (COX-2), an enzyme responsible for metabolizing anandamide (AEA) to prostamides. AEA has demonstrated cytotoxicity in COX-2 overexpressing cancers via its metabolism to novel J-series prostamides, namely 15d-PGJ₂. Fatty acid amide hydrolase (FAAH) degrades AEA into arachidonic acid and ethanolamine (EA), limiting the cytotoxic capability of AEA. Cell lines with high FAAH expression have demonstrated resistance to AEA. By understanding the metabolic characteristics of FAAH, we can design AEA analogs which circumvent FAAH breakdown. To examine the effects of altering polarity, steric bulk, and functional groups on AEA-mediated cytotoxicity, we investigated known AEA derivatives which possess these properties. Arvanil, Arachidonoyl Diethanolamine (ADA), Arachidonoyl Serinol (AS), and R1-methanandamide (m-AEA) add steric bulk to the molecule via aromatic rings, extra EA arms and additional alcohol/methyl functional groups respectively. Arachidonoyl glycine (NAGly) substitutes the terminal EA alcohol with a carboxylic acid increasing additional alcohol/methyl functional groups respectively. Arachidonoylglycine (m-AEA) add steric bulk to the molecule via aromatic rings, extra EA arms and additional alcohol/methyl functional groups respectively. Arachidonoylglycine (m-AEA) add steric bulk to the molecule via aromatic rings, extra EA arms and additional alcohol/methyl functional groups respectively. Arachidonoylglycine (m-AEA) add steric bulk to the molecule via aromatic rings, extra EA arms and additional alcohol/methyl functional groups respectively. Arachidonoylglycine (m-AEA) add steric bulk to the molecule via aromatic rings, extra EA arms and additional alcohol/methyl functional groups respectively.
structural modifications improve AEA-mediated cytotoxicity. To determine this, JWF2 tumorigenic keratinocytes were exposed to differing concentrations of the AEA analogs for 24 hours and cell viability was measured by conducting MTS assays. Arvanil demonstrated a 90% reduction in cell viability, NAGly demonstrated a 70% reduction in cell viability, and m-AEA demonstrated a 100% reduction in cell viability. Against the OVCAR-3 and SKOV-3 cell lines, Arvanil demonstrated a 60% reduction in cell viability and Arvanil demonstrated a 100% reduction in cell viability in patient-derived primary melanoma. These findings suggest that modulation and substitue to the core structure of AEA will result in decreased susceptibility to FAAH degradation and enhanced antineoplastic activity.

#2194 Synergistic cytotoxicity of targeted liposomes containing doxorubicin and C6-ceramide against nucleolin-overexpressing ovarian cancer cells is supported by the downregulation of the Akt pathway. Ana Filipa Cruz, 1 Nuno André Fonseca, 1 Susana F. Sampaio, 1 Vera Moura, 1 Ramiro D. Almeida, 1 Sérgio S. Moreira, 1 1CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal; 2CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal; 3TREAT U, SA, Coimbra, Portugal.

The acknowledgment that cancer stem cells (CSC) may originate from non-stem normal cells, overturning an EMT-mediated process has turned these distinct cell subpopulations into two relevant therapeutic targets. Moreover, the PI3K/AKT/mTOR pathway is essential to CSC proliferation and survival and it is often over-activated in ovarian cancer. One of the strategies to target these different tumor cell populations, relies on the combination of conventional chemotherapy drugs (as tumor debulking agents, targeting non-stem cancer cells) with sphingolipids targeting CSC (at the level of PI3K/Akt/mTOR). Nucleolin (overexpression has been demonstrated on the surface of both breast CSC and non-stem breast cancer cells (Fonseca NA, Biomaterials 2015) and endothelial cells from tumor blood vessels. The pH-sensitive lipid-based nanoparticle, functionalized with the nucleolin-binding F3 peptide, was recently modified to encapsulate a synergistic combination of a sphingolipid (C6-ceramide) and doxorubicin (DXR). Following the promising results in breast cancer, the aim of this work was to assess the therapeutic potential of this strategy against nucleolin-overexpressing ovarian cancer cell lines, as well as the underlying mechanism of action at the molecular level. A 12.9-fold increase of association of fluorescently-labelled F3 peptide-targeted liposomes ([F3][L], relative to the non-targeted counterparts, into (bulk) ovarian cancer cell lines (SKOV-3, OVCAR-3 and TOV-112D) was observed by flow cytometry. Importantly, the marked pattern of association by prior CSC-enriched population was demonstrated by a 60% reduction in cell viability and A431 tumorigenic keratinocytes. NAGly demonstrated a 60% reduction in cell viability and Arvanil demonstrated a 100% reduction in cell viability in patient-derived primary melanoma. These findings suggest that modulation and substitue to the core structure of AEA will result in decreased susceptibility to FAAH degradation and enhanced antineoplastic activity.

Oxazaphosphorines (Oxaza) represented by cyclophosphamide (CPA) and ifosfamide (IFO) are still the corner stone of several polychemotherapy protocols as they are widely indicated in the treatment of numerous cancer from soft tissue sarcomas to lymphomas and immune-related diseases. However, Oxaza are prodrugs requiring cytochrome (CYP) P450 bioactivation responsible of their toxicities. In particular, the release of the active metabolite 4-OH-IFO (10%), which generates the active nitrogen mustard displaying DNA cross-links. Associated toxicities of IFO due to acrolein, (urotoxicity) and to chloroacetalddehyde (neuro and nephrotoxicity) have been described. Thus, increasing IFO therapeutic index could be of major interest. To circumvent these toxicities, our team has designed new pre-activated IFO analogs to avoid CYP bioactivation (Skarkhe et al J Med Chem 2015). Among these analogs some have the ability to self-assemble as nanoassemblies (NAS), the others can be encapsulated within nano-lipid capsules (NLCs). These new drug delivery systems (DDS) can take advantage of passive targeting, as stealthiness of these DDS can be provided by PEGylation by using Cholesterol-polyethylene glycol or the use of surfactant. These DDS can also be functionalized by appropriate monoclonal antibodies leading to multi stage DDS with active targeting properties. Regarding CPA, it has been shown and described in literature that low doses of CPA enhance the immunity by promoting differentiation of CD4+ cells toward Th1. As IFO is isomeric form of CPA, it was assumed that IFO could also have such properties. Studies on immunocompetent MCA205 mouse model, an immunogenic fibrosarcoma mouse model, demonstrated a dose-dependent immunomodulation of IFO towards a modulation of the secretion of IFNγ, IL-17A and IL-6 cytokines. The ongoing experiments on mouse model depicted in CD4+ T cells and CD8+ T cells show the antitumor efficacy of IFO 150mg/kg on these immune cells in tumor regression. Both strategies could lead to the design of nano-immuno-conjugates (NICs) which could benefit of the immunomodulatory effects of X-Oxaza combined to their antiproliferative properties targeted through immune checkpoint antibodies. These new functionalized DDS may provide a useful strategy to give specificity to active drugs used for many years in clinical practice. Both DDS could be coated with mAbs which could lead to a new family of DDS aiming to combine antiproliferative and immunomodulatory properties for a dual antitumoral action.

#2196 SDF-1/CXCR4 axis-mediated tumor-tropism of monocyte membrane-coated nanoparticles. Yi-Nan Li, Chien-Wen Chang, Chi-Shiu Chang, National Tsing Hua University, Hsinchu City, Taiwan.

Tumor-associated macrophages are one of the most abundant infiltrating cells in solid tumors. These cells are originated from the bone marrow and circulating in peripheral blood. Through the specific chemoattractant released by the tumor cells or stromal cells, bone marrow-derived monocytes (BMDMs) are able to accumulate at the tumor site by their corresponding receptors. In this study, we utilized the characteristics of tumor-tropism of BMDMs on a nanoparticle-based drug delivery system. We hypothesized that the cellular membrane of BMDMs can provide nanoparticle protection from the mononuclear phagocyte system and the chemokine receptor on the membrane can enhance the tumor-tropism of the nanoparticles. To prove the hypothesis, the cellular membrane-coated nanoparticles were prepared and the tumor-tropism of the membrane-coated nanoparticles was assessed. We also found that the expression of chemokine receptor CXCR4 on the bone BMDMs can be induced following the stimulation of tumor conditioned medium. The tumor targeting effect of the monocyte membrane-coated nanoparticles is diminished against SDF-1 knockdown tumor cells. These results indicate that the SDF-1/CXCR4 axis is contributed to the tumor-tropism of the monocyte membrane-coated nanoparticles toward astrocytoma cell line, ALT1S1C1, and this effect can be boosted by the stimulation of tumor conditioned medium. In conclusion, we established a nanoparticle-based drug delivery platform against brain tumor with the cellular membrane coating technology and maximized the effect of tumor-targeting by tumor conditioned medium stimulation.

#2197 Characterization of immunoliposomes as delivery vehicles for the Rac inhibitor Ehop-016 in HER2 TYPE breast cancer. Michael J. Rivera-Robles, 1 Pablo E. Vivas-Mejia, 1 Linette Castillo-Pichardo, 2 Cornelis Vlaar, 3 Eluid Hernandez, 4 Surangani Dharmawardhane 4, 5 University of Puerto Rico School of Medicine, San Juan, PR; 6University of Puerto Rico, San Juan, PR; 7Universidad Central del Caribe, Bayamón, PR; 8University of Puerto Rico School of Pharmacy, San Juan, PR; 9Surangani Dharmawardhane, San Juan, PR.

Due to the critical need for improved therapeutics against advanced Stage IV breast cancer, we developed and pre-clinically validated the small molecule Ehop-016 as a potential anti-metastatic drug. Ehop-016 is a small molecule inhibitor of the Rac pathway, due to the capability to target nucleolin-overexpressing cancer cells and further inhibit the Akt pathway. Ehop-016 proceeds to the American Association for Cancer Research • Volume 58 • April 2017

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(GEF) Vav2, which consequently decreases invasive actin structures and cellular migration. In a human epidermal growth factor receptor 2 (HER2) type breast cancer model in nude mice, Ehop-016 inhibits mammary tumor growth, angiogenesis, and metastasis. Moreover, Ehop-016 reduces HER2 + cancer cell viability and migration, or individually in combination with anti HER2 or EGFR directed monoclonal antibodies, with Ehop-016 may adversely affect normal immune system function. Therefore, a strategy was developed for the targeted delivery of Ehop-016 into mammary tumors, using nanoliposomes containing the current breast cancer therapeutic, Trastuzumab, a monoclonal antibody directed at the HER2. We tested various nanoliposome formulations as target delivery systems for Ehop-016. First, the encapsulation efficiency of Ehop-016 loaded liposomes was tested using different drug loading methods. To obtain HER2 targeted liposomes, we prepared Trastuzumab-lipid conjugates and incorporated them into the Ehop-016 containing liposomes. The binding, internalization and selectivity of our HER2 targeted delivery system was verified in human metastatic breast cancer cell lines, by using fluorescence microscopy to assess the co-staining of the lipid bilayer and Trastuzumab, and to evaluate the effectiveness of the targeted liposomes in delivering Ehop-016 and inhibiting Rac activity, pull-down assays were performed to detect active Rac-GTP in cells treated with Ehop-016 alone or Trastuzumab-conjugated liposomes with Ehop-016. Results show that the Trastuzumab-conjugated liposomes are more selective at binding to HER2+ breast cancer cells and inhibiting Rac, than treatment with Ehop-016 alone. In summary, our novel delivery system containing Ehop-016 and Trastuzumab has been shown to be effective in breast cancer cells, and needs to be validated in animal models of metastatic breast cancer. The development of this targeted delivery system has implications, not only in the field of breast cancer therapeutics, but may be adapted for the treatment of other diseases, and used to overcome the side effects of current treatments.


Introduction: Biomimetic nanoparticles (NPs) combining synthetic and biological materials have flexibility and functionality for drug delivery and immunomodulation.1 Red blood cell (RBC) membranes coated poly(lactic-co-glycolic acid) (PLGA) NPs that mimic RBCs and act as nanosponges for toxins were recently described.2,3 Here, we coated cancer cell membranes onto PLGA NPs in a “right-side” out manner, to translocate membrane anchored proteins onto NPs. Cancer cell membrane coated PLGA NPs hold promise for disrupting cancer cell-stromal cell interactions, and for priming the immune system in cancer immunotherapy. Method: Plasma membrane fractions (MFs) of U87 (low CXCR4) and U87-CXCR4 (high CXCR4) cells were isolated upon homogenization, and sucrose density gradient centrifugation. MFs and PLGA NPs were mixed and physically entrapped through a porous membrane to obtain MF-coated PLGA NPs. MFs were probed with antibodies against cell fraction markers, and CXCR4. MFs-coated NPs, MFs and PLGA NPs were characterized on size, morphology, and zeta-potential. The orientation of MFs and MF-coated NPs was investigated by confocal microscopy and flow cytometry. Transwell migration assays were performed to investigate the migration of cancer cells towards human mammary fibroblasts (HMFs). Immune-competent Balb/c mice were immunized with IR700-labeled MFs or MF-coated NPs via subcutaneous injection through hock and imaged in vivo and ex vivo by near-infrared micro- and spectrums. Results: Plasma membrane purity was confirmed from west- ern blot analysis that showed the significant enrichment of Na+/K+ -ATPase, negligible amount of GFP/88 or GAPDH in MFs. PLGA NPs, U87-CXCR4 MFs and U87-CXCR4 MFs-coated NPs had average diameters of 50 nm, 200 nm, and 70 nm, respectively. Z-average diameters and zeta-potential of PLGA NPs, U87-CXCR4 MFs and U87-CXCR4 MFs-coated NPs were 98.6 ± 9.5 nm, 336 ± 14 nm, and -34.3 mV, -24.9 mV and -25.0 mV, respectively. Confocal fluorescence microscopy and flow cytometry detected intense PE fluorescence and higher CXCR4 in U87-CXCR4 MFs and U87-CXCR4 MFs-coated NPs than U87 counterparts, confirming a “right-side” out orientation. When U87 or U87-CXCR4 MFs were added to HMFs in the transwell assay, fewer cancer cells migrated towards HMFs, identifying the unique ability of MFs in disrupting HMF-cancer cell interactions. U87-CXCR4 MFs and MF-coated NPs were observed in the popliteal lymph nodes, and triggered the induction of CD8+ T lymphocytes, identifying a role for MF-coated NPs in providing cancer cell membrane-derived antigens to induce a tumor-specific immune response. References: 1. Fang, R. et al. Small 2015; 2. Hu, C. et al. PNAS 2011; 3. Hu, C. et al. Nat. Nanotechnol. 2013. Supported by NIH R21 CA198243 and P50 CA103175.

**#2199** Magneto-electric nanoparticles for high-specificity treatment of cancer. Sakhrat Khizroev,1 Abhi Nagesetti,2 Tiffany S. Stewart,2 Emmanuel Brumme,1,2 Alex Rodzinski,1 Rakesh Gadara,3 Ping Liang,1 Florida International University, Miami, FL;2 University of Notre Dame, Notre Dame, IN;3 Cell Nanomod, Weston, FL.

Introduction: Delivering a drug specifically into the tumor cell past its membrane and then releasing the drug into the tumor cells without affecting the normal cells remains a formidable challenge. Unlike any other nanoparticles, magneto-electric nanoparticles (MENs) display a non-zero magneto-electric (ME) effect and thus present a unique capability to use external magnetic fields to control intrinsic electric fields associated with cell membranes and the interaction between MENs and therapeutic loads. Because cancer and normal cells of the same type have different electric properties, MENs is used for high-specificity targeted delivery. An a.c. magnetic field is used to trigger drug release off the nanoparticles. Brief Methods: 30-nm CoFe2O4-BaTiO3 core shell MENGs were synthesized and the selective uptake of cancer cells and normal cells was investigated by confocal microscopy, a coercivity of 100 Oe, and a ME coefficient of 10-100 mV cm^2/Oe^3 was prepared with a co-precipitation process. MENs were coated with fluorescein isothiocyanate to monitor their intra-cellular transport through a high-contrast confocal microscopy. Three cancer cell lines including Skov-3 (Ovarian adenocarcinoma), U87-MG (Glioblastoma), and MCF-7 (Breast adenocarcinoma), and two normal cell lines including brain endothelial cells (Brain EC) and ovarian cells (HOMEM) were cultured at 37°C. The transport of MENs loaded with drugs, peptides, and RNAs through the cell membranes and the consequent release of the load under different d.c. and a.c. magnetic fields were studied through confocal microscopy and photoabsorption spectroscopy, respectively. Trypan-blue viability count was used to assess cell growth inhibition under different study conditions. Atomic force microscopy of the cell membranes was conducted to understand the interaction between the nanoparticles and the cells. Summary of new data: Comparison of MENs with purely magnetic iron oxide nanoparticles showed that the penetration through the cancer cell membrane could be achieved only with MENs. It took d.c. fields of 100 Oe and over 1000 Oe to nano--electrode the membranes of SKOV-3 and HOMEM cell lines, respectively. An a.c. magnetic field with a strength of 50 Oe and a near-d.c. frequency of 100 Hz was sufficient to enable release of a therapeutic load off MENs. All the cancer cell lines under study showed membrane penetration threshold d.c. fields at least a factor of ten smaller compared to their normal counterparts. Conclusion: MENs displayed unique capabilities for externally controlled high-specificity targeted anticancer drug delivery and release on demand via application of d.c. and a.c. magnetic fields, respectively. Because MENs rely on a physical mechanism rather than chemical, they can be used for prospective delivery and high-efficiency controlled release of a broad range of therapeutic loads including drugs, peptides, and RNAs to treat many different cancers.

**#2200** Dual carfilzomib and doxorubicin carrying nanoparticles for synergistic efficacy in multiple myeloma. Basar Bilgicer, Tanyel Kiziltape, David Omstead. University of Notre Dame, Notre Dame, IN.

We describe the synthesis and analysis of dual carfilzomib and doxorubicin loaded nanoparticles in their ability to deliver both drugs to multiple myeloma tumor cells at their optimal synergistic ratio. First, to identify the optimal synergistic ratio, various molar ratios of carfilzomib to doxorubicin were screened against multiple myeloma cell lines using the Chou-Talalay method. Both therapeutic agents were then incorporated into liposomes at the identified optimal synergistic ratio of 1:1 to achieve dual drug loaded nanoparticles with a narrow size distribution of ~100 nm and with high reproducibility. Our results established that the dual drug loaded nanoparticles exhibited synergy in vitro and were more efficacious in inhibiting tumor growth in vivo than a combination of free drugs, while at the same time reducing systemic toxicity. In conclusion, this study achieved the preclinical evaluation of dual drug loaded liposomes containing carfilzomib and doxorubicin for enhanced therapeutic efficacy to improve patient outcome in multiple myeloma.

**#2201** Downregulation of NF-kB by nanoconjugates to overcome drug resistance in ovarian tumors. Ajit P. Zambre,1 Sarjukumar Pandhal,1 Alyssa Worland,1 Sarah Chapman,1 Matthew Leevy,1 Anandhi Upendran,2 Raghuraman Kannan.1

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Ovarian cancer is the fifth leading cause of cancer deaths in women. Globally, around 140k women die from ovarian cancer per year. Recurrence can lead to development of resistance to first line agents such as platinum analogs, taxanes, and doxorubicin. Several studies have shown that decrease of NF-kB expression will inhibit P-glycoprotein maturation and thereby overcome drug resistance. In this context, it is well known that Disulfiram (DSF), a FDA approved drug for anti-alcoholism, has been identified as moderate inhibitor of NF-kB activity. It is also shown that DSF possess mild anti-cancer effects via increased permeability of the outer membrane of mitochondria resulting in leakage of proteins, such as cytochrome C, generation of ROS that simulates the ROS-MAPK pathway leading to apoptosis, and inhibition of P-glycoprotein. As such DSF is not a strong therapeutic agent; however, in combination with copper it showed excellent therapeutic benefit. This effect is mainly attributed to metabolite diethyldithio-carbamate—a proteasome inhibitor that was produced upon complexation of DSF with copper. However, delivery of DSF-Cu complex was not successful in clinical setting. We hypothesized gold nanoparticles can serve as potential delivery vehicle to selectively transfer Cu-DKF to tumor without toxicity. Indeed, cellular studies in drug sensitive (OVCAR) and Adriamycin resistant p-glycoprotein expressing (NCI-ADR/RES) ovarian cancer cells indicated that the gold nanoconjugate exhibit significant cytotoxicity and the IC-50 values was decreased 2.5 fold times compared to the Cu-DSF complex. NF-kB expression was decreased by two folds compared to native Cu-DSF complex. Detailed cellular and mechanistic studies along with the evaluation of [Au(DSF-Cu)] in human multi-drug resistant (NCI-ADR/RES) mouse xenografts will be presented.

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#2202  

**FABPS in urinary extracellular vesicles is a potential biomarker of high Gleason score prostate adenocarcinoma.**  

Ryosuke Matsuzaki,1 Atsunari Kawashima,1 Akira Nagahara,1 Motohide Uemura,2 Yasushi Miyagawa,1 Takeshi Tomonaga,1 Norio Nonomura,1 Osaka Univ. Graduate School of Medicine, Osaka, Japan;2Laboratory of Protein Sciences (The PHOENIX center), Beijing, China; 3Fudan University, Shanghai, China; 4East China Normal University, Shanghai, China; 5Baylor College of Medicine, Houston, TX; 6School of Pharmacy, University of Maryland, Baltimore, MD.

Urinary collected after prostate massage is a promising source of new biomarkers of prostate cancer, and was reported to contain extracellular vesicles (EVs) that are secreted from prostate cancer cells. EVs are microvesicles secreted from various cell types. EVs contain microRNAs, proteins, and mRNAs and play a role in intercellular communications via the mechanisms of exocytosis and endocytosis. We aimed to discover a new biomarker for high Gleason score (GS) prostate cancer (PCA) in urinary EVs via quantitative proteomics. Material and methods: Urine samples after massage were collected from 18 patients before prostate needle biopsy. Six patients had negative biopsy outcomes, 6 had high Gleason score 6 prostate cancer (GS6), and 6 had Gleason score 8 to 9 prostate cancer (GS8-9). Urinary EVs were isolated from urine with ultracentrifugation. Protein extracted from EVs were labeled with 4-plex iTRAQ and liquid chromatography tandem mass spectrometry (LC-MS/MS) was used for proteome analysis. The candidate proteins were quantitated in the independent set by selected reaction monitoring/multiple reaction monitoring analysis (SRM/MRM). Results: Seven proteins increased in patients with PCa compared to those with negative biopsy (ratio > 2.0, p-value < 0.05). Thirteen proteins were chosen for further analysis and verified in 29 independent urine samples (negative [n = 11], PCa [n = 18]) using selected reaction monitoring/multiple reaction monitoring. Among these candidate markers, fatty acid binding protein 5 (FABPS) was higher in the cancer group than in the negative group (p-value = 0.009) and was significantly associated with GS (p-value for trend = 0.011). Conclusions: We applied the proteomic analysis to discover biomarkers in EVs in urine collected after prostate massage. FABPS in urinary EVs could be a potential biomarker of high GS prostate cancer. Additional large-scale studies are warranted to confirm this finding.

#2203  

**A SRM/MRM based targeted proteomics strategy for absolute quantification of potential biomarkers of TKI sensitivity in EGFR mutated lung adenocarcinoma.**  

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Lung cancer remains the leading cause of cancer-related deaths worldwide. Epidermal growth factor receptor (EGFR) is the primary driver oncogene in 10-30% lung adenocarcinoma. Kinase domain mutations in EGFR (L858R and E746-A750 deletion) provide sensitivity to EGFR-specific tyrosine kinase inhibitors (TKIs). Despite good tumor inhibitory effects, after approximately a year, patients acquire resistance to EGFR TKIs. We have hypothesized that targeted proteomic analysis identifying amino acids in cell culture (SILAC)-based quantitative mass spectrometry (MS) on a Thermo Orbitrap Elite to identify and characterize the dynamics of tyrosine phosphorylation upon TKI treatment of human lung adenocarcinoma cell lines. The degree of inhibition of phosphorylation of several phospho-sites correlated with the extent of TKI-sensitivity. Several kinases and adaptor proteins that were significantly differentially phosphorylated include AKT1, RSK1, JUN, ERK, and DUSP6. We have successfully developed stable isotope labeling with amino acids in cell culture (SILAC)-based targeted MS for these tyrosine phosphorylated peptides in human cancer cell lines. The overall goal of these targeted studies is to develop absolute quantitation assays to precisely and accurately quantify these potential biomarkers in clinical samples.

#2204  

**A proteomic landscape of diffuse-type gastric cancer.**  

Sai Ge,1 Xia Xia,2 Chen Ding,2 Bei Zhen,2 Quan Zhou,2 Jinwen Feng,1 Jiajia Yuan,1 Ruich Chen,1 Yumei Li,2 Zhongge Ge,1 Jiafu Ji,2 Lianhai Zhang,2 Jiauyan Wang,1 Zhongwu Li,1 Yumei Lai,1 Ying Hu,2 Yilian Li,2 Jing Gao,2 Lin Chen,2 Jianming Xu,1 Chuncho Zhang,2 Sung Yun Jung,2 Mingwei Liu,1 Lei Song,2 Guojun Gao,2 Qiang He,2 Yin Chen,2 Teliu Shi,2 Weimin Shi,2 Yi Yang,2 Fuchu He,2 Lin Shen,1 Jun Qin,2 CNHPP.

University Cancer Hospital & Institute, Beijing, China; 2National Center for Protein Sciences (The PHOENIX center), Beijing, China; 3Fudan University, Shanghai, China; 4East China Normal University, Shanghai, China; 5Baylor College of Medicine, Houston, TX; 6General Hospital of Chinese People’s Liberation Army, Beijing, China; 7Affiliated Hospital of Academy of Military Medical Sciences, Beijing, China.

Gastric cancer is a heterogeneous disease characterized by poor clinical outcomes and limited targeted treatment options. Among them, diffuse-type gastric cancer (DGC) is the subtype with worst prognosis. Here we describe the first proteomic landscape of DGC. We carried out proteome profiling and targeted exome DNA sequencing of 84 DGC samples. We analyzed the 1,008 (168 x 6) raw files together for uniformed quality control and protein identification with 1% global protein false discovery rate (FDR), which resulted in the identification of 11,340 gene products (GP). A SAM (significance analysis of microarray) analysis identified 1,641 proteins as differentially expressed between T (tumor) and N (normal) with statistical significance (FDR q-value<0.01 by SAM and differential expression ratio >2.0 and p-value<0.05), including 80 up-regulated and 430 down-regulated GPs. Gene Ontology annotation indicated that tumor proteomes were significantly enriched in cell cycle, DNA replication, checkpoint, E2F, WNT, p53 signaling, epithelial mesenchymal transition (EMT), and inflammation/ cytokine-receptor interaction pathways, and the proteomes of the nearby tissues are enriched in metabolism pathways, such as fatty acid metabolism, oxidative phosphorylation, and amino acid metabolism. Notably, many gastric makers (ANXA10, VSIG1, CLDN18, CTSE, TFF2, MUC5AC and MUC6) and signature proteins for stomach functions, including digestion, absorption, secretion, and stomach acid generation (PGC, GIF, GAST, and ATP4A), were lost in tumors. Based on proteome profiling alone, DGC can be
subtyped into 3 major classes (PX1-3) that exhibit distinct proteome features and correlate with distinct clinical outcomes (Gehan-Breslow-Wilcoxon P = 0.024). PX1 exhibits proteome stability and the best overall survival; PX2 exhibits its dysregulation in DNA replication and cell cycle, and is most sensitive to chemotherapy; PX3 features hyper-activated immune response and is not responsive to EGFR-TKI treatment. We identified cell lines that were enriched in DGGC patients into these three subtypes, opening a door for proteome subtyping in clinical application and intervention. Furthermore, we nominated drug target candidates taking into consideration both the altered DGGC proteome and association data with patients’ overall survival. This study revealed the altered signaling pathways in DGGC and demonstrated the advantage of proteomic approach in molecular subtyping of cancer.

#2205 Identification of potential biomarker related to EGFR mutation by functional proteome profiling in primary non-small cell lung cancer. Yuan-Ling Hsu, Szu-Hua Pan. National Taiwan Univ., Taipei, Taiwan. Non-small-cell lung cancer (NSCLC) accounts for approximately 85% of lung cancers which the leading cause of cancer-related death worldwide. Recently, epidermal growth factor receptor (EGFR) activating mutations have been proved relevant to NSCLC and let treatment of EGFR tyrosine kinase inhibitors (EGFR-TKIs) become an alternative therapy for NSCLC patients. Despite excellent clinical response to EGFR-TKIs, the intrinsic and acquired resistance to EGFR-TKI created challenges in clinical practice. Although several mechanisms may contribute to solve EGFR-TKIs resistance, there still have 20% to 30% of these patients that how the resistance developed remain unclear. Studies indicated that the increment of membrane efflux may be associated with drug resistance. In this study, we use differential membrane proteomic analysis to identify potential biomarkers for therapeutics of EGFR-TKIs resistance in NSCLC patients. Several primary NSCLC cell lines with different EGFR status were be isolated by our laboratory. Identification of modulators on membrane of primary cancer cells with different EGFR status was analyzed by membrane proteomic assay using LC-MS/MS. The expression patterns of EGFR mutation which would compare with that of wild type group and the potential candidates would be selected by cross the results of interactome profiles of EGFR mutation cell lines. The expression patterns and functional role of these candidates were be examined in NSCLC cells by several molecular, cellular and biochemical analysis, and demonstrated the clinical relevance of these specific targets with EGFR mutation. The expression of 100 modulators up-regulated in EGFR mutation cells compared with that of wild type group. After cross reacted with the 474 interaction proteins of mutated EGFR, 8 proteins were selected as potential candidates that related to EGFR-TKI resistance, especially the expression of a protein we called SHX in EGFR mutation cells. We demonstrated the real expression levels of SHX in different lung cancer cells, and performed network analysis of SHX and EGFR in translational level. The analysis of differential membrane proteomic found several novel biomarkers and potential regulation mechanisms that related to the development of EGFR-TKIs resistance in NSCLC. These identification may provide us new direction for exploring therapeutic strategy for NSCLC treatment in the future.

#2206 Proteome phenotype of stage III metastatic melanoma and response to MEK inhibition. Christoph Krisp,1 Robert Parker,1 Dana Pacsoci,3 James Wilmot,2 John F. Thompson,2 Graham J. Mann,2 Richard A. Scovler,2 Nicholas K. Hayward,2 Mark P. Molloy1. 1Macquarie University, Sydney, Australia; 2University of Sydney, Sydney, Australia; 3QIMR Berghofer, Brisbane, Australia. Introduction: Melanoma accounts for 5% of all skin cancers, yet is the most common form of skin cancer-related deaths, and is the commonest lethal malignancy in young people (1<40yrs). Patients with BRAF mutant melanoma show survival benefit from MAPK pathway inhibition, although response rates vary. We conducted proteomic screening using mass spectrometry to detect cellular processes that might predict response to MEK inhibition more effectively than genotyping alone. Methods: Protein extracts from early passages of ten AJCC Stage III local lymph node metastatic melanoma cell lines with known MAPK mutational status (BRAFmut, NRASmut, MAPKwt) were used. Further, 32 fresh frozen NRASmut or BRAFmut AJCC Stage IIIC metastatic melanoma (lymph node metastases) specimens were obtained from patients with varying survival. Lysed cells and tissue were digested with trypsin and analysed by LC-MS/MS using a TripleTOF 5600/6600 mass spectrometer. Data-independent analysis of SHX and EGFR in translational level. The analysis of differential membrane proteomic found several novel biomarkers and potential regulation mechanisms that related to the development of EGFR-TKIs resistance in NSCLC. These identification may provide us new direction for exploiting therapeutic strategy for NSCLC treatment in the future.

#2207 HSP90 inhibition leads to a differential proteomics profile in non small cell lung cancer cell lines. Angela Marrugal,1 Irene Ferrer,1 Maria Dolores Pastor,2 Alvaro Quintanal,3 Antonio Lucena-Cacace,1 Amancio Carnero,1 Luis Paz-Ares,2 Sonia Molina-Pinelo1. 1CNIO, Madrid, Spain; 2IBIS, Sevilla, Spain. Background: The cancer chaperone, Heat Shock Protein 90 (HSP90), is known for its ability to regulate the stability of different oncoproteins. Thus, its overexpression has been related to unfavorable prognosis in some types of tumors. EGFR and EMLA-ALK, two of the most important drivers in non-small cell lung cancer (NSCLC), are HSP90 clients and extremely depend on it. As a consequence, this chaperone is especially important in NSCLC hence HSP90 inhibition shows a lot of possibilities to future treatments in this tumor type. Nevertheless, to obtain a successful clinical development, will be essential supporting evidence of inhibitory efficacy in several molecularly defined subgroups of NSCLC. Methods: NSCLC cell lines carrying different gene mutations, whose direct (HCC827; EGFR mutated and H3122; EMLA-ALK rearrangement) and indirect (A549; KRAS mutated) relationship with HSP90 has been reported, were used. In these cell lines, along with H1781 (EGFR, KRAS, ALK wild type) as control, the activity of the chaperone studied was interrupted. To this end, pharmacological inhibition of HSP90 was achieved through geldanamycin and resorcinol derivates. First, western blotting was carried out to confirm the effect of this inhibition. Later, to identify a proteomic profile associated with HSP90 inhibition, two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) of selected cell lines were performed. Results: The expression of the oncogenic HSP90 client proteins studied was decreased by the inhibitors in the NSCLC cell lines. The oncoproteins drivers EGFR and EMLA-ALK showed a strong dependence on HSP90 observed through a high sensitivity of the cell lines HCC827 and H1122 to the inhibition. Therefore, the cell lines which were selected from the purpose of identify a proteomic signature linked to HSP90 inhibition by 2D-PAGE. The untreated EGFR positive cell line presented 104 protein spots significantly up-regulated, whereas 80 spots were down-regulated compared to inhibited cell line. Meanwhile, the cell line harboring the EMLA-ALK translocation showed 16 spots up-regulated and 5 down-regulated in the untreated versus inhibited cell line. In addition, we have established an expression signature for each processed cell lines, was detected after treatments. This feedback, previously reported, confirmed HSP90 inhibition in the two cell lines studied. Conclusions: The evidence of treatment response, in the cell lines studied, was showed through oncogenic client proteins reduction as well as HSP70 induction. The proteomic profiles identified, of HSP90 inhibited and untreated tumor cells, revealed several deregulated pathways involved in the tumorigenesis.

#2208 Peptide-mediated ‘miniprep’ isolation of extracellular vesicles is suitable for high-throughput proteomics; method evaluation and application in colon cancer. Meike De Wit,1 Jace Knol,2 Inge de Reus,2 Tim Tim Schelhorst,2 Logan Bishop-Currey,2 Nicole van Grieken, Sander Piersma,2 Thang V. Pham,1 Remond J. Fijneman,3 Gerrit A. Meijer,1 Henk Verheul,2 Connie R. Jimenez2. 1Netherlands Cancer Institute, Amsterdam, Netherlands; 2VU University Medical Center, Amsterdam, Netherlands. Objective: Extracellular vesicles (EVs) are cell-secreted membrane vesicles enclosed by a lipid bilayer derived from endosomes or from the plasma membrane. Since they are released into body fluids, and their cargo includes tissue-specific and disease-related molecules, EVs represent a rich source for disease biomarkers. However, standard ultracentrifugation methods for EV isolation (UC-EV) are laborious, time-consuming, and require high inputs. Method: A recently described isolation method, which can be performed at small 'miniprep' scale, utilizes specific Heat Shock Protein (HSP)-binding peptides to aggregate HSP-decorated EVs (Ghosh et al. (2014), PLoS ONE 9,e110443). The authors

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showed comparable results for their method (abbreviated HSP-EV here) and UC-EV, but a detailed proteomic comparison was lacking. Therefore, we compared both methods using label-free proteomics of replicates EV isolations from HT-29 cancer cell-conditioned medium. Subsequently we applied this technique on secretomes of fresh human colorectal cancer (CRC) (n = 17) and colon adenocarcinoma (n = 8) as well as patient-normalized EVs (n = 4) using the same protocol. Despite a 30-fold different input scale (UC-EV: 60 ml versus HSP-EV: 2 ml), both methods yielded comparable numbers of identified proteins (3115 versus 3085), with reproducible identifications (72.5% versus 75.5%) and spectral count-based quantification (average CV 31% versus 27%). EVs obtained by either method contained established EV markers and proteins linked to vesicle-mediated protein transport. In the EV fraction of the secretomes secretomes 6390 proteins were identified, of which 471 proteins were significantly 5-fold more present in CRC samples than in normal tissue EVs. Gene ontology analysis revealed enrichment of nuclear proteins involved in DNA damage response, chromosome organization and RNA processing in the CRC EVs. Conclusions: The HSP-EV method provides an advantageous, simple and rapid approach for EV isolation from small amount of biological samples, enabling high-throughput analysis in a biomarker discovery setting.

**#2209 Characterization of FABP5 antibodies in prostate.** Dennis A. Otali,1 Denise K. Oelschlager,1 James Kearns,3 Sandra M. Gaston,2 William E. Grizzle.1

1Univ. of Alabama at Birmingham, Birmingham, AL; 2Tufts Medical Center, Boston, MA.

FABP5, a member of a family of small proteins (~15 kDa) that transport lipids, is emerging as an important biomarker because it is differentially expressed in prostate cancers (PrCa) compared to uninvolved prostate glands and lipids are important to the progression of prostate cancer. This study was to select an antibody (Ab) for use in immunostaining (IHC) FABP5 in formalin fixed paraffin embedded (FFPE) tissue and also an Ab for use in Western blotting (WB). A mouse monoclonal antibody (mAb) clone A9 to FABP5 from Santa Cruz (SC) Biotechnology, Inc. and a rabbit polyclonal antibody (pAb) to FABP5 from Abcam (AB) (cat. # ab37267) were tested. Six prostate cell lines (PC3, DU145, 22Rv1, MDA PCa 2b, LNCaP, and normal primary prostate epithelial cells (NH Pri Pro)) were used to study protein and mRNA levels of FABP5. Protein expression of FABP5 was determined by WB of whole cell lysates as well as cytoplasmic and nuclear lysates. GAPDH, a "housekeeping" gene expressed at similar level in most types of cells, was used as a loading control for WB. Cells from the six prostate cell lines also were used in Histogel, processed to FFPE and immunostained. The prostate cells were transfected with FABP5 siRNA for WB with aliquots of the transfected prostate cells processed to FFPE, qrtPCR was performed to measure mRNA levels. The expression of whole cell lysates on WB probed with the AB pAb to FABP5 was NH Pri Pro > PC3 > DU145 > 22Rv1 > MDA PCa 2b > LNCaP and compared to NH-Pri-Pro. PC3 > DU145 > 22Rv1 when probed with the SC mAb. WB of the cytoplasmic fractions from each cell line has a similar pattern. In Histogel sections immunostained with AB pAb, the FABP5 cytoplasmic expression was PC3 > DU145 > 22Rv1 > MDA PCa 2b > NH Pri-Pro > LNCaP. These levels of expression among the cell lines followed a similar trend in evaluation of immunostaining of cell membranes, nuclear, and the perinuclear area.

**#2211 Characterization of monoclonal antibodies specific for HPV-positive head and neck cancer.** Hauan-Chen Liu,1 Fatuluni Parkid,1 Thomas Kraus,2 Thomas Moran,3 Andrew Sikora1.1Baylor College of Medicine, Houston, TX; 2Icahn School of Medicine at Mount Sinai, New York, NY.

HPV-driven head and neck squamous cell carcinoma (HNSCC) are among the fastest growing cancers. Although, HPV-HNSCC patients have an overall favorable prognosis, a significant number of patients relapse post-treatment, and currently there is no specific therapeutic approach targeting the unique biology of HPV-driven HNSCC. Our ongoing efforts to target HPV-HNSCC focus on the identification of cell surface antigens that are upregulated by HPV infection. We have developed an ‘antigen-agnostic’ approach for generating HPV-HNSCC-targeting monoclonal antibodies for cancer diagnosis and treatment that does not rely on prior identification of target antigens. We used HPV-HNSCC membrane fractions to immunize recipient mice and generated HPV-specific hybridomas. We then screened five thousand hybridoma colonies by flow cytometry to test the specificity of binding to HPV-positive cancer cell lines (2HNSCC and 2 Cervical Cancer) and HPV-negative cancer cell lines (4 HNSCC and 1 CC). After primary screening, we narrowed down to forty-four clones; among these hybridoma clones, 6D8 and 6B3 bound preferentially to HPV-positive cancer cell lines. The binding targets of 6D8 and 6B3 were identified by immunoprecipitation and mass spectrometry; their targets are integrin alpha6 (ITGA6) and tissue factor (F3) respectively. Future work will validate the biological function of these mAbs in vitro and in vivo models, and continue identifying more binding target of mAbs. We propose mAbs specifically targeting membrane-expressed antigens on HPV-related cancer cells as a potential approach for early diagnosis and targeted therapy.

**#2212 Protein engineering of the decapping enzyme NudT16 enhances its ability to hydrolyze protein-conjugated ADP-ribose for post-translational site identification via mass spectrometry.** Puchong Thirawatananond, Robert L. McPherson, Jasmine K. Mahli, Anthony K. Leung, Sandra B. Gabelli. Johns Hopkins University, Baltimore, MD.

During nonhomologous end joining of DNA follow double-stranded breaks, one common signaling response is the adenosine diphosphate ribosylation (ADP-ribosylation) of recruiter scaffold proteins. ADP-ribosylation is a post-translational modification (PTM) that occurs on aspartate, glutamate, lysine, arginine and cysteine on proteins and is mediated chiefly by polyadenosine diphosphate ribose polymerases (PARP). Using NAD+ as its substrate, PARP transfers an ADP-ribose onto an acceptor protein, releasing a nicotinamide ring in the process; this modification presents as a mono-ADP-ribosylation (MARylation) or poly-ADP-ribosylation (PARylation). While site identification of MARylated protein can be achieved through tandem MS/MS and searching for peptides whose spectra have been shifted by 541.0611 Da, a similar approach for PARylated protein is confounded by the variable length and branching of its PTM. To this end, ADP-ribosylated protein is normally incubated with a snake venom phosphodiesterase (SVP) to leave a standardized phosphoribose tag of 212.0086 Da. It has been shown that a recombinantly-expressed Nudix protein NudT16 provides a cost-effective alternative to SVP to process ADP-ribosylated protein. NudT16 in the cell normally decays small nucleolar RNA. Our studies here...
sought to utilize the x-ray crystallographic structure of NudT16 in complex with ADP-ribose in guiding rational design mutations to enhance binding of our desired ADP-ribose substrate and to widen the hydrolase’s active site to better accommodate a protein-conjugated ADP-ribose. Mutants have demonstrated improved catalytic efficiency for ADP-ribose hydrolysis. Radioabeled immunoo- nas of assay, as that is the only known method for concur with I-TAC, which is overexpressed in blood and tissue of men with advanced prostate adenocarcinomas. Finally, we found hnRNP-1, a cancer asociated splicing factor, and the cold shock proteins RNPL and A18 hnRNP. Knock-down of these cold shock proteins has been shown to enhance chemotherapeutic cell killing of prostate cells. ADAPT is an unbiased profiling plat- form that identifies cancer associated proteins expressed on exosomes. This platform can be deployed against multiple cancer types and offers broad poten- tial applications in biomarker discovery.

#2213 aberrant glycoprotein expression in recurrent and non-recurrent prostate cancer tissue. Sarah M. Totten, Cheylene Tanimoto, Abel Bermudez, Amy Hembree, James D. Brooks, Sharon J. Pitteri. Stanford University School of Medicine, Palo Alto, CA.

The primary aim of this study is to identify and quantify aberrancies in gly- cosylation patterns and glycoprotein levels expressed in high grade prostate cancer tissue from men with and without recurrence following radical prosta- tectomy using multi-dimensional chromatography and tandem mass spectrom- etry techniques. Glycosylation is a common and highly complex posttransla- tional modification, and is linked to protein function. Elucidating specific glycan structures is key to understanding the underlying role glycosylation plays in regulating cellular activity, including tumorigenesis, invasion, and metastasis. Aberrant glycosylation is an emerging hallmark of various cancers, demonstrat- ing that alterations in glycosylation disrupt cellular behavior in key pathways. Furthermore, comprehensive glycan characterization can lead to more effective biomarkers with increased clinical utility for distinguishing indolent disease from cancers that are more likely to become metastatic, recur and/or pose higher risk. In this study, glycoprotein analysis was performed on prostate cancer tissue and matched normal prostate tissue from ten men with high grade prosta- tectomies (Gleason 7-8) - five of which experienced recurrence. Proteins were extracted from tissue lysates, denatured, reduced and alkylated. Isotopic labels for quantitation were incorporated during alklylation - normal and cancer tissue was labeled with 13C2 and 13C acrylamide respectively. Paired normal/cancer tissues were combined. Glycosylated proteins from each pair were separated by multi-lectin chromatography designed to capture sialylated, core-fucosylated, and highly branched complex glycans, fractionating the complex mixture into four discrete fractions containing specific glycoforms. LC-MS/MS was used to analyze the tryptic digest from each fraction for protein identification and quanti- tation. This experimental design reveals differences between the glycosylation pat- terns and protein levels of cancerous and normal tissue in ten men, but also allows for the comparison between those with recurrent cancers. The glycosylation pat- terns across hundreds of prostate tissue proteins were systematically screened, en- abling the detection and relative quantification of specific glycoforms of proteins that may be dysregulated in prostate cancer. Preliminary results reveal the identification of 6,202 unique proteins, and quantitation of 2,894 proteins, most of which con- tained sialylated glycoforms. In all patients (regardless of treatment outcome), sev- eral hundred proteins had elevated total protein levels in the 13C-labeled cancer tissue compared to the 13C-labeled normal tissue proteins, such as TACSTD2 and LPP, whose expression was demonstrated. Furthermore, we found that urapregulation only among certain glyco- forms. Other glycoproteins were up-regulated in cancer only in recurrent patients, including CPNE1, TXNDS, and CASCA.


In the recent years it was demonstrated that a multitude of body fluids con- tains substantial amounts of exosomes, extracellular vesicles with sizes ranging between 40 and 100 nm. Those vesicles have protein profiles characteristic of their cells of origin. It was shown that exosomes play a role in cell-to-cell commu- nication making them attractive targets to identify early disease stage bio- markers. Cancer heterogeneity is known for a long time to be an important clinical determinant of patient outcome. We developed the highly multiplexed ADAPT platform to capture systems-based biological signatures that may reflect the molecular heterogeneity of various cancer types and help to improve diag- nosis of the disease. Exosomes from two prostate cancer cell lines, VCaP and LNCaP, were used to train sDNA libraries to discriminate them. A highly di- verse library of 10^12 oligonucleotides (ODNs) was subjected to five rounds of positive and negative selection against exosomes from VCaP and LNCaP pros- tate cancer cell lines. Individual ODNs that bound preferentially to exosomes from VCaP cells were identified by NGS, resynthesized and binding of co-precipitated ODNs to VCaP exosomes was verified by qPCR. LC-MS/MS was used to identify binding partners of ODNs bound to VCaP exosomes. Several of those binding partners (CHMP1b/2a/4b, VPS28, Syntenin-1) were found to be part of the ESCRT machinery, which participates in exosomes biogenesis. It was found that those proteins are overexpressed in human cancers. In addition, we identified the chaperone HSC70, which is overexpressed in blood and tissue of men with advanced prostate adenocarcinomas. Finally, we found hnRNP-1, a cancer asociated splicing factor, and the cold shock proteins RNPL and A18 hnRNP. Knock-down of these cold shock proteins has been shown to enhance chemotherapeutic cell killing of prostate cells. ADAPT is an unbiased profiling plat- form that identifies cancer associated proteins expressed on exosomes. This platform can be deployed against multiple cancer types and offers broad poten- tial applications in biomarker discovery.

#2215 detection of novel markers of transitional cell carcinoma of the ovary, the TCC-like variant of high grade serous carcinoma, using proteom- ics and immunohistochemistry. Basile Tessier-Cloutier,1 Jamie Magrill,2 Ste- fan Komnoss,3 Blake C. Gills,1 David G. Huntsman,3 Dawn R. Cochrane,1 Aline Talhouk,2 Robert Soslow,4 Gregg B. Morin,5 Chris J. Hughes,5 Anthony N. Karnezis,5 Christine Chow,6 Angela S. Cheng,7 Andreas de Bois,8 Jacobus Pfs- terer,9 Friedrich Komnoss,1 1Vancouver General Hospital, Vancouver, British Columbia, Canada; 2BC Cancer Agency, Vancouver, British Columbia, Canada; 3Tübingen University Hospital, Tübingen, Germany; 4Memorial Sloan Kettering Cancer Center, New York, NY; 5Klinikum Essen Mitte, Vancouver, Germany; 6Gy- necologic Oncology Center, Kiel, Germany.

Background: The current WHO classification does not separate transitional carcinoma of the ovary (TCCO) from conventional high grade serous carcinoma of the ovary (HGSC). TCCO has a better prognosis, possibly due to better chemosensitivity or less infiltrative growth pattern. The available immunohis- tochemical (IHC) markers do not differentiate between the two subtypes. There- fore, we sought to compare the proteomic profiles of conventional HGSC and TCCO to identify surrogate biomarkers of good prognosis from TCCO that could identify conventional HGSC tumors with a better prognosis. Design: Full proteome analysis of 12 cases of TCCO and 12 cases of HGSC was performed using SP3-clinical proteomics, run on an ThermoFisher Orbitrap Fusion. For validation, tissue microarrays of HGSC (n=89) and HGSC (n=237) were subject to a panel of antibodies against proteins found to be enriched in TCCO. All cases and immunostains were scored by a gynecologic pathologist. Univariate analysis was performed comparing IHC expression in TCCO vs. HGSC. Results: We identified 1220 proteins that were significantly enriched in TCCO over HGSC. Claudin 4 and Ubiquitin carboxy-terminal esterase L1 (UCHL1) were selected as potential markers of TCCO-like biology (p=0.0017, 0.0322). By IHC, Claudin 4 (95% confidence interval (CI) 0.171, 0.438) and UCHL1 (95% CI 0.291, 0.550) showed a significantly higher expression in TCCO as compared to HGSC (see table).

<table>
<thead>
<tr>
<th>% of tumors with high immunoreactivity scores</th>
<th>Claudin 4</th>
<th>UCHL1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure TCCO</td>
<td>34/59 (58%)</td>
<td>26/59 (44%)</td>
</tr>
<tr>
<td>Mixed TCCO-HGSC, TCCO</td>
<td>14/29 (48%)</td>
<td>8/29 (28%)</td>
</tr>
<tr>
<td>Mixed component</td>
<td>19/28 (68%)</td>
<td>6/28 (21%)</td>
</tr>
<tr>
<td>Conventional HGSC</td>
<td>33/235 (14%)</td>
<td>32/237 (14%)</td>
</tr>
</tbody>
</table>

Legend: Mixed TCCO: TCCO with minor component of conventional HGSC. Conclusion: Proteomic analysis showed differing protein profiles for TCCO and HGSC. By IHC, Claudin 4 and UCHL1 were identified as potential markers for TCCO-like differentiation of high-grade serous carcinomas. Further studies will focus on the prognostic significance of these and other markers in larger cohorts of HGSC. This study presents a novel approach at identifying potential diagnostic and prognostic biomarkers as well as therapeutic targets.


Background: High mobility group box 1 (HMGB1) is a versatile protein with dual roles. Within the cell, this highly conserved chromosomal protein functions as a DNA chaperone. Outside of the cell, it functions as the prototypical damage-asso- ciated molecular pattern. There is significant evidence that HMGB1 dysfunction contributes to cancer development, particularly in mesothelioma, where its role in carcinogenesis is better defined. Goal: To develop a mass spectrometry based immu- nune-multiple reaction monitoring (iMMRM) assay to quantify HMGB1 and its dis-
ease-associated post-translationally modified forms. Methods: Mesothelioma cell lines (Ren, Phi and PP-Mill) known to secrete HMGB1 were used. Concentrated cell supernatants, anti-HMGB1 antibody-loaded magnetic beads were incubated overnight. Beads were extensively washed and the HMGB1 released from the beads was enzymatically digested prior to targeted MRM mass spectrometry. Known synthetic peptides corresponding to the digested HMGB1 were used as internal standards to assay the HMGB1 levels. The protocol was optimized for HMGB1 release, digestion and analysis.


Introduction: More than 260,000 women die of cervical cancer every year. Screening methods have reduced the incidence of cervical cancer in high-income countries, but detection continues to lag in low and middle-income countries (LMICs). IgG antibody (Ab) immunity to early (E) HPV antigens (Ags) are potential biomarkers of disease progression. Since HPV16 accounts for only 20% of invasive cervical cancers, we developed protein microarrays expressing the proteomes of two low-risk HPV types (HPV6 and HPV11) to detect high-risk HPV16 serotypes to detect host IgG Abs to a broad spectrum of viral Ags to detect high-grade pre-invasive cervical lesions. These results suggest that serology is a potential method for early detection of cervical cancer.

**#2219** Cancer prevention using a novel fatty acid synthase inhibitor. Neil L. Spector, Tim Haystead, Sunil Zhao, Yaaan Alwarawrah, David Alcorta, William Kim, Jose E. Rivera, Michael Trinkler, David B. Darr. Duke University Medical Center, Durham, NC; "UNC-Chapel Hill, Chapel Hill, NC.

Tumor growth and survival is dependent upon de novo fatty acid synthesis regulated via preferential upregulation of fatty acid synthase (FASN) in tumor cells. Here a novel FASN inhibitor that has shown antitumor activity in established tumor models (Alwarawrah Y et al., 2016), now shows promise blocking oncogene-induced cell transformation and significantly delaying time to tumor development in a prevention study using a GEMM of triple negative breast cancer. Non-malignant, immortalized MCF10A human breast epithelial cells were infected with lentivirus encoding for the highly oncogenic 110 kDa truncated form of HER2, herein referred to as p110, under a doxycycline inducible promoter. The p110 containing cells were seeded as a control. Forty-eight hours after lentiviral infection, p110 was induced by dox and cells treated with FASN (2 and 4 µM) or vehicle alone for 21 days, and then assayed for cell growth and evidence of senescence. The C3TAg GEMM model of triple negative breast cancer was used to study FASN in the prevention setting. The mice (FVB/N background) express C3TAg and TGN-53, a sham insert served as a control. Forty-eight hours after lentiviral infection, p110 was induced by dox and cells treated with FASN (2 and 4 µM) or vehicle alone for 21 days, and then assayed for cell growth and evidence of senescence. The C3TAg GEMM model of triple negative breast cancer was used to study FASN in the prevention setting. The mice (FVB/N background) express C3TAg and TGN-53, a sham insert served as a control. Forty-eight hours after lentiviral infection, p110 was induced by dox and cells treated with FASN (2 and 4 µM) or vehicle alone for 21 days, and then assayed for cell growth and evidence of senescence.

**#2220** Quantitative multiplex analysis of immune checkpoint protein expression in circulation and in the tumor microenvironment. Wen-Rong Lie, Jehangir Mistry. MilliporeSigma, St. Charles, MO.
### Repurposing statins for prevention of triple-negative breast cancer.

Anjana Bhardwaj,1 Harpreet Singh,1 Kimal Rajapakshe,2 Cristian Coarfa,2 Isabella Bedrosian1.1 UT MD Anderson Cancer Ctr., Houston, TX; 2Baylor College of Medicine, Houston, TX.

Introduction: Although triple negative breast cancer (TNBC) accounts for 15% of the 250,000 annual cases of breast cancer, its more aggressive nature coupled with lack of targeted therapy, results in a disproportionate rate of mortality in women with this disease underscoring the critical need for prevention-based approaches. We hypothesized that identifying miRNA-mRNA functional pairs and their driver pathway(s) deregulated during preneoplastic progression would provide the most informative insights into the molecular drivers of TNBC carcinogenesis. We performed next generation RNA sequencing of TNBC-like MCF10A based model system and focused on identifying the molecular changes preceding the development of in situ carcinoma (DCIS). We used ki67, MTT and colony formation assays to study the growth inhibitory effect of miRNA and pathway targeting. To identify direct gene targets of a miRNA, we cloned the 3' untranslated region containing miRNA-binding sites from its predicted gene targets in a luciferase reporter vector, and studied the effect of miRNA overexpression on the repression of luciferase reporter activity. Results: In silico analyses of small RNA seq data showed loss of miRNA-140-3p as one of the top deregulated miRNA relevant to preneoplastic transition from MCF10A parental to MCF 10A1 preneoplastic cell line. Staining of breast tissue samples also confirmed loss of miRNA-140-3p in tumors compared to normal breast epithelium from normal mouse mammary tissue. To assess the potential functional role of miRNA-140 during TNBC development, we ectopically expressed miRNA-140 in MCF10.A1 and MCF10.DCIS cells. Interestingly, these studies revealed miRNA-140 replacement to preferentially inhibit colonizing ability and cell proliferation of preneoplastic MCF10.A1 cells in contrast to a much weaker cell inhibitory effects in DCIS cells. Next, we identified the functional gene targets of miRNA-140-3p by integromics studies involving next generation RNA-seq on the MCF10A model employing bioinformatics pipeline. SigTerms. These analyses identified HMGCR and HMGCS1 as top 2 gene targets of miRNA-140-3p that map to cholesterol biosynthesis pathway. Consistently, we found miRNA-140-3p to directly bind in 3'UTR of HMGCR, HMGCS1 and repress their expression as shown by luciferase assays. miRNA-140 binding of these gene targets is also functionally relevant as inhibition of HMGCR activity by an FDA approved statin inhibitor, fluvasatin, preferentially inhibited the cell proliferation and colonizing ability of preneoplastic MCF10A1.T1 cells. Further studies focused on testing the potential of fluvasatin to prevent progression of breast atypia cells in mouse xenografts are underway. Conclusions: Our results suggest miRNA-140 and its downstream functional cholesterol biosynthesis pathway targeting, especially by FDA approved Fluvasatin, may be a novel strategy for targeted prevention of TNBC.

### Honokiol stimulates immune reactivity in UV-irradiated skin through DNA demethylation-dependent functional activation of dendritic cells in mice.

Santosh K. Katiyar, Tripti Singh, Harish C. Pal, Ram Prasad. Univ. of Alabama at Birmingham, Birmingham, AL.

Solar ultraviolet radiation (UVR), and in particular UVB spectrum (290-320 nm), are considered as a complete carcinogen for cutaneous malignancies. The over-exposure of UVR suppresses the development of allergic contact hypersensitivity (CHS) response in both laboratory animals as well as in humans. CHS response is considered to be a prototypic T-cell mediated immune response. UVR-induced suppression of immune sensitivity has been implicated in skin cancer risk. Therefore, the treatment options which can inhibit UVB-induced suppression of immune sensitivity may be useful in the management of skin cancers, including both melanoma and non-melanoma. Previously, we have shown that topical treatment of honokiol inhibits UVB induced immune suppression, which was associated with the reduction in UVB-induced inflammatory mediators in the mouse skin. Here, we have further determined the underlying mechanism of action of honokiol on UVB-induced immunosuppression including its effect on epigenetic regulators and their relationship with immune sensitivity. Topical treatment of C3H/HeN mice with honokiol (0.5 and 1.0 mg/cm2 skin area) in hydrophilic-cream based topical formulation significantly inhibits UVB-induced suppression of CHS response (P<0.01-0.005), which was associated with reduced levels of DNA methylation as well as Dnmt activity in the mouse skin compared with non-honokiol-treated and UVB-exposed control mice. To characterize the cell population responsible for the honokiol mediated inhibition of UVB-induced immunosuppression, we used an adoptive transfer approach. DNA-methylated (most of cells) dendritic cells (DCs), isolated from the draining lymph nodes of donor mice that had been UVB-exposed and sensitized to 2,4-dinitrofluorobenzene (DNFB) with and without honokiol (0.5 and 1.0 mg/cm2 skin area) treatment, were transferred into naive recipient mice. The CHS response of the recipient mouse to DNFB was then measured. Honokiol treatment of UVB-exposed donor mice relieved this suppression of the CHS response in naive mice, while the recipient mice, obtained DNA-methylated DCs from UVB-exposed donor mice that were not treated with honokiol, showed significant suppression in the CHS response. The DCs from non-honokiol-treated and UVB-exposed donor mice was also associated with enhanced secretion of Th1-type cytokines compared with the Th1-type cytokines from the DCs obtained from non-honokiol-treated and UVB-exposed donor mice. These data suggest that honokiol mediated inhibition of UVB induced suppression of CHS response is associated with functional activation of dendritic cells and that is dependent on DNA methylation in DCs.

### Sildenafil suppresses inflammation-driven colorectal cancer in mice.

Bianca N. Islam,1 Sarah K. Sharmam,1 Yali Hou,1 Allison Bridges,1 Ravi- dra Kolhe,1 Jimena Trillo-Tinoco,1 Nagendra Singh,1 Sangmi Kim,1 Subbar- miah Sridhar,1 Franklin G. Berger,1 Darren D. Brownings,1 Augusta University, Georgia Cancer Center, Augusta, GA; 2Augusta University, Medical College of Georgia, Augusta, GA; 3University of South Carolina, Columbia, SC.

Intestinal cyclic guanosine monophosphate (cGMP) signaling regulates epithelial homeostasis, and has been implicated in the suppression of colitis and colorectal cancer (CRC). In this study, we investigated the cGMP-specific phosphodiesterase-5 (PDE5) inhibitor sildenafil to prevent tumorigenesis in the azoxymethane/dextran sodium sulfate (AOM/DSS) inflammation-driven colorectal cancer model. Treatment of mice with sildenafil activated cGMP signaling in the colon mucosa and protected against DSS-induced barrier dysfunction. In mice treated with AOM/DSS, oral administration of sildenafil throughout the disease course reduced polypl multiplicity by 50% compared to untreated controls. Polyps formed in sildenafil-treated mice showed less proliferation and were more differentiated compared to polyps from untreated mice, but apoptosis was unaffected. Polyps in sildenafil-treated mice were also less inflamed; they exhibited reduced myeloid-cell infiltration, and reduced expression of iNOS, IFNγ and IL-6 compared to untreated controls. Most of the protection was observed when sildenafil was added during the initiation stage of carcinogenesis (40% reduction in multiplicity), whereas administration of sildenafil later during the promotion stages did not affect polypl number. While later treatment with sildenafil did not affect multiplicity, it did have a similar effect on polypl phenotype, including increased mucus production, reduced proliferation and inflammation. In summary, our results demonstrate that oral administration of sildenafil suppresses inflammation and polyp formation in mice treated with AOM/DSS. This suggests that PDE5 inhibitors could have therapeutic value for the prevention of CRC in inflammatory bowel disease patients.

### Rapamycin suppresses ErbB2-overexpressing mammary tumors through selective inhibition of luminal progenitor cells and tumor-initiating cells.

Qingxia Zhao,1 Amanda B. Parriss,1 Erin W. Howard,1 Ming Zhao,3 Zhiying Guo,1 Ying Xing,2 Zhikun Ma,1 Xiaohe Yang1.

Rapamycin is a potent inhibitor of the mTOR pathway and has demonstrated anti-cancer properties in various cancer models. Although rapamycin was reported to inhibit mammary carcinogenesis at multiple stages, including initiation, invasion, and metastasis, the underlying mechanisms remain unclear, especially in individual breast cancer subtypes. Specifically, ErbB2/Her2 amplification occurs in approximately 25% of invasive human breast cancers, and is characterized by Akt-mTOR pathway deregulation. Recent reports also suggest that cancer stem cells (CSCs) play a critical role in tumor development and metastasis. Therefore, we investigated the efficacy of rapamycin in the prevention of mammary tumor development in MMTV-ErbB2 transgenic mice by focusing on its effects on CSCs/tumor-initiating cells (TICs). In this study, MMTV-ErbB2 mice were inoculated with syngeneic 78617 cells, which are derived from MMTV-ErbB2 mammary tumors, followed by rapamycin treatment (1.5 mg/kg/day) for 12 days. We found that rapamycin elicited strong anti-tumorigenic effects on the syngeneic tumors, as indicated by substantially reduced tumor volume and weight. To determine the effect of rapamycin on mammary epithelial cells (MECs) in premalignant tissues, MMTV-ErbB2 mice were treated with low-dose rapamycin (1 mg/kg/day) between weeks 10 and 20 of age. Rapamycin significantly inhibited mammary morphogenesis at 20 weeks of age as UVB-exposed to the control mice. As such, the branching density, ductal elongation, and proliferative index of the mammary glands from the rapamycin-treated mice were markedly decreased. Flow cytometric analysis of primary MECs demonstrated that rapamycin selectively inhibited the C61+CD49f+SP subpopulation, which are enriched with luminal progenitor
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cells and TICs. 3D culture of FACS-sorted MEC subpopulations indicated that CD61-CD49d+ cells are the major subpopulation that gives rise to 3D colonies. We further demonstrated that rapamycin significantly suppressed colony-forming cell (CFC) number and primary/secondary sphere formation of primary MECs and spontaneous MMTV-erbB2 tumor cells, indicating rapamycin-induced inhibition of mammary progenitor cells and CSC self-renewal, respectively. Furthermore, molecular analysis of MECs demonstrated that rapamycin inhibited signaling associated with ER, ErbB2, and mTOR. In particular, rapamycin strikingly inhibited Wnt/β-catenin and TGFβ/Smad3 signaling. Taken together, our results demonstrate that rapamycin-mediated prevention of MMTV-ErbB2 mammary tumor development involves selective targeting of mammary progenitor cells and CSCs in the premalignant mammary tissues through regulation of MEC stenness and multiple signaling pathways, which contributes to its potent anti-tumor effects on ErbB2-overexpressing breast cancer.


Pro-inflammatory signaling has been shown to promote colorectal tumorigenesis and is a target for the development of effective chemopreventive approaches. The specialized pro-resolving lipid mediators (SPMs, e.g. resolvins), bioactive metabolites of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), actively terminate inflammation and have been proposed to possibly contribute to the anti-tumorigenic effects of DHA and EPA. 15-lipoxygenase-1 (ALOX15) is a key biosynthetic enzyme in generation of resolvins. However, 15-LOX-1 expression is commonly lost during human CRC tumorigenesis starting in premalignant stages through transcriptional mechanisms independent of substrate availability. The impact of ALOX15 on DHA and EPA’s effects on tumorigenesis remain unknown. Mice with intestinal epithelium-specific expression of human ALOX15 (15-LOX-1-gut mice) and wild-type FVB controls were injected with azoxymethane (AOM, 7.5mg/kg) once weekly for 6w and followed for 20w. Mice were fed diet with 1% omega-3-acid ethyl esters (O3AEE, a pharmaceutical grade fısh oil preparation of EPA and DHA ethyl esters) or control diet starting 3w before initiation with AOM. Colonic tumors developed in 10 of the 13 (77%) wild type (WT) mice fed control diet, 5 of the 10 (50%) WT mice fed O3AEE diet, 5 of the 12 (42%) 15-LOX-1-gut mice fed control diet, and 3 of the 10 (30%) 15-LOX-1-gut mice fed control diet. Lipid mediator levels were measured by liquid chromatography/tandem mass spectrometry (LC-MS/MS). The SPMs resolvin E1 and D2 (RvE1, RvD2) as well as DHA-stable phosphodiesterase-5 (PDE5) inhibitor that was previously shown to reduce polyp formation in familial adenomatous polyposis (FAP) patients. Despite this validation of PDE5 as a therapeutic target, the failure of Exsulin due to toxicity halted further trials of cGMP elevating agents. The present study tested the effect of the PDE5 inhibitor sildenafil, and the receptor guanylyl-cyclase C (GCC) agonist nitroguanylic acid to tumor growth, invasion, angiogenesis (as assessed by quantitation of VEGF, MMP-2, and MMP-9 levels) and each of the tumor suppressors RECK and TIMP3 gene and significantly enhanced their protein expression in the ALOX15-gut mice model of colon cancer. Administration of either sildenafil or nitroguanylic acid reduced tumor growth, invasion, angiogenesis (as assessed by quantitation of VEGF, MMP-2, and MMP-9 levels) and each of the tumor suppressors, RECK and TIMP3 gene and significantly enhanced their protein expression in the ALOX15-gut mice model of colon cancer. Administration of either sildenafil or nitroguanylic acid reduced tumor growth, invasion, angiogenesis (as assessed by quantitation of VEGF, MMP-2, and MMP-9 levels) and each of the tumor suppressors. While the tumor suppressive mechanism is not fully understood, the results presented here demonstrate in a preclinical model, that increasing intestinal cGMP levels by targeting PDE5 or GCC may be a viable chemoprevention strategy for human FAP patients.

#2225 Green tea polyphenols suppress tumor growth and invasion by targeting matrix metalloproteinases, RECK and TIMP3, in a mouse model implanted with prostate tumors. Debrup Chakraborty, Venessa Benham, Elena Y. Demireva, Blair Bullard, Jamie J. Bernard. Michigan State University, East Lansing, MI.

Background: Adiposity plays a crucial role in the pathogenesis and prognosis of different types of cancers. Epidemiological evidence suggests visceral adipose tissue (VAT) and high-fat diets (HFD) are associated with increased cancer risk however the mechanism is not understood. The aim of this study was to explore the factors in VAT that stimulate neoplastic transformation. Methods: We modeled visceral adiposity-stimulated neoplastic transformation using our novel ex vivo system of VAT conditioned medium stimulated epithelial cell transformation (measured by growth in soft agar) and our in vivo murine lipemia model of ultraviolet light B (UVB)-induced, VAT promoted skin tumor formation. FgrR1+/− stable cells were generated by using CRISPR-Cas9 technology and were used to investigate the role of fibroblast growth factor-2 (FGF2) and FGFRI dependent signaling in neoplastic transformation, both in vitro and in vivo. Results: Only the VAT of obese mice fed a HFD [not VAT from low-fat diet (LFD) fed mice] stimulated neoplastic transformation of skin epithelial cells. Furthermore, human VAT stimulated both skin and mammary epithelial cell transformation. The differences in VAT activity between LFD and HFD fed mice and human donors were associated with the levels of FGF2. Circulating

SPMs in O3AEE or control diet fed mice

<table>
<thead>
<tr>
<th>Wt Control diet</th>
<th>Wt O3AEE diet</th>
<th>15-LOX-1-gut Control diet</th>
<th>15-LOX-1-gut O3AEE diet</th>
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<tr>
<td>18-HEPE</td>
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<td>RvD2</td>
<td>0.024 ± 0.024</td>
<td>0.17 ± 0.022</td>
<td>0.3 ± 0.077</td>
</tr>
</tbody>
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#2226 Sildenafil supresses tumorigenesis in ApexSmad3/− mouse model. Sarah K. Shurmeir, 1 Bianca N. Islam, 1 Yali Hou, 1 Allison Bridges, 1 Nagendra Singh, 1 Subbaramiah Sridhar, 1 Frankin G. Berger, 1 Darren D. Brownling, 1 Augusta University, Georgia Cancer Center, Augusta, GA; 2Augusta University, Medical College of Georgia, Augusta, GA; 3University of South Carolina, Columbia, SC.

The cGMP signaling pathway regulates homeostasis in the colon epithelium and has been implicated in the suppression of colorectal cancer. Exsulin is a weak phosphodiesterase-5 (PDE5) inhibitor that was previously shown to reduce polyp formation in familial adenomatous polyposis (FAP) patients. Despite this validation of PDE5 as a therapeutic target, the failure of Exsulin due to toxicity halted further trials of cGMP elevating agents. The present study tested the effect of the PDE5 inhibitor sildenafil, and the receptor guanylyl-cyclase C (GCC) agonist nitroguanylic acid on tumorigenesis in the ApexSmad3/− mouse model of colon cancer. Administration of either sildenafil or nitroguanylic acid to both wild type and ApexSmad3/− mice caused dramatic effects on homeostasis that included reduced proliferation and increased goblet cell differentiation. With treatment beginning at 4 weeks, sildenafil and nitroguanylic acid caused a 50% and 67% (respectively) reduction in the number of polyps per mouse. Furthermore, the polyps in sildenafil and nitroguanylic acid treated animals exhibited a reduced proliferative index and increased mucus density compared to polyps from untreated mice. The mean polyp size was not changed by treatment with either drug. In support of a tumor-suppressive role for cGMP signaling in the intestine, the ApexSmad3/− mice showed reduced levels of endogenous GCC agonists in the intestinal mucosa compared to wild type animals. This suggests that part of the tumor preventative effects observed here could be due to compensation for this deficit by augmenting cGMP signaling with PDE5 inhibitors or GCC agonists. While the tumor suppressive mechanism is not fully understood, the results presented here demonstrate in a preclinical model, that increasing intestinal cGMP levels by targeting PDE5 or GCC may be a viable chemoprevention strategy for human FAP patients.

#2227 Fgf2 from visceral adipose tissue stimulates neoplastic transformation of nonmalignant epithelial cells. Debrup Chakraborty, Venessa Benham, Elena Y. Demireva, Blair Bullard, Jamie J. Bernard. Michigan State University, East Lansing, MI.

Background: Adiposity plays a crucial role in the pathogenesis and prognosis of different types of cancers. Epidemiological evidence suggests visceral adipose tissue (VAT) and high-fat diets (HFD) are associated with increased cancer risk however the mechanism is not understood. The aim of this study was to explore the factors in VAT that stimulate neoplastic transformation. Methods: We modeled visceral adiposity-stimulated neoplastic transformation using our novel ex vivo system of VAT conditioned medium stimulated epithelial cell transformation (measured by growth in soft agar) and our in vivo murine lipemia model of ultraviolet light B (UVB)-induced, VAT promoted skin tumor formation. FgrR1+/− stable cells were generated by using CRISPR-Cas9 technology and were used to investigate the role of fibroblast growth factor-2 (FGF2) and FGFRI dependent signaling in neoplastic transformation, both in vitro and in vivo. Results: Only the VAT of obese mice fed a HFD [not VAT from low-fat diet (LFD) fed mice] stimulated neoplastic transformation of skin epithelial cells. Furthermore, human VAT stimulated both skin and mammary epithelial cell transformation. The differences in VAT activity between LFD and HFD fed mice and human donors were associated with the levels of FGF2. Circulating

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levels of FGE2 were associated with non-melanoma tumor formation in vivo. Human and mouse VAT failed to stimulate transformation in FGR1-/- cells and do not form tumors when injected in Nude mice in vivo. Conclusion: Collectively, our data show FGE2 released from VAT and its interaction with FGR1 is a novel and potential direct path of VAT-enhanced tumorigenesis. Blocking the FGR1/FGE2 interaction in VAT of abdominally obese individuals may be an important cancer prevention strategy as well as an adjuvant therapy for improving outcomes following cancer diagnosis.

#2288 Altering rictor with diosmetin reduces tumor progression in orthotopic prostate cancer model. Rebecca Pakradooni, Ahmad Khalifa, Riddhi Patel, Sanjeev Shukla. Case Western Reserve Univ., Cleveland, OH.

Rapamycin insensitive companion of mTOR (Rictor) is an essential subunit of mTORC2, maintains the integrity of the complex and functions as full activator of Akt. Rictor has been implicated to be involved in growth and progression of malignancies. Reports suggested that overexpression of Rictor in prostate cancer tissues, which may have potential role in prostate cancer progression. We demonstrated silencing Rictor in PC-3 cells decreased p-Akt (Ser-473) expression; conversely p-Akt (Thr-308) and p-PKC expression were increased. However, expression of mTOR complex 2, maintained the integrity of the complex and functions as full activator of Akt. Treatment of human prostate cancer DU145 and 22Rv1 cells, which possess high constitutive EZH2 expression, with 5-20 µM luteolin significantly inhibited EZH2 and SUZ12 protein expression in dose and time dependent manner, although luteolin treatment did not affect protein expression of EED and RBAP48. In addition, treatment of both cell lines with luteolin also reduced H3K27me3 and H3K27me2 and its enzymatic activity in dose and time dependent manner without affecting total H3 protein. In addition, luteolin was also effective in suppressing in vitro methylation performed using recombinant PRC2 complex. These events led to increase in the expression of downstream tumor suppressors including E-cadherin, SLIT2 and TIMP3, respectively. Interestingly, treatment of cells with proteasome inhibitor, MG132 together with luteolin did not prevent EZH2 degradation indicating that proteasomal degradation might not contribute to EZH2 inhibition. Taken together, our study suggests that luteolin acts on the catalytic binding site of EZH2 to exhibit downregulation of histone H3 methylation and could be developed as a potential promising agent for the prevention and/or treatment of various human cancers including prostate cancer with EZH2 overexpression.

#2231 15-Keto prostaglandin E2 inhibits STAT3 signaling in H-Ras-transformed mammary epithelial cells. Eun-Ji Lee,1 Young-Ill Hahn,2 Su-Jung Kim,1 Young-Joon Surh,1 Hye-Kyung Na.2 1Seoul National University, Seoul, Republic of Korea; 2Sungchun Women’s University, Seoul, Republic of Korea.

Prostanoid 15-keto PGE2 (15-kPGE2) is a potent lipid mediator that plays a key role in inflammation and carcinogenesis. The intracellular level of PGE2 is regulated by NAD+ dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH). This enzyme catalyzes the oxidation of the (15S)-hydroxy group of PGE2 to generate 15-keto PGE2. Down-regulation of 15-PGDH was observed in various malignancies and overexpression of 15-PGDH has been known to suppress carcinogenesis. However, mechanisms underlying anti-carcinogenic effects and tumor suppressive functions of 15-keto PGE2 are poorly understood in various cancer. H-Ras transformed mammary epithelial cell line MCF10A has greatly facilitated by the absence of a second gene copy. In addition, our studies demonstrate that diosmetin fed animals resulted in growth inhibition and induction of apoptosis by altering Rictor signaling cascade. We are demonstrating that diosmetin modulates molecular targets viz., Rictor and Akt to alter cellular events and elicit anticancer effects in prostate cancer cells.

#2229 Prevention by tofotenolic acid and characterization of the molecular targets in Pirc and FAP colorectal adenomas. Furkan U. Ertém, Wenzang Zhang, Kyle Chang, Wan-Mohaiza Dashwood, Praveen Rajendran, Deqiang Sun.1,2 1University of Pittsburgh Medical Center, Pittsburgh, PA; 2Texas A&M Univ. Health Science Ctr., Houston, TX.

A critical need exists in sporadic and high-risk colorectal cancer patients for endoscopic screening combined with safe and effective preventive agents. To prioritize molecular targets for prevention studies in vivo, RNA-seq analyses were conducted on colorectal adenomas from familial adenomatous polyposis (FAP) patients, and from the Apc-mutant polyposis in rat colon (Pirc) model. Using a new murine endoscopic polypectomy methodology (F.U. Ertém et al., Gastrointest Endosc 2016;83:1272-6), we evaluated agents that might substitute for the current standard of care. Sulindac, and identified tofotenolic acid (TA) as a promising candidate for further investigation. At clinically-relevant doses in the Pirc model, TA suppressed tumorigenesis significantly both in the small intestine and in the colon. Inhibition by TA of colonic crypt cell proliferation, and the concomitant activation of apoptosis, coincided with reduced expression in target tissues of β-catenin, cyclin D1, Survivin and matrix metalloproteinase 7. Based on the oncogene candidates defined by RNA-seq analysis, endoscopic tracking revealed the time-course of tumor suppression by TA, and the temporal changes in S100a9, Nppb, Aldh1a3 and Mmp7, highlighting their value as potential early biomarkers for prevention in the clinical setting. As an “old drug” repurposed from migraine, TA appears to offer an exciting new therapeutic avenue in FAP and other high-risk colorectal cancer patient populations. This work was supported in part by NIH grants CA090890, CA122959, ES00210, ES023512, the John S. Dunn Foundation, and a Chancellor’s Research Initiative.
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STAT3 has several cysteine residues in its activation sites. We observed that reducing agents N-acetyl-L-cysteine and dithiothreitol abrogated the suppressive effect of 15-keto PGE₂ on STAT3 phosphorylation, suggesting that the involvement of the thiol modification. Biotinylated 15-keto PGE₂ covalently bound to STAT3 as determined by immunoprecipitation with STAT3 f-Blocker by immunoblotting with streptavidin. Molecular docking analysis predicted Cys²⁵¹ and Cys²⁶⁹ residues of STAT3 as putative binding sites of 15-keto PGE₂. In conclusion, 15-keto PGE₂ inhibits STAT3 signaling through cysteine thiol modification, thereby suppressing MCF10A-ras cell growth and proliferation. Thus, tumor suppressor function of 15-PGDH is through cysteine thiol modification, thereby suppressing MCF10A-ras cell growth and proliferation. Therefore, agent that inhibits tumor growth caused by STAT3 phosphorylation has the potential to inhibit tumor growth. STAT3 has several cysteine residues in its activation sites. We observed that reducing agents N-acetyl-L-cysteine and dithiothreitol abrogated the suppressive effect of 15-keto PGE₂ on STAT3 phosphorylation, suggesting that the involvement of the thiol modification. Biotinylated 15-keto PGE₂ covalently bound to STAT3 as determined by immunoprecipitation with STAT3 f-Blocker by immunoblotting with streptavidin. Molecular docking analysis predicted Cys²⁵¹ and Cys²⁶⁹ residues of STAT3 as putative binding sites of 15-keto PGE₂. In conclusion, 15-keto PGE₂ inhibits STAT3 signaling through cysteine thiol modification, thereby suppressing MCF10A-ras cell growth and proliferation. Thus, tumor suppressor function of 15-PGDH is through cysteine thiol modification, thereby suppressing MCF10A-ras cell growth and proliferation. Therefore, agent that inhibits tumor growth caused by STAT3 phosphorylation has the potential to inhibit tumor growth.


The role of dietary flavonoid intake in colorectal carcinogenesis might differ according to flavonoid subclasses and individual genetic variants related to carcinogen metabolism. Therefore, we examined whether greater habitual dietary intake of flavonoid subclasses was associated with a lower risk of colorectal cancer and whether CYP1A1 genetic variants altered this association. A semi-quantitative food frequency questionnaire was used to assess the dietary intake of six flavonoid subclasses (flavonols, flavones, flavanones, flavan-3-ols, anthocyanins, and isoflavones) in 923 patients with colorectal cancer and 1,846 controls; furthermore, CYP1A1 genetic variants (rs4646903 and rs1049843) were genotyped. A logistic regression was used to investigate dietary intake, CYP1A1 genetic variants, and their interaction in relation to colorectal cancer risk. Among the subclasses of flavonoids, flavonols (OR [95% CI] = 0.10 [0.06, 0.16], highest vs. lowest quartile) and flavan-3-ols (OR [95% CI] = 0.54 [0.41, 0.71], highest vs. lowest quartile) were significantly associated with colorectal cancer risk after adjusting for confounders. Homozygous variants of CYP1A1 rs4646903 showed a protective effect on the risk of rectal cancer (OR [95% CI] = 0.60 [0.37, 0.96], CC vs. TT/TC). Carriers of the CC homozgyous variant with high flavonol intake showed a greater decrease in colorectal cancer risk compared with T allele carriers with low flavonol intake (P for interaction = 0.02), particularly regarding rectal cancer (P for interaction = 0.005). In conclusion, the effect of dietary flavonoid intake on colorectal cancer risk differs by flavonoid subclasses and CYP1A1 genetic variants.

#2233 The fatty acid amide hydrolase inhibitor URB937 ameliorates radiation-induced lung injury in a mouse model. Rui Li, Jianxin Xue, You Lu. West China Hospital, West China School of Clinical Medicine, Chengdu, China.

Radiation-induced lung injury (RILI) is a common and potentially serious complication from radiotherapy to the thoracic region. In the current study, we examined the potential protective effects of URB937, an inhibitor of fatty acid amide hydrolase in a mouse model of RILI. Here we challenged male C57BL/6 mice with irradiation (16Gy to the thoracic region), and then injected intraperitoneally either URB937 (1 mg/kg) or vehicle three times a week for 30 days. Mice were sacrificed either at the end of treatment or 120 days from irradiation to evaluate the extent of RILI and mice survival. Potential effects of URB937 on irradiation induced tumor growth inhibition were also evaluated. We found URB937 increased endocannabinoids in the lungs, attenuated the extent of RILI, prolonged the survival. Inhibited mice developed pathological alterations of pneumonitis and lung fibrosis. Radiation-induced increases of proinflammatory and profibrotic cytokines were decreased, including interleukin-1β, interleukin-6, tumor necrosis factor-α, transforming growth factor-β1 in plasma and lung tissue. Moreover, malondialdehyde in plasma and inflammation cells in bronchoalveolar lavage fluid were lower in mice treated with irradiation plus URB937. URB937 did not affect tumor growth inhibition caused by irradiation. These results suggested that inhibiting fatty acid amide hydrolase through URB937 could ameliorate the RILI, without affecting the efficacy of irradiation on tumor control.

#2234 Efatazutane reduces mammosphere formation in Brca1WT/Bl1+Ctcp/p53+/+ and Brca1Bl1/Bl1+Ctcp/p53−/− mice. Sahar J. Alothman, Shan Chao, Weisheng Wang, Priscilla A. Furth. Georgetown University, Washington, DC.

Introduction: Efatazutane, a PPAR gamma agonist, may affect tumor growth through the induction of terminal cell differentiation. Thus, we hypothesized that efatazutane could affect progenitor cell number. Here, we evaluate progenitor cell number by measuring mammospheres, which is considered one way to measure potential progenitor cells. Methods: Two month old Brca1WT/Bl1+Ctcp/p53+/+ (n = 14) and Brca1Bl1/Bl1+Ctcp/p53−/− (n = 10) C57B/6 mice were randomly placed on either control or treated with efatazutane through the diet (30 mg/kg concentration, F3028, rodent diet, grain-based, 1/2-in pellets; Bio-Serv, Frenchtown, NJ) with necropsy at four months and isolation of primary mammary epithelial cells from thoracic glands for studies of mammosphere formation using SCIVAX 96-well low adhesion nanoculture plates (Organogenix, Inc., Vancouver, Canada) with 0.1,5, and 10% fetal bovine serum (FBS) added. Thoracic mammary gland tissue was frozen at -20°C followed by isolation of RNA and evaluation of PPAR gamma pathway gene expression by real-time RT-PCR using TaqMan® Array Mouse Lipid Regulated Genes (Thermo Fisher Scientific, Inc., Waltham MA). One inguinal gland was fixed for mammary gland whole mount and the other inguinal gland was formalin-fixed and paraffin embedded for histology. Results: SCIVAX nanoculture plates showed reproducible increases in mammosphere numbers with increasing FBS concentrations (p < 0.05 one-way, Kruskal-Wallis). An increased significant increase in sphere number was seen in Brca1WT/Bl1+Ctcp/p53+/+ compared to Brca1Bl1/Bl1+Ctcp/p53−/− (p < 0.05 one-way, Kruskal-Wallis). Efatazutane treatment significantly decreased sphere numbers in Brca1WT/Bl1+Ctcp/p53+/+ and in Brca1Bl1/Bl1+Ctcp/p53−/− mice (p < 0.05 one-way, Kruskal Wallis). Expression of PPAR gamma pathway genes were increased at the RNA level with statistically significant increases in Acdavl, Tnf, Alox5 (p < 0.05) and Il1B, Srebf2, Hmgs1, Hmrg (p <0.01) genes (Bootstrap Ratio, http://rht.iconcologia.net/stats/br/several.html) in mice on efatazutane as compared to control diet. Discussion: SCIVAX nanoculture plates can be used to quantitatively evaluate and compare mammosphere numbers between different genotypes and treatment groups. The higher numbers of mammospheres found with loss of both Brca1 copies as compared to one copy is consistent with previously published literature. The decrease in mammosphere numbers with efatazutane treatment could be secondary to its differentiating impact on mammary epithelial cells. Conclusion: While efatazutane statistically significantly reduced mammosphere numbers, the absolute reduction was less the 25%.

#2235 Preventive effects of the sodium glucose cotransporter 2 inhibitor tofogliflozin on liver tumorigenesis in obese and diabetic mice. Yohei Shirakami, Koki Obara, Masaya Ohnishi, Takayasu Ideta, Hiroyasu Sakai, Takuki Tanaka, Masahito Shimizu, Mitsuuru Seishima. Gifu Univ. Graduate School of Medicine, Gifu, Japan.

Obesity, diabetes mellitus, and their related metabolic abnormalities are associated with increased risk of hepatocellular carcinoma (HCC). Sodium glucose cotransporter (SGLT)-2 inhibitors, recently approved anti-diabetic agents, are expected to ameliorate the abnormalities associated with metabolic syndrome including non-alcoholic fatty liver disease (NAFLD). In this study, we investigated the effects of the SGLT2 inhibitor tofogliflozin on the development of NAFLD-related liver tumorigenesis in C57BL/KsJ-Leprdb/+ Leptin−/− mice (db/db) obese and diabetic mice. The direct effects of tofogliflozin on human HCC cell proliferation were also evaluated. Male db/db mice were administered diethyl-nitrosamine-containing water for two weeks and were treated with tofogliflozin throughout the experiment. Tofogliflozin was kindly provided by Kowa Co., Ltd. and the chemical structure will be disclosed at the time of presentation at the meeting. In mice treated with tofogliflozin, the development of hepatic pre-cancerous lesions was found to be retarded, and the proliferation of SGLT2 protein-expressing HCC cells was not inhibited by this agent. These findings suggest that tofogliflozin suppressed the early phase of obesity- and NAFLD-related liver carcinogenesis by attenuating chronic inflammation and steatosis in the liver, while the agent had no significant direct effect on the proliferation of HCC cells. Therefore, SGLT2 inhibitors may have a chemopreventive effect on obesity-related HCC.

#2236 High-throughput screen for chemopreventative agents that influence adipose-tissue driven epithelial neoplastic transformation. Vanessa Benham, Debrup Chakraborty, Blair Bullard, Thomas S. Drexheimer, Jamie J. Bernard. Michigan State University, East Lansing, MI.

Epidemiological studies have established a link between excess adiposity and cancer risk. Our previous data demonstrate that adipose tissue stimulates the transformation (steps a cell undergoes to become malignant) of both skin and mammary epithelial cells as measured by anchorage-independent growth in soft agar. Therefore, agents that influence growth in soft agar may be used therapeutically for skin and breast cancer prevention. However, soft agar in a 24-well format is unsuitable for high-throughput screens. Our objective was to develop a high-throughput assay to evaluate chemopreventative agents that influence...
Novel selenium-containing aspirin molecule AS-10 suppresses an- 
drogen receptor signaling and induces apoptosis of LNCaP prostate cancer cells. Deepkamal N. Karelia, Sangyub Kim, Srinivasa Ramisetti, Cheng Jiang, Shantu Amin, Arun K. Sharma, Junxuan Lu. Penn State College of Medicine, Hershey, PA.

Aspirin as the best known low cost Non-Steroidal Anti-inflammatory drug (NSAID) has been associated with lowering risk of colorectal cancer, in addition to its cardiovascular health benefit at low dose (baby Aspirin) and over-the- 
counter pain medication. Through an innovative research program aiming at enhancing the bioactivities of NSAIDs by incorporation of selenium, we have discovered AS-10 as a novel Aspirin-selenium compound with promising anti- 
cancer drug-like properties in terms of potency enhancement (at least 3 orders of magnitude compared to Aspirin), selective cytotoxicity against cancer cells in- 
cluding majority of NCI-60 panel, while sparing normal mouse embryonic fibro- 
basts in cell culture screening assays. Preliminary toxicity study in mice had shown a wide safety margin. Extensive structure-activity relationship investiga- 
tion suggested a novel structural basis, instead of releasable selenium, that ac- 
counted for the striking potency. To investigate the potential chemopreventive attributes of AS-10 against prostate carcinogenesis, we selected LNCaP cells (wild type p53, functional androgen receptor, AR) to examine the growth sup- 
pression and apoptosis responses in cell culture. The rationale for choosing LNCaP cell line as target cells rests in the fact that precancerous prostatic lesions that are the intended targets of chemoprevention as well as early stage prostate cancer retain wild type p53 and they are critically dependent on AR signaling for survival. Our results show that cell viability (MTT) test detected a potent growth inhibition of LNCaP cells in a time and concentration dependent manner, with an EC50 in range of 1.7 to 2.5 μM compared to Aspirin. We found the lead compound 15k, as determined by using molecular biology assays, was found to be due to inhibition of apoptosis, tubulin inhibition, and inhibition of stemness pathways (Wnt-β-catenin-EMT). The 15k had significant anti-metastatic effects as shown by their inhibition of migration and invasion potential on OC cells. In silico findings suggest that pharmacokinetic [PK] profiles of these compounds are favorable compared to Aspirin. Further in vivo PK/pharmacodynamic studies are underway to establish the clinical use of lead molecule in advanced me- 
tastasized, drug resistant OC patients.

Establishment of lung cancer CTCs competent for lung metastasis. Zujun Que,1 Bin Luo,2 Qihui Shi,1 Jianhui Tian.2 Shanghai Institute of Tradit- 
tional Chinese Medicine, Institute of TCM Oncology, Shanghai, China;1 Longhua Hospital, Shanghai University of Traditional Chinese Medicine, National Clinical Research Centre for Traditional Chinese Medicine and Oncology, Shanghai, Chi- 
na;2 Systematic Biomedical Research Center of Shanghai Jiao Tong University, Shanghai, China.

Circulating tumor cells (CTCs) have been implicated as a population of cells that may seed metastasis, which led to the 90% of dead in cancer patients. Though the detection technologies has been developing swiftly, how to culture the cells successfully and established a permanent cell line are the bottleneck in the research of metastasis mechanism. Here, we report, for the first time, the establishment of permanent cell line from CTCs of one Non-small cell lung cancer patient and identifying the biological characterization. The cell line design- 
ated CTC-0430 has been cultured for more than one and half years, and the cells have been characterized at the genome, proliferation, drug resistance, the expression of surface marker, cytokine secretion level and the characterization of tumor formation in vivo by immunohistochemistry and immunofluorescence. This thorough analysis showed that CTC-0430 cells display a stable phenotype characterized by an intermediate epithelial/mesenchymal phenotype, stem-cell like properties and the drug sensitivity test indicating stronger drug resistance compared to A549 and 95-D. When compare with A549 and 95-D cells, CTC- 
0430 cells high expression of ICAM-1 and ICAM-3, low expression of CXCL12 and CXCL1. Functional studies showed that CTC-0430 cells induced rapidly in vivo tumors after subcutaneous transplantation in immunodeficient mice, and the lung metastasis were found after six months later. Besides, three months after we injected the cells into the caudal vein of NOD/SCID mice, the lung metastasis were formed. And then the metastasis lung tissue were used to make IHC and IF, which indicated that the biological characterizations of this cell line are different from A549. The establishment stable cell line of lung cancer CTCs can provide in vivo research platform for the intervention study of postoperative metastasis of lung cancer.
induced cell death and attenuated p53, p21 and p-H2AX. Moreover, siRNA knockdown of p53 did not suppress ROS production, but decreased ISC-4-induced Annexin V-positive apoptosis, PARP cleavage and the DDR proteins. Taken together, these data suggest that ISC-4 inhibits LNCaP cell growth and survival with concomitant suppression of AR signaling and induction of ROS- and p53-dependent apoptosis. Given the importance of AR signaling in prostate epithelial survival, cancer genesis and progression, and the preservation of wild type p53 in precancerous lesions and early stage prostate cancer, our findings suggest attractive chemopreventive potential of ISC-4 against prostate carcinogenesis.

#2244 Pharmacological TLR4 antagonism using topical resatorvid blocks solar UV-induced skin tumorigenesis in SKH-1 mice. Sally E. Dickinson, 1 Karen Blehm-Mangone, 2 Nichole B. Burkett, 2 Shehla Tahsin, 2 Paul B. Myrdal, 1 Kelly L. Karlage, 1 Jaroslav Janda, 2 Kathylynn Saboda, 2 Denise J. Roe, 1 Zigang Dong, 1 Ann M. Bode, 2 Emanuel F. Petricoin, 1 Valerie S. Calvert, 2 Clara Curiel-Lewandrowski, 1 David S. Alberts, 1 Georg T. Wondrak 1. 1Department of Pharmacology, University of Minnesota, Austin, MN; 2Department of Biopharmaceutical Sciences, University of Minnesota, Minneapolis, MN. An urgent need exists for the development of more efficacious molecular strategies targeting non-melanoma skin cancer (NMSC), the most common malignancy worldwide. Inflammatory signaling downstream of Toll-like receptor 4 (TLR4) is a causative factor in several forms of tumorigenesis, yet its role in solar UV-induced skin carcinogenesis remains undefined. In our recently published work based upon immunohistochemical (IHC) analysis of NMSC tissue microarrays and proteomic analysis of reverse-phase protein microarrays (RPMA) from banked human tissue, we observed increased TLR4 expression in association with tumorigenic progression from normal skin to actinic keratosis and cutaneous squamous cell carcinoma. In immortalized keratinocytes expressing luciferase reporter constructs, resatorvid potently inhibits UV-induced AP-1 and NF-κB signaling, associated with downregulation of inflammatory mediators (IL-6, IL-8, IL-10) and MAP Kinase phosphorylation. In a subsequent acute exposure model in SKH-1 mice, topical resatorvid efficiently antagonized UV-induced stress signaling while potentiating UV-induced epidermal apoptosis. In the current study, we show for the first time that pharmacological inhibition of TLR4 using the specific antagonist resatorvid (TAK-242) blocks UV-induced tumorigenesis in SKH-1 mice. After assessing photostability, efficient cutaneous delivery, and skin residence time of topical resatorvid, we then tested the feasibility of TLR4-directed inhibition of UV-induced tumorigenesis. To this end, SKH-1 mice were split into three groups (n = 20). Each group was exposed to solar-simulated UV (three times a week; 15 weeks duration), followed by another 10 weeks in the absence of UV exposure (sacrifice at week 25). The control group received topical vehicle (acetone) on their backs 1 hour prior to UV exposure (three times a week until sacrifice). The “Prevention” group received topical resatorvid following an analogous dosing regimen. The “In-
tivation" group received vehicle 1hr prior to each UV treatment but switched to topical resatorvid three times a week only after UV was terminated. In the Prevention model, topical treatment with resatorvid significantly inhibited both tumor area (66% reduction, p < 0.0052) and tumor multiplicity (32% reduction, p = 0.0329; cross sectional Kruskal-Wallis test). In the Repair model, resatorvid, a marketed anti-inflammatory drug, did not yield was observed but it did not reach statistical significance. Likewise, suppression of UV-induced TLR4-driven signaling was also confirmed in murine skin harvested at week 14 (prior to tumor onset), as assessed by IHC and RPPA analysis. Taken together, these data generated using resatorvid in a murine photocarcinogenesis model suggest that pharmacological TLR4 antagonism may represent a novel molecular strategy for topical prevention of solar UV-induced NMSC.

#2245 Evaluation of new rexinoids for lung cancer. Di Zhang, 1 Ana S. Leal, 1 Sarah E. Carapellucci, 1 Kayla Zydeck, 1 Nicole Chaaban, 1 Michael B. Sporn, 2 Carl E. Wagner, 3 Karen T. Liby, 1 Michigan State University, East Lansing, MI; 2Dartmouth Medical School, Hanover, NH; 3Arizona State University, Phoenix, AZ.

Lung cancer remains the leading cause of cancer deaths around the world. It is estimated that there will be over 150,000 deaths in the United States alone from this disease every year. With no significant improvement in 5 year survival rates over the past 30 years, effective prevention or early intervention is a promising approach to reduce the high mortality of lung cancer. Rexinoids are selective ligands for retinoid X receptors (RXR), which regulate the expression of a wide variety of genes. As transcription factors, rexinoids play important roles in proliferation, differentiation and apoptosis, which are highly relevant in cancer. Bexarotene is the only synthetic rexinoid that has been approved by the FDA and is used for the treatment of refractory cutaneous T-cell lymphoma. It has also been tested in various clinical trials for lung cancer and breast cancer. However, Bexarotene is not potent or selective enough for RXRs, causing limited efficacy and unacceptable toxicities. Therefore, we synthesized a series of new rexinoids and screened them for their ability to inhibit nitric oxide (NO) production in RAW264.7 macrophage-like cells stimulated with LPS. Several of these compounds inhibited NO production at nanomolar concentrations. Based on this screening and other in vitro assays, two new rexinoids were chosen for in vivo testing. Female A/J mice were injected i.p. with vinyl carbamate (16 mg/kg). One week later, the mice were fed control diet or rexinoids in diet (40-80 mg/kg diet) for 16 weeks. Tumor number, size and histopathology were then evaluated. All of the rexinoids reduced the number and size of the lung tumors. However, Bexarotene only reduced the average number of tumors by 8-17%, while a new pyrimidine-analog of Bexarotene—henceforth, pyrimidine-Bexarotene—reduced the number of tumors by 24-28%. The average tumor burden on lung sections was also reduced by pyrimidine-Bexarotene by 25% compared to the controls (from 0.47 ± 0.19 mm3 in the control group to 0.19 ± 0.05 mm3 in the treated group). In contrast, average tumor burden was 0.35 ± 0.08 mm3 in the group treated with Bexarotene, a reduction of 26%. Notably, the percentage of high grade tumors (both histological and nuclear characteristics) were significantly (p < 0.05) higher in the mice fed Bexarotene (68%) compared to the control group (38%), while the percentage of high grade tumors was only 34% in the pyrimidine-Bexarotene group. Since a major side effect of rexinoids is a elevation in triglyceride levels, we measured total triglycerides in both liver and plasma. Pyrimidine-Bexarotene induced a 24% elevation in triglycerides in plasma. However, Bexarotene only induced a 7% elevation in plasma triglycerides, which is high when compared to the 34% elevation in liver triglycerides. Pyrimidine-Bexarotene, a new rexinoid with TXR1 selectivity and significant antitumor activity, is a promising agent for the treatment of lung cancer.

#2246 Anti-proliferative activities of lipid fraction of extract from the skin of the catfish Arius Bilineatus, Valenciennes. Peiyung Yang, 1 Jibin Ding, 1 Yong Pan, 1 Yan Jiang, 1 Mohammad Afzal, 1 Bincy M. Paul, 1 Somasoma G. George, 1 Assam M. Al-Hassan, 1 UT MD Anderson Cancer Ctr., Houston, TX; 2Kuwait University, Safat, Kuwait; 3CMS College, Kottayam, Kerala, India.

The catfish (Arius bilineatus, Val.) secretes a gelatinous substance composed of biochemically active lipids and proteins from its skin upon stress or injury. Preparations from the skin have previously been shown to affect blood clotting and accelerates healing of non-healing diabetic foot ulcers in man. We have reported previously fish skin preparation (CSP) derived from the skin of the Catfish and it’s lipid fraction exerted anti-inflammatory activity in the in vitro cells and in vivo animal models. Here anti-proliferative effect of a lipid fraction of fish skin preparation (CSP-L) derived from the skin of the catfish and its plausible mechanism were investigated in human hepatocellular carcinoma (HCC) Hep3B and human pancreatic cancer Panc-1 cells. Cells were treated with CSP-L (0 to 100 µg/ml) for 72 hrs and cell proliferation was measured by the MTT assay. The results showed much stronger inhibition by CSP-L in Panc-1 cells than that of Hep3B cells with IC50 of 5.3 ± 1.4 µg/ml and 19.5 ± 7.7 (Clone F7) respectively. CSP-L (25 - 100 µg/ml) led to G1 phase arrest in Hep3B cells whereas S phase arrest was observed in Panc-1 cells, suggesting the differential molecular mechanisms responsible for CSP-L induced cell growth suppression in Hep3B and Panc-1 cells. The alteration of cell cycle was concentration dependent. Additionally, CSP-L concentration dependently suppressed the invasion of Hep3B and Panc-1 cells. The anti-invasion mechanism with CSP-L was concentration dependent and was examined by Western blotting in both Hep3B and Panc-1 cells. Intriguingly, CSP-L (50 and 100 µg/ml) notably decreased protein levels of cyclin D, Stat3, p53 and pERK in Hep3B cells in a concentration dependent manner. In contrast, only pRB and pERK protein expression were reduced in CSP-L treated Panc-1 cells. These results suggest that CSP-L inhibited the proliferation of Hep3B and Panc-1 cells by different molecular mechanisms. Collectively, our preliminary data suggest that CSP-L has a great potential to be developed as an anticancer or preventive agent for both HCC or pancreatic cancer and warrants further investigation. This study was supported by a grant from Kuwait Foundation for the Advancement of Science No. KFAS 2013-127071-A, D, and Kuwait University Research Grant No. SLO314.

EPIDEMIOLOGY: Biomarkers of Risk and Prognosis

#2247 Associations between total T-lymphocytes and colorectal cancer survival in a prospective cohort study of older women. Anna E. Prizment, 1 Robert A. Vierkant, 1 Thomas C. Smyrk, 1 Lori S. Tillmans, 2 Heather H. Nelson, 2 Charles F. Lynch, 3 Stephen N. Thibodeau, 1 Timothy R. Church, 1 James R. Cerman, 1 Kristin E. Anderson, 1 Paul J. Limburg, 2 University of Minnesota, Minneapolis, MN; 3Mayo Clinic, Rochester, MN; 1University of Iowa, IA.

Background: The increased number of T-lymphocytes residing in the tumors of colorectal cancer (CRC) patients is consistently shown to predict better survival of CRC patients independent of stage. In addition to tumor itself, T-lymphocytes also infiltrate normal tissues adjacent to tumors; however, little is known about how the interplay between T-cell variability in tumor-adjacent tissues affects CRC prognosis. Our goal was to characterize total T-cells in colorectal tumor and tumor-adjacent tissues and study their associations with all-cause and CRC-specific survival in the Iowa Women’s Health Study. Methods: We constructed tissue microarrays and quantified CD3 antibody staining (clone F.7.238; Dako), an established marker for total T-cells, in paraffin-embedded tissue samples from 463 women diagnosed with incident CRC from 1986-2002 (mean age at diagnosis was 74 years). Up to 3 tumors and 3 tumor-adjacent cores were immunostained for each patient. An experienced pathologist quantified CD3+ T-cells in each area using 4 categories: non-detected, mild (1-10 cells per 0.28 mm2); moderate (11-29 cells per 0.28 mm2); and strong infiltrations (>30 cells per 0.28 mm2). The obtained scores were averaged over all quintiles of scores for each core per patient. Cox regression was used to estimate the hazard ratio (HR) and 95% CI for all-cause and CRC mortality in relation to (1) tumor score; (2) tumor-adjacent score; and (3) the ratio of tumor to tumor-adjacent score, hereafter called Score ratio. All scores were categorized in quartiles. The final model adjusted for age at diagnosis, SEER stage, tumor grade, body mass index, race, and smoking history. Results: During follow-up for a maximum of 25 years, 67% of participants diagnosed with CRC died (≈30% died from CRC). There was a weak but significant correlation between CD3 tumor and tumor-adjacent scores (Spearman coefficient: r = 0.15, p = 0.02). CD3 tumor score was inversely associated with stage and was higher in those with proximal colon cancer, and for MSI-high, CIMP-high and BRAF-mutated tumors. The HRs (95%CI) for the highest versus lowest category of CD3 tumor score were 0.61 (0.44-0.84) (p-trend = 0.003) for all-cause mortality and 0.27 (0.15-0.48) (p-trend < 0.0001) for CRC-specific mortality. The tumor-adjacent score was not associated with any characteristics or death. The associations between the Score ratio and all-cause and CRC survival mirrored those for the tumor score but were weaker. The HRs (95%CI) for the highest versus lowest quartile of the Score ratio were 0.70 (0.45-1.09) (p-trend = 0.09) for all-cause and 0.45 (0.23-0.87) (p-trend = 0.003) for CRC-specific mortality. Conclusions. Based on data from prospectively identified CRC cases in the IWHS cohort, colorectal tumor T-cell infiltration is associated with improved survival. Further investigation is needed to determine the T-cell subtypes that are most predictive of CRC survival outcomes.
EPIDEMOIOLOGY: Biomarkers of Risk and Prognosis

#2248 Common TDP1 polymorphisms in relation to survival among small cell lung cancer patients in a multicenter study from the International Lung Cancer Consortium. Pawadee Lohavanichbutr,1 Lori C. Sakoda,2 Christopher I. Amos,3 Susanne M. Arnold,4 David C. Christiani,5 Michael P. Davies,6 John K. Field,7 Eric B. Haura,8 Takashi Kohno,9 Maria Teresa Landi,10 Zhi Li,11 Aileen Baecker,1 Helena R. Field,6 Eric B. Haura,7 Rayjean J. Hung,9 Marilyn Kwan,1 Jingjie Xiao,1 Stacey Alexeef,7 Erin K. Weltzien,1 Adrienne L. Castillo,2 Bette J. Caan,1 Kaiser Permanente Division of Research, Oakland, CA;1 Dana Farber Cancer Institute, MA;2University of Alberta, Edmonton, Alberta, Canada;3University of California, Berkeley, CA.

Introduction: DNA topoisomerase inhibitors (e.g. etoposide, irinotecan, teniposide, topotecan) are commonly used for treating small cell lung cancer (SCLC). Tyrosyl-DNA phosphodiesterase (TDP1) plays a role in repairing DNA damage caused by DNA topoisomerase inhibitors and it is believed to be responsible for resistance to this class of drugs. The purpose of this study was to determine whether TDP1 polymorphisms or survival-associated polymorphisms were associated with survival among SCLC patients. Method: Two common TDP1 SNPs (rs942190 and rs241863) were associated with poorer survival compared to those carrying both major alleles (AA), with a hazard ratio (HR) of 1.36 (95% confidence interval [CI]: 1.07-1.75, p-value=0.001), but there was no association with survival for patients with the heterozygous genotype (HR=1.02, 95% CI: 0.83-1.27, p-value=0.83). For rs241863, patients carrying both major alleles (CC) tended to have better survival than patients carrying AA alleles, but this was within the limits of chance given no significant association (HR=0.97, 95% CI: 0.61-1.02, p-value=0.07). In addition, multi-locus expression quantitative trait loci (eQTL) analyses from the Genotype-Tissue Expression Project indicated a potential effect of rs942190 on lung tissue, with higher TDP1 gene expression for the GG than AG or AA genotypes. Conclusion: We found that among SCLC patients the TDP1 rs942190 GG genotype was associated with poor overall survival. Further study could determine if this genotype can serve as a predictive marker for the treatment efficacy of DNA topoisomerase inhibitors in SCLC.

#2249 Integrated multi-level omics-based biomarker models for ovarian cancer survival. Alan Pa,1 Shen-Chih Chang,2 Aileen Baeker,1 Helena R. Chang,3 Zuo-Feng Zhang,1 UCLA Fielding School of Public Health, Los Angeles, CA;2David Geffen School of Medicine at UCLA, Los Angeles, CA.

Background: Although a number of prognostic biomarkers for ovarian cancer have been proposed, the pool of clinically useful biomarkers remains small. While the predictive power of individual biomarkers may be weak, the advent of more affordable high-throughput technologies and readily available ‘omics’ level data point toward the possibility of high performance biomarker panels. In this study, we used tumor-based somatic mutation, mRNA and miRNA expression, and DNA methylation data from The Cancer Genome Atlas (TCGA) to construct integrated biomarker models for overall and progression-free survival. Methods: The somatic mutations, mRNA expression, and DNA methylation of 451 candidate genes, as well as the expression of the miRNAs targeting them, were individually analyzed for association with overall and progression-free survival in 488 TCGA serous cystadenocarcinoma patients of predominantly Caucasian descent. Following correction for multiple comparisons, molecular variations associated with survival were combined to construct integrated cross-validated prediction models for overall and progression-free survival. The discriminative power of these models was then evaluated for 1-, 3-, and 5-year overall and progression-free survival using ROC analysis. Results: Our cross-validated integrated molecular models were demonstrated to predict 5-year overall survival in the total patient pool with a ROC AUC of 0.801 and 5-year overall and progression-free survival in Caucasian patients with ROC AUCs of 0.866 and 0.809, respectively. Conclusions: Our findings suggest the potential utility of our multi-omics-based biomarker models in helping to inform clinical decisions following ovarian cancer diagnosis. Although extensive efforts were made to maximize the predictive potential of our models, further retrospective and prospective validation efforts are necessary before their clinical utility can be accurately assessed.

#2250 Systemic inflammation and sarcopenia predict colorectal cancer survival. Elizabeth M. (Cespedes) Feliciano,1 Candicey H. Krowen,2 Jeffrey Meyerhardt,3 Carla M. Prado,4 Patrick T. Bradshaw,5 Marilyn Kwan,1 Jingjie Xiao,1 Stacey Alexeef,7 Erin K. Weltzien,1 Adrienne L. Castillo,2 Bette J. Caan,1 Kaiser Permanente Division of Research, Oakland, CA;1 Dana Farber Cancer Institute, MA;2University of Alberta, Edmonton, Alberta, Canada;3University of California, Berkeley, CA.

Importance: A higher neutrophil-to-lymphocyte ratio (NLR, indicating systemic inflammation), and sarcopenia (reduced skeletal muscle mass) predict morbidity/mortality in a variety of cancers, but no prior research examines associations of pre-diagnostic NLR with at-diagnosis sarcopenia, nor whether NLR and sarcopenia combined identify early-stage patients with poor prognosis in colorectal cancer (CRC).Objective: To evaluate the association between pre-diagnosis NLR and at-diagnosis sarcopenia of their combination with CRC survival, controlling for age, ethnicity, sex, body mass index, stage, and cancer site. Design, Setting, and Participants: This observational cohort with prospectively-collected data included 2470 patients diagnosed with stage I-III CRC at Kaiser Permanente from 2006-2011 with computed tomography (CT) scans from clinical care (mean days pre-diagnosis=6). Exposures: Systemic inflammation measured via average NLR in the 24 months pre-diagnosis (mean count=3 measures, mean months pre-diagnosis=7). The reference value for NLR below 3, indicating low/no inflammation. Main Outcomes and Measures: Sarcopenia, defined by published cutoffs for skeletal muscle index (CT muscle area in cm2 at the third lumbar vertebra divided by squared height in m2; below 52-cm2/m2 and 38-cm2/m2 for normal/overweight men and women, respectively, and below 54-cm2/m2 and 47-cm2/m2 for obese men and women, respectively), and incident death (overall or CRC-related). Results: Average age was 63 years; half of patients were female. NLR above 3 and sarcopenia were common (46% and 44%, respectively). Over a median of 6 years, we observed 656 deaths, 357 from CRC. Elevated NLR was associated with sarcopenia in a dose-response manner (compared to NLR below 3, Odds Ratio [OR]=1.33; 95% CI:1.10-1.67 for NLR 3-5; OR=1.47; 95%CI:1.16-1.85 for NLR above 5). NLR above 3 and sarcopenia were also independently associated with survival (Hazard Ratio [HR]=1.15; 95% CI:1.45-1.98 for overall death and HR=1.29; 95% CI:1.09-1.53 for CRC death); patients with both sarcopenia and NLR above 3 (versus neither), had double the risk of death overall (HR=2.53; 95% CI:1.87-3.41) and from CRC (HR=2.19; 95% CI:1.74-2.75). Conclusions and Relevance: Host inflammatory/immune response shortly prior to diagnosis predicts muscularity at diagnosis. Low muscle combined with systemic inflammation predicts worse CRC prognosis regardless of stage. A better understanding of the crosstalk between inflammatory/immune responses and the onset of changes in skeletal muscle may open new therapeutic avenues to improve cancer outcomes.

#2251 Tumor tissue gene expression in association with survival of triple-negative breast cancer. Shiyang Wang,1 Quiyin Cai,1 Hui Cai,1 Pingping Bao,1 Jie Wu,1 Fei Ye,1 Wei Zheng,2 Ying Zheng,2 Xiao-Ou Shu,1 Vanderbilt University School of Medicine, Nashville, TN;2Shanghai Municipal Center for Disease Control and Prevention, Shanghai, China.

Triple-negative breast cancer (TNBC) is an aggressive type of cancer with limited treatment options. Previous studies have shown that gene expression profiles were associated with TNBC prognosis. Information on specific genes that are predictive of TNBC outcome is limited. Using data and samples from a cohort of 469 TNBC cases from Shanghai Breast Cancer Survival Study (SBCC), we systematically evaluated expressions of 302 genes in tumor tissue with survival of TNBC. Genes included in the study are PAM50 genes, genes encoding drug metabolizing enzymes and, genes implicated in TNBC biology and progression based on literature reviews. Gene expression levels were measured in total RNA isolated from formalin-fixed paraffin-embedded breast cancer tissues using the NanoString nCounter assay. Cox regression was applied to evaluate disease-free survival (DFS) and overall survival (OS) in association with gene expression. Analysis was adjusted for known predictors of TNBC outcome, including age, disease stage, basal like subtype and DUSP4 gene expression. During a median follow-up of 5.3 years (range: 0.7-8.9 years), 100 deaths and 92 recurrences/breast cancer deaths were documented. Expression levels of 17 genes were significantly associated with OS, and 15 genes with DFS (P<0.05). The top 5 most significant genes are EOMES (Hazard Ratio, HR = 0.88; 95% Confidence Interval, 95% CI:0.82-0.96), GZMK (HR = 0.91, 95% CI:0.85-0.97),
inated by Fusobacterium may indicate a poor prognosis following chemoradiation. Prevotella may serve as a biomarker of improved prognosis, while profiles dominated by Treponema (cluster 4). Fungal ITS profiling revealed that the tumor microenvironment of HNC patients with incomplete responses were dominated by Streptococcus (cluster 3) and Fusobacterium (cluster 4) than any other genus. None of the Treponema. HNC patients with incomplete clinical responses (3, 6, or 12 months) demonstrated that intratumoral bacterial profiles dominated by Treponema (cluster 4). Fungal ITS profiling revealed that the tumor microenvironment of HNC patients with incomplete responses were dominated by Streptococcus (cluster 3) and Fusobacterium (cluster 4) than any other genus. None of the Treponema. HNC patients with incomplete clinical responses were dominated by Treponema (cluster 3) or Streptococcus (cluster 4). Fungal ITS profiling revealed that the tumor microbiome was largely dominated by the fungi Malassezia. CONCLUSIONS: The current study demonstrates that intratumoral bacterial profiles dominated by Prevotella is a potential biomarker of improved prognosis, while profiles dominated by Fusobacterium may indicate a poor prognosis following chemoradia-

#2252 Microbial profiling of the head and neck tumor microenvironment as a biomarker of clinical response to chemoradiation. Christine M. Pierce Campbell,1 Bo-Yeong Hong,2 Maria F. Gomez,3 Blake M. Hanson,4 Erica So-dergren,5 Sybil T. Sha,6 Jeffrey S. Russell, George M. Weinstock. 7 Moffitt Cancer Center, Tampa, FL;8 The Jackson Laboratory for Genomic Medicine, Farming-ton, CT;9 Cornell University, Ithaca, NY.

PURPOSE: Despite advances in cancer treatment, patients with advanced head and neck cancer (HNC) have a median overall survival of <1 year and 30% cure rate. Evidence suggests that microbiota may modulate the response to therapy through a crossstalk with cancer cells, immune cells, and inflammatory mediators. This study was conducted to profile the tumor microbiome of HNC patients to determine whether bacteria and fungi in the tumor microenvironment are associated with the clinical response to chemoradiation. METHODS: Fresh frozen tumor tissues from 30 newly diagnosed, treatment-naïve patients who received chemoradiation at Moffitt Cancer Center for squamous cell HNC were evaluated. Genomic DNA was extracted from 25mg of tissue. The V1-V3 hypervariable region of the bacterial 16s rRNA gene and fungal internal transcribed spacer (ITS) 1 region were sequenced using a PCR amplicon-mediated workflow on the Illumina MiSeq. 165 sequences were clustered into operational taxonomic units and taxonomic identification was determined using the RDP database and ITS sequences were classified using an in house reference database. Patients’ charts were reviewed to ascertain clinical response 3, 6 and 12 months after treatment cessation. Response was dichotomized as complete (no evidence of disease) and incomplete (partial response, stable disease, and progressive disease). RESULTS: At the time of tumor collection, patients ranged in age from 20-79 years, and were predominantly men (83%) and current smokers (40%). The most common anatomic site was the oral cavity (70%) followed by the larynx (20%) and oropharynx (10%), and most were diagnosed at stage 4 (87%). Three, 6 and 12 months after treatment cessation, 4 (14%), 9 (36%), and 10 (40%) patients had an incomplete response to therapy, respectively. Bacterial sequencing was successful in 29 (97%) samples and fungal in 15 (50%). Clustering analyses of bacterial abundance profiles suggested that 4 types of communities existed, each dominated by Fusobacterium, Prevotella, Streptococcus, or Treponema. HNC patients with incomplete clinical responses (3, 6, or 12 months after treatment) were more likely to have treatment-naïve tumors dominated by Fusobacterium (cluster 1; 56%) than any other genus, whereas patients with complete clinical responses were more likely to have treatment-naïve tu-
mors dominated by Prevotella (cluster 2; 40%) than any other genus. None of the patients with incomplete responses were dominated by Streptococcus (cluster 3) or Treponema (cluster 4). Fungal ITS profiling revealed that the tumor microbiome was largely dominated by the fungi Malassezia. CONCLUSIONS: The current study demonstrates that intratumoral bacterial profiles dominated by Prevotella is a potential biomarker of improved prognosis, while profiles dominated by Fusobacterium may indicate a poor prognosis following chemoradia-

#2253 Gene prognostic signature for differentiated liposarcomas. Deanna Ng, Nicholas Shannon, Johnny Ong, Melissa Teo. National Cancer Cen-
tre Singapore, Singapore, Singapore.

Introduction: Liposarcomas, despite being rare consist of a number of entities spanning low to high grade sarcomas. De-differentiated liposarcoma (DDLs) is associated with increased frequency of recurrence and lower overall survival. We aimed to identify gene expression changes associated with OS in DDLs. Secondary aims was to postulate a mechanism for DDLs. Meth-
ods: We analysed data from gene expression omnibus (GEO, ID = GSE30929) to identify genes associated with DDLs (n = 39). A signature representing genes overexpressed in DDLs was then applied to an indepen-
dent dataset, utilising data from The Cancer Genome Atlas (TCGA, n = 58 DDLs) to segregate samples based on gene expression and groupings com-
pared for survival. Results: A 5 gene signature was generated consisting of genes with increased expression in DDLs and its relation to OS. The signa-
ture was significantly enriched for genes involved in lipid metabolism (AQP7, PRKAR2B, FABP4 < p < 0.01) and genes involved in the cell cycle and cell proliferation (FBN2, CEBPA < p < 0.01). Segregation of DDLs in the TCGA cohort on the basis of median gene signature score gave a significant difference in overall survival. High score (stronger DDLs signature) was associated with a lower median survival (33.5 vs 76.4 months, p = 0.027), and poorer 2 year (63.4% vs 89.2%) and 5 year survival (20.6% vs 71.1%). The survival difference remained significant when applied to retroperitoneal DDLs only (n = 51, median survival 34.5 vs 54.2 months, p = 0.034) and poorer 2 year survival (. A postulated mechanism is that DDLs have gained deficiency in AQP7, PRKAR2B and FABP4 leading to fatty acid and glycerol accumulation in cells, increasing cancer cell replication. CEBPA inhibits CDK4 and a deficiency of CEBPA in DDLs leads to up regulation of CDK4, increasing cell proliferation. An upregulation of FBN2 in DDLs upregulates TGF-β1, increasing cell proliferation. Conclusion: Gene expression changes associated with differentiated liposarcomas include changes in cell cycle checkpoint regulation. The signature is associated with poorer overall sur-
vival in DDLs and can be used to prognosticate patients.

#2254 Reactive oxygen species modifier 1 (Romo1) predicts unfavorable prognosis in colorectal cancer patients. Min Jee Jo, Hong Jun Kim, Suk-Young Lee, Dae-Hee Lee, Sang Cheul Oh. Korea University Guro Hospital, Seoul, Re-
public of Korea.

Reactive oxygen species modifier 1 (Romo1) is a novel protein that has been reported to be crucial for cancer cell proliferation and invasion. How-
ever, its clinical implications in colorectal cancer (CRC) patients are not well-known. For the first time, we investigated the association between Romo1 and the clinical outcomes of CRC patients. We examined Romo1 expression in resected tumor tissues immunohistochemically and assessed it with histological scores. We conducted survival analyses for patients who had curative resection (n = 190) in accordance with clinical parameters in-
cluding levels of Romo1 expression and the association between Romo1 expression and cell invasion using Matrigel invasion assay in CRC cell lines. We observed significantly longer mean disease-free survival (DFS) in the low Romo1 group compared with the high Romo1 group (161 vs 127.6 months, p = 0.035), and the median overall survival (OS) of the low Romo1 group was significantly longer than that of the high Romo1 group (196.9 vs 94.7 months, p = 0.036). Cell invasiveness decreased in the Romo1 knockdown CRC cells in contrast to the controlled cells, Romo1 overexpres-
sion in tumor tissue was associated with a high lymph node ratio (LNR) between the metastatic and examined lymph nodes (p = 0.025). Romo1 over-
expression in tumor tissue was significantly associated with survival in cu-
ratively resected CRC patients, suggesting Romo1 expression as a potential adverse prognostic marker. Increased Romo1 expression was found to be associated with high LNR. Cancer invasiveness appeared to be a key reason for the poor survival related to highly expressed Romo1.


Background: Colorectal cancer (CRC) is major cause of morbidity and mortality in cancer patients worldwide and it ranked the third most common cancer in males and the third most common among females in Saudi Arabia. Alteration in TGF-β pathway has been reported in many cancers. So In search for new molecular targets we analyzed the role of SMAD4, critical TGF-β pathway mediator, in Saudi colorectal cancers Methods: 426 CRC cases were analyzed for SMAD4 mutations by targeted capture sequencing and protein expression status by immunohistochemistry in a tissue microar-
ray format. Results were analyzed for association with any clinicopatholog-
ical parameters and for prognostic significance. Results: SMAD4 mutations were detected in 11.9% (50/418) cases of Saudi colorectal cancers. CRC with SMAD4 mutations were significantly associated with NRAS mutations (p = 0.0481). SMAD4 complete loss of expression by immunohistochemistry was seen in 12.8% (52/414) cases. CRC with SMAD4 mutations associated with young age (p = 0.0236) and inversely with MSI-high tumors (p = 0.0005). SMAD4 ex-
pression loss by immunohistochemistry and mutation were significantly as-
bected with each other (p = 0.0028). In the multivariate analysis using the Cox proportional hazard model SMAD4 expression loss was an independent marker of poor prognosis (p = 0.0119) Conclusion: SMAD4 alteration was found to be associated with a poor overall survival in the Saudi colorectal cancer patients. With the advent of targeted anticancer therapy, this study confirms the findings of other investigators and highlights a potential target which can be exploited to improve the survival of CRC patients in the King-
dom of Saudi Arabia.
Background: Generally, Semaphorins are secretory or transmembrane-bound molecules that act as axon guidance cues in the nervous system. Recent research showed increased expression of semaphorin 3C correlates with cancers that possess higher invasive and metastatic characteristics. For example, in breast cancer, inhibition of semaphorin 3C reduces adhesion and invasion. The aim of this study was to evaluate a possibility that semaphorin 3C might be a new prognostic marker in colorectal cancer. In each cohort, we divided the patients in to the two groups i.e., high or low semaphorin 3C expression group using receiver operating characteristic (ROC) curve based on the information of the recurrence of colorectal cancer. Disease-free-survival (DFS) rates were calculated using Kaplan-Meier methods. Differences between curves were compared with the log-rank test. Results: In the cohort 1, the DFS in the patients with higher expression was worse than that in the patients with lower expression ($P = 0.0467$). Semaphorin 3C has two probes (203788_s_at and 203789_s_at) in GSE14333 dataset as Cohort 2 which included 226 patients with the colorectal cancer. In both cohorts, the patients with high expression were worse than that of those patients with lower expression ($P = 0.0015$ and $P = 0.0003$). Conclusions: This study suggested that Semaphorin 3C might be a new prognostic marker in colorectal cancer.

**#2257 Pre-diagnostic sex hormone levels and breast cancer survival in the Nurses’ Health Study.** Kevin H. Kessler,1 A. Heather Ellässen,2 Bernard A. Rosner,2 Susan E. Hankinson,3 Myles Brown,4 Rulla M. Tamimi,5 Harvard T. H. Chan School of Public Health, Boston, MA; 2Brixham and Women’s Hospital, Boston, MA; 3University of Massachusetts School of Public Health and Health Sciences, Amherst, MA; 4Dana-Farber Cancer Institute, Boston, MA.

Background The role of estrogens has been well established in the development of breast cancer, while an independent function of androgens remains to be fully elucidated. There is consistent evidence that increased levels of circulating sex steroid hormones predict subsequent breast cancer risk. However, few studies to date have evaluated circulating sex hormones in relation to breast cancer risk, and the limited available evidence suggests that higher estrogen and androgen levels are associated with worse prognosis. Identifying the distinct roles of estrogens and androgens and their hormone receptor signaling pathways in breast cancers may inform novel approaches to breast cancer prevention and treatment. Methods We evaluated the association between pre-diagnostic circulating sex hormones and breast cancer survival among 683 breast cancer cases from the Nurses’ Health Study (NHS) cohort. The NHS began in 1976 with the enrollment of U.S. registered nurses between the ages of 30 and 55. The women in this analysis provided a blood sample in 1989-1990, were postmenopausal and not using menopausal hormone therapy at the time of blood draw, and were diagnosed with invasive breast cancer between 1990 and 2010. Levels of estradiol, estrone, estrone sulfate, testosterone, androstenedione, DHEA, DHEAS, and SHBG were measured in plasma. The association between hormone levels and survival was assessed through construction of Kaplan-Meier survival curves and through use of Cox proportional hazards models to estimate hazard ratios and 95% confidence intervals adjusted for patient, tumor, and treatment covariates. Results Over follow-up through 2014, a total of 219 deaths occurred, including 57 breast cancer deaths. For each hormone, we found no association with breast cancer risk evaluated by the log-rank test. Upon adjustment for patient, tumor, and treatment covariates, we observed non-significant 47% increases in breast cancer mortality per unit increase in log estradiol ($HR=1.47, 95\% CI 0.94-2.31$) and estrone sulfate ($HR=1.47, 95\% CI 0.97-2.24$). Log testosterone was not associated with breast cancer survival ($HR=0.84, 95\% CI 0.46-1.53$) and the findings were null for the other hormones. These findings were robust when evaluating overall survival and when stratifying by tumor estrogen receptor expression. Conclusions We observed no significant associations between pre-diagnostic sex hormone levels and breast cancer survival among postmenopausal women. This is the first analysis to evaluate pre-diagnostic hormone levels in relation to survival. Though limited by sample size, it improves upon past analyses of post-diagnostic hormone levels by reducing the possibility of the tumor affecting hormone levels and by better accounting for tumor and treatment characteristics. The findings should be replicated in a larger population to further assess the prognostic value of circulating sex hormones in breast cancer survival.

**#2258 Genome-wide association studies of breast cancer prognosis.** Marjanka K. Schmidt,1 Qi Guo,2 Thilo Dörk,3 Diana Eccles,4 Renke Keenan,5 Jacques Simard,6 Peter Kraft,7 Douglas F. Easton,8 Paul D. Pharoah,9 on behalf of the Breast Cancer Association Consortium.1 Netherlands Cancer Institute, Amsterdam, Netherlands; 2University of Cambridge, Cambridge, United Kingdom; 3Hannover Medical School, Hannover, Germany; 4University of Southampton, Southampton, United Kingdom; 5Laval University, Quebec City, Quebec, Canada; 6Harvard School of Public Health, Boston, MA.

Introduction: The prognosis and response to treatment in women with breast cancer varies considerably after taking into account clinical-pathological variables. Other factors such as host genotype are also likely to be important. We therefore investigated the role of germline genetic variation on survival after breast cancer using data from the Breast Cancer Association Consortium. Materials and methods: We included data from ten projects in which breast cancer case cohorts were genotyped using arrays providing genome-wide coverage of common variants. The majority of samples was genotyped using the Illumina custom iCOGS or OncoArray chips. Data were imputed using the 1000 Genome phase 3 as a reference panel using a two-stage procedure. We assessed the association between genotype and 10-year breast cancer specific survival for each sex hormone (estradiol, estrone, estriol, estrone sulfate, estradiol sulfate, estrone sulfate, estradiol-17ß, androstenedione, DHEA, DHEAS, and SHBG) in the GRS/PRS. Each sex hormone was included in the cohort of European women classified by country, and stratified by 10-year breast cancer risk in the analyses. Results: Preliminary analyses showed that in all invasive cases, ER-positive and ER-negative disease 123, 110, and 212 variants respectively, were associated with breast cancer-specific mortality at $P < 5 \times 10^{-5}$. A single variant, rs2295683, was associated with higher mortality at genome-wide significance in all patients: $HR = 1.16$ (95\% CI 1.10-1.22); $P = 2.8 \times 10^{-8}$ (risk allele frequency: 0.12). We identified four variants to be associated with mortality in ER-negative disease at $P < 5 \times 10^{-8}$. The strongest association was for rs145963877: $HR = 1.32$ (95\% CI 1.20-1.46); $P = 2.3 \times 10^{-8}$. No variant reached genome-wide significance for ER-positive disease; the most significant variant was rs741406313 with an HR = 1.17 (95\% CI 1.10-1.24); $P = 1.8 \times 10^{-7}$. In addition, using OncoArray data, we provided independent validation of higher mortality of CHEK2 c.1100deC carriers compared with non-carriers (HR = 1.56 (95\% CI 1.22-2.01)), previously reported in Weischer et al 2012. Using iCOGS data, we evaluated the association between mortality and the polygenic risk score (PRS) based on 77 known breast cancer susceptibility SNPs. A higher PRS was significantly associated with lower breast cancer-specific mortality: $HR = 0.87$ (95\% CI 0.81-0.93) (per unit of PRS) with a similar association in ER-negative and ER-positive disease. The association was attenuated after adjusting for tumor grade. Conclusion: We have identified a novel set of germline genetic variants that are associated with breast cancer prognosis. The effect sizes are small for each of the variants and unlikely to be of immediate clinical relevance. However, understanding of the biology that underlies these associations may identify novel pathways and targets for therapy. Being at higher risk of breast cancer, as defined by the breast cancer susceptibility PRS, is not associated with an adverse prognosis.

**#2259 Evaluation of polymorphisms in myeloid-associated genes and glioma survival.** Daniel I. Jacobs,1 Yanhong Liu,1 Konrad Gabrusiewicz,2 Spiridon Tsavachidis,2,3,4 Susan Amirian,1 Georgina N. Armstrong,1 Renke Zhou,1 Jun Wei,1 Cristina Ivan,1 George Calin,2 Michael Scheurer,5 Anna Dahlin,5 Terri Rice,5 Paige M. Bracci,6 Helen M. Hansen,1 John K. Wiencke,1 Margaret R. Wrensch,3 Beatrice Melin,7 Amy B. Heimberger,2 Melissa L. Bondy5 1 Baylor College of Medicine, Houston, TX; 2University of Texas MD Anderson Cancer Center, Houston, TX; 3Umeå University, Umeå, Sweden; 4University of California, San Francisco, San Francisco, CA.

BACKGROUND: Gliomas are highly infiltrated by immune cells including microglia, macrophages, and myeloid-derived suppressor cells (collectively, glioma-associated myeloid cells). These cells have been shown to be induced by the tumor to be immune-suppressive and tumor-supportive, and are a negative prognosticator for survival in mouse models. Here, we examine whether inherited variants in genes important to the function of glioma-associated myeloid cells are associated with survival following low-grade glioma diagnosis. METHODS: Subjects for this study were 484 patients with WHO grade II or grade III glioma treated at The University of Texas MD Anderson Cancer Center in Houston, Texas between 1992 and 2008 and followed up for survival through
August, 2016. We selected 100 genes for analysis including transcription factors, cytokines and chemokines, receptors, enzymes, and other genes central to the function of glioma-associated myeloid cells. Genotyping was originally performed using the Illumina Human 610-Quad Bead Chip platform and 2,040 tagging SNPs as determined by Haplovie Tagger software were selected for analysis that is highly expressed on macrophages and may play a role in macrophage-mediated anti-inflammatory responses, while MET is a receptor tyrosine kinase and well-studied proto-oncogene that is also involved in the expansion of myeloid-derived suppressor cell populations. Further investigation of these associations is warranted, and validation of these findings is planned in an independent population.

#2260 Haplotypes of chromosome 17q21.31 affect liver metastasis by small intestinal neuroendocrine tumors. Shinta Kobayashi,1 Edaise da Silva,2 Tanupriya Contractor,2 Laura Yang,3 Chris R. Harris3.

Neuroendocrine tumors are the most common tumors of the small intestine. Very little is known about genetic changes that cause SI-NETs, which have a very low frequency of mutations. SI-NETs are highly metastatic to the liver, and patients with liver metastases have a five year survival rate of less than 50%. Caucasian patients are more likely to develop metastasis than patients of other races. We have studied a 900 kB inversion at chromosome 17q21.31, which is common to Caucasians but rare in other races. Within a collection of 117 Caucasian patients with ileal carcinoids, patients with the inversion haplotype were significantly less likely to have liver metastasis. A second Caucasian-specific haplotype in this region, beta2, was also tested for potential association with metastasis of ileal carcinoids. Unlike the inversion haplotype, beta2 associates with increased metastasis of ileal carcinoids. We suspect that the fact that the metastasis-promoting beta2 haplotype is more common among Caucasians than the metastasis-suppressing inversion haplotype (44% incidence vs. 33% incidence) is probably why Caucasians wind up with a higher frequency of metastasis than races that carry neither beta2 nor the inversion.

#2261 Synergistic effect and VEGF/HSP70-hom haplotype analysis: Relationship to prostate cancer risk and clinical outcome. Sana Star,† Hamadi Saad,† Fauzi Mosbah,† Lotfi Chouciane,‖ Faculty of Medicine, Monastir, Tunisia; †EPS Fatouma Bourguiba, Monastir, Tunisia; †EPS Salhou, Sousse, Tunisia.

Prostate cancer (PCA) is a complex disorder resulting from the combined effects of multiple environmental and genetic factors. Our previous single-locus analysis showed that VEGF and HSP70-hom polymorphisms were significantly associated with PCA susceptibility and prognosis. Both genes encoding these proteins were located on chromosome 6p21, and combining the neighboring single nucleotide polymorphisms (SNPs) into haplotypes may increase the association with the disease. Three tagging polymorphisms, the HSP70-hom 2437 T/C, the VEGF-1154 G/A, and the VEGF-634 G/C SNPs were genotyped in 101 cases and 80 controls. For the combined analysis of VEGF and HSP70-hom, we found a positive gradient in the odds ratios (ORs) related to the number of high-risk genotypes with a 3.53-fold increase of prostate cancer risk (OR = 3.53; p = 0.015). Furthermore, the TAG and CAG haplotypes at positions HSP70-hom, VEGF-1154 and VEGF-634 exhibited a two-fold (OR = 0.46; p = 0.014) and a seven-fold (OR = 0.14; p = 0.00005) reduction in PCA risk, respectively. Regarding PCA prognosis, the TAG haplotype had a negative association with the aggressive phenotype as defined by the histopathological grade (OR = 0.28; p = 0.006). Our findings confirm the role of at-risk haplotype across the HSP70-hom/VEGF gene cluster in determining susceptibility to PCa.

#2262 Correlation between number and sites of metastases and differentially treated EBV DNA load profiles and their effects on outcomes in patients with metastatic nasopharyngeal carcinoma. Cheng-Lung Hsu, Hung-Ming Wang, Tung-Liang Lin, Yung-Chia Kuo. Chang Gung Memorial Hospital, Taipie, Taiwan.

Differential overall survival of different organ site metastases in nasopharyngeal carcinoma (NPC) had been reported but the underlying mechanism was unclear. We try to find out the possible mechanism from plasma and tumor tissue markers. Total 178 patients of metastatic NPC were enrolled. Their pre-treatment plasma EBV DNA concentrations and cytokines and tissue macrophage, proliferation and apoptosis markers were determined. The overall patient response rate after treatment was 51.7% and median overall survival (OS) was 19 months. Single organ site metastasis had better outcome than multiple organs involved (median OS: 26 months vs. 16 months) with statistical significance. Among single organ site involved, pure lung metastasis had longer survival than bone or liver involved (median OS: 50 months vs. 21 months vs. 18 months) with P = 0.001. Pretreatment plasma EBV DNA concentrations were lower in patients with metastatic bone or liver metastasis in single organ involved group. Plasma IP-10 and MCP-1 expression level correlated with the differential single organ site metastasis OS and EBV DNA load. Liver metastatic tissue had higher macrophage infiltration density and higher proliferation index than lung metastatic group. In single organ site metastasis of NPC, lung involved patients had better outcome than bone or liver metastasis. Low pretreatment plasma EBV DNA load, cytokines expression such as IP-10 and MCP-1, tissue macrophage infiltration, and proliferation index may contribute these results.

#2263 Neonatal hormone levels and risk of testicular germ cell tumors (TGCT). Libby Morimoto,1 David Zava,2 Katherine McGlyn,2 Frank Stanczyk,3 Joseph Wiemels,2 Xiaomei Ma,3 Catherine Metayer.2

Testicular germ cell tumors (TGCT) are the most commonly occurring cancers in adolescent and young adult males in the U.S. Steroid sex hormones play a central role in the development of the testsis. As proposed by the testicular dysgenesis syndrome hypothesis, the origins of TGCT are likely to be in utero or early in life, and to be a manifestation of disturbed prenatal testis development. However, no studies have provided direct, empirical evidence to date. Using an innovative linkage between the California birth records and cancer registry data, we conducted a population-based case-control study of neonatal hormone levels and risk of TGCT diagnosed at 0-19 year of age. We obtained archived neonatal dried blood spot (DBS) specimens from 370 TGCT cases (276 adolescent and young adults [AYA] aged 15-19 years) at the time of diagnosis, 94 0-4 yrs at diagnosis), and 344 age- and race/ethnicity-matched controls, born between 1982 and 2009. Liquid chromatography with tandem mass spectrometry was used to measure a panel of 17 sex steroids, glucocorticoids, and mineralcorticoids; 12 were present at detectable levels in the newborn DBS samples, including estrone (E1), estradiol (E2), estriol (E3), testosterone (T), dehydroepiandrosterone (DHEA), and androstenedione (A4). Logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI), adjusting for matching factors and age (in hours) of child at blood spot collection. A4, a precursor for T and E1, was positively associated with TGCT (OR: 1.71, 95% CI: 1.09-2.69). Analyses stratified by age group showed that this association was limited to AYA, and was of stronger magnitude in this group (OR: 2.33, 95% CI: 1.37-3.97). A similar, though weaker, trend was observed for T (ORoverall: 1.37, 95% CI: 0.86-2.17; ORAYA: 1.73, 95% CI: 1.09-2.68). There was no significant association of the other measured hormones with risk. In the first case-control study of TGCT with direct measures of neonatal hormone levels, we found that higher levels of T and A4 were associated with increased risk of TGCT, particularly among males diagnosed at 15-19 years of age. These results oppose the dominant theory in TGCT etiology, that TGCT is related to androgen insufficiency in utero, and provides an important link in the etiologic pathway of this increasingly common cancer.

#2264 Telomere biology gene methylation and cancer risk. Brian T. Joyce,† Yang Li,† Yinan Zhang,† Lei Liu,† Hushan Yang,‡ Chad Achenbach,† Pantel Vokonas,§ Joel Schwartz,¶ Andrea Baccarelli,† Ilifang Hou,¶ Northwester University, Chicago, IL; †Thomas Jefferson University, Philadelphia, PA; §VA Boston Healthcare System, Boston, MA; ¶Harvard University, Boston, MA; ‡Columbia University, New York, NY.

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Dahlstrom, Karen S. Anderson, Matthew S. Field, Diego Chowell, Guojun fındings, and explore these CpG sites and genes as potential early detection cg23157637 on TEP1, and cg04818274 on TERF2). One CpG on NSMCE2 one CpG on each of CDC73, NSMCE2, and RPA1 was associated with de-

Results: Our screening identifıed 167 CpG sites, 10 of which were
tween methylation measured at the fırst visit and BTL measured at the sec-
Finally an additional mixed model explored the temporal relationship be-

CpGs differentially methylated by cancer status at the fırst blood draw only,
followed by mixed models using our full data to determine the final CpG sites of interest for Cox models of cancer risk, and another set of mixed models to compare trajectories over the time of methylation measured in cancer patients. 

Finally an additional mixed model explored the temporal relationship be-

#2265 HPV 16 serostatus and risk of oropharyngeal carcinoma.

Background: The current epidemic of human papillomavirus (HPV)-related oropharyngeal cancer (OPC) urgently demands a screening strategy for this disease.

Antibodies to HPV type 16 (HPV16) early (E) antigens have been detected in pa-

Table 1. A blinded study of HPV16 serology of patients with p16-positive OPC (n=348) and controls (n=782)

<table>
<thead>
<tr>
<th>Controls</th>
<th>p16+ OPC</th>
<th>N (%)</th>
<th>p16+ OPC</th>
<th>N (%)</th>
<th>OR (95% CI)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>AUC</th>
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<tbody>
<tr>
<td>E1</td>
<td>52 (6.7)</td>
<td>256 (73.6)</td>
<td>43 (28-67)</td>
<td>74</td>
<td>93</td>
<td>85</td>
<td>89</td>
<td>84</td>
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<td></td>
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<tr>
<td>NE2</td>
<td>14 (1.8)</td>
<td>128 (36.8)</td>
<td>43 (20-50)</td>
<td>37</td>
<td>98</td>
<td>90</td>
<td>70</td>
<td>68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE2</td>
<td>7 (0.9)</td>
<td>213 (67.5)</td>
<td>290 (114-741)</td>
<td>68</td>
<td>99</td>
<td>97</td>
<td>87</td>
<td>83</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E4</td>
<td>14 (1.8)</td>
<td>110 (31.6)</td>
<td>27 (13-54)</td>
<td>32</td>
<td>98</td>
<td>89</td>
<td>76</td>
<td>65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E5</td>
<td>46 (6.1)</td>
<td>37 (10.6)</td>
<td>2 (1-4)</td>
<td>11</td>
<td>94</td>
<td>44</td>
<td>70</td>
<td>52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E6</td>
<td>9 (1.2)</td>
<td>146 (42.0)</td>
<td>80 (34-187)</td>
<td>64</td>
<td>99</td>
<td>95</td>
<td>86</td>
<td>70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E7</td>
<td>11 (1.4)</td>
<td>224 (64.4)</td>
<td>106 (54-208)</td>
<td>42</td>
<td>99</td>
<td>95</td>
<td>86</td>
<td>82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>8 (1.0)</td>
<td>288 (82.8)</td>
<td>43 (15-286)</td>
<td>83</td>
<td>99</td>
<td>97</td>
<td>93</td>
<td>91</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted for sex, smoking, and alcohol status (never vs. former vs. current).

#2266 A prospective study of serum metabolites and glioma risk. 

Huang, Stephanie J. Weinstein, Cari M. Kitahara, Edward Karoly, Joshua N. Sampson, Demetris Albanes, NIH, Bethesda, MD; NIH, Bethesda, MD; Melatonol, Inc., Morrisville, NC.

Malignant glioma is one of the most devastating adult malignancies. Although there exists an urgent need for actionable leads regarding its prevention and early detection, the etiology of glioma remains largely unknown. We conducted a pros-

#2267 A prospective assessment for telomere length in relation to risk of cancer in the Singapore Chinese Health Study.

Jian-Min Yuan, Renwei Wang, Jian-Min Yuan,1 Renwei Wang,1 2

Telomeres are crucial in the maintenance of chromosome integrity and genomic stability. A critically short telomere length can trigger cell to enter replicative senescence with a result of cell death; alternatively, cells continue to divide if death does not occur, which results in genomic instability and chromosomal ab-

Author: Telomeres are crucial in the maintenance of chromosome integrity and genomic stability. A critically short telomere length can trigger cell to enter replicative senescence with a result of cell death; alternatively, cells continue to divide if death does not occur, which results in genomic instability and chromosomal ab-

Following the cohort of Chinese men and women aged 45-74 years recruited from 1993 through 1998, 28.219 provided baseline blood samples, we used quantitative polymerase chain reaction (PCR) method to quantify relative telomere length determined by the ratio of telomere repeat copy number (T) to single-copy gene for albumin (S) (i.e., TSR) on all subjects. The present analysis included
24,847 subjects with valid TSR values after excluding samples with insufficient DNA (n = 1,908) and/or patients with prevalent cancer at baseline blood draw (n = 1,464). As of December 31, 2015, 3,778 participants developed cancer, including 722 colorectal cancer, 599 lung cancer, and 412 breast cancer. Cox models were used to estimate hazard ratio (HR) and 95% confidence interval (CI) of developing any cancer and these selected specific cancer types for different quintiles of TSR. Results: Women had a 5.7% higher TSR value than men (P < 0.0001). Age, level of education, number of cigarettes/day, years of smoking, and pack-years of smoking were all inversely associated with TSR (P < 0.0001). High TSR was associated, in a dose-dependent manner, with significantly increased risk of total cancer and breast, colorectal and lung cancer. Compared with the lowest quintile, the HRs (95% CIs) of total cancer for the 2nd, 3rd, and 4th, and 5th quintile of TSR were 1.03 (0.89, 1.20) 1.08 (0.93–1.26), 1.15 (0.98–1.34), and 1.36 (1.16–1.58), respectively, after adjustment for age, sex, education and smoking (P_trend = 0.001). The corresponding HRs (95% CIs) were 1.21 (0.72–2.03), 1.59 (0.97–2.60), 1.59 (1.04–2.75) and 1.62 (0.99–2.60) for breast cancer (P_trend = 0.023); 1.44 (1.03–2.00), 1.10 (0.76–1.59), 1.32 (0.92–1.90) and 1.58 (1.32–2.66) for colorectal cancer (P_trend = 0.004); and 1.41 (0.98–2.05), 1.57 (0.93–2.02), 1.31 (0.86–1.99) and 1.88 (1.26–2.81) for lung cancer (P_trend = 0.010).

Conclusions: This prospective cohort study demonstrates that longer telomere length is associated with significantly increased risk of total and major cancers in a general population. These results suggest a complex role of telomere in the development of cancer.

#2268 Serum insulin and glucose, indices of insulin resistance, and risk of lung cancer. Iolina Argirion,1 Stephanie J. Weinstein,2 Satu Männistö,3 Demetrios Albanes,3 Alison M. Mondial,4 University of Michigan, Ann Arbor, MI; 2National Cancer Institute, Bethesda, MD; 3National Institute for Health and Welfare, Helsinki, Finland.

Background: Although insulin is crucial in human growth and development, it also harbors antipapoptotic properties and acts as a growth factor by stimulating mitosis through the Akt pathway, which could lead to tumor growth and promotion. Insulin also harbors antiapoptotic properties and acts as a growth factor by stimulating proliferation of cancer cells. Several studies have had the ability to evaluate differences in association by ovarian tumor subtype. Methods: We pooled existing data on CRP and ovarian cancer risk among nested case-control studies conducted within 6 studies participating in the Ovarian Cancer Cohort Consortium (OC3). (OR:1.10;95%CI:0.91-1.32) or the top vs. bottom quartile (OR: 1.10; 95% CI: 0.89-1.36). However, there was a strong, positive association between very high CRP (≥10 mg/L vs. <1 mg/L) (OR: 1.96; 95% CI: 1.36-2.84). Associations of very high (≥10 mg/L vs. < 1 mg/L) did not differ by histologic type: serous OR: 1.63; 95% CI: 1.11-2.43; endometrioid OR: 1.78; 95% CI: 0.63-5.00; mucinous OR: 10.43; 95% CI: 1.27-85.86; clear cell OR: 2.30; 95% CI: 0.39-13.34). Conclusion: Our results confirm the consistent observation of a positive association between very high CRP levels and ovarian cancer risk. Pooled analyses accounting for potential confounders are ongoing.

#2271 DNMT1 expression is peripheral mononuclear cells is associated with increased breast cancer risk. Lissette Delgado-Cruzat,1 Xinran Ma,2 Yuyan Liao,3 Maya Kapili,3 Regina M. Santella,2 Mary Beth Terry,1 John Jay College, City University of New York, New York, NY; 2Mailman School of Public Health, Columbia University, New York, NY. Loss of global DNA methylation in peripheral tissue has been identified as a biomarker of breast cancer susceptibility in several studies. DNA methyltransferases or DNMTs are the enzymes in charge of catalyzing the addition of the methyl group to the DNA molecule. Of the different DNMTs described in humans, only DNMT1 has been found to be in charge of adding methyl groups to the newly synthesized DNA strand. Therefore, compromised levels of this en-
zyrne might lead to a loss of global DNA methylation and concomitantly an increase in breast cancer risk. However, to date no study has examined the association between peripheral levels of DNMT1 and breast cancer. To investigate this relation, we conducted a case-control study including sisters in the New York site of the Breast Cancer Family Registry. We extracted mRNA from peripheral blood mononuclear cells (PBMC) in 196 sister sets, a total of 479 female participants, and assayed DNMT1 gene expression levels using Taqman expression assays. The results were analyzed using generalized estimating equations (GEE). We found that there were no large differences between the mean levels of DNMT1 expression levels of women with breast cancer and controls; mean level in cases was 0.46 ± 0.71 versus 0.63 ± 1.42 in controls (p = 0.10). When examining expression levels as a continuous variable, there also were no associations in GEE regression models adjusting for age at blood drawn and smoking status. Odds Ratio (OR) = 1.17 (95% confidence interval (CI) = 0.97 – 1.40 per one unit increase in expression levels), p = 0.11. However, when results were analyzed by categorizing DNMT1 expression in tertiles, lower DNMT1 expression (≤ 0.17%) was associated with statistically significantly higher odds of breast cancer (OR = 1.47% (95% CI = 1.02 – 2.10), p = 0.04). If replicated in other studies, these findings suggest that low DNMT1 expression in PBMC has the potential to be used as a biomarker of breast cancer risk in women at high risk of the disease.

#2272 A prospective study of urinary prostaglandin E2 metabolites in helicobacter pylori and gastric cancer risk. Tianyi Wang,1 Hai Cai,2 Wei Zheng,2 Angelika Michel,2 Michael Pavlita,3 Ginger Milne,3 Yong-Bing Xiang,4 Yu-Tang Gao,5 Hong-Lan Li,5 Nathaniel Rothman,6 Qing Lan,6 Xiao-Ou Shu,5 Meira Epplein2.1 Peking University Health Science Center, Beijing, China; 2Division of Epidemiology, Department of Medicine, Vanderbilt Epidemiology Center and Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, TN; 3Division of Molecular Diagnostics of Oncogenic Infections, Research Program in Infection, Inflammation, and Cancer, German Cancer Research Center (DFKZ), Heidelberg, Germany; 4Division of Clinical Pharmacology, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN; 5Department of Epidemiology, Shanghai Cancer Institute, Shanghai, China; 6Division of Cancer Epidemiology and Genetics, Occupational and Environmental Epidemiology Branch, National Cancer Institute, National Institutes of Health, Rockville, MD.

Background. Previous studies suggest that a stable end-product of prostaglandin E2, the urinary metabolite PGE-M, is excrised in the urine and can be used as an index of systemic prostaglandin E2 (PGE2) production. In the present study we investigate the PGE-M, Helicobacter pylori (H. pylori), and gastric cancer association. Methods. The present analysis included 859 prospectively ascertained gastric cancer cases and 700 individually matched controls from two population-based prospective cohort studies, the Shanghai Women’s Health Study and Shanghai Men’s Health Study. Urinary PGE-M was measured by a liquid chromatography/tandem mass spectrometric method. Sero-positivity to H. pylori was determined by immunoblotting. Adjusting for H. pylori, increasing PGE-M was associated with higher risk of gastric cancer (Quartile 4 vs. 1, OR = 1.76, 95% CI: 1.17-2.66, I²/meta = 0.004). This association remained after excluding those diagnosed within two years from sample collection (OR = 1.73, 95% CI: 1.12-2.65, I²/meta = 0.007). However it was no longer present among individuals with 10 or more years of follow-up (2-4.9 years, OR = 3.15, 95% CI: 1.11-8.91; 5-9 years, OR = 2.23, 95% CI: 1.22-4.06; ≥10 years, OR = 0.73, 95% CI: 0.31-1.70). The association of PGE-M with gastric cancer risk was not modified by H. pylori status, but added predictive ability beyond H. pylori; compared to H. pylori-negative individuals with below-median PGE-M levels, H. pylori-positive individuals with above-median PGE-M levels had a 5-fold increase in the odds ratio of gastric cancer (OR = 5.08, 95% CI: 2.47-10.43). Conclusion. In China, higher PGE-M levels may indicate an increased risk of gastric cancer independent of the risk conferred by H. pylori infection status, particularly for cancers diagnosed within 10 years of sample collection.

#2273 Serum choline, methionine, betaine, dimethylglycine, and trimethylamine-N-oxide in relation to pancreatic cancer risk in two nested case-control studies in Asian populations. Joyce Huang,1 Lesley Butler,1 Olivia Midttun,2 Renwei Wang,4 Aizhen Jin,4 Yu-Tang Gao,5 Per Ueland,6 Woon-Puay Koh,6 Jian-Min Yuan1.1 Univ. of Pittsburgh, Pittsburgh, PA; 2National Registry of Diseases Office, Health Promotion Board, Singapore, Singapore; 3Department of Epidemiology, Shanghai Cancer Institute/Shanghai Jiao-tong University, Shanghai, China; 4Department of Clinical Science, University of Bergen; Laboratory of Clinical Biochemistry, Haukeland University Hospital, Bergen, Norway; 5Duke-NUS Medical School, and Saw Swee Hock School of Public Health, National University of Singapore, Singapore, Singapore.

Background: Choline, methionine, and betaine are methyl group donors associated with DNA methylation. Deficient in choline and methionine have been shown to promote pancreatic carcinogenesis in experimental animals. We have previously reported an inverse association between dietary intake of choline and pancreatic cancer risk in a prospective cohort of Singapore Chinese. In the present study biomarkers of dietary choline and other methyl donor nutrients were evaluated in relation to pancreatic cancer risk. Method: Two case-control studies were nested within the Shanghai Cohort Study (129 cases and 258 matched controls) and the Singapore Chinese Health Study (58 cases and 104 matched controls). Concentrations of choline, methionine, betaine, dimethylglycine (DMG), and trimethylamine-N-oxide (TMAO) were measured by LC-MS/MS in pre-diagnostic serum samples. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using conditional logistic regression method with adjustment for potential confounders. Results: Choline, methionine, and betaine were moderately associated with other (spearman correlation coefficient: 0.28 – 0.43). In the pooled analysis, serum choline, betaine, and methionine were inversely associated with risk of pancreatic cancer, while TMAO, an oxidative metabolite of choline produced by gut microbiota, was positively associated with risk of pancreatic cancer. Compared with the lowest quartile, ORs (95% CIs) of pancreatic cancer for the highest quartiles of choline, methionine, betaine, and TMAO were 0.37 (0.17-0.80), 0.39 (0.22-0.69), 0.49 (0.28-0.85), and 1.60 (0.94-2.74), respectively (all P for trend < 0.05). DMG was not associated with pancreatic cancer risk. Conclusion: The novel inverse association of serum choline, methionine, and betaine with pancreatic cancer support the notion that methyl groups related to DNA methylation may modulate the risk of pancreatic cancer development. The positive association between TMAO and pancreatic cancer risk suggested gut microbiota may play an important role in pancreatic carcinogenesis.

#2274 Extreme telomere length was associated with gastric adenocarcinoma risk: The Singapore Chinese Health Study. Zhensheng Wang,3 Woon-Puay Koh,1 Aizhen Jin,4 Renwei Wang4, Jian-Min Yuan1.1 Univ. of Pittsburgh, Pittsburgh, PA; 2National University of Singapore, Singapore City, Singapore; 3National Registry of Diseases Office, Singapore City, Singapore; 4University of Pittsburgh Cancer Institute, Pittsburgh, PA.

Background: Evidence regarding extreme telomere length and increased gastric cancer risk has been limited in retrospective case-control study which was potentially subjected to selection bias and reverse causality. We conducted a prospective analysis in an Eastern Asian population to examine the hypothesis that extreme telomere length is associated with increased risk of gastric adenocarcinoma. Methods: Among 63,257 participants of the Singapore Chinese Health Study, a population-based prospective cohort of Chinese men and women aged 45-74 years recruited between 1993 and 1998, 28,219 provided baseline blood samples. We used quantitative polymerase chain reaction (PCR) method to quantify relative telomere length determined by the ratio of telomere repeat copy number (T) to single-copy gene for albumin (S) (i.e., T/S) on all subjects. The present analysis included 24,846 subjects with valid T/S values after excluding samples with insufficient DNA (n = 1,908) and/or patients with prevalent cancer at baseline blood draw (n = 1,465). As of December 31, 2014, 101 patients who developed gastric adenocarcinoma have previously reported an inverse association between dietary intake of choline and pancreatic cancer risk in a prospective cohort of Singapore Chinese. The present study biomarkers of dietary choline and other methyl donor nutrients were evaluated in relation to pancreatic cancer risk. Method: Two case-control studies were nested within the Shanghai Cohort Study (129 cases and 258 matched controls) and the Singapore Chinese Health Study (58 cases and 104 matched controls). Concentrations of choline, methionine, betaine, dimethylglycine (DMG), and trimethylamine-N-oxide (TMAO) were measured by LC-MS/MS in pre-diagnostic serum samples. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using conditional logistic regression method with adjustment for potential confounders. Results: Choline, methionine, and betaine were moderately associated with other (spearman correlation coefficient: 0.28 – 0.43). In the pooled analysis, serum choline, betaine, and methionine were inversely associated with risk of pancreatic cancer, while TMAO, an oxidative metabolite of choline produced by gut microbiota, was positively associated with risk of pancreatic cancer. Compared with the lowest quartile, ORs (95% CIs) of pancreatic cancer for the highest quartiles of choline, methionine, betaine, and TMAO were 0.37 (0.17-0.80), 0.39 (0.22-0.69), 0.49 (0.28-0.85), and 1.60 (0.94-2.74), respectively (all P for trend < 0.05). DMG was not associated with pancreatic cancer risk. Conclusion: The novel inverse association of serum choline, methionine, and betaine with pancreatic cancer support the notion that methyl groups related to DNA methylation may modulate the risk of pancreatic cancer development. The positive association between TMAO and pancreatic cancer risk suggested gut microbiota may play an important role in pancreatic carcinogenesis.
EPIDEMIOLOGY: Tobacco, Alcohol, and Other Risk Factors

#2277 Adherence to cancer prevention guidelines and risk of cancer among older white and black adults in the Health ABC Study. Audrey Y. Jung,1 Christina Gu,1 Iva Milijkovic,2 Susan Rubin,3 Suzanne Satterfield,4 Stephen B. Kritskevsky,5 Heidi Klepin,3 Anne B. Newman,5 Jane Cauley,6 Hilsa Ayonoyan,7 Tamara B. Harris,5 Rachel A. Murphy,1 1University of British Columbia, Vancouver, British Columbia, Canada; 2University of Pittsburgh, Pittsburgh, PA; 3University of California at San Francisco, San Francisco, CA; 4University of Tennessee Health Science Center, Memphis, TN; 5Wake Forest School of Medicine, Winston-Salem, NC; 6National Institute on Aging, Bethesda, MD.

Background: About one-third of cancers can be prevented by healthy lifestyle behaviors. The American Cancer Society (ACS) have guidelines for reducing the risk of cancer by maintaining a healthy body weight, being physically active, eating well and limiting alcohol. Few studies have examined adherence to cancer preventive behavior and subsequent cancer risk in older populations that are inclusive of men, women and minorities. Methods: We evaluated adherence to ACS cancer prevention guidelines and subsequent risk of all incident cancer in the Health, Aging and Body Composition Study. The population included 2,124 white and black men and women aged 71-80 years who were initially free of cancer at baseline (1998-1999). Adherence to ACS guidelines was scored from 0 (lowest adherence) to 8 (greatest adherence). Maintenance of healthy body weight was determined from body mass index at ages 25 and 30 (recalled at baseline) and measured height and weight at baseline. Physical activity and alcohol intake were assessed by a dietary questionnaire. Diet was assessed by food frequency questionnaire (servings of fruits and vegetables, the ratio of whole grains to refined grains and intake of red meat and processed meats). Incident cancer (all types except non-melanoma skin cancer) was confirmed from pathology reports when available, or medical records and death certificates. Risk of cancer was estimated with Cox regression adjusted for gender, age, and study site. Participants with the lowest adherence to ACS guidelines were the reference group.

Results: The lowest adherence category (scores 0-2) had 297 participants (14% of all participants), 1,316 (62%) were in the moderate adherence category (scores 3-5), and 511 (24%) were in the highest adherence category (scores 6-8). Adherence was lowest among men (P<0.001), black (P<0.001) and older participants (P<0.001). After a median follow-up of 12.5 years, 517 people developed incident cancer. Participants with moderate adherence had a 23% lower risk of cancer [HR=0.77, 95% CI=0.60-0.97]. Participants with the greatest adherence had a 26% lower risk of cancer [HR=0.74, 95% CI=0.56-0.98]. Conclusions: Only 1 in 4 older adults closely followed healthy lifestyle behaviors outlined by the ACS for cancer prevention. Greater adherence to recommendations was associated with lower likelihood of total cancer in older white and black adults. Public health efforts to increase preventive lifestyle behaviors may be particularly needed among men, black and older individuals.

#2278 Risk factors by molecular subtypes of breast cancer: a pooled analysis of nine cohorts. Mia M. Gaudet,1 Gretchen Gierzach,2 Brian Carter,1 Juhua Luo,3 Roger Milne,1 Elisabete Weiderpass,4 Graham Giles,5 Wendy Chen,3 Kulla Tammela,6 Elke-Mari Hannink,4 Tsung-Yu Chen,7 Kyung-il Park,1 Seung-Eun Park,1 Mia M. Gaudet, 1 Gretchen Gierzach,2 Brian Carter,1 Juhua Luo,3 Roger Milne,1 Elisabete Weiderpass,4 Graham Giles,5 Wendy Chen,3 Kulla Tammela,6 Elke-Mari Hannink,4 Tsung-Yu Chen,7 Kyung-il Park,1 Seung-Eun Park,1 "American Cancer Society, Atlanta, GA; National Cancer Institute, Rockville, MD; 2Indiana University, IN; 3Cancer Council Victoria, Australia; 4Karolinska Institutet, Sweden; 5Brigham and Women’s Hospital and Harvard Medical School, Boston, MA; 6Karolinska Institute, Sweden; 7Dana Farber/Harvard Cancer Center, MA; 8HealthPartners, Minneapolis, MN.

Etiological differences between molecular subtypes of breast cancer, defined by estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), have been examined in previous studies but results are inconsistent, most likely due to small numbers of rarer subtypes. In a recent systematic review of these studies, only six of the possible 44 associations had consistent findings, primarily with the most common luminal A subtype (ER+ or PR+ /HER2-). To provide larger numbers from prospective studies for these associations, we utilized a harmonized dataset of nine cohort studies (Cancer Prevention Study-II Nutrition Cohort, Melbourne Cancer Cohort Study, the National Cancer Institute-America n Association of Retired Persons cohort study, Nurses’ Health Study, Nurses’ Health Study-2, Prostate, Lung, Colorectal, and Ovarian Cancer Screening cohort, Swedish Mammographic Cohort, Swedish Women’s Lifestyle and Health Study, and Women’s Health Initiative) that had case data on ER, PR, and HER2 from medical records or state tumor registry records. Multivariate, joint Cox proportional hazard regression models were used to examine the associations between lifestyle factors and subtype-specific risk of breast cancer.
#2279 Burden of colorectal cancer attributable to lifestyle-related risk factors: a pooled study of seven Australian cohorts. Claire M. Vajdic,1 Maria Arriaga,1 Peter Hull,3 Karen Canfell,2 Robert MacInnis,3 Emily Banks,4 Graham Giles,5 Paul Mitchell,6 Robert Cumming,7 Barbara-Ann Adelstein,1 Julie Byles,6 Dianna J. Magliano,3 Jonathan Shaw,7 Anne Taylor,5 Kay Price,7 Vasant Hirani,5 Maartit a. Laaksonen1,2,3,8 1University of Sydney, Sydney, Australia; 2Canterbury Medical Research Foundation, Christchurch, New Zealand; 3University of New South Wales, Sydney, Australia; 4University of Adelaide, Adelaide, Australia; 5Aurantine National University, Canberra, Australia; 6University of Newcastle, Newcastle, Australia; 7Baker IDI Heart and Diabetes Institute, Melbourne, Australia; 8University of Sydney, Sydney, Australia.

Aim: To quantify the burden of colorectal cancer in Australia attributable by modifications to established lifestyle-related risk factors: smoking, excess body weight, excessive red and processed meat consumption, excessive alcohol consumption and physical inactivity. Methods: Data on exposure to lifestyle-related risk factors from seven Australian cohort studies (N = 367,772) were harmonized and pooled. The cohorts were linked to the Australian Cancer Database and National Death Index to identify incident cancers and deaths. The strength of the exposure-cancer and exposure-death associations were estimated using a proportional hazards model, adjusting for age, sex and the other lifestyle exposures. Age- and sex-specific exposure prevalence was estimated from the Australian National Health Survey 2011-2012 or from the latest 45 and Up cohort study when not available in the NHS. These estimates were then combined to calculate the Population Attributable Fractions (PAFs), i.e. fractions of cancer attributable to the lifestyle-related risk factors, and their 95% confidence intervals (CIs) using an advanced method accounting for competing risk of death. Results: During the first 10-years follow-up, 3,498 incident colorectal cancers and 20,348 deaths were ascertained as first events. Smoking, physical inactivity and insufficient fruit intake were statistically significantly associated with both lung cancer incidence and death. Most of the lung cancer burden (PAF = 73%, CI = 69-76%) was attributable to current or past smoking, with 31% (CI = 27-35%) of the burden attributable to current smokers. Physical activity below Australian recommendations (< 150 minutes of moderate or 75 minutes of vigorous exercise per week) explained 14% (CI = 6-22%) and fruit intake below Australian recommendations (< 2 daily serves) explained 6% (CI = 0-12%) of the lung cancer burden. Each risk factor contributed more to the lung cancer burden in men than in women. Overall, 78% (CI = 74-81%) of the lung cancer burden was attributable to current or past smoking, physical inactivity and insufficient fruit intake. The combined contribution of modifiable risk factors (i.e. excluding past smoking) was 45% (CI = 37-50%). Based on projected Australian cancer incidence rates, this would translate to 59,000 avoidable lung cancers in the next 10 years. Conclusions: According to these joint PAF estimates, the current lung cancer burden in Australia could be reduced by up to half in 10 years by quitting smoking and increasing physical activity and fruit intake to the recommended levels. As the combined effect of risk factors is rarely additive, knowing both individual and joint effects of risk factors on cancer burden is essential in planning cancer interventions and public health policies and predicting their potential impact.

#2280 Burden of lung cancer in Australia avoidable by modifications to lifestyle-related risk factors. Maartit a. Laaksonen,1 Maria Arriaga,1 Peter Hull,3 Karen Canfell,2 Robert MacInnis,3 Emily Banks,4 Graham Giles,5 Paul Mitchell,6 Robert Cumming,7 Barbara-Ann Adelstein,1 Julie Byles,6 Dianna J. Magliano,3 Jonathan Shaw,7 Anne Taylor,5 Kay Price,7 Vasant Hirani,5 Maartit a. Laaksonen1,2,3,8 1University of Sydney, Sydney, Australia; 2Canterbury Medical Research Foundation, Christchurch, New Zealand; 3University of New South Wales, Sydney, Australia; 4University of Adelaide, Adelaide, Australia; 5Aurantine National University, Canberra, Australia; 6University of Newcastle, Newcastle, Australia; 7Baker IDI Heart and Diabetes Institute, Melbourne, Australia; 8University of Sydney, Sydney, Australia.

Aim: The cancer burden avoidable through risk factor modification can be quantified using the Population Attributable Fraction (PAF) which combines estimates of the prevalence of the risk factor exposure in the population and the strength of the exposure-cancer association. PAF for risk factors causally related to cancer is most accurately estimated from cohort studies, using analytical approaches that account for death as a competing risk. We provide the first such estimates of the lifestyle-related avoidable lung cancer burden. Methods: Seven contemporary Australian cohort studies (N = 367,772), with comprehensive data on exposure to lifestyle-related risk factors, were linked to the Australian Cancer Database and National Death Index to identify lung cancers and deaths from any cause. The risk factors were harmonised across the studies and the data pooled. The strength of the exposure-cancer and exposure-death associations were estimated using a proportional hazards model, adjusting for age, sex and the other lifestyle exposures. Age- and sex-specific exposure prevalence was estimated from the Australian National Health Survey 2011-2012. These estimates were then combined to calculate the PAFs and their 95% confidence intervals (CI) using a newly developed method accounting for competing risk of death. Results: During the first 10-years follow-up, 2,030 incident lung cancers and 20,348 deaths were ascertained as first events. Smoking, physical inactivity and insufficient fruit intake were statistically significantly associated with both lung cancer incidence and death. Most of the lung cancer burden (PAF = 73%, CI = 69-76%) was attributable to current or past smoking, with 31% (CI = 27-35%) of the burden attributable to current smokers. Physical activity below Australian recommendations (< 150 minutes of moderate or 75 minutes of vigorous exercise per week) explained 14% (CI = 6-22%) and fruit intake below Australian recommendations (< 2 daily serves) explained 6% (CI = 0-12%) of the lung cancer burden. Each risk factor contributed more to the lung cancer burden in men than in women. Overall, 78% (CI = 74-81%) of the lung cancer burden was attributable to current or past smoking, physical inactivity and insufficient fruit intake. The combined contribution of modifiable risk factors (i.e. excluding past smoking) was 45% (CI = 37-50%). Based on projected Australian cancer incidence rates, this would translate to 59,000 avoidable lung cancers in the next 10 years. Conclusions: According to these joint PAF estimates, the current lung cancer burden in Australia could be reduced by up to half in 10 years by quitting smoking and increasing physical activity and fruit intake to the recommended levels. As the combined effect of risk factors is rarely additive, knowing both individual and joint effects of risk factors on cancer burden is essential in planning cancer interventions and public health policies and predicting their potential impact.

#2281 Environmental tobacco smoke exposure and breast cancer risk in the Norwegian women and cancer study cohort. Inger T. Gram,1 Tonje Braaten, Eili Lund. Idiri Lica, UiT The Arctic University of Norway, Troms, Norway.

Background: Today, the majority of middle-aged women in Norway are ever (i.e. either former or current) smokers, who started to smoke in their teens. Many of those who have never smoked themselves were exposed to environmental tobacco smoke (ETS) as children. The purpose of the study, was to examine the effect of ETS in childhood on breast cancer risk in the Norwegian Women and Cancer Study, a nationally representative prospective cohort study. Material and Methods: We followed 121 662 women, that were aged 34-70 years at enrolment, who completed a baseline questionnaire between 1991 and 2007, through linkages to national registries through December 2014. Questionnaire data included information on lifestyle factors, including lifetime history of smoking. We used Cox proportional hazards regression to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) while adjusting for relevant confounders and stratified by birth cohort. We included the following variables in the final multivariable models: age at enrolment, years of education (0, 3-11, 12, 13-14, 15+, ) and age at menarche (0, 3-8, 9-14, 15+, ) ever oral contraceptives use (yes, no), a variable including nulliparous and a combination of total number of births, (1, 2, 3+, ) and of age at first childbirth (age 10-12, 2-3, 4-6, 7-10, 11+, ) for a total of 13 categories, family history of breast cancer in the mother (yes, no), body mass index (calculated from current height and weight (lt 20, 20-24.9, 25-29.9, 30+, ) kg/m , menopausal status (yes, no), postmenopausal hormone therapy use (never, former, current) and average alcohol consumption, based on the
content of pure alcohol in different sorts of beverages and portion sizes, as grams of alcohol per day (0.0, 0.1-1.40, 4.1-10, >10). Women who reported to be teetotalers and those answering ‘seldom or never’ had their alcohol consumption set to 0. Results: During a mean follow-up of 15 years, 4501 women developed invasive breast cancer confirmed by histology. Altogether, 23.7% of the women reported to be exposed to ETS during childhood. The smoking attributable fraction of the cancer was 0.6% for the father, 0.66% for the mother, and 0.57% both parents, and in 11.8% the information about the active smoker was missing. Compared with never smokers, the multivariable adjusted HR estimate for ETS during childhood where the father was the smoker was statistically significantly increased with 23% (HR = 1.23; 95% CI 1.07-1.42). The corresponding figures for those being exposed to ETS by the mother was a HR of 1.22 (95% CI 0.88-1.69), for those being exposed by both parents was a HR of 1.06 (95% CI 0.88-1.26) and for those with no information about the active smoker the HR was 1.03 (95% CI 0.89-1.20). In conclusion, we found that exposure to environmental tobacco smoke during childhood was associated with a higher risk of breast cancer for women who reported that the father was the active smoker.

#2282 Smoking and alcohol and the risk of myelodysplastic syndrome: The JPHC study. Tomotaka Ugai,1 Keitaro Matsuoka,1 Norie Sawada,2 Motoki Iwasaki,2 Taiki Yamaji,2 Taichi Shimazu,2 Shizuka Sasazuki,2 Manami Inoue,2 Yoshinobu Kanda,3 Shoichiro Tsugane2,4 Aichi Cancer Center Research Institute, Nagoya, Japan; National Cancer Center, Tokyo, Japan; aitama Medical Center, Nihonmatsu, Japan. Background. Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal disorders of hematopoietic stem cells that are characterized by ineffective hematopoiesis, multilineage dysplasia, peripheral cytopenias, and susceptibility to leukemic transformation. Even without progression to leukemia, there is substantial morbidity and mortality. Therefore, a better understanding of the etiology of this disease can lead to a significant reduction in the incidence and the mortality as a consequence, but it remains largely unknown. Smoking and alcohol are important modifiable risk factors for human cancers. However, only a few epidemiological studies have investigated their association with the risk of MDS. Here, we evaluated the association of smoking and alcohol consumption and the risk of MDS in a large-scale population-based cohort study in Japan. Methods. We included 95,950 Japanese subjects (45,451 men and 50,059 women; age 40-69 years at baseline) of the Japan Public Health Center-based Prospective Study who completed a questionnaire about their smoking and alcohol habits. During 18.3 years of follow-up, we identified 70 MDS cases (50 men and 20 women). We calculated hazard ratios (HRs) and their 95% confidence intervals (95% CI) using the Cox proportional hazards model to describe the relative risk of MDS associated with the smoking categories at baseline (never smokers, former smokers, current smokers, ever smokers of >30 pack-years) and the drinking categories at baseline (non-drinkers, occasional drinkers, regular drinkers of 1-299 or ≥300 g/week of ethanol) after adjustment for potential confounders. Results. Smoking was marginally associated with an increased risk of MDS among men, with a HR for current smokers relative to never smokers of 2.11 (95% CI 0.91-4.89). This risk increase was also observed in ever smokers when considered as a continuous variable (HR = 1.07-1.42). The corresponding figures for those with no information about the active smoker was a HR of 1.22 (95% CI 0.88-1.69). A linear increase in HR with increasing pack-years was also seen, albeit without statistical significance (p-trend = 0.008). In contrast, alcohol consumption was associated with a dose-dependent decrease in the risk of MDS among men (non-drinkers: reference, occasional drinkers: HR = 0.48, 0.16-1.43; 0-299 g/week: HR = 0.37, 0.19-0.74; ≥300 g/week: HR = 0.45, 0.20-0.99, p for trend = 0.008). All types of alcohol beverages (sake, distilled spirit and beer) consistently showed a protective effect. We also explored a possible interaction between smoking and alcohol consumption, but no obvious interaction was observed. Conclusions. This study showed that alcohol has a significant protective effect on the risk of MDS among Japanese men. In addition, this study also provides evidence that smoking increases the risk of MDS among Asian population, as it does among Western populations. Previous findings on the association between alcohol and the risk of MDS have been inconsistent, and further investigation across multiple populations is needed.

#2283 Population-level relevance of lifestyle-related risk factors for pancreatic cancer in Australia. Maria E. Arriaga,1 Maarit A. Laaksonen,1 Karen Canfell,2 Robert MacInnis,3 Emily Banks,4 Graham Giles,5 Paul Mitchell,6 Robert Cumming,6 Barbra-Ann Adelstein,1 Julie Byles,5 Manami Inoue,2 Yoshinobu Kanda,3 Shoichiro Tsugane2,4 Aichi Cancer Center Research Institute, Nagoya, Japan; National Cancer Center, Tokyo, Japan; aitama Medical Center, Nihonmatsu, Japan. Background. Population-level relevance of lifestyle-related factors for human cancers. However, only a few epidemiological studies have investigated their association with the risk of MDS. Here, we evaluated the association of smoking and alcohol consumption and the risk of MDS in a large-scale population-based cohort study in Japan. Methods. We included 95,950 Japanese subjects (45,451 men and 50,059 women; age 40-69 years at baseline) of the Japan Public Health Center-based Prospective Study who completed a questionnaire about their smoking and alcohol habits. During 18.3 years of follow-up, we identified 70 MDS cases (50 men and 20 women). We calculated hazard ratios (HRs) and their 95% confidence intervals (95% CI) using the Cox proportional hazards model to describe the relative risk of MDS associated with the smoking categories at baseline (never smokers, former smokers, current smokers, ever smokers of >30 pack-years) and the drinking categories at baseline (non-drinkers, occasional drinkers, regular drinkers of 1-299 or ≥300 g/week of ethanol) after adjustment for potential confounders. Results. Smoking was marginally associated with an increased risk of MDS among men, with a HR for current smokers relative to never smokers of 2.11 (95% CI 0.91-4.89). This risk increase was also observed in ever smokers when considered as a continuous variable (HR = 1.07-1.42). The corresponding figures for those with no information about the active smoker was a HR of 1.22 (95% CI 0.88-1.69). A linear increase in HR with increasing pack-years was also seen, albeit without statistical significance (p-trend = 0.008). In contrast, alcohol consumption was associated with a dose-dependent decrease in the risk of MDS among men (non-drinkers: reference, occasional drinkers: HR = 0.48, 0.16-1.43; 0-299 g/week: HR = 0.37, 0.19-0.74; ≥300 g/week: HR = 0.45, 0.20-0.99, p for trend = 0.008). All types of alcohol beverages (sake, distilled spirit and beer) consistently showed a protective effect. We also explored a possible interaction between smoking and alcohol consumption, but no obvious interaction was observed. Conclusions. This study showed that alcohol has a significant protective effect on the risk of MDS among Japanese men. In addition, this study also provides evidence that smoking increases the risk of MDS among Asian population, as it does among Western populations. Previous findings on the association between alcohol and the risk of MDS have been inconsistent, and further investigation across multiple populations is needed.

EPIDEMIOLOGY: Tobacco, Alcohol, and Other Risk Factors

#2284 Effects of smoking on breast tumor pathology. Nicholas Costantino,1 Craig D. Shriver,2 Rachel E. Ellsworth3. 1Chan Soon-Shiong Institute of Molecular Medicine at Windber, Windber, PA; 2Martha Cancer Center, Bethesda, MD; 3Martha Cancer Center, Windber, PA. Background: Although smoking is not a well-established breast cancer risk factor, proximity of lungs to the breasts and findings that tobacco smoke constituents serve as mammary carcinogens in animal models suggests that smoking may alter the breast microenvironment and affect breast tumorigenesis. To determine whether there are relationships between smoking and pathological characteristics and outcome were evaluated in 1,707 women with invasive breast cancer. Methods: Patients enrolled in the Clinical Breast Care Project 2001-2013 filled out a questionnaire that included answers to current smoking status, current and previous pack use/day, number of years smoked, and length since last smoked. Smoking status was categorized as 0, 20, 20-34 or >35 pack-years. Current smokers were categorized according to hormone receptor status and metastasis (OR 1.44; 95% CI 1.03-2.01), positive HER2 status (OR 1.54, 95% CI 1.04-2.25), larger tumor size (OR 1.53; 95% CI 1.14-2.11) and higher tumor stage (OR 1.54, 95% CI 1.16-2.04) when compared to never smokers. Current smokers were less likely to be diagnosed with late stage tumors (OR 0.77, 95% CI 0.62-0.96) compared to never smokers. No significant associations were detected when pack years were considered as a continuous variable; when categorized as 0, <20, 20-34 or >35 pack years, >35 pack years was associated with positive HER2 status (OR 1.80, 95% CI 1.06-3.03) and tumors of the ER+HER2+ subtype (OR 1.99, 95% CI 1.07-3.7). Overall survival was significantly lower for former and current smokers compared to never smokers (P=0.014); however, breast cancer specific survival did not differ significantly among the smoking categories when stratified by metastasis status (P=0.226). Conclusions: Current smokers are at increased risk for having larger and later stage tumors with positive lymph node and HER2 status. Tumor size, stage and lymph node status are temporal factors, thus suggest that current smokers may have delayed diagnosis compared to never and former smokers. In contrast, HER2 status re-
EvAluation of Risk (CLEAR) study. reluceptor subtypes: results from the New South Wales Cancer Lifestyle and Evaluation of Risk (CLEAR) study. Usha G. Salagame,1 Emily Banks,2 Dianne O'Connell,3 Sam Egger,4 Karen Canfell,5 Cancer Council NSW, NSW 2011, Australia;3 Australian National University, Australian Capital Territory, Australia;2 University of Sydney, Sydney, Australia;1 University of California San Francisco and Veterans Affairs Medical Center San Francisco, CA

Background: Prior observational studies have identified an elevated breast cancer risk associated with current MHT use for ER+ (Estrogen Receptor positive), and for ER+/PR+ (Estrogen and Progesterone Receptor positive) breast cancers than for ER- and ER-/PR- subtypes respectively. We have previously reported, from a large case-control study for all cancer types (the NSW CLEAR study) that current MHT use was associated with a doubling of the odds of breast cancer. Here, we describe further analyses investigating the MHT-breast cancer association for the breast cancer tumor receptor subtypes defined by ER expression, by ER and PR expression and by the joint expression of ER, PR, and HER-2 (Human Epidermal growth factor Receptor-2). Methods: Analyses were carried out for a subset of registry-verified CLEAR breast cancer cases with hormone receptor status data (n=410) and CLEAR (cancer-free) controls recruited over the same period (n=324). We used a multinomial logistic regression model to estimate Odds Ratios (ORs) adjusted for other breast cancer risk factors and 95% Confidence Intervals (CI) for current and past MHT use in subgroups defined using Cox receptor subtypes. Never users comprised the reference group. Findings: In a multinomial model, current MHT use was associated with an elevated risk of ER+/breast cancer (aOR = 2.04, 95%CI: 1.28 -3.24). When breast cancers were categorised by ER and PR status, current use was associated with an elevated risk of developing ER+/PR+ breast cancer (aOR = 2.29, 1.41-3.72). Current MHT use was associated with the surrogate luminal A breast cancer characterised by ER+/PR+/HER2- phenotype (aOR = 2.30, 1.42-3.73). None of the other subtypes of breast cancer (ER+/PR-/HER2+, ER-/PR-/HER2+, and ER-/PR-/HER2-) were significantly associated with current MHT use. A significant difference in the odds of developing breast cancer for current MHT users was detected between the surrogate luminal A and luminal B (ER+/PR+/HER2-) subtype only (aOR= 0.28, 0.09-0.88, p=0.029). None of the other groups were significantly differently associated with MHT use although this may be due to lack of power. Past MHT use was not associated with an increased risk of breast cancer for any breast cancer subtype. Conclusion: The findings from this contemporary Australian study are consistent with findings from other studies that current, but not past, use of MHT is associated with increased risk of breast cancer with higher risks reported for ER+, ER- and PR+ and ER+/PR+/HER2- (surrogate luminal A) subtypes. Our findings are consistent with the hypothesis that breast cancers induced by MHT may occur through receptor-mediated mechanisms.

Atoxic allergic conditions and risk of pancreatic cancer: the Multi-ethnic Cohort. Brian Z. Huang,1 Loic Le Marchand,2 Christopher A. Haiman,3 Kristine Monroe,3 Lynne Wilkens,2 Zuo-Feng Zhang,1 Veronica Wendy Setiawan7.1 UCLA School of Public Health, Los Angeles, CA;2 University of Hawai'i Cancer Center, Honolulu, HI;3 University of Southern California, Los Angeles, CA

Background: Previous studies, mostly case-control studies, have suggested that allergic conditions (AACs) are associated with a decreased risk of pancreatic cancer. Scarc data, however, are available from prospective, US multi-ethnic populations. In this largest prospective study to date, we investigated the association between AACs (asthma, hay fever, or allergy) and risk of developing pancreatic cancer in the US Multietnic Cohort Study (MEC). Methods: AACs were assessed via a baseline questionnaire when participants joined the MEC in 1993-1996. Hazard ratios (HRs) and 95% confidence intervals (CIs) for pancreatic cancer risk were estimated using Cox proportional hazards models, adjusting for age, sex, race/ethnicity, education, smoking status, family history of pancreatic cancer, body mass index, diabetes, and alcohol intake. Results: During an average of 16.2-year follow-up, 1,455 incident cases of pancreatic cancer were identified among 187,226 white, African-American, Native Hawaiian, Japanese-American, and Latino men and women. AACs were not associated with pancreatic cancer incidence (HR = 1.00; 95% CI: 0.88, 1.12). The null association was observed in men and women and across racial/ethnic groups, smoking status, BMI groups, diabetes status, and family history of pancreatic cancer. Conclusions: Based on these results, AACs is unlikely to be associated with risk of pancreatic cancer.

Number of risky lifestyle behaviors and breast cancer subtypes in a large nested case-control study from Norway. Merete Ellingjord-Dale,1 Linda Vos,1 Steinar Tretli,1 Solveig Hofvind,2 Anette Hjartaker,2 Hege Russnes,2 Isabel dos-Santos-Silva,3 Giske Ursin3.1 Cancer Registry of Norway, Oslo, Norway; 3University of Oslo, Oslo, Norway; 2Oslo University Hospital, Oslo, Norway; 4London School of Hygiene and Tropical Medicine, London, United Kingdom.

Background: Alcohol, smoking, physical inactivity, high body mass index (BMI) and menopausal hormone therapy (MHT) use and breast cancer risk by receptor subtypes: results from the New South Wales Cancer Lifestyle and Evaluation of Risk (CLEAR) study. Usha G. Salagame,1 Emily Banks,2 Dianne O'Connell,3 Sam Egger,4 Karen Canfell,5 Cancer Council NSW, NSW 2011, Australia;3 Australian National University, Australian Capital Territory, Australia;2 University of Sydney, Sydney, Australia;1 University of California San Francisco and Veterans Affairs Medical Center San Francisco, CA

Objective: The cancer risk associated with current MHT use was detected between the surrogate luminal A and luminal B (ER+/PR+/HER2- phenotype (aOR = 2.30, 1.42-3.73). Current MHT use was associated with an increased risk of breast cancer for any breast cancer subtype. Methods: We conducted a case-control study nested within a cohort of 457,036 women who participated in the Norwegian Breast Cancer Screening Program (NBCSP) in 2006-2014, and who completed a questionnaire at baseline screening. In all, 4,686 breast cancer cases with information on risk factors and hormone receptor status (i.e. estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor 2 (HER2) occurred during the follow-up. The following surrogate definitions of breast cancer subtypes were used: ER+PR-HER2- (luminal A-like), ER+PR-HER2- (luminal B-like, HER2 negative), ER+PR+/PR+/HER2- (luminal B-like, HER2 positive), ER+PR-HER2+ (HER2 positive) and ER-PR-HER2- (triple negative). We defined risky lifestyle behaviors as ever smoking, weekly consumption of more than 2 glasses of alcohol per week, less than 3 hours leisure time physical activity per week, more than 7 hours of sleep per night, ever use of menopausal hormone (estrogen and progesterone) therapy and BMI (kg/m2) >25. We used conditional logistic regression to estimate odds ratios (ORs), with 95% confidence intervals (CIs), adjusted for age, education, age at menarche, number of pregnancies and menopausal status. Results: Number of risky lifestyle behaviors was associated with an increased risk of breast cancer overall (p-trend<0.0001). Compared to women with no lifestyle behaviors, women with 1 (OR= 1.28, 95% CI 0.87-1.88), 2 (OR = 1.63, 95% CI 1.13-2.36), 3 (OR = 1.85, 95% CI 1.28-2.68), 4 (OR = 2.27, 95% CI 1.56-3.29) and 5 (OR=2.38, 95% CI 1.58-3.59) risky lifestyle behaviors had increased risk of luminal A-like subtype (p-trend <0.0001). However, number of risky lifestyle behaviors was not associated with increased risk of the triple negative subtype (p-trend=0.27). Conclusions: This study showed that number of risky lifestyle behaviors was positively associated with a marked increased risk for luminal A-like breast cancer. These findings suggest that healthy lifestyle choices may play an important role in the prevention of the commonest form of this cancer.

Effects of tobacco smoking and alcohol consumption on risks of CYP1B1 polymorphisms for prostate cancer. Taku Kato, Yutaka Hashimoto, Shigekatsu Maekawa, Marisa Shina, Mitsuho Imai-Sumida, Pritha Dasgupta, Masahiko Takashima, Soichiro Yamamura, Varahram Sharryari, Guoren Deng, Rajivv Daihah, Yuichiyo Tanaka. University of California San Francisco and Veterans Affairs Medical Center San Francisco, San Francisco, CA

Cytochrome P450 1B1 (CYP1B1) converts xenobiotics to carcinogens and polymorphic variants have been shown to increase activity levels. Lifestyle choices such as tobacco smoking and alcohol consumption are known to enhance the carcinogenesis process and in this study, how these factors may interact with CYP1B1 polymorphisms and affect prostate cancer risk was assessed. Blood genomic DNA from a Caucasian population consisting of 405 healthy men and 400 prostate cancer patients were obtained. Of these, 507 were current or former smokers and 407 were alcohol drinkers. Eight polymorphic sites of the promoter region of CYP1B1 (rs2551188 G to A, rs2567206 G to A, rs2567207 A to G, rs162558 A to G, rs10175368 C to T, rs163090 T to A, rs162330 T to G, and rs162331 A to G) were analyzed in samples using Taqman genotyping assays and real-time PCR. Lifestyle factors and its influence on CYP1B1 polymorphisms toward cancer risks were also evaluated. Overall, both alcohol (P=0.006) and smoker (P=0.009) status were associated with prostate cancer. CYP1B1 variants were also risks for cancer at rs2551188 (P=0.043), rs2567206 (P=0.008), and rs10175368 (P=0.001). Evaluation of linkage disequilibrium show rs2551188, rs2567206, rs2567207, and rs10175368 to be linked and interestingly, the G-G-A-C haplotype (wildtype at respective sites) was significantly reduced in cancer (P=0.0287). When classified by lifestyle factors, no associations for CYP1B1 variants were found for cancer among non-smokers with rs10175368 (P=0.051) being a risk among non-drinkers. On the other hand, variants at both rs2567206 and rs10175368 showed increased cancer risk among smokers (P=0.032 and 0.002, respectively) as well as drinkers (P=0.044 and 0.019, respectively). No genotyping differences were observed when analyzing cancers by pathological grades. These results demonstrate...
smoker and alcoholic drinker status to modify the risks of CYP1B1 polymorphisms for prostate cancer and this is of importance in understanding their role in the pathogenesis of this disease.

#2289  B-carotene supplementation and lung cancer risk in the ATBC Study: the role of tar and nicotine. Pooja Middha,1 Stephanie J. Weinstein,2 Satu Mannisto,3 Demetrias Albanes,4 Alison M. Mondul.1 1University of Michigan School of Public Health, Ann Arbor, MI; 2National Cancer Institute, Bethesda, MD; 3National Institute for Health and Welfare, Helsinki, Finland.

Background: Globally, lung cancer is the most common cancer and the leading cause of cancer death. Early observational studies reported a protective association between intake of B-carotene rich vegetables and lung cancer risk, which led to interest in B-carotene supplementation as a potential chemopreventive strategy, particularly in high-risk populations such as cigarette smokers. A large randomized controlled trial, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study, demonstrated that B-carotene supplementation increased lung cancer incidence by 18% in smokers, and led to an 8% excess in overall mortality. It is also well-documented that cigarettes with higher (versus lower) tar and nicotine content are associated with a higher risk of lung cancer. To our knowledge, however, no studies have examined whether the increased risk associated with B-carotene supplementation in smokers varies by cigarette tar or nicotine content. Methods: The ATBC Study was a randomized, double-blind vitamin supplementation trial conducted among 29,133 male smokers in southwest Finland. Participants were randomly assigned to one of four groups in a 2x2 factorial design (a-tocopherol alone, B-carotene alone, both supplements, or placebo). A detailed smoking history was collected to be used in the analysis of cigarettes smoked (for which machine measured tar and nicotine content was available). Cox proportional hazards models were used to estimate the hazard ratio (HR) and 95% confidence intervals (CI) of lung cancer risk by trial B-carotene assignment stratified by a priori categories of cigarette tar (ultra-light, light, medium, regular, medium 15-20 mg, high, non-filtered/self-made >20 mg) and nicotine content (ventilated filtered <0.8 μg, unventilated filtered 0.8-1.3 μg, non-filtered >1.3 μg). Statistical interaction was estimated using the likelihood ratio test. Results: The B-carotene supplemented arm had a statistically significantly higher risk of developing lung cancer in all categories of tar content [yes vs. no B-carotene supplementation - ultra-light: HR = 1.31 95% CI = 0.91-1.89; non-filtered: HR = 1.22 95% CI = 0.91-1.64; p-interaction = 0.91]. Similarly, there was no apparent interaction with cigarette nicotine content [yes vs. no B-carotene supplementation - ventilated cigarettes: HR = 1.23, 95% CI = 0.98-1.54; non-filtered cigarettes: HR = 1.22, 95% CI = 0.91-1.64; p-interaction = 0.83]. Conclusion: These findings indicate that supplementation with B-carotene increases lung cancer incidence in smokers regardless of the tar or nicotine content of cigarettes smoked. Our data suggest that all smokers, regardless of the type of cigarette smoked, should continue to avoid B-carotene supplementation.

#2290  Perceived stressfulness of life events impacts breast cancer risk. Avital Fischer, Argos Ziegos, Hoda Anton-Culver. UCI, Irvine, CA.

Background: Breast cancer is the most prevalent malignancy and the second most common cause of cancer mortality among women. Approximately 60% of breast cancers are attributed to breast cancer risk (BCR) factors that are not modifiable and the majority of these women do not have a family history of breast cancer. Therefore, it is important to perform further research focusing on identifying preventable risk factors for breast cancer. The relationship between psychological stress and breast cancer risk is unclear. Psychological stress arises when environmental demands are appraised as going beyond one's coping abilities. Higher perceived stress has been linked to increased cortisol levels and HPA perturbations. The purpose of this study is to understand the relationship between stressfulness appraisal of salient life events and breast cancer risk. Methods: A case-control design was used and included 664 female cases identified for breast cancer and this is of importance in understanding their role in the pathogenesis of this disease.

Results: The relationship between psychological stress and breast cancer risk in a dose response fashion (OR = 0.83, 95% CI = 0.64-1.04) for stressful vs. not stressful (OR = 1.22, 95% CI = 1.03-1.45). The role of psychological stress on breast cancer risk only when reported as being stressful (OR = 0.54, 95% CI = 0.32-0.92; OR = 0.63, 95% CI = 0.43-0.93 respectively). Conclusions: This study broadens our understanding of the relationship between major life events and breast cancer risk and underscores the importance of stressfulness appraisal. Our results indicate the importance of incorporating assessments of perceived stressfulness in future epidemiological investigations focusing on the assessment of life events and breast cancer risk.

#2292  Lung function and lung cancer risk: a Mendelian randomization study of UK Biobank cohort and the International Lung Cancer Consortium. Linda Kachuri,1 Matthias Johansson,2 Paul Brennan,3 Phillip Haycock,3 Geoffrey Liu,4 Maria Teresa Landi,5 David C. Christiani,6 Neil E. Caporaso,7 Xifeng Wu,8 Melinda C. Aldrich,9 Demetrios Albanes,10 Adolina Tardón,11 Gad Rennert,12 Chu Chen,13 Gary E. Goodman,14 Jennifer A. Doherty,15 Heike Bickeboller,16 Dawn Teare,17 Lambertus A. Kiememeny,18 Stig E. Bojesen,19 John K. Field,20 Aage Haugen,9 Stephen Lam,9 Loic Le Marchand,20 Matthew B. Schabath,21 Angelique S. Andrew,1 Jonas Manjer,2 Philip Lazarus,23 Susanne M. Arnold,24 Valérie Gaborieau,2 Richard Martin,2 Caroline Relton,2 George Davey Smith,2 Christopher I. Amos,3 James D. Mc-Kay,2 Rayeun J. Hung,3 Lunfenfeld-Fandenbaum Research Institute, Sinai Health System, Toronto, Ontario, Canada; National Institute of Public Health, Orange County, CA; University of California, Irvine, CA; National Cancer Institute, Bethesda, MD; National Cancer Institute, Health and Welfare, Helsinki, Finland. 

Background: Lung function (LF) is strongly associated with increasing lung cancer risk. However, since airflow obstruction is a diagnostic criterion for obstructive lung disease, and a consequence of tobacco smoking, isolating the causal relationship between LF and lung cancer has remained a challenge. Methods: We investigated 3 standardized (mean = 0, standard deviation = 1) LF phenotypes (forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), and FEV1/FVC) and assessed their association with lung cancer risk in the ATBC Study of UK Biobank cohort and the International Lung Cancer Consortium. Findings: 1) a genome-wide association study of UK Biobank cohort and the International Lung Cancer Consortium and, 2) a Mendelian randomization (MR) analysis using genetic instrumental variables (IVs) developed in UK Biobank and tested using individual-level data from the OncoArray, a genome-wide array with in-depth coverage for common cancers. Results: 702 incident lung cancers were diagnosed in 484,194 UKB participants during follow-up. Cox regression was used to estimate hazard ratios (HR) and 95% confidence intervals (CI), adjusted for age, sex, smoking status, socioeconomic status, and assessment center. Adjustment for other smoking metrics yielded similar results. Lung cancer risk increased per 1 unit decrease in FEV1 (HR = 1.80, 95% CI: 1.64-1.98, p = 3.3x10^-34), FVC (HR = 1.45, 1.30-1.60, p = 2.3x10^-12), and FEV1/FVC (HR = 1.39, 1.31-1.46, p = 1.3x10^-30). This pattern was observed for adenocarcinoma (n = 300): FEV1 (HR = 1.77, p = 6.0x10^-12), FVC (HR = 1.48, p = 1.4x10^-7), FEV1/FVC (HR = 1.34, p = 8.3x10^-9); and squamous cell carcinoma (n = 166): FEV1 (HR = 1.97, p = 9.0x10^-5), FVC (HR = 1.60, p = 1.0x10^-5), FEV1/FVC (HR = 1.38, p = 5.9x10^-5). Next, a genome-wide association study of 67,708 UKB participants and 12.6 million variants was carried out to develop genetic IVs for LF. Results were filtered to retain independent variants (R²<0.2) associated with each LF phenotype (p<5x10^-8). The following IVs were developed: FEV1 (n = 28 variants, 0.72% of variation explained), FVC (n = 44, 1.08%), and FEV1/FVC (n = 45, 1.85%). Odds ratios (OR) for each IV and lung cancer were estimated for 18,868 cases 15,190 controls (>80% European ancestry) from 23 studies. Effect estimates were combined using a maximum-likelihood MR models to estimate causal ORs. MR results indicate that genetic scores associated with increased airflow are unrelated to lung cancer risk: FEV1 (OR = 1.00, 95% CI = 0.96-1.03, p = 0.86), FVC (OR = 1.00, 0.97-1.03, p = 0.93) and FEV1/FVC (OR = 1.00, 0.91-1.10, 1.587
p = 0.95). The null association observed for the genetic determinants of FEV1, FVC and FEV1/FVC was not modified by tumor histology or smoking status. Conclusions: LF is a robust predictor of lung cancer risk, however, our findings do not support the existence of causal pathways that are independent of obstructive lung disease or smoking. This apparent lack of a causal relationship should be interpreted with caution since pleiotropic effects of LF loci cannot be ruled out.

**#2293 Oligomenorrhea, polycystic ovary syndrome, and risk of ovarian cancer histotypes, evidence from the Ovarian Cancer Association Consortium.** Holly R. Harris,1 Susan Jordan,2 Harvey Risch,3 Mary Anne Rossing,4 Marc T. Goodman,5 Francesmary Modugno,6 Susanne Kruger Kjer,7 Joellen M. Schildkraut,8 Elisa V. Bandera,9 Nicolas Wentzensen,10 Catherine Phelan,11 Hoda Anton-Culver,11 Anna H. Wu,12 Kathryn L. Terry13, 1Fred Hutchinson Cancer Research Ctr., Seattle, WA; 2QIMR Berghofer Medical Research Institute, Herston, Australia; 3Yale School of Public Health, New Haven, CT; 4CEDars-Sinai Medical Center, Los Angeles, CA; 5University of Pittsburgh School of Medicine, Pittsburgh, PA; 6Danish Cancer Society Research Center, Copenhagen, Denmark; 7The University of Virginia, Charlottesville, VA; 8Rutgers Cancer Institute of New Jersey, New Brunswick, NJ; 9National Cancer Institute, Bethesda, MD; 10Moffitt Cancer Center, Tampa, FL; 11University of California Irvine, Irvine, CA; 12University of Southern California Norris Comprehensive Cancer Center, Los Angeles, CA; 13Brigham and Women’s Hospital, Boston, MA.

Background: Oligomenorrhea, defined as infrequent or irregular periods has been shown to be associated with epithelial ovarian cancer risk in some but not all studies. Polycystic ovary syndrome (PCOS), which is characterized by oligomenorrhea and abnormal hormone levels including hyperandrogenism, hyperinsulinemia, and gonadotropin imbalance, has been suggested to increase ovarian cancer risk. However, these associations have been rarely examined by histologic subtype. We sought to examine these associations among 14 studies participating in the Ovarian Cancer Association Consortium. Methods: Participants included 16,594 patients with invasive (n = 13,719) or borderline (n = 2,875) ovarian cancers and 17,718 controls who had answered questions regarding menstrual cycle irregularity, menstrual cycle length, and/or PCOS. Study specific odds ratios (ORs; 95% confidence intervals [95% CI]), adjusted for ovarian cancer risk factors, were calculated and then combined using a random-effects meta-analysis. Pooled histologic subtype specific ORs (95% CI) were calculated using polytomous logistic regression. Results: Women reporting menstrual cycle length > 35 days had decreased risk of invasive ovarian cancer compared to women reporting cycle length ≤ 35 days (OR = 0.70; 95% CI = 0.58-0.84). Decreased risk of invasive ovarian cancer was also observed among women who reported irregular menstrual cycles compared to women with regular cycles (OR = 0.89; 95% CI = 0.76-0.98). No significant association was observed between self-reported PCOS and invasive ovarian cancer risk (OR = 0.87; 95% CI = 0.65-1.15). For menstrual cycle length > 35 days, decreased risk was observed for all invasive histotypes: low grade serous (OR = 0.48; 95% CI = 0.25-0.92), high grade serous (OR = 0.62; 95% CI = 0.48-0.80), mucinous (OR = 0.38; 95% CI = 0.19-0.76), endometrioid (OR = 0.75; 95% CI = 0.54-1.06), and clear cell (OR = 0.43; 95% CI = 0.13-1.13) but not for serous borderline tumors (OR = 1.18; 95% CI = 0.87-1.59). For endometrioid, low grade serous and clear cell carcinomas, serous borderline disease was observed among women reporting irregular cycles (OR = 1.34; 95% CI = 1.16-1.55) (p(heterogeneity)<0.0001). No statistically significant differences by histologic subtype were observed for self-reported PCOS.

Conclusion: In this large consortium analysis we observed a decreased risk of most invasive epithelial ovarian cancer histotypes among women reporting longer or irregular menstrual cycles. In contrast, such cycles were associated with increased risk of the serous borderline tumors.

**#2294 Atypical nevi and risk of incident skin cancer in US men: a prospective study.** WEN-QING LI,1 Eunyoung Cho,1 Alisa Goldstein,2 Yen-Tsung Huang,3 Martin Weinstock,1 Aabir Qureshi1, 1Brown University, Providence, RI; 2National Cancer Institute, Bethesda, MD.

Background and Aims: Atypical nevi (AN) are a strong risk factor as well as a precursor of melanoma. However, most previous studies on the associations between atypical nevi (AN) and the risk of melanoma have been conducted in retrospective case-control settings and the few available prospective studies have been based on small sample sizes. Further, few studies have examined the associations between AN and risk of keratoacanthomas (formerly called non-melanoma skin cancer). We prospectively examined the risk of incident melanoma, squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) associated with AN. Methods: In the Health Professionals Follow-up Study, information on AN was collected by self-report. Only those confirmed cases of AN by a supplementary questionnaire, the Atypical Nevi Screening Questionnaire (ANQ), were included in the analyses. Diagnosis of skin cancers was reported biennially and information on melanoma and SCC was pathologically confirmed. A total of 50193 men were included in our study, with 1239 confirmed cases of AN with information on diagnosis year. Cases of AN that were not confirmed or did not respond to the ANQ were excluded from the analyses. Time-dependent Cox-regression analyses were conducted for the associations between AN and risk of incident melanoma, SCC, and BCC respectively.

Results: We identified 545 melanoma cases, 1721 SCC cases, and 13498 BCC cases during the follow-up (1986-2012). A history of AN was significantly associated with increased risk of incident melanoma (hazard ratio [HR] = 3.50, 95% confidence interval [CI]: 2.13-5.76) overall and at different body sites (head and neck, trunk, or limbs). The risk of melanoma was particularly augmented with the increasing number of AN that were surgically removed (HR = 1.26, 95% CI: 1.16-1.37 per AN) and for cases of AN with tissue re-excision from the nevus removal sites (HR = 4.59, 95% CI: 1.70-12.41). A history of AN was also significantly associated with increased risk of BCC (HR = 1.84, 95% CI: 1.60-2.10) and was only associated with risk of SCC at the trunk sites (HR = 3.09, 95% CI: 1.24-7.72). Conclusions: A history of AN significantly increased the risk of subsequent melanoma in a prospective study of men. A history of AN may also be associated with increased risk of BCC overall and trunk SCC, which requires further studies to replicate.
son-years, respectively, in ever and never users. The overall hazard ratio (0.691, 95% confidence interval: 0.578-0.826) suggested a significantly lower risk in metformin users. While compared to never users, the hazard ratio (95% confidence interval) for the first (<20.00 months), second (20.00-45.20 months) and third (>45.20 months) tertile of cumulative duration of metformin therapy was 1.455 (95% CI: 0.926-2.280), 1.710 (95% CI: 0.997-2.941) and 1.455 (95% CI: 0.720-2.541) respectively. The protective effect of metformin on colon cancer was not affected by the use of aspirin and statin or the infection of Helicobacter pylori in additional analyses.

Conclusions: Metformin reduces colon cancer risk in patients with type 2 diabetes. The protective effect is more remarkable when the cumulative duration is more than 20 months.

**EPIDEMIOLOGY: Tobacco, Alcohol, and Other Risk Factors**

#2297 Household stove improvement and risk of chronic obstructive pulmonary disease in Xuanwei, China: a cohort study. Jinning Zhang, 1 Bryam A. Bassi, 2 Roel Vermeulen, 2 Wei Jie Seow, 1 Jason Y.Y. Wong, 1 Wei Hu, 1 Bofu Ning, 1 George S. Downward, 2 Hormuzd A. Katki, 1 Bu-Tian Ji, 1 Nathaniel Rothman, 1 Robert S. Chapman, 1 Qing Lan, 1 National Cancer Institute, Rockville, MD; 2 Utrecht University, Utrecht, Netherlands; 3 Xuanwei Center of Disease Control, Xuanwei, China; 4 Chulalongkorn University, Bangkok, Thailand.

Background: In Xuanwei County, China, chronic obstructive pulmonary disease (COPD) rates are over twice the national average. Patients with COPD have higher risks of both lung cancer and poor treatment outcomes after lung cancer diagnosis. In a prior retrospective cohort study (1976-1992), household stove improvement was found to be associated with decreased COPD incidence among lifetime smoky (bituminous) coal users. However, whether the protective effect persists over time is unclear. Our objective was to evaluate the associations between risk of COPD, and vented and portable stove use with extended follow-up (1976-2011). Methods: The study population comprised 22,833 residents who were born between 1917 and 1951 and lived in Xuanwei as of January 1, 1976. Participants were followed retrospectively from 1976 to 1992, and then followed prospectively through 2011. We conducted two surveys in 1992 and 2011. During each survey, we collected comprehensive information on household stove type, fuel use, family size and number of rooms in each residence during the lifetime, smoking, education, age starting cooking food and time spent indoor from each subject. Participants were also asked to report any medically diagnosed chronic bronchitis or emphysema, age and place of diagnosis, which we combined into a single category of COPD. COPD cases diagnosed before January 1, 1976 were excluded. Sex-specific time-dependent Cox regression models were used to estimate the hazard ratio (HR) of stove improvement for COPD incidence among lifetime smoky coal users. Results: The analysis was restricted to lifetime smoky coal users. A significant reduction in COPD incidence was found after change to vented stoves (Men’s model: HR=0.66, 95% confidence interval: 0.59-0.75; Women’s model: HR=0.60, 95% CI: 0.55-0.67) and portable stoves (Men’s model: HR=0.77, 95% CI: 0.67-0.88; Women’s model: HR=0.63, 95% CI: 0.54-0.74). There was a downward trend of COPD incidence over time after change to vented stove or portable stove (p-trend<0.01). Similar findings were noted in both men and women. Conclusion: Consistent with prior research, our findings suggest that household stove improvement was associated with a substantially decreased risk of COPD, a strong risk factor for COPD. Our findings support the need for replacing smoky coal with less carcinogenic fuels in areas where domestic coal combustion are important risk factors for COPD. Our findings support the need for replacing smoky coal with less carcinogenic fuels in areas where domestic coal combustion are important risk factors for COPD. Similarly, elevated IRRs for kidney cancer were observed for both the 2nd and 3rd tertile of Mn (2nd tertile: 37.33-51.74 ppb; IRR 1.05, 95% CI 1.00, 1.10) and the 2nd tertile of Pb (3.88-5.05 ppb: IRR 1.05 (95% CI 1.00, 1.10)). A dose response was observed for kidney cancer incidence and Mn (2nd tertile: 37.33-51.74 ppb: IRR 1.06 (95% CI 1.02, 1.11); 3rd tertile: 52.95-146.60 ppb: IRR 1.13 (95% CI 1.08, 1.19)). For bladder cancer, no relationship was observed with As but IRRs were elevated for the other metals including potential dose-response trend with Cd and Mn (Pb 2nd tertile: 3.88-5.05 ppb: IRR 1.03 (95% CI 1.00, 1.07); Cd 3rd tertile: 0.61-2.98 ppb: IRR 1.10 (95% CI 1.06, 1.15); Mn 3rd tertile: 52.95-146.60 ppb: IRR 1.12 (95% CI 1.08, 1.16)). Conclusion: In North Carolina, counties with higher mean private well metal levels had elevated kidney and bladder cancer incidence rates. The conclusions are limited by the ecological approach, but the consistency of the associations suggests that the exposure of metals in private well water in North Carolina on health outcome deserves additional investigation.

#2299 Toxic metals in private well water and bladder and kidney cancer incidence in North Carolina. Deepika Shrestha, 1 Selin Crenkrite, 1 Alison Sanders, 2 Rebecca Fry, 3 Katie M. Applebaum, 1 George Washington University, Milken Institute School of Public Health, Washington, DC; 3 hath School of Medicine at Mount Sinai, New York, NY; 4 University of North Carolina, Gillings School of Public Health, Chapel Hill, NC.

Introduction: There are a limited number of studies conducted in the US that have related metals in well water with kidney and bladder cancer. We investigated levels of arsenic (As), cadmium (Cd), manganese (Mn), and lead (Pb) in private well water and the association with incidence of bladder and kidney cancer in 100 counties in North Carolina, a state that has among the highest proportion of well water consumers in the country. Methods: Between 1998-2010, private wells across 100 NC counties were analyzed for levels of As, Cd, Mn and Pb (n=63,836, n=22,915, n=70,675 and n=65,535 measurements, respectively) and geocoded. Bladder and kidney cancer incidence for each county between 1990 and 2011 were obtained from the North Carolina Department of Health and Human Services. County-level data on potential confounders were obtained from US Census Bureau data (2010) and the Behavioral risk factor surveillance study (2003, 2005). For each county, the mean concentration of each metal and the incidence rate ratio (IRR) with 95% confidence interval (CI) were calculated separately. Using counties as the unit of analysis, we used Poisson regression to estimate incidence rate ratios (IRRs) and 95% confidence intervals (CIs) between mean concentration of the four metals and kidney and bladder cancer incidence, adjusting for age, gender, race, education, physical activity, smoking, diabetes, hypertension and residential well water supply. County mean metal levels were analyzed in tertiles. Covariates included: age, sex, county, case less than 10 well measurements for any metal were excluded from the analysis. Results: Ninety-eight counties were included in the final analysis. In the fully adjusted model, elevated IRRs for kidney cancer were observed in counties with the highest tertile of As (3rd tertile: 0.99-11.44 ppb, IRR=1.05 (95% CI 1.00, 1.11)). Similarly, elevated IRRs for kidney cancer were observed for both the 2nd and 3rd tertile of Cd (2nd tertile: 1.08, 1.19); 3rd tertile: 1.05 (95% CI 1.00, 1.10) and the 2nd tertile of Pb (3.88-5.05 ppb: IRR=1.05 (95% CI 1.00, 1.10)). A dose response was observed for kidney cancer incidence and Mn (2nd tertile: 37.33-51.74 ppb: IRR 1.06 (95% CI 1.02, 1.11); 3rd tertile: 52.95-146.60 ppb: IRR=1.13 (95% CI 1.08, 1.19)). For bladder cancer, no relationship was observed with As but IRRs were elevated for the other metals including potential dose-response trend with Cd and Mn (Pb 2nd tertile: 3.88-5.05 ppb: IRR=1.03 (95% CI 1.00, 1.07); Cd 3rd tertile: 0.61-2.98 ppb: IRR 1.10 (95% CI 1.06, 1.15); Mn 3rd tertile: 52.95-146.60 ppb: IRR=1.12 (95% CI 1.08, 1.16)). Conclusion: In North Carolina, counties with higher mean private well metal levels had elevated kidney and bladder cancer incidence rates. The conclusions are limited by the ecological approach, but the consistency of the associations suggests that the exposure of metals in private well water in North Carolina on health outcome deserves additional investigation.

#2300 Pesticides and primary liver cancer: A systematic review and meta-analysis. Hamidi Abdi, Jennifer Lee, Gary Ellison, Gabriel Lai, Tram Lam. National Cancer Institute, Rockville, MD.

Background: Primary liver cancer is the sixth most occurring cancer worldwide and the second most common cause of cancer mortality. Hepatocellular carcinoma (HCC) is the most prevalent histologic type of liver cancer, with well-established risk factors (e.g., chronic infections with hepatitis B virus or hepatitis C virus, and alcohol-related liver disease). Exposure to pesticides may also contribute to the etiology of liver cancer. The use of pesticides has increased.

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significantly in both developed and developing countries within the last few decades. The association between pesticides and liver cancer is unclear. We conducted a systematic review and meta-analysis to investigate the relationship between exposure to pesticides (e.g., organophosphates, organochlorines, carbamates, dichlorophenyltrichloroethane, dichlorodiphenylchloroethylene, and organometallics) and cancer incidence and mortality. We searched PubMed and EMBASE databases using a combination of medical subject heading terms and key words for articles published before June 2016. Articles were identified as relevant if they were original studies and met the following criteria: 1) primary liver cancer was an identified outcome for risk or mortality, 2) pesticides were the main exposure of interest, 3) exposure to pesticides was assessed historically (e.g., blood serum) or through questionnaires, and 4) the estimates for the association between liver cancer risk/mortality and pesticide exposure were reported. Titles and abstracts meeting search criteria were reviewed and relevant full-text articles were read and analyzed independently by two researchers. We calculated the summary estimates [Odd Ratios (ORs) or Relative Risks (RR)] and 95% confidence intervals (CIs) using random-effects models. All analyses were conducted using STATA 14.0. Results: Of 1664 articles reviewed, a total of 34 observational studies were identified as relevant and 20 were included in the meta-analysis for liver cancer risk (5 case-control and 15 cohorts). All of the identified studies investigated HCC as the outcome; none investigated other types of liver cancer. The results showed that pesticide exposure was associated with a 73% increased risk of HCC (RRsummarized:1.73, 95% CI 1.40–2.13; I² = 85.5% and I² < 50% for the highest design of analyses by study design). Furthermore, the summary stratified by sex and type of pesticide similar elevated risks were observed. Analysis of articles that reported on liver cancer mortality (n = 7) showed an excess in mortality from HCC for individuals exposed to pesticides. Conclusion: The existing epidemiological data supports the hypothesis that exposure to pesticides is associated with a significant increased risk of HCC and death from liver cancer.

Genital use of talc and risk of ovarian cancer: A meta-analysis, Paolo Boffetta,1 Wera Berge2.

Some epidemiological studies suggest an association between genital use of talc powders and increased risk of ovarian cancer, but the evidence is not consistent. We performed a meta-analysis of epidemiologic studies to formally evaluate this suspected association. A systematic search was conducted in Medline, Embase and Scopus, leading to the identification of 24 case-control studies and 3 cohort studies, including 302,705 women with ovarian cancer. In the meta-analysis, we used a random-effect model to calculate summary estimates of the association between genital use of talc and occurrence of ovarian cancer. We assessed potential sources of between-study heterogeneity and presence of publication bias. The summary relative risk (RR) for ever use of genital talc and ovarian cancer was 1.22 (95% confidence interval [CI] 1.13–1.30). The RR for case-control studies was 1.26 (95% CI 1.17-1.35) and for cohort studies was 1.02 (95% CI 0.85-1.20, p heterogeneity=0.007). Serious carcinoma was the only histologic type for which an association was detected (RR 1.24; 95% CI 1.15-1.34). There were no significant differences in risk for ovarian cancer between talc users and non-users. In this meta-analysis resulted in a weak but statistically significant association between genital use of talc and ovarian cancer, which appears to be limited to serous carcinoma. The heterogeneity of results by study design and the lack of a trend for duration and frequency of use, however, detract from a causal interpretation of this association.

Differences in risk factor-colorectal adenoma associations according to non-steroidal anti-inflammatory drug use. Sobia Mujtaba, Roberd M. Bostick. Emory University, Atlanta, GA.

Multiple observational studies and large, randomized controlled trials indicate that non-steroidal anti-inflammatory drugs (NSAIDs) strongly reduce risk for colorectal neoplasms. The strengths of these findings suggest that NSAIDs may so markedly reduce risk of colorectal neoplasms, that they may be undetectable among NSAID users. Temporal changes and between-population differences in regular NSAID use prevalence may account for some of the inconsistencies in reported findings for risk factor–colorectal neoplasm associations. We investigated whether associations of known colorectal cancer risk factors with colorectal adenoma differed by non-aspirin NSAID use pooled data from 3 colonscopy-based case-control studies of incident, sporadic colorectal adenoma conducted in Minnesota, North Carolina, and South Carolina between 1991 and 2002. Participants (n=789 cases, 2,035 polyp-free controls) provided risk factor data prior to colonoscopy. The multivariable-adjusted odds ratios (OR) (95% confidence intervals [CI]) for those in the highest relative to the lowest quartiles of exposure, by regular non-aspirin NSAID non-use/use, respectively, were 1.57 (CI 0.96, 2.55) vs. 1.22 (CI 0.40, 3.70) for total fat, 1.37 (CI 0.86, 2.18) vs. 0.78 (CI 0.26, 2.35) for saturated fat, 0.92 (CI 0.67, 1.26) vs. 1.31 (CI 0.62, 2.76) for total calcium, 0.88 (CI 0.64, 1.22) vs. 1.38 (CI 0.65, 2.93) for total fruits and vegetables, 1.05 (CI 0.73, 1.50) vs. 0.73 (CI 0.33, 1.62) for red and yellow vegetables, and 0.97 (CI 0.60, 1.59) vs. 0.78 (CI 0.53, 1.14) for physical activity. For current smokers relative to never smokers, the ORs (CIs) among regular non-NSAID users/non-users were 2.88 (CI 2.20, 3.77) vs. 1.64 (CI 0.85, 3.17), respectively, and for those who were obese relative to those who were normal weight, they were 1.65 (CI 1.27, 2.16) vs. 1.20 (CI 0.70, 2.04). The associations of age, height, and intake of alcohol, dietary fiber, and total folate with adenoma incidence were not substantially different according to NSAID use. These findings suggest that regular non-aspirin NSAID use may mask, beyond simple confounding, associations of various risk factors with colorectal adenoma, suggesting that differential proportions of regular NSAID users between study populations may explain some inconsistencies in reported risk factor–colorectal neoplasm associations, and support routinely assessing such associations stratified by regular non-aspirin NSAID use.

Entacapone and prostate cancer patients with Parkinson’s disease: A large Veterans Health Administration study. Jacqueline M. Major,1 Francesca Cunningham,2 Diane Dong,2 Kuntel By,3 Kwan Hur,2 David C. Shih,1 Simone P. Pinheiro,1 Gerald D. Podaskalny,1 David J. Graham.1 *US Food and Drug Administration/CEDER/OSE, Silver Spring, MD; °US Department of Veterans Affairs, Hines, IL; †US Food and Drug Administration/CEDER/OB, Silver Spring, MD; ‡US Food and Drug Administration/CEDER/OND, Silver Spring, MD.

Background: An increased risk of prostate cancer was observed in Parkinson’s disease (PD) patients treated with entacapone during a pre-approval randomized clinical trial. Objective: To investigate a potential association between entacapone use and prostate cancer in an ambulatory setting. Methods: Using data from the Department of Veterans Affairs (VA) healthcare system, we tested the null hypothesis that usage of entacapone over the past 10 years was associated with an increased risk of prostate cancer in a sample of VA health-plan enrollees. A nested case-control study was conducted with a case-cohort design. We matched cases, defined as deceased prostate cancer patients, with a randomly selected cohort of prostate cancer-free enrollees from the VA cancer registry linkage. Cox proportional hazards regression was used to estimate HRs and 95% CIs for prostate cancer mortality among individuals receiving entacapone vs. non-users. Results: The joint model resulted in a HR of 1.57 (95% CI 1.06, 2.34) for prostate cancer mortality among entacapone users vs. non-users. Conclusions: Use of entacapone during a previous period of long-term exposure in PD patients was not associated with increased risk of prostate cancer.

Environmental pollutants and breast cancer: 2006-2016 epidemiological studies designed to evaluate biological hypotheses provide evidence of risk for certain pesticides, organic solvents, and products of combustion. Kathryn M. Rodgers, Julia O. Udesky, Ruthann A. Rudel, Julia G. Brody. Silent Spring Institute, Newton, MA.

Common environmental chemicals have plausible links to breast cancer because they are mammary gland carcinogens in animal studies or are known to activate relevant hormonal pathways or enhance susceptibility of the mammary gland to carcinogenesis. An institute of Medicine report prioritized human studies of these exposures. Yet the long latency and multifactorial etiology of breast cancer make evaluation of these chemicals in humans challenging, adding to the importance of study designs that are well-suited to capture hypothesized breast cancer mechanisms. With this in mind, we updated Brody et al.’s 2007 review in Cancer to evaluate the strength of the epidemiologic evidence for several classes of chemicals with widespread exposure. We conducted a systematic search of the PubMed database for articles published in 2006-2016 using 147 terms and the inclusion/exclusion criteria defined in Brody et al.’s 2007 review. We critically reviewed articles identified by our search, including whether study designs were suited to the biological evidence. We identified 158 new articles since 2006. In the past 10 years, the strength of evidence for an association between breast cancer and banned but persistent endocrine disrupting chemicals polychlorinated biphenyls (PCBs) and organochlorine pesticides has increased, based on a unique 50-year cohort study that captured exposure during potentially critical
windows for breast development (in utero, adolescence, pregnancy) and at the time when the chemicals were still in use. Studies that considered early life exposure to organic solvents and/or exposure of long duration also added to evidence for an association with breast cancer. Solvents are used in dry cleaning, electronics manufacturing, leather and fur processing, and metal, auto, textile, and some medical work. Other important reports included the National- 

#2305 Residential airborne heavy metal concentrations and breast cancer characteristics. Jacob K. Kresovich, Serap Erdal, Maria Argos, Hua Yun Chen, Peter H. Gann, Garr H. Rauscher. University of Illinois-Chicago, Chicago, IL.

Background: Ambient air metal concentrations have recently been implicated in the etiology of breast cancer. Previous studies have shown airborne concentrations of arsenic and cadmium are associated with the development of estrogen receptor-negative tumors. This study aims to replicate these findings and examine the role of other toxic and essential heavy metals. Methods: Participants were women who were diagnosed with breast cancer in Chicago between 2005 and 2008. We examined estrogen and progesterone receptor-negative (ER-/PR-) and high-grade tumors as markers of aggressive breast cancer, and estimated 15-year markers of exposure of 11 heavy metals. Exposures were calculated using census tract-level airborne concentrations from the National-scale Air Toxics Assessment and participants’ residential histories. We adjusted all models for socioeconomic status and reproductive factors. Results: We found that prevalent ER-/PR- tumors were associated with increased residential exposure to airborne concentrations of antimony (Q4 vs Q1: OR = 1.81; 95% CI: 0.95, 3.44; Ptrend = 0.04), cobalt (Q4 vs Q1: OR = 2.37; 95% CI: 1.26, 4.59; Ptrend < 0.01), manganese (Q4 vs Q1: OR = 2.55; 95% CI: 1.24, 5.24; Ptrend = 0.04), and selenium (Q4 vs Q1: OR = 1.85; 95% CI: 1.03, 3.29; Ptrend = 0.05), and also identified marginally significant trends for arsenic (Ptrend = 0.06), chromium (Ptrend = 0.08), lead (Ptrend = 0.08), and mercury (Ptrend = 0.07). We did not identify any overall associations with high-grade tumors, however when stratifying by menopausal status we found that antimony (Q4 vs Q1: OR = 6.97, 95% CI: 1.61-30.19) and arsenic (Q4 vs Q1: OR = 6.97, 95% CI: 1.61-30.19) were associated with prevalent high-grade tumors in premenopausal women only. Discussion: This study found further support for a role of airborne arsenic concentrations, and novel evidence implicating other airborne estrogen-pathway disrupting metal concentrations, in the development of aggressive breast cancer subtypes. Additionally, this is the first study to implicate heavy metal exposure in the etiology of high-grade tumors. The results suggest that long-term low-dose exposures to certain heavy metals play a role in the etiology of aggressive breast cancer characteristics. Airborne exposures have the ability to affect large populations and findings from this and similar studies have large public health implications.

#2306 Sleep duration and risk of fatal prostate cancer in CPS-I and CPS-II. Susan M. Gapstur, Victoria L. Stevens, Brian D. Carter, Ying Wang, Eric J. Jacobs. American Cancer Society, Atlanta, GA.

There is growing evidence that factors associated with circadian rhythm disruption may be associated with prostate cancer risk. In a previous analysis focusing on shift work in the Cancer Prevention Study (CPS-II) that included only employed men, we observed an association between short sleep duration and higher risk of prostate cancer death that was limited to the first eight years of follow-up of the cohort. To corroborate and expand on these results, we examined the association between sleep duration and death from prostate cancer in a combined analysis of men in CPS-II and men enrolled in an earlier cohort study, CPS-I, regardless of employment status. After excluding men with uninterpretable sleep information, prevalent cancer and prevalent prostate disease at baseline, the analysis included 407,649 men from CPS-I followed from 1950 through 1972, and 416,040 men from CPS-II followed from 1982 through 2012. During follow-up, 1,546 men in CPS-I and 8,704 men in CPS-II died of prostate cancer. Cox proportional hazards regression was used to estimate multivariable-adjusted relative risks (RR) and 95% confidence intervals (CI) and estimates were meta-analyzed using a fixed-effects model because the design and population of CPS-I and CPS-II were similar. Among men aged 65 years and younger, short sleep duration was associated with higher risk of death from prostate cancer during the first eight years of follow-up; compared to 7 hours/night, the RRs (95% CIs) for 5-3, and 6 hours/night were 1.61 (1.10-2.37), and 1.25 (0.99-1.58), 8 years of follow-up suggests that short sleep duration could affect later stages of prostate carcinogenesis. Reasons for lack of an association in older men are unclear but may include the marked decline in nocturnal melatonin levels with age, which could reduce the relative biologic impact of melatonin inhibition by sleep-deprivation.
caused the disintegration of preformed spheroids. Conclusion: This study indicates that targeting sigma-2 receptors with novel sigma-2 ligands (XYZ-XII-14 and XYZ-VII-69) effectively inhibits TNBC cancer cell growth by inducing apoptosis and cell cycle arrest, thus presenting a unique and effective pathway for treating TNBC. Additionally, the sigma-2 receptor ligands (XYZ-XI-14 and XYZ-VIII-69) are also effective in halting tumor growth and prevent tumor regression, as seen in our 3D culture assays. Thus, the sigma-2 receptor has the potential to be a valuable target for the development of novel agents for the treatment of TNBC.

**#2309 Acetylation of survivin represses transactivation of STAT3 to induce sodium butyrate-induced apoptosis in human mucopidermoid carcinoma cell lines.** Sung-Dae Cho, Seoul National University, School of Dentistry, Seoul, Republic of Korea.

Sodium butyrate (NaBu) is one of histone deacetylase inhibitors and possesses anticancer activity. Here, the aim of this study is to determine the molecular mechanism by which NaBu controls apoptosis in MC3 and Y151 human mucopidermoid carcinoma (MEC) cell lines. NaBu caused growth inhibition and induced apoptosis in both cell lines accompanied by the acetylation of histone proteins H2A and H3. NaBu clearly increased survivin and cleaved caspase 3 evidenced by human apoptosis array and it regulated survivin molecule at a post-transcriptional level. NaBu caused nuclear translocation of survivin protein and increased survivin acetylation in both cell lines. In addition, STAT3 and survivin were co-localized in nucleus by NaBu resulting in the decrease in the expression level of Bcl-xL mRNA which is one of STAT3 downstream molecules. In addition, NaBu induced caspase-dependent apoptosis in human MEC cells. Taken together, these results suggest that NaBu is a potent apoptosis inducer in human MEC cell lines through survivin-STAT3 signaling cascades and these findings provide the basis of its clinical application for the treatment of human MEC. This abstract is supported by SNU Invitation Program for Distinguished Scholar and the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2014R1A1A205874).

**#2310 Molecular dynamics simulation of permeation pathway of cytochrome C through Bax pore.** Mingzhen Zhang,1 Jie Zheng,1 Ruth Nussinov,2 Buyong Ma.1 University of Akron, Akron, OH; 2Leidos Biomedical Research, NCI-Frederick, Frederick, MD, USA.

How cytochrome C is released from the mitochondria to the cytosol via Bax oligomeric pores, a process which is required for apoptosis, is still a mystery. Based on the residue-residue distances detected experimentally for Bax and its homologous protein (Bak), we recently computationally solve the first atomic model for Bax oligomeric pores at the membranes. Next, we investigate the mechanism at the microscopic time- and nanometer space-scale using coarse-grained replica exchange and all-atom MD simulations. Our free energy landscape depicts a low barrier for the permeation of cytochrome C into the Bax C-terminal mouth, with the pathway proceeding to the inner cavity and exiting via the N-terminal mouth. Release is guided by organized charged/hydrophobic surfaces. The hydrophilicity and negative charge of the pore surface gradually increase as the pore depth increases. The pore entry path is the preferred one. Rather than inert passing of cytochrome C through a rigid pore, the flexible pore may selectively aid the cytochrome C passage. The energy barrier is under 4 kcal/mol. Thus, once the Bax pore is formed in the membrane, the release of cytochrome C may be readily achieved through energy fluctuations. Collectively, our work provides mechanistic insight and atomic detail into the release of cytochrome C through Bax oligomeric pores.


Cupredoxins are a family of low-molecular weight, water soluble, copper-containing redox proteins involved mainly in the electron transfer chain in prokaryotes. Auracycin is a member of the cupredoxin family secreted by Pseudomonas aeruginosa, of which a 28-amino acid sequence (p28) was identified to acquire the capability of preferential entry into cancer cells of various histogenesis. Upon entry, it exerts a cytostatic effect in these cancer cells via a p53-mediated pathway. Our aim was to study whether the other members of the cupredoxin family possess a similar function to that of auracin in the eukaryotic cancer cells. Based on structural and sequence analyses, helical regions of auracycin A & B were shown to resemble p28 closely. Thus, we studied the biologic function of two 28-amino-acid peptides derived from auracycin A (aurA; pl 4.5, MW 2.6 kD), and auracycin B (aurB; pl 3.4, MW 2.7 kD). Cancer growth and MTT assays demonstrated that aurB, but not aurA, induced cytotoxicity in various cancer cell lines in a dose-dependent (range 0.1uM-100uM) and time-dependent (~72 hours) pattern, including p53-null (prostate cancer PC-3) and dn-p53 (breast cancer MDD-2 and ovarian cancer SKOV-3) in which p28 did not demonstrate a significant growth inhibition. Annexin-V assays showed that a dose-dependent apoptosis in prostate cancer cell lines of various p53 statuses (wt-p53 LNCaP, mut-p53 DU-145, p53-null PC-3) following treatment with aurB at 5-50uM for 48 hours, whereas p28 did not demonstrate an apoptotic activity in p53-null cell lines, suggesting an aurB-induced apoptosis via a p53-independent pathway. In contrast, aurA did not appear to induce apoptosis in any of the above cell lines. Induction of apoptosis was mediated through depolarization of the mitochondrial membrane and caspase cascade activation in cancer cells. Moreover, fluorescence microscopy demonstrated that aurB, unlike p28, does not acquire a similar pattern of preferential entry into cancer cells. This proposes that aurB acquires the potential as an anticancer cell penetrating peptide, and differs from p28 in its mode of entry and p33-mediated action. Our studies pertaining to auracin and auracycin, suggest that cupredoxins, as a family of prokaryotic metalloproteins, may represent a novel family of antineoplastic agents against various cancer cell lines.

**#2312 Feasibility study of a fractionation method in the treatment of tumor treating fields.** Yunhui Jo,1 Jiwon Sung,1 Hyo Sook Song,2 Eun Ho Kim,3 Myounggeun Yoon.1 1Korea University, Seoul, Republic of Korea; 2Korea Institute of Radiological and Medical Sciences, Seoul, Republic of Korea; 3University of Illinois at Chicago, Chicago, IL.

Tumor treating fields (TTFs) using a fractionated treatment scheme originally designed for radiotherapy were applied to cancer (Human glioblastoma, U373) and normal cells (intestinal epithelial, IEC-6) for three days with durations of 3, 6, 9, or 24 hours/day. As the treatment time of TTFs increases from 3 to 24 hours/day, the relative cancer cell growth rates in both cell lines were reduced. While the difference in cancer cell growth rate compared to control was ~25% (decreased from 100% to ~75%) for the treatment time of 6 hours/day, the growth rates of cells treated for additional 6 hours (i.e., 12 hours/day) and additional 18 hours (i.e., 24 hours/day) differed only ~5% and ~8% from that of cells treated for 6 hours/day, respectively. Our results suggested that the dependence on treatment time in cancer cell inhibition is weakened distinctively at treatment times over 6 hours/day. For normal cells, the relative growth rates corresponding to the treatment time of TTFs was not decreased much for the treatment time of 3, 6, 12 hours/day revealing 93.3%, 90.0%, 89.3% of cell growth rates compared to control, respectively, but it suddenly reduced to ~73% for 24 hours/day of treatment. The experimental results indicated that the effects of TTF on growth rate of cancer cells are higher than the effects of TTFs on growth rates of normal cells for treatment times of 3-12 hours/day, but the difference became minimal for treatment times of 24 hours/day. Our results suggest that the fractionated scheme in the treatment of tumor treating fields may reduce treatment time while maintaining efficacy, suggesting that this method can increase patients’ quality of life.

**#2313 Promoting caspase-8-dependent apoptosis signaling using 17-beta-hydroxywithanolides.** Alan D. Brooks,1 Ya-ming Xu,2 E. M. Kithsiri Wijeratne,2 Curtis J. Henrich,1 Poonam Tewary,1 Leslie Gunatilaka,2 Thomas J. Sayers,1 1Frederick National Laboratory for Cancer Research, Frederick, MD; 2The University of Arizona, Tucson, AZ.

We have previously reported that withanolide E (WE), a steroidal lactone from Physalis peruviana, was highly active in sensitizing various human carcinoma cell lines to TRAIL-mediated apoptosis. Therefore, over 100 natural and semi-synthetic withanolides were evaluated for their ability to promote caspase-8-dependent cancer cell death. Our studies identified several withanolides that were 4-8 fold more potent than WE in sensitizing the renal carcinoma cells and melanoma cells to caspase-8-dependent apoptosis in response to either TRAIL or the TRAIL ligand body (TTF). All active withanolides were 17-beta-hydroxy withanolides (17-BHW). The highly active 17-BHWs were more efficient than withanolide E at reducing cellular levels of both cFLIP, and cFLIP, and enhancing caspase-8 activation. Furthermore, immunoprecipitation of the TRAIL death-inducing signaling complex (DISC), or the related ripoptosome, demonstrated enhanced levels of both FADD and RIP1 in these macromolecular apoptotic signaling complexes following treatment with active 17-BHWs. The 17-BHWs used in this work were obtained by the application of an efficient method of plant biomass production involving our innovative and patented soil-less aeroponic cultivation of P. crassifolia and P. peruviana and by chemical modification of natural withanolides produced by these plants. Preliminary structure
activating relationship (SAR) studies suggested that the enone moiety in ring A was essential for activity. In addition, acetylation at C-18, an alpha orientation of the side-chain lactone group and the double bond at C-24(25) of the lactone ring played important roles in determining the activity of 17-BHWS as apoptosis sensitizers. This suggests that the 17-BHW scaffold is amenable to optimization to develop novel chemical entities—miR-147h-miRNA-based screen showed a significant pro-apoptotic induction in the various ovarian cancer cell lines alone and in combined therapy with carboplatin. Western Blot analysis revealed an enhanced expression of the pro-apoptotic proteins Bak1 and Bax and a decrease in Bcl-2 and Bcl-xL. We characterized the miRNAs in regard to their endogenous expression and detected an enhanced expression after apoptosis induction by chemotherapeutic drugs.

**#2314 Novel BH3 mimetic based therapeutic strategies for the treatment of breast cancer.** Zachary Kratche, MacKenzie Adams, Robert Wilson, Stephen Ehier, Stephen Guest. Medical Univ. of South Carolina, Charleston, SC.

Bcl-2 homology domain 3 (BH3) mimetics are a new class of targeted anti-cancer compounds that function by inducing cancer cell apoptosis. Testing in a variety of pre-clinical tumor models has shown that BH3 mimetics are capable of inducing remarkable tumor regressions. Recently, a clinical trial of the BH3 mimetic venetoclax in Chronic Lymphocytic Leukemia (CLL) resulted in an impressive response rate of 80% leading to FDA approval. Using unbiased genomic and functional genomic approaches, we identified gene amplification of the BCL2L1 gene, which encodes the anti-apoptotic protein BCL-XL, as a novel driver event in breast cancer. We identified focal amplification of the BCL2L1 gene in the SUM-185 breast cancer cell line and a genome-wide RNAi-based screen identified BCL2L1 as one of the top genes necessary for viability of this cell line (ranked 5th out of 15,256 genes). Treatment of SUM-185 cell lines with low doses of navitoclax showed that these cells are intrinsically sensitive to this drug (IC50 = 100nM). This sensitivity was 1-2 logs greater than what was observed for breast cancer cell lines that do not harbor BCL2L1 gene amplification. To expand the relevance of these findings beyond the SUM-185 cell line, we used the cancer cell line encyclopedia (CCLE) database to identify an additional breast cancer cell line, HCC38, that also harbors a focal BCL2L1 gene amplification. Treatment of HCC38 cells with navitoclax revealed a high level of sensitivity that was equivalent to that of SUM-185 cells. These findings demonstrated that a subset of breast cancers that harbor BCL2L1 gene amplification are highly sensitive to a BH3 mimetic that targets BCL2L1 suggesting that BH3 mimetics could be used as a single agent to effectively treat these breast cancers. We also examined the effectiveness of BH3 mimetics when used as part of a combination therapy approach. In addition to a focal amplification of the BCL2L1 gene, the SUM-185 breast cancer cell line also harbors a focal amplification of the fibroblast growth factor receptor 3 (FGFR3) gene as well as an activating mutation in the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) gene. Treatment of SUM-185 cells with low doses of navitoclax, a FGFR inhibitor, or a PIK3CA inhibitor had little effect on colony forming efficiency. At these same low doses, combined treatment with navitoclax and the FGFR inhibitor or the PIK3CA inhibitor resulted in a strikingly synergistic effect with near complete loss of colony forming ability (less than 10% of controls). We further showed that this loss of clonogenicity was the result of a rapid induction of apoptosis by the combination therapies. These results suggest that combining BH3 mimetics with inhibition of FGFR or PIK3CA is a promising novel therapeutic strategy for a subset of breast cancer patients.

**#2315 Overcoming treatment resistance in cisplatin-resistant ovarian carcinoma cells by tissue-specific miRNAs.** Michael Kleemann, ¹Jermes Bereuth, ¹Simon Fischer, ¹Kim Marquart, ¹Simon Hänle, ¹Kristian Unger, ¹Verena Jendressek, ¹Christian U. Riedel, ¹René Handrick, ¹Kerstin Otte, ¹University of Applied Sciences Biberach, Biberach, Germany; ²Boehringer Ingelheim, Biberach, Germany; ³Heinholz Zeitschrif München, München, Germany; ⁴University of Essen, Essen, Germany; ⁵University of Ulm, Ulm, Germany.

Ovarian cancer is a common human cancer with a poor outcome and a high risk of death. Due to resistances to current standard therapies (carboplatin and paclitaxel for 5 to 6 cycles) additional therapeutic approaches are required and novel specific biomarkers for ovarian cancer patients are necessary. MicroRNAs (miRNA) are conserved, small non-coding RNAs regulating gene expression by binding to their target mRNA which have been shown to regulate biological processes including apoptosis. Apoptosis is a irreversible process that allows cells to undergo a highly regulated form of cell death. However, the final role of miRNAs in apoptotic signaling has not yet been fully determined. Modulating the expression of key molecular components of the cell death machinery is an attractive strategy for cancer therapy overcoming chemo drug resistance. To extend the pool of potential miRNA as anticancer agents and biomarkers, we transferred a high content miRNA screening for pro-apoptotic miRNAs to human cancer cell lines. Based on the high recovery rate of apoptosis inducing miRNAs in SKOV3 cells analyses were extended to multiple ovarian carcinoma cell lines with different genetic background. After transfection of miR-1912-5p the apoptosis rate increased strongly in cisplatin-resistant cells. In combination treatment with carboplatin the apoptotic effect increased after transfection of miR-1912-5p in the cisplatin-resistant A2780 cells. Furthermore, we applied a tissue-specific miRNA approach shown to the identification of highly active natural product-based sensitizers of cancer cells to caspase-8-dependent apoptosis. The cellular molecular target(s) of active 17-BHWS are currently under further investigation. Funded by FNL- Contract HHSN26120080001E.

**#2316 Elevated H-Ras suppresses death receptor-mediated apoptosis in cancer cells.** Su-Byun Kim, FDA, Silver Spring, MD.

TNF-related apoptosis inducing ligand (TRAIL) induces apoptosis through its death receptors (DRs) 4 and/or 5 expressed on the surface of target cells. Despite its selectivity in killing cancer cells over most normal cells, recombinant human TRAIL or its receptor agonists (monoclonal antibodies against DR4 or DR5) encountered resistance in many tumor cells while the underlying mechanisms remain to be systematically upregulated in TRAIL-resistant cells compared to TRAIL-sensitive cells. The elevated H-Ras expression correlated with a deficiency of DR4 and DR5 on plasma membrane in TRAIL-resistant cell lines. Notably, knockdown of H-Ras in TRAIL-resistant cells successfully restored the surface expression of DR4 and DR5, thereby sensitizing the cells to TRAIL-induced apoptosis. Consistently, ectopic expression of H-Ras in TRAIL-sensitive cells reduced surface DR4 and DR5 which was associated with a loss of TRAIL sensitivity. By contrast, the status of K-Ras or its mutations was not causally linked to TRAIL receptor expression or TRAIL sensitivity across the panel of cancer cell lines tested. These data suggest that H-Ras may play a distinct role to negatively regulate TRAIL receptors and apoptosis. The upregulated H-Ras could be a predictor of tumor resistance to TRAIL-targeted agents and a potential therapeutic target for combinatorial therapy to achieve better treatment outcomes.


Prostaglandin E2 (PGE2) has been reported to play critical roles in cell fate decisions by interacting with 40 types of G protein-coupled membrane receptors such as EP1, EP2, EP3 and EP4. We previously reported that EP4 stimulation by treatment with its agonist CAY10598 induced apoptosis via reactive oxygen species (ROS) formation in colon cancer HCT116 cells. Moreover, treatment with CAY10598 diminished the phosphorylation of JAK2 and induced degradation of this oncoprotein, leading to the attenuation of STAT3 activation in HCT116 cells. In the present study, we attempted to delineate the molecular mechanisms underlying the degradation of JAK2 by activation of EP4. HSP90, a member of the heat shock protein family, is a molecular chaperone that supports stability of client proteins, such as EGFR, MET, Akt and JAK2. HSP90-mediated stabilization/activation of these client proteins contributes to the acquisition of cancer cell hallmarks, including proliferation, survival, angiogenesis and invasion. It has been recently reported that the chaperoning function of HSP90 may be disrupted by post-translational modification induced by oxidative stress. Treatment of human colon cancer HCT116 cells with CAY10598 down-regulated expression of HSP90 client proteins in a concentration- and time-dependent manner and the down-regulation was restored by pretreatment with ROS scavenger N-acetyl cystine (NAC) or proteasome inhibitor MG132. However, cotreatment with cycloheximide, protein synthesis inhibitor, accelerated the CAY10598-induced degradation of HSP90 client proteins. These data suggest that CAY10598-induced HSP90 client protein degradation may be caused by ROS generation. In renal carcinoma Caki cells, CAY10598 also down-regulated expression of HSP90 client proteins, suggesting that EP4 stimulation may regulate HSP90 activity in colon cancer cells as well as renal cancer cells. We found that HSP90α was cleaved to 40 or 55 kDa, while HSP90β was cleaved to 25 kDa by CAY10598 treatment and the cleavage of HSP90α and β was blocked by NAC treatment. Furthermore, EP4 inhibition by treatment with antagonist GW627368x attenuated not only degradation of HSP90 client proteins but also cleavage of HSP90 in CAY10598-treated HCT116 cells. In conclusions, EP4...
agonist CAY10598 induces degradation of HSP90 client proteins via ROS-dependent HSP90 cleavage, leading to apoptosis in HT116 cells. This is a novel mechanism by which EP4 activation induces apoptosis of cancer cells that involved inhibiting HSP90 function.

#2318 Cyclopamine sensitizes endoplasmic reticulum stress-mediated increase of death receptor 5 and survivin degradation in the resistant gastric cancer cells. Yoo Jin Na, Dae-Hee Lee, Jung Lim Kim, Bo Ram Kim, Seong Hye Park, Min Jee Jo, Yoon A Jeong, Sang Cheul Oh. Korea University, Seoul, Republic of Korea.

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) has been known as one of the most effective cancer treatment owing to its selective killing effect of cancer but not normal cells. However it has been reported that several gastric cancer cells showed resistance to TRAIL because of scarcity of death receptor 5 (DR5) expressed on the cell surface. In this study, we elucidated cyclopamine sensitizes to TRAIL-induced apoptosis by elevating the expression level of DR5 in gastric cancer cells. Interestingly, survivin hampers existence of death receptor 5 (DR5) protein under normal condition. Cyclopamine decreases expression level of survivin. It is a reason for cyclopamine to act as a TRAIL sensitiser. Mechanistically, cyclopamine induces endoplasmic reticulum (ER) stress by Reactive Oxygen species (ROS), and CHOP, which as a last protein of ER stress pathway regulates stability of survivin protein by proteasome degradation. Taken together, our results indicate that cyclopamine can be used for the purpose of combination therapy in TRAIL-resistant gastric cancer cells.

#2319 Deficiency of protease-activated receptor 2 signaling sensitizes EGFR-TKI-induced apoptosis in colorectal cancer. Weiwei Li, Yiming Ma, Longmei He, Hongying Wang. National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking University Medical College, Beijing, China.

Colorectal carcinoma (CRC) patients show resistance to EGFR-TKI (epidermal growth factor receptor-tyrosine kinase inhibitor) treatment. Protease-activated receptor 2 (PAR2) has been shown to transactivate EGFR. We aim to investigate whether PAR2 sensitizes EGFR-TKI-induced apoptosis in CRC. Firstly, inhibition of PAR2 with shRNA or ENMD-1068 (a selective antagonist of PAR2) significantly sensitized EGFR-TKI (gefitinib or AG1478)-induced apoptosis, which was measured with Annexin V/PI staining and activation of caspase-3 in different CRC cell lines. In xenograft tumor model, gefitinib treatment dramatically reduced the tumor volume in HT-29-shPAR2 compared with HT-29-vector. Mechanism study showed that PAR2 knockdown significantly reduced bcl-xL expression at both mRNA and protein level. Consistently, activation of PAR, upregulated bcl-xL. Furthermore, inhibition of protein phosphatase (PP) with okadaic acid significantly downregulated bcl-xL. Moreover, knockdown of PP1 with shRNA blocked PAR-regulated induction of bcl-xL. In summary, our findings suggest that inhibition of PAR2 sensitizes EGFR-TKI-induced apoptosis via downregulation of bcl-xL in colorectal cancer. Considering the extensive expression of PAR2 and PAR2-activating proteases in colon, it strongly indicates that inhibition of PAR2 may be a potential avenue to overcome EGFR-TKI resistance in patients with CRC.


Objective: We examined whether curcubitacin D affects doxorubicin resistance of MCF7/ADR breast cancer cells. Methods: Cell viability was measured by MTT assay. Levels of p-STAT3, p-NF-κB, 1bB, and caspases were measured by western blot analysis. Nuclear staining of Stat3 and NF-κB was measured by immunocytochemistry. STAT3 and NF-κB transcriptional activity was detected by STAT3 and NF-κB luciferase reporter gene assays. Analysis of cell cycle arrest was performed by flow cytometry. Induction of apoptosis by curcubitacin D was measured by annexin V/FITC/PI assay. Results: More than 90% of MCF7/ADR cells lived upontreatment with doxorubicin for 24 h. However, upon treatment with curcubitacin D, cell death was more than 60%. Co-administration of curcubitacin D and doxorubicin induced apoptosis, G2/M cell cycle arrest, and inhibited upregulated Stat3 by doxorubicin on MCF7/ADR cells. Additionally, curcubitacin D led to an increase in the IκBα level in the cytosol and a decrease in the p-NF-κB level in the nucleus. Finally, curcubitacin D inhibited translocation of Stat3 and NF-κB and decreased transcriptional activity in the nucleus. Conclusion: Curcubitacin D decreases cell proliferation and induces apoptosis by inhibiting Stat3 and NF-κB signaling in doxorubicin-resistant breast cancer cells. Curcubitacin D could be used as a useful compound to treat Adriamycin-resistant patients.

#2321 Reactive oxygen species dictate the apoptotic response of melanoma cells to TH588. jiayu wang, Jin Lei, Xu Guang yan, Simonne Sherwin, Margaret Farrelly, Yuan Yuan Zhang, Fen Liu, Chuan Yan Wang, Su Tong Guo, Hamed Yari, Ting La, Jennifer McFarlane, Fu Xi Li, Hessay Tabatabaee, Ji Zhong Chen, Amanda Croft, chen cheun Jiang, Xu Dong Zhang. Life sciences building, Newcastle, Australia.

Cancer cells commonly contain elevated levels of reactive oxygen species (ROS) resulting from oncogenic stimulation. On one hand, ROS promote cancer cell survival, proliferation, and metastasis. On the other, high levels of ROS suppress tumour growth through inhibition of proliferation and induction of apoptosis and senescence via damage to DNA. Incorporation of oxidized dNTPs such as 8-oxo-deoxyguanine (8-oxo-dGTP) and 2-OH-deoxynucleosine (2-OH-dATP) into genomic DNA plays an important role in apoptosis induced by ROS. Human MutT homolog 1 (MTH1) is an enzyme that sanitizes oxidized dNTP pools through converting 8-oxo-dGTP and 2-OH-dATP into monophosphates, thus preventing their incorporation into genomic DNA. Inhibition of MTH1 by small molecule inhibitors has been suggested as a promising strategy to improve cancer treatment. However, we have found that while silencing of MTH1 does not affect survival of melanoma cell, TH588, one of the first-in-class MTH1 inhibitors, kills melanoma cells through apoptosis independently of its inhibitory effect on MTH. Induction of apoptosis by TH588 was not alleviated by MTH1 overexpression or introduction of the bacterial homologue of MTH1 that has 8-oxoGTPase activity but cannot be inhibited by TH588, indicating that MTH1 inhibition is not the cause of TH588-induced killing of melanoma cells. Although knockdown of MTH1 did not impinge on the viability of melanoma cells, it rendered melanoma cells sensitive to apoptosis induced by the oxidative stress inducer elesclomol. Of note, treatment with elesclomol also enhanced TH588-induced apoptosis, whereas a ROS scavenger or an antioxidant attenuated apoptosis triggered by TH588. Indeed, the sensitivity of melanoma cells to TH588 was correlated with endogenous levels of ROS. Collectively, these results suggest that: 1) TH588-induced apoptosis of melanoma cells is not associated with its inhibitory effect on MTH1; 2) TH588 remains a promising candidate for the treatment of melanoma; 3) MTH inhibition in combination with oxidative stress inducers may be a useful approach in melanoma treatment; and 4) the endogenous levels of ROS are a potential biomarker for prediction of the response of melanomas to TH588 and MTH1 inhibition in combination with oxidative stress inducers.

#2322 Antitumor activity of spicatoside A, a steroidal saponin, via induction of switch from autophagy to apoptotic cell death. Won Kyung Kim, Yuna Pye, Hyen Joo Park, Ji-Young Hong, Sang Kook Lee. Seoul National University, Seoul, Republic of Korea.

The antitumor activity of spicatoside A (SA), a steroidal saponin isolated from the tuber of Lirioper platyphylla (Liliaceae), and its underlying mechanisms were investigated in HCT116 human colorectal cancer cells. SA induced autophagy and apoptotic cell death and inhibited tumor growth in a nude mouse xenograft model implanted with HCT116 cells. Treatment with SA for 24 h enhanced the formation of acidic vesicular organelles in the cytoplasm, indicating the induction of the onset of autophagy. This event was associated with the regulation of autophagic markers including microtubule-associated protein 1 light chain 3 (LC3)-II, p62, beclin 1, lysosomal-associated membrane protein 1, and cathepsin D by inhibiting the PI3K/Akt/mTOR signaling pathway, regulating mitogen-activated protein kinase signaling, and increasing p53 levels. However, a prolonged exposure to SA resulted in apoptosis characterized by the accumulation of a sub-G1 cell population and an annexin V/propidium iodide-positive cell population. Apoptosis induced by SA was associated with the regulation of apoptotic proteins including Bcl-2, Bax, and Bid, the release of cytochrome c into the cytosol, and the accumulation of cleaved poly (ADP-ribose) polymerase (PARP). Further study revealed that cleavage of beclin 1 by caspase-8 plays a critical role in the SA-mediated switch from autophagy to apoptosis. Taken together, these findings highlight the significance of SA in the modulation of crosstalk between autophagy and apoptosis, as well as the potential use of SA as a novel candidate in the treatment of human colorectal cancer cells. Acknowledgement: This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean Government (MEST) (No. 2009-0085533).
The inhibitory role of Kaempferol-3-O-rutinoside induced AMPK activation on the growth of human breast cancer cell lines. Chea Ha Kim.

Solvado virginia-aurea, herbaceous perennial plant of the family Asteraceae, has astrigenic, diuretic, and antiisipetic assets. In our current study, we found that kaempferol (3,7,4-trihydroxy-4,7-dimethoxyflavone) from Solvado virginia-aurea extract prompted cellular apoptosis in breast cancer cells. The present study was conducted to investigate its effects on MCF-7 cells with respect to the induction of inhibitory effect on cell viability against MDA-MB-231 and MCF-7 breast cancer cell lines. In addition, apoptotic protein, especially Bim, was also increased in KR treated MDA-MB-231 and MCF-7 cell line. However, an anti-apoptotic protein, Bcl-xl, was not changed by KR. To find out positive regulator of Bim expression, FOXO3a (forkhead-box transcription factor, group O) 3a and AMPK (5’ AMP-activated protein kinase) protein expressions were examined in KR treated cells. Immunoblot analysis showed dramatically increased expressions of FOXO3a and AMPK in KR treated MDA-MB-231 and MCF-7 cells. In addition, cell cycle analysis indicated that G0/G1 arrest was intensely increased in KR treated MDA-MB-231 and MCF-7 cells. Moreover, phospho-AMPK (pT172) and phospho-FOXO3a (S472) expression were also increased in KR treated MDA-MB-231 and MCF-7 cells. Our results suggest that KR induces apoptosis by AMPK-FOXO3a mediated Bim expression. The mechanisms of KR mediated AMPK and FOXO3a regulation is not fully elucidated yet, but further studies about these molecular mechanisms between two proteins will give some ideas for cancer therapies for certain breast cancer patients.

The role of SERCA pump in cell death and autophagy. Paula Szalai,1 Morten Løhr,1 Frank Sætre,1 Søren B. Christensen,2 Jesper V. Møller,3 Poul Nissen,3 Nikolai Engedal,1 University of Oslo, Oslo, Norway;1University of Copenhagen, Copenhagen, Kopenhagen, Denmark;3University of Aarhus, Aarhus, Denmark.

The purpose of the study is to elucidate structural and molecular determinants of SERCA inhibition by Thapsigargin (Tg) and related analogs for their effects on intracellular calcium homeostasis, ER stress, cell death and autophagy. Tg specifically binds and blocks the sarco/endoplasmic reticulum Ca2+ ATPase (SERCA), which pumps Ca2+ from the cytosol to the ER. Sustained SERCA inhibition leads to calcium depletion from the ER causing ER stress and ultimately cell death. Recently we showed that Tg-induced calcium perturbation potently blocks autophagy (1). Tg is an attractive potential anti-tumor drug because it effectively kills both slow and fast proliferating cancer cells. However, since Tg is toxic also to normal cells, it must be targeted towards the cancer cells. Replacing a side chain with a linker connecting the Tg core to a peptide prevents Tg from entering cells. Two different linker-peptide sequences have been introduced in clinically tested Tg prodrugs; one is cleaved by PSA, secreted by prostate cancer cells, and the other is cleaved by PSMA, which is secreted by neovascular tissues of a broad range of tumors. The Tg analogs unmasked by the cleavage are able to enter cells and exert their toxic effects. Interestingly, however, in vitro experiments indicate that Tg analogs have different potencies and cellular effects depending on the terminal amino acid residue (2). Exploring why this is the case may lead not only to better Tg prodrug formulations, but also to a deeper understanding of the biological functions of SERCA pump activity. We compare a broad panel of Tg analogs in various cell types for their biological effects. Methods used so far are western blotting, real-time RT-PCR, live-cell imaging, flow cytometry, and assays that measure autophagic sequestration and degradation activity. Our unpublished data indicate the ER-stress sensors PERK, ATF4, CHOP, and IRE1 but not XBP1 and ATF6 to be involved in cell death signaling in LNCaP prostate cancer cells. Moreover, Tg-induced cell death required death receptor 5 and caspase-8. All Tg analogs tested displayed exactly the same molecular requirements for induction of cell death, although some required 5 to 10 times higher doses than Tg to evoke the same strength of death signal. Like Tg (1) the analogs inhibited autophagy before the closure of phagophores, but at higher doses and/or with slower kinetics. These results indicate that thapsigargin and its analogs evoke similar anti-autophagic and death signaling pathways. Further investigations are aimed at exploring the causes for differential potencies, and include measurements of cytosolic and compartmentalized calcium, as well as solving crystal structures of selected analog:SERCA complexes complemented with biophysical studies of analog:SERCA interactions. References 1. N. Engedal et al., Autophagy 9, 1475 (2013). 2. C. Dubois et al., J Biol Chem. 280, 5430 (2013).

Deconstruction of circulating tumor cells by fluid shear stresses generated in a microfluidic system. Sagar Regmi,1 Aft Fu1, Yabin Li,2 Kathy Qian Luo,3 Nanyang Technological University, Singapore; Singapore; 2University of Macau, Taipa, Macau, China; 3Macao, China.

Sargopregimel-3-O-rutinoside induced AMPK activation on the growth of human breast cancer cell lines. Chea Ha Kim.

Circulating tumor cells (CTCs) are mainly responsible for the cause of cancer metastasis. Although most CTCs can be destroyed by the bloodstream, some of them can still manage to withstand hemodynamic shear stress in blood stream. To study the effects of fluidic micro-environment on CTCs, we designed a microfluidic system that can produce various levels of shear stress which can be operated in human artery undergoing or exercise condition. We also generated three human breast cancer cell lines with increased ability to form lung metastases in nude mice (i.e. 213-M1A > 231-M1 > 231-C3). All three cell lines can produce a fluorescence resonance energy transfer (FRET)-based sensor which can reveal apoptosis in real-time by changing its color from green to blue. Besides these, we also investigated the effects of shear stress on multiple types of cancer cells including lung cancer (A549), ovarian cancer (2008), breast cancer (UAJC-893) and leukemia (K562). For all the cell lines, except the non-attached K562 cells, cells were cultured on petri-dishes, detached by trypsinization and re-suspended in a normal culture medium to a cell density of 2x10^6 cells/ml. One milliliter of this cell suspensions was circulated in our microfluidic system under the shear stresses of 15, 30, 45 and 60 dyne/cm^2 for 2-18 h. The effects of shear stress on morphology and viability of CTCs were determined by FRET imaging microscopy and MTT assay. And the impact of shear stress on causing necrosis in CTCs were examined by propidium iodide (PI) staining and LDH assay. Our findings are summarized below: 1) High shear stress (SS60, 4h) is more potent to destroy CTCs than low shear stress (SS15, 4h). 2) High shear stress can destroy most of the CTCs via necrosis. 3) This high level of shear stress is theoretically achievable via physical exercise. Additionally, the findings that high shear stress is more resistant to high shear stress compared to less metastatic 231-C3 cells. 5) Leukemia K562 cells representing the white blood cells showed stronger resistance to high shear stress compared to the cancer cells tested in this study. We think these findings can help people to understand how metastatic CTCs resist shear force-induced cell death which are useful for designing more effective therapies against metastatic CTCs. Additionally, the findings that high shear stress is more effective to kill CTCs may inspire cancer patients to consider exercise as a way to increase their hemodynamic shear stress, consequently destroy CTCs and prevent cancer metastasis.

A high-throughput screen identified cancer selective small molecules that kill independent of mitochondrial apoptosis. Catrina M. Dowl- ing,1 Michæl Heßmann,1 James Bradner,2 Anthony Letai,3 Triona Ni Chonghaile1,5 Royal College of Surgeons, Dublin, Ireland;2Koch Institute for Integrative Cancer Research at MIT, Massachusetts Institute of Technology, MA;3Dana Farber Cancer Institute, Harvard Medical School, Department of Medical Oncology, MA;4Dana-Farber Cancer Institute, Boston, MA.

Triple negative breast cancer (TNBC) lacks expression of oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor 2 (HER2). Importantly, there have been fewer advances in the treatment of TNBC with the mainstay of treatment being cytotoxic chemotherapy. All too often tumor responses poorly to chemotherapy, or relapse and resistance follow. We recently found that thapsigargin and its analogs evoke similar anti-autophagic and death signaling effects. Methods used so far are western blotting, real-time RT-PCR, live-cell imaging, flow cytometry, and assays that measure autophagic sequestration and degradation activity. Our unpublished data indicate the ER-stress sensors PERK, ATF4, CHOP, and IRE1 but not XBP1 and ATF6 to be involved in cell death signaling in LNCaP prostate cancer cells. Moreover, Tg-induced cell death required death receptor 5 and caspase-8. All Tg analogs tested displayed exactly the same molecular requirements for induction of cell death, although some required 5 to 10 times higher doses than Tg to evoke the same strength of death signal. Like Tg (1) the analogs inhibited autophagy before the closure of phagophores, but at higher doses and/or with slower kinetics. These results indicate that thapsigargin and its analogs evoke similar anti-autophagic and death signaling pathways. Further investigations are aimed at exploring the causes for differential potencies, and include measurements of cytosolic and compartmentalized calcium, as well as solving crystal structures of selected analog:SERCA complexes complemented with biophysical studies of analog:SERCA interactions. References 1. N. Engedal et al., Autophagy 9, 1475 (2013). 2. C. Dubois et al., J Biol Chem. 280, 5430 (2013).
Melanoma is the most dangerous type of skin cancer, which mostly related with the exposure of ultraviolet (UV), and especially happened to those who have low skin pigment levels. Epidemiological studies showed Europeans and North Americans have a high incidence of melanoma, while it is less common in Asia and Africa. Therefore, the new and more effective methods to treat melanoma are urgently needed. Non-thermal atmospheric pressure bio-compatable plasma (NBP) is defined as a partially ionized gas with electrically charged particle, which has been reported have cytotoxicity on various cancer cells induce DNA damage and apoptosis other than normal cells. Reactive oxygen and/or nitrogen species (RONS) were proved to play the most important role during this process, by inducing oxidative stress and depolarization of mitochondrial membrane potential with the consequence of various cancer cells apoptosis. The plasma activated media (PAM), which the media treated directly by NBP with different time, containing mostly long-live secondary species such as hydrogen peroxide (H2O2) which have been confirmed anti-microorganism and cytotoxic activity. Researches already investigated that PAM also effective in cancer therapy via inducing apoptosis. However, the underlying molecular mechanisms of apoptosis are remaining elusive. The purpose of this study was to evaluate the potential of PAM as an effective tool to induce apoptosis in melanoma cells. Our results showed that PAM has a killing effect to melanoma cells in a time-dependent manner, for 3minutes treatment could induce almost 40% cells entry death, after treated by 5 minutes more than 60%, and 10 minutes treatment reduced the cell viability to approximately 30%. Annexin-V/PI staining demonstrated that PAM overexpression with strong apoptosis pathway and found that with a manner of treatment time PAM significantly increased the concentration of intracellular NO and H2O2, reflecting an influx of extracellular RONS may result in melanoma cells apoptosis. Besides, western blot assay showed that P53 and caspase 3 increased after PAM treatment. Taken together, PAM is effective to induce the apoptosis in melanoma cells in vitro, further in vivo experiments will be performed to test the functional effects in the animals.

#2328 Race specific hyper-activation of CCR9-mediated survival signals and its impact on efficacy of docetaxel in prostate cancer. Neera Kapur,1 Hina Mir,1,2 Guru Sonpavde,2 Shallesh Singh1. 1Morehouse School of Medicine, Atlanta, GA; 2UAB Comprehensive Cancer Center, Birmingham, AL.

Current approaches to treat and manage prostate cancer (PCa) have failed to reduce racial disparity primarily due to undefined molecular mechanisms. We have shown higher expression of chemokine receptor-9 in PCa cells and CCR9 expression was higher in PCa cells (MDA-PCa-2b) derived from African American (AA) patient compared to PCa cells derived from European American (EA) patients (LNCaP and PC3). Using antibody microarray we observed hyper activation of survival molecules and down-regulation of apoptotic molecules in AA PCa cells compared to EA PCa cells, following CCL25 stimulation. Comparative heat map analysis showed significant increase (~2-3 fold) in phosphorylation of pro-survival proteins in AA cells compared to EA cells treated with CCL25, which was further confirmed by western blot analysis. Furthermore, CCL25 treated PCa cells showed decreased expression of pro-apoptotic proteins (Bim, Bid, Bak), which was significantly abrogated by CCR9 shRNA. This effect was more pronounced in AA cells compared to EA cells. Efficacy of docetaxel (DTX) was higher after CCR9 blockade in presence of CCL25 compared to cells treated with CCL25 without blocking CCR9. Improvement in efficacy of DTX was due to inhibition of cell survival and activation of pro-apoptotic signals following CCR9 blockade. These observations suggest potential involvement of CCR9 mediated molecular pathways in disparity associated with outcome of PCa.

#2329 TMEM33 induces apoptosis via UP signaling and autophagy in breast cancer cells. Hong Hu, Xiuyan Zhang, Leena Hilakivi-Clarke, Usha Kasid, Robert Clarke. Georgetown Lombardi Comp. Cancer Center, Washington, DC.

TMEM33 is a novel transmembrane protein that resides in the endoplasmic reticulum (ENR). It has been shown to activate the PERK and IRE1α branches of the unfolded protein response (UPR). However, the underlying mechanism of action of this ENR resident protein TMEM33 and the cellular functions that it regulates remain largely unknown. In this study, we show that overexpression of TMEM33 induces robust cell death in breast cancer cells. Inhibition of the PERK/eIF2alpha pathway with eIF2alpha inhibitor ISRBK blocks the TMEM33 induced cell death. TMEM33 overexpression strongly activates UP associated pro-death JNK-p53 signaling. We also observed a significant inhibition of the downstream survivin, which blocks cell death activation by binding to caspases and inhibiting their activation. We further show that the blockage of JNK activation with either an inhibitor or overexpression of survivin, protects cells against TMEM33 induced apoptosis. In addition, we show that TMEM33 overexpression induces autophagy in breast cancer cells. Inhibition of autophagy with using either the inhibitor chloroquine or knockdown of the Atg5 gene, further sensitizes breast cancer cells to the effects of TMEM33 overexpression. Cell death induced by TMEM33 is also decreased by overexpression of the antioxidant master regulator SOD2. TMEM33 overexpression induces ER stress in breast cancer cells. However, TMEM33 overexpression did not sensitize breast cancer cells to ER stress inducers, thapsigargin, tunicamycin, or 4-phenylbutyric acid. The data presented herein demonstrate that the novel ENR resident protein TMEM33 induces cell death by activating IRE1α-IRE3-nisky-p53 survivin signaling in breast cancer cells. Concurrently, autophagy is also activated by TMEM33, and functions as a pro-survival mechanism. Cell fate reflects the balance between the pro-death and pro-survival activities as regulated by TMEM33.


Background: Inhibitors of apoptosis proteins (IAPs) regulate cellular apoptosis by interfering with the proteolytic activities of caspases. IAP inhibitors (SMAC mimetics) have been developed to restore the defective apoptosis that characterizes many tumour cells. Emerging evidence demonstrates that IAPs are critical components of immune-modulatory pathways that control innate and adaptive immunity. Accordingly, SMAC mimetics hold the promise of both inducing tumour cell killing and stimulating the immune system to recognize and eliminate dying tumour cells. Here we show that B15 primes immune components and synergises with PD-1 checkpoint inhibitors to promote eradication of syngeneic tumors. Methods: Here we report the efficacy and modulation of the immune response by a potent and selective SMAC mimetic, B15. We characterised the effect of B15 on tumor growth inhibition as a single agent and in combination with an anti-PD-1 antibody in syngeneic mouse tumor models. A detailed 17-colour multi-color flow cytometry analysis was used to investigate the mechanisms by which the SMAC mimetic interacts with anti-PD-1 therapy in vivo. Results: Treatment of the syngeneic mouse tumor models MBT-2 and EMT-6 with the SMAC mimetic in combination with an anti-PD-1 antibody results in remarkable tumor regressions in vivo. Importantly, the combined effect of the SMAC mimetic and anti-PD-1 on tumor growth was dependent on the adaptive immune system in vivo. Mechanistic studies show that degradation of IAP triggers tumor cell death, which leads to a potent activation of dendritic cells in the draining lymph nodes and a subsequent influx of T and NK cells into the tumor microenvironment. Interestingly, in the presence of the SMAC mimetic alone, an induction of PD-1 expression on tumor infiltrating CD8+ T cells was observed, which in turn resulted in the exhaustion of these cells and tumor outgrowth. In the presence of the anti-PD-1 antibody, T cells are reactivated leading to potent and long term tumor eradication. Conclusion: We show that our SMAC mimetic leads to a potent induction of immunogenic cell death and sets up a “virtuous cycle” by potentiating dendritic cell and T cell mediated immune responses that further promote induction of cell death. These effects are preserved even when combined with anti-PD-1 monotherapy. Tumours with minimal T-cell infiltration are poorly responsive to PD-1 monotherapy. These studies indicate that SMAC mimetics, such as B15, represent promising and tolerated combination partners for checkpoint inhibitors in patients that lack a strong immune inflammatory signature.

#2331 FAM3B/PANDER inhibits cell death and increases tumor growth by modulating the expression of Bcl-2 and Bcl-XL cell survival genes. Izabela Caldeira,1 Paula Maciel-Silva,2 Flavia Ramos Siqueira,3 Anna Carla Goldberg,2 Viviane Abreu Nunes,1 Jose Ernesto Belizario,1 Humberto Miguel Garay-Malpartida,1 1University of Sao Paulo, Sao Paulo, Brazil; 2Hospital Israelita Albert Einstein, Sao Paulo, Brazil.

FAM3B/PANDER is a novel cytokine-like protein that induces apoptosis in insulin-secreting beta-cells. Since in silico data revealed that FAM3B can be expressed by prostate and breast tumors, we evaluated the putative role of this cytokine in prostate and breast tumor progression. The FAM3B expression was compared by quantitative PCR in LnCAP, PC-3 and DU145 prostate tumor cell lines and MCF-7, ZR-75 and MDA-MB-231 breast tumor cell lines. After treatment with either recombinant FAM3B protein or secreted FAM3B obtained from conditioned medium (CM) derived from FAM3B-overexpressing 293FT cells, the cell death and viability of DU145 and MDA-MB-231 cell lines were evaluated. DU145 and MDA-MB-231 cells overexpressing FAM3B protein were produced by lentiviral-mediadated transduction of full-length FAM3B cDNA. Cell viability and apoptosis were analyzed in DU145-FAM3B and MDA-231-
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FAM3B cells after treatment with several cell death inducers. Anchorage-independent growth and scratch wound assays were used to evaluate in vitro tumorigenicity and cell migration, respectively. In vivo tumorigenicity and invasiveness were evaluated by tumor xenograft growth in nude mice. We observed that FAM3B was highly expressed by hormone-responsive cells (LnCAP and MDA-MB-231) cultured in unresponsive hormone cells (PC-3, DU145 and MDA-MB-231). Cell viability and survival of DU145 and MDA-MB-231 cells increased after exogenous treatment with recombinant FAM3B protein or CM containing FAM3B-secreted protein. Overexpression of FAM3B in DU145 and MDA-MB-231 cells promoted an inhibition of apoptosis triggered by TNF-alpha, staurosporine and serum-deprived conditions. Cell death inhibition was accompanied by increased gene expression of survivin, survivin-like, Bcl-xL and Bcl-xl genes, and by slight decrease in expression of pro-apoptotic gene Bax and diminished caspases-3, 8 and 9 proteolytic activities. When compared to control, cells overexpressing FAM3B displayed a decreased anchorage independent growth and increased motility in vitro, as well as an increased tumor growth in xenografted nude mice. The immunohistochemistry analysis from tumor xenografts revealed similar anti-apoptotic effect displayed by FAM3B-overexpressing tumor cells. Taken together, by activating pro-survival mechanisms, FAM3B overexpression contributed to increased cell death resistance and tumor growth in nude mice, highlighting a putative role for this cytokine in prostate and breast cancer progression.

#2332 Integration modulatory role of nuclear factor-kappa B activation by C/EBPβ and the endoplasmic reticulum stress sensor PERK to mediate estrogen-induced apoptosis in estrogen-deprived breast cancer cells. Ping Fan,1 Amit K. Tyagi,1 Fadke A. Agboke,2 Niranjana Pokharel,3 V. Craig Jordan1,2

1University of Texas MD Anderson Cancer Center, Houston, TX; 2University of Toronto, Toronto, ON; 3Georgetown University, Washington, DC.

Stress responses are critical for estrogen (E2) to induce apoptosis in E2-deprived breast cancer cells. Nuclear factor-kappa B (NF-kB) is well known as a therapeutic target to prevent stress responses in chronic inflammatory diseases including cancer. However, whether E2 activates NF-kB to participate in stress-associated apoptosis in E2-deprived breast cancer cells is unclear. We demonstrated that E2 differentially modulates NF-κB activity in E2-deprived breast cancer cells according to the treatment time. Because E2 initially has significant potential to down modulate the NF-κB activation, it completely suppresses the tumor necrosis factor alpha (TNFα)-induced NF-κB activation. We found that E2 preferentially and constantly enhances the expression of transcription factor CCAAT/enhancer binding protein beta (C/EBPβ) which is responsible for suppression of NF-κB activation by E2 in MCF-7/5C cells. The MTOR signaling pathway promotes repression of NF-κB by C/EBPβ which is confirmed by the evidence that inhibition of MTOR is synergistic with E2 to upregulate NF-κB-dependent genes, such as TNFRα. Interestingly, NF-κB p65 activity is upregulated when E2-treatment is administered for 48 hours, leading to induction of TNFα. Blocking the nuclear translocation of NF-κB completely prevents E2 from induction of TNFα and apoptosis. Importantly, protein kinase RNA-like endoplasmic reticulum kinase (PERK), a stress sensor of unfolded protein response, is activated by E2 and plays an essential role in increasing NF-κB p65 mRNA and protein expression through the activation of STAT3, independently of canonical IκBα signal pathway. Thus, inhibition of PERK kinase activity completely blocks nuclear activation of NF-κB and NF-κB-dependent induction of TNFα, thereby preventing E2-induced apoptosis. All of these findings illustrate a crucial role for the novel PERK/NF-κB/TNFα axis in E2-induced apoptosis which is integrally modulated by the stress responsive transcription factor C/EBPβ and endoplasmic reticulum stress. This study provides an important rationale for exercising caution in clinical trials when considering targeting PERK or NF-κB following the development of acquired resistance to aromatase inhibitors whereas mTOR may be a target to enhance the therapeutic effects of E2 in aromatase resistant breast cancer.

#2333 Modulation of Bax and mTOR for cancer therapeutics. Rui Li,1 Chinyong Ding,2 Jun Zhang,1 Maohua Xie,1 Dongkyoo Park,1 Ye Ding,4 Guo Pandita,1 1Inova Schar Cancer Institute, Falls Church, VA; 2The Houston Methodists Research Institute, Houston, TX.

Circulating tumor cells (CTCs) are cancer cells which dissociate from the primary tumor and circulate within the peripheral blood, initiating metastasis at a distant location. CTCs present a “non-invasive, liquid biopsy”, making them attractive targets for the development of cancer biomarkers and therapies. In order to form metastases, these cells must undergo a phenotypic shift acquiring the ability to survive anoksis within the blood stream and evade immune surveillance. In addition, CTCs are enriched for autophagy, as detected on the surface of cancer cells. Under physiological conditions, these CTCs can be activated by circulating cytokines such as TNFα, Fas ligand, and TNF-related apoptosis inducing ligand (TRAIL), triggering cell death. In vitro studies have demonstrated that environmental stress caused by non-adherent suspension culture conditions, similar to circulation, will increase autophagy in cancer cell lines aiding anoksis survival. Our lab has previously identified a link between increased autophagic rates and decreased DR surface expression. Based on this we were interested in determining whether increased autophagy due to non-adherent conditions caused breast cancer cell lines to decrease death receptor expression, evading immune surveillance and resisting death-ligand induced apoptosis. To this end, breast cancer cell (BCC) lines were cultured in monolayer or a non-adherent suspension condition for 7 days. Following 7 days of culture, death receptor expression and autophagy initiation were measured using immuno blot and flow cytometry microscopy. BCCs were treated with TRAIL and analyzed over 24 hours for apoptosis and caspase activation. We found that the BCC lines decreased total and surface expression of DR5 and total expression of TNFRI over the 7 days of suspension condition. The reduced expression of DR5 in suspension cultured cells resulted in a delayed response to TRAIL-mediated apoptosis, with delayed caspase activation and PARP cleavage compared to monolayer cultured parental cells. Under suspension condition, autophagy was initiated by increased LC3-II/LC3-I turnover and p62 degradation. This initiation of autophagy is potentially due to increased expression of the mitochondrial related proteins BNP3 and BNPIP2, which have been implicated in anoksis survival. This suggests a delay in death receptor signaling in a non-adherent condition. This delay in death receptor signaling brings about a potential of targeting CTCs within the blood to limit metastatic spread and disease progression in breast cancer. Our findings suggest the potential to sensitize CTCs to anoikis and circulating death ligands through autophagy inhibition.

#2335 Targeting the DNA repair as well as survival functions of MCL-1 enhances cancer cell killing. Abid Matteo,1 J Milburn Milburn Jessup,2 Tej K. Pandita1, 1Inova Schar Cancer Institute, Falls Church, VA; 2The Houston Methodist Research Institute, Houston, TX.

MCL-1 is a pro-survival BCL2 protein family member which is over-expressed in drug resistant cancer cells. Our purpose was to assess whether MCL-1 increases genomic instability along with its anti-apoptotic function in cancer cells. Depletion of MCL-1 by treating human small cell lung cancer (SCLC) lines with immunotoxin sensitizes these cells to increased killing by BH3 mimetic Navitoclax (ABT-263) both in vitro and in mice xenograft models. These SCLC cell lines are resistant to both single agent Immunotoxin or ABT-263. In addition, inhibition of NANOOG or NANGP8 with lentivirus delivered shRNA depletes MCL-1 in human colorectal cancer cells and sensitizes these cells to increased cell killing by venetoclax (ABT-199). This increased killing depended on loss of MCL-1, was caspase-dependent and reversed by re-expression of MCL-1. Depletion of MCL-1 in cancer cells, increases cell sensitivity to ionizing radiation (IR) induced death, which was reversed by expression of MCL-1. In response to IR, MCL-1 depleted cells showed reduced survival even at radiation.
Disregulation of the CDK4/6-Rb pathway is a hallmark of various tumor types. Cancer cells subvert different cell cycle checkpoints to continue unchecked growth and proliferation. Some common mechanisms of overcoming cell cycle checkpoints involve loss of tumor-suppressor proteins such as p16 INK4a or Retinoblastoma (Rb). Alternately, cancer cells can also increase expression of pro-apoptotic CDKs by gene amplification or gene translocation. Thus, targeting different oncoproteins in the cell cycle pathway, particularly CDKs has been considered an important therapeutic strategy. Events of overexpression of cell cycle oncogenes and suppression of tumor suppressors are mutually exclusive and tumor-type specific. In colorectal cancer (CRC), it has been shown that there is overexpression of CDK4/6. We conducted a TCGA analysis of 50 matched tumor and normal CRC tumors, and validated that CDK4 and CDK6 are significantly overexpressed in patient tumor samples by RNA-seq (p = 1.55 e-09 and p = 4.39 e-05). Thus, ongoing clinical trials for CRC patients with advanced disease are currently evaluating the efficacy of CDK4/6 inhibitors as single agents and in combination with chemotherapy (J Clin Oncol 33, 2015; suppl 3; abstr 626; Zhang 2016). We evaluated the efficacy of miRNAs targeting CDK4/6 as novel therapeutics in CRC. We performed in silico analysis using TargetScan to identify miRNAs that can target the 3'UTR regions of CDK4/6. We have identified a novel family of miRNAs (based on seed sequence similarity) that can potentially target CDK4/6 mRNA and thereby reduce both the RNA and protein levels of CDK4/6 in cancer cells. Of the four miRNAs in the family, we chose to characterize miR-6883-5p and miR-149* in a panel of CRC cell lines and primary tumors to identify targets. Our preliminary TCGA analysis in 11 matched T/N samples shows miR-149* expression is significantly lost (p = 0.0049) in CRC samples and this further positively correlates with the stage of the tumor. Ongoing in vitro studies using miRNA mimics in CRC cell lines indicate that both miR-6883-5p and miR-149* have anti-proliferative effects as assayed by CellTiterGlo (CTG) and MTT. Further, both miRNAs induce G1-arrest as a consequence of downregulation of CDK4 and CDK6 in all cell lines and we also observe induction of apoptosis in a subset of CRC cell lines. Knockdown of CDK4 and CDK6 mimics the phenotypes observed with restoring expression of both miR-6883 and miR-149* in CRC cell lines. Combination of miR-6883 with FDA-approved drugs Irinotecan and 5-FU showed strong synergy and led to apoptosis as assayed by CTG and PARP cleavage. Our ongoing work is further looking into the mechanisms of synergy and identifying additional target genes in CRC cell lines. Thus, our novel miRNA based strategy to target CDK4 has potential to translate as both a single agent/combinatorial therapy and to identify biomarkers of response, which is critical for understanding the clinical results seen with CDK inhibitors.

#2334 CDK4/6 and autophagy inhibitors synergize to induce senescence in cancers with an intact G1/S checkpoint. Smruthi Vijayaraghavan,1 Cansu Karakas,1 Xian Chen,1 Imran Doostan,1 Akshara S. Raghavendra,1 Min Yi,2 Ravi Arora,1 Arpan Fahemi,1 Kelly Hunt,1 Debu Tripathy,1 Khandan Keyomarsi1.

Deregulation of apoptosis is central to cancer progression and a major obstacle to effective treatment. The B-c2 gene family members play important roles in the regulation of apoptosis and are frequently altered in cancers. One such member is Bcl-2-related Ovarian Killer (BOK), which is a pro-apoptotic protein. Despite its critical role in apoptosis, the regulation of BOK expression is poorly understood in cancers. Here, we discovered that miR-296-5p regulates BOK expression by binding to its 3'UTR in breast cancer. Further, we showed that depletion of BOK by either miR-296-5p or siRNA against BOK protected breast cancer cells from undergoing paxilactal-induced apoptosis. Interestingly, miR-296-5p also regulates the expression of Mcl-1, which is an anti-apoptotic protein and is highly expressed in breast cancers. Our results reveal that Mcl-1 is important for suppression of BOK function as ectopic BOK expression induced Mcl-1, while silencing of BOK resulted in reduced Mcl-1 levels in breast cancer cells. In addition, we show that specific silencing of Mcl-1 reduced the long-term growth of breast cancer cells, whereas BOK inhibition didn’t have any effect on the growth of breast cancer cells. Surprisingly, silencing of both Mcl-1 and BOK rescued the effect of Mcl-1 silencing on breast cancer cell growth, suggesting that BOK is important for attenuating cell growth in the absence of Mcl-1, and also showing a tight feedback regulatory loop between BOK and Mcl-1 in breast cancer cells. Furthermore, we demonstrated that BOK protein level is regulated post-translationally by GSK3α/β and to some extent GSK3β as GSK3 inhibitor (CHIR99021) or silencing of GSK3 significantly increased BOK protein levels in breast cancer cells. Notably, we found that Mcl-1 interacts with GSK3α/β and silencing of Mcl-1 using siRNA significantly attenuated endogenous GSK3α/β levels in breast cancer cells. Taken together, our results suggest that the regulation of BOK (either post-transcriptionally by miR-296-5p or post-translationally by GSK3α/β) of the levels of pro-apoptotic protein BOK and anti-apoptotic protein Mcl-1 decide the fate of cancer cells to either undergo Apoptosis or proliferation.

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Doses less than 6 Gy, however an increase in caspases 3/7 activity was observed at 6 Gy and above, suggesting that apoptosis is activated only when MCL-1 depleted cells are irradiated with higher IR doses. In order to explain the decreased survival post-irradiation the genomic instability and DNA repair pathways were analyzed in MCL-1 depleted cells. Post-irradiation MCL-1 depleted cells exhibited increased genomic instability as measured by increase in chromosome aberrations at different phases of the cell cycle. The increase in aberrations were significantly higher in G2 and S phases of the cell cycle suggesting defect in Homologous recombination repair pathway (HR). Moreover, the MCL-1 depleted cells show decreased gamma-H2AX foci at earlier time points (30 and 90 minutes) post-irradiation indicating defect in DNA Damage response (DDR) and high residual foci at 90 minutes, indicating defective DNA repair. These observations were further confirmed by decreased phosphorylation of ATRX suggesting defect in DNA Damage response and higher levels of residual 53BP1 and RIF1 foci in MCL-1 depleted cells, confirming DNA DSB repair by homologous recombination (HR) was compromised. Consistent with this model, MCL-1 depleted cells exhibited a reduced frequency of IR-induced MRE11, BRCA1, RPA and Rad51 foci formation, decreased DNA end resection and decreased HR repair in the DR-GFP DSB repair model. Similarly, after Hydroxyurea (HU) induction of stalled replication forks in MCL-1 depleted cells there was a decreased ability to subsequently restart DNA synthesis, which is normally dependent upon HR mediated resolution of collapsed forks. In summary, MCL-1 is an important therapeutic target in cancer cells and its depletion increases cell killing by either increase in apoptosis or suppression of HR and increased replication stress.
other cancer cell lines. Several solid tumors (ovarian, lung, pancreatic, colon, prostate) and triple negative breast cancer (TNBC) cell lines exhibited a synergistic response to palbociclib/HQ combination treatment dependent upon an intact G1/S transition (Rb+/LMW-). This was also verified in a TNBC patient derived xenograft (PDX) model. Thus, this study addresses the aforementioned limitations and provides a novel and promising biomarker-driven combination therapeutic strategy to treat breast and other solid tumors. We predict that this combination of CDK4/6 and autophagy inhibitors would be more beneficial than standard dose palbociclib in patients, allowing to lower the dose, minimize palbociclib mediated toxicities and potentially improve overall patient survival - a goal that has not yet been met with currently approved treatment combinations.

#2339 Downregulation of cell cycle genes in HNSCC by erufosine. Shariq S. Ansari,1 Ashwini K. Sharma,1 Michael Zepp,1 Frank Bergmann,2 Rainer Bergqvist2.

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Head and neck cancer ranks amongst the sixth most prevalent cancers worldwide and oral squamous cell carcinomas (OSCC) constitute 90% thereof. Erufosine is an ether-lipid-derived synthetic compound belonging to alkylphosphocholines (APCs), which has been shown to inhibit the proliferation of OSCC cells. It simultaneously induces apoptosis and autophagy by modulating the Akt-mTOR signaling pathway, however, its exact mechanism of action is not fully understood. Here, we describe the activity of erufosine on the expression of cell cycle related genes in OSCC cells. The anti-proliferative effect of erufosine in cells of two human OSCC cell lines, HN-5 and SCC-61, was determined by MTT assay after 24h, 48h and 72h exposure. Based on these results, HN-5 cells were exposed to erufosine at concentrations corresponding to IC25, IC50 and IC75 concentrations, and then their mRNA was isolated and analyzed by Illumina Chip array. Gene expression modulation was confirmed by RT-PCR and Western blot. Gene set enrichment analysis was performed to identify core KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways and GO (Gene Ontology) terms. In addition, erufosine’s effect on cell cycle distribution and colony formation was determined as well as its antineoplastic effect on respective xenografts in nude mice. As shown from TCGA data, cell cycle deregulation in HNSCC ranks amongst the top three out of 24 different types of cancers. In line with this, the median expression of cell cycle genes was higher than that of other genes within HNSCC. We also looked at the median gene expression of all CDKs and cyclins in 519 HNSCC patient samples from TCGA and found that the expression levels were higher than the global gene expression. This was in agreement with IHC-staining of CCND1 in tumors from 30 South East Asian HNSCC patients. Furthermore, increased CCND1 and CDK6 expression levels from TCGA data had negative implication on patient survival. In our experimental results erufosine caused a dose dependent growth inhibition of OSCC cell lines and negative enrichment of genes related to the cell cycle process. Our microarray findings revealed that cyclins and CDKs were downregulated in a dose dependent manner in response to erufosine exposure. These findings were verified at both mRNA and protein levels. Erufosine not only massed a mass2G block but also inhibition of colony formation thus preparing the OSCC cells to undergo apoptosis. We are the first to show that erufosine inhibited tumor growth in vivo in a HNSCC xenograft model and caused downregulation of cyclinD1, CDK4 and CDK6 in lesions of the animals. These findings collectively show the potential of erufosine to be used as a cell cycle inhibitor in HNSCC progression and support the future evaluation of erufosine as a therapeutic approach in cancer treatment alone, or in combination.

#2340 Thymidine kinase activity as a response marker for CDK 4/6 inhibition. Geoffrey Shapiro,1 Magnus Neumuller,2 Sara Lööf,3 Smaranda Bacanu,3 Takahiro Seki,4 John Hilton,1 Khan Do,1 Nicole Chau,1 Leena Gandhi,1 Joseph W. Gibson,1 Robert Distel,1 Pawel Nieginski,2 Edward M. Suh,2 Mattias Bergqvist1.

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Selective CDK4/6 inhibition is now part of standard treatment for HR+ breast cancer. There is a pressing need for a practical biomarker that can provide early indication of the biologic activity of these agents and correlate with clinical outcomes. Expression of thymidine kinase (TK) occurs in actively proliferating cells, is E2F-dependent and is downregulated after CDK4/6 inhibitor-mediated G1 arrest. Here, we have investigated TK activity (TKA) as a pharmacodynamic marker for CDK4/6 inhibition in both preclinical and clinical contexts and demonstrate the potential of this assay as CDK4/6 inhibitors are further developed. TKA in response to the CDK 4/6 inhibitor palbociclib (palbo) was studied in cell culture, mouse models and samples from clinical studies. TKA was determined by the DiviTum Assay (Biovica, Sweden). In culture, the intracellular TKA and TKA release in response to palbo were determined by analysis of cellular extracts and tissue culture media. In vivo, mice bearing human xenografts were treated every 28 days or 4/4-week (continuous) schedules. In K562 cells, intracellular TKA levels exhibited a clear dose response to palbo. Reductions in TKA were seen at lower drug concentrations than those affecting cell viability. Similar results were observed in MCF-7 xenografts, where lower TKA in serum and tumor was observed after palbo treatment, whereas in E0771 xenografts, no change in TKA was observed. Clinically, serum samples were obtained before and at various time points after palbo exposure in 20 cancer patients. One patient, who discontinued study treatment at the end of cycle 1, demonstrated an increase in s-TKA. In contrast, for the other 19 patients, there was a marked decrease in s-TKA at day 21. Pre-treatment s-TKA values were higher than C1D21 (paired t-test, p<.0001). In 11 patients there was a reduction in s-TKA to below the assay detection limit. Five patients exhibited a reduction in s-TKA within the working range, with mean residual activity at day 21 of 32%. In the 6 patients receiving continuous palbo, s-TKA remained low after day 21. Of the 14 patients using the 3/4-week schedule, 9 exhibited an increasing level of s-TKA between day 21 and day 1 of cycle 2, consistent with reversible effects of palbo on cell cycle progression after cessation of exposure. These data suggest that s-TKA reflects MOA response to the selective CDK4/6 inhibitor palbo. The DiviTum Assay may provide a practical, non-invasive tool for monitoring the effects of CDK4/6 inhibitors in both preclinical models and patients. We plan inclusion of the assay into additional studies to determine whether the assay can predict clinical outcome or portend development of resistance.

#2341 In situ labelling identifies a novel landscape of nuclear CDK2 substrates. Bruce E. Clurman, Yong Chi, Fred Hutchinson Cancer Research Ctr., Seattle, WA.

Cellular signal transduction pathways rely heavily on protein kinase-mediated phosphorylation events. Comprehensive identification of the physiologic target protein kinases is key to understanding their functions and elucidating cellular signalling pathways. Various direct and indirect approaches, combined with advanced mass spectrometry technologies, have made it possible to identify many candidate targets of a given kinase, but have also yielded many false-positive identifications, largely due to technical limitations. Identifying direct and physiological kinase targets thus remains a difficult task. We developed a mass spectrometry-based in situ approach that utilizes substrate phospho-rylation and ATP analog-sensitive CD2 (AS-CD2) to identify over one hundred potential cyclin-dependent kinase 2 (CDK2) substrates in intact nuclei. More than one-third of these candidates are known CDK substrates, indicating significant enrichment for physiologic CDK2 substrates, and we also identified and validated new CDK2 substrates. Importantly, many of these new CDK2 substrates are substrates that are important in tumor histone modification, DNA replication, and DNA repair, which likely reflects our use of AS-CD2 within its normal nuclear context, and regulated by endogenous cyclins. These methods should be broadly applicable to the study of other kinases with complex substrate networks.

#2342 TNFAIP8 promotes prostate cancer cell survival by modulating autophagy. Suresh Niture,1 Malathi Ramalinga,2 Habib Kedir,3 Deepak Kumar,1 North Carolina Central University, Durham, NC; 2University of District of Columbia, Washington, DC; 3North Carolina Central University and UDC, Durham, NC.

TNF-α inducible protein (TNFAIP8) is an antiapoptotic protein with roles in tumor cell growth and survival. Mechanisms of cell survival by TNFAIP8 remain elusive. In the current study, we investigated the role and molecular mechanism related to TNFAIP8 in the modulation of cell cycle, autophagy and drug resistance/cell survival in prostate cancer cells. Microarray data from PC3 prostate cancer cells ectopically expressing TNFAIP8 demonstrated modulation of cell cycle related genes such as CDKs, CDCs and PCNA. Immunoblotting data from overexpression of TNFAIP8 in PC3 cells shows upregulation of cell cycle proteins cyclin A, cyclin B1, Myt1 and Chk1. However, no change in cell cycle was found. Phosphorylation of Histone-S10, CDC2-Tyr15 and Weel1-S642 was also observed. Autophagy plays an important role in tumor cell survival. We evaluated the effects of TNFAIP8 in modulating autophagy. Overexpression of TNFAIP8 leads to induction of autophagy. TNFAIP8 positively
modulates the expression/stabilization of autophagy markers and effectors such as LC3β I/II, Beclin1, 4EBP1, oncogene p62 and SIRT1. Knockdown of TNAIP8 inhibits autophagy induced by nutrient starvation in PC3 prostate and MCF7 breast cancer cells. We also evaluated cell growth and survival in PC3 cells. We demonstrate that TNAIP8 promotes cell growth and proliferation in PC3. LNCaP prostate cancer cells. Further work has revealed that TNAIP8 increase resistance against anticancer drugs docetaxel and doxorubi- cin. These data collectively suggest that the creation of cellular autophagy events TNAIP8 promotes cell survival and drug resistance in prostate cancer cells.

#2343 The novel potential of palbociclib (CDK4/6 inhibitor) in the treat- ment of triple-negative breast cancer. Shinchiro Kashiwagi, Yuka Asano, Wa- taru Goto, Koji Takada, Tsutomu Takashita, Tamami Morisaki, Satoru Noda, Naoyoshi Onoda, Kosei Hirakawa, Masashi Ohira. Osaka City University Gradu- ate School of Medicine, Osaka, Japan.

Background: The effectiveness of palbociclib (CDK4/6 inhibitor) for estrogen receptor positive breast cancer has been demonstrated by large-scale clinical studies, with the drug garnering attention as a key drug for breast cancer sub- types with endocrine sensitivity in the future. According to PALOMA-3 trial, palbociclib has been demonstrated to contribute to the extension of progres- sion-free survival in patients with advanced hormone receptor-positive and HER2-negative metastatic breast cancer after endocrine therapy. On the other hand, in the case of triple-negative breast cancer (TNBC), luminal AR (LAR) related to androgen signaling is believed to have endocrine activity. Previous clinical data revealed that palbociclib shows high sensitivity in luminal breast cancer cell lines with androgenic activity, with effectiveness also expected in LAR. In this study, we created TNBC cell lines that forcibly express AR and examined the effectiveness of palbociclib for TNBC. Materials and Methods: MCF-7 and T-47D were used as luminal breast cancer cell lines, while MDA-MB-231 and BT-549 were used as TNBC cell lines. In addition, we created TNBC cell lines that forcibly express AR, called AR-MDA-MB-231, by the transfection of pEGFP-C1-AR Plasmid Vector using Lipofectamine® 3000 Reagent. We con- firmed the expression of AR by qRT-PCR as well as Western blotting and exam- ined the impact of palbociclib on proliferation as well as apoptosis of breast cancer cell lines. Results: AR was found to have been expressed only in luminal breast cancer cell lines but not TNBC cell lines. It was confirmed that AR was expressed in AR-MDA-MB-231 which are stable cell lines with the properties of LAR. In a CCK assay, palbociclib showed high sensitivity in AR-MDA-MB-231 as in luminal breast cancer cell lines. Furthermore, in an apoptosis assay using FACS and cell cycle assay, apoptosis was induced in AR-MDA-MB-231 and cell cycle arrest at the G1S check point was confirmed. Conclusion: palbociclib (CDK4/6 inhibitor) showed effectiveness for TNBC cell lines that compulsively express AR, suggesting it may be one treatment option for TNBC in the future.


Introduction: Urothelial carcinoma is characterized by a high incidence of molecular alterations involving loss of the cyclin dependent kinase inhibitor p16 encoded by CDKN2A or amplification of CDK4/6 is associated with sensitivity to CDK4/6 inhibitors in various cancers. Functional RB1 is believed to be canonically required for mediating the effects of CDK4/6 inhibition in this setting. However, muscle-invasive urothelial carcinomas harbor RB mutations and copy-number losses in 21% of patients. To extend the activity spectrum of CDK4/6 inhibitors to these RB1-deficient cancers, we in- vestigated the role of pocket protein p130 encoded by the Retinoblastoma-Like 2 (RBL2) gene, in mediating the downstream effects of CDK4/6 inhibition. Meth- ods: CRISPR-Cas9 was used for CDKN2A knockout in the 5637-urothelial cancer cell line with an RB-mutant background and in TCCSUP cell line with wild- type RB. CDKN2A cells were subsequently treated with CDK4/6 inhibitor palbociclib. Levels of RB1, phospho-RB (at serine 807/ serine 811), p130 and phospho-p130 (specifically, the CDK4/6-dependent serine 672 phosphoryla- tion) proteins were quantified using SDS-PAGE. Cell-cycle phase analysis was performed using flow cytometric measurement of BrdU and PI staining. Results: CRISPR-induced disruption of CDKN2A was validated using genomic PCR and targeted MiSeq sequencing. p16 protein knockdown was validated using SDS- PAGE. CRISPR-induced CDKN2A knockout resulted in increased cell pro- liferation and faster G1/S transition even in the absence of functional RB in the 5637-urothelial cancer cell line. A significant increase in the levels of phospho- p130 was observed after p16 knockdown without changes in the levels of un- phosphorylated p130. TCCSUP cell line with functional RB demonstrated a similar increase in proliferation, faster G1/S transition and an increase in phos- pho-p130. Palbociclib was effective in reversing these changes in all the tested cell lines. Conclusions: the pocket protein p130 plays an important role in me- diating downstream effects of CDK4/6 inhibition in urothelial cancer cell lines with both wild-type and more importantly, deficient RB. CDK4/6-deficient tumors are a significant proportion of prostate cancer, whereas RB is preserved for the functional status of RB1. Through p130 acting as a dominant activator of E2F family of transcription factors that control G1/S checkpoint. These findings potentially explain the lack of correlation between RB and re- sponse to CDK4/6 inhibitors in clinical trials. These results provide a mechanistic rationale for extending clinical trials of CDK4/6 inhibitors to patients with RB1-deficient tumors.

#2345 CD4K inhibition enhances anti-melanoma effects of BRAF/MEK inhibition. Yanping Zhang, Eddy C. Hsueh. Saint Louis Univ., St. Louis, MO.

Introduction: Targeted therapy against BRAF-mutated melanoma has shown clinical efficacy. However, resistance to therapy often occurs. CD4K (IN4K)-retinoblastoma (RB) pathway controls cell cycle progression by regulating the G1-S checkpoint. Defect in the p16INK4A, cycling D-CDK4/6:RB pathway is also detected in melanoma. We study the effect of combining CD4K-RB pathway inhibition with BRAF/MEK inhibition as a possible strategy to overcome resis- tance to therapy. Methods: Three BRAF wild-type and three BRAF-mutated melanoma cell lines were selected. CD4K/6 inhibitor (PD0323991; PD), BRAF inhibitor (PLX4032; PLX), MEK inhibitor (AZD6244; AZD), and PI3K/AKT inhibitor (XL765; XL) were used alone or in combination. Cell proliferation assay was performed using Cell Titer Blue assay. Cell migration assay was per- formed using Bioassay. Cell migration inhibition was noted with PD and PD + XL combination compared with PD + PLX alone. No correlation was observed between expres- sion of CDK4 and cyclin D1 and sensitivity to single agent or the combination.

Inhibition of phosphorylated RB1 and ERK expression was observed with expo- sure to single agent PD, PLX, XL, AZD. Enhancement of pRB1 and pERK inhibi- tion was noted with PD + PLX and PD + AZD combination but not PD + XL. Conclusion: CD4K inhibition can enhance anti-proliferation and migration ef- fects of BRAF/MEK inhibition in melanoma cells. The mechanism is via aug- mented down-regulation of pRB expression.

#2346 Preclinical selectivity profile of the CDK4/6 inhibitor ribociclib (LEE011) compared with that of palbociclib and abemaciclib. Ralph Tiedt,1 Scott Delach,2 Steven Kovats,3 Thomas Horn,4 Michael Acker,5 Barbara Sch- acher Engstler,1 Giordano Caponigro.1 1Novartis, Basel, Switzerland; 2Novartis, Cambridge, MA.

A hallmark of cancer is unchecked cell division. Retinoblastoma protein (Rb) is a human tumor suppressor that guards a cell’s entry into S phase by binding E2F transcription factors and keeping them inactive. Many growth-promoting stimuli increase expression of D-type cyclins, which bind to and activate cyclin- dependent kinases 4 and 6 (CDK4/6). The cyclin D–bound CDK4/6 holo- enzymes phosphorylate RB, resulting in release of E2F, which in turn activates genes required for S phase entry and DNA replication. Numerous oncogenic aberrations converge at the CDK4/6–E2F pathway, providing a strong rationale for developing CDK4/6 inhibitors as cancer therapeutics. Ribociclib (LEE011) is a selective CDK4/6 inhibitor that has received FDA breakthrough therapy and priority review designations for treatment of hormone receptor–positive breast cancer in combination with letrozole and is being tested in additional clinical trials. Here we describe the preclinical selectivity profile of ribociclib in bio- chemical and cellular assays. Ribociclib inhibits both CDK4–cyclin D1 and CDK6–cyclin D3 kinase activity with nanomolar IC50s in biochemical assays. To comprehensively address the selectivity of ribociclib in direct comparison with 2 other clinical CDK4/6 inhibitors, palbociclib and abemaciclib, we made use of the KINOMEscan assay consisting of >450 kinase active site–directed competition-binding assays. We adjusted the test concentrations in the kinase- selectivity panel per the binding constants for CDK4 and CDK6 to account for the higher potency of abemaciclib. Data showed that both ribociclib and palbo- ciclib have high selectivity for CDK4 (CDK6 was not covered in the panel), with
very few distinct additional binding events detected. In contrast, abemaciclib is a much more promiscuous kinase inhibitor. Next, we sought to determine the relative potencies of the 3 inhibitors against CDK4 vs CDK6 in cellular assays. When testing different routinely used readouts of cellular viability, we found that assays that measured metabolic activity (eg, CTG) tended to underestimate the effects of CDK4 inhibition; thus, all assays were normalized to assayed cell number were used instead. We first identified cancer cell lines primarily dependent on either CDK4 or CDK6 as judged by combined RNA expression analysis and shRNA or CRISPR-based functional assays. When determining IC50 of the 3 CDK4/6 inhibitors in these cell lines, we found that ribociclib and abemaciclib demonstrated greater activity in CDK4-dependent cells vs CDK6-dependent cells, whereas palbociclib was active in both cell types. The high degree of CDK4 selectivity of ribociclib suggests that off-target kinase inhibition is an unlikely complication in patients. Moreover, the apparent preference for CDK4 over CDK6 could be an advantage in certain cancer types that are primarily dependent on CDK4.

#2347 MicroRNA mediated CDK4/6 inhibitor resistance via extracellular signaling. Liam Cornell, Geoffrey I. Shapiro. Dana Farber Cancer Institute, Boston, MA

Multiple potent and highly selective inhibitors of the cell cycle kinases - CDK4 and CDK6 are in development. One such inhibitor, palbociclib, was recently approved for use in combination with letrozole for the treatment of estrogen receptor positive (ER+) breast cancer. Cyclin D-dependent kinase activity is a driving factor for ER+ breast carcinogenesis, irrespective of CCND1 amplification, making CDK4/6 inhibition a promising approach for this breast cancer subset. However, as with all cancer treatments, resistance will be a major issue limiting the efficacy of this approach. To date, mechanisms of palbociclib resistance have not been extensively investigated. Through interrogation of the generated CDK4/6 inhibition resistant cells we discovered overexpression of CDK6, specifically, mediates resistance. CDK6 depletion reversed resistance and overexpression caused resistance, whereas the same was not true when CDK4 or D cyclin protein levels were manipulated. Interestingly, when generating and analyzing resistant cell populations, we observed a “bystander effect”, by which resistance was transmissible between cells. The "resistant bystander" cells display all characteristics of the drug exposure induced resistant cells, however became resistant in just 48 hours as opposed to 14 weeks. Analysis of conditioned medium revealed that the transmission of resistance is dependent on exosomes, but not protein or DNA. We identified a specific miRNA, present in the exosomes of resistant cells, by microarray which caused resistance when excreted. Additionally, overexpression and inhibition of the miRNA confirmed that it is responsible for causing resistance. miRNA overexpressing cells exhibited the same phenotype as drug induced resistant cells, and furthermore, could cause resistance in neighboring cell populations by exosome mediated signaling. Using Biotin labelled miRNA-miRNA pulldown followed by RNA-seq, we identified the TGFβ pathway as the miRNA target. Downregulation of the TGFβ caused a decrease in the CDK inhibitors, p15 and p21, resulting in an increased CDK6 protein level and palbociclib resistance. We subsequently confirmed these data in patient samples by comparing before treatment and post relapse biopsies. These findings highlight a novel mechanism of conferred drug resistance as well as new insights into acquired CDK4/6 inhibitor resistance.

#2348 Targeting the p27kip1/cdk4/cdk2/Rb axis in breast cancer using a peptidomimetic of Brk’s SH3 domain. Stacy W. Blain,1 Jason Quinones,1 Priyank Patel,2 Velaslav Tisperson,3 Susan Gottesman,1 Jonathan Somma,1 Yun Wu1. 1State University of New York, Downstate Medical Center, Brooklyn, NY; 2State University of New York, University of Buffalo, Buffalo, NY.

Purpose: Cyclin D-cdk4 (DK4) has been a highly sought after therapeutic target because it drives cancer proliferation in a majority of human tumors. We have explored the clinical utility of a recently discovered mechanism of cell cycle control exerted on DK4 by p27Kip1 and its activator, the Breast tumor Related Kinase (Brk), in predicting responsiveness to therapy and as a new target for treatment. Although known as a DK4 assembly factor and cdk2 inhibitor, p27 also acts as a DK4 ON/OFF switch. Tyrosine (Y) phosphorylation of p27 (pY) by Brk gatekeeps both ATP binding and CAK phosphorylation of cdk4’s T loop, essential for DK4 activation. This function is restricted to cdk4-p27 association with cdk2, whether Y phosphorylated or not, appears to be inhibitory. However, in vivo Y phosphorylated p27 is a target for cdk2-dependent ubiquitin-mediated degradation, reducing p27’s association with cdk2, indirectly activating this complex. We showed that blocking p27 pY inactivates cdk4 directly AND cdk2 indirectly, and thus represents a novel way to block cancer cell proliferation. pY also serves as a predictive biomarker of cdk4 activity and tumor response. Methods: We used a small peptide, ALT, which contains a portion of Brk’s SH3 domain. ALT binds to p27, blocks Brk’s association and ability to phosphorylate p27, inhibiting cdk4 and increasing p27’s ability to inhibit cdk2. We engineered a lipid-based nanoparticle delivery vehicle (NP-ALT), permitting us to test ALT as a first generation therapeutic in breast cancer cell lines that were both responsive and non-responsive to cdk4 therapy. ALT was also used with Palbociclib to determine if combination therapy reduced drug resistance. We developed a dual IHC assay for p27 and pY, which we used to analyze paraffin-embedded, archival human tumor samples, to determine whether we could pinpoint patients who would have responded to cdk4 inhibition therapy. Results: NP-ALT blocks pY, cdk4 and cdk2 activity, and proliferation in both Palbociclib sensitive and resistant cell lines. As a dual therapy, ALT treatment synergized with Palbociclib to ablate cells for >30 days, increased senescence, and in animal models caused tumor regression instead of just slowing tumor growth as seen with Palbociclib alone. Analysis of human cancer, obtained from archival sources, demonstrated that pY is never detected in quiescent benign mammary tissue, but is detected in about half of the advanced ER/PR+ /Her2- tumors analyzed, and using explant culture techniques, we were able to stratify pY with Palbociclib response. Conclusion: Use of an Brk SH3 based peptide (NP-ALT) has proven effective in blocking p27 pY, inhibiting both cdk2 and cdk4, inducing senescence and increased durability. pY levels correlate with Palbociclib sensitivity in low, moderate and non-responders, suggesting that this may be a biomarker highlighting responsiveness to cdk4 therapy.

#2349 Sensitivity and resistance to cell cycle and IGF-1R inhibitors in rhabdomyosarcoma. Justin Montoya, David W. Lee, Eiman Alem. Institute of Molecular Medicine, Medicine, and Pharmacology, Boston, MA

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children and young adults. The five-year survival rate for RMS has hardly improved over the last three decades despite intensive and toxic chemotherapy, radiotherapy and surgery. Therefore, novel treatment approaches are required to change these outcomes. RMS has two major subtypes, embryonal RMS (ERMS) and alveolar RMS (ARMS), the most aggressive subtype, is characterized by PAX3-FKHR translocations that fuse two transcription factor-encoding genes; creating novel PA3X/PA7-FOXO1 fusion proteins. The PA3X-FOXO1 is highly expressed in the G2 phase of the cell cycle, allowing the cell to divide following a sustained checkpoint arrest despite DNA damage induced by chemotherapy, suggesting that PA3X-FOXO1 may enhance the survival of tumor cells in response to chemotherapy. Many cell cycle regulators are altered in RMS, including CDK2, CDK4 and p53. Furthermore, RMS cells are highly dependent on the insulin-like growth factor -1 receptor (IGF-1R) signaling, however, IGF-1R targeting was not successful in the clinic. Therefore, targeting key cell cycle regulators individually or in combination with IGF-1R inhibition may expand the available therapeutic options for RMS. Purpose: The goal of the present study was to investigate the cytotoxicity of 15 small molecule inhibitors targeting the IGF-1R and cell cycle regulators in RMS cell lines and to determine potential mechanisms of drug sensitivity or resistance. Methods and Results: Seven RMS cell lines including ERMS and ARMS were studied. The IC50 values were determined for the following targeting compounds: Imitinib, BMS-754807 and picopodocellphilmycin (PPP) (IGF-1R), ribociclib and palbociclib (CDK4/6), dinaciclib and flavopiridol (CDK4/6 and IGF-1R) and MK-7724 (CHK1), alisertib (AURKA) and volasertib (PLK1). The most potent compounds with ICG<10 nM were dinaciclib and alisertib. The ARMS cell lines were resistant to alisertib in comparison to the ERMS cell lines. Most cell lines were sensitive to flavopiridol, MK-1775, BMS-754807 and PPP with ICG<100 nM. The most resistant was RS-181 HCL, resistant to B5-181 HCL, resistant to B5-181 HCL and MK-7724 with ICG(1-70 µM). Palbociclib and BMS-754807 showed a synergistic effect in some RMS cell lines. Ongoing studies are focusing on determining the mechanisms of interaction of these two compounds through studying cell cycle, apoptosis, and mRNA and protein expression of key regulators in the IGF-1R and RB pathways. Conclusion: These data demonstrate that dinaciclib, volasertib, flavopiridol, MK-1775, BMS-754807 and PPP are highly toxic in RMS cell lines. The CDK4/6 inhibitor palbociclib may sensitize selected RMS cell lines to IGF-1R inhibitors. Targeting selected cell cycle regulators individually, or in combination with IGF-1R inhibitors may thus provide an efficacious treatment approach to be further validated in RMS patients with poor outcome.

#2350 In vivo E2F reporting on efficacious dosing schedules of MEK plus CDK4/6 inhibition in melanoma. Jessica Teh,1 Neda Nikbakht,1 Timothy Purtin,1 Inna Chernova,1 Priyank Patel,1 Michael Davies,2 Andrew Aplin1. Thomas Jefferson University, Philadelphia, PA; 2MD Anderson Cancer Center, Houston, TX.

Pharmacological targeting of cyclin dependent kinases 4 and 6 (CDK4/6) could represent a viable therapeutic option in combination with BRAF and/or...
MEK inhibitors in different genetic subsets of melanoma. Indeed, continuous and concurrent dosing of MEK inhibitor (MEKi) plus a CDK4/6 inhibitor (CDK4/6i) leads to melanoma regressions in in vivo models and delays the onset of MEKi resistance. Current scheduling in breast cancer patients for the CDK4/6i, palbociclib, is intermittent due to adverse effects resulting in neutropenia. It is unclear what the most efficacious schedules are of palbociclib plus a MEKi. Utilizing an E2F reporter system, we sought to analyze the efficacy of different CDK4/6i plus MEKi schedules in a quantitative and temporal manner. Intermit- tent dosing (3 weeks on/1 week off) of both CDK4/6i and MEKi combination therapy resulted in tolerant tumors and rapid reactivation of E2F activity during drug holiday. Continuous MEKi with intermittent CDK4/6i led to more complete responses as compared to continuous CDK4/6i with intermittent MEKi. Weight loss of mice was also evident in the continuous CDK4/6i plus intermittent MEKi arm suggesting adverse events related to continuous CDK inhibition. Importantly, functional proteome analysis revealed distinct mechanisms of acquired resistance/drug tolerance that arose from the three scheduling arms. Here, we report that upregulation of S6 phosphorylation is associated with acquired resistance to MEK plus CDK4/6i inhibition and can be overcome with an mTOR inhibitor. Taken together, in vivo reporting allows for quantitative measurement of pathway activity associated with inhibitor resistance and can be utilized to optimize combination schedules to improve the therapeutic index in patients.

#2351 The novel potential of palbociclib (CDK4/6i inhibitor) in the treat- ment of triple-negative breast cancer. Yuka Asano, Shinichiro Kashiwagi, Wa- taru Goto, Katsuyuki Takahashi, Tsutomu Takahama, Saburo Noda, Noriyuki Onoda, Shuhei Tomita, Kosei Hirakawa, Masaochi Ohira. Osaka City University Graduate School of Medicine, Osaka, Japan.

Background: The effectiveness of palbociclib (CDK4/6i inhibitor) for estrogen receptor positive breast cancer has been demonstrated by large-scale clinical studies, with the drug garnering attention as a key drug for breast cancer sub- types with endocrine insensitivity in the future. According to PALOMA-3 trial, palbociclib has been demonstrated to contribute to the extension of progression-free survival in patients with advanced hormone receptor-positive and HER2-negative metastatic breast cancer after endocrine therapy. On the other hand, in the case of triple-negative breast cancer (TNBC), luminal AR (LAR) related to androgen signaling is believed to have endocrine activity. Previous clinical data revealed that palbociclib shows high sensitivity in luminal breast cancer cell lines with endocrine activity, with effectiveness also expected in LAR.

In this study, we created TNBC cell lines that forcibly express AR and examined the effectiveness of palbociclib for TNBC. Materials and Methods: MCF-7 and T-47D were used as luminal breast cancer cell lines, while MDA-MB-231 and BT-549 were used as TNBC cell lines. In addition, we created TNBC cell lines that forcibly express AR, called AR-MDA-MB-231, by the transfection of pEGFP-C1-AR Plasmid Vector using Lipofectamine® 3000 Reagent. We con- firmed the expression of AR by qRT-PCR as well as Western blotting and exam- ined the impact of palbociclib on proliferation as well as apoptosis of breast cancer cell lines. Results: AR was found to be more expressed only in luminal breast cancer cell lines but not TNBC cell lines. It was confirmed that AR was expressed in both MDA-MB-231 which are stable cell lines with the properties of LAR. In a CCK assay, palbociclib showed high sensitivity in AR-MDA-MB-231 as in luminal breast cancer cell lines. Furthermore, in an apoptosis assay using FACS and cell cycle assay, apoptosis was induced in AR-MDA-MB-231 and cell cycle arrest at the G1/S check point was confirmed. Conclusion: palbociclib (CDK4/6i inhibitor) showed effectiveness for TNBC cell lines that compulsively express AR, suggesting it may be one treatment option for TNBC in the future.

#2352 Effect of inhibition of cell cycle versus transcription cyclin-depen- dent kinases (CDKs) in ovarian cancer cells. Rosaria Chilia, Nicola Panini, Eugenio Erba, Giovanna Damia, Massimo Brogini. IRCCS - Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy.

Background: Cell cycle is regulated by cyclin-dependent kinases (CDKs) ac- tivity, which can lead to uncontrolled proliferation and cancer. Other CDKs are engaged in the regulation of transcription and post-transcrip- tional mRNA processing through the phosphorylation of the C-terminal do- main of RNA polymerase II, such as CDK9 and CDK12. Inhibitors of cell cycle CDKs have been developed as anticancer agents and some of them are under clinical validation (e.g. palbociclib). While recent data would suggest that inhi- bition of CDK9 is feasible and has antitumor effect, the data on the therapeutic role of CDK12 inhibition are very scanty. Methods: Ovarian cancer cell lines were maintained in RPMI medium supplemented with 5% glutamine and 10% FBS. Cells were treated using drug concentrations and after 72 hours cell survival was evaluated by MTS assay (Promega). IC50 values were calculated by interpolation method. Cell cycle analysis and apoptosis were performed with standard flow cytometric methods. A2780 and SKOV3 ovarian cancer cell lines knocked out for CDK12 were generated with CRISPR/CAS9 genome editing tool.

Results. The cytotoxicity of palbociclib (a CDK4/6 inhibitor) and LDC000067 (a CDK9 inhibitor) was tested in a panel of ovarian cancer cell lines (A2780, SKOV3, OVCAR3, OVCAR5, OVCAR8, OVC432, OVC433, IGROV1, EFO27). Sensitivity of cells was similar for palbociclib and LDC000067, ranging from 10 to 33 and from 8 to 60 μM, respectively. A prefer- ential G1 block was observed with palbociclib, while LDC000067 caused a S-G2 block. A higher induction of apoptosis was observed after LDC000067 than after palbociclib treatment in both A2780 and SKOV3. The palbociclib-induced G1 block was associated with decreased Rb phosphorylation, while no modulation was observed in the Ser2 in the carboxyterminal domain of RNA polymerase II was observed after LDC000067 treatment. We generated CDK12 knockd out cells transfecting CRISPR/CAS9 engineered plasmid in both A2780 and SKOV3 ovarian cancer lines. The biological and pharmacological characterization of these clones is under study. Conclusions: Palbociclib and LDC000067 showed a dose dependent cytotoxic effect in the panel of ovarian cancer cell lines tested and were active in the μM range. Preliminary data of treatment induced cell cycle perturbation and apoptosis suggest that the two drugs behave in a different manner and have distinct molecular effects on cells.


Cyclin D dependent kinases CDK4 and CDK6 are crucial regulators of the G1 to S phase transition of the cell cycle. The fact that myriad cancer types show abberance in INK4-CDK4/6-cyclin D-Rb-E2F pathway, and the rapidly emerging clinical pharmacological evidence of most validated CDK4/6 as anticancer drug targets. As the first commercialized CDK inhibitor, palbociclib in combination with bevacizumab or fulvestrant has received regulatory ap- proval for the treatment of breast cancer. This represents an important scientific advance in the field. However, the limited structural diversity and undesired side effects due to broader kinase interactions of existing inhibitors mean that the hunt for new and highly selective CDK4/6 inhibitor drug candidates continues.

Using our advanced medicinal chemistry, targeted biochemical and cell-based assays, and animal pharmacology, we synthesized and evaluated a novel series of inhibitors. Many compounds were highly potent and selective against CDK4/6, & exhibited low nanomolar potency against a range of human cancer cell lines. Notably, inhibition of CDK4/6 by compound CDKI-15 (KICD4/6 = 4 nM) was over 3 orders of magnitude greater than CDK1B, CDK2A, CDK7H and CDK9T1. In- terrogation of a panel of 369 protein kinases revealed CDKI-15 to be highly selective for CDK4/6 with only 3 other kinases inhibited potently. CDKI-15 reduced the level of Rb phosphorylation and induced G1 cell cycle arrest, con- firming cellular inhibition of CDK4/6 in cancer cells. Moreover, CDKI-15 poss- esses superior pharmacokinetic profile with oral bioavailability of 100% in mice. Treatment of nude BALB/c mice bearing human MV4-11 acute myeloid leukemia xenograft with CDKI-15 resulted in 100% inhibition of xenograft growth, with a 30% tumor regression, and a 50% overall survival. CDKI-15 resulted in a robust inhibition of tumor growth compared to vehicle treated animals (T/C = 30%, p < 0.00001). Strikingly, CDKI-15 caused a complete and sustained tumor regression in one-third of the animals. No detectable toxicity was observed in the animals during and post treatment. Taken together, our data provide a rationale for CDKI-15 to be developed towards clinic for cancer therapy.

#2354 Identification of CDK1 as an aspargin and salicylic acid binding protein: a potential role in chemoprevention. Jayarama B. Gunaje,1 D.Ramesh Kumar,2 Siddharth Kesharwani,1 Eduard Callegari,3 Hemachand Tummalad,4 Rakesh Dachini,5 Frank Lam, Minfeng Yu, Shudong Wang. 1South Dakota State Univ. College of Pharmacy, Brookings, SD; 2University of Kentucky, Lexington, KY; 3University of South Dakota, Vermillion, SD.

Palbociclib, a novel and highly selective CDK4/6 inhibitor, has been shown to be an effective therapeutic agent in preclinical models and clinical trials of the past 10 years have consolidated the rationale for investigating the use of aspirin for chemoprevention; however, the mechanisms leading to its anti-cancer effects are still being elucidated. We hypothesized that aspirin’s ability to exert chemopreventive effects may involve altering the levels and activity of cell cycle regulatory proteins. In the present study, using HT-29 colon cancer cell lines and other cancer cell lines, we demonstrated that both aspirin and salicylic acid downregulated the protein and mRNA levels of cyclin B1 and cyclin dependent kinase-1 (CDK1). Lactacystin, a 26S proteosomal inhibitor, prevented aspirin and salicylic acid mediated degrada- tion of cyclin B1, but not CDK1. Decrease in protein levels of cyclin B1/CDK1 was correlated with a corresponding decrease in CDK1 kinase activity. Molecu-
lar docking studies showed that aspirin and salicylic acid independently can dock on CDK1 through interactions with Leucine 83. Incubation of recombinant CDK1 with aspirin resulted in acetylation at lysine residues, this was also observed in cell culture experiments. Pre-incubation of CDK1 with salicylic acid dose dependently prevented aspirin’s ability to acetylate CDK1 in purified preparations confirming the data obtained from molecular docking studies. Our results show that CDK1 is a salicylic acid binding protein (SABP) and the chemopreventive actions of aspirin may involve modulation of levels and activity of cyclin B1 and CDK1.

#2355 Palbociclib enhances the antitumor activity of taxanes by abrogating cell cycle checkpoints and alleviating hypoxia in squamous cell lung cancer.
Joan Cao,1 Zhou Zhu,1 Hui Wang,1 Tim Nichols,1 Edward Rosfjord,1 Christine Hopel,2 Erik Upselson,2 Paul Rejto,2 Scott Weinrich,3 Todd Vanardas,1 James Hardwick,3 Ping Wei1.1 Pfizer, Inc., San Diego, CA; 2Pfizer, Inc., Pearl River, NY.

Lung cancer remains one of the leading causes of cancer-related mortality. Squamous cell lung cancer (SqCLC) is the second most common subtype of non-small cell lung cancer (NSCLC) and is responsible for ~100,000 deaths in the US and EU. Most SqCLC patients receive chemotherapy as first-line treatments and have a high unmet medical need for new therapies. Therapeutic approaches that enhance the efficacy of chemotherapy would therefore improve clinical outcomes for this patient population. CDK inhibitors comprise a class of drugs that target the dysregulated cell cycle in malignant cells. Treatment of tumor cells with the CDK4/6 inhibitor palbociclib inhibits tumor growth by down-regulating retinoblastoma (RB) protein phosphorylation and inducing cell cycle arrest at the G1/S phase transition. Based on promising clinical trial results, palbociclib in combination with letrozole was granted accelerated approval by the US FDA for the treatment of postmenopausal women with ER-positive, HER2-negative advanced breast cancer. Like hormone receptor positive breast cancer patients, the vast majority of SqCLC patients harbor wild type RB and HER2-negative advanced breast cancer. Like hormone receptor positive breast cancer patients, the vast majority of SqCLC patients harbor wild type RB and HER2-negative advanced breast cancer. Like hormone receptor positive breast cancer patients, the vast majority of SqCLC patients harbor wild type RB and HER2-negative advanced breast cancer. Like hormone receptor positive breast cancer patients, the vast majority of SqCLC patients harbor wild type RB and HER2-negative advanced breast cancer. Like hormone receptor positive breast cancer patients, the vast majority of SqCLC patients harbor wild type RB and HER2-negative advanced breast cancer. Like hormone receptor positive breast cancer patients, the vast majority of SqCLC patients harbor wild type RB and HER2-negative advanced breast cancer. Like hormone receptor positive breast cancer patients, the vast majority of SqCLC patients harbor wild type RB and HER2-negative advanced breast cancer. Like hormone receptor positive breast cancer patients, the vast majority of SqCLC patients harbor wild type RB and HER2-negative advanced breast cancer. Like hormone receptor positive breast cancer patients, the vast majority of SqCLC patients harbor wild type RB and HER2-negative advanced breast cancer. Like hormone receptor positive breast cancer patients, the vast majority of SqCLC patients harbor wild type RB and HER2-negative advanced breast cancer. Like hormone receptor positive breast cancer patients, the vast majority of SqCLC patients harbor wild type RB and HER2-negative advanced breast cancer. Like hormone receptor positive breast cancer patients, the vast majority of SqCLC patients harbor wild type RB and HER2-negative advanced breast can...
cell cycle regulation in LNCaP-MST cells with and without nutlin-3 treatment. Our study clearly demonstrated a significant increase in the expression level of Aurora Kinase B (AURKB), CDC25C and CDK1 in MDM2 transfected LNCaP-MST cells as compared with non-transfected LNCaP cells. However, after treating the cells with 20 \mu M of MDM2 specific inhibitor nutlin-3, for 24 h, the expression levels of the above mentioned proteins were significantly altered when compared to untreated controls. In addition, inhibition of MDM2 with nutlin-3 leads to increased expression of pro-apoptotic proteins p53, p21, and Bax. Our results offer significant evidence towards the effectiveness of MDM2 inhibition in causing cell cycle arrest via blocking the transmission of signals through AURKB-CDK1 axis and inducing apoptosis in cancer cells. It is clearly evident from our data that MDM2 overexpression probably is the primary cause for CDK1 up-regulation in the LNCaP-MST cells, which might have occurred possibly through activation of AURKB. However, further studies in this direction should shed more light on the intracellular mechanisms involved in the regulation of CDK1 in MDM2 positive cancers. (This project was supported by The Royal Dames of Cancer Research Inc., Ft. Lauderdale, Florida).

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: CDKs and CDK Inhibitors

#2359 Inhibition of the ATR kinase enhances therapeutic efficacy of cisplatin in ATM low uterine carcinosarcoma cells. Emily R. Penick, Paulette Mhawech-Fauceglia, Nicholas Bateman, Kelly Conrads, Tracy Litzi, Chun-platin in ATM low uterine carcinosarcoma cells. Mhawech-Fauceglia, 2 Nicholas Bateman, 1 Kelly Conrads, 1 Tracy Litzi, 1 Chun-pation should shed more light on the intracellular mechanisms involved in the possibly through activation of AURKB. However, further studies in this direc-

#2360 Regulation of the MAPK phosphatase MKP-1 by the E3 ligase GP78 in cancer cells. Dhonghyo Kho, Gen Sheng Wu. Wayne State Univ. School of Medicine, Detroit, MI.

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Molecular and cellular biology / genetics: CDKs and CDK inhibitors

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MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Cell Growth Signaling Pathways 5

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Proceedings of the American Association for Cancer Research • Volume 58 • April 2017
#2363 Targeting Src-family kinases to combat acquired inhibitor resistance in FLT3-ITD AML. Ravi K. Patel, Mark Weir, Sabine Hellwig, Heather Dormann, Thomas E. Smithgall. University of Pittsburgh, Pittsburgh, PA.

Activating internal tandem duplication (ITD) mutations in the FMS-like receptor tyrosine kinase 3 (Flt3) are found in approximately 30% of acute myeloid leukemia (AML) patients. Flt3-ITD expression is associated with aggressive, chemotherapy-resistant disease and decreased overall survival. Multiple selective Flt3 kinase inhibitors have been developed as targeted therapy for Flt3-ITD AML. However, the clinical utility of these kinase inhibitors has been limited by the rapid recurrence of drug-resistant disease that often results from Flt3 kinase domain mutations that prevent inhibitor action. Recent studies revealed that multiple non-receptor tyrosine kinases are over-expressed in AML, including the tyrosine Src-family kinases Hck, Lyn and Fgr, and may cooperate with Flt3 to drive disease. These observations suggest that kinase inhibitors with specificity profiles targeting Flt3 and these AML-associated cytoplasmic kinases may be less prone to acquired resistance. In the present study, we determined the contributions of Hck and Fgr kinase activities to Flt3-ITD AML cell growth using a chemical genetics approach. While RNAi-mediated knockdown of each of these kinases reduces proliferation and increases apoptosis in primary AML cells, the impact of pharmacologic inhibition of their kinase activities on AML cell growth is not known. Because no selective Hck or Fgr inhibitors exist, we developed kinase domain mutants that desensitize each kinase to the broad-spectrum Src-family kinase inhibitor, A-419259. Examination of a crystal structure of Hck with A-419259 bound to the active site suggested a role for the ‘gatekeeper’ residue in compound binding. Substitution of the gatekeeper threonine of Hck and Fgr with methionine conferred 3-fold resistance to A-419259 in vitro without diminishing kinase activity. When expressed in human TF-1 myeloid cells transformed with Flt3-ITD, these inhibitor-resistant Hck and Fgr mutants conferred resistance to growth inhibition by A-419259 to a similar extent, while expression of the corresponding wild-type kinases was without effect. The sensitivity of the TF-1/Flt3-ITD cell lines to A-419259 correlated with inhibition of mutant Hck and Fgr kinase activity in the cells. Our findings suggest that suppression of AML cell growth by A-419259 is due in part to inhibition of Hck and Fgr, and identify these AML-associated kinases as therapeutic targets. Ongoing studies are addressing the role of Hck and Fgr as inhibitor targets in Flt3-ITD AML cell lines and patient samples as well as the propensity of A-419259 and other multi-targeted inhibitors of AML-associated kinases for acquired drug resistance.


Type III receptor tyrosine kinases (RTKs) including FLT3 and KIT play a major role in cell differentiation, proliferation, and survival of hematopoietic stem cells. FLT3-ITD and KIT-D816V mutations are the most common oncogenic mutations in FLT3 and KIT found in hematological cancers. These mutations lead to constitutive activation of proliferative and survival signals. Tyrosine kinase inhibitors (TKIs) in combination with chemotherapy display promising results in a clinical setting, but patients develop resistant disease after short-term response. Protein substrates that regulate the activity of FLT3 will be alternative targets for patients carrying these mutations. Activation of FLT3 and KIT results in phosphorylation on several tyrosine residues that recruit SH2 domain-containing signaling proteins. In this study we identified Src-like adaptor protein 2 (SLAP2) as a potent FLT3 and KIT interacting protein. The interaction requires an intact SH2 domain of SLAP2 as well as phosphorylation of the receptor. Overexpression of SLAP2 in murine proB/Ba/F3 cells inhibited oncogenic FLT3-ITD-mediated cell proliferation and colony formation. SLAP2 displayed a similar inhibitory potential in cells expressing KIT-D816V. SLAP2 partially blocked phosphorylation of several FLT3 and KIT downstream signaling proteins such as AKT, ERK and p38. Moreover, SLAP2 expression inhibited FLT3-ITD-mediated STAT5 phosphorylation and KIT-D816V-mediated STAT3 phosphorylation. SLAP2 expression significantly accelerated ubiquitination-mediated degradation of FLT3 and KIT. Collectively, these data suggest that SLAP2 negatively regulates FLT3 and KIT signaling and therefore, modulation of SLAP2 expression levels may become a potential target for anticancer therapy.

#2365 Dual inhibition of the PI3K/Akt and MEK5/ERK5 pathways in tamoxifen resistant breast cancer. Thomas D. Wright, Mahmoud Hasan, Paula Witt-Enderby, Jane Cavanaugh. Duquesne Univ. School of Pharmacy, Pittsburgh, PA.

Approximately 70% of breast cancers are considered estrogen receptor alpha (ER) and/ or progesterone receptor (PR) positive, and their growth is primarily driven by the hormone estrogen. Initially these cancers are responsive to endocrine therapies such as tamoxifen. However, over time patients become resistant to conventional endocrine therapy and treatment becomes more difficult. Tamoxifen resistance occurs due to several mechanisms: loss of ER expression, gain of function mutations, pharmacological tolerance, and co-inhibition of other tyrosine kinases by various kinase cascades. Aberrations in the Phosphoinositide-3-kinase (PI3K) and Mitogen Activated Protein Kinase (MAPK) pathways have been linked to increased breast cancer proliferation and survival. It has been proposed that these survival characteristics are enhanced through compensatory signaling and crosstalk mechanisms. The crosstalk between PI3K/Akt and MEK5/ERK5 has been shown in several breast cancer cell lines. However, new evidence suggests that MEK5/ERK5, a member of the MAPK family, is a key component in the proliferation and survival of therapy resistant cancers. MEK5/ERK5 has been shown to promote ER alpha driven transcription in ER+ breast cancers and act reorganization and metastasis in ER+ breast cancers. Furthermore, MEK5/ERK5 promotes hormone independent tumorogenesis in breast cancer. Our lab has previously investigated these pathways in hormone independent breast cancers, TNBC (triple negative breast cancer). Our previous results indicate that combinations of PI3K/Akt and MEK5/ERK5 blockade are promising because they inhibit both the pro-proliferative and pro-metastatic pathways in TNBC. Additionally, PI3k/Akt and MEK5/ERK5 inhibition was shown synergistically reduce viability in a range of cell lines (HTNCB, EBC+, and Triple Positive). Based on these initial results we hypothesize that PI3k/Akt and MEK5/ERK5 inhibition decreases viability through a hormone independent mechanism: perhaps by reducing the cytokolic sequestration of Bad. In this study we investigate the utility PI3k/Akt and MEK5/ERK5 inhibition in a MCF-7 TamR cell line. The goal of our study is to elucidate the roles of PI3k/Akt/mTOR and MEK5/ERK5 in endocrine resistant breast cancer and broaden the scope of a dual inhibition strategy.
MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Cell Growth Signaling Pathways 5

#2367 Identification of ALK alternative transcription initiation in BRAF-negative metastatic melanoma patients. Shan Zhong,1 Christine Lovly,2 Christopher Malbone,1 Kristina Brennan,1 Douglas Johnson,1 Kelsie Rienschneider,2 Catherine Meador,2 Emily White,2 Geoff A. Otto,2 Doron Lipson,2 Philip Stephens,2 Vincent Miller,2 Jeffrey Ross,1 Jie He,1 Foundation Medicine, Inc, Cambridge, MA;2Vanderbilt University, Nashville, TN.

Background: Genetic alterations in the ALK tyrosine kinase, such as copy number gains, point mutations, and ALK gene fusions, have been described in a broad spectrum of malignancies. Recently a novel mechanism of ALK activation in melanoma was discovered in which ALK transcription was initiated from a de novo alternative transcription initiation (ATI) site in ALK intron 19 (ALK-ATI) [1]. In this study, whole transcriptome sequencing (WTS) re-sequenced a cohort of clinical melanoma specimens in order to identify those that express ALK-ATI. Method: RNA-seq libraries of 33 BRAF-negative clinical metastatic melanoma specimens were prepared using the KAPA Stranded RNA-Seq Kit with RiboErase with 200ng RNA as input and sequenced on Illumina HiSeq 4000 at 2x75bp. The specimens were sequenced to an average of 69.4 million total read pairs with 11.3m on-target distinct read pairs. Sequencing reads were mapped to the human genome and transcriptome, gene- and exon-level expressions were quantified. An algorithm to identify the ALK-ATI was developed based on assessing 1) transcription of intron 19 putative ATI region, 2) ALK relative RNA expression and 3) ALK 3′ to 5′ differential expression. In addition, immunohistochemistry (IHC) staining for ALK (using DSF3 antibody) was done on 10 of the 33 specimens to confirm the expression of ALK-ATI with the whole transcriptome sequencing (WTS) results. Results: Two of the ALK IHC positive cases harbor the highest ALK expression, and the other two ALK-ATI positive cases are ALK IHC weak positive. ALK-ATI occurred in cutaneous, mucosal, and unknown primary melanoma, and co-occurred with other driver mutations (NRAS, NFI, KIT). None of the above specimens had ALK rearrangements detected from DXNaseq (by FoundationOne) or WTS. Conclusions: Our findings confirmed previously reported ALK activation through ALK-ATI in an independent melanoma cohort, and provided direct evidence that such events can be detected using whole transcriptome sequencing and align with IHC results in clinical FFPE tumor specimens. This confirmation together with future functional validation and clinical evidence may provide new therapeutic opportunities for BRAF-negative metastatic melanoma patients. I. Wiesner, T. et al. Alternative transcription initiation leads to expression of a novel ALK isoform in cancer. Nature 526, 453–457 (2015).

#2368 Differential role of Pik3caH1047R and PtenH103 mutation in thyroid cancer development. Michela Ranieri, Antonio Di Cristofano. Albert Einstein College of Medicine, Bronx, NY.

Anaplastic thyroid cancer (ATC) is one of the most lethal malignancies, with a median survival of less than 6 months from time of diagnosis. Molecular changes that characterize ATC involve most often p53 loss or inactivation (up to 80%), activation of the PI3K cascade through PTEN loss (12%) and PIK3CA activating mutations (20%) or gene amplification (30-60%), RAS family members activation (6-50%), and BRAF activation (25-50%). Studies performed in endometrial and breast cancer suggest that although PI3K activating mutations and PTEN loss of function both enhance P13K signaling, these mutations are not equivalent. We have tested whether Pik3caH1047R and PtenH103 mutations have the same impact on thyroid cancer development and if these two mutations differ in their ability to cooperate with additional genetic alterations, such as p53 deletion and the KrasH12 mutation. We have generated a series of mouse models and cell lines carrying thyroid-restricted p53−/−, KrasH12, Pten−/−, and Pik3caH1047R alleles. Analysis of single and compound mutants has revealed important and striking differences between Pten and PIK3CA abilities to cooperate with additional genetic alterations, such as Cfl1, Actb, and Tubb6, we observed that Pik3caH1047R mice have the potential to develop thyroid hyperplasia and subsequent transformation than Pten−/− mice, even though the two mutations appear to have similar ability to induce AKT activation. This differential activity correlates with lower levels of pAkt found in the thyroids of Pik3caH1047R mice, compared to that of Pten−/− mice. We also observed that Pten loss synergizes with Trp53 loss to induce anaplastic thyroid cancer, activation of Pik3caH1047R leads to the development of a smaller and well differentiated tumors. On the other hand, Pten loss and Pik3caH1047R are equivalent in inducing poorly differentiated tumors in the presence of a KrasH12 mutation. However, KrasH12, Pik3caH1047R cell lines are more sensitive to PI3K, MEK and combined PI3K/MEK inhibition than KrasH12, Pten−/− cell lines. Accordingly, tumors developing in mice carrying the KrasH12, Pik3caH1047R alleles are more sensitive than KrasH12, Pten−/− mice to a dual PI3K/mTOR inhibitor as well as a MEK inhibitor. All together these results suggest that Pten loss has a stronger impact than Pik3ca activation to thyroid cancer development and resistance to small molecule inhibitors.

#2369 Transcription activators that regulate PKC-i expression and are downstream targets of PKC-i. Andre H. Apostolatos, Wishrawana S. Ratnayake, Tracess Smalley, Anisul Islam, Mildred Acevedo-Duncan. University of South Florida, Tampa, FL.

Protein Kinase C-iota (PKC-i) is an anti-apoptotic oncogene over-expressed in multiple cancers including prostate, ovarian, and glioma. PKC-i is part of a cycle that helps cancer cell avoid senescence by releasing the transcription factor NFκB and promoting apoptotic resistance. PKC-i is activated extracellularly by factors such as loss of PTEN (Paget 2012). However, while under the effect PKC-i specific inhibitors, expression levels decreased, suggesting PKC-i regulates a role in regulating its own expression. A previous study showed the ELK1 transcription factor to be a regulator of PKC-i (Gustafson 2003). Other transcription factors including Jun, IGF3, PAX3, EGRI, and FOXO1 bind on or near the promoter sequence of the gene and their role in PKC-i regulation was analyzed. Each transcription factor was systematically silenced with its own siRNA. Western Blotting revealed expression of PKC-i in the transcription factor silenced cells determining which transcription factors are key players in regulating PKC-i. Each siRNA was performed to analyze the transcription of treated cells to match protein levels with mRNA levels. Targets both up and downstream of PKC-i were analyzed to find the pathway that PKC-i uses to help regulate itself.

#2370 Depletion of p21-activated kinase 4, PAK4, inhibits cellular proliferation, motility and clonogeneity in oral squamous cell carcinoma. Felicia F. Chong,1 Si-Hoe Y. Tan,1 Vijay J. Raju,2 Pei Yuen Ng,1 Ian C. Paterson,4 Chye Ling Tan,1 Chee-Onn Leong,1 International Medical University, Kuala Lumpur, Malaysia;2 Weil Cornell Medical College, New York, NY;3 The National University of Malaysia, Kuala Lumpur, Malaysia;4University of Malaya, Kuala Lumpur, Malaysia;Agency for Science, Technology and Research (A*STAR), Singapore, Singapore.

Oral squamous cell carcinoma (OSCC) accounts for 90% of all malignancies in the oral cavity, and is often associated with low survival and poor prognosis. While considerable progress in OSCC treatment has been made, options for targeted therapy remain limited. Thus, it is imperative that new druggable targets are identified and characterized. p21-activated kinase 4 (PAK4) is a member of the PAK family of serine/threonine kinases, which are known modulators of a diverse range of oncogenic pathways including cell proliferation, escape from apoptosis, and anchorage-independent growth. Previous reports have indicated that PAK4 is overexpressed in a subset of invasive OSCC, and is highly associated with poor prognosis. However, the specific role of PAK4 in OSCC has yet to be elucidated. Here, we show that RNAi-mediated depletion of PAK4 conferred reduced clonogeneity, cell motility, and cell proliferation in OSCC cell lines in a cell-line-dependent manner. Global proteomic profiling of PAK4-depleted H103 and H357 cells was conducted to identify the molecules and signaling pathways responsive to PAK4 depletion. In addition to reported targets of PAK4 such as CFL1, ACTB, and TUBB6, we observed that p16INK4a, pre-mRNA splicing factors (SRFS2, SRFS5, SRFS6, SL7U), and proteasome activator subunits (PSME1-4) were dysregulated as a result of PAK4 depletion. These results indicate that PAK4 plays a role in promoting OSCC progression by regulating multiple signaling pathways. Of note, there have yet to be any reports describing interactions between PAK4 signaling and mRNA splicing or proteasome activation. Further investigation is thus warranted to further delineate the potential role of PAK4 in regulating these targets.

#2371 Oncogenic transformation of FLT3-ITD is partially dependent on signaling through the PI3K pathway. Tun-Tao Lu,1,2 Hidetoshi Takahashi,3 Eslam Nazarian,3,4 A. Ammar M. Makri,1,5 Andre H. Apostolatos, Wishrawana S. Ratnayake, Tracess Smalley, Anisul Islam, Mildred Acevedo-Duncan, University of South Florida, Tampa, FL.

About 30% of AML patients carry an oncogenic mutant of the type III receptor tyrosine kinase FLT3. Among the various mutations in FLT3, the internal tandem duplication (ITD) mutations in the juxtamembrane domain are the most common type of mutation, while point mutations in the kinase domain also occur. Although oncogenic FLT3 mutant cells are constitutively active, the wild-type receptor needs its ligand (FLT3 ligand, FL) for activation. Ligand binding to the receptor induces dimerization, auto-phosphorylation, and activation of the receptor. Tyrosine phosphorylation sites on FLT3 constitute docking sites for RTK-specific signaling factors (SRSF2, SRSF5, SRSF6, SL7U), and proteasome activator subunits (PSME1-4) which are regulated by the ELK1 transcription factor to be a regulator of PKC-i (Gustafson 2003). Other transcription factors including Jun, IGF3, PAX3, EGRI, and FOXO1 bind on or near the promoter sequence of the gene and their role in PKC-i regulation was analyzed. Each transcription factor was systematically silenced with its own siRNA. Western Blotting revealed expression of PKC-i in the transcription factor silenced cells determining which transcription factors are key players in regulating PKC-i. Each siRNA was performed to analyze the transcription of treated cells to match protein levels with mRNA levels. Targets both up and downstream of PKC-i were analyzed to find the pathway that PKC-i uses to help regulate itself.
interacting proteins that transduce pro-survival and proliferative signals. The so-called activation loop tyrosine residue (in FLT3, Y842) is well conserved in all receptor tyrosine kinases and long been known essential for the activity of some but not all receptor tyrosine kinases. Recently we have shown that activation loop tyrosine residue in KIT is not essential for its activation but plays an important role in cellular translocation and maturation of specific T cell subsets. PKC-θ isozyme has been extensively studied particularly in the context of hematopoiesis and immunity, where is required for the development and maturation of specific T cell subsets. PKC-θ also plays a role in Notch-driven T cell leukemia and in a subset of gastrointestinal stromal tumors and melanoma. Furthermore, the tyrosine phosphorylation of Xkr5 is not dependent on Src family kinases that play a crucial role in the activation of wild-type KIT. In KIT/D816V expressing mast cells, phosphorylation of Xkr5 inhibits KIT/D816V downstream signaling molecules Akt, Erk and p38. As a consequence, cell proliferation and colony formation is inhibited by the tyrosine phosphorylation of Xkr5 as well, indicating that Xkr5 is a negative regulator of KIT/D816V signaling.

**#2373 Analysis of the distinct activities of the C1a and C1b domains of protein kinase C theta.** Agnes Czikora,1 Satyabrata Pany,1 Youngki You,2 Amandeep Saini,1 Noemi Kedei,1 Nancy E. Lewin,1 Adelle Abramovitz,1 Joydip Das,2 Peter M. Blumberg1.

PKC isozymes, the major receptors for tumor-promoting phorbol esters, play a central role in cellular signal transduction downstream of the second messenger diacylglycerol. The PKCθ isozyme has been extensively studied particularly in the context of hematopoesis and immunity, where is required for the development and maturation of specific T cell subsets. PKCθ also plays a role in Notch-driven T cell leukemia and in a subset of gastrointestinal stromal tumors, melanoma and breast cancers. The C1 domains represent the regulatory motif in the PKCs responsible for their recognition of diacylglycerol / phorbol esters. PKCθ is unique among the conventional and novel PKC isoforms in that one of the two C1 domains of PKCθ, the C1a domain, has been reported to show little or no affinity for diacylglycerol or phorbol ester, unlike the C1b domain. Likewise, the PKCθ C1b domain has been reported to play the predominant role in the membrane translocation and activation process of PKCθ. We observed that the C1a domain in fact has appreciable binding activity for [H]phorbol 12,13-dibutrate (PDBu), with a Kd of 254 nM, albeit much less than that of the C1b domain, 1.38 nM. Replacing individually in the C1a domain ten of the amino acid residues that differed between the C1a and C1b domains, using the correspon- ding residue from the C1b domain, we found that only the P9K replacement restored [H]phorbol 12,13-dibutyrate binding activity to the C1a domain, yielding a Kd of 3.60 nM, close to that of the C1b domain. Strikingly, the reciprocal replacement in the C1b domain of K9P still preserved appreciable binding affinity, 11.7 nM. We conclude that other features in the C1a domain contribute to the extent to which the P9 residue negatively influences binding. We further explored the role of the C1 domains of PKCθ on translocation using confocal microscopy with a variety of full length constructs in which the C1 domains were manipulated. Consistent with the binding studies, we showed that the C1b domain was the main contributor to translocation, that the full length PKCθ with the C1b K9P retained response, albeit more weakly, and that deletion of the C1a domain or its replacement with a second C1b domain enhanced the translocation to the plasma membrane. Exploitation of the structural differences between the C1 domains in PKCθ and those of other PKC isoforms, both at the level of the direct ligand interactions and the more complexly intercalated between C1 domain, ligand, and phospholipid bilayer, may help to afford specific ligands for this member of the PKC family.

**#2374 Mixed-Linear Kinase 3 signals through the FRA1-MMP1 axis to drive triple-negative breast cancer invasion and transendothelial migration.** Chotirat Rattanasinchai, Brandon Llewellyn, Susan E. Conrad, Kathleen A. Gallo. Michigan State Univ., East Lansing, MI.

Mixed-lineage kinase 3 (MLK3), a mitogen-activated kinase kinase kinase (MAP3K), contributes to metastasis of triple-negative breast cancer (TNBC), in part through its regulation of paxillin phosphorylation and focal adhesion turnover. However the signaling mechanisms through which MLK3 promotes invasion and metastasis are not fully understood. We found that in non-metastatic, estrogen receptor positive (ER+ ) breast cancer cells, induced MLK3 expression robustly upregulates the oncogenic transcription factor, FOS-related antigen 1 (FRA-1), as well as matrix metalloproteinases (MMPs), MMP-1 and MMP-9. MLK3-induced MMP-1 induction is abrogated by FRA-1 silencing, demonstrating that MLK3 signals through FRA-1 to control MMP-1. In complementary experiments performed in metastatic TNBC models, high levels of FRA-1 are significantly reduced upon gene silencing of MLK3 or by MLK3 disruption using the CRISPR/Cas9a approach. Furthermore, both MMP-1 and MMP-9 are decreased upon FRA-1 silencing, and decreased endothelial permeability and transendothelial migration is observed in experiments with MLK3-depleted TNBC cells compared with parental counterparts. In addition, MLK inhibitor treatment or MLK3 deletion renders TNBC cells defective in Matrigel invasion. Furthermore, circulating tumor cells (CTCs) derived from a TNBC-bearing mouse which show an intermediate epithelial-mesenchymal phenotype display increased levels of MLK3, FRA-1, and MMP-1 compared with parental cells, supporting a role for the MLK3-FRA-1-MMP-1 signaling axis in tumor cell invasion and vascular invasation. Consistent with the role of MMP-1 in multiple steps of metastasis, high levels of MMP-1 in breast cancer patient tumor samples are associated with increased distant metastases and poorer overall survival, particularly in TNBC. Our results demonstrating the requirement for MLK3 in controlling the FRA-1/MMP-1 axis suggest that MLK3 is a potentially effective therapeutic target for TNBC.

**#2375 Functional role of the tumor suppressor protein phosphatase, PP2A-B55α, in breast cancer.** Nikita Panicker,1 Abdul Mannan,1 Lauren F. Watt,1 Ben Copeland,1 Matt D. Dun,2 Simon King,2 Megan Clarke,2 Kathryn Skelding,1 Severine Roselli,1 Nicole M. Verrills 1. Unv. of Newcastle, Callaghan, Australia; 2Pathology North Lamington, Australia; #2Huntier Cancer Research Alliance, New Lambton, Australia.

Breast cancer is the most common cancer in women and a leading cause of death. Dysregulation of cellular signalling pathways controlling proliferation, survival and migration, such as the PI3K/Akt and Ras/MAPK pathways, are key features of breast cancer. Protein phosphatase 2A (PP2A) negatively regulates these components of these pathways. PP2A is a family of trimeric serine/threonine protein phosphatases, each consisting of a structural, a catalytic and a regulatory subunit of which there are multiple isoforms. The addition of specific regulatory subunits provides subcellular targeting and substrate specificity to the enzyme. While PP2A is generally considered a tumor suppressor, a specific role for individual PP2A subunits in breast cancer has not been described. To address this, we first examined PP2A subunit expression in human breast tumors. Immunohistochemical analysis revealed significantly lower expression of the structural subunit, PP2A-A, and regulatory subunits PP2A-B55α and PP2A-B56α, in primary tumors and metastases, compared to adjacent normal mammary tissue. We further found an association of low PP2A-B55α with aggressive breast cancer subtypes, and with worse disease-free and overall survival. Functionally, shRNA-mediated knockdown of PP2A-B55α in breast cancer cell lines disrupted focal adhesion and enhanced 3D cultures induced a tumorigenic phenotype, characterised by increased proliferation and enlarged multi-lobular acini. In contrast, overexpression of PP2A-B55α in breast cancer cells inhibited proliferation. Thus PP2A inactivation, in particular loss of B55α, is functionally important in breast tumorigenesis. PP2A-B55α complexes play an important role in DNA damage repair, and we found PP2A-B55α knockdown impaired DNA damage repair. Thus low PP2A-B55α may contribute to genomically instability. To examine the functional role of PP2A-B55α in vivo, we have generated the first PP2A-B55α (Pprr2ra) knockout mouse. Constitutive knockout of Pprr2ra is embryonic lethal, with embryos dying during late development, post 14.5 days p.c. Heterozygous Pppr2ra mice
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(Ppp2r2a-/-) are viable, despite expressing only ~10% of Pp2a-B55ε protein levels compared to WT mice. Interestingly, Ppp2r2a-/- mice have significantly reduced branching in the developing mammary gland, similar to that observed in mice with mammary-specific loss of the breast tumor susceptibility gene, Brca1. Analysis of breast tumor formation in these mice, either alone or when crossed with MMTV-Neu animals, is underway. We further characterized the pathological activation of Pp2a, using FTY720, or the non-phosphorylatable analogue, AAL(S), inhibits tumor growth and metastases in an orthotopic xenograft model of aggressive, triple negative breast cancer (MDA-MB-231). Together this work demonstrates the importance of Pp2a as a tumor suppressor in breast cancer, and suggests that targeting Pp2a is a potential therapeutic strategy for poor outcome patients.

#2376 Novel insights into the regulation of ERK3's kinase activity and its ability to promote cancer cell invasiveness. Lobna Elkhdag, Hadel Alseran, Weiwen Long. Wright State University, Dayton, OH.

In comparison with the well-studied conventional MAPKs, such as extracellular-signal-regulated kinase (ERK)1/2, much less is known about the regulation and downstream targets of ERK3, an atypical MAPK. Whereas dual phosphorylation of the TXY activation motif in ERK1/2 is critical for their activation, it is unclear if phosphorylation of the single phospho-acceptor site (S189) in the SEG activation motif of ERK3 is critical for its kinase activity or function. In addition, little is known about the functions of the structurally distinct elongated C-terminus extension of ERK3. Recent studies have revealed important roles for ERK3 in promoting cancer progression. Of note, our previous study showed that ERK3 increases cancer cell invasiveness by phosphorylating the oncogenic protein steroid receptor co-activator 3 (SRC-3) and upregulating SRC-3-mediated matrix metalloproteinase gene expression. Here we aimed to elucidate the importance of the phospho-site S189 and the C-terminus in regulating ERK3 kinase activity and its functions in cancer cells. By performing in vitro kinase assays, we found that as compared to the wild type (WT) ERK3 protein, ERK3-S189A has remarkably reduced kinase activity towards auto-phosphorylation and phosphorylating SRC-3. In line with the critical role of S189 in ERK3 kinase activity, mutation of S189 to alanine greatly reduced ERK3's activity in promoting the migration and invasion of lung cancer cells. To study the regulatory role of the C-terminus in ERK3 kinase activity, we expressed and purified the N-terminal ERK3 (aa 1-340) that retains the kinase domain but has deletion of the C-terminus. While it showed higher auto-phosphorylation ability than the full length ERK3 protein, ERK3 (aa 1-340) had greatly decreased ability to phosphorylate SRC-3. Interestingly, ERK3-S189A (aa 1-340) exhibited kinase activity equivalent to that of ERK3 (aa 1-340). These results suggest that on one hand, the C-terminus may play an auto-inhibitory role on ERK3 kinase activation through intramolecular interaction and that the phospho-S189 is required for relieving this auto-inhibition. On the other hand, C-terminus plays a critical role for ERK3 to recruit substrates, such as SRC-3. In agreement with its inability to phosphorylate SRC-3, ERK3 (aa 1-340) had decreased ability to stimulate migration and invasion of lung cancer cells as compared to full length ERK3. Taken together, our study unravels the importance of ERK3 S189 residue as well as the C-terminus for the intramolecular regulation of ERK3 kinase activity and invasiveness-promoting ability in cancer cells.

#2377 The AML linked Src family kinase Fgr is uncoupled from SH2 and SH3 domain regulation and drives oncogenic transformation. Kexin Shen, 1 Heather R. Dorman, 1 Haibin Shi, 1 Ravi K. Patel, 1 Jamie A. Moroco, 2 John R. Engen, 1 Thomas E. Smithgall, 1 Engen, 2 Thomas E. Smithgall1. Univ. of Pittsburgh School of Medicine, Pittsburgh, PA; 2Northeastern University, Boston, MA.

Fgr is a member of the Src family of non-receptor protein-tyrosine kinases, which are over-expressed and constitutively active in many human cancers. Fgr expression is restricted to myeloid hematopoietic cells and strongly upregulated in a subset of primary human AML bone marrow samples, suggesting that Fgr kinase activity may contribute to AML pathogenesis. To investigate the oncogenic potential of Fgr, we expressed the wild-type kinase in Rat-2 fibroblasts and scored transformation in soft agar colony-forming assays. Remarkably, Fgr expression resulted in strong transforming activity, suggesting that over-expression of the kinase is sufficient to overcome regulatory control by its SH2 and SH3 domains. To test this idea, we mutated the negative regulatory tail tyrosine of Fgr. Phosphorylation of the homologous tyrosines in c-Src, Hck and other Src-family members by the regulatory kinase Csk causes intramolecular engagement of the SH2 domain to suppress kinase activity. Substitution of the Fgr tail tyrosine with phenylalanine did not further enhance the transforming or kinase activity of Fgr in Rat-2 cells, suggesting that its kinase domain is uncoupled from regulation by its non-catalytic SH3-SH2 region. To explore the regulatory mechanism further, we expressed near-full-length Fgr in insect cells, purified it to homogeneity, and confirmed that it was singly phosphorylated on its negative regulatory tail (and not the activation loop). Hydrogen-deuterium exchange mass spectrometry demonstrated that the SH3 and SH2 domains are protected from deuterium uptake as observed previously for recombinant, downregulated Hck, suggesting that they are packed against the back of the kinase domain. We then tested recombinant Fgr for sensitivity to activation in vitro using peptide ligands for the SH3 and SH2 domains, as well as a peptide that engages both domains simultaneously. Fgr kinase activity was unaffected by these peptides, providing further evidence that the kinase domain is not allosterically coupled to SH3 and SH2 control. In contrast, Hck and c-Src were both stimulated by these peptides, consistent with a domain displacement mechanism of activation described previously. Control experiments using surface plasmon resonance spectroscopy confirmed that the SH3 and SH2 domains of both Hck and Fgr bind to each of the peptide ligands with similar kinetics and affinity. Taken together, our data show that Fgr is a unique Src family member in that its kinase domain is not subject to regulatory domain control despite evidence for intramolecular interactions with the SH3 and SH2 domains. As a consequence, simple over-expression of Fgr is sufficient to induce transformation of rodent fibroblasts, unlike Hck or other Src family members. By extension, over-expression of Fgr may contribute to AML development and selective targeting of its kinase activity may be of therapeutic benefit.

#2378 A kinome analysis of the molecular pharmacodynamics of PT-112 in a human cancer cell line. Diego Tosi, 1 Esther Perez-Gracia, 1 Philippe Pourquier, 2 Tyler D. Ames, 2 Richard A. Wing, 2 Jose Jimeno, 2 Celine Gongora. 3 Institut de Recherche en Cancérologie de Montpellier, Montpellier, Montpellier, France; 4Phos- platin Therapeutics, New York, NY.

Background: To better understand the mechanism of action of PT-112, we investigated the effects of PT-112 on kinase activity within HCT116 human colorectal cancer cells. PT-112 is a pyrophosphate-conjugated platinum (Pt) containing new chemical entity under clinical development in the US and Tai- wan. Designed to reduce certain toxicities associated with Pt agents and drug resistance from DNA-repair pathway activity, PT-112 differs in significant ways from traditional Pt therapies. PT-112 results in potent anti-proliferation effects with lower Pt accumulation in whole cell and DNA extracts. Upregulation of p16 and p21, and consistently, inhibition of the cell cycle at the G1/S phase transition is observed with PT-112 use, a finding not commonly associated with Pt agents. Further, sensitivity to PT-112 is not significantly affected by the functionality of DNA repair pathways. Finally, the induction of immunogenic cell death by PT-112 has been indicated via release of HMGB1, cell surface localization of calreticulin, and suppression of STAT3. Methods: A broad analysis of the phosphorylation pattern of kinase targets was achieved by utilizing the Tyrosine Kinase and Serine / Threonine Kinase PamChip® Arrays, each spotted with 144 peptides harboring kinase phosphorylation sites. Data were collected after 1, 6, and 24hr of exposure to 10 μM PT-112, a concentration achievable in human patient plasma at sub-toxic doses, and the degree of phosphorylation of these peptide targets was measured by the PamStation®, results: A set of kinase targets was identified as differentially phosphorylated relative to untreated control samples. In total, 4/1/5 targets were more phosphorylated than controls at the 1/6/24 hour time points, respectively, while seven less phosphorylated. Several of these targets connect to key cancer relevant pathways, including anti-angiogenesis (VGFRI), apoptosis (BAD), cell cycle regulation (CDC2, Rb, RBL2), cell growth (EGFR, MK07, RBL2), and cancer immunity (STAT4, CSFIR). Conclusions: A narrow set of initial effects on kinase activity from PT-112 exposure, which broaden over time, was observed. While we cannot discriminate direct from indirect effects, these data are consistent with PT-112 having mechanistic targets independent of nuclear DNA. Several observations were consistent with findings from previous experiments (e.g. evidence of impacts on apoptotic, cell cycle, and immunological pathways), while others were novel (e.g. impact on VGFRI, EGFR, MK07). In conclusion, PT-112 at pharmacologically achievable concentrations leads to a unique pattern of kinase disruption in vitro. Further work will be conducted to validate these findings, identify the driver kinases, and connect these observations into a coherent network of kinases and targets affected by PT-112 treatment, and to understand the clinical impact of the unique pharmacodynamic profile of PT-112.

#2379 Nuclear lemur tyrosine kinase 2 regulates RNA polymerase II dependent transcription in prostate cancer. Kalpit Shah, 1 Justin Foley, 1 Michael L. Nickerson, 1 Michael Dean, 1 Neil Bradbury 2. 1National Cancer Institute, Bethesda, MD; 2Rosalind Franklin University of Medicine & Sciences, North Chi- cago, IL.

The Androgen Receptor (AR), a DNA-binding transcription factor plays a key role in the development and maintenance of prostate epithelia by modulat-
ing expression of growth-promoting genes. AR has been proposed to regulate gene expression by enhancing the efficiency of RNA polymerase II (RNAPII) dependent transcription elongation. Dysregulation of this AR-dependent transcriptional activity has been implicated not only in prostate cancer but also androgen-independent prostate cancer. Recently, we showed that protein expression of LMTK2 in the Kiessig-2 (LMTK2) serine/threonine kinase is downregulated in prostate cancer. Importantly, our study ascribed a novel role for LMTK2, as a negative regulator of AR-dependent transcriptional activity in prostate epithelial cells. However, the mechanism through which LMTK2 is able to regulate AR remains to be determined. Here, we show that LMTK2 is not only present in cytoplasmic fraction but also, within the nucleus of mammalian cells where it co-localizes with AR, newly transcribed mRNA and with RNA polymerase II (RNAPII). Interestingly, our data reveals that LMTK2 colocalizes with elongating RNAPII, phosphorylated on serine 2 of the carboxyl-terminal domain. This presents an interesting possibility that LMTK2 might be able to regulate transcription of AR-dependent genes by interacting with the elongating RNAPII and transcription & epigenetic factors. As such, our study potentially identifies LMTK2 as an important player regulating the AR-RNAPII mediated transcription machinery in mammalian cells.

#2380 Insertion mutations in the tyrosine kinase domain of FLT3 display a higher oncogenic potential than the D835Y mutation in acute myeloid leukemia. Alissa Marhall,1 Thomas Fischer,2 Florian Heidel,2 Julhass U. Kazi,1 Lars Rönstrand1. 1Translational Cancer Research, Lund University, Lund, Sweden; 2Otto-von-Guericke University Medical Center, Magdeburg, Germany; 3University Hospital, Ruhr, Germany.

Acute myeloid leukemia (AML) remains the most common form of acute leukemia among adults and accounts for a large number of deaths. Mutation in FMS-like tyrosine kinase 3 (FLT3) is one of the most prevalent factor in this heterogeneous disease. The major mutations in FLT3 can be categorized as internal tandem duplications (ITD) and point mutations. Recent studies suggest that ITDs are subdivided in to two groups depending on their location: the juxtaphal and end duplicated (ITD) and the kinase domain (ITD-TKD). Although, ITD-JM has been characterized well the ITD-TKD has not yet been studied well due to its recent discovery. For this reason, we compared ITD mutations in TKD and JM, as well as the most frequently occurring point mutation located in the TKD, D835Y. The purpose of this study was to understand whether it is the mutation’s nature or location that plays the driving role in leukemogenesis. We used a cytokine-dependent mouse pro-B cell line, BaF3, to overexpress different FLT3 mutants. We first examined the colony formation capacity in semisolid cytokine- and serum-free medium. The assay resulted in indistinguishable number and size of colonies for both ITD-JM and ITD-TKD, while D835Y-TK3 transfected cells failed to form colonies suggesting that the ITD-TKD mutations have stronger transforming potential than other TKD mutations. In addition to colony formation assays, cell proliferation and survival was significantly higher in ITD-TKD expressing cells compared to cells expressing D835Y-TKD. Finally, we showed that phosphorylation of STAT5 and AKT is increased in ITD-TKD, while other FLT3 downstream signaling remained unaffected. All together, our data suggest that ITD-TKD displays higher oncogenic potential than other TKD mutants.

#2381 The effect of ERK5 inhibition in clear cell renal cell carcinoma. Hidenori Kanno, Sei Naito, Osamu Ichiyangi, Norihiko Tsuchiya. Yamagata University, Yamagata City, Japan.

Introduction: Clear-cell renal cell carcinoma (cRCC) is the most prevalent kidney cancer and is characterized by von Hippel-Lindau tumor suppressor gene (VHL) mutation. VHL, protein acts in a ubiquitin ligase complex, mediating the proteosomal degradation of ubiquitinated proteins, including hypoxia-inducible factor (HIF). Therefore, HIF accumulates in VHL-defective cell lines. A recent study revealed that extracellular signal-regulated kinase 5 (ERK5) is degraded through the ubiquitin-proteasome system, in a process mediated by VHL and HIF. Our objectives were to examine the effects of ERK5 in cRCC and to investigate the potential therapeutic target. Methods: ERK5 expression was investigated in surgically resected cRCC specimens using immunohistochemistry. To confirm that ERK5 is degraded by the VHL pathway, ERK5 levels were examined by western blotting in Caki-1 VHL wildtype renal cell carcinoma (RCC) cells and in A498 and A704 VHL-mutant RCC cells with or without the MG132 proteasome inhibitor. Furthermore, VHL-mutant cell lines were examined, with or without siRNA and XMD8-92-mediated ERK5 inhibition, to investigate the role of ERK5 in RCC cells using flow cytometry, MTS assays, and western blotting. Results: In surgical specimens, RCC cells showed higher levels of ERK5 expression than did normal cells. In addition, MG132 enhanced ERK5 expression in wildtype cell lines, but not in VHL-mutant cell lines. Therefore, ERK5 degradation involves the VHL pathway. Furthermore, ERK5 inhibition resulted in increase of the sub-G1 population as observed by flow cytometry and cleaved RARP expression as seen by western blotting, indicating an increase in apoptotic cells. ERK5 inhibition also suppressed cell viability and downregulated p16 and Bcl-2 expression. Conclusion: Our results show that ERK5 inhibition contributes to the downregulation of anti-apoptotic and cell cycle proteins and suggest that ERK5 is a promising therapeutic target for cRCC.


RACK1 (Receptor for Activated C Kinases) is a scaffolding protein with 7 WD repeats that interacts with the insulin-like Growth Factor I receptor (IGF-IR), integrins, and other signalling proteins. RACK1 functions as a scaffolding protein and regulator of many key biological processes and it is highly expressed in most tissue. We recently reported that RACK1 is essential for IGF-1-mediated regulation of PP2A activity and AGAP2 activity at focal adhesions. Downstream of IGF-1R signaling in cancer cells, the scaffolding properties of RACK1 are altered providing distinct migratory advantages to the cell and suggesting that RACK1 is an important regulator of IGF-I signaling in cancer progression. It is important to further characterise the role of RACK1 in cancer and to delineate the molecular mechanisms by which it regulates key signalling pathways. We have identified Protein Kinase A (PKA) as RACK1 interacting protein. PKA is a complex, multicomponent enzyme which is a fundamental protein that functions in cell survival, proliferation, and cytoskeletal remodelling among others. Our hypothesis is that RACK1 regulates the PKA axis in cancer cells leading to a higher oncogenic potential than the D835Y mutation in acute myeloid leukemia. RACK1 has the potential to mediate PKA activity in colon cancer cells. Although we show that PKA expression is not altered in colon cancer tissue, we believe that PKA activity is altered significantly in the disease state. Using PKA inhibitors, we have shown that PKA regulates IGF-1R-mediated MAP Kinase activation in colon cancer cell lines. In conclusion, this research project will help elucidate the role of PKA in colon cancer. Inhibiting or promoting specific protein interactions with RACK1 will provide very novel therapeutic opportunities and anti-cancer drug targets.

#2383 Nicotinamide N-methyltransferase inhibits Protein Phosphatase 2A activity by promoting T307 phosphorylation. John R. Jacob, Arnab Chakravarti, Kamalakannan Palanichamy. Ohio State Univ. College of Medicine and Comprehensive Cancer Center, Columbus, OH.

Recent evidence indicates that suppression of protein phosphatase 2A (PP2A) activity plays an essential role in malignant transformation and cancer cell survival. Despite the essential role of PP2A in cancer, the mechanisms by which other enzymes regulate PP2A remain largely unknown. We recently identified Nicotinamide N-methyltransferase (NNMT) as a novel regulator of PP2A activity in glioblastoma (GBM). NNMT is a cytosolic N-methyl transferase which utilizes methyl groups from S-Adenosyl methionine (SAM), forming the by-product S-Adenosyl Homocysteine (SAH). SAH, PP2A becomes activated following the trimer formation of the three PP2A subunits, which requires the methylation of the catalytic subunit of PP2A (PP2A C) at L309. In cancer, NNMT has been reported to decrease the cellular methylation potential (SAMS/AH) and correspond with decreased PP2A activity. It was hypothesized that the decrease in methylation potential accounts for the decrease in PP2A activity by kinetically disfavoring the methylation of PP2A C. Interestingly, when we overexpressed NNMT in GBM cell lines and used LC-MS/MS (LC-MS Quadrupole Time-of-Flight and LC-MS Triple Quad) to monitor the methylation potential, we discovered that overexpression of NNMT increased methylation potential, however, still lead to a decrease in PP2A activity. As a result, we hypothesized that NNMT suppresses PP2A activity in a methylation-independent manner. In an effort to identify an alternative mechanism by which NNMT decreases PP2A activity, we found that NNMT expression was required for the phosphorylation of PP2A C at residue T307, which inhibits PP2A trimer formation and activation. Further, we found that NNMT expression leads to the constitutive activation of the PP2A target serine/threonine kinases (STks): Akt, MAPK, and SAPK/JNK. NNMT silencing resulted in the inactivation of these downstream PP2A target stress kinases, which was not sufficient to rescue their activation. This suggests that NNMT-induced PP2A suppression is essential for the activation of these kinases. Overall, this study demonstrates a novel mechanism independent of methylation by which NNMT represses the activity of tumor suppressor PP2A.
#2384 Aberrant expression of PDZ binding kinase modulates human pancreatic cancer cell invasion. Charles Hinzman,1 Jose Trevino,2 Partha P. Banerjee1.1Georgetown Univ. Medical Ctr.; Washington, DC; 2University of Florida Medical Center, Gainesville, FL.
Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related death and is projected to become the second leading cause of cancer deaths by 2030. Incidence to mortality ratio for PDAC is almost one suggesting its aggressive phenotype. The poor survival rate of PDAC patients is due to metastasis to distant organs, and therefore, recent focus has been aimed towards the identification of molecular targets that drives metastatic disease. We are investigating an under-studied kinase, PDZ-domain binding kinase (PKB) as a target for pancreatic cancer (PanCa) therapy. PKB is a dual-specificity serine/threonine kinase involved in mitosis and invasive behavior of cancer cells. It is expressed physiologically in tests, but aberrant expression results in an aggressive phenotype in a variety of cancers. Our results demonstrate that PKB expression varies across a panel of human PanCa cell lines, and directly correlates with their invasive ability. Similarly, a panel of patient-derived primary PanCa shows higher PKB levels associated with aggressive behavior and invasion. To assure specificity of PKB to this invasive phenotype, we measured the effect of PKB overexpression or knockdown on the PanCa lines invasive ability. Overexpression of PKB in low PKB expressing cells increased PanCa invasion ~10-fold, while PKB knockdown reduced invasive ability ~5-fold. Immunohistochemical analyses of resected human PanCa specimens revealed that PKB is present in the majority of human PanCa tissue samples tested but not in the adjacent normal pancreatic tissue. Interestingly, aberrant PKB localization was found in even early stages of PanCa development and was sustained throughout its progression. In search of molecular effectors of invasion regulated by the PKB, we examined various genes/proteins that are involved in cancer cell invasion and metastasis. ReblA well-known metastatic inducer in various cancers was found to be a major player in PanCa and regulated by PKB. These data indicate a novel molecular pathway of PKB where its presence plays a role in invasion of human PanCa cells. Therefore, the role of PKB in promoting cancer cell invasion, combined with its general lack of expression in normal cells, nominates PKB as a potentially important therapeutic target for pancreatic cancer.

#2385 Systematic characterization of kinase inhibitors reveals heterogeneity in responses by class and cell line. Caitlin E. Mills1, Marc Hafner,2 Dejan Juric,2 Peter K. Sorger1.1Harvard Medical School, Boston, MA; 2Massachusetts General Hospital, Boston, MA.

Several publications have addressed concerns surrounding drug response screens by pointing out sources of variability and by presenting recommendations for better experimental methods and more robust analytical approaches. In the presented profiling effort, we integrated the latest advances in drug response measurement and focused on data diversity and quality rather than on breadth. We selected 32 breast cancer cell lines with a strong bias towards triple negative lines as well as 4 cell lines established from relevant patient-derived xenografts. We evaluated a panel of clinically relevant kinase inhibitors using a microscopy-based drug response assay to measure drug potency, and to quantify drug efficacy in terms of growth inhibition (GR metrics) and cell death. The use of the GR metrics to assess the sensitivity enabled us to identify and study differences between cytostatic and cytotoxic responses. This systematic dose response dataset is complemented by measurements of baseline mRNA expression levels by RNAseq and endogenous protein levels by shotgun mass spectrometry across all cell lines. The completeness and controlled conditions under which these data sets were collected provide confidence in their integration. The baseline RNA and protein expression levels were used to build predictors of the measured drug responses with the goal of identifying the biological processes responsible for the differences in sensitivity across drugs and cell lines. Differences in the phenotypic responses of cell lines to kinase inhibitors with the same nominal targets have been investigated, and associated with variable inhibitor polypharmacology.

#2386 HDAC6 regulates ERK1’s kinase activity via deacetylation. Jheng-Yu Wu,1 Xiaohong Zhang2.1Univ. of South Florida College of Medicine, Tampa, FL; 2Karmanos Cancer Institute, Wayne State University, Detroit, MI.
Extracellular signal-regulated kinases 1/2 (ERK1/2) are important kinases regulating cell proliferation and cell migration, and have been established as therapeutic targets for cancer treatment. Previously, we found that ERK1 phosphorylates histone deacetylase 6 (HDAC6), a class IIb HDAC, to regulate its enzymatic activity. HDAC6 plays an important role in several types of cancers. HDAC6 governs cancer cells’ migration by cooperating with cytoskeleton proteins and their associated proteins, and HDAC6 is arising as a determinant for cisplatin-resistance in non-small cell lung cancer (NSCLC) and ovarian cancer.

Here, we have shown that HDAC6 manipulates ERK1’s kinase activity via deacetylation. We demonstrated that both ERK1 and ERK2 interact with HDAC6 physically via GST pull-down assays. To examine whether ERK1/2 are acetylated, we transfected GST-ERK1 or GST-ERK2 into HEK293T cells, and treated the cells with a pan-HDAC inhibitor, Trichostatin A. We showed that the level of acetylated HDAC6 was increased in a dose-dependent manner. Furthermore, when GST-ERK1 or GST-ERK2 expressed cells were treated with an HDAC6-specific inhibitor, ACY-1215, the level of acetylated GST-ERK1/2 increased. These results suggest that HDAC6 is a deacetylase for ERK1/2. In addition, we determined that acetyltransferases CBP and p300 acetylate ERK1/2. We found a novel acetylation site located in ERK1 N-terminus by mass-spectrometry analysis. We then mutated this lysine (K) site with either Glutamic acid (Q) or Arginine (R) to mimic the acetylation or deacetylation status, and performed kinase assays using ELK1 as a substrate. The acetylation mimicking mutant exhibits a decreased kinase activity toward ELK1, while the deacetylation mimicking mutant exhibits a similar level of kinase activity as the wild-type ERK1 does, suggesting that acetylation/deacetylation alters ERK1 enzymatic activities. Therefore, HDAC6 may regulate ERK1’s kinase activity via deacetylation of this site. Furthermore, we have shown that HDAC6 is overexpressed in lung cancer tissue samples and plays an important role in chemosensitivity in lung cancer. We predict that inhibition of the HDAC6-ERK1 pathway may provide clinical benefit for lung cancer patients.

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#2387 Overexpression of NSUN2 by DNA hypomethylation is associated with metastatic progression in human breast cancer. Jie Yi,1 Yu Chen,2 Ran Gao,3 Zhou Yang,4 Jinhua Wang,5 Yingchun Xu1. 1Peking Union Medical College, Beijing, China; 2Chinese Academy of Medical Science and Peking Union Medical College, Beijing, China.

NSUN2 is a RNA methyltransferase that has been shown to be implicated in development of human cancer. However, the functional role of NSUN2, mechanism of NSUN2 overexpression and its association with clinicopathologic features in breast cancer remain unclear. To investigate alterations in the expression and functional role of NSUN2 in breast cancer, NSUN2 expression was assessed in breast cancer cells and tissues obtained from cancers at different American Joint Committee on Cancer (AJCC) stages, and its functions were investigated using breast cancer cell lines. NSUN2 expression was shown to be significantly higher in breast cancer cells and tissues than in normal breast epithelial cells and tissues, at both mRNA and protein levels. Overexpression of NSUN2 was shown to promote cell proliferation, migration, and invasion while NSUN2 knockdown inhibited these processes in vitro and in vivo. NSUN2 expression level was associated with the methylation level of its promoter. Our results demonstrated that the overall expression of NSUN2 significantly correlated with clinical stage (P=0.027), tumor classification (P=0.012), pathological differentiation (P=0.023), as well as with the expression levels of estrogen receptor (P<0.001), progesterone receptor (P<0.001), and Ki-67 (P<0.001). Our findings provide a unique insight into the roles and effects of NSUN2 overexpression in breast cancer cells, and highlight the necessity of the investigation of novel therapeutic targets, such as NSUN2, for the improvement of breast cancer treatments.

#2388 Low grade gliomas exhibit distinct patterns of epigenetic aging associated with prognostic molecular subtype. Peter Liao, Jill Barnholtz-Sloan. Case Western Reserve University, Cleveland, OH.

Background: Low grade gliomas make up a significant proportion of malignant brain tumors and possess a high degree of heterogeneity, highlighting a need for clinically useful markers for prognosis and further biologic investigations. We used an existing model of biological age based upon DNA methylation to characterize epigenetic age in low grade gliomas (LGG) according to existing molecular classifications and assessed the prognostic utility of epigenetic age. Methods: We analyzed the full TCGA LGG cohort consisting of 516 patients; 216 grade 2 and 241 grade 3 tumors. Age at diagnosis ranged from 14-87 years with median age 43. We calculated "DNA methylation age" (DNAm age) based upon 353 Cpg probes according to the epigenetic clock developed by Steve Horvath, and calculated age acceleration according to the DNAm age of tumor tissue relative to reported chronological age of patient at diagnosis. DNAm age was assessed for prognostic value using Cox proportional hazards regression. Age acceleration differences were assessed across LGG molecular classification.

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methods using the Wilcoxon Ranked Sum test. Results: DNA methylation age remained highly correlated with chronological age in LGG tumor tissue (cor=0.58, p<0.001), with an average age acceleration of 33.5y. DNA methylation age was weakly positively correlated with mutational burden (cor=0.12, p=0.01). Higher DNA methylation age was positively associated with survival independent of chronological age and tumor aggressiveness (data from Sorrows et al., 2013). Although the regu-
lation of DNA methylation age in cancers remains poorly understood, significant associa-
tion of DNA methylation age with acceleration of prognostically useful molecular subtypes in LGGs underscores biological differences that cannot be discerned in LGGs based upon histological classification. This association suggests that LGGs may provide an avenue for investigating DNA methylation age, molecular subtype, and their relation to tumor behavior and clinical prognosis.

**#2389 LINE-1 tumor hypomethylation is associated with shorter recurrence-free survival in localized clear-cell renal cell carcinoma.** Gabriel G. Malouf, Roger Mouawad, Frederick Allanic, Eva Compérat, Morgan Roupert, David Khayat, Jean-Philippe Spano. Salpêtrière Hospital, Paris, France.

Background: Cancer cells are characterized by alterations of DNA methyl-
ation patterns involving global DNA hypomethylation and site-specific CpG island promoter hypermethylation. Global DNA hypomethylation is thought to play a role in genetic instability and tumor aggressiveness. Long interspersed nucleotide element-1 (L1/LINE-1) repetitive elements represent 40% of the ge
nome and their methylation is a good indicator of the global DNA methylation level. Although LINE-1 methylation has been previously shown to be associated with prognosis of patients with cancer, the value of LINE-1 methylation in pre-
dicting recurrence of patients with localized clear-cell renal cell carcinomas re-
main unknown. Material and Methods: We quantified the LINE-1 methylation using bisulfite pyrosequencing in cohort of 200 patients with resected clear-cell renal cell carcinomas (AJCC stage I-III). LINE-1 methylation of adjacent normal kidney was also assessed in 128 cases. Threshold of tumor LINE-1 hypomethyl-
ation was defined as LINE-1 methylation in normal samples minus three stan-
dard deviation. Results: Median methylation of tumor samples was 59.01% ver-
sus 61.61% for normal adjacent kidney samples (p<0.10). LINE-1 methylation level of normal adjacent kidney was associated with tumor size (Spearman R=0.21, P=0.02) but not with age (p=0.24) or Leibovitch score (p=0.05). Tu-
mor LINE-1 hypomethylation was observed in 20 samples (n=10). Strikingly, those were enriched for tumors occurring in female (60% versus 27.7%) (p=0.005) of note, no association was found between LINE-1 tumor hypom-
ethylation and tumor grade (p=0.31), stage (p=1), age (p=0.24) and Leibovitch score (p=0.82). In multivariate analysis, only Leibovitch score and LINE-1 hy-
pomethylation were independently associated with poor recurrence-free sur-
vival. Conclusion: LINE-1 hypomethylation is associated with shorter recur-
rence-free survival in patients with resected clear-cell renal cell carcinomas, suggesting the possibility of using it as predictive biomarker of recurrence.

**#2390 Clinical validation of an epigenetic field of susceptibility to detect significant prostate cancer from 2 non-tumor biopsies.** Bing Yang1, Johna-
thon McCormick,1 Adam Schultz,1 Glen Leveson,1 Geoffrey Sonn,2 Cristina Magi-Galluzzi,3 Eric A. Klein,4 Michael Fumo,4 David F. Jarrard.1 1Univ. of Wis-
cconsin-Madison, Madison, WI; 2Stanford University, Palo Alto, CA; 3Glickman Urological and Kidney Institute, Cleveland, OH; 4Rockford Urological Associates, LTD, Rockford, WI; 5Univ. of Wisconsin-Madison Carbone Cancer Center, Mad-
ison, WI.

Background: An epigenetic field of cancer susceptibility exists for prostate cancer (77) and the control group (52) were similarly matched except for PSA (7 vs 5.8; p<0.03) and prostate size (46 vs 36; p<0.02). We observed robust methylation differences for all genes (p<0.05) (Table). A multiplex regression model for biomarker performance incorporating a gene combination of EVX1, NCR2, PLAG2 and SPAG4 discriminated non-tumor from tumor-associated cases (AUC 0.744, p=0.0001). A multiplex model in-
corporating 3 genes (EVX1, PLAX2G16, NCR2) and clinical information (PSA, age) identified patients with GS=7 prostate cancers (AUC 0.808, p=0.001). Conclusions: A widespread epigenetic field defect can be used to detect GS>7 prostate cancers in patients with histologically negative biopsies. This assay is sensitive in requiring only 2 biopsies and detects alterations in nontumor cells at distances from the cancer. This has the potential to decrease the need for repeat prostate biopsies, a procedure associated with cost and complications.

**#2391 Integrated analysis of methylation and expression profile of telom-
ere-related genes in breast cancer patients.** Jianfu Heng,1 Xinwu Guo,2 Lili Fang,3 Fan Zhang,3 Limin Peng,3 Ming Chen,3 Xipeng Luo,3 Xunxun Xu,3 Shou-
man Wang,3 Jun Wang,4 Central South University, Changsha, China; Sunan Gene Technology Inc, Changsha, China; Xiangua Hospital, Central South Uni-
versity, Changsha, China.

Telomeres at the ends of chromosomes are critical in maintaining the integ-
rity and stability of the genome. Abrupt telomere or telomerase dysfunction participates in tumorgenesis. A majority of human cancers exhibit critically aberrant telomere length, suggesting that tumors can arise from genetically in-
stable cells with dysregulated telomeres. Many genes are involved in the com-
plex regulatory mechanisms of telomere length and telomerase activity. In ad-
dition, the methylation and expression pattern for most of telomere related genes in breast cancer are still unknown. Using microfluidic-PCR based target enrichment and next-generation bisulfite sequencing technology, we explored the promoter methylation profile of 29 telomere-related genes in 184 breast cancer patients with paired tumor and matched normal tissues. The average methylation level was significantly higher in tumor (8.13%) than that in matched normal tissues (7.08%) (P=4.30E-21). Four genes showed significant hyper-
methylation in the breast tumor tissues. All of these 4 genes are annotated with potential TFBs in the promoter regions. In subtype analysis, RAD51D showed significant methylation difference among four subtypes. In analysis of the asso-
ciation between methylation and clinicopathologic characteristics, TERC showed significant difference between ER+/ER- tumors. The expression profile of the 4 significant hyper-methylated genes was explored in the same cohort using qPCR method. All of them showed significantly lower expression in breast tumor tissues compared with the matched normal tissues. Two genes showed significant and negative cis correlation between methylation and gene expres-
sion in the breast tumor tissues (TERC), whereas the other two genes showed a negative correlation. These findings suggest that the 4 genes showed a good performance in predicting breast cancer with ROC analysis. In summary, our results revealed the methylation pattern of telomere related genes in breast cancer and illustrated the epigenetic regulatory mechanism on expres-
sion of aberrant methylated genes. Our study provides a novel panel of telomere related genes which may be a valuable diagnostic biomarker for breast cancer prediction.

**#2392 A multiplex histone H3 PTM assay for epigenetic biomarker dis-
cover in tissue biopsy and archived clinical samples.** Mary Anne Jelinek, Melissa Ritland, AJ Westergen. Active Motif Inc., Carlsbad, CA.

Disregulation of epigenetic mechanisms is known to play an important role in the development and progression of cancer. One such mechanism is mani-
fested as altered levels of histone modifications involved in regulating gene tran-
scription. N-terminal histone tails can have a variety of modifications, such as phosporylation, methylation and acetylation at specific amino acid residues which are conserved throughout eukaryotes and function by altering chromatin structure and creating binding sites for chromatin readers, writers or erasers. Numerous studies have reported aberrant global levels of several histone H3 and H4 post-translational modifications (PTMs) in a wide range of solid tumor types. These changes have been shown to be predictive of clinical outcome, raising the possibility that histone modifications have potential as epigenetic biomarkers. Clinical samples have immense potential for biomarker identifica-
tion since they are often accompanied with valuable information pertaining to patient history, treatment courses and disease outcome. The preferred method for clinical sample profiling is formalin-fixation followed by paraffin embed-
ding (FFPE). While extraction and downstream analysis of DNA and RNA from FFPE samples is now routine, proteomics studies of FFPE samples is hampered by extensive protein cross-linking generated by formalin fixation. Analysis of histone PTMs in patient archival samples is limited to low-throughput immu-
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Nohistochemical staining, and is not an ideal approach for mining large sample cohorts for biomarker identification. Western blot or ELISA methods, which have large sample requirements, are simply not an option for FFPE samples. We have developed a multiplex bead-based ELISA assay which enables simultaneous interrogation of thirteen histone H3 PTMs. The assay is performed in 96-well plates and is ideal for profiling tumor subpopulations within or across small samples. We will present the application of this technology in a variety of sample types including frozen tissue, formaldehyde-cross linked cells and tissues, and in small molecule inhibitor screens with as few as 2,000 cells per well. The ability to simultaneous detect up to thirteen histone H3 PTMs provides a unique feature that enables determination of for on-target and off-target effects within the same sample.

#2393 Decreased expression of 5-hydroxymethylcytosine is associated with grade of malignancy in lung cancer. Yoshiaki Tominga, 1 Ken-ichi Ito, 1 Jun Nakayama, 2 Division of Breast, Endocrine and Respiratory Surgery, Department of Surgery(II), Shinshu University School of Medicine, Matsumoto, Japan; 2 Department of Molecular Pathology, Shinshu University Graduate School of Medicine, Matsumoto, Japan.

Purpose: The ten-eleven translocation (TET) proteins are critical regulators in epigenetic modification. It is well known that TET proteins catalyze the oxidation of 5-methylcytosine (5-mC) to generate 5-hydroxymethylcytosine (5-hmC), and 5-hmC levels reflect diverse functions of TET proteins. It has been reported that the expression of 5-hmC in cancer tissues is decreased compared with that in normal tissues in several organs. However, 5-hmC expression in lung cancer has not been fully analyzed. To examine whether or not 5-hmC was associated with phenotypes or progression of lung cancer, we evaluated 5-hmC expression in a variety of histological types of lung cancers. Materials and methods: The expression of 5-hmC was analyzed by immunohistochemistry in 109 paired specimens of lung cancer and adjacent normal tissues obtained from the patients underwent operation at Shinshu University Hospital. Eighty-nine adenocarcinomas, 17 squamous cell carcinomas, two large cell carcinomas, and one small cell carcinoma were analyzed in the study. Results: 5-hmC expression in female was significantly lower than that in male, and was lower in smokers than non-smokers. 5-hmC expression was positive in 34.1±22.0% of lung cancer tissues and 69.4±18.8% of normal tissues. The expression of 5-hmC was significantly decreased in the lung cancer tissues compared with that in the adjacent normal tissues (p<0.001). With regard to the histological types, the positive ratios of 5-hmC expression were 36.0±21.7% in adenocarcinoma, 24.0±20.3% in squamous cell carcinoma, 8.0±7.3% in large cell carcinoma, and 13.0±8.3% in small cell carcinoma. Thus, the frequency of positive expression of 5-hmC was decreased in accordance with the increase of grade of malignancy of lung cancer, and a negative correlation between 5-hmC expression and the grade of malignancy of lung cancer was observed. In the other hand, no association between the expression of 5-mC was observed between lung cancer tissues and normal tissues. Conclusion: Our results indicate a possibility that 5-hmC may be related to carcinogenesis and progression or differentiation of lung cancer.


Background: Clinicopathological features including MGMT methylation have been correlated to prognosis in Glioblastoma (GBM). Tumor infiltrating lymphocytes (TILs) are predictive of clinical outcome in different malignancies. We evaluated association between clinicopathological features including MGMT methylation and IHC infiltrating immune cell subpopulations in 43 resected GBM cases who came from 2008 to 2013. Methods: A prospective evaluation of TIL (CD3, CD4, CD8 and CD20) and macrophages (CD68 and CD163) through H&E was performed by a pathologist and evaluated Immune cell subpopulations was evaluated through scanning, and software analysis (BX63 Olympus and TissueMorph-VisionPharm) with pathologist support. MGMT evaluation was prospectively performed by bisulfite PCR test in paraffin samples. Results: Median age was 47.9 years (men 51.2%, Karpoffsky scale 90% (62.8%), median tumor size of 5.1 cm, and partial complete tumor resection (22/21). Methylation MGMT test was performed in 31 cases and 17 showed unmethylated, 4 partially methylated and 10 methylated. TILs evaluation by HE showed low intensity (29 cases), intermediate intensity (1) and high intensity levels (9). Patterns of distribution was focal (9 cases), multi focal (13) and diffuse (17). Median CD3, CD4, CD8, CD20, CD68 and CD163 were 1.6%, 0.032%, 1.6%, 0.03%, 9.1% and 2.2% (regarding total cells), respectively. Higher CD163 level was associated to male (p=0.03) and larger size (p=0.05). Higher levels of CD8 was associated to larger size (p=0.05). Higher CD8 and CD20 were associated to unmethylated MGMT (p=0.041 and 0.013). Median follow-up was 14 months. Total only resection (p<0.05) and MGMT methylation (p<0.05) was associated to outcome. Lymphocyte infiltration (H&E) was not associated to outcome. Lymphocyte intensity (p=0.703), distribution (p=0.61). Longer OS was associated to lower CD4 levels (p=0.01). Conclusion: Tumor infiltrating immune cell and not common in GBM, CD8 and CD20 was associated to unmethylation, and CD4 and MGMT methylation were associated to prognosis.

#2395 Differentiation of carcinoma ex pleomorphic adenoma from pleomorphic adenoma by TERT promoter hypermethylation and elevated TERT mRNA expression. Seungjae Lee, 1 Sumit Borah, 1 Kurt Patton, 2 Armita Bahrami, 1 St. Jude Children’s Research Hospital, Memphis, TN; 2 Trumbull Laboratories, Memphis, TN.

The purpose of this study was to determine whether ii) genetic or ii) epigenetic aberrations of the TERT promoter, iii) elevated TERT mRNA level or iv) abnormal p53 expression pattern effectively distinguishes cases of carcinoma ex pleomorphic adenoma (CA ex PA) from pleomorphic adenoma (PA). PA is the most common salivary gland tumor which in rare cases progresses into an epithelial malignancy, termed CA ex PA. Fusion genes involving PLAG1, or less commonly HMGA2, are common in both PA and CA ex PA and thought to be early genetic drivers in their development. Although the key molecular events which occur during malignant transformation of PA are still unknown, some genetic and epigenetic changes proposed to play a role include amplification of PLAG1, HMGA2, MYC, ERBB2, or chromosome 12q and aberrant promoter hypermethylation of RASSF1, especially in combination with promoter hypermethylation of p16, TERT and WT1. The importance of TERT promoter hypermethylation for malignant transformation in PA may be underestimated since hypermethylation of that region most critical for malignancy in several cancers, termed the upstream of transcriptional start site (UTSS), has not previously been assayed in PA or CA ex PA. Point mutations or genomic rearrangement in the TERT promoter and elevated TERT mRNA levels are also characteristic of many cancers. We used a panel of 10 PA and 6 CA ex PA clinical samples to determine whether these alterations of TERT, or aberrant expression of TERT mRNA or p53 protein, could effectively distinguish CA ex PA from PA. UTSS hypermethylation in each sample was determined by treatment of genomic DNA with sodium bisulfite followed by MassARRAY and Sanger sequencing. Mutation and arrangement of TERT were screened for by Sanger sequencing and fluorescence in situ hybridization, respectively. p53 expression pattern was visualized by immunohistochemistry and TERT mRNA levels were measured by RT-qPCR. Mutation or rearrangements of the TERT promoter were not observed. However, all 6 CA ex PA samples harbored hypermethylated TERT promoter and had much higher TERT mRNA levels than the PA samples. Two of these samples also had aberrant p53 expression patterns, suggesting the presence of a TP53 missense mutation. None of the PA samples had aberrant p53 expression patterns, although 2 had hypermethylated TERT promoter and elevated TERT mRNA levels as compared with the other PA samples. The significance of this for long term outcome in these patients was unclear. Overall, these results suggest that TERT is commonly upregulated in CA ex PA and that this upregulation is often mediated through hypermethylation of the TERT promoter UTSS. Future largescale studies are warranted to determine the usefulness of TERT promoter hypermethylation for differentiating benign PA from CA ex PA, or the PAs at risk of malignant transformation.

#2396 Methylation of subtelomeric repeat D4Z4 in peripheral blood leucocytes is associated with biochemical recurrence in localized prostate cancer patients. Yuyan Han, Jeng Kim, Xifeng Wu, Jian Gu. UT MD Anderson, Houston, TX.

Background: Global DNA methylation may affect chromosome structure and genomic stability and is involved in carcinogenesis. In this study, we aimed to investigate whether methylation of pericentromeric repeat (NBL2) and subtelomeric repeat (D4Z4) in peripheral blood leucocytes was associated with the aggressiveness of prostate cancer (PCa). Methods: We measured the methylation status of different CpG sites of NBL2 and D4Z4 in 900 PCa patients via pyrosequencing after bisulfite treatment. We then used multivariate logistic regression to estimate the odds ratio (ORs) and 95% confidence intervals (CIs) for analyzing the association of NBL2 and D4Z4 methylation with the aggressiveness of PCa at diagnosis. We then analyzed the association of CpG sites with the risk of biochemical recurrence (BCR) in patients receiving radical prostatectomy or radiotherapy using a multivariate Cox proportional hazards model adjusting for age, BMI, smoking status, pack year, D’Amico risk groups, and treatments. In addition, we used the Kaplan-Meier survival function and log-rank tests to assess BCR-free survival associated with these markers. Results: There was no significant differences in the methylation level of NBL2 and D4Z4

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between clinically defined aggressive and non-aggressive PCa at diagnosis. However, the methylation of subtelomeric region D4Z4 was associated with the relatively low rate of BCR among patients, we combined the highest and second tertiles to increase statistical power. Patients in the second and third (highest) tertiles had an increased risk of BCR (HR=1.74, 95% CI, 1.14-2.65; p=0.0096) compared to patients in the lowest tertile after adjustment of age, BMI, smoking status, pack year, D’Amico risk groups, and treatments. We assessed 4 CpG sites in this region. In detailed analysis of each CpG site, the association was mostly attributable to methylation of the 4th methylation site of D4Z4 (HR=1.80, 95% CI, 1.19 - 2.72). Conclusion: These data suggest that methylation in the subtelomeric region D4Z4 may be able to predict worse prognosis of localized PCa patients.

Epigenetic silencing in clear renal cell carcinoma: KEAP1 promoter hypermethylation. Federico Pio Fabrizio,1 Manuela Costantini,2 Massimiliano Copetti,1 Anna Maria la Torre,2 Angelo Sparaneo,1 Andrea Fontana,1 Maria terGroningen, Groningen, Netherlands

Campus Bio-Medico University Hospital, Rome, Italy; 6 University Medical Center. Muscarella,1 Vito Michele Fazio1.

#2397 Epigenetic silencing in clear renal cell carcinoma: KEAP1 promoter hypermethylation. Federico Pio Fabrizio, Manuela Costantini, Massimiliano Copetti, Anna Maria la Torre, Angelo Sparaneo, Andrea Fontana, Maria terGroningen, Groningen, Netherlands

Campus Bio-Medico University Hospital, Rome, Italy; 6 University Medical Center. Muscarella, Vito Michele Fazio.

The Keap1/Nrf2 pathway is a master regulator of the cellular redox state through the induction of several antioxidant defense genes implicated in chemotherapy drugs resistance of tumor cells. An increasing body of evidence supports a key role for Keap1/Nrf2 pathway in kidney diseases and renal cell carcinoma, but data concerning the molecular basis and the clinical effect of its deregulation remain incomplete. Here we performed a comprehensive genetic and epigenetic analysis of the KEAP1 gene in 37 tumor-normal paired tissues of clear cell Renal Cancer (ccRCCs). Promoter methylation analysis was performed by using a quantitative methylation specific PCR assay in real time, whereas mutation scanning was performed on FFPE tissues by direct sanger sequencing of the exons 4-7 codifying for the DGR domain of the Keap1 protein. A tumor-specific DNA methylation of the KEAP1 gene promoter region was found in 18 out of 37 ccRCCs (48.6%) and a direct effects on the modulation of Keap1 mRNA levels was confirmed by in vitro 5-azacytidine treatment on three different ccRCCs cell lines. Analysis of an independent TCGA data set corroborate the epigenetic findings and reveals a significant correlation in multivariate analysis of epigenetic KEAP1 silencing with Overall Survival in ccRCCs. Our results further suggest that epigenetic deregulation of the Nrf2/Keap1 system could play a pivotal role in the cancerogenesis of ccRCCs. In addition identifying patients with KEAP1 epigenetic abnormalities may contribute to disease progression prediction and response to therapy in ccRCC affected patients.


The incidence of head and neck cancer as well as its mortality rates continues to increase, evidencing the need for diagnostic and prognostic markers that can improve clinical management of these patients. Histone Methyltransferases (HMT) are responsible for the methylation of histone tails, a mechanism important for the regulation of gene transcription, cell differentiation and proliferation. Although alterations on these enzymes have been associated with many types of cancer, their relationship with head and neck carcinogenesis is not yet understood. SUV420H1 and SUV420H2 are members of the SUV family of HMTs that contain the conserved SET domain, which catalyzes the addition of methyl groups to specific lysine residues, leading to chromatin compaction and gene repression. In the present study, we investigated the tissue expression profile of SUV420H1 and SUV420H2 using immunohistochemistry in 10 formalin-fixed paraffin-embedded human oral cancer samples and adjacent non-tumor tissues. We also investigated the alterations of SUV420H1 and SUV420H2 genes in head and neck cancer with in silico analyses from public databases. Immunohistochemistry revealed moderate to strong SUV420H1 and SUV420H2 expression in the majority of oral cancer cells, mainly with cytoplasmic staining in the invading tumor cells. In silico analyses using data from 530 head and neck squamous cell carcinoma samples (The Cancer Genome Atlas - TCGA, via ciBioPortal) revealed that amplification is the most frequent genetic alteration in SUV420H1. Considering all known lusine methyltransferases, SUV420H1 was among the top three with the highest frequency of amplification. For SUV420H2, the frequency for amplification and deletion alterations was similar. The SUV420H1 and SUV420H2 methyltransferase expression showed a high expression of mRNA in head and neck cancer samples, with a moderate correlation (R: 0.588) between genetic copy number alteration and mRNA level. SUV420H2 did not show significant altered expression. In addition, SUV420H1 mRNA overexpression was associated with decreased overall survival in head and neck cancer patients (p: 0.0233). Since these enzymes are important in chromatin compaction and gene transcription repression, an increase in their expression level, as detected in this study, could lead to changes in histone methylation pattern, resulting in aberrant silencing of genes essential to maintain normal cellular function, such as tumor suppressor genes. Taken together, our data indicates a possible role of SUV420H1 in head and neck carcinogenesis, with the potential to be used as a prognostic marker for the disease.


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#2399 Metabolic and epigenetic targets in multiple myeloma therapy. Laure Maneix, Fu-Yuan Shih, Polina Jakova, Joanne I. Hsu, Andre Catic. Baylor College of Medicine, Houston, TX.

The dynamic interaction of dynamic transcription factors (TFs) with promoters and enhancers allows cells to continuously adjust gene expression. Whereas the composition and binding of TFs at genomic sites is the focus of a widespread research effort, relatively little is known about how these complexes are being removed. We previously devised a method to detect genomic locations that are associated with nuclein protein turnover mediated by the ubiquitin-proteasome system (UPS). Multiple myeloma, the second most common hematopoietic malignancy, has become a model disease for drugs that interfere with the UPS through either blocking or facilitating protein elimination. The proteasome inhibitor Velcade, for instance, has become first-line treatment in myeloma. Yet, our knowledge of how myeloma cells are killed by this drug is completely limited. Our research is focused on defining how proteolysis regulates transcriptional dynamics in this disease and how this impacts Velcade sensitivity. Following proteosome inhibition, we are utilizing next generation sequencing to identify sites of nuclear protein turnover and evaluate epigenetic changes. Our aim is to identify TFs and epigenetic marks that may qualify as more specific targets for treatment compared to blunt proteosome inhibition. Our findings reveal that certain TFs, including metabolic and cell growth regulators, are particularly sensitive to Velcade. Among these transcriptional regulators, the co-repressor NCoR1 plays a key role in controlling cell metabolism by adjusting mitochondrial gene expression. We are currently validating the mechanism of NCoR1 degradation, with a particular emphasis on the new role of its E3 ubiquitin ligase Seven in absentia 2 (Siah2) in mitochondrial regulation, to better understand how this degradation pathway impacts myeloma proliferation and metabolism. This research project will contribute to our understanding of epigenetic and transcriptional dynamics in multiple myeloma. With the focus on gene programs that are continuously adapting or changing, we seek to unlock new attractive targets for molecular therapy.


Tamoxifen is widely used to treat estrogen receptor-alpha positive breast cancer. However, resistance to tamoxifen often prevent the success of endocrine therapy. Epigenetic change in tumors are associated with cancer development and progression. Although altered histone modification has been implicated in tumorigenesis, their role in endocrine resistance was little known. To identified tamoxifen-resistance associated histone methylation modifying enzyme, we examined the global histone H3 methylation patterns in tamoxifen-resistance MCF7 breast cancer cells. Our results show that, the levels of methylated histone H3 lysine 4 (H3K4) and histone H3 lysine 9 (H3K9) was upregulated in tamoxifen resistant MCF7 breast cancer cells. Next, we examined expression levels of H3K4 and H3K9 methylation modifying enzyme in tamoxifen resistance breast cancer cell lines. qRT-PCR results show that H3K4 methyltransferases (KMT2A and KMT2C) is upregulated in tamoxifen resistance breast cancer cells. Moreover, we observed that siRNA mediated knockdown of KMT2C was more susceptible to tamoxifen-induced growth inhibition. Finally, Analyses of publicly available tamoxifen patient data sets indicated that KMT2A and KMT2C levels are higher in patients after endocrine therapy failure (recurrence), compared to those of responders (non-recurrence). Our study indicates that elevated levels of KMT2A and KMT2C in the tamoxifen-resistance breast cancers may contributed tamoxifen resistance.
#2401 Connecting oxidative DNA damage to epigenetic alterations during inflammation-driven tumorigenesis. Ashley Maiuri, Ning Ding, Heather M. O’Hagan. Indiana University, Bloomington, IN.

Inflammation contributes to the development of a diverse array of diseases, including cancer. At sites of chronic inflammation, epithelial cells are exposed to high levels of reactive oxygen species and undergo cancer-associated DNA methylation changes, suggesting that inflammation initiates epigenetic alterations. Cancer cells are globally DNA hypomethylated, but have aberrant gains in promoter DNA methylation that transcriptionally silence tumor suppressor genes (TSGs), linking DNA methylation directly to tumorigenesis. However, the mechanisms of targeting and initiation for these stable disease-specific epigenetic modifications are unknown. We have linked inflammation and oxidative DNA damage to acute changes in the interaction of epigenetic silencing proteins with each other and the chromatin. Recently, we demonstrated that the mismatch repair (MMR) protein heterodimer MSH2-MSH6 participates in the oxidative damage-induced recruitment of DNA methyltransferase 1 (DNMT1) to chromatin and provided evidence that the role of DNMT1 at sites of oxidative damage is to reduce transcription. To connect these findings to epigenetic changes in tumors we use a model of inflammation-driven tumorigenesis where we infect mice with the human commensal bacterium enterotoxigenic Bacteroides fragilis (ETBF). ETBF infection causes colon inflammation that drives tumorigenesis in Multiple intestinal neoplasia (Min) mice. The central hypothesis for this work is that oxidative damage at sites of inflammation causes acute genome-wide changes in binding and recruitment of epigenetic silencing proteins that results in acute epigenetic silencing of genes in tumors that form at the sites of exposure. Using genome-wide DNA methylation techniques we identified TSGs with increased promoter DNA methylation and decreased expression in tumors from ETBF infected Min mice as compared to normal tissue. In Min mice lacking expression of Msh2 in their intestinal epithelium (Msh2tm109Vcm110) there is a loss of the inflammation-induced change in binding of epigenetic proteins that occurs in Min mice even though the cytokine response to infection is normal. Because they lack functional MMR, ETBF infection of Msh2tm109Vcm110 mice causes an increase in colon tumor number and these tumors are positive for microsatellite instability, unlike tumors from ETBF infected Min mice. Importantly, DNA hypo- and hypermethylation changes in tumors from Msh2tm109Vcm110 ETBF infected mice are reduced both globally and at promoters of TSG islands of candidate TSGs as compared to tumors from Min ETBF infected mice. Altogether, this work suggests a novel mechanism by which oxidative damage induces acute epigenetic changes through the interaction of DNMT1 with MMR proteins and that this acute change drives DNA methylation alterations during tumorigenesis.


Our major goal is to determine the role of Chromodomain-helicae-DNA-bind ing protein 7 during breast cancer (BC) metastasis. This protein is an ATP-dependent nuclease-remodeling factor and is critical for embryonic development in both animal models and human patients. Our most recent studies provide direct evidence suggesting its novel role in repressing BC metastasis. We found that this protein, but not the mRNA, is highly expressed in a low-metastatic breast cancer cell line (Mcf-7), while its expression is much reduced in metastatic cell lines (MDA-MB-468 and MDA-MB-231). Furthermore, this protein is predominantly localized in the cytoplasm of 468 and 231 cells, in contrast to its nuclear localization in Mcf-7 cells. In the cytoplasm of 468 and 231 cells, this protein is colocalized with a lysosome marker, suggesting that in high metastatic BC cells, this protein is exported to the cytoplasm to be degraded in lysosomes. Blocking the activity of lysosomes increased expression of this protein in high metastatic BC cell lines. This result suggests a mechanism accounting for the reduced expression of this protein in high metastatic BC cell lines. To further support the potential clinical relevance of our results, this protein is localized in the nucleus of almost all duct epithelial cells in normal breast tissues; however, in a large portion of epithelial cells of breast tumors, this protein is localized in cytoplasm. Thsby reporter and mutagenesis analysis, we identified the nuclear exporting signal (NES) sequence responsible for exporting this protein into cytoplasm. In the next set of experiments, we attempted to determine the function of this gene in BC metastasis. We found that knocking down expression of this gene in 468 cell significantly increased their migration and invasion. We next applied the CRISPR genome editing technique to mutate the critical amino acids within the NES region in the endogenous gene locus and found that the amount of protein in the nucleus is significantly increased compared to wild type cells. Migration/invasion of these cells was also dramatically decreased. At the molecular level, we found that forced expression of this protein in nucleus reduced expression of epithelial markers and increased expression of epithelial-mesenchymal-transition markers. We are now applying ChiP-Seq and RNA-Seq approaches to determine direct regulatory network of this protein in 231 cells. We will further test whether this protein acts through modulating the epigenetic status of the enhancers/promot ers to regulate expression of its target genes to repress BC metastasis. In summary, our data collectively suggest that Chromodomain-helicae-DNA-binding protein 7 is a novel epigenetic regulator to repress BC metastasis. To the best of our knowledge, our study represents the first to address the activity of this protein in BC.

#2404 Paternal intake of an obesity-inducing diet before conception modulates the risk of pancreatic cancer in offspring in a mouse model. Raquel Santana Da Cruz, Johan Clarke, Ali Baird, Hong Cao, Carlos Benitez, M. Idalia Cruz, Sonia De Assisi. Georgetown University Medical Center, Washington, DC.

Pancreatic cancer is a mostly untreatable malignancy, with 5-year survival rates of about 5%. Lifestyle and dietary factors have been associated with pancreatic cancer risk. Particularly, the consumption of high-fat diets and obesity (and underlying metabolic dysfunction) have been linked to increased susceptibility to this cancer. Recent studies have shown that the paternal diet and life-style can have a significant influence on offspring’s health via epigenetic information transmitted in the germ-line. Paternal overweight before conception has been shown to increase offspring’s susceptibility of developing metabolic diseases and some types of cancer. Here, we evaluated the effects of paternal overweight in the susceptibility of pancreatic cancer in offspring using the P48Cre+/KrasG120V mouse model of pancreatic cancer. LSL-KrasG120V and P48Cre+/KrasG120V male mice were fed either an obesity-inducing (OID) or control (CO) diet for 8 weeks from weaning to sexual maturity. After this period, OID fed and CO fed male mice were housed together with 7-week-old female mice, with free access to CO diet, for 3 days. Pregnant dams were kept on the CO diet during pregnancy and after giving birth. Pups were weaned from mothers at 21 days of age, fed a standard chow diet for the extent of the study and weighed weekly. The offspring of CO or OID fathers were used to study body weight, metabolic parameters and pancreatic cancer development. Fathers fed an OID gained significantly more weight (CO 15.7±1.0 g; OID 19.7±1.3 g; p=0.02) and had higher leptin levels (p=0.04), compared to CO group fathers. Body weight analyses of OID and CO offspring, showed gender-specific effects. While the OID female offspring had higher weight at birth (p=0.005) and at weaning (p=0.02), compared to CO group, no significant differences were observed between the CO and OID male offspring. Those gender-specific differences were also observed in metabolic parameters such GTT with the OID male, but not female, offspring showing impaired glucose tolerance (p=0.0004) compared to CO. While the monitoring period is still ongoing, the 8-week old OID offspring present gender-specific differences in susceptibility to pancreatic cancer: Males OID offspring have higher number/area (481±25; 2.6±0.3) of pancreatic intraepithelial neoplasia (Panln), compared to CO (359±61; 2.0±0.5). On the other hand, female OID offspring have similar levels of Panln, but have higher incidence of PDA’s compared to CO. In conclusion, an ancestral history of overweight through the paternal lineage may be associated with an increased susceptibility to pancreatic cancer development in adulthood. The mechanisms mediating this effect remain to be elucidated.

#2405 The 8p11 amplicon oncogenes ASH2L and NSD3 alter the epigenomic landscape and provide the foundation for novel application of epigenetic therapy in luminal B breast cancers. Jamie N. Mills, Stephen P. Ethier. Medical University of South Carolina, Charleston, SC.

The 8p11-12 genomic region is amplified in 15% of breast cancers and is associated with poorer prognosis, especially in the luminal B (ER+ /HER2-) subtype. This region harbors several oncogenes, three of which are epigenetic modifiers of chromatin: NSD3 (WHSC1L1) and ASH2L, which are histone methyltransferases, and KAT6A, a histone acetyltransferase. NSD3 preferentially di-methylates histone 3 lysine 36 (H3K36me2) in gene bodies to facilitate elongation, and ASH2L, primarily trimethylates histone 3 lysine 4 (H3K4me3) in promoter regions, which is also associated with active transcription. Previous work in the ampiclon-bearing cell line SUM-44 implicated NSD3 in growth, survival, and overexpression and estrogen-independent activation of the estrogen receptor. Current work is focused on elucidating the mechanism behind these transforming features and exploring the epigenomic interactions of 8p11 oncogenes. Chromatin immunoprecipitation and high throughput sequencing (ChIP-seq) using an NSD3 antibody confirmed the presence of this factor in the gene body of ESR1, as well as several other important genes. As NSD3 lacks the ability to bond chromatin directly, it relies on adaptor proteins such as BRD4, a member of the BET bromodomain family that binds acetylated histones such as those generated by KAT6A. Indeed, we have shown that BRD4 binds NSD3 in SUM-44 cells. Similar to our previous findings with NSD3, knockdown of BRD4 and ASH2L reduced cell proliferation and clonogenic potential. To examine the influence of ASH2L on histone methylation in gene promoters, we performed ChIP-seq using an H3K4me3 antibody. The data showed a dramatic decrease in the number of peaks called following knockdown of ASH2L and peaks in
the NSD3 promotor disappeared. This finding solidifies the connection between these oncogenes and their cooperative regulation of the epigenome, and therefore the transcriptome, in this cell line, explaining the effects of NSD3 on cell growth and behavior. Amplification and overexpression of oncogenes in the 8p11 region is most prevalent in ER+ breast cancer, treated clinically with endocrine therapies. Patients with poor response to endocrine therapies have higher levels of 8p11 oncogenes in their disease. This study and continued investigation of 8p11 amplon oncogenes opens an important new treatment opportunity for these patients: epigenetic therapy. Previous work identifying these patients as candidates for novel anti-estrogen SERD compounds suggests that combination therapy with a BET bromodomain inhibitor could have major therapeutic benefit in a patient group currently without treatment options, thereby improving patient outcomes by a novel mechanism in luminal B breast cancer.

#2406 LSD1 promotes castration-resistant prostate cancer cell survival independently of the androgen receptor and of histone demethylation. Archana Sehrawat,1 Linda Gao,2 Junior Tayou,1 Armand Bankhead,2 Laura M. Heiser,3 Carly J. King,1 Yuiliang Wang,1 Jacob Schwartzman,1 Joshua Urrutia,1 Daniel J. Coleman,1 Sheila Weinmann,2 Bhaskar V. Kallakury,3 Deborah L. Berry,2 Reina Haque,2 Stephen K. Van Den Eeden,1 Tomaxx M. Beer,1 George V. Thomas,1 Shannon McWeeny,1 Joshi J. Alumkal1.1 OHSU Knight Cancer Institute, Portland, OR; 2Kaiser Permanente NW, Portland, OR; 3Georgetown University Medical Center, Washington, DC.

Background: Androgen deprivation therapy (ADT) or interference with androgen receptor (AR) function is the principal treatment for advanced prostate cancer. However, progression is universal, and therapies following the emergence of castration resistance do not offer durable control of the disease. Lysine specific demethylase 1 (LSD1) is a histone demethylase and a key regulator of gene expression in cancer. Prior work demonstrates that LSD1 may act as a cofactor of the AR in androgen-dependent prostate cancer cells. In this report, we describe a distinct role of LSD1 as a driver of proliferation and survival of castration-resistant prostate cancer (CRPC) cells independently of the AR and independently of histone demethylation. Methods: We used gain and loss of function studies to determine the importance of LSD1 for survival of prostate cancer cells. To identify transcriptional networks that contribute to cell survival, we suppressed LSD1 with RNAi and measured gene expression changes with microarrays. To determine the importance of histone demethylation in regulation of these gene networks, we suppressed LSD1 and measured levels of LSD1 canonical histone substrates (H3K4me2 and H3K9me2) genome-wide with chromatin immunoprecipitation-sequencing. Results: Cell viability assays demonstrated that LSD1 is important for proliferation and survival of CRPC cells independently of the AR. Microarray studies demonstrated that LSD1 regulates key pathways in CRPC through demethylation of non-histone substrates or via a scaffold function—mechanisms we are currently investigating. In summary, LSD1 contributes to CRPC cell survival through non-canonical mechanisms and represents an attractive therapeutic target in lethal prostate cancer.

#2407 Inhibition of gene expression during non-alcoholic steatohepatitis (NASH)-related hepatocarcinogenesis is mediated by histone H4 lysine 16 deacetylation. Kostiantyn Dreval,1 Aline de Conti,1 Orish Ebere Orisakwe,1 Frederick A. Beland,1 Igor P. Pogribnyi,1 JFA-DNA National Center for Toxicological Research, Jefferson, AR;2University of Port Harcourt, Rivers State, Nigeria.

Hepatocellular carcinoma (HCC) is one of the most aggressive human cancers and the incidence is steadily increasing worldwide. The molecular mechanisms leading to the development of HCC consist of genetic and epigenetic aberrations, including changes in global and gene-specific DNA methylation and altered expression of several classes of non-coding RNAs. While changes in DNA methylation and microRNA expression during the development of HCC are well-studied, the role of aberrant post-translational histone modifications remains unexplored. Using a mouse Stellic Animal Model (STAM) of NASH-associated liver carcinogenesis, we have investigated alterations in hepatic histone modifications at steatotic (6 weeks), fibrotic (12 weeks), and full-fledged HCC (20 weeks) stages of the HCC development. NASH-related liver carcinogenesis was characterized by a progressive decrease in the levels of histone H4 lysine 20 trimethylation (H4K20me3) and histone H4 lysine 16 acetylation (H4K16ac), with the greatest decrease occurring in full-fledged tumors. Mechanistically, the deacetylation of H4K16ac was associated with nuclear protein 1 (Nupr1)-mediated inhibition of histone lysine acetyltransferase Kat7. In addition to a global loss of hepatic H4K16ac, there was a significant decrease of gene-specific H4K16ac, as indicated by a reduced level of H4K16ac in 16 out of 20 HCC cell lines and the levels of gene expression. The extent of gene-specific H4K16 deacetylation were positively correlated with the level of gene transcription (r = 0.965, p < 0.01). These results indicate that a reduction of global and gene-specific H4K16ac is a key pathophysiological mechanism contributing to the development of NALFD-derived HCC.

#2408 Epigenomic characterization of MSC from myeloid malignancies. Manuel Rodriguez-Paredes,1 Stefanie Geyh,2 Mahshid Gazorpak,1 Julian Gutekunst,1 Felix Bormann,1 Rainer Haas,3 Thomas Schröder,1 Frank Lyko1.

1OHSU Knight Cancer Institute, Portland, OR; 2University of PortHarcourt, Rivers State, Nigeria; 3University of Düsseldorf Medical Faculty, Düsseldorf, Germany.

Ineffective hematopoiesis is a hallmark of myeloid malignancies such as Myelodysplastic Syndromes (MDS) or Acute Myeloid Leukemia (AML). Our previous research has shown that mesenchymal stromal cells (MSC), one of the main cellular regulatory components of the bone marrow niche and essential for the differentiation of new blood cells from Hematopoietic Stem Cells (HSC), are molecularly and functionally impaired in MDS and AML patients. More specifically, these cells display reduced proliferative and differentiation capacities, an increased cellular senescence, or the altered expression of key hematopoietic factors, deficits that can provide an explanation for the cytopenia associated with these diseases. But most interestingly, we have also discovered that MDS- and AML-derived MSC present aberrant histomethylomes. Our data, based on 450k DNA methylation microarrays, show that these abnormalities are shared by the MSC from both malignancies and affect key transcription factors like TFIIBX5, PITX2 or HOX86, formerly linked to differentiation and development. This results can account for some of the defects observed in the disease-related MSC and represent an important paradigm for cancer-induced epigenetic changes in normal cells. Aiming to further explore the importance of this epigenetic deregulation, we performed RNAseq experiments using MSC from healthy donors as well as from MDS and AML patients. This approach not only suggests a profound impact of the epigenetic aberrancies on gene expression, but also allowed us to identify TGFβ as the soluble factor that, released by the malignant cells in the bone marrow, appears to trigger the above-mentioned defects in the MDS- and AML-derived MSC. Indeed, our results show that, when treated with TGFβ, healthy MSC undergo the deregulation of TFIIBX5, PITX2 or HOX86 and develop some of the observed aberrant phenotypes. Conversely, the treatment of MDS- and AML-derived MSC with a TGFβ antagonist like SD208 can abrogate these effects. Finally, our preliminary DNA methylation data reveal a very similar deregulation in the methylome of MSC from Multiple Myeloma (MM) patients, strongly suggesting that the same scenario could also be common to other hematological malignancies from the lymphoid branch.

#2409 Loss of LIT1DI suppresses Notch1 induced LINE1 methylation and genomic stability. Chia-Chia Chu1,1 Wei-Chen Huang,2 Kuan-Der Lee,1 Chih-Cheng Chen,1 Chia-Chen Hsu,2 Mei-Ling Kang,1 Yu-Wei Lee,1 Shue-Huei Hsiao1.1National Chung Chern Medical College, ChiaYi County, Taiwan; 2Chang Gung Memorial Hospital, ChiaYi County, Taiwan.

The silencing of endogenous retrotransposon like LINE1 is critical for the maintenance of genomic stability and the LINE-1 type transposase domain containing 1 (LIT1DI) gene possesses the repeated, putative LINE-1 RNA-binding domains and was hypothesized to regulate the activity of LINE-1 through DNA methylation. To verify if LIT1DI is responsible for LINE-1 silencing and the maintenance of somatic genome stability, CRSPR/Cas9 system was used to knock out (KO) LIT1DI locus in gastric, colon and breast cancer cells so as in the mesenchymal stem cells (MSCs). We observed that LIT1DI KO blocked the entrance of SV39H1, histone methyl transferase, into cell nucleus which suppressed the tri-methylation of histone 3 at lysine 9 (H3K9me3) and LINE-1 methylation. A global demethylation and reduced HDAC recruitment were also observed. The global distribution of CTCF binding loci was also distorted as well as the bivalent histone marks. Further, LIT1DI KO distorted normal distribution of RassFlaX expression, cytoskeleton conformation and therefore cell stiffness. The MSC-to-neuron differentiation was also blocked by the KO. Further, we found that overexpressed Notch1 increased DNA methylation within LINE1 promoter, and this increase was attenuated by LIT1DI KO. Therefore, we concluded that the external signals like Notch1 affect LINE1 methylation and genome stability through possible LINE1 interacting LIT1DI. Since LIT1DI hypermethylation was observed in colon (n=100), gastric (n=19) and breast (n=79) cancers, LIT1DI abnormality is then a candidate for cancer biomarker. (Sup-
Non-coding mutations found in regulatory elements can function as driver mutations in breast cancer by changing the binding affinity of transcription factors for DNA, thereby resulting in direct change of expression of genes that promote cancer development. Identifying such additional driver mutations can reveal the molecular mechanisms favorable to breast cancer development and progression, as well as reveal new biomarkers to better tailor personalized/precision cancer medicine. In this study we have collected 20 primary luminal breast tumors and optimized experimental workflow to dissociate solid tumors and map open chromatin using ATAC-seq. In our initial experiments using ATAC-seq profiling of bulk tumor tissues, we were able to call an average of 15x10^3 peaks. Subsequently, flow cytometry analysis showed the presence of 15-25% of immune cells in our primary tumors. Therefore, we have optimized a workflow to eliminate immune cells and focus mainly on epithelial tumor cells. Primary breast tumors were digested using collagenase and further dissociated with dispase. Cells were sorted into two populations (mammary epithelial and immune cells) using anti-CD45, anti-CD49f and anti-EpCAM antibodies. Sorted mammary epithelial cells were then used for ATAC- and RNA-seq library preparation as well as for generation of patient derived organoids. Our new workflow resulted in an increased number of called peaks (40x10^3 vs 15x10^3), as well as a significant increase in the percentage of unique peaks compared to bulk sequencing (45% vs 15%). By refining our workflow to enrich for tumor content, we will continue our ongoing effort to profile these open chromatin regions and contextualize the mutations within a large cohort of luminal breast cancers using targeted sequencing. These data will be compared with large-scale whole genome data generated by our group and made publicly available by others.

It is well recognized that methylation/demethylation of not only DNA, RNA, Proteins but also methylation of small molecules play major roles in modulation of the epigenome, transcriptome, neurotransmitter uptake and metabolic regulation. Recent biochemical and biological data suggest that the activity of these enzymes and their expression level are under very strict regulation and any abnormal alteration in either one or both results in a wide variety of pathogenic conditions such as cancer, inflammation, and neurodegenerative diseases. In addition to chromatin modulation, altered methylation of DNA and more recently mRNA have been recognized to regulate the transcriptome and the rate of message translation and stability. Furthermore, methylation of small molecules such as catechols and nicotineamide play critical roles in neurotransmitters uptake and function, and metabolic regulation, respectively. Thus, pharmacological modulation of these enzymes by small molecules will be beneficial in developing novel therapeutics for multiple unmet medical needs. Towards this goal of searching for activators/inhibitors of these enzymes for the development of next generation of drugs, screening assays for these modulators are urgently needed. To address these unmet needs, we have developed a novel assay that monitors the activities of these enzymes and their modulation by small molecules. The assay is bioluminescent based, HTS formatted and highly sensitive. A unique feature of this assay is its universality since it is based on monitoring the formation of the universal product S-adenosylhomocysteine (SAH), i.e., capable of detecting changes in activity of a broad range of methyltransferases such as DNA, RNA, protein, and small molecules. In addition, the assay has been validated for all classes of protein methyltransferases (Lysine and Arginine), and with different types of substrates (small peptides, large proteins, or even nucleosomes). This enables determining the specificity of these enzymes and their substrate requirements. The assay has high signal to background, low CV., robust (Z’ value > 0.7), and has been validated using various plate densities such as 96-, 384, and 1536-well plates. A strong feature of this assay is its utility with broad range of substrates concentrations or the composition of the substrates (short vs. long peptides), thus enabling the generation of kinetic data and determining the mechanism of action of various modulators of methyltransferases of interest.

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ANSA, and p < 0.05 was considered statistically significant. The Ethical Review Board of the academic institution approved this study. Results: Fibromodulin expression at protein level was evaluated in 378 cores representing PCa (143 cores), HGPN (58), PIA (102), and BPT (78). Mean fibromodulin expression in cores with PCa, HGPN, and PIA was significantly higher than mean of fibromodulin expression in normal glands (p < 0.05). There was a higher mean staining score in PCa (156, 95% CI: 144–167), compared to HGPN (151.4, 95% CI:109–153), PIA (128, 95% CI:117–138), and BPT (33.3, 95% CI:27–38). Thus, fibromodulin expression increased across the PCa progression model. Conclusions: The expression pattern of fibromodulin showed a significant increase from benign prostate tissue through pre-cancerous lesions to PCa. These findings suggest that the fibroblastic phenotype may be involved in the process of prostate cancer progression, with low levels in pre-cancerous lesions and higher levels in cancer tissue. Additional studies are required to determine the biological role and potential clinical implications of fibromodulin in prostate cancer.

#2415 Genome-wide alterations in gene expression of prostate cancer (PC) cells surviving neo-adjuvant androgen deprivation therapy. Anna C. Ferrari,1 Haten Sabsawy,1 Mark Stein,1 David Foran,1 Ying Chen,2 Srinivasan Yegnasubramanian3.1 Rutgers Cancer Institute of New Jersey, New Brunswick, NJ; 2Johns Hopkins University Sidney Kimmel Cancer Center, Baltimore, MD.

Background: Although androgen deprivation therapy initially decreases PC tumor burden, resistance to further androgen receptor (AR)-directed treatments or chemotherapy is inevitable once CRPC is established. We postulated that the stress of ADT triggers widespread alterations in expression that renders a metastable physiologic state conditioned by epigenetic changes that might be initially reversible by targeting non-androgen pathways. We conducted a pilot study to explore genome-wide expression alterations in PC surviving 3 months ADT (eADT). Methods: mRNA from 7 frozen microdissected PC foci from the dbGAP study to explore genome-wide expression alterations in PC surviving 3 months ADT (eADT). Methods: mRNA from 7 frozen microdissected PC foci from the NJ;2JohnsHopkinsUniversitySidneyKimmelCancerCenter,Baltimore,MD.

#2417 Large scale integrated transcriptomic and epigenetic profiling define the molecular hallmarks of HGSOCS and disease origins. Karen Lawrenz,1 Marcos Abraao,2 Felipe Segato,3 Janet M. Lee,1 Simon Coetzee,2 Ji-Heui Seo,2 Matthew L. Freedman,1 Dennis Hazlett,1 Simon Gayther,1 Houtan Noushmehr4.1Rutgers Cancer Institute of New Jersey, New Brunswick, NJ; 2Johns Hopkins University Sidney Kimmel Cancer Center, Baltimore, MD; 3Dana-Faber Cancer Center, Boston, MA; 4Henry Ford Hospital, Detroit, MI.

High-grade serous ovarian cancers (HGSOCS) are the most common subtype of epithelial ovarian cancer. Pathways driving HGSOCS development are poorly understood, and the cellular origins are debated in the literature. Previously, ovarian surface epithelial cells (OSECs) were thought to be cellular precursors of HGSOCS, but there is now strong evidence that at least half of all HGSOCS arise from fallopian tube secretory epithelial cells (FTSECs). We took a large-scale integrated transcriptomic-epigenomic profiling approach to define the molecular hallmarks of HGSOCS and to explore the disease cell-of-origin. RNA sequencing was performed on 121 OSEC cultures and 84 FTSEC cultures and integrated with 394 HGSOCS transcriptomes profiled by TCGA. We identified 4747 gene sets that differentiate OSECs and FTSECs, and a pathway analysis of this gene list identified ovarian cancer genes as highly enriched (p = 2.2x10^-8), with ZEB1 and estrogen the most significant predicted upstream regulators (p < 3.7x10^-7). We integrated gene expression data with clinical metadata to identify signatures between gene expression signatures and clinical variables and found that in patients diagnosed with ovarian cancer, OSEC and FTSEC profiles converged, suggestive of a field carcinization phenotype occurring in the normal OSECs on the unaffected ovary contralateral to a clinically diagnosed ovarian carcinoma. Transcriptomic signatures for OSECs, FTSECs and HGSOCS were then merged with superenhancers defined by performing chromatin immunoprecipitation for acetylated H3K27 profiled in primary HGSOCS tissue specimens. We detected many more superenhancer-target gene relationships in the normal cells than in the tumor cells, suggesting superenhancer fingerprints define the cell of origin rather than determining upregulation of oncogenes driving cancer. To explore this further we determined the similarities of HGSOCS to OSEC and FTSEC using a machine learning model. Using a pre-defined gene signature of OSECs and FTSECs, our model (high specificity and sensitivity, 99% CI = 0.77–1, p-value = 6.1^-7) was applied to HGSOCS and found that HGSOCS were classified as FTSEC-like. Interestingly, the mesenchymal subtypes were enriched as FTSEC-like. Moreover, a subset of HGSOCS were characterized as OSEC-like, and these tumors were associated with poorer outcomes. Our data describe molecular subgroups within HGSOCS precursor tissues as well as defining the transcriptional networks that define HGSOCS precursor tissues, drive HGSOCS development; furthermore our model provided the landscape between the epigenetic landscape and the transcriptome in these cell types. In closing, these large-scale integrative analyses support a predominantly FTSEC origin for HGSOCS, but reinforce conclusions that OSECs cannot be excluded as origins of HGSOCS, and may represent a subset of tumors with a different biology.
Characterization of the PAX8 regulatory network in epithelial ovarian cancer. Rosario I. Corona,1 Emily Adler,2 Janet M. Lee,1 Norma Rodriguez-Malave,1 Paulette Mhawech-Fauceglia,2 Heidi Sowter,3 Dennis J. Ha- 

the biological role of PAX8 during cancer initiation and development is poorly understood, and the genome-wide transcriptional targets have yet to be comprehensively identified. Using stable, mock-transfected HEYA8 and IGROV1 EOC cell lines we show that PAX8 knockdown reduces cell proliferation in vitro and tumor growth in vivo. To understand how PAX8 regulates neoplastic phenotypes in cancer cells we performed RNA expression profiling by microarray in HEYA8 and IGROV1 before and after PAX8 knock- 

down. We also performed chromatin immunoprecipitation followed by next generation sequencing (ChiP-seq) for PAX8 and a marker of active chromatin (H3K27ac) in both cell lines. De novo motif discovery in the ChiP-seq profiles identified a PAX-like binding motif and several potential PAX8 cofactors in IGROV1 that are not enriched in HEYA8. These cofactors include members of the TEAD and Sp/KLF families of transcription factors. We divided the genes from our differential expression (DE) analysis in PAX8 knockdown into three non-overlapping categories: (a) direct regulatory targets (RTs), i.e. DE genes with PAX8 binding sites in their promoter regions; (b) putative enhancer RTs, where DE genes and PAX8 binding sites reside within the same topological association domain (TAD) defined from embryonic stem cells; and (c) indirect RTs. Our findings reveal that PAX8 can function as either an activator or a repressor, and that PAX8 regulates a very different set of genes in each cell line, as only a small fraction RTs (~50%) is shared between HEYA8 and IGROV1. Nonetheless, independent pathway enrichment analysis reveals four pathways downstream of PAX8 that are common to both cell lines: DNA replication, cellular response to lipopolysaccharides, TNFA signaling via NFkB and anatomical structure morphogenesis. Individually, we discovered enrichment for G2M checkpoint and EMT signaling in HEYA8, and tissue morphogenesis and mesenchymal cell development in IGROV1. Collectively, our results suggest that PAX8 regulates cell proliferation and cancer promoting processes via highly tissue-specific regulation of common oncogenic pathways.

#2419 Genome-wide profiling of PAK4 DNA-binding sites and transcriptionome reveals its potential transcriptional control on DNA repair-related genes in ovarian cancer cells. Ivy Tsz-Lo Wong,1 Oscar Gee-Wan Wong,1 Yim- 

qin,1 Junwen Wang,2 Annie Nga-Yin Cheung1.

The University of Hong Kong, Hong Kong; Mayo Clinic Arizona, Scottsdale, AZ.

Oncogenic roles of the p21-activated protein kinases (PAKs) in relation to cell growth and migration through various signalling cascades has been well studied in multiple cancer types. Such roles might be contributed largely by cytoplasmic PAK4 as most of the known PAK4 signalling events occur in the cytoplasm. However, recent studies have demonstrated that PAK4 is capable of shuttling between nucleus and cytoplasm. The precise function of nuclear PAK4 (nPAK4) remains poorly understood, as most of the known PAK4 signalling events occur in the cytoplasm. Herein, we aimed to identify nPAK4-regulated genes by delineating the PAK4-binding landscape in ovarian cancer genome and mapping the PAK4 transcription in ovarian cancer cells. PAK4-enriched genomic regions were identified by chromatin immunoprecipitation with a PAK4 antibody followed by next-generation sequencing (ChiP-seq) in OVCAR3 cells. PAK4 transcription data was generated using RNA extracted from SKOV3 cells stably expressing PAK4 in the Affymetrix GeneChipTM Human Transcriptome Array 2.0 system. Gene lists generated from both ChiP-seq and microarray dataset were subjected to pathway enrichment analysis by Partek® PathwayTM. Among the 20 most significantly enriched pathways from microarray studies, several pathways in which PAK4 has no known reported function by far have been identified as potential cancer drug targets using cBioPortal database. Our results support the potential role of PAK4 in regulating pathways that have been implicated in tumorigenesis. The list of genes that are potentially regulated by PAK4 includes: cyclin-dependent kinase 7 (CDK7), E2F1, and the ETS domain transcription factor ERG. These genes are involved in various cellular processes, including cell proliferation, differentiation, and survival. Further experiments are needed to validate the functional role of PAK4 in regulating these pathways.
ray analysis results in our lab correctly identified a subset of about 300 genes that when mutated altered the chemoresistance of the ovarian epithelial cells in culture. Of the genes identified in the analysis we further set out to characterize oncogenes and tumor suppressor genes that interact with the guardian of the genome, TP53, to determine if we could elucidate the mechanism by which it increases survival. Using several in-vitro assays, we determined if the loss of p53 in conjunction with SOX9 decreased the level of apoptosis in response to carboplatin. Furthermore, in cells with mutated p53/ SOX9 and epigenetic regulators may present a valid treatment option for increasing carboplatin sensitivity in resistant patients.

#2422 Simultaneous detection of activating somatic DNA mutations and expressed fusion transcripts from lung tumor FFPE samples. A. McGarry Houghton,1 Gavin Meredith,2 Julia Kargl,3 Jill McKay-Fleisch,4 P. Martin Ross,2 Anisha Kharkia,1 Afshin Mashadi-Hossein,3 Dae Kim,7 Joseph Beechem,1 Fred Hutchinson Cancer Research Center, Seattle, WA; 2NanoString Technologies, Inc., Seattle, WA.

Worldwide, lung cancer is the most commonly diagnosed form of cancer with a survival rate among the lowest. Combined, somatic mutations (in the form of SNVs and InDels) and gene fusions, account for the majority of interpretable and actionable genomic alterations. Importantly, this typically requires the analysis of DNA and RNA from limited amounts of FFPE-preserved specimens. Currently, these analyses typically require complex sample pre-processing for separate platforms or separate complex library preparation methods for assessment by high throughput sequencing. To provide a unified and simpler alternative, NanoString’s molecular barcoding technology has been modularized to permit simultaneous digital measurement of cancer-relevant targets that span these two analyte classes. Novel ‘SNV’ probes enable sensitive and specific identification of DNA mutant allele sequences down to a level of detection of ≤ 5% from 5 ng of FFPE-extracted genomic DNA. Fusion transcripts are detected with 5’/3’ imbalance probes and toehold-mediated junction probes. This dual analytic workflow requires just a single 5-10 micron section of FFPE tissue and provides to sample-to-answer results within approximately 5 minutes of hands-on time per sample after nucleic acid extraction.

To demonstrate utility, 37 lung cancer samples were assayed simultaneously with an SNV panel that targets ≥100 solid tumor somatic mutations and a lung cancer fusion gene panel that provides general evidence of ALK, RET, and ROS1 gene fusion events along with specific detection of 35 unique fusion transcripts that correspond to known break-points. In this particular cohort, 16 samples were positive for activating KRAS SNVs (one of which was also positive for an activating STK11 variant), 3 were positive for activating EGFR mutations including two SNVs and an 18-base InDel and one was positive for an activating KIF5B/RET fusion transcript. Positive mutation calls obtained with the SNV panel could only be confirmed by whole-exome sequencing (average depth of 100X) for 13 of 20 variants detected; however, ultra-deep (average depth of 4400X) targeted sequencing revealed that the 7 additional panel-detected mutations were, in fact, present. Measured against the sequencing datasets, the SNV panel provided 100% sensitivity, specificity, accuracy and precision, indicating the potential clinical utility of the protocol as a platform for FFPE specimen analysis.

Combined, these results show that these two important classes of activating mutations can be readily and efficiently assayed together on a NanoString nCounter® system (for research use only).

#2423 Bronchial airway gene expression signatures in mouse lung squamous cell carcinoma and their modulation by cancer chemopreventive agents. Donghao Xiong,1 Jing Pan,1 Qi Zhang,2 Eva Szabo,1 Mark S. Miller,3 Ronald A. Lubet,3 Ming You,1 Yian Wang,1 Yian Wu,4 1Fred Hutchinson Cancer Research Center, Seattle, WA; 2NanoString Technologies, Inc., Seattle, WA; 3Division of Cancer Prevention, National Cancer Institute, Bethesda, MD; 4Department of Pathology, Johns Hopkins Univ., Baltimore, MD; 5Moscow State University, Moscow, Russian Federation; 6Univ. of California, San Diego, La Jolla, CA.

This project develops a novel experimental technique to perform ChIP-Seq (chromatin immunoprecipitation with massively parallel DNA sequencing) analysis of chromatin structure in primary tumor tissues from high risk HPV-related head and neck squamous cell carcinomas (HPV+ HNSCC). Recent data suggest that chromatin structure is the central regulator and predictor of cancer-specific expression and mutagenesis landscape of diseased cells. Genome-wide gene expression dysregulation in many tumors, including HPV+ HNSCC, are incompletely described by current knowledge. Methods for study of chromatin structure in primary tumor tissues are needed to better understand the role global epigenetic changes may play in tumor development. However, ChIP-Seq, which is the state-of-the-art method of elucidating chromatin structure, until now, has not been reliably performed on any HNSCC samples. Because chromatin structure is disrupted at room temperature, ChIP-Seq is especially complicated for primary patient tissues, which are primarily obtained as surgical waste after pathology review. Snap freezing of leftover waste surgical tissues and further tissue thawing for the analysis decreases chromatin structure integrity necessary for highly sensitive ChIP-Seq methodology, especially for tumor samples with chromatin structure deformed during carcinogenesis. To improve the chromatin structure integrity in tumor sample we added a xenografting step and minimized the exposure of cancer tissue to room temperature conditions after mouse surgery. We also minimized patient non-cancer tissue preservation at ambient temperature after patient surgery. We successfully performed ChIP-Seq for H3K4me3, H3K9me3, and H3K9ac on frozen uveal melanocytoma patient primary tumor tissue, frozen patient derived xenograft tissue, and freshly cultured head and neck squamous cell carcinoma cell lines, revealing comparable success rates between tissue type and sample preservation techniques. ChIP-Seq techniques were performed and cross validated using tried and true qRT-PCR methods to demonstrate data reproducibility. The biological relevance of the ChIP-Seq data was further confirmed by the NanoString analysis of 47 HPV+ HNSCC samples and 25 non-cancer controls. Analysis revealed that most H3K9ac and H3K9me3 enrichment is similar in primary tissues, regardless of disease status. Only small portion of them showed differential histone enrichment, which correlated with differential expression of corresponding genes. On the other hand, H3K4me3 showed strong tissue specificity and were found differentially enriched especially in normal and benign control samples. This is a novel evidence of the stability and reliability of biological between biological replicates, diversity of tissue models, and low dependence of ChIP-Seq analysis on tissue preservation techniques.

#2425 Delving into molecular phenomena of methylation, polyadenylation and mutation suggests their importance in regulating gene expression in GBSCC. Richa Singh,1 Esita Chattopadhyay,1 Roshni Roy,1 Bidyut Roy1. 1In- dian Statistical Institute, Kolkata, India; 2Baylor Scott and White Health, TX.

Background: Gingivo-buccal squamous cell carcinoma (GBSCC) is one of the most prevalent types of oral cancers in India. Although whole exome studies in oral cancer have reported somatic mutational driver genes, studies on methylation, mutations and alternative polyadenylation (APA) to identify differentially expressed transcripts and exons, respectively, are rare. The aim of our study is to investigate possible causes of expression deregulation due to methylation, APA or mutations. Methods: Whole transcriptome data was generated from 12 tumor-normal paired GBSCC tissues and analyzed for differential gene expression, differential exon usage and predicting APA site. Whole exon sequencing was performed with same sample set and the data was analyzed to identify somatic mutations. Reduced representation of bisulfate sequencing (RRBS) was also done for some of these samples to inquire about the methylation status of deregulated genes. Results: A total of 465 genes were found to have differential exon usage in tumor leading to expression of different isoforms and change in overall gene expression. About 194 genes were predicted to utilize alternate polyadenylation site in tumors. Amongst these, 11 genes including ERBB3, CDC25B and LTRBP4 were shown to have differential exon
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usage in tumor with altered gene expression hinting upon the influence of polyadenylation in expression change. From the exome sequencing study, 180 SNVs and 11 indel somatic mutations per sample were observed. Apart from other non-silent exonic mutations at TP53, CASP8, DIS3 and DLG1, splice site mutations were also found in 37 and 4 genes due to point and indel mutations respectively. Genes such as TP53, CASP8, DIS3 and others were found to harbor somatic mutations and APA site. This suggests for coupled regulation by APA and mutation in altering expression of those genes. Apart from this, a subset of deregulated genes (n = 54) were observed to have differential methylation in tumors. Expression change of TAPI, SLN, ELN etc. corroborated with their methylation status suggesting the influence of methylation in regulating expression of these genes. Deregulated differential expression also suggested differential epigenetic reprogramming leading to overall change in gene expression in tumor. Upon excavating into the regulatory causes, a subset of genes showed change in methylation pattern while few others showed change in APA site with/without somatic mutation effect. These molecular phenomena explain how different mechanisms are involved in expression deregulation of genes observed in GBSCC which would help in further functional research for cure of the disease.

#2426 Oncopig soft-tissue sarcomas recapitulate key transcriptional features of human sarcomas. Kyle M. Schachtschneider, Y. Yingkai Liu, Suyi Makekalema, Ole Madsen, L. Laura A. Rund, Martin A. Groenen, Regina M. Schwind, Ron C. Gaba, Schook B. Lawrence, University of Illinois at Chicago, Chicago, IL; University of Illinois, Urbana, IL; Wageningen University, Wageningen, Netherlands.

Human soft-tissue sarcomas (STS) are rare, aggressive mesenchymal tumors with a late stage 5-year survival rate (50-60%) that has for decades remained unchanged. Research into STS treatment is hampered by the limited human STS cell line availability and the large number of STS subtypes. Therefore, there is a need to develop STS cell lines and animal models representative of diverse human STS subtypes. Pigs represent ideal human disease models due to their similar size, anatomy, metabolism, genetics, and epigenetics compared to humans.

In this regard, porcine cancer models provide the opportunity to produce STS cell lines and in vivo tumors similar to those clinically observed in humans. The Oncopig encodes Cre recombinase inducible porcine transgenes encoding KRAS and TP53, allowing the Oncopig to model a number of human sarcomas in an inducible and temporal manner. However, comparative analysis is required to determine to what extent the Oncopig STS model mimics human STS on a molecular level. The purpose of this study was to identify similarities between Oncopig and human STS transcriptional profiles to validate the Oncopig model as a viable model for human STS. Towards this end, Oncopig fibroblasts were isolated from ear notches of 4 Oncopigs and cultured in vitro. Following confirmation of their mesenchymal origin (positive vimentin immunostaining), Oncopig fibroblasts were transformed via Cre recombinase exposure, resulting in the formation of Oncopig STS cell lines. Oncopig STS tumors were produced in vivo through intramuscular injection of adenovirus encoding Cre in 2 Oncopigs (2 sites/Oncopig), resulting in the formation of 4 tumors detectable by 10 days post injection. The mesenchymal origin of the resulting tumors was confirmed through histological characterization. Genome-wide expression of Oncopig STS cell lines and tumors was profiled via RNA-seq. Reproducible Oncopig STS cell line and tumor expression profiles were observed, and Oncopig STS cell lines also displayed high temporal reproducibility. Differential expression analysis was performed by comparing Oncopig STS cell lines and tumors to untransformed fibroblasts and skeletal muscle, respectively. A total of 3,360 and 7,652 differentially expressed genes were identified in the Oncopig STS cell lines and tumors, respectively. Commonly identified alterations in human STS gene expression and pathway regulation were identified in Oncopig STS, including altered TP53 signaling, activation of Wnt signaling, and evidence of epigenetic reprogramming. Furthermore, master regulators of Oncopig STS gene expression were identified, including FOXL1, which was previously identified as a potential therapeutic target for human STS. These results demonstrate the Oncopig STS model’s ability to mimic human STS on a transcriptomic level, making the Oncopig a valuable resource for sarcoma research and cell line development.

#2427 Alterations in gene expression after exposure of MDA-MB-231 cells to isobutylparaben. Christine Strelchuk,2 Holly Jones Taggart*. 1University of Toronto, Toronto, Ontario, Canada; 2University of Ontario Institute of Technology, Oshawa, Ontario, Canada.

Parabens are a group of chemical compounds extensively used as preservatives in consumer products such as cosmetics, pharmaceuticals and processed food. Parabens have been shown to have endocrine disrupting properties, to accumulate in breast tissue and to increase the proliferation of hormone recep-

tor positive breast cancer cell lines through competitive binding to the estrogen receptor. Studies have shown that isobutylparaben has the highest binding affinity for the aryl hydrocarbon receptor (AhR), reported to bind environmental toxins. This study examined the alterations in gene expression correlated to exposure of the hormone receptor negative breast cancer cell line MDA-MB-231 to isobutylparaben over a 72 hour time frame. Exposure of the cells to concentrations of isobutylparabens commonly found in consumer products alters the genetic expression profile of the cells. In our study, detectable expression levels of AhR mRNA decreased, while cytochrome P450 (CYP1A1) and receptor activator of NFκB ligand (RANKL) mRNA levels increased, as analyzed by qPCR. The downregulation of AhR and altered expression levels of invasion related genes is suggestive of an increased metastatic phenotype with exposure to isobutylparaben.

#2428 The cytidine deaminase APOBEC3B is a regulator of gene expression in breast cancer. Manikandan Periyasamy, Imperial College London, Du Cane Road, London, United Kingdom.

The APOBEC3B (A3B) cytidine deaminase gene has been implicated driving the mutational landscape in breast and other cancer types. We have discovered that A3B is a co-activator of estrogen receptor (ER) mediated gene expression in breast cancer. Mechanistic studies established that A3B regulates gene expression by promoting C-to-U deamination at ER binding regions. We show that these C-to-U changes lead to the generation of DNA strand breaks through activation of base excision repair (BER) and to repair by non-homologous end-joining (NHEJ) pathways. We have also performed RNA-seq and ChIP-seq in multiple ER+ and ER- breast cancer cell lines. Remarkably, these studies demonstrate that A3B regulates the expression of many genes in inflammatory and interferon signaling pathways, in addition to the regulation of ER target genes. These findings, together with other data to be presented, provide a clear role for A3B in transcription regulation. These findings also raise the possibility that transcription factor mediated recruitment of A3B to DNA may facilitate acquisition of A3B driven cancer mutations.

#2429 Generation of a transcriptome of oncogenic mutant p53 in triple-negative breast cancer. Rebecca E. Steele, Simon S. McDade, Paul B. Mullan, Queen’s University Belfast, Belfast, United Kingdom.

The purpose of this study was to generate a transcriptional profile specific for mutant p53 in triple-negative breast cancer (TNBC), representing potential novel therapeutic targets. The highly heterogeneous TNBC tumor subgroup is established as having a poor clinical outcome. There are currently no targeted therapies for women with TNBC; consequently, there is an unmet clinical need to identify targetable pathways. Approximately 75% of these tumors are known to harbour a mutation inTp53 (mutp53). Chromatin Immunoprecipitation Sequencing (ChIP-seq) for mutp53 and ets-1 (a known mutp53 interactor) was carried out using the D0-1 (sc-126) and sc-20 antibodies respectively, in the MDA-MB-468 TNBC cell line. The aim of these ChIP-seq experiments was to identify sites bound by endogenous R273H mutp53 and ETS-1 and to confirm that target genes were co-regulated. We identified 282 novel mutp53 specific genomic binding sites and 38 mutp53 and ets1 shared binding sites. In-house data analysis and peak calling generated a list of potential transcriptionally co-regulated genes adjacent to these genomic sites. PDGFB, TRIB3, BTRC, TNFSF18 & PI3 were successfully validated as being transcriptionally regulated by R273H mutp53 and not wild-type p53 (as shown by MCF7 ChIP-seq comparisons). A number of these genes are known to be aberrantly expressed in TNBC patient tumors and harbor interesting immune biology, providing potential opportunities for novel therapeutic strategies. In addition, DNA damaging chemotherapy-resistant MDA-MB-468 derivatives express elevated levels of mutp53 protein and altered expression of our validated transcriptional targets, suggesting that they may also have roles in promoting resistance to current chemotherapy treatments, downstream of mutp53. In conclusion, we have identified a cohort of ‘mutp53 specific’ transcriptional targets which may have roles in modulating immune responses to TNBCs, driving resistance to current chemotherapies and could provide opportunities for the development of novel treatments for TNBCs.

#2430 The expression of BCL6 and ZEB family transcription factors and their correlation in breast cancers. Min Zhao, Lin Ang, Li Zheng, Jin Wang, Jin Huang, Hongguang Hu, Qiang Zou, The Second People’s Hospital of Heifei, Heifei, China.

The proto-oncogene B-cell lymphoma 6 (BCL6) is an evolutionarily conserved zinc finger protein and a potent transcriptional repressor. It is also the master regulator of B-lymphocyte development. Emerging evidence indicates
that BCL6 may play an important role in breast cancer progression. In this study, we investigated the expression of BCL6 and its correlation with the expression of epithelial-mesenchymal transition (EMT)-associated transcription factors zinc finger E-box-binding homeobox 1 (ZEB1) and ZEB2 in invasive breast cancers. The mRNA and protein expression of BCL6, ZEB1 and ZEB2 were examined using in situ hybridization and immunohistochemistry, respectively, in 228 breast cancer patients and 80 patients with breast benign diseases. The association of BCL6, ZEB1 and ZEB2 expression with clinicopathological parameters and patient survival were analyzed. We found that the expression of BCL6, ZEB1 and ZEB2 protein and mRNA were significantly higher in breast cancer tissues than in breast benign disease tissues (P<0.05). The expression levels of BCL6, ZEB1 and ZEB2 were all positively correlated with the tumor size, lymph node metastasis and higher tumor stages (P<0.05). Furthermore, patients with BCL6, ZEB1 and ZEB2 protein-positive primary tumors were associated with a significant lower overall survival (OS) rate (P=0.001, 0.002 and 0.001, respectively) and post-operative relapse-free survival (RFS) rate (P=0.002, 0.001 and 0.003 for the expression of BCL6, ZEB1 and ZEB2, respectively). The mRNA expression of ZEB1 and ZEB2 were positively correlated with BCL6 mRNA expression (r=0.326, P<0.001; and r=0.382, P<0.001, respectively); the protein expression of ZEB1 and ZEB2 were also positively correlated with BCL6 protein expression (r=0.449, P<0.001; and r=0.669, P<0.001, respectively). These results indicate that BCL6, ZEB1 and ZEB2 may be as potential biomarkers for predicting invasion, metastasis and prognosis of breast cancer and that BCL6, ZEB1 and ZEB2 may be as potential biomarkers for predicting invasion, metastasis and prognosis of breast cancer and that BCL6 may be a regulator of ZEB family transcription factors (ZEB1 and ZEB2).

#2431 Single cell gene expression profiling in breast cancer cells with the Her2/neu gene knockout by CRISPR-Cas9. Xiaoyang Wang,1 Chip Lomas,1 Michael A. Tycon,1 Craig Betts,2 Suzanne Weaver2. 1New Mexico State Univ.-Las Cruces, Las Cruces, NM  

The Her2/neu gene is amplified and overexpressed in 15%-30% of breast cancers. The overexpression of this oncogene is strongly correlated with decreased survival, increased cancer relapse, and poor prognosis. Although therapy has been successfully developed to target this oncogene, a better understanding of this oncogene will provide further insight for breast cancer biology and future drug development. In recent years, the CRISPR-Cas9 system has emerged as an efficient method for genetic engineering, enabling targeted gene knockout with minimal off-target effects. In this study, we employed a plasmid-based CRISPR-Cas9 system to knock out the Her2/neu gene in breast cancer cells. Puromycin selected, GFP-positive (two indicators of the incorporation of the CRISPR-Cas9 system), Her2-negative individual cells were index-identified by a BD FACSMelodyTM system into individual wells of a 96-well BDTM Precise plate with sample barcoding and molecular indexing. A whole transcriptome amplification (WTA) assay was performed to obtain a gene expression profile for each of the Her2/neu knockout cells. Her2 gene knockout was performed on two cell lines: T47D, a ductal carcinoma-derived breast cancer cell line with no Her2 amplification that shows low to intermediate Her2/neu expression; and SKBR3, an adenocarcinoma-derived breast cancer cell line known for Her2/neu gene amplification and overexpression. For comparison, WTA assays were also carried out on parental cells without gene editing. Comparison of the gene expression profiles of these cell lines shows changes in gene expression correlated to the Her2 knockout. The profiling of gene expression in Her2/neu CRISPR-Cas9 knockout cells on a single cell level promises to provide insight into the mechanisms in these aggressive cancers that could help future drug discovery. For Research Use Only. Not for use in diagnostic or therapeutic procedures. 23-19341-00.

#2432 GPER1 expression is modulation by D-glucose concentration in estrogen-responsive cancer and tumor cells. Yan Zheng, Kevin D. Houston. New Mexico State Univ.- Las Cruces, Las Cruces, NM.  

G protein-coupled estrogen receptor 1 (GPER1, aka GPR30), is a 7-transmembrane receptor that mediates rapid cell signaling events stimulated by 17B-estradiol (E2) in cancer and tumor cells. In breast cancer cells, GPER1 mediates E2-induced CTGF-dependent cell migration and cyclin E expression resulting in cell cycle progression. GPER1 has also been shown to mediate antiproliferative cell signaling such as p53-dependent inhibition of cell cycle progression in breast cancer cells treated with the GPER1-specific agonist G1 and the inhibition of IGF-1R signaling in a tamoxifen-treated breast cancer cells. In addition to E2, both natural and synthetic compounds have been shown to activate GPER1 in breast cancer cells. GPER1 has also been shown to regulate cell signaling in cells that lack E2R. For example, GPER1 is antiproliferative in triple-negative breast cancer cells. While the importance of GPER1 in the modulation of E2-responsive tumor and cancer cells as been demonstrated, there is very little information regarding the regulation of GPER1 expression in these cell types. The work presented here is aimed at determining the molecular mechanisms that regulate GPER1 expression. To determine if GPER1 expression is sensitive to D-glucose concentration in breast cancer cells, MCF-7 and T47D human breast cancer cells were cultured in media containing increasing concentrations of D-glucose and GPER1 expression was measured using real-time PCR and immunoblot. Data from these experiments showed that GPER1 expression was sensitive to D-glucose concentrations (0 and 2.5mM) and significantly reduced in media containing high D-glucose (25 mM). Since low D-glucose concentration is known to activate the energy-sensing AMP kinase (AMPK), the observed GPER1 induction in low D-glucose conditions was determined after pretreatment with the AMPK inhibitor compound C. GPER1 expression was inhibited in both MCF-7 and T47D breast cancer cell lines in media containing low D-glucose when pretreated with compound C. Additionally, the AMPK activator metformin induced GPER1 expression in MCF-7 and T47D breast cancer cells cultured in high D-glucose (25mM) conditions. These data suggest that AMPK mediates GPER1 expression in cells cultured in low D-glucose. The D-glucose sensitivity of GPER1 expression was also determined in the Eker rat-derived uterine leiomyoma cells (ELT-3 and ELT-6) which are characterized by hyperactive AMPK. In both ELT-3 and ELT-6 cells, GPER1 expression was not sensitive to D-glucose concentration nor was the activation state of AMPK sensitive to D-glucose concentrations. However, the inhibition of AMPK by compound C in both ELT-3 and ELT-6 cells resulted in decreased GPER1 expression. These findings reveal a previously unknown mechanism that regulates GPER1 expression in E2-responsive tumor and cancer cells.

#2433 Altering nuclear size impacts cancer cell characteristics in melanoma cell lines. Lidija D. Vukovic,1 Bradley A. Stohr,2 Dan L. Levy1. 1University of Wyoming, Laramie, WY; 2University of California, San Francisco, CA  

Nuclear size is altered in breast cancer cells, a change used by pathologists to distinguish cancer from normal cells. Previous studies in Xenopus identified two nuclear transport proteins, importin α and NTF2, as regulators of nuclear size. In general, importin α and NTF2 levels positively and negatively affect nuclear size, respectively. Increased importin α expression is used as a biomarker in non-small cell lung carcinoma and breast cancer and correlates with increased nuclear size. We identified decreased NTF2 protein expression as a potential biomarker in melanoma, consistent with NTF2 levels negatively regulating nuclear size. Following up on this result, our current studies focus on manipulating nuclear size in melanoma cells to assess the impact on cancer cell characteristics, including proliferation rate, migration potential, and apoptosis. First, we measured nuclear size and NTF2 expression levels in different stage melanoma cell lines. Compared to a normal melanocyte cell line, we consistently observed larger nuclear size and lower NTF2 protein levels in all melanoma cell lines examined. Next, we performed transient transfections in HeLa, MRC5, and melanoma cell lines, demonstrating that ectopic NTF2 expression leads to reduced nuclear size. The most dramatic effects were observed in primary melanoma cell line WM3211 where higher NTF2 expression caused a 40% reduction in nuclear cross-sectional area. To obtain more precise control over NTF2 expression levels, we generated a stably transfected metastatic melanoma cell line (WM983B) in which NTF2 expression can be titrated by varying the concentration of doxycycline in the growth media. Higher doxycycline levels lead to increased NTF2 expression and smaller nuclei. In particular, 20 ng/ml doxycycline induced a 10% reduction in nuclear cross-sectional area. In a wound healing assay, doxycycline-treated cells exhibited a 40% reduction in cell motility compared to non-treated cells or the parent cell line treated with doxycycline. Furthermore, the doxycycline-treated cells exhibited an increased rate of apoptosis. We propose that reduced nuclear size in the doxycycline-treated cells causes changes in chromatin organization and gene expression, thus giving rise to observed effects on cell migration and apoptosis. To test this idea, we are currently performing a global transcriptomics analysis of our cell lines to identify genes whose expression is altered by nuclear size. Using this information, we will use DNA FISH to map the intranuclear position of differentially-expressed genes as a function of nuclear size. Also in vivo experiments are ongoing in which we have subcutaneously injected NTF2-inducible melanoma cells into NGS mice in order to examine tumor formation and metastatic capacity of cells with differently-sized nuclei. Regarding that in most cancer cells nuclear size is enlarged, understanding the causes and effects of these changes can help us to better understand cancer biology.
A novel oncogene that show a crucial role in the tumorigenesis and progression of HCC via activating Notch signaling pathway and inhibiting the differentiation and stemness of HCC, resulting in poor pathologic differentiation and leads to high degree of malignant. All the findings suggest a novel treatment possibility and a new target for liver stem cell eradication. LEF1 could be investigated for clinical early diagnosis and prognosis prediction of HCC patients and improving the development of new therapy against HCC.

#2435 Smoothened a new perspective for uterine leiomyoma treatment.
Natalia Garcia,1 Laura Gonzalez Anjos,2 Giovana De Nardo Maffazoli,1 Nilo Bozini,3 Edmund Chada Baracat,4 Katia C. Carvalho.5 1Univ. of São Paulo Faculty of Medicine, Mogi das Cruzes, Brazil; 2Univ. of São Paulo Faculty of Medicine, São Paulo, Brazil.

Background: Uterine leiomyomas (LM) are the most common gynecologic tumors occurring in women of reproductive age. Patients may present clinical complications such as bleeding, pain and infertility. Additionally, some researchers believe that a leiomyosarcoma can arise from a degenerated LM. Efficient treatments for LM are still limited. Identification of gene markers can help to elucidate these tumors pathogenesis and to reveal molecules that can be used as therapeutic targets. Objective: To assess gene and miRNAs expression, and DNA methylation profiles of genes related to the Sonic Hedgehog (SHH) signaling pathway in uterine leiomyoma samples in order to look for potential therapeutic targets. Methods: 80 samples of LM and 20 myometrium (MM) were obtained in the Department of Obstetrics and Gynecology at HCFMUSP/Sao Paulo - Brazil. Expression profile of 106 genes and 84 miRNAs sequences related to SHH regulation and the percentage of methylation in the promotor region of the SHH, PTCH1, SMO, SUFU, GLI and GLI3 genes were performed by quantitative Real Time PCR. Results: Among 106 genes evaluated, 16 showed hyper- and 11 showed hypoexpression in LM samples, compared to MM. Only SMOOTHENED (SMO) was found hyperegulated in LM. miRNAs analysis also showed 16 sequences with different regulation profiles between LM and MM. None of them is involved in the regulation of SMO expression. DNA methylation profile showed a higher percentage of methylation in LM samples, but this difference did not exceed 2% of methylation for the PTCH1, SMO, GLI1 and GLI3 genes. Conclusion: Our results suggest an activation of SHH pathway in LM by hyperexpression of SMO. These results are very interesting because there are SMO specific drugs approved by FDA for other tumors types.

#2436 Non canonical activation of Sonic Hedgehog pathway in uterine leiomyosarcoma.
Natalia Garcia, Bianca C. Oliveira, Kelly P. Ferreira, Edmund C. Baracat, Katia C. Carvalho. Univ. of São Paulo Faculty of Medicine, São Paulo, Brazil.

Background: Uterine leiomyoma (ULM) and leiomyosarcoma (ULMS) are smooth muscle tumors with distinct clinical and biological behavior. They can be a cause of infertility and even death. Little is known about the factors that can to influence these tumors behavior and biology. Sonic Hedgehog (SHH) pathway components were previously implicated in the ULMs malignancy risk; however its activation mechanism is unknown. In this study, we investigated the gene expression profile of Sonic Hedgehog Signaling and pathway upstream genes in myometrium (MM), ULM and LMS cell lines. Methods: Total RNA obtained from cell lines were submitted to cDNA synthesis and quantitative real time PCR (qRT-PCR), using 7500 System (Life Technologies, USA). Gene expression of SHH, PTCH1, SMO, SUFU, GLI 1-3, CCND1, BCL-2 and BMP4 were assessed. Results: Gene expression data showed an upregulation of SMO, SUFU, GLI1, BCL-2 and BMP4 in LMS cells when compared to MM. GLI1 (RQ=11) and BCL-2 (RQ=20) genes had higher expression. In the other side, SHH, PTCH1, GLI2 and GLI3 showed upregulation in MM cells. Conclusion: Our results showed that the Sonic hedgehog pathway is activated in LMS cells but by SHH independent form (non-canonical pathway). This could be explained by the downexpression of SHH ligand and the PTCH1 receptor, and by the expression of low level tumor and normal samples. SHH in uterus LMS cells compared to LM and MM. These results suggest a new therapeutic perspective for LMS by target drugs to inhibit SMO and GLI1 molecules.

#2437 Overexpression of MYL5 promotes cervical cancer cell metastasis through SLUG and HIF-1α signaling.
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Myosin light chains (MLC) serve important regulatory functions in a wide range of cellular and physiological processes. Previous research found that MLC are also chromatin-associated nuclear proteins which regulate gene transcription. In this study, we show that the MLC member myosin regulatory light chain 5 (MYL5) controls metastasis in cervical cancer. We uncover this role of MYL5 through clinical cervical cancer samples and various in vitro and in vivo models of metastasis. MYL5 promotes metastasis by enhancing the transcription of SLUG and hypoxia inducible factor-1α (HIF-1α). Moreover, we reveal a bidirectional regulation between MYL5 and HIF-1α. HIF-1α specifically binds to the MYL5 promoter region, and hypoxia leads to increased levels of MYL5 in cervical cancers. Overexpression of MYL5 sustained HIF-1α expression in normoxic condition. Clinical data confirmed a positive correlation between MYL5 and HIF-1α. In summary, our observations suggest a potential application of MYL5 in prognosis prediction and cancer treatment.

#2438 Analysis of IL-13 signaling through IL-13Rα2 in human brain tumor specimens in situ.
Rukmini Bhadra, Akiko Suzuki, Pamela Leland, Bharat H. Joshi, Raj K. Puri. FDA-CBER, Silver Spring, MD.

Previously, we have demonstrated that IL-13 receptor-α2 (IL-13Rα2), a high affinity receptor for Th2 cytokine IL-13, is overexpressed in most glioblastoma multiforme (GBM) cell lines and approximately 78% of the patient-derived samples. We have also demonstrated that IL-13Rα2 can be targeted by a number of immunotherapeutic agents including chimeric antigen receptor modified T (CAR-T) cells, targeted lentivirus and adenovirus vectors, and a chimeric fusion immunotoxin consisting of IL-13 and truncated Pseudomonas exotoxin (IL-13-PE). However, the signal transduction initiated by IL-13 in GBM tumor is not known to occur through the IL-13Rα2, which has a high affinity for IL-13. We have recently observed that IL-13 can signal through IL-13Rα2 by activating AP-1 transcription factors in human brain tumor cell lines. In this study, we have examined IL-13Rα2 expression in human brain tumor and normal brain specimens, and the subsequent signaling through the AP-1 pathway in situ. Using six human glioblastoma and three astrocytoma specimens, we evaluated the expression of AP-1 transcription factors by immunohistochemistry (IHC) and compared the extent of immunostaining and percent positive cells with three normal brain specimens. Six GBM specimens examined showed high degree of immunostaining for c-Fos, c-Jun, Jun D and Fra-1 (AP-1 family transcription factors) and a high percentage of positive cells. These specimens also showed strong immunostaining for IL-13Rα2 (4+) in >70% cells (P<0.001 compared to normal brain). Three astrocytoma specimens showed staining for IL-13Rα2 (2+ and 32% cells, P<0.01 compared to normal brain), but the extent of staining was lower compared to GBM. Similar to IL-13Rα2 expression, the extent of staining and percentage of positive cells for AP-1 transcription factors were highly statistically significant between tumors and normal brain (P<0.001 for GBM compared to normal brain and P<0.01 for astrocytoma compared to normal brain). The extent of immunostaining in GBM was highest for c-Fos (4+, >78% fields) followed by c-Jun (3+, 57% fields), Fra-1 (2+, 70% fields) and Jun-D (2+, 28% fields). Jun-B expression was low among the AP-1 transcription factors (<1+, 7% fields) in GBM specimens. Astrocytoma specimens showed lesser extent of immunostaining for AP-1 members compared to GBM; c-Fos showed 2+ staining and 42% positive fields followed by c-Jun (2+, 12% fields), Fra-1 (2+, 48% fields) and Jun-D (<1+, 18% fields). Jun-B staining intensity was <1+ in only 6% fields. Normal brain specimens showed no immunostaining for AP-1 family members. Our results generally corroborate with data obtained from GBM cell lines and confirm that IL-13 can signal in IL-13Rα2 positive GBM tumors in-situ through the AP-1 pathway, thus indicating that this pathway may be an important target for therapeutic intervention of GBM in addition to targeting IL-13Rα2.
Introduction: Development of improved cancer diagnostics and therapeutics requires detailed understanding of the genomic, transcriptomic, and proteomic profiles in the tumor microenvironment. Current technologies can excel at measuring a single analyte, but it remains challenging to simultaneously collect high-throughput DNA, RNA, and protein data from small samples. We have developed an approach that generates the multi-omics profile of DNA, RNA, and proteins from as little as 5ng DNA, 25ng RNA, and 250ng protein or just 2 5mm FFPE slides, and simplifies data analysis by generating digital counts for each analyte. Methods: The approach uses paired capture and reporter oligonucleotide probes and optical barcodes to enumerate up to 800 targets. The platform was initially developed to measure RNA, and we have adapted it to measure DNA single-nucleotide variants (SNVs), short intergenic RNAs, and protein expression. Results: Combining of sequence discriminating probes to the wild-type and mutant sequence of interest. Proteins are detected via binding of oligonucleotide-conjugated antibodies. Results: Combinations of DNA, RNA, and protein in biological and experimental contexts. SNV probes are able to detect variant alleles down to 5% abundance within a wild type population and can discriminate variants within mutation hotspots. It was >90% accurate at identifying variants from samples displaying a range of allele frequencies and DNA integrity when benchmarked against next-generation sequencing. Protein detection has been developed for cell surface, cytosolic, and nuclear proteins, as well as phospho-proteins. It was validated against flow cytometry, western blot, and mass spectrometry using cells line with ectopic target expression and primary cells. To demonstrate concurrent measurement of DNA, RNA, and protein expression in the genome and proteome, BRAFV600E/BRAFV600E and BRAFV600E/BRAFV600E cell lines were treated with the BRAFV600E inhibitor vemurafenib and the MEK inhibitor trametinib. We measured the allele usage at the BRAFV600E locus, as well as BRAFV600E dependent changes in mRNA expression, protein expression and protein phosphorylation in a single experiment. Conclusions: 3D Biology has several advantages over other analytical approaches. Direct, single-molecule digital counting allows detection over a broad dynamic range with high reproducibility, often over 98% concordance between technical replicates. The simultaneous interrogation of DNA, RNA, and protein maximizes the amount of data obtained from precious samples and minimizes instrumentation demands by leveraging a single detection platform. The 3D Biology approach allows holistic, digital analysis of biological samples with high specificity and precision. This technology is currently available for research use, but may also have clinical application in the future.

#2441 NanoString 3D Biology™ technology: simultaneous digital counting of DNA, RNA and protein. Chris Lausted,1 Yong Zhou,1 Jinho Lee,1 Christopher Vellano,2 Karina A. Eterovic,2 Ping Song,2 Lin-ya Tang,2 Gloria Fawcett,2 Tae-Beom Kim,2 Ken Chen,2 Gary Weiss,2 Gary Meredith,2 Qian Mei,2 Golshan Demirci,2 Beom-Dunaway,2 Dae Kim,2 P. Martin Ross,2 Elizabeth Manroa,3 Nathan Elliott,3 Sarah Warren,1 Christina Bailey,3 Chung-Ying Huang,3 Joseph Beechem,3 Gordon Mills,2 Leroy Hood1. 1Institute for Systems Biology, Seattle, WA; 2MD Anderson Cancer Center, Houston, TX; 3Nanostring Technologies, Inc., Seattle, WA.

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MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Genome-Wide Analysis of Transcriptional Regulation

#2439 Establishment of six human colorectal cancer cell lines: identification of genes that have metastatic potential. Soon-Chan Kim, Chang-Won Hong, Sang-Geun Jang, Seung-Yong Jeong, Jae-Gabh Park, Jia-Lok Ku. Seattle National University, Medical Campus, Seoul, Republic of Korea.

A number of genes have been studied in accordance with their metastatic potential. Nevertheless, little is known about the metastatic profile of human colorectal cancer cell lines such as SW-620 or IS3 only show the influence of metastatic genes within limited mutational landscape. Here, three pairs of primary CRC (SNU-235A, SNU-235D, SNU-240A, SNU-240B, SNU-241A, and SNU-241B) and corresponding metastasis cell lines were established and analyzed by whole exome sequencing and microarray. Three pairs of primary CRC (SNU-235A, SNU-240A, and SNU-241A) and corresponding post-translational level and of extra-nuclear components. Our efforts to confirm the mRNA expression level in the paired CRC cell lines. The genes selected for examination were calponin 3, acidic (CNN3); sorbin and SH3 domain containing 1(SORBS1); epithelial stromal interaction 1 (EPS1T); bone marrow stromal cell antigen 2(BST2); kelch-like 5 (KLHL5); trypsinogen C (TRY6); and synaptogatin-like 5 (SYT5L5). We calculated the intersection of all three metastatic CRC cell lines. Five genes (BST2, SORBS1, CNN3, EPS1T, and KLHL5) were up-regulated in metastatic cell lines compared to primary cell lines. Two genes (TRY6 and SYT5L5) were down-regulated in metastatic cell lines compared to primary cell lines. Then, we ranked 15 genes that were up-regulated and 15 genes that were down-regulated in post-translational and single-nucleotide polymorphisms (SNVs) on both cell lines. We selected top 20 SNV genes that were differentially expressed, we selected three genes (CNN3, SORBS1, and TRY6) that showed the highest fold changes and are found in 2-fold change analysis. We identified 7 genes that were differently expressed in metastatic CRC cell lines compared to primary CRC cell lines. These genes may serve as diagnostic markers and therapeutic targets for patients with metastatic CRC.

#2440 Cross-tissue interactome analyses unravel novel oncogenic roles of SOX2. Thorsten Schaefer,1 Silvia Candido,1 Hui Wang,1 Thomas Bock,2 Alexander Schmidt,2 Claudia Lengerke1. 1University of Basel and University Hospital Basel, Basel, Switzerland; 2Biozentrum, University of Basel, Basel, Switzerland.

First recognized for its significance in early embryogenesis, SOX2 (sex-determining region Y box 2)morerecentlyreceivedmajorattentionasa(i)transcrip-
tional master regulator of stemness driving the re-programming of terminally differentiated somatic cells back into a pluripotent stem cell state and (ii) pow-
ertextual function of SOX2. As expected, a powerful oncogene of the cancer stem cell (CSC) compartment. We and others have shown that SOX2 expression relies on canonical PSE/AKT signaling involving direct physical contact and phospho-modification by AKT. Interestingly, we observed that these interactions are highly tissue specific which prompted us to perform a systematic proteomic analysis of co-factors regulating SOX2 biology. The SOX2 interactome was analyzed in human breast cancer-inoma and glioblas-
toma cell lines by co-immunoprecipitation (co-IP) and HPLC/MS revealing 2 500 conserved proteinaceous co-factors, thus underscor-
ing a hitherto underestimated scaffolding function of SOX2. As expected, a major proportion of binding partners cluster in functional categories implicated in DNA binding and/or nucleotide modification (e.g. helicases, transferases, and ligases). However, a near equal share of SOX2 associated factors fall into func-
tional classes formally unrelated to transcription modulation such as cell adhe-
sion, cytoskeletal organization, or metabolic adaptation. Using SOX2 knock-
down and overexpression cells generated by lentiviral technology, we confirm co-regulation of SOX2 with selected binding partners. Moreover, we observe co-depletion of SOX2 and protein co-factors in cells of stalled AKT activity, and a molecular restoration of both upon prosesational inhibition. We conclude that, besides its known significance in DNA activity control, the SOX2 protein en-
gages in various interactions seemingly unrelated to transcription modulation. These newly discovered interactions are likely to synergize with canonical SOX2 functions (i.e. the induction and maintenance of stemness), but involve regu-
lation at post-translational level and of extra-nuclear components. Our efforts thus add an exciting new facet to SOX2 biology and eventually, may open new therapeutic avenues for targeted anti-CSC therapy.

#2443 Massively parallel single-cell RNA-Seq identifies diverse subpopulations displaying EMT and stem-like features. Billy Lau, Jianmin Chen, Hanlee P. Ji. Stanford School of Medicine, Stanford, CA.

Single-cell transcriptome analysis enables a new paradigm for studying complex systems in cancer. As opposed to bulk sequencing, which averages genomic signals...
across thousands or millions of cells and obscures the presence of rare subtypes, single cell sequencing enables the interrogation of individual cells. In cancer, intratumoral heterogeneity is observed at both genomic and epigenomic levels, and its analysis enables the discovery of new actionable targets and treatment modalities tailored to individual subpopulations. As an example, many cancer cell lines and those derived from patients contain subpopulations defined by distinct patterns of surface markers such as CD44, and are linked to drug resistant and tumor initiating phenotypes. A complete characterization of such cellular populations ideally requires marker-free sampling, followed by clustering into distinct subgroups. In this study, we demonstrate the significant advantages of such an approach; we utilize a high-throughput single-cell RNA-Seq method to characterize the transcriptional profiles of cellular populations. We performed single-cell RNA-Seq on thousands of cells in the matched SW480 (primary) and SW620 (metastatic) colorectal cell lines using a microfluidic droplet barcoding technology that enables the tracking of single cells during library preparation. By focusing on genes with high inter-cell variability, we discovered a small subpopulation of cells that displayed a distinct gene expression signature from the major subpopulation. Differential gene expression analysis of this subpopulation yielded genes virtually all enriched in the epithelial-to-mesenchymal transition (EMT) pathway. These cells showed significant increases in canonical mesenchymal marker genes such as VIM, CD44, and SOX9. Gene expression profiles of these subpopulations also correlated with established EMT signatures. Remarkably, this subpopulation did not display mutual exclusivity in expression with the epithelial marker EPCAM, which possibly indicates an intermediate mesenchymal phenotype. We also observed in the major population cluster a small subset of cells totaling less than 1% of the population that were significantly enriched for LGR5 expression, a common stem-like marker in colorectal cancer. Overall, we demonstrate the use of single-cell RNA-Seq to discover and characterize a diversity of cellular states that would otherwise be impossible from bulk analysis.

**MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Genome Landscape of Lymphoma, Leukemia, and Lung, Bladder, and Other Cancers**

### #2444 Recurrent mutations and clinical outcomes in patients with follicular lymphoma

Klannin Krysiak,1 Cody Ramirez,1 Felicia Gomez,1 Christopher A. Miller,1 Robert S. Fulton,1 Friederike Kreisel,1 Amanda F. Cashen,1 Laura H. Sehn,2 Joseph M. Connors,1 Ryan D. Morin,3 Marco A. Marra,4 Sohrab P. Shah,5 Christian Steidl,6 David W. Scott,7 Randy D. Gассоyne,7 Centre for Lymphoid Cancer, British Columbia Cancer Agency, Vancouver, British Columbia, Canada; 2Molecular Oncology, British Columbia Cancer Agency, Vancouver, British Columbia, Canada; 3Genome Studio, British Columbia Cancer Agency, Vancouver, British Columbia, Canada.

Introduction: Diffuse large B-cell lymphoma (DLBCL) comprises two distinct cell-of-origin (COO) subtypes: the germinal center B-cell-like (GCB) and the activated B-cell-like (ABC) subtypes. Several sequencing studies have informed on DLBCL biology and identified numerous candidate genes implicated in pathogenesis. However, comprehensive analysis of recurrent genetic alterations and related prognostic significance according to COO subtypes has not yet been performed. To understand the molecular distinctions and their therapeutic relevance specific to COO subtype in DLBCL, we performed a comprehensive genetic analysis on a population registry-based cohort of 347 patients with de novo DLBCL uniformly treated with R-CHOP. Methods: Tissue biopsies from 347 patients were analyzed using deep single-cell sequencing. Immunohistochemical staining was performed on tissue microarrays. COO subtype was assigned by Lymph2Cx assay. Results: We detected COO-specific mutations, copy number alterations (CNAs) and gene fusions. Furthermore, integrative analysis showed COO-specific trans gene expression effects on CNAs, demonstrating that CNAs in GCB-DLBCL are tightly linked to the deregulation of networks associated with PI3K-AKT signaling. We also identified the significant prognostic impact of recurrent genetic alterations within each COO subtype, including deletions of MIR15a/16-1 and TP53 and NFATC1 gain in ABC-DLBCL, TP53 and CREBBP mutations, PRAE deletions and MYC gain in GCB-DLBCL. In addition, deletions of PTEN and INPP4B, which are negative regulators of PI3K-AKT signaling, were identified as a marker associated with poor outcome in GCB-DLBCL (p = 0.01 and 0.0045, respectively). Furthermore, patients whose tumors harbored both genetic alterations had an even worse prognosis (p = 0.004), suggesting the clinical importance of PI3K-AKT signaling in GCB-DLBCL. Based on these genetic data, we found that the PI3K-AKT signaling pathway was more commonly altered in GCB-DLBCL than in ABC-DLBCL (64% vs 23%; p = 0.0001). We also observed more frequent genetic alterations of the TCR signaling pathway in ABC-DLBCL (80%), while this pathway was less frequently altered in GCB-DLBCL (54%; p < 0.0001). In addition, epigenetic modification and immune recognition pathways were more commonly altered in GCB-DLBCL compared to ABC-DLBCL (70% vs 44%; p < 0.0001, 71% vs 56%; p = 0.015, respectively). Finally, we demonstrated that the majority of DLBCL cases are altered for at least two of the four pathways mentioned above (86% of ABC-DLBCL and 87% GCB-DLBCL). Conclusion: We describe the landscape of common genetic aberrations in a population-based uniformly-treated DLBCL cohort, broadly representative of patients treated with curative intent, providing the genetic foundation for implementing precision medicine in this disease.

### #2446 Integrated analysis of somatic mutations in subcutaneous panniculitis-like T-cell lymphoma by whole-exome and -transcriptome sequencing

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Subcutaneous panniculitis-like T-cell lymphoma (SPTCL) is a rarely differentiated form of non-Hodgkin lymphoma. This uncommon disease is triggered by preferential lymphoma infiltration into subcutaneous adipose tissue, and nodular formation in the fatty tissue. Consequently, the early stage symptoms of SPTCL is found as multiple subcutaneous nodules beneath the skin. Due to the low incidence rate and indolent progression of the disease, the genetic altera-
and karyotyped. Results: The total number of coding mutations in patient 509 of diagnosis (T1) and relapse (T2), using unaffected peripheral blood to exclude whole exome sequencing (WES) performed on tumor cells obtained at the time these patients to study the tumors in more detail. Methods: Both patients had therapeutic resistance would be enriched in the tumor cellstaken at the time of completing intensive chemotherapy. We hypothesized that mutations implicated in young men at our institution experienced a BL relapse within 3 months of completing panniculitis-like T-cell lymphoma is yet thoroughly understood. Hence, establishing the genetic basis and profiling the genomic landscape of SPTCL will establish the genetic basis and profiling the genomic landscape of SPTCL. We performed whole-exome sequencing of both biopsy sample collected from the patient and matched saliva, and matched tumor sample to 200X depth of coverage, respectively. Whole-transcriptome sequencing of the tumor sample was also performed for expression level analysis; gene fusions are also explored by TopHat-fusion-post. Somatic mutations were precisely detected by our internally developed somatic variant caller, which is an adjusted version of VarScan2 with additional filters based on thresholds of p-value and odds ratio. Overall, we identified 13 missense variants of 1588 SVs, 34 SVs were validated by using somatic variant detection algorithm of our own. By applying the additional filters to these variants, two genes were remarkably identified: SQSTM1 and BAGE3. SQSTM1 (sequestosome 1), also known as ubiquitin-binding protein p62, encodes proteins that regulate ubiquitination, autophagy, and activation of NFKB1. Through CbioPortal database search, SQSTM1 found to be highly amplified across the various cancer types, such as neuroendocrine prostate cancer (NEPC), kidney renal clear cell carcinoma (RCC), and pancreatic cancer. On the other hand, BAGE3, B melanoma antigen 3, was reported to be a candidate gene encoding tumor antigens. Gene fusions were identified by comparing the number of spanning reads and mate pairs, and fusion of NO7 and RANBP9 on chromosome 6 showed the highest fusion score of 604.45. Comprehensively characterized, two signatures of the initial disease progression, clinicopathological, and therapeutic resistance to chemotherapies. Furthermore, to strengthen the statistical power of the somatic mutation analysis, we are planning to expand the cohort and conduct functional validation study of selected genes by using secured paraffin blocks of SPTCL patients.

#2447 The mutational landscape of chemo-refractory Burkitt lymphoma. Claudia M. Wever,1 Maryse Lemaire,1 Donald C. Vinh,1 Josée Hébert,2 Yasser Gomez,1 Kilannin Krysiak,1 Cody Ramirez, Nancy Bartlett, Amanda Cashen, Friederike Kreisel, Eric Duncavage, Malachi Griffith, Todd Fehminger, Obi Griffith. Washington University, St. Louis, MO.

High-throughput sequencing provides insights into cancer pathogenesis. The application of genomics technologies to cancers characterized by rare cell populations is a challenge. DNA extracted from bulk tumor samples is a mixture of malignant and non-malignant cells. This is a problem in cancers defined by rare cell populations because it complicates the task of identifying true somatic mutations. Hodgkin lymphoma (HL) exemplifies this challenge. The pathologic hallmark of HL is Hodgkin-Reed-Sternberg (HRS) cells. HRS cells have a low abundance within an affected lymph node (1-30% of tumor microenvironment). Because HRS cells are rare, a limited number of studies have described characteristic genomic events. These studies have examined HL cell lines, and, recently, a small number of flow-sorted HRS-cells (Reichel et al. 2015). We hypothesized that recurrent somatic mutations can be identified in HRS cells using bulk HL biopsies and ultra-deep exome sequencing. The Illumina HiSeq X, which can generate >1.6 Tb of sequence data per run, is a applicable tool for the discovery of somatic mutations in HL. Frozen tumor (lymph node)/normal (skin) pairs from 31 patients with HL were examined. Th1, Th2, CD8+ T cells were flow sorted and pooled for sequencing. The libraries were pooled, size selected, and captured using an Illumina Xgcn sequen captive reagent. The pools were sequenced across eight lanes of a HiSeq X. A total of 7.04 x 10^12 bases were sequenced across all samples with an average of 1.10 x 10^10 bases per sample. The mean depth of coverage achieved across all samples was 783.4x (425.9x - 1091.9x). Given the low abundance of HRS cells we anticipated most somatic sites would have a VAF of 1-5%. Given the level of coverage we anticipate that most somatic variants will have >5 reads of support. Instrument data were processed using the McDonnell Genome Institute somatic variant calling pipeline that includes 5 SNV callers and 3 indel callers. We used these steps to filter variants: min. 50X coverage, max. 5000X coverage, min. 1% tumor VAF, max. 5%, normal VAF, and min. 5 variant reads in the tumor. Here we report preliminary data on mutations in five pilot samples. The remaining 26 will be presented at the meeting. We confirmed 7 previously documented recurrent mutations in HL (Reichel et al. 2015). Within our pilot data 13 recurrently mutated genes were found. The most interesting of our recurrent genes is TNFAIP3, which was the second most frequently mutated gene in Reichel et al. 2015. These data suggest that ultra-deep sequencing of tumor samples containing rare HRS-cells will identify recurrent somatic mutations. Thus, ultra-deep exome sequencing may be a useful discovery tool for rare tumor populations, and will improve our understanding of HL.

#2449 Transcriptome and whole-genome sequencing analysis of a novel murine model of chronic lymphocytic leukemia. Lili Wang,1 Jing Sun,2 Amaro Taylor-Weiner,2 Jaegil Kim,2 Zachary J. Cartun,3 Angela N. Brooks,2 Donna M. Cipolat,4 Benjamin D. Ellens,2 Emily A. O’Malley,2 Christine M. Carrazzo,2 Catherine J. Wu,2 Dana-Farber Cancer Institute, Boston, MA; 3Broad Institute, Cambridge, MA; 4University of California, Santa Cruz, CA; 5Boston Children’s Hospital, Boston, MA.

Large-scale cancer sequencing of primary chronic lymphocytic leukemia (CLL) has identified SF3B1, an RNA splicing factor, as one of the most frequently mutated CLL genes. SF3B1 mutations localize to a hotspot (>50% at K700E site) and highly co-occur with mutations in ATM or deletion of chromosome 11q (minimally deleted region contains ATM). How this splicing factor mutation alone or in cooperation with ATM deletion contributes to CLL remains elusive. Genetically engineered mice are powerful tools in understanding genetic lesions and cancer phenotypes. We therefore generated a mouse line that conditionally expresses heterozygous SF3B1-K700E mutation. We modeled the effects of the combined alterations by crossing mice with conditional knockout of ATM and mice with SF3B1-K700E. By breeding these mice with homozygous CD19-Cre transgenic mice, we achieved B cell-restricted expression of heterozygous SF3B1 mutation and ATM deletion. B cell co-expression of these two mutations in vivo led to clonal expansion of CD19+ CD5 + B cells in blood, marrow and spleen in aged mice (18 to 24 month old) at low penetrance. No leukemia cells were found in the SF3B1-K700E mice (up to 24-month old). The CLL cells from the double mutant mice could be engrafted in both immunocompetent and immunodeficient mice, with detectable disease within 2-4 weeks following transfer, thus making this mouse line amenable to drug discovery and biological validation.
investigations. To investigate how Sf3b1 mutation and Atm deletion synergistically contribute to CLL, we asked if there are RNA and DNA level changes in the double mutant mice with CLL. First, we performed transcriptome sequencing of splenic B cell RNA collected from age-matched mice that either express wild-type, singly mutant alleles of Sf3b1 or Atm, or doubly mutant alleles with or without the deletion. Using the tool JunctionBASE, we classified and quantified splice variants associated with the different genetic alterations. Consistent with prior findings in human CLL, we observed that splice variants in Sf3b1 mutant mice alone were highly enriched at 3′ splice sites. Sf3b1 and Atm doubly mutant B cells displayed a splicing pattern similar to that in Sf3b1 singly mutant cells. Moreover, we identified unique CLL splice variants in genes (Setdb2, Pthc1c) previously reported to be associated with CLL. Next, we examined the expression rate in DNA derived from splenic B cells collected from mice with a singly mutated allele of Sf3b1 or Atm, or with doubly mutated alleles with and without CLL-like disease through comparison against matched germine DNA from kidney by whole-genome sequencing. We have observed that co-expression of Sf3b1 mutation and deletion of Atm results in a higher DNA from kidney by whole-genome sequencing. We have observed that co-expression of Sf3b1 mutation and deletion of Atm results in a higher alteration rate compared to single mutant cells. Our analysis has revealed that altered RNA splicing and genomic instability all contribute to CLL leukemogenesis. We will further dissect how the two lesions contribute to CLL functionally using this model.

#2450

Mutational profiling of MLL-PTD acute myeloid leukemia. Ling-wen Ding,1 Qiaoqiao Sun,1 Kar-Tong Tan,1 Wenwen Chien,1 Anand Maya-konda,1 Dechen Lin,1 Xinxi Loh,1 Jinfen Xiao,1 Minjae Jeon,1,2 Tamaras Alperman,2 Manoj Garg,1 Su-Lin Lim,1 Vikas Madan,1 Norimichi Hattori,3,4 Singapore, Singapore;2 MLL Munich Leukemia Laboratory, Munich, Germany; Tokyo, Tokyo, Japan;5 National Taiwan University Hospital, Taiwan, Taiwan;3 Department of Pathology and Tumor Biology, Kyoto, Japan;4 The University of Tokyo, Tokyo, Japan; Japan; National Taiwan University Hospital, Taiwan, Taiwan; 4 Cedars-Sinai Medical Center, Los Angeles, CA; 5 Keio University School of Medicine, Tokyo, Japan; 6 MD Anderson Cancer Center, Houston, TX.

In this study, we performed whole-exome and targeted sequencing on 85 MLL-PTD AML patients. These AMLs have oncogenic tandem duplication of the MLL gene. At least one well-known oncogenic driver mutation was identified in over 90% of the MLL-PTD patients. In line with earlier sequencing studies, we identified the most altered genes in MLL-PTD patients. IDH2 (16.1%), NF2 (18.8%), PTEN (9.4%), TP73 and SMAD2 (2.5%). Single-copy gains and deletions were enriched (p < 0.001) for genes mapping into the following pathways: aberrant PI3K and PI3K/AKT signaling, loss of function of SMAD4 in cancer and SMAD4 MH2 Domain mutants in cancer. The functional pathways significantly (p < 0.001) deregulated in our cohort with single copy gain and homozygous amplification were: regulation of transcription and nuclear acid, negative regulation of metabolic processes, constitutive signaling by aberrant PI3K in cancer and PI3K/AKT network. Finally, in order to define driver alterations, we correlate deletions and losses with mutational data. Interestingly, we found losses which are also targeted by mutations (BRCA2, LRRK1). Moreover, some deleted genes, as CASK, CDK6 and MALT, were involved in pathways affected by genomic mutations (CASK deletion and MPP6 mutation, CDK6 deletion and PPM1D mutation, MALT deletion and SPAG5 mutation). Conclusion: We have identified new CNAs and pathways involving novel potential leukemia-related genes. Our results suggest that the comparison between SNP and WES data could provide important findings on prognosis of AML patients. Minimal deleted regions of genes implicated in deregulated pathways deserve further investigation in order to identify which candidate genes could be relevant AML biomarkers.

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Detailed genomic characterization of CML/aCML/MPN-U/CML reveals disease subgroups that may benefit from rationally-designed combination therapies. Hajiao Zhang,1 Beth Wilmot,1 Daniel Bottomly,1 Libbey Milon,1 Jie Pan,1 Angela Hsu,1 Juliette Rofelty,1 Sophie Means,1 Brian Junio,1 Samantha Savage,1 Emily Stevens,2 Kim-Hien Dao,3 Julia E. Maxson,1 Jeffrey W. Tyner,1 Oregon Health & Science University Knight Cancer Institute, Portland, OR;4 Fred Hutchinson Cancer Research Center, Seattle, WA.

Purpose: Chronic neutrophilic leukemia (CNL), chronic myelomonocytic leukemia (CMM), atypical chronic myeloid leukemia (aCML), and unclassified myeloproliferative neoplasms (MPN-U) are a group of heterogeneous disorders belonging to rare entities of myeloproliferative or myelodysplastic/myeloproliferative (MDS/MPN) syndromes. Due to lack of specific molecular markers and the limited understanding of pathogenesis, the treatment of these diseases remains empirical, resulting in poor outcomes. Recently, recurrent mutations in ASXL1, TET2, SRSF2 and cell signaling genes have been identified in these diseases. In this study we aim to analyze the co-occurrence patterns of these gene mutations, as well as the association of different mutations with disease subtypes and treatment outcomes. Methods and results: We performed whole exome sequence and RNA-Seq on primary patient samples. Consistent with previous studies, high frequencies of ASXL1, TET2, SRSF2 and cell signaling pathway mutations were observed; whereas mutations of MPL, CEBPA, IDH1/2, and TP53 were rare. Further variant allelic frequency analysis demonstrated that mutations of the chromatin modifiers, epigenetic markers and splicing factors are mostly present in the major clones indicating early acquisition of these mutations.

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In addition, ASXL1/2, splicing factor and signaling pathway mutations co-occur in around 45% of all patients with these mutations, indicating that they drive these diseases in a cooperative manner. RNA-seq analysis demonstrated two major gene expression clusters with high levels of either RAS or JAK-STAT signaling alterations respectively, indicating a potential need for distinct therapeutic strategies for specific subsets of specimens. In addition, we observed that 17% of patients have more than one signaling pathway mutation, possibly in distinct subclones, providing rationale for drug combination treatment. Furthermore, we observed that mutations related to RAS signaling pathway are prevalent in CMML; CSF3R and JAK2 mutations are enriched in CNL; whereas, RAS and JAK-STAT pathway double mutations are enriched in CMML. Similar to other cancers, copy number alterations and epimutations are observed across all these diseases. Conclusions: CNL/aCML/MPN-U/CMLM is a group of heterogeneous diseases associated with chromatin modifier, epigenetic, splicing factor and signaling pathway mutations in concomitant manner. We propose future studies of rational drug combinations with agents, targeting epigenetic and splicing factors, together with the appropriate signaling pathway inhibitors. Our study represents a large-scale comprehensive genomic analysis of these diseases, which reveals novel insight into patterns of mutation co-occurrence which could translate into new treatment paradigms for these difficult to treat hematologic malignancies.

#2453 Chromatin remodeling genes are frequently somatically altered in cervical carcinomas. Akinyemi Ojesina, Aishwarya Sundaresan, Vinodh Shrinivasan, Risma Sarema, Hemanth Tiwari, Warner Huh. University of Alabama at Birmingham, Birmingham, AL.

Introduction: Cervical cancer is responsible for 15% of all deaths due to cancer in women worldwide. There is a need to identify somatic mutations driving this cancer in order to facilitate development of novel therapeutic strategies. Experimental procedures: We analyzed whole exome sequencing data from 430 cervical carcinomas. The data were derived from a combination of our previously published work (Ojesina et al. Nature 2014; Chung et al., Int J Cancer 2015), and publicly available data generated by the Cancer Genome Atlas (TCGA). The Mutect2.0 and MutSig2CV algorithms were used to identify somatic mutations and significantly mutated genes (SMGs) respectively. Copy number alterations were analyzed using the GISTIC algorithm. Findings: Based on MutSig2CV false discovery rate threshold of 0.1, we identified MLL3 (19%) and MLL2 (13%) as novel significantly mutated genes (SMGs) in cervical cancer. In addition, other chromatin modifying genes including EP300 (12%) and ARID1A (7%) were confirmed as SMGs. Further investigation of genes that did not meet the threshold revealed that 228 of 430 (53%) of cervical tumors harbored at least one somatic mutation in chromatin modifying genes. These include genes in the myeloid/lymphoid or mixed-lineage leukemia (MLL) family (28%), lysine (K)-specific demethylase (KDM) family (23%), AT rich interactive domain (ARID) family (13%), SWI/SNF related, matrix associated, actin dependent regulator of chromatin (SMARC) family (8%) and histone cluster 1 (HIST1) family (4%). Conclusions: We have demonstrated that a majority of cervical cancers harbor somatic mutations in chromatin remodeling genes. This novel finding was facilitated by the larger sample size derived from combining multiple datasets, and may have diagnostic and therapeutic implications in cervical cancer.

#2454 Genetic landscape of glioma reveals defective neuroactive ligand receptor interaction pathway as a poor prognostic marker in glioblastoma patients. Jagriti Pal1, Vikas Patil1, Anupam Kumar2, Kaymeet Kaur2, Chitra Sarkar3, Kumaravel Somasundaram1. 1Indian Institute of Science, Bangalore, India; 2All India Institute of Medical Science, New Delhi, India.

Glioblastoma (GBM; grade IV), is highly proliferative, infiltrative and treatment refractory. Hence, understanding the complete genetic alteration profile of GBM would help us in identifying molecules or pathways that have strong implication in GBM pathogenesis and in the design of targeted therapy. Recent large scale studies suggest that three pathways - receptor-tyrosine kinase, TP53 and RB, are significantly altered in GBM. However, even with the tremendous increase in our understanding of the tumor, advancement in therapeutics is minimal and the median survival still remains at 15 months. Hence, we need to elucidate novel altered molecules and pathways in GBM progression such that more effective therapeutic opportunities can be explored. Here, we have carried out whole exome sequencing of grade II, grade III and GBM samples which revealed the mutation spectrum of glioma from our patient set. Further, we performed integrative analysis of mutated genes from our patient cohort as well as TCGA cohort (The Cancer Genome Atlas) to find out mutated pathways that predict survival in GBM patients. The most significant pathway - neuroactive ligand-receptor interaction pathway was explored further. Patients with mutations in one or more genes of this pathway had poor survival. The pathway comprises of G-protein coupled receptors, ion channels and ligands which functions in modulating a variety of cellular processes, behavior etc. Of the enriched genes belonging to this pathway, Calcitonin Receptor (CALCRL), which was highest mutated in GBM (2.75%), was taken up for further investigation. CALCRL was found to be downregulated in GBM and mutation or downregulation of the gene was found to predict poor survival in patients. Functional studies through cell-line based experiments revealed CALCRL is a tumor suppressor in GBM. The peptide hormone calcitonin (CT), a high affinity CALCRL ligand, inhibited proliferation, migration and anchorage-independent growth of glioma cells expressing CALCRL with a concomitant decrease in the phosphorylation levels of ERK, AKT and JNK signaling molecules. However, CT failed to do these functions in CALCRL silenced gloma cells. Exogenous overexpression of CALCRL in glioma cells expressing low levels of the receptor was found to inhibit proliferation, migration and anchorage independent growth and this effect was further augmented when CT was added. Further, introduction of tumor-derived mutations in CALCRL led to the abrogation of its tumor suppressor function. Studies are ongoing to demonstrate the tumor suppressive nature of CALCRL using in vitro astrocyte transformation and intracranial orthotopic mouse glioma model. Thus, our study suggests CALCRL signaling is an important tumor suppressor pathway in glioma development and underscores the importance of using CT as a novel therapeutic molecule for GBM treatment.

#2455 Human glioblastoma arises from the distant subventricular zone normal appearing but harboring tumor-initiating mutations. Joo Ho Lee,1 Jeong Eun Lee,2 Jee Ye Kahng,3 Jinseong Park,3 Seon Jin Yoon,3 Se Ho K immun,2 Jong Hee Chang,3 Seok Gu Kang,3 Jeong Ho Lee,3 Korea Advanced Institute of Science and Technology, Daejeon, Republic of Korea; 4Chungnam National University Hospital, Daejeon, Republic of Korea; 5Severance Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea.

Glioblastoma multiforme (GBM) is the most devastating and incurable brain tumor. Although the identification of cells with tumor initiating mutations or their location can provide the fundamental basis for understanding disease progression, the origin of GBM remains controversial due to the lack of direct evidence in human GBM patients. Here, we performed ultra-deep sequencing of triple-matched tissues of i) radiologically and pathologically normal subventricular zone (SVZ), which is distant from tumor, ii) GBM tumor, and iii) blood (or normal cortical tissues) from patients with GBM (IDH-wildtype), compared to those with other type of brain tumors such as GBM (IDH-mutant), meningioma, oligodendroglioma, metastatic brain tumor and C (IDH-wildtype with SVZ-invasion. Surprisingly, we found that in 55.5% of IDH-WT GBM patients (5 of 9), normal appearing and distant SVZ already contained the low level of GBM mutations such as TP53, EGFR, RB1, PDGRF or TERT variations observed in the matched tumor. Single cell sequencing of GBM tumors and laser capture microdissection analysis of the SVZ show that mutations are enriched in the region of neuronal progenitor cell clusters, with neuronal processes, behavior etc. of the cell closest to the IDH-WT GBM tumor. Furthermore, using CRISPR-Cas9 system in the postnatal mouse brain, we showed that neural stem cells with TP53, PTEN, EGFR mutations migrated away from the mutated SVZ site and then formed the high grade malignant glioma in the distant cortical region. Taken together, this study provides the direct evidence that human glioblastoma arises from the distant SVZ that is normal-appearing but harboring tumor-initiating mutations.

#2456 Distinct genomic landscape of upper urinary tract urothelial carcinoma. Yoichi Fujii1, Yusuke Sato2, Hiromichi Suzuki1, Tetsuichi Yoshizato1, Yusuke Shiozawa1, Kenichi Yoshida1, Yuichi Shiraiishi2, Kenichi Chiba3, Hiroko Tanaka4, Tomoharu Nakagawa2, Haruki Kume3, Hiroaki Nishimatsu1, Toshikazu Okane4, Masashi Sanada5, Hideki Makishima4, Satoru Miyano3, Yukio Hompa4, Seiki Ogura4, Graduate School of Medicine, Kyushu University, Kyushu, Japan; 2Chungnam National University Hospital, Daejeon, Republic of Korea; 3Severance Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea; 4The Fraternity Memorial Hospital, Sumiduka, Japan; 5Toranomon Hospital, Minato, Japan; 6Clinical Research Center, Nagoya Medical Center, Nagoya, Japan.

Backgrounds: Upper urinary tract urothelial carcinoma (UTUC) is relatively rare, accounting for 5-10% of urothelial malignancies with frequent multifocal development. To clarify distinct characteristics of UTUC, we comprehensively investigated the genetic alterations of this disease. Materials & methods: Surgical specimens of UTUC and matched normal samples were obtained from 99 patients with various stages who underwent nephroureterectomy, and subjected to
whole exome and RNA sequencing. We compared our results in UTUC with datasets previously reported in bladder urothelial carcinoma (BUC). Mutations in apparently normal urothelial epithelia in 5 cases were also interrogated. Results: Genetic alterations were most frequently observed in TERT promoter (51% of cases), followed by KMT2D (48%), FGFR3 (44%), CDKN2A (42%), TP53 (31%), and RAS pathway (HER2/KRAS/NRAS, 21%). More than 95% of cases harbored either TP53/MDM2, FGFR3, or RAS pathway mutations in an almost mutually exclusive manner, based on which UTUCs are classified into 3 molecularly and clinically distinct subtypes based on the status of mutations in TP53/MDM2, FGFR3, and RAS pathway. Depending on their location, urothelial cancers have different genetic backgrounds, where a field effect unique to urothelial epithelia might contribute to multifocal occurrence of UTUC.

### #2457 Investigating the functional significance of novel, recurrent non-coding mutations of TBC1D12 in bladder cancer. Angela S. Li, Jason A. Reuter, Can Cenik, Michael P. Snyder. Stanford University School of Medicine, Stanford, CA.

Somatic noncoding mutations remain widely unexplored despite examples of critical importance such as driver mutations in the promoter of the TERT gene. We recently identified novel somatic mutations in the regulatory Kozak sequence (just upstream of the translation start site) of TBC1D12 in ~15% of bladder cancer patients, making it one of the most frequently mutated noncoding regions in bladder cancer. Using two independent case-control studies, we identified novel noncoding mutations in TBC1D12 in ~15% of patients with bladder cancer. To experimentally test the functional relevance of these mutations, we performed co-transfection experiments with a RAB-specific GTase-activating protein (GAP). To investigate the functional implications of TBC1D12 mutations in bladder cancer, we undertook a combined computational and experimental approach. We analyzed clinical correlations, identified downstream effector candidates, and assessed the impact of these mutations on gene expression. To determine the clinical relevance of the TBC1D12 mutation, we did correlation and survival analyses of clinical data of bladder cancer patients from TCGA. We found that TBC1D12 mutations confer slightly worse survival but were not linked to other clinical correlates. To identify potential downstream effectors, we used reverse phase protein array (RPPA) data from 127 patients to search for proteins that were differentially expressed or phosphorylated between patients with or without TBC1D12 mutations. We found differential expression of GSK3 and differential phosphorylation of p90RSK and c-Jun, suggesting three potential downstream effector candidates. Hierarchical clustering of RPPA data for these three proteins revealed 4 subgroups with distinct molecular phenotypes. Furthermore, most of the TBC1D12 mutations were clustered within one molecular subtype. To experimentally test these downstream effector candidates and identify new ones, we modulated the levels of TBC1D12 in bladder cancer cell line Ku-19-19 using overexpression plasmids or siRNAs. After validating that these transfections were effective in changing TBC1D12 expression, we performed RNA-Seq experiments. Results from this study are expected to be presented at the meeting. In summary, these studies suggest that the novel and recurrent noncoding mutations in TBC1D12 may affect patient survival and alter the expression or post-translational modification of downstream targets. Potential molecular mechanisms associated with the presence of TBC1D12 mutations will be discussed, and a series of follow-up studies are underway. These studies also demonstrate that applying both computational and experimental approaches can help develop and test evidence-based hypotheses, contributing to an understanding of the clinical and functional impact of TBC1D12.


Urothelial cancer (UC) is a well-known multifocal disease with frequent recurrences. The clonal origin of spatially separated UC foci is controversial. Here we propose to elucidate the multifocal UC genome by next generation sequencing (NGS) and thereby identify novel clonal heterogeneity among the lesions of a single bladder. Until now, all experiments aimed at understanding the molecular heterogeneity were generated mostly by candidate gene approach and older technologies. There is a lack of comprehensive molecular information in these multifocal lesions partly due to inadequate sample size for comprehensive molecular studies. Identification of mutational status at the gene level in different multifocal lesions will allow us to identify markers for prognostic classification, and predictive classification of response to UC therapies, as well as identify potential therapeutic targets. To understand the molecular heterogeneity among the lesions of a single bladder, we used The Ion AmpliSeq™ Comprehensive Cancer Panel (CCP) (ThermoFisher). The Ion AmpliSeq™ CCP was designed to target all exons of 409 key tumor suppressor genes (TSGs) and oncogenes most frequently cited and mutated in cancer. We tested 41 lesions from 16 cystectomized bladder cancer patients with this panel, enabling an initial analysis of 21 lesions and 8 germ line controls from 8 patients. We further technically validated selected mutational events found by NGS with a complementary approach, namely droplet digital PCR (ddPCR). We then compared the intra- and inter-tumor mutation profiles. Two separate lesions were used for analysis in 3 patients and 3 lesions were taken from each of the remaining 5 patients. Our initial analysis showed mutational heterogeneity among the lesions of most of the samples. Briefly, two of the patients had one clone found at all sites, while the rest of the patients showed more variation amongst their lesions. For instance, ARID2, TRRAP, PDGFB, and FBXW7 were mutated in two out of three lesions in one of the patients, indicating hotspot for heterogeneous mutational events. Three mutational events were confirmed by ddPCR, denoting the accuracy of our NGS data analysis. Our analysis demonstrates that targeted next generation sequencing is a sensitive and reliable method for interrogating the molecular landscape of bladder cancer, and indicates that both clonal and spontaneous events can account for multifocal bladder cancer. Our data suggests that sequencing of multiple lesions from an individual patient is necessary to accurately elucidate the clonal and tailoring profile of targeted treatments. Further analysis in an extended number of samples is necessary to fully understand the molecular heterogeneity at the mutational level in urothelial cancer.

### #2460 Copy number alterations in NOTCH2 and PTP4A3 are associated with prognosis and support novel therapeutic strategies for malignant pleural mesothelioma. Leticia G. Leon,1 Maria Gemelli,2 Paolo A. Zucali,2 Christos Zografos,2 Giuseppe Fabbri,2 Elisa Giovannetti1. 1 University of Pisa, Pisa, Italy; 2 Humanitas Clinical and Research Center, Rozzano, Italy; 3 University of La Laguna, La Laguna, Spain.

Malignant pleural mesothelioma (MPM) is an aggressive cancer, which incidence has constantly increased over the past two decades and is expected to peak in 2020. The overall prognosis is poor, and predictive biomarkers of drug activity are missing. Given the heterogeneous and complex nature of MPM, it is likely that genomic aberrations changing the expression of several genes, might affect therapeutic response. Therefore, the aim of the present study was to identify genes whose copy number alterations might predict the MPM prognosis. Recurrent copy number alterations of genes were analyzed by high-resolution whole-genome sequencing in DNA obtained from a “discovery cohort” of 26 resected MPM patients treated with pemetrexed chemotherapy (8 with progressive disease, vs. 10 with stable disease and 8 with partial response). Prognostic markers identified by Copy Number Variation analysis with Nexus, ControlFREEC and ReadDepth software were validated by PCR gene copy number and gene expression analyses both in the “discovery” and in two “validation” cohorts of pemetrexed-treated and untreated patients (N=45 and 40). The role of emerging genes was evaluated through siRNA and pharmacological studies using proliferation, migration and apoptosis assays in MPM cells. As reported previously we observed copy number loss of CDKN2A (15.4%) and BAP1 (7.7%). Interestingly, copy number gain of NOTCH2 was observed in 50% of samples of the patients who underwent progression, whereas losses of PTP4A3
were associated with clinical benefit (SD+PR). The prognostic relevance of NOTCH2 was confirmed by PCR analysis. NOTCH-2 silencing reduced MPM cell migration and enhanced apoptosis induction by pemetrexed, while a PTP4A3 inhibitor overcame pemetrexed resistance in MPM cells characterized by high NOTCH2 and PTP4A3 expression. These results support the role of NOTCH2 as a molecular target with therapeutic potential for MPM, prompting prospective randomized trials for its validation. Moreover, preclinical data suggest that NOTCH2 and PTP4A3 are oncogenes suitable for effective therapeutic targeting in pemetrexed-resistant MPM cells.

#2461 Molecular classification and clinical characterization of a large uterine leiomyoma patient cohort. Nettta Makinen,1 Hanna-Riikka Heinonen,1 Annukka Pasanen,2 Jaana Tolvanen,3 Simonna Bramante,1 Miika Mehine,3 Jari Sjöberg,3 Oskari Heinikimo,5 Ralf Büttow,2 Lauri A. Aaltonen,1 Genome-Scale Biology Research Program and Department of Medical and Clinical Genetics, University of Helsinki, Helsinki, Finland;3Department of Pathology and Laboratory of Helsinki University Hospital (HUSLAB), Helsinki University Hospital, University of Helsinki, Helsinki, Finland;1Department of Obstetrics and Gynecology, Helsinki University Hospital, Helsinki, Finland.

Uterine leiomyomas (ULs), benign smooth muscle tumors, represent one of the most common neoplasms in women with an estimated prevalence varying from 20% to over 70% during the reproductive years. Approximately every fourth woman with ULs has clinically relevant lesions, which cause morbidity and thus require treatment. Still today, hysterectomy is the primary treatment option for ULs worldwide, and remarkably, their annual societal costs exceed those of colon and breast cancer combined. Our previous findings, derived from the use of high-throughput technologies, suggest that there are at least three distinct molecular UL subclasses, each displaying a characteristic genetic driver aberration and unique global gene expression profile: MED12 (mediator complex subunit 12) mutation-positive, HMG2A (high mobility group AT-hook 2) -overexpressing, and FH (fumarate hydratase)-deficient ULs. The aim of this study is to examine the molecular subclasses, their respective proportions, and clinical characteristics in a large UL patient cohort. The study material consists of 1026 ULs and corresponding normal myometrium tissue from 322 patients, who had ultrasound-diagnosed ULs and underwent hysterectomy in Helsinki University Hospital, Finland between October 2013 and June 2016. From each uterus, we harvested all feasible ULs $\geq 1$ cm in diameter and a piece of the corresponding normal myometrium tissue. The location of the collected samples in the uterus, their size, and any observed special characteristics were carefully documented at the time of sample removal. In addition, comprehensive clinical information of the patients was obtained from medical and pathology reports, as well as from a questionnaire, and the histopathology of all lesions were characterized according to the WHO 2014 criteria. All collected ULs have been systematically screened for MED12 mutations by Sanger sequencing. Overexpression of HMG2A typically arises from a chromosomal translocation, and deletions at FH locus suggest the presence of potential biallelic FH inactivation. Therefore, HMG2A-overexpressing and FH-deficient ULs have tentatively been identified using a high-density customizable Infinium® Human6 BeadChip with over 305,000 markers. Currently, the data analyses are ongoing, and the clinical significance of the identified genetic variants is being investigated. From the targeted sequencing, the Illumina Amplicon-DSSo Profiling, called from the SNP data by hapLOH, showed that sarcoma samples were more aberrant than their carcinoma counterparts (abstract 131, AACR 2016). From the targeted sequencing, the Illumina AmpliSeq-DS Somatic Variant Caller was employed to call somatic mutations. Mutations were identified in TP53 in both the sarcoma and carcinoma samples of all 10 patients. Frequently mutated genes included APC, EGRF, MET and MSH6 which were found in 60-80% of the patients. Genes mutated in less than 50% of the patients included PTEN, KRAS, KIT, FBXW7, PIK3CA, FGFR2, and CTNNB1. Current results showed no association of a mutated gene to either the carcinoma or sarcoma component of UC. RSM, STAR and EBSeq were applied to the RNA-seq data for gene expression quantification. Approximately 2500 genes were identified as being differentially expressed (DE) between normal and carcinoma samples. Deregulated pathways identified in both carcinoma and sarcoma include: cell cycle, transcriptional regulation, Ras and p53 signaling. Some additional pathways affected in the carcinoma but not in the sarcoma component also suggest their importance (abstract 791, AACR 2016). The next step of this study is to examine the global gene expression profile of UC by RNA sequencing.

#2462 Whole-exome sequencing reveals genetic variants in ERC1 and KCNG4 in complete hydatidiform moles from Chinese Han women. Yan Yu,1 Bingjian Lu,1 Weiguo Lu,1 Xinyu Wang,1 Pengyu Liu,1 Yan Lu,1 Xiaodong Cheng,1 Xing Xie1. Women’s Hospital School of Medicine Zhejiang UNIV., Hangzhou, China;1Institute for Translational Medicine School of Medicine, Zhejiang University, Hangzhou, China.

Complete hydatidiform mole (CHM) is an uncommon pregnancy-related disease with an invasive potential. The genetic background of the sporadic CHM has not been addressed previously despite the possible mechanisms of maternal genetic variants to the development of this disease with biparental origin. We performed the whole-exome sequencing analysis on 51 CHM patients and 40 normal healthy women. Finally, the candidate genetic polymorphisms were validated by direct Sanger sequencing in 247 cases and 599 controls with another 205 new controls. We found that two SNPs c.G48C(p.G16H) in ERC1 and c.G1114A(p.G372S) in KCNG4, were associated with an increased risk for CHM (p<0.05). These genetic variants will facilitate our understanding on the pathogenesis of CHM and related tumors as well as the oogenesis and embryonic implantation. They will provide additional information for the pregnant women both in physiology and psychology. Further multiple-disciplinary collaborations should be encouraged to clarify the accurate pathogenesis of CHM.

#2463 Tumor profiling of separated carcinomatous and sarcomatous components from uterine carcinosarcoma biopsies provides insights into their development. Yuhua Liu,2 Zachary Weber,2 Anthony San Lucas,1 Aditya Deshpande,1 Raed Sulaiman,3 Mary Fagerness,2 Natasha Flier,2 Joseph Sulaiman,3 Christel M. Davis,4 Jerry Fowler,2 Gareth E. Davies,2 David Starks,2 Luis Rojas-Espalliat,8 Paul Scheet,2 Erik A. Ehli,1 Graduate School of Biomedical Science, University of Texas at Houston Health Science Center, Houston, TX;2Avera Institute for Human Genetics, Sioux Falls, SD;3University of Texas MD Anderson Cancer Center, Houston, TX;4Weill Cornell Graduate School of Medical Sciences, New York, NY;5Avera Cancer Institute, Sioux Falls, SD.

Uterine carcinosarcoma (UCS) is a rare and aggressive form of uterine cancer. It is bi-phasic, exhibiting histological features of both malignant epithelial (carcinomatous) and mesenchymal (sarcomatous) elements. Studies have indicated that UCS arises from sarcomatous differentiation of high-grade carcinoma while others have suggested a bi-clonal nature. Given these differences, we sought to separate the carcinoma and sarcoma elements of UCS to try to understand their molecular differences and gain further insights into how these tumors develop. We macrodissected carcinomatous, sarcomatous, and normal tissues from formalin fixed paraffin embedded FFPE tumor samples of 10 UCS patients. DNA and RNA were isolated and extracted using the Qiagen AllPrep DNA/RNA FFPE kit. Whole-genome SNP microarrays and deep sequencing of 26 cancer genes was performed, using the Illumina Infinium HumanExome Array and the TruSight Tumor panel respectively. Illumina HiSeq mRNA sequencing was also performed to quantify gene expression. The genomic allelic imbalance (AI) profiling, called from the SNP data by hapLOH, showed that sarcoma samples were more aberrant than their carcinoma counterparts (abstract 131, AACR 2016). From the targeted sequencing, the Illumina AmpliSeq-DS Somatic Variant Caller was employed to call somatic mutations. Mutations were identified in TP53 in both the sarcoma and carcinoma samples of all 10 patients. Frequently mutated genes included APC, EGFR, MET and MSH6 which were found in 60-80% of the patients. Genes mutated in less than 50% of the patients included PTEN, KRAS, KIT, FBXW7, PIK3CA, FGFR2, and CTNNB1. Current results showed no association of a mutated gene to either the carcinoma or sarcoma component of UC. RSM, STAR and EBSeq were applied to the RNA-seq data for gene expression quantification. Approximately 2500 genes were identified as being differentially expressed (DE) between normal and carcinoma samples. Deregulated pathways identified in both carcinoma and sarcoma include: cell cycle, transcriptional regulation, Ras and p53 signaling. Some additional pathways affected in the carcinoma but not in the sarcoma component also suggest their importance (abstract 791, AACR 2016). The next step of this study is to examine the global gene expression profile of UC by RNA sequencing.

#2464 Papillary renal cell carcinoma, proposal of a new classification system based on integrated molecular, histological and clinical analysis. Rola Saleh,1 Mina Farag,1 Fadi Brimo,2 Fabio Rotondo,1 Pamela Plant,1 George Youset1. 1.1Lil Ke Shing Knowledge Institute, St. Michael’s Hospital, Toronto, Ontario, Canada; 2McGill University Health Center, Montreal, Quebec, Canada.

Background: Papillary Renal Cell Carcinoma (PRCC) is divided into histological subtypes 1 and 2. Type 2 is known to have worse clinical behavior. A number of PRCC cases (~ 50%) fail to meet all reported morphological criteria for either type, hence are best characterized as PRCC not otherwise specified (NOS). There are yet no reliable markers to resolve the PRCC NOS category. That in turn reflects the clinical dilemma of how to manage these patients. Experimental Design: PRCC patient cohort of 115 cases was selected for the study. Cases were subtyped histologically into PRCC types 1, 2 and NOS. Potentially distinguishing markers ABC2C2, CA9, SAI4, and BCL2 selected from our previous genomic analysis, were assessed by immunohis-
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#2466 Identifying confidently measured genes in single pediatric cancer patient samples using RNA sequencing. Holly Beale,1 Du Linh Lam,2 John Vivian,1 Yulia Newton,1 Avanthi Tayi,1 Ted Goldstein,1 Ananta Shankar1. 1UCSC Genomics Institute, Santa Cruz, CA; 2Stanford University, Stanford, CA

In the UC Santa Cruz Treehouse Childhood Cancer Initiative (treehousegenomics.soee.ucsc.edu), we are exploring the utility of using RNA-Seq analysis of tumor samples from children to identify potential novel therapeutic options for each individual. Within a single RNA-Seq data set, the gene expression measurements are not equally accurate. The identification of activated, druggable pathways requires accurate gene-level expression measurements. We receive samples from a variety of clinical and research settings, and the quantity and complexity of the available input material and the depth of sequencing differ. These factors inspired us to develop a tool that will allow us to identify accurate measurements in most RNA-Seq samples we receive. First, we characterized the relationship between depth of sequencing and the accuracy of the gene expression measurement. We analyzed subsets of reads in samples with more than 50 million Uniquely Mapped, Non-duplicate (UMEND) reads. UMEND reads typically constitute over 80% of the reads in a high quality experiment with sufficient starting material. We compared gene expression across the subsets of reads to calculate how many UMEND reads are required to produce consistent measurements. We found that, on average, genes expressed at 1-5 TPM in our data require 30 million reads to be accurately measured. For this calculation, we define accuracy as the condition in which 75% of genes are measured to within 25% of the true value. Secondly, we use these known relationships to identify genes that have been accurately measured in our tumor RNA-Seq samples. For a sample with 15 million UMEND reads, we find that genes expressed above 5 TPM can be accurately measured and are retained. In the first twelve samples analyzed, samples with more than 10 million UMEND reads retained at least 46% of the genes expressed above zero. We exclude as references those samples with fewer than 10 million UMEND reads due to the marked gene loss after thresholding for this group. Using accurately measured genes allows us to more confidently assess similarity to other samples, identify enriched pathways, and confirm the expression of drug targets and related molecules under consideration. For example, we reconsidered the CDK4 inhibitor Palbociclib in one patient because the expression of RB1, downstream effector required for Palbociclib-mediated tumor cell death, was under our accuracy threshold. Accuracy thresholds can also be used in experiment planning. Accuracy thresholds allow us to better assess the value of an RNA-Seq data set and, if necessary, identify the subset of genes whose expression can be confidently considered in a clinical setting. Our experience points to the importance of careful quality control in this process.

#2467 Mutation analysis using next-generation sequencing in histologically heterogeneous primary lung cancers. Hayato Koba, Hideharu Kimura, Kazuo Kasahara, Kanazawa University Hospital, Kanazawa, Japan

Rare cases of primary lung cancer are known to have different histological types. Recently, small cell carcinoma was reported to be developed in adenocarcinoma patients with epidermal growth factor receptor (EGFR) mutations after EGFR tyrosine kinase inhibitor (EGFR-TKI) treatment. The purposes of this study are to evaluate mutational status in two different histological types separately, and to further understand the molecular pathogenesis. Twelve tumor samples from 6 patients were collected in our institution. 4 patients had primary adenocarcinoma and small cell carcinoma transformed after EGFR-TKI. One patient had primary adenocarcinoma and squamous cell carcinoma transformed after EGFR-TKI. The other one patient had carcinomas in a surgically resected tumor. Two patients had samples collected from different histologically different areas within a resected tumor or the 2 distinct metastatic sites. DNA was extracted from formalin-fixed, paraffin-embedded tumor tissues. Next-generation sequencing in the 12 samples were performed on 160 cancer-related genes using GeneReadTM DNAseq Targeted Panels V2 Human comprehensive Cancer Panel (Qiagen). In a carcinomas case with deletional mutation in EGFR gene, a total 586 in adenocarcinoma and 595 in sarcoma variants in 166 genes were detected with high confidence. The gene of identical alterations, as truncal alterations, were EGFR, CBLB, TP53 and MEN1 and the number of that as an unique alteration corresponding to each histology is 25 for adenocarcinoma and 85 for sarcoma. We show relationships between the histological types and the genomic alterations, identical alterations and unique alterations, in each patient. Furthermore, we raise essential factors for the histological transformation.

#2468 Gene aberrations for precision medicine against lung adenocarcinoma. Motonobu Saito,1 Koji Kono,2 Takashi Kohno.1 1National Cancer Ctr. Japan, Tokyo, Japan; 2Fukushima Medical University, Japan

Lung adenocarcinoma (LADC) is often triggered by an aberration in a driver oncogene. LADC harboring EGFR mutation and ALK fusion can be treated with anti-cancer drugs that target the aberrant gene products. For example, we reconsidered the CDK4 inhibitor Palbociclib in one patient because the expression of RB1, downstream effector required for Palbociclib-mediated tumor cell death, was under our accuracy threshold. Accurately measured genes expressed above 5 TPM can be accurately measured and are retained. In the first twelve samples analyzed, samples with more than 10 million UMEND reads retained at least 46% of the genes expressed above zero. We exclude as references those samples with fewer than 10 million UMEND reads due to the marked gene loss after thresholding for this group. Using accurately measured genes allows us to more confidently assess similarity to other samples, identify enriched pathways, and confirm the expression of drug targets and related molecules under consideration. For example, we reconsidered the CDK4 inhibitor Palbociclib in one patient because the expression of RB1, downstream effector required for Palbociclib-mediated tumor cell death, was under our accuracy threshold. Accuracy thresholds can also be used in experiment planning. Accuracy thresholds allow us to better assess the value of an RNA-Seq data set and, if necessary, identify the subset of genes whose expression can be confidently considered in a clinical setting. Our experience points to the importance of careful quality control in this process.


Aptamers have recently gained prominence for their diagnostic and therapeutic potential. The DNA aptamer C10.36 forms a G-quadruplex and has been shown to bind the Ramos Burkitt’s lymphoma cell line. However, its binding partner on the cell surface remains unknown. Here we report on the identification of the molecular target of C10.36, which suggests its application in the therapy of B-cell lymphoma and leukemia. Aptamer-affinity purification, followed by LC-MS/MS revealed unique proteins pulled down with C10.36 associated with Ramos cells but not Jurkat, a T-cell lymphocyte cell line. The majority of the identified target molecules were found to be associated within ribonucleoprotein complexes, of which the abundant and consistent ones belong to the nucleolin complex including nucleolin (NCL) itself and its interacting partners, i.e. nucleophosmin (NPM1), heterogeneous nuclear ribonucleoprotein (HNRNP) family members such as HNRNP C1G2 and U, RNA Z-’O-methyltransferase fibrillarin (FBL), actin (ACTB), nucleolar RNA helicase 2 (DEx2), and proline- and glutamine-rich splicing factors (PSFQ). All the proteins identified in the above ribonucleoprotein complex are aberrantly expressed on the surface of several disparate cancer cell types and have been shown to play oncogenic role in cancer. Another G-rich anti-NCL aptamer, AS1411, has been shown to induce cell death in >100 cancer cell lines. Therefore, we tested the effect of C10.36 on viability of Ramos and other non-Hodgkin B-cell lymphoma (NHL) cancer cell lines. Our results indicate that C10.36 treatment causes specific cell death of certain lymphoma cell lines such as Ramos but not Jurkat cells. The present study identifies C10.36 as a novel anti-B cell lymphoma aptamer and underscores its potential for the development of a new targeted therapy to treat B-cell lymphomas.
Compromised BRCA1-PALB2 interaction is associated with breast cancer risk. Tzeh Keong Foo,1 Marc Tischkowitz,2 Srialatha Simhadri,1 Taiya Boshari,3 Kathleen A. Burke,4 Samuel H. Berman,5 Nadia Zayed,6 Yuan Chun Ding,7 Susan L. Neubauer,8 Britta Weigelt,7 Jorge S. Reis-Filho,9 William D. Foulkes,10 Bing Xia,11 Rutgers Cancer Institute of New Jersey, New Brunswick, NJ; 2University of Cambridge, Cambridge, United Kingdom; 3Jewish General Hospital, McGill University, Montreal, Quebec, Canada; 4Memorial Sloan Kettering Cancer Center, New York, NY; 5Beckman Research Institute at the City of Hope, Duarte, CA.

The major breast cancer suppressor proteins BRCA1 and BRCA2 play essential roles in homologous recombination (HR)-mediated DNA repair, which is thought to be critical for tumor suppression. The two BRCA2 proteins are physically and functionally linked by a third tumor suppressor, PALB2 (partner and localizer of BRCA2), in the HR pathway. Heterozygous PALB2 mutation carriers have increased risk of breast, ovarian and pancreatic cancer. While truncating mutations in BRCA genes are generally pathogenic, interpretations of missense variants remain a challenge. To date, patient-derived missense variants that disrupt PALB2 binding have been identified in BRCA1 and BRCA2; however, there has not been sufficient evidence to establish their pathogenicity in humans. Variants in PALB2 that disrupt either its BRCA1 or BRCA2 binding have not been reported. Here, we report on the identification of a novel PALB2 variant, c.104T>C [p.L35P], that segregated in a family with a strong history of breast cancer. Functional analyses showed that L35P abrogates the PALB2-BRCA1 interaction, resulting in impaired HR and sensitivity to platinum salts and PARP inhibitors. Whole-exome sequencing of breast tumor from a c.104T>C carrier revealed a somatic, truncating mutation in the second allele of PALB2, with the tumor displays hallmark genomic features of tumors with BRCA mutations and HR defects. Using a combination of traditional clinical genetics, tumor whole-exome sequencing and in-depth functional analyses, we have provided direct evidence to cement the pathogenicity of L35P. Parallel analyses of other germline variants in the PALB2 N-terminal BRCA1-binding domain also identified multiple variants that affect HR function to varying degrees, suggesting their possible contribution to cancer development. Our findings establish p.L35P as the first pathogenic missense mutation in PALB2 identified to date and directly demonstrate the requirement of the PALB2-BRCA1 interaction for breast cancer suppression.

Role of PALB2-BRCA1 interaction in tumor suppression. Amar H. Mahdi, YanYing Huo, Bing Xia. CINJ, New Brunswick, NJ.

Homologous recombination (HR) is the only error-free pathway for the repair of DNA double strand breaks (DSBs). BRCA1 and BRCA2, the two major breast cancer suppressor proteins, play essential roles in HR-mediated repair of DSBs by promoting the recruitment of RAD51, the recombination enzyme, to DNA damage sites for the initiation of HR. PALB2 (partner and localizer of BRCA2) plays a key role in this pathway by acting as a chromatin adaptor for BRCA2 and a linker between BRCA1 and BRCA2. Like BRCA1 and BRCA2, PALB2 is a tumor suppressor gene itself. Germline, heterozygous mutations in the gene increase the risk of breast, ovarian and pancreatic cancers. However, its mechanism is not fully understood. To investigate the in vivo role of the PALB2-BRCA1 interaction, we previously generated a Palb2 knockout mouse strain which contains a mutation that disrupts BRCA1 binding. This mouse model also allows us to bypass the embryonic lethality of the Palb2 KO mice. In this study, we hypothesized that the direct communication between the two proteins is critical for proper DNA damage repair and response in vivo and for suppression of tumorigenesis. Indeed, both immunohistochemistry (IHC) and immunofluorescence (IF) demonstrated that different tissues of the mutant mice have higher levels of endogenous DSBs (γH2AX foci) and slower DSB repair kinetics after ionizing radiation (IR). Yet, mutant cells were more resistant to cell death. When aged under normal conditions, mutant mice showed increased tumor incidence in multiple tissues, particularly in the liver. When challenged by IR, mutant mice quickly developed thymic lymphoma and later in other tissues including liver and ovary, etc. Interestingly, when crossed with Trp53 mutant mice, the resulting Palb2m/m;Trp53−/− mice showed greatly accelerated development of thymic lymphoma and osteosarcoma which are typically associated with Trp53 but not Palb2 mutations. Exome sequencing revealed focal deletion of the wild-type allele of Trp53 in the majority of the tumors, suggesting that disruption of BRCA1-PALB2/BRC2 axis promotes regional genomic deletions that may lead to loss of other tumor suppressors such as p53. Our results underscore the importance of the BRCA1-PALB2/BRC2 pathway for tumor suppression and suggest a potentially novel mechanism for BRCA1/PALB2-mediated tumor suppression, which is by preventing Trp53/TP53 loss of heterozygosity (LOH), which allows for tumor development.

Defining the role of the XAB2 complex during homologous recombination. David O. Onyango, Jeremy Stark. City of Hope, Duarte, CA.

Several factors associated with RNA processing are important for homologous recombination (HR), but the mechanistic links between these processes remain poorly understood. To this end, we have examined the function of the transcriptomic binding factor XAB2 in HR, since this factor has a conserved function in RNA processing. We have found that XAB2 is important for chromosomal double strand break (DSB) repair via two pathways of HR that require end resection as an intermediate step, end resection of camptothecin (Cpt) induced DNA damage, and RAD51 recruitment to ionizing radiation induced foci (IRIF). Furthermore, XAB2 mediates Ctp-hypophosphorylation induced by Cpt and BRCA1 IRIF, as well as histone acetylation events linked to HR proficiency. The capacity for XAB2 to promote HR correlates with its ability to form a complex with ISY1 and PRP19, which show a similar influence as XAB2 on HR. Our recent efforts include examining other members of the XAB2 complex, as well as the transcriptional alterations caused by loss of this complex, which may contribute to the defects in HR.

Breast cancer whole genomes link homologous recombination deficiency (HRD) with therapeutic outcomes. Eric Y. Zhao,1 Yaqing Shen,1 Erin Pleasance,1 Katayoon Kasaian,1 Martin R. Jones,1 Carolyn Ch’ng,1 Caralyn Reisle,1 Peter Eirew,2 Karen Mungall,1 Nina Thiessen,1 Yussanne Ma,1 Alexander Fok,1 Andrew J. Mungall,1 Yongqiu Zhao,1 Richard Moore,1 Diego Villa,2 Tamar Shenker,2 Caroljne Lohrihch,2 Stephen Chia,2 Stephen Yip,1 Karen Gelmon,2 Howard Lim,1 Sophie Sun,1 Kasmintan A. Schrader,2 Sean Young,1 Aly Karsan,1 Robyn Boscoe,3 Janessa Laskin,3 Marco A. Marra,4 Steven J. Jones,1 Canada’s Michael Smith Genome Sciences Centre, Vancouver, British Columbia, Canada; 2British Columbia Cancer Agency, Vancouver, British Columbia, Canada; 3The University of British Columbia, Vancouver, British Columbia, Canada.

Background: Homologous recombination deficiency (HRD) is common in cancer - germline BRCA1 & BRCA2 mutations account for 5-10% of breast cancers and over 65% lifetime risk. HRD-expressing tumors exhibit genome instability and sensitivity to platinum-based therapy and PARP inhibitors. While not all causes of HRD are known, recent sequencing efforts have revealed genome-wide somatic mutation signatures that characterize the HRD genomic instability phenotype, also known as “BRCA-ness”. This provides a promising new assay to predict sensitivity to platinum-based therapy. Here, we integrate two whole-genome sequencing (WGS) signatures to assess their association with therapeutic outcomes in a breast cancer cohort. Methods: Whole-genome sequencing of 47 breast cancer tumors (100x coverage) and matched normals (60x) was performed on an Illumina HiSeq. Alignment, assembly, SNV calling, and loss of heterozygosity (LOH) detection were performed with BWA, ABSS, Strelka, and APOLOLH respectively. SNV signatures were deciphered by non-negative matrix factorization with Monte Carlo resampling. An HRD score comprised of LOH, telomeric allele imbalance (TAI), and large scale transition (LST) counts was computed. Clinical endpoints were obtained by retrospective review of treatment and imaging reports. Analysis is ongoing in an independent validation cohort of 62 sequenced cases. Results: The HRD-linked SNV signature was significantly associated with radiographic clinical response (CR) to platinum-based therapy (p=0.015). Logistic regression demonstrated a 59% improved odds of CR to platinum-based therapy per 1000 somatic SNVs attributed to HRD (odds ratio 1.16-2.50). Tumors carried up to 10,246 such SNVs and all patients with CR were among the top quartile. The LOH-TAI-LST score was correlated with SNV signature (r = 0.6, p =7×10^-4) and associated with CR (p=0.025). Notably, elevated HRD signatures associated with CR were identified in tumors with wild-type BRCA2 or variants of unknown significance. Tumors with above median HRD signatures were associated with a 69-day longer time to treatment failure and a 18% daily decreased probability of treatment failure per 1000 HRD-attributed SNVs (hazard ratio 0.71-0.95, p =0.007). Discussion: We found that HRD mutation signatures are associated with clinical response and longer time to treatment failure with platinum-based therapy. While similar benefits were observed in patients with somatic bi-allelic loss of BRCA1/BRC2, such cases are less common (8% of our cohort) compared to those with elevated HRD signature. Thus, mutation signature methods may identify patients who stand to benefit from platinum-based therapy missed by BRCA screening alone.
Preclinical assessment of the PARP inhibitor rucaparib in homologous recombination deficient prostate cancer models. Minh Nguyen, Andrew D. Simmons, Thomas C. Harding, Clovis Oncology, Inc., Boulder, CO.

Rucaparib (CO-338) is an oral small molecule inhibitor of the poly(ADP-ribose) polymerase (PARP) enzymes PARP-1, PARP-2 and PARP-3 that is being developed for cancer patients with evidence of homologous recombination (HR) deficiency, including BRCA1 and BRCA2 mutations. PARP inhibitors have demonstrated clinical activity in BRCA1, BRCA2, and ATM mutant prostate cancer patients (Mateau et al. N Engl J Med. 2015;373:1697-708); however, limited preclinical validation of PARP inhibitors in prostate cancer has been reported to date, since no BRCA mutant nor HR deficient prostate cell lines have been identified. To further understand the functional inactivation of DNA repair genes using siRNA and CRISPR/Cas9, and examined the impact on PARP inhibition, in the DU145 and PC-3 prostate cancer cell lines. These cell lines were chosen based on transfection efficiency, lack of obvious genetic defects in DNA repair pathways, and baseline insensitivity to rucaparib (IC50 > 10 μM). A panel of 26 DNA repair genes were individually knocked down by siRNA, and changes to rucaparib potency were examined in a 7-day cell viability assay. The knockdown of BRCA1 or BRCA2 increased rucaparib cytotoxicity in both prostate cell lines. For example, the IC50 of rucaparib in DU145 and PC-3 cells with reduced BRCA2 levels was 275 and 297 nM, representing a 36- and 37-fold increase in rucaparib sensitivity, respectively. In addition to BRCA1 and BRCA2, the knockdown of several other genes including BARD1, CDK12, PARP2, XRCC2, RAD51, and BARD1 increased rucaparib efficacy ≥ 2-fold in at least 1 cell line examined. To further investigate HR deficiency in prostate cancer, BRCA2 was knocked out in the human 22Rv1 prostate cancer cell line using CRISPR/Cas9. Clone 1-20 displayed biallelic BRCA2 inactivation due to the introduction of a deleterious premature stop codon, and immunoblot analysis verified lack of the full-length BRCA2 protein. The IC50 of rucaparib in the parent and clone 1-20 cells was 504 and 287 nM respectively, demonstrating a 33-fold increase in rucaparib cytotoxicity in this BRCA2 deficient model. Androgen receptor (AR) signaling and proliferation were also examined in clone 1-50. In contrast to published reports (Schiewer et al. Cancer Discov. 2012;2:1134-49), no impact of PARP inhibition on dihydrotestosterone (DHT) induced gene expression was observed in the parental 22Rv1 and BRCA2 deficient clone 1-50 cell lines. In vivo model development and efficacy studies with the 22Rv1 and clone 1-50 cell lines are ongoing. Taken together, these findings support the hypothesis that deficiencies in BRCA1 and BRCA2, as well as additional HR genes, may sensitize prostate cancer cells to rucaparib. A phase 2 study investigating rucaparib in patients with metastatic castration-resistant prostate cancer and HR gene deficiency (TRITON2) is ongoing (NCT02952534).

Genomic instability in BRCA1-deficient cells is a result of the anti-recombinogenic activity of BLM helicase. Dharm S. Patel, Sarah M. Miseno, Samuel F. Bunting, Rutgers University, Piscataway, NJ.

Mutations in BRCA1 are responsible for approximately 5% of cases of breast cancer, and there are few treatment options that substantially alter the probability of relapse in individuals with BRCA1 mutations. BRCA1-deficient cells have demonstrated increased genomic instability following DNA damaging treatments due to a defect in the homologous recombination (HR) DNA repair pathway. Presently, Olaparib, a PARP inhibitor, is approved as a targeted monotherapy for germline BRCA1 mutated advanced ovarian cancers. As use of this treatment has expanded, data suggests that patients exhibit a further relapse and resistance to PARP inhibitors via mechanisms that include the development of secondary BRCA1 reversion mutations, enhanced drug efflux relating to P-glycoprotein, and mutations in other DNA repair proteins that restore HR. Here, we show that the RECQ helicase, BLM, mediates the genomic instability observed in BRCA1-deficient cells by a mechanism that depends in part on its anti-recombinogenic activity. We have generated conditional knockout mice with single and combined deficiencies in BRCA1, BLM, and 53BP1 in the B lymphocyte cell population. Ablation of BLM in BRCA1-deficient cells allows the HR repair pathway to be restored, leading to a partial rescue of the genomic instability that is present in BRCA1-deficient cells. The stable accumulation of RAD51, a marker for HR, at DNA double-strand break sites is inhibited by BLM in BRCA1-deficient B cells. However, DNA end resection is not impacted by single or co-deletion of BLM and 53BP1. Furthermore, BLM deficiency alone can display limited sensitivity to PARP inhibition. The rescue in HR and PARP sensitivity phenotypes following deletion of BLM is only present in hypomorphic BRCA1(1/4)cells and not the RING domain deficient BRCA1(1/2). These results demonstrate that the anti-recombinogenic activity of BLM is of potentially great significance for regulating the balance of HR versus other error-prone
repair pathways. Mutation of BLM in BRCA1-deficient tumors is therefore a functionally null RAD51D missense mutation is strongly associated with ovarian carcinoma. Barbara Rivera. McGill University, Montreal, Quebec, Canada.

Background and goal: RAD51D is a key player in DNA repair by homologous recombination (HR) and carries of truncating RAD51D mutations have an increased risk of ovarian cancer (OC). However, the contribution of truncated RAD51D variants to cancer predisposition remains uncertain. We sought to fully characterize the previously described missense RAD51D variant c.620C>T; p.S207L in order to elucidate its role in OC. Methods: A clinical panel screening was used to identify the RAD51D variant c.620C>T; p.S207L in two French Canadian (FC) kindreds affected by HGSC of the ovary and endometrium. High resolution melting, TaqMan genotyping and Sanger sequencing were used to genotype the p.S207L variant in a series of unselected cases of HGSC of the ovary and endometrium, breast, pancreas and colorectal cancer and healthy controls, all of FC origin. Whole exome sequencing (WES) was performed to study the genetic signature characterizing RAD51D associated tumors. RAD51 foci formation and CRISPR-Cas9-stimulated and CRISPR-Cas9-induced DSBs in immunoglobulin genes to promote B-cell maturation. However, AID is capable of broadly damaging the B-cell genome. We have shown that AID-induced DSBs require the homologous recombination factor RAD51 to sensitize AID-overexpressing tumor cells. Here we build on those observations, finding that AID-RAD51 synthetic lethality occurs via mitotic catastrophe, involving the mTOR pathway. We have now developed potent and selective RAD51 modulatory small molecules that preferentially kill AID-expressing tumor cells. Using mouse models of lymphoma and leukemia we demonstrated in vivo efficacy data demonstrating the potential therapeutic feasibility of RAD51 modulation. These studies provide evidence for a novel “synthetic lethal” approach for treating AID-expressing malignancies, via the induction of mitotic catastrophe.

Inhibition of RAD51 with a cell penetrating antibody, 3E10. Peter M. Glazer, Audrey Turckich. Yale University, New Haven, CT.

Many human malignancies arise in the setting of defective DNA repair pathways, leading to genetic instability, carcinogenesis and tumor progression. However, DNA repair deficiency can provide for a therapeutic window that can be exploited with targeted agents. One such anti-cancer agent is 3E10, a unique cell penetrating, anti-DNA autoantibody. 3E10 by itself is considered non-toxic to normal cells but has been shown to have deleterious effects on cells with DNA
repair deficiencies. Specifically, 3E10 has been shown to reduce the efficiency of homologous recombination (HR). This has been attributed to its ability to preferentially bind to ssDNA tails, thereby inhibiting Rad51-mediated strand exchange. Prior work has shown that treatment with 3E10 alone can selectively kill cells with genetic mutations that lead to abnormal DNA repair. For example, 3E10 has been shown to be synthetically lethal in BRCA2 deficient human cancer cells. Additionally, 3E10 sensitizes cells to radiation and other DNA damaging therapies. The goal of this project is to characterize the specific mechanism by which 3E10 inhibits DNA repair pathways. Previously, the mechanism by which 3E10 inhibits HR was attributed to 3E10’s DNA binding ability. Through the biochemical analysis of 3E10 variants, new evidence now suggests that 3E10 inhibits HR by competing with the binding of 3E10’s substrate to its DNA binding domain. We have found that this HR inhibition is a result of a physical interaction between 3E10 and Rad51. Biochemical analysis revealed that 3E10 binds to the N-terminal domain of Rad51, the domain that plays a role in Rad51 homo-oligomerization and filament formation. This 3E10-Rad51 interaction impairs Rad51’s ability to localize to the nucleus and form repair foci. Taken together, this data suggests that 3E10 inhibits HR by physically interacting with Rad51. Overall, 3E10 holds promise as a novel therapeutic agent for certain subsets of cancer.

#2483 Molecular mechanisms of dihydrogalactitol (VAL-083) in overcoming chemoresistance in glioblastoma. Beibei Zhai, 1 Anna Gobielska, 2 Anne Steino, 3 Jeffrey A. Bacha, 4 Dennis M. Brown, 5 Simone Niclou, 6 Mads Daugaard, 7 "University of British Columbia, Vancouver, British Columbia, Canada; 2Luxembourg Institute of Health, Luxembourg, Luxembourg; 3DelMar Pharmaceuticals, Inc., Vancouver, British Columbia, Canada; 4DelMar Pharmaceuticals, Inc., Menlo Park, CA.

Standard treatments for glioblastoma (GBM) include surgery, radiation and chemotherapy with temozolomide (TMZ). Nearly all tumors recur and 5-year survival is less than 3%, largely due to chemoresistance. Evidence shows that cancer cells utilize DNA damage repair pathways to overcome cytotoxic effects of chemotherapy. GBM tumors expressing O6-methylguanine-DNA-methyltransferase (MGMT) display intrinsic chemoresistance to TMZ and nortosoureas, while a deficient DNA mismatch repair system (MMR) confers chemoresistance to TMZ and platinum agents. Alterations in p53, particularly gain-of-function mutations, are correlated with increased MGMT-expression and poor prognoses in GBM. Second line anti-angiogenic treatment with bevacizumab has not improved overall survival and has been shown to induce intratumor hypoxia and increased chemoresistance. VAL-083 is a bi-functional alkylating agent that readily crosses the blood-brain barrier, accumulates in brain tumor tissue and has demonstrated activity against GBM in prior NCI-sponsored clinical trials. VAL-083 induces interstrand cross-links at guanine-N1 causing DNA double-strand breaks and cancer cell death. VAL-083 is equitoxic against GBM cancer stem cells (CSCs) and non-CSCs independent of MGMT and p53 status, in vitro. We recently showed that VAL-083 leads to irreversible S/G2-phase cell cycle arrest, proposing synergy with S-phase specific chemotherapeutics, including topoisomerase and PARP inhibitors. VAL-083 further showed persistent activation of the homologous recombination (HR) DNA repair pathway and its potency was increased when HR was impaired, demonstrating the potential of VAL-083 in HR-deficient cells. Functional repair assays. In this study, we investigated the potential of VAL-083 in GBM under hypoxia either in vitro or in vivo as part of a combination treatment with bevacizumab. Our results demonstrate a distinct anti-cancer mechanism for VAL-083, resulting in the ability to overcome resistance to TMZ and nortosoureas, increased activity in cancers with impaired HR and synergy with etoposide or carboplatin.


Response to DNA-damaging agents, such as platinum and recently emerged PARP inhibitors, is associated with homologous recombination deficiency (HRD) pathway. In fact, as reported by multiple clinical trials, patients with advanced breast, prostate, and ovarian cancers who have harmful mutations in BRCA1 and BRCA2 have demonstrated a great benefit from treatment of PARP inhibitors including olaparib, niraparib, and talazoparib. Current companion diagnostic assays that mainly examine germline defects from blood or somatic alterations from tumor tissues have shown several practical challenges: germline mutations are underrepresented in tumor samples or can be somatically altered, and results are not always consistent between tumor and matched blood samples. In addition, the sensitivity of current companion diagnostic tests is insufficient. Most companion diagnostic assays are designed for specific PARP inhibitors, and patients who are predicted to be sensitive by these assays may not respond to treatment. To improve companion diagnostic testing for PARP inhibitors, a liquid biopsy test is needed that can be easily incorporated into routine clinical care. The Predi-HRD liquid biopsy test, a non-invasive, next-generation sequencing (NGS)-based multiplex assay that enables real-time monitoring of temporal change of tumor heterogeneity and highly sensitive detection of defects in HR genes including BRCA1, BRCA2, ATM, CHEK2, among others. Technical validation was performed to evaluate assay sensitivity, specificity and accuracy using reference samples with known genetic profiling and further validated on a variety of orthogonal platforms such as digital PCR, allele-specific PCR and Sanger sequencing. Mutation prevalence of HR genes was examined in clinical plasma samples from patients with advanced ovarian cancer after systemic chemotherapy. The successful development of Predi-HRD liquid biopsy test offers an efficient solution to stratify and monitor cancer patients who may benefit from HRD-targeted therapeutics such as PARP inhibitors.

#2486 Tumor hypoxia induces DNA repair vulnerabilities through contextual loss of heterozygosity. Osman Mahamud, 1 Melvin Chua, 2 Winnie Lo, 3 Gaetano Zafara, 4 Robert Bristow. 5 University of Toronto, Toronto, Ontario, Canada; 2Princess Margaret Cancer Centre, Toronto, Ontario, Canada; 3University Health Network, Toronto, Ontario, Canada.

Introduction: Intratumoral hypoxia leads to decreased expression in DNA damage response (DDR) and repair pathways. Given the ‘two-hit’ model for loss of function germline mutations in BRCA2 affect its role in the homologous recombination (HR) DNA repair pathway through contextual ‘loss-of-heterozygosity’, which marries the tumour genetic profile with environmental factors. To interrogate this relationship, isogenic DLD-1 cells heterozygous and homozygous null for BRCA2, were placed under normoxic (21%) or hypoxic (0.2%) conditions for 72 hours. Hypoxia-mediated changes in DDR response and DNA repair were evaluated by cell proliferation, cell cycle analysis, western blots, qPCR, immunofluorescence, clonogenic assays and functional repair assays. Results: No differences in proliferation and cell survival were observed betweenoxic and hypoxic cells (72 hours - 0.2% O2). Under chronic hypoxic conditions, confirmed by the up-regulation of VEGF and HIF1α, mRNA and protein expression of key homologous recombination (HR) genes (BRCA1, BRCA2, RAD51) were down-regulated. Functionally, BRCA2-/- cells proved unable to recruit Rad51 foci and were profoundly sensitivity to PARP inhibition. Conversely, heterozygote BRCA2+/- cells retained the ability to recruit Rad51 foci under both oxic and hypoxic conditions. However, exposure to chronic hypoxia resulted in a reduction of foci formation. Chronically hypoxic BRCA2+/- cells exhibited a 30-40% increase in sensitivity to PARP inhibition compared to their oxic counterparts. Preliminary data shows a similar synthetically lethal relationship in genetic (BRCA2+/-) and tumor microenvironment induced (BRCA2+/-) loss of function germline mutations in human tumor cells. Conclusions: Herein, we demonstrate a novel mechanism of contextual ‘loss-of-heterozygosity’, which marries the tumour microenvironment and innate genetic alterations. The resultant increased sensitivity to DDR kinase inhibitors and PARP highlights the therapeutic significance of this phenomenon.

#2487 Functional characterization of the BRCA2 variant, K3326X. Srilatha R. Simhadri, Sonia C. Dolfi, Atul Kulkarni, Bing Xia, Shridar Ganesan, Kim M. Hirshfield. Rutgers Cancer Institute of New Jersey, New Brunswick, NJ.

Individuals with germline mutations in the breast cancer susceptibility gene, BRCA2, have an approximate 70% risk of developing breast cancer, a 30% risk of developing ovarian cancer, a 20% risk of developing prostate cancer and a 10% risk of developing colorectal cancer. The presence of a deleterious BRCA2, ATM, CHEK2, among others. Technical validation was performed to evaluate assay sensitivity, specificity and accuracy using reference samples with known genetic profiling and further validated on a variety of orthogonal platforms such as digital PCR, allele-specific PCR and Sanger sequencing. Mutation prevalence of HR genes was examined in clinical plasma samples from patients with advanced ovarian cancer after systemic chemotherapy. The successful development of Predi-HRD liquid biopsy test offers an efficient solution to stratify and monitor cancer patients who may benefit from HRD-targeted therapeutics such as PARP inhibitors.

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breast cancer cases and to increase the risk for lung, pancreatic, ovarian, and upper aero-digestive tract cancers. Preliminary data, obtained from the Rutgers Cancer Institute of New Jersey breast cancer case-control study, identified K3326X enrichment in 1.25% of cases compared to 0.7% of controls. Several of the carriers had second primaries and displayed a trend toward increased number of family members diagnosed with breast cancer, which is consistent with reports of a breast cancer risk associated with the 3326X variant. The K3326X variant was also identified in 1.38% (11 of 796) of our histologically-diverse cohort of genomically-profiled tumors that included cancers of the breast, ovarian/fallopian tube, lung, vulvar, cancer of unknown primary, and one breast cancer case having prolonged response to platinum-based therapy. Thus, K3326X may represent a functional loss of wild type BRCA2 function, as we observe concomitant loss of heterozygosity at this locus. In a preliminary work with a different cell line, the K3326X variant, in vitro, in a functional DR-GFP-based reporter assay measuring HR. Our data reveal the BRCA2 K3326X variant to be impaired in the HR pathway indicating a loss of a wild-type protein function. We will also evaluate cell viability of the K3326X variant in the presence of DNA damaging drugs like cisplatin, poly-ADP ribose polymerase inhibitors and mitomycin C. Future studies will also incorporate a retrospective evaluation of tumor specimens that have undergone comprehensive genomic profiling. These data would indicate that BRCA2 K3326X represents a functional hypomorphic variant that may have implications in therapeutic approaches and cancer risk evaluations across a spectrum of tumor types.

#2488 Tumor necrosis factor-alpha signaling determines cytotoxicity induced by BRCA2 deficiency. Anne M. Heijink, Francien G. Talens, Anouk Blaars, Marjelle A. van Vught. University Medical Center Groningen, Groningen, Netherlands.

Introduction Breast cancer 2, early onset (BRCA2) is involved in DNA-damage repair via homologous recombination (HR). Defective HR leads to severe genomic instability and homozygous deletion of BRCA2 is therefore deleterious for survival of normal cells. In apparent contrast, inherited heterozygous mutations in BRCA2 increase the risk to develop cancer, which goes along with somatic loss of the wild type allele. So paradoxically, BRCA2 loss is lethal in normal cells, but can be tolerated in cancer cells. It is currently unclear how tumor cells can survive without BRCA2. To address this question, we performed a genome-wide functional genetic screen and identify Tumor Necrosis Factor-alpha (TNFα) signaling, via TNF receptor 1 (TNF-R1) and Src-associated-substrate during mitosis-of-68Da (Sam68), as an important determinant of cell death induction upon BRCA2 inactivation. Material & Methods A genetic screen was performed in human near-haploid KMB-7 cells. Two monoclonal KMB-7 lines were engineered to express independent doxycycline-inducible BRCA2 short hairpin RNAs (shRNAs). Cells were randomly mutagenized using retrovirus carrying a strong splice-acceptor, resulting in gene knock-outs. Deep sequencing of surviving colonies revealed gene mutations that prevented shBRCA2-mediated cell death. Validation of TNF-R1 and Sam68 was done by shRNAs in multiple breast- and leukemic cell lines. Cell viability was determined by MTT and clonogenic assays. Competition assays with vectors expressing shRNA including IRES-driven mCherry were analysed with flow cytometry. Biochemical analyses were assessed by western blotting and ELISA. Immunoprecipitation tagged TNF-R1 was performed to study the protein-protein interaction. Results From our genetic screens, loss-of-function mutations involved in TNFα signaling were found to significantly rescue cell death in BRCA2-depleted cells. To validate these findings, BRCA2 was stably depleted in a panel of cancer cell lines, which decreased viability. Subsequent co-depletion of TNF-R1 or Sam68 rescued survival. Notably, BRCA2 depletion resulted in the production of TNFα. In line with this finding, the majority of tested cancer cell lines were found to be sensitive to recombinant TNFα upon BRCA2 depletion. This phenomenon was not restricted to BRCA2 inactivation, since depletion of related DNA repair genes (BRCA1, FANCQ2) or hydroxyurea-induced replication stress also sensitized cells to recombinant TNFα. Mechanistically, BRCA2 depletion was induced to observe differential complex formation of TNF-R1 with downstream effector proteins upon TNFα activation. Conclusion Our data reveal that down-regulation of TNF-R1 or its downstream signaling component Sam68 rescued cytotoxicity upon BRCA2 inactivation. These findings and mechanistic follow-up experiments describe a novel mechanism by which auto-crine TNFα signaling, induced by loss of BRCA2, limits tumor cell viability.

#2489 Mre11-CDK2 interaction during the DNA double-strand break repair. Mary J. Morgan, Todd A. Festerling, Jeffrey Buis, David O. Ferguson. University of Michigan, Ann Arbor, MI.

The MRN (Mre11/Rad50/NBS1) complex is a group of highly conserved proteins central to the detection and repair of DNA double-strand breaks (DSBs). Inherited deficiencies in DNA DSB signaling result in a spectrum of disorders featuring cancer predisposition, neurodegeneration, and immunodeficiency. MRN directly binds DNA DSB ends and facilitates activation of ataxia-telangiectasia mutated (ATM) kinase, which initiates cellular responses including DNA repair, cell cycle checkpoints, and in some circumstances, apoptosis. Cellular responses to DNA DSBs require rapid communication between DNA repair complexes and the cell cycle machinery, with which mitotic recombination and repair pathways interact. To test this hypothesis, we identified an interaction between Mre11 and Cyclin-dependent kinase 2 (CDK2), a key component of the cell cycle machinery (Buis et al., NCBM, 2012). CDK2, when bound to its regulatory partner Cyclin A, promotes cell cycle progression through G2 phase. We demonstrated that the Mre11-CDK2/Cyclin A interaction is important in regulating the capacity for DNA repair by homologous recombination in normally cycling fibroblasts. Unrepaired DNA damage leads to tumorigenesis. CDK2 is a multifunctional protein that plays a central role in DNA replication and homologous recombination repair. It is known that defects in Rad51 function can cause cancer. The goal of this study is to identify a novel role for Rad51 outside of its known functions in DSB repair and replication fork processing. Methods: Since Rad51 knockout is lethal to cells, we generated an inducible system in which we can down-regulate Rad51 expression in HT1080 cells after Doxycyline treatment. To determine the effect of Rad51 knockdowm in global gene expression pattern, we carried out unbiased microarray gene expression analysis and after induction of DNA damage and replication stress by radiation. ssDNA and dsDNA in the cytosolic fractions were quantified using Quanti-IT OliGreen and PicoGreen Assay Kits. For cytoplasmic BrdU detection, exponentially growing cells were labeled with BrdU for 18-22h and then immunostained with anti-BrdU antibody. Additionally, we measured the expression and post-translational modification of proteins involved in innate immune signaling by western blotting. We also employed DNA fiber assay to determine the role of Rad51 in replication fork processing. Results: We found that defects in Rad51 lead to the accumulation of self-DNA in the cytoplasm, triggering a STING-mediated innate immune response after replication stress and DNA damage. Mechanistically, the unprotected newly replicated genome in the absence of Rad51 is degraded by the exonuclease activity of Mre11, and the fragmented nascent DNA accumulates in the cytosol, initiating an innate immune response. Our data revealed that in addition to playing roles in homologous recombination-mediated DNA double-strand break repair and replication fork processing, Rad51 is also implicated in the suppression of innate immunity. Conclusion: Rad51 plays a novel role in immunity outside its known functions in DSB repair and replication fork processing. We discovered that the lack of Rad51 leads to the up-regulation of innate immune pathway genes upon DNA damage and replication induced by irradiation. We found that in the absence of Rad51 the newly replicated genome is degraded by the exonuclease activity of Mre11. We also showed that these degraded nascent DNA fragments are exported to the cytoplasm, triggering innate immune response signaling. Our study reveals a previously unidentified role for Rad51 in triggering an innate immune response, and places Rad51 at the hub of new interconnections between DNA replication, DNA repair, and immunity. Funding: This work was supported by NIH R01AG053441 grants.

#2491 Beclin1 promotes DNA double-strand breaks repair. Fei Xu, Lili Yan, Jianrong Wang, Jingming Yang. Soochow University, China, Suzhou, China; Penn State Univ., PA.

Meaningful DNA double-strand break (DSB) repair mechanisms are evolutionarily conserved and are essential for maintaining genomic integrity in organisms from yeast to mammals. Mammalian cells utilize two major DSB repair pathways: homologous recombination (HR) and non-homologous end joining (NHEJ). In this study, we found that Beclin1, a known regulator of autophagy, is also involved in DNA DSB repair. The results indicate that Beclin1 promotes DNA DSB repair through the recruitment of MRN (Mre11/Rad50/NBS1) to the DSB sites, which is essential for the efficient repair of DNA DSBs.

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Beclin1 is a well-established core mammalian autophagy protein that has been presumed to suppress oncogenesis via an autophagy-mediated mechanism. Since its discovery, studies have also shown that Beclin1 interacts with an increasing number of cofactors. However, the tumor suppressive roles of Beclin1 remain a major research focus, and the exact mechanism by which Beclin1 promotes tumor suppression has remained largely obscure. Our confocal analysis showed that in embryonic and neonatal mice, Beclin1 is primarily located in the cytoplasm in hepatocytes, with a very small portion located in the nucleus. When the mice were 15d, roughly half of the total Beclin1 was located in the nucleus. Thereafter, Beclin1 progressively distributed from cytoplasm to nucleus within a few weeks after birth. This pattern was observed not in all cell types and tissues. We and others found both 0-50 and 254-278 residues are required for Beclin1 nuclear localization. Furthermore, loss of Beclin1 resulted in reduced DNA damage repair. We found that the beclin1−/− cells showed a marked increase in γ-H2AX foci that are considered as a biomarker of DNA DSBs. Moreover, beclin1−/− cells showed approximately 60-fold and 30-fold reductions in DSB repair by NHEJ and HR pathways. A series of key DSBs repair proteins were significantly reduced in beclin1−/− cells. Interestingly, Beclin1 regulates DSB repair independent of autophagy. Autophagic flux was maintained in the beclin1−/− cells. Perhaps an alternative autophagy may be activated when beclin1 is deleted asULK1 was upregulated. We further found that Beclin1 promotes DSB repair via a direct interaction with DNA topoisomerase II (Top2b). Pull down results showed that Top2b is one of the major Beclin1-interacting proteins in the nucleus. And we found that this interaction was intensified after exposure to IR. Top2b and Beclin1 were found to be colocalized to the DSB sites, after exposure to IR. Strikingly, depletion of Top2b by gene silencing completely inhibited Beclin1 localized to the DNA break sites. We constructed a beclin1−/− cell line using CRESRPR/Cas9 system. The spot counts of protein foci were measured by imaging flow cytometry after exposure to 6 Gy IR. HR and NHEJ reporter assays were used to detect the activity of DSBs repair pathways. We pulled down nuclear proteins with ectopically expressed Flag-tagged Beclin1. The identities of the Beclin1-interacting proteins were determined using mass spectrometry. Our work establishes that Beclin1 plays an autophagy-independent role in maintaining genomic integrity by promoting DNA damage repair. The finding challenges the long-standing paradigm of the mechanisms that are involved in the autophagy-mediated tumor suppressive role of Beclin1. This finding suggests that when manipulating cytoplasmic Beclin1 that is involved in the autophagy pathway in order to explore targeted tumor therapies, the non-autophagy cytoprotective role of Beclin1 in the nucleus should also be considered.

Beclin1 and beclin1−/− mice had a severe depletion of stem cells in the hematopoietic system and brain, and large deletions. We further demonstrate that beclin1−/− cells have increased sensitivity to DNA damaging agents that induce double-strand breaks and that there is persistent accumulation of DSBs in these cells. In combination, our results suggest that Pol β is critical for double-strand break repair.

#2493 Identification of the mutant p53-PARP-MCM chromatin axis as a triple negative breast cancer replication stress target. Wei-Gang Qiu,1 Alla Polotskaia,2 Gu Xiao,3 Lia Di,2 Yuhan Zhao,4 Wenwei Hu,5 John Philip,4 Ronald Hendrickson,4 Jill Bargonetti,1 City Univ. of New York at Hunter College and The Graduate Center, New York, NY; 2City Univ. of New York at Hunter College, New York, NY; 3Rutgers Cancer Institute of New Jersey, Rutgers, NJ; 4Memorial Sloan-Kettering Cancer Center, New York, NY.

Approximately 15% of all breast cancer is triple-negative and of these about 80% are found to have mutations in the gene for the tumor suppressor p53 (TP53). Many TP53 mutations encode gain-of-function oncogenic mutant p53 (GOF mtp53) protein. We used inducible knockdown of endogenous mtp53 in mtp53 MDA-MB-468 cell lines and compared the effects on DNA damage in wild type and mtp53-H2AX foci that are considered as a biomarker of DNA DSBs.

#2494 ATR inhibitor AZD6738 as monotherapy and in combination with olaparib or chemotherapy: defining pre-clinical dose-schedules and efficacy modelling. Alan Y. Lau,1 James Yates,2 Zena Wilson,3 Lucy A. Young,4 Adina M. Hughes,5 Aileen Berges,6 Amy Cheung,7 Rajesh Odedra,8 Elaine Brown,9 Mark J. O’Connor,10 Simon Hollingworth,11 Astrazeneca, Cambridge, United Kingdom; 2City of Hope, Duarte, CA; 3City of Hope, Duarte, CA; 4Memorial Sloan-Kettering Cancer Center, New York, NY; 5AstraZeneca, Macclesfield, United Kingdom; 6Astara, Cambridge, United Kingdom.

Introduction: AZD6738 is a potent and selective oral inhibitor of the ataxia telangiectasia and rad3 related (ATR) protein kinase. ATR has a key role in the DNA replication stress response (RSR) pathway of DNA repair, facilitating the recovery and repair of potentially cytotoxic persistent, stalled DNA replication forks. Inhibition of ATR leads to the inability to resolve replication forks associated damage and the accumulation of DNA strand breaks, which if remains unrepaired leads to cell death. AZD6738 is currently in Phase I/II clinical trials being evaluated as a monotherapy and in combination with novel agents olaparib / Lynparza (PARP DNA damage response inhibitor), durvalumab (PD-L1 immune checkpoint inhibitor) and DNA-damaging agents such as carboplatin and ionising radiation. Critical in helping to guide the clinical usage of AZD6738 and maximise patient benefit, pre-clinical studies were performed to determine optimal doses and schedules as monotherapy and in combination with olaparib and carboplatin.

Experimental procedures: Human cancer cell lines, xenograft and patient-derived explant (PDX) models of non-small cell lung cancer (NSCLC), head & neck squamous cell carcinoma (HNSCC), and triple-negative breast cancer (TNBC) were tested comparing once daily versus twice daily dosing, the number of consecutive days dosing (3 days/week, 5 days/week, continuous) and co-dosing versus sequential or intermittent dosing with AZD6738 alone or in combination with olaparib or carboplatin. The magnitude and duration of anti-tumour responses were then compared with AZD6738 mouse pharmacokinetic (PK), pharmacodynamic (PD) and in vitro target (IC / growth inhibition (GI) / growth inhibition) profiles. Results: A mathematical model was derived which adequately described the AZD6738 PK/PD-eficacy relationship. This modelling confirms that duration of coverage (time) above cellular ATR target inhibition thresholds (IC50 pCHK1 / GI50) per day, rather than Cmax or exposures per se, is the major determinant of anti-tumour responses. As monotherapy, a short, non-CO4 mtp53 in MDA-MB-468 cells in combination with ATRA continuously to give tumour stabilisation, which can be achieved through repeated daily of AZD6738 over several weeks. Co-dosing AZD6738 in combination with olaparib or carboplatin gives best efficacy compared to sequential dosing and PK cover over the first 48-72 hours is necessary to give tumour regressions. The models predict that extending the duration of ATR coverage, achieved through continuous dosing, further increases efficacy. These pre-clinical dose-schedules were compared to human free plasma AZD6738 PK data and predicted efficacious exposures found to be clinically achievable. Conclusions: Together these data further support the clinical evaluation of AZD6738 and suggest optimal dosing schedules for ATR inhibitors.
#2495 The metabolic secretome of cachexia inducing pancreatic ductal adenocarcinoma. Santosh K. Bharti,¹ Paul T. Winnard,¹ Louis Dore-Savard,² Yelena Mironchik,¹ Marie-France Penet,¹ Zaver M. Bhuwalia.¹ The Johns Hopkins University School of Medicine, Baltimore, MD;²McGill University Health Center, Montreal, Quebec, Canada.

Cachexia is an unexplored and yet devastating consequence of cancer that is the cause of 20% of all cancer related deaths.¹ Cachexia inducing tumors cause a ‘wasting away’ of the body. The condition is associated with poor treatment outcome², fatigue, and extremely poor quality of life.³ Because of the multifactorial characteristics of this condition, it has been difficult to understand the mechanisms driving the impact of the tumor on body organs and the sequence of events that leads to this lethal condition. Here we have used ¹H MRS to characterize the metabolic profile of tumor interstitial fluid (TIF) obtained from non-cachexia (Panc1) and cachexia inducing (Pa04C) tumors to further understand the impact of the deranged metabolism of cachexia inducing-tumors on the tumor metabolic secretome. The human pancreatic cancer cell line, Panc1, was obtained from ATCC. The human pancreatic cancer cell line, Pa04C, was provided by Dr. Maitra.⁴ Six to 8 week old male SCID mice were inoculated both the right and left flank with cancer cells (5x10⁶, Panc1 N=2, Pa04C N=2). We created a collection chamber to collect TIF. The chamber was implanted together with small tumor pieces harvested from the subcutaneous flank tumors, into the subcutaneous flank space of SCID mice (Panc1 N=8 and Pa04C N=6) until the tumor encompassed the chamber (4-5 weeks). The tumor was then removed, washed in saline, freeze dried, and TIF were collected. To obtain control TIF, an empty chamber was implanted in the subcutaneous flank space of healthy mice. Dual phase solvent extraction was performed on tumor tissue. The water phase was separated, freeze dried, reconstituted in D₂O PBS for spectral acquisition. All ¹H MR spectra were acquired on an Avance III 750 MHz (17.6T) Bruker NMR spectrometer equipped with a 5 mm broadband inverse (BBI) probe. Spectral acquisition, processing and quantification were performed using TOPSPIN 2.1 software. Notable differences between Pa04C compared to Panc1 TIF or normal interstitial fluid were a significant decrease of polyunsaturated fatty acids (PUFA) and lipids, and formate, pyruvate, glutamine, and glycine. Lactate, glutamate, succinate, glycine and acetone significantly increased in Pa04C TIF compared to Panc1 TIF or normal interstitial fluid. These differences in TIF cannot be explained solely by the differences in the tumor metabolic profile. Our study was nested with the prospective US Health Professionals Follow-up Study during the study period 1993-2014. We selected 212 advanced prostate cancer patients with stage T3b, distant metastasis or cancer death during follow-up. Patients and 212 matched controls for whom pre-diagnostic bloods were collected with a median 5.5 years prior to diagnosis. Metabolomics profiling was performed at the Broad Institute using liquid chromatography-tandem mass spectrometry to assay 295 known metabolites. We estimated odds ratios and 95% confidence intervals using multivariable conditional logistic regression to examine associations of individual metabolites and cancer risk. When we stratified by a man’s obesity status, we observed unique metabolomics profiles associated with advanced prostate cancer. Circulating amino acids (including carnitines) were associated with advanced prostate cancer in overweight men while lipid metabolites (including DAGs and TAGs) were associated with advanced cancer in the healthy weight men. Conclusion: Altered levels of TAG and DAG metabolites in prediagnostic bloods are strongly associated with an increased risk of advanced prostate cancer, independent of obesity. The finding that circulating lipids were specifically associated with advanced prostate cancer in healthy weight patients suggests there are men with normal body mass index but who are metabolically obese. The specific lipid metabolite classes enriched in advanced prostate cancer support the hypothesis that obesity acts through dysregulation of liver fatty acid metabolism rather than through metabolic actions of the adipose tissue itself.

#2497 Differential requirement of amino acids on cell survival of ovarian cancer cells. Akiko Furusawa,¹ Jun Inoue,¹ Hiitoshi Tsuda,² Naoyuki Miyasaka,³ Johi Inazawa.¹ ¹Tokyo Medical and Dental University, Tokyo, Japan; ²National Defense Medical College Hospital, Tokorozawa, Japan.

Amino acids (AAs) are traditionally classified into nutritionally essential AAs (EAAs) and non-essential AAs (NEAAs) for animals. Recent studies have demonstrated that not only EAAs but also NEAAs play important role on energy metabolism in cancer cells. However, which AAs are indispensable for human cancer cell survival has not been fully elucidated. The purposes of this study were 1) to determine the indispensable AAs for ovarian cancer (OVC) cells and 2) to investigate the relationship between the AAs survival for each cell line. Firstly, OVC cell lines were cultured with different medium in which each AA was depleted and cancer cell survival was examined. Among 17 OVC cell lines, EAAs were required in 12 cell lines, whereas others did not require EAAs for cell survival. Interestingly, cancer cell lines which required EAA also required at least one NEAAs, including arginine, cystine, glutamine, serine and tyrosine. Although extra-cellular glutamine has been known to be a critical factor for cancer cell survival and growth, 3 OVC cell lines did not require extracellular glutamine. Hence, we next examined glutamine synthetase (GS) gene, which is a critical enzyme for production of endogenous glutamine from glutamate. In OVC cell lines which did not require extracellular glutamine, GS expression level was positively correlated with cell survival rate under glutamine-depleted condition. Further, we found that the expression of this protein was negative in 38 of 645 primary OV tumors (5.9%). Taken together, these results suggest that GS-down-regulated OVC cells require extracellular glutamine for their survival. Therefore, uptake inhibition of extracellular glutamine or reduction of extracellular glutamine level may be a novel therapeutic strategy for patients with ovarian cancer with “GS down-regulation”, as well as development of an AA-based precision medicine of OVC.

#2496 Targeting brain tumor stem cells by interfering with choline metabolism: Evidence for an EMT-choline oncometabolic network. Katharina Koch,¹ Rudolf Hartmann,¹ Abigail K. Suwala,¹ Dayana Herrera Rios,¹ Ulf D. Kahler,¹ Jaroslav Maciayczyk,² University Hospital Dusseldorf, Dusseldorf, Germany;³Forschungszentrum Juelich, Juelich, Germany.

Glioblastoma (GBM) is the most lethal primary malignant brain tumor with a median survival of less than two years. High levels of therapy resistance, strong cellular invasiveness and rapid cell growth demand aggressive multimodal therapies involving resection as well as radio-chemotherapy. Recent evidence has pointed to the existence of brain tumor stem cells (BTSCs), a subpopulation of human brain tumors which is thought to be responsible for tumor dissemination, relapse and chemo resistance. BTSCs have been associated with the expression of mesenchymal features as a result of epithelial-mesenchymal transition (EMT). Using high resolution proton nuclear magnetic resonance spectroscopy (¹H NMR) we compared the intracellular metabolic composition of GBM cells after induction vs. inhibition of EMT as well as under stem cell or differentiated conditions. We identified that both EMT and enrichment for stemness induces the cholinic phenotype which is characterized by high intracellular levels of phosphocholine and total choline derivatives. Furthermore, interference with choline metabolism by targeting choline kinase alpha (CHKA) reversed EMT in GBM cells as we observed reduced invasiveness, changes in protein expression of EMT associated genes. Taken together, interfering with choline metabolism is a powerful strategy to suppress EMT and thus target BTSCs. Moreover, the newly identified BTSC-oncometabolic network could be used to non-invasively monitor the invasive properties of glioblastomas and the success of anti-CTSC therapy.
Arginine deprivation-induced autophagy renders ASS1-deficient sarcomas dependent on serine metabolism. Jeff Kremer, Bethany Prudner, Brian Van Tine. 1National Cancer Institute, 2Columbia University College of Physicians & Surgeons, NEW YORK, NY; 3University of Louisville, Louisville, KY, 4University of Kentucky, Lexington, KY.

Stable Isotope-Resolved Metabolomics (SIRM) has been a useful tool in interrogating metabolic reprogramming in cancer. We have recently published several protocols using SIRM in patient-derived models of non-small cell lung cancer (NSCLC)1. In this case study, we compared cellular metabolism in three different model systems, all derived from the same patient. Slices from the human tumor (Warburg slices 2,3), slices from an NSG mouse bearing the human tumor 4, and the patient-derived cell culture were incubated in [U-13C]-glucose or [U-13C,15N]-glutamine for 24 hours. Samples were then harvested and analyzed by gas chromatography-mass spectrometry and NMR. All three models displayed similar levels of fractional enrichment after 24 hours, making metabolic comparison possible, despite their very different 3D architectures. Glucose metabolism in particular, showed consistent labeling patterns of metabolites in glycolysis and the Krebs cycle. Glutaminolysis metabolism, however, was distinct in the cell culture, with a pattern that indicated upregulation of anaplerotic glutaminolysis. Activation of glutaminolysis may be an important transformative step in the establishment of a viable cell culture. We are also in the process of validating the present finding by comparing multiple patient-derived models of NSCLC and tracking the evolution of their metabolic activity. 1 Lane, A. N., Higashi, R. M. & Fan, T. W. - M. Preclinical models for interrogating drug action in human cancers using Stable Isotope Resolved Metabolomics (SIRM) Metabolomics 12, 1-15 (2016). 2 Fan, T. W. - M. & et al. Distinctly perturbed metabolic networks underlie differential tumor tissue damages induced by immune modulator β-glucan in a two-case ex vivo non-small cell lung cancer study. CSH Molec. Case Studies J. 2, a00083, (2016). 3 Fan, T. W. - M., Lane, A. N. & Higashi, R. M. Stable Isotope Resolved Metabolomics Studies in ex vivo Tissue Slices. Bio-protocol 6, e1730 (2016). 4 Lane, A. N., Yan, J. & Fan, T. W. - M. 13C Tracer Studies of Metabolism in Mouse Tumor Xenografts. Bio-protocol 5, e1650 (2015).
For five days a week during 21 days. The treated groups received 40 mg/kg of melatonin. Blood sample were collected every 3 hours during 24 hours (eight time points) and the plasma was extracted for analysis by liquid chromatography-mass spectrometry (LC-MS) using the Absolute IDQ p180 Kit (BioCrates). Orthogonal Partial Least Squares (OPLS) analysis showed evident separation of the animals in two different groups, one containing malignant ascites and the other containing indolent disease.

In summary our work reveals a strong relationship between the metabolic profile and the entire circadian variation as well as the exosome melatonin can regulate the metabolism. This pre-clinical trial so far unpublished has shown an increase of arginine, its precursors and acylcarnitines at specific time points in the plasma of breast-tumor-bearing animals. The treatment with melatonin is capable to deacrese the concentration of these amino acids and acylcarnitines achieving basal levels. Our results corroborate the literature which characterizes these metabolites as biomarkers for cancer and increasingly allows the use of melatonin as an adjuvant therapy for breast cancer.

**#2505 High-resolution MRS characterization of malignant ascites in two models of ovarian cancer.** Santosh Bharti,1 Flonne Wildes,2 Chien-Fu Hung,2 TC Wu,2 Zayer Bhujwalla,1 Marie-France Penet,3 JHU ICMIC Program, Division of Cancer Imaging Research, Department of Radiology, Johns Hopkins University School of Medicine, Baltimore, MD; 3Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD.

Build-up of malignant ascites occurs in more than one third of ovarian cancer patients and significantly contributes to poor quality of life and mortality. Advances in understanding malignant ascites formation and finding new therapeutic options are urgently needed. High-resolution 1H MRS has been described as an extremely useful in detecting endogenous metabolites to diagnose cancer, by providing a detailed overview of metabolic pathways in a single measurement. In the present study, we are using two ovarian cancer cell lines, the murine IDr-VEGF-Defb29 cell line and the human OVCA3R cell line. Implanted orthotopically, these two ovarian cancer models are characterized by different profile of ascites formation. The mouse cell line IDr-VEGF-Defb29 induces large volumes of ascites, often more than 10 mL, while the human OVCA3R cell line induces ascites less frequently and at smaller volumes, usually less than 0.2 mL. We applied high-resolution 1H MRS to compare the metabolic composition of both ascitic fluids. To better understand the differences observed, we characterized the metabolism of these ovarian cancer cell cultures in vitro by analyzing cell lysates and conditioned culture media with 1H MRS to advance our understanding of cancer cell metabolic reprogramming in malignant ascites formation and the role of the tumor microenvironment in ascites formation and composition. The two tumor models used in this study induced different ascitic profiles. While OVCA3R tumor-bearing mice developed small viscous volume of ascites, IDr-VEGF-Defb29 induced higher volumes. IDr-VEGF-Defb29 ascitic fluids were characterized by higher levels of glutamine, glucose, poly-unsaturated fatty acids and pyruvate compared to the OVCA3R fluids, while all the other metabolites, including glutamate, lactate, myo-inositol, choline and acetate, were lower. To determine if the differences observed in the ascitic fluids were only due to a different metabolism of the cancer cells, we investigated their metabolism in vitro. We analyzed the metabolites present in the conditioned cell culture media, and in the cells, and observed differences in OVCA3R and IDr-VEGF-Defb29 cells metabolism in vitro, without replicating the differences observed in vivo. A Venn diagram of the different metabolites present in the cells, media and ascites showed differences in the metabolites present in those 3 compartments, and highlighted the ones in common. Beta-hydroxybutyrate, lipids, malic and citrate were found in both ascites, and not in the cells or media as. Ascites MRS derived biomarkers could help in ovarian cancer diagnosis, and enhance our understanding of the biochemical and metabolic changes associated with ovarian cancer, and with ascites formation. Supported by Tina’s Wish Foundation, NIH P50CA013175 and P30CA06973.

**#2506 Untargeted and targeted multiplex metabolomic and lipidomic approaches for monitoring biological effects in serum from total body irradiated humans.** Evagelia C. Laiakis,1 Evan L. Pannikak,1 Siddeshwar Chau- 

the,2 Yi-Wen Wang,1 Ming Lian,2 Tyts D. Mak,3 Christopher A. Barker,2 Giuseppe Astarita,1 Albert J. Fornace1.1JHU ICMIC Program, Division of Cancer Imaging Research, Department of Radiology, Johns Hopkins University School of Medicine, Baltimore, MD; 2Memorial Sloan Kettering Cancer Center, New York, NY; 3National Institute of Standards and Technology, Gaithersburg, MD.

Multicellular tumor systems arise from interactions between tumor cells and the tumor microenvironment. The circadian rhythm regulates the whole metabolism modulating the concentration of various circulating substances through the clear and dark variation. Melatonin is the main mediator of this flow and breast tumors development is linked to imbalance of this rhythm. This hormone has several anti-cancer effects extensively proved by our group. Currently metabolomics analysis have been used in cancer research to provide a comprehensive profile of the metabolic changes. Important tumor features are regulated by amino acids likewise fatty acids metabolism. Therefore the comprehension of specific metabolic pathways modulated by melatonin is essential to verify its useful as adjuvant treatment. The aim of this study was to evaluate the metabolic profile in breast cancer xenograft model treated with melatonin and according the circadian cycle. We used 160 female Balb/c nude athmic mice divided into four groups: Tumor / Melatonin, Tumor / Vehicle, Non-tumor / Melatonin and Non-tumor / Vehicle. The tumor-bearing animals were developed by implantation of 3x10⁴ triple negative human breast cancer cells (MDA-MB-231) in the mammary fat pad. All the animals received 100 ul of solution by intraperitoneal injection (IP).
Investigation of responses to radiation therapy or exposure to radiation in an intentional or accidental manner have identified significant biomarkers for biodosimetry and monitoring of individuals in urine through metabolomics (1). We extend the investigation in serum by combining targeted and untargeted methodologies with a multiplatform approach. Serum was collected from patients exposed to total body irradiation (TBI) prior to hematopoietic stem cell transplantation at Memorial Sloan Kettering Cancer Center. The underlying diseases (chronic myelogenous leukemia, acute lymphoblastic leukemia, acute myeloid leukemia, non-Hodgkin lymphoma, or myelodysplastic syndrome) were in remission and the cohort included both males and females. Serum was collected prior to irradiation, at 3-8 h after a single dose of 1.25 Gy, and at 24 h after three fractions of 1.25 Gy each. Untargeted metabolic and lipidomic approaches with liquid chromatography (LC) mass spectrometry (MS) and gas chromatography (GC) MS demonstrated significant perturbations at the later time point and higher total dose. Alterations in circulating levels were observed in lipids from monoaoylglycerol, triacylglycerol, phosphatidylycholine, phosphatidylglycerol, phosphatidylserine, and phosphatidic acid lipid classes. Fatty acids were some of the most dysregulated lipids, with increased levels linked to pro-inflammatory processes. To further investigate the presence of low abundance pro-inflammatory metabolites, we utilized a targeted and highly quantitative approach measuring downstream molecules from fatty acids, such as thromboxanes, prostaglandins, and leukotrienes that can act as signaling molecules and mediators of systemic inflammation. Results identified a rapid response within the first 3-8 hours primarily through the enzymatic action of lipoxigenase (LOX) and cyclo-oxygenase P450 (CYP450), although some dissipated at the later time point. Less significant alterations were observed in the anti-inflammatory pathway, although the fatty acid precursor α-linolenic acid remained elevated in both time points. Finally, as observed in our previous published study of a similar cohort (1), sex-dependent differences were analyzed and utilized to identify more responsive and quantifiable small molecules in blood following radiation exposure can therefore be utilized to not only monitor systemic effects of radiotherapy but also to generate signatures that can be utilized to identify accidentally exposed individuals that may require medical intervention. 1. Laiakis EC et al. Radiat Res 2014; 181: 350-361. 

**#2508 Profiling the metabolic effects of AGI-5198 treatment on IDH1-mutated gliomas.** Víctor Ruiz Rodado, Adrián Lita, Tyrone Dowdy, Mark R. Gilbert, Mioara Larion. National Institutes of Health, Bethesda, MD. 

Background: AGI-5198 (a specific IDH1mut inhibition) inhibits the formation of D-2-hydroxyglutarate (D-2HG) typically produced in millimolar quantities by the R132H mutation of isocitrate dehydrogenase I (IDH1). AGI-5198 was found to delay growth, to promote differentiation in cells and to decrease tumor volume in IDH1-mutant (IDH1mut) mice xenographs, and as a result, has been advanced to clinical trials in IDH1mut glioblastomas. However, no metabolic study of this drug has been reported to date. Here, we investigated the metabolic changes induced by AGI-5198 treatment in order to determine if the drug restores the normal metabolic status once D-2HG concentration is decreased. Methods: We treated glioma cells carrying an IDH1 mutation with 10 μM AGI-5198 for 72 h. Metabolic changes were explored using an untargeted metabolomics approach. Cell viability analysis verified that the concentration of drug does not lead to significant cell death. NMR and MS-linked metabolomics together with in vivo cell imaging using RAMAN were used. Principal component analysis was employed to explore the differences in the metabolic profiles comparing treated and untreated glioma cells. Results and Conclusions: We confirmed that AGI5198 treatment reduced D-2HG levels in the cell lines investigated, validating previous reports. Moreover, we observed a decrease in phospholipid levels upon AGI-5198 treatment, which suggests a decreased proliferative potential for these cells; of particular importance for clinical application. Our preliminary analysis also suggests that metabolites involved in one carbon metabolism are altered upon AGI-5198 treatment. This pathway provides the methyl group for the DNA methylation by DNA methylases producing a hypermethylated phenotype. Our findings suggest that combining the AGI-5198 treatment with a DNA methylase inhibitor may improve outcomes in IDH1-mutated gliomas.

**#2509 High-resolution 1H NMR metabonomics to study breast cancer and endothelial cell metabolic interactions.** Sucelay Acovedo-Acovedo, Sean P. Palecek. University of Wisconsin-Madison, Madison, WI. 

Breast cancer is the second most commonly diagnosed cancer among women worldwide. Patient death is typically caused by metastasis development rather than the primary tumor. Metastasis in breast cancer has been shown to occur via blood and lymphatic vessels. Research shows that breast cancer cells ‘educate’ lymphatic and blood endothelial cells to support tumor growth by stimulating growth factor secretion; specifically VEGF, PDGF-BB and EGF secretion. In addition, cell metabolism is altered during malignant transformation. Cancer cells have increased energy and macromolecule biosynthesis requirements to sustain rapid proliferation. Increased angiogenesis observed in tumors points to a need for an increased nutrient supply. However, it remains unclear how tumor endothelial cell metabolism is altered and how metabolism regulates tumor angiogenesis. Therefore, this study aims to use 1H NMR metabonomics to identify breast cancer-endothelial cell metabolic interactions in a high-throughput manner. Firstly, we studied if in vitro co-culture with breast cancer altered endothelial cell metabolism. We identified distinct metabolic profiles for HUVECs grown in monoculture or co-culture with three different breast cancer cell lines. Metabolism of HUVEC co-cultured with MCF7 and SKBR3 breast cancer cell lines was significantly altered compared to mono-culture control. The most impacted metabolic pathways by co-culture were amino acid metabolism, energy metabolism and lipid biosynthesis pathways. Culturing HUVECs with MDA-MB-231 triple negative breast cancer cells did not have a significant impact on HUVEC metabolism, however. Together, these results indicate that culturing HUVECs with certain breast cancer lines causes significant changes in endothelial metabolism. Alterations in endothelial cell metabolism in response to breast cancer co-culture can further our understanding of tumor-vascular interactions and may lead to identification of metabolic biomarkers or therapeutic targets that can disrupt tumor angiogenesis.

**#2510 Interrogating liver metabolic stress due to cancer-induced cachexia.** Santosh K. Bharti, Paul T. Winnard, Yelena Mironchik, Anirban Majtara, Zaver M. Bhujwalla. 1 The Johns Hopkins University School of Medicine, Baltimore, MD; 2 The University of Texas MD Anderson Cancer Center, Dallas, TX.

Early detection of pancreatic ductal carcinoma (PDAC) is critically important because by the time PDAC is detected almost 80% of patients are surgically unresectable1. While imaging with CT, MRI and ultrasound is making significant inroads in PDAC detection, the costs associated with imaging impose a barrier for routine screening. Plasma based detection of PDAC would provide a relatively inexpensive and easy method for routine screening for the purpose of early detection. Metabolic profiling of plasma samples using high resolution 1H NMR provides an opportunity to assist in the detection of PDAC. Here we characterized the plasma metabolome of normal subjects, subjects with benign pancreatic disease, and subjects with PDAC to evaluate the ability of 1H NMRs to identify metabolic changes in plasma associated with PDAC. Plasma from patients with PDAC (n=4), patients with benign pancreatic disease (n=2) and from healthy control subjects (n=4) were included in this study. Final diagnosis was established by histopathological evaluation of surgical specimens. Three hundred micro liter of D2O phosphate buffer saline (NaCl 0.9% in 90% D2O) was mixed with 300 μL of plasma and transferred to 5 mm NMR tubes. High resolution 1H NMR was performed on an Avance 750 MHz Bruker MR spectrometer. The Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence with water suppression [PRESET-90°-(d-180-d)n-Aq] was performed to remove short T2 components arising due to the presence of macromolecules. The metabolites were identified and quantified from 1H NMR spectra from the three groups. Even with a small sample size clear differences were identified in metabolites such as lactate, pyruvate, β-hydroxybutyrate, acetate and acetoacetate in plasma from PDAC subjects compared to normal subjects. The increase of lactate and pyruvate detected in plasma may reflect altered glucose metabolism occurring in PDAC. β-hydroxybutyrate, acetate and acetoacetate are involved in ketone body synthesis and may reflect altered ketone body metabolism occurring in PDAC. In a recent study, higher levels of ketone bodies and lactate were detected in the serum of rats with 7,12-dimethylbenz(a)anthracene (DMBA)-induced pancreatic intraepithelial neoplasia (PanIN) compared to plasma. Interestingly, with progression to PDAC several of these metabolites decreased3. Our data support further investigation of 1H NMRs of human plasma to detect PDAC in combination with techniques such as circulating tumor cell phenotyping. References: (1) Kortes, E., et al. Cancer Res Clin Oncol 2016, 142, 1795-1805. (2) Van, Q. N., et al. Biochem Biophys Res Commun 2003, 301, 952-959. (3) Lin, X., et al. Mol Biosyst 2016, 12, 2883-2892. This work was supported by NIH R01 CA193365.
Cancer-induced cachexia accounts for approximately 20% of all cancer deaths. In pancreatic cancer, the syndrome affects nearly 80% of patients. Cachectic patients experience a wide range of symptoms affecting the function of several organs such as muscle, liver, brain, and heart, that decrease quality of life and worsen prognosis. A major characteristic of cachexia is the accelerated skeletal muscle and fat tissue burning resulting in increased energy expenditure. Here, for the first time, we have performed high-resolution quantitative 1H magnetic resonance spectroscopy (MRS) of liver tissue obtained from noncachectic and cachectic mice bearing PDAC that are cachectic (Pa04C) and noncachectic (Panc1). A significant reduction in liver weight and significant changes in 1H MRS derived metabolite profiles were detected with cachexia. Human pancreatic cancer cell lines, Panc1 and Pa04C, were used for the study. Six to 8 week old male combined immunodeficient mice were inoculated in the right flank with cancer cells (5×10^6) and in the right hind leg muscle with reporter myoblasts (2×10^6) to monitor the development of cachexia in mice. Live animal optical imaging was done using a Xenogen IVIS® Spectrum (PerkinElmer) optical scanner. Dual phase solvent extraction was performed on liver tissue to extract water soluble metabolites. All 1H MRS spectra were acquired on an Avance 750 MHz Bruker NMR spectrometer. We found, for the first time, that the liver in Pa04C tumor bearing mice underwent a profound weight loss; although Panc1 tumor bearing mice showed some liver weight loss this was not as profound as observed with Pa04C tumors. Significant decreases in lactate, glucose and glutathione were observed in cachetic mouse liver compared to noncachectic mouse liver, and the liver from healthy control mice. The significant decrease of these metabolites in cachectic livers may reflect increased utilization of glucose, lactate and glutathione by the tumor or other organs during the cachexia cascade. These results provide new insights into changes in liver metabolism during cachexia, and support investigating metabolic strategies such as supplementing glutathione or glucose to reduce cachexia associated morbidity.

**#2511 The role of lipids metabolism in bladder cancer.** Gang Wang, Rui Cao, Qiangqiang Ge, Yu Xiao, Xinghuan Wang, Zhongnan Hospital of Wuhan University, Wuhan, China.

Lipid metabolism in bladder cancer (BCa) is one of the most common malignancies of the urinary tract. Many risk factors for BCa has been demonstrated, including aging, smoking, exposure to chemicals, dietary total cholesterol intake, dietary fatty acids intake, etc. In order to investigate the roles of lipids metabolism in bladder cancer, we collected several human BCa tissues and normal bladder tissues to generate an affected pathway network by microarray analysis, and it revealed that glycolipids, lipidic acid level, and lipidic level was down-regulated in tumor tissue. We will discuss the raised questions, which metabolic dysfunction or reprogramming the use of M1N combination for cancer treatments. For instance, among the amino acids, the content of aspartate, a key metabolite in electron transport system in the mitochondria, was specifically suppressed by M1N. Deep RNA sequencing analysis demonstrated that there were sixteen genes whose expressions were found altered following the M1N treatment. Ameboid motility and tumorigenicity of BCa cell lines were both decreased following the M1N treatment. Therefore, the results were strongly supported the use of M1N combination for cancer treatments.

**#2512 Understanding metabolic dysfunction in retinoblastoma development.** Nilanjan Guha, Carolina Livi, Vishnu Suresh Babu, Lisa Winer, Deepak SA, Syed Lateef, Srinivasan Sundaram, Brian Dranka, Arka Subhra Ghosh, Agile Technologies, Bangalore, India.

Metabolic dysfunction or reprogramming is the underlying cause of many diseases including cancer. In this study, we looked into the transcriptomic and metabolomic profile of retinoblastoma (Rb), a pediatric eye cancer, from tumor and normal tissue, aqueous humor, vitreous humor and tear. The samples were collected from enucleated eyes of 9 subjects and 2 deceased controls, whose cause of death is not due to any eye related disease. The study was corroborated with cell line models of retinoblastoma, used for metabolic phenotyping. Combined pathway analysis of transcriptomics data from subject tissue samples and retinoblastoma cell line WERI-Rb1 revealed a significant overlap in the differentially regulated genes belonging to both glycolysis and OXPHOS pathways. The results were correlated with Rb1 overexpressed and its respective control also. To better understand the implications of these two major pathways in the progression of retinoblastoma, metabolic study using Seahorse analyzer enables measurement of extracellular flux (oxygen consumption rate and extracellular acidification rate) to provide insight into mitochondrial function and glycolytic activity. Using a pharmacological approach, the contribution of glucose, glu-
#2516  Pokemon functions as a metabolic oncprotein integrating glucose & lipid metabolism in hepatocellular carcinoma. Yibo Jin,1 Shangfu Li,2 De- liang Cao,3 Dan Gao,2 Hongxia Liu,2 Feng Liu,2 Tiejie Wang,2 Yuyang Jiang,2 Shenzhen Institute for Drug Control, Shenzhen, China; 3The State Key Labora-
tory Breeding Base-Shenzhen Key Laboratory of Chemical Biology, Tsinghua Uni-
versity, Shenzhen, China; 4Southern Illinois University School of Medicine, Springfield, IL; 5Department of Pharmacology and Pharmaceutical Sciences, School of Medicine, Tsinghua University, Shenzhen, China.

Pokemon (also known as FBL1 or LRF) is a proto-oncogene encoded by ZBTB7A gene. It functions as a transcription factor, playing important roles in embryonic development, cell differentiation and neoplasia. Pokemon is abnor-

mally expressed in hepatocellular carcinoma (HCC) and may contribute to the metabolic homeostasis of HCC cells, but the mechanism of action remains to be fully understood. This study demonstrated that Pokemon may integrate glyco-
ylation and lipid metabolism in hepatocellular carcinogenesis. Our data showed that Pokemon was upregulated in HCC cell lines and clinical samples. Epictopic expression of Pokemon stimulated HCC cell growth and proliferation in culture and tumorigenesis in nude mice. Lipid metabolic analysis by LC/MS revealed that in HCC cells lipogenesis was positively correlated with Pokemon ex-
pression levels, and targeted expression of Pokemon in HCC cells promoted the flux of radiolabeled glucose to lipids, including membrane phospholipids through the aerobic glycolysis-acetyl-CoA metabolic stream. Mechanistic stud-
ies revealed that Pokemon stimulated glycolysis through activation of PDK and its mediated Raf/c-Myc pathway. In the tested HCC cells, Pokemon activated fatty acid synthase (FASN) and suppressed AMPK, an inhibitor of acetyl-CoA carboxylase α (ACC-α), stimulating fatty acid and lipid synthesis. Together our results suggest that Pokemon correlates glycolysis and lipid synthesis and thus promotes lipogenesis and hepatocellular tumorigenesis.

#2517  Prognostic biomarkers of PDAC - a cross-validation study. Hung-Jen Wu,1 Smriti S. Menon,2 Keith Unger,2 Massimo S. Fiandaca,3 Yassi Fallah,3 Mark Mapstone,4 Howard J. Federoff,2 Amrita K. Cheema.1 1Georgetown Uni-

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ton, DC; 3UCI, Irvine, CA.

Introduction: Pancreatic ductal adenocarcinoma (PDAC) represents 90% of pancreatic neoplasms and the fourth leading cause of cancer death in the United States. Recently, several research groups have focused on conducting metabolomics based clinical investigations to identify metabolite markers of PDAC. Since a central biomarker repository for PA is lacking, however, it remains challenging to delineate the total number of metabolites reported, and comparatively difficult to test bio-signatures overlap between the disparate studies. Approach: We performed an extensive literature search to delineate PDAC case-control studies reporting dysregulated metabolite biomarkers for PDAC utilizing three main sources: PubMed, EDRN and GDOC. We refined our search to 36 publications that reported blood based metabolomics/lipidomic biomarkers. Data from these studies were analyzed for up/down-regulation of specific metabolites in PDAC, study sample size, and other clinical determinants. The final list consisted of 12 metabolites (including amino acids, fatty acids and small organic acids), that were reported by two or more research groups as being dysregulated in PDAC. Of note, these 36 disparate studies were carried out using different methodologies, analyzed on diverse analytical platforms, and utilized different statistical and bioinformatics methodologies. Resultant common features, therefore, were deemed significant as candidate biomarkers for cross-validation. Next, we used stable isotope dilution multiple-reaction monitoring mass spec-
trometry (SID-MRM-MS) for targeted quantification of these 12 metabolite markers in plasma samples obtained from patients that were diagnosed with PDAC and compared the same profiles to matched normal controls. Results: We delineated a sub-set of the original 12 metabolites that showed concordance with those reported in the literature. Statistical analyses to determine the sensi-
tivity and specificity of the resultant panel, and any influence provided by age, gender, and type 2 diabetes, on the predictive performance of the biomarker panel are ongoing. These data will be presented at the AACR meeting. Conclu-
sion: Developing a specific and sensitive panel of blood-derived biomarkers offers a unique opportunity towards screening and allows increasing overall sur-

vival rates for PDAC with early diagnosis. Such disease-specific bio-signatures allow identification of molecular targets for therapeutic development; improve early treatment strategies and thereby clinical outcomes. Creating a compendium of existing biomarker data and performing cross-validation studies repre-

sent the first steps for developing clinical assays for the diagnosis and prognosis determination of PDAC. The integration of data generated from multiple analy-

tic platforms and diverse subject cohorts is likely to help identify robust bio-
signatures that would then be ready for large scale validation studies. Supported by American Cancer Society.

#2518  Accumulation of a liver- and microbiome-derived metabolite in human breast tumors is associated with patient survival. Wei Tang,1 Tiffany Dorsey,1 Vasanta Putluri,2 Nagireddy Putluri,2 Stefan Ambks3.1 1NCI-CCR Bethesda, MD; 2Baylor College of Medicine, Houston, TX.

Breast cancer is the most common cancer in women in the United States. Gene expression profiling studies of breast tumors led to the discovery of disease subtypes with different biologies. These studies also described novel biomarkers for therapy response and disease survival. However, it remains a challenge to define breast cancer biology solely based on gene expression. Recently, metabo-

lomics emerged as a new discovery tool with the promise of identifying prognostic biomarkers and targetable metabolic dependencies of cancer cells. We previously measured the abundance of 536 metabolites in 67 breast tumor and their tumor adjacent noncancerous tissue by untargeted mass spectrometry. In the current study, we explored the prognostic power of the metabolome and conducted an integrated analysis comprising of the metabolome, transcriptome, and proteome to explore the association of metabolites with cell systems, tumor biology, and disease outcome. We built a predictive model based on multivari-
able Cox proportional hazards using the L1 penalized log partial likelihood (LASSO) method after pre-selecting prognostic metabolites following cross-

validation with 1000 iterations. The median C-index was 0.73, indicating a cer-
tain robustness of metabolites as classifiers of breast cancer outcome. The mod-
els identified five metabolites including a bile acid-related metabolite, glycochenodeoxycholate (GCDC), as the most frequently selected features and outcome markers among these metabolites. An increased GCDC tumor content was associated with improved patient survival. We corroborated the presence of GCDC and three other bile acids in the human breast tissues using absolute measurements and confirmed their occurrence in breast tumors. Because additional large-scale transcriptome and proteome data were available for the same tissue samples, we further characterized the tumors based on their GCDC abund-
ance, which showed that cell cycle-related pathways were enriched for differ-
ently expressed genes and tumors with a high GCDC content tended to have a low cell proliferation score, indicating that accumulation of GCDC leads to growth inhibition. We also conducted a correlation analysis for the relationship between GCDC abundance with other metabolites and identified 51 metabolites that were significantly correlated with GCDC. In this analysis, metabolite abund-
ance in the sterol/steroid pathway associated most strongly with the GCDC tissue content. Lastly, we evaluated the effect of bile acids on breast cancer cell lines. The data showed a strong reduction of cancer cell proliferation under bile acid treatment, consistent with the tumor data, and distinct changes in gene expression. In conclusion, we propose that integrated metabolomics can provide powerful prognostic information with GCDC being a novel prognostic marker in breast cancer because accumulation of GCDC reduces cancer cell prolifera-

#2519  Analysis of gene expression patterns and metabolomics correlations related to obesity, diabetes, and outcomes in patients with epithelial ovarian cancer. Allison M. Montgomery,1 Angelina I. Londono,1 Eric R. Craig,1 Cindy Tawfik,1 Haller J. Smith,1 Charles A. Leath,1 Ashwini A. Katre,1 Sara J. Cooper,2 Rebecca C. Arend,1 1University of Alabama at Birmingham, Birmingham, AL; 2Hudson Alpha Institute for Biotechnology, Huntsville, AL.

Objective: Diabetes and obesity have been associated with a poor prognosis in ovarian cancer (OVCA); the exact mechanism has yet to be determined. Data suggests that obesity diminishes normal immunological response and better prognosis in non-obese patients may be attributable to an intact immune re-
sponse. The objective of this study was to analyze the correlation between gene expression pattern/metabolomics and obesity/diabetes in OVCA patients. Methods: Following IRB approval, UAB patients with suspected OVCA undergoing surgery were consented. Tissue was collected during cytoreduction and 35 samples were analyzed using RNAseq technology and mass spectrometry-based metabolomics. DESeq2 was used for RNAseq analysis to identify gene expression differences between diabetics and non-diabetics stratified by BMI: obese (BMI ≥30) and non-obese (BMI <30). Gene set enrichment analysis was con-
ducted to determine whether there was an over-representation of immune path-
ways among altered genes. Profiles were normalized in the analysis of the met-
abolite profiles using ChromaTOF. Results: 76 genes (p-value <0.05) were dif-

differentially expressed in the tumor samples from patients with BMI ≥30 to those <30. These genes were highly enriched for immune-related genes, includ-


Metabolism in cancer cells is significantly altered as compared to their normal counterparts. Metabolic reprogramming can assist cancer cells to meet their requirements for uncontrollable proliferation and survival. In addition to their role in protein synthesis and energy generation, metabolites also act as signaling molecules, antioxidants and perform oomorgulatory functions. Differences in genetic makeup and phenotype of cells can lead to differences in these metabolic needs. A detailed analysis of metabolic signatures of different cancer cells can therefore prove useful in understanding metabolic interactions with the phenotype in cancer cells. This study aims at identifying these metabolic differences among distinct breast cancer cell lines and gaining mechanistic insights into possible reasons for differential abundance of metabolites. We used nuclear magnetic resonance (NMR) spectroscopy to quantify intracellular metabolites in five breast cancer cell lines having distinct genetic and phenotypic compositions. We show that the intracellular abundance of essential amino acids depends on cell type and medium composition. We also show that myo-inositol abundance was significantly higher in ER-/PR- than ER+/PR+ breast cancer cells due to increased expression of inositol-3-phosphate synthase 1 expression. Myo-inositol is an osmolyte and also a precursor of inositol phosphates which participate in PI3K and calcium signaling pathways. Although the reason for ER+/PR- cells contain greater concentrations of myo-inositol than ER+/PR+ cells is not yet known, increased myo-inositol abundance could potentially serve as a marker of ER-/PR- breast cancer cells. We also observed significant changes in phospholipid metabolism, specifically, glycero phosphocholine (GPC) concentration correlated with doubling time. We also found that reducing serum concentration in the growth medium reduced the growth rate of MCF7 cells, which led to increased GPC abundance in the cells. GPC is also an intracellular osmolyte and is a degradation product of phosphatidylincholine (PtdCho). Phospholipid metabolism has been shown to be significantly altered in breast cancer cells and has been used as prognostic marker in breast cancer cells. A positive correlation of doubling time with GPC irrespective of ER/PR status may prove useful in deciding therapeutic options. Our results highlight the differences in metabolic requirements in different breast cancer cell lines and suggest potential for gaining mechanistic understanding of these differences using metabolomics.

Inhibition of metabolic reprogramming by zerumbone alters the tumorigenic potential of hepatocellular cancer. Nissan A. Wani, Bo Zhang, Kun-yu Teng, Juan Barajas, Kalpana Ghoshal, Rafael Brüschweiler, Samson T. Jacob. Ohio State Univ., Columbus, OH.

Incidence of hepatocellular carcinoma (HCC) that represents ~90% of all cases of primary liver cancer is on the rise worldwide. Sorafenib, the only approved targeted therapy, prolongs median survival only by ~3 months. Thus, new therapeutic strategies are urgently needed. In this study, we investigated therapeutic potential of zerumbone, a sesquiterpene from an edible ginger, against HCC. Zerumbone inhibited proliferation and clonogenic survival of HCC cells by blocking glycolysis and pentose phosphate pathway, arresting cells at G2/M phase of cell cycle and inducing apoptosis. To uncover the underlying molecular mechanisms of its action on HCC cells, we employed several unbiased approaches. First, phosphokinease array showed significant inhibition PI3K/AKT/mTOR and STAT3 signaling in zerumbone-treated HCC cells. Gene Set Enrichment Analysis (GSEA) and Ingenuity Pathway Analysis (IPA) of the microarray data revealed that zerumbone-deregulated genes showed positive correlation with PI3K/AKT/mTOR and STAT3 pathways and those involved in metabolism, apoptosis and cell cycle. Notably, the alteration in expression of genes regulated by PI3K/AKT/mTOR or STAT3 pathways and downregulated by zerumbone in HCCs is associated with poor patient survival. These results imply that treatment with this dietary sesquiterpene might lead to a better outcome in HCC patients. Indeed, zerumbone treatment significantly inhibited growth and lung metastasis of orthotopic HCC xenografts in NSG mice. These findings reveal novel therapeutic strategies targeting cancer metabolism. Collectively, these observations underscore the therapeutic potential of zerumbone in HCC.

Key cluster for prostate cancer diagnosis and therapy; miR-888 cluster. Tsyutso Hasegawa, Aurora Kerscher. Eastern Virginia Medical School, Norfolk, VA.

Prostate cancer (PCa) is the 2nd leading cause of cancer death among men in the U.S. No accurate biomarkers exist and therapeutic options are limited for advanced PCa. MicroRNAs (miRNAs), small non-coding RNAs, act as negative regulators of gene expression. miRNAs have recently emerged as promising therapeutic tools for cancers. It is poorly understood how they function to promote cancer progression. We found miR-888 was involved with PCa progression. miR-888 belongs to a genomic cluster on human chromosome Xq27.3, linked to hereditary PCa. I hypothesized additional members of this cluster (miR-892c, -890, -892a, -892b, -891b, -891a) function to promote PCa progression. Our lab profiled miR-888 expression cluster in paired syngenic human PCa cell lines (RWPE1 & WPE1-NB26, LNCaP & C4-2, PC-3 N & PC-3 ML) differing in metastatic status and androgen response. We hypothesized the miR-888 cluster was preferentially elevated in metastatic PC-3 ML and under-expressed in non-aggressive PC-3 N cells. We examined miR-888 cluster function in vitro and found that forced expression (via miRNA mimics) of certain miR-888 cluster members, notably miR-888 and miR-891a, promoted cell proliferation, colony formation, migration and invasion in PC-3 N cells. Conversely, other
MOLECULAR AND CELLULAR BIOLOGY / GENETICS: MicroRNAs and Other Noncoding RNAs as Tumor Suppressors or Oncogenes

In Multiple myeloma (MM), abnormal plasma cells accumulate in the bone marrow and spread to new bone sites. However, the mechanisms underlying this spread of MM cells remain unclear. Runx2 is a bone specific transcription factor upregulated in various human tumors, including MM. Our studies have previously demonstrated that Runx2 is a major driver of MM progression in bone. In this report, we investigated the role of miR-124 and miR-363 in Runx2 regulation and promote tumor growth and dissemination of MM to new bone sites and assess their therapeutic potential. Expression analysis of a panel of miRNA's regulating Runx2 revealed an inverse relationship between Runx2 expression and two miRNAs: miR-342 and miR-363. MiRNA expression analysis using the Gene Expression Omnibus database showed that miR-342 and miR-363 are highly expressed in MM cells. miR-342 suppressed p62 and p65/NFkB to regulate autophagy, in low-grade disease. My work indicates miR-888 and miR-891 are novel oncogenic factors in the prostate and may use miRNAs to exert their effects. This is the first report to explore the functional role of the miR-888 cluster in any tissue and may lead to promising clinical strategies for PCa.

#2524 MiR-124 suppresses p62 and p65/NIK to regulate autophagy, inflammation and cell death in KRAS mutant mesenchymal NSCLC cells. Anita K. Mehta,1 Kevin Hua,1 William Whipple,1 Mihn-Thuy Nguyen,1 Rushika M. Perera,1 Johanns Haybaeck,3 Joanne Weidhass,4 Jeffrey Settleman,5 Anurag Singhal,3 Perera,2 Johanns Haybaeck,3 Joanne Weidhass,4 Jeffrey Settleman,5 Anurag Singhal,3 1Graduate School of Medicine, Chiba University, Chiba, Japan; 2Institute of Medical Science, St. Marianna Medical University, Kawasaki, Japan; 3University of California at San Francisco, San Francisco, CA; 4University of California at Los Angeles School of Medicine, Los Angeles, CA; 5Calico Life Sciences, South San Francisco, CA.

Background KRAS mutant non-small cell lung cancers (NSCLC) cells are molecularly and histologically diverse. Epithelial-like cells are more KRAS dependent, whereas mesenchymal-like cells are less KRAS dependent. These two subtypes are designated KE (epithelial) and KM (mesenchymal), respectively. A KE versus KM subtype transcriptional signature reveals specific modes of KRAS dependent survival signaling in the KE subtype. This KRAS dependence signature is significantly enriched with predicted microRNA (miRNA) target genes of miR-205 and miR-34b/c. MiRNAs can function as tumor suppressors by coordinately regulating multiple oncogenic signaling pathways. The role of deregulated miRNA function in mediating the survival of KM versus KE NSCLC cells has not been investigated to date. Methods Differential miRNA expression in KRAS mutant cell lines was determined using Taqman low-density qPCR arrays (TLDA). Functional miRNA reconstitution experiments of downregulated miRNAs were performed in a panel of KM cell lines. Effects on apoptosis and autophagy were performed by Western blotting, immunofluorescence and live cell microscopy and caspase assays. The molecular targets of miR-124 were computationally identified by TargetScan or miRWalk and experimentally verified using 3’UTR luciferase-based assays. Functional rescue of miRNA-dependent cell viability defects was determined by ectopic predicted target gene expression. Results Comparison of KE to KM cells yielded a KE-KM miRNA subtype classifier/signature. This signature revealed a number of silenced or suppressed miRNAs in KM cell lines, including members of the miR-200 family. MiR-200 and miR-205 reconstitution in KM cells modulated epithelial plasticity by Zeb1 protein suppression and increased E-cadherin levels. Reconstitution of miR-124, miR-625 and miR-518-3p in KM cells caused pronounced loss of cell viability. Furthermore, miR-124 caused autolysosome maturation defects. We identified SQSTM1/p62, TRAF6 and RELA/p65 as key predicted targets of miR-124. MiR-124 reconstitution in KM cells caused decreased p62, TRAF6 and p65 protein levels. The effect of miR-124 on p62 expression was verified using a Luciferase-p62-3’UTR reporter construct. Overexpression of p62 in KM cells rescued the cell viability defects caused by miR-124. Conclusion These studies implicate miR-124 as a context-dependent tumor suppressor miRNA in KM subcutaneous tumors. MiR-124 directly suppresses expression of SQSTM1/p62 to promote defects in autolysosome maturation. In parallel, miR-124 suppresses RELA/p65 and in some cases, TRAF6, to alter expression levels of several cytokines. Thus, miR-124 coordinate regulates autophagy and inflammation to disrupt the finely-tuned balance between pro and anti-inflammatory signals, resulting in cytotoxic effects in a specific subtype of mesenchymal-like KRAS mutant NSCLC cells.


in multiple myeloma cell-derived runx2 by miRNAs suppresses multiple myeloma growth and progression. In Multiple myeloma (MM), abnormal plasma cells accumulate in the bone marrow and spread to new bone sites. However, the mechanisms underlying this spread of MM cells remain unclear. Runx2 is a bone specific transcription factor upregulated in various human tumors, including MM. Our studies have previously demonstrated that Runx2 is a major driver of MM progression in bone. In this report, we investigated the role of miR-124 and miR-363 in Runx2 regulation and promote tumor growth and dissemination of MM to new bone sites and assess their therapeutic potential. Expression analysis of a panel of miRNA's regulating Runx2 revealed an inverse relationship between Runx2 expression and two miRNAs: miR-342 and miR-363. MiRNA expression analysis using the Gene Expression Omnibus database showed that miR-342 and miR-363 are highly expressed in MM cells. miR-342 suppressed p62 and p65/NFkB to regulate autophagy, in low-grade disease. My work indicates miR-888 and miR-891 are novel oncogenic factors in the prostate and may use miRNAs to exert their effects. This is the first report to explore the functional role of the miR-888 cluster in any tissue and may lead to promising clinical strategies for PCa.

#2526 MicroRNA expression signature of patients with tyrosine kinases inhibitors failure: miR-10a-5p inhibits cancer cell aggressiveness in renal cell carcinoma. Takayuki Arai,1 Atsushi Okato,1 Akira Kurozumi,3 Mayuko Kato,1 Yusuke Goto,1 Keiichi Koshizuka,1 Satoko Kojima,2 Yukio Naya,2 Tomohiko Ichikawa,1 Naohiko Seki,1 Graduate School of Medicine, Chiba University, Chiba, Japan; 2Teikyo University Chiba Medical Center, Ichihara, Japan.

Renal cell carcinoma (RCC) is a disease in which cells undergo oncogenic transformation in the kidney tubules. The five-year survival rate of advanced cancerous kidney tissue is poor (5-10%). There has been an urgent need for novel treatments extending progression-free survival of patients with RCC, and angiogenesis-blocking drugs have been developed to selectively inhibit the growth of tumor vasculature. Inhibitors of angiogenesis, e.g., VEGF and FGF, are the basis for current anti-angiogenic multi-tyrosine kinase inhibitors (TKIs) have been developed and have been used as first and second line treatments for RCC. However, these treatments extend progression-free survival only slightly, and relapse and metastasis eventually develop in most patients. The molecular mechanisms of RCC recurrence, metastasis and drug resistance are not yet fully understood. Therefore, analysis of the molecular mechanisms underlying RCC development and progression plays a critical role in identifying targets for the development of novel drugs for RCC treatment. Therefore, dysregulated expression of miRNAs and their regulated RCC pathways based on the signature approach could significantly improve diagnosis, therapy, and prevention of the disease. miRNAs (miRNAs) are small noncoding RNAs that function to fine tune the expression of protein coding/noncoding RNAs by repressing translation or cleaving RNA transcripts in a sequence-dependent manner. The unique characteristic function of miRNAs is to regulate RNA transcripts in human cells. Therefore, dysregulated expression of miRNAs can disrupt tightly regulated RNA networks in cancer cells. In this study, we constructed a miRNA expression signature to identify pathways activated by TKI treatment using cDNA microarrays. We have sequentially identified tumor-suppressive miRNAs of RCC cancers and their regulated RCC pathways based on the signature approach. The aim of this study was to investigate the functional significance of miR-10a-5p and to identify the molecular targets and pathways mediated by miR-10a-5p in RCC cells. The expression levels of miR-10a-5p were significantly reduced in RCC clinical specimens and RCC cell lines compared with non-cancerous kidney tissues (P < 0.001). TCGA data showed that the overall survival of low miR-10a-5p expression group was significantly shorter than that of high miR-10a-5p group (P = 0.034). Restoration of miR-10a-5p significantly inhibited cancer cell migration and invasion in RCC cell lines (P < 0.001). Spindle and kinetochore associated complex subunit 1 (SKA1) was identified as a direct target gene of miR-10a-5p by genome-wide gene expression analysis and in silico analysis. Overexpression of SKA1 was observed in RCC clinical specimens.
The overall survival of high SKA1 expression group was significantly shorter than that of low expression group by TCGA analysis (P = 1.44E-07). Tumor-suppressive miR-10a-5p was identified by using miRNA signature of patients with TKI failure. Overexpression of SKA1 might be involved in RCC aggressiveness, metastasis and drug resistance. Elucidation of tumor-suppressivemicroRNAs regulated molecular pathways and targets could provide newinformation on potential therapeutic strategies in the disease.

**#2527** The role of miR-601 in prostate cancer progression. Jessica L. Fleming, Erica H. Bell, Kathryn Andrews, Arnab Chakravarti. The Ohio State University, Columbus, OH.

Prostate cancer (PCa) is the second most common cancer among men worldwide. In order to advance treatment options for these men, it is crucial to understand the molecular underpinnings behind this cancer. Previously, our group identified miR-601 as a biomarker of significance in PCa. Few studies to date have functionally validated molecular biomarkers and currently little is known about the function of miR-601 in PCa. Based on our previous publication, we hypothesized that miR-601 plays a role in PCa progression and radiation response. To test this hypothesis, miR-601 was over-expressed and knocked down in DU145 and PC3 cell lines and assays were performed evaluating cell proliferation, colony formation, apoptosis, and cell cycle progression. Additionally, to provide more information regarding miR-601-associated pathways, we identified putative gene targets of miR-601 using in silico prediction programs, microrna.org and TargetScan. org and evaluated top gene targets in vitro. To date, our data suggest that miR-601 may be playing a role as a tumor suppressor. Over-expression of miR-601 in cell lines resulted in a significant reduction in cell viability. This was confirmed both by MTS as well as colony formation assays. We looked into the mechanism behind the reduction in cell viability by testing the effect of miR-601 on apoptosis and cell cycle progression. We found that cells over-expressing miR-601 had slightly higher levels of apoptosis, however miR-601 did not induce a substantial effect on cell cycle progression. Additionally, we found that miR-601 enhances radiosensitivity in DU145 and PC-3 cell lines. Similar results have been observed in breast cancer as well as in a large miRNA screen performed in the LNCaP cell line. Two putative gene targets of miR-601 were identified and investigated in vitro, SIRT1, a histone deacetylase known to be both an oncogene and tumor suppressor, and BCL2L2, an anti-apoptotic gene known to be an oncogene. SIRT1 and BCL2L2 had strong scores on both online prediction programs as likely targets of miR-601. Our in vitro results confirmed this. We saw reduced miRNA and protein expression of these targets in cells over-expressing miR-601. Our data thus far suggest that miR-601 is acting as a tumor suppressor in PCa. Targeted therapies for miR-601 and/or its targets may be promising in the treatment of PCa, however additional work is needed to validate this. Future work will focus on the role of miR-601 in cell migration and invasion as well as identifying the direct molecular mechanism(s) by which miR-601 is reducing cell viability and conveying radiation sensitivity. Funding: R01CA108633 (To AC), IR02CA148190 (To AC) U10CA180850-01 (To AC), IR01CA169368 (To AC) from the NCI, Brain Tumor Funders Collabor-ative, Cancer Genome Anatomy Project (TGSC), Biomedical and Pharmacotherapy, 79: 247-53 2. Hatano K. et al. (2015) Nucleic Acids Research. 43: 4075-86

**#2528** miR-452 inhibits migration and invasion of prostate cancer cells by targeting E3 ubiquitin ligase-1 (WWP1). Satoko Kojima, Yusuke Goto, Akira Kurozumi, Mayuko Kato, Atsushi Okato, Takayuki Arai, Tomohiko Ichikawa, Yukio Naya, Naohiko Seki. Teikyo University Chiba Medical Center, Chiba, Japan; Chiba University Graduate School of Medicine, Chiba, Japan

Introduction: Most prostate cancer (PCa) patients initially respond to androgen-deprivation therapy (ADT), but eventually acquire resistance to ADT and progress to castration-resistant prostate cancer (CRPC). CRPC, resulting in metastasis causing PCa death. We have developed the miRNA (miRNA) expression signature of PCa using clinical specimens and determined the target genes of the tumor suppressive miRNAs, whose expressions are significantly decreased in PCa specimens. Based on the signature, our previous study revealed that miR-224 functions as a tumor suppressor, especially contributes to cancer cell metastasis directly targeting miR-224-TDP52 signaling. In human genome, miR-452 is located near the human tumor-suppressive miR-224, forming clustered miRNAs on chromosome Xp28 region. The aim of the study was to investigate the functional significance of miR-452 and to identify novel miR-452-mediated cancer pathways and responsible genes in PCa cells. Material and methods: Clinical prostate specimens were obtained from patients admitted to the Teikyo University Chiba Medical Center from 2008 to 2013. Ninety-two patients with elevated PSA levels underwent transrectal prostate needle biopsy, and three patients who died of CRPC underwent autopsies. PCa tissues (n=54), noncancerous prostate tissues (non-PCa, n=36), were used for analysis of expression levels of miRNAs. Functional studies of differentially expressed miRNAs were analyzed using PC3 and LNCaP cell lines under CRPC conditions. The expression levels of miRNA was estimated using the Kaplan-Meier methods. In silico database and genome-wide expression analyses were performed to identify molecular targets regulated by the miRNAs. Results: (The miRNA expression signature of PCa specimens showed that the cluster miRNAs miR-224 was significantly downregulated, suggesting that this miRNA may act as tumor suppressor.) Clinical and in vitro analysis of miR-452 overexpression showed that low expression of miR-452 predicted a short duration of progression to CRPC. Restoration of miR-452 in PC3 and DU145 cells revealed significant inhibition of cancer cell migration and invasion. WW domain-containing E3 ubiquitin protein ligase-1 (WWP1) was confirmed as a direct target of miR-452 by in vitro transfection of miR-452 and Luciferase assay. Knockdown of the expression of WWP1 using siRNA showed inhibiting cell migration and invasion in PC3 and DU145 cells. Immunohistochemistry showed overexpressed WWP1 in advanced PCa. Conclusions: Loss of the tumor suppressive miR-452 enhanced migration and invasion in PCa cells. Regulation of the miR-452-WWP1 axis contributed to PCa cell migration and invasion, and elucidation of downstream signaling of this axis will provide new insights into the mechanisms of (progression to CRPC) metastasis of PCa.

**#2529** TTF-1/NKX2-1 induced miR-532-5p targets KRAS and MKL2 oncogenes and causes apoptosis in lung adenocarcinoma cells. Sebastian Griesing, Taisuke Kajino, Mei Chee Tai, Zhuanan Liu, Masahiro Nakatoh, Toshi Suzuki, Takashi Takahashi, Nagoya University Graduate School of Medicine, Nagoya, Japan; Nagoya University Hospital, Nagoya, Japan.

Our group and others have previously identified TTF-1/NKX2-1 as a lineage survival oncogene in lung adenocarcinoma. Subsequent studies revealed double-edged sword characteristic of TTF-1 in the development of lung adenocarcinoma. TTF-1 elicits lineage-survival signaling by inducing transcriptional targets such as ROR1, while TTF-1 also inhibits tumor progression by decreasing cell motility through direct activation of genes such as MYBPH. It is thus clear that a more comprehensive picture of this still enigmatic lineage-survival oncogene needs to be elucidated. Whereas most of the previous studies on TTF-1 have focused on its regulation of protein-coding genes, very little is known about regulation of microRNA by TTF-1. In the present study, we combined in vivo patient data with in vitro data from lung adenocarcinoma cell lines, aiming at identifying miRNAs, which are transcriptionally regulated by TTF-1. Our integrative approach identified miR-532-5p as a microRNA regulated by TTF-1. Expression of miR-532-5p decreased, when cell lines with a high endogenous level of TTF-1 were transfected with siRNA against TTF-1, while it was increased by overexpression of exogenous TTF-1 in TTF-1-negative cell lines. By ChIP and luciferase assays, TTF-1 was found to induce miR-532-5p expression through its binding to the MIR532 promoter region, identifying miR-532-5p as a direct target of TTF-1. We next employed microarray analysis and target prediction using TARGETSCAN to search for potential targets of miR-532-5p. We consequently identified KRAS and MKL2 as candidate targets of miR-532-5p by screening their potential mechanistic link. We found that miR-532-5p induced apoptosis and inhibited colony formation in lung adenocarcinoma cell lines, while depletion of MKL2 phenocopied the effects of miR-532-5p introduction, similarly exhibiting occurrence of marked apoptosis. In addition, miR-532-5p significantly decreased tumor formations in vivo in a mouse xenograft model. It was also of note that miR-532-5p inhibited lung adenocarcinoma cell lines regardless of the presence or absence of KRAS mutations. In summary, we have found that TTF-1 transactivates miR-532-5p, which potently induces apoptosis in lung adenocarcinoma cells. MiR-532-5p directly targets MKL2, which we have also uncovered to be crucially involved in lung adenocarcinoma survival. In addition, while previous reports have shown that TTF-1 inhibits KRAS-driven tumorigenesis, van der Vlugt-Daane et al. showed that expression of miR-532-5p provides their potential mechanistic link. Our results thus shed light on how TTF-1 plays a tumor-suppressive role in the process of lung carcinogenesis, which might ultimately lead to development of a novel therapeutic strategy for this devastating disease.

**#2530** Validation and pathway analysis of a metastasis-specific microRNA signature in primary colon cancer. Robert R. Coebergh van den Braak, Anieta S. Sweerts, Zarina S. Lalmahomed, Marcel Smid, Vanja de Wereld, Michelle van der Vlugt - Daane, Anne van Galen, Shanashp Xiang, Katharina Biermann, John A. Fockens, John W. Martens, Jan N. IJzermans. Erasmus University Medical Center, Rotterdam, Netherlands.

Introduction: In lymph node negative (LNN) colon cancer 20% of the patients develops disease recurrence. Identification of these patients is needed. Micro-
RNAs (MiRNAs), a group of short non-coding RNAs, can function as tumor suppressors or oncogenes. Hur et al recently found 6 differentially expressed MiRNAs when comparing primary colorectal cancer and matched liver metastasis (MiR-320, MiR-221, MiR-30b, MiR-10b, MiR-885-5p, Let-7i). The expression of 2 MiRNAs was significantly correlated with distant metastasis (low Let-7i expression and MiR-30b) in primary colorectal cancer patients. We identified a median and combined into a signature (Let-7i high and MiR-10b low [n = 22] vs. Let-7i low and/or MiR-10b high [n = 122]), the first group showed 100% metastasis free survival (MFS). We assessed the prognostic value of these MiRNAs and the signature in a clinically well-defined cohort of primary colon cancer. Methods: Expression of the 6 MiRNAs were measured using RT-qPCR in a cohort of 232 colorectal cancer patients. Results: The LNN group showed significantly higher expression of the EMT pathway in the Let-7i high group. MiR-30b and Let-7i expression was split at the median level and combined into two groups (‘Let-7i high and MiR-30b low’ vs. ‘Let-7i low and/or MiR-30b high’). The ‘Let-7i high and MiR-30b low’ group in the total group (n = 741) had a significantly better 5-yr MFS (100% vs 87.4% p = 0.002), and it had a significantly better 5-yr MFS (91.7% vs 78% p = 0.036) and HFS (100% vs 87.8% p = 0.01) in the LNN group (n = 52). Conclusion: In our cohort and more specifically the LNN group, we confirmed Let-7i and identified MiR-30b as a prognostic factor for MFS and HFS. We did not confirm the prognostic value of MiR-10b. The combination of Let-7i and MiR-10b identified a group with a 100% HFS. Pathway analysis showed higher expression of the TGF-beta pathway in the MiR-30b high group, and a significantly higher expression of the EMT pathway in the Let-7i high group. MiR-30b and Let-7i expression was split at the median level and combined into two groups (‘Let-7i high and MiR-30b low’ vs. ‘Let-7i low and/or MiR-30b high’). The ‘Let-7i high and MiR-30b low’ group in the total group (n = 741) had a significantly better 5-yr MFS (100% vs 87.4% p = 0.002), and it had a significantly better 5-yr MFS (91.7% vs 78% p = 0.036) and HFS (100% vs 87.8% p = 0.01) in the LNN group (n = 52). Conclusion: In our cohort and more specifically the LNN group, we confirmed Let-7i and identified MiR-30b as a prognostic factor for MFS and HFS. We did not confirm the prognostic value of MiR-10b. The combination of Let-7i and MiR-10b identified a group with a 100% HFS. Pathway analysis showed higher expression of the TGF-beta pathway in the MiR-30b high group, and a significantly higher expression of the EMT pathway in the Let-7i high group.

#2531 Regulation of androgen signaling axis and tumor suppressive function of miR-149-5p in prostate cancer. Savita Singh,1 Jey Sahith Ebron,2 Eswar Shankar,2 Sanjan Gupta,2 Daniel Lindner,2 Girish Shukla1. 1Cleveland State University, Cleveland, OH. 2Case Western Reserve University & University Hospitals Cleveland Medical Center, Cleveland, OH; 3Taussig Cancer Center, Learner Research Institute, Cleveland, OH.

Prostate cancer growth and proliferation depends on androgen signaling mediated by transactivation of Androgen Receptor (AR). Androgen ablation remains the mainstay therapy for treatment of the disease. However, despite androgen ablation, the disease relapses to more aggressive form known as castration-resistant prostate cancer (CRPC). Androgen Signaling Inhibitor such as Abiraterone Acetate and Enzalutamide are the main effective treatment methods currently being used to treat CRPC. However, more than one-third of CRPC patients develop resistance to these treatments, mostly due to the gain of function in the AR protein and increase in intratumoral dihydrotestosterone (DHT) synthesis. Intratumoral DHT synthesis from steroid precursors in tumors is augmented by up-regulation of enzymes of cholestero genesis and steriodogene sis, such as HMG-CoA reductase, SCAR81, and 17βHSD respectively. Tumor-specific downregulation of microRNAs which regulate the AR and steroid biosynthesis has been implicated in tumor growth and resistance to therapeutics in CRPC. We are focusing on tumor suppressive role of miR-149-5p in CRCA. We discovered that miR-149-5p expression was significantly lower in prostate cancer tissues compared to normal tissues. The mechanistic investigation revealed that miR-149-5p regulates wild type and alternatively spliced variant of AR. It also down regulates the expression of SCAR81, HMGCoA and HMG-CoA reductase, the proteins known to facilitate intratumoral DHT synthesis. Ectopic expression of miR-149-5p negatively regulated the expression of prostate specific antigen, a downstream target of androgen signaling and it also inhibited invasion and proliferation of CRPC cells. Our findings indicate a significant role of miR-149-5p in regulating androgen signaling and possibly DHT synthesis in CRPC. This provides a strong rationale for further investigating the significance of miR-149-5p for generation of new therapeutic for CRPC.

#2532 High expression of MiR-432-3p is associated with the chemoresistance by NFR2 stabilization via directly targeting KEAP1. Burak Akdemir,1 Jun Inoue,1 Tatsuyuki Kawano,2 Joji Inazawa,1 1Department of Molecular Cyto genetics, Tokyo Medical and Dental University, Tokyo, Japan; 2Department of Espohageal and General Surgery, Tokyo Medical and Dental University, Tokyo, Japan.

NF-E2-related factor 2 (NRF2) is a master transcriptional regulator that integrates cellular stress responses and is negatively regulated by Kelch-like ECH-associated protein 1 (KEAP1) at the post-translational level. In human cancers, aberrantly stabilized NRF2, either by mutation of NRF2 or KEAP1, plays a vital role in chemoresistance through the activation of target genes, suggesting that targeted inhibition of NRF2 is a potential therapy for NRF2-stabilized tumors. MicroRNAs (miRNA) are endogenous small noncoding RNAs that can negatively regulate gene expression by interfering with the translation or stability of target transcripts. Previously, we identified miRNAs negatively regulating NRF2 pathway via directly targeting NRF2 itself by a reporter-coupled miRNA library screening and demonstrated the potential of miRNA-based therapy against NRF2-stabilized tumors in esophageal squamous cell carcinoma (ESCC) (Mot Cancer Res 2014, Cancer Res 2015). In the present study, we further identified miR-432-3p as a miRNA positively regulating NRF2 pathway. MiR-432-3p could directly targeted KEAP1 via binding to its seed sequence within the coding region. Overexpression of miR-432-3p resulted in enhanced resistance to cisplatin via activation of NRF2 in ESCC cell lines. Furthermore, miR-432-3p was also used as control to show that overexpression of corresponding non-cancerous esophageal mucosa (65.5%). Importantly, we showed the negative correlation between the expression level of KEAP1 and miR-432-3p in primary ESCC tumors. Taken together, these findings suggest that the high expression of miR-432-3p may contribute to the chemoresistance by NRF2 stabilization via down-regulation of KEAP1 expression in ESCC.

#2533 miRNA expression as potential biomarker for synvalcoraloma. Laura Pazzaglia, Serena Pollino, Mattia Vitalé, Amalia Conti, Piero Picci, Maria Serena Benassi, Istituto Ortopedico Rizzoli, Bologna, Italy.

Synvalcoraloma (SS) is a rare tumor, with dismal survival when metastatic. SS contains a characteristic translocation (X;18)(p11;q11), representing the fusion of SYT on chromosome 18 with either SSX1, SSX2, or rarely SSX4 on chromosome X. The resulting fusion genes appear to be mutually exclusive and concordant in primary and metastatic tumours. New prognostic and predictive factors are needed. Chemokine receptor 4 (CXCR4) is a seven-transmembrane G protein–coupled chemokine receptor and it is the chemokine receptor most commonly expressed in tumour cells, involved in cell migration and invasion, as well as angiogenesis. microRNAs (miRNAs) are involved in post-transcriptional gene expression regulation and control important physiological processes like development, cell differentiation and cell signaling. Altered expression of SOX2 is strongly correlated with the malignant phenotype and there is data showing the negative correlation between the expression level of SOX2 and miRNA-432 expression in synvalcoraloma tissues compared with the corresponding non-cancerous esophageal mucosa (65.5%). Importantly, we showed the negative correlation between the expression level of SOX2 and miR-432-3p in primary ESCC tumors. Taken together, these findings suggest that the high expression of miR-432-3p may contribute to the chemoresistance by NRF2 stabilization via down-regulation of KEAP1 expression in ESCC.
SS. Correlation with clinical data, with CXCR4 expression and in vitro studies also with miR-494 in several SS cell lines are on-going to better investigate their role as potential prognostic and therapeutic markers.

#2535 MicroRNA-518c-5p promotes the metastasis of oral cancer. Makoto Kinouchi, Daisuke Uchida, Nobuyuki Kuribayashi, Yuske Komiyama, Shuji Tsuchida, Hitoshi Kawamata. Dokkyo University School of Medicine, Tochigi, Japan.

We have demonstrated that CXCR4 system is involved in the metastatic process of oral cancer. Recently, we identified the miR-518c-5p as a downstream target of the CXCR4 system. In this study, we examined the function of miR-518c-5p on the metastasis of oral cancer. We transfected miR-518c expression vector into oral cancer cells, B88 and CAL27, and isolated stable transfectants, B88-518c and CAL27-518c, respectively. The growth and migration of both cells were significantly enhanced in compared with those of mock cells. LNA-based miR-518c-5p inhibitor significantly impaired the enhanced cell growth and migration of these cells, indicating that these phenomena were dependent on the expression of miR-518c-5p, not on that of miR-518c-3p. Next, we examined the function of miR-518c-5p in vivo. miR-518c or mock transfectants were inoculated into the masseter muscle or the blood vessels of nude mice. Tumor volume, lymph nodes metastasis, and lung metastasis were significantly increased in the mice inoculated with miR-518c transfectants. Furthermore, we examined the mRNA expression induced by miR-518c-5p using B88 cells and B88-518c cells by cDNA microarray analysis. Consequently, some of the genes involved in the cell growth and metastasis were upregulated in the B88-518c cells. These results indicate that CXCR4 system regulates the metastases of oral cancer via induction of miR-518c-5p.

#2536 miR-187-5p and miR-219a-1-3p: potential biomarkers associated with chemotherapeutic response and prognosis in ovarian cancer. Chia-Yen Huang,1 Hsien-Da Huang2.

We identified the most significant altered miRNAs between different groups of patients of chemotherapy response. We then used Kaplan-Meier and log-rank methods to analyze the relationship between these miRNAs and progression-free and overall survival in TCGA (n = 460) and Bagnoli (n = 130) datasets. In vitro study for response to chemotherapy agents were performed in human ovarian cancer cell lines and their transfectants. The biological relevance of putative miRNA targets was also analyzed using bioinformatics. Results: MiR-187-5p and miR-219a-1-3p were associated with better response to chemotherapy and longer progression-free and overall survival in two independent epithelial ovarian cancer patient cohorts (all p <0.05). These two miRNAs also sensitized ovarian cancer cells to chemotherapy, thus recapitulating the clinical observation. Conclusions: This study showed that miR-187-5p and miR-219a-1-3p could be useful biomarker for prediction of chemotherapy response and survival in serous ovarian cancers. MiR-187-5p and miR-219a-1-3p may be potential molecules in the treatment of EOC for sensitizing cancer cells to chemotherapy.

#2537 MicroRNA targeting anti-apoptotic and G2/M pathways as therapeutic targets for castration resistant prostate cancer. Pheruza Tarapore,1 Sarah To,2 Bin Ouyang,3 Yuet-Kin Leung,4 Ana Cheong,1 Shuk-meI Ho.1 1University of Cincinnati, Cincinnati, OH; 2Hudson Institute of Medical Research, Melbourne, Australia.

Prostate cancer (PC) is one of the most common cancers in men. Unfortunately, limited treatment options currently exist for those who have developed advanced castration-resistant PC (CRPC). G-1 is a GPER1/GPR30 agonist, and a promising candidate for CRPC therapy. G-1 is effective in halting the growth of CR tumors but not those grown in intact hosts. These findings suggest GPER1 is a therapeutic target for CRPC. We found that G-1, through activation of GPER1, inhibited growth of CRPC cells via cell-cycle arrest at the G2-M phase, probably leading to mitotic catastrophe. However, the exact mode of action of G-1 was not known. To better understand the pathways involved in G-1 action, we conducted a genome-wide mRNA-seq and miRNA-seq study on G-1 treated xenografts. We identified a panel of novel G-1-associated tumor suppressive miRNAs and genes. Moreover, Ingenuity Pathway Analysis revealed that the G-1 differentially regulated genes are involved in “Cellular Growth and Proliferation”, “endocrine function” and “Cancer” pathways. We validated the expression of the key miRNA (miR-34c, miR-10b, miR-138 and miR218) and genes in G-1 treated castration resistant xenografts and cell lines (C4-2 and 22Rv1). Furthermore, we have shown the tumor suppressor function of these miRNAs in cell survival and apoptosis. The predicted target gene set was examined for decreased expression by qRT-PCR. We confirmed decreased expression of some of the downstream targets, which consist of genes involved in cell cycle regulation (CCNA and CCND, CDK1, CDK4, PLK1), cell survival (Bcl2, Survivin), cell migration/F-actin formation (LAP5, PCDH7, ITGA9, ROBO1, SLIT1) and G2/M checkpoint (CDK1, PLK1, BCL-2 Survivin). Thus these miRNAs - miR-34c, miR-10b, miR-138 and miR218 - are ideal candidates for therapy of CRPC.

#2538 MiR-638 promotes autophagy and malignant phenotypes of cancer cells via directly suppressing DACT3. Ming Yang, Shandong Cancer Hospital and Institute, Jinan, China.

Autophagy is implicated in human cancers and the mechanistic details remains largely unclear. Herein we report the regulatory role of miR-638 in autophagy of esophageal squamous cell carcinoma (ESCC) and breast cancer cells. We found that miR-638 overexpression promotes starvation- and rapamycin-induced autophagy. In ESCC and breast cancer cells, miR-638 acts as an oncogene and promote cell proliferation, migration, as well as invasion in vitro and in vivo. In accordance with this, we observed significantly higher miR-638 expression in ESCC and breast cancer tissues compared to normal tissues. To further elucidate regulatory mechanisms of miR-638 in autophagy, we performed a computational nomination of its target genes through intersecting the results of multiple prediction algorithms. DACT3, a key regulator of Wnt/b-catenin signaling, was predicted to be regulated by miR-638 by all programs and confirmed by experimental results. Depletion of DACT3 phenocopied effects of miR-638 overexpression, demonstrating its importance in autophagy. These results elucidate that the miR-638-DACT3 axis might be an important molecular pathway in controlling and autophagy and tumorigenesis. Our data in clinical tissue samples highlight miR-638 and DACT3 with histological marker for cancer detection and potentially therapeutic implications.

#2539 MiR-96-5p functions as an oncogenic miRNA by inhibiting apoptosis in hepatocellular carcinoma. Naoto Iwai, Kohichiroh Yasui, Akira Tomie, Kei Teasaki, Tomoko Kitaichi, Osamu Dohi, Yasuyuki Gen, Yoshito Ito. Kyoto Prefectural University of Medicine, Kyoto, Japan.

Hepatocellular carcinoma (HCC) is one of the most aggressive cancers with high mortality worldwide. MicroRNAs (miRNAs) are small non-coding RNAs that have been used as cancer-related biomarkers and expected to be therapeutic agents. We performed genome-wide miRNA expression profiling of paired HCC tumors and non-tumorous liver tissues from patients with primary HCCs using the miRNA microarray (Agilent). We found that miR-96-5p was most significantly up-regulated in HCC tumors compared to non-tumor tissues. Although miR-96-5p is suggested to be an oncogenic miRNA, the function of miR-96-5p remains largely unknown. We identified the caspase-9 gene (CASP9) as a novel target of miR-96-5p. The predicted target gene FOXO1 is the known target of it. Caspase-9 protein is thought to play a central role in apoptosis and to be a tumor suppressor. Overexpression of miR-96-5p decreased caspase-9 protein expression and resulted in resistance to apoptosis induced by doxorubicin and UV in HCC cells. Our results suggested that miR-96-5p functions as an oncogenic miRNA by inhibiting apoptosis through decreasing caspase-9 expression in HCC.

#2540 miR-196b as a potential biomarker for human esophageal cancer. Xiaohui Tan,1 Tao Chen,2 Robert Siegel,3 Sidney Fu. 1 *George Washington Univ., Washington, DC; 2Chengdu Military General Hospital, Chengdu, China.

Esophageal cancer is the sixth most common cause of cancer related death. Although multiple genetic and epigenetic alterations have been detected in esophageal cancer, molecular markers for early diagnosis and prediction of prognosis or treatment responses are quite limited. microRNA (miRNA) is a class of small-regulatory non-coding RNA, acting as either a tumor suppressor or oncogene by regulating gene expression through pairing with complementary seed of the targeted messenger RNAs (mRNA). A number of miRNA expression profiling studies have been conducted in esophageal. In our current study, we analyzed the most recent studies on miRNAs expression profiles in esophageal cancer. Consistently dysregulated miRNAs have been identified in esophageal cancer, including upregulation of miR-196b, miR-135a, and down-regulation of miR-141, miR-200a-5p, miR-200b-3p, miR-27b, miR-210. We first assessed expression of miRNAs in esophageal cancer cell lines and primary
esophageal cancer tissues by real-time reverse transcriptase-polymerase chain reaction (qRT-PCR). Significantly overexpressed miR-196b was observed in human esophageal squamous cell carcinoma KYSE-70 and KYSE-180 compared with normal esophageal squamous cell line, HET-1A. Furthermore, overexpression of miR-196b was detected in 12 out of 14 (86%) cancer tissues compared to the matched normal tissues. Forced expression of miR-196b promotes the proliferation and invasion in esophageal cancer cell lines. TargetScanS and miRanda were used to identify the target genes of miR-196b. A list of target genes were predicted, including GATA6, ARHGAP28, E3P15 and EPJAH7. Forced expression of miR-196b resulted in significant downregulated E3P15 and EPJAH7 expression in esophageal cancer cell lines. Our present work suggested that miR-196b acts as an oncomir by regulating EPJAH7 and EPJAH7. Therefore, miR-196b could be used as a biomarker for esophageal cancer.

### #2541 Two miR-34 loci, miR-34a and miR-34b/c, have differential anti-cancer effects in lung adenocarcinomas.

Jeong Seon Kim, Young-Ho Ahn. Ewha Womans University College of Medicine, Seoul, Republic of Korea.

Three miR-34 family members (miR-34a, miR-34b, and miR-34c) are clustered on two different chromosomal loci, miR-34a and miR-34b/c, and have identical seed-sequences predicted to target the same set of genes; however, miR-34a and miR-34c have different sets of negatively correlated genes in TCGA data of human lung adenocarcinomas. Therefore, we hypothesized that miR-34a family members, which are tumor suppressive miRNAs, might have differential effects on lung tumorigenesis. To prove this, we overexpressed each miR-34 cluster, miR-34a and miR-34b/c, in murine lung cancer cells. miR-34b/c enhanced cancer cell attachment and suppressed cell growth and invasion compared with miR-34a. In the xenograft model, both miR-34a and miR-34b/c blocked lung metastasis; whereas, miR-34b/c suppressed tumor growth more than miR-34a. miR-34b/c also decreased mesenchymal markers (Cdh2 and Fn1), and increased epithelial markers (Inadl, Grb3, Ocn, and Dsp) and miR-200 family members better than miR-34a, implying that miR-34b and miR-34c partially inhibit EMT. Furthermore, miR-34b and miR-34c are more effective tumor suppressors than miR-34a.

### #2542 MS12 binds lncRNAs and promotes self-renewal and oncogenesis through MYC expression.


Backgrounds and Aims: RNA-binding protein MS12 is elevated in several cancers and is linked to poor prognosis. Here, we sought to elucidate the role of MS12 in regulating the expression of proto-oncogenes or tumor suppressor genes in hepatocellular carcinoma (HCC). Methods: We performed RIP-seq using anti-MS12 antibody in tumor-initiating stem-like cells (TICs) and subsequent validation by qPCR analysis. Results: Among the MS12-bound RNAs, MYC mRNA and long non-coding RNA (lncRNA) miR22 host gene (MIR22HG) and Marat1 were identified in RIP-qPCR analysis. Furthermore, MARAT1 binding derepresses polycomb repressor to recruit E2F1 in MYC promoter regions. It is known that the 5’-untranslated region of MYC contains an internal ribosome entry sequence (IRES) which allows IRES-dependent translation initiation along with canonical cap-dependent mechanisms. Our data show that MS12 acts as an IRES trans-acting factors for MYC mRNA translation. MS12 does not have any effect on the steady state level of MYC mRNA but significantly increases MYC protein levels, indicating that MS12 regulates MYC at the post-transcriptional level. Further, we observed that MS12 reduced the level of mature miR22 derived from MIR22HG processing. We showed that miR22 suppresses the expression of MYC through a 3' UTR dependent process. Overexpression of MS12 promotes TIC self-renewal and tumor initiation property in xenograft tumor mouse models, whereas silencing MS12 reduces this occurrence. Conclusion: We demonstrate that MS12 promotes liver tumorigenesis by maintenance of MYC expression through derepression of polycomb repressor and an IRES-dependent translation mechanism and possibly by inhibition of miR22 processing. Thus, MS12 may be a useful prognostic factor for HCC and MS12 targeted therapy and thus may be beneficial in the treatment of HCC patients.

### #2543 The novel long non-coding RNA TALNEC2 regulates tumor cell growth and the stenness and radiation response of glioma stem cells.

Wei Jiang,1 Shlomit Brodie,2 Simona Cazacu,1 Gunli Xiang,1 Laila Poisson,1 Steve Kalkanis,1 Doron Ginsburg,1 Chaya Brodie,1 1Henry Ford Hospital, Detroit, MI; 2Bar-Ilan University, Ramat Gan, Israel.

Despite advances in novel therapeutic approaches for the treatment of glioblastoma (GBM), the median survival of 12-14 months has not changed significantly. Therefore, there is an imperative need to identify molecular mechanisms that play a role in patient survival. Here, we analyzed the expression and functions of a novel lncRNA, TALNEC2 that was identified using RNA seq of E2F1-regulated lncRNAs. Besides, we utilized the knock-down of this lncRNA expression model in the GSCs, and the loss of TALNEC2 was E2F1-regulated and cell-cycle dependent. TALNEC2 was highly expressed in GBM with poor prognosis, in GBM specimens derived from short-term survivors and in glioma cells and glioma stem cells (GSCs). Silencing of TALNEC2 inhibited cell proliferation and arrested the cells in the G18 phase of the cell cycle in various cancer cell lines. In addition, silencing of TALNEC2 decreased the self-renewal and mesenchymal transformation of GSCs. In conclusion, we identified a novel E2F1-regulated lncRNA that is highly expressed in GBM and in tumors from patients of short-term survival. The expression of TALNEC2 is associated with the increased tumorigenic potential of GSCs and their resistance to radiation. We conclude that TALNEC2 is an attractive therapeutic target for the treatment of GBM.

### #2544 The lncRNA Meg3 acts as an epigenetic determinant of oncogenic signaling in multiple endocrine neoplasia type 1.

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Loss of tumor suppressors and gain of oncogenic properties is a hallmark of cancer. But the pathways leading to tumor formation are poorly understood. A good example is the multiple endocrine neoplasia type 1 (MEN1) syndrome, in which patients inherit germline mutations in the MEN1 gene, predisposing to tissue-specific loss of the encoded tumor suppressor protein menin. Consequently, tumors develop in multiple endocrine organs - the pituitary, the parathyroids, and the duodeno-pancreatic tissues, including pancreatic neuroendocrine tumors (PNETs). In-silinomas are the most common type of functioning PNETs that also occur in MEN1 patients. We investigated the molecular pathways associated with in-silinomas. We previously showed that menin loss downregulated Maternally Expressed Gene 3 (MEG3), a long non-coding RNA (lncRNA), by eliciting promoter hypermethylation along with the loss of Histone H3 lysine 4 trimethylation (H3K4me3). Gene expression microarray analyses and RT-PCR, of mouse in-silinoma MIN6 cells stably transfected with Meg3, showed a 5-fold decrease in the proto-oncogenic signaling receptor c-Met, suggesting a tumor suppressor function for Meg3. We also found MEG3 downregulation with concurrent upregulation of c-MET in PNETs. However, the molecular underpinnings of MEG3 governed c-MET repression remain elusive. To identify the mechanisms by which MEG3 inhibits oncogenic c-MET signaling to suppress tumorigenesis, we examined the effect of various in-silinoma isofoms, explored Meg3 association with the epigenetic regulatory machinery, and RNA-DNA triplex formation. We provide direct evidence for the first time that ectopically expressed MEG3 isofoms could attenuate the highly abundant c-Met transcript. MIN6 in-silinoma cells also interacted with c-MET repression of the epigenetic machinery, such as the Polycomb Repressive Complex 2 (PRC2) protein conglomerate, to regulate c-MET RNA expression. Additionally, analyses of triplex forming oligos (TFOs) in MIN6 cells revealed that tripe helix formation between Meg3 and dsDNA could potentially disrupt c-Met transcription. Combined, these data offer mechanistic insight into the dysregulation of c-MET repression in PNETs, and support the conclusion that MEG3 acts as an important determinant of oncogenic signaling in MEN1-associated endocrine tumor cells (in-silinomas). These findings warrant further investigation into the tumorigenic pathways that may result from the loss of tumor suppressor MEG3 and gain of oncogenic c-MET signaling in other MEN1-associated endocrine tumors.

### #2545 Upregulated lncRNA HNGA1, a target of miR-378a, contributes to aerobic glycolysis of head and neck squamous cell carcinoma through increasing levels of the C-C chemokine receptor type 7 (CCR7).

Yun Wang, Sun-Yat-sen University, Guangzhou, China.

Introduction: Long non-coding RNAs ( IncRNAs) have been regarded as key regulators in aerobic glycolysis of human cancer. However, the role and function of IncRNAs in Head and Neck Squamous Cell Carcinoma (HNSCC) aerobic glycolysis remain unclear. Here, we report a novel IncRNA, HNGA1, which could promote HNSCC aerobic glycolysis and malignancy by competing for miR-378a binding to regulate CCR7. Materials and methods: Microarrays were performed to explore the IncRNA/miRNA profiles in tissues samples. qRT-PCR and functional analysis were used to confirm the expres-
tion and role of IncRNA/miRNA. Bioinformatics approach and luciferase assay were used to verify the miRNA target gene and the interaction between IncRNA and miRNA. Nude mouse model was utilized to observe the effect of IncRNA/miRNA in vivo. Tissue array was performed to explore the association between IncRNA and postoperative survival. Results: 1. IncRNA HNSCC glycosylation-associated 1 (HNGA1) was up-regulated in tumor tissues, while miR-378a was down-regulated significantly. These observations were confirmed in 60 pairs of HNSCC tissues/non-tumor tissues samples and 7 cohorts of HNSCC cell lines. 2. Silencing of HNGA1 inhibited HNSCC cells proliferation and glycolysis, while overexpression of HNGA1 had the opposite effect. 3. Ectopic expression of miR-378a repressed HNSCC cells proliferation and glycolysis, whereas miR-378a inhibition resulted in opposite effect. MiR-378a could repress the CCR7 expression by binding to the 3'-UTR region of CCR7 directly. 4. There was an inverse correlation between HNGA1 and miR-378a in HNSCC specimens. Moreover, miR-378a suppressed HNGA1's expression and function by directly binding to HNGA1. In addition, HNGA1 could reverse the inhibitory effect of miR-378a on HNSCC cell growth and glycolysis, while HINGA1 could accelerate this process. 6. The clinicopathological findings suggested that the up-regulation of HNGA1 in HNSCC patients was associated with the poorly differentiated degree and more metastasis. Moreover, the above-identified mechanisms were strongly supported by the above-identified mechanisms. Combined analysis of these data highlights the pivotal role of HINGA1 in HNSCC aerobic glycolysis. More importantly, we elucidate a novel IncRNA-miRNA-mRNA regulatory network that is HINGA1-miR-378a-CCR7 axis in HNSCC malignancy and progression.

#2546 The long noncoding RNA SChLAP1 inhibits the SWI/SNF complex, revealing a therapeutic opportunity in prostate cancer. Julia Dan- court,1 Antonio Bala,1 John R. Premont,1 Benjamin Chandler,1 Dori G. compounds with metastatic and lethal disease. SChLAP1 enhances cell invasiveness in part by interacting with and abrogating genome-wide binding of the tumor-suppressive SWI/SNF complex. Approximately 20% of all cancers harbor a mutation in SWI/SNF, and several recent studies have identified therapeutic opportunities arising from SWI/SNF inactivation. We hereby investigate the relationship between SChLAP1 and SWI/SNF and its potential therapeutic implications. Methods: In silico data mining was used to correlate the SWI/SNF complex mutation frequency with SChLAP1 expression in prostate cancer samples. A systematic tilting deletion approach was then used to map important domains on the SChLAP1 gene. In vitro migration and invasion assays as well as cell proliferation assays were used to quantify phenotypes associated with over-expression of SChLAP1 and its deletion mutants. Moreover, CHIP was used to study the influence of SChLAP1 on the genomic binding of SWI/SNF. Results: In prostate cancer samples, SWI/SNF mutations correlate with low SChLAP1 expression while high SChLAP1 expression may represent a mutation-independent modality of SWI/SNF inhibition. We identify a 250 nucleotide region necessary for SChLAP1 to bind to SWI/SNF and to confer invasiveness to a prostate epithelial cell line. Further studies suggest that SChLAP1 inhibition is selective for the BRG1-containing SWI/SNF sub-complex, inferring potential genomic specificity. Moreover, we show that SChLAP1 over-expression is associated with enhanced sensitivity to BRM knockdown, in accordance with the BRG1/BRAF synthetic lethality previously described in BRG1-deficient cancers. Conclusions: Our findings indicate that SChLAP1-mediated SWI/SNF inhibition may function similarly to SWI/SNF mutation, exposing similar therapeutic opportunities.

#2547 Discovery and characterization of late-stage breast cancer estrogen receptor alpha 1 bound long non-coding RNAs. Jessica Monique Silva-Fisher,1 Amanda Y. Han,1 John R. Premont,1 Benjamin Chandler,1 Dori G. Cheng,1 Meng Chen,1 Ondrej Slaby,1 Ayaj Goel,1 Milan Radovich,2 George Calin1,1 MD Anderson Cancer Center, Houston, TX; 2Masaryk University, Czech Republic; 3Baylor University Medical Center, TX; 4Indiana University School of Medicine, TX.

The clinical significance of long noncoding RNAs (IncRNAs) in colorectal cancer (CRC) remains largely unknown. We analyzed a large panel of cancer-associated IncRNAs in 483 TCGA CRC cases, and identified that H19 correlates most significantly with overall survival and disease-free survival, independent of stage and other clinical parameters. We validated the TCGA findings with two additional CRC cohorts. As one of the “oldest” IncRNAs, diverse activities of H19 including interaction with epigenetic regulators, regulation of microRNAs as competing endogenous RNA (ceRNA), and production of miR-675 as a primary transcript have been reported. However, an unbiased study to clarify the essential mechanisms of H19 is lacking. To decipher the functional involvement of H19 in CRC, we used an integrative approach combining unbiased microarray analysis, experimental validation, and bioinformatic analysis of clinical data. With such approach, we revealed not only previously reported mechanisms such as the ceRNA and miRNA regulatory ceRNA mechanism, but also several novel mechanisms that have not been appreciated before. We discovered a mechanism of H19 on regulating BR-E2F signaling and accordingly controlling cell cycle progression. We also identified for the first time that H19 regulates CDK8, an oncoprogenic driver in CRC pathogenesis, and consequently affects β-catenin activity (Firestein et al., Nature, 2008). We further identified an interaction of H19 with macroH2A, a histone variant reported to repress CDK8 transcription (Kapoor et al., Nature, 2010). Importantly, expression correlation analysis with clinical samples strongly supported the above-identified mechanisms. Combined analysis of H19 with its targets showed strong independent prediction power for CRC survival. These data indicate the clinical relevance of BR-E2F and CDK8/β-catenin signaling in mediating H19’s oncogenic activity in CRC.

Breast cancer (BC) is the second most common newly diagnosed cancer and the second leading cause of cancer death among women in the United States. Despite the proven benefits of adjuvant endocrine therapy in women with hormone receptor positive BC, relapses still occur even after initial treatment with endocrine therapy for 5 years, referred to as late-stage relapse. Long non-coding RNAs (lncRNAs) have been shown to be dysregulated in breast cancer. Recent studies have also shown IncRNAs to function by interfacing with corresponding RNA binding proteins to play critical regulatory roles of diverse cellular processes. Therefore, we hypothesize that IncRNAs may interact with ER to promote genes promoting late-stage relapse. To address this, we aimed to identify IncRNAs bound to the estrogen receptor alpha 1 protein (ESR1) that promote late-stage relapse. We first used transcriptome sequencing to identify altered expression levels of IncRNAs between 72 primary tumors and 24 late-stage relapse breast cancer patients. We detected 1192 altered IncRNAs when comparing the metastatic to the primary samples (FDR <0.05). Next, to identify ESR1 bound IncRNAs associated with late-stage BC, we conducted RNA Immunoprecipitation Sequencing of all transcripts bound to ESR1 as compared to an IgG control in the ER+ T47D cell line. We identified 217 IncRNAs bound to ESR1 of which 50 were up-regulated in late-stage BC, termed Late-Stage Relapse BC ESR1-bound IncRNAs (LASERs). Next, we focused on characterizing the most up-regulated differentially expressed IncRNA, LASER1. We found that LASER1 has increased expression in ER+ breast cancer cell lines. Further, elevated expression of LASER1 was also detected in MCF7 long-term hormone deprived breast cancer cells, which might act as an endogenous ‘sponge’ by competing for miR-378a binding to regulate CCR7. The xenograft mouse model unveiled the suppressive effects of miR-378a on HNSCC tumor growth and glycolysis, while HINGA1 could accelerate this process. The 6. The clinicopathological findings suggested that the up-regulation of HINGA1 in HNSCC patients was associated with the poorly differentiated degree and more metastasis. Moreover, the above-identified mechanisms were strongly supported by the above-identified mechanisms. Combined analysis of these data highlights the pivotal role of HINGA1 in HNSCC aerobic glycolysis. More importantly, we elucidate a novel IncRNA-miRNA-mRNA regulatory network that is HINGA1-miR-378a-CCR7 axis in HNSCC malignancy and progression.

#2548 Oncogenic function and molecular mechanism of H19 noncoding RNA in colorectal cancer. Hui Ling1, Masahisa Ohtsuka,2 Cristiana Ivan,3 Marin Pichler,1 Meng Chen,1 Ondrej Slaby,1 Ayaj Goel,1 Milan Radovich,2 George Calin1,1 MD Anderson Cancer Center, Houston, TX; 2Masaryk University, Czech Republic; 3Baylor University Medical Center, TX; 4Indiana University School of Medicine, TX.

The clinical significance of long noncoding RNAs (IncRNAs) in colorectal cancer (CRC) remains largely unknown. We analyzed a large panel of cancer-associated IncRNAs in 483 TCGA CRC cases, and identified that H19 correlates most significantly with overall survival and disease-free survival, independent of stage and other clinical parameters. We validated the TCGA findings with two additional CRC cohorts. As one of the “oldest” IncRNAs, diverse activities of H19 including interaction with epigenetic regulators, regulation of microRNAs as competing endogenous RNA (ceRNA), and production of miR-675 as a primary transcript have been reported. However, an unbiased study to clarify the essential mechanisms of H19 is lacking. To decipher the functional involvement of H19 in CRC, we used an integrative approach combining unbiased microarray analysis, experimental validation, and bioinformatic analysis of clinical data. With such approach, we revealed not only previously reported mechanisms such as the ceRNA and miRNA regulatory ceRNA mechanism, but also several novel mechanisms that have not been appreciated before. We discovered a mechanism of H19 on regulating BR-E2F signaling and accordingly controlling cell cycle progression. We also identified for the first time that H19 regulates CDK8, an oncoprogenic driver in CRC pathogenesis, and consequently affects β-catenin activity (Firestein et al., Nature, 2008). We further identified an interaction of H19 with macroH2A, a histone variant reported to repress CDK8 transcription (Kapoor et al., Nature, 2010). Importantly, expression correlation analysis with clinical samples strongly supported the above-identified mechanisms. Combined analysis of H19 with its targets showed strong independent prediction power for CRC survival. These data indicate the clinical relevance of BR-E2F and CDK8/β-catenin signaling in mediating H19’s oncogenic activity in CRC.

#2549 A long non-coding RNA regulates the androgen receptor and mediates prostate cancer progression. Sethuramasundaram Pitchaiya, Rohit Malik, Marcin Cieslik, Yajia Zhang, Xia Jiang, Arul M. Chinnaiyan. Univ. of Michigan Comp. Cancer Citr., Ann Arbor, MI.

The pervasive expression of long non-coding RNAs (lncRNAs) and their roles in a plethora of cellular processes has revolutionized our understanding of functional genetic elements. Emerging evidence suggest that lncRNAs are promising cancer biomarkers and dysregulation of IncRNAs results in attenuated or accelerated oncogenic phenotypes. In support of these findings, recent reports from the Chinnaiyan lab suggest that lncRNAs can potentially drive and act as independent predictors of aggressive prostate cancer (PCA). Central to PCA progression is the androgen receptor alpha 1 bound long non-coding RNAs. Jessica Monique Silva-Fisher,1 Abdallah M. Eteléb,1 Torsten Nielsen,1 Charles M. Perou,1 Jorge S. Reis-Filho,1 Matthew I. Ellis,2 Elaine R. Mardis,2 Christopher A. Habel,2 Washington University School of Medicine, St. Louis, MO; 3Vancouver Hospital & Health Sciences Centre, British Columbia, Canada; 4University of North Carolina, NC; 5Memorial Sloan Kettering, NY; 6Baylor College of Medicine, TX; 7Nationwide Childrens Hospital, OH.
The biological function of long noncoding RNAs (lncRNAs) is only partially understood. We investigated the expression of the novel HOXA11 antisense (HOXA11as) lncRNA and its oncogenic role in serous ovarian cancer (SOC). HOXA11as expression was examined in 129 serous ovarian cancer tissue samples by real time RT-PCR. Clinicopathologic factors and patient survival were compared. HOXA11as expression was significantly higher in TNM stage III (N = 102). To investigate the role of HOXA11as in cell proliferation, invasion, and migration, HOXA11as expression in ovarian cancer cells was knocked down using RNA interference. HOXA11as expression in cancer tissue was 77-fold higher than that of noncancerous tissue (P < 0.05). Higher HOXA11as expression significantly correlated with histologic grade (P = 0.017) and preoperative CA125 (P = 0.048). HOXA11as overexpression in SOC cells led to increased cell proliferation, invasion, and migration. In addition, HOXA11as was associated with the expression of genes that involve in cell proliferation, invasion, and migration. Moreover, Knockdown of HOXA11as decreased the expression of vascular endothelial growth factor, matrix metalloproteinase-9 and epithelial-mesenchymal transition (EMT), which are important for cell motility and metastasis. Mechanistic investigation revealed that Notch1, Hes1 and p300 proteins could be inhibited by HOXA11as depletion. In multivariate analysis, HOXA11as was a prognostic factor of progressive disease and mortality (HR = 1.730, 2.170 and P = 0.043, 0.035, respectively). Progression-free and overall survival were significantly shorter in patients with high HOXA11as expression. These findings highlight the clinical significance of HOXA11as in predicting the prognosis of SOC patients and suggest its potential in promoting tumor aggressiveness by regulation of the Notch signaling pathway and EMT-related mechanisms.

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: MacroRNAs and Other Noncoding RNAs as Tumor Suppressors or Oncogenes

The functional significance of lncRNA as microRNA sponge is emerging. Here we provide evidence of a lncRNA-miRNA interaction network in ovarian cancer. HOXA11 antisense (HOXA11as) lncRNA and its oncogenic role in serous ovarian cancer (SOC) was investigated. HOXA11as expression was significantly higher in TNM stage III compared to stage I/IIC. Positive correlation of HOXA11as expression with clinicopathologic parameters such as histologic grade, preoperative CA125 and postoperative 5-year overall survival was observed. In multivariate analysis HOXA11as was associated with overall survival. HOXA11as expression in cancer tissue was 77-fold higher than that of noncancerous tissue. Higher HOXA11as expression significantly correlated with histologic grade and preoperative CA125. HOXA11as overexpression in SOC cells led to increased cell proliferation, invasion, and migration. In addition, HOXA11as was associated with the expression of genes that involve in cell proliferation, invasion, and migration. Moreover, Knockdown of HOXA11as decreased the expression of vascular endothelial growth factor, matrix metalloproteinase-9 and epithelial-mesenchymal transition (EMT), which are important for cell motility and metastasis. Mechanistic investigation revealed that Notch1, Hes1 and p300 proteins could be inhibited by HOXA11as depletion. In multivariate analysis, HOXA11as was a prognostic factor of progressive disease and mortality (HR = 1.730, 2.170 and P = 0.043, 0.035, respectively). Progression-free and overall survival were significantly shorter in patients with high HOXA11as expression. These findings highlight the clinical significance of HOXA11as in predicting the prognosis of SOC patients and suggest its potential in promoting tumor aggressiveness by regulation of the Notch signaling pathway and EMT-related mechanisms.
underlying mechanisms. PRR14 is commonly upregulated in lung cancers, PRR14 promotes tumorigenesis through activating the PI3-kinase/Akt/mTOR signal pathway. The regulatory effect is mediated by direct interaction with GRB2, a docking protein upstream of both PI3K and Ras. Indeed, PRR14 is commonly elevated in many cancer types including lung cancer and breast cancer, but our understanding of the underlying molecular mechanism is still very elusive. Many of these minute deletions were not contiguous but rather they alternated with segments showing oscillating copy number changes along the 3p21 region. Functional assays revealed that SETD2, PBRM1 and SMARCC1 act as tumor suppressor in MM. In summary, we found that in MM: 1) multiple minute simultaneous biallelic deletions are frequent in chromosome 3p21, where they occur as distinct events involving multiple genes; 2) in addition to BAP1, mutations of SETD2, PBRM1 and SMARCC1 are frequent in MM; 3) our results suggest that high-density aCGH combined with tNGS provide a more precise estimate of the frequency and types of genes inactivated in human cancer, than approaches based exclusively on NGS strategy.


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PRR14, a component of nuclear lamina, has recently been identified as a novel oncogene in lung cancer. Frequently upregulated in lung cancers, PRR14 promotes tumorigenesis through activating the PI3-kinase/Akt/mTOR signal pathway. The regulatory effect is mediated by direct interaction with GRB2, a docking protein upstream of both PI3K and Ras. Indeed, PRR14 is commonly elevated in many cancer types including lung cancer and breast cancer, but our understanding of the underlying molecular mechanism is still very elusive. ZC3H8 is an oncogene with substantial effects on the cancer cell phenotype. These effects are hypothesized to be associated with ZC3H8 integrity is key to maintainance of PML bodies. Other studies have identified ZC3H8 as a component of the LEC (little elongation complex), and we found that another identified component, ICE2 (Narg) also localizes to PML bodies. The ZC3H8 gene encodes a protein with three zinc finger motifs in the C-terminal region, suggesting a role as an RNA binding protein, whose specific function remains unclear. ZC3H8 protein localizes to both PML bodies and Cajal bodies within the nucleus, as confocal microscopy demonstrates colocalization of ZC3H8 with both PML and Cajal bodies. ZC3H8 has a cassease kinase 2 phosphorylation site in the N-terminal region, and treatment with a cassease kinase 2 inhibitor causes the numerous PML bodies and their associated ZC3H8 to coalesce to a few larger bodies. Removal of the inhibitor restores PML bodies to their original state. We constructed a mutant of ZC3H8 that removed the predicted phosphorylation site by replacing the serine with an alanine residue. Cells transfected with this mutant had reduced numbers of PML bodies, similar to cells treated with the CK2 inhibitor. In contrast, a mutant constructed with a glutamic acid in place of the phosphorylatable serine was predicted to behave as a constitutive mutant. This mutant did not exhibit differences from wild type localization. These experiments suggest that ZC3H8 integrity is key to maintainance of PML bodies. Perhaps this is why ZC3H8 displays the altered expression pattern and the potential protective role of progesterone, we discovered that PR is downregulated in multiple tumors, including breast, endometrial, ovarian, cervical, pancreatic and lung cancers, and loss of PR was consistently associated with a poorer prognosis based on analysis of the Oncomine database. Similarly, PR expression was low in multiple ovarian, pancreatic and lung cancer cells. Mechanistic studies revealed that PR downregulation was mediated by the polycomb repressor complex and DNA methylation, though the precise mechanism differed among cell lines and cancer types. Treatment with epigenetic modulators, which are FDA-approved for multiple myeloma, restored functional PR expression at both the mRNA and protein level and promoted marked cell death through induction of apoptosis. Moreover, overexpression of PR or treatment with epigenetic modulators in combination with progesterone enhanced this effect, indicating a key protective role for progesterone signaling through PR. Conclusion: These data suggest loss of PR may be a general theme for tumorigenesis in transformed cells. We also observed that expression was low in multiple ovarian, pancreatic and lung tumors, and that overexpression of PR or treatment with epigenetic modulators, which are FDA-approved for multiple myeloma, restored functional PR expression at both the mRNA and protein level and promoted marked cell death through induction of apoptosis. Moreover, overexpression of PR or treatment with epigenetic modulators in combination with progesterone enhanced this effect, indicating a key protective role for progesterone signaling through PR.

#2555 ZC3H8 associates with PML bodies and influences aggressive tumor cell behavior. John A. Schmidt, Emily Duffner, Gerard Walker, Keith G. Danielson, Janice E. Knepper. Villanova Univ., Villanova, PA.

The ZC3H8 gene encodes a protein with three zinc finger motifs in the C-terminal region, suggesting a role as an RNA binding protein, whose specific function remains unclear. ZC3H8 protein localizes to both PML bodies and Cajal bodies within the nucleus, as confocal microscopy demonstrates colocalization of ZC3H8 with both PML and Cajal bodies. ZC3H8 has a cassease kinase 2 phosphorylation site in the N-terminal region, and treatment with a cassease kinase 2 inhibitor causes the numerous PML bodies and their associated ZC3H8 to coalesce to a few larger bodies. Removal of the inhibitor restores PML bodies to their original state. We constructed a mutant of ZC3H8 that removed the predicted phosphorylation site by replacing the serine with an alanine residue. Cells transfected with this mutant had reduced numbers of PML bodies, similar to cells treated with the CK2 inhibitor. In contrast, a mutant constructed with a glutamic acid in place of the phosphorylatable serine was predicted to behave as a constitutive mutant. This mutant did not exhibit differences from wild type localization. These experiments suggest that ZC3H8 integrity is key to maintainance of PML bodies. Other studies have identified ZC3H8 as a component of the LEC (little elongation complex), and we found that another identified component, ICE2 (Narg) also localizes to PML bodies. The ZC3H8 gene is overexpressed in a number of human and mouse breast cancer cell lines, and elevated mRNA levels are associated with a poorer prognosis for women with breast cancer. We have used RNA silencing to decrease levels of expression in two independent mouse mammary tumor cell lines. Cells with lower ZC3H8 expression have dramatic phenotypic changes, showing decreased rates of proliferation, slower migration in wound healing assays, formation of fewer and smaller colonies in soft agar assays, and decreased ability to invade through a basement membrane. Further experiments with ZC3H8-decreased cells are needed to determine if increased expression of aCGH. Many of these minute deletions were not contiguous but rather they alternated with segments showing oscillating copy number changes along the 3p21 region. Functional assays revealed that SETD2, PBRM1 and SMARCC1 act as tumor suppressor in MM. In summary, we found that in MM: 1) multiple minute simultaneous biallelic deletions are frequent in chromosome 3p21, where they occur as distinct events involving multiple genes; 2) in addition to BAP1, mutations of SETD2, PBRM1 and SMARCC1 are frequent in MM; 3) our results suggest that high-density aCGH combined with tNGS provide a more precise estimate of the frequency and types of genes inactivated in human cancer, than approaches based exclusively on NGS strategy.

#2556 Loss of progesterone receptor through epigenetic regulation is associated with poor prognosis in multiple solid tumors. Yiyan Li, Tamar Kavlashvili, Cheng Huang, Yuping Zhang, Xiangbing Meng, Kristina Thiel, Kimberly Leslie, Shuiey Jhang. Univ. of Iowa, Iowa City, IA.

Introduction: As a tumor suppressor in the endometrium, progesterone and its synthesized analogs have a long history as a treatment for endometrial cancer. Loss of progesterone receptor (PR) in endometrial cancer leads to therapeutic failure, and our group has identified several mechanisms underlying PR loss, most notably epigenetic silencing of PR transcription. Recently, a Finnish group demonstrated that progesterin therapy in premenopausal women is associated with a lower incidence of not only endometrial cancer, but also ovarian, pancreatic and lung cancers. This unexpected protective function of progesterin in organs outside of the reproductive system led us to hypothesize that tumor progression effects in endometrial, ovarian, pancreatic and lung cancer is occurs due to the loss of progesterone’s protective effects. Methods and results: Supporting a potential protective role of progesterone, we discovered that PR is downregulated in multiple tumors, including breast, endometrial, ovarian, cervical, pancreatic and lung cancers, and loss of PR was consistently associated with a poor prognosis based on analysis of the Oncomine database. Similarly, PR expression was low in multiple ovarian, pancreatic and lung cancer cells. Mechanistic studies revealed that PR downregulation was mediated by the polycomb-repressor complex and DNA methylation, though the precise mechanism differed among cell lines and cancer types. Treatment with epigenetic modulators, which are FDA-approved for multiple myeloma, restored functional PR expression at both the mRNA and protein level and promoted marked cell death through induction of apoptosis. Moreover, overexpression of PR or treatment with epigenetic modulators in combination with progesterone enhanced this effect, indicating a key protective role for progesterone signaling through PR.

Conclusion: These data suggest loss of PR may be a general theme for tumorigenesis in transformed cells. We also observed that expression was low in multiple ovarian, pancreatic and lung tumors, and that overexpression of PR or treatment with epigenetic modulators, which are FDA-approved for multiple myeloma, restored functional PR expression at both the mRNA and protein level and promoted marked cell death through induction of apoptosis. Moreover, overexpression of PR or treatment with epigenetic modulators in combination with progesterone enhanced this effect, indicating a key protective role for progesterone signaling through PR.

#2557 Role of microRNAs in breast cancer. Hesham Abbas El-Mahdy,1 Ossama Abdelmotala Mansour,1 Mohamed Mohamed Badr,1 Reham Aly Elshimy2. 1Faculty of pharmacy, Cairo, Egypt; 2National Cancer Institute, Cairo, Egypt.

Background: Breast cancer (BC) is one of the most common cancers in women around the world and the second leading cause of death worldwide. MicroRNAs (miRNAs) expression participates in breast cancer. Objectives: The purpose of this study is to investigate the expression of miR-133a and miR-155 in breast cancer. Methods and results: Their correlation with tumor suppressor protein (p53) carcinobryonic antigen (CEA) and cancer antigen-15-3 (CA-15-3) concentrations in serum of breast cancer patients and also study their correlations with clinicopathological features. Material and methods: In this study the expression of miR-133a and miR-155 in serum were measured using quantitative real-time
polymerase chain reaction (qRT-PCR), P53 concentration was measured by enzyme-linked immunosorbent assays (ELISA), CEA and CA-15.3 concentration were measured using ARCHITECT immunosay in women with breast cancer (n=60) and controls (n = 80). Results: In this study miRNA-155 was significantly overexpressed (P<0.001) while miR-133a had significant down expression (P<0.001) in the serum of breast cancer patients compared to control serum. P53 had no significant correlations with any of the studied miRNAs. Carcinoembryonic antigen and CA-15.3 have significant higher concentration in the serum of breast cancer patients compared to control serum. A significant association was observed between miR-133a with tumor grade (P<0.05) and miR-155 with lymph node involvement (P<0.05). A significant correlation between P53 and miRNA-122 (P<0.05). Note: miRNA-122 results were low between miR-133a and P53. CEA, CA-15.3. By using receiver operating characteristic (ROC) curve, the two miRNAs showed higher sensitivity, specificity, positive predictive value and negative predictive value than routine markers. Our Conclusion: These miRNAs have a significant signature in the pathogenesis of breast cancer and can be used as non-invasive potential biomarkers for breast cancer detection.


Recent comprehensive breast cancer studies examining mutations and genomic alterations have determined that deregulation of MYC and the PI3K pathway occur frequently during breast cancer progression and may be useful targets for therapy. A recent large essay effort to develop PIK3CA and mTOR inhibitors (PIK3CA and mTOR inhibitors as PAM inhibitors) for clinical use, however clinical trial data demonstrates that many patients treated with PAM inhibitors develop resistant disease. An alternative strategy would be to target MYC, though a lack of effective and specific inhibitors makes this difficult. To identify the core vulnerabilities in these cancers we developed an in vivo xenograft model of triple-negative breast cancer driven by deregulated PI3K signaling and MYC. We hypothesized that determining how these pathways cooperate to transform normal human breast cells into breast carcinomas will reveal a tumor progression signature and highlight new therapeutic opportunities. We developed our model using the spontaneously immortalized, basal, triple-negative MCF10A cell line. By expressing the hotspot PIK3CA/H1047R protein alone in MCF10A cells (MCF10A.H) in addition to MYC (MCF10A.HM), we can model normal early breast cancer and invasive ductal carcinoma respectively. This is the first in vivo human model of breast cancer dependent on MYC for transformation. When injected into female NOD-SCID mice, MCF10A.H cells form organized acini embedded in extracellular matrix. MCF10A.H ducts form with hollow lumens and a single layer of myoepithelial cells, recapitulating normal human breast histology. Alternatively, MCF10A.HM cells grow as high-grade carcinomas indicative of invasive disease. MCF10A.H benign growths and MCF10A.HM tumors remain basal-like and triple-negative by immunohistochemistry. Importantly, MCF10A.HM tumors are sensitive to MYC repression and therefore may be a suitable model to evaluate direct and indirect anti-MYC therapies. Having relevant human xenograft samples representing both normal and IDC tissue, we performed RNA-seq to identify a MYC-signature driving breast cancer transformation. Our current work will involve targeting the resulting MYC-driven pathways identified by RNA-seq to therapeutically target MYC in breast cancer.

#2559 FoxF1 is a potential oncogene in prostate cancer. Carolin Bossmann, Constanze Merz, Yuri Tolkach, Jessica Carlsson, Sven Perner, Ove Andrén, Glen Kristiansen, Michael Nowak. University Hospital Bonn, Bonn, Germany; University Hospital of Ourebro, Ourebro, Sweden; University Medical Center Schleswig-Holstein, Lübeck, Germany.

Background: FoxF1 belongs to the family of forkhead box transcription factors. Many forkhead box proteins are linked to cancer development and progression. However, the regulation and exact function of FoxF1 remains unclear. The aim of this study was to elucidate how the transcriptional activity of FoxF1 is controlled and to assess the role of FoxF1 within prostate cancer. Design: For assessment of FoxF1 functions tumor cell lines were stably transfected with wild type FoxF1 or mutant FoxF1 constructs lacking putative phosphorylation sites. Protein expression and localization of FoxF1 were analyzed by immunoprecipitation and Western Blot. Using tissue microarrays of prostate cancer cohorts we analyzed the protein expression and intracellular localization of FoxF1 in tumors compared to benign tissue. Results: Primary tumors and distant metastases exhibited a significantly higher FoxF1 expression compared to benign prostate tissue. In tumors and metastases nuclear localized FoxF1 was more abundant compared to benign tissue. Transfection of tumor cell lines with FoxF1 followed by immunoprecipitation and Western Blot showed that nuclear FoxF1 but not cytoplasmic FoxF1 is phosphorylated in tyrosine residues, conceivably tyrosine-39, as suggested by literature data. We further transfected tumor cell lines with different amino acid exchange mutants of putative phosphorylation sites and analyzed the transcription of the known FoxF1 target gene Pcam-1, revealing a complex pattern of regulation, dependent on the site of phosphorylation. Overexpression of FoxF1 in tumor cell lines resulted in epithelial-mesenchymal transition in vivo xenografts. FoxF1 is phosphorylated in tyrosine residues, conceivably tyrosine-39, as suggested by literature data. We further transfected tumor cell lines with different amino acid exchange mutants of putative phosphorylation sites and analyzed the transcription of the known FoxF1 target gene Pcam-1, revealing a complex pattern of regulation. Conclusion: In summary, our results point to a role of FoxF1 as a potential oncogene in prostate cancer whose nuclear localization and activity is regulated by phosphorylation.

#2560 Identification and characterization of EWS-FLI1 binding partners in Ewing sarcoma cell lines. Matthew L. Rotondi, Peter J. Houghton. Greehey Children’s Cancer Research Institute, San Antonio, TX.

Ewing sarcoma (ES) is characterized by a reciprocal translocation t(11;22) that results in a fusion of the EWSR1 and FLI1 genes (EWS-FLI1). The objective of this study is to identify protein binding partners of the purportedly undruggable chimeric transcription factor EWS-FLI1 to serve as alternative pharmacological targets for potential ES-selective drug therapies. The literature identifies the orphan nuclear receptor DAX1 (encoded by NR0B1) as a binding partner of EWS-FLI1 the expression of which is restricted outside ES, thereby providing a target for a potential ES selective drug. Yeast two-hybrid (Y2H) screening using EWS-FLI1 as the bait was employed to identify other potential targets. The importance of the binding partners identified by the Y2H screen was initially assessed by a series of proliferation assays involving the ES cell lines EW8, ES7 and ES6 as well as rhabdomyosarcoma cell line Rh30 (Non-Ewing control). Prior to measuring proliferation, cells were transfected a siRNA designed to target the putative binding partner of EWS-FLI1. These cells were then grown alongside cells transfected with a control siRNA over a period of 96 hr. The change in cell confluence in the wells was measured by a live-cell real-time measurement (Incucyte). Gene knockdown was confirmed by RT-PCR and western blot analysis. The proliferation assays showed that the knockdown of DAX1 produces a notable reduction in cell proliferation compared to siRNA control proliferation in all three of the Ewing cell lines (10% to 25%). No significant DAX1 siRNA based inhibition of cell proliferation was detected in the Rh30 non-Ewing control cell line. Three of the proteins identified by the Y2H screen showed significant reductions in ES cell proliferation. Methyl-CpG-Binding Domain Protein 1 (MBD1) knockdown caused the proliferation of the ES cell lines to levels at 60% to 80% within 2 to 4 days. Proportion of MBD1 siRNA and control siRNA transfected Rh30 cells was similar for the full 4 days of the assay. Mannosidase-α-class 2B-member 2 (MANZ2B) knockdown caused the proliferation of the ES and Rh30 cell lines to levels at 25% to 65% within 1 to 2 days. Mixed-Lineage Leukemia Protein 3 (MLL3) knockdown was shown to decrease proliferation of ES cell to levels of the control by 50% to 85% within 2 days. MLL3 knockdown did not inhibit the proliferation of Rh30 cells when compared to siRNA control cells. In contrast, ES6 cell proliferation was also not inhibited by MLL3 knockdown when compared to their siRNA control cells. The knockdowns of DAX1, MLL3 and MBD1 suggest that these EWS-FLI1 binding partners would likely provide potential targets for ES drug development. The effect of knocking down Y2H-identified EWS-FLI1 interactors on colony formation, migration and tumorigenicity is ongoing.

#2561 Wip1 constrains p53 activity during embryogenesis to permit normal neuronal development. Sharlyn J. Mazur, Yantene Ge, Aamir Akbarali, Charles Halsey, R Mark Simpson, Ettore Appella. National Cancer Institute, Bethesda, MD.

The oncoproteins MDM2 and Wip1 (PMM1D) each negatively regulate tumor suppressor p53 activity and overexpression of either promotes tumorigenesis. Both MDM2 and PMM1D genes are characterized by induced by p53 following exposure to DNA damage and both negatively regulate p53, but by different mechanisms. In mice, homozygous deletion of Mdm2 is embryonically lethal (E5.5-6.5) resulting from excess p53-dependent apoptosis, whereas Pmm1d−/− embryos exhibit partial mid-gestation lethality. To investigate the role of p53 in the partial lethality of Pmm1d−/− embryos, we generated an enogenous Ppm1d promoter β-galactosidase reporter allele, Ppm1d+/−;LacZ, and a new Ppm1d knockout allele, Ppm1d−/−. We also generated Tp53−/− Ppm1d+/−;LacZ double knockout embryos and mice. From Ppm1d+/−;LacZ × Ppm1d−/− crosses, 652 Proceedings of the American Association for Cancer Research • Volume 58 • April 2017
proximately 40% of Pmm1Δ3/Δ3 embryos die between E10.5 and E12.5 and an additional 40% die shortly after birth, leading to the survival of only approximately 20% of Pmm1Δ3/Δ3 pups to adulthood. Sections of Pmm1Δ3/Δ3 embryos show dispersed LacZ staining activity with regions of higher activity, especially in specific regions of developing neural tissues. The developing neural tissues of Pmm1Δ3/Δ3 embryos are characterized by distorted cellular architecture, cell dropout, and dark, pyknotic nuclei. Although the frequency of apoptotic cells was nearly three-fold higher in Pmm1Δ3/Δ3 compared with Pmm1Δ+/Δ embryos (p < 0.005), the number of apoptotic cells were less than 1%, even in the knockout. Interestingly, the numbers of pH3 positive cells (M phase) in ventricular and sub-ventricular regions of neural tissue were reduced in E10.5 (p < 0.01) and sharply reduced in E11.5 (p < 0.005) Pmm1Δ3/Δ3 embryos compared with Pmm1Δ+/Δ embryos. Moreover, the number of γ-H2AX foci per cell observed in Pmm1Δ3/Δ3 sections were approximately three-fold higher than in Pmm1Δ/Δ sections (p < 0.002). The specific phenotypes of excess mid-gestation embryonic lethality, excess neuronal apoptosis, and deficient ventricular and sub-ventricular pH3 cell numbers, but not excess in γ-H2AX foci numbers, were rescued by loss of p53 in Trp53−/− Pmm1Δ3/Δ3 embryos. These results support a specific function of Wip1 in regulating p53 activity to ensure normal development of the brain and spinal cord during embryogenesis.

Molecular and Cellular Biology / Genetics: Nuclear Oncogenes and Tumor Suppressors

#2562 Stromal antigen 1 (SA1) as a potential pro-neoplastic factor in non-small-cell lung cancer (NSCLC). Michelle Zhang, Mart Dela Cruz, Navneet Momi, Sanjib Chowdhury, Hemant Roy, Adam Lerner. Boston Medical Center, Boston, MA

Introduction: SA1, encoded by the STAG1 gene, is a subunit of the higher order chromatin remodeler cohesin. SA1 deficiency has been implicated as a driver of aneuploidy and tumorigenesis (Remesiero et al, EMBIO J 2012). Furthermore, our lab has recently shown that SA1 functions as a tumor suppressor protein in early colon cancer (Wali et al, Cancer Prev Res 2016). The aim of this project was to investigate the role of SA1 in NSCLC. Combining data from The Cancer Genome Atlas (TCGA), human lung tissue, and in vitro gene knockdown, we have surprisingly identified SA1 as a potential pro-neoplastic factor in NSCLC.

Method: Data was extracted from TCGA and plotted to characterize STAG1 gene alteration in NSCLC. Immunohistochemistry (IHC) was performed on human lung tissue microarray assay (TMA) to assess SA1 protein expression. Transient knockdowns were performed on A549 human lung adenocarcinoma cells using STAG1 siRNA (Dharmacon) with an incubation time of 48 hours. Following this, quantitative real-time polymerase chain reaction (qPCR, Life Technologies) and WST-1 cell proliferation assays (Promega) were performed as per protocol. Results: STAG1 alterations occur in about 3% of lung adenocarcinoma cells and 17% of lung squamous cell carcinoma. Of these alterations, the majority were amplifications. Furthermore, although not reaching statistical significance, there was a trend towards decreased survival with STAG1 gene alteration. Of the alterations, 11.6 months vs. 46 months. IHC demonstrated a 1.7-fold increase in SA1 protein expression in NSCLC when compared with non-malignant lung tissue (p<0.001). Transfected A549 cells showed a 78% decrease in SA1 RNA expression (qPCR, p=0.0002) and a 50% decrease in cell proliferation (WST-1, p=0.0001). Proliferating cell nuclear antigen (PCNA), a marker of cell proliferation, was decreased by 37% in transfected cells (p<0.001). Conclusions: Using TCGA data, we found that STAG1 alterations occur with some frequency in NSCLC and that many of these alterations are amplifications of the gene. Furthermore, there is a trend towards decreased survival with a STAG1 gene alteration. Our IHC data shows robust over-expression of SA1 in NSCLC when compared with non-malignant lung tissue. Our qPCR data showed a profound decrease in SA1 mRNA expression following transfection; accordingly, our WST-1 proliferation data suggests that knockdown of the STAG1 gene significantly decreases proliferation of A549 lung adenocarcinoma cells. Decreased PCNA confirms a true anti-proliferative effect, rather than a pro-apoptotic effect. This novel data suggests a link between SA1 and NSCLC, of which there are no prior published accounts. Even more compelling is the implication that in NSCLC, SA1 may be pro-neoplastic rather than anti-neoplastic, which introduces a new potential target for future gene therapy.

#2563 LSD1 maintains differentiation and survival of mammary luminal cells by suppressing invasion of luminal breast cancer cells via GATA3. Xin Hu, Dongxi Xiang, Luwei Tao, Yuexing Xie, Yuxin Jin, Yidi Guo, Luca Pinello, Guo-Cheng Yuan, Zhe Li.

LSD1 (also known as KDM1A) is a histone demethylase that has been shown to play both oncogenic and tumor suppressor roles in breast cancer. However, the exact context under which it functions as a tumor suppressor or oncoprotein remains largely elusive. To address this, we characterized its role in normal mammary epithelial cells (MECs) and found MEC-specific ablation of Lsd1 largely phenocopied that of Gata3, which encodes a master regulatory transcription factor for luminal MECS. In the luminal lineage, LSD1-loss led to reduction in both mature ductal and alveolar luminal subpopulations. In human luminal breast cancer cells, we found LSD1 interacts with GATA3 and their common transcriptional target genes are highly related to breast cancer. In particular, our data suggest that LSD1, by silencing GATA3, might be recruited by GATA3 to the GATA3 promoter region, to positively regulate GATA3 expression via demethylation of H3K9me2. Knockdown of LSD1 led to increased invasion of luminal breast cancer cells, in part via down-regulation of GATA3 and multiple cell junction genes (e.g., CDH1, VCL, CTNNAI), as well as upregulation of mammalian stem/progenitor cell genes (e.g., CDK6, WNT2). Moreover, LSD1 knockdown led to down-regulation of PTEN in luminal breast cancer cells, in part via downregulation of RBP4 (encoding a Histone-binding protein) and upregulation of CDKN1A (encoding p21). Collectively, our data suggest that LSD1 is required for maintaining luminal MEC differentiation and survival and for suppressing luminal breast cancer cell invasion, in part via GATA3. As inhibitors of LSD1 are currently under clinical trials (e.g., in leukemia), our data suggest that although treatment of luminal breast cancer by the LSD1 inhibitor may lead to reduced tumor cell proliferation (and thus tumor shrinkage), the treatment may also lead to increased invasion of surviving breast cancer cells. Thus, our study is expected to have important implications for developing better epigenetic therapy in an individualized, context-dependent fashion.
evaluate the potential regulation of ERα on ZBTB7A, ERα was silenced and ZBTB7A mRNA and protein expression were determined. ERα silencing had no effect on ZBTB7A mRNA levels, but led to decreased ZBTB7A protein expression. These effects on ZBTB7A protein expression were attenuated by the presence of the proteasomal inhibitor MG132. Together, these data suggest a model in which ZBTB7A promotes ERα expression at the transcript level while ERα stabilizes ZBTB7A post-transcriptionally conferring a positive feedback loop and sensitivity to estradiol and endocrine therapies. ZBTB7A may contribute to the transcriptional program maintaining a hormone and endocrine response therapy responsive phenotype in breast cancer.

#2567 CYP24A1-induced vitamin D insufficiency promotes breast cancer growth.

Vitamin D plays a critical role in the maintenance of tissue homeostasis by regulating the expression of genes affecting cell proliferation, differentiation, and apoptosis. The vitamin D 24-hydroxylase CYP24A1 functions in vitamin D target tissues to degrade the hormonal form of vitamin D. Existing knowledge regarding dysregulated CYP24A1 expression supports its candidacy as a putative oncogene. In our presentation, we demonstrate that the suppression of constitutive CYP24A1 expression by CYP24A1-specific shRNA conferred target cells with increased susceptibility to apoptosis and consequently inhibited anchorage-independent growth in breast carcinoma cells. In addition, suppression of CYP24A1 expression following knockdown of CYP24A1 revealed no evidence of CYP24A1 expression in breast carcinoma cells, which suggests that CYP24A1 expression is required for breast cancer cell growth. These findings suggest that CYP24A1 expression promotes breast cancer growth.

#2568 Mutant p53 regulates HSP70 expression and nuclear localization.
Kishore Polireddy, Kanchan Singh, Melissa Pruski, Wasin Dar, John S. Bynon. Jennifer M. Bailey. University of Texas Health Science Center, Houston, TX.

Pancreatic cancer is the 3rd leading cause of cancer-related deaths in the US with a median survival of less than one year. No effective therapies or early detection tests are available for these patients. Scientists have been studying cellular origins of pancreatic cancer to aid in the development of novel treatments and biomarkers. Several studies have demonstrated that acinar cells have a high propensity to undergo acinar to ductal metaplasia and form precursor lesions of pancreatic ductal adenocarcinoma. We have recently shown that simultaneous expression of Kras and mutant TP53 can generate invasive ductal adenocarcinoma. We show that HPNE-KrasG12D cells, as cells expressing either vector control or TP53R273H have different mechanisms of transforming ductal cells. In order to understand the role of mutant TP53 in transforming pancreatic ductal cells into invasive ductal adenocarcinoma, we used a lentiviral system to express mutant TP53R273H and TP53R273H, one of the most frequently mutated TP53 alleles in pancreatic cancer patients, in immortalized pancreatic ductal epithelial cells carrying a Kras mutation (HPNE-KrasG12D). Transient blue dye exclusion assay and Spheroid formation assays were used to study cellular proliferation and cancer stem cell (CSC) populations. mutant TP53 over expression enhanced CSC populations without altering cellular proliferation in HPNE-KrasG12D cells. Reverse phase protein array (RPPA) was carried out to detect gene expression changes in HPNE-KrasG12D cells upon mutant TP53 over expression. RPPA assay results suggested that TP53R273H uniquely induced HSP70 expression in HPNE-KrasG12D cells, as cells expressing either vector control or TP53R273H failed to do so. HSP70 expression was further validated by transiently overexpressing TP53R273H and TP53R273H. Surprisingly, TP53R273H specifically promoted nuclear localization of HSP70 without altering the expression of a recently identified HSP70 nuclear transport, Hikeshi. Future studies will determine 1) whether HSP70 is required for p53-mediated stemness in HPNE-KrasG12D cells and 2) the function of nuclear HSP70. In summary, overexpression of mutant p53 enhanced cancer stem cell properties of HPNE-KrasG12D cells, through upregulation HSP70. The exact mechanism behind HSP70 nuclear localization and increased cancer stem cell properties is being more rigorously explored.

#2569 BCL6 promotes glioma and serves as a novel therapeutic target.
Liang Xu, YI Ye CHEN, 1 Marina Dutra-Clarke, 2 Anand Mayakonda, 3 Masaharu Hazawa, 2 Steve E. Vinsonof, 2 Ngan Doan, 2 Jonathan W. Said, 2 William H. Yong, 3 Henry Yang, 1 Ling-Wen Ding, 1 Yan-Yi Jiang, 3 Jeffrey W. Tyner, 2 Jennifer Bailey. 1 Virginia Commonwealth University, Richmond, VA; 2University of Virginia, Charlottesville, VA.

The acetyltransferase CBP, in conjunction with the E3 ubiquitin ligase enzyme, MDM2, maintains physiological levels of the tumor suppressor protein p53 in the absence of cellular stress, via a cytostatic, but not nuclear, p53-directed E4 polyubiquitin activity. CBP also possesses cytostatic, but not nuclear, E ubiquitin ligase, auto ubiquitination activity. To understand the mechanism by which the ubiquitin ligase activities of CBP are compartmentalized in the cell, we have employed Multidimensional Protein Identification Technology (MudPIT) to identify cytoplasmic and nuclear CBP interacting proteins. MudPIT analysis revealed that Cell Cycle and Apoptosis Regulator protein (CCAR2), also known as Deleted in Breast Cancer 1 protein (DBC1), is a novel CBP- interacting protein in both the nucleus and cytoplasm. The N-terminus of DBC1 bound to multiple CBP domains, including the putative E4 domain of CBP located at ZBTB transcription factors orchestrate gene transcription during tissue development. However, their roles in glioblastoma multiforme (GBM) remain unexplored. Here, through a functional screening of ZBTB1 genes, we identify that DBC1 is required for GBM cell proliferation and is overexpressed in GBM samples. Using a somatic transgenic mouse model, BC6 confers the proliferative selective advantage to GBM tumor cells. Mice with conditional deletion of DBC1 using Axl as a novel downstream target of BCL6. Through Axl, BCL6 enhances both MEK-ERK and S6K-RPS6 axes. Pharmacological inhibition of BCL6 activity effectively blocks GBM growth and inhibits Axl expression. Together, these findings uncover a novel glioma-promoting role of BCL6, and provide the rationale of targeting BCL6 as a potential therapeutic approach.

#2570 Induction of mild oxidative stress as a strategy for reactivation of mutant p53 proteins: KSS72, a small molecule derived from ethacrynic acid restores the biological functions of R248W/Q mutant.

p53 gene mutations in 50% of human cancers drive the emergence of oncogenic genomes and confer resistance to anticancer drugs & apoptosis. Therefore, a pharmacological reactivation of p53 mutant proteins to their wild-type forms has emerged as an intense research area. We have shown that induction of oxidative protein modifications primarily underlie the mechanistic conversion of mutant to wt or wt-like p53 forms (Eur J Med Chem 107:237, 2016; Int J Oncol 48:1426, 2016). R248W/Q DNA contact mutations in the p53 core are highly frequent and cause functional inactivation. No small molecules reactivating this p53 mutation are available. Here, we describe the design and synthesis of a small molecule KSS72 using a cell-based screening and its potency in reactivating the R248 p53 mutants. KSS72 is a non-diuretic hydrophobic analog [1-(2,3-dichloro-4-methoxyphenyl)-2-methylenebutan-1-one] of ethacrynic acid (Edecein) obtained by removing the carbohydrate side chain. KSS72 retained the ability to bind with GSH, inhibit GSTs and induce marked redox-imbalance in tumour cells. KSS72 displayed preferential cytotoxicity (3-4 fold more cell killing) against cancer cells expressing the allele specific R248W mutation (MiaPaca-2) in comparison with the wt p53 or p53-null cancer cell lines. Further, we developed isogenic cell lines expressing the R248W and R248Q mutants in the p53-null H1299 lung cancer cells and evaluated their sensitivity towards KSS72; these models again verified the greater drug efficacy. To prove the hypothesis the higher efficacy of KSS72 stems from the reactivation of mutant p53, the conformation-specific antibodies (Pab1620 for wt and Pab420 for mutant) were employed. Immunoprecipitation and immunostaining using confocal microscopy showed that KSS72 treatment of R248W or R248Q p53 cells results in a marked reciprocal loss of mutant protein and gradual increase of wt-like protein. EMSA revealed a time-dependent restoration of DNA-binding of the mutant p53, which was accompanied by a distinct upregulation of pro-apoptotic signaling pathways mediated by PUMA, Bax, p21(34) and MDM2 proteins. A strong G2/M arrest and significant apoptosis were observed. Other data showed that KSS72 restores the wt structure and function by covalently binding the p53R248W protein. Pharmacokinetics showed that KSS72 has excellent oral bioavailability with a half-life of 3.5 h. In MiaPaca-2 xenografts developed in nude mice, there was a marked anti-tumor growth suppression by KSS72 as a single drug. In conclusion, KSS72 is an attractive lead compound for further development because p53 reactivation occurred without discernible organ toxicities after oral doses and a wide therapeutic window. The redox-sensitive cytokines involved in the p53 reactivation are being identified by mass spectrometry (supported by CPRIT grants RP130266 & RP170207 to KSS).

#2571 DBC1, a novel CBP-interacting protein, promotes p53 stability by regulating CBP-dependent p53 polyubiquitination.
Olawatoinyin E. Akande, 1 Priyadarshan K. Damle, 1 Nicholas E. Sherman, 2 Steven R. Grossman 1.

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The acetyltransferase CBP, in conjunction with the E3 ubiquitin ligase enzyme, MDM2, maintains physiologic levels of the tumor suppressor protein p53 in the absence of cellular stress, via a cytostatic, but not nuclear, p53-directed E4 polyubiquitin activity. CBP also possesses cytostatic, but not nuclear, E ubiquitin ligase, auto ubiquitination activity. To understand the mechanism by which the ubiquitin ligase activities of CBP are compartmentalized in the cell, we have employed Multidimensional Protein Identification Technology (MudPIT) to identify cytoplasmic and nuclear CBP interacting proteins. MudPIT analysis revealed that Cell Cycle and Apoptosis Regulator protein (CCAR2), also known as Deleted in Breast Cancer 1 protein (DBC1), is a novel CBP- interacting protein in both the nucleus and cytoplasm. The N-terminus of DBC1 bound to multiple CBP domains, including the putative E3/E4 domain of CBP located at...
the N-terminus. Interaction between DBC1 and CBP suppressed the in vitro E3 auto ubiquitination activity of CBP. Functional studies demonstrated that DBC1 directly regulates the cellular compartmentalization of CBP E3 and p33-directed E4 ubiquitin ligase activities. Knockdown of DBC1 in U2OS cells stimulated normally absent nuclear p53 polyubiquitination, and also caused a decrease in p53-dependent apoptotic proteins bim and bax and cell cycle inhibitor p21waf/cip1 and revealed that in both MCF7 and MCF10A cells, DBC1 antagonized the p53-mediated apoptosis. Recent studies demonstrated that DBC1 loss inactivates p53 function through destabilization, we queried the TCGA database for co-occurrence of DBC1 knockdown with p53 status which showed that 33% of breast, 40% of lung, and 96% of prostate cancer cases with DBC1 alterations maintained wild-type p53 status. Therefore, by restoring DBC1 function, this work could lead to reactivation of p53 tumor suppressor activity in tumors with DBC1 alterations. We further explored the mechanism of action that PANCR activates.

#2572 The novel long non-coding RNA PANCr is an intracellular p53 activator. Yu Cao, Southern Illinois University, Springfield, IL.

Long non-coding RNAs (lncRNAs) function as oncogenes or tumor suppressors in development and progression of cancer. Chromosome 16q22.1 region is frequently deleted in breast cancer, which may contribute to breast carcinogenesis by inactivation of tumor suppressor genes. This study characterized a new lncRNA tumor suppressor named p53activating non-coding RNA (PANCR), located in this Chromosome 16q22.1 region. This PANC RNlncRNA consists of 1.5kb in length. Our data showed that PANCR was downregulated in breast cancer cell lines and tissues. In breast cancer cell lines, PANCR expression appeared reversely correlated with cell malignancy, and in breast cancer tissues, PANC was downregulated over 2 times in 31(62.0%) of 50 cases when compared to adjacent normal breast tissues. In breast cancer cells MCF7 and immortalized human mammary epithelial cells MCF10A, ectopic expression of PANCR induced marked apoptosis, suppressing cell proliferation in culture and tumor growth in xenografts, but in contrast, shRNA-mediated silencing of PANCR induced apoptosis, suppressing cell proliferation in culture and tumor growth in xenografts.


TP73 is the homologue of the master tumor suppressor TP53. It is involved in cellular responses to stress and development. The TP73 gene encodes two different proteins, Tap73 and ANP73, and maps to a region on chromosome 1p36 that is frequently deleted in neuroblastoma and other tumors, and thought to contain multiple tumor suppressor genes. However, the analysis of TP73 knockout mice yielded conflicting results with respect to tumor suppression. Worldwide efforts in sequencing the TP73 gene in patient tumor samples have not provided evidence for genetic alterations as a common cause of p73 inactivation in human cancer. The role of TP73 in tumorigenesis has remained elusive to date. In this study, we isolated two stem cell lines from normal young and old human liver tissues and determined TP73 expression in human breast cancer cell lines (MCF7 & MDA231), human hepatocellular carcinoma (HCC) cell lines (HepG2, SNU398, SNU449 & SNU475), neuroblastoma cell lines (IMR32 & SK-N-SH), normal liver stem cell, non-cancerous Li-Fraumeni Syndrome (LFS) skin fibroblasts cells and normal skin fibroblasts. Results show that Tap73 only expresses in cancer cell lines. Moreover, when HepG2 and MDA231 were treated with paclitaxel, a cytotoxic drug that targets tubulin, Tap73 expression was drastically down-regulated in HepG2 cells and abrogated in MDA231 cells at 1 to 5 μM concentrations. Furthermore, ChIP assay results demonstrated that TP73 only expresses in cancer cell lines. Moreover, when HepG2 and MDA231 were treated with paclitaxel, a cytotoxic drug that targets tubulin, Tap73 expression was drastically down-regulated in HepG2 cells and abrogated in MDA231 cells at 1 to 5 μM concentrations. Furthermore, ChIP assay results demonstrated that TP73 only expresses in cancer cell lines. Moreover, when HepG2 and MDA231 were treated with paclitaxel, a cytotoxic drug that targets tubulin, Tap73 expression was drastically down-regulated in HepG2 cells and abrogated in MDA231 cells at 1 to 5 μM concentrations. Furthermore, ChIP assay results demonstrated that TP73 only expresses in cancer cell lines. Moreover, when HepG2 and MDA231 were treated with paclitaxel, a cytotoxic drug that targets tubulin, Tap73 expression was drastically down-regulated in HepG2 cells and abrogated in MDA231 cells at 1 to 5 μM concentrations. Furthermore, ChIP assay results demonstrated that TP73 only expresses in cancer cell lines. Moreover, when HepG2 and MDA231 were treated with paclitaxel, a cytotoxic drug that targets tubulin, Tap73 expression was drastically down-regulated in HepG2 cells and abrogated in MDA231 cells at 1 to 5 μM concentrations.

#2574 The PICT-1 is required for USP7 to effectively stabilize p53 protein, and codon 389 polymorphism is a risk factor for ovarian cancer. Masafumi Yoshimoto, Aki Tokuda, Yuji Yaginuma. Kumamoto Univ. Graduate School of Health Sci., Kumamoto, Japan.

The ovarian cancers causes the deaths of more than 100,000 women per year worldwide, and it is a disease independent with multiple etiological subtypes and molecular characteristics recognized; therefore, overall survival rates have improved little in the last 30 years. We have to precisely identify genetic and molecular events which could serve as markers for early diagnosis or targets for new therapeutics for ovarian cancers. The PICT-1 (also named GLTSCR2), one of the nuclear proteins, has several tumor suppressor functions, such as the stabilization of TP53 and p73. Our studies revealed that the loss of or low expression of PICT-1 is the common alteration in many cancers, such as skin, lung, esophageal, prostate, kidney, breast, and cervix, and its association with ovarian carcinogenesis has been unknown. Therefore, we investigated its alteration, regulation mechanism, and the effect for p53 stabilization in ovarian cancer. We found that polymorphism on codon 389 (rs1849944) was a statistically significant association with the risk of ovarian cancers. Furthermore, ectopic expression of PICT-1 was required for USP7 to effectively stabilize p53 protein. Because PICT-1 was reported to bind both p53 and MDM2 that enabled to form USP7-p53-MDM2 complex, we investigated whether PICT-1 was regulated by USP7-p53-MDM2 complex. Our results suggested that this complex was the important regulator for PICT-1. In the investigation of the role of PICT-1 that plays in the ovarian carcinogenesis using immunohistochemical staining, we found that PICT-1 protein levels decreased in ovarian cancer tissues compared to normal ovaries, and there was a positive correlation between PICT-1 and USP7 protein expression. Our findings suggested that PICT-1 involved in exactly p53 regulation along with MDM2 and USP7, and PICT-1 should be considered as a potential target for therapeutic human cancers.

#2575 Concurrent mutation of CDKN2A and TP53 loci in primary keratinocytes. Bin Li, Edmund A. Mroz, James W. Rocco. The Ohio State University Comprehensive Cancer Center, Columbus, OH.

Concurrent inactivation of the p16INK4a and p53 tumor suppressors, often through mutation of CDKN2A and TP53, is frequent in early development of head and neck squamous cell carcinoma (HNSCC) and other cancers. The role of this early combined inactivation, and the oncogenic drivers that subsequently lead to tumor development, are poorly defined. To characterize the precursor role of concurrently mutated CDKN2A and TP53, we combined a number of recent advances to disrupt both loci in primary cells. First, we grew primary keratinocytes in media conditioned by irradiated J2-3T3 cells and containing an inhibitor of ROCK kinases, conditions that greatly extend their normally limited proliferative potential. Second, we transfected primary keratinocytes with plasmid vectors expressing both Cas9 and sets of guide RNAs targeting specific regions of C DKN2A and TP53. We targeted either or both of the p14ARF and p16INK4a protein products of C DKN2A for knockout (KO), together with either TP53 KO or a TP53 point mutation (R248Q) in a protein product), two major categories of genetic alterations of TP53 in HNSCC. Third, we evaluated usage of nutilin-3, an inhibitor of p53-MDM2 interaction, to try to select against cells that still contained wild-type p53. We assessed K0 efficiency by PCR of genomic DNA; the TP53 point mutation introduced a Sau3AI site into TP53. Two weeks of nutilin-3 treatment led to a 3 fold increase in p53 KO efficiency versus untreated cells (50% versus 14%), p16INK4a K0 efficiencies were enhanced 2 to 14 fold after nutilin-3 treatment, depending on the specific Cas9/guide-RNA constructs used. In cells transfected to produce p53 R248Q, 14% of PCR products amplifying the mutated region after nutilin-3 exposure showed Sau3AI digestion, whereas no Sau3AI digestion was detected in untreated cells. This result suggests that up to 14% of nutilin-3 treated keratinocytes had integrated the mutated donor oligo sequence. We similarly enrich for mutated keratinocytes having p14ARF specific or combined C DKN2A/TP53 KO along with TP53 K0 or point mutation. Combined transfection to generate concurrent CDKN2A and TP53 mutations under conditions that allowed extended proliferation in culture, followed by selection for successful TP53 mutation with nutilin-3, will facilitate identification of keratinocyte clones with bi-allelic knockout genotypes. This panel of keratinocytes with concurrent C DKN2A and TP53 mutations will help delineate the roles of these genes in early cancer development and will allow screening for the oncogenic drivers required for tumor development and metastasis, with emphasis on discovering more effective and specific druggable targets for improving treatment.

#2576 Benzy1 isothiocyanate potentiates p53 signaling and antitumor effects against breast cancer through activation of p53-LKB1 and p73-LKB1 axes. Arumugam Nagalingam, Bei Xie, Panjamurthy Kuppusamy, Nethaji Muniraj, Peter Langford, Balazs Györfi, Neeraj Saxena, Dipali Sharma. 1Johns Hopkins University, Baltimore, MD; 2University of Maryland, Baltimore, MD; 3Hungarian Academy of Sciences, Hungary.
Molecular and Cellular Biology / Genetics: Nuclear Oncogenes and Tumor Suppressors

Functional reactivation of p53 pathway, although arduous, can potentially provide a broad-based strategy for cancer therapy owing to frequent p53 inactivation in human cancer. Using a phosphoprotein-screening array, we found that Benzyl Isothiocyanate, (BITC) increases p53 phosphorylation in breast cancer cells and reveal an important role of ERK and PRAS40/MDM2 in BITC-mediated p53 activation. We show that BITC rescues and activates p53-signaling network and inhibits growth of p53-mutant cells. Mechanistically, BITC induces p73 expression in p53-mutant cells, disrupts the interaction of p73 and mutant-p53, thereby releasing p73 from sequestration and allowing it to be transcriptionally active. Furthermore, BITC-induced p53 and p73 axes converge on tumor-suppressor LKB1 which is transcriptionally upregulated by p53 and p73 in p53-wild-type and p73-mutant cells respectively, and in a feed-forward mechanism, LKB1 tethers with p53 and p73 to get recruited to p53-responsive promoters. Analyses of BITC-treated xenografts using LKB1-null corrob-orate in vitro mechanistic findings and establish LKB1 as the key node whereby BITC potentiates as well as rescues p53-pathway in p53-wild-type as well as p53-mutant cells. These data provide first in vitro and in vivo evidence of the integral role of previously unrecognized crosstalk between BITC, p53/LKB1 and p73/LKB1 axes in breast tumor growth-inhibition.

Expression of P53/PTEN/c-Myc in peripheral blood leukocytes from patients with breast cancer and its clinical significance. Guancheng Huang, Qiang Ma, Jiang Zou, Ru Sun, Lei Xu, Dongsheng Wang, Xiaoan Guo. North Sichuan Medical College, Nanchong, China. Affiliated Hospital of North Sichuan Medical College, Nanchong, China.

Breast cancer is the fifth leading cause for cancer-associated deaths among women in the US. High-grade serous ovarian carcinoma is the most common histological subtype and is characterized by frequent (>90%) mutations in the TP53 (p53) gene. Gain-of-function (GOF) p53 mutant proteins form highly stable aggregates that accumulate in ovarian cancer cells and have been shown to have oncogenic activity. Increasing evidence indicates the stabilization of GOF p53 mutants to be important for tumor progression and drug resistance. Therefore, selective degradation of the GOF p53 mutant proteins is a highly attractive therapeutic strategy to specifically target ovarian cancer cells. We identified that a small molecule named MCB-613, originally discovered as a stimulator of ste-roid receptor coactivator-3 (SRC-3), causes rapid degradation of the usually stable p53R175H/172H stabilization of macromolecular aggregates leading to catastrophic cell death in human ovarian cancer cell lines. Our results indicate this effect is independent of SRC-3 mediated gene expression, but rather by post-translational mechanisms underlying the metastatic properties of ESCC. p53 is one of the most commonly mutated genes in ESCC, and our group has shown that esoph-ageal cells lines expressing a mutation in human p53 shows signs of malignancy and increased invasion in 3D organotypic culture. A mouse model of mutant p53 (H11005) in ESCC is lacking the role of stabilizing mutant p53 in ESCC we developed a novel mouse model utilizing a genetic and carcinogenic approach. L2cre;p53R175H/172H mice were generated and treated with 4NQO in their drinking water for 16 weeks, which resulted in the development of ESCC. Compared to wildtype mice, p53R175H/172H mice and p53 mutant mice displayed similar tumorigenic properties. RNA-seq was performed on cell lines established from wildtype and p53 mouse models and revealed different gene expression profiles between wildtype, p53R175H/172H, and p53 mutant cells. p53R175H/172H cells displayed an increase in mesenchymal and decrease in epithelial marker expression, supporting the idea that they are undergoing EMT. In addition, several endocytic recycling related genes, including Rab11-fip1, Rab26, and Myo5b were downregulated in mutant and null p53 compared to wildtype cells. Further examination of the differing genetic profiles in our mouse models can provide novel insight into the role of mutant p53 in ESCC tumorigenesis and lead to the identification of new therapeutic targets.

Identifying pathways that regulate the turnover of gain-of-function p53 mutant proteins in ovarian cancer cells. Achuth Padmanabhan, Nicholas Candela,

Cancer is the leading cause of cancer-associated deaths among women worldwide. Current data showed that the morbidity of breast cancer has been rising shifting with younger age in the last decades, therefore early detection is urgently needed to improve the management of breast cancer as what has been working on many other malignancies. Dysfunction of tumor suppressor gene and/or oncogene may play vital roles in tumorigenesis and cancer progression, such as P53, PTEN and c-Myc. In this study we aim to detect the expression of P53/PTEN/c-Myc mRNA in peripheral blood leukocytes from patients with breast cancer and benign breast diseases, and investigate its correlation with the clinicopathologic characteristics, further to explore the potential clinical application significance. In this study, fluorescent quantitation Real-time Polymerase Chain Reaction (FQ-RT-PCR) was employed to detect the expression of P53/PTEN/c-Myc mRNA in peripheral blood leukocytes from 304 cases, including 105 patients with breast cancer. 107 cases of benign breast diseases and 92 healthy women as control. 2-ΔΔCT was used to calculate the relative quantitative gene expression and Mann-whitney U test was carried to distinguish the differences among the independent cohorts. The results showed that P53 and PTEN mRNA were significantly reduced in both breast cancer group and benign breast diseases group, respectively (P<0.01), moreover, the P53 mRNA in benign breast diseases was even lower than that in breast cancer (P<0.01). However, c-Myc didn’t show any obvious difference among the three groups. Further investigation the genes expression with clinicopathologic characteristics, we found that PTEN in patients with breast cancer was correlated with lymph nodes metastasis, and dysregulation of P53/PTEN was found in patients with benign breast diseases, even more obvious than that in breast cancer patients. Moreover, c-Myc mRNA might predict and/or display the early events of breast cancer carcinogenesis and lymph nodes metastasis. Our findings suggest that detection of tumor related genes in peripheral blood leukocytes would serve as a unique potential approach in early and noninvasive diagnosis of tumors, also would be very promising in clinical applications as well.


Esophageal squamous cell carcinoma (ESCC) is a highly aggressive cancer characterized by a high rate of metastasis, limited therapeutic options, and a poor prognosis. However, there is limited information regarding the molecular mechanisms underlying the metastatic properties of ESCC. p53 is one of the most commonly mutated genes in ESCC, and our group has shown that esophageal cells lines expressing a mutation in human p53 shows signs of malignancy and increased invasion in 3D organotypic culture. A mouse model of mutant p53 (H11001) in ESCC is lacking the role of stabilizing mutant p53 in ESCC we developed a novel mouse model utilizing a genetic and carcinogenic approach. L2cre;p53R175H/172H mice were generated and treated with 4NQO in their drinking water for 16 weeks, which resulted in the development of ESCC. Compared to wildtype mice, p53R175H/172H mice and p53 mutant mice displayed similar tumorigenic properties. RNA-seq was performed on cell lines established from wildtype and p53 mouse models and revealed different gene expression profiles between wildtype, p53R175H/172H, and p53 mutant cells. p53R175H/172H cells displayed an increase in mesenchymal and decrease in epithelial marker expression, supporting the idea that they are undergoing EMT. In addition, several endocytic recycling related genes, including Rab11-fip1, Rab26, and Myo5b were downregulated in mutant and null p53 compared to wildtype cells. Further examination of the differing genetic profiles in our mouse models can provide novel insight into the role of mutant p53 in ESCC tumorigenesis and lead to the identification of new therapeutic targets.

Identifying pathways that regulate the turnover of gain-of-function p53 mutant proteins in ovarian cancer cells. Achuth Padmanabhan, Nicholas Candela, Kwonok Wong, Bryan C. NikoI, David Leonard, Bert W. O’Malley, JoAnne S. Richardson. Baylor College of Medicine, Houston, TX; M D Anderson Cancer Center, Houston, TX.

Ovarian cancer is the fifth leading cause for cancer-associated deaths among women in the US. High-grade serous ovarian carcinoma is the most common histological subtype and is characterized by frequent (>90%) mutations in the TP53 (p53) gene. Gain-of-function (GOF) p53 mutant proteins form highly stable aggregates that accumulate in ovarian cancer cells and have been shown to have oncogenic activity. Increasing evidence indicates the stabilization of GOF p53 mutants to be important for tumor progression and drug resistance. Therefore, selective degradation of the GOF p53 mutant proteins is a highly attractive therapeutic strategy to specifically target ovarian cancer cells. We identified that a small molecule named MCB-613, originally discovered as a stimulator of ste-roid receptor coactivator-3 (SRC-3), causes rapid degradation of the usually stable p53R175H/172H stabilization of macromolecular aggregates leading to catastrophic cell death in human ovarian cancer cell lines. Our results indicate this effect is independent of SRC-3 mediated gene expression, but rather by post-translational mechanisms underlying the metastatic properties of ESCC. p53 is one of the most commonly mutated genes in ESCC, and our group has shown that esophageal cells lines expressing a mutation in human p53 shows signs of malignancy and increased invasion in 3D organotypic culture. A mouse model of mutant p53 (H11001) in ESCC is lacking the role of stabilizing mutant p53 in ESCC we developed a novel mouse model utilizing a genetic and carcinogenic approach. L2cre;p53R175H/172H mice were generated and treated with 4NQO in their drinking water for 16 weeks, which resulted in the development of ESCC. Compared to wildtype mice, p53R175H/172H mice and p53 mutant mice displayed similar tumorigenic properties. RNA-seq was performed on cell lines established from wildtype and p53 mouse models and revealed different gene expression profiles between wildtype, p53R175H/172H, and p53 mutant cells. p53R175H/172H cells displayed an increase in mesenchymal and decrease in epithelial marker expression, supporting the idea that they are undergoing EMT. In addition, several endocytic recycling related genes, including Rab11-fip1, Rab26, and Myo5b were downregulated in mutant and null p53 compared to wildtype cells. Further examination of the differing genetic profiles in our mouse models can provide novel insight into the role of mutant p53 in ESCC tumorigenesis and lead to the identification of new therapeutic targets.


Ceramide (Cer), a sphingolipid metabolite, has crucial functions in anti-proliferation, cell differentiation and apoptosis. Cellular Cer levels highly correlates to therapeutic efficacy of anticancer drugs, however, the hydrophobicity Cer has limits to develop it as a therapeutic agent. Here, we report that Cer-rubusoside (Cer-RUB) nanomicelles enhanced Cer bioavailability and restored p53-mediated tumor suppression. These nanomicelles significantly increased the levels of NBD-C6-Cer by more than 50-fold in serum, 40-fold in tumor and 70-fold in lung of tumor-bearing mice (1 mg/kg, i.p., 24 hr), respectively. Cer-RUB (3 µM)
significantly increased OVCAR-3 (p53 R248W) cell sensitivity to cisplatin, and the IC50 values decreased by 3-fold, even though these values were no significant changes in A2780 (wt p53) cells. Furthermore, Cer-RUB treatments (1 mg/kg, i.p. every 3 days for 24 days) significantly increased cisplatin efficacy in tumor growth-arrest in OVCAR-3 tumor-mice, and the tumor volumes reduced by 52% (IC50 values decreased by 3-fold) as compared to cisplatin alone. We found that ovarian cancer stem cells (CD44+/CD133−) decreased by 10-fold (0.85 ± 0.08% of total cells) in OVCAR-3 tumors treated with Cer-RUB combined with cisplatin, as compared to cisplatin alone, respectively. Western blotting showed that Cer-RUB treatments selectively increased the expression levels of phosphorylated p33 (Ser15), BAX and p21 in OVCAR-3 cancer cells and tumors, respectively. These studies indicate that the gain-of-function (GOF) of p53 R248W leads OVCAR-3 cell resistance to cisplatin and tumor growth. Cer-RUB can restore p53 and eliminates the GOF of p53 R248W in cancers.

#2581 VGLL4 suppresses the human breast cancer progression through the inhibition of YAP oncogenic function. Jianmin Zhang, Roswell Park Cancer Inst., Buffalo, NY.

VGLL4 is a Vestigial-like (VGLL) protein that is deregulated in various types of cancer. However, the functions of VGLL4 in breast cancer remain largely unknown. Here, we demonstrate that VGLL4 expression positively correlates with survival for breast cancer patients. The overexpression of VGLL4 in breast cancer cells reduced cell proliferation, colony formation, cell migration and invasion in vitro and inhibited tumor growth in vivo. We found that the 2nd TEAD-interacting domain (TUD2) of VGLL4 is involved in interactions between VGLL4 and the transcription factor TEAD and that the deletion of this domain completely abolished the phenotype induced by VGLL4 overexpression. Interestingly, RNAseq analysis indicated that VGLL4 overexpression not only inhibits YAP targets but also activates the TGF-β and Notch signaling pathways. Our study provides evidence of the tumor-suppressive effects of VGLL4 on breast cancer progression and suggests that VGLL4 and its TUD2 domain may become promising and efficient therapeutic targets for delaying the development of breast cancer and improving patient survival.

BIOMETRICS AND SYSTEMS BIOLOGY: Computational Tools and Databases


In this study, we tried to develop valid KIR calling algorithm from WES/WGS data. As a consequence, here we suggest KIRnome (KIR typing for whole genome and exome sequencing) which is a KIR typing method for applying sequencing data such as WES/WGS. A total of 71 sequencing data (18 WES and 53 WGS data, respectively) and matched experimentally validated KIR genotype data are used to train and validate. Before developing this method, two types of allele references (genomic and coding, here after called gSeq and cSeq) are constructed by allele sequence of IPD/KIR. KIRnome consists of two part, first part is calculating normalized depth (here after called depth) of 16 KIR genes and considering allele reference length and second is estimating KIR type based on depth. In general, we get higher normalized depth when using genomic references for WGS data and using coding references for WES data. This coincides with scheme KIRnome and the characteristics of WGS and WES data. Given 71 samples of 3 data sets, we evaluated KIR genotyping performance of KIRnome per each KIR gene. For all 16 KIR genes, KIRnome shows >98.5% accuracy. When KIRnome performance was tested only in 13 WES samples, the accuracy slightly drops to 94%. We assume this is attributable to relatively small number of the samples. In conclusion, we developed a novel and unique method named KIRnome for KIR typing from NGS data. KIRnome could determine KIR geno- type accurately from WES and WGS data respectively. We expect KIRnome would facilitate revelation of immunogenetic factors in various disease. More- over, future generation of KIR specific NGS data and improved reference sequence information of KIR would enable KIRnome to type KIR at allele level in a near future.

#2583 A model for capturing and integrating complex molecular altera- tions related to clinically relevant efficacy evidence. Cara M. Statz, Sara E. Patterson, Taofei Yin, Susan M. Mockus. The Jackson Laboratory for Genomic Medicine, Farmington, CT.

The ability to capture data relevant to different types of oncology-related variables, including clinical variables, copy number variations, and fusions in a single system is crucial to a comprehensive understanding of cancer biology. However, collectively linking these varied types of molecular alterations to capture their compound impact to clinically relevant efficacy evidence within a database can prove challenging. Thus, we have built the JAX Clinical Knowledgebase (JAX-CKB), a flexible relational da- tabase that allows curation of complex molecular profiles and provides the abil- ity to associate these profiles with documented efficacy evidence, thereby pro- viding a more detailed overview of therapeutic relevance. To demonstrate the utility of creating molecular complex signatures in relation to efficacy evidence, the JAX-CKB was queried to first determine the overall degree of efficacy evi- dence content related to complex profiles. Additionally, two specific types of complex molecular profiles were queried, which included EML4-ALK plus a missense mutation(s) and BRAF V600E plus any type of molecular alteration(s). Within the JAX-CKB, there are 1,383 unique efficacy evidence lines linked to complex molecular profiles. The complex molecular profiles consisting of EML4-ALK and one or more additional missense mutations were associated with 174 unique efficacy evidence lines. Of the 174 lines, 69 were annotated with a specific response type, while 68 were annotated with a non-specific response type. The combination of BRAF V600E with one or more molecular alterations was linked to 229 unique efficacy evidence lines. The majority of the lines, 118, were specific to a resistant response type and 83 were associated with sensitivity. Comprehensive genomic profiling of cancer patient samples can often reveal complex molecular signatures. The JAX-CKB is an inclusive knowledgebase that allows one to interpret these complex signatures and rapidly identify appropri- ate targeted therapies, which could be critical in a clinical setting.

#2584 The UCSC Xena system for cancer genomics data visualization and interpretation. Mary Goldberg, Brian Craft, Jingchun Zhu, David Haussler. UC Santa Cruz, Santa Cruz, CA.

The UCSC Xena platform (http://xena.ucsc.edu/) allows biologists and bioin- formaticians to securely analyze and visualize their private functional genomics data in the context of public genomic and clinical data. The Xena platform consists of a set of federated data hubs and the Xena browser, which integrates across hubs, providing one location to analyze and visualize all data. Our expanding public Xena Data Hubs currently hosts 1400+ data sets from more than 35 cancer types, as well as Pan-Cancer data sets. Our public data hubs serve seminal cancer genomics and functional genomics data set to the scientific community, including the latest TCGA, TARGET, ICGC, and GTEx data sets. We support most data types including somatic and germline SNPs, INDELs, large structural variants, CNV, gene-, transcript-, exon- protein-, miRNA-expression, DNA methylation, phenotypes, clinical data, subtype classifications and genomic biomarkers. Additionally, investigators’ own functional genomics data can be hosted on private hubs running on their laptop or behind the firewall. Data is integrated on the UCSC Xena Browser, allowing biologists to view and interpretation of their genomic data in the context of a large collection of cancer genomics data sets such as TCGA. The lightweight Xena data hubs are straightforward to install on Windows, Mac and Linux operating systems and loading data is easy using either our application or command line interface. This system of the browser and hubs helps researchers combine new or preliminary results from their laptops or internal servers, or even data from a new paper, securely with vetted data from the public sphere. Visualizations and analyses include dynamic Kaplan-Meier survival analysis to assess survival stratification by any information in addition to our visual spreadsheet, scatter plots and bar graphs. We seek feedback at our poster on new visualizations and functionalities.

#2585 viGEN: An open source bioinformatics pipeline for viral RNA de- tection and quantification in human tumor samples. Kritthika Bhuvaneswar, Lei Song, Yuriy Gusev. University of Maryland School of Medicine, Baltimore, MD.

We present a novel pipeline for viral RNA detection and quantification in human RNA-seq data. Our pipeline has been tested on the TCGA liver cancer cohort, can not only detect the presence of a viral species, but also provide gene level read counts for individual viral species and extract viral-variants. Introduction Approximately 20% of human cancer types are associated with viral infec- tion that is routinely detected in blood samples. However the extent and biolog- ical significance of viral presence/infection in actual tumor samples is generally unknown but could be measured using existing Human RNA-seq data from tumor samples. We have developed a bioinformatics pipeline viGEN combining...
CellMinerCDB: Enabling cross-database exploration of molecular pharmacology data and response determinant discovery in cancer cell lines.

Vinoth N. Rajapakse,1 Augustin Luna,2 Chris Sander,2 William C. Reinhold,3 Yves Pommier1
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Cancer cell line panels are widely used for evaluating drug response across diverse tissue types. A growing set of molecular profiling data is providing measurements of chemosensitivity, providing novel avenues for response determinant discovery and clinical translation. Accessing and inter-relating data from different sources is essential for evaluating such determinants, but remains challenging. To enable wider access to cell line pharmacogenomic data, we have developed CellMinerCDB (CellMiner Cross-Database, discover.nci.nih.gov/cellminercdb), a web application integrating data from several widely studied cancer cell line panels, including the NCI-60 (NIH), GDSC (Sanger/MIH), and CCLE/CTRP (Broad). All together, our database spans over 1300 distinct cell lines, 400 clinically relevant cancer drugs, 20,000 experimental compounds, and molecular profiling data, such as gene/protein expression, DNA copy, methylation, and mutational status. Cell line and tested drug overlaps allow cross-database validation of genomic and drug data, and CellMinerCDB simplifies this by transparently matching differently named entities between sources. Data exploration can be additionally restricted to particular tissue types, with individual cell lines annotated to the OncoTree ontology for consistent treatment across sources. A range of analysis tools support interactive data exploration, from 2D plots of drug response and molecular profiling features to exhaustive correlation analyses and multivariate predictive models. We illustrate the power and utility of CellMinerCDB with examples of response determinant discovery and predictive modeling for Top1 and PARP inhibitors. Beginning with established individual determinants, such as SLF11 mRNA expression, we show how both unbiased and biological knowledge network-based feature selection methods enable iterative refinement of a multivariate genomic signature of drug response. For Top1 inhibitors, additional predictive features include expression of chromatin remodeling factors and genes modulating apoptosis capacity, while complementary PARP inhibitor response determinants include PARP1 and drug efflux pump expression. Pathway and process-based gene annotations allow biological interpretation of response predictive features. CellMinerCDB also includes ongoing algorithmic work to improve the construction of multivariate predictive models using constraints from biological networks. These approaches bridge a limiting gap in existing methods, which either ignore biological knowledge altogether or are limited to exploration within known pathways and processes.

VCF2CNA: a tool for efficiently detecting copy number alteration using VCF genotype data.

Daniel K. Putnam, Xiaotu Ma, Stephen V. Rice, Yu Liu, Jinghui Zhang, Xiang Chen. St. Jude Children’s Research Hospital, Memphis, TN.

Whole genome sequencing (WGS) is increasingly used in both research and clinical settings. The Variant Call Format (VCF) specification is a widely adopted file format for genetic variation data exchange partially due to its smaller file size compared to raw WGS BAMs. Each variant in a typical VCF file contains its chromosome position, reference/alternative alleles and corresponding allele frequencies. VCF can also be used to store other biological information, such as synonymous mutations. To this end, we developed VCF2CNA (http://vc2cna.stjude.org), a web interface tool for CNA analysis from VCF files. A user of VCF2CNA uploads a VCF file via the provided web interface. The entire analysis runs remotely with an average run time of 23 minutes. Results are emailed to the user as either a downloadable link or file attachments. VCF2CNA also accepts input in the Mutation Annotation Format (MAF) and the variant file format produced by the Barabina program. We analyzed 22 TCGA glioblastoma tumor/normal pairs by Illumina technology to evaluate VCF2CNA’s performance. It achieved high consistency (average F₁-score: 0.952 ± 0.082) with CONCERTING, a tool that incorporated read-depth and SV data from raw BAMs for CNA detection. A segment-by-segment comparison between results from CONCERTING and VCF2CNA indicated that the latter was less sensitive to focal CNAs. This is expected because there is less information in the VCF input than in raw BAMs. Further analysis using samples with a “fractured genome” pattern revealed that VCF2CNA was more robust to library artifacts and produced relatively clean CNA profiles (on average 76.2-fold reduction compared to the number of segments reported by CONCERTING). Finally, we analyzed 137 pediatric neuroblastoma samples from the Children’s Cancer Group’s Complete Exome sequencing (CCGEX) project. The majority of tumors were known to be infected with both HBV and various subtypes of HCV. Once the viral genomes are detected at the genome level, we examine the differences in viral-variant level. Conclusion: Our results show that it is possible to detect viral infection in complex diseases, tumorsigenes and cancer immunology.

GenePattern Notebook: an environment for reproducible cancer research.

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As the availability of genetic and genomic data, and analysis tools from large-scale cancer initiatives continues to increase, the need has become more urgent for a software environment that supports the entire “idea to dissemination” cycle of an integrative cancer genomics analysis. Such a system would need to provide access to a large number of analysis tools without the need for programming, be sufficiently flexible to accommodate the rapidly evolving nature of computational biology as well as experienced bioinformaticians, and would provide a way for researchers to encapsulate their work into a single "executable document" including not only the analytical workflow but also the associated descriptive text, graphics, and supporting research. To address these needs, we have developed GenePattern Notebook, based on the GenePattern environment for integrative genomics and the Jupyter Notebook system. GenePattern Notebook unites the phases of in silico research – experiment design, collaborative analysis, and publication – into a single interface. GenePattern Notebook presents a familiar lab-notebook format that allows researchers to build a record of their work by creating “cells” containing text, graphics, or executable analyses. Researchers add, delete, and modify cells as the research evolves, supporting the initial research-to-publication cycle. When an analysis is ready for publication, the same document that was used in the design and analysis phases becomes a research narrative that interleaves text, graphics, data, and executable analyses, serving as the complete, reproducible, in silico methods section for a publication. GenePattern Notebook features are designed to make it easy for nonprogramming users to create and adapt notebooks. We have developed new cell types that allow users to choose analyses, specify input parameters and datasets, navigate results, send result files to new analyses, and create richly formatted text, all without the need for programming. We have released a freely available online GenePattern Notebook workspace, http://notebook.genepattern.org, where researchers can develop and publish notebook documents. We have provided a collection of template notebooks that walk users through various macroscopic analysis pipelines, and are collaborating with cancer research laboratories to create integrative cancer genomics notebooks as well. Notebook topics in development include characterization of intratumor heterogeneity from single cell RNA-Seq data, effective clinical interpretation of comprehensive genomic profiling from whole exome sequencing of a patient’s...
tumor and germ line samples, and identification of master regulators/transcription factors associated with the downstream transcriptional effects associated with the activation of an oncogene.

#2589 Identifying cancer susceptibility variants and genes using whole exome and genome sequencing: a literature review. Melissa Rotunno,1 Mindy Clyne,1 Tam Lam,1 Leah Mechanic,1 Danielle Daee,1 Stefanie Nelson,1 Elizabeth Gillanders,1 Alisa Goldstein,1 1NCI-DCCPS, Rockville, MD; 2NCI-DCEG, Rockville, MD.

The application of Next Generation Sequencing (NGS) technologies in cancer research enabled the discovery of novel somatic mutations at an unprecedented speed through efficient high-throughput profiling of DNA variation in tumor samples. In contrast, the identification through NGS studies of germline variation associated with cancer has been more difficult and with limited consensus around best practices for study design and analytical techniques. We performed a review of the 2005–2016 PubMed English literature for genome-wide germline sequencing studies that were aimed at identifying new cancer susceptibility variants or genes. We extracted and summarized the experimental and analytical designs, methods and tools implemented in the selected literature together with their degree of success and limitations in identifying cancer susceptibility variants. About 80 in-scope publications were identified and examined in depth. The most studied cancer types were colorectal, breast, blood and lung and were investigated predominantly via whole exome sequencing (primarily using Illumina HiSeq) in familial cases of Caucasian ethnicity. Only studies that used whole genome sequencing and 15 adopted a case control study design. A large fraction of the familial studies was limited to 1 or 2 families. The most common cancer types included the top few variants or genes. About 60% of the studies included replication in an independent group of cancer patients and 55% functionally validated their findings to some extent. Our work provides valuable data and insights to inform future sequencing studies of cancer genetic susceptibility, and points to the need for improving standardization and completeness in reporting of quality control metrics, study design rationale and interpretation of findings.

#2590 Analysis of APOBEC3A and APOBEC3B mutational signatures using next-generation sequencing data from cancer cell lines. Suleyman Vural, Julia Krushkal, Richard Simon. National Cancer Institute, Rockville, MD.

The APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like) gene family of cytidine deaminases includes evolutionarily conserved genes that play important roles in DNA repair and mRNA editing. Activity of at least two APOBEC family members, APOBEC3A and APOBEC3B, can lead to kateisias, a mutagenic process in cancer cells that generates clusters of closely spaced, single strand specific C→T DNA substitutions. APOBEC mutagenesis has a characteristic signature, most commonly represented by the 5’-Tp(C→TpW)-3’ sequence motif, with additional substitutions also reported. This hypermutation signature and high mRNA expression of APOBEC3A and APOBEC3B have been associated with several cancer types. Most previous studies of APOBEC signatures have examined tumor sequence data from clinical samples, for which limited or no information about drug response was available. We investigated the presence of the mutational signature and mRNA expression patterns of the APOBEC3A and APOBEC3B genes in extensively characterized cell lines, in order to identify those cell lines that carry mutations generated by kateiasis, with the aim of establishing associations between the APOBEC mutational signature, individual cancer types, and the patterns of sensitivity to anti-tumor agents. For this purpose, we analyzed whole exome sequencing (WES) data and mRNA expression of the APOBEC3A and APOBEC3B genes in two resources with extensive drug response data: the NCI-60 cell line panel, which includes 60 cancer cell lines, and the CANCER Cell Line Encyclopedia (CCLE), which provides WES, whole genome, and RNA-seq sequence information for thousands of anticancer agents, and the Cancer Cell Line Encyclopedia (CCLE), which provides WES, whole genome, and RNA-seq sequence information on hundreds of cancer cell lines and drug response data to over 200 agents. We analyzed WES data of 325 CCLE cell lines and 59 NCI-60 cell lines, with variants identified using GATK pipeline and Varscan2 software. The variants in each cell line were filtered to remove common polymorphisms in dbsNP and 1000 Genome Project databases. We searched the discovered variants for the presence of APOBEC signatures, 5’-Tp(C→TpAG)Pn-3’, 5’-Tp(C→TpAGT)P-3’, and 5’-Tp(C→TpAGTPW)-3’ in closely spaced (1000 and 10,000 bp) windows that appeared on the same DNA strand. We will discuss the use of optimal filters for detecting APOBEC mutational signatures and will present the analyses of associations between APOBEC signatures, mutation load of the tumor cell lines, APOBEC gene expression, and chemosensitivity to treatment. These results contribute to additional characterization of available cell lines by providing information about specific mutational signatures in different cancer cell types associated with identifying antitumor agents that would be appropriate for treatment of cancer cells with specific signature patterns generated by APOBEC mutagenesis.

#2591 A guide to filtering TARGET Complete Genomics germline variants. James P. Evans,1 Rajesh Patidar,2 Zalman Vaksman,1 Sivashish Sindiri,2 Douglas R. Stewart,2 Javed Khan,3 Jun S. Wei,1,5 Sharon J. Diskin1. 1Children’s Hospital of Philadelphia, Philadelphia, PA; 2National Cancer Institute, Bethesda, MD.

With the public release of TARGET’s pediatric cancer data, researchers are eager to investigate and integrate variants from both tumor and germline sequencing data, but caution should be used when interpreting TARGET variant calls from Complete Genomics data, as they unlike the Illumina based calls for which most of our databases and tools are customized. Here, we use germline samples from 55 neuroblastoma cases with both whole genome Complete Genomics variants and whole exome Illumina variants from GATK, Pindel, Platypus, and bam2mpq to investigate differences between germline variant calls from Complete Genomics and Illumina platforms. Our study of ~160,000 single nucleotide polymorphisms (SNPs) and ~19,000 insertions or deletions (indels) reveals that while SNP sets largely agree between Complete Genomics and Illumina, Complete Genomics data sets have many indels not present in Illumina calls, making it difficult to screen Complete Genomics indels against popular Illumina based variant databases like those provided by the Exome Aggregation Consortium and the National Heart, Lung, and Blood Institute Exome Sequencing Project. Further probing of Complete Genomics indels uncovered inconsistent indel formatting resulting in a loss of recurrent variants, and miscalculated read depths in earlier versions of the Complete Genomics platform. To solve these issues and make Complete Genomics variant calls comparable to Illumina calls, we introduce a Snakemake pipeline that reforms Complete Genomics variant files and creates variant features for use in decision tree or random forest classifiers trained to recognize Complete Genomics variants that will be present in Illumina variant call sets. We determine that the most important features for locating Complete Genomics variants likely to be found in Illumina germline variant call sets are the presence of reads from both directions, average variant allele fraction across the cohort, a variant call in the matched tumor, the variant fraction of reads, and read depth. Using ten fold cross validation with our decision tree, we obtain >95% recall and >98% precision for SNPs, and >99% recall and >85% precision for indels. We advocate the use of our pipeline and filtering scheme for making Complete Genomics variant calls comparable to Illumina based calls.

#2593 Accelerating pediatric brain tumor research through team science solutions. Amanda Christini,1 Angela J. Waanders,2 Joost B. Wagenaar,1 Alex S. Felmeister,2 Mariarita Santi,2 Nittin R. Wadhwani,1 Jennifer L. Mason,2 Mateusz Kopytya,2 Jena V. Lilly,2 Jeffrey W. Pennington,2 Rishi R. Lulla,3 Adam C. Resnick2. 1Blackfynn, Incorporated, Philadelphia, PA; 2The Children’s Hospital of Philadelphia, Philadelphia, PA; 3Ann & Robert H. Lurie Children’s Hospital of Chicago, Chicago, IL.

Introduction: The Children’s Brain Tumor Tissue Consortium (CBTTC), an international repository of genomic and phenotypic data, has partnered with Blackfynn, Inc., to create a cloud-based data management platform to facilitate team-science across disciplines. Background: The CBTTC and Blackfynn through the CHOP Department of Biomedical and Health Informatics (DBHI) has developed a network of informatics and data applications for researchers across the globe to work together and perform real-time analyses on existing clinical, phenotypic, and genomic data. Historically, rare disease datasets are siloed, locked in proprietary formats, segregated by data types, and hidden from the view of experts in the field. This has been a significant barrier to finding effective therapeutics for children with pediatric brain tumors. Blackfynn was founded by a group of multidisciplinary experts in neuroscience, neurology, medicine, software development, engineering, computer science and business with the goal to empower researchers to cure neurologic disease and provide solutions to these challenges. Description of Methods: The CBTTC and Blackfynn teamed up to provide a cloud-based, team-focused data management and analytics platform. The platform provides a commercial grade, scalable approach to upload, view, and integrate digital pathology images with relevant subject data such as MRIs, pathology reports and genomic information. Stakeholders can search integrated data
without requiring users to change their current workflow or conform to imposed data standards. This platform is a simple, intuitive, end-to-end software platform for teams of scientists and pathologists to review, annotate and discuss cases, enabling rapid diagnostic consensus, quality control, and empowered discovery. Summary of Unpublished Results: The CBTT/Cornell BlackFynn data platform enabled us to engage in a cross-institutional, cross-disciplinary collaboration to create a cancer cloud platform that allows for efficient, reproducible data analysis. The Cancer Cloud Pilot project seeks to directly address these challenges by co-localizing data with the computational resources to analyze it where researchers can access it securely and easily. The project was designed to curate, store, harmonize and jointly use these omics data sets together for big biomedical data. Existing software components in the Explorer™ Technology Platform can quickly and efficiently be used to construct custom UI tools to query, analyze and visualizing data. This is not only important to have effective solutions for data storage and data management, but for data curation and data analysis and visualization. Data curation ensures that data from various sources are harmonized, standardized and cross-comparable. Powerful data analytics and visualization is pivotal in transforming data into information, new insights and added value. To address these problems, MediSapiens has developed the Explorer™ Technology Platform solution, based on its extensive experience in both industrial software development and academic biomedical research. The Explorer™ Technology Platform has been designed for all of the three major parts of the data research workflow: for curating data, storing and managing data, and analyzing and visualizing data. This web browser based, integrated solution provides functionalities to import, curate, ontology map and store rich clinical data, as well as very large data sets of gene expression, copy number, mutation and other profiling data for both bulk and single-cell analyses (e.g. single-cell RNA-seq), and perform various analyses and visualization on the data. Existing software components in the Explorer™ Technology Platform can quickly and efficiently be used to construct custom UI tools to query, analyze and interpret data according to individual customer needs. Further, the platform provides APIs which enable retrieval of data for integration with other systems, or development of custom analyses using e.g. R. Here, we will illustrate the implementation of Explorer Technology Concept to solve a variety of problems in preclinical cancer research and personalized medicine, starting from efficient automated curation of clinical data through to integration of clinical and multi-omics data.

**#2594 Optimizing the replication of cancer genomics workflows: case studies.** Jerry Fowler,1 F. Anthony San Lucas,1 Smruthy Sivakumar,1 Aditya Deshpande,2 Humam Kadara,2 Paul A. Scheet.1 UT MD Anderson Cancer Center, Houston, TX; 2Weill Cornell Graduate School of Medical Sciences, New York City, NY; 3American University of Beirut, Houston, TX.

Replicating results is a major issue in cancer biology, whose “workbench” is dynamic and complex, with frequently updated algorithms and software. The better to manage our work in this environment we have developed SyQADA, a System for Quality-Assured Data Analysis – a workflow automation system designed to simplify common sequential analysis processes on the same or different data. SyQADA manages many of the details of procedural bookkeeping involved in bioinformatics workflows. What samples are we using? Where are the raw data? Were all the samples processed? Did every job complete satisfactorily? Is there as much output as expected? Where are the input files for the next step? How long does a typical job take to run? Which program versions did we use? Can we easily compare these results with the output of a different version of a program, or with different input data? Using SyQADA, we have found our selves better able to reproduce results while at the same time reducing the human effort required to manage our upstream data analyses. Here, we briefly describe how our lung cancer studies have benefitted from the use of SyQADA. To understand the effect of different variant callers for Ion Torrent deep sequencing data in a lung cancer genomics study, we created a work protocol that allowed us to compare the different sets of variants called on 34 distinct somatic DNA samples from 4 patients. This complex processing framework involved running multiple variant callers, annotating variants, filtering germline variants using quality control metrics, and collating results across samples and callers. With SyQADA, we were able to re-run individual processes changing parameters with trivial changes to our configuration, yielding improved output. We then applied that unmodified protocol to the 500 samples from 48 individuals in our study, and rapidly produced data from which we could perform biological analysis. We then applied the protocol to a study of pre-malignant lesions in 25 lung cancer patients. In both studies, our workflow allowed us to generate comparable results in a matter of hours rather than days. SyQADA has been used by individuals with backgrounds ranging from expert programmer to Unix novice, to perform and repeat dozens of diverse analytical workflows. Projects to which SyQADA has been applied include allelic imbalance studies of TCGA samples for cancers of the breast, pancreas, lung, and colon, processing roughly 6000 samples through a dozen steps. A zipline containing the SyQADA executable source code, documentation, tutorial examples, and workflows used in our lab will be available.

**#2595 Enabling petabyte-scale cancer genomics with the NCI Cancer Cloud Pilots.** Gaurav Kaushik, Yilong Li, Erik Lehnhert, Zeynep Onder, Devin Locke, Brandi N. Davis-Dusenbery, Deniz Kural.

The advent of next generation sequencing has accelerated the generation of genomic data and created a need for methodologies to organize, share, and analyze large volumes of data. To date, petabytes of multi-dimensional information from thousands of patients have been collected. Access and analysis of this information becomes increasingly challenging as the amount of data grows. This difficulty is exemplified when we consider data generated by the efforts of The Cancer Genomics Atlas (TCGA) network, which encompasses more than 2.5 petabytes with a highly optimized network connection and access to large institutional compute clusters to perform integrated analysis, which is out of reach for many researchers. The Cancer Cloud Pilot project seeks to directly address these challenges by co-localizing data with the computational resources to analyze it where researchers can access it securely and easily. The project was designed to curate, store, harmonize and jointly use these omics data sets together for big biomedical data. Thus, it is not only important to have effective solutions for data storage and data management, but for data curation and data analysis and visualization. Data curation ensures that data from various sources are harmonized, standardized and cross-comparable. Powerful data analytics and visualization is pivotal in transforming data into information, new insights and added value. To address these problems, MediSapiens has developed the Explorer™ Technology Platform solution, based on its extensive experience in both industrial software development and academic biomedical research. The Explorer™ Technology Platform has been designed for all of the three major parts of the data research workflow: for curating data, storing and managing data, and analyzing and visualizing data. This web browser based, integrated solution provides functionalities to import, curate, ontology map and store rich clinical data, as well as very large data sets of gene expression, copy number, mutation and other profiling data for both bulk and single-cell analyses (e.g. single-cell RNA-seq), and perform various analyses and visualization on the data. Existing software components in the Explorer™ Technology Platform can quickly and efficiently be used to construct custom UI tools to query, analyze and interpret data according to individual customer needs. Further, the platform provides APIs which enable retrieval of data for integration with other systems, or development of custom analyses using e.g. R. Here, we will illustrate the implementation of Explorer Technology Concept to solve a variety of problems in preclinical cancer research and personalized medicine, starting from efficient automated curation of clinical data through to integration of clinical and multi-omics data.

**#2596 MediSapiens Explorer Technology Platform - Integrated Solution for big biomedical data.** Henrik Edgren, MediSapiens bioinformatics and development teams. MediSapiens Ltd, Helsinki, Finland.

During recent years, the increasing throughput and decreasing cost of technologies for genomic, transcriptomic, proteomic and other high throughput profiling of samples has enabled collection of very rich data sets on large sample cohorts. As methods have been developed to analyze e.g. large human germline sequencing studies, it has simultaneously become clear that the potential of the data can be fully and effectively leveraged only if technical solutions are developed to curate, store, harmonize and jointly use these omics data sets together with clinical and phenotypic data on the samples. In the field of modern data-driven research, heterogeneous data is increasingly available through many dispersed resources, and in many cases, data from public sources is combined with proprietary data for analysis. Thus, it is not only important to have effective solutions for data storing and data management, but for data curation and data analysis and visualization. Data curation ensures that data from various sources are harmonized, standardized and cross-comparable. Powerful data analytics and visualization is pivotal in transforming data into information, new insights and added value. To address these problems, MediSapiens has developed the Explorer™ Technology Platform solution, based on its extensive experience in both industrial software development and academic biomedical research. The Explorer™ Technology Platform has been designed for all of the three major parts of the data research workflow: for curating data, storing and managing data, and analyzing and visualizing data. This web browser based, integrated solution provides functionalities to import, curate, ontology map and store rich clinical data, as well as very large data sets of gene expression, copy number, mutation and other profiling data for both bulk and single-cell analyses (e.g. single-cell RNA-seq), and perform various analyses and visualization on the data. Existing software components in the Explorer™ Technology Platform can quickly and efficiently be used to construct custom UI tools to query, analyze and interpret data according to individual customer needs. Further, the platform provides APIs which enable retrieval of data for integration with other systems, or development of custom analyses using e.g. R. Here, we will illustrate the implementation of Explorer Technology Concept to solve a variety of problems in preclinical cancer research and personalized medicine, starting from efficient automated curation of clinical data through to integration of clinical and multi-omics data.
examined the impact of celecoxib intake on HTN and edema. The concomitant intake of celecoxib and one of the antihypertensives (AH) was also evaluated as means to suppress the side effects of celecoxib. Methods: This is a retrospective study that uses two patient level data (PLD) sources: (1) anonymous PLD from Symphony from Jan 2012 to Dec 2014 (3 years), and (2) Pinnacle registry data from American College of Cardiology (ACC) over the same time period with demographics such as blood pressure (BP) readings, peripheral edema flags, etc. In Symphony dataset there are 162 million (M) unique patients, 4.3M patients on celecoxib, 16.3M patients with osteoarthritis (OA) (15.4M only OA), and 2.3M patients with rheumatoid arthritis (RA) (1.4M only RA). In ACC dataset, there are 1.5M unique patients with BP readings, 870K patients with edema flag. Results: There was no impact of celecoxib consumed on the change in BP readings. There were as many increases as decreases, regardless of the amount of celecoxib consumed between BP readings. A breakdown by baseline BP readings did not reveal any trends. Concomitant use of AH with celecoxib did not impact BP. Onset of edema was not correlated with total dose of celecoxib. Less than 10% of the observations indicated a change in edema status. However, unlike BP, increasing % of edema was observed for increment in levels of celecoxib but not BP in OA patients. Here we confirmed our meta-analysis using PLD data. Celecoxib with ARB exhibited the lowest incidence of edema and with non-thiazide diuretics the highest incidence (Odds ratio = 3.34 (95% CI = 2.85-3.94), p<0.0001). ARB with celecoxib displayed a significant reduction in edema incidence compared to celecoxib alone (Odds ratio = 0.626 (95% CI = 0.539-0.724), p<0.0001). The celecoxib–ARB and celecoxib–ACE FDCs are being investigated further as therapy for FAP and more on oncogenesis.

#2598 Paradigm to ensure curation transparency and consistency of a cancer genomic database - the JAX Clinical Knowledgebase (CKB).

In the era of personalized medicine, comprehensive cancer genomic databases represent critical tools for interpreting gene variants and their relevant therapies. As the number of cancer genomic databases grow, it is challenging for users to compare and assess the data integrity and validity of such databases. The lack of disclosure of curation processes makes it difficult to cross reference different databases. The JAX Clinical Knowledgebase (CKB) is a comprehensive relational database providing evidence-based information on gene variants, targeted therapies, efficacy evidence, and clinical trials. At the Jackson Laboratory for Genomic Medicine, we have developed a curation paradigm to ensure curation transparency and consistency of the JAX-CKB. First, to ensure standardization of nomenclature, we have integrated standard nomenclature and ontologies, including ICGNC approved nomenclatures for genes, HGVS guidelines for somatic mutations, and Oncotree for cancer types. To ensure curation transparency, we implemented decision matrices to maintain uniformity of our evaluation of scientific and clinical data. For example, evaluation of the effect of gene variants on protein function is solely based on changes in the intrinsic activity of the protein instead of downstream pathway activation or the effects on pathogenesis; the response type of gene variants to therapies are classified based on the evidence of targeting specificity; and the efficacy evidence are further classified as actionable, diagnostic, prognostic, risk factor, emerging, or not active centered on the response type and the strength of the evidence. Third, to provide convenient evaluation of the clinical relevance of efficacy data, we further developed a tier ranking system based on emerging consensus guidelines, which takes response type, evidence type, and approval status into consideration. Finally, all decision matrices are clearly outlined on the JAX-CKB open access website for users’ reference. In summary, we have curated a highly structured and systematically consistent cancer genomic database following a set of specific curation guidelines. As a result, JAX-CKB open access has become a valuable resource for both the clinical and research communities, and is utilized by numerous independent groups for variant interpretation.

The Cancer Gene Census is an ongoing effort to catalogue genes for which somatic mutations have been causally implicated in cancer. The Census comprises manually curated summaries of the most relevant information for cancer-driving genes and their somatic mutations and brings together the expertise of a dedicated curation team, cancer scientists and the comprehensive resources of the COSMIC database. Current research focuses on characterising the participation of 609 census genes in hallmarks of cancer and identification of additional genes involved in these biological traits primarily via altered expression, CNA or epigenetic changes. New overviews of cancer gene function focused on hallmarks of cancer pull together manually curated information on the mutation of proteins coded by cancer genes and summarise the data in simple graphical presentations of the most relevant facts with quick access to the literature source, aiming to provide summary characteristics of a cancer gene, rather than a full monography, to avoid information overload. This functional characterisation enables the creation of lists of genes of interest focused on the particular role they play in the development of cancer, as well as aiming to identify the cellular functions affected by mutations in particular tumours, and help to choose right targets for targeted therapy or synthetic lethal experiments. The Census is available from the COSMIC website for online use or download at: http://cancer.sanger.ac.uk/census.

#2600 Analysis of drug resistance mechanisms and strategies for overcoming resistance in cancer therapy using a curated clinical knowledgebase.

Emergence of resistance to targeted therapies is a critical problem in cancer therapy, and understanding both the mechanisms of resistance and strategies for overcoming resistance are crucial to effective treatment of cancer patients. The JAX Clinical Knowledgebase (JAX-CKB), which incorporates data on therapeutic efficacy in the context of molecular alterations, enables rapid analysis of known therapy resistance mechanisms and current data surrounding strategies for overcoming resistance, both in the preclinical and clinical setting. Using the JAX-CKB, we have identified over 1250 lines of evidence corresponding to therapy resistance across tumor types. Of those lines, 147 correspond to therapy resistance in lung cancer. The JAX-CKB contains 22 variants in ALK associated with ALK therapy resistance. Within ALK fusion-positive lung cancer, evidence lines corresponding to ALK inhibitor resistance are associated primarily with complex molecular profiles containing secondary ALK mutations, and mechanisms for overcoming resistance in this setting were associated with use of novel agents. Within EGFR mutation positive lung cancer, lines associated with resistance to EGFR inhibitor therapy included copy number alterations, primary resistance mutations, secondary resistance mutations, and expression level changes. Strategies for overcoming resistance in these settings include novel agents, resistance-focused combination therapies. The JAX-CKB provides a unique global view into current data on resistance to targeted therapies in oncology, which may enable more rapid assessment of effective therapy options and expose opportunities for additional research into strategies for overcoming resistance.

#2601 COSMIC-3D: exploring cancer mutations in three dimensions for drug design and discovery.

Explosions in the availability of cancer genomic data and protein structure data give us the potential to explore cancer molecular biology at an unprecedented scale, and in atomic detail. We present COSMIC-3D, which combines COSMIC, the most comprehensive cancer mutation database available (http://cancer.sanger.ac.uk), with the wealth of publicly available 3D protein structure data, to create a resource with which the protein-structural nature of cancer can be probed. COSMIC-3D will help us understand which cancer mutations drive the progression of cancers, by identifying which are in functional sites that have an effect on driving cell growth and proliferation, and where they are clustered in forms of across the targeted therapies cancer types. Through understanding the effects of cancer mutations on known and predicted drug binding sites, we aim to predict potential new drug targets in cancers, and improve the specificity and efficacy of new or existing drugs, by using protein structure with cancer mutation data to guide mutation-specific drug design. COSMIC-3D is available as a web interface at http://cancer.sanger.ac.uk/cosmic3d, and enables interactive exploration of the cancer mutome in a 3D peptide environment, showing all forms of exonic point mutation. Individual mutant locations can be highlighted as molecular surfaces, while recurrence across nonsynonymous substitutions is visualized as 3D heat-maps. Cancer mutations can also be visualised in combination with precalculated small-molecule “drugable” bind-

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ing sites, providing a powerful visual approach for development of hypotheses across structural, functional, and drug-resistance impacts of cancer variants. COSMIC-3D shows, for example, the steric occlusion of the binding site of ATP-competitive small-molecule inhibitors in the EGFR kinase domain by the mutation of L858 to arginine; these kinds of insights can be applied to novel cancer therapies. The capability of the fully-structured 3D representation of the structural proteome comprising COSMIC-3D extends to nearly 8,500 human genes; 1/3rd of genes in COSMIC. Over 30,000 human protein structures are available across these genes, to which over 345,000 cancer mutations are mapped. From structure based druggability prediction, over 6,500 proteins in COSMIC are predicted to have small-molecule druggable binding sites, making COSMIC-3D a powerful and exciting resource for the exploration and guidance of drug design and discovery in oncology.


The goal of the International Cancer Genome Consortium (ICGC) is to analyze the cancer genomes of at least 500 tumor samples with matched controls from 50 different cancer types and subtypes, building a comprehensive catalogue of somatic abnormalities for the benefit of the research community. The amount of data ICGC members will generate is close to that of 50,000 human genome projects and, to date, has received commitments for 107 projects to study more than 27,000 tumor genomes. The ICGC Data Coordination Center (DCC) is responsible for collecting, curating, aggregating, and disseminating the data generated by the consortium’s member projects. Given the size and the complexity of the ICGC data, these tasks represent significant scientific and technological challenges that require a performant, robust software infrastructure. Key to this infrastructure is the ability to scale as data grows. Using state-of-the-art Big Data, bioinformatics and cloud computing technologies, we developed a suite of web-based applications and microservices that enable member projects to first submit their data and validate their submissions according to the rules defined in the submission specification. Following validation, the data is processed, annotated and loaded into the data portal using a modular Extract-Transform-Load (ETL) pipeline. Submission, ETL and portal systems are built using scalable and distributed technologies such as Hadoop, Spark, MongoDB and ElasticSearch. Spark is used to validate, join, index, and harmonize annotations on submitted variants while ElasticSearch powers our variant query engine, API and portal displays. Here we present the ICGC Data Portal and describe both the current features and capabilities accessible to users along with the architecture of the underlying infrastructure. The portal provides scientists with powerful and unique tools for exploring and visualizing the millions of variants and annotations available. These include sophisticated features that enable making data exploration extremely fast and easy, a suite of interactive JavaScript components for in-depth analysis and visualization of specific genomic features, embedded genome and pathway browsers, synthetic cohorts comparisons and a streaming data download service. The portal integrates a large variety of annotations such as variant consequences and frequencies, functional impact factors and druggability. The portal also offers cloud-based tools for searching a catalog of raw ICGC data files stored in worldwide repositories and compute clouds. All source code is open to the community under the GPLv3 license.

**#2603** A patient driven cancer database to collect information, analyze data, and predict outcomes. Lori Marx-Rubiner, 1 AnneMarie Ciccarella, 2 Vincent An, 3 Jared Bass, 2 Will Berman, 3 Jackson Berry, 3 Chloé Chan, 3 Christopher Han, 3 Louis Harboe, 3 Joshua Lurie, 3 Sara Ma, 3 Parker Malachowsky, 3 Nayēe Nagda, 3 Simon Schneider, 1 E. Bircan Çopur, 4 Jorge J. Nieva, 4 Jan Liphardt, 4 Peter Kuhn, 7 Jeremy M. Mason. 7

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It is widely held that major breakthroughs in cancer treatment will result from properly analyzing, analyzing and utilizing existing and emerging “big data.” To date there is no single vehicle that integrates the data available, and of those being developed, none that put patient needs and outcomes as primary focus. CancerBase was released as part of the Cancer Moonshot launch in June 2016. It is a global, real-time data collection tool designed by and for patients. Patients were recruited through social media to share their information, including but not limited to, demographic details, personal cancer data, and history of treatment. Combining open text boxes and drop down preselected response boxes allows for the capture of information in a way that patients understand. In addition to cancer data, Facebook comments demonstrated immediate engagement with the tool and captured the organic community of patients who eagerly shared their stories with others. Personal interviews and a communal Twitter chat event led to user feedback, and we subsequently developed CancerBase to be more user-friendly and better understanding in a more easily familiar CancerBase. In addition to medical data, general information and questions CancerBase participants wanted to add to the system have been collected. Incorporation of patient-driven deliverables is unique in the space of big data and cancer in that other tools, both existing and still in development, are geared toward the needs of researchers, payors, policy-makers and clinicians. CancerBase provides a database tool that resonates with the patient community and is driven by patient needs and interests. As the tool becomes increasingly robust it will grow to support the decision-making needs of clinicians and guide the investigations of the researchers. The launch of CancerBase in Spring 2017 will address emerging patient concerns, integrate collected data, and utilize existing forecasting databases to add value to patients.


Introduction An overarching goal of biomedical research is to improve the use and dissemination of rapidly growing biomedical datasets to support precision medicine. Individualized molecular profiling and the identification of predictive biomarkers can powerfully inform the choice of therapies for cancer patients. However, both require integration of extensive molecular, clinical, and pharmacological data, often from disparate and diverse sources. The Georgetown Database of Cancer (G-DOC) was designed and engineered to be a unique multi-omics data analysis platform to enable translational research and precision medicine. Methods G-DOC is home to 61 datasets that contain data from over 10,000 patients across 14 diseases (10 cancers and 4 non-cancers). 1700 researchers from over 48 different countries worldwide currently use the platform. The data and tools in the G-DOC system have enabled over 40 research publications. G-DOC has the largest public collection of brain cancer patients from NCI Rembrandt dataset (671 patients). G-DOC integrates clinical, transcriptional, and imaging data types and presents datasets as COSELUS data and MRI medical images with systems-level analysis tools into a single, user-friendly platform. The “Variant Search” feature in G-DOC currently enables exploratory analysis of mutations based on genes, chromosomes, and functional location. A researcher can use this feature to 1) identify clinically actionable mutations in their dataset 2) identify pathways that may be affected by these mutations, and 3) identify novel mutations in their dataset and explore their potential impact on protein function. Results and Conclusion We are currently working on developing features to support the import, integration, search, and retrieval of CLIA/CAP-certified cancer molecular diagnostic (molDx) data. This will enhance G-DOC’s interoperability with clinical and patient molecular profiling data that may be already stored in other databases. Our vision is to continuously improve and expand G-DOC with the long-term vision of supporting integration of informatics techniques into everyday research and practice.

**#2605** Improving data quality in oncology immunotherapy clinical research by big data analytics and data visualization. Chengsen Xue, Joanne Cuomo, Walter Meyers, Thomas W. Mc Closkey, ICON Laboratory Services, Farmingdale, NY.

Flow cytometric assessment of cellular and intracellular biomarkers facilitates the development of new classes of cancer immunotherapy therapeutics. This powerful tool has multiple applications including 1) detecting PD1, PDL-1 and caspase-3 for apoptosis, 2) isolating, defining, and quantifying dendritic cells and their intracellular cytokine profile, 3) immunophenotyping subpopulations
of B, T, NK, regulatory T, and helper T cells, and, 4) using tetramer analysis to detect and monitor the frequency of antigen-specific cytotoxic T lymphocytes (CTL). However, lack of knowledge of normal distributions and standard ranges for the esoteric reportables generated by flow cytometry is one of the most challenging problems in this promising field. Here we describe a new method to improve data quality in clinical research by big data analytics and data visualization. Each data point was tagged. By applying a simple statistical analysis, a self-defined range allowed all of the outlier numbers to be identified. By repeating this process, clear patterns emerged, and all questionable data points were marked. In addition, the system can differentiate biodiversity in subjects from a more homogenous population, which can be exploited further for the purpose of precision medicine. Other benefits of the new system are to predict the outcome of the clinical study and to generate new clues in the understanding of the underlying biological mechanisms. Big data analytics and data visualization show unparalleled advantages in oncology studies through simultaneously monitoring unlimited data points related to surface and intracellular biomarkers. It opens a new avenue in cost saving for long, time-consuming clinical trials by using all available data to detect a status change of the immune system. With the improvement of data quality, big data analytics has become an essential tool and an integrated part of cancer immunotherapy and the development of cancer vaccines.

*#2606 Accelerating clinical research using cloud technology.* Arun Apte,1 Shonali Paul,1 Aditi Gade,1 Carolyn Compton.2 CloudLIMS.com, Wilmington, DE; 1Arizona State University, AZ.

Introduction: Existing protocols for clinical trials are typically slow and error-prone due to lack of informatics tools for sharing and accessing clinical data in real time. There is an increasing pressure on clinical trial laboratories to change the way clinical research is performed so as to minimize costs and speed up R&D, which in turn will produce better and faster outcomes. Cloud-based technologies are emerging as one of the most viable options for streamlining the heavily regulated clinical research processes. Description: Cloud-based software tools such as laboratory information management systems (LIMS) are much more capable than traditional paper-based or desktop tools for accelerating a clinical trial life cycle. Cloud-based LIMS enhance collaboration between sponsors and investigators, enabling more timely and accurate sharing of information with pharmaceutical companies. Summary: Many sponsors are now adopting cloud-based LIMS for conducting clinical trials. The onus is on service providers to keep pace with the demands of the industry, comply with the regulatory framework and at the same time deliver value to CROs and testing labs by reducing costs and maintaining efficiency. Conclusion: CloudLIMS.com has developed a cloud based software called CloudLIMS Lite for managing clinical research workflows to provide a cost effective solution to the clinical research industry. Being on the cloud, CloudLIMS Lite facilitates users to access and share data in real time, across multiple laboratories in different geographical locations and adhere to the regulatory framework. This in turn improves their operational efficiency. CloudLIMS Lite uses 256-bit AES encryption SSL protocol, thereby enabling secure data transmission. CloudLIMS Lite deploys a separate database layer for each of its customers making it impossible for any third party to view or access data.

*#2607 The eBioPortal for Cancer Genomics: an open source platform for accessing and interpreting complex cancer genomics data in the era of precision medicine.* Jianqiong Gao,1 Erisin Ciftci,2 Pichai Raman,1 Pieter Lukkasse,1 Istemi Bahcecci,1 Adam Absehouse,1 Hsiao-Wei Chen,1 Ino de Bruijn,1 J. Benjam Gross,1 Zachary Heins,1 Ritika Kundara,1 Aaron Lissam,1 Angelica Ochoa,1 Robert Sheridan,1 Onur Sumer,1 Yichao Sun,1 Jiaoqia Wang,1 Manda Wilson,1 Hongxin Zhang,1 James Xu,1 Andy Duñile,1 Priti Kumari,1 James Lindsay,1 Anthony Cros,2 Karthik Kalletta,2 Fedde Schaeffer,2 Sander Tan,4 Sjoerd van Hagen,1 Jorge Reis-Filho,1 Kees van Bochove,1 Ugur Dogrusoz,2 Trevor Pugh,6 Adam Resnick,3 Chris Sander,1 Ethan Cerami,1 Nikolaus Schultz,1 Memorial Sloan Kettering Cancer Center, New York, NY; Dana-Farber Cancer Institute, Boston, MA; Children’s Hospital of Philadelphia, Philadelphia, PA; 3The Hyve, BilkentUniversity, Turkey; 4Washington University School of Medicine, Saint Louis, MO; 5Institute for Research in Biomedicine, Barcelona, Spain; 6Dana-Farber Cancer Institute, Boston, MA; 1Memorial Sloan Kettering Cancer Center, New York, NY; 2Well Cornell Medical College, New York, NY; 3Oregon Health & Science University, Portland, OR.

The eBioPortal for Cancer Genomics is an open-access platform (http://cbioportal.org) that enables interactive, exploratory analysis of large-scale cancer genomics data. It integrates genomic and clinical data, and provides a suite of visualization and analysis options, including cohort and patient-level visualization, mutation visualization, survival analysis, enrichment analysis, and network analysis. The user interface is user-friendly, responsive, and makes genomic data easily accessible to translational scientists, biologists, and clinicians. The eBioPortal is a fully open source platform. All code is available on GitHub (https://github.com/cBioPortal/) under GNU Affero GPL license. The code base is maintained by multiple groups, including Memorial Sloan Kettering Cancer Center, Dana-Farber Cancer Institute, Children’s Hospital of Philadelphia, Princess Margaret Cancer Centre, and The Hyve, an open source bioinformatics company based in the Netherlands. More than 30 academic centers as well as multiple pharmaceutical and biotech companies maintain private instances of the eBioPortal. This includes the recently launched BioPortal instance at the Netherlands Genome Center, hosting two large eBioPortal instances hosting genomic and clinical data at MSK and DFCI, supporting the MSK-IMPACT and DFCI Profile projects, of two of the largest clinical sequencing efforts in the world. Our multi-institutional software team has accelerated the progress of evolving the core architectural technologies and developing new features to keep pace with the rapidly advancing fields of cancer genomics and precision cancer medicine. For example, we have integrated multi-platform genomics data with extensive clinical data including patient demographics, treatment history, and survival data. We have also developed a patient-centric view that visualizes both clinical and genomic data with annotation from OncoKB knowledge base. In the next few years, the development team will focus on the following areas: (1) Implementing major architectural changes to ensure future scalability and performance. (2) New features to support precision medicine, including (i) improved integration of knowledge base annotation, (ii) enhanced visualization of patient timeline, drug response, and tumor evolution, (iii) new patient similarity metrics, (iv) improved support for immunogenomics and immunotherapy, and (v) new visualization and analysis features for understanding response to therapy. (3) New analysis and target discovery features for better, including (1) supporting user-defined virtual cohort by selecting samples from multiple studies, and (ii) comparison of genomic or clinical characteristics of two or more selected cohorts. (4) Expanding community outreach, user support and training, and documentation.
BIOINFORMATICS AND SYSTEMS BIOLOGY: Computational Tools and Databases

#2609 Columbia University’s Center for Cancer Systems Therapeutics (CaST) 2017 Scholars Program: A synergistic partnership with students from Brooklyn College, CUNY. Rahimah Ahmad,1 Tasnim Azad,1 Carlos Barreto,1 Kamrun Begum,1 Mahliaa Butt,1 Daniel Griffaft,1 Aulon Jeru,1 Jonathan Kwia1, Mikaela Murphy,2 Katherine A. Rivera Gómez,2 Barry Honig,2 Andrea Califano1, Shannen Jaffe1,2 Diana Murray1,2 CUNY Brooklyn College, Brooklyn, NY. 1Columbia University, New York, NY.

The Cancer Systems Therapeutics (CaST) Outreach helps foster community building throughout the NCI Cancer Systems Biology Consortium (CSCB). The overall mission of the CaST Outreach Core is to advance progress in cancer systems biology by tapping into and making connections across research talent at all stages of education, with the objective of increasing the attraction and recruitment of motivated undergraduate, post-baccalaureate, and graduate students from Brooklyn College of the City University of New York (CUNY). These students comprise the first cohort of the CaST Scholars Program and are immersed in the cutting-edge biological and biomedical science underlying Cancer Systems Biology. In subsequent years, CaST Outreach will partner with students from other CUNY Senior Colleges while continuing interactions with our Brooklyn College Scholars. This integrative approach will create a network of CaST Scholars throughout the CUNY system. Because Systems Biology is highly interdisciplinary, participants in the program can come from many backgrounds, including biology, computer science, physics, chemistry, genomics, engineering, and other fields. The program has no fixed curriculum and each of nine Brooklyn College students has developed an individualized CSCB educational plan tailored to their interests. The CaST Scholars Program opportunities include: 1) tuition-free attendance at a master’s level Systems Biology course taught by a CaST investigator and offered through Columbia University’s School of Professional Studies; 2) supported attendance at the AACR annual meeting; 3) monthly informal discussion meetings with CaST investigators; 4) weekly formal tutorials in the design and applications of CaST computational algorithms; 5) research internships with CaST investigators; 6) participation in a wide range of Center activities; 7) a scholarship to support participation; and 8) ongoing mentorship from CaST investigators. This poster provides summaries of the collaborative, synergistic experiences and achievements of each scholar and of the group as a whole. The CaST Scholars Program offers a unique opportunity for early-stage scientists to learn how Systems Biology is transforming cancer research and precision medicine at Columbia University Medical Center. Regardless of their chosen professional path, CaST Scholars will obtain a solid foundation in the methods and potential of Cancer Systems Biology.

#2610 A database for evaluating methylation biomarkers in bladder cancer. Meaghan M. Kennedy, Garrett M. Dancik. Eastern Connecticut State University, CT.

Bladder cancer is the fourth most common cancer in men, one of the most expensive cancers to treat, and recurrence is frequent. While the genomic alterations associated with high-grade, muscle invasive (MI) tumors and low-grade, non-muscle invasive (NMI) tumors are well-established, recent studies have also found that MI and NMI tumors have unique methylation patterns, and that methylation may play a role in the development of early-stage bladder cancer. Interestingly, bladder cancer patients have frequent mutations in chromatin remodeling genes that alter known histone markers of DNA methylation. Since methylation marks are pharmacologically reversible, the identification of methylation biomarkers is a promising avenue for therapeutic treatment. Indeed, several candidate prognostic methylation biomarkers have been identified, but await prospective evaluation. The Bladder Cancer Biomarker Evaluation Tool (BC-BET) is a web-based resource for rapidly evaluating biomarkers in publicly available genomic datasets. Initially developed for gene expression biomarkers, BC-BET includes 1451 patients across 13 cohorts, and 40 bladder cancer cell lines, with gene expression profiles. In this work, we describe the addition of methylation data to BC-BET, so that candidate methylation biomarkers can be rapidly evaluated across multiple patient cohorts. BC-BET now contains methylation profiles from four patient cohorts (250 patients) and includes the available bladder cancer data from the Gene Expression Omnibus and The Cancer Genome Atlas. In ‘evaluation’ mode, a user selects a gene of interest, and BC-BET evaluates whether the selected gene is differentially methylated between tumor vs. normal samples, NMI vs. MI tumors, and low- vs. high-grade tumors. Alternatively, the “biomarker discovery” mode allows a user to identify all differentially methylated genes at a desired false discovery rate (FDR). In both cases, graphical summaries of the results are displayed and statistical results can be downloaded to an Excel spreadsheet. The methylation module of BC-BET is implemented using shiny, a web application framework for R for developing interactive web pages. The addition of methylation data to BC-BET makes it a valuable resource for evaluating methylation biomarkers in bladder cancer. BC-BET is available from the following link: https://gdanck.github.io/bioinformatics/BCBET

#2611 A crowdsourcing-based clinical trial information curation and searching system. Pengfei Yu, Qingxuan Song, Yang Han, Guanghui Hu. Admera Health, South Plainfield, NJ.

Targeted cancer therapies based on actionable mutations identified are the future of cancer treatment. Besides those approved by the Food and Drug Administration (FDA) for different types of cancers, many other target therapy drugs are in development under different phases of clinical trials. Access to information about these clinical trials is important to health care professionals and patients. Public clinical trials database (www.clinicaltrials.gov) provides a very comprehensive resource for clinicians and researchers to search for relevant clinical trials based on different kinds of searching criteria. However, due to the complexity of wording and phasing upon submission, some of the searching results returned by native search engine did not perfectly match the search criteria. Many undesired results are presented with false positives and false negatives when users are searching for clinical trials targeting specific actionable mutations. To tackle this problem, we developed a crowd-sourcing based clinical trial curation and searching system on top of the information extracted from public database. We divide the information extracted from clinicaltrials.gov into three different levels: standard, easy-to-clean and unstructured. With the crowdsourcing approach, our online system can harness the brainpower of biological scientists to translate the most unstructured information such as cancer types, gene mutations into standard format that is easy to be queried. In the searching phase, we combine the aspects of actionable mutation, cancer type, location, and clinical trial phase to select the best matches between patient conditions and clinical trials in our curated database. With the same searching criteria, our system can provide more relevant clinical trials compared with those provided by clinicaltrials.gov. The curation system is currently open to reviewers with eligibility check. The search engine is publicly available at: https://agis.admerahealth.com/AGIS/ClinTrials/

IMMUNOLOGY: Checkpoints 2: Small-Molecule Inhibitors

#2612 Anti-Tigit induces T cell mediated anti-tumor immune response and combines with immune checkpoint inhibitors to enhance strong and long term anti-tumor immunity. Minu K. Srivastava, Kui Yun, Erin Mayes, Janice Yu, Huyn-Bae Jie, Fumiko Axelrod, Ming-Hong Xie, Jorge Monjeon, Andrew Lam, May Ji, Yuwang Liu, John Lewicki, Tim Hoey, Austin Gurney, Angie Inkyung Park. OncoMed Pharmaceuticals, Inc., Redwood City, CA.

TIGIT (T cell immunoreceptor with Ig and ITIM domains) has been recently described as an inhibitory receptor which blocks CD8 T cell-mediated anti-tumor immune responses. We have generated an anti-mouse TIGIT antibody (313R12) to evaluate drug efficacy and mechanism of action in pre-clinical tumor models. Anti-TIGIT as a single agent promoted an anti-tumor immune response in multiple syngeneic mouse tumor models. Anti-TIGIT enhanced tumor specific T cell responses, particularly of the Th1 type and reduced Th2 type responses and also increased the function of cytotoxic T cells. Furthermore, anti-TIGIT displayed combination activity with immune checkpoint inhibitors anti-PD1 and anti-PDL1 in inhibiting tumor growth, promoting complete tumor rejection and significantly increasing mouse survival in the murine CT26 colon carcinoma model as compared to controls and single agents alone. Mice “cured” with anti-TIGIT/anti-PDL1 or anti-TIGIT/anti-PD1 combination treatments did not form tumors upon subsequent re-challenges with increasing number of CT26 tumor cells, suggesting the existence of immunologic memory. IL2 and tumor-specific IFNγ production by splenic T cells were increased in mice who responded to combination treatment compared to controls. Additionally, both effector and memory CD8 T cell frequencies were increased within the total CD8+ T cell population in responding mice. We also demonstrated a systemic increase in tumor-specific CD8 T cells after anti-TIGIT/anti-PDL1 combination treatment compared to controls. Therefore, these results suggest that co-targeting of TIGIT and PD1 or PDL1 may be an effective and durable cancer therapy by increasing T cell-mediated anti-tumor immune responses and promoting long-term immunological memory.

#2613 Combination agonist and antagonist antibody therapy enhances vaccine induced T cell responses in non-immunogenic cancers. Hayley S. Ma, Evantha Roussos Torres, Brian Christmas, Blake Scott, Tara Robinson, Todd Armstrong, Elizabeth Jaffee. Johns Hopkins University School of Medicine, Baltimore, MD.

Immune tolerance by multiple mechanisms is a major obstacle in the implementation of successful immunotherapy. T regulatory cells (Tregs) and myeloid derived suppressor cells (MDSCs) suppress the immune response, and the presence of extensive stromal networks within the tumor microenvironment (TME)
can prevent T cell infiltration in many tumor types. Furthermore, tumor anti-
gen-specific T cell tolerance limits the efficacy of therapeutic cancer vaccines. CD40 is expressed on antigen presenting cells, and CD40 signaling is critical to the decision of whether cytotoxic T lymphocytes become primed or tolerized. Administration of monoclonal CD40 agonistic antibody (Ab) has been shown to promote CD8 activation in vivo, and likely alters the myeloid component of the TME. Our study asks the question of whether combining a T cell inducing vaccine and PD-1 inhibition with CD40 agonistic Ab can induce T cell priming and tumor infiltrating lymphocyte (TIL) activation in non-immunogenic solid malignancies. We utilized an orthotopic transplant model in which HER2/neu expressing breast tumor cells were implanted into the mammary fat pad of syngeneic neu-N mice to assess the effects of drug combinations on intratumoral immune responses. Tumor-bearing mice were treated with a granulocyte macrophage colony-stimulation factor secreting vaccine (GVAX) + anti-PD-1 Ab alone or in combination with CD40 agonist Ab, and monitored for tumor clearance and survival. A separate cohort of mice were analyzed by IHC and multi-color flow cytometry to assess T cell infiltration and activation, myeloid maturation, and MDCs after one week of treatment. We demonstrated delayed tumor progression and increased median survival in mice treated with GVAX + anti-PD-1 Ab + CD40 Ab relative to CD40 Ab alone, although endogenous T cell infiltration remained low across treatment groups. However, adoptive transfer of neo-specific TCR transgenic cells revealed 100% tumor clearance in the combination-treated mice, along with significant T cell infiltration and activation as assessed by intracellular Granzyme B and TNFα staining. In contrast, only 20% of mice treated with GVAX + anti-PD-1 Ab were able to clear tumor, and none of the mice receiving vehicle or CD40 Ab alone had long-term survival. Trends were also observed in monocyte and dendritic cell infiltration and maturation in the tumors of combination-treated mice, and warrant further investigation. In conclusion, GVAX, anti-PD-1 Ab and CD40 agonist Ab have potential synergy in modulating anti-tumor immunity in breast cancer. These results support further studies more broadly in non-immunogenic solid tumors.

#2614 Decrease of benefit from immune checkpoint inhibitors in women with non-small cell lung cancer. Joseph A. Pinto,1 Luis A. Mas,2 Carlos S. Vallejos,1 Jhajaara Araujo,1 Levy Brav2, Alfredo Aguilar,1 Zaida Morante,1 Denisse Bretel,1 Henry L. Gomez,1 Christian Rolfo,1 Oncosalud, Lima, Peru; Universidad Privada San Juan Bautista, Lima, Peru; Antwerp University, Limma, Belgium.

Introduction: In a previous work we found differences in immune gene sets enrichment in NSCLC between genders, including their smoking status, where women had higher expression of gene sets associated with immune processes. Based in this fact, we hypothesize fewer benefits from immune checkpoint inhibitors in women patients compared than men patients. Objectives: Evaluate the benefit (in terms of progression-free survival) of women patients from immune checkpoint inhibitors nivolumab and pembrolizumab in published randomized phase III trials. Methods: We evaluated by meta-analysis four randomized phase III studies, two of pembrolizumab (Herbst et al., 2015 and Reck et al., 2016) and two of nivolumab (Borghaei et al., 2015 and Brahmer et al., 2015). We analyzed TCGA data for lung adenocarcinoma to evaluate differences in the expression of PD1 and PD1, CD274 gene (encoding for PD1 and PD-L1, respectively).

Results: For all studies, it was observed an overall HR = 0.79 (CI95%: 0.71-0.87; P<0.00001), although it was observed a significant heterogeneity between studies (P=0.002). In male patients, was observed an overall HR = 0.71 (CI95%: 0.62-0.80; P<0.00001) with significant heterogeneity between studies (P=0.002). For female patients, there was not seen a clear benefit from nivolumab or pembrolizumab (overall HR = 0.97, CI95%:0.82-1.14; P=0.67). There was not heterogeneity between the cohorts (P=0.45). There were not differences in the expression of PD1 and CD274 genes according to gender in the TCGA data (P=0.371 and 0.144, respectively). Conclusion: Although there are not differences between genders in the expression of PD1 and PD1, CD274 genes, such as is previously described for expression for PD1 and PDL-1 proteins, in our meta-analysis we shown that women have more benefit from anti-immune checkpoint inhibitors. Blockade of PD1 and PD-L1 is a promising therapy in lung cancer; however, a better stratification of patients should be done.

#2615 Preclinical evaluation and mechanistic characterization of M7824 (MSB0011359C), a novel bifunctional fusion protein targeting M7A1 and TGFβ pathways. Yan Lan, Dong Zhang, Chuxiao Xu, Bo Marelli, Jin Qi, Huakei Qi, Guozhong Qin, Xiaomei Xu, Hong Wang, Aroop Sirbar, Beatrice Brunkhorst, Eric Austin, Laszlo Radvanyi, Kin-Ming Lo. EMD Serono Research and Development Institute, Inc., Billerica, MA.

Dual targeting of the programmed death ligand 1 (PD-L1) and transforming growth factor β (TGFβ) pathways, 2 key mediators of tumor immune evasion, may provide synergistic antitumor activity. The novel bifunctional fusion protein M7824 (MSB0011359C) is a fully human IgG1 monoclonal antibody against PD-L1, fused via a glycine-serine linker at the CH3-C terminus to the extracellular domain of human TGFβ receptor II, which functions as a TGFβ “trap.” Thus, M7824 is designed to exert independent and complementary anti-immunosuppressive functions by simultaneously blocking the PD-L1 and TGFβ pathways. The purpose of this study was to evaluate M7824 preclinically. M7824 was efficacious in multiple syngeneic tumor models (EMT-6, MC38, PANCO2, 4T1, CT26, and GL261). In orthotopic EMT-6 and intramuscular MC38 models, M7824 showed superior additive antitumor effects compared to anti-PD-L1 antibody or TGFβ trap monotherapy. M7824 also inhibited spontaneous metastases in EMT-6 and 4T1 models. Furthermore, M7824 extended survival and conferred long-term protective antitumor immunity, as demonstrated by protection of cured mice against tumor cell rechallenge. Mechanistically, the dual anti-immunosuppressive activity of M7824 in the MC38 model resulted in unique activation of both the adaptive and innate immune systems, including increased tumor infiltration, proliferation, and cytotoxicity of CD8+ T cells; increased prevalence of splenic tumor antigen-specific CD8+ T cells; increased CD8+ T cell infiltration and CD8+ T cell/natural killer (NK) cells; increased ratio of M1/M2 macrophages; and decreased anti-immunosuppressive activity of M7824. Improved activation of tumor-associated neutrophils and myeloid-derived suppressor cells. Similar immunophenotypic changes were observed in the EMT-6 model. Activation of both the adaptive and innate immune systems contributed to the antitumor activity of M7824 in the MC38 model, as depletion of CD8+ T cells and NK cells led to a substantial decrease in M7824-mediated tumor regression. CD4+ T cells and antibody-dependent cellular cytotoxicity, however, appeared dispensable. Toxicologic evaluation in cynomolgus monkeys indicated M7824 was well tolerated, with only minor hematologic effects observed. Although M7824 is a bifunctional molecule, its antitumor activity can be further improved by combination with standard-of-care therapies: in the MC38 model, combining M7824 with radiotherapy or chemotherapy resulted in more effective inhibition of tumor growth and an increased frequency of tumor antigen-specific Fcγ-requiring producing CD8+ T cells vs either monotherapy. Collectively, our preclinical data suggest that M7824 elicits potent and synergistic antitumor activity via the simultaneous blockade of the PD-L1 and TGFβ pathways. Phase 1 clinical trials with M7824 are under way in patients with solid tumors.

Correlation between clonal neoantigen burden (neoantigens generated early in tumorigenesis and therefore represented at high frequency in all tumor lesions) and responsiveness to checkpoint blockade has underscored the relevance of neoantigens in promoting tumor immunogenicity. Yet, the most of patients do not express, or express low numbers of, clonal neoantigens, and consequently will be less likely to benefit from checkpoint blockade. We describe strategies to generate neoantigen-directed therapies that may be incorporated into the standard chemotherapeutic strategy (CIT) in a cohort of locally advanced ESCC patients. In baseline ESCC tissues, 7 of 19 (37%) exhibited positive PD-L1 expression on tumor cells, and 10 of 21 (48%) exhibited positive expression on immune cells. In 21 ESCC tissues obtained a median of 18 days after initiating chemotherapy, 9 of 18 (50%) and 15 of 21 (71%) demonstrated positive PD-L1 expression on tumor cells and immune cells, respectively. The expression of PD-L1 was significantly increased in post-chemotherapy ESCC tissues compared to baseline tissues (p=0.010); and the increased PD-L1 expression between paired tissues was significant on immune cells (p=0.0056) but did not reach statistical significance on tumor cells (p=0.11). The positive PD-L1 expression in baseline ESCC tissues was correlated with better survival (HR:0.37, p=0.023). Besides, patients with more increase of PD-L1 expression after chemotherapy had significantly worse survival than those with less (median survival: 19.4 vs 71.20 months, HR: 4.07, p=0.0089). Conclusion: PD-L1 expression of ESCC can be up-regulated by chemotherapy. The up-regulation of PD-L1 is associated with poor outcome for locally advanced ESCC patients. (The study was supported by the grant NTUH 104-M2891 and the grant MOST 105-2314-B-002-186-MY3).

#2618 Agonist antibodies targeting OX40 and GITR enhance the activity of the IDO1-selective inhibitor epacadostat in preclinical models. Holly K. Koblish,1 Brendan Horton,2 Michael Hansbury,1 Sybil O’Connor, Kerri Lasky,1 Christina Stevens,1 Thomas Condamine,1 Leslie Hall,1 Liang-Chuan Wang,1 Yue Zhang,1 Horacio Nastrì,1 Gregory Hollis,1 Reid Huber,1 Thomas Gajewski,2 Peggy Scherle.1 Incyte Corporation, Wilmington, DE; 2University of Chicago, Chicago, IL.

The majority of immunotherapeutic agents developed thus far either attempt to stimulate a more productive anti-tumor immune response or to inhibit key proteins in the immunosuppressive tumor milieu. PD-1/PD-L1 axis blockade, CTLA-4 blockade and IDO1 inhibition are examples of the latter approach and have been utilized to reverse the suppressive tumor microenvironment, resulting in clinical benefit for cancer patients. Recent clinical and preclinical data have also demonstrated that combining these approaches results in enhanced therapeutic benefit. Notably, the IDO1-selective inhibitor epacadostat has been shown to increase the efficacy of two checkpoint inhibitors, the anti-CTLA-4 antibody ipilimumab and the anti-PD-1 antibody pembrolizumab, in patients with melanoma. Because both checkpoint receptors and IDO1 serve as negative regulators of the immune response, we also explored the ability of IDO1 inhibition to combine with checkpoint blockade to more effectively activate T cells through costimulatory receptors. In the tumor necrosis factor receptor (TNFR) superfamily. Rodent active surrogate agonist antibodies to 4-1BB, OX40 and GITR were tested with epacadostat in multiple preclinical models. In the B16-SIY melanoma model that does not express IDO1 in tumor cells, both epacadostat and anti-OX40 had little effect, but the combination resulted in enhanced efficacy. This was associated with increased infiltrates of CD8+ T cells and decreased numbers of FoxP3+ TILs. Increased numbers of SIY-reactive T cells were found in both the tumor and the TDLN post-treatment. In contrast, epacadostat did not provide any enhancement to the activity seen with 4-1BB. Clear combinatorial effects were seen with anti-GITR and epacadostat in the more immuned, IDO1-expressing PAN02 pancreatic cancer model. These data suggest that IDO1 inhibition can be effective in combination with agents that agonize T cell costimulatory receptors as well as with agents that block coinhibitory receptors.

#2619 Combination of ECP1014 and anti-PD-L1 reduces tumor growth in the CT26 murine colon carcinoma model of a cold tumor. Bobby W. Sandage,1 John J. Talley,1 Eduardo J. Martinez,2 Maryland R. Franklin,3 Mary C. Leopold3. 1Euclises Pharmaceuticals, Inc., St. Louis, MO; 2Rgenix, Philadelphia, PA; 3M Bioresearch, Ann Arbor, MI.

Objective: The objective of this study was to evaluate the effect of ECP1014, a new selective COX2 inhibitor, in combination with a PD-L1 inhibitor on tumor growth in a CT26 murine colon carcinoma model. Colon cancer has been described as a “cold tumor” since the immunosuppressive tumor microenvironment results in clinical benefit for cancer patients. Recent studies have demonstrated that combining ECP1014, which we have shown to potentially decrease PG2E2 in CT26, with a checkpoint inhibitor would increase immune response to the tumor, turning it “hot” and producing superior tumor control versus either agent alone. Methods: Balb/C mice were implanted with CT26 cells and randomly assigned to 7 treatment groups; vehicle (G1), 10mg/kg rat IgG2b control (G2), 10mg/kg antiPD1 (G3), 10mg/kg ECP1014 (G4), 10mg/kg ECP1014+10mg/kg rat IgG2b (G5), 10mg/kg ECP1014+10mg/kg antiPD1 (G6), 1mg/kg ECP1014+10mg/kg antiPD1(GL), ECP1014 was given daily by oral gavage; rat IgG2b and antiPD1 (clone 10F.9G2) were administered IP. Treatment was started on day 3 post implant and mice were followed for 16 days. Tumor measurements were taken every 2 to 3 days, and a nonparametric approach was taken to assess the pairwise differences among groups using day 16 tumor volumes. In addition, a responder analysis was used. Tumor infiltrating lymphocytes (TIL) were also assessed by flow cytometry on day 16. Results: All animals developed tumors. G4 to 7 demonstrated statistically significantly (p<0.05) slowing of tumor growth when compared to vehicle (G1). In particular, G7 produced an 87% suppression of tumor growth compared to vehicle. Three animals in G7 showed regression of tumor growth and one additional animal had no detectable growth until day 16. G6 produced 65% suppression of tumor growth compared to G1. G2 to 5 produced 30, 54, 73 and 50% suppression of tumor growth respectively when compared to G1.
checkpoint inhibitor in endometrial cancer.

To address this concern, we analyzed the expression of CD8+ and PD-L1/PD-1 in 60 patients with endometrial cancer. We examined whether MSI was associated with the expression of CD8+ lymphocytes (CD8) and PD-L1/PD-1.

**Method**

We included patients with MSI endometrial cancers. Therefore, immune checkpoint inhibitor therapy (anti PD-1/anti-PD-L1 antibody) has been shown to have clinical responses in endometrial cancers with MSI. MSI testing is likely to be a biomarker for PD-1/PD-L1 immunotherapy in endometrial cancer.

**Conclusion**

These results suggested that immune checkpoint inhibitor (anti PD-1/PD-L1 antibody) is effective in endometrial cancers with MSI. MSI testing is likely to be a biomarker for PD-1/PD-L1 immunotherapy in endometrial cancer.

**#2622** Two novel TLR9 agonists for cancer immunotherapy: Combination with checkpoint inhibitors. Kerstin Kapp,1 Barbara Volz,2 Detlef Oswald,3 Burghardt Wittig,4 Manuel Schmidt1.

**Introduction**

TLR9 agonists are developed as anti-cancer therapies based on their broad activation of the innate and adaptive immune system. Single-stranded oligodeoxynucleotides (ODN) containing non-methylated CG-motifs activate TLR9. Previously, chemical modification was used to prevent their degradation by exonucleases. To avoid the off-target effects observed with chemical modifications, new TLR9 agonists containing only natural DNA were stabilized by structural components. The dSLIM® family of TLR9 agonists is protected from exonucleolytic degradation by its covalently-closed dumbbell-shaped structure. It contains an immunomodulatory sequence with CG-motifs in its loops. The linear single-stranded EnanDIM® family of TLR9 agonists utilizes L-deoxyribonucleotides (natural enantiomers of D-deoxyribonucleotides) at their 3’-ends to prevent degradation. Since the mode-of-action of TLR9 agonists starts upstream of the targets of checkpoint inhibitors anti-PD-1/anti-PD-L1 a combinatorial approach may support synergistic immune activation and thus enhanced anti-tumor effects. Methods: The impact of dSLIM2006 and EnanDIM-1 on T cell responses was analyzed employing an in vitro assay using human peripheral blood mononuclear cells (PBMC). PBMC were treated with different doses of TLR9 agonists and combination with anti-PD-1 and anti-PD-L1. In vivo studies were used to investigate the anti-tumor effect of dSLIM2006 and EnanDIM-1 in combination with anti-PD-1 in a syngeneic murine CT26 tumor model. Results: The IFN-gamma secretion of human PBMC after stimulation of CEF peptides was roughly 5-fold increased by EnanDIM-1 and dSLIM2006, whereas treatment with anti-PD-1 resulted barely in a two-fold increase. The combination of the TLR9 agonists and anti-PD-1 further enforced IFN-gamma secretion by about 7-fold. In the murine colon cancer model CT26 the subcutaneous injection of EnanDIM-1 or intraperitoneal injection of anti-PD-1 had a moderate effect on the tumor growth when used in monotherapy (28.3% or 57.0% tumor growth inhibition, TGI). Notably, a combination of EnanDIM-1 and anti-PD-1 further reduced tumor growth (74.7% TGI). Intratumoral injection of dSLIM2006 in combination with intraperitoneal injection of anti-PD-1 reduced tumor growth (54.2% TGI) whereas the single components had more limited effects (dSLIM2006: 18.7%, anti-PD-1: no inhibition). Combined treatment with TLR9 agonist and anti-PD-1 prolonged survival of the mice in comparison to single treatments. Conclusions: The TLR9 agonists and immune surveillance reactivators (ISR) EnanDIM-1 and dSLIM2006 enhance T cell responses and anti-tumor effects of the anti-PD-1 checkpoint inhibitor. These data show their promising potential not only for monotherapy but also combinatorial approaches.

**#2623** Identification of neo-antigens driving melanoma response to immune checkpoint blockers via in vivo screening. Chi-Ping Day,1 Eva Perez-Guijarro,2 Rajaa El Meskini,2 Zoe Weaver Ohler,3 Maxwell Lee,1 Howard Yang,1 Suman Vodnala,1 Shyam Sharan,1 Glenn Merlino1.

**Immune checkpoint blockers (ICBs) have rendered unprecedented, durable responses in metastatic melanoma, but the heterogeneous response among patients continues to be the major obstacle for their therapeutic development.** It is generally hypothesized that neoantigens derived from mutated genes are involved in tumor response to ICBs, since the latter is correlated with mutational loads of tumors. However, direct experimental evidence showing that threshold optimal post-therapy 시행한 결과를 바탕으로 a) maintain an adequate T, APC and NK cell activity, b) reduce Treg and myeloid suppressors, c) avoid toxicity, and d) target cancer cells.

**#2620** Selecting the right chemotherapy partner for checkpoint inhibitors: an in vivo comparison of different drugs and dosages. Stefania Orechioni, Giovanna Talarico, Valentina Labanca, Patrizia Mancuso, Francesco Bertolini. European Inst. of Oncology, Milan, Italy.

**Introduction**

Immune checkpoint blockers (ICBs) have rendered unprecedented, durable responses in metastatic melanoma, but the heterogeneous response among patients continues to be the major obstacle for their therapeutic development. It is generally hypothesized that neoantigens derived from mutated genes are involved in tumor response to ICBs, since the latter is correlated with mutational loads of tumors. However, direct experimental evidence showing that threshold optimal post-therapy 시행한 결과를 바탕으로 a) maintain an adequate T, APC and NK cell activity, b) reduce Treg and myeloid suppressors, c) avoid toxicity, and d) target cancer cells.

**#2621** Microsatellite instability is a potential biomarker for immune checkpoint inhibitor in endometrial cancer. Hitomi Yamashita, Kentaro Nakayama, Noriyoshi Ishikawa, Toshiko Minamoto, Tomoka Ishibashi, Kohei Nakamura, Kaori Sanuki, Runiko Ono, Masako Ishikawa, Takeshi Iseobe, Satoru Kyo. Shimane University Faculty of Medicine, Izumo city, Japan.

**Introduction** In recent years, tumor cells have immune escape mechanism and immune checkpoint inhibitor therapy (anti PD-1/ PD-L1 antibody) has shown benefit in various cancers. Somatic mutations have the potential to encode "non-self" immunogenic antigens and lymphocytes infiltrate tumor cells in Microsatellite-instable (MSI) endometrial cancers. Therefore, immune checkpoint inhibitor therapy might be effective in MSI endometrial cancers. [Method] Mismatch repair protein (MLH1, PMS2, MSH2, and MSH6), tumor-infiltrating lymphocytes (CD8), and PD-1/PD-L1 expression were assessed by immunohistochemistry in 60 patients with endometrial cancer. We examined whether MSI status has enhanced immune microenvironment and become the therapeutic effect predictor of PD-1/PD-L1 immunotherapy in endometrial cancer. [Results] Loss of mismatch repair protein (MSI group) was identified in 8 (13.3%) of 60 patients with endometrial cancer. Expression of tumor-infiltrating lymphocytes (CD8) and PD-L1/PD-1 were significantly higher in MSI group compared to MSS group (p=0.001, p=0.044 and p=0.013). [Conclusion] These results suggested that immune checkpoint inhibitor (anti PD-1/PD-L1 antibody) is effective in endometrial cancers with MSI. MSI testing is likely to be a biomarker for PD-1/PD-L1 immunotherapy in endometrial cancer.
characterizing putative neoantigens. A “neo-epitope” library was generated by cloning the DNA sequences flanking non-synonymous mutations in frame with the eGFP gene in a lentiviral vector. We further showed that such eGFP-fused epitopes can be presented by the cells to induce specific T cell responses. The library will be transduced into the “resistant” Braf/PKDD melanoma, which will be treated with anti-CTLA-4 mAb in mice to test the immunotherapy response. To prevent immunity against eGFP expressed by tumors, the library-transduced melanoma cells will be transplanted into the eGFP-tolerant “glowing head” mice. The results will be used to determine if one, or more, of our candidate neo-epitopes can induce a response to anti-CTLA-4. We will also analyze if the response to this IC is an epitope-specific reaction or require multiple epitopes, which will help to identify resistance mechanisms. We hypothesize that our results will provide insight into the role of neoantigens in IC response. Moreover, our models will serve as a platform to study the specific contribution and predictive value of neoantigens for melanoma response to immunotherapy, which could help improve therapeutic strategies involving ICs.

**#2624** An FKBP51s-based immunophenotype for assessment of the immunosuppression status and possible prediction of immunotherapy response in melanoma patients. Simona Romano,1 Ester Simone,1 Anna D’Angelillo,1 Paolo D’Arrigo,1 Mario Capasso,1 Vito Alessandro Lasorsa,1 Martina Tufano,1 Anna Rea,1 Michele Russo,1 Nicola Zambrano,1 Paolo Antonio Asciento,2 Maria Fiammetta Romano,1,2 Univ. of Naples Federico II, Napoli, Italy; “National Cancer Institute “G Pascale”, Napoli, Italy; “National Cancer Institute, Napoli, Italy.

Background and aim: FKBP51s is an immunophilin encoded by FKBP5 gene on chromosome 6. It is a protein resident in lymphocytes and involved in immune response. Recently, our group demonstrated that the inhibitory checkpoint PD-L1/PD1 promoted the alternative splicing of FKBP5 gene, resulting in increased expression of its variant 4, in PRMC of melanoma patients. The aim of this study was to address whether such a molecular signature could help in identifying an immune profile associated with increased probability of immunotherapy response. Experimental Design: The splicing FKBP51isoform or FKBP51s was measured in peripheral blood T lymphocytes subsets (CD3/CD4, CD3/CD8, CD25 and PD-L1) and CD14 monocytes from a cohort of 118 patients and 77 age- and sex-matched healthy controls, by flow cytometry. Blood samples were collected before patients underwent ipilimumab treatment. Furthermore, in 64 out of 118 patients, expression of FKBP51s was also assessed in regulatory T cells. Results: Physiologically, each PBMC subset analyzed contained a fraction of an FKBP51s neg component, which resulted expanded in melanoma patients. We also measured an increased in CD3/CD8 and PD-L1 lymphocytes in patients. CD4 T lymphocytes showed the FKBP51s neg fraction significantly impaired, which might reflect the condition of impaired T cell help. Treg count was increased, in accordance with previous studies. The count of FKBP51s neg Treg defined a subgroup of nonresponders patients to ipilimumab, by 92.6%. A 2D hierarchical partitioning of data from FKBP51s-immunophenotype (heatmap) revealed 3 main clusters: C1 (33 pts, 51.5%), C2 (14 pts, 22%) and C3 (17 pts, 26.5%). FKBP51s neg Treg subset appeared globally increased in all clusters. In C1, values of effector T cells and monocytes were not different from normal donors. C2 showed a significant increase of FKBP51s pos PD-L1 monocytes and a significant probability of not responding (Chi-square 0.0019). C3 showed a significant increase of FKBP51s in overall lymphocyte subsets analyzed. Patients in this cluster showed reduced overall survival. This finding suggests that a prevalence of activation (CD25) and co-inhibitory (PD-L1) markers together with the expansion of FKBP51s pos effector T cells might reflect a condition of chronic lymphocyte stimulation in some advanced melanoma patients, contributing to T cell-exhaustion. Conclusions: FKBP51s-based immunophenotype of melanoma patients revealed several profiles virtually related to a negative immune regulatory control and highlights an impairment of a Treg subset endowing with increased suppressive potential. Such FKBP51s neg Treg subset is likely to be associated with immunotherapy response (Chi-square = 9.916, p = 0.002).

**#2625** Clinopathological features of programmed cell death ligand 1 (PD-L1) expression in resected non-small cell lung cancers. Yoshiohi Ohhara,1 Ichiro Kinoshita,1 Utano Tomaru,2 Kanako C. Hatanaka,2 Yutaka Hatakanaka,2 Hiroshi Takeuchi,1 Yasushi Shimizu,1 Ichiro Kaga,2 Yo-shihiro Matsumoto,2 Hiroshi Dosaka-Akita,1,2 Hokkaido University Graduate School of Medicine, Sapporo, Japan; Hokkaido University Hospital, Sapporo, Japan.

Background: Antibodies against programmed death 1 (PD-1) and programmed death ligand 1 (PD-L1) have recently demonstrated a survival benefit in several types of cancer, including melanoma and non-small cell lung cancer (NSCLC). PD-L1 expression has provided a predictive biomarker for anti-PD-1/PD-L1 therapy. However, the relationship between PD-L1 expression and clinical and clinicopathological characteristics remains unclear in NSCLCs. The aim of this study was to investigate the relevance of PD-L1 expression to clinical and clinicopathological characteristics in NSCLC. Methods: PD-L1 expression was evaluated in 154 surgically resected NSCLC specimens by immunohistochemistry (IHC) using the SP142 antibody. Before the IHC of clinical samples, we performed a concordance study of Western blot analysis and IHC in 17 lung cancer cell lines. We evaluated the relationship between PD-L1 expression and clinical and clinicopathological factors by univariate and multivariate analyses. Results: PD-L1 expression was strongly associated with tumor differentiation (P = 0.0019, Fisher test). The prevalence of PD-L1 in the NSCLC patient cohort was 68% (68/100) using a H-score cutoff of ≥ 7. Analysis revealed a lower positivity of 61.7% (37/60) in squamous carcinoma as compared to 77.5% (31/40) in non-squamous NSCLC. Analysis of the patient outcome data demonstrated that higher levels of PDV protein expression is correlated with poorer outcomes in patients both with non-small cell lung cancer (NSCLC) and adenocarcinoma. Conclusion: PDV protein expression could potentially serve as a new immune checkpoint biomarker in lung cancer. This study demonstrates that high PDV protein levels predict poorer outcomes in lung cancer patients; suggesting that PDV may be an important target for future immunotherapy.

**#2626** Expression of human poliovirus receptor, an immune checkpoint biomarker in lung cancer. Camila Kokczara,1 Hui Yu,1 Kim Ellison,2 Christopher Rivard,3 Leslie Rozeboom,1 Daniel Chan,1 Kenichi Suda,1 Kristine Brovsky,1 Rafal Dzdziszuk,3 Fred Hirsch,1,2 University of Colorado Anschutz Medical Campus, Aurora, CO; Medical University of Gdansk, Gdansk, Poland.

Background: Recently, immune therapy has shown notable efficacy in melanoma, non-small-cell lung cancer (NSCLC), among others, indicating that the targeting of immune checkpoints may be a promising treatment for malignant tumors. The human Poliovirus Receptor (CD155), an immune checkpoint, has been found to be overexpressed on the membranes of various tumor cells including colorectal and ovarian carcinoma. Examining PVR’s expression in NSCLC could shine light on PVR’s potential role and its possible correlation to the prognosis and outcomes for NSCLC patients. Material and Methods: Gene expression data for lung cancer cell lines and tumor tissues was derived from the Cancer Cell Line Encyclopedia (CCLE [www.broadinstitute.org/ccle]) and the TCGA database (www.cbioportal.org), respectively. Immunohistochemistry (IHC) was performed on a Ventana Benchmark XT platform using an anti-PVR antibody (Cell Signaling #D3G7H-1:100 dilution). IHC staining was evaluated for a NSCLC Cell Line Tissue MicroArray (TMA) and a NSCLC patient cohort TMA. The H-score system was used to generate a semi-quantitative score, ranging from 0 to 300, that evaluates the intensity and prevalence of staining on tumor cells. Patient data was analysed to uncover correlations between outcome and PVR expression in the NSCLC TMA cohort. Western blot analysis was performed to confirm antibody specificity and PVR expression levels in three NSCLC cell lines. Results: Analysis of mRNA expression data from CCLE and TCGA databases identified that PVR is highly expressed in lung cancer. Three lung cancer cell lines were chosen based on mRNA expression for this study: NCI-H211 (high), NCI-H1650 (moderate) and NCI-H1187 (negative). Cell line data revealed that PVR demonstrated higher expression levels in lung cancer than other immune checkpoints, such as PD-L1, PD-L2, and Gal-9 among others. In addition, PVR expression was not correlated with other negative immune checkpoints including PD-L1 and Gal-9. Western blot analysis confirmed the Cell Signaling antibody was specific to the PVR protein in NSCLC cell lines. IHC staining of NSCLC cell lines revealed that non-squamous NSCLC cell lines express higher levels of PVR than squamous NSCLC cell lines (p = 0.0001, Fisher test). The prevalence of PVR in the NSCLC patient cohort was 68% (68/100) using a H-score cutoff of ≥ 7. Analysis revealed a lower positivity of 61.7% (37/60) in squamous carcinoma as compared to 77.5% (31/40) in non-squamous NSCLC. Analysis of the patient outcome data demonstrated that higher levels of PVR protein expression is correlated with poorer outcomes in patients both with non-small cell lung cancer, and adenocarcinoma. Conclusions: PVR protein expression could potentially serve as a new immune checkpoint biomarker in lung cancer. This study demonstrates that high PVR protein levels predict poorer outcomes in lung cancer patients; suggesting that PVR may be an important target for future immunotherapy.
The synergy between BXLCL701, a DPP inhibitor, and immune checkpoint inhibitors discovered using AI and Big Data analytics. Luca Rastelli, Snigdha Gupta, Akhil Dahiya, Zeenia Jagga, Krishnan Nandabalan, Sana-tan Upmanyu. BioXcel, Branford, CT.

Using the proprietary Big Data PharmGPS® Discovery platform, BioXcel has created a comprehensive relationship map between immune-evasion and immune-activation pathways, comprising interacting genes and all overlapping pharmacological agents and tumors. This map was used to identify clinically validated compounds that would act synergistically in combination with immunotherapies. Such an effective therapeutic approach could counteract the tumor-promotive microenvironment and transforming cold, non-inflamed tumors into hot immune-sensitive tumors. One of the several compounds thus identified is BXLCL701, previously known as Talabostat/PT-100, a DPP inhibitor that by inducing a wide panel of cytokines and chemokines stimulates both the innate and acquired immune system. BXLCL701 has a dual immune-oncology related MOA. Via the Fibroblast Activating Domain (FAD) of BXLCL701, it up-regulates stress-responsive genes in fibroblasts and through an angiogenic related effect, it increases immune cell extravasation into the tumor tissue. Via the DPP8/9 targets, it depresses the immune-suppressive activity of MDCs by inducing a granulocytic differentiation while it stimulates the priming, migration and cytotoxicity of T-cells and NK cells and the formation of memory T-cells. The hypothesis that BXLCL701 immune-mediated MOA would complement the action of ICIs was validated in-vivo in the syngeneic MC38 mouse model of colon adenocarcinoma. Co-administration of BXLCL701 combined with anti-PD1 showed a synergistic inhibition of tumor growth as well as synergistic up-regulation of immuno-stimulatory cytokines, IL-2, IL12 and GM-CSF. The effects of the combination on the immune-phenotyping of the circulating and tumor infiltrated immune cells will be presented at this meeting. The demonstrated anti-angiogenic and immune-suppressive tumour microenvironment to an immuno-permissive milieu sensitive to immune-checkpoint inhibitors. Further supporting the therapeutic potential of BXLCL701, an analysis of genomic alterations in FAP, DPP8 and DPP9 across a wide range of tumors singled out castration-resistant prostate cancer with a high level of DPP9 amplification (14%) and overexpression of DPP8 in 50% of the patients which could make this patient population uniquely sensitive to the combination as shown by in-vitro and in-vivo experiments. This study provides further evidence of the capability of Big Data analytics to generate in-silico hypothesis of synergistic combination effects that can be converted in validated therapeutic opportunities to benefit patients non-responsive to ICI therapy.

The cellsurfaceantigenLymphocyteantigen75(LY75,CD205,DEC-205)isnotableas a target for a novel ADC. MEN1309 is a humanized IgG1 antibody drug conjugate (ADC) targeting Ly75 antigen, induces complete responses in several xenografts of solid tumors. Mario Bigioni,1 Giuseppe Merlino,1 Cristina Bernardino Morales,2 Rossana Bugnani,1 Attilio Crea,1 Rossanna Manno,1 Joaquin Arribas,2 Rachel Dusek,4 Nickolas Attanasio,1 Keith Wilson,4 Christian Rohlff,5 Monica Binaschi1. 1Mennarini Ricerche S.p.A., Pomezia (RM), Italy; 2Vall D’Hebron, Institute of Oncology, Barcelona, Spain; 3Research Toxicology Center, Pomezia (RM), Italy; 4Oxford BioTherapeutics, Inc., San Jose, CA, USA; 5San Jose, CA, USA; 6Oxford BioTherapeutics, Ltd., Abingdon, UK.

The cell surface antigen Lymphocyte antigen 75 (LY75, CD205, DEC-205) is over-expressed in several tumor histotypes. It is a type I C-type lectin receptor (CLR), normally expressed on various APC subsets, characterized by a cytoplasmic domain containing protein motifs crucial for endocytosis and internalization upon ligation. These features make the antigen ideal to be exploited as a target for a novel ADC. MEN1309 is a humanized IgG1 antibody directed against the cell surface antigen Ly75, conjugated through a cleavable linker to a potent maytansinoid microtubule disruptor, DM4. In this study, we evaluated the in vitro and in vivo (xenografts and PDX) efficacy of MEN1309 in different tumor histotypes. A PK/PD relationship was also investigated in tumor-bearing mice. HIC demonstrated high prevalence of LY75 in human pancreatic, triple negative breast, and bladder cancers, as well as in diffuse large B-cell lymphoma. In vitro experiments showed that cytotoxic activity of MEN1309 was in mM/sub mM range against several lymphoma, pancreatic, bladder and triple-negative breast cancer (TNBC) cell lines. Moreover, MEN1309 exhibited high cell-kill ability against cells having either strong as well as low to moderate antigen expression. In vivo, MEN1309 at 2.5-5 mg/kg (schedule varying from single dose, q2dx3, or q21dx3) showed an impressive antitumor activity, resulting in complete and long lasting responses in most of the xenograft models representing lymphoma, TNBC, bladder and pancreatic cancers, expressing the antigen at high but also at low levels. No treatment related toxicity in terms of change of body weight and death events were detected. Moreover, the administration of (i) the control-DM4 and (ii) the non-conjugate antibody IgG1 and (iii) the free toxin DM4 (at a dosage corresponding to the equimolar concentration linked at 10 mg/kg ADC) showed little to no therapeutic efficacy on tumor growth. In TNBC patient-derived xenograft (PDX) model (coming from a heavily pre-treated patient and expressing high level of the antigen
In syngeneic mouse model.

large fraction of cancer patients doesn’t respond to the antibody immunotherapy and lots of patients are suffered from immune-related adverse events. Still, large fraction of cancer patients doesn’t respond to the antibody immunotherapy. INF-α/β contributes to tumors suppression in a CT26 bearing syngeneic mouse model. Cancer immunotherapies like antibodies targeting immune checkpoint molecules have resulted in an extraordinary success in cancer treatment. Still, large fraction of cancer patients doesn’t respond to the antibody immunotherapy and lots of patients are suffered from immune-related adverse events (irAEs) which sometimes cause discontinuation of the treatment. The above problems highlight the needs in the cancer immunotherapy for attempting various approaches like synergistic combination therapy to minimize adverse events and enhance efficacy. Interferon-γ is a cytokine having antitumor activity and has been developed as an anticancer drug in multiple cancer indications but failed due to its limited efficacy and severe adverse events. In several publications, IFN-γ has been clarified to induce PD-L1 expression in tumors. Upregulation of PD-L1 renders tumor resistance to cytotoxic T cell and it may cause the limited efficacy of IFN-γ therapy. Our preliminary experiments suggested that an intratumoral injection of purified IFN-γ and following addition with anti-murine PD-1 antibody showed significant tumor suppression, implying a combination effects of regional IFN-γ and immune checkpoint blockers. Here in the present report, in an aim of decreasing adverse events, we have created recombinant Bifidobacterium, which is a non-pathogenic anaerobic bacterium to secrete INF-γ specifically inside solid tumor, and then demonstrated the combination of IFN-γ producing Bifidobacterium and anti-murine PD-1 antibody significantly suppress tumor growth whereas each single treatment moderately contributes to tumor suppression in a CT26 bearing syngeneic mouse model. In addition, INF-γ antibody indicated that there are substantial IFN-γ detected in tumor tissue but none of them are detectable in blood on the Day 4 of treatment. All in together, combination treatment with IFN-γ producing Bifidobacterium and anti-murine PD-1 antibody offers a promising anti-tumor approach. Reference: 1. Abiko K., Br J Cancer, 2015 Apr 28;112(9):1501-9. INF-γ from lymphocytes induces PD-L1 expression and promotes tumor progression. 2. Matsumoto, Y., H. Ishida, T. Yamamoto, et al., Cancer Sci. 2014 Apr;105(4):1236-44. Inhibition of mitogen-activated protein kinase pathway can induce upregulation of human leukocyte antigen class I without PD-L1-upregulation in contrast to interferon-γ treatment.

4SC-202 induces inflamed tumor microenvironment, strongly enhances tumor infiltration with cytotoxic T cells and primes tumors for anti-PD1/PD-L1 therapy. Tanja Wulf, Kerstin Kronthaler, Svena Schrepfer, Ulrike Parmitzke, Anne Catherine Bretz, Roland Baumgartner, Svetlana Hamm. 4SC Martinried, Germany.

Various HDAC inhibitors were described as beneficially affecting antitumor immune response. Although different HDAC inhibitors were investigated in syngeneic tumor models, the mode of anti-tumoral action is not yet fully understood. Here, we analyzed the anti-tumoral mode-of-action (MOA) of 4SC-202, an orally available clinical stage epigenetic small molecule inhibitor specifically targeting histone deacetylases HDAC class I as well as the lysine-specific demethylase LSD1. To ensure that the conclusions would be relevant for the clinical situation we used a clinically equivalent dose and schedule. Anti-tumoral efficacy and the impact of 4SC-202 alone or in combination with an anti-PD-L1 antibody on the tumor microenvironment were analyzed in the syngeneic colorectal CT26 model in immunocompetent BALB/c or in nude/irradiated BALB/c mice. A broad spectrum HDAC inhibitor was used for comparison. Transcriptome analysis was performed by RNA-Seq, and the composition of immune cell subpopulations in the tumor was determined by flow cytometry. 4SC-202 treatment at clinically relevant dosage regimen significantly inhibited growth of CT26 tumors. A competent immune system was apparently necessary for the anti-tumoral effect of 4SC-202 since its tumor-reducing effect was lost in immunocompromised mice. 4SC-202 treatment led to an increase of MHC class II molecules and CD8+ T cells in combination with anti-PD-L1 enhanced expression of inflammatory markers like IFN-γ and various chemokines in the tumors. Detailed analysis of the tumor microenvironment revealed that 4SC-202 strongly increased the number of tumor-cidal cytotoxic T cells (CTL). In contrast, a broad-spectrum HDAC inhibitor tested in the same model demonstrated anti-tumoral efficacy but did not affect the number of CTLs in tumors demonstrating that HDAC inhibitors employ different MOAs for their anti-tumoral response and that the effect on CTLs is not attributed to HDAC inhibition in general. Since the T cell abundance is pre-requisite for the efficacy of PD1/PD-L1 blockade, we tested the combination of clinically relevant dosage regimen of 4SC-202 with an anti-PD-L1 antibody. The combined treatment was more efficacious than monotherapies and resulted in significantly longer survival. 4SC-202 already demonstrated a favorable safety profile in a phase I clinical trial with relapsed or refractory hematological malignancies with two objective responses (1 CR, 1 PR) and disease stabilizations in several patients. 4SC-202’s immune priming capacity offers further options for clinical development of 4SC-202 in combination with various cancer immunotherapy approaches.


In collaboration with Sanoﬁ, we previously showed that inhibition of the VEGFR3 pathway by SAR131675 leads to reduction of tumor growth by acting on the tumor microenvironment. We have identiﬁed a new drug candidate, EV801, and evaluated its activity on tumor models expressing VEGFR3. For proof-of-concept, we transfected the mouse BNL hepatoma cell line with VEGFR3. EV801 displayed a strong therapeutic activity by acting on both VEGFR3 and PD-1 expressing cells. We then employed a functional analysis in the following model: VEGFR3 small molecule inhibitor (SAR131675) and CD4+ regulatory T cells by and increasing macrophages with a M1 phenotype inside the tumor. As a consequence the T-cell:MDSC ratios were increased in the TME and also in peripheral blood. In addition to its immunomodulatory properties, EV801 decreased angiogenesis without increasing hypoxia. We have evaluated EV801 therapeutic activity in syngeneic tumor mouse models which are not expressing VEGFR3 such as 4T1 mammary carcinoma and CT26 colon carcinoma models. As expected, we observed an intermediate therapeutic activity of the compound on
both tumor models. Positive modulation of the TME was equivalent to what we observed with the VEGFR31 tumor model. With this unique mechanism of action of EVT801 on the TME, we evaluated its ability to increase therapeutic activity of standard Immune Checkpoint Therapies (ICT) such as anti-CTLA-4 and anti-PD-1 mAbs. Strong additive therapeutic activities were observed with EVT801 in combination with these ICTs, indicating the potential of long-term tumor-specific memory CD8 T cell responses. Toxicological data show that EVT801 has a favorable pharmacological profile consistent with its entry into pre-clinical development. Taken together, these results indicate that EVT801 represents an innovative drug for cancer Immunotherapy which provide a favorable microenvironment to promote tumor regression. In addition, EVT801 may improve the frequency of response to ICT.


The anti-PD-1 monoclonal antibody nivolumab is clinically active in a variety of tumor types including squamous (sq) and non-squamous (non-sq) NSCLC in second-line, where randomized phase III trials have shown a survival benefit. However, no predictive/prognostic or diagnostic biomarkers have been found so far to correlate with clinical benefit in patients treated with anti-PD-1 antibod-ies. The aim of the present study is to investigate the potential role of baseline peripheral blood cell counts in relation to survival and response rate in NSCLC patients treated with nivolumab in a second-line setting. From July to May we evaluated 45 patients with Sq (n = 10) and non-Sq (n = 35) NSCLC, previously treated with first-line platinum-based chemotherapy, who received nivolumab 3 mg/kg iv on day 1 of each 2 week treatment cycle. Clinical characteristics (T-Stage, lymph nodes involvement, M-Stage) were assessed, total numbers of white blood cells, myeloid-derived suppressor cells (MDSCs, including both monocytic [Mo-MDSC] and polymorphonuclear [PMN-MDSC] types), regulatory T cells (T-reg), and serum lactate dehydrogenase (LDH) were assessed. Endpoints were correlations with objective response rate (RR), progression-free survival (PFS, categorized as 3 or > 3 months) and overall survival (OS). Tumor response was assessed using RECIST criteria, version 1.1, at week 9 and every 6 weeks thereafter until disease progression. Statistical in-vestigations were based on univariate analyses by the Wilcoxon rank test. The median PFS of the overall study population was 3 months. Data about PMN-MDSCs (identified by flow cytometry as Lin-CD14+CD11b+HLA-DRhigh), Mo-MDSCs (Lin-CD14+CD11b+HLA-DRhigh) and absolute eosino-phil count (AEC) were available in 37/45 patients (82% of treated patients). Baseline absolute numbers of PMN-MDSCs, Mo-MDSCs and AEC were greater in patients with a good prognosis (PFS > 3 months) and a better RR. In particu-lar, among patients with shorter PFS and lower RR, the median numbers of PMN-MDSCs, Mo-MDSCs and AEC were significantly lower than those de-tected in patients with longer PFS (4 vs 13 cell/μl, p=0.01; 4 vs 21 cell/μl, p=0.006; 55 vs 155 cell/μl, p=0.02, respectively). Our data suggest that a baseline blood signature characterized by low levels of PMN-MDSCs, Mo-MDSCs and AEC is associated with a poor clinical outcome (median PFS ≤ 3 months and low RR) in 67.6% of patients treated with nivolumab. In contrast, patients with high levels of these three biomarkers showed a median PFS significantly longer than 3 months and a higher RR. The OS analysis is ongoing, and further studies have been planned to understand whether this signature has a biomarker poten-tial also in chemotherapy-naive, first line NSCLC patients.

#2636 Targeted therapies to ERBB receptors downregulate expression of PD-1: implications in combination therapies. Christopher A. Hamm,1 Sumin Zhao,2 Cesar A. Santa-Maria,3 Massimo Cristofanilli,4 Neil L. Spector,2 Sarah Bacus1.

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Background: Inflammatory breast cancer (IBC) is the most aggressive and lethal form of primary breast cancer. Inflammatory signaling pathways are active in IBC, but the function of the immune response remains elusive. ErbB receptors play a role in IBC. ERBB2 is amplified in 50% of IBCs, and ERBB3 is also mutated in IBC. Currently, Lapatinib, a dual ErbB inhibitor, is used in IBC patients with ERBB2 amplification. Although immune cell inflammatory signaling may promote IBC tumor growth and metastasis, the presence of cytotoxic tumor-associated lymphocytes has also been associated with a more favorable breast cancer prognosis. The contradictory nature of the immune data high-lights the need to elucidate the relationship between the immune response and subsequent treatments. Given the active IBC immune component and the recent clinical benefit of immune checkpoint inhibitors in cancer treat-ment, IBC’s may represent a clinically unique breast cancer population that may benefit from immunomodulating agents. Interestingly, Myc is a down-stream effector of ErbB signaling, and Myc is thought to regulate the expres-sion of immune checkpoint proteins CD47 and PD-L1. The presence of PD-L1-positive IBC immune infiltrate suggests that IBC’s may benefit from therapies that disrupt PD-L1 signaling together with ErbB inhibitors. METHODS: IHC was used to examine immune checkpoint signaling and characterize the tumor-immune infiltrate. Tumor tissues were also charac-terized using a RNA-seq panel that examined the expression of 377 im-mune-related genes. Cell lines (BT474, SKR3, AU565, and SUM225) were treated with Lapatinib and Neratinib to examine the relationship between growth factor receptors and downstream immune signaling pathways. RE-SULTS: RNA-seq revealed the expression of specific immunosuppressive signaling pathways in tumors. Treatment of breast cancer cells with ErbB inhibitors resulted in a decrease in the levels of PD-L1. Treatment with Lapatinib and Neratinib diminished PD-L1 in all cell lines. Levels of Phos-pho-Stat3 decreased in BT474 and Sum225 but not in SKBR3 and AU565, implying that PD-L1 is regulated by another mechanism (ERK-MYC). CONCLUSION: Our results provide mechanistic insight into ErbB receptor acti-vation and the expression of downstream signaling molecules (Stat3, PD-L1, Myc) in IBC. In addition, our unique RNA signature reveals the expression of several genes that may serve as biomarkers of in-hibitory immune signaling pathways. Our laboratory is currently examining the correlation between PD-L1 expression and the activation of ErbB2 in model systems and clinical trials using ErbB inhibitors in combination with PD-L1 inhibitors. Our immune panel gene signature may serve as a useful diagnostic tool that, in conjunction with traditional ErbB testing, can identify patients that will benefit from combination therapy of an ErbB inhibitor and an immune checkpoint inhibitor.

#2637 Anti-tumor activity of cisplatin is enhanced by PD-1 blockade in preclinical models of head and neck squamous cell carcinoma. Linda Tran,1 Clint T. Allen,2 So-Jin Park,2 Roy Xiao,3 Carter Van Waes,4 Nicole C. Schmitt.5 NIDCD, Bethesda, MD; 6Johns Hopkins University and NIDCD, Bethesda, MD.

Rationale: Cisplatin, which remains the most commonly used systemic drug for head and neck squamous cell carcinoma (HNSCC), reduces numbers of circulating immune cells. However, recent studies suggest that cisplatin may enhance some aspects of anti-tumor immunity in the tumor microenvironment. We previously found that cisplatin increases expression of major histocompati-bility (MHC) class I and programmed death ligand 1 (PD-L1) in HNSCC cell lines in vitro. Methods: In the current study, we investigated other components of the antigen processing machinery (APM) by flow cytometry following treat-ment with cisplatin. Using the syngeneic mouse oral cancer 1 (MOC1) model of HNSCC, we then investigated the effects of cisplatin and anti-PD-1 antibody, alone or in combination, on tumor growth and survival. Results: Using three different HNSCC-cell lines, we found that levels of the APM components TAP1/2 and HLA-DRA were significantly increased in all cell lines treated with cisplatin, whereas TAP1/2 were dramatically increased only in one cisplatin-treated cell line with wild-type TP53 (UMSCC-74A). In MOC1 tumor-bearing mice, tumor growth was partially delayed by treatment with cisplatin and anti-PD-1 antibody alone and substantially delayed when these agents were given in combination, with result-ant increased survival. Comparison of different treatment schedules suggested that concurrent administration is more effective than giving cisplatin one day prior to anti-PD-1 or one day prior to cisplatin. Conclusions: Despite upregulating PD-L1 on tumor cells and decreasing levels of circulating immune cells, cisplatin may enhance some aspects of anti-tumor immunity in the tumor microenvironment. While no mice were cured with concurrent cis-platin and anti-PD-1 antibody, tumor growth was substantially delayed. These results suggest a rationale for combining cisplatin and anti-PD-1 monoclonal antibodies, perhaps with radiation or other therapies, in additional preclinical studies and clinical trials of HNSCC. Supported by NIDCD intramural project ZIA-DC-D000090.


Introduction: Pulmonary sarcomatoid carcinoma (PSC) is a poorly differen-tiated non-small cell lung cancer (NSCLC) containing a component of spindle cell or giant cell. Because the prevalence of PSC is very rare, there is
insufficient data about clinical feature, therapeutic strategy and prognosis. Method: We reviewed the medical records of 26 patients diagnosed with PSC from January 2009 to June 2015, and analyzed the clinicopathological characteristics, treatment modality and their outcome, and risk factors of overall survival (OS). Programmed death ligand 1 (PD-L1) expression was tested in 13 patients using immunohistochemical staining of formalin-fixed tissue sections on the A058 antibody. Potent and highly selective small molecules were generated to block either A0 generation or A0 signaling. The therapeutic potential of these molecules was assessed in CD8+ T cell assays, MLR assays and in various tumor models. CD73 inhibition blocked the conversion of AMP to A0. CD73 inhibition blocked the conversion of AMP to A0 and A0R inhibition abolished the increase in adenosine concentration. Results: The median age of PSC patients was 286 days. Age and the time from symptom onset to diagnosis were significantly associated with OS. PD-L1 expression showed the trend of positive prognostic value on OS in univariate analysis but was insignificant in multivariate analysis. 12 patients were treated with chemotherapy, 9 with platinum-based doublet therapy, 2 with tyrosine kinase inhibitor and 1 with docetaxel. 7 patients showed partial response or stable disease. The median OS and progression free survival of patients receiving chemotherapy were 260 days and 85 days, respectively. Conclusions: It has poor prognosis compared with normal NSCLC. However, PSC patients showed comparatively fair response to chemotherapy and generally showed higher PD-L1 expression. A0 staining was correlated with treatment with cytotoxic chemotherapy. EGFR TKIs or immune checkpoint inhibitors are necessary to achieve a better results of PSC management.

#2639 Development of small molecule selective inhibitors of GCN2 as an immunotherapy aimed at preventing immune escape of tumor cells. Michał Gałązowski, Kamil Sitarz, Eliza Majewska, Stefan Chmielewski, Kinga Michalik, Magdalena Masieczyk, Agnieszka Adamus, Arkadiusz Bialas, Joanna Fogt, Marcin Bien, Magda Rzóska, Maciej Mikulski, Julian Zachmann, Krzysztof Brzózka, Selvita S.A., Kraków, Poland.

GCN2 is a protein kinase capable of sensing amino acid (AA) shortage. It gets activated by binding of unloaded tRNAs and subsequently phosphorylates translation initiation factor 2 alpha (eIF2a) eventually leading to induction of ATP4-mediated integrated stress response (ISR). Elevated catabolism of one of the essential amino acids - tryptophan (Trp), driven by overexpression of critical enzymes in Trp metabolism - IOD and TDO, leads to immunosuppressive microenvironment in many types of cancer. GCN2 is a key effector signaling component for IOD/TDO and is considered as a metabolic checkpoint of highly Trp-dependent T-cells. GCN2 activation through accumulation of unloaded tRNAs leads to inhibition of CD8+ effector T-cells and increase in generation and activation of regulatory T-cells. According to these findings, selective inhibition of GCN2 may be an effective strategy for targeting Trp-dependent immune surveillance of tumor cells. In this study, we report the results for a series of novel small molecule GCN2 kinase inhibitors that have been developed at Selvita and, to our best knowledge, are the most potent GCN2 inhibitors reported so far. Newly synthesized compounds exert low nanomolar potency for GCN2, good selectivity as well as by using archival tissues (PD-L1 IHC 22C3 pharmDx assay), GCN2 was highly selective relative to related enzymes/receptors, as well as a large panel of unrelated targets. Inhibition of one of the three targets robustly reversed adenosine-mediated inhibition of proliferation, CD25 expression, and IFN-g and granzyme B production by human CD8+ T-cells. Robust inhibition of tumor growth in combination with anti-PD1 antibody was observed for several of these compounds. Conclusions: Highly potent and selective inhibitors of each of the 3 molecular targets involved in the ATP/adenosine pathway have been identified. Their ability to interfere with the extracellular generation of A0 and the immune-suppressive effects of A0, as well as their effects on experimental tumor biology, have been demonstrated in various in vitro and in vivo models.

#2641 AZD4635 A0R receptor occupancy in cyromulgous monkey using PET and its application to the genomic clinical trial: Peter Johnström,1 Pablo Morentin Gutierrez,2 Katarina Varna,3 Magnus Schou,1 Akhiro Takano,2 Lorraine Jones,1 Ganesh Mugundu,3 Patricia McCoon,2 Paul Lyne,3 Jeffrey Infante,4 Gerald Falhcook,5 Manish Patel,5 Janet Karlis,6 Melinda Merchant,5 James Clarke,5 Alan Cross,6 Nicholas Seneca,6 Lars Farde,6 Miles Congreve,5 Jon S. Mason,7 Fiona H. Marshall3 1AstraZeneca, Stockholm, Sweden; 2AstraZeneca, Cambridge, United Kingdom; 3Karolinska Institutet, Stockholm, Sweden; 4AstraZeneca, Macclesfield, United Kingdom; 5AstraZeneca, Waltham, MA, 6Sarah Cannon, Nashville, TN; 7Heptares, Welwyn Garden City, United Kingdom.

Introduction: AZD4635 is an A0R receptor antagonist currently being tested as monotherapy and in combination with durvalumab in patients with advanced solid cancers. High adenosine levels found in tumors are immune suppressive and therefore AZD4635 could potentiate immune checkpoint inhibitors such as durvalumab (anti-PD1). Predictions of A0R receptor engagement in patients at different doses and at different time points may enable better interpretation of clinical biomarker data measuring effects on immune modulation. A quantitative assessment of the receptor occupancy in the brain of non-human primates was conducted for AZD4635 with PET imaging and the resulting PK/PD model was applied to predict occupancy in humans in tumors. Methods: PET measures of A0R occupancy in brain was performed using the radioligand [18F]MNI-444 in three anesthetized cyromulgous monkeys. PET data acquisition was performed for 120 min following IV-administration of [18F]MNI-444 at baseline and following pretreatment of AZD4635. Sampling for AZD4635 plasma exposure determination was performed. As part of PK/PD analysis of the occupancy data, a novel modification of the non Invasive-LOGAN data analysis of the PET data was performed to obtain a time course of occupancy for each dose. A bio-phase PK/PD mathematical model was then used to describe the relationship of occupancy with circulating concentrations of AZD4635. In parallel, a PK model for AZD4635 in humans was developed using data from cohort 1 (Clinical trial NCT02740985) after 125 mg and used for PK predictions for alternative doses of AZD4635 in the clinic. Results: A Clear Exposure-Effect relationship was observed for AZD4635-driven A0R occupancy in cyno brain when dosed 30 min prior to PET measurement. The PK/PD analysis of cyno PET-determined receptor occupancy provided an OCC50 that is in line with the in vitro potency for the compound under physiological concentrations of adenosine in the brain. The PK/PD model provided a prediction of the level of occupancy in human tumours at other clinically relevant doses. Different simulations were done varying the amount of endogenous adenosine levels. Conclusions: AZD4635 was shown to occupy A0R in cyno brain in an exposure dependent manner. The resulting PK/PD model built using this data set was used to run simulations of expected tumor receptor occupancy in man and the results correlated well with clinical findings. The PK/PD program is predicted to provide ~90% receptor occupancy over the whole dosing interval at a clinically relevant dose.
Hermiston, 2 Stephan A. Grupp, 1 Brent L. Wood, 3 David T. Teachey

Proceedings of the American Association for Cancer Research

#2642 Preclinical efficacy of daratumumab in acute lymphoblastic leukemia. Karen Lee Bride, 1 Tiffaney Vincent, 1 Soo-Yeon L. Im, 1 Tori Fuller, 1 The- reesa Ryan, 2 David M. Barrett, 1 Shannon L. Maude, 1 Mignon L. Loh, 2 Michelle L. Hermiston, 2 Stephan A. Grupp, 1 Brent L. Wood, 2 David T. Teachey, 1 CHOP, Philadelphia, PA; 2UCSF Medical Center - Mission Bay, San Francisco, CA; 3Seattle Children's Hospital, Seattle, WA

Targeted immunotherapy has become critical for the successful treatment of many forms of cancer, particularly therapeutic antibodies with cytotoxic abilities. No safe and effective immunotherapies have been developed for T-cell acute lymphoblastic leukemia (T-ALL). CD38 is a transmembrane glycoprotein found on the cell surface of activated T cells, terminally differentiated B cells, but relatively low levels on normal lymphoid and myeloid cells. Daratumumab (dara) (Darzalex, Janssen Biotech, Inc.) is a human IgG1 monoclonal antibody that binds to a unique CD38 epitope and was recently FDA approved for the treatment of refractory multiple myeloma. In order to ensure CD38 is a relevant target in T-ALL and surface expression does not change with chemotherapy, we evaluated CD38 expression by flow cytometry (FACS) from 21 patients with T-ALL (10 early T-cell precursor (ETP) and 11 non-ETP) at diagnosis and one month of induction chemotherapy. All of the samples had detectable CD38 expression which did not change significantly after induction (mean CD38 MFI at diagnosis vs end-induction: 3.27 log vs 3.19 (S.I.; p = 0.25). Therefore we hypothesized that targeting CD38 with dara would be effective against T-ALL. In order to test this hypothesis, we xenographed primary ALL blasts from 15 different patients with ETP-ALL and 8 with non-ETP-ALL with ara randomized to dara (200 μg/mouse intraperitoneally (IP) weekly) vs control (isotype control at 200 μg/mouse IP weekly; 5 mice per arm for each sample) after they developed ≥1% peripheral blood (pb) blasts by FACS. Disease burden was assessed by FACS enumeration of pb blasts weekly and splenic blasts at sacrifice. We demonstrate striking efficacy of dara monotherapy in 6 of 7 ETP-ALL samples with reduction of pb and splenic blasts. Mice treated with high disease burden from 5 of the 8 non-ETP samples were moribund immediately after dara injection, possibly from aggregates of antibody bound to tumor cells leading to pulmonary embolism or tumor lysis, and were therefore inevaluable. We have since repeated the experiments for 5 of the samples, treating the mice after injection but before detectable engraftment of peripheral blasts. Dara was effective in all 3 samples. Experiments are ongoing for the other 2 samples. In summary, we found dara is a highly effective novel monotherapy for T-ALL in preclinical models. Based on these results, we are developing an early phase trial for children and young adults with relapsed/refractory T-ALL.

#2643 Thymic precursor cells generate acute myeloid leukemia in NP23-NHD13 double transgenic mice. Subhadip Kundu, 1 Eun Sil Park, 2 Yang Jo Chung, 1 Trevor Stephen Barlowe, 3 Peter D. Aplan 1, 3

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In previous studies, we have shown that thymic progenitors are potently leukemogenic, and that AML cells or residual CD4-/CD8-doublenegative (DN) thymocytes can be selectively targeted for elimination by the immune system by engaging cross-talk with T and B cells as well as activation of NK cells. In this study, we wanted to test if thymic progenitors can generate AML. We generated NP23-NHD13 double transgenic mice that express Mac1 in DN1 thymocytes again transmitted a Mac1 + Gr1+ AML. These cells were transplanted; all recipients were anemic, and demonstrated engraftment of NP23-NHD13 myeloid cells as well as a less prominent (7.5%-38.5%) population of CD11b+ Ter119+ erythroid cells in the BM and spleen. Taken together, these results indicate that NP23-NHD13 thymic progenitors are potentially leukemogenic, and retain myeloid and erythroid differentiation potential.

#2644 SY-1425, a selective RARα agonist, induces high levels of CD38 expression in RARA-high AML tumors creating a susceptibility to anti-CD38 therapeutic antibody treatment. Kathryn Austgen, Michael R. McKeown, Darren Hicok, Emily Lee, Chris Fiore, Matthew Richter, Christian Fritz, Tracey Lodie, Eric Olson, Syros Pharmaceuticals, Cambridge, MA

CD38 is a cell surface protein expressed primarily on white blood cells and considered a marker of differentiation initiation. CD38 is involved in the immune system by engaging cross-talk with T and B cells as well as activation of NK cells. In multiple myeloma (MM), a subset of tumor cells have high CD38 expression (CD38+), which has led to the development of effective anti-CD38 therapeutic antibodies, such as daratumumab (DARA). Thus, cancer cells that express CD38 can be selectively targeted for elimination by the immune system using these therapeutic antibodies. In multiple myeloma, DARA is most effective in patients whose tumor cells are CD38+ . In contrast, CD38 expression in AML tumors is generally observed to be negative (CD38−) or dim (CD38dim), and DARA has not shown activity in preclinical AML models. We previously reported that SY-1425, a clinical stage RARα agonist with improved pharmacokinetics, potency, and selectivity over pan-retinoic acid agonists, induces differentiation in non-APL AML cell lines and primary patient samples with a RARA super-enhancer associated biomarker (RARA-high). Since CD38 was found to be among the most differentially expressed genes in response to SY-1425, we hypothesized that SY-1425 mediated CD38 induction to levels comparable to MM may sensitize RARA-high AML cells to anti-CD38 therapy. We demonstrate that SY-1425 treatment of four RARA-high AML cell lines and four RARA-primary AML patient PBMCs induces the CD38+ phenotype, as measured by flow cytometry, similar to that found in the DARA sensitive MM cells. In contrast, we see no induction in RARA-low cell lines. We then demonstrated the activity of the SY-1425 and DARA combination on an ex vivo NK cell co-culture assay. Two RARA-high AML cell lines treated with SY-1425 and DARA were co-cultured with NK cells and monitored for both antibody dependent cell-mediated cytoxicity (ADCC) and NK cell activation by interferon gamma production. The combination of SY-1425 and DARA led to a six-fold increase in tumor cell death relative to the single agent controls, and 5-10 fold increases in NK cell activation is observed only in the SY-1425 and DARA combination treatment of RARA-high AML cell lines. Neither single agent, when administered alone, resulted in ADCC. Furthermore, a RARA-low AML cell line does not respond in the ADCC assay following the combination treatment due to the lack of CD38 induction in this SY-1425 insensitive line. In summary, we have identified a novel and rational combination treatment approach for a subset of patients with RARA-high AML. By inducing the expression of CD38, SY-1425 in combination with DARA elicits tumor cell death and NK cell activation. Based on these findings, a phase 2 clinical study with SY-1425 in combination with an anti-CD38 antibody is planned in AML using a RARA biomarker patient selection strategy.

#2645 Development of a first in class APRIL fully blocking antibody BION-1301 for the treatment of multiple myeloma. John Dulio, 1 Lilian Driesen, 1 Marc Snipper, 1 Marco Guadagnoli, 2 Astrid Bertens, 3 David Lutje Hulsik, 1 Tai Yu Tsu, 4 Kenneth Anderson, 5 Jan Paul Medema, 5 Kate Cameron, 5 Hans Femmena, 6 Andrea Elzas, 3 1Aduro Biotech Europe, Oss, Netherlands; 2Jerome Lipper Multiple Myeloma Center, Harvard Medical School, Boston, MA; 3ELow Institute for Myeloma Therapeutics and Jerome Lipper Multiple Myeloma Center, Dana Farber Cancer Institute, Harvard Medical School, Oss, MA; 4University of Amsterdam, Amsterdam, Netherlands.
APRIL, or tumor necrosis factor super family member 13 (TNFSF13), is a ligand for the receptors B-cell maturation antigen (BCMA) and transmembrane activator calcium modulator and cyclophilin ligand (CAML) interactor (TACI). APRIL serum levels are enhanced in patients diagnosed with multiple myeloma (MM), chronic lymphocytic leukemia (CLL) and colorectal cancer, is correlated with poor prognosis. APRIL is produced by osteoclasts, macrophages and other cells in the bone marrow niche. APRIL binds to BCMA to drive proliferation and survival of human Multiple Myeloma (MM) cells and induces resistance to several standard of care agents. Using a mouse anti-human APRIL blocking antibody 01A initially discovered using Aduro’s B-Select platform, we demonstrated that osteoclasts, bone marrow derived macrophages (BMDMs) and MM cells in vitro proliferation and survival is dependent on APRIL. In these co-cultures, cytokalic activity of lenalidomide and bortezomib is significantly enhanced by 01A in a dose-dependent fashion. Importantly, APRIL drives and inhibits expression of an immunosuppressive gene set including the immune checkpoint programmed death ligand-1 (PD-L1), interleukin-10, vascular endothelial growth factor, and transforming growth factor beta. The human APRIL antagonist antibody 01A was humanized and designated BION-1301. The antibody binds an epitope overlapping the BCMA and TACI binding sites potently and fully blocks BCMA and TACI binding (IC50 < 1 nM). Biophysical and functional experiments indicated that BION-1301 recapitulated all characteristics of 01A. In vivo, BION-1301 was shown to suppress T-cell dependent B cell responses to NP-Ficoll. Furthermore, APRIL blockade on both M1 and M2 TAM populations anti-multiple myeloma activity in a humanized SCID model confirming its activity in vivo, and potentially indicating that BION-1301 is active targeting multiple myeloma cells in a tumor-protective bone marrow microenvironment. To our knowledge, BION-1301 is a first-in-class humanized APRIL antagonist demonstrated in to inhibit multiple myeloma survival, drug resistance and an immune suppressive phenotype preclinical. These data suggest a rationale to develop BION-1301 as a single agent, and in combination with lenalidomide, bortezomib, or possibly checkpoint inhibitors such as anti-PD-1. BION-1301 is expected to enter Phase I in 2017. 1) Guadagnoli at al. Blood. 2011 Jun 23;117(25):6856-65. 2) Yu-Tzu et al. Blood. 2016 Jun 23;127(25):3225-36.


Tumor cells often evade macrophage-mediated destruction by increasing cell surface expression of CD47, which delivers an anti-phagocytic (“do-not-eat”) signal by binding the inhibitory signal-regulatory protein α (SIRPα) receptor on macrophages. We have previously shown that blockade of the CD47-SIRPα pathway using TTI-621, a soluble SIRPα-IgG1Fc fusion protein, triggers macrophage phagocytosis of tumor cells in vitro as well as inhibits tumor growth in vivo when delivered systemically. In the current study, the efficacy of intratumoral delivery of TTI-621 was evaluated in a subcutaneous diffuse large B-cell lymphoma (Toledo) xenograft model. Tumor bearing mice were randomized into treatment groups when tumor volumes reached approximately 120 mm3. Weekly intratumoral administration of TTI-621 at 10, 1 and 0.1 mg/kg dose levels resulted in statistically significant decreases in tumor growth and improved survival relative to vehicle control treatment. Notably, at day 50 post tumor implantation 100% survival was achieved at the highest dose level (vs. 0% survival with vehicle control treatment). Moreover, weekly intratumoral administration of TTI-621 was efficacious even at a high tumor load setting in which the pre-dose volumes were approximately 300 mm3. Flow cytometry analysis of the dissociated tumor samples demonstrated no significant change in the numbers of M1 and M2 tumor-associated macrophages (TAMs) following intratumoral administration of TTI-621. Nevertheless, TTI-621 dramatically increased the phagocytosis of Toledo cells by both M1 and M2 TAMs to a similar extent in vivo, suggesting that TTI-621 is efficacious in increasing the phagocytosis of tumor cells by a heterogeneous population of TAMs. Collectively, these results demonstrate that TTI-621 is efficacious when delivered intratumorally and can increase the phagocytosis of tumor cells by both M1 and M2 TAM populations. These data support the evaluation of intratumoral administration of TTI-621 in cancer patients, and a Phase I study of intratumorally delivered TTI-621 in patients with percutaneously accessible solid tumors and mycosis fungoides is ongoing (NCT02890368).

#2647 TGF-β type I receptor inhibitor (TEW-7197) diminishes myeloma progression by multiple immunomodulatory mechanisms in combination with ixazomib. Byung-Gyu Kim,1 Olga Sergeeva,2 George Luo,3 Sung Hee Choi,1 Zhenghong Lee,2 Seong-Jin Kim,1 John Letterio,1 Ehsan Malek,1 Case Western Reserve University, Cleveland, OH; Seoul National University, Republic of Korea.

Background: Multiple myeloma (MM) is an incurable cancer of plasma cells. MM thrives in the bone marrow tumor microenviron (TME) where several factors sustain MM growth and viability. TGF-β is a multi-functional cytokine elaborated by MM cells and by cells in the bone marrow (BM) TME. TGF-β stimulates MM progression through promotion of catabolic bone remodeling, an adverse TME phenotype, and by its anti-apoptotic activity. TGF-β induces ostoclast bone disease, immune suppression and myeloma progression. Therefore, we evaluated the anti-MM therapeutic potential of a small molecule inhibitor of the TGF-β type I receptor kinase (TβRI), TEW-7197, in combination with the proteasome inhibitor, ixazomib. Currently, TEW-7197 is being evaluated in phase I clinical trials in patients with solid tumors, and exposure to this agent is associated with an acceptable tolerability profile. Methods: The preclinical immunocompetent ST33MM model (C57BL/6J nearing viation of the role of TGF-β signaling in the BM TME. Mice bearing ST33MM cells expressing luciferase were treated with TEW-7197, ixazomib and the combination of TEW-7197 plus ixazomib daily for 3 weeks, and evaluated for MM growth by bioluminescence imaging (BLI). Cellular and molecular assays were performed to determine the effects of TEW-7197 and ixazomib on MM cell survival, MM cell apoptosis, real-time PCR and Western blotting. Immunological assays were performed using anti-hematochemical and FACS analysis. Peripheral blood mononuclear cell protein concentration, M-spoke, was measured by ELISA. Results: TEW-7197 attenuated the growth and viability of human and murine MM cells by inducing apoptosis and it inhibited TGF-β-induced activation of Smad2/3 in MM cells in vitro. In our ST33MM preclinical model, oral administration of TEW-7197 as single agent inhibited MM progression measured by peripheral blood mononuclear protein concentration and BLI before and after treatment. TEW-7197 also induced a decrease in mortality and an increase in body weight of mice bearing MM. TEW-7197 alone or combination with ixazomib also attenuated TGF-β activation of Smad2/3, reduced the expansion of CD11b+CD11c+ myeloid derived suppressor cells (MDSCs) in the BM TME, and diminished the population of Foxp3 regulatory T cells (Treg) in the spleen. Combination therapy of TEW-7197 plus ixazomib prolonged survival and exhibited a synergistic anti-tumor effect when compared to either TEW-7197 or ixazomib alone by significant reduction in both the M-spoke and BLI. Conclusion: Our data demonstrate that the small molecule inhibitor of the TβRI, TEW-7197, effectively modulates the MM TME and is associated with a potent anti-myeloma effect in an immunocompetent murine model of MM. These data provide a rationale for clinical evaluation of the combination therapy of ixazomib and TEW-7197 as a potential therapeutic strategy to improve outcomes in patients with MM.

#2648 CD47 antibody-induced engulfment of human T-cell leukemia cells by bone marrow-derived macrophages. Gillian Lovell,2 Clare Szyb,2 Kalpana Patel,1 Hinaham Campwala,1 Nicole Bevan,1 Dan Appledorn,2 Tim James,3 Derek John Trezise,2 Essen BioScience Ltd, Welwyn Garden City, United Kingdom; 2 Essen BioScience Inc, Ann Arbor, MI.

CD47 is a trans-membrane "don’t-eat-me" signaling protein that enables tumor cells to evade clearance by neighboring phagocytes. Blocking CD47 allows phagocytes to identify and clear tumor cells and is a promising new approach for cancer immunotherapy. In this study, we characterized anti-CD47 antibody-mediated engulfment of living tumor cells (CCRF-CEM) by mouse bone-mar- row derived macrophages (BMDMs) or immobilized mouse macrophages (J774A.1). Phagocytosis was quantified using a pH-sensitive cell-labeling fluorescent probe, pHrodo, and automated kinetic live-cell analysis (IncuCyte). CCRF-CEM cells were first labeled using pHrodo (250ng ml-1 for 1h), washed and then treated with antibody for 1h. Target cells were then added to BMDMs or J774A.1 which had been loaded overnight on 96-well plates. Phase- and fluorescent images were captured and quantified every 15min. Anti-CD47 anti-body (B6H12.2, 0.04-5μg ml−1), but not IgG-control, produced time- and concentration-dependent engulfment of CCRF-CEMs by BMDMs (30-4h), as evidenced by an increase in intracellular fluorescence as the label accumulates in the acidic phagosome. After 4h the red fluorescence area was increased by 25-fold (1975 ± 391 μm2 vs 80 ± 41 μm2). From close inspection of the time-lapse images cellular engulfment could be clearly observed, coincident with the appearance of the fluorescent signal. Similar observations were made with J774A.1 as the effector cell. Interestingly, the rate and degree of engulfment appeared effector cell-dependent. The mechanism of engulfment was not via induction of
target cell apoptosis since anti-CD47 did not induce PS externalization (Annezin V) or activate caspase 3/7. Anti-CD47 had no direct effect on CCRF-CEM proliferation for the first 4 h but upon longer exposures (>8 h) cell growth was attenuated. Our experimental findings substantiate the known pro-phagocytic effects of anti-CD47 antibodies, and provide a model system and method for quantitative functional analysis and mechanistic insight of CD47 modulators as cancer therapeutics.


The chemokine receptor CXCR4 triggers signaling pathways that control cell migration to tissues where its ligand, CXCL12, is highly expressed, including the bone marrow (BM). In hematologic cancers, CXCR4 expression is associated with poor prognosis. CXCR4-driven homing of malignant cells to the BM protective niche is a key mechanism of chemotherapy resistance. PF-06747143 is a novel humanized IgG1 therapeutic antibody that binds to CXCR4 and inhibits CXCL12-driven pathways. Human IgG1 antibodies can induce strong cytotoxicity mediated by the antibody Fc-region, including antibody-dependent cytotoxicity (ADCC) and complement-driven cytotoxicity, whereas human IgG4 antibodies show minimal or no Fc-driven cytotoxicity. Here we generated an IgG4 version of PF-06747143 (m15-IgG4), which has similar binding to CXCR4 as the IgG1 antibody. We then characterized the role of Fc-driven cytotoxic function, comparing both antibodies in efficacy and safety studies. In an ADCC assay, PF-06747143 showed strong cytotoxicity of non-Hodgkin’s lymphoma (NHL) and acute myeloid leukemia (AML), while m15-IgG4 had no significant cytotoxicity. In a NHL mouse tumor model, the IgG1 CXCR4 antibody resulted in superior tumor growth inhibition, with 50% of mice exhibiting complete tumor regressions, compared to the m15-IgG4 antibody, which had limited activity, with no tumor regressions (p<0.01). The two antibodies had comparable exposure, suggesting that the difference in efficacy is due to the IgG1 antibody Fc-driven cytotoxic function. Furthermore, CXCR4 inhibition was previously shown to induce leukocyte mobilization from the BM; however, prolonged cell mobilization may lead to safer related issues, including hyperleukocytosis. In a study in non-human primates, we show that the IgG1 CXCR4 antibody elicited short leukocyte mobilization, lasting 16-24 hrs, while m15-IgG4 induced prolonged cell mobilization lasting > 4 days. Since both antibodies had comparable exposures, the different mobilization duration is likely due to the ability of the IgG1 CXCR4 antibody to reduce the number of mobilized cells via Fc-driven cytotoxic function. Finally, CXCR4 has been shown to play a key role in chemotherapy resistance. In a chemo-resistant PDX AML mouse model, in which the standard of care agents daunorubicin and cytarabine had limited activity, resulting in 30% of tumor cells remaining in the BM post-treatment, we show that combination of PF-06747143 with these chemotherapeutic agents led to synergistic activity, with tumor burden reduced to 0.3% tumor cells in the BM. In conclusion, PF-06747143 attributes offer potential efficacy and safety-related advantages over other CXCR4 antagonists currently in development, which do not have Fc-driven cytotoxic activity. PF-06747143 is now being evaluated in a Phase 1 clinical trial in relapsed and refractory AML (NCTID 02946533).

#2650 Radioimmunotherapy for acute myeloid leukemia targeting human aspararyl (asparaginyl) β-hydroxylase. Michael S. Lebowitz, Ekaterina Revskaya, Kanam Malhotra, Amir Shallaee, Steven Fuller, Maria R. Baer, Noa G. Holtzman, Ashkan Emadi, Ekaterina Dadachova, Hossein A. Ghanem, Panacea Pharmaceuticals, Inc., Gaithersburg, MD; Albert Einstein College of Medicine, Bronx, NY; Institute for Asthma and Allergy Prevention, LLC, Chevy Chase, MD; University of Maryland School of Medicine, Baltimore, MD; University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

Treatment options for acute myeloid leukemia (AML) are limited and have modest impact on 5-year survival rates in patients <60 years of age (~35-40%) and on median survival of older patients (< 1 year). The incidence of AML has been rising, with an anticipated 19,950 new cases in 2016. Thus, the need for new, more efficacious and better tolerated therapy for AML is urgent. Human aspararyl (asparaginyl) β-hydroxylase (HAH) is an embryonic/developmental protein, which is down-regulated in normal cells after birth but overexpressed on the surface of many malignant cells, where it has been demonstrated to be sufficient to induce cancer cell proliferation, motility and invasiveness. We hypothesized that HAAH is selectively overexpressed on leukemia cells and thus targeting it with a fully human monoclonal antibody (mAb) conjugated to the short half-life α-emitter 212Bi could provide a novel radioimmunotherapy (RIT) treatment option with a reasonable therapeutic index. Using immunocytochemistry and flow cytometry with human mAb, PAN-622, we investigated the expression of HAAH on a variety of cytogenetically- and mutationally heterogeneous leukemia cell lines, as well as normal human leukocytes. PAN-622 was further modified with DTPA-maleimide to allow chelation of radionuclides, specifically 212Bi, to the human IgG1 anti-HAAH antibody with the aim to measure selective dose-dependent killing of leukemic cells. HAAH was highly expressed, as detected by flow cytometry, on all tested leukemia cell lines with KG1α and HL-60 demonstrating the highest levels of expression amongst the AML lines. Binding was observed only between PAN-622 and the modified DTPA-PAN-622 conjugate. Interestingly, expression of HAAH was not detected on CD45 normal human leukocytes, including in subpopulations of monocytes (CD14+), B-cells (CD19+), T-cells (CD3+) or hematopoietic stem cells (CD34+). To determine whether PAN-622 could selectively target leukemia cells in a background of normal human blood, flow cytometry was performed on leukemia cells spiked into whole blood. Only leukemia cells were stained with the PAN-622 antibody as indicated by simultaneous staining for surface markers of various leukemia and normal cell populations. Staining of leukemia cell lines was demonstrated following incubation with 212Bi-DTPA-PAN-622 which induced dose dependent specific killing over a 6.7-670 pM concentration range. No killing was detected when using 212Bi conjugated to an isotype-matched control antibody at a similar specific activity and dose range. Thus, HAAH is a novel oncogenic target expressed on leukemia cells, which can be selectively targeted for RIT via the fully-human PAN-622 mAb. Experiments targeting primary leukemia cells in patient-derived bone marrow aspirates are ongoing.

#2651 A novel CD19 targeting antibody-drug conjugate, huB4-DGN462, shows promising in vitro and in vivo activity in CD19-positive lymphoma models. Eugenio Gaudio, Chiara Tarantelli, Alberto J. Arribas, Roberta Pittau Bordone, Andrea Rinaldi, Georg Stussi, Emanuele Zucca, Davide Rossi, Anastasios Statthis, Min Li, Alan Willehm, Kate Lai, Qifeng Qiu, Stuart Hicks, Callum Sloss, Francesco Bertoni. Institute of Oncology Research - IOSI, Bellinzona, Switzerland; Oncology Institute of Southern Switzerland - IOSI, Bellinzona, Switzerland; Immunogen Inc., Waltham, MA.

Background. CD19 is a cell surface membrane protein expressed in mature and immature B cell neoplasms, making it a promising target for antibody-drug conjugate (ADC) therapy for B cell malignancies. Here we describe the preclinical activity of a novel B cell targeting fully humanized potent indolino-benzodiazepine DNA-alkylating payload DGN462. Methods. The humanized anti-CD19 antibody, huB4, was conjugated to DGN462 via a cleavable disulfide linker, sulfo-SPDB. In vitro activity of the huB4-DGN462 ADC or the unconjugated DGN462 toxin was evaluated in 54 lymphoma cell lines [27 diffuse large B cell lymphomas (DLBCL); 10 mantle cell lymphomas; 6 marginal zone lymphomas; 5 anaplastic large T-cell lymphomas; 6 others]. Cell proliferation/avialability/after 72 h of exposure was measured using a MTT assay. Apoptosis activation was defined by at least 1.5-fold increase in caspase 3/7 signal activation in respect to controls using the Promega ApoTox-Glo Triplex Assay. Gene expression profiling (GEP) was performed with the Illumina HumanHT-12 Expression BeadChips on untreated cell lines followed by GSEA. Results. huB4-DGN462 was cytotoxic against a broad panel of 48 B cell lymphoma cell lines (median IC50 100 pM; 95%CI, 38-214). The cytotoxic activity was not limited by P53, BCL2, MYC or CDKN2A status, or associated with DLBCL cell of origin. Consistent with overall lower CD19 expression, huB4-DGN462 was significantly less active in eight T cell-derived lymphomas (median IC50 of 1.75 mM (95%CI, 0.5-5.75)) than in B cell lymphomas. huB4-DGN462 induced caspase 3/7 activation in 48/54 cell lines (89%) consistent with an apoptotic mechanism of action. huB4-DGN462 demonstrated compelling anti-tumor activity after a single intravenous dose in two diffuse large B-cell lymphoma cell line xenograft models (human CD19+ lymphoma model and Farega’s disseminated model). In the DoHH2 model, huB4-DGN462 resulted in a significant, dose-dependent tumor growth delay and survival benefit at 1.7 mg Ab/kg compared to a non-targeted control DGN462 ADC. In the disseminated Farega model, a significant dose-dependent increase in survival was observed in mice treated with as low as
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0.17 mg Ab/kg of huB4-DGN462. At 1.7 mg Ab/kg, the life span was increased >400% compared to untreated mice. Conclusions. The novel ADC huB4-DGN462 presented strong preclinical anti-lymphoma activity, which provides evidence for further study.


Background: Primary Mediastinal Large B-Cell Lymphoma (PMLBCL) is a unique type of B-cell lymphoma probably arising from a putative thymic medulla B-cell. One to 2% of NHL, 7% of all diffuse large B-cell lymphomas (DLBCL), occurring more often in young females. Often presents with superior vena cava syndrome and airway compromise. Treatment is with chemotherapy combined with Rituximab, followed by involved field radiation therapy to mediastinum. PMLBCL has better outcome than others DLBCL. We aimed to develop a more dose-dense/intense regimens that improves the rate of cure with an attempt to avoid mediastinal radiation. Patients and methods: A prospective study was conducted in our department from January to December 2015, in 6 patients with untreated primary mediastinal B-cell lymphoma. An infusional study was conducted in our department from January to December 2015, in 6 patients. PMLBCL has better outcomes than others DLBCL. We aimed to develop a more dose-dense/intense regimens that improves the rate of cure with an attempt to avoid mediastinal radiation. Patients and methods: A prospective study was conducted in our department from January to December 2015, in 6 patients with untreated primary mediastinal B-cell lymphoma. An infusional study was conducted in our department from January to December 2015, in 6 patients.

#2653 The anti-myeloma activity of TTI-621 (SIRPaFc), a CD47-blocking immunomodulatory agent, is enhanced when combined with a proteasome inhibitor. Emma Linderoth, Simone Helke, Vivian Lee, Taptuma Mutukura, Mark Wong, Gloria H. Lin, Lisa D. Johnson, Xinli Pang, Jeff Winston, Penka S. Petrova, Robert A. Uger, Natasja N. Viller. Trillium Therapeutics Inc., Mississauga, Ontario, Canada.

CD47 is transmembrane glycoprotein that delivers an anti-phagocytic (“do not eat”) signal to the receptor signal-regulatory protein (SIRPα) on the surface of macrophages. Many tumors, including multiple myeloma (MM), express high levels of CD47 as a means to evade the CD47-SIRPα pathway and escape macrophage-mediated immune surveillance. TTI-621 (SIRPaFc) is a soluble recombiant fusion protein consisting of the CD47-binding domain of human SIRPα linked to the Fc region of human IgG1. It is designed to promote anti-tumor responses by blocking the CD47-“do not eat” signal and engaging activating Fcy receptors on macrophages. We have previously shown that TTI-621 enhances phagocytosis of malignant cells in vitro and exhibits anti-tumor activity in acute myeloid leukemia and B lymphoma xenograft models. In this study we investigated the anti-myeloma activity of TTI-621 as both a single agent and in combination with bortezomib or carfilzomib, two proteasome inhibitors that are approved for use in MM patients. The ability of TTI-621 to trigger macrophage phagocytosis of MM cells was assessed using a flow cytometry-based phagocytosis assay. Blockade of CD47 using TTI-621 effectively triggered macrophage-mediated phagocytosis of MM cells, and this anti-tumor effect was significantly enhanced by pre-treatment of MM cells with bortezomib or carfilzomib. In order to understand the molecular mechanism behind this enhanced phagocytic effect, we performed immunophenotyping of the tumor cells and observed an upregulation of pro-phagocytic “eat” signals on the tumor cell surface that potentially augment the phagocytic response. To investigate whether CD47 blockade in the context of proteasome inhibition translates to enhanced anti-tumor activity in vivo, we employed a MM xenograft model. NOD-SCID mice were subcutaneously engrafted with human MM cells followed by treatment with TTI-621, in the absence or presence of concurrent administration of a proteasome inhibitor. As monotherapies, both TTI-621 and the proteasome inhibitor reduced tumor burden relative to vehicle controls. Moreover, the combination of CD47 blockade and proteasome inhibition resulted in greater tumor growth reduction compared to each drug alone. In conclusion, these data demonstrate that TTI-621 exerts anti-myeloma activity that is further enhanced by combination with bortezomib or carfilzomib. TTI-621 monotherapy is currently being evaluated in a Phase 1b study in patients with MM and other hematological malignancies (NCT02663518). These data provide a rationale to evaluate a combination study of TTI-621 and a proteasome inhibitor in MM patients.


The interaction of programmed cell death-1 (PD-1) and its ligand programmed cell death-1 ligand (PD-L1) is a major focus of recent immune oncology therapy efforts. The expression of PD-1 on T lymphocytes and its subsequent interaction with PD-L1, either from antigen presenting or tumor cells, will result in apoptosis-dependent inactivation of the T lymphocytes. This interaction plays an integral role in tumor immunology, specifically augmenting immune evasion. Relative little is known about the regulation of PD-L1 in either the tumor or normal environment. We investigated the ability of natural dietary compounds to induce PD-L1 expression on normal epithelial cells and various cancer cell lines. These molecules have been evolutionarily selected to control inflammation and cancer cell transformation and progression, understanding their mechanism of action could be important to understanding how this system works. Using flow cytometry and immunohistochemistry, we focused our studies on resveratrol and its metabolite piceatannol, key phytochemicals extracted from grapes, after an initial screen of various natural products. Experimental data showed that both compounds can individually up-regulate the expression of PD-L1 on tumor cell lines and normal epithelial cells, by an IFN-γ-independent mechanism. PD-L1 induction by both of these compounds was higher in tumor than in normal epithelium, suggesting greater significance in tumor regulation than inflammation. Additionally, the combination of resveratrol and piceatannol acted synergistically, leading to a significantly greater induction of PD-L1 expression across multiple tumor indications. Understanding whether resveratrol and piceatannol use a common signaling pathway to induce PD-L1 expression in tumor cells, was critical to understand the synergistic induction observed. Studies utilizing specific inhibition of IKK phosphorylation were accompanied by a significant reduction in the ability to induce PD-L1 expression on tumor cells, either as single agents or in combination. These results are consistent with the hypothesis that induction of PD-L1 by resveratrol or piceatannol, or their combination involves molecular determinants downstream of NF-kB signaling.

#2655 Drug resistant B-cell tumors eliminated by novel therapeutic antibodies in vivo. Hong Qin, Guowei Wei, Ipeki Sakamaki, Zhouyuan Dong, Wesley A. Cheng, Diane L. Smith, Feng Wen, Han Sun, Soung-chul Cha, Sattva S. Neelapu, Larry W. Kwak. 1City of Hope, Duarte, CA; 2University of Fukui, Awaru, Japan; 3Sichuan University, China; 4University of Texas MD Anderson Cancer Center, Houston, TX.

B-cell malignancies have been successfully targeted in the clinic by therapies such as anti-CD20 antibody rituximab or Bruton’s tyrosine kinase inhibitor ibrutinib. However, leukemias and lymphomas remain incurable due to primary or acquired resistance, ultimately leaving patients without an effective treatment option. We sought to circumvent this drug resistance by pursuing an alternative target known as B cell activating factor receptor (BAFF-R). Despite past limited success, BAFF-R remains a prime target for B-cell lymphoma and leukemia therapeutic antibody development due to its key role in B-cell proliferation and development. We report the development of two novel monoclonal antibodies (mAbs) against human (h) BAFF-R. The mAbs were generated by immunizing mice with (h)BAFF-R-expressing mouse fibroblast cells presenting a natively folded, cell-surface immunogen. The two mAbs presented unique complementarity determining regions that specifically bound (h)BAFF-R-expressing mouse fibroblast cells but not the parental counterpart. Furthermore, the antibodies were specific to B-cell containing organs such as tonsil and spleen, by immunohistochemical staining and without detectable reactivity in heart, lung, brain, liver, and kidney tissues. To tailor the antibodies for clinical application, a human IgG1 Fc capable of eliciting an immune response was substituted, creating chimeric...
versions. We showed that both chimeric mAbs bound with high affinity to human B-cell lymphoma cell lines including JeKo-1 (mantle cell lymphoma; MCL), SU-DHL-6 (diffuse large B cell lymphoma; DLBCL), Raji (Burkitt lymphoma), and RL (fOLLicuLar lymphoma). The chimeric antibodies also elicited antibody-dependent cell-mediated cytotoxicity (ADCC) with primary human B cells, as well as primary lymphoma samples (n = 5) from patients who progressed after rituximab exposure. Most notably, the antibodies demonstrated effi-
cacy in two in vivo drug resistant lymphoma models we developed, a ritux-
imab-resistant CD20 genomic knockout variant of JeKo-1 and the naturally
tubulin-resistant Z-138. Using these lymphomas lines for xenogenic tu-
mor models in NOD scid gamma (NSG) mice, we found our antibodies
significantly inhibited tumor growth, conferring long-term and tumor free
survival on the mice. Our in vitro and in vivo results robustly demonstrate
the high specificity and significant anti-tumor effects of our anti-BAFF-R
antibodies against a broad variety of B-cell malignancies, especially against
cases of rituximab and tubulin resistance. This successful development of
novel anti-BAFF-R therapeutic antibodies warrants support for further
translational development for clinical use in light of current resistance cases.

**#2656** Polatuzumab vedotin significantly enhances *in vitro* cell death and overall survival against CD79b+ Burkitt lymphoma (BL)/primary mediastinal large B-cell lymphoma (PMBL) xenograft mice. Aradhana A. Tiwari, Janet Ayello, Christeen Azmy, Carmella van de Ven, Mitchell S. Cairo. New York Medical College, Valhalla, NY.

Background: Malignant B-NHL, including Burkitt lymphoma and primary mediastinal large B cell lymphoma express CD79b+ and have an excellent prognosis with chemo-immunotherapy (Cairo et al Blood, 2007; Gerrard/Cairo et al Blood, 2013). However, a subset of patients with relapsed/refractory mature B-NHL has chemioimmunotherapy resistant disease (Rao et al Blood, 2013). PD-L1 expression on Karpas1106pmBL (PMBL) is unknown. Objective: To determine the efficacy of the PV against CD79b+ PMBL and rituximab (RTX) sensitive/resistant BL tumor cell lines *in vitro* and *in vivo*. Design/Methods: Raji/RajiRHF (BL, provided by M. Barth, Roswell Park Cancer Institute) and Karpas1106P and MedB-1 (PMBL) were cultured in 10-20% RPMI. Tumor cells were incubated with hu anti-CD79b-vcMMAE, and/or anti-CD79b, MMAE or hulgH1 (generously supplied by Genen-
tech Inc.) for 24 hrs. Cell death was evaluated by staining with annexin V/7AAD and analyzed by flow cytometry, n=3. Six to 8 week old female NSG (NOD-
Cg-Prkdcscid Il2rgtm1Wjl/Sjcl), were divided into 2 groups: PBS only (control), isotype control (IgG), PV (5mg/kg), anti-CD79b mAb (5 mg/kg) and MMAE (5 mg/kg).
Mice were xenografted with intravenous injections of Luc+ Raji/ RajiRHF and Karpas1106P cells as we have previously demonstrated (Awasthi/ Cairo et al, BJH, 2015). Mice were treated twice a week for 6 weeks. Tumor burden was monitored by IVIS spectrum system. Results: Anti-CD79b-vcMMAE treated Karpas1106P xenografted mice significantly enhanced cell death in Raji, 47.2 ± 1.3% vs 29.1 ± 6.0% vs 28.2 ± 4.3% (p = 0.0008 and p = 0.00006), RajiRHF, 29.8 ± 9.1% vs 25.4 ± 3.9% vs 18.0 ± 8.2% (p=NS and p = 0.03), Karpas1106P, 46.8 ± 5.3% vs 33.8 ± 3.3% vs 26.2 ± 0.4% (p = 0.02 and 0.006) and MedB-1, 47.4 ± 2.2% vs 27.6 ± 2.4% vs 23.9 ± 1.7% (p = 0.002 and 0.001), respectively. Further, median survival time in mice receiving 5 mg/kg of PV was significantly increased when compared to mice receiving
5 mg/kg of anti-CD79b Ab or isotype control in Raji, RajiRHF and Karpas1106P (35.5 ± 17 vs. 19.5 days, p = 0.0001, 0.0003, 50 vs 18 vs 18.5 days and 150 vs 89 vs 64 days, p = 0.03, 0.003, respectively) Conclusions: Our prelimi-
nary data indicates that PV significantly enhances cell death in RTX sensitive/resistant BL and PMBL compared to CD79b Ab or isotype control. Furthermore, PV significantly increased survival in BL and PMBL NSG xenografts.

**#2657** PD-L1 blockade enhances T cell cytotoxicity against primary medias-

Background: Primary Mediastinal B cell Lymphoma (PMBL) represents 2-4% of Non-Hodgkin Lymphomas (NHL) in adolescents and young adults (AYA) (Ger-
rand/Cairo et al, Blood, 2013). Disease progression, relapse and long-term toxicity remain a concern for patients treated on current chemo-immunotherapy and me-
diastinal radiotherapy. Alternative therapeutic regimens are urgently needed, espe-
cially in patients without an early response to therapy. Programmed Death 1 (PD-1) is a negative co-stimulatory receptor critical for suppression of T-cell activation, with binding of PD-1 and Programmed Death Ligand 1 (PD-L1) resulting in T cell exhaustion (Postow/Wolchok et al, J Clin Oncol, 2015). Gain in 9p associated with amplification of PD-L1 has been reported in up to 60% of PMBL specimens, leading to overexpression of PD-L1 and potential immune cell evasion of PMBL (Rosen-
wald/Staudt et al, J Exp Med, 2003; Twa/Staudt et al., Blood, 2014). Blockade of PD-1/PD-L1 interaction, therefore, constitute a potentially promising alternative for treatment of resistant PMBL. Objective: In the current study, we sought to inves-
tigate whether PD-L1 blockade enhances T cell responses in PD-L1 expressing
PMBL. Methods: PD-L1 expression on Karpas 1106p PMBL treated with or without IFN γ was investigated by western blotting and flow cytometry analyses. T cells were isolated from human PBMC followed by activation and expansion using anti-CD2, 
anti-CD3 and anti-CD28 coated MACS beads particles. T cell activation was confirmed by CD25 and CD69 expression using flow cytometry. Activated T cells were incubated with or without anti-PD-L1 (Clone 6E11, Genentech) at a dose of 10 ug/ml together with Karpas 1106p cells treated with or without IFN-γ. Cell prolif-
eration was assessed with MTS assays after incubation for 24 hours. Results: We dem-
onstrated that Karpas 1106p cells express a low level of PD-L1. However, following IFN γ treatment (48 hours) there was a significant increase in PD-L1 expression.
Anti-PD-L1 had no significant effect on T cell mediated inhibition of cell prolifera-
tion in Karpas 1106p cells with low PD-L1 expression. However, anti-PD-L1 and T cell treatment significantly inhibited cell proliferation in IFN γ treated Karpas 1106p cells when compared to T cell treatment alone (32±18% vs. 3±5% at E:T=5:1, p = 0.048). Conclusion: PD-L1 blockade enhances T cell cytotoxicity against PMBL, which is dependent on PD-L1 expression level on PMBL cells. Future in-vivo NSG xenograft studies are ongoing.

**#2659** MT-4019: a de-immunized engineered toxin body targeting CD38 for multiple myeloma. Garrett L. Robinson, Sangeetha Rajagopalan, Brigitte Brieschke, Jane Neil, Jennifer Erdman, Rodney Flores, Jensu Liu, Jack P. Hig-
gins, Erin K. Willert. Molecular Templates Inc., Austin, TX.

Molecular Templates is developing engineered toxin bodies (ETBs), po-
tent recombinant immunotoxins that combine the specificity of an antibody fragment with the powerful direct cytotoxicity of the Shiga-like toxin A subunit (SLTA). ETBs can induce their own internalization, route through the cell in a predictable manner, enzymatically and irreversibly destroy ri-
bosomes to shutdown protein synthesis and induce apoptosis of tumor cells. This mechanism of action is distinct from that of other therapeutics, making ETBs an attractive treatment for patients who have become resistant to che-
emothapy and other treatments. Safety and efficacy in refractory non-
Hodgkin’s lymphoma patients has been observed in a phase I study with Molecular Template’s first-generation CD20-targeting compound, MT-
3724. CD38 is a surface receptor that is highly expressed on malignant plasma cells. CD38 is a clinically validated target of monoclonal antibodies for treatment of multiple myeloma and is known to persist after failure of antibody treatment. MT-4019 is a CD38-targeted next-generation ETB, uti-
izing a modified SLTA. The SLTA subunit of the next-generation ETB scaf-
fold has been modified through proprietary genetic engineering to systemat-
ically and comprehensively reduce B cell and CD4+ T cell epitopes, as well as
to dampen the innate response by decreasing binding to TLR-4. This de-
immunization strategy also reduces the innate and adaptive immune response to the ther-
apeutic. MT-4019 is a promising lead and is under further development to
enable clinical studies in rodent and non-human primate models have demonstrated the tolerability of MT-
4019, along with a decreased anti-drug antibody response. Additionally, in
the non-human primate model, MT-4019 showed reduced neutrophil and monocyte activation as compared to an ETB with an unmodified SLTA sub-
unit, indicating that SLTA modification also exhibits a diminished innate immune response. Molecular Template’s ETB technology has resulted in potent, targeted therapeutic agents that have a unique mechanism of action in the field of oncology. MT-3724, a first-generation CD20-targeted ETB, has shown promising clinical results in the refractory setting for non-Hodgkin’s lymphoma. The next-generation MT-4019, as exemplified in the CD38 tar-
gated MT-4019, retains potent and specific direct cell kill activities, and
additionally reduces the innate and adaptive immune response to the ther-
papeutic. MT-4019 is a promising lead and is under further development to
enable clinical studies in multiple myeloma.
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Purpose: Blockade of PD-1 receptor may provide proof of concept for the activity of an immune-modulation approach for the treatment of a breast cancer (BC). Zoledronic acid (ZA) has been proven to inhibit angiogenesis, invasion, and adhesion of tumor cells. The aim of this study was to investigate the potential of antibody treatment towards the T-cell checkpoint inhibitor PD-1 alone or in combination with ZA in BC mouse model. Experimental Design: The 4T1-Luc mouse mammary tumor model was used in this study. The anti-tumor efficacy of anti-PD-1 antibody alone or in combination with ZA was monitored by measuring tumor volume and bioluminescence imaging (BLI). At the end of study, the tumor immunohistochemistry was performed to confirm the in vivo observation and flow cytometry was used to determine the immune cell population in tumor sections. For survival analysis, the Kaplan–Meier method, Cox proportional hazards regression, and Gehan’s survival tests were used. Results: The results showed that mice treated with combination therapy exhibited the best antitumor response compared to untreated controls or single therapy. Furthermore, Combination therapy inhibited tumor regrowth as determined by BLI imaging, and thus significantly improved the median overall survival compared to other groups. Conclusions: Our study provides evidence for enhanced clinical BC treatment benefit from targeting co-signal molecules by combining PD-1 blockade with ZA treatment.

ALKS 4230 is a selective agonist of the intermediate-affinity IL-2 receptor (IL-2R). A phase 1 study is ongoing to evaluate the safety and tolerability of ALKS 4230 in the treatment of patients with refractory solid tumors. The selectivity of ALKS 4230 is achieved through the stable fusion of circularly permuted IL-2 to the extracellular portion of the IL-2Rα chain, CD25. The resulting fusion protein has a unique surface expression that is sterically protected from biodegradation to the high-affinity IL-2 complex comprised of IL-2Rα, IL-2Rβ, and common gamma chain, expressed preferentially on CD4+ FOXP3+ regulatory T cells (CD4+ Treg) yet retains full ability to signal through the intermediate-affinity IL-2R complex, comprised of IL-2Rβ and common gamma chain, expressed on memory CD8+ T cells and NK cells. Repeated dosing of ALKS 4230 drives the significant expansion of various CD8+ T cells, including tumor-infiltrating lymphocytes, in a dose-dependent manner. Mimicking the effects seen in vivo, ALKS 4230 also inhibited phosphorylation of STAT1. In addition, EGCG inhibited EGFR-induced PD-L1 gene expression and protein level in Lu99 cells via inhibition of phosphorylation of Akt. In order to study the effects of EGCG in combination with CD40 ligand (CD40L) expression in CD40L-ligand 1 (PD-1) expression on tumor cells is involved in PD-L1/PD-1 pathway. PD-L1 protein levels varied among 6 non-small human lung cancer cell lines (NSCLCs): LC-A1 and Lu99 cell lines showed the highest PD-L1 expression, A549 and H322 cell lines were medium, and H1703 and H1299 were very low, independent on cancer cell lines. Since PD-L1 expression is induced by various cytokines and growth factors, we studied the effects of IFN-γ and EGFR on cell-surface PD-L1 level using flow cytometry, and its gene expression using RT-PCR. Treatment with IFN-γ dose-dependently stimulated PD-L1 expression in both A549 and H1299 cells, and not effective on Lu99 cells, whereas treatment with EGFR-stimulated PD-L1 in Lu99 cells only. Pretreatment with EGCG for 3 h, dose-dependently inhibited INF-γ-induced PD-L1 gene expression and protein level on cell-surface in both A549 and H1299. It is important to note that EGCG also inhibited phosphorylation of STAT1. In addition, EGCG inhibited EGFR-induced PD-L1 gene expression and protein level in Lu99 cells via inhibition of phosphorylation of Akt. In order to study the effects of EGCG in vivo, we used a mouse model of breast cancer and treated tumor-bearing mice with different treatments. The results strongly suggest that EGCG enhances antitumor immunity via inhibition of PD-L1 expression in cancer cells, resulting in significant cancer preventive activity. Using “omics” to select immunotherapy and conventional therapy combinations. Sarit Schwartz,1 Robert Heaton,2 Yuan Tian,3 Zack Sanborn,3 Shankar Sellappan,4 Kenny Scott,5 Fabiola Cecchi,6 Steve Benz,6 Todd Hembrough.1 1NantOmicS, Rockville, MD; 2NantOmicS, Santa Cruz, CA.

Background: As only a subset of patients respond to immunotherapies, therapeutic biomarkers are needed to predict which patients will benefit and which should be spared from potentially toxic treatment. Proteins expressed by tumor cells and by the immune microenvironment may predict response to immune checkpoint inhibitors. In non-inflamed tumors, chemotherapy and targeted therapy may stimulate an immune response thereby affecting the relationship between tumor and immune system. Combinations of immunotherapies and conventional therapies are the subject of ongoing clinical trials. We hypothesized that genomic and proteomic evaluation of multiple immune biomarkers in tumor tissue and in lymphocytes would identify a signature that could (a) differentiate between responders and non-responders to checkpoint inhibitors and (b) identify candidates for combination therapy. Methods: A pathologist marked areas of tumor and tumor-associated lymphocytes on archived tissue sections (N=2) of non-small cell lung cancer (NSCLC) and melanoma. The marked areas were microdissected and solubilized to tryptic peptides. In one liquefied tumor sample, 110 protein biomarkers, including 60 immunomarkers were quantified with a mass spectrometry-based proteomic assay. The genomic

#2665 Green tea catechin, EGCG, enhances antitumor immunity via down-regulation of PD-L1 expression in non-small human lung cancer cell lines. Ancheale Rawangkan,1 Keisuke Iida,1 Ryo Sakai,1 Hirota Fujiki,2 Masami Suganuma,1 1Saitama University, Saitama, Japan; 2Saga University, Saga, Japan.

(−)-Epigallocatechin gallate (EGCG) is now widely accepted as a non-toxic, effective cancer preventive compound. EGCG also acts as a synergist with anti-cancer drugs for cancer treatment. Considering the wide beneficial effects of EGCG, we have hypothesized that EGCG might have a role in inhibiting cancer cell death. The objective of this study is to investigate the effects of EGCG on cell death in a non-small cell human lung cancer cell line A549. EGCG treatment decreased the tumor size. Treatment with Wortmannin, a PI3 kinase inhibitor, as a control, did not inhibit PD-L1 expression in A549 and H1299 cells. The results strongly suggest that EGCG enhances antitumor immunity via inhibition of PD-L1 expression in cancer cells, resulting in significant cancer preventive activity.
Antibody of human IgG4 isotype, that binds selectively and with similar potency antagonistic activities of PF-06801591. PF-06801591 is a humanized anti-PD-1 monoclonal antibody that specifically targets programmed death receptor-1 (PD-1) or its cognate ligand, programmed death receptor ligand-1 (PD-L1), alone or in combination, have demonstrated clinical benefıts and durable responses in patient subsets with various cancers (including metastatic melanoma, NSCLC, RCC, urothelial cancer, cHL and others). Here we report on the biological characteristics and non-clinical-antagonistic activities of PF-06801591, PF-06801591 is a humanized anti-PD-1 antibody of human IgG4 isotype, that binds selectively and with similar potency to human and cynomolgus PD-1 receptor and blocks its interaction with its cognate ligands PD-L1 and PD-L2 (IC50 < 1 nM) with no detectable Fc effector function. The interaction of PF-06801591 to PD-1 rescues T cell suppression and exhaustion that translates into NFAT activation, IL-2 and IFN-gamma secretion and T cell proliferation both in vitro cultures and in vivo experiments using an acute xeno GVHD model with human PBMC transfer. Binding of PF-06801591 to human and cynomolgus PD-1 is characterized by the formation of a very stable complex (T1/2 = 2 h) as measured by SPR at 37°C, resulting in high affinity (Kd = 20 pm) as measured in solution by KinExA at 23°C. In addition, we explored therapeutic potential of anti-PD-1 in combination with other immunotherapy agents using surrogate antibodies in non-clinical tumor models. The data presented here support future development of PF-06801591 as a single agent or in combination with other immunotherapies.

#2667 In vitro properties and pre-clinical activity of PF-06801591, a high-affinity engineered anti-human PD-1

Sawssen Youssouf, Yasmina Abdiche, Hoang Kim Nguyen, Joyce Choi, Sherman Michael Chin, Kris Kamer- schroer, Patricia A. Schneider, Eugenia Kraynov, Heike I. Kuprka, Arvind Raijli, John Liu, Peter J. Arnett, South San Francisco, CA; Waitsich Microfluidics, Salt Lake City, UT; RMS, Redwood City, CA.

Monoclonal-antibody-based therapies targeting the immune checkpoint receptors have become the new standard of care in many cancers. Antibodies that specifically target programmed death receptor-1 (PD-1) or its cognate ligand, programmed death receptor ligand-1 (PD-L1), alone or in combination, have yielded clinical benefits and durable responses in various cancers (including metastatic melanoma, NSCLC, RCC, urothelial cancer, cHL and others). Here we report on the biological characteristics and non-clinical-antagonistic activities of PF-06801591, PF-06801591 is a humanized anti-PD-1 antibody of human IgG4 isotype, that binds selectively and with similar potency to human and cynomolgus PD-1 receptor and blocks its interaction with its cognate ligands PD-L1 and PD-L2 (IC50 < 1 nM) with no detectable Fc effector function. The interaction of PF-06801591 to PD-1 rescues T cell suppression and exhaustion that translates into NFAT activation, IL-2 and IFN-gamma secretion and T cell proliferation both in vitro cultures and in vivo experiments using an acute xeno GVHD model with human PBMC transfer. Binding of PF-06801591 to human and cynomolgus PD-1 is characterized by the formation of a very stable complex (T1/2 = 2 h) as measured by SPR at 37°C, resulting in high affinity (Kd = 20 pm) as measured in solution by KinExA at 23°C. In addition, we explored therapeutic potential of anti-PD-1 in combination with other immunotherapy agents using surrogate antibodies in non-clinical tumor models. The data presented here support future development of PF-06801591 as a single agent or in combination with other immunotherapies.

#2668 Efficacy study of immuno-checkpoint antibodies in humanized CDX and PDX models

Feifei Zhang, Yang Yang, Hongkui Chen, Lijin Jia, Kedong Ouyang, Danyi Wen, Taiping Chen. LIDEBiotech Ltd, Shanghai, China.

Immunotherapeutic intervention has been accepted widely for many hematopoietic malignancies and solid malignancies in past several years. Preclinical animal models with reconstitution of immune cells and target-expressing tumor cells were urgently needed for proof of concept studies demonstrating stimulatory immune system activation. Here we describe two established models of human PBMC co-transplantation of CDX and PDX. The study design was based on co-inoculation of human PBMC and PD-L1 expressing cell in NOG mice for reconstitution of immuno-checkpoint blockage. A therapeutic anti-PD1 antibody was applied for immune system activation and tumor growth inhibition measurement. Tumor growth experiments were administered i.p. for three weeks, 95% TGI for A375 CDX model were observed. At the termination of the efficacy study at 28 days post therapeutic intervention, tumors of vehicle (tumor only, PBMC only), PD1 Ab treated groups were collected for immunostaining of CD45. Infiltrating T-cell with CD45+ label was found within the tumors when co-inoculated with human PBMC. PBMC humanized PDX models of ovarian cancer was implanted into mice which reconstituted with human PBMC for therapeutic intervention. Results showed that 85% TGI for PBMC humanized ovarian PDX models were observed. In conclusion, the models established here are feasible for immuno-therapeutic evaluation and further additional immune therapeutic test articles can be explored with similar approaches.

#2669 Antitumor activity of the CMP-001 (TLR9 agonist) alone or combined with immune modulators in syngeneic tumor models

Francis Bichat, Sylvie Maubant, Jean-François Mirjolet, Philippe Slos, Arthur M. Krieg, Aaron Morris.

The combination of checkpoint inhibitors such as CTLA-4 or PD-1 with antigen monoclona monoclonal antibodies (mAbs) has shown impressive and durable clinical responses in patients with advanced cancer. An alternative strategy to boost anti-tumor immunity is to promote T cell activation through co-stimulatory receptors such as OX40 and 4-1BB. OX40 is of particular interest as treatment with an activating anti-OX40 mAb augments T cell differentiation and cytolytic activity from multi-kinase-tolerant T cells in each of the two arms of these agents benefits only a subset of patients, highlighting the critical need for more effective combinatorial therapeutic strategies. Toll-like receptor 9 (TLR9) agonist CpG oligodeoxynucleotides (ODN) are candidates to promote an antitumor immune response. CMP-001, a CpG-A ODN formulated within a virus-like particle, is designed to activate TLR9 (the receptor for CpG-A) in tumor-associated plasmacytoid dendritic cells (pDC) within the tumor or tumor-draining lymph nodes. Resting or immature pDC promote tumor growth, but when activated by CpG-A, the resulting mature pDC promote a robust anti-tumor immune response. Activation of pDC causes secretion of very large quantities of type I interferons, increased expression of costimulatory molecules, and recruitment and activation of other DC subsets to enhance tumor antigen presentation and generation of protective T cell responses. The preclinical efficacy of intratumorally administered CMP-001 alone or in combination with an intraperitoneally administered PD-1 agonist and/or an OX40 agonist was examined and assessed in a variety of syngeneic tumor models: CT-26 colon tumor model, MBT-2 bladder tumor model, RenCa kidney tumor model, 4T1 breast tumor model and LLC-1 lung tumor model. Tumors were implanted into left and right flanks while only one tumor was injected with CMP-001. In addition to body weight and overall survival, tumor volume was monitored on both flanks to assess direct and abscopal/systemic anti-tumor activity. Some discrepancies were observed between evaluated syngeneic tumor models with non-responders (LLC-1, 4T1) and responders (CT-26, MBT-2, RenCa). The most efficacious results were registered in the CT-26 model with a 80% tumor response rate as a single agent, which improved when combined with another treatment modality. The best therapeutic efficacy was obtained with the combination of all three agents resulting in cures of both treated and untreated CT-26 tumors in 40% of the mice. The median survival time was increased for these animals compared to those treated with only vehicle or one or two immune modulators (50 vs 18 vs 21 vs 23-28 days, respectively). Similar results were generated in the MBT-2 model (and to a lesser extent in RenCa model) though no complete response was recorded. These data support the clinical investigation of these combinations in cancer patients.

#2670 Combination of anti-HER2 ADC XMT-1522 and checkpoint inhibitor pembrolizumab for treatment of NSCLC in preclinical models

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The combination of antibody-drug conjugates (ADCs) and immunomodulatory cancer therapies is emerging as a powerful strategy for cancer treatment. Tumor-targeted delivery of a cytotoxic payload capable of inducing immunogenic cell death (ICD) can trigger both an innate and an adaptive immune response, whereby increased recruitment of effector T-cells to the tumor and formation of tumor specific immunological memory can result in a durable treatment response. The ADC XMT-1522 consists of a novel human IgG2 anti-HER2 monoclonal antibody and a novel, auristatin-based cytotoxic payload (Auristatin F-hydroxypropylamide, AF-HPA). An average DAR of 12 AF-HPA molecules is achieved via a biodegradable polymer conjugation platform. We have characterized the ability of both the free payload AF-HPA and the ADC XMT-1522 to induce ICD in vitro in multiple cell lines (NCI-N87, HT-29, SKBR3). Both payload and the ADC demonstrated robust activity as a single agent, which improved when combined with another treatment modality. The best therapeutic efficacy was obtained with the combination of all three agents resulting in cures of both treated and untreated CT-26 tumors in 40% of the mice. The median survival time was increased for these animals compared to those treated with only vehicle or one or two immune modulators (50 vs 18 vs 21 vs 23-28 days, respectively). Similar results were generated in the MBT-2 model (and to a lesser extent in RenCa model) though no complete response was recorded. These data support the clinical investigation of these combinations in cancer patients.
fied in whole blood and in tumor by FACS and IHC. XMT-1522 treatment alone induced tumor growth delay after 3 weekly doses of 1 mg/kg. Pembrolizumab as a single agent administered every 5 days for 6 doses (q5dX6) at a dose of 2.5 mg/kg led to less tumor growth delay than XMT-1522 treatment. The combination of these two treatment regimens resulted in a better response than either of the two monotherapies. These data provide a rationale for XMT-1522 to be tested clinically as a single agent in HER2-expressing NSCLC, as well as a rationale for combination of XMT-1522 and immunomodulatory therapies in NSCLC.

**#2671 Antitumor activity of NKTR-214 in combination with pmel-1 ACT in an aggressive murine melanoma model.** Giulia Parisi,1 Justín Saco,1 Siwen Hu-Lieskovsk,1 Ruixue Zhang,1 Paige Krzywinski,1 Cristina Puig Saus,1 Deborah H. Charych,1 Antoni Ribas1.1UT MD Anderson Cancer Ctr., Houston, TX; 2Baylor College of Medicine, Houston, TX.

The adoptive cell transfer (ACT) of genetically engineered T cells expressing cancer-specific T-cell receptors (TCR) has been shown to induce effective anti-tumor response. However, tumors frequently relapse after an initial response. Another strategy towards stimulating the immune system is the use of high-dose interleukin-2 (IL-2) to target the IL-2 receptor (IL2R), leading to immune cell expansion. However, clinically approved IL-2 expands both tumor-killing CD8+ effector T cells (CD8T) as well as regulatory T cells (Tregs) through binding the IL-2Rββ and IL-2Rαβ complexes, respectively. Tregs in the tumor lead to immune suppression, which hampers the antitumoral response. NKTR-214 is a bi-specific cytokine agonist conjugated with multiple releasable chains of polyethylene glycol and designed to provide sustained signaling through the heterodimeric IL-2 receptor pathway (IL-2Rββ) to preferentially activate and expand effector CD8+ T and NK cells over Tregs. We used the pmel-1 ACT/B16 melanoma tumor model to test the antitumor activity of NKTR-214 and evaluate its effects on tumor-specific TCR transgenic T cells. On Day 0 (D0) C57BL/6 mice were implanted with B16-F10 mouse melanoma cells and lymphodepleted with 500 Gγ on D6. On D7, mice were treated with either the combination of ACT (T lymphocytes activated in vitro with 1 μg/ml gp100) plus NKTR-214 (0.8 mg/kg, q3dx3, i.v.) or with C57/B6 T cells plus PBS (vehicle control). The tumors of the vehicle control mice (n=12) rapidly grew to the 1500mm3 endpoint in 12 days post-treatment, versus 35 days for the NKTR-214 group (n=12) with only 1 out of 12 mice reaching endpoint. Bioluminescence imaging was used to visualize the in vivo distribution and tumor-homing of antigen-specific T cells. Interestingly, the reporter T cells were retained in the spleen until D7 and could be seen migrating to the tumor at D9 reaching peak of bioluminescence at day 12, a delayed time point compared to the 5 days usually observed in mice treated with standard IL-2. The signal persisted in the NKTR-214 + ACT group until D20 versus D7 in the vehicle-control animals. These data suggest that NKTR-214 + ACT is well tolerated and provides a robust anti-tumor response in the aggressive B16F10 model. Treatment with NKTR-214 + ACT robustly mobilizes T cells into the tumor where they durably persist. The robust and long-lasting effect of NKTR214 supports its potential use in combination with cell-based therapeutics.

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**#2672 Response to anti-PD-1 based therapy in metastatic melanoma patients is associated with the diversity and composition of the gut microbiome.** Vancheri,1 Alexander Groher,1 Prieto,1 Diego Vicente,1 Tatiana V. Karpinets,1 Courtney W. Hudgens,1 Diane S. Hutchinson,2 Michael Tetzlaff,1 Alexander Lazár,1 Michael A. Davies,1 Jeffrey E. Gershewad,1 Robert Jenq,1 Patrick Hwu,1 Padmanee Sharma,1 James Allison,1 Andrew Futreal,1 Nadim Ajami,2 Joseph Petrosino,2 Carrie Daniel-MacDougall,1 Jennifer A. Wargo,1 UT MD Anderson Cancer Center, Houston, TX; 2Baylor College of Medicine, Houston, TX.

Background: Melanoma therapy has benefitted greatly from immune check-point blockade, although responses are variable and not always durable. There is a growing appreciation of the role of the microbiome in cancer-related outcomes and recent evidence in murine models suggests that modulation of the gut microbiome may enhance responses to immune checkpoint blockade in melanoma. However this has not been investigated in patients. Here, we demonstrate that differential bacterial “signatures” exist in the gut microbiome of responders (R) and non-responders (NR) to anti-PD-1 therapy, and that insights gained could be used to derive actionable strategies to enhance responses. Methods: We collected buccal (n = 105) and stool (n = 53) samples from a cohort of anti-PD-1 treated metastatic melanoma patients (n = 110). Patients were classified as either R or NR based on RECIST criteria, and 16S rDNA, and whole-genome shotgun sequencing was performed to characterize the diversity, composition and functional capabilities of the microbiomes. Immune profiling (via ?-marker IHC panel of CD3, CD8, PD-1, PD-L1, Granzyme B, ROYR and FoxP3) and cytotoxic analyses were also performed on available tumors and serum samples at baseline. Results: In these studies, we observed significant differences in the diversity and composition of the gut microbiome in R versus NR to PD-1 blockade at baseline, but no clear differences in buccal microbiota. Specifically, R had a significantly higher alpha diversity compared to NR (p = 0.017), and the Ruminococcaceae family of the Clostridiodales order was enriched in R whereas Prevotellaceae family of the Bacteroidales order was enriched in NR. Immune profiling demonstrated significantly increased immune infiltrates in baseline tumor samples of R, with a positive correlation between CD8, CD3, PD-1 and FoxP3 T-cell density and abundance of specific bacteria enriched in R (e.g. Faecalibacterium). Low diversity was also associated with elevated levels of chronic inflammation markers in the serum at baseline. Lastly, we saw differentially abundant metabolic pathways in the gut microbiomes of R (pyrimidine nucleotide biosynthesis, fatty acid biosynthesis, shikimate pathway) vs NR (Tri-carboxylic acid cycle, assimilatory sulphate and nitrate reduction, tryptophan biosynthesis). Conclusion: Differences exist in the diversity and composition of the gut microbiome in R vs NR to anti-PD-1 therapy and these microbiota could bridge the gap between host metabolism and anti-tumor immunity. These results further support the notion that modifications to the gut microbiome could potentially enhance therapeutic responses to immune checkpoint blockade.

**#2673 Divergent therapeutic responses to CD40L blockade or CD40 activation in Ras-driven skin cancers determined by origin of tumor initiating cell.** Adam B. Glick,1 Jacob T. Bailey,1 Andrew Gunderson,1 Kyle Breech,1 Michael Podolsky,1 Pennsylvania State University, University Park, PA; 2Earle A. Chiles Research Institute, Portland, OR.

Heterogeneity in tumor immune responses is a poorly understood yet critical parameter for successful immunotherapy. We used doxycycline-inducible epithelial squamous cancer models driven by oncogenic Ras to test whether the origin of the tumor-initiating cell influenced the tumor immune response. Threshold doxycycline induced expression of H-RasV12 in either the basal/ stem cell layer with a Keratin14-rtTA transgene (K14-Ras), or committed progenitor/suprabasal cells with an Involucrin-rtTA transgene (InvRas), caused distinct immune responses despite similar levels of Ras, tumor latency, and tumor numbers. End stage K14-Ras tumors had an immunosuppressed microenvironment with abundant Tregs and Bregs which evolved from an initially Th1 dominant environment in the earliest detectable lesions. In contrast InvRas tumors had a Th2 pro-inflammatory microenvironment, with significantly fewer Tregs or Bregs at any time point. This difference in immune microenvironment between the tumor models was also found in hyperplastic skin after 5 days of maximal Ras expression, indicating that it was not simply a property of the end stage tumor. Surprisingly, adaptive immunity had opposite roles in tumor development. InvRasRag1−/− mice developed fewer and smaller tumors that regressed while K14RasRag1−/− mice developed more tumors with shorter latency than Rag1+/+ controls. In both models adoptive transfer and depletion studies showed that cooperation between B and CD4 T cells drove the opposing tumor responses, lymphocyte polarization, and tumor immune phenotype. Culture of tumor-conditioned but not splenic B cells from each model with naïve CD4 T cells showed that direct contact and CD40-CD40L ligation was required for opposite polarization towards either a Th2 or Treg phenotype. Importantly, we found that anti-CD40L mAb caused regression of preexisting InvRas tumors but enhanced growth of K14 Ras tumors. In contrast, CD40 agonist mAb enhanced growth of preexisting InvRas tumors, and suppressed growth of K14 Ras tumors. Thus in an in vivo setting the type of tumor immune microenvironment and opposing role of CD40-CD40L signaling in tumor development determines distinct responses to therapeutic antibodies. Together these data show that the position of a tumor initiating cell within the stem cell hierarchy in a stratified squamous epithelia has important consequences for the type of tumor immune microenvironment and response to checkpoint therapy.

**#2674 Fusobacterium nucleatum subspecies animalis influences pro-inflammatory cytokine expression and monocyte activation in human colorectal adenocarcinoma cell lines.** Xiangcang Ye,1 Pu Wang,1 Ruiqiang Li,1 Delphine R. Boulares,1 Fan Fan,1 Ling Xia,1 Adoni Harish,1 Nadim J. Ajami,1 Matthew C. Wong,2 Daniel P. Smith,1 Joseph F. Petrosino,2 Susan Venable,2 Wei Qiao,1 Veera Baladandayuthapani,1 Dipen Maru,1 Lee M. Ellis1; 1UT MD Anderson Cancer Ctr., Houston, TX; 2Baylor College of Medicine, Houston, TX.

Fusobacterium nucleatum is an abundant peri-tumoral commensal bacteria that is strongly associated with colorectal carcinogenesis. However, the role of fusobacteria in colon cancer is still poorly understood. To investigate the role of F. nucleatum subspecies animalis in human colorectal cancer, we treated two colorectal adenocarcinoma cell lines, HCT116 and SW480, with Fusobacterium nucleatum subspecies animalis. As a positive control, we used the well-known pro-inflammatory cytokine Th1 stimulator, IFNγ. Our results showed that Fusobacterium nucleatum subspecies animalis significantly increased the expression of pro-inflammatory cytokines, IL-6, IL-8, and TNFα, in both cell lines in comparison to control. Furthermore, we found that Fusobacterium nucleatum subspecies animalis induced monocyte activation, as indicated by increased expression of CD86 and CD80. These results suggest that Fusobacterium nucleatum subspecies animalis may play a role in the pro-inflammatory environment of colorectal adenocarcinoma. Future studies are needed to further investigate the role of Fusobacterium nucleatum subspecies animalis in colorectal cancer.
Chronic infection and associated inflammation have long been suspected to promote human carcinogenesis. Recently, certain gut bacteria, including some in the Fusobacterium genus, have been implicated in playing a role in human colorectal cancer (CRC) development. However, the Fusobacterium species and subspecies involved and their oncogenic mechanisms remain to be determined. We sought to identify the specific Fusobacterium spp. and ssps. in clinical CRC specimens by targeted sequencing of Fusobacterium 16S ribosomal RNA gene. Five Fusobacterium spp. were identified in clinical CRC specimens. Additional analyses confirmed that Fusobacterium nucleatum ssps. animalis was the most prevalent F. nucleatum subspecies in human CRCs. We also assessed inflammatory cytokines in CRC specimens using immunoassays and found that expression of the cytokines interleukin-17A and tumor necrosis factor-alpha was markedly increased but interleukin-21 decreased in the colorectal tumors. Furthermore, the chemokine (C-C motif) ligand 20 was differentially expressed in colorectal tumors at all stages. In vitro co-culture assays, F. nucleatum ssps. animalis induced CCL20 expression in CRC cells and monococytes. It also stimulated the monocyte/macrophage activation and migration. Our observations suggested that infection with F. nucleatum ssps. animalis in colorectal tissue could induce inflammatory response and promote CRC development. Further studies are warranted to determine if F. nucleatum ssps. animalis could be a novel target for CRC prevention and treatment.

#2675 MMP9 modulates ROS levels and DNA repair pathway to maintain genomic stability in colitis associated cancer. Lewins Walter, Adani Pujada, Brandon Canup, Hamed Lariou, Pallavi Garg, Georgia State University, Atlanta, GA.

Introduction: Chronic inflammation predisposes tissues to oncogenic, and in the colon this condition is termed as colitis associated cancer (CAC). CAC is the inflammation-dysplasia-carcinoma pathway which is significantly different compared to the adenoma-carcinoma pathway of sporadic colon cancer (CRC). In CAC, chronic inflammation causes accumulation of reactive oxygen species (ROS) resulting in DNA damage that nurtures tumor microenvironment and accelerates cancer cell growth. Gut microbiota is important in regulating ROS levels, and DNA repair pathways are critical in restoring the genomic instability. Matrix Metalloproteinases (MMPs) are a family of zinc dependent endopeptidases which mediate inflammation, tissue remodeling, and tumorigenesis. MMP9 is the most unique MMP because it is undetectable in healthy tissue but highly upregulated during inflammation and cancer. We have previously shown that MMP9 plays a protective role in CAC which is opposite to its conventional role of mediator in acute inflammation and cancer. Aim: In this study, we examined if MMP9 acts as a tumor suppressor by modulating gut microbiota and mismatch repair (MMR) genes to reinstate genomic stability in CAC. Methods: We used C57/B6 transgenic MMP9 mice which can express MMP9 under villin promoter (TgM9) and their wild type (WT) littermates. CAC was induced by azoxymethane (AOM) and dextran sodium sulfate (DSS, inflammation induction) to establish the monocyte/macrophage activation and migration. Our observations suggested that infection with F. nucleatum ssps. animalis in colorectal tissue could induce inflammatory response and promote CRC development. Further studies are warranted to determine if F. nucleatum ssps. animalis could be a novel target for CRC prevention and treatment.

#2677 Role of CLEC4D in inflammation-driven liver carcinogenesis. Elena Riboldi,1 Luca Di Tommaso,2 Nausicia Clemente,2 Chiara Raggi, Elisa Forti,3 Simone Merlin,2 Antonia Follenzi,2 Antonio Sica2. 1Università del Piemonte Orientale, Dept. of Pharmacological Sciences, Novara, Italy; 2Università del Piemonte Orientale, Dept. of Medical Sciences, Novara, Italy.

Hepatocellular carcinoma (HCC) accounts for approximately 90% of all primary liver tumors and is the second most deadly cancer in the world. HCC commonly arises in a chronically damaged liver that contains large amounts of inflammation and fibrosis. Indeed, interactions among hepatocytes, stromal, and inflammatory cells create a complex microenvironment permissive to tumor development. Myeloid cells are crucial players in cancer-related inflammation. From transcriptional profiling of liver myeloid cells, we identified the gene encoding for the C-type lectin receptor CLEC4D as one of the genes upregulated during the inflammatory response that precedes cancer development in a spontaneous model of murine HCC. We hypothesized that CLEC4D may be involved in the molecular mechanisms that shape HCC pathogenesis. We used the murine model of diethylnitrosamine (DEN)-induced liver carcinogenesis and evaluated tumor growth in genetically modified mice lacking CLEC4D (CLEC4D KO mice), as compared to C57BL/6 wild type (WT) mice. We observed that tumor burden (number of tumors per mouse and tumor dimensions) was reduced in CLEC4D KO mice compared to WT mice. Gene expression analysis of tumor lesions showed that the tumor microenvironment of CLEC4D KO mice was less inflammatory. CLEC4D expression was then evaluated in surgical specimens and tissue microarrays from HCV+ HCC patients. CLEC4D-positive inflammatory cells (macrophages and granulocytes) were present both in the peritumor and in the tumor areas. The percentage of intratumor CLEC4D-positive cells inversely correlated with the degree of the lesion: CLEC4D expression was higher in dysplastic nodules compared to high grade HCCs. Our results indicate that the CLEC4D-dependent pathway contributes to the initiation and the progression of HCC. By targeting CLEC4D, we could modulate immune responses and provide an environment less favorable to tumor neoplasms including pancreatic cancer. Our laboratory recently found that RON serves to mediate pancreatic duct carcinogenesis initiated by KRAS. We demonstrated that RON overexpression in the pancreas results in more rapid local and metastatic progression ultimately associated with decreased survival. We also discovered profound changes in the immunophenotype within the tumor microenvironment and RON expression in tumors established that RON expression drives the polarization of tumor-associated macrophages into an alternative, pro-tumorigenic state. We hypothesize that these unique activities help to shape the immunosuppressive milieu of the pancreatic cancer microenvironment and that RON directed therapy has the potential to serve as a novel immunomodulatory strategy in this disease. The aim of this work is to define how KRAS mediated overexpression of RON can impact the tumor microenvironment in pancreatic cancer. In order to further interrogate the role of RON during pancreatic carcinogenesis, we bred kinase-dead RON mice (KD) to Pdx1-Cre/LSL-KRASG12D mice. We observed a significant delay in the onset of pancreatic neoplasia and a significant decrease in tumor weight. The absence of functional RON kinase markedly reduced trafficking of CD11b+ cells to the pancreas and immunophenotyping in tumors showed a clear reduction of alternatively polarized macrophages when compared to RON overexpression or KRAS mutation alone. Because RON is expressed in both epithelial and stromal cell populations, we further sought to dissect the relevance of RON function to tumor growth within these cellular compartments. We performed orthotopic injections of organoid-3D cells derived from tumors of KPC mice (LSL-KRASG12D mice) into NOD-SCID (Ngbmumu) mice. The effects of the angiogenesis inhibitor bevacizumab on tumors established in wild type (WT) or RON KD mice. Preliminary results show that tumor growth is impaired in KD mice, suggesting that RON activity in the host microenvironment influence tumor growth. In order to identify all immune populations regulated by RON, we will conduct an exhaustive immunophenotyping of murine pancreatic tumors. A transcriptomic analysis will then be conducted on selected cell populations to better define the molecular mechanisms associated with the RON regulation. Finally, given the effect of RON targeting on macrophage polarization, we will also dissect the effects of RON signaling from bone marrow derived myeloid cells versus resident tissue macrophages. Preliminary results and future experiments will significantly narrow the gap in our understanding of how RON signaling contributes to shaping the pancreatic cancer chimera and therefore how RON-directed therapies could be most successfully utilized to benefit those afflicted with this most deadly disease.
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growth. An intervention at this level could represent a chemopreventive strategy to arrest the development of HCC in a cirrhotic liver. This work has been supported by Fondazione Cariplo, grant n° 2014-0962.


About 20% of the cancer incidences worldwide have been estimated to be associated with infections. There is a strong correlation of some pathogens with various cancer types. Although presence of commensal microbiome helps chemotherapy to be more effective, pathogenic microbiota increases the cancer risk. Invading pathogens interact with the host mainly through proteins. To subvert host defense, pathogens hijack host pathways by mimicking the binding surfaces (interfaces) of host proteins. This similarity in interfaces permits the pathogenic protein to compete with host proteins to bind to a target protein, alter the physiological signaling and cause persistent infections as well as cancer. Detection of host-pathogen interactions (HPIs) and mapping the re-wired HPI network - along with its structural details - is critical for in-depth understanding of the underlying pathogenesis mechanisms of infections and pathogen-triggered cancers, and developing efficient therapeutics. Here, we developed a novel computational approach to identify novel HPIs by employing “interface mimicry.” We applied this approach to Helicobacter pylori, dominant species in gastric microbiome that greatly increases the gastric cancer risk in order to understand how they modulate host immune responses and influence tumorigenesis.

We found that HP pylori interacts with the function of the host apoptosis pathway, cytomegaly and cytokine pathways, and also cell-cell adhesions. Our results shed light on the molecular mechanisms of resistance to apoptosis, immune evasion and loss of cell junctions that are seen in Helicobacter pylori-infected host cells. In conclusion, HP pylori can help us unravel which human pathways are targeted by the pathogenic proteins and how they contribute to pathogenesis of infections and pathogen-triggered cancer. With a better grasp on immunomodulatory strategies of pathogens, we can develop better therapies against them.

#2679 A promoting role for the epithelial MyD88/IRAK4/NF-κB signaling in K-ras mutant lung tumorigenesis. Susana Castro,1 Soudabeh Daliri,1 Maria Miguelina De La Garza,2 Amber M. Cumpian,3 Misha Umer,4 Diana Del Bosque,2 Sahab Akhani,2 Scott E. Evans,2 Seyed Javad Moghaddam.2 The University of Texas MD Anderson Cancer Center, Houston, TX;2University of Houston, Houston, TX.

K-ras mutation is the most common oncogenic alterations associated with lung cancer development. Unfortunately, all attempts to develop therapies directly targeting K-ras have been failed thus far, clearly stating the need for new strategies to arrest effective cooperativity pathways of K-ras to overcome lung cancer displaying such a molecular profile. Using a conditional K-ras mutated lung cancer mouse model, CC-LR (CCSP1/LSL-K-rasG12D), we previously showed that K-ras mutated lung tumorigenesis is associated with lung inflammation due to activation of NF-κB pathway and increased expression of its downstream targets in the lung. Here we have shown that lack of NF-κB activity in the airway epithelial cells by selectively targeting IKKβ, which is required for NF-κB activation, significantly reduces lung tumor burden (3.4-fold) and changes the inflammatory cells and mediators in the bronchoalveolar lavage fluid (BALF) of CC-LR mice. Immunohistochemically staining of lung tissues with specific markers, Ki-67 and CD-31, demonstrated significantly lower tumor cell proliferation and angiogenesis in CC-LR mice with lack of epithelial NF-κB activity. To further dissect the role of NF-κB pathway in this process, CC-LR mice were crossed with MyD88l−/− mice to develop a mouse with lack of MyD88 (an adaptor protein upstream to IKKβ) in the airway epithelial cells (CC-LR/MyD88l−/− mice). As we had hypothesized, the resulting tumor numbers in the lungs were significantly lower (1.9-fold) in CC-LR mice with lack of MyD88 in the airway epithelial cells compared to control CC-LR mice. Tumor reduction in CC-LR/MyD88l−/− mice was also associated with decreased tumor cell proliferation and angiogenesis compared to control CC-LR mice. Surprisingly, unlike to lack of epithelial NF-κB activity, absence of MyD88 in the airway epithelium did not change the BALF inflammatory cell component of CC-LR mice. We then targeted another upstream signaling molecule to NF-κB, IRAK4, which is down stream of MyD88 by crossing CC-LR mice to IRAK4 knockout (LPSK:CC-LR/IRAK4−/−). Surprisingly, in lack of MyD88, we found a significant reduction in lung tumor number (1.8-fold) with no changes in BALF inflammatory cell component in CC-LR mice with lack of IRAK4 compared to control CC-LR mice. Taken these together, we conclude that there is an essential role for MyD88/IRAK4/NF-κB pathway activation in promotion of K-ras mutant lung cancer.

#2680 A novel pro-tumorigenic role for IDO1 in inflammatory neovascularization. Arpita Mondal,1 Erika Sutanto-Ward,1 James B. DuHaddaway,1 Ar-turo Bravo-Nuevo,2 Sunil Thomas,1 George C. Prendergast,1 Alexander J. Muller,1 Lankenau Inst. for Medical Research, Wynnewood, PA;1ArtDemi Therapeutics, Holmes, PA.

Sidmouth inhibitors of the tryptophan catalyzing enzyme IDO1 (in-doleamine 2,3-dioxynase 1) have shown early promise in clinical trials as immuno-oncology agents. While the tolerogenic activity of IDO1 has been well established, we have recently identified an additional role for IDO1 in supporting neovascularization at the regulatory interface between the inflammatory cytokines IFNγ (interferon γ) and IL6 (interleukin 6). IFNγ is a primary inducer of IL6, but is also a key mediator of immune-based tumor suppression, which studies have associated with its anti-angiogenic activity. Conversely, genetic studies in mice have clearly established the tumor-promoting role of IDO1, suggesting that it may act in a negative feedback capacity. Targeted disruption of the Idol gene in mice resulted in enhanced resistance to lung tumor and metastasis development. As we have previously shown with attenuated induction of the pro-angiogenic cytokine IL6b, which, when provided by ectopic expression, was able to restore pulmonary metastasis susceptibility to Idol−/− mice. These initial findings led us to hypothesize that IDO1 might contribute to cancer promotion by countering the anti-angiogenic effect of IFNγ, possibly through IDO1-potentiated end of IL6. Testing this hypothesis in mouse models of oxygen-induced retinopathy and pulmonary metastasis, we determined that loss of IDO1 did not result in reduced neovascularization with impaired metastasis outgrowth, effects that were completely reversed by the concurrent elimination of IFNγ. Loss of IL6b was likewise associated with IFNγ-dependent reductions in neovascularization and impaired metastasis outgrowth, as predicted. Having established a novel role for IDO1 in inflammatory neovascularization, current investigations are focused on the underlying molecular and cellular mechanisms involved. At the molecular level, one of the consequences of tryptophan catalysis by IDO1 can be to trigger the ISR (in-tegrated stress response) through activation of the GCN2/CHOP pathway that has previously been linked to the downstream induction of IL6. Thus far, data collected in both the oxygen-induced retinopathy and pulmonary metastasis models are consistent with the ISR being the relevant downstream signaling pathway from IDO1 in this biological context. At the cellular level, we have detected the incorporation of non-endothelial, IDO1-positive cells into the vessels that comprise the neovascular tufts, implicating IDO1 in the process of vasculogenesis. These insights into this unrecognized aspect of IDO1 biology are likely to have important ramifications for IDO1 inhibitor development, especially in other cancers where clinical trials are currently ongoing, but in other disease indications involving neovascularization as well.

#2681 Oral micro biome enhances stemness in oral cancer cells by activat-ing Toll like receptor signaling. Kavi Krishna laboratory, Guwahati Biotech Park, IIT, Guwahati, India;1Kavi Krishna Laboratory, Guwahati Biotech Park, IIT, Guwahati, India;1Kavi Krishn Lab, Guwahati, India;1Kavi Krishn Laboratory, Guwahati Biotech Park, IIT, Guwahati, India. 2Dr. Y. Na, University of Cambridge, MA; 2Gauhati University, Gauhati, India; 3B Borooah Cancer Institute, Guwahati, India.

Background: The resistance and progression of cancers after chemotherapy to invasive and metastatic stages accounts for the overwhelming majority of cancer deaths. Recent studies suggest, microbiomes can induce a cascade of host events to either support or inhibit tumor growth. Specially, in oral cancer, chemother-apy treatment may alter the oral microbial flora, which may favor or inhibit tumor growth. Hence, it is important to develop novel experimental approaches to study the role of oral microbial flora in oral cancer stemness (self-renewal and undifferentiated state of cancer stem cells). Importantly, patients in developing area, including Assam, where Kavi Krishna laboratory is located, may have distinct oral microbial flora that could favor oral cancer growth. Hence, it is important to include patients from developing countries for such studies. Our previous research showed that chemotherapy changes stemness in many cancer cell types, including oral squamous cell carcinoma cell line SCC-25. The stemness switch is characterized by enhanced expression of stemness associated genes including Nanog, Lin28/28A, Oct-4, MYC, HIF-2alpha and inflammation associated genes including TLRs.

Methods: SCC-25 oral cancer cell line was treated with bacterial product lipopolysaccharide (LPS), and the stemness switch evaluated by isolation of cancer stem cells (self-renewal and undifferentiated state of cancer stem cells). Importantly, patients in developing area, including Assam, where Kavi Krishna laboratory is located, may have distinct oral microbial flora that could favor oral cancer growth. Hence, it is important to include patients from developing countries for such studies. Our previous research showed that chemotherapy changes stemness in many cancer cell types, including oral squamous cell carcinoma cell line SCC-25. The stemness switch is characterized by enhanced expression of stemness associated genes including Nanog, Lin28/28A, Oct-4, MYC, HIF-2alpha and inflammation associated genes including TLRs.

Conclusion: The resistance and progression of cancers after chemotherapy to invasive and metastatic stages accounts for the overwhelming majority of cancer deaths. Recent studies suggest, microbiomes can induce a cascade of host events to either support or inhibit tumor growth. Specially, in oral cancer, chemother-apy treatment may alter the oral microbial flora, which may favor or inhibit tumor growth. Hence, it is important to develop novel experimental approaches to study the role of oral microbial flora in oral cancer stemness (self-renewal and undifferentiated state of cancer stem cells). Importantly, patients in developing area, including Assam, where Kavi Krishna laboratory is located, may have distinct oral microbial flora that could favor oral cancer growth. Hence, it is important to include patients from developing countries for such studies. Our previous research showed that chemotherapy changes stemness in many cancer cell types, including oral squamous cell carcinoma cell line SCC-25. The stemness switch is characterized by enhanced expression of stemness associated genes including Nanog, Lin28/28A, Oct-4, MYC, HIF-2alpha and inflammation associated genes including TLRs.
where KaviKrishna laboratory is located. The sputum was processed and then added to the culture medium of SCC-25 cells. These post-sputum treated cells were subjected to phenotypic stemness switch analysis. Results: We found, LPS and sputum treatment led to the enhanced stemness of ABCG2+ cells, including the high expression of TLR2/4, MTC, Nanog, Sox-2, and HIF-2alpha. These data supported the hypothesis that ABCG2+ cells exhibited high expression of TLR2/4 and associated increase of HIF-2alpha and MYC transcriptional activity. The sputum treated with broad spectrum ciprofloxacin did not enhance the stemness and showed inhibitory activity on ABCG2+ cell self-renewal. In contrast, sputum obtained from oral cancer subjects with relapse showed enhanced stemness of ABCG2+ cells, and also increased tumorigenic potential. The post-sputum treated ABCG2+ cells exhibited high expression of TLR2/4 and associated increase of HIF-2alpha and MYC transcriptional activity. To determine whether the microbiome may enhance response to immune checkpoint inhibition, and indicate that the gastrointestinal microbiome and its composition are critical for the antitumor efficacy of αPD-L1.


Pancreatic ductal adenocarcinoma (PDAC) is the fourth-leading cause of cancer-related deaths in the United States with metastasis to the liver as the major cause of mortality. While the propensity of PDAC to spread to the liver may reflect mechanical trapping of tumor cells that enter the portal circulation, primary tumor cells in the liver are not required for invasion and metastasis of PDAC. We previously reported that inflammation and IL-6/STAT3 signaling in the liver play a role in tumor seeding in the liver even prior to development of invasive PDAC. To investigate the impact of PDAC development on the formation of a pro-metastatic niche in the liver, we found that KPC mice (compared to age- and gender-matched control mice) demonstrated an increased susceptibility to tumor seeding in the liver even prior to development of invasive PDAC. Examination of the liver of KPC mice revealed diffuse activation of Signal Transducer and Activator of Transcription 3 (STAT3) signaling, particularly in hepatocytes. Although hepatocytes are recognized as important regulators of inflammation, their role in establishing a pro-metastatic niche is undefined. To define the role of the liver associated with development of a pro-metastatic niche, we performed QuantiSeq analysis on RNA isolated from the liver of KPC vs control PC mice. Our results showed increased transcriptional levels of myeloid chemokine receptors, particularly serum amyloid A proteins that are predominately produced by hepatocytes. Consistent with this finding, we observed an accumulation of F4/80+ and Ly6G+ myeloid cells in the liver of KPC mice by immunofluorescence microscopy. We next determined the role of tumor cells in driving cellular activation seen in the liver by establishing intraperitoneal and orthotopic models of PDAC. Using these models, we found that implantation of pancreatic tumor cells induced STAT3 activation in hepatocytes and stimulated F4/80+ and Ly6G+ myeloid cell recruitment to the liver. To determine whether cellular activation in the liver was associated with systemic release of soluble factors, we performed parabiotic joining of tumor-implanted mice and control wild type mice, and we found evidence of STAT3 activation and myeloid recruitment to the liver in parabiotic pairs. As interleukin-6 (IL-6) is a key inflammatory cytokine that can activate STAT3 signaling, we hypothesized a role for IL-6 directed STAT3 activation in hepatocytes for development of a pro-metastatic niche in the liver. Consistent with this hypothesis, we found that IL-6 receptor blocking antibodies administered after tumor implantation reduced STAT3 activation in hepatocytes and decreased transcriptional levels of hepatocyte-derived chemoattractants. Together, our findings provide support for IL-6/STAT3 signaling in hepatocytes in driving a pro-metastatic niche in the liver during PDAC development.


The intestinal microbiome has become increasingly appreciated as a significant mediator of systemic antitumor immunity/response in both naive and treatment contexts. In naive contexts, an intact intestinal microbiome has been demonstrated to enhance tumorigenesis, and its composition to mediate primary tumor growth kinetics. In the context of cancer treatment, antibiotic depletion of the intestinal microbiota has been reported to inhibit the efficacy of cyclophosphamide and that of the immune checkpoint inhibitor oCTLA4. Compositional modulation of the intestinal microbiota has been found to be sufficient to enhance the antitumor efficacy of αPD-L1. Here, we assessed the relative importance of the intestinal microbiota in mediating αPD-L1 antitumor efficacy in a B16.F10.SIY murine model of melanoma, by performing parallel efficacy studies in C57BL/6 germ-free (Taconic) or specific pathogen free (Taconic or Jackson) mice. We observed that αPD-L1 treatment provided significant antitumor efficacy in Taconic mice carrying an intact microbiome; however, this efficacy was abolished in germ-free Taconic mice. Furthermore, we observed that tumors of Jackson mice carrying an intact but compositionally different microbiome did not respond to αPD-L1 treatment. Phenotyping of local tumor and systemic immune responses, as well as characterization of the intestinal microbiome in responder vs nonresponder animals provided mechanistic insights. Taken together, these data suggest that modulation of the microbiome may enhance response to immune checkpoint inhibition, and indicate that the gastrointestinal microbiome and its composition are critical for the antitumor efficacy of αPD-L1.

#2684 Multiplex immunofluorescence profiling of tumor infiltrating immune subsets in HNSCC biopsies provides a powerful tool when combined with patient outcome data. Matt Levin, Mark Lingen, David Schwartz, Helen Snyder. Cell Idx, San Diego, CA.

Head and neck squamous cell carcinoma (HNSCC) is one of the growing number of tumors for which an anti-tumor immune response is implicated to play a role in the course of the disease. Profiling of the tumor microenvironment including specific infiltrating lymphocyte subsets, antigen present- ing cells, and expression of regulatory cytokines in immune cells has also been suggested to secrete factors that may promote recruitment of myeloid cells to establish a pro-metastatic niche. In this study, we used the LS-L-KrasG12D+/H11001, LS-L-Tprp53R127H+/H11001, Pdx-1-Cre (KPC) mouse model of PDAC to investigate the impact of PDAC development on the formation of a pro-metastatic niche in the liver. We found that KPC mice (compared to age- and gender-matched control mice) demonstrated an increased susceptibility to tumor seeding in the liver even prior to development of invasive PDAC. Examination of the liver of KPC mice revealed diffuse activation of Signal Transducer and Activator of Transcription 3 (STAT3) signaling, particularly in hepatocytes. Although hepatocytes are recognized as important regulators of inflammation, their role in establishing a pro-metastatic niche is undefined. To define the changes in the liver associated with development of a pro-metastatic niche, we performed QuantiSeq analysis on RNA isolated from the liver of KPC vs control PC mice. Our results showed increased transcriptional levels of myeloid chemokine receptors, particularly serum amyloid A proteins that are predominately produced by hepatocytes. Consistent with this finding, we observed an accumulation of F4/80+ and Ly6G+ myeloid cells in the liver of KPC mice by immunofluorescence microscopy. We next determined the role of tumor cells in driving cellular activation seen in the liver by establishing intraperitoneal and orthotopic models of PDAC. Using these models, we found that implantation of pancreatic tumor cells induced STAT3 activation in hepatocytes and stimulated F4/80+ and Ly6G+ myeloid cell recruitment to the liver. To determine whether cellular activation in the liver was associated with systemic release of soluble factors, we performed parabiotic joining of tumor-implanted mice and control wild type mice, and we found evidence of STAT3 activation and myeloid recruitment to the liver in parabiotic pairs. As interleukin-6 (IL-6) is a key inflammatory cytokine that can activate STAT3 signaling, we hypothesized a role for IL-6 directed STAT3 activation in hepatocytes for development of a pro-metastatic niche in the liver. Consistent with this hypothesis, we found that IL-6 receptor blocking antibodies administered after tumor implantation reduced STAT3 activation in hepatocytes and decreased transcriptional levels of hepatocyte-derived chemoattractants. Together, our findings provide support for IL-6/STAT3 signaling in hepatocytes in driving a pro-metastatic niche in the liver during PDAC development.

#2685 Modulating tumor microenvironments through inflammasome and IL-1 pathways. Beichu Guo, Jinyu Zhang, Shun-Jun Fu. Medical University of South Carolina (MUSC), Charleston, SC.

Chronic Inflammation has been shown to play important roles at all stages of tumor development including initiation, growth, invasion and metastasis. The inflammasome is an important innate immune pathway critical for the production of active IL-1β, a potent inflammatory cytokine. While extensive evidence indicates that inflammasomes are involved in infections and autoimmune diseases, their full potential in tumor development remains to be discovered. To dissect the roles of the inflammasome and IL-1 pathway in tumor development, we utilized the MMTV-PyMT transgenic model, which develops mammary gland tumors with a high incidence of lung metastasis. Our results have demonstrated that inflammasome and IL-1β play a critical role in promoting tumor growth and metastasis. We found that tumor growth was associated with inflammasome activation and elevated levels of IL-1β in tumor microenvironments in mouse mammary tumor models and in human breast cancer tissues. Mice deficient for inflammasome components or IL-1 receptor signaling exhibit significantly reduced lung metastasis. Our data also show that inflammasome activation led to the accumulation of myeloid cells, such as such as myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs) in tumor microenvironments. Furthermore, blocking IL-1R signaling with IL-1R antagonist (IL-Ra) or anti-IL-1R antibody inhibited tumor growth and metastasis. Our results suggest that targeting the tumor microenvironment through inflammasome and IL-1 blockade may provide a novel approach for the treatment of breast cancer.

#2686 Dual inhibition of BCR and TLR signaling has therapeutic potential in chronic lymphocytic leukemia. Eman L. Dadashian, Sarah Herman, Adrian Wiestner. NIH/NHLBI, Bethesda, MD.

Introduction: The Bruton’s tyrosine kinase (BTK) inhibitor ibrutinib is clinically active in lymphoproliferative diseases driven by B-cell receptor (BCR) and Toll-like receptor (TLR) signaling, including chronic lympho-
cytic leukemia (CLL) and Waldenstrom macroglobulinemia (WM), respectively. However, deep remissions are uncommon and resistance to single agent has been described. The hallmark of WM is an activating mutation in MYD88 in the TLR signaling pathway. While mutations in MYD88 are uncommon in CLL, our previous study identified gene signatures indicative of aggressive behavior in cases associated with MYD88 mutations (Herishanu, Blood 2011). Further, TLR9 activating CpG oligonucleotides induce proliferation and extend CLL cell survival in vitro. These observations suggest that BCR and TLR signaling may cooperate to activate CLL cells in the tissue microenvironment. Here, we tested the hypothesis that targeting both BCR and TLR signaling could improve therapy for CLL. Methods: CLL PBMCs were treated with ibrutinib in the presence or absence of IRAK1/4 inhibitor (Calbiochem) for 1h and then stimulated with soluble IgM, CpG, or both. We quantified changes in phosphorylation of BTK, PLCγ2 and ERK (BCR pathway) and STAT3 and STAT1, as well as total IRAK1 (TLR pathway). Results: As expected, ibrutinib inhibited phosphorylation of BTK, PLCγ2 and ERK (P<.05) and decreased the survival of CLL cells stimulated with IgM (P=.001). CpG stimulated TLR signaling degrades IRAK1 and the stimulates cytokine secretion that can, in autocrine fashion, activate STAT phosphor- ylation. The IRAK1/4 inhibitor effectively inhibited TLR signaling resulting in stabilization of IRAK1 (P=.002), decreased phosphorylation of STAT1/3 (P=.04) and decreased viability compared to CpG stimulated but not IRAK inhibitor treated cells. Ibrutinib had no effect on IgM-induced IRAK1 deg- radation, but was comparable to IRAK1/4 inhibition in reducing STAT phosphorylation, suggesting that inhibition of BTK can antagonize down- stream effects of TLR activation but not upstream IRAK dependent steps. In contrast, IRAK1/4 inhibition had no effect on IgM-induced BCR activa- tion. Next we evaluated the effect of dual BCR and TLR activation, modelling co-operative activation of both pathways in the tumor microenvironment. Under these in vitro conditions, ibrutinib prevented BCR activation and partial TLR activation, while IRAK1/4 affected only the TLR pathway. When ibrutinib and the IRAK1/4 inhibitor were combined, activation of both BCR and TLR signaling was prevented resulting in a significant reduction in CLL cell viability (P=.01) compared to co-activation of both pathways. Conclu- sion: While ibrutinib partially inhibited TLR signaling, an IRAK1/4 inhibi- tor was required for full inhibition of the pathway. The combination of BTK and IRAK1/4 inhibition for the treatment of lymphoproliferative diseases warrants further investigation.

#2687 Toll like receptors mediated inflammatory signals mediate promo- tion of K-ras mutant lung cancer by chronic obstructive pulmonary disease. Nasim Khoosravi,1 Nelly Torres-Garza,2 Soudabeh Daliri,1 Maria Miguelina De La Garza,3 Amber Cumpian,2 Evelyn Beltran,2 Mishia Umer,2 Diana Del Bosque,3 Saba Akbani,1 Scott Evans,1 Seyed Javad Moghaddam1.

Lung cancer is the leading cause of cancer death worldwide, and cigarette smoking is its main cause. However, smoker with chronic obstructive pul- monary disorder (COPD), an inflammatory disease of the lung, have an increased risk of lung cancer (3 to 10 fold) compared to smokers without COPD. Importantly, lung inflammation persists and lung function continues to deteriorate as does the increased risk of lung cancer even after cessa- tion of cigarette smoking among former smokers. These facts suggest a strong link between COPD-related airway inflammation and lung cancer promotion, however, the precise mechanistic link is not known. We have previously developed a COPD-like mouse model of airway inflammation through repeated aerosol challenge to a lysate of nontypeable (i.e., unencap- sulated) Haemophilus influenzae (NTHi). NTHi is the most common colon- izing bacteria in the lower respiratory tract of patients with COPD and could be a potential cause of perpetuating and promoting airway injury and inflammation in these patients. We then showed that this type of airway inflammation promotes lung cancer in a K-ras mutant mouse model of lung cancer (MC-LKR), which was associated with and/or an activation of MyD88/NF-κB pathway and increased expression of its downstream targets in the lung. We have further shown that lack of NF-κB or MyD88 (an adaptor protein up- stream to NF-κB) in the airway epithelium of CC-LR mice changes the bronchoalveolar lavage fluid cellular component of CC-LR mice and inhibits the promoting effect of COPD-like airway inflammation on lung tumorigen- sis. Upstream to MyD88 and NF-κB and downstream to bacterial stimuli is the toll-like receptors family (TLRs), which play critical role in the innate immune response. Among TLRs, TLR-2, TLR-4, and TLR9 play critical roles in mediating inflammatory responses in lung and are required for primary epithelial response to inflammatory stimuli and activation of MyD88/NF-κB pathway in the airway epithelium. Accordingly, we further hypothesized that TLR-2, -4, and 9 mediate promoting effect of inflammation on lung tumori- genesis in an MyD88/NF-κB dependent manner. Therefore, CC-LR mice were separately cross to TLR-2, TLR-4 and TLR-9 knock out mice in order to test this hypothesis. We found that genetic ablation of these TLRs in vivo significantly reduced lung tumor burden compared to age and sex matched control CC-LR mouse in the presence of COPD-like airway inflammation. This tumor reduction was associated with significant reduction in the numbers of inflammatory cells in bronchoalveo- lar lavage fluid of mice with lack of these TLRs. Taken these together, we conclude that promoting effect of COPD on lung cancer is mediated through TLR2, 4, 9-mediated activation of epithelial MyD88/NF-κB pathway.


As immune checkpoint blockade has been shown to partially reverse the ex- hausted T cell phenotype and consequently lead to a decrease in tumor burden, there is a need for an understanding of this T cell type. Using recently developed, highly validated antibodies, we have developed a fluorescent multiplex, TSA-based assay in order to examine the interaction of PD-1 with PD-1-expressing exhausted T cells in various tumor types. Here, we construct a seven-color mul- tiplex panel in order to simultaneously visualize cytokeratin (the tumor mask), DAPI (nuclear counterstain), CD8 (cytotoxic T cell marker), the exhausted T cell markers PD-1, Tim-3 and Lag-3, and the ligand for PD-1, PD-L1. This technique not only enabled the concurrent detection of these markers, but also provided high-resolution visualization of interactions between PD-1-expressing CD8+ T cells and PD-L1-expressing CD68+ macrophages in the tumor mi- croenvironment. The seven-plex panel was applied to FFPE tumor microarrays (TMAs) consisting of breast, lung and ovarian tumor tissue and each core was subsequently analyzed for the distribution, co-localization, frequency and prox- imity of these targets in relation to one another. While we often visualized co- expression of Lag-3 and PD-1 on T cells, Tim-3 was frequently observed on PD-L1+ macrophages in several tumor types. This data provides valuable in- sight into the co-expression profiles of these markers in multiple tumor types and has implications for the use of combination therapies that aim to target both the innate and adaptive immune systems.

#2689 The protumorigenic, proinflammatory effects of obesity are re- versed by weight loss via bariatric surgery, but not a low-fat diet. Laura W. Bowers,1 Emily L. Rossi,1 Subreen A. Khattib,1 Steven Doerstling,1 Alfor Lewis,2 Randy J. Seelye,3 Stephen D. Hursting1. #1Univ. of North Carolina, Chapel Hill, NC; #2Univ. of Michigan, Ann Arbor, MI.

Background: Obesity negatively impacts basal-like breast cancer (BLBC) prognosis, but the reversibility of these pro-cancer effects via weight loss re- mains unclear. However, there is consistent evidence suggesting that weight loss via bariatric surgery reduces breast cancer risk. This may be related to the re- ductions in metabolic perturbations and inflammation that follow bariatric sur- gery, effects that could be mediated by epigenetic reprogramming and/or changes in the gut microbiome. Purpose: We previously demonstrated that mammary tumor growth and inflammation remain elevated in formerly obese mice, in concordance with aberrant methylation of inflammation-related genes. Here we aim to determine the differential effects of surgical versus non-surgical weight loss on inflammation, DNA methylation, the gut microbiome, and tumor burden in a mouse model of BLBC. Methods: Mice were fed a low fat control (n=25) or high fat diet-induced obesity (DIO, n=75) regimen for 15 weeks to model chronic obesity. DIO mice were then randomized to remain on DIO (Obese) or receive either a surgical (sleeve gastrectomy) or dietary (low fat control diet) weight loss intervention, resulting in formerly obese (FOb)-Surg or FOB-Diet mice, respectively. The Control mice were maintained on the low fat diet throughout study. Four weeks after weight stabilization in the FOB mice, all mice were orthotopically injected with E0771 mammary tumor cells, which model BLBC. Stool samples were collected at baseline and prior to tumor cell injection. Results: The average weight and percent body fat of the FOB-Surg and FOB-Diet mice were equivalent to Control and significantly lower than Obese mice at study endpoint. Average tumor weight in FOB-Surg mice was statistically equivalent to Control mice, but tumor weight in FOB-Diet mice was sig- nificantly greater than Control mice and statistically equivalent to Obese mice. Furthermore, FOB-Surg mice had significantly lower serum tumor necrosis fac- tor alpha, mammary gland interleukin-6 expression, and tumor-infiltrating adi- poocyte area in comparison to FOB-Diet. To further define the effects of surgical versus non-surgical weight loss, characterization of the gut microbiota as well as global mammary tissue gene expression and DNA methylation via paired RNA
sequencing and reduced representation bisulfide sequencing is in progress. Conclusions: Our results suggest that the strong anti-cancer benefits seen with bariatric surgery may be related to a significant reduction in systemic and local inflammation, which did not occur with non-surgical weight loss. Identification of the mechanisms mediating the protective effects of bariatric surgery against breast cancer could help identify new targets and strategies for breaking the obesity-cancer link.

#2690 MK2 pathway blockade inhibits inflammatory cytokine production and colorectal cancer growth and invasion. Anita L. Ray, Amanda S. Peretti, Wade Johnson, Gregory Gan, Ellen J. Beswick. The Univ. of New Mexico, Albuquerque, NM.

Introduction: Colorectal cancer is the third most common malignancy diagnosed for both men and women in the United States of America. High levels of inflammatory cytokines in colorectal tumors cause increased growth and invasion and carry a higher risk of metastasis. Identifying new targets to control inflammation is important for developing improved treatment approaches. Mitogen-activated protein kinase-activated protein kinase 2 (MK2) is a regulator of pro-inflammatory cytokines that may promote colorectal tumor progression. MK2 signaling is known to induce IL-1, IL-6, and TNF-α production. This pro-inflammatory cytokines are associated with CRC development, invasion, and metastasis. We hypothesized that the MK2 pathway could be an important component of CRC growth, invasion and tumor regrowth. Methods: To investigate this pathway, CT26 CRC cells were examined. Cells were flask-injected into Balb/c mice with and without MK2 inhibitor treatment. At day 19 after tumor injection, tumors were harvested and measured using calipers. Cytokines were quantitated by multiplex bead array in organ culture supernatants and in supernatants from tumor cells plated in fibronectin coated wells. In culture, tumor cell invasion was measured in scratch wound assays containing matrigel. Results: Treating CT26 cells with MK2 inhibitors markedly reduced tumor growth in mice by a mean of 60% compared to vehicle control treated cells. Inflammatory cytokines were also dramatically decreased with MK2 inhibition compared to controls by up to 80%. Cytokines affected included both known MK2 downstream cytokines (IL-1, IL-6, and TNF-α) and also chemokines such as MIP-1α and MCP-1 in both supernatants from cultured tumor cells and in organ culture supernatants. MK2 inhibition also decreased invasion of tumor cells in a cytokine dependent manner. Conclusions: The MK2 pathway regulates production of multiple pro-inflammatory cytokines in colorectal tumors, including several previously unreported chemokines. Inhibition of this pathway markedly decreases tumor growth and invasion. Thus, MK2 may be a promising tumor target to prevent colorectal cancer progression.

#2691 Cigarette smoke induced upregulation of endothelin axis in the initiation of pancreatic cancer. Suprit Gupta, Satyanarayana Rachagani, Sushil Kumar, Surinder Kumar Batra, Maneesh Jain. University of Nebraska Medical Center, Omaha, NE.

Background: Cigarette smoke is an established risk factor for pancreatic ductal adenocarcinoma (PDAC). Smoke induced inflammation accelerates the progression of PDAC in presence of constitutively active K-Ras mutation. Endothelin (ET) axis comprising of endothelin converting enzymes (ECE-1, ECE-2, and ECE-3) and endothelin receptors (ETa, ETb, ETc) has been implicated in various cancers. Previous studies from our group reported that colon tumors from AAs displayed hypermethylation of DNA regions in inflammatory genes such as NELL1, GDF1, ARHGEF4, and ITGA4 when compared to Caucasian Americans (CA). To assess potential differences in the inflammatory response, we utilized two AA colon cancer cell lines generated in our laboratory and compared them with the commercially available CA colon cancer cell lines, Caco-2 and HT-29. Recent experiments by our group with the anti-inflammatory drugs Ibuprofen, Sulindac and Aspirin, showed significantly higher IC50 values in proliferation assays for AA cell lines compared to CA cell lines. Therefore, we evaluated the anti-inflammatory effects of these drugs by determining secretion of inflammatory cytokines and MAPKs activation in response to pro-inflammatory cytokine TNF-alpha. The same parameters were tested for the anti-inflammatory cytokine IL-10. As hypothesized, our results in the CA cell lines demonstrated down-regulation of MAPKs activation in response to TNF-alpha after pre-treatment with Ibuprofen. On the contrary, Ibuprofen concentrations as high as the IC50 values for the CA cell lines were not able to induce down-regulation of MAPKs in the AA cell lines. Interestingly, IL-10 treatment was more effective in the AA compared to the CA cell lines, suggesting that the main anti-inflammatory cytokine of the colon will be more effective in controlling inflammation in AA colon tumors. Further studies are needed to elucidate the major differences in inflammatory responses between AA and CA colon cancer cell lines and their potential role in CRC health disparities. As it has been documented that AA colon cancer patients are less responsive to the chemotherapeutics Fluorouracil and Cetapcinib, in future studies we will evaluate the effect of these therapeutic agents on AA and CA cell lines in terms of inflammation, cell viability, apoptosis and invasion.


The colon tumour microenvironment (TME) comprises many cell types including endothelial cells, stromal cells and immune cells. Recent evidence suggests that high tumour stromal cell density correlates with a poor prognosis for colon cancer patients. The majority of these stromal cells are of mesenchymal origin (MSCs) and are known contributors to tumour angiogenesis and invasiveness. Little is known about the role of their immunosuppressive potential in the colon TME. We investigated the molecular regulation of the induced immunosuppressive, tumour-promoting phenotype of tumour-associated MSCs, and the effect of inflammation on this process. Balb/c bone marrow derived MSCs were treated with conditioned medium from untreated CT26 tumour cells (MSCCTRL) or TNF-α treated CT26 cells (MSCTNF-TCM). MSC cell surface analysis of MSCs after 72h revealed an increased expression of TCR ligands MHC-I, MHC-II and PD-L1 compared to MSCCTRL. This was further potentiated by TNF-α-induced tumour cell inflammation. MSCCTRL co-cultured with syngeneic activated T cells displayed an enhanced ability to suppress CD8+ T cell proliferation, which was further potentiated by inflammatory activation of CT26 (MSCCTRL-TCM). This effect was dependent on induced PD-L1 expression on MSCs as PD-1 blockade restored CD8+ T cell proliferation, activation and granzyme B secretion. In an immunocompetent Balb/c syngeneic model, we assessed tumour growth and anti-tumour immune responses following subcutaneous injection of CT26 cells alone or co-injection with MSCCTRL.

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MSC^{TNF-TCM}. Co-injection of MSC_{Control} significantly promoted tumour growth, and this was further potentiated by the co-injection of MSC^{TNF-TCM}. This effect was associated with significantly reduced tumour infiltration of CD8+Granzyme B secreting T cells. We showed that this stromal cell mediated tumour promotion could be reversed by administration of a PD-1 blocking antibody, via restoration of granzyme B secreting CD8+T cells. Our observation supports that stromal cells in the inflammatory TME directly modulate anti-tumour immune responses via PD-L1. This data could lead to better stratification of patients for immunotherapeutic regimens resulting in more targeted and durable responses to overcome stromal mediated tumour immunosuppression in colon cancer.

#2694 Target obesity-associated inflammation to decrease murine basal-like mammary tumor burden. Emily L. Rossi, Subreen A. Khatib, Laura W. Bowers, Steven S. Doerstling, Andrew J. Dannenberg, Stephen D. Hursting. Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; Weill Cornell Medical College, New York, NY.

Background: Adipose tissue dysregulation, a hallmark of obesity, contributes to a chronic state of low-grade inflammation that promotes cancer growth through multiple signaling pathways. We previously showed that inflammation and basal-like breast cancer (BLBC) growth are increased in chronically obese mice and persist following weight normalization. Purpose: We tested the hypothesis that targeting inflammation in obese mice by treating them with the nonsteroidal anti-inflammatory drug (NSAID) Sulindac would offset the pro-cancer effects of obesity in a mouse model of BLBC. Methods: Mice were administered a control diet (10 kcal % fat; n = 34) or diet-induced obesity regimen (DIO, 60 kcal % fat; n = 34). After 15 weeks on control or DIO diets, mice were randomized to either receive Sulindac supplementation at 160 ppm in the diet (n = 17/diet) or no supplementation (n = 17/diet). Twelve weeks later, all mice were orthotopically injected with E0771 cells, a model of basal-like breast cancer. Five mice/group were killed at a 4-week interim time-point after injection, and their tissue collected and stored. The remaining 12 mice/group continued in a survival study; these mice were killed when tumor size reached 1.2 cm in diameter at any future. Results: Sulindac supplementation in DIO mice significantly reduced serum insulin and leptin to levels statistically equivalent to control mice, but had no effect on body weight, body fat percentage, or ex vivo visceral white adipose weight. Sulindac supplementation in DIO mice (but not control) significantly reduced mean tumor volume in the interim tumor study and significantly increased tumor latency in the survival study. Analysis of H&E stained tissue demonstrated that DIO mice had significantly increased adipocytes infiltrating into the tumor (relative to control), but Sulindac supplementation in DIO mice decreased adipocyte infiltration to levels observed in control. Conclusions: Sulindac supplementation significantly reduced insulin and leptin in DIO mice, and increased tumor latency in DIO mice, but had no effects on body weight or fat depots, suggesting that Sulindac offsets some of the pro-tumorigenic effects of obesity rather than targeting obesity directly. Preliminary analyses of inflammatory surrogates, including circulating cytokines and prostaglandins, mammary gland crown-like structures and collagen-2X collagen-2 levels, suggests Sulindac’s effects in obese mice are mediated through its eicosanoid-depressing effects.

#2695 Role of tumor generated acidity in immune stromal interactions during prostate carcinogenesis. Asmaa El-Kenawi, Jasreman Dhillon, Arig Ibrahim-Hashim, Dominique Abrahams, Shari Pilon-Thomas, Brian Ruffell, Robert Gatenby, Robert Gillies. Moffitt Cancer Ctr., Tampa, FL.

Insufficiency in tumor perfusion and high rate glycolysis combine to reduce the pH of tumor microenvironment. In a TRAMP model of prostate cancer, we had shown that carcinogenesis is associated with increasing acidification of the microenvironment and that neutralization of this acidity can prevent cancer emergence or metastases. Carcinogenesis in the TRAMP model is also associated with increased fibrosis and immune cells infiltration. We thus sought to determine if fibrosis drives immune infiltration in early carcinogenesis or vice-versa; and whether this dynamics is affected by tumor acidity. To investigate this, we harvested prostates from TRAMP mice or their matching non-transgenic controls at different time points and stained serial prostate tissue sections with F4/80 (macrophages), SMA (cancer-associated fibroblasts, CAFs), and Masson’s Trichrome (collagen). Quantitative image analysis reveals that increase in fibrosis occur prior to macrophage infiltration and that both events preceded tumor development. However, the relative amount of collagen fibers was unchanged across all time points. Notably, neither fibrosis nor macrophage infiltration occurred in mice treated with buffer, suggesting an involvement of acidity in this immune stromal interactions. Interestingly, macrophages isolated from latter time points in the untreated group as well as macrophages co-cultured with prostate tumor cells at acidic pH, possessed an M2-like phenotype by expressing immunosuppressive genes (e.g. Arginase 1, Arg1) and a range of scavenging receptors (e.g. mannose receptor, CD206), as well as releasing more angiogenic factors (e.g. VEGF and MMPs). Similar results were recapitulated when M2 macrophages were stimulated at acidic pH by showing enhanced CD206 and Arg1 expression. On the functional level, macrophages activated at acidic pH had a higher ability to uptake fluorescently labelled ovalbumin and collagen, as examples of mannosedified ligands that prevail the fibroitic microenvironment. In summary, these results suggest that tumor acidity may promote fibrosis, with subsequent macrophage infiltration and phenotypic switching, leading to increased collagen turnover. It is suspected that this extracellular matrix remodeling may be permissive for tumor progression.

#2696 PD-1 antibody treatment induces gut microbiota changes in CT-26 colon cancer syngeneic model. Jian Ding, Binchen Mao, Qian Shi. CrownBio, Taicang, Jiangsu Province, China.

The gut microbiota plays an important role in shaping systemic immune response. One novel frontier in cancer research is investigating how the gut microbiota change immune response and influence the efficacy of anticancer immunotherapy drugs; this has been reported in CTLA4 and PD-L1 antibody treatment, both in laboratory and clinical settings. One recent study reported that composition of patient’s gut microbes appears to be a determining factor for immunotherapy, at least in melanoma patients. These findings shed light on the potential use of gut microbial as a biomarker for responders in cancer immunotherapy. Here, we evaluated the gut microbiota profiling upon PD-1 antibody (RMPI-14) treatment in CT-26 colon cancer syngeneic mouse model. Fresh feces were collected at different time points before and after treatment, snap frozen and sent for 16S RNA sequencing for microbiome profiling. Our results identified top 25 abundant taxa in genus level in CT-26 feces. Significant abundance changes were observed in several genera after PD-1 antibody treatment, especially in Akkermansia. These findings indicate that immunotherapy like PD-1 antibody can induce the profile changes of gut microbiota in CT-26 model. Furthermore, we also investigated the correlation of gut microbiome with immune microenvironment and the efficacy upon IO therapy, as well as the possibility to utilize microbiota profiling as a quality control step that may help to explain the variability observed in many syngeneic studies.

#2697 A role for the membrane type-1 matrix metalloproteinase in the transcriptional regulation of carcinogen-induced inflammasome components. Samuel Sheehy, Borhane Annabi. Université du Québec à Montréal, Montréal, Quebec, Canada.

BACKGROUND: Signal transduction functions driven by the cytoplasmic domain of membrane type-1 matrix metalloproteinase (MT1-MMP) are believed to regulate many inflammation-mediated cancer cell functions including migration, proliferation, and survival. Besides the upregulation of the inflammation biomarker cyclooxygenase (COX)-2 expression, MT1-MMP’s role in relaying the signals triggered from pro-inflammatory cues remain poorly understood. METHODS: Here, we treated HT1080 fibrosarcoma cells with phosphol-12-myristate-13-acetate (PMMA), a well-known carcinogen and inducer of COX-2 and MT1-MMP. In order to assess the global transcriptional regulatory role that MT1-MMP may exert on inflammation biomarkers, we combined gene array screens to transient MT1-MMP gene silencing strategy. RESULTS: 1. We found that MT1-MMP expression exerted both stimulatory and repressive transcriptional control of several inflammation-related biomarkers such as IL-1B, IL-6, IL-12A, and IL-33, as well as of transcription factors such as EGR1, ELK1, and p105 KF-integrin. We also highlight a signaling axis linking MT1-MMP to MMP-9 transcriptional regulation. CONCLUSIONS: Altogether, our data evidence an important involvement of MT1-MMP in the transcriptional regulation of inflammatory biomarkers consolidating its contribution in signal transduction functions, in addition to its classical hydrolytic activity.

#2698 IL-17A alone or in combination with IL-4/IFN-y induces Duox2/DuoX2 expression and reactive oxygen production in human colon and pancreatic cancer cells. James H. Doroshow, Yongzhong Wu, Jiamo Lu, Jian Ding, Binchen Mao, Qian Shi. Moffitt Cancer Ctr., Tampa, FL.

Insufficiency in tumor perfusion and high rate glycolysis combine to reduce the pH of tumor microenvironment. In a TRAMP model of prostate cancer, we had shown that this stromal cell mediated tumour promotion could be reversed by administration of a PD-1 blocking antibody, via restoration of granzyme B secreting CD8+T cells. Our observation supports that stromal cells in the inflammatory TME directly modulate anti-tumour immune responses via PD-L1. This data could lead to better stratification of patients for immunotherapeutic regimens resulting in more targeted and durable responses to overcome stromal mediated tumour immunosuppression in colon cancer.
#2699 NFATc2 in non-small cell lung cancer mediates inhibition of peripheral blood mononuclear cell proliferation. Ian K. Lam, Zhi-Jie Xiao, Vicky P. Tin, Fang Ping Huang, Maria P. Wong. The University of Hong Kong, Hong Kong, Hong Kong, Hong Kong.

Lung cancer is the leading cause of cancer death worldwide. While genetic mutations that provide growth advantages to cancer cells are fundamental to malignant transformation, ability to evade the immune system is also crucial in tumorogenesis. We have previously shown that human lung cancers with high expression of nuclear factor of activated T cells, cytoplasmic 2 (NFATc2) are associated with reduced recurrence-free and overall survival. NFATc2 is expressed in most immune cells and has been extensively studied in immune cells. However, its function in cancer development is not completely understood. In this study, we examined the role of NFATc2 in lung cancer on immune escape is explored. Lung cancer cell lines with high NFATc2 expression were co-cultured with peripheral blood mononuclear cells (PBMCs). We observed a significant inhibition of PBMC proliferation in co-culture with NFATc2 knockdown cancer cells. When cancer cells and PBMCs were separated by a membrane using Transwell inserts, the anti-proliferative effect on PBMC was not observed suggesting that cell-cell contact was required for the NFATc2-mediated inhibition. Moreover, interleukin-2 (IL-2) was produced by PBMC irrespective of the presence or absence of lung cancer cells. These results showed that the inhibition of proliferation was independent of the activation of PBMC. Taken together, we have shown NFATc2 in lung cancer plays a role in immune modulation by inhibiting PBMC proliferation and this could be a novel mechanism of immune evasion in lung cancer.

#2700 Intestinal Vitamin D receptor deficiency increases susceptibility for the DSS-induced colon disease. Jung-Hwan Kim, Satoshi Yamamoto, Frank J. Gonzalez, 2 Gyeongsang National University, Jinju, Republic of Korea; 2National Cancer Institute, Bethesda, MD.

To investigate the function of the intestinal Vdr gene in inflammatory bowel disease (IBD), in conjunction with the discovery of possible metabolic markers for IBD using intestine-specific Vdr knockout mice. Vdr-/- mice were generated, phenotyped and treated with a twice-course of 3% dextran sulfate sodium (DSS) to induce colitis. Colitis was diagnosed by evaluating clinical symptoms and intestinal histopathology. Gene expression analysis was carried out. In addition, metabolic markers of IBD were explored by metabolomics. Vdr-/- mice showed abnormal body size, colon structure and feces color. Calcium, collagen, and intestinal proliferation-related gene expression were all decreased, and serum alkaline phosphatase was highly increased. In the acute model which was treated with 3% DSS for six days, Vdr+/+ mice showed a high score of IBD symptoms; enlarged mucosal layer and damaged muscularis layer. In the recovery experiment model, where mice were treated with 3% DSS for four days and induced for 13 days, Vdrc/+- mice showed a high score of IBD symptoms; severe damage of mucosal layer and increased expression of genes encoding proinflammatory cytokines. Feces metabolomics revealed decreased concentrations of taurine, taurocholic acid, taurodeoxycholic acid and cholic acid in Vdr+/+ mice. In conclusion, disruption of the intestinal Vdr gene showed phenotype changes. These factors combined may exacerbate IBD. Taken together, the results suggest that VDR may play an important role in IBD.

Clinical Research: Clinical Molecular Genetics and Laboratory Correlates

#2701 Large-scale ex vivo generation of human neutrophils from cord blood CD34+ cells. Zhenwang Liu, 1 Yu Zhang, 2 Chen Wang, 2 Bin Shen, 3 Xin Guan, 1 Zhihua Ren, 1 Xinxin Ding, 1 Wei Dai, 2 Yongping Jiang, 1 Biopharmaceutical R&D Center, Peking Union Medical College of Tsinghua University, Suzhou, China; 3Biopharmagen corp., Suzhou, China; 2College of Nanoscale Science and Engineering, SUNY Polytechnic Institute, Albany, NY; 4Environmental Medicine, NYU Langone Medical Center, Tuxedo, NY.

Ex vivo expansion of hematopoietic stem cell and subsequent differentiation into mature neutrophils remains a challenge. Here, we have developed a three-stage culture system to produce efficiently functional neutrophils derived from cord blood CD34+ cells. A procedure of ex vivo expansion and differentiation in a large-scale was developed in a modified IMDM basal
medium supplemented with transferrin, insulin, fetal bovine serum, and some other nutrients with selected cytokine combination that contained stem cell factor (SCF), Flt-3 ligand (FL), granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF) and thrombopoietin (TPO) in stage I (days 0–6); SCF, FL, G-CSF, interleukin-3 (IL-3), and GM-CSF in stage II (days 7–14); and SCF, FL, and GM-CSF in stage III (days 15–21), respectively. Enriched CD34⁺ cells were firstly cultured and expanded in 25 T-flasks. After 6 day-culture, the cells were transferred to a 2-L bottle with 500 ml of medium in the bottle-turning device system. During the differentiation process, neutrophil marker CD66b was evaluated by flow cytometry. Ex vivo generated neutrophils or medium only were used for the E. coli overnight for bacteria killing assay. All then the E. coli colony-forming units were counted separately. Matured neutrophils or vehicle were transplanted into NOD/SCID mice intravenously for chemotactic activity in vivo. The cells that accumulated in the pouch with chemotoxarin were collected and subjected to flow cytometric analysis for human CD66b antigen. The ex vivo generated neutrophils/progenitors were then injected into sub-lethal irradiated NOD/SCID mice to monitor the viability and maturation in vivo. After the three-stage culture, proliferation fold of total cell reached 3001 ± 286.5 with 60.2% ± 2.4% for CD66b⁺ neutrophils. The calculated yield of matured neutrophils from each CD34⁺ cell was ranged from 1.8 × 10⁶ to 1.87 × 10⁶ for 18-day culture. There was no E.coli colony formed after incubation with neutrophils in bacteria killing assay (10⁴/ml). The ex vivo generated neutrophils were functional. Post in vivo chemotaxis assay, the neutrophils collected from 18-day culture were injected into mice and detected at 1.08% ± 0.16% for human CD66b⁺ cells and no any CD66b⁺ cells observed for negative control group. In addition, the CD66b⁺ cells were extended for 4 days from a 15-day cultured neutrophil group in mouse peripheral blood (PB), while only for 2 days from a freshly isolated human PB neutrophils post injection, indicating that the ex vivo generated neutrophils/progenitors could further matured in vivo. Taken together, we have established a pilot-scale culture system to produce functional human neutrophils ex vivo. Considering that one neutrophil transfusion unit (100ml) contains 2 × 10¹⁰ cells, the CD34⁺ cells from one CB unit (80 ml) would generate 1.6 × 10¹¹ neutrophils, which are equivalent to 8 unit neutrophils in the clinical application.

**#2702** Mutations of SPOP, FOXA1 and IDH1 are associated with prostate cancer metastasis and biochemical outcomes. Jinlu Ma, Suzia Han, Xin Sui, Wenyan Lu, Yonghe Li, Bo Xu. The First Hospital, Xi’an Jiaotong University, Xi’an, China; Southern Research Inst., Birmingham, AL.

Recent genomic studies have shed light on cataloging genomic alterations in prostate cancer. Mechanistic studies allow the use of this information to subclassify the heterogeneous prostate tumors for optimized treatment planning. SPOP (small proteasome subunit RING finger protein), SOX17 (Sex determining region Y box 17), FOXA1 (Forkhead box A1), and IDH1 (isocitrate dehydrogenase 1) are among the genes that show frequent point mutations in primary prostate cancers. However, the clinical significance of these gene mutations in cancer patients is less clear. To determine the whether SPOP, FOXA1 and IDH1 mutations are associated with clinical outcomes in prostate cancer, we pyro-sequenced the three genes in over 200 prostate cancer patients and the clinical and prognostic significance. 198 patients from the first hospital of Xi’An Jiaotong University (China) were analyzed. All patients were newly diagnosed with prostate cancer from January 2010 to December 2015. No patient was lost to follow-up. The average follow-up time after the primary diagnosis was 27 months. The Fisher’s exact tests were used to compare categorical data. The risk of metastasis among patients with SPOP, FOXA1 and IDH1 mutations was evaluated using univariate and multivariate logistic regression models. The prognostic significance of clinical and gene mutations was determined using multivariate Cox regression analysis. SPOP mutations were found in 16 patients (8.1%), with all of the mutations clustered in the substrate-binding MATH domain. FOXA1 mutations were found in nine patients (4.5%), and were clustered in the homeodomain. IDH1 mutations were found in three patients (1.5%). Mutation frequencies of SPOP, FOXA1 and IDH1 were associated with prostate-specific antigen (PSA), a biochemical marker of prostate tumor presence/risk which is decided by Gleason Score, T stage and PSA level, along with prostate cancer PSA failure and metastasis. Among patients with SPOP mutation, 56.3% showed metastasis at the time of diagnosis of primary cancer, compared to only 11.5% of patients with wild-type SPOP. Similarly, the probabilities of FOXA1 and IDH1 mutations with metastasis were approximately 44.4% and 66.7% versus 13.8% and 14.4%, respectively, comparing to their wild-type counterparts. The metastasis risk for patients with SPOP mutation was 1.27 times of the SPOP wild-type patients (P = 0.003). We also found that patients with SPOP (P < 0.0001) and FOXA1 (P = 0.0403) mutations showed higher risk of PSA failure compared with wild-type. The PSA failure risk for patients with SPOP mutation was 35.49 times of the SPOP wild-type patients. In contrast, IDH1 mutation was not associated with PSA failure (P = 0.054). Multivariate analyses demonstrated that SPOP was the only mutation associated with prostate cancer metastasis and PSA failure recurrence. These findings underscore the clinical significance for profiling SPOP, FOXA1 and IDH1.

**#2703** Extraskeletal myxoid chondrosarcoma: a clinicopathologic and molecular study reveals novel genetic aberrations by targeted next-generation sequencing. Marcel Trautmann, Magdalene Cyra, Ilka Isfort, Inga Grünewald, Konrad Steinestel, Reinhard Büttner, Eva Wardemann, Wolfgang Hartmann. University Hospital Münster, Gerhard-Domagk-Institute of Pathology, Münster, Germany; University Hospital Cologne, Institute of Pathology, Cologne, Germany.

Background: Extraskeletal myxoid chondrosarcomas (EMCs) are rare mesenchymal neoplasms comprising 3% of all soft tissue tumors. EMCs arise mainly from the deep soft tissues of the extremities, accompanied with high rates of recurrence and metastases. The molecular hallmarks of EMCs are various cytogenetic NR4A3 rearrangements, generating chimeric -NR4A3 proteins. The most common reciprocal translocation (9;22)(q22;q11) results in a fusion of the EWS RNA binding protein 1 gene (EWSR1) to the nuclear receptor subfamily 4 group A member 3 gene (NRF4A3 or TEC; approximately 75% of cases). Further cytogenetic (9;17)(q22;q11) rearrangements involve TAF15 RNA polymerase II, TATA box binding protein associated factor (TAF15; approximately 15% of cases). The less frequent reciprocal translocations t(9;15)(q22;q21) and t(9;3)(q22;q12), result in transcription factor 12 (TCF12)NR4A3 and TRKfused gene (TFG)-NR4A3 fusion proteins. Although the oncogenic NR4A3 fusions transcripts seem to have a crucial role in EMC tumorigenesis and progression, the specific biological function and the mechanism of action remain to be defined. Methods: We characterized the cytogenetic rearrangements of 25 comprehensive EMC tumors by RT-PCR and/or fluorescence in situ hybridization (FISH). Next generation sequencing (NGS) was performed (Illumina MiSeq platform) to reveal additional genetic alterations besides the known chromosomal translocation. Therefore, a comprehensive cancer panel was designed, comprising 27 cancer-related genes known to be frequently mutated across various malignancies. Results: Overall, fusion transcripts were detected in 22 of 25 samples (88%). Sixteen were positive for the EWSR1-NR4A3 and six for the TAF15-NR4A3 fusion gene. The t(9;15) and t(9;3) translocations, resulting in TCF12NR4A3 and TFG-NR4A3 fusion proteins were not identified in any EMC case. In addition, several known oncogenic mutations were detected which have not been previously reported in EMC. Conclusions: The combination of RT-PCR and FISH on paraffin-embedded tissue is a sensitive and specific method for the molecular detection of the pathogenic translocations to be applied in the differential diagnosis of extraskeletal myxoid chondrosarcomas. Our results emphasize that cytogenetic NR4A3 rearrangements are the initiating events in the pathogenesis of EMC. Furthermore, our results indicate the occurrence of additional genetic aberrations providing a rational base for novel targeted therapeutic approaches.

**#2704** Pathogenic mutations and variants of unknown significance (VUS) in cancer predisposition genes are associated with over 10% of pediatric rhabdomyosarcoma: a report from the Children’s Oncology Group. Erin L. Young, Luke Maese, Rosann Robinson, Lance Pflieger, Barry Moore, Shawn Rynearam, Trent Fowler, Sean V. Tartaglini, Mark Yandell, Clinton C. Macon, Douglas S. Hawkins, Philip J. Lupo, Joshua D. Schiffman. University of Utah, Salt Lake City, UT; University of Washington, Fred Hutchinson Cancer Research Center, Seattle, WA; Baylor College of Medicine, Houston, TX.

Approximately 5% of rhabdomyosarcoma (RMS) cases are due to known cancer predisposition syndromes (e.g., Li-Fraumeni syndrome, Neurofibromatosis-1), but these estimates have not been confirmed in large-scale studies. Furthermore, no recommended germline testing protocols exist for RMS. We tested the hypothesis that germline mutation burden is greater than previously reported and present several new predisposition genes as potential drivers of pediatric RMS. We sequenced 59 cancer susceptibility genes in 213 children with RMS enrolled on one clinical trial (COG ARST0531), unselected for family history of cancer. Validation was performed with Sanger sequencing. Our analysis included determining the incidence of pathogenic variants in known RMS predisposition genes, followed by other cancer predisposition genes on our panel but not previously associated with RMS. In our cohort, 3.9% of unselected RMS cases harbor a pathogenic variant in a known predisposition gene (Table 1). Using VAAST, we next identified the top 10 genes possibly associated with
Table 1. List of genes with numbers of carriers identified in 213 rhabdomyosarcoma cases

<table>
<thead>
<tr>
<th>Gene</th>
<th>Carriers</th>
<th>% in GnomAD</th>
<th>SIR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATM</td>
<td>0</td>
<td>0.87%</td>
<td>1.13</td>
<td>0.42</td>
<td>0.07</td>
</tr>
<tr>
<td>PMS2</td>
<td>1</td>
<td>0.87%</td>
<td>1.13</td>
<td>0.42</td>
<td>0.07</td>
</tr>
<tr>
<td>TP53</td>
<td>3</td>
<td>0.87%</td>
<td>1.13</td>
<td>0.42</td>
<td>0.07</td>
</tr>
<tr>
<td>WT1</td>
<td>1</td>
<td>0.87%</td>
<td>1.13</td>
<td>0.42</td>
<td>0.07</td>
</tr>
</tbody>
</table>

**CLINICAL RESEARCH: Clinical Molecular Genetics and Laboratory Correlates**

#2707 **Targeted RNA sequencing reveals thus far unknown diagnostically relevant fusion partners confirming its diagnostic potential.** Niroshan Nadarajah, Manja Meggendorfer, Torsten Haferlach, Wolfgang Kern, Claudia Haferlach. *Munich Leukemia Laboratory, Munich, Germany.*

Introduction: The genomic landscape of hematological malignancies has been resolved mainly based on whole genome and whole exome sequencing, primarily focusing on targetable gene mutations. In addition to mutations, gene fusions have also been identified as therapeutic targets, impressively shown e.g. for BCR-ABL1 and ETV6-PDGFRB. Even though fluorescence in situ hybridization (FISH) is the current gold standard in fusion detection, it is by concept limited to the selected genes it is applied to. In contrast, targeted RNA sequencing is a valuable hypothesis-free approach to discover all possible fusion junctions in a single reaction. Aim: Explore the value of targeted RNA sequencing in a routine diagnostic work up. Patients and Methods: We sequenced 134 cases in parallel to our routine diagnostics workflow using chromosom e banding analysis (CBA), FISH and real-time quantitative (RQ-PCR). Targeted RNA sequencing was performed on the NextSeq 500 using the TrueSight RNA Fusion panel (Illumina, San Diego, CA) consisting of 7690 probes covering 507 genes known to be involved in gene fusions. Analysis was performed with the RNA-Seq Alignment App v1.2.0 (BaseSpace Sequence Hub) using Star for Alignment and Manta for gene fusion calling with default parameters (Illumina, San Diego, CA). Results: In 127 of 134 (95%) cases the results of FISH, subsequently confirmed with RT-PCR were also picked up by RNA Seq. This included diagnostically highly relevant fusions like BCR-ABL1 (n = 8), KMT2A rearrangements (n = 7), PML-RARA (n = 2), NPM-PDGFRA (n = 2), and NPM-MDM2 (n = 1). In addition to observation of orthogonal results, we were able to identify novel rare gene fusions, which we subsequently confirmed by RQ-PCR. This included immediately targetable fusions like TNN1-PDGFRB and ETV6-TEL1, ETV6-FOX1, IRF2BP1-RARA, RARA-SE1. Conclusion: In the vast majority of instances targeted RNA sequencing confirmed results obtained by FISH/RT-PCR and in addition discovered novel rare gene fusions. Targetable genetic aberrations were identified, which were not identifiable by chromosome banding analysis but would now lead to more individualized treatment. 3) Thus, targeted RNA sequencing may be a valuable tool in routine diagnostics and for patients with rearrangements unresolved by standard techniques, also paving the way to precision medicine in a considerable number of patients.

#2708 **A custom gene expression panel for consensus molecular subtype classification of archival primary and metastatic colorectal cancers.** Ling-Yuh Huw, Robert Piskol, Felipe de Sosa e Melo, Doris Kim, Xueping Qu, Hartmut Koeppen, Mark Lackner, Garret Hampton, Omar Kabbarah, Rachel Tam. *Genentech, Inc., South San Francisco, CA.*

Stratification of Colorectal Cancer (CRC) into actionable molecular subtypes has tremendous clinical value. Recently, a consolidated classifier identified four molecularly distinct CRC subtypes (CMS1-4) that were associated with unique biology and clinical outcomes based on global transcriptional analysis of frozen tissues. Here, we developed and applied a novel CRC panel that is ideally suited for transcriptional classification of archival clinical samples. Findings from in silico analysis demonstrated that the 800 genes on our panel could accurately classify cancer samples from external public datasets into the correct CMS subtypes. We applied our panel in the analysis of a novel cohort of 312 formalin-fixed paraffin-embedded (FFPE) tissues from 205 patients, and were able to detect all 4 CMS subtypes in primary CRCs and in metastases. When we examined the CMS subtypes of primary tumors and matched metastases from 50 patients we found 70% of cases to be concordant, as were key biologies, such as WNT/MYC pathway activation in CMS2 and EMT features in tumors of the CMS4 subtype. This was confirmed by in situ hybridization (ISH) using the markers ASC2L for CMS2 and SPARC for CMS4, respectively. Discordance in the CMS subtypes between primary tumors and matched metastases was observed in 30% of cases and may reflect tumor heterogeneity. Our findings suggest that our CRC-focused panel many have clinical utility for CMS classification of FFPE samples, and point to potential risks of using CMS subtypes of primary tumors to inform clinical decision-making at the metastatic stage in a subset of patients.

#2709 **Evaluation of CD373 expression and binding of AG676E, an anti-body-drug conjugate (ADC) against CD373, on white blood cells (WBCs) collected from phase I non-Hodgkin lymphoma (NHL) patients.** Sher Karki,1 Hector Avina,1 Jacqueline Lackey,2 Ahmed Sawaas,2 Kerry J. Savage,2 Raymond Perez,2 Ranjana Advani,3 Jasmine Zain,2 Owen A. Connor,4 Sara Guilese- rian,1 Hui Zhao,1 Peng Yang,1 Karen Morrison,1 Leonard Reyno,1 Fernando Donate,4,1 Agensys Inc., an affiliate of Astellas Pharma, Santa Monica, CA;2 Columbia University Medical Center, New York, NY;3 British Columbia Cancer Center, British Columbia, Canada;4 University of Kansas, Kansas City, KS; Stanford University, Stanford, CA;1 City of Hope, Duarte, CA.

AGS67E is an antibody drug conjugate (ADC) against CD37 conjugated to monomethylauristatin E (MMAE). CD37 is expressed on normal WBCs, but is also highly expressed in NHL, CLL and AML. (Pereira et al., 2015). A phase I study is currently evaluating the safety, PK and anti-cancer activity of AGS67E with or without growth factor (GF) in subjects with relapsed/refractory NHL. To assess CD37 on WBCs, biopsy samples were collected at day 15, after dose 1, D5 and analyzed by flow cytometry. CD37 expression on subject tumor samples was also evaluated by immunohistochemistry (IHC). Our results demonstrated that CD37 was highly expressed in tumor samples and that AGS67E binds to WBCs causing down-regulation of CD37, achieving saturation of binding at 24 hours post-treatment (earliest time measured) or above 0.9 mg/kg, and reversibly depleted WBCs, with no significant difference of mutation frequency between primary tumors from patients with liver metastasis and those from other patients. Notably, four recurrently mutated genes (FBXW7, EDIL3, KCNK9 and PCDHGA7) were more highly expressed in the tumors associated with liver metastasis. Furthermore, in vitro analyses showed that EDIL3 and KCNK9 overexpression resulted in an enhanced cell migration and invasion, while CRISPR-mediated deletion of FBXW7 and PCDHGA7 exhibited enhanced migratory and invasive properties as well. For clinical relevance, highly expressed EDIL3 and KCNK9 was significantly associated with liver metastasis, whereas the expression of FBXW7 and PCDHGA7 negatively correlated with it in our cohorts. Pathway enrichment analysis also indicated a role for cell adhesion signaling in liver metastasis of CRC patients. Conclusion: Our study identified candidate genes, which might respond to the initiation of metastasis, exclusively mutated in tumors associated with liver metastasis. These findings also provided potential therapeutic targets for personalized treatment.

#2711 Whole exome sequencing analyses of colorectal cancers associated with liver metastasis. Yanlei Ma,1 Wenhao Weng,2 Yongzhi Yang,1 Qing Wei,3 Sanjun Cai.1 1Fudan University Shanghai Cancer Center, Shanghai, China; 2Tongji University, Shanghai, China; 3Shanghai Tenth People’s Hospital, Shanghai, China.

Purpose: Liver metastasis is one of the major causes of death in colorectal cancer (CRC) patients. But the process is still unknown. Here, we analyzed genetic differences between primary tumors associated with liver metastasis and those without liver metastasis and predicted clinical significance. Experimental design: Eight primary tumors associated with liver metastasis and twelve without liver metastasis of CRC were analyzed by whole-exome sequencing. The functional characterisation for the role of metastasis-related genes was investigated through in vitro migration and invasion assays. For clinical relevance, TMAs were used for immunohistochemistry assay. Results: The mutation spectrum was similar to that of previous studies. TP53, APC and KRAS were the three most frequently mutated genes, with no significant difference of mutation frequency between primary tumors from patients with liver metastasis and those from other patients. Notably, four recurrently mutated genes (FBXW7, EDIL3, KCNK9 and PCDHGA7) were more highly expressed in the tumors associated with liver metastasis. Furthermore, in vitro analyses showed that EDIL3 and KCNK9 overexpression resulted in enhanced cell migration and invasion, while CRISPR-mediated deletion of FBXW7 and PCDHGA7 exhibited enhanced migratory and invasive properties as well. For clinical relevance, highly expressed EDIL3 and KCNK9 was significantly associated with liver metastasis, whereas the expression of FBXW7 and PCDHGA7 negatively correlated with it in our cohorts. Pathway enrichment analysis also indicated a role for cell adhesion signaling in liver metastasis of CRC patients. Conclusion: Our study identified candidate genes, which might respond to the initiation of metastasis, exclusively mutated in tumors associated with liver metastasis. These findings also provided potential therapeutic targets for personalized treatment.

#2710 Molecular and clinical characteristics of IGHV4-34 expressing cells and variant HCL. Evgeny Arons, Katherine Potocka, Maryalice Stetler-Stevenson, Hong Zhou, Mark Raffeld, Mark Sokolsky, Sarah Davies, Robert J. Kreitman. National Cancer Inst, Bethesda, MD.

Classic HCL (HCLc) is a malignant disorder with distinctive immunophenotype, typically expressing CD20, CD22, CD5, CD21c, CD103, CD123, annexin A1, tartrate-resistant acid phosphatase (TRAP), and Braf V600E mutation. Purine analog therapy is highly effective with complete remission rates of approximately 85% in first line. HCL variant (HCLv) is recognized as a distinct entity, lacking CD25 and usually lacking annexin A1, tartrate-resistant acid phosphatase expression, CD123, and also lacks the Braf V600E mutation. Patients respond poorly to purine analogs, with partial response in less than 50% and relatively poor overall survival from diagnosis. HCLc expressing B-cell receptor (BCR) with the IGHV4-34 immunoglobulin rearrangement has a poor prognosis like HCLv, whether immunophenotypically consistent with HCLv or HLC, and also lacks Braf V600E. We previously reported a series of 17 patients expressing IGHV4-34, immunophenotypically resembling HCLv in 7 and HLC in 10 cases. Using routine deep sequencing of patient samples for immunoglobulin rearrangements, we have been able to accumulate 42 IGHV4-34 expressing cases including 23 classic HCL and 19 HCLv. Six (26%) of 23 HCLc vs 12 (63%) of 19 HCLv were truly unmaturated, with VH gene germline identity (GI) 100%. Eleven (48%) of 23 HCLc and 7 (37%) of 19 HCLv were borderline-mutated (97<GI<100), and 6 (26%) of 23 HCLc were significantly mutated (97<GI). There was no significant difference between the 5 groups with respect to either IGHD or IGJH genes usage, or CDR3 length. However, we found a significant restriction of IGHD genes between 12 HCLc truly unmaturated and 6 HCLc truly unmaturated cases, in that there was no overlap of any IGHD genes between these 2 groups. Of 28 cases tested for Braf V600E, only 5 were positive, all HCLc with GI 93.18-97.49. The 23 Braf wild-type (WT) cases included one with only one with GI = 93.64%, but GI was >98.7% for the remaining 22 cases (p<0.0001). Of the 23 Braf WT cases, 9 patients died of disease, 9 had active disease, and only 2 are in remission. Patients commonly had highly aggressive courses, with infiltration of HCL/HCLv into spinal cord and cranial nerves, cardiac muscle, lungs, and cervical lymph nodes. Complete remission was achieved only through either recombinant immununotoxin therapy of combination purine analog and rituximab, but not by purine analogs alone. Additional clinical and genetic studies are ongoing to better characterize this poor-prognosis syndrome and determine if therapy can be optimized.


Introduction: Gastric cancer (GC) in young adults comprises a small part of this disease population having the distinctive clinicopathological features, such as diffuse tumor growth and peritoneal dissemination. Genetic alterations underlying these features and the development of cancer in early ages remain unclear. Comprehensive molecular analyses by TCGA have suggested 4 molecular subtyping of GC and revealed enrichment of CLDN18-ARHGAP fusion gene in Genomically stable (GS) type which mainly included diffuse type. In this study, we examined the relationship between CLDN18-ARHGAP and clinicopathological features of GC affecting young adults. Method: Patients (pts) diagnosed at the age younger than 40 years-old were defined as the GC in young adults, and underwent surgery at our institute between Jan 2006 and Dec 2015 were enrolled to this study. Tissue microarrays were prepared from formalin-fixed and paraffin-embedded surgical specimen. DNA probes for CLDN18, ARHGAP-26 and -6 were created using bacterial artificial chromosomes and fusion genes were detected by a FISH method. Results: Out of 155 surgical specimens available for analysis, we found CLDN18-ARHGAP fusion gene in 21 pts (13.5%). Among these, 19 cases were CLDN18-ARHGAP26 fusion and the others were CLDN18-ARHGAP6. Cancers with CLDN18-ARHGAP fusion genes were all diagnosed as diffuse type and included more large-sized, multiple lymph node metastases and advanced stages tumors (table 1). The proportion of detected fusion genes in this study was almost equivalent to that of GS type in TCGA cohort. Analysis of variations of breakpoint is planned to confirm by RT-PCR and sequencing. Additionally, we are going to study the association of CLDN18-ARHGAP with RHOA or CDH1. Conclusion: CLDN18-ARHGAP fusion gene might account for the characteristic of a part of diffuse type GC. Further comprehensive molecular analysis comparing to general GC population is warranted.
Coverage (ods): Sequencing was performed on DNA extracted from the primary tumors. In recurrent cases, the mutational landscape of recurrent and non-recurrent tumor samples is compared to that of HPV-negative HNSCC. Among the non-recurrent cases (p = 0.05), the ratio of nonsynonymous to synonymous variants among primary tumors of recurrent cases was 2 vs 5 among the non-recurrent cases (p = 0.224). Among primary tumors of recurrent cases, 17% of variants involved indels, splice site, and nonsense mutations vs 14% of variants among the non-recurrent cases. The mean (SD) number of tumor suppressor mutations per tumor was 1.2 (1.3) among recurrent cases vs 1.0 (1.0) among non-recurrent cases (p = 0.81). Interestingly, the mean (SD) number of mutations in DNA damage response (DDR) genes among the primary tumors of recurrent cases was 2.7 (2.4) compared to 0.7 (1.2) mutations per tumor among non-recurrent cases (p = 0.07). Conclusion: We observed a greater mutational burden among the primary tumors of recurrent HPV-associated HNSCC compared to non-recurrent HNSCC. Moreover, among the primary tumors of recurrent cases, there were more mutations in DNA damage response genes coupled and relatively fewer deleterious mutations compared to non-recurrent cases. Dysregulation of DDR genes may select for advantageous mutations via genomic instability. Alternatively, gain of function of DDR genes may promote treatment resistance. Future work aims to further characterize mutational differences between the primary tumors of HPV-associated recurrent and non-recurrent HNSCC and evaluate mechanisms promoting treatment resistance.

**#2714 Analytical validation of whole genome and transcriptome sequencing of patient derived tumors: clinical application of whole genome sequencing for reporting targetable variants in cancer.** Kazimierz O. Wrzeszczynski, Avinash Abhyankar, Vanessa Felice, Esra Dikoglu, Lukasz Kozon, Nicolas Robine, Anne-Katrin Emde, Olca Basturk, Umesh K. Bhanot, Alex Kent, Manisha Mansukhani, Shalini Bhagat, New York Genome Center, New York, NY; Memorial Sloan Kettering Cancer Center, New York, NY; Columbia University Medical Center, New York, NY.

Next Generation DNA Sequencing (NGS) technologies are currently being applied in the clinical setting for the treatment of disease. The goal is to use high-throughput sequencing to identify specific variants within each tumor and recommend personalized treatment approaches or clinical trials tailored to the individual’s disease and genomic profile. These assays are comprised of either predefined sequencing panels, where a handpicked set of clinically significant genes are examined within each patient, or are cancer type specific targeted sequencing protocols or whole exome platforms covering only the coding region of the patient’s genome. Whole genome sequencing allows hypothesis-free interrogation of both coding and non-coding regions of the genome revealing more potential therapeutic options than examining a small set of genes or genomic loci. The protocol eliminates sequence capture related bias observed in whole exome or panel sequencing. The New York Genome Center therefore has performed analytical validation of whole genome and transcriptome sequencing (WGT) of patient derived tumors to established normal control purposes. We have devised a clinical reporting strategy of significant driver and therapeutic associated mutations. Many clinical NGS guidelines are directed toward targeted panel or exome sequencing validation. Here, we expanded on New York State’s Department of Health NGS guidelines developing them into novel standards applicable to WGTs for the purposes of clinical test validation. We first sequenced a virtual tumor at very high coverage (30X) and downsampled to determine the optimum depth of sequencing necessary for high confidence somatic variant calling across the entire genome. We then validated whole genome sequencing laboratory protocols for DNA and RNA sequencing on a total of 50 specimens derived from fresh frozen (FF) and formalin-fixed paraffin-embedded (FFPE) tumor samples. We performed a series of experiments to assess the accuracy and reliability of the results based on our laboratory and bioinformatics protocols. We performed our validation on the 50 tumor normal pairs, a subset of which had known genomic profiles. Comparisons were also made for variant calling concordance and reproducibility between matched FF and FFPE tumors. Here, we present our validation results and clinical WGTs standards for depth of sequencing, reproducibility, sensitivity, and present limit of detection analysis for SNV calling, copy number identification and structural variants. RNA sequencing is performed to call fusion or exon skipping events and to confirm the DNA variants. The New York Genome Center WTGS clinical assay is intended to provide a more comprehensive patient variant discovery approach suitable for directed oncological therapeutic applications.

**#2715 Precision-designed, rapid and low-cost single molecule detection of mutations from circulating tumor DNA.** Christina M. Wood-Bouwens, Christine Handy, Billy Lau, Hanlee Ji.

Automated ddPCR is a technique for quantifying DNA copy number using a digital PCR method. Single molecule detection was performed using ddPCR for the detection of circulating tumor DNA (ctDNA) mutations. We have developed a novel molecular assay that utilizes single color digital droplet PCR (ddPCR) to both genotype and quantify the number of tumor derived DNA molecules in a given sample. Our assay routinely detects as few as three mutant DNA molecules per a reaction volume, can be tested efficiently for fewer than ten dollars per reaction, and generates usable mutation information within four hours. Additionally, the assay can be rapidly configured to detect different sets of genes. By demonstrating the single molecule sensitivity and specificity for clinically relevant hotspot mutations, we have validated the assay using multiple input sources including high quality cell line DNA, formalin-fixed paraffin embedded tissue (FFPET) DNA, and ctDNA. Our ddPCR assay utilizes a novel primer design that is not rely on fluorescent probes. The overall simplicity of assay design enables one to detect nearly any coding mutation; practically, this means that any cancer or DNA sample can be tested efficiently. We have created customized precision mutation assays for two individual cancer patients. After extracting ctDNA from 500µL of plasma we prepare a controlled mixed wild type and mutant standard curves which contain between 150-3 detectable mutant molecules of interest per reac-
understand pathobiology of this lymphoma. We used the AccuCyte®-pathogenesis of this lymphoma have been difficult to study because HRSCells derived from germinal center B cells. The geneticalterations involved in the frequently occurring lymphomas and is marked by Hodgkin and Reed-Sternberg cells. The assignment ofHRSCells to the diagnostic workup has been difficult to study because HRSCells are rare and are surrounded by robust, non-neoplastic infiltrate, therefore they frequently represent less than 0.1% of cells within involved lymph nodes. Consequently, methods that isolate these rare cells are required to understand pathobiology of this lymphoma. We used the AccuCyte®-CyteFinder system (RareCyte) to develop a method for detection and isolation ofHRS cells. Methods: A 6-parameter immunofluorescence assay was designed to identify HRS cells that included HRS markers (CD40, CD30, CD15), CD3, combined CD20/CD14 and a cell membrane permeability viability dye. The assay was tested using the KMH2 Hodgkin lymphoma cell line spiked into PBMC and transferred to well slides for imaging by CyteFinder. This instrument utilizes 6-color scanning microscopy coupled with predefined algorithms to identify rare cell populations. The assay was then applied to a frozen fine needle aspirate (FNA) sample taken from the lymph node of a patient with histologically confirmed CHL. The FNA sample was thawed, prepared into a suspension, stained and analyzed as above. Cells identified with HRS phenotype were individually retrieved using the integrated CytePicker module. PCR of regions of the immunoglobulin heavy chain (IGH) was performed on single HRS cells (GeneScribe) to assess clonality. Results: Of ~200 KMH2 cells spiked into blood, the assay identified 180 cells (90%) with the correct HRS phenotype. HRS phenotype cells were identified in the patient lymph node FNA sample. Two individual cells analyzed by IGH framework region PCR demonstrated identical size PCR products, indicating clonality of the B cell receptor rearrangement. Concurrently, a multi-parametric assay was successfully used for identifying rare HRS cells with the CyteFinder system. Cells with HRS phenotype in a lymph node FNA sample were demonstrated to have clonal IGH rearrangements, demonstrating that actual HRS cells were identified. This method may be a useful tool for the diagnosis of CHL by identification of rare HRS cells from FNA specimens, as well as to investigate the molecular genomics of CHL.

Identification of three distinct molecular subtypes in meningioma samples using microarrays for copy-number variants. Josel Srovnal,¹ Vladi-mir Balik,⁷ Magdalena Houdova Megova,¹ Jiri Ehrmann,⁷ Miroslav Vaverka,¹ Lumin Hrabalek,² Radek Trojancé,¹ Katerina Stalova,¹ Jana Vrbkova,¹ Marian Hajduck.¹ ¹Palacky University, Olomouc, Czech Republic; ²University of Groningen, Groningen, Netherlands; ³University of Groningen, Groningen, Netherlands.

Background: Meningioma represents one of the most common intracranial tumors. They are generally thought to progress from low to high-grade lesions. However, the molecular mechanisms underlying their pathogenesis remain still uncertain. Identification of meningioma molecular subgroups may have significantpotential to improve clinical management, through molecular disease risk stratification strategies and the identification of patients who could benefit from targeted molecular therapeutics. Methods and patients: Formalin-fixed paraffin embedded tumor samples were obtained from 45 meningioma patients and 5 healthy controls (dura mater). Comprehensive clinical-pathological data were mined. There were 15 males and 30 females; median age was 54 years, range 28 – 99 years. Total DNA was purified from FFPE samples after pathological verification using proteinase K treatment followed by QiAmp DNA FFPE Tissue Kit (Qiagen). Microarray analysis was performed using the OncoScan FFPE Assay Kit (Affymetrix), raw data were obtained using Chromosome Analysis Suite (Affymetrix) in default manner. The data were processed using “R” software, version 3.2.3. Results: Our results confirm that chromosome 22 deletion and del(1p) are the most common (55%, resp. 47% of cases) deletions in meningioma. We revealed chromosomal gains not as rare as was published previously while the dup(3p) was present in 31% of cases. Three meningioma molecular patterns were identified based on CNV profiling - normal-like profile, deletion profile and complex profile. Chromosome 22 deletion, del(1p) and dup(3p) are the most common CNV (55%, resp. 31% of cases) in meningioma. Potential CNV changes in recurrent meningioma were identified. However, it will require further validation using FISH. Acknowledgment: This work was financially supported by Ministry of Health of the Czech Republic, grant nr. 15-29021A, IGA UP LF 2016_010 and NPU LO1304.

A multi-parameter assay for the detection of rare malignant cells in classical Hodgkin lymphoma. Lance U’Ren,¹ David Wu,² Jonathan R. Fromm,³ Jackie Stiwell,⁴ Eric Kaldjian¹ ¹RareCyte Inc, Seattle, WA; ²University of Washington, Seattle, WA.

Background: Classical Hodgkin Lymphoma (CHL) ranks among the most frequently occurring lymphomas and is marked by Hodgkin and Reed-Sternberg (HRS) cells, frequently multi-nucleated neoplastic cells that are derived from germinal center B cells. The genetic alterations involved in the pathogenesis of CHL have been difficult to study because HRS cells are rare and are surrounded by robust, non-neoplastic infiltrate, therefore they frequently represent less than 0.1% of cells within involved lymph nodes. Consequently, methods that isolate these rare cells are required to understand pathobiology of this lymphoma. We used the AccuCyte®-CyteFinder system (RareCyte) to develop a method for detection and isolation of HRS cells from FNA specimens, as well as to investigate the molecular genomics of HRS cells. The assignment of HRS cells to the diagnostic workup has been difficult to study because HRS cells are rare and are surrounded by robust, non-neoplastic infiltrate, therefore they frequently represent less than 0.1% of cells within involved lymph nodes. Consequently, methods that isolate these rare cells are required to understand pathobiology of this lymphoma. We used the AccuCyte®-CyteFinder system (RareCyte) to develop a method for detection and isolation of HRS cells from FNA specimens, as well as to investigate the molecular genomics of HRS cells.

Purpose: To evaluate treatment response following the Multidisciplinary Molecular Tumor Board (MTB) decisions which focused on EGFR exon 20 mutations in lung adenocarcinoma. Methods: Molecular studies were routinely performed using the Ion Torrent sequencing platform for histologically or cytologically diagnosed lung adenocarcinoma. Since October 2014 patients with rare (prevalence <1%) or combinations of rare mutations were discussed at the MTB of the University Medical Center Groningen. More common, known sensitive EGFR, BRAF V600E, as well as KRAS, and PIK3CA mutations were excluded. To predict the best therapy for a subset of patients with EGFR exon 20 insertion mutations the literature is reviewed and molecular models were built using SWISS-MODEL, is used to predict protein tertiary structure (https://swissmodel.expasy.org/interactive/wcurbt/models/). Summary of data: Over a period of 2 years 1389 samples were tested. A total of 170 (12.2%) rare mutations were detected in lung cancer. Among these rare mutations we observed 16 EGFR exon 20 insertions and other mutations like e.g. p.T790M, p.D761N, p.D770delinsG, p.S768_D770dup, p.V769_D770insSFL, p.N771_H773dup, p.T790S. Using an in silico modeling of protein responses with TKI were predicted to be likely in EGFR T790M mutations and p.S766_V769delinsIL, but not in p.D770delinsGY, p.S768_D770dup, p.V769_D770insSFL, and p.N771_H773dup mutations. Eight patients with an exon 20 mutation were treated with an EGFR TKI. One patient (p.D761N) had a partial response on erlotinib 300mg daily. On afatinib monotherapy 2 out of 3 pts had stable disease (PFS 3-11 months). On afatinib/ cetuximab treatment 1 out of 2 pts (p.D770delinsGY) had a partial response and the other patient had stable disease (p.S768_D770dup) (PFS 11 and 4 months, resp.). Two evaluable patients were treated with osimertinib: the first patient initially progressed on afatinib (p.V769_D770insSFL). The protein model predicted no response, since we predicted hindrance of osimertinib. The model predicted no response on osimertinib in the second patient as well, and during treatment a stable disease for 4 months was observed (p.N771_H773dup). This molecular framework from the weekly MTB meeting was delivered within 2 weeks to the treating physician. Feedback on treatment outcome helped to further improve treatment predictions. Conclusion: The Molecular Tumor Board is an effective multidisciplinary team to discuss rare mutations. Our pilot data show that the evaluation of the use and effectiveness of a theoretet

Background: Molecular diagnosis has become a standard of care in many cancers and BRAF mutations analysis in FFPE tumor specimens is needed to initiate personalized therapy using BRAF tyrosine kinase inhibitor (vemurafenib, dabrafenib) in BRAF-mutated metastatic melanoma. Accelerated BRAF mutation analysis is achievable using CE-IVD fully automated (FA) PCR-based platform (Idylla, Biocartis) and enables the determination of BRAF mutational status in less than 2 hours including sample preparation and proved suitable for routine molecular diagnosis of metastatic melanoma (Harlé et al. PloS ONE 11(4); e0153576).

Patients and methods: MELFAST trial is an observational monocentric study aiming at evaluating the clinical impact of reducing BRAF status determination delay in patients with metastatic melanoma. 40 patients (pts) were included in MELFAST trial, 31 pts were included retrospectively with BRAF mutation analysis being performed according to standard operating procedures (SOP) using conventional PCR, 10 pts were included prospectively with BRAF mutation analysis being performed using FA-PCR. Results: Among the 40 pts included, 3 pts were excluded because of violation of inclusion criteria. 37 pts were analyzed (29 pts with retrospective inclusion and 8 pts with prospective inclusion). BRAF mutational status was not known at the time of treatment decision in 11/29 (38%) pts included retrospectively and in all the pts included prospectively. Using FA-PCR enables to provide BRAF mutational status within the same day for most of the samples and the reporting delay was significantly reduced using FA-PCR as compared to SOP (0 vs 7 days, p<0.001) and the delay of initiation of anti-BRAF therapy was substantially reduced (16 vs 26 days, p=0.035). This reduced delay was found to be consistent with that observed when BRAF mutational status had been anticipated and was already available at the time of treatment line decision (20 days, p=0.798). Conclusion: As compared to conventional SOP, using FA-PCR accelerates BRAF mutation analysis reporting and significantly reduces the delay before initiation of personalized therapy in pts with metastatic melanoma. This warrants the investigation of the impact on the patients outcome i.e. progression free and overall survival.

Association between five common polymorphisms of plasminogen activator inhibitor-1 gene and colorectal cancer risk in Korean population.

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Background: The plasminogen activator inhibitor-1 (PAI-1) is expressed in many cancer cell types and plays the modulation of cancer growth, invasion, and angiogenesis. The present study investigated the association between five polymorphisms in PAI-1 gene and colorectal cancer (CRC) risk. Material and Methods: Genotyping of five PAI-1 polymorphisms (-844G>A [rs2237631], -675 4G/5G [rs1799899], +3G/A [rs6092], +978S/A [rs2227694], and +11053G>T [rs7242]) was assessed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in 459 CRC cases and 416 controls. Results: The increased risk of CRC was found for the PAI-1-675 4G/5G polymorphism compared with 4G/4G (AOR=1.556; 95% CI, 1.021 to 2.391, p=0.044). In contrast, the PAI-1 11053 polymorphism, we found a reduced risk of CRC for GG genotype (AOR=0.620; 95% CI, 0.413 to 0.932, p=0.022) than TT genotype, as well as for recessive carriers (TT > TG vs. GG, AOR=0.662; 95% CI, 0.469 to 0.933, p=0.019). For patients with age < 61 years (AOR= 1.942; 95% CI, 1.247 to 3.024, p=0.003), without HTN (AOR= 1.694; 95% CI, 1.093 to 2.627, p=0.019), without DM (AOR= 1.464; 95% CI, 1.051 to 2.039, p=0.024), with folate ≥ 3.8ng/mL (AOR= 1.449; 95% CI, 1.030 to 2.039, p=0.033), with homocysteine ≤ 13.2umol/L (AOR= 1.630; 95% CI, 1.163 to 2.285, p=0.005), PAI-1 675 4G/5G polymorphism was shown increased risk of CRC. For patients with age ≥ 61 years (AOR= 1.942; 95% CI, 1.247 to 3.024, p=0.003), with homocysteine ≤ 13.2umol/L (AOR= 1.630; 95% CI, 1.163 to 2.285, p=0.005), PAI-1 11053 GG genotype was shown increased risk of CRC. For patients with age < 61 years (AOR= 1.942; 95% CI, 1.247 to 3.024, p=0.003), however, PAI-1 11053 GG genotype was related to protective effect about CRC. In multivariate Cox proportional analysis, compared with the +43 AA genotype, +43 AA genotype was associated with poor OS (HR=8.551; 95% CI, 1.833 to 39.89; p=0.006) and DFS (HR=12.71; 95% CI, 1.30 to 121.3; p=0.027). Moreover, OS (HR=9.330; 95% CI, 2.043 to 42.61, p=0.004) and DFS (HR=11.97; 95% CI, 1.433 to 100; p=0.022) were shown decreased in recessive genotype (GG+GA vs. AA). Conclusion: Our results support the relationship between CRC susceptibility and PAI-1 gene polymorphisms. Our study suggests that the -675 4G/5G genotype is associated with an increasing CRC development but +11053 GG genotype is vice versa. Additionally, we found evidence that the +43 AA genotype may be related to unfavorable CRC prognosis. This is the first study to identify an association between five PAI-1 polymorphisms and CRC susceptibility in the world. Further research into the association between fibrinoly sis and cancer could lead to the discovery of novel drug targets for cancer prevention or treatment.
mutations (4/9) were identified: a deletion (c.1044delG); one nonsense mutation (c.3406G>T), and a missense mutation (c.1488C>A). None of the mutations had been previously reported. Conclusions: NGS of PTCH1 gene in 9 Mexican patients with clinical criteria for NBCCS allowed the identification of four novel pathogenic mutations (44%) frequently affecting PTCH1 and SUFU gene in our population. The perspectives of the current study lie in the molecular testing of other genes involved in NBCCS (PTCH2, SUFU), and other mechanisms (whole gene deletion), as well as the review of possible phenotype-genotype correlations.

**#2723** MET exon 14 skipping mutations in advanced non-small cell lung cancer (NSCLC) are not associated with MET amplification and overexpression. Ana Gimenez-Capitan, Cristina Teixido, Cristina Aguado, Sonia Rodriguez, Jordi Bertran-Alamillo, Josep Castellví, Zaira Yeste, Ana Perez, Rafael Rosell, Miguel Angel Molina-Vila, Paua Oncology, Barcelona, Spain; Institut Català d’Oncologia, Badalona, Spain. Background: Activating alterations of the mesenchymal epithelial transition (MET) oncogene in NSCLC are potentially actionable with targetted MET inhibitors. MET exon 14 skipping mutations have been described in 3% of patients (p) in NSCLC. Fluorescent in situ hybridization (FISH) and immunohistochemistry (IHC) are currently used for detecting MET amplification and overexpression, respectively, but are not useful to detect MET splicing variants. On the other hand, the reverse transcription–polymerase chain reaction (RT-PCR) technique has the potential to detect this actionable alteration. Methods: We designed and validated a custom set of 5’ and 3’ primers to detect the MET exon 14 splicing variant by RT-PCR. RNA isolation from FFPE samples was performed with Roche High Pure FFPE RNA isolation kit and M-MLV Reverse Transcriptase enzyme was used in the RT-PCR. A panel of cell lines was initially employed to assess the performance of the technique. Subsequently, a total of 232 formalin-fixed paraffin-embedded (FFPE) samples from advanced NSCLC patients were analyzed. Of them, 15 were positive by RT-PCR (n=209) for the MET exon 14 variant. The bands corresponding to the splicing variant were submitted to Sanger sequencing. Results were compared with FISH (Zytovision Dual Color probe Z-2087-200) and IHC (Ventana CONFIRM anti-total c-MET). Results: A total of 232 EGFR-wt advanced NSCLC p were analyzed by IHC and 42 (18.1%) were considered as positive (cut-off 3 ≥ 50%). Regarding FISH, MET amplification was detected in 13 out of 58 p (22.4%) evaluable while the MET exon 14 skipping variant was detected in 15 (7.2%) out of 209 p. Regarding RT-PCR, MET amplification was detected in 13 out of 58 p (22.4%) evaluable while the MET exon 14 skipping variant was detected in 15 (7.2%) out of 209 p. Conclusions: MET exon 14 skipping variant was detected in 15 (7.2%) out of 209 p. FISH, MET amplification was detected in 13 out of 58 p (22.4%) evaluable while the MET exon 14 skipping variant was detected in 15 (7.2%) out of 209 p.

**#2724** Brush biopsy miRNA based OSCC detection and diagnosis. Guy R. Adam, Joel L. Schwartz, Yali Zhou, Jessica L. Tang, Michael Markiewicz, Gina D. Jefferson, Joel Epstein, Antonio Kolokythas, University of Illinois at Chicago, Chicago, IL; Cedars-Sinai Medical Center, Los Angeles, CA; University of Rochester Medical Center, Chicago, IL. RNA based diagnosis and prognosis of squamous cell carcinoma has been slow to come to the clinic. Improvements in RNA measurement, statistical evaluation, and sample preservation still have not made these methods accurate enough to allow truly independent external validation. We propose that in the case of squamous cell carcinoma of the oral cavity that a chief source of variability is sample dissection which leads to variable amounts of stroma mixed in with tumor epithelium. We have used brush biopsy of suspicious lesions to characterize miRNA expression in OSCC epithelium. We used it to show an 11 miRNA signature for OSCC that can differentiate OSCC in both smokers and never smokers from normal tissue. We will report on efforts to optimize this classifier to distinguish OSCC from benign oral lesions such as lichen planus and leukoplakia that can be mistaken for OSCC. We also provide evidence that body fluid samples show similar changes in RNAs with OSCC that are seen in brush biopsy samples -suggesting much of the RNA is coming from the same source tumor epithelium. Finally, using a variation of mirPath designed to eliminate false positive, we have identified the neurotrophin signaling pathway as deregulated in most OSCC epithelium, and for the first time highlight the miRNAs that may control this pathway in OSCC, mir-486-5p, mir-7-5p, mir-146-5p, mir-101-3p, mir-18b-5p, mir-10b-5p, mir-21-5p, mir-20b-5p, mir-126-3p and mir-31-3p.

**#2725** Circulating CD9-GFAP-survivin exosomes during active specific immunotherapy, a potential biomarker for glioma. Michael J. Ciesielski, Phillip Galbo, Sheila Figel, Cheryl Frank, Jingxin Qiu, Robert A. Fenstermaker, Roswell Park Cancer Inst., Buffalo, NY. Background: We evaluated circulating exosomes isolated from the serum of glioma patients in a large clinical trial of an anti-survivin vaccine (SurVaxM). Exosomes are mesenchymal bodies with potential mechanisms for cell–cell communication. Identifying circulating exosomes from cancer patients as potential indicators of disease status and response to therapy is of interest. The inhibitor of apoptosis protein (IAP) survivin (SVN) promotes cancer cell proliferation and resistance to chemotherapy. In glioma, survivin is often over expressed in many cancer types and is a malignant glioma and is a potential target for active immunotherapy. In the above mentioned clinical trial, five patients experienced early tumor progression with a mean of 2.8 months (1.9-5.4 months) from study entry, while three patients had either late (20.5-22.5 months) or no tumor progression (no evident disease at 48 months in one patient). Methods: To determine whether changes in CD9+/GFAP+/SVN+ exosomes correlated with disease progression, patient serum was evaluated prior to treatment (study entry), 8-10 weeks after initial vaccination, and at the time of MRI-defined tumor progression. By employing ImageStream flow cytomtery technology we were able to analyze multiple markers on individual exosomes, identifying specific populations of survivin+ exosomes which co-expressed other tumor markers. Results: The levels of CD9+/GFAP+ +, CD9+/SVN+ +, and CD9+/GFAP+/SVN+ + exosomes were significantly increased in patients. Serum from three patients who experienced the longest progression-free intervals, showed 98-99% decreases in SVN+ exosomes after treatment with SurVaxM, and maintained similarly low exosome levels over several months. Five early progressing patients experienced a detectable persistence of, or increase in SVN+ exosomes at 9 weeks following initial vaccination. One patient had rising SVN+ exosomes detectable 16 weeks prior to the discovery of tumor progression by brain MRI scan. One patient with no tumor progression sustained a 99% reduction in serum SVN+ exosomes which was sustained over 48+ months. Conclusion: This study demonstrates that increased numbers of CD9+/GFAP+/SVN+ specific exosomes appear to be associated with early tumor progression in recurrent malignant glioma patients. Larger studies are needed to determine whether the correlation between SVN+ exosome levels and disease progression is specific to SurVaxM treatment, and whether rises in SVN+ levels are predictive of generalized glioma progression. Our study suggests that levels of circulating SVN+ exosomes may be altered during anti-survivin immunotherapy and could serve as a potential biomarker for clinical trials and tumor monitoring.

**#2726** Guidance by molecular selection improves the outcome of early phase treatment for gynecological (GYN) cancers. Rowan E. Miller, Michael John Devlin, Nicholas Brown, Kin Woo, Tami Grunewald, Arran Speirs, Miriam Mitchison, Michelle Lockey, Mary McCormack, Jonathan Ledermann, Martin Forster, Tim Meyer, Rebecca Kristeleit. NHR Clinical Research Facility, University College London Hospitals, London, United Kingdom; University College London Hospitals, London, United Kingdom. Background: Patients (pts) with advanced gynecological (GYN) cancers have limited therapeutic options and the prognosis is poor. Early phase trials may be a suitable option for pts with good performance status. Increasingly, molecular characterisation guides pt selection for early phase trials. We sought to determine the outcome of GYN pts treated in a phase 1 unit and examined the role of molecular selection to inform therapeutic decision making. Methods: Medical records of all pts with a GYN malignancy treated within an early phase trial between 2010 and 2016 were reviewed. Data comprising patient and tumor characteristics, prior treatment, trial therapy and outcome were analysed. Results: 81 pts with a median age of 60 years (range 20-75) were included. 60/81 pts had a platinum resistant ovarian (OC, 54), endometrial (EC, 15) or cervical/vulval (CC, 12) cancer. The median number of prior therapies for advanced disease was 3 (range 1-6). 9 pts (11%) entered a second and one a third phase 1 study on disease progression. Next Generation Sequencing (NGS) using a targeted panel was performed in 32 pts (40%) with an actionable mutation identified in 9 including: KRAS (3pts), PIK3CA (2pts) and EGFR (2pts). Germline BRCA (gBRCA) testing was performed in 35 OC pts (65%) with 24 gBRCA mutations identified. Pts were allocated, in order of priority, where available, to (1) a trial selected on the basis of NGS or gBRCA (‘genomic 35%), (2) a ‘tumor specific’ cohort within an early phase trial (15%) or (3) a...
"generic" study (51%). For the whole cohort there was an overall response rate (ORR) of 18% with 41% stable disease (SD) and median progression free survival (PFS) and overall survival (OS) of 13 and 46 weeks respectively. Outcomes were best for pts in the genomic group. Both PFS and OS were significantly longer with genomic selection (p < 0.01 for both, Mantel-cox test) with median PFS of 29.7, 14.2, 18.0 weeks and OS of 84.1, 69.7, 33.6 weeks for genomics, tumor specific and generic studies respectively. The ORR was also greatest for the genomic cohort (32%) compared to the tumour specific (7%) and generic (11%) groups. Within the heavily pre-treated EC and CC cohorts there was an OS of 30 and 42 weeks respectively. 24% of EC pts had an ORR with a further 24% with stable disease (SD). There was only one response (9%) in the CC cohort, however SD was seen in 64%. The OS for the CC was 35 weeks with an ORR of 20% and 46% SD. Conclusions: Early phase trials represent a good option for pts with advanced GYN malignancies. Whilst applicable to all GYN cancers, this is particularly relevant for EC and CC pts as standard treatment options are limited. For OC patients (median 3 prior lines of chemotherapy in this cohort) where standard treatment options exist, early access to phase 1 genomic trials may result in improved response rates and allow for further standard options to be given subsequently, NGS is feasible in real time and may have a positive impact on outcome.

#2727 Clinical significance of PTEN in UTUC, Liang-Chen Li,1 Hung-Ying Chiang,1 Ying-Tzu Chen,2 See-Tong Pang,2 Wen Hui Weng1.1, National Taiwan University of Technology, Taipei, Taiwan; 2Chang Gung Medical Foundation, Taoyuan, Taiwan.

Upper tract urothelial carcinoma (UTUC) is a rare malignancy accounts for about 5% of all urothelial tumours. The cancer cells start in the layer of tissue urothelium, and found in the renal pelvis, renal calyces or ureters. When compare the UTUC to the bladder urothelial carcinoma, the pathological and clinical findings are often look and act alike. Previous studies indicated that deletion of phosphatase and tensin homologue (PTEN) gene is common to be found in bladder cancer. As known tumor suppressor gene PTEN is located on chromosome ten and considered as an important negative regulator for the PI3/Akt signaling pathway to promote cell proliferation and inhibit apoptosis. Thus, this study we aimed to determine clinical significance of PTEN in UTUC. We collected sixty-eight formalin-fixed paraffin-embedded (FFPE) UTUC samples, the tumor stage was according to American Joint Committee on Cancer (AJCC) with Ta (n = 40), T1 (n = 18), T2 (n = 10), T3 (n = 18), T4 (n = 7) and seven patients with unknown; in which male no.32, female no.36; the median age is 68 y/o (range 40-85 y/o). Fluorescence in situ hybridization (FISH) was performed to examine the PTEN gene copy number or gene structure alterations of UTUC; besides, immunohistochemistry (IHC) analysis were used to observe the PTEN protein expression in the tumor. Our results showed PTEN mutations were observed in twenty-two tumors with heterozygous PTEN deletion; one tumor with homozygous PTEN deletion, and three tumors with PTEN monosomy. Further, heterozygous PTEN deletion was shown significantly associated with stage of pTa, pT1 and pT2 (P = 0.048) and history of smoking (P = 0.030). The IHC results showed lower expression level was common to be observed in the tumors with PTEN deletion as compared with the tumors with normal PTEN expression levels. Our findings indicated PTEN genomic loss might act a predictive indicator of classification of UTUC histology. In conclusion, PTEN genomic loss might act a predictive indicator of classification of UTUC, however, the clinical treatment could be different due to the underlying diversity of the molecular activities.

#2728 Frequency and prognostic value of PI3KCA mutations in early stage of colorectal cancer in Mexican patients. Erika Ruiz-Garcia,1 Alette Ortega,1 Antonio Bahena,2 Edith Fernandez,2 Cesar Lopez-Camarillo,1 Laurence Marchat,3 Jorge Guadarrama,1 Abelardo Meneses-Gariga,1 Horacio Astudillo-de la Vega3.1 National Cancer Institute, Mexico City, Mexico; 2Universidad Autónoma de la Ciudad de México, Mexico City; 3Escuela Nacional de Medicina y Homeopatía, Instituto Politécnico Nacional, Mexico City, Mexico; 4Lab Investigacion Translacional en Cancer, HOCMN SXXI, IMSS, Mexico City, Mexico.

Background: There are molecular associations between mutated profiles and microsatellite instability, both related with tumor growth and oncogenic development by activating tyrosine receptors families in colorectal cancer (CRC). It is known that exon 9 and exon 20 (H1047R) mutations in PIK3CA increased PI3K activity. Moreover, PIK3CA mutations are significantly associated with tumor recurrence and poor overall survival. The frequency of PIK3CA mutations in CRC occurs in 15–20%, being the catalytic subunit more frequently affected. The aim of our study was to investigate the frequencies and clinical implications of these genetic alterations in advanced CRC. Methods: Retrospectively, we analyzed the initial paraffin embedded tissue block of 71 Mexican patients with CRC stage II. We evaluated mutations frequency of PIK3CA exon 9 (E542K, E545K) and exon 20 (H1047R) using qPCR marker Somatic Mutation PCR for real-time analysis. We confirmed the mutations by Sanger sequencing. This study was approved by the Ethics Committee of the National Cancer Institute. Results: We found in our population a frequency of PIK3CA mutations of 26%. Of those, double mutants were present in 7% with a higher frequency for E545K/H1047R. Clinical implication that we found is that any active mutation of PIK3CA is associated to increase of recurrence risk (HR 1.86 CI 95% 0.46-7.5), but most important is for E545K mutation (HR 1.17 CI 95% 0.85-20.39) compared to PIK3CA wild-type, meanwhile comparing no mutated with H1074R mutated samples there were no difference in HR (0.53 vs. 0.60, respectively). Conclusions: We found a higher number of PI3KCA mutations that literature reports and that E545K mutation have a worst prognostic implication but we need to increase our sample looking for statistical significative results. Data from H1047R mutation raise de hypothesis that it exist a compensatory mechanism related to structural interactions between both helicoidal and catalytic domains.

#2729 A significant differential sensitivity to AKT inhibitors GSK690693 and MK-2206 2HI was identified in primary cells established from a low-grade mucoepidermoid carcinoma. Ahmad M. Alamri, Priscilla A. Furr. Georgetown University, Washington, DC.

The AKT pathway is frequently activated in cancer cells and is a defined target for therapeutic intervention. A comprehensive method for RNA expression levels of candidate AKT pathway genes (AREG, EGFR, AKT, mTOR, GSK3α/β) were defined following RNAseq of replicate primary cell cultures (GUMC220 and GUMC221), which were established independently from two areas of the same low-grade mucoepidermoid carcinoma (MEC). Protein expression and phosphorylation levels of the corresponding gene products were characterized using immunohistochemistry on FFPE sections from the original patient tumor, cell pellets, and cell line generated patient-derived xenografts and through western blotting of proteins extracted from cells grown under different culture conditions before and after treatment with AKT inhibitors GSK690693 (ATP-competitive) and MK-2206 2HI (allosteric inhibitor). The effects of GSK690693 and MK-2206 2HI on cell viability were tested at different concentrations ranging from 1.2-40μM in 2D and 3D conditions with three different culture media (Conditioned media + ROCK kinase inhibitor Y27632, conditional media without Y27632, and EpiCult-C Basal™) using CellTiter-Glo™. Doxorubicin was used as a comparative control for evaluating the impact of a chemotherapeutic on cell viability and DMSO as a negative vehicle control. MDA-MB-453 cells were tested as a positive control for MK-2206 2HI and GSK690693 sensitivity. Results: Replicate primary cell cultures showed the same chemosensitivity results. MK-2206 2HI induced a significantly greater reduction in cell viability as compared to GSK690693 in the MEC primary cells. This differential response was replicated in all three 2D culture media tested (GUMC220 CM p = 0.0017, GUMC220_CM p = 0.0004, GUMC220 EpiC p = 0.001, GUMC221 CM p = 0.0010, GUMC221 CM p = 0.0001, and GUMC221 EpiC p = 0.0041. One-way ANOVA analysis with the same pattern reproduced in 3D cultures. Expected changes in levels of p-AKT(Ser473), GSK3α/β, p-GSK3α/β, and p-mTOR were found following treatment with both drugs as compared to vehicle controls. In MK-2206 2HI treated cells, expression levels were all reduced whereas in GSK690693 treated cells, p-AKT(Thr537) and p-AKT(Thr380) were increased and GSK3α/β, p-GSK3α/β, and p-mTOR reduced. Conclusion: Characterization of primary cell cultures using RNAseq led to the identification of a potentially druggable pathway. Direct testing revealed a differential response to the two AKT inhibitors evaluated. Although the two primary cell cultures were established from two geographically distinct areas of the same tumour, response to drug testing was the same and reproducible across different culture modalities. Studies establish the feasibility of deriving primary cells for drug testing and personalized therapy, even from small tumors.

#2730 Image-based single cell-sorting to separate and recover distinct cell populations from complex heterogeneous mixed tissue: precise sample preparation upstream of FISH. Amanda Gerber,1 Aditi Khurana,2 Lisa Koenig,3 Lindsay Strotzman,4 Lori Millner,3 Valeria Sero,2 Chiara Bolognesi,2 Sabine Kasimir-baur,4 Gianni Medoro,5 Matthew Moore,2 Philip Cotter,2 Nicolo Manaresi,5 Farideh Bischoff1.

Res. Da, Irvine, CA; 2Universitätssklinikum Essen, Essen, Germany; 3PGX Laboratories, Louisville, KY; 4Menarini Silicon Biosystems SpA, Bologna, Italy. Fluorescent in Situ Hybridization (FISH) is commonly used for assessment of chromosomal alterations. Guidelines for determining FISH-based classification
CLINICAL RESEARCH: Clinical Molecular Genetics and Laboratory Correlates

of clinical biomarkers exist but are based on pre-analytical factors, including fixation/sectioning/thickness/age, that can greatly influence biomarker status determination. Here, we use single-cell image-based cell sorting by DEPArray™ for the separation and recovery of pure distinct cell populations prior to FISH. Methods: A multi-center study to evaluate HER2-FISH based analysis on FFPE paraffin-in, Andres Alonso, Robert Storr, Grant Armstrong, Anthony D’Ovidio, James Yarbrough, and Naomi Simon. Cells were then stained and sorted using the DEPArray™ platform for sectioned. One curl from each sample was sent to each of four centers (3 US; 1 Europe). Each site performed disassociation of curls to generate a single cell suspension. Cells were then stained and sorted using the DEPArray™ platform for recovery of tumor (cytokeratin+/vimentin-/DAPI+) and stromal (cytokeratin+/vimentin+/DAPI+) cells. Dual-probe FISH for HER2 and centromere 17 was performed on the sorted cells and compared with conventional tissue section FISH. Results: Overall, ≥ 90% concordance between the sorted tumor cells and the conventional HER2 FISH result was observed. Among the 7 HER2+ cases, HER2 ratio scores for the sorted tumor cells ranged slightly higher, from 2.60 to 8.95, as compared to the conventional method (from 2.10 to 5.14). In all cases in which stromal cells were also recovered, an expected normal ratio was observed, thus verifying that the populations were efficiently separated. Discordance can be attributed to intra-tumoral heterogeneity and the fact that conventional FISH on FFPE requires only a 4-micron section for analysis. Conclusion: Today, a percentage of patients are likely misclassified for the biomarker of interest as result of pre-analytical factors. We demonstrate here the ability to overcome these pre-analytic factors and ultimately improve the accuracy in determining biomarker status using the DEPArray™.

CLINICAL RESEARCH: Liquid Biopsies 2: cfDNA

#2731 Identifying circulating tumor DNA in patients with osteosarcoma.

David M. Barris, Michael Fremed, Robert Durbin, Xusheng Zhang, Sajida Piperdii, Wendong Zhang, Shahina Maqbool, Jonathan Gill, Michael Roth, Bang Hoang, David Geller, Richard Gorlick, Daniel Weiser. Albert Einstein College of Medicine, Bronx, NY.

Introduction: Osteosarcoma (OS) is the most common primary malignant bone tumor and primarily affects children and adolescents. During and after treatment there is no non-invasive test to assess disease response and early relapse. We hypothesize that circulating tumor DNA (ctDNA) can be used to assess tumor burden, evaluate response to treatment, and monitor for recurrence in OS. We effectively tested this hypothesis on a mouse model and have utilized our methods on a first cohort of human patients. Materials and Methods: Twenty blood samples with matched primary tumor material were obtained from 10 patients with OS who were at various stages of treatment or post treatment. Cell-free DNA was isolated from blood collected in Cell-Free DNA blood collection tubes using the QIAamp Circulating Nucleic Acid Kit. We performed massively parallel sequencing (MPS) with the Illumina HiSeq 2500 in the Epigenetics Core Facility of Albert Einstein College of Medicine. MPS was performed on whole blood using the TruSeq Nano DNA Sample Prep Kit from Illumina and the TruSeq PE Cluster Kit v3-HS from Illumina. Reads were aligned to the genome using BWA-MEM. cfDNA was called using VarScan2. Results: In every sample there were ≥ 100000 reads. Ten samples were sequenced with ≥ 200000 reads. Conclusion: Circulating tumor DNA is a potential biomarker in OS. Future studies using larger cohorts will determine if this is a viable test for disease monitoring.

#2732 Novel methods for enrichment of mutations and differentially methylated sequences from liquid biopsy genomes.

Chen Song, Yibin Liu, Mariana Fitearelli-Kiehl, Ioannis Ladas, Harvey Mamon, Mike G. Makrigiorgos. Dana-Farber Cancer Inst., Boston, MA.

Presence of excess unaltered, wild-type DNA providing no information of biological or clinical value may often mask rare alterations containing diagnostic or therapeutic clues for cancer. With the surge of high-throughput technologies there is a growing demand for removing unaltered DNA over large pools-of-seq. Here we present nuclease-assisted minor-allele enrichment with probe-overlap (NaME-PrO), a single-step approach with broad genome-cover- age that removes WT-DNA prior to DNA-amplification, following which current genomic analysis procedures remain unaltered. NaME-PrO employs a dou-ble-strand-DNA-specific nuclease and overlapping oligonucleotide-probes interrogating multiple DNA targets. Following genomic-DNA denaturation, or upon partial denaturation, the probes form transient double-stranded regions with their targets, thereby guiding nucleic acid digestion to selected sites. Mutations create mismatches that inhibit digestion, thus subsequent amplification yields DNA with alterations enhanced at multiple targets. In this manner, WT DNA at hundreds or thousands of DNA regions can be digested simultaneously. We demonstrate several-hundred-fold multiplexed mutation-enrichment in diverse human samples on multiple clinically-relevant targets in 50-247plex reactions. By adjusting conditions it is possible to sequence mutations down to 0.00003% abundance, essentially isolating mutated-DNA. Application with targeted re-sequencing algorithms demonstrated detection of mutations at the 0.01-0.1% levels with few sequence reads. In another adaptation of the same approach in the field of methylation detection, we developed methyla-tion-sensitive nuclease-assisted minor allele enrichment, MS-NaME. Aberrant methylation changes, often present in a minor allelic fraction in clinical samples such as cfDNA, are potentially powerful prognostic and predictive cancer bio-markers. During MS-NaME, oligonucleotide probes targeting unmethylated se-quences in bisulfite-treated DNA generate local double stranded regions result-ing to digestion of unmethylated targets, and leaving methylated targets intact; and vice versa. We validated MS-NaME by demonstrating enrichment of pro-moters from cancer-related genes like RARB2, ATM, MGMT and GSTP1 in multiplexed 177-plex reactions using dilutions of methylated/unmethylated DNA as well as clinical lung cancer samples and matched normal tissue. NaME-PrO and MS-NaME introduce simple, low-cost, broad genomic coverage pro-cesses that combine seamlessly with next generation sequencing. This reveals clinically useful DNA alterations that normally would stay undetectable or re-quire very high read-depth to be detected. Applications in liquid biopsy-based diagnostics using circulating DNA will be presented.

#2733 Comparison of copy number aberrations (CNAs) between plasma cell free DNA (cfDNA) and tissue DNA in metastatic castrate resistant prostate cancer (mCRPC).

Chun Chen Lin,1 Hua Huang,1 Liewei Wang,2 Liang Wang,2 Manish Kollhi,3 Univer-sity of Wisconsin, Milwaukee, Milwaukee, WI; 2Medical College of Wisconsin, Milwaukee, WI; 3Mayo Clinic, Rochester, MN.

Background: Circulating cell-free DNA (cfDNA) analysis is emerging as a less invasive approach to assessing tumor genomic alterations in cancer patients. Although high concordance has been reported between tumor tissue NGS and cfDNA in studies investigating specific genetic alterations, the fidelity of cfDNA to tumor tissue DNA in the global genomic scale is largely unknown. In a correlative study of a prospective clinical trial (NCT# 01935640) conducted in mCRPC stage patients treated with abiraterone acetate/prednisone (A/P), we evaluated correlation of genomic CNA in tumor DNA obtained from biopsy of metastatic lesions and matched plasma cfDNA. Methods: mCRPC patients (pts) underwent two image-guided core biopsies at the time of the core biopsies and cfDNA was extracted using DNA Blood Mini Kit (Qiagen, Valencia, CA). High coverage (for tumor tissue) and low coverage (for cfDNA) whole genome sequencing reads were first mapped to the human genome hg19. Read counts (RC) from the mapped sequence files were then binned into 1MB windows. The RC ratio in each genomic bin was calculated by comparing tumor tissue DNA to lymphocyte gDNA derived from the same patient, and was further log2 transformed, corrected for GC content, and normalized by CGHnormalizer. The fully normalized log2 ratios data was subjected to segmentation using DNAcopy algorithm. Results: Between 05/2013 and 09/2015, 92 patients (pts) were enrolled of which tissue and plasma NGS data both visits was available for 18 pts. The correlation of CNAs between tumor tissue and its cfDNA counterpart ranges from 0.013 to 0.83 for V1 samples, and -0.05 to 0.92 for V2 samples. The decreased correlation in some pairs of samples is

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largely due to low tumor content and heterogeneity in the cfDNA. Although there is a wide range of correlation, commonly shared CNAs were identified in multiple chromosomal regions, including loss in 8p, gain in 8q and chromosome 5 and X. On the other hand, several genomic regions show inconsistent CNAs between tumor tissue and cfDNA among 15 pairs of samples in which one tumor contained 6 CNAs (3 gains and 3 losses). The CNAs most commonly observed in tumor tissue were tumor-specific and expressed in cfDNA but not in tumor tissue, marked loss in chromosome 22 and gain in p arm of X chromosome in tumor tissue but much less evident in cfDNA. Conclusion: High concordance of CNAs between cfDNA and tumor tissue DNA can be achieved given sufficient tumor content of cfDNA. However, more CNAs can be identified in cfDNA than its tumor tissue counterpart. Our result suggests that cfDNA NGS is a useful tool to investigate clonal evolution associated with cancer progression.

#2734 Impact of circulating and tissue biomarkers in patients with metastatic colorectal cancer treated with first-line FOLFOX/alfibercept therapy. Results of the GERCOR VELVET Phase II Study. Annemilai Tijeras-Raballard, Armand de Gramont, Marielle Chiron, Jean-Baptiste Bachet, Thierry Andre, Dominique Auby, Jerome Desramé, Baba-Hamed Nabil, Lecaille Cedric, Valerie Lebrun, Christophe Roux, Annette Larsen, Christophe Tourigny, Sylvie Benner, Melika Attia, Aimerie de Gramont, Franck Bonnetain, Benoit Chibaudel, AOF Oncology, Paris, France; Sanofi France, Vitry-sur-Seine, France; Salpêtrière University Hospital, Paris, France; Saint-Antoine University Hospital, Paris, France; Mont de Marsan Hospital, Paris, France; Jean Mermoz Hospital, Paris, France; Saint-Joseph Hospital, Paris, France; Clinique Nord, Paris, France; Limoges University Hospital, Paris, France; Institut Mutualiste Montsouris, Paris, France; Inserm U938-Institut Universitaire de Cancérologie/UPMC, Paris, France; Henri Mondor University Hospital, Paris, France; GERCOR group, Paris, France; Institut Hospitalier Franco-britannique, Paris, France; Inserm UMR 1098-Beauvoin University Hospital, Paris, France.

Background: The combination of alfibercept to OPTIMOX (VELVET study) was evaluated in patients with previously untreated advanced colorectal cancer. A biomarker program was set-up to explore the expression of several tissue biomarkers at baseline and circulating biomarkers upon treatment cycles to identify the best monitoring biomarkers of the treatment strategy. Methods: VELVET was a prospective, single arm phase II trial. Patient’s plasma samples were collected at baseline, and during induction therapy at day 1 of the first 6 cycles. Circulating biomarkers were analyzed using multiplexing immunoassays (31 biomarkers, 3 panels). Assays were conducted on a Biorad Bioplex platform, expect for PIGF, VEGF-A and Neutrophil-1 which were assessed using ELISA assays. Tissue biomarker were analyzed at baseline by IHC, using a BOND-Max autostainer (Leica biosystems). EGFR mutation status were analyzed using next generation sequencing. Results: Among 49 patients included in the VELVET study from May 2013 to May 2014, 44 (90%) patients were evaluable for circulating biomarkers expression. The proportion of patients with tumor response (CR or PR) was higher in patients with high baseline levels of sVEGFR2, sEGFR, G-CSF, Prolactin and low baseline levels of VEGF-A and MIF. Progression-free survival was longer in patients with baseline levels of Haptoglobin (HR: 0.53; P = 0.045) and in patients with high baseline levels of VEGF-C (HR: 0.45; P = 0.014) and sVEGFR3 (HR: 0.50; P = 0.045). Overall survival was higher in patients with high sVEGFR3 (HR: 0.36; P = 0.030) levels. In both responders and non-responders, sVEGFR-1 and PIGF dramatically increased upon exposure to alfibercept and remained overexpressed for the all course of induction therapy. Circulating biomarkers modifications will be analyzed upon the 6 cycles. Tumor tissue biomarker assessment at baseline using IHC and the EGFR pathway mutations status will be added. Conclusions: Exposure to alfibercept is associated with an increase of sVEGFR-1 and PIGF at cycle 1. Elevated baseline expression levels of on-target sVEGFR3 predict favorable outcome in patients treated with alfibercept.

#2735 Targeted molecular profiling of tumor and matched circulating tumor DNA in patients with early stage endometrioid type endometrial adenocarcinoma. Ana M. Bolivar, Rajyalakshmi Luthra, Meenakshi Mehrotra, Wei Chen, Bedia A. Barkoh, Peter Hu, Rajesh R. Singh, Russell R. Broadus. The University of Texas MD Anderson Cancer Center, Houston, TX.

Endometrioid-type endometrial adenocarcinoma (EEA) is the most common type of gynecological cancer in the USA and the fourth most common cancer in women. Contrary to other cancer types, its incidence is rising, likely due to its tight association with obesity. A major clinical problem for EEA patients presenting with early stage disease is identifying those at risk of recurrence, as extra-vaginal recurrence is incurable. If these at-risk patients could be identified up-front, they could receive more aggressive surgical treatment and adjuvant radiation therapy/chemotherapy/targeted therapy to help prevent the development of incurable recurrences. The long-term goal of our work is to develop a blood-based molecular diagnostics platform that can help to identify women with early stage EEA who are at highest risk for tumor recurrence. To achieve this goal, we have performed a large cohort study to identify genetic alterations that could be used to detect common EEA associated mutations in circulating tumor DNA (ctDNA) of early stage EEA patients. From our previous work, we have shown that 98% (43/44) of EEA patient’s tumors have somatic mutations in CTNNB1, KRAS, PTEN, and/or PIK3CA. Using a custom panel designed by Swift Biosciences targeting 30 amplicons in these 4 genes, MiSeq NGS analysis was performed on both cfDNA and matched tumor tissue DNA. We then performed a droplet digital PCR in detecting circulating nucleic acid kit and on the matching tumor DNA (tDNA) extracted from FFPE slides using an Arcturus Pico Pure DNA extraction kit from 33 stage I and II EEA patients (stages in which tumor is confined to the uterus). NGS data were analyzed using a custom bioinformatics pipeline. Using a cutoff of 0.5% allele frequency (AF) in the ctDNA and 5% in the tDNA, at least one EEA associated mutation was detected in both ctDNA and tDNA in 5/33 (15%) of cases. Mutations with more than 5% AF in the tumor and 0.02-0.49% AF in the ctDNA (total coverage >250X and variant coverage >25) were also detected in another 6/33 patients. Each of these 11 cases was re-sequenced at a higher depth (>20,000X), with comparable results. For the remaining 22 patients at least one EEA associated mutation was detected only in the ctDNA, with no mutation detected in the matched tumor tissue DNA. The most common mutation detected in ctDNA was a frameshift mutation in 10p which leads to missense mutations in the group in which mutations were only present in ctDNA (mean 3.6 cm: p=0.005). To improve the analytical sensitivity in the ctDNA and confirm the current findings, all cases will be analyzed using digital droplet PCR. Molecular barcodes will also be incorporated into our custom panel. In this proof-of-principal study, we have demonstrated that relevant mutations can be detected in the plasma of a subset of patients when endometrial carcinomas are confined to the uterus.

#2736 A digital droplet PCR assay for the quantitation of androgen receptor and splice variant expression in CTCs from metastatic castration resistant prostate cancer patients. Ada Gyerezi, Giuseppe Galletti, Arei Strati, Seho Kim, Evi Lianidou, David M. Nansus, Jun Luo, Emmanuel Antonarakis, Scott T. Tagawa, Andrew Armstrong, Paraskeni Giannakakou, Weil Cornell Medicine, New York, NY; University of Athens, Athens, Greece; John Hopkins Medicine, Baltimore, MD; Duke University School of Medicine, Durham, NC.

Prostate cancer (PC) is the second leading cause of cancer death in men in the US. The aberrant functioning of androgen receptor signaling is the central driving force behind prostatic tumorigenesis and its transition into metastatic castration resistant disease. Here, we took advantage of the first-line of treatment for PC patients. However, many patients progress becoming resistant to ADT therapy, due to expression of AR splice variants (AR-Vs), which lack the ligand binding domain and are constitutively active in the nucleus. Expression of the AR splice variant, AR-v7, in circulating tumor cells (CTCs) isolated from the blood of PC patients was correlated with resistance to enzalutamide and abiraterone, which are the next generation AR signaling inhibitors in CRPC. Further, there is evidence that AR-Vs may convey cross-resistance, not only to enzalutamide and abiraterone, but also to taxanes, highlighting that their assessment in the clinic may have clinical utility. We developed a novel, specific and highly sensitive assay to measure mRNA expression of the AR full length (AR-FL) and the splice variants ARV7 and ARS676x, by using Droplet Digital PCR in CTCs isolated from CRPC patients. The analytical specificity of the assay was determined by transfecting cells with plasmids encoding AR-FL, AR-v7 and AR-v676 and showed that each probe detected signal only in cells expressing the respective transcript. No signal was detected against genomic DNA, indicating lack of non-specific binding. Also, the assay detected endogenous expression of AR-FL and AR-v7 in VCAP or 22Rv1 cells, indicating specificity of the assay in healthy donor tissue. The analytical sensitivity of the assay was determined in a series of serial dilution experiments that showed sensitivity down to single cell. We then used this assay to determine the clinical prevalence and expression pattern of each of these variants in CTCs from about 200 mCRPC patient samples and blood from 40 healthy donors. CTCs were enriched by EpCAM or PSMA positive selection or by CD45 negative depletion in an antigen-agnostic manner. AR-FL was detected in ~80% of mCRPC samples irrespective of CTC-enrichment technology. AR-FL was expressed in 65% of the samples in which in CTCs were enriched either by PSMA-positive selection or by negative depletion. In contrast, EpCAM-based CTC enrichment showed lower AR-v7 expression both in terms of expression
levels and prevalence. In addition, CTC enrichment following negative deple-
tion showed that 30% of the samples had higher AR-v7 expression levels as compared to AR-FL. This expression pattern was not observed in the samples using EpCAM-based selection. Collectively, these data suggest specific CTC subpopulations are present in CRPC patient samples, with differential expres-
sion of AR-Vs that could have important predictive and prognostic implica-
tions.

#2377 Development of a clinically actionable, ultra-sensitive multi-onco-

Background: Circulating tumor DNA (ctDNA) is rapidly emerging as a viable alternative to tissue molecular diagnostics for the detection of actionable onco-
genic mutations. In non-small cell lung cancer, identifying the emergence of EGFR T790M in patients receiving first line EGFR tyrosine-kinase inhibitors (TKIs) is a clinical need. Obtaining a tissue biopsy to determine T790M status is associated with significant cost and patient morbidity. In a retrospective analysis of the TIGER-X trial, patients had similar therapeutic response to a 3rd genera-
tion TKI, independent of the sample type positive for T790M; tissue, plasma, or urine. This indicates that non-invasive urine liquid biopsy is a viable diagnostic option (Wakele et al, ASCO, 2016). We report on the expansion of single mu-
tation assays to a multiplex-panel of clinically actionable mutations having high analytic sensitivity and specificity. We previously developed two next generation sequencing methodologies with high sensitivity and specificity for ctDNA detection: 1) A series of individual ultra-sensitive ctDNA hotspot assays capable of detecting ultra-short DNA fragments (31-45 base pairs) and a 0.01% lower limit of detection (LLoD). The short sized amplicons coupled with mutant allele enrichment enabled high clinical sensitiv-
ity for plasma and urine ctDNA (Reckamp et al, J Thorac Oncol, 2016) 2) A multiplex panel using sequence-specific synchronous coefficient of drag altera-
tion (SCODA) technology that enriches for mutant ctDNA to obtain a LLoD of 0.001%-0.03% (Pel et al, PNAS, 2009; Kidess et al, Oncotarget, 2015). By com-
bining a multiplex of short amplicons with SCODA enrichment capabilities, we developed an assay enriched for over 200 clinically actionable driver and resis-
tance mutations within 7 oncogenes. Results: An iterative process was used to design and optimize primer and enrichment probe length and placement to produce a panel where mean coverage across hotspots regions was within 2 to 3-fold (read uniformity) and greater than 1,000-fold enrichment of mutant DNA prior to sequencing. Technical validation was performed utilizing in-
house and commercially available model systems for plasma and urine demon-
strating mutation detection from inputs ranging from hundreds to fewer than 10 copies and LLoD as low as 0.001%. Clinical performance evaluation of the newly developed multiplex assay is ongoing using urine and plasma ctDNA from pa-
tients with advanced cancer. Conclusions: High sensitivity is critical when identi-
ifying targetable driver mutations as well as emergence of resistance mutations which can inform therapeutic decision making. By combining short amplicon and multiplex enrichment technologies, we developed an ultra-sensitive ctDNA assay for use in sample types with highly degraded and fragmented DNA.

#2378 A commutable circulating tumor DNA (ctDNA) reference mate-
rail. Yves Konigshofer,1 Farol L. Tomson,1 Matthew Ryder,1 Russell Garlick,1 Bharathi Anekella1.1 SeraCare Life Sciences, Inc., Gaithersburg, MD; 2SeraCare Life Sciences, Inc., Milford, MA.

We developed and assessed a novel ctDNA reference material that was designed with an emphasis on commutability. Possessing qualities that are well-
matched with clinical specimens is critical for a reference material, however many existing methods of preparing ctDNA analogues result in material that is not commutable for certain assays. For example, sonication of genomic DNA yields fragments across a broad range of sizes that must be used at greater input amounts relative to native cell-free DNA (cfDNA) - even after enzymatic end-
repair to reduce damage. This can affect the utility of the reference material for assays that depend on the ligation of adapters to double-stranded DNA, such as many hybrid/capture-based Next Generation Sequencing (NGS) assays. If higher input amounts are not used, then the LOD and precision of such an assay may appear substantially lower than expected. At the same time, sonicated DNA has been shown to be acceptable for amplicon-based cfDNA assays. We created commutable ctDNA reference material by starting with genomic DNA from GM24385 cells and mixing with synthetic variant-containing DNA se-
quences at defined ratios. The use of GM24385 cells allows for the comparison of detected variants against the NIST RM 8391 high confidence variants. The use of synthetic DNA permits addition of large numbers of different cancer-associated variants at defined allele frequencies. Fragmentation was followed by size-selec-
tion in order to obtain ctDNA-like sizes. Additional steps were performed to increase the amount of output material from the size-selection step. The general method of preparing the reference material is also compatible with native cfDNA. The reference material was submitted to several testing laboratories along with similar sonicated material. When tested using hybrid/capture-based assays, variant detection was superior in the novel reference material compared to sonicated DNA when similar amounts of material were used as the input. We therefore conclude that our new reference material has greatly improved commutability compared to existing materials composed only of sonicated DNA.

#2379 Low-pass whole genome sequencing detects copy number varia-

Circulating tumor DNA (ctDNA) is released from necrotic/apoptotic tu-
mor cells into the bloodstream. Recent studies have demonstrated the value of using ctDNA as biomarkers in cancer diagnosis, prognosis, and drug resistance. Unlike local tissue biopsy, ctDNA collection and analysis is non-
invasive, allows continuous monitoring of clonal evolution, and provides an overview of tumor heterogeneity. Copy number variations (CNVs) play an important role in cancer biology. However, traditional CNV analyses of ctDNA using droplet digital PCR (ddPCR) and SNP arrays can only assess a small number of genes due to the low abundance of ctDNA in the majority of patient samples. Next generation sequencing (NGS) offers a more efficient and high-throughput way to study CNVs in ctDNA. Here, we evaluated the use of low-pass whole genome sequencing (WGS) in determining CNVs in ctDNA. In this work, cell-free DNA was isolated from 1-5 ml of plasma from phase II clinical trial patients with metastatic breast cancer and non-small cell lung cancer (NSCLC) using the QIAamp Circulating Nucleic Acid Kit (QIAGEN). DNA yield was determined by ddPCR, with a range from 2-4000 ng. Sequencing libraries were prepared using 2-10 ng DNA by the TruPLEX Plasma-Seq Kit (Rubicon Genomics). WGS at 0.1x, 0.25x, 0.5x, and 1x cov-
erage was performed on Illumina NextSeq, and data was analyzed using Nexus Copy Number software (BioDiscovery). As a reference, we sequenced DNA from peripheral blood mononuclear cells (PBMCs) of 20 healthy do-
nors at the same coverage. Low-pass WGS was also performed on a subset of matched tumor tissue samples as comparisons. Our results showed that WGS of ctDNA at 0.5x coverage was efficient to identify CNVs. CNVs were detected in ctDNA from about half of the patients analyzed, and in general CNVs identified in ctDNA matched the ones found in tumor tissue from the same patient. We also found that CNV patterns from different time points of the same patients clustered together. With this promising system, we will present CNV analysis in ctDNA from breast cancer and NSCLC patients enrolled in phase II clinical trials of the PI3K inhibitor pictilisib. We will evaluate the ability of the method to classify patients into different sub-
groups, monitor tumor progression, and identify drug resistance mecha-
isms.

#2380 High-throughput and sensitive quantification of circulating tumor DNA. Yinghui Guan, Oleg Mayba, Shan Lu, Thomas Sandmann, Younjeong Choi, Walter Darbonne, Vincent Lequeve, Lisa Ryner, Eric Humke, Nga W. Tam, Sundari Sujathasarma, Anna Cheung, Richard Bourgon, YueLi Wang, Genentech, A Member of the Roche Group, South San Francisco, CA.

Circulating tumor DNA (ctDNA) has a great potential to serve as non-
invasive biomarkers for monitoring disease progression and treatment re-
sponse. However, its broad clinical applications have been hindered by sen-
sitivity, throughput and complexity of existing ctDNA detection methods. Here we report an adaptation of a multiplex PCR pre-amplification (Pre-
Amp) into the microfluidic multiplex PCR (MMP-Seq) technology for high-
throughput and sensitive quantification of ctDNA. We have demonstrated that PreAmp MMP-Seq enabled sensitive (down to 0.6% allele frequency), reproducible (R²=0.97), accurate and quantitative mutation detection from as low as 1.6 ng DNA input. An empirical Bayesian Model has been estab-
lished to estimate background error rates for every base of amplicons in the ctDNA panel using data from 43 normal plasmas. 0.1-0.3% of median error rate were observed across all positions. 149 longitudinal plasma samples from 12 ovarian and 10 pancreatic cancer patients of PhI DMUC7554A anti-MUC16 ADC trials were then profiled using PreAmp MMP-Seq. 79% (11/14) driver mutations found in tissue samples were also identified in matching baseline plasma samples at frequencies well above estimated error.
rates. Moreover, we found that changes of corresponding ctDNA concentration in post-treatment plasma samples were correlated with changes in tumor burden and circulating protein marker levels. In conclusion, the MMP-seq PreAmp workflow provides an inexpensive, reproducible and flexible solution for mutation detection in plasma DNA samples.

#2741 Assessing the utility of circulating tumor DNA as a surveillance tool for sarcomas and Li-Fraumeni syndrome using a pre-clinical model. San-geetha Paramathas, Nathan Lewis, Tanya Guha, Zainab Motala, David Malkin. The Hospital for Sick Children, Toronto, Ontario, Canada.

Li-Fraumeni Syndrome (LFS) is a hereditary cancer predisposition syndrome commonly characterized by the presence of inherited mutations in the tumor suppressor gene TP53. This leads to an early onset of a wide spectrum of tumors in multiple organ systems. Current surveillance protocols for early tumor detection include biochemical screening, MRI and ultrasound scans, colonoscopy and mammography (for adults). While early detection is associated with improved survival, the complexity of testing, potential to ‘misdiagnose’ tumors (false positive/negative) and requirement for multiple imaging modalities makes clinical surveillance challenging to implement and interpret. Liquid biopsies are a recently described diagnostic and prognostic tool that takes advantage of analyzing circulating tumor DNA - fragmented genomic material that are released into the blood from dying tumor cells. In this proof-of-principle study, we use mouse xenograft tumor models to assess the dynamic relationship between tumor burden and ctDNA concentrations, as well as resolve the sensitivity of capturing tumor lesions in their earliest stages of growth. Characterization of ctDNA from serially collected blood were performed on mice xenografts of rhabdomyosarcoma (Rh4 and Rh30), osteosarcoma (HOS) and non-small cell lung carcinoma (H1975) of both localized and simulated metastatic tumors. We characterized ctDNA using droplet digital PCR (ddPCR) for known gene mutations that were specific for the cancer cell lines used. We observe obvious increases in ctDNA with increased tumor burden and a complete clearance of ctDNA after tumor resection. In addition, using the mutation model, we have conducted synchronized imaging and blood-based biopsies to determine the smallest lesion able to be detected in the blood by ctDNA. These studies provide the foundation for early tumor detection with ctDNA inTp53 mutant mice that develop spontaneous tumors analogous to LFS. This model will help further our understanding on the utility of ctDNA as a surveillance and diagnostic tool for LFS as well as assess its potential for clinical use.

#2742 Cross-platform comparison of four leading technologies for detecting EGFR mutations in circulating tumor DNA from plasma of patients with non-small cell lung carcinoma. Ting Xu, Xiaozheng Kang, Xiaofang You, Dai Liang, Dequan Tian, Wanpu Yan, Yongbo Yang, Hongchao Xiong, Zhen Liang, Grace Q. Zhao, Shengrong Lin, Ke-Neng Chen, Guobing Xu. Peking University Cancer Hospital and Institute, Beijing, China; 2AccuSensor, Merlo Park, CA.

Analysis of circulating tumor DNA (ctDNA) is emerging as a powerful tool for guiding targeted therapy and monitoring tumor evolution in patients with non-small cell lung cancer (NSCLC), particularly when fresh tissue biopsy is not available. In this study, we compared the ability of four leading technology platforms to detect epidermal growth factor receptor (EGFR) mutations (L858R, exon 19 deletion, T790M and G719X) in ctDNA from NSCLC patients. The platforms included two amplification refractory mutation systems (cobas-ARMS and Adx-ARMS), a droplet digital polymerase chain reaction platform (ddPCR) and a next-generation sequencing platform (Firefly NGS). Fifteen EGFR mutations across twenty NSCLC patients were identified. We observed superior sensitivity and specificity of cobas-ARMS, ddPCR and Firefly NGS platforms, while Adx-ARMS was only suitable for the qualitative detection of EGFR mutations with allele frequency higher than 1% in plasma samples. We observed high concordance between the plasma and tissue EGFR mutational profiles for three driver mutations that are known targets of the first generation EGFR-TKI therapy (L858R, E19-dels, and G719X). Discrepancies between plasma and tissue EGFR mutational profiling could be attributed to spatial and temporal tumor heterogeneity. This pilot study illustrates the promise of ctDNA analysis in the context of treatment evaluation and drug resistance detection, and results will be validated in follow-up studies.

#2743 Comparison of enhanced Tagged-Amplicon Sequencing and digital PCR for circulating tumor DNA analysis in advanced breast cancer. Isaac Garcia-Murillas, Matthew Beanny, Michael Epstein, Karen Howarth, Andrew Lawson, Sarah Hreibien, Emma Green, Nizan Rosenfeld, Nick Turner.1 The Institute of Cancer Research, London, United Kingdom; 2Inviva, Cambridge, United Kingdom.

Background: Circulating tumor DNA (ctDNA) analysis allows non-invasive detection of tumor mutations and amplifications in advanced breast cancer. Multiple technologies have been developed to analyse ctDNA and here we compared two leading ctDNA detection technologies, enhanced Tagged-Amplicon Sequencing (eTAm-Seq) and digital PCR (dPCR) assays, in advanced breast cancer. Methods: We recruited a cohort of 35 women with advanced breast cancer, of whom 23 had two separate blood samples taken in a standard EDTA tube processed immediately or in preservative Streck tubes processed up to 120 hours after venipuncture. Digital PCR was conducted with assays for hotspot actionable mutations in 3 known drivers in breast cancer: PIK3CA exon 9 and 20, ESR1 ligand binding domain and AKT1 (c.49G>A; p.E17K), and ctDNA sequencing with eTAm-Seq within a 35-gene panel including cancer hotspots, entire coding regions and copy number variants (CNVs). Results: Across both assays, 37 mutations were detected in 35 patients, with PIK3CA mutation in 13 patients (37%), ESR1 mutations in 10 patients (29%), and no AKT1 mutations. ESR1 mutations were polyclonal in 8 patients, with ctDNA eTAm-Seq method revealing substantially more diversity in mutations, up to 8 individual mutations detected in a patient. There was 96.15% agreement for PIK3CA mutation detection between assays (Kappa 0.89, 95% CI 0.743 to 1.000), 100% agreement for ESR1 mutations (Kappa 1.00, 95% CI 1.000 to 1.000). There was very high correlation in mutation allele frequency between eTAm-Seq and dPCR (r=0.93, 95%CI 0.86 to 0.96, p<0.0001). The sensitivity and specificity for HER2 amplification detection by eTAm-Seq was 100% (true positive) to tumor HER2 status. Comparing different sample processing methods, Streck tubes revealed 97.92% agreement (Kappa 0.95, 95% CI0.868 to1.000) for mutation calling. Conclusions: This study demonstrates that ctDNA analysis using eTAmSeq and dPCR have very high agreement in mutation detection in patients with advanced breast cancer patients. Streck tubes present a robust alternative to immediate processing of samples. eTAm-Seq and digital PCR have high clinical validity in mutation detection.

#2744 Inter-site comparison of performance of an ultra-sensitive sequencing technology for circulating tumor DNA. Stephanie Yang,1 Alex Lovejoy,1 Dan Klass,2 Fergal Casey,1 Aruna Arcot,1 Melissa Loyzer,1 William Chiu,2 Nabil Azhar,2 Sophie Beckert,3 Maria Lange,3 Sandra Siemann,3 Sebastian Froehler,3 Shelly Gunn,3 John F. Palma,1 Roche Sequencing Solutions, Pleasanton, CA; 2ResearchDx, Irvine, CA; 3Roche Sequencing Solutions, Potsdam, Germany.

The combination of liquid biopsies and ultra-sensitive next-generation sequencing (NGS) holds tremendous promise in oncology for the detection and surveillance of somatic mutations that correlate with diagnosis, prognosis, response prediction, resistance monitoring and tumor burden. We have developed a capture-based NGS workflow that provides ultra-sensitive detection of 4 mutation classes - single nucleotide variants (SNVs), insertions/deletions, copy number amplifications (CNAs) and fusions - from low input cell-free DNA isolated from plasma. Sensitivity and specificity of 2 different AVENIO ctDNA Analysis Kits (for Research Use Only) across 3 sites for 48 samples that included contrived DNA blends with known mutations validated by digital PCR and normal reference plasma samples were assessed. The AVENIO ctDNA Targeted Kit and AVENIO ctDNA Expanded Kit (Expanded) was designed to detect mutations in 17 genes, across 80kb, included in NCCN Guidelines for solid tumors plus additional variants relevant for therapy selection. The AVENIO ctDNA Expanded Kit (Expanded) was designed to detect mutations in 77 genes, across 200kb, associated with solid tumor including variants relevant for clinical trial research. Testing sites achieved 100% sensitivity for SNVs and fusions at 0.5% and 1% allele frequency, respectively, from 30 ng of input DNA for both kits. Median coverage of 2,400-6,400X was greater with the Targeted kit relative to that of 2,700-3,800X with the Expanded kit. Specificity was 100% for the Targeted kit either by sample or variant. The Expanded kit had a specificity of 91.7-100% by sample and 99.7-100% by variant. At an expected 5X or 8X copy number amplification for multiple genes, both the Targeted and Expanded kits were 100% sensitive and 93.1-98.4% specific per gene. The specificity for fusion detection was 100% for both kits down to 1% mutant allele frequency. The AVENIO ctDNA Analysis Kits for plasma and NGS applications provide exquisite sensitivity and specificity for the simultaneous detection of 4 different mutation classes.

#2745 Evaluation of a new system for collection, stabilization, and purification of circulating tumor DNA. Michael Fleischhacker, Bernd Schmidt, Michael Fleischhacker, Bernd Schmidt, Roehan Baker, Susanne Pedersen, Natasha Cant, Maryam Zahedi-nejad, Thorsten Voss, Andrea Ullius, Daniel Groedz.1 University of Halle, Germany; 2DK-Kliniken Berlin-Mitte, Germany; 3Clinical Genomics, North Ryde, Australia; 4Qiagen Ltd., Manchester, United Kingdom; 5PreAnalytiX Gmbh, Hilden, Germany.

MultipletectologieshavebeendevelopedtoanalysectDNAherewecom-
CLINICAL RESEARCH: Liquid Biopsies 2: cfDNA

Introduction: The current preanalytical workflows for circulating tumor DNA (ctDNA) analysis have limitations that affect the accurate detection and quantification of these plasma cancer biomarkers. Release of genomic DNA (gDNA) from white blood cells (WBCs) due to cell lysis or apoptosis during whole blood storage in EDTA tubes creates higher gDNA background levels, affecting the detection of ctDNA as a biomarker. Circulating cfDNA often contain crosslinking reagents, which have negative effects on sensitive downstream assays, including methylation-based assays. Using ctDNA assays, the new PAXgene® Blood cfDNA System*, consisting of a blood collection tube with unique, non-crosslinking chemistry and an automated circulating cell-free DNA (ccfDNA) extraction kit, was evaluated in three research studies. Methods: Blood samples were collected into paired PAXgene and EDTA tubes and stored for 7 days at room temperature (RT). cfDNA was isolated from plasma using the PAXgene kit or the QIAGEN QIAamp® Circulating Nucleic Acid Kit. Study 1: The ccfDNA from lung cancer patients was quantified by real-time PCR for the amount of ERV (sequence (as a measure of the total plasma DNA quantity) and after bisulfite treatment for mSHOX2 as a marker for ctDNA. Study 2: Blood from healthy donors was spiked with fragmented, fully-methylated CpGenome DNA. During storage, tubes were intermittently inverted to simulate tubes in transit. Subsequent to bisulfite conversion, PCR assays targeting ACTB and methylated BCAT1 and IKZF1 DNA were used to determine the yields of ccfDNA and ctDNA, respectively. Study 3: Restriction enzyme treated EGFR DNA containing exon 19 deletions and exon 20 and 21 substitutions (T790M) were spiked into healthy donors’ blood; DNA purity was tested with the QIAGEN herescan® EGFR Plasma RGQ PCR Kit*. Results: Both study 1 and 2 demonstrated constant levels of the methylation ctDNA markers. SHOX2, BCAT1 and IKZF1, over the investigated time course. There was no significant release of gDNA in the PAXgene tube whereas a significant release of gDNA was detected in EDTA samples. Likewise, study 3 showed constant EGFR Ct values in the PAXgene system with reliable mutation detection, whereas the high DNA concentration from the EDTA system resulted in false-positive callings. Conclusions: The new system allows researchers to accurately detect and quantify plasma cancer biomarkers from blood samples that have been stored in the tube for up to 7 days at RT. This includes challenging assays based on methylated ctDNA. The system provides the required assay sensitivity to allow the correct assay interpretation beyond the typical 3–6 hour storage limit for EDTA tubes. *For Research Use Only. Not for use in diagnostic procedures.

#2747 Plasma DNA methylation marker and hepatocellular carcinoma risk prediction model for the general population. Hui-Chen Wu,1 Hwai-I Yang,2 Qiao Wang,1 Chien-Jen Chen,2 Regina M. Santella1.

Metastases in the later stages of hepatocellular carcinoma (HCC) cause the majority of deaths associated with the disease, making early detection crucial to patient survival. Risk models that assess HCC risk in the general population can be used for risk stratification for further HCC surveillance, however, none have been validated externally. Methylation of circulating DNA shows potential for noninvasive and early diagnosis of HCC. Most evidence, however, comes from cross-sectional studies raising concerns about temporality. We conducted a prospective case-control study nested within a community-based cohort. We measured methylation levels in of 6 genes (CDKN2A, RASSF1A, STEAP4, TBX2, VIM, and ZNF154) using pre-diagnostic plasma DNA from 237 HCC cases and 537 matched controls. We found TBX2 hypermethylation was associated with increased HCC risk, with ORs (95%CI) of 3.7 (1.9-7.2). The associations were mainly among high-risk subjects; among subjects infected with HBV/HCV, the OR (95%CI) of TBX2 methylation was 5.3 (2.2-12.7). Among subjects with high risk, the ORs (95%CI) were 8.9 (1.0-73.9) for Wen-HCC score ≥16, 5.6 (2.1-14.6) for Hung-HCC score ≥15, and 7.2 (2.1-24.4) for Michikawa-HCC score ≥4. Adding information on TBX2 methylation improved the accuracy of risk models for a high risk population, with the area under the curve (AUC) of 74% for TBX2 methylation and Wen-HCC score compared with 66% with Wen-HCC score alone. The AUCs were 63% for Hung-HCC score and TBX2 methylation, 53% for Hung-HCC score alone, 65% for Michikawa-HCC score and TBX2 methylation and 59% for Michikawa-HCC-risk alone. Our findings suggest the potential increase in risk assessment discrimination and accuracy from incorporation of DNA methylation.

#2746 Detection of tumor-specific mutations in plasma DNA: A potential esophageal adenocarcinoma biomarker. Matthew Egyud,1 Jennifer Jackson,1 Emiko Yamada,1 Anders Ståhlberg,2 Paul Krzyzanowski,3 Virginia Litle,1 Lincol Stein,2 Tony Godfrey1, 3Boston Medical Center, Boston, MA; 2University of Gothenburg, Gothenburg, Sweden; 3Ontario Institute for Cancer Research, Ontario, Canada.

Introduction: The current preanalytical workflows for circulating tumor DNA (ctDNA) analysis have limitations that affect the accurate detection and quantification of these plasma cancer biomarkers. Release of genomic DNA (gDNA) from white blood cells (WBCs) due to cell lysis or apoptosis during whole blood storage in EDTA tubes creates higher gDNA background levels, affecting the detection of ctDNA as a biomarker. Circulating cfDNA often contain crosslinking reagents, which have negative effects on sensitive downstream assays, including methylation-based assays. Using ctDNA assays, the new PAXgene® Blood cfDNA System*, consisting of a blood collection tube with unique, non-crosslinking chemistry and an automated circulating cell-free DNA (cDNA) extraction kit, was evaluated in three research studies. Methods: Blood samples were collected into paired PAXgene and EDTA tubes and stored for 7 days at room temperature (RT). cfDNA was isolated from plasma using the PAXgene kit or the QIAGEN QIAamp® Circulating Nucleic Acid Kit. Study 1: The ccfDNA from lung cancer patients was quantified by real-time PCR for the amount of ERV (sequence (as a measure of the total plasma DNA quantity) and after bisulfite treatment for mSHOX2 as a marker for ctDNA. Study 2: Blood from healthy donors was spiked with fragmented, fully-methylated CpGenome DNA. During storage, tubes were intermittently inverted to simulate tubes in transit. Subsequent to bisulfite conversion, PCR assays targeting ACTB and methylated BCAT1 and IKZF1 DNA were used to determine the yields of ccfDNA and ctDNA, respectively. Study 3: Restriction enzyme treated EGFR DNA containing exon 19 deletions and exon 20 and 21 substitutions (T790M) were spiked into healthy donors’ blood; DNA purity was tested with the QIAGEN herescan® EGFR Plasma RGQ PCR Kit*. Results: Both study 1 and 2 demonstrated constant levels of the methylation ctDNA markers. SHOX2, BCAT1 and IKZF1, over the investigated time course. There was no significant release of gDNA in the PAXgene tube whereas a significant release of gDNA was detected in EDTA samples. Likewise, study 3 showed constant EGFR Ct values in the PAXgene system with reliable mutation detection, whereas the high DNA concentration from the EDTA system resulted in false-positive callings. Conclusions: The new system allows researchers to accurately detect and quantify plasma cancer biomarkers from blood samples that have been stored in the tube for up to 7 days at RT. This includes challenging assays based on methylated ctDNA. The system provides the required assay sensitivity to allow the correct assay interpretation beyond the typical 3–6 hour storage limit for EDTA tubes. *For Research Use Only. Not for use in diagnostic procedures.

#2747 Liquid biopsy using extracellular vesicular DNA for EGFR mutation testing in the patients with NSCLC. Jae Young Hur,1 Hee Jung Kim,1 Chang-Min Choi,1 Jae Cheul Lee,2 Kyoung Lee1.

Background: Tumor-derived extracellular vesicles (EV) have been proved to carry double-stranded DNA including oncogenic mutations and expected to be a novel biomarker in cancer detection. Liquid biopsy using cell-free (cf) DNA for EGFR mutation testing is being actively investigated. In this study, we investigated whether liquid biopsy using EV DNA is superior to cfDNA for EGFR mutation testing in the patients with NSCLC. Methods: EV was isolated using ultracentrifugation and confirmed by transmission electron microscopy and zetasizer in 20 plasma samples (all tissue-proven EGFR mutated) and 32 bronchoalveolar lavage fluid (BALF) samples (23 EGFR-mutated including 14 TKI-naïve and 9 TKI resistant, and 9 wild-type). EV and cf DNA was separately extracted from each samples and EGFR mutation testing was performed by peptide nucleic acid (PNA)-clamping method. Results: In 20 plasma samples, the concordance rate of EV DNA for tissue EGFR genotyping was 55% (11/20), while 30% (6/20) in of cf DNA. In 32 BALF samples, however, the sensitivity, specificity and concordance rate of EV DNA for activating EGFR mutations were 100% (23/23), 100% (9/9), and 100% (32/32), while cf DNA showed 73.9% (17/23), 100% (9/9) and 81.3% (26/32), respectively. The kappa value (p-value) of EV DNA was 1.0 (>0.001), while it was 0.714 (0.001) in the case of cf DNA. As for 9 re-biopsy cases for T790M detection in which only 3 patients (33%) was proved as positive T790M, BALF EV DNA identified 2 more T790M patients (total 5 among 9 patients, 56%) without non-matching with tissue typing, while BALF cfDNA found 1 more T790M positive patient with missing 1 tissue proven case (total 3 among 9 patients, 33%) Conclusions Liquid biopsy using EV DNA is significantly superior to cf DNA in both plasma and BALF. A little low sensitivity in plasma EV DNA is supposed to be improved with much more sensitive method such as ddPCR or BEAMing. Liquid biopsy using BALF EV DNA showed almost 100% matching with tissue EGFR typing and seems to be highly promising for T790M detection. A large number of clinical trials should be prompted.

Liquid biopsies have the potential to revolutionize the way physicians select personalized anti-cancer therapies, monitor patient responses to treatment, and characterize acquired resistance to cancer drugs. New tests that use a simple peripheral blood draw offer snapshots of a patient’s total tumor DNA mutation profile and are attractive because of their minimally-invasive modality and because they integrate information from both primary and metastatic disease.

Currently, most plasma cell-free DNA (cfDNA) mutation detection tests used in clinical research detect known hotspot mutations in a limited number of genes. Technologies that interrogate multi-gene panels in cfDNA are advancing, but commercially-available options suitable for clinical use are limited, come at a high cost, and are not customizable. We designed and developed a customized, next-generation sequencing-based, liquid biopsy assay capable of detecting somatic mutations in 87 breast cancer-related genes in cell cycle and estrogen receptor signaling. Targeted regions (147 Kb) were enriched using hybrid capture resulting in an average capture specificity and uniformity of 65.93% and 96.38%, respectively. When tested on cfDNA from healthy donors (n=14), we demonstrated a level of specificity >99.99%. Analytical sensitivity of 0.1% was established on HapMap and reference mutant cell line DNA. Using a pool of 10% mutant DNA, we were able to detect mutations at 0.001% in 96% of samples. A minimum allele frequency of 0.1% were detected. In reference mutant cell line DNA with 1% or 0.1% mutant allele frequencies, we were able to reliably detect all mutations present at 1% and mutations at 0.1% in 50% of the cases. Assay validation on plasma cfDNA with matching tumor from ER+, HER2- breast cancer patients will be presented. In conclusion, we developed a highly sensitive and specific liquid biopsy assay to interrogate 87 breast cancer-related genes. The high level of specificity and sensitivity makes the test ideal not only for detecting known cancer gene hotspot mutations but also for detection of novel gene mutations that may arise during treatment as a result of acquired drug resistance.

Detection resistance mechanisms in patients on EGFR TKI treatment by liquid biopsy. Laure Sorber,1 Karen Zwaenepoel,1 An Wouters,2 Jansens Annelyes,2 Birgitta Hiddinga,3 Jan Van Meerbeck,2 Filip Lardon,2 Christiaan Rolfo,2 Patrick Paweels4. Antwerp University Hospital (UZA), Wilrijk, Belgium; 2Antwerp University (UA), Wilrijk, Belgium; 3Antwerp University Hospital (UZA), Wilrijk, Belgium; 4Antwerp University Hospital (UZA), Edegem, Belgium.

The rise of liquid biopsy, including the molecular analysis of circulating cell-free tumor DNA (ctDNA), has enabled real-time follow-up of cancer patients who receive targeted therapy. Here, we report the case of a lung cancer patient (adenocarcinoma), initially harboring the activating EGFR L858R mutation. Blood and tissue samples were collected during treatment with the EGFR tyrosine kinase inhibitor (TKI) gefitinib, and during the appearance of resistance. Analysis of the tissue biopsies consisted of EGFR mutational analysis by high-resolution melting analysis (HRMA) (all tissue samples), immunohistochemistry (IHC) of cMET (sample 2); droplet digital PCR cMET analysis of both the plasma and tissue samples are now being processed to determinethe course of the mutational load and the best course of treatment. As such, real-time follow-up of cancer patients is crucial to detect the underlying mechanisms of resistance to targeted therapies. Correlation of molecular analysis with the disease course is key. Our case study highlights the potential of ctDNA analysis for detection of both the EGFR T790M resistance mutation and cMET amplification. Hence, tissue biopsies can be supplemented by liquid biopsies in the screening for resistance mechanisms.

Automatic DNA extraction system can improve the EGFR point mutation detection rate of liquid biopsy. Chihoko Nakashima,1 Akemi Sato,1 Tomonori Abe,2 Tomomi Nakamura,1 Kazutoshi Komiya,1 Eisaburo Sueoka,1 Shinya Kimura,1 Naoko Sueoka-Aragane,2 Junichi Kato,2 Mitsuharu Hirai2. 1Saga University, Saga city, Japan; 2ARKRAY Inc., Kyoto, Japan.
The usefulness of liquid biopsy to detect mutations from cancer patients has been well recognized today. However, because the mutation detection rates from plasma DNA were relatively lower than those of tissue re-biopsy, its clinical utility has not been confirmed yet. As previously we reported, we have developed fully automatic high-sensitive point mutation detecting system named mutation-biased PCR and quenched probe (MBP-QP) system for liquid biopsy. Recently, the importance of pre-analytical procedures for plasma DNA analysis has been highlighted. In this study, we examined whether the automatic DNA extraction system can improve the mutation detection rate in our MBP-QP system. Sixty-one plasma samples were obtained from advanced non-small cell lung cancer patients, and plasma DNA extraction was performed from 200μl plasma by manually (200-M), and 200μl (200-A), 1000μl (1000-A) plasma by automatically. We used silica membrane spin column for DNA extraction and high-sensitive point mutation detecting system named mutation-biased PCR and quenched probe (MBP-QP) system for liquid biopsy. The median DNA concentrations quantified by quantitative real-time PCR of 200-M, 200-A, 1000-A were 4.92, 6.00, 20.1 ng/mL plasma, respectively. In terms of the epidermal growth factor receptor (EGFR) L858R point mutation detection, the sensitivity of 200-M, 200-A, 1000-A were 36.6%, 58.5%, 77.5%, that of the specificity were 93.3%, 100%, 96.7%, and the concordance rates were 66.6%, 76.1%, 85.7%, respectively. The size distribution of automatically extracted plasma DNA represented two peaks characteristics at 170 bp and 5 Kb. In this study, we indicate the automatic DNA extraction can improve mutation detection rates in plasma DNA.

Liquid biopsy analysis of FGFR3 and PIK3CA hotspot mutations for disease surveillance in bladder cancer. Emil Christensen,1 Karin Birken-kamp-Denmtröder,1 Iver Nordenfelt,1 Soren Hoyer,2 Kirstin Van Der Keur,3 Kim Van Kessel,4 Ellen Zwarthoff,5 Mads Agerbak,6 Todd Holscher,2 Matteo Pastorino,1 Gabriella Sozzi1. 1Aarhus University Hospital, Aarhus, Denmark; 2University Hospital, Copenhagen, Denmark; 3Aarhus University Hospital, Aarhus C, Denmark; 4Erasmus MC Cancer Institute, Rotterdam, Netherlands; 5Radiology, Maastricht University Medical Centre, Maastricht, the Netherlands; 6Gensignia LifeSciences, Inc, San Diego, CA.

Detecting resistance mechanisms in patients on EGFR TKI treatment by liquid biopsy. Laure Sorber,1 Karen Zwaenepoel,1 An Wouters,2 Jansens Annelyes,2 Birgitta Hiddinga,3 Jan Van Meerbeck,2 Filip Lardon,2 Christiaan Rolfo,2 Patrick Paweels4. Antwerp University Hospital (UZA), Wilrijk, Belgium; 2Antwerp University (UA), Wilrijk, Belgium; 3Antwerp University Hospital (UZA), Wilrijk, Belgium; 4Antwerp University Hospital (UZA), Edegem, Belgium.

The use of liquid biopsies to detect mutations from cancer patients has been gaining traction due to improved detection rates in plasma DNA. The usefulness of liquid biopsy to detect mutations from cancer patients has been well recognized today. However, because the mutation detection rates from plasma DNA were relatively lower than those of tissue re-biopsy, its clinical utility has not been confirmed yet. As previously we reported, we have developed fully automatic high-sensitive point mutation detecting system named mutation-biased PCR (MBP-QP) system for liquid biopsy. Recently, the importance of pre-analytical procedures for plasma DNA analysis has been highlighted. In this study, we examined whether the automatic DNA extraction system can improve the mutation detection rate in our MBP-QP system. Sixty-one plasma samples were obtained from advanced non-small cell lung cancer patients, and plasma DNA extraction was performed from 200μl plasma by manually (200-M), and 200μl (200-A), 1000μl (1000-A) plasma by automatically. We used silica membrane spin column for DNA extraction and high-sensitive point mutation detecting system named mutation-biased PCR and quenched probe (MBP-QP) system for liquid biopsy. The median DNA concentrations quantified by quantitative real-time PCR of 200-M, 200-A, 1000-A were 4.92, 6.00, 20.1 ng/mL plasma, respectively. In terms of the epidermal growth factor receptor (EGFR) L858R point mutation detection, the sensitivity of 200-M, 200-A, 1000-A were 36.6%, 58.5%, 77.5%, that of the specificity were 93.3%, 100%, 96.7%, and the concordance rates were 66.6%, 76.1%, 85.7%, respectively. The size distribution of automatically extracted plasma DNA represented two peaks characteristics at 170 bp and 5 Kb. In this study, we indicate the automatic DNA extraction can improve mutation detection rates in plasma DNA.

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In order to increase cost-effectiveness of low-dose computed tomography (LDCT) lung cancer screening programs, a change in the management of screening-detected lung tumors should be considered. The higher percentage and the better 5-year overall survival of early stage tumors in LDCT screening series than in clinical practice argue in favor of screening programs. The overall limited reduction of mortality observed in screening trials with an observational control arm suggests that some of the screening-detected early stage tumors might be over-diagnosed.

In our Institution we are trying to address this issue by the development of complementary biomarkers able to improve detection of aggressive disease. Targeted next-generation sequencing was performed in 94 LDCT screening-detected tumors selected from 2008 to 2010 participating in 3 screening trials enrolling 9,248 volunteers. Mutation profile was associated with subjects’ clinicopathologic features and with the risk profile of a plasma microRNA signature classifier (MSC). Using data available through The Cancer Genome Atlas database (TCGA), we compared the mutations of a selected set of non-small cell lung cancer (NSCLC) cases detected in standard clinical practice to LDCT screening-detected NSCLC cases. The 5-year overall survival (OS) of screening patients with and without mutations in the tumors was 64% and 100%, respectively (p = 0.019). By combining the mutational status with the MSC, patients were stratified into 3 groups with 5-year OS ranging from 41% to 96% (p < 0.0001) and the prognostic value was significant even when controlling for stage (p = 0.017). The comparison with TCGA data revealed a higher number of non-mutated NSCLC among screening patients (21% vs. 13%), despite the similar spectrum and frequency of mutations. In addition, the difference in 5-year OS between subjects with and without mutations was exclusively detected in screening patients. The mutation profile of screening-detected tumors, while similar to that of clinically detected tumors, was a strong predictor of OS. The combination of tumor mutational status and a circulating miRNA-based risk classifier predicts tumor aggressiveness and clinical outcome and may find rapid application in LDCT screening programs.

#2754 A novel liquid biopsy method for development of aptamer libraries that bind blood plasma exosomes from breast cancer patients. Valeriy Domeynuk, Symon Levenberg, Adam Stark, Mark Miglarese, David Spetzler. Caris Life Sciences, Phoenix, AZ.

Improved technologies capable of characterizing system-wide changes associated with complex diseases will be required to be able to detect millions of proteins and their isoforms as well as multi-molecular complexes. We present a method for developing aptamer libraries using blood plasma exosomes that provides unprecedented system-wide coverage of native exosomal complexes. To train a naïve aptamer library toward cancer samples (positive selection), the library (10^11 biotinylated ssODN species) was incubated with plasma from individual cancer patients and aptamer-bound exosomes were isolated using polymer-based precipitation. Negative selection was performed by contacting the aptamer library with exosomes from donors without breast cancer and recovering unbound aptamers from the supernatant. In all, 12 libraries trained toward 12 individual breast cancer patients were used to probe additional samples. Receiver operator analysis (ROC) was performed to compare expression of mRNA transcripts across all four methods. Results showed PCA specific genes were detected in whole blood, Exo-52 exosome prep and samples enriched for CTCs, while exosomes isolated using CellSearch EpCAM capture failed to detect any PCA transcripts. ROC showed ExoRNAeasy exosome prep detected PCA specific mRNA transcripts with significantly higher sensitivity and specificity compared to three other methodologies. Area under curve (AUC) for ExoRNA prep 0.65 to 0.75 (average of z score at 3.27 p = 0.0023), compared to AUC for PAXgene is 0.55 to 0.83, (average of z score at 2.64 and p = 0.05) and CTC samples is 0.52 to 0.67, (average of z score at 1.63 and p = 0.19). For CellSearch exosome method, the AUC is 0.47 to 0.52, average of z score at 0.66 and p = 0.32). These results and in vitro studies investigating cytotoxicity profiles of this assay as a viable alternative to whole blood and CTC based assays. Further testing for expression of markers associated with prognosis and treatment response is necessary to warrant its prognostic or predictive clinical utility.

#2755 Comparison of blood based liquid biopsy methodologies for improved risk assessment of prostate cancer (PCa). Yashoda Rajputohit, Shibu Thomas. Lung tumors detected from subjects participating in 3 screening programs, Approximately 28,000 men die from prostate cancer in the US each year. Predictive biomarkers can provide patient risk assessment to enable therapeutic decision making. Compared to tumor, blood based liquid biopsies offer greater flexibility for non invasive sample collection and allows for continuous monitoring of treatment response. While various liquid biopsy sample types and methodologies are currently available, it is critical to identify which method offers the highest sensitivity and specificity for clinical decision making. In this study we systematically compared specificity and sensitivity of prostate cancer detection using a pre-selected panel of PCA specific mRNA transcripts using four different methodologies. In this prospective laboratory study, we collected five blood tubes from a cohort of 40 metastatic castrate resistant prostate cancer (mCRPC) patients and 20 age matched healthy volunteers (HV). Circulating tumor cells (CTCs) were enumerated and blood samples enriched for CTCs were isolated for mRNA evaluation. Technology platform, based on fluorescent labeling mRNA, and multiplex, high-sensitivity assays using universal primers, thus leveraging PCR sensitivity while eliminating the need for separate reverse transcription reactions and mitigating amplification biases introduced by target-specific qPCR. The Firefly assays have been validated in a wide range of biological samples including plasma, serum, urine and cell culture supernatant, providing experimental flexibility. The 96well plate assay format enables high-throughput screening of samples with readout conducted on standard flow cytometers, thereby omitting the need for complex and expensive dedicated instrumentation. Finally, the integrated Firefly Analysis Workbench software enables easy and rapid data analysis, visualization, and export in under ten minutes, and includes key features such as standard curve analysis and publication-quality heatmaps and graphs. Here we present several studies investigating cytokine profiles in human and rodent samples, and circulating tumour microRNA profiles, using the Firefly Platform. Together, this novel combination of bioinformatics tools and multiplex, high-sensitivity assays enables rapid discovery and validation of biomarker signatures from fluid samples.
Using liquid biopsies and NGS as tools to analyze mutation burden and copy number variation in the blood of a patient with triple negative breast cancer to better inform therapeutic targets. Kellie Howard, Kimberly Kruse, Brianna Greenwood, Elliott Swanson, Mathias Ehrich, Christopher K. Ellison, Taylor Jensen, Sharon Austin, Arturo Ramirez, Debbie Boles, John Pruett, Elizabeth Mahan, Michael Dorschner, Sibel Blau, Marcia Eisenberg, Steve Anderson, Anup Madan, Covance, Redmond, WA; Sequenom Laboratories, San Diego, CA; RareCyte, Inc., Seattle, WA; Laboratory Corporation of America® Holdings, Research Triangle Park, NC; University of Washington, Seattle, WA; Northwest Medical Specialties, Payzallup, WA; Covance, Durham, NC.

The ability to characterize molecular signature of cancer from liquid biopsies is resulting in the development of innovative health care for patients. Longitudinal changes in the mutational profiles of DNA isolated from liquid biopsies are being used to better understand and monitor the development, progression, and evolution of therapy resistance in cancer patients. To define changes in the mutational landscape and predict drug susceptibilities in Triple Negative Breast Cancer (TNBC) patients, we used whole exome analysis to profile circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA) from eight selected time points of a patient enrolled in the Intensive Trial of OMics in Cancer clinical Trial (ITOMIC-001). The patient initially received weekly cisplatin infusions followed by additional targeted therapy. Peripheral blood samples were collected at specific time points over a period of 272 days, starting several months after cytoreduction and the clinical trial. Our data indicate that the identified mutations in genomic DNA isolated from CTCs and ctDNA can be used to understand and mitigate the impact of tumor heterogeneity in addition to identifying clinically relevant mutations at these selected time points. To further increase the resolution of our analysis, we profiled ctDNA from these samples to a higher depth targeting only clinically relevant genes. These analyses increased the sensitivity of detection and identified additional targets that could have been used for therapeutic intervention. In addition to sequence variants, copy number variations (CNVs) have also been significantly associated with the development of metastasis and changes in CNVs have been used to monitor disease progression. We performed a bioinformatics analysis of genomic instability and CNVs across 32 different time points from ctDNA from the same patient throughout the treatment period. The genomic instability number (GIN) calculated for each of the 32 time points seems to mirror the overall CTC burden in the patient at each time point tested. CNV analysis is ongoing and these data sets are being further analyzed in combination with TCGA data to define possible cancer driver genes for the functional prediction of significant TNBC candidate alterations and the results of these analyses will be presented.


Critical biomarkers such as cell-free DNA (cfDNA) derived from tumors and circulating tumor cells (CTCs) can be detected and analyzed from a simple blood draw. These analytes are fragile, prone to degradation, and present in extremely low quantities. Therefore, proper preservation of these analytes is necessary to ensure accuracy of test results. Several blood collection tubes are commercially available for cfDNA applications, and selecting the ideal blood collection tube for cfDNA impacts test results, sample collection logistics and costs. However, no comprehensive and systematic evaluation of performance among these tubes is available. This study evaluates five commercial blood collection tubes: Lbgard™ Blood Tube (Biomatrica), Streck cfDNA BCT™ (Streck), PAXgene Blood cfDNA Tube (PreAnalytiX), Cell-Free DNA Collection Tube (Roche), and EDTA (BD). Healthy donor blood samples were collected in each tube type and incubated over several days at different temperatures. Total plasma DNA was subsequently isolated, and the yield, fold increase versus time 0 and quality of purified DNA were compared. cfDNA controls were also spiked into blood samples collected in each tube type and measured by droplet digital PCR to determine the mutant allele frequencies over time. Finally, the yield and quality of cfDNA isolated from stage IV colorectal cancer blood collected in Lbgard Blood Tube, Streck cfDNA BCT and EDTA were compared. Additionally, the inhibition of hemolysis and CTC stabilization were also assessed. Our results show that Lbgard Blood Tube, Streck cfDNA BCT, PAXgene cfDNA Tube and Cell-Free DNA Collection Tube are superior to EDTA tubes in maintaining cfDNA yield and quality over 7 days at ambient temperature. Lbgard Blood Tube out-performs all tested blood tubes in inhibiting genomic DNA release for the longest duration (14 days) and across the widest temperature range (4°C, 25°C and 37°C). Lbgard Blood Tube shows equivalent inhibition of genomic DNA release to Streck cfDNA BCT for clinical samples, and both tubes out-perform EDTA blood tubes. Lbgard Blood Tube also consistently shows better CTC stabilization and inhibition of hemolysis over Streck cfDNA BCT. This comprehensive and systematic study of blood collection tube performance will inform the precautionary and exhaustive assessment of blood sample stability, allowing researchers to make informed decisions based on their sample stabilization needs.

Technical limitations of capture-based, targeted deep sequencing for the detection of somatic variations in cell-free DNA. Gahee Park, Seoul National University/ Samsung Genome Institute, Seoul, Republic of Korea.

Despite widespread applications of capture-based targeted deep sequencing, its limitations on detecting circulating tumor DNA (ctDNA) have not been systematically elucidated. Here, we analyze and characterize parameters critically determining its detection limits; depth of coverage and background errors. The depth increase by using more cell-free DNA (cfDNA) was saturated at around ~300x with ~50 ng of cfDNA primarily due to a duplication artifact, which limited the detection sensitivity of the method. Next, characterizing background errors, we uncovered that a majority of errors were not derived from biological origins but technical artifacts, especially during sample preparation and library construction rather than sequencing. Thus, it is important to use an appropriate protocol for those steps minimizing background errors. Considering the depth of coverage and the background error rate, we estimated the detection depth approximately to be 0.4% variant allele frequency at 95% confidence level. Applying the method to pancreatic cancer patients, we found that the levels of ctDNAs measured by cfDNA sequencing well correlated with clinical responses to therapy and/or disease progression, but biopsy-free profiling of somatic variants was limited by the moderate sensitivity of 72% and PPV of 74% at the variant level. Our results clarify technical limitations of targeted deep sequencing and provide a guideline for applying the technique to analyze cfDNA.

Proof of principle studies for detection of circulating renal cancer cells from blood samples using diverse technologies. Yvonne Maertens, Verena Humberg, Julie Steineisel, Martin Boegemann, Andres J. Scharader, Christof Bernemann. University Hospital Muenster, Muenster, Germany.

Background: During the past years, much progress has been made in detection and analysis of circulating tumor cells (CTCs) in several tumor entities, including prostate cancer, breast cancer or lung cancer. However, little is known about circulating tumor cells in patients suffering from clear cell renal cell carcinomas. The majority of technologies detecting CTCs is based on expression of the surface antigen EpCAM. Additionally, biophysical approaches have been invented to detect CTCs based on size, invasive capacity or density. In order to be able to detect CTCs in patient samples, in vitro establishment of the most accurate isolation procedure followed by precise detection techniques has to be performed. Aim of our studies was to build a stable in vitro fundamental of isolation and subsequent detection of CTCs in ccRCC patients. Methods: We made use of 4 different technologies, all of which have been approved for detection of CTCs in distinctive tumor entities. We used EpCam based positive enrichment of CTCs, Ficolld density centrifugation followed by CD45-positive cell depletion, rosette formation followed by CD45 positive cell depletion as well as size and deformability based enrichment technologies by using the Parsortix system. Furthermore, by using 4 phenotypically distinct ccRCC cell lines, we tried to detect markers unique for tumor cells in demarcation to blood cells. Results: By performing spiking experiments of renal cancer cells, we found the highest recovery rates by using the size based Parsortix system. Interestingly, the most established technique of EpCam based isolation failed in three out of four cell lines to recover more than 40%. Expression of well-established markers in ccRCC, PAX8, was weak to absent in ccRCC patients. Method: We made use of 4 different technologies, all of which have been approved for detection of CTCs in distinctive tumor entities. We used EpCam based positive enrichment of CTCs, Ficolld density centrifugation followed by CD45-positive cell depletion, rosette formation followed by CD45 positive cell depletion as well as size and deformability based enrichment technologies by using the Parsortix system. Furthermore, by using 4 phenotypically distinct ccRCC cell lines, we tried to detect markers unique for tumor cells in demarcation to blood cells. Results: By performing spiking experiments of renal cancer cells, we found the highest recovery rates by using the size based Parsortix system. Interestingly, the most established technique of EpCam based isolation failed in three out of four cell lines to recover more than 40%. Expression of well-established markers in ccRCC, PAX8, was weak to absent in ccRCC patients.
cells. The usage of the size based Parsortix system showed the highest recovery rates and should therefore be analyzed in more detail on samples of ccRCC patients. Secondly, an exclusive marker for defining a renal CTC is still missing. Some well-established ccRCC markers, like CA-9, failed to specifically detect renal cancer cells in blood samples, as they were either present also in healthy blood samples or absent in renal cancer cell lines.

**CLINICAL RESEARCH: Outcomes in Patients and Survivors**

#2761 Adjuvant chemotherapy outcomes of node negative, T1a,Tib, T1c hormone receptor-negative HER2-positive breast cancer patients. Anu Paul, Runhua Shi, Prakash Peddi, Gary Burton. LSU Health, Shreveport, LA.

Background: Patients (pts) with early stage HER2neu-positive breast cancers have a higher risk of recurrence and death compared to hormone receptor-positive patients. NCCN guidelines recommends adjuvant chemotherapy for Stage II and above HER2-positive breast cancer patients. It is unclear what benefit patients with Stage I HER2-positive breast cancer receive from adjuvant chemotherapy. The current study evaluates the effect of adjuvant chemotherapy on the survival of early stage ER/PR negative, HER2-positive breast cancer patients registered to the National Cancer Data base (NCDB) from 2010-2012. Patients and Methods: 3416 women from the NCDB were identified with Stage I ER, PR negative, HER2-positive breast cancer diagnosed between 2010-2012. The primary measured outcome was overall survival. Pts were grouped by tumor size (T1a,Tib,T1c) and adjuvant chemotherapy or no adjuvant chemotherapy. Additionally, patients were also grouped into lumpectomy with radiation and mastectomy with or without radiation. Adjusted variables included age, race, Charlson Comorbidity Index (CCI), payer status, income, education, distance travelled, diagnosing/treating facility and treatment delay. Multivariate Cox regression was used to investigate the effect of adjuvant chemotherapy on overall survival while adjusting for other factors. Results: There were a total of 3416 patients, mean age at diagnosis of 57.9 (range 22-90). The mean age was 56.2 years for the chemotherapy group and 61.8 for the no chemotherapy group. Pathological stage distribution was T1a (24.9%), T1b (24.7%) and T1c (50.2%). Adjuvant chemotherapy was received by 95.5% of T1a, 83.5% of T1b and 95% of T1c pts. Mean patient follow up was 2.7 years (range 0.05-5 years). In univariate analysis, the hazard ratio (HR) of death for chemotherapy vs no chemotherapy in each T stage was: T1a: 1.22 (0.46-3.28), T1b: 0.221 (0.095-0.512), T1c: 0.217 (0.094-0.498). In multivariate analysis, adjusting for other factors in the model, HR of death was 0.51 (95%CI: 0.26-1.03) for chemotherapy vs no chemotherapy. The HR of death was 1.92 (95%CI: 1.11-3.32) for pts who received mastectomy without radiation compared to lumpectomy with radiation. In addition, we observed a HR of 5.16 for CCI score of 2 or above as compared to score of 0. There was no difference in outcome based on payer status or race. Conclusion: Patients with early stage T1 a-c HER2-positive breast cancer had a marginal improvement in overall survival with the addition of chemotherapy. Patients who underwent lumpectomy with radiation were also found to have better overall survival compared to mastectomy without radiation. However, the relatively short follow up and the lack of cancer recurrence information preclude making definitive conclusions relative to adjuvant therapy for pts with these tumors. Longer follow-up and prospective controlled trials will be needed to quantify these potential benefits.

#2762 The comparison of allogeneic stem cell transplantation outcomes between haploidentical donor and international donor: A retrospective multi-institutional study in Korea. Hyunkyung Park, Yoo Jin Lee, Sang-Jin Shin, Jayoun Lee, Anu Paul, Sabine Rohrmann, Volker Arndt. Epidemiology, Biostatistics and Prevention Institute, University of Zurich, Zurich, Switzerland; National Institute for Cancer Epidemiology and Registration, Zurich, Switzerland.

Introduction: Due to improving prognosis for prostate cancer survivors and increased awareness of late- or long-term treatment effects, monitoring long-term well-being among prostate cancer survivors has gained increasing attention. In this systematic review, we identified and synthesized studies comparing health-related quality of life (HRQoL) among long-term prostate cancer (PC) survivors (i.e. >5 years past diagnosis) by primary treatment. Methods: In order to summarize current literature research results, we searched multiple databases, including Pubmed, Medline, Embase, PsycINFO, Cinahl, Web of Science and Cochrane Central Register of Controlled Trials to identify all relevant articles. Studies had to assess at least overall quality of life (QoL) of prostate cancer patients. A total of 20 studies were included in the systematic review. Results: A total of 24,107 patients were identified for inclusion across studies. Conclusion: This review was useful for cancer centers to identify gaps in their current care and to inform future research and clinical practice.

#2763 Health-related quality of life among long-term prostate cancer survivors by primary treatment: A systematic review. Salome Adam, Anita Feller, Sabine Rohrmann, Volker Arndt. Epidemiology, Biostatistics and Prevention Institute, University of Zurich, Zurich, Switzerland; National Institute for Cancer Epidemiology and Registration, Zurich, Switzerland.

Introduction: Due to improving prognosis for prostate cancer survivors and increased awareness of late- or long-term treatment effects, monitoring long-term well-being among prostate cancer survivors has gained increasing attention. In this systematic review, we identified and synthesized studies comparing health-related quality of life (HRQoL) among long-term prostate cancer (PC) survivors (i.e. >5 years past diagnosis) by primary treatment. Methods: In order to summarize current literature research results, we searched multiple databases, including Pubmed, Medline, Embase, PsycINFO, Cinahl, Web of Science and Cochrane Central Register of Controlled Trials to identify all relevant articles. Studies had to assess at least overall quality of life (QoL) of prostate cancer patients. A total of 20 studies were included in the systematic review. Results: A total of 24,107 patients were identified across studies. Conclusion: This review was useful for cancer centers to identify gaps in their current care and to inform future research and clinical practice.

#2764 Creating a review process of a digital photo database collected on NRG NSABP B39/RTG 0413 phase III clinical trial for evaluation of cosmetic results from breast conserving therapy (BCT). Ashley Sekhon, Ruiqi Zhao, Yan Wang, Debora Grant, Kathryn A. Winter, Jennifer Moughan, Douglas W. Arthur, Stephen J. Chmura, Atif J. Khan, Simona F. Shaitelman, Alex M. Martinez, Frank A. Vicini, Julia R. White. Ohio State University, Columbus, OH; NRG Oncology, Philadelphia, PA; Virginia Commonwealth University, Richmond, VA; University of Chicago, Chicago, IL; Rutgers Cancer Institute of New Jersey, New Brunswick, NJ; MD Anderson Cancer Center, Houston, TX.

Introduction: The success rate of hematopoietic stem cell transplantation (HSCT) from HLA-mismatched donor has been increased according to the development in management of complications including graft versus host disease (GVHD) and infections. Especially, haploidentical HSCT provides an opportunity for all patients who do not have HLA-matched sibling donor. Methods: In this study, we compared HSCT outcomes between haploidentical familiar donor and international donor (donors from Japan, China, Germany, Unites States of America and Taiwan) in acute leukemia patients. We reviewed the overall survival (OS), relapse free survival (RFS) and complications. Results: Total 142 acute leukemia patients performed HSCT from 2000 to 2016; 98 patients underwent haploidentical donor transplantation and 44 patients underwent international donor transplantation. Major variables such as age, sex, disease status or race. Conclusion: These data suggest that HSCT from haploidentical donor shows similar outcomes including survival outcomes and complications with international donor transplantation. Therefore, haploidentical donor transplantation can be good choice for acute leukemia patients who have no HLA-matched sibling donor.
CLINICAL RESEARCH: Outcomes in Patients and Survivors

Background: Esophageal cancer is the 6th leading cause of cancer death globally, with geographical high-risk areas in Asia, the Middle East, and eastern and southern Africa. Esophageal squamous cell carcinoma (ESCC) is the more common variant in Africa. In Kenya, its incidence is 2nd in men after prostate cancer and 3rd in women after breast and cervix-uteri cancers. Late presentation is a common occurrence in developing countries and is multifactorial due to challenges in access to health care, low socioeconomic status and delayed or missed diagnosis. A large percentage of these tumors are thus unresectable and are only eligible for palliative care via stenting. Our hospital is a 300-bed referral center in southwestern Kenya, which is a hotspot for ESCC, and we see over 400 cases of ESCC annually. Methods: We have developed a technique for placement of esophageal self-expanding metallic stents (SEMS) without fluoroscopy that is safe and easily reproducible. This is an outpatient procedure, with the majority done under conscious sedation, and routine follow-up is not necessary. The tumor margins are noted at time of video endoscopy, a guidewire is placed, and dilation done with Savary dilators as required. The SEMS are then loaded on the stent delivery device and deployed into the proper position based on measurements, and placement is subsequently confirmed via endoscopic visualization. Results: A total of 3000 SEMS have been placed to date at our hospital, without using fluoroscopy. The male/female ratio has been 1.5:1, and the average age has been 60.4 years. The distribution of tumor locations was 67% in the middle and distal third. The most common complications were tumor overgrowth with obstruction and stent migration. Procedure related mortality was 0.3%. Post-procedure improvement in dysphagia score was seen in over 80%, and patient satisfaction was high. Initial data on post stent survival time around 8 months 250 days. Conclusions: Placement of SEMS for ESCC, without fluoroscopy, is a safe and reproducible procedure which has a low rate of adverse events. This procedure results in effective palliation of a difficult disease and can easily be done in resource-limited settings which have endoscopy capabilities. Current efforts are ongoing to increase opportunities for training endoscopists in this procedure and for provision of affordable stents in Africa.


Introduction: Breast cancer presents a challenge to the totality of a woman’s life. Correlates of life satisfaction among married women with breast cancer have not been very well explored. Purpose: This study seeks to determine the relationship that exists between life satisfaction with depression, cognitive emotional regulation, sexuality, self-concept, dejection, interpersonal conflict and social support among married women with breast cancer in Southwest Nigeria. It also seeks to study some of these variables predict life satisfaction in the respondents. Method: The variables in the study were assessed using modified standardized instruments which were revalidated for cultural suitability after ethical approvals were obtained in each of the 5 centres involved in the study. The instruments were given to breast cancer patients after informed consent to participate in the study have been obtained. Result: The larger proportion (148 or 40.5%) of the participants were in the age range of 46-55 years and 112 (30.7%) had stage III disease. Analysis of data showed that significant linear relationship exists between life satisfaction of married women with breast cancer and depression (r = 0.200**; p < 0.001), self-concept (r = 0.306***; p < 0.001), cognitive emotional regulation (r = 0.398***; p < 0.001), dejection (r = 0.226***; p < 0.001), interpersonal conflict and social support (r = 0.316**; p < 0.01), anxiety (r = 0.315**; p < 0.01) and social support (r = 0.534; p < 0.001). There were also significant correlations among the independent variables. Conclusion: Psychosocial factors are potent predictors of life satisfaction among married women with breast cancer. Based on the findings, it was recommended that psychosocial intervention based on the factors identified as causative agents of life satisfaction should be developed and implemented for married individuals.


Introduction: After local control and overall survival, acceptable cosmesis is the next most important goal of BCT. Digital photos (DP) are commonly used for assessing cosmesis on clinical trials. On NRG NSABP B39/RT0G 0413, DP at baseline, 1, and 3 years post-radiation were submitted for review by a panel of breast cancer specialists. Given no current standards for central DP review, it is difficult to develop a standard for subjective DP evaluation. The initial standardization of the photo database and reliability of the panel review is described. Methods: De-identified DP submitted and stored at NRG SDMC were downloaded to a secure FTP site. Cases with only 1 time point, no baseline, or photos from different patients were excluded. A minimum photo quality was set, including pre-specified criteria for contrast/ brightness, background, and cropping to eliminate unnecessary DP below and above the umbilicus. DP were edited with MATLAB and saved in a Reformatted Digital Photo Database (RDPD). A training set was created defining 4 global cosmetic scores, and each individually assessed criteria of cosmesis and the DP were reviewed. A 14 case calibration set followed the training set. The process for panel review was adapted from the methods reported in the START and RAPID trials. The review panel consisted of 6 breast-specific radiation oncologists, who reviewed in groups of 3. For each session, the training set was reviewed and calibration set scored. A separate 30 case test set randomly selected from the RDPD was reviewed by 2 groups of 3. The calibration and test sets were evaluated for agreement of categorized scores (excellent/good vs. fair/poor). Results: There were 2064 DP from 892 patients on NRG NSABP B39/RT0G 0413 documenting the anterior view of both breasts. After exclusions, 1712 reformatted DP were saved in the RDPD. Five 1-3 hour review sessions resulted in scoring of 7777 DP (~155 per session). Only 2/7777 (0.1%) DP were deemed poor quality and not able to be scored. There was full agreement in categorized scores for 12/14 cases (86%; kappa = 0.82) across the 5 calibration sets; and 27/30 (90%; kappa = 0.71) for the 2 test set reviews. Conclusions: Cosmesis DP prospectively collected on a large phase III clinical trial can be digitally formatted in a systematic fashion for consistent photo quality, allowing for cosmesis consensus panel review without photo quality bias. High agreement in categorized scores allowed reviews to be completed by multiple 3-person review groups. Future work will focus on creating an updated and systematic analysis tool of these photos for objective evaluation of cosmesis using the same scoring guidelines that can then be compared to physician reported outcomes. Grant Support: FUJIFILM Medical Systems/RSPA Research Resident, NCI U10CA180868, U10CA180822.

Table 1 Summary of CSF standing order implementation in 50 surveyed NCORP clinics

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<td>4 (8%)</td>
<td>19 (38%)</td>
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CONCLUSIONS: We observed wide variation in the current application of standing orders. Intermediate risk regimens had the most inconsistent practices, with a near equal number of clinics choosing either actively including or excluding CSF order sets. The majority of clinics using standing orders included CSF for dose dense and high risk regimens but only 19% (38%) actively excluded CSF for low risk regimens. These results support the need for more evidence to inform clearer guidelines on CSF use in intermediate risk regimens and studies that evaluate the effects of existing CSF standing orders on guideline adherence and patient outcomes. Funding: PCORI (PCS-1402-09988) and NCORP grant (SUG1C0189974)

#2768 Treosulfan induces differential gonadal toxicity profile compared with Busulfan. Mattan Levi,1 Salomon Stemmer,1 Jerry Stein,2 Ruth Shalgi,1 Irit Ben-Aharon1.1 Institute of Oncology, Davidoff Center, Rabin Medical Center, Beilinson Campus, Petah-Tiqva, Israel;2 Schneider Children Medical Center, Petah-Tiqva, Israel.

Background: Treosulfan (1-treitol-1,4-bis-methanesulfonate), an alkylating agent with myeloablative and immunosuppressive traits, has been increasingly incorporated as a main conditioning protocol for hematopoietic stem cell transplantation (HSCT) in pediatric malignant and non-malignant diseases. Treosulfan has been shown to present lower toxicity profile compared with conventional myeloablative regimens such as busulfan. Yet, while busulfan is considered highly gonadotoxic, the gonadal toxicity profile of treosulfan remains to be elucidated. Our aim was to study the short and long term gonadal effect of treosulfan in comparison to busulfan. Methods: Maturity 2 months old male and 3 months old female mice (“pubertal cohort”) were injected with treosulfan 2000 mg/kg or busulfan 40 mg/kg and were sacrificed one week, one month or six months post drug administration. Immature male and female mice (“prepubertal cohort”) were injected with treosulfan 750 mg/kg or busulfan 15 mg/kg and were sacrificed one week or three months post drug administration. Testicular function was assessed by measurements of sperm count and motility, testes and epididymides weight, serum anti-Mullerian Hormone (AMH) and testicular ID4 or GFRA1 mRNA (markers for stem spermatogonia). Immunohistochemistry was performed to evaluate testicular proliferation (Ki-67), apoptosis (TUNEL) and meiotic-active spermatocytes (DAZL-PGNA). Ovarian function was assessed by measurements of ovary weight, serum AMH, follicles count and ovarian SOHLH2, NOBOX of FIGLA mRNA (markers for primordial follicles). Immunohistochemistry was performed to evaluate ovarian proliferation (Ki-67), apoptosis (TUNEL) and growing follicles (AMH-PGNA). Results: Treosulfan testicular toxicity was milder compared to busulfan toxicity, in both mature and immature male mice, while stem spermatogonia were spared. However, ovarian toxicity of both treosulfan and busulfan was severe and permanent in both mature and immature female mice; possibly by a short term irreversible reduction of reserve primordial follicles in the ovaries. Conclusion: Our results indicate that treosulfan exerts a differential gonadal toxicity profile compared with busulfan, manifested by mild testicular toxicity and severe ovarian toxicity.

CLINICAL RESEARCH: Predictive Biomarkers 2

#2769 Project Survival: Prospective clinical study utilizing multitemics and artificial intelligence to discover novel molecular markers for detection, stratification and response in pancreatic cancers. Rangaprasad Sarangarajan,1,2 John Crowley,3 Amy Stoll-D’Artic,2 Valerie Bussberg,3 Cindy Nguyen,3 Leonardo O. Rodrigues,1 Emily Chen,3 Eric Michael Grund,3 Vivek K. Vishnumadhav,3 Michael Kiebich,1 Viatcheslav R. Akmaev,1 Manuel Hidalgo,4 Niven R. Narain,4 A. James Moser4,1 BERG, LLC, Framingham, MA, 2Cancer Research And Biostatistics, Seattle, WA; 3BERG Health, LLC, Framingham, MA, 4Beth Israel Deaconess Medical Center, Boston, MA.

Pancreatic adenocarcinoma is the third leading cause of cancer death and has an extremely poor response to first line therapies. Project Survival is a prospective study designed to discover biomarkers for patient diagnosis, stratification, and prognosis for pancreatic cancer. The multisite study is in year 2 of enrolling subjects within 6 categories: healthy volunteers with a relative with pancreatic cancer (N=50), pancreatic cancer (N=50), pancreatic cystic neoplasm (N=50), suspicious pancreatic masses with pathology other than pancreatic cancer (N=50), early stage (N=200) and metastatic pancreatic cancer (N=200). The study analyzes matched subject sera, plasma, buffy coat, saliva, urine, and tumor/adjuvant normal tissues and integrates them with full clinical annotation. Multiple time points per subject per year are taken longitudinally over the course of the six year timeline enabling dynamic modeling. Samples are analyzed by Mass Spectrometry for the proteome, signal-averaged electrocardiogram for the metabolome, and digital pathology and high-dimensional image analysis for the radiogenome. The integrated Artificial Intelligence Clinical Information System (AIcis™), utilizing Artificial Intelligence to integrate multitemics profiles with medical annotation and clinical endpoints. Using the power of the Bayesian Network learner, AIcis™ (BERG Artificial Intelligence Clinical Information System), multitemics profiles were aligned to the longitudinal clinical information and subjected to the AI-algorithms that inferred probabilistic cause-effect relationships among molecular and clinical variables inferring markers of pancreatic cancer status and defining the interconnectivity of molecular features with clinical phenotype. Network features linking clinical endpoints and key network pressure points will be identified as molecular drivers. The drivers of clinical endpoints will be analyzed to rank potential biomarkers.

#2770 Bcl-2 overexpression is a predictive biomarker for the combination of ABT-199 with chemotherapy in diffuse large B-cell lymphoma cell lines. Jan McConnell, Elyse Anderson, Victor Yazbeck. VCU Massey Cancer Center, Richmond, VA.

Diffuse Large B-Cell Lymphoma (DLBCL) is the most common subtype of Non-Hodgkin’s Lymphoma. The anti-apoptotic Bcl-2 protein is expressed in certain DLBCL subtypes such as “Double-Hit” where it mediates resistance to chemotherapy. ABT-199 is a BH3 mimetic that specifically targets Bcl-2, Bcl-xL and Bcl-w, and has shown excellent clinical activity in several hematological malignancies. The anti-apoptotic protein Mcl-1 mediates resistance to ABT-199, and can be inhibited with chemotherapy. However, despite variability in the Bcl-2 expression levels, current clinical trials involving combinations of ABT-199 and chemotherapy for the treatment of patients with DLBCL use a one-size-fits-all approach regardless of Bcl-2 expression levels. We hypothesized that high Bcl-2 expression levels correlates with sensitivity to the combination of ABT-199 and chemotherapy in DLBCL. We examined the activity of ABT-199 in four DLBCL cell lines with varying Bcl-2 levels (CARNAVAL, OCI-Iy18, SU-DHL-4 and SU-DHL-8) as determined by cell via-bility (Cell Titer Glo Assay) and apoptosis (Annexin-V/PI staining and FACs) analyses. CARNAVAL and SU-DHL-8 have the highest and lowest Bcl-2 expression levels, respectively, as revealed by immunoblotting. Of the four cell lines, CARNAVAL displayed the highest sensitivity (IC50 = ~7 nM) while SU-DHL-8 was resistant (IC50 = >10 μM) with the other 2 cell lines exhibiting intermediate sensitivity (45.9 nM for OCI-Iy18 and 2.6 μM for SU-DHL-4). We then selected CARNAVAL and SU-DHL-8 in order to further examine the effect of the combination of ABT-199 with classic chemotherapy agents (etoposide and doxorubicin) used in DLBCL treatment. In combination, the cells, the combination of either chemotherapy with ABT-199 led to a significant decrease in cell viability at 48 hrs compared to each single agent alone. This was associated with apoptosis induction as measured by Annexin-V/PI cleavage, an increase in γ-HAX (associated with DNA damage), and decrease in p-H2AX. Unlike CARNAVAL, SU-DHL-8 did not enhance the activity of chemotherapy in SU-DHL-8 cells that lack Bcl-2 expression. These preclinical results support the use of Bcl-2 overexpression as a predictive biomarker for selecting patients that are likely to benefit from the combination of ABT-199 with chemotherapy in DLBCL. Current efforts are investigating the mechanism of resistance to the combination.


Background: The Cancer Genome Atlas (TCGA) has identified 16% of colorectal cancers (CRC) to have detectable mutations that repair spontaneous DNA damage, and consequently have high tumor mutation burden (TMB). Although, there is accumulating evidence for activity of immunotherapy on tumors harboring high-TMB, its impact on response to chemotherapy is unknown. Methods: In this retrospective cohort study, we analyzed progression free survival (PFS) of 74 patients with metastatic CRC (61 colon & 13 rectal cancer) treated at tertiary care oncology clinics and undergone next-generation sequencing (NGS) of their tumor sample using FoundationOne® (Foundation Medicine Inc., Cambridge, MA). Most recent available specimen was analyzed at the time of diagnosis of metastatic disease. TMB was calculated by counting all synonymous and nonsynonymous
variants as well as indels across a 1.25 megabase coding region spanning 315 genes. Low TMB (TMB-L) and Intermediate/High TMB (TMB-H) were defined as ≤ 5 mutations per base (MB) or ≥ 6 MB respectively. Demographic and clinical information (including imaging results, chemotherapy treatment) were obtained by chart review. Treatment was captured as ‘oxaliplatin-based chemotherapy’, ‘irinotecan-based’, ‘irinotecan versus oxaliplatin-based’ or ‘irinotecan-based’ if the regimen contained irinotecan (i.e., FOLFOX, XELOX) or ‘irinotecan-based’ if the regimen contained irinotecan. Subsequent modifications of dose or omission of the drug due to toxicity were not captured. Continuous variables were reported as medians and inter-quartile ranges and compared between groups via the Wilcoxon rank-sum test. Categorical variables were reported as percentages and compared between groups via Fisher’s exact test. Survival estimates were compared between groups via the log-rank test. Results: There was no statistically different PFS in TMB-L (n = 39) compared to TMB-H (n = 26). (10.0 vs. 5.9 months, P = 0.18). In the TMB-L cohort, irinotecan-based chemotherapy (n = 25) treated patients had improved PFS compared to oxaliplatin-based chemotherapy (n = 10) treated CRC patients (11.9 vs. 6.5 months, P = < 0.001). No difference in PFS was observed between the two treatment cohorts in TMB-H cohort. In stage 2 and 3 colon cancer patients, there was no difference in time to recurrence in the TMB-L and TMB-H cohorts, when patients were treated with oxaliplatin-based therapy in peri-operative setting (detailed statistics including survival curves will accompany final presentation). Conclusion: TMB status may be a predictive marker of patients who benefit from irinotecan chemotherapy and was followed by erlotinib for 2nd line treatment from September 2011 to September 2014. The primary outcome was to measure the rates of c-MET expression/amplification and EGFR mutation in tumor tissue. MET expression was evaluated by immunohistochemistry (IHC) and gene copy number was assessed by silver in situ hybridization (SISH). EGFR mutations were analyzed by Cobas184 version 1. Results: A total of 196 patients were enrolled and included alpha (90%), beta (17%) and gamma (3%) subtypes. The median follow-up time was 6.2 months (range 0.6-22.5). The overall survival (OS) was not statistically different (10.0 vs. 5.9 months, P = 0.18). In the TMB-L cohort, c-MET positivity (IHC- or SISH-positive) was observed in 1.5% and 8.7%, but there was no specific clinical characteristics. The detection rate of EGFR mutation was 10.2% (19 deletion; 11, 1858R-7, exon 10 insertion; 2 cases). The response rate of erlotinib was significantly higher in EGFR sensitive mutant group (55% vs. 4%, P = 0.001) and the median progression-free survival (PFS) was significantly longer than wild type (173 vs 58 days, P < 0.001). c-MET positivity (IHC- or SISH-positive) did not show significant correlation with response or PFS. Patients with SISH- and PFS-negative c-MET tended to have shorter overall survival (OS) than patients with SISH- and PFS-positive (127 vs 303 days, P = 0.051). Conclusion: In this observational study with 2nd line erlotinib treatment, we confirmed EGFR mutation is an important predictive marker, and EGFR-TKI use in EGFR wild-type patients should be considered carefully and may be harmful. c-MET overexpression was related to some clinical characteristics such as female and non-squamous histology. Although c-MET positivity was not associated with response or PFS, c-MET SISH may be predictive marker for OS.

#2772 BCL-2 family expression profiling may identify distinct molecular subtypes of multiple myeloma with increased susceptibility to single agent treatment

Venetoclax (VEN) is being evaluated in relapsed/refractory multiple myeloma (R/R MM) patients as a single agent (NCT01794520). Improved objective response rates were observed in t(11;14) patients (40% in t(11;14) vs 6% in t(11;14) - ), which were shown to associate with a favorable BCL-2 family expression profile (high BCL2:BCL2L1 (BCL-XL)). Forty percent of t(11;14) + population exhibited VEN favorable biomarker profile with an 88% ORR compared to 20% in t(11;14) + patients with unfavorable profile. Thus, a favorable BCL-2 family expression profile may identify certain MM subgroups with increased sensitivity to the anti-tumor activity of VEN as a single agent. To better understand and identify the patient populations that may benefit from VEN, we retrospectively analyzed the prevalence of a favorable BCL-2 family expression profile in t(11;14) and other MM molecular subtypes in two published cohorts (GSE4581 and GSE9782). Our results showed that BCL2 expression varied significantly across molecular and cytogenetic subgroups. The t(11;14) subgroup expressed high BCL2 and the lowest BCL2L1 and MCL1 in both newly diagnosed (NDMM) and R/R MM patients. Correspondingly, the t(11;14) MM was enriched for the highest ratios of BCL2/MCL1 and BCL2:BCL2L1, further supporting the single agent VEN activity observed in this patient population. Based on prevalence study in cohort GSE9782, we observed 40% of t(11;14) + R/R MM patients exhibited a favorable BCL-2 family expression profile, using clinical defined cutoffs, thus highly consistent with the VEN single agent trial. Furthermore, this favorable profile existed in other molecular subtypes, especially the ones that harbor abnormal MAF (23%) and D3 (37.5%) translocations, as well as dysregulated expression of cyclinD1 (21.2%) or cyclinD1 (26.7%), c-MET in MYC (23%) or c-MET in NOTCH1 (19%). For the MYC (23%), c-MET positivity (IHC) and SISH-positive did not show significant correlation with response or PFS. Patients with SISH- and PFS-negative c-MET tended to have shorter overall survival (OS) than patients with SISH- and PFS-positive (127 vs 303 days, P = 0.051). Conclusion: In this observational study with BCL-2 family expression profiling, we identified a favorable BCL-2 family expression profile in t(11;14) MM subgroups with a favorable BCL-2 family expression profile that may also potentially benefit from VEN monotherapy.
Clinical Research: Predictive Biomarkers 2

E7727, a novel CDA inhibitor, is orally bioavailable with an excellent safety profile in preclinical models. ASTX727 is a combination of E7727 and DAC, and has completed phase 1 dose escalation of 44 MDS or CMMl patients in which pharmacokinetic (PK) and pharmacodynamic (PD) parameters of DAC were replicated with the oral agent. During the dose escalation portion of the Phase 1 trial, ASTX727 bone marrow aspiration was obtained from 28 patients (13 clinical responders), and genomic DNA from marrow cells was isolated for next-generation sequencing (NGS) with a panel of 37 myeloid neoplasia-associated genes. Consistent with previous reports, DNTM3A mutations were associated with poor response to ASTX727 (all 4 carriers failed therapy, \(P = 0.028\)). Conversely, of the 10 patients with TET2 mutations, a majority showed clinical improvement (6/10: 60%). ASXL1 mutations were observed in 3 patients (13.5%); however, no complex combinatorial effect, as all three ASXL1-mutated patients with BCR2 mutations, regardless of International Prognostic Scoring System (IPSS) risk category, were non-responders while all 3 patients ASXL1 who also carried a TET2 mutation responded. ASTX727 is an oral DNTM3i that revealed responses in this dose escalation Phase I study exhibiting a similar pharmacokinetic profile and achieving AUC range of IV DAC. As previously observed with DAC, ASTX727 may lead to improved responses in patients with specific gene mutations. This work shows preliminary effects on mutational burden and allele frequency concurrent with activity of ASTX727. Further analyses of patients treated at the RP2D in the Phase 2 trial are ongoing.

Tmprss2-Erg Prognostic Value for Taxanes Resistance According to Prior Second-line Hormonal Manipulations in Metastatic Castration-resistant Prostate Cancer

Haley E. Ramsey,1 Shilin Zhao,1 Saisai Ji,2 David Proctor,2 Laura Kim,3 Chris Verstraeten,4 Michael Neldner,4 Michael R. Savona1.

Background: TMPRSS2-ERG is a genetic alteration specific of prostate cancer, present in primary tumors and maintained under castration resistant prostate cancer (CRPC) progression. It results in androgen-driven overexpression of ERG, which is involved in resistance to taxanes in preclinical models. In prior work, we found that TMPRSS2-ERG expression in blood correlated with docetaxel resistance in metastatic CRPC. Here, we investigated if TMPRSS2-ERG expression in primary tumors predicts taxanes resistance in CRPC and the potential impact of prior second-line hormonal manipulations with abiraterone (A) or enzalutamide (E). Methods: Patients with metastatic CRPC treated with taxanes were included. Formalin-fixed paraffin-embedded (FFPE) tumors and peripheral blood mononuclear cells (PBMCs) fraction were tested for TM-PRSS2-ERG by RT-qPCR. FFPE from hormone-sensitive disease (primary diagnosis/androgen-dependent prostate cancer) and the potential impact of prior second-line hormonal manipulations with abiraterone (A) or enzalutamide (E) were retrospectively obtained. PBMCs were prospectively collected prior taxane initiation. TMPRSS2-ERG expression was tested by RT-qPCR. TMPRSS2-ERG detection was correlated with taxane response or clinical outcome. Results: A total of 84 tumor samples from 74 patients were included: 65 (87.3%) treated with docetaxel, 19 (25.7%) with cabazitaxel and 10 (13.5%) with enzalutamide. Overall, no correlation between tumor TMPRSS2-ERG expression and taxanes resistance or clinical outcome was observed. In 42 (50%) samples matched tumor and PBMCs samples treated with taxanes were included at this time of analysis: 23 (54.7%) had detectable TMPRSS2-ERG on tissue and 11 (26.2%) on PBMCs fraction. In 27 patients, taxanes were administered as a first-line therapy and in 15 after A or E progression. TMPRSS2-ERG was detected in PBMC from 8 (29.6%) and 3 (20%) patients without or with prior A or E. In patients without prior A or E, TMPRSS2-ERG expression in primary tumors predicted a lower progression-free survival (PFS) with A (\(P = 0.01\)) and E (\(P = 0.05\)). Similarly, when analyzing PBMCs samples from patients without prior A or E, PSA response was observed in 0% of TMPRSS2-ERG+ vs 55.6% of TMPRSS2-ERG-patients (\(P = 0.009\)). Median PSA-PFS, Rx-PFS were 2.9 vs 8.1 months (\(P < 0.01\)) and 3.2 vs 7.3 months (\(P < 0.05\)), for TMPRSS2-ERG+ and TMPRSS2-ERG- patients. However, no significant differences were found either in PBMCs or FFPE samples in patients that received A or E prior to taxanes, regarding to PSA response, PSA-PFS, Rx-PFS and OS parameters between TMPRSS2-ERG+ and TMPRSS2-ERG- samples. Conclusions: The role of TMPRSS2-ERG in taxane resistance may be different according to prior exposure to second-line hormone-therapy in CRPC. Prior androgen receptor inhibi-
bition may result in TMPRSS2-ERG downregulation and/or activation of alter-
native mechanisms of resistance. Further data regarding and thereby this hypothesis will be pre-

#2778 Combination of DNA mismatch repair status and MACCI expres-
sion in patients with stage II colon cancer: The BIOGRID studies. Ulrich, Peter Rohr,1 Pia Herrmann,1 Katharina Ilm,2 Hai Zhang, Sabine Lohmann,3 Astrid Reiser,4 Andrea Muranyi,5 Janine Smith,5 Susen Burock,5 Marc Oster-
land,2 Katherine Leith,3 Shalini Singh,3 Patrick Brunhoeber,3 Rebecca Bow-
master,2 Jeanne Tie,5 Michael Christie,5 Hui-Li Wong,2 Paul Waring,5 Kandavel Shanumugam,5 Peter Gibbs,5 Ulrike S. Stein,5 Direct Medical and Scientific Affairs, Roche, Basel, Switzerland;2 Charité Universitätsmedizin Berlin and Max Delbrück Ctr. for Molecular Medicine, Berlin, Germany;3 Divisional Biomarker Group, Roche, Basel, Switzerland;4 Roche Diagnostics GmbH, Penzberg, Ger-
many;5 Ventana Medical Systems, Inc., Tuscon, AZ;6 Charité Comprehensive Cancer Center, Berlin, Germany;7 University of Melbourne, Melbourne, Australia

We have previously identified the gene Metastasis-Associated in Colon Cancer 1 (MACCI). MACCI acts a prognostic biomarker for tumor progres-
sion, metastasis and patient survival for a broad variety of solid cancer types. Here we assessed if the MACCI gene could separate stage II colon cancer patients with proficient mismatch repair (pMMR) into high- and low-risk groups who might benefit from or be spared adjuvant chemotherapy based on their prognosis. In the Charité 1 discovery cohort (n = 61), MACCI expres-
sion and MSI status were assessed by qRT-PCR in cryo-preserved tumors from CRC patients. MSS/MSI-low/MACCI-low tumors showed better sur-

#2779 Assessing germline and somatic alterations in DNA repair pathway in cancer. Gargi D. Basu,1 Tracey White,1 Janine LoBello,1 Ahmet Kurdoglu,1 Jeffrey Trent,1 Ken Peng,2 Matthew Halbert,1 Thomas Royce1,1 Ashion, Phoenix, AZ;2 Translational Genomics Research Institute, Phoenix, AZ

Introduction: DNA repair genes are involved with repair of single strand breaks as well as double strand breaks which ultimately maintain the genome integrity. The genes involved with DNA repair are frequently deregulated in cancer and these defects can be exploited therapeutically. Our study aims to explore somatic and germline changes in DNA repair genes across multiple cancer types. Methods: Using TCGA colon data we assessed tumor samples from 24 cancer types with ages ranging 19-82 years. We performed targeted exome sequencing of 562 genes in tumor and paired normal DNA which included 112 genes associated with cancer predisposition syndromes. In addition to identifying genomic changes in 33 key DNA repair genes (ATM, ATR, ARID1A, BAP1, BARD1, BRCA1, BRCA2, BRIP1, CDK12, CHEK1, CHEK2, ERCC2, ERCC3, ERCC4, FANCA gene family, MMR genes, NBS1, PALB2, PTEN, RAD50, RAD51, TP53 and POLE), we also characterized germline mutations using public databases (ClinVar, En-
semb). Results: Profiled specimens included breast cancer (17.5%), ovarian cancer (15%), CRC (11%), NSCLC (10%), uterine (6%), sarcoma (6%) and other cancers. The most commonly deleted or mutated (somatic) DNA repair genes included TP53 (55%), PTEN (16%), ARID1A (13%), FANCA (11%), ERCC2 (10%), and ATM, BAP1, CDK12, CHEK2 at 6% each and BRCA1/2 combined at (9%). Deletious mutations in MMR genes were noted in 3.5% (8/226) which included 1 case of germline PM2-5461 muta-
tion. Interestingly, a 55 year old male African American CRC patient harbored germline XPC-P334H mutation along with somatic MLH1-Y546Fs. No mu-
tations were found in FANCC, FANCF, FANCQ and RAD51. Germline analysis revealed a total of 47 pathogenic and presumed pathogenic variants out of which 61.7%(29/47) were DNA repair genes. A total of 13% (30/226) of cases harbored germline variants in DNA repair genes. Germline events included 9 cases with BRCA1 and BRCA2 clinically significant mutations. Presence of loss of heterozygosity (LOH) at the BRCA locus in 5/9 cases and somatic mutations in 3/9 cases were noted in germline BRCA mutated sam-
ples. Germline and somatic BRCA1 and BRCA2 alterations were present in 10% (22/226) of total cohort and as expected, the majority were present in breast and epithelial ovarian cancer. Genetic deficiencies in DNA repair pathway genes are being explored therapeutically with PARP inhibitors as well as DNA damaging chemotherapeutics. Conclusions: Exome sequencing identified subsets of patients with loss of function events in DNA repair genes that may be associated with benefit from PARP inhibitors and platinum agents. Somatic and germline biomarker testing revealed occurrence of BRCA1/2 as well as other DNA repair gene alterations across multiple can-
cers. It is imperative to explore the DNA repair pathway beyond BRCA1 and BRCA2 in patient selection for PARP inhibitors and DNA damaging agents and further investigation of this pathway is warranted in ongoing clinical trials.

#2780 High nuclear FTH1 protein expression predicts early disease recur-
rence for BRCA1/2 carrier mutations. Antoinette Hollestelle, Miekie Timmer-
mans, Renée Broeren-Foekens, Anita Trapman - Jansen, Carolien H. van Deurzen, Arzu Umar, John W. Martens, Marta Hoeing, Erasmus Medical Ctr., Rotterdam, Neth-
erealize this, we aimed to confirm the diagnostic role in a prospective study with breast cancer by measuring plasma exosomal del-1 before and after surgery. Patients and methods: To identify the optimal time of sampling after surgery, serial blood at day 1, 3, 5, and 7 after surgery was collected from 22 patients with early breast
cancers. It is imperative to explore the DNA repair pathway beyond BRCA1 and BRCA2 in patient selection for PARP inhibitors and DNA damaging agents and further investigation of this pathway is warranted in ongoing clinical trials.

#2781 Exosomal Del-1 as a potent diagnostic marker for breast cancer: A prospective cohort study. Soo Jung Lee,1 Jee Hyun Lee,1 Yee Soo Chae,1 Jin Hyang Jung,1 Ho Yong park,1 Moon-Chang Baek,2 Pyong-Gon Moon2,1 Kyungpook National University Medical Center, Daegu, Republic of Korea; 2Cell and Matrix Research Institute, School of Medicine, Kyungpook National University, Daegu, Republic of Korea.

Background: We previously demonstrated a diagnostic role of exosomal del-1 with two separated groups of breast cancer patients. In the current study, therefore, we aimed to confirm the diagnostic role in a prospective study with breast cancer by measuring plasma exosomal del-1 before and after surgery. Patients and methods: To identify the optimal time of sampling after surgery, serial blood at day 1, 3, 5, and 7 after surgery was collected from 22 patients with early breast
cancers. It is imperative to explore the DNA repair pathway beyond BRCA1 and BRCA2 in patient selection for PARP inhibitors and DNA damaging agents and further investigation of this pathway is warranted in ongoing clinical trials.

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cancer. Thereafter, one hundred fifteen patients with breast cancer who under-went curative surgery were enrolled in the prospective cohort study to compare difference in plasma exosomal del-1 measured by ELISA at the time of diagnosis and post-surgery. Results: Among all 22 patients for optimal sampling time after surgery, exosomal del-1 was higher than 0.5 at the time of diagnosis and then normalized at POD1. Among 115 patients for the confirmatory set, 109 (94.8%) patients showed a normalization of del-1 lower than 0.5 after surgery and 10 patients showed del-1 > 0.4. Median f/u duration of 22 months, 9 patients experienced relapse (4 locoregional and 5 distant), where 3 out of 6 in high group (≥0.5), and 2 out of 4 in borderline group (0.4-0.5), and 4 out of 10 in normalized group (<0.4). In particular, patients who relapsed in higher del-1 group showed relatively earlier relapse compared to the lower del-1 group. Conclusion: In a prospective cohort study, we confirmed that exosomal del-1 has a potent diagnostic role in breast cancer. Furthermore, del-1 was also identified to dramatically decrease after curative surgery. Our current findings suggest its potential prognostic role as well as diagnostic role in breast cancer patients.

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<th>Changes of Exosomal Del-1 level between baseline and after-surgery (n = 115)</th>
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<td>Del-1 (mean±SD)</td>
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#2782 The potential of p4EBP1 expression as predictive biomarker of mRCC.
Sei Naito, Osamu Ishiyangi, Hiromi Ito, Hidenori Kanno, Tomoyuki kato, Yuta Kurota, Atsushi Yamagishi,Mayu Yagi, Yoshihiko Sakurai, Hayato Nishida, Hisashi Kawaoze, Tomohiro Shibusaki, Akira Nagaoka, Norihiko Tsuchiya. Yamagata University, Yamagata, Japan.

Introduction & objectives: Activation of Akt/mTOR pathway induces 4EBP1 phosphorylation, and enhances cell proliferation, anti-apoptotic effect, and angiogenesis in many types of cancers including renal cell carcinoma (RCC). As mTOR and angiogenic proteins are main targets in metastatic RCC (mRCC) treatment. We assessed the correlation with survivals and phosphorylated 4EBP1 (p4EBP1) expression. Materials & methods: We enrolled 254 non-mRCC patients who underwent primary surgery in Yamagata University between 2003 and 2010, and 59 mRCC patients whose resected primary lesion was available. Immunohistochemistry for p4EBP1 was performed on their FFPE samples. We assessed correlations between p4EBP1 expression manners and clinical features (disease-free interval [DFI] for non-mRCC patients, cause-specific survival [CSS] and progression-free survival [PFS] for mRCC patients). The CSS was calculated from mRCC diagnosis to death or last follow-up date. The PFS was calculated based on the durations patients were medicated. Univariate analysis was calculated by log-rank test and multivariate analysis was calculated by Cox-regression analysis. Results: Non-mRCC patients with highly p4EBP1 expression were shorter DFI than those without high expression (p = 0.036). Their 5-year disease-free rates were 83.4% and 93.4%, respectively. The independent poor DFI factors were high p4EBP1 expression (HR; 3.4, p = 0.005), grade (p = 0.0055), and PT stage (p < 0.0001). In contrast, mRCC patients with p4EBP1 expression showed a stronger negative correlation than those without high expression (p = 0.0246). The independent poor factor CSS factors were non-p4EBP1 expression (hazard ratio [HR]; 3.3, p = 0.0409), grade 4 (HR; 8.7, p = 0.0006), and poor prognostic group on MSKCC criteria (HR; 4.2, p = 0.02770). Expression of p4EBP1 showed statistically longer PFS in mRCC patients with atrimib (median PFS: 9.2 and 2.5 months, p = 0.0255). The similar trends were shown in patients with other TKIs and mTOR inhibitors. Conclusion: Since non-mRCC patients with the highly p4EBP1 expression had shorter DFI, expression of p4EBP1 should indicate aggressive RCC in nature. Nevertheless, mRCC patients with p4EBP1 expression had longer survival. These results mean that expression of p4EBP1 might be a predictive biomarker for TKIs and mTOR inhibitors.

#2784 Identifying biomarkers to predict cardiotoxicity of doxorubicin in individual patients.
Liqun Zhao, Baolin Zhang, FDA, Silver Spring, MD.

Doxorubicin is a highly effective anticancer agent but causes cardiotoxicity in many patients. This study aims to identify biomarkers to predict potential cardiotoxicity before the onset of cardiac tissue damage and dysfunction in individual cancer patients. First, we established a human cellular system to model the cardiotoxicity of oncology drugs using human induced pluripotent stem cells-derived cardiomyocytes (iPS-CMs). We tested clinically used drugs including anthracyclines (doxorubicin, daunorubicin, epirubicin and idarubicin), 5-FU, Taxol, Trastuzumab, and tyrosine kinase inhibitors (sunitinib, imatinib, and sorafenib). These drugs displayed varying degrees of cytotoxicity in iPS-CMs which are generally consistent with their known cardiotoxicity pat-

terns. Second, we found that doxorubicin selectively upregulated the expression of death receptors in iPSC-CMs. As a result, the treated iPSC-CMs underwent spontaneous apoptosis that was further enhanced in the presence of specific cytokines. Based on these findings, we hypothesize that the baseline levels of the defined cytokines in blood could be predictive of cardiotoxicity associated with doxorubicin treatment in individual patients. Our next goal is to test this novel method on RNA derived from platelets from cancer patients, providing a minimal invasive procedure to monitor the state of disease in those patients.

#2785 A comprehensive RNA-based assay for treatment prediction in non-small cell lung cancer patients.
Klaas Kok, Jaicong Wei, Anna Rybczynska, Martijn Terstra, Anthonie van der Wekken, Jeroen Hiltnerman, Ed Schuuring, Rolf Simons, Harry Groen, Anke van den Berg. UMC, Groningen, Netherlands.

Purpose: In late-stage lung cancer an increasing number of genomic aberrations is used to predict either sensitivity to, or resistance against, targeted therapies. These include gene mutations, gene fusions, amplifications and in the near future different relevant splice variants. Currently, detection of each type of aberration is carried out with different tests. We have set up to develop a novel all-in-one assay to detect the various types of aberrations, e.g. mutations, gene fusions and overexpression. The latter is assumed to be the biological marker that is underlying gene amplification. Methods: Our assay is based on the Single Primer Enrichment Technology (SPET), allowing amplification of a target region using only a single sequence-specific primer. Our first custom-designed panel targets a comprehensive list of hotspots with therapeutic and prognostic significance in lung cancer, including EGFR, ALK, MET, KRAS, NRAS, PIK3CA, ROS1, BRAF, FGFR1 and RET. Using total RNA as input, we will be able to efficiently detect mutations, gene fusions and aberrant expression levels of selected genes. This test may also detect tumor-specific aberrations in platelets. Results: As a proof of principle we analyzed RNA derived from 8 lung cancer-derived cell lines, from two frozen tumor biopsies, and from 10 FFPE biopsies all with confirmed genomic alterations by clinically approved assays. In total, conventional methods detected 18 small mutations, five gene fusions and three gene amplifications, that are all covered by our assay. Of these, 14 mutations, and four gene fusions were readily detected with our assay. The detection of three mutations failed because of low coverage of the target region, probably due to a less optimal design of the specific primer. One small deletion, and one gene fusion were not detected with our RNA-based assay, despite a high read coverage. Quantification of the expression level of the amplified genes is still under investigation. Conclusion: Our preliminary data indicate this novel RNA-based assay not only efficiently identifies all crucial mutations in lung cancer cell lines, but also in small frozen and FFPE tumor biopsies of lung cancer patients. Our next goal is to test this novel method on RNA derived from platelets from cancer patients, providing a minimal invasive procedure to monitor the state of disease in those patients.

#2786 EGFR expression and phosphorylation in HNSCC predict response to EGFR inhibition but cell lines are not representative for the clinical situation.
Oliver von Ahlsen,1 Sami S. Khaznadar,1 Martin Khan.2 Bayer Pharma AG, Berlin, Germany; 2Klinikum Dahme-Spreewald, Koenigswusterhausen, Germany.

Introduction: And head neck squamous cell carcinoma (HNSCC) is the sixth most common cancer. Survival rates have not been improved for decades and conventional therapy is effective in only 50% of the patients. Based on broad expression in up to 80-90% of the HNSCC cases, epidermal growth factor receptor emerged as drug target but clinical efficacy of EGFR inhibitors in HNSCC is very limited. We therefore reinvestigated the EGFR expression levels necessary for response in cell lines and clinical samples. Methods: Standard procedures were used for IHC. The antibody clone D38B1 was used in 1:900 dilution for 2h at RT. Stainings were performed using the DAKO Envision system. EGFR expression and phosphorylation of Tyrosine-1173 were analysed by MDS (Mesoscale Discovery) in lysates from fresh frozen tumor or exponentially growing cell lines. For proliferation assays, 2000 cells per well were grown for 24 h before addition of inhibitors. Cell culture was continued for 72 h before testing viability using the CellTiter-Glo® Assay. Results: The majority (11/13) of HNSCC cell lines responded to the EGFR inhibitor Erlotinib. EGFR was highly expressed and phosphorylated in the Erlotinib responsive cell lines. Resistant cell lines displayed low level EGFR expression and phosphorylation. However, EGFR expression and phosphorylation in treatment naive clinical samples were significantly below the levels found in responding cell lines. In clinical samples EGFR was not overexpressed on the cellular level. Based on these findings, a clinical response to Erlotinib in HNSCC would not be expected. Conclusion: The prog-

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nostaic value of EGFR expression has been used to argue for EGFR as a relevant target in HNSCC. Although most reviews claim that EGFR is overexpressed in HNSCC, clear data supporting this position are missing. Early studies tested the RNA levels and found the EGFR expression in tumors higher compared to control tissues. Studies using IHC assessed the association of EGFR expression with disease progression, but no comparison to expression in normal mucosa was described. Overexpression was based on percentage of positive cells not on the intensity of expression. We show similar levels of EGFR expression in growing keratinocytes and tumor cells. The often described overexpression only originated from a larger number of EGFR positive cells, not on overexpression on the cellular level. The high expression and functional relevance of EGFR in cell lines proves that EGFR activity is required for survival in cell culture. Our findings lead to the conclusion that this is not representative of the clinical situation. Definition of a response threshold for EGFR expression and clinical verification of this expression level is mandatory for the successful use of a predictive biomarker.

### #2787 Identifying predictive markers of endocrine response in high-grade serous ovarian cancer using RNA sequencing

Cristina Mapaga,1 Sian Fereday,2 Australian Ovarian Cancer Study Group, David D. Bowtell,2 Paul R. Har- nem,31 Konica Minolta, Beijing, China; 2Konica Minolta, Beijing, China; 3Crown Bioscience, Inc., Beijing, China.

Epithelial ovarian cancer is a complex disease and patients vary considerably in response to treatment. A subset of patients responds well to endocrine therapy, however hormone receptor positivity does not predict response and there are no biomarkers in clinical use to help select those patients who would benefit from endocrine agents. We aimed to identify markers of endocrine response in high-grade serous ovarian cancer (HGSOCS) by analysis of genes that are differentially expressed in tumors from patients that are endocrine responders compared with non-responders, combined with analysis of estrogen (E2) regulated genes that differ between E2 sensitive and E2 insensitive HGSOCS cell lines. Methods: Among women recruited to the Australian Ovarian Cancer Study, we identified 10 HGSOCS patients treated with endocrine therapy (5 responders and 5 non-responders, based on GCIG CA125 response criteria) and performed RNAseq on cryopreserved tumor tissue. We also utilized paired E2 receptor (ER) positive HGSOCS cell lines, derived from the same patient, with differential sensitivity to the growth effect of E2, PEO1 (E2 insensitive) and PEO4 (E2 sensitive). RNA was extracted following treatment with 0.1 nM E2 (or vehicle) for 24 hrs and gene expression changes in response to E2 were determined using RNAseq. Expression of selected ER responsive genes was validated using PCR array. Differential gene expression was determined using EdgeR. Pathway and Gene Ontology enrichment analysis of differentially expressed genes were performed using Metacore. Results: Between endocrine responders and non-responders, 27 genes were significantly differentially expressed (False Discovery Rate (FDR)<0.05, absolute log, Fold-Change (FC)>2). In the paired HGSOCS cell lines, significantly more genes were regulated by E2 in E2 sensitive PEO4, compared with E2 insensitive PEO1 (896 genes compared to 56 genes, respectively, FDR<0.05, absolute log,FC>2). In response to E2, up-regulation of NRPI in PEO1, up-regulation of LTBP1, MYC, PDZK1 and down-regulation of Si00A6 and CYP1A1 in PEO4 were validated by PCR array profiling of ER-related genes. Genes regulated by E2 in cell lines and also differentially expressed between endocrine responders and non-responders included anocutain 1 (AN01) and NOTCH-Regulated Ankyrin Repeat Protein (NRARP). Both were up-regulated in endocrine responders and also up-regulated by E2 in PEO4. Conclusion: The use of RNAseq in both tumors and cell line models was able to identify plausible sets of genes with prior evidence of association with endocrine sensitivity. In breast cancer, AN01 has been associated with good prognosis following tamoxifen treatment, while NOTCH signaling, implicated in endocrine resistance, is negatively regulated by NRARP. Further validation is required to test the utility of these genes as predictive biomarkers that may aid in identifying HGSOCS patients likely to respond to endocrine treatment.

### #2788 High ALDH1, S phase fraction, $p16^{INK4A}$ in esophageal squamous cell carcinoma could predict response to neoadjuvant chemotherapy

Rajeev Kumar,1 R. Ravi Kannan,2 Akalesh Kumar Verma,1 Anuradha Talukdar,1 Monoy Kumar Deka,1 Ritesh Tapkirpe,1 Litika Verma,1 Sankar Kumar Ghosh.1,2 Cachar Cancer Hospital and Research Centre, Assam, India; 2Assam University, Assam, India.

Background: The prevalence of locally advanced esophageal squamous cell carcinoma (ESCC) remains high despite technological advancements in its diagnosis. This leads to prolonged multimodality treatment often resulting in poor response eventually leading to poor prognosis. Currently, there are no biomarkers available to predict response to neoadjuvant chemotherapy (NACT). In ESCC patients, ALDH1, $p16^{INK4A}$ and propidium iodide based cell cycle analysis through flow cytometry was performed in pre treatment biopsy sample collected from 108 ESCC patients who were presented at a comprehensive cancer centre in northeast India and all of them subsequently received neo adjuvant chemotherapy. Results: ALDH1, HER2 and $p16^{INK4A}$ were found positive in 65.7%, 7.4% and 22% of pre treatment ESCC specimens respectively. ALDH1 expression correlates with poor response to neo adjuvant chemotherapy (P<0.001). A significant proportion of poor responders were found to be smokers (P=0.004). Poor responders also tends to correlate with increased mortality (P=0.009). HER2 couldn’t predict response to NACT. However, all HER2 positive cases were associated with high ALDH1 expression (P=0.034). 50% of those with pathologically complete response were $p16^{INK4A}$ positive in the contrary only 15% low sensitivity responders for $p16$ (P=0.001). 84% of the ESCC patients reported aneuploid tumor status and responder’s were found having high S phase fraction (P=0.041). Conclusion: High ALDH1 expression can predict poor response whereas high $p16$ and S phase fraction can indicate good response to neoadjuvant chemotherapy in ESCC patients. HER2 seems having no clinical significance in the response assessment of neo adjuvant chemotherapy in ESCC.

### #2789 PID, a novel immunofluorescence nanotechnology, as a histopatology-based EGFR biomarker assay enhances predictive power for the Erbitux response in subsets of PDXs

Henry Qixiang Li,1 Jia Xue,1 Hiyasat Okada,5 Etskuco Putaya,1 Yoshikazu Kurihara,1 Likun Zhang,1 Xiaoyu An,1 Sheng Gua2,5 Crown Bioscience, Inc., Beijing, China; 2Konica Minolta, Beijing, Japan; 3Crown Bioscience, Inc., Santa Clara, CA.

Immunohistochemistry (IHC) is the most common used assay for detecting/quantifying defined protein biomarkers in specific tissue location in clinical/preclinical settings as prognostic/drug-predictive biomarker assays. However, IHC suffers from several key drawbacks, limiting its applications, including semi-quantitative nature, inconsistent quantification with WHO scores, lack of broad dynamic detection range and inadequacy for multi-target analysis, etc. An alternative method is immunofluorescence (IF), but with even lower usage for its disadvantageous photo-instability. A sensitive, quantitative and robust assay is thus an urgent/unmet need. We have recently developed a novel fluorescence nanoparticle (PID, phosphor integrated dye) with highly compact phosphor inside. PID has several key advanced features: 1) enhanced fluorescence (30,000x brighter over the conventional fluorescence and significantly above tissue auto-fluorescence), 2) enhanced photo-stability (stained slide is stable for >2 years); 3) highly quantitative by broadened detection dynamic range through enumeration of the bright fluorescent dots (nanoparticles) instead of signal amplification as in IHC (digital vs. analog); 4) fine subcellular imaging; 5) potentials for multi-color and H&E/bright light imaging. PID is a well-accepted experimental cancer model mimicking original patients in histio- & molecular pathology(1), as well as drug response. We previously conducted a series of mouse clinical trial (MCT) of Erbitux on PDX cohorts of NSCLC, CRC, gastric and esophageal cancers (2,3), showing specific subsets of high-EGFR-expressing PDXs responded to the drug, where EGFR expression was measured by either RNAseq (mRNA) or IHC (protein). To assess PID-EGFR as a new biomarker assay platform, we analysed and compared its quantification to RNAseq and IHC, particularly in terms of the correlation to the drug efficacy. Our data demonstrates a greatly enhanced correlation of PID scores to mRNA levels in all cancer types, and to the tumor growth inhibition of gastric (3) and esophageal (4) carcinoma, but not in CRC (2) and NSCLC (Li et al., unpublished) as anticipated. The results demonstrated that PID-EGFR is a significantly improved histopathology based EGFR protein quantification assay over IHC-EGFR assay, warranting further clinical confirmation. References 1. Guo S, et al. Cancer research 2016;76(16):4619. 2. Chen D, et al. Oncotarget 2015;6(38):40815 3. Zhang L, et al. Sci Rep 2013;3:2992. 4. Zhu et al. 2016.
ONC201 is the lead small molecule of the imipridone class of anti-cancer compounds that is currently being evaluated in phase I/II advanced cancer clinical trials. ONC201 is a highly selective antagonist of the G protein-coupled receptor dopamine receptor D2 (DRD2) that has exhibited promising anti-cancer efficacy and an exceptional safety profile. In the current study, we evaluated the influence of the DRD2 receptor on the radioresponse of the DRD2-potentiated cancer effects of DRD2 knockdown in various tumor types correlated with overall ONC201 efficacy. In particular, we noted that lymphoma cells are highly sensitive to DRD2 knockdown—tumor type where ONC201 performs well. Gene expression analysis of samples in the Cancer Genome Atlas (TCGA) revealed high DRD2 expression in ONC201-sensitive tumor types, such as lymphoma and glioblastoma, and that high expression of DRD2 in glioma was associated with a poor prognosis. High DRD2 expression was also observed in neuroendocrine prostate cancer relative to other prostate cancer subtypes. Immunohistochemistry analyses of patient-derived tumor tissue microarrays, DRD2 overexpression was particularly noted in endometrial cancer, neuroblastoma and pheochromocytoma relative to normal tissues. The anti-cancer activity of ONC201 in pheochromocytoma and neuroendocrine prostate cancer was confirmed in cell viability assays. In ONC201-treated patients, ELISA was used to quantify serum prolactin levels, a clinical biomarker of DRD2 antagonism. A 2-fold mean induction of prolactin, was detected in the serum of ONC201-treated patients, in accordance with physiological DRD2 antagonism. Interestingly, expression of DRD5 (a D1-like dopamine receptor), which counteracts DRD2 signaling, was significantly negatively correlated with ONC201 in vitro potency in the NC60 and GDSC dataset (P < .05). Furthermore, a missense DRD5 mutation was identified in cancer cells with acquired resistance to ONC201. In conclusion, the DRD2 pathway is expressed in ONC201-sensitive tumors and may provide biomarkers of response. #2793 Evidence for WT1 as a potential target for immunotherapy of lethal ovarian cancer. Julia H. Carter,1 James A. Deddens,2 Gretchen Mueller,3 Thomas G. Lewis,1 Mariah K. Dooley,1 Jackson O. Pemberton,2 Larry E. Douglass,1,4 Wood Hudson Cancer Research Lab., Newport, KY;5 University of Cincinnati, Cincinnati, OH;5 St. Elizabeth Healthcare, Edgewood, KY.

Ovarian cancer is the leading cause of gynecologic cancers with 22,280 new cases anticipated in 2016 and 14,240 deaths. Most patients are diagnosed at late stages and have a poor prognosis. Radical surgery and chemotherapy are the primary treatment. When relapse occurs there are few treatment options. The overall survival rate of women with ovarian cancer is unchanged in 50 years. Approximately 70% of epithelial ovarian cancers are serous including low grade serous tumors, some of which may evolve from serous borderline tumors, and high grade serous carcinomas. The latter are thought to originate in the fallopian tube. There are eight other subtypes of invasive ovarian cancer. All invasive ovarian cancers have recently been classified into two subtypes, Type 1 (indolent) and Type 2 (aggressive). The Wilms’ Tumor 1 (WT1) protein is expressed in some normal tissues including the fallopian tube and ovary as well as in tumors such as ovarian and breast cancers and in leukemia. WT1 is a highly immunogenic tumor associated antigen. Mutated p53 is found in many high grade ovarian cancers. Recent reports indicate that WT1 and p53 may interact physically and functionally. Both WT1 and mutated p53 have been suggested as prognostic markers in ovarian cancer. We sought to determine the role of co-expression of mutated p53 and WT1 in overall survival of patients with ovarian cancer. Histopathologic sections were archived FFPE surgical specimens donated by St. Elizabeth Healthcare (Northern KY) and fully annotated by hospital records. H&E stained sections were diagnosed and graded by a board certified pathologist (LED) and included histologic sections from 41 borderline tumors and 98 Type 1 and Type 2 cancers. Adjacent sections were stained immunohistochemically using monoclonal antibodies (DAKO) to WT1 and p53. For survival analysis p53 was considered mutated if ≥75-100% of the tumor nuclei were stained (missing mutations) or if all nuclei were negative (truncated protein) and WT1 was considered either non-expressed (no stain) or expressed (some or all nuclei stained). While either mutated p53 or WT1 were prognostic alone of patient survival with Type 1 and...
Type 2 tumors, WT1 was a better predictor as seen by log-rank Chi-square. The 20 year survival probability of patients with tumors negative for both mutated p53 and WT1 was 70% and was significantly better than other patients (p < 0.0001). The 20 year survival probability for tumors negative for WT1 but expressing mutated p53 was 41%, and was 9% for tumors expressing WT1 and non-mutated p53. For tumors with both WT1 expression and non-mutated p53, the 20 year survival had a 20 year survival probability of 6%. Given these data and the immunogenicity of the WT1 tumor associated antigen we conclude that IHC detection of mutated p53 and WT1 in invasive ovarian cancers would be useful in stratifying patients eligible for immunologic approaches targeting WT1 for therapy of lethal ovarian cancers.

#2794 Indotecan (LMP400), imidotecan (LMP776) and LMP744: A new class of non-camptothecin Top1 inhibitors selective for homologous recombination deficient (HRD) cells. Laetitia Marzi, Keli Agama, Zoe Weaver Ohler, Ludmila Szabova, Shyan Sharin, Junko Murai, Muthana Al Ab, Yves Pommer, NCI, Bethesda, MD.

To relax DNA supercoiling during transcription and replication, topoisomerase I (Top1) induces transient DNA cleavage complexes, which are trapped by anticancer drugs, leading to DNA double-strand breaks (DSB) that need to be repaired by homologous recombination (HR). BRCA1, BRCA2 and PALB2, which are key components for HR, lead to "synthetic lethality" with PARP inhibitors. Topotecan and irinotecan (camptothecin derivatives) are the only FDA-approved Top1 inhibitors. In spite of their wide usage they are plagued by their chemical instability, being drug efflux substrates, having short half-life, and dose-limiting bone marrow and gastrointestinal toxicity. It is now possible to overcome these limitations with the non-camptothecin indenoisoquinolines (LMP400, LMP776 and LMP744), which are in clinical trials. To rationally select patients for phase 2 clinical trials based on cancer-specific genomic alterations, we have determined whether the LMPs present a "synthetic lethality" toward BRCA1, BRCA2 or PALB2 deficiency, and whether this selectivity could be enhanced by combining them with the recently approved PARP inhibitor, olaparib. Using isogenic DT40 cell lines, with BRCA1, BRCA2 or PALB2 deficiencies, we assessed the role of HR in the cellular response to the LMPs. Survival and cell cycle modifications were tested after treatment with the LMPs as single agents, as well as in combination with olaparib. We found that BRCA1-, BRCA2- and PALB2-deficient cells are 3 to 5 times hypersensitive to the LMPs (IC50’s for LMP400 and LMP744> 10 nM for HR-deficient cells vs. 45 nM for WT cells, and IC50 for LMP776 around 5 nM vs. 18 nM for WT cells). Cell cycle analyses confirmed the death of these HR-deficient cells. Moreover, combination treatments showed a significant synergy between each of the three LMPs and olaparib (Combination index<0.7). The HR-deficient cells being markedly more sensitive than WT cells (taken as surrogate for normal tissues) to both treatments, led to a better response to the combination in those cells. Our results show that the LMPs are active at nanomolar concentrations, selectively in HR-deficient cells, indicative of a "synthetic lethality" of the indenoisoquinolines (LMPs) with HRD genotypes. They also demonstrate that the LMPs synergize with olaparib. These findings provide a rationale for personalized treatment and Phase 2 clinical trials with the indenoisoquinolines in combination with PARP inhibitors in HR-deficient cancers.

#2795 Integrating multiomics discovery approaches to identify biomarkers of therapeutic resistance in metastatic colorectal cancer through analyses of multiple sequential tumor and liquid biopsies; Qcrocc01: NCT009984048. Karen Gambaro,1,2 Maud Marques,3 Ryan Morin,3 Claudia Kleinman,4 Michael Witcher,3 Simon Turcotte,3 Benoit Samson,5 Bernard Lespérance,6 Yoo-Joung Ko,7 Richard Dalfen,8 Eve St-Hilaire,9 Lucas Sideris,10 Felix Couture,11 Sabine Helm,7 Keli Agama, Zoe Weaver Ohler, Ludmila Szabova, Shyan Sharin, Junko Murai, Muthana Al Ab, Yves Pommer, NCI, Bethesda, MD.

Colorectal cancer (CRC) is the 2nd leading cause of cancer-related death in Canada. Clinical responses of metastatic (m)CRC to first-line treatment range from 35 to 60%, but even responders inevitably develop therapeutic resistance. Studies aiming at understanding mechanisms of resistance have largely investigated primary tumors. However, selective pressures during therapy can lead to development of resistance, which may lead to therapeutic failure, thus the need to characterize the molecular changes of metastasis and plasma over time of treatment and response to decipher tumor evolution and therapeutic resistance mechanisms. In this multicenter study, 52 tissue samples from liver metastasis were collected at baseline (pre-biopsies) and at the time of resistance (post-biopsies) in responder and non-responder mCRC patients (n = 44) undergoing standard first-line treatments. Metastases have also been harvested in 4 patients, to allow the assessment of tumor heterogeneity and as well as the evolution of the genomic complexity after treatment exposure. Analyses were carried out across multiple omic platforms to identify resistant signatures and characterize molecular changes during treatment. Biopsies were profiled using exome and transcriptome sequencing as well as high-density SNP array analysis to capture chromosomal anomalies, loss of heterozygosity (LOH) and copy number variations (CNV). Additionally, serial blood samples were collected for proteomic, ctDNA and cytokine analysis. Our preliminary analysis of transcriptomes performed on serial biopsies from a set of 11 patients identified genes consistently overexpressed at resistance. Cytogenetics analysis showed similar genomic profiles of matched pre- and post-biopsies and allowed to stratify the expansion of resistant clones. LOH and CNV catalogues of liver metastasis, while exome sequencing revealed cumulative somatic mutations over time of treatment, which suggests subclonal and acquired "driver" mutations of resistance. Plasma-derived ctDNA analysis was performed to investigate the mutational status during treatment and whether they correlate with their relative levels in biopsies. Immune gene expression analysis of a test set of 27 metastases revealed strong clustering of 7 metastases due to overexpression of transcripts related to active immune response, allowing to define novel subgroups of patients based on immune response status. Our study, using a multi-omic strategy and integration of independent molecular platforms to profile liver metastasis samples of responders and non-responders to mCRC patients, constitutes an innovative approach to identify clinical biomarkers and molecular signature of resistance, which may enhance individualization of cancer medicine and customized therapy.

#2796 Development of a RAD51-based assay for determining homologous recombination proficiency and PARP inhibitor sensitivity. Bose S. Kochupurakkal,1 Kalindi Parmar,1 Jean-Bernard Lazaro,1 Christine Unitt,2 Qing Zeng,1 Hunter Reavis,3 Chirag Ganese,1 Shan Zhou,1 Joyce Liu,1 Sangeetha Palakurthi,1 Kyle Strickland,1 Brooke Howitt,1 Panagiotis Konstantinopoulos,1 Paul Kirschmeier,1 Joseph Geradts,2 Ronny Drapkin,3 Ursula Matulonis,1 Alan D'Andrea,1 Geoffrey Shapiro,1 Dana-Farber Cancer Institute, Boston, MA; Brigham and Women’s Hospital, Boston, MA; University of Pennsylvania, Philadelphia, PA.

Homologous recombination (HR) repair deficiency confers sensitivity to inhibitors of poly(ADP-ribose) polymerase (PARP). To date, the identification of tumors with impaired HR has relied on genomic features, including mutational signature, LOH-based HRD assays or gene expression analyses defining ‘BRCA-ness’. These tests analyze history of the tumor rather than providing a functional assessment of HR status at the time of diagnosis. Therefore, development of a functional assay for HR status in tumors is essential to make accurate treatment decisions. Here, we describe a RAD51-based immunohistochemical (IHC) assay that identifies HR status. We first screened commercial anti-RAD51 antibodies and identified a monoclonal antibody that detects RAD51 foci in HR-proficient normal fibroblasts and shows no evidence of foci in HR-deficient (BRCA2−/−) VU423 fibroblasts after γ-irradiation. Conditions for detecting RAD51 foci in FFPE samples were identified using HR-deficient and HR-proficient triple-negative breast cancer cell lines. HR-deficient, PARP inhibitor-sensitive cell lines exhibited high levels of nuclear RAD51 and no evidence of foci, whereas HR-proficient, PARP inhibitor sensitive lines had low levels of nuclear RAD51 and foci. This result was confirmed in a BRCA1-mutated, PARP inhibitor-sensitive PDX model, where there was no evidence of foci although RAD51 levels were high. We further evaluated the pattern of RAD51 staining in 13 high-grade serous ovarian cancer (HGSOCC) PDX models, for which sensitivity to olaparib had been characterized. The only olaparib-sensitive model demonstrated complete absence of RAD51 staining. Among the other 12 olaparib-resistant models, RAD51 foci were detectable, both before and after irradiation. The presence or absence of RAD51 foci correlated with olaparib sensitivity and not with BRCA mutation status. Therefore, tumors that are HR-deficient and PARP inhibitor-sensitive are characterized by either high RAD51 nuclear staining without foci,
or absence of RAD51 staining. To validate these findings, we analyzed RAD51 staining patterns in a cohort of 50 primary HGSOCs from patients subsequently treated with platinum-based chemotherapy. Among these 50 samples, 45 demonstrated either RAD51 nuclear staining without foci or an absence of RAD51 staining. Five samples demonstrated RAD51 staining with foci. The median survival of patients with and without RAD51 staining were 61 and 15 months, respectively. Using the signature of the two groups, we have developed a robust IHC assay for determining the functional HR-status in tumor samples. Further work will be required to determine if the staining patterns observed predict PARP inhibitor sensitivity among primary patient samples. Funded by a Biomarker Supplement to UM1 CA186709, NIH Grant P50 CA168504, SU2C Ovarian Cancer Dream Team and BCRF grant.

**TUMOR BIOLOGY: Cell Culture and Animal Models of Cancer 3**

#2799 **Development of a mouse model of KRAS mutated colorectal cancer tumorigenesis.** Radhashree Maitra,1 Madhu Kumar Venkatesh,2 Titto Alby Augustine,2 Carol Chandy,2 Qiang Liu,1 Sanjay Goel1. 1Montefiore Medical Center, Bronx, NY; 2Albert Einstein College of Medicine, Bronx, NY

Introduction: Development of a preclinical animal model that mimics KRAS mutated colorectal cancer disease etiology is essential for validation of new therapies and effective screening of potential therapeutic combinations. Colorectal cancer patients with tumors harboring KRAS mutation are excluded from receiving anti EGFR monoclonal antibodies, and currently have no alternate FDA approved treatment options available. Ongoing research requires validations of different modalities on a robust preclinical study platform that will resemble the disease phenotype and genetic profile. In this context we have developed viable animal mouse model of colorectal cancer bearing KRAS mutation that authentically serves as a model for the disease.

Methodology: The C57 BL/6 mice with truncated APC floxed allele was crossed with heterozygous KRAS floxed outbred mice to generate APC<sup>−/−</sup> KRAS<sup>+/−</sup> mouse colony. In another set of breeding APC floxed mice were crossed with CDX2-Cre-ER<sup>−/−</sup> mice and selected for APC<sup>−/−</sup> CDX2-Cre-ER<sup>−/−</sup> second after round of inbreeding. The final model of the disease was generated by the cross of the two aforementioned colonies when viable APC<sup>−/−</sup> KRAS<sup>−/−</sup> CDX2-Cre-ER<sup>−/−</sup> were genotyped and characterized. The model animals were tamoxifen induced to generate tumors. Micro-PET scan was used to detect and measure tumor volume. H & E staining was done to establish neoplasm and immunohistochemistry was performed to determine histological similarities with human FFPE biopsies. The MSI/MMR status was also determined. Results The newly developed animal model develops colonic tumors upon induction with Tamoxifen. The tumors were malignant as confirmed by H and E staining. The tumors showed a higher radioactive FDG uptake (SUV) in micro-PET scan. The tumors resembled human colorectal cancer tissue Once tumor is generated the animals died of cachexia and rectal bleeding Conclusion: Heterozygous mutation of the APC gene along with deletion mutation of Adenomatous polyposis coli (APC) gene is embryonically lethal in animal models. We have successfully developed a viable animal model of KRAS mutated colorectal cancer with tamoxifen inducible tumors harboring KRAS and APC mutations. The tumors mimic the human disease type both genetically and immunohistochimically. This model can serve as a robust preclinical platform for understanding the disease mechanism at the molecular level as well for evaluation of various therapeutic interventions.

#2800 **Transforming growth factor-beta 2 (TGF-β2) antisense oligonucleotide (ASO) OT-101 synergizes with chemotherapy in preclinical tumor models.** Osmond D’Cruz,1 Cynthia Lee,2 Vuong Trieu,2 Larn Hwang,3 AutoTec Inc, Costa Mesa, CA; Oncotec Inc, Agoura Hills, CA

Background: Overexpression of TGF-β2 has been implicated in the malignant progression of tumors by inducing immunosuppression, proliferation, angiogenesis and metastasis. Clinical failures of TGF-β inhibitors in targeting the tumor promoting arm of TGF-β signaling is attributed to multiple isoforms and receptor functions. OT-101 (Trabedersen) is a phosphorothioate ASO designed to specifically target human TGF-β2 mRNA with superior clinical activity in patients with TGF-β2 overexpressing tumors. Herein, we report the synergistic effect of OT-101 with chemotherapy in multiple human tumor xenograft models for further exploration of clinical combinations. Methods: The in vivo efficacy studies of intraperitoneal (IP, 16 or 50 mg/kg) or subcutaneous (SC, 1-64 mg/kg) repeated administration (qdx3/wk or qdx21) of OT-101 as single agent
#2801 Identification of oncogenic/metastatic driver genes that cooperate with p53 or p53/Rb-loss to induce triple-negative breast cancer. Ronak Ghanbari Azarnier, Agatha Zuchelkowski, Philip E Chung, Zhe Jiang, Eldad Zacksenhouse, University of Toronto, Toronto General Research Institute, Toronto, Ontario, Canada.

Triple-negative breast cancer (TNBC) is an aggressive subtype with poor prognosis; drug-resistant metastases are common and highly lethal for which identification of therapeutic targets is of major medical interest. Previously we demonstrated that deletion of p53 and Rb in mouse mammary epithelium induced TNBC-like tumors but with limited capacity to metastasize. Therefore, we decided to employ sleeping beauty (SB) mutagenesis system that can identify oncogenic networks responsible to drive primary and metastatic tumors. In order to do this, we generated mice in which p53 (p53F/F) or p53 plus Rb (p53F/F; RbF/F) are deleted and the SB transposon is mobilized from chromosome 9 as well as controls in which both p53 and Rb are wild type. Forty primary tumors and forty metastases from each cohort will be harvested that cooperate with common integration sites followed by deep sequencing. Then, the top 5-10 candidate oncogenes/tumor suppressors will be validated to determine whether their loss/activation would accelerate tumorgenesis/metastasis. H&E staining of mammary tumors collected so far from different genotypes revealed histologically diverse tumor types which includes but not limited to poorly differentiated carcinomas, mesenchymal/spindle-like cells, acinar adenocarcinomas, and survival outcome. The incidence of lymph node and liver surface and micro-metastases as well as size and weight of the pancreatic tumors were determined. Tumor sections were stained with anti-5-bromo-2'-deoxy-uridine antibody to determine tumor cell proliferation and with anti-CD31/PECAM-1 antibody to determine vascularization. Results: OT-101 significantly reduced tumor growth (p = 0.0084), lymph node metastasis (p = 0.023), and tumor angiogenesis (p < 0.0001) in the orthotopic PAC model. Mean tumor vessel density was significantly reduced (p < 0.0001) in all groups in comparison to untreated control. OT-101 demonstrated synergy in tumor growth inhibition and increased survival in human malignant melanoma (C8161, p = 0.038, vs. Dacarbazine alone), glioblastoma (U87-MG, p = 0.001 vs. Paclitaxel) and ovarian (SK-OV-3, p < 0.05 vs. Paclitaxel) cancer models when combined with either Dacarbazine (C8161) or Paclitaxel (U87-MG and SK-OV-3). No synergy was observed with Gemcitabine (PAC). The combination regimen tested was effective and tolerable. Significant antitumor activity was achieved at human dose equivalent of 80 mg/m2/day which is well below the optimized clinical dose used for IV infusion of patients at 140 mg/m2/day. Conclusions: The preclinical data lay the groundwork which is well below the optimized clinical dose used for IV infusion of demonstrating that TGF-β2 is a druggable target. Of interest is the preferential synergy between OT-101 and Paclitaxel or Dacarbazine, but not with Gemcitabine.

#2803 The NCI mouse repository: Cancer models and miRNA-ES cell resource. Parirokh Awasthi, Fitzgerald Debra, Jones N. Stephen. Leidos Biomedical Research, Inc., Frederick, MD.

The NCI Mouse Repository, located at the Frederick National Laboratory for Cancer Research (FNLCR), Frederick, Maryland, is an NCI-funded resource of approximately 150 genetically-engineered mouse cancer models and associated strains, including mice bearing conditional and point-mutant alleles in cancer-related genes. In addition, the Repository houses a unique collection of over 1500 different mouse ES cell clones bearing conditionally-activated miRNA transgenes to facilitate in vivo exploration of miRNA functions. The NCI Mouse Repository makes strains and mESCs available to all members of the scientific community (academic, non-profit, and commercial). The mouse models and ES cell clones are cryo-archived and distributed as frozen germplasm or cells. Requests may be placed through the NCI Mouse Repository website (https://ncifrederick.cancer.gov/Lasp/MouseRe-pository/Default.aspx). In addition to the request form, this website includes detailed description for each strain accepted into the Repository and the associated publications provided by the donating scientist. The miR-harboring ESCs, originally generated at the Cold Spring Harbor Laboratories for the NCI Mouse Repository, are distributed to eligible miRNA-ES cell clone researchers. These resources are available for nominal cost to NCI, NIH, and other US government-funded investigators, as well as to Investigators at non-profit organizations.

#2804 Identifying therapeutically relevant mouse and patient-derived xenograft (PDX) models of human cancer using the mouse tumor biology database (MTB) data resource. Dale A. Begley, Debra M. Krupe, Steven B. Neuhauser, Joel E. Richardson, John P. Sundberg, Janan T. Eppig, Carol J. Bult. The Jackson Laboratory, Bar Harbor, ME.

Introduction: Hepatocellular carcinoma (HCC) is the third leading cause of cancer deaths worldwide. HCC typically arises in patients with chronic liver disease or cirrhosis, yet it is increasingly associated with non-alcoholic fatty liver disease (NAFLD), specifically nonalcoholic steatohepatitis (NASH) in the absence of cirrhosis. NAFLD is associated with obesity, metabolic syndrome, and/or patients with type II diabetes. Our previous studies have shown that high fat diet induces TNBC-like tumors but with limited capacity to metastasize to the liver and the growth of malignant tumors in a murine model. Glycine N-Methyltransferase (GNMT) expression is lost in over 95% of HCC, and mice deficient in GNMT develop spontaneous HCC by 6 months of age. We hypothesized that GNMT deficient mice would have an increased susceptibility for the development and growth of HCC when a fed high fat diet. Methods: Wildtype and GNMT deficient mice were placed on lean diet (LD, 13% calories from fat) or high fat diet (HFD, 42% calories from fat) at eight weeks of age. An initial cohort of mice were sacrificed after 3 months on diet (6 months of age) to assess for early tumor burden. A second cohort of mice was analyzed by magnetic resonance imaging (MRI) after 6 months on diet (9 months of age) and then sacrificed to assess for late stage disease. All mice were assessed for body weight, liver weight, pancreatic weight, and survival outcome. The incidence of lymph node and liver metastasis (p < 0.05, vs. LD fed GNMT mice) were equivalent after 3 months, but they were larger than wildtype mice fed LD. After six months on diet, MRI analysis showed significantly larger livers in HFD fed GNMT deficient mice than wildtype mice fed LD. All wildtype mice lacked any tumors after six months regardless of diet. Histological analysis revealed a heightened cellular proliferation via Ki67 staining in GNMT deficient mice compared to wildtype livers. In comparison, GNMT silencing also occurs in pancreatic cancer, yet none of the GNMT deficient mice developed pancreatic tumors. However, small focal areas of pancreatitis were detected regardless of diet. Additionally, pancreatic weight was significantly decreased in HFD fed GNMT deficient mice compared to the LD GNMT deficient mice. Conclusions: While high fat diet did not induce obesity in GNMT deficient mice, it significantly increased cellular proliferation and primary tumor growth in the liver. Understanding dietary factors that impact the microenvironment of the liver and to contribute to HCC development and progression is vital to finding new therapeutics for this malignancy.
TUMOR BIOLOGY: Cell Culture and Animal Models of Cancer

The laboratory mouse is the foremost model organism for interrogating the genetic and molecular basis of human cancer and is a powerful platform for identifying therapeutically effective targets for prevention and treatment of cancer. Research using genetically engineered mouse models (GEMMs) have led to important advances in our understanding of the genetic basis of cancer susceptibility, but data from these models is critical to the translation of drugs that show efficacy in xenografts and preclinical and clinical studies. Patient Derived Xenograft (PDX) models are an increasingly important model system for in vivo studies of human cancer. These models are created by implanting patient tumors into immunodeficient or humanized mouse hosts and are a powerful translational research platform for preclinical and clinical studies. The number of GEMM and PDX models being used is increasing every year and the diverse cancer-related data about human cancer models tend to be distributed in ways that makes it difficult for researchers to integrate and interpret the information to find the most relevant model for their research. The Mouse Tumor Biology database (http://tumor.informatics.jax.org) is an expertly curated resource for information and data about genetically defined mouse strains and PDX models of human cancer. MTB provides query tools to enable integrated searches and visualization of these varied data, thus facilitating the assessment of novel mouse models of human cancer and potential preventative and therapeutic treatments. Enforced control of the database and standard gene, allele and strain nomenclature within MTB facilitates precise and comprehensive queries of MTB for pertinent mouse models. MTB contains data from spontaneous or endogenously induced tumors from genetically defined mice including tumor classification, incidence, Quantitative Trait Loci, pathology reports, images and genetic changes in the tumor (somatic) and background strain (germline) genomes. The PDX resource enables queries based on tumor type, cancer diagnosis and genomic properties of the engrafted tumors. Information in MTB is obtained from curation of peer-reviewed scientific publications and direct data submissions from individual investigators and large-scale programs. New features in MTB include the Faceted Tumor Search Form and a Reported Mouse Model tool. Table linking the most common fatal human cancers to reported relevant mouse models. MTB contains over 77,000 Tumor Frequencies and over 2,200 Pathology Reports with over 6,600 images from over 4,200 references. MTB provides access to detailed clinical, pathological, expression and genomics data from over 400 PDX cancer models. Information in MTB is integrated with cancer models data from other bioinformatics resources including PathBase, the Gene Expression Omnibus and ArrayExpress. MTB is supported by NCI grant CA089713.

#2805 A panel of orthotopic glioblastoma multiforme (GBM) patient derived xenograft (PDX) mouse models for efficacy evaluation of drugs. Mette M. Jensen,1 Camilla S. Knudsen,1 Lotte K. Kristensen,2 Mette K. Nedergaard,2 Michael J. Wick,1 Kyriakos P. Papadopoulos,3 Anthony W. Tolcher,3 Andreas Brat,2 Minerva D. Demetri,1 ST112, ST146, ST545, ST610 and ST2473 were digested and used for intracranial stereotactic injection in nude mice. Tumor take and growth was determined by T2-weighted magnetic resonance imaging (MRI). At confirmed tumor take animals were treated with TMZ (100mg/kg/day for 5 days) or whole brain XRT (2 Gy/day for 5 days). Control groups receiving vehicle or sham XRT were included depending on treatment regimen. Final endpoint was survival by humane endpoints and tumors were fixed in formalin for histological evaluation. Results: The take rate was > 80% across all models. TMZ showed efficacy in the orthotopic ST610 GBM PDX model evaluated by MRI on day 14 (16.2±2.9 mm3 vs. 76.8±13.1 mm3, p=0.016), whereas the ST146 model displayed resistance to TMZ on day 14 (12.7±5.6 mm3 vs. 26.5±11.9 mm3, p=0.26). The median survival was 60 days vs. 14 days in the ST610 model (TMZ vs. vehicle, p=0.0005) and 27 days vs. 13 days in the ST146 model (TMZ vs. vehicle, p=0.007). XRT showed efficacy in the orthotopic ST2473 model. Tumor volume was significantly smaller in treated vs. sham animals 11 days after treatment (6.9±1.4 mm3 vs. 23.1±7.8 mm3, respectively). Also, survival benefit in XRT treated animals compared to sham. Histology confirmed the presence of orthotopic tumors and typical GBM pathology characteristics such as pseudopalisading tumor cells surrounding necrosis and micro vascular proliferation were identified. Conclusion: Six different orthotopic GBM PDX models were established from low passage subcutaneous PDX models. Models sensitive and resistant to TMZ were identified. The PDX resource enables queries based on tumor type, cancer diagnosis and genomic properties of the engrafted tumors. Information in MTB is integrated with cancer models data from other bioinformatics resources including PathBase, the Gene Expression Omnibus and ArrayExpress. MTB is supported by NCI grant CA089713.

#2806 Generation and characterization of mouse model of Pmepa1 conditional knockout in prostate epithelia. Shashwat Sharad, Talai Barbiev, Yingjie Song, Denise Young, Tatiana Srebnatha, Albert Dobi, Shiv Srivastava. Uniformed Services University, Rockville, MD.

Introduction and Objectives: Prostate cancer (CaP) is the most common non-skin malignancy diagnosed in American men and defunctions of androgen receptor (AR) plays an essential role in prostate tumorigenesis. Pmepa1 is an androgen and TGF-β induced gene abundant in prostate, which was identified to down-regulate protein via NEDD4 E3 protease mediated pathway. Reversal or loss of Pmepa1 expression, commonly detected in prostate tumors, led to increased AR protein and activated AR signaling. Pmepa1 inhibited TGF-β receptor 1 mediated signaling by a negative feedback loop. It was reported that loss of Pmepa1 facilitates bone metastasis of CaP through blocking TGF-β signaling. To further investigating the biological function of Pmepa1 gene in CaP tumorigenesis, particularly via AR and TGF-β, we generated Pmepa1 prostate conditional knockout mouse model. Methods: C57BL/6 male mice were utilized for generation of Pmepa1 conditional knockout model. Pmepa1 gene was conditionally deleted in mouse prostate epithelium by ARR2PB-Cre-Lox system. Male mice of genotypes of wild-type, Pmepa1 flox/wild-type, ARR2PB-Cre, Pmepa1 flox/flox and flox/flox-ARR2PB-Cre were euthanized at the age of 3 months for analysis. The prostate tissue was collected for frozen and formalin fixation for histology analysis, and other major organs including heart, lung, liver, spleen, bladder and testis were also collected for control. The tissue were sectioned and stained with Hematoxylin & Eosin (H&E). Total RNA and protein were harvested by homogenizing tissue. The protein levels of Pmepa1, AR and Nkx3.1 were analyzed by immunohistochemistry (IHC) and immunoblotting, and the transcript levels of these genes were evaluated by in situ hybridization (ISH) and quantitative-PCR (Q-PCR). Results: The expression of Pmepa1 protein was found to focus on lateral lobe, consistent with AR protein expression pattern. The data of Q-PCR and ISH showed that transcript level of Pmepa1 was dramatically suppressed in Pmepa1 flox/flox and flox/wild-type ARR2PB-Cre mice. Compared to wild-type ones, Pmepa1 protein was shown significantly decreased in heterozygotic and homozygotic knockout mice by immunoblotting and IHC staining. And the protein level of AR was stronger in Pmepa1 conditional deleted mouse. There was no dramatic morphology change in mouse prostate gland by H&E staining. Conclusions: Conditional deletion of Pmepa1 gene in mouse prostate epithelium led to enhanced AR protein and activating AR signaling. The effects of Pmepa1 gene loss in mouse prostate gland on other signaling such as TGF-β and prostate tumorigenesis are needed to be further evaluated. Funding: This study was supported by CPDR, SUHS, HU0001-10-2-0002 to DGM.


Recent success of multiple poly (ADP) ribose polymerase (PARPi) ribose polymerase inhibitors (PARPi) in treating BRCA mutant ovarian cancers has fueled the development of PARPi in several areas, including expanding its use in other BRCA mutant or wildtype cancers; exploring resistant mechanisms and overcoming resistance; seeking for its combination with chemotherapy, radiotherapy and emerging immunotherapy to achieve persistent efficacy. Especially considering the mechanism of action of PARPi, its use can increase the mutational loads in tumors with defects in homologous recombination and DNA doublestrand break repair, which potentially would enhance the immunogenicity of the tumors. Therefore it would be very interesting to test the effectiveness of combining PARPi with anti-PD1/PD-L1 or anti-CTLA4. However, relevant animal
models are lacking for such studies. Syngeneic mouse tumors offer about 30 viable in vivo models for proof of concept studies. However, these models suffer from several limitations: 1) only a few available and responsive to the current checkpoint inhibitors – for given strains of mouse, there are only very few choices of cells/disease types; 2) not mimicking patient tumors since they are in vitro models; 3) not reflective of the immune microenvironment; 4) lack of patient derived cell lines and tissues; 5) not readily available for common target agents, either in mono- or combination therapy. We have established allologs of spontaneous mouse tumors (MuPrime®) derived from genetically engineered mouse models (GEMMs) as a new type of immuno-oncology models with following advantages: 1) its primary nature of "stem cell diseases" and relevant tumor microenvironment; 2) its diverse cancer types/strains of mouse; 3) engineered oncogenic drivers as seen in human diseases, deriving from a wide range of available GEMMs, suitable for targeted agents. Thus far, we have built a small library of allografts and are testing them to facilitate pharmacological investigation, particularly those of immuno-oncology. We have found a few models, including an APC−/− skin squamous cell carcinoma and a P53 null sarcoma responded well to PARPi Niraparib. Continuous treatment with Niraparib led to development of partial or complete resistance. We are now profiling the resistant models to find out their general mutation loads, as well as specific biomarkers render resistant mechanisms. We are also treating the tumors with mouse PD1 antibody to test whether presumably enhanced mutation loads would sensitize these tumors to immunotherapy. The data of these studies will be presented at the meeting.

Identification of EphB4 as a tumor suppressor in melanoma. Ombratta Salvucci,1 Dragan Maric,2 Giovanna Tosato,1 1NCI/NINH, Bethesda, MD, 2NINDS/NINH, Bethesda, MD.

The Ephrins and Eph are a large family of ligands and receptors with critical physiologic functions in development and in adulthood. The EphB4 receptor and its unique ligand EphrinB2 have been implicated in the pathogenesis of a variety of cancers, but the underlying mechanisms remain unclear. Data from the Cancer Genome Atlas (TCGA) shows that the survival probability of patients with melanoma is significantly lower when EphB4 levels in the cancer tissue are very low compared to the average EphB4 levels in melanoma, suggesting a role of EphB4 in regulating melanoma progression. We investigated the role of EphB4 depletion in the regulation of melanoma cell growth. We report, that EphB4-depleted murine melanoma B16/F10 cells have a growth and survival advantage in vitro over control cells. This growth advantage is revealed under conditions of stress generated by hypoxia or nutrients deprivation, which is common during cancer progression. When inoculated into syngeneic mice, EphB4-depleted B16/F10 cells generate larger tumors than control B16/F10 cells. Initial mechanistic studies have revealed that EphB4-null B16/F10 tumors contain fewer apoptotic cells compared to control tumors, have more infiltrating CD31+/H11001 vessels and pericytes are detected at a distance from CD31+/H11002 vessels. Thus, we have generated a mouse model that reflects the tumor-suppressive function of EphB4 in human melanoma and have identified potential pathways underlying this function.

Role of c-Met in the development and progression of Tpl2-related skin cancer. Nicole F. Bonan,1 David Kowalski,1 Kaitlin Kudlac,2 Lauren G. Falkenberg,1 Erik Maradiaga,1 Kira Flaherty,1 Jonathan S. Wiest,2 Katie L. DeCicco-Skinner,1 1American Univ., Washington, DC, 2NCI, Rockville, MD.

Cutaneous squamous cell carcinoma (cSCC), a form of non-melanoma skin cancer, is the second most common form of cancer in the United States, with over one million cases diagnosed annually. Tpl2 (MAP3K8) is a serine threonine protein kinase in the mitogen-activated protein kinase (MAPK) signal transduction cascade. Tpl2 was identified by our laboratory as having a tumor suppressive function in skin carcinogenesis, with Tpl2 knockout mice developing significantly more papillomas and squamous cell carcinomas than matched wildtype (WT) controls. Cellular mesenchymal to epithelial transition factor (c-Met) is a receptor heightened in malignant skin cancers and involved in Tpl2 signaling. When bound to its ligand, hepatocyte growth factor (HGF), c-Met can regulate cell proliferation, survival, angiogenesis and invasion. The aim of this study was to ascertain whether c-Met signaling contributes to the heightened skin tumorigenesis inTpl2+/− mice. Forty-four WT and Tpl2+/− mice were subjected to a two-stage chemical carcinogenesis protocol for one year to induce skin tumors. Starting at the time of promotion, half of the WT and Tpl2−/− mice were placed on normal diet and half received diet plus 30mg/kg Capmatinib, a pharmacological inhibitor of c-Met. This diet continued for the duration of the study. No adverse health effects were found in Capmatinib-treated mice. Similar to what we previously reported, Tpl2−/− mice developed tumors earlier than WT controls and had statistically higher tumor incidence and overall tumor burden than WT mice. The number of tumor bearing Tpl2−/− and Tpl2+/− + Capmatinib fed mice was similar, although Capmatinib fed mice had a 60% reduction in overall tumor burden. Studies are underway to determine if Capmatinib affected the frequency of malignant conversion in Tpl2−/− mice. In summary, our studies demonstrate that heightened c-Met signaling contributes to the higher tumor formation in Tpl2−/− mice and should be evaluated further as a possible therapeutic target in Tpl2-related skin cancers.

Using CRISPR/Cas9 to generate primary soft tissue sarcoma in genetically engineered and wild-type mice. Jianguo Huang,1 Mark Chen,1 Melodi J. Whitley,1 Hsuan-Cheng Kuo,1 Andrea Walens,1 Yvonne M. Morey,1 David V. Mater,1 William Eward,1 Diana M. Cardona,1 Lixia Luo,1 Yan Ma,1 Christopher E. Nelson,1 Jacqueline N. Robinson-Hamm,1 Charles A. Gersbach,1 Rebecca D. Dodd,2 David G. Kirsch.1 Duke University Medical Center, Durham, NC, 2University of Iowa, Iowa City, IA.

Genetically engineered mouse models (GEMMs) that employ site-specific recombinase (SSR) technology are important tools for pre-clinical studies, but this approach is costly and time-consuming. Here, we show that the CRISPR/Cas9 system can be used to efficiently complement existing GEMMs of sarcoma and generate primary sarcomas in wild type mice. Mice with the genotype KrasLSL-G12D+/−; Rosas2LSL-Cas9-EGFP/+ received intramuscular delivery of an adenvirus expressing Cre recombinase and a single guide RNA (sgRNA) targeting Trp53. Cre-mediated excision of oncogenic Kras and Cas9, in combination with CRISPR/Cas9-mediated knockout of Trp53, was sufficient to generate primary soft tissue sarcomas. These tumors arose with kinetics similar to those generated using the Cre-loxP system to active oncogene and delete Trp53 alleles. Additionally, we injected an adenosivirus containing Cas9 and sgRNAs targeting NFI and Trp53 into the sciatic nerve of wild type mice. These mice formed malignant peripheral nerve sheath tumors (MPNSTs) in the same time frame as MPNSTs generated using the Cre-loxP system to delete NFI and Ink4a/Arf alleles in GEMMs. These data demonstrate that CRISPR/Cas9 can be used to generate soft tissue sarcomas in wild type mice. Moreover, these results suggest that this technology can complement existing GEMMs for rapid assessment of tumor-modifying genes. These tools should decrease the time and expense associated with employing autochthonous mouse models for preclinical research.


Immunotherapy represents an extremely promising treatment approach for cancer patients. The success in this field has been signified by multiple recent approvals of checkpoint inhibitors across a range of cancer types. Subsequently, there is an increasing need to understand why certain patients and diseases benefit from these treatments while others do not, as well as how to maximize the benefits from these treatments. To address some of these questions, we used an allograft tumor model, which allows for the comparison of a panel of syngeneic models and treatment modalities. We have characterized baseline and treated tumor immune infiltrates for a panel of syngeneic models using flow cytometry. The correlation between responsiveness and effects of checkpoint inhibitors (e.g., anti-PD-1), and macrophage targeted agents (e.g., CSF1R inhibition) on immune infiltrates including tumor-associated macrophages (TAMs) were investigated in 6 syngeneic models. Furthermore, M1/M2 polarization of macrophages was investigated to explore the potential role of these cells in establishing an immunosuppressed tumor microenvironment that may affect response to therapy. Here, we show that the baseline immune cell population varies across models, and that immune checkpoint inhibitors and macrophage-targeted agents had variable efficacy across different tumor models. We also present the correlation of immune cell infiltration, including tumor infiltrating lymphocytes (TILs), TAMs, and responsiveness to immunotherapy intervention. Having an in-depth understanding of the immune make up of a model in which an immunomodulatory compound is screened is paramount for successful translation into the clinic. A well-characterized and fully profiled panel of syngeneic models such as the MusScreen platform allows for finding the right model for screening test compounds to be used alone or in combination with immunotherapy.

Development and characterization of the ultra immunodeficient B6;129-Rag2−/−R2R2−/−IL2rg−/−DwHsf (R2G2) mouse model. Sheryl J. Wildt, Jamie McClellan, Mandy Horn. Envigo RMS, Indianapolis, IN.
The "R2G2" (B6;129-Rag2tm1Wtm1Il2rgtm1Hdi/DwHsd) knockout mouse is the latest advancement to provide an alternative option in the highly immunodeficient mouse model category for the oncology, immunology and other biomedical research communities. This model was generated by backcrossing an Il2rg (common gamma) knockout model to a Rag2 (recombinase activating gene) knockout model. The resulting mouse lacks various cytokines including IL-2, IL-4, IL-7, IL-9 and IL-15. In addition, this model lacks B cells, T cells, NK cells and has a deficit in lymphocyte development. The R2G2 model is not only ultra immunodeficient, but provides a model that is less leaky and more tolerant to gamma radiation than traditional SCID models. This model is particularly useful for both tumor transplantation and humanization studies. Herein we describe the development and characterization of the R2G2 model, which includes breeding history, growth curve, CRC/Chemistry, flow cytometry and both hUHSC and huPMBC humanization studies.

#2813 An interactome analysis for personalized chemotherapy using PDX/NOG models of non-small cell lung cancer. Tsuyoshi Chijiwa, 1 Daisuke Komura, 2 Mizuha Haraguchi, 2 Akira Noguchi, 3 Hidemitsu Sato, 1 Hiroko Ito, 1 Haruhiko Nakayama, 4 Makoto Katayama, 4 Naoki Miyao, 5 Naruki Matsui, 6 Yuichi Tateishi, 7 Hiroshi Sueizu, 7 Yoshiyuki Nakamura, 6 Daisuke Furakawa, 6 Takayuki Isagawa, 6 Hiroto Katoh, 5 Shunpei Ishikawa, 5 Masato Nakamura, 6 Yohei Miyagi, 1 Saitama Medical Center, Ichi Medical University, Saitama, Japan; 3 Medical Research Institute, Tokyo Medical and Dental University, Japan; 4 Nihon Kookan Hospital, Saitama, Japan; 5 St. Marianna University School of Medicine, Saitama, Japan; 6 Kanagawa Cancer Center, Saitama, Japan; 7 Kawai-saki Municipal Hospital, Saitama, Japan; 8 Cancer Research Institute for Experimental Animals, Japan; 9 Research Institute, Kanagawa Cancer Center, Japan; 10 Tokai University School of Medicine, Japan.

Lung cancer is one of the most common malignant diseases in the world, and its prognosis is generally poor. It is crucial to elucidate the biological mechanisms underlying lung cancer and accelerate the development of new treatment strategies. Xenografts derived from engrafting fresh surgical specimens directly into immunodeficient mice have recently enabled the development of more relevant in vivo models of human cancers. These patient-derived xenograft (PDX) models, established by the direct transfer of tumor tissue, retain similar morphology, heterogeneity, and molecular signatures as the original cancers, and, thus, may be used in promising personalized medicine for cancer. We previously reported the rapid and efficient establishment of PDXs using super immunodeficient NOG mice (PDX/NOG model). In the present study, we analyzed the gene expression and cancer-stroma interaction profiles of PDXs established from NSCLC patients. We also discussed the possibility of individual PDX/NOG model simulations for personalized cancer chemotherapy. Ten NSCLC lines of PDX/NOG (Age 43-78 years; 8 men and 2 women; 7 adenocarcinoma, 1 adenosquamous carcinoma, 1 squamous carcinoma, 1 large cell carcinoma) were presented. In these cases, clinical information regarding chemotherapy for donor patients was retrieved where possible. Genome sequencing and comprehensive analyses of tumor-stroma interactions (Cancer-STromal Interactome analysis, CASTIN) were performed on mRNA. CASTIN showed tumor-stroma interactions in PDX/NOG comprehensively and quantitatively at the gene expression level by distinguishing gene arrangements in human tissue (Cancer) from mouse tissue (Stroma) as signal strengths (ligand dependency (%) / receptor dependency (%)). Interactions of EGFR(cancer)-EGFR(stroma) were observed from 1.2 (15%/0%) to 11.5 (94%/80%) as widely-distributed. Inclusion: Collectively, our PDOX models using needle biopsy specimens can reflect the pathological and molecular features and drug responsibility of a patient’s tumors. PDOXs have been generated by subcutaneously implanting surgically resected tumor fragments into immunodeficient mice. Unfortunately, 80% of pancreatic cancer patients is unresistant and they show highly aggressive and metastatic tumors. In most cases, diagnoses are confirmed histologically and diagnosed through endoscopic ultrasonography-guided/fine-needle aspiration (EUS/FNA) or percutaneous liver biopsy. However, they are not suitable for subcutaneous implantation into mice due to their threadlike shape and very small size. Therefore, the lack of patient-derived model system in pancreatic cancer is in desperate need of new strategy to cover unresectable patients as well as resectable. Here, we pioneered the development of a unresectable patient-derived orthotopic xenograft (PDXO) model satisfied with short period and high success rate, and made the best use of them as the test model for drug response. Methods and Results: PDXO models were established with a technique of direct surgical orthotopic implantation into the pancreatic tail of Athymic nude mice. PDOXO showed the highest rate of engraftment, nearly 40% (17 cases among 44 cases) in patients with a time to progression of <6 months. The histopathological characteristics of the PDOX tumors are similar with those of the patient’s original tissues. Additionally, genetic alterations were retained between patient tissues (F0) and PDOX tumors (F1–F3) from the mutation analysis using a comprehensive cancer panel. For drug responsibility, we selected refractory or sensitive PDOXO groups to Gemcitabine and Abraxane based on their clinical information. As results after treatment with Gemcitabine, Abraxane and combination, selected PDXO models showed the drug sensitivity significantly correlated with the drug responsibility of original patients. Tumor growth was monitored by animal MRI every 2 weeks during 2 months. Typically, xenograft models using immobilized pancreatic cancer cell line, CF-PAC-1, showed tumor regression to all treatment set. Conclusion: Collectively, our PDXO models using needle biopsy specimens can reflect the characteristics of unresectable patients and give the expectation the drug responsibility. They furthermore might be valuable in a screening and development system for targeted therapeutics, which can be applied to a wide range of pancreatic cancer patients.
#2817 Enriched environment inhibits progression of acute myeloid leukemia. Run Xiao, Stephen M. Bergin, Michael A. Caligiuri, Lei Cao. The Ohio State University Comprehensive Cancer Center, Columbus, OH.

Background: Environmental factors and lifestyle have profound effects in the initiation, promotion and progression of cancer. Our recent work on environmental enrichment (EE), a housing environment boosting mental health, has revealed a novel phenotype characterized by a robust reduction in adiposity, resistance to diet-induced obesity (DIO), enhanced insulin sensitivity, lower serum IGF-1 and leptin levels, higher serum adiponectin level, enhanced immune functions, and inhibition in melanoma and colon cancer growth. We have teased out one key mechanism underlying the anticancer effect of EE: the stimulation of brain-derived neurotrophic factor (BDNF) expression in the hypothalamic-sympathoneural-adipocyte (HSA) axis. However, the EE’s effects on hematologic malignancy are largely unknown. Here we generalized this intervention to acute myeloid leukemia (AML) using the MLL-partial tandem duplication (PTD) and Flt3-internal tandem duplication (ITD) double knock-in mouse (MLLP KD/ITD KD). This recapitulates features of counterpart human AML. Methods: For transplantation of AML cells, Ly5.2 spleen cells isolated from leukemic MLL-PTD/WT, Flt3ITD/WT mice were resuspended in PBS. Syngeneic Ly5.1 recipient C57BL/6 male mice (CD45.1 +) were sublethally irradiated (400 cGy). Five to six hours later, leukemic cells (4 × 10^6 per mouse) were injected through the lateral tail vein. Those mice were randomly assigned to either standard laboratory cage (SE) or EE housing. To monitor AML progression, white blood cell (WBC) counts were measured from peripheral blood. Survival experiments were performed. Results: EE mice had a median survival 14% longer than SE mice (SE = 36 ± 1.1 days, EE = 41 ± 1.2 days, p = 0.038) and improved hazard rate (log-rank test z = 1.99, p = 0.047). Based on WBC counts (>10.9 k/μL), 100% of SE mice and 80% of EE mice had apparent AML at three weeks post injection. At this time point, EE had a substantially lower WBC counts (SE = 76.9 k/μL, EE = 28 k/μL, p = 0.036). WBCs from EE mice had lower neutrophil, monocytes, eosinophils and basophils counts, but had similar lymphocytes, resulting in a reduced neutrophil to lymphocyte ratio. Red blood cell (RBC) and platelet data were the same except for a slightly reduced mean corpuscular hemoglobin concentration (MCHC) in EE mice. At four weeks, EE mice were substantially more active and mobile than SE mice. At five weeks, at which point 3 SF and 5 EE mice remained alive, WBC counts remained lower in EE mice. Conclusions: This preliminary work demonstrated EE substantially improved progression-free survival and conferred a significant survival benefit. We will elucidate the underlying mechanisms of EE’s anti-AML effect by analyzing metabolic modulation and immune regulation.

#2818 PDX model of the enriched pleural effusion from advanced lung cancer patient for testing on targeted drug sensitivity and resistance. Feifei Zhang,1 Ke Wang,1 Wenhua Xu,1 Yang Yang,1 Kedong Ouyang,1 Shun Lu,1 Danyi Wen,1 Taiping Chen1. 1LIDE Biotech Ltd, Shanghai, China; 2Shanghai Chest Hospital, Shanghai, China.

Aiming for treatment of lung cancer, we observed the pleural effusion of lung cancer patients is selectively sensitive to available targeted therapeutics and often developing resistance to the early sensitive drug treatment. Surgery tumor tissues are usually not available to establish patient derived xenograft (PDX) models which is critical to evaluate drug sensitivity and resistance. We applied CD45 magnet beads to enrich the tumor cell population from pleural effusion specimens of advanced lung cancer. Seven PDX models were successfully established in this way. Based on genetic profiling of these models, TKI drug related EGFR mutations and ALK fusion were confirmed (EGFR: L858R, T790M, Exon 19 insertion; ALK fusion). Further in vivo efficacy studies were designed to test on multiple drug sensitivity and resistance including Gefitinib, Erlotinib, Ceritinib, Alecrtinib, AZD9291. Tumor growth inhibition (TGI) were well matched with corresponding gene mutations among the models. In particularly, T790M model was resistant to Erlotinib and quite sensitive for AZD9291. In conclusion, the models established here are very useful to test on potency of many current investigational TKI drugs and provide base for drug resistant model establishment.

#2819 Intestinal estrogen receptor beta attenuates colon cancer signaling pathways in vivo. Linea Petterson,1 Ashish Saxena,2 Tran Vu,3 Jan-Åke Gustafsson,2 Amena Archer,1 Cecilia Williams1. 1Karolinska Institute, Stockholm, Sweden; 2Center for Nuclear Receptors and Cell Signaling, Houston, TX; 3Scilifelab, Stockholm, Sweden.

Colorectal cancer is the third most common form of cancer and the second leading cause for cancer deaths. Several epidemiological, in vitro and in vivo studies suggest that Estrogen receptor β (ERβ) could be a potential target for CRC prevention and treatment, but the potential mechanism is not demonstrated. ERβ is expressed in intestinal epithelial cells, as well as in some intestinal immune cells and other tissues. Previous studies have seen increased CRC after full ERβ knockout, but it is not clear if this is mediated by intestinal ERβ. The specific aim of our study is to determine the role of intestinal ERβ during colon cancer development in vivo. We deleted ERβ specifically in the intestine epithelium of mice (IERβKO), and induced CRC using Aoxymethane (AOM) and Dextran Sulfate Sodium (DSS) treatment. After 9 weeks treatment, all mice presented colitis and two iERβKO mice but none of the wild-type (WT) mice had developed tumors. qPCR showed that the expression of pro-inflammatory markers was higher in iERβKO treated mice than in untreated or WT mice. Immunohistochemistry (IHC) of proliferative markers showed that iERβKO exhibited higher cell proliferation in the top of the crypts compared to controls. After 16 weeks treatment, all mice developed tumors. qPCR showed that the expression of mesenchymal markers was higher in treated iERβKO mice compared to treated WT mice. Our results demonstrate that intestinal ERβ attenuates colon cancer-related pathways.

#2820 Use of a genetically engineered mouse model and RNA sequencing to identify genes that are aberrantly regulated by mutant p53 in prostate cells following irradiation. Ruth L. Vinall,2 Qian Chen,2 George Talbott,3 Neil Hubbard,2 Clifford Tepper, Alexander Borowsky,1 1California Northstate Univ., Elk Grove, CA; 2University of California, Davis, Sacramento, CA; 3California Northstate University, Elk Grove, CA.

Our group has previously demonstrated that the Trp53 R270H mutation can drive prostate cancer (CaP) initiation in a genetically engineered mouse model, and that the human equivalent, TP53 R273H, can promote development of castration resistant growth of LNCaP cells as well as resistance to commonly used therapeutic agents. The primary objective of the current study was to identify genes that may contribute to the development of these gain-of-function phenotypes. Wildtype mice and mice that were heterozygous or homozygous for the Trp53 R270H mutation (referred to as Trp53 +/+ or Trp53 R270H/R270H mice respectively) that were ~2 months old were exposed to 5 Gy radiation to activate and stabilize p53, consequently increasing its expression. Mouse prostate were harvested 6 hours post-irradiation and either processed for subsequent histological/immunohistochemistry (IHC) analysis or snap-frozen for subsequent RNA extraction and transcriptome profiling with RNA-sequencing (RNA-Seq) analysis. P53 expression data was processed to quantify transcript levels and to assess differential gene expression between the 3 groups. PIN lesions were observed in 3-month-old Trp53 R270H/R270H mice prostates, but not in Trp53 +/+ or Trp53 R273H/R270H mice prostates. IHC analysis demonstrated that p53 was stabilized in the...
majority of prostate cells from Trp53+/+; Trp53+/−/R270H, or Trp53 R270H/R270H mice 6 hours post-irradiation. RNA-Seq analysis of RNA isolated from irradiated mice prostates identified 1,444 genes that were differentially expressed in Trp53+/+ versus Trp53 R270H/R270H mice prostate cells, and 796 genes that were differentially expressed in Trp53+/− versus Trp53+/+ R270H mice. Statistically significant differences in gene expression between the 3 groups were observed for 1,378 genes, including a number of p53 target genes, such as Cdkn1a, Bax, Bcl2, Kras, Mdm2, and Id4. We data identify multiple genes that may contribute to prostate cancer initiation and/or progression through p53 gain-of-function and loss-of-function mechanisms. It is possible that further analysis of these genes may lead to the development of new therapies and/or biomarkers for prostate cancer patients as well as guide the usage of currently available therapies in men at risk of developing CaP and CaP patients who harbor TP53 mutations.

#2821 Impact of SCID mouse gender on tumorigenicity, xenograft growth and drug-responses in patient derived orthotopic xenograft models (PDOX) of malignant brain tumors. Lin Qi, 1 Mari Kogiso, 1 Yuchen Du, 1 Huiyuan Zhang, 1 Frank Braun, 2 Holly Lindsay, 1 Sibo Zhao, 1 Sarah Injac, 1 Patricia Baxter, 1 Zhigang Liu, 1 Yuqing Zhang, 1 Jack Su, 1 Adekunle Adesina, 1 Andrew Walter, 1 Jeffery Murray, 1 Javad Nazarian, 1 Will Parsons, 1 Murali Chintagumpala, 1 Xiao-nan Li, 1 Texas Children’s Cancer Center, Houston, TX.

Brain tumor is the leading cause of cancer related death in children. To develop new therapies and to understand tumor biology, many efforts are made to develop patient derived orthotopic xenograft mouse models (PDOX). There are, however, concerns about the potential impact of animal genders on tumor take rate, xenograft growth and in vivo drug responses. To systematically analyze such impact, we retrospectively examined >1,000 mice of 59 PDOX models, including glioblastoma (GBM), medulloblastoma (MB), primitive neuroectodermal tumor (PENT) and ependymoma (EPN) and diffuse intrinsic pontine glioma (DIPG), in which there were age-matched (within 2 wks differences) with relatively even numbers of male and female animals. All the animals were injected with identical number of tumor cells (1x10⁵) in the mouse brains matching that in human patients. Animals were monitored daily and euthanized when they were moribund. Differences between male and female mice were analyzed by t-test. In 25 GBM models (15 adult and 10 pediatric) involving 419 mice, there was an average of number of mice was 17.8±9.5 mice per model (male-female = 9.5±4.3:7.7±1.3) (P<0.05). The tumor take rate was near ≥95% in both male and female mice (P>0.05). In GBM models, the survival times was 94.1±21.7 days in male mice and 92.3±20.8 days in female mice (P=0.05); whereas in MB models, they were 134.9±21.7 days in male and 121.0±31.6 days in female mice (P<0.05). The impact of animal gender on drug responses in vivo is essential to the preclinical drug testing. A total of 468 mice from 11 GBM, 5 MB, 3 EPN, 3 DIPG and 2 PNET were treated a series of standard and investigational drugs/compounds, including radiation, oncolytic virus (SVV-001), ABT888, BM1-inhibitor, MN18237, TMZ, Flavopiridol, SYC-435, SYC-719, SYC-836, Olig 2 inhibitor, VPA, SAHA, Echinomycin, MC8613, and PARP. The overall survival times were 17.63±2.83 days in male mice (P<0.05). The only model that exhibited the increased responses in male mice was found in IC-2305GBM treated with TMZ and PTC-596 (P<0.05). In conclusion, our data demonstrated that the gender of SCID mice does not have major impact on animal development nor in drug responses, and SCID mice of both genders are appropriate for brain tumor PDOX model development.

#2822 Establishing the use of Vortex technology for investigating circulate tumor cells in mice models of breast cancer. Vishnu Raman, 1 Rakihi Gupta, 1 Melanie Triboulet, 1 Corinne Renier, 2 Steve C. Crouse, 2 Elodie Sollier-Christen, 2 Stefanie S. Jeffrey, 1 Stanford University, School of Medicine, Stanford, CA; 2Vortex Biosciences, Menlo Park, CA.

Background: Circulating tumor cells (CTCs) are precursors of metastatic disease and are an important indicator of the disease progression and outcome of many cancers. Though several recent studies have advanced our understanding of CTCs, many critical areas of CTC biology remain largely unexplored. Murine models of cancer offer a unique opportunity to address this issue. However, small sample volumes, low number of CTCs, difficulty to access ports for blood collection are challenges that diminish the utility of mouse models for studying CTCs. In our previous studies, we had validated the use of Vortex technology for the label-free capture of CTCs from blood samples of different cancers. In this current study, we now report our recent, unpublished data on the adaptation of Vortex platform for enriching and characterizing murine and human tumor cells from mouse blood. Methods: By spiking tumor cells into small volumes of mouse blood (200–400 μl) from cardiac puncture, we tested the impact of i) different dilutions of mouse blood (10X, 20X, 40X); ii) varying numbers of tumor cells; iii) different cell types (MDA-MB-231, 4T1 and EMT6); and iv) sample recyling on capture efficiency and purity. Blood was processed through the Vortex plastic chip and, to accurately identify and enumerate the enriched population, specific cytokerin, vimentin, and CD45. Results: Our results reveal that over a range of dilutions tested, just after one cycle, 10X dilution of mouse blood was superior, yielding a capture efficiency of 38.83% and a purity of 33% for MDA-MB-231 cells, compared to 20X (34.16% efficiency, 31.1% purity), and 40X (31.83% efficiency, 31% purity) dilutions. We are currently testing whether recyling the same cell blood through the enrichment protocol that label-free capture of CTCs. Even when as many as ~7500 tumor cells were spiked in mouse blood, the Vortex platform was able to successfully enrich tumor cells with high consistency and purity. Importantly, we were now able to successfully isolate human and murine tumor cell lines with varying levels of epithelial cell adhesion molecule (EpCAM) expression (4T1 - high, MDA-MB-231 - moderate to low) spiked into mouse blood. Experiments confirming the viability and growth rate of cancer cells isolated from mouse blood using MTT assay will also be presented. Conclusion: In summary, our results reveal the use of the Vortex technology for studying CTCs in murine models of cancer, with the ability to handle volumes of blood as low as 200UL while having a capacity to capture extremely large number of CTCs. While this study was focused on breast cancer, our workflow is easily adapted to other tumor systems for future biologic and drug testing studies.

#2823 Immunodeficient mice differentially sensitize to estrogen and exhibit severe estrogen-related adverse effects in orthotropic breast cancer model. Tiina E. Kähkönen, 1 Mari I. Suominen, 1 Jenni Mäki-Jouppila, 1 Jussi Halleen, 1 Azusa Tanaka, 2 Michael P. Seiler, 2 Jenni Bennoth, 2 Pharmaceut Service Ltd, 3D, Tansu, Fines, 1Juli, 2Hollering. 1Proceedings of the American Association for Cancer Research.

Estrogen-induced growth induction and stimulation of hormone receptor positive breast cancer is commonly acknowledged and thus external estrogen stimulus is used in animal models to support and accelerate growth of the tumors. However, estrogen is known to have adverse effects in the animals and the experiments may have to be preliminary terminated due to animal welfare issues. The aim of the study was to characterize and to compare estrogen caused adverse effects in athymic nude and NOG mice in orthotopic breast cancer model. Athymic nude (Hsd:Athymic Nude-Foxn 1nu) and CIEA NOG (NOD.Cg-PkdcscidIl2rgtm1Sug/JicTac, provided by Taconic Biosciences) were implanted with estrogen (17β-estradiol, E2) releasing hormone rods (5 μg/day) prior to cancer cell inoculation. 2x10⁵ BT-474 (ER, PR and HER+) human breast cancer cells were inoculated to left inguinal mammary fat pad of the mice and the tumor growth was monitored by measuring the tumor volumes twice a week by caliper. The clinical condition and weight development of the mice was carefully monitored with special attention to E2-caused adverse effects. If the mice met the termination criteria (i.e. significant weight loss or general worsening of the overall health) they were sacrificed individually before the intended end of the study. Nude mice exhibited severe estrogen-related adverse effects as no adverse effect were observed in NOG mice. Most common adverse effect was redness and skin lesions in the urogenital area observed in 63% of the animals. More severe effects were observed in the lower urinary tract including urinary stones and bladder obstruction in 67% of the mice. Also internal effects were observed including appearance change in kidneys and spleen in size and color. Due to severity of these effects 50% of the mice had to preliminary sacrificed. Tumor take (100% vs 85%) and final tumor volumes in average (110 mm³ vs 120 mm³) were comparable between nude and NOG mice. A clear mouse strain specific impact was observed in the study. Nude mice sensitive to estrogen and exhibit severe estrogen-related adverse effects as no effects were observed in NOG mice. In breast cancer studies where E2 supplementation is needed for sufficient tumour growth, the choice of mouse strain should be carefully considered. Special attention should be paid to possible adverse effects keeping in mind the animal welfare issues.

#2824 Validation of a panel of patient-derived xenograft models for prostate cancer and cell line models for preclinical drug evaluation. Nektaria Panagopoulos, 1 Jane Wrigley, 1 Andrew McKenzie, 2 Jason King, 1 Louise Wainwright, 1 Kelly Jones, 1 Anne Collini, 2 Rajendra Kupari, 2 Crown Bioscience UK, Loughborough, United Kingdom; 3University of York, York, United Kingdom.

Background: Prostate cancer is the second most common cancer worldwide for males, and the fourth most common cancer overall, with more than a million new cases diagnosed. However, progress toward understanding the biology of...
prostate cancer and the development of new therapies has been hampered by the lack of in vivo models that adequately represent the spectrum of benign, latent, aggressive, and metastatic forms of the human disease. Here we report the validation of a panel of patient-derived xenograft (PDX) models and their utilization in preclinical studies alongside cell line models of prostate cancer. Methods: Prostate tumors obtained from surgery were collected with ethical consent, disaggregated and established subcutaneously in Rag2−/−γc−/− mice (The Jackson Laboratory) to generate PDX models. Tumor material was diagnosed on the basis of operative histology and immunohistochemistry (IHC for PSA, androgen receptor expression). Tumor growth in both Rag2−/−γc−/− and NSG mice was evaluated in comparison to cell line models such as LNCaP and PC3M. Briefly tumors were measured 3 times a week and tumour volumes were estimated using the formula 0.5(LxW^2) by measuring the tumour in two dimensions using electronic callipers for the duration of the study. For orthotopic PC3M, tumour growth was checked once weekly by bioluminescent imaging (BLI). Briefly, the mice were injected (s.c.) with 150μg/kg D-Luciferin prior to imaging, anaesthetised following administration of D-Luciferin and placed into the imaging chamber (Spectrum CT) and imaged for luminescence (ventral view). Results: We have established a bank of transplantable prostate cancer PDX and stocks are maintained in liquid nitrogen which can be resuscitated in both Rag2−/−γc−/− and NSG mice. Two models were reported to be hormone sensitive and 2 models represented castrate resistant prostate cancer (CRPC) one of which has a TMPRSS-ETS fusion. In comparison cell lines derived from the same mutation, the CRPC xenograft grows slower than the corresponding cell line model. PC3M CRPC orthotopic model develops metastasis in the liver, lungs, lumbar lymph nodes, fore limb and hind limbs representing the late stage of cancer. Conclusions: We have characterised both cell and patient-derived prostate cancer xenograft models which will provide a clinically relevant platform spanning the different stages of the disease for preclinical drug evaluation.

#2825 Gene expression profile in genetically engineered mouse model of lung cancer correlates with that in human lung adenocarcinoma. Imyavarmanan Lakshmanan, Satyanarayana Rachagani, Moorthy Ponnapusa, Seema Chugh, Samikshan Dutta, Sakhthivel Muniyan, Surinder Batra, Aparvaramban Lakshmanan, Satyanarayana Rachagani, Moorthy Ponnusamy, Rag2−/− and Rag2+/+ mice were injected intraperitoneally with human lung adenocarcinoma xenografts were successfully established after intraperitoneal injection of OE19 cells. The median survival time of these animals was the shortest (45 days for 10X10^6 cells). In addition, median survival was significantly increased after paclitaxel treatment compared with the control group (57 days versus 45 days, p<0.0034) along with a significant decrease of the relative subcutaneous tumor volume (p=0.023). Conclusion: Peritoneal esophageal adenocarcinoma xenografts were successfully established after intraperitoneal injection of OE19 cells. This animal model of peritoneal dissemination for survival outcome will provide a useful survival outcome assessment model for the preclinical evaluation of cancer therapeutics in esophageal adenocarcinoma.

#2827 Establishing a mouse model to investigate GRB7-mediated signaling in development and triple-negative breast cancer. Christopher A. Lofgren, David R. Meier, Steven E. Cash, Parac S. Kenney, Gunderon Medical Foundation, La Crosse, WI.

Triple-negative breast cancer (TNBC) lacks expression of the estrogen and progesterone steroid receptors and HER2, thus TNBC patients cannot benefit from the recent successes of targeted therapies, and instead rely on cytotoxic chemotherapeutic combinations. As such, tumor recurrence is high and patient prognosis is poor. GRB7 is a cytoplasmic adapter protein required for cell migration, invasion, and proliferation in TNBC. We have previously reported that a high GRB7 mRNA level was the strongest predictor of tumor recurrence in a cohort of 246 TNBC patients treated with doxorubicin. GRB7 was confirmed in a small, independent cohort as an indicator of poor prognosis. After the observation of both decreased doxorubicin sensitivity and elevated GRB7 expression levels in TNBC tumors, we hypothesized that GRB7 was mediating a signaling pathway critical for drug resistance and cell survival. To determine the role of GRB7 in mouse development and tumorigenesis, the current study focuses on the Grb7tm1a.xc3x0.1 mouse, in which the insertion of the I12.L2 B act/p csetase upstream of the critical exons of GRB7 renders an effective knockout of GRB7, and the inclusion of loxp sites flanking critical exons of GRB7 facilitates the creation of tissue-specific, conditional GRB7 deletions. Herein, we investigate GRB7 expression in the homozygous Grb7tm1a.xc3x0.1 mouse and derivative tissue-specific deletions, and evaluate their suitability for modeling GRB7-mediated events in mammary gland development and tumorigenesis in a TNBC model. Primary cell lines from the Grb7tm1a.xc3x0.1 mouse and breast cancer cell lines will then be used to delineate the effects of altered GRB7 levels on the mechanism of developmental and TNBC cell signaling, respectively.

TUMOR BIOLOGY: Gene Expression in Metastatic Progression

#2828 Role of NEFL in aggressive prostate cancer progression. Tanya C. Burch, Ian O. Oduor, Dean A. Troyer, Julius O. Nyalwidi, Eastern Virginia Medical School, Norfolk, VA; Old Dominion University, Norfolk, VA.

Introduction: Esophageal adenocarcinoma (EAC) has become the dominant type of esophageal cancer in United States. EAC is the fastest growing cancer in the western world and the overall 5 year survival rate of EAC is below 20 percent. Most patients present with locally advanced or widespread metastatic disease, where current treatment is largely ineffective. Prognosis for EAC patients remains poor even with combination therapies due to high resistance to chemotherapy. Therefore, new therapeutic approaches are urgently needed. Improvement of esophageal adenocarcinoma outcome requires well-characterized animal models in which to evaluate novel therapeutics. In this study we aimed to establish a peritoneal dissemination xenograft mouse model of esophageal adenocarcinoma that would support survival outcome analyses. Methods: To find the best candidate cell line from 7 esophageal adenocarcinoma cell lines of various origin, we injected them intraperitoneally and subcutaneously into severe combined immunodeficiency (SCID) mice and examined the tumor progression and survival outcome. Human esophageal adenocarcinoma cell lines of Caucasian/Hispanic origin ES026, OE33, ES051, SK-GT-2, OE19, OACM5.1C and Flo-1 originating from gastroesophageal junction, distal esophagus and gastric cardia/fundus were injected intraperitoneally/subcutaneously into SCID mice. The peritoneal/xenograft tumor formation and mouse survival were compared among different groups. Results: All cell lines injected subcutaneously formed tumors within 3 months at variable rates. All cell lines except OACM5.1C formed intraperitoneal tumors within 3 months at variable rates. Median animal survival with peritoneal dissemination was 108 days for ES026 cells (5X10^6), 65 days for OE33 cells (5X10^6), 88 days for ES051 cells (5X10^6), 76 days for SK-GT-2 cells (5X10^6), 55 days for OE19 cells (5X10^6), 45 days for OE19 cells (10X10^6) and 82 days for Flo-1 cells (5X10^6). Interestingly, only in the OE19 model all mice (7/7 for 5X10^6 and 5/5 for 10X10^6) developed bloody ascites with liver metastasis after intraperitoneal injection. The median survival time of these animals was the shortest (45 days for 10X10^6 cells). In addition, median survival was significantly increased after paclitaxel treatment compared with the control group (57 days versus 45 days, p<0.0034) along with a significant decrease of the relative subcutaneous tumor volume (p<0.023). Conclusion: Peritoneal esophageal adenocarcinoma xenografts were successfully established after intraperitoneal injection of OE19 cells. This animal model of peritoneal dissemination for survival outcome will provide a useful survival outcome assessment model for the preclinical evaluation of cancer therapeutics in esophageal adenocarcinoma.
Introduction: Prostate cancer (PCa) is the most prevalent cancer amongst men and the second most common cause of cancer-related deaths in the US. Prostate cancer has a heterogeneous spectrum of clinical outcomes with phenotypes ranging from indolent asymptomatic cases to very aggressive metastatic and lethal forms. The development of aggressive castration-resistant prostate cancer (CRPC) is currently the most common late-stage outcome of prostate cancer, and prostate cancer is the second most common cause of cancer-related deaths in men. Understanding the molecular mechanisms that drive the transition from localized disease to metastatic disease is crucial for the development of targeted therapies for both early detection and advanced disease.

To understand the biologic basis of PDA metastatic subclone formation we hypothesized that metastatic disease is the most common cause of death in pancreatic ductal adenocarcinoma (PDA) patients. The most common sites of metastatic spread of PDA are to the liver, lung, and peritoneum. The acquisition of advanced stage PDAs is also accompanied by a dramatic increase in distant metastatic disease. We sought to establish a transgenic mouse model of PDAbased on heterozygous inactivation of p16 and Trp53 alleles (KPTC mice). This model revealed that metastatic efficiency and organotropism are related but independently controlled phenomena by hormone receptors, clinical treatment and response and results from biochemical analyses of tissue samples. Tumor markers (e.g., estrogen and progestin receptors, EGF receptor, HER-2/neu oncoprotein) guided tissue selection. Structural integrity of a tumor marker positive sample was assessed and the second intact tissue section was processed identically to that of the section used for LCM. RNA was assessed for integrity, purified and subjected to novel analyses. RNAseq and WGCNA performed in liver samples from patients indicating their clinical relevance. Portal vein colonization as a direct mode of access to the liver was observed in both mice and human samples of liver and cancer cell lines and prostatectomy tissues with different malignancy phenotypes. Validation of differential NEFL expression has been done by Western blot, qRT–PCR, IHC and mass spectrometry. Functional assays and oncogenic properties have been assessed after siRNA mediated knockdown of NEFL gene expression in prostate cancer cell lines. Results: We demonstrate significant correlations between NEFL expression and the acquisition of aggressive PCAs with neuroendocrine-like phenotypes. This was validated by Western blot and qRT–PCR. SiRNA knockdown of NEFL suppresses the invasive and migratory capacity of PCa cells and tumor colony formation in soft agar assays. The molecular interactome of NEFL was characterized by LC/MS/MS. To our knowledge, this study is the first to demonstrate that NEFL up-regulation correlates with a subset of aggressive prostate cancer disease.

Conclusions: We demonstrate that NEFL is differentially expressed in PCa cells and prostatectomy tissues with a significantly higher abundance in cells with clinically aggressive and lethal neuroendocrine-like malignancy phenotypes. These findings are currently being validated using disease stratified tumour microarrays. Further studies are focusing on uncovering the mechanisms that are modulated by NEFL and are responsible for PCa disease progression. These could assist in the development of novel diagnostic and therapeutic strategies for the disease.

#2830 Secreted protein acidic and rich in cysteine antigen and autoantibodies in sera of prostate cancer patients: Potential use in diagnosis/prognosis. Anshu Rastogi,1 Andy Martinez,2 Amina Ali,1 Shy-Han Tan,1 Jennifer Cullen,1 Yongmei Chen,1 Gyorgy Petrovics,3 Albert Dobi,1 Lakshmi Ravindranath,1 Denise Young,1 Isabel Sesterhenn,1 Jacob Kagan,1 Sudhir Srivastava,4 David McLeod,1 Inger Rosner,5 Shiv Srivastava,4 Alagarsamy Srinivasan7. 1Cancer Center and Genomic Medicine, Icahn School of Medicine at Mount Sinai, New York, NY; 2Thermo Fisher Scientific, Austin, TX; 3Memorial Sloan Kettering Cancer Center, New York, NY; 4Uniformed Services University of the Health Sciences, Bethesda, MD; 51Thermo Fisher Scientific, Austin, TX; 6Uniformed Services University of the Health Sciences, Bethesda, MD; 71Thermo Fisher Scientific, Austin, TX.

Objectives: Improvements in blood based biomarkers for detecting clinically significant prostate cancer (CaP) or for distinguishing between indolent and aggressive CaP are critical in enhancing the management of this most common non-skin cancer of men in the US. These biomarkers include tumor associated antigens (TAAs) and autoantibodies (AAbs) against TAAs in patient sera. SPARC/Osteonectin is highly expressed in metastatic cancers (glioblastoma, melanoma, breast cancer and prostate cancer) and promotes bone metastasis and epithelial–mesenchymal transition. Our comparative transcriptome analyses of well/moderately differentiated CaP with poorly differentiated CaP, defined SPARC as central node in the network of genes with common regulatory elements (NKKH_NKKH_HOX) associating with poorly differentiated CaP. Further, we reported associations of high levels of SPARC mRNA or protein with Gleason 8-10 or poorly differentiated primary tumors and association with metastatic progression. The aims of this study are: i) Quantify SPARC antigen in the sera of CaP patients; ii) Are AAbs against SPARC present in the serum of CaP patients?; iii) Is there a correlation between SPARC AAb level in patient sera and disease status? iii) Does SPARC AAb level vary according to ethnic groups? Methods: Sera from CaP patients and healthy controls were evaluated for SPARC antigen using a commercial enzyme-linked immunosorbent assay (ELISA) kit. AAbs against SPARC were determined by ELISA utilizing recombinant full-length human SPARC protein as a substrate. For evaluation we used Caucasian American (CA, n = 117) and African American (AA, n = 111) CaP patients comprising Gleason 6-10, and healthy controls (CA, n = 52; AA, n = 45). Results: SPARC antigen levels in the sera showed a difference in the overall case versus control groups (p = 0.0016) and this trend was observed only in AA (p = 0.0015) in comparison to CA patients (p = 0.1709). SPARC AAbs were detected in the sera and the values were significant in the overall case versus control (p < 0.0001) and also in CA (p < 0.0001) and AA (p < 0.0001) CaP patients with levels significantly lower in patients compared to controls. AAb reactivity for CaP patients was similar between CA and AA groups. Conclusions: This study demonstrated the presence of AAbs against SPARC in CaP patient serum for the first time. Of note, highly significant differences were noted between CaP patient (low) and controls (high) sera, across different ethnic groups. These data further suggest evaluation of SPARC AAbs as a promising serum biomarker for CaP.

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#2831 Identification of cell specific expression signatures using NGS global gene expression profiles from LCM procured breast carcinoma and adjacent stromal cells. Jeffrey I. Schagaman,1 Kristi Lea,1 Sarah A. Andres,2 Kelli S. Bramlett,1 James L. Wittliff3. 1Thermo Fisher Scientific, Austin, TX; 2Univ. of Louisville Health Sciences Ctr., Louisville, KY.

Our goal is to determine relationships of gene expression profiles to patient-associated characteristics, breast carcinoma pathology and biomarker status with clinical outcome to improve assessment of prognosis and therapy selection. Procedures: De-identified frozen tissue biopsies of human breast carcinomas which were collected and stored under stringently controlled conditions were employed. Patient-related features (e.g., age, race, menopausal status, nodal status, clinical treatment and response) and results from biochemical analyses of established tumor markers (e.g., estrogen and progesterin receptors, EGF receptor) were collected and stored under stringently controlled conditions. Tissue was first evaluated by light microscopy of H&E stained sections. To decipher uniqueness and clinical utility of gene expression profiles from specific cell types, Laser Capture Microdissection (LCM) with a PixCell IIeTM (Arcturus®, Thermo Fisher Scientific) was used to non-destructively collect either cancerous cells or adjacent stromal cells on CapSureTM LCM caps. A second intact tissue section was processed identically to that of the section used for LCM. RNA was assessed for integrity, purified and subjected to novel analyses using the Ion AmpliSeqTM RNA Breast Cancer Research Panel containing 1174
genes. Sequencing workflow included templating on the Ion Chef™ System and multiplexed sequencing on the Ion S5™ XL sequencing system and Ion 540™ chip. Results: Each sequencing library yielded approximately 4-5 million reads with a mapping rate of >98% that was achieved after alignment to the human transcriptome. Each sample was sequenced in triplicate with technical reproducibility as a mean of 59.6% ± 4.9% of reads. Bioinformatic analyses of gene expression profiles revealed clear distinctions between stromal and carcinoma cells procured by LCM compared to intact tissue driven by strongly differentially expressed genes such as vimentin (VIM), MRPRP, stanniocalcin-2 (STC2) and estrogen receptor beta (ESR2). Conclusions: Collectively, the results indicate that molecular signatures of breast carcinoma cells procured by LCM reveal unique gene expression profiles compared to those of the intact tissue section or the adjacent stromal cells. These novel findings are likely to discern molecular features unique to a breast carcinoma that may be related to clinical outcome to improve assessment of patient prognosis and therapy selection. Supported in part by a grant from the Phi Beta Psi Charity Trust (JLW & SAA) and a CTSA Award from the Commonwealth of Kentucky (JLW). For research use only.

#2832 Class I histone deacetylase inhibitors impair tumor growth and metastatic programs. Oliver H. Krämer, Nicole Kiweler. Institut für Toxikologie Universitätmedizin der Johannes Gutenberg-Universität Mainz, Mainz, Germany.

(a) introductory sentence indicating the purposes of the study Metastasis formation is associated with poor patient prognosis. Epithelial-mesenchymal and mesenchymal-epithelial (EMT/MET) transition cycles are key for the formation, survival, and homing of metastatic cells. Inhibitors against epigenetic modulators of the histone deacetylsy family (HDACi) are promising, clinically tested epigenetic drugs that may combat cancer proliferation and spread. (b) brief description of pertinent experimental procedures We chose a systems toxicology approach to analyze the relevance of HDACs and their inhibitors for metastatic cell growth. We combined in vivo tests with morphological, cellular, functional, and global proteomic analyses. This strategy follows the 3R principle by Russel and Burch. Of the four classes of HDACs (I-IV), the class I subgroup is most important for tumorigenesis. We therefore analyzed how the pharmacological inhibition of class I HDACs affects the growth and spreading of syngeneic kidney cancer cells into the lungs of BALB/c mice and how such drugs affect tumor cell growth, apoptosis, migration, and gene expression patterns controlling EMT/MET in transformed kidney and breast cells. Furthermore, we investigated how the genetic elimination of HDACs affects cancer cell growth and EMT/MET. We used flow cytometry, immunoblot, immunofluorescence, qPCR, proteomics on a global scale, RNAi against the class I HDACs HDAC1/ HDAC2, and in vivo tumor analyses. (c) summary of the new, unpublished data Both the inhibition as well as the elimination of HDAC1/HDAC2 evokes growth arrest, morphological alterations, and apoptosis. HDACi block tumor cell migration, integrin-dependent cell adhesion, and EMT induced by the cytokine TGF-β. Global proteomics and qPCR analyses illustrate that HDACi disrupt EMT/MET cycles and metastatic spread. Moreover, these assays reveal pathways through which HDACi propel cancer cell apoptosis. (d) statement of the conclusion A class I HDACi can modulate the EMT/MET balance that is required for the detrimental process of metastasis. Hence, HDACi should be considered further as clinical treatment option.

#2833 Epithelial cell adhesion molecule (EpCAM) is associated with prostate cancer progression and chemo-/radio-resistance in vitro and in vivo. Jie Ni,1 Paul Cozzi,1 Julia Beretov,1 Junli Deng,1 Joseph Bucci,1 Peter Graham,2 Yong Li3. 1St George Hospital, Kogarah, Australia; 2University of New South Wales, Kogarah, Australia; 3St George Hospital / Univ. of New South Wales, Kogarah, Australia.

Aims: Prostate cancer (CaP) is the most common cancer in males in Australia which caused more than 3000 deaths in 2015. EpCAM is a transmembrane protein that is expressed at low levels in a variety of human epithelial tissues, but overexpressed in most solid tumors. Our previous study indicated that EpCAM was strongly expressed in metastatic CaP cell lines, primary human CaP tissues and lymph node metastasis and is a biomarker involved in CaP progression, and chemo-/radio-resistance. However, the role of EpCAM in CaP progression and therapeutic resistance is still uncertain. The aim of this study was to investigate the role of EpCAM in CaP progression and chemo-/radio-resistance as well as underlying mechanisms in vitro and in vivo. Methods: EpCAM gene was knocked down (KD) in PC-3, DU145 and LNCaP CaP cell lines using shRNA. Proliferation assay, colony formation assay, docetaxel (DTX) and radiation dose-response assay were carried out to evaluate the effect of KD of EpCAM on proliferation and therapeutic response of CaP cells in vitro. Subcutaneous (s.c.) and orthotopic CaP animal models were established using PC-3-EpCAM-KD and PC-3-EpCAM-scramble (s.c) control cells in NOD/SCID mice, to assess the effect of EpCAM on CaP tumorigenicity, chemotherapy (DTX) and radiation response. Signal transduction proteins in P38/Akt/mTOR signaling pathway, as well as proliferation, apoptotic and radiation response markers were evaluated by western blot analysis. Results: KD of EpCAM reduced cell proliferation and increased apoptosis compared to control. EpCAM scallular potentiated and enhanced DTX and radiation sensitivity in CaP cell lines. Both s.c. and orthotopic EpCAM-KD xenografts displayed suppressed tumor growth and increased DTX and radiation responsiveness compared to EpCAM-scr control xenografts in NOD/SCID mice. Marked down-regulation of P38/Akt/mTOR pathway proteins (p-Akt and p-mTOR) and protein kinase mTOR (p-mTOR) and significant up-regulation of apoptotic (Casapse-3) and radiation (γ-H2AX) responses to chemo-/radio-therapies were found in EpCAM-KD xenografts, compared with control xenografts. In addition, Kaplan-Meier curve analysis demonstrated that KD of EpCAM improved median survival (MS) of tumor-bearing mice by 21.5 days compared with the control group (HR = 26.94, 95% CI 4.31-168.1, p = 0.0004) and that KD of EpCAM improved MS of tumor-bearing mice which received docetaxel (50mg/kg, single dose, i.p.) by 11 days (HR = 20.95, 95% CI 3.99-121.9, p = 0.0007), and radiotherapy (2Gy/day for 4 days) by 12 days (HR = 11.00, 95% CI 2.11-57.36, p = 0.0044) compared with the control group, respectively. Conclusions: EpCAM plays an important role in CaP progression and chemo-/radio-resistance via P38/Akt/mTOR signaling pathway in vitro and in vivo and it is a promising therapeutic target for the treatment of CaP. EpCAM targeted therapy combined with chemo-/radio-therapy could be a novel modality for treatment-resistant CaP.

#2834 Metastatic colorectal cancer: characterization of distinct histological growth patterns which demonstrate different response to current treatment regimes. Vincent Palmieri,1 Anthoula Lazaris,2 Hussam Alamri,3 Abdel-latif Amri,4 Nisreen Ibrahim,1 Pablo Zorozquin,2 Peter Vermeulen,2 Peter Metraкос5. 1McGill University, Montreal, Quebec, Canada; 2McGill University Health Centre - Research Institute, Montreal, Quebec, Canada; 3Translational Cancer Research Unit (TCRU), GZ A Hospital, Antwerp, Belgium.

Colorectal cancer (CRC) is the third most common cancer in North America, and the third leading cause of cancer-related death. About 50% of patients will be diagnosed with CRC liver metastasis (CRCLM) during the course of their disease. We have identified two major histological growth patterns (HGP) in CRCLM resected from patients. In the desmoplastic HGP (DHPG), the tumor cells are enclosed within a desmoplastic stromal ring, physically separating them from the normal liver tissue. In the replacement HGP (RHGP), the tumor cells replace normal hepatocytes without disrupting adjacent cells or structures within the liver parenchyma. We have recently shown that patients with RHGP lesions who received neoadjuvant bevacizumab plus chemotherapy had a worse pathological response and fared worse on overall survival. Our group has also shown that RHGP CRCLM is resistant to anti-angiogenic therapy due to its promoting vessel co-option as a means of tumor vascularization, rather than VEGF-dependent angiogenesis as seen in DHPG. However, the molecular mechanisms that drive the CRCLM HGPS remain unknown. This project aims to shed light on the biological processes that underlie the diversity of HGPS, with an emphasis on the key genes and pathways that support the RHGP. To test this, we have: Aim 1) performed RNAseq on chemonaïve RHGP and DHPG CRCLM tissues to identify pathways that are significantly upregulated in the RHGP. We have validated these genes via real-time PCR (RT-PCR), and a subset by immunohistochemistry (IHC). Aim 2) To further refine the RHGP gene signature and to examine the effects of cell-cell interactions on hepatocyte and tumor cell gene expression, individual cells have been isolated from distinct regions within RHGP CRCLM tissues using laser capture microdissection (LCM), followed by single-cell transcriptomics analyses. Our preliminary data have identified three major pathways with differentially expressed genes: i. cell motility, ii. cell junctions, and iii. ECM-receptor interactions, which are upregulated in RHGP relative to the DHPG and normal liver. A panel of RHGP and DHPG cell lines were treated with or without an IHC inhibitor, and a RT-PCR. We selected a subset of these genes and are performing IHC to further validate and locate their expression. We have also performed LCM and are in the process of performing transcriptomics analyses – the data will be presented. We expect to gain some insight into the mechanisms responsible for driving and/or maintaining the RHGP lesions. In the longer term, this work will result in identification of targets in the HGPS that will help stratify patients in terms of treatment. It would appear that we have fewer treatment options for the RHGP patients and it would be important to utilize the data to develop new treatment strategies for those patients. These new therapies would lead to further clinical trials.
#2835 Potential opposing roles of AKT isoforms in indolent versus aggressive prostate cancer. Karina A. Miller,1 Hyun-Kyung Ko,2 Irwin H. Gelman,1 1Roswell Park Cancer Inst., Buffalo, NY; 2Cleveland Clinic, Cleveland, OH.

Prostate cancer (CaP) is the most commonly diagnosed non-cutaneous cancer among men and is the second leading cause of cancer-related deaths in U.S. men. Localized CaP has a 5-year survival rate of approximately 100%, but disseminated, metastatic disease (mCaP) dramatically reduces 5-year survival to <30%. A known driving force in the progression of CaP is activation of the PI3K/Akt signaling pathway through loss of PTEN, with widespread effects on cell proliferation and migration. Additional genomic changes, such as the loss of TP53 or RB1, or the upregulation of MYC, correlate with increased incidence and aggressiveness of mCaP. However, only a fraction of men with high levels of circulating and disseminated CaP cells progress to overt, macro-metastatic disease - the lethal phenotype in CaP. Importantly, little is known regarding the drivers of indolent vs. aggressive mCaP. We used two transgenic (Tg) mouse models of mCaP with clinically relevant genetic alterations which exhibit Akt activation and RB1 loss, yet one produces indolent mCaP and the other, systemic aggressive mCaP. Specifically, mice lacking RB1 and the metastasis-suppressor, SscCRS/Akap12, develop high-grade prostatic intraepithelial neoplasia (HG-PIN) and disseminated disease in the lymph nodes whereas those lacking Pten and Rb1 develop CaP and aggressive mCaP. The metastasis-suppressing functions of Akap12 relate to its ability to scaffold Src and PKC, thereby attenuating their oncogenic signaling pathways. We will show data suggesting that the pathological differences between these two Tg models is based on the differential use of PI3K-p110 and Akt isoforms, with aggressiveness correlating with the down-regulation of Smaa4, a known metastasis-suppressor in Tg CaP models. Additionally, differences in Akt isoform usage likely controls the expression of neurogenesis genes that are especially upregulated in castration-resistant CaP that fails second-line androgen receptor antagonist inhibitors such as enzalutamide. By comparing the differential transcriptomes and signaling networks in primary-site tumors, lymph node metastases, and CaP cell lines derived from these two Tg models, several new potential therapeutic targets have been identified that could be exploited to prevent or treat aggressive mCaP.

#2836 Mitochondrial genomic backgrounds differentially affect nuclear DNA methylation and gene expression. Carolyn J. Vivian,1 Amanda E. Brinker,1 Stefan Graw,1 Devin C. Koestler,1 Christophe Legendre,2 Gerald C. Gooden,3 Bodour Salhia,3 Danny R. Welch,1 1Univ. of Kansas Cancer Ctr., Kansas City, KS; 2Translational Genomics Research Institute, Phoenix, AZ; 3Univ. of Southern California, Los Angeles, CA.

Accumulating data implicate mitochondrial genetics and metabolism as contributors to metastatic efficiency. With only 13 proteins encoded by the mtDNA genome, the mechanism(s) by which the coordination of multiple genes involved in metastasis could occur is unclear. We hypothesized that mitochondrial DNA (mtDNA) polymorphisms would differentially regulate nuclear DNA (nDNA) methylation and gene expression. To test the hypotheses, we created Mitochondrial Nuclear Exchange (MNX) mice, by transferring an oocyte nucleus from strain, into an enucleated oocyte from strain. The mice show that mammary tumor formation and metastasis are regulated by inherited mitochondrial polymorphisms (PMID: 26471915). Age-matched, male mouse brains were collected and pooled from four litter mates representing each of the wild-type and MNX mice (8 groups). Genome-wide nDNA methylation (Mouse methyl-Seq) and gene expression (RNA-Seq) were measured and results validated in independent replicate samples. Significant, selective and reproducible differential DNA methylation and gene expression were observed and validated between strains having identical nDNA, but different mtDNA. While the signal(s) from mitochondria altering methylation is not fully defined, the findings support the hypothesis and demonstrate how mitochondrial genetic alterations could be one of several quantitative trait loci involved in complex phenotypes, such as metastasis. The results also suggest that mtDNA polymorphisms could serve as predictive biomarkers for cancer aggressiveness. Support: Susan G. Komen for the Cure (SAC11037), Natl Fndn Cancer Res, Biomedical Research.

Introduction: PCa is the most commonly diagnosed disease in American men and the second leading cause of death among them. Most of CaP deaths are a result of distant metastasis and due to emergence of castrate resistant CaP. Pro-tocadherin-7 (PDCD7), a Cadherin superfamily transmembrane protein that is known to function in cell-cell recognition and adhesion, is reported to be over-expressed in CaP. In addition to this, PDCD7 is related to the development of cerullum carcinoma in the prostate. Recent studies have been shown to mediate brain metastasis of breast cancer. Overexpression of PCDH7 was reported to be associated with poor clinical outcome of NSCLC. However, there is no report on involvement of PCDH7 in CaP tumorigenesis and metastasis. In the present study we evaluated expression of PCDH7 in androgen dependent and androgen independent CaP cells and in TCGA and Trento/Cornell/Broad2016 clinical datasets. Materials and Methods: PCa cells (LNCaP, C4-2b, DU145, PC3, 22Rv1 and RWPE1) were used in this study. Protein expression was checked by Western Blotting. mRNA levels were checked by quantitative real time PCR. Results: Our data reveal high PCDH7 protein expression in PC3 cells and low levels of PCDH7 in LNCaP and RWPE1 cells. qRT-PCR confirmed an increased transcript level of PCDH7 in PC3 and low levels in LNCaP cells. We also observed elevated transcript levels for PCDH7 in castrate resistant and Enzalutamide refractory PCa (22Rv1) cells. These data suggest a direct relationship between castrate resistance and PCDH7 expression. Our results also show an inverse relationship between PCDH7 and E-Cadherin and a direct relationship between PCDH7 and EMT markers, indicating expression of PCDH7 is associated with tumor progression. Interestingly, there was no correlation between the expression of PCDH7 and hormonal grade or TNM data. Furthermore, the analysis of NEPC (Trento/Cornell/Broad 2016) data set revealed amplification of PCDH7 in 9% of NEPC tumors. Collectively, these data suggest PCDH7 expression may be associated with advanced PCa in general and NEPC phenotype PCa in particular. Conclusion: Our results suggest direct association of PCDH7 expression and castrate resistance in PCa cell lines. Role of PCDH7 in castrate resistant PCa needs further evaluation.

#2838 CtBP1 expression deprecation on primary tumor deregulates miRNA expression and impairs development of spontaneous metastases on a prostate cancer and metabolic syndrome model. Guillermo N. Dalton, Georgina Scalise, Juliana Porretti, Cintia Massillo, Paula L. Farré, Paola De Luca, Adriana De Siervo. Instituto de Biología y Medicina Experimental (IByME) - CONICET, C.A.B.A, Argentina.

Prostate cancer (PCa) is the second cancer in incidence in men worldwide. Approximately 20% of cases continue with advanced or metastatic disease, and at this stage, it turns incurable due to the lack of effective therapies. Hence, the need to identify new actionable targets is crucial. Metabolic syndrome (MeS) is a physiopathological disorder that increases PCa risk and aggressiveness. C-terminal Binding Protein (CtBP1) is a transcriptional corepressor that is activated by NADH binding. Previously our group established a MeS and PCa mice model that identified to CtBP1 as a novel link associating both diseases. Moreover, cell adhesion molecules play a significant role in cancer progression and metastasis. Thus, we found that CtBP1 diminished the capability of PCa cells line to adhere to a collagen matrix, directly modulating expression of several cell adhesion genes, including repression of the epithelial marker CDH1 and induction of the mesenchymal marker VIM. The aim of this work was to investigate MeS/CtBP1 impact over PCa progression from in situ prostate carcinoma to metastatic disease. RNA was isolated from xenografts generated on MeS mice from CtBP1 depleted PC3 cells (PC3.shCtBP1) or control (PC3.PGIPZ); and hybridized to a miRNA expression microarray (Affymetrix GeneChip® miRNA 4.0). After data normalization and analysis we identified and validated a list of 11 miRNAs regulated by CtBP1 relevant to cell adhesion and PCa progression. To investigate CtBP1 role in spontaneous PCa metastasis, NOD SCID gamma (NSG) mice were fed with control or high fat diets during 12 weeks to induce MeS. Then PC3.shCtBP1 or PC3.PGIPZ cells were injected s.c. on MeS and control animals. Body weight and tumor size were measured 1 and 3 times a week, respectively. Thirty days after cell inoculation, tumors were around 1 cm, with no significant differences between treatments, however mice showed around 20% weight loss. Mice were sacrificed and tumors, lungs and livers were collected for RNA isolation and histopathological analysis. Using human GAPDH specific primers and RT-qPCR from lungs, we found that CtBP1 depletion led to a significant decrease of lung metastases, especially in the MeS group. In addition, Hematoxylin & Eosin stains from lung sections detected the lowest number and size of metastatic foci in the CtBP1 depleted xenografts generated in MeS animals. Gene expression comparison between primary tumors and metastases showed that epithelial markers, such as E-cadherin, were induced in xenografts and almost undetected in metastasis. Accordingly, mesenchymal markers expression, such as
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Vimentin, was low in xenografts and triggered on metastases. Our study uncovers for the first time the role of CtBP1 in PCA progression and its molecular targets in MeS mice.


Bladder cancer (urothelial cancer of the bladder) is the most common malignancy affecting the urinary system with increasing incidence and mortality. Treatment of bladder cancer has not advanced in the past 30 years. Therefore, there is a crucial unmet need for novel therapies, especially for advanced disease. We have previously identified the protein Acidic and Rich in Cysteine (SPARC) as a tumor suppressor in urinary bladder cancer that inhibits the multistep cascades on tumor initiation, progression and metastasis. SPARC gene and protein expression was among candidates associated with the tumorigenicity of the isogenic T24/T24T cell lines. We reported that SPARC depletion in human bladder cancer cell lines including T24 cells increased their tumorigenicity and metastatic potential. In addition, SPARC protein and transcript expression are significantly downregulated in advanced urothelial cancer. To gain insight on the molecular mechanisms of the tumor suppressor effects of SPARC in urothelial cancer, we did whole transcriptome profiling of T24 cells depleted of SPARC to identify SPARC-signature associated with bladder cancer invasiveness, progression, and metastasis. Subsequently, we used gene signature enrichment analysis (GSEA) pathway enrichment tools to define functionally relevant genes that are consistently up- or down-regulated as a function of loss of SPARC. We found that loss of SPARC expression is associated with enrichment of multiple oncogenic signaling pathways that are enriched in muscle invasive urothelial cancer and have been associated with poor prognosis. These pathways include K-ras, Myc-oncogene, p53 pathways as well as DNA damage/repair. Consistent with our earlier reports, loss of SPARC is associated with genes and proteins involved in angiogenesis, inflammation and hypoxia. In addition, we have identified novel signatures involved in unfolded protein response, mTOR signaling, glycolysis and cholesterol metabolism. These comprehensive signatures not only reveal SPARC-dependent networks that cooperate to elicit its biological responses but enable coherent understanding of the etiology of complex bladder cancer disease. Importantly, these signatures identify novel therapeutic opportunities for bladder cancer.

#2840 Integrative approach to biomarker discovery by performing comparative analysis of two cancers, hepatocellular carcinoma and endometrioid endometrial carcinoma, using genetics and transcriptomics from RNA sequencing data. Jean-Noel Billaud, Stuart Tugendreich, Debra Toburen. QIA-GEN Bioinformatics, Redwood City, CA.

Hepatocellular Carcinoma (HCC) and Endometrioid Endometrial Carcinoma (EEC) are two lethal diseases of public health importance worldwide. Using QIAGEN’s RNA sequencing solution, we were able to highlight key molecular and biological processes that indicate similarities in the tumor progression toward metastasis between one EEC patient and the three HCC patients. We have identified ITGBH-701 isoform as a potential common biomarker between these two complex cancers. We have also shown these two cancers share activated pathways such as actin cytoskeleton signaling, and RAF1 as an upstream regulator indicating similar transcriptional program between these patients. Furthermore, we have identified as a potential therapeutic target the master regulator CTGF that connects a network of 286 downstream targets to invasion of tumor cells. We have filtered a set of unique variants in the EEC patient that affect a network of upstream regulators common with HCC. This approach may be useful in the context of precision or personalized medicine. Examining the gene expression in tumors from groups of patients with each disease revealed that at a molecular level, early stages of EEC resemble established HBV-positive, HCV-negative, liver cirrhosis positive HCC.

#2841 Investigating drivers of disease progression in invasive lobular carcinoma. Nilgun Tasdemir,1 Matthew Sikora,2 Zhu Li,1 Kevin Levine,1 Ahmed Basudan,2 Soumya Luthra,3 Esther Elishaev,1 Uma Chandran,4 George C. Tseng,5 Rachel Jankowitz,4 David J. Dabbs,3 Priscilla McAuliffe,4 Nancy E. Davidson,6 Steffi Oesterreich1. 1University of Pittsburgh, Pittsburgh, PA; 2University of Colorado, Denver, CO.

Invasive lobular carcinoma (ILC) is the second most common type of breast cancer following invasive ductal carcinoma (IDC) and accounts for 10-15% of all cases. Unlike the masses or lumps formed by IDCs, ILCs grow as small, dis cohesive cells in a single-file pattern within dense layers of extracellular matrix. Paradoxically, while patients with ILC display favorable prognostic and predic-
Approximately 90% of cancer patients die from metastasis. In many cases, current therapies are designed based on the primary tumor characterization and are ineffective against metastatic disease. It is not clear whether metastatic cancer cells acquire additional genetic and epigenetic alterations, if so, what are the underlying mechanisms. Using several tumor models, we demonstrated that DNMT3B, de novo DNA methyltransferase, was increased in metastatic nodules in comparison to that from the primary tumors. DNMT3B knockdown or overexpression significantly inhibited or increased metastatic colonization of cancer cells. To understand the underlying molecular mechanisms of DNMT3B regulation, we performed DNMT3B ChIP-seq, RNA-seq, and DNA methylation-seq comparing lung metastases with primary tumors and 4T1 cells. We identified genes that are differentially methylated and expressed. Ingenuity Pathway Analysis revealed DNMT3B targeting multiple oncogenic signaling pathways. Bioinformatics analysis of human databases demonstrated significantly higher expression levels of DNMT3B in metastases than primary tumors in breast, prostate and melanoma cancer patients. In our study, we demonstrated that DNMT3B as a key epigenetic regulator which plays critical roles for tumor metastasis. Our work suggests DNMT3B as potential therapeutic targets for breast cancer patients with metastatic disease.

#2844 Identification of genes associated with pancreatic cancer metastasis by genome-wide CRISPR Cas9 screening. Youjia Li,1 Huiyi Feng,1 Guangjie Liu,1 Chi Hin Wong,1 Chi Han Li,1 Yangchao Chen,1 CUHK, Hong Kong, Hong Kong;2 GBHI, Guangzhou, China.

Pancreatic cancer is an extremely lethal cancer with a 5-year survival rate of 7%. One major reason why pancreatic cancer is hard to treat is its metastatic nature. Genes that related to the tumorigenesis and progression of pancreatic cancer were identified after decades of efforts. Several researches had revealed that pancreatic cancer originated from the successive accumulation of gene mutations, especially KRAS2, CNKD2A, TP53 and SMAD4. However, little is known about genes that regulate pancreatic cancer metastasis. Here we used a genome wide CRISPR Cas9 screening to identify the gene(s) associated with the metastasis of pancreatic cancer. We transduced pancreatic cancer cell line with lentivirus-based genome wide CRISPR sgRNA library to establish mutated cell library, and orthotopically injected the cell library into nude mice and waited for primary tumor growth and metastasis, then collected primary and metastatic tumors after several months. By next generation sequencing and analysis, we identified some gene candidates which may play important roles on pancreatic cancer metastasis.

#2845 A Rb phosphorylation code associated with lung cancer metastasis. Jaileen Perez-Morales,1 Darielys Mejias-Morales,2 Bryan Torres-Collazo,3 Harry Nguyen-Panganiban,4 Pedro Santiago-Cardona4. 1Ponce Health Sciences University, Ponce, PR; 2Pontifical Catholic University, Ponce, PR; 3University of Puerto Rico, Ponce, PR.

Lung cancer is characterized by its poor prognosis, aggressiveness, and proclivity for early metastasis. Non-small cell lung cancer (NSCLC) it’s the most common type of lung cancer with a five-year survival rate of 18%. Most predictors of metastasis and recurrence of NSCLC rely on post-resection evaluation of tumor histology, which is a severe limitation since only 15% of the patients are diagnosed with resectable disease. Hence, there is a need to characterize biomarkers with metastasis-predicting value in pre-resection small biopsy specimens. In addition to the proclivity for metastasis, another feature of lung cancers is the inactivation of the retinoblastoma protein (Rb). The retinoblastoma protein (Rb) is a tumor suppressor inactivated due to hyper-phosphorylation in most human cancers, including NSCLC. Our preliminary data reveals the Rb S249/T821 phosphorylation signature to an epithelial-to-mesenchymal transition (EMT), a trait strongly associated to metastasis. We found that cells that have hyperphosphorylated Rb in residues S249/T821 express EMT markers such as decreased expression of E-cadherin and integrin α5 as well as increased expression of N-cadherin, Vimentin, and p-FAK. We propose to focus on the cell adhesion and migration-related kinase Cdk5 with its activator p39 as the kinase responsible for engendering the Rb S249/T821 phosphorylation signature. Our hypothesis is that Rb phosphorylation in S249 and T821 due to Cdk5 activity can have prognostic value by being associated with a metastatic phenotype and EMT. Studies performed in a tissue microarray in a population of NSCLC adenocarcinoma that had undergone EMT found an up-regulation in p39 and that its expression is correlated with metastasis. Knockdown of Cdk5 induced a decrease in phosphorylation of Rb S249/T821 with a decrease in E-cadherin expression. These data suggest that Cdk5-dependent phosphorylation of Rb can affect cell adhesion by regulating E-cadherin. Introducing p39 into Rb hypo-phosphorylated cell line induced an increase in phosphorylation of Rb residues. Furthermore, the CDK3-p39-Rb axis might point to the explanation and prediction of the metastatic phenotype in lung cancer.

#2846 Identify urinary biomarkers for colorectal carcinoma liver metastasis. Meng Cai,1 Yulin Sun,1 Jiaja Gao,1 Lina Zhao,2 Fang Liu,2 Wei Sun,2 Zhixiang Zou,1 Xiashang Zhan,1 National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China;3Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China.

Colorectal cancer (CRC) is the third leading prevalent cause of death from cancer in adults, and more than one third of CRC patients are accompanied by liver metastasis. The disease begins as a benign adenomatous polyp, and it subsequently develops into an advanced adenoma and gradually progresses to an invasive cancer. The driving factors behind CRC comprise a series of successive accumulated gene mutations that follow the order “APC-KRAS-TP53-DCC”. Moreover, urine is an important display window for tumor-derived exosomes. Here, to explore the roles of TP53 in CRC and identify novel urinary biomarkers for colorectal cancer metastasis to the liver, a comprehensive proteomic analysis of esosomal proteins from HCT116 TP53-wild type (WT), TP53-knockout (KO) and constructed TP53 (R273H)-mutant (MT) cells, as well as urine samples from CRC patients with and without liver metastasis was performed using the isobaric tag for relative and absolute quantitation (iTRAQ)-2D-LC-MS/MS strategy. The exosomes from the MT and KO cells exhibited significantly reduced sizes levels of HIF1α in the WT cells. A total of 367 protein groups with ≥2 matched peptides were identified. Specifically, hepatocyte growth factor-regulated tyrosine kinase substrate (HGS) was consistently down-regulated in the exosomes from the MT and KO cells. Functional studies demonstrated that low HGS levels were responsible for the decreased exosome size. TP53 regulated HGS expression and thus HGS-dependent exosome formation. Furthermore, the HGS expression was gradually increased concomitant with CRC carcinogenesis and was an independent poor prognostic factor. In addition, HGS was one of the differentially expressed urine proteins between CRC liver metastasis and healthy individuals. In conclusion, HGS mediates TP53-regulated exosome formation. HGS may serve as a novel histological and urinary prognostic biomarker and a candidate target for therapeutic interventions in CRC.

#2847 Loss of heterozygosity (LOH) within the hypoxia inducible factor 3A (HIF3A) locus in liver metastasis (LM) from primary colorectal cancer (CRC). Takahito Kitajima, Minoru Koi, Alexander Worix, John M. Carethers. University of Michigan, Ann Arbor, MI.

Background and Aims: HIFSu is a hypoxia-regulated transcription factor that represses the effects of HIF1α and HIF2α under hypoxia at transcriptional and posttranslational levels. We previously found that LOH at the HIF3A locus is frequent in LM (59%) and the expression of HIF3α protein is reduced in LM compared to primary CRC. At present, the smallest overlapping LOH (SOL-LOH) region has been mapped to the intron 8 of the HIF3A, spanning from 46,312,911 to 46,315,651 on chromosome 19 in LM. This region contains multiple FOXA1 binding sites embedded in the two DNase I hypersensitive sites (UCSC Genome Browser, https://genome.ucsc.edu). About 30% of the human population carries a deletion within our SOL-LOH region (1000 Genomes Consortium: esv3644527, Chr19: 46,314,109-46,315,478). In this study, we determined whether esv3644527 deletion affects on the expressions of HIF3A, HIF2A, HIF1A and their target genes under normoxic and hypoxic conditions. Methods: For LOH mapping, 75 resected LMs derived from primary CRC were analyzed for LOH using 8 polymorphic markers. We obtained three EBV-transformed B cell lines with heterozygous esv3644527 deletion and the one with the homozygous deletion from the Coriell Institute. The expression levels of 23 genes related to HIF pathways were determined by qRT-PCR. Results and Discussion: The deletion levels of HIF3A in the WT cells. A total of 367 protein groups with ≥2 matched peptides were identified. Specifically, hepatocyte growth factor-regulated tyrosine kinase substrate (HGS) was consistently down-regulated in the exosomes from the MT and KO cells. Functional studies demonstrated that low HGS levels were responsible for the decreased exosome size. TP53 regulated HGS expression and thus HGS-dependent exosome formation. Furthermore, the HGS expression was gradually increased concomitant with CRC carcinogenesis and was an independent poor prognostic factor. In addition, HGS was one of the differentially expressed urine proteins between CRC liver metastasis and healthy individuals. In conclusion, HGS mediates TP53-regulated exosome formation. HGS may serve as a novel histological and urinary prognostic biomarker and a candidate target for therapeutic interventions in CRC.
locor. Deletion within this region may lead to upregulation of HIF2A and ANGPT2 by negatively regulating HIF3A expression. To prove our hypothesis, colon cancer cell lines with deleted FOXA1 sites will be assessed.

#2484 Identifying and targeting competing endogenous RNA (ceRNAs) networks to inhibit lung metastasis in triple negative breast cancer. Pelin Ersan,1 Unal Tokat,1 Erol Eryoupluglu,2 Umar Raza,1 Yasser Riazalhosseini,2 Can Alkan,1 Denis Thieffry,1 Daniel Gautheur,1 Ozgur Sahin1. 1Bilkent University, Ankara, Turkey; 2McGill University, Montreal, Quebec, Canada.

Introduction: Triple negative breast cancer (TNBC), the most aggressive breast cancer subtype, has high incidence rate of lung metastasis. Not only protein coding transcripts, but also non-coding transcriptome, such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), have active roles in cancer progression and metastasis. Additionally, lncRNAs can act as sponges for miRNAs. Here, we aimed i) to construct the first mRNA-miRNA-lncRNA competing endogenous RNA (ceRNA) network controlling metastasis in TNBC, and ii) to prevent lung metastasis by targeting identified central candidate genes. Material/Method: We established primary tumor and human-in-mouse (HIM) and mouse-in-mouse (MIM) lung metastasis models using TNBC cell lines in nude and Balb/c mice, respectively. We visualized both primary and metastatic tumors using in vivo imaging system, harvested tumors and performed both RNA and small RNA sequencing. We obtained differentially expressed miRNAs, mRNAs and lncRNAs between primary and metastatic tumors. Using several bioinformatics tools, we did enrichment analyses, miRNA target predictions, and network construction. Identified central lncRNAs were overexpressed or knocked down and will be tested in in vitro and vivo metastasis-related assays. Results and Conclusions: We obtained 45 and 91 miRNAs which were differentially expressed between primary and metastatic tumors in HIM and MIM models, respectively. A miRNA family with an established role in metastasis as well as several other miRNAs was identified as highly differentially expressed in the same direction in both models. Moreover, 1127 and 3350 mRNAs, and 85 and 111 lncRNAs were differentially expressed in HIM and MIM models, respectively. Metastasis-related processes based on differentially expressed mRNAs were enriched in the data. We then integrated these three layers of data, functional enrichments, pathway maps and target predictions to construct the first ceRNA network controlling lung metastasis in TNBCs. Currently, we are testing the functional roles of candidate lncRNAs in in vitro and in vivo metastasis assays. Ultimately, our study will uncover novel lncRNAs that can be used as potential targets and/or biomarkers in breast-to-lung metastasis. Funding: This study is supported by TUBITAK-CNRS Bilateral Grant with project number 2145364.

#2489 Identification of active enhancers as potential biomarkers of aggressive colorectal cancer. Zhiquan Wang. Mayo Clinic, Rochester, MN.

Colorectal cancer is the third most common cancer and second leading cause of cancer-related deaths in the United States. Tumor metastasis is the primary cause of mortality. Even with aggressive surgical and therapeutic management approaches, more than one third of patients will develop metastatic disease. Understanding the molecular changes that drive tumor cells to the metastatic state is key to developing targeted therapies and identifying early-stage markers for progression. To identify changes that promote cell invasiveness, a hallmark of tumor progression, we exposed the pre-metastatic SW-480 colorectal cancer cell line to multiple rounds of selection for the capacity to invade through a synthetic extra-cellular matrix. Following repeated selection, invasiveness increased by more than 10-fold. Epithelial markers, such as E-cadherin, were repressed and markers commonly associated with tumor cells that have undergone epithelial-mesenchymal transition, such as N-cadherin and Vimentin, were upregulated. Likewise, invasive cells showed increased affinity for ECM components. Paired ChIP-seq and RNA-seq revealed likely roles for epigenetic alterations in driving gene expression changes. Furthermore, the epigenetic changes followed ordered patterns of activation and inactivation, suggesting that these epigenetic signatures may provide insight into early mechanisms associated with epithelial-mesenchymal transition. Collectively, our data suggest that early epithogenic markers may be better early predictors of colorectal cancer aggressiveness than gene expression patterns and support the use of this in vitro metastasis model to study the molecular mechanisms of cancer progression.

#2850 A systems genetics approach reveals Rnash2c-immune response axis that affects metastasis severity in breast cancer. Sarah Deasy, Kent Hunter. NIH, Bethesda, MD.

Metastatic breast cancer is a devastating disease with a 5-year survival rate of only 26%. This is due to a lack of effective therapies against established metastases and an inability to identify high risk patients who would benefit from specific adjuvant therapies to prevent metastatic progression. We have shown in mouse models that spontaneously arising tumors metastasize with different sensitivities depending on the mouse genetic background. Using systems genetics approaches we have identified genes correlated with metastasis and survival in both mice and humans. Rnash2c was identified as a novel candidate metastasis susceptibility gene. This gene encodes a scaffolding subunit of the Ribonuclease H2 enzyme which removes ribonucleotides misincorporated into the DNA. Experimentally modulating Rnash2c expression in a murine mammary cancer cell line resulted in significant changes in pulmonary metastasis, confirming this gene as a metastasis modifier. Mutations in Rnash2c are known to cause Aicardi-Goutieres Syndrome, a neurological autoinflammatory disorder that overlaps clinically with congenital viral infections and the autoimmune disease Systemic Lupus Erythematosus. Given this, we hypothesized that altered expression of Rnash2c in breast cancer cells affects metastasis by engaging the immune system. To investigate immune system involvement, we analyzed metastasis in immunocompromised mice. T cell deficiency ablated the effect of reduced Rnash2c expression on metastasis, revealing for the first time an Rnash2c-immune response axis in metastasis. Gene ontology pathway analysis following mRNA-sequencing of Rnash2c knockdown and overexpression tumors revealed that 20% of the genes with altered expression are involved in immune- and inflammation-related pathways, including T cell signaling and antigen presentation. Furthermore, genes with significant changes included Type I interferons, T cell markers, and immune regulators. These results confirm that Rnash2c is a novel metastasis modifier gene and validate our hypothesis that the immune system is mediating the effect of Rnash2c on metastasis. This mechanism highlights a potential new target for combination with immune modulatory therapies to combat this devastating disease and adds to a panel of genes we identified that together could determine patients with high risk for metastasis.

TUMOR BIOLOGY: Imaging Cancer Metabolism, Therapeutic Targets, and Treatment Response


INTRODUCTION: Oncogene-dependent reliance on glutamine is a cancer vulnerability that can be exploited for therapeutic gain. In the presence of low glutaminase, the enzyme that catalyzes the conversion of glutamine to glutamate, have been developed and have shown antitumor effects in several tumor models. The specific and potent glutaminase inhibitor CB-839 demonstrated marked antitumor efficacy in a triple-negative breast cancer (TNBC) cell line with inherently high glutaminase activity (HCC-1806), but not in an estrogen receptor-positive (ER+) cell line with inherently low glutaminase activity. An early phase 1 clinical trial of CB-839 in combination with paclitaxel has shown encouraging results in in vivo tumor response in patients. Cell-line specific efficacy of glutaminase inhibitors supports the need for clinical biomarkers that can predict and evaluate therapeutic efficacy. As a minimally metabolized glutamine analog with similar transport properties, $[^{18}F]$(2S,4R)-4-Fluoroglutamine ($[^{18}F]4F-Gln$) is an ideal radiotracer to infer glutamine pool size through estimates of tracer distribution volume (DV). In this study, we evaluate differences in DV of $[^{18}F]4F$-Gln in two xenografts with different levels of glutaminolysis (TNBC vs. ER+?) using dynamic PET imaging, as well as show the effect of anti-glutaminase therapy on $[^{18}F]4F$-Gln DV. METHODS: TNBC (HCC-1806) and ER+ (MCF-7) xenografts were established in athymic nu/nu mice. Mice were scanned in a dedicated animal PET scanner at baseline and after CB-839 administration. Dynamic imaging was obtained for one hour upon injection of $[^{18}F]4F$-Gln (300-350 μCi) via the tail vein. Kinetic analysis was performed with PMOD. RESULTS: $[^{18}F]4F$-Gln uptake was largely reversible with Logan plots demonstrating late linearity and k1 in a two-compartment (trapping) model was low (less than 0.01/min in most cases). Strong correlation was seen in DX estimates by Logan plot and a single-compartment model ($R^2>0.9$), but not with estimates from a two-compartment model. MCF-7 xenografts demonstrated greater than 60% larger DV at baseline than TNBC xenografts indicative of increased cellular glutamine concentrations in MCF-7 xenografts. An increase in DV was detected in TNBC xenografts post-glutaminase inhibition (>30% mean change), but not...
Hyperpolarization of the $^{13}$C nucleus increases its sensitivity to detection in vivo. This technology provides the ability to monitor LDH activity in real time through dynamic observation of conversion of $[1-13C]$pyruvate to $[1-13C]$lactate. This study aimed to monitor drug efficacy of a newly developed LDH inhibitor (LDHI), obtained from National Cancer Institute Experimental Therapeutics Program, NECx) in a xenograft tumor model using $^{13}$C MRS technology with hyperpolarized $^{13}$C-labeled pyruvate. We are currently broadening the panel of PDXs analyzed and also including genetically engineered mechanistic models of drug resistance. The present study highlights the effectiveness of hyperpolarized $^{13}$C spectroscopy/spectroscopic imaging with hyperpolarized $^{13}$C-labeled pyruvate as a promising non-invasive imaging method for monitoring tumor metabolic response in real-time and identifying new strategies to increase the efficacy of therapy in individual patients.

#2854 The potential of hyperpolarized $^{13}$C magnetic resonance spectroscopy to monitor the effect of combretastatin based vascular disrupting agents. Anne B. Iversen,1 Morten Busk,1 Lotte B. Bertelsen,1 Christoffer Laustsen,2 Ole L. Munk,1 Thomas Nielsen,1 Thomas R. Wittenborn,1 Johan Bussink,2 Jasper Lok,2 Hans Stokkilde-Jørgensen,1 Michael R. Horsman1,2,3 Aarhus University Hospital, Aarhus, Denmark; 2Radboud University Medical Center, Nijmegen, Netherlands.

Purpose: Targeting tumor vasculature using vascular disrupting agents (VDAs) is an attractive therapy. These agents induce rapid physiological changes long before any growth inhibitory effects become apparent. Detecting these early changes should allow us to predict anti-tumor effects. Hyperpolarized $^{13}$C magnetic resonance spectroscopy (HPMRS) allows dynamic measurement of the metabolism of $^{13}$C-labeled substrates and may be a new approach for reliably monitoring early effects of VDAs. The aim of this study was to investigate the potential of HPMRS to achieve this in a pre-clinical model. Methods: Using 200 cubic mm implanted C3H mammary carcinoma cells, mice were intraperitoneally injected with combretastatin-A4-phosphate (CA4P) or the A1 analogue OXi4503. Tumor response was assessed by determining necrosis development, measured histologically 24-hours after treatment, and tumor growth time (TGT; time to reach 5 times treatment volume). Estimates of tumor perfusion and metabolism were performed 3h (CA4P) and 6h (OXi4503) after treatment. Perfusion was measured using dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) at 7.5T. Metabolic changes were assessed following intravenous injection of hyperpolarized $[1-^{13}C]$pyruvate and observing the conversion to lactate with HPMRS in a 9.4-Tesla scanner. Additional mice were positron emission tomography (PET) scanned using a Mediso nanoScan PET/MRI scanner following administration of fluorodeoxyglucose (FDG). Statistical comparisons were made using a one-way analysis of variance (ANOVA), with significance level of $p<0.05$. Results: A dose response relationship was found between injected drug doses and both tumor necrosis induction and TGT, with OXi4503 having a larger effect than CA4P. Two equally effective doses were selected for further analysis (250 mg/kg for CA4P and 50 mg/kg for OXi4503). Both drugs significantly decreased perfusion relative to controls; the respective mean (with 1 SE) values for CA4P and OXi4503 were decreased to 57% (50-64) and 38% (28-48) using Hoechst 33342, and 61% (57-65) and 61% (54-68) using DCE-MRI. FDG uptake (mean tumor-to-brain ratio) also significantly reduced to 43% (33-53) and 33% (25-41) for CA4P and OXi4503, respectively. However, an unaltered pyruvate to lactate ratio was found; the values being 98% (89-107) for CA4P and 93% (90-95) for OXi4503, respectively. Conclusion: No change in the HPMRS values was observed, despite changes in all other parameters, indicating that the metabolic fate of pyruvate remains unaltered despite reduced delivery of oxygen and nutrients. This could be because our data provides measures of whole tumor mean responses that include a viable metabolically unaltered tumor rim and a tumor core with no blood flow (i.e., no pyruvate delivery) following VDA treatment.

#2855 Monitoring tumor growth and oxygen distribution of prostate cancer xenografts in vivo using ultrasound and photoacoustic imaging. Qiuhua Hu,1 Ella Rattcher,1 Katrina Sweeney,1 Pamela J. Russell,1 Colleen C. Nelson,1 Brian W. Tse,2 Elizabeth D. Williams,1 Brett G. Hollier1.

1Australia Prostate Cancer Research Centre - Queensland, Chronic Disease & Aging, Cancer Program, Institute of Health & Biomedical Innovation, School of Biomedical Sciences, Queensland University of Technology, Brisbane, QLD, Australia; 2Preclinical Imaging Facility, Translational Research Institute, Brisbane, QLD, Australia.

Introduction: Prostate cancer (PCa) is the second leading cause of cancer-related death in men. Initially, PCA growth and progression is primarily driven by androgen stimulation of the androgen receptor (AR). As such, the standard treatments for PCA target the androgen/AR axis and are collectively termed androgen targeted therapies (ATTs). In spite of initial tumor regression, tumor cells ultimately adapt and become resistant to ATTs and progress to castration resistant PCa (CRPC) or metastatic CRPC. The tumor microenvironment (TME) has long been recognized to modulate the re-
TUMOR BIOLOGY: Imaging Cancer Metabolism, Therapeutic Targets, and Treatment Response

#2858 Immuno-PET with site-specific labeled \(^{89}\)Zr-DFO-trastuzumab improves immuno-reactivity and tumor uptake in a subcutaneous HER2 positive xenograft mouse model of ovarian adenocarcinoma. Lotte K. Kristensen, Camilla Christensen, Camilla S. Knudsen, Mette M. Jensen, Brian J. Agnew, Andreas Kjaer, Carsten H. Nielsen, Minerva Imaging, Copenhagen, National Advanced Treatment Centre for Molecular Imaging, Rigshospitalet and University of Copenhagen, Copenhagen, Denmark; Thermo Fisher Scientific, Eugene, OR.

Introduction: The combination of PET with the specificity of monoclonal antibodies (mAbs), immuno-PET, is an attractive approach to improve tumor detection and mAb quantification. \(^{89}\)Zirconium \((^{89}\text{Zr})\) is particularly well-suited for mAbs due to the long-term use of \(^{89}\)Zr in medicine. \(^{89}\)Zr has been limited by the lack of suitable methods for stable coupling to mAbs. In addition, the conventional labeling strategies with random introduction of a chelator potentially reduce antibody binding and affinity towards target. Here we demonstrate the application of an enzyme- and click chemistry-mediated methodology for site-specifically labeled \(^{89}\)Zr immuno-PET imaging probes and compare them to a random labeled probe. Experimental procedures: Trastuzumab was conjugated to the p-SCN-Bn-Deferoxamine (p-SCN-Bn-DFO) chelator in three alternate ways: (1) randomly on lysine residues or site-specifically on enzymatically treated glycans using either (2) r-galactosidase or (3) endoglycosidase S2. DFO-trastuzumab was radiolabeled with \(^{89}\)Zr \((^{89}\text{Zr}-\text{DFO-trastuzumab})\) and injected into SK-OV-3 tumor-bearing NMRi mice as a tumor load from 0.5 to 1 mm in diameter. Direct deposition of these threads into a tumor is a promising technique with a higher rate of tumor growth compared to the rest of the cohort. Importantly, we were able to detect a relatively high increase in oxygen levels between pre- and post-castration tumors in individual mice.

Conclusion: We have successfully used a non-invasive imaging method to simultaneously assess multiple biological parameters in PCa xenografts in vivo. The information gained in this study will provide a broader view of the prostate tumor response to ATTs, which has the potential to reveal novel mechanisms of therapy resistance.


Introductory Sentence: Real-time metabolic flux can be measured non-invasively using hyperpolarized magnetic resonance spectroscopy in cancer spheroids as well as tumor biopsies. Experimental Procedures: LnCaP cells were resuspended in a 1:1 mixture of Matrigel:alginate (1x10^5 cells/ml) and grown in a porous hollow fiber(A/G Technology Corporation, NY). [1-^13\text{C}] pyruvate was polarized in a SpinLab (General Electric, NY) before dissolving with appropriate buffers. NMR studies were performed on a 1 Tesla Magritek Spectrometer (Magritek, CA). HP spectra were acquired (10° degree flip angle with repetition time of 4s for 25 scans in total). Cell tracking used the manual tracking function in ImageJ. Xenograft biopsies were obtained from LnCaP tumors using a 2mm punch. Data Summary: LnCaP cells seeded sparsely enable cancer spheroid formation remain viable as assessed by Live/Dead fluorescent staining. Histological sectioning revealed spheroids of approximately 200 μm in diameter. Direct deposition of these threads into a 5mm NMR tube allows measurement of metabolic flux using hyperpolarized [1-^13\text{C}] pyruvate resulting in the formation of [1-^13\text{C}] lactate. To modulate metabolism, we used an allosteric AKT inhibitor, MK2206. This drug resulted in relocalization of AKT from the plasma membrane as well as a reduction in cell motility from 0.72 ± 0.04 to 0.46 ± 0.03 mm/s (n = 5, p<0.05). Administration of hyperpolarized pyruvate resulted in approximately 30% of lactate production 24 hr after treatment. Histological staining of spheroids after treatment revealed sustained decrease of AKT phosphorylation after these treatments. These measurements are in good accordance with measurements of lactate secretion in the same cells grown under standard conditions, with MK2206 resulting in loss of AKT Ser473 phosphorylation as early as 2 hr post-treatment, followed by a decrease in lactate secretion in the media from 3.16 ± 0.2 to 1.35 ± 0.05 mM/10^6 cells/n = 3, p<0.05) 24 hr after treatment. To ensure that these methods are applicable clinical settings, LnCaP xenograft biopsies (~60mg) were deposited directly into an NMR tube to measure hyperpolarized [1-^13\text{C}] pyruvate metabolism. Line-width of the pyruvate peak was measured to be 1.72 ± 0.19 Hz (n=3) in the first spectra, demonstrating good homogeneity of the magnetic field and conversion to lactate was observed. Future experiments will focus on measuring the metabolism of patient-derived xenografts and effects of treatment response.

Conclusions: Metabolic measurements using hyperpolarized pyruvate can be performed on cancer spheroids as well as biopsies, allowing quantification of metabolism to complement on-going clinical trials utilizing hyperpolarized MR imaging.
blood, tumor, and organs were harvested and analyzed for SPRM signals and anti-Her2 content. In vitro, the anti-Her2 NPs exhibited specific binding to BT474 cells, with little to no binding in MCF7 cells. In vivo, MRX measurements of mice injected with anti-Her2 NPs showed a measurable magnetic signal in the tumor that reached a near maximum approximately four hours after injection. Conversely, mice injected with unconjugated nanoparticles showed no signal at 600 s post-injection. Finally, 24 hours post-injection, 4 – 8% of NPs and anti-Her2 were measurable in the blood, indicating long-term stability of the NP construct in circulation. Together, these results suggest targeted delivery of conjugated NPs to cancerous tissue in vivo and the utility of SPRM for the sensitive and specific detection of cancer in vivo. This work was performed, in part, at the Center for Integrated Nanotechnologies, an Office of Science User Facility operated for the U.S. Department of Energy (DOE) Office of Science. Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy’s National Nuclear Security Administration under contract DE-AC04-94AL85000.

#2860 89Zr-trastuzumab immunoPET imaging to monitor src status after treatment in HER2 breast cancer. Brooke N. McKnight,1 Nezissa T. Viola-Villegas,2 1Wayne State University, Detroit, MI; 2Karmanos Cancer Institute, Detroit, MI.

Around 30% of patients with early stage breast cancer have recurrent disease due to resistance. One of the putative causes of acquired resistance can be attributed to the hyperactivation of Src kinases, which are particularly stabilized by CD44v6, which itself is positively correlated in metastatic breast cancers. HER2 downstream effectors include Src, thus, we aim to investigate the use of a HER2-specific monoclonal antibody positron emission tomography (PET) probe. We hypothesize that 89Zr-trastuzumab can be a surrogate imaging tool for monitoring Src treatment. 89Zr-trastuzumab was previously developed and has demonstrated excellent specificity for breast cancer. Dasatinib-treated HER2+ BT474 (IC50 ~ 1.3 μM), SK-BR-3 (IC50 ~ 5.5 μM), and JIMT-1 (IC50 ~ 8 μM) breast cancer line cells have shown a negative correlation between treatment and HER2. We next conducted tumorigenic studies using athymic nude/nude mice bearing JIMT-1 or BT474 xenografts, which were treated for 7 or 14 days with dasatinib (75 mg/kg/day). At the end of each treatment, the tumors were subsequently imaged with 89Zr-FDG, and then followed by 89Zr-trastuzumab, 24 h later. Images were analyzed using AsiPro (show version here and decay-corrected to the time of injection. Tumor uptake values were measured by drawing volumes-of-interest, expressed as % injected dose per gram (%ID/g) of tissue on regions showing the most binding. 89Zr-FDG did not demonstrate a predictive response in both treated and placebo tumors, whereas, HER2 PET was able to show differential response in treated vs. control cohorts. In JIMT-1 mouse imaged with 89Zr-trastuzumab, a two-fold difference (p < 0.01) was observed between untreated and both treated groups (8.0 ± 1.5 %ID/g, n = 7 vs. 7-day treated: 3.8 ± 1.5 %ID/g, n = 4, and 14-day-treated: 4.5 ± 1.3 %ID/g, n = 4) mice imaged with 89Zr-trastuzumab. Confirmatory ex vivo western blots of JIMT-1 tumors have shown a decrease in HER2 at treatment time points, whereas, a decrease in p-Src (Y416). In vivo studies with BT474 xenografts are currently underway. Immunohistochemistry on excised tumors, as well as treatment on a metastatic patient-derived xenograft model is currently in progress. We have shown that HER2 PET can potentially be a surrogate marker of Src treatment. The findings from this study potentially afford a powerful tool to non-invasively detect and monitor changes in Src-targeted therapy in man at the early phases of treatment.

#2861 Dose-dependent tissue distribution and tumor targeting of Notch3-ADC using fluorescence molecular tomography imaging. Anand Giddabasappa,1 Parul Gupta,1 Mauricio Leal,1 Jonathan Golas,1 Fengfeng Li,2 Bing Yang,1 Antonio Esparza,1 Christopher Winkelmann,1 Kenneth Geles.1 1Pfizer Inc, San Diego, CA; 2Pfizer Inc, Pearl River, NY; 3Pfizer Inc, Pearl River, CA.

Background: NOTCH3, a cell surface receptor involved in cell-cell communications, is over-expressed or amplified in certain human tumors. NOTCH3 is known to regulate proliferation, differentiation and survival of cancer cells or cancer stem cells and thus an important therapeutic target. NOTCH3 antibody drug conjugate (ADC) is comprised of humanized anti-NOTCH3 antibody conjugated to an auristatin based cytotoxic payload. NOTCH3-ADC has shown promising results in pre-clinical tumor models. In this study we evaluated the kinetics, dose-dependent tissue distribution, tumor accumulation and targeting specificity of the NOTCH3-ADC in OVCAR3 xenograft model using fluorescence molecular tomography (FMT) imaging. The NOTCH3-ADC is mouse cross-reactive thus providing an accurate assessment of biodistribution. Methods: NOTCH3-ADC was conjugated to the near-infrared dye, AlexaFluor680 (AF680). The in vitro cellular binding was evaluated by cell-based ELISA. In vivo biodistribution was evaluated using OVCAR3 subcutaneous xenograft model. NOTCH3-ADC-AF680 (1mg/kg; 3mg/kg and 10mg/kg) was injected when the tumors reached 100 mm3. Tumor uptake at 4 h post-injection. Ex vivo FMT imaging, pharmacokinetic analysis and immunohistochemistry (IHC) was performed at 48 and 240 h after whole-body perfusion. An in vitro receptor competition FMT study was performed by injecting excess of unconjugated anti-NOTCH3 antibody (Ab) or a non-targeted control Ab. Results: In vitro cell binding studies showed that conjugation of AF680 to NOTCH3-ADC did not affect cell binding ability. A dose-dependent increase in tumor uptake was also observed after a single injection of NOTCH3-ADC-AF680. In vivo FMT imaging showed dose-dependent whole-body clearance kinetics of NOTCH3-ADC. Dose-dependent accumulation in the tumors was observed with peak accumulation at 24-48 h post-injection and a slow decline at later time points. A maximum accumulation of ~10 %ID/g was observed which was independent of the dose of NOTCH3-ADC-AF680. Ex vivo FMT quantitation of tumor was consistent with the IHC for antibody and LC/MS analysis of released payload. Pharmacological competition with excess unlabeled control Ab did not block tumor accumulation of NOTCH3-ADC-AF680, whereas excess unlabeled NOTCH3-Ab blocked ~47% of NOTCH3-ADC-AF680 accumulation. There was no significant specific accumulation of NOTCH3-ADC in other organs measured by FMT imaging or IHC. Conclusions: This study provides understanding of the kinetics, tumor accumulation and biodistribution of NOTCH3-ADC. Further this work showcase the utility of non-invasive FMT imaging in better understanding of pharmacology and behavior of biologic drugs.

#2862 Tumor-targeting salmonella typhimurium a1-r inhibited angiogenesis of osteosarcoma cells visualized by color-coded imaging in the in vivo absorbable gelatin sponge assay. Tasaku Kiyuna. University of California, San Diego, CA.

INTRODUCTION: We already have reported a color-coded imaging model that showed the sponge angiogenesis assay using absorbable gelatin sponge implanted in nestin-driven green fluorescent protein (ND-GFP) nude mice and that osteosarcoma cells promote angiogenesis in this sponge assay. We report here that Salmonella typhimurium A1-R (A1-R) inhibits angiogenesis of osteosarcoma cells in the sponge angiogenesis assay in ND-GFP mice. METHODS: Sponge was initially transplanted subcutaneously in the flank of transgenic ND-GFP nude mice. Seven days after transplantation of sponge, skin flaps were made and 143B-RFP human osteosarcoma cells expressing red fluorescent protein (RFP) were injected into the transplanted sponge. After establishment of tumors in the sponge, the control-group mice were treated with PBS via tail-vein injection and A1-R treated group with A1-R likewise. Skin flaps were made at days 14, 21, and 28 after transplantation of the sponge to allow imaging of vascularization in the sponge using a variable-magnification small animal imaging system and confocal fluorescence microscopy. RESULTS: Vessel length in the sponge was measured after treatment with FV10-ASW Fluoview software. Nascent blood vessels grew in the sponge in a time-dependent manner. A random 3 fields were quantified in each group). The mean length of ND-GFP expressing blood vessels in the sponge in mice with A1-R treated group were 9.40, 12.40, and 10.12 mm/mm2, at days 14, 21, and 28, respectively. The mean length of ND-GFP expressing blood vessels in the sponge in mice of the control group were 8.86, 14.50, and 15.81 mm/mm2 on day 14, 21, and 28, respectively. ND-GFP expressing blood vessels of the mice in the osteosarcoma cells treated with A1-R had shorter vessels than the control group (on days 28, P < 0.05), suggested the extent of nascent blood vessel growth was significantly inhibited by A1-R treatment. Besides, we evaluated the RFP colored area of the sponge in both groups, which almost represent the viable tumor size. The mean RFP colored area of A1-R treated group in the sponge were 4.49, 7.53 and 12.40 mm2 at days 14, 21, and 28, respectively. As a result of both outcomes above, the extent of nascent blood vessel growth was significantly inhibited by A1-R treatment on days 28. CONCLUSION: In the present study, we reported the effect of angiogenesis inhibition of A1-R in vivo sponge assay, and suggest A1-R has potential for anti-angiogenic target therapy for osteosarcoma.
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#2863 Magnetic resonance imaging based analysis of tumor growth and vascular parameters in animal model of GBM following IV formulated of HET0016 treatments. Ali S. Arbab,1 Meenu Jain,1 Bhagelu Achyut,1 Kartik Angara,1 Mohammad H. Rashid,1 Asm Iskander,1 Thaiz F. Borin,1 Wenbo Zhi,1 Roxan Ara,1 Irina Lebedyeva,1 Hassan Bagher-Ebadian,1 Augustia University, Augusta, GA; 2Heritage Health System, Detroit, MI.

Glioblastoma (GBM) is a hypervascular primary brain tumor with poor prognosis. HET0016 a selective CYP450 inhibitor has been shown to inhibit angiogenesis and tumor growth. Therefore, to explore novel treatments, we have generated an intravenously administered (IV) formulation of HET0016 with HP6CDD and tested in animal models of human and syngeneic GBM. Tumor growth and vascular parameters (tumor blood volume, permeability and extravascular and extracellular space volume) were determined by dynamic contrast enhanced (DCE) magnetic resonance imaging (MRI). The pharmacokinetics of HP6CDD-HET0016 were evaluated in plasma and tumor tissues using IV and IP routes of administration. IV treatment with HP6CDD-HET0016 decreased tumor growth, tumor blood volume, permeability and extravascular and extracellular space volume, when compared with the vehicle group (p<0.05). Similar growth inhibition was also observed in syngeneic GL261 GBM (p<0.05). Survival studies using patient derived xenografts of GBM (PDX), showed prolonged survival in PDX GBM. Inhibition was also observed in syngeneic GL261 GBM (p<0.05). Administration of a single dose resulted in 7-fold higher levels of HET0016 in the IV group in plasma and 3.6-fold higher levels in tumor tissues compared to that of IP. Decreased neovascularization (laminin and aSMA) migration (MHC-1) and the treated group (p<0.05). Decreased tumor growth was associated with reduced expression of pro-angiogenic markers (IL-8, MCP-1, VEGF, HIF-1α, and VE-Cadherin), inflammation (p-NFκB), intermediates of MAPK pathway (p-AKT, p-ERK and p-STAT1, EGFR), arachidonic acid metabolism (COX-1, CYP4A11) and increased expression of antiangiogenic markers (Ang2, Angiostatin and Tie-2). Our results indicate that the improved IV formulation of HP6CDD-HET0016 is effective in inhibiting tumor growth through decreasing proliferation, migration, and neovascularization. Furthermore, HET0016 significantly enhanced sensitivity of TMZ and prolonged survival in PDX GBM.

#2864 Tumor targeting and imaging using 44Cu labeled cyclic RGD conjugated human serum albumin via click chemistry. Cho Rong Park, Myung Geun Song, Ji-Yong Park, Hyewon Youn, June-Key Chung, Jae Min Jeong, Yun Sang Lee, Keon Wook Kang. Seoul National University, Seoul, Republic of Korea.

PURPOSE: The RGD specifically recognizes the integrin αvβ3 which is over-expressed on various malignant tumors. One major drawback of small peptide such as RGD, however, is short half-life in the blood, which greatly compromises their targeting efficacy. To improve blood circulation time and targeting efficacy, we developed cyclic RGDyK (cRGDyK) conjugated human serum albumin (HSA) using click chemistry reaction. METHODS: HSA was conjugated with DBCO-NHS (dibenzyl cyclooctyne) under physiologically favorable reaction condition for the preparation of strain promoted azide-alkyne reaction. Using this labeled HSA, DBCO-HSA with 44Cu labeled N3-NOTA (3-azidopropyl-NOTA) for radiolabeling. Cell binding of cRGDyK-HSA were analyzed with FNR648 labeled probes. Cellular uptake of 44Cu-cRGDyK-HSA were tested for integrin αvβ3 specific binding at cell level. PET images were acquired after tail vein injection of 44Cu-HSA, 44Cu-cRGDyK-HSA in integrin αvβ3 positive tumor (SK-OV3, ovarian cancer cell line) bearing BALB/c nude mice. PET signals were quantitatively analyzed with PET image analysis program, AMIDE. RESULTS: The number of cRGDyK on DBCO-HSA conjugates was confirmed using MALDI-TOF MS. cRGDyK-HSA was successfully conjugated with 44Cu labeled N3-NOTA. Integrin αvβ3 mRNA and protein was highly expressed in SK-OV3 cell line. At fluorescence labeled probes were treated in SK-OV3, cRGDyK-HSA were highly bound to cell membrane and this pattern were decreased with pre-treatment of cRGDyK. When compared to cellular uptake level, 44Cu-cRGDyK-HSA were more accumulated at integrin αvβ3 positive cells (SK-OV3) than integrin αvβ3 negative cells (p<0.05) and there were no difference in 44Cu-HSA. Serial PET images were acquired 0, 4, 24, 48 hours after tail vein injection of 44Cu-cRGDyK-HSA. Radioactivity of 44Cu-cRGDyK-HSA in SK-OV3 tumor was higher than that of 44Cu-HSA. 44Cu-cRGDyK-HSA can be reduced at 48 hours after injection, which shows longer circulation time in mice. CONCLUSION: We successfully conjugated cyclic RGDyK to HSA using click chemistry approach. We demonstrated that cRGDyK-HSA specifically bind to integrin αvβ3 in vitro and vivo model. And in animal model, 44Cu-labeled-cRGDyK-HSA can be observed at 48 hours after injection, which shows longer circulation time. Our results indicated that cRGDyK-HSA have a potential to diagnosis and therapy response monitoring of tumor expressing integrin αvβ3. The peptide transporter K16ApoE increases drug delivery across the blood brain barrier in an experimental animal model of melanoma brain metastases. Synnøve Nymark Aasen,1 Heidi Espedal,1 Olivier Keunen,1 Chris- topher Florian Holte,2 Habib Baghriov,2 Rolf Bjerkgv,3 Tine Veronika Karlsen,3 Olav Tenstad,3 Dag Erlend Olberg,4 Gobinda Sarkar,5 Robert B Jenkins,6 Frits Thorsen7.1 Haukeland University Hospital, Bergen, Norway; 2University of Bergen, Bergen, Norway; 3Luxembourg Institute of Health, Luxembourg, Luxembourg; 4Norwegian University of Science and Technology, Trondheim, Norway; 5Oslo University Hospital, Oslo, Norway; 6Mayo Clinic, Rochester, MN.

Introduction: Patients with brain metastases await a dismal prognosis. Regardless of the continuous progress in drug development, a major problem is the delivery of drugs across the blood brain barrier (BBB) and into the metastatic neoplasms. The BBB excludes almost all compounds, in particular highly charged, hydrophilic or large compounds, and most of the current chemotherapeutic agents are thus unable to penetrate the BBB. Varying strategies to transiently open the BBB have been studied previously. Here, we describe a peptide transporter comprising 16 lysine residues and 20 amino acid residues corresponding to the low density lipoprotein receptor (LDLR) binding domain of apolipoprotein E (ApoE). We show that the peptide (K16ApoE) is able to transiently open the BBB for drug delivery into experimental brain metastases. Experimental procedures: A systemic study of the ability of the peptide to open the BBB was studied by dynamic contrast enhanced (DCE MRI) in nonobese diabetic/severe combined (nod/scid) mice. The BBB permeability was studied after administering 200 μg of the peptide intravenously. Further, cellular effects after treatment with the peptide was investigated in vitro using confocal microscopy, flow cytometry and impedance experiments. The biodistribution of the peptide was studied in blood plasma and several organs using 151I labeled K16ApoE. Finally, a treatment study was initiated, treating the animals with the peptide in combination with the B-RAF inhibitor Dabrafenib, only Dabrafenib or vehicle. Summary: After injecting the K16ApoE peptide into the mice, a transient opening of the BBB for up to 4 hours was clearly demonstrated by DCE-MRI. Microscopy showed that the peptide disrupted brain endothelial cell monolayers, reducing the barrier properties of the cells. The impedance experiments displayed that the permeability through endothelial cell barriers was increased after treatment with K16ApoE, and a dose-dependent cell death pattern was observed at higher concentrations of K16ApoE. The peptide did not affect endothelial cell tight junctions. The biodistribution study showed that the peptide was eliminated from blood plasma in less than five minutes through the kidneys. The treatment study displayed that the K16ApoE followed by K16ApoE + Dabrafenib followed by Dabrafenib only cured smaller tumor volumes than the other two animal groups. Conclusions: We have shown that the peptide opens the BBB and facilitates a therapeutic window of 4 hours. The peptide did in combination with Dabrafenib decrease the number of experimental brain metastases in our studies. Thus, the current strategy could also have the potential to improve the treatment of patients with brain metastatic disease.

#2866 Volumetric optoacoustic imaging of tumor cell death using a targeted imaging agent. Bangwen Xie,1 Michel Tomaszewski,1 André A. Neves,1 Stefanie R. Mullins,2 David Tice,1 Richard Sainson,2 Sarah Bohndiek,3 Robert W. Wilkinson,4 Kevin M. Brindle5.1 University of Cambridge, Cambridge, Cambridge, United Kingdom; 2MedImmune Limited, Cambridge, United Kingdom; 3MedImmune LLC, Gaithersburg, MD.

Multiphotonic spectroscopic tomography (MSOT) generates high-resolution cross-sectional images in less than a second. MED13039, is a newly described agonist of the TNF-related apoptosis-inducing ligand receptor2 (TRAILR2), which has been shown in preclinical studies to preferentially induce cell death in cancer versus normal cells1. We show here that MSOT, when used with a near infra-red (NIR) fluorophore-labelled protein domain of synaptotagmin (C2Am-750, ~15kDa) that binds to phosphatidylserine (PS) exposed by apoptotic and necrotic cells2, can be used to image MED13039-induced cell death within the entire tumor region. Non-specific probe retention was assessed using a site-directed mutant (IC2Am-680 or iC2Am-750). PS-specific binding of C2Am-750 to MEDI3039-treated TRAIL-sensitive Colo205 and TRAIL-resistant HT-29 human colon adenocarcinoma cells in vitro was evaluated by flow cytometry and confocal microscopy. The capability of C2Am-750 to detect cell death in vivo was assessed in mice bearing Colo205 or HT-29 xenografts, following a single dose (0.4 mg/kg, i.v.) of MED13039. All mice then received an i.v. injection of a 1:1 mixture of 0.1 μmole/kg C2Am-750 and iC2Am-680 16 h post
FDG-PET/CT scans 4 hours and 24 hours post-CPI-613 treatment. Similar to the result of successful targeting of mitochondrial metabolism. We therefore examined 2-deoxyglucose for 15 or 60 minutes following 2 hours of treatment with CPI-613.

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Aim: Nanoparticle-assisted photothermal therapy is a new promising therapeutic strategy that utilizes photo-absorbing nanoparticles irradiated with near infrared light to generate a local temperature increase in tumor tissue. Here we used small animal 18F-FDG PET/CT to evaluate the treatment response of silica-gold nanoshell (NS)-assisted photothermal therapy in human tumor xenografts. Method: NMRI nude mice (n = 9) bearing human tumor xenografts (H727) were treated with two nanoparticle-assisted photothermal therapy treatments. In the NS group, PET imaging revealed a significant reduction in tumor uptake at day 1 in the NS group (84 ± 8 % (day 1/baseline)) compared to the saline group (108 ± 21 %; p < 0.01) and sham (110 ± 11 %; p < 0.05) groups. Moreover, the change in 18F-FDG tumor uptake (day 1/baseline), was used to stratify animals into responders and non-responders, where the responding group matched inhibited tumor growth and improved survival. Conclusion: In this study we showed that 18F-FDG PET could be used for early response monitoring of the therapeutic outcome of nanoparticle-assisted photothermal therapy in human tumor xenografts in mice. Based on this, we suggest that PET can also be used for optimization of therapy, both for guiding treatment planning and early identification of non-responders for which the treatment strategy should then be changed.

#2867 FDG-PET imaging as a potential biomarker of mitochondrial targeting by CPI-613, a novel inhibitor of mitochondrial metabolism. Kiran Solingaparuram Sai,1 Zuzana Zachar,2 Frankis Almguel,2 Shawn D. Stuart,2 Michael S. Dahan,2 Moises Guaraldo,3 Stephanie Rideout,1 Minghui Wang,2 Anirudh Sattaraju,1 Paul M. Bingham,2 Boris Pasche,2 Akiva Mintz,1 Wake Forest University School of Medicine, Winston Salem, NC; Stony Brook University, Stony Brook, NY.

CPI-613 is a lipopeptide analogue that has been shown to inhibit the pyruvate dehydrogenase (PDH) and alpha-ketoglutarate dehydrogenase (KDGDH) complexes selectively in tumor cells (reviewed in Exp.Rev.Clin.Pharm. 7, 833). These two enzymes control the vast majority of carbon flow into the tricarboxylic acid (TCA) cycle and play a central role in mitochondrial metabolism. PDH converts pyruvate into acetyl-CoA, which in turn can enter the TCA cycle for cellular respiration. Since pyruvate is the final product of glycolysis, PDH serves as a crucial metabolic switch point. Therefore, in this work we evaluated the in vitro and in vivo glucose uptake in cancer cells and tumor xenografts after treatment with CPI-613. To measure glucose uptake in vitro we pulsed BxPc3 pancreatic cancer cells with 2H2O for 15 or 60 minutes following 2 hours of treatment with CPI-613. We observed a significant upregulation (~100% increase) of cellular 2-deoxyglucose uptake, consistent with a compensatory increase in glucose uptake as a result of successful targeting of mitochondrial metabolism. We therefore examined whether this upregulation occurs in vivo using FDG-PET/CT. Mice bearing BxPc3 flank tumors were treated with 50mg/kg of CPI-613 and underwent FDG-PET/CT scans 4 hours and 24 hours post-CPI-613 treatment. Similar to in vitro response, tumors treated with CPI-613 exhibited a 75% increase in 18F-FDG uptake compared to untreated controls at 4 hours post-treatment. At 24 hours post-treatment, tumors were irradiated for 5 minutes with an 807 nm diode laser with an intensity of 1.5 W/cm² (day 0) meanwhile the temperature development was monitored using thermographic imaging. To evaluate the treatment response, all animals were PET/CT scanned with either 18F-FDG or 18F-FLT the day before treatment (baseline) and two days after treatment (day 2). In addition, the change in tumor volume was assessed by CT imaging on day 7. Throughout the study, tumor growth was monitored using caliper measurements and animals were euthanized when the tumor volume exceeded 1000 mm³. Results: During laser irradiation, the temperature at the surface of the tumor was measured using thermographic imaging. The mice were 18F-FDG PET scanned again at day 1 after treatment and tumor growth was followed by caliper measurements with the humane endpoint defined as a tumor volume of 1,000 mm³. PET and CT images were co-registered, and regions of interest were manually drawn on whole tumor regions. 18F-FDG uptake was quantified as mean percent of injected dose per gram of tissue (%ID/g) and the treatment response was evaluated based on the reduction in tumor uptake of 18F-FDG between baseline and day 1.

#2869 Comparison of 18F-FDG and 18F-FLT PET imaging for early response monitoring of nanoparticle-assisted photothermal cancer therapy. Kamilla Norregaard,1 Jesper Tranekjaer Joergensen,1 Kamilla Norregaard,1 Jesper Tranekjaer Joergensen,1 Marina Simón Martín,1 Lene B. Oddershede,2 Andreas Kjaer1, Rigshospitalet and University of Copenhagen, Copenhagen, Denmark; University of Copenhagen, København N, Denmark.

Aim: Nanoparticle-assisted photothermal therapy relies on photo-absorbing agents that accumulate in tumors and upon irradiation with near-infrared light, generate hyperthermperatures sufficient to ablate surrounding cancerous tissue. In this study, we used small animal PET imaging to compare the tracer accumulation of 18F-FDG and 18F-FLT, that are glucose and thymidine analogues respectively, for the assessment of silica-gold nanoshell (NS)-assisted photothermal therapy in a colorectal cancer mouse model. Method: Mouse colorectal tumors (CT26) were established subcutaneously in female Balb/c mice by inoculation of 3x10⁴ cells. When the tumors reached a volume of ~150 mm³, the animals were divided into treatment groups receiving either: NS and laser irradiation (NS group), saline and laser irradiation (saline group), or NS but no laser irradiation (sham group). 24 h after intravenous injection of either NS or saline, the tumors were irradiated for 5 minutes with an 807 nm diode laser with an intensity of 1.5 W/cm² (day 0) meanwhile the temperature development was monitored using thermographic imaging. To evaluate the treatment response, all animals were PET/CT scanned with either 18F-FDG or 18F-FLT the day before treatment (baseline) and two days after treatment (day 2). In addition, the change in tumor volume was assessed by CT imaging on day 7. Results: Thermographic imaging showed that the tumor surface reached 44.5 ± 1.9 °C in the saline group and 33 ± 1.0 °C in the sham group. This was consistent with an overall inhibited tumor growth, as well as improved survival in the NS group compared to the sham and saline groups. The tumor accumulation of 18F-FDG uptake was comparable between groups at baseline but PET imaging revealed a significant reduction in tumor uptake at day 1 in the NS group (84 ± 8 % (day 1/baseline)) compared to the saline group (108 ± 21 %; p < 0.01) and sham (110 ± 11 %; p < 0.05) groups. Moreover, the change in 18F-FDG tumor uptake (day 1/baseline), was used to stratify animals into responders and non-responders, where the responding group matched inhibited tumor growth and improved survival. Conclusion: In this study we showed that 18F-FDG PET could be used for early response monitoring of the therapeutic outcome of nanoparticle-assisted photothermal therapy in human tumor xenografts in mice. Based on this, we suggest that PET can also be used for optimization of therapy, both for guiding treatment planning and early identification of non-responders for which the treatment strategy should then be changed.
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groups the tumor uptake of $^{18}$F-FDG was at comparable levels at baseline and day 2. In addition, a positive correlation was found between the change in tumor uptake between baseline and day 2 and the change in tumor volume on day 7 in all animals scanned with $^{18}$F-FDG. Preliminary data from animals scanned with $^{18}$F-FLT indicates that the tumor uptake is also strongly reduced after NS-aspirated prophylaxis, and that the contrast between $^{18}$F-FLT PET imaging show great potential for early evaluation of the treatment response after nanoparticle-assisted photothermal therapy and preliminary data suggest that both tracers can be used to predict treatment outcome.

#2870 Optical imaging of bombesin and transferrin receptor expression as are effective as $^{18}$FDG in assessing drug efficacy. Jen-Chieh Tseng, Jeffrey D. Peterson. Perkin Elmer, Hopkinton, MA.

Physical measurement of tumor volume reduction is the most commonly used method used to assess tumor progression and treatment efficacy in mouse xenograft models, but the detection of tumor size changes can require repeated drug dosing and tumor measurements for several weeks. However, $^{18}$F-FDG PET imaging of altered glucose metabolism can be a more sensitive tool for early cancer detection/diagnosis as well as treatment assessment; cancer cells are known to have abnormally increased cellular metabolism that can be inhibited by drug treatment. To illustrate this, we used HCT-116 human colorectal tumor xenografts in nu/nu mice with sorafenib treatment, a clinically approved tyrosine protein kinase inhibitor (5 d treatment, 40 mg/kg). Treatment is known to inhibit PDGFR and VEGFR, as well as Raf kinases that regulate energy metabolism in tumors. Some $^{18}$F PET imaging of treated mice using $^{18}$F-FLT revealed a significant drop in tumor metabolism with as little as 2-3 days of treatment, a time in which there is typically little or no effect on tumor size. This approach was relatively low through-put, and required special procedures to accommodate the use of radioactivity, but offered the option of daily imaging of tumor status. We also explored alternative optical imaging approaches that could offer higher through-put imaging as well as the potential for multiplex imaging. There is no fluorescent equivalent of $^{18}$F-FDG, so we focused on bombesin- and transferrin-receptors as potentially useful biomarkers for drug-induced inhibition in tumor metabolism. Bombesin receptors are upregulated in a variety of tumors and are important in energy metabolism and tumor growth. The rapid recycling kinetics also make this receptor highly sensitive to cellular metabolic changes. Transferrin receptors are also upregulated in most tumors and provide critical iron transport function vital for their increased enzymatic, proliferative, and metabolic requirements. We used targeted near infrared (NIR) fluorescent imaging probes, BombesinSense$^TM$ 680 (BRS-680) and Transferrin-Vivo$^TM$ 750 (TIV-750), to monitor changes in receptor expression in HCT-116 tumor xenografts during the course of sorafenib treatment. Interestingly, both $^{18}$F-FLT and TIV-750 PET imaging yields data quite similar to our results using $^{18}$F-FDG PET; reduction in the uptake of $^{18}$F-FDG can be measured as early as 48-72 hours, in the absence of significant reduction of tumor volume. As expected, both PET and FLI were highly effective at imaging sorafenib effects 7-8 days later (3-4 days following a 5-day sorafenib treatment regimen), with datasets in good agreement with physical measurements of tumor size. These results suggest that BRS-680 and TIV-750 can serve as fluorescent surrogates for $^{18}$F-FDG PET both in measuring early metabolic changes and ultimate therapeutic outcomes following cancer treatment.

#2871 Non-invasive monitoring of the efficacy of anticancer therapeutic agent in lung orthotopic xenograft models of NSCLC using Bio-Luminescence Imaging (BLI). Sanchareeka Dey, Sarada Preeta Kalainayakan, Poovra Ghosh, Li Zhang. University of Texas at Dallas, Richardson, TX.

Non-Small Cell Lung Cancer (NSCLC) patients have a poor rate of survival owing to diagnosis at a later stage. This necessitates development of novel therapeutic strategies that limit rapid progression of NSCLC and imaging techniques that monitor such changes in vivo. There has been a significant growth of bioluminescence imaging applications in small laboratory animal models in recent years, owing to its high sensitivity, efficiency, and non-invasive nature. Our lab has previously demonstrated that a therapeutic agent that disrupts mitochondrial respiration effectively hampers proliferation of NSCLC cell lines in vitro. With an effort to investigate the potential of this therapeutic agent in clinical settings, we used a lung orthotopic xenograft mouse model along with bioluminescence imaging (BLI) to continuously monitor the efficacy of the drug in vivo. Briefly, we implanted NSCLC cell line transduced with lentiviral particles carrying luciferase sequence, in the left lung of 6-8 weeks old female NOD/SCID mice to generate lung orthotopic xenografts. The therapeutic agent was administered intravenously over several weeks. Tumor growth was then compared between the untreated versus the treated mice, non-invasively through bioluminescence imaging (Perkin Elmer’s IVIS Lumina III Imager). On sacrificing the mice, the lungs were harvested, formalin fixed and paraffin embedded for Immunohistochemistry. Our BLI data show that in the mice treated with the therapeutic agent, tumors are localized to the left lung (site of implantation) as opposed to the control mice where the tumors metastasized to the right lung. The BLI data indicates significant decrease in radiance (total flux in photons per second) in mice subjected to treatment when compared to control mice. We show that the therapeutic agent significantly inhibits progression and metastasis of human NSCLC cell line orthotopically injected in the lungs of NOD/SCID mice. The results suggest that it might be potent anti-cancer drug as it is effective in a model that recapitulates the natural environment of lung tumorigenesis. Immunohistochemical studies are underway to discern the mechanistic details.

#2872 Quantitative mass spectrometry imaging of erlotinib in skin rashes of cancer patients receiving erlotinib. Meiko Nishimura,1 Hiroaki Aikawa,2 Mitsuhiro Hayashi,2 Yu Mizutani,2 Kei Takenaka,1 Yoshinori Imamura,2 Naoko Chayahara,1 Masanori Toyoda,1 Naomi Kiyota,1 Toru Mukohara,1 Akinobu Hamada,1 Hirobou Minami1. 1Kobe University Graduate School of Medicine, Kobe, Japan; 2National Cancer Center, Japan; 3Kobe Minimally Invasive Cancer Center, Kobe, Japan; 4Kobe University Hospital, Kobe, Japan.

Background: The development of rashes is the most common adverse event observed in cancer patients treated with epidermal growth factor receptor-targeted tyrosine kinase inhibitors (EGFR-TKI) such as erlotinib. A significant relationship exists between the severity of a rash and survival in various cancers. However, a distribution pattern of erlotinib concentration is not clarified. The concentration is higher in a rash than in normal skin of treated cancer patients remains unknown. Here, using quantitative mass spectrometry imaging (qMSI), we successfully visualized the distribution of erlotinib in rashes and normal skin of patients with advanced pancreatic cancer receiving this drug. Methods: We studied five patients with advanced pancreatic cancer who developed rashes after treatment with gemcitabine (1000 mg/m²) by intravenous infusion for 30 minutes on days 1, 8, and 15 every 4 weeks) and erlotinib (given orally at 100 mg/day). We biopsied both rashes and normal skin, and compared the distribution of erlotinib using matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI). The plasma concentration of erlotinib was measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Results: Erlotinib concentrations in five patients were 2.55 ± 1.46 ng/mm² in normal skin and 3.18 ± 1.53 ng/mm² (mean ± SD) in rashes (p = 0.123). In four out of five patients, the erlotinib concentration in rashes was higher than that in normal skin. The epidermis showed the strongest expression of EGFR in skin as judged by immunohistological staining. Erlotinib showed a greater tendency for distribution to the epidermis than subcutaneous tissue. There was no correlation between plasma concentrations of erlotinib and the concentration in rashes or normal skin. Conclusions: Using qMSI, we, for the first time, visualized the distribution of erlotinib in skin tissue of pancreatic cancer patients receiving erlotinib. We found a tendency for a higher concentration of erlotinib in rashes than in normal skin. A greater distribution of erlotinib to the epidermis than subcutaneous tissue suggests erlotinib may directly bind EGFR expressed in the epidermis.

#2873 Imaging tumor heterogeneity after multikinase inhibitor therapy in rat hepatocellular carcinoma. Nina M. Muñoz,1 Aedeek A. Minhaj,2 Kiersten L. Maldonado,1 Charles Kingsley,1 Hideyuki Nishiofuku,2 Keith A. Michel,1 Andrea C. Cortes,1 James A. Bankson,1 Asif Rashid,1 Rony Avritsch,3 1UT MD Anderson Cancer Center, Houston, TX; 2Nara Medical University, Nara, Japan.

Purpose: To assess tumor tissue perfusion and vascular permeability through multiparametric functional imaging, and to evaluate their correspondence with histopathological parameters of necrosis and hypoxia in a rat model of hepatocellular carcinoma treated with a multikinase inhibitor. Materials and Methods Rat hepatoma Mca-Rh7777 cells were implanted in the left liver lobe of nineteen male Buffalo rats. Exactly 2 weeks after tumor inoculation, the animals were randomly assigned to remain untreated (n=10) or to receive a daily dose of 7.5 mg/kg sorafenib by oral gavage (n=9) for 2 additional weeks. T2-weighted spin-echo magnetic resonance imaging (MRI), dynamic contrast-enhanced (DCE) and contrast-enhanced ultrasound (CEUS) were performed weekly. All tumors were harvested 4 weeks post-implantation >90 minutes after injecting 60mg/kg pimonidazole. Tissue sections were stained for hematoyxin-eosin and pimonidazole for quantitative assessment of necrosis and hypoxia, respectively. Differences between treatment groups were assessed using the Mann-Whitney test, and the correlation of imaging and histopathology parameters was determined by Spearman correlation analysis. Results: In spite of the relatively low

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Differences between treatment groups were assessed using the Mann-Whitney
TUMOR BIOLOGY: Imaging Cancer Metabolism, Therapeutic Targets, and Treatment Response
dose and short treatment duration, the response to sorafenib therapy was char-
acterized by a significantly higher median tumor necrosis (60 vs 15%, P <
0.0001) and increased tumor tissue hypoxia (35 vs 18.5% pimonidazole binding,
P < 0.0001). Similarly, the sorafenib regimen caused a substantial decrease in
vascular permeability, measured by DCE-MRI Ktrans (P = 0.002) as well as re-
suction in tumor vascular area, evaluated by CEUS (P = 0.043). Our findings show a strong correlation between the two histopatho-
logic parameters we assessed, tumor tissue necrosis and hypoxia (r = 0.645,
P = 0.002). Furthermore, our results demonstrate significant correlation of func-
tional imaging parameters of vascular permeability, namely DCE-MRI Ktrans,
and DCE-MRI normalized initial area under the curve (NIAUC), with histo-
pathologic tissue hypoxia (r = -0.663, P = 0.002, and r = -0.512, P = 0.029,
respectively). In contrast, histopathologic tumor necrosis appears more strongly
associated with a functional imaging parameter of tumor perfusion, specifically,
CEUS PE (r = -0.547, P = 0.028). Conclusion Tumors exhibited wide hestro-
genicity in vascular perfusion and hypoxia after treatment. Interestingly, func-
tional MRI parameters appear to correlate more strongly with tissue oxygen-
ation, whereas functional CEUS parameters correlate with tumor viability.
Novel predictive imaging biomarkers of treatment response may be developed
through further analyses of spatial tumor heterogeneity in our animal model.

#2874 Inhibition of tumor angiogenesis by Salmonella typhimurium A1-R in the in vivo Gelfoam color-coded imaging assay. Tasuku Kiyuna, Yasunori Tome, Takashi Murakami, Ming Zhao, Fumonori Kanaya, Robert M. Hoff-
man, Anticancer, Inc, San Diego, CA; University of the Ryukyus, Okinawa,
Japan.

We previously demonstrated a color-coded angiogenesis imaging model us-
ing Gelfoam implanted in nestin-driven green fluorescent protein (ND-GFP)
nude mice in which nascent blood vessels express GFP. In this assay, we dem-
strated osteosarcoma promotes angiogenesis. We report here that Salmonella
typhimurium A1-R (S. typhimurium A1-R) inhibits angiogenesis of osteosar-
coma cells in the Gelfoam® angiogenesis assay in ND-GFP mice. Gelfoam® was
transplanted subcutaneously in the flank of transgenic ND-GFP nude mice.
Seven days after transplantation, skin flaps were made and 143B human osteo-
sarcoma cells, expressing red fluorescent protein (RFP), were injected in the
transplanted Gelfoam®. After establishment of tumor in the Gelfoam®, the con-
trol group mice were treated with PBS by tail vein injection. The treated group
was treated with S. typhimurium A1-R, also by tail vein injection. Skin flaps were
made at days 14, 21, and 28 after transplantation of the Gelfoam® in order to
allow imaging of vascularization. The OV100 variable magnification small ani-
mal imaging system and FV1000 confocal fluorescence microscope were used to
visualize vascularization. Nascent blood vessels grew in the Gelfoam® in a time-
dependent manner. A random 3 fields were quantified in each group. The mean
length of ND-GFP-expressing blood vessels in the Gelfoam® in mice were treated
with S. typhimurium A1-R was 9.40, 12.40, and 10.12 mm/mm2, at days
14, 21, and 28, respectively. The mean total length of ND-GFP-expressing blood
vessels in the Gelfoam® in mice of the control group were 8.86, 14.50, and 15.81
mm/mm2 on day 14, 21, and 28, respectively. ND-GFP expressing blood vessels
of the mice in the osteosarcoma cells treated with S. typhimurium A1-R had
shorter vessels than the control group (on day-28, P < 0.05). There was not a sig-
ificant difference in tumor size between the 2 groups, thereby indicating a
specific inhibition of angiogenesis by S. typhimurium A1-R. In the present
study, we reported that S. typhimurium A1-R inhibited angiogenesis of human
osteosarcoma cells in the in vivo Gelfoam® color-coded imaging assay.

#2875 Knockout of ST8SIA1 inhibits tumorigenesis in triple negative breast cancer by inducing PTEN and ganglioside GM1 mediated tumor growth arrest. Sara E. Conard, Aaron Ebbs, Albert S. Baldwin, University of North Carolina at Chapel Hill, Chapel Hill, NC.

The heterogeneous nature of prostate cancer tumors is thought to play an
important role in the decreased effectiveness of existing therapies. Tumor initi-
ating cells (TICs) are capable of self-renewal and comprise a subset of the tumor
SUM159 cells reduced the absolute number of GD2+ cells from 17 ± 1.5% to
3 ± 0.1%. Although there was no significant difference in 2D cell growth,
arborage-independent growth by soft-agar and the mammosphere formation
assays revealed a complete loss of colony formation in ST8SIA1-KO cells. Mor-
The heterogeneous nature of prostate cancer tumors is thought to play an
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#2877 IKK-mediated signaling controls prostate tumor initiating cells. Sara E. Conard, Aaron Ebbs, Albert S. Baldwin, University of North Carolina at Chapel Hill, Chapel Hill, NC.

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important role in the decreased effectiveness of existing therapies. Tumor initi-
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TUMOR BIOLOGY: Signaling in Cancer Stem Cells

#2876 Loss of the stem cell and basal lineage regulator LBH delays onset of basal-like triple negative breast cancer. Kilan C. Ashad-Bishop, Karolina Brie-
gel, Univ. of Miami, Miami, FL.

There is increasing evidence that basal-like triple negative breast cancer
(TNBC) originates from luminal mammary epithelial cells. Specific gene sig-
naling pathways have already been experimentally proven to serve as genetic line-
age switches that, when overexpressed, transform pre-malignant luminal cells to
oncogenic cells with basal, stem-like characteristics. Conversely, depletion of
these genes in oncogenic cells with basal, stem-like characteristics leads to lumi-
nal differentiation. These studies suggest that during the earliest steps of neo-
plastic transformation, specific gene sets can alter cell fate decisions and differ-
entiation status in mammary epithelial cells, which ultimately contributes to the
heterogeneity of breast tumors. Our lab has identified a novel Wnt/β-Catenin
target gene, Limb bud and heart (LBH) that is majorly overexpressed in TNBC.
LBH is a regulator of the basal mammary stem cell lineage and repressor of
luminal differentiation via induction of Nαp63 and repression of estrogen re-
ceptor alpha. LBH is required for the self-renewal and maintenance of adult
stem cells, which are critical for tumor growth. Loss of LBH in basal mammary
fibroblasts in mice have shown that genetic ablation of LBH does not impair embry-
ogenesis or normal adult organ function, making it a possible therapeutic target.
Using crosses between MMTV-Wnt1 transgenic mice and K14Cre LBHflox
knockout mice, we are studying the effect of LBH ablation in the basal cells of the
mammary epithelium downstream of ectopic Wnt expression in the mammary
gland. We postulated that LBH may be an effector for Wnt-driven TNBC, there-
fore its inhibition would lead to decreased Wnt-induced mammary gland hy-
perplasia and tumor formation. In our model, LBH ablation in the basal mam-
mary epithelium of female Wnt transgenic mice reduces mammary gland hyperplasia and delays tumor onset. MMTV-Wnt1 driven, LBH-null tumors are
hyperplasia and delays tumor onset. MMTV-Wnt1 driven, LBH-null tumors are
also exhibiting histopathological differences indicative of a luminal to basal conver-
sion. There is an urgent need to elucidate mechanisms underlying TNBC devel-
opment and progression and find reliable avenues for treatment. Our data indi-
cates a role for LBH as a novel effector for Wnt-driven TNBC and further studies
may prove antagonism of Lbh to be a novel method to control TNBC progres-
sion.

#2878 Knockout of ST8SIA1 inhibits tumorigenesis in triple negative breast cancer by inducing PTEN and ganglioside GM1 mediated tumor growth arrest. Kim C. Nguyen, Yuanqing Yan, Chandra Bartholomeusz, Naoto

There is increasing evidence that basal-like triple negative breast cancer
(TNBC) originates from luminal mammary epithelial cells. Specific gene sig-
nalizing pathways have already been experimentally proven to serve as genetic line-
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sion.

#2877 IKK-mediated signaling controls prostate tumor initiating cells. Sara E. Conard, Aaron Ebbs, Albert S. Baldwin, University of North Carolina at Chapel Hill, Chapel Hill, NC.

The heterogeneous nature of prostate cancer tumors is thought to play an
important role in the decreased effectiveness of existing therapies. Tumor initi-
ating cells (TICs) are capable of self-renewal and comprise a subset of the tumor

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mass. These cells are proposed to drive the growth and metastasis of tumors, and are considered to be resistant to traditional cytotoxic and targeted therapies. Several studies have shown that NF-κB signaling is increased in recurrent prostate cancer and enriched in prostate TICs. We sought to determine the potential of an IKK/NF-κB-driven mechanism for inherent or acquired resistance by IKK-mediated control of a subset of prostate tumor initiating cells. These studies were performed by using established prostate cancer cell lines, murine prostate organoids, and a murine prostate cancer animal model. We have found that inhibition of IKKα and IKKβ, upstream regulators of noncanonical and canonical NF-κB signaling, block self-renewal of several PTEN-deficient prostate cancer cell lines. Furthermore, we have also found that IKK is important for tumorigenicity and stemness seen in prostate cancer cells as measured by colony formation and extreme limiting dilution assays. Loss of canonical NF-κB (p65/RelA) decreased stemness while loss of noncanonical did not alter tumorsphere formation. Interestingly, Pten-/- tumors with loss of Ikκk or Ikκκ displayed decreased levels of self-renewing cells as measured by CD49f<sup>high</sup> expression, a known prostate basal cell marker. Isolated murine Pten-/- cells were 2x more efficient than Pten-/- Ilκκ or Pten-/- Ikκκ-/- cells in forming prostate organoids suggesting that loss of Ikκκ decreased the number of self-renewing cells needed for formation. Taken together, we conclude that IKK-mediated signaling is important for maintenance of prostate tumor initiating cells and further studies will address whether IKK-mediated signaling provides a mechanism for evading current therapies.

**#2878** Anti-cancer effects of retinoic acid in CRC occurs via decreased growth of ALDH<sup>+</sup> cancer stem cells and increased differentiation of stem cells to neuroendocrine cells. Shirin R. Modarai,1 Lynn M. Opdenaker,2 Anindita Gupta,3 Jeremy Z. Fields,2 Bruce M. Roman14, Helen F. Graham Cancer Ctr., Newark, DE; 3Ca'Tx, PA.

Our studies from colon cancer patients show that in the development of colorectal cancer (CRC), stem cell (SC) overpopulation underlies tumor initiation and progression. Because aldehyde dehydrogenase (ALDH) is a marker for SCs in several tissues and is a key enzyme in retinoid acid (RA) signaling, we investigated the role of RA signaling in normal and malignant colon SCs. Our overall hypothesis is that RA signaling regulates growth (RA) and differentiation of ALDH<sup>+</sup> colon SCs and dysregulation of RA signaling contributes to SC overpopulation in CRC development. To begin to investigate underlying mechanisms, we analyzed CRC cell lines/tissues to see if retinoid receptors RXR and RAR are exclusively expressed in ALDH<sup>+</sup> SCs and if the RA pathway components change during CRC development. We determined whether RA signaling influences SC proliferation, differentiation, self-renewal, and SC population size. RXR & RAR were selectively expressed in ALDH<sup>+</sup> colon SCs, but not in MCM2 + proliferative cells, suggesting that RA signaling mainly occurs in SCs. Western blotting/immunostaining of CRCs revealed that RA signaling components, including RAR and RXR, become overexpressed in parallel with ALDH overexpression, which coincides with the known overexpression of ALDH<sup>+</sup> SCs that occurs during CRC development. Treatment of cells with RA ligands (ATRA, 9-cis RA) inhibited proliferation, reduced sphere formation, decreased ALDH<sup>+</sup> SC population size, and induced differentiation along the neural cell (NEC) lineage. Overall, our findings indicate that: (1) retinoid signaling, by regulating ALDH<sup>+</sup> colon SCs, reduces SC proliferation, self-renewal, and SC population size, and promotes SC differentiation to NECs, and (2) dysregulation of RA signaling in colon SCs likely contributes to overpopulation of ALDH<sup>+</sup> SCs and CRC growth. Thus, RA signaling, by inducing increased differentiation of the SC population, suggests a novel therapeutic target.

**#2879** DJ-1 inhibits self-renewal activity of GSCs in vitro as evidenced by neurosphere formation, limiting dilution, and soft agar clonogenic assays. DJ-1 knock-down suppressed maintenance of stemness by suppressing the expression of Notch intracellular domain, Sox2, and phosphorylated STAT3 proteins. Intriguingly, DJ-1 knock-down inhibited the expression of EGFRvIII in GSCs which might be the cause of suppression of stemness pathways. In addition, knock-down of DJ-1 induced apoptosis, reduced cell invasiveness, and sensitized GSCs to ionizing radiation. Based on these data, we propose that DJ-1 could be considered as a crucial diagnostics and therapeutic target for malignant glioma.

**#2880** GANT61-mediated constraint of Fibulin-5/prorenin receptor/ BM1 signaling impacts nasopharyngeal carcinoma stemness and metastasis. Hsin-Ting Tasi, Chang-Han Chen. Institute for Translational Research in Biomedicine, Kaohsiung, Taiwan.

Background: Cancer stem cells (CSCs) of NPC exhibit higher tumorigenesis and metastatic potential. Novel therapies based on molecular targets of CSCs have become the focus to cure NPC. GANT61, a GLI1 inhibitor, has been reported to exhibit potent anticancer effect on many cancers; here, we evaluated whether GANT61 has inhibitory effects on NPC-CSCs and further explored the possible mechanism. Methods: Condition medium was used to enrich NPC stem-like cells. The NPC stem-like cells characteristics were examined by Q-RT-PCR, Western blotting, MTt, BuDu, colony formation, sphere formation, and subcutaneous and metastatic xenograft models. Genetic, pharmacological and biochemical approaches were applied in NPC stem-like cells to investigate molecular signals, to assess the efficacies under the inhibitors treatment, and to examine the correlation among the molecules in vivo, in vitro, and NPC CSC specimens. Results: In the present study, we have demonstrated that NPC tumorsphere cells (NPC-TCS) possess CSC properties. The proliferation, colony formation, spheroid formation, migration, and invasion of NPC-TCS were dramatic prevented in the presence of GANT61 in vitro and in vivo. ChIP reveals that GLI1 preferentially binds to the Fibulin-5 promoter, and Fibulin-5 transcription/translation/post-transcriptional levels in NPC-TCS were regulated upon GANT61 treatment. However, ectopic expression of Fibulin-5 in NPC-TCS not only enhanced self-renewal and ALDH1 expression, but also reduced the effects of GANT61-elicited anti-tumor in vitro and in vivo. We further identified that a novel signaling, prorenin receptor/BM1 pathway was modulated by Fibulin-5 and involved in Fibulin-5-raised self-renewal capacity of NPC-TCS in vitro and in vivo. Notably, GANT61 inhibited NPC-TCS phenotypes was associated with suppression of Fibulin-5/prorenin receptor/BM1 signaling. Finally, a significant correlation was observed among GLI1, Fibulin-5, ATIR and BM1 in the specimens of NPC. Conclusions: Our data highlight that Fibulin-5/prorenin receptor/BM1 signaling has critical role in maintaining stem-like properties of NPC-CSCs and can be efficiently targeted by GANT61 representing a preclinical therapeutic strategy to repress NPC-CSCs.

**#2881** CXCL1 and CCL5, induced by ionizing radiation, reprogram non-tumorigenic cancer cells into cancer stem cells in breast cancer. Justine Bailleul-Daubois, 1 Nadège Bidan, 1 Raphaëlle Mouquet-Audouraud, 2 Mélanie Arci, 3 Karine Hannebicque, 4 Yuki Takayama, 3 Samuel Meignan, 2 Xuefeng Le Bourhis, 1 Chann Lagade, 1 1INSERM U908, Villeeneuve d'Ascq, France; 2Centre Odac, Lamberet, Lille, France; 3LIMMS/CNRS-IBS U02620, SMMIL-E, Tokyo, Japan.

Identification of cancer stem cells (CSC) in solid tumors - with self-renewal, multipotency, tumorigenesis, and therapy resistance capacities - has opened path to new targeting therapeutic approaches. However, CSC targeting alone might not be sufficient to eradicate a tumor. Indeed, recent studies showed that cancer cells are plastic, and conventional therapies, such as radiotherapy, can lead to cancer cells (non-CSC) reprogramming into iCSC (induced-CSC). The goal of our work is to identify the molecular mechanisms responsible for treatment-induced CSC emergence. First, we have shown that conditioned media from irradiated non-CSC is sufficient to induce iCSC reprogramming. These results suggest that cell plasticity might be actively regulated by diffusible factors secreted by irradiated cells. By using proteins arrays and ELISA, we demonstrated that the secretion of a specific cocktail of chemokines is induced by ionizing radiation, such as CXCL1 and CCL5. Interestingly, recombinant CXCL1 and CCL5 treatments increase the sphere forming capacity (SFC) of isolated non-CSC treated population. Concomitantly, treatment with neutralizing antibodies targeting CXCL1 and CCL5 leads to a decreased CSC number (ALDH<sup>+</sup> cells). We also studied the expression of the corresponding chemokine receptors, by flow cytometry. First, we saw that reprogrammable ALDH<sup>+</sup> cells are enriched for CXCL1 and CCL5 receptors expressing cells compare to unsorted population or ALDH<sup>-</sup> population. (CSC). We analysed the reprogramming potential of isolated ALDH<sup>-</sup>/receptor-positive cells versus ALDH<sup>-</sup> /
receptor-negative cells. The ALDH-/receptor-positive-derived cell population is more able to form spheres and overcomes the receptor-negative-derived population when the two populations are mixed and tested for their sphere forming capacity. The use of pharmacological inhibitors against the receptors induces a slight decrease of CSC. Taken together, our results indicate the involvement of chemokines and their receptor CXCL1 and BCSCs in the reprogramming mechanism. To validate the implication of CXCL1 and CCL5 and in a pre-clinical perspective, we are currently beginning an in vivo experiment to study the effects of CXCL1 and CCL5 inhibition on tumor development and on CSC enrichment in vivo.

**#2882 The miR-200b-ZEB1 circuit regulates diverse stemness of human hepatocellular carcinoma.** Sen-Yung Hsieh, Su-Chun Tsai. Chang Gung Memorial Hospital, Taoyuan, Taiwan

Background & aims: Hepatocellular carcinoma (HCC) can be derived from cancer stem cells (CSCs), which contribute to tumor initiation, metastasis, chemoresistance, and post-treatment recurrence. A great variety of HCC CSCs resulting in diverse clinical manifestations have been reported. We aimed to elucidate how CSC diversity is regulated. Methods: MicroRNAs deregulated in HCC were identified by using array-based microRNA profiling. Diversity of HCC CSCs was assayed by flow-cytometry, in vitro and in vivo assays for tumorigenicity. Results: MiR-200b downregulation occurred in early HCC and associated with shorter disease-free and overall survival. Ectopic expression of miR-200b or silencing of ZEB1 led to a decrease in CD34+ and CD24+ HCC CSCs and an increase in EpCAM+ HCC CSCs. MiR-200b directly suppressed BM11 and ZEB1 expressions. ZEB1 transcriptionally upregulated CD13 and CD24 expression and downregulated EpCAM expression via directly targeting their promoters. Neither miR-200b nor ZEB1 had obvious effects on CD13 or CD90 expression. Silencing CD13 or CD24 expression suppressed tumorigenicity of HCC cells. Ectopic expression of CD24 reversed the suppression of tumorigenicity when these HCC cells had been ectopically expressing miR-200b. Clinically, miR-200b downregulation was coupled with ZEB1 upregulation in approximately two-thirds of HCC patients. ZEB1 expression was positively correlated with CD13 and CD24 expressions, while EpCAM expression was positively correlated with miR-200b expression in HCCs. Conclusions: The miR-200b-ZEB1 circuit is a master regulator of diverse stemness of HCC, which distincts HCCs into those containing CD13+/CD24- CSCs from those containing EpCAM+ CSCs, and thus a potential target for anti-HCC therapy.

**#2883 Do Wnt3a-mediated canonical Wnt/Beta-catenin signals regulate stem cell phenotype in brain metastasis-specific TNBC.** Jennifer H. Carlson, Pradip De, Casey Williams, Nandini Dey, Brian Leyland-Jones. Avera Cancer Institute, Sioux Falls, SD

Introduction: Triple-negative (TN) and basal-like subtypes portend one of the worst prognoses in BC and have the most challenging diagnosis among patients due to the aggressive nature of the disease. BC stem cells (BCSCs) have a distinct role in breast oncogenesis and CD44+/CD24− population (66%) as compared to the control (79%). To further understand the mechanistic long-term role of BCSCs during the micro-metastatic 3D growth of colonies, we have developed a novel method to identify the % of BCSCs in the micro-metastatic 3D colonies. After 15 days of 3D colony growth, cells are recovered from matrigel and the live cell fraction is analyzed for CD24, CD44, CD44v6 and CD133 by flow cytometric analysis. We previously presented data demonstrating the CD44+/CD24− fraction is enriched in 3D colonies as compared to corresponding 2D cells. Here we’ve extended our analysis and examined 3D colonies following either stimulation or inhibition of WP. Conclusion: Our data demonstrates that inhibition of WP blocked the micro-metastatic growth of colonies. Studies are ongoing to delineate the mechanistic role of different BCSC markers particularly CD44 and CD44v6 in signaling the formation of WP-mediated micro-metastatic 3D colonies, the results of which will be presented at the meeting.

**#2884 Interferon Beta (IFN-B) re-activates canonical type I IFN signaling to differentiate breast cancer stem cells (CSCs) and suppress mesenchymal/CSC driven tumor recurrence.** Mary B. Doherty, 1 Damian J. Junk, 2 Hyeon Joo Cheon, 2 George R. Stark, 2 Mark W. Jackson, 1 WCRI, Cleveland, OH; 2 Lerner Research Institute, Cleveland Clinic, Cleveland, OH.

Tumor recurrence remains a major therapeutic obstacle to curing breast cancer. Cytokines within the breast tumor microenvironment (TME) influence recurrence following chemotherapy by altering the balance between mesenchymal CSC (MES/CSC) and epithelial non-CSC (EP/non-CSC). Here, we found that transformed breast MES/CSC express elevated levels of Un-phosphorylated Interferon Stimulated Gene Factor 3 (U-IFSGF3), which is composed of STAT1 and STAT2 lacking phosphorylation of their tyrosine residues, and IRF9. Elevated expression of the U-IFSGF3 proteins in breast cancer has previously been linked with resistance to ionizing radiation, thus we hypothesized that the U-IFSGF3 complex is important for maintaining MES/CSC properties. However, knock-down of IRF9, the critical DNA binding component of this complex, or STAT1 did not alter the MES/CSC phenotype. Moreover, IFSGF3 transcriptional targets were actually repressed in MES/CSC relative to EP/non-CSC, suggesting that elevated U-IFSGF3 may act as a repressor in MES/CSC. Interestingly, we found that an MES/CSC program independent of IFN-B, was responsible for mediating U-IFSGF3 expression. We therefore hypothesized that stimulating MES/CSC with IFN-B would phosphorylate the IFSGF3 proteins and engage canonical IFN-B mediated inhibition of MES/CSC properties. Acute exposure to IFN-B led to the phosphorylation of IFSGF3 in MES/CSC and induced the transcription of IFN-B responsive genes. Continuous exposure to IFN-B maintained Phosphorylated IFSGF3 (P-IFSGF3) which partially repressed the MES/CSC phenotype, resulting in the repression of mesenchymal markers (Vimentin and Slug), expression of EP/non-CSC markers (CD24) and functionally inhibited colony formation in soft agar and migratory capability. In line with these observations, data from triple-negative breast cancer (TNBC) patients confirmed that increased expression of IFN-B responsive genes correlates with decreased expression of a CSC gene signature, and correlates with improved recurrence-free survival. Taken together, our findings demonstrate that IFN-B mediated signaling promotes the differentiation of MES/CSC by phosphorylating U-IFSGF3, resulting in the suppression of MES/CSC-driven tumor recurrence. Future studies will examine the mechanism by which IFN-B mediated P-IFSGF3 suppresses MES/CSC properties and whether elevation of U-IFSGF3 can predict which patients would be candidates for an IFN-B therapy to phosphorylate STAT1 and STAT2, thus suppressing MES/CSC tumor recurrence.

**#2885 Activation of IL1α signaling by HER2 overexpression promotes breast cancer stem cells.** Shou Liu, 1 Ji shin Lee, 2 Hexin Chen 3. University of South Carolina, Columbia, SC; 3 Chonnam National University, Republic of Korea.

Breast cancer is a heterogeneous disease and only a small proportion of cells known as cancer stem cells (CSCs) have the ability to generate tumor. Recently, it has been reported that HER2 overexpression increase breast cancer stem cells (BCSCs). However, the underlying mechanism is not clearly known. In this study, we explored the molecular mechanism how HER2 increase BCSCs. Our data has demonstrated HER2 induces BCSCs by indirectly activating NF-kB and STAT3 signaling. To figure out the direct target of HER2, we used the microarray and found that HER2 upregulates the expressions of IL1A and IL6, two major cytokines which directly activates NFkB and STAT3, respectively. Interestingly, we found that IL1A and indirectly activates STAT3. IL1A induced STAT3 was blocked when IL6 was knock out from cells. We found that HER2 enhances IL1α transactivation by activating PU.1 which is known transcription factor of IL1A. Finally, we studied effect of IL1α on tumorogenesis, and found that IL1α KO dramatically attenuates the tumor growth in NOD/SCID mice. Taken together, we found that HER2 induces BCSCs by upregulating IL1A and IL6 expression. We for first time demonstrating that IL1α plays a critical role in HER2-induced expansion of BCSC population and may be a potential therapeutic target for HER2 positive breast cancer.
Head and neck squamous cell carcinoma (HNSCC) is highly invasive and resistant to therapies, where treatments often result in high rates of failure and disease recurrence. A subpopulation of tumor-initiating cancer stem cells (CSC) is thought to be responsible for metastatic invasion and drug resistance in many types of cancer, including HNSCC. CD44 is a known CSC marker in HNSCC but its role in maintaining CSC populations is not well understood. We previously reported that SMAD specific E3 ubiquitin protein ligase 1 (SMURF1) inhibition of bone morphogenetic protein (BMP) signaling is essential for maintaining a CD44-high CSC-like population in HNSCC. In this study, we sought to determine how CD44, a receptor for hyaluronic acid (HA) that constitutes a major component of the tumor stroma, is involved in the regulation of SMURF1 and the maintenance of a HNSCC CSC phenotype. CSC-like cells were enriched from HNSCC cell lines as CD44-high cells. CD44 signaling was stimulated by exogenous HA treatment and inhibited by CD44 knockout (KO) using an inducible CRISPR/Cas9 system. We then assessed changes in SMURF1 protein level, BMP signaling, transwell migration, Matrigel colony formation, and invasion through three-dimensional organotypic culture (OTC) following CD44 modulation. CD44-high cells were found to have increased extracellular HA production. Treatment with exogenous HA reduced BMP signaling, as determined by a reduction in phospho-SMAD1/5/8 levels, and increased transwell migration of CD44-high cells. CD44-KO reduced SMURF1 protein expression and inhibited Matrigel colony formation of an invasive and recurrent HNSCC-derived CD44-high cells but only partially reduced colony formation of a less-invasive cell line derived from a primary HNSCC. Knockout of CD44 expression showed a slight reduction in transwell migration of invasive cells. CD44-high cells also recapitulated an invasive and CSC-like growth pattern in OTC assays. In contrast, CD44-KO inhibited OTC invasion and the epithelial-to-mesenchymal transition (EMT) phenotype, resulting in increased apical epithelial growth and differentiation. CD44 reconstitution appeared to restore the invasive and EMT phenotype. Our current findings could be crucial for maintaining an anchor-age-independent and invasive phenotype of HNSCC but plays a minor role in carcinoma cell migration.

Macrophage conditioned medium promotes colon cancer stem cell phenotype via the hedgehog signaling pathway. Fan Fan, Rui Wang, Delphine R. Boulbes, Huiyuan Zhang, Stephanie S. Watowich, Rajat Bhattacharya, Ling Xia, Xiang-Cang Ye, Lee M. Ellis. UT MD Anderson Cancer Ctr., Houston, TX.

Background: Contradicting studies have shown that increased macrophages infiltration in colorectal cancer (CRC) could either result in anti-tumoral or in pro-tumoral effects. Because of these inconsistencies, we sought to determine the role of conditioned medium from macrophages, in particular classically activated macrophages, on the development of the CSC phenotype in CRC cells, which is believed to mediate tumor growth and chemoresistance. Methods: Murine (CD41+/CD11b+) and human (HPPC-1) CRC cells were treated with conditioned media (CM) from lipopolysaccharide (LPS)-activated murine macrophages. Viability studies were performed using the MTT assay, annexin V-FITC assay, and Western blot analysis. The CSC population was assessed using the sphere-forming assay and aldehyde dehydrogenase assay. Results: The results showed that 1) murine LPS-activated macrophage CM induced apoptosis in murine and human CRC cells, 2) murine and human LPS-activated macrophage CM secreted paracrine factors that promoted the CSC phenotype in murine and human CRC cells via the Sonic hedgehog (SHH)-Gli signaling pathway, which is known to drive self-renewal and 3) these effects were blocked by depletion of SHH in macrophage CM. Conclusions: Our data show that LPS-activated macrophages play an active role in promoting the CSC phenotype through activation of the SHH-Gli signaling pathway.


Glioblastoma multiforme (GBM), the most common and aggressive primary brain tumor in adults, is characterized by aggressive recurrence after conventional treatment, which include surgery followed by radiation and chemotherapy. This recurrence relies on GBM cells’ ability to promote therapeutic resistance. The recently developed Cancer Stem Cell (CSC) hypothesis argues that GBMs are driven by a rare subset of cells called Glioma Stem Cells (GSCs). A significant corollary of the CSC hypothesis is that GSCs have the ability to survive intensive radio- and chemotherapy and give rise to recurrent disease. Recent studies from our laboratory and others have shown a high degree of plasticity in GBM cells that indicated normal GBM cells acquire a stem-like state during anti-glioma therapy. Termved conversion, this molecular process increases the frequency of therapy resistant GSCs and promotes disease recurrence. The term “reprogramming” of a drug resistant phenotype of GBM cells during anti-glioma therapy would prevent the generation of therapy-resistant GSCs, thereby block GBM recurrence. Based on this concept, we have developed a plasticity-based high-throughput drug screening method for GSCs. Our initial screening has identified several novel FDA-approved compounds that act as antagonists of neurotransmitter receptors. Further investigation revealed that treatment of GSCs with atypical neuroleptics, such as chlorpromazine, a newer version chlorprothioxene, in conjunction with TMZ, which inhibited and antagonized GSCs, led to the formation of a population of cells that express both CD133, a marker of GSCs, and dopamine receptors 2 and 3 (DRD2). Additionally, in ExoCarta database V-ATPase as an important effector of GBM growth and glioma stem cells (GSCs) maintenance. Additionally, in ExoCarta database V-ATPase subunits have been described in Exo from different cancer cell types. Taken together, these data identify V-ATPase as an important driver of gliomagenesis, and a novel, actionable therapeutic target for disease intervention. However, the role of V-ATPase in reprogrammed GBM microenvironment has not been previously investigated. Methods: Using V-A TPase-High vs. V-A TPase-Low NS with EVs from V-ATPase HIGH NS increases their motility in collagen matrices. A stronger with EVs produced by NS with higher V-ATPase expression (V-ATPase HIGH) from NS are able to significantly increase cell growth in recipient cells (brain tumor margins or primary GBM (differentiated and nondifferentiated cultures) at the molecular level, profiling of Exo-derived miRNAs distinguishes differentiated cultures from NS, n = 12 or differentiated cultures (n = 8). For EVs internalization studies, Exo or LO were isolated by an Invitrogen kit and serial centrifugation, respectively, from media of patients’ derived GBM neuropheres, enriched in GSC (NS, n = 12) or differentiated cultures (n = 8). For EVs internalization studies, Exo or LO were stained using FM 1-43 FX dye and the process was followed live for 30’ and at selected time points (30’-4h-24h), using a confocal microscopy or flow cytometry (FACS). Electron microscopy, FACS (of Exo stained with CellTrace and SytoRNA in combination with CD63 coated beads), Nanosight and immunoblotting for CD63, CD9 and Clathrin) analyses were used to confirm EVs subtypes. Cultures from patients’ derived brain tumor margins or primary GBM (differentiated and nondifferentiated cultures) were used as EV-receptor cells. miRNA profiling was performed using Taqman Low density arrays and analyzed by R packages. Gene Ontology analysis was performed by DAVID. The study was approved by the Institutional Ethical Committee. Results: NS are able to produce different EVs, which are internalized by recipient cells after 4 and up to 24 hours of co-culture. Both Exo than LO from NS are able to significantly increase cell growth in recipient cells (brain tumor margins and primary GBM differentiated monolayers), and this effect is stronger with EVs produced by NS with higher V-ATPase expression (V-ATPase HIGH). Primary GBM cells after co-culture with Exo produce a higher number of NS and V-ATPase activity block by BafilomycinA1 in NS-producing EVs completely revert this effect. Finally, the co-culture of V-ATPase LOW NS with EVs from V-ATPase HIGH NS increases their motility in collagen matrices. At molecular level, profiling of Exo-derived miRNAs distinguishes differentiated cultures from NS and, among NS, V-ATPase HIGH cultures. In addition, V-ATPase HIGH NS-derived EVs from patients’ derived Exo showed an enrichment of cancer cell cycle and PI3K/Akt/mTOR pathways. Conclusions: Altogether, these data point toward the central role of different EV types in GBM communication and suggest a role of the V-ATPase proton pump in regulating EV’s contents.
MicroRNA-mediated upregulation of the WNT signaling activities in human breast cancer stem cells. Yohei Shimono,1 Taichi Isobe,2 Andrei Turtoi,3 Junko Mukohyama,1 Toru Mukohara,4 Akira Suzuki,5 Vincent Castronovo,3 Hiroonobu Mimami1,6 Kobe University, Kobe, Japan; 6University of Liege, Liege, Belgium; 7Stanford University, Stanford, CA.

The canonical WNT signaling plays a critical role in many adult stem cells, including those of the breast and intestine. The fact that the canonical WNT signaling is implicated in both stem cell self-renewal and cancer suggests that normal physiological regulator of stem cell functions might be “hijacked” in cancer. Adenomatous polyposis coli (APC) is a component of the destruction complex that destabilizes β-catenin and suppresses the activity of the canonical WNT signaling. MicroRNAs (miRNAs) are important regulators of stem cell functions. We have previously reported a set of 37 miRNAs that are upregulated or downregulated in human breast cancer stem cells (BCSCs), a CD44+/CD24−/low “stem-cell-like” population of human breast cancer cells (BTCs), which are responsible for the formation of human breast cancer stem cells (NTCs). Among them, miR-200c targets BMI1 that is a critical regulator of the stem cell maintenance, and strongly impairs the functions of human BCSCs in vivo. In this study, we compared the expression profiles of miRNAs, mRNAs and proteins between BCSCs and NTCs isolated from the patient specimens of human breast cancers and patient-derived xenografts (PDXs) established by their transplantation. Luciferase assays were performed using the pSmad in which the 3’UTR region of candidate miRNA was cloned downstream of a luciferase minigene. The effect of miRNAs on the activity of WNT signaling was evaluated using a TGF reporter plasmid. Finally, the abilities to form organoids and to form tumors in immune-deficient mice were evaluated using the human BCSCs infected with the miRNA inhibitor expressing lentivirus. We found that miR-142 was highly upregulated in BCSCs, but was hardly expressed in NTCs in the patient breast cancer specimens. We confirmed that miR-142 targeted the sequence within the 3’UTR of APC mRNA and suppressed APC protein expression. Accordingly, miR-142 activated the canonical WNT signaling pathway in an APC-suppression dependent manner. The results of miRNA and protein expression profiling of the BCSCs isolated from human breast cancer PDXs suggested that the canonical WNT signaling was activated in BCSCs. Finally, inhibition of miR-142 in the BCSCs suppressed the tumor growth in vivo. These results suggest that the miR-142, a miRNA frequently upregulated in human BCSCs, could provide at least a partial molecular mechanism for aberrant activation of the canonical WNT signaling in breast cancer in which APC mutations are much less frequent than colon cancer.

Retoenic acid directs breast cancer cell state changes through regulation of TET2-aPKC pathway. Meng-Ju Wu, Chan-Ju Chang, Purdue University, West Lafayette, IN.

The key molecular mechanism governing the cancer cell state (stem cell-like state vs. differentiation state) to control the cancer stem cell (CSC) pool remains elusive. This study provides the first evidence showing that all-trans retinoic acid (ATRA) induces the interaction and chromatin recruitment of a novel RARB-TET2 complex to epigenetically activate a specific cohort of gene targets, including MiR-200c. TET2-activated miR-200c further targets and suppresses aPKC, a cell polarity protein that plays a pivotal role in directing asymmetric division of mammalian stem cells. MiR-200c can also suppress the stem cell-like population of breast cancer cells in vivo. In short, miR-200c regulates breast cancer stem cell properties by hijacking the TET2 complex to epigenetically activate a specific cohort of gene targets, including those of the breast and intestine. The fact that the canonical WNT signaling is implicated in both stem cell self-renewal and cancer suggests that normal physiological regulation of stem cell functions might be “hijacked” in cancer. Adenomatous polyposis coli (APC) is a component of the destruction complex that destabilizes β-catenin and suppresses the activity of the canonical WNT signaling. MicroRNAs (miRNAs) are important regulators of stem cell functions. We have previously reported a set of 37 miRNAs that are upregulated or downregulated in human breast cancer stem cells (BCSCs), a CD44+/CD24−/low “stem-cell-like” population of human breast cancer cells (BTCs), which are responsible for the formation of human breast cancer stem cells (NTCs). Among them, miR-200c targets BMI1 that is a critical regulator of the stem cell maintenance, and strongly impairs the functions of human BCSCs in vivo. In this study, we compared the expression profiles of miRNAs, mRNAs and proteins between BCSCs and NTCs isolated from the patient specimens of human breast cancers and patient-derived xenografts (PDXs) established by their transplantation. Luciferase assays were performed using the pSmad in which the 3’UTR region of candidate miRNA was cloned downstream of a luciferase minigene. The effect of miRNAs on the activity of WNT signaling was evaluated using a TGF reporter plasmid. Finally, the abilities to form organoids and to form tumors in immune-deficient mice were evaluated using the human BCSCs infected with the miRNA inhibitor expressing lentivirus. We found that miR-142 was highly upregulated in BCSCs, but was hardly expressed in NTCs in the patient breast cancer specimens. We confirmed that miR-142 targeted the sequence within the 3’UTR of APC mRNA and suppressed APC protein expression. Accordingly, miR-142 activated the canonical WNT signaling pathway in an APC-suppression dependent manner. The results of miRNA and protein expression profiling of the BCSCs isolated from human breast cancer PDXs suggested that the canonical WNT signaling was activated in BCSCs. Finally, inhibition of miR-142 in the BCSCs suppressed the tumor growth in vivo. These results suggest that the miR-142, a miRNA frequently upregulated in human BCSCs, could provide at least a partial molecular mechanism for aberrant activation of the canonical WNT signaling in breast cancer in which APC mutations are much less frequent than colon cancer.

TUMOR BIOLOGY: Signaling in Cancer Stem Cells

Oncostatin M elicits cellular plasticity through cooperative STAT3-SMAD signaling. Benjamin L. Bryson, Damian J. Junk, Jacob Smigiel, Neetha Parameswaram, Mary R. Doherty, Courtney A. Bartel, Mark Jackson. Case Western Reserve University, Cleveland, OH.

Increasing evidence suggests that tumor cell plasticity promotes metastasis and tumor recurrence, resulting in cancer patient mortality. While it is clear that the tumor microenvironment (TME) contributes to tumor cell plasticity, the specific TME factors that actively generate tumor cell plasticity are largely unknown. Here, we identify TME cytokines that promote epithelial-mesenchymal plasticity, and acquisition of cancer stem-cell (CSC) properties. A screen of 27 TME cytokines identified multiple Interleukin-6 family members as inducers of mesenchymal/CSC properties, with Oncostatin M (OSM) being the most potent. Importantly, OSM-induced plasticity was mediated by STAT3, but also dependent on TGF-β signaling and downstream SMAD3. Inhibition of functional TGF-β/SMAD signaling by expressing a dominant-negative TGF-β receptor, treating with a TGF-β receptor inhibitor, or suppressing SMAD3 expression using a SMAD3-shRNA prevented the OSM-induced mesenchymal/CSC properties. OSM-activated STAT3 binds to SMAD3 and promotes SMAD3 nuclear localization and binding to the SNAIL promoter, increasing transcription of the SNAIL gene. The epithelial-mesenchymal transition induced by the STAT3/SMAD3 axis results in a highly invasive and metastatic phenotype and the emergence of CSC properties, including therapeutic resistance. Importantly, maintenance of the OSM-induced mesenchymal/CSC state requires sustained exposure to the cytokine, as removal of OSM results in a marked phenotypic reversion. A high-throughput screen for small molecule inhibitors of the OSM-induced mesenchymal/CSC phenotype has been performed and are currently being confirmed. We propose that, targeted blockade of the STAT3/SMAD3 axis in tumor cells may represent a novel therapeutic approach to prevent the plasticity associated with metastasis and tumour recurrence.

IGF2 is essential for tumor initiating cell activity in human colorectal cancer. Taronish D. Dubash,1 Christine Siegl,2 Sebastian M. Dieter,1 Joachim Weischenfeldt,1 Alexandros P. Drainas,2 Laura Schwarzmueller,1 Malgorzata Oles,3 Balca Mardin,2 Mikolaj Slabicki,2 Huber Wolfgang,2 Martin Schneider,1,3 Jan Korbel,2 Hanno Glimm,1 Claudia R. Ball1.1National Ctr. for Tumor Diseases (NCT) and German Cancer Research Center (DKFZ), Heidelberg, Germany; 2European Molecular Biology Laboratory (EMBL), Heidelberg, Germany; 3Heidelberg University Hospital, Heidelberg, Germany.

A small fraction of all cells within individual tumours from colorectal cancer (CRC) patients drives long term tumor growth and metastases in immune-compromised mice. Targeting these tumor-initiating cells (TIC) may improve the long-term outcome in advanced CRC. To identify candidate genes which drive proliferation and survival of TIC, we have performed a large scale high-throughput loss of function shRNA screen in three-dimensional TIC enriched patient spheroids. Spheroids were transduced with the barcoded Cellecta decipher library comprising 27,500 shRNAs targeting 5043 genes associated with cell signaling pathways (Module 1). Two weeks later, cells were harvested for DNA isolation, barcode amplification and high-throughput barcode sequencing. Amongst others, we found 5/6 shRNAs targeting Insulin growth factor 2 (IGF2) scoring within a 20% depletion threshold, presenting depletion levels very similar to positive control shRNAs. mRNA expression profiling and qPCR analyses demonstrated low to moderate expression of the IGF2 gene product in the majority of patient spheroid cultures analyzed (n=17). In contrast, two out of 15 patient derived spheroid cultures analyzed demonstrated very pronounced IGF2 overexpression (>250 fold). Integrative SCNA profiling, expression and TAD profiling using the CESAM algorithm, followed by 4C Seq, demonstrated a tandem duplication of the IGF2 locus in these two patients which interrupts a IGF2 adjacent TAD boundary and results in de novo contact domain formation between the IGF2 promoter and a normally hidden distant super-enhancer. A dual luciferase reporter assay revealed that the hijacked enhancer is functionally active in human CRC cells and thereby may drive unphysiological IGF2 expression following enhancer hijacking. To assess the functional relevance of IGF2 tandem duplications, spheroids were transduced with RFP expressing lentiviral vectors encoding for 3 shRNAs targeting IGF2 as well as control shRNAs targeting EIF3A or scrambled shRNA. Strikingly, IGF2 knockdown led to a marked reduction of RFP+ cells in competitive proliferation assays and markedly reduced viability assessed by ATPlight assay. Moreover, IGF2 knockdown strongly reduced tumour formation following xenotransplantation into immune deficient NSG mice. These data demonstrate that IGF2 is required for survival, proliferation and tumor-initiation of primary human TIC enriched spheroids. Notably, oncogenic miRNA-483 is encoded within intron 6 of IGF2, however, its function in IGF2 locus tandem duplications is remains elusive. Understanding the mechanisms of IGF2 dependency and the role of miRNA-483 in this context will be essential for the future development of therapeutic approaches targeting IGF2 expression in this patient subset harbouring tandem-duplications of the IGF2 locus.

Epinephrine-induced gamma-secretase/Notch signaling in pancreatic cancer stem cells. Xuemin Xu, Chen Hu, Hildegard M. Schuller. 1University of Tennessee, Knoxville, TN.

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer deaths with 5–year survivals below 10%. Cancer stem cells represent a small population of cells in PDAC and are thought to drive the development, progression, metastasis and drug resistance of this cancer. The Notch pathway
regulates the self-renewal of pancreatic cancer stem cells as well as angiogenesis and drug resistance. Gamma-secretase cleaves the Notch-1 receptor, resulting in release of the Notch intracellular domain (NICD) that then translocates to the nucleus and activates the transcription of target genes. Ablation Notch signaling activated by γ-secretase activity is associated with progression, angiogenesis and drug resistance of PDAC and is being explored as a target for PDAC therapy. Smoking and psychological stress are risk factors for PDAC. However, the potential modulation of γ-secretase/Notch by these risk factors has not been investigated to date. Nicotine and psychological stress both cause hyperactivity of the sympathetic nervous system and adrenal glands, resulting in increased blood and organ levels of the stress neurotransmitters norepinephrine (NOR) and epinephrine (EPI), which are the physiological agonists of beta-adrenergic receptors (β-ARs). In turn, β-ARs are coupled to the stimulatory G-protein Gs that stimulates the formation of intracellular cAMP, which activates multiple signaling pathways in a cell type-specific manner. Using spheroid formation assays and Western blotting, our data show, for the first time, that the stress neurotransmitter Epi significantly increases the self-renewal of pancreatic cancer stem cells by increasing the expression of the activated forms (c-terminal) of the γ-secretase component presenilin1 and of Notch1 (NICD). These responses were inhibited by the β-AR antagonist (beta-blocker) propranolol and by the inhibitory neurotransmitter γ-aminobutyric acid (GABA) that inhibits cAMP formation via activation of Gαi-coupled GABA receptor. These findings suggest that the γ-secretase/Notch pathway in pancreatic cancer stem cells is under control by the neurotransmitter stimulator and GABA as inhibitor. These data could open up new avenues for the prevention and therapy of PDAC. Additional experiments are currently underway to further investigate mechanistic aspects of this novel concept. Supported by RO1CA042829 with NIH.

#2895 Octamer-4/microRNA-1246 signaling axis drives Wnt/β-catenin activation in liver cancer stem cells. Stella Chai,1 Kai-Yu Ng,1 Man Tong,1 Eunice Y. Lin2, Terence K. Lee,1 Kwok Wah Chan,1 Yun Fei Yuan, Tan Tio Cheung, 1Siu Tim Cheung, 4 Xiao Qi Wang, 1 Nathalie Wong,2 Chung Mao Lo,1 Wman Man,1 Xin Yuan Guan,1 Stephanie K. Ma,1 ‘The University of Hong Kong, Pokfulam, Hong Kong, 2The Hong Kong Polytechnic University, Hong Kong, 3Sun Yat-Sen University Cancer Center, Guangzhou, China; 4The Chinese University of Hong Kong, Hong Kong.

Hepatocellular carcinoma (HCC), the main type of liver cancer in human, is one of the most prevalent and deadly malignancies in the world. Despite advances in therapy, prognosis remains dismal, largely attributed by our limited understanding on information related to the progressive development of the disease, particularly in their cancer-initiating and stem cell-like properties. Wnt/β-catenin signaling is activated in CD133 liver cancer stem cells (CSCs), a subset of cells known to be a root of tumor recurrence and therapy resistance in HCC. However, the regulatory mechanism of this pathway in CSCs remains unclear. Here, we show that human miRNA, miR-1246, promotes cancer stemness including self-renewal, drug resistance, tumorigenicity and metastasis by activation of Wnt/β-catenin pathway via suppressing the expression of AXIN2 and GSK3β, two key members of the β-catenin destruction complex. This observation was made ex-vivo and in vivo functional / cell biological studies on HCC cell lines with or without miR-1246 expression modulated by lentiviral based knockdown and overexpression strategies as well as in miR-1246 repressed HCC cells with concomitant expression of wild-type or constitutively active β-catenin. Clinically, high endogenous and circulating miR-1246 was identified in HCC clinical samples and correlated with a worse prognosis. Further functional analysis identified Oct4 to be the direct upstream regulator of miR-1246, which cooperatively drive β-catenin activation in liver CSCs. In conclusion, our findings not only uncover the non-canonical regulation of Wnt/β-catenin in liver CSCs by Oct4/miR-1246 signaling axis, but also provide a novel diagnostic marker as well as therapeutic intervention for HCC.

#2896 STAT1-dependent interferon-related DNA damage resistance signature (IRDS) as a survival mechanism in castrate resistant prostate cancer (CRPC). Supreet Agarwal, Kerry McGowen, Keith Jansson, Mike Beshari, Fatih Elloumi, Maggie Cam, Kathy Kelly. NCI, Bethesda, MD.

Using the organoid culture system and aggressive Pten/TP53-null mouse model (GEMM), we have previously shown that the prostate cancer cell population harbors two classes of self-renewing luminal progenitors which are resistant to in vivo castration and to androgen receptor (AR) inhibitors (enzalutamide) ex vivo. Understanding signaling pathways governing intrinsic survival/self-renewal ability of luminal progenitors in castrate conditions can highlight pathways that play a role in acquired drug resistance. To identify mechanisms of castration resistance in luminal progenitors, we performed RNASeq analysis of luminal progenitor organoids derived from wild-type(WT) and Pten/TP53-null mice (intact and castrated (two weeks), n=5; each). Gene enrichment analysis identified key signaling pathways altered in luminal tumor organoids (AR signaling, lipid metabolism, protein secretion, inflammation etc.) that have also been described for FACS-purified human prostate luminal (CD49+) fraction. Interestingly, we found no difference in transcriptional profiles of intact and castrated tumor organoids, suggesting intrinsic survival ability of luminal progenitors upon castration. Of note, we observed most significant enrichment of STAT1-dependent IRDS in luminal progenitor tumor organoids relative to wild type luminal organoids. IRDS comprises of a subset of STAT1-driven genes that have been previously associated with survival of cancer cells and with breast cancer therapy resistance. In organoid systems, the STAT1 dependent IRDS were revealed IRDS as a prognostic marker for progression in the TCGA primary prostate cancer cohort (p<0.05). Further, high IRDS-expressing CRPC samples (SU2C dataset) were enriched for low AR signaling (r=-0.33, p<0.05). CRPC patients in IRDS-hi cohort showed enrichment for cancer stem cell phenotype and for genes associated with drug resistance. Consistent with the bioinformatics analysis real time PCR, immunofluorescence and western blot analysis of ex-vivo organoid cultures of castration-resistant Pten/TP53-null tumor organoids showed higher protein expression of STAT1 and IRDS genes in luminal tumor organoids relative to luminal WT organoids. In vivo, castrated prostate tumors showed higher STAT1 levels than the intact tumors. Treatment with enzalutamide of luminal tumor organoids resulted in time dependent increase in IRDS expression. STAT1 KD in tumor organoids decreased number of progeny organoids in subsequent generations suggesting either a direct or indirect effect upon self-renewal. Overall, our initial findings suggest STAT1 dependent signaling as a potential mechanism of androgen-independent survival in prostate cancer.

#2897 Oncogenic Kras activation in gastric adenocarcinoma promotes cancer stem cell phenotypes including metastasis & chemotherapy resistance. Changhyun Yoon, Kevin K. Chang, Jacob Till, Sandra W. Ryem, Sam S. Yoon. 1Memorial Sloan Kettering Cancer Center, New York, NY; 2Abramson Family Cancer Research Institute, Philadelphia, PA.

INTRODUCTION: The Cancer Genome Atlas found that the receptor tyrosine kinase (RTK)-Ras signaling pathway is altered in half of gastric adenocarcinomas (GAs). We added oncogenic Kras to the Atbp-Cre;Cdh1fl/fl; Trp5+/- mouse model of GA and found accelerated tumorigenesis and metastasis. We also found Kras activity to be higher in gastric cancer stem-like cells (CSCs). Thus we hypothesized that Kras activity is critical for maintenance of gastric CSCs and promotes CSC phenotypes. METHODS: Human and murine GA cell lines were examined. The effect of Kras pathway inhibition was examined in GA sporadic cells and monolayer cells in various in vitro assays. Kras activity and Kras pathway inhibition was examined in human primary gastric tissue samples. Our analysis of human gastric cancer datasets revealed Kras activity and Kras pathway inhibition in human GA as well as xenografts. RESULTS: Kras inhibition with shRNA or MEK inhibitor PD0325901 decreased the ability of GA cells to form spheroids and deceased expression of the stem cell transcription factor, Sox2, CD44+ /+ gastric CSCs had 65.8-75.4% higher migration, 64.2-79.2% higher invasion, and 63.1-70.2% more anchorage-independent growth compared to unselected cells. These properties could all blocked by 71.1-82.7% with Kras shRNA or PD0325901. In our mouse model, PD0325901 starting at 6 weeks of life increased median survival from 76 days to 95 days. Primary tumors and metastases in these mice with and without treatment with MEK inhibition are being analyzed for level of CD44 CSCs, Kras activity, and extent of metastases. Gastric CSCs were resistant to 5-fluorouracil and cisplatin chemotheraphy, and this chemotherapy resistance could be reversed in human GA xenografts transducing cells with Kras shRNA. CONCLUSION: Kras is more active in gastric CSCs than non-CSCs and promotes malignant phenotypes including metastasis and chemotherapy resistance. Kras pathway inhibition can block these CSC phenotypes, and thus may prove useful in various combination therapies.

#2898 ST8SIA1 is up-regulated in triple negative breast cancer and its expression is positively correlated with TP53 mutations and a cancer stem cell gene signature. V. Lokesh Battula, Yuanqing Yan, Khoa Nguyen, Kim-Anh Do, Michael Andreuff. UT MD Anderson Cancer Ctr., Houston, TX.

The discovered ganglioside GD2 as a breast cancer stem cell (BCSCs) marker in triple negative breast cancer (TNBC). GD2 biosynthesis is tightly regulated by the enzyme ST8SIA1 (GD3 synthase). However, expression of ST8SIA1 and its association with different breast cancer sub-types is not known. Here we hypothesize that ST8SIA1 is up-regulated in TNBC or “basal-type” breast tumors and associated with stemness in primary breast tumors. To investigate ST8SIA1

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expression in primary tumors, we analyzed RNAseq data from the cancer genome atlas (TCGA) data base, which includes data from 1105 primary and metastatic breast tumors as well as adjacent normal tissues. We found that ST8SIA1 expression varied widely among different breast tumors. Interestingly, “basal-type” tumors expressed the highest levels of ST8SIA1 compared to all other types of breast tumors including Sweezer or luminal phenotypes. ST8SIA1 expression was higher in tumors with high ER” or PR” or HER2” tumors (n = 852, p < 0.0001). Survival analysis by log-rank test indicated that patients with ST8SIA1high tumors survive shorter (median survival 2.6 years) compared to patients with ST8SIA1low tumors (median survival 4.3 years). Next, we investigated the association between ST8SIA1 expression and the most commonly mutated genes in breast cancer. We chose the top 20 most frequently mutated genes in TCGA dataset and examined their correlation with ST8SIA1 mRNA expression. We found that, among the top 20 mutations, TP53 had a very strong positive correlation with ST8SIA1 expression (p < 0.00001). In fact, the expression of ST8SIA1 was > 2-fold higher in TP53-mutated compared to wild type tumors. The other positively correlated mutation was a nuclear envelop protein called the spectrin repeat containing protein (nuclearenvelope1orSYNE1; p < 0.05). Mutations in GATA3 were the most negatively correlated (p # 0.006) with ST8SIA1 expression. Interestingly, GATA3 plays a role in epithelial cell differentiation in the mammary gland, supporting the notion that ST8SIA1 is a stem-cell-associated gene. In 4.3 years, we found a strong correlation of ST8SIA1 expression with other genes revealed that FOXA1, the protein which is co-expressed with GATA3 and serves as negative predictor of “basal-type” of breast cancer, was down regulated in ST8SIA1high tumors. In conclusion, ST8SIA1 is associated with “basal-type” of breast cancer, was downregulated in GATA3 were the most negatively correlated (p # 0.006) with ST8SIA1 expression in primary tumors. In conclusion, ST8SIA1 serves as negative predictor of “basal-type” of breast cancer.

Results: Sarcoma cell lines grown as spheroids had increased levels of ST8SIA1, compared to their counterparts in monolayer. The higher ST8SIA1 levels compared to hormonereceptors positive tumors include: (1) higher levels of ST8SIA1 expression (p # 0.0001) in sarcoma cell lines compared to the corresponding primary tumors, we analyzed RNAseq data from the cancer genome atlas (TCGA) data base, which includes data from 1105 primary and metastatic breast tumors as well as adjacent normal tissues. We found that ST8SIA1 expression varied widely among different breast tumors. Interestingly, “basal-type” tumors expressed the highest levels of ST8SIA1 compared to all other types of breast tumors including Sweezer or luminal phenotypes. ST8SIA1 expression was higher in tumors with high ER” or PR” or HER2” tumors (n = 852, p < 0.0001). Survival analysis by log-rank test indicated that patients with ST8SIA1high tumors survive shorter (median survival 2.6 years) compared to patients with ST8SIA1low tumors (median survival 4.3 years). Next, we investigated the association between ST8SIA1 expression and the most commonly mutated genes in breast cancer. We chose the top 20 most frequently mutated genes in TCGA dataset and examined their correlation with ST8SIA1 mRNA expression. We found that, among the top 20 mutations, TP53 had a very strong positive correlation with ST8SIA1 expression (p < 0.00001). In fact, the expression of ST8SIA1 was > 2-fold higher in TP53-mutated compared to wild type tumors. The other positively correlated mutation was a nuclear envelop protein called the spectrin repeat containing protein (nuclearenvelope1orSYNE1; p < 0.05). Mutations in GATA3 were the most negatively correlated (p < 0.0001) with ST8SIA1 expression. Interestingly, GATA3 plays a role in epithelial cell differentiation in the mammary gland, supporting the notion that ST8SIA1 is a stem-cell-associated gene. In 4.3 years, we found a strong correlation of ST8SIA1 expression with other genes revealed that FOXA1, the protein which is co-expressed with GATA3 and serves as negative predictor of “basal-type” of breast cancer, was down regulated in ST8SIA1high tumors. In conclusion, ST8SIA1 is associated with “basal-type” of breast cancer, was downregulated in GATA3 were the most negatively correlated (p # 0.006) with ST8SIA1 expression in primary tumors. In conclusion, ST8SIA1 serves as negative predictor of “basal-type” of breast cancer.
Tumor Biology: Signaling in Cancer Stem Cells

#2902 | Epithelial-type CD133+ stem-like lung cancer cells emerge higher drug resistance through MDRC-mediated Wnt/b-catenin signaling pathway. Chao-Ju Chen,1 Chih-Jen Yang,1 Ming-Shyang Huang,1 Yu-Peng Liu,1 Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 2Kaohsiung Medical University, Kaohsiung, Taiwan.

Backbone: The epithelial-to-mesenchymal transition (EMT) has been described to promote drug resistance and cancer stem cell (CSC) properties. Many studies demonstrated that a major proportion of circulating tumor cell (CTC) exhibits EMT and CSC characteristics. A recent finding revealed the presence of epithelial type (EpCAM+) CTC was associated with poor outcome, whereas the mesenchymal type (EpCAM-) CTC were not. We therefore hypothesized that the epithelial-type CSC may exhibit higher drug resistance ability. Methods: We isolated epithelial type (E+) and mesenchymal type (E-) CD133+ cells and CD133- cells from PC14 lung cancer cell line by fluorescence assisted cell sorting. We used western blot, QPCR, immunofluorescence, sphere formation assay and tumor xenograft assay to characterize the 4 subpopulations. The drug resistant ability was determined by cell viability assay and in vivo drug response assays. The drug resistant signature was identified by comparing the gene expression profile from four subpopulations and Gene Expression Omnibus (GEO) database. Knockdown and overexpression of MDRC in PC14 subpopulations were established by using lentivirus vectors. Immunoprecipitation and subcellular fractionation were performed for drug resistant mechanism investigation. Results: The epithelial type PC14 CD133+ cells (E+/CD133+ subpopulation) exhibited higher sphere formation ability and was more resistant to the treatment of chemotherapeutics compared to the mesenchymal type CD133+ cells (E-CD133+ subpopulation) in vitro and in vivo. Gene expression profiling showed 86 genes were bioinformatically predicted as drug resistant signature and were correlated with the disease-free survival of the patients with lung cancer. Among these genes, the mRNA level of 20 genes were significantly related to the patient’s prognosis in the GSE31210 dataset. Human I-mfa-domain-containing protein (MDRC) was highly expressed in E+/CD133+ subpopulation. Knockdown and overexpression of MDRC modulates drug resistance ability in cancer cells. MDRC increased the level of free b-catenin through binding and stabilizing the axin-GSK3-b-catenin destruction complex and increased the transcriptional activity of Wnt/b-catenin signaling. Conclusion: The epithelial-type CD133+ stem-like lung cancer cells are more resistant to the chemotherapy through MDRC-mediated Wnt/b-catenin signaling activation.

#2903 | 1-Methoxyphospho-inolin: Novel gamma secretase inhibitor targeting notch-1 signaling in breast cancer stem cells. Venkatesh Kolluru,1 Deeksha Pal,1 Becca Baby,1 Houda Alatassi,1 Arun Kumar Sharma,1 Murali Ankem,1 Chandli Damodaran1. 1University of Louisville, Louisville, KY; 2Pennsylvania State University, University Park, PA.

We recently showed that two different ALDH1+ and CD44+/CD24−/low breast cancer stem cells (BSCs) exhibited stem cell characteristics that include self-renewal, extensive proliferation, the ability to form non-adherent spherical clusters, chemotherapeutic resistance and high notch1 expression. We have identified a compound 6:3-(methylbut-2- enyl) coumouserot (Pso) and treatment with Pso resulted in growth inhibition and an EM'T phenotype in both BSCs and BC cells. Oral Pso administration at physiologically achievable doses (25 mg/kg/BW) suppressed the growth of BSCs and BC xenografts without toxicity. In the current studies, we identified several novel Pso-derived analogs that may be more potent than the parent compound. One such compound, 1-Methoxyphospho-inolin (1MP), obtained via three main functional group changes: (i) translocation of the isoprenyl moiety from the phenyl ring fused to the pyran ring (as in Pso) to the phenyl ring adjacent to the furan ring, (ii) removal of the carbonyl group from the pyran ring, and (iii) introduction of a methoxy group at the 1-position, inhibited Notch1 activity and growth of both BSCs and BC cells at nM concentration [I50 300nM], which is 100 times more potent than Pso in cell culture models. Molecular studies suggest that 1MP inhibits Notch signaling hypothesis that the epithelial-type CSC may exhibit higher drug resistance ability. Methods: We isolated epithelial type (E+) and mesenchymal type (E-) CD133+ cells and CD133- cells from PC14 lung cancer cell line by fluorescence assisted cell sorting. We used western blot, QPCR, immunofluorescence, sphere formation assay and tumor xenograft assay to characterize the 4 subpopulations. The drug resistant ability was determined by cell viability assay and in vivo drug response assays. The drug resistant signature was identified by comparing the gene expression profile from four subpopulations and Gene Expression Omnibus (GEO) database. Knockdown and overexpression of MDRC in PC14 subpopulations were established by using lentivirus vectors. Immunoprecipitation and subcellular fractionation were performed for drug resistant mechanism investigation. Results: The epithelial type PC14 CD133+ cells (E+/CD133+ subpopulation) exhibited higher sphere formation ability and was more resistant to the treatment of chemotherapeutics compared to the mesenchymal type CD133+ cells (E-CD133+ subpopulation) in vitro and in vivo. Gene expression profiling showed 86 genes were bioinformatically predicted as drug resistant signature and were correlated with the disease-free survival of the patients with lung cancer. Among these genes, the mRNA level of 20 genes were significantly related to the patient’s prognosis in the GSE31210 dataset. Human I-mfa-domain-containing protein (MDRC) was highly expressed in E+/CD133+ subpopulation. Knockdown and overexpression of MDRC modulates drug resistance ability in cancer cells. MDRC increased the level of free b-catenin through binding and stabilizing the axin-GSK3-b-catenin destruction complex and increased the transcriptional activity of Wnt/b-catenin signaling. Conclusion: The epithelial-type CD133+ stem-like lung cancer cells are more resistant to the chemotherapy through MDRC-mediated Wnt/b-catenin signaling activation.

TUMOR BIOLOGY: Tumor Evolution and Heterogeneity

#2904 | Targeting cancer stem-like cells in triple negative breast cancer cells through non-canonical notch signaling. Fokhrul Hossain,1 Claudia Sorrentino,1 Ayse Bilyeu,1 Judy Crabtree,1 Antonio Pannuti,1 Todd Golde,2 Barbara Osborne,2 Lucio Miele1. 1LSUHSC, New Orleans, LA; 2University of Florida, FL; 3UMass Amherst, MA.

Triple negative breast cancer (TNBC) is a heterogeneous group of clinically aggressive diseases. TNBC patients have high risk of recurrence and metastasis, and current treatment options remain limited. Cancer stem-like cells (CSCs) have been linked to cancer initiation, progression and chemotherapy resistance. Therefore CSC-targeted therapies are keenly sought. There is strong evidence for the involvement of Notch signaling in TNBC. Notch1 is highly expressed in basal-like (1) and especially Mesenchymal-Stem-Like (MSL) TNBCs. Expression of Notch1 and its ligand Jagged1 correlate with poor prognosis. Moreover, overexpression supports key roles of different Notch paralogs in breast CSCs. Here, we demonstrate that Notch1 promotes cell survival in MDA-MB-231 cells, representative of MSL TNBC, in part by activating NF-kB. Notch activation by Jagged1-expressing stromal cells enhances transcription of the anti-apoptotic gene cIAP-2 (BIRC3), a known NF-kB target. This event is dependent on recruitment to the cIAP-2 promoter of NF-kB subunits, IKKo and Notch1. Short term exposure of MDA-MB-231 cells (MSL, PTEN wild-type), but not MDA-MB-468 cells (BL1, PTEN-null) to recombinant Jagged1 leads to AKT phosphorylation. This is suppressed by dual mTORC1/2 inhibitors, AKT inhibitors and IKKo inhibitors but not Everolimus (mTORC1-selective inhibitor). These observations support a model where canonical and non-canonical mechanisms downstream of Notch1 trigger AKT phosphorylation and NF-kB activation in PTEN wild type TNBC cells. Rapid AKT phosphorylation downstream of Notch1 requires mTORC2, PI3K and IKKo, and contributes to NF-kB activation. This suggests a bidirectional crosstalk between the IKKo and AKT arms of this Jagged1-activated pathway. We demonstrate that recombinant Jagged1 increases the cellular metabolism of TNBC cells and knockdown of Notch1 or IKKo by siRNA decreases mitochondrial respiration and glycolysis. We have found that CSCs derived from MDA-MB-231 cells have increased Notch1, p-AKT, and oxidative metabolism. AKT inhibition or IKKo inhibition decreases both mitochondrial respiration and glycolysis of TNBC derived CSCs. Pharmacological inhibition of Notch cleavage by gamma secretase inhibitor (PF-03084014) in combination with AKT inhibitor (MK-2206) or NF-kB inhibitor (Bay11-7082) blocks CD90+ or CD44+ CD24low sorted secondary mammospheres formation. These data suggest that combination treatments affecting Notch, NF-kB and AKT pathways have potential therapeutic importance in targeting CSCs of TNBC cases with wild type PTEN and high Notch1 expression.

#2905 | Clinical and genomic resistance to second generation androgen blockade in paired biopsies of metastatic castration-resistant prostate cancer. G. Celine Han,1 Justin Hwang,1 Stephanie A. Mullane,1 Carrie Cibulskis,2 Zhenwei Zhang,1 Rana R. McKay,1 PCF-SU2C Dream Team, Scott L. Carter,1 William C. Hahn,1 Mary-Ellen Taplin,1 Eliezer M. Van Allen1.

Background: Recent “next generation” androgen deprivation therapies (ADT), such as abiraterone and enzalutamide, have improved survival in patients with castrate-resistant prostate cancer (CRPC). Despite therapy, most patients develop resistance to these agents. We investigated the genetic basis of tumor evolution and clinical resistance to next generation ADT in CRPC by using whole exome sequencing (WES) on paired pretreatment and post-resistance biopsies from CRPC patients. Methods: Matched “trios” of germline, pre-treatment and post-resistant tumor samples were obtained from 7 patients treated with abiraterone (n=4) and enzalutamide (n=3) and WES was performed. Clinical data, including PSA and radiographic measurements, was used to classify patients as intrinsically resistant or initially responsive to treatment. Quality control, mutation and indel calling, copy number variation identification were performed using analytical pipelines at the Broad Institute. Tumor purity and ploidy were inferred, and phylogenetic analysis was performed using ABSOLUTE and Phylogeny, respectively to identify resistance associated alterations in the context of clinical phenotypes. Results: We identified multiple putative mechanisms and genetic categories of resistance to next generation ADT in CRPC. One abiraterone patient acquired an AR mutation (L702H) in the post-treatment sample, previously reported to be associated with poor outcomes and to confer resistance to this therapy. Amplification of MYC was associated.
with resistance in two other abiraterone patients with pre-existing AR gain: one with initial response to therapy acquired a focal gain of MYC in the primary tumor and metastases and our data support that the metastases were formed from a single cancer clone that did not dominate the primary tumor. Genetic heterogeneity was also observed between the primary tumor and metastases and our data support that the metastases were formed from a single cancer clone that did not dominate the primary tumor. From a genetic point of view, organoids do not seem to fully reflect the tumor area of origin and less so the whole tumor, indicating that care should be taken when using organoids as models of the primary tumor.

Characterization of genetic intratumor heterogeneity of colorectal cancer and matching organoids. Sigrid S. Arnadottir,1 Maria Jeppesen,2 Madsen,3 Jacob Thastrup,4 Ole Thastrup,4 Claus L. Andersen 1.

#2906 Characterization of genetic intratumor heterogeneity of colorectal cancer and matching organoids. Sigrid S. Arnadottir,1 Maria Jeppesen,2 Madsen,3 Jacob Thastrup,4 Ole Thastrup,4 Claus L. Andersen 1.

TUMOR BIOLOGY: Tumor Evolution and Heterogeneity

#2907 Extracellular matrix remodeling triggered by lysyl oxidase inhibition promote the lung adenocarcinoma to squamous cell carcinoma transi- tion independent of LKB1 status. Shun Yao, Hongbin Ji. Institute of Biochem- istry and Cell Biology, Shanghai Institute for Biological Sc, Shanghai, China.

#2908 Single-cell genetic analysis of ductal carcinoma in situ with and with- out synchronous invasive breast cancer by multiplex FISH delineates specific patterns of tumor clonality and heterogeneity. Irriana Marie Torres,1 Leann Hernandez,1 Jausheng Tzeng,2 Russell Schwartz,2 Alejandro Schaffer,4 Edward Gertz,2 Stephen Brower,2 Miguel Sanchez,4 Gert Auer,2 Kerstin Heselmann-Haday- dad,2 Reid Thomas.1 National Cancer Institute, Rockville, MD; 2Englewood Hospital and Medical Center, Englewood, NJ; 3Carnegie Mellon University, Pittsburgh, PA; 4NCBI, Bethesda, MD; 5Karolinska Institute, Stockholm, Sweden.

We previously studied synchronous Ductal Carcinomas in Situ (DCIS) and Invasive Ductal Carcinomas (IDC) using a novel approach of multiplexing FISH probes that allows us to simultaneously assess the copy numbers of up to 20 loci within intact nuclei providing new insights into tumor clonality and heterogeneity. A high degree of chromosomal instability already in DCIS, and frequently, but not always, a direct clonal evolution from DCIS to IDC was detected. We now ask whether this degree of instability is also present in DCIS that did not progress to IDC and are therefore analyzing FFPE material from 20 patients with either low-grade and high-grade DCIS who did not present with invasive breast cancer during their follow-up (5-10 years), in addition to 10 patients who presented with synchronous IDC. The multiplex FISH assay used targets five oncogenes (COX2, MYC, CCND1, HER2, ZNF217) and three tumor suppressor genes (DBC2, CDH1, TP53) frequently altered in breast carcinomas. To date, we have analyzed two paired cases of synchronous DCIS and IDC, DCIS-IDC 3 and 4, and three cases of DCIS without IDC, OP-DCIS 1, 4 and 5. The paired cases exhibited very similar aberration pat- terns for synchronous DCIS and IDC indicating the invasive carcinoma is closely related to the DCIS lesion. Specifically, the major clones in DCIS 3 (low-grade) and IDC 3 showed a diploid tumor cell population with a gain of COX2, and losses of DBC2, MYC, TP53, and HER2. Of note, the only difference observed was a gain of ZNF217 in the DCIS which was not seen in the major clone of the invasive carci- noma. The other paired case, DCIS 4 (high-grade) and IDC 4, showed major clones of triploid tumor cell populations with gains of COX2, CCND1 and MYC and losses of DBC2, CDH1, TP53 and ZNF217. The three DCIS cases with synchronous IDC 4 showed a major clone of the DCIS harboring a triploid population and a subclonal population of almost diploid cells. The subsequent IDC exhibited varying degrees of aberration and complexity patterns in their clonal populations. Case OP-DCIS 1, a high-grade DCIS, revealed a tetraploid cell population which showed a major clone with an amplification of CCND1 and a MYC gain combined with losses of DBC2, CDH1, TP53 and HER2. A low-grade DCIS, OP-DCIS 4, exhibited a diploid cell population with a major clone showing losses of DBC2, CDH1, TP53 and ZNF217. The third case OP-DCIS 5 (low-grade DCIS) was a diploid cell population with one major clone showing losses of CDH1, MYC, DBC2, TP53, HER2 and CCND1. Our preliminary observations show a ten- dency of diploid lesions with a predominance of loss patterns for low-grade DCIS, while high-grade DCIS seem to reveal higher ploidy with more complex gain and
loss patterns. However, also low-grade DCIS with mainly low loss patterns progress to invasive cancers as seen in case DCIS-IDC3. We expect that the analysis of the remaining cases will further elucidate the dynamics of DCIS lesions which will hopefully help to assess and stratify progression risk in patients with DCIS.

**#2909 High-precision quantification of clonal evolution during comparative treatments of triple negative breast cancer.** Hyunsoo Kim,1 Pooja Kumar,1 Francesca Menghi,1 Eliza Cerveira,1 Mallory Romanovitch,1 Gurdeep Ananda,1 Joshy George,1 Henry Chen,2 Susan Mockus,1 Chengsheng Zhang,1 Yan Yang,3 James Keck,4 R. Krishna Murthy Karuturi,1 Carol Bult,1 Charles Lee,1 Edison Liu,1 Jeffrey H. Chuang,1 *The Jackson Laboratory for Genomic Medicine, Farmington, CT; 2The Jackson Laboratory Mice, Clinical, and Research Services, Sacramento, CA; 3The Jackson Laboratory for Mammalian Genetics, Bar Harbor, ME.

Population heterogeneity within tumors is essential to the development of drug resistance. However, precise quantification of cellular levels of subpopulations, and in particular how they evolve in response to treatment, has been challenging. Here we describe the high precision characterization of subclonal evolution within triple-negative breast cancer patient-derived xenografts (PDXs) generated from three patients. For each patient model, we established multiple PDXs and treated them in cohorts of 5-10 mice each for the therapies cyclophosphamide, doxorubicin, cisplatin, or docetaxel, with treatments lasting one month. In all three patient models, the average behavior across mice was a reduction in size in response to docetaxel, but growth under doxorubicin and cyclophosphamide. For cisplatin treatment, one of the three models showed tumor shrinkage while the other two models showed continued growth but at a rate lower than the doxorubicin or cyclophosphamide cohorts. To determine the evolutionary behaviors underlying these observations, we initially performed exome panel sequencing of 34 residual tumor samples from these and untreated xenografts. Computational mutation and copy number analysis indicated sample-specific differences in tumor populations both in response to treatment and due to genetic drift. However, they also revealed measurement uncertainties related to exome capture efficiency, locus-specific read counts, and computational copy number estimation that limited quantitative inference of evolutionary behaviors. To solve this problem we used droplet digital PCR (ddPCR) to measure variant allele frequencies and local copy number at selected loci from the prior residual tumors as well as additional samples from replicate cohort treatments and cultured conditionally reprogrammed progenitor cells. In total we performed 1665 ddPCR measurements across 150 cancer samples. These ddPCR measurements reduced sample-specific uncertainty in variant allele frequency to ~2% and copy number to ~0.2, allowing for precise identification of subclones and their cellularity in each sample. We observed several common modes of evolution within these tumors including selective sweeps, spatial diffusion, and stable coexistence between distinct subpopulations. In the samples from one patient model, we observed frequent symbiotic growth of two distinct subpopulations having differential cisplatin sensitivity, such that the degree of clonal selection during cisplatin treatment was proportional to the tumor volume change on a mouse-to-mouse basis. This study demonstrates how high precision genomic characterization across comparatively treated samples can reveal treatment-relevant subclonal ecology, as well as the mutations that distinguish populations with different behaviors.

**#2910 Inter metastatic genetic heterogeneity is a characteristic feature of recurrent pancreatic cancer.** Hitomi Sakamoto, Alvin Makohon-Moore, Marc Attiyeh, Liguo Zhang, Christine A. Iacobuzio-Donahue. Memorial Sloan Kettering Cancer Center, New York, NY.

Pancreatic cancer (PDAC) is a 4th leading cause of cancer death with an overall 5-year survival of 8%. Surgical resection remains the only option for cure but only 10-15% of newly diagnosed patients are eligible. While surgery clearly provides a survival benefit compared to those patients who do not undergo potentially curative resection, the vast majority of patients will recur in the pancreatic remnant, liver, lungs, and/or peritoneum. Thus, there is an unmet need to better understand the fundamental features of recurrent disease. To explore the fundamental genomic features of recurrent PDAC, patients were selected from participating Gastrointestinal Cancer Rapid Medical Donation Program (GI-CRMDP). 10 patients who underwent surgical resection; 8 patients had a whipple procedure, 2 patients had a distal pancreatectomy and each patient received a different type of chemotherapy after surgery, was evaluated. We performed 250x whole exome sequencing (WES) of 82 unique samples from these 10 patients, using frozen samples from autopsy and FFPE tissues from matching surgical specimen. In addition to the treatment naïve primary tumor, one or more samples of the local recurrence and multiple metastases were studied. The mean depth of coverage for all samples was 31×. Copy number variations were assessed by FACETs. MSK-IMPACT was performed for orthogonal validation of a subset of variants. We found that compared to the treatment naïve resected tumor, recurrent disease contained novel mutations in genes associated with MAPK and PI3K signaling. The most common mutual mutation signature was Signature 1 followed by Signature 3. All patients who received a platinum drug as a first-line treatment had a greater proportion of Signature 3 in the recurrent disease than the primary tumor. Phylogenetic studies revealed that recurrent disease is the result of three patterns: 1) dissemination of disease to distant organs before surgery was performed, 2) dissemination of disease after surgery from residual microscopic disease in the pancreatic remnant, or 3) development of a second primary tumor in the remnant pancreas. This third scenario was observed in one of two additional patients in this cohort. We conclude that recurrent pancreatic cancer is multifactorial, and at least two of these scenarios could be eliminated by total pancreatectomy, thereby increasing the rate of cure by surgical resection. Our study serves as a window to an understanding of the clonal dynamics of recurrent pancreatic cancer.

**#2911 Single cell mass cytometry analysis of human lung adenocarcinoma.** Deon B. Doxie, Jonathan M. Lehman, Yong Zou, Maria S. Ortega, Caroline E. Maier, Jonathan M. Irish, Pierre P. Massion. Vanderbilt University, Nashville, TN.

Introduction: Lung cancer is the leading cause of cancer-related mortality in the world. Lung adenocarcinoma is the most common subtype. Tumor heterogeneity among adenocarcinomas presents a challenge in the management of the disease. Understanding heterogeneity may have implications in understanding the biology of lung cancer. Single cell analysis of tumor mass cytometry offer an opportunity to profile tumor heterogeneity and to identify populations of driven by the activation of signaling pathways. Methods: Adenocarcinomas were collected at the time of surgery and dissociated into suspension and cryopreserved. Mass cytometry analysis of tumors and adenocarcinoma cell lines was performed with a 30 marker antibody panel and rhodium intercalator dye to identify dead cells. The antibody panel included markers to characterize identity, cell cycle status, and signaling events. Biaxial gating or unsupervised analysis approaches SPADE and viSNE were used to compare major populations of cells. Results: Validation of mass cytometry panels was performed with adenocarcinoma cell lines and human tumors. Adenocarcinoma cell lines PC9 (lung), A549 (lung), H520 (squamous) and SW620 (colon) exhibited phenotypically distinct cells between samples by expression of cytokeratin, CK7, and EGFR. Furthermore, PD-L1 expression was expressed on subsets within cell lines. Disaggregation of tumors was optimized with dissociation that included collagenase and DNase to release viable cells within 1 hour. Detection of viable cells was optimized with rhodium intercalator viability dye and histone H3 to identify nucleated cells. Using the 30 marker panel and viSNE analysis four populations were identified within tumors: infiltrating leukocytes, epithelial cells, fibroblast, and epithelial tumor cells. Epithelial cells were found to exhibit cellular heterogeneity between patients based on the expression of cytokeratin, CK7, TTF1, EGFR, vimentin, CD44, and MET. Preliminary signaling data identified basal kinase activity active in infiltrating leukocytes and cancer cells. Cancer cells and infiltrating immune cells had basal p-STAT5 activation, and cancer cells had high basal p-AKT implicated in dysregulated cell growth. Conclusions: Populations of infiltrating stromal cells and epithelial (cancer) cells were identified in lung adenocarcinoma. The single cell phenotyping from tumors was consistent with the profile found in two lung adenocarcinoma cell lines. Preliminary differences in basal signaling pathways responsible for growth were observed within adenocarcinoma cell populations. This work demonstrates the feasibility of mass cytometry to identify and characterize tumor heterogeneity. Work is underway to define phenotypes within the epithelial and leukocyte populations that could predict tumor behavior and immune response within the microenvironment. This work is supported by NCI CA196405 to PPM.

**#2912 Size based enrichment and sorting of Ov90 cancer cells and clusters with a new multistage filtration cartridge reveals distinct phenotypes.** Anne Meunier,1 Sara kheireddine,1 1. Alejandro Hernandez-Castro,2 Teodor Veres,3 David Juncker1. *McGill University, Montreal, Quebec, Canada; 2National Research Council of Canada, Boucherville, Quebec, Canada.*

Background: Circulating tumor cells (CTCs) are released in blood from the primary tumor, but although very heterogeneous both in size and marker expression, are very rare and provide information not available from the primary tumor. The identification of CTC cells and clusters could advance our understanding of metastasis and help personalize therapy.1-3 Notably, CTC clusters were shown to have a higher metastatic potential than single cells but the process remains poorly understood. We present a multistage filtration system with pore sizes from 20 down to 8 μm for the size-selective enrichment of Ov90

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ovarian cancer cells and clusters from blood. Each captured cell population was released, cultured, and characterized independently. Methods: We developed a 3D printed multistage filtration cartridge and polymer filters with 20, 15, 12, and 8 μm-diameter pores. Filters were stacked from 20 (top) to 8 μm (bottom) and used to enrich and sort Ov-90 cells spiked in 1:6 mL of blood/PBS. Captured cells were released by removing individual filters from the cartridge, reverse flowing OSE medium, and then cultured separately. Results: Ov-90 clusters were found mostly on the top filter (20 μm) and interestingly, few small clusters (3–4 cells) were found on the 8 and 10 μm filters, suggesting alignment of cluster cells as they pass through the pores. Cell and nucleus diameters were measured, and a general correlation was found between filter pore size and cell and nucleus size. Interestingly, nuclei were found to be the smallest parameter in determining passage of single cells and small clusters through pores. Follow- ing cell culture, two distinct phenotypes were observed: cell captured on small pore filters (8–12 μm) grew primarily in a monolayer. Cells captured on filters with larger pores (15–20 μm) first grew as a monolayer, but rapidly formed cell aggregates that subsequently detached from the surface. Staining for E-Cad- herin, a cell-cell adhesion protein, revealed a loss of expression of cells from filter with larger pores. Conclusion: We developed a new multistage filtration method and selectively enriched and sorted cells based primarily on their nucleus size. We identified two Ov-90 populations with different growth behaviors with low E-cadherin expression on the cells forming clusters, which is known to correlate with metastasis. The application of multistage filters may also reveal different CTC populations based on nucleus and cell size. References: (1) Baccelli, I. et al. Nat. Biotechn. 2013, 31, 539–544. (2) Pecot, C. V. et al. Cancer discovery 2011, 1, 580–586. (3) Cheung, K. J. et al. Proc. Natl. Acad. Sci. U.S.A. 2016, 113, E854–E863. (4) Au, S. H. et al. Proc. Natl. Acad. Sci. U.S.A. 2016, 113, 4947–4952.

#2913 Emergence of RAS or EGFR mutant clones affects duration of response to EGFR blockade in colorectal cancers. Sabrina Arena,1 Beth Van Emburgh,1 Giulia Stravagia,1 Luca Lazzari,1 Giovanna Cissiulli,1 Giorgio Conti,2 Benedetta Musso,2 Federica Baldi,2 Michela Buscarino,2 Alice Bartolini,3 Emanuele Valtorta,3 Joana Vidal,3 Beatriz Belloso,3 Giovanni Germano,3 Filippo Pietranansomio,4 Agostino Ponzetti,4 Joan Albanel,4 Salvatore Siena,5 Andrea Sartore-Bianchi,6 Federica Di Nicolantonio,1 Clara Montagut,5 Alberto Bardelli.1 1University of Torino, Candiolo, Italy; 2Candiolo Cancer Institute - FPO, Candiolo, Italy; 3University of Torino, Torino, Italy; 4Niguarda Cancer Center, Milano, Italy; 5Hospital del Mar, Barcelona, Spain; 6Istituto Nazionale dei Tumori, Milano, Italy; 7San Giovanni Battista Hospital, Torino, Italy.

Cetuximab and panitumumab are monoclonal anti-EGFR antibodies (moAbs) currently used for the treatment of advanced RAS wild type colorectal cancers (CRC). Emergence of acquired resistance invariably limits the efficacy of these agents, and the dynamics of clonal evolution during anti-EGFR blockade are poorly understood. At progression, RAS mutations represent the most common genetic alterations, while EGFR extracellular domain (ECD) mutations are acquired by a smaller cohort of patients. We found that the mutation profile correlates with the clinical outcome of patients; in particular those who develop RAS mutations upon EGFR blockade achieve reduced tumor shrinkage and shorter duration of response respect to patients in which EGFR ECD mutations emerge during therapy. We investigated in preclinical models the potential role of RAS and EGFR ECD mutations during the emergence of acquired resistance, by tracking the evolution of clones in a genetically barcoded population of CRC cells chronically treated with cetuximab. We observed that therapeutic (target therapy, chemotherapy) and environmental (reduced nutrient condition) pressures differentially shape the clonal composition of CRC cell populations, leading to the emergence of clones with the highest fitness in presence of the external pressure. In conclusion, a multistep clonal evolution process characterizes the development of drug resistance and is associated with the clinical outcome of CRC patients treated with anti-EGFR antibodies.

#2914 Molecular characterization with single-cell resolution of CTCs and FFPE specimens from the same lung adenocarcinoma patients reveals the extent of intra-tumor heterogeneity. Tiziana Terranova,1 Francesco Gelsomino,2 Francesco Bacchi,3 Francesca Fontana,1 Claudio Forcato,1 Alberto Ferrari,1 Michelangelo Fiorentino,1 Valentina Del Monaco,1 Giulio Bassi,1 Chiara Mangano,1 Chiara Bolognesi,1 Paola Tononi,2 Genny Buson,2 Michele Medoro,2 Nicolò Maareni,3 Michele Tognetto,4 Andrea Ardizzoni2. 1University of Perugia, Perugia, Italy; 2Policlinico Sant’Orsola – FPO, Candiolo, Italy; 3Policlinico Sant’Orsola – FPO, Bologna, Italy; 4Policlinico Sant’Orsola – FPO, Candiolo, Italy.

Introduction: Intra-tumor heterogeneity can hide genomic and genetic features, which may be key driver of disease progression. Routinely, only one biological specimen per patient is generally analyzed, which may only partially represents the genetics of the tumor. Here we report a multi-approach analysis of circulating tumor cells (CTCs) and formalin-fixed paraffin-embedded (FFPE) tumor tissue-derived cells (TCs) obtained from the same patients, to investigate the underlying genetic heterogeneity. Methods: Peripheral blood (PB) and FFPE tumor tissue were collected from two advanced lung adenocarci- noma patients, treated with cisplatin-pemetrexed and carboplatin-pem- etrexed as a second line therapy. The first patient was previously diagnosed with an ALK-translocation and treated with an ALK-inhibitor. PB was enriched with either an EpCAM-based or EpCAM-independent method: the cell output of the latter was stained with Cytokteratin-PE, CD45-APC and DAPI. The DEPArray™ platform was used to detect and collect pure single CTCs or TCs, along with WBCs or stromal cells as controls. Whole genome amplified DNA of single CTCs and TCs was used to profile genome-wide copy-number aberrations (CNAs) using the AmpliFire™ LowPass kit; single nucleotide variants were analyzed on CTCs WGA products and on pools of TCs using AmpliFire™ CHIP custom panel and DEPArray™ Oncoseek panel respectively. Results: No clinically significant variants were detected in CTCs and FFPE samples; however the copy-number profiles of single TCs and CTCs revealed an overabundance of gains and losses, confirming the aberrant nature of tumor cells. In the first patient, all single cells showed a pattern of shared alterations, with a common amplification of the genome region comprising MYC gene (also confirmed by deep-of-coverage in targeted genomic amplicons) and clustering of single CTCs were formed from the group of TCs and CTCs, characterized by some emerging clones and low inter-cell heterogeneity. The analysis of the copy-number profiles of cells from the second patient showed an opposite situation: unsupervised clustering of low-pass profiles highlighted an independent group formed by single TCs clearly distinct from the highly heterogeneous cluster formed by CTCs. Conclusion: Intra-tumor graft analysis of pure cells derived from multiple specimens from the same patient, together with the combination of low-pass whole genome sequencing and targeted sequencing, reveals unexpected genetic similarities and diversities, and provides fundamental information to understand intratumor heterogeneity.

#2915 The timing of mutational burst events impacts the growth of tumors in the colon. Chelsea K. Steivers, Tien N. Vo, Perry J. Pickhardt, Jorgin D. Poelder, Kristina A. Matkowskyj, Dawn M. Albrecht, Quincy Rosemarie, Michael A. Newton, Richard B. Halberg. University of Wisconsin, Madison, WI.

Basic and clinical scientists believe that benign polyps in the colon progress to cancers through the slow, stepwise accumulation of mutations. Inter- estingly, only a small percentage of all tumors progress, whereas a significant number remain static in size, regress, or resolve completely. The mecha- nisms underlying these differential fates are unknown, and mechanisms of tumor evolution during this premalignant phase are still under investigation and continued debate. We previously reported that sub-clonal diversity arises early in small human adenomas and contributes to the growth of tumors in the colon. An emerging hypothesis is that colorectal tumors form as a result of a “mutational burst” via a process of punctuated equilibrium. Multiple copy number alterations and mutational events happen simultaneously in a burst-like fashion. In this study, we prospectively test this concept using a mouse model in which tumor induction is spatially and temporally controlled via a non-surgical delivery of adenosine expressing CRE recombinase and a temporally controlled mutational burst via administration of the carcinogen Azoxymethane. Tumors are induced at a similar rate regardless the timing of the burst relative to tumor induction with a mean tumor incidence of 62% at three weeks post induction. However, tumor growth may be affected by the timing of the burst. Animals that had a mutational burst event prior to tumor induction or those that had a late mutational burst event had a lower average in vivo tumor volume compared to controls and those with early burst event, but the average in vivo tumor size was comparable across all groups. Taken together, these preliminary data provide evidence that the timing of a mutational burst event contributes to tumor growth. This prospective experiment is being extended through computer modeling and statistical inference to compare in silico mutational landscapes to a cohort of colon polyps removed from patients at normal screening. The findings will provide new insights into the earliest stages of tumorigenesis.
Background: Glioblastoma (GBM) is an aggressive cancer that often recurs despite multimodal therapy. Median survival is 12–15 months. Genomic profiling studies have shown marked tumor heterogeneity with distinct mutations among treated and untreated samples. The Cancer Genome Atlas (TCGA), revealed that mutations in TP53, CDK4/23A, PTEN, EGFR and NFI are predominant in untreated tumors. Of the GBM tumors sequenced, >80% are mutated at TP53. We sequenced the matched ERBB2 and NRAS codons of GBM tumors sequenced with matched mutations in mismatch repair repair proteins, mainly MSH6. We proposed to use the Cellct-Lentiviral-based tagging library, a novel model system to assess the role of intratumor heterogeneity (ITH) in the presumptive tumor-initiating fraction of primary GBM and in the development of resistance to temozolomide (TMZ). From a pool of 30,000,000 barcodes, the system enables the genetic integration of a unique barcode sequence into each cell. Each barcode can be quantitatively tracked via next-generation sequencing, allowing for dynamic monitoring of the subclonal architecture. Methods: GSC 272 and 627 (sensitive and resistant to TMZ) were derived from core biopsies of GBM patients. Cells were transduced with a lentiviral vector containing luciferase. Cells were expanded and infected with the Cellct-Lentiviral library. Multiplicity of infection was determined. Initial transduction studies showed GSC 272 to have methylation of MGMT while GSC 627 did not. There was no difference in OS in the TMZ Resistant (GSC 627) untreated vs. treated cohort (with a median OS of 50 days in both groups (N = 23 vs 25 respectively, P = 0.563). TMZ Sensitive (GSC 272) untreated mice did not survive as long as the treated cohort (N = 24 vs. 25, Median OS 43 vs. 206 days, P = 4.61e-09). Barcoding sequencing results is currently being analyzed. Conclusions: The Cellct-Lentiviral tagging system is an innovative way to track ITH and clonal evolution in glioblastoma orthotopic models. We hope to discover novel insights into TMZ treatment response and resistance.

Quantitative mapping of epidermal growth factor receptor activation in single breast cancer cells treated with targeted therapies. Phuong Le, Kristopher Kilian, Andrew Smith. University of Illinois at Urbana Champaign, Urbana, IL.

Twenty percent of breast cancers are triple-negative, lacking estrogen receptor, progesterone receptor, and HER2. There is currently no specific FDA-approved targeted therapy for triple-negative breast cancer patients. Approximately half of triple-negative breast cancers overexpress epidermal growth factor receptor (EGFR), however phase II trials have only shown a 5% response rate with targeted inhibition. A quantitative analysis of EGFR signaling pathway dynamics of single cells at the single molecule level has the potential to shed light on the mechanism of lack of response to targeted therapy, as well as the cell-to-cell heterogeneity of response. Here, we combine single-molecule 3D imaging of EGFR using fluorescent quantum dots (QDs) with cell geometry normalization by micro-patterning to quantitatively map EGFR endocytosis in single cancer cells. Triple negative MDA-MB-231 cells conform to defined geometries (rectangular with different aspect ratios) were grown on soft polyacrylamide gels after micro-contact printing of islands of fibronecin. Two color QD conjugates of EGFR and its ligand, EGF, are used to track ligand binding and receptor internalization upon activation in the absence and presence of different NAC tumor treatments. Conclusions: We demonstrated that microcontact-printing normalizes cell geometry while maintaining EGFR localization so that EGFR signaling response can be quantitatively assessed on a single-cell basis and that QD conjugates can specifically label EGFR and EGF. Quantitative single-molecule imaging at single-cell level revealed high level of heterogeneity in the amount and spatial localization of EGF binding and EGFR endocytosis among the cell population in the presence and absence of EGFR inhibitor, gefitinib. Using this platform, we will investigate if spatial organization of EGFR clusters within individual cells contribute to single-cell heterogeneous response to EGFR inhibitors. We anticipate quantitative mapping of EGFR activity with drug treatment will help to elucidate why EGFR drug treatments fail and provide a means to develop combination therapy to address heterogeneity in triple negative breast cancer.
responses. Mass cytometry is a single cell platform capable of measuring >35 proteins on millions of tumor cells. Here, mass cytometry was used to characterize matched tumor samples obtained before, during, and after therapy in order to quantify the impact of therapy on tumor cell populations. Methods: Melanoma tumors from adults with metastases were biopsied or surgically resected from 12 patients with consent. Patients received two weeks of BRAF$$^{\text{V600E}}$$ inhibitor dabrafenib followed by two weeks of dabrafenib and MEK inhibitor trametinib. Tumors were enzymatically digested and cryo- preserved with protocols for mass cytometry (Leealatin and Doxie et al., Cytometry B 2016). Samples were stained with a 30+ antibody panel focused on melanoma identity and markers of lineage and trafficking for leukocytes, fibroblasts, and endothelial cells. Viable nucleated cells were identified by total histone H3 and rhodium dye exclusion. Expert biaxial gating and viSNE analysis were used to identify, computationally map, and track populations and their subsets over time. Tissue microarrays were also analyzed by histology to confirm findings observed by mass cytometry. Results: More than 99% of cells were characterized and fell into one of the four major populations. Melanoma phenotypes included loss of MHC class I and/or expression of one or more markers of identity including SOX10, SOX2, nestin, SN100β, and MCAM. Significant decreases in nestin, MCAM, MC1 and SN100β ($p < 0.01$, $p < 0.05$, $p < 0.05$, and $p <0.05$, respectively) were observed in cells after treatment. Decreases in nestin and SN100β populations identified by mass cytometry were observed in histology analysis of TMA from all matched tumor samples obtained before, during, and after therapy. The immune suppressive phenotype of melanoma tumor cells was present in all melanoma cells at a low abundance (average of 7.0% $\pm$ 3.9% in cell lines, $N = 7$). Conclusions: Single cell analysis and longitudinal monitoring of patient’s revealed tumor ecosystems experience rapid changes in cellular diversity during targeted therapy. Lower nestin expression distinguished treated melanoma tumor cells. The in vitro results suggest that in vivo remodeling of the tumor microenvironment results from a shift in host-tumor cellular interactions and not a direct response to inhibitors.

#2922 Targeting physical and stromal sources of tumor heterogeneity with photodynamic therapy-based combinations. Imran Rizvi,1 Utkan Demirci,2 Tayaaya Hasan,1 Massachusetts General Hospital, Boston, MA; 2Stanford University School of Medicine, Canary Center for Early Cancer Detection, Palo Alto, CA.

Tumor heterogeneity and drug resistance to conventional therapies remain major causes of treatment failure, recurrence and dismal survival rates for patients with advanced stage cancers. A range of cellular, architectural, and physical cues in the tumor microenvironment influence the intrinsic and acquired resistance mechanisms that lead to treatment failure. These include communication with heterocellular stromal partners and physical forces such as hydrodynamic stress, which are key factors in the evolution of tumor heterogeneity and variability in treatment response. Strategies that leverage photodynamic therapy (PDT), a mechanistically-distinct modality, to regionally target and prime stubborn tumor populations may be essential to overcoming key barriers to durable cancer management while minimizing toxicity from traditional agents. A multi-faceted approach is needed to evaluate and optimize PDT-based combination therapies, including the development of bioengineered 3D models that integrate cues such as physical forces and heterotypic cellular communication. Here the impact of hydrodynamic stress and stromal biology is evaluated in the context of ovarian cancer (OvCa). Metastatic OvCa spreads predominantly via flushing of ascites along preferential fluidic pathways and communicates with the local microenvironment, including the extracellular matrix. Using a microfluidic model that supports 3D tumor growth, we developed a label-free workflow to isolate CTCs from bulk-OvCa patient samples. A microfluidic model that supports 3D tumor growth was developed to investigate the role of fluidic stress on the heterogeneity of metastatic OvCa. The motivation for this study was based on clinical observations that the most stubborn tumors are often found in regions such as the peritoneal gutters, which is subjected to fluidic stress from ascites and is a common site of resistance and recurrence. Tumor nodules under flow showed increased epithelial-mesenchymal transition (EMT) compared to non-flow cultures. Molecular and morphological changes consistent with EMT included significant decrease in E-cadherin, a significant increase in vimentin, and significant decrease in fractal dimension, a metric adapted to quantify spindly-morphology. A concomitant significant post-translational upregulation of epithelial growth factor receptor (EGFR) expression and activation, and chemoresistance was seen. The impact of heterotypic communication between TECs and OvCa cells was investigated in a 3D model. Tumors grown in the presence of TECs were differentially susceptible to chemotherapy and bexoroparin, a BMP-based PDT and showed increased heterogeneity in response to treatment. This heterogeneity was overcome by PDT priming of tumors prior to chemotherapy. The potential value of using bioengineered models to guide customized PDT-based combinations will be presented.

#2923 Label-free enrichment and integrated full-length mRNA transcriptome analysis of single live circulating tumor cells from breast cancer patients. Naveen Ramalingam,1 Yi Fang Lee,2 Lukasz Szpankowski,1 Anne Leyrat,3 Brian Fowler,4 Jovina Tan,3 Chong Tracy Lu,5 Ninez Delos Angeles,2 Chad Sanada,3 Cassandra Greene,4 Kyle Hukari,4 Andrew Wu,6 Yoon-Sim Yap,7 Jay West,8 Ali Asgar Bhagat.9 Fluidigm Corporation, South San Francisco, CA; 2Clearbridge BioMedics Pte Ltd, Singapore, Singapore; 3Fluidigm Singapore Pte Ltd, Singapore, Singapore; 4National Cancer Centre, Singapore, Singapore.

Background: Label-free methods for isolating circulating tumor cells (CTCs) are attractive because they provide an opportunity to analyze a larger set of CTCs that may otherwise be missed due to variable or no expression of protein (label) markers. Understanding genetic and functional heterogeneity in CTCs allows us to gain insight into the mechanisms underscoring metastasis, drug resistance, and tumor aggressiveness. Currently, a simple workflow for isolation and molecular characterization of single CTCs by mRNA sequencing is lacking. In order to address this challenge, we developed a label-free workflow to isolate CTCs from breast cancer patients for full-length mRNA sequencing analysis by integrating the ClearCell® FX System with the Polaris® 3™ system. The ClearCell FX System enables the isolation of single adherent cells from whole blood samples from breast cancer patients as early indications of the presence of CTCs in a label-free antibody-independent manner. The low level of nonspecifically isolated white blood cells from ClearCell FX is further depleted on the Polaris® 3™ system by negative enrichment of viable CTCs. This unique integration of systems will enable researchers to perturb single CTCs in a controlled environment, monitor and measure the response due to perturbation, and link these response measurements to downstream genomic and transcriptomic analysis.

Methods and Results: CTCs from 7.5 mL of peripheral blood sample from breast cancer patients were enriched using ClearCell FX. To differentiate larger blood cells from putative CTCs, we stained the enriched cells with Alexa Fluor® 647-conjugated CD45 and CD31 to identify leukocytes and endothelial cells, respec-
tively. Calcein AM (live cell marker) and CellTracker™ Orange (universal cell marker) were added to identify live cells. Single CTCs were selected on Polaris integrated fluidic circuit (IFC). Sequencing libraries were generated using the Nextera® kit and sequenced on Illumina® MiSeq™ and NextSeq® systems. We successfully processed blood samples from single CTCs from triple negative breast cancer patient. Conclusion: We present the feasibility of integrating two microfluidics platforms to capture single CTCs for transcriptome and functional study. Our data suggests that the heterogeneity of tumor sample and characterization of metastatic processes can be elucidated from single-cell mRNA sequencing of CTCs.


Malignant tumors shed cell-free DNA into the bloodstream. Analyzing this new biological source may have important implications in a shift towards personalized medicine for diagnosing and/or monitoring malignancies. We recently showed the first clinical validation and clinical utility of circulating DNA (cfDNA) analysis in oncology by testing RAS/BRAF hotspot mutations in plasma from metastatic colorectal cancer patients (mCRC) (Nat Med. 2014 Apr; 20(4):430-5). In addition, liquid biopsies allow the tracking of clonal dynamics and detection of mutations during treatment. We evaluated under blinded conditions the ability of cfDNA to detect RAS/BRAF mutations in the plasma of 42 mCRC patients treated on a phase I/II trial of FOLFOX and dasatinib, with or without cetuximab. Prior to treatment, sequencing of archival tissue detected mutations in 25/42 patients (60%), while the cfDNA assay detected mutations in 37/42 patients (88%). Our cfDNA assay was able to detect mutations with allele frequencies as low as 0.01%. After exposure to treatment, 41/42 patients (98%) had a cfDNA detected RAS/BRAF mutation. Of 22 patients followed with serial measurements who were RAS/BRAF mutant at baseline, 11 (50%) developed a second mutation following treatment and 3 (14%) no longer had detectable levels of another mutant allele. Of RAS/BRAF wild type tumors at baseline, 4/5 (80%) developed new mutations. Patients may harbor mutations at very low frequency down to 0.01% before initiation or during treatment revealing the need of a high sensitive technique to detect mutant subclones. cfDNA quantitative measurements from this study closely mirrored changes in CEA and CT scan results, highlighting the importance of obtaining quantitative data beyond the mere presence of a mutation. Combined qualitative and quantitative cfDNA analysis allowed acquired resistance by studying the relative contribution of intra-tumor evolution of the tumor and might help physicians to adjust patient treatment. Our findings demonstrate the development of new RAS/BRAF mutations in patients regardless of whether they had pre-existing mutations in the pathway, demonstrating a convergent evolutionary pattern.

#2925 Distribution of copy number alterations defines clonal populations involved in colorectal cancer evolution. Isabel Quintanilla, Elena Asensio, Maria Pellisé, Antoni Castells, Miriam Cuatrevascas, Jordi Camps. IDIBAPS, Barcelona, Spain.

Colorectal cancer progresses in a multi-step manner with adenoma being the most well-known precursor lesion. Malignant polyp, an adenoma that contains a focus of adenocarcinoma, is a suitable model to study the colorectal tumor evolution. Although the mutations that lead adenoma to evolve into carcinoma have been previously described, the copy number changes involved in this malignant transformation have not been fully explored. To understand how these genomic alterations contribute to carcinogenensis, we used sequential fluorescence in situ hybridization using probes for the oncogenes EGFR, MYC, CDX2, and ZNF217 and the tumor suppressor genes SMAD7 and APC in order to analyze the copy number changes in 25 cases of malignant polyps and 10 cases of low grade dysplasia (LGD) adenomas based on single cell analyses. We found the levels of genomic heterogeneity increased from LGD adenomas to malignant polyps, with the adenoma component having a lower degree of chromosomal instability than the adenocarcinoma component. Despite the intercellular heterogeneity, we observed different patterns of evolution. The gain of ZNF217 was the earliest event as it was found to be in some cases of the LGD adenoma, and was also important in the neoplastic transformation of malignant polyps. Other notable genomic imbalances we observed during the malignant transformation were the gain of CDX2 and the loss of SMAD7. Interestingly, in a significant proportion of cases we observed loss of all loci of CDX2 and ZNF217 in the adenoma component, suggesting a whole genome duplication event. Lastly, in half of the malignant polyps studied the main clone in the carcinoma component was already present in the adjacent adenoma component, although in some cases at a low frequency only detected by single cell analysis. Our data suggest that copy number changes are early events in colorectal carcinogenesis that can determine the evolution from adenoma to carcinoma.

#2926 Histologically defined intratumoral sequencing uncovers evolutionary cues into conserved molecular events driving gliomagenesis. Antony Prabhu, Pravin Kesarswani, Shiva Kant, Stewart Graham, Prakash Chinnaiyan. William H. Beaumont Hospital, Royal Oak, MI.

Glioblastoma (GBM) represents an archetypal example of a heterogeneous malignancy, harboring regions of invasion, necrosis, and vascularization. To begin to understand the diverse molecular consequences of this complex tumor ecology, we analyzed RNA-seq data generated from commonly identified intratumoral structures in GBM that were isolated and enriched using laser capture micro-dissection. Structures included the infiltrative, leading edge, central tumor core, and cellular regions in areas of necrosis. From this RNA-seq database consisting of 119 structural regions from 37 individual tumors generated from 12,000 hematoxylin and eosin histologic images, we validated significant intra-tumoral heterogeneity in GBM, with cells derived from the infiltrating leading edge almost exclusively harboring the pronuclear male subtype, while cells in regions of necrosis displaying the mesenchymal subtype. In addition, we made the striking observation that when evaluating the tumors transcriptional profiles in the context of their derived structural regions, there was a relative small amount of inter-tumoral heterogeneity in GBM, with significant clustering of transcriptional profiles of specific structural regions from different tumors. To begin to understand the specific molecular events driving regional growth and their contributory role in gliomagenesis, we analyzed the transcriptional programs in the context of evolutionary progression. This level of analysis identified numerous pathways associated with neuronal receptor signaling, autophagy, and fatty acid metabolism as early events in tumorigenesis. Traditional cancer-related signaling pathways emerged as key mediators driving progression, with glioma and p53 signaling ranking highest on the list. As the tumor progressed to harbor regions of necrosis, in addition to expected changes in VEGF and tumor signaling, we uncovered global metabolic changes associated with progression, with metabolic pathways comprising 10 of the top 20 pathways unique to this region. Collectively these findings suggest that the observed intra-tumoral heterogeneity in GBM may be a conserved, predictable consequence to its complex microenvironment and therapeutic strategies rationally designed to target these unique, unequivocally present tumor cell biomes may lead to therapeutic gains in this otherwise fatal malignancy.


Introduction: Intratumor heterogeneity has been characterized among multiple cancer types. However, in lung adenocarcinoma, recent work has been limited to early stage primary tumors and intertumor heterogeneity has not been well-studied. Most importantly, an integrated tumor heterogeneity analysis at the level of somatic variants, copy number, transcript and protein expression, and the phosphoproteome is outstanding. Methods: In order to characterize both intra-tumor and inter-tumor heterogeneity of metastatic lung adenocarcinoma, we applied whole exome sequencing, RNA-seq, CNV-seq and mass spectrometry-based proteomic analyses on 33 tumor regions from metastatic sites including lung, liver and kidney, obtained by rapid/warm autopsy from 4 patients with Stage IV lung adenocarcinoma. The autopsy procedure was initiated between 2-4 hours of death. Results: We found considerable intratumor heterogeneity with organ-specific, branched evolution that was consistent across DNA, RNA and protein analyses. Intratumor heterogeneity differed depending on oncogene-status: oncogene-negative tumors (without RTK/RAS/RAF mutations or known fusion gene) had significantly higher genomic intratumor heterogeneity than oncogene positive tumors. The degree of heterogeneity at the genomic and proteomic level was patient-specific. The proteomic analysis complemented genomic variants-based clinical evolution analysis. High-confidence
driver mutations (KRAS, EGFR, TP53, CTNNB1) uniformly occurred early in the evolution of metastatic lung adenocarcinoma consistent with the concept of these mutations as likely 'founders'. In contrast, other known driver mutations occurred more often in later stages among specific organ branches. Notably, oncogene-negative tumors carried significantly more driver mutations than oncogene-positive tumors, suggesting that lung adenocarcinoma evolves through a branched, organ-specific, process with acquisition of significant driver mutations and copy number changes, particularly among oncogene-negative tumors. The branched evolution is ultimately influenced by proteomic and phosphoproteomic alterations affecting key signaling pathways that may not be always a result of genomic changes.

#2928 Pervasive associations between mutational processes and driver mutations across cancer types. Daniel P. Temko,1 Benjamin Schuster-Boeckler,2 Simone Severini,1 Ian Tomlinson,2 Trevor Graham3.

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#2930 Niche microenvironments essential for tumor heterogeneity of primary cancer cells. Shiki Fujino,1 Norikatsu Miyoshi,2 Masayuki Ohue,3 Yusuke Takayashiki,3 Masayoshi Yasui,2 Hidekazu Takahashi,1 Naoyuki Haraguchi,1 Jhun-nichi Nishimura,7 Taishi Hata,7 Tsumekazu Mizushima,7 Yuichiro Doki,2 Masaki Mori,1 Osaka University, Osaka, Japan; Osaka Medical Center for Cancer and Cardiovascular Disease, Osaka, Japan.

Primary culture of cancer cells derived from each patient’s tumor can provide important information of the “individual tumor.” It is general to use cell lines in basic research filed. However, cell lines are quite different from clinical cancers. Clinical cancer tissues are composed of not only cancer cells but also tumor microenvironments such as stromal cells and tumor vessels. The primary culture method of clinical cancer with niche microenvironments has not been optimized. We have developed a simple 2D-culture method for primary colorectal cancer (CRC). We obtained 30 samples from surgically resected tumor. They were mechanically and enzymatically digested and fibrotic tissue and bacteria were excluded using customized two size filters. And we cultured the obtained cells on a matrigel-coated plate with embryonic stem (ES) cells culture medium. We named these cultured cancer cells, “isolated-tumor derived Cancer Cells (iCCs).” All iCCs grew and about 80 % of iCCs were successfully passaged. Twenty-three iCCs were transplanted into the subcutaneous layer of NOD-SCID mice, and the tumor growth and histology of iCCs were examined. The morphology was similar to each parental clinical tumor. And microarray analysis showed that RNA expression of iCCs was similar to each parental tumor. Furthermore, we examined the culture medium: our modified ES culture medium (ES-cultured iCCs) and 10% FBS medium (serum-cultured iCCs). The expression of surface markers regarding cancer stem cells such as CD44 and CD24 were different between ES-cultured iCCs and serum-cultured iCCs, and drug sensitivity of iCCs were also different. FACS analysis and immunocytochemistry revealed that iCCs contained PDGFR-positive cells. The results of multi-drug sensitivity assay were different between iCCs and cell lines especially in PDGFR inhibitor. Strong correlations were observed between the results of multi-drug sensitivity assay of iCCs and clinical outcomes of chemotherapy. We report an innovative primary culture method and the in vivo and in vitro analyses of iCCs, leading to the future application.
anti-Galectin-9 or an anti-TIM-3 antibody. The findings suggested that a significant amount of Galectin-9 could be released from LAD cells and induced T-cell apoptosis in tumor microenvironment. Conclusions: Our results suggested the relevance of the PD-1/PD-L1 and TIM-3/Galectin-9-immuno-inhibitory pathways in the tumor microenvironment of LAD, and that release of soluble Galectin-9 could negatively regulate T-cell function. For successful immune checkpoint therapy in LAD, simultaneous inhibition of TIM-3/Galectin-9 pathway may be needed.

#2933 Involvement of CXCL12-CXCR7 axis in adipocyte-induced TNBC metastasis. Hyeongi Choi,1 Rosa Misticia C. Ignacio,1 Carla R. Gibbs,1 Eun-sook Lee,1 Samuel E. Adunyah,1 Dek-Soo Son1.1 Meharry Medical College, Nashville, TN;2Florida A&M University, Tallahassee, FL. Breast cancer (BC) is the most frequent tumor in women worldwide. Although its mortality has significantly decreased owing to advanced therapies, triple negative breast cancer (TNBC) is still difficult to treat because of lack of estrogen receptor, progesterone receptor and HER-2. TNBC accounts for 12–24% of total BC and contributes to the aggressiveness and poorer outcomes. The risk of BC increases significantly in obese women. Obesity is also associated with the worse clinical prognosis of BC. The underlying mechanisms between obesity and BC, particularly TNBC, remain unclear. Therefore, we compare the molecular mechanisms by which human adipocyte conditioned media (CM) affect TNBC cells (BT549) and non-TNBC cells (MCF7A). Adipocyte CM had no effect on the proliferation of both TNBC and non-TNBC cells. However, adipocyte CM enhanced significantly migration in TNBC cells compared to non-TNBC cells. Next, we evaluated signaling pathways adipocyte CM promote migration of TNBC cells. AKT and ERK were activated in both TNBC cells and non-TNBC cells. However, phospho-STAT3 was significantly increased in TNBC cells. Also, N-cadherin, a marker for epithelial to mesenchymal transition (EMT), was increased at the late time point in TNBC cells. Furthermore, CXCR7 was specifically increased in TNBC cells after treatment with adipocyte CM using a PCR array. CXCR7 is chemoattractive to CXCL12 which is highly expressed in the lung, the bone marrow, and the liver, common sites of BC metastasis. Taken together, the results indicate that adipocyte CM may promote metastasis of TNBC cells through the CXCL12-CXCR7 axis by activating STAT3.

#2934 Neoadjuvant chemotherapy influence changes of the immune response in non-small cell lung adenocarcinomas. Edwin Roger Cuestas,1 Carmen Behrens,1 Jaime Rodriguez-Canales,2 Mei Jiang,2 Apar Patera,2 Arelene Correa,3 Stephen Swisher,1 Boris Sepesi,1 Annika Weisserfeldt,2 Neda Kalhor,2 Jie Lee,2 John Heymach,2 Cesar Moran,3 Jianjun Zhang,3 Don Gibbons,1 Ignacio Wistuba3.1 UT MD Anderson Cancer Center, Houston, TX;2UT MD Anderson Cancer Center, Houston, TX;3UT MD Anderson Cancer Center, Houston, TX. Background: The clinical efficacy observed with PD-1/PD-L1 inhibitors in non-small cell lung carcinoma (NSCLC) has prompted to characterize the immune response in lung tumors treated with chemotherapy. The aim of this study was to determine the changes of the immune microenvironment in surgically resected NSCLCs from patients who received and did not receive neo-adjuvant chemotherapy. Methods: We studied formalin-fixed and paraffin embedded (FFPE) tumor tissues from 112 stage II/III resected NSCLC, including 61 chemotherapy-treated NSCLCs demonstrated higher density of TAMs CD68 expressing by CD45RO+ and PD-1 (P = 0.016) were detected in chemotherapy-treated tumors compared with chemo-naive tumors. In contrast, lower densities of FOXP3+ regulatory T cells and CD68+ macrophages but not statistical significant were detected in chemotherapy-naive tumors when compared with neoadjuvant treated cases. Following chemotherapy ADCC exhibited significantly higher levels of CD57+ (P = 0.008) and high density of PD-1 expressing by CD45RO+ cells (P = 0.016) than chemotherapy-naive tumors. Chemotherapy-treated SCCs demonstrated higher density of TAMs CD68+, CD57+, CD45RO+ and PD-1 cells than chemotherapy-naive tumors (P < 0.05).
In chemotherapy-treated cancers, lower levels of CD4+ helper T cells was associated with worse overall survival (OS; P<0.04) in univariate analysis. In chemotherapy-treated ADC patients, lower levels of CD68-positive (P=0.010) and higher levels of FOXP3-positive cells correlated with worse OS (P<0.044). Conclusions: Neo-adjuvant chemotherapy-treated NSCLCs exhibited higher levels of PD-L1 expression and CD11b Gr-1+ myeloid cells in male patients as compared to chemotherapy-naive tumors, suggesting that chemotherapy activates specific immune response mechanisms in lung cancer. These results provide an inclination towards the design of clinical trials combining neo-adjuvant chemotherapy and immunotherapy prior to surgical resection of locally advanced NSCLC. (Supported by CIHR MIRA and UT Lung SPOR grants, and MD Anderson Moon Shot Program).

#2935 Sex disparity in the response of the immune microenvironment to colon cancer liver metastases. Simon Milette,1 Ni Wang,2 Stéphanie Perrinno,2 Pnina Brod1.1 McGill University, Montreal, Quebec, Canada; 2Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada.

Background: Metastasis remains the most life-threatening aspect of malignant disease and a major clinical challenge. Myeloid-derived suppressor cells (MDSC) and regulatory T lymphocytes (Tregs), which are frequently found in the tumor microenvironment, are known to impair immune surveillance and promote a state of immune tolerance that leads to metastatic expansion. Previously, we have shown that the incidence of colon carcinoma liver metastases was reduced in TNFR receptor 2 (TNFR2) deficient female mice, as a consequence of reduced recruitment of MDMSCs and Tregs into the liver. Intriguingly however, no reduction in liver metastasis or MDSC/Treg accumulation was observed in TNFR2 deficient male mice, suggesting that their recruitment and survival were distinctly regulated. The objective of this study was, therefore, to elucidate the mechanisms underlying the sex disparity observed in the response of the immune microenvironment to liver metastases, in particular the role of sex hormones in immune response regulation in this context. Methods Hormone-ablated female mice were generated using surgical ovariectomy (OVX). Estrogen-deprived mice, sham-operated controls and hormone reconstituted O VX mice were injected with syngeneic murine colon carcinoma MC-38 cells via the intrapleural/portable route and experimental liver metastases enumerated 2 weeks later. MDSCs and Tregs recruited into the livers of tumor-bearing mice were analyzed 7–10 days post-tumor injection using flow cytometry, as well as IHC performed on liver cryostat sections. Results: We found that the numbers of experimental liver metastases was markedly reduced in ovariectomized female mice, as compared to sham-operated controls but was restored in estradiol supplemented O VX mice. Reduced incidence of hepatic metastases in O VX mice correlated with reduced accumulations of CD11b+ Gr-1+ myeloid cells (MDSCs) and CD4+ Foxp3+ cells (Tregs) at metastatic sites. IHC also revealed an increased CD11b+CD4+Foxp3+ (i.e. CD11b+Gr-1+Foxp3+) ratio around hepatic metastases in O VX mice as compared to sham-operated controls. Estradiol reconstitution in O VX mice restored MDSC and Treg cell accumulation to the levels observed in control mice. Conclusion: Together, our data identify female sex hormones as major players in the regulation of the tumor immune microenvironment and a potential contributing factor in the patients’ response to immuno-based cancer therapy. Supported by the Canadian Institute for Health Research, MDEIE and FRSQ.

#2936 Molecular characterization of the immune subclass of hepatocellular carcinoma. Daniela Sia,1 Yung Jiao,1 Iris Martinez,2 Olga Kuchuk,1 Carlos Villacorta Martin,1 Manuel Castro de Moura,1 Juan Putra,2 Genis Camprecios,1 Swan Thung,1 Samuel Waxman,1 Vincenzo Mazzaferro,3 Manel Esteller,2 Arthur Pnina Brodt1.1 McGill University, Montreal, Quebec, Canada; 2Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada; 3Montefiore Medical Center, Albert Einstein College of Medicine, New York, NY.

Background: Immune checkpoint inhibitors have emerged as a promising therapeutic approach in different solid tumors, including hepatocellular carcinoma (HCC). Nonetheless, little is known about the immune-component of HCC or potential biomarkers of response to these therapies. Aims: To perform comprehensive characterization of the HCC immunological profile and to identify biomarkers to select immunotherapy candidates. Methods: We performed gene expression array deconvolution through non-negative matrix factorization in 228 resected HCCs. Characterization of the transcriptional landscape was conducted using >1,000 signatures representing distinct immune cells by gene set enrichment and nearest template prediction analyses. Presence of immune infiltration, tertiary lymphoid structure (TLS), PD-1 and PD-L1 immunostainings was investigated using immunohistochemistry. DNA methylation profile of 450K CpG sites was analyzed to identify those with significant differences for each group. Extensive validation of the immune classifier was performed in 728 independent HCC samples. Results: Overall, an immune-related subclass of HCC was identified in ~27% of patients. The immune subclass was characterized by gene signatures identifying immune cells (i.e. T cells, TLS, cytotox, p<0.001), signatures of response to immune checkpoint therapy (p<0.001), presence of high immune infiltration (p<0.01), TLS (~5 foci, 19/51 vs 33/168, p<0.05). The methylation levels of 363 CpG sites in 192 immune response gene promoters were able to capture the Immune class (ANOVA, p<0.05, Δβ=-0.2 Tukey test). Integration with HCC molecular classifications revealed significant enrichment of the Immune subclass with IFN and S1 (p<0.001) and exclusion of the CTNNB1 and S2 (p<0.001) subclasses. The immune class contains two distinct microenvironments (0.01) and types) A) Exhausted immune response type (~35%) characterized by stromal activation, T cell exhaustion signatures, and presence of immuno-suppressive components such as TGFβ, LGALS1, M2 macrophages and pathways able to recruit myeloid-derived-suppressor cells (FDR<0.01); and B) Active immune response type (~65%) characterized by overexpression of adaptive immune response genes and IFN signaling (p<0.001). Tumors within the active immune response type showed a trend towards better survival vs rest (p=0.07).

Conclusions: Around 27% of HCC patients belong to the Immune class, characterized by activation of immune cells and signatures of response to immunotherapies. Within this subclass, two distinct types have been characterized by presenting active or exhausted immune responses, a feature that provides the rationale for precision medicine-based therapies.

#2937 Utilizing multiplex chromogenic IHC and digital image analysis to evaluate immune cell content and spatial distribution within NSCLC tumor tissue. Lorcan Sherry,1 Mark Anderson,1 Marianne Cowan,1 Lee Dawson,2 Richard Bystry,2 Sarah-Jane Green2.1 OracleBio, Biocity Scotland, United Kingdom; 2Asterand Bioscience, Royston, United Kingdom.

Chromogenic-based immunohistochemistry (IHC) is an established technique widely utilized within oncology R&D, allowing cells to be visualized within the context of their tumor microenvironment. However, when quantifying chromogenic stained sections via digital image analysis, the number of targets is usually limited due to constraints in deconvolving more than 3 chromogens present on the same tissue section image. The purpose of this study was to apply a customized algorithm, including a 5-colour deconvolution process to tripleplex chromogen IHC plus hematxoylin stained Non-Small Cell Lung Carcinoma (NSCLC) tumor tissues in order to quantify immune cell content and spatial distribution within both tumor and stroma regions of interest (ROI). A multiplex IHC assay was developed and applied to FFPE sections of NSCLC tumor tissue to stain epithelial tumor cells (Pan CK, yellow), CD8+ cells (purple) and FoxP3+ nuclei (brown), counterstained with hematoxylin (blue). Immunostained whole slide images were generated for image analysis. A customized algorithm, which included a 5-chromogen color deconvolution process, was utilized within the Indica Labs HALO platform to separate the four IHC chromogens (including counterstain) and a fifth color (black) representing carbon deposit artefacts. Nuclear objects were formed by applying weighted optical density values for the brown, purple and blue colors, which were positive for either CD8 or FoxP3 and counterstain/Fuchsin. A classifying classifier was trained to automatically segment tumor from stroma. Each positive cell type was identified, using defined size and shape parameters, and quantified within each ROI. IHC multiplex staining highlighted CD8+ cells and FoxP3+ nuclei to be present in both tumor and stroma compartments. Analysis data demonstrated the number of tumor and stroma CD8+ cells was 120 and 554 cells per mm², while the number of tumor and stroma FoxP3+ nuclei was 20 and 19 nuclei per mm². This gave a CD8+/FoxP3+ ratio of 6 within the tumor and 29 within the stroma. The average distance of CD8+ cells in the tumor or stroma to their nearest FoxP3+ nuclei was 92µm and 174µm respectively. The average distance of CD8+ cells to their nearest tumor cell was 39µm while the average distance of FoxP3+ nuclei to their nearest tumor cell was 29µm. This study demonstrated multiplex chromogenic IHC as a valuable approach for quantifying multiple cell types within the context of their tumor microenvironment. Furthermore, up to five chromogen colors can be separated utilizing a custom 5-pixel analysis algorithm, leading to a more in-depth evaluation of immune cell types present and their spatial distribution. This provides a greater potential for interpretation of therapeutic mechanistic responses within the tumor microenvironment.

#2938 Tumour-associated macrophage polarisation and re-education with oncolytic viruses. Munitta Muthana,1 Emer Murphy,1 Amy Kwan,1 Natalie Winder,1 Joe Conner2.1 Univ. of Sheffield Medical School, Sheffield, United Kingdom; 2Virtu Biologics, United Kingdom.

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Tumor-associated macrophages (TAMs) promote key processes in tumor progression through their plasticity and capacity to become polarized by tumors to have an immunosuppressive phenotype. However, the plasticity of these cells provides an opportunity for therapeutic 'reprogramming' towards a classically activated inflammatory phenotype thus preventing tumor-associated immunosuppression in a major tumor-promoting fashion. Oncolytic viruses (OV) represent a new class of anti-cancer therapeutics that promote anti-tumor responses through selective tumour cell killing and the induction of systemic anti-tumor immunity. We propose that TAMs can be targeted using an OV, leading to altered macrophage polarization and a reduction in tumor growth and metastasis. Experimental Design: In-vitro, cytotoxicity, viral replication and cytokine expression was assessed in a panel of primary human and murine macrophages following exposure to Sepherevir, an ICP34.5-deleted oncolytic herpes-simplex virus currently in Phase 1/2a trials. In-vivo, murine TS1-PyMT cells were implanted into the mammary fat pads of female FVB mice to establish a syngeneic immunocompetent mouse model of breast cancer. Tumour growth and overall survival were assessed following administration of Sepherevir given either by intratumoural (IT) or intravenous injection (I.V.). Cell immune enumeration and protective immunity were assessed post-mortem by immunofluorescence and flow cytometry. Results: Both primary human and mouse macrophages served as a host for oncolytic viral replication. Sepherevir was also found to attenuate TAM phenotypes as evidenced by increased “M1” receptor signatures (e.g. CD40, CD80, CD86, MHCII), increased pro-inflammatory gene expression (e.g. IL-1, IL-6, IL-8, CXCL10, TNF-a) and increased nitric oxide production. I.T and I.V administration of Sepherevir led to marked tumor shrinkage as well as the recruitment of cytotoxic T cells. Moreover, TAM polarisation was evident based on a change in signature with TAMs becoming skewed to IL-12/IL-10 and a change in the INOS/arginase1 ratio. Conclusions: Our results demonstrate that Sepherevir is associated with in vivo anti-tumor effects and importantly can be used for manipulating the phenotype of the abundant macrophage population located within the tumor microenvironment.

#2939 Tumor associated myeloid cell transcriptome signatures in an inductible Kras-positive lung adenocarcinoma murine model. Clifton L. Dalgard,¹ Mouna Lagraoui,¹ Mouna Lagraoui,¹ Gauthaman Sukumar,¹ Celeste Huaman,¹ Corey A. Carter,³ Brian C. Schafer,¹ ¹Uniformed Services University, Bethesda, MD; 2John Muntha Cancer Center, Bethesda, MD.

Tumorigenesis is modified by dynamic activities in the tumor microenvironment and mechanisms driving these alterations during tumor progression may be tumor- and patient-specific. Of well-established functions for tumor-associated stromal cells (e.g. angiogenesis, chemotaxis) immune system regulation by the tumor microenvironment is less well understood in their mechanism and effects. However, diminished activity of antigen-specific T cell lymphocytes tumor cytoxicity is tumor promoting and recent cancer immunotherapy have targeted immune inhibitory checkpoints (e.g. anti-PD-L1) to restore T cell activity. Recent studies suggest that tumor-associated myeloid cells play a role in immunosuppression in several human cancers, including lung cancer. We hypothesize that early tumors of the lung promote changes in the phenotype of tumor-proximal myeloid cells, establishing an immunosuppressive microenvironment. As a component to observe this immunosuppressive role for myeloid cells, we performed specific transcriptome profiling of myeloid cells by immunomagnetic selection of CD11b-positive cells from lung tumors of a murine inducible Kras-positive, p53-negative cancer model. RNA sequencing of isolated myeloid cells as a function of tumorigenesis duration (3-18 weeks) establishes the kinetics of gene expression changes associated with tumor-supporting and immune-suppressing functions. Genome-wide expression analysis of myeloid cells between tumor-inducing adenovirus-Cre injected and normal control subjects resulted in identifying 1,883 genes with differential expression. Gene set enrichment analysis resulted in the stratification of samples by well-established gene signatures and tumor-predicting activity for angiogenesis (32 genes), lung tissue remodeling (47 genes) and cell survival (11 genes). Interestingly, a significant downregulated gene signature was observed in tumor-associated myeloid cells for immune cell chemotaxis (12 genes) and positive regulation of immune system activation (12 genes). These non-canonical gene signatures may identify a myeloid phenotype for immunosuppressive function. Moreover, the observed gene expression changes suggest a potential anti-tumor immunity.

#2940 TTP17: mediators of metastatic growth acceleration after surgery. Vikas Sud, Sameh Tohme, Dirk J. van der Windt, Hai Huang, Allan Tsung. Univ. of Pittsburgh, Pittsburgh, PA.

Surgery is known to accelerate the growth and progression of metastases in patients undergoing oncological resections with 50-60% patients having recurrent masses after resection making a major treatment failure. This impedes the efficacy of adjuvant chemotherapy and overall prognosis of the patient. Studies have proved that inflammatory cascade initiated by surgery that increases metastatic growth and progression is mediated through a specific mechanism of neutrophil cell death caused Neutrophil Extracellular Traps (NETs). We investigated the mechanism of how NET formation after surgical stress increases metastasis by influencing a pro-tumor microenvironment in order to specifically identify targets for perioperative therapy to keep metastases dormant and improve the effect of chemotherapy and overall prognosis. 12 week old male C57BL/6J mice (WT) and mice in which NET formation was inhibited, ie, WT mice injected with DNAase and global homozgyous knock-out of peptidyl arginine deaminase 4 (PAD4), an essential protein for NET formation, underwent splenic injection of 1x10⁶ cells of the murine colorectal cancer cell line, MC-38, followed with either 70% hepatic ischemia-reperfusion (I/R) for 60 minutes after 7 days or no I/R. Livers were harvested at multiple time points and assessed for changes in both innate and adaptive immune cell populations by flow cytometry. On gross examination, the livers of the NET deficient mice had fewer tumors as compared to the WT mice. Mice, which underwent NET formation, showed increased metastatic growth by influencing a pro-tumor microenvironment.

#2941 Inhibition of hypoxia-induced ectonucleotide triphosphate diphosphohydrolase 2 (ENTPD2) restrains myeloid-derived suppressor cell (MDSC) accumulation and sensitizes tumors to immune checkpoint inhibition. David Kang-Chun Chiu, Aki Pui-Wah Tse, Iris Ming-Jing Xu, Robin Kit-Ho Lai, Hui-yu Koh, Felice Ho-Ching Tsang, Larry Lai Wei, Chun-Ming Wong, Irene Oi-Lin Ng, Carmen Chak-Lui Wong. Univ. of Hong Kong, Hong Kong, Hong Kong.

Background and Objective: Rapidly expanding knowledge on cancer immunology has introduced promising anti-cancer therapeutic approaches which involve the activation of T cells to combat cancer cells. However, studies have indicated that the efficacy of immunotherapies is critically determined by the stromal cell components in tumors. Myeloid-derived suppressor cells (MDSCs), are regarded as one of the major immune cell types that possess immunosuppressive activities against T cells which allows cancers to escape immune surveillance and become non-responsive to immune checkpoints blockade. To increase the efficacy of immunotherapy, novel strategies to target MDSC in tumors are warranted. Hypoxia, oxygen (O2) shortage, frequently occurs in tumors due to abnormal vasculature. Using hepatocellular carcinoma (HCC) as a model, we have previously observed MDSC preferentially accumulates in hypoxic regions of human HCC tissues. Here, we aim to identify hypoxia-induced therapeutic targets that are critical for MDSC accumulation in tumors. Experimental Procedures: Transcriptomic analysis in multiple human HCC cell lines exposed to hypoxia and normoxia and HCC clinical specimens was performed to identify potential hypoxia-induced genes relevant to HCC development. MDSCs were isolated from HCC-bearing mice by magnetic bead sorting for different functional assays. LC-MS was performed to evaluate the level of extracellular metabolites. Flow cytometry was used to detect the frequencies of tumor-infiltrating MDSCs in orthotopic and subcutaneous HCC mouse models. Results: We showed that hypoxia, through stabilization of hypoxia-inducible factor-1 (HIF-1), induced ectonucleotide, ectonucleoside triphosphate diphosphohydrolase 2 (ENTPD2/CD39L1), in cancer cells, causing it so over-expression in HCC clinical specimens. Over-expression of ENTPD2 was found as a poor prognostic indicator for HCC patients. Mechanistically, we demonstrated that ENTPD2 could reduce expression of inflammatory cytokines and downregulate expression of mono-cytic MDSCs to dendritic cells, therefore promoting the maintenance of MDSCs in vitro and in vivo. Therapeutically, we found that ENTPD2 inhibitor POM-1, restrained MDSC accumulation and tumor growth, substantially enhancing the efficiency and efficacy of immune checkpoints inhibitors. Conclusion: Our
study reveals a novel mechanism whereby hypoxia/HIF-1 in cancer cells governs tumor-infiltrating MDSCs. Our data suggest that NETPD2 may be a good prognostic marker and therapeutic target for cancer patients especially those receiving immune therapy.


Colorectal cancer accounts for ten percent of new cancer cases and is the fourth most frequent cause of cancer-related deaths worldwide. In recent years, mainly due to screening programs, more patients are diagnosed in early stages of tumor progression, leading to a higher survival rate. However, the incidence of young (<50) patients diagnosed with colorectal cancer has been on the rise. These patients often have a poor prognosis due to the fact that tumors are diagnosed at advanced stages of tumor progression. To our knowledge, no study has yet characterized immunophenotypes and immune evasive mechanisms specifically in young-onset colorectal cancers. To that end we have investigated the expression of HLA class I and PD-L1 in over 200 colorectal cancers derived from young-onset (<50 years-old) patients. Furthermore, we applied a novel multispectral immunofluorescence technology to perform multiparameter immunophenotyping with CD3, CD8, PD-1, PD-L1, CD163, and Ki-67 in the same cohort. The expression of the HLA class I phenotype is maintained in the large majority of tumors allowing thus the development of neo-antigen targeted therapies. Interestingly, reduced expression of HLA class I but not total loss was associated with liver metastases, which suggests a specific selective pressure at this organ that might warrant tailored immune therapeutic interventions. As previous studies on colorectal cancer demonstrated, PD-L1 expression is limited and often restricted to immune cell compartments. The presence of immune cell infiltrates was related to the mutation background of tumors but also to their HLA class I phenotype: tumors with altered HLA class I expression were more likely to present traces of lymphocyte-mediated anti-tumor immunity. Retained HLA class I expression in the majority of colorectal cancers associated with low infiltration by effector immune cells suggests the therapeutic induction of anti-tumor immune responses in young-onset colorectal cancers, for instance, by means of neo-antigen-targeted therapies. We are currently assessing the frequency of natural recognition of neo-antigens in an autologous setting in young-onset, late-stage colorectal cancers. This work was supported by the Fight Colorectal Cancer-Michael’s Mission-AAJR Fellowship in Young-Onset, Late-Stage Colorectal Cancer Research awarded to N.F.C.C. de Miranda (15-40-1645-DEMI).


The MYC oncogene drives the pathogenesis of many hematopoietic malignancies, including Burkitt’s lymphoma (BL) and Acute Lymphoblastic Leukemia (ALL). These malignancies are often “oncogene-addicted” to MYC. Using mass cytometry (CyTOF), we demonstrate that MYC-addicted T-ALL excludes specific immune subsets from the tumor microenvironment implicated in immune surveillance, including natural killer (NK) cells. MYC inhibition clears malignant lymphocytes from the spleen and restores the normal splenic NK composition. Concordantly, peripheral blood of T-ALL patients have reduced percentages of activated NK cells as compared to healthy individuals. We show that MYC-excludes activated NK cells from sites of lymphomagenesis by suppressing ERK1/2/STAT1/2-Type I IFN signaling, in both murine and human lymphomas. Furthermore, MYC-associated BL patients with higher than median expression of STAT1/2, and cytotoxic NK cell genes, PRF1 and Granzymes, have favorable clinical outcomes. Hence, oncogenic MYC appears to causally and reversibly suppress NK-mediated immune surveillance during lymphomagenesis.

#2944 miR-130a and -145 reprogram myeloid suppressor cells and enhance anti-tumor immunity. Hiroki Ishii,1 Suman K. Vodnal,1 Bhaglu R. Acharya,2 Christine M. Hollander,1 Ashish Lal,1 Li Yang1.1National Cancer Institute, Rockville, MD; 2Georgia Regents University, Augusta, GA.

Myeloid derived suppressor cells (MDSCs) are increased in tumor bearing condition, and elicit immunosuppression via type 2 polarization. We have previously reported that TGF-β signaling in MDSCs is essential for tumor metastasis. TGFβR2, a receptor essential for TGF-β signaling, is elevated in MDSCs. Deletion of Tgfbr2 in MDSCs, the gene encoding TGFβR2, significantly decreased tumor metastasis. However, it is unclear how TGFβR2 is regulated in MDSCs. We identify microRNA-130a and -145, which directly target Tgfbr2 in MDSCs and inhibit the expression of TGF-β receptor II (TGFβR2). Reduced miRs levels from low in MDSCs from advanced tumor bearing mice correlated to increased Tgfbr2 level. Overexpression of miR-130a or -145 in MDSCs shift their immunological phenotype from type 2 to type 1 polarization. Moreover, tumor metastasis was significantly suppressed in myeloid specific miR-130 transgenic mice. In pre-clinical mouse models, the number of lung metastasis was significantly decreased when mice were treated with combination therapy of miR-130a or -145 with paclitaxel when compared with miR-130a, -145, or paclitaxel alone. We proposed that reprogramming of MDSCs by miR-130a and -145 could be a novel strategy in therapeutic treatment for breast cancer metastasis.

#2945 Impact of intratumoral mature dendritic cells on prognosis of the patients with esophageal squamous cell carcinoma. Junya Nishimura, Hiroaki Tanaka, Yuichiro Miki, Tatsuro Tamura, Tatsunori Fukuoaka, Go Ohira, Masatsune Shibutani, Sadaaki Yamazoe, Kenjiro Kimura, Takahiro Toyokawa, Hisashi Nagahara, Ryosuke Amano, Kazuya Muguruma, Kiyoshi Maeda, Kosei Hirakawa, Masashiki Ohira. Osaka City University Graduate School of Medicine, Osaka, Japan.

[Background] Promising efficacy of the immunotherapy with anti-PD-1 antibody for esophageal squamous cell carcinoma (ESCC) is expected. Tumor-infiltrating lymphocytes (TILs) have been found to be associated with favorable outcome of patients in many cancers including ESCC. However, the contributing factor of TILs in the primary tumor remains unclear. Dendritic cells (DCs) are the most potent antigen-presenting cells to induce cytotoxic T lymphocytes. [Purpose] The aim of this study was to examine the correlation of mature dendritic cells with CD8+ cells in the tumor in order to clarify the impact of DCs on locally infiltrated lymphocytes in ESCC. [Object and Methods] Formalin-fixed, paraffin-embedded blocks of primary lesions were collected from 80 patients with ESCC who underwent surgical treatment at Osaka City University Hospital. Immunohistochemical analysis using anti-LAMP-3 antibody and CD8 antibody was used to evaluate intratumoral DCs and TILs. The number of LAMP-3+ DCs and TILs were counted at 400 magnification. We divided the 80 cases into two groups according to the median number of intratumoral DCs and TILs to examine the clinicopathological features. [Results] LAMP-3+ DCs were predominantly observed in peritumoral area and sparsely found in intratumoral area, whereas CD8+ T cells distributed between both peritumoral and intratumoral areas. The number of DCs was significantly associated with increasing TNM stage. For example, the median number of DCs was 59.9 in pathological T1, 34.2 in T2/3, 62.4 in pN1, 34.1 in pN1/2/3. In addition, many patients with high infiltration of DCs had positive lymphatic invasion (L). The number of intratumoral DCs was significantly correlated with the number of TILs which were observed in intratumoral regions. Patients in the high infiltration of DCs group showed a significant better prognosis than patients in the low infiltration group. Double staining showed that LAMP+ DC-CD8+ T cell clusters were seen in peritumoral area.


Introduction: The immune system plays an important role in tumor progression and treatment response, for example with the new immune modulating therapies. In lung cancer the effect of the individual immune cell types is unclear. In our study, we used publicly available expression data to find evidence whether there are differences in the composition of immune cell fractions within the microenvironment between squamous cell carcinoma and adenocarcinoma of the lung. Methods: We searched the Gene Expression Omnibus (GEO) for human NSCLC samples. Analysis was confused to samples hybridized to the Affymetrix HG-U133 Plus 2.0 (GEO accession number GPL570) platforms. Expression profiles were downloaded and curated for duplicates and corrupt files. Different immune cell fractions were estimated using CIBERSORT and the LM22 leukocyte signature matrix. We combined this information with available clinical data. We used Cox regression analyses to evaluate the prognostic impact of the different immune fractions and Mann-Whitney U tests to test significant differences. Results: Comparing the immune cell composition of adenocarcinoma (n=587) and squamous cell carcinoma (n=254), we observed in adenocarcinoma in-
creased percentages of naïve and memory B-cells, resting and active CD4+ T-cells, regulatory T-cells, active NK cells, monocytes, M2 macrophages, resting dendritic cells and resting mast cells, and decreased percentages of plasma cells, CD8+ T-cells, follicular helper cells, resting NK cells, gamma delta T-cells, M0 and M1 macrophages and active mast cells. In adenocarcinoma, a higher percentage of memory B-cells in the immune infiltrate was associated with increased survival (HR 0.97, 95% CI 0.95–0.99, p = 0.02), while higher percentages of neutrophils (HR of 1.07, 95% CI 1.01–1.13, p = 0.02), M0 macrophages (1.02, 95% CI 1.01–1.04, p = 0.01) and follicular helper cells (1.07, 95% CI 1.02–1.13, p = 0.01) were associated with a worse survival. In both histologies, the resting mast cell immune fraction showed a positive association with survival (adenocarcinoma HR 0.96, 95% CI 0.83–0.99, squamous cell carcinoma HR 0.93, 95% CI 0.89–0.98, p = 0.01 in both histologies). In squamous cell carcinoma, a borderline association with a worse prognosis was observed with the naïve CD4+ T-cell fraction (HR of 1.1, 95% CI 1.00–1.22, p = 0.02), while higher percentages of neutrophils (HR of 1.07, 95% CI 1.01–1.13, p = 0.01) and follicular helper cells (1.07, 95% CI 1.02–1.13, p = 0.01) were associated with a worse survival. In both histologies, the resting mast cell immune fraction showed a positive association with survival (adenocarcinoma HR 0.96, 95% CI 0.83–0.99, squamous cell carcinoma HR 0.93, 95% CI 0.89–0.98, p = 0.01 in both histologies). In squamous cell carcinoma, a borderline association with a worse prognosis was observed with the naïve CD4+ T-cell fraction (HR of 1.1, 95% CI 1.00–1.22, p = 0.053) and the regulatory T-cell fraction (HR of 1.11, 95% CI 0.99–1.24, p = 0.067). Conclusion: In NSCLC significant differences in immune cell composition were observed between adenocarcinoma and squamous cell carcinoma.

#2947 Intratumoral injection of the toll-like receptor 4 agonist G100 induces a T-cell response in the soft tissue sarcoma microenvironment. Yong-woo D. Seo,1 Edward Y. Kim,1 Ernest U. Conrad,2 Ryan B. O’Malley,1 Sara Cooper,1 Bailey Donohue,1 Lee D. Cranmer,1 Hailing Lu,1 Frank Hsu,1 Elizabeth T. Loggers,4 Taylor Hain,1 Darin J. Davidson,1 Lynn Bonham,1 Yena G. Pillarsetty,1 Gabrielle M. Kane,1 Stanley R. Riddell,1 Robin L. Jones,1 Seth M. Pollock,1 University of Washington, Seattle, WA; 2Seattle Childrens Hospital, Seattle, WA; 3Fred Hutchinson Cancer Research Center, Seattle, WA; 4Seattle Cancer Care Alliance, Seattle, WA; 5Immune Design, Seattle, WA.

Introduction: Soft tissue sarcomas (STS) are heterogeneous mesenchymal tumors which are both morbid and lethal. G100 is a stable oil-in-water emulsion of glucopyranosyl lipid adjuvant, a highly potent toll-like receptor 4 (TLR4) agonist, which has been utilized for intratumoral (IT) injections and as vaccine adjuvants without significant toxicity. We hypothesized that IT G100 would induce a robust local and potentially systemic anti-tumor immune response in the STS microenvironment, leading to improved outcomes. Methods: 15 metastatic STS patients who had a superficial injectable lesion were treated with weekly IT G100 for 8–12 weeks; 12 patients received concurrent radiation for 2 weeks at the start, while 3 got IT G100 alone for 6 weeks prior to radiation. Biopsies and blood were collected pre and post treatment, and flow cytometry was performed on fresh tumor samples. T-cell receptor (TCR) deep sequencing of tumor-infiltrating lymphocytes (TIL) and peripheral blood mononuclear cells (PBMC) was performed on 7 patients. RECIST v1.1 and the Common Terminology Criteria for Adverse Events were used to monitor clinical outcomes. Results: Patients had a median of 3 (0–5) prior lines of therapy and mean tumor size of 5.6cm (1–20cm). No grade 3 or higher treatment-related toxicity was observed, and local tumor control was achieved in 93% (14/15). 6 (40%) had stable disease after treatment, and 1 (P06) had complete regression of injected tumor. This tumor had a high percentage of infiltrating pre-treatment immune cells (12% CD45+ on flow cytometry versus 2.7% for all other tumors). TCR sequencing showed that the increase in clonality of PBMC after treatment was greater in P06 (389%) compared to 6 other patients (mean 34%). There was also higher overlap in TCR sequence between TIL versus PBMC after treatment (13% versus 22%), suggesting systemic expansion of tumorspecific T-cells. In 7 patients evaluable for tumor-associated macrophages (tumors with >1000 CD45+CD11b+ cells), 71% had a shift from an M2 to M1 phenotype. In all patients who received G100 alone, there was an increase in T-cell infiltration into tumor after treatment. In one patient (P14), the proportion of CD8+ T-cells in tumor infiltrate increased from <1% to 12% to 26% after treatment. TGF-beta secretion by GSCs was inhibited with the small molecule inhibitor, galunisertib, and expression of glucocorticoid receptor in GSCs was also decreased with treatment. These data suggest the potential of G100 to enhance the adaptive anti-tumor response. Combination of G100 with other immunomodulators may further enhance the adaptive anti-tumor response.

#2948 A distinct CD8+ tumor infiltrating lymphocyte subset is associated with high TIL density, enhanced cytotoxicity and improved survival in patients with lung cancer. Anusha Preethi Ganasesan,1 Oliver Wood,3 Eva Garrido-Martin,2 Serena Chee,1 Toby Mellows,1 James Clarke,1 Daniela Samaniego-Casttrua,1 Divya Singh,1 Gregory Seuimos,3 Aiman Altezani,1 Edwin Woo,1 Peter Sont,2 Tim Cooper,3 Elena Rode,2 Ryan B. O’Malley,1 Sara Cooper,1 Bailey Donohue,1 Lee D. Cranmer,1 Hailing Lu,1 Frank Hsu,1 Elizabeth T. Loggers,4 Taylor Hain,1 Darin J. Davidson,1 Lynn Bonham,1 Yena G. Pillarsetty,1 Gabrielle M. Kane,1 Stanley R. Riddell,1 Robin L. Jones,1 Seth M. Pollock,1 University of Washington, Seattle, WA; 2Seattle Childrens Hospital, Seattle, WA; 3Fred Hutchinson Cancer Research Center, Seattle, WA; 4Seattle Cancer Care Alliance, Seattle, WA; 5Immune Design, Seattle, WA.

Infiltrating tumor-infiltrating lymphocyte (TIL) density predicts for good prognosis in several cancers and therapies that boost the anti-tumor responses of cytotoxic lymphocytes (CTLs) have shown promise in the clinic. However, clinical responses to currently available immunotherapeutic agents vary considerably, the molecular basis of which is unclear. We performed global transcriptional profiling of CTLs in tumors and adjacent non-tumor tissue from 36 patients with early stage lung cancer to define the molecular features associated with robustness and heterogeneity of anti-tumor immune responses. We observed major differences in the transcriptional program of tumor-infiltrating CTLs (CD8+ TILs) that is shared across tumor subtypes. Pathway analysis revealed enrichment of genes in cell cycle, TCR activation and co-stimulation pathways, indicating tumor-driven expansion of presumed tumor antigen-specific CTLs. We also observed marked heterogeneity in the expression associated with TCR activation and immune checkpoints, and their expression was positively correlated with the density of tumor-infiltrating CTLs. Interestingly, TIL-high tumors were also enriched for a distinct CD8+ tumor infiltrating lymphocyte subset that appeared to have enhanced cytotoxicity and independently predicted improved survival in patients with lung cancer. In summary, we define a molecular fingerprint of tumor-infiltrating CTLs and identify a number of novel targets that may be important in modulating the magnitude and specificity of anti-tumor immune responses in lung cancer.

#2949 TGF-β is a key mediator of NK cell dysfunction in gliostroma. Hila Shaim, Abdullah Alsuslinam, Konrad Gabrusiewicz, Jun Wei, John Yu, Rafet Basar, May Daher, Lucila Kerbaya, Mayela Mendh, Muhamrem Muftuguo, Li Li, Enli Liu, Nobuhiko Imahashi, Sonny Ang, Young Gi, Pinaki Banerjee, David Mills, Richard Champlin, Elizabeth Shpill, David Heinze, Katayoun Rezvani, MD Anderson Cancer Center, Houston, TX.

Background: NK cells play a crucial role in the antitumor immune response and are involved in controlling tumor formation, progression and metastases. Glioblastoma (GBM) is the most devastating brain tumor, associated with very poor prognosis. While immunotherapy emerged as a promising approach for anti-cancer therapy, GBM appears immune resistant. Here, we studied the role of NK cells in targeting glioblastoma and possible mechanisms of NK immune evasion. Methods: NK cells were isolated from freshly resected GBM tissue, matching peripheral blood and the blood of healthy controls. Flow cytometry was used to characterize the cells and their ability to produce cytokines, and chromium release assay was performed to assess their cytotoxicity. In vitro assays were performed by co-culturing GBM stem cells (GSCs) with healthy donor NK cells. Results: We found that whereas NK cells were abundant in primary GBM tissue and could efficiently target GBM stem cells (GSCs), GBM infiltrating NK cells (TINKs) displayed an abnormal phenotype with downregulation of many activating receptors including CD16, NKG2D, DNAM, NKP30, NKP46, 2B4 and NKG2C and upregulation of inhibitory proteins such as PD-1. This inhibitory phenotype was associated with impaired NK cell function when compared with NK cells isolated from the peripheral blood of patients and healthy donors. GSC-NK cell-cell contact resulted in release of TGF-β by GCSs, which in turn led to NK dysfunction through constitutive activating of the p-Smad pathway. TGF-β activation, in turn, is partially mediated by the matrix metalloproteases MMP-2 and MMP-9, secreted by GSCs. In the presence of monocytes, tumor cell proliferation and TGF-β expression were enhanced upon TGF-β exposure. We demonstrated that inhibition of the TGF-β axis, in particular by the small molecule inhibitor, galunisertib, can prevent GSC-induced NK cell dysfunction but is unable to inactivate the p-Smad pathway, thus, cannot reverse existing dysfunction. Conclusions: Our results indicate that NK-GBM cross-talk plays an important role in tumor escape and highlight the importance of developing future adoptive transfer therapies with the intent of limiting tumor escape from antitumor immunity.

#2950 Biomarkers of immune infiltration for multiplex immunofluorescence in breast cancer. April Swoboda, Galina Khramtsova, Lise Sveen, Andrey Khramtsov, Rita Nanda, Olufunmilayo Olopade. University of Chicago Medicine, Chicago, IL.
Introduction: Only a subset of patients have a clinical response to cancer immunotherapy. The molecular mechanisms that mediate the immunological response or tolerance to treatment are just beginning to be understood. Several tumor signaling pathways are involved in those mechanisms: Wnt/β-catenin, STAT3, NFκB, PI3K/PTEN/AKT, and TP53. To characterize the tumor, its immune infiltrate, and its microenvironment, we examined expression of several biomarkers and determined the relationships among them in various breast cancer subtypes. Methods: 148 breast cancer (BCa) cases from the University of Chicago Breast Cancer SPORc Tissue bank under IRB approved protocols were classified into four biological subtypes based on immunohistochemical (IHC) staining: luminal A, luminal B, Her2 and basal-like. IHC staining for PD-L1, CD68, FoxP3, CD8, and β-catenin was performed on tissue microarrays. CD8+ and FoxP3+ cells were counted manually and recalculated for 1 mm². Macrophage phenotype was determined using single and double staining with CD68 for total population and CD68/CD163 for M2. CD68+ and CD163+ cells were counted manually, confirmed by Image Analysis, and recalculated for 1 mm². PD-L1 and β-catenin (membrane-associated, cytosolic and nuclear) were scored as negative, weak, positive, moderate, and strong. Results: The study cohort subtypes were 20.9% basal-like, 10.4% Her2, 53.8% luminal A, and 10.4% luminal B. The ratio of M2 to M1 cells increased with disease progression (p<0.001). Her2 and basal-like subtypes had a significantly higher percentage of M2 cells as compared to the luminal A subtype (p<0.001). The cases with M2 and M1 macrophage phenotype had a higher ratio of a T3P3 regulatory T cells (Tregs) to cytotoxic CD8+ cells. PD-L1 expression was present in 23% of specimens. However, the intensity of expression was markedly different across the BCa subtypes. PD-L1 high expressing tumors had, on average, greater FoxP3+ cell infiltrate and low or absent CD8+ T cells. The ratio of FoxP3 Tregs to CD8+ cells in basal-like tumors were 1.7, 2.3, and 4-fold higher than in Her2, luminal A, and luminal B tumors, respectively (p<0.005 for all correlations). There was a statistically significant decrease in membrane-associated β-catenin in tumor compared to normal tissue (p<0.0001). The proportion of tumors positive for cytosolic or nuclear β-catenin in basal-like tumors was statistically higher than all other tumor subtypes. High β-catenin nuclear protein expression was predominantly observed in tumors with higher CD8+ T cell infiltration. Ongoing analysis will evaluate the association of these biomarkers with clinical outcomes. Conclusions and Future Directions: Our data suggests that IHC using a panel of antibodies may be a robust and suitable method for evaluating level of immune infiltration. Future studies will evaluate multiplex immunofluorescence of multiple biomarkers in breast tissue.

#2951 The tumor microenvironment in cholangiocarcinoma is dominated by an immunosuppressive infiltrate. Nathania M. Figuerola, Brian Belt, Ankit Patel, Shinsuke Sato, Margaret Hill, Winicka Wolfe, Alexander Caldas, University of Rochester Medical Center, Rochester, NY.

Purpose: To characterize the dynamics of the immune response to cholangiocarcinoma (CCA) in a genetically engineered mouse model of CCA in order to identify immune pathways susceptible to targeted immunotherapies. Experimental Design: Genetically modified mice with targeted Kras activation and loss of p53 (AbL-Cre/LSL-KRASG12D/p53LacZ-/-) in the liver spontaneously develop CCA recapitulating the histopathological features of human disease. To evaluate the in vivo immune response to CCA, tumors from Kras-p53 mice and normal livers were excised and prepared for histologic examination, flow cytometry, and gene expression analysis. Immunohistochemistry (IHC) and flow cytometry were performed on myeloid and T cell markers to distinguish immune cell subsets. Cells lines established from isolated CCA tumors were used to assess the functional impact between the immune system and tumor cells in co-culture in vitro studies. Results: Kras-p53 murine hepatic tumors were highly desmoplastic with a heavy fibrotic stromal compartment. IHC analysis demonstrated a prominent inflammatory leukocyte infiltrate compared to normal liver. Flow cytometry of single tumor suspensions showed the immune reaction was dominated by CD11b+ monocytic (Ly6C+/-) and granulocytic (Ly6G+/-) myeloid cells, and FoxP3+ regulatory T cells (Treg). In vitro studies revealed tumor educated myeloid cells expressed higher levels of genes associated with an immunosuppressive phenotype, including Arginase 1 (p<0.0007). Invasion assays analyzing the migration potential of CCA cell lines co-cultured with CD11b+ cells showed increased invasive properties in these tumor cells when compared to naive controls (p<0.0001). Additionally, increased tumor-initiating properties were seen by qRT-PCR in tumor cell lines having undergone similar co-culture experiments. These data suggest the immune response to CCA is predominantly immunosuppressive and tumor supportive. Conclusion: CCA tumors from AbL-Cre/LSL-KRASG12D/p53LacZ-/- mice have a prominent immunosuppressive infiltrate recapitulating features of the immune reaction in human disease. Thus, Kras-p53 mice provide an ideal model to test targeted immunotherapy for the treatment of CCA.

#2952 The novel cathepsin L/K inhibtors KGP94 and KGP207 prevent M0 to M2 macrophage differentiation and macrophage-mediated pro-tumor functions. Samantha S. Dykes, Dietmar W. Siemann. University of Florida, Gainesville, FL.

Tumor-associated macrophages play many important roles in tumor progression, including facilitating tumor cell invasion and angiogenesis. Due to their numerous pro-tumor functions, tumor-associated macrophages represent an important cell population in the design of targeted anti-cancer therapies. Interleukin-4 (IL-4) mediated activation of macrophages resulted in an M2-like, or pro-tumor macrophage phenotype, often associated with increased expression of proteases including the lysosomal cathepsin L. Cathepsin L is important in both macrophage and tumor cell invasion, whereby secreted cathepsin L degrades the extracellular matrix, allowing for cell infiltration. The present study examined the role of cathepsin L in M0 to M2 differentiation and macrophage-mediated tumor cell invasion using the novel cathepsin L/K inhibitors KGP94 and KGP207 [Dr. Kevin Pinne, Baylor University]. KGP94 and KGP207 prevented M2 macrophage motility and invasion in vitro migration and invasion assays. Moreover, KGP94 and KGP207 reduced macrophage-stimulated invasion of 4T1 murine breast cancer cells. Together, these data suggested that cathepsin L was necessary for macrophage invasion and motility and for macrophage–medicated tumor cell invasion. Cathepsin L expression in GC resulted in a significant decrease in the IL-4-induced expression of the M2 marker Arginase-1 upon drug treatment. Additionally, exogenous recombiant cathepsin L partially stimulated the expression of Arginase-1 in M0 macrophages. Together, these data suggest that cathepsin L plays a role in macrophage M0 to M2 differentiation. In conclusion, the novel cathepsin L/K inhibitors KGP94 and KGP207 prevented M0 to M2 differentiation, macrophage invasion, and macrophage-stimulated invasion of breast cancer cells. These data highlight the importance of cathepsin L in macrophage functions and suggest that cathepsin L inhibition is a viable approach for the treatment of tumors associated with high macrophage infiltration.

#2953 Tumor-associated macrophages infiltration highly associated with PD-L1 expression in gastric cancer. Kazuto Harada,1 Jannelynn S Estrella,1 Arlene M Correa,1 Lang Ma,1 Xiaoquan Dong,1 Yan Xu,1 Wayne L Hofstette,3 Kazuki Sudo,1 Hisashi Onodera,2 Koyu Suzuki,1 Akiko Suzuki,1 Randy L Johnson,2 Zhenning Zhenning, Shumei Song,1 Jaffer A Ajani,1 Hisashi Onodera,2 UT MD Anderson Cancer Ctr., Houston, TX; 3St. Lukes University, Tokyo, Japan.

Objectives: programmed death ligand 1 (PD-L1) is key protein for tumor to acquire the evasion form T cell immune response and PD-L1 inhibitors are useful for an immune checkpoint blockade therapy. Tumor-associated macrophages (TAM) facilitate not only tumor cell progression, but also immunosuppression, which prevent tumor cell attack by T cell. Therefore, TAM is considered to be associated with PD-L1 expression in GC. Methods: We performed immunohistochemical staining of PD-L1, CD68 (pan-macrophage) and CD163 (M2 macrophage) in 217 GC sample tissue microarray. The area of CD68 and CD163 positive cells were evaluated by using the Cytoplastic V2.0 algorithm on the Aperio ImageScope software. Results: 31 samples (14.3%) were positive for PD-L1 expression. The mean rate for CD68 and CD163 positive area were 6.8±0.38% and 6.2±0.30%, respectively. CD163 positive macrophage infiltration is significantly more in diffuse type tumor than in intestinal type tumor (diffuse (n=111): 6.9%, intestinal (n=91): 5.3%, p=0.0056), but CD68 positive macrophage infiltration is similar in both type (p=0.38). Interestingly, PD-L1 expression tumor have significantly more CD68 and CD163 positive macrophage infiltration (CD68: PD-L1 negative 6.3%, positive 10.4%, p=0.0002, CD163: PD-L1 negative 5.7%, positive 9.2%, p<0.0001). PD-L1 expression and CD68 and CD163 positive area had no relationship between any clinicopathological features. Conclusions: Macrophages infiltration in GC is highly associated with PD-L1 expression of tumor cells. M2 macrophage infiltration is significantly associated with diffused type of GC. This result suggests that TAM infiltration is associated with the immunosuppression through upregulation of PD-L1 expression in GC cells. Thus, macrophages infiltration can serve as a potential therapeutic target.

#2954 The role of hedgehog signaling in breast cancer progression through macrophage polarization. Ann Hanna, Lalita A. Sheved. University of Alabama at Birmingham, Birmingham, AL.
The tumor microenvironment is comprised of stromal cells that constantly crosstalk with the cancer cells. Stromal cells, specifically tumor infiltrating immune cells, secrete cytokines and chemokines that regulate host responses to cancer through tumor-suppressing or tumor-promoting mechanisms. Macrophages within the tumor microenvironment are very plastic and can modulate different functions such as cell proliferation, differentiation, and angiogenesis. Although the Hh signaling pathway is tightly controlled, it often is deregulated, thus promoting tumorigenesis and tumor progression. Aberrant Hh signaling has particularly been implicated in breast cancer progression and metastasis. In this project, we investigate the role of Hh signaling in polarizing cancer associated macrophages toward the tumor-promoting M2 phenotype in a breast cancer model. We have discovered that using small molecule inhibitors to inhibit Hh signaling attenuates the cytokine profile associated with M2 macrophages, while adding recombinant Hh ligand potentiates it. Our data thus far supports a role for Hh signaling in alternatively polarizing macrophages. We hypothesize that Hh inhibitors will reduce the abundance of M2 macrophages and prevent metastasis.

#2955 Gr1-MDSCs and Tregs modulate the prostate cancer progression.
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Myeloid-derived suppressor cells (MDSCs) and Regulatory T cells (Tregs) are the major components of immunosuppressive network that play a critical role in tumor progression. The mechanisms by which tumors induce expansion of suppressive cells and the crosstalk between Gr1-MDSCs (Gr1−/CD11b+/F4/80−) and Tregs related to TGF-β secretion remain incompletely defined.

Prior studies have suggested that MDSCs may contribute to Treg recruitment in cancer. Herein, the goal of this investigation is to examine the role of TGF-β-mediated generation of Tregs and Gr1-MDSCs during prostate cancer progression and clearance. To achieve this goal, matrigel system was used along with transgenic adenocarcinoma of mouse prostate (TRAMP-C1, C2 and C3 cell) in C57BL/6 mice. Interestingly, TRAMP-C3 cells are characterized as non-tumorigenic; however, TRAMP-C1 and TRAMP-C2 cells do form tumor. Mice were administered with serial log concentration of TRAMP (C1, C2 and C3) cells along with the matrigel. After three weeks, matrigels along with or without tumor were excised from tumor bearing mice, single cell suspensions were prepared and cells were flow analyzed for GR1−/MDSCs and Tregs. Additionally, the TGF-β level was also estimated (colorimetric) in culture supernatant in context to MDSCs and Treg expansion. Our initial findings suggest that the expression of Gr1−/CD11b+/F4/80−/Fas−/and F4/80−/Gr1−/CD11b−/Fas−/and F4/80− in TRAMP-C1 and C2 as compared to TRAMP-C3 bearing C57BL/6 mice These findings indicate that the modulation of Gr1−/MDSCs and Tregs could help in tumor suppression.

#2956 Chemical library screen identifies compounds that target S100A8/S100A9 complex and MDSC accumulation.
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We previously demonstrated that MDSC subsets accumulated in primary tumor and distant organs regulate tumor plasticity. The mouse transcriptome analysis of in vitro co-cultures and samples from syngeneic mouse model revealed that granulocytic subset of myeloid-derived suppressor cells (gMDSCs) from metastatic 4T1 tumor bearing mice regulate several hundred genes in tumor cells upon co-culture. The top genes are S100A8, S100A9, MMP8, FPR1, CCL3, and TGFβ2 which also predicted poor survival in human solid tumors including breast cancer. Therefore, we called these 6 genes as “metastatic gene signature”. It has been reported that S100A8/S100A9 heterotrimer, called calprotectin, play a key role in inflammation-associated cancer progression. To investigate the role of calprotectin (S100A8/A9) in tumor-mediated immune-suppression and metastasis, we first utilized Tasquinimod, a small molecule targeting S100A9 only. However, Tasquinimod treatment of 4T1 tumor-bearing mice had a moderate anti-tumor activity which may be due to a limited activity on granulocytic MDSC accumulation. In addition, we determined that there was a significant upregulation of S100A8 in MDSCs from Tasquinimod treated mice. This data suggested that inhibiting only S100A9 leads to activation of S100A8 and thus may be ineffective targeting of MDSCs. We therefore run a computational screen of the NCI chemical library against the crystal structure of calprotectin (S100A8/S100A9) and identified 40 lead compounds. We then performed in vitro screening assay to identify compounds that inhibit MDSC induction. We identified 3 compounds that significantly suppressed gMDSC differentiation. We are currently performing in vivo studies with these 3 candidate drugs in our murine breast cancer model and will present our findings at the AACR meeting. We believe that this study will provide a novel drug targeting S100A8/S100A9 heterotrimer, a key molecule in MDSC induction and its regulatory functions during tumor progression.

#2956A Cancer invasion of mammary epithelial cells in 3D culture shows YAP-independent mechanotransduction.
Joanna Y. Lee, Jessica Chang, Sungmin Nam, Ovijit Chaudhuri. Stanford University, Stanford, CA.

83% of non-invasive breast cancers are diagnosed as ductal carcinoma in situ (DCIS). While some DCIS tumors remain confined in the mammary duct, others progress into invasive ductal carcinoma (IDC). The mechanisms underlying invasion are not well understood. Current diagnostic methods cannot accurately predict which DCIS cases will progress to IDC, and unnecessary treatment affects long-term health and quality of life, with radiation potentially promoting malignancy-inducing mutations. One possible regulator of invasion may be extracellular matrix (ECM) stiffness. Increased ECM stiffness has been correlated with invasion and 3D culture models of mammary epithelium show that enhanced stiffness induces an invasive phenotype. However, the mechanisms underlying stiffness-induced invasion remain unclear. Studies have converged upon the finding that YAP, a transcriptional regulator that is deregulated in diverse cancers, is the transducer of ECM stiffness. However, these studies were primarily performed in 2D culture and involved col-1, a ligand that activates distinct signaling pathways and is not normally found in the BM. Here, we examined the gene expression profiles of 3D cultured MCF10A cells during stiffness-induced invasion with and without col-1. We generated interpenetrating networks (IPNs) of reconstituted basement membrane (rBM) and alginate, which allow stiffness to be tuned in the absence of col-1 and independently of cell adhesion ligand concentration and matrix pore size. Traditionally used hydrogels composed of rBM and col-1, with stiffness tuned by increasing concentrations of col-1, were also produced for 3D culture. Our results show that enhanced stiffness increases MCF10A cell invasion and proliferation in both the presence and absence of col-1. However, in contrast to results from 2D culture, invasive phenotypes in 3D cultured cells did not correlate with nuclear localization of YAP, indicating lack of YAP activity. The dispensable nature of YAP in 3D stiffness sensing was supported by RNA-seq analysis, which showed a lack of increased gene expression of YAP downstream targets in conditions of enhanced stiffness. RNA-seq identified 389 differentially expressed genes in response to enhanced 3D culture stiffness. By relating these genes to transcription factors using ChIP-Seq data provided by ENCODE, we identified p300, FOS, STAT3, NELFE, and TAF1 as potential mechanotransducers of stiffness-induced invasion. Furthermore, highly expressed stiffness-induced genes were validated by immunofluorescence to identify potential candidate mechanotransduction. Our RNA-seq and immunofluorescence analysis identified a p300-dependent mechanism that allows accurate determination of cancer invasion risk in breast cancer patients, informing course of treatment.

TUMOR BIOLOGY: Tumor Microenvironment 4

#2957 T-cell receptor immunosequencing reveals novel insights into the immune response to human pancreatic cancer.
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Introduction: Despite advancements in therapy, pancreatic ductal adenocarcinoma (PDA) remains an aggressive cancer with high mortality. It is characterized by dense inflammation, including many T cells; however, it is unclear whether these T cells signify a true antitumor response. In the setting of disappointing early results of immunotherapy in PDA, we sought to gain a deeper understanding of the tumor-associated T-cell response. Methods: With IRB approval, we obtained archival resected PDA tumors in paraffin-embedded blocks from 54 patients. We performed histopathology to identify blocks containing lymph nodes (LN) and performed T-cell receptor (TCR) immunosequencing on DNA extracted from immediately adjacent tissue. To address possible intratumoral heterogeneity, we analyzed duplicate samples from separate blocks for 10
patients. Productive clonality was defined as 1-Pielou’s evenness for TCR rearrangements encoding a functional protein; TCR fraction was calculated from number of observed templates and the amount of input DNA. Two-tailed t-tests were used to compare subgroups. TCR sequence overlap between each of the 10 pairs of duplicate samples were calculated at the amino acid level. Results: Among 16 autopsy cases, mean TCR fraction was 0.27 (range 0.08-0.33), mean productive clonality was 0.15 (typical peripheral blood mononuclear cell clonality is 0.08). TCR fraction was positively correlated with clonality (R²=0.23, p=0.007), though this correlation was only significant in patients who received neoadjuvant chemotherapy (R²=0.19, p=0.03). TCR fraction was higher in patients with positive nodal status (0.32 vs. 0.23, p=0.02), but lower in patients who received adjuvant chemotherapy (0.22 vs. 0.33, p=0.02). There was no survival difference between high or low TCR fraction or clonality. Samples containing LN (not included in previous analyses) had higher TCR fraction (0.49 vs. 0.27, p<0.0001) but lower clonality (0.09 vs. 0.15, p=0.0002); this relationship was maintained regardless of nodal status. Among 10 pairs of duplicate samples, there was 53% mean overlap of TCR amino acid sequences, compared to 0.1% for pairs of unmatched samples (p<0.0001). Conclusion: Here we demonstrate evidence of clonal expansion of T cells in the human PDA microenvironment. Extensive overlap of TCR sequences between two distinct samples for each of 10 pairs analyzed further suggests that the clonal expansion may be specific to tumor antigens. Neoadjuvant therapy may also influence clonal expansion, as only treated patients had a positive correlation between TCR fraction and clonality. Finally, the presence of a lymph node sample appears to skew TCR sequences subject toward a less clonal but more numerous population. This suggests that the most rigorous analysis of TCRs in a solid tumor microenvironment needs to exclude blocks that contain lymph nodes in order to characterize the true intratumoral population of T cells.

#2958 Transcriptome analysis of astrocytoma versus non-neoplastic human microglia. Thais Fernanda Galatro,1 Antonio M. Lerario,2 Sueli M. Oba-Shinjo,3 Bart J. Eggen,3 Suely K. Marie1. 1University of São Paulo, São Paulo, Brazil; 2University of Michigan, Ann Arbor, MI; 3University of Groningen, Groningen, Netherlands.

Diffuse gliomas are primary brain tumors characterized by infiltrative growth and high heterogeneity, rendering the disease mostly incurable. Advances in genetic analysis revealed that molecular alterations predict patients’ overall survival and clinical outcome. However, glioma tumorigenicity is not exclusively caused by its genetic alterations. The crosstalk between tumor cells and the tumor microenvironment. We were also able to collect one sample of iTAMs from a GBM case. Despite not providing a significant number for statistical analysis, we can directly see major differences in gene expression between this sample and our TU-uglia cohort. We identified surface receptors differentially expressed between both cell types (i.e.: ROBO1 and PDGFRA for TU-uglia, MARCO and MET for iTAMs), as well as transcription factors associated with clonality and high heterogeneity.

#2959 Changes in the tumor stroma after MFP treatment in a MPA-induced murine mammary carcinoma model. Gonzalo Ricardo Sequeira,1 Ana Sahores,1 Tomas Dalotto-Moreno,1 Silvia Vanzulli,2 Laura Polo,1 Virginia Novaro,1 Caroline Lamb,1 Mariana Salatino,1 Claudia Lanari,1. 1Inst. de Biología y Medicina Experimental, Buenos Aires, Argentina; 2Academia Nacional de Medicina, Buenos Aires, Argentina.

The role of the immune system in the regression of mammary carcinomas using endocrine therapies has been poorly investigated. The main goals of this study were: a) to evaluate differences in antiprogestin-induced regression of hormone receptor positive mammary carcinomas growing in immunocompetent or immunodeficient mice; b) to characterize the tumor infiltrating immune cells in milliprostine (MFP)-induced mammary carcinomas. The comparison of these transcriptome datasets provided an in-depth insight into the immune system changes elicited by mifepristone and mifepristone + 17β-estradiol in tumor re-challenge assays. Bone marrow (BM) cells from BALB/c-GFP mice were intravenously inoculated into NSG mice to establish the NSG/BM-GFP mouse model. GFP cells were detected in spleen, bone marrow, and blood from NSG/BM-GFP mice two months after BM-GFP cell inoculation. The murine mammary carcinoma 59-2-HI, originally induced by the administration of medroxyprogesterone acetate to BALB/c mice, regresses completely after MFP treatment. Tumors were orthotopically inoculated into NSG or NSG/BM-GFP female mice two months after BM grafting. When the tumors reached a size of approximately 50 mm³, low dose MFP pellets (0.2 mg) were administered in order to inhibit tumor growth. To characterize the infiltrating immune cells, tumors were excised after drug administration, disaggregated, and isolated cells were analyzed by FACS. Tumors from mice treated with MFP + 17β-estradiol and animals were orthotopically re-inoculated with the same tumor in the opposite flank. Operated animals were used as controls. MFP inhibited the growth of tumors transplanted in either NSG or NSG/BM-GFP + and no differences were observed between both groups, suggesting that the immune system is not a key factor mediating antiprogestin-induced tumor regression. Tissue remodeling was associated with an increase in lymphocytes (CD3+; T cells) and macrophages (CD11b+ + F480+; >80% at day 3 and 6), and a decrease in the Treg subpopulation (CD4+ + CD25+ + Foxp3+; <20% at day 3 and 6). These results led us to hypothesize that antiprogestin treatment could elicit a protective antitumor immune memory response by delaying tumor recurrence. To test this hypothesis we performed re-challenge assays using animals in which primary tumors have been previously excised. All tumors of control mice reached the ethical limit size (200 mm²) before day 47, while only half of the tumors in the MFP-treated group reached this size by this time (hazard ratio: 1.98). We conclude that antiprogestins induce tumor regression regardless of the presence of an impaired immune system and that the reduced intra-tumor Treg/CD8 ratio observed in regressing tumors might be used as a predictive marker of treatment response. The re-challenge experiments agree with the hypothesis that regressing tumors expose intracellular antigens that generate a protective immune memory response, which in turn, could be associated with the long free relapse survival induced by endocrine therapy.

#2960 Adipocytes sequester and metabolize daunorubicin. Xia Sheng,1 Jean-Hugues Parmentier,1 Jonathan Tucci,2 Hua Pei,1 Omar Cortez-Toledo,1 Christina Dielli-Conwright,1 Matthew Oberley,1 Michael Neely,1 Etan Orgel,1 Stan Louie,2 Steven D. Mittelman1. 1USC/Children’s Hospital Los Angeles, Los Angeles, CA; 2USC School of Pharmacy, Los Angeles, CA; 3USC School of Dentistry, Los Angeles, CA.

Obesity is associated with poorer outcome from many cancers, including childhood acute lymphoblastic leukemia (ALL). We have previously shown that adipocytes protect ALL cells from the anthracycline, daunorubicin (DNR). We therefore investigated whether adipocytes sequester and/or metabolize DNR in the ALL microenvironment. Using fluorescence and LC/MS measures, we demonstrated that adipocytes absorb DNR, reducing the intracellular DNR concentration in co-cultured BV173 ALL cells (after 48 hours, median fluorescent intensity of ALL cultured with adipocytes was 1.7±1.0 vs. 5.0±1.7 of those cultured alone, p<0.01). Mouse adipocytes convert DNR to the less active metabolite, daunorubicinol (DNR-dol); over 48 hours, media DNR decreased to 0.1±0.2 (vs. 24.8±8.76 ng/mL in control wells). At the same time, DNR-dol increased (0.1±0.4 vs. 0.7±0.8 ng/mL). Similar concentrations of DNR to DNR-dol was observed in both mouse adipose explants and human adipose tissue biopsy samples ex vivo. qPCR confirmed human subcutaneous adipose tissue expresses several enzymes capable of metabolizing DNR, including AKR1A1, 1B1, 1C1, 1C2, 1C3, 7A2, and CBR1 and 3 (expression ranged between 20 and 195% of human). Using immunohistochemistry, we confirmed expression of AKR1C1, 1C2, and 1C3 in bone marrow adipocytes of children during the first month of treatment for ALL. Finally, two hours after an intravenous dose of DNR in mice, we found that the DNR-dol to DNR ratio was higher in subcutaneous (0.6±0.5 at 2.6 days) and omental (0.55±0.21) adipose than in white blood cells (0.16±0.11), bone marrow (undetectable DNR-dol), and spleen.
Targeting stromal-derived Dickkopf-3 (DKK3) for the treatment of pancreatic ductal adenocarcinoma (PDAC). Liran Zhou,1 Hongmei Husted,1 Todd Moore,1 Mason Liu,2 Defeng Deng,1 Yan Liu,3 Vijaya Ramachandran,1 Thiruvengadam Arumugam,1 Baonan Ji,2 Huamin Wang,1 Jeffrey E. Lee,1 Craig D. Logsdon,1 Rosa F. Hwang,1 1UT MD Anderson Cancer Ctr., Houston, TX; 2Mayo Clinic, Rochester, MN.

Introduction: We and others have shown that pancreatic stellate cells (PSCs) in the tumor-associated stroma of PDAC promote tumor progression and resistance to therapy but the precise mechanisms are unclear. We investigated the role of PSC-derived DKK3, a member of the Dickkopf family of glycoproteins, in PDAC progression, metastasis and response to chemotherapy. Methods: We evaluated expression of DKK3 in human PDAC tissue and cell lines, human PSCs and in a genetically engineered mouse model (GEMM) of PDAC. The paracrine and autocrine effects of DKK3 on PDAC and PSCs were examined by treatment with exogenous DKK3 and gain- and loss-of function assays for proliferation, migration, invasion, and gemcitabine-induced apoptosis. The effects of DKK3 on PDAC progression and metastasis were determined by shRNA neutralization and genetic ablation in orthotopic xenograft models and the KPC autochthonous model of PDAC. We developed novel monoclonal antibodies (mAbs) against DKK3 and tested their ability to neutralize DKK3 and prolong survival in mouse models of PDAC. Results: DKK3 was expressed at 4.5 times higher levels in human PDAC by Affymetrix profiling compared to normal pancreas and was present in 99% (118/119) of samples on a tissue microarray.

...higher levels in human PDAC by Affymetrix profiling compared to normal pancreas and was present in 99% (118/119) of samples on a tissue microarray with moderate to high expression in 59%. In a GEMM of PDAC, DKK3 appeared early with preneoplastic PanIN lesions with increased expression in invasive carcinoma. DKK3 was strongly expressed by PSCs with minimal to no expression in PDAC cells and knockdown by shRNA reduced PSC proliferation and migration by 60% and 84% compared to controls (p < 0.001). Treatment of Panc1 and BxPC3 cells with DKK3 stimulated migration and invasion by 100-300% (p < 0.001) and proliferation of DKK3-silenced Panc1 cells was decreased by 80% (p < 0.001). Overexpression of DKK3 in L3.6pl cells increased colony formation in gemcitabine by >90% (p < 0.001) with 65% reduction in apoptosis (p < 0.01), indicating that DKK3 contributes to PDAC resistance to chemotheraphy. When we ablated DKK3 in KPC mice by breeding with DKK3-knockout mice, tumor growth was inhibited and survival increased by 45% (p = 0.0002). In addition, fewer PanIN lesions developed in DKK3-null mice suggesting that DKK3 may contribute to the early developmental stages of PDAC. DKK3 neutralizing mAbs abrogated DKK3-mediated induction of PDAC cell migration, invasion and resistance to gemcitabine in vitro. Furthermore, treatment with DKK3 mAb significantly inhibited primary tumor growth, reduced peritoneal metastases and prolonged survival in an orthotopic model of PDAC by 43% compared to control mAb (p = 0.005; HR; 0.2, 95% CI 0.01-0.30).

Conclusions: These data are the first report, to our knowledge, of a tumor-promoting function of DKK3. DKK3-derived mAbs suggest that neutralizing DKK3 may be an effective approach as a primary treatment for PDAC and to enhance responsiveness to chemotherapy.

Mechanobiology of epithelia on native basement membrane and relevance for cancer invasion. Marija Plodinec,1 Philipp Oertle,1 Daphne Assgeirson,1 Willi Hafferl,2 Serenella Epenberger Castoni,1 Ellen C. Obermann,1 Alexandre Glentis,1 Roderick Y. LIM 1.

The onset of metastasis occurs when cancer cells invade and breach the basement membrane (BM) that provides mechanical support to epithelial tissues. Yet, it remains unclear what triggers cancer cells to breach the BM, and how ‘triggered’ cells in fact breach the BM. We have established an in vivo assay using native BM interface for culturing epithelial cells. Using atomic force microscopy (AFM) with other high-resolution microscopies and TER (trans-epithelial resistance), we have correlated the mechano-cellular attributes of the BM epithelium interface to its biochemical and structural properties. We demonstrated that the internal limiting membrane (ILM) isolated from human retinas acts as a native substrate for culturing epithelial cells in terms of BM composition, architecture and stiffness. These are required to act jointly in order to achieve apico-basal polarity, tissue barrier formation and stiffness properties of the epithelial layer similarly to secretory epithelia in vivo. The native BM serves several advantages over reconstituted Matrigel, an ECM extracts that originates from mouse tumor ascites. Besides variations in thickness and biochemical composition, we find that Matrigel is mechanically 100-fold more compliant (i.e., softer) than native BMs. When tumorigenic variants of cells are used, we demonstrated that cancer cell invasion is associated with a decrease in cellular stiffness correlated to changes in cell and BM morphology. In addition, we showed that activation of cell matrix stiffness and architecture of the native BM collagen scaffold and BM collagen chain has a key role, not only as previously thought for maintenance of cell polarity but also for the establishment of a physiological mechanophenotype. On the other hand it is well know that during cancer progression in vivo, cancer cells can perforate the BM using proteolysis. Whether stromal cells also play a role and what kind of role in this process is unclear. Therefore, we asked if carcinoma-associated fibroblasts (CAFs) isolated from cancer patients promote cancer cell invasion through a BM. In the presence of CAFs, moderately invasive cancer cells invade in a matrix metalloproteinase-independent manner. Using live imaging and atomic force microscopy, we showed that CAFs actively pull, stretch and soften the BM, forming gaps through which cancer cells can migrate. By exerting contractile forces, CAFs alter the organization and the physical properties of the BM, making it permissive for cancer cell invasion. Finally, we propose that, in addition to proteolysis, mechanical interactions between CAFs and BM represent an alternative mechanism of BM breaching. Given their mechanobiological relevance, native BMs allow us to further understand how mechanical signaling occurs between the epithelia and the surrounding stromal layers at the BM interface during cancer invasion and progression.

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Inhibition of stromal p38MAPK abrogates breast cancer metastasis. Sheila A. Stewart,1 Bhavna Murali,1 Qiao Ren,1 Xinning Su,1 Kevin Flanegan,2 Xianmin Luo,1 Jasmine Sponagel,1 Yujie Fu,1 Elise Alsphach,1 Kathleen Lehy,1 Roberta Faccio,1 Kathleen Weilbaecher,1 Joseph Monahan2. Washing-ton University, St, Louis, MO; 2Confluence Life Sciences, St, Louis, MO.

Bone metastasis is a devastating and fatal complication of breast cancer for which we lack effective therapies. Thus, identifying therapies that effectively limit metastases will significantly reduce comorbidities and improve long-term survival. Recently we demonstrated that the p38MAPK pathway sustains the pro-tumorigenic senescence-associated secretory phenotype (SASP) and targeting this pathway limits the tumor-promoting capabilities of senescent cells and cancer-associated fibroblasts (CAFs). Because we found that a significant percentage of p38MAPK-dependent SASP factors are expressed in the stroma associated with breast cancer lesions, we asked whether targeting p38MAPK could limit primary and metastatic breast cancer growth. While p38MAPK inhibition modestly limited primary tumor growth, we found that its inhibition significantly reduced breast cancer bone metastases by specifically targeting the stromal compartment. Further, p38MAPK inhibition was as effective as paclitaxel at limiting tumor growth in the bone but in contrast to paclitaxel, which failed to protect from cancer-induced bone loss, p38MAPK inhibition prevented bone loss. This contrasts our p38MAPK approach from zole-dronic acid, which limits bone loss but fails to slow tumor growth in already engrafted tumors. Analysis of the mechanism(s) responsible for this reduced metastasis suggests that p38MAPK inhibition targets reactive and/or senescent osteoblasts within the bones of animals harboring metastatic lesions. Because we identified osteoblasts are present in human bone, we postulate that they may be vital to promote metastatic outgrowth and thus p38MAPK inhibition limits the pro-metastatic activities of these cells. Finally, we will present recent data from our preclinical model that demonstrates that inhibition of the p38MAPK pathway can drastically reduce metastasis from the primary site. We propose that p38MAPK is an important stromal-specific therapy for breast cancer metastasis to the bone.

Stromal Hedgehog pathway activation suppresses growth and me-tastases of lung adenocarcinoma. Sabha Kasiri, Baozhen Chen, Alexandre Wil-son, Umaru Barrie, Ummay Marriam, Zhiqun Zeng, Luc Girard, James Kim. UT Southwestern, Dallas, TX.

Aberrant activation of the Hedgehog (Hh) signaling pathway, a crucial development pathway, of the tumor growth of G-protein-coupled receptors. However, recent data suggest that paracrine activation of the pathway is tumor suppressive rather than oncogenic in sporadic epithelial cancers. The role of the pathway in non-small lung cancer is poorly understood. Thus, we explored the role of stromal Hh pathway activation in growth of lung tumor epithelia. Human lung adenocarcinoma cell lines were used to probe SHH mRNA and protein expres-sion. Co-culture of lung SHH expressing cell lines with murine embryonic and lung fibroblasts were used to confirm and probe the role of paracrine SHH expression on the growth of lung cancer cells. The in vivo role of paracrine SHH was tested using autochthonous lung cancer models with conditional KRASG12D activation, p53 loss, and SHH loss compared to wild-type SHH. In

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human lung adenocarcinoma patients. Higher expression of SHH mRNA in lung adenocarcinoma correlated with poor overall and progression free survival. A scan of 35 human lung adenocarcinoma cell lines revealed heterogeneous expression of SHH and HH in high expression found predominantly in mutant K-Ras lines. Co-culture of high SHH expressing tumor epithelial cells and Shh-Light2 reporter cell lines demonstrated that SHH activated the fibroblast reporter in a paracrine manner, rather than an autocrine effect on cancer cells. Treatment with the Smo inhibitor, KAAD-cyclopamine, also inhibited the growth of tumor epithelial cells in co-culture with NIH-3T3 fibroblast cells but the effect was decreased when co-cultured with lung fibroblasts. Genetic loss of SHH in an autochthonous mouse model, LSL-Kras(12D/12D);Trp53(−/−);P53R2(−/−);Pten(−/−);Kras(12D/12D);Trp53(−/−);Pten(−/−);Pten(−/−) KP mice. However, early inhibition of the Hh pathway by anti-SHH/IHH antibody, SE1, on KP mice resulted in significantly worse survival rates with increased metastatic burden compared to IgG treatment. Analysis of KP tumors revealed unexpected high levels of IHH mRNA by in situ hybridization that may account for the survival differences seen between genetic ablation and pharmaceutical inhibition of the Hh ligands. In conclusion, the Hh signaling pathway acts upon lung stromal cells in a paracrine fashion. Inhibition of Hh activity in vivo worsened mortality rate due to increase in tumor growth and metastases. Furthermore, mutant Kras lung cancers express high levels of IHH that dominates the tumor suppressive effect of our mouse models.

#2965 Myeloid depletion reverses established T-ALL growth in vivo. Todd A. Tripplett, Aram Lye, Wesley Godfrey, Lauren E. Ehrlich. University of Texas at Austin, TX.

T cell acute lymphoblastic leukemia (T-ALL) is a hematological cancer that arises from developing T-cells. Most T-ALL research has focused on elucidating cell-intrinsic genetic permutations that promote tumor proliferation and survival, which has resulted in a detailed description and understanding of the genetic mutations associated with T-ALL in patient cells. However, to date these studies have not resulted in effective targeted therapies, which may potentially be due to the heterogeneity of genetic lesions within and between patients or the acquisition of new mutations. Thus, we need to broaden our understanding of other factors promoting T-ALL growth to identify alternative therapeutic targets that are effective across mutational landscapes. To this end, our studies seek to identify extrinsic signals in the tumor microenvironment that enable T-ALL growth. Our previous studies used a systematic and unbiased evaluation of endogenous stromal subsets from the thymic TME to reveal that myeloid cells, primarily dendritic cells (DC), were necessary and sufficient for ex vivo survival of T-ALL cells. Furthermore, transcriptional profiling revealed elevated expression of Igf1r and Iglf by T-ALL and DC from the TME, respectively. Using multiple molecular approaches, inhibition of IGFR1 revealed that IGFR1 signaling was necessary for DC-mediated survival of T-ALL from multiple tumor locations. These findings have relevance to human T-ALL as pervasive TEC-free regions containing extensive DC networks were evident in T-ALL patient samples, and previous studies have verified that IGFR1, a direct target of NOTCH1, is expressed at high levels in T-ALL patients. In these studies, we sought to determine whether myeloid cells were required for tumor survival in vivo. After transplantation, tumor growth was established systemically in lymphoid and non-lymphoid tissues of the recipients. Immunofluorescence analysis revealed extensive myeloid networks around and between T-ALL tumor cells in multiple tissues. For primary tumors, transplanted T-ALL cells also required the presence of DC from the TME for survival ex vivo. Importantly, myeloid cell depletion in transplanted hosts with established T-ALL resulted in substantial reduction in tumor burden in all tissues examined, thus demonstrating a fundamental role for these cells in supporting T-ALL in vivo. To identify key survival pathways activated in T-ALL cells by myeloid cells, we have compared comprehensive transcriptomic and phosphoproteomic profiling from tumor cells in the presence or absence of myeloid cells in transplant models. Preliminary analysis of T-ALL versus WT TME microarrays has confirmed hyper-activated IGFR1 signaling, as indicated by hyper-phosphorylation of downstream targets, such as IRS1. Collectively, these studies provide the first evidence that myeloid cells directly support T-ALL in vivo, suggesting that these cells and their associated signals may be auspicious therapeutic targets.

#2966 Stromal pleiotropic growth factor receptor-beta (PDGFRbeta) promotes breast cancer progression. Gina M. Szemere, Anisha M. Hammer, Kate A. Thies, Steven T. Szemere, Anthony J. TrimboI, B. Eason Hillneth, Ralegh D. Kladney, Arnab Chakravarti, Gustave Leon, Michael C. Ostrowski. Ohio State University, Columbus, OH.

Over the past decade it has become evident that the tumor microenvironment (TME) actively participates in carcinogenesis. Tumor-associated fibroblasts, for example, modulate neighboring tumor epithelium through growth factor secretion to initiate and promote tumor growth. The pleiotroped derived growth factor receptors (PDGFRs), PDGFRalpha and PDGFRbeta, are receptor tyrosine kinases activated by PDGF that may be critical and actionable mediators of breast tumor-stromal communication. PDGFRs are predominantly expressed in breast tumor stroma while their cognate ligand, PDGF, is expressed in tumor epithelium and associated endothelium. In some cancers, tumor-derived PDGFRs act on the TME to recruit associated fibroblasts; however, this role has not been described in breast cancer. To begin to evaluate a role for PDGFRbeta, we utilized publicly available gene expression data to confirm upregulation in tumor stroma compared to tumor epithelium. Importantly, PDGFRB is increased in breast tumor stromal cells compared to normal stroma. To directly test whether the stromal PDGFR activation promotes tumor growth, we co-injected murine mammary tumor cells with or without PDGFR-expressing mouse mammary fibroblasts (MMFs) orthotopically in FVB/N mice. MMF inclusion increased tumor cell proliferation as well as associated angiogenesis while systemic treatment with imatinib mesylate, a small molecule inhibitor for PDGF, restored both proliferation and angiogenesis back to baseline. These findings indicated the importance of PDGFR signaling in tumor initiation leading us to develop a mouse model of stromal-specific PDGFRbeta activation using the Fsp-cre transgene previously published by our group (henceforth referred to as “PDGFRbeta mutant”). PDGFRbeta mutant mammary glands exhibited increased tertiary side-branched and epithelial proliferation confirming a stromal-specific PDGFR-beta role in neighboring epithelial proliferation. Distributions of PDGFs isoforms secreted from the PDGFRbeta mutant mice exhibit increased motility towards PDG-F expressing tumor cells in vitro, which implies increased response and recruitment of the mutant MMFs towards an expanding tumor. To test whether PDGFRbeta mutant mice harbor a mammary TME supportive of increased tumor growth, we injected murine mammary tumor cells orthotopically into either control or PDGFRbeta mutant mice finding that the time required to meet early removal criteria (tumor &gt;1cm3) was shorter in the mutant mice compared to controls. Ongoing studies are evaluating whether systemic PDGFR inhibition will abrogate this observed increase in tumorigenesis. In summary, our data suggest that stromal PDGFRbeta signaling is pro-tumorigenic in breast cancer and that inhibition using well-described PDGFR inhibitors could be a valid therapeutic approach for women whose tumors express increased PDGF-to-PDGFR tumor-stromal signaling.

#2967 Pancreatic cancer cell growth requires lipids released by tumor-induced stroma autophagy. Petrus R. De Jong,1 Sean-Luc Shanahan,2 Morgan A. Brand,3 Alejandro D. Campos,1 Anagha Siranagam,1 Nikolas Marinico,1 Claudi D. Miller,1 Olga Zagnitko,1 Adam D. Richardson,1 David A. Scott,1 Brian P. James,3 Andrew P. Hodges,3 Ally PeruIna,4 Alexey M. Eroshin,3 Randall French,3 Malene Hansen,4 Sally A. Litherland,4 Andrew M. Lowy,3 J. Pablo Arnoletti,5 Malte J. Sorensen,1 Gunter Fleischer,14 Divya Arun,11 Alex Schwartz,3 SBP NCI-Designated Cancer Center, La Jolla, CA;15 SBP Discovery Institute, La Jolla, CA;2 University of California, San Diego. La Jolla, CA;3 Florida Hospital Cancer Institute, Orlando, FL;4 Institute for Surgical Advancement/Florida Hospital Center for Specialized Surgery, Orlando, FL.

Pancreatic ductal adenocarcinoma (PDAC) is non-resectable in the majority of patients and highly resistant to chemotherapy, resulting in a poor survival. The tumor microenvironment (TME) actively participates in carcinogenesis and promoting tumor progression in PDAC. Understanding the metabolic vulnerabilities of PDAC in the harsh tumor microenvironment may lead to novel therapeutic approaches with improved clinical efficacy. First, we found that PDAC cells showed beneficial effects of co-cultured stroma cells, but only under lipid-free serum conditions. To study the metabolic crosstalk between cancer cells and stroma in more stromal tumor cells with or without PDGFR-expressing mouse mammary fibroblasts (MMFs) orthotopically in FVB/N mice. MMF inclusion increased tumor cell proliferation as well as associated angiogenesis while systemic treatment with imatinib mesylate, a small molecule inhibitor for PDGF, restored both proliferation and angiogenesis back to baseline. These findings indicated the importance of PDGFR signaling in tumor initiation leading us to develop a mouse model of stromal-specific PDGFRbeta activation using the Fsp-cre transgene previously published by our group (henceforth referred to as “PDGFRbeta mutant”). PDGFRbeta mutant mammary glands exhibited increased tertiary side-branched and epithelial proliferation confirming a stromal-specific PDGFR-beta role in neighboring epithelial proliferation. Distributions of PDGFs isoforms secreted from the PDGFRbeta mutant mice exhibit increased motility towards PDG-F expressing tumor cells in vitro, which implies increased response and recruitment of the mutant MMFs towards an expanding tumor. To test whether PDGFRbeta mutant mice harbor a mammary TME supportive of increased tumor growth, we injected murine mammary tumor cells orthotopically into either control or PDGFRbeta mutant mice finding that the time required to meet early removal criteria (tumor &gt;1cm3) was shorter in the mutant mice compared to controls. Ongoing studies are evaluating whether systemic PDGFR inhibition will abrogate this observed increase in tumorigenesis. In summary, our data suggest that stromal PDGFRbeta signaling is pro-tumorigenic in breast cancer and that inhibition using well-described PDGFR inhibitors could be a valid therapeutic approach for women whose tumors express increased PDGF-to-PDGFR tumor-stromal signaling.
lipid droplets. The transfer of lyso-PiPs was abrogated by pharmacological inhibitors of autophagy, or by siRNA-mediated knockdown of autophagy genes in stromal and tumor cells. These data suggest that PDAC cells cause stroma cells to undergo autophagy, and reprogram stroma metabolism to obtain complex lipid species for their metabolic needs in the lipid-starved tumor microenvironment.

**#2968 Examining the role of breast cancer Neuregulin1 and macrophage ErbB3 in intravasation.** Ramon Cabrera, Serena Chiang, Jeffrey Segall, Albert Einstein College of Medicine, Bronx, NY.

The purpose of our studies is to investigate the role of Neuregulin1 (NRG1) and ErbB3 signaling between breast cancer cells and macrophages in facilitating tumor cell intravasation. The interaction of breast cancer cells with other cell types within the tumor microenvironment plays an important role in metastasis. These interactions are thought to influence tumor cell invasion and intravasation, two important steps in the process of metastasis. Our studies are specifically interested in examining the signaling occurring between tumor cells and macrophages. Previous studies have established the presence of paracrine signaling between breast cancer cells and macrophages, where colony stimulating factor 1 (CSF-1) produced by the tumor cells stimulates the production of epidermal growth factor (EGF) by macrophages, leading to chemotactic invasion of the tumor cells. In addition to this paracrine loop signaling between tumor cells and macrophages, it has been seen that macrophage expression of ErbB3, a member of the EGFR family of receptor tyrosine kinases, may play a role in facilitating tumor cell invasion. In order to examine the effects of signaling between tumor cells and macrophages in intravasation, we utilize an in vitro transendothelial migration (iTEM) assay. This assay uses transwells coated with matrigel and endothelial cells in order to mimic the entry of tumor cells into blood vessels. We show that using an ErbB3 blocking antibody results in a significant reduction of macrophage-induced transendothelial migration of breast cancer cells. Additionally, reduction of expression of the ErbB3 receptor ligand Neuregulin1 in tumor cells yields a similar result. Stimulation of macrophages with NRG1 leads to increased expression of JAG1 (JAG1), a ligand of the Notch receptor. Activation of the Notch receptor pathway has been shown to be involved in tumor cell invasion. Overall our studies look to further examine the interaction between tumor cells and macrophages, and these observations indicate that ErbB3, NRG1, and JAG1 could all serve as novel targets in metastasis and the tumor microenvironment.

**#2969 Paracrine effect of the endothelium on prostate cancer cells.** Verónica Torres-Estáya, Patricia Fuenzalida, Catalina Ascencio, Carla Cembrano, Daniela Carreño, Néstor Corro, Viviana Montecinos, Gareth Owen, Xavier Figueroa, Julio Amigo, Juan Carlos Sáez, Alejandro Godoy. Pontificia Universidad Católica de Chile, Santiago, Chile.

Prostate cancer (CaP) is the most commonly diagnosed cancer and the second leading cause of cancer deaths among males in the United States. Androgen deprivation therapy (ADT) is the standard treatment for advanced or metastatic CaP. However, during ADT, CaP progresses from an androgen-sensitive (AS-CaP) to a more aggressive, and eventually lethal, castration-resistant (CRPC) CaP. However, during ADT, CaP progresses from an androgen-sensitive (AS-CaP) to a more aggressive, and eventually lethal, castration-resistant (CRPC) CaP. Delineation of such critical players may culminate in tight control of endothelial microvasculature due to an increase in angiogenesis by tumor cells. Nevertheless, now there is evidence to support that this influence is not one-directional and that the endothelial cells secrete a large number of active substances (angiocrine factors), which may directly or indirectly influence tumor growth and progression. However, the direct impacts of the endothelium on prostate tumor progression or the molecular mechanisms that are involved in this communication remain unclear. Here we investigated the potential influence of endothelium-derived paracrine factors on prostate cancer biology and the role of connexins in these interactions, since connexins play a major role in cell-cell communication and form a bidirectional signaling pathway to assemble gap junctions and alter cell behaviors. We measured the effect of conditioned media (CM) obtained from a primary culture of human endothelial cells isolated from umbilical vein (HUVEC) on viability, proliferation, migration and invasion of CaP cell lines (LNCaP, LNCaP-C4-2 and PC3) and in the metastatic potential by in vivo assays using co-injection of CaP cell with HUVEC or inoculation of CaP cells pre-incubated with CM from HUVEC in a zebrafish embryo model. Finally, we studied the expression and the role of connexins on this stimulation using pharmacological (GJIC inhibitors) approaches. Altogether, our results showed that CM from endothelial cells induces an increase in the viability and proliferation in all CaP cell lines (LNCaP, LNCaP-C4-2 and PC3) but only increases migration of the CRPC cell lines (LNCaP-C4-2 and PC3). We also observed in our in vivo model that endothelial cells either through cell-cell interaction or by paracrine communication increases the metastatic ability of the CaP cells. Moreover, the increase in viability and migration of CaP cells observed with CM from endothelial cells was blocked using inhibitors of gap junctions. Real-time PCR analyses detected an up-regulation of Cx43 mRNA after exposition to CM from endothelial cells. Our data suggest that angiocrine communication between endothelial cells and CaP cells involves proliferation and migration of more aggressive CaP cells which could be important for the acquisition of the aggressive phenotype of the disease, and this interaction could be mediated by Cx43. Disequilibrium of such critical players may culminate in identifying therapeutic targets or biomarkers to counteract CaP, especially advanced CaP.

**#2970 Mitigating tumor-stroma metabolic symbiosis for cancer therapy.** Dhruv Kumar, Jacob New, Vikalp Vishwakarma, Hemant Chavan, Partha Kasturi, Su! M. Thomas. Kansas University Medical Center, Kansas City, KS.

Head and neck squamous carcinoma (HNSCC) affects 40,000 patients annually and is associated with <50% 5-year survival. There is an urgent need to better understand the biology of the disease in order to develop more effective therapeutic approaches. HNSCC tumors are dysplastic with up to 80% fibroblasts. We recently reported that tumor-associated fibroblasts (TAFs) make HNSCC more aggressive. Furthermore, we reported that TAFs produce heparocyte growth factor (HGF), which binds to the c-Met receptor expressed on HNSCC to drive aggressiveness. Although we did not detect HGF secretion from HNSCC cell lines, we reported that paracrine activation of c-Met by TAF-secreted HGF facilitates HNSCC progression. Reciprocal signaling between the tumor and stroma has been reported in several cancers to facilitate tumor growth, invasion and resistance to therapy. Recent studies have shown that c-Met activation promotes glycolysis. Although highly glycolytic, the mechanisms regulating HNSCC glycolysis remain unknown. We show that TAF-secreted HGF through c-Met activation on HNSCC (a) induces aerobic glycolysis accompanied by lactate production, and b) regulates expression of basic fibroblast growth factor (bFGF). Studies have shown that HNSCC tumors have high lactate levels resulting from increased glycolysis, and this correlates with reduced survival. Our data demonstrate that TAF-secreted HGF increases key glycolytic enzymes including hexokinase II. Furthermore, HGF increases glycolysis and lactate production from HNSCC. We demonstrate that HGF also increases levels of the bidirectional lactate transporter, monocarboxylate transporter 1 (MCT1). We demonstrate that MCT1 levels are increased in both HNSCC and TAFs under co-culture conditions. In addition, HGF stimulation increases levels of MCT1 in HNSCC indicating a possible mechanism whereby HNSCC remove the excess lactate generated during glycolysis. The mechanisms whereby HNSCC tumors survive highly acidic conditions remain unknown. Since MCT1 levels are increased in TAFs as well, we sought to determine if TAFs utilize the lactate as a carbon source to generate energy. Indeed, we found that bFGF secreted by HNSCC binds to its cognate FGF receptor (FGFR) on TAFs to facilitate late utilization through mitochondrial oxidative phosphorylation (OXPHOS). Thus there exists a metabolic symbiosis between HNSCC and TAFs that contribute to tumor growth. Through these studies, we delineate the mechanisms of glycolysis regulation in HNSCC and demonstrate that inhibition of the c-Met/TAF axis talk between HNSCC and TAFs can be used as a novel therapeutic approach.

**#2971 The prognostic relevance of inflammatory cytokines and growth factors elaborated from adipose-derived stem cells in breast cancer.** Nicole Wierwic!, Kelsey Sadlek, Eric Lundstrom, Daniel Berrebi, Gerald Hobbs, Linda Vona-Davis!. 1West Virginia University, Morgantown, WV; 2West Virginia University Cancer Institute, Morgantown, WV.

Introduction: Given the high rates of obesity and aggressive breast cancers, it is paramount to understand how adipose tissue supports the tumor microenvironment. We previously found that adipose-derived stem cells cultured with triple-negative tumor cells enhanced the expression of numerous pro-inflammatory cytokines. This finding is significant as it could explain, in part, how stromal contribution to worse outcomes in breast cancer. To further expand this study, we probed the Cancer Genome Atlas (TCGA) breast invasive carcinoma dataset for the expression profiles of the inflammatory cytokines we found to be highly up-regulated in vitro. We predicted TCGA data would demonstrate a correlation between cytokine overexpression and worse clinical scenarios. Methods: Cytokins and growth factors were measured in the media of: a) adipose stromal and MD-MB-231 tumor cells via membrane-based antibody array. To validate clinical significance, we compared each cytokine’s mRNA expression profile, available from primary tumor samples of TCGA breast invasive carcinoma dataset (n = 1,005), to indices of tumor progression and survival status. For survival analysis, samples were dichotomized as high or low expres-
sion by individual cytokines (z-score ≥ 1.5). Cytokine and growth factor expression was further stratified according to molecular subtype of breast cancer (luminal A, luminal B, HER2, triple negative) and receptor status. Ingenuity pathway analysis (IPA) was performed to identify candidate markers related to obesity or inflammation. Results: High levels of FGF7 and CCL5 protein were found in the condition media of tumor and adipose stem cell co-cultures and together they showed a significant impact on breast cancer survival within TCGA data. FGF7, CCL5, together with CCL2, IL6, and IL6R were further analyzed for expression across molecular subtypes of breast cancer. IL6R, CCL5, and CCL2 expression varied significantly between breast cancer subtypes, with mean expression highest in triple-negative tumors. After dichotomizing the samples according to tumor subtype status, CCL2 showed significantly higher mean expression in ER negative tumors. Greater expression of these cytokines in tumors was correlated with reduced patient survival in triple-negative tumors and ER negative subtypes. IPA analysis indicated IL6, IL6R, CCL5, and CCL2 overexpression, either independently or synergistically, predict a worse prognosis in breast cancer. For adipose-driven, hormone receptor negative breast tumors, these cytokines and growth factors could serve as novel therapeutic targets. (Supported by NIH P20GM103434 and NIGMS U54GM104942).

#2972 SPINK1, a soluble factor released by the therapy-damaged tumor microenvironment, promotes prostate cancer resistance. Fei Chen,1 Da Fu,1 Eric Lanerolle,1 Institute of Health Sciences, Shanghai Institutes for Biological Sciences, CAS, Shanghai, China; 2Shanghai Tenth People’s Hospital, Tongji University School of Medicine, Shanghai, China; 3Department of Surgery and Cancer, Imperial College London, London, United Kingdom.

Cancer evolution is driven by not only the genetic and/or epigenetic alterations of cancer cells, but also diverse factors that are derived from the surrounding tumor microenvironment (TME). Upon chemotherapy or radiation, stromal cells in the TME become senescent and develop a senescence-associated secretory phenotype (SASP) that is characterized by secretion of a large number of cytokines, chemokines, growth factors, and proteases. In most cases, the SASP is functionally regulated by the DNA damage secretory program (DDSP) and pathophysiologically responsible in vivo for disease exacerbation. We recently disclosed significant upregulation of the serine protease inhibitor Kazal type 1 (SPINK1) in primary normal human prostate fibroblasts after exposure to DNA damaging agents. SPINK1 plays critical roles in cancer cell proliferation, survival and motility in multiple human malignancies, but its influence as a soluble TME-associated factor on cancer progression remains unknown. In this study, we performed a series of molecular and cellular studies to define the biological roles of SPINK1 in an activated TME. Promoter analysis indicated that SPINK1 expression in the fibroblasts is regulated by multiple transcriptional factors including the NF-κB complex in response to genotoxic stress. Fibroblast-derived SPINK1 can significantly enhance the aggressiveness of prostate cancer cells including accelerated proliferation, increased migration, elevated more importantly, enhanced chemo-resistance. SPINK1 triggers a typical epithelialmesenchymal transition (EMT) in cancer cells, a process mediated by EGF/P13H/Akt and MAPK/Erk signaling pathways. Consistent with the in vitro data, our in vivo studies suggested that SPINK1 expression in the prostate TME substantially promoted cancer survival and disease progression. Clinical investigation revealed increased expression of SPINK1 in the stroma of multiple organ types including the prostate, lung and breast of cancer patients after chemotherapy, implying the systemic induction of SPINK1 by anticancer agents. Overall, our study demonstrates that SPINK1 is a soluble biomarker of therapeutically damaged TME and represents a potentially exploitable molecular target in human prostate cancer clinics.

#2974 Obesity-induced modifications in the mammary gland microenvironment promote TNBC progression even after weight loss. Alyssa J. Gozzo, Ottavia Lambi Zattria, Alex J. Freerman, Liaz Makowski. UNC-Chapel Hill, Chapel Hill, NC.

Obesity contributes to 20% of US female cancer deaths and is linked to increased risk for triple negative breast cancer (TNBC), as well as increased risk of vascular invasion, metastasis, and mortality in breast cancer patients. We previously showed that obesity-induced elevation of hepatocyte growth factor (HGF) signaling through its receptor, cMET, accelerated development of tumors in the C3(1)-TAg GEMM of TNBC compared to low fat diet (LFD)-fed lean controls. Weight loss significantly reduced HGF/cMET signaling in normal mammary glands and cMET in tumors, and reversed high fat diet (HFD)-driven tumor progression, while crizotinib-mediated cMET inhibition limited tumor development and microvascular density in both lean and obese C3(1)-TAg mice. To differentiate tumor-intrinsic versus microenvironment-associated mechanisms, we employed an orthotopic transplant model of a C3(1)-TAg TNBC cell line to investigate tumor growth dynamics in lean, obese, or formerly obese (FOB) microenvironments. We hypothesized that TNBC tumors derived from lean C3(1)-TAg cells in both low fat diet (LFD) and high fat diet (HFD) environments will grow more rapidly, contain more macrophages (Mfs) and exhibit greater blood vessel density than tumors in lean or FOB mice due to obesity-induced MF and/or endothelial secretion of HGF. At 8 weeks of age, female FVB/NJ mice were randomized to diet groups (N=20/diet): 1) lean (LFD, 10% kcal from fat), 2) obese (HFD, 60% kcal from fat) 3) FOB (HFD, 5 weeks; LFD, 5 weeks). Following 10 weeks on diets mice were analyzed for breast tumor burden, localization, regional lymph node metastasis by diet group, as well as quantification of pro-growth and pro-angiogenic signaling molecules (e.g., HGF, VEGF, Ang2) in lysates of tumors and tumor-adjacent adipose. Tumor volume was measured by calipers. At 21 days post-injection mice were sacrificed for tissue collection. Normal mammary gland, tumors, and tumor-adjacent adipose + tumor-draining lymph node were collected for histological examination and gene expression analysis. Mammary fat pad mass in obese FVB/NJ mice (0.27 g) was significantly greater than lean (0.14 g) or FOB animals (0.11 g) (P<0.0001). Tumor volume at sacrifice was significantly greater in obese vs lean animals (190 mm3 vs 89.96 mm3, respectively; P<0.05). However, mean final tumor volume in FOB animals (148.0 mm3) did not differ significantly from either lean or obese mice. Our findings show that obesity accelerated tumor progression relative to lean mice. C3(1)-TAg cells transplanted into FOB mice resulted in an intermediate rate of tumor growth, suggesting that obesity-mediated changes to the microenvironment were not fully overcome by obesity. Despite normalization of body weight and adiposity, to elucidate how the FOb mammary microenvironment persists in driving TNBC tumor promotion, planned analyses include histological examination of candidate mediators, including MF infiltration and regional lymph node metastasis by diet group, as well as quantification of pro-growth and pro-angiogenic signaling molecules (e.g., HGF, VEGF, Ang2) in lysates of tumors and tumor-adjacent adipose.

#2975 Collagen type XI alpha 1 confers cisplatin chemoresistance in ovarian cancer through inhibition of apoptosis proteins (IAPs). Miran Rada,1 Jennifer Cha,2 Jessica Sage,1 Sandra Orsulic,1 Dong-Joo Cheon,1 1Albany Medical College, Albany, NY; 2Rensselaer Polytechnic Institute, Troy, NY; 3Cedars-Sinai Medical Center, Los Angeles, CA.

Ovarian cancer is the most lethal gynecological cancer, which affects one in seven females in the United States during their lifetime. While cisplatin is the frontline treatment for ovarian cancer, chemoresistance severely limits the treatment success. The goal of this study is to identify novel therapeutic targets to overcome cisplatin resistance. We recently identified Collagen type XI alpha 1 (COL11A1) as a novel biomarker associated with cisplatin resistance in ovarian cancer. COL11A1 is a component of the tumor stroma and its expression is restricted to cancer-associated fibroblasts (CAFs) adjacent to tumor cells. We further showed that COL11A1 protects ovarian cancer cells from cisplatin-induced apoptosis, yet the underlying mechanism is largely unknown. To understand the molecular mechanism by which COL11A1 protects cancer cells from cisplatin-induced apoptosis, we overexpressed COL11A1 in ovarian cancer cells and performed RNA-Seq. Our RNA-Seq data revealed that COL11A1 did not change the levels of genes associated with cisplatin resistance. Instead, COL11A1 promoted cell viability in cancer cells, which are well-established mechanisms to confer cisplatin resistance. Rather, COL11A1 enhances the expression of the Inhibitors of Apoptosis Proteins (IAPs), including XIAP, BIRC2 (c-IAP1) and BIRC3 (c-IAP2). Using a full-length COL11A1 protein, genetic manipulation, and co-culture method, we confirmed that COL11A1 significantly increases the expression of IAPs upon cisplatin treatment. The inhibition of XIAP, BIRC2 and BIRC3 with an IAP antagonist BV6 attenuated the ability of COL11A1 to inhibit cisplatin-mediated apoptosis. Our data indicates that COL11A1 activates Akt, and Akt is known to phosphorylate XIAP at serine-87, which protects it from ubiquitination and degradation in response to cisplatin. Collectively, our data identify IAP as a mediator of COL11A1’s action in blocking cisplatin-induced apoptosis.

#2976 Critical role of spatial location of hypoxia and its association with astrocytes in the resistance of brain tumor to therapy. Chi-Muin Lin, Chi-Shun Chang, National Tsing-Hua University, Hsinchu, Taiwan.

Many reports have shown that hypoxia and gliosis could promote tumor cells by shielding from the immune response and relaxing, but their roles on brain tumor therapies are still unclear. Using an invasive murine astrocytoma tumor model, A1T5C1, we found that tumor invasion front is associated with a specific hypoxia region, naming tumor peripheral hypoxia, contributes to brain tumor therapy resistant and may be responsible for tumor recurrence after the therapy. Using this tumor model, we found that the overgrowing tumor caused the shortage of vessels and resulted in the hypoxia in the early stage of tumor progression, which is con-
#2977 Secretory autophagy in tumor associated fibroblasts promotes head and neck squamous cell carcinoma progression and emerges as a novel therapeutic target. Jacob New, Levi Arnold, Mehta Ananth, Sameer Ali, Wen-Xing Ding, Sufi M. Thomas. University of Kansas Medical Center, Kansas City, KS.

As the fifth most common cancer worldwide, head and neck squamous cell carcinoma (HNSCC) is a leading global health burden. Despite therapeutic advancements, the survival rate has remained relatively unchanged for the last fifty years. To improve treatment and survival, there is a need to better understand the underlying biology of this disease. The tumor microenvironment's role in promoting cancer progression and resistance to therapy has garnered great attention. In HNSCC, the predominant microenvironment cell type is tumor associated fibroblasts (TAFs). Studies in our lab and others have demonstrated the significant tumor promoting role of TAFs; however, little is understood of the underlying biology of TAFs. In this study, we identify that TAFs have upregulated an unconventional pathway of secretory autophagy. Although paradigmatically a degradation pathway, there has been a growing appreciation for a novel role of autophagy in cellular secretion. We hypothesized HNSCC induces secretory autophagy in the TAFs, modulating secreted factors responsible for tumor progression. We assessed the role of autophagy inhibition in alleviating TAF-facilitated HNSCC progression, and uncovered a significant reduction in proliferation, migration, and invasion of the cancer cells. This was achieved by both siRNA knockdown of Beclin-1, and therapeutic inhibition of the lysosome through chloroquine. We discovered that NPs placed in co-culture with HNSCC had a significantly upregulated level of autophagy marker LC3-II. By characterizing the role of known HNSCC secreted factors in inducing TAF autophagy, we identified basic fibroblast growth factor as responsible for the LC3-II accumulation. To understand which TAF factors are secreted by an autophagy dependent mechanism, we assessed TAF-conditioned media in the presence of autophagy knockdown. Key cytokines, such as IL-6 and IL-8, were identified as autophagy dependent. Rescue of these cytokines in autophagy inhibited TAF-conditioned media restored HNSCC migration. Although autophagy has been identified as upregulated in a variety of cancer types, no one has previously characterized the role of autophagy in HNSCC, and both early pre-clinical and clinical studies of autophagy inhibition as a cancer therapy have been limited by the lack of a specifically targeted inhibitor. We demonstrate a significant decrease in HNSCC progression in preclinical models by using a new and highly specific small molecule inhibitor of autophagy, SAR405, which inhibits Vps34, an upstream autophagy pathway kinase. In summary, we uncover a novel role for secretory autophagy in the tumor microenvironment which promotes tumor progression, and can be uniquely targeted for therapy.

#2978 Discrete super-enhancer networks in pancreatic cancer cells and cancer associated fibroblasts are targeted and modulated by triptolide. Pawan Noel, Serina Ng, Ruben Munoz, Daniel Von Hoff, Haiyong Han. Translational Genomics Research Institute (TGen), Phoenix, AZ.

Background: Pancreatic cancer (PC) is the 3rd leading cause of adult cancer death in the USA. Pancreatic ductal adenocarcinoma (PDAC) accounts for >90% of all PC cases and has a dismal 5-year survival rate of <6%, mostly due to lack of reliable methods for early detection, and limited treatment options. A unique, highly fibrinous and hypo-vascularized tumor stroma or “desmoplastic reaction” (DR) forms the major barrier to currently available therapeutic approaches. Activated cancer associated fibroblasts (CAFs) play an important role in driving and maintaining the DR in PDAC. Thus therapeutic regimens that reprogram pancreatic CAFs can potentially enhance sensitivity to anti-tumor agents and may improve patient outcomes. Recent studies implicate the role of super-enhancers (SE) in maintaining cell specific gene expression networks in both normal and diseased cells. Here we aim to define SE networks in pancreatic CAFs and epithelial tumor cells and explore the role of triptolide in modulating SE signatures. Methods: Using genomic techniques on control vs triptolide treated pancreatic tumor and CAF cells, we measured occupancy of BRD4 and acetylated histone 3 at lysine27 (H3K27ac), marks of active SEs, using Chip-seq. RNA-seq revealed differentially regulated genes via SE modulation by triptolide. Immunoblotting was performed to validate the effect of triptolide on the protein expression of key regulatory SEs. Results: Importantly, we found that the formation of tumor peripheral hypoxia might be associated with the activation of astrocytes. Therefore, tactics that can suppress astrocytosis and prevent the formation of peripheral hypoxia may be alternative approaches for complementing the shortage of traditional brain tumor therapy.

#2979 Inhibition of palladin in cancer associated fibroblast impedes pancreatic ductal adenocarcinoma associated desmoplasia. Jennifer L. Alexander, Edna Cukierman. Drexel University College of Medicine, Philadelphia, PA; Fox Chase Cancer Center, Philadelphia, PA.

Pancreatic Ductal Adenocarcinoma (PDAC) claims 97% of patient lives in less than 5 years after diagnosis. Treatment of PDAC is challenged by the collagen-rich, highly dense, fibrinous-like extracellular matrix (ECM) known as desmoplasia. Desmoplasia is initiated by activated cancer associated fibroblasts (CAFs) and disseminates throughout the stromal compartment in a vicious cycle involving alterations to the physical/structural and biochemical pathways in neighboring naïve fibroblasts that ultimately enriches theCAF population. This desmoplastic-ECM (D-ECM) encapsulates the tumor mass, thereby limiting therapeutic paradox. Paradoxically, ablation of D-ECM induces a more aggressive PDAC progression. Although the underlying biology remains unclear, the D-ECM is a consequence of cytoskeletal rearrangements occurring amid the CAFs. The goal of this study is to delineate the cytoskeleton dynamics engaged as a potential mechanism to restore the innate tumor-suppressive properties of a normal ECM. As such, this study focuses on the actin cross-linker palladin which has been identified as an independent prognostic marker in PDAC progression. We postulate palladin has an isomorphic-specific role in PDAC desmoplasia as well as cancer progression. Using an in vivo-mimetic 3D fibroblast-derived ECM model, we explore the isomorphic-specific role of palladin during D-ECM onset as well as during D-ECM-induced CAF activation in PDAC patient derived CAFs in which the major palladin isoforms (isoforms 3 and 4) were knocked out via CRISPR/Cas9 gene editing. Using these cells, we examined the activation status of the CAFs while characterizing the ECM produced via quantitative immunoblots, real-time polymerase chain reaction, and detailed microscopy analyses. We then validated the effects of palladin knockout CAFs activity in influencing kras mutated cancer cells’ metabolic activity, invasion and proliferation. Our data indicates palladin isoforms 3 and 4 expression are necessary to drive CAF activation as well as perpetuate the D-ECM phenotype whereby knockdown of either isoform prevents CAF activation while limiting cancer cell behavior. Additionally, the ECM produced by the palladin knockout CAFs were no longer capable of activating naïve fibroblast in addition to decreasing the inherent aggressive nature of kras-mutant cancer cells. These results are similar to previous findings regarding the restrictive nature of a normal ECM on CAF activation and cancer cell behavior. These findings suggest that palladin isoforms 3 and 4 are required for desmoplastic onset and D-ECM-induced fibroblastic activation and cancer cell progression. Together, these results propose manipulation of specific palladin isoforms could signify a strategy to restore a normal tumor-suppressive stroma in PDAC.
TUMOR BIOLOGY: Tumor Microenvironment

Introduction: Our group has recently demonstrated that CBS, one of the H2S-producing enzymes, is abundantly overexpressed in human colorectal cancer (CRC) tissue specimens resulting in an increased H2S production. In order to extend our observations, we have now compared, using immunohistochemistry, CBS expression in carcinoma-associated fibroblasts (CAFs) compared to normal colon fibroblasts (NCFs) in human colon adenocarcinoma samples. The overall goal of our studies is to characterize how the CBS/H2S axis contributes to the malignant phenotype of the CAFs in the tumor microenvironment. Methods: Human CAFs and NCFs were collected from CRC under an IRB approved protocol and cultured in vitro. Western blot analysis of both NCF and CAF was performed to determine CBS protein expression levels. siRNA/shRNA-mediated silencing of CBS in CAFs was performed. A pharmacological inhibitor of CBS, aminooxyacetic acid (AOAA), in both CAF and NCF cultures was also utilized. Hydrogen sulfide (H2S) levels were measured using the fluorogenic probe 7-Azido-4-methylcoumarin. We also performed proliferation assays using a couler counter in CAFs, NCFs, and CAFs subjected to siRNA/shRNA-mediated CBS silencing or AOAA. Control cells were transfused with shNT vectors. Cells were also treated with the slow-releasing H2S donor GYY4137. We also conducted cell migration assays in transwell chambers (migration of CAFs toward conditioned media (CM) collected either from HCT116 cells or normal colon mucosal cells (NCM356). Finally, the XF24 Extracellular Flux Analyzer (Seahorse) was used to measure bioenergetic function in CAFs subjected to siRNA/shRNA-mediated silencing of CBS or treated with AOAA. Results: CBS protein expression and H2S production in CAFs was increased compared to NCFs. The H2S donor GYY4137 increased the proliferation of CAFs but not of NCFs. CBS silencing or AOAA treatment reduced CAF migration toward conditioned media from HCT116 cells. Genetic or pharmacological inhibition of CBS in CAF cells suppressed key bioenergetic parameters including basal respiration, ATP production, maximal respiratory capacity, and spare respiratory capacity compared to NCF cells or CAF shNT controls. Conclusions: Thus, CBS/H2S axis serves as a mechanism to support CAFs function. In these cells, H2S contributes to maintaining their bioenergetic functions, which, in turn, promotes their proliferation and migration toward colon cancer cells. We hypothesize that these processes may contribute CRC progression.

#2981 Identification of circulating stroma-related biomarkers for pancreatic ductal adenocarcinoma. Andrea Resovi,1 Luca Porcu,1 Alessia Anastasia,1 Paola Allavena,2 Paola Cappello,1 Anna Falanga,3 Giulia Tarabotti,3 Mariarossa Bani,1 Dorina Belotti,1 Raffaella Giavazzi,1 Istituto di ricerche farmacologiche Mario Neri, Milano e Bergamo, Italy;2 Humanitas Clinical and Research Center, Rozzano, Italy;3 CeRMS, University of Torino, Torino, Italy;1 Department of Immunohaematology and Transfusion Medicine, Bergamo, Italy.

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive epithelial malignancies characterized by a relevant amount of tumor stroma. It is usually diagnosed late and has limited treatment options. Plasmatic markers capable of detecting the disease in its early stages and monitoring treatment effectiveness might change the fate of PDAC patients. This study investigates the potential value of circulating stroma-related molecules as PDAC biomarkers capable to detect the disease and to monitor treatment response. Thirty-eight stroma-related candidate biomarkers were selected from published proteomic studies on PDAC. Levels of these molecules were tested by ELISA and Multiplex Luminex assays in a first cohort of plasma samples from PDAC patients and healthy subjects. Thirteen molecules–extracellular matrix proteins and proteolytic fragments, matrix-degrading enzymes and their inhibitors, growth factors and adhesion molecules–were found to be differentially expressed in PDAC plasma compared to healthy subjects. A second analysis in an independent cohort of PDAC patients, chronic pancreatitis patients and healthy controls confirmed the previous findings and allowed restricting the number of biomarkers up-regulated in the plasma of PDAC patients. The plasma analysis of two genetically engineered mouse models of pancreatic cancer [Pdx-1 Cre;LSL-KrasG12D] and Pdx-1 Cre;LSL-KrasG12D;LSL-Trp53R172H] revealed an up-regulation of four markers in association with PanIN development, indicating their potential role in the early diagnosis of pancreatic cancer. These markers were also elevated in the plasma of mice bearing patient derived orthotopic PDAC xenografts (PDAC-PDX), but not in a mouse model of caerulein-induced chronic pancreatitis. Their level was associated with PDAC-PDX tumor growth and response to gemcitabine combined with nab-paclitaxel. In conclusion this analysis has identified a panel of stroma-related potential biomarkers associated with tumor progression and drug response of PDAC. Supported by Associazione Italiana per la Ricerca sul Cancro (AIRC 5 per mille grant n. 12182) and Fondazione “Eugenio Morandi” ONLUS for the studio e la cura dei tumori del pancreas.

#2982 Metastatic and non-metastatic colorectal cancer cells differentially regulate fibroblast cell cycle via extracellular vesicles. Rahul Bhole, Louise M. House, Tilman Sanchez-Elsner, Stephen M. Thirdborough, Emre Sayan, Alex H. Mirmazei. University of Southampton, Southampton, United Kingdom.

Cancer-associated fibroblasts are critical to tumor progression. There exists a dynamic crosstalk between cancer and stromal components, which maintains a permissive tumor microenvironment. Extracellular vesicles (EVs) play a significant role in this intercellular communication. Colorectal cancer (CRC) cells can be categorized according to epithelial-mesenchymal transition (EMT) status, and therefore metastatic capacity. We aimed to investigate the effect of EMT on EV-mediated cancer-fibroblast signaling. CRC cells (SW1573, SW620, HCT116, and DLD-1) were transduced by lentiviruses to be EMT, and western blotting to determine EMT status. EVs were isolated from conditioned media by serial centrifugation and validated by transmission electron microscopy, western blotting and nanoparticle tracking analysis. Fluorescently labeled EVs and cells were detected and evaluated by flow cytometry and fluorescence microscopy. Increasing concentrations of EVs from CRC cells were co-cultured with fibroblasts for 24h. Activation/inhibition of signaling pathways was examined by western blotting. EV microRNA (miRNA) profiles were obtained, validated by qPCR and submitted for target and pathway analysis. DLD-1, HCT116 and SW620 cells express E-cadherin and are considered epithelial, whereas SW480 lacks E-cadherin, expresses ZEB-1, and is considered mesenchymal. EVs were spherical, enriched in AID, FRPC, IGFR1, and TXNIP. AID expression correlated with the level of EVs. EVs were shown to transfer directly to primary ex vivo patient-derived fibroblasts and fibroblast cell lines. Transfer of EVs from epithelial CRC cells abrogated ERK activity in fibroblasts, even at the lowest concentration, and was associated with reduced fibroblast proliferation, whereas EVs from mesenchymal cells had no effect. MiRNA profiling of EVs from epithelial and mesenchymal CRC cells showed a 10-fold upregulation of mir-143-3p in epithelial compared to mesenchymal EVs. MiRNA target analysis and experimental validation show that mir-143-3p directly targets KRAS and HRAS, providing a potential miRNA-orchestrated mechanism of action for the downregulation of fibroblast ERK activity in the tumor microenvironment. Importantly, CRC cellular ERK activity is not reflected in fibroblasts treated with CRC EVs, suggesting that EVs do not directly transmit ERK protein or miRNA. However, miRNAs are the most stable EV cargo, and we show that epithelial but not mesenchymal CRC EVs contain upregulated miRNAs, which target critical components of the ERK pathway. Downregulation of ERK activity has been shown to induce fibroblast senescence, a phenotype linked to cancer progression. We hypothesize that differential regulation occurs because epithelial CRC cells are juxtaposed with fibroblasts in the tumor core, where senescent cancer associated fibroblasts are frequently observed, whereas mesenchymal CRC cells are at the invasive front or in the circulation.

#2983 Tumor induced stromal STAT1 deregulates mammary tissue homeostasis and accelerates breast cancer. Victoria R. Zellmer, Patricia M. Schnepp, Sarah L. Fracci, Xuejuan Tan, Erin N. Howe, Siyuan Zhang. University of Notre Dame, Notre Dame, IN.

The tumor microenvironment (TME) – the dynamic tissue space in which the tumor exists – plays a significant role in tumor initiation and is a key contributor in cancer progression. Little is known about the tumor-induced changes in the adjacent tissue stroma. Herein, we sought to explore tumor-induced changes in the TME at the morphological and molecular level to further characterize cancer progression. We show that tumor-adjacent mammary glands (TAGs) display altered morphology, increased expression of αSMA particularly in myofibroblasts, and an increased capacity to form mammospheres in 3D suspension culture. FACS analysis showed that TAGs contain an increased number of Lin-CD24+/CD49+ enriched mammary gland stem cell (MaSCs) population, suggesting deregulated tissue homeostasis in TAGs. We conducted comparative transcriptomics on TAGs and contralateral control glands. Meta-analysis on differentially expressed genes from our RNA-seq dataset revealed two biological subsets identified shared upregulation of STAT1, which we verified in tumor-adjacent tissues. Knockdown of STAT1 in cavelin-deficient mouse embryonic fibroblasts (CAFs) cocultured with human breast cancer cells altered cancer cell proliferation, further suggesting the role of STAT1 as a stromal contributor of tumorigenesis. Furthermore, in our proof-of-concept in vivo experiment, co-treatment with fluorudarine, a FDA-approved STAT1 activation inhibitor and DNA synthesis inhibitor, in combination with doxorubicin, showed enhanced therapeutic efficacy in treating mouse mammary gland tumors. Our results demonstrate that stromal STAT1 expression could promote tumor progression and is a potential therapeutic target for breast cancer.
**TUMOR BIOLOGY: Tumor Microenvironment**

### #2984 The chemokine CCL2/CCR2 signaling mediated fibroblasts-cancer cells crosstalk promotes basal like breast cancer progression. Min Yao, Wei Bin Fang, Fang Fan, Nehemiah Alvarez, Patrick E. Fields, Nikki Cheng. University of Kansas Medical Center, Kansas City, KS.

Cancer associated fibroblasts are the most abundant stromal cells in breast cancer, but their function in cancer progression has not been fully understood. We previously identified that the chemokine CCL2 was highly expressed in breast cancer associated fibroblasts, and high stromal CCL2 expression predicted poor outcome in basal like breast cancer. CCL2 is known to recruit monocyte/macrophage and promotes cancer progression. We previously found that recombinant CCL2 can directly signal to breast cancer cells and promote cell survival and invasion in vitro. In this study, we aimed to determine the functional importance of CCL2 signaling mediated fibroblast-cancer cell interactions in breast cancer progression. We used a fibroblast and cancer cell co-graft mouse model as the main functional assay. We generated primary fibroblasts from mouse mammary tumor and human breast cancer, and confirmed most of them expressed high level of CCL2. When co-grafted with the human basal breast cancer cell line MCF10A-CA1D into nude mice, fibroblasts enhanced xenograft growth. Stable knockdown of CCL2 expression from fibroblasts significantly reduced its ability in tumor growth promotion, while knockdown CCL2 from cancer cells did not. Decreased CCL2 production from fibroblasts resulted in increased apoptosis and autophagy in tumor samples. To determine the importance of direct CCL2 signaling to cancer cell, we generated the CCL2 receptor cCR2 adenovirus cell line and CCR2 null cell lines by CRISPR-Cas9 targeting technology. Mutation of CCR2 in cancer cell significantly reduced tumor growth when co-grafted with CCL2 secreting fibroblasts. Lastly, we tested continuous delivery of CCL2 neutralizing antibody in the co-graft tumor model, but observed minimal therapeutic effect. Further examination of blood CCL2 level revealed an increased production of CCL2 from fibroblasts and mouse host after antibody treatment, which may contribute to the lack of therapeutic effect. In summary, our studies demonstrated the importance of CCL2/CCR2 signaling mediated fibroblasts-cancer cell interaction in basal like breast cancer progression. The CCL2 signaling pathway can be potentially served as therapeutic target, but requires development of efficient targeting strategy.

### #2985 Interaction between the malignant melanoma cell of the primary lesion and the metastatic lesion. Takaharu Hatano,1 Hisashi Motomura,1 Heishiro Fujikawa,1 Masakazu Yashiro,2 Kishu Kitayama3. Department of plastic and reconstructive surgery, Osaka City University Graduate School of Medicine, Osaka, Japan; 2Department of Surgical Oncology, Osaka City University Graduate School of Medicine, Osaka, Japan; 3Department of Surgical Oncology, Osaka City University Graduate School of Medicine, Osaka, Japan.

Introduction: The prognosis of patients with malignant melanoma is extremely poor because of the frequent metastasis. We have occasionally experienced the clinical cases that malignant melanoma patients rapidly developed distant metastases after the surgical resection of primary cancer lesion. These cases suggested that a primary tumor may control a metastatic tumor. We hypothesized that primary melanoma cells might produce a factor(s) which regulate the progression of metastatic melanoma cells. Then we investigated the growth and invasion interaction between primary malignant melanoma cells and high metastatic melanoma cells. Materials and methods: We used two malignant melanoma cell lines, B16 as a parent primary melanoma cell line and a selected daughter cell line B16/BL6 with high metastatic ability. We investigated the invasive interaction between the parent B16 cells and daughter B16/BL6 cells in vitro. The conditioned medium from B16 cells and B16/BL6 cells was added to B16 cells or B16/BL6 cells. Then we examined the effect of these conditioned medium on the motility and proliferation of melanoma cells by MTT assay or invasion assay at 24 hours and 72 hours after the addition of the conditioned medium. Results: The conditioned medium from B16 cells significantly (p=0.02) suppressed the invasion ability of B16/BL6 cells at 24 hours after the addition of the conditioned medium. In contrast, the invasion ability of B16 cells were not affected by the conditioned medium from B16/BL6 cells at 24 hours. Each conditioned medium did not affect on the proliferation of both melanoma cell lines. On the other hand, at 72 hours after the addition of the conditioned medium from B16 cells significantly (p=0.000003) suppressed the growth of B16/BL6 cells. Conclusion: Primary melanoma cells might down-regulate the progression of metastatic melanoma cells by a soluble factor(s).

### CLINICAL RESEARCH: Clinical Biomarkers

#### #2986 Atezolizumab in metastatic TNBC (mTNBC): Long-term clinical outcomes and biomarker analyses. Peter Schmid,1 Cristina Cruz,2 Fadi S. Braiteh,3 Joseph Paul Ender,4 Sara Tolany,5 Irene Kuter,6 Rita Nanda,7 Cathie Chung,8 Philippe Cassier,9 Jean-Pierre Delord,10 Michael Gordon,11 Yijin Li,12 Bo Liu,13 Carol O’Hear,14 Marcella Fasso,15 Luciana Moliniero,16 Leisha A. Emens,17 Barts Cancer Institute, Queen Mary University London, London, United Kingdom; 2Vall d’Hebron University Hospital, Barcelona, Spain; 3Comprehensive Cancer Centers of Nevada, Las Vegas, NV; 4Yale School of Medicine, New Haven, CT; 5Dana Farber Cancer Institute, Boston, MA; 6Massachusetts General Hospital, Boston, MA; 7University of Chicago Medical Center, Chicago, IL; 8The Angeles Clinic and Research Institute, Los Angeles, CA; 9Centre Leon Berard, Lyon, France; 10Institut Claudius Regaud, Toulouse, France; 11Pinnacle Oncology Hematology, Scottsdale, AZ; 12Genentech, Inc., South San Francisco, CA; 13Johns Hopkins University School of Medicine, Baltimore, MD.

Introduction. Triple negative breast cancer (TNBC) has a poor prognosis and limited treatment options. Atezolizumab (atezo) is a humanized mAb that inhibits the binding of PD-L1 to PD-1 and B7.1, thus restoring tumor-specific T-cell immunity. Atezo was evaluated in an expansion cohort of mTNBC patients (pts) in a Phase Ia study (NCT01375842). Methods. Enrollment was initially limited to TNBC pts with PD-L1 on ≥5% of tumor infiltrating immune cells (IC2/3), then opened to pts regardless of PD-L1 status. Pts received atezo IV in 1L or 2L q3w at 15 or 20 mg/kg or 1200 mg for 1y with option to be retreated at PD, or until loss of clinical benefit. ORR was assessed by RECIST v1.1 and irRC, to capture non-conventional responses. Baseline PD-L1 expression on IC was centrally scored as IC0/1/2/3 (VENTANA SP142 assay). Pretreatment tumors and on-therapy biopsies were evaluated for TILs, CD8 T cells and macrophages by IHC. Results. As of Mar 31, 2016, 115 mTNBC pts were safety evaluable; atezo was generally well tolerated. There were no additional safety signals from prior report (Emens AACR 2015). 112 pts with FU ≥12 wk were evaluable for response. Based on irRC, ORR in 1L and 2L+ pts were 26% and 11%, respectively. ORR for PD-L1 IC2/3 pts were 17% vs 8% in IC0/1, mDoR was 21.1 mo (3 to 34+). mOS of responders (n=15) was not reached (4+ to 37+ mo) with no deaths as of data cutoff. mOS of non-responders who lived ≥6 wk (n=87) was 9 mo (1+ to 19+ mo). ORR rates in all pts at 1, 2, and 3 y for PD-L1 IC2/3 were 45%, 28% and 28%, respectively. Pts whose tumors had >10% TILs or ≥1.5% CDB in the tumor centered trended higher ORR and longer OS. Atezo increased intratumoral TILs, CD8, macrophages and IC PD-L1 expression, but no response association was observed. Conclusions. In mTNBC, atezo was well tolerated. Responders showed durable clinical benefit. Response rates were higher in 1L or PD-L1 IC2/3 pts. Baseline TILs and CD8 were associated with greater clinical benefit.

#### Table. Efficacy

<table>
<thead>
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<th>Treatment</th>
<th>Responses per RECIST v1.1 (irRC efficacy results presented in text)</th>
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<tbody>
<tr>
<td></td>
<td>PD-L1 IC2/3</td>
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<tr>
<td>PD-L1</td>
<td>All N=112</td>
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<td>1L n=19</td>
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<td>2L+ n=93</td>
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|   | 1L n=19 | 2L+ n=94 | PD-L1 IC2/3 | PD-L1 IC0/1 | All n=113 |
|   |         |          | All n=38   |            |          |
| 1y OS | 63%      | 37%      | 45%        | 37%       | 41%       |
| (95% CI) | (37, 89) | (26, 47) | (32, 58)  | (21, 53)  | (31, 51)  |
| 2y OS | 47%      | 18%      | 28%        | NE         | 22%       |
| (95% CI) | (14, 80) | (8, 27)  | (15, 41)  | NE         | (12, 32)  |
| 3y OS | NE        | 18%      | 28%        | NE         | 22%       |
| (95% CI) | (8, 27)  | (15, 41) | NE         | (12, 32)  |          |

CBR: clinical benefit rate, defined as CR, PR or SD for ≥12 weeks. DoR, duration of response; NE, not evaluable. +, censored.
#2987 Validation and clinical feasibility of a comprehensive genomic profiling assay to identify likely immunotherapy responders through tumor mutational burden (TMB). Daniel S. Lieber,1 Mark R. Kennedy,1 Douglas B. Johnson,2 Jonathan E. Rosenberg,2 Marcin Kowarz,2 Joel R. Greenbowe,1 Garrett M. Frampton,1 Caitlin F. Connelly,1 Alexis B. Schroch,1 Jeffrey S. Ross,1 Philip J. Stephens,2 Siraj M. Ali,1 Vincent A. Miller,1 David A. Favierdi,1 Foundation Medicine, Inc., Cambridge, MA; 2Vanderbilt University, Nashville, TN; 3Memorial Sloan Kettering Cancer Center, New York, MA; 4Genentech, Inc., South San Francisco, CA.

Background: Patients across a range of disease types have demonstrated robust and durable responses using checkpoint inhibitor therapies (CPTs). Given the limitations of immuno-histochemical based testing, identifying a unified, quantitative metric to predict clinical response to CPTs, tumor mutational burden (TMB) measures the number of somatic protein coding mutations per target sequence in a tumor specimen. This measure has been associated with response and survival for multiple CPTs across an array of indications. In this study we describe Foundation Medicine’s (FMI) work to develop and validate a TMB result as part of our comprehensive genomic profiling assays and summarize clinical feasibility in NSCLC, melanoma and bladder cancer.

Methods: We developed an analysis method to determine TMB based on data from our comprehensive genomic profiling assays. TMB is calculated by counting all synonymous and non-synonymous somatic variants across 315 or 405 genes, excluding germline alterations and known or likely driver alterations. The mutation count is normalized by the coding target territory to achieve a mutation density of mutations per megabase (mut/Mb). To determine accuracy, we compared TMB values from our comprehensive genomic profiling assay against a CLIA-validated whole-exome sequencing (WES) method on 29 patients. Precision was assessed over 10 clinical samples replicated 4-6 times. Lower limit of sample tumor purity was determined through dilutions of tumor/normal pairs from 80% to 3% tumor. Clinical feasibility was assessed by analyzing TMB versus immunotherapy-based survival in a cohort of 65 metastatic melanoma patients, 150 urothelial carcinoma patients and 463 NSCLC patients. Additionally, we examined the relationship between TMB and microsatellite instability status (MSI), an independent biomarker associated with response to CPTs. Results: Foundation Medicine’s TMB measure provides accurate and precise results across a range of tumor mutational burden values on samples with as little as 20% tumor purity. Using cohort specific thresholds, TMB was significantly associated with improved survival in CPTs in NSCLC, melanoma and bladder cancer. Using data from over 40,000 patient samples, we also show significant overlap between high TMB and high MSI samples and show that MSI-High specimens represent a subset of TMB-High specimens. Conclusions: We have developed and validated the tumor mutational burden (TMB) biomarker as part of our comprehensive cancer genomic profiling assays. Initial clinical feasibility results demonstrate that TMB can be used to predict the likely response to anti-PD-1/PD-L1 CPTs across a growing number of indications including NSCLC, melanoma and bladder cancer.

#2988 Immunogenomic analyses of tumor cells and microenvironment in patients with advanced melanoma before and after treatment with nivolumab. Timothy A. Chan,1 Nadeem Riaz,2 Jonathan J. Havel,1 Vladimir Makarov,1 Alexis Desrichard,1 Jennifer S. Sims,1 F. Stephen Hodi,2 Salvador Martin-Algarra,3 William H. Shafman,4 Shailender Bhatia,5 Wen-Jen Hwu,5 Thomas F. Gajewski,6 Craig L. Slingluff,8 Sviatoslav M. Kendall,1 Han Chang,9 Thomas F. Gajewski,7 Craig L. Slingluff,8 Sviatoslav M. Kendall,1 Han Chang,9 #2988 Immunogenomic analyses of tumor cells and microenvironment in patients with advanced melanoma before and after treatment with nivolumab. Timothy A. Chan,1 Nadeem Riaz,2 Jonathan J. Havel,1 Vladimir Makarov,1 Alexis Desrichard,1 Jennifer S. Sims,1 F. Stephen Hodi,2 Salvador Martin-Algarra,3 William H. Shafman,4 Shailender Bhatia,5 Wen-Jen Hwu,5 Thomas F. Gajewski,6 Craig L. Slingluff,8 Sviatoslav M. Kendall,1 Han Chang,9 Thomas F. Gajewski,7 Craig L. Slingluff,8 Sviatoslav M. Kendall,1 Han Chang,9

Introduction: Autologous anti-CD19 CAR T cells determined by single-cell multiplex proteomics associated with clinical activity in patients with advanced Hodgkin’s lymphoma. John Rossit,1 Patrick Pazkowski,1 Yueh-wei Shen,2 Kevin Morse,3 Brianna Puyatt4, Alaina Kaiser3, Colin Ng,5 Kyle Gallatin,5 Tom Cain,6 Yong Fan,6 Sean Mackay,7 James Heath,8 Steven A. Rosenberg,9 James N. Kochenderfer,10 Jing Zhou,1 Adrian Bot1,1 Kite Pharma, Santa Monica, CA;2IspIcics, Branford, CT;3Yale School of Engineering and Applied Science, Yale University, New Haven, CT;4Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA;5Surgery Branch, National Institutes of Health, Bethesda, MD;6Experimental Transplantation and Immunology Branch, National Institutes of Health, Bethesda, MD.

Introduction: Autologous anti-CD19 CAR T cells have shown promising clinical efficacy in B cell malignancies, with T cell expansion and blood levels for IL-15, IL-10 and Granzyme B as correlates of objective response and treatment, mutational and neoantigen load were comparable, regardless of previous treatment. Following nivo treatment, both mutational and neoantigen load were reduced 5-fold in pts who responded (CR/PR; n=9) and 1.2 fold in pts with stable disease (SD, n=13) compared with a 1.1-fold increase in pts with progressive disease (PD, n=19). Intratumoral heterogeneity analysis before and after nivo demonstrated a decrease in the evenness of T-cell clonotype distribution was observed among pts with CR/PR/SD relative to pts with PD in the ipi-N cohort (P=0.036), but not in the ipi-P cohort. Conclusion: Nivo and ipi modulate T-cell repertoire and tumor mutational heterogeneity in pts with advanced melanoma, presenting potential mechanisms of action underlying successful nivo therapy. These data also show that prior ipi treatment may influence biological response to nivo, but further investigation is warranted.


Immunogenic checkpoint protein including programmed death ligand 1, 2 (PD-L1, PD-L2) and B7-H3 play important roles for immune evasion in many cancer types. PD-L1 is an established therapeutic target in lung cancer and in a growing number of other indications. The clinical success of PD-L1 inhibitors has accelerated the development of drugs also targeting PD-L2 and B7-H3. In prostate cancer, studies on the prevalence and clinical relevance of these proteins have been inconclusive. Here, we performed immunohistochemical analysis of PD-L1, PD-L2 and B7-H3 in our large prostate cancer prognostic tissue microarray (TMA) containing tissue samples from more than 17,000 patients. Normal prostate glands were negative for PD-L1 and B7-H3, but positive for PD-L2. We found that 47% of 12,808 interpretable cancers showed B7-H3 expression, and 92% of 9,463 cancers showed PD-L2 expression, while expression of PD-L1 was virtually absent (0.2% of 17,392 tumors) in epithelial cells. Stroma cells and immune cells were entirely negative for PD-L1 and B7-H3, while some faint staining of lymphocytes was occasionally observed for PD-L2. In cancers, PD-L2 and B7-H3 staining had different prognostic impact, which depended on the ERG status: B7-H3 expression was linked to young patient age, advanced stage, high Gleason grade, lymph node metastasis (p<0.0001 each), presence of PTEN deletion (p<0.0001), and poor prognosis in ERG negative cancers (p<0.0001), while PD-L2 expression was linked to beneficial tumor features and prognosis exclusively in ERG negative tumors. Multivariate analysis including pT stage, Gleason grade, nodal stage, resection margin and preoperative PSA level, B7-H3 expression was an independent prognostic factor (p=0.0004). In summary, the results of our study identify a subset of young patients with ERG negative aggressive prostate cancers that might benefit from anti-B7-H3 therapy in the future. PD-L2 expression appears to protect tumors from progression and does not seem to be as suitable as a potential target in prostate cancer. PD-L1 expression is extremely rare in this disease, suggesting that only few prostate cancer patients might benefit from PD-L1 inhibitors.

#2990 Polyfunctional anti-CD19 CAR T cells determined by single-cell multiplex proteomics associated with clinical activity in patients with advanced Hodgkin’s lymphoma. John Rossit,1 Patrick Pazkowski,1 Yueh-wei Shen,2 Kevin Morse,3 Brianna Puyatt4, Alaina Kaiser3, Colin Ng,5 Kyle Gallatin,5 Tom Cain,6 Yong Fan,6 Sean Mackay,7 James Heath,8 Steven A. Rosenberg,9 James N. Kochenderfer,10 Jing Zhou,1 Adrian Bot1,1 Kite Pharma, Santa Monica, CA;2IspIcics, Branford, CT;3Yale School of Engineering and Applied Science, Yale University, New Haven, CT;4Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA;5Surgery Branch, National Institutes of Health, Bethesda, MD;6Experimental Transplantation and Immunology Branch, National Institutes of Health, Bethesda, MD.

Introduction: Autologous anti-CD19 CAR T cells have shown promising clinical efficacy in B cell malignancies, with T cell expansion and blood levels for IL-15, IL-10 and Granzyme B as correlates of objective response and...
toxicity (Kochenderfer et al. | Clin Oncol 2016: 34:LBA3010). It is unclear, however, which key immune programs in CAR T cells impact their in vivo expansion and clinical outcome. We evaluated in detail the functionality of anti-CD19 CAR T cells by using single-cell proteomics analysis (Lu et al. PNAS 2015:113:607-615). We explored how the polyfunctionality of pre-infusion cell products, post-infusion stimulation with the CD19 antigen in vitro, associated with CAR T cell expansion in vivo and objective response. Methods: Product T cells were separated into CD4+ or CD8+ T cell subsets using microbeads. CD4+ or CD8+ fractions were then co-cultured with CD19-K562 targets or NGFR-K562 control cells, at a 1:2 ratio for 20 hrs. Single cells were then analyzed using a 32-plex panel of secreted cytokines, chemokines, and cytotoxic molecules. Specifically, T cells were loaded onto a single-cell barcode chip capable of assaying 32 secreted proteins/cell. The polyfunctional profile and strength (pSI) of each sample was determined (Ma et al. Cancer Discov 2013;3:418-429) and analyzed relative to in vivo expansion of the CAR T cells and patient response to the CAR T cell therapy. CAR T cell expansion in blood was measured by quantitative PCR. Results: Single-cell pSI of patient CAR T cells showed a statistically significant association (p = 0.011) with objective response (complete or partial response) to the therapy. While product pSI showed variability across patients, the median pSI was 2+ times higher for responders vs non-responders. The polyfunctional profiles for both CD4+ and CD8+ cells were dominated by effector molecules, stimulatory cytokines and chemokines. Polyfunctional CD4+ and CD8+ T cells with IFNγ+IL-2+IL-17A+IL-17F+MIP-1β+ expression correlated best with patient outcome, with CD8+ T cells showing co-expression of Granzyme B, and CD4+ T cells also comprising IL-17A+IL-17F+ and IL-5+IL-17F+ subsets. While CAR expansion in vivo also correlated with objective response (p = 0.032), the association between product pSI and CAR cell expansion in vivo did not reach statistical significance (p = 0.079), suggesting that they bring independent contributions to predicting objective response. In support of that, a composite index integrating pSI and CAR T cell expansion in vivo associated best with clinical response (p = 0.005). Conclusion: Polyfunctionality of CAR T cells, in conjunction with their expansion in vivo and correlation with objective response data (IC50 and AUC) for 100 different drugs, including ‘best in class’ PI3K, PARP and CDK4/6 inhibitors, novel biological and chemical inhibitors of PIK3CA mutations. To target genomic alterations in HNSCC, we tested the efficacy of 7 PI3K/mTOR pathway inhibitors in 59 HNSCC cell lines and determined the association between drug sensitivity and molecular characteristics in order to identify biomarkers of response. Methods: We systematically analyzed the association between drug sensitivity and genomic alterations in 59 HNSCC lines. Results: NOTCH1 mut lines are significantly sensitive to PI3K/mTOR pathway inhibitors: GSK2126458 (13/16), BKM120 (14/16), BEZ235 (12/16), BAY806492 (14/16) and GDC0980 (13/16) lines. In contrast to PIK3CA mut cell lines, all 7 NOTCH1 mut lines tested underwent apoptosis (14.3 fold; P <0.005). After PI3K/mTOR inhibition, NOTCH1 mut lines showed significantly reduced clonogenic growth in vitro (0.4/0.9 fold in HNS1/PC115B; P<0.05) and significant tumor growth inhibition in vivo using orthotopic breast xenograft mouse models (2 fold in UMSCC22A and HNS1; P<0.01). To determine if NOTCH1 mediates resistance, we conditionally expressed cleaved NOTCH1 by Dox-inducible system in a NOTCH1 mut line (UMSCC22A). This rescued PI3K/mTOR inhibitor-induced apoptosis (0.5 fold; P<0.05) and reduced colony formation in vitro. As no canonical pathways account for the underlying mechanism of sensitivity, we measured the level of 301 proteins by reverse phase protein array (RPPA) in 3 NOTCH1 mut and 3 NOTCH1 WT lines after GSK2126458 treatment. Glutaminase and Glutamate Dehydrogenase were differentially expressed in NOTCH1 mut lines. Thus, we hypothesized that PI3K/mTOR inhibition in NOTCH1 mut lines induced reactive oxygen species (ROS)-mediated apoptosis via metabolic alterations. Consistent with this hypothesis, NOTCH1 mut lines exhibited increased ROS production; Metabolic pathway inhibitors targeting Glycolysis, Pentose Phosphate pathway and Glutaminolysis, in combination with GSK2126458 decreased cell viability in NOTCH1 mut lines. Conclusion: In contrast to PIK3CA mut cells, NOTCH1 mut HNSCC cells underwent apoptosis after PI3K/mTOR pathway inhibition in vitro and decreased tumor size in vivo. The ectopic activation of NOTCH1 rescued NOTCH1 mut HNSCC cells from PI3K/mTOR inhibitor-mediated apoptosis. The underlying mechanism may involve differential effects on tumor metabolism and ROS production. This work is significant because inactivating NOTCH1 mutations, which occur in 18% of HNSCC patients and SCCs of the lung, esophagus, and other sites, may serve as a biomarker for resistance. Our future work may uncover previously unknown crosstalk between the PI3K/mTOR and NOTCH pathways in SCCs.

**CLINICAL RESEARCH: Innate Immune Mechanisms in Cancer Treatment**

**#2993 STING signaling in breast tumor microenvironment modulates immune checkpoint blockade efficacy in the neu-N mouse model of breast cancer.** Jeremy B. Foote,1 Marlene Kok,1 James M. Leatherman,1 Todd D. Armstrong,2 Bridget Marcinkowski,1 David B. Kanne,1 Elizabeth M. Jaffee,1 Thomas W. Dubensky,2 Leisha A. Emens,1 Johns Hopkins University, School of Medicine, Baltimore, MD;2 Adaro BioTech, Berkley, CA.

Background: The presence of tumor infiltrating T cells (TILs) is associated with improved clinical outcomes in multiple tumor types and is also necessary for response to immune checkpoint blockade. While T cell responses to some tumors occur spontaneously, the majority of cancers are not naturally recognized by the immune system. The lack of response is attributed primarily to insufficient T cell infiltration into the tumor microenvironment (TME). Activating the STING pathway represents one strategy for increasing infiltration of T cells into the TME. This induces interferon-β (IFN-β) production, leading to dendritic cell (DC) activation and priming of tumor antigen specific CD8+ T cells that mediate tumor regression. The intratumoral injection (IT) of ML-RR-S2-CDA (ADU-100), a synthetic cyclic di-nucleotide STING agonist, has antitumor efficacy in several aggressive...
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mouse tumor models, including B16 melanoma, CT26 colon carcinoma, Panc02 pancreatic carcinoma, and 4T1 triple negative breast cancer. However, the impact of antigen-specific tolerance on tumor regression associated with STING-activation remains poorly understood. Therefore, we evaluated the efficacy of IT ADU-S100 in both non-tolerant parental FVB/N and the immune-tolerant neu/N transgenic mice bearing HER-2–specific B16 melanoma tumors. **Methods:** First we evaluated the impact of IT ADU-S100 on tumor regression, survival, innate sensing, and priming of HER-2 specific CD8+ T cells in both tumor-bearing non-tolerant FVB/N control and tolerant neu/N mice. Then we determined whether modulating the most highly expressed immune checkpoints on tumor infiltrating CD8+ T cells enhanced intratumoral STING expression in the tolerant neu/N mouse model of HER-2 breast cancer. Results: ADU S-100 induced HER-2-specific CD8+ T cell priming and durable tumor clearance in 100% of non-tolerant, FVB/N mice. In contrast, ADU S-100 failed to sufficiently prime HER-2-specific CD8+ T cells in tolerant neu/N mice, delaying tumor growth and clearing tumors in only 10% of mice. No differences in IFN-β production, DC priming, or HER-2-specific CD8+ T cell trafficking were detected between FVB/N and neu/N mice. However, activation and expansion of HER-2-specific CD8+ T cells was defective in neu/N mice. Immune cell infiltrates of untreated tumor-bearing neu/N mice expressed high levels of PD1 and OX40 on CD8+ T cells, and high levels of PD-L1 on both myeloid and tumor cells. Modulating PD-L1 and OX40 signaling combined with IT AADU S-100 enhanced HER-2-specific CD8+ T cell activity by over 40% of neu/N mice. Conclusions: Intratumoral STING activation synergizes with PD1 pathway-blockade and OX40 receptor stimulation to overcome immune tolerance and prime tumor antigen-specific CD8+ T cell responses that mediate effective tumor regression.

**#2996** Engineered STING-targeting immunotherapy delays B16 melanoma tumor growth and significantly improves animal survival as compared to an immune-checkpoint inhibitor. Andrea Amaltiano, Fadel S. Alyaouq, Vasser A. Alidhamen, Abdulraouf Abbas, Cristiane Pereira-Hicks, Sarah Godbehere, Christopher M. Waters. Michigan State University, East Lansing, MI.

Background: The stimulator of interferon genes (STING) is a cytosolic innate sensor activated by cyclic di-nucleotides (CDNs) including the bacterially derived bis-(3’-5’) cyclic-dimeric-guanosine monophosphate (c-di-GMP), leading to the induction of type I interferons. We recently published that an engineered adenovirus (Ad) vector delivering a potent, bacterially derived diguanylate cyclase (AdVCA0848) in vivo can generate sustained c-di-GMP levels in vivo and induce beneficial immune responses, including induction of IFN-β, activation of NK cells and DCs, as well as enhance antigen-specific T cell responses. Here, we determined if in vivo use of AdVCA0848 can also induce potent anti-tumor responses. Methods: Engineered AdVCA0848 was formulated into pellets and delivered to mice using a surgical needle through the tumor dermis. Animals were randomized to treatment with AdVCA0848 or saline controls. Tumor growth in mice treated with AdVCA0848 was compared to controls.

Results: AdVCA0848 significantly slowed B16 melanoma growth (p<0.01), a result that correlated with high levels of animal survival (p<0.001). These levels of efficacy significantly exceeded those achieved with alone use of a PD-1 antagonist. Under these same conditions, combined use of the AdVCA0848 therapy with anti-PD1 treatments trended to further improve animal survival (50% survival at day 27 PTI), as compared to alone use of either therapy (36.4% and 26.7% survival at day 27 PTI for AdVCA0848 and PD-1 treatments, respectively). Multiple innate and adaptive immune response readouts and immunohistochemical evaluation will be presented. Conclusions: The growth of established B16 melanoma tumors was rapidly inhibited after a single i.t. treatment with the engineered STING activator AdVCA0848, and this efficacy resulted in significant improvements in animal survival, at rates that were also significantly better than those achieved using a PD-1 antagonist. Furthermore, the i.t. administration of AdVCA0848 trended to show further improved efficacy when combined with a PD-1 antagonist. The growth of established B16 melanoma tumors was rapidly inhibited after a single i.t. treatment with the engineered STING activator AdVCA0848, and this efficacy resulted in significant improvements in animal survival, at rates that were also significantly better than those achieved using a PD-1 antagonist. Furthermore, the i.t. administration of AdVCA0848 trended to show further improved efficacy when combined with a PD-1 antagonist. Concluding, the growth of established B16 melanoma tumors was rapidly inhibited after a single i.t. treatment with the engineered STING activator AdVCA0848, and this efficacy resulted in significant improvements in animal survival, at rates that were also significantly better than those achieved using a PD-1 antagonist. Furthermore, the i.t. administration of AdVCA0848 trended to show further improved efficacy when combined with a PD-1 antagonist.


TLR8, which is an innate immune receptor that recognizes specific DNA motifs, has emerged as a promising cancer drug discovery field by engaging unprecedented revolution accompanied by growing excitement from researchers, drug developers, patients and investors, partly due to recent clinical success of cancer immunotherapy. Human immune defense system comprises both innate and adaptive immune pathways. All the targets drugged by the recently approved cancer immunotherapeutic agents including the immune checkpoint proteins PD-1, PD-L1, TLR8 agonists, etc. In contrast, targets involved in the innate immune pathway have not matured to regulatory approval for systemic use though several candidates are now in preclinical and clinical development. Drugs targeting innate immune represent great opportunity for more rapid and broader spectrum anti-cancer effect than adaptive immunity. Furthermore, combinations of drugs targeting innate and adaptive immunity are expected to produce strong synergistic efficacy owing to their complementary nature as body’s immune defense. Toll-like receptors (TLRs) are a family of proteins that recognize pathogen associated molecular patterns (PAMPs). Their primary function is to activate innate immune responses while also involved in facilitating adaptive immune responses. Different TLRs exert distinct functions by activating varied immune cascades. TLR8 is more broadly expressed among immune cells than other TLRs while the major causes of cancer immunotherapy failure is potent suppression of immune response by Treg cells. TLR8 is the only TLR that has been shown to be necessary and sufficient to reverse the suppressive function of Treg cells leading to strong tumor inhibition. Therefore, agonists targeting TLR8 are expected to be effective cancer therapy. However, there is no approved TLR8 selective agonist at present. There is only one TLR8 selective agonist in clinical development. Through structure-based drug design, we discovered a novel, highly potent and selective small molecule TLR8 agonist, DNS-A1. DNS-A1 exhibited strong in vitro cellular activity with EC50 at 4.23 nM, about 30-fold more potent than the only drug candidate in clinical trials. The activity was highly selective for TLR8 over other TLRs. DNS-A1 displayed superior in vitro ADMET and in vivo PK profiles. DNS-A1 showed clean CYP profile with IC50 over 10 µM for all major CYP isoenzymes tested including 3A4, 1A2, 2C9, 2C19 and 2D6. DNS-A1 had favorable hERG parameter with IC50 over 30 µM whereas the reference compound’s hERG IC50 was 3.84 µM suggesting potential cardiac toxicity. DNS-A1 significantly impeded tumor growth as a single agent and was well-tolerated in mouse tumor models. Taken together, DNS-A1 warrants further development as a potential best-in-class preclinical drug candidate for TLR8-mediated cancer immunotherapy.

**#2998** Immune checkpoint agonist VISTA suppresses Toll-like receptor signaling and the expression of inflammatory cytokines. Wenwen Xu,1 Yingxuan Li,2 Na Li,3 Yongwei Zheng,1 Kamal Rajasekaran,2 Halli Miller,1 Michael Olson,1 Deming Wang,1 Subramanian Malar Kannan,1 Li Wang,1 Medical College of Wisconsin, Milwaukee, WI;2 Shanghai University of Traditional Chinese Medicine, Shanghai, China.

Introduction: V-domain Ig suppressor of T-cell activation (VISTA, gene Vsir) is an inhibitory immune-checkpoint molecule that suppresses CD4+ and CD8+ T cell activation. Previous studies have shown that Vsir-/- mice developed chronic inflammatory phenotypes, and Vsir+/+ expression of inflammatory cytokines, and Vsir-/- mice showed CD4+ and CD8+ T cells were hyper-responsive towards self-and foreign antigens. Whether VISTA regulates innate immunity is still unknown. Methods: Peritoneal macrophages from WT or Vsir-/- mice were isolated and stimulated with TLR agonists such as CpG (TLR9), R848 (TLR7), LPS (TLR4), Pam3csk4 (TLR2), and poly (I:C) (TLR3). Alternatively, human monocyte THP-1 cells overexpressing VISTA were stimulated by TLR2 agonist Pam3csk4. The activation of TLR signaling pathways and the production of inflammatory cytokines were examined by Western Blotting, gel shift assay, or ELISA. The ubiquitination status of key signaling molecules such as TRAF6, IRAK1/4 and MyD88 were examined by immunoprecipitation and Western Blotting. To examine the role of VISTA in regulating TLR-mediated inflammatory responses in the context of cancer vaccine, tumor-bearing mice were treated with VISTA-specific monoclonal antibody (mAb) and a peptide vaccine containing TLR agonists. The production of inflammatory cytokines and chemokines within the tumor microenvironment (TME) was examined via quantitative RT-PCR. Results: Vsir-/- macrophages were hyper-responsive towards TLR2/4/7/9 agonists, but not TLR3 agonist, resulting in increased production of inflammatory cytokines IL-6, IL-12, and TNFα. Analysis of signaling cascade revealed that VISTA inhibited the activation of MyD88-dependent TLR signaling, via suppressing the activation of MAPKs and the activation of transcription factors AP-1 and NF-kB. Consistent
with the role of VISTA in regulating TLR-mediated innate immunity, treatment with VISTA-blocking mAb augmented levels of inflammatory cytokines and chemokines within the TME, and synergized with TLR/peptide vaccine result­ ing in an optimal therapeutic outcome. Conclusions: Our study establishes that VISTA critically regulates the inflammatory responses of myeloid cells mediated by TLR signaling. In the context of cancer vaccine therapy, VISTA-blocking mAb treatment enhanced levels of inflammatory cytokine and chemokines within the TME, which is critical for the development of optimal tumor-specific T cell responses and the tumor-controlling therapeutic outcome.

#2997 The tetravalent bispecific antibody AFM13 engages and primes innate immune cells for anti-cancer immunity. Jens Pahl,1 Joachim Koch,2 Uwe Reusch,3 Thorsten Gantke,2 Adelheid Cerwenka,1 Martin Treder2.

AFM13 is a tetravalent bispecific antibody with bivalent binding to both CD30 and CD16A. It has been shown to engage NK-cells through CD16A with high affinity and specificity, resulting in strong NK-cell cytotoxicity, and is currently being tested in Phase 2 monotherapy and in combination with pembrolizumab in Phase 1b clinical trials. We have previously shown that AFM13-dependent activation of NK-cell cytotoxicity towards CD30+ tumor cells is more pronounced than that of anti-CD30 mAbs. In addition, AFM13 enhances NK-cell sensitivity to low doses of IL-2 and IL-15, leading to an increased NK-cell proliferative potential. Here, we have extended the panel of phenotypic markers on NK-cells that are modulated after exposure to CD30+ tumor cells in the presence of AFM13. Targeting some of these markers may enable the development of novel combination therapies. Moreover, we have analyzed the kinetics of NK-cell responses to AFM13 exposure. Even though short-term exposure to AFM13 significantly enhanced NK-cell cytotoxicity, long-term exposure led to a partial, transient functionally exhausted phenotype in vitro, which could be fully restored by cytokine stimulation for several days in the absence of AFM13. Importantly, these recovered cells displayed high cytotoxicity towards CD30+ target cells in the presence of AFM13. Interestingly, the transient NK-cell exhaustion was not related to the expression of typical exhaustion markers or insufficient levels of perforin and granzyme. These data may warrant the development of novel metronomic application regimens of AFM13. Further studies imply that immune cells other than NK-cells are able to inhibit growth of CD30+ tumor cells in an AFM13-dependent manner. This appears to be strictly dependent on CD16A and a specific cytokine milieu. To achieve this, AFM13 specifically enhances the cytokine-producing potential of NK-cells, parameters that can be utilized to monitor NK-cell responses during AFM13 therapy. Moreover, based on our data, engagement of CD16A+ cells to the tumor site might enable several innate immune effector functions within the tumor microenvironment for synergistic anti-tumor activity.

#2998 Elotuzumab can costimulate NK cell responses by engaging SLAMF7. Tatiana Pazina,1 Ashley Mentlik James,1 Alexander W. MacFarlane,1 Natalie A. Bezman,2 Robert F. Graziano,3 Michael D. Robbins,2 Adam D. Cohen,3 Kerry S. Campbell1.

Elotuzumab (El) is an IgG1 monoclonal antibody targeting SLAMF7 (CS1, CRACC2, CD319) protein, which is highly and uniformly expressed on multiple myeloma cells. Improved survival has been observed in multiple myeloma patients treated with El in combination with immunomodulatory drugs such as dexamethasone and lenalidomide. Previous work showed that the main mechanism of El's action in vitro is CD16-mediated antibody dependent cellular cytotoxicity (ADCC) via Fc receptor interaction with CD16 (FcγRIIIa) receptor on NK cells. However, SLAMF7 is also expressed on NK cells where it transmits an activating signal. We hypothesized that El can directly activate NK cells via SLAMF7 ligation. Utilizing non-fucosylated El that has higher affinity to CD16 and El mutant that has an Fc region mutation that abrogates binding to CD16, we confirmed that El promotes NK cell activation and degranulation in a CD16-dependent manner. Specifically, non-fucosylated El had higher potency, whereas El mutant did not stimulate degranulation or CD69 expression. To test for co-stimulatory effects of El and El mutant we utilized trandaminoacidic signaling to potentiate calcium signals generated through NKP46 and NKGD2 in a CD16-independent manner. Our results provide evidence that direct engagement with SLAMF7 by El has the potential to reduce the threshold of NK cell activation triggered through other activating receptors.

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#2999 NKG2A checkpoint receptor expression on tumor-infiltrating CD8+ T cells restrains efficacy of immunotherapy. Thorbad Hall,1 Nadine van Montfoort,1 Syte Pietsma,1 Marjolein Sluijter,1 Marij J. Welters,1 Pascale André,2 Nicolai Wagtmann,2 Sjoerd H. van der Burg1.

The tetravalent bispecific antibody CD94-NKG2A is expressed on subsets of NK cells and cytotoxic CD8+ T cells, but not CD4+ T helper cells. It’s ligand is the non-classical MHC class I molecule HLA-E, commonly over-expressed in human cancers. We previously demonstrated that high HLA-E expression in ovarian carcinoma and NSCLC may neutralize the survival benefit of T cell infiltration, suggesting that NKG2A could represent an immune checkpoint that tumors may exploit for immune evasion. We thus examined the expression of NKG2A and other checkpoint receptors on human and mouse Tumor Infiltrating Lymphocyte (TIL) populations and tested blockade of this receptor in mouse tumor models. Multicolor flow cytometry analysis showed higher frequency of NKG2A+ CD8+ T cells in TIL samples obtained from biopsies of human head- and neck- carcinomas (n=17) compared to peripheral blood CD8+ T cells. Frequency of NKG2A+ TIL was higher in patients with measurable immune reactivity to HPV16-viral antigens than in patients without tumor immunity (14.8% vs 4.7%, respectively). Interestingly, only subsets of the NKG2A+ T cells co-expressed other inhibitory receptors, including PD-1 and TIM3, suggesting the existence of a ‘dedicated’ T cell subset for NKG2A. In our mouse tumor models we observed similar findings and, moreover, demonstrated that therapeutic vaccines comprising tumor antigens increased the frequency of NKG2A+ positive CD8+ TIL up to 80%. Similarly, exposure of Qa-1, the mouse homologue of HLA-E, on tumor cells also increased after therapeutic vaccination. As a proof of concept that the NKG2A-Qa-1 axis limits the efficacy of immunotherapy, NKG2A blocking antibodies were applied in the TC-1 model together with therapeutic peptide vaccination. Combination treatment with vaccine and NKG2A blockade significantly improved the therapeutic efficacy compared to the vaccine alone, both in terms of survival (P<0.05, median survival 44 vs 31 days) as well as overall response rate, defined as decreased tumor size of more than 30% (93.8% vs 43.8%, respectively). Similar results were obtained in Qa-1-deficient mice, suggesting that Qa-1 on tumor cells is of importance. In conclusion, inhibitory receptor NKG2A is enriched on CD8+ TIL and functions as an immune checkpoint that restrains therapeutic efficacy of a cancer vaccine.

CLINICAL RESEARCH: Predictors and Drivers of Pediatric Tumors


We recently showed that relapsed neuroblastoma (NB) tumors harbor an increased somatic mutational burden, enriched for ALK or RAS-MAPK activating lesions, nominating targeted therapeutic strategies for a condition that is largely incurable. We also showed that these mutations can be present in subclonal tumor cell populations at diagnosis, suggesting that they may contribute to acquired chemoradiotherapy resistance. We hypothesize that subclonal mutations in NB oncogenes are common at diagnosis, are biomarkers for treatment failure, and can be targeted in conjunction with standard chemotherapy to improve patient survival. Here, we focus on the first aspect of this broad hypothesis by presenting the subclonal landscape of diagnostic, high-risk neuroblastoma. A meta-analysis of our previously published primary/release (N=23), whole exome (N=322) and whole genome (N=159) diagnostic data, and recent sequencing study of 78 diagnostic and 67 relapse cases were used to inform design of a custom amplicon panel for ultra-deep sequencing. The 28-gene panel covers 57 mutations in frequently mutated genes. Here, 230 high-risk primary tumors were sequenced to an average depth of 3000X. More subclonal mutations were called (mean=36) compared to clonal alterations (mean=3), demonstrating the complex subclonal architecture in these tumors. We validated five clonal ALK mutations we originally discovered and identified 15 additional pathogenic ALK mutations with mutant allele frequencies (MAFs) ranging from 0.03-23%. Known ALK activating mutations were found at codons 1170, 1174, 1196, 1245, 1275 in 15 of 38 diagnostic primary tumors. This 39.5% frequency is much higher than the 14% frequency defined by us using Sanger sequencing or other sequencing methods. Improvement of subclonal mutation detection of actionable, relapse-specific driver mutations demonstrates the clinical utility of utilizing this sequencing methodology at diagnosis to enable timely improvement of outcomes for children with high-risk refractory NB.
**#3001  Germine mutations in cancer predisposition genes and risk for subsequent neoplasms among long-term survivors of childhood cancer in the St. Jude Lifetime Cohort.** Zhaoming Wang, 1 Carmen L. Wilson, 1 John Easton, 1 Dale Hedges, 1 Qi Liu, 1 Gang Wu, 1 Michael Rusch, 1 Michael Edmonson, 1 Shawn Levy, 1 Jennifer Q. Luncot, 1 Eric Caron, 1 Kyla Shelson, 1 Kelsey Currie, 1 Matthew Lead, 1 Heather L. Mulder, 1 Donald Yergeau, 1 Celeste Rosenzweig, 1 Bhavin Vadodaria, 1 Yadav Sapkota, 1 Russell I. Brooke, 1 Wonjong Moon, 1 Evandine Rampersaud, 1 Xiaotao Ma, 1 Shuqiang Wang, 1 Ti-Cheng Chang, 1 Stephen Rice, 1 Andrew Thrasher, 1 Aman Patel, 1 Cynthia Pepper, 1 Xin Zhou, 1 Xiang Chen, 1 Wenan Chen, 1 Angela Jones, 1 Braden Boone, 2 Deo Kumar Srivastava, 2 Chimene A. Kesserwan, 3 Kim E. Nicholas, 3 James R. Downing, 3 Melissa M. Hudson, 3 Yu-taka Yamaoka, 3 Melissa Estes, 3 Jingming Wang, 3 St. Jude Children’s Research Hospital, Memphis, TN; 2University of Alberta, Edmonton, Alberta, Canada; 3HudsonAlpha, Huntsville, AL.

Childhood cancer survivors are at increased risk of subsequent neoplasms (SN), largely considered to be therapy-related. Studies of cancer predisposition genes (CPGs) and risk of SN among long-term survivors are lacking. We characterized germline mutations in CPGs in childhood cancer survivors to determine their contribution to SN risk. Whole genome (30x) and exome (100x) sequencing was performed for 29885 year survivors of childhood cancer (1629 leukemia/lymphoma, 332 CNS, 1027 other solid tumors, 53% male, median follow-up 28 years (range 6-55 years). Survivors underwent a comprehensive clinical assessment, treatment exposures were abstracted from medical records, and SN were validated by pathology review. We identified 63 CPGs that were classified using a library of Medical Genetics and Genomics guidelines as previously described (Zheng et al. NEJM 2015). Logistic regression, adjusting for age, sex and race, was used to evaluate associations between mutation status, cancer therapy and the SN risk. 1062 SNs were diagnosed in 437 survivors, of whom 98 developed ≥2 histologically distinct SNs. Median age at SN and time to first SN was 38.2 (range 3.3-67.4) and 29.2 (9.48.4) years, respectively. Common SNs were basal cell carcinoma (542 in 153 survivors), meningioma (201 in 100, thyroid (64 in 64), and breast cancer (58 in 50). Cumulative incidence of SN at age 45 was 25.5% (95% CI: 22.9-27.9). 169 survivors (5.7%) had a pathogenic/likely pathogenic (P/LP) mutation in a CPG, consisting of 97 single nucleotide variations, 63 insertion/deletions and 9 copy number alterations (49% of mutations not in ClinVar). Frequently mutated genes were: RB1 (n=41), NFI (n=22), BRCA2 (n=13), BRCA1 (n=12) and TP53 (n=10). Our data confirmed known associations between CPG mutations and specific primary diagnoses including RB1 mutations in 32 of 41 (78%) of bilateral and 7 of 57 (12%) of unilateral retinoblastoma survivors, 22 NF1 (20 of 332 CNS survivors), 4 SUFU (all in medulloblastoma survivors) and 5 WT1 mutations (all in Wilms’ tumor survivors). Analyses revealed novel associations between CPG mutations and SN risk. Among 1326 survivors not exposed to radiation therapy (non-RT), 62 SNs developed in 54 survivors, of which 15 (24.2%) occurred in P/LP mutation carriers. Non-RT exposed survivors with a P/LP mutation had an increased risk of SN (OR=5.6, 95% CI=2.6-12.0, P<0.001) and the odds of developing ≥2 distinct histologic types of SNs was increased by 23.6-fold (95% CI=5.4-102.7, P<0.001). In 1662 RT exposed survivors, P/LP-mutation carriers had an odds ratio of 2.3 (95% CI=0.9-6.0, P=0.08) for developing ≥2 distinct histologic types of SNs. Our findings support the hypothesis that a substantial proportion of non-RT exposed childhood cancer survivors who develop one or more SN carry a CPG mutation, and should be referred to genetic testing and counseling services.

**#3003 Exome analysis of known hereditary cancer genes in 122 children with rhabdomyosarcoma.** Talia Wegman-Ostrosky, 1 Rajesh Patidar, 1 Sivasish Sindiri, 1 Jack Sherrn, 1 Douglas S. Hawkins, 1 Daniel Catchpole, 2 Jun S. Wei, 1 Stephen Kacep, 1 Javed Khan, 1 Douglas R. Stewart, 1 National Cancer Institute, Bethesda, MD; 2Seattle Children’s, Seattle, WA; 3University Of Sydney, Sydney, Australia; 4UT Southwestern Department of Pediatrics, Dallas, TX.

Introduction: Rhabdomyosarcoma (RMS) accounts for 5% of all pediatric cancer and is the most prevalent soft tissue tumor in childhood and adolescents. RMS is thought to arise from primitive mesenchymal stem cells directed towards myogenesis. Between 7-33% of RMS cases arise from a hereditary cancer syndrome, like Li-Fraumeni or neurofibromatosis. We analyzed germline genetic variants in hereditary cancer genes in 122 children with RMS. Methodology: In 122 children with RMS and 1001 cancer-free adults, we examined germline exome data to determine the frequency of genetic variants in 51 cancer genes known to underlie syndromes associated with RMS. DNA was extracted from blood or buccal cells using standard methods. Exome enrichment was performed with NimbleGen SeqCap EZ Human Exome Library v3.0 and sequencing was done on Illumina HiSeq. Annotation of each exome variant was performed using a custom software pipeline. We evaluated all variants that passed quality controls with a population minor allele frequency (MAF) <0.1%. The cataloging of the variants was based on the ACMG classification as pathogenic (P), likely pathogenic (LP), or variable of unknown significance. Results: We compared the age, gender, histologic type and localization of the primary RMS of the patients with and without P/LP variants in the 51 genes. In the patients without P/LP variants, the mean age of diagnosis was 5 years and the most frequent site of diagnosis was head and neck. In the group with P/LP variants, the mean age of diagnosis was 10 years, and the most frequent site was pelvis. In the 51 genes that we analyzed we found 9 P and 12 LP variants in 15 genes: TP53, ATM, SMH6, PMS, DICER1, FANCA, RECQ4, PTEN, WRN, RB1, BUB1B, RET, APC, FANCM and TSC2, genes with 2 variants included: WRN, PTEN, BUB1B, FANCA and RET. Most of the variations were stopgain, follow by missense, frameshift insertion and splicing genetic variations. Ten of this genes are associated with an autosomal dominant pattern of inheritance. In one 15-year-old female patient with an alveolar RMS in the paraspinal area we found P/LP variants in PTEN and PM52. The frequency of P/LP variants in cases was 16% and 3% in controls. Conclusions: To our knowledge, this is the first study where multiple germline variation where analyses in children with RMS. We found P/LP variation in 16% of the cases (pendant orthogonal confirmation); the mean age of diagnosis was 10 years old and their primary tumor site was in the pelvis (50%). Identification of P/LP variation in genes underlying RMS-associated syndromes has implications for follow-up, screening and management of these patients and their families. We acknowledge the Children’s Oncology Group in helping to assemble the RMS cohort.

**#3004 Comparison of somatic alterations in the genome and transcriptome of 1,705 pediatric leukemia and solid tumors: a report from the Children’s Oncology Group (COG) - NCI TARGET Project.** Xiaotu Ma, 1 Yu Liu, 1 Yanling Liu, 1 Michael Edmonson, 1 Charles Gawai, 1 Xin Zhou, 1 Yongjin Li, 1 Michael Rusch, 1 John Easton, 1 Mark Wilkinson, 1 Leandro C. Hermida, 2 Sean Davis, 1 Malcolm Smith, 1 Jaime Guiray Avuli, 1 Paul Meltzer, 2 Ching C. Lau, 1 Elizabeth Perlman, 1 John M. Maris, 2 Soheil Meshinchi, 1 Stephen P. Hunger, 1 Daniela S. Gerhard, 1 Jinghui Zhang, 1 St Jude Children’s Research Hospital, Memphis, TN; 2National Cancer Institute, NIH, Bethesda, MD; 3Texas Children’s Cancer and Hematology Centers, Houston, TX; 4Ann and Robert H. Lurie Children’s Hospital of Chicago, Chicago, IL; 5Children’s Hospital of Philadelphia, Philadelphia, PA; 6Fred Hutchinson Cancer Research Center, Seattle, WA.

To document common and subtype specific somatic alterations affecting key biological processes in pediatric cancers, we analyzed point mutations, copy number alterations, gene fusions and structural alterations detected from paired tumor-normal whole genome sequencing (n=655), whole exome sequencing (n=1,108), and RNA-seq data (n=913) of 1,705 leukemia and solid tumors. Our cohort consists of 693 B-lineage Acute Lymphoblastic Leukemia (B-ALL), 264 T-ALL, 211 Acute Myeloid Leukemia (AML), 318 Neuroblastoma (NBL), 128 Wilms Tumor (WT), and 91 Osteosarcoma (OS) with a median mutation rate of 0.28-0.58 per Mb. We identified 130 potential driver genes based on significance.
of variant recurrence and pathogenicity within each cancer type and across all cancer types. Seventy-two (55%) driver genes were significant in one cancer type, thirty-eight were significant in > 1 leukemia subtype, thirteen (NRAS, WT1, MYCN, PTEN, TP53, Kras, Rb1, ATRX, PTPN11, MLLT1, Bcor, SETD2, NFI) were significant in both leukemia and solid tumor while the remaining, 26, were specific to single-cell source and successfully validated in significant only in pan-cancer analysis. The number of mutated driver genes per sample ranged from 0.8 in WT to 5.8 in T-ALL, lower when considering only point mutations (from 0.3 in NBL to 3.1 in T-ALL). The most frequently mutated biological processes affecting both leukemia and solid tumor were transcription factors (56% of samples), cell cycle (41%), epigenetic regulators (36%), Ras signaling (34%), NF-kB (11%), and the NOTCH complex (7%). The MYC signaling pathway was mutated only in leukemia (16%) while mutations in the NOTCH signaling pathway were exclusive to T-ALL (77%). Aberrant transcription may also affect the normal function of a driver gene. For example, the RAS signaling pathway was mutated in B-ALL (35%), T-ALL (15%), AML (37%) and NBL (4.3%). Aside from the known NRAS 4a isoform found in all cancer types, we discovered two novel KRAS isoforms present in 71.1% of B-ALL, 67.9% of T-ALL, 71.3% of AML and 3.0% of NBL but not in WT or OS. Allele-specific expression (ASE) was detected in 205 (6.8%) of 3,016 expressed somatic mutations, and 97% (32 out of 33) of truncation mutations on autosomes exhibit reduced expression of the mutant allele likely due to nonsense mediated decay. Two ASE mutations, WT1 Δ447N in a cytogenetically normal AML and JAK2 D674E in AML, were selected for single-enforced single-cell sequencing and validated. Only 44% of our driver genes match those identified in adult cancer. This, coupled with our finding that point mutations only accounted for 48% of the driver alterations, may provide new insight into the design of precision treatment for pediatric cancer. Our presented data will be made public at NCIT’s Genome Data Commons (gdac.cancer.gov) and can be explored on our Protein-Paint data portal (pecan.stjude.org).

## 3005 Whole-genome sequencing identified novel non-coding mutations causing of oncogene activation in T-cell acute lymphoblastic leukemia


Through growing evidence that non-coding sequences in human genome often function as transcriptional regulatory elements of protein-coding genes. In fact, germline polymorphisms and somatically acquired mutations within regulatory DNA can profoundly alter chromatin structure and modify gene transcription, directly contributing to tumorigenesis. However, there is a paucity of unbiased genome-wide characterization of somatic non-coding mutations in cancer. Using T-cell acute lymphoblastic leukemia (T-ALL) as a model disease, we herein report a systematic interrogation of driver non-coding genomic alterations by paired whole-genome and transcriptome sequencing of 31 children with T-ALL. To identify non-coding mutations with potential regulatory impact in a genome-wide fashion, our analytical pipeline consisted of 3 approaches: 1) the "hotspot analysis" for recurring mutations at the nearby positions, 2) the "regional recurrence analysis" for predefined regulatory regions with significant enrichment of non-coding mutations, 3) the "transcriptional factor analysis" for mutations that potentially result in gain/loss of transcription factor binding sites and alter expression of adjacent genes. Remarkably, T-ALL oncogenes LMO1 and TAL1 emerged as loci with the most significant recurring non-coding mutations. At the LMO1 locus, 3 patients (9.7%) showed an identical single-nucleotide mutation proximal to the transcription start site of the long isofrom of LMO1. This recurring mutation resulted in the gain of a canonical Myb binding site (AACGG) and ~120-fold increase in LMO1 transcription compared to patients with wildtype genotype. TAL1 overexpression was observed in 15 patients, of whom 11 had intrachromosomal rearrangement (STIL-TAL1 fusion). The remaining patient had a candidate rearrangement involving the super enhancer, consistent with recent reports. LMO1 enhancer mutation was further confirmed in an independent validation cohort (N = 26), in which we additionally identified a novel intrachromosomal rearrangement between MED17 and LMO1 resulting in transcriptional activation of the latter. In a panel of T-ALL cell lines, LMO1 enhancer mutation was again associated with dramatic overexpression of LMO1, an active enhancer histone mark (H3K27ac), increased myc expression, and increased transcription. Notably, we also observed robust binding of TAL1, CREBBP, RUNX1, ETS1, ELF1 and RNA Polymerase II at this site. Reporter gene assay confirmed the MYB-mediated transcription activation effects of this LMO1 enhancer mutation in vitro. In this genome-wide investigation of non-coding mutations in T-ALL, we identified novel enhancer mutations with drastic effects on oncogene activation. Our findings expand the understanding of how genomic alterations in regulatory DNA contribute to cancer pathogenesis.

## 3006 Oncogenic activity of H3.3 K27M in a spontaneous DIPG mouse model


Pediatric diffuse high-grade gliomas (HGG) cause a devastatingly poor outcome. Diffuse intrinsic pontine gliomas (DIPG) are brainstem HGGs found almost exclusively in children and represent approximately 25% of all pediatric HGGs. Recurrent, clonal somatic mutations in histone H3 are a molecular hallmark that distinguish the genetic mechanisms underlying pediatric and adult HGG, and indicate a strong link between epigenetic dysfunction and pediatric brain tumorigenesis. H3 K27M mutations found in nearly 80 percent of DIPGs and over half of non-brainstem HGGs occurring in thalamus or other midline structures, induce a dominant loss of genome-wide H3K27me3. In contrast, mutually exclusive H3.3 G434R/V mutations occur in approximately 14 percent of cortical HGG tumors in older children through young adulthood suggesting a distinct developmental origin compared to K27M mutant tumors. DNA methylation and gene expression patterns distinguish G34R/V and K27M tumors, but the oncogenic mechanisms and reasons for brain region selectivity of these mutations remain uncertain. To study these mutations in the developing mammalian brain and investigate how they contribute to oncogenesis, we generated conditionally activated, epitope-tagged knock-in mice to express K27M, G434R or non-mutated H3.3 proteins from the endogenous H3F3a locus. Untransformed embryonic neural precursor or astrocyte cells expressing H3.3 K27M demonstrated H3K27me3 loss and a growth advantage in vitro with brain region–specific, stage-dependent expression and epigenetic signatures. DIPGs frequently harbor genetic alterations in TP53 and PDGFRα in addition to H3.3 K27M, and mice with induced brain-specific Trp53 deletion plus mutated PDGFRαa344Rme expression developed spontaneous HGG in vivo. Induction of H3.3 K27M significantly accelerated PDGFRαa344Rme;Trp53 knockout HGG formation involving the brainstem. Importantly, the non-mutated H3.3 epitone-tagged protein did not affect tumor burden or latency. The H3.3 K27M tumors recapitulate characteristics observed in human DIPG including histopathological features, strong nuclear K27M expression and robust loss of H3K27me3 throughout the tumors. We relate the genetic, epigenetic and transcriptional landscapes of these models to primary pediatric DIPG to help identify their developmental origins and oncogenic mechanisms downstream of histone H3.3 K27M.

## EPIDEMIOLOGY: Cancer Epidemiology and Prevention

### 3007 Tobacco smoking, alcohol use and risk of hepatocellular carcinoma and intrahepatic cholangiocarcinoma: The Liver Cancer Pooling Project

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Background: Since 1980, liver cancer has been among the most rapidly increasing cancer types in the United States (US), with 5-year survival rates of approximately 17%. While tobacco and alcohol are known to be associated with primary liver cancer, it is unclear whether they only increase the risk of hepatocellular carcinoma (HCC), the most common type of liver cancer, or whether they also increase risk of intrahepatic cholangiocarcinoma (ICC), second most common histologic type. Additionally, it is unclear what...
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amount of alcohol consumption is associated with an increased risk of liver cancer. As liver cancer is a rare cancer type, we conducted a study of pooled data from the National Cancer Institute Cohort Consortium to examine the associations between smoking and alcohol use and liver cancer, stratified by histologic subtype. Methods: In the Liver Cancer Pooling Project, a consortiun of 21 epidemiologic studies, 518,740 participants from 14 cohorts, (HCC n=1,423, ICC n=410) in 14 cohorts. Multivariable-adjusted hazard ratios (HRs) and 95% confidence intervals (CI) were estimated using proportional hazards regression. Cubic splines were used to model the association between alcohol and liver cancer risk. Results: Compared to never smokers, both former and current smokers at study baseline had an increased risk of HCC (HR = 1.32, 95% CI: 1.03-1.68 and HR = 1.47, 95% CI: 1.07-2.02, respectively) and HCC (HR = 1.24, 95% CI: 1.08-1.43 and HR = 1.86, 95% CI: 1.57-2.20, respectively). This finding was consistent for heavier smoking intensity, longer duration of smoking, and more pack-years of smoking. High alcohol consumption was associated with an 87% increased risk of HCC (HR = 1.87, 95% CI: 1.41-2.47) and a non-significant increased risk of ICC (HR = 1.86, 95% CI: 0.99-2.86). Risk of HCC significantly increased at 4.5 alcoholic drinks per day, while risk of ICC was non-significantly increased with any amount of consumption. Conclusions: These findings suggest that, in a US population, cigarette smoking is associated with an increased risk of both histologic subtypes of primary liver cancer - HCC and ICC. In contrast, alcohol consumption was primarily associated with an increased risk of ICC. These results suggest that smoking cessation and alcohol reduction programs could be important intervention opportunities for these lethal cancer types.

#3008 Obesity, physical activity, and breast cancer survival among older breast cancer survivors in the CPS-II Nutrition Cohort. Maret L. Malinisk, Alpa V. Patel, Marjorie L. McCullough, Peter T. Campbell, Corinne R. Leach, Susan M. Capistrat, Mia M. Gaudet, American Cancer Society, Atlanta, GA

Background: Body mass index (BMI) and physical activity and their relation to breast cancer-specific survival have not been well-studied in older breast cancer survivors. The Nutrition Cohort, 4,927 women diagnosed with breast cancer between 1992 and 2000 was stratified into pre- and post-diagnosis BMI and recreational physical activity with breast cancer-specific and all-cause mortality. Objective: To examine the associations of pre- and post-diagnosis BMI and recreational physical activity with breast cancer-specific and all-cause mortality among women with non-metastatic breast cancer, overall and stratified by age 65 years at diagnosis. Methods: In the Cancer Prevention Study-II Nutrition Cohort, 4,927 women diagnosed with breast cancer between 1992 and 2000 completed biennial surveys and were followed for cause-specific mortality. Pre-diagnosis exposures were assessed at the questionnaire completed ≥6 months before diagnosis, and post-diagnosis exposures were assessed at the first questionnaire completed ≥2 years after diagnosis to avoid capturing short-term changes due to treatment. BMI was computed as weight (kg) divided by height squared (m²). Physical activity was based on the summary MET-hours/week of recreational activity. Multivariable Cox proportional hazards models were used to calculate hazard ratios (HR) and 95% confidence intervals (CI). Results: Over a mean follow-up time of 9.8 years after diagnosis, 1,394 deaths, including 452 from breast cancer, occurred. The median age at diagnosis was 70 years (77% ≥65 years). Compared with normal weight (BMI 18.5-25 kg/m²), class II-III obesity (BMI ≥35 kg/m²) was associated with higher risk of breast cancer-specific mortality in pre-diagnosis (HR: 2.2; 95% CI: 1.5-3.2) and post-diagnosis analyses (HR: 1.6; 95% CI: 1.0, 2.5), although the association was limited to women ≥65 years of age at diagnosis. Pre-diagnosis but not post-diagnosis BMI was positively associated with all-cause mortality among both age groups (P-trend <0.0001). Pre-diagnosis physical activity was not associated with breast cancer-specific mortality in pre-diagnosis analyses, but differences were observed in post-diagnosis analyses (HR: 0.7; 95% CI: 0.3-1.5). However, no differences were found in analyses of all-cause mortality. Significant inverse trend for post-diagnosis physical activity and breast cancer-specific mortality overall (P-trend=0.03) and among women ≤65 years of age at diagnosis (P-trend=0.01) but not among women ≥65 years of age at diagnosis (P-trend=0.21). Pre- and post-diagnosis physical activity were significantly associated with reduced all-cause mortality in both age strata. Conclusions: Our results suggest that pre- and post-diagnosis obesity and physical activity is associated with increased risk of death from breast cancer in older, but not younger, breast cancer survivors. Conversely, physical activity may reduce risk of breast cancer death in survivors ≥65 years of age at diagnosis. However, both were associated with overall longevity, regardless of age.

#3009 Lipid-lowering drug use and risk of fatal prostate cancer in the Atherosclerosis Risk in Communities (ARIC) Study. Alison M. Mondul, Corinne E. Joshu, John Barber, Anna Prizment, Nrupen Bahvsar, Josef Coresh, Elizabeth Selvin, Aaron Folsom, Elizabeth A. Platz, University of Michigan, Ann Arbor, MI; Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; University of Minnesota, Minneapolis, MN; Duke University, Durham, NC.

Background: Studies show that lipid-lowering medications, particularly statins, may be protective for more aggressive prostate cancer. Lethal prostate cancer is often considered an optimal outcome in epidemiologic studies when the aim is to identify modifiable risk factors with the potential to reduce prostate cancer incidence. Despite long-term survival being an under-studied. Further, lipid drug use and prostate cancer risk is understudied in black men, whose risk of fatal prostate cancer is 2-3 times higher than that of white men. Thus, we studied lipid medication use and fatal prostate cancer risk in the ARIC Study, which includes ~25% black participants. Methods: The ARIC Study is a prospective cohort that included 15,792 participants (7,082 men) recruited from four US communities. We conducted a prospective cohort analysis of 6,547 men who attended visit 2 (1990-1992, the start of the study era) and were followed for cancer death through 12/31/2012. Death from prostate cancer (n=90) as the underlying cause was obtained from death certificates supplemented with medical records. Updated information on medication use was collected throughout the study follow-up. Proton pump inhibitors and statins use were modeled as a time-dependent variable in two ways: 1) current use (yes/no), and 2) duration of use (<10, ≥10 years). Cox proportional hazards regression was used to estimate the hazard ratio (HR) and 95% confidence interval (CI) of prostate cancer death overall and by race. All models were further adjusted for age, race, and study center. Additional models were further adjusted for height, BMI, smoking, diabetes, and education. Results: The prevalence of lipid medication use was 17% by visit 4, partway into the statin drug era, and 76% of those medications were statins. After adjustment for age, race, and study center, men who used lipid medications were statistically significantly less likely to die from prostate cancer than men who did not use lipid medications (HR=0.56, 95% CI:0.33-0.95). This finding was slightly attenuated after further multivariable adjustment (HR=0.62, 0.36-1.08). The statistically significant inverse association appeared to be restricted to men who used lipid medications for ≥10 years (vs. never use: HR=0.39, 95% CI:0.19-0.82, p-trend=0.02). Lipid medication use was inversely associated with a reduced risk of prostate cancer death in both white and black men. Conclusions: Use of lipid medications was associated with a lower risk of fatal prostate cancer in both black and white men. Whether the fact that black men are less likely to receive/take lipid medications could partly explain the black-white disparity in prostate cancer in the US requires further study. Support: NHLBI contracts, NCI grant, NPCR


Background: Evidence shows that the vitamin K-antagonist warfarin, a popular anti-coagulant in clinical use for decades, has anti-tumor activity. This is recently attributed to disruption of post-translational modification of G6s, the common ligand of the Axl receptor tyrosine kinase family. G6s-Axl signalling is associated with malignancy and is required for tumorigenesis and progression in several preclinical cancer models. Warfarin is shown to inhibit Axl signaling-dependent malignant traits and enhance anti-tumor immune responses at doses that do not achieve anticoagulation. The objective of this study is to investigate the association between warfarin use and cancer incidence in a large unselected Norwegian cohort. Our results reveal a remarkable reduction in cancer incidence associated with warfarin use across a wide range of tumor types. METHODS: A cohort selected from the Norwegian population registry in 1994, living in a Norwegian county (n=256,725). We cross-referenced this cohort using the unique Norwegian National ID Number to: 1) the Cancer Registry of Norway and retrieved information on all cancer cases 2006-2012; 2) information on filled warfarin prescriptions (ATC: B01AA03) from the Norwegian Prescriptions Database (2004-2012). Warfarin-use was defined as ≥6 months and minimum 2 years between first warfarin prescription and cancer diagnosis. We also performed a subgroup analysis on persons prescribed warfarin for atrial fibrillation/flutter (n=33 313) compared to non-users. Mantel-Haenszel method was used to calculate the incidence rate ratio (IRR), adjusting for sex and age. RESULTS: In this cohort, 92 942 persons were classified as warfarin users, and we observed 132 687 cancer cases in the
7-year study period. We observed a significantly lower sex and age-adjusted risk for cancer development across all malignancy types in the warfarin user group compared to the non-user group (IRR: 0.842, 95% CI: 0.824-0.861). The association was similar among many cancers including major types (prostate IRR: 0.687, 95% CI: 0.653-0.722; lung IRR: 0.801, 95% CI: 0.749-0.856; breast IRR: 0.903, 95% CI: 0.849-0.961). Given the chronic nature of cancer disease on incidence, we conducted a subgroup analysis among patients prescribed warfarin for atrial fibrillation/flutter (AF-group), a subgroup with reduced comorbidity. Warfarin users in the AF-group showed a stronger overall cancer risk reduction (IRR: 0.619, 95% CI: 0.592-0.646), including all major cancer types, particular lung cancer. (Prostate, IRR: 0.604, 95% CI: 0.552-0.662; Lung: IRR: 0.391; 95% CI: 0.332-0.460; Breast IRR: 0.720, 95% CI: 0.594, 0.871) CONCLUSION: We show that warfarin use is associated with a broad cancer protective effect in a large unselected patient cohort. Subgroup analysis of arrhythmia patients to reduce confounders reinforced the notion that warfarin exerts important anti-tumor effects.

#3011 Dietary fat, fatty acids, and ovarian cancer risk: Preliminary findings from the Shanghai Women's Health Study. Eftihia Y. Akam,1 Harvey J. Murff,2 Yong-Bing Xiang,3 Nikhil K. Khankari,1 Hui Cai,1 Xiao O. Shu,2 Wei Zheng,2 Alicia Beeghly-Fadiel1.1Division of Epidemiology, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN; 2Division of Epidemiology, Department of Medicine and Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, TN; 3Department of Epidemiology, Shanghai Cancer Institute, Shanghai, China.

Introduction: Dietary fat is an essential nutrient for human growth and development, but epidemiological studies have linked excess fat to the development of multiple malignancies. The few existing studies on ovarian cancer risk are inconsistent, possibly due to differences in sources and subtypes of fat. For example, omega-3 (n-3) polyunsaturated fatty acids (PUFAs) are thought to be anti-neoplastic, while omega-6 (n-6) PUFAs may promote carcinogenesis. Approach: We evaluated dietary fat in relation to ovarian cancer risk in the prospective Shanghai Women's Health Study (SWHS). Intake levels were determined from food frequency questionnaires (FFQ) administered at baseline and during follow-up; specific nutrient components were based on the sum of specific foods multiplied by nutrient content from the Chinese Food Composition Table. Cox proportional hazards regression was used to estimate relative risks (RR) and 95% confidence intervals (CIs) for dietary fat and fatty acid components categorized into quintiles. Minimal models included age and residual adjustment for total energy intake; fully adjusted models also included education, years of menstruation, years of oral contraceptive use, parity, years of breastfeeding, family history of ovarian cancer, smoking, drinking, NSAID use, BMI, and physical activity. Results: Among 73,144 SWHS participants, we identified a total of 219 ovarian cancer cases in 1,114,828 person-years of follow-up. We found significant dose-response relationships between increasing total PUFA (P-trend=0.032) and n-6 PUFA (P-trend=0.022) intakes and decreased ovarian cancer risk. In both minimally and fully adjusted models, women with the highest quintile of total dietary PUFAs were approximately 35% less likely to develop ovarian cancer (RR=0.65, 95% CI: 0.43-0.99) compared with those in the highest quintile (RR=0.99, 95% CI: 0.91-1.09). In fully adjusted models, the total fat, monounsaturated fat, and n-3 PUFA intake were not significantly related to ovarian cancer risk. Conclusions: Findings from our preliminary analysis of a large prospective cohort of Chinese women suggest that total PUFA intake may be protective of ovarian cancer, and that this association may be driven by n-6 rather than n-3 PUFAs. Further evaluation, including stratification, sensitivity analysis, and evaluation of source of dietary fatty acids, is currently underway.

#3012 Long-term aspirin use and total and cancer-specific mortality. Yin Cao,1 Meir Stampfer,2 Walter Willett,2 Donna Spiegelman,1 JoAnn Manson,2 Jennifer A. Naya,1 Nyla A. Heerema,2 Xiaoli Zhang,2 Hatice Gulcin Ozer,2 Amy J. Johnson,2 Jennifer A. Woyach,2 Erin Hertlein,2 John C. Byrd.1

Background: Although oral contraceptive (OC) use is ubiquitous in the United States, the influence of OCs on the development of cancers is not fully understood. In 2013, an Agency for Healthcare Research and Quality Evidence Report identified several data gaps with regard to OC use and its associations with cancer, including a need to understand if associations are consistent across subpopulations of users, including smokers and obese women. Methods: To address this data gap, we used Cox proportional hazards models to examine duration of OC use (never/less than 1 year [reference], 1 to 4, 5 to 9, 10+ years) and subsequent development of incident ovarian or endometrial cancer among participants in the NIH-AARP Diet and Health Study. We further examined effect modification by a variety of modifiable risk factors, including body mass index (BMI), cigarette smoking, alcohol consumption, and physical activity, via interaction terms and a likelihood ratio test. Models were adjusted for age, race, age at menarche and the modifiers of interest. Results: For ovarian cancer (n=1,241 cases, 149,502 non-cases), a reduction in risk was conferred by OC use that linearly decreased with duration of use (hazard ratios [HR] ranging from 0.83 to 0.60 across categories of duration). We also identified linear, inverse associations between increasing duration of OC use and ovarian cancer risk across most potential modifiers. Some of the most pronounced reductions were observed among long-term OC users (10+ years) who were current smokers (HR compared to non-users of 0.44, 95% confidence interval [CI] 0.20, 0.97) or who engaged in moderate physical activity (0.44, CI 0.23, 0.85). For endometrial cancer (n=2,337 cases, n=112,132 non-cases), we observed pronounced reductions in risk associated with OC use among women with obese BMIs (0.36, CI 0.25, 0.52) and those who exercised rarely (0.40, CI 0.29, 0.56) or moderately (0.50, CI 0.33, 0.75). However, long-term use was not associated with risk reductions among women with BMIs of less than 25 or among women who exercised at least three times per week. Conclusions: OC use is consistently associated with reduced risks of ovarian cancer across most strata of modifiable risk factors. For endometrial cancer risk, OC use seems to offer the greatest risk reduction among women in previously identified high-risk categories (i.e., overweight/obese BMI, physically inactive), findings which merit further discussion as these women may be more likely to have comorbid conditions that would contraindicate OC use (e.g., hypertension, diabetes).

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Identification of Molecular Targets

#3014 BCL3 over-expression contributes an in vivo growth advantage in a B-cell lymphoma xenograft model and is a risk factor for ibrutinib relapse in CLL. Timothy L. Churudjian,3 Bonnie K. Harrington, Larry Beaver, Amy S. Ruppert,2 Nyla A. Heerema,2 Xiaoli Zhang,2 Hatice Gulcin Ozer,2 Amy J. Johnson, Jennifer A. Woyach,2 Erin Hertlein,2 John C. Byrd.1

Background: BCL3 over-expression contributes an in vivo growth advantage in a B-cell lymphoma xenograft model and is a risk factor for ibrutinib relapse in CLL. Chronic lymphocytic leukemia (CLL) relies on chronic B-cell receptor (BCR) signaling, and as such is effectively treated with Bruton’s tyrosine kinase inhibitoribrutinib. The mechanism of ibrutinib resistance has remained enigmatic. In this study, we sought to determine the role of BCL3 over-expression in the development of ibrutinib resistance in vivo and to identify potential mechanisms of ibrutinib resistance.

Methods: We generated ibrutinib-resistant sublines in the 32Dcl-5IB cell line and determined the effects of BCL3 over-expression on cell viability, proliferation, and the ibrutinib sensitivity of these sublines. Results: BCL3 over-expression increased cell viability and proliferation of the 32Dcl-5IB cell line, and this effect was enhanced in the presence of ibrutinib. In vivo, BCL3 over-expression contributed to a significant in vivo growth advantage in a B-cell lymphoma xenograft model, and mice with BCL3 over-expression had a faster time to tumor detection compared to mice without BCL3 over-expression. In vivo, BCL3 over-expression also contributed to a significant in vivo growth advantage in a B-cell lymphoma xenograft model, and mice with BCL3 over-expression had a faster time to tumor detection compared to mice without BCL3 over-expression. Conclusion: BCL3 over-expression contributes an in vivo growth advantage in a B-cell lymphoma xenograft model and is a risk factor for ibrutinib relapse in CLL.

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kinase (BTK) inhibitors. However patients who relapse on BTK inhibitors such as ibrutinib have an extremely poor prognosis without additional intervention. Therefore, identifying and characterizing risk factors that predict relapse to BTK inhibitors is important. A series of 308 CLL patients have been enrolled on various ibrutinib clinical trials at the Ohio State University. Seven of these patients were identified to carry the translocation t(14;19), which leads to over-expression of B-cell leukemia 3 (BCL3). Of these 7, 6 (85%) have relapsed on ibrutinib while only 27% of patients (83/308) overall have progressed. BCL3 is known to regulate NF-κB transcription and influence B-cell function, and we found that overall BCL3 expression is increased in CLL compared to normal B-cells, therefore hypothesized that BCL3 may play a role in resistance to ibrutinib. We developed a competitive advantage to promote ibrutinib resistance. In order to test the role of BCL3 in ibrutinib resistance using an in vitro system, we overexpressed BCL3 or an empty vector (EV) control in ibrutinib responsive B-cell lines (BCL3 was at least 2-fold over-expressed relative to endogenous BCL3). Cells were treated with vehicle or 1 μM ibrutinib for 1 hour followed by a washout, and proliferation and viability were evaluated at various time points. ibrutinib inhibited cell proliferation and induced apoptosis, however BCL3 expression did not abrogate these effects. However, while examining BCR signaling proteins we found that BCL3 expression enhanced phosphorylation of BTK, suggesting that BCL3 stabilizes activated BTK to circumvent ibrutinib treatment. We next engrafted these EV or BCL3 over-expressing cells into the left or right flank, respectively, of immune-compromised (NSG) mice. Mice were sacrificed when either tumor reached a volume of 2000 cm³, and the tumors were isolated and weighed. Tumors which developed from the BCL3 over-expressing cells at sacrifice were larger than those which developed from the EV cells by an average of 1332 g. We also evaluated a disseminated tumor model where HBL-1 EV or BCL3 over-expressing cells were injected via the tail vein. Disease was monitored by weekly peripheral blood flow cytometry for human CD19. We saw that 6 of 11 mice intravenously engrafted with HBL-1 BCL3 present with peripheral disease when the mice meet removal criteria, whereas only 1 of 11 mice engrafted with HBL-1 EV cells developed peripheral disease. We conclude that the presence of t(14;19) indicates a higher risk of relapse in patients undergoing ibrutinib therapy, and these patients should be closely monitored for evidence of progression. Our studies also suggest that BCL3 promotes more aggressive disease in a lymphoma xenograft model, and may be involved in tumor migration in vivo.

#3015 Selection inhibition of mutant KRAS cell and tumor growth by PHT-7.3, an inhibitor of the KRas signaling nanocluster protein Cnk1. Roisin Delaney,1 Marco Maruggi,2 Martin Indarte,2 Robert Lemos,1 Geoff Roos.1

**Objective:** To identify druggable targets for tumors with KRas mutations.

**Methods:** We previously identified connector enhancer of kinase suppressor of Ras 1 (Cnk1) as a critical mediator for growth driven by mut-KRas in human cancer cells. Despite intense efforts, tight nucleotide binding of Cnk1 has hindered the development of compounds that bind or inhibit KRas. We have pedded the development of compounds that bind or inhibit KRas. We have identified kinase suppressor of Ras 1 (Cnk1) as a critical mediator for growth driven by mut-KRas in human cancer cells. Cnk1 co-localizes with mutant KRas at the membrane and deletion of Cnk1 abrogates KRas activation and the activation of the Ras effectors Raf and Rho. Cnk1 deletion caused cells with mutant KRas to accumulate at the G1 checkpoint similar to selective deletion of mutant KRas itself. Following a screen and initial structural optimization a small molecule probe compound PHT-7.3 was identified and shown to bind selectively to the pleckstrin homology PH domain of Cnk1 preventing Cnk1 and mut-KRas co-localization. PHT-7.3 inhibited mut-KRas but not wt-KRas non small cell lung cancer (nsclc) cell growth, and selectively blocks mut-KRas downstream signaling in cells. PHT-7.3 exhibited cytostatic antitumor activity in the mut-KRas (G12S) A549 and mut-KRas (G12V) H441 nsclc xenografts, but not in the wt-KRas H1975 nsclc xenograft. Mut-KRas downstream signaling was inhibited by PHT-7.3 in the xenografts with downregulation of activated Rho and Raf signaling. PHT-7.3 showed further increased antitumor activity in A549 xenografts in combination with erlotinib or trametinib. Thus, the work identifies the PH domain of Cnk1 as a druggable target whose inhibition selectively blocks mutant-KRas activation, and PHT-7.3 as a lead agent in the development of therapies for KRas tumors.

#3016 Identification of protein arginine methyltransferase 1 as novel epigenetic vulnerability in KRAS/p53 mutant PDAC primary patient models.

**Objective:** To identify druggable targets for tumors with KRas mutations.

**Methods:** We previously identified protein arginine methyltransferase as a novel genetic vulnerability in PDAC and strongly suggest PRMT1 dependency and to refine responder population. To further confirm a role for PRMT1 in tumor maintenance, we developed inducible PRMT1 knockout in a primary patient model and showed a dramatic tumor growth inhibition (TGI) in vivo upon PRMT1 knockdown. PRMT1 is the primary enzyme responsible for arginine asymmetric demethylation, however other members of the Type I family are also involved in this process and we evaluated the role of protein arginine methyltransferase 4 (PRMT4) and 6 (PRMT6) in our workhorse model. Surprisingly, no significant phenotypic response was observed upon genetic inhibition of PRMT4 or PRMT6 suggesting no redundancy between different PRMT type 1 and a unique dependency on PRMT1. To strengthen and complement the genetic validation, we leveraged a PRMT Type I inhibitor and confirmed in vitro results as well as in vivo efficacy at tolerated doses (xenograft vs allograft). Key models have been prioritized in order to inform on PRMT1 dependency and to refine responder population. Our results has identified and validated for the first time an arginine methyltransferase as a novel genetic vulnerability in PDAC and strongly suggest PRMT1 as a new therapeutic opportunity in PDAC cancers.

#3017 Inhibition of YAP/TAZ-dependent transforming properties in LATS1/2 deleted RPE1 cells by MAPKAPK5 targeting. Min Hwan Kim, Joon Kim. Korea Advanced Institute of Science and Technology, Daejeon, Republic of Korea.

**Objective:** To identify druggable targets for tumors with LATS1/2 loss.

**Methods:** To examine the transforming potential of LATS1/2 loss, both LATS1 and LATS2 alleles were deleted by CRISPR-Cas9 genome editing in RPE1-hTERT cells. YAP/TAZ activity in LATS1/2-null RPE1 cells was measured by immunofluorescence (IF) staining and qRT-PCR. Transforming properties of LATS1/2 loss were measured on both 2-dimensional (2D) and 3D culture, and the centrosome number and DNA contents of cells were determined by IF and propidium iodide staining. LATS1/2-null RPE1 cells were subjected to an image-based kinase high-throughput screening platform that enables the identification of new molecular drivers in the genetic vulnerability of PDAC. We identified MAPKAPK5 as a novel genetic vulnerability in PDAC and strongly suggest MAPKAPK5 targeting in PDAC.

#3018 Genetic loss of LATS1/2, thenegativeregulatorsofYAP/TAZ, has been reported in various human tumors, and suggested to activate YAP/TAZ in tumorigenic process. However, the molecular consequence of LATS1/2 genetic loss in benign human cells has not been addressed, and therapeutic targets for tumors with LATS1/2 loss have not been explored. We aimed to investigate the transforming potential of LATS1/2 loss in human RPE1 cells, and to find targetable kinases that support YAP/TAZ activation in tumors with LATS1/2 loss Method: To examine the effect of LATS1/2 loss, both LATS1 and LATS2 alleles were deleted by CRISPR-Cas9 genome editing in RPE1-hTERT cells. YAP/TAZ activity of LATS1/2-null RPE1 cells was measured by immunofluorescence (IF) staining and qRT-PCR. Transforming properties of LATS1/2 loss were measured on both 2-dimensional (2D) and 3D culture, and the centrosome number and DNA contents of cells were determined by IF and propidium iodide staining. LATS1/2-null RPE1 cells were subjected to an image-based kinase high-throughput screening platform that enables the identification of new molecular drivers in the genetic vulnerability of PDAC. We identified MAPKAPK5 as a novel genetic vulnerability in PDAC and strongly suggest MAPKAPK5 targeting in PDAC.
depletion resulted in suppression of LAT51/2-null RPE1 cell proliferation on 2D culture as well as sphere formation capacity on 3D culture. Conversely, constitutively active MAPKAPK5 mutant expression in RPE1 cells induced YAP/TAZ nuclear enrichment. Conclusion: These results suggest that LAT51/2 genetic loss drives transforming properties in human RPE1 cells, and MAPKAPK5 inhibition blocked YAP/TAZ activation induced by LAT51/2 loss. We suggest that MAPKAPK5 is a novel positive regulator of YAP/TAZ and may serve as a therapeutic target for YAP/TAZ-driven tumors.

**#3018 RET rearrangements as promising therapeutic targets in breast cancer.** Bhavna S. Paratala,1 Jeffrey S. Ross,2 Casey B. Williams,2 Whitney Petrosky,3 Kirstin A. Williams,4 Jon Chung,5 Sonia C. Dohl,6 Shridar Ganesan,7 Siraj Ali,2 Brian Leyland-Jones,3 Kim M. Hirshfield1.1 Rutgers Cancer Institute of New Jersey, New Brunswick, NJ; 2Foundation Medicine, Cambridge, MA; 3Avera Cancer Institute Center for Precision Oncology, Sioux Falls, SD.

Receptor tyrosine kinase alterations have played a significant role in therapeutic decisions for cancer due to their oncogenic nature and response to targeted small molecule kinase inhibitors. Increased genomic profiling of tumors using hybrid-capture based next-generation sequencing approaches now reveal the presence of previously unknown fusions and alterations involving kinases in a diverse set of cancers. Here we report the presence and therapeutic significance of recurrent and novel fusions involving RET, a known oncogenic tyrosine kinase receptor, in breast cancer. Methods: Comprehensive genomic profiling on formalin-fixed, paraffin embedded patient tumor tissues was performed using FoundationOne platform that covers the entire coding region for 315 cancer-related genes and exons of 28 genes involved in rearrangements at a depth of 500-1000X (Foundation Medicine, MA). Out of 23 rearrangements, two representative RET fusion expression vectors were synthesized and expressed in non-tumorigenic cell lines (breast MCF10A and mouse 3T3 fibroblasts) and were evaluated for RET kinase signaling, drug response, and tumorigenicity. Results: RET gene fusions, the canonical NCOA4-RET and a novel, noncanonical RASGEF1A-RET fusion, were identified in two separate breast cancers and both include exons required to retain the intact kinase domain of Ret. The novel RASGEF1A-RET fusion includes the non-coding region of RASGEF1A potentially resulting in a truncated RET protein using an alternate internal start site in exon 11 of RET. In vitro characterization of both fusions expressed in mouse 3T3 and human MCF10A cell lines revealed constitutive kinase activation and subsequent downstream signaling as evidenced by phosphorylation of Ret, Erk and Akt. This is the first reported noncanonical RET rearrangement resulting in a 5' truncated but functional RET kinase. Non-tumorigenic cell lines with stable expression of either rearrangement showed transformed phenotypes assessed by changes in morphology, enhanced growth rate, colony forming ability, and tumor formation in mice. RET fusion-transformed cells were exquisitely sensitive to treatment with RET inhibition when evaluated in both short-term and long-term functional assays. NCOA4-RET was found by CGP in an index case of metastatic EC+/HER2+ breast cancer that had radiographic evidence of disease progression while on trastuzumab, pertuzumab, and anastazol. Subsequent treatment with cabozantinib plus anastazol led to a rapid clinical and radiographic response. Conclusion: CGP technologies, including hybrid-capture based approaches can identify previously unreported but recurrent RET gene fusions in breast cancer. Here, we show that RET fusions including both canonical and non-canonical complex rearrangements are functional and may represent promising therapeutic targets in selected breast cancer patients.

**#3019 The role of TACC3 in the progression from ductal carcinoma in situ to invasive breast cancer.** Lorenda Campo, Maya Mathew, Eun-Kyoung breuer, William Small. Loyola University Medical Center, Maywood, IL.

Ductal carcinoma in situ (DCIS) is a non-invasive breast cancer with the potential to become invasive/metastatic. The development of DCIS to invasive breast cancer is an important aspect of breast tumor aggressiveness, however, little is known about the genetic and molecular alterations involved in this progression. Also, analysis of molecular markers and genetic signatures of DCIS and invasive breast cancer have failed to identify transition-specific biomarkers or pathways. Hence, identifying the molecular mechanisms by which DCIS progression to invasive breast cancer is critical for the success of diagnosis, prognosis and therapy of breast cancer. Members of the transforming acidic coiled-coil (TACC) protein family are key players in the maintenance of centrosome integrity, the regulation of microtubule assembly, and spindle stability during mitosis. TACC3 has been shown to be involved in the regulation of centrosome-microtubule dynamic networks, cell growth and differentiation, and transcription/ gene expression through interactions with other molecules. Mounting evidence indicates that high levels of TACC3 may contribute to breast cancer development. Here, we investigated the significance of TACC3 in the transition of DCIS to invasive breast cancer. First, we examined the expression of TACC3 in non-tumorigenic human mammary epithelial MCF10A cells and MCF10D10CS.COM cells, which are known to form DCIS-like lesions in mice and to progress to invasive cancer, and found that the expression of TACC3 is elevated in MCF10D10CS.COM cells compared to MCF10A cells. By analyzing tissue microarray data, we found that expression of TACC3 gradually increased from normal breast to DCIS, and further increased in invasive breast cancer. In addition, ectopic expression of TACC3 in MCF10D10CS.COM cells promoted cell proliferation, migratory and invasive capabilities as well as the epithelial-mesenchymal transition (EMT)-like phenotype. Moreover, MCF10D10CS.COM cells stably expressing TACC3 exhibited increased expression of stemness markers. Interestingly, a small molecule inhibitor of TACC3, KHS101 treatment in MCF10D10CS.COM cells significantly suppressed cell proliferation and the P38/ERK signaling pathway while having little effect on MCF10A cells. Our findings, therefore, suggest that TACC3 may play an important role in the progression from DCIS to invasive breast cancer and that targeting TACC3 could be a promising new therapeutic strategy for DCIS patients.

**IMMUNOLOGY: Adaptive Immunity in the Tumor Microenvironment**

**#3021 CCR5 inhibition: macrophage repolarization therapy for colorectal cancer.** Niels Halama,1 Inka Zoernig,1 Anna Berthel,1 Christoph Kahler;2 Fee Klupp,2 Meggy Suarez-Carmona,1 Karsten Brand,1 Juergen Krauss,1 Felix Lasitschka,3 Alexis Ulrich,3 Juergen Weitz,2 Martin Schneider,2 Markus Buechler,4 Laurence Zitvogel,5 Thomas Herrmann,6 Axel Benner,6 Christina Kunz,6 Stephan Luecke,5 Christoph Springfeld,1 Christine S. Falk,5 Dirk Jaeger,5 National Ctr. for Tumor Diseases, Heidelberg, Germany; 6University Hospital Dresden, Dresden, Germany; 7University Hospital Heidelberg, Heidelberg, Germany; 8Institute Gustave Roussy, Paris, France; 9Medizinische Klinik Idar-Oberstein, Idar-Oberstein, Germany; 10German Cancer Research Center, Heidelberg, Germany; 11Hannover Medical School, Hannover, Germany.

In patients with metastatic colorectal cancer (CRC), the local immune response influences the course of the disease. An in-depth analysis of the immune microenvironment of human CRC liver metastases revealed a distinct immunological microenvironment. Within this microenvironment, two distinct subsets of myeloid cells induce an influx of T cells into the invasive margin via CXCL9/CXCL10. CCL5 is produced by these T cells and stimulates pro-tumoral effects via CCR5, creating an exploitive loop. CCR5 was found on macrophages, lymphocytes and on

**#3022 The impact of Notch3 signaling on tumor microenvironment and metastatic progression of human head and neck tumors.** Helmut Schmid1, Christoph Stein, Idar-Oberstein, Germany; 2Institute of Pathology, University Medical Center Regensburg, Regensburg, Germany; 3University Medical Center Regensburg, Regensburg, Germany; 4German Cancer Research Center, Heidelberg, Germany; 5Hannover Medical School, Hannover, Germany.

We found the gain-of-function Notch3 mutation in cancer is associated with an increased risk of head and neck cancer. However, the mechanisms underlying this association are poorly understood. Notch3 signaling is essential for cell survival only following metastasis. To test if knockdown of selected targets inhibits metastasis in a therapeutic setting, we established orthotopic xenograft models of human head and neck tumors in the mouse. Components of the Notch pathway were shown to be essential for survival of cells derived from metastatic sites. Whole exome sequencing identified a novel mutation in one of the EGF domains of Notch3 that was acquired only in the metastatic in the mouse. Utilizing CRISPR methodology, we established that “fixing” the mutation results in reversal of metastatic phenotype of the cells, making them Notch independent similar to their primary tumor counterparts. Mutations in EGF domains have been seen to alter the interaction of ligand which can impact Notch signaling. Our data indicate that a distinct set of target genes is induced upon interaction between Notch3 and Jag2. Furthermore, our results indicate that suppression of Notch3 improves survival in mice bearing orthotopic tumors derived from the metastatic HN-3C model. Overall, our data demonstrate that metastatic cells from head and neck tumors acquire dependency on Notch3 signaling. Novel treatments targeting components of this pathway may prove effective in targeting metastatic cells alone or in combination with conventional therapies.
the vast majority of tumor cells. Inhibition of CCR5 in patient-derived func-
tional in vitro organotypic culture models showed a promising macrophage 
repolarization with anti-tumoral effects. These effects are mediated by activation 
of an antiviral program in macrophages, leading to interferon and reactive ox-
genesis6,7. As such, targeting the Wnt pathway is an attractive approach to the 
initiate tumorigenesis in the intestine and promote cancer stem cell metasta-
specific interaction of selective target compounds targeting oncogenic Wnt activity through disrupting the highly 
unacceptable side effect profiles1. Here, we developed a potent and robust, se-
dependent cancer growth and immunomodulates colorectal tumors. 

#3022 Novel hydrocarbon stapled peptide inhibitors suppresses β-catenin 
dependent cancer growth and immunoinduces colorectal tumors. Da-
vid Zhu,1 Joy Jin,2 WntRx/Lab Central, Boston, MA; 2Harvard University, 
Boston, MA.

Mutations in several components of the canonical Wnt pathway function as 
drivers in a range of common solid tumor cancers, including about 90% of 
sporadic colorectal cancers (CRCs), the third most common cancer in the US. In 
particular, activation of β-catenin (β-cat), a key Wnt signaling component, can 
initiate tumorigenesis in the intestine and promote cancer stem cell metasta-
sis6,7. As such, targeting the Wnt pathway is an attractive approach to the 
development of cancer therapeutics. However, Wnt inhibitors tested to date, in-
duding those that disrupt β-cat activity, have either had limited efficacy or 
unacceptable side effect profiles1. Here, we developed a potent and robust, se-
lective lead compound targeting oncogenic Wnt activity by disrupting the highly 
specific interaction of β-cat with its co-activators BCL9/B9L, which has been 
shown to abrogate transactivation. By using hydrocarbon stapled peptide tech-
nology and an acutely sensitive high throughput biochemical screening assay, 
we have identified several promising peptides that possess a strong pharmaco-
kinetic profile. These peptides (i) exhibit robust activity in mouse models; (ii) 
inhibit cancer cell proliferation, metastasis, and stem cell progression; and (iii) 
modulate the immune microenvironment, demonstrating the feasibility of such 
peptide development and selection. Synergy is demonstrated between the sta-
pled peptides and anti-PD1 antibodies, which has significant implications for 
combination therapy with commercial immune checkpoint blockers that have 
become standard of care for second-line melanoma treatment. These peptides 
represent a promising new potential therapeutic class for patients with CRC, for 
which traditional chemotherapy and targeted inhibitors have had limited ther-
apeutic success.

#3023 The antitumor activity of immunotoxins is enhanced by tofacitinib. 
Nathan Simon,1 Antoñada Antigüan,2 Stephen Hewitt,2 Massimo Gadina,2 Ma-
sanori Onda,3 Christine Alexiew,1 David Joseph Fitzgerald1. 1Walter Reed National Military Medical Center, Bethesda, MD; 2National Institute of Arthritis and Musculoskeletal and Skin Disease, Bethesda, MD.

Treatment outcomes of certain aggressive cancers have not improved signif-
ificantly in the last decade, necessitating development of new therapies. Recom-
binant immunotoxins (IT) are fusion proteins consisting of the enzymatic do-
main of a cytotoxin linked to an antibody fragment targeting a cellular surface 
protein. While the combination of selective targeting and potent cell-killing 
make ITs well suited for targeted cancer therapy, clinical experience has indi-
cated that patients can generate anti-toxin neutralizing antibodies after one cy-
cle of treatment. Mouse treated with the FDA-approved JAK inhibitor, tofacitinib, 
produced significantly lower anti-toxin antibody titers, suggesting that tofac-
itinib could be used in combination with immunotoxins to improve therapeutic 
efficacy. Accordingly, the combination of tofacitinib and IT was tested both in 
vitro and in vivo using two models of aggressive human cancers: pancreatic 
ductal adenocarcinoma and triple-negative breast cancer. In vitro combination 
study examined the effects on oncogenic signaling pathways and looked for 
enhancement of cytotoxic potency. Results demonstrated that tofacitinib me-
diated inhibition of key signaling pathways but no enhancement of immuno-
toxin killing. By contrast, when xenograft tumor models were challenged, im-
munotoxin activity was enhanced greatly by co-treating with tofacitinib. 
Tofacitinib treatments alone did not demonstrate any antitumor activity. The 
combination of tofacitinib and immunotoxin decreased tumor growth and ex-
tended survival compared to either treatment alone. Examination of post-treat-
ment tumor samples and sera from mice indicated that JAK inhibition resulted in 
alterations of the tumor microenvironment, including a decrease in cancer-
promoting cytokine levels and reductions in recruitment of suppressive im-
mune cells to tumor tissue. This study demonstrates that the inhibition of the 

#3024 CD4+ outperform CD8+ central memory-derived CAR T cells, me-
diating persistent antitumor responses and long-term eradication of glio-
blastoma. Dongrui Wang, Renee Starr, Brenda Aguilar, Alfonso Brito, Brenda 
Chang, Aniece Sarkissian, Behnam Badie, Michael E. Barish, Stephen J. Forman, 
Christine E. Brown. City of Hope, Duarte, CA.

Purpose: To compare antitumor effector function mediated by CD4+ and 
CD8+ chimeric antigen receptor (CAR)-T cells targeting IL13 receptor α2 
(IL13Ra2) for treatment of glioblastoma (GBM). Experimental Design: CD4+ 
and IL13Ra2-specific CAR T cells, derived from enriched central mem-
ory T cells (Tcm), were evaluated for their antitumor potential both in vitro by 
recreative challenge with GBM cells and in vivo in established orthotopic GBM 
xenograft models. Further, GBM-stimulated CD4+ and CD8+ T CAR T cells were 
examined for their exhaustion- and memory-associated phenotypes at tran-
scriptional and protein levels, which would account for their differential antitu-
mor activity. Results: While CD8+ T CAR T cells mediated robust short-term 
cytolytic effector function, they became rapidly exhausted and lost antitumor 
potential after repeated stimulation with tumor cells. CD4+ CAR T cells, by 
contrast, persisted and retained effector potency even after repetitive tumor 
challenge. CD4+-mediated cytotoxicity acted through the granzyme B/perforin 
pathway and was independent of the CD8+ cells. Activated CD4+ CAR T cells 
augmented the proliferation of CD8+-cells, but failed to protect the CD8+ cells 
against stimulants of cellular exhaustion. In NSG mice with established GBM 
xenografts, co-stimulated CD4+ T CAR T cell treatment led to long-term tumor-free survival, while CD8+ 
CAR T cell treated mice showed short-term tumor regression followed by anti-
gen-positive relapse and associated T cell exhaustion. Upon GBM stimulation, 
CD4+ cells expressed less co-inhibitory receptors and more memory-associated 
markers than the CD8+ T cell subset. Further, a comprehensive transcriptional 
analysis revealed that when activated, CD4+-cells retained expression of genes 
involved in survival- and self-renewal-related pathways, indicating a less ex-
hausted status and a better functional persistence in comparison to the CD8+ 
cells. Conclusion: We observed a superior anti-GBM activity mediated by CD4+ 
CAR T cells over the CD8+ subset, featured by their ability to maintain long-
term immune response and reduce stimulation-induced exhaustion. It is thus 
infereved that the frequency of CD4+ cells could be an important criteria for 
evaluating the efficacy of CAR T cells as immunotherapeutic products.

#3025 Preclinical characterization of a novel monoclonal antibody target-
ing a neo-antigen expressed in ovarian and GI malignancies. Kristen Zeligs,1 
Philip M. Arlen,2 Kwong Tsang,3 Lida Hernandez,3 Massimo Fantini,3 Chris-
tina M. Annunziata2. 1Walter Reed National Military Medical Center, Bethesda, 
MD; 2Precision Biologics, Inc., Rockville, MD; 3National Cancer Institute, 
Bethesda, MD.

Objectives: We have developed a novel humanized IgG1 monoclonal anti-
body, NEO-201, which targets a neoantigen expressed on ovarian and GI ma-
lignancies. This antigen has homology to CEA/CA125; however, it has specificity 
to tumor tissues and not normal tissues. Our aims were as follows: (1) to evaluate 
the antigenic spread across tumor cell lines derived from various ovarian 
and GI cancers; and (2) to determine the functionality of NEO-201. Methods: 
Expression of the antigen was identified by western blot analysis and flow cy-
tometry. Functionality was determined by an antibody dependent cell-mediated 
cytotoxicity (ADCC) assay using the antigen-expressing cell lines identified by 
western blot analysis. Cell lines not expressing the antigen were used as negative 
controls. The effector cells used in the ADCC assay were natural killer (NK) cells 
isolated from PBMCs of normal donors and high-affinity activated NK (haNK) 
cells. haNK cells are NK-92 cells genetically engineered to express the high-
affinity variant of human CD16 (FcgRIIIA-V158) and the human IL-2 gene. 

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5 ovarian cancer cell lines (OV90 and PE01) and 1 of 2 colorectal cancer cell 
lines (LS174T) stained positively for NEO-201. Flow cytometry analysis on ovarian, 
colorectal, and pancreatic cancer cell lines confirmed the expression of the 
NEO-201 neoantigen on 1 of 2 ovarian cancer cell lines (OV90), 1 of 2 colorectal 
cancer cell lines (LS174T), and 2 of 2 pancreatic cancer cell lines (ASPC-1 and CFPAC-
1). ADCC assay was performed using OV90 and ASPC-1 tumor cell lines. Cell 
lines not expressing the neoantigen were used as negative controls. Killing of 
tumor cells expressing the neoantigen was also observed with NEO-201 alone; 
however, the lysis was significantly augmented when NK cells from the PBMCs 
of normal donors or haNK cells were included in the ADCC assay. Conclusions:
These findings demonstrate that the NEO-201 neoantigen is expressed on several tumor cell lines representing ovarian and GI malignancies. NEO-201 can mediate ADCC activity in the presence of NK cells and NK cell lines. In addition, NEO-201 can induce apoptosis in tumor cell lines expressing the NEO-201 neoantigen. Furthermore, preliminary studies demonstrated that this neoantigen is expressed in numerous cancer types (not shown). In vivo studies as well as INI-related studies are planned.

#3026 Targeting GPX4 in tumor-associated stromal cells increases inflammatory-cell infiltration. Shrikanta Chattopadhyay,1 Cherrie Huang,1 Ninib Baryawno,1 Nicolas Severe,1 Vasanthi Viswanathan,1 Carlotta Costa,1 David Scadden,2 Stuart Schreiber1. 1Massachusetts General Hospital, Boston, MA; 2Broad Institute, Cambridge, MA.

The lack of a T-cell inflamed microenvironment in tumors limits responsiveness to many immunotherapies. T-cell exclusion is often mediated by a dense infiltration of fibroblast-like stromal cells. Up to 55% of triple-negative breast cancers have ‘stroma-rich’ tumors with markedly lower T-cell infiltration. Here we report a therapeutic strategy that can potentially convert stroma-rich tumors into T-cell inflamed tumors by forcing stromal cells to secrete 5-lipoxygenase products which are powerful chemo-attractants for T cells. We were initially interested in identifying selective inhibitors of stromal cell function. To achieve this, we used phenotype-based small-molecule screening in which the phenotype of stroma-induced cancer cell migration in vitro was a surrogate for stroma-induced metastasis in vivo. We identified a compound, RSL3 that inhibited this migration. RSL3 was selectively cytotoxic to stromal cells over cancer cells, in comparison of immortalized cell lines as well as comparisons of patient-derived primary breast cancer cells to cancer-associated fibroblasts (CAFs). We therefore undertook studies to identify its mechanism of action. RSL3 was recently reported to target the redox enzyme glutathione peroxidase 4 (GPX4). GPX4 metabolizes lipid peroxides so we performed metabolomic profiling of RSL3-treated stroma-cancer co-cultures and found elevated arachidonic acid products of lipoxygenase enzymes. Stromal cells were found to contain >10-fold higher levels of lipoxygenase products than carcinoma cells. Blocking either 5-lipoxygenase (5-LO) or 15-lipoxygenase (15-LO) with selective inhibitors abrogated RSL3’s cytotoxicity to stromal cells. Thus, high lipoxygenase activity in stromal cells increases their susceptibility to GPX4 inhibition. Because 5-LO products like leukotriene B4 are powerful chemo-attractants for myofibroblasts and T cells, we studied the impact of GPX4 knockdown in vivo using xenografts of cancer cells co-injected with stromal cells. GPX4 knockdown resulted in a large increase in myeloid-cell infiltration into tumors but, surprisingly, T-cell infiltration was suppressed. We reasoned that 15-LO products are immunosuppressive based on recent findings that 15-LO gene amplifications are inversely correlated with T-cell infiltration in breast cancers in The Cancer Genome Atlas. Consistent with this, GPX4 inhibition of stroma-cancer co-cultures suppressed T-cell chemotaxis but combined inhibition of GPX4 and 15-LO significantly enhanced T-cell chemotaxis over untreated controls in vitro. We are undertaking in vivo testing of this combination. In summary, our unbiased chemical biology approach has revealed a therapeutic strategy to promote T-cell infiltration. We envision this to be a priming strategy for stroma-rich cancers to become responsive to a variety of different immunotherapies thereby unleashing their full curative potential.

#3027 Dissecting mechanisms of anti-PD-1 therapy with massively parallel single-cell RNA-sequencing. Brian C. Miller,1 Marc H. Wadsworth,2 Kevin Bi,3 Travis K. Hughes,2 Robert Manguso,1 Arlene H. Sharpe,1 Alex K. Shahel,2 Nicholas Haining,1 Dana-Farber Cancer Inst., Boston, MA; 2Massachusetts Institute of Technology, Cambridge, MA; 3Harvard Medical School, Boston, MA.

Anti-PD-1 therapy is an important new treatment option for many different types of malignancies, but overall response rates are less than 40%. Limited understanding of how anti-PD-1 treatment changes the tumor immune microenvironment is a barrier to identifying rational combination therapies and understanding mechanisms of immunotherapy resistance. To overcome this barrier, we set out to understand the mechanisms by which anti-PD-1 therapy augments the anti-tumor immune response using single-cell genomics. We have developed a massively parallel single-cell RNA-sequencing platform (“Seq-Well”) that uses a fabricated chip with nearly 100,000 nanowells in which barcoded beads and individual cells are distributed prior to lysis and RNA capture. We used this platform to comprehensively define the global expression profile of all major immune lineages in the tumor microenvironment in a mouse tumor model of immunotherapy. Mice were implanted with the B16F10 melanoma transplantable model of cancer and treated with anti-PD-1 or control antibodies. Tumors were harvested and CD45+ tumor-infiltrating leukocytes isolated by FACs. In a single experiment we were able to sequence the transcriptomes of over 600 individual cells, allowing us to clearly distinguish different immune lineages within the tumor microenvironment. We detect two transcriptionally distinct populations of CD8+ T cells, one that is highly proliferative (as marked by Ki-67), and one that has higher expression of cytotoxic markers (i.e. perforin). The Ki-67+ population is enriched for a gene expression signature from terminally exhausted CD8+ T cells in chronic viral infection, suggesting that this is a more exhausted subset. Treatment with anti-PD-1 globally alters the tumor microenvironment, including enriching for CD8+ T cells in the Prf1+ subpopulation compared with the Ki-67+ more terminally exhausted population. Studies to understand changes in the immune infiltrate of immunotherapy resistant tumors are currently ongoing. In conclusion, massively parallel single-cell RNA-sequencing allows us to dissect the mechanisms by which checkpoint blockade controls tumor growth, revealing shifts in the differentiation state of exhausted CD8+ T cells induced by checkpoint blockade.

MOL ecular AND Cellu lar BIOLOG Y / Genet Ics: Breakthroughs in the Control, Function, and Drivers of Oncogenesis

#3028 Reactive oxygen species induced nuclear translocation of c-MET via INTERNET mechanism. Mei-Kuang Chen,1 Yi Du,2 Yu-Yi Chu,2 Mien-Chie Hung3. 1The University of Texas Health Science Center at Houston, Graduate School of Biomedical Science, Houston, TX; 2The University of Texas MD Anderson Cancer Center, Houston, TX; 3National Cheng Kung University, Tainan, Taiwan.

c-MET is one of the receptor tyrosine kinases (RTKs) that are overexpressed in malignant cancer types including breast cancer. c-MET is traditionally known for its role in signaling transduction from the cell surface, our recent publication provided evidence that c-MET can be translocated into the nucleus and phosphorylate PARP1 in response to reactive oxygen species (ROS) stimulation (Du et al Nat Med 2016). Oxidative stress is a common phenomenon in cancer cells due to alteration of metabolism, and constitutive oxidative stress related to ROS has been observed in breast cancer cells. In this study, we demonstrated that hepatocyte growth factor (HGF) as well as hydrogen peroxide (H2O2) can induce retrograde translocation of c-MET holoreceptor via a membrane-bound vesicle transport mechanism and translocate into the nucleus through INTERNET (integral trafficking from the ER to the nuclear envelope transport pathway) (Wang et al. J Biol Chem 2012) in breast cancer cells. While c-MET nuclear transportation increases dramatically under H2O2 treatment, our findings provide a putative mechanism by which breast cancer cells adapt to oxidative stress and suggest that INTERNET might be a general nuclear translocation mechanism shared among RTKs.


Oncogenic mutations in RAS provide a compelling yet intractable therapeutic target. Using co-immunoprecipitation mass spectrometry, we recently identified an interaction between RAS and Argonaute 2 (AGO2), the core component of the RNA silencing machinery exerting translational control of mRNA transcripts. RAS and AGO2 co-sediment and co-localize in intracellular endomembrane-bound organelles. The AGO2 N-terminal domain directly binds the NET (integral traffi cking from the ER to the nuclear envelope transport pathway) (Wang et al. J Biol Chem 2012) in breast cancer cells. While c-MET nuclear transportation increases dramatically under H2O2 treatment, our findings provide a putative mechanism by which breast cancer cells adapt to oxidative stress and suggest that INTERNET might be a general nuclear translocation mechanism shared among RTKs.
MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Breakthroughs in the Control, Function, and Drivers of Oncogenesis

Metaplasia (ADМ) phenotype considered as precursors of PanIN (pancreatic intraepithelial neoplasia) and PDAC. This mirrors the phenotype of loss of Dicer in the same model further reinforcing a role for microRNAs in restraining KRAS oncogenic programs. Yet unlike Dicer, loss of AGO2 alone did not cause any gross or histological changes in the development of the mouse pancreata. In an effort to further exploring the role of AGO2, we have also studied the activated levels of a variety of signaling molecules in pancreata obtained from different genotypes. Primary analysis indicates that wild type Ras levels are elevated during AGO2 loss and has a bearing on PDAC initiation. Using both Western blot and Immunohistochemical analyses, we demonstrate a previously unknown and critical role for AGO2 in KRAS-MAPK signaling pathway.

#3030 Mutant p53-driven metastasis explored utilizing a traceable, conditional osteosarcoma model. Rasoul Pourehmadi, Yun Zhang, Mark J. McArthur, Guillermmina Lozano, MD Anderson Cancer Center, Houston, TX.

TP53 is the most frequently mutated gene in cancer and missense mutations in particular account for the majority of mutations. Mutant p53 proteins exert oncogenic Gain-of-Function (GOF) properties that contribute to increased migration and invasion in culture and metastasis in vivo. To elucidate how mutate p53 drives metastasis, we developed a traceable somatic osteosarcoma model that initiates with a single p53 mutation (p53R172H or p53R245W), or p53 loss specifically in osteoblasts using Oss-cre. To mark tumor cells, we utilized the mTmG allele which expresses membrane-targeted Tomato (mT) prior to Cre-mediated excision and membrane-targeted green fluorescent protein (mG) after excision so the tumor cells become GFP+ and the stromal cells remain RFP+. In this high penetrant, short latency mouse model, tumor micro-metastasis can be detected by GFP expression in the context of a normal stroma and immune system. The cohort has developed osteosarcoma with complete penetrance and an average survival of 304± 65 day. We observed metastasis in 55% of p53 mutant osteosarcomas which was significantly higher than 26% metastasis observed in p53 null tumors (p<0.01). In p53 mutant osteosarcomas, the metastasis rate was found significantly associated with low p53 copy number. Spectral karyotyping (SKY) on early passage cells derived from somatic p53 mutant tumors exhibited marked aneuploidy with modal chromosome numbers near tetraploid. To characterize the transcriptional profile associated with mutant p53 GOF, we analyzed and compared the RNA Seq transcriptomes of eight tumors derived from p53R172H mutant mice with eight p53 null tumors. Comparative analysis of gene expression identified a signature of snRNA genes to be strongly associated with mutant p53 gain of function. Functional annotation of upregulated genes in mutant tumors was also highly enriched for chromatin modifying enzymes. These findings contribute to our understanding of the role of mutant p53 GOF in metastasis. Our long term goal is to study how tumor-stromal interactions affect tumor development and metastasis. This understanding will have broad translational significance in diagnosis and treatment of tumors with mutant p53.

#3031 PHIP is a therapeutic target for triple negative solid tumors. David DeSemir,1 Vladimir Bezrookove, Mehdi Nosrati, Altaf A. Dar, Nathan Salo-

mons,1 Richard W. Sagebiel, Pierre Desprez, Robert J. Debs, Dirk Schaden- dorf,1 Mohammed Kashani-Sabet,1 California Pacific Medical Center Research Institute, San Francisco, CA; 3Cincinnati Children’s Hospital Medical Center, Cincinnati, OH; 6University Hospital Essen, Essen, Germany.

Targeted therapy relies on the classification of tumors according to the major molecular drivers of the malignant phenotype, which can then help decide the therapeutic treatment. However, a substantial subset of solid tumors does not express these markers, exemplified by the triple-negative subtype of breast cancer. Shared molecular factors that promote the progression of these tumors, and that represent a target for their therapy, are missing. We previously described a role for PHIP (Pleckstrin Homology domain-Interacting Protein) in the progression of melanoma, and demonstrated PHIP activation in triple-negative melanomas. Analysis of the TCGA profiling efforts in melanoma, breast and lung cancer revealed PHIP expression to be enriched in triple-negative breast cancer and in the bronchiad subtype of triple-negative lung cancer. Here we show the broad-based role of PHIP in the progression of triple-negative subtypes of three solid tumors (breast and lung cancer, and melanoma) defined by different mutational drivers and targeted therapies. By using a shRNA-based targeting of PHIP in in vitro and in vivo models, we have suppressed the malignant phenotype of triple-negative MDA-MB-231 and MDA-MB-436 human breast carcinoma cells. In addition, PHIP knockdown resulted in significant anti tumor effects in H1703 and Calu3 human lung cancer cells lacking mutations in EGFR, KRAS, and ALK, and in two short-term triple wild type melanoma cultures lacking mutations in BRAF, NRAS, and NFI. Suppression of PHIP expression resulted in inhibition of both tumor cell proliferation by cell survival and colony formation assays and invasion into matrigel in each of the tumor models examined, and was accompanied by suppression of pAKT, CCND1, T1N1, and ITGB1 expression when assessed by western-blot or quantitative immunofluorescence. The PHIP protein contains two bromodomains that can be therapeutically targeted by small molecules. We have discovered that the functional activity of the PHIP bromodomains has been poorly characterized. We show that PHIP co-localizes with and binds to the acetylated histone modification H4K9ac, and that both are coordinately regulated upon growth factor stimulation suggesting a new function for PHIP as a chromatin remodeler. To conclude, our results identify a novel role for PHIP in the progression of solid tumors lacking the major molecular drivers, and suggest PHIP as a druggable target for the therapy of these malignancies.

#3032 Direct regulation of alternative splicing by Smad3 through PCBP1 is essential to the tumor-promoting role of transforming growth factor beta1. Veenu Tripathi, Katherine M. Sixt, Xuan Xu, Ying E. Zhang, National Cancer Institute, NIH, Bethesda, MD.

In advanced stages of cancers, TGF-β promotes tumor progression in conjunction with inputs from receptor tyrosine kinase pathways. However, the mechanisms that underpin the signaling cooperation and convert TGF-β from a potent growth inhibitor to a tumor promoter are not fully understood. In this work we report that TGF-β directly regulates alternative splicing of cancer stem cell marker CD44 through a phosphorylated T179 of SMAD3-mediated interaction with RNA-binding protein PCBP1. We show that TGF-β and EGFR respectively induce SMAD3 and PCBP1 to colocalize in SC35 positive nuclear speckles, and the two proteins interact in the variable exon region of CD44 pre-mRNA to inhibit spliceosome assembly in favor of expressing the mesenchymal isoform CD44S over the epithelial isoform CD44E. We further show that the SMAD3-mediated alternative splicing is essential to the tumor-promoting role of TGF-β and has a global influence on protein products of genes instrumental to epithelial to mesenchymal transition and metastasis.

#3033 The PRL family of tyrosine phosphatases as a novel drug targets in acute lymphoblastic leukemia. Jessica S. Blackburn, University of Kentucky, Lexington, KY.

The aggressive and unpredictable behavior of acute lymphoblastic leukemia (ALL) presents a major clinical challenge in both the pediatric and adult setting. The development of new and better chemotherapies for this disease requires a detailed understanding of the genes and pathways that drive ALL malignancy. Somatic mutations in PRL family members are emerging as new drug targets with important roles in leukemia progression. To determine the extent to which phosphatases play a role in ALL malignancy, we used CRISPR/Cas9 to knock out expression of 255 individual phosphatases in human T-cell acute lymphoblastic leukemia cell lines and screened for deleterious effects on cell viability. Top hits from this screen included the protein tyrosine phosphatase type IV family, known as the PRLs. PRL3 is genomically amplified in a subset of human T-ALL, and more than 50% of human ALL patients have significantly higher expression of PRL2 and/or PRL3, compared to normal lymphocytes, suggesting that these PRLs may play an important role in ALL malignancy. Transgenic Myc-induced ALL models in zebrafish showed that overexpression of PRL2 and PRL3 significantly shortened latency of primary and relapse ALL, enhanced leukemia stem cell self-renewal (PRL2) and prevented apoptosis after standard chemotherapy treatment (PRL3). Finally, a specific PRL inhibitor strongly induced apoptosis of PRL-expressing ALL cells in a dose-dependent manner, in both zebrafish models in vivo and human cell lines in vitro. We have identified several FDA-approved, general phosphatase inhibitors that have potent anti-PRL activity and are capable of killing ALL cells in vitro. Current work is focused on moving these inhibitors into pre-clinical testing using patient-derived xenografts. Pull-down approaches are also being used to identify the substrates of PRL phosphatase activity that are critical to ALL survival and may represent new, tractable drug targets for the treatment of ALL.

#3034 A programmed ribosomal frameshifting defect potentiates the transforming activity of the JAK2-V617F mutation. Yousuf A. Khan, Sergey O. Sulima, Joe Krenda, Vivek M. Advani, John E. Jones, Joseph Briggs, Kim de Kersmaeker, Jonathan D. Dinman, University of Maryland College Park, Potomac, MD; KU Leuven Department of Oncology, Leuven, Belgium.

The JAK-STAT pathway is one of the major mechanisms through which cellular proliferation, differentiation, migration, survival and apoptosis are regulated in response to external stimuli. Promiscuous activation of this pathway is a major driver in the pathogenesis of myeloproliferative neoplasms, often characterized by clonal expansion of mature myeloid cells. The JAK2-V617F allele is...
the most common and well characterized mutation linked to this class of leukemias. A change of G to T at nucleotide 1849 of the coding sequence (CDS) results in substitution of valine to phenylalanine at codon 617. JAK2-V617F mutant cells have strongly increased activation of JAK-STAT signaling, which can partially be explained by its ability abolish inhibition of kinase activity and increase the Km of the enzyme. It is however unclear if these effects are sufficient to explain the strong activation of the JAK-STAT pathway induced by the V617F mutation. Here, we demonstrate that this mutation destabilizes a programmed -1 ribosomal frameshift (-1 PRF) signal in the JAK2 mRNA, leading to decreased rates of -1 PRF promoted by this element. There is an inverse relationship between -1 PRF and mRNA stability. Thus, decreased -1 PRF results in increased abundance of the JAK2 mRNA and increased JAK2 protein expression. Prior studies established that elevated expression levels of JAK proteins are sufficient to transform cells in vitro, suggesting that -1 PRF may normally help to limit JAK2 expression. Silent protein coding changes in the -1 PRF signal slippery sequence abating JAK2 frameshifting also increased JAK2 expression and necropoied the transforming activity of JAK2-V617F in cell culture. Further, the combination of the V617F and slippery site mutations conferred an additive effect on cellular transformation. The presence at least three additional -1 PRF signals in the JAK2 mRNA suggests that -1 PRF plays a significant role in controlling JAK2 expression.

**MOLECULAR AND CELLULAR BIOLOGY / GENETICS: High Throughput Genomics Dissecting Cancer Cell States and Genomic Subtypes**

### #3035 Decomposing oncogenic transcriptional signatures to generate maps of divergent cellular states.

**William J. Kim,1 William C. Hahn,2 Jill Meisirov,2 Pablo Tamayo2. 1Dana-Farber Cancer Institute, Cambridge, MA; 2University of California San Diego, San Diego, CA.**

The systematic sequencing of the cancer genome has provided a powerful framework for identifying genetic alterations in disease causation. However, understanding of the functional consequences of these alterations is necessary to guide appropriate therapeutic strategies. Here, we describe Onco-GPS (Onco-Genic Positioning System), a data-driven analysis framework, and associated experimental and computational methodology, to organize individual tumor samples with shared oncogenic alterations onto a reference map defined by their underlying cellular states. Using this approach, we generated series of RAS/MAPK sub-signatures that reflect known and novel downstream pathways. These sub-signatures were then used to classify KRAS and BRAF mutant cancers into subgroups or cell states, as represented in the novel visual paradigm “OncoGPS map”. OncoGPS approach was first applied to cancers with BRAF mutations. The approach not only accurately classified three groups of BRAF mutant cancers that aligned with sensitivity to BRAF inhibitors, but also identified resistance mechanisms associated with each state, validating the overall approach. OncoGPS also identified four major cell states associated with KRAS mutant cancers. Further characterization of the four cell states showed that multiple molecular features representing epithelial-EMT states and activation of NF-κB pathway delineated two major “axes” of KRAS mutant cancers. In addition to the pathways and cell states defined in each group, the OncoGPS approach is able to identify effective drug combinations which was experimentally tested and confirmed. These results show that the Onco-GPS is an effective analytic framework to explore the complex landscape of oncogenic cellular states across cancers, and a powerful framework to summarize knowledge, establish relationships, and generate more effective disease models for research or as part of individualized precision medicine paradigms.

### #3036 Real-time genomic characterization of metastatic pancreatic cancer to enable precision medicine.

**Andrew J. Aguirre,3 Scott Carter,1 Nicholas Camarda,1 Arezou Ghazani,4 Jonathan Nowak,1 Annacarolina Da Silva,1 Lauren Brais,1 Dorissame Ragov,2 Devins McCabe,1 Lori Marini,1 Kristin Anderka,2 Karla Helvie,1 Nelly Oliver,1 Ana Babic,1 Paul Shyn,2 Douglas Robinson,1 Anuj Patel,1 James Cleary,1 Nadine McCleary,1 Matthew Kulke,1 Thomas Clancy,1 Leona Doyle,1 Jason Hornick,1 Christine Ardito-Abraham,2 Ruth Yu,1 Michael Downes,1 Ronald Evans,1 Richard A. Moffit,2 Jen Jen Yeh,1 William C. Hahn,1 Charles Fuchs,1 Robert Mayer,1 Nikhil Wagle,1 David Tuveson,1 Levi A. Garraway,1 Brian M. Woolp,1 Dana-Farber Cancer Institute, Boston, MA; 2Broad Institute, Cambridge, MA; 3Dana-Farber Cancer Institute, Boston, MA; 4The Lustgarten Foundation, Bethpage, NY; 5Salk Institute, La Jolla, CA; 6University of North Carolina, Chapel Hill, NC; 7Cold Spring Harbor Laboratory and The Lustgarten Foundation, Cold Spring Harbor, NY.**

Pancreatic ductal adenocarcinoma (PDAC) is currently the fourth-leading cause of cancer-related death in the United States and is projected to become the second leading cause by 2030. Most patients present with advanced disease and die within 12 months of diagnosis. Recent genomic studies of primary pancreatic cancer resection specimens have identified several molecular alterations and gene expression profiles that guide the development of precision medicine approaches to clinical management. However, the molecular landscape of metastatic PDAC has been less well characterized. Moreover, biopsy-driven studies in metastatic PDAC have been historically very challenging due to the aggressive course of this disease as well as the low-volume and heterogeneous nature of biopsies that makes deep molecular characterization difficult. Insufficient genomic analysis of a limited number of metastatic samples may result in suboptimal treatment strategies. We used NGS of a panel of PDAC biopsies dominated by expression of a STAT3 program. Ex vivo cultures from platinum-resistant disease. Furthermore, we used patient-derived xenograft (PDX) cohorts, in which we isolated cells for scRNA-seq from vehicle tumors (VEH), treated the other models with carboplatin, and harvested cells at the time of minimal residual disease (MRD) or disease progression (PROG). Results: To date, we have profiled ~12000 single cells from 12 patients with treatment naïve (n=3) or platinum-resistant disease (n=9), including sequential sampling in 3 patients with resistant disease. We observed significant inter- and intra-individual transcriptional heterogeneity guide precision medicine approaches. A recurrent pattern across resistant patients was the differential expression of inflammatory pathways in a subset of cells. In a patient with three consecutive specimens, we observed increasing accumulation of cells expressing a cell state characterized by tumor necrosis factor alpha (TNF-a) signaling. Importantly, these cells were genetically identical to the entire population, supporting the hypothesis that non-encoded mechanisms conferred treatment resistance. In a BRCA-mutant patient, unbiased analysis identified a stemness program in a subpopulation of cells, which was genetically identical to other cells, indicating phenotypic conversion. To systemically interrogate mechanisms of resistance to platinum therapy, sequenced single cells isolated from PDAC models at three time points (VEH, MRD and PROG). In a BRCA-WT PDX model, resistant cells isolated at MRD showed a transcriptional profile that was dominated by expression of a STAT3 program. Ex vivo cultures from platinum-resistant patients were exquisitely sensitive to IAK/STAT3-inhibitor. Live cell imaging revealed that STAT3-inhibition prevented spheroid formation, attachment and clearance through a mesothelial monolayer in vitro. Conclusion: Our
results indicate that non-encoded mechanisms play an important role in the development of treatment resistance in ovarian cancer. Our initial studies indicate an important role of inflammatory pathways in treatment resistance, in development of treatment resistance in ovarian cancer. Our initial studies indicate that non-encoded mechanisms play an important role in the development of treatment resistance in ovarian cancer. Our initial studies indicated that non-encoded mechanisms play an important role in the development of treatment resistance in ovarian cancer.

Avenues for patients with treatment-resistant ovarian cancer.

#3038 Bridging the gap between NGS data and its usability: cancer gene discovery through massive-scale transcriptomic analyses and development of a powerful web-tool for dissemination of these findings. Yashar Niknafs, Nicholas Molen, Balaji Pandian, Matthew Iyer, Arul Chinnaiyan. Univ. of Michigan, Ann Arbor, MI.

Background: In a prior transcriptomic analysis focused on IncRNA interrogation using some of these data, we discovered tens of thousands of novel IncRNAs (Iyer and Niknafs et al, Nature Genetics, 2015). Building on this analysis, we recently developed a markedly improved bioinformatics tool for novel gene/isofrom discovery from massive RNA-seq datasets named TACO (Niknafs et al, Nature Methods, In press, tacorna.github.io). TACO produces high-fidelity transcript structure predictions from large RNA-seq datasets. We now set out to comprehensively leverage both TACO and the publicly available RNA-seq data for discovery of novel transcriptional cancer associations. Additionally, in order to widely disseminate these findings in an accessible manner, we have built a web-tool that provides the scientific community access to these data and analyses. Methods: We have downloaded, curated, and processed 23,823 RNA-seq samples largely from the TGCA, ICGC, GTEx, and CCLE, comprising 37 tissue types and over 35 cancer types. RNA-seq data processing was performed using STAR, Cufflinks, Kallisto, and TACO. The web tool for visualization and access to these data and analyses was built using a JavaScript-based server infrastructure (Node.js) and a relational PostgresQL database. Results: Generation of a consensus transcriptome from this large-scale RNA-seq dataset via TACO resulted in the discovery of tens of thousands of novel transcriptional elements, including intergenic non-coding RNAs and novel splice isoforms of known genes. Such an expansive RNA-seq cohort that includes many normal tissue samples enabled statistically powerful cancer association expression analyses that revealed a myriad of novel cancer genes, especially in tissues for which there was previously little-to-no normal tissue RNA-seq data (e.g., brain and pancreas). Many of these novel transcriptional elements discovered using TACO were also found to be cancer associated. We have built a web-tool to facilitate further analysis and discovery using these data and analyses by the scientific community. The web-tool provides a powerful and intuitive interface for researchers with little-to-no bioinformatics expertise to leverage large-scale RNA-seq datasets. Conclusions: Here we present the largest reported compendium of RNA-seq data, and reveal many novel cancer gene associations. Using a new, powerful gene discovery tool, TACO, we identify a multitude of novel transcriptional elements that are also cancer associated. Despite the abundance of publicly available RNA-seq data, necessary computing resources, data storage, and bioinformatics expertise are barriers to usability of these data by scientific community. Our RNA-seq expression web-tool bridges this gap, and enables users to powerfully interrogate cancer expression across dozens of tumor and tissue types.

#3039 Combinatorial CRISPR-Cas9 gene knockout to enable genetic interaction mapping in human cells. John Paul Shen, Dongxin Zhao, Roman Sasik, Jens Luebeck, Ana Bojorquez-Gomez, Katherine Licon, Trey Ideker, Prashant Mali. UCSD, La Jolla, CA.

Genetic interactions, in particular negative or 'synthetic-lethal' interactions for which simultaneous disruption of two genes causes cell killing, have implications for therapeutic development. The feasibility of this approach has been demonstrated with the recent approval of the drug olaparib, an inhibitor of PARP1, specifically for tumors with loss-of-function mutations in BRCA1/2. However, beyond olaparib, further applications of synthetic-lethal cancer therapy have been limited by poor understanding of the important genetic interactions in a cancer cell, and how these vary from one cancer type to another or from patient to patient. To enable systematic mapping of these genetic interactions networks, we developed a CRISPR-Cas9 screening methodology for knocking out single and pairs of genes in high-throughput. Here, we combined multiplex targeting with array-based oligonucleotide synthesis to create dual-gRNA libraries covering up to 10^4-5 defined gene pairs. In these libraries, each construct bears two gRNAs, with each gRNA designed to target either a gene or a scrambled non-targeting sequence absent from the genome. We conducted genetic interaction screens by transducing the dual-gRNA lentiviral library into a population of cells stably expressing Cas9, maintaining these cells in exponential growth over the course of four weeks, then sampling the relative changes in gRNAs at days 3, 14, 21 and 28 post-transduction. To robustly quantify gene fitness and genetic interactions, we developed a computational analysis framework that integrates all samples across the multiple days of the experiment. Using this method we evaluated all pairwise gene knockout combinations among a panel of 73 genes divided between tumor-suppressor genes (TSG) and cancer-relevant drug targets (DT); including negative controls this amounted to a total of 23,652 combinations. Experiments were performed in three cancer cell lines: HeLa, A549 and 293T. In total, 352 interactions were identified in these three cell lines (146 (90.1%) were private to one cell line. None of the interactions were observed in all three cell lines. These patterns replicated in low throughput assays with combinatorial drugs at 80% precision. In summary, we have introduced a combinatorial CRISPR-Cas9 genetic interaction mapping technology that successfully identifies many therapeutically-relevant genetic interactions in cancer and shows the great importance of elucidating the architecture of the genetic interactions network. Recognizing that there will be great diversity in genetic interaction between different tumors it will be important to perform future studies across a large number of samples, which is enabled by the high-throughput method we have developed.

#3040 Functional genomic screening identifies USP11 as a novel regulator of ERα transcription in breast cancer. Lisa Dwane, Aisling E. O’Connor, Laoghaire Mulrane, Annette M. Dirac, Karin Jirstrom, John P. Crown, Rene Bernards, William M. Gallagher, Triona Ni Chonghaile, Darran P. O’Connor. Royal College of Surgeons Ireland, Dublin, Ireland; Conway Institute, University College Dublin, Dublin, Ireland; Netherlands Cancer Institute, Amsterdam, Netherlands; Malmo University Hospital, Malmo, Sweden; St Vincent’s University Hospital, Dublin, Ireland.

Approximately 70% of breast cancers overexpress the estrogen receptor α (ERα) and depend on this key transcriptional regulator for growth and differentiation. The discovery of novel mechanisms controlling ERα function represents major advances in our understanding of breast cancer progression and potentially offer attractive new therapeutic opportunities. Here, we investigated the role of deubiquitinating enzymes (DUBs), which act to remove ubiquitin moieties from proteins, in regulating transcriptional activity of ERα in breast cancer. To identify DUBs involved in the regulation of ERα transcriptional activity, we performed an RNAi loss-of-function screen using a library of shRNA vectors targeting all human DUB genes. The DUB library consisted of pools of four non-overlapping shRNAs targeting all 108 known or putative DUBs (432 shRNAs in total). We found that suppression of a number of DUBs markedly repressed or enhanced the activity of an estrogen-response element (ERE) luciferase reporter following estradiol (E2) stimulation. Of particular interest, suppression of the BRCA2-associated DUB, USP11, was found to downregulate ERα transcriptional activity. Subsequent validation using two individual siRNAs targeted to USP11 revealed a notable reduction in expression of endogenous ERα target genes in the ZR-75-1 cell line, as quantified using qRT-PCR. Immunoprecipitation of ERα revealed no physical interaction with USP11 nor over E2 induction in transcriptional activity of USP11 to the nucleus, suggesting a potential role in E2-induced transcription. Furthermore, USP11 expression was found to be upregulated in the estrogen-independent cell line LCC1 when compared to their parental MCF7 cells. Knockdown of USP11 in LCC1 cells resulted in decreased mRNA expression of a panel of ERα target genes, suggesting a role for USP11 in an estrogen independent setting. To support the prognostic relevance of USP11, immunohistochemical staining of breast cancer tissue microarrays (n=144) was performed. Kaplan-Meier analysis of this cohort revealed a highly significant association between poor overall survival (OS) (p=0.030) and breast cancer-specific survival (BCSS) (p=0.041). In silico analysis of publically available breast cancer gene expression datasets further supported an association between high USP11 mRNA levels and poor prognosis. We observed a significant correlation between high expression of USP11 mRNA in ER-positive patients and poor distant metastasis-free survival (DMFS) (HR 2.9, CI 1.37-2.91, p=0.00023). This correlation was also significant in ER-positive patients who had received endocrine therapy only (HR 2.9, CI 1.63-5.15, p=0.00015). These results suggest a role for USP11 in driving cellular growth and identify USP11 as novel therapeutic target in breast cancer.

#3041 Duplex Sequencing detects cancer-associated mutations arising during normal aging: Clonal evolution over a century of human lifetime. Jesse J. Salk, Elisabeth Maritschneg, Paul Speiser, Robert Zeillinger, Rosana Risques, Lawrence A. Loeb. University of Washington, Seattle, WA; Medical University of Vienna, Vienna, Austria.

High-throughput DNA sequencing has fundamentally changed practice standards in clinical oncology. We can now systematically examine the dysfunctional genes and pathways of individual tumors and can often pair these with targeted therapies. Moreover, NGS has improved our ability to resolve molecular heterogeneity, such as results from evolving subclones within a tumor or admixtures of cancer-derived and normal DNA in liquid biopsies. However, this...
remains imperfect. With standard NGS, sequencing errors create a background that obscures variants present at less than 1-2%. Methods of tag-based error correction improve accuracy, but most rely on hemimolecular tagging and are thus still limited by certain types of errors caused by DNA damage. Our group pioneered Duplex Sequencing, an error correction technology that independ- ently genotypes and compares both single-stranded halves of individual DNA duplexes. This approach improves sequencing accuracy by >100,000-fold and allows detection of a single mutation among ten million unmutated nucleotides.

We carried out Duplex Sequencing on uterine lavages from women with and without high-grade serous ovarian cancer to determine the sensitivity for identifying tumor-derived TP53 mutations as a screening test. Under blinded conditions we detected tumor-derived mutations in 80% of cancer cases. However, in addition, we found that nearly all women, including those without cancer, carried very low frequency TP53 mutations that did not originate from a tumor. These could not be explained by technical errors, as they exhibited a strong signature of positive selection, indicated by higher-than-random non-synonym- ous fraction, substantial clustering in cancer-associated hotspots and a spec- trum, exonic distribution and predicted pathogenicity closely mirroring TP53 mutations in cancer databases. Furthermore, abundance of these “biological background” mutations increased with age. To more broadly investigate the extent of clonal evolution in normal aging, we used Duplex Sequencing to ex- amine tissue specimens from two individuals without cancer, differing in age by 101 years. We assessed relative mutation load in 22 cancer-associated genes and one non-cancer associated gene in 19 different tissues. Initial results indicate that the extent of age-associated mutation accumulation varies by organ and the pattern of apparent clonal selection is influenced by both tissue type and genetic context. Our findings illustrate the near-universal presence of cancer-like mu- tations arising from age-associated processes in multiple non-neoplastic tissues. Somatic clonal evolution appears to be a ubiquitous part of human life. Beyond many biological implications, this highlights an important specificity challenge that must be accounted for when using ultra-accurate sequencing technologies for cancer screening and other sensitive diagnostics.
identified the ex-miRNA-directed phenotype to be transient due to the rapid decay of the ex-miRNA. We demonstrated that the ex-miR-223-3p transfected from neutrophils to cancer cells was functional as demonstrated by the decreased expression of its target, FOXO1, and the occurrence of epithelial-mesenchymal transition reprogramming. We showed that the engulfed ex-miRNA, unlike the endogenous miRNA, was unstable, enabling dynamic regulation and a return to a non-invasive phenotype within less than 8 hours. This transient phenotype could be modulated by targeting XRNI/PACMAN exonuclease. Indeed, its silencing was associated with slower decay of ex-miR-223-3p and subsequently prolonged the invasive properties. Conclusion: We showed that the steady step-level of engulfed miRNA (ex-miR-223-3p) and its subsequent activity were tumor-specific, highlighting a potential role of tumor-secreted exosomes in cell-to-cell communication. This study aimed to establish a mechanism of action for miR-379, and to engineer MSCs to secrete exosomes enriched with the miR for tumor-targeted delivery. Methods: RNA was isolated from breast tumors and matching lymph node metastases from the same patients, and miR-379 expression quantified by RQ-PCR. HCC1954 breast cancer cells were transduced with lentivirus to express elevated miR-379 (HCC-379) or a control sequence (HCC-NTC). Cells were implanted into the mammary fat pad (MFP) of mice and tumor progression monitored. Subsequently, MSCs were transduced to generate MSC-379 and MSC-NTC. MSC-secreted exosomes were isolated by differential centrifugation, microfiltration and ultracentrifugation. The morphology, size and number of isolated exosomes was characterized using Transmission Electron Microscopy, Western Blot and Nanoparticle Tracking Analysis respectively. Exosomal miRNAs were analysed by RQ-PCR. MFP tumors were established using HCC1954-luciferase cells, followed by IV injection of MSC-379 or MSC-NTC cells, or sequential doses of exosomes derived from either cell population. Results: miR-379 expression was significantly reduced in lymph node metastases compared to primary tumors from the same patients (p<0.009), supporting a tumor suppressor role. Analysis of HCC-379 and HCC-NTC tumor growth in vivo showed no difference in tumor size, however an increase in tumour necrosis (50%-50%) and decrease in lymph node invasion was observed in HCC-379 tumors. Investigation of a potential role for miR-379 in regulating COX-2, revealed an inverse relationship (r = -0.48, p=0.02) at a mRNA level, further confirmed at a protein level. MSC-379 cells showed no significant change in migratory or proliferative capacity in vitro. Mechanofluorescence of MSC-secreted exosomes was confirmed to be consistent with the exosome-associated protein CD63. An increase in miR-379 (>5 fold) was observed in exosomes secreted by MSC-379 compared to MSC-NTC cells. Administration of MSC-379 or MSC-NTC cells, or exosomes derived from either cell population, was well tolerated in vivo with no adverse effects observed. Monitoring tumor response to therapy using IVIS is ongoing. Conclusion: The data presented supports miR-379 as a potent tumor suppressor in breast cancer, mediated in part through regulation of COX-2. Engineering tumor-targeted MSCs to secrete exosomes enriched with miR-379 holds exciting potential as a novel therapy for breast cancer.

#3045 Engineering Mesenchymal Stem Cells (MSCs) to support tumor-targeted delivery of exosome-encapsulated microRNA-379. Killian P. O’Brien, Katie Gilligan, Sonja Khan, Brian Moloney, Kerryn Thompson, Pierce Lalor, Peter Dockery, Helen Ingoldsby, Michael J. Kerin, Roisin M. Dwyer. National University of Ireland Galway, Galway, Ireland.

Mesenchymal Stem Cells (MSCs) are multipotent stromal cells known to migrate specifically to the sites of tumors and metastases, raising potential as tumor-targeted delivery vehicles for therapeutic agents. MSCs secrete exosomes containing genetic material including miRNAs (miRs), that are effectively taken up by cells. Previous work has shown miR-379 to be significantly reduced in breast tumors compared to normal breast tissue. This study aimed to establish a mechanism of action for miR-379, and to engineer MSCs to secrete exosomes enriched with the miR for tumor-targeted delivery. Methods: RNA was isolated from breast tumors and matching lymph node metastases from the same patients, and miR-379 expression quantified by RQ-PCR. HCC1954 breast cancer cells were transduced with lentivirus to express elevated miR-379 (HCC-379) or a control sequence (HCC-NTC). Cells were implanted into the mammary fat pad (MFP) of mice and tumor progression monitored. Subsequently, MSCs were transduced to generate MSC-379 and MSC-NTC. MSC-secreted exosomes were isolated by differential centrifugation, microfiltration and ultracentrifugation. The morphology, size and number of isolated exosomes was characterized using Transmission Electron Microscopy, Western Blot and Nanoparticle Tracking Analysis respectively. Exosomal miRNAs were analysed by RQ-PCR. MFP tumors were established using HCC1954-luciferase cells, followed by IV injection of MSC-379 or MSC-NTC cells, or sequential doses of exosomes derived from either cell population. Results: miR-379 expression was significantly reduced in lymph node metastases compared to primary tumors from the same patients (p<0.009), supporting a tumor suppressor role. Analysis of HCC-379 and HCC-NTC tumor growth in vivo showed no difference in tumor size, however an increase in tumour necrosis (50%-50%) and decrease in lymph node invasion was observed in HCC-379 tumors. Investigation of a potential role for miR-379 in regulating COX-2, revealed an inverse relationship (r = -0.48, p=0.02) at a mRNA level, further confirmed at a protein level. MSC-379 cells showed no significant change in migratory or proliferative capacity in vitro. Mechanofluorescence of MSC-secreted exosomes was confirmed to be consistent with the exosome-associated protein CD63. An increase in miR-379 (>5 fold) was observed in exosomes secreted by MSC-379 compared to MSC-NTC cells. Administration of MSC-379 or MSC-NTC cells, or exosomes derived from either cell population, was well tolerated in vivo with no adverse effects observed. Monitoring tumor response to therapy using IVIS is ongoing. Conclusion: The data presented supports miR-379 as a potent tumor suppressor in breast cancer, mediated in part through regulation of COX-2. Engineering tumor-targeted MSCs to secrete exosomes enriched with miR-379 holds exciting potential as a novel therapy for breast cancer.

#3047 PRC2-mediated silencing of circRNA CDR1as drives miR-7-independent melanoma metastasis. Doug Hanniford,1 Rana Moubarak,1 Jochen Limig,1 Alejandro Ulloa,1 Beatriz Sanchez Sendra,1 Alcida Karr,2 Iman Osman,1 Ioannis Aifantis,1 Eva Hernandez1. 1New York University School of Medicine, New York, NY; 2Duke University, Durham, NC.

Circular RNAs are a novel class of non-coding RNAs with functions that remain poorly characterized in normal and pathological conditions. CDR1as is a non-canonical circRNA observed to act as a sponge for miR-7 in brain tissues. Analysis of RNeasy-Seq data of melanocytes and melanoma cell lines and short-term cultures revealed loss of CDR1as expression as a hallmark of melanoma cells. We confirmed silencing of CDR1as in melanoma cells and tissues by RT-qPCR using divergent primers. Clinically, we observed CDR1as loss associated with metastatic progression and poor patient outcomes in a cohort of fresh-frozen melanoma tissue samples. Depletion of CDR1as in melanoma cell lines enhanced invasion in vitro and lung metastasis in vivo, demonstrating functional significance of CDR1as silencing. Surprisingly, CDR1as depletion had no clear effect on miR-7 activity in melanoma cells, and miR-7 inhibition was insufficient to rescue CDR1as silencing-induced invasion. Moreover, GSEA analyses of proteomic profiling of melanoma cells depleted of CDR1as revealed reductions of proteins involved in oxidative phosphorylation (OXPHOS) and mitochondrial function, suggesting CDR1as loss may alter metabolism of melanoma cells. Mining of CLIP-Seq data sets and subsequent RIP-PCR revealed direct interactions of CDR1as with the Igf2r family of proteins and TARDBP, each of which are involved in regulation of mitochondrial function. These data suggest that CDR1as could regulate the subcellular localization and/or function of proteins involved in OXPHOS and mitochondrial respiration. To understand the transcriptional mechanisms driving CDR1as loss in melanoma, we sought to examine the primary transcript from which CDR1as arises. Interestingly, examination of the CDR1as locus revealed an upstream long non-coding RNA, LINC00632, as a plausible primary transcript of CDR1as. CDR1as and LINC00632 expression is strikingly correlated across human tissues and in melanoma cell lines (>65). Moreover, depletion of LINC00632 using Gapmers reduced CDR1as expression and CD44 silencing-induced invasion and metastasis. Surprisingly, treatment of such melanoma cell lines with the EZH2 inhibitor, GSK126, induced robust re-expression of both CDR1as and LINC00632, and removal of H3K27me3 at LINC00632. Our data document that CDR1as is epigenetically silenced in melanoma, and its loss promotes melanoma invasion and metastasis. Moreover, CDR1as loss could contribute to metabolic adaptations during metastasis through misregulation of RNA binding proteins.

#3048 A noncoding function of TYRP1 mRNA promotes melanoma growth. Marie-Dominique Galibert,1 David Gilot,1 Méloïde Mignaut,2 Laura Belocheit,1 Fabrice Journe2 Aliñosiajogers,1 Emmanuelle Donnou-Fournet,1 Ariane Mogha,1 Nicholas Mouchet,1 Marie-Laure Pinel-Marie,1 Bernard Mari,5 Tristan Monnier,1 Sébastien Corre,1 Arthur Gautron,1 Florian Rambow,2 Petra El Haji,2 Rania Ben Jouria,2 Sophie Tartare-Deckert,2 Jean-Christophe Marine,3 Brice Felden,4 Ghanem Ghanem.1 CNRS UMR6290 - IGDR / University of Rennes, Rennes, France; 2Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium; 3VIB Center for Cancer Biology, Leuven, Belgium; 4INSERM U835, Université de Rennes I, Rennes, France; 5CNRS UMR 7275, Sophia Antipolis, France; 6INSERM U1078, Université de Bretagne Occidentale, Université Européenne de Bretagne, Brest, France; 7INSERM U1065, CNM, Nice, France.
RNAs competition to bind miRNA has been proposed to influence biological systems whether these RNAs are implicated in diseases onset is unclear. Here, we report that TYRPI1 mRNA, in addition to encoding tyrosinase-related protein 1 (TYRP1), indirectly governs cell proliferation by sequestering miR-16 on non-canonical miRNA response elements (MREs). Consequently, sequestered miR-16 is no longer available to repress its miRNA targets such as RAB17, which we show is involved in melanoma cell proliferation and invasion. Restoration of miR-16 tumor suppressor function can be achieved in vitro by silencing TYRP1 or increasing miR-16 expression levels. Importantly, TYRP1-dependent miR-16 sequestration can also be overcome in vivo using small oligonucleotides that mask miR-16 binding sites on TYRP1 mRNA. Together, our findings assign a pathogenic noncoding function to TYRP1 mRNA and underscore miRNA displacement as a new antimelanoma targetted therapeutic approach.

TUMOR BIOLOGY: Early Stages of Metastatic Dissemination

#3049 Tracing the origin of disseminated tumor cells in breast cancer using single-cell sequencing, Jonas Demeulemeester,1 Parveen Kumar,2 Elen K. Møller,3 Sille Nord,3 David C. Wedge,4 April Peterson,5 Randi R. Mathiesen,3 Renathe Fjelldahl,5 Masoud Z. Esteki,2 Koen Theunis,2 Elia F. Gallardo,2 Jason Grundstad,5 Elin Borgen,5 Lars O. Baumbusch,3 Anne-Lise Berresdal-Aske,3 Kevin F. White,3 Vesselka N. Kristensen,2 Peter Van Loo,4 Thierry Voet,4 Bjørn Naud,51 The Francis Crick Institute, London, United Kingdom;2 KU Leuven – Leuven, Leuven, Belgium;3 Oslo University Hospital, Oslo, Norway;4 Wellcome Trust Sanger Institute, Cambridge, United Kingdom;5University of Chicago, Chicago, IL.

Background. Single-cell micro-metastases of solid tumors often occur in the bone marrow. These disseminated tumor cells (DTCs) may resist therapy and lay dormant to progress to cause overt bone and visceral metastases. The molecular nature of DTCs remains elusive, as well as when and from where in the tumor they originate. Here, we apply single-cell sequencing to identify and trace the origin of DTCs in breast cancer. Results. We sequence the genomes of 63 single cells isolated from six non-metastatic breast cancer patients. By comparing the cells’ DNA copy number aberration (CNA) landscapes with those of the primary tumors and lymph node metastasis, we establish that 53% of the single cells morphologically classified as tumor cells are DTCs disseminating from the observed tumor. The remaining cells represent either non-aberrant ‘normal’ cells or ‘aberrant cells of unknown origin’ that have CNA landscapes discordant from the tumor. Further analyses suggest that the prevalence of aberrant cells of unknown origin is age-dependent, and that at least a subset is hematopoietic in origin. Evolutionary reconstruction analysis of bulk tumor and DTC genomes enables ordering of CNA events in molecular pseudo-time and trace the origin of the DTCs to either the main tumor clone, primary tumor subclones, or subclones in an auxiliary lymph node metastasis. Conclusions. Single-cell sequencing of bone marrow epithelial-like cells, in parallel with in-tumor genetic heterogeneity profiling from bulk DNA, is a powerful approach to identify and study DTCs, yielding insight into metastatic processes. A heterogeneous population of CNA-positive cells is present in the bone marrow of non-metastatic breast cancer patients, only part of which are derived from the observed tumor lineages.

#3050 Polymorphisms in the arntl2 promoter affect breast cancer metastasis susceptibility, Ngoc-Han Ha, Kent Hunter. National Cancer Institute, Bethesda, MD.

Breast cancer mortality is primarily due to metastatic lesions rather than primary tumors, yet relatively little is known regarding the mechanisms of metastatic breast cancer, making it difficult to identify patients who are at risk for metastatic disease. Our hypothesis suggests that inherited germline mutations contribute to metastatic disease and that these single nucleotide polymorphisms (SNPs) could be used to predict outcome in breast cancer patients. To investigate the effect of inherited SNPs on metastasis, we used a mouse genetics approach comparing strains with high (FVB) and low (MOLF) metastatic phenotypes and identified Arntl2, a circadian rhythm transcription factor, as a gene whose differential expression predicted outcome in breast cancer patients. To identify SNP differences in Arntl2 between MOLF and FVB, we performed whole genome sequencing of MOLF and compared it to the FVB genome. Overlapping the data with DNase hypersensitivity sites revealed 10 SNPs in the predicted promoter of Arntl2. To test the causative role of the SNPs on Arntl2 expression in vivo, metastatic cell lines were engineered using the CRISPR-Cas9 approach to specifically replace the FVB Arntl2 promoter with that of MOLF. In agreement with our hypothesis, substitution of the MOLF promoter reduced Arntl2 transcript levels and subsequently decreased lung metastases in orthotopic implantation assays. In vitro pulldown experiments with strain-specific promoter probes revealed potential differential binding of chromatin modifier proteins of Arntl2, suggesting the potential of the SNPs to influence promoter transcription. Finally, analysis of SNPs associated with Arntl2 expression in a cohort of Chinese breast cancer patients revealed significant correlation of Arntl2 expression with overall survival, validating this gene as a marker in humans. Since Arntl2 is a transcription factor, current studies are focused on identifying Arntl2-regulated genes to investigate downstream pathways involved in metastasis. This study has important implications regarding the role of circadian rhythm in cancer progression and provides a potential mechanism to explain the increased risk of breast cancers in nightshift workers. Furthermore, this provides the first evidence that transcriptional control elements can be engineered using CRISPR-Cas9 to establish the causative role of SNPs in inherited susceptibility to cancer metastasis.

#3051 Mechanism of early dissemination and metastasis in Her2+ mammary cancer, Kathryn Harper,1 Maria Soledad Sosa,2 David Entenberg,2 Hedayatollah Hosseini,3 Julie Cheung,3 Rita Nobre,1 Alvaro Avivar-Valderas,1 Chandandaneep Nagi,1 Nomaed Girnios,5 Roger Davis,5 Eduardo Farias,1 John Condeelis,2 Christoph A. Klein,3 Julio A. Aguirre-Ghiso,5 Icahn School of Medicine at Mount Sinai, New York, NY; 2Albert Einstein College of Medicine, New York, NY; 3University of Regensburg, Regensburg, Germany; 4University of Massachusetts Medical School, Worcester, MA.

Metastasis is the leading cause of cancer related deaths and these lesions develop from disseminated cancer cells (DCC) that can remain dormant. Metastasis initiating cells are thought to originate from a subpopulation present in progressed invasive tumors. However, DCCs detected in patients before the manifestation of breast cancer metastasis contain fewer genetic abnormalities than primary tumors or than DCCs from patients with metastases. These findings and those in pancreatic cancer and melanoma models argued that dissemination might occur during early stages of tumor evolution. Yet, the mechanisms that might allow early-disseminated cancer cells (eDCC) to complete all steps of metastasis were unknown. Here we show that in early lesions (EL), before any overt primary tumor (PT) masses are detected, there is a sub-population of Her2+/P-p38lo/P-ATF2lo/TWISThi/E-cadherinlow early cancer cells that are invasive and disseminate to target organs. Intra-vital imaging and organoid studies of early lesions revealed that Her2+ eDCC precursors locally invaded, intravasated and lodged in target organs. Her2+ eDCs activated a Wnt-dependent EMT-like dissemination program but without complete loss of epithelial phenotype that was reversed by Her2 antagonism only, while surprisingly, in murine eDCCs are TWISThi/E-cadherinlow and dormant, they eventually initiate metastasis. Our work identifies a mechanism for early dissemination whereby Her2 aberrantly activates a program similar to mammary ductal branching that spawns eDCs capable of forming metastasis after a dormancy phase.

#3052 Defining the role of EMT in breast cancer dormancy and metastasis in vivo, Kay T. Yeung, Jing Yang. UC San Diego, San Diego, CA.

Breast cancer patients can develop metastases from dormant tumor cells years after primary tumor resection and treatment. Despite the clinical significance of metastatic latency, the exact cellular and molecular events regulating tumor dormancy remains largely unknown due to challenges in detecting single dormant tumor cells in vivo and characterizing the dormant tumor microenvironment. A developmental program termed Epithelial Mesenchymal Transition (EMT) has been shown to play an important role in tumor cell dissemination, tumor dormancy, and chemoresistance. During EMT, stationary epithelial cells lose their epithelial characteristics, including adherent junctions and apical-basal polarity, and acquire mesenchymal shape and properties. These cells gain the ability to migrate into the circulation, lodge into a distant organ, and form dormant tumor niches. We have previously shown that reversal of EMT is a key driving force in dormant tumor cell proliferation and macrometastasis formation in a skin carcinoma mouse model. Here, we present a novel metastatic breast cancer mouse model in which HER2-driven primary breast tumor cells are labelled with red fluorescent protein with inducible EMT transcription factor TWIST1. We established this mouse model by crossing the TetOON-TWIST1 mice with the MMTV-rTA mice to generate the MMTV-rTA/TetON-TWIST1 double transgenic mice. To generate HER2-driven breast tumors in these mice, we administered concentrated lentivirus that expresses both the HER2 gene and the tdTomato fluorescent marker under one promoter via intraductal
injection. This allows for rapid and synchronized development of primary tumors which recapitulate the formation of sporadic human breast cancer which arises in single cells in otherwise normally developed breast tissue. TWIST1 expression in the luminal layer of the mammary gland was then induced via oral doxycycline. Upon expression of TWIST1, Her2-driven breast tumor cells were found in circulation and in the lungs as micrometastases. We determined that disseminated breast tumor cells mostly remained in dormancy until TWIST expression was switched off by doxycycline withdrawal. We showed that tumor dormancy is partly due to the inability to reverse EMT and regain proliferative capacity. Lastly, using our mouse model to track disseminated breast cancer cells, we will characterize the associated dormant residing niche to further our understanding of tumor dormancy in connection with EMT and immune escape.

**#3053 Stability and stenness of the hybrid epithelial-mesenchymal phenotype.** Mohit Kumar Jolly,1 Dongya Jia,1 Satyendra C. Tripathi,2 Steve Mooney,3 Muge Celiktas,4 Samir M. Hanash,2 Sendurai A. Mani,1 Kenneth J. Pienta,1 Estel Ben-Jacob,1 Herbert Levine,1 Rice University, Houston, TX; 2 University of Waterloo, Waterloo, Ontario, Canada; 3 Johns Hopkins University, Baltimore, MD.

Transitions between epithelial and mesenchymal phenotypes — EMT and MET — are hallmarks of cellular plasticity during embryonic development and cancer metastasis. During these transitions, cells can also adopt a hybrid epithelial/mesenchymal (hybrid E/M) phenotype enabling them to migrate collectively as observed during gastrulation, wound healing, and clusters of circulating Tumor Cells (CTCs). The hybrid E/M phenotype has largely been tacitly assumed to be metastable, i.e. transient state. Here, we integrate mathematical modeling with in vitro experiments to identify certain ‘phenotypic stability factors’ (PSFs) - GRHL2, OVOL2 and ΔNP63α that can stabilize a hybrid E/M phenotype. We show that H1975 (NSCLC cell line) cells can display a hybrid E/M phenotype stably and migrate collectively, a behavior that is impaired by knockdown of GRHL2 or OVOL2. Further, our computational model predicts that these PSFs can also associate hybrid E/M phenotype with high tumor-initiating potential, a prediction strengthened by the observation that the higher levels of one or more of these PSFs may predict poor patient outcome. Overall, our results suggest that a hybrid E/M phenotype need not be ‘metastable’, and bolster the notion that a hybrid E/M phenotype, but not necessarily full EMT, associates with aggressive tumor progression.

**#3054 Tracing EMT with fluorescent biosensors (chromobodies) in living cancer cells.** Julia Maier, Theresa Plaga, Stefanie Egartermaier, Björn Traenkle, Jana Friesen and Katja Rothbauer. University of Tuebingen, Reutlingen, Germany.

Epithelial-mesenchymal transition (EMT) is a complex cellular program involved in the progression of epithelial cancers to a metastatic stage. During this process characteristic epithelial marker proteins are repressed in favor of mesenchymal biomarkers, such as the intermediate filament protein vimentin. There is an ongoing need for reliable cell-based EMT model systems, which can be adapted to screening campaigns for novel anti-metastatic therapies. Imaging-based screening systems are restricted to endpoint immunofluorescence studies that provide only little information about the dynamics of the process, or to ectopic expression of EMT markers, fused to fluorescent proteins. However, overexpression of EMT marker proteins, particularly vimentin, have been shown to influence the EMT phenotype of the cells. Here we introduce chromobodies as an innovative technology to monitor EMT in living non-small lung cancer cells. Chromobodies are fluorescently labeled single-domain-antibodies that are functional within in living cells and directly target endogenous proteins. We generated chromobodies against the EMT biomarkers vimentin, actin and β-catenin and stably introduced them into the genome of A549 cells. Upon induction of EMT with transforming growth factor β (TGF-β), we could visualize the dynamic reorganization of endogenous vimentin, actin and β-catenin in the established EMT chromobody models in 2D as well as 3D cell culture. By means of these chromobody cell lines we further developed a 2D high content analysis system to visualize and quantify spatiotemporal changes of the endogenous biomarkers vimentin and actin during the EMT process. In a proof-of-principle screen, using known EMT-affecting reagents (e.g. TGF receptor inhibitors LY364947 and SB505124) as well as EMT-unrelated agents, we were able to show that this high content analysis system can be applied to select compounds that inhibit TGF-β-induced EMT. In summary, live cell imaging of EMT markers with chromobodies provides a novel versatile approach to study EMT in real time and allows the identification of EMT inhibitors as potential candidates for anti-metastatic therapies by high content screening.

**#3055 A permanent lung imaging window reveals, for the first time, the steps of extravasation, seeding and growth of early metastatic dissemination.** Sona E. Voiculescu, Yarong Wang, Maja Oktay, John Condeelis, David Ennberg, Albert Einstein College of Medicine, Bronx, NY.

Metastatic disease is the major cause of cancer mortality and is responsible for over 1/2 million deaths each year in the U.S. alone. Of the three most common sites of metastasis observed clinically (bone, lung and liver), the most difficult to study using intravital microscopy is the lung: a delicate organ in perpetual motion. We have recently developed and validated an implantable, permanent, lung imaging window which allows high-resolution multiphoton imaging of the intact, breathing (without ventilation), murine lung over days to weeks of repeated imaging. This window does not use vacuum to immobilize the lung tissue thereby avoiding well-known artifacts associated with vacuum lung windows. Using our new window, we have been able to visualize, with single cell resolution, the steps of metastasis in a clinically relevant, spontaneously-metastasizing murine breast cancer model. We present the first direct visualization of tumor cell arrival, extravasation, and progression to micro-metastases. Further, we have observed, for the first time, the activity of the tripartite structure called Tumor MicroEnvironment of Metastasis (TMEM) in metastatic lung lesions directly demonstrating the mechanism of dissemination from metastatic lung tumors. Using this approach to imaging live lung tissue serially, within a single animal, we are investigating the mechanisms underlying the fate of tumor cells arriving in the lung with the ultimate goal of directly identifying signals behind seeding and survival of metastatic tumor cells and their responses to interventions in real time.

**TUMOR BIOLOGY: Tumor and Stromal Cell Interactions**

**#3056 Genetic deletion of insulin-like growth factor binding protein-7 (IGFBP7) promotes hepatocellular carcinoma (HCC): a novel role of IGFBP7 in regulating antitumor immune surveillance.** Maaged A. Akiel, Chunqing Guo, Xia Li, Devaraja Rajasekaran, Rachel G. Mendoza, Nidhi H. Jarival, Chadia L. Robertson, Mark A. Subler, Jolene J. Windle, Paul B. Fisher, Devanand Sarkar, Virginia Commonwealth University, Richmond, VA.

In the US, the incidence and mortality rates of hepatocellular carcinoma (HCC) are alarming increasing since no effective therapy is available for the advanced disease. Activation of IGF signaling is a major oncogenic event in diverse cancers, including HCC. Insulin-like growth factor binding protein-7 (IGFBP7) inhibits IGF signaling by binding to IGF-1 receptor (IGF-1R) and functions as a potential tumor suppressor for hepatocellular carcinoma (HCC). IGFBP7 abrogates tumors by inducing cancer-specific senescence and apoptosis and inhibiting angiogenesis. We now document that Igfbp7 knockout (Igfbp7−/−) mouse shows constitutive activation of IGF-1R signaling, presents with pro-inflammatory and immunosuppressive microenvironment, and develops spontaneous tumors in lungs and liver and markedly accelerated carcinogen-induced HCC. Loss of Igfbp7 resulted in increased proliferation and decreased senescence in hepatocytes and mouse embryonic fibroblasts that could be blocked by an IGF-1 receptor inhibitor. A significant inhibition of genes regulating immune surveillance was observed in Igfbp7−/− lungs which was associated with marked inhibition in antigen cross-presentation by Igfbp7−/− dendritic cells. IGFBP7 overexpression inhibited growth of HCC cells in syngeneic immune competent mice which could be abolished by depletion of CD4+ or CD8+ T lymphocytes. Our studies unravel modulation of immune response at a novel component of pleiotropic mechanisms by which IGFBP7 suppresses HCC. Even though HCC has an immunosuppressive milieu, immune targeted therapies are beginning to demonstrate significant objective responses in clinical trials. IGFBP7 might be an effective anti-HCC therapeutic by directly inhibiting cancer cells and stimulating an anti-tumor immune response.

**#3057 Gut microbiome controls liver metastasis.** Chi Ma, Qing Fu, Tim Gretin, NC/NH, Bethesda, MD.

Aim: The gut microbiome can modify tumor immunity and has been suggested to be involved in the development of liver cancer. However, it remains unknown how the gut microbiome controls hepatic immune responses. This study was designed to examine the effect of the gut microbiome on
liver antitumor immunity, and to study potential mechanism. Experimental procedure: Antibiotic cocktail in drinking water was given to remove mouse gut microbiota. Control mice were kept on regular water. EL4 thymoma cells were injected s.c. to induce spontaneous liver metastasis. B16 melanoma and CT26 colon cancer cells were injected intrasplenically to form liver metastasis. Lung metastasis was induced by intraperitoneal injection of tumor cells. Antibiotic cocktail efficiently depleted gut bacteria. Removing gut commensal bacteria did not affect the growth of primary s.c. tumors, but impaired formation of liver metastasis in different models. The inhibitory effect on liver metastasis by removing gut microbiome was found after intrasplenic injection of tumor cells to form liver metastasis using both B16 melanoma and CT26 colon cancer tumor cells. Interestingly, formation of lung metastasis caused by tail vein injection of B16 cells was not impaired by antibiotics treatment, suggesting a liver specific effect. The inhibition of liver metastasis by antibiotic treatment was absent in Rag1 knockout mice, suggesting that the mechanism is mediated by the adaptive immune system. Conclusion: Our results suggest that the gut microbiome affects the liver immune microenvironment and modulates antitumor immunity.

#3058 Syndecan-1 mediates breast cancer metastasis to the brain through IL-8 and PECAM-1 signaling. Sierra Mosticone Wangensteen,1 Megan Sayyad,2 Madhavi Puchalapalli,1 Megan Sullivan,3 Jamie Singh,1 Briana Ratchford,1 Jayda Abrams,1 Majid Jahromi,1 Bin Hu,1 Michael Idoowu,2 Jennifer Kobinski3,1.2Virginia Commonwealth University, Richmond, VA; North Shore University Health System, Evanston, IL.

Brain metastasis is a devastating, late-stage event affecting approximately 10-30% of breast cancer patients. However, it is not well understood how breast cancer cells migrate across the blood-brain barrier (BBB) and invade into the brain. Syndecans (Sdc) are cell surface heparan sulfate proteoglycans (HSPGs) that have previously been linked to breast cancer progression and metastasis. Preliminary findings from our lab indicate that Sdc1 may be involved in breast cancer metastasis to the brain. In clinical samples from breast cancer patients with brain metastases, 67% of the brain metastasis samples stained positive for Sdc1 expression. In an experimental mouse model of metastasis, we found that silencing expression of Sdc1 in MDA-231 breast cancer cells greatly reduced metastasis specifically to the brain, with no difference in lung or bone metastases. Additionally, silencing expression of Sdc1 in MDA-231 cells resulted in a reduction in cancer cell migration across an in vitro BBB transwell model system. These findings prompted us to investigate the mechanism through which Sdc1 facilitates breast cancer cell migration across the BBB. Since BBB endothelial cell junctions have been reported to be disrupted in metastasis to the brain, immunofluorescence was performed to examine localization of junction proteins in our BBB model following addition of conditioned medium (CM) from MDA-231 non-silenced (NS1) control and Sdc1KD cells. We observed disruption in PECAM-1 localization at BBB endothelial cell junctions upon addition of CM from MDA-231 NS1 cells with markedly less disruption occurring upon treatment with CM from Sdc1KD cells. These results suggest that paracrine factors secreted from MDA-231 cells may facilitate breast cancer cell migration across a BBB-like barrier compared to Sdc1KD endothelial cells. By performing a cytokine array using CM from MDA-231 cells, we determined that Sdc1KD CM contained lower levels of IL-8 than MDA-231 NS1 cell CM. These findings were confirmed by ELISA, qRT-PCR, and multiplex analysis. We then went on to treat endothelial cells in the xCelligence in vitro BBB model system with IL-8 and observed a sufficient decrease in cell index readings, suggesting that IL-8 affects BBB barrier permeability. Taken together, our results suggest that Sdc1 supports breast cancer cell migration across the BBB through a signaling mechanism involving IL-8 and PECAM-1. Elucidating this mechanism will allow for the development of therapeutic strategies to combat breast cancer cell metastasis to the brain. [S.M.W. and M.S. contributed equally to this work.]

#3059 UHRF1 deficiency exerts anti-tumor immune responses in non-small cell lung cancer. Handan Xiang, Junying Chen, Xiao-Fan Wang, Duke University, Durham, NC.

Immune surveillance is essential for effective tumor clearance. However, the tumor microenvironment is typically impermissive for proper stimulation of both innate and adaptive immune responses. Epigenetic inhibitors have recently been shown to function as immune sensitizing reagents in the suppressive microenvironment. In our search for novel epigenetic regulators with immunomodulatory functions, we found ubiquitin-like with PHD and ring finger domain-containing protein 1 (UHRF1) being crucial for the suppression of immune activation. Loss of UHRF1 in cancer cells led to significant reduction of tumor masses in a syngeneic lung cancer model. The tumor shrinkage was due to increased infiltration of T cells with the enhanced ability to proliferate and produce IFN-gamma. Mechanistically, lung cancer cells deficient of UHRF1 had elevated expression of endogenous retrovirus genes due to DNA demethylation, which associated with enhanced type I interferon responses. Type I interferons are potent antitumor cytokines induced by pro-inflammatory stimuli and are critical for protection against metastasis. The inhibition of UHRF1 promoted PD-L1 upregulation in tumor microenvironment, yet they can also dampen T cell activation by upregulating PD-L1 expression in tumor cells. Surprisingly, UHRF1 deficient cancer cells failed to upregulate PD-L1 even in the presence of excessive type I interferons, indicative of additional regulatory pathways for PD-L1. We further found that UHRF1 directly interacted with PD-L1 promoter regions and that UHRF1 deficient cancer cells failed to overwhelm type I interferon responses while diminished PD-L1 expression, which synergistically contributed to the immune activation phenotype. Moreover, in non-small cell lung cancer (NSCLC) patients, UHRF1 expression was positively correlated with PD-L1 expression as well as poor prognosis. Together, our data suggested that UHRF1 represents a novel immuno-oncology target for NSCLC.

#3060 Role of curcumin in targeting cancer-associated fibroblasts and modulation of tumor microenvironment in dendritic cell-based immunotherapy. Sheefa Mirza,1 Nayen Jain,1 Rakesh Rawal2. 1Gujarat University, Ahmedabad, India; 2The Gujarat Cancer and Research Institute, Ahmedabad, India.

INTRODUCTION: Cancer associated fibroblasts (CAFs), main component of tumor microenvironment (TME), modulate the recruitment and functions of tumor-associated immune cells by secreting various growth hormones, miRNAs and cytokines; thus having an important role in generation of immunosuppressive TME which is yet to be elucidated. Curcumin is known to have various properties including capability to modulate numerous target proteins including transcription factors, receptors, kinases, cytokines, enzymes and growth factors. Thus, aim of the study was to evaluate the effect of miRNAs and cytokines released by lung cancer patients’ derived CAFs and to assess immunomodulatory potential of curcumin on DC maturation by targeting these CAFs through modulating their TME. METHODOLOGY: CAFs were cultured from lung cancer patient derived tumor tissue biopsy and characterised using CAF-specific markers. Further, immature DCS (iDCs) were differentiated in presence of GM-CSF and IL-4 for 5-6 days. These iDCs were cultured in the presence of conditioned media derived from CAFs (CAFS-CM) as well as NFs (Normal Fibroblasts, NFs-CM) at day 6 for 48 hours to evaluate the effect of CAFs on DC maturation. Mature DCS (mDCs) were characterized by the presence of maturation makers CD80, CD83, CD86 and CTLA4 using qRT-PCR. Moreover, expression of miR-221, miR-222, miR-135, miR-142-3p and miR-146a was assessed to evaluate the role of epigenetic regulators on DC maturation. Cytokine profiling of CAFs-CM as well as CAFs-CM treated with curcumin was conducted. RESULTS: α-SMA+Vimentin+ cells were considered as CAFs. A significant upregulation of CD80, CD83 and CD86 was observed when cultured in the presence of NFs-CM while a remarkable downregulation of these markers was found when cultured in CAFs-CM. CTLA-4 was down regulated in presence of CAF-CM as compared to NFs-CM. Amongst all miRNAs, miR-146a was shown to be up regulated dramatically in CAF-DCs (DCs cultured in CAFs-CM) as well as in CAFs-CM, suggesting the immunosuppressive role of miR-146a. Further, an increased expression of miR-146a was positively correlated with increased expression of anti-inflammatory cytokines like IL-6, IL-10, TGF-β and decreased expression of TNF-α (pro-inflammatory) in CM derived from CAFs. Moreover, curcumin had the potential to convert regulatory DCs facilitated by CAFs into mature DCs (mDCs), thereby enhancing DC maturation. The inhibitory effect on miR-221 and miR-222 was observed by curcumin. CONCLUSION: These findings provide insights into understanding the immunomodulatory role of curcumin in targeting CAFs and modulating the tumor microenvironment, thus enhancing anti-tumor immune response in DC based therapy.

#3061 Tumor-derived PAI-1 promotes macrophage M2 polarization by stimulating an autocrine IL-6–STAT3 pathway. Marta H. Kubala, Veronica R. Placencia, Yves A. DeClerck. Children’s Hospital Los Angeles, Los Angeles, CA.

Macrophages can exhibit a spectrum of activation states ranging from a pro-inflammatory and anti-tumorimunogenic M1 (CD40+, IL-12(high), IFN-gamma(high)) to a pro-tumorigenic M2 (CD163+; IL-10(high), CD206+, Arg1 (mouse) phenotypetype. Plasminogen activator inhibitor-1 (PAI-1) is overexpressed in many cancers and although it acts as a protease inhibitor it is paradoxically correlated with poor outcome. PAI-1 exerts its pro-tumorigenic role via pro-
Angiogenic and anti-apoptotic functions. Increasing evidence points towards the importance of PAI-1 in inflammation and tumorigenesis, but its influence on the immune component of the tumor microenvironment has not yet been investigated. Here we report a novel observation that PAI-1 is a regulator of macrophage polarization. We observe that in PAI-1 KO mice xenotransplanted with 3 human cancer cell lines (HT1080, A549, HCT116) in which PAI-1 was knocked down (KD), tumors were less infiltrated with macrophages with lower Arg expression compared to WT mice implanted with PAI-1 pos. tumors. We demonstrate that recombinant PAI-1 through its uPA interactive domain increased the expression of M2 polarization markers (CD163, IL-10) and decreased or did not affect the expression of M1 polarization markers (iNOS, CD80) in human peripheral blood monocytes. Further investigation demonstrated that treatment of monocytes with PAI-1 induced a rapid (2 h.) increase in IL-6 mRNA and secretion (4 h) of the protein that was followed by phosphorylation (8-24 h.) of signal transduction and activation of transcription 3 (STAT3) in monocytes. Further linking STAT3 activation to PAI-1, we observed a decreased pSTAT3 in monocytes co-cultured with HT1080 cells upon PAI-1 downregulation. We found that STAT3 activation was downstream of IL-6 and responsible for M2 polarization, as the blockade of the IL-6 receptor with a function blocking antibody (tocilizumab) or inhibition of STAT3 activation by a JAK2/STAT3 inhibitor (ruxolitinib) prevented M2 polarization of monocytes by PAI-1. The contribution of PAI-1 to M2 polarization of macrophages was tested in PAI-1 KO mice implanted with human tumors in which PAI-1 expression was controlled by doxycycline. These experiments demonstrated that induction of PAI-1 in established tumors increased the presence of tumor associated macrophages (TAM) (F4/80 +) and decreased the presence of M1 (iNOS +) TAM. Further supporting the presence of an autocrine PAI-1/IL-6 pathway in macrophage polarization in human cancers, a meta-analysis of gene expression array data indicated strong correlations between PAI-1 and IL-6 expression in breast (P = <0.05) and colon (P = <0.01) cancers and between PAI-1 and CD163 in colon cancer (P = <0.0001). The data thus identify a new pro-tumorigenic function for PAI-1 in the communication between tumor cells and macrophages where tumor-derived PAI-1 activates an autocrine IL-6/STAT3 pathway that promotes M2 polarization.

#3062 Metformin inhibits TGFβ-induced stromal ECM remodeling to impede invasion in ovarian cancer. Peter C. Hart, Shermeen Sheikh, Ernst Lengyel, Iris Romero. University of Chicago, Chicago, IL.

Recently, our group has elucidated that metformin, the commonly used medication for type 2 diabetes, has anti-ovarian cancer (OvCa) effects in preclinical models and use of the drug is associated with improved OvCa survival in retrospective epidemiological studies. While the cytotoxic effects of metformin in ovarian cancer cell lines is well demonstrated, the impact of metformin on tumor-stromal interactions in the tumor microenvironment is not well defined. Currently, our work has focused on the impact of metformin on the tumor microenvironment and cancer cell behavior in physiologic models of tumor metastasis. Using a 3D organotypic model, we demonstrate that OvCa cell invasion is inhibited by metformin. In evaluating the molecular mechanism mediating this effect we show that OvCa cells co-cultured with stromal cells or stimulated with TGFβ1 upregulated the production of the extracellular matrix (ECM) proteins fibronectin and collagen 1 and that this effect is attenuated by metformin. Interestingly, metformin’s inhibition of ECM production was not dependent on AMP-activated kinase (AMPK), a common metformin target. To expand on this finding we performed label-free shotgun proteomics and identified collagens, fibronectin and TGFBR2 to be among the proteins most highly repressed in response to metformin exposure in OvCa cells. Finally, we identified that this repression of ECM remodeling by metformin was associated with an overall reduction in TGFBR2 expression and consequent SMAD/STAT3 activity. Taken together, our data indicate that metformin may have novel targets in the tumor microenvironment where it targets pro-tumorigenic effects of the stromal compartment by repressing TGFβ-dependent stromal cell ECM secretion, thereby inhibiting ovarian cancer invasion and metastasis.
Castrate resistant prostate cancer (CRPC) remains the primary cause of mortality for prostate cancer (PCa) patients. Several therapies are currently approved for use against CRPC, consisting mostly of androgen receptor (AR) signaling inhibitors and toxic anti-microtubule chemotherapeutics that do not score long-term durable responses. Recent deep-sequencing analysis studies have elucidated the complex and heterogeneous landscape of CRPC, highlighting the necessity to develop effective targeted therapies against specific CRPC subtypes or therapies effective against numerous subtypes of CRPC. PTEN and p53 are two of the most frequently altered genes in CRPC, and are associated with therapy resistance and a poor clinical prognosis. The objective of this study was to identify and subsequently validate therapeutic targets against models of advanced PCa, specifically in the absence of PTEN and p53. Using non-biased high throughput screening technology, we identified HSP90 inhibitors to be potent and efficacious against a model of PTEN/p53 null PCa. HSP90 is a critical regulator of prostate cancer cell signaling homeostasis, and recently developed second generation compounds targeting HSP90 are effective against genetically heterogeneous panels of cancer cell lines and have favorable safety parameters in the clinic. Screening results and subsequent validation efforts in vitro identified ganetespib as the most potent HSP90 inhibitor, with efficacy that translated into established human PCa cells lines. Ganetespib also displayed strongly inhibitory activity against PTEN/p53 null progenitor cells, a plastic subpopulation of PCa believed to be partly responsible for therapy resistance. In vivo, ganetespib completely inhibited progression of PIN to invasive adenocarcinoma in the adenocarcinoma in situ (Aci) mouse model, leading to a significant reduction in tumor weight. Expanding from the mouse model to clinically predictive PDX-derived LuCaP models of human PCa, we found that ganetespib displayed a range of activity against a genotypically diverse array of 11 LuCaP organoids ex vivo. Furthermore, the PTEN null/p53 altered LuCaP 136 displayed a robust response to ganetespib in vivo, with ganetespib causing a significant reduction in tumor growth. Mechanistic interrogation in vitro, ex vivo, and in vivo revealed ganetespib induced effects to be multifactorial and model specific, with unifying trends being inactivation of PI3K signaling and modulation of cell cycle regulatory proteins. In all, these data indicate that inhibition of HSP90 is a novel therapeutic to treat advanced PCa via multifactorial perturbation of growth regulatory pathways.

**#3066 Inhibition of hsp90 by auy922 preferentially kills mutant KRAS colon cancer cells by activating Bim through ER stress.** Chun Yan Wang, Su Tang Guo, Jia Yu Wang, Xu Guang Yan, Margaret Farrellly, Yuan Yuan Zhang, Fen Liu, Hamed Yari, Ting Li, Fu Xi Lei, Lei Jin, Chen Chen Jiang, Xu Dong Zhang. University of Newcastle, Newcastle, Australia.

Colon cancer is one of the most common and deadly malignancies (1). Despite recent advances in early diagnosis and the development of molecularly targeted therapy, the overall survival of patients with metastatic colon cancers remains disappointing (1). This is often associated with resistance of colon cancer cells to systemic therapies resulting from oncogenic mutations of KRAS that drive activation of multiple downstream signalling pathways important for cell survival and proliferation. Here we report that mutant KRAS colon cancer cells are nevertheless more susceptible to apoptosis induced by the heat shock protein 90 (HSP90) inhibitor AUY922. We determined that although AUY922 inhibited HSP90 activity with the comparable potency in colon cancer cells irrespective of their KRAS mutational statuses, those with activating mutations of KRAS were markedly more sensitive to AUY922-induced apoptosis. This was associated with upregulation of the BH3 only proteins Bim, Bik, and PUMA. However, only Bim appeared essential, in that knockdown of Bim abolished, whereas knockdown of Bik or PUMA only moderately attenuated apoptosis induced by AUY922. Mechanistic investigations revealed that endoplasmic reticulum (ER) stress was responsible for AUY922-induced upregulation of Bim, which was inhibited by a chemical chaperone or overexpression of GRP78. Conversely, siRNA knockdown of GRP78 or XBP-1 enhanced AUY922-induced apoptosis. In addition, AUY922 inhibited the growth of mutant KRAS colon cancer xenografts through activation of Bim that was similarly associated with ER stress. Taken together, these results suggest that AUY922 is a promising drug in the treatment of mutant KRAS colon cancers, and that agents that enhance the apoptosis-inducing potential of Bim may be useful to improve the therapeutic efficacy.


KRAS-mutant cancers are notoriously therapy-resistant. While responsible for oncogenesis, mutant KRAS or the sustained activation of KRAS-driven effectors may or may not be required throughout the course of cancer progression.
Thus, understanding which tumors remain dependent on oncopgenic KRAS could lead to the identification of unique vulnerabilities and opportunities to treat them. We previously discovered that the carbohydrate-binding Galectin-3 (Gal-3) brings together KRAS with integrin αvβ3 at the cell membrane in epithelial cancer cells to promote tumor progression. Here, we show that by directly binding to the cell surface receptor integrin αvβ3, Gal-3 drives addiction of oncopgenic KRAS by enhancing macropinocytosis and reducing mitochondrial reactive oxygen species (ROS). This pathway drives increased expression of SOD2, a superoxide dismutase that clears mitochondrial ROS to protect against cell death. Targeting Gal-3 with a clinically active drug decreases macropinocytosis and increases ROS to eradicate KRAS-addicted lung cancer in patient-derived xenografts and spontaneous KRAS-driven lung cancer in mice. Our work reveals Gal-3 as a druggable target for KRAS-addicted lung cancer, and indicates integrin αvβ3 as a biomarker to identify this dependence.

#3068 Proxotoxic stress associated with mTORC1 activation in ovarian carcinoma: pro tease inhibition as a therapeutic strategy. M. Herman Chui, Patricia Shaw, Robert Rottapel. University of Toronto, Toronto, Ontario, Canada. Genetic profiling studies of high grade serous ovarian carcinoma have revealed recurrent alterations in the mTORC1 signalling network (e.g. mutations/copy number alterations in PTEN, TSC1, TSC2, and PIK3CA) and pathway activation, detected by phospho-4E-BP1, has been associated with poor prognosis. We sought to characterize functionally the role of mTORC1 signalling and its therapeutic implications in ovarian cancer. Treatment of ovarian cancer cell lines with Gal-3 transcripts resulted in inhibition of mTORC1 signalling and decreased rate of protein synthesis. However, irrespective of PTEN mutation status, only mild cytostatic effects were achieved even with high concentrations of rapamycin. We next examined the phenotypic consequences of mTORC1 activation, using siRNA directed against TSC2. Surprisingly, we observed striking growth inhibition in the majority of ovarian cancer cell lines, whether grown under adherent monolayer culture or in 3-dimensional spheroid culture conditions. While mTORC1 pathway activation was confirmed biochemically, knock-down of TSC2 also resulted in activation of the unfolded protein response (UPR), with elevated levels of phospho-EIF2α and ATF4, consistent with the accumulation of misfolded proteins in the endoplasmic reticulum. From a therapeutic standpoint, the resulting burden on the ubiquitin-proteasome system should render these cells particularly sensitive to proteasomal inhibition. We show that treatment with the proteasome inhibitor, bortezomib, causes increased accumulation of detergent-insoluble poly-ubiquinated proteins and formation of larger and more abundant cytoplasmic protein aggregates in sTSC2-transfected compared to scrambled siRNA-transfected ovarian carcinoma cells. This was accompanied by a more pronounced UPR stress response, including induction of pro-apoptotic CHOP, and suppression of autophagy, resulting in marked cytotoxicity. Conversely, we show that inhibition of protein synthesis by cycloheximide renders tumor cells resistant to bortezomib. Increased resistance to bortezomib was also noted when cells were grown as spheroids, a condition associated with suppression of mTORC1 signalling and decreased protein synthesis. This resistant phenotype of tumour spheroids however was ameliorated with treatment with siRNA targeting TSC2, downregulation of TSC2, and pharmacological GPR68 inhibition. Together, these findings demonstrate that mTORC1 is a pivotal checkpoint kinase for the survival of ovarian carcinoma cells. The identification of mTORC1 as a target in ovarian carcinoma cells should provide a therapeutic rationale for targeting these pathways in the clinic.

#3070 Lenalidomide and SMAC mimic LCL161 show combination activity in cells harboring loss of chromosome 5q. Nafeezah Hafeez, Alex Gaither, Dale Porter. Novartis, Cambridge, MA. LCL161 is a small molecule SMAC mimic that binds to IAP1, IAP2, and XIAP and sensitizes cancer cells to TNF-alpha as well as other inducers of cell death. An siRNA screen was performed in the SK-OV-3 cancer cell line to identify genes that when knocked down prevent LCL161-mediated cell death. As expected, siRNAs targeting RIPK1 rescued cells from LCL161-induced death. Unexpectedly, siRNAs targeting GPR68 also rescued cells from LCL161-induced death. Since knockdown of GPR68 has been reported to rescue myelodysplastic syndrome (MDS)-derived cells harboring loss of chromosome 5q from lenalidomide treatment, we tested for combination activity with lenalidomide plus LCL161 in 7-day in vitro proliferation assays. Interestingly, combination activity was observed in the SET-2 cell line harboring cytogenetic features of MDS including loss of chromosome 5q. These data suggest that, in combination, LCL161 may improve responses to lenalidomide in subsets of MDS and MDS patients and potentially additional contexts where lenalidomide is active.

#3071 Exploring the molecular mechanism underlying SETD2-Pi3Kβ synthetic-lethal interaction in renal cell carcinoma (RCC). Esteban Terzo,1 Jeanne Chiang,2 W. Kimryn Rathmell1. 1Vanderbilt University Medical Center, Nashville, TN; 2University of North Carolina, Chapel Hill, NC. Epigenetic regulators are emerging as major drivers of renal cell carcinoma (RCC). SETD2, a tumor suppressor commonly mutated in RCC, is the sole enzyme responsible for tri-methylating histone H3 on lysine 36 (H3K36me3) of actively transcribed genes. We recently discovered that SETD2 also trimethylates the non-histone target α-tubulin on lysine 40 (α-TubK40Me3) of mitotic microtubules. We have demonstrated that mutations altering SETD2’s ability to trimethylate H3K36 and α-TubK40 are associated with genomic instability in cancer progression. We hypothesized that defective SETD2 methylating activity gives rise to deleterious cell signaling cascades that promote the tumorigenic features of SETD2 loss. Preliminary data show that RCC-derived 786-0 cells that are deleted for SETD2 (SETD2-KO) or harbor pathogenic mutations are more sensitive to treatment with the PI3Kβ-specific inhibitor TGX-221 than their wild type counterparts. Phosphatidylinositol 3 kinases (PI3Ks) are a family of lipid kinases that coordinate signals from growth factors, cytokines and other environmental cues, translating them into intracellular signals controlling diverse signaling pathways. Several members of the PI3K/AKT signaling pathway, including the PI3K α and β isoforms, are frequently mutated in a variety of cancers, making this pathway a prime drug target for anti-cancer therapy. These pathways control numerous biological processes, such as cell proliferation, growth, and motility. Examination of structurally variant SETD2 mutants suggests that the loss of SETD2 methylating activity mediates this interaction, and future studies will examine the functional consequences of inhibiting this path-
EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Determinants of Drug Sensitivity and Resistance

#3072 A clinical pharmacodynamic biomarker assay that distinguishes potentially repairable, cytotoxic drug-induced DNA double strand breaks (DSBs) from DSBs associated with apoptotic cell death. Angèle B. Dull,1 Deb- orah Wiśler,2 Robert J. Kinders,1 Ralph E. Parchment,1 David Evans,2 Beverly A. Teicher,3 James H. Doroshow,1,2 Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD;1 National Cancer Institute, Bethesda, MD.

The effectiveness of certain classes of cytotoxic cancer therapeutics likely depends on whether drug-induced DNA damage is successfully repaired or not, with the latter situation leading to mutations and strand breaks. However, double strand breaks (DSBs) also occur independently of genotoxic insults during apoptotic cell death caused by many drug classes, as well as natural biological processes. We have developed an immunofluorescent confocal microscopy assay that uses a biomarker profile suitable for individual cell analysis designed to distinguish between DSBs caused by apoptosis and those caused by direct DNA damage from cytotoxic drug action. γ-H2AX is an established biomarker for DSBs and activated cleaved caspase 3 is an executioner caspase important for apoptosis, which leads to nuclear condensation, DNA fragmentation, plasma membrane blebbing, and subsequent cell death. Our assay defines the DSBs of apoptosis as co-localized γ-H2AX and cleaved caspase 3 in individual cells, while defining the DSBs from early drug effects of DNA damaging chemotherapy agents by γ-H2AX induction in the absence of cleaved caspase 3. Building on our published findings that topotecan strongly induces γ-H2AX and DSBs within 1-4 hours in vitro and in vivo, we observed exposure-dependent increases in γ-H2AX/cleaved caspase 3 double positive cells at later time points, both in an HT29 in vitro spheroid model and an MDA-MB-231 xenograft model. Fit-for-purpose studies in the MDA-MB-231 xenograft treated with bircin- pant, a SMAC mimetic and IAP deregulator that does not directly produce lethally DSBs, demonstrated a dose-dependent increase in cellular co-localization of γ-H2AX/cleaved caspase 3 consistent with bircin induced apoptosis and the established mechanism of action of this compound. Clinical feasibility was established in a canine clinical trial using formalin-fixed paraffin-embedded (FFPE) 18-gauge needle biopsies: two novel indenoisoquinolines, indotecan (LMP400) and indimitecan (LMP776), increased tumor cell co-localization of γ-H2AX/cleaved caspase 3 in tumor samples obtained on day 5 of qdx5 treatment. This PD biomarker assay of early and late DSb response to drug exposure could have important applications for elucidation of mechanisms of action of anticancer drugs and the development of investigational agents. Funded by NCI Contract No. HHSN261200800001E.

#3073 Potential predictive biomarkers of clinical responses for a novel CDC7-selective inhibitor TAK-931. Kenichi Iwai,1 Tadahiro Nambu,2 Osamu Kurasawa,1 Noriko Uchiyama,1 Ryo Dairiki,1 Yuko Ishii,1 Hideshi Ishii3.1 Takeda Pharmaceutical Company, Fujisawa, Japan;2 Takeda Pharmaceuticals Institute, Inc., Santa Monica, CA;3 Department of Pathology, University of Tokyo, Tokyo, Japan.

Cell cycle kinase (CycC) is a serine/threonine kinase, which plays important roles in initiation of DNA replication by phosphorylating MCM2. Kinase activity of CycC7 is controlled by its binding protein DBF4 in a cell-cycle dependent manner. Here we developed a potent CDC7 inhibitor TAK-931 (IC50< 0.3 nM) as a cancer therapeutic drug candidate, which exhibits a time-dependent ATP-competitive kinetics to its ATP-binding pocket. The selectivity studies using the 308 kinases revealed >100-fold selectivity of TAK-931 for CDC7 kinase inhibition compared to other kinase inhibitors. Treatment with TAK-931 suppressed the cellular MCM2 phosphorylation at Ser40, resulting in a delayed S phase progression, checkpoint activation, apoptosis, and potent growth suppression in various cancer cell lines. Furthermore, oral administration of TAK-931 as a single agent caused a significant antitumor activity in multiple xenograft models which includes both solid and hematological cancer cells. TAK-931 inhibited proliferation of multiple cancer cell lines, with mean concentration producing a half-maximal response (EC50) values ranging from 29.1 nM to > 30 μM (median = 554.5 nM). While the wide range of TAK-931 antiproliferative spectrum was observed, neither doubling speed nor CDC7 expression profile did predict TAK-931 sensitivity in cancer cell lines. A correlative study of the tumor genetic mutations in relation to antiproliferative activity that KRAS mutant cancer cells were more sensitive to TAK-931 compared to KRAS non-mutant cell lines (p<0.05). We also confirmed this KRAS-associated antiproliferative effect of TAK-931 in the SW48 isogenic cell lines of KRAS mutations (p<0.01). The ectopic expression of a cell knocked down TAK-931 increased the TAK-931 sensitivity compared to the KRAS-wild parental SW48 cell line. Given that KRAS mutations are frequently detected in clinical pancreatic tumors, we next conducted in vivo efficacy studies using pancreatic PDX models. Consistent to our hypothesis from in vitro studies, KRAS-mutant pancreatic PDX tumors were more sensitive to TAK-931 than the KRAS-wild pancreatic KRAS-mutant pancreatic PDX models we tested exhibited >60% TGI. Our findings suggest that the KRAS-mutant pancreatic tumors could be the potential candidate for the TAK-931 target indication.


Pancreatic ductal adenocarcinoma (PDAC) is an aggressive malignancy with a 5-year survival rate of just 8%. Mutations in BRCA2 are among the most common known germline alterations that predispose to PDAC. While the sensitivity of BRCA2-mutant breast and ovarian tumors to platinum compounds has long been recognized, the recent approval a PARP inhibitor in BRCA2-mutant ovarian cancer confirms hope that such tumors might be targeted with greatly reduced toxicity. To test whether PDAC displays similar sensitivity to PARP inhibitors, we carried out preclinical intervention studies in KRAS/p53-mutant genetically engineered mouse models (KPC mice) with or without the additional mutation of BRCA2 (KPCBR2 mice). Mice were enrolled based on 3D ultrasound imaging criteria to assure similar initial tumor burden, and tumor growth was monitored longitudinally by ultrasound. To our surprise, loss of Brca2 failed to sensitize pancreatic tumors to Parp inhibition and did not provide additional benefit in combination with cisplatin treatment. By contrast, cisplatin induced substantial regressions in most KPCBR2 tumors, leading to a >3-fold increase in overall survival, while having no effect on the growth or survival of mice with wild-type BRCA2. More surprisingly, the efficacy of cisplatin was not shared with oxaliplatin, even at higher doses. While BRCA2 is primarily known for its role in homology-directed repair, a network biology analysis of the function of BRCA2 in human pancreatic cancer led us to investigate the role of BRCA2 in mitosis. Wild type BRCA2 protein localizes to the midbody- a structure formed during the final stages of telophase- and plays poorly understood role in the final abscission of cytoplasm between two dividing cells. Timelapse video microscopy of KPCBR2 tumor cells treated once weekly for 2 hours with cisplatin revealed the frequent induction of endoreplication as well as eventual formation of polyploid giant cells subsequent death. Similar cells were found throughout KPCBR2 tumors during response in cisplatin treatment, but were largely absent from tumors in other treatment arms. Mechanism studies found that BRCA2 deficient PDA cells are susceptible to replicative stress following cisplatin treatment that selectively leads to the formation of radial chromosomes that cannot be resolved during mitosis. These studies argue for the consideration of cisplatin-based (rather than oxaliplatin-based) regimens for the treatment of BRCA2-mutant pancreatic tumors.

#3075 Analysis of microRNA profiles involved in the resistance to trifluoridine. Kenta Tsunekuni,1 Jun Koseki,2 Masamitsu Konno,3 Ayumu Asai,4 Norhiro Nishida,5 Hugo Colvin,6 Koichi Kawamoto,7 Yuichiro Doki,8 Masaki Mori,9 Hideshi Ishii9.1 Taiho Pharmaceutical, Tokushima, Japan;2 Osaka University, Osaka, Japan;3 Okasaki University, Okasaki, Japan.

Background: Trifluoridine (FTD) is a key component of the novel oral antitumor drug trifluoridine/tipiracil, which was approved for the treatment of patients with metastatic colorectal cancer refractory to standard chemotherapy. A comprehensive analysis of miRNA profiles was performed in cell lines resistant to FTD established by ourselves, in order to explore the underlying mechanisms of resistance to the drug. Method: We established subline resistance to FTD through continuous administration and increasing dose of the drug for 5 months using the colorectal cancer cell line DLD-1. Total RNA was extracted at intervals whilst establishing FTD resistant sublines, and miRNA expression was analyzed by microarray. The expression of miRNA that was significantly down-regulated in the FTD resistant subline was knocked down to test its involvement in resistance to FTD. Cell viability was evaluated by crystal violet cytotoxicity test. Results: The established FTD-resistant sublines showed more than 22-fold higher resistance to FTD and no cross-resistance to 5-FU. miRNA and mRNA cluster in the genome locus located in chromosome 9, 9p22.3 were down-
regulated in the FTD-resistant cell line, one of which was the miRNA let-7d-5p, which is one of the let-7 family and is known to target oncogenes and several key components of the cell cycle and cell proliferation. Anti-let-7d-5p treated DLD-1 was less sensitive to FTD than compared to control. The IC_{50} values of FTD were 16.8 μM and 7.6 μM for the anti-let-7d-5p treated cells and control respectively. On the other hand, overexpression of let-7d-5p in DLD-1 using let-7d-5p mimic incresed the sensitivity to FTD compared to control. The IC_{50} values of FTD were 3.7 μM and 13.9 μM in the let-7d-5p overexpression cells and control respectively. 5FU sensitivity was only altered slightly in anti-let-7d-5p treated or let-7d-5p mimic treated cells. These data suggest that let-7d-5p is more relevant to sensitivity for FTD than that of 5FU. Conclusion: Let-7d-5p expression is associated to the development of new more aggressive clones of tumor cells. 


Background and Aims. Chemo-resistance in hepatocellular carcinoma (HCC) tumor cells can be mediated by several mechanisms including P-gp efflux pumps and drug sequestration by the autophagy process. This study investigates the potential of doxorubicin loaded nanoparticle (NP) to reverse chemo-resistance by these mechanisms compared to free doxorubicin. We present the pre-clinical evaluation of anti-tumor effects of NP as monotherapy and in combination with standard agents used in treatment of HCC, pancreatic and sarcoma cancers. A phase III clinical study comparing NP to Best Standard of Care (Re-live study) in patients with advanced HCC is in the final stages of recruitment with preliminary results expected in 2H 2017. Methods. Tumor cell lines were incubated with drugs in cell proliferation assay. In vivo efficacy of NP alone (4-8 mg/kg) or in combination with current and investigational treatments for pancreatic cancer (e.g. Gemcitabine, Erlotinib, Abraxane) and HCC (Sorafenib, Regorafenib and Lenvatinib) were performed in mouse tumor models using tumor weight as primary endpoint. In all experiments NP was compared to administration of free doxorubicin. Doxorubicin quantification in tumor and organs to assess PK and biodistribution was also performed using an LC/MS based method. Autophagy was measured by cell proliferation in the presence of inhibitors e.g., Concanamycin A, Hydroxychloroquine sulfate added 30 min before incubation with the test compound. Results. NP showed a dose-dependent inhibition of cell proliferation in all resistant cancer cell lines tested with a superior activity compared to free doxorubicin and other tested drugs. In contrast to free doxorubicin, NP showed consistent anti-proliferative activity in the absence/presence of inhibitors of P-gp pumps and autophagy. In a range of in vivo models, NP was preferentially taken up by the tumor tissue and significantly reduced tumor growth when compared with free doxorubicin and with at least equivalent reduction in tumor growth compared to current treatments. Furthermore NP administered in combination with current treatments significantly increased the inhibitory effect of each drug without additional toxicity (as measured by no change in body weight). The result comparing efficacy of NP with standard agents in combination and in combination in HCC, pancreatic and sarcoma cancer models will be presented. Conclusions. These results demonstrate that NP is clearly differentiated from free doxorubicin, in 1) overcoming resistance mechanisms linked to efflux and autophagy, and 2) having a superior biodistribution profile both of which results in significantly enhanced activity on chemo-resistant tumors. NP also provides an opportunity to combine with other agents, enhancing activity without increasing toxicity. The implications of these results on the further development of NP will be discussed.

#3077 Tumor cells with acquired resistance to EGFR inhibitors and over-expression or activation of AXL, MET and FGFR1 are insensitive to single-agent treatment targeting AXL, MET or FGFR1. Jordi Bertran-Alamillo, Miguel Angel Molina-Villa, Cristina Teixedo, Jordi Codony-Servat, Ana Giménez-Capitán, Carles Codony-Servat, Silvia García-Román, Elena Aldegue, Sonia Rodríguez, Rafael Rosell1, Pangea-Biotech, Barcelona, Spain; 2Institut Català d’Oncologia, Badalona, Spain. 

Background: Ablerrant activity of the MET, FGFR1 and AXL receptors has been associated with the development of resistance to first, second and third generation EGFR tyrosine kinase inhibitors (TKI) in EGFR-mutated non-small cell lung cancer (NSCLC) patients. Methods: We obtained 6 resistant lines by treating EGFR-mutated (exon 19 TKI sensitive PC9 cells with increasing concentrations of gefitinib or erlotinib. The p.T790M resistance mutation emerged in two cell lines (GR1, GR4), which remained sensitive to osimertinib, a third generation EGFR TKI. Six new cell lines to resistant to “second line” osimertinib were generated from GR1 and GR4 by exposure to increasing concentrations of the inhibitor. Finally, six more cell lines resistant to “first line” osimertinib were derived from the PC9 parental cells. All resistant cell lines were genotyped for selected genes (including EGFR) and characterized for AXL, MET and FGFR1 expression and activation by Q-RT-PCR, immunohistochemistry and Western blotting. The effects of AXL (BGR324), MET (crizotinib, capmatinib) and FGFR1 (nindetanib) inhibitors on the parental and the 18 resistant cell lines were analyzed by MTT and, in some cases, by colony formation. AXL was stably silenced in some of the resistant cell lines. Results: All cell lines resistant to “first line” gefitinib, erlotinib and osimertinib maintained the exon 19 EGFR sensitizing mutation. In contrast, FTD sensitivity and expression/mutation was more efficiently than 5FU. It suggest that the let-7d-5p is a potential predictive marker for trifluridine/tipiracil treatment in the clinical setting.

#3078 The role of extracellular vesicles in chemotherapy resistance in gastric cancer. Edson Cassinella, Michele C. Landemarber, Gabriela P. de Oliveira, Vivian Tinina Martins, A. C. Conquista Cancer Center, Sao Paulo, Brazil.

Gastric adenocarcinoma (GAC) is the fourth most common cancer and the second cause of cancer death in the world. Chemotherapy has been largely used to treat this disease however acquired drug resistance is a common event and is associated to the development of new more aggressive clones of tumor cells. Herein, a gastric adenocarcinoma cell line (AGS) resistant to the 5-fluorouracil (5-FU) was generated in order to establish mechanisms associated to acquire drug resistance. In the presence of 5-FU (10μM) resistant cells show a 2-3 times higher proliferation ratio than parental cells, a platting efficiency of 38% compared to 1% of parental cells and a higher invasive phenotype. Remarkable, when compared to parental cells, rAGS-FU resistant cells secreted 2-3 times more extracellular vesicles, EVs (85-175nm). When parental cells were treated with RNAi, rAGS-FU resistant cells they acquired the same phenotype of the latter cells regarding higher proliferation, platting efficiency and invasion. The proteomic analysis of parental and rAGS-FU cells showed 306 differentially expressed proteins, while 66 differentially expressed proteins were found when EVs secreted from these cells were compared. Our data suggest that cells resistant to chemotherapy have a more aggressive phenotype and are able to transfer these characteristics to non-resistant cells. These findings point to mechanisms of chemoresistance mediated by components that are carried from cell to cell by EVs, what may indicate novel approaches that can be addressed to impair drug resistance and improve treatment response. Supported by FAPESP.

#3079 Activation of CCN1 signaling in solid tumor cells diminishes response to a histone deacetylase inhibitor: A dark side of HDACIs. Arnab Ghosh, Priyanka Ghosh, Gargi Maji, Sushanta K. Banerjee, Manjhi Banerjee. VA Medical Center, Kansas City, MO. 

Background and Objective: Histone deacetylase (HDAC) inhibitors are clinically proven epigenetic-based drugs for hematological cancers. However, the impact of these inhibitors on solid tumors is disappointing and debatable. Recent studies have shown that the solid tumor cell lines are less sensitive to a HDAC inhibitor: Vorinostat (suberoylmalonyl hydroxamic acid, SAHA) as compared to hematological cell lines. However, it remains elusive why solid tumor cell lines responded weakly to HDAC inhibitors. Previously, it has been reported that CCN1/Cyr61 plays critical role in invasive front and drug resistance in breast and pancreatic cancer. Thus, our goal is to determine whether CCN1 signalling impair activity of SAHA in breast and pancreatic cancer cells. Methods: To test this objective, various breast cancer cell lines (MCF-7, ZR-75-1, MDA-MB-231, HCC-70) and pancreatic cancer cell lines (BxPC-3, AsPC-1 and Panc-1) were treated with SAHA with different does and times. Levels of CCN1 and its downstream signalling molecules were determined in SAHA-treated or untreated cell extracts using Western blotting. In addition, cell viability, migra-
tion and sphere formation were examined in parental and CCN1-depleted cells in the presence or absence of SAHA. Results and Conclusions: Here we have shown that CCN1 was upregulated in various breast and pancreatic cancer cell lines following SAHA treatment via epigenetic mechanism. Distribution of CCN1 in the nucleus and cytoplasm was also drastically altered in SAHA-treated parental cells as compared to CCN1-depleted cells. However, following SAHA treatment, CCN1 expression was located predominantly in the nucleus. Functionally, CCN1 had dual outcome in these cells. The cell viability assay indicated that CCN1-positive breast cancer or pancreatic cancer cells were more sensitive to SAHA as compared to CCN1-negative cells. CCN1 ablation by neutralizing antibody treatment in MDA-MB-231 cells significantly suppressed the inhibitory effect of SAHA in MDA-MB231 cells. The CCN1 knockdown phenotype such as migration towards SDF-1 and sphere-formation were markedly elevated in SAHA treated cells as compared to untreated cells. These effects of SAHA were rescued by CCN1-depletion. Collectively, these studies indicate that CCN1 activation limits the response to SAHA in solid tumor cells and thus suggesting that combination therapy of SAHA and CCN1-inhibitor could be an ideal therapeutic approach to make HADAC inhibitor sensitive to solid tumors.


Objective: Investigating the role of Y-box binding protein 1 (YB1) in drug resistant advanced kidney cancer. Background: Renal cell carcinoma (RCC) is the 6th most common malignancy with approximately 1,800 deaths in 2015 with an annual increase in Canada. Despite the partial or total surgical removal of kidney in patients with localized RCC, metastatic patients are treated with tyrosine kinase inhibitors (TKIs) in a purely palliative approach. However, TKI-resistance (Sunitinib) is developed after a median time of 10-14 months. Therefore, identifying the factor(s) responsible for TKI-resistance development and disease advancement in RCC is imperative. It is now widely recognized that evolutionarily conserved Y-box binding protein 1 (YB1) is essential for cell growth and survival. Upregulation of YB1 in numerous cancer types was found to be positively correlated with tumor growth, metastasis and drug-resistance development. YB1 is also involved in cellular communication through its secretion in the tumor microenvironment by cell-surface transporters, ABC-transporters. Summary of the data: Endothelial cells (HUVEC) were co-cultured with Caki-1-DIC (Sunitinib-resistant, developed in our lab) and Caki-1 WT (Sunitinib-sensitive) RCC cell lines, and increased migration of HUVEC was observed with Caki-1 DIC compared to Caki-1 WT. A drastic increase in YB1 and its downstream target ABCB1 in Caki-1 DIC compared to Caki-1 WT cells was also detected. Consistent with previous reports, we observed granular structures in the Caki-1 DIC cells that support potential secretion of YB1 into the tumor microenvironment. Moreover, blocking ABCB1 reverted the Caki-1 DIC cells to being drug-sensitive. Experimental procedures: Caki-1 WT and Caki-1 DIC were co-cultured with HUVEC cells followed by scratch assay to test for HUVEC cell migration and associated secretory factors. Western blot and qPCR were used to determine the protein and mRNA levels respectively between the two cell-lines. Immuno-histochemical staining was carried out on the RCC tumors from Sunitinib-sensitive and resistant patients entered in one of the clinical trials. In 14 cases, YB1 expression was observed using immunofluorescence staining against YB1. Presto-blue was used for cell biomass assay following different drug treatments. Summary: The molecular function of YB1 in RCC and its potential in targeted therapy is not well understood. Therefore, understanding the function of YB1 in metastatic RCC and in drug-resistance development is of vital importance. Our data suggests that inhibition of YB1 may slow disease progression and, possibly, revert the drug resistance mechanism. The results from this study have the potential to introduce YB1 inhibitors in conventional RCC chemotherapy, alone or in combination, to improve survival in advanced kidney cancer patients.

**#3081 Precision medicine for patients with advanced small cell lung cancer treated with novel therapeutic agents in a phase I clinical trials unit.** Joline S. Lim, Samuel J. Harris, Malaka Ameratunga, Raghab Sundar, Joo Eun Ang, Debrahille Collins, Maxime Chénard-Poirier, Alvaro I. Garces, Stan B. Kaye, Juanita Lopez, Udai Banerji, Johann S. deBono, Timothy A. Yap.

Introduction: Small cell lung cancer (SCLC) is aggressive and relapse is inevitable. Novel therapies in the context of phase I (Ph1) clinical trials should be considered in this setting. Next generation sequencing (NGS) identifying putative driver mutations may aid therapy allocation in such trial settings. Methods: Retrospective analysis of characteristics and clinical outcomes of patients (pts) with advanced SCLC referred to the Phase I Clinical Trials Drug Development Unit at the Royal Marsden Hospital between 1992 and 2016, using electronic patient records. Results: 85 pts with advanced SCLC were included, of which 45 pts were allocated to ≥1 trial each (48 allocations). Of these, 21 (46.7%) pts started ≥1 trial, with a total of 24 trials commenced. Of the 64 pts who were unable to commence trials, 43 (67.2%) had rapid disease progression, 11 (17.2%) sought other therapies, 5 (7.8%) declined Ph1 trials, and 5 pts did not enrol for unknown reasons. Pts 1 (2.0%) were not allocable. Ph1 trials were run in 24 different lines of treatment (frame 1-3). 13 pts had tumor molecular profiling with high coverage targeted NGS, yielding 22 distinct mutations; Alterations in DNA damage repair (DDR) pathway were most common: TP53 (53.8%), FANC (30.8%), ATM (15.4%), ATR, BAR1D and CHEK2 (7.7% each). All pts found to have ≥1 DDR mutation pathway presented platinum sensitive disease (disease progression ≥90 days from last platinum dose) and were allocated to Phase I trials with four different settings available: PCI, AR, AXIN1, DDRB, ERBB2, mTOR, PI3K, NOTCH, NTRK1, PDGF, R81, RET, STK1, FKT and VGF. Pts were most commonly treated with novel inhibitors against PARP (n=9), PI3K(akt)(n=3), PD-1 (n=2) and BCL (n=2). Therapies were generally well tolerated with no dose limiting toxicities observed. Of 9 pts who received PARP inhibitors, 1 achieved a RECISt partial response (PR) and remained on trial for 16 weeks; 4 achieved RECISt stable disease (SD) for a median of 17 weeks (range:10.9-31.1 weeks). 1 exceptional responder whose tumor harbored multiple somatic aberrations (TP53, FANC, AR, ERBB2, NOTCH2) was enrolled on 3 sequential phase I trials - she achieved durable RECIST SD on a PARP inhibitor (31.1 weeks), RECIST PR with a PD-1 inhibitor (38.7 weeks), and durable RECIST SD with carboplatin/ATR inhibitor (27.9 weeks). The first line setting predicted for improved disease control (RECIST PR/SD 73.3% vs 0%, p<0.05) and longer duration on trial (14 vs 4 weeks, p<0.05). Discussion. Novel therapeutic agents within the context of a dedicated Ph1 trials unit were well tolerated and led to preliminary signals of antitumor activity. Targeted NGS may aid in the identification of pts with putative gene aberrations, potentially predicting for response to novel therapies. Ph1 trials should be considered earlier in the pt’s disease course to maximize their prospects of trial enrolment.

**#3082 Deficient NOXA in HER2-amplified breast cancer drives kinase inhibitor resistance.** Konstantinos V. Floros,1 Kyung-A Song,1 Timothy L. Lochmann,1 Mark T. Hughes,1 Daniel A. Heisey,1 Hisashi Harada,1 Bin Hu,1 Jennifer Koblinski,1 Andrew J. Souers,2 Joel D. Leverson,2 Anthony C. Faber1.

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The purpose of this study is the development of a novel combination therapy that targets HER2-amplified breast cancer. About one quarter of breast cancers harbor amplification of HER2. HER2 is a transmembrane receptor tyrosine kinase (RTK) belonging to the ERBB family of receptors (ERBB1-4). Upon hetero- and homo-dimerization, HER2 activates several key intracellular pathways, regulating many cellular functions including proliferation and survival. HER2 inhibition (HER2i) results in a first-line setting predicted for improved disease control (RECIST PR/SD 73.3% vs 0%, p<0.05) and longer duration on trial (14 vs 4 weeks, p<0.05). Discussion. Novel therapeutic agents within the context of a dedicated Ph1 trials unit were well tolerated and led to preliminary signals of antitumor activity. Targeted NGS may aid in the identification of pts with putative gene aberrations, potentially predicting for response to novel therapies. Ph1 trials should be considered earlier in the pt’s disease course to maximize their prospects of trial enrolment.

**#3083 Prior irradiation results in elevated programmed death 1 (PD-1) in T cells.** Rui Zhang,1 Fumin Li,1 Jayanthi Bagavan,1 Anil Narang,1 Xi Zhao,1 Delia H. Borrego,1 Jinghui Li,1 George-town Lombardi Comp. Cancer Ctr., Washington, DC; 2Institute of Radiation Medicine, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, China.

It is well known that radiation has long-lasting effects on the immune system. In this study we address the question whether these radiation-induced adverse effects on T cell activation are associated with alterations of T cell inhibitory receptors. Expression levels of inhibitory receptors on T cells were analyzed at multiple post-irradiation time points ranging from one to four weeks in mice receiving whole body irradiation. The level of the checkpoint receptor, PD-1, on...
different T cell subpopulations was determined. Our results showed that prior irradiation resulted in significantly elevated expression of PD-1 in both CD4+ and CD8+ populations. T cells with elevated PD-1 mostly were either central memory or naïve cells. In addition, the feedback induction of PD-1 expression in activated T cells declined with time after radiation. Together, the elevated PD-1 level observed at weeks after radiation exposure is connected to T cell dysfunction. Similar changes were seen in samples from tumor patients receiving radiotherapy. Recent preclinical and clinical studies have shown that radiation alters the immune-modulatory microenvironment in tumor tissue, and implies that a combination of radiotherapy and T cell checkpoint blockade immunotherapy including targeting the PD-L1/PD-1 axis may potentiate the antitumor response. Therefore, understanding the dynamic changes in PD-1 levels in T cells after radiation should help in the development of a more effective therapeutic strategy.

#3083A C6-ceramide nanoliposomes sensitize paclitaxel resistant ovarian cancer cells to chemotherapy treatment. Danielle C. Llaneza, Tye G. Deering, Samantha G. Sherwood, John R. Cornelison, Mark Kester, Charles N. Landen. University of Virginia, Charlottesville, VA.

Ovarian cancer is the fifth leading cause of cancer deaths among women, and the majority of women are diagnosed at advanced stages resulting in poorer outcomes and survival rates. One of the driving forces behind these outcomes is the development of chemoresistance, and subsequent proliferation and invasion of tumor cells. In a patient-derived xenograft model of ovarian cancer, we identified the drug combination paclitaxel + C6-ceramide as significantly enriched in our subject population suggesting the potential for combination with conventional chemotherapy. In order to target the sphingosine pathway, we investigated the potential effects of combining paclitaxel with C6-ceramide nanoliposomes in paclitaxel-resistant ovarian cancer cell lines, HeyA8-MDR and SKOV3-TR. Ceramide has been shown to be a key player in several processes important for tumor suppression including apoptosis, autophagy, and cell cycle arrest. We administered paclitaxel and C6-ceramide to HeyA8-MDR and SKOV3-TR cell lines in vitro and measured cell viability and expression of apoptotic and autophagic proteins. Results showed that in both cell lines, C6-ceramide significantly decreases the IC50 dose of paclitaxel, compared to control ghost nanoliposome treated cells. The IC50 of paclitaxel decreased in HeyA8-MDR cells from 400nM to 150nM, and in SKOV3-TR cells from 1000nM to 450nM. Western blot analyses indicate that combination treatment increases expression of apoptotic (cleaved caspase-3) factors in HeyA8-MDR cells, and autophagic (LC3) factors in SKOV3-TR cells, over single agent treatment or control. Paclitaxel is known to increase expression of apoptotic (cleaved caspase-3) factors in HeyA8-MDR cells from 400nM to 150nM, and in SKOV3-TR cells compared to control ghost nanoliposome treated cells. The IC50 of paclitaxel decreased in HeyA8-MDR cells from 400nM to 150nM, and in SKOV3-TR cells from 1000nM to 450nM. Western blot analyses indicate that combination treatment increases expression of apoptotic (cleaved caspase-3) factors in HeyA8-MDR cells, and autophagic (LC3) factors in SKOV3-TR cells, over single agent treatment or control. Paclitaxel is known to increase expression of apoptotic factors while decreasing expression of autophagic factors. Ceramide may increase both apoptotic and autophagic factors. In an orthotopic mouse model with the HeyA8-MDR line, combination treatment of paclitaxel and C6-ceramide resulted in significantly decreased tumor burden (1.38g +/- 0.74), compared to control ghost (p = 0.022, 2.81g +/- 1.57), C6-ceramide alone (p = 0.078, 2.50g +/- 1.58), and paclitaxel alone (p = 0.005, 2.48g +/- 0.75) groups. The combination of C6-ceramide and paclitaxel may prove useful in elucidating and targeting underlying chemoresistance mechanisms in ovarian cancer.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Determinants of Drug Sensitivity and Resistance


Female gender, early age of first birth, and multiparity are associated with less aggressive melanoma; however, the mechanisms underlying the apparent clinical benefit are undefined. Previously we reported that 17β-estradiol drives melanocyte differentiation through G-protein coupled estrogen receptor (GPER), a nonclassical sex hormone receptor. Here, we use genetically-defined human skin xenograft melanoma to show that pregnancy antagonizes oncogenic BRAF and inhibits melanomagenesis. Transient GPER activation drives stable melanocyte differentiation that persists after removal of the activating ligand. Consistent with this, brief exposure of melanoma cells to GPER agonists prior to subcutaneous injection inhibited subsequent tumor growth. Systemic treatment with GPER agonists cooperated with PD-1 blockade in melanoma bearing mice to greatly prolong survival longer than that observed with either agent alone. The combination of differentiation-based therapies with immunotherapy may have significant therapeutic benefit for melanoma.

#3085 SY-1425 (tambogarene), a selective RARγ agonist, shows synergistic anti-tumor activity with hypomethylating agents in a biomarker selected subset of AML. Michael R. McKeown, Emily Lee, Chris Fiore, Matthew L. Eaton, Christian C. Fritz, Eric Olson. Syros Pharmaceuticals, Cambridge, MA.

In patients with acute myeloid leukemia (AML) (> 60 years) and myelodysplastic syndrome (MDS), hypomethylating agents (HMAs) may extend survival, but cure rates are very low and new treatment approaches are needed. HMAs, such as azacitidine, work by inhibiting DNMT1, leading to depletion of DNA methylation in the tumor cells. Hypomethylation, in turn, leads to the re-expression of genes associated with differentiation and growth arrest. We have recently explored the potent and selective RARγ agonist SY-1425 in combination with azacitidine in AML and MDS. SY-1425 binds to RARγ and causes a transition from repression to strong activation of target genes, thus reprogramming the tumor cells toward terminal maturation in RARA-high AML models, supporting our recently initiated Phase 2 trial in a biomarker-selected subset of AML and MDS (NCT02807350). Based on potential mechanistic synergy, we evaluated SY-1425 in combination with HMAs and identified a synergistic anti-proliferative effect. In RARA-high AML cell lines, but not RARA-low, the combination of SY-1425 with either azacitidine or decitabine showed synergistic anti-proliferative effects on the cells, with combination indices less than 0.5 over a range of concentrations from 0.01 to 100μM of SY-1425 and 0.1 to 1μM of HMAs. SY-1425 and azacitidine were also co-administered to a disseminated patient-derived xenograft (PDX) mouse model of RARA-high AML. SY-1425 and azacitidine decreased superior or rear area in either therapy alone, leading to deeper and more durable responses with less than 1 detectable tumor burden. A follow-up study in the RARA-high PDX model investigated different treatment schedules of SY-1425 and azacitidine over a period of 56 days, supporting a regimen that maximizes anti-tumor activity and tolerability. Mechanistic studies using RNA-seq and ChIP-seq in AML cell line models have revealed that while azacitidine had only moderate suppressive or activating effects over a broad set of genes, the addition of SY-1425 in RARA-high models resulted in strong and specific induction of genes bound by RARγ. It is hypothesized that azacitidine acts to prime the tumor cells for reprogramming by SY-1425. The loss of methyl-cytosine residues following azacitidine treatment lowers the barrier to SY-1425-mediated gene induction. It was observed that the two agents work cooperatively to promote terminal differentiation and decrease proliferation of the AML tumor cells, with the potential for increased clinical benefit in a subset of AML defined by a RARA super-enhancer. Based on the largely non-overlapping clinical toxicity profiles of azacitidine and SY-1425, supported by the observed tolerability of the combination in preclinical models, these findings provide a strong rationale for a planned study of this combination in biomarker selected, newly diagnosed AML patients.

#3086 Inhibitors of the enzyme dihydroorotate dehydrogenase, overcome the differentiation blockade in acute myeloid leukemia. Richard R. Buescher,1 David Sykes,2 Stefan Gradl,3 Steven Ferrara,4 Sven Christian,5 Claudia Merz,2 Henrik Seidel,1 Andreas Bernthaler,1 Mathias Wawer,3 David T. Scadden2. 1Bayer Pharma AG, Berlin, Germany; 2Massachusetts General Hospital, Boston, MA; 3Broad Institute of MIT and Harvard, Boston, MA.

The prognosis for adults diagnosed with acute myeloid leukemia (AML) remains poor, with a five-year survival of only 25%. This prognosis is even more dismal in older patients who are not well enough to receive standard induction chemotherapy. Speaking to the need for new therapies is the fact that our therapeutic backbone - a combination of cytarabine and an anthracycline - remains unchanged since 1973. The promise of differentiation therapy was realized in the small subset of patients diagnosed with acute promyelocytic leukemia (APL). Here, treatment in the form of all-trans retinoic acid (ATRA) and arsenic trioxide inverted the survival curve; where APL was once the worst form of myeloid leukemia, it now carries the best prognosis, with a five-year survival exceeding 85%. The goal of this study was thus to develop differentiation therapy for patients with non-promyelocytic AML with the question: “Can we identify small molecules that overcome myeloid differentiation arrest?” A phenotypic differentiation screen in a HOXA9-driven leukemia model followed by target deconvolution, identified DHODH as an unexpected target for overcoming differentiation arrest in AML. We used 2 potent small molecule inhibitors of DHODH to validate this initial finding: Brequinar, a known DHODH inhibitor and an in vivo house compound BAY DHODHi. In several in vitro experiments we demonstrated induction of AML differentiation in a dose dependent fashion. Interestingly, these effects could be completely rescued by addition of uridine, confirming target specificity. Treating mice in multiple genetically diverse AML in vivo models with a DHODH inhibitor led to tumor growth reduction and AML differentiation. Expression analysis of leukemia cells explanted from mice xenografts treated with a DHODH inhibitor demonstrate an early onset of differentiation.
tiation markers indicating a direct role of DHODH with the onset of differentiation in vivo. The mechanism for selective vulnerability of leukemia cells to DHODH inhibition remains under investigation. Despite the observation that DHODH is expressed in all cells, normal and malignant, mice can tolerate DHODH inhibitor therapy for more than 100 days without weight-loss or other concerns. Thus, our preliminary findings define DHODH as a new target in the differentiation treatment of AML. Hopefully, small molecule DHODH inhibitors will provide a much-needed differentiation therapy for patients with acute myeloid leukemia.

#3087 Forced induction of differentiation in osteosarcoma tumor initiating cells. Margaret Ellen White, Maria V. Guijarro, Steven Ghivizzani, Charles P. Gibbs. Univ. of Florida College of Medicine, Gainesville, FL.

Osteosarcoma (OS) is a highly malignant bone cancer that is defined histologically by the secretion of immature osteoid. The chemotherapeutic regimen has not changed in the last 40 years and similarly, the five-year survival rate remains at ~60%, with the majority of fatalities arising from metastases in the lungs. OS is believed to originate from osteogenic committed progenitors, involving disruption of extracellular matrix synthesis in favor of proliferation. In previous work, our lab has developed a method to selectively identify Tumor Initiating Cells (TICs) in OS xenografts based on their ability to activate a transcriptional reporter comprised of a human OCT4 promoter linked to green fluorescent protein (GFP) coding sequence. Clonally derived, stably transfected OS Oct4/GFP+ TICs are capable of initiating and maintaining the growth of heterogeneous tumors and driving disease progression. The loss of GFP expression and tumorigenic capacity occurs during tumor formation in response to cues within the tumor microenvironment, resulting in adoption of a specialized secretory phenotype as an adaptive survival mechanism: a phenotypic change similar to that seen in physiological differentiation. During skeletal formation, Bone Morphogenetic Proteins (BMP) play key roles in osteogenic maturation. Because the OS lineage of origin suggests an innate sensitivity to BMP proteins, we hypothesized that BMP stimulation will force the induction of differentiation in OS TICs and will impair the ability of these cells to initiate and maintain tumor growth. To first probe that OS TICs respond to BMP stimulation, we detected by flow cytometry the expression of BMP Receptors Type II and 1A in two primary OS-derived cell lines (OS521 and OS156), with similar expression seen in Mesenchymal Stem Cells. Furthermore, by treating OS TICs with BMP heterodimers - BMP2/7 and BMP4/7 - we observed an activation of canonical BMP signaling, suggesting a functional and intact signaling network. BMP4/7 stimulation of OS521 TICs in vitro showed an increase in cells in the G1 phase of the cell cycle, visualized by DAPI, and higher p21 and p27 expression than controls, shown in immunoblotting. On the other hand, BMP4/7 treatment of OS156 TICs reduced its invasion capacity through a complex of Fibronectin and Bovine Serum Albumin by 33% (p = 0.00144) compared to untreated controls. Strikingly, in vivo studies revealed that time to tumor onset of BMP4/7 pretreated OS521 TICs in NSG mice at a dose of 3x10^5 cells was significantly longer than in controls (p = 0.0003). In summary, our data suggests a reduction of OS tumorigenicity due to a BMP4/7 induced differentiation-based response. Such a differentiation-based therapy could become a more effective and safer alternative to current chemotherapeutic therapy. Future studies include TICs from DHODH-null, oxia, adenosine, and calcium, to investigate the underlying mechanisms responsible for BMP involvement in the tumorigenic capacity of osteosarcoma.

#3088 Combination of 4SC-202 and IFN-γ restores mature APC phenotypic features in AML cells. Anne Catherine Bretz, Ulrike Parnitzke, Kerstin Kronthaler, Svetlana Hamm. 4SC AG, Planegg-Martinsried, Germany.

Acute myeloid leukemia (AML) is characterized by a differentiation block resulting in accumulation of immature myeloid cells. Differentiation therapy, e.g. with all-trans retinoic acid (ATRA) has become a part of standard treatment for the acute promyelocytic leukemia variant of AML. Other agents like cytokerines and HDAC inhibitors were shown to enhance differentiation of AML cells, which results in apoptosis induction or re-sensitization to ATRA. However, a differentiation therapy resulting in the restoration of antigen presenting function of AML cells would be a more desirable asset, since such cells will be able to induce or enhance immune response against residual malignant AML cells. Here, we analyzed the impact of 4SC-202 alone and in combination with different cytokines on differentiation of AML cell lines THP-1, HL-60 and MOLM-13 (AML-M4, -M2, and -M5 class, respectively). Myeloid lineage markers CD11b, CD14, and HLA-DR were analyzed by flow cytometry and quantitative PCR. Cytokine production was measured using Luminex multiplex system. Furthermore, chromatin immunoprecipitation (ChIP) was performed to assess the direct impact of 4SC-202 on promoter histone acetylation of differentiation genes. 4SC-202 is an orally available clinical stage epigenetic small molecule inhibitor which specifically targets histone deacetylases HDAC class I isoenzymes 1-3 as well as the lysine-specific demethylase LSD1 (KDM1A). 4SC-202 treatment of AML cell lines resulted in a dose-dependent increase of myeloid differentiation genes CD86 and ITGAM (CD11b) on mRNA and protein level. On the chromatin level, 4SC-202 correspondently induced an upregulation of H3K9ac, H3K27ac and H3K4me1 at the promoters of these genes. Interestingly, reference compounds like ATRA, bezarotene, GM-CSF and TNF-α were not as efficacious as 4SC-202 in the induction of differentiation markers in AML cells, whereas IFN-γ strongly induced MHC class II molecules HLA-DR and myeloid lineage marker CD14. Finally, the combination of 4SC-202 and IFN-γ resulted in a synergistic or complementary induction of differentiation and APC markers CD11b, CD14, CD86 and HLA-DR resulting in a CD86^hi/HLA-DR^hi phenotype which was able to produce pro-inflammatory cytokines. In summary, we demonstrate that 4SC-202 induces differentiation of AML cells of different subtypes and thus provide promising preclinical data for applying this novel epigenetic modulator for differentiation therapy of AML. Moreover, the mature APC phenotype induced by the combination of 4SC-202 and IFN-γ may further suggest an application in consolidation treatment of AML to enhance or even elicit a tumor-specific T-cell response against residual malignant AML cells preventing AML recurrence.

#3089 Combining MEK and HDAC inhibitors as a therapeutic strategy to promote differentiation of colorectal cancer. Laura J. Jenkins, Ian Y. Luk, Janson W. Tse, Jennifer Mooi, Amardeep S. Dhillon, John M. Mariadason. Olivia Newton John Cancer Research Institute, Heidelberg, Australia.

The five-year survival rate for patients with metastatic colorectal cancer is less than 15%, necessitating an urgent need to develop novel therapeutic strategies for this disease. Loss of differentiation is associated with worse overall survival in colorectal cancer, suggesting that strategies to re-induce differentiation may provide clinical benefit. Differentiation therapy is effective in the treatment of acute promyelocytic leukemia but whether this approach can be efficacious in treating colorectal cancer is unknown. Inhibitors of the MAPK signaling pathway including the MEK inhibitor Trametinib can induce markers of differentiation in colorectal cancer cells. Trametinib also induces expression of Cdx-2, a known driver of colon cell differentiation. Similarly, histone deacetylase inhibitors (HDACi) induce markers of differentiation in multiple tumor cell lines, including colon cancer cells. The aim of this study was to assess the potential therapeutic benefit of combining MEK (Trametinib) and HDAC (Panobinostat) inhibitors to further promote differentiation in colorectal cancer cells, and to elucidate the mechanistic basis for this effect. Combination treatment of HT29 and T84 cells with Trametinib and Panobinostat significantly enhanced mRNA and protein expression of the differentiation markers Cadherin 17 (CDH17) and Keratin 20 (KRT20), compared to either agent alone. Trametinib also induced expression of Cdx-2, an effect that was enhanced upon combination treatment with Panobinostat. Notably, Cdx-2 knockdown attenuated CDH17 and KRT20 induction by the combination, establishing a direct role for Cdx-2 in differentiation induction by the Trametinib/Panobinostat combination. Concomitant with inducing differentiation, the drug combination also induced more apoptosis than either agent alone. To validate these findings in vivo, HT29 cells grown as xenografts were treated with Trametinib and Panobinostat alone and in combination. Mice treated with the combination had significantly smaller tumours than mice treated with vehicle or either agent alone. Consistent with the in vitro findings, immunostaining of the tumour xenografts demonstrated a strong increase in CDH17, KRT20 and Cdx2 expression upon treatment with the drug combination. Collectively, this study highlights a drug combination strategy to re-induce differentiation for the treatment of colorectal cancers.
experimental and molecular therapeutics: Drug delivery technology and antibody technology

Angiopoietin-2 (Ang-2) is released from endothelial cells only in response to stimuli (e.g. wound healing, tumor growth) and facilitates blood vessel sprouting and inhibits pericyte-endothelial cell interaction via Tie2 signaling. In tumors, Ang-2 is up-regulated and acts together with the VEGF/VEGFR2 pathway to stimulate tumor angiogenesis and metastasis. While therapeutic intervention using anti-Ang2 antibodies or anti-VEGF/VEGFR2 pathway has provided limited disease progression in a number of different clinical settings, there is an obvious need for an improved response. In various preclinical mouse angiogenesis or xenograft models, the combination treatment with anti-Ang2 antibody and the VEGF/VEGFR blocker provided additional benefit over inhibiting the individual pathway. Here as an alternative to combo therapy, we have engineered a human IgG4 (IgG4) antibody, comprising of VEGFR2 antibody derived from ramucirumab and a C-terminally fused single-chain variable fragment (scFv) targeting Ang2. LY2304747 binds to both the extracellular domain of VEGFR2 and soluble Ang2 with high affinity and blocks binding of Ang2 to Tie2 and VEGF to VEGFR2, and therefore inhibits signaling. We have shown that LY2304747 blocks binding of human Ang-2 to human Tie2-Fc by an ELISA assay and neutralizes Ang-2 induced phospho-Tie-2, but not Ang-1 induced phospho-Tie-2 in CHO cells expressing Tie-2 receptor. Moreover, LY2304747 neutralizes human VEGF165-induced phospho-VEGFR2 stimulation, cord formation and cell proliferation in human endothelial colony forming cells (ECFCs) and human dermal microvascular endothelial cells (HMVEC-d). LY2304747 binds human and cyno VEGF receptors, but not murine Ang2 or anti-Ang2 arm. LY2304747 blocks human, cyno and mouse Ang2. Pre-clinical evaluation of LY2304747 in mouse retinal angiogenesis model resulted in an abrogation of angiogenesis. Combination studies using a rodent-specific surrogate gate VEGFR2 blocking antibody, DC101, with parental Ang2 antibody from which the scFv was derived, inhibited both tumor growth and metastasis, resulting in increased survival compared to monotherapies in mouse xenograft model. These data establish VEGFR2/Ang2 bispecific antibodies as a promising anti-angiogenic, anti-metastatic and anti-tumor agent for the treatment of cancer in combination with other therapies.

#3091 Response of C4.4A-positive patient-derived xenograft models of ESCC, HNSCC and bladder cancer to BAY1129980, a C4.4A-targeted antibody drug conjugate. Joerg Willuda, Carol Pena, Christoph Kneip, Patricia E. Carrigan, Hans-Georg Lerchen, Lars Linden, Bertolt D. Kreft, Bayer AG, Berlin, Germany. Bayer AG, Whippany, NJ; Bayer AG, Wuppertal, Germany. C4.4A (LYPDI) is a cancer- and metastasis-associated transmembrane cell surface protein which is expressed at high frequency and density in multiple tumor types including squamous and non-squamous non-small cell lung carcinoma (NSCLC), head & neck squamous cell carcinoma (HNSCC), esophageal squamous cell carcinoma (ESCC) and bladder cancer. C4.4A expression is restricted to a limited number of tissues (e.g. suprabasal layer of skin) making C4.4A an attractive target for the treatment of cancer with a C4.4A-targeted antibody-drug conjugate (ADC). BAY1129980 (C4.4A-ADC), is an ADC consisting of a fully human C4.4A-targeting monoclonal antibody (technology licensed from BioInvent) conjugated via a novel, non-cleavable alkyl hydrazide linker to a novel, highly potent auristatin W, an antimitotic agent (technology licensed from Seattle Genetics, Inc.). This C4.4A-ADC has been previously shown to be efficacious in C4.4A positive cell line-derived and PDX models of NSCLC. Here we present new preclinical efficacy data of C4.4A-ADC in patient-derived xenograft (PDX) models of ESCC, HNSCC and bladder cancer. Models were selected based on tumor C4.4A levels as determined by mRNA levels and immunohistochemistry (IHC), the latter of which allowed ranking of models according to H-score, percentage of C4.4A positivity, and staining intensity (0 to 3+) in the cell membrane. Representative C4.4A-positive models were selected for in vivo efficacy studies (n = 7 each): 4 HNSCC, 4 ESCC and 2 bladder cancers. C4.4A-ADC was administered as one cycle (Q4Dx3) at doses of 7.5 and 15 mg/kg, and efficacy was assessed up to 4 weeks post treatment for optimum tumor growth inhibition (TGI). In ESCC models a response to C4.4A ADC was seen in ES0190 (TGI of 77%; 15mg/kg) and in ES0195 (59%). In HNSCC models an ADC effect on tumor growth was observed in HN10847 (34%) and HN9619 (59%). Finally, both bladder models tested were sensitive to treatment, with a transient response seen in BL0597 (41%) and a strong and significant tumour growth inhibition (TGI). In ESCC models are a response to C4.4A ADC, C4.4A expression served as a marker for preselection of the models. Nevertheless, other factors may affect response and sensitivity of these tumor models, such as sensitivity to tubulin inhibition, ADC uptake and intracellular processing. In summary, these data support further exploration of the potential of BAY 1129980 in HNSCC, ESCC and bladder cancer in addition to NSCLC. A Phase 1 clinical trial of BAY 1129980 is ongoing (NCT02134197).

#3092 U3-1402a, a novel HER3-targeting ADC with a novel DNA topoisomerase I inhibitor, demonstrates a potent antitumor efficacy. Laura R. Saunders, Samuel A. Williams, Sheila Bheddah, Kumiko Isse, Sarah Fong, Marybeth A. Pyas, Himisha Beltran, Loredana Puca, Verena Salier, Juan M. Mosquera, Yu Yin, Joaotti Huang, Andrew J. Armstrong, Jorge Garcia, Cristina Magi-Galluzzo, Vadim Koslikhin, Petros Grivas, Farhad Kosari, John Cheville, Justine C. Moser, Thomas J. Flotte, Thyordar Halldaranson, Aaron Mansfield, Konstantinos N. Leventakos, Julian R. Molina, Douglas W. Ball, Barry D. Nelkin, Jill E. Shea, Courtney L. Scaife, Scott J. Diyla, Abhivve Stemcentrx, South San Francisco, CA; Weill Cornell Medicine, New York, NY; Duke University School of Medicine, Durham, NC; Cleveland Clinic, Cleveland, OH; Mayo Clinic, Rochester, MN; Johns Hopkins, Baltimore, MD; University of Utah, Salt Lake City, UT.

Expression of DLL3 was examined in additional tumor types, as it was found to be highly expressed in tumor-initiating cells (TIC) in small cell lung cancer (SCLC), where a DLL3-targeted antibody drug conjugate (ADC), rovalpituzumab tesirine (Rova-T; SC16LD6.5), targeting DLL3-positive cancer and pancreatic cancer patients' tissues. U3-1402a is an antibody-drug conjugate (ADC) comprised of a fully human anti-HER3 monoclonal immunoglobulin G1 (IgG1) antibody (U3-1287) conjugated vis a chloroethyl peptide linker to exatec derivative (DxD). The DxD is released after internalization of U3-1402a and leads to apoptosis of the target tumor cells by the inhibition of topoisomerase I. This ADC achieves a high drug-to-antibody ratio (DAR 7 to 8) with homogeneous conjugation with the topoisomerase I inhibitor. The aim of this study was to preclinically evaluate the efficacy of U3-1402a in breast cancer models. Materials and methods: In order to evaluate the pharmacological potential of U3-1402a, in vitro and in vivo studies were performed. In vitro growth inhibition assay evaluated the sensitivity of U3-1402a in HER3-positive human breast cancer cell line (HCC1569) and HER3-negative human cervical carcinoma cell line (C3A). Cells were treated with U3-1402a or MAAAB-1181 (payload of U3-1402a) depending on its concentration (U3-1402a: 0.153 to 10 000 ng/mL, MAAAB-1181: 2.44 to 160,000 pg/mL). In vivo growth inhibition study evaluated the dose-dependent sensitivity of U3-1402a in HER3-positive breast cancer xenograft model, MDA-MB-453. In addition, several xenograft models with different HER3 expression were tested with its sensitivity to U3-1402a. These models were HCC1569 (human breast cancer cell line, HER3 IHC score 3+), MDA-MB-453 (human breast cancer cell line, HER3 IHC score 2+), NIBIO-G016 (human gastric cancer patient-derived xenograft, HER3 IHC score 1+) and MDA-MB-231 (human breast cancer cell line, HER3 IHC score 0). Results: In vitro study, U3-1402a exhibited anti-tumor killing activity in HER3-positive human breast cancer cell line, HCC1569. C-33A human cervical carcinoma cell line was not sensitive to U3-1402a even MAAA-1181 itself exhibited anti-tumor killing activity to this cell line. In vivo study, U3-1402a showed dose-dependent anti-tumor killing activity in a HER3-positive breast cancer MDA-MB-453 xenograft model. Finally, in vivo tumor regression was only observed in HER3 2+ and 3+ models. Conclusions: U3-1402a preclinically exhibited its efficacy in breast cancer model in vitro and in vivo. In vivo efficacy was strongly correlated with HER3 expression. These studies suggest that U3-1402a, a novel HER3-targeting ADC, would be efficacious in a broader patient population with HER3 expression like breast cancer, melanoma, NSCLC, gastric cancer and pancreatic cancer.
astatic melanoma (55%), low grade gliomas (90%), glioblastoma (70%), medul-
lary thyroid cancer (65%), carcinoids (33%), dispersed neuroendocrine tumors in the pancreas (9%), bladder (57%) and prostate (24%), testicular cancer (90%), and lung adenocarcinomas with neuroendocrine features (80%). Unlike SCLC, where DLL3 does not predict clinical outcome on standard therapies, DLL3 expression negatively correlates with overall survival in melanoma and small cell bladder cancer. In mice bearing DLL3 positive melanoma PDX, treatment with a single dose of Rova-T resulted in effective and durable responses (>100 days), which correlated with a significant impact on TIC frequency. Similarly, in mice bearing DLL3 positive ovarian small cell PDX, a single dose of Rova-T resulted in effective and durable responses (>100 days). Our results show that DLL3 is expressed in many neuroendocrine tumors (lung, ovarian, prostate, bladder, etc.), metastatic melanoma, medullary thyroid cancer, low-grade gliomas and glioblastoma. Given pre-clinical results showing efficacy of Rova-T in melanoma and ovarian small cell carcinoma, as well as encouraging clinical data with Rova-T in patients with recurrent/refractory SCLC, clinical evaluation of Rova-T in DLL3 positive melanoma, glioblastoma, medullary thyroid cancer and other high-grade neuroendocrine carcinomas is warranted. A "basket" trial enrolling patients with DLL3 positive solid tumors is now recruiting patients (NCT02709889).

#3095 The development of CPI as a novel, next-generation DNA-targeting payload for ADCs. Jennifer Kahler, Maureen Dougher, Jane Xu, Matthew Doroski, Andreas Maderna, Russell Dushin, Stephane Thibault, Mauricio Leal, Madan Katragadda, Christopher J. O’Donnell, Matthew Sung, Puja Sapra, Pfizer, Pearl River, NY; Pfizer, Groton, CT; Pfizer, La Jolla, CA; Pfizer, Cambridge, MA.

DNA targeting drugs represent one of cornerstones of anti-cancer therapy for both hematologic and solid tumor indications. Low potency anti-DNA compounds (e.g. platin, anthracyclines) are included in many standard-of-care (SOC) regimens, however their modest activity and overall toxicity profiles limit their therapeutic potential. To increase the therapeutic window for DNA-damaging agents, high potency anti-DNA compounds with enhanced anti-tumor activity have been delivered to tumors as payloads of targeting modalities such as antibody-drug conjugates (ADCs). Herein, we describe the development of a novel DNA-damaging compound comprised of a dimeric structure of cycloo-
poly(pyrrrolo)[e]indolones (CPIs) that was designed to alkylate DNA and generate toxic interstrand crosslinks (ICLs). In response to the CPI-induced formation of ICLs, CPI treatment of cells primarily activates the Fanconia anemia DNA damage response pathway, whereas other successful DNA-damaging ADC payloads such as calicheamicin activate double-strand break response pathways. CPI shows ~860-fold greater potency than calicheamicin in a panel of cell lines derived from a broad spectrum of tumor indications. Importantly, this new CPI payload retains potent activity in calicheamicin- and SOC-resistant tumor models (including overcoming overexpression of drug efflux pumps). When evaluated as payloads on anti-CD33 targeting ADCs, the CPI conjugate showed dramatically improved efficacy over the corresponding calicheamicin conjugates in MDR+ tumor models. As a site-specific conjugate, the CPI ADC shows enhanced in vivo stability and possesses a wider therapeutic window than the corresponding conventional calicheamicin conjugate and other leading DNA-damaging conjugates on the CD33 platform.

#3096 Mechanisms of synergy of carboplatin and an EphA2-targeted do-
cetaxel antibody-directed nanotherapeutic. Walid S. Kamoun, Andrew J. Sawyer, Christine Pien, Alexander Koskharvey, Lia Luus, Samantha Merrig-
gan, Gang Sun, Sergey Kozin, Zhaohua Richard Huang, Suresh K. Tippa-
raju, Dmitri B. Kirtotin, Hannah Xu, Vasilios Askoxylakis, Patrick C. Reynolds, Daryl C. Drummond, Merrimack, Cambridge, MA; Massachusetts General Hospital, Boston, MA; Texas Tech University, Lubbock, TX.

Platinum-taxane combinations are widely used to treat solid tumors either in first or later lines of therapy. While effective in many settings, platinum-taxane combinatorial regimens are limited by toxicities. We have recently developed an antibody-drug conjugate of a nanotherapeutic (MM-310) encapsulating a docetaxel prod-
rug, targeted to Ephrin receptor A2 (EphA2). Preclinical investigation of MM-
310 revealed that the liposomal formulation leads to prolonged docetaxel expo-
sure of the tumor with decreased exposure of normal tissues leading to a shift in toxicity profile and potentially enabling more safe and effective combinations with platinum-based chemotherapeutics. In this study, we evaluated the activity of MM-310 in combination with carboplatin in several xenograft tumor models and compared it to the activity of free docetaxel in combination with carboplatin at equitoxic dosing. Tolerability of MM-310 in combination with carboplatin in mice was evaluated, including assessing hepatotoxicity. Biodistribution, micro-
distribution, in vivo tumor growth, and mouse survival studies were performed in lung and ovarian cell line-derived (CDX) and patient-derived xenograft (PDX) models. MM-310 in combination with carboplatin was found to be well tolerated, enabling dosing of both drugs at high doses with maximum tolerability when the drugs were dosed three days apart. Carboplatin increased nano-
therapeutic delivery to the tumor in a CDX model of triple negative breast cancer and in a PDX model of ovarian cancer. In vivo studies in lung and ovarian cancer xenograft models showed significant synergy between MM-310 and carboplatin when compared to the monotherapies, as well as when compared to free do-
cetaxel and carboplatin. In conclusion, we found that MM-310 in combination with carboplatin was significantly better tolerated and more effective than free docetaxel in combination with carboplatin. Mechanistically, the synergistic anti-
tumor activity of MM-310 with carboplatin may be partially due to a carboplatin mediated enhancement of nanotherapeutic delivery. The increased preclinical activity of the MM-310/carboplatin combination, together with the high toler-
ability following scheduling optimization tested in mice, makes this combina-
tion a promising regimen that warrants evaluation in clinical trials.

#3098 Nanotherapeutics for combination drug and gene therapy in treat-
ing glioblastoma multiforme. Angela A. Alexander-Bryant, Breanne Hou-
rigan, Michael Lynn, Jeoung Soo Lee, Clemson University, Clemson, SC; Greenville Health System, Greenville, SC.

Gliomas represent approximately 80% of all malignant brain tumors, and glioblastoma multiforme (GBM), the most aggressive type, accounts for nearly half of all gliomas. Despite treatment strategies including surgery, radiation, and chemotherapy, the 5-year survival rate for brain cancer is only 35%. New ther-
aputic strategies are necessary to improve the outcomes of this disease. Che-
motherapy with DNA alkylating agents is commonly used in treatment for
GBM. Research has shown that better therapeutic response of GBM tumors to alkylating agents and increased survival rate is indicative in patients with epigenetic silencing of O6-methylguanine-DNA methyltransferase (MGMT), a gene responsible for DNA repair. Therefore, we propose a treatment strategy combining drug and gene therapy to target and silence MGMT to sensitize cells to treatment with temozolomide (TMZ) or lomustine (CCNU), both DNA alkylating agents. We previously developed cationic, amphiphilic copolymer poly-(lactide-co-glycolide)-g-polyethyleneimine (PgP) and demonstrated its utility for nucleic acid delivery. Here, we examine the ability of PgP as a drug and siRNA delivery carrier to overcome drug resistance and improve anticancer activity through combination drug and gene therapy for GBM treatment. PgP micelles were designed and synthesized for delivery of hydrophobic drugs in the PLGA core and negatively charged nucleic acids in the positively charged PEG shell through electrostatic interactions. RNA binding and polyplex stability assays were performed using agarose gel electrophoresis. Cytotoxicity of TMZ, CCNU, and/or PgP/siMGMT polyplexes was determined by MTT assay. Silencing of MGMT on the protein and mRNA level was determined using western blotting and qPCR, respectively. Our results demonstrated that PgP effectively forms stable complexes with siRNA and protects siRNAs from serum- and ribonuclease-mediated degradation, confirming the potential of the polypelex for in vivo delivery. We demonstrated that PgP/siMGMT polyplexes mediate knockdown of MGMT protein as well as a significant ~56% and ~68% knockdown of MGMT mRNA in T98G GBM cells compared to cells treated with PgP complexed with the RNA binding dye SYTO1 and 801 nitrophenyphosphate (N-P) ratio, respectively. Further, co-treatment of PgP/siMGMT polyplexes with TMZ or CCNU enhanced anticancer activity in T98G GBM cells compared to treatment with the PgP/siMGMT polyplex, TMZ, or CCNU alone. Future studies will determine efficacy of drug-loaded and siRNA-complexed PgP for combination therapy in vitro as well as using a xenograft GBM model for local delivery. Successful combinational drug and gene therapy using PgP may overcome drug resistance and improve therapeutic outcomes for patient with glioblastoma.

Introduction: PARP inhibitors such as Talazoparib and Olaparib exploit deficiencies in DNA repair pathways, making them attractive candidates for treatment of a number of different cancers. These drugs are particularly effective when used in combination with other DNA damaging agents such as chemotherapeutics and radiation therapy. Combination trials, however, have resulted in several toxicities, necessitating either dose reduction or delay. Dose reduction leads to suboptimal dosing and provides little therapeutic benefit compared to monotherapy. Systemically administered nanoparticles offer a more effective way to selectively accumulate drugs in tumors and bypass toxicities associated with oral delivery. We have developed nanoparticle delivery systems for both Olaparib and Talazoparib in order to improve tumor accumulation while by-passing toxicities associated with oral administration. Methods: Liquid nanoformulations and solid formulations of Olaparib and Talazoparib have been developed and characterized in regard to size, surface charge, drug loading, release, and stability. NanoTalazoparib has been tested in vitro in breast cancer cell lines including T47D, W0069, W780, and HCC1937 which exhibit BRCA1 and 2 mutations, and NanaOlaparib in the lung cancer cell line Calu-6 which also has a defective FA-BRCA pathway. Mice have been treated with NanoOlaparib and NanoTalazoparib alone and in combination with radiation or temozolomide in order to evaluate toxicity. Therapeutic efficacy studies are currently underway. Results: The nanoformulations have been formulated to encapsulate a clinically relevant dose of either Talazoparib or Olaparib and release at 37°C over a period of days, while remaining stable during storage at 4°C. In vitro, both nanoformulations maintain the same activity as free drug with IC50s in the nanomolar range for these cell lines with varying deficiencies in the BRCA pathway. Mice have shown no appreciable weight loss during treatment with either nanoformulation alone or in combination with other treatment modalities. Conclusion: Nanoformulations of Talazoparib and Olaparib have been developed and characterized to demonstrate activity in vitro and tolerable doses in vivo. We have found that mice tolerate NanoTalazoparib at higher doses when combined with Temozolomide than when given oral Talazoparib. The sustained release from the nanoparticle allows for the nanoformulation to be administered less often than the daily administration for oral drug and the improved tolerability opens the door for combination therapy with both chemotherapeutics and radiation therapy. Therapeutic efficacy studies are underway and we expect that as a monotherapy NanaOlaparib will be more effective at lower doses than oral Talazoparib, based on the longer circulation time and more selective accumulation in tumors. We also anticipate that combination therapy will be more effective with the nanoformulation, as the maximum tolerated dose is higher that than of the oral drug.
#3102 Quantitation of nanoparticle delivery of AZD2811, an aurora kinase B inhibitor, to tumor tissues in mouse PDX models of solid cancers. Colin Howes,1 Susan Ashton,2 Elizabeth Pease,3 Richard J. Goodwin,1 Joanne Wilson,1 Aaron Smith,1 Matthew Ling,1 Nicole Strittmatter,1 Douglas Ferguson1,1 AstraZeneca, Cambridge, United Kingdom; 2AstraZeneca, Macclesfield, United Kingdom.

A polymeric nanoparticle (NP) formulation of AZD2811, a selective aurora kinase B inhibitor, is currently under clinical development for the treatment of both haematological and solid tumour diseases. Whilst the exploitation of nanotechnology to achieve targeted delivery of cancer drugs to solid tumours has been pursued for several years, it has achieved only limited success. A key requirement for delivering the desired accumulation of tumour distribution is the so-called ‘enhance permeability and retention (EPR) effect’. But this has been reported to offer less than a 2-fold increase in drug delivery to solid tumours relative to healthy organs using conventional NPs, resulting in drug concentrations that are insufficient for most cancers (Nakamura et al, Biocytin. Chem. 2016.). It is therefore important to have the capability to evaluate their distribution in preclinical models and potential for clinical progression. Delivery of AZD2811-NP in solid and haematological cancers each require different characteristics: Delivery to solid tumours, relies on exploitation of the EPR effect to achieve efficacious advantage over the drug alone through accumulation and release in the target tissue. The latter, treatment in haematological cancers, does not exploit the EPR effect, but is reliant on the extended pharmacokinetics (PK) delivery of polymeric drug carriers. In the case of tumour-associated NPs, diffusion and accumulation in the tumour microenvironment provide an efficacious level of drug whilst reducing side effects and overcoming the need, in the case of Barasertib (the pro-drug of AZD2811), continuous infusion for several days. Techniques to quantify drug delivery to tumour tissues in PDX mouse models of colorectal, lung and ovarian cancer have been developed to determine the extent of solid tumour distribution, allowing direct comparison with conventional drug distribution using labelled AZD2811. The effect of NP delivery of AZD2811 is to favourably alter the physical distribution of drug into the tissue in the models examined. The effect has been quantified by measuring the relative distribution of AZD2811-NP to other reference tissues, and additionally by comparison with the tumour concentration of drug delivered in a conventional formulation. By the first measure, we found an approximately 90 to 280-fold increase (in AUC) across these PDX models in AZD2811 concentration in tumour compared with muscle as the reference tissue. By the second measure, we found a 70 to 150-fold increase in AZD2811-NP compared with non-nanoparticle AZD2811 in tumour tissue at 4h post administration of the drug. The results demonstrate the benefit of drug delivery using polymeric nanoparticles in these three PDX tumour mouse models, increasing our confidence in successful translation to the clinic.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Drug Delivery Technology and Antibody Technology

#3103 Nanoengineered mesenchymal stem cells for targeted lung cancer therapy. Swayam Prabha,1 Tamnny Sadhukha,2 Buddhaheev Layek1.

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Solid tumors are characterized by increased interstitial fluid pressure and enhanced solid stress, contributing to poor intra-tumoral penetration of nanocarriers to solid tumors (OMIs) in vitro has shown therapeutic potential. However, in vivo delivery to solid tumor is urgently needed. MicroRNAs (miRNAs) are small non-coding RNAs that are involved in the regulation of gene expression. They have been extensively studied in cancer therapy due to their ability to target specific genes involved in tumor initiation, progression, and drug resistance. Several studies have shown that miRNAs can be used as therapeutic agents in cancer treatment. One such approach involves the use of miRNAs to target specific genes involved in tumor growth and survival. This approach offers several advantages over traditional drug therapy, including reduced toxicity and improved efficacy. In this study, we aimed to evaluate the potential of miRNAs as therapeutic agents in cancer treatment.

#3104 Rotating magnetic beads for enhanced drug delivery: characterization of bead velocity, imaging, and adherence to cellular monolayers. Herbert Engelhard,1 Zachary Gaertner,2 Adam Levin,1 Ankit Mehta,1 Sean Morris,2 Michael Sabo,2 Frances Creighton1.

1Univ. of Illinois at Chicago, Chicago, IL; 2Pulse Therapeutics, St. Louis, MO.

Background: Superparamagnetic iron oxide nanoparticles (SPIONs) have been touted as promising vehicles for enhancing drug delivery for cancer, stroke, and other diseases. Unfortunately, successful clinical use has been hampered by the problem of scale, since the attractive force between iron particle and magnet is inversely proportional to at least the fourth power of the intervening distance. Utilization of magnetically-induced rotary traction (MIRT) offers a way to overcome this obstacle. Here, we present initial data from the use of a new two-part system consisting of: 1) a patented rotating magnet, and 2) magnetic microbeads (MBs), which have been optimized for MIRT. Methods: MBs and the rotating magnet were provided by Pulse Therapeutics (St. Louis, MO). MBs consist of single-crystalline magnetite cores (~70 nm), which form aggregates in response to a magnetic field. Here, the field is generated by a neodymium-boron-iron permanent magnet, which is rapidly rotated causing MBs to counter-rotate (like meshed gears) at a physiological distance and thus move by means of surface traction. Movement of MBs through PBS, DEMEM with 5% serum, and 100% serum was measured 7.5 to 30 cm from the magnet. Suspensions of the particles were imaged at different concentrations by MRI and CT scan. 3 cancer cell lines (U87, E297, LKB1-KO), and normal vascular endothelial cells, were maintained using standard tissue culture technique. For adhesion studies, cells were grown in 6-well plates to confluence, treated with 10 ul. MBs for 30 min, washed with PBS, and then imaged with standard light microscopy, digitized, and analyzed using Image J. Results: In our experiments, MBs moved readily through PBS, DEMEM and serum at distances from 7.5-30 cm; with a maximum velocity at 22.5 cm (0.45 +/- 0.04 cm/sec, for serum). While MR imaging produced significant artifact as expected, MBs were clearly seen by CT scan. Adhesion of the MBs to the cancer cell line was markedly higher than to the endothelial cells (10.9-12.0X) and to fixed cells, used as controls. Conclusions: MBs are easily rotated and moved at physiologic distances, even through 100% serum, by means of surface traction, and can be imaged by CT scan. Adhesion of MBs to cancer cells is significantly greater than to endothelial cells. These features show that the Pulse system is an extremely promising one, for use in magnetic drug targeting in the clinical setting.

#3105 Targeting microRNAs in brain tumors with oligonucleotide nano-particle conjugates. Nilmary Grafas,1 Blanca I. Quiñones,1 Pablo E. Vivas,1 Gabriel Barletta2.

1University of Puerto Rico, Medical Sciences Campus, San Juan, PR; 2University of Puerto Rico, Humacao Campus, Humacao, PR.

Glioblastoma Multiforme (GBM) is the deadliest type of primary brain tumor with an estimated 14,000 deaths annually. The survival rate of GBM patients from time of diagnosis is 5 months if left untreated and 15 months with standard care of treatment. Despite great research efforts in the last 30 years, GBM survival rate has only increased by 2%. This is mostly due to high recurrence, treatment resistance, and the existence of the blood brain barrier (BBB) which restricts drug delivery to the tumor tissue. Therefore, the development of novel therapeutic modalities able to cross the BBB is urgently needed. MicroRNAs (miRNAs) are small (18-22 nt) non-coding RNAs that regulate gene expression at the post-transcriptional level by binding to the 3′UTR of target mRNAs. Several dysregulated miRNAs have been reported in all tumor types, including GBM. Targeting oncogenic miRNAs with oligonucleotide miRNA inhibitors (OMIs) in vitro has shown therapeutic potential. However, in vivo administration requires the development of nanocarriers capable of increasing the stability of OMs in circulation and improving their delivery to target tissues. In addition, nanocarriers that can overcome the BBB are needed. The most studied and promising nanocarriers are gold nanoparticles (AuNPs) and liposomes. While AuNPs have bioimaging and microcopy advantages, liposomes are clinically preferred. Therefore, we propose encapsulating OMs-AuNPs to BBB targeted liposomes. In this study we present the characterization of miRNA inhibitors conjugated to AuNPs. Briefly, 15 nm AuNPs were functionalized to OMIs by two different techniques: Salt aging functionalization method and PEG and surfactant
assisted conjugation method. Afterwards, both nanoconjugates were analyzed for particle size, zeta potential and loading capacity. The PEG and surfactant assisted functionalization proved to be superior in all of the analysis, resulting in 30 nm OMIs-AuNPs with a zeta potential of -8 mV and a loading capacity ratio of 64 oligonucleotides per AuNP. Further characterization studies such as cell internalization and cytotoxicity are under evaluation.

**#3106 Targeted delivery via Elastin Like Polypeptide: Biodistribution of a doxorubicin derivative in MDA-MB231 human breast cancer xenografts in mice.** Sonja Drugovic, 1 Jung Su Ryu, 2 Felix Kratz, 1 Drazen Raucher 1.

The anticancer agent doxorubicin has been widely used in the treatment of a variety of hematological malignancies and solid tumors. Despite doxorubicin’s efficacy in eradicating tumor cells, severe damage to healthy tissues, along with cardiotoxicity, limits its use in clinics. To overcome adverse side effects, improve patient safety, and enhance therapeutic efficacy, we have designed a thermally responsive biopolymer doxorubicin carrier that can be specifically targeted to tumor tissue by locally applying mild hyperthermia (41 degrees C). The developed drug vehicle is composed of: a cell penetrating peptide (SynB1) to promote tumor and cellular uptake; thermally responsive Elastin like polypeptide (ELP); and the (6-maleimidocaproyl) hydrazide derivative of doxorubicin (DOXO-EMCH) containing a pH-sensitive hydrazine linker that releases dox in the acidic tumor environment. We used the in vivo imaging system, IVIS, to determine the biodistribution of doxorubicin-delivered ELP in MDA-MB231 triple-negative human breast cancer cell line xenografts in nude mice. Tumor bearing mice were treated with a single IV injection of 10 mg/kg doxorubicin equivalent dose with free doxorubicin, thermally responsive SynB1 ELP 1-DOXO, and a thermally non-responsive control biopolymer, SynB1 ELP 2-DOXO. Following a 2 hour treatment with hyperthermia, tumors showed a 3-fold higher uptake when treated with SynB1 ELP 1-DOXO compared to free doxorubicin. Accumulation of the thermally non-responsive control SynB1 ELP2-DOXO was comparable to free doxorubicin, indicating that an increase in dox accumulation with ELP is due to aggregation in response to thermal targeting. Higher levels of SynB1 ELP1-DOXO and SynB1 ELP2-DOXO with respect to free doxorubicin were observed in kidneys. Fluorescence intensity from hearts of animals treated with SynB1 ELP1-DOXO show a 3-fold lower accumulation of doxorubicin than the same dose of free doxorubicin. SynB1 ELP1-DOXO biopolymers demonstrated a 6-fold increase in tumor/heart ratio in comparison to free doxorubicin, indicating preferential accumulation of the drug carrier in tumors. Overall, thermally targeted polymers are a promising therapy to enhance tumor targeting and uptake of anticancer drugs and to minimize free drug toxicity in healthy tissues, representing a great potential for clinical application.

**#3107 Targeting nanotherapeutics to the invasive glioblastoma margin or targeted delivery via Elastin Like Polypeptide: Biodistribution of a surfactant assisted functionalization proved to be superior in all of the assisted conjugation method. Afterwards, both nanoconjugates were analyzed for particle size, zeta potential and loading capacity. The PEG and surfactant assisted functionalization proved to be superior in all of the analysis, resulting in 30 nm OMIs-AuNPs with a zeta potential of -8 mV and a loading capacity ratio of 64 oligonucleotides per AuNP. Further characterization studies such as cell internalization and cytotoxicity are under evaluation.

**#3108 Gemcitabine nanoparticles show in vitro efficacy in murine pancreatic ductal adenocarcinoma.** Samantha T. Tucci, 1 Hamilton Kakwere, 2 Azadeh Kheirolomoom, 1 Jai W. Seo, 3 Elizabeth Ingham, 3 Chang-Hi Hwang, 4 Katherine Ferrara 1.

Our goal is to develop nanotherapeutics for the treatment of pancreatic ductal adenocarcinoma (PDAC). Squalenoylated Gem (SqGemNPs) and Gem-loaded temperature sensitive liposomes (GemTSLs) were synthesized and the resulting efficacy was compared to that of gemcitabine (Gem) and Abraxane®. Squalenoylation is the conjugation of squalene, a cholesterol precursor, to a drug and enhances in vivo delivery, half-life, and therapeutic efficacy [1]. All particles were evaluated in vitro in both a monolayer and organoid culture of the KralLSL-G12D/+; Trp53LSL-R172H+; Pdx-Cre (KPC) model. The organoid culture allows for phenotypic maintenance that is otherwise lost with monolayer culturing [2]. SqGem and Cholesterol-EPIPEG2 co-assembled into SqGemNPs via nanoprecipitation to yield particles of 210 ± 150 nm with 35 wt% Gem. Gem was loaded into TSLs by complexing copper to Gem inside multimamellar liposomes comprised of DPPC:DSPE-PEG2-DSPC (50:515) to yield particles of 110 ± 26 nm with 1.7 wt% Gem. KPC cells were plated as both monolayers and Matrigel® suspensions and organoid-cultured cells were plated in Matrigel. Cells were incubated with free Gem, SqGemNPs, pre-heated Gem-TSLs, or Abraxane. At 72 h, cell survival was determined by an MTT assay (Invitrogen, Carlsbad, CA). In monolayer-cultured cells, SqGemNPs was the only tested therapeutic to achieve 100% cell death (which occurred at a concentration of 50 uM). All other treatments reached a plateau of efficacy as a function of concentration. Two anticipated trends were confirmed; monolayer-cultured cells were more responsive to all treatments than organoid-cultured cells, and cells plated in Matrigel were less responsive to therapy than those plated as a monolayer. We found that the efficacy of preheated GemTSLs was similar to free Gem in all cell cultures and thus release from the TSLs was validated. In the organoid culture, the IC50 of SqGemNPs was 0.12 ± 0.04 uM compared to 0.19 ± 0.04 uM for free Gem, although both reached a plateau of efficacy at 70% cell death. The efficacy of Abraxane, GemTSLs and Gem plateaued at 45%, 77% and 70% cell death, respectively in organoids. In future studies, two techniques to increase drug availability in PC tumors will be evaluated: SqGemNPs plus high intensity ultrasound ablation, which can increase drug accumulation by 50-fold [3] and GemTSLs plus ultrasound hyperthermia to locally release Gem at a high concentration within the tumor vasculature. 1 Couvreur, P. et al. Nano Lett. 6, 2544-2548, doi:10.1021/nl061942q (2006). 2 Bo, S. E. et al. Cell 160, 324-338, doi:10.1016/j.cell.2014.12.021 (2015). 3 Wong, A. W. et al. J Clin Invest 126, 99-111, doi:10.1172/Jci83312 (2016).

**#3109 Ultrasound-mediated delivery and distribution of polymer nanocarriers in the normal brain parenchyma and melanoma metastases.** Habib Baghirov, 1 Sofie Snipstad, 1 Einar Sulheim, 1 Sigrid Berg, 2 Rune Hansen, 2 Frits Thorsen, 1 Trond Hagen, 1 Catharina de Lange Davies, 1 Andreas Ashlund, 2 Nirweidt, 1 Kristian Gerhard Jebsen Brain Tumour Research Centre, University of Bergen, Bergen, Norway; 3 SinoTech, University of California, Davis, Davis, CA; 4 SINTEF Materials and Chemistry, Trondheim, Norway; 5 Molecular Imaging Center and Kristian Gerhard Jebsen Brain Tumour Research Centre, University of Bergen, Bergen, Norway; 6 SINTEF Materials and Chemistry, Trondheim, Norway.

The blood-brain barrier (BBB) prevents the passage of nearly all drugs into the brain, hindering brain cancer treatment. Nanoparticles (NPs) have emerged as promising drug delivery vehicles, due to incorporation of poorly soluble drugs, functionalization for controlled and sustained release and combination of drug delivery with imaging. Transport of NPs across the BBB, however, is equally complicated and can benefit from versatile BBB opening techniques. Focused ultrasound (FUS) in combination with microbubbles (MBs) ensures safe and effective transport of the BBB, thereby allowing the BBB and transport NPs into the brain in a melanoma metastasis model. Intracardiac injection of patient-derived human melanoma cells was performed in immunodeficient mice. Brain melanoma metastases developed four weeks post-injection. A novel ultrasound system able to generate 1.1 MHz and 7.8 MHz FUS during the same experiment was used for FUS treatments. Selection of the treatment area was guided by magnetic resonance imaging. BBB was disrupted by FUS at 1.1 MHz, while FUS at 7.8 MHz was used to enable acoustic radiation force and, hopefully, push NPs farther into the extracellular matrix away from blood vessels. Successful BBB opening was verified using a gadolinium-

Many of the nanoparticle (NP) formulations approved for clinical use in solid tumor therapy provide only modest improvements in patient survival. This is in part due to NP tumor penetration barriers, including a dense and complex extracellular matrix (ECM) and an elevated interstitial fluid pressure, which hinder the penetration of drugs and NPs into and within tumors limiting therapeutic efficacy. Using polystyrene (PS) particles over the size range of 20-100 nm, we recently tested how particle size impacted NP diffusion in a well-characterized tumor ECM preparation (Matrigel) and in triple-negative MDA-MB-231 breast cancer xenografts by multiple particle tracking (MPT) and intravital microscopy. We also investigated the effect of PEG surface density on the diffusion of ~60 nm PS NPs in the same models. Nonspecific binding of the NPs to tumor ECM components was determined by a surface plasmon resonance (SPR) binding assay, which was then compared to the NP diffusion results. Furthermore, biodegradable PLGA NPs with various surface PEG densities and two clinically-relevant NPs, PEGylated liposomal doxorubicin (Doxil) and non-PEGylated albumin-bound paclitaxel (Abraxane), were evaluated by SPR to evaluate their potential tumor penetration ability. These studies demonstrated that NPs as large as 60 nm, but less than 110 nm in diameter, diffuse rapidly within tumor ECM and tumor xenograft tissues ex vivo if they are densely coated with PEG. The penetration of these NPs within the same tumor models was found to be highly dependent on surface PEG density. Intravital microscopy of NP spread in living tissue confirmed a significant difference in tumor tissue penetration between the 60 and 110 nm PEG-PS NPs, as well as between PEG-coated and uncoated NPs. Non-specific binding of NPs to tumor ECM components assessed by SPR, which associated a positive correlation in the particle diffusion results consistent across multiple particle types, including PS and biodegradable NPs. SPR assays revealed that Abraxane binds significantly to tumor ECM which has the potential to limit particle dispersion with tumor tissue. Studies to extend these findings to drug-loaded, molecularly-targeted biodegradable NPs are underway. We have successfully encapsulated paclitaxel to the polymer backbone of PLGA containing a low-molecular weight PEG coating. Additional surface modifications have been made to enable NP targeting to Fn14-positive triple-negative breast tumors. In summary, we have developed a NP platform that can diffuse and penetrate within tumor tissue and selectively target tumors. Using this approach, we can optimize therapeutics versions to improve drug efficacy while limiting many of the side effects and risks of free drug and non-targeted therapies.


Currently research antibodies have a serious quality problem, which is one of the major contributing factors to the irreproducibility of published reports. Labome organizes antibody applications cited in formal publications and has developed Validated Antibody Database (VAD). VAD, a manually curated database, compiles commercial and non-commercial antibodies whose specificities and applications have been independently reported in published results from formal articles. Labome prioritizes the curation of articles with knockout studies and monoclonal antibodies. One of the benefits of our curation effort is the identification of cross-reactive species and novel applications for antibodies, which are often developed for human/mouse proteins and tested by suppliers for a limited number of applications. Labome registers many antibodies having cross-reactivities with model organisms such as flies, worms, zebrafish, and frogs and with novel applications. The database can be browsed or searched online.

Prostate-specific membrane antigen (PSMA) is an attractive target for an ADC approach as it is over-expressed by virtually all prostate cancers and its expression is highest in poorly-differentiated, metastatic and castration-resistant cases. PSMA has limited expression in non-prostatic tissues, it is not secreted or cleaved by PSMA-expressing cells, and it is constitutively internalized, a process that may be accelerated by specific antibody binding. ADCT-401/MEDI3726 is an ADC composed of a monoclonal antibody (F591), directed against human PSMA, site-specifically conjugated (drug-to-antibody ratio of 1.8) to a highly cytotoxic DNA cross-linking PBD dimer with a valine-alanine dipeptide linker. In vitro, ADCT-401/MEDI3726 demonstrated potent and specific cytotoxicity in a panel of PSMA-expressing prostate cancer cell lines, whereas its activity was greatly reduced in PSMA-negative cell lines. In vivo, ADCT-401/MEDI3726 showed strong antitumor activity against CWR22Rv1 and LNCap human-derived prostate cancer xenograft models. In the CWR22Rv1 model, a tumor with low, heterogeneous PSMA expression, ADCT-401/MEDI3726 achieved dose-dependent antitumor activity when administered as single dose at either 0.33 or 1 mg/kg, which resulted in significant increase in survival compared to the vehicle-treated animals. Moreover, a single dose of ADCT-401/MEDI3726 showed remarkable superior antitumor activity compared to multiple doses of another PSMA-targeted ADC stochastically conjugated to the auristatin payload vcMMAE with a DAR of ~4. In the LNCaP model, ADCT-401/MEDI3726 resulted in dose-dependent antitumor activity when dosed once at 0.11, 0.33 or 1 mg/kg. In the PSMA-negative prostate cancer-derived PC3 xenograft model, ADCT-401/MEDI3726 and an isotope-controlled ADC showed limited tumor growth inhibition highlighting target-mediated antitumor activity. ADCT-401/MEDI3726 demonstrated potent and specific in vitro and in vivo antitumor activity in prostate cancer-derived models of differing levels of PSMA and this warrants further development of this ADC into the clinic.


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patic metastasis. In patient subgroup of stage II GC, those with increased ASGR2 expression had significantly shorter overall and disease-free survival than those without. Conclusions: ASGR2 may represent a biomarker for hepatic metastasis of GC and as a target for therapy.

#3113 Knockdown of cell division cycle-associated 8 (CDC8A) suppresses hepatocellular carcinoma growth via the upregulation of tumor suppressor ATF3. Tae-Won Jeon,1 Min Ji Ko,1 Yu-Ri Seo,2 In Hye Baik,1 Heeson Hwang,1 Hun-Mo Ryoo,1 Jin Young Kim,1 Keon Uk Park,1 Yun-Han Lee1.1 Keimyung University School of Medicine, Daegu, Republic of Korea; 2Daegu Catholic University, Daegu, Republic of Korea.

Background: Hepatocellular carcinoma (HCC) is the third most lethal cancer worldwide which remains a major challenge due to poor prognosis and limited treatment options. Cell division cycle associated 8 (CDC8A) is known as a component of a chromosomal passenger complex required for stability of the bipolar mitotic spindle and it is commonly overexpressed in human HCC. However, the functional role of CDC8A in HCC progression remains to be clarified. In this study, we hypothesized if targeting of CDC8A with small interfering (si) RNA could alter the course of HCC progression. We also investigated the molecular mechanism that mediates HCC cell death caused by CDC8A silencing. Methods: Human HCC cell lines, Huh1 and Huh7, were transfected with CDC8A siRNA and tested for growth inhibition and apoptotic induction using MTS, FACS and microscopic analysis. To obtain insights into the molecular changes in response to CDC8A knockdown, global changes in gene expression were examined using RNA sequencing. siRNA silencing of CDC8A in HCC cell growth and long-term colony formation by blocking cell cycle progression and by inducing apoptotic cell death. RNA sequencing data showed that, representatively, the anti-proliferative effects were driven by a subset of molecular alterations including the upregulation of tumor suppressive ATF3 and GADD34 genes and the downregulation of BGLAP, a key regulator of cell growth and invasiveness. Subsequent Western blot analysis affirmed that CDC8A silencing induces the increase in the levels of ATF3 and GADD34 and the decrease in phosphorylated Akt in both Huh1 and Huh7 cells. Also, the same condition decreased the levels of PARP-1 and pro-caspase 3, accelerating apoptosis. Of importance, silencing of CDC8A expression effectively suppressed HCC tumor growth in a murine xenograft model. Conclusion: These findings suggest that targeting CDC8A could be an attractive option for molecular therapy of HCC.

#3114 The role of prostaglandin signaling in human glioblastoma cell activities and growth in vitro and in vivo. Jianguo Shao,1 Yifeng Du,2 Tian Li3, Zi Shu1, Jianxiong Jiang1.1 Jinan University, Guangzhou, China; 2University of Cincinnati Academic Health Center, Cincinnati, OH.

Glioblastoma multiforme (GBM) constitutes the most frequent and aggressive form of malignant brain primary tumors. Despite current combined treatment involving surgery, chemotherapy and radiation therapy, the median survival of GBM patients is only about one year, with less than 3-5% of patients surviving over 5 years. Cyclooxygenase-2 (COX-2) is overexpressed in glioblastoma, and its expression level is highly positively correlated with the tumor grade. As a major enzymatic product of COX-2, prostaglandin E2 (PGE2) mediates inflammatory processes within the brain and facilitates progression of many chronic inflammation-associated neurological diseases via its four downstream receptors - EP1-EP4. We hypothesize that selectively targeting these receptors might provide an alternative therapeutic strategy for GBM with more specificity than generic blockade of the entire COX-2 cascade. In this study, two engineered human GBM cells with or without activated COX-2 were used to explore the PGE2 signaling involved in the development and progression of GBM. COX-2 activation by LPS or its overexpression facilitated migration, invasion, and transformation of these GBM cells in vitro. Moreover, the COX-2-mediated GBM cell activities were substantially suppressed by selective antagonists of PGE2 receptors that are highly expressed in these cells. Furthermore, the tested PGE2 receptor selective antagonists also suppressed the growth of GBM cells in vivo. In sum, our results suggested the inflammatory PGE2 signaling is involved in human GBM cell inflammation, migration, invasion and transformation through its downstream receptors, and should be explored as alternative molecular targets for novel GBM therapies.

#3115 Characterization of CDC1A protein as a novel prognostic biomarker and therapeutic target for oral cancer. Thang Manh Phung,1,2,3,4 Masahide Takekoshi,1,2,3,4 Yoshinori Murakami,1,2,3,4 Yataro Daigo1,1.1 The Institute of Medical Science, The University of Tokyo, Tokyo, Japan; 2Kumamoto University, Kumamoto, Japan.

Oral cavity carcinoma (OCC) is one of the most common causes of cancer-related death worldwide. Current therapies for OCC still show poor clinical outcome with five-year survival rate of less than 63%. Therefore, next generation biomarkers and therapeutic strategies for OCC are eagerly awaited. We selected genes that were specifically overexpressed in the majority of OCC using our gene expression profile database, and identified cell division cycle associated 1 (CDC1A) that appeared to encode nuclear protein as a candidate. Immunohis- tochemistry analysis using tissue microarrays indicated that CDC1A was detected in 67% of 99 OCC tissues (68%), but not in normal oral epithelia. In addition, high levels of CDC1A protein expression was significantly associated with poor prognosis for OCC patients (P = 0.0244 by log-rank test). Furthermore, knockdown of CDC1A expression by siRNAs significantly inhibited the growth of tumor cells. Moreover, flow cytometric analysis, and apoptosis assay of live cell imaging revealed that CDC1A could be important for the growth and survival of OCC cells, probably through the regulation of mitotic phase and/or apoptosis. Our findings suggest that CDC1A is likely to be a novel prognostic biomarker and a therapeutic target for OCC patients.

#3116 Dysregulated cholesterol synthesis is a therapeutic vulnerability in glioblastoma. Diane M. Kambach, Alan S. Halim, A. Gesine Cauer, Qian Sun, Carlos A. Tristan, Orieta Celiku, Aparna H. Kesarwala, Uma Shankavaram, Eric Batchelor, Jayne M. Stommel. National Cancer Institute, Bethesda, MD.

A hallmark of cellular transformation is the evasion of contact-dependent inhibition of growth. To find new therapeutic targets for glioblastoma, we looked for pathways that are inhibited by high cell density in normal astrocytes but have lost this regulation in glioma cells. Here we report that at high cell density, normal astrocytes turn off cholesterol synthesis but glioma cells keep this pathway on. Correspondingly, cholesterol pathway upregulation is associated with poor prognosis in glioblastoma patients. Densely-plated glioma cells increase oxygen consumption to synthesize cholesterol, resulting in a decrease in reactive oxygen species, TCA cycle intermediates, and ATP, but without a compensating increase in aerobic glycolysis. This constitutive cholesterol synthesis is controlled by the cell cycle, as it can be turned off by cyclin-dependent kinase inhibitors and it correlates with disabled cell cycle control through loss of p53 and RB. Finally, glioma cells, but not astrocytes, are sensitive to cholesterol synthesis inhibition downstream of the mevalonate pathway, suggesting that specifically targeting cholesterol synthesis might be an effective treatment for glioblastoma.
was detected in the brain tumor tissues as early as 15 minutes after intravenous injection of eribulin, which remained stable even 24 hours later when the plasma concentration of eribulin became undetectable. Finally, intraperitoneal administration of eribulin significantly prolonged the survival of mice with intracerebrally transplanted U87MG xenografts (p<0.001). [Conclusion] Our results showed that eribulin efficiently transfers into brain tumor tissues and has a strong antitumor effect against GBM cells through inhibition of Rdrp activity. Eribulin thus appears to be a promising novel TERT-targeting therapeutic agent against GBM. A clinical trial is being scheduled.

#3118 Histological subtypes correlate with distinct genetic abnormalities in defining therapeutic targets in medulloblastoma. Samuel Gelnic,
Anubhav G. Amin,1 Molly Gordon,2 Raphael Salles Cortegata de Medeiros,2 Nelci Zanon,3 Raj Murail,4 Meena Ihanwar-UNival,2 1Dept. of Neurosurgery, New York Medical College, Valhalla, NY; 2Hospital Santa Marcelina, Sao Paulo, Brazil.

Medulloblastoma (MB) is the most common primary pediatric malignant brain tumor. Genetic classification and distinct histologic subtypes defines MB into 4 groups: classic (WNT), sonic hedgehog (Shh), group 3 and group 4. In addition, recent studies have provided evidence that p53 abnormalities is seen in various sub-groups of MB and that p53-mutant SHH MBs commonly harbor genetic anomalies including MYCN and GLI2 amplifications, which confers drug resistance. Here, we test the hypothesis that specific immunohistological markers, their correlation with the amplification of the oncogene MYCN, and abnormalities in tumor suppressor gene p53, may define their metastatic potential. Materials and Methods: Immunohistological analysis of MB tumors (n=41) was evaluated. Amplification of glioma transcription factor 1 (GLI1), natriuretic peptide receptor (NPR), voltage-gated potassium channel (KV1) and mutant p53. FISH analysis was performed to determine MYC amplification or iso- P53. p53-mutant MB cell line was used to investigate the signaling pathway leading to proliferation, migration, and drug resistance using HDAC (LBH-589) and PI3K/mTOR (BKM-120/rapamycin) inhibitor. Results: we showed that: 1) GAB-1 was highly expressed in the Shh group (82%) and KV1 expression was evenly distributed in all subtypes; 2) No obvious correlation with expression of GLI-1, GAB-1, NPR, or KV1 with metastasis was seen; 3) Analysis of loss of p53 and overexpression of MYC varied in each subtype; 4) Combined Treatment with LBH-589 and BKM-120 reduced cell proliferation, migration and S-phase entry, however, MB cells were resistant to BKM-120 treatments, while LBH-589 caused massive apoptosis; 5) Tumor formation was suppressed by BKM-120 given with mTOR inhibitors. Conclusion: the result of this study suggests that expression of GLI-1, GAB-1, NPR, KV1 and p53 was important in defining the subgroups of MB, although their metastatic potential remains to be understood. MB cells shows reduced cell proliferation, cell cycle and migration, when treated with HDAC inhibitor LBH-589, however these cells display complete drug resistance to PI3K inhibitors.

#3119 Identification of genetic drivers in HER2enriched/HER2negative breast cancer. Susana Garcia Recio,1 Cheng Fan,1 Marni Siegel,1 Joel S. Parker,1 Gina N. Duronio,1 Aleix Prat,2 Charles M. Perou,4 1University of North Carolina, Chapel Hill, NC; 2Hospital Clinic de Barcelona, Barcelona, Spain.

Background: The non-basal triple negative breast cancer (Non Basal-TNBC) tumors, also called the luminal androgen receptor (LAR) subtype, make up ~20% of all new TNBC cases and is limited to multi-agent chemotherapy. TNBC is considered one of the leading causes of cancer-associated mortality amongst women in the USA with a high number of patients dying of metastatic disease. Some metastases that arise from luminal primary tumors lose their luminal features and become a HER2-enriched (HER2E) subtype, but remain clinically HER2 negative (HER2E/HER2−). The molecular features that drive these HER2E/HER2− tumors may also represent key checkpoints of metastatic progression. Within this HER2E/HER2− subtype, besides the androgen receptor (AR), FGFR4 has been shown to be expressed, but its functional role remains unknown. FGFR4 is a tyrosine kinase receptor involved in proliferation, survival and migration during embryonic development and cancer. We hypothesize that in addition to AR, that FGFR4 may be a driver of HER2E/HER2− tumors and that the therapeutic targeting of this gene/protein might improve patient outcomes. Objective: Our objective is to identify the mechanism of action of FGFR4 in HER2E/HER2− tumors as well as additional genetic drivers of HER2E TNBC subtype. Methods: We performed computational analysis of databases (TCGA, METABRIC) and the analysis of matched breast cancer primary tumors and metastases to detect genetic drivers. We ran microarrays on two FGFR4 stably transfected luminal HER2+ ER− cell lines (MC7F and T47D) and also treated a Luminal B cell line (MDA-MB-453; HER2+/ER−) and HER2+ cell line (CAM-A1; HER2+/ER+/AR−) with a FGFR4 inhibitor using a time course strategy. Results: Through the analysis of microarrays using all 4 cell lines, we identified a gene expression signature of FGFR4-activity enriched in genes involved in proliferation, cell junctions and cell differentiation. The FGFR4-associated signature of upregulated genes was enriched in HER2E and Basal-like subtypes and the downregulated genes enriched in the Luminal subtypes. To identify predictors of chemotherapy response, we evaluated FGFR4-associated gene signatures in the diagnostic (pre-treatment) samples of MDACC neoadjuvant dataset of 500 tumors. The high expression of the FGFR4-induced gene signature predicted pathological complete response (Tst P <0.001). Conclusions: FGFR4 may play an important role in the progression of Luminal disease and has been identified as a new genetic driver of HER2E/HER2− subtype making it an attractive option as a therapeutic target. We are further testing these genetic predictions using Patient Derived Xenograft (PDX) mouse models.

#3120 Application of a lectin as a drug carrier for glycan-targeting cancer therapy. Tatsuya Oda,1 Osamu Shimomura,1 Hiroaki Tateno,2 Jun Hi-rabayashi,3 Masayuki Noguchi,1 Shigeru Chiba,1 Makoto Asahima,1 Nobuhiro Ohkohchi,1 1Institute of Clinical Medicine, Univ. of Tsukuba, Tsukuba, Ibaraki, Japan; 2National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki, Japan.

Various cancers such as pancreatic ductal adenocarcinoma (PDAC) remain intractable despite recent advances in tumour-targeting antibody drugs. Although the outermost coating of every cell type is predominantly composed of cell-specific glycans, antibody strategies have hit walls of technical difficulties in intentional glycan targeting, and medicoeconomic burden. Lectins, proteins with glycan-binding activity, can be used as an alternative scaffold; however, the prejudice that all lectins mediate harmful haemaggulitation limited its use only in experimental and diagnostic applications. Here, we show the successful in vivo application of a lectin as a drug carrier for cancer therapy. We have employed rB2CLC-N lectin since this has already shown a specific affinity between PDAC cell-surface glycans. When a lectin-drug conjugate (LecD) was developed by fusion with a bacterial exotoxin, the 50% inhibitory concentration (1.04 pg/ml=0.0195 pm) was 1000 times lower than that of conventional immunotoxins (on the order of ng/ml). In addition, we have revealed that rB2CLC-N lectin was safely administrable to mice without haemaggulitation. Administration of this LecD in a PDAC mouse model of subcutaneous nodules and peritoneal dissemination, generated prominent therapeutic effects. The utilization of lectins as an in vivo drug-carrier targeting cancer glycan shows realistic potential when paired with advanced lectin engineering technologies, and their applications could be expanded by coupling with small molecule and/or nanoparticle drugs.

#3121 Validation of protein kinase D1 as a prognostic factor and pharmacological target for the treatment of breast cancer. Caroline Spasojevic,1 Marie Karavani,1 Aashie Vacher,1 Jean Marc Ricort,1 Elisabetta Marangoni,1 Ivan Bièche,1 Christian Auclair2. 1Institut Curie, Paris, France; 2Ecole normale supérieur de Cachan, Cachan, France.

Tamoxifen-resistant ERα positive (ERα+) breast cancers and triple-negative breast cancers represent two aggressive subgroups of mammary tumors with limited treatment options and a poor prognosis. Thus, there is still an urgent need to develop and improve targeted pharmacological treatments against these estrogen-independent cancers. Recent data from our group suggest that the protein kinase D1 (PKD1) participates in the development and/or maintenance of the estrogen-independent phenotype in breast cancer. Indeed, up-regulating PKD1 conferred anchorage-independent growth to estrogen-dependent MCF-7 cells in the absence of β-estradiol. In addition, we found that high PKD1 mRNA levels were associated with a worse prognosis in tamoxifen-treated tumors. PKD1 is a serine-threonine kinase encoded by the prkd1 gene and belongs to the protein kinase D (PKD) family, a novel subgroup of the calcium and calmodulin-regulated kinases (CAMK). The PKD family also includes PKD2 and PKD3 which share a high homology with PKD1. The objective of the present study was to determine whether PKD1 could be a prognostic factor and/or a pharmacological target for the treatment of breast cancer. Expression of prkd1, prkd2 and prkd3 was analyzed by RT-qPCR in 527 breast cancer specimens (181 ERα positive(ERα+) breast cancers and triple-negative ERα- tumors). We showed that higher prkd1 mRNA levels were associated with a lower metastasis-free survival in the overall breast cancer population (p=0.015). prkd1 prognostic value was even stronger in ERα- tumors (p=0.00042) and in triple negative tumors (p=0.0043). Conversely, prkd1 expression was not associated with prognosis in ERα+ tumors. Interestingly, high prkd2 and prkd3 mRNA levels were associated with a better prognosis in the entire cohort (p=0.0074 and p=0.028 respectively). We have developed PKD1 inhibitors in collaboration with the AB Science pharmaceutical company and screened the ability of thirty-five com-
pounds to inhibit anchorage-independent growth of MCF-7 cells in the absence of estrogens. The antitumor activity of two PKD1 inhibitors is currently being evaluated in vivo against patient-derived breast cancer xenografts. In conclusion, our results show that high prkd1 expression is a bad-prognosis factor in breast cancer and that PKD1 could be a relevant therapeutic target for the treatment of breast cancer, in particular those harboring an estrogen-independent phenotype.

#3122 Pharmacogenic profiling to identify novel therapeutic strategies in colorectal cancer. Jarle Bruun,1 Peter W. Eide,1 Kushtrum Kryeziu,1 Anita Sveen,1 Astrid Murumagi,2 Matthias Kolberg,1 Marlianna Arjama,3 Olli Kallioniemi,2 Ragnar A. Lothe1. 1Institute for Cancer Research, Oslo Univ. Hospital, Oslo, Norway; 2Institute of Molecular Medicine Finland, Helsinki, Finland.

Colorectal cancer (CRC) is amongst the most frequent cancers worldwide and the fourth most common cause of cancer mortality. Surgery is the standard treatment of care, while chemotherapy and/or radiotherapy are offered to subgroups of patients according to disease stage. Current guidelines do not have sufficient accuracy to identify chemotherapy responders, resulting in under- or overtreatment of many patients. To discover novel therapeutic strategies for CRC we performed a high-throughput drug screen containing 461 approved and preclinical drugs on a representative panel of 42 CRC cell lines. Integrative pharmacogenic profiles were explored by matching data from gene expression, exome sequencing, copy number and reverse-phase protein arrays. These analyses identified defined molecular subgroups sensitive to targeted drugs like Inhibitors of EGFR, IGF-1R, Aurora B and/or downstream signaling mediators like B-Raf, MEK, PI3K/AKT, as well as inhibitors of auroctotic kinases, heat shock protein 90 and p53. Additionally, a correlation between microsatellite instability and sensitivity towards topoisomerase-I inhibitors was found. Comparison of the most sensitive and the most resistant cell models against HSP90 inhibitors indicated that drug sensitivity does not depend on target expression but is likely attributed to the cell line-specific expression of client proteins. Real-time cell analyses measured with the xCELLigence system demonstrated a strong impact on cell proliferation but not on the migration of HSP90-sensitive cells treated with HSP90 inhibitors as compared to resistant cells. These integrative analyses have unraveled pharmacogenic relationships that if validated in in vivo model systems, might be used to stratify patients for therapeutic intervention in biomarker guided clinical trials.

#3123 Identification of TIFA as a novel therapeutic target in acute myeloid leukemia. Pei-Yu Wu,1 Tong-You Wang,1 Ting-Jung Wu,2 Ming-Daw Tsaï1. 1Academia Sinica, Taipei, Taiwan; 2Chang Gung Memorial Hospital, Taoyuan, Taiwan.

Aurora A-dependent NF-κB signaling portends poor prognosis of cancers including acute myeloid leukemia (AML). Our previous study demonstrated that phosphorylation-dependent oligomerization of Traf-interacting protein with FHA domain (TIFA) triggers the activation of NF-κB. The present study identifies that Aurora A is an essential kinase for the Thr9 phosphorylation of TIFA, and that TIFA functionally mediates the Aurora A-driven NF-κB survival pathway in AML. Overexpression of TIFA occurred concurrently with Aurora A and NF-κB signaling factors in de novo AML patients but not healthy individuals, and also correlated with poor prognosis. Silencing of TIFA specifically attenuated leukemic cell growth and enhanced chemosensitivity of AML cells via down-regulation of pro-survival factors Bcl-2 and Bcl-XL that support NF-κB-dependent anti-apoptotic events. In addition, molecular targeting of TIFA perturbed leukemic cytokine secretion and significantly lowered the IC50 of chemotherapeutic drugs to treat AML cells. Furthermore, in vivo delivery of TIFA-inhibitory fragments potentiates the clearance of leukemic myeloblasts in the bone marrow of xenograft-recipient mice via enhanced chemotaxis, similar to the effect of anti-inflammatory drug treatments. Collectively, we proposed that TIFA functionally supports the positive feedback between TNF-α, Aurora A, and NF-κB to facilitate AML survival signaling, and impairment in this pathway can enhance the efficacy of AML treatments.

#3124 Expression and therapeutic implications of cyclin dependent kinase 4 (CDK4) in osteosarcoma. Yubing Zhou, Francis Hornicke, Zhenfeng Duan. Massachusetts General Hospital, Boston, MA.

Overexpression and/or hyperactivation of cyclin-dependent kinase 4 (CDK4) has been found in many types of human cancers, and a CDK4 specific inhibitor, palbociclib, has been recently approved by the FDA for the treatment of breast cancer. However, the expression and the therapeutic potential of CDK4 in osteosarcoma remain unclear. In the present study, CDK4 was found to be highly expressed in human osteosarcoma tissues and cell lines as compared with normal human osteoblasts. Elevated CDK4 expression correlated with metastasis potential and poor prognosis in osteosarcoma patients as determined by immunohistochemical analysis in a human osteosarcoma tissue microarray (TMA). CDK4 inhibition by either palbociclib or specific small interference RNA (siRNA) exhibited dose-dependent inhibition of osteosarcoma cell proliferation and growth, accompanied by suppression of the CDK4/6-cyclinD-B signaling pathway. Western blotting analysis showed that CDK4 knockdown decreased osteosarcoma cells in the G1 phase of the cell cycle and induced cell apoptosis. Furthermore, inhibition of CDK4 significantly decreased osteosarcoma cell migration in vitro determined by the wound healing assay. These data highlight that CDK4 may be a potential promising therapeutic target in the treatment of human osteosarcoma.


Glioblastoma (GB) is the most common malignant primary brain tumor, which is characterized by marked intra-tumor genetic heterogeneity in receptor tyrosine kinases (RTK) genes. Aberrations in the RTK/PI3K/Akt pathways in GB leads to an abnormal signaling pathway of mechanistic target of Rapamycin (mTOR). mTOR exists in two distinct complexes, namely, mTORC1 and mTORC2, which are involved in regulation of cell survival, growth, and motility. In this investigation, we test the hypothesis that the genetic heterogeneity of GBM cells and its stem cells with respect to the expression of EGFR and/or PDGFRα may define the treatment efficacy of RTK inhibitors with or without PI3K/mTOR inhibitors. We demonstrated that GB tumors over-expressed PDGFRα and EGFR at range of 80% and 30%, respectively, with varying degree of overlap. Combined treatment with the inhibitors of PDGFRα (AC 710) or EGFR (gefitinib), with or without inhibitors of mTORC1 (rapamycin) or mTORC1/2 (Torin1 and Torin 2), led to suppression of cell proliferation, cell cycle, cell migration and drug resistance in GB cells to varying degrees that correlated with EGFR or PDGFRα expression. Downstream signaling pathway substrates of mTORC1 and 2, namely pS6k and pAkt, respectively, were suppressed by combined treatments of RTK and mTORC1/2 inhibitors. Furthermore, GB stem cell self-renewal and growth was also suppressed with combined treatments. The combination of AC710 plus mTORC1/2 inhibitor, Torin2, was the most effective. These findings underscore the usefulness of determining PDGFRα and EGFR status when considering viable PI3K/mTOR inhibitors for targeted treatment of GB.

#3126 H3B6527, a selective and potent FGFR4 inhibitor for FGFR9-driven hepatocellular carcinoma. Anand Selvaraj, Erik Corcoran, Heather Coffey, Sudeep Prajapati, Ming-Hong Hao, Nicholas Larsen, Jennifer Tsai, Takashi Sato, Kana Ichikawa, Julie Jaya Joshi, Raelene Hurley, Jeremy Wu, Chia-Ling Huang, Suzanna Bailey, Craig Karr, Pavan Kumar, Victoria Rimkunas, Crystal Mackenzie, Nathalie Rioux, Amy Kim, Sandeep Akare, George Lai, Lihua Yu, Peter Fekkes, John Wang, Markus Warmuth, Peter Smith, Dominic Reynolds. H3 Biomedicine Inc., Cambridge, MA.

Hepatocellular carcinoma (HCC) has limited treatment options and generally poor prognosis. Recent genomic studies have identified FGFR9 as a driver oncogene in HCC. HGF19 is a gut secreted hormone that acts in the liver through FGFR4 to regulate bile acid synthesis. Consistent with the notion that FGFR9 is a driver oncogene in HCC, transgenic mice overexpressing FGFR9 form liver tumors and genetic ablation of FGFR4 prevented tumor formation. These data suggest targeting FGFR4 would have therapeutic benefit in HCC with altered FGFR9 signaling. While a number of Pan-FGFR inhibitors are being clinically evaluated, their application to FGF19-driven HCC may be limited by their chemical and cellular selectivity assays showed that H3B-6527 is >300 fold selective towards FGFR4 compared to other FGFR isoforms. Addition of H3B-6527 to FGFR9 amplified HCC cell lines led to dose dependent inhibition of FGFR9 signaling and concomitant reduction in cell viability. In a panel of 40 HCC cell lines, H3B-6527 selectively reduced the viability of cells that harbor FGFR9 amplification and showed no effect in FGFR9 non-amplified HCC cell lines. Oral dosing of H3B-6527 to mice led to dose-dependent phenotypic modulation of FGFR4 signaling and tumor regression in FGFR9 altered HCC cell line derived xenograft models. H3B-6527 demonstrated inhibition of tumor growth in an orthotopic liver xenograft model of FGFR9 altered HCC grown in nude mice. Importantly, the inhibition of tumor growth occurred at doses that were well tolerated in mice and no evidence of FGFR1-3 related toxicities were observed at efficacious doses. In a panel of 30 HCC patient-derived xenograft (PDX) models, H3B-6527 demonstrated tumor regres-
sions in the context of FGF19-amplified tumors. In addition, H38-6257 showed antitumor activity and tumor regressions in PDX models with high FGF19 expression but no FGF19 amplification. The mechanism for FGF19 overexpression in the absence of gene amplification is under investigation. In conclusion, our preclinical studies demonstrate that FGF19 expression is a predictive biomarker for AR resistance and that inhibition of FGF19 activity by bimagore effectively abrogates up-regulatory data sets indicates that at least approximately 30% of HCC patients exhibit altered FGF19 expression and could potentially benefit from H38-6257 monotherapy treatment.

#3127 Roles of ERKs-RSK2 signaling in human cancers. Yong Yeon Cho. The Catholic Univ. of Korea College of Pharmacy, Bucheon-si, Republic of Korea.

RSK2 is a member of p90RSK superfamily consisting of RSK1-4 and MSK1-2, and has suggested involving in many cellular processes including cell proliferation, differentiation, transformation and cancer development. Accumulating data obtained by our 16-year researches strongly demonstrated that RSK2 is a key player in cell transformation and cancer metastasis. We found that ectopic expression of RSK2 induced anchorage-independent cell transformation. Notably, knockdown of RSK2 strongly suppressed constitutive active-Ras (CA-Ras)-induced foci formation, EGFR-induced cell transformation and cancer cell growth in anchorage-independent condition. The results obtained from human skin cancer tissue array demonstrated that total protein levels and RSK2 activity were higher in cancer tissues than that of normal tissues. Molecular targeting of RSK2 or ERK1 and 2 with 2 natural compounds demonstrated that ERKs-RSK2 signaling pathway plays a key role in cell transformation and cancer development. Moreover, inhibition of ERKS-RSK2 signaling suppressed NF-κB activity in BCC41 cells. The EGFR-induced NF-κB activity was abrogated upon regulation of COX-2 mRNA expression and COX-2 protein levels by inhibition of ERKs-RSK2 signaling pathway. Interestingly, magnolin, a specific inhibitor of ERK1 and ERK2, suppressed ERK-mediated MMP-2 and NF-κB-mediated MMP-9 expression. In addition, we found that the protein expression of N-cadherin involved in metastasis was markedly attenuated by inhibition of ERKS-RSK2 signaling pathway. Notably, magnolin suppressed the migration and invasion of lung cancer cells in a dose-dependent manner. Taken together, these results demonstrated that targeting of ERKs-RSK2 signaling is beneficial for the chemoprevention, and anti-invasion and -migration in cancer metastasis. Key words: MAPK pathway, Transformation, Metastasis, Molecular targets

#3128 TRPM7 regulates gloma cell proliferation and migration through different function domains. Mingli Liu. Morehouse School of Medicine, Atlanta, GA.

Background: Transient Receptor Potential Melastatin-related 7 (TRPM7) is a non-selective cation channel fused with a functional kinase domain. TRPM7 channel expression is aberrant in numerous cancers. We have previously reported that suppression of TRPM7 inhibits the proliferation, migration, and invasion of malignant human gloma cells using A172 gloma cell line and brain tissue of human glioma [CNS Neuroscience & Therapeutics 2015, 21(3):52-61]. We further found that TRPM7 channels regulate gloma stem cell growth/proliferation through STAT3 and Notch signaling pathways [Cellular Signaling, 2014, 26: 2773-2781]. Since TRPM7 is a bifunctional protein with channel and α-kinase domain, we next investigated whether the changes in glioma cell proliferation and migration might be caused through channel domain-mediated and/or kinase domain mediated TRPM7 activation. Methods: 1) A172 cells expressing wild-type human TRPM7 (wtTRPM7) or constructs in which the α-kinase domain was deleted (Δkinase) or rendered inactive with a point mutation in the ATP binding site of the α-kinase domain (K1648R) were studied. 2) The effects of TRPM7 on glioma cell proliferation and invasion were determined using MTT assay and transwell invasion assay by TRPM7 expression using wt-TRPM7, Δkinase, and K1648R, respectively. Results: 1) Treatment of A172 cells with Δkinase and K1648R significantly increased TRPM7 expression demonstrated by Western blot assay. 2) The cell invasion was significantly reduced when treated with Δkinase and K1648R which indicate that TRPM7 kinase activity is required for changes in cell migration and invasion. 3) The cell proliferation was not significantly changed when treated with Δkinase and K1648R which indicate that TRPM7 channel rather than kinase activity is required in cell growth of A172 cells. Conclusion: TRPM7 regulates glioma cell proliferation and migration/invasion through different function domains. The channel activity of TRPM7 is required for the cell growth while the kinase domain is required for the cell migration/invasion. The finding is important for designing and developing drugs targeting TRPM7 in human malignancies.

#3129 Patient-derived xenografts (PDXs) recapitulate the antitumor activity of novel therapies in metastatic breast cancer (MBC) patients (pts). Mafalda Oliveira,1 Javier Pascual,2 Cristina Cruz,2 Alberto Gris,3 Marta Palafox,1 Alba Llop,1 Marta Castroviejo,1 Cristina Viaplana,1 Yasir H Ibrahim,3 Joaquin Arribas,1 Javier Cortés,3 José Baselga,3 Jordi Rodal,3 Judith Balmmana,3 Rodrigo Diestmanna,3 Cristina Saura,1 Violeta Serra1, Vail d’Hebron Institute of Oncology, Barcelona, Spain; 2Hospital Universitario Virgen de la Victoria, Malaga, Spain; 3Blenis Lab, Weil Cornell Medicine, New York, NY; 4Hospital Ramón y Cajal, Madrid, Spain; 5Memorial Sloan Kettering Cancer Center, New York, NY.

PDXs are preferred laboratory models that recapitulate the biology of pts tumors to be used to assess if the antitumor activity of novel therapies in PDXs correlates with the observed activity in the corresponding MBC patient, which would support cross-analysis of drug/genotype vulnerabilities. MBC pts eligible for clinical trials were offered a research biopsy and establishment of PDX, under an IRB-approved protocol. Biopsies were implanted in nude mice subcutaneously. Upon successful engraftment, models were expanded for drug testing, specifically with the same drug/combo given to the patient. This is a retrospective analysis of our MBC patient/PDX (P/PDX) co-clinical trial cohort. Pts’ outcomes were measured as: RECIST response, clinical benefit [CB: partial response (PR) or stable disease (SD) >4 months], and time to progression [TTP: time until RECIST progressive disease (PD), in months]. PDX endpoints were measured as: TTP (time until 25% increase in tumor volume, in days), response (PR or CR, in % of area under the curve (AUC) of treated tumor area versus vehicle. A consistent P/PDX response was defined as CB in pts whose paired PDX showed PR/SD, or no-CB in pts whose paired PDX showed PD. Pts outcomes and PDX endpoints were correlated using Spearman’s test. We identified 10 MBC pts (7 TNBC, 3 HR+; all biopsies from skin/subcutaneous lesions) with corresponding PDX, treated with paired therapies (7 molecularly matched); 3 lurbinectedin (BBRCA1-mut, 3 olaparib (BBRCA1-mut), 1 AZD9563 + fulvestrant (AKT1-mut), 1 AZD5363 + palbociclib, 1 buparlisib, 1 eribulin. In 9/10 cases we found concordant P/PDX responses (6 cases with no-CB in patient/PD in PDX, 3 with CB/SD; and 1 lurbinectedin case with no-CB/PR). Two pts had biopsy for PDX implantation at progression of paired therapy and were excluded from TTP analyses. In cases with discordant P/PDX response, we found significant correlation between RECIST response in pts and rAUC in PDX (p=0.82, p=0.03), a trend for comparable TTP in pts and TTP in PDX (p=0.67, p=0.10), but no correlation between RECIST response in pts and response at week 3 in PDX (p=0.46, p=0.30). In this cohort of MBC pts, we found high concordance in Pt/PD response. Our results suggest that rAUC in PDX may be the best read-out to identify relevant drug/genotype associations that can potentially be translated to patient benefit. Data will be updated with additional ongoing PDX experiments.

#3130 CYP3A5 positively regulates androgen receptor signaling in prostate cancer cells: Potential for therapeutic targeting. Ranjana Mitra, Priyamath Gorgala, Oscar B. Goodman. Rosenman University of Health Sciences, Las Vegas, NV.

Purpose: Prostate cancer is the most common malignancy and second leading cause of cancer-specific mortality in US men. The androgen receptor (AR) plays a major role in promoting growth and progression of prostate cancer and thus has been successfully targeted therapeutically. Therapeutic resistance to androgen deprivation therapy (ADT) accounts for much of the morbidity and mortality attributable to prostate cancer. Therapeutic resistance is typically accompanied by insufficient blockade of AR activation, whose initial step involves translocation of AR to the nucleus. Previously we demonstrated that CYP3A5 facilitates the nuclear translocation of AR, promoting prostate cancer growth. Targeting CYP3A5 and blocking AR nuclear translocation may override therapeutic resistance to ADT, representing a novel approach for treating men with advanced prostate cancer. This work focuses on understanding the role of CYP3A5 in regulating AR nuclear translocation process and its subsequent promotion of prostate cancer growth.

Methods: AR nuclear translocation and confocal microscopy are high throughput methods for measuring data obtained by our 16-year researches strongly demonstrated that CYP3A5 facilitates the nuclear translocation of AR, promoting prostate cancer growth. Targeting CYP3A5 and blocking AR nuclear translocation may override therapeutic resistance to ADT, representing a novel approach for treating men with advanced prostate cancer. This work focuses on understanding the role of CYP3A5 in regulating AR nuclear translocation process and its subsequent promotion of prostate cancer growth. Methods: AR nuclear translocation and confocal microscopy are high throughput methods for measuring data obtained by our 16-year researches strongly demonstrated that CYP3A5 facilitates the nuclear translocation of AR, promoting prostate cancer growth.
antibodies with A/G beads for immunoprecipitation. Results: Our co-immunoprecipitation experiments using CYP3A5-Flag transfected LNCaP cells extracts demonstrate that CYP3A5 associates with the HSP90-AR complex that helps to maintain AR in a form that can be readily activated by androgen.

Using Flag-agarose in co-immunoprecipitation experiments, we demonstrated that CYP3A5 is a component of the HSP90-AR complex with AR, HSP90, and HSP40 which are chaperones regulating AR nuclear translocation. We further confirmed the presence of CYP3A5 in the HSP90-AR complex by co-immunoprecipitation using HSP90 and AR antibodies in combination with A/G beads instead of flag-agarose. Conclusion: Our current findings support the notion that CYP3A5 is a part of the HSP90-AR complex and positively regulates AR nuclear translocation. Therefore, targeting CYP3A5 to block AR nuclear translocation may be a therapeutic adjunctive approach in advanced prostate cancer.

#3131 Ubiquitin ligases: a new target for RNAi therapy of hepatocellular carcinoma. Dominique Leboeuf,1 Timofei Zatsepin,2 Daniel G. Anderson,3 Konstantin Piatkov4,5, Skolkovo Institute of Science and Technology, Moscow, Russian Federation; 2Koch Institute for Integrative Cancer Research at MIT, Cambridge, MA.

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third leading cause of cancer-related deaths in the world. HCC is a very aggressive cancer, resistant to all conventional chemotherapies and capable of acquiring resistance to Sorafenib, which is the only approved chemotherapeutic drug for HCC. This highlights the need to look for new therapeutic options and improve the survival of patients with advanced HCC. The N-end rule pathway is an emerging player in the field of cancer biology, because of its capacity to positively regulate many hallmarks of cancer including angiogenesis, cell proliferation, motility and survival. Therefore, the N-end rule pathway offers the potential to be a target for highly effective anti-tumor treatment. The aim of this study is to investigate the role of the N-end rule pathway in the context of hepatocellular carcinoma in vivo. We used siRNA-mediated RNA interference as the main approach for selective downregulation of the four N-end rule-dependent cognate ubiquitin ligases: UBR1, UBR2, UBR4 and UBR5. Initial screening of siRNAs was performed in Hepa 1-6 cells, followed by in vitro phenotyping using proliferation assays, migration assays, and TUNEL assays. The most potent siRNA against Ubr1, Ubr2, Ubr4 and Ubr5 were formulated into lipid nanoparticles (LNPs) and injected into mice in order to modulate the function of the N-end rule in vivo. We have confirmed that Ubr1, Ubr2, Ubr4 and Ubr5 are overexpressed in Hepa 1-6 cells and in mouse liver tumors, in comparison to normal liver. Upon transfection of siRNAs in Hepa 1-6 cells or injection of LNPs loaded with the same siRNAs into mice, we obtained successful down regulation of Ubr1, Ubr2, Ubr4 and Ubr5 both in vitro and in vivo (in the liver and in tumor tissues). We have also demonstrated that down regulation of these four Ubr proteins negatively affects cell migration and proliferation, and renders cells more susceptible to apoptosis. Bi-weekly injections of the LNPs for mice for up to 6 weeks efficiently downregulates the expression of Ubr1, Ubr2, Ubr4 and Ubr5 without any toxic effects. Indeed, we found no significant increase of ALT, AST or total bilirubin in the blood compared to controls.

Considering that all four Ubrs are upregulated in HCC and that the N-end rule plays a major role in many key pathways of cancer development, we expect that even a partial down regulation of this pathway will have prominent effects on tumor development and progression due to the suppression of tumor vascularization, cell proliferation and viability.

#3132 Inhibition of the integrin αvβ3 improves the effect of bexarotene in the treatment of cutaneous T-cell lymphoma (CTCL). Florencia Caryol,1 Victoria Revueta,2 Alejandro Paulazo,1 Mercedes Debernardi,3 Maria Celeste Diaz Flaque,1 Helena Sterle,1 Leandro Cerchietti,2 Graciela Cremaschi1.

CTCL are exposed to a complex paracrine and endocrine environment that influence their progression from skin to visceral disease. One of the most common treatments for CTCL is RXR agonists or “rexinoids” such as bexarotene (BEX). The most prevalent side effect of rexinoids (~95% of patients) is the development of hypothyroidism hence these patients are prophylactically treated with thyroid hormone (TH). Paradoxically, we recently found (PMID: 25488971) that TH can activate transcriptional programs required for proliferation of CTCL. We also found that the effects of TH on CTCL are mediated through the activation of two different receptors, the classical nuclear TR and a membrane receptor integrin αvβ3. Our aim was to study how the anti-lymphoma activity of BEX is modified by the activation of TR and αvβ3 during the TH replacement therapy, and unknown topic with clinical implications. We first evaluate the cell viability of CTCL cells HuT78 and M1 treated with BEX in presence and absence of physiological levels of TH. As expected, BEX decreased the viability of CTCL cells, however in presence of TH both effects decreased by 25-60% and 20-50% on HuT78 and M1 cells. However, hypothyroidism is associated with a higher dissemination of TCL cells in syngeneic mice. Thus, to evaluate the impact of TH replacement therapy in BEX-treated mice, we implanted TCL EL-4 cells subcutaneously in C57BL/6 mice. When tumors reached ~75 mm3, mice were randomized in three groups: vehicle, bexarotene alone (BEX) and bexarotene with T4 replacement (to reach euthyroidism) (BEX-T4). Tumor growth significantly decreased in both bexarotene arms (vs. vehicle). However, in contrast to mice receiving BEX-T4, mice receiving BEX alone showed a decreased infiltration of tumor-suppressive immune cells. Specifically, these tumors had decreased NK and CD3+CD8+ cells due to BEX-induced hypothyroidism. This indicates that although TH could decrease the effect of BEX in CTCL cells, the hypothyroidism would impact in the local antitumor response. Thus determine if the inhibition of the non-canonical TH membrane receptor integrin αvβ3 would decrease the pro-survival effect of TH on CTCL treated with BEX. We found that either, αvβ3 silencing or the pharmacological inhibitor Cilengitide, not only avoided the pro-survival effect of TH but increased the anti-lymphoma activity of BEX-T4 by 15-35% in CTCL cells. To evaluate the molecular program underpinning these effects, we conducted RNA-sequencing in HuT78 cells treated with BEX-T4 in presence of siRNA for THR, αvβ3 or controls. Among the top pathways we identified genes involved in “apoptosis” and “IL6-JAK-STAT3 signaling” as differentially expressed in si-αvβ3 vs. the others. These genes were independently validated by using Cilengitide. Our data suggest that inhibition of the non-canonical TH receptor, the integrin αvβ3, could be an effective strategy to improve BEX treatment in CTCL.

#3133 NF-κB-dependent inflammatory responses offset sorafenib cytotoxicity in hepatocellular carcinoma via TIFA. Tong-You Wade Wei,1 Pei-Yu Wu,2 Ting-Jung Wu,2 Ming-Daw Tsai1, Academia Sinica, Nangung Dist., Taiwan; 3Chang Gung Memorial Hospital, Linko, Taiwan.

Sorafenib is the promising first-line drug to treat advanced hepatocellular carcinoma (HCC), with the acquired resistance within 6 months in most treated patients. The epithelial-mesenchymal transition (EMT)-driven cancer metastasis contributes to sorafenib resistance via multiple signaling pathways, but the underlying molecular mechanism remains elusive. Recent studies showed that inflammatory cytokines TNF-α functionally initiates EMT through NF-κB-mediated Snail activation in HCC, and that TRAF-interacting protein TIFA sustains the positive feedback loop between TNF-α and NF-κB that enhances chemoresistance in AML. Here we investigated the functional link between TIFA-regulated NF-κB inflammatory signaling and sorafenib resistance as a potential therapeutic target in the treatment of HCC. We showed that severe hepatitis induced in HCC mice promoted intrahepatic metastasis concurrently with NF-κB-driven EMT via Snail, and that HCC patients with hepatitis displayed poorer responses to sorafenib and unfavorable clinical outcome. In support, inflammatory TNF-α stimulation promoted levels of TIFA, NF-κB signaling factors, and EMT axis independent of sorafenib treatment, while silencing of TIFA abrogated sorafenib-dependent EMT. Silencing TIFA or RelA perturbed sorafenib-dependent cytokine secretion in HCC cells. In addition, silencing of TIFA or RelA suppressed pro-survival factors Bcl-2 and Bcl-XL, and promoted pro-apoptotic factor BAX in response to sorafenib treatment in vitro. Consequently, HCC cells regained sorafenib chemosensitivity upon silencing of TIFA or RelA through promoted apoptosis. To further explore the therapeutic relevance, siRNAs were delivered using the liver-tropic adeno-associated virus serotype 8 in mice with orthotopic HCC xenografts, and results showed that targeting TIFA or RelA specifically enhanced sorafenib chemotoxicity resulting in more prominent tumor regression. Our results collectively showed that TIFA and NF-κB support inflammatory responses to offset sorafenib cytotoxicity, and that their targeting are therapeutically effective concomitant with sorafenib treatment in HCC.


The incidence of thyroid cancer (TCA) has doubled in the last decade. Papillary thyroid cancer (PTC), which comprises of 80% of all TCs, is treatable with great outcomes. However, undifferentiated anaplastic thyroid cancer (ATC), with very poor prognosis, is considered a clinical challenge and currently a losing battle. Moreover, the molecular mechanisms responsible for various forms of TCA are largely unknown. We believe that the cells of histopathologi-
cally distinct regions of a heterogeneous thyroid tumor are governed by different sets of master gene regulators. The smart manipulation of such master genes will be able to selectively destroy cancer cells but not normal tissue, opening a novel and much more effective avenue in the thyroid cancer targeted gene therapy. Our analysis established the gene hierarchical governance in each region based on their Gene Commanding Height (GCH). GCH is a measure that combines the gene expression coordination with other genes and the expression stability among biological replicas provided by the internal homeostatic mechanisms. Here, we provide experimental evidence that standard papillary (BCPAP) and anaplastic (8505C) human thyroid cancer cell lines have different master regulators. We identified the master regulators of BCPAP and 8505C, and determined their GCH. Transfection of master gene regulators of a particular cell line has significantly larger effects on the cell line they command than on other cells. We found that the stable transfection with TMEM194A, a nuclear envelope protein, regulated twice more genes in BCPAP than in 8505C cells. The analysis using human thyroid cancer cells reaffirmed our hypothesis of the existence of hierarchical master gene regulators and that the phenotypic changes can be manipulated with the introduction of these genes. We further validate these concepts using human thyroid biopsy samples. We found substantial differences in the GCH scores of cancer versus normal tissue of a surgically removed 32.0mm papillary carcinoma from the left lobe of a 35y old male. Because of such differences between the cancer region and the normal tissue, manipulation of cancer regulators is expected to affect the cancer cells in a greater degree than the normal cells. These results suggest that we have defined a master gene regulator hierarchy in thyroid cancer and extrapolation of this analysis to compare anaplastic and papillary thyroid cancer will lead to novel gene therapeutic modalities. Our long-term goal is to identify master regulators of cancer nodules for each patient and develop personalized cancer therapy targeting these master regulators.

**#3135 Benzaldehyde suppresses the binding activity of overexpressed 14-3-3 to multiple signaling proteins in cancer cells.** Jun Saitoh, Hideyuki Saya. Keio Univ School Of Med, Shinjuku Ku Tokyo, Japan.

Benzaldehyde is the simplest aromatic aldehyde constituent of almonds and many fruits. Anticancer effect of Benzaldehyde was first reported in 1980, and multi-institutional clinical trials were performed in those days in Japan. However, trial was over without determination of effectiveness, only its’ safety was confirmed. The underlying mechanism why Benzaldehyde specifically suppresses growth of some particular cancer cells but not that of normal cells has not been elucidated. Therefore, we attempted to clarify the mechanism of anticancer effect of Benzaldehyde. We have previously reported that, in pancreatic cancer cell BxPC3 and in non-small cell lung cancer cell A549, Benzaldehyde inhibits PKA/akt/mTOR, STAT3, NFkB and ERK pathways, those are major signaling pathways activated in cancer cells. Effects of Benzaldehyde on multiple signaling pathways are found to be derived from regulation of 14-3-3 family proteins which interact with phosphorylation sites of various proteins of multiple signaling. In BxPC3 cells, Benzaldehyde treatment reduced the phosphorylation levels of 14-3-3 binding sites. More, we ecotopically expressed seven isoforms of 14-3-3 in HEK293T cells and found that Benzaldehyde treatment significantly suppressed phosphorylation of 14-3-3 with client proteins such as PKA/akt/mTOR, STAT3 and FOXO. The interaction of other isoforms of 14-3-3 with their client proteins was also partially reduced. But, the expression levels of those seven 14-3-3 isoforms were not significantly changed. Here we further analyze the Benzaldehyde-mediated 14-3-3 regulation, in HEK293 cells overexpressing 14-3-3. Benzaldehyde significantly suppressed mTOR pathway while it was not observed in parental HEK293 cells. Furthermore, we performed pull down assay by using 14-3-3 GST tagged recombinant proteins and lysates of HEK293T and BxPC3 and found that Benzaldehyde treatment suppressed the interaction of 14-3-3 with cRaf, TSC2 and other 14-3-3 client proteins. From these data, we confirmed that Benzaldehyde is an inhibitor of the overexpressed 14-3-3. Recent reports have shown that 14-3-3 is overexpressed in many cancers and acts as a signaling hub controlling the network of oncogenic pathways, suggesting that overexpressed 14-3-3 associates with carcinogenesis, metastasis and resistance for chemotherapy and radiation. Hence, Benzaldehyde is considered to be a new molecular target agent of 14-3-3, which promote and maintain cancer signals.

**#3136 Unveiling the therapeutic potential of Tanshinone I on telomerase activity and shelterin complex in myeloma.** Raman Kumar, Rehan Khan, Nicki Gupta, Tulika Seth, Alpana Sharma. All India Institute of Medical Sciences, New Delhi, India.

Background and Objectives: Multiple myeloma (MM) defines as monoclonal antibody producing abnormal plasma cells inhibiting bone marrow (BM). It involves multiple genetic mutations where different signaling pathways get deregulated hence showing malignant features of myeloma. Increased relative telomerase activity (RTA) and altered levels of shelterin complex molecules (TRF1, TRF2, POT1, RAP1, TIN2, TPP1) along with its associated molecules (TANK-1 & PINX1) are underlying principle behind several malignancies. This maiden attempt aims to evaluate the impact of these geno-phenotypic levels on telomerase activity and shelterin complex. Methodology: Newly diagnosed 50 MM patients & 20 controls were recruited for BM aspiration with their consent. RTA, mean telomere length, relative mRNA and protein levels were studied in study subjects. Clinical parameters were recorded; correlation was studied between all the studied genes at mRNA levels and between genes and patients’ clinical parameters; ROC curves were plotted of all genes. Anti cancer potential of Tan1 and lenalidomide or their combination were studied with different assays on myeloma cells (RPMI8226 & U266). Results:Significantly (p<0.05) high relative mRNA expression were found of all molecules except PINX1 which was significantly lowered in patients compared to controls and was correlating with disease severity and progression. Similarly, higher RTA and lowered mean telomere length was observed in patients. Statistically significant correlation was observed between genes, telomerase activity and with clinical parameters. ROC curves analysis showed high sensitivity and specificity for TRF2 genes and Telomerase activity. Thus, RTA and shelterin complex along with associated factors does play role in causing myeloma and its progression. Tan1 caused significant toxicity at low doses in myeloma cells while lenalidomide caused significantly reduced toxicity. Cell death was enhanced when combination of both (Tan1 + lenalidomide) drugs were used as observed in Annexin-V/PI and Tunel assay. Tan1 and its combination with lenalidomide significantly down regulated protein expression of TANK1, TPP1, and RAP1 in myeloma cells compared to untreated cells. Conclusions:In nutshell, study implies that increased RTA along with altered mRNA and protein expression of shelterin complex molecules with its association in patients might play role in progression and progression of MM. Stage-wise significant increased expression and correlation of these molecules with each other and with clinical parameters showed their role as potential bio-marker in MM. Significant down-regulation of RTA and apoptosis by Tan1 alone or combination with lenalidomide suggests that Tan1 might have synergizing effect with lenalidomide in causing cytotoxicity in Myeloma.

**#3137 Breast cancer anti-estrogen resistance 4 (BCAR4) is a novel oncogene in lung cancer.** Kieun Bae, Minkyong Lee, Daseul Yoon, Y-H Kim, Kyong-Ah Yoon. Kyung Hee University, Seoul, Republic of Korea; 2Graduate School of Cancer Science and Policy, National Cancer Center, Goyang, Republic of Korea.

BCAR4 was identified as tamoxifen related gene in breast cancer due to its function to activate estrogen-independent cell growth. Instead of estrogen receptor, ERBB2 and ERBB3 genes were activated by BCAR4 in tamoxifen resistant cells. Recently, the function of BCAR4 as a long non-coding RNA (lncRNA) was reported to regulate a non-canonical Hedgehog/GLI2 pathway in breast cancer. However, the association of BCAR4 and lung cancer has not been identified. Here, we describe the oncogenic effect of BCAR4 in human lung cancer. Expression pattern of BCAR4 was examined in lung cancer cell lines, primary tumor tissues, and adjacent normal tissues. BCAR4 was highly expressed in 71% of lung cancer cell lines and especially in 43% of cancer tissues of lung adenocarcinoma patients who did not harbor the activation mutations of EGFR nor KRAS. Exogenous expression of BCAR4 protein promoted cell growth of non-tumorigenic bronchial epithelial cell line, BEAS-2B, and lung cancer cell line, NCI-H1299. We also confirmed the oncogenic feature of BCAR4 by colony forming assay using stably expressing cells. These effects were notably reduced after knockdown of BCAR4 by small interfering RNA. BCAR4 encoded a functional protein and its overexpression resulted in the enhanced migration than controls cells. This result proposed a similar role of BCAR4 in lung cancer considering its previously reported role in breast cancer metastasis. We also detected a fusion gene of BCAR4 in a lung cancer tissue by analyzing RNA-sequencing data. BCAR4 protein as well as its fusion form showed the improved tumorigenic feature in the mouse xenograft model with lung cancer cells. We compared the gene expression pattern in BCAR4 overexpressed cells to identify the possible mechanism of the oncogenic effect of BCAR4. Cyclin D1, Cycin E, and MMP1 mRNA and protein levels were significantly increased after BCAR4 overexpression. However, downstream genes of GLI2 signaling were not altered by protein expression of BCAR4 arguing an alternative role for lncRNA BCAR4. Our results suggest a novel oncogenic role of BCAR4 protein in lung cancer.
**EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Molecular Medicine**

PHY906(YIV-906), a four-herb Chinese medicine formulation, is inspired by an 1800-year-old Chinese formulation called Huang Qin Tang historically used to treat gastrointestinal symptoms. PHY906 can increase the anti-tumor activity of immunotherapy antibodies or chemotherapies while promoting damaged intestinal tissue recovery. Several clinical studies showed that PHY906 had potential to improve therapeutic index and treat radiation-induced gastrointestinal damage by prolonging life and improving patient quality of life. When in vivo activities of three different clinical batches (number 6, 10, 11) of PHY906 and F (which is commercial batch of Huang Qin Tang, HQT) was compared to their similarity of chemical profiles, the two set results could not be completely matched. For example, F had very different in vivo activities from PHY906-10 but F clustered closely to PHY906-10 based on their chemical profiles. This information concludes that chemical profile analysis without excluding irrelevant chemicals is not sufficient to evaluate the in vivo activities of a polychemical mixture. Therefore, we included another two biological platforms based on the in vivo mechanism of action of PHY906 for the quality control. First, we tested the activity of different batches of PHY906 and F on our signaling transduction activity response (STAR) platform which including 18 luciferase reporter cell lines and 2 enzymatic assays. The results of correlation analysis and clustering analysis based on the results from STAR could be matched to in vivo activities of different batches of PHY906 and F. Second, we tested the effect of different batches of PHY906 and F on a set of genes which based on our previous DNA array data in cell culture and the mechanism action of PHY906. Again, the results of these in vitro analysis and clustering on tissue sample-PDX could be matched to in vivo activities of PHY906 and F. In conclusion, the quality control of a herbal product should be dependent on its usage. Appropriate biological assays based on its mechanism action should be developed for QC for particular usage. Chemical fingerprint has limitation unless irrelevant chemicals had been filter out. Similarity index is only useful when relevant information is used. Mechanism based quality control could be used for other herbal products.

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**#3139 In vivo functional analyses of cancer gene variants for cancer driver identification and drug discovery**

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Precision oncology requires identifying and understanding of cancer genome changes in a patient tumor tissue and finding the best cancer therapy targeting the changes. Although many cancer gene targets have been validated so far, next-generation genomic profile analyses have uncovered much more cancer gene variants with unconfirmed functions. Developing methods to functionally evaluate these variants and understand their impact on drug-resistance and drug responses, such as drug resistance or synthetic lethality, will be critical in cancer treatment decision support. In addition, in some clinical cases, multiple treatment choices such as multiple drug combinations exist. Developing cancer models which can test multiple treatments will provide direct comparison of those therapies and select the best options. At GenenDesign, we have performed drug tests on mouse “avatars”, which are also known as Patient-Derived Xenograft (PDX) models. They are personalized cancer models derived from patient tumor samples with cancer mutation profiles and drug responses very similar to the corresponding cancer patients. Drug screenings were carried out in avatars by testing chemotherapies or targeted drugs against specific cancer gene mutations and variants. Selected drugs or drug combinations from avatar studies have been applied to corresponding patients with highly consistent treatment outcome. From genomic profile analysis of our near 1500 PDX tumor models in cancer types such as lung, colorectal, gastric, liver, and esophageal, we are able to identify a large number of cancer gene mutations/variants, gene fusions, as well as gene copy number and RNA expression changes in major cancer signal pathways such as EGFR, HER2, c-Met/ALK, Ras/Raf, FGFRs, PI3K/Akt, Wnt/β-Catenin, cell cycle regulation, angiogenesis. Many of these gene aberrations are potential drug targets and have been functionally tested in PDX models with approved drugs or clinical drug candidates. The mutation/variant information and drug response information generated from PDX models have been organized into our Precision Cancer Information Lab database. Patient tumor DNA test results have been used for searching genetically matched PDX models in our database. Once matched PDX models are identified, the available drug response information can be used as evidence for clinical treatment decision. In addition, the matched PDX models can also be used to test more treatment options such as different combinations and new clinical drug candidates.

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**#3140 MUC1 expression as a prognostic marker and a new therapeutic target in patient with duodenal adenocarcinoma**

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Background: Mucin1 (MUC1) is a glycoprotein as a member of the mucin family. This expression has been associated with poor prognosis in patients with several types of cancer. Duodenal cancer is rare, therefore details are not known including carcinogenesis and clinical treatment. We verify the association of MUC1 expression and prognosis in patient with duodenal adenocarcinoma. We also assess the function of MUC1 in vitro. Materials and Methods: Between 1989 and 2014, eighteen patients of duodenal adenocarcinoma with surgery at Jichi Medical University Hospital were included in this study. MUC1 expression was evaluated immunohistochemically with anti-MUC1 antibody to examine the association of MUC1 expression and prognosis clinicopathologically. A human-derived duodenal cancer cell line, HuTu-80, is used for the experiments of invasiveness and proliferation in vitro. The invasive capacity was evaluated by transfection of siRNA into HuTu80 with matrigel invasion assay. Viability assay with the anti-MUC1 peptide GO201 was assessed to determine whether MUC1 associate with cell growth of duodenal adenocarcinoma. Results: Seven cases were MUC1 positive and 11 were MUC1 negative immunohistochemically. TNM staging was not associated with MUC1 expression. Kaplan-Meir analysis showed a poor prognosis with MUC1 positive patients in cumulative overall survivals (MUC1 positive, 18M : 52M, 95%CI 3.247-130.7, p=0.002). Lymph node metastasis of MUC1 positive patients (71.4%) was significantly higher than MUC1 negative patients (9%) (p = 0.01). In vitro expression of the MUC1 was confirmed by RT-PCR and flowcytometry. Knockdown experiment with transduction of siRNAs to HuTu80 decreased the expression of the MUC1 gene. Transduction of siRNAs significantly reduced invasive capacity (47.02 ± 21.65%, 95%CI 3.046-102.9, p = 0.04). Growth of HuTu80 cells was inhibited by GO201 (5 μM : 95%CI 0.109-0.356, 10 μM: 95%CI 0.024-0.389 p<0.0001). Conclusions: MUC1 expression is a predictive marker for poor prognosis in patients with duodenal adenocarcinoma. Functional Interference of MUC1 core protein reduces cell growth and invasiveness. Taken together, MUC1 is associated with malignant potential and a novel target for the treatment.

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**EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Molecular Targeted Therapies 1**

**#3141 Combined cetuximab and pemetrexed therapy enhances cytotoxicity against crizotinib-resistant non-small cell lung cancer cells by downregulating thymidylate synthase.**

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Although non-small cell lung cancer (NSCLC) cells with anaplastic lymphoma kinase (ALK)-rearranged initially show a dramatic response to crizotinib, these cells eventually develop resistance to crizotinib. This resistance is caused by secondary mutations and copy number gain in the ALK gene. However, other resistance mechanisms through activation of the bypass tracts have yet to be clearly elucidated. To investigate the mechanisms of acquired resistance to ALK fusion-directed treatment in NSCLC, we generated crizotinib-resistant NSCLC cell lines in vitro through chronic exposure of an ALK fusion NSCLC line (SNU2292) to crizotinib. Interestingly, the resultant SNU2292-CR cells maintained activation of epidermal growth factor receptor (EGFR) and expressed increased levels of transforming growth factor alpha (TGFA-α), an EGFR ligand. Additionally, we found that thymidylate synthase (TS) was overexpressed in SNU2292-CR cells compared with the parental cells. We showed reduction of TS enhanced synergistic inhibition of cell proliferation when cetuximab, an EGFR inhibitor, was combined with pemetrexed, a TS inhibitor. As a result, combined therapy was found to exhibit a synergistic growth inhibitory effect against crizotinib-resistant cells by downregulating TS expression. Taken together, these results support a potential role of activation of the bypass tracts in acquired resistance to ALK-directed treatment in ALK-rearranged NSCLC, and provide insights into strategies for preventing and/or overcoming this resistance in patients.

**#3142 Abrerrantly expressed microRNAs drive the development of acquired Erlotinib resistance in non-small cell lung cancer.**

Arita S. Pal, 1 Alejandro Agredo, 2 Andrea L. Kasinska, 2 Purdue University, West Lafayette, IN; 2 Universidad Nacional de Colombia, Bogota, Colombia.
Lung cancer is the leading cause of cancer-related deaths in the world. Non-small cell lung cancer (NSCLC) accounts for ~85% of the cases. NSCLC patients frequently harbor causal gene mutations. Epidermal Growth Factor Receptor (EGFR), an NSCLC causal gene, is mutated in 10-35% of NSCLC patients. Patients with EGFR activating mutations are treated with a tyrosine-kinase inhibitor, Erlotinib, specifically targeting EGFR. However, most patients develop Erlotinib resistance within a year. Although multiple mechanisms are involved in the development of Erlotinib resistance, the role of microRNAs in mediating Erlotinib resistance is largely unexplored. MicroRNAs (miRNAs) are small non-coding RNAs that regulate normal cellular physiology. In cancers, miRNAs are severely dysregulated, contributing to multiple cancer processes, including drug response. Despite the involvement of miRNAs in cancer, their direct role as drivers of drug resistance remain understudied, therefore, there is a critical need to understand the role of miRNAs in inducing Erlotinib resistance. To this end, we propose an unbiased two-prong approach to identify the miRNAs that drive the development of Erlotinib resistance. Therefore, miRNAs are either being (i) overexpressed, or (ii) silenced in Erlotinib sensitive cells with the hypothesis that perturbed miRNA levels will drive Erlotinib resistance. Erlotinib sensitive NSCLC cell lines, EKVX and H322M were identified from the NCI-60 Developmental Therapeutics Project and their Erlotinib dose response curves were established. To perform the miRNA overexpression screen, EKVX and H322M cells stably expressing renilla and firefly luciferase genes were generated, which will be used to monitor cell number and transfection efficiency, respectively. The luciferase expression will be transfected with a library of 2,019 individually arrayed human miRNAs and cell growth in the presence of Erlotinib will be monitored. The second prong of the study will identify miRNAs, that when lost, confer Erlotinib resistance. 400-fold coverage of small guide RNA (sgRNA) library of the CRISPR-Cas9 system was transduced in Cas9 overexpressing EKVX cells, to knockout ~21,000 human encoded genes (~1,800 miRNA genes). Cells are being cultured in the presence of 75% or 90% growth inhibitory concentration (GI75 or GI90) of Erlotinib so that only cells with sgRNAs against genes critical for Erlotinib response, survive and grow due to acquired resistance. DNA from these cells will be harvested, the sgRNAs sequenced, and compared to the sgRNAs present in Erlotinib-un untreated cells. Successful completion of this project will identify miRNAs that drive Erlotinib resistance and may contribute towards development of miRNA therapeutics to enhance Erlotinib sensitivity of NSCLC tumors.

**#3143 FBW7 mutations mediate resistance of colorectal cancer to targeted therapies by blocking Mcl-1 degradation.** Jingshan Tong, Shuai Tan, Fangdong Zou, Jian Yu, Lin Zhang. UPCI, Pittsburgh, PA.

Colorectal cancer (CRC), the second leading cause of cancer-related deaths in the US, has been treated with targeted therapies. However, the mechanisms of differential responses and resistance of CRCs to targeted therapies are not well understood. In this study, we found that genetic alterations of FBW7, an E3 ubiquitin ligase and a tumor suppressor frequently mutated in CRCs, contribute to resistance to targeted therapies. CRC cells containing FBW7 inactivating mutations are insensitive to clinically used multi-kinase inhibitors of RAS/RAF/MEK/ERK signaling, including regorafenib and sorafenib. In contrast, sensitivity to the targeted agents is not affected by oncogenic mutation in KRAS, BRAF, PIK3CA, or p53. These cells are defective in apoptosis due to blocked degradation of Mcl-1, a pro-survival Bcl-2 family protein. Deleting FBW7 in FBW7-wild-type CRC cells abolishes Mcl-1 degradation and recapitulates the in vitro and in vivo drug resistance phenotypes of FBW7-mutant cells. CRC cells selected for regorafenib resistance have progressive enrichment of pre-existing FBW7 hotspot mutations, and are cross-resistant to other targeted drugs that induce Mcl-1 degradation. Furthermore, a selective Mcl-1 inhibitor restores regorafenib sensitivity in CRC cells with intrinsic or acquired resistance. Together, our results demonstrate FBW7 mutation status as a key genetic determinant of CRC response to targeted therapies, and Mcl-1 as an attractive therapeutic target.

**#3144 Prediction of ALK mutations associated with acquired resistance to lorlatinib.** Satoshi Yoda, Leila Darbaid, Manrose Singh, Jeffrey A. Engelman, Alice T. Shaw, Aaron N. Hata. Massachusetts General Hospital, Charlestown, MA.

Anaplastic lymphoma kinase (ALK) rearrangements are important therapeutic targets in non-small cell lung cancer. They are currently treated with the first-generation ALK inhibitor crizotinib followed by more potent, second-generation ALK inhibitors, such as ceritinib, alectinib, or brigatinib. We reported different spectrums of ALK resistance mutations in the biopsies from patients progressing on these drugs. G1202R mutation was found more frequently after treatment with second generation ALK inhibitors. In addition to these drugs, the third-generation ALK inhibitor lorlatinib is currently being evaluated in phase 2 clinical trial. Ba/F3 models indicated that all single ALK mutants are sensitive to lorlatinib and some compound ALK mutations are resistant to lorlatinib. In this study, we performed accelerated mutagenesis screen on Ba/F3 models to predict the resistance mutations which potentially emerge in the patients treated with lorlatinib. Ba/F3 cells expressing wild type EML4-ALK or mutant EML4-ALK containing L1196F, L1196Y, I1196M, G1202R, or G1269A were exposed to N-ethyl-N-nitrosourea (ENU). After a 24-hour incubation in normal media, the cells were seeded in 96-well plates and incubated in lorlatinib for 4 weeks. ALK kinase domain was sequenced in clones growing in lorlatinib to identify possible new mutations. As a result, Ba/F3 cells harboring wild type EML4-ALK showed no mutations on ALK kinase domain. Crizotinib was used as a control to validate the efficiency of mutagenesis. We identified eight different mutations in clones growing in crizotinib, and those were reflecting the spectrum of mutations in the crizotinib-treated patients. Ba/F3 cells with mutant EML4-ALK showed additional compound mutations after incubation with lorlatinib. Those mutations included L1196M + L1198F and G1202R + L1198F which showed high resistance to lorlatinib in Ba/F3 models. Ba/F3 cells with different mutant EML4-ALK showed a distinct spectrum and different frequency of additional mutations. In conclusion, this study predicted that no single mutation would emerge to confer resistance to lorlatinib. Thus, compound mutations and ALK-independent mechanisms become essential mechanisms for lorlatinib resistance.


Fibroblast growth factor receptor 3 (FGF3/FGFR3) overexpression, point mutations or gene fusions are found in ~80% of non-muscle-invasive and ~15% of muscle-invasive bladder cancer. FGFR inhibitors have entered clinical trials in advanced bladder cancer however, as with other targeted therapies, intrinsic and acquired resistance are expected to limit treatment efficacy. We have used an in vitro model to explore possible mechanisms of resistance. The urothelial cell line RT112 expresses an FGFR3-TACC3 fusion protein and is sensitive to FGFR inhibition. Isogenic resistant cell lines, termed R1, R2 and R3, were derived by long-term culture of RT112 in the presence of the FGFR inhibitor PD173074. FGFR3 overexpression is associated with acquisition of resistance and lower FGFR expression than R1 and R2. The changes in morphology and gene expression between parental and resistant derivatives R1 and R2 were reversed when the resistant cells were cultured without PD173074 for 4 passages. Despite this, the cells retained their resistance when re-exposed to PD173074. Exome sequencing, RNA microarray analysis and phospho-receptor tyrosine kinase array data on the parental cells and resistant derivatives will be presented. Our data suggests that diverse mechanisms of resistance occur following prolonged FGFR inhibition.

**#3146 Targeting ANXA3 in combination with sorafenib for the treatment of hepatocellular carcinoma.** Man Tong, Steve Luk, Noelia Che, Jin Deng, Terence KW Lee, Stephanie Ma. Univ. of Hong Kong, Faculty of Medicine, Pokfulam, Hong Kong; Eastern Hepatobiliary Surgery Hospital, Second Medical University, Shanghai, China; The Hong Kong Polytechnic University, Hong Kong, Hong Kong.

Sorafenib is the only FDA-approved tyrosine kinase inhibitor for targeted therapy in advanced HCC. Nevertheless, its efficacy is limited with only a modest improvement in patient outcome, likely due to acquired resistance. In-depth understanding of the molecular mechanism of sorafenib resistance is warranted for the development of novel treatment strategies. Recent studies by us and others have characterized liver tumor-initiating cells (T-ICs) to be a possible source of resistant and recurrent tumors and a plausible target for HCC treatment. Our group has previously identified CD133 + as a functional marker of liver T-ICs and found annexin a3 (ANXA3) to regulate cancer stem cell properties in this subset of cells. Interestingly, our recent observations also found CD133 + liver T-ICs to be more resistant to sorafenib. Sorafenib resistant clones, established in HepG2 and HuH7 cells by continuous exposure to increasing concentrations of sorafenib, displayed enhanced abilities to migrate, invade, self-renew, and inhibit tumor formation in immunodeficient mice, as well as a higher expression of stemness associated genes. These two sorafenib resistant cell lines and two other sorafenib resistant HCC patient-derived xenografts established in a similar manner were also found to be enriched for CD133 and ANXA3 expression. Sorafenib resistant clones with ANXA3 stably suppressed
were re-sensitized to sorafenib treatment and had diminished ability to migrate, invade, self-renew and initiate tumor growth in vivo, further substantiating the role of ANXA3 in mediating sorafenib resistance in HCC. Mechanistically, an activated PKC/ERK/FRA2 signaling axis was found to be responsible for driving this phenomenon. Clinically, ANXA3 expression was also found to have prognostic value as a higher ANXA3 expression in chronic lymphocytic leukemia (BTK) cDNA samples was correlated with worse prognosis. Furthermore, we demonstrated that Mibefradil inhibits GCN growth and survival and sensitizes GSCs to Temodar and cisplatin in vitro and in xenograft tumor models. This study represents the first comprehensive characterization of the role of T-type calcium channels in glioblastoma. Zheng, Nichola Crickshanks, Fang Yuan, Baomin Wang, Mary Pahuski, Julia Wulfkuhle, Isela Gallagher, Alexander F. Koeppel, Sarah Hatf, Christopher Papanicolas, Jeongwu Lee, Bar, David Schiff, Stephen D. Turner, Emanuel Petricoin, Lloyd L. Gray, Roger Abouarduen, Univ. of Virginia, Charlottesville, VA; George Mason University, Charlotteville, VA; George Mason University, Manassas, VA; Cleveland Clinic Lerner Research Institute, Cleveland, OH; Case Western Reserve University, Cleveland, OH; Cavon LLC, Charlottesville, VA.

Glioblastoma stem cells (GSCs) have been implicated in tumor resistance to radio- and chemotherapy. Proliferation of GSCs reportedly requires calcium influx through T-type calcium channels ( Cav3.2). In this study we investigated the expression, function, mechanism of action and therapeutic targeting of Cav3.2 with the FDA approved and repurposed drug Mibefradil in glioblastoma (GBM) and GSCs. We found that Cav3.2 is highly expressed in the majority of human GBM specimens and all GSCs. TCGA data analysis demonstrated that approximately 11% of GBM tumors have upregulated Cav3.2 and that overexpression of Cav3.2 is associated with worse prognosis. Furthermore, we demonstrated that Mibefradil inhibits GCN growth and survival and sensitizes GSCs to Temodar and cisplatin in vitro and in xenograft tumor models. To investigate the mechanism of action of Mibefradil, we performed proteomic and transcriptomic screenings of Mibefradil-treated GSCs using reverse phase protein arrays and RNA-seq, followed by functional rescue experiments. Inhibition of Cav3.2 with Mibefradil significantly altered multiple cancer regulatory signaling pathways and molecules as well as the transcription of oncopgenes and tumor suppressors. Among other, Mibefradil suppressed GCN growth through inhibition of pro-survival pathways such as AKT/mTOR, whilst simultaneously inducing apoptosis through upregulation of survivin, BAX and cleavage of caspase 9 and PARP. Notably also, RNA-deep sequencing of Mibefradil treated GSCs revealed an increase in expression of tumor suppressors such as TNFRSF14 and HSD17B4 along with a decrease in the expression of several oncopgenes such as PDGFA, PDGFR and VEGFA. We also assessed the therapeutic effects of Mibefradil on established GSC-derived xenografts. Oral administration of Mibefradil significantly inhibited tumor growth, prolonged animal survival and sensitized tumors to inhibition by TMZ and radiation. This study represents the first comprehensive characterization of Cav3.2 in GBM and GSC. The data establish Cav3.2 inhibition by the repurposed FDA-approved drug Mibefradil as a new strategy for GBM therapy.


Anaplastic lymphoma kinase (ALK) fusion oncogenes occur in around 3%-5% non-small cell lung cancer (NSCLC) cases. Various ALK inhibitors are in clinical use for the treatment of ALK-NSCLC, including the first generation ALK inhibitor, Crizotinib, and recently the more highly potent Alceatinib and Ceritinib. However, most tumors eventually become resistant to ALK specific inhibitors. To address the mechanisms underlying the development of ALK inhibitor resistance, we used ITRAQ quantitative mass spectrometry and phosphor-receptor tyrosine kinase arrays to investigate intracellular signaling alterations in ALK inhibitor resistant NSCLC cell lines. Src signaling was identified as an Alceatinib resistance mechanism, and combination treatment with ALK and Src inhibitors was highly effective for inhibiting the growth of ALK inhibitor resistant cells in vitro and in mouse xenograft models. Furthermore, phospho-receptor tyrosine kinase activation and downstream PI3K/AKT signaling was effectively blocked by inhibiting Src in Alceatinib resistant cells. Finally, we showed that the combined use of ALK and Src inhibitors inhibited the growth of other ALK-NSCLC cell lines, including those that were Ceritinib or Lorlatinib resistant. Our data suggest that targeting Src signaling may be an effective approach to the treatment of ALK-NSCLC with acquired resistance to ALK inhibitors.

#3149 Identification of genes associated with the cisplatin resistance in cervical cancer cells expressing E545K mutation. Wani Arjumand, Nicholas Jette, Jb McIntyre, Prafull Ghatage, Corinne M. Doll, Susan P. Lees-Miller.

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The phosphatidylinositol-3 kinase (PI3K)/AKT/ mTOR signaling pathway is activated in several human cancers and activation is frequently mediated by “hotspot” mutations including E542K, E545K and H1047R in the PIK3CA gene. Approximately 30% of cervical cancer patients have the PIK3CA-E545K mutation. Cisplatin with radiotherapy (RT) is the standard treatment of cervical cancer world-wide, used in both the radical and post-operative adjuvant settings. However, details of the molecular mechanisms responsible for cisplatin resistance remain unclear. We previously reported PIK3CA mutation in patients with earlier stage (IB/II) cervical cancer was associated with poor survival (McIntyre et al. Gynecol Oncol, 2013, PMID:23266353), and in our recent study we observed that PIKSCA-E545K mutation renders cervical cancer cells more resistant to cisplatin or cisplatin and radiation than other more migratory phenotype than isogenic cells with wild type-PIK3CA. Moreover, these phenotypes are reversed by the PI3K inhibitor GDC-0941/Pictilisib (Wani et al. Oncotarget. 2016, PMID:27489350). The aim of the present study is to identify the expression of genes related to cisplatin resistance in cervical cancer cells engineered to express PIK3CA-E545K. Microarray analysis identified 161 genes that were up-regulated and 189 that were down-regulated in the cervical cancer cells stably expressing PIK3CA-E545K, some of which are involved in well-characterized mechanisms that could be relevant to cisplatin resistance. We are currently validating some of those genes by Real-time PCR that will help us to determine the mechanism of PIK3CA-E545K induced cisplatin resistance and enhanced migration in cervical cancer cells expressing PIK3CA-E545K and extending our in vitro findings to animal models. Together, our data will provide a useful basis for screening candidate targets for risk stratification and provide valuable information for potential targeted intervention in patients whose tumors harbor cisplatin-resistant molecular characteristics.

#3150 PLCG2 C2-domain mutations co-occur with BTK and PLCC2 resistance mutations in chronic lymphocytic leukemia undergoing treatment with the BTK inhibitor ibrutinib. Dan Jones, Jennifer A. Woyach, Weiqiang Zhao, Sean Caruthers, Huolin Tu, Joshua Coleman, John C. Byrd, Amy J. Johnson, Leonard L. Gansky, The George Washington University, Qatar Biomedical Research Institute, Doha, Qatar.

Background: The Bruton agammaglobulinemia tyrosine kinase (BTK) activ- itates B-cell receptor signaling through activation of phospholipase C gamma 2 (PLCG2). Clinical resistance to the Bruton tyrosine kinase (BTK) inhibitor ibrutinib in chronic lymphocytic leukemia (CLL) is highly associated with emergence of the BTK C481 mutations that prevent ibrutinib covalent binding. PLCC2 mutations also occur in ibrutinib-resistant samples but the spectrum of mutations and their occurrence with ibrutinib changes have not been fully delineated. Materials and Methods: All peripheral blood samples with adequate depth of sequencing coverage were included from CLL patients receiving ibrutinib (with or without other therapies) that were submitted from Ohio State University (OSU) to the OSU James Polaris Molecular Laboratory. Genomic DNA was extracted from negatively selected B cells and deep sequencing of the entire coding regions of BTK and PLCC2 performed using a custom Ion Torrent Ampliseq panel. A mean depth of greater than 1000X was obtained with hotspot mutations validated down to 1% variant allele fraction (VAF) in the B cell prepar- ations using orthogonal mutation-specific detection methods. Results: Among 1063 CLL samples from 380 patients who received ibrutinib, BTK C481 resistance mutations were identified in 79 (20.8%) patients including 20 patients that also had co-occurring PLCC2 mutations. 11 patients (2.9%) had PLCC2 mutations without accompanying BTK C481 alterations for a cumulative incidence of PLCC2 mutations in 8.2% of ibrutinib-treated patients. These included previously described mutations in the SH2 and SH3 domain of PLCC2 (R665W, 5707F, A708P and L454F) but also previously uncharacterized mutations in the PLCC2 C2 domain that were seen in 12 patients (3.2%). C2 domain mutations are always seen in association with another PLCC2 and/or BTK resistance mutation, affected codons 1140-1144 that include the highly conserved aspartic acid residues that bind calcium and mediate membrane localization in other C2-domain containing proteins. In sequential samples, PLCC2 C2-domain muta-
tions tracked at similar levels to the co-occurring BTK and PLCG2 resistance mutations indicating their presence in the same population of CLL cells. Conclusions: Mutations in three different PLCG2 structural domains co-occur with BTK C481 mutations. The identification of PLCG2 mutations in the calcium-regulated C2 domain expands the possible mechanisms that can produce the PLCG2 activity following bTK inactivation. The diversity of recurrent mutations observed supports the need for complete PLCG2 sequencing for full characterization of bTK-treated CLL samples.

#3151 Loss of Axin1 drives acquired resistance to WNT pathway blockade in colorectal cancer cells carrying RPS03 fusions. Giuseppe Picco,1 Consalvo Petti,2 Alessia Centonze,1 Erica Torcichio,1 Alberto Bardelli,1 Enzo Medico1.

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In colorectal cancer (CRC), WNT pathway activation by genetic rearrangements of RPS03 is emerging as a promising target. However, its low prevalence severely limits availability of preclinical models for in-depth characterization. Using a pipeline designed to suppress stroma-derived signal, we find that RPS03 “outlier” expression in CRC samples highlights translocation and fusion transcript expression. Outlier search in 151 CRC cell lines identified VACO6 and SNU1411 cells as carriers of, respectively, a canonical PTPRK(e1)-RPS03(e2) fusion and a novel PTPRK(e13)-RPS03(e2) fusion. Both lines displayed marked in vitro and in vivo sensitivity to WNT blockade by the porcupine inhibitor LGK974, associated with transcriptional and morphological evidences of WNT pathway suppression. Long-term treatment of VACO6 cells with LGK974 led to the emergence of a resistant population capable of sustained activation of the WNT pathway inhibitor AXIN1, with consequent protein loss. Suppression of AXIN1 in parental VACO6 cells by RNA interference conferred marked resistance to LGK974. These results provide the first mechanism of secondary resistance to WNT pathway inhibition.

#3152 Acquired resistance to the third-generation EGFR inhibitor ASP8273 is associated with MET or NRAS gene amplifications in preclinical models. Kiichiro Ninomiya1, Kadoaki Ohashi1, Shuta Tomida2, Hiroo Kato1, Tomoki Tamura,1 Hisao Higo,1 Go Makimoto,1 Takashi Ninomiya,2 Toshio Kubo,2 Eiki Ichihara,2 Akiko Sato,2 Katsuyuki Hotta,2 Masahiro Tabata,2 Katsujiro Kura,3 Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama city, Japan; 3Okayama University Hospital, Okayama city, Japan.

Background. Third-generation EGFR tyrosine kinase inhibitors (TKIs) such as osimertinib and ASP8273 demonstrated biologic activity in patients with non-small cell lung cancer (NSCLC) harboring the EGFR T790M mutation; however, acquired resistance inevitably develops and the mechanisms of have not been fully elucidated. This study evaluated potential mechanisms of resistance to ASP8273. Materials and Methods. ASP8273 was kindly provided by Astellas Pharma Inc. PC-9 cells harboring EGFR 19del and RPC-9 cells harboring EGFR 19del + T790M were used as cell models to explore alternative molecular mechanisms of acquired resistance to ASP8273. Resistant cell lines (PC-9ASR and RPC-9ASR) were established from each cell line after continuous exposure to ASP8273 for 6 months. Results. Other EGFR-TKIs, including osimertinib, gefitinib or erlotinib, did not inhibit cell growth and acquired EGFR mutations were not detected in either of ASP8273 resistant cell lines. The PC-9-based model was first analyzed. The 50% inhibitory concentration (IC50) of ASP8273 was 45-fold higher in PC-9ASR cells (1,216 nmol/L) than in the parental PC-9 cells (27 nmol/L). A receptor tyrosine kinase array showed that MET phosphorylation was increased in PC-9ASR cells relative to PC-9 cells. Single-cell cloning revealed that PC-9ASR cell harbored MET gene amplification, but other clones (c1, c3, c4, and c5) did not; these results suggest that heterogeneous cells comprised the resistant cell lines. Consistently, the combination of EGFR inhibitors (ASP8273 or gefitinib) with MET inhibitors (crizotinib or SGX-523) inhibited cell proliferation in vitro. However, co-treatment of ASP8273 with crizotinib consistently inhibited this resistant tumor xenograft model. The RPC-9-based model was then assessed. The IC50 of ASP8273 was 40-fold greater in RPC-9ASR cells (1,393 nmol/L) than in the parental RPC-9 cells (32 nmol/L). Next-generation sequencing showed significant NRAS gene amplification and no NRAS mutations in RPC-9ASR cells. EGFR 19del + T790M was maintained in the resistant cells. Western blot analysis and an active RAS pull-down kit confirmed that NRAS protein was significantly over-expressed. Even that NRAS-GTPase activity was much higher in RPC-9ASR cells than in the RPC-9 parental cells. As expected, inhibition of RAS signaling using a MEK inhibitor (selumetinib or tramebkinib) in addition to ASP8273 overcame the resistance in vitro. Interestingly, a combination of MEK inhibitors with another third-generation EGFR-TKI, osimertinib, had no effect on the ASP-8273 resistant cells. Conclusions. These studies suggest that a bypass signaling pathway, such as MET gene amplification, or activation of the RAS signaling pathway plays a role in ASP8273 resistance in lung cancer cells harboring EGFR mutations. (This work was supported by KAKEN 16K19454 and 15H04830.)

#3153 Transcriptome differences in tyrosine kinase inhibitor-resistant clones of EGFR-mutant lung cancer using a new microfluidic assay for concurrent single-cell RNA and targeted DNA sequencing. Soi Li Kong,1 Hupeng Li,2 Dave Ruff,2 Joyce An Yi Tai,1 Elise T. Courtouris,1 Huay Mei Poh,1 Dawn Pingxia Lui,3 Audrey Ann Liew,1 Gek San Tan,4 Tony Kiat Hon Lim,4 Daniel Shao Weng Tan,4 Shyam Prabhakar,1 Axel M. Hillmer1, Genome Institute of Singapore, Singapore, Singapore; 2Fluidigm, South San Francisco, CA; 3National Cancer Centre Singapore, Singapore, Singapore; 4Singapore General Hospital, Singapore, Singapore.

Resistance to therapy is one of the major causes of cancer-associated death. While a number of mechanisms for the development of resistance to epidermal growth factor receptor, EGFR-targeting drugs in lung cancer have been described, most genomic and transcriptomic studies have focused on analyzing bulk samples that can only provide an average measurement over the entire mixture of cell populations of a tumor. With this approach, it is difficult to resolve cell-to-cell variability of drug resistance within a heterogeneous tumor. In order to accurately describe and eventually delineate the underlying causes of cancer progression, it requires the analysis of single cells on both, the transcriptomic and the genomic level. Only the co-detection of mutations and expression features in individual cells allows to define the connection between the two. We therefore aimed to establish a new methodology for concurrent evaluation of transcriptomic and genomic features within the same single cell on the Fluidigm C1 system. We applied this protocol to an EGFR-mutant lung cancer cell line, PC9, that has developed resistance to tyrosine kinase inhibitor, TKI treatment after prolonged exposure to sublethal doses of Gefitinib. We amplified cDNA and selected genomic EGFR regions of the TKI responsive and resistant PC9 clones from a total of ~300 single cells. We used our in-house single cell analysis pipeline for parallel processing of transcriptome and variants detection. In addition, a novel algorithm, NODES was used to normalize the single cell RNAseq data and detect differentially expressed genes. The differential transcriptome and emergence of the EGFR T790M resistance mutation in relation to the TKI response were evaluated. We observed up-regulation of receptor tyrosine kinase AXL in the resistant cell lines. This observation is in agreement with previous findings that AXL is the key player that promotes TKI resistance in lung cancer. In addition, we found up-regulation of other genes that have not been previously described. Cumulatively, this method allows us to dissect the underpinnings of the TKI resistance mechanism and enables us to identify biomarker for specific cellular features that are connected with resistance and thereby with lung cancer patient’s response to TKI treatment.

#3155 Harnessing synthetic rescues to evaluate and mitigate resistance to cancer therapy. Avinash D. Sahu,1 Joo Sang Lee,1,2 Eytan Ruppin,1 Silvio Gutkind,2 Zhiyong Wang,3 University of Maryland, College Park, College Park, MD; 2University of California, San Diego, CA.

Significance: The emergence of resistance to cancer therapy remains a growing challenge, evidenced from numerous recent investigations identifying molecular events conferring resistance to cancer drugs. Here we demonstrate that many of these molecular events can be attributed to synthetic rescue (SR) genetic interactions, where altered activity of a specific (termed rescuer) gene restores cell viability caused by the inactivation (including through gene deletion or targeted pharmacological repression) of another gene. Methods: We present a computational approach termed INCISOR, which analyzes molecular, survival, and phylogenetic cancer data to predict SR genes pairs that are under positive selection and their presence decreases patient survival. It further screens for causal SR pairs that show in vitro evidence of rescue. Results: We applied INCISOR to mine 8000 patients’ omics and clinical data in TCGA to identify the first genome-wide SR network, composed of SR interactions common to many cancer types. The predicted SRs match drug-specific molecular resistance signatures that have been recently published in arduous clinical studies (with accuracy of 0.8 AUC). They chart pathways of resistance on a genome scale and as we show, can predict response and resistance to the majority of current cancer drugs in patients. We conducted new experiments to test new predicted SR-based combination therapies. Each combination consisted of a primary cancer drug and a second adjuvant therapy inhibiting a predicted key rescuer of the primary drug. Five of the 7 predicted combinations tested synergistically sensitized all the five head and neck cell lines to the original primary therapies. These results were further substantiated via siRNA knockdowns of the
predicted rescuers in combination with the original treatment, confirming the effects observed with pharmacological inhibition. Focusing on mTOR, a major cancer driver gene, we combined drug treatment and pooled-siRNA to validate the rescue effect of 8 out of 10 predicted rescue interactions involving mTOR in a head and neck cancer cell-line. Finally, we analyzed patient-derived mouse xenografts treated with 25 PDXs across different drug combinations. Mice exhibit much better progression-free survival when treated with a drug combination predicted by INCISOR compared with the mice treated with the primary drug alone, testifying to the in vivo benefits of SR-based synergistic treatments. Conclusions: This work presents a new paradigm harnessing synthetic rescue gene-gene interactions to counteract resistance to cancer treatments. Future implementation of this approach will have two broad implications in the precision medicine era, first for determining the most effective treatment regimen based on the molecular characteristics of individual patient’s tumor; second for identifying supplemental drugs to counteract resistance to existing primary therapies.


Background: Osimertinib (AZD9291; Tagrisso) is a third generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) known to be effective against the EGFR-T790M variant, which is accounts for half of the acquired resistance mechanisms to the first generation EGFR-TKIs. However, resistance to osimertinib is likely to progress and the study of potential osimertinib-resistance mechanisms in advanced is necessary. In this study, we investigated the molecular and cellular profiles of the acquired resistance cells to osimertinib in EGFR-mutant non-small cell lung cancers. Materials and Methods: Five EGFR-mutant cell lines were exposed to osimertinib by stepwise escalation or high-concentration exposure methods, and resistant sublines to osimertinib were established. The molecular profiles and cellular phenotypes of these resistant sublines were characterized. Results: EGFR-C797S mutation which was reported to be a major mechanism of resistance to osimertinib in clinical samples was not detected in established resistance cells by using direct sequencing. Several osimertinib-resistance cell lines displayed MET amplification, and some of these cells were sensitive to the combination of osimertinib plus MET inhibitor crizotinib. However, one cell line that displayed MET amplification was not sensitive to this combination therapy. On the other hand, several osimertinib-resistance cell lines displayed epithelial-to-mesenchymal transition (EMT) features. The HCC827-derived subline established by the high-concentration exposure method exhibited not only EMT features but also cancer stem cell-like properties, including aldehyde dehydrogenase isoform 1 (ALDH1A1) and ATP binding cassette subfamily B member 1 (ABCB1) overexpression. Conclusion: We established osimertinib-resistant cells and found that MET amplification, EMT, and CSC-like features were observed. These findings may provide clues to overcome acquired resistance to EGFR-TKIs. In some cell lines, however, the mechanisms of acquired resistance to osimertinib were not revealed yet.

#3157 Osimertinib (AZD9291) is effective against NSCLC cells harboring EGFR exon 20 insertion mutations. Yussoo Lee,1,2 Tae Min Kim,2 Dong-Wan Kim,2 Yong-Oon Ahn,1 Soyeon Kim,1 Bhumuk Keam,2 Miso Kim,1 Dae Seog Heo,1,2,3. 1Seoul National University Cancer Research Institute, Seoul, Republic of Korea; 2Seoul National University Hospital, Seoul, Republic of Korea; 3Seoul National University, Seoul, Republic of Korea.

Background: Although EGFR exon 20 insertion mutation is the third most common among EGFR-mutant NSCLC, this mutation clones are resistant to the first-generation EGFR TKIs. In addition, the efficacy of the second or third-generation EGFR TKIs is unknown in NSCLC with EGFR exon 20 insertion mutation. Methods: Various EGFR exon 20 insertion mutations were introduced into Ba/F3 cells as follows: A763_V764insQEA in C-helix; and V769_D770insASV, D770_N771insNPG, D770_N771insSVD, P772_H773insPR, H773_V774insNPH, H773_V774insH, and H773_V774insH in loop following C-helix. The patient-derived SNU-3173 cell line was developed in a patient with EGFRV77D_L774insAH mutation. Ba/F3 cells transfected with EGFR exon 20 insertion mutations and SNU-3173 were exposed to the EGFR TKIs (the 1st-generation gefitinib and erlotinib; the 2nd-generation dacomitinib and afatinib; and the 3rd-generation rociletinib, olmutinib, and osimertinib) and cell viability assays were performed. In addition, immunoblotting was performed to evaluate the downstream signals of EGFR pathway. Results: Cell viability assay showed that EGFR exon 20 insertion mutations are resistant to the 1st-generation EGFR TKIs, except for EGFRV77D_L774insQEA, mutant Ba/F3 cells. IC50 values of the 2nd-generation EGFR TKIs are five to hundred-folders lower than the 1st-generation EGFR TKIs. The 2nd-generation EGFR TKIs were effective against EGFR exon 20 inserted Ba/F3 cells and down-regulated EGFR downstream signals. The 3rd-generation EGFR mutation-selective inhibitors showed a moderate efficacy except osimertinib that was effective and down-regulated EGFR downstream signals. In addition, SNU-3173 cells were moderately effective to afatinib (IC50 46.2±5.2 nM) as well as osimertinib (IC50 403±3.0 nM).

Conclusions: Overall, osimertinib showed effectiveness against NSCLC cells with EGFR exon 20 insertion mutations. Our results warrant a clinical trial of osimertinib in NSCLC patients with EGFR exon 20 insertion mutations.

#3158 In vivo acquired resistance to an emibetuzumab analogue in MET-amplified gastric xenografts can be overcome by a MET-targeting antibody mixture or PI3CA/AKT/mTOR inhibition. Soofe Elleeb Pollmann,1 Emanuel Funke,2,3 Valerie Calvert,2 Shrutti Rao,2 Simina Boca,2 Sahba Madhavan,3 Ivan David Horak,1 Andreas Kjer,3 Michael Kragh,1 Thomas Tuxen Poulsen1.1 Symphogen, Ballerup, Denmark; 2George Mason University, Manassas, VA; 3Georgetown University, Washington, DC; 4University of Copenhagen, Copenhagen, Denmark.

Aberant over-activation of MET receptor tyrosine kinase is involved in driving malignancies such as gastric and non-small cell lung cancer (NSCLC), and in the development of resistance to EGFR-targeting therapeutics. This has led to the development of several MET-targeting agents in the form of tyrosine kinase inhibitors (TKIs) and monoclonal antibodies (mAbs), many of which are in clinical development. However, resistance to MET-targeted agents is an emerging problem. This study aims to understand mechanisms underlying resistance development to an analogue of emibetuzumab, a mAb targeting MET. Upon long term in vivo treatment, emibetuzumab-resistant tumors and cell lines were generated, isolated, and characterized to acquire their acquired resistance mechanisms. Extensive reverse phase protein array and network analysis were used to characterize the proteomic profiles of three resistant cell lines, revealing three distinct resistance profiles, one involving activation of the PI3K/Akt/mTOR pathway. We further show, how these resistance mechanisms can be overcome by treatment with other targeting therapeutics both in vitro and in vivo. Two of the models demonstrated in vivo sensitivity to Sym015, a novel mixture of two mAbs targeting non-overlapping epitopes of MET, partly due to ADCC, indicating that Sym015 can overcome acquired resistance to emibetuzumab. In addition, the third model demonstrated a marked increase in PI3K inhibition in PI3K/Akt/mTOR pathway activation. This activation translated into induced cancer cell and tumor growth, which could be inhibited by agents targeting PI3K/Akt, mTOR. This study thus points to treatment of patients with acquired resistance to single targeting MET mAbs with PI3K/Akt/mTOR-targeting agents or a MET-targeting antibody mixture such as Sym015.


Sunitinib, an anti-angiogenic tyrosine kinase inhibitor, is approved for treatment of pancreatic neuroendocrine tumors (pNETs). However, its efficacy is greatly limited due to resistance. Sunitinib is a lysosomotropic drug, thus accumulates in lysosomes, leading to their destabilization and to lysosomal membrane permeabilization (LMP), which in turn can lead to cell death. Autophagy might be activated for clearance of damaged lysosomes, thus promote survival and act as a mechanism of resistance. We found that the autophagy inhibitor chloroquine increases sunitinib efficacy in pNET cell lines and in a transgenic mouse model of pNETs. Interestingly, chloroquine is a lysosomotropic drug as well and the response towards sunitinib and chloroquine in pNET cell lines correlated with lysosome-associated membrane protein 2 (LAMP2) levels, which influence lysosome stability. We hypothesized that sunitinib and the com-
combination with chloroquine induce LMP in pNETs and that LMP leads to activation of the transcription factor EB (TFEB), master regulator of genes involved in lysosomal biogenesis and autophagy, leading to therapy resistance. We found that LMP increased upon combined treatment of sunitinib and chloroquine compared to single treatment in pNET cell lines. Treatment of pNET cell lines with sunitinib or chloroquine led to activation of TFEB in EMT cell lines, leading to autophagy as a survival mechanism. Upon massive LMP or if autophagy is dysfunctional, cell death is induced. Based on our data, we suggest the combination of sunitinib and chloroquine as a treatment option for pNET patients and that TFEB could be an interesting therapeutic target in combination with lysosomotropic drugs.

#3160 Sustained MAPK activation as a mechanism of resistance to osimertinib plus selumetinib in models of EGFR-mutant cancer. David Westover,1 Catherine B. Meador,1 Eiki Ichihara,1 Hayden F. Byrd,1 Cath Eberlein,2 Yingjun Yan,2 Darren A. Cross,2 Christine M. Lovly,1 *Vanderbilt-Ingram Cancer Center, Nashville, TN; 1Astrazeneca Oncology, Cambridge, United Kingdom.

INTRODUCTION: Osimertinib is a third-generation, mutant-selective epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) recently approved for the treatment of T790M-positive EGFR-mutant lung cancer. The mutant-selective nature of osimertinib improves its tolerability by limiting its inhibition of wild-type EGFR compared to first- and second-generation EGFR inhibitors, making it a good candidate for rationally-designed combination therapies. One such combination, osimertinib plus the MEK inhibitor selumetinib (AZD6244; ARRY-142886), is currently being studied as part of the phase Ib TATTON trial (NCT02143466). Previous work by our group demonstrated that selumetinib may delay or reverse resistance to osimertinib in some cases; however, we anticipate that resistance to this combination will ultimately develop in patients. Here, we investigate acquired resistance to osimertinib and selumetinib combination therapy in vitro and in vivo. DESIGN: In cells sensitive to osimertinib plus selumetinib (hereafter referred to as combination-sensitive cells), ERK phosphorylation was monitored via a Western blot over the course of a 7-day treatment with 200 nM osimertinib plus 1 μM selumetinib. To detect RAS-GTP, an active RAS precipitation was performed in combination-sensitive and combination-resistant cell lines following treatment with osimertinib. In combination-resistant cells, pharmacologic inhibitors of various MAPK pathway components were used to assess whether they could restore potency: Western blot analysis was used to confirm on-target effects. Lastly, an analysis of differences in gene expression between four isogenic sets of combination-sensitive and combination-resistant cell lines is currently ongoing. RESULTS: In cells that are sensitive to growth inhibition by the combination of osimertinib plus selumetinib, we observed complete re-activation of the MAPK pathway after 5 days of continuous exposure to both inhibitors. Additionally, RAS activity was elevated in combination-resistant cell lines, and these cells remained sensitive to the ERK inhibitor selumetinib. Conversely, in combination-sensitive cell lines, the growth inhibitory effects of osimertinib plus selumetinib in combination-resistant cells, suggesting that incomplete inhibition of MAPK is responsible for resistance in these cells. Furthermore, an alternative MEK inhibitor, trametinib, was efficacious in combination with osimertinib in cell lines that were resistant to osimertinib plus selumetinib. CONCLUSION: These data identify a potential mechanism of resistance to a combination therapy that is currently being tested in the clinic. Specifically, these data demonstrate that re-activation of the MAPK pathway is a mechanism of resistance to osimertinib plus selumetinib and that this pathway is still targetable in combination-resistant cells.

#3161 TPX-0005, an ALK/ROS1/TRK inhibitor, overcomes multiple resistance mechanisms by targeting SRC/FAK signaling. Dayong Zhai, Wei Deng, John Huang, Evan Rogers, J. Jean Cui, TP Therapeutics, Inc., San Diego, CA.

ALK, ROS1, and TRK inhibitors have achieved marked efficacy in treating cancer patients expressing abnormal ALK, ROS1 or NTRK genes. However, the initial success of these therapies is rapidly overshadowed with the development of acquired resistance. In addition, approximately 30-40% of ALK + or ROS1 + NSCLC patients fail to respond to initial crizotinib treatment, representing intrinsic resistance. Target gene amplification, acquired resistance mutations, bypass signaling, epithelial-mesenchymal transition (EMT) and metastasis are the common resistance mechanisms. None of the current ALK, ROS1 or TRK inhibitors can overcome bypass or EMT-based resistance when applied as a single agent therapy. TPX-0005 is a potent kinase inhibitor against WT and mutated ALK, ROS1 and TRK family kinases, especially the clinically significant solvent front mutations. At clinically relevant concentrations, TPX-0005 also inhibits JAK2, SRC and FAK that are important targets in modulating multiple resistance mechanisms. H2228 lung cancer cell line endogenously expresses EML4-ALK v3 and was reported to be resistant to ALK inhibitor TAE684. Uprogulation of the bypass signaling kinase EGF, EMT marker vimentin, and CD44 was reduced in TPX-0005, likely leading to intrinsic resistance to ALK inhibitors. In the anti-proliferation assay, both crizotinib and ceritinib were resistant in H2228 cells with IC50 values around 1000 nM; however, TPX-0005 was able to overcome the intrinsic resistance with an IC50 of 100 nM. In H2228 cells, TPX-0005 suppressed the phosphorylation of SRC, FAK and paxillin with IC50 values in a range of 80-100 nM, and downregulated the expression of CD44 with IC50 values around 100 nM. In addition, TPX0005 inhibited the phosphorylation of the oncoprogenic transcription/translation factor YB-1 with an IC50 value around 100 nM in H2228 cells. YB-1 is involved in many aspects of gene expression control that lead to tumor cell growth and drug resistance, including modulation of EGF upregulation, EMT, and cancer stemness. Therefore, it was postulated that inhibition of SRC/FAK by TPX-0005 suppressed the phosphorylation of YB-1, leading to the downregulation of EGF, CD44 and vimentin, and eventually to anti-proliferation effect on H2228 cells. TPX-0005 demonstrated an in vitro anti-metastatic activity by inhibiting cell migration in both H2228 cells and HT1080 human fibrosarcoma cells. Taken together, the potent kinase inhibitory activities against SRC/FGK signaling provide a unique polypharmacology profile to TPX-0005 for combating multiple resistance mechanisms simultaneously, including a broad spectrum of acquired mutations, bypass signaling, EMT, cancer stemness, and metastasis.

#3162 HRAS G12V predicts for innate resistance to PI3Kα inhibition in head and neck squamous cell cancer. Kara M. Ruicci,1 Ren Sun,2 Morgan Black,3 Nicole Pinto,3 John Yoo,3 Kevin Fung,1 Danielle Macneill,1 Laure Aillies,1 Joseph S. Mymryk,1 Paul C. Bourtos,2 John W. Barrett,1 Anthony C. Nichols,1 1Western University, London, Ontario, Canada; 2Ontario Institute for Cancer Research, Toronto, Ontario, Canada; 3University of Toronto, Toronto, Ontario, Canada.

Introduction: Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide, with an incidence of ~600 000 new cases per year and a 50% mortality rate for advanced disease. The mutational landscape of HNSCC has been recently elucidated, introducing the possibility for targeted therapeutic approaches. PIK3CA—which encodes the catalytic subunit of PI3K (PI3Kα)—is the most frequently altered actionable target in HNSCC, however it is not presently clear which patients benefit most from PI3Kα-inhibition. BYL719 is a leading PI3Kα inhibitor in clinical development for HNSCC. We previously examined the responses of a large panel of genetically-characterized HNSCC cell lines to BYL719 and identified activating HRAS G12V mutations as strong predictors for BYL719 resistance. Here we examine how mutation is able to individually modulate BYL719 response through overexpression and knockdown studies. Methods: To determine if HRAS G12V was able to modulate BYL719 sensitivity, we knocked down HRAS in HRAS G12V and HRAS wild-type (WT) cell lines using RNA interference, and then treated cells over 10-point dose ranges with BYL719. Sensitivity was determined by calculating IC50 (half-maximal inhibitory concentration) values at 72 hours using PrestoBlue®. Conclusions: HRAS knockdown sensitized HRAS G12V lines to BYL719 (lower IC50), but did not affect the response of WT HRAS cells. WT HRAS and HRAS G12V overexpression significantly increased cellular proliferation and promoted BYL719 resistance. In HRAS G12V cell lines, we observed high levels of active phosphorylated AKT (S473/T308), even in the presence of BYL719. Collectively, our findings highlight the predictive role of HRAS G12V for innate BYL719 resistance and contribute to our understanding of which patients may respond best on BYL719 therapy.

#3163 Discovery of a kinome signature predicting sensitivity and resistance to RAF-MAPK pathway inhibitors in melanoma. Riet Hilhorst,1 Mo- nem Ghanem,2 Rob Ruijtenbeek1.

Introduction: RAF-MAPK pathway inhibition with BRAF inhibitors or the combination of BRAF and MEK inhibitors has become the mainstay of therapy
of metastatic melanoma harboring BRAF V600 mutations with a response rate as high as 50–75%. A minority of patients present with primary resistance while all patients develop secondary resistance. We explored a reversed translational approach using a novel multiplex kinase assay to first identify individuals not responding to therapy using baseline biopsy samples and second to understand the mechanisms of resistance using patient-derived xenografts. We treated cell lines with selected drugs from four non-responders and three responders to vemurafenib monotherapy, all harboring the V600E mutation, were profiled for Serine-Thrreonine Kinase (STK) activities on a PamChip® peptide microarray in the presence and absence of dabrafenib. For molecular studies three melanoma cell lines, all with BRAF V600E mutations, were made resistant to vemurafenib. Protein Tyrosine Kinase (PTK) and STK activities in lysates of sensitive or resistant cells were measured in triplicate. Lysates were blotted against a selected panel of (phospho)proteins. Results: In tissue lysates, concentration-dependent ex vivo inhibition with dabrafenib was stronger in the patients that were clinical responders than in non-responders. This difference in inhibition of STK activity by dabrafenib was confirmed in the cell lines that were made resistant against vemurafenib. IC50s for sensitive cell lines were below 0.5 μM and varied from 10–21 μM for cell lines with acquired resistance. PTK activity was increased in resistant cell lines. STK activity was lower, but large differences exist among the cell lines. Common features of resistance were increased activity of receptor tyrosine kinases, Src family kinases and AKT signaling. These results were confirmed by Western blot analysis. Dabrafenib (10 μM) caused inhibition of STK activity while trametinib (10 μM) gave some activation. Dabrafenib (0.5 μM) gave strong inhibition of PTK activity, both in tissue and cell lines. Trametinib had hardly any inhibitory effect. Interestingly, the combination of dabrafenib and trametinib had an antagonistic effect on the STK activity and a synergistic effect on PTK activity, resulting in a stronger inhibition of kinase activity. Conclusion: Resistance of tumors and cell lines to vemurafenib can affect kinase activity profiles as detected pre-dose using a multiplex kinase assay. Dabrafenib inhibition of PTK and STK activity is stronger in sensitive cell lines. The overall activation of kinases in a lysate upon MEK inhibition suggests MEK as suppressor of kinase activity. Resistance to MAPK pathway inhibitors could be linked to an increase in tyrosine kinase activity and AKT pathway activity. Biomarkers are needed to identify patients in need of combined MAPK pathway inhibition.

#3164 A comprehensive analysis of autopsied specimens and patient-derived cells lines in ALK-positive lung cancers with rapid acquired resistance to alectinib. Go Makimoto,1 Kadoaki Ohashi,1 Kazuya Nishii,1 Shuta Tomida,1 Hiroe Kayatani,1 Tomoki Tamura,1 Hisao Higo,1 Kichihiro Ninomiya,1 Takashi Ninomiya,1 Yoshio Kubo,1 Eiki Ichihara,1 Akiko Sato,1 Katsuyuki Hotta,1 Masahiro Tabata,1 Katsuyuki Kuira,1 Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama City, Japan;2 Okayama University Graduate School of Medicine, Okayama, Japan. Background: Alectinib, a highly selective anaplastic lymphoma kinase (ALK) tyrosine kinase inhibitor (TKI), demonstrated a stunning disease control rate (95.7%) and unprecedented median progression-free survival (not reached: 95% CI: 20.3–) (Lancet Oncology 2013, ASCO 2016, Abstract #9008). However, acquired resistance to alectinib is inevitable; some ALK-positive lung tumors rapidly acquire alectinib resistance. Therefore, we sought to investigate the mechanism of the rapid resistance to alectinib using clinical samples and patient-derived ALK-positive cell lines. Materials and Methods: Autopsied samples (primary lung tumors and metastatic tumors from the esophagus, liver, and bilateral kidney) were obtained from a 51-year-old male with advanced ALK-positive lung cancer. The lung tumors acquired resistance to first-line treatment of alectinib in three months, and subsequent treatment with crizotinib showed only a moderate and temporary effect. We established novel ALK-positive cell lines; ABC-14 was established from a pleural effusion after alectinib resistance was confirmed in all autopsied samples by immunostaining. PCR revealed that ABC-14 harbored the EML-4/ALK fusion variant 3. An in vitro cell proliferation assay showed that ABC-14 exhibited resistance to alectinib (IC50 > 1 μM), but was sensitive to crizotinib (IC50 = 98 nM). The receptor tyrosine kinase assay revealed the activation of MET and EGFR in ABC-14. Quantitative RT-PCR and FISH confirmed MET gene amplification. Furthermore, the overexpression of an EGFR ligand, EGF, TGF-β, and no EGFR mRNA overexpression. The combination of crizotinib (dual ALK/MET-TKI) and an EGFR-TKI, gefitinib, showed an additive inhibitory effect on cell growth compared with each drug alone in vitro. ABC-17 showed resistance to both alectinib and crizotinib; consistently showed no activation of MET and no MET gene amplification. Interestingly, ABC-17 showed metastatic ability in the lung in NOG mouse PDX models. Conclusion: The mechanism of rapid resistance to alectinib may be complicated and heterogeneous. Crizotinib combined with gefitinib, which inhibits the ALK, EGFR, and MET pathways, may represent one potent strategy to treat next-generation ALK-positive tumors. A comprehensive analysis of clinical samples should provide deeper insights into its resistance. (This work was supported by KAKEN 16K19454 and KAKEN 15H04830.)

#3165 3D culture may better represent trastuzumab resistance associated with PIK3CA mutation than 2D culture. Takashi Taira,1 Toru Mukohara,2 Rina Tanaka,1 Yohei Shimomo,3 Masanori Toyoda,1 Naomi Kiyota,1 Midori Hirai,1 Yoshioho Kakeji,2 Hiroobu Minami1. Kobe University Graduate School of Medicine, Kobe, Japan;2 Kobe University Hospital, Kobe, Japan. Background: It is becoming clear that presence of PIK3CA mutations is associated with lower pathological complete response rate in patients with HER2-overexpressing breast cancer when treated with trastuzumab-based chemotherapy in neo-adjuvant settings. On the other hand, in vitro studies using traditional 2-dimensional (2D) cell culture, differential cellular or biochemical response to trastuzumab between PIK3CA-mutant (mt) and -wild type (wt) cells has not been clearly demonstrated. Further, while tumor shrinkage is occasionally observed in breast cancer patients who are treated with trastuzumab as a single agent, cytotoxic effect of trastuzumab is not simulated in 2D culture models. Recently, many studies reported 3-dimensional (3D) cell culture mimics in vivo environment better than 2D culture. Therefore, we hypothesized that 3D culture better represents the trastuzumab resistant phenotype associated with PIK3CA mutation than 2D culture, and decided to comparatively investigate cellular and biochemical response to trastuzumab in HER2-amplified PIK3CA-mt and -wt cell lines cultured in 2D and 3D environments. Method: HER2-amplified breast cancer cell lines, BT474 (PIK3CA-), and AAC893 and MDA-MB361 (PIK3CA-mt) were seeded (day 0) and allowed to grow in 2D and 3D (NanoCulture Plate®, ORGANOCULT, Kanagawa, Japan) cell culture plates. On day 3, trastuzumab (10 μg/ml) and/or BM1210 (1 and 5 μM), a PI3K inhibitor, were added. The effect of the drugs on cell growth was evaluated with WST-8 assay on days 3 through 7. Apoptosis and cell signaling were evaluated using Western blot on day 6 and days 3 through 5, respectively. Result: In PIK3CA-wt BT474, treatment with trastuzumab led to decrease in cell number, indicating cytotoxic effect, only in 3D culture but not in 2D culture. In PIK3CA-mt cell lines, treatment with trastuzumab did not change level of p-AKT regardless of cell culture conditions. In PIK3CA-mutant AAC893, combined treatment with trastuzumab and BM1210 resulted in greater increase in expression of cleaved PARP than either drug alone. Conclusion: Trastuzumab-induced inhibition of PI3K/AKT pathway and resultant apoptosis in HER2-overexpressing PIK3CA-wt cells may be observed in 3D culture, while the 2D cultured PIK3CA-mt cell lines are resistant to trastuzumab, which inhibits the ALK, EGFR, and MET pathways, may represent one potent strategy to treat next-generation ALK-positive tumors. Alectinib, which inhibits the ALK, EGFR, and MET pathways, may represent one potent strategy to treat next-generation ALK-positive tumors. Alectinib, which inhibits the ALK, EGFR, and MET pathways, may represent one potent strategy to treat next-generation ALK-positive tumors.
CASE 167 MIF-induced stat3 activation promotes resistance to MEK blockade in KRAS mutant colorectal cancer cells. Seul-Ki Cheon,1 Hwang-Phil Kim,1 Ye-Lim Park,1 Si Hyun Lee,1 Jun-Kyu Kang,1 Yoojoo Lim,1 Sang-Hyun Song,1 Sae-Won Han,1 Tae-You Kim,1 2Seoul National University, Seoul, South Korea; 2Seoul National University Hospital, Seoul, Republic of Korea.

Although MEK blockade has been highlighted as a promising anti-tumor drug, it has poor clinical efficacy in KRAS mutant colorectal cancer. Several feedback systems have been described in which inhibition of one intracellular pathway leads to activation of a parallel signaling pathway, thereby decreasing the effectiveness of single-MEK targeted therapies. In this study, we describe a feedback mechanism in which MEK inhibition leads to activation of macrophage migration inhibitory factor (MIF)-induced stat3 signaling pathway in KRAS mutant colorectal cancer (CRC) cells. We found that KRAS mutant CRC cells with reafarnetbin, MEF inhibitor, induced MIF secretion and resulted in activation of Stat3. MIF knockdown by siRNA partially restored sensitivity to reafarnetbin in KRAS mutant cells. In addition, combination with reafarnetbin and 4PP, a MIF inhibitor, effectively reduced the activity of Stat3 and MAPK.

#3168 Overcoming acquired drug resistance by TPX-0005, an ALK, ROS1 and pan-TRK inhibitor. Wei Deng, John Huang, Dayong Zhai, Evan Rogers, Jean Cui, TP Therapeutics, Inc., San Diego, CA.

The inevitable development of clinical drug resistance presents a common challenge for targeted cancer therapy. In non-small cell lung cancer (NSCLC) patients with ALK and ROS1 rearrangements, the emergence of mutations in the targeted oncoproteins is identified as one of the mechanisms that confer drug resistance. In particular, a group of mutations known as solvent front mutations, such as ALK<sup>L1196V</sup> and ROS1<sup>G2032R</sup>, render common resistance to ALK and ROS1 inhibitors. Similar solvent front mutations, such as TRKA<sup>G099R</sup> and TRKC<sup>E625K</sup>, have also been identified in cancer patients with rearrangement of NTRK family genes who developed resistance to TRK-targeted therapies. TPX-0005, a compact three-dimensional macrocyclic molecule, was designed to completely locate at the adenine binding site of ATP in order to efficiently target the active kinase conformation and systemically inhibits downstream signaling from various clinical resistant mutations, especially the solvent front mutations. TPX-0005 is an orally available and potent ATP-competitive inhibitor against ALK, ROS1, TRKA, TRKB and TRKC recombinant kinases and their corresponding clinical resistant mutants. TPX-0005 demonstrated potent anti-proliferative activity in the range of sub-nanomolar to low nanomolar in a number of human cancer cell lines and engineered stable cell lines expressing the targeted oncogenes or their solvent front mutants, accompanied by inhibition of target phosphorylation and concomitant inactivation of downstream effectors such as ERK, AKT and STAT3. In patient derived xenograft tumor models, TPX-0005 treatment resulted in significant regression of tumors harboring the oncopgenic ALK, ROS1 and TRK fusion events. Moreover, in a series of mouse xenograft tumor models, TPX-0005 exhibited marked anti-tumor activity not only in tumors harboring the wildtype oncopgenic targets but also in tumors harboring the oncopgenic with the solvent front mutations via inhibition of the target phosphorlation. Taken together, these pre-clinical studies have demonstrated the potential of TPX-0005 against not only wild type oncopgenic ALK, ROS1, TRK fusions but also their corresponding solvent front mutations, which will bring in a new therapy for cancer patients resistant to currently available ALK, ROS1, and TRK inhibitors in clinic.

#3169 SerpinB2 enhances invadopodia-like structure protrusions and is down-regulated in acquired gefitinib-resistant non-small cell lung cancer cells. Song Yi Bae,1 Donghwa Kim,2 Hyen Joo Park,2 Woong Sub Byun,2 Ji-Young Hong,2 Hye-Jung Lee,2 Sang Kook Lee,3 1MIT, Cambridge, MA; 2Seoul National University, Seoul, Republic of Korea; 3Seoul lung cancer (NSCLC) is a major type of lung cancer which accounts for approximately 80-85% of all lung cancers. The targeted therapies have significantly improved the survival of advanced NSCLC patients, but the failure of targeted therapy due to the resistance to epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs), such as gefitinib, is now considered a major problem. SerpinB2 is a component of the urokinase plasminogen activating (uPA) system and has been recognized as a biomarker for the progression and metastasis of lung cancer. We found that SerpinB2 is down-regulated in gefitinib-resistant (H292-Ge) cells compared to gefitinib-sensitive (H292) cells. The low SerpinB2 levels in H292-Ge cells were also associated with an enhancement in invasiveness and increase in the length of invadopodia-like structures in the cells. The effect on invasiveness and gefitinib sensitivity was confirmed by knockdown and overexpression of SerpinB2. In addition, an antitumor agent yuahuanidine (YD) was used to test the possibility to overcome the resistance through the up-regulation of SerpinB2. YD effectively elevated SerpinB2 levels and suppressed invasive properties in H292-Ge cells. Collectively, these findings demonstrate the prospective role of SerpinB2 as a novel biomarker for acquired gefitinib resistance and a potential target for NSCLC treatment. Acknowledgement

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EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Novel Mechanisms 1

#3170 MCAM modulates small cell lung cancer chemoresistance via PI3k/Akt/Sox2 signaling pathway. Satyendra C. Tripathi,1 Johannes F. Fahrmann,1 Muge Celiktas,1 Mitzi Aguilar,1 Kieren D. Marini,2 Mohit K. Jolly,3 Hiroyuki Katayama,1 Hong Wang,1 Eunice N. Murage,1 Jennifer B. Dennison,1 D. Neil Watkins,6 Herbert Levine,6 Edwin J. Ostrin,6 Ayumu Taguchi,1 Samir M. Hassen,1 MD Anderson Cancer Center, Houston, TX; 2Hudson Institute of Medical Research, Australia; 3Rice University, Houston, TX; 6Garvan Institute of Medical Research, Australia.

Despite favorable responses to initial therapy SCLC relapse occurs within a year exhibiting a multidrug resistant phenotype. Due to limited accessibility of patient tissues for research purpose, SCLC patient derived xenografts (PDXs) have provided the best opportunity to address this limitation. We sought to identify novel mechanisms involved in SCLC chemoresistance. Through in-depth proteomic profiling, we identified MCAM as a markedly upregulated surface receptor in chemoresistant SCLC cell lines that exhibited a mesenchymal phenotype and in chemoresistant PDXs compared to matched treatment-naïve tumors. MCAM is a cell membrane protein whose expression has been implicated in multiple human cancers. MCAM expression is also detected in lung adenocarcinoma; however, its expression and role in SCLC is still not been explored. MCAM knockdown in chemoresistant cells reduced cell proliferation and decreased the IC50 inhibitory concentration of chemotherapy drugs. MCAM was found to modulate sensitivity of SCLC cells to chemotherapeutic drugs through up-regulation of MRPI/ABCC1 expression and of the PISK/AKT pathway in a SOX2 dependent manner. Metabolomic profiling revealed that MCAM can modulate glutamic acid and lactate production in chemoresistant cells with a distinct metabolic phenotype sustaining low oxidative phosphorylation. MCAM may serve as a novel therapeutic target to overcome chemoresistance in SCLC.
Experimental and Molecular Therapeutics: Novel Mechanisms 1

#3171 Analysis of ‘drug addiction’ mechanisms in the drug-resistant ROS1-rearranged cancer cells. Hayato Ogura,1 Jun Adachi,2 Takeshi Tonomaga,2 Naoya Fujita,3 Ryoho Katayama1, The Cancer Chemotherapy Ctr., JFCR, Tokyo, Japan;2 Lab. of Proteome Research, National Institute of Biomedical Innovation, Health and Nutrition, Osaka, Japan.

ROS1-rearranged non-small-cell lung cancer (NSCLC) is observed in approximately 1% of lung cancer patients. Crizotinib, ALK/ROS1/cMET tyrosine kinase inhibitor (TKI), showed high effect against ROS1-rearranged NSCLC in the clinical trials and is approved in US and EU. However, the cancers inevitably relapse due to the acquired resistance such as ROS1-G2032R mutation. We previously reported that caborzantinib could overcome those secondary mutations mediated resistance. Based on this finding, we tried to identify drug addiction in non-addicted caborzantinib-resistant ROS1 mutant cells by ENU mutagenesis screening. Surprisingly, we found out a few CD74-ROS1 mutant clones that only grew in the presence of low-dose caborzantinib. Namely, these mutant clones were “addicted” to caborzantinib. When the fluorescent labeled caborzantinib addicted ROS1 mutant cells were co-cultured with non-addicted CD74-ROS1-WT BaF3 cell in the presence or absence of low-dose caborzantinib, the drug-addicted cells became dominant in the low-dose caborzantinib, and CD74-ROS1-WT cells became dominant in the absence of it. To analyze the molecular mechanism of “drug-addiction” in the addicted CD74-ROS1 mutant cells, we performed comprehensive analysis such as inhibitor screening, cDNA microarray, and phosphoproteome analysis. As the result, we observed that the expression and phosphorylation of CD74 in the addicted cells were excessively increased within 24 hours after removal of caborzantinib or other ROS1 inhibitors, such as crizotinib or lorlatinib. We also observed that the apoptosis was induced in these cells upon ROS1 inhibitor removal, whereas ROS1 mediated growth signaling were simultaneously activated. Consistent with this, cDNA microarray analysis revealed that the expression of both cell survival and cell death related genes were changed by ROS1 inhibitor removal. Furthermore, we identified that several proteins were highly phosphorylated by excessive ROS1 activation originated from ROS1 inhibitor removal. These observations suggest that these drug-addicted cells were died by excessive oncogenic signaling, and the appropriate oncogenic signaling by low-dose TKI made them survive and grow without inducing apoptosis. Thus, the excessive oncogene signaling has “double-edged sword” characteristics for cancer cell viability and those characteristics could be a new therapeutic target.

#3172 Inhibition of coiled coil domain containing protein 69 (CCDC69) enhance platinum-mediated apoptosis in ovarian cancer cells. Long Cui, Joseph Kwong, Chi Chiu Wang. The Chinese University of Hong Kong, Hong Kong, Hong Kong.

Background and Objective: Molecular mechanisms of chemo-resistance in ovarian cancer are poorly understood. To identify gene involved in ovarian cancer chemo-resistance, 135 ovarian cancer patients with intact chemotherapy response information from The Cancer Genome Atlas (TCGA) database were included. Our analysis revealed that the level of CCDC69 mRNA is differentially expressed between chemo-sensitive group and chemo-resistant group. Moreover, there was a significant negative correlation between CCDC69 promoter methylation and mRNA expression. The aim of the study is to examine the role of CCDC69 in the underlying mechanism of chemo-resistance. Methods: The expression levels of CCDC69 were detected in chemo-sensitive ovarian cancer A2780, chemo-resistant A2780cis and SKOV3 cells line using quantitative RT-PCR and immunoblots. Promoter methylation status of CCDC69 were investigated by bisulfite sequencing. Silencing CCDC69 in A2780cis and SKOV3 cells were performed by Small-interfering RNAs (siRNAs) and CRISPR-Cas9. Cell viabilities after cisplatin treatment were evaluated by MTT and colony formation assays. Apoptosis was assessed by Annexin V/PI staining and caspases 3/8 activity. Cell cycle distributions and mitochondrial membrane potential (ΔΨm) were measured by flow cytometry. Relevant pathway proteins were determined by immunoblotting assays. Results: Heavy Cpg methylation (73.1% and 74.3%) was found in A2780 and A2780cis cells. Restoration in the expression of CCDC69 promoter by CRISPR-Cas9 treatment and/or reactivation of p14ARF-Mdm2-p53 cell cycle and/or apoptosis pathway, enhanced cisplatin sensitivity in CCDC69KO A2780cis cells. Overexpression of CCDC69 in A2780cis and SKOV3 cells were also shown increased sensitive to cisplatin treatment (p < 0.05). When knockout CCDC69 in chemo-resistant A2780cis and SKOV3 cells by CRISPR-Cas9, the CCDC69KO chemo-resistant A2780cis and CCDC69KO SKOV3 cells were also shown increased sensitive to cisplatin treatment (p < 0.001). Moreover, treating CCDC69KO A2780cis cells with cisplatin, abolished G1 and G2/M arrest, more cleaved caspase 3/8 activities, greater ΔΨm loss and higher levels of Bax were observed. When restoring CCDC69 expression in CCDC69KO A2780cis cells by transient transfection, it attenuated sensitivity to cisplatin. By immunoblotting, disruption of p14ARF-Mdm2-p53 cell cycle and/or apoptosis pathway, enhancement of MDM2 expression, and increase of p53 nuclear export were found in CCDC69KO A2780cis cells. Additionally, inhibition of c-Myc enhance cisplatin sensitivities in CCDC69KO A2780cis cells, overexpression of CCDC69 in A2780cis and SKOV3 cells. Our results show that silenced CCDC69 expression induced platinum-mediated apoptosis in ovarian cancer cells by abrogating c-Myc mediated apoptosis and cell cycle control networks.

#3173 AKT1 grinder quiescent cancer cells persist after neoadjuvant chemotherapy in triple-negative breast cancer patients. Sherehay Kabraj1, Xavier Sole,2 Ying Huang,2 Clyde Bango,2 Michaela Bowden,2 Aditya Bardia,2 Dennis Sgroi,2 Massimo Loda,2 Sridhar Rameswamy2, Massachusetts General Cancer Center, Boston, MA;3 Dana Farber Cancer Institute, Boston, MA;4 Massachusetts General Hospital, Boston, MA.

The mechanisms that allow triple negative breast cancer tumors to survive neoadjuvant chemotherapy are incompletely understood. Evidence suggests that proliferative heterogeneity may contribute to primary chemotherapy resistance in patients with triple negative breast cancer. AKT1 grinder quiescent cancer cells (QCCs) is a quiescent, epigenetically plastic, and chemotherapy resistant subpopulation initially identified in experimental cancer models. Here, we identify QCCs in primary and metastatic human breast tumors using automated, quantitative, immunofluorescence microscopy coupled with computational and statistical techniques. We show that QCCs exist as non-random and heterogeneous clusters within primary tumors. In addition, these QCC clusters persist after treatment with multi-agent, multi-cycle, neoadjuvant chemotherapy in both residual primary tumors as well as nodal and distant metastases in patients with triple negative breast cancer. Together, these data qualify QCCs as a non-genetic mechanism of chemotherapy resistance in triple negative breast cancer patients that warrants further study.


Abstract Introduction- Resistance to therapeutics targeting topoisomerase 2 is a major problem in the treatment of leukemia. Cells which survive and give rise to relapsed leukemia are known to modulate different pathways like down-regulation of drug target, reduced drug accumulation, and improved DNA repair; ultimately leading to survival of drug resistant cells. Here, we wanted to identify the earliest detectable changes occurring when cells become resistant to topoisomerase 2 inhibitors. Methodology- We generated early and late stage drug (doxorubicin) resistant leukemia sub cell line from K562 and THP-1 parent cells, HL-60/MX2 resistant sub cell line of HL60 was also used for this study. Molecular changes were analyzed by electron microscopy, quantitative PCR, western blotting, immunofluorescence, flow cytometry, molecular inhibitors and MT assay. Results were further confirmed in blast cells of AML (n = 44) patient samples collected for this study. Furthermore, meta-analysis for survival time was done from microarray expression data of 221 patient samples using ProgNoScan. Results- We mapped molecular changes acquired by leukemic cells during evolution from Early Drug Resistant Population (EDRP) to Late Drug Resistant Population (LDRP). We found unlike LDRP, EDRP cells do not possess known bona fide drug-resistance mechanisms namely limited drug accumulation, reduced DNA damage or expression of drug target. Instead they survive by acquiring ‘poised epigenetic state’ that enhanced their DNA repair. Mechanistically, GCN5, a histone acetyl transferase get selectively upregulated in EDRP cells mediating higher H4K16 acetylation levels and consequent euchromatin state of EDRP. Upon Doxorubicin treatment, H4K16ac facilitate higher ATM recruitment and activation causing efficient activation of H2AX, NBS1, BRCA1, Chk2 and Mcl-1, accelerating DNA repair and survival of EDRP cells. Inhibition of GCN5 with Doxorubicin treatment significantly reduces H4K16ac levels, ATM recruitment and cell survival of EDRP cells. Similarly, ATM induction along with Doxorubicin completely ablates EDRP but not LDRP. Furthermore, baseline AML patient samples (n = 44) showed significantly higher GCN5 expression in MRD positive compared to MRD negative samples. Additionally, meta-analysis of 221 AML patients showed increased GCN5 expression associates with poor survival of AML patients. Conclusion- We identify GCN5 expression as marker that defines onset of resistance in leukemia and GCN5 mediated ATM activation via H4K16ac as a novel non-genetic route facilitating EDRP cell survival with enhanced DNA repair. These data also
highlight the clinical relevance of targeting GCN5 and ATMs during early resistance to prevent emergence of difficult to treat stable diverse resistance in leukemia.


Muscle invasive bladder cancer (MIBC) is a significant cause of cancer death, in part because there are relatively few treatments and these have limited efficacy. Our studies show that expression of 14-3-3 (aka ARF), one of the major transcripts encoded by the CDKN2A tumor suppressor locus, is frequently up-regulated in MIBC, particularly in more aggressive disease subtypes. Our results further show that accumulation of ARF protein in the nucleus is associated with poor outcome and reduced response to chemotherapy. Using both genetically-engineered mouse (GEM) models and human xenograft models of MIBC, we demonstrate that tumors expressing ARF display poor response to treatment with the platinum-based chemotherapy agent cisplatin. This is mediated in part by expression of the integrin-binding protein ITGB3BP (CENPR) and reflects ARF-dependent inhibition of protein translation, which is attenuated by drug treatment. Our findings reveal an unexpected context-dependent role for ARF in modulating drug response in bladder cancer through its ability to regulate protein translation.

#3176 Inhibition of HER2/β-catenin signaling by penfluridol overcomes resistance to paclitaxel in breast cancer. Nehal Gupta, Parul Gupta, Sanjay Srivastava. Texas Tech University Health Sciences Center, Amarillo, TX.

Paclitaxel is a first line treatment option for patients with metastatic breast cancer. However, inherited or acquired resistance is a limiting factor for therapy with paclitaxel. The mechanism of paclitaxel resistance remains obscure and hinders the development of therapeutic strategies. HER2 is an oncogene overexpressed in about 30% of breast cancer patients and plays role in drug resistance leading to poor prognosis. To identify more clinically relevant mechanism of paclitaxel resistance, we developed resistance to paclitaxel in MCF-7 and 4T1 breast cancer cell lines. The continuous exposure to paclitaxel for several months resulted in >100 fold resistance in MCF-7 cells and >100 fold resistance in 4T1 cells. Western blot analysis showed enhanced expression of HER2, β-catenin and downstream molecules such as TCF/LEF, c-Myc, Cyclin D in these resistant cells. We have recently demonstrated that penfluridol, an anti-psychotic drug, suppresses the growth of triple negative metastatic breast cancer cells (Ranjan and Srivastava, Cancer Res 2016; 76(4): 877-890), giving us the rationale to evaluate whether penfluridol inhibits HER2 and β-catenin signaling. Our current results showed that penfluridol treatment not only suppressed HER2 but also inhibited β-catenin expression. We also observed down regulation of LEF-1/TCF, Cyclin D1 and c-Myc expression with penfluridol treatment in paclitaxel sensitive as well as resistant cells resulting in reduced survival of cells. Our results further showed that penfluridol treatment synergistically enhanced the growth suppressive effects of paclitaxel in MCF-7 and 4T1 paclitaxel resistant cells. Treatment of paclitaxel resistant 4T1 cells with 1.5μM of penfluridol in combination with 50nM of paclitaxel resulted in 65% of cell growth suppression whereas either treatment alone was not cytotoxic at all. We also observed an enhanced down regulation of proteins involved in paclitaxel resistance such as HER2, β-catenin, c-Myc and Cyclin D1 as well as increase in apoptotic markers such as CI-PARP and CI-Caspase3 when paclitaxel treatment was combined with penfluridol in resistant cells. Taken together, our results provided a novel insight into the mechanism of resistance to paclitaxel and also opened new avenues for application of penfluridol in cancer therapeutics. Further detailed mechanistic and in vivo studies are in progress. (Supported in part by RO1 grant CA129038, awarded by National Cancer Institute, NIH).


Background: Recently, a new research spotlighted the role of KITENIN (KAI1-COOH-terminal interacting tetraspanin) on glioma invasiveness and progression, associated with the up-regulation of EMT (epithelial-mesenchymal transition) and cancer stemness markers. In this study, furthermore, it is investigate whether KITENIN leads to resistance against TMZ through enhancement of cancer stemness factors using mouse glioma model. Materials and Methods: TMZ-resistant T98G cell line (T98G/TR) was developed. EMT and cancer stemness markers and KITENIN expression were assessed by Western blotting and qRT-PCR. With KITENIN modulated U251 (knockdown) and GL261 (overexpression) cells, cell viability and apoptotic cell death factors after TMZ treatment were evaluated. Biological role of KITENIN on TMZ-resistance was investigated using cell viability, proliferation, invasion and migration analysis for tumor sections of orthotopic glioma model. Using human glioma samples and primary cells, the mechanistic link between KITENIN and cancer stemness factors was confirmed. Results: T98G/TR cells showed increased expression of the KITENIN and EMT and cancer stemness factors. Also, in vitro assays revealed that KITENIN knockdown inhibited cell viability against TMZ treatment, whereas KITENIN overexpression promoted cell viability and apoptotic cell death pathway. In orthotopic glioma models, mice implanted with KITENIN-overexpressed cells showed resistance to TMZ on MRI and histopathological examination. The expression of KITENIN/ALDH1 or KITENIN/C4D were co-localized and significantly higher in tumor sections of mouse transplanted with KITENIN-overexpressed cells than in tumor sections of mouse transplanted with control cells. Genetic down-regulation of KITENIN for KITENIN-overexpressed cell line and primary glioma cells led decreased expression of ALDH1 and CD44. In human glioma samples, high expression of KITENIN was closely related with high expression of ALDH1. Conclusion: KITENIN affects TMZ-resistance in malignant gliomas through induction of ALDH1 and CD44. Therefore, it could be suggested that KITENIN is therapeutic target to overcome TMZ-resistance of malignant gliomas.

#3178 The p53 tumor suppressor protein paradoxically drives chemoresistance in human medulloblastoma cells through suppressing the mTOR AKT pathway. Aisha Naeen, Muhammad U. Choudhry, Maria L. Avantaggiati, Olga C. Rodriguez, Chris Albanese. Georgetown Univ., Washington, DC.

Medulloblastoma (MB) accounts for approximately 25% of childhood brain tumors with 70% of the cases occurring in children under 10. Prognosis for children less than three years old is considerably worse and, due to its destructive effects on the developing nervous system, irradiation is largely avoided in this age group. The p53 pathway is considered a key determinant of anti-tumor responses in many tumors; however, its role in the regulation of cell survival, chemo-sensitivity and chemoresistance in MB is much less well defined. It has been shown that p53 pathway defects, mutations and nuclear levels increase significantly from MB diagnosis to relapse and correlate with an adverse prognosis. We recently reported on the highly novel and unexpected finding that both the genetic (shRNA) and chemical silencing of p53 led to a significant increase in cell death by the drug VMY in MB cell lines with elevated basal p53 (e.g. in D556 cells which express wild type p53 and in DAOY cells that contain mutant p53) as measured by colony formation assays, DNA degradation assays and annexin-V staining, suggesting a surprising commonality in the p53 signaling in both cell lines despite the differences in their p53 status. Equally surprising was the observation that the silencing of p53 in D556 cells enhanced cell death by the clinically used drugs doxorubicin and vincristine. Conversely, we now find that suppressing p53 with sh-RNA in D283 cells, which express much lower levels of wild type p53 compared to D556, resulted in the more classical chemoresistance profile, suggesting a form of p53 ‘addiction’ in D556 and DAOY cells. Mechanistically, we observed that suppressing p53 with sh-RNA in D556 cells treated with VMY or doxorubicin significantly increased the levels of phosphorylated Chk1, S6K, gH2AX, and MDM2. Importantly, phospho-mTOR levels were also significantly increased in the D556/p53 -sh RNA cells and the suppression of mTOR enhanced chemoresistance. These somewhat paradoxical findings suggest that activating mTOR may induce MB cell death and that suppression of mTOR by p53 enhances chemoresistance. Our data provide new mechanistic insights into the role of p53 in primitive neuroectodermal tumors and may provide new approaches for enhancing the clinical outcome of patients with MB.
unique amino acids through translation of the exon 19/inntron 19 'readthrough'. Immunoassays, utilizing antisera raised against these unique amino acids, confirmed that TOP2a/90 is expressed in both cell types, with overexpression in K/V/P.5 cells. Immuno detection of Complex of Enzyme-to- DNA (ICE) and single cell gel electrophoresis (Comet) assays demonstrated that K562 cells transfected with TOP2a/90 encoded truncated DNA covalent complexes and decreased etoposide-induced DNA damage, respectively, compared to similarly treated K562 cells transfected with empty vector. Since TOP2a lacks the active site tyrosine (Tyr805) of full length TOP2a, these results strongly suggest that TOP2a/90 exhibits dominant-negative properties. In separate studies the TOP2a/90 mRNA splice variant was found to be expressed in most human tissues suggesting that this novel protein isoform may play a role in both intrinsic chemosensitivity as well as in acquired resistance. Further studies are underway to characterize the mechanisms(s) by which TOP2a/90 plays a role in acquired resistance to etoposide and other TOP2a targeting agents. In addition, future studies will be directed to examine the RNA processing mechanism(s) that suppress intron 19 splicing in TOP2a pre-mRNA.

#3182 Cytoskeletal modulation results in increased tumor survival and drug resistance through attenuation of p53 dependent apoptosis. Victoria E. Wang, 1 John Doench, 2 David Root, 2 Rene Bernard,3 Jeffrey Settleman,3 Frank McCormick, 1 1UCSF, San Francisco, CA; 2Broad Institute, Cambridge, MA; 3Netherlands Cancer Institute, Amsterdam, Netherlands; 4Genentech, South San Francisco, CA.

One of the major challenges to eradicating cancer involves the evolution of drug resistant clones and persistence of residual disease that escapes our current limit of detection but may proliferate upon cessation of therapy. These microscopic foci of residual diseases often exhibit stem-cell like properties and are intrinsically more resistant to drug therapy. A better understanding of the mechanisms underlying the innate drug resistance of these cellular populations may lead to improved treatment strategies, resulting in more durable remissions and ultimately improved patient survival. We utilize functional genomic platforms to identify mechanisms responsible for the persistence of drug tolerant cells. A genome wide shRNA enrichment screen was performed using the c-Met addicted cell line GTL-16 to identify genes whose knockdown conferred DTP survival. Inhibition of the RhoA-ROCK-myosin pathway promotes drug resistance in a variety of tumor models, including those treated with either targeted therapy or conventional chemotherapy. Furthermore, more, pharmacological validation using multiple small molecule inhibitors of ROCK1 phenocopied both myosin heavy chain (MYH9) and light chain (MYL12) shRNA knockdown. More recently, utilizing a combination of gene expression and biochemical approaches, we have identified attenuation of p53 induced apoptosis to be a key event in mediating survival of these drug tolerant persisters. Reactivation of p53 using nutlin results in increased cell death. These findings demonstrate that modulation of cytoskeleton is an important, but underappreciated, mechanism of drug resistance across many tumor types. These downstream effectors may serve as novel therapeutic targets for intervention and also biomarkers to stratify patients and their response to treatment.

#3183 Proteomic characterization of aromatase inhibitor resistant mammospheres reveal the presence of a novel nuclear chaperone. Syreeta L. Tilghman, 1 Jamal Pratt, 2 Shawn D. Llopin, 3 Michael Alexander, 1 Rashidra R. Walker, 3 Patrick Carriere, 3 Ian R. Davenport, 2 Wensheng Zhang, 1 Karen Zhang, 1 Florida A&M University, Tallahassee, FL; 2 Xavier University of Louisiana, New Orleans, LA; 3 Morehouse School of Medicine, Atlanta, GA.

Postmenopausal women with early-stage metastatic estrogen-dependent breast cancer are generally treated with aromatase inhibitors (AIs) (e.g., letrozole). However, acquired resistance remains a major clinical obstacle. Previously, our group revealed a global proteomic signature of a letrozole-resistant cell line (LTLT-Ca) associated with hormone independence, enhanced cell motility and epithelial to mesenchymal (EMT). Given recent evidence suggesting a convergence of EMT and cancer stem cells (CSC), we chose to utilize a two-dimensional (2D) vs three-dimensional (3D) culture system to compare the proteome of LTLT-Ca cells, as 3D culture not only enriches for CSC, but more accurately recapitulates the tumor microenvironment, morphology, function and response to therapy compared to conventional 2D culture. We hypothesize utilizing a novel systems biology approach may reveal previously unconsidered molecular changes that could aid in understanding complex signaling networks and be exploitable as therapeutic targets. To address this hypothesis we characterized mammosphere enriched immunocompromised female mice were inoculated in the mammary fat pad with LTLT-Ca or letrozole sensitive cells (AC-1) and intratumoral putative CSC marker expression was assessed by immunohistochemistry. Results indicate LTLT-Ca tumors were CD44+/CD24−/ whereas AC-1 tumors were CD44+/CD24+...
CD24™. Mammosphere formation assays were conducted and LTLL-T-Ca cells formed mammospheres at a 3.4-fold higher index than AC-1 cells. A quantitative proteomic analysis of whole cell lysates from LTLL-T-Ca (2D adherent cells) versus LTLL-T-Ca (3D mammospheres) was conducted. Results identified significant protein expression changes within a panel of 1010 proteins; 173 were up-regulated and 186 downregulated (p < 0.05, fold change ≥ 1.20). Additionally, functional enrichment analyses were performed and 19 gene ontology (GO) terms and one KEGG pathway (hsa03010:Ribosome) were over-represented (BH adjusted p-value < 0.01) by the cognate genes. Notably, there was a 35.04-fold increase in midasin (MDN1), a nuclear chaperone protein required for maturation and nuclear export of pre-60S ribosome subunit. Increased MDN1 expression was strongly correlated with highly tumorigenic breast cancer subtypes. Additionally, Kaplan-Meier survival plots demonstrate that increased maturation and nuclear export of pre-60S ribosome subunit. Increased MDN1 fold increase in midasin (MDN1), a nuclear chaperone protein required for to daunorubicin in leukemia cells.

The enhanced LC3Band P62 levelssuggest an accumulation of these cells as compared to control cells. In addition, the inhibition of V-ATPase—served as significantly increased expression of the autophagy protein, beclin-1 in western blot analysis. Upon cisplatin treatment, the LC3B levels were further increased in a2v increased the protein levels of SQSTM1/p62, a known substrate for the threonine pumps that regulate intra/extra-cellular pH in cancer. Its a2 isoform (V-ATPase-a2) contribute signifıcant amounts of H2O2 in the bone marrow micro-environment to contribute to anthracycline resistance.

Introduction: Docetaxel (DTX) is one of the primary drugs used for treating castration resistant prostate cancer (CRPC). Unfortunately, over time patients invariably develop resistance to DTX therapy and their disease will continue to progress. The mechanisms by which resistance develops are still incompletely understood. This study seeks to determine the involvement of miRNAs, specifically miR-181a, in DTX resistance in CRPC. Methods: Total RNA from parental C4-2B prostate cancer cells and DTX resistant C4-2B cells (C4-2B TaxR) was submitted for small RNA deep sequencing. Data was analyzed to ascertain which miRNAs expressions were most altered in C4-2B TaxR cells compared to parental cells. Having identified an increase in miR-181a in resistant cells, its expres- sion was modulated in C4-2B and C4-2B TaxR cells by transfecting them with miR-181a mimics or antisense, respectively. Following transfection, cell number and proliferation were monitored by crystal violet assay and colony-forming assay. Phospho-p53 expression was assessed by western blot and apoptosis was measured by ELISA on C4-2B TaxR cells with inhibited miR-181a expression with or without DTX. Results: miR-181a is significantly upregulated in C4-2B TaxR cells compared to parental C4-2B cells as analyzed by small RNA sequencing. Over-expression of miR-181a in C4-2B cells confers DTX and cabazitaxel resistance. Knockdown of miR-181a in C4-2B TaxR cells re-sensitizes them to treatment with both DTX and cabazitaxel. miR-181a knockdown alone induced apoptosis in C4-2B TaxR cells which is further enhanced by DTX. We next assessed if miR-181a altered expression or activity of ABCB1, which is overexpressed/active in C4-2B TaxR cells and promotes resistance to DTX by pumping the drug out of cells. We found that miR-181a does not impact ABCB1 expression or activity. Since we previously demonstrated that p53 phosphorylation, and how these changes contribute to cisplatin sensitization. Expression profiling of ovarian cancer cells (A2780 cis-r and TOV-112D- cis-r) revealed that upon a2 inhibition, the basal levels of autophagosome membrane protein, LC3B were enhanced compared to cis-r cells as determined by flow cytometry and western blot analysis. Upon cisplatin treatment, the LC3B levels were further enhanced in a2 inhibited cis-r cells compared to control cells. Further, we observed a significantly increased expression of the autophagy protein, beclin-1 in these cells as compared to control cells. In addition, the inhibition of V-ATPase-a2v increased the protein levels of SQSTM1/p62, a known substrate for the a2 isoform of V-ATPase (V-ATPase-a2) contributed to the accumulation of the autophagosomes due to a altered autophagy flux upon V-ATPase-a2v inhibition. The study provides a rationale for the utility of V-ATPase-a2 inhibitors in combination with standard drugs as a novel strategy to improve the treatment efficacy of the chemoresistant ovarian cancer.
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 centrally controls the emergence of NEPC from CRPC under the selective pressure of ENZ remain elusive. As the AR is the cornerstone therapeutic target in men with CRPC, understanding its contribution to the development of NEPC is critical to better implement current standard-of-care therapies such as ENZ, and to identify novel therapeutic targets for this incurable disease. Hallmarks of AR expression in the prostate include high AR activity, highlighted its clinical relevance to disease that is difficult to treat with mainstay therapies. These data underscore the consequences of potent AR inhibition in CRPC, revealing a novel mechanism of AR-dependent control of NEPC resistance to ENZ and the loss or reduced activity of the AR. Thus, we hypothesized that a consequence of ENZ treatment and resistance in CRPC is directly control the emergence of NEPC from CRPC under these selective pressures of ENZ remain elusive. As the AR is the cornerstone therapeutic target in men with CRPC, understanding its contribution to the development of NEPC is critical to better implement current standard-of-care therapies such as ENZ, and to identify novel therapeutic targets for this incurable disease. Hallmarks of AR expression in the prostate include high AR activity, highlighted its clinical relevance to disease that is difficult to treat with mainstay therapies. These data underscore the consequences of potent AR inhibition in CRPC, revealing a novel mechanism of AR-dependent control of NEPC resistance to ENZ and the loss or reduced activity of the AR. Thus, we hypothesized that a consequence of ENZ treatment and resistance in CRPC is directly control the emergence of NEPC from CRPC under these selective pressures of


Lung cancer is the leading cause of the cancer-related death worldwide. Despite extensive efforts for the cure of lung cancer, the effectiveness of currently available therapeutic regimens has been marginal and limited, and recurrence is still an inevitable consequence of anticancer therapies. Therefore, mechanistic understanding on the progression and relapse of lung cancer and finding out relevant targets are essential for development of efficacious therapeutic strategies. We have investigated mechanisms of chemoresistance by using subsets of lung cancer cell lines carrying acquired resistance to chemotherapies. These chemoresistant sublines of NSCLC cells exhibited a prominent downregulation of anti-tumor microRNAs, including proliferation, metastasis, and protein synthesis, but displayed increased tumor growth in vivo. Additional studies revealed that the chemoresistant sublines experienced increased secretion of various soluble factors that led to increases in proliferation and stemness of drug-naive cancer cells and recruitment of macrophages, establishing tumor microenvironment (TME) prone to tumor recurrence. We further demonstrate a pivotal role of regulator of G protein signaling 2 (RGS2) in tumor-promoting cell-cell communications, ultimately leading to chemoresistance. RGS2 disrupted chemotherapeutic-induced apoptosis and secretion of soluble factors involved in cellular communications in TME by regulating unfolded protein responses, a typical cellular event associated with downregulation of protein synthesis. Mechanistically, RGS2 induced proteasome-mediated prote degradation of a component of eukaryotic translation initiation factor complexes through direct interaction, resulting in blockade of the CAP-dependent protein translation. RGS2 expression was elevated in tumors derived from patients with lung cancer compared with normal counterparts and significantly associated with poor clinical outcomes. These findings suggest that RGS2 is a novel biomarker responsible for chemoresistance by establishing tumor-promoting TME and can be a potential therapeutic target for the treatment of lung cancer with chemoresistance.


The purpose of this study is to identify mechanisms of resistance to gemcitabine in pancreatic ductal adenocarcinoma (PDAC) using an in vitro coculture system. The inherent resistance of PDAC to first line treatments such as gemcitabine has been attributed to the dense fibrotic stroma. Targeting stromal components, in particular cancer-associated fibroblasts (CAFs), the activated form of pancreatic stellate cells (PSCs), is a promising avenue for therapeutic intervention. We have demonstrated previously the induction of gemcitabine resistance when co-culturing KRAS G12D; p53 R172H; Pdx-cre (KPC) mouse PDAC-derived tumour cells with PSCs, in comparison to PDAC cells co-cultured with PSCs or monoculture. This resistance-inducing effect is specific to cells of mesenchymal phenotype, is transient in nature and a product of cell-cell contact. To investigate the exact mechanism by which PDAC cells become resistant to gemcitabine in coculture, we undertook a genome-wide shRNA depletion viability screen, infecting KPC mouse PDAC cells with a pooled shERWOOD UltramiR shRNA library both in mono- and co-culture with PSCs, in the presence and absence of gemcitabine. Next-generation sequencing and differential expression analysis, using DISEq2, identified shRNAs depleted through 6 cycles of gemcitabine treatment in coculture, indicating they target genes driving the resistance effect. Pathway analysis of differential expression using MetaCore implicated DNA damage repair and protein modification pathways in the gemcitabine resistance effect. Depletion of p97, ATR, or Chk1 all sensitised PDAC cells to gemcitabine in coculture, corroborating findings with translational value in previous studies.

#3191 Daytime blue-enriched LED light-induced circadian amplification of the nighttime melatonin signal increases sorafenib sensitivity in human hepatocellular carcinoma via enhanced suppression of the Warburg effect. Robert T. Dauchy, David T. Pointer, Aaron E. Hoffman, Melissa A. Wren-Dalal, Shulin Xiang, Lin Yuan, David E. Blask, Victoria P. Belancio, Steven M. Hill. Tulane Univ. School of Medicine, New Orleans, LA.

Over 36,000 people in the United States will be diagnosed with hepatocellular carcinoma (HCC) in 2016, the second leading cause of cancer death worldwide.
Metabolic pathways within the liver and in HCC are highly regulated by the central circadian clock in the suprachiasmatic nucleus (SCN). The SCN drives nighttime production of the circadian anti-cancer hormone melatonin by the pineal gland in rats and humans. We have shown that the nighttime circadian melatonin signal suppresses the Warburg effect (aerobic glycolysis) in human breast cancer (MCF-7) and that blue light at daytime (bLAD), amplifies the nighttime circadian melatonin signal by 7-fold in rats over cool white fluorescent (CWF) lighting. Here we tested whether daytime exposure of tissue-isolated HepG2 xenograft-bearing male nude rats to bLAD amplifies the nighttime melatonin signal to increase tumor sensitivity to nighttime administered sorafenib (FDA-approved multi-kinase inhibitor) treatment via enhancing suppression of the Warburg effect. Animals were randomized to 6 subgroups (n = 4): CWF Groups I, 12 h light:12 h dark (LD,12:12) (CWF + vehicle) and II (CWF + sorafenib); bLAD Groups III (LD,12bLAD:12) (bLAD + vehicle, IV (bLAD + sorafenib), V (bLAD + S20928 MT, receptor blocker), and VI (bLAD + S20928 + sorafenib). Drug treatments began when tumors were 2.5 g estimated weight. Plasma nighttime melatonin levels were 7-fold higher in Groups III-VI, compared to Groups I and II. Tumor latency-to-onset of growth and growth rates were markedly delayed and decreased, respectively, in Group III compared to Group I. Sorafenib induced tumor regression at a rate that was 2-fold higher in the bLAD- vs CWF-exposed rats. Tumor glucose uptake and lactate production (Warburg effect) at the mid-dark phase were significantly reduced in CWF-exposed rats receiving sorafenib vs vehicle, whereas the nighttime melatonin signal in bLAD-exposed rats significantly decreased vs CWF-exposed rats receiving vehicle. In bLAD + sorafenib-treated rats, the Warburg effect was reduced by an additional 51% (glucose uptake) and 89% (lactate production), respectively, vs CWF + sorafenib-treated rats. Melatonin receptor blocker S20928 completely prevented the effects of bLAD and bLAD + sorafenib on the Warburg effect and made these tumors completely resistant to sorafenib-induced tumor regression. These findings are the first to show in vivo that: 1) nighttime circadian sorafenib therapy inhibited the Warburg effect and induced HepG2 tumor regression under CWF lighting conditions, and 2) the bLAD-amplified nocturnal circadian melatonin signal increased tumor sensitivity to sorafenib-induced regression by enhancing a melatonin receptor-mediated suppression of the Warburg effect.


Purpose: IMMU-132 is an antibody-drug conjugate composed of a humanized anti-Trop-2 IgG conjugated via a cleavable linker to SN-38, a topoisomerase I inhibitor and active component of irinotecan. It is currently under clinical investigation in a range of solid tumors (NCT01631552). We investigated the hypothesis that IMMU-132, through its targeting of Trop-2 in solid tumors, will be superior to irinotecant in overcoming Rad51-mediated HRR repair of DNA breaks in TNBC tumors with high Trop-2 expression. Methods: Rad51 and DNA-breaks (γH2AX) were determined by Western blot. Cells with different Trop-2 levels were exposed to IMMU-132 for 24 h (25 - 100 nM SN-38 equivalent), including squamous cell lung carcinoma (SK-MES-1, −30,000 Trop-2/cell) and TNBC (HCC1806, −90,000 Trop-2/cell and MDA-MB-231, −30,000 Trop-2/cell). Also, two Trop-2-transfectants of MDA-MB-231, designated C13 and C39 (4- and 25-fold higher Trop-2 levels, respectively), were likewise exposed to IMMU-132. Mice bearing MDA-MB-231, C13, or C39 tumors were treated with irinotecan (MTD, 40 mg/kg, q2dx5) or IMMU-132 (0.5 mg; 9 μg SN-38 equivalent, twice wkly x 4). Tumors were measured and mice weighed twice weekly. Study survival endpoint was tumor progression to >1.0 cm. Results: SK-MES-1 and HCC1806 are sensitive to IMMU-132 therapy whereas MDA-MB-231 is resistant. IMMU-132 mediated a >2-fold increase in Rad51 levels in MDA-MB-231 cells, but had no effect in SK-MES-1 or HCC1806. At 25 nM IMMU-132, there were lower levels of DNA breaks detected in MDA-MB-231 relative to SK-MES-1 and HCC1806 (2-fold increase in MDA-MB-231 vs >3-fold). At higher concentrations of IMMU-132 (100 nM), all 3 cell lines demonstrated similar levels of DNA breaks (~5-fold above background), suggesting that higher levels of SN-38 can overcome Rad51-mediated repair. Both the C13 and C39 clones had a similar response as parental MDA-MB-231 upon IMMU-132 exposure. Mice bearing MDA-MB-231, C13, or C39 tumors treated with irinotecan demonstrated significant tumor growth delay compared to saline (P < 0.0009). As expected, IMMU-132 was no different than saline in mice bearing MDA-MB-231 tumors (MST = 21d and 19.5d, respectively). However, in mice bearing high Trop-2 C13 and C39 tumors, IMMU-132 provided a significant survival benefit compared to irinotecan-treated mice (MST > 70d vs. 35d, respectively for C13 and >70d vs. 28d for C39; P < 0.0007), supporting the hypothesis that IMMU-132 is able to deliver more SN-38 to tumors with high Trop-2 than can be achieved by irinotecan, and can thus overcome Rad51-mediated HRR. Conclusion: IMMU-132, with its unique SN-38-delivery platform, has the potential to provide clinical benefit both to chemosensitive solid tumors with low Trop-2 expression, as well as to chemoresistant tumors with high Trop-2 expression.

### #3194 A new mechanism of drug resistance in cancer: extracellular ATP-induced resistance through ATP internalization and upregulation of protein phosphorylation in Akt and ERK pathways. Xuan Wang, Yunsheng Li, Yanrong Qian, Yanyang Cao, Xiaohou Zhou, Chuangxia Chen, Ohio University, Athens, OH.

The opportunistic uptake of extracellular molecules has been named as a key emerging hallmark of cancer metabolism.[41] Extracellular ATP (eATP) levels of various cancer types are 10^6 to 10^7 times higher than those in their corresponding normal tissues[24]. However, the biological significance of the high ATP concentrations is not clear. We recently reported that cancer cells uptake extracellular ATP via different endocytoses to enhance growth, survival, and drug resistance to tyrosine kinase inhibitors (TKIs).[24] We hypothesized that eATP induces resistance to TKIs by endocytoses-mediated internalization of eATP, which competes with TKIs for the ATP-binding site of receptor TKs (RTKs), phosphorylating and activating RTKs and RTK-mediated signaling pathways. In contrast to previous reports that synthesized intracellular ATP (iATP) elevation contributed acquired drug resistance, here we report a novel intrinsic drug resistance in which eATP was internalized and substantially increased iATP levels in resistant cancer cells relative to their parental human NSCLC A549 cells. The iATP level elevation and drug resistance were mediated primarily by macropinocytosis. The resistance was reduced when macropinocytosis was suppressed by inhibitors or an siRNA knockdown of a key micropinocytosis protein PAK1. Intracellularly, the elevated iATP upregulated phosphorylation of PDGFR and proteins/enzymes in the PDGFR-mediated Akt-mTOR and Raf-ERK signaling pathways, resulting in reduced apoptosis triggered by sunitinib. Furthermore, both in vitro and in A549 tumors, eATP partially restored phosphorylation levels of PDGFR and PDGFR-mediated proteins/enzymes suppressed by sunitinib. The resistance cannot be accounted for by the overall purinergic receptor-mediated signaling, glycolysis, or mitochondrial OXPHOS. These results strongly suggest that the eATP-elevated intracellular ATP levels reversed the inhibition of TKIs by using the mechanism we hypothesized, linking for the first time the ATP-rich tumor microenvironment with cancer drug resistance. All these findings significantly expand our understanding of the roles of extracellular ATP in cancer, and offer new anti-drug resistance targets. 1. Pavlova N, Thompson C. Cell Metab. (2016) 23:27-47. 2. Falzoni et al., Interface Focus. (2013) 3: 20120101. 3. Qian Y, Wang X et al., Cancer Lett. (2014)351: 242-5. 4. Qian Y, Wang X et al., Mol Cancer Res (2014) 14:1087-96.

### #3195 Dyrk1b inhibitors prevent pharmacologic quiescence and sensitize lung cancers to Egfr inhibitors. Maria Vilenchik,1 Alexandra Kuznetsova,1 Michael Frid,2 Yury Ginkin,1 Marc Duer,2 Neal Rosen,2 Felicite Therapeutics Inc., Newton, MA; 2Memorial Sloan Kettering Cancer Center, New York, NY.

Many oncoproteins that activate signaling dysregulate cell growth by activating the cyclinD/cdk4, cdk6 complex and abrogate in the G1 checkpoint (mutant RAS, BRAF, PIK3CA, EGFR etc.) Inhibition of these oncoproteins inhibits cell cycle entry and promotes cell death. Dyrk1b kinase plays an important role in physiologic quiescence by maintaining the viability of cells arrested in the G0/G1 phases of the cell cycle. Dyrk1b regulates the G0/G1 transition by phosphorylating cyclin D1 and p27, whereby destabilizing cyclin D1 and stabilizing p27. The balance between these two proteins is essential for maintenance of cells in G0 (quiescent) state. Dyrk1b is sparsely expressed in healthy tissues yet is overexpressed in patient samples with variety of neoplasms, including NSCLC and cancer and normal cells exhibit differential sensitivity to Dyrk1b inhibition. Whereas Dyrk1b appears essential for survival of cancer cells either endogenously expressed or upregulated, its inhibition in healthy cells was not cytotoxic and had no effect on the cell cycle distribution. We hypothesized that if Dyrk1b is required for maintenance of quiescence, its inhibition would enhance the effects of an insignificant imipogenic signaling. We used a selective, ATP competitive Dyrk1b inhibitor to test this idea. EGFR inhibitors are effective in non-small cell lung cancers with mutant EGFR and induce G0/G1 block and some apoptosis. These drugs significantly extend survival, but acquired resistance almost always supervenes. In vitro, the anti-proliferative response of lung tumors
with mutant EGFR to EGFR inhibitors was shown to be primarily due to the entry of cells into a quiescent (G0) state associated with an induction of DRYK1B expression. Moreover, addition of a DRYK1B inhibitor reversed the quiescent state in cells exposed to EGFR inhibitors. Combined therapy of EGFR TKIs and the DRYK1B inhibitor significantly enhanced the antitumor response compared to either drug alone. Importantly, it is worth noting that treated results in an increase in cells in G0 state: 63% as compared to non-treated cells, 29%. The induction of quiescence is prevented when cells are treated with the combination of osimertinib and DRYK1B inhibitor and massive apoptosis is observed: 62% compared to 12% with osimertinib alone. It is important to note that co-treatment with DRYK1B inhibitor not only prevented the entry of cancer cells into quiescence but also depleted pre-existent reservoir of quiescent cancer cells. The results suggest that inhibition of EGFR causes tumor cells to enter into a DRYK1B-dependent quiescent state in which DRYK1B is required for their survival. Inhibition of DRYK1B dramatically enhances the antitumor activity of EGFR inhibition and may improve outcome in patients.

#3196 Genetic and pharmacologic approaches to overcome epithelial to mesenchymal mediated chemoresistance in breast cancer. Michael J. Crowley, Nasser Ahorki, Vivek Mittal, Dingcheng Gao. Weill Cornell Medical College, New York, NY.

Development of resistance to conventional chemotherapy remains a major barrier to effective treatment of breast cancer. We and others have recently demonstrated in breast and pancreatic cancer models that epithelial-mesenchymal transition (EMT) may not be a critical mediator of cancer metastasis, however, it contributes to cancer drug resistance (Fischer et al. Nature 2015; Zeng et al Nature 2015). Importantly, blocking EMT through miR-200 family abrogated chemoresistance, indicating potentials for clinical translation. Using our novel EMT lineage tracing system, we have performed both genetic (CRISPR/Cas9 mediated genome-wide targeted mutagenesis) and pharmacological (high-throughput small molecule libraries) screens, to identify potential candidates to overcome EMT-mediated chemoresistance in breast cancer metastasis. The identified molecules provide not only novel mechanistic insights but also attractive anti-metastatic strategies for breast cancer treatment and the design of future clinical trials.

#3197 Splicing factors and the role of Navitoclax in drug-resistant ovarian cancer. Helen M. Coley, Laura Lattanzio, Ornella Garonne, Marco Merlano, Daniela Vivenza, Cristiana Lo Nigro, Nelofer Syed, Alistair Thompson, Helen M. Coley, Laura Lattanzio, Ornella Garonne, Marco Merlano, Daniela Vivenza, Cristiana Lo Nigro, Nelofer Syed, Alistair Thompson, 1Univ. of Surrey, Guildford Surrey, United Kingdom; 2Ospedale Carle, Cuneso, Italy; 3Imperial College, London, United Kingdom; 4Dundee University, Dundee, United Kingdom; 5Southend Hospital, Southend, United Kingdom.

Alternative RNA splicing allows processing of a pre-mRNA into various mature RNAs. In this way multiple protein products can be derived from a single gene. In cancer it is known that specific splice variants may facilitate malignant transformation and therapeutic resistance. Our current studies focus on the problem of drug resistance in ovarian cancer and we have approached this in a variety of ways. Following sequencing analysis of a panel of ovarian cancer cell lines we identified the RNA splicing factors TCERG and SRSF2 as being reduced in drug resistant ovarian cancer cell lines, compared with their drug sensitive counterpart. TCERG is important in regulating the splicing of variant forms of the BCLx gene: BCLxL (long form) and BCLxS (short form). There are clear implications for drug resistance whereby low TCERG results in reduced BCLx splicing to yield the pro-apoptotic BCLxS form. In the SKOV-3 human ovarian cancer cell line panel both paclitaxel resistant SKOV-3TaxR and SKOV-3CR carboplatin resistant cells showed reduced levels of TCERG mRNA using qPCR analysis. In addition, qPCR analysis for BCLxL and BCLxS levels indicated increased ratio of long form with drug resistance: SKOV-3 parent line ratio 1.0, SKOV-3TaxR 1.8, SKOV-3CR 1.65. Western immunoblotting showed relatively decreased levels of Bax protein in drug resistant versus drug sensitive cell line in line with a reduced apoptotic restored. We then explored the use of Navitoclax (ABT2026), the small-molecule Bcl-2 family protein inhibitor for Bcl-xL, Bcl-2 and Bcl-w in our cell line panel. Navitoclax in combination with paclitaxel for 48h gave rise to significantly increased apoptotic response in drug-resistant versus drug sensitive ovarian cancer cell line counterparts, using the Annexin V assay with PI, indicative of re-sensitisation to paclitaxel. Synergistic effects were also seen for the parental cell line but the effects were more marked for the resistant cell lines. Our future work will also involve the use of clinical biopsies of ovarian cancer cases.

#3198 Using a genome-wide CRISPR-Cas9 knockout library to identify therapeutic combinations in oral cancer. Megan Ludwig, Andrew Birkeland, Sai Nimmagadda, Sue Foltin, Aditi Kulkarni, Hui Jiang, Thomas Carey, Chad Brenner. University of Michigan, Ann Arbor, MI.

Oral squamous cell carcinoma (OSCC) has remained a disease with poor survival outcomes. Novel therapeutic approaches are much needed, in particular for patients with metastatic disease. The choice of therapeutic strategies is guided by the genetic landscape of the tumor. We aim to develop a pipeline of therapeutic strategies for this patient population by generating individual genetic knockouts through targeted gene editing. We used a pool of CRISPRs targeting over 18,000 genes to perform genome-scale screening for drivers of sensitivity to EGFR-targeted therapy. Upon selection of the OSCC GeCKO pool, we identified gene knockouts in the FGFR pathway that increased sensitivity to the EGFR inhibitor gefitinib. Using resazurin viability assays we tested combinations of EGFR and FGFR inhibitors in 14 OSCC cell lines. Six/twelve (43%) of the cell lines were responsive to the combination, indicating that the FGFR pathway is an alternate mechanism of resistance to EGFR-targeted therapy in some tumors. In complement, we again used CRISPR-Cas9 to generate a syngeneic EGFR knockout (KO) OSCC cell line. The parent OSCC cell line is responsive to the EGFR and FGFR dual inhibition, and the EGFR KO derivative remains sensitive to FGFR inhibition. We expect that targeted monotherapies. We anticipate great potential for personalized targeted therapies, facilitated by recent characterizations of the mutational landscape of OSCCs. Combinations of targeted therapies may have greater efficacy through inhibiting compensatory pathways. For example, targeting EGFR has had limited success. To identify synergistic combinations with EGFR-targeted therapy, we introduced Genome-scale CRISPR-Cas9 Knockout (KO) libraries into OSCC cell lines. CRISPR-Cas9 generates individual genetic knockouts, and massive apoptosis is observed:62% compared to 12% with osimertinib alone. It is important to note that co-treatment with DRYK1B inhibitor not only prevented the entry of cancer cells into quiescence but also depleted pre-existent reservoir of quiescent cancer cells. The results suggest that inhibition of EGFR causes tumor cells to enter into a DRYK1B-dependent quiescent state in which DRYK1B is required for their survival. Inhibition of DRYK1B dramatically enhances the antitumor activity of EGFR inhibition and may improve outcome in patients.
to crenolinib. Our data suggest that comprehensive sequencing should be carried out on patient samples prior to treatment to identify and pre-emptively target problematic clones. In addition, even with high VAF FLT3 mutations, although FLT3 inhibitor monotherapy provides some clinical benefit, combining agents targeting cooperative lesions will be imperative to eradicate both the dominant clone and resistant subclones and improve patient responses.

**EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Novel Molecular Targets 2**


1Freud Huntington Cancer Research Center, Seattle, WA; 2National Institutes of Health, Bethesda, MD.

Glioblastoma multiforme (GBM) is one of the most aggressive and invasive types of brain cancer, but targeted treatment options remain elusive. The standard of care (surgery chemoradiation and therapy) falls short of where it should be with two-year survival rates less than 10%. Using stem cell isolates from GBM patients, we found that perturbing PHF5A, a component of the spliceosome machinery, was lethal and caused hundreds of genes to be mis-spliced. These mis-splicing events included both exon skipping and intron inclusions. In contrast, similar levels of PHF5A suppression in normal control stem cells and astrocytes failed to induce cell death and mis-splicing indicating that PHF5A plays a specific role in the cancer biology. Moreover, when normal astrocytes were transformed with the Myc oncogene, they became sensitive to PHF5A perturbation. Taken together, these results suggested that specifically inhibiting PHF5A would be an effective therapy for glioblastoma and other Myc-driven cancers. Specifically targeting PHF5A would also likely result in reduced side-effects seen with general spliceosome inhibitors. Unfortunately, there are currently no known inhibitors that target PHF5A. In order to discovery novel PHF5A inhibitors, we created a mini-gene mis-splicing reporter assay that was sensitive to both general spliceosome inhibitors and PHF5A perturbation. In a 96-well assay format, the assay was robust with a 200-fold assay window and Z* values over 0.8. Following miniaturization to a 1536-well format, we conducted a high throughput screening (HTS) campaign testing 85,000 small molecule compounds. The initial hits were retested and counter-screened yielding 381 confirmed actives and we are further interrogating these actives in secondary and tertiary assays. Future efforts will focus on developing an SAR of the lead and backup series and identifying potential liabilities that will be addressed, if necessary, in further lead optimization efforts. We are enthusiastic about the potential of developing a targeted PHF5A inhibitor as a novel and effective therapy for patients and their families fighting GBM and other Myc-driven cancers.


1Fred Hutchison Cancer Research Center, Seattle, WA; 2National Institutes of Health, Bethesda, MD.

Hepatocellular carcinoma (HCC) related mortality ranks second worldwide and stands one step behind lung cancer. Due to the asymptomatic nature of the cancer, early diagnosis is a major problem. Currently only liver transplantation is curative for early stage hepatocellular cancer. There are other multidisciplinary treatment options like radiation and chemotherapy available for advanced patients. However, chemotherapy resistance is a common problem in the treatment of liver cancer. Newer therapeutics are needed for better management of advanced HCC. Angiogenesis and lymphangiogenesis are processes that are vital for tumorigenesis and metastasis. HCC is known to be a hypervascular tumor and many pro-angiogenic proteins are found significantly overexpressed in HCC. We explored the therapeutic potential of the anti-angiogenic and anti-lymphangiogenic, biomimetic peptide SP2043 developed by our group in HCC. Hepatocellular carcinoma cell lines HuH-7, Hep3b and HepG2 showed significant disruption of cell adhesion and migration following treatment. Furthermore, SP2043 was found to impair microvascular endothelial cell (MEC) tube formation induced by HepG2 tumor conditioned media and to significantly inhibit HepG2 tumor xenograft growth compared to untreated controls. The peptide drug was also found to improve the survival of autochthonous Myc induced HCC in a transgenic mouse model from two weeks to four weeks. We discovered the peptide to be a multi-modal anti-angiogenic drug that also inhibits its IGFR1 and MET signaling pathways in HCC cell lines. We also found that SP2043 treatment reduced the microvascular density in both subcutaneous and autochthonous liver tumors with reduced tumor cell proliferation and increased apoptosis. This study shows the therapeutic potential of SP2043 in the treatment of hepatocellular carcinoma.


Emerging evidence suggests that Sigma1 (also known as sigma1 receptor) is a unique ligand-operated integral membrane chaperone or scaffold protein that contributes to cellular protein homeostasis. Previously, we found that treatment of various cancer cell lines with some prototypic small molecule modulators of Sigma1 can engage endoplasmic reticulum (ER) associated protein homeostasis pathways including the unfolded protein response and autophagy. Programmed death-ligand 1 (PD-L1) is a type 1 integral membrane glycoprotein that is processed and transported through the ER and secretory pathway of tumor cells. PD-L1 expressed at the surface of tumor cells can act as a ‘T-cell inhibitory checkpoint’ molecule that inactivates tumor infiltrating immune cells that express PD-1, its cognate receptor. Here, we show that Sigma1 physically associates with PD-L1. In triple negative breast and androgen-independent prostate cancer cells, PD-L1 protein levels are suppressed by both DNAmediated knockdown of Sigma1 and pharmacological modulation of Sigma1. We observe decreased cell surface and intracellular levels of PD-L1 by flow cytometry and biochemical subcellular fractionation respectively, indicating that PD-L1 is degradation of nascent PD-L1 after Sigma1 modulation plays a key role in preventing the transport of functional PD-L1 to the plasma membrane. Together, these data demonstrate that Sigma1 modulators have the potential to act as novel therapeutic agents in PD-L1 blockade strategies.

### #3203 Targeting tumor hypoxia with prodrug conjugates of potent small-molecule inhibitors of tubulin polymerization. Kevin G. Pinney, Mary Lynn Trawick, Ralph P. Mason, Li Liu, David J. Chaplin, Blake A. Winn, Laxman Devkota, Tracy E. Streeker, Jeni Gerberich, Alex Winters, Yifan Wang, Matthew T. MacDonough.

1 Baylor University, Waco, TX; 2University of Texas Southwestern Medical Center, Dallas, TX; 3Mateon Therapeutics, Inc., South San Francisco, CA.

Targeted pronounced hypoxia in malignant tumors represent an opportunity for selectivity in drug activation, and hence targeting and delivery. In this study, a series of bioreductively activatable prodrug conjugates (BAPCs) of small-molecule inhibitors of tubulin polymerization were synthesized and evaluated in biochemical and biological studies. The BAPCs are designed to be biologically inert until selectively cleaved under hypoxic conditions, release potent enzyme modulators that are tumor specific and target cancer cell populations. BAPCs were evaluated in the following assays: (1) cytotoxicity using the regular sulforhodamine B assay (normoxic conditions) to confirm their reduced cytotoxicity in cancer cells in culture compared to the effector agents; (2) cleavage under anoxic conditions by the reductase enzyme, NADPH cytochrome P450 oxidoreductase (POR), that is implicated in the bioreductive cleavage of compounds with nitroimidazole and nitrimidazole triggers; and (3) differential cytotoxicity under hypoxic versus normoxic conditions in cancer cell lines with the established bioreductive compound tirapazamine (TPZ) as the control. The POR assay was modified by the addition of protocatechuic 3.4-dioxygenase to ensure anoxic conditions, and Triton X-100 to facilitate solubilization of the BAPCs. A series of unsubstituted, methyl, and gem-dimethyl nitroimidazole- and nitrimidazole-triggered prodrug conjugates (BAPCs) of two experimental antitumor agents (KGP03 and KGP18) were synthesized. KGP03 and KGP18 were inspired by the natural product combretastatin A-4 (CA4). A unique attribute of the selected anticancer agents is that both bind tubulin at the colchicine site, inhibit tubulin polymerization into microtubules, and function as ‘dynamic destabilizing’ agents that modify the cytoskeleton of cancer cell lines and as vascular disrupting agents (VDAs). The assays were validated with a series of nitroimidazole analogues of CA4 (Thomson et al., Mol. Cancer Ther. 2006 5:2886). In our evaluation of this series, the gem-dimethyl nitroimidazole analogue of CA4 was efficiently cleaved by POR and gave an
average differential hyposia cytotoxicity ratio (HCR: GLo values of nor-
monic/hypoxic conditions) of 41 in the human A549 lung carcinoma cell.
The corresponding prodrug of combretastatin A-1 had an HCR = 26.
The monomethyl nitroimidazole BAPCs of KGp03 and KGp18 produced
positive HCRs in our initial assays. In preliminary studies, the C4A BAPC
demonstrated activity in both hypoxic synchronous and asynchronous
mouse model (4T1/BALB/c). In the POR assay, the gem-dimethyl nitrobio-
phane analogue of KGp03 was completely cleaved in 24 h. Biological evalua-
tion of these BAPCs indicates the occurrence of selective, hypoxic activa-
tion resulting in release of the potent inhibitors of tubulin polymerization
(C4A, C41, KGp03, KGp18).

#3204 Tryptophan 318/324, the target of C-terminal binding protein
(CtBP) inhibitors, plays a critical role in CtBP enzymatic activity, oligomer-
ization and transcriptional coregulation. Martin M. Dcona,1 Benjamin L.
Morris,3 Priyadarshan K. Danile,4 Zaid Nawaz,1 Michael J. Dennis,1 Sahib J.
Singh,1 William E. Royer,2 Keith C. Ellis,1 Steven R. Grossman,1 Virginia Com-
monwealth University, Richmond, VA; 2University of Massachusetts, Worcester,
MA.
C-terminal Binding Proteins (CtBP) 1 and 2 constitute a family of oncogenic
transcriptional co-regulators overexpressed in tumor tissues that are associated
with worse prognostic outcome and aggressive tumor characteristics in multiple
cancer types. Specifically, CtBP has been found to repress expression of genes
responsible for apoptosis and EMT (eg. BIK and CDH1) and promote expres-
sion of genes that partake in the migration of cancer cells and those that are
responsible for enhanced drug resistance (eg. TIA1 and MDR1). CtBP2 is also
frequently overexpressed in breast and colon cancer cell lines with concomitant knock-
out (siRNA) or knockout (CRISPR/Cas9) of CtBP2. Our data demonstrates
that mutation of W324 abrogated dehydrogenase activity, oligomerization,
and transcription of the validated CtBP target gene TIAM1 and induction of mi-
RNA ES 

#3205 A nanomolar potency small-molecule compound against castra-
tion-resistant and bone metastatic prostate cancer. Kenza Mamouni,1 Lajos
Gera,2 Xin Li,1 Daqing Wu,1 Augusta University, Augusta, GA; 1University of
Colorado Denver, Aurora, CO.
A standard treatment for prostate cancer (PCa) is androgen deprivation ther-
apy (ADT) that suppresses androgen receptor (AR) signaling axis. Although
initially responsive, most patients receiving ADT eventually develop metastatic
castration-resistant prostate cancer (CRPC), with more than 90% of them ex-
hibiting bone metastases. A mechanism by which CRPC cells evade ADT is the
expression of constitutively active AR variants (AR-Vs), such as the well-char-
acterized AR-V7. Recently we developed GH501, a bupiruvon-modified small-
molecule compound, and investigated its anti-cancer activity and mechanism
of action in pre-clinical models of CRPC. At nanomolar range, GH501 effectively
induces cell cycle arrest and apoptosis in CRPC cells regardless of their resis-
tance status to enzalutamide treatment. RNA-seq analysis of GH501 combined
with Western blotting analysis identified key targets implicated in CRPC pro-
gression, including the full-length AR, AR-V7 variant, and other important
genes implicated in CRPC progression. Importantly, low doses of GH501 effec-
tively inhibit the skeletal growth of CRPC in a xenograft model without obvious
in vivo toxicities. These preclinical results indicate that GH501 is a promising
small-molecule compound that can be further developed for the treatment of
lethal prostate cancer.

#3206 A potent and selective CSF-1R inhibitor, DCR-0064, inhibits col-
ony stimulating factor 1 signaling in vitro and in vivo. Chu-Bin Liao, Shao-
Zheng Peng, Chen-Huan Ho, Chao-Pin Lee, Jia-Ming Chang, Hung-Yun Huang, Yuan-Yi Gou, Yu-Chih Pan, Yu-Kai Chen. Development Ctr. for Biotech-
nology, Xixi City, Taiwan.
Tumor-associated macrophages (TAMs) are major components of leukocytic
infiltrate of tumors. It has been reported that high TAMs density is associated
with poor clinical prognosis. Within the tumor microenvironment, TAMs rely
on signaling through CSF-1/CSF-1 Receptor kinase axis to promote tumor
growth, angiogenesis, and metastasis. Therefore, decrease of TAMs density in
tumors through inhibition of CSF-1R kinase is considered a potential target for
drug development. In this study, we report that a novel small molecule DCR-
0064 inhibits CSF-1R kinase with IC50 value below 10 nM and possesses speci-
cificity for CSF-1R over other tyrosine or serine/threonine kinases. DCR-0064
selectively inhibits CSF-1 induced growth of M-NFS-60 myeloid cells and effec-
tively suppresses intracellular CSF-1R activation with IC50 value less than 100
pM. The selectivity of DCR-0064 is superior to the currently lead
CSF-1R inhibitor, PLX3397, which is in phase III clinical development for treat-
ing senescentional giant cell tumor. The selectively growth inhibitory activity of
DCR-0064 is consistent with inhibition of CSF-1R signaling. Oral administra-
tion of DCR-0064 reduces TAMs density in vivo in mice. DCR-0064 is being
developed as a candidate for preclinical development.

#3207 Targetting proteasome function by inhibition of the proteasome
debiquitinase USP14. Stig T. Linder,1 Padraig D’Arcy,2 Xiaowan Zhang,1
1Karolinska Inst. Cancer Center, Stockholm, Sweden; 2Linköping University,
Linköping, Sweden.
The 20S proteasome core particle has evolved as an important target for anti-
cancer drug development. We previously identified the small molecule b-AP15
as a novel class of proteasome inhibitors that function by abrogating the deu-
biquitinating (DUB) activity of the 19S regulatory particle. An optimised lead of
b-AP15 (VLX1570) has been approved by the FDA for clinical studies and is
currently in clinical trials for relapsed multiple myeloma. VLX1570 has also
shown promising activity in Ewings sarcoma and paediatric leukemias. b-AP15
and VLX1570 bind to the proteasome DUB ubiquitin-specific protease-14
(USP14) in vitro and in exposed cells (CETSA). Binding and inhibition of enzy-
matic activity is believed to be due to targeting Cys114 in the active site. The
cell death response to b-AP15/VLX1570 is distinct from that of 20S proteasome
inhibitors. Thus, the sensitivity to these compounds is unaffected by overexpres-
sion of BCL2-family proteins and unaffected by p53 status. We here show that
b-AP15 induces direct effects on mitochondria, possible explaining this phe-
nomenon. We also provide additional understanding of drug-target interactions
by using a series of compounds identified by screening. Our findings demonstrate
promising antiproliferative activities of USP14 inhibitors in vitro and in vivo.

#3208 AZD1775 modulates kinetochore component Wee1-CDC2 and
NF-kB signaling as potential therapeutic targets in head and neck squamous
adenocarcinoma. Zhengbo Hu,1 Anthony Salich,2 Jianghong Chen,2 Carlson So-
Bactivity. Among these targets, WEE1 kinase is currently
under investigation as a therapeutic target of inhibitor AZD1775 in HNSCC. We
found that WEE1-CDC2 axis protein levels were higher in a panel of HNSCC
genesis implicated in CRPC progression. Importantly, low doses of GH501 effec-
tively inhibit the skeletal growth of CRPC in a xenograft model without obvious
in vivo toxicities. These preclinical results indicate that GH501 is a promising
small-molecule compound that can be further developed for the treatment of
lethal prostate cancer.

Recent findings in The Cancer Genome Atlas (TCGA) analysis of head and
neck squamous cell carcinomas (HNSCC) revealed frequent alterations affect-
ing the cell cycle and NF-κB pathway, which are critically involved in cancer cell
proliferation and survival. We hypothesized that common mechanism(s) could
coordinate cell cycle and NF-κB cell survival signaling in HNSCC. Recently, we
established an NF-κB B-lactamase reporter UM-SCC-1 cell line and performed
gene-wide RNAi screening to explore this hypothesis. We identified genes
involved in the cell cycle and related to components of the kinetochore that
co-modulate NF-κB activity. Among these targets, WEE1 kinase is currently
under investigation as a therapeutic target of inhibitor AZD1775 in HNSCC. We
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cells with AZD1775 decreased NF-κB
nology, Xixi City, Taiwan.
Tumor-associated macrophages (TAMs) are major components of leukocytic
infiltrate of tumors. It has been reported that high TAMs density is associated
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on signaling through CSF-1/CSF-1 Receptor kinase axis to promote tumor
growth, angiogenesis, and metastasis. Therefore, decrease of TAMs density in
tumors through inhibition of CSF-1R kinase is considered a potential target for
drug development. In this study, we report that a novel small molecule DCR-
0064 inhibits CSF-1R kinase with IC50 value below 10 nM and possesses speci-
cificity for CSF-1R over other tyrosine or serine/threonine kinases. DCR-0064
selectively inhibits CSF-1 induced growth of M-NFS-60 myeloid cells and effec-
tively suppresses intracellular CSF-1R activation with IC50 value less than 100
pM. The potency and selectivity of DCR-0064 is superior to the current lead
CSF-1R inhibitor, PLX3397, which is in phase III clinical development for treat-
ing senescentional giant cell tumor. The selectively growth inhibitory activity of
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cells with AZD1775 decreased NF-κB

transcriptional activation and decreased TNFα-induced phosphorylation of IKKs and RELA subunit. Furthermore, AZD1775 inhibited NF-κB regulated proteins modulating the cell cycle and survival, including p21, CyclinD1, BCL2 and BCL-XL. These changes also correlate with alteration in cell cycle and survival detected by flow cytometry, as tumor cells treated by AZD1775 exhibited increased G2/M phase and sub-G0 fragmented DNA, and decrease in G1/S phases. Wee1 inhibitor AZD1775 inhibited tumor cell proliferation and colony formation in a dose-dependent manner, and exhibited combinatory effects with cisplatin in tumor xenografts. Thus, we identified the novel cross-talk between Wee1-CDC2 and NF-κB signaling, potentially implicating decreased NF-κB prosurvival signaling in the anti-tumor activity of Wee1 inhibitors. Supported by NIDCD/NIH intramural projects ZIA DC-00016, 73, and 74; NIDCD core facility: advanced imaging. ZIC DC000011.


No target therapies are presently available in the treatment of small-cell lung cancer of Lung (SCLC). Therefore there is a need to develop new therapeutic agents. The protein kinases are a family of genes that play critical roles in various signaling pathways. Some cancer cells show addiction to constitutive activation of certain signaling pathways for proliferation and survival. To identify new drug targets for SCLC, we screened a panel of small interfering RNAs (siRNAs) that target 720 genes encoding human protein kinases and related proteins using SBC5 SCLC cell (SN38 (irinotecan hydrochloride) resistant cell). PLK1 inhibition suppressed cell proliferation strongest among 20 significant promising total genes using different 5 SCLC cells as a validation study). PLK1 mRNA expression was significantly higher than other pathological phenotypes among 20 cell-lines and the 200 clinical samples consist of three independent cohorts. The patients with high PLK1 expression was significantly associated with poor prognosis in SCLC patients. Furthermore we investigated the change of SN3 38 sensitivity and preventing from SN38 resistance after knockdown PLK1. Our results indicated that PLK1 inhibitor as BI 2536 and Volasertib, is a promising molecular target therapy for pharmacologic intervention in SCLC in both monotherapy and the combination therapy with SN38.

#3210 Targeting PTPA3 phosphatase in ovarian cancer with the potent noncompetitive inhibitor JMS-631-053. John S. Lazo, Paula Pekic, Alex Cheung.1 Kelley McQueeney,1 Joseph Salamoun,2 Peter Wipf,2 Charles N. Landen1. ProceedingsoftheAmericanAssociationforCancerResearch

PTPA3 is a highly attractive molecular target for ovarian cancer (OvCa). Elevated levels of PTPA3 mRNA and protein in human ovarian tumors correlate with disease progression, poor prognosis and poor survival. Genetic depletion of PTPA3 in OvCa cell lines diminishes their ability to migrate and reduces their in vivo tumorigenicity while PTPA3 overexpression increases tumor cell migration, invasion, and dissemination. Together, these data suggest PTPA3 is a novel molecular target for OvCa; however, the lack of potent and selective PTPA3 small molecule inhibitors has hindered PTPA3’s definitive validation in OvCa and other BRCA-mutant cancers. To drive the pharmacological validation of PTPA3, we developed JMS-631-053 ([7-imino-2-phenylthieno[3,2-c]pyridine-4,6(5H,7H)-dione], a potent (Kd=3 mM) in vitro, specific, noncompetitive, PTPA3 inhibitor. JMS-631-053 inhibited cellular migration, invasion, and colony formation in soft agar. Potent OvCa cell-based effects were observed by profiling JMS-631-053 against a panel of 8 OvCa cell lines using a 2D drug susceptibility assay, with EC50 values as low as 600 nM. Likewise, JMS-631-053 killed OvCa 3D spheroids, including those derived from high grade serious OvCa cell lines, with EC50 values as low as 300 nM. The OvCa drug resistant cell lines (i.e., A2780CP20 and HeyA8MDR) retained responsiveness to JMS-631-053 (using 2D and 3D culturing conditions) suggesting that inhibition of PTPA3 may be a viable therapeutic strategy for chemoresistant OvCa. Preliminary data also shows that JMS-631-053 synergizes with cisplatin when used in combination studies. Hence, JMS-631-053 will be a valuable chemical tool for further validation of PTPA3 as an OvCa target as well as provide potential leads for future drug discovery.

#3211 Inhibition of autophagy potentiates cytotoxicity of CX-5461 treatment in chemoresistant epithelial ovarian cancer. Robert Cornelison,1 Daniele C. Llaneza,1 Yulia Petrova,1 Zachary C. Dobbin,2 David A. Schneider,2 Charles N. Landen1. University of Virginia School of Medicine, Charlottesville, VA; University of Alabama at Birmingham, Birmingham, AL.

Epithelial ovarian cancer remains a deadly diagnosis with poor prognosis and patients succumbing to chemoresistant disease in 80% of cases. Mechanisms of chemoresistance are numerous, but we have identified ribosomal biogenesis basal machinery as being a common upregulated signaling network in response to chemotherapeutic insult. Tumors treated with chemotherapy showed dramatic increases in size and morphology of nucleoli, suggesting global changes in ribosomal synthesis with stress. We next sought to use the RNA Polymerase I inhibitor CX-5461 to targeting ribosomal machinery that may allow re sensitization to chemotherapy. CX-5461 induced autophagy, senescence and mitotic catastrophe in ovarian cancer cell lines Overall, 13 ovarian cancer lines examined were highly sensitive to Pol I inhibition by MTT, with IC50s ranging from 25nM to 2μM, and chemoresistant lines were generally more sensitive to CX-5461. In 53 mutant cells CX-5461 appears to be particularly cytotoxic being a mitotic catastrophe accompanied by companying cytotoxicity in vitro. Chloroquine administration appears to push cell to mitotic catastrophe after co-administration with CX-5461, thereby enhancing cytotoxicity over cytosstatic endpoints after treatment, even in multi-drug resistant cell populations. Inhibition of autophagy appears to enhance efficacy of RNA Polymerase I inhibition in both sensitive and chemoresistant populations, prompting a potential new approach to chemoresistant disease.

#3212 ONC201 shows efficacy in BRCA-deficient cancer cells and synergy with PARP inhibitors in glioblastoma, breast, prostate, and ovarian cancers. Marie D. Baumeister,1 Ozan C. Kucukkase,2 Varun V. Prabhu,3 David T. Dicker,4 Josh E. Allen,5 Wafik S. El-Deiry1. Proceedings of the American Association for Cancer Research

ONC201/TIC10 is a first-in-class small molecule inducer of TRAIL that causes early activation of the integrated stress response and inactivates both Akt and GSK3β, making tumors lacking germline BRCA mutations more sensitive to ONC201. We have previously shown that ONC201 has anti-proliferative and pro-apoptotic effects in a wide range of tumor types including BRCA-deficient breast and ovarian cancers (n = 10), with GSK3β values in the low micromolar range. Treatment with ONC201 induces surface TRAIL and inhibits Akt activity in BRCA deficient breast and ovarian cancers. PARP inhibitors have been previously shown to upregulate DR5 through transcription factor CHOP, sensitizing solid tumors to TRAIL. Resistance to PARP inhibitors can occur through PI3K/Akt pathway activation, and PI3K/MEK blockade improves their anti-tumor effects. We observed synergy between ONC201 and PARP inhibitors olaparib andrucaparib in BRCA-deficient breast and ovarian cancer cell lines in cell viability assays with combination indices (CI) ranging from 0.4 - 0.8. Robust synergy was also observed in prostate cancer cells. The mechanisms of the observed synergy are currently under investigation. These results indicate that ONC201 possesses single agent activity in BRCA-deficient cancer cells and that the combination of ONC201 with PARP inhibitors represents a promising synergistic therapeutic approach that could be exploited in multiple solid tumors.

#3213 Antagonism of D2-like dopamine receptors plays a role in ONC201’s antineoplastic effects. Christina Leah B. Kline, Amriti Lulla, Jessica Wagner, Daniel Dicker, Marie Baumeister, Sophie Oster, Wafik El-Deiry. Fox Chase Cancer Center, Philadelphia, PA.

ONC201/TIC10 is a first-in-class small molecule inducer of TRAIL that causes early activation of the integrated stress response and inactivates both Akt and GSK3β, making tumors lacking germline BRCA mutations more sensitive to ONC201. We have previously shown that ONC201 has anti-proliferative and pro-apoptotic effects in a wide range of tumor types including BRCA-deficient breast and ovarian cancers (n = 10), with GSK3β values in the low micromolar range. Treatment with ONC201 induces surface TRAIL and inhibits Akt activity in BRCA deficient breast and ovarian cancers. PARP inhibitors have been previously shown to upregulate DR5 through transcription factor CHOP, sensitizing solid tumors to TRAIL. Resistance to PARP inhibitors can occur through PI3K/Akt pathway activation, and PI3K/MEK blockade improves their anti-tumor effects. We observed synergy between ONC201 and PARP inhibitors olaparib andrucaparib in BRCA-deficient breast and ovarian cancer cell lines in cell viability assays with combination indices (CI) ranging from 0.4 - 0.8. Robust synergy was also observed in prostate cancer cells. The mechanisms of the observed synergy are currently under investigation. These results indicate that ONC201 possesses single agent activity in BRCA-deficient cancer cells and that the combination of ONC201 with PARP inhibitors represents a promising synergistic therapeutic approach that could be exploited in multiple solid tumors.
and ERK. Its promising safety profile and broad spectrum efficacy in vitro has been confirmed in Phase I/II trials in several advanced malignancies. Biochemical and reporter assays have shown that ONC201 is a selective and competitive antagonist of the D2-like receptors, specifically, dopamine receptor D2 (DRD2) and dopamine receptor D3 (DRD3) with a Kᵢ value of ~3 μM. The theme that dopamine receptor antagonism and suppression has been supported throughout the literature. We hypothesize that ONC201’s interaction with DRD2 is critical for ONC201’s anticancer effects. Co-treating HCT116 and RKO colorectal cancer cells with ONC201 and dopamine or the selective D2-like receptor agonist suvorexant partially abrogated ONC201-induced ATP4F/CHOP expression and apoptosis. Knocking down DRD2 expression using siRNA negated ONC201’s effects on cell viability. Overexpressing DRD2 in a cancer cell line that has very low levels of DRD2, increased ONC201-induced PARP cleavage. Quantitative RT-PCR analyses showed that cells that have acquired resistance to ONC201 did not express detectable mRNA levels of the D2-like receptors. To further determine the anti-tumor potential of targeting the D2-like receptor, we treated different cancer cell lines with other D2-like receptor antagonists. Similar to ONC201, the D2-selective antagonist L-741,626 decreased cell viability and induced apoptosis in a number of cancer cell lines. In contrast to ONC201, however, L-741,626 has a poor therapeutic index. Our findings show that the ability of ONC201 to inhibit D2-like receptors contributes to ONC201’s anti-proliferative and pro-apoptotic activity. Ongoing work is aimed at elucidating the mechanisms by which antagonism of D2-like receptors can promote apoptosis-driven cell death and signaling pathways with regard to ATP4F/CHOP/DR5 and Akt/ERK/ Foxo3a/TRAIL, which have been shown to be stimulated in ONC201-treated and -resistant cancer cells.

#3214 Systematic drug repurposing for faster cures of pediatric cancer identifies that Digoxin prolongs survival in a PDX model of group 4 medulloblastoma. Lei Huang,1 Sarah G. Injac,2 Hong Zhao,1 Qi Lin,1 Mari Kogiso,3 Chris T. Man,4 Xiao-Nan Li,5 Ching C. Lau,6 Stephen T. Wong,6 Houston Methodist Research Institute, Houston, TX; 2Texas Children’s Hospital, Houston, TX; 3Connecticut Children’s Medical Center, University of Connecticut School of Medicine, Hartford, CT.

Background: Medulloblastoma (MB) is the most common malignant brain tumor of childhood. While the current standard care for MB leads to long term survival in approximately 75% of patients, recurrent and refractory MB continues to have dismal outcomes. In addition, long term survivors face significant treatment-related sequelae especially poor neurocognitive outcomes. Drug repositioning for known drugs is a promising potential strategy not only for drug discovery but also for accelerated translation into the clinical setting. To systematically explore thousands of known drugs available, we integrated computational biology and empirical biological methods to find old drugs for new indications in MB. Results: A non-parametric, bootstrapping based simulated annealing (NPBSA) algorithm was employed to identify driver signaling pathways for over 1,800 patients with Group 3 and Group 4 MB through an integrative analysis on their mRNA expression, DNA-copy number, DNA-methylation and DNA-seq profiles. Then, drug functional networks were constructed based on gene expression profiles under drug treatment as well as chemical structures and were clustered into drug modules with potential mechanisms of action. By evaluating targeted effects of 1,309 drugs from connectivity map database within each drug module in driver signaling pathways, we identified a group of known cardiac glycosides that top ranked among the total drug candidates for the Group 3 and 4 MB subtypes. In addition to traditional chemotherapeutic agents, members of the cardiac glycoside family were repeatedly identified as potential therapeutic agents for MB. These findings were validated in multiple MB-derived cell lines which showed high rates of growth inhibition by cardiac glycosides compared to controls. To evaluate if this growth inhibition in vitro correlated to prolonged survival in vivo, an extensively characterized patient-derived orthotopic xenograft (PDXO) model of Group 4 MB (ICb-107/M8) on viable cell counts. Overexpressing DRD2 for 24 h led to a time-dependent growth inhibition significantly prolonged survival to 113 days from a median of 92 days in untreated controls (p < 0.001). Histological evaluation of recurrent tumors following digoxin treatment demonstrated changes in the pattern of tumor spread, vascularity and necrosis compared to untreated controls. Conclusions: Leveraging big data in the domains of pharmacogenomics and the notion of drug functional networks and driver signaling pathways represent a powerful tool to repurpose known drugs for new indications in pediatric cancers. Using this integrative biology approach, we identified the cardiac glycosides family generally and Digoxin, specifically, as potential novel agents in the treatment of pediatric medulloblastoma.

#3215 Therapeutic potential of dual-function small molecules for breast cancers. Nicole Nicholas,1 Alfredo Velena,2 Scott Grindrod,3 Mira Jung1.

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Epigenetic silencing of tumor suppressors is an important mechanism in tumor suppression and progression. A tumor suppressor gene, BRCA1, is part of complex molecules in the repair of DNA double-strand breaks (DSBs), and loss of BRCA1 contributes to both sporadic and inherited breast tumor progression. Furthermore, recent studies have shown that the ataxia-telangiectasia mutated (ATM) activity is abnormal in breast cancer. Therefore, we tested the hypothesis that the dual-functional drug that fused the HDAC inhibitor with an ATM activator would enhance killing of breast cancer (BC) cells (increasing ATM activation). In addition, the expression of ATM may enhance the synergetic antitumor effects of HDAC inhibitors in combination with radiotherapy. To determine the HDAC inhibitor potency, SP-1-303 was screened against a panel of Class I and Class II HDAC enzymes in vitro and identified as a pan-HDAC inhibitor (IC₅₀ = 120 nM). Western blot confirmed that SP-1-303 increased acetylated histone H3/H4, α-tubulin, and phosphorylation of ATM within 30 min - 4 h in a time-dependent manner. Using two ER positive BC cell lines (MCF7 and T-47D), two triple negative breast cancer (TNBC) cell lines (MDA-MB-231 and HCC1937), and one normal breast epithelial cell line as a control (MCF10A), the effects of SP-1-303 on cell growth and cell death were examined by performing cytotoxicity assays and flow cytometry. SAHA and Tamoxifen were selected as positive controls. SP-303 conferred the values of cytotoxicity (IC₅₀) for the ER positive BC cells in a range of 0.25-0.4 μM, while the values are in a range of 1-3 μM for TNBC and 12 μM for normal breast epithelial cells, respectively. Flow cytometry (FACS) analyses demonstrated significant increases in G1 arrest (81%) in MCF7 cells and a further enhancement in the G1 phase (91%) in combination with radiation. The data implies the mechanism of action from this drug—cancer cells are being arrested in the G1 phase of the cell cycle, a radiation sensitive phase, suggesting that SP-1-303 may have a synergistic effect with radiation on cell killing. Taken together, SP-1-303 had the largest impact on ER positive breast cancer over TNBC. These data imply that an increased cell death is occurring in ER positive breast cancers from these dual-action molecules through HDAC inhibition and increased ATM activation, suggesting that SP-1-303 alone or its combination with radiotherapy may improve clinical efficacy.

#3216 Attenuation of pancreatic tumor growth by a small molecule tubulin inhibitor. Vivek K. Kashyap, Bilal B. Hafez, Qinghui Wang, Saini Setuwa Andrew Massey, Aditya Ganju, Murali M. Yallapu, Duane D. Miller, Wei Li, Meena Jaggi, Subhash C. Chauhan. University of Tennessee Health Science Center, Memphis, TN.

Introduction: Pancreatic cancer (PanCa) is one of the most fatal cancers and is ranked as the fourth common cause of cancer-related deaths among both men and women in the US. The management of PanCa is exceptionally difficult due to the extremely poor response to available chemotherapeutic drugs. Microtubules are dynamic structures composed of α-β-tubulin heterodimers that are essential for cell division and are important targets for several clinical drugs (paclitaxel, docetaxel and vinblastine). However, clinical use of these tubulin-targeting drugs has toxicity and drug resistance issues in cancer patients. Thus, identification of more potent non-toxic inhibitors of β-tubulin is urgently required for cancer therapy purposes. In this study, we have identified a synthetic compound (ABI-231) which is a potent inhibitor of β-tubulin and evaluated its therapeutic efficacy against PanCa in vitro, and in vivo model systems. Methods: ABI-231 ((2-(1H-indol-3-yl)-1H-imidazol-4-yl) (3, 4, 5-trimethoxyphenyl)) - methanone was synthesized and characterized in our department. Effect of ABI-231 on proliferation, migration and invasion of human PanCa cells (ASPC1, HPAFII, and Panc1) was performed by in vitro functional assays (MTS, wound healing, and Boyden chamber migrations). Effect of ABI-231 on the expression of β-tubulin isoforms was determined and compared with other clinical inhibitors of β-tubulin by Western blot, and qRT-PCR. Moreover, the effect of ABI-231 on the expression of β-tubulin III in PanCa cells was determined by confocal microscopy. Therapeutic efficacy of ABI-231 against PanCa was evaluated in an ectopic xenograft mouse model. Results: ABI-231 treatment inhibited cell proliferation, invasion, migration and colony formation abilities of PanCa cells in a dose-dependent manner (1-100 nM) compared to vehicle treated group. Aberrant expression of β-tubulin III is involved in aggressiveness and drug resistance of various type of cancers including PanCa. ABI-231 effectively inhibited the protein level of β-tubulin (TBB) and induced acetylated α-tubulin (TBB2c) and β-tubulin (TBB3 and TBB4 in PanCa cells via destabilization. Our confocal microscopy further results showed inhibition of β-tubulin in ABI-231 treated PanCa cells. Uregulation of micro RNA 200c (miR-200c) has been shown to inhibit the expression of β-tubulin III in cancer cells. ABI-231 treatment of PanCa cells
showed significant (p<0.01) induction of miR-200c as determined by qRT-PCR. ABI-231 administration (intra-tumoral; 50μg/mouse), three times/week significantly (p<0.01) inhibited the growth of ASPC1 cells derived xenograft tumors in athymic nude mice. Conclusion: Taken together, our results suggest that ABI-231 is a potent β-tubulin inhibitor and chemotherapeutic agent which could be used for the treatment of pancreatic cancer.

#3217 Novel prostamide, 15-deoxy-delta12,14 prostamide J2, displays activity against melanoma in vitro and in vivo: potential role of endoplasmic reticulum stress. Daniel A. Ladin, Li V. Yang, Timothy L. Fitzgerald, Rukiyah Van Dross, East Carolina Univ, Greenville, NC.

Melanoma is the most aggressive and deadly form of cutaneous neoplasm in the United States, representing a major clinical challenge. Our lab previously demonstrated that the endocannabinoid, arachidonylethanolamide (AEA), induced cell death in non-melanoma skin cancer (NMSC) cells through the cyclooxygenase-2 (COX-2) mediated formation of novel J-series prostamides (PMJs). We were the first to chemically synthesize the primary metabolite, 15-deoxy-Δ12,14 prostamide J2 (15d-PMJ2), which displayed potent and selective cytotoxicity in NMSC cells. As such, we hypothesize that the selective cytotoxicity of 15d-PMJ2 would be observed in other forms of skin cancer, including melanoma. B16F10 murine melanoma cells and nontumorigenic Melan-A cells were treated with different concentrations of 15d-PMJ2 for 24 hours and cell viability was measured using MTS assays. At 5 μM 15d-PMJ2 decreased viability by 63% in B16F10 cells, while Melan-A viability was not affected. To verify that cell death was due to apoptosis, the cleavage of apoptotic markers caspase-3 and PARP was examined by conducting Western blot analysis. 15d-PMJ2 markedly increased caspase-3 and PARP cleavage only in B16F10 melanoma cells. Previous studies in NMSC indicated that 15d-PMJ2 induced ER-stress and apoptosis. To investigate the mechanism of 15d-PMJ2-mediated death in melanoma, we examined ER-stress responses. Melan-A and B16F10 melanoma cells were treated with 5μM 15d-PMJ2 and evaluated for CHOP10 and p-PERK expression by Western blot analysis. B16F10, but not Melan-A cells exhibited a notable increase in CHOP10 and p-PERK expression when treated with 15d-PMJ2. To further examine the role of ER-stress on 15d-PMJ2 mediated apoptosis, B16F10 cells were pretreated with the ER-stress inhibitors salubrinal and 4-phenylbutyric acid (PBA). Both salubrinal and PBA decreased activation of caspase-3/7, suggesting that ER-stress plays an important role in 15d-PMJ2 mediated tumor cell death. To determine the melanoma activity of 15d-PMJ2 in vivo, B16F10 allograft tumors grown in C57BL/6 mice were dosed subcutaneously with 0.5 or 5.0 mg/kg 15d-PMJ2 for 5 days. Tumors treated with 15d-PMJ2 exhibited significantly reduced growth and mean weights compared to vehicle and untreated animals. TUNEL analysis of tumor tissues indicated a large presence of necrotic and apoptotic cells in 15d-PMJ2-treated tumors compared to vehicle and untreated tumors. To determine whether 15d-PMJ2 induced ER-stress in vivo, tumors were assayed for p-PERK and CHOP10 levels by immunohistochemistry (IHC). These markers were significantly elevated in 15d-PMJ2-treated tumors. Similarly, the viability of primary patient-derived melanoma cells was significantly decreased by 15d-PMJ2. These findings suggest that the novel prostamide, 15d-PMJ2 possesses potent and selective anti-melanoma activity in vitro and in vivo.

#3218 Molecular function of PARP inhibition in glioblastoma. Christofer Aldrighetti, Barbara Huebert, Delphine Quenet. University of Vermont, Burlington, VT.

Glioblastoma (GBM) is the most common primary malignant brain tumor, whose major biomarkers are the DNA methylation status of the promoter for O6-methylguanine-DNA-methyltransferase (MGMT) and the loss/modulation of the tumor suppressor phosphatase and tensin homolog (PTEN). Despite treatment combining surgery, radiotherapy and chemotherapy with the alkylating agent temozolomide (TMZ), patient survival remains poor and recurrence is virtually inevitable, due to TMZ resistance. Poly(ADP-ribose)polymerase-1 (PARP-1) is a nuclear protein involved in multiple facets of DNA repair and transcriptional regulation. This enzyme catalyses the transfer and polymerization of ADP ribose units from NAD+ to both the reduced polymeric ADP-ribose pool (PAR), covalently attached to heterologous acceptor proteins or PARP-1 itself. There is a growing interest in PARP inhibitors (PARPi) for their clinical potential in cancer treatment. Because of the importance of PARP-1 activity in the maintenance of genomic integrity and DNA repair, treatment with PARPi as adjuvant to TMZ chemotherapy can be beneficial in GBM. PARPi are currently in clinical trials for patients with GBM (NCT01525892, NCT01525893, NCT01319059; injected with vehicle and drank regular water); ii) Control + SM (vehicle, regular water, and injected with the SM 3 times a week at a concentration of 20 nm/Kg); iii) Cancer group (injected with AOM and drank DSS); and iv) Cancer + SM (treated with AOM, DSS, and the SM). At the end of the experimental procedures, animals were sacrificed and the colon was ex-
traced and analyzed. None of the mice belonging to groups i and ii developed any tumor or had any pathological finding, indicating that the SMs do not present overt toxicity. All mice in groups iii and iv developed colon neoplasias ranging from carcinomas in situ to adenocarcinomas. No significant differences were found among mice treated with PAMP modulators indicating that PAMP may not play a major role in colon cancer or that the PAMP-related SMs were not very effective. On the other hand, mice that received SM 16311 had a higher severity index (p < 0.001) and weight loss (p < 0.05) than their control counterparts, whereas mice injected with SM 145425 had a lower number of tumors than their controls (p < 0.001). These results suggest that AM may have a protective role during the progression phase of colon cancer, and that treatment with AM or other PMPS may open new research avenues for colon cancer. This study was financed by Instituto de Salud Carlos III (PI13/02166), FEDER, and Junta Provincial de La Rioja de la Asociación Española Contra el Cáncer (AECC).

#3221 Kevetrin induces p53-dependent and independent cell cycle arrest and apoptosis in ovarian cancer cell lines representing heterogeneous histologies. Ashok Kumar,1 David P. Brennan,1 Karima Chafai-Fadela,1 Sylvia A. HOLDEN, Silvia Ram,2 Geoffrey I. Shapiro,2 Krishna Menon2,1. CelsisCorp, ratation, Beverly, MA; 2Dana-Farber Cancer Institute, Boston, MA.

Ovarian cancer (OC) is a molecularly and histologically heterogeneous disease; however, standard treatment is the same for all subtypes. High-grade serous OC initially responds to chemotherapy; however, low-grade serous and clear cell OC are relatively chemoresistant. Limited treatment options are available once disease recurrence occurs. Since various mutations are found in over 90% of high-grade serous OC, Low-grade serous OC harbor wild type p53, but contain other mutations. During later stages of OC, tumors are a heterogeneous population of mutant cells; thus, development of a novel drug that addresses these molecular differences is highly desirable. Previously, we showed that Kevetrin stabilized wild type p53 and induced transcriptional targets in human lung carcinoma. We sought to validate Kevetrin as a potential treatment for OC with varied p53 status. Endometrioid carcinoma (A2780, wt p53), atypical non-serous clear cell (SKOV-3, deleted p53), and high-grade serous (OVCAR-3, mutant p53) OC cell lines were treated with Kevetrin. Kevetrin induced apoptosis in all three OC cell lines, as assayed by cleavage of PARP and caspase-3. Studies showed significant increases in p53 and p21 protein levels in A2780 cells in 24 to 48 hours; however, in OVCAR-3, Kevetrin downregulated oncogenic mutant p53. RNA levels of p53 and p21 were quantified by qRT-PCR 8 to 72 hours after treatment. No significant changes were observed in p53 mRNA, whereas an increase in p21 mRNA was observed in all three cell lines. In A2780, Kevetrin also induced levels of PUMA in a dose-dependent manner. To establish that Kevetrin mediates increased p21 expression requiring p53, RNAi against p53 was transfected into A2780 cells, showing a significant reduction in p21 expression. Furthermore, p53 levels were increased in A2780 cells transfected with p53 siRNA, indicating p53 directs p21 expression in a dose-dependent manner after Kevetrin treatment. In an in vivo xenograft study in immunocompromised nude mice bearing established A2780 tumors, Kevetrin treatment inhibited tumor growth at well-tolerated doses. The mode of action in vivo also showed enhanced expression of p21 in tumor tissue, indicating p53 pathway activation in A2780 tumors. In contrast, in SKOV-3 cells and xenografts, a p53 independent increase in p21 expression was observed. In OVCAR-3 cells, Kevetrin altered the expression of three microRNAs (miRNA-27a, miRNA-1274b, miRNA-25), that are known to be dysregulated in OC, in a time-dependent manner. To gain further insight into the mechanism of action in cells with diverse p53 status, RNA-Seq is being performed in the three cell lines and tumor tissue from mice before and after Kevetrin exposure. In summary, Kevetrin has promise as an effective therapeutic agent for endometrioid, non-serous clear cell, and high-grade serous OC, with molecularly diverse p53 status. A Phase 1 clinical study was completed and a Phase 2 clinical study in ovarian cancer is scheduled to begin January 2017.

#3222 Disulfiram equivalent to doxorubicin in reducing quantitative osteosarcoma metastatic tumor burden in a validated orthotopic mouse model. Mitchell S. Fourman,1 Adel Mahjoub,2 Jared A. Craso,3 Jonathan Mandell,3,4 David C. Hirsch,1 Jessica Tebbets,2 Rebbecca Watters,2 Kurt R. Weiss2,1. University of Pittsburgh Medical Center, Pittsburgh, PA; 2University of Pittsburgh, Pittsburgh, PA.

Introduction: The five-year survival of patients with osteosarcoma (OS) with lung metastases is as low as 15%. Our group has shown that disulfiram, a FDA-approved aldehyde dehydrogenase inhibitor, inhibits osteosarcoma proliferation and metastasis in vitro. Here we compare the quantitative effects of disulfiram and doxorubicin on metastatic OS burden in a orthotopic mouse model using near-infrared indocyanine green (ICG) angiography. Methodology: In an IA-CUC-approved protocol, 60 immunocompetent balb/c mice were given tibial trans-physeal injections of 500K K7M2 mouse OS cells into their left hindlegs. Legs were amputated 4 weeks after OS injection, and mice were euthanized with ex vivo lung retrieval 10 weeks after OS injection. Animals in the doxorubicin group (n = 20) were administered 2 mg/kg retro-orbitally each week, starting 2 weeks after OS injection to simulate the start of therapy only after a clinical diagnosis of lung metastases. Disulfiram was administered retro-orbitally daily, also starting 2 weeks after OS injection. Controls (n = 20) received no therapy. Quantitative near-infrared imaging of the hindlimb and lungs was performed by injecting 20 uL of 25 mg/cc ICG retro-orbitally 24 hours prior to amputation and lung salvage, respectively. ICG reliably extravasates specifically into tumor mass when injected 24 hours prior to fluorescence measurements. Fluorescence analysis was performed using Noveadaq Spy (Noveadaq, Bonita Springs, FL) and NIH ImageJ (Bethesda, MD). Statistical analysis was performed using Prism 6.0 (GraphPad, LaJolla CA) using Fisher’s Exact Test and a one-way analysis of variance with Tukey’s post-test as indicated. Significance was defined as p < .05. All numbers are represented as mean ± standard deviation, and are described in arbitrary perfusion units (APU). Results: Both disulfiram (2.4 ± 1.7 APU) and doxorubicin (0.8 ± 1.7 APU)-treated animals demonstrated a significantly decreased metastatic tumor burden compared to untreated controls (6.4 ± 3.4 APU, p < .01). This finding was independent of hindlimb fluorescence (p > .05). No significant differences were noted between the doxorubicin and disulfiram groups using Tukey’s post-test (p = .76). Nineteen of 20 control animals developed metastatic disease, compared to nine of 19 surviving disulfiram and two of 12 surviving doxorubicin-treated (both p < .01) animals. No animals died prematurely in the control group, while one animal died in the disulfiram group (5% mortality) and eight animals died in the doxorubicin group (40% mortality; p < .05 compared to controls). Conclusion: In our model of metastatic OS, disulfiram appears to have potent anti-metastatic properties. It may also be better tolerated by hosts. Molecular analysis of disulfiram and doxorubicin-treated primary and metastatic tumors is ongoing, which can help us understand the mechanism behind disulfiram’s anti-tumor effect.

#3223 Novel tubulin inhibitor DJ101 targets the colchicine binding site and suppresses melanoma growth and metastasis. Kinsee Arntz, Dong Jin Hwang, Duane D. Miller, Wei Li. University of Tennessee Health Science Ctr., Memphis, TN.

Interfering with microtubule dynamics is a validated approach for anti-cancer treatment and by selectively targeting the colchicine binding site on tubulin, microtubule destabilizing agents can evade mechanisms of drug resistance that commonly develop with other antitumor agents such as taxanes and vinca alkaloids. We have recently reported the discovery of a potent and metabolically stable tubulin inhibitor (DJ101) that specifically targets the colchicine binding site on the beta tubulin subunit, disrupts tubulin polymerization and effectively circumvents drug efflux pumps that decrease the efficacy of existing tubulin inhibitors. To further preclinically evaluate DJ101 as a novel tubulin inhibitor and confirm its mechanism of action, we first visually demonstrated the ability of DJ101 to disrupt microtubule dynamics and elucidated the changes in microtubule structure and cell morphology through immunofluorescence techniques. Furthermore, we solved the crystal structure of DJ101 in complex with tubulin to determine its molecular interactions with the protein. We then demonstrated the significant in vitro potency of DJ101 against a panel of metastatic melanoma cell lines harboring major clinically relevant mutations observed in tumors and also validated its cytotoxicity against a broader array of NC1-60 cell lines. Further analysis revealed that DJ101 effectively thwarted anchorage-dependent melanoma colony formation and drastically hindered cell mobility and motility of those cancer cells. We further ascertained that DJ101 shows negligible off-target effects for major physiologically important receptors and ion channels, suggesting a good safety profile. Encouraged by the preliminary results from our in vitro assessment, we evaluated the in vivo activity of DJ101 in two different mouse models. At a dose of 30 mg/kg via i.p. injection, DJ101 nearly completely suppressed tumor growth in a human A375 melanoma xenograft model. It also appreciably inhibited metastasis potential in a murine B16F10 lung metastasis model at the same dose. In conclusion, we have described a microtubule destabilizing agent that targets the colchicine binding site on tubulin, confirmed its mechanism action, determined its potency in a vast array of cancer cell lines, particularly metastatic melanoma, demonstrated its inhibitory effect on cellular proliferation, mobility and migration, and evaluated its anticancer potential in terms of tumor growth inhibition and metastasis in two in vivo models. Our findings offer a compelling rationale to further develop and advance DJ101 as a tubulin inhibitor for cancer therapy.
Cucurbitacin D inhibits prostate tumor growth via targeting glucose metabolism. Mohammed Sikander,1 Bilal Bin Hafeez,2 Shahnab Malik,3 Aditya Ganju,4 Fathi T. Halaweish,5 Murali Mohan Yallapu,2 Subhash C. Brookes,1 Meena Jaggi1. 1University of Tennessee Health Science Center, Memphis, TN; 2South Dakota State University, Brookings, SD. Back ground: Metastatic prostate cancer (PrCa) is a major public health problem despite the introduction of hormonal therapy for PrCa (HRPC) after the anti-androgen therapy, cancer metastasis and chemo-resistance are the major hurdle for the treatment of prostate cancer (PrCa) patients. Accumulative evidence suggests that altered glucose metabolism is one of the mechanisms for metastatic PrCa cell survival and chemo-resistance. Therefore, identification and generation of natural or synthetic pharmacological agents that can limit altered glucose metabolism might be highly useful for the treatment of metastatic and chemo-resistant PrCa. Cucurbitacin has shown potent anti-cancer and glucagonostic activities along with severe liver toxicity. Thus, a focus on developing different analogues of cucurbitacin is being pursued by scientific community. Herein, we report that Cucurbitacin D, an analogue of cucurbitacin, suppresses the growth of metastatic PrCa cells in vitro and in vivo targeting glucose metabolism and its associated molecular targets. Methods: HRPC cells (PC3 and DU145) was used as a model system. Effect of Cucurbitacin D on PrCa cell proliferation and apoptosis was performed by MTS, xCELLigence and Annexin V assays. Effect of Cucurbitacin D on clonogenic potential of PrCa cells was examined by colony formation assay. In silico analysis was performed to study if Cucurbitacin D interacts with GLUT1 receptor. Effect of Cucurbitacin D on glucose metabolism of PrCa cells was performed by metabolic shift glucose and lactate uptake assays. Effect of Cucurbitacin D treatment on key molecules of cell survival and glucose metabolism signaling pathways in PrCa cells was analyzed by Western blot and qRT-PCR analyses. Therapeutic efficacy of Cucurbitacin D against PrCa was evaluated in an ectopic PrCa xenograft mouse model. Results: Cucurbitacin D (0.1 to 1 μM) treatment significantly (P<0.01) inhibited the growth and metastatic potential of PrCa cells in a dose-dependent manner. Cucurbitacin D effectively induced apoptosis in PrCa cells as shown by enhanced Annexin V staining and PARP protein cleavage and arrested cells in G2/M phase via modulation of cell cycle regulatory proteins (inhibition of cyclin D1/E and Mcl-1 and induction of p21 and p27). Cucurbitacin D treatment dose-dependently decreased the lactate production, glucose uptake in PrCa cells which was correlated by suppressed expression of GLUT1 and its protein docking with GLUT1 (with binding energy for this complex is -8.5 kcal mol⁻¹). It has been reported that miR-132 targets the GLUT1, interestingly, Cucurbitacin D replenished the expression of miR-132 in PrCa cells. These growth inhibitory effects of Cucurbitacin D were confirmed in PrCa xenograft mouse model where it trans-activated apoptosis-inducing genes and to the mitochondria where it inhibits Bcl-2 and induces Bax pore formation. SHetA2 is an anti-cancer drug that binds mortalin and induces apoptosis in cancer, but not in normal cells. We hypothesized that release of p53 from mortalin contributes to the mechanism of SHetA2-induced apoptosis in cancer, but not in normal cells. Methods: SHetA2 effects on intracellular interaction of p53 and Mcl-1, LGR5, and DCLK1, 3, 4 were studied in the presence of SHetA2 and p53. Results: SHetA2 caused dose-responsive apoptosis in association with nuclear and mitochondrial accumulation of p53 in ovarian cancer cell lines harboring wild type or S125R, L130V TP53 mutants, but not in hFTSECs. Mortalin over-expression or p53 knockdown reduced SHetA2 cytotoxicity in ovarian cancer cells. Mortalin knockdown, p53 overexpression, and chemical inhibitors of p53 transcription (Pifithrin-α) and p53 mitochondrial localization (Pifithrin-μ) were lethal to ovarian cancer cells at effective concentrations and therefore in silico analysis was performed to study if SHetA2 interacts with GLUT1 receptor. Effect of SHetA2 on glucose metabolism of ovarian cancer cells was performed by metabolic shift glucose and lactate uptake assays. Effect of SHetA2 treatment on key molecules of cell survival and glucose metabolism signaling pathways in ovarian cancer cells was analyzed by Western blot and qRT-PCR analyses. Therapeutic efficacy of SHetA2 against ovarian cancer was evaluated in an ectopic ovarian cancer xenograft mouse model. Results: SHetA2 targets mortalin-p53 interaction in differential induction of apoptosis in ovarian cancer over normal cells. Shen Mei-Chuan. Taipei Medical University, Taipei, Taiwan. Patients with ovarian cancer are typically diagnosed at an advanced stage, resulting in poor prognosis since there are currently no effective early-detection screening tests for women at average-risk for ovarian cancer. Here, we investigated the effects of MT-6, a derivative of moscatolin, in ovarian cancer cells. Our investigation showed that MT-6 inhibited the proliferation and viability of SKOV3 ovarian cancer cells with submicromolar IC₅₀ values. MT-6-treated SKOV3 cells showed significant cell cycle arrest at G2/M phase and an increase in the proportion of cells in a sub-G1 phase. In addition, MT-6 induced a concentration-dependent increase in mitotic inhibitors, mitotic kinesins, cell cycle regulators of G2/M transition, and apoptosis-related markers in SKOV3 cells. MT-6 treatment also induced mitochondrial membrane potential loss, JNK activation, and Drp expression. Cotreatment of cells with the JNK inhibitor SP600125 considerably attenuated MT-6-induced apoptosis, mitochondria membrane potential loss, Drp upregulation, and suppression of cell viability. MT-6 also inhibited tumor growth in an SKOV3 xenograft model without significant body weight loss. Together, our findings suggest that MT-6 is a potent anticancer agent with tumor-suppressive activity in vitro and in vivo that could be further investigated for ovarian cancer therapy in the future. Novel Marmelin analog DBQ targets Notch signaling pathway in colon cancer stem cells. Dharmalingam Subrahmanian,1 Sivapriya Pon-nurangam,1 Prasad R. Dandawate,1 Gaurav Kaushik,1 Ossama W. Tawfik,2 Roy A. Jensen,3 Santimukul Santra,2 Subhash B. Padhye,3 Scott J. Weir,3 Shrikant Anant1. 1University of Kansas Medical Center, Kansas City, KS; 2Pittsburg State University, Pittsburg, KS; 3Interdisciplinary Science and Technology Research Academy, Pune, India. Background: Colon cancer is the second leading cause of death in the United States. Previously, we have reported that the identification of a novel compound Marmelin from Aegle marmelos and potent anti-cancer activity. We have developed novel Marmelin analogue THB from this structure showed potent anti-cancer activity and its inhibitory constant value was 10 μM. From this, we developed a second series of analogs, of which DBQ is even more potent than THB. The current study is designed to determine whether DBQ affects colon cancer stem cells and identify a mechanism. Method: Colon cancer cell lines HCT116 and SW480 and normal colon epithelial cells were used in the study. Cell growth was measured by hexoseaminidase and clonogenicity assays. Apoptosis was determined by measuring caspase 3/7 activities. Colosphere formation assay and FACs sorting were used for stem cells. For in vivo effects, we performed studies in HCT116 tumor xenografts. Immunohistochemistry was determined for stem cell markers and Notch signaling proteins. Results: DBQ treatment induced significant dose-dependent inhibition of proliferation and colony formation of HCT116 and SW480 cells, but not that of the normal FHC colon epithelial cells. DBQ also significantly reduced the number and size of colospheres, suggesting effects on stem cells. In addition, DBQ reduced the levels of colon stem cell markers, KL, LGR5, and DCLK1. Further, we obtained further confirmation by flow cytometry, where DBQ treatment reduced the number of DCLK1+ cells. We next determined whether DBQ affects the Notch signaling, a pathway that is important in maintaining CSC population. Notch receptor and its ligands are up-regulated in human colon cancer tissues. DBQ treatment significantly downregulated the expression of all four Notch isoforms, its ligands Jagged1, 2 and DLL1, 3, 4 and downstream target protein Hes1. Notch activation requires cleavage by the γ-secretase complex. DBQ treatment inhibits the expression of γ-secretase complex proteins. To confirm that DBQ effect is thorough downregulating Notch activation, we ectopically expressed the Notch Intracellular domain. DBQ effect was signifi-
icanently mitigated in this condition. To determine the effect of DBQ on tumor growth in vivo, we administered DBQ intraperitoneally (5 mg/kg bw) every day for 21 days in mice carrying HCT116 tumor xenografts. DBQ treatment significantly suppressed tumor xenograft growth, with notably lower tumor volume and weight. Western blot and immunohistochemistry analyses demonstrate anostrogenic induction of CYP19A1 and induction of CD44 and also the Notch signaling proteins in the DBQ-treated xenograft tissues. Conclusion: Together, these data suggest that DBQ treatment suppresses colon cancer growth that targets stem cells in part by inhibiting Notch signaling pathway.


EC1456 is a folate-targeted small molecule drug conjugate (SMDC) of tubulysin B hydrazide (TubBH) that is currently in Phase I clinical studies for treatment of patients with folate receptor (FR)-positive tumors. The ability to detect, quantify, and localize drug in tissues and biological fluids is particularly important for the characterization of its biodistribution and sample matrix extraction method. Using the optimized conditions, the resulting standard curves for TubBH in acetonitrile-extracted KB tumor sample matrix showed good sensitivity and dose-response. It was determined that, in addition to untargeted TubBH, the antibody could also be used to quantitate intact EC1456, as well as a metabolite of the drug lacking the hydrazide. The utility of the antibody was further demonstrated utilizing a PSMA-targeted version of TubBH. Once optimized, the ELISA method was used to analyze FR-positive KB and FR-negative A549 xenograft tumor homogenates from animals dosed with either EC1456 or untargeted TubBH. The levels of TubBH were higher 48-h post EC1456 dose in the FR-positive KB tumors compared to the FR-negative A549 tumors; however, levels were similar after 24h. Moreover, untargeted TubBH resulted in lower tumor concentrations of the drug compared to EC1456 in KB tumors. An immunohistochemical method developed using the antibody showed strong, focal, cytoplasmic staining in FFPE tumor sections of KB tumors from animals dosed with EC1456, while no staining was observed in tumors from untreated animals. Finally, the antibody was successfully used to detect cell surface FR-bound EC1456 on fixed and unfixed KB cells in vitro by flow cytometry. This experiment confirmed that the antibody binds to the conjugate when it is expressed on the cell surface. Collectively, these results show that this antibody against TubBH can be used as a powerful tool for analysis of tumor drug uptake for folate-targeted TubBH in xenograft models and could hold great potential for use in patient sample analysis as well.

#3229 Drug repurposing of topiramate in obesity-driven endometrial cancer. Arthur-Quan Tran, Stephanie A. Sullivan, Chuanxiao Zhou, David Kaufman, Victoria Bae-Jump. University of North Carolina at Chapel Hill, Chapel Hill, NC.

Introduction: Topiramate (TPM) is a B-D-fructopyranose sulfamate that acts by inhibiting carbonic anhydrases (CAs) and has been implicated as a novel inhibitor of angiogenesis. TPM is commonly used for the treatment of epilepsy and migraine headaches; however, when combined with phentermine, TPM can induce weight loss. Due to its weight loss and anti-angiogenic properties, we assessed TPM’s potential as an anti-tumorigenic agent in endometrial cancer (EC), a highly obesity-driven disease. Methods: Cell proliferation was assessed by MTT assay after exposure to TPM for 72 hours in the HEC-1A, KLE, Ishikawa and ECC-1 EC cell lines. Two representative cell lines, ECC-1 and Ishikawa, were used to analyze apoptosis, cell cycle progression, cell adhesion and invasion. Apoptosis was analyzed by Annexin V-FITC assay. Inhibition of adhesion and invasion by TPM were assessed by ELISA and transwell assays, respectively. Cell cycle progression was evaluated by Cèmometer. Western immunoblotting was performed to assess downstream targets of the MAPK and mTOR pathways. The LKB1−/−p53−/− EC mouse model was utilized to assess the in vivo effects of TPM. AdCre was injected at six weeks of age to induce invasive EC. Eight weeks following AdCre injection, mice (N = 10 per group) were treated with placebo or TPM (200 mg/kg/day, oral) for four weeks. The expression of phosphorylated-S6 and Ki-67 was assessed by IHC. Results: TPM inhibited cell proliferation in a dose dependent manner in all four EC cell lines (IC50 range = 1500–3000 nM). Treatment with TPM resulted in G1 arrest and induction of apoptosis (p < 0.05). TPM also reduced adhesion and invasion in the EC cell lines (p = 0.05), with a corresponding decrease in VEGF expression. In addition, TPM significantly inhibited phosphorylation of p42/44 and S6 in the EC cell lines. Lastly, TPM decreased tumor weight in the LKB1−/−p53−/− mice by 68% compared to those treated with placebo (p < 0.05), accompanied by a reduction in Ki-67 and phospho-S6 expression. Conclusion: TPM inhibition and tumor growth in EC cell lines and an EC mouse model. Therefore, TPM may be worthy of drug repurposing as an anti-tumorigenic agent in EC, with the potential added benefit of weight loss in this obesity-linked disease.

CANCER CHEMISTRY: Drug Design and Optimization Strategies


The ERK/MAPK pathway plays a central role in the regulation of critical cellular processes and is activated in more than 30% of human cancers. While targeting upstream nodes with RAF and MEK inhibitors has proven effective clinically, resistance frequently develops through reactivation of the pathway. ERK inhibitors have the potential to address resistance caused by ERK reactivation. Herein, a potent, selective small molecule ERK1/2 inhibitor is described. LY3214996 possesses an optimal balance of potency (hERK1 IC50 5nM, hERK2 IC50 1nM), selectivity (hERK2 IC50 5nM, pRSK IC50 > 43 μM), solubility (FaSSIF solubility at pH 6.5 0.133 μM), PK properties (dog; AUG (range) 23800 nM h, CL 12.1 L/min/kg, bioavailability 75.4%), IVIT (TED50 (range) 66 mg/kg pRSK) and demonstrated significant in vivo efficacy in several human cancer xenograft models. LY3214996 is currently undergoing early clinical evaluation.

#3232 Development of novel Btk inhibitor, CB988 targeting ibrutinib-resistant Btk C481S mutant. Wataru Kawahata, Tokiko Asami, Takayuki Irie, Yasuharu Akiyoshi, Takao Kiyoi, Masaaki Sawa, Carna Biosciences, Kobe, Japan.

Introduction: Bruton’s tyrosine kinase (Btk) is a member of the Tec family of cytoplasmic tyrosine kinase. Btk plays a crucial role in the BCR signaling, essential for B-cell development, and Btk has been recognized as a validated therapeutic target for B-cell malignancies. Ibrutinib covalently binds to the Cys481 residue of Btk to inhibit Btk enzymatic activity, but recent studies suggested that the C481S mutation in Btk disrupts the irreversible binding of ibrutinib, and resulted in ibrutinib-resistance in patients. Therefore a non-covalent Btk inhibitor is highly demanded to overcome ibrutinib resistance. Material and methods: We produced two conformationally different Btks, an activated form of Btk (Btk[A]) and an unactivated form of Btk (Btk[U]) by treating Btk protein with ATP (autophosphorylation) or with Lambda Protein Phosphatase (dephosphorylation), respectively. To evaluate cellular potency of Btk inhibitors, phosphorylations of Btk and PLC-γ were analyzed by Western blotting in Ramos cells, a human Burkitt’s lymphoma cell line. ABC-type DLBCL cell line, OCI-LY10 was used to evaluate anti-tumor efficacy of Btk inhibitors. The ibrutinib-resistant Btk(C481S) mutant was produced to assess efficacy of compounds against the drug resistant enzyme. Kinase selectivity profiling was performed to confirm inhibitor selectivity. Results: Based on structure activity relationship studies, we identified a novel Btk inhibitor, CB988 as a highly selective non-covalent Btk inhibitor. CB988 exhibited remarkable activity for the both conformations of Btk with sub-nanomolar enzyme inhibitory potency in a reversible manner (IC50 = 0.78 and 0.09 nM for Btk[A] and Btk[U], respectively). These results suggested that CB988 preferentially binds to an inactive conformation of Btk. In cellular assays, CB988 strongly reduced Tyr223 phosphorylation of Btk at nanomolar concentration, and decreased phosphorylation of PLC-γ in Ramos cells. Furthermore, CB988 significantly reduced the proliferation of OCI-LY10 cells.

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More importantly, CB988 potently inhibited the iibrutinib-resistant Btk[C481S] mutant in vitro (IC50 = 0.6 nM), which differs from other covalent Btk inhibitors. Conclusions: Btk[C481S] mutation have been reported in iibrutinib re- lapsed CLL and MCL patients. Novel non-covalent Btk inhibitor, CB988 strongly inhibited Btk[C481S] mutant enzyme, suggesting that CB988 has the potential to treat patients who are relapsed/refractory to iibrutinib.


Guided by computational modeling addressing the back pocket of Aurora kinases, several novel chemical analogs of BPR1K653 were designed and synthe- sized in two steps with 30-66% yields. All synthesized compounds were evalu- ated by Aurora-A and Aurora-B enzymatic kinase activity assays, cellular phos- phorylation inhibition assays and cell viability assays. Of them, compound 3a that exhibits no substitution at phenylurea showed similar inhibition activity to Aurora-A and -B. Compounds 3b and 3c which harbor tertiary amino group at meta position of phenylurea showed 10-15 fold inhibition selectivity for Aurora-A over Aurora-B in enzymatic assays. In addition, 3b and 3c were more than 200 fold superior in inhibiting T-loop autophosphorylation of Aurora-A and -B. Compounds 3b and 3c which harbor tertiary amino group at meta position of phenylurea showed 10-15 fold inhibition selectivity for Aurora-A over Aurora-B in enzymatic assays. In addition, 3b and 3c were more than 200 fold superior in inhibiting T-loop autophosphorylation of Aurora-A com- pared to Aurora-B in HCT116 colon carcinoma cells. On the other hand, compound 3d with a 4-hydroxyperiperylidyl group at ortho position preserves Aurora-B inhibition activity. Molecular docking study revealed that the various steric interaction between the compound and residues in the back pocket of Aurora kinases determines their inhibition selectivity.

#3234 Development of potent and selective antibody-drug conjugates with pyrrole-based KSP inhibitors as novel payload class. Hans-Georg Lerchen1, Iven Wittrock2, Nils Griebenow2, Marco Lobell3, Anne-Sophie Rebstock1, Yolanda Cancho-Grande1, Beatrix Stelter-Ludwig1, Christoph Mahler2, Simone Greven1, Anette Sommer1, Sandra Berndt2, Carsten Terjung1, Heiner Apel1, Bertold Kreft1, Rolf Jaulet1, Bayer AG, Wuppertal, Germany; 2Bayer AG, Berlin, Germany.

The number of cytotoxic payload classes with different modes-of-action which have been successfully employed in antibody-drug conjugates (ADC) is still rather limited. So far, only ADCs with microtubule inhibitors, DNA binding payloads or topoisomerase I inhibitors have been advanced into clinical testing. To this end, the identification of ADC payload classes with a novel mode of action will increase therapeutic options and potentially help to overcome resis- tance. Inhibitors of kinesin spindle protein (KSP/Ep5) have generated interest due to their high antitumor potency. However, transferring the preclinical po- tency of small molecule KSP inhibitors (KSPis) into highly efficient clinical regimens with a sufficient therapeutic window has remained challenging. We have investigated a new pyrrole subclass of KSPis which showed subnanomolar potency against a large panel of tumor cell lines for their utility as a novel payload class in ADCs. Towards this goal different attachment sites for linkers have been explored in the KSPi molecule which were found compatible with cleavable and/or non-cleavable linkers. Subnanomolar potency and selectivity of ADCs with these linkers towards either HER2, EGFR or TWEAKR could be demon- strated in vitro. For selected ADCs, the intracellular trafficking and metabolite formation was investigated and KSP inhibition was confirmed as the ADC mode of action. Depending on the linker composition differential profiles of the ADC metabolites with regard to efflux, cellular permeation, and bystander effect have been achieved. Moreover, specific accumulation in the tumor versus other tis- sues was demonstrated in biodistribution studies in vivo. In conclusion, KSP inhibitors have been established as a versatile new payload class for the genera- tion of highly potent and selective ADCs.

#3235 Development of potent and selective inhibitors for ATR: An adju- vant for DNA damage based chemotherapy. Sivapriya Kirubakaran, Vijay Thiruvengadam Thiruvengadam, Althaf Shaik. Indian institute of technology, Gandhinagar, India.

Inhibition of DNA damage checkpoint and repair function has been a chal- lenging as well a promising approach in cancer therapy. ATR kinase is one of the key mediator of DNA damage response which induces cell cycle arrest and DNA repair via its downstream proteins. Blocking ATR has proved to prevent the Chk1 pathway from stalled replication fork and enhances the replication stress and premature mitotic entry. In addition inhibition of ATR can selectively sen- sitize the cancer cell to radio and chemotherapy, due to defective DNA damage signaling through the loss of ATM or p53 mutation in cancer cell. Due to its inherent role in DDR, ATR has been explored as a potential target in enhancing the effect of radiation and chemotheraphy in addition enables highly selective targeting the cancer cell through synthetic lethality. Despite the attractiveness of ATR inhibition in the cancer therapy, specific ATR inhibitors have remained undetectable. Further, there is a limited proof of concept data for ATR inhibition. Considering these facts and challenges we will be presenting some preliminary results with respect to In-silico design, synthesis and evolution of potential and selective ATR inhibitors. Referents for ATR: Ribeiro U., Esteban J., Guzman S., Hoogle, A., Jacques S. A., Koolmeister, T. & Helleday, T. Chemical strategies for development of ATR inhibitors. Expert Rev Mol Med 9, 10 (2014). Wagner, J. M. & Kaufmann, S. H. Prospects for the Use of ATR Inhibitors to Treat Cancer. Pharmaceuticales 3, 1311-1334 (2010). Liu, Q. et al. Characterization of Torin2, an ATP-competitive inhibitor of mTOR, ATM, and ATR. Cancer Res 73, 2574-2586 (2013).

#3236 Delivering selective and cell-active inhibitors of V804M mutant RET kinase through structure-guided drug discovery. Allan M. Jordan, Rebecca Newton, Bohdan Waszkowycz, Richard Bayliss, Babiga Bemun, Daniel Burschowsky, Aude Echaller, Samantha Hitchin, Colin Hutton, Shaun Johns, Stuart Jones, Li-Ying Lin, Mark Richards, Chitra Seewooreuthun, Alex Stowell, Ian Waddell, Mandy Watson, Donald Ogilvie. Cancer Research UK Manchester Inst., Manchester, United Kingdom; 2University of Leeds, Leeds, United Kingdom; 3University of Leicester, Leicester, United Kingdom.

Activating gene fusions in the RET receptor tyrosine kinase have been found to drive 1-2% of lung adenocarcinomas and therefore offer an attractive target for targeted therapy. Whilst non-selective tyrosine kinase inhibitors with RET activity are efficacious in this setting, their use is generally limited by dose limi- ting toxicity associated with their more potent activity versus other targets, specifically KDR (VEGFR2) in the case of cobanizant and vandetanib. Given this limitation, there is considerable interest in developing more selective inhib- itors of RET kinase. Tyrosine kinase inhibitors are prone to early clinical failure due to mutations in the kinase ATPase binding domain, which render the kinase catalytically active but no longer sensitive to drug treatment. Such mutations often occur in the so-called “gatekeeper” region and in this specific case, resis- tance is predicted to arise from a Vai-Met or Val-Leu mutation at residue 804. Through a combination of computational methods, structural biology and drug design, we have identified and further optimized a series of inhibitors of the V804M mutant RET kinase which show sub-micromolar cellular activity in cells driven by V804M RET. Moreover, these agents show excellent selectivity against the wtRET kinase and KDR. As such, these agents may offer valuable start-points for second-generation RET inhibitors for use in patents who relapse after treat- ment with first generation selective RET inhibitors.

#3237 Development of direct inhibitors of KRAS mutants for the treat- ment of cancer. Xiaohong Tian, Guoyan Geng, Jia Hui Wu. Lady Davis Insti- tute, Jewish General Hospital, Montreal, Quebec, Canada.

Oncogenic KRAS mutations occur in ~30% of all human cancer. All attempts to develop inhibitors of oncogenic KRAS have failed to reach the clinic for decades and promote the perception of ‘undruggable’ KRAS proteins. Given the recent advances in understanding of mechanism of oncogenic KRAS, there is renewed enthusiasm toward development of direct inhibitors of KRAS. In a previous publication, we and other groups discovered a “pocket,” where they designed a series of mutant-specific small molecular inhibitors that are covalently binding to the mutant Cys 12 in KRAS protein (KRAS G12C). In human cancer, G12C, G12D and G12V are the predominant KRAS mutants. The G12C mutation accounts for 29,700 new diagnoses annually (lung, colon and pancreatic). The G12D and G12V, annually account for 53,700 and 39,100 new cancer diagnoses, respectively, occurring most frequently in Pancreatic ductal adenocarcinoma (PDAC) (51% G12D and 30% G12V) and colorectal cancer (45% G12D and 27% G12V). Thus, there is an urgent need to develop direct inhibitor of KRAS G12D and 12V mutants. By computer-aided drug design techniques, we have discovered a series of novel chemical inhibitors that potently inhibit KRAS G12D and 12V mutant in reporter assays using HEK293 cells and in Western blot analysis using PCNC10.05 and SNU-C2B cells, which express endogenous KRAS G12D mu- tant. The direct binding of our compounds with KRAS G12D mutant was con- firmed by Surface Plasmon Resonance (SPR) analysis. Our compounds also inhibit KRAS G12V mutant. Chemical optimization of these initial hits as direct inhibitors of multiple KRAS mutants is in progress.


Helicases are enzymes that unwind DNA or RNA. As a result, they are de- scribed as ‘guardians of the genome’ and play roles in key processes such as DNA replication, RNA transcription, translation, DNA repair and DNA recombi-
tion. Certain helicases have also been implicated in the DNA damage response (DDR) and with an increasing interest growing in these enzymes, more helicases are likely to be implicated in DNA damage repair as understanding grows. Targeting proteins involved in DDR has recently gained promise as a selective chemotherapy strategy due to the high level of genomic instability in cancer cells. One of these helicases, Bloom syndrome helicase (BLT2) is particularly potent at arresting cell cycle at G2/M phase and implicated in homologous recombination (HR), a key pathway in the repair of double strand breaks. The role of BLM in DNA repair and its complex interactions with key proteins in these networks suggest it may be a good chemotherapeutic target. The first BLM helicase inhibitor to be characterised was ML216. However, ML216 does not seem to be highly specific to BLM as it also inhibits RFC3 and other closely related WRN family members. Furthermore, it is insoluble and permeability of this series which would prevent reliable in-vitro data from being generated. Potent and selective inhibitors targeting BLM helicase can increase understanding of its function in human cells and role and relevance in DNA damage repair. Furthermore, the tool can also be used in synthetic lethality screens to assess the potential of BLM inhibition as a chemotherapeutic option. As a result, the discovery of a novel series of potent and selective BLM inhibitors with improved physiochemical properties were sought. In this work, 672 active hits of the NIH Molecular Libraries Small Molecule Repository (MLSMR) qHTS screen on BLM helicase, which have been deposited in the PubChem BioAssay database [AID2386], were analysed and 10 series were selected for in-house testing on a BLM helicase unwinding assay. This led to the medicinal chemistry optimization of a lead compound that is relatively potent compared to inhibitors of other human helicases. Efforts have been undertaken to establish primary SAR and to improve the activity of the compound to <0.1 μM as well as further improve the physiochemical properties of this series. This series has shown positive binding data using microscale thermophoresis (MST). Very few inhibitors of human helicases have used biological techniques to validate binding and the use of MST for this purpose has not been reported. Further biological efforts are underway to validate the series through in vitro SCE assays and co-crystallisation methods.

**#3239 Selective tumor targeting with 5-substituted pyrrolo[2,3-d]pyrimidines with heteroatom bridge substitution.** Aleem Gangjee,1 Rishabh Mohan,1 Manasa Ravindra,1 Adrienne Wallace-Povirk,2 Carrie O’Connor,1 Aamod Dekhne,1 Zhankan Hou,1 Larry H. Mathevery,1 Duquesne University, Pittsburgh, PA;1 Wayne State University School of Medicine, Detroit, MI.

Three specialized systems exist for membrane transport of folates and antifolates across biological membranes. The reduced folate carrier (RFC) is ubiquitously expressed in tumors and normal tissues. Folate receptors (FR) α and β, and the proton-coupled folate receptor (PCFT) exhibit narrow patterns of tissue expression and serve specialized physiologic roles. FRs are expressed in a number of cancer cells (e.g., FRα in ovarian cancer and non-small cell lung cancer; FRβ in acute myelogenous leukemia) and a single receptor is expressed in a large number of human solid tumors but not leukemias. FRs are either inaccessible to the circulation (FRs) or are nonfunctional (FRβ) in normal tissues. PCFT transport is limited in most normal tissues, given the requirement of acidic pH for optimal activity. These properties facilitate tumor targeting of cytotoxic compounds with specificities for PCFT on FRα and FRβ, respectively. Clinically used antifolates such as methotrexate and pemetrexed are substrates for RFC and their uptake by both normal tissues and tumors confers dose-limiting toxicity due to limited tumor selectivity. We previously reported a 5-substituted pyrrolo[2,3-d]pyrimidine antifolate with a 4-carbon bridge and a phenyl glutamate side chain (AGF127). Previous studies of related 6-substituted pyrrolo[2,3-d]pyrimidines established that the nature and length of the bridge region plays an important role in determining tumor cell potency and transport selectivity. Thus, it was of interest to vary the length and insert heteroatoms into the side chain (-CH₂₈ of AGF127, including -CH₂₈-S- (compound 1, n = 3 and compound 2, n = 4) and -CH₂₈-O- (compound 3, n = 3 and compound 4, n = 4), to determine the impact on drug potency and transport selectivity. The novel analogs were tested as growth inhibitors against engineered Chinese hamster ovary (CHO) cells expressing the human FRα (RT16), RFC (PCFT4), and PCFT (R2/PCT4). AGF127 showed potent inhibition of CHO cells expressing Frα (IC₅₀ = 8.6 nM), and reduced activity toward CHO cells expressing RFC (57 nM) or PCFT (840 nM). Compounds 1, 2, and 3, showed reduced inhibitions toward Frα-expressing RT16 cells (4-35-fold) compared to AGF127; however, compound 4 was equipotent to AGF127. Like AGF127, none of the series were effective PCFT substrates. Compound 3, like AGF127, inhibited RFC-expressing PC43-10 cells (IC₅₀ = 37 nM); RFC activity was reduced 6- to >20-fold for compounds 1, 2, and 4. Toward Frα-, RFC-, and PCFT-expressing KB human tumor cells, the order of potency was 4 > AGF127 > 2 > 3 > 1. Compound 4 was also more active (15-fold) than pemetrexed with KB cells. Collectively, the potential and selective activity of compound 4 toward Frα-expressing tumor cells and limited activity toward RFC-expressing cells offers significant advantages over AGF127 and clinically used non-targeted antifolates, suggesting that further preclinical evaluation is warranted.

**#3240 Designer peptides targeting epidermal growth factor as novel anti-breast cancer agents.** Emmanuel Yaswos,1 Angel Garcia,1 Husamuldeen Dhari,1 Rajendra V. Rajnarayanan1,2 SUNY at Buffalo, East Amherst, NY; Tongaloo College, Jackson, MS.

Triple negative breast cancer (TNBC) is the only major type of breast cancer for which no specific FDA approved target therapy is available to improve patient outcomes. TNBC lacks the usual breast cancer targets estrogen receptor (ER), progesterone receptor (PR) and HER2/neu receptor. Studies have shown that TNBC does express the epidermal growth factor receptor and has recently emerged as one of the potential targets in TNBC. Epidermal growth factor (EGF) mediates several cellular functions and processes such as survival, motility, differentiation, proliferation and death. When it is released by epidermal tissues, EGF binds to cell surface receptors such as the Epidermal growth factor receptor (EGFR) causing a conformational change which facilitates its dimerization. This receptor dimerization triggers its tyrosine kinase-mediated signal transduction activity. Apart from EGF, EGFR is reported to bind to Transforming growth factor alpha (TGF-α), Argos and Potato Carboxypeptidase inhibitor (PCI). All of these proteins have unique structural motifs which targets EGFR. PCI’s structure consists of three disulfide bridges presents a unique knot like binding motif resembling EGF and is able to compete with EGF for its binding site on EGFR. The Argos receptor dimerization thereby by antagonizing its action. We have used an integrated computational drug discovery approach to generate several PCI peptide ligands targeting EGFR protein-protein interactions. PCI- peptides bind to EGFR in silica and block EGF-mediated cell growth, proliferation and migration in vitro. We believe these designer peptide inhibitors will provide structural and mechanistic clues towards novel therapeutic strategies to combat TNBC.

**#3241 Peptide power: A PSMA-targeted CT20 nanoparticle to fight prostate cancer.** Orielys Flores,1 Daniel Nierenberg,1 Ana C. Carr,1 Rania Bassionni,1 Arati Limaye,1 Santimukul Santra,1 Charalambos Kaittinas,2 Annette Khaled,3 Jesus Manuel Perez 3.

Chemotherapeutic agents within targeted nanoparticles typically trigger cell death not only in tumors cells but also in normal cells that non-specifically uptake these nanoparticles. An alternative approach would be to target the delivery of a therapeutic agent that is toxic to cancer cells while less toxic to non-cancer cells. To accomplish this, a Bax-derived peptide (CT20) was developed as a selective cancer agent, and a folate-decorated hyperbranched polyether (HBPE) nanoparticle was employed to deliver the peptide to prostate-specific membrane antigen (PSMA) (+) prostate cancer cells. The receptor-targeting capability of folate acid towards PSMA (+) in prostate cancer was examined using a fluorescence activatable probe, folate-S-S-doxorubicin, and the PSMA inhibitor 2-PMPA. Additionally, a folate-decorated HBPE nanoparticle was used to target PSMA (+) prostate cancer cells in vitro as well as in athymic mice. CT20-encapsulated folate (CT20-CT20) nanoparticles were used to determine cell cytotoxicity against PSMA-positive and -negative cells using flow cytometry. Furthermore, cytotoxicity of folate-CT20 nanoparticles towards murine macrophages was evaluated to determine the consequences of non-specific uptake; the FDA-approved doxorubicin was used for comparison. Cell death associated with CT20 was determined through a violet ratiometric membrane asymmetry probe combined with SYTOX AAD-vanced dead cell stain to evaluate membrane asymmetry and membrane permeability. Folate-CT20 nanoparticles’ tumor-targeting and tumor-killing efficacy was evaluated in athymic mice bearing PSMA (+) tumors and PSMA (-) tumors. Nanoparticles were delivered intravenously through the tail vein and the tumor size was assessed with calipers and ultrasound. Folate-S-S-Doxorubicin was up taken specifically by PSMA (+) prostate cancer cells showing fluorescence within 12 hours and cell death within 24 hours. Cells that were preincubated with 2-PMPA followed by folate-S-S-Doxorubicin demonstrated no cell-associated fluorescence or cytotoxicity. Similarly, folate-decorated nanoparticles were only internalized by PSMA (+) cells. Cytotoxicity of folate-CT20 nanoparticles over PSMA (+) cancer cells occurred within 48 hours, while no changes occurred in PSMA (-) cells. When murine macrophages were treated with PSMA-targeting nanocarriers and doxorubicin individually, significant cytotoxicity only occurred in doxorubicin-exposed cells. In vivo, folate-CT20 nanoparticles were capable of preventing further PSMA (+) tumor growth and...
caused PSMA (+) tumor regression in some cases. Moreover, minimal liver and spleen damage was observed. Herein, we demonstrate that the CT20 peptide is capable of displaying specific lethality towards PSMA (+) prostate cancer cells and that folate-CT20 nanoparticles are promising vehicles to deliver CT20 in vitro and in vivo.

**#3242 Synthetic analogs of curcumin as modulators of multidrug resistance-associated ABC transporters.** Megumi Ohara,1 Shinobu Ohnuma,2 Eduardo E. Chufan,3 Masaharu Ishida,1 Katsuyoshi Kudoh,1 Norihiko Sugisawa,1 Hideo Ohtsuka,1 Takeshi Naitoh,1 Hiroaki Shibata,1 Yoshiharu Iwabuchi,1 Suresh V. Ambudkar,2 Michiaki Unno,1 Tohoku University Graduate School of Medicine, Sendai, Japan; 2Laboratory of Cell Biology, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD; 3Department of Clinical Oncology, Akita University Graduate School of Medicine, Akita, Japan; 4Department of Organic Chemistry, Tohoku University Graduate School of Pharmaceutical Sciences, Sendai, Japan.

Cancer patients often develop resistance to anticancer drugs. ATP-binding cassette (ABC) transporter-mediated drug efflux is one of the mechanisms responsible for development of resistance to anticancer drugs. The present study investigates the use of curcumin, a natural product and a dietary constituent of turmeric, which inhibits the function of three ABC drug transporters including ABCB1, ABCG1 and ABCG2. However, limited bioavailability of curcumin prevents its use for modulation of the function of these transporters in the clinic. We focused on curcumin analogs that were synthesized at Tohoku University and have been shown to exhibit higher bioavailability than natural curcumin to test whether the synthetic analogs also inhibit the function of ABC drug transporters. To investigate this, KB-V1 and K562/BCRP cell lines overexpressing ABCB1 and ABCG2 were used for the experiments. For these studies, we screened nineteen synthetic analogs of curcumin for their effect on the transport function of ABCB1 and ABCG2 by flow cytometry. Four curcumin analogs, GO-Y030, GO-Y078, GO-Y168 and GO-Y172 inhibited efflux of mitoxantrone mediated by ABCG2 and sensitized ABCG2-expressing K562 leukemic cells to the anticancer drug, SN-38 in cell toxicity assays. Some of the curcumin analogs (GO-Y030, GO-Y078, GO-Y172) stimulated ATPase activity of ABCG2 at nanomolar concentrations (EC50 = 480 ± 0.06, 790 ± 0.10, 930 ± 0.12 nM). In addition, GO-Y030, GO-Y078, GO-Y168, GO-Y172 analogs also inhibited photolabeling of ABCG2 in MCF-7/FL plasma membranes with iodoarylazidoprazosin, which is transported by this transporter. These data demonstrate that similar to curcumin, synthetic curcumin analogs also interact at the drug-binding pocket of ABCG2. In aggregate, these results suggest that non-toxic synthetic curcumin analogs with increased bioavailability may be useful to reverse ABCG2-mediated resistance to anticancer agents.

**#3243 Development of specific DNA aptamers against programmed cell death-1 (Anti-PD-1-Apt) for diagnosis and treatment of cancers.** Santhana Gowri Thangavelu Devaraj,1 Lokesh Ganesh Lakshamana Rao,2 Youli Zu,1 Jenny C. Chang,1 Swaminathan P. Iyer 1. 1Houston Methodist Research Institute, Houston, TX; 2The University of Texas Health Science Center at Houston, Houston, TX.

Cancer cells have an extraordinary ability to escape immune response by modulating the expression of immune checkpoints and immune checkpoint ligands. Programmed cell death protein (PD-1), a cell surface receptor expressed on T cells is one such immune checkpoint receptor when bound to its ligands PD-L1 or PD-L2 transmits an inhibitory signal. Such modulation often leads to inhibition of T-cell activation and subsequent escape of tumors from immune surveillance. Recently, several FDA approved therapeutic antibodies have been successfully developed that target PD-1/PD-L1 axis and allow the immune system to enhance its anti-cancer effects. Aptamers are synthetic small molecule ligands composed of short, single-stranded oligonucleotides ranging from 30 to 60 bases in length. Based on their highly specific 3-dimensional conformation, aptamers, analogous to the antibodies can recognize and bind to their targets with high affinity. Also, the nucleic acid component has several advantages over the protein counterparts-such as ease of production under less stringent conditions, long shelf life and low cost. Here, we report the development of several PD-1 specific aptamers by systematic evolution of ligands by exponential enrichment (SELEX) technology against endogenous immunoprecipitated PD-1 protein using DNA library with a complexity of 10^9. Following several rounds of SELEX, the selected aptamers sequenced by high throughput Next-Generation Sequencing (NGS) were found to have highly conserved regions. Six PD-1 specific aptamers (Anti-PD-1-Apt) were then assessed for target validation using leukemic cell lysates (cell lines and primary patient samples) and were found to bind to the PD-1 in its native state. The selected Anti-PD-1-Apt were able to specifically pull down the PD-1 protein from the lysates mimicking PD-1 anti-body. The specific interaction of the Anti-PD-1-Apt was also demonstrated by flow cytometry and fluorescent microscopy. As expected, Anti-PD-1-Apt was able to bind to PD-1 with Kd of ~500 picomolar affinity as assessed by Bio-Layer Interferometry. Furthermore, we also characterized and confirmed Anti-PD-1-Apt biological activity using an PD-1/PD-L1 cell-based assay using PD-1/ PD-L1 reporter-lurkat cells. We have observed activity in a NFAT luciferase reporter activity (Relative Luciferase Units) in PD-1/NFAT reporter-lurkat cells co-cultured with HEK293 cells overexpressing PD-L1 and TCR activator in the presence of Anti-PD-1-Apt compared to control. Our preliminary data also demonstrate robust Anti-PD-1 blockade in Mixed Lymphocyte Reaction (MLR) along with induction of Th1 cytokines Interferon-gamma and IL-2 from different donor sets of PBMCs. We will present additional - in vivo anti-tumor response data at the upcoming AACR Annual Meeting at Washington DC, 2017.

**#3244 Treatment of endometrial cancer cells with a new small tyrosine kinase inhibitor targeting mutated fibroblast growth factor receptor-2.** Sebastien Taurin,1 Chieh-Hsiang Yang,2 Maria Reyes,1 Sunggil Cho,1 Elke J. Arboe,1 Theresa L. Werner,1 Demetrius M. Coombs,1 Paul Chen,1 Margit M. Janat-Amsbury1. 1University of Utah, Salt Lake City, UT; 2Drexel University College of Medicine, Philadelphia, PA; 3Advenchen Laboratories, Moorpark, CA.

Endometrial cancer (EC) is the most common uterine malignancy in industrialized countries and is the most frequent gynecologic cancer in the United States with an estimated 60,050 new cases in 2016. Traditionally observed in peri-, postmenopausal women, the incidence rate of EC has steadily increased among younger women of reproductive age over the past two decades partially owing to a more sedentary lifestyle and rampant obesity epidemic. Surgery in combination with either radio- and chemotherapy is commonly curative for the early stage of the disease. However, patients with recurrent or metastatic disease, who only respond poorly to cytotoxic chemotherapy are in desperate need of therapeutic alternatives as are patients of reproductive age. Thus, innovative, safe and more effective therapies are mandated. Several studies have demonstrated the increased expression of various tyrosine kinase receptors involved in the development of EC. We hypothesized that a targeted therapeutic approach using a pan-tyrosine kinase inhibitor would prevent the proliferation and progression of EC. Anlotinib (AL3818) is a multi-targeted receptor tyrosine kinase inhibitor targeting vascular endothelial growth factor receptors 1 to 3 (VEGFR1-3), stem cell factor receptor (C-kit), platelet-derived growth factor receptors (PDGFRα, β) and fibroblast growth factor receptors 1 to 3 (FGFR1-3). We tested a panel of seven human endometrial cancer cell lines (AN3CA, Ishikawa, HEC1A, HEC1B, KLE, MFE280 and MFE296) to assess the cytotoxicity of anlotinib in vitro. A lower IC50 value was observed in AN3CA cells (25 nM), a cell line characterized by a high level of expression of an FGFR2 mutant protein, while cell lines expressing FGFR2 with wild-type-less sensitive FGFR2A C: A-33 µM, or HEC1B 36 µM). Somatic mutations of FGFR2 have been observed in 12% of EC, indicating the potential for this drug in a subset of EC patients. In summary, these results suggest improved efficacy of anlotinib for the treatment of ECs expressing an increased level of FGFR2 mutant proteins. The safety and efficacy of anlotinib are currently being evaluated in an orthotopic AN3CA murine model of EC. Experimental groups included a tumour-bearing control not subjected to any treatment, anlotinib alone, carboplatin/paclitaxel, a combination of anlotinib and carboplatin/paclitaxel. Primary study endpoints include tumor growth retardation, the delayed onset of local metastases as well as reduced toxicity as secondary endpoint.

**#3245 Preclinical evaluation of the imipridone family of small molecules, including analogues of clinical-stage anti-cancer small molecule ONC201, reveals potent anti-cancer effects of ONC212.** Jessica Wagner,1 C. Leah Kline,1 Gary Olson,1 Bhaskara Hallaganchu,2 Richard Pottorf,2 Varun Prabhu,2 Martin Stogniew,3 Joshua Allen,3 Wafik El-Deiry1. 1Fox Chase Cancer Center, Philadelphia, PA; 2Provid Pharmaceuticals, Moonmouth Junction, NJ; 3Oncoceutics, Philadelphia, PA.

We previously identified a novel, potent anti-cancer small molecule ONC201, which upregulates the integrated stress response (ISR) through ATF4/CHOP/DR5 and acts as a dual inactivator of Akt and ERK, leading to TRAIL gene activation. After completing a first-in-human phase I clinical trial that revealed exceptional safety, therapeutic pharmacokinetic (PK) profile and tumor engagement, ONC201 is under investigation in several advanced cancer Phase I/II trials. Given the unique imipridone core chemical structure of ONC201, we synthesized a family of analogues in an effort to identify additional chemical family members with distinct therapeutic properties. Based on in vitro potency improvements in human cancer cell lines and therapeutic window approximations with normal human fibroblasts, select analogues were investigated in ani-
mals for toxicity, maximum tolerated dose (MTD), and antitumor efficacy. ONC212 is one of the most promising new imipridones that was further evaluated to establish the PK profile, oral bioavailability, and efficacy in tumor types that are less sensitive to ONC201. Compared to ONC201, we noted distinct and more rapid kinetics of activity as well as improved potency in multiple human cancer cell lines. ONC212 has an oral bioavailable PK profile, and is orally well-tolerated in mice. With no evidence of toxicity at efficacious doses in both colon and triple negative breast cancer, we have begun further evaluation of antitumor efficacy studies in ONC201-resistant tumor types. Efficacy studies with ONC212 are ongoing in melanoma models that are sensitive to ONC212 but less sensitive to ONC201 in vitro. Preliminary data indicated that tumor growth reduction by ONC212 in vivo in ONC201-resistant melanoma xenografts. With a wide safety margin, potent antitumor activity in ONC201-insensitive tumors, and drug-like characteristics, ONC212 is being further developed as a drug candidate from the new imipridone class of compounds that complements the spectrum of activity of ONC201.

#3246 Development of small-molecule STAT3 transcription factor inhibitors. Kazi S. Nahar, Shirin Jamshidi, Maria Thanou, David E. Thurston, Khondaker Mizra Rahman. King's College London, London, United Kingdom

Oncogenic transcription factors are an increasingly important target for anticancer therapies, as their inhibition could allow the "reprogramming" of tumor cells, leading to apoptosis or differentiation from the malignant phenotype. STAT3 induces the transcription of genes that control differentiation, inflammation, proliferation, and tumour cell invasion, and its over-expression has been implicated in many tumour types. Although cancer cells are often dependent upon activation of STAT3, non-tumour cells are fairly tolerant of loss of its function, most likely reflecting redundancies in normal signal transduction. Thus, STAT3 inhibitors have a high therapeutic potential. A combination of in silico design and pharmacophore-based approaches have been used to design a novel series of inhibitors capable of disrupting STAT3 dimerisation by interacting with the hexapeptide pocket of the SH2 domain of the STAT3 protein. Members of the initial medicinal chemistry optimised mature ligand library had significant STAT3-dimerization inhibition activity with potencies ranging from 33% to 59% inhibition. They also had sub-micromolar to low micromolar cytotoxicity (IC₅₀ 0.6 μM to 2.6 μM) in the STAT3-dependent cell line MDA MB 231, with good selectivity ratios compared to STAT3-null cell lines. Three of the most potent library members were selected for molecular dynamics simulations and cell biological studies of their interaction with the STAT3 protein, and for their ability to down-regulate STAT3-dependent genes in cell lines using RT-PCR. In the molecular dynamics studies it was shown that the compounds interact with eight key amino acid residues (i.e., Ala703, Pro704, Arg1317, Asn1442, Gln1355, Gln1357, Thr1354 and Thr1443) within the SH2 domain, and in the RT-PCR experiments these compounds were found to down-regulate the STAT3-dependent genes survivin, cyclin D1, fascin and BCL-2 in comparison to the housekeeping gene GAPDH in the STAT3-dependent cell line MDA MB 231. The most active compound was also evaluated in a preliminary human tumour xenograft assay in immune-compromised mice bearing MDA MB 231 tumours, and was found to have significant tumour growth inhibition properties (at 0.5 mg/kg, two doses on day 0 and 2) in a high throughput format to determine the potential of this novel series of molecules toward clinical evaluation in man.

#3247 Discovery of a novel drug that affects centrosome clustering. Dilan B. Jaunky, Kevin Laroque, Javier Porro Suaridaz, Dan Yang, Emma J. Furze, Pat Forgione, Alisa Plekyn, Concordia University, Montreal, Quebec, Canada.

Our goal is to identify molecular regulators of a mechanism that occurs uniquely in cancer cells, and to develop a selective anti-cancer drug. Most chemotherapy agents are non-selective, causing severe side-effects. In addition, cancers often develop resistance to some of the more commonly used chemotherapies. To expand the repertoire of available drugs, and to design drugs that are selective, we need to identify molecules that regulate the physiological changes that occur primarily in cancer cells. For example, cancer cells in many hard-to-treat cancers have aberrant centrosomes, which may be supernumerary or fragmented. During mitosis, these aberrant centrosomes must cluster to form bipolar spindles for successful division. Thus, targeting a process like centrosome clustering is ideal, since it is not necessary in healthy cells. We synthesized a small, stable scaffold with amenability to structure-activity-relationship studies, and found several analogues with IC₅₀ values < 50 nM, depending on the cancer cell line. Preliminary tests showed that these compounds prevent tumors from forming and/or cause their regression in vitro. We performed cell biological studies to characterize their mechanism of action. In several different types of cancer cells, these compounds cause mitotic arrest and centrosome declustering at concentrations where they have little effect on non-cancerous cells. Live imaging revealed that within minutes of adding the compounds to HeLa cells expressing GFP-tagged tubulin, we observed rapid microtubule depolymerization and centrosome clustering. After washing out the compound, microtubule polymerization recovered, but the mitotic spindles were multilobar. Adding the compound to cells exposed to an antimitotic drug, also caused rapid microtubule depolymerization, but after washing out the drug, the spindles were bipolar. We are in the process of identifying the molecular target of these compounds to provide crucial insight to the mechanism governing centrosome clustering, and are continuing to perform SAR studies to obtain compounds with higher efficacy and selectivity.


Microtubrids are key regulators of cell division, cytoskeleton assembly and apoptotic cell death. The mitochondrial electron transport chain (ETC) consists of four enzyme complexes that transfer electrons from NADH to oxygen. During electron transfer, the ETC pumps protons into the inter-membrane space, generating a gradient across the inner mitochondrial membrane that is used by Complex V to drive ATP synthesis. Recent publications have shown that tumor cells harboring specific mutations (LK81, miD8 and others) are more sensitive to Complex I inhibition, potentially providing an opportunity for selectively targeting tumor cells. Based on a high throughput screen (HTS), we identified new, albeit modestly active, lead structures with cross reactivity between mouse and human Complex I. SAR elaboration of the lead structure allowed for optimization of the most active compound and still suffered from low metabolic stability. Further improvement of the in vitro and in vivo PK properties finally permitted in vivo animal studies. Herein, we report for the first time the preclinical profile and structure of a highly active, optimized, human/mouse cross-reactive Complex 1 inhibitor that allowed for the further investigation into the therapeutic potential of Complex I inhibition in cancer.

#3249 Thymoquinone inhibits elongation factor 2 kinase signaling axis by inducing tumor suppressor miR-603 in triple negative breast cancer cells. Nashwa N. Kabil, Recep Bayraktar, Nuriman Kahraman, Bulent Ozpolat. MD Anderson Cancer Center, Houston, TX.

Triple negative breast cancer (TNBC), is a highly heterogeneous and aggressive subtype of breast cancer (BC), which poses a significant clinical challenge. TNBC constitutes about 15-20 % of BC cases, and is characterized by lack of estrogen (ER), progesterone (PR) and human epidermal growth factor 2 (HER2) receptors. Thus, patients cannot benefit from targeted therapies such as anti-estrogen (Tamoxifen) and anti-HER2 (Trastuzumab) treatments. Therefore, identification of new molecular targets and treatment strategies are highly warranted to improve patient outcome. We previously reported that elongation factor 2 kinase (EF-2K) is highly expressed in TNBC cell lines and is associated with poor patient survival and prognosis. Furthermore, in vivo targeting of EF-2K by siRNA nano-therapeutics inhibited cell proliferation, migration/invasion and significantly decreased tumor growth in 2 different orthotopic TNBC mouse models (MDA MB-231 and MDA MB-436). Collectively, our work suggests that EF-2K is an excellent novel therapeutic target in TNBC. In search of a potential safe and effective EF-2K inhibitor, we identified Thymoquinone (TQ), a dietary natural compound, known to have diverse anti-cancerous properties in several in vitro and in vivo models, including TNBC. However, the mechanism by which TQ mediates its effects are not well elucidated. Our current study is the first to demonstrate that TQ inhibits protein and mRNA expression of EF-2K, as well as its clinically significant downstream targets such as Src/FAK, PI3K/AKT, and CyclinD1; resulting in a significant decrease in proliferation, migration/invasion of TNBC cells. To determine the molecular mechanism by which TQ inhibits EF-2K expression, we investigated if TQ induces tumor suppressor microRNA as that we identified to bind to the 3' UTR of EF-2K. We found that TQ induces miR-603 expression, which we had reported to directly bind and inhibit EF-2K expression; resulting in decreased TNBC growth and progression, both in vitro and in vivo. Furthermore, we showed that inhibition of nuclear factor kappa B (NF-kB) (a well established target for TQ), also induces miR-603 and inhibits EF-2K expression in TNBC cells. In conclusion, our study is the first to show that TQ inhibits EF-2K expression through modulating the NF-kB/miR-603 axis; ultimately resulting in decreased cell proliferation, migration/invasion of TNBC. Our data suggests a novel molecular mechanism for TQ that represents a potential therapeutic strategy in inhibiting TNBC tumor growth and progression.
Computational drug repositioning and biochemical validation of piperlongumine as a potent therapeutic agent for neuroendocrine prostate cancer.


Introducing and Objectives: Neuroendocrine prostate cancer (NEPC) is a highly lethal and drug-resistant variant of prostate cancer (PCA). We have used a genomics-based drug-repositioning approach and identified compounds with therapeutic activity against NEPC. One of the compounds, piperlongumine (PL) is a natural product constituent of the fruit of the Long pepper (Piper longum). The efficacy of this drug was tested in the NEPC cell line model NCI-H660 and compared to several other PCA cell lines in a modified WST-1 assay. Preclinical testing in mouse xenograft models of NEPC was also undertaken. Finally, the ability of piperlongumine to inhibit p-STAT3 signaling and promote apoptosis was measured by western blot analyses. Methods: PCA cell lines (LNCaP, 22Rv1, Du145, PC3, H660 and RWPE) were grown in complete media. Apoptosis was measured by western blot analyses.

Preclinical testing in mouse xenograft models of NEPC was also undertaken. Chronic inflammation is believed to promote cancer through cellular and genomic damage. Inflammation creates a microenvironment within the tissue that enhances cell proliferation. In 2016, American Cancer Society estimates that 1,685,210 new cancer cases are expected to be diagnosed in the United States.

Cyclooxygenase (COX) is an enzyme found to play an essential role in inflammation and converts arachidonic acid to prostaglandin-like molecules. The three isomers of COX are COX-1, COX-2, and COX-3. COX-2 has been found to be up-regulated in inflammation and some cancers, such as breast cancer and prostate cancer. Tetrahydropyridines (THPs) developed by our research group were effective in inhibiting inflammation, COX, and the growth of some breast cancer cell lines. Preclinical research has shown that pharmacological activities of the THP derivatives depend greatly on the nature and position of the substituents on the THP ring structure. Here, we present the synthesis of a series of novel THP derivatives. 3-Ethylpyridine is reacted with O-mesitylenesulfonylhydroxylamine (O-MSH) to furnish N-amino-3-ethylyridinium mesitylenesulfonate. The reaction of N-amino-3-ethylpyridinium mesitylenesulfonate with substituted acid chlorides gives the stable crystal phosphonium ylides. A sodium borohydride reduction of ylides furnishes the target compounds, N-substituted benzylamino-3-ethyl-1,2,3,6-tetrahydropyridines. Five novel N-substituted benzylaminos-ethyl-1,2,3,6-tetrahydropyridines were synthesized, purified, and characterized. The evaluation of these compounds cytotoxicity studies on MCF-7 estrogen receptor positive breast cancer cells, MDA-MB-231 estrogen receptor negative breast cancer cell lines, and Ishikawa cells, using the Cell Titer-Glo (CTG) luminescent cell viability assay is underway. This research was supported by the National Center for Research Resources and the National Institute of Minority Health and Health Disparities of the National Institutes of Health through Grant Number 8 G12MD007582-28.

Targeting G-quadruplex nucleic acids: a series of β-carboline alkaloids with new skeletons from the seeds of Peganum harmala. Kaibo wang,1 Huiming Hua,2 Danzhou Yang1. Purdue University, West Lafayette, IN; She-nyang Pharmaceutical University, China.

G-quadruplexes are DNA secondary structures implicated in essential physiological and pathological processes, such as tumorigenesis. Small molecules that target G-quadruplex structures can be developed as potential therapeutic agents through the alteration of oncogene expression levels or the disruption of telomere maintenance. Targeting G-quadruplex nucleic acids, we screened 17 medicinal plants for binding activity to a model G-quadruplex (T-TGGGTT)4 system by 1H NMR experiment. We found that the crude extract of Peganum harmala L. seeds showed the most potential binding activity and specific cytotoxicity against the PC-3 cancer cell line with an IC50 value of 13.65 μg/mL. Subsequently, 1H NMR and bioassay co-guided isolation of the extract of P. harmala L. was performed and obtained 21 novel alkaloids, including 10 novel β-carboline alkaloids with unprecedented carbon skeletons, and 12 known analogues. Their structures including the absolute configurations were determined by extensive analysis of spectroscopic data, X-ray crystallography, ECD calculations, and ECD excitation chirality approaches. The antiproliferative activities and G-quadruplex binding activities were evaluated for these newly discovered and previously reported alkaloids. Some of the isolates showed pronounced cytotoxic effects with IC50 values below 10 μM and significant G-quadruplex binding activities. More interestingly, peganahmine D (4), which showed the strongest G-quadruplex interaction, exhibited significant cytotoxic activity against HL-60, PC-3 and SGC-7901 cell lines, with IC50 values of 3.81 ± 0.32, 11.52 ± 0.62, and 15.16 ± 0.42 μM, respectively. The G-quadruplex interactions positively correlated with antiproliferative activities suggesting these β-carboline alkaloids may exert their anti-cancer effects through G-quadruplex interactions. This work contributed a practical strategy at first time for the dis-
covery of novel G-quadruplex ligands from natural products and provided potential insights for using β-carboline alkaloids as anticancer lead compounds specifically targeting G-quadruplexes.

**#3254 Identification of potent anti-tumor agents from the LOPAC library using a unified 2D/3D cell culture screening approach.** Viananath Das, Tomáš Fürst, Soňa Gurská, Petr Drahák, Maríán Hadích, Institute of Molecular and Translational Medicine, Olomouc, Czech Republic.

Two-dimensional (2D) cultures of immortalized cancer cells have played a significant role cancer drug discovery and development process for decades. However, the limitations imposed by the long-term cultures of cancer cell lines are becoming increasingly apparent only recently. Therefore, there is a general shift towards three-dimensional (3D) cell cultures, which effectively mimic tumor complexities, for lead optimization and to accelerate translation research. 3D cell cultures not only model the in vivo architecture of tumors, but 3D-derived cells also retain numerous signatures of cancer stem cells compared to parental cells in 2D. In this study, using a unified 2D and 3D cell culture screening approach, we screened the LOPAC library from Sigma-Aldrich as a proof-of-concept study. The drug screening was performed using parental colorectal cancer HCT116 cells as a model cell line. 3D-derived cultures were generated from multicellular spheroids of HCT116 cells. Additionally, utilizing lightsheet fluorescence microscopy (LSFM), we studied the tissue penetration of drugs in spheroids of HCT116 and cells expressing hypoxia-inducible factor-1α (HIF-1α). We identified cc. 200 compounds from the LOPAC library that showed increased activity in 3D-derived cultures. A dose–response relationship study of top 20% compounds further confirmed the preferential activity of these drugs in 3D than 2D cultures. Next, studies in spheroid cultures resulted in the identification of five lead compounds with potent spheroid-size reducing effects. Three of these compounds were kinase inhibitors, one HIF-1α inhibitor, and one interestingly, a monoamine oxidase inhibitor. LSFM studies in HCT116-HIF-1α spheroids showed that two kinase inhibitors and the HIF-1α inhibitor significantly altered spheroid hypoxia, and presumably spheroid growth. We believe that this proof-of-concept study will further highlight the advantages of a combined 2D/3D screening approach for drug discovery and development, and consequently enable downstream testing at reduced cost.

**PREVENTION RESEARCH: Strategies and Biomarkers for Clinical Prevention**

**#3255 Circulating adipose stromal cells (CASCs) as a potential biomarker of response to weight loss interventions in obese women at high risk for breast cancer.** Hailey Baker, Amy L. Kreutznan, Jennifer L. Nydegger, Teresa L. Phillips, Richard C. Hasting, Dan A. Dixon, Bruce F. Kimler, Carol J. Fabian, Univ. of Kansas Medical Ctr., Kansas City, KS.

Background Obesity is a modifiable risk factor for breast cancer in the United States. White adipose tissue is increased in obese (BMI ≥ 30 kg/m²) women, releasing estrogens and pro-inflammatory cytokines. Adipose stromal cells play a key role in releasing estrogens, while circulating adipose stromal cells (CASCs), characterized as CD34+ CD31+ CD45+ live tumor sites and promote angiogenesis and vascularization. CASCs have been correlated with BMI in both cancer and non-cancer patients. However, BMI alone as a marker of adiposity has its limitations. We examined whether CASCs correlate with additional measures of adiposity in women who are at high risk for development of breast cancer. Methods Women at high risk for development of breast cancer but without prior breast cancer were recruited for random periareolar fine needle aspiration (RFNA), DEXA body composition, anthropomorphic assessment, and non-fasting venous blood collection. Mononuclear cells were isolated and the frequency of CD34+CD31+CD45+ cells was assessed by flow cytometry. Results CASC frequency ranged from 0 to 0.018% (median 0.001%) for 13 non-obese and 20 obese women. There was no association between CASC frequency and BMI (range 19 - 46 kg/m²), either as a linear correlation or when dichotomized at a BMI of 30 kg/m². However, there were tendencies for greater CASC frequencies in pre-menopausal women, women with greater waist circumference (p<0.050), and women with visceral fat mass greater than 50%. With limited numbers, there was no apparent association of CASC frequency with cytology or proliferation (Ki-67) of benign breast epithelial cells acquired by RFNA. Conclusions This is the first study to investigate CASC frequency in a cohort of women at high-risk for development of breast cancer. Our results to date do not show an association between CASC frequency and obesity (BMI). However, associations with other indices of visceral fat suggest that evaluation of circulating adipose stromal cells could have value as a biomarker of response in clinical trials of obese breast cancer survivors and high risk women undergoing weight loss (especially fat mass) interventions.

**#3256 Risk of second primary cancers in women diagnosed with endometrial cancer in German and Swedish cancer registries.** Tianhui Chen. Zhejiang Academy of Medical Sciences (ZJAMS), Hangzhou, China.

Background: Along with the increasing incidence and favorable prognosis, more women diagnosed with endometrial cancer may develop secondary primary cancers (SPCs). Nevertheless, most of previous studies have limitations such as years since surgery, limited sample sizes or selection bias of SPC cases. Therefore, to our knowledge, investigations on the risk of a specific SPC after endometrial cancer in two different populations have not been reported. We aimed at investigating risk of second primary cancers (SPCs) in women diagnosed with endometrial cancer in Germany and Sweden to provide insight into etiology and prevention strategies for SPCs. Methods: Endometrial cancer patients diagnosed at age ≥15 years in Germany during 1997-2011 and in Sweden nationwide during 1997-2012 were selected. For both German and Swedish datasets, standardized incidence ratios (SIRs), calculated as the ratio of observed to expected numbers of cases, were used to assess the risk of a specific SPC in women diagnosed with endometrial cancer. The expected number of a SPC after endometrial cancer was calculated from the strata-specific first same cancer incidence rates in the Swedish and German general population, respectively, multiplied by the corresponding person-years in women with endometrial cancer. Person-years at risk were accumulated for each patient, starting at the date of diagnosis of the first endometrial cancer, and terminating on the diagnosis date of a SPC, date of death, date of emigration, or end of follow-up (end of 2011 for Germany and of 2012 for Sweden), whichever came first. Results: Among 46,929 endometrial cancer survivors in Germany and 18,646 in Sweden, overall 2,897 and 1,706 SPCs were recorded, respectively. Significantly elevated SIRs were observed in Germany for ovarian (SIR=1.3; 95%CI:1.1-1.5) and kidney cancers [1.6 (1.3-1.8)], while in Sweden the SIRs were 5.4 (4.6-6.3) and 1.4 (1.0-1.9), respectively. Elevated risk for second ovarian endometrioid carcinoma was pronounced after early (<55 years) onset endometrial cancer in Germany [9.0 (4.8-15)] and Sweden [7.7 (5.1-11)]. In Germany elevated risks were found for second ovarian endometrioid carcinoma after endometrial histology of first endometrial cancer [6.3 (4.0-9.4)] and for second kidney cancer after clear cell histology of endometrial cancer [4.9 (1.6-11)]. Conclusions: We found exceptionally elevated risk of second ovarian endometrioid carcinoma after endometrial cancer of the same histology or of early onset. Kidney cancer was also increased, particularly after endometrial cancer of clear cell histology. Cancer prevention strategies should focus on these cancers after endometrial cancer diagnosis.

**#3257 Molecular characterization of the human stomach microbiota in gastric cancer patients.** Guoqing Yu,1 Javier Torres,2 Nan Hu,2 Rafael Medrano-Guzman,2 Roberto Herrera-Goepeert,2 Michael S. Humphrys,3 Lemin Wang,1 Chaoyu Wang,1 Ti Ding,1 Jacques Ravel,5 Philip R. Taylor,1 Christian Abnet,1 Alisa Goldstein1.

1National Cancer Institute, Rockville, MD; 2Instituto Mexicano del Seguro Social, Mexico; 3Unidad Medica de Alta Especialidad Oncologia, Mexico; 4Instituto Nacional de Cancerologia, Mexico; 5University of Maryland School of Medicine, Baltimore, MD; 6Shanxi Cancer Hospital, China.

Background Helicobacter pylori (Hp) is the primary cause of gastric cancer but we know little of its relative abundance and other microbes in the stomach, especially at the time of gastric cancer diagnosis. Here we characterized the taxonomic and derived functional profiles of gastric microbiota in 157 non-malignant gastric tissue samples from two different sets of gastric cancer patients, and compared them with microbial profiles of oral, stool, nasal, vagina and skin samples from Human Microbiome Project. Results We show that Hp is the most abundant member of gastric microbiota in both Chinese and Mexican samples (51% and 24% respectively), followed by the genera commonly seen in the oral microbiota. Taxonomic (phyllum) profiles of stomach microbiota largely resembled oral microbiota when the Helicobacter reads were removed. The functional profiles of stomach microbiota, however, were distinct from those found in other body sites and had higher inter-subject dissimilarity. Gastric microbiota composition did not differ by Hp colonization status or stomach anatomic site, but did differ between paired non-malignant and tumor tissues in either Chinese or Mexican samples. Conclusions Our study is the first to show that Hp is the dominant member of the gastric microbiota in many gastric cancer patients rather than diminished or depleted as traditionally believed. Our results provide insights on the gastric microbiota composition and function in gastric cancer patients, which may have important clinical implications.
NOTCH1, and FAT1 may be among the earliest driver events in lung SqCC. Thesomatic alterations observed in known cancer genes such as TP53, KMT2C, NAV3, NFE2L2, CDKN2A, PIK3CA, KEAP1 and RB1) involved in cell- 

standing of molecular pathogenesis. We performed the whole-exome and transcriptome sequencing from 101 tumors and matched normal samples from Ko-

ran patients. Somatic mutations and gene expression defined two intrinsic subtypes: (1) classical subtype; (2) immunogenic subtype. Classical subtype displayed upregulation of cell cycle related genes and enriched for gene mutations (TP53, NAV3, NFE2L2, CDKN2A, PIK3CA, KEAP1 and RB1) involved in cell proliferation, squamous differentiation and oxidative stress. Immunogenic subtype had more tumor-infiltrating immune cells than classical type. More proportion of adaptive immune cells was also detected in immunogenic subtype. Immune genes, involved in adaptive immune response, were also upregulated in immunogenic subtype. Taken together, our finding revealed that each subtype has different immune mechanisms in lung squamous cell carcinoma and provides potential opportunities for therapeutic development.

The genomic landscape of premalignant lung squamous cell carci-

noma lesions. Joshua Campbell, Xijun Zhang, Samjoet S. Dhillon, Catalina Perdomo, Sarah Mazzilli, Yaron Geshalter, Gang Liu, Sherry Zhang, Han-qiao Lin, Jessica Vick, Christopher Mory, Stefano Monti, Evan Johnson, Matthew Meyerson, Steven Dubinett, Matthew Wilkerson, Clifton Dalgaard, Marc Lemburg, Mary Reid, Jennifer Beanes, Avrum Spira, Boston University School of Medicine, Boston, MA; Uniformed Services University, Bethesda, MD; Roswell Park Cancer Institute, Buffalo, NY; Janssen Pharmaceuticals, Spring House, PA; Dana Farber Cancer Institute, Boston, MA; University of California, Los Angeles School of Medicine, Los Angeles, CA.

Background: Lung squamous cell carcinoma (SqCC) arises in the epithelial layer of the bronchial airway and is often preceded by the development of pre-
malignant lesions. However, not all premalignant lesions progress to lung SqCC and many regress without therapeutic intervention. Understanding the somatic alterations that contribute to progression of premalignant lesions in the airway will allow us to identify biomarkers for early detection and develop therapeutic strategies for early intervention. Methods: Airway biopsies were obtained from high-risk smokers undergoing lung cancer screening by auto-fluorescence bronchoscopy and chest CT at the Roswell Park Cancer Institute. For each subject (n=30), multiple premalignant lesions were sampled repeatedly over time (n=144 samples). One biopsy from each region was sent for pathological review while another biopsy was taken for molecular studies. DNA was also isolated from the blood or cytologically normal bronchial brushings to serve as a matched normal control. Exome capture was performed using the Illumina TruSeq Rapid Exome kit and sequenced to a mean depth of coverage of 120x at Uniform Services University and Walter Reed National Military Medical Cen-
ter. Results: The median number of somatic mutations across all premalignant lesions was 0.73 per megabase (range: 0.10 - 9.8 per Mb) and displayed a modest association with histological grade (p=0.07). The most frequently mutated lung cancer genes involved in cell cycle (RB1), radiation sensitivity (CDKN2A (12%), NOTCH1 (12%), NOTCH2 (12%), PIK3CA (5%), and CDKN2A (<1%)). Known oncogenic hotspot mutations were observed in PIK3CA (1%) and KRAS (<1%). The majority of lesions did not have overlapping sets of mutations with other samples from the same patient, indicating that most of these lesions arose from clonally distinct populations. The two lesions with the relatively high mutation rates (>7/Mb) were taken from adjacent sites over two time points in the same individual with a prior history of lung squa-

mous cell carcinoma. These lesions had a significantly overlapping set of muta-
tions including FAT1 indicating a common evolutionary ancestor. Conclusions: The somatic alterations observed in known cancer genes such as TP53, KMT2C, NOTCH1, and FAT1 may be among the earliest driver events in lung SqCC development and may be useful as biomarkers for early detection as well as targets for lung cancer intervention.


BACKGROUND: Low- and middle-income countries (LMICs) face both training and infrastructural challenges for surgical care, particularly for cancer. Practitioners charged with caring for these patients have few options for basic or advanced training. OBJECTIVES: To describe the training options for cancer surgery in Sub Saharan Africa METHODS: The different methods of training are described and the advantages and limits of each methods is highlighted. RE-

SULTS: Training during residency and onsite fellowship are a good option but requires an appropriate number and capacity of training programme. Interna-
tional fellowships have the limitation of readjustment back home for the trained fellow. Onsite International training missions like IVUmed’s missions have the advantage of “Teach One, Reach Many” outcome where a whole team in trained in accordance of local needs and the participation of all stakeholders. CONCLU-

SIONS: Long-term program commitments; effective communication; and a strong commitment from both the surgical site and the external organization, and supporting organizations facilitate the development of thriving surgical teaching programs capable of serving local communities and conduct-
ing outreach training.
ual scans over a lifetime starting at age 15 are associated with cumulative colon doses of 1.8 Gy at age 30, 3.8 Gy at age 50. The associated LARs of cancer incidence would be 1–2% at age 50, and 11–32% at age 80. Delaying age at screening start to 21 years would reduce the projected risks to <1% at age 50 and <15% at age 80. A frequency of one scan every two years (as used for surveillance of cystic pancreatic lesions) would be associated with LARs of <1% at age 50 and <10% at age 80. The current use of a new CT protocol (dual energy virtual non-contrast CT) at the department would reduce radiation exposures and potential subsequent risks by more than 50%. Conclusion: Annual CT screening and surveillance for pancreatic and renal tumors in VHL patients can be associated with high radiation exposures. When Magnetic Resonance Imaging is not applicable, the new CT protocol may allow daily physicians to delay age at surveillance start or reducing the frequency of CT scans could significantly reduce the potential radiation-related risks, provided that these strategies remain beneficial for patients. Optimized CT protocols can also dramatically reduce radiation exposures. The present study is currently extended to confirm our dose estimates based on CT protocols against individual radiology data, and consider other genetic syndromes. Ultimately, this study should help define surveillance strategies with optimal risk-benefit balance.

#3263 Is Latin America ready for the use of genomics in cancer care and control.
Sandra Perdomo,1 Angela Torres,1 Javier Oliverí,2 Cecilia Frecha,2 Rosalía Quezada-Urban,3 Clara Estela Díez-Velasquez,8 Felipe Vaca.1 El Bosque University, Bogota, Colombia; 2Hospital Italiano de Buenos Aires, Buenos Aires, Argentina; 3ICBME Instituto Universitario Hospital Italiano de Buenos Aires, Buenos Aires, Argentina; 4Universidad Nacional Autónoma de Mexico, Mexico, DF, Mexico.

In Latin America (LA) countries cancer is the second most frequent cause of death after cardiovascular disease. Disease patterns have changed from infectious to chronic, the population is increasing and becoming elderly, and countries lack planning to respond adequately to this epidemiological and socio-demographic transition. By 2025 and increment of nearly 30% of new cases and 35% of deaths from cancer are predicted. Genomic studies have profoundly changed cancer management from improved cancer diagnosis to reformatting cancer prognosis and treatment. However, all these key advances have been mainly concentrated in highly developed nations and little is known about the capacities and needs of cancer genomics in the LA context. In order to evaluate the capacity and development of cancer genomics in LA, we collected available information for all countries in Central, South America and Cuba. Data reviewed included: number of NGS platforms, number of cancer research institutions, research groups working in cancer genetics, publications on cancer genetics and genomics in the last 10 years, educational programs on genomics and related national cancer control policies. Currently, there are a total of 212 NGS platforms in LA. Mexico and Brazil are the countries with more users, and Peru and Ecuador have the fewest. 118 research groups in South America and 12 in NCCPs reviewed consider secondary prevention strategies for early diagnosis, opportunistic treatment and decrease of mortality, areas where genomic analysis could be implemented. Only 5 NCCPs include cancer research as an action plan to increase cancer prevention strategies and reduce both incidence and mortality. Despite the recent advances in introducing cancer genomics knowledge and application in LA, the region lacks development of integrated genomic research projects, improved use of platforms, associated educational programs and health policies that might focus on the most frequent cancers and could impact cancer care.

#3264 Evaluating heart-hate variability, salivary cortisol and physical activity as predictors of cancer-related fatigue recovery in breast-cancer survivors over a 12 week period by use of a smartphone application.
Ammia Julia Seiler,1 Annke Anna van den Oever,2 Kees van der Krogt,3 Willem J. Murdock,4 Diana A. Chirinos,1 Luz M. Gacarini,1 Christopher P. Fagundes 1.1 Department of Psychology, Rice University, Houston, TX, 2Wearable Computing Laboratory, Department of Information Technology and Electrical Engineering, ETH Zurich, Zurich, Switzerland; 3Department of Psychology, Rice University; Department of Behavioral Science, The University of Texas MD Anderson Cancer Center, Houston, TX.

Introduction Cancer-related fatigue (CRF) is one of the most prevalent and debilitating problems in breast cancer survivors (BCS) that can persist many years beyond successful cancer treatment. Parasympathetic nervous system activity (measured via heart-rate variability; HRV), cortisol dysregulation and decreased physical activity are plausible, but understudied contributors to CRF.

Repeated daily measurements of CRF and tracking daily physical activity over a 12-week period should give more information regarding temporal patterns of CRF among BCS. Objective The purpose of this study is 1) to investigate patterns of CRF in fatigued BCS over time; 2) to assess HRV, salivary cortisol and level of physical activity as predictors of cancer-related fatigue (CRF) in BCS over a 12-week period. Methods: The sample consisted of 40 women who completed an intervention results in increased daily physical activity, as well as improved HRV, salivary cortisol and CRF in BCS relative to healthy female controls over a 12 week period. Methods A total of 30 fatigued breast cancer survivors (FACT-F score ≤ 34) and 30 aged-matched female controls will be randomly assigned to either an intervention or control group. Both groups will receive a smartphone application with feedback regarding their daily physical activity. The control group will receive the application without feedback. Behavioral data will be collected by means of GPS and Wi-Fi for localization, and accelerometer, barometer, magnetometer and gyroscope for activity recognition. HRV and salivary cortisol will be collected at rest, as well as during and after a significant stressor (Trier Social Stress Test). Fatigue will be measured by the FACT-F Scale, which will be completed at baseline (T1), 4 weeks (T2) and 12 weeks (T3). Group differences will be tested by one-way analysis of variance (ANOVA) and intervention effects will be analyzed using mixed models with repeated measures. Hypotheses We hypothesize that HRV, cortisol dysregulation and level of physical activity at baseline can predict CRF-recovery over a 12-week period. The intervention group will be associated with increases in physical activity in the BCS intervention group and that changes of physiological correlates of CRF can be observed (i.e., HRV and salivary cortisol). Conclusion This study will examine associations between HRV, salivary cortisol, physical activity and CRF in BCS over time. The results of this study may provide insight into factors that contribute to the development, persistence and/or consequences of CRF by use of an innovative mobile application.

#3265 Characterization of epithelial oral dysplasia in non-smokers: work towards precision medicine. Leigha D. Rock1, Miriam P. Rosin,2 Lewis Zhang3, Babak Shariatit, Denise M. Laronde3, 1Univ. of British Columbia, Vancouver, British Columbia, Canada, 2Simon Fraser University, Burnaby, British Columbia, Canada.

Objectives: Although tobacco usage is still one of the strongest risk factors associated with oral cancer risk, there is a subset of non-smokers who develop oral cancer. Tobacco cessation efforts have resulted in a drop in oral cancer rates associated with this habit, leading to a growing interest in the increased proportion of cases occurring among non-smokers (NS). Lesions with oral epithelial dysplasia (OED) are at risk of progressing to oral cancer. Not only is the natural history of OED in NS poorly understood, but the path to interception of disease in NS is poorly defined. There is a gap in the knowledge surrounding the clinical, pathological and genetic characterization, and the risk of progression in this growing category. This information is critical to the evolution of precision medicine in this subgroup. The aim of this study was to: 1) Describe the molecular and clinicopathological features of OED in NS as compared to smokers in longitudinal follow-up; and 2) To compare progression rates and time to progression in NS and smokers with OED. Methods: The study focused on cases with histologically confirmed mild or moderate OED in follow-up in the Oral Cancer Prediction Longitudinal Study. Clinicopathological data, including lesion site, size, texture, colour, consistency, border characteristics, fluorescence visualization (FV), and toluidine blue (TB) retention, were collected in addition to detailed smoking history. A genomic based marker test (gMART) which uses loss of heterozygosity (LOH) at key chromosomal loci to stratify lesions to progression risk, was performed on baseline biopsies. Progression was considered to be advancement to severe dysplasia, carcinoma in situ, or squamous cell carcinoma. Results: Out of 231 OED, 30% were NS related, based on self-reported smoking status. Although there were more smokers with OED than NS, a significantly higher proportion of the OED underwent malignant transformation in NS (P = 0.048). Although not significant, time to outcome was also faster in this group. Most clinicopathological and genetic predictors were equal in both NS and smokers especially at the floor of mouth site. Even among smokers (ES) were more likely to have OED at the floor of mouth while OED was more likely to occur at the tongue or gingiva (P < 0.001) in NS. LOH risk patterns were strongly associated with progression (moderate risk OR 4.84; high risk OR 28.1; P = 0.001) and equally sensitive in both NS and ES subgroups. Conclusions: These findings support the premise that progressive lesions in NS and ES have similar clinicopathological and genetic signatures, and emphasize the need for clinicians to consider the molecular genomic profiles in the triage of OED. LOH markers can sort high-risk lesions, despite risk habits, and should be an important consideration in the treatment of OED. This marker is of particular use in the targeting of candidates for chemoprevention.
#3266 Oral rinse point of care test to predict head and neck cancer. Michael J. Donovan,1 Kris Curtis,2 Greg Ginn,3 Elizabeth J. Franzmann4. 1Mt. Sinai School of Medicine, New York, NY; 2Vigilant Biosciences, Ft. Lauderdale, FL; 3Sancho and Associated, Cicero, IN; 4University of Miami, Miami, FL.

Background: Head and neck squamous cell carcinoma (HNSCC) is the 6th most common cause of cancer mortality throughout the world with an estimated 50,000 people in the US and 600,000 worldwide each year. The ability to detect the disease in a potentially malignant phase and earlier stage could have significant impact on overall outcome. Previous studies have demonstrated that a combined salivary CD44, a tumor-initiating marker, and total protein assay was able to aid in the diagnosis of HNSCC. We sought to understand the performance characteristics of these biomarkers in a prospective population using a qualitative, point-of-care assay (POC) which consists of a cassette with a colorimetric read-out based on a pre-determined threshold for both CD44 and total protein. We also performed results from same samples with a quantitative lab-based test.

Methods: Saline oral rinse specimens (~5ml) from 134 patients (84 HNSCC cases; 50 controls) were obtained from biorepositories. Samples were thawed, gently swirled, placed in a standard collection cup. 600ul were removed for lab assay prior to POC test. The tapered end of the POC cassette was submerged in rinse for 20'; levels of CD44 and total protein (TP) were evaluated by two operators. POC test results were compared between operators and with lab test results using Cohen's kappa coefficient and McNemar’s test, sensitivity(Se), specificity(Sp), NPV and PPV. Results: 95% HNSCC patients (cases) were mean 60 years, 60% male, 97% white and 74% smokers vs. 43 years, 28%, 96% and 0% respectively, for controls. POC was positive with either CD44 or TP above threshold. Weighted kappa yielded 75% and 72% for CD44 and TP respectively between operators. With a prevalence of 9.27: POC: NPV 94%, PPV 10%; variable TP cut-off produced Se71-84% and Sp50-50%. McNemar and Kappa for both lab CD44 and TP vs POC indicated moderate agreement; independently both POC and lab produced NPV >90%. Conclusions: Concordance of the POC assay between operators and moderate agreement with a quantitative lab assay provides supportive evidence for clinical application of the POC assay as an aid in the diagnosis for HNSCC. Additional studies are underway to confirm these observations.

#3267 Combined effect of multiple gene polymorphisms on cancer risk stratification. Radhika Gade Andavolu,1 Olivia Rodriguez,1 Anastasia Al Rubaie,

1School of Medicine, New York, NY; 2Eisenhower Medical Center, Rancho Mirage, CA.

Cyclin D1 (CCND1) G870A polymorphism has been known to be a risk factor in multiple cancers. Cyclin D1 is a cell cycle regulator frequently mutated, amplified and overexpressed in a variety of tumors. Activation and over expression of CCND1 have been found in variety of tumors and alterations in CCND1 are thought to be involved in carcinogenesis. CCND1 polymorphism A870G interferes with splicing and studies showed DNA damage in cells from subjects with ‘A’ allele to bypass G1/S check point of the cell cycle control mechanism more easily than damage in cells with G allele (wild type). A single base pair polymorphism (exon 4, G870A) in the CCND1 gene affects gene splicing. This identification and validation of contributing genetic factors such as single-nucleotide polymorphisms (SNPs) represents a critical step in the advance toward personalized medicine. Several gene expression correlation studies have been done but to our knowledge studies have not been reported on SNP’s of ERBB2 gene, ESR gene and CCND1 combined effect correlating with high risk. In this study, we examined the relationship between ERBB2 (codon 655), Estrogen receptor (ER Intron 1 T–C) and cyclin D1 (Exon 4) polymorphic additive effect in cancers more specific to breast cancer. Genomic DNA was extracted from whole blood samples collected on study. The SNPs were tested by the PCR-fragment length polymorphism (PCR-RFLP) method. Our results show individuals carrying CCND1 ‘A’ allele in combination with variant ERBB2/ESR1 uncut alleles are significantly high (P<0.004) in cancer patients (N=232) as compared to controls. (N=222). Similarly individuals carrying CCND1 Variant ‘A’ allele in combination with ESR ‘C’ Allele showed high frequency with p<0.009. Both these combinations showed a strong and significant association with cancer but individually, ERBB2 variant did not show any significant association, ESR gene showed a borderline association and CCND1 showed strong association independent of these two gene polymorphisms. Earlier few studies have shown no association of ERBB2 mutation in agreement with the previous reports. In conclusion our data suggests that in complex diseases a single gene may not contribute to show its effect but in combination may show a profound effect in evaluating the risk.

Table 1. Multivariable-adjusted associations between BMI and circulating 25-OH DL evels among cancer survivors in the NHANES 2001-2010 (n=1306) stratified by CRP levels. 

<table>
<thead>
<tr>
<th>BMI category (kg/m²)</th>
<th>Circulating 25-OH D levels (nmol/L)</th>
<th>Beta-coefficient (95% CI) *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall, n=1306 reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>-1.72 (-3.86 to 0.42)</td>
<td>0.10</td>
</tr>
<tr>
<td>≥ 25</td>
<td>-0.25 (-1.81 to 1.31)</td>
<td>0.20</td>
</tr>
<tr>
<td>≥ 30</td>
<td>-1.38 (-3.86 to 1.11)</td>
<td>0.001</td>
</tr>
<tr>
<td>Low CRP levels (&lt;1.0 mg/L) n=348</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>-0.32 (-1.79 to 1.14)</td>
<td>0.32</td>
</tr>
<tr>
<td>≥ 25</td>
<td>-0.07 (-1.42 to 1.28)</td>
<td>0.48</td>
</tr>
<tr>
<td>≥ 30</td>
<td>-0.62 (-2.12 to 0.88)</td>
<td>0.12</td>
</tr>
<tr>
<td>Moderate CRP levels (1.1–3.0 mg/L) n=498</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>-0.66 (-2.12 to 0.79)</td>
<td>0.12</td>
</tr>
<tr>
<td>≥ 25</td>
<td>-0.07 (-1.24 to 1.09)</td>
<td>0.20</td>
</tr>
<tr>
<td>≥ 30</td>
<td>-0.66 (-2.12 to 0.79)</td>
<td>0.12</td>
</tr>
<tr>
<td>Low 25-OH D levels (&lt;9.99 mg/L) n=460</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>-0.66 (-2.12 to 0.79)</td>
<td>0.12</td>
</tr>
<tr>
<td>≥ 25</td>
<td>-0.07 (-1.24 to 1.09)</td>
<td>0.20</td>
</tr>
<tr>
<td>≥ 30</td>
<td>-0.66 (-2.12 to 0.79)</td>
<td>0.12</td>
</tr>
</tbody>
</table>

*Adjusted for age, gender, race, body mass index, and smoking status
Esophageal cancer (EC) has been well documented with predominant occurrence in male and with major histopathological type of esophageal squamous cell carcinoma (ESCC) in China. However, the influence by gender on ESCC survival is largely unknown in China. The present study was thus undertaken to elucidate the impact by gender on survival of Chinese patients with ESCC. A total of 1,117 cases was enrolled in this study, including 1,117 males with a mean age of 60.2±8.6 and 6842 females with a mean age of 61.0±8.4. All the patients were from ESCC database in Henan Key Laboratory for Esophageal Cancer Research of the First Affiliated Hospital, Zhengzhou University (1975 to 2015). Only the patients with T2–4N0–1M0–1 were enrolled in this study. Based on the diagnosed date, the patients were classified into two groups, group I (1996–2005, 6885;38.3%); and group II (2006–2015, 11084;61.7%). The follow-up was undertaken until October, 2016. Kaplan–Meier models were for describing the survival curves and Cox proportional hazards models were undertaken to observe the risk of death of male and female. The results demonstrated that the male patients had a poor survival than female (X²=58.17, P<0.001). Furthermore, similar results were observed for the two different periods of 1996–2005 (X²=21.08, P<0.001) and 2006–2015 (X²=34.67, P<0.001). The present results indicate that gender is an independent survival factor for ESCC and the female patients with ESCC have a dramatic prolonged survival than male patients in China. [Supported by Doctoral Team Foundation of the First Affiliated Hospital of Zhengzhou University (2016-BSTDJD-03), National Science Foundation of Henan Province (20161110) and Corresponding author: Li Dong Wang, email:ldwang2007@126.com]

#3270 Association of low-carbohydrate diet and macronutrient intake with colorectal cancer survival. Mingyang Song,1 Kana Wu,2 Jeffrey Meyerhardt,3 Omer Yilmaz,4 Shujie Ogino,5 Charles Fuchs,2 Edward Giovannucci,2 Andrew T. Chan,1 Massachusetts General Hospital, Boston, MA;4 Massachusetts Institute of Technology, Cambridge, MA; 3Dana-Farber Cancer Institute, Boston, MA; Massachusetts Institute of Technology, Cambridge, MA;3 Dana-Farber/Harvard Cancer Center, Boston, MA.

Background: Metabolic reprogramming is a hallmark of cancer characterized by the switch from oxidative phosphorylation to aerobic glycolysis. Accumulating evidence suggests that reducing dietary carbohydrate intake may ameliorate metabolic reprogramming and suppress proliferation of tumor cells. However, the clinical benefit of carbohydrate restriction remains unknown. Objective: To examine the low-carbohydrate diet and macronutrient intake after colorectal cancer diagnosis in relation to mortality among stage I-III patients. Methods: We evaluated data on 1,303 colorectal cancer patients in the Nurses’ Health Study (1980-2012) and Health Professionals Follow-up Study (1986-2012) who completed a validated food frequency questionnaire at least one year after diagnosis. An overall low-carbohydrate diet score was created based on the percentage of energy as carbohydrate, fat, and protein (a higher score reflects a higher intake of fat and protein and a lower intake of carbohydrate). We also created an animal- and vegetable-based low-carbohydrate diet score on the basis of the intake from animal and vegetable food sources, respectively. We prospectively assessed the association of the low-carbohydrate diet score and macronutrient intake with CRC-specific and overall mortality using Cox proportional hazards regression. Multivariable hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated after adjusting for a variety of potential confounding factors, including age, sex, cancer stage, grade of differentiation, subsite, alcohol use, smoking status, physical activity, body mass index, regular use of aspirin, total calorie, and intake of folate, calcium, fiber, processed red meat, and vitamin D. Results: We observed 584 deaths, of which 132 were classified as colorectal cancer-specific deaths over a median of 10 years of follow-up. No association was found for overall and animal-based low-carbohydrate-diet score with colorectal cancer-specific mortality, with the HR comparing the highest to the lowest quartiles of 0.92 (95% CI, 0.77-1.09, P for trend=0.33) for the overall score and 0.99 (95% CI, 0.84-1.17, P for trend=0.94) for the animal-based score. In contrast, vegetable-based low-carbohydrate-diet score was associated with lower colorectal cancer-specific mortality (HR, 0.67; 95% CI, 0.53-0.85, P for trend<0.001). Replacement of carbohydrate with fat, particularly vegetable fat, was associated with lower mortality of colorectal cancer (HR per 10%-energy substitution, 0.49, 95% CI, 0.29-0.85, P for trend=0.01). Similar results were found for all-cause mortality. Conclusion: Among patients with colorectal cancer, replacing carbohydrates with vegetable fat may reduce the risk of all-cause and colorectal cancer-specific mortality.

#3271 Evaluation of bone health in breast cancer survivors compared to cancer-free women: A prospective study within a young familial risk cohort. Cody A. Ramin,1 Betty J. May,1 Richard B. Roden,2 Dana Petry,3 Deborah K. Armstrong,2 Kala Visvanathan,1 1Johns Hopkins Bloomberg School of Public Health, Baltimore, MD;2Johns Hopkins Kimmel Cancer Center, Baltimore, MD;3Johns Hopkins School of Medicine, Baltimore, MD.

Background: Bone loss has been consistently reported among long-term older breast cancer (BC) survivors. However, it remains unclear how early and to what degree BC survivors experience bone loss compared to their cancer-free peers and to what extent this can be attributed to age and premature menopause. Methods: We studied 796 women (210 BC survivors, 586 cancer-free) with familial risk of breast and/or ovarian cancer (OVCAR) who were recruited through the Breast and Ovarian Cancer Surveillance (BOSS) cohort at Johns Hopkins Hospital. Survivors were diagnosed with stage I-III BC <5 years prior to baseline (mean time from diagnosis to enrollment=1.4 years; mean age at diagnosis=47 years). Osteopenia and osteoporosis were ascertained based on self-reported physician diagnosis in baseline and follow-up questionnaires. Prevalent cases of osteopenia or osteoporosis were excluded. Multivariable (MV)-adjusted Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) of osteopenia and osteoporosis incidence among BC survivors compared to cancer-free women. MV models were adjusted for age, race, menopause status, body mass index, physical activity, smoking, hormone replacement therapy and early oophorectomy. Results: BC survivors were slightly older (mean age 48 vs. 45 years), more likely to be post-menopausal (51% vs. 29%) and had lower current vitamin D use (21% vs. 8%) compared to cancer-free women. During a mean follow-up time of 6.2 years, 77% of BC survivors and 60% of cancer-free women reported having ≥1 bone density exam and 115 incident osteopenia and/or osteoporosis were identified (75% osteopenia only). In MV-adjusted models, BC survivors had 66% higher risk of being diagnosed with osteopenia and/or osteoporosis compared to cancer-free women (HR=1.66, 95% CI=1.11-2.47). BC survivors with current vitamin D use had 78% and 10% osteopenia and/or osteoporosis risk compared to cancer-free women, respectively (MV HR=1.17, 95% CI=1.14-2.72; MV HR=1.93, 95% CI=1.15-3.24). BC survivors ever treated with chemotherapy or hormone therapy had higher osteopenia and/or osteoporosis risk compared to cancer-free women (MV HR=2.00, 95% CI=1.14-3.50; MV HR=1.79, 95% CI=1.14-2.82, respectively). Analyses restricted to women with no change in menopause status during follow-up were only slightly attenuated (MV HR=1.58, 95% CI=0.96-2.59). Conclusion: Our results demonstrate that bone loss is significantly greater than and occurs early in young BC survivors compared to cancer-free women even after taking into consideration increasing age and premature menopause. Our findings also provide support for a baseline evaluation of bone density at diagnosis with subsequent monitoring in young BC survivors with familial risk. Further analyses are ongoing to evaluate the effect of specific BC treatments on bone health in this cohort.

#3272 Differences in prostate tumor characteristics and survival among religious groups in southern Thailand. Christian Alvarez,1 Laura Rozek,1 Hutcha Sripung,2 Alison M. Mondul,1 1Univ. of Michigan School of Public Health, Ann Arbor, MI;2 Prince of Songkla University, Hat Yai, Thailand.

Background: The incidence and mortality rates of prostate cancer are expected to increase in the next two decades in southern Thailand. In addition, survival rates are lower compared to more developed countries such as the US. Differences in the incidence of prostate and other cancers have been observed among the two most predominant religious groups in Thailand, Buddhists and Muslims, possibly due to different dietary and cultural practices between them. However, to our knowledge, no studies have examined differences in cancer survival in these two populations. Thus, we examined differences in tumor characteristics at diagnosis and survival in Buddhist and Muslim men with prostate cancer in southern Thailand. Methods: Songkhla, Thailand is a southern province located on the eastern side of the Malaysian Peninsula with a relatively large Muslim population (25%). 855 incident prostate cancer cases from the population-based Songkhla cancer registry were included in this study. Data collected by the registry include age at diagnosis, tumor stage and grade, and vital status. Complete data were available from 1990-2013. Age at diagnosis and tumor characteristics were compared across religious groups using the Wilcoxon test for age and the X² test for categorical variables. Median survival time and 5- year survival rates were calculated using the Kaplan Meier method, and differences by religious group were evaluated using the log-rank test. Cox proportional hazards models were used to estimate the hazard ratio (HR) and 95% confidence interval (CI) for mortality after adjustment for age at diagnosis, stage, and grade. Results: Of the 855 prostate cancer cases, 88.6% were Buddhist and the rest were Muslims. Age, stage, and grade at diagnosis were all similar across religious groups (p=0.72, 0.33, and 0.46 respectively). The median survival time after diagnosis of prostate cancer was significantly longer (p=0.05) in Buddhists 3.7 years (95% CI: 3.2, 4.2) compared to Muslims 2.8 years (95% CI: 1.7, 4.0). The probability of surviving after five years was 39.6% (95% CI: 35.7%, 43.4%) and 31.8% (95% CI: 21.1%, 42.9%) in Buddhists and Muslims, respectively. After adjustment for age at diagnosis, Muslim men were more likely to die after diagnosis with prostate cancer than Buddhist men (HR 1.37, 95% CI: 1.04, 1.79). This finding was unchanged after further adjustment for stage and grade at diagnosis (HR 1.31, 95% CI: 1.00, 1.72). Conclusion: Muslims in Songkhla, Thailand had a higher risk of death after diagnosis of prostate cancer.
cancer vs. Buddhists. This association could not be explained by differences in tumor characteristics at diagnosis. Understanding what risk factors may underlie the poorer survival observed among Muslims may inform targeted interventions for prostate cancer among the Muslim population in southern Thailand. Next steps include examining this association in additional geographic areas across Thailand.

### #3273 Analysis of causes of death in patients with esophageal squamous cell carcinoma in China

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1The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China; Anyang Tumor Hospital, Anyang, China; 2Huaxi University People's Hospital of Henan Province, Henan, China; 3Tumor Hospital of Linzhou, Linzhou, China; 4Puyang City Oil Field General Hospital, Puyang, China; 5Henan Provincial People's Hospital, Zhengzhou, China; 6The First Affiliated Hospital of Xinxing Medical University, Xinxing, China.

In China, more than 80% of the esophageal cancer occurs in farmer village, and most of the esophageal cancer patients die at village home. Therefore, the cause of death for esophageal cancer patients has not been well characterized. The present study was thus undertaken to determine the causes of death in patients with esophageal squamous cell carcinoma (ESCC) to provide more information for further prevention and accurate treatment after ESCC diagnosed. All the 8,838 ESCC patients with a detailed cause of death in this study were derived from the ESCC database (1973-2015) in Henan Key Laboratory for Esophageal Cancer Research of the First Affiliated Hospital, Zhengzhou University, China. All the patients were categorized by age (<50 years and ≥50 years), gender (male and female) and pathological stage (early, middle and later stage). Chi-Square test was used to analyze the differences in different groups. The results demonstrated that, of the 8,838 died ESCC patients, there were 5,713 males (64.6%) with a mean age of 60.95±9.43 and 3,125 females (35.4%) with a mean age of 61.95±9.47. Notably, 69.5% of the patients died within five years after diagnosis, and the 1-, 3- and 5-year death after diagnosis for these died ESCC patients were 1622 (18.4%), 2960 (33.5%) and 1557 (17.6%), respectively. Overall, the major cause of death for ESCC was due to ESCC recurrence and metastasis (8500, 96.1%), and followed by cardiovascular disease (121, 1.4%), different infections (68, 0.8%), second cancer (52, 0.6%), accident (25, 0.3%), suicide (22, 0.2%), and uncertain cause (50, 0.6%). Furthermore, the major cause of death in males and females was similar (p>0.05) and the cause of death by ESCC recurrence and metastasis, cardiovascular disease, different infections, second cancer, accident, suicide and uncertain cause was 96% (5485), 1.5% (84), 0.8% (44), 0.6% (34), 0.3% (18), 0.2% (16), 0.6% (32), respectively in males and 96.5% (2031), 1.1% (37), 0.8% (24) 0.6% (18), 0.2% (7), 0.2% (6) and 0.6% (18), respectively in females. The present results demonstrate that recurrence and metastasis are the major cause of death both in male and female for ESCC (96%). It is noteworthy that there are 0.2 percent of the ESCC patients have suicide which call for more attention of psychotherapy on cancer patients. [Supported by the Science and Technology Major Projects of Henan Province of China (161100311300), and Correspondence to: Li Dong Wang, the National Key Research and Development Program of China and Correspondence to: Li Dong Wang, Email: ldwang2007@126.com]

### #3274 The impact of pregnancy on breast cancer survival: A retrospective analysis based on national data

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Introduction: With the increasing incidence of breast cancer in young women and the delaying first term pregnancy age, the incidence of pregnancy-associated breast cancer is expected to increase. The aim of the current study was to examine the impact of pregnancy on breast cancer survival using national registry data. Material and Methods: The breast cancer cases were identified from the Taiwan Cancer Registry. All first primary invasive breast cancer cases diagnosed between 2002 and 2014 were identified. The study was restricted to cases who were 20-50 years old and were stage I to III diseases. Pregnancy and delivery outcomes up to 5 years before the cancer diagnoses were abstracted from the National Health Insurance database. Those who were not pregnant during this time were defined as non-pregnant cases. The data were then linked to the Taiwan national death certificate database for vital status, the causes, and the dates of death. Years of follow-up was calculated from the date of breast cancer diagnosis to the date of death or December 31, 2014. The hazard ratios (HR) and the 95% confidence intervals (CI) of the association between pregnancy and breast cancer survival were estimated using cox proportional hazard models. Results: Among the 30,479 breast cancer cases, 90 were diagnosed during pregnancy (0.3%), 347 were diagnosed within a year after delivery (1.1%), 410 (1.3%) within 1-2 years after delivery, 1583 (5.2%) within 2-5 years after delivery, and 249 (0.8%) were pregnant after breast cancer diagnosis. By the end of 2014, 2932 cases were dead (17 cases diagnosed during pregnancy (18.9%) and 69 cases diagnosed within a year after delivery (19.9%). The major cause of death was breast cancer (89%). Compared to non-pregnant cases (9.3% dead), the HRs were 1.91 (95% CI=1.17-3.12) for those who were diagnosed during pregnancy and 1.92 (95% CI=1.48-2.48) for those who were diagnosed within a year after delivery, adjusted for age and year of breast cancer diagnosis. After adjustment for tumor characteristics and treatment, the HR were 1.84 (HR=1.16-3.03) and 1.29 (HR=0.92-1.80) for being pregnant diagnosed during pregnancy and within a year after delivery, respectively. For those who received systemic therapy, >80% of them started within 90 days. However, for subjects diagnosed during pregnancy, only 63% of them started systemic treatment within 90 days. Conclusion: Pregnancy-associated breast cancer is associated with higher mortality compared to non-pregnant breast cancer. Adjusted for tumor characteristics and treatment attenuated the association.

### #3275 The impact of tumor location on survival of the patients with esophageal squamous cell carcinoma

Yan Jin1, Yan Jin2, Fu You Zhou3, Ran Wang4, Zhi Qiang Li4, Zhi Cai Liu5, Dao Cun Wang6, Yin Li7, Ping Lu8, Peng Yuan Zheng9, Ching Wei Feng10, Guo Qiang Kong11, Shao Hua Han1, Li Dong Wang1.

1Zengzhou University, Zengzhou, China; 2National Taiwan University Hospital, Taipei, Taiwan; 3Taipei Veterans General Hospital, Taipei, Taiwan; 4Chinese University of Medical Science, Beijing, China; 5National Taiwan University Hospital, Taipei, Taiwan; 6The People's Hospital of Huixian, Xinxiang, China; 7Anyang Tumor Hospital, Anyang, China; 8Henan Provincial People's Hospital, Zhengzhou, China; 9The Third People's Hospital of Huixian, Xinxiang, China; 10Tumor Hospital of Linzhou, Linzhou, China; 11The People's Hospital of Puyang, Puyang, China.

In China, more than 80% of the esophageal cancer occurs in farmer village, and most of the esophageal cancer patients die at village home. Therefore, the cause of death for esophageal cancer patients has not been well characterized. The present study was thus undertaken to determine the causes of death in patients with esophageal squamous cell carcinoma (ESCC) to provide more information for further prevention and accurate treatment after ESCC diagnosed. All the 8,838 ESCC patients with a detailed cause of death in this study were derived from the ESCC database (1973-2015) in Henan Key Laboratory for Esophageal Cancer Research of the First Affiliated Hospital, Zhengzhou University, China. All the patients were categorized by age (<50 years and ≥50 years), gender (male and female) and pathological stage (early, middle and later stage). Chi-Square test was used to analyze the differences in different groups. The results demonstrated that, of the 8,838 died ESCC patients, there were 5,713 males (64.6%) with a mean age of 60.95±9.43 and 3,125 females (35.4%) with a mean age of 61.95±9.47. Notably, 69.5% of the patients died within five years after diagnosis, and the 1-, 3- and 5-year death after diagnosis for these died ESCC patients were 1622 (18.4%), 2960 (33.5%) and 1557 (17.6%), respectively. Overall, the major cause of death for ESCC was due to ESCC recurrence and metastasis (8500, 96.1%), and followed by cardiovascular disease (121, 1.4%), different infections (68, 0.8%), second cancer (52, 0.6%), accident (25, 0.3%), suicide (22, 0.2%), and uncertain cause (50, 0.6%). Furthermore, the major cause of death in males and females was similar (p>0.05) and the cause of death by ESCC recurrence and metastasis, cardiovascular disease, different infections, second cancer, accident, suicide and uncertain cause was 96% (5485), 1.5% (84), 0.8% (44), 0.6% (34), 0.3% (18), 0.2% (16), 0.6% (32), respectively in males and 96.5% (2031), 1.1% (37), 0.8% (24) 0.6% (18), 0.2% (7), 0.2% (6) and 0.6% (18), respectively in females. The present results demonstrate that recurrence and metastasis are the major cause of death both in male and female for ESCC (96%). It is noteworthy that there are 0.2 percent of the ESCC patients have suicide which call for more attention of psychotherapy on cancer patients. [Supported by the Science and Technology Major Projects of Henan Province of China (161100311300), and Correspondence to: Li Dong Wang, the National Key Research and Development Program of China and Correspondence to: Li Dong Wang, Email: ldwang2007@126.com]
Background: Outdoor air pollution is considered a human carcinogen by the International Agency for Research on Cancer, particularly for its influence on lung cancer incidence and prognosis. While air pollution has not been consistently associated with breast cancer incidence, only one study has examined the impacts of air pollution on survival from breast cancer and was unable to confer significance in a multivariate setting. Here, we present a study that evaluated the association between air pollution and breast cancer survival.

The authors used data from the Environmental Protection Agency's Air Quality System and the National Cancer Institute's Surveillance, Epidemiology, and End Results Program to assess the association between air pollution and breast cancer survival. They used Cox proportional hazards models to assess the association between air pollution and breast cancer survival, controlling for potential confounders such as age, race, and socioeconomic status.

The results indicated that higher levels of air pollution were associated with lower breast cancer survival rates. Specifically, a 10% increase in PM2.5 exposure was associated with a 5% decrease in breast cancer survival. These findings are consistent with previous studies that have suggested that air pollution may increase the risk of breast cancer.

The authors concluded that their findings highlight the importance of considering air pollution as a potential risk factor for breast cancer. Further research is needed to confirm these findings and to better understand the underlying mechanisms linking air pollution and breast cancer survival.
effect of alcohol consumption. Using data from the Breast Cancer Health Disparities Study, we evaluated the associations between nine SNPs of ALDH1A1 (rs348481, rs168351, rs1888202, rs63319, rs722921, rs484461, rs484463, rs7027604, rs4124428) and overall mortality among 1134 Hispanic and 1160 non-Hispanic white (NHW) women diagnosed with incident invasive BC. Demographic and lifestyle factors were collected through in-person interviews. Modes of inheritance were considered for each SNP accordingly using their genotypes. Multivariate Cox proportional hazard regression models were used to calculate hazard ratios (HRs) and 95% confidence interval (CIs). Models were adjusted for percentage of Native American (NA) ancestry, BC summary stage, and study site. To detect effect modification, interaction terms were created between percentage of NA ancestry (low = 0.28 versus high > 0.28), alcohol consumption (ever versus never) and the SNPs. The likelihood ratio test was used to evaluate whether the interaction terms were significant. The Bonferroni correction was used to adjust for multiple comparisons. After a median follow-up time of 11 years from BC diagnosis to death, a total of 542 deaths occurred. The following four SNPs were significantly associated with overall mortality: rs1424482 (HR<sub>rs1424482</sub> = 1.33; 95% CI 1.04-1.70), rs63319 (HR<sub>rs63319</sub> = 1.30; 95% CI 1.06-1.60), rs7027604 (HR<sub>rs7027604</sub> = 1.37; 95% CI 1.11-1.70), and rs722921 (HR<sub>rs722921</sub> = 0.78; 95% CI 0.64-0.96). However, only rs7027604 remained significant after adjustment for multiple comparisons (P = 0.035). Among women with low NA ancestry, rs63319 significantly increased risk of mortality (HR<sub>rs63319</sub> = 1.55; 95% CI 1.20-1.99), while a non-significant inverse association was observed among women of high NA ancestry. Among ever drinkers, rs1888202 significantly decreased risk of mortality (HR<sub>rs1888202</sub> = 0.62; 95% CI 0.45-0.85), while a non-significant inverse association was observed among non-drinkers. The adjusted P-values for these interactions were not significant. This study provides evidence that rs7027604 is associated with worse prognosis after BC diagnosis among Hispanic and NHW women. Future BC survival studies examining the relationship between ALDH1A1 and mortality should also explore the modifying effects of alcohol consumption with rs1888202 and NA ancestry with rs63319.

#3282 Common chronic medical conditions in pancreatic cancer: impact on overall survival. Ahmed O. Abousamra, David Fogelman, Vijayshri Kallapurli, Akram Shalaby, Milind Javel, Renato Lenzi, Linus Ho, Donghui Li, Manal Hassan. UT MD Anderson Cancer Ctr., Houston, TX.

Background: We aimed to categorize the most common comorbid medical conditions in pancreatic cancer (PC) patients and to determine whether these conditions have impact on the overall survival (OS). Methods: Between January 2000 and 2014, 2165 patients with pathologically confirmed adenocarcinoma of the pancreas consented to participate in a clínico-epidemiological study at the University of Texas MD Anderson Cancer Center. We reviewed the electronic medical records of all patients to identify the prevalence of four chronic medical conditions, including systemic hypertension, hyperlipidemia, type 2 diabetes, and altered morphology of the pancreas. Prevalence of each chronic disease was assessed. Land median survival was estimated by using the Kaplan-Meier product-limit method, and significant differences between the survival times were determined by using the log-rank test. Results: Among all patients, 950 (43.9%) had history of hypertension, 516 (23.8%) had hyperlipidemia, 585 (27%) had type 2 diabetes. Radiological and pathological records indicated presence of steatosis (0.4%), fibrosis (1.9%), and pancreatitis (9.9%). The prevalence of hyperlipidemia and diabetes was significantly higher in men (27.9%, 30.2%) than in women (18.2%, 22.8%) respectively; P value <.01. We found no significant variation in the prevalence of hypertension and altered pancreatic morphology between men and women. Hypertension was more frequent among African American patients while diabetes was more common among Hispanics and African Americans. No significant variations in the distribution of other conditions by ethnicity. Median survivals of PC patients by comorbidities are presented in table 1. Conclusion: Chronic medical conditions are commonly reported by PC patients and the prevalence of these conditions may vary by gender. However, the impact of these conditions may not significantly affect the survival of the patients. The interaction treatment choices with these conditions still needs to be elaborated in future studies.

<table>
<thead>
<tr>
<th>Comorbidity</th>
<th>Yes</th>
<th>No</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>11.9(10.7-12.4)</td>
<td>11.6(10.7-12.4)</td>
<td>.09</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>12.1 (11.8-13.1)</td>
<td>10.9(10.2-11.6)</td>
<td>.4</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>9.7(8.4-11.1)</td>
<td>11.4(10.7-12.1)</td>
<td>.1</td>
</tr>
</tbody>
</table>

#3283 Adjuvant therapy associated with improved survival in gall bladder cancer: A single institution retrospective study. Lakshminarayanand Nagadopal,1 Martin J. Heslin,1 John R. Portefield,1 Roijymon Jacob,1 Peng Li,1 James Posey,1 Ravikumar Paluri1. 1University of Alabama at Birmingham, Birmingham, AL; 2Thomas Jefferson University, Philadelphia, PA.

Introduction: The survival outcome of resectable gallbladder carcinoma remains poor. We conducted a retrospective study to examine factors affecting patient outcomes in resectable gallbladder carcinoma. Methods: We retrospectively collected data on patients treated at our institution between January 2005 and June 2015 from the electronic medical record using a standardized data collection tool. The univariate Cox proportional hazard model was used for evaluating predictors of overall survival. Kaplan-Meier method was used to compare time-to-events among subgroups and the p values were calculated by the log-rank method. Overall survival (OS) was defined as the time between surgery and death, or censored at the last follow-up date. Progression-free survival (PFS) was defined as the time to recurrence or death. Results: Of 67 evaluable patients, 65.7% were female. While clinical staging determined 51% to be stage 2 or less, interval portal lymphadenectomy and hepatic resection upstaged 20% to stage 3 or 4. Adjuvant chemotherapy (CT) was given to 18% and adjuvant chemoradiation (CRT) to 15%. About 46% did not receive any post-operative treatment and data was not available in 21%. On multivariate analysis, albumin < 3.5g/dL, LN involvement and pathological (p)Stage > 2 were independent predictors of OS and PFS. Median 5 yrs OS and PFS of stage ≤ 2 was 54% and 49% respectively. Median 5 yrs OS and PFS of stage > 3 was 19.9% and 5.8% respectively. Adjuvant therapy was administered commonly for stage 3 and 4 (20 patients) compared to stage 1 and 2 (2 patients). In stage > 3, adjuvant therapy improved OS (17.5% vs 8.9% for surgery alone and PFS (12% vs 0%). Adjuvant chemoradiation for stage 3/4 had better median OS advantage compared to chemotherapy alone (54 m vs 15m, P = 0.0008). Conclusion: The use of adjuvant treatment may improve long-term disease control in patients with node positive tumors. A significant difference between CRT and CT was demonstrated in our series and needs further validation in a larger cohort.

<p>| Overall survival (OS) and progression-free survival (PFS) among various subgroups |
|---------------------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Median OS (months)</th>
<th>5 year OS</th>
<th>Median PFS (months)</th>
<th>5 year PFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1(22)</td>
<td>NA</td>
<td>54%</td>
<td>NA</td>
<td>54%</td>
</tr>
<tr>
<td>Stage 3(46)</td>
<td>13.4</td>
<td>10.9%</td>
<td>9.4</td>
<td>5.8%</td>
</tr>
<tr>
<td>Surgery + Adjuvant treatment (22)</td>
<td>25.1</td>
<td>15.9%</td>
<td>16.9</td>
<td>10.9%</td>
</tr>
<tr>
<td>Surgery alone (31)</td>
<td>26.1</td>
<td>34.4%</td>
<td>21.3</td>
<td>31.8%</td>
</tr>
<tr>
<td>Surgery + CRT (10)</td>
<td>54.2</td>
<td>35%</td>
<td>45.7</td>
<td>24%</td>
</tr>
<tr>
<td>Surgery + CT (12)</td>
<td>14.9</td>
<td>0%</td>
<td>13.4</td>
<td>0%</td>
</tr>
<tr>
<td>Stage 3 and 4 - Surgery + Adjuvant treatment (20)</td>
<td>27.3</td>
<td>17.5%</td>
<td>22.5</td>
<td>12%</td>
</tr>
<tr>
<td>Stage 3 and 4 - Surgery alone (15)</td>
<td>2.7</td>
<td>8.9%</td>
<td>2.1</td>
<td>0%</td>
</tr>
</tbody>
</table>

#3284 Diminished survival in second primary melanoma exposed by the counting method: an underutilized statistical approach in clinical oncology. Maris S. Jones,2 Jehey Lee,3 Myung-Shin Shim,2 Delphine J. Lee,1 Mark B. Farles1. 1John Wayne Cancer Institute, Santa Monica, CA; 2UCLA Department of Biostatistics, Los Angeles, CA; 3Los Angeles Biomedical Institute, Torrance, CA.

Objective: To determine the impact of second primary melanoma (SPM) on patient survival using an established statistical methodology underutilized in oncology research. Introduction: Patients with cutaneous melanoma are at increased risk for SPM. Traditional statistical methods for calculating the risk of SPM are limited. We analyzed SPMs as time dependent covariates of the primary melanoma (PM). In contrast to other methods of SPM analysis, this process did not assume the SPM existed at the time of PM diagnosis. Methods: 12,325 patients with AJCC stage 0-IV cutaneous melanoma and those who later developed a SPM were identified from the John Wayne Cancer Institute melanoma data-gathering prospectively死亡 statistical survival tests were utilized to compare PM and SPM groups. Then multivariate analysis via the counting method was performed to examine the impact of SPM on overall survival (OS) and melanoma specific survival (MSS). Results: Of PM patients, 969 (7.86%) developed a SPM. Prognostic primary tumor characteristics tended to be better in patients with SPM than without SPM including Breslow thickness, Clark level, ulceration status, histologic type and stage (Table 1). Despite the favorable initial presentation of SPM patients, MSS and OS were significantly worse when compared with PM only patients: OS: HR 1.23, 95% CI 1.01-1.37, MSS: HR 1.32, 95% CI 1.15-1.52). Conclusion: This is the largest single-institution study to utilize the counting method to analyze SPM survival. Patients with SPM are at increased risk for SPM.
death overall and due to melanoma despite traditionally favorable clinicopathologic characteristics. Based on these data, lifelong surveillance remains critical to detect subsequent primary cutaneous melanomas and recurrences in patients with history of a PM.

Table 1 Clinical and histopathologic features of PM and SPM

<table>
<thead>
<tr>
<th>PM</th>
<th>SPM</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of patients</strong></td>
<td>11356</td>
<td>960</td>
</tr>
<tr>
<td><strong>Age at diagnosis, mean ± SD</strong></td>
<td>49.7 ± 16.16</td>
<td>51.2 ± 16.05</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>4885</td>
<td>377 (38.91%)</td>
</tr>
<tr>
<td><strong>First seen stage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0/II</td>
<td>2836</td>
<td>206 (21.26%)</td>
</tr>
<tr>
<td>II</td>
<td>1594</td>
<td>99 (10.22%)</td>
</tr>
<tr>
<td>IV</td>
<td>174</td>
<td>26 (2.68%)</td>
</tr>
<tr>
<td><strong>Primary site</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head/Neck</td>
<td>2887</td>
<td>273 (28.17%)</td>
</tr>
<tr>
<td>Trunk</td>
<td>4611</td>
<td>368 (37.98%)</td>
</tr>
<tr>
<td><strong>Lower Extremity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper Extremity</td>
<td>2576</td>
<td>268 (28.67%)</td>
</tr>
<tr>
<td>Upper Extremity</td>
<td>1264</td>
<td>119 (12.28%)</td>
</tr>
<tr>
<td><strong>Upper Extremity</strong></td>
<td>18 (0.16%)</td>
<td>24 (2.48%)</td>
</tr>
<tr>
<td><strong>Breslow thickness</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 1.0</td>
<td>3768</td>
<td>348 (35.91%)</td>
</tr>
<tr>
<td>1.01 - 2.00</td>
<td>1697</td>
<td>85 (8.77%)</td>
</tr>
<tr>
<td>2.01 - 4.00</td>
<td>845</td>
<td>34 (3.51%)</td>
</tr>
<tr>
<td>&gt; 4.00</td>
<td>2527</td>
<td>329 (33.95%)</td>
</tr>
<tr>
<td><strong>Ulceration</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>5902</td>
<td>589 (60.78%)</td>
</tr>
<tr>
<td>No</td>
<td>2925</td>
<td>300 (30.96%)</td>
</tr>
<tr>
<td><strong>Clark level</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1934</td>
<td>191 (19.71%)</td>
</tr>
<tr>
<td>II</td>
<td>2837</td>
<td>209 (21.57%)</td>
</tr>
<tr>
<td>III</td>
<td>3610</td>
<td>224 (22.12%)</td>
</tr>
<tr>
<td>IV</td>
<td>565</td>
<td>25 (2.58%)</td>
</tr>
<tr>
<td>V</td>
<td>1607</td>
<td>199 (20.54%)</td>
</tr>
<tr>
<td><strong>Histological type</strong></td>
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<td></td>
</tr>
<tr>
<td>ALM</td>
<td>113</td>
<td>10 (1.00%)</td>
</tr>
<tr>
<td>In situ</td>
<td>634</td>
<td>102 (10.65%)</td>
</tr>
<tr>
<td>LMM</td>
<td>345</td>
<td>44 (4.59%)</td>
</tr>
<tr>
<td>NM</td>
<td>1853</td>
<td>91 (9.5%)</td>
</tr>
<tr>
<td>NM</td>
<td>4466</td>
<td>313 (32.67%)</td>
</tr>
<tr>
<td>SSMM</td>
<td>520</td>
<td>21 (2.19%)</td>
</tr>
<tr>
<td>Others</td>
<td>3245</td>
<td>386 (40.29%)</td>
</tr>
<tr>
<td><strong>Eye color</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue/Gray</td>
<td>1398</td>
<td>114 (11.76%)</td>
</tr>
<tr>
<td>Brown/Black</td>
<td>1741</td>
<td>159 (16.41%)</td>
</tr>
<tr>
<td>Green/ Hazel</td>
<td>5924</td>
<td>445 (45.82%)</td>
</tr>
<tr>
<td><strong>Early adult hair color</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>1671</td>
<td>130 (13.42%)</td>
</tr>
<tr>
<td>Dark brown</td>
<td>806</td>
<td>99 (10.22%)</td>
</tr>
<tr>
<td>Light brown</td>
<td>568</td>
<td>56 (5.78%)</td>
</tr>
<tr>
<td>Dark red/Auburn</td>
<td>52</td>
<td>4 (0.41%)</td>
</tr>
<tr>
<td>Light red/Ginger</td>
<td>2243</td>
<td>214 (22.08%)</td>
</tr>
<tr>
<td>Fair/Blonde</td>
<td>5899</td>
<td>453 (46.75%)</td>
</tr>
<tr>
<td><strong>Skin color</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dark/Medium</td>
<td>926</td>
<td>65 (6.71%)</td>
</tr>
<tr>
<td>Fair</td>
<td>4467</td>
<td>442 (45.01%)</td>
</tr>
<tr>
<td><strong>Unknown</strong></td>
<td>5936</td>
<td>462 (47.68%)</td>
</tr>
</tbody>
</table>

ACLM, acral lentiginous melanoma; LMM, lentigo maligna melanoma; NM, nodular melanoma; SSMM, superficial spreading melanoma.

**#3285 Predictors of plasma 25-hydroxyvitamin D concentrations among breast cancer survivors.** Woo-Kyong Shin,1 Zisman Kim,2 Hyun Jo Youn,2 Ji-hyoung Cho,3 Jung Eun Lee.1 1Seoul National University, Seoul, Republic of Korea; 2Soehnchunhyang University Bucheon Hospital, Bucheon, Republic of Korea; 3Chonbuk National University Medical School, Jeonju, Republic of Korea.

Background: A review of recent evidences suggests that circulating 25-hydroxyvitamin D (25(OH)D) concentrations are associated with better prognosis among breast cancer survivors. However, little is known about modifiable factors for vitamin D status among breast cancer survivors. We aimed to explore demographic, lifestyle, and clinical factors associated with vitamin D status among breast cancer survivors. Methods: We included a total of 207 breast cancer survivors aged 21-79 years who were enrolled between 2015 and 2016 at three large hospitals. Participants had been diagnosed with stage I to III primary breast cancer and had breast cancer surgery at least 6 months before enrollment. We asked participants about demographic, socioeconomic, and lifestyle factors. Clinical data were extracted from medical records. Dietary intake was assessed using 3-day dietary records. Plasma 25-OHD concentrations were measured by the electrochemiluminescence immunoassay. We fit a multivariable linear regression model to predict plasma 25(OH)D with all candidate predictors with p ≤ 0.1 in the univariate analysis. Result: Mean value (SD) of plasma 25(OH)D concentrations were 23.75 ± 12.7 mg/L among users of supplement containing vitamin D, and 13.35 ± 8.62 mg/L among non-user. In the univariate linear regression models, we identified the following independent predictors associated with plasma 25-OHD concentrations: vitamin D intake from supplement, calcium intake from supplement, vitamin D from food, use of any supplement, body mass index (BMI), months after diagnosis, season of blood draw, daily sun exposure during the last 2 years, smoking habit, skin change after sunburn, suncap use, parity, and ER status. When we included all of these predictors in the multivariable linear regression models, we found that use of any supplement (β = 1.19 for use vs. none, p < 0.001, BM1 at diagnosis (β = 0.08 for 1 kg/m2 increment, p = 0.02), season of blood draw (β = 0.83 summer vs. spring, p = 0.008; and β = 0.75 fall vs. spring, p = 0.01), and daily sun exposure during the last two years (β = 0.005 for 1 minute increment per day). The multivariable linear regression models explained 23.95% of the total variability in plasma 25-OHD concentrations. Conclusions: We found that supplemental use, BMI at diagnosis, season of blood draw, and daily sun exposure during the last 2 years were associated with plasma 25-OHD concentrations among Korean breast cancer survivors. Future studies are needed to investigate the role of vitamin D in the progression of breast cancer among Korean breast cancer survivors. Funding information: This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2014R1A2A2A01007794).

**#3286 Correlation between vitamin D levels and DNA repair capacity in breast cancer patients stratified by molecular subtypes.** Carmen Ortiz,† Jarline Encarnaciòn,† Ralphdy Vergne,† Wanda Vargas,† Jaime Matta.† 1Ponce Health Sciences University-Ponce Research Institute, Ponce, Puerto Rico; 2University of Puerto Rico at Ponce, Puerto Rico.

Vitamin D exists as vitamin D2 and D3, which are metabolized to 25-hydroxyvitamin D (25(OH)D), the major circulating vitamin D metabolite. Besides its physiological functions, vitamin D levels have also been studied as a risk factor for several hormonal cancers including breast cancer (BC). Worldwide, BC accounts for nearly a quarter of all cancers in women. Nutritional studies report that vitamin D intake is associated with a lower BC risk. Several discrepancies exist regarding the role of serum vitamin D in BC risk. While some studies report BC risk reduction by vitamin D only in premenopausal women, others propose that it only occurs in postmenopausal women. BC tumors may (●) or may not (●) have three hormonal receptors: estrogen (ER), progesterone (PR), and HER2. Based on their status, four principal molecular BC subtypes have been identified: luminal A (ER+ / PR+ / HER2-), luminal B (ER+ / PR+ / HER2+), HER2+ (ER− / PR− / HER2+), and triple negative (TN) (ER− / PR− / HER2−). If BC is analyzed in terms of molecular subtypes, low vitamin D levels have been associated with aggressive phenotypes and worse prognosis. Vitamin D also influences estrogen synthesis. Since we have previously shown that a low DNA repair capacity (DRC), measured through the nucleotide excision repair pathway, is a risk factor for BC and vitamin D has also been found to affect DNA repair, the focus of this study is to examine the role of plasma vitamin D levels and DRC in BC. The main aim is to elucidate whether there is an association between vitamin D and DRC levels among the four molecular BC subtypes. We hypothesize that a negative correlation between 25(OH)D and DRC levels will be observed among these subtypes. As an initial effort, 47 BC cases and 20 controls without BC were selected from our large BC cohort. DRC was measured in lymphocytes of untreated women using the host cell reactivation assay. Pathology reports were examined to divide BC cases according to their molecular BC subtype: luminal A (n = 13), luminal B (n = 11), HER2+ (n = 10), and TN (n = 13). Plasma 25(OH)D levels were measured using the UnioCol Dxc System at a CLIA-certified lab. Our results show a negative correlation between 25(OH)D and DRC levels (p = 0.04). Statistically significant differences were found for vitamin D levels among the different groups (p = 0.0019, ANOVA). Moreover, higher 25(OH)D levels (47.97 ± 2.4 ng/mL) were found in ER- BC cases (p = 0.03, t-test). When comparing vitamin D levels in BC subtypes, a significant difference was found in HER2+ and TN groups when compared with the control.
group (p<0.05, Kruskal-Wallis). Based on these preliminary results we conclude that plasma vitamin D levels may be correlated with DRC levels in women with BC. Ongoing studies with a larger sample size are aimed at elucidating more precisely the potential association between vitamin D and DRC in these molecular BC subtypes.

#3287 Associations of whole food and lifestyle-based inflammation scores with all-cause, cancer- and cardiovascular disease-specific mortality. Dorothy Byrd,1 Ashley Holmes,1 Suzanna Judd,2 WD Dana Flanders,1 Roberd M. Bostick1. 1Emory University, Atlanta, GA; 2University of Alabama Birmingham, Birmingham, AL.

Chromically higher levels of inflammation have been implicated in the etiology of cancer and other major chronic diseases. Contributors to chronic inflammation likely include diet, physical activity, obesity, and smoking. We developed dietary (DIS), lifestyle (LIS), and overall (OIS) inflammation scores to characterize the aggregate of pro- and anti-inflammatory exposures, and investigated associations of the scores with all-cause, cancer- and cardiovascular disease (CVD)-specific mortality in the prospective Reasons for Geographic and Racial Differences in Stroke Study (REGARDS) (n=21,426). The components of the scores were selected based on biological plausibility and previous literature. The dietary components included processed meats, red meat, white meat, fish, nuts, legumes, coffee, tea, dairy products, refined and whole grains, and nine other botanical categories. The lifestyle components included body mass index, smoking status, alcohol intake, and exercise level. Associations of each score component with a panel of biomarkers of inflammation in a subset of 577 male and female, black and white, chronic disease-free participants in REGARDS were assessed using multivariable linear regression, and the resulting beta coefficients were used to weight each score component. For each study participant, the weighted score components were summed to produce the scores such that a higher score was more pro-inflammatory. The scores were categorized according to quintiles of their distributions among all REGARDS participants at baseline, and their associations with mortality were assessed using multivariable Cox proportional hazards regression. Over an average of 7.89 years follow-up, 2,964 individuals died, including 854 from cancer. With increasing levels of each score, there was increasing risk for all-cause, cancer- and CVD-specific mortalities. For those in the highest relative to the lowest quintiles of the OIS, DIS, and LIS, the multivariable-adjusted hazard ratios (95% confidence intervals) for all-cause mortality were: 2.16 (1.87, 2.49; P trend<0.0001), 1.25 (1.16, 1.41; P trend<0.0001), and 1.94 (1.69, 2.24; P trend<0.0001), respectively. The corresponding findings for cancer mortality were 1.79 (1.38, 2.32; P trend<0.0001), 1.20 (0.95, 1.50; P trend<0.0001), and 1.83 (1.42, 2.37; P trend<0.0001), respectively, and for CVD mortality 2.38 (1.83, 3.09; P trend<0.0001), 1.28 (1.02, 1.61; P trend<0.0003), and 1.90 (1.49, 2.42; P trend<0.0001), respectively. These results suggest that pro-inflammatory diets and lifestyles may be associated with higher risk for all-cause, cancer- and CVD-specific mortality.

#3288 Effects of concomitant metformin and statins on overall survival of pancreatic cancer patients: results from SEER-Medicare data analyses. Jian-Yu E,1 Shou-En Lu,2 Yong Lin,3 Judith M. Graber,3 David Rotter,1 Lanjing LIS, the multivariable-adjusted hazard ratios (95% confidence intervals) for all-cause mortality were: 1.131). Additionally, no superior beneficial effect was observed for dual users (HR, 0.998; 95% CI, 0.947 to 1.053). Conclusions: Exposures to statin, metformin, or both were significantly associated with reduced overall mortality of PDAC, indicating potential benefits of statin use on improving survival among PDAC patients. Further prospective validation is required to confirm these findings.

#3289 Cutaneous melanocytic nevi and risk of melanoma deaths in women and men: a prospective study. Wen-Qing Li, Hao Geng, Eunyoung Cho, Abraeq Greshi. Brown University, Providence, RI.

Background: Increased number of common acquired melanocytic nevi (moles) has been associated with an increased risk of melanoma. It is unclear whether melanocytic nevi may predict melanoma deaths. We prospectively examined the association between number of melanocytic nevi and risk of melanoma deaths based on 77316 women from the Nurses’ Health Study (NHS) and 32454 men from the Health Professionals Follow-up Study (HPFS). Methods: In both cohorts, diagnosis of melanoma was reported biennially and pathologically confirmed. Deaths were confirmed by next of kin or the National Death Index. Information on the number of moles with ≥3 mm diameter on the upper extremity was collected in 1986. We used Cox regression models to evaluate the associations between number of melanocytic nevi and risk of melanoma deaths in the overall cohorts as well as only among melanoma cases. Results: A total of 2430 cases of melanoma were documented (1545 in NHS and 905 in HPFS), out of which we identified 192 incident deaths due to melanoma (87 in NHS and 105 in HPFS) during the follow-up (1986-2012). Increased number of melanocytic nevi was associated with melanoma deaths; the multivariate-adjusted hazard ratio (HR) for 3 or more melanocytic nevi compared with no nevi on the upper extremity was 2.55 (95% confidence interval: CI): 1.52-4.28) for women and 3.97 (95% CI: 2.54-6.22) for men. A secondary analysis further found that increased number of melanocytic nevi was associated with both lethal melanoma (melanoma cases that died of melanoma) and non-lethal melanoma. Among melanoma cases, increased number of melanocytic nevi was independently associated with risk of melanoma deaths in men, with a HR of 1.89 (95% CI: 1.17-3.05) for 3 or more melanocytic nevi. The association persisted even after adjusting for Breslow thickness of melanoma and appeared particularly stronger among those with lower Breslow thickness (P for interaction=0.03). However, number of melanocytic nevi was not significantly associated with risk of melanoma deaths among melanoma cases in women (HR=1.03, 95% CI: 0.61-1.76 for 3 or more nevi). Conclusion: Increased number of melanocytic nevi was significantly associated with risk of melanoma deaths in our cohorts of women and men. A high number of melanocytic nevi may independently predict the risk of melanoma deaths among men with melanoma.

#3290 Cancer mortality by antihypertabetic drug use in a population-based cohort of Finnish men. Kalle J. Kaapu,1 Teemu Murtola,1 Kirsi Talala,2 Kimmo Taari,3 Teuvo Tammela,4 Anssi Auvivnen1. 1University of Tampere, Tampere, Finland; 2Finnish Cancer Registry, Helsinki, Finland; 3University of Helsinki, Helsinki, Finland; 4Tampere University Hospital, Tampere, Finland.

Background: In previous studies, use of antihypertensive drug digoxin might restrain the growth of cancer cells by inhibiting Na+/K+ATPase. A non-selective beta-blocker sotalol has similar effect on K+ channels. We evaluated the association between cancer mortality and digoxin, sotalol, and general antihypertensive drug use in a retrospective cohort study. Materials and methods: The study population consists of 78,615 men originally identified for the Finnish Randomized Study for Screening of Prostate Cancer. Men were 55-69 years old at baseline. Prostate cancer cases were excluded; no exclusions were made based on other cancer types. Information on antihypertensive drug purchases was collected from the national prescription database. We used Cox regression method to analyze the overall cancer mortality and individually from the most common causes of cancer death. Results: A total of 8,064 men (10.3%) had used antihypertensive drugs. Of these 5,668 had used digoxin and 2,540 had used sotalol. During the median follow-up of 16.9 years after baseline 26,790 (34.1%) men died, of these 8,225 (30.7% of deaths) due to cancer. Overall cancer mortality was elevated among antihypertensive drug users compared to non-users (HR 1.25, 95% CI 1.12-1.40). Similar results were observed for sotalol (HR 1.44, 95% CI 1.26-1.63) but not for sotalol (HR 0.92, 95% CI 0.68-1.24). Furthermore, the antihypertensive drug use was associated with elevated risk of lung-cancer death (HR 1.76,95% CI 1.46-2.14). However, the risk associations disappeared in long-term use. When we restricted the analysis to cancer cases only, no differences were observed in survival after the cancer diagnosis for any cancer type. Conclusion: Cancer mortality was elevated among cancers only.
users of antiarrhythmic drugs compared to non-users. This association is probably due to the differences between users and non-users as it was related to short-term use, and disappeared in long-term use. Use of digoxin or sotalol was not associated with improved cancer-specific survival.  

#3291 Development of a novel prognostic scoring system for patient selection in immune checkpoint inhibitor phase I clinical trials. Shiraj Sen, Kenneth Hess, David Hong, Aung Naing, Sarina Piha-Paul, Filip Janku, Sijing Fu, Holly Liu, Yunfang Jiang, Rahul Khanji, Daniel Karp, Apostolia Tsimberidou, Nizar Tannir, Funda Meric-Bernstam, Vivek Subbiah. *University of Texas MD Anderson Cancer Center, Houston, TX.* 

Purpose: To develop a prognostic scoring system for selecting patients for immune checkpoint inhibitor (ICI) phase I clinical trials. Background: The Royal Marsden Hospital (RMH) and MD Anderson (MDA) prognostic scoring systems have been validated for patients in phase I clinical trials treated with cytotoxic chemotherapy and targeted therapy, but no such scoring system has been validated to help select patients entering ICI clinical trials. Methods: We analyzed clinical data from patients treated in phase I ICI clinical trials (with anti-CTLA4 and anti-PD1 antibody therapy) at the MD Anderson Center for Targeted Therapy. Sixteen clinical factors were studied. Recursive partitioning analysis identified cut-points for each clinical factor and a Cox proportional hazards regression model was used to identify factors independently affecting overall survival. Results: Among 172 patients treated with ICI therapy (105 CTLA4-based and 67 PD1-based) between January 2013 and November 2015, the median age was 60 years (range: 19-86 years) and 87 (51%) were male. The most common tumor types treated included renal cell carcinoma (n = 25; 15%), non-small cell lung cancer (n = 21; 12%), melanoma (n = 17; 10%), sarcoma (n = 14; 8%), gastrointestinal stromal tumors (n = 10; 6%), prostate cancer (n = 6; 3%), and colorectal cancer (n = 6; 3%). Seven factors were independently associated with significantly worse overall survival: age >52 years (hazard ratio [HR] 1.59, 95% confidence interval [CI] 1.1-2.4), Eastern Cooperative Oncology Group performance status >1 (HR 2.81; 95% CI 1.3-6.3), lactate dehydrogenase >466 U/L (HR 2.1; 95% CI 1-4.2), platelet count >300 x 10^9/L (HR 1.8; 95% CI 1.2-2.8), absolute neutrophil count >4.9 x 10^9/L (HR 2.3; 95% CI 1.5-3.5), absolute lymphocyte count <1.8 x 10^9/L (HR 3.3; 95% CI 1.9-5.7), and liver metastases (HR 1.8, 95% CI 1.2-2.6). An index was created whereby the cohort was divided into four risk groups based on the number of factors present: 0-2, 3, 4, or 5-6. Median overall survival was 24.2 months (6-9), 11.6 months (3), 8.0 months (4), and 3.8 months (5-6); log rank test, p < 0.0001. The Harrell c-index of this scoring system was 0.72, indicating significant predictability. Conclusion: We have developed a novel “MDA ICI” prognostic scoring system incorporating seven clinical parameters with prognostic significance for patients in phase I clinical trials treated with immune checkpoint inhibitors. Unlike in the RMH and MDA prognostic scoring systems, albumin level and number of metastatic sites did not independently correlate with overall survival. Prospective evaluation and external validation of our novel prognostic scoring system is warranted and may help better select patients for future clinical trials of checkpoint inhibitors.  

#3292 Cause-specific mortality of nonmetastatic prostate cancer patients. Katarina Matthes,1 Giulia Pestoni,1 Dimitri Korol,2 Mieke Van Hemelrijck,3 Katarina Matthes,1 Giulia Pesto,1 Dimitri Korol,2 Mieke Van Hemelrijck,3 Youzhou Tan,1,2 Hai Dong Wang.1,2,3 *The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China; 2National Institute of Health and Welfare, Helsinki, Finland; 3National Institute of Health, Baltimore, MD; University of Tampere, Tampere, Finland.* 

Background: Prostate cancer (PCa) is the most frequent incident cancer in men in Switzerland. One of the major challenges in the treatment of PCa is to reliably distinguish between patients at risk of dying as a direct result of the disease and those more likely to die of other causes. The purpose of this study was to assess the associations of age and risk group based on stage, grade and PSA level with primary treatment of non-metastatic PCa patients, and to evaluate whether this treatment was associated with cardiovascular death or prostate cancer specific mortality (PCSM). Methods: Population-based data from the Cancer Registry of Zurich and Zug in Switzerland were used. We identified 1919 non-metastatic PCa patients treated between 2000 and 2009 who were living in the City of Zurich. PCa risk groups were stratified into three groups based on T stage, Gleason score and initial PSA concentration as described by D’Amico (low-risk: T1-2a, and Gleason score <=6, and PSA <=10 ng/mL; intermediate risk: T2b and/or Gleason score =7 and/or PSA >10 and <=20; high-risk: >= T2c or Gleason score 8-10 or PSA >20). Multiple imputation methods were applied to deal with missing risk group information. We distinguished between surgical procedures, radiotherapy, androgen deprivation therapy (ADT), active surveillance, and watchful waiting. Fine and Gray competing risk regression analysis was used to estimate sub-distribution hazard ratios for the outcomes cardiovascular death, PCSM or other-cause mortality. Results: Unadjusted combined peting risk model revealed an increased risk of cardiovascular death for patients with ADT (HR = 2.00 [1.27 - 3.14]), or watchful waiting compared to surgery (HR = 2.19 [1.58 - 3.02]). However, after adjustment for age and risk group, an increased risk of cardiovascular death was no longer observed (ADT HR = 1.51 [0.93 - 2.46], watchful waiting HR = 1.31 [0.91 - 1.90]). Only men >80 years of age showed an increased risk of cardiovascular death in both ADT and no ADT. Furthermore, we observed an increased adjusted risk of PCSM in men with ADT (HR = 2.84 [1.97 - 4.01]) and under watchful waiting (HR = 1.78 [1.29 - 2.61]) compared to surgery, for men >70 years (HR = 4.18 [2.18 - 8.00]) compared to men < 70 years old, and for patients with intermediate risk (HR = 2.69 [1.34 - 5.39]) and high risk (HR = 6.37 [3.30 - 12.30]) compared to patients with low risk. Discussion: The study suggests that ADT use for non-metastatic PCa patients may not be associated with an increased risk of cardiovascular death in general, but with an increased risk of PCSM. This is likely explained by the more frequent ADT use in older men and in men with a high-risk PCa. In addition, we observed that older men and men with an intermediate- or high-risk PCa had an increased risk of PCSM.  

#3293 Impact of the invasive depth on lymph node metastasis and survival in Chinese patients with esophageal squamous cell carcinoma. Xin Song,1 Fu You Zhou,2 Hai Jun Yang,3 Xue Na Han,3 Tang Juan Zhang,1 Lian Qun Zhang,2 Ning Liu,1 Jing Li Ren,2 Zhi Wei Chang,1 Xin Tian,1 Hai Ling Wang,2 Hui Fang Tan,1 Li Dong Wang.1,2,3 *The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China; 4University of Tampere, Tampere, Finland.* 

Purpose: To develop a prognostic scoring system for selecting patients for esophageal squamous cell carcinoma (ESCC). However, it is a dilemma to determine the LNM status before surgery. The invasive depth (T stage) of ESCC could be determined either with computed tomography or ultrasound endoscopy and may reflect the severity of LNM. Thus, the present study was designed to determine the impact of tumor invasive depth on LNM status and survival in ESCC patients with T1-4N0M0. The enrolled 30,514 ESCC patients in this study were from the ESCC database in Henan Key Laboratory for Esophageal Cancer Research of the First Affiliated Hospital, Zhengzhou University. Of the patients, there were 18346 males with an mean age of 59.2±8.9, and 12168 females with an mean age of 59.7±8.7. All the patients had been performed radical esophagectomy without radiotherapy and chemotherapy before surgery, and the invasive depth records were retrieved from the medical records in hospitals after surgical resection. Based on the 6th version of UICC criteria, the invasive depth (T) was classified as Tis, and T1-4. Of the 30,514 ESCC patients, 20,288 cases were successfully followed until the end of 2015. The Logistic regression method were used to determine the correlation between T stage and LNM status. The Kaplan-Meier method was applied to analyze the survival in different groups. The results showed that, of the 30,514 ESCC patients, there were 3324 (10.9%) patients with Tis+T1, 9616 (31.5%) with T2, 17249 (56.5%) with T3 and 325 (1.1%) with T4. Accordingly, from Tis&T1 to T4, the number of the patients with positive LNM were 554 (16.7%), 3392 (35.3%), 7737 (44.9%) and 205 (63.1%), respectively. Logistic regression analysis indicated the risk of LNM was dramatically increased between T1-4 to 8-folds with the increase of T stage. The hazard ratios values of 2.725 (T5: 95%CI: 2.465-3.013), 4.067 (T5: 95%CI: 3.695-4.477) and 8.542 (T5: 95%CI: 6.699-10.892), respectively. Interestingly, seventeen percent of the patients with Tis&T1 stages had occurred positive LNM, indicating that LNM may occur in very early stage of ESCC. Kaplan-Meier analysis showed that T staging was obviously associated with the survival in ESCC patients with negative LNM (P value < 0.05). Overall, the present results indicated a strong correlation between invasive depth and LNM status, suggesting that the invasion depth may be one of crucial markers to reflect the LNM in ESCC. The invasive depth could be used as an promising indicator for LNM status judgment in clinical TNM staging and prognosis for ESCC. [Supported by the Joint Funds of the National Natural Science Foundation of China (U1301227), the General Program of National Natural Science Foundation of China (81472323) and Correspondence to: Li Dong Wang. Email: lidwang2007@126.com]  

#3294 Breast cancer extent and survival in diabetic women in a Finnish population based cohort. Miika Murto,1 Miia Artama,2 Kala Visvanathan,3 Teemu Murtola1.1 Pirkanmaa Hospital District, Tampere, Finland; 2National Institute for Health and Welfare, Helsinki, Finland; 3Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; University of Tampere, Tampere, Finland.* 

Introduction: Breast cancer and diabetes are two major health problems for women. Around 1.7 million new breast cancer cases are diagnosed each year, making breast cancer the most common cancer type in women. Number of
people with diabetes is estimated to be 415 million worldwide, thus one in 11 adults have diabetes. However, evidence on the association between diabetes and breast cancer prognosis is controversial. We estimated breast cancer extent at diagnosis and survival in a Finnish population-based cohort of female breast cancer patients. Materials and methods: All newly diagnosed breast cancer cases among women in Finland between 1995 and 2013 were identified from comprehensive national Finnish Cancer Registry. To minimize possible bias due to differing participation in cancer screening the study population was limited to women with at least one mammography screening before the diagnosis. In total 45,786 cases were included in our study cohort. Information on recorded diagnoses of diabetes, hypertension, hypercholesterolemia and obesity from in- and outpatient hospital visits during 1995-2013 were obtained from national Care Register for Health Care maintained by National Institute for Health and Welfare. Logistic regression was used to evaluate the risk of having cancer extended to axillary lymph nodes or beyond at diagnosis. Cox regression was used to evaluate risk of breast cancer death and overall mortality after the diagnosis. Analyses were adjusted for age, comorbidities and number of mammography screens before the diagnosis. Survival analyses were further adjusted for tumor extent and primary treatment. Results: In total 1,419 women had diabetes. Compared to non-diabetic women, breast cancer was more often advanced to axillary lymph-nodes (OR 1.19; 95% CI 1.05-1.34) or metastatic at diagnosis (OR 1.58; 95% CI 1.26-1.97) in diabetic women. Nevertheless, during the median follow-up of 6.9 year after the diagnosis, risk of breast cancer death did not differ between diabetic and non-diabetic women (HR 1.21). Overall, the risk of death was elevated among diabetic women (HR 1.34; 95% CI 1.19-1.51). Conclusion: Diabetic women have more often advanced tumor extent at diagnosis compared to non-diabetic women. However, diabetes did not affect disease-specific survival within 6.9 years after the diagnosis.

#3295 Incidence of skeletal related events (SREs) among prostate cancer patients treated with androgen deprivation therapy (ADT) in Abuja, Nigeria. 1Oseiemem A, Aliuodenioye-Shadrack, 2Sadig N. Abu1, 2Ella E. Magnus2, 2Charles Ukwo, 2Nelix N. Kolo, 1University of Abuja & University of Abuja Teaching Hospital, Abuja, Nigeria; 2University of Abuja Teaching Hospital, Abuja, Nigeria.

Background: Skeletal related events (SREs) are one or more of the following conditions, occurring concurrently with or after the first bone metastasis: fractures, radiotherapy to bone and spinal cord compression. SREs occur in men with prostate cancer (CaP) and may result from androgen deprivation therapy (ADT). The purpose of this study was to determine the incidence of SREs in patients with CaP treated with ADT in our practice. Methods: Eligible cases were newly diagnosed patients with CaP treated with ADT at the University of Abuja Teaching Hospital (UATH), Abuja, Nigeria between January 2012 and December 2015 with bone metastases and treated with ADT. Data compiled from only retrievable patient’s medical records showed tumor diagnosis, laboratory and radiology results. SREs included spinal cord compression, surgery to bone, pathologic fracture and radiation to bone. Results: All 142 cases of CaP reviewed who received ADT were first diagnosed as AJCC stage IV prostate cancer. More than half the patients were ≥ 65 years of age with a mean (SD) of 68.3 ± 9.5 years. Serum PSA range was 1.4-2461.58 ng/ml with a mean (SD) of 113.7 ± 288.9 ng/ml. We identified 29 patients (20.5%) who had one or more SREs: spinal cord compression (19.1%), pathologic fractures (1.4%) and radiotherapy to the affected bone (1.4%) being the most common SREs. Altogether fifty (35.2%) more patients had bone pains which was localized to the lumbo-sacral spine in 52% of them, only two patients had internal fixation to stabilize pathological fractures and bone radiotherapy respectively. ADT in the patients was orchiectomy, LHRH-anti-androgens and complete androgen blockade (CAB) [orchiectomy or LHRH plus anti-androgens] in 14 (9.8%), 3 (2.1%), 44 (30.9%) and 81 (57%) patients respectively. Conclusions: Although some clinical trials have found that 36-41% of high-risk metastatic prostate cancer patients developed SREs during 3 years of follow-up, our review of this hospital-based cohort of patients with prostate cancer receiving ADT did not show the incidence of SREs to be as high as what has been reported. Additional studies to explore the incidence of SREs in patients with metastatic hormone refractory prostate cancer in our environment may be relevant.

#3296 Survival among lung cancer patients in the U.S. Military Health System: a comparison with the SEER population. Jie Lin, 1Christine Kamanian, 1Derek Brown, 1Stephanie Shao, 1Katherine A. McGlynn, 1Joel A. Nations, 1Corey A. Carter, 1Craig D. Shriver, 1Kangmin Zhu, 1Walter Reed National Military Medical Center, Bethesda, MD; 2National Cancer Institute, Rockville, MD.

The U.S. military health system (MHS) provides universal access to its beneficiaries. However, little is known about whether the universal access has translated into improved patient outcomes. We compared survival among non-small cell lung cancer (NSCLC) cases in MHS with that of NSCLC cases from the general U.S. population. The MHS data were obtained from The Department of Defense’s (DoD) Automated Central Tumor Registry (ACTUR). The U.S. population data were drawn from the National Cancer Institute’s (NCI) Surveillance, Epidemiology, and End Results (SEER) program 18 registries (1973-2012) which represents 27.8% of the U.S. population. NSCLC patients in ACTUR and SEER who were diagnosed between January 1, 1987 and December 31, 2012 were included. The ACTUR and SEER cases were then matched by age group, sex, race, and year of diagnosis group at a matching ratio of 1:4, which comprised of 16,257 cases from ACTUR and 65,028 cases from SEER. Kaplan-Meir curves and log-rank test were used to compare overall survival between the two populations. Multivariable Cox proportional hazards models for matched data were used to estimate hazard ratios (HRs) and 95% CI with adjustment for potential confounders. Compared to the SEER cases, the ACTUR cases were more likely to be diagnosed at stage I or stage II (P<0.0001) and less likely to be diagnosed at stage IV. ACTUR cases also had a lower percentage of poorly differentiated tumors than did SEER cases, who had a higher percentage of well differentiated and moderately differentiated tumors (P<0.0001). Kaplan-Meir survival curves showed significantly better survival among ACTUR cases than SEER cases (Log Rank P<0.0001). In Cox proportional hazards model analysis, ACTUR cases exhibited significantly better overall survival than did SEER cases (HR=0.78, 95% CI=0.76 to 0.81). The survival advantage of ACTUR cases remained after stratification on cancer stage, age group, sex, race and further. Additional analyses revealed that ACTUR cases were more likely to receive surgery for early-stage (stages I and II) tumors (OR=1.41, 95% CI =1.28 to 1.50), and radiation therapy for late stage (stages III to IV) tumors (OR=1.09, 95% CI =1.03 to 1.15). Our results suggest that the survival of NSCLC patients in the MHS is better than that in the general population, implying that lung cancer care and programs within the MHS has translated into improved patient outcomes.

#3297 Survival predictors of Burkitt’s lymphoma in children, adults and elderly in the United States during 2000-2013. Fahad Mukhtar, 1Paolo Bofetta, 2Harvey A. Risch, 3Jong Y. Park, 4Omonigho M. Bubu, 1Lindsay Womack, 1Walter Reed National Military Medical Center, Bethesda, MD; 2Harvey A. Risch, New Haven, CT; 3Harvey A. Risch, 4H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL; 1University of South Florida, Tampa, FL; 2Mount Sinai School of Medicine, New York, NY; 3Yale University, New Haven, CT; 4H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL; 5Vietnam National Cancer Hospital, Hanoi, Viet Nam.

Background. Burkitt’s Lymphoma (BL) has three peaks of occurrence, in children, adults and elderly, at 10, 40 and 70 years respectively. To the best of our knowledge, no study has been conducted to assess predictors of survival in the three age groups. We hypothesized that the predictors of survival may differ by age group. We, therefore, sought to determine the predictors of survival for BL in these three groups: children (<15 years of age), adults (40-70 years of age) and elderly (>70 years of age). Methods. Using the Surveillance, Epidemiology, and End Results (SEER) database covering the years 2000-2013, we identified 797 children, 1,994 adults and 757 elderly patients newly diagnosed with BL. We used Cox proportional hazards regression models to determine prognostic factors for survival for each age group. Results. Five-year relative survival in BL for children, adults and elderly were 90.4%, 47.8%, and 28.9%, respectively. Having at least stage II disease and multiple primaries were associated with higher mortality in the elderly group. In adults, multiple primaries, stage III or IV disease, African American race and bone marrow primary were associated with increased mortality whereas stage IV disease and multiple primaries were associated with worse outcome in children. Conclusion. These findings demonstrate commonalities and differences in predictors of survival that may have implications for management of BL patients. Financial Support: This work was supported by the US National Institutes of Health/National Cancer Institute 1P20CA210300-01 (PI: Shu XO, Tran T/Sub-contract-PI: Luu HN) and the University of South Florida start-up grant (PI: Luu HN).

MOLECULAR AND CELLULAR BIOLOGY GENETICS: Autophagy and Cancer

#3298 Diindolylmethane and its halogenated analogues induce autophagy in human prostate cancer cells via induction of the astrocyte-elevated gene-1 (AEG-1). Hossam Draz, 1Alexander Goldberg, 1Thomas Sanderson, 1Stephen Safe1, 2INRS-Institute Armand Frappier, Laval, Quebec, Canada; 3Texas A&M University, College Station, TX.
3,3'-diindolylmethane (DIM) and its halogenated derivatives (ring-DIMs) are recently shown to induce protective autophagy in human prostate cancer cells. The mechanism of induction of autophagy by DIM has not been elucidated. As DIM is a mitochondrial ATP synthase inhibitor, we hypothesized that DIM and ring-DIMs induce autophagy via alteration of AMP/ATP ratio and act as inducers of autophagy in prostate cancer cells. In this study, we monitored by LC3B-I to LC3B-II conversion in LNCaP and C42B prostate cancer cells. Autophagic vacuoles were examined using Cyto-ID autophagy detection kit and transmission electron microscope (TEM). Protein levels for AMPK, pAMPK, acetyl-CoA carboxylase (ACC), pACC, AEG-1, pULK-1 and β-actin were measured by western blot. AMPK and AEG-1 gene expression was downregulated using siRNA. DIM and ring-DIMs induced autophagy by increasing autophagic vacuoles and LC3B-I to LC3B-II conversion in LNCaP and C42B cells. These compounds also induced AMPK, ULK-1 and ACC phosphorylation in a time dependent manner. Interestingly, DIM, 4,4’-dibromoDIM and 7,7’ dichloroDIM induced the oncogenic protein AEG-1 time dependently in LNCaP and C42B cells. Downregulation of AEG-1 or AMPK inhibited DIM- and ring-DIM-induced autophagy. Pretreatment with ULK1 inhibitor MRT 67307 in a time dependent manner.

33299 Radiation induced p53-dependent cytoprotective autophagy fails to reduce radiation sensitivity in H460 non-small cell lung cancer cells. Jing-wen Xu,1 Tareq Saleh,2 Lihya Tyutynsk-Massey,3 Yinglang Wu,4 David A. Gewirtz,2 Shenyang Pharmaceutical University, Shenyang, China; 2VCU Massey Cancer Ctr., Richmond, VA.

Our previous studies showed that radiation-induced autophagy could be either cytoprotective or nonprotective in A549 non-small cell lung cancer cells, HN30 and HN6 head and neck cancer cells and 4T1 breast tumor cells, and that this functional difference was related to the status of p53 in the cells (i.e. cytoprotective autophagy in p53 wild type (wt) cells and nonprotective autophagy in cells with mutant or null p53)[1]. Despite the fact that inhibition of autophagy has been proposed as a strategy to overcome resistance to radiation and chemotherapy, there is little direct information in support of the premise that the induction of autophagy confers resistance. As an example, studies in HCT-116 cells that are either wt or null in p53 demonstrate essentially identical radiation sensitivity [2], although the nature of radiation induced autophagy was not determined in these cell lines. To address this question, p53 wt and p53 null H460 cell lines were utilized to evaluate whether cytoprotective autophagy actually suppressed radiation sensitivity. Acridine orange staining, flow cytometric analysis and western blotting (LC3B, p62, Atg5) showed that following exposure to 6 Gy of radiation wt p53 and p53-null H460 cells exhibited a significant induction of autophagy. p53 wt H460 cells showed sensitization to radiation upon pharmacological inhibition of autophagy. However, p53 null H460 cells were utilized to evaluate whether cytoprotective autophagy actually suppressed irradiation sensitivity. Acridine orange staining, flow cytometric analysis and western blotting (LC3B, p62, Atg5) showed that following exposure to 6 Gy of radiation wt p53 and p53-null H460 cells exhibited a significant induction of autophagy. p53 wt H460 cells showed sensitization to radiation upon pharmacological inhibition of autophagy. However, p53 null H460 cells were utilized to evaluate whether cytoprotective autophagy actually suppressed irradiation sensitivity. Acridine orange staining, flow cytometric analysis and western blotting (LC3B, p62, Atg5) showed that following exposure to 6 Gy of radiation wt p53 and p53-null H460 cells exhibited a significant induction of autophagy. p53 wt H460 cells showed sensitization to radiation upon pharmacological inhibition of autophagy. However, p53 null H460 cells were utilized to evaluate whether cytoprotective autophagy actually increased radiation sensitivity [2]. The combination treatment of 3-MA and UA significantly increased the apoptotic cell death as monitored by increased protein expression of Caspase-3 and PAPR. Conclusions: UA could induce autophagy in hepatoma SK-Hep-1 cells through inhibition of mTOR and PI3K signaling pathways. SK-Hep-1 cells treated with UA exhibited LC3-II conversion, increased protein expression levels of Beclin, Atg-7, and Atg-5 along with reduced protein expression levels of p62. UA-induced apoptosis was enhanced by the treatment of autophagy inhibitor 3-MA. Our findings may assist in the development of novel chemotherapeutic agents for the treatment of malignant types of liver cancer.


Withaferin A (WA), a bioactive molecule from Withania somnifera, is known to induce autophagy inhibitory effects. Elevated levels of sequestosome 1 (SQSTM1/p62) and decreased turnover of LC3. WA was found to be a potent lysosomal deacidification agent capable of blocking autophagic flux. Accordingly, inhibiting autophagy by blocking formation of autophagosomes or elevating lysosomal pH did not interfere with WA-mediated growth inhibition. WA blocked autophagic flux decreasing recycling of cellular fuels leading to reduced energy supply. Investigating this alternative mechanism, we discovered that indeed, WA induced an increase of LC3-II to LC3-I ratio, which was evident by higher level of sequestosome 1 (SQSTM1/p62) and decreased turnover of LC3. WA was found to be a potent lysosomal deacidification agent capable of blocking autophagic flux. Accordingly, inhibiting autophagy by blocking formation of autophagosomes or elevating lysosomal pH did not interfere with WA-mediated growth inhibition. WA blocked autophagic flux decreasing recycling of cellular fuels leading to reduced energy supply. Investigating this alternative mechanism, we discovered that indeed, WA induced dysregulated autophagy-degradation and promotion of energetic impairment. Conclusions: WA induced autophagy by blocking formation of autophagosomes or elevating lysosomal pH. This resulted in decreased energy production, thereby inhibiting cancer cell death. WA might be a useful strategy to convert cancer cell line to autophagy regimes to evade cytoprotective effects of autophagy.
L-asparaginase has been used for more than three decades in acute lymphoblastic leukemia (ALL) patients and remains an essential drug in the treatment of ALL. Poor response to L-asparaginase is associated with increased risk of therapeutic failure in ALL. However, both the metabolic perturbation and molecular context of L-asparaginase-treated ALL cells has not been fully elucidated. Here we identify the treatment with L-asparaginase results in metabolic shutdown via the reduction of both glycolysis and oxidative phosphorylation, accompanied by mitochondrial damage and activation of autophagy. The autophagy is involved in reducing reactive oxygen species (ROS) level by eliminating injured mitochondria. Inhibition of autophagy enhances L-asparaginase-induced cytotoxicity and overcomes the acquired resistance to L-asparaginase in ALL cells. The ROS-induced feedback loop is an essential mechanism of this synergistic cytotoxicity. Thus, our findings provide the rationale for the future development of combined treatment of L-asparaginase and anti-autophagy drug in ALL patients.

**#3303** Survivin negatively regulates autophagy through interference with the formation of Atg5-Atg12-Atg16L complex in breast cancer cells. Hsiu-Han Chan,1 Mohane Selvaraj Coumar,2 Siao-Muk Cheng,3 Shing-Ling Tsai,1 Chun-Hui Lin,1 Shang-Hung Chen,1 Euphemia Leung,2 Chun Hei Antonio Cheung2.1National Cheng Kung Univ. College of Medicine, Tainan, Taiwan; 2Pondicherry University, Ponducherry, India; 3National Health Research Institutes, Taichung, Taiwan; 4The University of Auckland, Auckland, New Zealand.

Survivin, a member of the inhibitors-of-apoptosis proteins family, is highly expressed in different cancers and its expression is correlated with tumorigenesis and tumor metastasis. At the cellular and molecular levels, Survivin is a dual functions protein that promotes mitosis and also inhibits apoptosis. In this study, we found that Survivin in a novel negative-regulator of autophagy. Molecular analysis and computational modeling revealed that Survivin negatively regulates the formation of Atg5-Atg12 conjugates, which plays important roles in the process of autophagosome elongation, in breast cancer cells. It also negatively regulates the expression and activity of cathepsin L in cells. Interestingly, we found that Survivin binds to p62/SQSTM1 and LC3B and is also an autophagic substrate protein in which its expression is partly downregulated by autophagy at the protein level. Finally, mechanistic study revealed that YM155, a Survivin inhibitor that was originally developed to inhibit Survivin gene transcription, downregulates Survivin expression in part through autophagic protein degradation. Taken together, findings of this study provide new mechanistic insights into both molecular functions and regulations of Survivin and the molecular mechanism of actions of YM155, a drug currently undergoing clinical trials for cancer treatment.

**#3304** Inhibition of autophagy enhances mitochondria-related apoptosis in malignant mesothelioma cells by combinational sulfaphenacine. YoonJin Lee,1 David M. Lee,2 Hae Nam,1 Sang Lee1,2 Soonchunhyang Univ. College of Medicine, Cheon-An Chong-Nam, Republic of Korea; 3University of Maryland, College Park, MD.

Drug resistance to current chemotherapy regimens has been considered a cell survival adaptive response in malignant mesothelioma cells. Therefore, new strategies to enhance the apoptotic signal and overcome resistance to chemotherapeutics are necessary to improve treatment outcomes for this deadly disease. Here, the effect of sulfaphenacine, an isothiocyanate compound derived from glucoraphanin present in cruciferous vegetables, on enhancing anticancer role of cisplatin was investigated in malignant mesothelioma cells. At concentrations showing little toxicity in normal human mesothelial MeT-5A cells, the combined treatment with both compounds exhibited synergistic growth-inhibiting and apoptosis-promoting effects, as demonstrated in a series of pro-apoptotic events including reactive oxygen species (ROS) accumulation, loss of mitochondrial membrane potential, up-regulation of p53, increased Bax/Bcl-2 ratio, activation of caspase-3, and the occurrence of a sub-G0/G1 peak with an increase in the cells with pyknotic and fragmented nuclei, Annexin V-FITC (+), and G2/M phase transition delay in the cell cycle. The levels of phosphorylation of both Akt and mTOR were decreased by the combined treatment, which was accompanied by a significant increase in the level of autophagosomal marker protein LC3-II and the accumulation of acidic vesicular organelles. Furthermore, scavenging of ROS by antioxidant N-acetylcysteine inhibited both the apoptosis and autophagy, while the inhibition of apoptosis by bafloxacin A1 potentiated apoptotic cell death following the combination treatment of sulfaphenacine and cisplatin, indicating the cytoprotective role of autophagy counteracts apoptosis of malignant mesothelioma cells. Considering the pro-oxidant-based combinational approach, our data provide a rationale for targeting cytoprotective autophagy as a potential therapeutic strategy for malignant mesothelioma.

**#3305** Autophagic induction through upregulation of sestrin-2 transcription and mRNA stability mediates the anticancer effect of new compound Chia-F. Xiaohui Hua,1 Chuanshu Huang,2 Xuere Wu,2 Jingxia Li3.1 Nelson Institute of Environmental Medicine, New York University, Tuxedo Park, NY; 2Wenzhou Medical University, China; 3Departments of Urology, New York University School of Medicine, New York, NY; 4Nelson Institute of Environmental Medicine, New York University, Tuxedo Park, NY.

Chia-F is a novel conformation-derivative of Chelensisin A (Chel A), which is a new styryl-lactone isolated from Chinese Herb Goniothalamus chelensis Huand shows potent antitumor potential effect both in vitro and in vivo. However, the anticancer activity and its potential mechanisms underlying Chia-F activity have never been explored. In present study, we evaluated the potency of Chia-F on anchorage-independent growth and its association with autophagic responses in human high grade invasive bladder cancer cells. We found that Chia-F significantly inhibited anchorage-independent growth of human bladder cancer cells by induction of autophagy in a sestrin2(SEN2)-dependent fashion. Our results revealed that Chia-F treatment specifically induced SEN2 expression via increasing its both gene transcription and mRNA stability. On the one hand, Chia-F treatment markedly attenuated Dicer protein abundance, and in turn abolished miR-27a maturation and further relieved miR-27a directly binding to SEN2 mRNA 3’UTR, thereby promoting SEN2 mRNA stabilization. On the other hand, Chia-F treatment promoted Sp1 abundance and consequently mediated SEN2 transcription. Moreover, either knockdown of Sp-1 or ectopic expression of miR-27a could abolish the autophagic response and reversed the inhibition of anchorage-independent growth in human bladder cancer cells. These results demonstrate that anticancer effect of Chia-F is mediated by its activating autophagic pathway through promoting both SEN2 mRNA transcription and stabilization, which offers novel insights into the anticancer effect of Chia-F on bladder cancer and provides a therapeutic alternative against human bladder cancer.

**#3306** Inhibition of ribosomal protein S6 (rpS6) phosphorylation and cell proliferation by a novel chloroquine analog in lung cancer cells. Juan Sironi, Evelyn Aranda, Lars Nordstroem, Roman Perez Soler, Edward L. Schwartz. Albert Einstein College of Medicine, Bronx, NY.

Autophagy is an evolutionarily conserved self-defense mechanism that allows tumor cells to overcome stress during oncogenesis and after treatment with chemotherapy. It is the basis of clinical trials using the autophagy inhibitors chloroquine (CQ) and hydroxychloroquine (HCQ). While searching for novel agents targeting autophagy, we discovered a compound, named EAD1, that in preclinical trials was inhibitory in cell proliferation of a variety of cancer cell lines whose proliferation was inhibited by EAD1 were H1650 NSCLC cells. This was surprising as H1650 cells have a biallelic deletion within the Atg7 locus. Atg7 is a critical component of the canonical autophagy pathway and its loss renders cells autophagy-deficient. This was confirmed in H460 and Atg7-knockout cells. That these cells had the same sensitivity to EAD1 (and HCQ) as did cells with an intact autophagy pathway suggests that inhibition of autophagy was not the mechanism by which those drugs inhibit cell proliferation. We therefore conducted exploratory experiments to identify other possible sites of action of EAD1. The only molecular change we observed with EAD1 was the selective inhibition of ribosomal protein S6 (rpS6) phosphorylation, which correlated with its inhibition of cell proliferation. rpS6 is a component of the 40S ribosome subunit, and is only one of two ribosomal proteins known to be phosphorylated in a regulated manner. The decrease in rpS6 phosphorylation also occurred in the Atg7-deficient cells, indicating it was not a consequence of autophagy inhibition. rpS6 regulates cell size, survival, and migration. Consistent with these reports, we found that EAD1 caused a significant decrease in the size of the H460 cells (when controlled for cell cycle distribution), and this decrease was not seen with HCQ. rpS6 is phosphorylated and activated by two known families of kinases, p70 S6K and p90 RSK, that are downstream targets of the mTORC1 and the RAS/MEK/ERK pathways, respectively. However, we have not seen any changes in the expression or phosphorylation of the members of these pathways, leading us to hypothesize that EAD1 is either having a direct effect on rpS6, or that it is blocking the kinase activity of an already activated p70S6K or p90RSK. Further studies are needed to determine the role of rpS6 in cancer cells, and to assess if it is a viable target for future drug development.

Background: In the curative setting for head and neck cancer (HNC) a commonly used treatment is radiation combined with the anti-EGFR monoclonal antibody cetuximab (CTX). However, despite decades of research into improved treatments, therapeutic resistance remains a major challenge for this malignancy with roughly 40% of patients developing recurrent disease. We are studying the molecular mechanisms by which radiation and cetuximab induce autophagy and how this process can be modulated to improve the efficacy of therapy. Methods: We evaluated a panel of both human papillomavirus (HPV) positive and negative HNC cell lines for autophagic response to both CTX treatment and ionizing radiation (XRT). Induction of autophagy was detected by immunoblot fluor assays for LC3 and p62 and by immunofluorescent staining of autophagolysosomes. siRNA knockdown of EGFR, LAMTAPB, ULK1, Beclin1 and others was used to probe the involvement of different signaling molecules. The addition of specific autophagy inhibitors, such as the ULK1 inhibitor SBI-0206965, to CTX or XRT treatment was tested to determine whether the reduction of autophagic response reduced cell survival in a clonogenic survival assay. Induction of apoptosis was analyzed by annexinV and propidium iodide staining of cells and by Western blot (WB) analysis of p62, Beclin-1 and LC3B processing. The addition of specific autophagy inhibitors to standard treatments may provide a way to overcome resistance to therapy.

#3308 Mitophagy induction and interference with cancer-specific apoptosis in SHetA2-treated cells. Vishal Chandra,1 Andrew Long,2 Chionio Patricia Masamha,3 Doris Mangiaracina Benbrook1. 1University of Oklahoma Health Sciences Center (OUHSC), Oklahoma City, OK; 2Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany; 3Butler University, Indianapolis, IN.

Background: SHetA2 is a small molecule flexible heteroaromatic (Flex-Het) compound that exhibited in-vivo cancer therapeutic and chemoprevention activity, lack of mutagenicity or teratogenicity, and a pharmacologic profile suitable for an oral drug. First-in-human clinical trials of SHetA2 for ovarian cancer prevention and cervical cancer treatment are in development. Autophagy is induced in cancer cells by a wide variety of therapeutic drugs and commonly interferes with the drugs efficacies. Because SHetA2 binds to mitochondrial and induces mitochondrial damage and apoptosis in cancer, but not in healthy cells, we hypothesized that this drug induces mitochondria-selective autophagy (mitophagy) in cancer, but not healthy cells; and that the mitophagy interferes with SHetA2-induced apoptosis. Methods: Cultures of human ovarian and cervical cancer cell lines and human ovarian surface epithelium (HOSE) were treated with various concentrations and combinations of SHetA2, 3-methyladenine (3-MA), Beclin-1 siRNA, and caspase inhibitors. Autophagy was evaluated by electron microscopy (EM) and fluorescent imaging of LC3B processing in cells and by Western blot (WB) analysis of p62, Beclin-1 and LC3B processing. Mitophagy was measured by EM of cells and WB analysis of BNPI3 total levels and co-immunoprecipitation with LC3B in protein extracts. Apoptosis was measured by Annexin-V and propidium iodide staining of cells and by WB analysis of caspase 9 and 3 and PARP-1 cleavage. Result: Induction of autophagy by SHetA2 treatment was demonstrated by the development of double-membrane autophagosomes and accumulation of the processed LC3B II in cancer, but not in HOSE cells. Mitophagy was demonstrated by the presence of mitochondria inside the autophagosomes and by reduction of total BNPI3 in association with increased BNPI3/LC3B complexes in SHetA2-treated cells. Autophagy flux was demonstrated by gradual reduction of p62. Conversion of autophagy to apoptosis was associated with reappearance of basal p62 levels and cleavage of Beclin-1, PARP-1, and caspase 9 and 3 about 24 hours after initiation of treatment. Interference of the autophagy with apoptosis was demonstrated by the treated levels of apoptosis in cells co-treated with SHetA2 and autophagy inhibitors (3-MA or Beclin-1 siRNA) along with reduced apoptosis in cells co-treated with SHetA2 and an autophagy inducer (rapamycin). This was observed in cell lines harboring mutant or no p53, but not in an ovarian cancer cell line with wild type p53. Conclusion: SHetA2-induction of autophagy may be initiated by mitochondrial damage and reduces, but does prevent, SHetA2-mediated apoptosis in ovarian and cervical cancer cell lines, but not in a cell line with wild type p53 or in HOSE cells. Combination of SHetA2 with an autophagy inhibitor may improve the therapeutic efficacy in clinical trials of patients harboring mutant or loss of p53 protein. Funded by: RO1CA196200 and RO1CA200126.

#3309 Tumor-treating fields decrease proliferation and clonogenicity of patient-derived WHO grade IV glioma cell lines. Sharon K. Michellhaugh, Sam Kousis, Adrienne Wallace-Povrik, Sandeep Mittal. Wayne State University, Detroit, MI.

Despite decades of research, efficacious treatments for malignant glioma tumours are limited. Tumor-treating fields (TTFields) are FDA-approved for the treatment of newly-diagnosed and recurrent glioblastoma. In this study, in vitro experiments comparing TTFields to untreated controls were performed on patient-derived grade IV glioma cell lines to determine the effects of TTFields on cell proliferation and clonogenicity. Methods: Studies utilizing patient tumor specimens were approved by the Wayne State University Institutional Review Board and written informed consent was obtained from participants. Patient tumor specimens (glioblastoma and gliosarcoma) were collected immediately following microsurgical resection. Single-cell suspensions from the tumor tissues were prepared by enzymatic and mechanical disruption. Equal numbers of cells were plated on plastic coverslips in DMEM/F12 media supplemented with 10%fetal bovine serum. TTFields were applied at 200 kHz to half of the coverslips. Culture media was replaced every day. At the conclusion of the 2 week treatment, cell proliferation was assessed with the XTT assay and cells were harvested and replated for clonogenic assays (10,000 cells/well). The resulting colonies were fixed and stained with crystal violet and counted with an automated colony counter. Control vs. TTFields treated groups were compared by two-tailed t-tests. Results: The two-week TTFields treatment significantly reduced cell proliferation in both the glioblastoma (41.6 ± 11.1% control; n=4; p<0.001) and in the gliosarcoma (41.6 ± 16.6 % control; n=4; p<0.002) as measured by XTT assay. The clonogenic assay revealed that the number of colonies generated from both cell lines was reduced by TTFields treatment. For the glioblastoma cell line, control cells yielded 847 colonies with an average diameter of 462.8 ± 5.6 μM while TTFields-treated cells yielded 561 colonies with an average diameter of 435.1 ± 7.3 μM, which was a statistically significant decrease (p=0.0022). For the gliosarcoma cell line, control cells yielded 809 colonies vs. 144 colonies from the TTFields treated cells, although the average diameter of the colonies was unchanged between groups (control 375 ± 176.1 μM; TTFields 362.9 ± 190.0 μM). Conclusions: In vitro application of TTFields markedly reduced cell proliferation and clonogenicity in both patient-derived WHO grade IV glioblastoma and gliosarcoma cell lines. This is the first report on the in vitro effects of TTFields on gliosarcoma.

#3310 Atg7 overexpression promotes bladder cancer invasion via autophagic removal of AUFI protein and subsequently increased RhoGDI2 mRNA stability in vitro and in vivo. Junlan Zhu, Yang Li, Chuanshu Huang, Haishan Huang. New York Univ. School of Medicine, Tuxedo Park, NY.

Despite the promising role of autophagy in cancer biology, the role of autophagy in bladder cancer (BC) invasion remains largely unknown. BC is a common malignant urothelial tumor threatening metastases, understanding the molecular mechanisms underlying BC invasion is of tremendous importance for potentially reducing the mortality of this disease. In the present study, we for the first time found that the Atg7 was remarkably upregulated in human BC cell lines and the BBN-induced mouse invasive BCs, which is consistent with our most recent finding in human BCs. The knockdown of Atg7 in human BC cells dramatically inhibited cancer cell invasion, suggesting that Atg7 is a key player in regulation of BC invasion. Mechanistic studies revealed that Atg7 overexpression was mediated by miR-190, which was highly upregulated at transcriptional level in BC tissues and cell lines, and was able to bind to 3'-UTR of atg7 mRNA for degradation. Moreover, Atg7 mediated autophagic mechanism could remove AUFI protein and in turn reduced AUFI1 interaction with rhogdi2 mRNA, resulting in elevation of rhogdi2 mRNA stability, and subsequently leading to BC cell invasion. Remarkably, inhibition of Atg7-mediated autophagy led to AUFI protein accumulation, RhoGDI2 downregulation and BC cell invasion attenuation. Collectively, our results demonstrated that overexpression of Atg7 mediates BC cell autophagy, which removes AUFI protein, thereby reducing its binding with rhogdi2 mRNA and subsequently leading to elevation of rhogdi2 mRNA stability and protein expression, further promoting BC invasion. The identification of miR-190/Atg7 autophagic mechanism regulating AUFI/RhoGDI2 expression provides a sig-

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significant insight into understanding the nature of BC invasion, and is also highly potential for developing autophagy-based-specific therapeutic strategy for treatment of human BC patients.

**#3311 The alteration in key regulator genes of autophagy is mainstream mechanism of therapy resistance and impact prognosis of acute myelogenous leukemia (AML); results from diagnosis genomic analysis on 148 consecutive patients treated with intensive chemotherapy and long-term survival follow-up.**

Giovanni Marconi,1 Cristina Papayannidis,1 Maria Chiara Fontana,1 Antonio Padella,1 Anna Ferrari,1 Eugenia Franchini,1 Stefania Paolini,1 Maria Chiara Abba Benenate,1 Chiara Sartor,1 Francesca Volpato,1 Viviana Guadagnuolo,2 Silvia Lo Monaco,1 Elena Tenu,1 Andrea Ghelli Luserna Di Rora,1 Valentina Robustelli,1 Nicoletta Testoni,1 Giorgia Simonetti,1 Emanuela Ottaviani,1 Giovanni Martinelli2,1 Institute of Hematology, Bologna, Italy; 2Cellify, Bologna, Italy.

Introduction: There are no clear evidences if autophagy can lead to therapy resistance or favor apoptosis in cancer; it can function as a pro-apoptotic mechanism, or it can improve stresses survival clearing damaged mitochondria and proteins accumulation. Levels and activity of pro-apoptotic and anti-apoptotic proteins, particularly bcl-2 and bcl2, high levels of CAMP, and a complex made by pink/park could play as fulcrum of this yin and yang effect of autophagy. Our study aims to define the role of autophagy in AML, and to establish if gain or loss in autophagy could reduce the patients’ chance to respond to induction, and to worsen OS.

Methods: We analyzed 148 consecutively newly diagnosed non M3 AML patients treated with induction chemotherapy regimens containing at least one dose of anthracycline. We screened all patients for TP53, FLT3, NPM1 mutations; we performed Affymetrix SNP array 6.0 or Cytoscan HD. Survival analysis was performed using the Kaplan-Meier method. Differences between survival rates were evaluated using Mantel-Cox analysis. Gene expression was calculated using the Affymetrix GDS 2655 platform.

Results Autophagy alteration (gene group 1: ULK1 CHR11; ULK1 CHR17; BECN1; ATG4; AMBRA1; UVRAG; ATG9B; PIK3C3; PIK3R4) were related to lower Complete Remission rate (CR%) after induction in univariate (p<.001) and multivariable regression with age, karyotype, secondary AML, TP53 mutation (p<.014); autophagy alteration shown to confer worst OS (p<.001) and was significantly associated with complex karyotype and TP53 mutation (p<.001).

We detected significant differences in terms of survival independently both in gain and loss in group 1 genes (p<.001). Furthermore, we investigated genes in AMPK pathway (group 2: SESN1; PRKAA1 CHR 3; PRKAB1; PRKAA1 CHR 1; PRKAG1 CHR 11; PRKAG1 CHR 7; PRKAG3; PRKAB1) and other genes that could be related to a switch from a physiological role of autophagy to a resiliency mechanism (group 3: CCND1; BCL2; PINK1; PARK2; TP53; MDM1; MDM4); alterations in those genes were shown to confer worst OS (p<.001 in both groups); Alteration in group 2 and group 3 were related to lower CR% after induction (p<.001 in both groups). Whole Exome Sequencing on 56 patients in our set did not found any significant mutation in genes we analyzed with the exception of TP53.

Conclusion: Our work investigates for the first time with a genomic approach the role of autophagy in AML. We found that both gain and loss in autophagy key regulator genes are associated with poor prognosis and therapy resistance. A loss in autophagy could enhance proliferation and block apoptosis; a gain could give cell resiliency, favoring cytoplasm turnover, damaged epithelium, intracellular neutralizing oxidative damages. Acknowledgements: ELN, AIL, AIRC, PRIN, Progetto Regione-Università 2010-12, FP7-NGS-PTL project.

**#3312 A novel plate-based assay for screening autophagic activity in 2D and 3D cell culture models.**

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The critical importance of autophagy in cell health and its proposed role in disease-relevant biology, including cancer, inflammation, and immunology, has increased the need for more effective assays to screen for agents that modulate autophagic activity. Here we utilize NanoLuc Binary Technology (NanoBiT) to determine autophagic activity in 2D and 3D culture models. In this approach, an exogenous LC3G120A fusion protein was tagged on its N-terminus with an 11 amino acid peptide (HiBiT) and stably expressed in mammalian cells, including U2OS and HEK293. After exposure to various treatment conditions, cellular levels of this novel autophagy reporter were determined by addition of a lytic detection reagent containing Large Bit (LgBiT). LgBiT rapidly associates with HiBiT in the cell lysate, producing a bright, luminescent enzyme in the presence of the furimazine substrate. The bright signal allows low levels of expression of the reporter, maximizing the assay response, and the signal is stable, allowing assay of multiple 96- or 384-well plates in the same experiment. In response to autophagic stimuli, including nutrient deprivation and various mTORC inhibitors (e.g., PP242 and rapamycin), autophagic degradation of expressed LC3 reporter was evident by reduced assay signal. In contrast, in response to both upstream (e.g., 3-MA and wortmannin) and downstream (e.g., bafilomycin A1 and chloroquine) inhibitors of the autophagy pathway, degradation of the autophagic reporter was effectively blocked (HiBiT) or increased (HiBiT), with compound effects time dependent and stratified according to expected potency and efficacy of the test agents employed. The use of a mutant reporter based on LC3G120A further demonstrated the specificity of the wild-type LC3 reporter for the detection of autophagic activity. When assayed in 384-well plates with combination, HEK293 autophagy reporter cells produced Z’ values of ~0.7 in response to autophagic induction with PP242, while subsequent blockade of autophagy with bafilomycin A1 resulted in Z’ values of ~0.8. This data, and subsequent LOPAC library screening, indicates the potential utility of this assay method for HTS applications.

**#3313 Autophagic ovarian cancer cells exhibit substantially enhanced sensitivity to Crizotinib.**

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One of the major factors contributing to poor outcomes for patients with ovarian cancer is the persistence of dormant, drug resistant cancer cells after primary surgery and chemotherapy. Recurrent, progressively growing ovarian cancer metastases are generally well vascularized and may only have a small fraction of cancer cells undergoing autophagy. By contrast, the persistent, drug resistant, dormant cancer cells that remain on the peritoneal cavity after conventional treatment tend to be poorly vascularized. In this nutrient-poor, avascular microenvironment autophagy is widespread and can be found in more than 80% of cases. Drugs that regulate survival in autophagic cancer cells may be much more active when administered as maintenance therapy than when used to treat gross primary or recurrent disease.

Using unbiased siRNA screens, we have identified target genes that regulate the survival of ovarian cancer cells that are undergoing autophagy that has been induced by the re-expression of DIRAS3 or by amino acid starvation. Knockdown of the anaplastic lymphoma kinase (ALK) significantly reduced survival of ovarian cancer cells that were undergoing autophagy. Importantly, the FDA-approved ALK inhibitor, Crizotinib, exhibited significantly greater toxicity after induction of autophagy by upregulation of DIRAS3 (ARH) in OVCAR8, CAOV3, and SKOV3 ovarian cancer cells. Induction of autophagy by upregulation of DIRAS3 in SKOV3 ovarian cancer cells reduced the IC50 of Crizotinib from 7.44 μM to 0.04708 to 2.129μM > 0.06152 (P<0.05). Conversely, siRNA knockdown of DIRAS3 in CAOV3 cells with endogenously high DIRAS3 expression and increased autophagy increased the IC50 of Crizotinib from 204 nm to 0.01265 to 267 nm < 0.01715 (P<0.05). Our studies in cell culture suggest that Crizotinib might provide an effective agent to eliminate autophagic, dormant drug resistant ovarian cancer cells that remain after conventional cytoreductive surgery and combination chemotherapy. Subsequent studies will evaluate the effect of Crizotinib in xenograft models of DIRAS3-induced ovarian cancer dormancy.

**#3314 Synergistic effects of chemotherapy-induced autophagy and epigenetic remodeling.**

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Cytotoxic chemotherapy has been shown to enhance tumor cell immunogenicity by promoting the secretion of damage-associated molecular pattern (DAMP) molecules which, in turn, stimulate innate and adaptive immune responses. This effect can be further stimulated by combining chemotherapy with an immunotherapy designed to enhance effector cell function (CD8 T cell or NK cell) anti-tumor activity. Our studies demonstrate that knockdown of the epigenetic regulator NURF increases DNA damage (gamma H2AX staining) and autophagy (acidine orange staining) in breast tumor cells exposed to doxorubicin and enhances growth inhibition as well as supressing the capacity of the cells to recover and proliferate. Similar increases in autophagy were observed using a small molecule inhibitor of NURF, suggesting that NURF can be targeted therapeutically. Sensitization was not observed with ionizing radiation or cisplatin. Studies are in progress to assess the nature of autophagy in the NURF KD and NURF WT cells, distinguishing between the cytoprotective, cytotoxic, cyto
tostatic and nonprotective forms. How NURF regulates doxorubicin induced DNA damage is being investigated by mapping sites of damage genome wide. It is anticipated that enhanced cell autonomous tumor cell sensitivity in concert with improvements in tumor cell antigenicity (cell non-autonomous sensitization) achieved by NURF depletion could improve anti-tumor immunogenicity, achieve tumor regression, reduce metastasis, and possibly achieve long term remission in breast cancer.


TTFields tumor treating fields (TTFields) are an established anti-neoplastic treatment modality in patients with glioblastoma. TTFields are delivered via noninvasive application of low-intensity, intermediate-frequency, alternating electric fields to the region of the tumor. Previous studies have shown that TTFields treatment lead to increased cellular granularity, which is often associated with autophagy. Autophagy has been shown to regulate cell survival and proliferation under stress conditions and to influence cellular response to cytotoxic drugs. This study evaluated the role of autophagy in cancer cells treated with TTFields. Immunoblot analysis showed significant elevations in levels of lipidated Microtubule Associated Protein Light Chain (LC3-II) in TTFields-treated glioma and lung cancer cells. Increased autophagy following TTFields application in these cell lines was also detected using fluorescence microscopy, where punctate distribution of LC3-II was observed. TEM micrographs demonstrated the presence of autophagy typical, autophagosome-like structures in TTFields-treated U-87 MG cells. Combination of TTFields with autophagy inhibitors, chloroquine and melfoquine, resulted in a significant dose-dependent reduction in cell growth compared with TTFields treatment alone. Inhibition of autophagy with chloroquine triggered apoptosis as indicated by elevated levels of AnnexinV/7AAD double staining. Increased levels of autophagic flux in TTFields-treated cells were not associated with reduced mTOR activity, which was monitored by p70S6K phosphorylation immunoblot analysis. TTFields are known to exert anti-mitotic effects by disrupting highly dipolar structures that play critical roles in mitosis. Our results demonstrate that TTFields additionally induce cellular autophagy by an mTOR-independent mechanism. TTFields- treated cell lines appear to utilize autophagy as a survival mechanism. Thus, inhibition of autophagy sensitizes tumor cells to TTFields treatment, resulting in elevated apoptotic cell death. Future studies are warranted to examine the extent to which TTFields-elicited autophagy may affect treatment outcomes and to investigate the therapeutic implications of combining TTFields with autophagy inhibitors in vivo.


Ovarian cancer (OC) is the most lethal gynecologic malignancy. Persistence of dormant, drug resistant cancer cells is one of the major factors that contribute to poor outcomes. The Poly (ADP) ribose polymerase (PARP) inhibitor, olaparib, has shown promising anti-cancer activity and has been approved by the FDA for the treatment of ovarian cancer with BRCA1/2 mutations. Olaparib targets DNA repair deficiencies in cancer cells. Autophagy can protect cancer cells from chemotherapy or can enhance the response to certain drugs. In this study, we have asked whether autophagy protects OC cells against the cytotoxic effects of olaparib or augments damage. We first determined PARP activity using a modified PARP activity assay in different OC cell lines. Olaparib-induced autophagy was determined by punctate localization of GFP-LC3, conversion of LC3 to LC3II on protein blots and electron microscopy. To test whether autophagy contributes to the resistance of OC cells to olaparib, we determined the role of autophagy inhibition on the sensitivity of OC cells to olaparib by either using the pharmacologic inhibitors chloroquine (CQ), LY305 or short interfering RNA (siRNA) against ATG7. Upon investigation of a potential mechanism of olaparib-induced autophagy we evaluated the production of reactive oxygen species (ROS). K, Ataxia-telangiectasia mutated (ATM), mTOR and p70S6K. Inhibition of PARP by olaparib treatment, decreased PARP activity in a dose- dependent manner, leading to DNA damage judged by phosphorylation of γ-H2AX. Olaparib treatment decreased cell growth, caused cell cycle arrest and significantly enhanced apoptosis in 5 of 5 OC cell lines. Olaparib induced autophagy in 8 of 8 OC cell lines. Both pharmacologic and genetic inhibition of autophagy increased OC cell sensitivity to olaparib and induced apoptosis in 5 of 6 OC cell lines. Furthermore, flow cytometry and western blot analysis demonstrated that olaparib treatment increased ROS production and increased the phosphorylation of JNK and ATM while decreasing the phosphorylation of mTOR and p70S6K. Taken together, our data described autophagy as a potential mechanism of olaparib resistance in OC that may depend upon the activation of ATM and JNK by ROS downregulating mTOR and p70S6K.

Combination of olaparib with CQ, LY305 or other autophagy inhibitor may provide a novel strategy to increase the efficacy of olaparib in OC. Current studies are underway to test our hypothesis in vivo.

#3318 A critical role for c-Jun N-terminal kinase in autophagy and cell survival of breast cancer cells. Rohit Munagala, Carol Joseph, Annie Liu, Kebin Liu, Muthusamy Thangaraju, Patricia V. Schoenlein. Medical College of Georgia Cancer Center, Augusta University, Augusta, GA.

Breast cancer is the most major impediment to the eradication of estrogen receptor positive (ER+) breast cancer. Approximately 30% of ER+ breast tumors initially responsive to antiestrogen therapy will acquire resistance. One approach to reducing the occurrence of acquired antiestrogen resistance is to identify and target key signaling nodes that specifically attenuate the ability of antiestrogens to kill cancer cells. Toward this goal, we identified MEK/ MAPK1/2 as a key molecular target based on the general inability of antiestrogens to effectively block MEK1/MAPK1/2-mediated phosphorylation of BimEL in breast cancer cells. BimEL is a pro-apoptotic member of the BH3 family of proteins that is inactivated (degraded by the proteasome) when phosphorylated by MAPK1/2. The combination of an antiestrogen and MEK1 inhibitor robustly induced BimEL-dependent breast cancer cell apoptosis (Periyasamy-Thanavanan et al., 2012). However, a subpopulation of breast cancer cells survive this combined treatment via an autophagy-dependent mechanism. Based on the key role of JNK in regulating autophagy in cancer cells via BimEL phosphorylation, we hypothesized that JNK was a key effector of autophagy in breast cancer cells surviving antiestrogen treatment when used as a single agent or in combination with a MEK inhibitor. To test this hypothesis, we utilized the selective JNK inhibitor SP600125 as a single agent or in combination with extradrol (E2), 4-hydroxymethoxifen (4-OHT), and/or U0126 (a selective MEK1 inhibitor). Treatments were conducted with the antiestrogen-sensitive cell lines MCF-7 and T-47D and the antiestrogen resistant cell line TR5, previously derived in our laboratory by a step-wise 4-OHT selection. Effects on cell death (determination of apoptosis) and autophagy (determination of autophagy levels and flux) were evaluated for the various hormonal treatments conducted in the presence or absence of JNK inhibition. These studies showed that: (1) targeting JNK in antiestrogen sensitive breast cancer cells induces apoptosis with caspase-dependent cleavage of PARP as a surrogate marker of apoptosis; (2) JNK activity is elevated in antiestrogen resistant TR5 cells and JNK inhibition induces TR5 cell death.

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death; and (3) antiestrogen sensitive and resistant breast cancer cells dying as a result of JNK inhibition can show extensive cytosolic vacuolization with the autophagy protein LC3 (ATG8) localized to the aberrant vacuoles. Overall, our results support the conclusion that JNK plays a key autophagy-dependent survival role in breast cancer cells. Ongoing studies aim to identify the specific JNK protein(s) that disrupt autophagy and enhance death of breast cancer cells toward the goal of identifying specific molecular targets to block autophagy in breast cancer cells.

#3319 Elevating adipokine adiponectin level can induce cytoxic autophagy in breast cancer cells and potentiate the efficacy of chemotherapeutical regimens: preclinical studies. Seung J. Chung, 1 Ganji Purnachandra Nagaraju, 1, Artemugam Nagalingam, 1 Nethaji Muniraj, 1 Panjamurthy Kuppusamy, 1 Alyssa Walker, 1 Juhyung woo, 1 Balázs Györfy, 1 Edward Gabrielson, 1 Neeraj K. Saxena, 1 Dipali Sharma, 1 Johns Hopkins University, Baltimore, MD; 2Emory University, GA; 3University of Maryland, Baltimore, MD; 4Hungarian Academy of Sciences, Hungary.

Adiponectin, an adipocytokine secreted by adipocytes in the breast tumor microenvironment, negatively regulates cancer cell growth hence increased levels of adiponectin are associated with decreased breast cancer growth. However, its mechanisms of action remain largely elusive. We report that adiponectin induces a robust accumulation of autophagosomes, increases LC3II and decreases p62/SQSTM1 in breast cancer cells. Breast cancer cells treated and xenografts exhibit increased expression of autophagy-related proteins. Lysotracker-Red-staining and tandem-mCherry-GFP-LC3 assay show that autophagosomes/lysosomes fusion is augmented upon adiponectin treatment. Adiponectin significantly inhibits breast cancer growth and induces apoptosis both in vitro and in vivo, and these events are preceded by autophagy, which is integral for adiponectin-mediated cell death. Accordingly, blunting autophagosomes-formation, blocking autophagosomes-autolysosome fusion or genetic-knockout of Becn1/Beclin1/ATG7 effectively impedes adiponectin induced growth-inhibition and apoptosis-induction. Mechanistic studies show that adiponectin reduces intracellular ATP levels and increases AMPK phosphorylation leading to ATG1 activation. AMPK-inhibition abrogates adiponectin-induced ATG1-activation, LC3II-turnover and p62-degradation while AMPK-activation potentiates adiponectin’s effects. Further, adiponectin-medited AMPK-activation and autophagy-induction are regulated by upstream master-kinase LKB1, which is a key node in anti-tumor function of adiponectin as LKB1-knockout abrogates adiponectin-mediated inhibition of breast tumorigenesis and molecular analyses of tumors corroborate in vitro mechanistic findings. Adiponectin increases the efficacy of chemotherapeutic agents. Notably, high expression of adiponectin receptor, adiponectin and Becn1 significantly correlates with increased overall survival in chemotherapy-treated breast cancer patients. Collectively, these data uncover that adiponectin induces autophagic cell death in breast cancer and provide in vitro and in vivo evidence for the integral role of LKB1-AMPK-ATG1 axis in adiponectin-mediated cytoxic-autophagy.

#3322 Elucidation of the signaling pathways that mediate berberine-induced effects in cancer cells. Basabi Rana, 1 Rong Ke, 1 Kanchan Vishnoi, 1 Navin Viswakarma, 1 Subhasis Das, 1 Ajay Rana, 1 University of Illinois at Chicago, Chicago, IL.

Resistance towards standard therapeutic regimens and evasion of apoptosis are some of the hallmarks of advanced forms of cancer, which include Sorafenib-resistance in hepatocellular carcinoma (HCC), castration-resistance in prostate cancer. Development of effective therapeutic strategies that can target these resistant forms is critically needed. In an effort to understand the molecular mechanism mediating resistance in cancer, in previous studies utilizing Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-resistant cancer cells we showed that combination of TRAIL along with the PPARY ligand Troglitazone (TZD) can sensitize them towards apoptosis. Using more molecular approaches these studies identified the serine/threonine protein kinase, AMP-activated protein kinase (AMPK) as a mediator of TRAIL-TZD-induced apoptosis. Since AMPK seemed to be a major mediator of this apoptosis, in the current studies we utilized the natural compound Berberine (BBR), a known activator of AMPK in combination with TRAIL. These demonstrated a significant reduction of cell viability (MTT assay) and induction of apoptosis (caspase activation) when treated with a combination of TRAIL and BBR. This apoptosis is attenuated in cells expressing AMPKα1 dominant negative (DN), suggesting an involvement of AMPK in mediating this. To understand the downstream targets and the mechanism involved, an apoptosis PCR array analysis was performed, which suggested induction of TNFRSF1B (DR5) gene expression by BBR. In addition, knockdown of DR5 expression attenuated TRAIL-BBR-induced apoptosis, suggesting DR5 to be a potential target of BBR in this apoptotic cascade. Future studies will include determining any crosstalk of AMPK in BBR signaling to modulate DR5 pathway. Our earlier studies also demonstrated an involvement of β-catenin in mediating cancer cell resistance. Stabilizing muta-
tion of β-catenin has been reported to be present in a number of tumors, most profoundly in HCC leading to an activation of Wnt/β-catenin signaling. To understand any role of β-catenin in TRAIL-BBR-induced apoptosis, we determined the effect of BBR on β-catenin pathway. These revealed a significant attenuation of β-catenin protein expression by BBR in various HCC cells in a time and dose-dependent manner. BBR treatment also attenuated β-catenin/ TCF-induced transcriptional activity, indicating antagonism of β-catenin pathway. Our studies indicate that combination of TRAIL and AMPK activator BBR might be an effective means of antagonizing β-catenin pathway and ameliorating TRAIL resistance via DR5 in advanced forms of cancer.

#3323 Anticancer activity of medicinal plant and neuroglioma. Michael Quincy Shivers, 1 Jackson State University, Jackson, MS.

Neuroglioma, also called gliocytoma, is one of the common malignant tumors in central nervous system at present. Neuroglioma can make aggressive growth around brain tissue, so complete radical treatment can be realized by single excision. Nutritional and botanical treatments have been found useful in treating neuroglioma and other types of cancers. Vernonia amygdalina (VA) is a valuable medicinal plant that is widespread in East and West Africa. It has been reported to have not only diverse therapeutic effects for many tropical diseases, but has recently been shown to possess anti-cancer properties. Therefore, the goal of this research was to determine the therapeutic mechanisms of VA leaf extracts in the management of brain tumor. To achieve this goal, Human H4 neuroglioma cells were treated with different concentrations of Vernonia amygdalina for 24 hr. Cell survival was determined by MTS assay. The extent of oxidative cell/tissue damage was determined by measuring malondialdehyde (lipid peroxidation biomarker) concentrations by spectrophotometry. Cell apoptosis was measured by flow cytometry assessment (Annexin V/PI assay). Data obtained from the MTS assay indicated that VA significantly (p < 0.05) reduced the viability of H4 cells in a concentration-dependent manner. We detected a significant (p < 0.05) increase in malondialdehyde (MDA) concentrations in VA-treated glioblastoma cells compared to the control. Flow cytometry data showed a strong concentration-response relationship between VA exposure and annexin V/PI positive cells. Taken together, our finding indicates that VA induced cytotoxicity and apoptosis in H4 cells is associated with the formation of MDA, a by-product of lipid peroxidation and biomarker of oxidative stress. At therapeutic concentrations, VA-induced cytotoxic and apoptotic effects in H4 cells is mediated by oxidative stress.

#3324 FATE1 promotes mitochondrial hyperfusion and supports maintenance of mitochondrial networks following apoptosis stimulation. Anne Hamacher-Brady, 1 Verena Lang, 1 Nathan R. Brady, 1 Johns Hopkins University, Baltimore, MD; 2German Cancer Research Center, Heidelberg, Germany.

FATE1 (fetal and adult testis expressed 1), also known as the cancer-testis antigen B1-HCC-2, is expressed in testis and tumor tissues. FATE1 was recently described as a major survival factor in tumor cells of various origins, by mediating the degradation of the pro-apoptotic BH3-only protein Bik (Maxfield et al., in Nat Commun 2015 Nov 16;6:8480) and through ER-mitochondrial uncoupling (Doughman-Bouguerra et al., in EMBO Rep 2016 Sep;17(9):1264-80). Interestingly, FATE1 shares high sequence homology with the mitochondrial Drp1 receptor MFF (mitochondrial fission factor). We thus investigated a possible role of FATE1 impact on mitochondrial morphology following the stimulation of apoptosis in cancer cells. We found that, similar to MFF, FATE1 is localized to outer mitochondrial membranes and, unlike MFF, additionally localized to the endoplasmic reticulum. Importantly, in contrast to MFF, FATE1 overexpression does not recruit Drp1 to mitochondria, and instead promotes hyperfusion of mitochondrial networks. Co-immunoprecipitation experiments and reconstitution of Mfn2 or Mfn1 in double knockout mouse embryonic fibroblasts indicate a role for the mitochondrial fusion protein Mfn2, but not Mfn1. As FATE1 overexpressing cancer cells were more resistant to mitochondrial fragmentation in response to TNF and valinomycin treatments, we propose FATE1 as a novel regulator of mitochondrial morphology changes occurring during apoptosis, with possible implications in FATE1-mediated resistance of cancer cells to chemotherapy.

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Ginkgetin, a biflavonoid derived from leaves of Ginkgo biloba, induces autophagic cell death in non-small cell lung cancer via p62. Jian Shu Lou, Cathy Bi, Gallant Chan, Tina Dong, Karl Tsim. The Hong Kong University of Science and Technology, Hong Kong, Hong Kong.

Chemotherapeutic drugs in the treatment of non-small cell lung carcinoma (NSCLC) are usually mediated by induction of apoptosis; this usually results in drug resistant during the therapy. Many compounds from natural resource have been shown to trigger autophagy in promoting cell death, and therefore which could be a novel treatment for cancer. Ginkgetin, a biflavonoid from Ginkgo biloba leaves (Ginkgoaceae), was shown its anticancer effects recently. The leaf of G. biloba is being recorded as a safe herbal drug in Pharmacopoeia from China and Europe. Here, the possible development of ginkgetin as an anticancer drug was demonstrated. Ginkgetin induced the death in A549 (NSCLC) with an IC50 lower than that of cisplatin. This anticancer effect of ginkgetin was also illustrated in NSCLC xenograft nude mice model. Transmission electron micro-scope revealed that ginkgetin-induced autophagy was the main cause of cell death. Autophagy inhibitors, 3-methyladenine and chloroquine but not apoptosis inhibitor Z-VAD-FMK, rescued ginkgetin-induced cell death. Reciprocally, the induction of autophagy by rapamycin promoted ginkgetin-induced cell death, as well as the formation of p62-TRAF6-mTOR. During autophagy, p62 is hypothesized to be a target of ginkgetin-induced autophagic cell death. This notion is supported by several lines of evidence. The application of ginkgetin blocked fully the expression of p62 in cultured A549, and in contrast the overexpression of p62 reduced ginkgetin-induced autophagy and cell death concomitantly with the decline on autophagosome formation. In parallel, the modulation on p62 expression could regulate Nrf2 activity and ROS level in the presence of ginkgetin. Thus, autophagy could act as an inducer in ginkgetin-induced cell death, and p62 was proposed to be a possible target during this process. Here, ginkgetin exhibited promising anticancer effect in NSCLC via autophagy, a signaling very different to orthodox chemotherapeutic drug cisplatin. Besides, ginkgetin is a phytochemical from G. biloba that is considered as a safe herbal supplement, and no obvious toxicity was observed in xenograft nude mice. Therefore, ginkgetin is proposed to be a potential compound for the development of novel anticancer drug. Acknowledgements: This study was supported by Hong Kong Research Grants Council Theme-based Research Scheme (T13-607/12R), GRF (603012C, 662713, M-HKUST04/13), TUYF15S01 and Foundation of The Awareness of Nature (TAON12SC01) to Karl Tsim.

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Identification of a novel androgen receptor co-regulator in prostate cancer. Aymen A. Shatnawi,1 Sridhar Acharya Malkaram,2 Efronski Tsouko,3 Daniel E. Frigo4. 1Univ. of Charleston, Charleston, WV; 2West Virginia State University, Charleston, WV; 3University of Texas MD Anderson Cancer Center, Houston, TX; 4University of Houston, Houston, TX.

Prostate cancer (PC) is one of the most frequently diagnosed tumors in the United States, affecting one in six men during their lifetime and second only to lung cancer as a cause of cancer death in men. One of the major drivers of prostate cancer is the androgen receptor (AR). It has been shown that deregulation of AR co-regulators play a crucial role in PC through modulating the transcriptional activity of the receptor. While the importance of the AR complex is well established, there is still an incomplete understanding of what all the associated co-regulators are and how they modulate AR signaling. Here, we have identified that the inhibitor of growth family member 4 (ING4), a protein that has been implicated in the pathogenesis of several solid tumors such as gastric and breast cancer, is a novel AR co-regulator. Overexpression of ING4 increased AR activity in an androgen-dependent manner as assessed using luciferase reporter gene assays. Supporting a role as a potential AR co-activator, RNA Next-Generation Sequencing of prostate cancer cells with ING4 knocked down revealed a repression of endogenous AR/R1881-activated genes and expression of AR/R1881 suppressed genes. Further, mining of existing clinical datasets revealed that the ING4 gene expression is de-regulated in PCs compared relative to normal tissue and tracks with poor patient prognosis. Collectively, the current findings strongly support the involvement of ING4 in AR signaling and PC progression and development.


The epidermal growth factor receptor (EGFR) deregulations play a central role in cancer progression. EGFR generates extra- and intracellular signals through various pathways, including its release in the tumor microenvironment through extracellular vesicles, as well as the nuclear EGFR signaling. That ultimately results in oncogenic signals promoting the tumor aggressiveness. Our previous report showed that sortilin, a member of the VPS10P sorting proteins, is associated with EGFR to allow its secretion through exosomes in non-small cell lung cancer (NSCLC) cells. Furthermore, sortilin is mainly present in the Golgi which is an essential compartment involved in the EGFR bypass towards the nucleus. Therefore, our aim is to investigate the potential role of sortilin in EGFR nuclear translocation. EGFR and sortilin translocation into the nucleus has been validated by proximity ligation assay and confocal microscopy analysis. Moreover, to study the sortilin function in the EGFR nuclear translocation, we resorted to the NSCLC cell line A549 in which the sortilin expression was silenced using a shRNA delivery. Subcellular fractionations and nuclear protein extractions were performed to study the impact of the sortilin loss on the EGFR-induced EGFR nuclear translocation. EGFR stimulation highly increases the nuclear translocation of the EGFR and sortilin complex. Interestingly, sortilin depletion significantly decreases the EGFR-induced EGFR nuclear translocation in the A549 cells. Strikingly, sortilin-depleted cells exhibit an overexpression of EGFR target genes (MYC, ARCG2, AURKA and CCND1) compared to the control cells. These results suggest that the overexpression of the EGFR target genes is independent of EGFR nuclear translocation in sortilin-depleted cells. Thus, the role of sortilin in the translocation of the known transcription factors was directly involved in the control of EGFR up-regulated genes to determine whether they are activated in sortilin-depleted cells. Moreover, to better understand the sortilin-dependent EGFR nuclear translocation, we compare functional properties (proliferation, migration and angiogenesis assays) of sortilin-depleted and control cells. Altogether, our findings suggest a key role of sortilin in the regulation of the EGFR nuclear transport, towards the control of target genes that are widely involved in cancer progression.

Protochadherin ? acts through SET and PP2A to potentiate MAPK signaling by EGFR and KRAS during lung tumorigenesis. Xiaorong Zhou,1 Barrett L. Updegraff,2 Yabin Guo,1 Michael Peyton,2 Luc Girard,3 Jill E. Larsen,4 Xian-Jin Xie,1 Yunyun Zhou,5 Tae Hyun Hwang,1 Yang Xie,4 Jaime Rodriguez-Canales,4 Pamela Villalobos,4 Carmen Behrens,4 Wistuba I. Ignacio,5 John D. Minna,4 Karl F. Kraut,2,4,5 and A. O’Donnell,6 1UT Southwestern Medical Center, Dallas, TX; 2QIMR Berghofer Medical Research Institute, Brisbane City, Australia; 3University of Mississippi Medical Center, Jackson, MS; 4MD Anderson Cancer Center, Houston, TX.

Non-small cell lung cancer (NSCLC) is the leading cause of cancer-associated deaths worldwide. Given the efficacy of membrane proteins as therapeutic targets in human malignancies, we examined cell-surface receptors that may act as drivers of lung tumorigenesis. Here we report that Protochadherin ? (PCDH7), a transmembrane receptor and member of the Cadherin superfamily, is frequently overexpressed in NSCLC tumors, and high expression of PCDH7 is associated with poor clinical outcome. PCDH7 overexpression synergizes with KRAS and EGFR to induce MAPK signaling and tumorigenesis. Conversely, PCDH7 depletion suppresses EGFR and KRAS-induced autophagy and reduces tumor growth. PCDH7 potentiates ERK signaling by facilitating interaction of protein phosphatase PP2A with its potent inhibitor, the SET oncoprotein. By establishing a previously unanticipated oncogenic role for PCDH7 in lung tumorigenesis, these results provide proof-of-concept support for the development of novel therapies that target PCDH7 at the cell surface of NSCLC cells. Experiments to evaluate the role of Pcdh7 in Kras-mediated lung tumorigenesis in vivo using autochthonous mouse models, which we expect to lend further support for therapeutic targeting of this protein, are in progress.

Combinatorial blockade of ERBB receptors in HER2 low breast cancer. Mireia Berdiel-Acer,1 Eileen Reinz,2 Khalid Abnoura,1 Sara Burnmester,1 Rainer Will,1 Ulrike Korf,1 Stefan Wiemann1. 1UT Southwestern Medical Center, Dallas, TX; 2QIMR Berghofer Medical Research Institute, Brisbane City, Australia.

Large number of breast cancer patients clinically classified as HER2 negative, show low/moderate levels of HER2 along with other ERBB receptors. Concomitant blockade of EGFR, ERBB2 and ERBB3 with specific therapeutic antibodies (cetuximab, trastuzumab/peruzumab and lumretuzumab, respectively) appears as a beneficial approach to improve survival of patients who have failed to previous treatment strategies. Additionally, ERBB3 expression has been reported as a trastuzumab resistance mechanism in HER2 positive subtypes. We have confirmed a specific pattern of ERBB receptors expression in different breast cancer subtypes. Individual and combined blockade of the receptors with...
therapeutic antibodies has been tested in vitro using a metabolic viability assay (Cell Titer Glo) and their effects in cell cycle status measured by BrdU staining. All four therapeutic antibodies bind to the respective extracellular domains inhibiting downstream signaling pathways. Response to individual treatment is cell dependent and correlates with EGFR expression in the triple negative MDA-MB-468 but not in luminal T47D cancer cell line. Response of T47D cells to lumretuzumab is higher when combined with pertuzumab or trastuzumab, suggesting ERBB2/ERBB3 dimer as the most relevant one, an effect more evident upon ectopic addition of NRG1 (only ligand for ERBB3). Long exposure of MDA-MB-468 cells to cetuximab induces ERBB3 expression and increases its sensitivity to cetuximab when combined. As well as in the HER2 + BT474 cancer cell line resistant to trastuzumab, higher levels of ERBB3 also make cells more sensitive to alternative anti-ERBB therapies. To establish the role of ERBB4 (no specific therapeutic antibody) in the whole ERBB network, stable knock-down regulation (shRNA) has been performed in T47D cells. Although treatment of HER2 overexpressing breast tumors has been successful, targeting other ERBB members in a HER2 moderate/low scenario seems to be a promising approach in combinatorial therapies; even in resistant cell lines. Thus, better characterization of ERBB network should help to pave the way for a personalized treatment of HER2 low breast cancer. [U.K.: Deceased March, 2016]


Purpose: Over 75% of all solid tumor cancers are characterized by aberrant expression of MUC1. Until date, no effective MUC1 targeting therapeutic has succeeded. The purpose of this body of work was to develop an antibody therapeutic that would effectively treat MUC1 positive solid tumor cancers, without damaging healthy MUC1 positive tissues. Experimental Procedures: MUC1 is a heavily glycosylated transmembrane protein whose expression is restricted to the apical border of healthy epithelial tissues but uniformly expressed over cancerous tissues. We discovered that the cancer-associated form of MUC1 is transmembrane cleavage product that remains after cleavage and release of the bulk of the extracellular domain. We named cleared MUC1, MUC1* (muk 1 star), and showed that it is a growth factor receptor that is activated by ligand-induced dimerization of its small extracellular domain. We also showed that 100% of pluripotent human stem cells express MUC1* but cleavage stops with the onset of differentiation. By studying stem cells and cancer cells in parallel, we sought to develop antibodies that would only recognize MUC1* as it is expressed on cancerous tissues but would not recognize MUC1* as it exists on some healthy cells and tissues. Summary: New Data: We previously showed that we had developed antibodies that recognized the cleaved, growth factor receptor form, MUC1* but not full length MUC1. However, MUC1 is cleaved to a healthy form of MUC1* on, for example, pluripotent stem cells, hematopoietic stem cells and stem cells of intestinal crypts. We now report that we have developed monoclonal antibodies that only recognize the cancerous form of MUC1* and do not recognize these healthy forms of MUC1*. To demonstrate that we have succeeded in deciphering the differences between cancerous MUC1* and healthy MUC1*, we also developed a set of monoclonal antibodies that only recognize healthy MUC1*. Thousands of human cancerous versus normal tissue specimens attests to the antibody’s specificity. Cancer specific and MUC1* but not full length MUC1 was blocked alone blocked the growth of MUC1* positive breast and hormone refractory prostate cancers in animals, with no detectable adverse effects. On the basis of tissue safety studies and efficacy studies, a clinical candidate antibody MNC2 has been identified, humanized and incorporated into more than 20 CAR constructs. huMNC2-scFv-CAR T cells display high cytolytic release cells, when co-cultured with MUC1* positive cancer cells. huMNC2-scFv-CAR T cells selectively kill MUC1* positive cancers, while stimulating T cell expansion. Conclusions: The relevant target for anti-cancer drugs is the extracellular domain of MUC1*, not full length MUC1. MUC1* is a growth factor receptor that is activated by NME family growth factors. Subtle differences between MUC1* as expressed on healthy stem-like cells and MUC1* expressed on cancerous cells has allowed the development of cancer-specific MUC1* antibodies, especially suited for cancer immunotherapies.

#3331 PID1, a candidate tumor suppressor in pediatric and adult brain tumors, is a novel interacting partner for a cell surface receptor. Anat Erdreich-Epstein, Xiuhai Ren. Children’s Hospital Los Angeles/University of Southern California, Los Angeles, CA.

BACKGROUND: PID1 is higher in fat from obese patients and is an inhibitor of insulin receptor signaling. We reported that PID1 is a novel inhibitor of medulloblastoma and glioma cell growth, and its mRNA level is highly correlated with survival across multiple independent datasets of these tumors. PID1 harbors a phosphotyrosine binding domain and is predicted to interact with proteins harboring NPYX motifs. We therefore hypothesized that PID1 will interact with phosphotyrosine-binding (PTB) domain containing protein(s) that may be important in brain tumors, and will modulate its/signaling. Here we report PID1 interactions with some of these proteins and determine its biological consequences. METHODS: Experiments consisted of immunoprecipitations, transient transfections, and western blotting. Functionally, assays included cell culture, flow cytometry for proliferation and migration, and reporter assays. RESULTS: The receptor tyrosine kinase AXL mediates nuclear translocation of the epidermal growth factor receptor. Toni M. Brand, Mari Izda, Kelsey L. Corrigan, Cara M. Braverman, John P. Coan, Bailey G. Flanagan, Andrew P. Stein, Ravi Salgia, Jana Roff, Randall J. Kimple, Deric L. Wheeler. Univ. of Wisconsin-Madison, Madison, WI; City of Hope, Duarte, CA; Experimental Pharmacology and Oncology Berlin-Buch GmbH, Berlin, Germany.

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The epidermal growth factor receptor (EGFR) is an important therapeutic target in patients with various cancers. Unfortunately, resistance to anti-EGFR therapeutics is common. Previous studies in our laboratory identified two mechanisms of resistance to the EGFR monoclonal antibody cetuximab: First, the nuclear translocation of EGFR bypasses the inhibitory effects of cetuximab; and second, the tyrosine kinase AXL mediates cetuximab resistance by maintaining EGFR activation and downstream signaling. On the basis of these findings, we hypothesized that AXL could mediate the nuclear translocation of EGFR in the setting of cetuximab resistance. We found that NSCLC cetuximab-resistant clones and patient-derived xenografts (PDxKs) had increased abundance of nuclear EGFR (nEGFR) and AXL. Cellular fractionation techniques, super resolution microscopy, and electron microscopy revealed that genetic loss of AXL diminished the nuclear translocation and accumulation of EGFR. Building on previous studies indicating that SRC family kinases (SFKs) and HER family ligands mediate the nuclear translocation of EGFR, we found that AXL knockdown down-regulated the expression of the SFKs YES and LYN, and the ligand neuregulin-1 (NRG1). Furthermore, AXL knockdown decreased the interaction between EGFR and HER3 and the nuclear abundance of HER3. Nuclear localization of EGFR could be rescued only upon simultaneous overexpression of Lyn and NRG1 in cells depleted of AXL. Collectively, these data uncover a previously unrecognized role for AXL in regulating the nuclear translocation of EGFR and suggest that AXL-mediated SFK activation and NRG1 expression are necessary and sufficient to regulate this process.

#3334 The FGF4R-388Arg variant exerts pro-tumorigenic effects in lung cancer by inducing an EMT phenotype. Álvaro Quintanal-Villalonga, Rocío Suárez, Laura Ojeda-Márquez, Santiago Ponce-Aix, Amancio Carnero, Luis Paz-Ares, Irene Ferrer, Sonia Molina-Pinel, H120-Cinto, Madrid, Spain; IBIS, Sevilla, Spain.

The FGF4R-388Arg allele, where an arginine substitutes a glycine in the 388 codon of the FGF4R gene, has been associated to poorer patient outcome in different tumor types. In lung cancer, the variant has been related to shorter overall survival in adenocarcinoma (ADC) patients, while no relationship with squamous cell carcinoma prognosis (SCC) has been reported. Mechanistically, the FGF4R-388Arg variant has been related to increased MAPK pathway activation and to EMT in prostate cancer in vitro models. Furthermore, the variant has been associated with higher STAT3 signaling in murine models of breast and lung cancer. However, so far the molecular biology for this FGF4R variant in lung cancer patients has not been addressed. We overexpressed the FGF4R-388Gly and FGF4R-388Arg variants in lung cancer cell lines from different histologic backgrounds and performed tumorigenicity surrogate assays and downstream signaling activation assessment. In the generated stable cell lines, we also determined the expression of EMT markers. Furthermore, we determined the FGF4R variant and the FGF4R and N-cadherin mRNA expression in a cohort of NSCLC patients (N=65) and correlated these data to patient outcome. We reported that FGF4R-388Arg increases tumorigenicity in lung cancer cell lines. Mechanistically, these functional effects seem to be mediated by MAPK and STAT3 overactivation and by the induction of an EMT phenotype, which includes N-cadherin increased expression. In fact, this induction of N-cadherin protein expression by the FGF4R-388Arg variant seems to be responsible for the pro-oncogenic effects reported. In NSCLC patient tumor samples, the FGF4R-388Arg variant correlates with higher N-cadherin expression and with poorer survival. In conclusion, the FGF4R-388Arg variant is a potential prognostic biomarker in NSCLC, including ADC and SCC patients. This variant increases tumorigenesis through the activation of MAPK and STAT3 signaling pathways and through the promotion of an EMT phenotype.


This study aims to investigate the role of Transforming Growth Factor-beta type III receptor on tumor metastasis through cell migration and invasion. Metastasis is the cause of 90% of cancer-related deaths. An important step in the metastatic process is the epithelial to mesenchymal transition (EMT) of cancer cells, which is stimulated by TGFβ. The binding of TGFβ to its type II receptor (TβRII) triggers the phosphorylation of ser/thr kinase TGFβ type I receptor (TβRI), and receptor-regulated Smad (R-Smad) which translocates to the nucleus and alters gene transcription. The activity of this pathway is affected by receptor localization on the cell membrane, while the presence of TβRII complex in clathrin-coated pits propagates TGFβ signaling. However, TβRII complex relocation to membrane lipid rafts reduces signaling. A TGFβ receptor that lacks kinase activity, type III (TβRIII), regulates TGFβ signaling through multiple roles. Previous investigations have determined TβRIII sequesters TGFβ ligand to reduce signaling, while also presenting TGFβ to TβRII to increase signaling. TβRIII has also been shown to bind type I and type II TGFβ receptors independently, and reverse their membrane partitioning from membrane lipid rafts to clathrin-coated pits. This TβRIII induced relocation of TβRII/II increases receptor half-life, altering TGFβ signaling. Many cancers demonstrate increased expression of TβRIII, suggesting that this protein is influential in the metastatic pathway. Interestingly, conflicting studies have shown that metastasis is stimulated when TβRIII is either overexpressed or knocked-down. Overexpression of exogenous TβRIII and knockdown of endogenous TβRIII using siRNA were used to explore alterations in cell migration and invasion potential. TGFβ signaling, receptor expression, membrane partitioning, and protein interaction studies were performed using Western blotting, sucrose-density ultracentrifugation, and immunoprecipitation techniques. Immunofluorescent transwell assays measured relative cell migration and invasion through Matrigel. Transfection of TβRIII demonstrated both the punctate and membrane localization of TβRIII, while inhibiting transwell migration. The ability of transfected cells to migrate and invade through transwell assays clarified the impact of TβRIII expression on metastatic processes. Finally, both the overexpression and silencing of TβRIII resulted in significantly different cancer cell migration and invasion profiles when compared to untransfected cell lines. TβRIII expression and activity modify cellular migratory and invasive potential through the modulation of the TGFβ signaling pathway, altering epithelial to mesenchymal transitioning and therefore tumor metastasis.

#3336 Comparison of epithelial mesenchymal transition mediated TKI resistance NSCLC cells containing wild type EGFR and mutant EGFR. Tsatsral Iderzorig, Sanjana Singh, Gagan Chhabra, Neelu Purri. Univ. of Illinois at Chicago, Rockford, IL.

Epithelial to mesenchymal transition (EMT) is a vital process in development and of metastasis and occurs when epithelial cell lose their polarized structure, by reducing adherent junction proteins E-cadherin, Claudin and ZO-1 on the membrane. Cells with EMT are elongated spindle like structures due to upregulation of mesenchymal markers Vimentin and N-cadherin. EMT may be responsible for resistance to molecular targeted therapies such as tyrosine kinase inhibitors (TKIs) against EGFR which is used in patients with activated EGFR mutations. However, these patients acquire resistance to TKIs after prolonged use. This acquired resistance to TKIs may also be due to a secondary T790M mutation in the kinase domain which could be responsible for inducing EMT. EMT is regulated by p120-catenin which interacts with the Kaiso factor in the cell nucleus and inhibits the transcriptional repressor activity of Kaiso factor. Kaiso factor represses Wnt target genes such as ZEB-1. The binding of p120-catenin to Kaiso factor also increases Wnt signaling which results in loss of E-cadherin. Cells undergoing EMT can acquire cancer stem-cell like characteristics by expressing stem-cell marker ABCB1. Thus we investigated EMT characteristics in TKI-resistant NSCLC cells, H2170 ER (Erlotinib resistant), H358 ER and H1975. To determine modulation of EMT biomarkers in TKI-resistant cells, H1975 with L858R and T790M mutations was compared to TKI-sensitive cell line H3255 with L858R mutation, using immunoblotting, and qPCR. Expression of stem-cell markers ABCB1 and EMT biomarker E-cadherin are measured using flow cytometry. Key EMT-related proteins such as PRMT-1, Slug, Snail, Twist, p120-catenin, and Vimentin were upregulated by 3.2, 3.18, 6.2, 1.68, 4 and 6.5 fold, respectively, and E-cadherin, Claudin and ZO-1 were down-regulated by 89%, 90% and 99% fold as compared to the H3255 TKI-sensitive cell line. We also observed upregulation of N-cadherin, ZEB-1 and Vimentin by 2.4, 2.6, and 12 fold and downregulation of E-cadherin by 50% in H1975 by qPCR. Immunofluorescence studies for Vimentin showed that H1975 cells were more elongated and stratified as compared to the H3255 that had polarized structures. 90% colocalization of p120-catenin and Kaiso factor was seen in H1975 whereas 10% colocalization was seen in H3255 cells. Flow cytometry results indicated that there was significant increase in expression of stem cell marker ABCB1 in TKI resistant cells H2170 ER, H358 ER and H1975 in comparison to H2170 P( Parental), H358 P and H3255 TKI sensitive cells. In conclusion, our results indicate that EMT is mediated through PRMT-1, which methylates Twist, transcriptional repressor of E-cadherin along with Snug and Snail in H1975 cells with T790M mutation. Cancer stem-cell marker ABCB1 is specific for TKI resistant NSCLC cells which exhibit EMT.

#3337 The G protein-coupled P2Y6 pyrimidoceptor increases tumorigenesis potential of epithelial cell in colorectal cancer associated to colitis. Morgan Placet, Caroline M. Mollé, Djordje M. Gribic Grbic, Guillaume Arguin, Sameh Geha, Fernand-Pierre Gendron. Université De Sherbrooke, Sherbrooke, Quebec, Canada.
BACKGROUND: Carcinogenesis is a complex process induced by various genetic mutations, which is characterized by 3 phases. The initiation phase, inflammation is predominant, as in colitis-associated carcinogenesis, and involves the active recruitment of immune cells like macrophages, dendritic cells and T cells. The promotion and progression phases are respectively tied to dysplasia and cancerous tissue and metasasis. Inflammatory cytokines, including colitis, induces an imbalance in pro- and anti-inflammatory cytokines, a breakdown in the epithelial barrier, pathogens infiltration and leukocytes recruitment and activation. During inflammation, nucleotides are released in a large amount in the extracellular environment. The P2Y6 receptor was associated with IBD where its expression was increased in T cells infiltrating inflamed colonic tissue and on the epithelial cells of the inflamed tissue. In this context, activation of the P2Y6 receptor may contribute to the formation of a tumor-promoting environment by modulating the immune response and establishment of tumors. METHODS: Colorectal cancer associated to colitis was induced in 2Pry6 gene knockout mice (P2Y−/−) using azoxymethane, as a carcinogen, and dextran sulfate sodium challenges as a promoting agent. Mice were euthanatized and the number and size of tumor measured. Tissues were fixed and stained prior to histological characterization. The impact of P2Y6R activation on apoptosis and NFκB pathway was determined by western blotting, and the impact on inflammatory cytokines balance and mucus layer were analyzed by histology and qPCR of different markers. RESULTS: Invalidation of the P2ry6 gene in mice lead to a reduction in the number of colorectal tumors in our AOM-DSS model. We linked this observation to the protective role of P2Y6R toward TNFα-induced apoptosis of cancerous epithelial cells. Furthermore, P2Y6R regulates the balance between pro- and anti-inflammatory cytokines in inflammatory conditions, and maintain the integrity of the epithelial barrier by modulating the density of the mucus layer. CONCLUSION: In this study, we have shown that P2Y6R could contribute to colorectal cancer colitis associated by blocking the apoptotic process, modulating the cytokine network, and regulating the integrity of the mucus layer. These results suggest that P2Y6R could be a prime target to reduce colorectal cancer colitis associated.

#3338 A role for FGFR4 in growth and survival of Ewing sarcoma cells. 
Justin J. Montoya,1 Daniel H. Wai,1 David W. Lee,1 Peter A. Azorsa,1 Oliver B. Pepper,2 Robert J. Arceci,1 David O. Azorsa1.

Ewing sarcoma is the second most common cancer of bone and soft tissue arising in children and young adults. Although the survival rate has improved for patients treated for localized disease, the survival rate for patients with metastatic tumor remains lower than 30%. In order to identify novel therapeutic targets and to better understand the genes involved in growth and survival of Ewing sarcoma, we employed a functional genomics approach based on siRNA screening. Four Ewing sarcoma cell lines, TC-32, TC-71, SK-ES-1 and RD-ES, were transfected with a library of siRNA targeting 287 cancer-associated genes. The resulting siRNA screening data for each cell line were normalized and statistically cut-offs were determined. The results indicated that siRNAs targeting Fibroblast Growth Factor Receptor 4, FGFR4 were among the most effective in reducing cell viability in all four of the Ewing sarcoma cell lines. Validation of the siRNA targeting FGFR4 reduced viability more than those to FGFR1, FGFR2, or FGFR3. Furthermore, siRNA targeting FGFR4 were able to induce caspase 3 activity. FGFR4 protein is expressed in Ewing sarcoma cells as determined by western blot analysis, although expression levels were lower compared to FGFR4 expression on rhabdomyosarcoma cells. Targeting FGFR activity in Ewing sarcoma cells using a pan-FGFR inhibitors PD-173074 and BGP-398 demonstrated that Ewing sarcoma cells were sensitive to FGFR inhibition. Furthermore, treatment of Ewing sarcoma cells with the selective FGFR4 inhibitor BLU9931 resulted in growth inhibition and decreased ERK signaling. These results indicate that FGFR4 may play an important role in growth and survival of Ewing sarcoma and could serve as a potential therapeutic target for this disease.

#3339 Muscarinic acetylcholine receptor subtype 3 regulates gastric stem cell number and gastric cancer cell proliferation by controlling YAP activation.

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Within the gastrointestinal, nerves help to regulate both normal and neoplastic stem cell dynamics. Several previous studies suggested that cholinergic nerve signaling plays an important role in gastrointestinal cancer development, but the exact underlying mechanism has not been clarified. In this study, we examined the role of muscarinic acetylcholine receptor subtype 3 (M3R) in gastric homeostasis and cancer development by using mouse models and human cancer cell lines. In situ hybridization revealed M3R expression in gastric stem cell zone, and its expression was markedly upregulated in gastric cancer cells. We knocked out M3R in Lgr5+ gastric stem cells in Lgr5-CreERT, M3Rflox/flox mice, and found that deletion of M3R inhibited clonal expansion of Lgr5+ cells in regenerative states. In a gastric tumor model of Mist1-CreERT, Apc/flox/flox mice, knockout of M3R dramatically suppressed tumor growth. RNA sequencing analysis of these tumors revealed that several important pathways were significantly inhibited in M3R knockout samples, including YAP/TAZ pathway. We established M3R-expressing gastric cancer cell lines, and western blotting, luciferase assay, and RT-PCR analysis confirmed that acetylcholine (ACh) agonist activates YAP pathway through M3R. YAP is upregulated in approximately half the gastric cancer cell lines with dysplasia or high-grade dysplasia in disease stage and histological form. This M3R-YAP axis activates the gastric stem cell niche and offers a compelling target for tumor treatment and prevention.

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Cell Growth Signaling Pathways

#3340 Identification of CARM1/PRMT4 as a novel therapeutic target for AML
Sarah M. Greenblatt, Pierre-Jacques J. Hamard, Takashi Asai, Na Man, Concepcion Martinez-Caja, Fan Liu, Stephen Nimer. University of Miami, Miami, FL.

Chromatin modifying enzymes, and specifically the protein arginine methyltransferases (PRMTs) have emerged as important targets in cancer. PRMT4, also known as CARM1, is overexpressed in a number of cancers, including breast, prostate, pancreatic, and lung cancer. Our lab reported the overexpression of PRMT4 in the context of acute myeloid leukemia (AML), showing that more than 70% of cytogenetically normal AML patients have up-regulation of PRMT4. Here, we investigated the role of PRMT4 in normal hematopoiesis and leukemia development. In order to study the role of PRMT4 in normal hematopoiesis, PRMT4-floxed mice were crossed with Vav1-cre mice purchased from the Jackson Laboratory. Inducible Prmt4 conditional KO mice were generated by crossing Prmt4-floxed mice with Mx1-Cre mice and Prmt4 gene excision was induced by poly(C:C). Using this hematopoietic specific knockout system, we show that loss of PRMT4 has little effect on normal hematopoiesis, but promotes the differentiation of hematopoietic stem and progenitor cells. Next we evaluated the role of PRMT4 in leukemia development using leukemia transplantation models driven by fusion oncoproteins. Strikingly, the knockout of PRMT4 completely abrogates leukemia initiation in fetal liver transplantation models driven by the AML1-ETO or MLL-AF9 fusion proteins. We further characterized the mechanism for the leukemia-specific dependence on PRMT4 using leukemia cell lines and found that knockdown of PRMT4 impairs cell cycle progression, decreases proliferation, and induces rapid apoptosis. To examine PRMT4 dependent changes in gene expression in a leukemia system, we used lentiviral vectors that express RFP and shRNAs directed against PRMT4. We knocking down PRMT4 in four leukemia cell lines or normal human cord-blood derived CD34+ cells. Gene set enrichment analysis showed that all four leukemia cell lines with knockdown of PRMT4 significantly down-regulated E2F target genes compared to the scrambled control. Chromatin immunoprecipitation analysis (ChIP) confirmed the presence of PRMT4 and H3R17 dimethylation at the promoter regions of E2F1 targets. The PRMT4 conditional knockout mice and PRMT4 knockdown experiments both suggest that the loss of PRMT4 protein has a selective effect on leukemia cells compared to normal hematopoietic stem and progenitor cells. Collectively, this work supports that targeting PRMT4 through chemical inhibition may be an effective therapeutic strategy for AML and other cancers with up-regulation of PRMT4.

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Epigenetics

#3341 Epigenetic deregulation of the Hippo pathway in muscle-derived sarcomas.
T. S. Karin Eisinger, Shuai Ye. Univ. of Pennsylvania School of Medicine, Philadelphia, PA.

Soft tissue sarcomas are an aggressive group of roughly 65 mesenchymal malignancies diagnosed in 200,000 people worldwide annually. The treatment approach for sarcomas has not changed significantly in 25 years. Furthermore, recent sequencing efforts have revealed that consistent oncogenic mutations are rare in muscle cancers. As a result, these tumors are not sensitive to most available targeted therapeutics, which specifically interface with the functions of mutant oncogenic pathways. We are investigating the possibility that together with p53 or RB1 mutation/loss, alterations in chromatin state and epigenetic regulation rather than oncogenic mutation, are required for sarcoma transformation. Based on our current findings we are testing the hypothesis that epigenetic deregulation of Angiomotin

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YAP1 activity promotes NF-κB activity. Our studies have revealed that YAP1 expression is epigenetically modulated through AKT regulation and caused re-expression of the muscle differentiation marker MYOD and p57 suggesting that epigenetic modulation promotes differentiation as well as inhibiting proliferation. Gene expression studies of control and YAP1-deficient tumors revealed that YAP1 controls NF-κB signaling. We have performed H3K27Ac ChIP-seq and super-enhancer analysis of human UPS tumors to determine whether NF-κB targets are important in human sarcomas. These analyses revealed that NF-κB is the most transcriptionally active pathway in UPS. Together these data suggest that persistent YAP1-mediated NF-κB signaling promotes sarcomagenesis. In normal undifferentiated muscle cells NF-κB signaling is essential for proliferation and inhibition of differentiation. We have now shown that SAHA/JQ1-mediated YAP1 inhibition subsequently decreases NF-κB activity, underscoring the ability of epigenetic therapies to decrease tumorigenesis and enhance differentiation. Our findings suggest that pharmacological modifier (Perifosine, 10mg/kg body weight daily for 4 weeks) and observed that AKT inhibition significantly induces di and tri methylation of H3K4, concomitant with decreased KDM5B levels. Overall, we observed that AKT activation negatively regulated miR-137 but not miR-138 levels, which further transcriptionally represses KDM5B expression. miR-138 and miR-137 as these have been shown to transcriptionally regulate H3K4 methylation. We observed that AKT activation negatively regulated miR-137 but not miR-138 levels, which further transcriptionally represses KDM5B expression. Overexpression of miR-137 significantly reduced KDM5B and increased H3K4 di and tri methylation levels but failed to change AKT phosphorylation. Genetic knockdown of AKT or inhibition of AKT phosphorylation increased miR-137 and reduced KDM5B expression. Overall, we observed that AKT transcriptionally regulates KDM5B mainly via repression of miR-137. Our data identify a mechanism by which PI3K/AKT signaling modulates the prostate cancer epigenome through regulating H3K4 methylation and KDM5B expression. Additional studies that assess the impact of AKT inhibition on genome occupancy of H3K4 methylation will help in the identification of targets and devise novel strategies to enhance the therapeutic efficacy of PI3K/AKT inhibitors that are currently in clinical development.


AlkB homolog 5 (ALKBH5) is one of the most prevalent internal modifications in eukaryotic messenger RNA. This dynamic and reversible modification is installed by methyltransferase complex consisting of three subunits: Methyltransferase-like protein 3 (METTL3), Methyltransferase-like protein 14 (METTL14) and Wilms tumor 1-associated protein (WTAP), and erased by two independent demethylases, Fat mass and obesity associated protein (FTO) and AlkB homolog 5 (ALKBH5). RNA demethylase ALKBH5 has been reported to play vital roles in several biological processes. However, very little is known about the role of ALKBH5 in cancer in general and breast tumorigenesis in particular. In this study, we report that ALKBH5 promotes breast cancer growth and progression. Using in vitro and in vivo models, we show that silencing of ALKBH5 inhibits breast cancer growth and invasion. Importantly, our studies reveal that ALKBH5 mediates its pro-tumorigenic function by regulating several microtubule associated genes including KIF2C, PLK1, MAPRE1 and RCC2 that are critical for spindle formation or assembly during mitotic progression. Interestingly, ALKBH5 target genes KIF2C and MAPRE1 are highly expressed in breast cancer patients and their higher expression is strongly correlated with lower survival of breast cancer patients. We believe that an optimal level of RNA modification of key target genes is critical for normal cell cycle progression and any alteration in the levels of RNA methylation may alter activity or expression of these genes resulting in unbalanced cell proliferation.

**#3344 AKT mediates transcriptional regulation of H3K4 demethylation in PTEN null prostate cancer.** Mohammad Imran Khan, Susvamita Rath, Qateeb Khan, Vagaa M. Adhami, Hasan Mukhtar. Unv. of Wisconsin-Madison, Madison, WI.

One of the common contributors to the pre-disposition of most human cancers is inappropriate activation of AKT due to loss of its negative regulator PTEN. Activated AKT regulates changes in metabolic processes such as acetyl-CoA production that impacts chromatin. AKT phosphorylates a wide variety of histone modifying enzymes including acetyltransferases and methyltransferases resulting in their hyperactivation or inhibition. Therefore, understanding the impact of AKT inhibition on global histone modification would be critical to identify novel histone modifiers regulated by AKT. We performed unbiased and comprehensive profiling of histone modifications in prostate specific PTEN knockout mice treated with an AKT inhibitor (Perifosine, 10mg/kg body weight daily for 4 weeks) and observed that AKT inhibition significantly induces di and tri methylation of H3K4, concomitant with reduction acetylation of H3K9. Changes in other histone modifications such as H3K6 and H3K9 methylation and acetylation were not observed. Mechanistically, we observed that AKT inhibition reduces the expression of H3K4 methylation related histone demethylases KDM5 family, especially KDM5B expression at transcriptional level. Similar observations were noted at translational level also. To further understand the transcriptional regulation of KDM5B by AKT we focused on miR-137 and miR-138 as these have been shown to transcriptionally regulate H3K4 methylation. We have observed that miR-137 knockdown or inhibition increased miR-137 but not miR-138 levels, which further transcriptionally represses KDM5B expression. Overexpression of miR-137 significantly reduced KDM5B and increased H3K4 di and tri methylation levels but failed to change AKT phosphorylation. Genetic knockdown of AKT or inhibition of AKT phosphorylation increased miR-137 and reduced KDM5B levels. Overall, we observed that AKT transcriptionally regulates KDM5B mainly via repression of miR-137. Our data identify a mechanism by which PI3K/AKT signaling modulates the prostate cancer epigenome through regulating H3K4 methylation and KDM5B expression. Additional studies that assess the impact of AKT inhibition on genome occupancy of H3K4 methylation will help in the identification of targets and devise novel strategies to enhance the therapeutic efficacy of PI3K/AKT inhibitors that are currently in clinical development.


The histone methyltransferase EZH2 is the enzymatic subunit of the polycomb repressive complex 2 (PRC2) that catalyzes the methylation of H3K27 thereby repressing target gene transcription. EZH2 is amplified, overexpressed, or mutated in multiple cancer types, most notably Follicular Lymphoma (FL) and germinal center Diffuse Large B-cell Lymphoma (GBL-DBCL). We previously reported that pre-
clinical models of malignant rhabdoid tumors, which are deficient in the SWI/SNF core component INI1 (SNF5, SMARCB1), are selectively killed by potent and selective inhibitors of EZH2. Here we report another class of SWI/SNF-altered cancers named small cell carcinoma of the ovary, hypercalcemic type (SCOHT) that is dependent on EZH2 activity. SCOHT is a very aggressive form of cancer that responds poorly to conventional therapy with a one-year overall survival rate of only 50%. Very few novel agents have been approved for this indication; thus there is a need for targeted therapeutics in SCOHT. SMARCA4 and SMARCA2 are co-inactivated in this tumor type that has many rhabdoid features. We demonstrate that tazemetostat, an EZH2 inhibitor currently in phase 2 clinical trials, induces potent and selective killing in SMARCA2 and SMARCA4-deficient ovarian cell lines. In addition to small molecule inhibitor data, we conducted functional genomic studies with CRISPR pooled screening, and confirmed that SCOHT is also sensitive to CRISPR-mediated EZH2 gene ablation. Dose-dependent anti-tumor effects were observed upon tazemetostat treatment in SCOHT xenografts deficient in both SMARCA2 and SMARCA4. We also report on additional non-ovarian tumor types with dual SMARCA2/SMARCA4 loss including NSCLC that exhibit EZH2 deficiency representing additional potential therapeutic indications for tazemetostat treatment.

#3346 Epigenetic downregulation of miRNA-34a and miRNA-449 induce epithelial-mesenchymal transition and acquired resistance to ceritinib. Sun Min Lim,1 Mi Ran Yun,2 Kyoung Ho Pyo,2 Seong Keun Kim,1 Seon-Kyu Kim,4 Yong Sung Kim,1 Mirang Kim,2 Byoung Chul Cho.1 Yonsei Univ. College of Medicine, Seoul, Republic of Korea; 1IE-UK Institute for Cancer Research, Seoul, Republic of Korea; 2CHA University, Seongnam, Republic of Korea; 3Korea Research Institute of Bioscience & Biotechnology, Republic of Korea.

Background: Treatment with ALK tyrosine kinase inhibitors elicits profound initial antitumor responses in ALK fusion-positive patients with lung adenocarcinoma. However, patients invariably develop acquired resistance to ALK inhibitors. Although the recent functional genetic studies characterized the mechanisms of resistance to ALK inhibition, epigenetic mechanism of acquired resistance is poorly understood. Method: We established in vivo and in vitro models of acquired resistance to ceritinib and crizotinib using H3122 and H2228 cells. For in vivo model, mice with established H3122-derived tumors were treated with ceritinib to derive ceritinib-resistant tumors. To investigate the epigenetic mechanisms of resistance, we performed methylated DNA binding domain sequencing (MBD-seq) for DNA methylation and chromatin immunoprecipitation-sequencing (ChIP-seq) for histone modifications associated with enhancers (H3K4me1 and H3K27ac) of H3122 and H3122 ceritinib-resistant (LR) cells. We also performed in situ hybridization of miR-34 and miR-449 in patient samples. Results: Epithelial-to-mesenchymal transition with AXL activation was found in multiple in vivo and in vitro ALK-rearranged lung cancer models with acquired resistance to ceritinib. Genome and epigenome-wide analysis identified that miR-34a and miR-449a are decreased with loss of H3K27ac, while AXL and mesenchymal genes are increased in the process of acquired resistance. We confirmed that miR-34a and miR-449a were involved in AXL-dependent epithelial-mesenchymal transition (EMT) by treating mimics and inhibitors of miR-34a and miR-449a. In addition, pharmacological inhibition of AXL activation of miR-34a or miR-449a restored sensitivity to ceritinib in H3122-LR cells. Histone deacetylase (HDAC) inhibitor, panobinostat, synergistically induced anti-proliferative effects with ceritinib in resistant cells. Treatment with panobinostat led to increased expression of miR-449a with increased H3K28ac signal in H3122 LR cells. Panobinostat also reversed EMT phenotype by downregulating N-cadherin and upregulating E-cadherin expression. Additionally, combination of panobinostat and ceritinib induced enhanced antitumor efficacy in acquired resistant xenograft models. To clinically validate our preclinical findings, we measured the expression of miR-34a and miR-449 by in situ hybridization in 5 matched ALK-rearranged lung cancers obtained from individuals both before and after ALK inhibitor treatment. We detected decreased expression of miR-34a and miR-449 in 3 of 5 samples (60%). Conclusion: Our findings demonstrate that H3K27ac remodeling is a crucial event in ceritinib resistance and inhibition of both ALK and HDAC could prevent or overcome acquired resistance to ALK inhibitors in individuals with ALK-rearranged lung cancer.

#3347 Exploring the role of ASXL family of genes in enabling oncogenic AR-signaling in prostate cancer. Abhijit Parolla, Xuhong Cao, Arul Chinnaiah Venkata, Anjali Seth, Anna Lippert, and Micah J. Bond.

Prostate cancer (PCa) is the second leading cause of cancer-related mortality in North American men. In recent years, there has been mounting evidence establishing the centrality of epigenetic mechanisms in PCA initiation and progression. Accordingly, various epigenetic genes have been described to collaborate with the androgen receptor (AR) in enabling its oncogenic transcriptional program and aberrantly restoring its activity in metastatic castration resistant PCa (mCRPC). Concordantly, our lab has recently described two epigenetic genes, BRD4 and MLL2, as key co-activators of the AR signaling complex. Furthermore, we have demonstrated the therapeutic potential of targeting these AR-collaborating genes, as part of a multi-institutional consortium, our lab had performed comprehensive molecular profiling of 150 mCRPC patient tumors. These analyses revealed the molecular mechanisms underlying the sensitivity of inhibited PCa. Thus, in a setting where invariably all metastatic PCa patients progress to evolve resistance to anti-AR therapy, epigenetic genes emerge as potent co-targets with the promise to improve patient outcomes. To To identify novel AR-collaborating genes, as part of a multi-institutional consortium, our lab had performed comprehensive molecular profiling of 150 mCRPC patient tumors. We have recently demonstrated that cooperative genetic alterations in chromatin modifiers are frequently detected in advanced mCRPC. To identify candidate genes, we performed a preliminary pipeline that integrates DNA methylation data from the AR-silenced PTEN knockout (PTEN-C) cell line and co-expression of AR and chromatin modifiers. We identified that the ASXL family of genes is the most enriched for co-expression with AR and is a candidate therapeutic target in mCRPC. To validate this hypothesis, we transfected PTEN-C cells with ASXL1, ASXL2, or ASXL3 and measured cell viability using the CellTiter-Blue assay. We observed a significant decrease in cell viability for all three ASXL family members, indicating their potential as therapeutic targets in mCRPC. To determine the mechanism of this effect, we performed RNA sequencing and ChIP-seq analysis to identify changes in gene expression and chromatin modifications. We found that ASXL1 and ASXL2 are both epigenetic modifiers and targets of AR signaling. ASXL1 is a known co-activator of AR, and our data suggest that it may play a role in mediating AR signaling in mCRPC. ASXL2, on the other hand, is a novel target of AR signaling and may represent a new therapeutic target in mCRPC. To confirm these findings, we performed a series of functional experiments, including xenograft models and reporter assays, which demonstrated that ASXL1 and ASXL2 are both critical for AR signaling in mCRPC. In conclusion, our findings identify the ASXL family of genes as potential therapeutic targets in mCRPC and suggest that targeting these genes may be a promising strategy for treating this aggressive cancer.

#3348 UnID: unified and integrated diagnostic pipeline for malignant gliomas based on DNA methylation data. Jie Yang, Jiangwu Wang, Lihong Long, Ravesankar Ezharlajaran, Erik Sulman. MD Anderson Cancer Center, Houston, TX.

Prognostic and predictive molecular diagnostics for patients with gliomas typically rely on multiple assays, requiring large amounts of tissue and high cost. DNA methylation has been utilized to identify prognostic subsets and high-throughput platforms exist that are suitable for archival tissue. Therefore, we developed a unified and integrated diagnostic pipeline that can assess multiple prognostic and predictive biomarkers using only the Illumina Infinium Methylation array. This pipeline includes two data processing and diagnostic biomarker pipelines. Data processing starts from the raw data and followed with quantitative sample, probe, and batch quality control. The diagnostic biomarkers include a glioma methylation assay that predicts radiation response (GaMA); tumor classification enriched for TCGA expression subclasses; copy number alterations including phosphatase and tensin homolog (PTEN) loss, epidermal growth factor receptor (EGFR) amplification, and chromosomal instability (CIN); isocitrate dehydrogenase (IDH) mutation, and 5′-methylguanine-DNA methyltransferase (MGMT) promoter hypermethylation. WHO grade II-IV gliomas were analyzed in both publicly available and institutional datasets. A signature was identified to effectively distinguished radiation resistant from radiation sensitive glioma stem-like cells (GSCs). Signature has been applied to 272 TCGA GBM samples from patients who received standard radiotherapy (RT). The survival analysis showed that the subgroup with RT-sensitive and RT-resistant have significant difference in survival time (log-rank test p-value = 0.0016). Gene expression subclasses predict biomarker was build by using the revised TCGA gene expression subclasses as gold standard. The prediction accuracy in test data set was 83.5% in the homogeneous subgroup, 71.0% in the semi-heterogeneous subgroup, and 62.1% in the heterogeneous subgroup. The prediction accuracy decreased as tumor heterogeneity increased. Certain copy number alteration events were predicted by developing specific signatures. Revised methylation signatures were developed for IDH mutation and G-CIMP status respectively, which can identified 99% of those samples. 238 GBM samples with 450k data available were tested with MGMT methylation-specific real-time PCR for MGMT methylation status. The methylation-based MGMT prediction accuracy reached about 90%. In summary, we have developed a single, FFPE-based pipeline for unified and integrated determination of multiple biomarkers of malignant glioma.

#3349 A regulatory circuit H1P1 gamma/miR-451a/c-Myc promotes prostate cancer progression. Ruimin Huang,1 Cunjie Chang,2 JiaJian Liu,3 Xiao-Jing Huang,2 Lan Shen,2 Dianzheng Zhang,3 Jun Yan.1 1Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China; 2Model Animal Research Center of Nanjing University, Nanjing, China; 3Philadelphia College of Osteopathic Medicine, Philadelphia, PA.

Heterochromatin protein 1 gamma (HP1 gamma), as a reader of the histone codes, is involved in the determination of higher order of chromatin structure. It
has also been implicated in carcinogenesis of various cancer types. However, the role of HP1 gamma in prostate cancer (PCa) progression and the underlying molecular mechanisms remain largely unknown. Using the public microarray data from GEO and TCGA database, we found that HP1 gamma was upregulated in PCa, comparing with benign prostate tissues. PCa patients with higher level of HP1 gamma had shorter disease-free survival, indicating that HP1 gamma level can serve as prognostic marker in PCa patients (p = 0.016). In addition, depletion of HP1 gamma by shRNA in PCa cells not only repressed proliferation and induced apoptosis, but also impaired tumorigenicity in vitro and in vivo. We also found that c-Myc was capable of upregulating HP1 gamma by directly binding to the E-box element in the first exon of HP1 gamma gene, and the upregulated HP1 gamma, in turn, repressed the expression of miR-415a by enhancing H3K9 methylation at the promoter region of miR-415a. Furthermore, reduction of miR-415a significantly reversed HP1 gamma loss-induced PCa cell apoptosis, whereas miR-415a overexpression repressed cell survival by targeting and downregulating c-Myc. The association among mRNA and protein expression levels of c-Myc, HP1 gamma and miR-415a were further confirmed in human clinical samples using Spearman correlation analysis. Therefore, we propose that an HP1 gamma/miR-415a/c-Myc regulatory circuit exists in PCa cells and this circuit plays a crucial role in PCa progression.

#3350 Cell-free DNA fragmentation patterns analyzed in over 15000 cancer patients reveal changes associated with tumor somatic mutations and result in improved sensitivity and specificity of somatic variant detection.


Background: Cell-free DNA (cfDNA) isolated from plasma consists of DNA fragments surviving clearance of dying cells and bloodstream trafficking. In cancer, these fragments carry the footprint of tumor somatic variation as well as its microenvironment. Since genomic distribution of cell free DNA fragments was shown to reflect nucleosomal occupancy in hematopoietic cells, we hypothesized that (a) heterogenous patterns of cfDNA positioning would be associated with distinct mutations in patient tumors and (b) integration of fragmentation patterns into analysis would allow increased sensitivity and specificity of somatic mutation detection. Methods: cfDNA fragment length and position distributions as well as associated somatic genomic profiles of over 15,000 patients with advanced-stage clinical cancer were determined by a highly accurate, deep-coverage (15,000x) cfDNA NGS test targeting 70 genes (Guardant360). An integrative data analysis of variant-free fragmentomics domain across different driver mutations was performed to identify patterns associated with detected somatic alterations. Results: We discovered distinct classes of fragmentomics subtypes significantly enriched in samples with different genomic subtypes. An independent cohort of samples with known HER2 immunohistochemistry status was interrogated to confirm discovered association between fragmentation patterns and HER2 status. Integrating fragmentation amplification signature with ERBB2 copy number analysis has resulted in 42% increase in the sensitivity and 7% increase in specificity of detection. Observed lung adenocarcinoma fragmentomics subtypes co-occurred with mutually exclusive genomic alterations and previously described intrinsic molecular subtypes of lung cancer. Conclusions: Fragmentomics classification of cancer cfDNA provides independent evidence for observed somatic variation and underlying tumor microenvironment, leading to higher sensitivity and accuracy of variant detection.

#3351 Aberrant RNA editing of GLI1 promotes malignant regeneration in multiple myeloma.

Elisa Lazzari, Nathaniel Delos Santos, Christina Wu, Lars Grontved, Gordon Hager. 1NCI/NIH, Bethesda, MD; 2Department of Pathology, Denver, CO.

In multiple myeloma, GLI1 promotes malignant regeneration. Procedures: Publicly available primary patient datasets were screened for HPV related oropharyngeal squamous cell carcinoma. Shuling Ren, Daria A. Gaykzlava, Theresa Guo, Zubair Khan, Mizuo Ando, Sunny Haf, Joseph A. Califano. 1University of California, San Diego, La Jolla, CA; 2Johns Hopkins Hospital, Baltimore, MD. HPV related head and neck squamous cell carcinoma (HNSCC) is known to occur in a separate patient population in comparison to tobacco related HNSCC, and also exhibits less mutational, genetic, and chromosomal alterations, and a different composition of epigenetic alterations. In particular, HPV is present in up to 70% of oropharyngeal squamous cell carcinoma (OPSCC), and is driving the increase in incidence rates of OPSCC. Given the precedent for successful screening for cervical cancer, it is attractive to consider similar population based screening for HPV related OPSCC. Here, we developed an epigenetic biomarker of ADAR1, or treatment with extrinsic pro-inflammatory stimuli, was utilized to probe the functional impact of RNA editing activity in MM models. Site-specific qPCR was used to quantify RNA editing in specific cancer stem cell-associated loci. Functional effects of ADAR1 activity were assessed in vitro survival and self-renewal assays, and in novel in vivo PCL xenografts. Results: Patients harboring 1q21 amplifications showed significantly increased IL-6 expression in vitro. Last, in vitro pro-inflammatory IL-6 stimulation, or continuous exposure to the immunomodulatory drug lenalidomide led to increased ADAR1 mRNA and protein levels, with a concomitant induction of RNA editing activity. Conclusions: In MM, 1q21 amplification has been linked to progression. We provide new evidence linking expression and activity of ADAR1, located on 1q21, and disease stage. Because ADAR1 induces transcript recoding, A-to-I editing could contribute to the marked transcriptomic diversity typical of advanced MM. While the Hh pathway has been linked to cancer stem cell generation in human MM, here we identified a primate-specific mechanism of Hh pathway activation in MM through RNA editing-dependent stabilization of GLI1. Together, both genetic and microenvironmental factors modulate epitranscriptomic deregulation of cancer stem cell pathways in MM.
Background and Aims: Accumulating evidence suggests that a subset of cancer stem cells (CSCs) influence various clinical outcomes in cancer, including tumor recurrence, metastasis and resistance to chemotherapy. Recently stemness has been recognized as a dynamic state governed by epigenetic modifiers including miRNAs. Despite identification of several DNA methylation-associated biomarkers, epigenetic regulation by miRNAs remains unclear. Herein, we characterized miRNA expression of CSCs with high vs. low CD44v6 expression through RNA-Seq. Subsequently, we investigated the clinical significance of a novel miRNA identified from this systematic discovery approach. Methods: Colon cancer CSCs from HCT116 and HT29 cell lines were grown as spheroid-derived cancer stem cells (CSCs). CD44v6+ and CD44v6- CSCs were subsequently confirmed in CD44v6+ and CD44v6- CSCs. qRT-PCR: Results: MiRNA profiling identified a unique overall pattern of CD44v6+ CSCs indicative of high degree of stemness features in cancer. In particular, we identified miR-1246, which is significantly overexpressed in CSCs as well as chemoresistant cells and its expression was associated with poor prognosis in CRC patients. Collectively, we have identified a unique group of previously unreported miRNAs which appear to have important mechanistic roles in CSCs and could serve as a promising predictive biomarker for recurrence and prognosis in patients with CRC.


Introduction: Colorectal cancer (CRC) is the third most frequent malignancy in males and second most common disease in females worldwide. CRC pathogenesis is intimately associated with lifestyles, such as diet, obesity, and smoking. Multiple evidences have revealed that these risk factors can trigger specific epigenetic alterations and subsequently promote carcinogenesis. Recently, adenosine (A)-to-inosine (I) RNA editing has been shown to be a potential epigenetic event in human cancers. One of the most important RNA editing gene targets is the antizyme inhibitor 1 (AZIN1), and edited AZIN1 promotes accumulation of ornithine decarboxylase and polyamines, leading to promotion and development of cancer.

**Molecular and Cellular Biology / Genetics: Epigenetics**

**#3354 Histone deacetylase-1 promotes urothelial cell migration and invasion by modulating p63-pMLC2 signaling.** Kirtiman Srivastava, Conor Brennan, Karen Mccloskey.

Background: Aberrant expression of histone deacetylases (HDAC) and p63 transcription factor are associated with poor prognosis in invasive urothelial tumors. The current study focused on HDAC-evoked epigenetic modification of p63 and its downstream effects. Methods: Primary human urothelial cells (HUC) and urothelial cancer cell lines (HT1376, T24, TCCSUP) were cultured either on human bladder fibroblast-embedded collagen-I to establish 3D-organotypic rafts or as 2D-monolayers. Scratch wound assay was performed on 300 μm and 700 μm gaps to study the effect of HDAC on cell migration and invasion. Treatment with HDAC inhibitors (vorinostat (pan) and tinininvasive cells indicating that cytoskeletal reorganization may be pivotal for cell migration. Treatment with HDAC inhibitors (vorinostat (pan) and entinostat (HDAC1, 3)) attenuated the number of invasive incidents in T24 and TCCSUP cells, restored expression of p63, p21, pMLC2-Ser18, pMLC2-Thr18, Ser18 and p21 in invasive cells compared to normal (N = 5, p < 0.05). These results coincided with the loss of stress fiber formations visualized by immunofluorescence detection of F-actin in invasive cells indicating that cytoskeletal reorganization may be pivotal for cell migration. Treatment with HDAC inhibitors (vorinostat (pan) and entinostat (HDAC1, 3)) attenuated the number of invasive incidents in T24 and TCCSUP cells, restored expression of p63, p21, pMLC2-Ser18, pMLC2-Thr18, Ser18 and p21 in invasive cells compared to normal (N = 5, p < 0.05), and normalized expression of 14-3-3 eta and p-p27-Thr157. Reappearance of stress fiber formations traversing across the cell after HDAC inhibitor treatments was supportive of these results, while nuclear enrichment of p27 indicated its enhanced cytoplasmic-to-nuclear trafficking. Transient knockdown of HDAC1 in the invasive population mimicked the effect of HDAC inhibitors (N = 5, p < 0.05).

Conclusions: Inhibition of HDAC1 activity attenuates urothelial cell migration and invasion by restoring the expression of p63 which, (a) upregulates the expression of ROCK1, and (b) suppresses the expression of ROCK1. ROCK1 expression was associated with poor prognosis in CRC patients comprising of 329 colorectal cancer and adenoma patients. In addition, we performed a series of functional assays to elucidate the functional role of AZIN1 RNA editing in CRC pathogenesis. Results: Using RESSeqPCR, AZIN1 RNA editing levels were analyzed in two CRC cohorts. AZIN1 editing levels were significantly higher in cancer tissues at all stages (I through IV) compared with normal mucosa. Additionally, AZIN1 was highly edited in colorectal adenomas. Differently expressed miRNAs were subsequently confirmed in CD44v6+ CSCs, and discovered upregulation of previously unreported miRNAs (miR-1265, 3605, 3182 and 4284). KEGG pathway analysis indicated that these miRNAs regulate Akt-MAPK and Wnt signaling pathways. Subsequently, we selected miR-1246 and validated its expression in CD44v6+ and CD44v6- CSCs and chemoresistant cells. Clinically, the expression of miR-1246 was significantly elevated in CD44v6- CSCs compared to corresponding normal mucosa and this occurred in a stage-dependent manner in primary CRCs. Furthermore, the expression of CD44v6 positively correlated with miR-1246 in CRC tissues. High miR-1246 expression resulted in poor disease free and overall survival. Conclusion: Using a systematic and comprehensive approach, we have identified a unique network of dysregulated miRNAs in CD44v6+ CSCs indicative of high degree of stemness features in cancer. In particular, we identified miR-1246 to be frequently overexpressed in CSCs as well as chemoresistant cells and its expression was associated with poor prognosis in CRC patients. Collectively, we have identified a unique group of previously unreported miRNAs which appear to have important mechanistic roles in CSCs and could serve as a promising predictive biomarker for recurrence and prognosis in patients with CRC.

**#3355 Identification of a novel network of miRNAs that regulate stemness in colorectal cancer.** Shusuke Toden, Takatoshi Matsuyama, Elizabeth Hutchins, Kendall Jensen, Ajay Goel.

Background: HPV infection is a well-known risk factor for the development of cervical cancer and adenocarcinomas. HPV infection is commonly associated with dysregulation of epigenetic modifications, leading to promotion and development of cancer. The role of miRNAs in the development and maintenance of stemness features is less understood. Herein, we characterized miRNA expression of CSCs with high vs. low CD44v6 expression through RNA-Seq. Subsequently, we investigated the clinical significance of a novel miRNA identified from this systematic discovery approach. Methods: Colon cancer CSCs from HCT116 and HT29 cell lines were grown as spheroid-derived cancer stem cells (CSCs). CD44v6+ and CD44v6- CSCs were subsequently confirmed in CD44v6+ and CD44v6- CSCs. qRT-PCR: Results: MiRNA profiling identified a unique overall pattern of CD44v6+ CSCs indicative of high degree of stemness features in cancer. In particular, we identified miR-1246 to be frequently overexpressed in CSCs as well as chemoresistant cells.

**Gene** | **Tissue Sensitivity** | **Tissue Specificity** | **Saliva Sensitivity** | **Saliva Specificity**
--- | --- | --- | --- | ---
EMB1 | 97.7% | 83.3% | 13.3% | 94.7% |
KCNA3 | 76.7% | 92.0% | 13.3% | 94.7% |
EMB1&KCNA3 | 100.0% | 84.0% | 13.0% | 84.2% |

**EMB1 Sensitivity Specifıcity Sensitivity Specifıcity**

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**Panel that can be combined with high risk HPV DNA detection with potential application for development of a population based screening test.**


Background: HPV infection is a well-known risk factor for the development of cervical cancer and adenocarcinomas. HPV infection is commonly associated with dysregulation of epigenetic modifications, leading to promotion and development of cancer. The role of miRNAs in the development and maintenance of stemness features is less understood. Herein, we characterized miRNA expression of CSCs with high vs. low CD44v6 expression through RNA-Seq. Subsequently, we investigated the clinical significance of a novel miRNA identified from this systematic discovery approach. Methods: Colon cancer CSCs from HCT116 and HT29 cell lines were grown as spheroid-derived cancer stem cells (CSCs). CD44v6+ and CD44v6- CSCs were subsequently confirmed in CD44v6+ and CD44v6- CSCs. qRT-PCR: Results: MiRNA profiling identified a unique overall pattern of CD44v6+ CSCs indicative of high degree of stemness features in cancer. In particular, we identified miR-1246 to be frequently overexpressed in CSCs as well as chemoresistant cells.

**Gene Sensitivity Specifıcity Sensitivity Specifıcity**

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<td>EMB1&amp;KCNA3</td>
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**EMB1 Sensitivity Specifıcity**
MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Epigenetics 3

groups. Taken together, these results highlight the oncogenic role of AZIN1 RNA editing in CRC. Conclusion: Our systematic and comprehensive study, which is first of its kind, reveals that AZIN1 RNA editing is novel epigenetic alteration that promotes an oncogenic behavior in colorectal cancer. In addition to its functional role, AZIN1 editing levels may be one of the important facilitators of adenoma-carcinoma sequence in CRC and serve as an important clinical biomarker in this disease.

#3357 Heritable methylation marks associated with breast cancer risk. Melissa C. Southey,1 Jhooon E. Joo,1 James G. Dowty,2 Roger L. Milne,2 EE Ming Wong,1 Pierre-Antoine Dugué,2 Dallas English,1 John L. Hopper,1 David E. Goldgar,2 Graham G. Giles*,1 The University of Melbourne, Melbourne, Australia; 2Cancer Council Victoria, Melbourne, Australia; 3Huntsman Cancer Institute, Utah, UT.

While most epigenetic marks are reprogrammed during early embryogenesis, some studies have reported Mendelian-like inheritance of germline DNA methylation in particular cancer susceptibility genes. We aimed to identify heritable methylation marks associated with breast cancer susceptibility by studying 25 Australian multi-generational families with multiple cases of breast cancer who were not known to carry mutations in breast cancer susceptibility genes. Peripheral blood DNA methylation was measured at approximately 480,000 genetic loci (methylation sites) for 210 members of these families using the Illumina HumanMethylation450 BeadArray. We developed and applied a new statistical method to identify heritable methylation marks based on complex segregation analysis. Carrier probabilities were estimated for the 1000 most heritable methylation marks, based on family structure but not disease status, and Cox proportional hazards survival analysis was used to test for associations between these carrier probabilities and breast cancer. After correcting for multiple testing, we identified 11 methylation marks whose corresponding carrier probabilities were significantly associated with breast cancer. Three marks clustered within 200 base pairs of the noncoding RNA miR886. We found evidence for these marks to be associated with aggressive (but not non-aggressive) prostate cancer risk in a population-based prostate cancer study. The association with breast cancer risk was replicated for three of the eleven marks in an independent population-based breast cancer study. Our work demonstrates the efficacy of our novel approach for identifying heritable methylation marks associated with breast cancer. This methodology may provide insights into other disease settings where known lifestyle exposures and genetic risk variants do not fully explain the familial relative risk.

#3358 Epigenetic signatures associated with patient outcome in thyroid carcinoma. Mariana Bisarro dos Reis,1 Caroline Moraes Beltrami,1 Mateus Camargo Barros-Filho,2 Fabio Albuquerque Marchi,2 Hellen Kuasne,2 Skirant Ambatipudi,2 Zdenko Herceg,2 Luiz Paulo Kowalski,2 Silvia Regina Rogatto*.1 Veje Hospital, Veje, Denmark; 2AC Camargo Cancer Center, Brazil; 3International Agency for Research on Cancer, France.

BACKGROUND: Thyroid cancer (TC) is the most frequent endemic neoplasia composed essentially by well-differentiated tumors (90%). These tumors generally are indolent and the patients show a favorable outcome. However, a set of TC patients presents aggressive outcome. Global deregulation of DNA methylation has been described as involved in thyroid cancer (TC) development. In this study, DNA methylation profile was performed aiming to identify a prognostic signature in TC. PATIENTS AND METHODS: The methylation profile of 50 non-neoplastic thyroid tissues (NT), 17 benign thyroid lesions and 74 TC (60 papillary, 8 follicular, 2 Hurthle cell, 1 poorly differentiated and 3 anaplastic thyroid carcinomas) were investigated using the Methylation 450 Human Infinium® BeadChip platform (Illumina). The data were normalized and analyzed using SVA, watermelon and LIMMA packages. The threshold delta beta of 0.2 and adjusted p-value < 0.05 were used to identify differentially methylated probes among the histological subtypes. The delta beta of 0.1 and adjusted p-value < 0.05 were used in the prognostic features analysis in WDTC (well differentiated thyroid cancer) cases. An epigenetic classifier according to WDTC was identified using diagonal linear discriminant analysis. The results were compared with The Cancer Genome Atlas (TCGA) database. RESULTS: Methylation analysis revealed a specific epigenetic profile according to the histological subtypes. A global hypermethylation was observed in benign lesions and follicular carcinomas, while papillary and undifferentiated carcinomas were widely hypomethylated compared with NT. An epigenetic signature comprising 21 probes differentially methylated (delta beta 0.1) was able to predict poor outcome in WDTC patients. This classifier revealed 63% of sensitivity and 92% of specificity, which was confirmed by TCGA database (64% of sensitivity and 88% of specificity). Using the established signature, we were able to confirm the involvement of poor prognosis markers with high-risk scores (multivariable analysis; P < 0.001). CONCLUSION: Thyroid tumors showed different methylational profile according to the histological subtypes. Genes regulated by methylation in TC and associated with the tumor development were identified and confirmed by TCGA. In addition, a meaningful algorithm was designed and confirmed as capable to predict recurrence in WDTC. FINANCIAL SUPPORT: FAPESP (2015/20458-5) and National Institute of Science and Technology in Oncogenetics (FAPESP 2008/57887-9 and CNPq 753589/08-9), CNPq (202608/2011-4).

#3359 TET1 mediated hypomethylation activates oncogenic signaling pathways in triple negative breast cancer. Charly Ryan Good, Andrew Kelly, Jozeč Madzo, Jaroslav Jelinek, Jean-Pierre Issa. Fels Inst. at Temple Univ. School of Medicine, Philadelphia, PA.

Triple negative breast cancer (TNBC) is a subtype of breast cancer that occurs in 15-20% of patients, and is defined by tumors that do not overexpress the estrogen, progesterone and HER2 receptors. This aggressive subtype has a significantly worse overall survival compared to non-TNBC and importantly, these patients lack options for targeted therapy. To identify novel subtype-specific therapeutic targets, we must first understand the biological underpinnings of the disease. DNA methylation is a hallmark of cancer, as it can regulate gene expression of both tumor suppressor genes and oncogenes. We have found that TN tumors have widespread genome-wide hypomethylation when compared to other breast cancer subtypes and normal breast controls. TET1 is a DNA demethylase that converts 5-methyl cytosine into 5-hydroxymethyl cytosine, which can be further oxidized into un-methylated cytosine. To determine if TET1 could possibly play a role in TNBC hypomethylation, we analyzed genome-wide DNA methylation, DNA mutation and RNA-seq datasets from the TCGA breast cancer cohort. We identified a subset of TN patients that upregulate TET1 and display DNA hypomethylation. To identify the hypomethylated TET1 target genes, we computed Spearman correlations between TET1 expression and methylation % for 450,000 sites (450K array) across 67 TNBC patients. Filtering for sites with r² < 0.3 revealed 42,559 sites negatively correlated with TET1 expression. Cluster analysis of the sites that lose methylation compared to normal breast (12,907 CpG sites) revealed two distinct clusters. Cluster 1 (43% of TNBC cases) were TET1 high and hypomethylated, while cluster 2 (58%) looked more like normal breast controls. Gene set enrichment analysis of the hypomethylated genes revealed Hippo Signaling, Pathways in Cancer and PI3K-Akt Signaling as significantly enriched, with p < 0.001. In addition, only 4% of patients in cluster 1 have mutations/genomic alterations in the PI3K pathway, compared to 29% of cluster 2 patients, p = 0.01. Most strikingly, analysis of phosphorylated EIF4EBP1/RPPA proteomic data revealed TET1 high patients have increased PI3K pathway activity, even though they lack mutations in the pathway, p = 0.02. We hypothesize that the T3PK hyper-activation, in part, can be explained by TET1 upregulation and target gene demethylation. In TET1 knock out MDA-MB-231 cells, we observed a reduction in phosphorylated 4E-BP1, suggesting loss of PI3K activity is concomitant with loss of TET1 as well as decreased cellular proliferation (p = 0.01). Furthermore, breast cancer cell lines with high TET1 (but not low TET1) are sensitive to drugs targeting the PI3K/ERK pathway (XMD8-85, AZ628, TGX221). In addition to explaining and predicting PI3K inhibitor sensitivity in breast cancer, our studies may establish TET1 as a TNBC specific oncogene that could serve as a novel druggable target for therapeutic intervention.

#3360 Targeted catalytic inhibition of EZH2 synergizes with low-dose HDACi in malignant rhabdoid tumors. Dean Popovski,1 Elizabeth M. Algar,1 Catherine R. Cochrane,1 Anette Szczepny,1 W. Samantha Jayasekara,2 David M. Ashley,2 Peter Downie,2 D. Neil Watkins,4 Jason E. Cain,1 Hudson Institute of Medical Research, Clayton, Australia; 2Barwon Health, Geelong, Australia; 3Monash Children’s Hospital, Clayton, Australia; 4Garvan Institute of Medical Research, Darlinghurst, Australia.

Malignant Rhabdoid Tumor (MRT) is a rare pediatric cancer of the kidney and CNS that is resistant to current treatment protocols. MRT is genetically characterized by homozygous inactivation of SMARCB1, a critical subunit of the SWI/SNF chromatin-remodeling complex. Next-generation sequencing data suggests that inactivation of SMARCB1 is the primary driver mutation, implicating epigenetic deregulation in the pathogenesis of MRT. Recently, we showed that sustained treatment of MRT cell lines with low-dose Panobinostat (LBH589), inhibited tumor growth by driving multi-lineage differentiation in vitro and in vivo. Furthermore, re-expression of physiological levels of SMARCB1 in G401 MRT cells phenocopied the low-dose LBH589 treatment and led to growth inhibition, senescence and terminal differentiation in vitro and in vivo. Enhancer of Zeste homolog 2 (EZH2), a core subunit of the Polycomb Repressive Complex 2 (PRC2), confers transcriptional silencing via the addition of methyl groups to Lysine 27 of Histone 3 (H3K27me3), and is a comb Repressive Complex 2 (PRC2), confers transcriptional silencing via the addition of methyl groups to Lysine 27 of Histone 3 (H3K27me3), and is a...
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#3361 Methylome differences in differentiated thyroid cancers and benign adenomas. Josena K. Stephen, Kang Mei Chen, Jason Merritt, Indrani Datta, Dhananjay Chitale, George Divine, Maria J. Worsham. Henry Ford Hospital, Detroit, MI.

Many recent studies examining aberrant DNA methylation in thyroid cancer are restricted to either candidate genes or genome-wide methylation in specific thyroid tumor subtypes. The goal of this study was to identify differentially methylated genes globally and their association with molecular pathways and signaling networks. Common mutations in papillary thyroid cancer (PTC), follicular thyroid cancer (FTC), follicular adenoma (FA) and normal thyroid were also examined. Genome-wide methylation profiling using the Infinium HumanMethylation450BEFORMALIN fixed paraffin embedded BeadChip array was performed on 24 thyroid cases (8 PTC, 8 FTC, 4 FA and 4 normal thyroid). Ingenuity Pathway Analysis (IPA) was utilized to assess the roles of significantly differentially methylated genes in biological functions, signaling/metabolic pathways, and networks. Common mutations in 4 genes (BRAF, NRAS, HRAS, and KRAS) were assessed using TaqMan Mutation Detection assay. Twelve genes were significantly differentially methylated among 4 comparison groups: cancer vs normal, cancer vs adenoma, PTC vs normal, and PTC follicular variant (PTC-FV) vs PTC-Classic. CTU1 and HLA-DPB1 were significantly hypermethylated and AARS2, TMSB10, RNFE216L, KIF15, KIAA1143, and SLC2A13 were significantly hypomethylated between cancer and normal. Significant differential hypermethylation was noted for PNPAL7 and NPC1L1 in cancer vs adenoma and SNX1 in PTC-FV vs PTC-Classic. NT5C1B was hypermethylated and AARS2 was hypomethylated in PTC vs normal. IPA identified 2 gene networks, involving 11/12 genes, characterized by 1) Cellular development, Cellular growth and proliferation, Connective tissue development and function and 2) Drug metabolism, Cell-mediated immune response, Cellular development. NT5C1B was involved in all 4 highly ranked canonical nucleotide degradation pathways. Several significant bio-functions involved NPC1L1. Mutations of NRAS codon 61 were identified in 1 sample each of FTC-Classic, PTC-FV and FA and BRAF V600E in one PTC-Classic sample. Differential methylation of AARS2, CTU1, HLA-DPB1, SLC2A13, PNPLA7, NPC1L1, NT5C1B and SLC2A13 were identified in 1 sample each of FTC-Classic, PTC-FV and FA and BRAF V600E in one PTC-Classic sample. Differential methylation of AARS2, CTU1, HLA-DPB1, SLC2A13, PNPLA7, NPC1L1, NT5C1B and SLC2A13 in FTC were characterized by a significant enrichment of genes involved in the above pathways.

#3362 PBRM1 regulates the transcription of cell adhesion genes in ccRCC. Basudev Chowdhury. Purdue Univ., West Lafayette, IN.

Advances in parallel sequencing have facilitated the recent identification of Polybromo-1 (PBRM1), a component of the PBAF (Polybromo-associated-BRG1- or BRM-associated factors) chromatin remodeling complex, as the second most frequently mutated gene in clear-cell renal cell carcinoma (ccRCC). While several studies have described the diversity of PBRM1 mutations in ccRCC and established the mutation of PBRM1 as a “driver” of ccRCC carcinogenesis, little is known about the biological significance of PBRM1. With the intent of shedding light on the mechanistic roles of PBRM1, we have employed Next Generation Sequencing to delineate the transcriptional profile of cellular models of ccRCC with or without PBRM1 expression and identified genes associated with cell adhesion to be regulated by PBRM1 in 2D monolayer cultures. Since 2D cultures lack the architectural complexities to support studies pertaining to cellular adhesion, we evaluated the role of PBRM1 in 3D culture on Basement Membrane Matrix to provide a physiologically relevant analysis of gene expression and the resulting cellular phenotype. The results showed that of the 97 cell adhesion genes identified to be upregulated by PBRM1 in 2D cultures, 45 were also upregulated in 3D cultures and a subset of 32/45 genes underwent increased gene expression in 3D with respect to 2D cultures. The gene expression profile of candidate genes were verified by qPCR and Western Blotting. Mining The Cancer Genome Atlas (TCGA) publicly available data, we were able to correlate the gene expression profiles of cell adhesion genes identified in 3D cultures with those of ccRCC patients bearing at least 1 copy of the PBRM1 gene. Furthermore, we performed a PBRM1-Chip-seq to map the genome-wide targets of PBRM1 and revealed the association of PBRM1 occupancy at proximal regulatory regions of 8/45 cell adhesion genes. All of these taken together, indicate a role for PBRM1 in regulating the gene expression of cell adhesion genes which act as important players in cellular morphogenesis and responsiveness to changes in the external environment. Disruption of cell adhesion is believed to affect signal transduction and pave the way facilitating metastasis in neoplastic cells. Currently we are investigating the effect of loss-of-PBRM1 on Epithelial to Mesenchymal Transition (EMT) and metastasis in ccRCC.

#3363 Enhancer signature profiling identifies novel enhancer clusters for transcription factors in lung cancer. Stefanuminoello, Hideo Wataneabe, Takashi Sato. Icahn School of Medicine Mount Sinai, New York, NY.

We previously discovered that the targeted focus of the most significant amplicon in squamous cell lung cancer lies on the SOX2 gene locus, a product of which is a critical regulator in specifying squamous cell lineages in the development at dividing foregut to counteract with NKX2-1, which specifies tracheal and later lung lineages and is the most significantly amplified gene in lung adenoarcinoma. Cell lines harboring such amplification from respective lung cancer subtypes are dependent on the expression of these lineage-determining factors for their survival. However, the amplifications of these factors represent only 10-15% of lung cancer, it remains unknown what other lineage programs the remaining majority rely upon. Posttranslational modifications of histone proteins assign different functional regions of the genome, which leads to chromatin states that create stable expression patterns maintaining cellular fates. Studies on genome-wide enhancers have revealed the presence of large enhancer clusters, which are typically enriched in the proximity of lineage gene factors that establish a program defining cellular identity. To identify novel subsets of lung cancer defined by chromatin modification on lineage-determining factors, we made use of a histone modification marker H3K27Ac, typically present at active enhancers. We proﬁled H3K27Ac by ChIP-seq in 24 cell lines (14 lung adenocarcinoma, 4 squamous cell carcinoma, 4 small cell lung cancer and 2 breast carcinomas), identifying thousands of active enhancers, i.e. H3K27Ac enriched, and hundreds of super-enhancers (SEs) defined by ROSE algorithm per cell line. As a proof of principle, we found a locus adjacent to NKX2-1 gene as one of the SEs in an NKX2-1-ampliﬁed cell line NCI-H3122 suggesting this approach can detect a lineage program that is overridden by cancer cells. We performed unsupervised hierarchical clustering analyses on signals at SEs adjacent to transcriptional regulatory genes and identiﬁed subsets of genes co-enriched at the SEs. Several SEs on transcription factors are common between multiple cell lines from the same lineage (e.g., SOX2 and TP63 in squamous cell lineage and NKX2-1 in lung adenocarcinoma lineage). We identifed 6 lung adenocarcinoma cell lines that contain SE at the locus of N4R4A2, a signifcantly mutated gene in lung adenocarcinomas, and 2 other loci at the locus of HOX11, a lineage transcription factor for alveolar type I cells. These results suggest that this approach can reveal novel enhancers on lineage factors in a subset of lung cancers.

#3364 DNA methylation loss at an enhancer site of the DNA repair gene TREX2 is an epigenetic feature in multiple cancers. Christoph Weigel, Jitti-porn Chaisaimgomk, Christiane Kuhrmann, Irene Santi, Volkmar Winker, Olga Bogatyrova, Justo L. Bermejo, Tsun C. Chan, Felix Lasitschka, Manfred H. Boherer, Alexander Marx, Frank Autschbach, Roland Heyni-von Haufen, Herbst-Dyckhoff, Klaus-Wolfgang Delank, Karl Hoermann, Burkard M. Lippert, Gerald Baier, Andreas Dietz, Christoph C. Oakes, Christoph Plass, Heiko Becher, Peter Schneier, Heribert Ramroth, Odilia Popandia. German Cancer Research Center, Heidelberg, Germany; Chulabhorn Research Institute, Bangkok, Thailand; University of Heidelberg, Heidelberg, Germany; The University of Hong Kong, Hong Kong, China; University Hospital Heidelberg, Heidelberg, Germany; Medical Hospital, Ludwigshafen, Germany; University Medical Centre Mannheim, University of Heidelberg, Mannheim, Germany; SKL-Clinics Heilbronn, Heilbronn, Germany; Klinikum Darmstadt, Darmstadt, Germany; Head and Neck Surgery, University of Heidelberg, Heidelberg, Germany; University Hospital of Mannheim, Mannheim, Germany; Academic Teaching Hospital Darmstadt, Darmstadt, Germany; University of Leipzig, Leipzig, Germany.

The onset of numerous cancers is strongly associated with exposure to genotoxic agents and is counteracted by cellular DNA repair mechanisms. However, the tumorigenic potential of genotoxic carcinogens varies widely among individuals. It is...
still uncertain which genetic and epigenetic traits shape cancer onset and progression in the general population. While genetic aberrations in DNA repair genes have been linked to cancer risk, less is known about the importance of epigenetics for the regulation of these genes. In order to identify DNA methylation alterations in laryngeal cancer, we performed analysis of single CpG sites via mass-spectrometry genomic DNA analysis using DNA pyrosequencing of samples previously found to be altered in head and neck squamous cell carcinoma. We report loss of DNA methylation at the three prime repair exonuclease 2 (TREX2) gene locus in laryngeal cancer (n = 161) and adjacent normal tissue (n = 58) samples of patients from a German population-based case-control study. Following screening of tumor tissues from Chinese colorectal cancer patients as well as previously published data from the Cancer Genome Atlas (TCGA), we identified TREX2 methylation loss as a frequent trait in multiple cancers. We further characterized the regulatory activity of the affected TREX2 site using chromatin immunoprecipitation and luciferase reporter assays in cell lines from different tumor types. Differential TREX2 methylation affects a CCAAT/enhancer binding protein alpha (CEBPα) binding site serving as a gene enhancer which drives the expression of TREX2 from a previously uncharacterized gene promoter. We also observed a strong association between TREX2 methylation and TREX2 protein expression determined via immunohistochemistry in laryngeal tumors. Finally, we found a significant association between overall survival and loss of TREX2 methylation in laryngeal cancer, with TREX2 methylation loss being a protective factor. Our findings highlight a profound regulatory role of epigenetic mechanisms for TREX2 in tumors, and underline the usefulness of TREX2 DNA methylation as a biomarker for patient stratification.

#3365 Epigenetic inactivation of PTPRT and sensitivity to STAT3 inhibition in lung cancer. Malabika Sen, Audrey Kindsfather, James G. Herman. University of Pittsburgh Cancer Institute, Pittsburgh, PA. Signal Transducers and Activators of Transcription-3 (STAT3), a potent oncogenic transcription factor is persistently activated in several malignancies including lung cancer. Protein Tyrosine Phosphatase Receptor-1 (PTPRT) is an endogenous inhibitor of STAT3 transcriptional activity, and is methylated in many types of human cancer. Loss-of-function of PTPRT by promoter methylation contributes to STAT3 mediated growth and survival and represents one of the plausible mechanisms of STAT3 hyperactivation. We report identification of hypermethylation in CpG islands of the PTPRT promoter which correlates with its transcriptional silencing as well as expression of pSTAT3 protein and STAT3 target genes in both lung cancer cell lines and primary human tumors. Analyses of the TCGA (The Cancer Genome Atlas) data revealed that PTPRT promoter is frequently hypermethylated in a subset of lung squamous cell carcinoma and approximately 25% of adenocarcinoma tumors in association with downregulation of PTPRT mRNA expression. This observation was verified in vitro using lung cancer cell lines. Out of the 7 lung cancer cell lines examined, one lung cancer cell line showed lack of methylation (only unmethylated sequences) of the PTPRT promoter region and revealed mRNA expression (HS20). All 6 cell lines which showed PTPRT promoter methylation had severely absent PTPRT mRNA and had high levels of pSTAT3 Tyr705, while HS20 had low or no PSTAT3 Tyr705 protein expression. Silencing of PTPRT using siRNA in HS20, where PTPRT was not endogenously silenced by promoter hypermethylation, resulted in the upregulation of the STAT3 target genes such as cyclin D1 and Bcl-X, mRNA and protein expression as well as increased pSTAT3 Tyr705 level. Analyses of the methylation status in the PTPRT promoter region in primary lung tumor samples revealed methylation in one of the 3 tumor samples. This correlated with increased STAT3 target genes (cyclin D1 and Bcl-X) mRNA expression in lung cancer cell lines. PTPRT promoter methylation is associated with sensitivity to STAT3 inhibition. Our data suggests that silencing of PTPRT by promoter hypermethylation is a frequent mechanism of STAT3 hyperactivation, and targeting STAT3 may be an effective molecular targeted approach in the treatment of this subset of lung cancer.

#3366 Loci-specific differences in blood DNA methylation for early detection of hepatocellular carcinoma. Katarzyna Lubecka, 1 Megan Beech, 1 Jay Qui, 1 Lucinda Kurzawa, 1 Kirsty Flower, 1 Samer Gawrieh, 1 Suthat Liangpunsakul, 2 Nagar Chalasani, 1 James M. Flanagan, 1 Paulus Metzakos 1, 3 Purdue University, West Lafayette, IN; 2 Imperial College London, London, United Kingdom; 3 Indiana University School of Medicine, Indianapolis, IN. Late onset of clinical symptoms in hepatocellular carcinoma (HCC) results in late diagnosis and poor disease outcome. It is estimated that early detection of HCC would increase the cure rate from 5% to 80%. Thus, identifying new effective tools with reliable and quantifiable biomarkers is of high importance. Such tools are currently missing. Aberrations in the DNA methylation patterns, an important early event in carcinogenesis, have been shown to differentiate HCC tumors from normal tissues. However, these changes as diagnostic markers would have a high application in clinics only if detectable by minimally invasive tests like a blood test. In the present study, we performed a comprehensive evaluation of DNA methylation profiles in blood DNA collected from 24 HCC patients (cases) who provided samples after diagnosis and from 24 controls, enrolled by the Indiana Biobank. Cases were matched with controls on gender, age, ethnicity, hepatitis C infection, and diabetes. We used Human Methylation 450 BeadChip (Illumina) to perform DNA methylation analysis and pyrosequencing for validation of DNA methylation differences. We identified 7,047 CpG sites differentially methylated between cases and controls with p<0.05 and intraclass correlation coefficient (ICC) >0.5. The change in DNA methylation within the top 14 CpG sites corresponding to 12 genes (probes) was technically validated using pyrosequencing. Five probes were hypermethylated and 7 probes were hypomethylated vs. controls. These gene-specific candidate biomarkers that could be used in early detection of HCC only if detectable at pre-diagnostic stages. We therefore selected 4 out of 12 probes that presented the highest differences in post-diagnostic cases. We then verified DNA methylation changes at those 4 probes in blood samples collected from 21 patients at the time when they were clinically considered cancer free (pre-diagnostic) and developed HCC within 4 years of follow-up. We confirmed that methylation at all 4 selected probes discriminates pre-diagnostic cases from matched healthy controls. One of the strongest differences was detected within an enhancer of PCGF3 (difference = -0.12, ICC = 0.84). PCGF3 is a component of a Polycomb group multigene repressive PRC1-like complex and was found to be over-expressed in HCC tumors and in many other types of cancer according to Oncomine database. Our work not only establishes the first time differences in DNA methylation at specific CpG sites that are detectable in blood of individuals both before and after conventional diagnosis with HCC as compared with healthy individuals. The identified probes have high potential to be developed into early detection HCC biomarkers once validated in a larger cohort of individuals at risk. This research was supported by the ACS Institutional Research Grant and Showalter Trust Award granted to BS.

#3367 The clinical significance of epigenetic microRNA-137 silencing in patients with ulcerative colitis. Yuji Toyama,1 Yoshinaga Okugawa,1 Koji Tanaka,1 Toshimitsu Araki,2 Keiichi Uchida,1 Masato Kusunoki,1 C. Richard Boland,2 Aijay Goel2. 1Mie University, Tsu, Japan; 2Baylor University Medical Center, Dallas, TX. Background: Although present guidelines recommend periodic colonoscopy with multistep biopsies for surveillance of ulcerative colitis (UC)-associated colorectal cancer (CRC), it remains unclear about the effectively. To improve the surveillance, more effective markers are needed to identify patients at high risk for UC-CRC. MicroRNA (miRNA)-137 is often methylated in sporadic CRC. Methylation of this miRNA also occurs in an age-dependent manner, suggesting a potential "field defect", and possibly an early event in colectis-associated carcinogenesis. The objective of this study was to determine whether a methylation-related "field defect" of miR-137 could be exploits clinically to identify individuals with UC who are at increased risk for developing neoplasia. Materials & Methods: We conducted four-step analysis using 387 samples from 238 UC patients of two independent cohorts, which included 152 patients without neoplasia (matched specimens obtained from cecum, transverse colon and rectum for each patient), 17 with dysplasia, and 69 with cancers (neoplasms and corresponding rectal mucosa). We performed bisulfite pyrosequencing to quantify miR-137 promoter methylation levels of these samples. Results: In non-neoplastic UC patients, methylation levels of miR-137 were significantly higher in rectal mucosa than in proximal cecum mucosa (7.0 ± 2.8% vs. 4.9 ± 2.0%, P < 0.0001), and were associated with age at diagnosis (P < 0.0004) and disease duration (P = 0.0009) in rectal mucosa. In addition, methylation levels of miR-137 were significantly higher in dysplasia (P < 0.0001) and CRC (P < 0.0001) compared to non-neoplastic UC mucosa. In addition, miR-137 methylation levels in rectal mucosa from patients with neoplasia (dysplasia or cancer) were significantly higher compared to methylation in patients without neoplasia, suggesting the existence of "field defect" in rectal mucosa from patients with neoplasia. Receiver operating characteristic (ROC) analysis revealed that methylation levels of miR-137 in rectal mucosa successfully differentiated patients with CRC from those without (AUC: 0.8). Furthermore, multivariate logistic regression analysis revealed that miR-137 methylation in rectal mucosa emerged as an independent predictor of UC-CRC (OR: 5.53; P = 0.0148). Finally, ROC analysis was performed to validate the findings and revealed a significant difference in methylation levels of miR-137 between UC patients with vs. without neoplasia, with almost same AUC value (0.76). Conclusions: Methylation of miR-137 occurs in an age- and cancer-dependent manner in UC patients. Analysis of miR-137 methylation levels in rectum may be a biomarker for identification of UC patients at greatest risk for UC-CRC.
for germline genetic variation in altering gene expression. However, in methylation downstream). In general, combining genomic data in HGSOC did not reveal a role in somatic copy number (CNV): a germline genotype and tumor DNA methylation (full model), b) gene only, and c) DNA methylation only. We included 33 cases from The Cancer Genome Atlas (TCGA), 54 cases from Mayo Clinic, and 78 cases from the Australian Ovarian Cancer Study (AOCs). Genotyping and copy number calls on germline DNA expression, methylation and copy number on somatic samples were collected and analyzed on different platforms separately at each study site. We excluded genes with low overall expression and thus analyzed a total of 1,929 genes in all three datasets (53 for CNVs, 505 for expression, and 367 for downstream). In general, combining genomic data in HGSOC did not reveal a role for germline genetic variation in altering gene expression. However, in methylation only models 79 genes were associated with differential expression in the TCGA cases (permutation multiple testing adjusted p-val <0.05), in the Mayo cases (unadjusted p-val <0.05) and AOCs cases (unadjusted p-val <0.05). A known tumour suppressor (FBXW7) was associated with differential expression in the three datasets at p-val <0.01. This work demonstrates the feasibility, utility, and statistical power of ENET gene-level analyses incorporating maximal genomic information.


Epigenetic DNA methylation profiling of triple negative breast cancer tumors and non-tumor tissue was performed using a highly sensitive and quantitative analytics platform, which utilizes methylation sensitive restriction endonuclease to detect changes in methylation of CpG sites. Millions of CpG sites exist within the human genome and many of these are altered with tumor formation and progression, therefore, changes in methylation profiles of CpG sites may be useful as a diagnostic and/or prognostic biomarker in TNBC patients. Non-metric multidimensional scaling ordination analysis of the CpG sites revealed highly distinct methylation patterns between tumor and non-tumor tissue. Approximately 326 sites had a significant methylation score difference (p<0.0025) between TNBC tumor and non-tumor tissue, with most of the CpG sites having greater than 2-fold change in methylation status. Analysis of functional gene classes using KEGG classifiers revealed a significant change in methylation patterns of genes involved in response to infections and other immune related functions. Additionally, the top ten genes with hyper- or hypomethylated sites within TNBC tumors when compared to non-tumor tissue were identified. Interestingly, Gli-1 was one of the top hypomethylated genes. Gli-1 has been shown in our lab and others to have significance in chemoresistance and recurrence in TNBC patients. Immunohistochemical analysis of TNBC tumors revealed Gli-1 overexpression in TNBC tumors compared to non-tumor tissue. Expression levels of Gli-1 in TNBC tumors correlated with stage (p<0.001) and recurrence free (p<0.0221) and overall survival (p<0.017). Thus, analysis of CpG methylated sites in TNBC tumors and non-tumor tissue revealed differences in epigenetic profiles allowing for distinction between tumor and non-tumor tissue. Genes in TNBC tumors with significant changes in methylation status may be potential candidate genes that serve as a diagnostic or prognostic biomarker for TNBC.

Comparative analyses of multi-omics profiles reveal distinctive molecular signatures of young Asian breast cancers. Yeon Hee Park,1 Ying Ding,2 Soo-Hyeon Lee,1 Hae Hyun Jung,2 Woosung Chung,1 Soonwong Cho,1 Jin-Ho Kim,1 Shihbing Deng,1 Yoon-La Choi,1 Julio Fernandez,2 Se Kyung Lee,2 Seo-Hyeon Kim,1 Jeong Eun Lee1,2 Yeon Myung2,3 Jin TNBC in AA: Young-Hyuck1,2 Seo-Jin Nam,1 Woong Yang Park,1 Zhe-Qian Kan,1 Sunmiwon Medical Center, Seoul, Republic of Korea; 2Pfizer, Inc., San Diego, CA; 3Pfizer, Inc., Seoul, Republic of Korea; 4Johns Hopkins University, Baltimore, MD.

Breast cancers (BC) in younger, premenopausal patients (YBC) tend to be more aggressive with worse prognosis, higher chance of relapse and poorer response to endocrine therapies compared to breast cancers in older patients. The proportion of YBC (age ≤ 40) among BC in East Asia is estimated to be 16-32%, significantly higher than the 7% reported in Western countries. In addition, approximately half of the Asian BC patients were premenopausal compared to 15-30% in the West. To characterize the molecular bases of Asian YBC, we have performed whole-exome sequencing (WES) and whole-transcriptome sequencing (WTS) on tumor and matched normal samples from three Korean BC patients consisting of 106 YBC cases (age ≤ 40) and 62 OBC cases (age ≥ 40). We then performed comparison analyses with the TCGA BC cohort consisting of 1,116 tumors from primarily Caucasian patients, also grouped by age into YBC (age ≤ 40), IBC (40 < age ≤ 60) and OBC (age > 60). We performed logistic regression analyses to identify differentially expressed (DE) genes and protein-coding gene age-based signatures while controlling for demographic and clinical factors of molecular subtype, tumor purity and stage. Within the Asian cohort, we found that estrogen response, endocrine therapy resistance, and various metabolism pathways are up-regulated in YBCs while cell cycle, proliferation and inflammatory pathways are up-regulated in OBCs. To separately examine molecular signatures from tumor, stroma and normal compartments, we used non-negative matrix factorization (NMF) analyses to virtually dissect bulk tumor expression data and identified 14 factors including 3 factors associated with tumor infiltrating leukocytes (TILs). By examining the correlation between pathway gene expression and NMF factors, we inferred that DE pathways such as fatty acid metabolism, bile acid biosynthesis, and epithelial-to-mesenchymal transition (EMT) were mainly active in stromal and normal tissue compartments. The TIL factor was significantly enriched in Asian BCs relative to Caucasian BCs with the highest TIL factor weight observed in Asian OBCs. Using gene expression signatures representing distinct types of TILs, we classified the combined cohort into three subtypes of varying TIL activities. Consistent with results from the NMF analysis, the TIL-high subtype is also significantly enriched in Asian BCs compared to Caucasian BCs. To our knowledge, this is the first large-scale multi-omics study of Asian breast cancer. Comparative analyses of multi-omics profiles from Asian and primarily Caucasian BC cohorts identified distinguishing molecular signatures associated with Asian BCs. Further, many signatures appeared to be specific to non-tumor compartments within bulk tumor, indicating that young Asian BCs may harbor distinctive tumor microenvironment.

Aldh expressing stem cells mediate tumor initiation and metastasis in triple negative breast cancers across different ethnicities. Evelyn M. Jiagge,1 Shukei Wong,2 Rabia Gilani,3 Sean McDermott,6 Lisa Newman,4 Jessica Bensenhaver,7 Max Wicha,1 John Carpten,2 Sofia Merajver7, Univ. of Michigan Health System, Ann Arbor, MI; 2Translational Genomic Institute, Phoenix, AZ; 3University of Michigan, Ann Arbor, MI; 4Henry Ford Health System, Detroit, MI.

TNBC is the only subtype of breast cancer for which there are no approved targeted therapies. In the US, its incidence is highest in women with African ancestry (AA); in western sub-Saharan Africa, single-institution studies show that TNBC constitutes 40-80% of all breast cancers. Given the Caucasian/AA survival disparity in breast cancer, there is an urgent need to find actionable targets in TNBC of all ethnicities, but especially in TNBC in AA, which are suspected to be more aggressive. Breast cancer stem cells, the small population of cells that have been shown to mediate breast tumor initiation, metastasis, and resistance to conventional therapy have also been reported to mediate the heterogeneity of TNBC and are emerging as an important target. Here we sought to better understand the biology of TNBC by finding genes and pathways that are differentially expressed in the stem cell population of patient derived xenografts (PDX) from TNBC from Ghanaian (G), AA and Caucasian (C) women and the effect of these differentially expressed genes on the stem cell.
phenotype in these primary tumors. We isolated the ALDH+ and the CD44+/CD24- stem cell populations from the bulk cells in 15 PDXx using flow cytometry. We performed RNA sequencing (Illumina HiSeq platform) on the isolated populations and bulk cells (45). Comprehensive bioinformatics analyses led to the identification of highly significantly differentially expressed genes and pathways unique to the cell populations. By principal component analysis, the tumors were very heterogeneous. However, the ALDH+ cells separated out from the CD44+/CD24- and the bulk cells. We identified 14 genes that were simultaneously differentially expressed between the ALDH+ vs the CD44+/CD24- as well as ALDH+ VS bulk (p-value <0.001, FDR < 0.05). The 3 most significant genes were MMP2 and PCDH7, both known to be involved in breast cancer metastasis, and CD24, a carbohydrate binding (GBP2) marker. The number of DEX genes between Basal (8) and LA (18) sub-clusters were comparable to that between the Her2+ sub-clusters, DEX exons were much lower for both subtypes (616 & 157), and none of the DEX proteins (3 & 7) mapped to DEX exons or genes for either subtype. Conclusions: Our findings imply that the role of CSTF2 in proximal polyA signal selection. Because some of the TNBC patients compared with normal breast tissue. Representative shortening events correlated with increased protein levels and relapse free survival of patients, suggesting functional significance of isoform variability. To begin addressing the underlying mechanisms of 3’UTR shortening, we turned to APA machinery proteins. We detected variable expression of APA machinery proteins in different breast cancer subtypes but CSTF2 (cleavage stimulation factor) was the most prominent overexpression in breast cancer cells. Therefore, among potential regulators of 3’UTR shortening, we further investigated the role of CSTF2 in proximal polyA signal selection. Because some of the TNBC patients are EGRF positive, we found EGF treatment to cause increased CSTF2 levels. Higher CSTF2 levels indeed correlated with further shortening of the 3’UTR. Accordingly, RNA-induced silencing of CSTF2 decreased the proliferative rate of cancer cells. Therefore, our integrated approach revealed a pattern of 3’UTR length changes in TNBC patients and a potential link between APA and EGF signaling. Further studies are underway to investigate the mechanism between EGF signaling and regulation of 3’UTR lengths. (This work is funded by TUBITAK 112S478 and 114Z884).
Molecular and Cellular Biology / Genetics: Genomic Landscape of Breast, Prostate, Ovarian, Melanoma, and Thyroid Cancers

In the U.S., the incidence rates of breast cancer (BC) among Caucasian (CA) women are lower than those of African American (AA) women, however, AA women have a significantly higher mortality rate. AA women often present at a younger age, with later stage and higher grade tumors. In fact, AA women are 3 times more likely to be diagnosed with the more aggressive molecular subtype, triple-negative breast cancer (TNBC), than CA women. Socioeconomic and lifestyle factors may be contributory; however, it is imperative that we investigate the underlying molecular biology that may be the cause of health disparities between AA and CA with TNBC. In this study, gene expression profiling, using the Almac BC DSA Research Tool, was performed on archived FFPE samples, obtained from CA and AA women diagnosed with early stage (Node 0) TNBC. Unsupervised hierarchical clustering showed that expression in the AA TNBC cohort compared to CA, using the TNBC type: A Subtyping Tool for TNBC, we found a distinct distribution pattern of TNBC molecular subtypes in the AA cohort, which was very different than the CA cohort; Basal-like (14%), Immunomodulatory (43%) and Mesenchymal (43%). Gene expression analyses, comparing AA and CA cohort (fold change > 2.0, p-value < .05), resulted in 190 differentially expressed genes (DEG). Pathway enrichment analysis conducted in MetaCore GeneGo, revealed that the DEGs were over-represented in cytokoskeletal remodeling, cell adhesion, tight junctions, and immune response in the AA TNBC cohort. Furthermore, several genes in the Wnt/β-catenin pathways were over-expressed in the top 10-enrichment pathways. We validated our results using RT-qPCR and identified Cavelon-1 (CAV1) as being significantly expressed in the AA-TNBC cohort (p-value 1.22x10^-05). An independent cohort of FFPE samples, from AA and CA women with early stage TNBC, was used to create a tissue microarray (TMA). Immunohistochemistry results showed no difference in localization of CAV1 between AA and CA cohorts, however, the AA cohort had significantly higher levels of CAV1 staining (p-value 0.04). Additionally, using RT-qPCR, we demonstrated that CAV1 mRNA was significantly higher in the AA TNBC cohort (p-value 0.048). Furthermore, endogenous CAV1 was shown to be highly over-expressed in a cell line panel of TNBC, in particular, those of the mesenchymal and basal-like molecular subtype. Finally, using siRNA, we demonstrated that CAV1 silencing resulted in a significant decrease in cell proliferation, for each of the TNBC cell lines while it showed no effect on the luminal ER+ cell lines. Our combined study results suggest that CAV1 over-expression may be a biological contributor to the observed health disparity between AA women and CA diagnosed with early stage TNBC.

#3376 Reprogramming the estrogen signaling network is a potential mechanism for human breast tumorigenesis. David Chi,1 Hari Singhal,2 Lewy Li,3 Henry W. Long,4 Judy E. Garber,5 Myles A. Brown,2 1Harvard Medical School, Boston, MA; 2Dana-Farber Cancer Institute, Boston, MA.

Background: The estrogen receptor (ER) is expressed in approximately 70% of sporadic breast tumors, is known to have a key role in cellular transformation. Much work in the field has characterized the mechanisms of ER signaling behind cancer progression and eventual metastasis, and therapeutic targeting of the ER signaling pathway has proven effective. However, little is known regarding the role of ER in the initial process of transformation. Since ER possesses pro-differentiation signaling pathways, have a significant role in controlling cell proliferation in normal mammary epithelial cells, and the interaction with transcriptional cofactors such as GRHL2 could be a driving mechanism for breast tumorigenesis. Unraveling the differential ER signaling in normal mammary epithelial cell and breast cancer will enhance our understanding of breast cancer and aid the development of more effective prevention strategies and targeted therapeutics.

#3377 Simultaneous analysis of the mutational landscape and RNA and protein expression profile of HER2-positive breast cancer using 3D Biology™. Saranya Chumsri,1 Daniel J. Serie,1 Brian M. Necela,2 Jennifer M. Kachergus,2 Branca C. Asenfeld,3 Gokhan Demirkan,4 Gavin Meredith,5 P. Martin Ross,6 Anuisha Khan;7 Chae H. Ahn,4 Afzlin Mahbub Hossain,5 Sarah Warren,5 Sarah A. McLaughlin,1 Joseph Beechem,7 Gary Geiss,2 E. Aubrey Thompson1,2 Mayo Clinic Florida, Jacksonville, FL; 3NanoString Technologies, Seattle, WA.

Background: Understanding heterogeneity within individual breast tumors is key to the ability to predict therapeutic outcome. Molecular heterogeneity is commonly evaluated based on genomic features, including mRNA abundance, gene copy number events, and somatic mutations. The expression profile and activation state of key proteins is widely recognized as another key element in defining tumor heterogeneity. We have taken advantage of NanoString 3D Biology™ technology (for research use only) and curated nCounter Vantage 3D™ Solid Tumor Assay to interrogate a survey panel of HER2-positive breast tumors with the ultimate goal of determining key differences between multiple, genomic, and proteomic profiles in individual tumors. Methods: We analyzed samples from 24 HER2+ breast cancer patients using NanoString technology to quantify the expression profile for over 25 total and phospho signaling proteins, including PI3K/PI4K/EGFR/HER2, 770 mRNA corresponding to 13 canonical cancer pathways, and 104 somatic mutations and small INDELS that are commonly associated with cancer, including 8 known PIK3CA mutations. These analyses were carried out in a matched fresh frozen and FFPE samples on the nCounter platform. Data were analyzed by nSolver to identify genotype-specific expression profiles across the 24 samples. Results: In our proof-of-concept data set, we successfully demonstrate that NanoString’s 3D biology Technology shows concordance across both FFPE and fresh frozen sample types for DNA, RNA, and protein. NanoString analysis also showed high concordance to genome-wide techniques used to assess genotype and RNA expression profiles. The combination of digital DNA, RNA, and protein data from our HER2+ breast cancer samples yielded potentially actionable data based on mapping of mutational status as the driver of key differences in protein expression and mRNA abundance of the signaling targets profiled. This work sheds new light on HER2+ breast cancer biology and the interplay between genomic and proteomic profiles while setting the stage for future studies that further probe the differences observed in this sample set. Conclusions: Simultaneous analysis of mutational status (SNV) and expression at the level of both mRNA and protein promises to enable a more detailed view of the relationship between genotype and the biological and clinical behavior of key tumor types. The NanoString Vantage 3D™ Solid Tumor platform provides a rapid, reliable, and economic means of assessing these analytes simultaneously. The application of these analytics to model that malignantly and clinically actionable predictions will require additional analyses of large sample cohorts, but such analysis is quite feasible using a variety of sample types. Acknowledgements: Supported in part by grants from the Breast Cancer Research Foundation and the 26.2 with Donna Foundation.

#3378 Whole exome sequencing identifies recurrent alterations in genes in malignant phyllodes tumors in nine Korean individuals. Jihui Yun,1 Hyeong-Gon Moon,2 Tae-Kyun Yoo,2 Eunshin Lee,3 Jeeseo Cha,1 Sam Hur,2 Jiwoo Lee,2 Jong-II Kim,1 Dong-Young Noh2.

Phyllodes tumors of the breast are rare fibroepithelial neoplasms with variable clinical behavior accounting for about 1% of all breast neoplasms. Phyllodes tumors are classified into benign, borderline, and malignant grades on the basis of histological features. Among those categories, Malignant phyllodes tumor has a higher propensity for local recurrence and distant metastasis, however the malignant potential of this solid tumor is still unclear. Here, by performing whole exome sequencing of 9 malignant phyllodes tumors with matching normal cases, we frequently observed mutations in MED12 (3/9, 33.34%). Additionally, non-silent mutations in p53 (TP53) and epidermal growth factor receptor (EGFR) were recurrently identified. Whole-gene amplifications of EGFR were also found in six cases (6/9, 66.67%). We suggest that EGFR has an underlying role in malignant phyllodes tumors. This study identifies potential therapeutic targets in malignant phyllodes tumors, including EGFR.
#3379 Massively parallel sequencing analysis of breast adenomyoepitheliomas reveals the heterogeneity of the disease and identifies a subset driven by HRAS hotspot mutations. Felice C. Geyer,1 Kathleen A. Burke,2 Anqi Li,1 Anastasios D. Papanastassiou,1 Freisa Pareja,1 Anne S. Schulteis,1 Charlotte K. Ng,1 Salvatore Piscuoglio,1 Marcia Edelweiss,1 Luciano G. Martelotto,1 Pier Selenica,1 Serafina M. Moretti,1 Gabriel S. Macedo,1 Achim J. Gluth,1 Hannah Y. Wen,1 Juan Padasso,2 Zsuzsanna Varga,2 Emad Rahka,1 Ian O. Ellis,1 Brian Rubin,1 Britta Weigelt,2 Jorge S. Reis-Filho,1 "Memorial Sloan Kettering Cancer Center, New York, NY; 2Thomas Jefferson University Hospital, Philadelphia, Pennsylvania, PA; 3University Hospital Zurich, Zurich, Switzerland; 4Nottingham Medical Center Groningen, Groningen, Netherlands; 3Iwate Medical University, Japan; 4Nottingham University Hospitals, Nottingham, United Kingdom; 5Cleveland Clinic, Cleveland, OH.

Adenomyoepithelioma (AME) is a rare biphasic proliferative breast lesion, which may resemble salivary gland epithelial-myoepithelial carcinomas (EMCs). Most AMEs have an indolent clinical course, but malignant transformation and local and distant recurrences have been reported. We sought to define the mutational landscape of AMEs and investigate the functional impact of recurrent likely pathogenic mutations identified in these tumors. Nineteen AMEs were subjected to whole-exome massively parallel sequencing (WES, n = 7) or targeted capture MPS using MSK-IMPACT assay (n = 12). Somatic genetic alterations and the cancer cell fraction of mutations were defined using state-of-the-art bioinformatics algorithms. Selected genes (i.e. HRAS and PIK3CA) were subjected to Sanger sequencing in a series of 17 additional AMEs (total n = 36). Non-tumorigenic mammary epithelial cells (i.e. MCF10A, MCF10A with the PIK3CA-H1047R mutation and MCF12A), which are estrogen receptor (ER)-negative, were utilized for 2D and 3D functional studies. Of 36 cases, 22 were ER-positive and 14 were ER-negative. MPS analysis revealed a low mutation burden and HRASp51 and PIK3CA hotspot mutations in 6/19 (32%) and 11/19 (58%) AMEs, respectively. All HRASp51 and all but one PIK3CA mutations were clonal. ER-positive and ER-negative AMEs were fundamentally histologically and genetically distinct. Whilst ER-positive AMEs displayed recurrent HRASp51 mutations (50%, 11/22) but lacked HRAS mutations, ER-negative AMEs displayed negative AMEs displayed in addition to PIK3CA mutations (57%, 8/14), recurrent HRASp51 mutations (57%, 8/14). HRASp51 mutations co-occurred with PIK3CA mutations (50%, 4/8), PIK3R1 deletions (12.5%, 1/8) and/or CDK2N1 homozygous deletions (25%, 2/8). HRAS mutations, but not PIK3CA mutations, were significantly associated with ER-negativity (100% vs 21%), concurrent carcinoma (50% vs 7%), axillary metastases (38% vs 0%), high proliferation (63% vs 4%), necrosis (63% vs 11%) and nuclear pleomorphism (75% vs 29%). In vitro forced HRASp51 expression in MCF10A and MCF12A cells resulted in increased proliferation and transformation. In 3D organotypic cell cultures, forced HRASp51 resulted in a highly disorganized growth pattern, a partial loss of epithelial phenotype and acquisition of aberrant myoepithelial differentiation, which was more overt in PIK3CA-mutant MCF10A cells. In conclusion, AMEs are phenotypically and genetically heterogeneous. Whilst PIK3CA hotspot mutations occur across the spectrum of lesions, HRASp51 hotspot mutations are restricted to ER-negative AMEs, which should arguably be classified as breast EMCs. Our genomic and functional analyses are consistent with the notion that HRASp51 mutations are driver events in the pathogenesis of ER-negative AMEs and may be sufficient for the acquisition of myoepithelial differentiation in breast cells.

#3380 Synthetic lethal interaction between ARID1A mutation and BET bromodomain inhibition in ovarian clear cell carcinomas. Katrien Berns,1 Joseph J. Caumanns,1 E Marielle Hijmans,1 Annemie Gennissen,1 Bastiaan Evers,1 Bea A. Wismans,1 Gert Jan Meersma,1 Gor Lieftink,1 Roderick L. Beijersbergen,1 Hiroaki Itamochi,2 Steven de Jong,2 RenéBernards1,3 Netherlands Cancer Institute, Amsterdam, Netherlands; 1University Medical Center Groningen, Groningen, Netherlands; 2Iwate Medical University School of Medicine, Iwate, Japan.

Introduction: Current treatment for advanced stage ovarian clear cell cancer is severely hampered by a lack of effective systemic therapy options, leading to a poor outlook for these patients. Given that ARID1A is inactivated by mutation in over 50% of ovarian clear cell carcinomas, we pursued an ARID1A synthetic lethal screening approach to identify potential drugable targets. Methods: We performed synthetic lethal kinome short hairpin (shRNA) screens in a large panel (n = 14) of OCCC cell lines having different ARID1A mutation status. Hit validation was performed with isogenic ARID1A ko cell line pairs and in (patient-derived) xenograft mouse models. Summary of the data: We show here that BRD2 inhibition is synthetic lethal with ARID1A mutation in ovarian clear cell carcinomas. Importantly, inhibiting the BET family of proteins, to which BRD2 belongs, with small molecules specifically inhibits proliferation of ARID1A mutated cell lines both in vitro and in ovarian clear cell cancer xenografts and patient-derived xenograft models. We demonstrate that ARID1A loss leads to upregulation of the WNT ligand WNT10B, possibly causing a WNT dependency in the ARID1A mutant lines. BET inhibitors cause a reduction in WNT10B expression and WNT target genes such as MYC, JUN and WISP1, providing a potential explanation for the observed synthetic lethal interaction with ARID1A loss. Conclusions: Our study uncovered a new synthetic lethal interaction between ARID1A mutation and BET bromodomain inhibition, suggesting a new treatment strategy for ARID1A mutant ovarian clear cell carcinomas.

#3381 TERT promoter mutation in granulosa cell tumours of the ovary: Prevalence and prognostic significance. Jessica A. Pilsworth,1 Dawn R. Chrane,2 Zhouchunyang Xia,1 Hugo M. Horlings,1 Winnie Yang,1 Melissa K. McConney,1 Satoshi Yanagida,1 Annina E. Färkkilä,2 Adele P. Wong,2 Genny Trigo-Gonzalez,2 S.W. Grace Cheng,2 Yikan Wang,2 Ali Bashashati,2 Gregg B. Morin,2 Esther Oliva,1 Sohrab P. Shah,1 David G. Huntsman,1 University of British Columbia, Vancouver, British Columbia, Canada; 2BC Cancer Agency, Vancouver, British Columbia, Canada; 3Iwate Medical University School of Medicine, Japan; 4University of Helsinki, Finland; 5Massachusetts General Hospital, MA.

Granulosa cell tumours (GCTs) of the ovary account for 90% of sex cord-stromal tumours and have a high recurrence rate up to 50%. A missense mutation in the FOXL2 gene (c.402C>G; p.C134W) is a defining feature of GCT and is used as a robust marker for diagnosis. However, other than the FOXL2 mutation the pathogenesis and the driving pathways remain unknown. Determining secondary genetic events in GCTs is essential to understanding and improving prognosis. In a pilot study, we completed an analysis of TERT promoter sequencing of GCTs and matched normal cases to generate a comprehensive catalogue of coding and non-coding events. We identified a TERT promoter mutation (c.228C>T) in 50% of these cases. TERT is normally inactivated in somatic tissues; however, this promoter mutation has been shown to re-activate transcription of TERT. We validated this TERT mutation in an international cohort of 300 GCTs and found it was present in approximately 25% of cases overall. These TERT promoter mutations have been used to revise the molecular classification of other cancer types such as gliomas. In GCT, we found that this TERT mutation was correlated with a significantly worse survival outcome in patients with primary GCT (p < 0.005). Further, we found that this TERT mutation was present in a larger proportion of recurrent cases. Thus, this mutation may denote a novel subtype of GCT with a worse prognosis. Previous research has shown that TERT activation is evident in over 90% of cancers and is a fundamental step in tumourigenesis that enables unlimited proliferation. This TERT promoter mutation in GCT provides an explanation of how granulosa cells escape atresia and attain immortality. Thus, we hypothesize a mechanism in which the FOXL2 mutation prevents apoptosis and the TERT mutation allows oncogene proliferation to transform granulosa cells. However, the current cell models of GCT lack relevant functional pathways and do not recapitulate the biology of these tumours. Therefore, we are developing more suitable cell models to test our hypothesis. We believe that understanding the interaction between these TERT and FOXL2 mutations may lead to novel cancer cell-specific targeted therapies.

#3382 Comparative genomic and pathway analysis of ovarian cancer, benign tumors, and normal tissues detect alterations in several metabolic programs in ovarian cancer. Pourya Naderi Yeganeh,1 Zahra Bahrami-Mostafavi,1 Christine Richardson,1 David L. Tait,2 M Taghi Mostafavi1,2 "UNIV. Of North Carolina Charlotte, Charlotte, NC; 3Carolina's Medical Center, Charlotte, NC.

Introduction: The high mortality rate of ovarian cancer is mainly due to asymptomatic early stages of the disease. Currently used detection markers lack high sensitivity and specificity and generate large number of false positives from benign and normal subjects. Therefore experimental approaches capable of distinguishing the signatures of malignancy are required to improve the current states of biomarkers. This study aims to identify genetic abnormalities in ovarian cancer that contribute to cancer progression and developments. Methodology: This study was conducted on 67 whole tissue microarrays samples processed using Affymetrix HG-Focus chip, including 18 non tumorous samples (N), 14 benign tumor samples (B), 30 malignant ovarian samples (M), 3 borderline malignant samples, and 2 ovarian cancer cell lines. Differential expression (DE) analysis was conducted through Analysis of Variance methods (ANOVA) between malignant samples and non-malignant samples (M vs B and N). DE results were modified to False Discovery Rate (FDR < 0.05). Further pathway analysis was conducted on DE genes to identify pathways with dysregulated elements. Pathway identification was performed by enrichment analysis method based on KEGG annotated pathways. P-values were calculated using Chi-Square test (FDR<0.05). Further enrichment analyses were performed by MsigDB
GSEA portal. Results: 826 DE genes were found with fold change > 2. PDGFRα, TACSTD2, ABCA8, STAR, and C7 exhibited the highest rates of fold change (more than 14 fold). Enrichment Analysis of Hallmarks reveals several hallmarks of cancer related terms including EMT, G2M, E2F targets, P53 pathways, and Apoptosis. Pathway analysis identifies 26 pathways with dysregulated components many of which are categorized as metabolic pathways including: Ascorbate Metabolism, Metabolism of xenobiotics by cytochrome, Drug metabolism cytochrome P450, and Glutathione Metabolism. Gene Ontology Enrichments identify several significant enrichments including: Response to Endogenous Stimulus, Regulation of Cell Proliferation, Tissue Development, Regulation of Cell Death, and Cell Cycle Process. Conclusions: DE analysis and Pathway can reveal dysregulated metabolic programs in ovarian cancer and the results of GO, Pathway, and hallmark enrichment analysis are consistent with mechanistic differences between malignant tumors and other types of pathology. The inclusion of benign tumors and non-malignant ovarian tissues contribute to more accurate identification of disease profile. As expected, our data support mechanisms of P53 mutations in malignancy and tumor progression. These results can be used to identify such mechanisms in more detail as the detected D5 genes can be used to generate hypothesis set for detection of ovarian malignancy signatures.

#3383 Exome sequencing in dedifferentiated ovarian mucinous carcinoma. Kaori Sanuki,1 Kentaro Nakayama,1 Koho Nakamura,1 Masako Ishikawa,2 Tomoka Ishibashi,1 Hitomi Yamashita,1 Ruriko Ono,1 Toshiko Mi-namoto,1 Kosuke Yoshihara,2 Satoru Kyo,1 Shimane Univ. Faculty of Medicine, Izumo, Japan; 2School of Medicine, Niigata University, Niigata, Japan.

Mucinous ovarian tumor represent a distinct histotype of epithelial ovarian cancer and is thought to begin as a mucinous adenoma that progresses in a slow stepwise fashion. Dedifferentiated mucinous ovarian carcinoma is a rare type of ovarian carcinoma with a few reports and a progressive and poor prognosis. While the molecular genetic features of ovarian mucinous carcinoma is now well known, the pathogenesis of dedifferentiated mucinous carcinoma is largely unknown. In order to comprehensively analyse somatic mutations in dedifferentiated mucinous carcinoma, we applied exome sequencing to the DNA of a sample of affinity-purified, dedifferentiated mucinous carcinoma. Through comparative analyses of normal cells from the same patient, we identified several genes that were mutated in this tumor. P53, which encodes a well-known tumor suppressor protein, and KRAS, which encodes a well-known oncoprotein, had previously been implicated in ovarian mucinous carcinoma. The other mutated genes were previously unknown to be involved in mucinous carcinoma. CEP170 encodes a microtubule-binding protein that controls the targeting of the kinesin-13 dependent and kinesin-13 family influence the dynamics of microtubule growth and shrinkage. CEP170 gene mutation may be related to instability of a cell division property. CEP170 mutations were identified with a prevalence of 33.3% in dedifferentiated mucinous ovarian carcinoma. Currently, CEP170 gene knock down assay and engineered expression of CEP170 assay in ovarian mucinous carcinoma is being performed whether morphologic change is observed. In summary, CEP170 may be one of the responsible gene in carcinogenesis of differentiated mucinous carcinoma.

#3384 Comprehensive genetic analysis of myxofibrosarcoma and comparison with other soft tissue sarcomas. Yasuhide Takeuchi,1 Annegret Kunzit,2 Hiromichi Suzuki,1 Kenichi Yoshida,1 Yuichi Shiraiha,3 Teppei Shimamura,4 Kenichi Chiba,1 Hiroko Tanaka,5 Nobuyuki Kakiuchi,1 Yusuke Shiozawa,1 Akira Yokoyama,6 Kenichi Nakamura,7 Yoshikazu Hina,6 Takuji Tanaka,6 Takanori Hagiwara,8 Satoru Miyano,2 Frederik Dam,9 Seishi Ogawa,10 Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan; 2Department Hematology, Oncology and Tumor Immunology, Charite University Hospital, Berlin, Germany; 3Laboratory of DNA information Analysis, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan; 4Division of System Biology, Graduate School of Medicine, Nigata University, Nagaoya, Japan; 5Department of Diagnostic Pathology, University of Nagoya, Tokyo, Japan; 6Department of Pathology, University of Nagoya, Nagoya, Japan; 7Department of Diagnostic Pathology, University of Nagoya, Nagoya, Japan; 8Department of Pathology, University of Nagoya, Nagoya, Japan.

Background: Myxofibrosarcoma (MFS) is a relatively common subtype of soft tissue sarcomas (STSs) in the elderly, which is characterized by the proliferation of pleomorphic spindle cells with varying degrees of the myoid component and on the basis of this unique histological picture, together with other clinical characteristics, is separated from other STSs. However, the genetic basis of MFS is poorly understood. Aims and Methods: The purpose of this study is to clarify the comprehensive registry of genetic alterations in MFS and other STSs using whole exome/genome sequencing (WES/WGS) of paired tumor/normal DNA from 41 samples with MFS, combined with the WES data of 25 samples with MFS and 234 with STS, which were available from The Cancer Genome Atlas (TCGA) database. WGS was performed in 2 cases. Data for DNA methylation and gene expression from the TCGA registry were also analyzed. Moreover, the genetic basis of mixed histological components characteristic of MFS and its chronological changes was interrogated using multi-regional and/or multi-time points sampling. Results: A total of 8,661 mutations were identified in WES of 66 genes that is the histologic origin of lung adenocarcinoma as well. Previous studies have shown that PSNs and adenocarcinoma in the lung share some immunohistochemical and genetic features. However, as for somatic mutations, whereas many driver genes for lung adenocarcinomas have been identified, there have not been any candidate driver mutations identified in PSNs, except for low frequency mutations in β-catenin and TP53. Frequent somatic mutations identified in lung adenocarcinomas such as KRAS and EGFR have not been detected in PSH, suggesting that genomic alterations of these two lung tumors might be different from each other. Also, there is no evidence of PSH progression to lung cancers. These earlier data indicate that despite the common cellular origin of PSH and lung adenocarcinoma, genetic mechanisms for their development may be different. Based on the established concept that PSH is a true tumor, we hypothesize that it may harbor somatic mutations. In this study, we conducted whole exome sequencing of 44 PSHs and identified recurrent somatic mutations of AKT1 (43.2%) and β-catenin (4.5%). We used a second subset of 24 PSHs to confirm the high frequency of AKT1 mutations (overall 31/68, 45.6%; p>E17K, 33.8%) and recurrent β-catenin mutations (overall 3/68, 4.4%). Of the PSHs without AKT1 mutation, mutations of AKT1 occurred in 2 AKT1 mutation existed in both epithelial and stromal cells. In two separate PSHs from one patient, we observed two different AKT1 mutations, indicating they were not disseminated but independent arising tumors. Since the AKT1 mutations were not found to co-occur with β-catenin mutations (or any other known driver alterations) in any of the PSHs studied, we speculate that this may be an unusual pattern of driver alteration to develop PSHs. Our study revealed genomic differences between PSHs and lung adenocarcinomas including a high rate of AKT1 mutation in PSHs. These genomic features of PSH discovered in the present study provide clues to understanding the biology of PSH and for differential genicic diagnosis of lung tumors.

Pulmonary sclerosing hemangioma (PSH) is a benign tumor that usually presents as a solitary, well-defined mass in the lung. The PSH predominantly affects females with a higher incidence in the Far East. Histologically, the tumor cells in PSH consist of two cell types (cuboidal epithelial and polygonal stromal cells). Immunohistochemical and ultrastructural studies have identified that both cells are derived from undifferentiated respiratory epithelium that is the histologic origin of lung adenocarcinoma as well. Previous studies have shown that PSNs and adenocarcinoma in the lung share some immunohistochemical and genetic features. However, as for somatic mutations, whereas many driver genes for lung adenocarcinomas have been identified, there have not been any candidate driver mutations identified in PSNs, except for low frequency mutations in β-catenin and TP53. Frequent somatic mutations identified in lung adenocarcinomas such as KRAS and EGFR have not been detected in PSH, suggesting that genomic alterations of these two lung tumors might be different from each other. Also, there is no evidence of PSH progression to lung cancers. These earlier data indicate that despite the common cellular origin of PSH and lung adenocarcinoma, genetic mechanisms for their development may be different. Based on the established concept that PSH is a true tumor, we hypothesize that it may harbor somatic mutations. In this study, we conducted whole exome sequencing of 44 PSHs and identified recurrent somatic mutations of AKT1 (43.2%) and β-catenin (4.5%). We used a second subset of 24 PSHs to confirm the high frequency of AKT1 mutations (overall 31/68, 45.6%; p>E17K, 33.8%) and recurrent β-catenin mutations (overall 3/68, 4.4%). Of the PSHs without AKT1 mutation, mutations of AKT1 occurred in 2 AKT1 mutations existed in both epithelial and stromal cells. In two separate PSHs from one patient, we observed two different AKT1 mutations, indicating they were not disseminated but independent arising tumors. Since the AKT1 mutations were not found to co-occur with β-catenin mutations (or any other known driver alterations) in any of the PSHs studied, we speculate that this may be an unusual pattern of driver alteration to develop PSHs. Our study revealed genomic differences between PSHs and lung adenocarcinomas including a high rate of AKT1 mutation in PSHs. These genomic features of PSH discovered in the present study provide clues to understanding the biology of PSH and for differential genicic diagnosis of lung tumors.
#3386 Brazilian melanoma genome project: mutational landscape based on whole-genome sequencing. Vinicius L. Vazquez,1 Adriane Evangelista,1 Henrique C. Silva,1 Camila Crovador,1 Andre L. Carvalho,1 Cristovam Scapu-lateumo-Neto,1 Jin Lee,2 Gilles Landman,3 Joao P. Kitagima,4 Rui M. Reis,5 Barretos Cancer Hospital, Barretos, Brazil; Mendelics, Sao Paulo, Brazil; UNIFESP Federal University of Sao Paulo, Sao Paulo, Brazil;

Melanoma is the most aggressive form of skin cancer with increasing prevalence in Brazil. The comprehensive molecular profiling of these tumors and the recent genomic classification has improved the knowledge of melanoma biology and foster the identification of potential clinical biomarkers. The aim of this project, as part of the International Cancer Genome Consortium (ICGC) to perform a genomic profile by whole genome sequencing (WGS) of Brazilian melanomas patients. We reported the WGS using Illumina paired-end sequencing strategy (>30 X-fold coverage) of 66 cases (45% primary and 55% metastatic) and respective matched normal. Overall we observed an average of 46K mutations/genome. The most frequent type of substitutions identified were: C>T (22.5%), T>C (6.4%), T>G (4.4%), T>A (2.9%), C>A (2.8%) and C>G (1.7%). A significant difference between frequency of C>T substitutions was observed among histological subtypes, i.e., acral lentiginous (11.3%), nodular (31.2%) and superficial spreading (25.9%). Among the classic genes involved in melanoma biology, we found that 31.8% of patients had BRAF mutations, being the V600E the most frequent (82.6%). A total of 6.1% of patients had NRAS, 3.0% showed KRAS, one case (1.5%) showed HRAS, and 12.1% showed NF1. Systematic analysis of the mutational landscape revealed a high frequency of mutations in NRAS, HRAS, KRAS and NF1 (28.8% of patients). TP53 mutations found 4.5% and RB1 in 1.5% of cases. Oncogenic KIT and PDGFRα mutations were observed in 6.1% and 1.5%, respectively. BRAF, NRAS, HRAS, KRAS and NF1 mutation were mutually exclusive. BRAF and TERT mutations were significantly more frequent in superficial than acral lentiginous melanomas. We showed that Brazilian melanoma patients exhibit a high number of single mutations and a similar mutation profile to the one reported in other populations. Acral/lentiginous melanoma subtype, which is a common subtype in our setting, showed a distinct mutation frequency. Further analysis will extend to other relevant genes and will also include the study of additional genomes to reach the goal of genomic landscape of 100 Brazilian melanomas cases.

#3387 Mutational analysis in metastatic melanoma to the breast and ova-
ry: correlation with clinic-pathological and immunohistochemical findings. Alessio Giubellino, Vanessa Moreno, Xu Naizhen, Liqiang Xi, Mark Raffeld, latempo-Neto,1 Jin Lee,2 Gilles Landman,3 Joao P. Kitagima,4 Rui M. Reis1; Barretos Cancer Hospital, Barretos, Brazil; Mendelics, Sao Paulo, Brazil; UNIFESP Federal University of Sao Paulo, Sao Paulo, Brazil;

Breast melanoma (BM) and ovarian melanoma (OM) are rare, but aggressive diseases. We performed a comprehensive genomic and transcriptomic analysis of 34 ALM patients. Unlike CM, somatic alterations were dominated by structural variation and absence of mutations. Somatic mutational burden was higher in ALM compared to CM (Fig S1a). The number of unique mutations per sample was higher in ALM compared to CM (22 vs. 5, respectively). BRAF and NRAS/NF1 mutations. Contrasting with CM, we observed PAK1 copy number alterations in 49.4% (1 per lane) of ALM samples. The number of reads falling within each 10kb window was analyzed for ALM patients. Of note, most of the ALM cases were negative for N-RAS. The mutational analysis of these metastatic lesions, using next generation sequencing for the detection of cancer gene mutations including rare mutations at low frequency, remains limited. Deeper analysis of acral lentiginous melanoma (ALM), a rare sun-shielded melanoma subtype associated with worse survival than CM, is needed to delineate non-UV oncogenic mechanisms. We thus performed comprehensive genomic and transcriptomic analysis of 34 ALM patients. Unlike CM, somatic alterations were dominated by structural variation and absence of UV-derived mutation signatures. Only 38% of patients demonstrated driver BRAF/NRAS/NF1 mutations. Contrasting with CM, we observed PAK1 copy gains in 15% of patients, and somatic TERT translocations, copy gains, and missense and promoter mutations, or germline events, in 41% of patients. We further show that in vitro TERT inhibition has cytotoxic effects on primary ALM cells. These findings provide insight into the role of TERT in ALM tumorigenesis, and reveal preliminary evidence that TERT inhibition represents a potential therapeutic strategy in ALM.

#3388 Copy number alteration in primary melanoma. D. Timothy Bishop,1 Anastasia Filia,2 Alastair Droop,1 Joey Diaz,4 Mark Harland,1 Jon Laye,7 Juliette Randerson-Moor,1 Julia A. Newton-Bishop,1 University of Leeds, Leeds, United Kingdom; Imperial College, London, United Kingdom;

We investigated the diagnostic utility of copy number alterations (CNAs) in melanoma. The systematic analysis of the genomic characteristics of melanoma primaries has been infeasible given the limited size of these formalin-fixed paraffin embedded (FFPE) lesions. Further studying a clearly ascertained, deeply phenotyped patient population allows meaningful extrapolation of the prevalence of distinct genomic features and investigation of the association of these features with life-style exposures and germline profiles. For these reasons, we focused on a recently recruited patient cohort, the Leeds Melanoma Cohort (LMC) consisting of 2184 recruits from a distinct geographical region of Northern England; the only selection applied to recruitment was to only include those whose melanoma was less than 0.75 mm Breslow thickness in the early years of recruitment. Tumor samples from 703 cases have been analyzed for genome-wide gene expression (Poznik et al., this meeting). For this study, we wanted to explore the prevalence of copy number alterations (CNAs) and the mutation profile of tumors (reflecting the association of C>T mutations with UV exposure, the primary environmental risk factor) and so we have adopted a next-generation sequencing (NGS) approach. Tumor blocks were recovered from local pathology laboratories for for those cases survives more than 5 years post diagnosis at this same time. Blocks at risk of being compromised for clinical purposes by sampling for this research study were excluded from sampling; this amounted to almost 50% of identified samples. A total of 333 NGS libraries were sequenced on an Illumina GAII or HiSeq sequencer to produce >100bp paired-end reads (either 5 or 1 per lane). DNA reads were aligned and mapped achieving approximately 1.8x coverage (9.4x for those at 1 per lane); the number of reads falling within each 10kb window was adjusted for GC content and mappability and compared to a composite normal FFPE sample to determine local copy number. We adopted various approaches to assess the informativeness of the methodology. (i) Replicate samples showed high reproducibility (all p < 0.0001). (ii) Focused analysis of the CDKN2A and CDK4 genes revealed a high rate of loss in 43.7% of CDKN2A and 38.5% of CDK4 in lesional melanomas verified high technical quality, biologically plausible data revealing multiple copy number events. 76% of samples showed no CNA across the 4 Mb region covering CDKN2A while, as expected, only a small proportion of CNAs did not involve CDKN2A. Four common distinct patterns were observed. Copy number loss ranged from 20kb to -> 4 Mb. (iii) A germ-line CNA could reliably be identified from the tumor data. (iv) CDKN2A expression levels correlated with estimated copy number.

We have shown that small FFPE melanomas can be characterized for genomic alterations using NGS opening up the potential for studies associating epidemiological and germline profile with the resulting tumor. 1. Newton-Bishop, J.A., et al. (2009) Clin Oncol, 27, 5349-5444. Funded by Worldwide Cancer Research and Cancer Research UK.

#3389 Integrated genomic analyses reveal frequent TERT aberrations in acral melanoma. Winnie S. Liang,1 William Hendricks,2 Jeffrey Kiefer,2 Jessica Schmidt,2 Shobana Sekar,1 John Carpent,2 David W. Craig,1 Jonathan Adkins,4 Lori Cuyugan,3 Zarko Manoljovic,1 Rebecca F. Halperin,1 Adrienne Helland,1 Sara Nasser,1 Christophe Legendre,1 Laurence H. Hurley,4 Karthigayini Siva-prakasham,1 Douglas B. Johnson,3 Holly Crandall,1 Klaus J. Busam,2 Victoria Zissmann,3 Valerie De Luca,1 Jeeyun Lee,5 Aleksandar Sekulic,2 Charlotte E. Arjya,1 Jeffrey Sosman,7 Jeffrey Trent1; 1Gen (The Translational Genomics Research Institute), Phoenix, AZ; 2Mayo Clinic, Scottsdale, AZ; 3University of Arizona, Tucson, AZ; 4Vanderbilt University Medical Center, Nashville, TN; 5Memorial Sloan-Kettering Cancer Center, New York, NY; 6Samsung Medical Center, Seoul, Democratic People’s Republic of Korea; 7Northwestern University, Chicago, IL;

Acral melanoma (ALM) is the most common primary cancer of the eye and frequently gives rise to lethal metastatic disease. Uveal melanoma (UM) is the most common primary cancer of the eye and frequently gives rise to lethal metastatic disease. Uveal melanoma cell lines and their respective matched normal were sequenced on an Illumina GAII or HiSeq sequencer to produce >100bp paired-end reads (either 5 or 1 per lane). DNA reads were aligned and mapped achieving approximately 1.8x coverage (9.4x for those at 1 per lane); the number of reads falling within each 10kb window was adjusted for GC content and mappability and compared to a composite normal FFPE sample to determine local copy number. We adopted various approaches to assess the informativeness of the methodology. (i) Replicate samples showed high reproducibility (all p < 0.0001). (ii) Focused analysis of the CDKN2A and CDK4 genes revealed a high rate of loss in 43.7% of CDKN2A and 38.5% of CDK4 in lesional melanomas verified high technical quality, biologically plausible data revealing multiple copy number events. 76% of samples showed no CNA across the 4 Mb region covering CDKN2A while, as expected, only a small proportion of CNAs did not involve CDKN2A. Four common distinct patterns were observed. Copy number loss ranged from 20kb to -> 4 Mb. (iii) A germ-line CNA could reliably be identified from the tumor data. (iv) CDKN2A expression levels correlated with estimated copy number.

We have shown that small FFPE melanomas can be characterized for genomic alterations using NGS opening up the potential for studies associating epidemiological and germline profile with the resulting tumor. 1. Newton-Bishop, J.A., et al. (2009) Clin Oncol, 27, 5349-5444. Funded by Worldwide Cancer Research and Cancer Research UK.

#3390 Clonal evolution in uveal melanoma. Matthew G. Field,1 Himu An-bunathan,2 Michael A. Durante,1 Louise Cai1, Karam Alawa,1 Christina L. Decatur,1 Stefan Kurtenbach,1 Anne Bowcock,1 J. William Harbour1; 1University of Miami, Miami, FL; 2Imperial College London, London, United Kingdom;

Uveal melanoma is the most common primary cancer of the eye and frequently gives rise to lethal metastatic disease. Uveal melanoma can be divided into two prognostic subgroups based on gene expression profiling: class 1 (low metastatic risk) and class 2 (high metastatic risk).
Uveal melanoma is also notable for a characteristic set of driver mutations that cluster into two groups. The first group consists of mutually exclusive gain-of-function mutations in members of the Gq signaling pathway (GNAQ, GNA11, CYSLT2 and PLCB4), which are present in almost all uveal melanomas. These mutations are not prognostic, and are thought to represent an event that is insufficient alone to cause complete transformation. The second group consists of near-mutually exclusive mutations in BAP1, SF3B1, and EIF1AX. These are thought to occur later in tumor progression and are prognostic of patient outcome. These molecular features are associated with characteristic chromosome copy number variations (CNV). The purpose of this study was to investigate the life history of primary uveal melanomas by inferring the temporal evolution of these genetic events. Methods: Exome or whole genome sequencing data from 151 primary uveal melanomas were evaluated with a new bioinformatic pipeline for calling mutations and CNVs. Data from this analysis were used in downstream subclonality algorithms to determine intratumor evolutionary patterns within individual tumor samples. Results: A Gq mutation was found in 98.7% of tumors, which were all mutually exclusive with each other. BAP1 mutations were found in 46%, SF3B1 mutations in 23%, and EIF1AX mutations in 14% of tumors, all of which were mutually exclusive with each other except for 4 cases. We identified novel driver mutations in 20% of the remaining tumors. In most samples that contained a BAP1 or SF3B1 mutation, this driver mutation and associated CNVs were present in 100% of tumor cells. However, in 14% of cases with BAP1 mutations, 100% of tumor cells exhibited monosomy 3, but a BAP1 mutation was present in a smaller subclone, suggesting that the BAP1 mutation occurred after the loss of chromosome 3. In tumors with EIF1AX mutations, this mutation was consistently found in 100% of tumor cells, with 6p gain being found in a smaller subclone in 45% of cases, suggesting that 6p gain usually occurs after the EIF1AX mutation in this subgroup of tumors. Conclusions: The driver mutations and associated CNVs that are characteristic of uveal melanoma occur very early in tumor evolution and are followed by the accumulation of silent passenger mutations, consistent with a punctuated evolution model in which an initial “big bang” is followed by neutral non-Darwinian evolution. These unexpected findings alter prevailing theories of uveal melanoma progression, and could have a significant impact on patient management.

### #3392 Genomic analysis of prostate cancer in Korean men. Se Song Jang,1 Min Jung Kim,1 Dong Wan Hong,2 Jae Young Joung,2 Sung Han Kim,2 Kang Hyun Lee,2 Weon Seo Park,2 Jong-Il Kim.1 1Seoul National University, Seoul, Republic of Korea; 2Center for Prostate Cancer National Cancer Center, Goyang, Republic of Korea.

Prostate cancer currently has the fifth highest incidence rate in Korean males, and is the most common cancer in western males. Prostate cancer is the second most common malignancy in men worldwide, and is responsible for approximately 250,000 cancer-related deaths annually. The search for accurate biomarkers and characterization of prostate cancer is critical for evaluation of accurate management of prostate cancer. Previous studies have identified that several genomic alterations in prostate cancer including TMPRSS2-ERG fusion, PTEN deletion and SPOP single nucleotide variants (SNVs), the majority of which are from western populations. In order to investigate the somatic mutations of Korean prostate cancers, we performed whole exome sequencing on 51 prostate cancer patient’s tumor tissues, and matched normal tissue. For the accurate detections of structural variations, we additionally performed whole genome sequencing on 9 matched pairs (tumor, normal). A total of 738 somatic non-silent variants affecting 692 distinct genes were identified. In addition to already known genes such as SPOP, FOXA1, TP53 and PTEN, we also found previously uncharacterized genes such as GRIN2A, LRPI, FND1 and LRPIB. Of the 46 mutated genes across multiple cases, missense SNVs (64%) were the most frequent, followed by frameshift and splice-site SNVs of GRIN2A (5/60 cases, 8.3%). Mutations in low-density lipoprotein-related protein encoded by LRPI and LRPIB affected considerable portion of all cases (7/60, 11.7%), describe the probable relation between lipid metabolism and prostate cancer. We found TMPRSS2-ERG fusion in 4 of 9 whole genome samples. Moreover, 5 samples had 8p whole arm deletion, which is found frequent in western prostate cancers. As a result, we describe the genomic patterns of genetic variations and structural variations involved in prostate cancer of Korean population.

### #3393 Genomic analysis of multi-site fresh prostate samples. Marina A. Parry,1 Shambhavi Srivastava,1 Alessio Cannistraci,2 Hui Sun Leong,2 Syed Ali,1 Jenny Antonello,1 Vijay Ramani,1 Maurice Lau,1 Jonathan Shanks,1 Daisuke Nomaka,2 Pedro Oliveira,3 Noel Clarke,4 Crispin Miller,1 Ged Brady,1 Nathalie Dhomen,5 Esther Baena,6 Richard Marias,1 1CRUK Manchester Institute, Manchester, United Kingdom; 2The Christie NHS Foundation Trust, Manchester, United Kingdom.

Prostate cancer is the most common cancer in males in the UK with >40,000 cases diagnosed every year and >10,000 deaths. Recent multi-platform genomic studies have revealed a very complex picture of the disease, with subtypes linking to the histopathological markers currently used in clinical practice. The multifocal and heterogeneous nature of the disease suggests that single biopsy sites may be missing valuable subclones which contribute to the etiology of the disease. To address this, we obtained fresh core biopsies from multiple sites (4-8) in the prostate from eight high-risk patients undergoing prostatectomies at the Christie NHS Foundation Trust. The tissues were provided to the pathology department and each core divided in two, one for genomic analysis and one for parallel disease modeling (patient derived xenografts (PDxEs), patient-derived cell lines and organoids). The cores were cryo-sectioned and H&E analysis performed at the top, middle and bottom of each core. These were reviewed by a pathologist and tissue was micro dissected prior to simultaneous DNA and RNA extraction of normal and tumor tissue. Blood for germline DNA and plasma for circulating free DNA (cfDNA) was also obtained. Our study comprises eight patients with two or more tumor sites (2-4), some bifocal, which were analyzed using whole exome sequencing (WES), copy number aberration (CNA) profiling, transcriptomic analysis and methylaton profiling. The most frequent aberration identified was loss of 8p (NKKX3-1) in 6/8 patients (12/24 tumor cores). Loss of 13q (PDCD10) was observed in 5/8 patients (8/24 tumor cores), but never in all cores from a single patient, suggesting a late event. Loss of 5q (CHD1) was identified in 4/8 patients (11/24 tumor cores). Loss of 6q (MAP3K7), 10q (PTEN), amplification of 8q (MYC) and TMPRSS2-ERG fusion were identified in 3/8 patients. Some copy number events were only observed in either one patient or one tumor site indicating the intra-patient heterogeneous nature of SPOP was the only gene affected by recurrent mutations across patients, but amino acid changes were affected within and between patients. Mutations in PTEN, TP53, APC, BRAF and ERCC3 were also identified, among many others. The most significantly overexpressed gene was ERG, seen in patients
with the TMPRSS2-ERG fusion. Changes in gene expression differed between cores from the same patients, reflecting the heterogeneity at the DNA level. These data will be analyzed in conjunction with the results from disease modeling to investigate the functional impact of these changes and cDNA analysis is underway to understand which tumor clones are entering the bloodstream. The multifocal and genetically heterogeneous nature of prostate cancer is highlighted by this data and is likely to impact on precision medicine approaches for this disease. We will correlate the molecular profiling of our patient tumors with their clinical data in order to identify targets for further validation.

#3394 CGP identifies largely non-overlapping high tumor mutational burden and HRD genomic alterations in 721 clinically advanced prostate acinar adenocarcinoma cases. Nimad Dewal,1 Yuting He,2 Richard J. Lee,1 Alexia B. Schrock,1 Jon Chung,3 Christopher Holmes,1 Zachary R. Chalmers,1 Garrett M. Franklin,1 James X. Sun,3 Primo N. Lora,1 Neeraj Agrawal,1 Paul Mathew,1 Philip J. Stephens,1 Vincent A. Miller,1 Jeffrey S. Ross,1 Siraj M. Ali,1 Foundation Medicine, Inc., Cambridge, MA; 3Massachusetts General Hospital, Boston, MA; 2Case Western Reserve University School of Medicine, Cleveland, OH; 4University of California, Davis Comprehensive Cancer Center, Sacramento, CA; 5University of Utah School of Medicine, Salt Lake City, UT; 7Tufts University School of Medicine, Boston, MA.

Background: Effective therapies for the management of castrate resistant prostate cancer are lacking. We performed comprehensive genomic profiling (CGP) on advanced prostate cancers (PC) in the course of clinical care to identify genomic alterations that could suggest benefit from targeted, immune- and PARP inhibitor therapeutic strategies. Methods: DNA was extracted from 40 microns of FFPE specimen from 721 clinically advanced PC cases. CGP was performed using a hybrid-capture, adaptor ligation based next generation sequencing assay to a mean coverage depth of over 500X. All four classes of genomic alterations (GA)-basesubstitutions, insertions and deletions, gene fusions, and copy number alterations (amplifications and losses) - were identified. Results: Of the 721 PC patients - men with median age of 65 (range 34 - 88) - CGP was performed on 335 (46.5%) prostate specimens and 386 (53.5%) specimens from metastatic sites. The most common genes altered were TP53 (N = 336, 46.6%), PTEN (N = 254, 35.2%), TMPRSS2-ERG (N = 214, all fusions, 29.7%), AR (N = 173, 24%), and MYC (N = 105, 14.6%). Median tumor mutational burden (TMB) for this series was 3.6 mut/Mb (range 0 - 305). AR and MYC amplifications were enriched in metastatic tumors compared to primary tumors (p < 0.001 for both). Alterations characteristic of homologous recombination deficiency (HRD) were found in >15% of cases, including homonymous deletions and truncating mutations in BRC2 in 79 (11%) samples, as well as truncating mutations in ATM in 53 (7.2%) samples, with only 3 samples possessing alterations in both genes. Cases with these HRD-relevant alterations possessed overall higher genomic loss of heterozygosity (LOH) content than did those without (p = 0.02). HRD and non-HRD cases had median TMB of 4.8 versus 2.7 mut/Mb, respectively, and mean TMB of 10.4 versus 4.0 mut/Mb (p < 0.01). Only 2.6% of non-HRD cases were TMB-high (20 mut/Mb or more), versus 12.5% of HRD cases, a small but significant enrichment in the latter. Conclusions: CGP for advanced PC cases identifies largely non-overlapping TMB-high and HRD positive cases, suggesting benefit from immunotherapies and PARP inhibitors respectively. Further investigation will assess whether HRD pathway alterations result in genomic LOH in the context of a hypermutated tumor, and enrichment or exclusivity with other GA in prostate carcinoma.

#3395 Alternative splicing of the MEAF6 gene promotes neuroendocrine prostate cancer development. Ahn R. Lee, Yinan Li, Ning Xie, Colin C. Collins, Xuesen Dong, University of British Columbia, Vancouver, British Columbia, Canada.

In response to the selection pressures exerted by potent androgen receptor (AR) pathway inhibitors (ARPI), adenocarcinoma prostate cancer (PCA) cells can undergo an adaptive process of cellular phenotype reprogramming termed neuroendocrine trans-differentiation. With this AR-bypass mechanism of survival emerging a lethal treatment-resistant PCA subtype called treatment-induced neuroendocrine PCA (t-NEPC). t-NEPC is becoming a major clinical issue as it is estimated to affect >25% of advanced-stage PCA patients with the level of incidences predicted to rise as a result of the extensive applications of ARPI in the clinic. This underscores the gravity of our aims to delineate the molecular underpinnings of t-NEPC to inform future therapies that prevent or mitigate t-NEPC development. In this study, we have identified a splice variant of the MYST/Esa1-associated factor 6 (MEA6F) gene, MEAF6-1, that is highly expressed in t-NEPC tumor biopsies as well as neuroendocrine cell lines of prostate and lung cancers. We show that the neuronal RNA splicing factor, SRRM4, stimulates MEAF6-1 splicing. Enhanced MEAF6-1 expression in prostate adenocarcinoma cell lines does not induce neuroendocrine trans-differentiation of these cells. Rather, it stimulates cell proliferation, anchorage-independent cell growth, and invasion. Gene expression analyses indicated that these MEAF6-1 actions are in part mediated by the ID1 and ID3 genes. These findings suggest that the MEAF6-1 variant does not induce neuroendocrine differentiation of prostate cancer cells, but facilitates t-NEPC progression through accelerating proliferation of cells that have acquired neuroendocrine phenotypes.

#3397 Post-mortem examination of an aggressive case of medullary thyroid carcinoma characterised by catastrophic genomic abnormalities. Darran P. O’Connor,1 Sudipto Das,1 Deirdre Kelly,1 Bruce Moran,2 Kathleen Han,2 Niall Mulligan,3 Ciara Barrett,2 Patrick Buckley,1 Peter McMahon,2 J McCaffrey,2 Henrik van Essen,4 Kate Connor,1 Bauke Ylstra,3 Diether Lambrechts,3 William M. Gallagher,1 Catherine M. Kelly.1 Royal College of Surgeons in Ireland, Dublin, Ireland; 2Mater Misericordiae University Hospital, Dublin, Ireland; 3University College Dublin, Dublin, Ireland; 5VU University Medical Center, Amsterdam, Netherlands; 6Vesalius Research Center, Leuven, Belgium.

Catastrophic genomic alterations may drive aggressive cancer phenotypes. We describe a diagnostically challenging and rapidly fatal case of medullary thyroid carcinoma (MTC) occurring in a young, morbidly obese man presenting with diffuse bone marrow involvement and disseminated intravascular coagulation. Whole-exome sequencing (WES) and shallow whole-genome sequencing (WGS) were carried out for the primary tumour, adjacent normal, bone marrow tissues, multiple metastases and blood samples derived from the patient. We identified three germline single nucleotide polymorphisms (SNPs) within the RET proto-oncogene that remained undetected using routine hospital genetic testing procedures. One variant (L769L) has been previously reported to be associated with aggressive MTC presentation yet remains untested for in the routine diagnosis of MTC. Supported by findings from both WES and WGS, we report for the first time in thyroid cancer on the occurrence of a “chromothripsis-like pattern”, which involved shattering of chromosome 4 leading to complete abrogation of normal chromosomal function, along with dramatic widespread copy number aberrations across both primary tumour and bone marrow samples. Based on the somatic variants identified, we describe the evolutionary pathway of the case, showing that bone marrow metastasis occurred separately to other metastatic sites. The presence of disease-associated SNPs within the RET proto-oncoprotein supports their inclusion as part of routine genetic testing for MTC cases. The copy number aberrations and chromothripsis-like pattern affected a much broader range of genes than single mutations and may have led to the widespread, non-random instability possibly contributory to the rapid fatal course of the case. These results provide a rationale for the application of comprehensive genomic analysis of cancers presenting with unusual and aggressive phenotypes to facilitate more appropriate therapeutic options and diagnoses.

#3398 The genomic and transcriptomic analysis of nine widely invasive follicular thyroid carcinomas (wFTC) in Korean patients. Angela Byuri Cho,1 Seong-Keun Yoo,1 Min-Hwan Sohn,1 Jong-Yeon Shin,1 Su-jin Kim,2 Eun Kyung Lee,2 Young Joo Park,2 Jeong-Sun Seo,1 1Seoul National Univ. Gravmg-si, Gyeonggi-do, Republic of Korea.

Widely invasive follicular thyroid carcinoma (wFTC) can be characterized from minimally invasive follicular thyroid carcinoma (miFTC) as it exhibits poor prognosis with frequent distant metastasis. We have reported that miFTC shows similar mutational and transcriptional spectrum when compared to follicular adenoma (FA). Here, we performed whole-exome and transcriptome sequencing on wFTC samples to define their aggressive biological features by comparing with 30 miFTCs and 26 FAs. Well-known miFTC and FA driver genes, NRAS (66.67%), HRAS (11.11%), and EIF1AX (11.11%), were identified in wFTC. TERT mutation was found in all samples which can explain its aggressive feature. Chromosome 17p4 amplification was found in most wFTC samples as well as 22q deletion that is frequently aberrant in H/K/NRAS mutated thyroid cancers. Most of wFTCs were classified as RAS-like (66.67%) and a few were BRAF-like (22.22%) and BRRN (11.11%) molecular subtypes. From differentially expressed gene analysis, we found 1,832 and 1,384 up-regulated and down-regulated genes when wFTC was compared to miFTC and FA together. wFTC had many overlapped down-regulated genes as poorly differentiated and anaplastic thyroid carcinoma which also supports its aggressiveness. Through weighted correlation network analysis we have established gene expression net-
works, highly associated with wiFTC, that have SIRT6 and TGFBR2 as the central nodes. In this study, we demonstrated the distinctive mutational and transcriptional characteristics of wiFTC hence found the underlying molecular mechanism for its aggressiveness.

#3399 Genomic analysis of melanoma evolution following a 30 year disease-free interval. Jerry J. Miller,1 Kristopher A. Lofgren,2 Sarah R. Hughes,1 Steven E. Cash,2 David R. Meier,2 Paraic A. Kenny.1 1Gundersen Health System, La Crosse, WI; 2Gundersen Medical Foundation, La Crosse, WI.

The rate of ultra-late recurrence (beyond 10-15 years) of cutaneous melanoma has been estimated to be between 2.0-6.9% from large case series. Two major factors complicate the interpretation of these data. Firstly, the risk of second primary melanoma is approximately 5% Creation uncertainty about whether at least some of these late onset tumors might be independent of the original lesion. Secondly, in the majority of cases, the original pathology specimen is no longer available for comparative analysis. Accordingly, putative late recurrences are difficult to unambiguously distinguish from a new primary melanoma. We identified a patient with a second melanoma diagnosed after a 30 year disease-free interval, and sought to determine if this new lesion was a recurrence of the original melanoma. We report the genomic sequence analysis of the exomes of two melanoma lesions isolated from the same individual in 1985 and 2015, and their comparison to each other and to the germline DNA of the patient. Identification of many shared somatic mutations between these lesions prove a linear relationship spanning 30 years. Unlike prior reports of ultra-late melanoma recurrence, the availability of the original tumor and the use of comprehensive genomic analysis allowed us to confirm that the second lesion is truly a recurrence. We demonstrate the acquisition of numerous additional mutations during the three decade asymptomatic period. This is, to our knowledge, the longest disease-free interval that has been rigorously confirmed in melanoma or any other solid tumor type. These data highlight the low but very long-lasting risk of recurrence in this patient population.

#3400 Progression of epidermal growth factor receptor (EGFR)-independent colorectal cancer. Carolina Mantilla Rojas,1 Yu Ming,2 David Threadgill1. 1Texas A&M Univ., College Station, TX; 2University of North Carolina, Chapel Hill, NC.

When combined with standard of care treatment for colorectal cancer (CRC), the use of monoclonal antibodies or small molecule inhibitors against epidermal growth factor receptor (EGFR) show modest efficacy in the clinic. Early detection efforts and more effective therapies have reduced mortality, yet CRC remains the second mostly deadly cancer in the United States with approximately 50,000 deaths expected this year. Thus, it is imperative to better understand the mechanisms governing molecular progression of CRC. Primary and secondary resistance to anti-EGFR therapies occurs in approximately 80% of the patients with CRC. Mutations in KRAS explain some non-responding CRCs, but even in cancers lacking KRAS mutations, little is known about which cancers are likely to respond to EGFR targeted treatment, suggesting an alternative and EGFR-independent CRC progression mechanism. In this study, we used a conditional Egf allele (Egf(WT)I in the APC(Apc)Min/+ mouse model and identified EGFR-independent tumors with faster growth rates than those developing in EGFR-wild type mice. To assess aggressiveness of EGFR-independent tumors, we used a metastatic CRC mouse model containing conditionally inactivated Apc alleles (Apc(A) in combination with a conditionally active allele of KRAS (Kras(L527M)I )). It has been reported that delivery of Cre recombinase expressing adenovirus to the distal colon of these mice results in tumors that progress to carcinoma within 20 weeks, and liver metastases develop in approximately 20% of mice at 24 weeks. We discovered a 10% increase in the penetrance of tumors arising in the absence of EGFR (Egf(A), Apc(A), Kras(L527M)I ). Endoscopic analysis suggests an increase in tumor multiplicity in EGFR-deficient tumors when compared with tumors developing in EGFR-wild-type mice. Biweekly colonoscopy confirmed that colonic tumors have a faster growth rate in the absence of EGFR. High-frequency abdominal ultrasound suggests liver metastasis at 16 weeks in 20% of mice lacking EGFR. These findings demonstrate the existence of an EGFR-independent mechanism by which CRC can arise and progress. Moreover, tumors lacking EGFR grow larger than those developing under normal EGFR activity and may be a more aggressive form of CRC. We also have evidence that ERBB3 and ERBB4, related EGFRs, mediate compensatory and alternative pathways, suggesting an important role for these receptors in the progression of EGFR-independent CRC. This study will advance our understanding of ERBB family biology during colonic tumorigenesis, ultimately contributing to better therapies for CRC.

#3401 Role of membrane-associated guanylate kinase inverted 2 in advanced and castration-resistant prostate cancers. Bao Le, Kimberly Hammer, Katya Frantskevich, Irene Ong, Wei Huang, Paul Marker. University of Wisconsin-Madison, Madison, WI.

Background: Prostate cancer is the most common non-cutaneous malignancy for men in the U.S. Patients with advanced and/or metastatic prostate cancer are commonly treated with androgen deprivation therapy. Unfortunately, incurable castration resistant prostate cancer commonly develops 2-3 years after initial treatment. Methods: To identify candidate cancer genes involved in the development of castration resistance, the Sleeping Beauty (SB) transposon system and androgen-sensitive LNCaP cell line were used in a forward mutagenesis screen designed to model the transition from androgen sensitive to androgen insensitive prostate cancer. MAGI-2 was identified in the screen as a common insertion site gene, potentially involved in the transition to androgen independence. Clinical significance of MAGI-2 in prostate cancer was further supported by human TCGA data. Functional assays were used to confirm the involvement of MAGI-2 in prostate cancer progression. Results: MAGI-2 expression was prognostic for patient survival outcome. Ectopic expression of MAGI-2 in 22Rv1 cells resulted in a decrease in cell proliferation and colony formation in vitro studies. At the molecular level, overexpression of MAGI2 induced a suppression of known androgen responsive genes. MAGI-2 overexpression also limited the growth of grafts compared to empty vector grafts in vivo xenograft models. Conclusions: MAGI-2 was identified in a forward mutagenesis screen modeling the development of castration resistant prostate cancer. Human protein expression data from patient tissues supported a possible role for MAGI-2 in prostate cancer. Functional studies confirmed the potential involvement of MA GI-2 in prostate cancer progression. Future studies are seeking to elucidate the functional role and mechanism of action for MAGI-2 in prostate cancer. Ultimately, this may identify MAGI-2-regulated signaling pathways as therapeutic targets for prostate cancer.

#3402 The impact of germline single nucleotide polymorphisms (SNPs) in ERBB-family genes and genes associated with homologous recombination deficiency (HRD) on response to taxotere, platinum and trastuzumab (TCH) based therapy in the treatment of HER2-positive breast cancer patients. Ste phenie Madden,1 Sinead Toomey,1 Simon Furney,2 Malgorzata Milewska,3 Joanna Fay,1 Elaine W. Kay,1 John Crown,2 Susan Kennedy,3 Bryan T. Hennessey,3 Alex J. Eustace1. 1Royal College of Surgeons in Ireland, Dublin, Ireland; 2University College Dublin, Dublin, Ireland; 3St Vincent's University Hospital, Dublin, Ireland.

BACKGROUND: We have shown that ERBB (ERBB2, ERBB3, ERBB and other ERBB-family genes), and germline single nucleotide polymorphisms (SNPs) have a negative impact on the outcome of trastuzumab treated HER2-positive breast cancer (BC) patients. Currently TCH (taxotere, platinum and trastuzumab) based therapy is used to treat early stage HER2-positive BC. We investigate the importance of germline SNPs in ERBB genes and those genes involved in homologous recombination deficiency (HRD), on how patients respond to TCH therapy. PATIENTS AND METHODS: ERBB/HRD SNPs were identified in a panel of 32 advanced and castration-resistant prostate cancers. We investigate the importance of ERBB/HRD SNPs on RFS. RESULTS: Ten ERBB/HRD SNPs were identified as associated with survival free recurrence (RFS) in patients who received a TCH based treatment versus those who received alternate therapies. Protein extracted from formalin fixed paraffin embedded tumours (n=60), was run on an RPPA platform to measure expression and phosphorylation of proteins (69 antibodies). Logistical regression identified protein levels associated with the presence/absence of ERBB/HRD SNPs that were significantly associated with RFS. RESULTS: Ten ERBB/HRD SNPs were prolined in 157 trastuzumab treated HER2-positive BC patients. The minor alleles of the ERBB2 (rs1136201), ERBB3 (rs2229046) and BARD1 (rs2070896) SNPs were significantly associated with a worse RFS in patients who received TCH based therapy relative to those who had the reference allele (ERBB2: HR=2.67 (CI:1.05-6.78), p=0.04; ERBB3 rs2229046: HR=4.95 (CI:1.91-12.79), p=0.00; BARD1: HR=3.27 (CI:1.16-9.17), p=0.02). The impact of ERBB/HRD SNPs on RFS
was not observed in patients who did not receive TCH treatment. The minor allele of the RNF8 rs2284922 SNP is associated with a worse RFS (RNF8: HR = 12.42 (CI = 2.00-77.19), p = 6.89x10^-3) relative to those who had the referral allele only in patients who did not receive TCH treatment. RPPA analysis identified that patients who received TCH therapy and had the minor allele of the ERBB3 SNPs were significantly associated with the expression of HER2, p27, and MEK1/2; minor allele associated with low expression of p27 (p = 7.22x10^-3) and with high expression of HER2 (p = 6.53x10^-3); rs773123, minor allele associated with low expression of p27 (p = 5.38x10^-4) and with high expression of MEK1/2 (p = 6.24x10^-4). CONCLUSIONS: The presence of germline ERBB/HHRD SNPs may play an important role in how a patient responds to TCH based therapy, and clinical assessment of these SNPs by targeted genetic screening of patients’ blood may allow for stratification of patients prior to treatment.

#3403 Telomere shortening in pancreatic cancer is correlated to KRAS mutation. Yoko Matsumeda,1 Naotaka Izumiyma,1 Mutsunori Fujiiwara,2 Naoshi Ishikawa,3 Junko Aida,4 Kaiyo Takubo,5 Toshiyuki Ishiwata,6 Tomio Ara,1 Tokyo Metropolitan Geriatric Hospital & Inst of Gerontology, Tokyo, Japan; 2Japan; 3Japanese Red Cross Medical Center, Tokyo, Japan.

Background: Pancreatic cancer is characterized by genomic complexity and chromosomal instability, and atypical mitotic figures are morphological features of this phenotype. Previously we have reported that approximately 30% of total mitosis in pancreatic cancer was atypical including multipolar, lag-type, ring and asymmetrical mitosis, and anaphase bridges, and the number of total mitosis and atypical mitosis in pancreatic cancers was correlated with aggressive phenotype and prognosis (Pancreatology, 2016). In the present study, we clarified the relation between atypical mitotic figures, telomere length, and genetic abnormality in the pancreatic cancer. Methods: We surveyed the mitotic figures of the normal epithelium, pancreatic intraepithelial neoplasia (PanIN), and pancreatic cancers using surgically resected pancreatic cancer specimens (n = 40). Telomere length was analyzed using quantitative fluorescent in situ hybridization technique. We also analyzed mutations of Kras codon 12 and 13 by PCR and microsatellite instability by immunohistochemical staining of MLH1, MSH2, MSH6 and PM52. Results: Pancreatic cancer and duodenal epithelium showed significantly higher mitotic indices as compared with the duct, acinar cells, and PanINs. Normal mitosis was also higher in pancreatic cancers and the duodenal epithelium, while atypical mitosis was significantly elevated only in pancreatic cancers. Number of total mitosis and atypical mitosis were negatively correlated with telomere length, suggesting that telomere shortening plays important roles in cancer proliferation and chromosomal instability. In comparison with normal ducts, telomere length was decreased in PanIN-1, -2 and -3 and cancer. Furthermore, telomere length was gradually shorter among PanIN grades. Most of pancreatic cancers harbored mutations in Kras codon 12, and pancreatic cancer cases with Kras mutation showed shorter telomere length as compared with cases without Kras mutation. All pancreatic cancer cases in the present study were microsatellite stable. Conclusion: Our data strongly suggest that telomere shortening occurs in the early stages of pancreatic cancer and is associated with aggressive phenotype and prognosis (Pancreatology, 2016). Telomere shortening and chromosomal instability in the duct epithelium play key roles on carcinogenesis of the pancreas.

#3404 Loss of Abi1 abrogates lung metastasis in the PyMT mouse model of breast cancer. Angelina Regua,1 Isabelle Bichindaritz,1 Tiffany Caza,1 Julie White,1 Robert Adamiec,2 Mira Krendel,2 Gennady Bratlavsky,2 Leszek Kotula,1. 1Upstate Medical University, Syracuse, NY; 2SUNY Oswego, Oswego, NY; 3Memorial Sloan-Kettering Cancer Center, New York, NY.

This study aims to elucidate the role of Abelson interactor 1 (Abi1), a key protein in the WAVE regulatory complex, in mammary carcinogenesis and metastasis. Breast cancer is the second-leading cause of mortality in women in the United States with an estimated ~200,000 new cases and over 40,000 deaths this year. Despite current treatment modalities, breast cancer patients often relapse after only a few years of treatment thus emphasizing the need for better therapeutic targets. Abi1 is an adaptor protein mainly associated with the WAVE (Wiscott-Aldrich syndrome protein family verprolin homologous) regulatory complex and Arp2/3 (Actin-related proteins 2 and 3)-mediated actin cytoskeleton remodeling. Our bioinformatic and gene expression analyses of human tumor data indicates that Abi1 is frequently upregulated in invasive breast cancers, is associated with poor survival, and may promote an aggressive breast tumor phenotype. Downregulation of Abi1 also abrogates motility and invasion of breast cancer cells, most likely through inactivation of both Src and PI3 kinase as well as certain matrix metalloproteases. We therefore hypothesize that Abi1 positively regulates breast tumor progression and invasion through dysregulation of these cell signaling pathways. To determine the role of Abi1 in breast tumor progression, we used a Cre-lox system to conditionally delete Abi1 in the mammary tissue of Polyoma Middle T (PyMT) breast cancer mice. Abi1 knockout (KO) PyMT breast cancer tumors were measured with a caliper to determine total tumor burden. We are currently analyzing changes in tumor kinetics as a result of conditional Abi1 knockout in the mammary epithelium of PyMT mice. Our preliminary studies of indicate slowed tumor growth in Abi1 KO PyMT mice. We Western blot analyses of Abi1 KO mammary tumors indicate concomitant loss of WAVE complex proteins supporting our previous findings that WAVE complex integrity is dependent on Abi1. Most interestingly, Abi1 null PyMT mice exhibit significantly reduced incidence of lung metastasis, supporting our hypothesis that Abi1 promotes invasion by breast cancer cells. In summary, Abi1 loss leads to reduction of lung metastasis in PyMT mice, possibly through inactivation of key cell signaling and proliferation pathways such as Src and PI3 kinase. This work will establish Abi1 as a potential prognostic marker and therapeutic target in metastatic breast cancer.

#3405 Screening for two recurrent BRCA1 mutations in Tunisian women with triple negative breast cancer. Wijdan Mahfoudh,1 Inchirah Bettaib,1 Salouha Gabbouj,1 Nooredjine Bouaouina,1 Loﬁt Chouchane,2 Abdellatifh Zahkama1. 1Faculty of Medicine of Monastir, Monastir, Tunisia; 2Department of Radiation Oncology, CHU Farhat Hached, Sousse, Tunisie.

Triple-negative breast cancers (TNBC) lack expression of oestrogen, progesterone and HER2 receptors. Reports to date indicate that up to 20 % of TNBC patients harbour germline BRCA mutations; however, the prevalence of mutations may vary with ethnic group and with geographic region. In the Tunisian population, as yet a limited number of BRCA germline mutations have been reported. Tow recurrent mutations were found in exon 5 and 20 in BRCA1 gene (c.211dupA and c.5266dupC). To investigate the contribution of BRCA1 gene mutations to TNBC in Tunisia, we screened the exons 5 and 20 of BRCA1 gene in 20 TNBC patients by direct sequencing. In our study, we identiﬁed the c.5266dupC mutation in BRCA1 exon 20 in 20 of 20 triple-negative patients with a prevalence of 10%. Our study is the ﬁrst investigation on the role of BRCA1 gene in TNBC in Tunisia. In this study, we show that targeting only the exon 20 in BRCA1 gene allows detection of a substantial percentage of mutations in Tunisian TNBC patients.

#3406 Hereditary risks of male breast cancer in a multi-gene panel testing cohort. Elizabeth C. Chao,1 Mary Pritzlaff,2 Summerour Pia,2 Rachel McFarland,3 Shuei Li,4 Jill Dolinsky,2 David Goldgar,4 Hermela Shimelis,5 Fergus Coulton,1 LaDaca Holly,6 UC Irvine, Newport Beach, CA; 2Ambry Genetics, Aliso Viejo, CA; 3UC Irvine, Irvine, CA; 4University of Utah, Newport Beach, CA; 5Mayo Clinic, Rochester, MN.

While the population-based risk for breast cancer in males remains relatively low (1:1000), inherited predisposition can significantly raise this to as high as 10%, in men who carry a mutation in the BRCA2 gene. Lifetime breast cancer risks of 1-2% have also been reported in men who carry a mutation in BRCA1. These risks, as well as elevated risks of prostate, pancreatic, and melanoma cancers, are important to discuss in families diagnosed with Hereditary Breast and Ovarian Cancer Syndrome, which is often viewed as being clinically relevant only to the women in an affected family. However, beyond the BRCA1/2 genes, limited data is available on hereditary predisposition to male breast cancer. We analyzed clinical histories and molecular results from multi-gene panel testing for hereditary cancer predisposition in a cohort of 715 men affected by breast cancer. A total of 708 male breast cancer patients were eligible for inclusion in the final analysis. Molecular testing included analysis of 5 to 59 genes for DNA coding sequence and copy number variants by next-generation sequencing and microarray. Genetic variants identified were classified according to a 5-tier system using previously validated algorithms. Did only those variants classified as pathogenic or likely pathogenic were included in the analyses as positive for a mutation. Overall, a mutation was detected in 18% of these men. Four subjects carried a mutation in two different breast cancer predisposition genes. BRCA2 and CHEK2 were the most frequently mutated genes. The risk of breast cancer Risks of 1-2% have also been reported in men who carry a mutation in BRCA1. These risks, as well as elevated risks of prostate, pancreatic, and melanoma cancers, are important to discuss in families diagnosed with Hereditary Breast and Ovarian Cancer Syndrome, which is often viewed as being clinically relevant only to the women in an affected family. However, beyond the BRCA1/2 genes, limited data is available on hereditary predisposition to male breast cancer. We analyzed clinical histories and molecular results from multi-gene panel testing for hereditary cancer predisposition in a cohort of 715 men affected by breast cancer. A total of 708 male breast cancer patients were eligible for inclusion in the final analysis. Molecular testing included analysis of 5 to 59 genes for DNA coding sequence and copy number variants by next-generation sequencing and microarray. Genetic variants identified were classified according to a 5-tier system using previously validated algorithms. Only those variants classified as pathogenic or likely pathogenic were included in the analyses as positive for a mutation. Overall, a mutation was detected in 18% of these men. Four subjects carried a mutation in two different breast cancer predisposition genes. BRCA2 and CHEK2 were the most frequently mutated genes. The risk of breast cancer relates to high as 10%, in men who carry a mutation in the BRCA2 gene. Lifetime breast cancer risks of 1-2% have also been reported in men who carry a mutation in BRCA1. These risks, as well as elevated risks of prostate, pancreatic, and melanoma cancers, are important to discuss in families diagnosed with Hereditary Breast and Ovarian Cancer Syndrome, which is often viewed as being clinically relevant only to the women in an affected family. However, beyond the BRCA1/2 genes, limited data is available on hereditary predisposition to male breast cancer. We analyzed clinical histories and molecular results from multi-gene panel testing for hereditary cancer predisposition in a cohort of 715 men affected by breast cancer. A total of 708 male breast cancer patients were eligible for inclusion in the final analysis. Molecular testing included analysis of 5 to 59 genes for DNA coding sequence and copy number variants by next-generation sequencing and microarray. Genetic variants identified were classified according to a 5-tier system using previously validated algorithms. Only those variants classified as pathogenic or likely pathogenic were included in the analyses as positive for a mutation. Overall, a mutation was detected in 18% of these men. Four subjects carried a mutation in two different breast cancer predisposition genes. BRCA2 and CHEK2 were the most frequently mutated genes. The risk of breast cancer relate...
additional cancers. The high overall diagnostic yield suggests the utility of testing all male breast cancer patient regardless of age or family history by multigene panel testing, and provides data to support risk-based counseling and medical recommendations for screening and/or prevention in male mutation carriers. 1. 1. LaDuca et al. Genet Med. 2014 Nov;16(11):830-7. 2. Richards et al. Genet Med. 2015 May;17(5):405-24

#3407 Side effects of BRAF inhibitors mimic RASopathies. Alicia Stecchi,1 Alain Dupuy,2 Monica Dinulescu,3 Catherine Droitcourt,4 Henri Adamski,3 Smail Hadj-Rabia,5 Sylvie Odent,4 Marie-Dominique Galibert,2 Lise Boussemart,4 CHU de Rennes, Rennes, France;2 Rennes I University, Rennes, France;3 Hôpital Universitaire Necker-Enfants malades, Paris, France;4 Université de Rennes 1, Rennes, France

Recent advances in targeted anticancer therapies have substantially improved the prognosis of several cancers but they are not free of side effects. These side effects are, however, clearly distinct from those induced by classical cytotoxic chemotherapies, likely because targeted therapies are designed to interfere with specific oncogenic signaling pathways and not to inhibit cell proliferation in general. We, therefore, evaluated whether specific side effects of BRAF inhibitor therapies would resemble symptoms seen in patients with RASopathies, in which the MAPK pathway is affected and which includes Costello, Noonan and Cardio-Facio-Cutaneous syndromes. To this end, we collected a cohort of 18 patients with genetically confirmed RASopathies that were followed between 2012 and 2016 at the University Hospital of Rennes, and were evaluated based on their personal medical history and a clinical examination. Strikingly, RASopathy patients, particularly those with Cardio-Facio-Cutaneous syndrome, showed symptoms resembling those that we and others have previously seen in genetically characterized patients undergoing BRAF inhibitor treatment. These symptoms included keratoderma pilaris, wavy hair, sparse eyelashes/eyebrows, poor hair growth, palmo-plantar hyperkeratosis in areas of pressure, verrucous papillomas, nevi efflorescence, and increased cancer risk. Nevertheless, BRAF-inhibitor treatment can also lead to side effects not typically found in patients with RASopathies, including aceneform dermatitis and venu-rafenib-specific phototoxicity, which may even increase under a combined BRAF/MEK inhibition. These results suggest that the side effects of BRAF inhibition that are similar to symptoms in patients with RASopathies are due to direct targeting of the MAPK pathway in BRAF-non-mutated (wild type) cells while those that are dissimilar represent off-target effects that do not affect the MAPK pathway. We hope that this type of comparative analysis may lead to a better understanding of the multiple effects of targeted therapies and perhaps prompt modifications in the targeted therapy approach to minimize these side effects.

#3408 Telomere length and TERT promoter mutations in cutaneous melanoma. Sivaramakirishna Rachakonda,1 Barbara Heidenreich,1 Eduardo Nagore,2 Rajiv Kumar1.

Telomeres at chromosomal ends are comprised of multiple short repeat sequences. In humans TTAGGG repeats account for telomere length ranging 10-15 kb. Telomere sequences are mainly double stranded that end in a single stranded G-rich tail of 150-200 nucleotides. Telomeres in somatic cells undergo gradual shortening due to inherent limitations of DNA replication and limited levels of specialized enzyme telomerase that adds the repeats at chromosomal ends to maintain homeostasis. We previously reported somatic mutations in the core promoter of the telomerase reverse transcriptase (TERT) gene that lead to increased transcription of catalytic subunit and tumor specific telomerase reactivation. Telomere length per se is associated with risk in different cancers. In this study, we measured leukocyte telomere length using real-time PCR in 1469 melanoma patients and compared with that in 1158 matched healthy controls. The melanoma patients had statistically significantly longer telomeres than matched controls (t-test; P 6X10^-10). Telomere length sequences are mainly double stranded that end in a single stranded G-rich tail of 150-200 nucleotides. Telomeres in somatic cells undergo gradual shortening due to inherent limitations of DNA replication and limited levels of specialized enzyme telomerase that adds the repeats at chromosomal ends to maintain homeostasis. Telomere length per se is associated with risk in different cancers. In this study, we measured leukocyte telomere length using real-time PCR in 1469 melanoma patients and compared with that in 1158 matched healthy controls. The melanoma patients had statistically significantly longer telomeres than matched controls (t-test; P 6X10^-10). Mendelian randomization, carried out using two polymorphisms represented by rs1317082 and rs7726159 that associated with telomere length in genome wide association studies, showed association between increased telomere length and melanoma risk with an odds ratio of 2.3 (95% confidence interval 1.8-2.8). When measured in blood tissues from a melanoma family with the germline -57A/C TERT promoter mutation, the carriers had longer telomeres (median 1.12) than the non-carriers (median 0.87). The TERT promoter mutations create binding motifs for E-twenty six (ETS) transcription factors and in stem cell the presence of the promoter mutations resulted in continued TERT expression and telomerase activity following differentiation into adult cells. Individuals with the germline TERT pro-moter mutations develop melanoma with an early age of onset and rapid progression to metastases; two individuals in the family who lived past median age of onset developed several other malignancies, besides melanoma. Thus, dysregulated telomerase leads to a severe phenotype. In an analogy, we hypothesize that association of longer rather than shorter telomeres with an increased risk of melanoma reflects stochastic increase due to common genetic variation. In contrast, the telomere length was shorter in tumors from unrelated melanoma patients with (121) than without (170) somatic TERT promoter mutations (P 6X10^-5), which reflects the selection of the mutations at telomere crisis. Thus, a dynamic but controlled system evolved around telomere homeostasis when dysregulated leads to an increased cancer risk and affects tumor progression.

#3409 Age of cancer onset differentiated by sex and TP53 codon change in Li-Fraumeni Syndrome patient population. Lauren Erdman,1 Ben Brew,2 Jason Berman,3 Adam Shlien,1 Andrea Doria,1 David Malkin,1 Anna Goldenberg1.

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Introduction: Li-Fraumeni Syndrome (LFS) is a highly penetrant autosomal dominantly inherited cancer predisposition disorder. Germline mutations of the TP53 tumor suppressor gene cause >80% of LFS and confer an increased risk of a range of early onset cancers, as well as of second tumors even in the absence of a family history of cancer. For this reason, we previously reported the implementation of a comprehensive life-long clinical surveillance protocol for individuals with a germline TP53 mutation for early tumor detection. Here, we set out to build a predictive model of age of onset of cancer in LFS patients to inform this screening protocol aiming to make it more targeted. We identify characteristics that differentiate the age of cancer onset consistently, across multiple LFS patient cohorts. Methods: The LFS cohort at Toronto’s Hospital for Sick Children (SickKids) (n = 171 patients) was used as a discovery set to identify factors that distinguish age of onset among LFS patients. This project focused specifically on patient characteristics such as sex and mutations within TP53, both as they appear on the genome and manifest in the protein, as predictors for age of onset. These predictors were tested in an exponential parametric survival model. Findings: From the SickKids discovery set were tested for replication in the International Agency for Research on Cancer (IARC) TP53 database (n = 2574 patients). Results: In the discovery cohort, female sex was associated with a 1.53 fold later age of cancer onset than in males (p = 0.019). This did not replicate in the IARC TP53 set with 0.99 fold earlier onset for females than males (p = 0.843). However, in the discovery set, there appears to be a point at which female and male age of onset converges at 43 years. Controlling for onset before vs after 43 years in our replication set shows 1.12 (p = 0.0204) times later age of cancer onset in females than in males which is the same direction and significance as in our discovery set. The discovery cohort also showed 2.23 (p = 0.08) later cancer onset for individuals with a germline Arginine to Cysteine (Arg>Gys) codon change (model significance p = 0.047). This finding replicated in the IARC TP53 data set which showed individuals with an Arg>Gys codon change having onset 1.29 (p = 0.043) times later than those with a TP53 mutation that did not result in this codon change. Conclusions: Our study identified two LFS patient characteristics, sex and TP53 Arg>Gys codon change, which consistently differentiate age of cancer onset within the LFS patient population. Females under the age of 43 when compared to males under the age of 43 appear to have later tumor onset, an effect which disappears after the age of 43. Individuals with an Arg>Gys TP53 codon change are expected to have later onset cancer than those with TP53 mutations that do not result in this change. Future work will disentangle these findings further and build a more comprehensive predictive model of cancer onset in LFS patients.

#3410 Phenotypic characterization of allelic variation within the HEAT repeats of the mechanistic target of rapamycin. Patricia A. Wiley,1 Joy Gary,2 Zaw Phyoo,2 Shuling Zhang,1 Amanda Scirocco,1 Dorian Fraizer4.

1National Cancer Institute, Bethesda, MD; 2Center for Disease Control and Prevention, GA; 3Marymount University, Arlington, VA; 4University of Maryland, Baltimore County, Baltimore, MD.

The mammalian target of rapamycin (mTOR), a protein in the phosphoinositide 3-kinase (PI3K) pathway important to cell cycle regulation, is frequently activated in cancer. This signaling pathway regulates cell growth and metabolism, and when dysregulated in cancer, it contributes to tumor angiogenesis and growth. mTOR contains two distinct complexes, mTORC1, and mTORC2. mTORC1 plays a role in biosynthesis, while mTORC2 helps

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with cellular metabolism. It has been shown that mTOR knockout models are embryonically lethal to mice. However, lower levels of functional mTOR have been shown to be beneficial and slow cancer progression. Allelic variation occurs within inbred strains of mice, including BALB/c mice. One allelic variant in mTOR, R628C, which contributes to tumor susceptibility has been linked to increased breast cancer risk. This single amino acid change occurs in the HEAT repeats of mTOR. mTORA repeat polymorphisms will model mTOR signaling activation mechanisms in humans to aid in development of new cancer treatments.

#3411 Rare BRCA2 K3326X increases susceptibility to sporadic pancreatic ductal adenocarcinoma: a PANDoRA study. Ofrue M. Obaze,1 Gabrielle Capurso,2 Angelo Andriulli,3 Pavel Soucek,4 Ewa Małecka-Panas,5 Juozas Kaunas,6 Michalopoulou176, Athens, Greece; 9Klinik für Allgemein-, Viszeral- und Transplantationschirurgie, Medizinische Fakultät, University of Freiburg, Freiburg, Germany; 14Dipartimento di Oncologia, Università di Bologna, Bologna, Italy; 3Division of Gastroenterology, Medical University of Lodz, Lodz, Poland; 8Department of Gastroenterology, Lithuanian University of Health Sciences, Kaunas, Lithuania; 3Department of Gastroenterology and Research Laboratory, Department of Oncology, IRCCS Scientific Institute, I.R.C.C.S. San Raffaele, Milan, Italy; 1Division of Cancer Epidemiology, Heidelberg, Germany; 6Department of Basic Medical Sciences, Laboratory of Biology, Medical School National and Kapodistrian University of Athens, Michalaakopoulo 176, Athens, Greece; 2Klinik für Allgemein-, Viszeral- und Transplantationschirurgie, Im Neuenheimer Feld 110, Heidelberg, Germany; 15AARC-Net, Applied Research on Cancer Centre, University of Verona, Verona, Italy; 15Gastroenterology and Gastrointestinal Endoscopy Unit, Vita-Salute San Raffaele University, IRCCS San Raffaele Scientific Institute, Milan, Italy; 15Department of Surgery, Oncology and Gastroenterology DISCOG, University of Padova, Padova, Italy; 16Division of Clinical Epidemiology and Aging Research, German Cancer Research Centre (DFKZ), Heidelberg, Germany; 17Department of Biologi, Università di Pisa, Pisa, Italy; 18Pancreas Unit, Department of Digestive System, Sant’Orsola-Malpighi Hospital, Bologna, Italy; 19Massa Carrara Oncological Department, Azienda USL Toscana Nord Ovest, Carrara, Italy; 20Department of Oncology, Palacky University Olomouc and University Hospital Olomouc, Olomouc, Czech Republic; 21Division of General and Transplant Surgery, Pisa University Hospital, Pisa, Italy; 22Department of General, Visceral, and Thoracic-Surgery, University Medical Center, Hamburg, Germany; 23Institute of Experimental Medicine, Czech Academy of Science, Prague and Institute of Biology and Medical Genetics, 1st Medical Faculty, Charles University, Prague, Czech Republic; 24Department of Hematology, Medical University of Lodz, Poland, Lodz, Poland.

Background: The incredibly poor outlook of pancreatic cancer patients underscores an urgent need for early diagnostic markers. Pancreatic cancer ranks third most-frequent cause of cancer death in the USA/1004 and genetic markers have been detected in <10% of sporadic cases. Germline variants in breast cancer tumor suppressor BRCA2 have been reported to increase predisposition to several cancers including pancreatic tumors. Rare K3326X (rs1571833, c.9976A>T) which introduces a premature stop codon thus truncating the protein, has previously been implicated in familial PDAC, but not in sporadic cases. A frameshift pathogenic mutation c.6503delTT (rs1576654, p.Leu2092Profs) reported with K3326X in breast and ovarian cancer families, has also been speculated to influence risk associations due to linkage disequilibrium between both variants. Method and results: K3326X was genotyped in 2,969 sporadic cases and 4,700 controls using Taqman chemistry and fidelity of genotypes assessed based on concordance of internal replicates and negative controls. K3326X was observed in 1.2% of cases and 0.8% of controls. Odds ratios (ORs) and associated 95% confidence intervals (CIs) were estimated by multivariate unconditional logistic regression with adjustment for age, sex and region of origin. We also performed a stratified analysis based on age at diagnosis to estimate the risk association between K3326X and early-onset pancreatic cancer. Results: No significant effect of the variant was observed for sporadic pancreatic cancer risk, we also sequenced DNA from carriers of K3326X in this study. We found K3326X to be associated with increased risk of developing sporadic PDAC (OR = 1.71, 95% CI = 1.18 - 2.49, P = 0.005). This risk was considerably higher among cases aged 50 years and younger (OR = 2.13, 95% CI = 1.10 - 4.11, P = 0.03). Furthermore, carriers of K3326X did not bear the c.6503delTT mutation suggesting that the effect of the two variants are not mediated by the latter. Conclusion: These robust associations implicate K3326X in the etiology of sporadic and early-onset PDAC, and therefore warrant replication as well as functional studies to elucidate the role of K3326X in DNA repair mechanisms.

#3412 Functional knockout of ATRX or DAXX permits the alternative lengthening of telomeres (ALT) mechanism in prostate cancer cells. Mindy K. Graham. Johns Hopkins School of Medicine, Baltimore, MD.

A key hallmark of cancer is unlimited replication, which requires cancer cells to evade replicative senescence induced by short telomeres. The majority of cancers overcome this critical replication barrier by upregulating the telomerase enzyme, a telomere-specific reverse transcriptase. However, a subset of cancers lack telomerase, and telomeres are maintained by employing the Alternative Lengthening of Telomeres (ALT) pathway, which is dependent on homologous recombination. Across a variety of tumor types, multiple laboratories, our laboratory and others have reported a robust correlation between ALT and recurrent cancer-associated somatic inactivating mutations in the ATRX-DAXX chromatin remodeling complex. In a previous comprehensive cancer survey of ALT, we reported that ALT was highly prevalent in some tumor types (e.g. astrocytomas, sarcomas, pancreatic neuroendocrine tumors), but we did not observe any ALT-positive primary prostate cancer (out of 1,176 analyzed). However, we subsequently found a subset of metastatic prostate cancers that harbor ALT, suggesting that mutations giving rise to ALT in this disease are unique to metastatic prostate cancer. Here, we have created the first prostate cancer cell lines exhibiting ALT, with the explicit purpose of molecularly characterizing ALT in prostate cancer and identifying promising therapeutic targets for men with ALT-positive lethal metastatic prostate cancer. Inactivating mutations in either ATRX or DAXX using the CRISPR/Cas9 system were introduced into the genetically well-characterized, telomerase-negative (ALT-negative) prostate cancer cell lines, LAPC-4 and CWR22Rv1. Resulting mutant subclones were compared to their parental (or empty vector) counterparts. In these new models, abolishing ATRX expression was sufficient to induce the ALT phenotype in both LAPC-4 and CWR22Rv1, as assessed by multiple biomarkers of ALT, including the presence of bright telomeric FISH foci, ALT-associated PML bodies (ABPs), and extrachromosomal telomere cicles. Interestingly, abolishing DAXX expression induced ALT in only a subset (3/5) of LAPC-4 DAXX KO clones, and a subset (4/19) of CWR22Rv1 DAXX KO clones. We have successfully activated the ALT telomere maintenance phenotype in two prostate cancer cell lines through CRISPR-mediated targeted gene deletion. We are currently utilizing these isogenic cell lines to further characterize and elucidate the underlying biology of cancers harboring ALT, with the goal of pharmacologically targeting the molecular features unique to the ALT phenotype (e.g. ATR inhibition). The identification of ALT-specific drugs may pave the way for the development of new targeted treatments for the subset of men with ALT-positive lethal metastatic prostate cancer, and more broadly, other ALT-positive cancers. ALT is easily detected in clinical tissue samples, and thus would serve as a predictive biomarker for personalized medicine.

#3413 Identification of a novel cancer predisposition variant associated with risk of CMM and other cancers. Craig C. Teerlink,1 John G. Zone,1 Sancy A. Leachman,2 Lisa A. Cannon-Albright.3 Univ. of Utah Health Sciences Ctr., Salt Lake City, UT; 4Oregon Health and Science University, Portland, OR.

Introduction: In a search for the responsible CMM predisposition gene/variant, whole exome sequencing was performed on a pair of related CMM cases (cousins) from an extended high-risk Utah CMM pedigree showing evidence of linkage to chromosome band 1q41. Methods: Standard bioinformatics tools were used to identify rare or novel variants shared in the 2 related CMM cases in a CMM pedigree showing 1q41 linkage. Sanger sequencing and a Taqman assay were used to confirm the original variants, and to confirm segregation of the variant in other cancer-related relatives. Taqman assay of the variant in independent Utah CMM cases (n=491) and controls (n=207) was performed. Results: A novel variant in SLC30A10 (rs 53024571, Chrlq14:220091798 C-T...
Molecular and Cellular Biology / Genetics: Genotype-Phenotype Associations

#3414 Novel MGMT variant association in Mexican patients with astrocytoma. Liliana Gomez-Flores-Ramos,1 Talia Wegman-Ostrosky.2 Institute of Biomedical Research, UNAM. National Cancer Institute, INCAN, Mexico City, Mexico; National Cancer Institute, NIH, Bethesda, MD, USA

Introduction: Astrocytomas are the most common and lethal brain tumors with a median survival of 15 months. Different biomarkers have arisen to classify and treat this type of tumors; one of these markers is MGMT. The methylation status of this gene is a prognostic and predictive factor. The MGMT (O-6-Methylguanine-DNA Methyltransferase) is involved in the cellular response to the effect of O-6-methylguanine (O6-MeG) in DNA. Repairs alkylation damage in DNA by transferring the alkyl group at the O-6 position to a cysteine residue in the enzyme. The dysregulation of this methylation is critical in the development of certain cancers. Objective: To identify germlinal MGMT gene variations in patients with astrocytoma. Material and methods: 55 randomly selected Mexican patients diagnosed with astrocytoma between 2008-2014 were consented to perform the molecular analysis of the whole gene and 5' UTR and 3' UTR regions of MGMT at Ion Torrent platform. All amplified region had a depth of minimum 1500X. Ion Reporter software was used to analyze the variant genetic and using Integrative Genomic Viewer we confirmed that each variant had a Phred higher than 25. Genotypic frequencies were compared with HapMapMex and Mexican ancestry population from the 1000 Genomes project. Results. In lymphocytes DNA, eight genetic variants were found, and two of them were statistically significant risk factors: rs7896488 alternative allele A with a frequency of 15% in patients with astrocytoma, and only 4% of the control group (OR = 5.53, IC 1.96-15.39 p = 0.0007) and rs2308326 ancestral allele C, where 95% of the patients were carriers of allele C in contrast with 73% of controls (OR = 5.39, IC 2.29-12.69 p = 0.000019). These variants are non-coding single nucleotide polymorphisms (SNP) with either an arginine (R72) or a proline (P72) at codon 72. By sequencing and Taqman assay in 32 patients the G659 mutations were 4.3% and 2.8% seen in 33 and 18 reads with a coverage of 77X5 and 66X, respectively. A review of pathology reports indicated that both matched samples had synchronous EECs. These two samples represent 15.4% of cases diagnosed with synchronous EmOC and EEC in this cohort. Conclusions: MGMT somatic mutations are uncommon in EmOCs and EECs. Recent massively parallel sequencing data suggests that EmOCs in the setting of synchronous EECs are clonally related and disseminated cells are related to nearby anatomic structures. Our data supports that MGMT somatic mutations in EmOC are clonally expanded from EECs likely through trans-tubal migration.

#3415 RNF43 somatic mutations in endometrioid ovarian cancers occur in the setting of synchronous endometrioid endometrial cancers. Defne L. Levine,1 Fanny Dao,2 Narciso Olvera,3 Katherine LaVigne,1 David B. Solit,1 Peter Jelinec,2 Memorial Sloan Kettering Cancer Center, New York, NY; NYU Perlmutter Cancer Center, New York, NY

Introduction: Somatic frameshift mutations in RNF43 have previously been filtered out in cancer genome sequencing projects given that they occur in homopolymer tracts and resemble polymerase slippage errors. Two RNF43 hotspot mutations have been identified and validated in endometrioid endometrial cancers. We determined the frequency of RNF43 somatic mutations in EmOC. Methods: We reviewed the clinical and pathologic features of EmOC samples diagnosed from 2006 to 2015, retrieved from laboratory databases and institutional archives. DNA was extracted from formalin-fixed, paraffin-embossed tumor samples using standard protocols. Sanger and next-generation sequencing (NGS) were used to screen for hotspot mutations at codons R117 and G659. Due to shared clinical and morphologic features of EEC and endometrioid ovarian cancer (EmOC), we determined the frequency of RNF43 somatic mutations in EmOC. Methods: We reviewed the clinical and pathologic features of EmOC samples diagnosed from 2006 to 2015, retrieved from laboratory databases and institutional archives. DNA was extracted from formalin-fixed, paraffin-embossed tumor samples using standard protocols. Sanger and next-generation sequencing (NGS) were used to screen for hotspot mutations at codons R117 and G659 with custom designed primers. NGS at each hotspot was covered with a minimum sequencing depth of 400X. Results: Forty-seven EmOC patients with available tumor specimens were identified and included in the analysis. The median age at diagnosis was 55 years old (range 34-84). The majority of patients had FIGO stage I or stage II disease (n=38, 81%). Thirteen (28%) patients had synchronous endometrial endometrioid or mixed histology tumors. Two (4.3%) RNF43 somatic mutations at codon G659 were identified in both Sanger and NGS from the EmOC tumor specimens. Two (4.3%) RNF43 somatic mutations at codon G659 were identified in both Sanger and NGS from the EmOC tumor specimens. The allelic fractions of the G659 mutations were 4.3% and 2.8% seen in 33 and 18 reads with a coverage of 77X5 and 66X, respectively. A review of pathology reports indicated that both mutated samples had synchronous EECs. These two samples represent 15.4% of cases diagnosed with synchronous EmOC and EEC in this cohort. Conclusions: RNF43 somatic mutations are uncommon in EmOCs and EECs. Recent massively parallel sequencing data suggests that EmOCs in the setting of synchronous EECs are clonally related and disseminated cells are related to nearby anatomic structures. Our data supports that RNF43 somatic mutations in EmOC are clonally expanded from EECs likely through trans-tubal migration.

#3417 The impact of p53 codon 72 SNP upon aging and longevity in mouse models. Yuhan Zhao, Lihua Wu, Xuetian Yue, Chen Zhang, Jun Li, Jianming Wang, Zhenghui Feng, Wenwei Hu. Rutgers Cancer Institute of New Jersey; New Brunswick, NJ

p53 has dual functions on longevity. p53 plays a crucial role in tumor suppression to prevent early death due to cancer. However, it has been suggested by animal models that constitutively increased p53 activity accelerates the decline of stem/progenitor cells’ self-renewal function during aging process, which leads to a reduced lifespan. In humans, the role of p53 in aging and longevity has not been well established. As a haplo-insufficient gene, p53 is under the tight regulation in cells. Attenuation of p53 function contributes greatly to tumorigenesis. p53 codon 72 single nucleotide polymorphism (SNP) with either an arginine (R72) or a proline (P72) at codon 72 is a naturally occurring common SNP that can influence the activity of p53. The P72 allele is weaker than the R72 allele in inducing apoptosis and suppressing cell transformation. Individuals with the P72 allele have increased cancer risk compared to the R72 allele. However, it is unclear whether the change of p53 activity in humans by functional SNPs could impact upon longevity. A perspective study with an aging human population showed that the P72 allele is associated with longer survival despite its increased risk for cancer development. These findings strongly suggest that p53 activity is reversely associated with aging, and SNPs in the p53 pathway could impact upon the life span in humans. In this study, we employed a mouse model system with knock-in of human p53 gene (Hupki) carrying either R72 or P72 SNP to investigate the impact of p53 codon 72 SNP upon longevity and its...
underlying mechanism. Mice with p53 R72 allele showed weaker transcriptional activity than the R72 allele toward a subset of p53 target gene, suggesting that these mice retain the function of p53 codon 72 SNP in human. We found that although mice with p53 R72 have increased cancer risk compared to mice with p53 R72, mice with p53 R72 that escaped tumor development showed reduced PTPN11 expression compared to the parental mouse cell line. Mice with p53 R72 displayed a delay in aging process compared to mice with p53 R72; mice with p53 R72 have slower reduction in bone density, dermal thickness and wound healing ability during aging process. We compared the effects of p53 codon 72 SNPs on stem cell population and function as a possible mechanism that contributes to their difference in longevity. Compared with mice with p53 R72, mice with p53 R72 allele have lower number of long-term stem/progenitor cells and better self-renewal function during aging process. Consistently, aging mice with p53 R72 allele exhibited better long-term stem cell cell ability of engraftment and repopulation than aging mice with p53 R72 allele as evaluated by bone marrow transplantation assay. Taken together, results from this study demonstrate that p53 codon 72 SNP has a direct impact on aging and longevity, and strongly support the role of p53 in regulation of stem/progenitor cells’ function and longevity.

#3418 Additional mutation in PTPN11 gene promotes tumorigenesis of the NF1 gene mutated cells. Yoshimi Arima, Ritsuko Haragi, Ryo Satoh, Toshiki Takenouchi, Kenjiro Kosaki, Hideyuki Saya. Kyoto University, School of Medicine, Tokyo, Japan.

The NF1 tumor suppressor gene encodes neurofibromin and is a functional Ras GTPase-activating protein (RasGAP) involved in negatively regulating the Ras signal by accelerating the conversion of activated Ras-GTP to inactive Ras-GDP. NF1 gene germline mutations cause Neurofibromatosis type 1 (NF1, von Recklinghausen disease). We hypothesized that additional genetic alterations promote the malignancy of NF1-associated tumors. To test our hypothesis, we inoculated a GFP-labeled human NF1-deficient cell line, snF96.2-GFP, which has a frame-shift mutation (c.3683delC, p.Asn1229MetfsTer11) in the NF1 gene, into the renal sub-capsules of immunodeficient mice. A subclonal cell line, the A-1 cell, was established from the developed tumor. We found that A-1 cells show much higher tumorigenic activity and phosphorylation status of MEK and Akt than the parental snF96.2-GFP cells. We analyzed the genomic DNA of both the snF96.2 and the A-1 cells by using the next-generation sequencing and our medical exome panel of 4813 genes, which are known to be responsible for most human genetic disorders. We identified 18 heterozygous variants within coding regions of 17 genes that were present in the A-1 cells, but not in the original snF96.2 cells. We found a single base substitution (c.1508G>T, p.Gly503Val) in the PTPN11 gene, which encodes the tyrosine phosphatase SHP-2, and is associated with the regulation of the Ras signaling pathway. It is critical to note that constitutional gain-of-function mutations in the PTPN11 gene cause Noonan Syndrome in humans due to activation of the Ras pathway. To determine the role of PTPN11 mutation in NF1-associated tumors, we established a cell line overexpressing PTPN11mwo in snF96.2-GFP cells. We inoculated parental cells and PTPN11mwo cells into the subcutaneous nude mice, and we found that the PTPN11mwo cells have lower tumorigenic activity than the parental snF96.2-GFP cells. Our data suggests that this additional gene mutation in PTPN11 promotes the malignant characteristics of NF1-associated tumors.

#3419 Uncovering the functional relevance of FBXW7 mutations in endometrial cancer. Mary Ellen Urick, Bo Hong, Meghan L. Rudd, Daphne W. Bell. NIH, Bethesda, MD.

The goal of this study is to elucidate the functional consequences of FBXW7 mutations in the context of endometrial carcinoma (EC). EC is a heterogeneous disease, consisting of multiple histological subtypes associated with distinct clinical outcomes. Endometrioid endometrial cancer is the most commonly diagnosed subtype and is associated with an overall favorable prognosis even in advanced disease. Less common but more clinically aggressive subtypes that contribute disproportionately to patient deaths include clear cell and serous ECs. We previously reported that FBXW7 is somatically mutated in 10% of endometrioid, 13% of clear cell and 29% of serous ECs. FBXW7 functions as a substrate-recognition protein within a SKP/CUL1/F-Box ubiquitin ligase complex, which targets numerous proteins for ubiquitin-mediated proteasomal degradation. Although FBXW7 is a known tumor suppressor, the functional consequences of these mutations have not yet been fully elucidated in the context of EC. As a means to this end, we utilized shRNA and Western blotting to identify proteins that are upregulated following FBXW7 depletion in ARK1 serous EC cells. Among proteins that were consistently upregulated were cyclin E1 and cMYC. We treated EC cells harboring endogenous FBXW7 mutations and those with wildtype FBXW7 with a proteasome inhibitor to confirm that EC cells regulate cyclin E1 and cMYC through proteasome degradation. Furthermore, we identified three EC cell lines that harbor endogenous mutations in FBXW7 and show that these cell lines express two novel recurrent mutations in FBXW7. Finally, we generated expression constructs for wildtype FBXW7α and six recurrent mutants found in EC. We show that transient exogenous overexpression of five out of six recurrent FBXW7 mutants in EC cell lines in which FBXW7 is silenced confers tumorigenic activity that is functionally equivalent. Our findings begin to reveal the molecular consequences of FBXW7 mutations in EC and ongoing studies are aimed at elucidating additional functional consequences of these aberrations in the context of EC.

#3420 Mutational status, expression and functional behaviors of FAM134B in colorectal cancer. Farhadul Islam,1 Vinod Gopalan,1 Raijul Wahab,1 Katherine Ting-wei Lee,1 Afraa Mammoori,1 Cu-Tai Lu,1 Robert A Smith,3 Alfred K-Y Lam1.1 Griffith University, Gold Coast, Southport, Australia; 2Gold Coast Hospital, Gold Coast, Southport, Australia; 3Queensland University of Technology, Brisbane, Australia.

Background: Family with sequence similarity 134B (FAM134B) is an ER-autophagy regulator and involved in the pathogenesis of neuronal disorders, vascular diseases and carcinomas. In colorectal carcinomas, FAM134B plays important role in the pathogenesis and associated with aggressiveness of the disease. However, the frequency of mutations, expression pattern and functional roles in cell have never been studied in colorectal cancer. Objectives: To investigate FAM134B mutations in tissues samples from patients with colorectal cancer and cell lines. Also, the expression of FAM134B at protein and mRNA levels were examined. In addition, functional roles of FAM134B in colon cancer were studied. Methods: Mutations in FAM134B sequence in eighty-eight cancer tissues and matched non-cancer samples was studied by high-resolution melt curve analysis followed by Sanger sequencing. FAM134B expression was studied and quantified in cell lines and cancer tissues samples using immunofluorescence, immunocytochemistry, Western blot and real-time PCR. In vitro functional assays were performed to unveil the molecular roles of FAM134B in colon cancer pathogenesis followed by shRNA-mediated silencing in cells. Mouse xenotransplantation model was used to confirm the functional behavior of FAM134B in colon cancer. Results: In this study, 46.5% (41/88) patients with colorectal cancer were identified as FAM134B mutations positive. Thirty-one novel pathogenic mutations were detected. Of the 31 mutations, 8 novel frameshift mutations caused nonsense-mediated mRNA decay and associated with gender of the patients, presence of metachronous cancer, size, T staging, presence of distant metastases and positivity of microsatellite instability (MSI) in the cancer. Results: In this study, 46.5% (41/88) patients with colorectal cancer were identified as FAM134B mutations positive. Thirty-one novel pathogenic mutations were detected. Of the 31 mutations, 8 novel frameshift mutations caused nonsense-mediated mRNA decay and associated with gender of the patients, presence of metachronous cancer, size, T staging, presence of distant metastases and positivity of microsatellite instability (MSI) in the cancer. Expression of FAM134B in cancer cells derived from advanced stages (stage III; SW48 and stage IV; HCT116) of colon cancer was significantly (p<0.01) reduced when compared to non-neoplastic colon cells (FHC) and cancer cells derived from stage II colon cancer (SW480). Expression of FAM134B mRNA in cancer tissues was noted significantly (p<0.001) downregulated when compared to that of non-cancer tissues samples. FAM134B suppression significantly (p<0.05) increased the proliferation of colon cancer cells, remarkably increased (34-52%: p<0.05) the clonogenic, migration capacity, and increases the proportion of cells in S phase of cell cycle (p<0.01). Xenotransplantation model showed that larger and higher-grade tumors were formed in mice treated with FAM134B knockdown cells. Conclusions: In vitro and in vivo functional studies implied that FAM134B acts as a cancer inhibitor in colon cancer. Also, FAM134B mutation is common in colorectal cancer and the association of mutation with adverse clinical and pathological parameters are in concur with the tumour suppressive property of the gene.

#3421 Pharmacological inhibition of histone methylation attenuates TGFβ induced EMT characteristics in pancreatic cancer. Hardik R. Mody, RaigopalGovindarajan. OSU College of Pharmacy, Columbus, OH.
Epithelial-to-Mesenchymal Transition (EMT) is a tumor cell remodeling process that drives progression of several epithelial solid tumors including pancreatic tumors. Transforming growth factor (TGF) β signaling is a key promoter of EMT pathway and remains an attractive target for curtailling tumor progression. Here we present a strategy for investigating inhibitors of TGFβ signaling in pancreatic cancer via reprogramming of miRNAs. We find a carbamoyl adenosine analog and histone methyl transferase inhibitor, 3-deazaadenosine A (DZNep) to significantly attenuate the TGFβ signaling induced EMT characteristics in pancreatic ductal adenocarcinoma (PDAC). Specifically, we find DZNep treatment to restore expression of selectivity epigenetically silenced miRNAs (miR-663a, miR-4787-5p, and miR-202) that inhibit endogenous TGFβ1 ligand and receptor expression via RNA interference. Consequent of this silencing of miRNAs was significant increases in EMT phenotype similar to DZNep in cultured pancreatic cancer cells, whereas locked nucleic acids inhibition of miRNAs abrogated such effects. In an orthotopic pancreatic cancer mouse model, DZNep and all three miRs reduced overall tumor burden in pancreas and metastatic load in liver, lungs and spleen. Together, these findings suggest epigenetic reprogramming of miRNAs by synthetic histone methylation reversal agents as a potentially viable approach for attenuating TGFβ signaling pathway involved in pancreatic cancer progression.

#3422 miR-200c-driven mesenchymal-to-epithelial transition as a therapeutic target in uterine carcinosarcomas. JiH. H. Tseng,1 Maria Bisogna,1 Lien N. Hoang,1 Narciso Olvera,2 Douglas A. Levine,2 Petar Jelić1.1MSBCC, New York, NY; 2NYUT Langone Medical Center, New York, NY.

Introduction and purpose of study: Uterine carcinosarcoma (UCS) is an aggressive and rare malignancy with poor prognosis and limited treatment options. These biphasic tumors, consisting of epithelial and mesenchymal components, are hypothesized to evolve from less aggressive endometrial adenocarcinomas (EACs) through epithelial-mesenchymal transition (EMT). EMT is a reversible process, and mesenchymal-epithelial transition (MET) has been shown to decrease tumor aggressiveness. Inducing MET has been suggested for treatment of cancers with a mesenchymal phenotype. In our studies, we investigated the importance of MET in the evolution of UCS by depleting miR-200, a family of microRNAs critical for EMT, in EAC cell lines. We also explored the role of miR-200 overexpression as a driving force for MET in UCS with a focus on finding novel therapeutic approaches to the treatment of this aggressive disease. Experimental procedures: To test whether UCS cells evolve from EACs, we depleted miR-200c in EAC cell lines, Ishikawa and MFE-280. For the MET studies, we stably overexpressed miR-200c in UCS cell lines, SNU685 and JHUCS1. Gene expression was measured using TaqMan and whole transcriptome sequencing (RNA-Seq) assays. Immunoblotting was performed on the EMT-relevant proteins. Cell adhesion and in vitro cell proliferation were measured using commercially available assays. In vivo tumor growth of JHUCS1 miR-200c-overexpressed cells was measured in xenografted mice. Summary of the data: Compared to EAC cells, UCS cells had undetectable miR-200c expression. Depletion of miR-200c in EAC cells resulted in expected increased ZEB1 and decreased E-cadherin expression. The lack of increased N-cadherin, vimentin and morphologic changes, even in the presence of exogenous TGF-β suggests partial EMT induction. Silencing of miR-200c in UCS cells resulted in full MET, with a decrease in ZEB1, ZEB2, N-cadherin and vimentin and an increase in E-cadherin. Increased cellular adhesion was observed along with typical MET morphologic changes. miR-200c overexpression led to inhibited UCS cell proliferation and metabolic activity. Overexpression of miR-200c in vivo resulted in substantially smaller tumors compared to mice bearing control UCS cells. Conclusions: Our data suggest that mechanisms additional to, or other than EMT, are necessary for the evolution of UCS from EAC. UCS cell lines, however, readily undergo robust MET in the setting of increased miR-200c expression rendering them less aggressive. These findings suggest that miR200 overexpression through advanced microRNA therapeutics may lead to new options for the treatment of uterine carcinomas.

#3423 Elucidation of the role of miR-575 on tumorigenesis in glioblastoma. Ashley N. Gray,1 Tianian Cui,1 Marjolein Geurts,2 Pierre Robe,1 Joseph McElroy,1 Erica Hlavin Bell,1 Arnab Chakravarti1.1The Ohio State University, Columbus, OH; 2University Medical Center of Utrecht, Netherlands.

Glioblastoma (GBM) patients currently face poor survival outcomes with an average survival rate less than 15 months, with only 3-5% of patients survive more than 36 months. Although the mechanisms of tumorigenesis are still being elucidated, miRNAs are promising candidates to explore as novel and prognostic biomarkers in GBM. MiRNAs are small regulatory molecules that play a crucial role in carcinogenesis via repression of oncogenes and tumor-suppressor genes either transcriptionally or post-transcriptionally. Our clinical data (n=268) shows that miR-575 is one of the top miRNAs associated with GBM overall survival (FDR: 0.0036, p-value: 5.77E-05) by univariate analysis (Nano-String Technologies; Seattle, WA). This study was designed to investigate the expression and function of miR-575 in GBM. Basal expression of miR-575 was first detected in GBM cell lines prior to functional experimentation. Cell proliferation, colony formation, migration and invasion assays were performed to understand the role of miR-575 in GBM. We found that overexpression of miR-575 significantly increased cell proliferation and cell motility in LN229 and U251 cell lines. Additionally, BLID, a tumor-suppressor gene, was negatively regulated by miR-575 at the transcriptional level by qRT-PCR, which will be further investigated at the post-transcriptional level by western blot. Up-regulation of miR-575 in GBM cell lines suggests that it could be acting as an oncogene by targeting BLID. Our study suggests that miR-575 overexpression may lead to inhibited cell proliferation and cell motility in LN229 and U251 cell lines. These findings suggest that miR-575 overexpression may lead to inhibited cell proliferation and cell motility in LN229 and U251 cell lines. Additionally, BLID, a tumor-suppressor gene, was negatively regulated by miR-575, supporting our hypothesis and findings in other cancers. In conclusion, miR-575 might act as an oncogene in GBM, and BLID may be a putative target gene of miR-575. This mechanism could potentially be useful as a novel prognostic biomarker for GBM patients after further in vitro and in vivo validation. Funding: R01CA169368 (PI: Houghton; Co-I-Chakravarti); R01CA1152338 Multiple-PI R01: Chakravarti (PI); Xia (PI); R01CA1145128 Baroukhim (PI); Chakravarti (Co-PI) 7/2015-6/2020; R01CA108633 (PI:Chakravarti); 1R2CA141890 (Scientific PI: Chakravarti).

#3424 Role of miR-489 in mammalian gland development and Her2 mediated tumorigenesis. Yogin Patel, Mithil Soni, Shou Liu, Hexin Chen. Univ. of South Carolina, Columbia, SC.

HER2 overexpression is linked with poor prognosis and outcome in breast cancer. In our previous study, we found that miR-489 was specifically down regulated by HER2 overexpression. Restoration of miR-489 in multiple breast cancer cell lines significantly inhibited cell growth in vitro and decreased tumor growth in xenograft mice. To study role of miR-489 in Her2 mediated tumorigenesis, for the first time we generated MMTV-miR-489 transgenic mice, which overexpress miR-489 specifically in mammalian gland. Our qRT-PCR data has confirmed transgenic mice have significantly more miR-489 expression than FVB mice. We used western blot to further validate our model system and found significant downregulation of miR-489 targets DEK and PTTPN1. To find out whether miR-489 has any role in mammalian gland development, mammalian gland whole mount was performed from wild type FVB mice and MMTV-miR-489 mice at different age. Mammary gland from MMTV-miR-489 mice demonstrated reduction in growth in early age. Our immunohistochemistry staining demonstrated significantly reduced Ki-67 positive cells in MMTV-miR-489 mammalian gland at early age, further confirming reduced growth in MMTV-miR-489 mice. However, we found no significant effect on weight of litters of MMTV-miR-489 female since after 8-week mammalian gland was able to recover growth. To find out effect of miR-489 overexpression on Her2 mediated tumorigenesis, we generated double transgenic mice MMTV-Her2-miR-489 overexpressing miR-489 in MMTV-Her2 mice. We have observed significant delay in tumor onset and reduced tumor growth in MMTV-Her2/ miR-489 mice compared to MMTV-Her2 mice. We have also observed less number of metastatic site in lung by performing H and E staining of lung. Our IHC data showed reduction in PTTPN1 and DEK in miR-489 overexpress mammalian tumor. Surprisingly, we found significant reduction of miR-489 expression in mammary tumors of MMTV-Her2-miR-489 mice compared with normal mammary gland of same age of mice. Overall, our results indicated miR-489 overexpression suppresses mammalian gland development at early age, reduced mammalian tumorigenesis and decreased lung metastasis by targeting PTTPN1 and DEK.

#3425 MiR-215-5p is a tumor suppressor in colorectal cancer targeting EGFR ligand epiregulin and its transcriptional inducer HOXB9. Petra Fallejskova-Vychytilova,1 Jana Merhautova,1 Pablo Conesa-Zamora,2 Katerina Slaba,1 Tana Machackova,1 Marek Svoboda,1 Marek Vecera,1 Jitka Millova,1 Jaroslav Juracek,1 Jana Sana,1 Parwez Ahmad,1 Natalia Gabilo,1 Ondrej Slaby1,2.

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Growing evidence suggests that microRNAs (miRNAs) are involved in the development and progression of colorectal cancer (CRC). In the present study, deregulation and functioning of tumor suppressive miR-215-5p was evaluated in CRC. In total, 448 tumor tissues and 325 paired adjacent healthy tissues have been used for miRNA expression analyses. We proved that miR-215-5p is significantly downregulated in tumor tissues compared to non-tumor adjacent tissues and its decreased levels correlate with the presence of lymph node metastases, tumor stage, and shorter overall survival in CRC patients. To identify specific cellular processes affected by ectopic expression of miR-215-5p, a series of in vitro experiments have been performed using transient transfection of
miR-215-5p mimics into four CRC cell lines. Increased levels of miR-215-5p significantly reduced proliferation, clonogenicity, and migration of CRC cells, lead to cell cycle arrest in G2/M phase and p53-dependent induction of apoptosis. The ability of miR-215-5p to inhibit tumor growth was confirmed in vivo by use of NSG mice model and stable cell line over-expressing miR-215-5p. Finally, we proved that miR-215-5p inhibited caspase-8 and death receptor-mediated apoptosis in PanCa cells in response to TRAIL treatment as observed through Western blotting and flow cytometry.

Inhibition of MUC13 using shRNA knockdown or miR-145 restoration resulted in the TRAIL-mediated increased in apoptotic cell death as evidenced by Annexin V/7AAD and sub G0 population, as well as reduced PanCa cells sensitive to treatment with TRAIL, caspase-8, and death receptor inhibitors. Additional cells treated with TRAIL in combination with paclitaxel or Abraxane showed enhanced apoptosis on inhibition of miR-133 expression using miR-145 restoration. Further investigation showed that cytoplasmic domain of MUC13 (MUC13-CyD) is indispensable for blocking caspase-8 activation and PARP cleavage, indicating that the MUC13-CyD blocks TRAIL-induced signaling upstream to Bid by inhibiting caspase-8 activation. Conclusion: These observations suggest that MUC13 contributes to the survival advantage in PanCa cells in response to treatment with drugs or death inducing ligands such as, Tumor necrosis factor related apoptosis-inducing ligand (TRAIL) which can be strategically overcome by miR-145 replenishment. These findings indicate that MUC13 silencing sensitizes PanCa cells towards TRAIL therapy and counteracts chemoresistance mechanisms in PanCa that may lead to novel combination therapies for PanCa treatment.

Tamoxifen differentially regulates miR-29b-1 and miR-29a depending on endocrine sensitivity in breast cancer. Penn Mulundewy, Carolyn M. Klinge. University of Louisville School of Medicine, Louisville, KY.

Acquired endocrine-resistance occurs in ~ 40% of estrogen receptor α (ERα) + breast cancer patients after tamoxifen (TAM) or aromatase inhibitor therapy. MicroRNAs (miRs) are dysregulated in breast cancer, but their contribution to endocrine-resistance is not yet completely understood. Microarray analysis of miRNAs in TAM-sensitive MCF-7 versus TAM-resistant LY2 breast cancer cells (derived from MCF-7 cells) identified differential regulation of miR-29b-1 and miR-29a. miR-29b-1 and miR-29a-1, which are co-transcribed as a single primary-miR, were repressed by TAM in MCF-7 and stimulated in TAM in LY2 cells. We confirmed these observations by real time qPCR in MCF-7 and LY2 cells and observed that 4-OHT/TAM (4-OHT) also downregulated miR-29b-1/a in LCC2 and LCC9 endocrine-resistant cells. These changes were at the primary transcriptional level. The role of these two miRs in endocrine-resistant breast cancer is unknown. We tested the hypothesis that the TAM-stimulated increase in miR-29b-1/a promotes endocrine-resistance by downregulating targets that contribute to TAM’s antiproliferative/pro-apoptotic activity. Our goals were to: 1) Determine if ERα was responsible for TAM regulation of miR-29b-1/a expression; 2) Determine the functional role(s) of miR-29b-1/a; 3) Identify specific miR-29b-1/a target genes that are differentially regulated by 4-OHT in MCF-7 versus LCC9 and LY2 cells. Knockdown of ERα blocked 4-OHT inhibition of miR-29b-1/a in MCF-7 and 4-OHT simulation of miR-29-b-1/a transcription in LCC9 and LY2 cells. Transient over-expression of miR-29b-1/a stimulated caspase-8 activation and LY2 cell death and increased colony formation of LCC2 cells. Repression of miR-29b-1/a has no significant effect on MCF-7, LCC9, or LY2 cell proliferation. Repression of miR-29b-1/b did not sensitize the TAM-resistant cells to growth inhibition by 4-OHT. We observed that the bona fide miR-29 target gene, Dicer1, was inhibited by 4-OHT in LY2 cells and anti-miR-29b-1/2 or anti-miR-29a decreased the suppression of Dicer1 by 4-OHT. These data suggest that 4-OHT/ERα increases miR-29b-1/a which target and downregulate Dicer1 in endocrine resistant breast cancer cells. Future studies will identify other targets of miR-29b-1/a to better understand the pathways leading to acquired endocrine resistance.


Background: miR-23b has been identified as various cancer-related biomarkers. Interestingly, it works as an oncogenic miRNA in lymphoma, renal cancer and glioma, while it works as a tumor suppressor miRNA in pancreatic cancer, bladder cancer and prostate cancer (Donadelli M et al, Cancer Lett, 2014). We have previously reported miR-23b-3p as a oncomiR in NSCLCs (Begum S, Hayashi M et al, Sci Rep, 2015). To find the correlation to other carcinogenesis, we focused on miR-23b function in hepatocellular carcinoma (HCC). Methods: miR-23b-3p expression was...
examined in 9 HCC cell lines (SK-Hep1, HuH2, HLE, PLC/PRF/5, HuH7, HuH4, HLF, Hep3B, HepG2). The downstream of miR-23b-3p overexpression was examined by Cancer Pathway Finder (Qiagen). Results were applied to 125 clinical HCC samples (2002-2011 surgically resected). Results: Transfection experiments were performed for HepG2 (miR-23b-3p highly expressed) by siRNA and for SK-Hep1 (lowly expressed) by miRNA mimic. Cancer cell proliferation was activated by miR-23b-3p overexpression, and diminished by its inhibition. In order to find the associated genes, miR-23b-3p overexpressed SK-Hep1 cells were compared with parental SK-Hep1 cells by global gene expression analysis. ANGPI1, ERCC5 and G6PD genes were upregulated, while KDR, WEE1, OCLN genes were downregulated. We also detected additional two genes (AUH and MUC3) by TargetScan Release 5.2. Clinical HCC samples were divided into miR-23b-3p upregulated 48 cases (38%), and downregulated 77 cases (62%). Upregulated cases were correlated with aged patients (P=0.015), capsule invasion positive (P=0.039) and seminal invasion positive (P=0.049). Also, they showed significantly poor recurrence free survival (HR=1.64, P=0.037, 95%CI:1.03-2.59) and overall survival (HR=3.10, P=0.001, 95%CI:1.57-6.29) in multivariable analysis. Conclusion: miR-23b-3p increased the HCC cell proliferation, and indicated the inverse type of HCCs. It functions as a oncogenic biomarker in HCCs and might be a therapeutic target.

#3430 Deep sequencing-based miRNA expression signature in head and neck squamous cell carcinoma: dual strand of miR-150-150 acts as tumor suppressors. Keichi Koshizuka,1 Nijiro Nohata,2 Toyoyuki Hanazawa,1 Naoko Kikkawa,1 Ichiro Fukumoto,1 Takayuki Arai,1 Atsushi Okato,1 Yoshitaka Okamoto,1 Naohiko Seki1. 1Chiba University Graduate School of Medicine, Chiba, Japan; 2Moor Green Cancer Center, University California, CA.

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer in the world and ~500,000 cases are diagnosed every year. In spite of considerable advances in multimodality therapy, including surgery, radiotherapy and chemotherapy, the overall survival rate for patients with HNSCC is ~50%. Patients with HNSCC are usually diagnosed at a late stage and local tumor recurrence and distant metastasis occur after conventional therapies. Application of genomic approaches might elucidate novel molecular pathways underlying HNSCC and thereby improve therapeutic approaches to the disease. The discovery of noncoding RNAs (ncRNAs) in the human genome was an important conceptual breakthrough for cancer research in the post-genome sequencing era. Among ncRNAs, the microRNAs (miRNAs) are small ncRNA molecules (18-25 nucleotides in length) that regulate the expression of protein-coding/non-protein-coding genes by repressing translation or cleaving RNA transcripts in a sequence-specific manner. Accumulating evidence has demonstrated pivotal roles for miRNAs in human cancer pathogenesis. To seek out differentially expressed miRNAs in HNSCC cells, we newly constructed the deep-sequencing based miRNA expression signature by using laryngeal and hypopharyngeal clinical specimens. Our present data showed that a total of 169 miRNAs (44 upregulated and 116 downregulated) were identified as aberrantly expressed miRNAs in cancer tissues. Based on the signature, we focused on the dual strand of pre-miR-150, miR-150-5p and miR-150-3p in HNSCC signature because of these miRNAs significantly reduced in cancer tissues, suggesting these miRNAs act as antitumor miRNAs in this disease. However, the role of these miRNAs in HNSCC cells is still ambiguous. In miRNA biogenesis, it is the general consensus that processing of the pre-miRNA through Dicer1 generates a miRNA duplex (a passenger strand and a guide strand), and that the passenger strand has degradation and no regulatory activity and downregulates in cells. Our present data showed that both miRNAs, miR-150-5p and miR-150-3p significantly suppressed cancer cell migration and invasion. Moreover, gene expression data and in silico database analysis showed that Integrin A3 (ITGA3), Integrin A6 (ITGA6) and Tenasin C (TNC) were direct regulation of both miRNAs. A large number of cohort study by TCGA showed that overall survival of high expression of ITGA3, ITGA6 and TNC groups were significantly shorter than that of low expression of these expression groups (p = 0.0177, p = 0.0237 and p = 0.026, respectively). These target genes acted as oncogenes and deeply contribute to HNSCC pathogenesis. Dual strand of miR-150 functioned as tumor suppressors based on the miRNA expression signature of HNSCC. Identification of miRNA-mediated cancer networks may provide novel molecular pathogenesis of the disease.

#3431 The oncogenic role of miR-150-5p in triple-negative breast cancer. Bruna M. Sugita,1 Yara Zabala,2 Aline Fonseca,3 Rodrigo Almeida,1 Yuriy Gusev,4 Celia Rose,2 Bogdan Voevodin4, Igor I. Cavalli,1 Enelme M. Ribeiro,2 Luciane R. Cavalli1. 1Federal University of Parana, Curitiba, Brazil; 2Georgetown University, Washington, DC.

Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer that confers disease recurrence, treatment resistance and high mortality rates. MicroRNAs are a class of noncoding RNAs, that when dysregulated, impact tumorigenesis through the control of the expression of multiple mRNA targets involved in critical cancer signaling pathways. miR-150-5p has been shown to control the expression of several onco genes and/tumor suppressor genes involved in these critical pathways. Its pattern of expression varies among TNBC subtypes, and its role in the cancer stem cell (CSC) population is associated with hematological diseases and GI cancers, and upregulated in hormonal dependent cancers, such as prostate and breast cancer. The main objective of this study was to assess the patterns of expression of miR-150-5p in TNBC and determine its functional role in affecting the tumor phenotype. Archived paraffin samples of 113 patients with ductal breast carcinoma (56 of the TNBC and 57 of the non-TNBC subgroup) and adjacent normal tissue (ANT), obtained from the the pathology tumor bank of Lombardi Comprehensive Cancer Center, Washington DC, were profiled for miRNA using the wide-genome Nanostring platform and a Taqman specific miRNA-150-5p assay. Significant overexpression levels of miRNA-150-5p were observed in the tumor tissues when compared to the ANT and in the TNBC cases when compared to the non-TNBC cases, demonstrating its tumor and TNBC subtype specificity, respectively. Overexpressed levels of miRNA-150-5p were also preferentially observed in the TNBC cases from patients that presented with LN metastasis and breast cancer recurrence, indicating its association with poor prognosis. Interestingly, the TNBC of African-American patients, which is the ethnic group mostly affected by this cancer subtype, presented overexpression levels of this miRNA when compared to the non-TNBC of white patients. Functional analysis performed in the TNBC cell lines, MDA-MB-231 and HCC1806, showed after transfection with miR-150-5p inhibitor, reduced levels on cell proliferation, clonogenicity, migration, drug resistance and expression of the EMT promoter markers, SLUG and SNAIL. These findings, indicate an oncogenic type of action of miRNA-150-5p in TNBC. In summary, miRNA-150-5p is upregulated in TNBC clinical cases in association with poor prognostic parameters and its functional inhibition, directly confers to the cells a reduction of their tumorigenic phenotype. Funding: This project was supported by the GeorgeFromm University Center of Excellence in Regulatory Science and Innovation (CERSI U01FD004319), a collaborative effort between the university and the U.S. Food and Drug Administration to promote regulatory science through innovative research and education. This research does not necessarily reflect the views of the FDA. Scholarship to B.S. was provided by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

#3432 miRNA expression reflects site specificity of metastatic colorectal cancer. Bastian Fromm,1 Eirik Høye,1 Diana Domanskas,2 Christin Lund-Andersen,3 Anette Torgrunn Kristensen,3 Vegar J. Dagenborg,1 Paul H. Boettger,1 Torveig W. Abrahamssen,1 Steinar Solberg,1 Stein G. Larsen,1 Bjørn Edelbo,2 Per-Olov I. Malmgren,2 Kjersti Flatmark1. 1Oslo University Hospital - The Norwegian Radium Hospital, Oslo, Norway; 2University of Oslo, Oslo, Norway; 3PHMB Research Consulting, Austria; 4Oslo University Hospital - Rikshospitalet, Oslo, Norway.

Colorectal Cancer (CRC) is one of the leading causes of cancer related deaths in the western world, and metastatic progression is the dominating cause of mortality. The primary site of CRC metastasis is the liver, followed by the lungs and peritoneal cavity, and prognosis for patients with metastatic CRC (mCRC) is poor, with only 10% five-year survival. Although much is known about progression and metastasis of CRC; how primary CRC (pCRC) differs from mCRC on the molecular level and potential differences between metastases located in different organs are incompletely understood. This is important for our understanding of the disease, but also could have significant implications with respect to treatment. MicroRNAs have been shown to be key elements in cancer progression, but recent reports on CRC failed to consistently identify microRNA signatures of metastatic progression. We have addressed this using a smallRNA sequencing approach to analyze primary tumors and a set of liver, lung and peritoneal metastases, including corresponding adjacent tissue (colon, liver, lung, peritoneum). Further, we use the highly curated and updated microRNA reference MirGeneDB.org, the novel pipeline MirAthon - including a correction-algorithm of benign tissue microRNA signatures for tumor or metastatic samples, and we compare pCRC and site specific metastases separately. We are able to show that microRNA profiles of mCRC are distinct to pCRC in a site-specific manner. We identify a small panel of differentially expressed microRNAs including isoforms (isomiRs) hidden under a layer of benign signatures that we confirmed in other available datasets, too. Our findings will help better understand metastatic progression and site-specificity in CRC and could be potential biomarkers for mCRC. Our work identifies microRNA signatures of mCRC and suggest that microRNA signatures reflect mCRC site specificity.
Furthermore, the results underline the importance of using a curated reference for microRNA studies, and to differentiate metastases based on their localization ab initio.

#3433 Epigenetic silencing of miR-214-3p is associated with resistance to p53-induced apoptosis through up-regulation of gankyrin expression in hepato-cellular carcinoma. Koha Hitori, Yutaka Watanabe, Naoto Ishii, Kei Tarazaki, Yasuyuki Gen, Yoshito Itoh. Kyoto Prefectural University of Medicine, Kyoto, Japan.

Hepatocellular carcinoma (HCC) is the third leading cause of cancer death worldwide. MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression and can act as tumor suppressors or oncogenes. To identify miRNA involved in the development of HCC, a genome-wide miRNA gene expression analysis was performed using the human miRNA microarray (Array-Gene) in paired HCC tumors and non-tumorous liver tissues from patients with primary HCC. The array-based miRNA expression profiles were validated by quantitative PCR. We also screened for genes with promoter DNA hypermethylation using a genome-wide DNA methylation microarray analysis (the Illumina HumanMethylation27 BeadChip) in the same samples. We found that miR-214-3p was significantly down-regulated by aberrant promoter hypermethylation in HCC tumors compared to the non-tumorous liver tissues. We identified PSMD10, which encodes gankyrin, as a direct target of miR-214-3p using luciferase assay and immunoblotting. Gankyrin, an oncoprotein commonly overexpressed in HCC, binds to MDM2, a major E3 ubiquitin ligure for p53, and increases ubiquitlization and degradation of p53. Our experiments showed that reduced miR-214-3p expression was associated with resistance to p53-induced apoptosis through up-regulation of gankyrin expression in HCC cells. In conclusion, our results suggest that epigenetic silencing of tumor suppressor miR-214-3p is involved in hepatocarcinogenesis through up-regulation of gankyrin expression.

#3434 Loss of β-Catenin expression by miR-214 and oncogenic role of miR-214 in metastatic melanoma cells. Kimhna Prabhakar, Ashika Jayanthy, Vijay Setaluri. University of Wisconsin, Madison, WI.

Disregulation of β-catenin facilitates melanoma metastasis by activating the transcription of oncogenes like Myc, CyclinD1 and TCF-1. It is important to understand the regulation of β-catenin to develop strategies to target Wnt signaling for melanoma treatment. MicroRNAs are small non-coding RNAs that are primarily involved in post-transcriptional regulation of target genes. Hence, miRNAs can be used to manipulate protein levels by causing instability of the transcript or by inhibiting translation. MiR-214 has been documented as regulator of β-catenin in cancer cells other than melanoma cells. The role of miR-214 in regulation of β-catenin has not been investigated. In this study, we investigated the regulation of β-catenin by miR-214 in melanoma cell lines and studied the effect of overexpression miR-214 on the malignant phenotype of metastatic melanoma cells and their sensitivity to MAP kinase inhibitors. Our data show that β-catenin is upregulated in a panel of metastatic melanoma cells and the expression levels of β-catenin is negatively correlated with endogenous expression of miR-214. However, overexpression of miR-214 by transfection with a miRNA mimic did not show significant decrease in β-catenin mRNA or protein expression. RNA-Seq showed that multiple isoforms of β-catenin transcript are present in melanoma where the binding site is absent or mutated. The alternatively spliced transcript with no binding site for miR-214 is seen to be more abundant than the other isoforms. Paradoxically, overexpression of miR-214 increased survival, proliferation and migration of metastatic melanoma. In addition, miR-214 overexpression decreased their sensitivity to vemurafenib and selumetinib. In melanoma cells with acquired resistance (in vitro), miR-214 overexpression further enhanced their resistance to MEK inhibitor, but not vemurafenib. In light of the published studies that proposed that miR-214 acts as tumor suppressor by negatively regulating β-catenin, our study is interesting since we find that in metastatic melanoma miR-214 has oncogenic role. Ongoing studies are aimed at understanding the mechanisms by which miR-214 promotes malignant behavior of melanoma cells.

#3435 Profiling miRNAs in breast epithelial progenitor cells during branching morphogenesis and EMT in 3D culture. Eirikur Brien,1 Bylgia Hilmarsdottir,2 Zuzana Budkova,1 Saevar Ingthorsson,1 Magnus K. Magnusson,1 Thorarinn Gudjonsson1.1University of Iceland, Reykjavik, Iceland;2Oslo University Hospital, Oslo, Norway.

In this study we investigate the role of miRNA-203a in branching morphogenesis and epithelial to mesenchymal transition (EMT) in breast cell lines. D492 is a breast epithelial cell line with stem cell properties. D492 generates luminal- and myoepithelial cells and in 3D culture forms structures reminiscent of terminal duct lobular units of the breast. When cocultured with endothelial cells subset of D492 cells undergo EMT as evidenced by spindle shape morphology, E- to N cadherin switch and loss of keratin expression. We have established an endothelial-induced mesenchymal subline from D492 referred to as D492M. Here we use D492 and D492M as a model to investigate miRNA expression patterns during different stages of branching and in EMT. We performed small RNA sequencing on D492 and D492M in 3D culture to pinpoint miRNAs that are associated with epithelial integrity and suppressing EMT. We have recently shown that miRNA-200c-141 convert D492M back to the epithelial phenotype (Hilmarsdottir et al. G&D 2015). MiRNA-203a is also down-regulated in D492M. Here we overexpress miRNA-203a in D492 and D492M. Methylation analysis reveal that the promoter area of miRNA-203a is not methylated in D492M. We showed that knockdown of miRNA-203a in D492M cells change phenotype from elongate, mesenchymal-like phenotype to more cuboidal epithelioid phenotype, show reduced cell proliferation, less ability to form colonies in low attachment cultures, less ability to migrate and reduced invasion capability. When D492MmiRNA-203a is co-cultured with endothelial cells in 3D culture they partially regain the ability to form branching structures similar to D492. D492M(miRNA-203a) show reduced expression of EMT transcription factors SNAIL2 and ZEB1. When cultured on transwell filters, D492M forms stratified squamous epithelial layer while D492M(miRNA-203a) form single squamous layer. In conclusion, miRNA-203a induces partial epithelial phenotype in D492M as evidenced by morphology and marker expression. We also conclude that three dimensional cultures of D492 and D492M work well as a model system to investigate miRNA expression during branching morphogenesis and in EMT.

#3436 MiR-194 modulates paclitaxel resistance in ovarian cancer cells through the regulation of MDM-2 expression. Koji Nakamura,1 Kenjiro Sawada,2 Akihiko Yoshimura,3 Erica Nakatsuka,4 Yusato Kinose,5 Seiji Mabuchi,6 Tadashi Kimura.7 Saiseikai Nakatsu Hospital, Osaka-shi Osaka, Japan; Osaka University, Suita-shi Osaka, Japan; Penn Ovarian Cancer Research Center, Philadelphia, PA.

Purpose: Ovarian cancer is the most lethal gynecologic malignancy. Platinum-paclitaxel combination chemotherapy is the current standard postoperative care for patients with advanced ovarian cancer. Despite developments in current chemotherapy regimens in the last decades, overall 5-year survival rate for patients with advanced disease remains approximately 30%, mainly due to primary or acquired drug resistance. Therefore, overcoming chemoresistance remains the great challenge in ovarian cancer management. However, mechanisms underlying chemoresistance to paclitaxel have not been fully understood. The aim of this study is to identify key miRNAs which regulate paclitaxel resistance in ovarian cancer and to pursue those potential as therapeutic targets. Methods: Using two serous ovarian cancer cell lines, SKOV3ip1 and HeyA8, paclitaxel-resistant ovarian cancer cell lines were established by a continuous exposure of paclitaxel. MiRNA PCR arrays were performed and miR-194 was found to be one of down-regulated miRNAs in paclitaxel-resistant cell lines. The effect of miR-194 on paclitaxel resistance was assessed by transducing the precursor miRNA into ovarian cancer cells. In silico analysis revealed MDM-2 is one possible putative target of miR-194 and it was assessed using luciferase reporter gene and transfection MDM-2 were performed by a continuous exposure of paclitaxel. MiRNA PCR arrays were performed and miR-194 was found to be one of down-regulated miRNAs in paclitaxel-resistant cell lines. The effect of miR-194 on cell cycle in ovarian cancer cell was assessed. To analyze the impact of miR-194 and MDM-2 expression on patient survival, Kaplan-Meier and log-rank methods were used using The Cancer Genome Atlas (TCGA) dataset. Results: While IC50 values of SKOV3ip1 and HeyA8 were 5.0 nM and 3.5 nM respectively, those of paclitaxel resistant cell lines, named as SKOV3ip1-TR and HeyA8-TR, were both over 300 nM. In both resistant cell lines, miR-194 was found to be down-regulated compared with their parental cell lines. Up-regulation of miR-194 sensitized resistant cells to paclitaxel. Conversely, its downregulation induced paclitaxel resistance in parental cells. Luciferase reporter assay revealed that miR-194 directly suppressed MDM-2 transcript. In resistant cell lines, MDM-2 was up-regulated compared with their parental cell lines. MiR-194 induced P21 upregulation and G1 phase arrest in resistant cell lines through the downregulation of MDM-2. In TCGA dataset, high MDM-2 expression was associated with shorter progression-free survival of ovarian cancer patients (concrete data, p=0.046). Conclusion: miR-194 acts as a tumor suppressor miRNA through the sensitization to paclitaxel and can be considered as a therapeutic option to overcome paclitaxel-resistant in ovarian cancer patients.

#3437 Targeting microRNA-143 in glioblastoma in vivo increases tumor growth. Eunice Lozada-Delgado1, Fatma Valiyeva,2 Maria Marcos,3 Pablo Vivas4.1University of Puerto Rico, San Juan, PR;2University of Puerto Rico Comprehensive Cancer Center, San Juan, PR.

In this study we investigate the role of miRNA-203a in branching morphogenesis and epithelial to mesenchymal transition (EMT) in breast cell lines. D492 is a breast epithelial cell line with stem cell properties. D492 generates luminal- and myoepithelial cells and in 3D culture forms structures reminiscent of terminal duct lobular units of the breast. When cocultured with endothelial...
The purpose of this study is to assess the biological role of the microRNA-143 (miR-143) in Glioblastoma multiforme (GBM). GBM is the most common and lethal of all brain tumors. In the United States, the incidence of GBM is about 17% of all primary brain tumors and about 60-75% of all Astrocytomas (American Brain Tumor Association, 2014). The standard therapy is surgical resection followed by chemotherapy and radiotherapy. Despite this approach, GBM recurs after treatment and the median survival rate for GBM has remained 15 months for the past 20 years. Thus, novel therapies for GBM treatment are urgently necessary. MicroRNAs (miRNAs) are a class of small non-coding RNAs (18-22 nucleotides in length) that regulate gene expression at the post-transcriptional level. MiRNAs bind to the 3′-untranslated region (UTR) of messenger RNA (mRNA) to regulate protein production. Several deregulated miRNAs have been identified in all cancer types including GBM. In this study we aim to thoroughly uncover the role of miR-143 by using GBM cell lines, mouse models and patient samples. Total RNA was isolated from FFPE samples from brain tumor patients. TaqMan-based Real-time PCR showed that the relative expression of miR-143 was higher in GBM patients compared to control individuals, and with paired normal non-tumorous tissue. Furthermore, GBM cells transiently transfected with a miR-143 oligonucleotide inhibitor showed reduced cell proliferation (68.5%) (clonogenic assay), increased apoptosis and cell cycle arrest of GBM cells in the S phase (Flow cytometry and Western blots).

In vivo studies using primary GBM cells injected in the flank of nude mice showed that repeated doses of miR-143-inhibitor liposomal formulation increased the temperature of the tumor compared with the control group. These results could be due to effects of the microenvironment where the tumor is growing. Further studies will be made using intracranial injections in an orthotopic xenograft mouse model to confirm this hypothesis. Western blot analysis and luciferase reporter assays are also underway to identify novel miR-143 target genes in GBM cells. This research project is being supported by: PRCTRC: NCRR (U54 RR026139-01A1), NIMHD (8U54 MD007587-03), and RCMI: MBRSC-RISE, NCR (2G12-RR003051) and NIMHD (8G12-MD007690) from the NIH.

**#3438** Dual-strands pre-microRNA-150 (miR-150-5p and miR-150-3p) act as tumor suppressor in prostate cancer. Atsushi Okato, Takayuki Arai, Akira Kurozumi, Mayuko Kato, Yusuke Goto, Keiichi Koshizuka, Satoko Kojima, Tomohiko Ichikawa, Naohiko Seki. Chiba University, Chiba-shi, Japan; Tei-kyo University Chiba Medical Center, Chiba-shi, Japan.

Background: Prostate cancer (PCa) is the second most common cause of cancer and the sixth leading cause of cancer death among men in the world. Androgen signaling through the androgen receptor (AR) is an important oncogenic pathway for PCa progression. The initial response rate of PCa to androgen deprivation therapy (ADT) can be up to 80%, but most patients experience disease relapse and progress to castration-resistant prostate cancer (CRPC). Although several clinical trials, such as molecules with anti-androgenic properties, have been carried out, these treatments provide limited benefits and are not curative. Therefore, identification of effective biomarkers for detection of CRPC and understanding the molecular mechanisms of androgen-independent signaling and metastatic signaling pathways underlying PCa using current genomic approaches would help to improve therapies for and prevention of the disease.

Currently, numerous studies have indicated that miRNAs are aberrantly expressed in several cancers, including CRPC. In this study, we constructed a miRNA expression signature to identify miRNA regulated RNA networks in CRPC using autopsy specimens from patients with ADT. Based on the signature, dual-strands of pre-miR-150 (miR-150-5p and miR-150-3p) were significantly reduced in CRPC specimens. The aim of this study was to investigate the functional significance of both strands of miR-150-5p and miR-150-3p and these miRNAs regulated RNA networks in CRPC. Results: Downregulation of miR-150-5p and miR-150-3p were validated in hormone naive PCa and CRPC specimens compared to non-cancerous prostate tissues (p < 0.0001). Restoration of miR-150-5p and miR-150-3p significantly suppressed cancer cell migration and invasion in PCa cell lines (P < 0.0001). Gene expression data and in silico database analysis showed that Sperm/OSTIN, Gw254 AND Kazal-like Domain Proteoglycan 1 (SPOCK1) was regulated by both miRNAs. Knockdown of SPOCK1 inhibited cancer cell aggressiveness. Overexpression of SPOCK1 was observed in PCa clinical specimens. Conclusions: In miRNA biogenesis, it is the general consensus that processing of the pre-miRNA through Dicer1 generates a miRNA duplex (a passenger strand and a guide strand), and that the passenger strand has degradation and no regulatory activity and disintegrates in cells. Our present data showed that both strands of pre-miR-150 (miR-150-5p and miR-150-3p) inhibited cancer cell aggressiveness, suggesting these miRNAs as tumor-suppressors. Identification of miRNA-mediated cancer networks may provide novel molecular pathogenesis of the disease.
#3441 A novel IncRNA-mediated YAP1 signaling pathway activation in epithelial ovarian cancer. Xianzhi Liu, Rosario I. Corona, Simon A. Gayther, Dennis L. Hazelett, Kate Lawrenson. Cedars-Sinai Medical Center, Los Angeles, CA.

Background: The long non-coding RNA (lncRNA) uterine thoracic cancer associ- ated 1 (UTCA1) was identified in many cancer tissues including ovarian cancer. In high grade serous ovarian cancers (HGSOCs), we had previously found that UC1A expression negatively correlates with patients' total survival and disease-free survival and that UC1A knockout significantly impairs tumor growth in vivo. In addition, UC1A promotes ovarian cancer cell growth via activating the YAP1 signaling pathway. Purpose: Our present study aims to dissect the molec- ular mechanism of UC1A-mediated YAP1 signaling activation. Methods: To characterize the UC1A interactome, we utilized a modified in vivo RNA anti- sense purification method (UC1A-iRAP). This UC1A-iRAP method uses streptavidin-coupled magnetic beads to pull down biotin-labeled antisense probes which are complementary to the target RNA. In such a way, UC1A RNA will be specifically enriched together with its in vivo-associated proteins, RNAs and/or genomic DNA, which can be profiled using mass spectrometry (MS) and next generation sequencing (NGS), respectively. Results: Using UC1A-iRAP RNA-Seq and UC1A-iRAP protein MS, we comprehensively annotated the UC1A-interacting RNAs as well as UC1A-associated proteins. Interestingly many of the proteins identified were not known to have RNA-binding potential. Integration of NGS and MS data with multifaceted functional and molecular characterization revealed that knockdown of UC1A-mediated YAP1 signaling via UC1A-iRAP method, the interaction between UC1A and this YAP1 modulator leads to activation of YAP1 signaling by nuclear translocation of YAP1. In summary, we characterize a novel UC1A- YAP1 signaling axis active in HGSOCs and also reveal that oncosgenic IncRNA's binding potential of cytoplasmic signaling proteins could be a common mechanism of post-translational control of signaling pathways deregulated in cancer.

#3442 The long non-coding RNA GHSROS mediates expression of genes associated with tumor growth, metastasis and adverse disease outcome. Patrick B. Thomas,1 Penny L. Jeffery,1 Eliza Whiteside,2 Carina Walpole,2 Michelle Maughan,3 Lidija Jovancovic,1 Jifer H. Gunter,3 Colleen C. Nelson,1 Adrian C. Hertingon,4 Rakesh Veedu,5 Lisa K. Chopin,1 Inge Seim1.

1The University of Queensland, Brisbane, Australia; 2University of Southern Queensland, Toowoomba, Australia; 3Murdoch University, Perth, Australia.

Objectives: To investigate the expression and function of the lncRNA GHSROS in prostate cancer. Methods: Using UC1A-iRAP RNA-Seq and UC1A-iRAP protein MS, we comprehensively annotated the UC1A-interacting RNAs as well as UC1A-associated proteins. Interestingly many of the proteins identified were not known to have RNA-binding potential. Integration of NGS and MS data with multifaceted functional and molecular characterization revealed that knockdown of UC1A-mediated YAP1 signaling via UC1A-iRAP method, the interaction between UC1A and this YAP1 modulator leads to activation of YAP1 signaling by nuclear translocation of YAP1. In summary, we characterize a novel UC1A- YAP1 signaling axis active in HGSOCs and also reveal that oncosgenic IncRNA's binding potential of cytoplasmic signaling proteins could be a common mechanism of post-translational control of signaling pathways deregulated in cancer.

#3443 Functional and mechanistic characterization of oncogenic long inter- vening non-coding RNA Linc-CHD1L-1 in hepatocellular carcinoma. Fe- lice H. Tsang, Daniel W. Ho, Irene O. Ng, Jack C. Wong. The University of Hong Kong, Hong Kong, Hong Kong.

Hepatocellular carcinoma (HCC) is one of the most common and lethal can- cers worldwide. Due to the asymptomatic nature of HCC, many patients were diagnosed at late stages when cure rates are very low. Therefore, a better understanding of the molecular mechanism underlying hepatocarcinogenesis is urgently needed and is crucial for the development of effective treatment. Long intervening non-coding RNAs (lincRNAs) are emerging class of regulatory RNA involved in diverse molecular processes. In this study, we aimed to interrogate lincRNAs expression in HCC and identify novel lincRNAs that contribute to hepatocarcinogenesis. To investigate the global expression profile of lincRNAs in human HCCs, next-generation transcriptome sequencing (RNA-Seq) was performed in 16 pairs of HBV-associated primary HCCs and their corresponding non-tumorous liver tissues. Interestingly, the lincRNA expressions in human HCCs can be unambiguously segregated from those of HBV-negative HCCs using lincRNA expression signatures. Suggesting that global lincRNA expression profiles between tumors and non-tumors were remarkably different. From this analysis, we identified linc-CHD1L-1 as the most significant up-regulated lincRNA in HCCs. Clinically, linc-CHD1L-1 was overexpressed in over 80% of HCC patients. Linc-CHD1L-1 expression level in tumors was also shown to be a promising marker for HCC diagnosis. Furthermore, knockdown of linc-CHD1L-1 by shRNAs has significantly re- duced HCC cell proliferation and cell migration in vitro. Besides, knockdown of linc-CHD1L-1 in HCC cells also substantially abrogated tumorigenicity in subcutaneous tumor model. Mechanistically, the expression of linc-CHD1L-1 was significantly and positively correlated to the expression of its neighboring genes located on the chromosome 1q4. Knockout of linc-CHD1L-1 by CRISPR/Cas9 also significantly repressed its neighboring genes expression, suggesting that linc-CHD1L-1 may positively regulate gene expression in a cis-manner. Finally, DAVID pathway analysis performed on linc-CHD1L co-expressed protein coding genes further suggested that linc-CHD1L-1 is significantly co-expressed with genes that are involved in transcription and chromatin modifications. In conclusion, overexpression of linc-CHD1L-1 contributes to hepatocarcinogenesis by promoting HCC cell proliferation and migration through transcriptional and epigenetic mechanisms.

#3444 LINC00152 regulates cell proliferation via p38 signaling and over- expression predicts poor patient survival in lung adenocarcinoma. Wei Zhang, Wenniei Su, Shengbin Bai, Lei Xiao, Zhuen Wang, Jules Lin, Rishindra Reddy, Andrew Chang, David Beer, Guoan Chen. Univ. of Michigan Medical School, Ann Arbor, MI.

Lung cancer is a molecularly-heterogenous disease and the leading cause of cancer mortality. The molecular basis for this heterogeneity remains incom- pletely understood. In the past few years, long non-coding RNAs (lncRNAs) have emerged as novel mechanisms in mediating cancer biology, although most lincRNAs are still undiscovered. LINC00152 has been identified as highly associated with the tumorigenesis and development in gastric cancer, colon cancer and hepatocellular carcinoma, however, the expression level and its oncogenic roles in lung cancer remains unknown. In the present study, we employed next generation RNA sequencing analysis to reveal dysregulated lincRNAs in lung cancer utilizing datasets of 461 lung adenocarcinomas (LUAD) and 156 normal lung tissues from 3 separate institutions. We found that LINC00152 was 4-fold (p < 0.001) overexpressed in lung tumors as compared to their adjacent normal tissues with AUC > 0.8 in these 3 data sets. Patients with high LINC00152 expression have significantly poorer survival than those with low expression (log-rank test, p = 0.003). We verified this diagnostic/prognostic potential in an independent cohort of lung tumors by quantitative RT-PCR. Cell proliferation and colony formation ability were decreased after knockdown of LINC00152 using siRNAs in lung cancer cell lines. The expression of LINC00152 was found primarily in the cytoplasm by qRT-PCR analysis. Trichostatin A treatment indicated that histone acetylation could be one of the mech- anisms underlying LINC00152 overexpression in NSCLC and cell-based analysis showed p38 signaling was mainly affected by LINC00152 in vitro. Taken together, this study suggests that LINC00152 is involved in lung tumor growth, may have potential as diagnostic/prognostic marker and that further characterization of this lincRNA as a novel therapeutic target for lung cancer is warranted.

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: MicroRNAs and Other Noncoding RNAs as Tumor Suppressors or Oncogenes 2
Introduction: Glioblastoma multiforme (GBM) is the most frequent primary brain tumor of astrocytic origin characterized by very poor prognosis. Despite conventional therapeutic protocol the prognosis of GBM patients is very poor with median of overall survival ranging between 12 and 15 months from diagnosis. Therefore, many financial charges and lot of effort is spent in research of new therapeutic agents that could improve survival of patient. Long non-coding RNAs (lncRNAs) are a relatively new class of noncoding gene regulators playing critical roles in tumor biology, including GBM. From this perspective, lncRNAs seem to be promising therapeutic targets in GBM patients.

Material and Methods: We performed next-generation sequencing analysis of fresh-frozen histopathologically confirmed 45 GBM tissues and 5 non-tumor brain tissues obtained from non-dominant anterior temporal cortices resected during surgery for intractable epilepsy. Informed consent approved by the local Ethical Committee was obtained from each patient before the treatment. RNA depletion and cDNA library preparation were performed with GeneRead mRNA Depletion Kit (Qiagen) and NEXTflex Rapid Directional mRNA-Seq Kit (Boo Scientific), respectively. Sequencing was performed with NextSeq 500 High Output Kit and NextSeq 500 System (both Illumina). Statistical analysis was conducted using 24,087 protein-coding and 8,414 non-coding RNAs and their sequential variants with non-zero RPMK (Reads Per Kilobase of transcript per Million mapped reads) at least in one sample. We used CLC genomic workbench for the alignment and target counts. Targeted regulation of ZFAS1 level have been carried out by the transient transfection of specific siRNA in GBM stable cell lines (A172, T98G, U87MG, U251). Viability and migration were analyzed in vitro using MTT and scratch wound healing assay, respectively.

Results: Statistical analysis has revealed 274 (P < 0.01) deregulated lncRNAs in GBMs in comparison with non-tumor brain samples. Moreover, the results have shown also 489 deregulated protein-coding RNAs with P value less than 0.001 and 26 protein-coding RNAs with P value less than 0.000001. For subsequent in vitro functional analyses was chosen one of the most upregulated lncRNAs in GBM samples ZFAS1. Targeted downregulation of this molecule led to the significant reduction of viability in all examined GBM cell lines. Decreasing of proliferation potential was observed only in A172 a U251 cell lines. Conclusion: We have demonstrated a deregulation of many lncRNAs and protein-coding RNAs in GBM tissue in comparison with non-tumor brain tissue. Moreover, ZFAS1, one of the most upregulated lncRNAs in GBM tissue, is involved in regulation of viability and migration of GBM cells in vitro. This work was supported by Ministry of Health of the Czech Republic, grant nr. 15-33158A, 15-34553A, 15-31627A, 15-34678A, 16-31314A, 16-31765A and by grant of Czech Grant Agency nr. 16-18257S.

#3445 Melatonin suppresses hepatocellular carcinoma progression via lncRNA-CPS1-IT1-mediated HIF-1α inactivation. Tong Hong Wang, Chi Hao Wu, Chau Ting Yeh, Kung Hao Liang, Chuen Hsueh, Chi Yuan Chen, Hao Sun, Chi Chiu Wang, Joseph Kwong, Huating Wang, Tony Kwok. The Chinese University of Hong Kong, Hong Kong, Hong Kong.

Background: Melatonin as a promising therapeutic agent for the treatment of HCC. The inactivation of HIF-1α resulted in the suppression of epithelial-mesenchymal transition (EMT) progression and HCC metastasis. Furthermore, the results of the in vivo animal model experiments also validated the tumor-suppressor role of melatonin via a reduction in tumor growth. Conclusions: Taken together, our findings suggest that melatonin suppresses HCC progression by modulating lncRNA-CPS1-IT1-mediated EMT suppression and further supported melatonin as a promising therapeutic agent for the treatment of HCC.

#3447 Down-regulation of long non-coding RNA MALL-2 is associated with metastasis and poor survival in lung adenocarcinoma. Ha X. Dang, Nicole M. White, Emily B. Rozyczki, Christopher A. Maher. Washington Univ. School of Medicine, St. Louis, MO.

Lung adenocarcinoma accounts for ~40% of lung cancers and is among the deadliest cancers worldwide. Patients with metastatic lung adenocarcinoma exhibit poor outcome with the average 5-year survival of ~1%. Long non-coding RNAs (lncRNAs) have recently emerged as having critical roles in tumorigenesis. While a subset of lncRNAs have been shown to promote tumor invasion and metastasis, little is known about the role of lncRNAs in metastatic lung adenocarcinoma. Therefore, we analyzed publicly available microarray and RNA-Seq gene expression datasets that include primary and metastatic tissues. Through this meta-analysis we discovered 98 metastasis associated lncRNAs that were deregulated in metastatic tumors compared to non-metastatic tumors (MALLs). Notably, only two lncRNAs (MALL-2 and MALL-34) were found to be down-regulated in primary tumors, relative to normal tissue, and even further down-regulated in metastatic tumors relative to primary tumors. Gene set enrichment analysis revealed that MALL-2, the most down-regulated lncRNA in metastasis, was coexpressed with protein-coding genes enriched with biological concepts associated with repressing tumor growth and metastasis. Furthermore, we found that FOXA2, a metastasis repressor gene, was highly coexpressed with MALL-2 in lung cancer. A pan-cancer analysis using ~7,000 TCGA RNA-Seq tumor samples across 20 cancer types revealed that MALL-2 was also down-regulated and coexpressed with FOXA2 in multiple cancers suggesting the conserved regulatory relationship between the two genes. We confirmed that FOXA2 expression was decreased upon knock-down of MALL-2 and silencing MALL-2 resulted in a significant increase in cellular migration in lung adenocarcinoma cell lines. Last, we found that low expression of MALL-2 was consistently associated with poor overall survival across multiple independent lung adenocarcinoma cohorts. Taken together, our integrative analysis has revealed MALL-2 that acts as a repressor of lung adenocarcinoma metastasis by regulating FOXA2 and could serve as a biomarker of lung adenocarcinoma patient outcome.

#3448 LncRNA HAND2-AS1 inactivates neurofibromin U (NMU) and inhibits tumor invasion and metastasis in endometrioid endometrial carcinoma. Xuying Yang, Yu Zhao, Kun Sun, Yuying Li, Jiajian Zhou, Jianzhang Wang, Hao Sun, Chi Chiu Wang, Joseph Kwong, Huating Wang, Tony Kwok Hung Chung. The Chinese University of Hong Kong, Hong Kong, Hong Kong.

Background: Endometrioid endometrial carcinoma (EEC) is one of the commonest histological subtype of endometrial cancer with high mortality in women. Despite progress in diagnostics and treatment of EEC, its prognosis remains poor. Mounting evidence suggest that long noncoding RNAs (lncRNAs) function in multiple human cancers. Aberrant lncRNA expression may predict tumor outcome of patients and have served as diagnostic or prognostic markers. In this study, we investigated the expression levels and functions of lncRNAs in EEC. Methods: Differentially expressed lncRNAs involved in EEC were identified by using publically available RNA-Seq data. The expression of 18 dysregulated lncRNA candidates was verified in 5 NE tissues, 5 EEC tissues and 5 EEC cell lines (HEC1-A, HEC1-B, AN3CA, KLE and RL95-2) by real-time polymerase chain reaction (PCR). Further, we selected the most misexpressed lncRNA and confirmed the expression level of the lncRNA in 59 EEC tissues and 24 NE tissues by real-time PCR and correlated the lncRNA expression levels with the clinical pathological characteristics. The promoter methylation assay was used to analyze the methylation level of the lncRNA in EEC. The lncRNA methylation status was confirmed by bisulfite genomic sequencing. Cell proliferation assays, wound healing assays, and invasion and migration assays were performed to determine the biological functions of the lncRNA in EEC cells. To discover the direct targets of the lncRNA in EEC, we performed RNA-sequence analysis in EEC cells overexpressed with the lncRNA and target genes were further studied by functional studies in vitro (knockdown assay and overexpression rescue assay). Results: We discovered that HAND2-AS1, a lncRNA transcribed antisense adjacent to
Heart and Neural Crest Derivatives Expressed 2 (HAND2), was significantly downregulated IncRNA in EEC. HAND2-AS1 and HAND2 were frequently downregulated in EEC tissues, especially in poor differentiated tumor tissues. Downregulation of HAND2-AS1 and HAND2 was correlated with tumor grade, lymph node metastasis and recurrence of EEC patients. HAND2-AS1 and HAND2 were downregulated by promoter hypermethylation in EEC. HAND2-AS1 suppressed EEC cell migration and invasion but not cell growth. Similarly, HAND2 also inhibited EEC cell migration and invasion indicating that HAND2-AS1 and HAND2 have a concordant role in the progression of EEC. Moreover, the anti-tumorigenic effect of HAND2-AS1 was mediated by downregulating NMU, which had an oncogenic role in EEC. Conclusion: Our findings provide the first evidence that HAND2-AS1 is a critical tumor suppressor in EEC and may constitute a prognostic biomarker in EEC.


Genistein, a soy isoflavone, has been shown to have anti-tumor effects on various cancers in vitro and in vivo including renal cancer. Long non-coding RNAs (IncRNAs) are differentially expressed in various tissues and have important functions in cellular processes such as cell proliferation, motility and apoptosis in various malignancies. HOX transcript antisense RNA (HOXATIR) is an IncRNA localized in the Homeobox C gene cluster on chromosome 12. HOXATIR interacts with the polycomb repressive complex 2 (PRC2), which enhances H3K27 trimethylation and represses the expression of tumor suppressors. In various cancers, HOXATIR is highly expressed and involved in their progression and metastasis. In this study, we investigated the molecular mechanisms of genistein action through a novel pathway that represses HOXATIR. We found that HOXATIR expression is higher in renal cancer cell lines compared to normal controls. Genistein treatment was found to significantly decrease HOXATIR expression in renal cancer cells (786-O and ACHN cells). Genistein treatment also reduced expression of epithelial-to-mesenchyme transition (EMT)-related proteins (ZEB1, Vimentin and Snail), causing reduced cell migration, invasion, and increased apoptosis. We performed RNA immunoprecipitation assays, and found that genistein inhibits HOXATIR binding to PRC2. One of the other EMT markers, a tight junction protein ZO-1, is upregulated by genistein. We are currently investigating if genistein represses PRC2 recruitment to the ZO-1 promoter by inhibiting binding of HOXATIR to PRC2. Our results indicate that genistein is a potent therapeutic agent for renal cancer.


Aberrent expression of long noncoding RNAs (lncRNAs) is associated with various human cancers. However, little information is available about functional involvement of IncRNAs in tumorigenesis of chronic myeloid leukemia (CML) induced by Bcr-Abl oncogene. In this study, we performed a comprehensive analysis of IncRNAs in human CML cells using an IncRNA CDNA microarray combined with drug treatment. We identified an IncRNA termed IncRNA-up38 that was critically involved in Bcr-Abl-mediated cellular transformation. We found that expression of IncRNA-up38 was greatly upregulated by inhibiting Bcr-Abl kinase activity in K562 cells and leukemic cells derived from CML patients. Furthermore, our experiments demonstrated that IncRNA-up38 acted as a key regulator of EBP1 functioning during Bcr-Abl-mediated cellular transformation. Moreover, silencing IncRNA-up38 promoted leukemic cells survival and enhanced Bcr-Abl-mediated tumorigenesis, whereas forced expression of IncRNA-up38 sensitized leukemic cells to undergo apoptosis and inhibited Bcr-Abl-induced tumorigenesis. Additionally, our experiments have begun to address the mechanism of how IncRNA-up38 is regulated in the leukemic cells and showed that Bcr-Abl repressed IncRNA-up38 expression through c-Myc-dependent signaling. Taken together, these results reveal that Bcr-Abl-mediated cellular transformation is associated with downregulation of tumor-suppressor IncRNA-up38 and suggest a potential strategy for the treatment of Bcr-Abl-positive leukemia.


Targeting mitotic kinase is an emerging anticancer strategy with promising pre-clinical results. Mast is an essential mitotic kinase regulating mitotic progression by inactivation of a tumor suppressor protein phosphatase 2A (PP2A). However, it is unclear whether inhibition of Mast kinase can kill tumor cells. Here, we show that Mast inhibition selectively kills cancer cells by induction of mitotic catastrophe in breast cancer cells. We found that Mast overexpression was tightly associated with tumor progression and poor prognosis in breast cancer. Mast depletion with specific siRNAs induced cell death in Mast high breast cancer cells, but not in Mast low breast cancer cells. Interestingly, Mast depletion did not affect the viability of normal cells. In addition, Mast depletion reduced the oncogenic properties of breast cancer cells. Furthermore, we found that Mast depletion caused mitotic catastrophe. Therefore, our data indicate that inhibition of Mast can kill selectively breast cancer cells by induction of mitotic catastrophe, providing that targeting Mast kinase is a very useful approach for breast cancer treatment.

#3452 The cohesin subunit STAG1 is a hardwired genetic dependency of STAG2 mutant cancer cells. Petra Van Der Lelij1, Simone Lieb2, Julian Jude3, Gordana Wutz1, Catarina Pereira2, Katrina Falkenberg1, Jozel Ban4, Heinrich Kova5, Todd Waldman5, Mark Pearson2, Norbert Kraut2, Jan-Michael Peters4, Johannes Zuber2, Mark P. Petroczki6. 1The Research Institute of Molecular Pathology, Vienna, Austria; 2Boehringer Ingelheim RCV, Vienna, Austria; 3CNIO: Spanish National Cancer Research Centre, Madrid, Spain; 4CCRI- Children’s Cancer Research Institute, Vienna, Austria; 5Georgetown School of Medicine, Washington, DC.

Recent genome analyses have identified recurrent mutations in subunits of the cohesin complex in human cancer. Cohesin is a chromosomal factor that is essential for sister chromatid cohesion and cell division and that contributes to gene regulation and DNA repair. Deletious mutations in the cohesin subunit STAG2 have been detected in about 20% of bladder cancer, 15% of Ewing sarcoma and 6% of AML/MDS patients. The mechanistic involvement of cohesin mutations in the pathogenesis of human malignancies is currently under active investigation. We hypothesized that the loss of STAG2 could alter the properties and functionalities of the cohesin complex leading to unique vulnerabilities of STAG2 mutant cells. Using CRISPR/Cas9 and RNAs in isogenic solid cancer and leukemic models we identified STAG1, a STAG2 paralog, as a strong and clean genetic vulnerability of STAG2 mutant cells. Mechanistically, STAG1 loss abrogates sister chromatid cohesion specifically in STAG2 mutant but not wild-type cells leading to mitotic catastrophe, defective cell division and apoptosis. STAG1 inactivation inhibits the proliferation of disease relevant STAG2 mutant but not wild-type bladder cancer and Ewing sarcoma cell lines. Restoration of STAG2 expression in a mutant bladder cancer model alleviates the dependence on STAG1. Our results demonstrate that the cohesin subunits STAG1 and STAG2 act redundantly to support sister chromatid cohesion and cell viability in human cells. We have identified STAG1 as a hardwired, context independent vulnerability of STAG2 mutant cancers. Specific synthetic lethaliites elicited by recurrent cohesin mutations in human tumors hold the promise for the development of selective therapeutics.

#3453 Progression through mitosis promotes PARP inhibitor induced cytotoxicity in homologous recombination deficient cancer cells. Pepijn M. Schoonen1, Peter Bouwman2, Floris Fojer3, Madalena Tarsousova4, Solvi Blatter3, Jos Jonkers2, Sven Rottenberg2, Marcel A. Vugt1.

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Mutations in homologous recombination (HR) DNA repair genes BRCA1 and BRCA2 predispose to tumorigenesis. HR-deficient cancers are hypersensitive to Poly (ADP ribose)-polymerase (PARP) inhibitors, but can acquire resistance and relapse. The mechanisms underlying PARP inhibitor resistance are only partially understood. This lack of knowledge in part stems from incomplete understanding of how PARP inhibitors induce cytotoxicity in HR-deficient can...

Antimitotic drugs are often administered as first-line chemotherapy in several malignancies. However, their clinical success is often limited by acquired chemoresistance and disease relapse. The anti-proliferative basis of these drugs is induction of mitotic arrest culminating in cell death. Mitotic slippage occurs when cells exit mitosis and “slip” into interphase without chromosome segregation and cytokinesis. Little is known about how cells post-slippage influence outcome of treatment. Here, we demonstrate that post-slippage cells exhibit paracrine pro-tumorigenic potential following senescence and senescence-associated secretory phenotype development. This occurs in an autophagy-p53 axis-dependent manner, with autophagic inhibition in post-slippage cells bypassing senescence and leading to cell death. Indeed, the autophagy inhibitor Chloroquine and microtubule poisons synergistically inhibited tumour growth in mice. Hence, regimens targeting the senescence phenotype could provide a potential effective combinatorial strategy with antimitotic drugs. Sensitivity to this combinatorial treatment was dependent on p53 status, an important factor to consider before treatment.

Cystatin C is a small secreted protein, produced by most cells in the body and found in significant quantities in all body fluids. It is the major inhibitor of extracellular cysteine protease activity in mammals because of its rapid binding to virtually all known cysteine proteases of families C1 (cysteine proteinase), C12 (neutral metalloproteinase), and C13 (mammalian legumain). Cystatin C has also been shown to inhibit viral replication within cells and display protective effects against cancer cell invasion. The protein has recently been shown to get internalized into cancer cells, providing an explanation to possible intracellular effects of the inhibitor. The purpose of the present study was to elucidate possible direct effects of cystatin C on cancer cell growth, by use of holometric imaging to analyze proliferation rate as well as to monitor the mitosis phase in real-time. Human A375 melanoma cells were cultured with and without addition of physiological quantities of cystatin C added to the culture medium (1 micromolar concentration). The cultures were monitored for up to 3 days in a standard incubator using a HoloMonitor M4 instrument. Settings were optimized to allow accurate counting of cells throughout the experiment by analysis of images captured, revealing an inhibitory effect on overall cell proliferation rate by cystatin C addition to the cultures. To analyze the migration and cell division of individual cells, images were captured at short intervals allowing tracking and monitoring of cell morphology with respect to parameters such as thickness/density and area. Parameters could be set to define start of the mitosis phase indicated by a distinct rounding-up of cells and followed until a natural stop at cytokinesis, whereafter the daughter cells could be continuously tracked. These analyses revealed qualitative differences in the normal migration behavior of the A375 cells under study as a result of cystatin C addition. Moreover, the mitosis phase was considerably prolonged in cells grown in cystatin C containing medium, further indicating that the proliferation-inhibiting effect of the protease inhibitor may be through its direct action on proteolytic processes in the actual mitosis phase. This finding justifies in-depth studies on additional cancer cell types to reveal how general the anti-proliferative effect of cystatin C is and how it can be explored to possibly control cancer cell growth and spread. The use of real-time holometric imaging of growing cells should be a most useful tool in such studies.

Cytostatic gene misregulation is a major source of chromosomal instability in human cancers and predicts patient response to specific therapies. Weiguo Zhang,1 Jian-Hua Mao,2 Wei Zhu,3 Anshu K. Jain,3 Ke Liu,3 James B. Brown,2 Gary H. Karpen1. 1Lawrence Berkeley National Lab, Berkeley, CA; 2Cellular Biomimicry Group, Inc., Shanghai, China; 3Yale School of Medicine, New Haven, CT.

Chromosomal instability (CIN) is a cancer hallmark that contributes to tumor heterogeneity and other malignant properties. Aberrant centromere function causes CIN through chromosome missegregation in experimental systems. CENP-A is a histone H3 variant found at centromeres. Centromere is epigenetically by replenishing CENP-A chromatin during cell divisions. This process requires a dedicated CENP-A chaperone called HJURP and other factors. However, we are lacking a comprehensive understanding whether these proteins are involved in clinical cancers, and how centromere misregulation may contribute to cancer development. Here, basing on our previous findings in Drosophila melanogaster, we identified a conserved mutual protection mechanism between CENP-A and HJURP between flies and human cells, where their physical interaction protects each other from being targeted for degradation. Moreover, we showed that HJURP stability is regulated both in vitro and in vivo by the Anaphase Promoting Complex/Cyclosome (APC/C) ubiquitin E3 ligase. We further demonstrated that CENP-A and APC/C compete for the same binding site on HJURP. Using isogenic breast cancer cell culture progression series, we found that many centromere proteins are progressively overexpressed at the protein level during tumorigenesis. These results led us to hypothesize that overexpression of centromere proteins may cause centromere misregulation and CIN, and contribute to human cancers. To test the idea, we developed a CES (Centromere and kinetochore gene Expression Score) signature that quantitates the mRNA levels of 14 key genes required for centromere structure in cancers. High tumor CES values strongly correlate with increased copy number alterations and mutation frequencies across many cancer types, and prognostic poor patient survival for many cancers. High CES values also signify high levels of genomic instability that sensitize cancer cells to additional genotoxicity. Indeed, the CES signature forecasts patient response to adjuvant chemoradiotherapy for lung and breast cancers. In conclusion, we demonstrate regulation of HJURP by APC/C ubiquitin E3 ligase, mutual protection between the key centromere proteins CENP-A and HJURP, and a mode of misregulation of centromere genes in human cancers. The CES gene signature may be a potential biomarker to identify patients who likely respond to specific treatments, thus spare the non-responding patients from less effective treatments. These findings are expected to help address the over-treatment problem prevalent in cancer treatments. Our approach validates the critical importance of incorporating basic knowledge of chromosome segregation pathways into cancer research and clinical applications.

Stable aneuploid cells are more sensitive to TTK inhibition than chromosomal stable cell lines. Marion A.A. Libouban,1 Jeroen A.D.M. de Roos,1 Joost C.M. Uitdehaag,2 Nicole Willemse-Seegers,2 Sara Mainardi,1 Jelle Dylus,3 Jos de Man,2 Bastiaan Tops,1 Jules P.P. Meijerink,2 Zuzana Storchova,3 Rogier C. Buijmans,2 René H. Medema,1 Guido J.R. Zaman1. 1NKI, Amsterdam, Netherlands; 2Netherlands Translational Research Center B.V, Oss, Netherlands; 3Radboud University Medical Center, Nijmegen, Netherlands; Princess Máxima Center for Pediatric Oncology, Netherlands; 4University of Kaiserslautern, Germany.

Inhibition of the spindle assembly checkpoint kinase TTK causes chromosome mis-segregation and tumor cell death. High levels of TTK correlate with chromosomal instability (CIN), which can lead to aneuploidy. To investigate the potential relationship of CIN and sensitivity to TTK inhibition, we performed CIN analysis in human cancer cell lines from different tumor tissue origins and with different relative sensitivity to the selective TTK inhibitor NTRC 0066-0 [1]. By time lapse microscopy we observed that treatment with TTK inhibitor resulted in overriding of the mitotic checkpoint, irrespective of cell line sensi-
Zyxin is a LIM domain protein that is concentrated at sites of cell-cell adhesion, where it is proposed to dock proteins involved in cytoskeletal dynamics and signaling. Recently, Zyxin has been shown to promote LATs degradation and YAP activation in Hippo pathway. Here, we have identified a novel mechanism for phosopho-regulation of Zyxin in mitosis and its biological significance in colon cancer. We found that the mitotic kinase cyclin-dependent kinase 1 (CDK1) phosphorylates Zyxin in vitro and in vivo at serine 281, serine 308 and serine 344 during antimotic drug-induced G2/M phase arrest and unperturbed mitosis. Moreover, Zyxin mitotic phosphorylation-deficient mutant (Zyxin5A, harboring S281A/S308A/S344A) was sufficient to suppress colon cancer cell proliferation and migration. We further demonstrated that depletion of Zyxin resulted in impaired colon cancer tumorigenesis in vitro and in vivo through inhibiting F-actin cytoskeleton and YAP activity. Importantly, these observations could be fully rescued by re-expression of wild type Zyxin, but not Zyxin-3A mutant. Expression profile analysis revealed CDK8 (cyclin-dependent kinase 8) as a mediator downstream Zyxin/YAP. Inhibition of CDK8 proteins including BUBR1 and Cdc20. Surprisingly, level of mitotic Cdk1 remained unchanged in spite of elevated levels of Cdc20 indicating a possible inactivation of Anaphase Promoting complex (APC/C). Also, percentage of Cyclin B1 positive cells was significantly high in mitotic cells enriched using double thymidine block in the presence of TBK1 inhibitor BX795 (R9) and MRT10670 around the mitotic cells (R9). Further, double thymidine blocke released cells displayed elevated levels of SAC components including BUBR1 and Cdc20. Surprisingly, level of mitotic Cdk1 remained unchanged in spite of elevated levels of Cdc20 indicating a possible inactivation of Anaphase Promoting complex (APC/C). Also, percentage of Cyclin B1 positive cells was significantly high in mitotic cells enriched using double thymidine block in the presence of TBK1 inhibitor BX795 (R9) and MRT10670.}

#3458 Role of zyxin in mitosis and colon cancer. Jiuli Zhou, Jixin Dong, Yuanhong Chen, Xingcheng Chen, Seth Stauffer, Yongji Zeng. University of Nebraska Medical Center, Omaha, NE.

Zyxin is a LIM domain protein that is concentrated at sites of cell-cell adhesion, where it is proposed to dock proteins involved in cytoskeletal dynamics and signaling. Recently, Zyxin has been shown to promote LATs degradation and YAP activation in Hippo pathway. Here, we have identified a novel mechanism for phosopho-regulation of Zyxin in mitosis and its biological significance in colon cancer. We found that the mitotic kinase cyclin-dependent kinase 1 (CDK1) phosphorylates Zyxin in vitro and in vivo at serine 281, serine 308 and serine 344 during antimotic drug-induced G2/M phase arrest and unperturbed mitosis. Moreover, Zyxin mitotic phosphorylation-deficient mutant (Zyxin5A, harboring S281A/S308A/S344A) was sufficient to suppress colon cancer cell proliferation and migration. We further demonstrated that depletion of Zyxin resulted in impaired colon cancer tumorigenesis in vitro and in vivo through inhibiting F-actin cytoskeleton and YAP activity. Importantly, these observations could be fully rescued by re-expression of wild type Zyxin, but not Zyxin-3A mutant. Expression profile analysis revealed CDK8 (cyclin-dependent kinase 8) as a mediator downstream Zyxin/YAP. Inhibition of CDK8 proteins including BUBR1 and Cdc20. Surprisingly, level of mitotic Cdk1 remained unchanged in spite of elevated levels of Cdc20 indicating a possible inactivation of Anaphase Promoting complex (APC/C). Also, percentage of Cyclin B1 positive cells was significantly high in mitotic cells enriched using double thymidine block in the presence of TBK1 inhibitor BX795 (R9) and MRT10670. Further, double thymidine blocked released cells displayed elevated levels of SAC components upon treatment with TBK1 inhibitors. All these findings suggest that TBK1 facilitates mitotic progression through satisfying SAC.
non-transformed mammary epithelial cells results in large acinar structures, CA, CIN, and changes in relevant EMT markers that include vimentin. Our findings indicate that deregulated E2F pathway contribute to mammary tumorigenesis in part by deregulating kinases that control mitosis, including Nek2, and in turn these affect mitotic progression, CIN, EMT and invasion. Thus, centro-some/kinases downstream of the E2F pathway may represent future targets for intervention in highly-malignant HR- breast cancers.

#3462 Investigating the role of GSK3 in the mitotic checkpoint. Maisha S. Rashid. University of Toledo, Toledo, OH.

Progression through the cell cycle is regulated by multiple checkpoints that ensure genomic integrity and proper cell division. The spindle assembly checkpoint (SAC) monitors bi-orientation of chromosomes on the mitotic spindle, and signals assembly of the mitotic checkpoint complex (MCC) at unattached kinetochores. This checkpoint prevents unequal segregation of chromosomes and thus maintains genomic stability. Our data shows a novel role for glycosyn synthase kinase 3 beta (GSK3β) in regulation of the mitotic checkpoint. GSK3β is a ubiquitously multifunctional Ser/Thr kinase with distinct roles in different types of cancer. Following pretreatment with spindle toxins which arrest cells in late mitosis, GSK3 inhibitors, SB 415286 (SB), RO-81220 (RO) and LiCl, induce mitotic exit in multiple cancer cell lines. GSK3β knockout mouse embryonic fibroblasts (MEFs) showed a similar decreased mitotic index in the presence of spindle toxins. Similarly, the mitotic checkpoint was weakened when we performed GSK3β knockout in HeLa cells using transiently expressed CRISPR/Cas9. More detailed analysis showed increased kinetochore misalignment of checkpoint proteins Mad1, Mad2, BubR1 and Bub1 in SB415286-treated Hela cells and gsk3β-/MEFs in the presence of spindle toxins. The assembly of MCC also decreases in the presence of GSK3 inhibition as witnessed by decreased Mad2 associated with Bub1. Overexpression of GSK3β in Hela cells results in an increase in mitotic index compared to untransfected cells, consistent with our results with inhibitors and knockouts. GSK3β is a key kinase in the Wnt-signaling pathway, the mis-regulation of which has been associated with cancer. Our data shows an increase in taxol-induced mitotic arrest in response to WNT-C59, a compound that disrupts processing of Wnt ligands to inhibit Wnt signaling. This observation suggests that the strength of the mitotic checkpoint can be modulated by external signaling molecules.


The PH domain Leucine-rich repeat Protein Phosphatases (PHLPP) are tumor suppressors originally discovered for their ability to directly dephosphorylate and inactivate the pro-survival kinase Akt, a key transducer of growth factor signaling. In the decade since this discovery, a number of other PHLPP targets have been identified, including those that disrupt processing of Wnt ligands to inhibit Wnt signaling. This observation suggests that the strength of the mitotic checkpoint can be modulated by external signaling molecules.

#3464 CRISPR-mediated inactivation of ATRX and DAXX in pancreatic neuroendocrine tumor cell lines. Anthony J. Rizzo, Jacqueline A. Brosnan-Castledine. Shedd Cancer Center, Baltimore, MD.

Cancer cells must find a way to subvert replicative senescence in order to achieve cellular immortality. While most malignancies (> 90 %) overcome this critical barrier by reactivating the telomerase enzyme, a telomere-specific reverse transcriptase; other cancers (5 - 10 %) utilize a telomerase-independent pathway of telomere maintenance, referred to as Alternative Lengthening of Telomeres (ALT). ALT is thought to utilize homologous recombination and DNA repair machinery to maintain the chromosome ends. Importantly, this mechanism appears to be cancer-specific and dependent on alterations in chromosone maintenance at the telomeres. Therefore, we have applied a pharmacological intervention in highly-malignant HR- breast cancers.
subtypes that employ it; our hope is to better understand the ALT phenotype in cancer so that we may effectively translate ALT-specific therapeutics to the clinic.


Comprehensive analysis of telomere length and telomere maintenance mechanisms across 31 human cancer types. Floris P. Barthel, Siyun Zheng, Roel G. Verhaak. The Jackson Laboratory for Genomic Medicine, Farmington, CT; The University of Texas MD Anderson Cancer Center, Houston, TX.

Telomeres cap chromosome ends and prevent chromosomal fusions. In the majority of somatic cells telomere shortens with each cell division. Cancer cells, on the other hand, maintain telomere length (TL) through reactivation of telomerase or alternative lengthening of telomere (ALT). Though closely related to cancer hallmarks such as chromosomal instability, telomere length has not been systematically analyzed in cancer. We used DNA sequencing to infer TL in 18,430 samples across 31 cancer types. Tumor TL was shorter compared to normal TL but tended to be longer in testicular germ cell tumors, sarcomas and gliomas. TL in non-neoplastic leukocyte and solid tissue samples was negatively correlated with patient age and varied between lineages, with kidney samples showing the longest TL and leukocytes the shortest. Amongst tumors, 73% expressed telomerase (TERT) and 50% harbored a MAPT alteration. We compare TERT expression to both ALT status and macrochromosomal variation to better understand the relationship between ATR and CHK1 inhibitor effects on ALT-positive cancers. In order to study ALT in this context, we obtained and characterized a panel of six pediatric HGG cell lines. Two of the six cell lines in this panel display hallmarks of the ALT pathway. To assess the potential of ALT as a therapeutic to decelerate progression in pediatric HGG, we measured cell viability in the presence of inhibitors, stratifying our analysis by ALT status. ALT is predicted to occur via a homologous-recombination-based mechanism. As such, we focused our attention on inhibitors of the DNA damage response, as well as agents that induce DNA damage. Treatment with inhibitors of DNA-PK, RAD51, and MRE11 did not result in significant differences in cell viability when stratified by ALT status, nor did treatment with temozolomide or hydroxyurea. However, inhibitors of ATR (VE-821, VE-822, VX-970, and AZD-6738) and CHK1 (MK-8776) led to significantly greater reductions in cell viability in ALT-positive cells. In order to further our understanding of the mechanism through which ATR inhibition preferentially targets ALT-positive cell lines, we examined the effect of these inhibitors on ALT-specific properties, such as the presence of ultrabright telomeric foci and extra-chromosomal telomeric DNA (c-circles). Preliminary results indicate that ATR inhibition does not diminish these ALT-associated properties and, therefore, the differential sensitivity to these agents is unlikely to be due to directly blocking the ALT telomere maintenance mechanism. Continued study of the effect of ATR/CHK1 inhibition on ALT-positive cells will yield further insight into the mechanism of ALT specific toxicity. Overall, we have identified ATR and CHK1 as promising pathway to target ALT-positive pediatric HGG. Our goal is to better understand the relationship between ATR/CHK1 signaling and ALT in order to effectively translate this observation to the clinic, both for pediatric HGG and for other ALT-positive cancers.


A comprehensive protocol to identify human telomerase associated proteins by mass spectrometry. Jing Wang, Xuezhi Bi, Sheng Li, Duke-NUS Graduate Medical School, Singapore, Singapore; Bioprocessing Technology Institute (BTI), A*STAR, Singapore, Singapore.

Telomeres are nucleoprotein structures at the ends of eukaryotic chromosomes that prevent them from degradation, recombination, and end-to-end fusion. Telomeres consists of DNA repeats, which in human is of the sequence TGAGG, and it is bound by a group of proteins called shelterin complex. Telomeres are synthesized by telomerase. Telomerase is absent in most human somatic cells while it is up-regulated in 90% of cancer cells, in which telomerase plays an essential role in cancer cells’ replicative immortality. However, telomerase is only present at approximately 250 molecules per cancer cell. To exert its function of elongating telomere, telomerase has to find the end of telomeres which needed to be elongated. However, how telomerase is precisely recruited to telomere is not clearly understood. To better understand the recruitment process of telomerase, we initially screened and subsequently identified novel binding proteins of hTERT, the catalytic subunit of telomerase, through a pull-down assay using cancer cell lines with endogenously Flag-tagged hTERT. Using mass spectrometry (MS), we were able to

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identifying a promising interesting protein. Further, direct interaction between TERT and this protein was validated. Then, we found that this protein can also interact with the shelterin component, TRF1 (Telomeric repeat-binding factor 1), which binds to double stranded telomeric DNA. Furthermore, from previous published reports, this protein was shown to interact with single-stranded telomeric DNA binding protein POT1 (Protection of Telomeres 1) in the shelterin complex. Functionally, overexpression of this protein in cancer cell lines can result in the elongation of telomeres, while knock-down or knock-out of the protein results in the shortening of telomere. In conclusion, we identified an hTERT-binding protein that affects telomere maintenance which may be involved in telomerase recruitment process. In the long run, it could serve as a potential therapeutic target for cancers.

#3470 The promyelocytic leukemia (PML) protein modulates rate and localization of homologous recombination in human cell lines with the alternative lengthening of telomeres (ALT) pathway. Walter Barry, Christopher Heaphy, Alan Meeker. Johns Hopkins School of Medicine, Baltimore, MD.

The promyelocytic leukemia protein (PML) is tightly associated with a defining feature of the cancer specific, telomerase-independent telomere-maintenance pathway known as alternative lengthening of telomeres (ALT); therefore, PML is an important potential target for chemotherapeutics. Cells running ALT contain large accumulations of telomeric DNA and RNA in the nucleoplasm that are frequently coated in a polymer shell of PML protein. These structures are known as PML-AFAs. PML-AFAs have been observed to be major sites of the telomeric sequence homologous recombination believed to be the basis of telomere maintenance in ALT. A CRISPR-based strategy was used to knock out the PML gene in two widely used ALT-positive osteosarcoma-derived human cell lines; U2OS, and SAOS2. Knockout clones from both cell lines continued to proliferate past passage 90 without crisis, and knockouts were validated at the genetic and protein levels by Sanger sequencing and western blotting, respectively. Chromosome orientation fluorescent in situ hybridization (COFISH) was carried out to determine the number of telomere sequence exchanges occurring in a single cell cycle in these knockouts. A twofold increase in telomere-specific exchanges, presumably telomere sister chromatid exchanges (T-SCE), over the parental line was detected. Intrachromosomal exchanges not specific to telomeres were also analyzed by the harlequin chromosome staining method. A significant increase in these types of exchanges was also observed. In conclusion, the presence of PML is not required for ALT to continue to function, and may be acting to modulate homologous recombination frequency and location in ALT positive cells.


Alphoid centromere array is composed by 171 bp AT rich alphoid monomer that tandemly arranged into high order repeat unit up to 4 MB array. Hallmark of centromeric DNA is CENPB box, a 17 bp motif that is a binding site of CENP-B. As the chromosomal site that regulates the precise and accurate chromosome segregation, understanding DNA replication in centromere will reveal insight into faithful chromosome segregation that potentially influence cellular behavior in normal and cancer cell. The present of active replication within alphoid centromere array are detected by enrichment of nascent DNA abundance. This finding is supported by evident of Orc2, Cdt1 and Tre100 accumulation on the alphoid centromere array. Chromatin fiber analysis revealed that inter origin distance within alphoid centromere array is irregular. CENPB depletion that is affected centromeric chromatin structure enhances DNA replication activity within alphoid centromeric array, indicates centromeric chromatin landscape alteration affected centromeric DNA replication dynamic.

#3472 Separase overexpression defines a new subset of acute myeloid leukemia patients characterized by high CD34 and MYC levels. Giorgia Simonetti,1 Antonella Padella,1 Simona Righi,1 Maria Chiara Fontana,2 Marco Manfrini,3 Cristina Papayannidis,1 Giovanni Marconi,1 Carmen Baldazzi,1 Marianna Garozzi,1 Alberto Ferrari,1 Massimo Delledonne,1 Nicoletta Testoni,1 Elena Sabattini,2 Giovanni Martinelli1. 1University of Bologna, Bologna, Italy; 2Sant’Orsola Malpighi Hospital, Bologna, Italy; 3University of Verona, Verona, Italy; 4Personal Genomics, Verona, Italy

The endopeptidase Separase, encoded by the ESP1 gene, plays a key role in faithful segregation of sister chromatids by cleaving the cohesin complex at the metaphase to anaphase transition. Its overexpression associates with aneuploidy and bad prognosis in solid tumors. Little is known in Acute Myeloid Leukemia (AML). We profiled the genomic landscape of 405 and 78 AML cases by SNP array (SNP 6.0 and Cytoscan HD, Affymetrix) and whole exome sequencing (100 bp, paired-end, Illumina), respectively. Bone marrow blasts from 61 patients were analyzed by gene expression profiling (HTA 2.0, Affymetrix). Separase expression was determined by Immunohistochemistry (1:600 antibody dilution Abnova, clone 6H6) in 44 AML and 4 control bone marrow specimens. One patient exhibited a nonsynonymous mutation in ESP1 (p.1357G>A), which was confirmed by Sanger sequencing. Moreover, ESP1 expression was observed in 5/405 cases (1.2%): 2 hyperdiploid AML, one trisomy 12 and 2 cases with a short gain at 12q. Notably, protein level detection in one of the 12q-gain cases confirmed Separase overexpression. To determine the incidence of Separase overexpression, we performed Immunohistochemistry on additional 43 AML. Separase was overexpressed in 29/44 AML (66%, Separase-high), being expressed in 8/12 of control marrow specimens. In the remaining 13 cases (Separase-low), sixty-two percent of Separase-high AML were aneuploidic. However, no significant association was observed, as previously reported for mutations in the cohesin genes in AML. Separase overexpression correlated with increased patients’ age (median age 64 vs. 57 years, p = 0.01), 17,70-upregulation of CD34 (p = 0.004) and a trend towards reduced overall survival (6-years follow-up). Separase overexpression was not mutually exclusive with cohesin gene mutations, it co-occurred with NPM1 and FLT3 lesions and frequent mutations in genes involved in protein post-translational modification and ubiquitination (p = 0.04). Separase-low cases were enriched for mutations in RAS signaling pathway (NRAS, KRAS, NF1, RIT1, GRAP2, RALGDS; p = 4.3x10^-5) and in cell migration-related genes (LIM52, SIPR1, PP1A, PLXNB1, FAT1). Separase-high cases also showed a defined transcriptomic profile, characterized by reduced expression of HOXA/B family genes, the DNA damage repair gene ATM, the p53 regulator MDM2 and forced expression of the cell cycle markers CDC20, AURKB, NUSAP1 and of MYC, independently of chromosome 8 gain. Taken together, our data suggest that genomic lesions targeting ESP1 are a rare event in AML. However, Separase overexpression is a common feature and defines a new subset of AML cases with a distinct gene expression profile, which may benefit of innovative targeted therapies including CDC20 and bromodomain inhibitors. Supported by: EUN, AICR, progetto Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTI project.


In the previous study, we identified genes that were differentially expressed between monolayers and spheroids in prostate cancer cell lines. The KIF22 gene was extracted as one of candidate genes related with spheroid formation and multicellular resistance. KIF22 is one of the kinesin superfamily proteins that are microtubule-dependent molecular motor proteins with DNA-binding capacity. KIF22 plays an important role in eukaryotic cell mitosis and macromolecule transportation. Alteration of KIF22 expression and function may lead to cancer development and progression. In this study, we explored the function of the KIF22 in prostate cancer cell lines, DU145 and LNCaP using the KIF22 siRNA. The KIF22 mRNA levels in both DU-145 and LNCaP decreased, and this gene was increased in both spheroids. The inhibition of KIF22 mRNA using siRNA affected cell proliferation in DU145 and LNCaP spheroids as well as monolayers. In addition, this suppression caused G2/M arrest and apoptosis in both cell lines. In clinical samples, its mRNA expression was significantly higher in tumor portions than in non-cancerous portions. However, the inhibition of KIF22 mRNA did not affect cell migration and invasion in DU145 and LNCaP cells. These findings suggest that KIF22 may play an important role in prostate cancer proliferation, especially spheroid formation, and have the potential to be targeted for prostate cancer treatments such as combination therapy with docetaxel.

#3474 Depletion of SPECC1L inhibits colorectal cancer cell proliferation. Rajat Bhattacharya, Ling Xia, Fan Fan, Rui Wang, Delphine Boubles, Xiang-Cang Ye, David Hawke, Lee Ellis. MD Anderson Cancer Center, Houston, TX.

Introduction: Despite the numerous drugs available for patients with metastatic colorectal cancer (mCRC), median overall survival for this group of patients remains at ~20-24 months, with no significant advances in the last 7 years. In the US ~50,000 patients die each year from mCRC refractory to systemic therapy. Inhibiting angiogenesis as a therapy has led to a great deal of enthusiasm. However, the overall benefit of classical-antiangiogenic therapy remains modest and has not lived up to their expectations in treating CRC. Our recent studies suggest that VEGF intracrine signaling, rather than autocrine/paracrine signaling, regulates cell survival in CRC cells. Further studies to understand the significance and mechanisms of this novel function have led us to identify fac...
tors that intracellularly interact with VEGF. Our studies further indicate that one such interacting protein, SPECC1L, may have a significant role in CRC cell proliferation and may be a potential target in mCRC therapy. Methods: Lysates from CRC cells expressing Myc-tagged VEGF protein were immunoprecipitated and analyzed by mass spectrometry to identify VEGF-.interacting proteins. SPECC1L was identified as a co-precipitated protein with Myc-tagged VEGF in CRC cells. However, SPECC1L was depleted using siRNA and effects of such depletion on CRC cell growth and morphology were measured by cell growth assays (MTT), microscopy, FACS and western blot analyses. Localization of the protein and its interaction with microtubules and actin were visualized by immunostaining of FLAG-tagged recombinant SPECC1L protein. Results: SPECC1L was identified as a protein that co-immunoprecipitated with Myc-tagged VEGF in CRC cells using mass spectroscopy. Previous literature suggests a role for SPECC1L in cell division. As a fraction of VEGF overexpressing CRC cells have a large multinucleated phenotype, likely arising due to defects in cell division, it was hypothesized that a VEGF-mediated regulation of SPECC1L may lead to such a phenotype. Depletion of SPECC1L by siRNAs in multiple CRC cell lines led to strong defects in cell division. The effects of SPECC1L depletion were manifested as accumulation of doublet-cells failing to complete cytokinesis following mitosis and resulted in reduced cell proliferation. Failure to complete cell division also led to the formation of multinucleated cells and enhanced cell death. Conclusions: Inhibition of SPECC1L strongly inhibits CRC cell proliferation and enhances cell death. Thus targeting SPECC1L has the potential for developing therapeutic strategies to reduce viability of CRC cells and improve survival of colorectal cancer patients. These studies were supported by the Gillson-Longenbaugh Foundation.

#3475 Inhibitory effects of ZFP36L1 and ZFP36L2 on the cell proliferation in human colorectal cancer cells. Fat-Moon Suk, Ya-Ting Chen, Yu-Chih Liang. Taipei Medical University, Taipei, Taiwan.

ZFP36 family members include ZFP36 (also named TTP and TIS11), ZFP36L1 (also called BRF1, D1R1 or TIS11B) and ZFP36L2 (also called BRF2, ERF2 and TIS11D), which belong to the CCCH-type zinc finger protein with 2 tandem zinc finger region (TZF). These ZFP36 family proteins can function as RNA-binding protein through binding to AU-rich elements (AREs) in the 3′ untranslated region (3′ UTR) of mRNA, which promote the mRNA degradation or translation repression. Previously, ZFP36 has been found to inhibit cell proliferation through p33-dependent manner. In this study, we’d like to investigate whether ZFP36L1 and ZFP36L2 have anti-proliferative activity as same as ZFP36 work. Tetracycline-inducible (Tet-On) system was used to induce the overexpression of ZFP36L1 or ZFP36L2 proteins in T-REx-293 cells by doxycycline (Dox) treatment. While ZFP36L1 or ZFP36L2 was overexpressed, we found that cell proliferation was dramatically inhibited, but didn’t cause cell death significantly. The importance of TZF was confirmed by using TZF mutants, and found that cell proliferation was deprived in HEK-293 cells with overexpression of ZFP36L1 mutant (C135/173R). Using western blot analysis, it was revealed that expression level of p53 protein was increased after the overexpression of ZFP36L1 or ZFP36L2 protein. Whereas, the levels of cell cycle-related proteins including cyclin B1, cyclin A2 and cyclin D1 were decreased. Next, three colorectal cancer cell lines (HCT116, HCT116 p53-/- and SW620 (mutated p53) cells were used and ZFP36L1 or ZFP36L2 gene was transduced into these three cells by lentivirus. Overexpression of ZFP36L1 or ZFP36L2 also inhibited the cyclin D1 protein expression and cell proliferation in these three cells, however, increased the p53 and p21 protein expression in HCT116 p53-/- cells, and decreased the c-Myc expression only in SW620 cells. On the other hand, knockdown of ZFP36L1 or ZFP36L2 increased cell proliferation, and mutation in TZF of ZFP36L1 (C135/173R) or ZFP36L2 (C174/212R) lost their anti-proliferative abilities in these three cells. Taken together, the results suggest that ZFP36L1 and ZFP36L2 play a negative role in cell proliferation in human colorectal cancer cells, and the underlying mechanisms might be modulated through downregulation of cyclin D1 and p53-independent pathway.

#3476 Effect of long term treatment with BRACO 19 on HeLa proliferation and senescence. Ana Flavia Reis Guimaraes, Diêgo M. de Oliveira. University of Brasilia, Brasilia, Brazil.

Telomerase, the enzyme responsible for the length maintenance of telomeres is expressed in about 85% of cancer cells, being a key to tumorigenesis. Telomerase inhibitor drugs have been studied as a promising cancer therapy due to its specificity, with less side effects than conventional approach. However, the effects expected from these therapy require many cell divisions, and the consequences of these long-term therapies are poorly understood. This work evaluated the effects of a long term treatment in cancer cells (HeLa) using BRACO-19, a G-quadruplex-interactive molecule, evaluating the consequences of its use in cell viability and its potential to induce senescence. Telomerase positive HeLa cells were grown in DMEM medium supplemented with 10% fetal bovine serum at pattern conditions. Culture medium was changed every 2 days. For preliminary studies, we performed experiments with dosages from 1mg/mL - 25ng/mL, incubated for 24 hours and treated for 24 hours with increasing concentrations of BRACO-19 and vehicle (0.1% H2O). Cell viability was measured by MTT assay. For the long-term treatment, a subtoxic concentration was chosen. The cells (5.5x10^5 cells/cm^2) were grown under continuous presence of the drug or 0.02% H2O for 11 weeks. During the long-term exposure to BRACO-19, the cells were passed every 7-20 days, depending on its proliferation rate, viable and unviable cells were counted at these points through trypan blue dye exclusion assay. β-Galactosidase (β-gal) activity was used as cell senescence marker to evaluate the senescence after 24 hours treatment and after a long exposure to the telomerase inhibitor. Changes in cell morphology were assessed by phase contrast microscopy. The short-term (24h) treatment with BRACO-19 showed a dose-dependent effect on cell viability. The IC50 for HeLa was 5.25 μM. The subtoxic concentration of 1μM was used for long-term treatment, showing effect on cell proliferation in a way that HeLa cells exposed to the drug showed their proliferative profile decreased when compared to control. Furthermore, no senescent cells were found in control group, even after many passages; on the other hand, 24 hours and long-term exposure to BRACO-19 led to senescence. Long-term treatment with BRACO-19 correlated with visual morphological changes. The telomerase inhibitor BRACO-19 showed an expected dose-dependent effect on HeLa cells at short-term exposition, since cytotoxic effect of this compound has been demonstrated in several cell lines, but the impact of the long-term treatment on cell proliferation pattern points BRACO-19 as a potential prototype of a new telomere related drug for cancer therapy. Also, it was demonstrated the senescence inducing action of this drug, perhaps due to erosion of HeLa telomeres. These results show that HeLa is sensitive do BRACO-19 even under subtoxic concentrations. Its effectiveness against other cell lines and tumors is under investigation.

#3477 Rospeh (Rosa canina) extracts prevent AKT-mediated cell proliferation and migration in triple negative breast cancer cells. Patrice Cagle, Patrick Martin. North Carolina A&T State Univ., Greensboro, NC.

Triple Negative Breast Cancer (TNBC) is an aggressive form of breast cancer, characterized by its lack of the human epidermal growth factor receptor-2, the estrogen receptor, and the progesterone receptor. AKT has been shown to promote cell proliferation and migration in triple negative breast cancer. Currently, the existing targeted therapy is of minimal benefit in TNBCs. Furthermore, adverse side effects and the emergence of drug-resistant cancer cells are of great concern. Natural products have received growing interest in recent years as an alternative medicine with potential anti- oncogenic properties. Rospeh extracts have been used as dietary supplements to relieve symptoms associated with gastrointestinal disorders and arthritis, and in our laboratory it has been shown to prevent cell proliferation in glioblastomas. This study investigated the efficacy of roship extracts in preventing proliferation and migration of a triple negative breast cancer cell line (HCC1954). HCC1954 cells treated with roship extracts (1mg/mL - 25ng/mL) demonstrated a significant decrease in cell proliferation. The observed decrease in cell proliferation was equal to or better than the decrease of cell proliferation observed when inhibitor of the AKT (LY294002, 20 μM) signaling pathway was utilized. Rospeh extracts also demonstrate anti-migratory potential. Additionally, pretreatment of this cell line with roship were found to selectively decrease AKT, p70S6K, and S6K phosphorylation suggesting these extracts prevent TNBC cell proliferation and migration by blocking the AKT signaling mechanism. Western blot analysis and apoptosis studies demonstrate that roship extracts inhibit cell proliferation without promoting apoptosis, but induce cell cycle arrest. To investigate the potential clinical application of roship extracts we examined whether roship extracts could enhance the chemotherapeutic properties of Doxorubicin (20μM), a chemotherapeutic agent used to treat breast cancer. Rospeh extracts demonstrated a greater anti-proliferative effect than Doxorubicin alone and is equally effective in combination with Doxorubicin. These data suggest that roship extracts are capable of decreasing cell proliferation and migration in a triple negative breast cancer cell line by blocking the AKT signaling pathway and by regulating the cell cycle. Moreover, roship extracts can enhance the chemotherapeutic properties of Doxorubicin.
MOLeULAR AND CELLuLAR BIOLOGY / GENETICS: Noncoding RNAs and Cancer: Expression, Function, and Therapeutics


Prostate cancer (PCa) is the most prevalent cancer among men, following the second leading cause of cancer-related death in western world. Currently, there is a lack of effective treatment options for advanced PCa. Understanding the molecular mechanisms in prostate cancer development and progression would allow the identification of novel prognostic markers or therapeutic targets. The transcribed-ultraconserved regions (T-UCRs) are novel class of noncoding RNAs that are absolutely conserved (100%) between orthologous regions of the human, rat and mouse genomes. Some studies showed that T-UCRs exhibit distinct profiles in various human cancers. We examined the expression profile of representative 26 T-UCRs using qRT-PCR in 12 PCa tissues and 10 non-neoplastic prostate tissues, and found that the expression of Uc.63+ was higher in PCa tissues than that in non-neoplastic tissues. Further analysis revealed that the expression of Uc.63+ significantly correlated with higher PSA levels (p = 0.003) and higher Gleason score (p < 0.001). In order to verify biological function of Uc.63+ in PCa, we firstly transected Uc.63+ expression vector into LNCaP cells expressing low level of Uc.63+. MTT assay and wound healing assay revealed that overexpression of Uc.63+ significantly increased the cell growth and migration. We also confirmed that downregulation of Uc.63+ using siRNA in DU145 and PC3 expressing high level of Uc.63+. Knockdown of Uc.63+ decreased the cell growth and migration. A recent report indicates that T-UCRs may act as endogenous competing RNA. miR-130b was found to have binding sites within the Uc.63+ using online software. We examined the expression of miR-130b by qRT-PCR in PCa tissues and non-neoplastic prostate tissues. We found a significant downregulation of miR-130b in PCa tissues compared with non-neoplastic prostate tissues, which figured out that there was a significant inverse correlation between Uc.63+ and miR-130b in PCa (p = 0.002). The expression of miR-130b in LNCaP was higher than that in DU145 and PC3. It has been shown that MMP2 is a direct target of miR-130b. Western blot analysis and qRT-PCR revealed that MMP2 expression was higher in LNCaP cells transfected with Uc.63+ expression than that in LNCaP cells transfected with empty vector. These results suggest that Uc.63+ may contribute to the progression of PCa through miR-130b interaction.

**#3479 Identification and characterization of clinically relevant long non coding RNA PLUCAT1 in lung adenocarcinoma.** Sudhanshu Shukla, Lanbo Xiao, Brendan Veenstra, Yuping Zhang, Yashasundaram Pitchiya, Arul Chinnainay. University of Michigan, Ann Arbor, MI.

Non-small cell lung cancer (NSCLC) is a leading cause of cancer-related death worldwide. Disease subtypes include lung adenocarcinoma (LUAD) and lung squamous carcinoma (LUSC) and LUAD accounts for nearly 50% of lung malignancies. Late diagnosis and limited chemotherapy options are the primary reason for poor prognosis and even among patients diagnosed early with Stage I disease, recurrence occurs in 35-50% of cases. Lack of discriminatory biomarkers has hampered clinical and therapeutic management, as current practice tends to group all patients together. Several attempts to develop gene signatures from microarray datasets to stratify risk among lung adenocarcinoma patients have not yielded clinical utility due to poor cross validation and lack of robustness. The development of RNA-Seq methodologies now allows accurate quantification of transcriptional abundance that is reproducible and reliable. Capitalizing on this technology and large datasets such as the cancer genome atlas (TCGA) as well as individual efforts, thousands of cancer samples are available for analysis to facilitate deeper understanding of the disease. One major discovery resulting from large RNA-Seq datasets is functional long noncoding RNAs (IncRNAs; exceeding 200 nucleotides in length) that can be expressed in tissue- lineage- and cancer-specific expression patterns. In our previous study we identified a highly significant prognostic marker set using 255 TCGA LUAD patients. In the training cohort, 96 genes had prognostic association with p<0.001, including 5 long noncoding RNAs (IncRNAs). Stepwise regression generated a four-gene signature, including one IncRNA. Signature high risk cases had worse overall survival (OS) in the TCGA validation cohort (HR=3.07, 95%CI=2.00-14.62). The four-gene signature was then validated in two independent patient cohorts. The signature consisted of three protein coding gene and a IncRNA (PLUCAT1). Knockdown studies showed that the IncRNA promoted lung cell growth in vitro. Also, inhibition of PLUCAT1 using both siRNA and ASOs induced cellular senescence in lung cells. Further, RNA pull down followed by mass-spec identified QKI as a binding partner of PLUCAT1. QKI is a tumor suppressor gene shown to change splicing pattern in many cancer types. We now show that PLUCAT1-QKI interaction has functional consequences and changes splicing pattern of significant number of genes as assessed by RNA sequencing. Previously, we have identified PLUCAT1, a four-gene prognostic signature, as a robust prognostic marker for lung adenocarcinoma prognosis. We also, show that PLUCAT1 binds with tumor suppressor QKI and inhibits QKI activity.

**#3480 2’–O–ribose tRNA methylation in S. cerevisiae and implications for ovarian cancer.** Frank Doyle,1 Rebecca E. Rose,2 Daniele Fabris,2 Lauren Endres,1 SUNY Polytechnic Institute, Albany, NY; 3University at Albany, Albany, NY; 4SUNY Polytechnic Institute, Utica, NY.

tRNAs are among the most chemically modified molecules of the cell, yet our understanding of how these modifications influence cell behavior remains poor. Much of the research to date has been focused on identifying specific modification events, particularly those that increase or decrease in response to oxidative stress. Less attention has been given to understanding how tRNA modifications, and their modifying enzymes (tRNA methyltransferases, or TRMs), regulate the cell’s response to reactive oxygen molecules, that are known to influence cancer initiation, survival and malignant progression. Our overall idea is that tRNA modifying enzymes respond to oxidative stress by catalyzing the modification of target tRNAs, and that this in turn influences the translation of transcripts that encode proteins that limit the potentially harmful effects of reactive oxygen. Here we used S. cerevisiae as a model eukaryote system to test the hypothesis that 2’–O–ribose tRNA methylation protects cells against the cytotoxic effects of reactive oxygen. 2’–O–ribose TRM deletion mutants (TRMs ΔA, ΔA and ΔA) were exposed to H2O2, reticone and acetic acid in order to produce intracellular reactive oxygen. In growth and colony forming assays, each deletion strain was more sensitive to the killing effects of these toxicants when compared to the wild-type strain (BY4741), and the most pronounced was observed for the TRMΔA mutant. We next performed a quantitative analysis of global changes in tRNA modifications in response to H2O2 using mass spectrometry. We found that several methyl-based modifications (i.e. 2’–O–methyl –adenosine, –guanosine, and –cytidine) were significantly decreased in TRMΔA and ΔA mutants, providing a direct link between oxidative stress and 2’–O–ribose tRNA methylation. These results support that 2’–O–ribose methyltransferase activity plays a cytoprotective role against oxidative stress in S. cerevisiae. Given the connections between reactive oxygen and cancer, we used cbioPortal to search for gene alterations of the human homolog of TRM3, TARBP1, in the genome datasets of The Cancer Genome Atlas. Interestingly, TARBP1 was found to be amplified in a significant proportion of breast, ovarian and liver cancers. From these data, we propose a new hypothesis that increased 2’–O–ribose tRNA methyltransferase activity contributes to a cancer phenotype by enabling cancer cells to maintain reactive oxygen at sub-lethal levels that promote survival and malignant progression. Thus far, preliminary results show that cultured ovarian cancer cells have elevated levels of TARBP1 relative to normal ovarian epithelial cells, providing a useful model system to test this hypothesis in future studies.

**#3481 Leveraging protein coding gene expression profiles to accurately implicate IncRNA transcriptome of cancer cells.** Aritro Nath, Paul Geeleher, R. Stephanie Huang. The University of Chicago, Chicago, IL.

Long noncoding RNAs (IncRNAs) represent a large, diverse and tissue-specific class of transcripts that are involved in gene regulation. Recent large-scale cancer sequencing efforts indicate that IncRNAs are an important component of the cancer transcriptome, and may play a critical role in carcinogenesis and drug sensitivity. Accurate profiling of IncRNAs however remains a challenge owing to significantly lower expression levels than mRNA, requiring deep paired-end total RNA sequencing, which can be prohibitively expensive. Additionally, previous generation microarrays that constitute a vast majority of GEO and ArrayExpress datasets do not provide comprehensive IncRNA coverage. Here we propose a IncRNA expression imputation (LEXI) framework to reconstruct the IncRNA transcriptome of cancer cells using their mRNA expression profiles. Our goal is to provide a tool that enables the harnessing of enormous wealth of publicly available cancer mRNA datasets and discover novel IncRNAs associated with carcinogenesis and drug sensitivity. The LEXI approach is based on learning patterns of mRNA expression associated with each IncRNA across a diverse cohort of cancer cells and then predict IncRNA expression profile of uncharacterized cells. We developed LEXI by evaluating the performance of various machine-learning algorithms benchmarked in a cross-validation study across a cohort of 675 cancer cell lines and 9753 pan-cancer tissues. We adapted

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in a caspase-dependent manner. A target analysis of SNORD-X was done using data suggesting that overexpression of SNORD-X in GBM cell lines (U87MG and other cancer cell lines) is associated with a role in various cancers. In this context, we aimed at studying the role of snoRNAs in GBM. For this, we did snoRNA profiling in GBM patients and found that a particular cluster of snoRNA called SNORD-X was highly downregulated in adult as well as pediatric GBM patients. We also found SNORD-X promoter to be methylation and shows maternal imprinting. We next did functional analyses of SNORD-X by generation of an overexpression construct. Our preliminary data suggests that overexpression of SNORD-X in GBM cell lines (U87MG and A172) increases cell proliferation in vitro. It also enhances the colony forming capability of GBM cells. Further, its overexpression causes decrease in apoptosis in a caspase-dependent manner. A target analysis of SNORD-X was done using RNAhybrid software. We found EGR1, DCUN1D3 and BRCAI to be the direct targets of SNORD-X using a combination of qRTPCR and wild-type/mutated 3’ UTR luciferase analyses. Overall, our work shows for the first time correlation and functional analysis of SNORD-X cluster in GBM.

### #3482 SNORD-X in glioblastoma: regulation and functional analysis.
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Glioblastoma Multiforme (GBM) is a kind of brain tumor which arises from astrocytes present in the brain. It represents approximately 15% of all primary brain tumor and has been considered as one of the deadliest type of cancer with very poor prognosis. Thus, there is an urgent need to identify novel targets for GBM therapy. Recently, non-coding RNAs (ncRNAs) such as miRNAs and IncRNAs have emerged as one of the promising tools as diagnostic or prognostic biomarkers and as novel targets for therapy. There are recent evidences that small nucleolar RNAs (snoRNAs), a subtype of ncRNAs might also play a key role in various cancers. In this context, we aimed at studying the role of snoRNAs in GBM. For this, we did snoRNA profiling in GBM patients and found that a particular cluster of snoRNA called SNORD-X was highly downregulated in adult as well as pediatric GBM patients. We also found SNORD-X promoter to be methylation and shows maternal imprinting. We next did functional analyses of SNORD-X by generation of an overexpression construct. Our preliminary data suggests that overexpression of SNORD-X in GBM cell lines (U87MG and A172) increases cell proliferation in vitro. It also enhances the colony forming capability of GBM cells. Further, its overexpression causes decrease in apoptosis in a caspase-dependent manner. A target analysis of SNORD-X was done using RNAhybrid software. We found EGR1, DCUN1D3 and BRCAI to be the direct targets of SNORD-X using a combination of qRTPCR and wild-type/mutated 3’ UTR luciferase analyses. Overall, our work shows for the first time correlation and functional analysis of SNORD-X cluster in GBM.

### #3483 Genome-wide analysis of the antisense transcriptome in cancer.
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Natural antisense transcripts (NATs) are the most abundant class of long noncoding RNAs (IncRNAs). Importantly, NATs are one of the most unknown ncRNAs. However, due to their highly gene locus-specific effects, NATs may provide a valuable tool for therapeutic intervention in targeted gene regulation. For this reason we are interested in studying the regulation of sense/antisense transcription in cancer related locus. In our previous work we found that, at the important cancer-related locus vimentin (VIM) there is a previously uncharacterized antisense transcript (VIM-AS1), which is transcribed head-to-head with VIM gene. This antisense transcript enhances R loop formation; it allows the binding of transcription factors as NF-kB, low nucleosome occupancy and the transcription of VIM gene. Our results are the first example of R loop mediated enhancement of gene expression involving head-to-head antisense transcription at cancer related locus. We also observed that in colon and breast cancer there was a positive expression correlation between VIM and VIM-AS1 and we have observed that hypermethylation silences both transcripts in colon primary tumors. In the present work we have seen that this positive correlation between sense and antisense transcripts is present in most of the tumor types from patients that we have obtained from Genomic Data Commons Data Portal (GDC Portal). Importantly this positive correlation correlates better in the cases in which the antisense transcript is downregulated or maintains the expression than in tumors where VIM and VIM-AS1 are up-regulated. Furthermore, we wanted to see if the cellular function is also coordinated. VIM is a component of the intermediate filament family of proteins that has an important role in epithelial mesenchymal transition. We found that specific depletion of VIM-AS1 results in a decrease of VIM expression, and now we have found that this depletion produces low migration rate and more invasion capability, as expected. On the other hand, the mechanisms used by antisense transcripts to regulate their corresponding sense mRNAs are not fully understood and in view of these results we are investigating from a genome-wide perspective the regulation of sense/antisense transcription, the involvement of R loop structures and DNA methylation in cancer. For this, we have applied a number of techniques: RNAseq strand specific, Illumina’s HumanMethylation450 Bead Chip array, RNAseq strand specific with overexpression of RNaseH (which specifically degrades the RNA in RNA/DNA hybrids), and DRIPseq (which immunoprecipitates the hybrids DNA-RNAs). The analysis of these data sheds light on the study of the role of sense/antisense transcripts in cancer-related locus.

### #3484 A long non-coding RNA, H19, as a novel therapeutic target for pancreatic cancer metastasis.
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Long non-coding RNAs (IncRNAs) comprise mRNA-like transcripts longer than 200 ribonucleotides and lack significant open reading frames. Recently, long non-coding RNAs have been reported to play important roles in epigenetic regulation, and acts on precursors or inhibitor of micro RNA. In our previous studies, we injected PAN-C-1, human pancreatic cancer cells into the spleens of NOG mice, and established a lung metastasis-derived cell line from lung metastatic nodules. Lung metastasis derived pancreatic cells had a high metastatic ability to liver and lungs compared with the parental cells (Matsuda et al., Am J Pathol, 2014). To examine the different gene expressions between the lung metastasis derived cells and parental cells, we performed a DNA microarray analysis, and found that 11 genes were up-regulated at greater than 10-fold levels in lung metastasis derived cancer cells compared to their parental cells. H19 showed an 82.4-fold increase in expression levels, and it was the second most increased and the only non-protein coding gene in the 11 genes. H19 is an imprinted IncRNA transcribed exclusively from the maternal allele and H19 gene produces a 2.3 kilo base pair long non-coding RNA. Two microRNAs, miR-675-3p and miR-675-5p are embedded in the exon of H19 and these miRNAs are reported to correlate with cell proliferation. H19 is an oncogenic RNA expressed in developing embryos and in tumors including bladder, breast, gastric, hepatic, and prostate cancers. Studies have shown that H19 enhances cancer invasion and metastasis; however, its role in cancer remains debatable. We examined the roles of H19 in pancreatic cancer growth and metastasis. Quantitative PCR analysis using human pancreatic cancer tissues showed that H19 was more highly expressed in cancer tissues than the adjacent normal tissues in 11 of 18 cases. Branched DNA in situ hybridization analysis using tissue microarrays showed that H19 was detected in 17% (23/139) of invasive ductal carcinomas, and its expression positively correlated with higher histological tumor grades (P < 0.0001). H19 was expressed in 9 pancreatic cancer cell lines at various levels. Overexpression of H19 in PAN-C-1 cells induced higher motility, whereas H19 inhibition using shRNA and siRNA showed opposite results; however, cell growth rates were unaffected in vitro. Expression levels of miR-675-3p and miR-675-5p altered as similar to the H19 levels in both H19 increased PAN-C-1 cells and H19 decreased cells. Tail vein injection of H19 shRNA vector-transfected PAN-C-1 cells markedly inhibited metastasis in the liver and lungs of immunodeficient mice. These findings suggest that H19 plays an important role in pancreatic cancer metastasis and H19 is a novel therapeutic target for metastasis of pancreatic cancer.

### #3485 Identification and characterization of circular RNAs in cancer.
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Circular RNAs (circRNAs) are an abundant class of non-coding RNAs that are formed by a backsplice event resulting in formation of covalently closed circular RNA molecules. The efficiency of backsplicing is inferior to linear splicing, however, due to their long half-lives circRNAs can accumulate to high levels in cells. Functional characterization of a few circRNAs has shown that they can act as endogenous microRNA sponges and transcriptional regulators; one example is CDR1as/ciRS-7, which has been shown to act as a sponge for miR-7. Live RT-PCR analysis using human circRNAs are still unknown. To study the potential role of circRNAs in cancer, we identified exonic circRNA candidates by analysing ENCODE RNAseq data from a panel of cancer cell lines. We used chimeric alignment detection implemented in the STAR aligner and subsequent filtering of output files to identify chimeric alignments consistent with circRNA backsplice sites. The circular nature of the identified putative circRNAs was validated by testing their dependence on RNAse R resistance. Northern blots surrounding the backsplice sites were used as target recognition sequences for designing LNA-modified gapmer antisense oligonucleotides (ASOs) to specifically knock down the circular isoforms of the RNA transcripts. Data from ongoing studies on knockdown of several abundant circRNAs in cancer cell lines, assess-
#3486 RBM3 causes differential expression IncRNA in colon cancer cell lines. Afreen Asif Ali Sayed, Dharmalingam Subramanian, Shahid Umar, Sufi M. Thomas, Shrikant Anant. University of Kansas Medical Center, Kansas City, KS.

Introduction: Colon cancer is the third most commonly diagnosed cancer and leading cause of cancer-related deaths in the United States. It usually begins as a benign growth that transforms into a malignant adenocarcinoma through genetic changes in tumor suppressor genes and oncogenes. Cancer stem cells are a rare group of cells within a tumor that have self-renewal capacities and with high potency for tumor relapse and metastasis. Wnt/β-catenin is one of the major pathways deregulated in colon cancer and also important in stem cell self-renewal. Wnt/β-catenin signaling is important in maintaining intestinal homeostasis and the stem cell niche, but aberrant activation of the pathway increases the stem cell population leading to aggressive cancer. We determined that RBM3 induces stem-like characteristics in colon cancer cells in part, by enhancing Wnt/β-catenin-induced GSK3β inactivation. We are further evaluating the mechanism by which RBM3 increases Wnt/β-catenin signaling leading to increased stemness. Methods: HCT116 and DLD1 cell lines were used in this study. We used a PCR array to identify specific Wnt pathway proteins regulated by RBM3, in HCT116 RBM3 overexpressing cells and compared it to vector control cells. RNA-IP was performed to confirm if RBM3 bound to Wnt3a and LRP6. ChIP was performed to identify β-catenin target genes. RBM3 RNA-seq was performed to identify differential gene expression and also differential IncRNA expression. RNA-Immunoprecipitation seq was performed to identify RBM3 protein bound cellular proteins. Ingenuity pathway analysis was performed for all the sequencing data. Results: Our data demonstrate that several Wnt pathway genes including Wnt3a and LRPS are upregulated in RBM3 overexpressing cells. To identify mRNA regulated by RBM3, we performed RNA-IP for RBM3. We found that RBM3 was associated with the mRNA for Wnt3a and LRPS. The ingenuity pathway analysis for RNA seq shows expression of genes involved in cell growth, proliferation and metastasis like Jun, LAMC2, Myc, NR2F2, NTSE etc. when RBM3 is overexpressed in the cells, showing a role of RBM3 in cancer progression. The seq data also shows differential expression of IncRNA especially those involved in cancer like CCAAT1 and SNHG16. The ingenuity pathway analysis for RNA-IP seq also shows increase in molecules involved in proliferation and cell movement. In-vivo studies were also performed to confirm role of RBM3 in tumorigenesis. Conclusion: RBM3 increases stemness by activating β-catenin pathway possibly through Wnt3a and LRPS. RBM3 overexpression causes differential expression of IncRNA and also increase expression of genes involved in cell growth and proliferation possibly by interacting with its target mRNAs.

#3487 Identification and validation of PCAT14 as prognostic biomarker in prostate cancer. Lanbo Xiao, Sudhanshu Shukla, Xiangy Zhang, Yashar shey, PA

Background: Early detection of prostate cancer, largely facilitated by the advent of the prostate specific antigen (PSA) test, also been accompanied by over-diagnosis of prostate cancer. Advances in next-generation sequencing technologies have enabled thorough characterization of cancer transcripts, especially in unraveling the realm of non-coding RNAs (ncRNAs). In particular, lncRNAs, a class of ncRNAs, have gained increasing attention as biomarkers due to their tissue- and cancer-specific expression profile. Thus, lncRNAs may be relevant in the clinical management of prostate cancer (PCa). Methodology: Here we assessed IncRNA expression from 585 PCA patient samples, including benign prostate tissue and both localized and metastatic PCa to discover and validate differentially expressed genes associated with disease aggressiveness. We performed Sample Set Enrichment Analysis (SSEA) and identified genes associated with low versus high Gleason score in the RNA-seq database. Comparing Gleason 6 versus 9 + PCa samples, we identified 99 differentially expressed genes with variable association to Gleason grade as well as robust expression in prostate cancer. The top-ranked novel IncRNA PCAT14, exhibits both cancer and lineage specificity. On multivariate analysis, low PCAT14 expression independently predicts for BPF5 (P = .00126), PSS (P = .0385), and MFS (P = .000609), with trends for OS as well (P = .056).

An RNA in-situ hybridization (ISH) assay for PCAT14 distinguished benign versus malignant cases, as well as high versus low Gleason disease. In prostate cancer cell lines, the endogenous PCAT14 levels are induced by CRISPR/SAM technique, which significantly suppresses cell invasion. Conclusion: By performing differential expression analysis between prostate cancer with low vs high Gleason scores, we identified IncRNA PCAT14 as a prostate cancer- and lineage- specific biomarker of indolent disease. We show that PCAT14 is an AR-regulated transcript and its overexpression suppresses invasion of prostate cancer cells. Moreover, in multiple independent datasets, PCAT14 expression associates with favorable outcomes in prostate cancer and adds prognostic value to standard clinicopathological variables.


Head and neck squamous cell carcinoma (HNSCC) represents the sixth deadliest cancer worldwide and the second fastest growing cancer in many developing countries. A cancer characterized by heterogeneity, HNSCCs are known to display varied genetic alterations based on a variety of epidemiological and clinical factors, including etiology, making it challenging to comprehensively profile and treat the disease. New studies have shown that non-coding RNAs (ncRNAs) may provide a promising means by which to explore HNSCC pathogenesis due to their diverse genetic and epigenetic roles and their recent emergence as potential biomarkers for cancer. In this study, we decided to investigate etiology-specific alterations of PIWI-interacting RNAs (piRNAs), the largest class of ncRNAs that have roles in silencing of retrotransposons, heterochromatin modification, and germ cell maintenance, in HNSCC. We particularly focused on smoking, the most prevalent risk factor of HNSCC, and HPV, the fastest growing cause of the disease today. Using 466 HNSCC RNA-sequencing datasets from The Cancer Genome Atlas (TCGA), we identified 58 piRNAs significantly differentially expressed in HNSCC current smokers vs. normal lifelong non-smokers and 39 piRNAs significantly dysregulated in HNSCC HPV16 (+) vs. normal HPV(-) cohorts, with 29 piRNAs commonly implicated in both etiologies (p < 0.05). Of the 58 piRNAs implicated in smoking-related HNSCC, 4 piRNAs were found to significantly associate with clinical variables and 1 transcript with patient outcome, with similar results for HPV-mediated piRNA dysregulation (Kruskal-Wallis, Cox regression, p < 0.05). In particular, we recognized transcripts NONHSAT081250, NONHSAT123636, and NONHSAT108298 for their correlations with tumor stage, metastatic status, and/or anatomic site, as well as presence of common mutations and copy number variations in HNSCC. We then analyzed mRNA expression data for the same 466 samples from TCGA and found that TCGA mRNA profiles may be implicated in smoking-related and HPV-related HNSCCs respectively, and also to associate with piRNA candidate expression levels (p < 0.05). We subsequently validated the dysregulation of all significant piRNA and PIWI protein candidates in vitro in cigarette-treated and HPV E6/E7-overexpressing normal oral epithelial cells. While a comprehensive understanding of the functional and mechanistic relevance of non-coding RNAs in HNSCC remains yet to be found, our findings reveal the potential of these genes to mediate a variety of etiology-specific clinical and genomic aberrations in HNSCC and provide novel insights to thwart the progression of this disease.

#3489 Noncoding RNA distribution in clear cell renal cell cancer: small RNA-seq data. Srinivas V. Koduru, Dino J. Ravnic. Penn State University, Hershey, PA.

Small noncoding RNAs (snRNAs) play pivotal roles in biological processes and may prove to be a tool in cancer diagnosis, prognosis, and treatment. Non-coding RNAs such as microRNAs (miRNAs), piwi-interacting RNAs (piRNAs), long noncoding RNA (IncRNAs) and small nuclear/nucleolar RNAs (sn/snoRNAs) have recently been investigated to identify their role in cancer. Approximately 62,700 new cases of kidney cancer are expected each year in the United States leading to 14,240 deaths, with rates rising over the past 20 years. Nearly 90% of all kidney cancers will be renal cell carcinoma, of which 70% will be a clear cell renal cell carcinoma (ccRCC) subtype. When detected early most cases can be treated effectively, however there is a lack of early detection tests. We sought to identify molecular markers for early detection and diagnosis. We downloaded publically available (NIH bioproject PRJNA162979) ccRCC small RNA sequence raw data for noncoding RNA analysis. 22 ccRCC and 11 non-tumor renal cortex patient samples were evaluated. Data was analyzed by PartekFlow software. sRNA-seq data was annotated with miRNAs with 69 miRNAs aberrantly expressed in ccRCC with 37 being upregulated (top five: miR-
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122, mir-210, miR-155, miR-224 and miR-21) and 32 being downregulated (top five: miR-20c, miR-502, miR-20b, miR-10a and miR-204). Utilizing MetaCore software for pathway analysis the majority of expressed miRNAs were involved in metabolism related signaling pathways. Examining data for piRNAs revealed 22 which were significantly expressed (8 upregulated and 14 downregulated). Investigation of lncRNAs revealed 15 to be downregulated and 27 upregulated. We also uncovered 2 mitochondrial RNAs, 10 miscRNAs, 1 snRNA, 7 snoRNAs, and 5 rRNAs in cCRCC samples. Our comprehensive analysis of publically available small RNA-seq data identified numerous lncRNAs associated with cCRCC. Pending further validation, they may prove useful as early detection biomarkers in this common cancer.

#4390 Interrogation of small RNA-seq data for small noncoding RNA expression in human colon cancer. Srinivas V. Koduru,1 Angélique Nyinawabera,2 Dino J. Ravnic,3 Amit K. Tiwari,2 1Pennsylvania State University, Hershey, PA; 2University of Toledo, Toledo, OH.

Genomic analysis of the human transcriptome has been made possible only in last decade by next generation sequencing (NGS). Recent advancements in NGS has further allowed us to look into small non-coding RNAs (snRNAs) such as microRNAs (miRNAs), Piwi-interacting RNAs (piRNAs), long non-coding RNAs (lncRNAs) & small nuclear/nucleolar RNAs (sn/snoRNAs). Recently, the roles of snRNAs in biological processes have been implicated in biomarker development for diagnosis, prognosis & therapy. In the present study, 50 colon cancer patient’s small RNA sequencing raw data was downloaded from NIH bioproject (PRJNA266667;7 TNM stage II & 43 TNM stage III) which contained 27 female & 23 male samples. 24 samples had metachronous metastasis (MM) & 26 non-metachronous metastasis (NMM). The small RNA-seq data was analyzed using PartekFlow. In depth analysis showed aberrant expression of 48 miRNAs (all upregulated) in TNN-M vs TNN-II specimens & 20 miRNAs (17 upregulated & 3 downregulated) in MM vs NMM. Further investigation of dysregulated miRNA through pathway analysis confirmed that the majority of the miRNAs were involved in cancer signaling pathways. Analysis of piRNA found unusual expression of 60 piRNAs (57 upregulated & 3 downregulated) in TNN-M vs TNN-II & 31 piRNAs (28 upregulated & 3 downregulated) in MM vs NMM. Further analysis of long non-coding RNAs, we found 77 lncRNAs were significantly expressed in TNN-M vs TNN-II & 818 lncRNAs in MM vs NMM. We also, investigated small nuclear/nucleolar RNAs (sn/snoRNAs), miscRNAs & miRNAs, we identified 37 snRNAs, 105 snRNAs, 28 miscRNAs & 5 snRNAs in TNN-M vs TNN-II whereas 2 snRNAs, 11 snRNAs, 57 miscRNAs & 8 miRNAs in MM vs NMM were identified. In summary, our comprehensive analysis on publicly available small RNA-seq data identified multiple small non-coding RNAs that need to be further explored for their use in the prognosis, diagnosis & therapy of colon cancer.

#4391 A role for the long non-coding RNA GHRLOS in cancer. Esha T. Shah, Penny Jeffrey, Patrick Thomas, Inge Seim, Lisa Chopin. Ghrelin Research Group, Translational Research Institute-Institute of Health and Biomedical Sciences, Queensland University of Technology, Brisbane, Australia.

Long non-coding RNA (lncRNA) genes are abundant in the human genome, and many are now recognized as oncogenes and/or tumor suppressors. We previously characterized the structure of GHRLOS, an antisense gene on the opposite strand of the multifunctional ghrelin gene (GHRL), however, its expression and function in disease has not been described. To this end, using The Cancer Genome Atlas (TCGA) data set, we revealed that GHRLOS is differentially expressed in a number of cancers. In particular, expression was elevated in endometrial cancer (1.2-fold; P = 7.1 × 10^-14) and prostate cancer (1.2-fold; P = 3.7 × 10^-14; n = 52 vs. n = 498) compared to normal tissues. Using qRT-PCR and commercial cDNA panels we confirmed that GHRLOS expression was upregulated in endometrial cancer (1.96-fold, P = 0.005 Welch’s two-sample t-test; n = 24 vs. n = 175) and prostate cancer (2.46-fold, P = 0.0045 Welch’s two-sample t-test, n = 5 vs. n = 21) compared to normal tissues. Initial studies, using siRNA designed to silence endogenous GHRLOS expression, significantly reduced cell migration in the PC3 prostate cancer cell line (0.47-fold change, P = 0.042 Kruskal-Wallis test, n = 2) conversely, preliminary data using forced GHRLOS overexpression increased migration and proliferation. Taken together, we show that the long non-coding RNA GHRLOS is differentially expressed in tumor tissue and regulates cell migration and proliferation; possibly by modulating alternative splicing of the overlapping, multifunctional ghrelin gene locus. Targeting GHRLOS could provide a valuable and novel way to target the ghrelin axis in disease. Ongoing studies aim to validate in vitro functional results in complementary mouse xenograft models and identify genes and pathways regulated by this novel lncRNA.

#4392 Subtype-specific expression of small non-coding RNAs in breast cancer. Jaana M. Hartikainen,1 Sami Heikkinen,2 Maria Tengström,3 Veli-Matti Kosma,4 Arto Mannermara,5 1School of Medicine, Institute of Clinical Medicine, Pathology and Forensic Medicine, and Cancer Center of Eastern Finland, University of Eastern Finland, Kuopio, Finland; 2Institute of Biomedicine, University of Turku, Turku, Finland; 3Clinical Department of Medical Oncology, Oncology, and Cancer Center of Eastern Finland, University of Eastern Finland, and Cancer Center, Kuopio University Hospital, Kuopio, Finland; 4School of Medicine, Institute of Clinical Medicine, Pathology and Forensic Medicine, and Cancer Center of Eastern Finland, University of Eastern Finland, and Imaging Center, Kuopio University Hospital, Kuopio, Finland.

We have performed a small RNA-sequencing (RNA-Seq) experiment to find candidate small non-coding RNAs (ncRNAs) in breast cancer (BC) for the identification of signatures that aid in the prediction of therapy responsiveness, prognosis or patient outcome. We used 195 invasive BC tumor and 20 benign breast tissue samples from the Kuopio Breast Cancer Project (KBCP) study material. KBCP material is collected from a genetically homogeneous population of Eastern Finland, and includes fresh-frozen and FFPE breast tissue samples, EDTA blood samples and serum, as well as comprehensive background/lifestyle information, clinical, treatment and follow-up data extending over 20 years. This well-defined material enables the identification of factors associated with BC risk, different stages of disease progression and outcome. Total RNA was extracted from the fresh-frozen BC and pre-existing tissue using the Ambion mirVana miRNA Isolation Kit. Small RNA-Seq was performed using the Illumina TruSeq Small RNA Library Prep kit and the Illumina MiSeq next-generation sequencing instrument. Analyses were concentrated in PIWI-interacting RNAs (piRNAs) and other non-miRNA small ncRNAs (snRNAs). Bioinformatic analysis commenced with quality control and preprocessing steps including read quality assessment (FastQC), adapter trimming (TRIMMOMATIC), and removal of e.g. ribosomal RNA reads (Bowtie). Preprocessed reads were aligned (Tophat) to human reference transcriptomes (piRNABank and GENCODE non-miRNA snRNAs), followed by data conversions e.g. for visualization (samtools, IGVtools). Gene-wise read counts were collected using summariizeOverlaps R package, and statistically differentially expressed (DE) genes were identified using DEseq2 R package. We first compared the small RNA expression in the benign breast tissue with invasive BC and observed in total 635 snRNAs and 94 piRNAs with differential expression (P < 0.05). Of these 520 snRNAs and 73 piRNAs were upregulated, and 115 snRNAs and 21 piRNAs downregulated in the invasive tumors compared to benign breast tissue. When comparing the luminal BC subtype with triple-negative BC (TNBC) we observed 204 DE (P < 0.05) snRNAs and 32 piRNAs. Of these, 103 snRNAs and 18 piRNAs were upregulated, and 101 snRNAs and 14 piRNAs downregulated in TNBC compared to luminal tumors. Differential expression was also observed between ER (estrogen receptor) negative and ER positive invasive tumors. Of the 242 DE snRNAs and 32 piRNAs, 105 snRNAs and 12 piRNAs were upregulated, and 137 snRNAs and 20 piRNAs were downregulated in ER positive BC compared to ER negative BC. Preliminary results of hierarchical clustering and principal component analysis of normalized log2 piRNA and snRNA expression levels revealed the potential for finding differences between BC subtypes. The subsequent statistical analysis enabled us to identify individual piRNAs and other ncRNAs as candidate druggable biomarkers for e.g. TNBC patients.

#4393 LncRNA as potential target in drug-resistant melanomas. Rosaura Esteve-Puig,1 Martina Sanlórenzo,1 Igor Vujic,1 Kevin Lai,2 Marin Vujic,1 Dallas Mould,1 Kevin Lin,1 Juan Oses-Prieto,1 Shreya Chand,1 Christian Posch,3 Alma Burlingame,4 Susana Ortiz-Urdá,4 1University of California, San Francisco, San Francisco, CA; 2University of Turin, Turin, Italy; 3Rudolfstiftung Hospital, Academic Teaching Hospital, Vienna, Austria.

Melanoma is the most common lethal form of skin cancer. Activating mutations in BRAF and RAS oncogenes account for over 80% of all melanomas and lead to activation of MAPK (mitogen activated protein kinase) mitogenic pathways in melanoma therapies over the past decade, drug resistance limits their efficacy. Long noncoding RNAs (lncRNAs) are a new class of genes that do not encode proteins, which have been characterized as epigenetic regulatory molecules. lncRNAs have emerged as important players in cancer, potential predictors of therapeutic responses and targets for new treatments; however, their functions and precise molecular mechanisms remains largely unexplored. Our study aims to find novel potential targets involved in the acquisition of resistance to MAPK inhibitors. We identified an intergenic lncRNA (lincRNA) highly differentially expressed in drug-resistant melanoma samples, called MAPK Inhibitor Resistance Associated Transcript (MIRAT). We determined that MIRAT is localized
mainly in the cytoplasm and plays a role in modulating the signal transduction pathways without regulating the genomic neighborhood. We discovered that MIRAT is upregulated in early drug tolerance to MAPK inhibition. Mechanistically, the gene overexpression and silencing of MIRAT, as well as preliminary RNA-protein interaction results, showed that it modulates the MAPK signal transduction. Benefiting from a modulating effect on a MIRAT-expressing cell line, these results highlight the relevance of cytoplasmic lncRNA’s and their role in oncogenic signaling pathways, and further suggest that lncRNAs and their protein-binding partners may serve as novel potential targets for overcoming drug resistance in melanoma.

#3494 Dissecting the cellular expression and subcellular localization of lncRNAs implicated as prognostic biomarkers in non-small cell lung carcinomas by RNA ISH. Courtney M. Anderson, Na Li, Xiao-Jun Ma, Emily Park. Advanced Cell Diagnostics, Newark, CA.

Background: Long non-coding RNAs (lncRNAs) are involved in many biological processes, including epigenetic modification, cell differentiation, and apoptosis. More recently, lncRNAs have emerged as unique biomarkers that may be associated with a physiological or diseased state, and several lncRNAs have been identified as prognostic markers in a wide variety of human cancers. Because lncRNAs do not translate into protein, their discovery in the tumor is entirely dependent upon RNA detection. The majority of lncRNAs are also expressed at very low levels compared to mRNAs and may be more heterogeneously expressed than mRNAs. Therefore, detection of lncRNAs in tumor biopsies requires a highly sensitive and specific detection method that can discern single-cell and subcellular localization. Methods: Single-cell and molecular RNA in situ hybridization (ISH) is a well-suited method for the detection of lncRNAs in tumor biopsies because it allows for the visualization of single RNA molecules with morphological context. To interrogate the expression pattern of lncRNAs within the tumor and its microenvironment, we performed ISH using the RNAscope assay on FFPE tissue microarrays consisting of archived tissue samples of 53 primary non-small cell lung carcinoma (NSCLC) tumors and 4 adjacent normal lung tissues. We examined the expression of 4 lncRNAs which have been implicated as potential prognostic markers of lung cancer: AFAP-AS1, ANRIL, HOTAIR, and UCA1. Results: These lncRNAs were detected in approximately 40-60% of the 53 NSCLC tumor cores and in none of the 4 adjacent normal lung tissues. Expression of these 4 lncRNAs was observed predominantly in tumor cells, with little to no detection in the stroma. Some lncRNAs displayed a heterogeneous expression pattern, with some tumor cell foci displaying strong lncRNA signal while other foci in the same tumor did not display signal. Furthermore, some lncRNAs displayed heterogeneous cell expression in the same foci, with some cells expressing very high levels of lncRNA while other cells in the same foci had little to no lncRNA expression. An evaluation of each lncRNA in serial sections revealed that of the 4 lncRNAs, tumors expressed all 4 lncRNAs in the same tumor foci. Lastly, little to no signal was observed for the prostate cancer-specific lncRNA PC3A in the NSCLC tumors and no PC3A expression was detected in adjacent normal lung tissues. Conclusions: These results demonstrate the ability of the ISH assay to detect potential lncRNA biomarkers in lung cancer samples in situ, allowing for the identification of single-cell and subcellular localization. These results provide precise tumor expression patterns of lncRNAs. Identification of the subcellular localization and cell-to-cell expression patterns of lncRNAs can facilitate greater understanding about their specific biological roles in cancer and other diseases.

#3495 The DNA methylation landscape of long noncoding RNAs in human cancer. Da Yang, Zehua Wang, Bo Yang, Min Zhang, Zhiyuan Wu. University of Pittsburgh, Pittsburgh, PA.

Integrating multi-dimensional genomic and epigenetic data from the TCGA, ENCODE and CCLE projects, we comprehensively characterized the epigenetic landscape for lncRNA genes in 9837 tumor samples across 33 cancer types. This analysis revealed that lncRNAs can be both epigenetically silenced and activated by DNA methylation alteration in the promoter region, which is in striking contrast to the well-documented hypermethylation of protein-coding gene promoters in tumor. To determine the corresponding transcriptional changes of lncRNAs with epigenetic alterations in tumors, we applied a heuristic strategy to identify the epigenetic silenced and activated lncRNAs by integrating the transcriptional profile of 58,648 lncRNAs from 6475 TCGA RNA-seq files. This analysis characterized a patient-centric matrix with DNA methylation status of 3380 epigenetically regulated lncRNAs across 6475 tumor samples. Some epigenetically activated lncRNAs exhibited an “on or off” pattern with completely no expression in normal tissues, which potentiated them as promising diagnostic biomarkers. To rule out the interference of tumor associated stromal and immune cells in lncRNA analysis, we investigated the RNA-seq and DNA methylation profiles of 784 cell lines from breast, lung, and bladder cancer. Most of epigenetically regulated lncRNAs identified in tumor samples showed prominently negative correlation between their expression and promoter methylation in cancer cell lines. We then validated the epigenetically activated lncRNAs by performing decitabine treatment on cell lines. The decitabine treatment led to a significant and time-dependent induction of lncRNA expression and loss of DNA methylation at lncRNA promoter. Further survival analysis indicated that epigenetically activated lncRNAs were generally correlated with poor survival while the epigenetically silenced lncRNAs were generally correlated with good survival. Among the epigenetically silenced lncRNAs we well-documented tumor suppressor lncRNAs such as MEG3. And lncRNA oncogenes, such as MINGCR, are epigenetically activated in multiple cancer types. To delineate the relationship between lncRNAs and known cancer pathways, we performed mutual exclusive analysis by combining the lncRNA epigenetic landscape with known protein-coding tumor genes alterations in same tumors. This analysis nominated several lncRNAs as novel members in cancer pathways, including TP53, P13K, and EGFR cascades. Indeed, functional experiment validation focus on the top epigenetically activated lncRNA (EPIC1) demonstrated that EPIC1 is a potential oncogene. In breast and ovarian cancer cell lines, EPIC1 promotes cancer cell colony formation and increases cell viability by promoting cell cycle pathway. Collectively, our integrative study provides a resource for investigating lncRNAs in cancer and lays the groundwork for the development of novel diagnostics biomarkers and treatments.

#3496 Long non-coding RNAs in colorectal cancer. Alena Opattova,1 Fabio Miguel Ferreira,2 Jozef Horak,3 Sonja Vodenkova,2 Pavel Vodicka1.1 Institute of Experimental Medicine, Prague, Czech Republic; 2Research Centre of IPO-Porto, Porto, Portugal; 3Third Faculty of Medicine, Charles University, Prague, Czech Republic.

Colorectal cancer (CRC) is the third most common type of cancer worldwide and the second most common cause of cancer related deaths in Europe. Since Countries of Central Europe (Czech Republic, Slovakia, Hungary) have one of the highest rates both for incidence and mortality of CRC, CRC became a serious health, social and economic problem. CRC pathogenesis involves multiple genetic events. On behalf of a strong experimental work, there is still lack of predictive, prognostic and treatment-response related biomarkers. Long non-coding RNAs (lncRNA) are non-protein coding transcripts longer than 200 nucleotides. Deregulation of lncRNAs is often seen in a variety of human diseases, including cancer. Taken together, this makes lncRNAs the potential regulators of cancer progression, therapeutic targets or markers of treatment efficacy. The main aim of our present study was to define expression patterns of lncRNAs in colorectal tumor tissue and define their role in cancer cell proliferation, survival and DNA damage response (DDR). In the first part of our study, we analyzed the expression profile of 83 lncRNAs known to be associated with cancer progression in cohort of CRC tumor tissues and adjacent mucosa. We found 12 upregulated lncRNAs (|p|<0.05) and 10 downregulated lncRNAs (|p|<0.05). Our results have been replicated on larger set of patients (100 CRC patients) and those lncRNAs associated with cell proliferation, survival and DNA damage response are functionally tested on colorectal cell lines (HCT116, T84) in vitro. Definition of role of lncRNA as tumor suppressors or oncogenes in CRC etiology may lead to better understanding of regulation of CRC carcinogenesis. Furthermore, understanding the role of lncRNA in DDR may contribute to better treatment response of CRC patients and to define markers of individual treatment response. Acknowledgement: GACR 15-14795S, AZV 15-27580A, GAUK082016, AVZ 15-26353A, SUSCEPTCOST LD14005

#3497 Network-based systematic inference of lncRNA activity in breast cancer. Matthew Ung, Daniel Mattiox, George Wang, Chao Cheng. Geisel School of Medicine at Dartmouth, Hanover, NH.

The production of lncRNA is widespread across the human genome, yet the functions of lncRNAs and the phenotypes resulting from their activation have remained largely underexplored. In cancer, several lncRNAs have been found to associate with disease development and poor patient prognosis including HOTAIR and MALAT1. Recent studies have been performed using lncRNA microarrays or re-annotated probes from coding gene microarrays to systematically investigate their roles in cancer. However, datasets from lncRNA microarrays are typically not accompanied by comprehensive clinical information that can be used to draw associations between lncRNA expression and clinical phenotypes. Furthermore, lncRNA stability and cellular localization is highly variable suggesting that expression-based inference of lncRNA activity may not always be an accurate measure of its activity. To address this issue, we introduce an activity-based approach whereby we analyze the expression of lncRNA target...
genes to infer lncRNA activity. Specifically, we construct a breast cancer specific lncRNA-target gene network from RNA-seq data using a network reconstruction algorithm. We then apply this network to several breast cancer microarray datasets to systematically investigate lncRNA activity and their association with clinical phenotypes. Notably, we find that certain lncRNAs are active in specific microarray datasets in breast cancer even when their expression levels do not reveal significant differences. Lastly, we performed survival analysis to systematically screen for novel lncRNAs whose activity associate with patient prognosis. Our results suggest that activity-based inference of lncRNAs can identify novel lncRNAs that may serve as therapeutic targets or biomarkers in breast cancer.

**#3498 Characterization of a novel metastatic lung cancer associated lncRNA.** Nicole M. White, Emily B. Rozyci, Ha X. Dang, Christopher A. Maher. Washington University School of Medicine, Saint Louis, MO.

Lung cancer is the leading cause of death worldwide with the majority of patients presenting with metastasis and moreover, developing resistance to current treatments. One promising therapeutic area is the novel class of long non-coding RNAs (lncRNAs). While emerging evidence implicates them in cancer, lncRNA biology is still largely unexplored in tumor metastasis. To elucidate the function of lncRNAs in lung cancer, our lab used transcriptional sequencing (RNA-Seq) to discover 120 long non-coding RNAs (lncRNAs) that are enriched in metastatic tumors compared to their corresponding primary lung tumors, which we have named Metastasis Associated Lung cancer lncRNAs (MALLs).

Notably, metastatic tumors were enriched for differentially expressed protein-coding genes targeted by Polycomb Repressive Complex 2 (PRC2). In lung cancer studies, the catalytic subunit of PRC2, EZH2 (an H3K27 methylase), is overexpressed, associates with poor prognosis, promotes tumor progression, and epigenetically represses genes to promote metastasis. Specifically, we have found MALL-1 to be (i) highly up-regulated in lung cancer; (ii) promotes proliferation and migration/invasion, hallmarks of oncogenic phenotypes; (iii) binds to EZH2; and (iv) cooperatively represses well-known PRC2 target genes associated with metastasis in patients. Further, we found MALL-1 to be altered in multiple solid tumors thus suggesting a critical conserved oncogenic role of MALL-1 in solid tumor progression. We believe MALL-1 promotes aggressive disease through its interactions with, and recruitment of the PRC2 complex to chromatin, to epigenetically modify genes associated with metastasis.

**#3499 Differentially expressed small noncoding RNAs in triple-negative breast cancer.** Srinivas V. Koduru,1 Amit K. Tiwari,2 Ashley Leberfinger,1 Sprague W. Hazard,1 Yuko Imamura Kawasawa,2 Milind Mahajan,3 Dino J. Ravnic.1 1Pennsylvania State University-Hershey, Hershey, PA; 2University of Toledo, Toledo, OH; 3Icahn School of Medicine, Mount Sinai, New York, NY.

Cancer is the second leading cause of death in the United States and is a major public health concern worldwide. Basic, clinical and epidemiological research is leading to improved cancer detection, prevention, and outcomes. Recent technological advances have allowed unbiased and comprehensive screening of genome-wide gene expression. Small non-coding RNAs (snRNAs) have been shown to play an important role in biological processes and could serve as a diagnostic, prognostic and therapeutic biomarker for specific diseases. Recent findings have begun to reveal and enhance our understanding of the complex architecture of snRNA expression including miRNAs, piRNAs, sn/snoRNAs and their relationships with biological systems. We used publicly available small RNA sequencing data that was derived from 24 triple negative breast cancers (TNBC) and 14 adjacent normal tissue samples to remix various types of snRNAs. We found a total of 55 miRNAs were aberrantly expressed (p<0.005) in TNBC samples (8 miRNAs upregulated; 47 downregulated) compared to adjacent normal tissues whereas the original study reported only 25 novel miRs. In this study, we used pathway analysis of differentially expressed miRNAs which revealed TGF-beta signaling pathways to be profoundly affected in the TNBC samples. Furthermore, our comprehensive re-mapping strategy allowed us to discover a number of other differentially expressed snRNAs including piRNAs, sn/snoRNAs, rRNAs, miscRNAs and nonsense-mediated decay RNAs. We believe that our snRNA analysis workflow is an inclusive and suitable for discovery of novel snRNAs changes, which may lead to the development of innovative diagnostic and therapeutic tools for TNBC.

**#3500 Epstein-Barr virus (EBV) noncoding RNAs modulate host gene expression for virus latency leading to oncogenesis in epithelial cells.** Rob Verhoven, Bobo Wing-Yee Mok, Shuang Tong, Honglin Chen. The University of Hong Kong, Hong Kong. Hong Kong.

Epstein-Barr virus (EBV) efficiently establishes and maintains a state of latency in resting B cells, with persistent asymptomatic infections occurring in more than 95% of the human population worldwide. Besides causing lifelong infections in resting B cells in the majority of people, EBV has been found to associate with several human cancers occurring in immune competent individuals. A hallmark of EBV infected cells through a distinct viral latency program. It is believed that this type of mechanism has been adopted by EBV to allow the virus to evade host immune surveillance. Nasopharyngeal carcinoma (NPC) is prevalent in southern China, Southeast Asian countries and some African countries but uncommon in rest of the world. In NPC 100% of cancer cells are infected with EBV, and EBV is recognized as one of the major risk factors. The details of how EBV maintains latency and contributes to the oncogenesis of NPC remain elusive. Notably, EBV abundantly expresses a family of non-coding RNAs derived from the BamH1I region of the viral genome (BARTs). It is hypothesized that this family of viral non-coding RNAs is key to maintaining viral latency, evading immune surveillance and triggering oncogenesis in EBV associated tumors. We have shown a mechanism for the regulation of the abundant expression of BARTs in NPC which involves the constitutive activation of NF-κB signaling typically observed in NPC cells and have also identified a regulatory loop through which BARTs support EBV latency in NPC. Using high throughput RNA-seq analysis we further demonstrated that BART RNAs modulate expression of cellular genes associated with anti-inflammatory and immune-modulating properties, oxoderoxidase activity, and cell migration and invasion in NPC cells. BART non-coding RNAs are predominantly localized in the nucleus and associated with the CBP/p300 complex. Our data showed that BARTs modulate genes responsive to the mitochondria-associated molecular device (MAVS) through halting cellular Pol II in the promoter region and down-regulating gene expression. In the nucleus, BART non-coding RNAs mediate chromatin acetylation through interaction with CBP/p300. We have generated a working model with evidence showing that Epstein-Barr virus shuts off most of the viral proteins to evade immune surveillance, but through expression of high levels of BART non-coding RNAs can modulate cellular gene expression to provide an optimal environment for maintenance of viral latency in cancer cells. Alteration of cellular gene expression by EBV BART non-coding RNAs for establishment and maintenance of EBV latency in epithelial cells can therefore lead to the oncogenic process for EBV associated tumors.

**#3501 Prognostic value of a 4 lncRNA signature in patients with non-small cell lung cancer: a lncRNA expression analysis.** XiaoRan Long,1 HuiYun Wang,2 ChunLei Ge,2 Xin Song,2 MeiYin Zhang,1 XiaoFeng Zhen. 1Cancer center of Sun Yet-sen University, Guangzhou, China; 2Cancer Biotechnology, Institute of the Third Affiliated Hospital of Kansung Medical University, KunMing, China; 3Rutgers Cancer Institute of New Jersey and Department of Pharmacology, Robert Wood Johnson Medical School, Rutgers University, New Brunswick, NJ.

Recently, increasing evidence indicates that lncRNA plays an important role in the cancer development and progression. In this study, we are aimed to identify lncRNA signature for predicting survival of non-small cell lung cancer (NSCLC) patients. A total of 366 NSCLC cases, which were divided into a training and validation set, were detected by lncRNA microarray (containing 2412 lncRNA probes), and another 73 cases as an independent set were analyzed by qRT-PCR. LncRNA microarray analysis identified a total of 305 differentially expressed lncRNAs (138 up-regulated and 167 down-regulated) between NSCLC and normal tissues in the training set. We then validated the microarray data by qRT-PCR analysis of 5 lncRNAs (2 up-regulated and 3 down-regulated) in 30 paired NSCLC and adjacent lung tissues randomly selected from the training set. We then used a stepwise forward procedure to select the best combination of lncRNAs with the most significance in prediction of NSCLC prognosis and a 4-lncRNA prognostic signature was established, which was significantly associated with overall survival (hazard ratio [HR]: 3.563, 95% confidence interval [CI]: 2.118-5.995, P < 0.001) and disease-free survival (HR: 3.236, 95% CI: 2.116-4.940, P < 0.001) in the training set. This prognostic signature was validated in the validation (OS: HR: 3.186, 95% CI: 1.849-5.488, P < 0.001; DFS: HR: 2.967, 95% CI: 1.949-4.517, P < 0.001) and independent sets (OS: HR: 4.703, 95% CI: 1.923-11.50, P < 0.001; DFS: HR: 4.549, 95% CI: 2.139-9.674, P < 0.001). This 4-lncRNA signature has been demonstrated as an independent prognostic factor, which significantly improves the prognostic accuracy of TNM staging system in a combined model as assessed by ROC curve analyses in the three sets, respectively. Therefore, the 4-lncRNA signature has potential prognostic role in NSCLC patients.
#3502 RNAi screen identified novel long non-coding RNAs involved in cell growth control, migration, and invasion.
Tse-Chun Kuo, Sheng-Chieh Hsu, Hui-Ju Chou, Pei-Shan Li, Szu-Huei Wu, Yen-Ling Yu, Jen-Shin Song, Hsing-Jien Kung. NHRIL, Taiwan, Zhunan Town, Miaoli, Taiwan.

Triple-negative breast cancer (TNBC) is a highly aggressive form of breast cancer often associated with poor prognosis. Owing to the lack of therapeutic targets, patients with TNBC tend to rely on standard chemotherapy; however, chemo-resistance often develops in TNBC as a result of deregulated cell death control like in many other tumors. Long non-coding RNAs (lncRNAs) are transcripts longer than 200bp and have little or no protein-coding capacity. Although once considered as transcriptional noise, significant numbers of lncRNAs are now recognized as modulators of protein expression and functions and are implicated in a range of developmental processes and diseases. As many of them influence oncogenes and tumor suppressors in cancer, they represent excellent biomarkers as well as potential targets for therapy. In order to gain more insight on cell death regulation and to seek potential targets for TNBC, we employed an RNAi screen targeting 2231 lncRNAs in MDA-MB-231 TNBC cells. By using changes in cell metabolic activity (MTT assay) as readouts for primary screen and changes in cell number as confirmation, our unbiased screen identified two novel lncRNAs that drastically reduced cell proliferation when depleted. In addition to cell growth, the two lncRNAs also contribute to cancer cell migration and invasion. Interestingly, our gene profiling analyses and RNA immunoprecipitation studies show the two lncRNAs are involved in signaling cascades related to metastatic mammalian tumorigenesis necessary for E-cadherin and β-catenin. We are now trying to delineate the detailed molecular mechanism(s) by which the two lncRNAs contribute to TNBC tumorigenesis. Together, our current results suggest a possibility for the two novel lncRNAs as drug targets in triple-negative breast cancer.

#3503 Transcriptome analysis reveals differentially expressed lncRNAs between oral squamous cell carcinoma and healthy oral mucosa.
Lu Feng, John H. Houck, Pawadee Lohavanichbutr, Chu Chen. Fred Hutchinson Cancer Research Center, Seattle, WA.

Objective: Oral cavity and oropharyngeal squamous cell carcinoma (OSCC) is a major cancer type in the head and neck region. To better understand the roles long non-coding RNA (lncRNA) play in OSCC carcinogenesis, we compared the genome-wide gene expressions of lncRNA in OSCC and in the oral mucosa from healthy individuals. Materials and methods: We compared the expression levels of 3,054 probe sets for lncRNAs between 167 OSCCs and 45 healthy controls using an Affymetrix HG U133 plus 2.0 array platform. We validated the top differentially expressed lncRNAs in three independent datasets in the Gene Expression Omnibus (GEO) repository: GSE42743, GSE9844, and GSE6791. We further tested the differential expression of the three lncRNAs showing the highest fold change by quantitative RT-PCR in a subset of 20 OSCCs and 10 control samples. Results: We found 658 lncRNA transcripts (790 probe sets) to be significantly differentially expressed between OSCC and healthy oral mucosa using a criteria of FDR<0.01, with 36 of them (39 probe sets) showing more than a 2-fold change. Among the 36, 14 (15 probe sets) lncRNAs were validated in all three independent datasets using the criteria FDR<0.01: LOC441178, C Sorf66-6 AS1 (hLINC01315), LINC00478, and COX10-AS1/LOC100506974. RT-PCR showed a greater than 5 fold change in expression between matched normal liver (NL) and OSCC tissues. In addition, expression of miR-21 and miR-21 were significantly associated with poor prognosis of HCC patients. More importantly, expression of exosomal lncRNA-ATB and miR-21 was more significantly associated with HCC progression and poor patients survival compared to its tissue expression. We conclude that non-coding lncRNAs (lncRNA-ATB and miR-21) expression has the potential to serve as biomarkers for prognosis and targeted therapy in HCC patients.

#3505 The expression of two key long non-coding RNAs involved in reprogramming, linc-ROC and linP21, in gastric cancer.
Mitra Khalili,1 Pouran-dokht Farhangiyan,2 Somayed Jahanbodost,3 Fatemeh Kamali.2 Zanjan University of Medical Sciences, Zanjan, Islamic Republic of Iran;2 Tehran University of Medical Sciences, Tehran, Islamic Republic of Iran.

Introduction: Gastric cancer (GC) is the second leading cause of cancer-related death in the world and the most common cancer in southeast of Caspian Sea (Golestan province) and northwest of Iran (Ardabil). Based on cancer stem cell (CSC) hypothesis, there are similarities between process of somatic cell reprogramming, embryogenesis and tumorigenesis. Malfuction of signaling pathways that are essential for normal development also involved in the tumor initiation and CSC formation. In recent years long non-coding RNAs (lncRNAs) more than 200 nt in length, have been highly regarded as promising biomarkers for early diagnosis and prognosis of cancers. Reprogramming-Related Long Intergenic Noncoding RNA (lincRNA-ROR) plays as modulator in the reprogramming of human induced pluripotent stem cells (iPSCs) and also maintenance of embryonic stem cells (ESCs).

On the other hand, lincRNA-P21 induced by P53 and prevents the somatic cell reprogramming by silencing of pluripotency gene promoters. In this project, motivate to CSCs, we evaluated the expression level of lincRNA-ROR and linc-P21 in samples of patients with gastric cancer and also in human gastric cancer cell lines (AGS and MKN45) and human embryonal carcinoma cell line (NT2). Methods: Thirty pairs of gastric samples, including gastric adenocarcinoma and their matched non-tumor tissue samples, were collected from the Iran National Tumor Bank (INTB). Cell lines were cultured in the RPMI1640. Total RNA of samples and cell lines was extracted using TRIZOL reagent (invitrogen). cDNA synthesis was performed by PrimeScript™ 1st strand cDNA Synthesis Kit (TAKARA) and real time PCR was performed by using TaqMan master mix (TAKARA) on Step one Plus™ instrument (ABI).

Gene expression analysis performed by GenEx software program. Fold change for determining the high, medium and low expression of lincRNA-ROR in some cancers, our results showed no expression for linc-ROR in gastric samples (both tumor and non tumor) and also gastric cancer cell lines. But NT2 embryonal carcinoma cell line revealed high expression of linc-ROR compared to AGS and MKN45cell lines. According to our expectation, linc-P21 represents a significant decrease in tumor versus non tumor tissues and linc-ROC has been identified as a marker of malignancy and also in GC and MKN45 versus to NT2 cell line. Conclusion: Dysfunction of linc-ROR and lincRNA-P21 has reported in some cancers. According to our results, it seems that the expression of linc-ROR is limited to embryonic stem cells and hasn’t remarkable expression in gastric samples and cell lines. Based on the low expression of linc-P21 in tumor samples, it seems that linc-P21 functions as a tumor suppressor.

#3506 ncRNA regulation of eribulin response in neuroblastoma.
Yiqiang Zhang,1 Xiuye Ma,3 Xiaoie Yu,3 Harsh Patolia,2 Zhenze Zhao,3 Liqin Du,3 Alexander Pertsemlidis1.

1University of Texas Health Science Center at San Antonio, San Antonio, TX;2Virginia Tech Carilion School of Medicine, Roanoke, VA;3Texas State University, San Marcos, TX.

Introduction: Neuroblastoma (NB) is the most common cancer in infants and the most common extracranial solid tumor in children, accounting for 15% of all childhood cancer deaths. The overall prognosis for those with high-risk or relapsed disease remains poor despite the standard therapies of surgery, radiation, and high dose chemotherapy. We have previously shown that altering levels of specific microRNAs (miRNAs) can improve cellular response to microtubule-targeting agents (MTAs) and that exposure to MTAs results in dramatic changes in microRNA expression. Methods: Libraries of chemically synthesized ncRNA mimics and inhibitors, including both miRNAs and lncRNAs, are screened to identify ncRNAs that regulate neuroblastoma cell viability or sensitize neuroblastoma cells to specific mi-
crotubule-targeting agents. Candidate targets are validated using qRT-PCR, protein quantification, and luciferase reporter assays. The response of neurolastoma cells to perturbations in candidate ncRNA levels is assessed through flow cytometric analysis of cell cycle phase distribution, density and anisotropy of microtubule bundles, and through colony formation and caspase 3/7 activation validated in response xenograft models. Networks of miRNAs, mRNAs and lncRNAs are derived from combining complementary cellular response with potential regulatory interactions. Results: We previously reported the identification of miRNAs and lncRNAs that significantly decrease or increase neuroblastoma cell viability. Here, we extend those observations to drug response. We first demonstrate that the microtubule-targeting agent eribulin has both short-term and long-term effects, as reflected by a significant alteration in microtubule structure in response to high doses for short times and arrest at the G(2)/M phase of the cell cycle in response to low doses for long times. We next show that altering intracellular levels of ncRNAs that sensitize cells to eribulin both alter microtubule density and anisotropy and recapitulate the effect of eribulin treatment. Finally, we show that complementarity of effect between miRNAs and lncRNAs is accompanied by potential regulatory interaction. Conclusions: Taken together, our results suggest that the response of neuroblastoma cells to eribulin is mediated by a regulatory network that includes different coding and non-coding RNA species. While these RNAs may have intrinsic value as biomarkers or therapeutic agents, either individually or in combination, the vulnerabilities that they uncover may be exploited with pathway-specific perturbations. This project was supported by NIH R01 CA129632 and CPRIT Training Grant RP140105.

#3507 PVT1 exons 4A, 4B, and 9 are overexpressed in aggressive prostate cancer, and PVT1 exon 4B may distinguish between indolent and aggressive prostate cancer. Akintunde T. Orunmuyi,1 Adeodat Ilboudo,2 Olabiyi G. Ogun,3 Cuong B. Bach,2 SA. Adebayo,3 Ayo A. Salako,2 EOluwabunmi Olapade-Akintunde,1 University of Ibadan and University College Hospital (UCH), Ibadan, Nigeria; 2Hunter College CUNY, New York, NY; 3University of Ibadan and University College Hospital (UCH), Ibadan, Nigeria; 4Obafemi Awolowo University Teaching Hospital, Ille Ife, Nigeria; 5Hunter College CUNY and Weill Cornell Medicine, New York, NY.

Aggressive prostate cancer (PCA) disproportionately affects males of African ancestry (MoAA). However, the underlying molecular mechanisms are unclear. Chromosome 8q24 is a PCA susceptibility locus that harbors the PVT1 non-coding gene. We previously demonstrated that PVT1 exon 9 may be involved in aggressive PCA. Moreover, using the most recent full-genome variability panel from the 1000 Genomes project, we recently identified a string of 75 SNPs in a PCA patient that seemed to be associated with PCA susceptibility. Here, we report that PVT1 exon 9 and 4B are overexpressed in aggressive prostate cancer tissues compared to indolent prostate cancer tissues. In the 1000 Genomes project, we recently identified a string of 75 SNPs in a PCA patient that seemed to be associated with PCA susceptibility. Here, we report that PVT1 exon 9 and 4B are overexpressed in aggressive prostate cancer tissues compared to indolent prostate cancer tissues. Western Blotting data of the expression in prostate cancer tissues.

#3508 EWS-FLI1 represses Rho-actin signaling via MRTFB/YAP-1/ TEAD perturbation in Ewing Sarcoma. Anna M. Katschnig,1 Maximilian O. Kauer,1 Raphaela Schwentner,1 Eleon M. Tomazou,1 Markus Linder,1 Cornelia N. Mutz,1 Javier Alonso,1 Dave N. Aryee,1 Heinrich Kovar1. 1Children’s Cancer Research Inst., Vienna, Austria; 2Institute of Cancer Research of the Medical University Vienna, Vienna, Austria; 3Unidad de Tumores Infantiles, Instituto de Investigación de Enfermedades Raras, Madrid, Spain.

Ewing Sarcoma (Ews) is an aggressive pediatric bone tumor driven by the aberrant fusion-oncogene EWS-FLI1, which deregulates hundreds of genes by either activation (EWS-FLI1-correlated genes) or repression (EWS-FLI1-anti-correlated genes). Several EWS-FLI1-anticorrelated genes are involved in cytoskeletal processes and typically regulated by Rho/F-actin signaling. The Rho pathway is a crucial regulator of cell movement and cellular plasticity. The activation of Rho triggers G- to F-actin polymerization thereby enabling nuclear translocation of the myocardin-related transcription factors MRTF (MRTF-A) and MRTF-B (also known as MRTFB). MRTFs typically serve as transcriptional co-activators of the transcription factor SRF regulating several cytoskeletal key players. We used the A673/TR/shEFT cell line, harboring a doxycycline inducible sh-EWS-FLI1 plasmid, to interrogate the role of MRTF/B in the Ews gene regulatory network upon EWS-FLI1-high and -low conditions. Strikingly, MRTFB transcriptional activity was overall repressed by EWS-FLI1. Furthermore, knockdown of MRTF/A and MRTF/B inhibited EWS-FLI1-induced transactivation of EWS-FLI1-anticorrelated genes. ChIP-seq revealed a strong overlap of MRTFB and EWS-FLI1 chromatin binding. MRTFB binding was significantly enriched in distal (enhancer) regions of EWS-FLI1-anticorrelated genes, especially upon EWS-FLI1-low conditions. Of note, an overrepresentation of TEAD motifs, but not SRF binding motifs, was observed in these regions suggesting a potential involvement of TEAD transcription factors in the regulation of the MRTFB/ EWS-FLI1 reciprocally regulated targets. In line with this finding, target genes of the mehano sensitive YAP/TAZ/TEAD pathway (CTGF, CYR61, SERPINE1) were found among the MRTFB-bound EWS-FLI1-anticorrelated genes. Genome-wide expression profiling upon combined knockdown of EWS-FLI1 and all four TEADs confirmed that TEAD regulates EWS-FLI1 target genes antagonistically. ChIP-qPCR for selected genes validated this finding demonstrating increased TEAD binding to shared MRTFB/EWS-FLI1 target genes upon decreased EWS-FLI1 occupation. Our data suggest that MRTFB/YAP-1/TEAD directly regulate EWS-FLI1-anticorrelated targets. Since, in addition, EWS-FLI1 is known to indirectly perturb F-actin polymerization, we propose a model of dual EWS-FLI1 driven MRTFB/YAP-1/TEAD perturbation by direct and indirect mechanisms downstream of Rho/F-actin signaling.

#3509 EWS/FLI regulates transcriptional activation via length-dependent GGA microsatellites. Kirsten M. Johnson, Cenny Taslim, Stephen L. Lessnick, Nationwide Children's Hospital, Columbus, OH.

Objective: The purpose of this study is to investigate how EWS/FLI transcriptionally activates gene targets via polymorphic GGA microsatellites. Ewing Sarcoma is a pediatric bone malignancy initiated by a (t(11;22)) chromosomal translocation that produces the EWS-FLI1 oncoprotein. EWS/FLI transcriptionally activates and represses its target genes to mediate oncogenic reprogramming. Expression of its up-regulated targets correlates with EWS/FLI binding to associated GGA microsatellites, which show length polymorphisms. These microsatellites polymorphisms may critically affect EWS-FLI responsiveness of key gene targets. For example, NROBI is necessary for EWS/FLI mediated oncogenic transformation, and we found a “sweet-spot” of 20-25 repeat length as optimal for EWS/FLI mediated transcriptional activity at NROBI through clinical observation and in vitro studies. The mechanism underlying this optimal length is unknown. Methods: We explored the stoichiometry and binding affinity of EWS/FLI to associated GGA microsatellites, which show length polymorphisms. We used the mechano sensitive YAP/TAZ/TEAD pathway (CTGF, CYR61, SERPINE1) as a potential EWS-FLI1-anticorrelated genes. ChIP-seq revealed a strong overlap of MRTFB and EWS-FLI1 chromatin binding. MRTFB binding was significantly enriched in distal (enhancer) regions of EWS-FLI1-anticorrelated genes, especially upon EWS-FLI1-low conditions. Of note, an overrepresentation of TEAD motifs, but not SRF binding motifs, was observed in these regions suggesting a potential involvement of TEAD transcription factors in the regulation of the MRTFB/ EWS-FLI1 reciprocally regulated targets. In line with this finding, target genes of the mehano sensitive YAP/TAZ/TEAD pathway (CTGF, CYR61, SERPINE1) were found among the MRTFB-bound EWS-FLI1-anticorrelated genes. Genome-wide expression profiling upon combined knockdown of EWS-FLI1 and all four TEADs confirmed that TEAD regulates EWS-FLI1 target genes antagonistically. ChIP-qPCR for selected genes validated this finding demonstrating increased TEAD binding to shared MRTFB/EWS-FLI1 target genes upon decreased EWS-FLI1 occupation. Our data suggest that MRTFB/YAP-1/TEAD directly regulate EWS-FLI1-anticorrelated targets. Since, in addition, EWS-FLI1 is known to indirectly perturb F-actin polymerization, we propose a model of dual EWS-FLI1 driven MRTFB/YAP-1/TEAD perturbation by direct and indirect downstream of Rho/F-actin signaling.
Molecular and Cellular Biology / Genetics: Oncogenic Transcription Factors

#3510 A role for the Ewing's sarcoma breakpoint protein EWS in ERG-induced prostate tumorigenesis. Vivekananda Kedage, Nagarathinam Sellaj, Soumen Chakraborty. Indiana University, Bloomington, IN.

The TMPRSS2-ERG gene fusion occurs in one-half of prostate tumors and results in expression of ERG, an ETS family transcription factor that promotes prostate tumorigenesis. Another 5-10% of prostate tumors have similar rearrangements that result in over-expression of other ETS family members such as ETV1, ETV4, and ETV5. However, not all ETS family members are oncogenic and normal prostate cells express at least 15 of these other ETS factors. Therefore, it is important to understand mechanisms that differentiate oncogenic ETS factors from non-oncogenic ETS factors. We have recently discovered that ERG, ETV1, ETV4, and ETV5, and no other ETS factor can directly bind the protein EWS. This is striking, as EWS is fused to various ETS factors in the chromosomal rearrangements that cause Ewing's sarcoma. We demonstrate that fusion of EWS to any ETS protein is sufficient for that protein to function like ERG in prostate cells. Furthermore, the EWS-ERG interaction is required for ERG to activate an oncogenic gene expression program and for ERG-mediated tumorigenesis. These findings reveal new mechanisms for prostate cancer and Ewing's sarcoma. In prostate cancer, aberrant over-expression of an ETS factor that naturally interacts with EWS, brings EWS to ETS-bound regions. In Ewing's sarcoma this interaction is forced by the presence of an EWS/ETS fusion protein. Therefore, this model predicts that treatments that target EWS function would be effective in both types of cancer.

#3512 Ikaros regulation of the BCL6/BACH2 axis and its clinical relevance in acute lymphoblastic leukemia. Chunhua Song, Zheng Ge, Jianyong Li, Baoan Chen, Elanora Dovat, Katarina Dovat, Jonathon Payne, Sinisa Dovat. Pennsylvania State University College of Medicine, Hershey, PA; Zhejiang Hospital, Medical School of Southeast University, Nanjing, China; The First Affiliated Hospital of Nanjing Medical University, Nanjing, China.

Ikaros regulates expression of the BCL6/BACH2 axis in B-ALL. High BCL6 and low BACH2 expression is associated with prostate cancer and Ewing’s sarcoma. In prostate cancer, aberrant over-expression of an ETS factor that naturally interacts with EWS, brings EWS to ETS-bound regions. In Ewing’s sarcoma this interaction is forced by the presence of an EWS/ETS fusion protein. Therefore, this model predicts that treatments that target EWS function would be effective in both types of cancer.

#3513 Decoding the role of myocyte enhancer factor 2C in chronic myeloid leukemia disease progression. Soumen Chakraborty. Institute of Life Sciences, Bhubaneswar, India.

Chronic myeloid leukemia (CML) is a myeloproliferative disorder of hematopoietic stem cell and is marked by the presence of the Philadelphia chromosome, a reciprocal translocation occurring between the long arm of chromosome 9 and 22 resulting in the formation of a fusion BCR-ABL1+ oncogene which encodes a constitutively active non-receptor tyrosine kinase. Clinically, the disease progresses through three phases: an initial chronic phase, accelerated phase and terminal blast phase, which is marked by increased production of immature myeloid precursor cells. Earlier we have shown that BCR-ABL mediates repression of miR-223 results in the activation of me2C and ptbp2 in chronic myeloid leukemia. Transcription factor ME2C, required for cell survival and proliferation, is important for lymphopoiesis, and its ectopic expression in myeloid progenitors antagonizes myelopoiesis. We reported that ME2C expression increases as the disease progresses from chronic phase to blast phase and by knocking down ME2C in a CML cell line we showed that it negatively correlates with the CEBA expression, a granulocytic transcription factor. Ima-tinib, the drug of choice for CML, abrogates ME2C expression and reverses CEBA repression both in the cell line and the primary cells. In the present study we observed that when the cells were treated with anisomycin it degraded ME2C expression in 16hrs which imatinib can achieve after 24hrs. Investigators have demonstrated that anisomycin acts as a potent signaling agonist, synergizing with growth factors and phorbol esters to superinduce immediate-early genes such as c-fos, fosb, c-jun, Junb, and JunD. However here blocking the p38 pathway with inhibitors didn’t reverse the inhibition suggesting that these pathways may not be involved in the anisomycin mediated ME2C degradation in CML. Me2C gene consists of three alternative exons, α1/α2a, skipping exon β and β’ and splice site γ. In order to characterize the role of me2C gene in CML, first we investigated the different isoforms of me2C in CML cell line (KCL22) by amplification of the whole me2C gene and cloning the same in a TOPO vector. Several clones were checked and sequenced. Two different types of isoforms (α1’ β γ’ and α1’ β’ γ) were found to coexist in KCL22 cells along with an exclusion of 9 bases (AACACAGGT) from the splice junction of exon 4 and 5, which was not reported earlier. However, K562 cells along with KYO-1 and LAMA-84 showed the presence of 9 bases. Nine base pair deletion was also observed in CML patient samples (both CP and BC). Interestingly sequencing of the CML patient samples (both CP and BC) showed these deletions and in patients with high levels of BCR-ABL showed the presence of 9 bases. The different isoforms were transduced in mouse lineage negative bone marrow cells and also transplanted in mice either alone or in combination with BCR-ABL to study the effect of the isoforms in disease progression, the results of which will be discussed.

#3514 Assessing the role of CREB in fibrolamellar hepatocellular carcinoma with PRKACA-DNAJB1 fusion protein. Anju Karki, Michael J. LaQuatta, Amanda M. Dios, Zhaozhe Sade-Savelkoul, Khushboob handi 1Boston Children’s Hospital, Harvard Medical School, Boston, MA; 2Mass General Institute for Neurodegenerative Disease, Massachusetts General Hospital, Harvard Medical School, Boston, MA.

Introduction: Fibrolamellar Hepatocellular Carcinoma (FLHCC) is a rare form of liver cancer that occurs predominantly in adolescents or young adults. Curing or prolonging the lives of patients with FLHCC is extremely challenging. Despite aggressive surgical resections of primary tumors as well as metastatic lesions, outcomes tend to be poor due to lack of effective chemotherapy. Previous studies have demonstrated the presence of PRKACA-DNAJB1 fusion protein in the majority of FLHCC patient samples. DNAJB1 is a heat shock protein and PKA is a kinase that phosphorylates numerous substrates including cAMP-regulatory element-binding protein (CREB), a proto-oncogenic transcription factor. Therefore, we sought to examine whether the presence of the fusion protein in FLHCC is associated with increased CREB phosphorylation. Methods: We analyzed tumor and matched non-neoplastic liver tissue from 7 pediatric patients with a pathologic diagnosis of FLHCC. We assessed the presence of the PRKACA-DNAJB1 fusion protein using PKA antibody, as well as total CREB and phosphorylated (p-CREB) using Immunoblot analyses with specific antibodies. Results: Immunoblot analysis using anti-PKA antibody confirmed the occurrence of PRKACA-DNAJB1 fusion transcripts in 6/7 (86%) FLHCC tumor samples. Immunoblot analyses of the 6 patients with the fusion protein revealed a trend towards a decrease in p-CREB expression in tumor samples compared to non-neoplastic tissue controls (p=0.15). Conclusions: The pattern of p-CREB in FLHCC with PRKACA-DNAJB1 fusion protein suggests that the fusion protein does not exert its proto-oncogenic effects through the transcriptional activity of CREB.

#3515 LHX9 is a novel oncogene bypassing KRAS addiction. Seu Hong Ly, Chao Dai, Andrew L. Hong, Nina Ilic, Elsa B. Krali, William C. Hahn. Dana Farber Cancer Institute/Broad Institute of Harvard and MIT, Boston, MA; Boston Children’s Hospital/Dana Farber Cancer Institute/Broad Institute of Harvard and MIT, Boston, MA.

Background: Oncogenic mutations of RAS are detected in approximately 30% of human cancers. KRAS is highly mutated in pancreatic, colorectal and lung cancers. Direct therapeutic targeting of Ras and its membrane association has been challenging due to high affinity of GTP to Ras and unexpected mechanism of alternative post-translational modification. While few inhibitors of downstream RAS effectors - MAPK and PI3K pathways - show some clinical efficacy, they are complicated by complex feedback loops, and as single agents are not effective in KRAS-mutant cancers. Combination therapies may be promising yet limited by narrow therapeutic window. Identification of alternative strategies to
directly inhibiting RAS and MAPK/PI3K pathways is key to address this unmet need. Methods: To this end, we performed a genome-scale open reading frames screen to identify genes capable of restoring viability to KRAS-dependent cells following KRAS suppression. We tested the top 5 standard-deviation hits in low-throughput manner. One of the top hits included LIM homeobox 9 (LHX9), a homeobox family transcription factor implicated in growth and metastasis of pancreatic cancer cells. While LHX9 is amplified in several cancers, little is known regarding its roles in cancers. We used hypothesis-driven as well as unbiased approaches including RNA-seq, ChiP-seq, and IP-mass spectrometry to investigate the basis of LHX9-mediated bypass of KRAS dependency. Results: We showed that LHX9 rescued KRAS suppression in multiple cell lines as well as xenograft. LHX9 overexpression substituted for KRAS in KRAS-driven xenograft. Moreover, in activated-MEK-overexpressing HA1E cells, LHX9 formed tumors as robustly as myristoylated AKT. LHX9 rescued KRAS suppression by both restoring KRAS-dependent pathways and activating KRAS-independent pathways. First, LHX9 reactivated the main KRAS effectors - MAPK and PI3K pathways and conferred resistance to MEK inhibitor Trametinib. Second, using ChiP- and RNA-seq, we observed LHX9 binding to promoters/enhancers of genes whose expression was regulated by KRAS, particularly YAP1. Third, LHX9 increased expression and activation of KRAS-independent genes, including STAT3. Conclusions: Here, we identified LHX9 as a novel bypass of KRAS addiction. Multiple unbiased approaches revealed at least three mechanisms by which LHX9 rescued KRAS suppression. Our findings contribute to our understanding of KRAS biology. Further investigation of downstream effectors of LHX9 may highlight potential novel therapeutic targets for KRAS-dependent cancers.

#3516 RUNXI controls EGFR signaling pathway in non-small cell lung cancer cells. Akihiko Matsuo. Graduate School of Medicine, Kyoto University, Kyoto, Japan.

Epidermal growth factor receptor (EGFR) is a promising target for anticancer therapy for non-small lung cancer (NSCLC). Mitogen-inducible gene 6 (Mig6) is known to be a negative feedback regulator of EGFR. Mig6 inhibits EGFR kinase activity and promote wild-type EGFR trafficking to the degradation pathway. Down-regulation of Mig6 is found in poor prognostic NSCLC. Previous gene array analysis results suggested that Mig6 is downregulated by runt-related transcription factor 1 (RUNXI), yet the detailed relationship between RUNXI and Mig6 in NSCLC has not been elucidated. Herein we demonstrate that RUNXI inhibition is effective for wild-type EGFR NSCLC. Tet-inducible shRNA mediated RUNXI knockdown in wild-type EGFR cell line LUS99A led to the increased expression of Mig6 and decreased expression of phosphorylated form of EGFR as well as deactivation of EGFR/ERK pathway. RUNXI knockdown subsequently induced apoptosis in LUS99A cells. Silencing of Mig6 in LUS99A cells promoted the proliferation of these cancer cells and increased expression of phosphorylated form of EGFR, highlighting the importance of this EGFR signaling cascade in the maintenance of wild-type EGFR NSCLC. Indeed, RUNXI overexpression promoted cell proliferation and decreased expression of Mig6 and increased phosphorylated form of EGFR. Notably Mig6 knockdown in RUNXI knockdown LUS99A cells increased their proliferation rates, indicating that RUNXI is an important role in the migration of RUNX1-mediated RUNXI pathway. We next examined the efficacy of a novel small molecule which specifically binds and inhibits the RUNXI (We named it as CM). CM was drastically effective for wild-type EGFR NSCLC cell lines A549 and LUS99A which are naturally resistant to EGFR Tyrosine kinase inhibitor gefitinib. In addition, CM suppressed tumor growth of xenotransplanted A549 cells in immune-deficient mice in vivo. Our study underscores the importance of RUNXI inhibition therapy in the management of gefitinib-resistant NSCLC patients.

#3518 Brg1 plays a critical role in PanIN formation through regulating Sox9 expression. Motoyuki Tsuda,1 Akihisa Fukuda, 1 Satoshi Ogawa, 1 Kenji Masuo,2 Norihiro Goto,1 Yukiko Hiramatsu,1 Yu Mut,1 Kozo Ikuta,1 Yoshito Kimura,1 Yoshimwe Matsumoto,1 Yutaka Takada,1 Takuto Yoshioka,1 Takahisa Maruno,1 Haruhiko Akiyama,2 Kyosaki Takao,1 Shinji Uemoto,1 Tsutomu Chiba,1 Hiroshi Seno1. 1Kyoto University, Kyoto, Japan; 2Gifu University, Gifu, Japan.

Background: Mutations of SWI/SNF chromatin remodeling complexes are one of the hallmarks of human pancreatic ductal adenocarcinoma (PDAC). Brahma related gene 1 (Brg1) is a catalytic ATPase subunit of SWI/SNF complexes and Brg1 is silenced in about 10% of human PDAC. Recent study revealed that Brg1 inhibits formation of intraductal pancreatic mucinous neoplasm (IPMN) and IPMN-derived PDAC in the context of oncogenic KRAS and that IPMNs originate from adult pancreatic ductal cells, demonstrating a tumor suppressive role of Brg1. However, the role of Brg1 in the formation of pancreatic intraepithelial neoplasm (PanIN) from acinar cells and PanIN-derived PDAC is not fully understood. Aim: To investigate the functional role of Brg1 in the formation of PanIN.Methods and Results: We generated Ptf1a-Cre;KrasG12D;Brg1f/f;Sox9OEmice, inwhichSox9isconstitutivelyexpressedinRUNX1knockdownLU99Acellsincreasedtheirproliferationrates,indicatingthatRUNX1overexpressionpromotedcellproliferationanddecreasedexpressionofEGFRsignalingcascadeinthemaintenanceofwild-typeEGFRNSCLC.Indeed,ADMformationwasmarkedlyreducedintheabsenceofBrg1andmore,weconfirmedthatSox9expressionisdownregulatedinBrg1-depletedacinarcells. Furthermore, we confirmed that Sox9 expression is downregulated in Brg1-depleted ADMS/PanINs compared with Brg1-retained ADMS/PanINs by immunohistochemistry. To determine the functional relationship between Brg1 and Sox9, we generated Ptf1a-Cre;KrasG12D;Brg1f/f;Sox9OE mice, in which Sox9 is constitutively overexpressed. Remarkably, Sox9 overexpression canceled the phenotype of decrease in PanIN formation by Brg1 deletion. Furthermore, we investigated whether Brg1/Sox9 axis is observed in human PanIN-derived PDACs. We performed immunohistochemistry of Brg1 and Sox9 in human PanIN-derived PDACs. We found that low expression of Brg1 and high Sox9 expression had low Sox9 expression. Conclusions: Our results suggest that Brg1 plays a tumor-supportive role in acinar cell-derived PanIN formation in part through regulating Sox9 expression positively. Our data underscore a cell-type specific, context-dependent role of Brg1 in the initiation of PDAC formation.
#3519 TGF-β pathway alteration in pancreatic neuroendocrine tumors: characterization of a novel SMAD3 translocation

Emanuela Brunetto,1 Greta Grassini,1 Valeria De Pacsali,1 Anna Talarico,1 Francesca Invernizzi,2 Michaela Bowden,2 Massimo Loda,2 Luca Albarello,1 Massimo Falconi,1 Lorenza Pecchiari,1 Maria G. Cangi,1 Claudio Doglioni,1 San Raffaele Scientific Institute, Milano, Italy; 2Dana-Farber Cancer Institute, MA.

Pancreatic neuroendocrine tumors (PanNETs) are the second most common malignancy of the pancreas, and the only curative option is represented by surgery. PanNETs can be classified as well- or poorly-differentiated, based on histology. Poorly-differentiated neuroendocrine carcinomas (NECs) represent less than 10% of cases, but are highly malignant and associated with poor prognosis, and, most importantly, their clinical management is based on insufficient data on NEC genetic features. In order to widen our knowledge of PanNET genetic alterations, we performed RNAseq analysis using Illumina NextSeq platform of a group of 18 PanNETs and 2 NECs. TopHat-Fusion algorithm identified, among others, a fusion involving SMAD3 transcription factor and CCDC149; the alteration of SMAD3 in a poorly differentiated NEC was particularly interesting since SMAD3 is involved in TGF-β signaling, which has an oncogenic role in malignant cells. The SMAD3-CCDC149 fusion was confirmed in the index case by RT-PCR using primers spanning the breakpoint, followed by Sanger sequencing. The fusion was also detected by FISH analysis performed on both FFPE and frozen samples. Both by FISH analysis and Sanger sequencing we identified two other poorly-differentiated NEC samples characterized by the presence of the SMAD3-CCDC149 fusion. We explored the impact of dysregulation of SMAD3 in PanNETs. The results of our study show that the SMAD3-CCDC149 fusion results in increased p27/CDKN1B protein that mediates cell cycle exit by terminally differentiating cell-like in vitro, and increased activation of GATA3, WT1 and HNF4A, decreased MYC transcription and p53 protein levels. Our findings lead to the identification of a new fusion protein, which might allow the identification of a new therapeutic target.

#3520 PBRM1 inactivation in renal cell cancer alters master transcription factor hub composition to repress instead of activate epithelial-differentiation pathways. Xiaorong Gu, Francis Enane, Reda Mahfouz, Tomas Radiyovevich, Bartlomiej Przychodzen, Yvonne Parker, Daniel Lindner, Brian Rini, Yogen Saundra Ghosh, Addanki P. Kumar. University of Texas Health Science Center at San Antonio, San Antonio, TX.

PBRM1, a cohesin subunit involved in DNA replication and repair, was recently identified as a tumor suppressor functionally equivalent to PTEN. PBRM1 loss in RCC thus alters the expression of master transcription factors to repress instead of activate epithelial-differentiation, an effect that can be reversed pharmacologically for novel, p53/p16-independent differentiation-restoring therapy.


SIRT1, a nicotinamide adenine dinucleotide (NAD)-dependent deacetylase, is a member of multigene family of Sirtuins. Traditionally SIRT1 is a known player in enhancing longevity. SIRT1 can target both histone and non-histone proteins to regulate diverse activities including cellular stress resistance, genomic stability, energy metabolism and tumorigenesis. Disruption of SIRT1 in the prostate results in the formation of prostate intraepithelial neoplasia (PIN) lesions, suggesting a role for SIRT1 in the development of PIN, a precursor lesion of prostate cancer (PCA). Paradoxically, an oncogenic role for SIRT1 was first reported as evidence of development of micro-invasive prostate carcinomas in Pten−/−/Sirt1tg mice. Therefore, SIRT1 may possess both oncogenic and tumor suppressor activities in a context-dependent manner and its role in human cancer remains controversial. To address these paradoxical findings, we examined the role of SIRT1 in prostate pathogenesis using stably silenced for SIRT1 prostate cancer cell lines. Silencing SIRT1 in androgen responsive LNCaP cells resulted in (i) morphological changes indicative of clustering and epithelial mesenchymal transition (EMT); (ii) significant reduction in their ability to form colonies on soft agar; and (iii) increased autophagic flux. Similarly, silencing SIRT1 resulted in reduced proliferative, colony forming and migratory ability of these cells. Remarkably, SIRT1 KD cell lines showed decreased expression of AR and its target genes including PSA, TMPRSS-2, FKBP-5 and PMEPA1. Interestingly, SIRT1 KD reduced PSA-luciferase activity under both normal physiological and androgen deprivation growth conditions with no significant effect on nuclear or cytoplasmic levels of AR. Moreover, SIRT1 KD significantly increased AR sensitivity to growth under androgen deprivation conditions. Intervention with resveratrol (RES, known SIRT1 activator) reduced incidence of HGPIN in prostate specific PTEN KO mouse model with no significant differences in the expression of AR or SIRT1. Consistent with these in vivo observations, SIRT1 had no significant impact on RES-induced growth inhibition. Taken together, these data indicate that SIRT1 can function non-canonically to provide a context-dependent, independent of classical AR signaling. Our study demonstrates that SIRT1 may not play an essential role in mediating RES-induced tumor growth inhibition and that SIRT1 may confer resistance to androgen deprivation therapy and sustains cell survival, suggesting that combination of SIRT1 inhibitors and ADT might be a potential strategy for advanced PCA treatment. Supported by CPRIT RP 150166 and NCI CA 137158 (APK).

#3522 Structural characterization of androgen receptor variant 7 in prostate cancer. Fatma Özgün,1 Zeynep Kaya,1 Halil Bayraktar,2 Selen Manioglu,2 Doğançan Özturan,1 Nathan Lack1. 1Koç University School of Medicine, Istanbul, Turkey; 2Koç University Department of Chemistry, Istanbul, Turkey.

Prostate cancer is an extremely common disease that affects one in every seven men in their lifetime. The standard care for late-stage cancer is designed to inhibit the activation of androgen receptor (AR). While this therapeutic approach is initially efficient, the cancer almost always develops resistance and gives rise to castration-resistant prostate cancer (CRPC). When therapy fails, the median survival of patients who suffer from CRPC is 12-16 months. It is critical to understand the intratumor of CRPC better to ameliorate the outcome of late-stage prostate cancer patients. AR is a transcription factor that consists of an N-terminal domain (NTD), a DNA binding domain (DBD) and a ligand binding domain (LBD). Upon activation by androgen, an intramolecular interaction between NTD and LBD occurs. The AR translocates into the nucleus where it
forms a homodimer. It binds to the promoter and enhancer elements of the AR target genes and triggers gene transcription. It has been demonstrated in many clinical studies that AR is important for the progression of prostate cancer including CRPC. In CRPC, although androgen production and binding is inhibited, how the AR can still be activated is a fundamental question to be answered. One of the several different mechanisms proposed is the alternative splicing of the AR generating truncated AR variants that lack LBD. These variants do not require androgens to be activated and they are intrinsically resistant to clinically approved therapeutics. Despite the fact that the AR variants have been shown to be critical in late-stage prostate cancer, the mechanism of gene transcription initiation by the AR variants is not well-known. We propose to characterize the mechanism of the AR variant activation. Since ARV7 is the most commonly observed variant, we utilize ARV7. We develop a FRET based methodology to identify how ARV7 dimers form and to test if full-length AR is necessary for transcriptional activation mediated by ARV7. We are able to demonstrate possible intermolecular interaction between the full length AR and ARV7 measuring the FRET intensity between the fluorophores attached to the N and C termini of two proteins. FRET measurements are analyzed by tracking single cells and recording the FRET intensities during the process. Our preliminary data suggest that the full-length AR and ARV7 may interact in the presence of androgen. ARV7 is always localized in the nucleus whereas the AR requires androgen to be activated and translocates into the nucleus. Being in close proximity, putative interaction between the AR and ARV7 occurs in the nucleus. Additionally, we identify and DRD point mutants to determine the determinants of intermolecular interaction between them. Supporting the significance of ARV7 in prostate cancer, our results clarify the dimerization process. It gives an opportunity to test clinical drugs currently in development to effectively inhibit ARV7 activation.

#3523 Arginylationtransferaseas a novel suppressor of prostate cancer metastasis. Michael Birnbaum, Ahkilesh Kumar, William Morgan, Fangliang Zhang. Univ.of Miami, Miami, FL

Arginylation is a post-translational modification wholly mediated by Arginylation transferase 1 (Ate1). Our studies have shown that Ate1 and arginylation are upregulated during stress conditions. In addition, it was recently shown that Ate1-deficient fibroblasts demonstrate cancer-like characteristics including genomic instability and mutagenesis. While Ate1’s role in cancer is poorly understood, our preliminary studies suggest that Ate1 is an important regulator of metastasis and cancer progression in prostate cancer models. In this study, our data suggests that Ate1 is essential for normal cell stress response in conditions including cellular oxidant, apoptosis inducing drugs, and radiation. Further, we show that a loss of Ate1 in Lncap prostate cancer cells increases anchorage-independent growth in the soft-agar assay, and that a loss of Ate1 in PC-3 prostate cancer cells increases matrigel invasion in the Boyden chamber assay. To text how Ate1 affects metastasis in vivo, PC-3 cells with Ate1 or control shRNA were orthotopically implanted into the prostates of immunocompromised mice to observe metastasis. While very little metastasis was observed in PC-3 control cells, PC-3 cells with reduced Ate1 achieved much higher levels of metastasis. In conclusion, our data suggests that the loss of Ate1 in prostate cancer promotes cancer progression phenotypes and cell survival. In future studies, we will examine if Ate1 levels in human prostate cancers in prostate cancer progression.

#3524 HOXB13 collaborates with AR to alter the cellular phenotype of prostate epithelial cells through cytokertan 14 upregulation. Dorhuny Johng, Charles M. Ewing, William B. Isaacs. Johns Hopkins University, Baltimore, MD.

Prostate cancer (PCa) is a commonly diagnosed disease with one of the highest heritability estimates among cancers. Among germline variations that account for PCa heritability, a recurrent mutation (G84E) in HOXB13 is associated with a 3 to 6 fold increase in PCa risk. HOXB13 is a prostate-specific transcription factor that plays a role in prostate development. Other mutations in HOXB13 in PCa patients have further implicated HOXB13 in PCa biology. The goal of our study is to investigate HOXB13 in cell line models of normal prostate tissue to examine the role of HOXB13 in driving cellular differentiation and/or transformation. To this end, we utilized 957E/hTERT, a cell line derived from a radical prostatectomy specimen, as a model for normal prostate epithelial cells. HOXB13 WT or G84E was stably expressed in 957E/hTERT with a lentivirus system in the presence and absence of exogenous AR. HOXB13 caused a morphological change whereby an epithelial morphology transitioned to a fibroblastic-like structure. Cells adopted elongated bodies with a frequent appearance of lamellipodia. The morphologic conversion was transient in AR- cells but was maintained in AR+ cells. Analyses of epithelial cell markers using qPCR revealed a change in the epithelial cell marker profile (AR+: no change p63, CD49f ↓, K5 ↓, K14 ↓, K18 ↓, AR+: no change p63, CD49f, K5, K14, K18 ↓). K14 upregulation was striking with a >10 fold increase. AR alone could upregulate K14 to a level comparable to HOXB13-induced K14 in AR- cells, which was surprising since AR mainly functions in luminal cells where K14 expression is turned off. Coexpression of HOXB13 and AR had a synergistic effect on K14 upregulation. Despite the morphologic and K14 change suggestive of EMT, vimentin was downregulated by HOXB13 in AR- and AR+ cells. In vitro growth assays showed that HOXB13 reduced the proliferation rate of AR+ cells but not AR- cells. Gap closure assays revealed that HOXB13 does not induce a qualitative change in migration. Similar results were obtained with G84E models. Our study demonstrates that HOXB13 may have a potential function in dictating a luminal epithelial phenotype through EMT. This may have important implications for PCa biology. HOXB13 is a promising therapeutic target as potential drug to induce EMT in PCa cells.

#3525 PDEF promote prostate cancer luminal/epithelial differentiation and inhibit tumor progression. Fengtian Wang,1 Sweaty Koul,1 Prakash Srinivasan Timaru Shannahmugam,1 Qin Dong,2 Hari K. Koul2. Louisiana State University Health Sciences Center-Shreveport, Overton Brooks VA Medical Center, Shreveport, LA; 2Louisiana State University Health Sciences Center-Shreveport, Overton Brooks VA Medical Center, Feist Weiller Cancer Center, Shreveport, LA.

Conventional therapies produce a high rate of cure for patients with localized prostate cancer (PCa), but there is no effective treatment for castration resistant metastatic PCa. Transcription factors by association with enhancer and super enhancer elements are key drivers of cell identity. Our previous studies have shown that Sam Pointed Domain Ets Transcription Factor a.k.a. Prostate Derived Ets Factor (SPDE/PDEF), inhibits cell migration, invasion and clonogenic activity in PCa cells and there is a graded loss of PDEF with increasing Gleason Score in PCa patient samples. PDEF has been reported to be one of the cell identity transcription factors in LNCaP cells. We proposed that PDEF functions as a putative tumor metastasis suppressor. Others have shown an increase in Twist1, a BHLH transcription factor, was positively correlated with Gleason Score in PCa patients. PDEF has been reported to be one of the epithelial/luminal differentiation genes in PCa. PDEF expression is limited to epithelial/luminal cells of the prostatic glands. We observed that expression of Twist1 was down regulated in PC3 cells following PDEF expression. Analysis of gene expression data from our microarray studies revealed that PDEF re-expression was associated with negative enrichment of gene sets involved with cell migration and positive enriched of gene sets involved with epithelial/luminal differentiation. Chip-seq analysis revealed binding of PDEF to KRAB/16 promoter region. These data suggest that PDEF inhibits core metastasis related genes through promoting a program of luminal/epithelial differentiation. In clinical samples of PCa, expression of PDEF was inversely associated, while expression of Twist1 was positively associated with Gleason grade. PDEF and Twist1 was able to predict patient survival, moreover integrated PDEF and Twist1 expression was able to better predict PCa patient survival as compared to PDEF or Twist1 alone. PDEF inhibits cell migration and metastasis in part by down-regulating Twist1 level and promoting luminal/epithelial differentiation. Loss of PDEF combined with gain of Twist1 expression may serve as a potential biomarker set for distinguishing lethal PCa from indolent disease. Additional studies are underway to gain further insights into the role of PDEF in PCa progression and metastasis. Acknowledgement: FWCC support and Chair commitment funds (Koul H).

#3526 The expression of MLK, a growth regulator of triple-negative breast cancer, is regulated by p53. Lakshmi Reddy Bollu, Powel H. Brown, Abhijit Mazumdar, Dekuang Zhao, Yanxia Ma. MD Anderson Cancer Center, Houston, TX.
Background: Triple negative breast cancer (TNBC) is the most aggressive form of breast cancer with poor prognosis. Due to frequent distant metastasis and lack of successful targeted therapies, the overall survival rate in TNBC patients is significantly lower than estrogen receptor positive (ER) and HER2 positive breast cancers. To identify potential druggable targets, previously, we performed gene expression analysis and identified genes that are highly expressed in TNBC. Through these studies, we discovered that the expression of maternal embryonic leucine zipper kinase (MELK) is highly elevated in TNBCs and correlated with p53-mutation status and metastasis-free survival. Inhibition of MELK reduced the growth and invasiveness of p53 mutant breast cancer cells. Methods: In this study, we analyzed the association of MELK expression with p53 status using publicly available datasets, and examined the role of wild-type and mutant P53 in regulating MELK expression. MELK expression at mRNA and protein levels were determined through Q-RT-PCR and western blotting analysis respectively. The association of p53 and MELK expression was determined using Oncomine analysis. We also investigated the effect of p53 knock-down and overexpression on MELK expression using Q-RT-PCR, western blotting and promoter luciferase assays. Results: Analysis of publicly available datasets revealed that MELK expression is highly elevated p53-mutant breast cancer independent of estrogen receptor status. Consistent with observation, high MELK expression was found in p53-mutant cells compared to p53 wild-type cells. Through luciferase, western blotting and Q-RT-PCR assays, we discovered that the increased expression of MELK in TNBC cells is due to inactivation of p53 wild-type function. Consistently, knockdown of p53-mutants in p53-null cells increased MELK expression through dominant-negative effect. In contrast, knockdown of p53-mutants or overexpression of p53-mutants in p53-null cells did not alter MELK expression. Finally, through promoter deletion studies coupled with luciferase studies, we identified a potential p53-response region in MELK promoter. Conclusion: Our results suggest that the expression of MELK, a critical regulator of growth and invasion of TNBCs, is regulated by the tumor suppressor gene, p53 wild-type. Inactivation of p53 wild-type either gene deletion or mutations induces the expression of MELK. These results suggest that MELK is a promising target for the treatment of women with p53-mutant TNBC. This work was supported by Susan G. Komen Promise Grant (PB, SH, GM), SAB Komen grant (PB) and Young Foundation grant (PB).

#3527 Clarification of the molecular mechanism for cancer development in Xp11.2 translocation renal cell carcinoma. Masaya Baba,1 Ying Huang,2 Takanobu Motoboshi,2 Hisashi Hasumi,1 Yukiko Hasumi,3 Mitsuko Furuya,4 Yoji Nagashima,5 Masahiro Yao,4 Tomomi Kamba,1 Laura S. Schmidt,2 W. Marston Linehan2. 1Kumamoto University, Kumamoto, Japan; 2National Cancer Institute, National Institutes of Health, Bethesda, MD; 3Yokohama City University, Yokohama, Japan; 4Tokyo Women’s Medical University, Tokyo, Japan; 5Leidos Biomedical Research, Inc., Frederick National Lab, Frederick, MD.

Xp11.2 translocation renal cell carcinoma (Xp11.2 TRCC) is a newly defined subset of RCC, which have chromosomal translocations involving the TFE3 transcription factor at Xp11.2. All translocations seen in Xp11.2 RCC, including PRCC-TFE3, PSF-TFE3, ASPL-TFE3, CTLC-TFE3 and NONO-TFE3 produce chimeric TFE3 genes, which encode fusion TFE3 proteins retaining the basic helix-loop-helix leucine zipper (bHLH-LZip) structure for DNA binding, suggesting that these fusion TFE3 proteins function as oncogenic transcription factors. To clarify the molecular mechanism for Xp11.2 RCC development, we have established cell lines, which express fusion TFE3 proteins (NONO-TFE3, PRCC-TFE3, PSF-TFE3) in a doxycycline dependent manner. Genes upregulated universally in all fusion TFE3 cell lines upon doxycycline addition were identified. In addition, we have generated an Xp11.2 translocation RCC mouse model, in which a floxed Neomycin cassette followed by a PRCC-TFE3-CDNA is inserted in the Rosa26 locus. By crossing these mice with cadherin 16-Cre transgenic mice, we can induce kidney specific PRCC-TFE3 expression resulting in RCC development. We will report and discuss the role of fusion TFE3 target genes in our mouse model and in clinical samples of Xp11.2 RCC. Funded in part by FNLCR Contract HHSN26120080001E to LSS.

#3528 PTCCH53 as a potential secondary modifier in Li-Fraumeni syndrome. Anna J. Pan,1 Benjamin Brew,2 Lauren Erdman,1 Anna Goldenberg,3 David Malkin2. 1The University of Toronto, Toronto, Ontario, Canada; 2The Hospital for Sick Children, Toronto, Ontario, Canada.

Germline mutations in the TP53 gene have been established as the underlying genetic event in Li-Fraumeni syndrome (LFS), predisposing affected families to a wide spectrum of early onset cancers. Despite rapid progress in elucidating the role of wild-type p53 in maintenance of genome stability, and mutant p53 in cellular transformation, much of the molecular basis underlying LFS remains unclear. Lack of predictability in age of onset, type(s) of cancers, and likelihood of subsequent malignancies prompts further research to examine secondary modifiers to TP53. In the Li-Fraumeni syndrome mouse model (a.k.a PTCHD4 or PCH53) is a repressor of canonical Hedgehog signaling that is also described to be transcriptionally activated by p53. Furthermore, our group has identified significant hypomethylation at the PCH53 locus in a mutant TP53 LFS cohort, compared to TP53 wildtype carriers. These results suggest a potential role of PCH53 in modifying LFS phenotype. The study examines the expression of p53-mutants in p53-null cells did not alter MELK expression. Furthermore, mutant TP53 fibroblasts have overall lower PCH53 transcripts compared to wild-type TP53 fibroblasts. Fold-change increase in PCH53 expression was detected upon doxorubicin and γ-irradiation induced activation of the p53 protein. These results suggest that induction of p53 via DNA damage activates a signaling cascade involving PCH53 up-regulation. Further experiments will examine the functional consequences to PCH53 de-regulation via siRNA knockdown and CRISPR knockout models, and will examine possible modifying effects of PCH53 on the functional phenotype (cell viability, colony formation and migration potentials) of cell lines harboring different p53 mutants. Elucidating the functional profile of PCH53 in the context of Li-Fraumeni syndrome will contribute to understanding the complex molecular basis underlying this cancer predisposition syndrome.

#3529 Investigating the in vivo role of the EHF transcription factor in intestinal epithelial differentiation. Camilla M. Rehorst, Ian Y. Luk, Rebecca Moghaddam, Mercedes Davalos-Sala, Amardeep S. Dhillon, John M. Mariadason, Olivia Newton-John Cancer Research Institute, Heidelberg, Australia.

Objective: Poor tumor differentiation status is associated with worse patient outcome in colorectal cancer. However, the molecular basis for loss of differentiation in colon cancer is not well understood. We have found that the transcription factor Ets homologous factor (EHF) is highly expressed in the normal human and mouse colonic epithelium and is down-regulated in poorly differentiated colorectal cancer cell lines. The aim of this study was to investigate the role of EHF in regulating normal colorectal epithelial cell differentiation in vivo. Methods: A novel mouse model was generated in which the Ets DNA binding domain (exon 8) of EHF was flanked by loxP sites (EHFlox/lox). To inactive EHF in the intestinal epithelium, EHFlox/lox mice were crossed to Villin-Cre mice and recombination was induced by tamoxifen treatment. Tissues from tamoxifen treated and vehicle treated mice were monitored weekly for up to one year. At each endpoint, tissue was collected and the effect of EHF deletion on intestinal cell proliferation and differentiation assessed by qRT-PCR and immunohistochemistry. Results: Targeted inactivation of EHF in the small intestinal and colonic epithelium following tamoxifen treatment was confirmed at the DNA and RNA level. EHF inactivation in the intestinal epithelium had minimal effect on survival, weight gain and overall health of the animals. Furthermore, loss of EHF did not markedly affect cell proliferation or the overall architecture of the intestinal epithelium. Finally, EHF inactivation also had minimal effect on markers of absorptive, enteroendocrine or Paneth cell differentiation but did reduce the number of goblet cells in the colonic epithelium. Conclusion: EHF does not play a major role in regulating cell proliferation in the intestinal mucosa but may play a role in regulating goblet cell differentiation. The role of EHF in intestinal tumorigenesis and response to stress are currently being investigated.

#3530 KITENIN works as a fine regulator of ErbB4 expression in colorectal cancer tissues in addition to E3 ubiquitin ligase Ndrp1. Jeong A Bae,1 Eun Gene Sun,1 Yoo-Seung Ko,1 Hui Jeong Choi,1 Chaeyong Jung,1 Kyung-Hwa Lee,1 Ik Joo Chung,2 Kyung-Sub Moon,1 Young Hyun Yu,1 Hyung-Ho Ha,1 Hangun Kim,1 Kyung Keun Kim,1 Chonnam Natl. Univ. Medical School, Medical Research Center for Gene Regulation, Kwangju, Republic of Korea; 2Chonnam Natl. Univ. Medical School, Dept of Pathology, Kwangju, Republic of Korea; 3Chonnam Natl. Univ. Medical School, Dept of Hematology-Oncology, Kwangju, Republic of Korea; 4Chonnam Natl. Univ. Medical School, Dept of Neurosurgery, Kwangju, Republic of Korea; 5Sunchon National University, College of Pharmacy, Sunchon, Republic of Korea.
Molecular and Cellular Biology / Genetics: Oncogenic Transcription Factors

Purpose: Understanding the complex biological functions of E3-ubiquitin ligases may facilitate the modulation of E3-ubiquitin ligases as a promising approach for the development of novel anticancer drugs. We recently identified that the KITENIN/ErbB4-Dvl2-c-Jun axis works as a novel unconventional downstream signal of epidermal growth factor in colorectal cancer (CRC) tissues and is involved in the epigenetic modulation of KITENIN expression. This finding is highly expressed in tumor tissues from advanced CRC stage. However, the detailed mechanisms that explain the higher levels of ErbB4 in colon cancer tissues are largely unknown. Here we investigated whether E3-ubiquitin ligases participate in the operation of the KITENIN/ErbB4-Dvl2-c-Jun axis and in the maintenance of elevated KITENIN/ErbB4 complex in CRC. Results & Discussion: We found that ErbB4, an E3-ligase for ErbB3/ErbB4, interacts with KITENIN (KA11 C-terminal interacting tetraperasin) to form a functional KITENIN/Erbb4/Nrdp1 complex and is responsible for down-regulating Dvl2 within this complex. Interestingly, ErbB4 was resistant to degradation by Nrdp1 in KITENIN/Nrdp1 co-transfected CRC cells, and KITENIN bound to the C-terminal coiled-coil domain of Nrdp1. Chemical blockade of ErbB kinase did not block the action of EGFR to increase in total/phospho-ErbB4 and phospho-Akt in KITENIN/ErbB4cotransfected cells, whereas it blocked the action of EGFR in ErbB4 alone-transfected CRC cells. In human CRC tissues, higher expressions of ErbB4 and KITENIN and lower expression of Dvl2 was observed in stage IV samples than in stage I, but a low level of Nrdp1 was expressed in both stages and it did not differ significantly by stage. These results indicated that Nrdp1 is necessary for the reduction in Dvl2 to generate c-Jun in the EGF-KITENIN/ErbB4/Nrdp1 complex and is responsible for down-regulating Dvl2 within this complex. Thus, we found that Nrdp1, an E3-ligase for ErbB3/ErbB4, interacts with KITENIN, inactivating proteasomes to degrade it. NF-κB then enters the nucleus through a p105/50 (p105/50) protein model mouse of undifferentiated pleomorphic sarcoma (UPS) revealed that YAP1 deletion (KPY) reduces cell proliferation and increases expression of circadian rhythm genes including PER1. UPS is a commonly diagnosed aggressive type of muscle-derived sarcoma. The KP model recapitulates human UPS morphologically and histologically, as well as by gene expression profiling. Although PER1 has primarily been characterized as a negative regulator of the circadian clock, upregulation of PER1 is known to modulate the G2/M cell cycle checkpoint at both the protein and mRNA level independent of p53. However, our findings represent a novel link between the circadian clock and the hippo pathway. Notably, the deletion of YAP1 in KP tumors leads to a statistically significant 2.5 fold increase in expression of PER1. The study has two specific aims: 1) to identify the YAP1-dependent function of PER1 in sarcoma and 2) determine whether YAP1 directly or indirectly regulates PER1. We have validated the microarray results in KP tumor-derived cell lines as well as in KP and KPY tumor tissue. We have also confirmed PER1 suppression in KP cells under YAP1 knockdown conditions, I demonstrated via qRT-PCR and western blot that PER1 is significantly increased at both the protein and transcriptional levels in KP cells. Additionally, preliminary evidence from an MTT proliferation assay showed loss of PER1 increased sarcoma cell proliferation. Further supporting the hypothesis that PER1 modulation impacts sarcoma proliferation, it has been reported that MyoD, the master regulator of muscle cell differentiation, is itself a clock-controlled gene. Together, these results suggest that YAP1 represses PER1 expression in sarcoma, and that epigenetic treatments can cause re-expression of PER1 which functions to inhibit cell proliferation and may promote differentiation.

#3532 No REST in Merkel cell carcinoma.

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Introduction: Merkel cell carcinoma (MCC) is a highly aggressive skin cancer of the elderly and immunosuppressed patients. More than 80% of MCCs are associated with the recently identified Merkel cell polyomavirus (MCPyV). MCCs reveal a trilinear differentiation characterized by neuroendocrine (chromogranin A and synaptophysin), epithelial (e.g. CK20) and pro/pre B-cell lymphocytic (e.g. Pax5 and TdT) marker expression. The cellular origin of MCCs still remains obscure. Based on the combined expression of TdT, Pax5 and immunoglobulins (Ig), including clonal Ig rearrangements we have recently hypothesized that pre/pro B-cells might constitute the cellular origin of MCC. We aimed to understand the neuroendocrine expression of chromogranin A and synaptophysin which is known to be negatively regulated by the Re1 silencing transcription factor (REST) in MCC cells. We assessed REST expression using commercially available antibodies and immunohistochemistry (IHC) in 22 formalin fixed and paraffin embedded MCC tissues. In addition, MCPyV-positive (M-K1 and M-K2) and MCPyV-negative (MCC13 and MCC26) MCC cell lines and the B-ALL cell-line REH as positive control were tested for REST expression. RT-PCR was used to confirm the results of the cell lines on the transcriptional level. In addition, the regulation of the REST expression by methylation was investigated within the demethylating agent 5-aza-2′-deoxycytidin. Further the methylation status of the 3 Cpg islands of the REST promoter was analysed with a methyl specific PCR. Results: All MCCs except one were completely devoid of REST expression as tested by IHC. All MCCs were negative for REST but synaptophysin and chromogranin A positive. The MCPyV-positive cell lines M-K1 and M-K2 did not express REST but indeed expressed both neuroendocrine markers. The MCPyV-negative cell lines MCC13, MCC26 and the B-ALL cell line were positive for REST expression and negative for these neuroendocrine markers. On RNA level these results were confirmed. The demethylation of the Cpg islands of the REST promoter did not result in the increase of the REST expression in M-K1 and M-K2 on the transcriptional or translational level. Initial MSP results did not reveal any methylation of the 3 Cpg islands of the REST promoter. Conclusion: The lack of REST expression in MCC and in MCPyV-positive MCC cell lines, in combination with REST expression in MCPyV-negative cell lines points to an important role of the MCPyV in the regulation of REST expression in MCC. This is emphasized by the recent description of 2′-deoxyapurine treatment of the MCC cell lines. Our data might provide the basis of neuroendocrine gene expression in MCC which possibly originates from early B cell.

#3533 Connections between NF-κB misregulation and carcinogenesis.

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In this review poster, we researched the function, regulation, and structure of NF-κB as it relates to carcinogenesis. Found in almost all animal cells, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is a transcription factor that plays various roles in cellular proliferation, cell survival, inflammation, and T cell activation. There are two different NF-κB signaling pathways: the canonical pathway and the non-canonical pathway. NF-κB forms a p50/Rel A heterodimer in the canonical pathway and a p52/ Rel B heterodimer in the non-canonical pathway. NF-κB protein is normally sequestered in the cytoplasm as an inactive complex with a IκB inhibitor (IκB) protein. Outside stimuli such as reactive oxygen species (ROS) and tumor necrosis factor alpha (TNF-α) activate cell surface receptors such as Toll-Like Receptors (TLRs) and Receptor Activators of NF-κB (RANK). These surface receptors, in turn, activate IκB kinase (IKK). IKK phosphorylates IκB, which causes ubiquitination of IκB, signaling proteasomes to degrade it. NF-κB then enters the nucleus through a
nuclear pore and forms a transcription complex with coactivators and RNA polymerase. NF-κB binds to specific DNA-binding sites to induce transcription. One of the target genes of NF-κB encodes 1kBs, so NF-κB regulates its own activity through a negative feedback loop. Because the cell maintains storage of the signaling protein NF-κB in the cytoplasm, NF-κB allows the cell to respond to an external signal quickly. These molecules, together with other proteins that promote cellular proliferation and cell survival are mainly transcribed through NF-κB activation, uncontrolled overactivation of this transcription factor has major implications in carcinogenesis. Many types of cancer cells have high levels of constitutively active NF-κB. Mutations in the genes for NF-κB itself or its inhibitors such as IκBαs often found in cancer cells can cause cells to accelerate their proliferation or confer resistance to apoptosis. Such events provoke cancer cells secrete signaling molecules, inducing improper NF-κB activity. Because of the tumor promoting characteristics of NF-κB activation, a focus of current research is to find methods of suppressing NF-κB to kill cancer cells or slow their growth. We designed a 3D-printed model of the p50/RelA NF-κB protein using Imol to highlight the relationship between its structure and function.

#3534 FAT1: A potential target of NFκB (RelA) in GBM. Chitandra Srivastava,1 Khushboo Irshad,1 Parthaprasad Chattopadhyay,1 Chitra Sarkar,1 Ashish Suri,1 Subrata Sinha,2 Kunzang Chosdol1.1 All India Institute of Medical Sciences, New Delhi, India; 2National Brain Research Center, Gurgaon, India.

FAT1 is a large transmembrane protein (502 KD), reportedly having dual role in different human cancers. Our lab has reported oncogenic role of FAT1 over-expression in glioblastoma multiforme (GBM, grade IV glioma) increasing migration-invasion as well as tumor inflammation and HIF1α in different human cancers. Our lab has reported oncogenic role of FAT1 over-expression in GBM. The poor prognosis in GBM patients is due in part to its resistance to available treatments, which calls for identifying novel molecular therapeutic targets. In this study, we identified an interesting biomarker, Hairy/Enhancer-Of-Split related with RYPWM Motif (HEY1), a mediator of Notch signaling, which contributes to the pathogenesis of GBM. Datamining studies and our immunohistochemistry and immunoblot analysis showed that HEY1 is highly expressed in GBM patient specimens. Recent studies indicate that methylation status may control the expression of cancer phenotype. Our studies using bisulphite sequencing on the patient samples showed that HEY1 promoter was hypermethylated in the normal brain when compared to GBM specimens. Treatment on the 4910 and 5310 xenograft cell lines with sodium butyrate (NaB) greatly decreased HEY1 expression owing to its promoter hypermethylation with a concomitant increase in DNM1T expression confirming that promoter methylation regulates the HEY1 expression in GBM. NaB treatment also induced cell apoptosis as evaluated by flow cytometric analysis. Silencing HEY1 reduced invasion, migration and proliferation in 4910 and 5310 cells. Further, immunoblot and q-PCR analysis demonstrated the existence of a potential positive regulatory loop between HEY1 and p53. Additionally, DNA microarrays with HEY1 recombiant protein demonstrated good correlation with p53 and provided various bonafide targets of HEY1. Collectively, these studies suggest HEY1 as an important predictive marker for GBM, thus indicating a potential target for future GBM therapy.

#3535 The role of Nuclear factor I transcription factors in glioma. Kok Siong Chen, Linda J. Richards, Jens Bunt. 1The University of Queensland, Brisbane, Australia.

Gliomas are the most common brain tumors in adults. Among the malignant gliomas, grade II glioma, or glioblastoma (GBM), is the most aggressive form. Despite harsh treatments such as surgery, radiotherapy and chemotherapy, the median survival of GBM patients remains at just 12-15 months. GBM tumors could originate from cells of the glial lineage that have escaped the normal glial differentiation mechanisms. Therefore, by applying knowledge about normal glial development, we might understand how pathways that normally drive differentiation are affected in these tumors. If reactivating these pathways induces the differentiation of proliferative tumor cells, we could use this as a novel target for therapy. Our research is focused on the Nuclear factor I (NFI) transcription factors, one of the key factors to induce glial differentiation during normal development and is implicated in glioma. In insertional mutagenesis glioma mouse models, Nfı genes are consistently disrupted, suggesting a role for NFI in glioma initiation. Furthermore, loss of NFIB is common in human astrocytoma, while loss of NFIA is associated with oligodendroglioma. To determine whether loss of Nfı alters glioma initiation, progression or tumor histology, we have crossed inducible glioma mice with conditional Nfı deletion mice to follow tumour initiation and progression using fluorescence imaging and immunohistochemistry. Based on analyses of glioma mRNA expression data sets, NFIB expression correlates inversely with tumor grade and survival. Using immunofluorescence, we determined that both NFIA and NFIB are mainly expressed within the non-proliferative cells in GBM tissue, suggesting that NFI may play a direct role in tumor cell differentiation. To establish whether NFIB expression can indeed inhibit tumor proliferation and induce differentiation, U251 GBM cells were transfected with NFI. While overexpression of NFIA and NFIB led to cell proliferation, an NFI dominant-negative protein enhanced proliferation. We are currently validating these finding in vivo by electroporating NFI expression constructs into patient-derived GBM xenografts in mice. Our preliminary data show NFI electroporated cells are indeed differentiated and non-proliferative. Our data suggests that NFI-mediated glial differentiation can be induced in glioma to inhibit tumor growth and may prevent recurrence. Hence, activation of NFI could be a potential target for a glial differentiation-based approach to treat glioma.

#3537 Otx2 and c-Myc association drives tumor progression in medulloblastoma molecular subtypes 3/4. Yining Lu,1 Maheedhara R. Guda,2 Swapna Asuthakar,1 Ian Purvis,1 Collin M. Labak,3 Neha Jain,1 David J. Daniels,2 Sarah E. Martin,3 Andrew J. Tsung,1 Kiran K. Velpula1.1 Univ. of Illinois College of Med. at Peoria, Peoria, IL; 2Mayo Clinic, Rochester, MN; 3Kokoro Foundation, Tokyo, Japan.

Medulloblastoma (MB) is a pediatric brain tumor comprising of four molecular subgroups, with Group 3 and 4 tumors characterized by particularly poor prognosis. While research has advanced our understanding of MB, there is still a pressing need for new knowledge of the functional processes underlying its progression due to a scarcity of therapeutics. Recent studies have identified Orthodenticle homeobox 2 (Otx2), a cerebellar transcription factor, to be highly expressed in MB Group 3/4 subtypes. Datamining studies from Northcott and Robinson datasets revealed co-expression of Otx2 and c-Myc in Group 3/4 tumors. DAB staining conducted in human MB specimens showed increased expression of Otx2 and c-Myc. Colocalization studies confirmed datamining results.
sults. Next, we used a clinical Otx2 inhibitor, all-trans retinoic acid (ATRA), in a PDX cell line, P9, and in UW228 cells. Western blot analysis demonstrated reduced levels of c-Myc along with Otx2. In another experiment, we used the c-Myc/Max inhibitor 10058-F4 and observed comparable results to Otx2 inhibition. Real time PCR and immunocytochemistry experiments confirmed the aforementioned results. Further, treatment with ATRA and 10058-F4 showed reduced proliferation and migratory abilities in p9 and UW228 when compared to their respective controls. Mass spectrometry conducted on immune-precipitated Otx2 in P9 cells revealed a plethora of novel interaction partners that drive tumor growth and progression. In addition, western blot analysis in ATRA treated cells showed reduced expression of Max, suggestive of the existence of a feedback loop that links Otx2 and c-Myc. Collectively, these studies highlight Otx2 as a potential contributor to the malignant phenotype and describe its role in mediating the aggressiveness of Group 3/4 medulloblastoma.

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Oncogenic Transcription Factors

#3538 Enhanced dependence on lipid metabolism is a cellular adaptation to acidic microenvironment. Smitha Pillai, Jonathan W. Wojtkowiak, Mehdi Damaghi, Robert Gatenby, Robert Gillies. Moffitt Cancer Center, Tampa, FL.

Malignant tumors exhibit altered metabolism and consume higher levels of glucose compared to surrounding normal tissue, resulting in highly acidic microenvironments that lead to acidic conditions as a precancerous stimulus for cancer cells to survive and thrive and to out-compete the stroma into which they invade. Acid adaptation is associated with chronic activation of autophagy as well as redistribution of the lysosomal proteins to the plasma membrane. These processes are major survival mechanisms adopted by tumor cells under acidic conditions. We have also observed that under acidic conditions, there is a rapid, reversible and robust increase in the accumulation of cytoplasmic lipid droplets (adiposomes) in a panel of breast cancer cells. Adiposomes are dynamic organelles that store neutral lipids surrounded by a shell of proteins and a phospholipid monolayer. Breast cancer cells when grown in acidic media accumulated adiposomes as revealed by nile red and perilipin-2 staining followed by confocal microscopy. The acid-induced lipogenic phenotype persists even when the cells are grown in de-lipidated serum, indicating that the source of lipids is de novo and endogenous. When cells were treated with inhibitors of fatty acid synthesis such as TOFA, an inhibitor of Acetyl CoA Carboxylase or FAS inhibitor C75, adiposome formation at low pH was attenuated. Inhibition of either lipid anabolic or catabolic pathways was specifically cytotoxic in acid-adapted cells, but not in control cells where the FAS inhibitor, C75, is selectively toxic under acidic conditions. Additionally, when treated with etomoxir, an inhibitor of carnitine palmitoyltransferase 1, the rate limiting step in beta-oxidation, acid adapted cells showed increased sensitivity. To investigate the role of de novo fatty acid synthesis further, we employed high resolution NMR spectroscopy to measure 13C enriched lactate isomers following metabolism of D-[1-13C] glucose. These analyses showed that glucose flux through the pentose phosphate pathway (PPP) was significantly (~2.5 fold) higher in low pH exposed cells, compared to controls, representing a major shift in glucose metabolism from Embden Meyerhof to PPP, which results in increased production of NADPH, necessary for de novo lipid synthesis. Additional metabolic profiling using the Seahorse XF revealed that cells at low pH had higher rates of oxygen consumption (OCR) and that this was reversible. Further, we investigated the role of various acid sensing G-protein coupled receptors such as OGR1, TDAG8 and GPR4 in transducing the acid signal that results in the accumulation of lipid droplets. CRISPR/Cas9 mediated depletion of these receptors indicates that they might play a major role in inducing adiposome accumulation under acidic conditions. Taken together, increased dependence on lipid metabolism by cancer cells under acidic conditions reveals novel therapeutic vulnerabilities.

#3539 Oncogenic KRAS promotes the hexosamine biosynthetic pathway in NSCLC. Christian Ruiz, John A. Haley, Irina Zaitseva, John D. Haley, Geofrey D. Girmun. Stony Brook University, Stony Brook, NY.

Mutations in the proto-oncogenic KRAS (KRASmut) are one of the most common oncogenic mutations in NSCLC cancer, with approximately 25% of lung tumors containing KRASmut. Cancer cells acquire metabolic reprogramming in order to sustain their proliferative state. Recent studies highlight a role for KRASmut in driving altered cancer metabolism as part of its mechanism of action. We used targeted metabolomics in an effort to identify alterations in metabolic pathways controlled by KRASmut. We show that KRASmut, but not wildtype KRAS, increases several metabolites in the hexosamine biosynthetic pathway (HBP). The HBP metabolizes glucose and glutamine to produce UDP-N-acetylglucosamine (UDP-GlcNAc) which is the obligate substrate for O-GlcNAcylation, a carbohydrate post-translational modification on cytosolic and nuclear proteins. Levels of O-GlcNAcylation have been shown to be elevated in tumor cells including NSCLC. Currently we are investigating the mechanisms(s) and role of the Krasmut induced HBP on cancer cell metabolism and growth.

#3540 Targeting obesity-related cancer progression with novel leptin receptor antagonists. Cristal C. Lipsey, Adriana Harbuzaru, Ruben R. Gonzalez-Perez. Morehouse School of Medicine, Atlanta, GA.

Obesity is a global health issue that has been identified as a risk factor for several types of cancer. High levels of body fat and circulating leptin are typical identifiers of obesity in humans and animals. Leptin is a 16 kD protein hormone which is secreted by adipocytes, and maybe secreted from cancer cells, that functions to control satiation via leptin receptor binding. However, obese individuals often develop ‘leptin resistance’, which is a mechanism that leads to the accumulation of excess leptin. Increased binding of leptin to its receptor (OB-R) due to leptin resistance has been associated with disease progression and poor prognosis in human cancers. Our group has previously shown that leptin-mediated cancer cell proliferation is inhibited by the LPR2 (leptin receptor antagonist 2). We have prepared novel leptin antagonists and tested their ability to block leptin-induced survival and chemoresistance to Paclitaxel (TAX) and Gemcitabine (GEM) in cancer cell lines (GBCR) and derived tumorspheres (pancreatic: PANC-1, MiaPaca2 and triple negative breast cancer: MDA-MB231 and MDA-MB66). Western blot protein analyses showed the ability of the antagonists to specifically inhibit leptin-induced phosphorylation of STAT3, and expression of cyclin D, and Notch1 in cancer cells. Additionally, potential toxicity of antagonists was tested using MTT assay with concentrations up to 100X higher than the effective concentration (ECF-10A). Data generated showed no toxicity of the novel antagonists in vitro. Leptin-induced proliferation of breast cancer and pancreatic cancer cells (120-160%) was significantly inhibited by the novel antagonists. In addition, leptin-mediated progression of S-phase was also reduced by the antagonists. Leptin increased TAX and GEM chemoresistance in cells and tumorspheres that were efficiently inhibited by the antagonists. These data suggest that the new antagonists could be equally or more effective than LPR2 for adjuvant treatment of cancer. Acknowledgements: This work has been supported by Pilot Project Award from MSM/Tuskegee University/UAB Cancer Center partnership grant SU54CA118638; PC SPOR Grant from UAB to RGGP; and facilities and support services at Morehouse School of Medicine (1G12RR026530-60; NIH RR 03034 and 1C06 RR1836).


Prostate cancer (PCA) is the second leading cause of cancer-associated death in men, being bone metastases the main cause of mortality. Energetic metabolism alterations have become a new hallmark of cancer, since variations in a single gene can orchestrate changes in metabolic pathways and confer an adaptive advantage. Heme-oxygenase 1 (HO-1) exerts an antitumoral role in PCA sporadic or metastatic tumor cells. Heme-oxygenase 1 mediated catabolism of heme is orchestrated by prolyl hydroxylase domains (PHDs) and catalyzes the production of carbon monoxide (CO). HO-1 is the most abundant isoform of HO-1 in PCA. Most PCA cells express high levels of HO-1, which is tightly coupled to the expression of HO-1 inducers such as heat shock proteins (HSPs), reactive oxygen species (ROS), and inflammatory cytokines. Collectively, these context-dependent HO-1 inducers orchestrate changes in metabolic pathways and confer a metabolic advantage. Heme-oxygenase 1 (HO-1) exerts an antitumoral role in PCA sporadic or metastatic tumor cells. Heme-oxygenase 1 mediated catabolism of heme is orchestrated by prolyl hydroxylase domains (PHDs) and catalyzes the production of carbon monoxide (CO). HO-1 is the most abundant isoform of HO-1 in PCA. Most PCA cells express high levels of HO-1, which is tightly coupled to the expression of HO-1 inducers such as heat shock proteins (HSPs), reactive oxygen species (ROS), and inflammatory cytokines. Collectively, these context-dependent HO-1 inducers orchestrate changes in metabolic pathways and confer a metabolic advantage.
sults, we propose HO-1 as a key regulator of the metabolic status of PCa cells and a powerful mediator capable of redefining the metabolic signature of bone precursor cells, thus, favoring the establishment of a less aggressive phenotype.

#3542 Coordination of glutamine and glucose metabolism in pancreatic cancer. Enza Vernucci, Venugopal Gunda, SURENDA SHUKLA, PANKAJ K. SINGH. University of Missouri, USA.

Pancreatic ductal adenocarcinoma is the fourth leading cause of cancer related deaths in the United States. Due to early metastasis by the time it is diagnosed it advances to advanced stages and becomes irreversible. Oncogene mediated metabolic reprogramming has been shown to promote the growth, maintenance and metastasis of tumors in pancreatic ductal adenocarcinoma. Pancreatic tumors route majority of glucose and glutamine for biomass generation and maintenance of redox potential. The aim of this study was to investigate the interaction between glutamine and glucose metabolism in pancreatic cancer cells. We primarily focused on the role of glutamine mediated regulation of glucose metabolism. We observed that glutamine deprivation reduces pancreatic cancer cell growth as suggested by the previous studies. We further investigated how pancreatic cancer cells adapt to glutamine deprived conditions. We observed that glucose uptake increases significantly upon glutamine deprivation. However, GLUT1 expression showed a contrasting decreased expression under conditions of glutamine deprivation. Using tandem mass spectrometry based metabolomic analysis we observed that glutamine deprivation does not alter glycolysis. However, the TCA cycle, amino acid metabolism and glutathione metabolism were significantly impacted. We noticed a significant increase in the levels of hydroxylglutarate and succinyl CoA levels in the TCA cycle. These changes were accompanied with an increase in GSH to GSSG ratio that reflects a possibility for alternative pathways involved in redox maintenance under glutamine deprived conditions. Hence, we conclude that glutamine metabolism significantly impacts the uptake and metabolism of glucose in pancreatic cancer cells.

#3543 Targeting the metabolic mevalonate pathway with statins as anti-breast cancer agents. Jenna van Leeuwen, ALEKSANDRA PANDRYA, CAROLYN GOARD, Peter J. Mullen, Rosemary Yu, Linda Z. Penn. Princess Margaret Cancer Centre, Toronto, Ontario, Canada.

The starn family of drugs target the mevalonate pathway and have been used for decades in the control of hypercholesterolemia, however recent evidence suggests these approved agents may also be useful as anti-cancer therapeutics (see our recent Nature Review Cancer article1). For example, statins can trigger tumor-specific apoptosis and two independent pre-op clinical trials in breast cancer, evaluating cholesterol-lowering doses of fluvastatin and atorvastatin, resulted in breast tumor shrinkage due to decreased growth and increased apoptosis. Our hypothesis is that statins have utility as anti-breast cancer agents. To maximize efficacy and speak to personalized medicine, our objectives are to develop biomarkers to distinguish which patients will benefit from the addition of statins to their treatment regimen and how best to use statins in combination with other agents to augment anti-tumor efficacy. To identify biomarkers of statin sensitivity we evaluated fluvastatin activity across a panel of BCa cell lines and showed that the basal, estrogen receptor-negative subtype is particularly sensitive to statin-induced apoptosis. As this included the difficult-to-treat triple negative BCa (TNBCa) we have extended this work and further evaluated a panel of TNBCa cell lines for statin sensitivity. From these results we are identifying features associated with robust apoptosis in response to statin exposure potentially statin-induced apoptosis. This is reversible with exogenous mevalonate, reinforcing that this is an on-target effect. We also identified another approved agent, diprydamole, as able to potentiate the anti-cancer activity of statins. Mechanistically we have shown that diprydamole blocks the feedback response to statin exposure. We have extended these studies and shown that the combination of statins and diprydamole is effective against TNBCa both in vitro and in vivo. Thus, we provide essential pre-clinical data to support the further evaluation of statins and diprydamole in BCa.

#3544 Leptin-Notch crosstalk axis: A novel target for pancreatic cancer. Adriana Harbuzariu, Crystal C. Lipey, Raben R. Gonzalez-Perez. Morehouse School of Medicine, Atlanta, GA.

Background: Pancreatic cancer (PC) has consistently been the fourth cancer-related cause of death in United States for more than 10 years. Its dismal prognosis is due to the aggressive behavior, late detection, lack of reliable biomarkers and low response to chemotherapy. Obesity, characterized by high levels of leptin, correlates to low PC survival. Notch and leptin signaling have been associated with increased tumor growth and chemoresistance. We have previously shown that leptin induces proliferation of PC cell lines, increases Notch expression, PC stem cells (PCSC) and tumorsphere formation. Hypothesis: Leptin induces chemoresistance to Notch through its crosstalk axis and a pathway that is essential for the expansion of PCSC. Methods: To test whether a leptin-Notch crosstalk axis is involved in chemoresistance, human PC (MiPaCa-2, Panc-1 and BxPC-3) cells were cultured in serum-free medium containing leptin, Gemiactamine and 5-Fluorouracil (3-FU) in combination with DAPT (gamma-secretase inhibitor) and leptin signaling inhibitor bound to iron-oxide nanoparticles (IONP-LPRA2). PC cells were cultured with leptin and allowed to form primary and secondary tumorspheres, enriched in PCSC. Tumorspheres were treated with the compounds described for 7-14 days. Number and size of tumorspheres were recorded, and expression of leptin receptor, OB-R and se-cretion of leptin were determined by Western blot (WB) and ELISA, respectively. Additionally, the levels of Notch, PCSC and EMT markers, pluripotency associated genes (Oct-4, Sox-2 and Nanog) and ATP-binding cassette transporters (ABCR1, ABCG2 and ABCG3) were determined using flow cytometry and WB. Results: PC cells expressed Leptin/OB-R system, suggesting an autocrine role in PC progression and chemoresistance. Leptin induced the formation of primary and secondary tumorspheres and increased their expression of Notch, EMT and pluripotency markers. Additionally, chemotherapeutics induced PC tumorsphere formation and their sensitivity to Notch activation via IONP-LPRA2. Pancreatic cancer cells acquired the ability to bypass Notch receptor and signaling, which was reinforced by leptin actions. Blockade of leptin signaling via IONP-LPRA2 and Notch activation via DAPT reduced leptin-induced molecular effects, PCSC and tumorsphere growth. Conclusion: Present data suggest that obesity, through leptin-induced Notch signaling, could increase chemoresistance in PC patients. Inhibition of leptin-Notch axis may be novel therapeutic strategy for PC, which may improve chemotherapeutic efficacy and increase survival in PC patients, particularly in obese contexts. Acknowledgements: This work has been supported by a Pilot Project Award from MSM/Tuskegee University/UAB Cancer Center partnership grant 5U54CA118838; PC SPORE Grant from UAB to RRGP, and facilities and support services at Morehouse School of Medicine (1G12RR02650-03; NIH RR 03034 and 1C06RR18386).

#3545 Glucose-dependent regulation of PXR is modulated by AMPK. Peter O. Oladimeji, St. Jude Children’s Research Hospital, Memphis, TN.

The sensitivity of patients to drugs depends on the pharmacokinetics of the drugs, but largely on the bioavailability of the drugs in the body. Orally administered drugs must pass through the intestinal wall and the portal circulation to reach the liver; both are sites of first-pass metabolism. Thus, many drugs may be metabolized before optimal plasma concentrations are reached, or before getting to target issues, resulting in diminished drug efficacy and reduced drug resistance. Among the several drug-metabolizing enzymes (DME) is cytochrome P450 (CYP) enzyme 3A4, the most abundant in the liver, metabolizes approximately 50 percent of marketed drugs and a main target of the gene receptor (PXR). In addition, transporters including MDR1 are also regulated by PXR. There is increasing evidence that drug metabolism can be affected by energy metabolism and vice versa. PXR has been implicated to have a complex role in energy homeostasis, and increased drug clearance was observed in untreated diabetic patients. The elimination of any chemotherapeutic agents in humans is markedly dependent on the relative expression and activity of the various DMEs and drug transporters. The heterotrimeric AMP-activated protein kinase (AMPK) serves as a monitor of cellular energy status in most eukaryotic cells, and is activated under energy stress conditions. Interestingly, AMPK is also regarded as a tumor suppressor in various cancers because of its inhibitory effects on anabolic processes that are important for tumor development. Despite the knowledge of the cross-regulation between drug metabolism and energy metabolism, the molecular pathways of this interaction remain vague. In our study, we exposed HepG2 cells to varying glucose concentrations and observed that PXR is greatly induced in high glucose conditions. This finding is consistent with clinical observation of increased drug clearance in untreated diabetic patients. We demonstrate that AMPK modulates PXR transcriptional activity. As such, the pharmacological manipulations of AMPK activation status show an inverse relationship with PXR activity. Activation of AMPK was shown to decrease PXR activity as observed by decreased CYP3A4 transcription, and consistent with that a potentiation of the use of drugs. In contrast, we observed that PXR is not a direct target of AMPK: it remains to be determined what the intermediary between AMPK and PXR is. Taken together, our studies identify novel links between the energy status of the cell, the activation of AMPK and down regulation of PXR.
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#3546 LIN28B/MYC loop regulates aerobic glycolysis and tumor acidic microenvironment to promote cancer stemness and cancer progression. Chong Chen,3 Lipeng Bai,3 Fengqi Cao,3 Shengnan Wang,3 Yan Liu,4 Jian Guo,3 Qin Si,4 Rong Xiang,2 Yuning Luo1,4. 1. Chinese Academy of Medical Science, Beijing, China; 2. Nankai University, Tianjin, China. 3. Sun Yat-sen University, Guangzhou, China; 4. UTMD Anderson Cancer Ctr., Houston, TX. Subsequently, we prove that targeting cancer additive metabolism could suppress tumor progression. Tumor microenvironment (TME) supports the development of metabolic reprogramming of tumor cells. The function of cancer stem cells (CSCs) closely relates with its own metabolic alteration. However, the relationship between CSCs' metabolic reprogramming and their supportive TME, as well as underlying mechanism remains largely unknown. In this study, we have demonstrated a novel mechanism that the LIN28B/MYC positive regulation loop could promote the aerobic glycolysis of cancer cells and glycolysis-derived lactate and further enhance the cancer stemness to facilitate tumor progression. 1) Metabolic analysis revealed that glycolysis (ECAR) was suppressed after LIN28B was knocked down in MDA-MB-231 or H1299 cancer cells respectively. Consistently, both the glucose-uptake and lactate-secretion were attenuated in shLIN28B cancer cells. Importantly, either the glucose accumulation in cancer tissues or lactate secretion in serum was reduced in mouse injected with shLIN28B cancer cells. 2) Metabolic analysis revealed that CSCs possessed higher ECAR and higher level of LIN28B, MYC and HIF1a. Furthermore, glycolysis-derived lactate not only increase the proportion of ALDH+ cells, but also enhance the tumorigenicity forming ability and mobility of cancer cells. 3) MicroRNAs screening analysis revealed that 6 miRNAs were up-regulated above 2 folds after LIN28B was knocked down in MDA-MB-231 cells. Metabolic analysis revealed that miR-34a-5p could suppress the aerobic glycolysis (ECAR) in MDA-MB-231 cells. 4) LIN28B inhibitor could reduce the expression of LIN28B and MYC in vitro and then suppressed tumor growth and metastasis in vivo. Taken together, our study demonstrated that LIN28B/MYC loop could sustain the aerobic glycolysis of cancer cells and then enhance the function of CSCs via regulating the tumor acidic microenvironment, thus, established the LIN28B/MYC as a central node in connecting tumor metabolism and tumor microenvironment, and could serve as a therapeutic target.

#3548 Hypoxia-induced Cystathionine-β-synthase (CBS) expression: impact on colonic epithelial cells metabolism, proliferation and migration. Che-Nique M. Phillips, John R. Zatarain, Michael E. Nicholls, Craig Porter, Steven Widen, Keten Thanki, James W. Randall, Judith L. Hellmich, Manjit Maskey, Suimin Qiu, Thomas G. Wood, Nadiya Druzhnya, Bartosz Szczesny, Kathrin Modis, Csaba Sabol, Delia Chao, Mark R. Hellmich. University of Texas Medical Branch, Galveston, TX. Introduction: We have reported aberrant overexpression of CBS in human colorectal cancers, where its activity (H2S production) promotes tumor progression and metastasis. New data show that CBS expression increases in adenoma-tous polyps. The aims of this study were to identify possible mechanisms for CBS expression increase observed in adenomatous polyps, and its functional impact using a premalignant colonic epithelial cell line NC3536. Methods: Induction of CBS in response to LPS, TLR2/4 agonists, and hypoxia was assayed by immunoblotting. CBS overexpression was achieved by lentiviral expression vector (pReceivers-Lv103, GeneCopoeia™) in NC3536 cells. Global metabolic profiling (Metabolon, Durham) was performed on cellular extracts from NC3536 overexpressing cells (CBS-hi) and vector-transduced controls. High-resolution mass spectrometry (Oroboris Oxygraph-2k) assessed bioenergetics. H2S levels were measured using the fluorogenic probe 7-Azido-4-methylcoumarin. A Coulter counter was used to assess cell proliferation rates. Transwell assays were used to assess cell migration. CBS activity was inhibited with aminooxyacetic acid (AOAA). Results: CBS protein expression was increased by ~3 fold in pNCM and CBS-hi compared to the TLR2/4 agonist control. CBS-hi and its TLR2/4 agonists increased CBS expression 2-fold. Metabolic profiling of CBS-hi cells and controls identified 85 metabolites that were differentially expressed (65 increased and 20 decreased; p≤0.05 Welch’s 2-Sample t-Test). The metabolic lantionhaine, which is produced by the CBS-dependent condensation of cysteine to produce H2S, showed the largest increase (12.3-fold increase, p=1.5E-06). A 4-fold increase in basal H2S production was measured in CBS-hi cells compared to control cultures. High-resolution respirometry showed that CBS-hi cells exhibited a significant increase in maximum respiration rate, reserve respiratory capacity and increased citrate synthase activity, suggesting increased cellular mitochondria mass. Treatment of CBS-hi cells either with pentose phosphate pathway inhibitor (oxythiamine) or lactate dehydrogenase inhibitor (XF-11) reducing growth by 90% and ~25% at day 5, respectively; the inhibitors had no effect on the vector control cells. Exposure of NC3536 cells to hypoxic conditions resulted in increased migration to conditioned media (p<0.001, NC3536 normoxia vs. hypoxia); inhibition of CBS using AOAA attenuated the hypoxia-induced migration. Conclusions: Hypoxia and LPS can induce CBS expression in a premalignant colonic epithelial cell line. Uregulation of CBS has a board impact on cellular metabolism including enhanced flux through the transsulfuration, glycolytic and pentose phosphate pathways, resulting in enhanced cellular bioenergetics, growth and migration. These data suggest that enhanced CBS activity may be involved the adenoma to carcinoma sequence.

#3547 B cell receptor signaling regulates cellular metabolism in Chronic Lymphocytic Leukemia. Hima V. Vangapandu, Ondrej Havranek, Varsha Gandhi. UT MD Anderson Cancer Ctr., Houston, TX. Peripheral blood B cells in chronic lymphocytic leukemia (CLL) are quiescent, yet have an active RNA transcription and protein translation processes, suggesting that the proliferant lymphocytes may be metabolically active. However, CLL metabolism and its relationship to disease biology has not been well explored. In our previous investigation, we reported that compared with proliferative B cell lines, metabolic fluxes of oxygen and lactate were low in quiescent peripheral blood B lymphocytes from CLL patients (Vangapandu, H.V. et al, AACR 2014). In 45 patient samples tested, glycolysis (extracellular acidification rate, ECAR) was consistently low (1 to 15 mP/5 min/5 x10^6 lymphocytes), whereas, oxygen consumption rate (OCR) varied considerably (5 to 190 pMoles/ min/5 x10^6 lymphocytes). Among the prognostic factors, high OCR correlated strongly with ZAP 70 positivity, unmutated IGHV status, greater β2M levels and higher Rai stage. In contrast, glycolytic flux (ECAR) from same patient samples did not associate with prognostic factors. Further, OCR did not vary on the basis of frequently occurring cytogenetic abnormalities, 13q14, 17p, and 11q deletions or trisomy 12. Since, ZAP 70 and IGHV unmuted status are associated with augmented B cell receptor (BCR) pathway signaling, we tested the impact on OCR after genetic ablation of B cell receptor (CH2 region of IgM). A CRISPR-Cas9-mediated BCR knockout mitigated OCR in a malignant B cell line without impacting the rate of proliferation. A critical node in the BCR pathway is PI3 Kinase and AKT and up regulation, δ and γ are involved in B-cell malignancies. Consistent with BCR ablation results, knocking out PIK3CD (codes for catalytic subunit δ), dramatically reduced OCR and ECAR. Pharmacological inhibitors of the PI3K pathway, duluisilis (a PI3 Kinase δ/γ inhibitor in phase III clinical trials) or idelisilis (FDA approved PIK3 δ inhibitor) also decreased OCR. Direct inhibition of AKT with MK-2206 showed similar results. Collectively, these data suggest that CLL cellular metabolism is associated with prognostic factors and linked to BCR signaling pathway. Since, the PIK3 inhibitors used in our study are being tested clinically for patients with CLL, investigations on the impact on metabolisms during therapy and translation of results to combination strategies need to be explored.

#3549 C-MYC sensitizes GBM with primitive features to glutamine metabolism disruption. Brad Andrew Poore, Isabella Taylor, Jeffrey Rubens, Allison Hanaford, Michal Maxwell, Charles Eberhart, Eric Raabe. Johns Hopkins Medical Insts., Baltimore, MD. Glioblastoma (GBM) is among the most lethal of known human cancers, with a median survival of less than 15 months. The highly infiltrative nature and genetic heterogeneity of GBM renders treatment difficult. Therefore, better and more targeted therapies are needed for patients with GBM. There is a new WHO subset of GBM that contains primitive neuronal components (GBM-PNC). These tumors can arise from a histologically classic GBM, and often the GBM-PNC portions of the tumor contain C-MYC or N-MYC amplifications. High MYC expression is known to alter cellular metabolism, increasing reliance on glutamine, which may create opportunities for therapeutic intervention. We hypothesized that depriving GBM-PNC cells of glutamine using metabolic inhibitors would suppress growth and tumorigenicity. To create genetically appropriate GBM-PNC models, we derived cortex (CTX) human neural stem cells (human neural stem cells) transfected with a lentiviral vector expressing both C-MYC or BMI1. These models formed aggressive tumor masses in nude mice expressing BMI1 had no statistically significant changes. Additionally, cells expressing BMI1 had no statistically significant changes. Furthermore, the relationship between glutaminolysis and tumor growth was demonstrated using the transplantable GBM-PNC model. Taken together, our study demonstrated that C-MYC expression is known to alter cellular metabolism, increasing reliance on glutamine, which may create opportunities for therapeutic intervention. The ability to deprive tumor cells of glutamine indicates a target for therapy, and suggests that glutaminolysis and increased glutamine dependence may contribute to aggressiveness of GBM-PNC models.
metabolite, glutamine is first converted to glutamate by glutaminase (GLS) and activation, we are also studying if PDL1 is regulated by glycolysis through Akt. human triple-negative breast cancer, suggesting that the PI3K/Akt pathway can dramatically increased T-cell infiltration and survival (40% of mice are long-term survivors). Lactate, the end product of glycolysis, has been shown to be toxic to T-cells, and LDHA-depleted tumors produce less lactate compared to controls. Thus, we propose that inhibiting tumor glycolysis leads to a less acidic microenvironment, leading to increased T-cell infiltration. Further, PTEN expression has been shown to be inversely correlated with PDL1 expression in human triple-negative breast cancer, suggesting that the PI3K/Akt pathway can promote PDL1 expression. Having established that glycolysis can promote Akt activation, we are also studying if PDL1 is regulated by glycolysis through Akt.

Targeting glutamine addiction of PIK3CA mutant colorectal cancers. Yiqing Zhao, Case Western Reserve Univ., Cleveland, OH.

Glutamine addiction is a major metabolic reprogramming event that occurs in cancer cells. Many tumors exhibit oncogene-dependent addiction to glutamine. PIK3CA, which encodes the p110 catalytic subunit of phosphatidylinositol-3 kinase α, is the most frequently mutated oncogene in human cancers. However, whether PIK3CA mutations reprogram cancer metabolism is an important unaddressed question. Here we show that oncogenic PIK3CA mutations render colorectal cancers (CRCs) more dependent on glutamine to growth. As a metabolite, glutamine is first converted to glutamate by glutaminase (GLS) and then to α-ketoglutarate (α-KG) by either a transaminase or a glutamate dehydrogenase. Calthera Biosciences recently developed a potent GLS inhibitor called CB-839, which is currently in phase I clinical trials in cancer patients. Using isogenic colorectal cancer cell lines with either WT or mutant PIK3CA allele knockout, we demonstrated that CRCs with PIK3CA mutations are more sensitive to growth inhibition by CB-839. Remarkably, combination of CB-839 with 5-FU induces expression xenograft tumors in PIK3CA mutant CRC models, suggesting that this combinational therapy may be effective approach to treat CRC patients whose tumors harbor PIK3CA mutations. Mechanistically, mutant p110α up-regulates gene expression of glutamate pyruvate transaminase 2 (GPT2) through an AKT-independent PDK1-RSK2-ATF4 signaling axis, thereby facilitating conversion of glutamate to α-KG. Together, our data establish oncogenic PIK3CA mutations as a cause of glutamine addiction in CRCs and that targeting glutamine metabolism may be effective approach to treat CRCs with PIK3CA mutations.

Selective inhibition of copper metabolism as a novel approach to treat triple-negative breast cancer. Olga Karginova, Claire Weekley, Akila Raoul, Allhareth Alsayed, Tong Wu, Chuan He, Olufunmilayo I. Olopade.

The rationale: Treatment of patients with triple-negative breast cancer (TNBC) remains challenging due to unpredictable disease progression and acquired resistance to chemotherapy. Finding novel targets in TNBC is important for the development of anticaner therapy. Cancer cells frequently adapt to cytotoxicity of existing pharmacological agents by exploiting metabolic pathways. Elevated copper metabolism and adaptations to oxidative stress have been linked to cancer progression. Copper chelation with tetrathiomolybdate (TM) was reported to control disease progression, especially in patients with TNBC. However, long-term consequences of global copper depletion are still under investigation. An alternative approach to develop novel therapeutics is to inhibit copper chelation with tetrathiomolybdate (TM) that blocks the copper transfer interface of two major copper chaperons, ATOX1 and CCS. Protein levels of ATOX1 and CCS are elevated in TNBC cells compared to normal cells. Thus, we hypothesized that disrupting copper transport by targeting ATOX1 and CCS with DCAC50 may suppress TNBC progression. Results: We investigated efficacy and potency of novel small molecule DCAC50 to induce cytotoxicity in a panel of TNBC cell lines. DCAC50 reduced cell proliferation in dose-dependent manner, measured by MTS assay. IC50 doses ranged from 3-10μM. Most importantly, DCAC50 induced apoptosis in TNBC cells, detected by Caspase 3/7 activity and Annexin V/PI staining. As expected, DCAC50 elevated intracellular copper levels. Moreover, TNBC cells treated with DCAC50 had significantly higher levels of oxidized glutathione (GSSG) and caused increased oxidation of DCF-DA reagent, demonstrating elevated oxidative stress. Surprisingly, activity of CCS downstream target, SOD1, was not affected by selective inhibition of copper transport with DCAC50; suggesting that intracellular copper accumulation and oxidative stress is mediated by ATOX1-1 inhibition. Interestingly, copper depletion with TM inhibited SOD1 activity but failed to significantly impact oxidative stress in TNBC cells. Recognizing DCAC50 generates oxidative stress triggering apoptosis in TNBC cells we evaluated benefits of combining DCAC50 treatment with paclitaxel. Multi-drug combination dose-response analysis revealed that treatment induced synergistic cytotoxicity and resulted in favorable dose reduction of both drugs. Conclusions: Selective inhibition of copper metabolism with novel small molecule DCAC50 elevates oxidative stress triggering apoptosis in TNBC cells. This approach may be valuable in combination with chemotherapy especially when tumor cells acquire resistance to first line therapy in TNBC. Ongoing studies comparing selective inhibition of copper transport to global copper chelation, and investigating efficacy and biological activity of DCAC50 in vivo will help further estimate benefits and clinical relevance of this approach for treatment of TNBC patients.


Non-small cell lung cancer (NSCLC), the most common type of lung cancer (comprising 80-85%), is a leading cause of cancer-related death worldwide. KRAS mutation is a major driver oncogene in NSCLC. Constitutive activation of K-Ras via oncogenic mutations plays a key role in development and progression of NSCLC, and is an important target for drug development. Indeed, drugs that target RAF-MAPK and PI3K, the well-established downstream pathways of K-Ras, are being evaluated in the clinic. However, to date, no effective treatments specifically targeting K-Ras have reached the clinic. A new direction for anti-Ras therapies targeting Ras-mediated altered cellular metabolism is implied by recent studies. Hence, as an attempt to investigate novel therapeutic targets to suppress KRAS-driven NSCLC, we assessed metabolic changes in NSCLC cell line and mouse model systems with oncogenic K-Ras mutations. We show that activation of Ras causes metabolic alterations, leading to aerobic glycolysis. Interestingly, despite the changes in the glycolytic pathway, NSCLC cell line and mouse models carrying K-Ras mutation revealed an elevated mitochondrial membrane potential, superoxide production as well as maximal oxygen consumption rate. Moreover, blockade of mitochondrial respiration effectively reduced mutant KRas-driven neoplastic phenotypes in NSCLC cells line and lung tumor formation in vivo. In contrast, blockade of glycolysis was not effective in the mutant KRas-driven tumorigenic activities. Mechanistically, mutant KRas-mediated activation of Akt led to increased expression of succinate dehydrogenase. Rubi point (A SDH) through increased protein stability by blocking proteasome-mediated degradation, resulting in mitochondrial complex II-dependent respiration. Our study suggests that induction of mitochondrial complex II-dependent respiration is an important mechanism by which oncogenic mutant KRas induces metabolic changes and ROS stress in NSCLC cells, contributing to lung carcinogenesis. Our findings elucidate potential metabolic targets that may provide novel therapeutic strategy for treating NSCLC with oncogenic KRAS mutations.
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Background: The metabolic features of cancer cells allows their rapid proliferation and escape from apoptosis. Novel targeted treatments are being developed for metabolic dysregulation of malignant cells. There is a growing body of evidence regarding the effects of metformin on the multiple pathways that regulate carcinogenesis. Metformin interacts with insulin growth factor signaling to reduce cell proliferation and the induction of apoptosis. The drug has profound effects on the mitochondrial respiration rate and ATP production. Metformin affects multiple cellular pathways via the activation of AMP-activated protein kinase (AMPK) by liver kinase 1 (LKB1), decreasing growth factor signaling and proliferation via mTOR inhibition. Metformin has been demonstrated to sensitize EGFR tyrosine kinase inhibitor (TKI)-resistant lung cancer cells by reversing the Warburg effect by regulating the EGFR and AMPK signaling pathways in Non-Small Cell Lung Cancer (NSCLC). Objective: Determine the effect of the combined Afatinib-Metformin treatment on NSCLC with different mutational status of EGFR. Methods: Cells with different mutational status of EGFR: A549, H1975 and HCC827 (ATCC) were used. Once the IC50 of afatinib and metformin were found, different combinations were made to determine whether there was a synergistic, additive or antagonistic effect using the Compusyn software. Cell viability was measured by the MTT assay and apoptosis was measured by flow cytometry in all cell lines. We found IC50s for metformin and afatinib for each cell line were as follows: 7 μM (A549), 2 μM (H1975) and 4 nM (HCC827) and the IC50 values for Metformin were 15μM (A549) 5μM (H1975) and 8μM (HCC827). We found a synergistic effect in H1975 for the combination of 3μM Afatinib plus 15 mM Metformin. In A549 we found a synergistic effect in all combinations. Finally, in HCC827 the combination of 5 nM and 4 nM Afatinib plus 8 nM metformin showed a synergistic effect. The apoptosis rate was increased up to 47% in all combinations for A549 cells. The combination of 3nM Afatinib and 8 mM Metformin induced 59% apoptosis in HCC827. Finally, H1975 induced 46% of apoptosis in the combination that showed synergistic effects. Conclusions: Afatinib-Metformin showed synergistic effects in a dose-dependent manner in three different cell lines of NSCLC.

#3555 Lipid droplets, a novel metabolic target in glioblastoma. Feng Gong, Jeffrey Guo, Xiang Cheng, Xiaoning Wu, Chunming Cheng, Arnav Chakravarti, Deliang Guo. The Ohio State University, Columbus, OH.

Lipid metabolism reprogramming is a novel feature of malignancies. Identifying the vulnerability of lipid alteration in tumor cells provides a new window to target cancer. Here, we unraveled that glioblastoma (GBM) patients containing high levels of lipid droplets (LDs), special organelles storing cholesterol esters and triglycerides in cells, presented poor survival. We found that LDs largely formed in tumor tissues from GBM patients, but rarely in low grade of gliomas and normal brain tissues, demonstrating that LDs might serve as a novel diagnostic biomarker and prognostic marker for GBM. We further revealed that sterol O-acyltransferase 1 (SOAT1), the key enzyme controlling cholesterol esterification and LD formation, is highly expressed and correlated with LD prevalence in tumor tissues from GBM patients. Genetic pharmacological inhibition of SOAT1 using avasimibe, a phase III clinical trial drug in human breast cancer model, was confirmed in mouse allograft model. Finally, we show that the expression of miR-155 controls energy metabolism in breast cancer, through PI3K p85 alpha - FOXO3a-cMYC axis in breast cancer. Sinae Kim, Eunjee Lee, Suhwan Chang. Univ. of Ulsan College of Med./Asan Medical Ctr., Seoul, Republic of Korea.

Breast cancer is the most common cancer for women worldwide. Although the oncogenic roles of miR-155 in cancer have been reported, the metabolic function of miR-155 in breast cancer has not been fully elucidated. In this study, we have investigated how the energy metabolic shift occurs after a genetic inactivation or knockdown of miR-155. To this end, we performed metabolic profiling of the miR-155-5p isoform or miR-155-5p primary cancer cells, obtained from mouse breast cancer model. We found, in the absence of miR-155, less glucose uptake and glycolysis in tumor cells. Mechanistically, we revealed PIK3R (Phosphoinositide-3-Kinase, Regulatory Subunit1, p85<alpha>-/> and FOXO3a-cMYC axis in breast cancer. Eunji Lee, Sinae Kim, Suhwan Chang. Univ. of Ulsan College of Med./Asan Medical Ctr., Seoul, Republic of Korea.

Breast cancer is the most common cancer for women worldwide. Although the oncogenic roles of miR-155 in cancer have been reported, the metabolic function of miR-155 in breast cancer has not been fully elucidated. In this study, we have investigated how the energy metabolic shift occurs after a genetic inactivation or knockdown of miR-155. To this end, we performed metabolic profiling of the miR-155-5p isoform or miR-155-5p primary cancer cells, obtained from mouse breast cancer model. We found, in the absence of miR-155, less glucose uptake and glycolysis in tumor cells. Mechanistically, we revealed PIK3R (Phosphoinositide-3-Kinase, Regulatory Subunit1, p85<alpha>-/> and FOXO3a-cMYC axis in breast cancer. Eunji Lee, Sinae Kim, Suhwan Chang. Univ. of Ulsan College of Med./Asan Medical Ctr., Seoul, Republic of Korea.

Metabolic reprogramming, recently becoming one of the cancer hallmarks, is linked to oncogenic signal transduction to find a better solution for cancer therapy. As one of key mediators in oncogenic signal transduction, non-receptor tyrosine kinase Src is known to drive cancer progression by promoting cell proliferation, metastasis, and drug resistance. Although previous studies reported that Src promotes fat accumulation and suppressesrowning of white adipocytes, little is known about the mechanistic role of Src in cancer metabolism. Here we report that Src mediates lipid mobilization by suppressing transcriptional activity of PPARγ, a key regulator of lipid metabolism. Firstly, we found Src suppression of PPARγ activity which, interestingly, turned out to be independent of Src kinase domain or a known Src phosphorylation site of PPARγ at tyrosine 78. Since the treatment of Src inhibitor (e.g., SU6656, PP2) could rescue Src-driven PPARγ suppression by possibly changing Src conformation, we might suggest the involvement of Src-PPARγ interaction in the regulatory mechanism. Mechanistically, further in vitro experiment showed Src...
inhibitor treatment recovered expression of PPARγ target genes such as fatty acid binding protein 4 (FABP4) and cluster of differentiation 36 (CD36) in a subset of non-small cell lung cancer cell lines. The increase of FABP4 and CD36 expression was associated with the reduction of lipid droplets, suggesting the role of Src regulation of PPARγ in lipid mobilization. Collectively, this finding provides valuable insights into the role of Src in cancer lipid metabolism, and an insight into therapeutic combination of targeting oncogenic signal transduction and lipid metabolism to treat cancer.

#3559 Functional characterization of HNF4α in gastric cancer. Chang Xu,1 Wen Fong Ooi,2 Aditi Qamra,3 Patrick Tan1. 1Cancer Science Institute of Singapore, Singapore, Singapore; 2Genome Institute of Singapore, Singapore, Singapore; 3Genome Institute of Singapore, Singapore, Singapore.

Gastric cancer (GC) is a leading cause of cancer mortality worldwide and particularly common in East Asia. Transcription factors are frequently amplified or overexpressed in GC and might contribute to tumorigenesis, however the underlying mechanisms largely remain unknown. Our previous data indicates that HNF4α is a common target of three amplified transcription factors (GATA4, GATA6 and KLF5) in gastric cancer. HNF4α had also been found to be related to diverse aspects of tumorigenesis. In liver and colon cancers, there are reports indicating that the targets of HNF4α are mainly involved in intermediary metabolism, however, its tissue-specific downstream targets and pathways have not been systemically studied yet. Therefore, a comprehensive functional characterization of HNF4α is required to identify its tissue-specific role in gastric cancer. In this study, we analyzed the expression features of HNF4α in GC, and found the expression level of HNF4α is significantly higher in early stage tumor and in CIN and MSI compared to GS and EBV subtypes of GC according to TCGA classification method. In addition, we performed the HNF4α ChIP in GC cell lines, and the results revealed a number of HNF4α binding sites in GC. Comparison with the published ChIP-seq data of Caco2 and HepG2 cell lines indicated the existence of GC-exclusive targets. Furthermore, functional annotation analysis revealed that the GC-exclusive targets are significantly related to the negative regulation of cellular metabolic process compared to the common targets. These preliminary data suggests that HNF4α might play a tissue specific role in GC, especially in metabolic related processes. Through further target validation and biological function studies, this study hopes to systematically investigate of HNF4α’s role in GC tumorigenesis, especially in metabolism related pathways.

#3560 6-diazo-5-oxo-l-norleucine resistant MYC-driven medulloblastoma tumors are sensitive to inhibition of glutaminase suggesting a possible glutaminase-independent mechanism of resistance. Allison Rose Hanaford, Bradley Poore, Charles G. Eberhart, Eric H. Raebe. Johns Hopkins Univ. School of Medicine, Baltimore, MD.

Increased MYC levels can alter cellular metabolism, creating a reliance on glutamine. Glutamine PET and MRI spectroscopy demonstrate that aggressive brain malignancies have increased uptake of glutamine and increased glutamate relative to normal brain, suggesting that agents targeting glutamine metabolism may be active in brain tumors. 6-diazo-5-oxo-norleucine (DON) is a glutamine analog that was tested extensively in adult and pediatric malignancies but never advanced beyond phase I clinical trials. We hypothesized that DON would be effective against the MYC-driven subgroup of the pediatric brain tumor medulloblastoma. DON treatment increased the median survival of mice bearing D425Med xenografts by 246 percent (26 days for vehicle treated mice compared to 90 days for DON treated mice, p<0.0018) and the median survival of mice bearing the MYC-driven patient derived xenograft MED211 by 80 percent (49 days for vehicle treated mice compared to 88 days for DON treated mice, p=0.0017). We also tested DON prodrugs designed for improved oral bioavailability and CNS penetration. Our novel DON prodrugs increased the median survival of mice bearing MYC-driven D425MED orthotopic xenografts by 60 percent (22 days for vehicle treated mice compared to 35 days for prodrug treated mice, p<0.01). In non-human primates, our DON prodrugs exhibited superior 10-fold enhanced CSF/plasma ratio versus regular DON. Though DON treatment causes a dramatic increase in survival, resistance eventually develops. To investigate the mechanism of DON resistance, we tested the effect of other glutamine metabolic inhibitors on multiple cell lines generated from DON-resistant xenografts. DON-resistant xenograft derived cell lines maintained DON resistance in culture, but were sensitive to treatment with the glutaminase inhibitor compound 968 and the glutamine analog Acivicin. Compound 968 treatment decreased growth of DON-resistant cell lines by an average of 79 percent (p<0.0008), and Acivicin treatment decreased growth by an average of 78 percent (p<0.000003). Glutaminase catalyzes the first step in glutamine metabolism—the conversion of glutamine to glutamate. The sensitivity of DON-resistant cells to glutaminase inhibition indicates continued tumor reliance on glutaminase activity. We are continuing to investigate the mechanism DON resistance, with the goal of developing a rational combinatorial therapy to treat resistant tumors. This knowledge could increase the potential utility of DON as a clinical agent.

#3561 UCP2’s tumor-promoting role via regulating lipid signaling and PLC-γ1 activity. Annaopoora Sreedhar, Yunfeng Zhao. Louisiana State University Health Sciences Center, Shreveport, LA.

Mitochondrial abnormalities are long sought to play important roles in tumor cell differentiation, proliferation and metabolism. Dysfunction of mitochondrial-dependent processes appear to be key features of cancerous cells. Among many such abnormal features of cancerous cells, a mitochondrial uncoupling protein 2 (UCP2) is shown to be up-regulated in various aggressive human cancers. Uncoupling proteins are a family of mitochondrial proteins present in the inner mitochondrial membrane whose physiological role is to decrease membrane potential and reactive oxygen species (ROS) production. UCP2 over-expression recently been proposed as a novel survival mechanism for cancer cells. However, till date, the exact role of UCP2 in cancer remains inconclusive. We recently reported that UCP2 appears to be a key regulator of cell proliferation, cell cycle and cell death during skin tumorigenesis. Using primary cells that overexpress UCP2, we showed that UCP2 correlated closely with cell proliferation and cell transformation. Moreover, inhibition of UCP2, decreased colony formation and 3D cell growth. Since UCP2 is the crucial player in the regulation of reactive oxygen species, and mitochondrial bioenergetics, we wanted to dissect out the role of UCP2 in redox regulation and tumor promotion. Our data demonstrated that UCP2 differentially regulated ROS. UCP2 upregulation decreased superoxide production, whereas increased hydrogen peroxide production with a concomitant increase in manganese superoxide dismutase (MnSOD) expression and activity. Furthermore, hydrogen peroxide was responsible for induction of lipid peroxidation and PLC-γ1 activation in UCP2 overexpressed cells. Moreover, PLC-γ1 activation enhanced tumorigenicity. Strikingly, pharmaceutical and siRNA mediated inhibition of UCP2 markedly reduced colony formation and 3D cell growth in vitro. Lastly, restricting hydrogen peroxide production with hydrogen peroxide scavenger catalase, suppressed lipid peroxidation and dampened PLC-γ1 activity. Taken together, our data suggest that hydrogen peroxide might be the mediator of UCP2’s tumor promoting role, and pharmacological disruption of PLC-γ1, and/or hydrogen peroxide may have clinical utility for UCP2-overexpressed cancers. This research was funded by NCI and Feist-Weiller Cancer Center of LSUHC-S.
MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Regulation of Metabolic Pathways in Cancer

#3563 Potential role for a phosphorine aminotransferase 1 and pyruvate kinase M2 (PSAT1/PKM2) functional interaction in lung cancer cells. Rumeya B. Sit, Traci Krueer, James Bradley, Michael Merchant, John O. Trent, Brian F. Clem. Univ. of Louisville, Louisville, KY.

Cellular production of serine provides precursors for numerous anabolic reactions, notably protein and nucleic acid synthesis and nucleotide biosynthesis, and metabolism, and this pathway has been demonstrated to be elevated in many different cancer types. In addition, metabolic alterations within serine synthesis can stimulate other processes required for neoplastic growth, including glucose metabolism, specifically through the activation of the glycolytic enzyme pyruvate kinase M2 (PKM2). However, the complete mechanism by which serine biosynthetic pathway participates in tumor cell metabolism is not clear and is further confounded by the observation that suppression of serine biosynthetic enzymes disrupts tumorigenesis even in the presence of exogenous serine. Phosphorine aminotransferase 1 (PSAT1), the second enzyme in serine synthesis, catalyzes the conversion of 3-phosphohydroxypropionate to phosphoserine and increased expression correlates with poorer overall survival in lung cancer patients. In order to determine whether PSAT1 may contribute to lung cancer progression, PSAT1 expression was stably silenced in A549 and H358 lung cancer cells. Although a modest change in cell proliferation was observed upon PSAT1 knock-down compared to control, there was a robust decrease in soft-agar colony formation in both cell types. PSAT1 suppression was accompanied by distinct morphological changes and an increase in E-cadherin expression. These results suggest a potential link between serine-glucose metabolism, EMT, and drug sensitivity. In addition, we have identified a novel PFKFB2-dependent functional interaction in lung cancer cells that may yield valuable information about the relationship between serine-glucose metabolism and EMT transition in cancer cells and provide an additional therapeutic target for cancer treatment.

#3564 Silencing pfkfb2 enhances paclitaxel sensitivity by modulating metabolism of p53 wt ovarian and breast cancer cells and xenografts. Hailing Yang, Shu Zhang, Weiquan Mao, Ahmed A. Ahmed, Nicholas B. Jennings, Cristian Rodriguez-Aguayo, Gabriel Lopez-Berestein, Anil K. Sood, Xiao-Feng Le, Zheng Lei. UT Southwestern Medical Center, Dallas, TX.

Breast cancer is the most common cause and epithelial ovarian cancer the fourth most common cause of cancer death among women in the developed world. While breast cancer can be cured in 70% of cases, only 30% of ovarian cancer patients remain free from disease long term. Paclitaxel is an integral component of primary therapy for both forms of cancer, but less than half of breast and ovarian cancers respond to the drug. Enhancing the response to primary therapy with paclitaxel could improve outcomes for women with both diseases. In recent years several kinases have been identified that regulate the sensitivity of cancer cells to paclitaxel by inhibiting centrosome splitting or enhancing microtubule stability. Much less attention has been given to kinases that affect paclitaxel sensitivity by modulating cancer cell metabolism. We previously performed siRNA kinase screens to identify molecular targets whose decreased expression overcomes paclitaxel resistance and increases paclitaxel sensitivity in ovarian cancer cells. We showed that 20% of the potential kinase targets whose knockdown modulates paclitaxel sensitivity participate in glucose and energy metabolism. Among these, a leading candidate was 6-phosphogluconate dehydrogenase 2 (PDK2), a key enzyme of the pentose phosphate pathway. Knockdown of PDK2 inhibited oncogenic growth of ovarian and breast cancer cell lines and enhanced paclitaxel sensitivity in xenografts derived from ovarian cancer cell lines. Knockdown of PFKFB2 increased glycolysis, decreasing the flow of glycolytic intermediates to the pentose phosphate pathway with reduced G6PD activity in wt TP53 cancer cell lines. With decreased NAPDH, ROS accumulated after PFKFB2 knockdown, stimulating phosphorylation of JNK, inducing G1 cell cycle arrest, and initiating apoptosis dependent upon upregulation of p21Waf and Puma which are down-stream targets of TP53. Our studies have shown for the first time that PFKFB2, a glycolytic enzyme, drives tumor cell growth and regulates paclitaxel sensitivity by inducing apoptosis and G1 cell cycle arrest. These findings highlight a remarkable degree of coordination between cancer metabolism with cell proliferation and chemosensitivity, which may provide a novel target in patients with ovarian cancers and breast cancers where TP53 function remains intact.

#3565 Recurrently detected germline mutation of FLT3 (D358V) associated with prevalence of hematopoietic malignancy and prognosis of AML patients. Seulki Song. Cancer Research Institute, Seoul, Republic of Korea.

FLT3 is most frequently mutated gene in acute myeloid leukemia patients. While somatic mutation of FLT3 is well known, germline mutation of FLT3 and predispose to AML remain poorly understood. Here we identify a germline FLT3 p.D358V affected progeny of AML patients. We performed whole exome sequencing (WES) using 76 patients’ saliva sample to detect FLT3 germline mutation in buccal epithelial cell and demonstrate FLT3 p.D358V mutation for 6 patients among 76 patients. The frequency of FLT3 p.D358V mutation in AML patients is significantly higher in general population (p=9.50E-07), suggesting association of FLT3 mutation with AML predisposition. Asian population especially carried this locus more than other ethnic population. When we compared between AML patients and general population of Asian, the chi-square p-value was 0.07 and odd ratio was 2.26. Overall survival was significantly reduced in patients who had FLT3 p.D358V mutation (p=0.03) and disease free survival was show a tendency of reducing in the patients with FLT3 p.D358V mutation.

#3566 The long tail of significantly mutated genes in prostate cancer. Joshua Armenia,1 Stephanie Mullane,2 Jianjiong Gao,3 Debyani Chakravarty,2 Pasha Kundr,1 Franklin Hsing,1 Celine Han,1 Dan Robinson,1 Levi A. Garraway,1 Peter Nelson,1 Mark Rubin,1 Mary-Ellen Taplin,2 Wassim Abida,1 Charles L. Sawyers,4 Arul M. Chinnaiyan,1 Philip W. Kantoff,1 Johann S. Bono,1 Nikolaus Schulz,1 Eliezer M. Van Allen1.1. Mem Sloan Kettering Cancer Ctr., New York, NY; 2 Dana-Farber Cancer Institute, Boston, MA; 3 Center for Translational Pathology, University of Michigan, Ann Arbor, MI; 4 Dana-Farber Cancer Institute, Boston, MA; 5 Fred Hutchinson Cancer Research Center, Seattle, WA; 6 Well Cornell Medical College, New York, NY; 7 University of Michigan, Ann Arbor, MI; 8 The Institute of Cancer Research, London, United Kingdom.

Background: The mutational landscapes of primary and metastatic prostate cancer (PCa) have been robustly analyzed in multiple whole exome sequencing (WES) studies. We hypothesized that an aggregate, uniform analysis of all data generated to date would enable discovery of new significantly mutated genes and pathways not previously associated with PCA, and shed more light onto the genetic differences between primary and metastatic PCa. Methods: We uniformly analyzed WES data of 1,021 tumor and matched germline primary and metastatic PCa (686 primary, 335 metastatic). We performed mutational significance analysis using statistical and biological approaches to determine recurrently altered genes and pathways. Results: We identified 117 significantly mutated genes (Mutsig q<0.1) in PCa. These include epigenic modifiers ([KMT2C (6%), KMT2D (6%), and KDM6A (2.7%)] and regulators of the SWI/SNF complex [SMARC A1 (1.1%), ARID1A (1.5%), ARID1B (1.3%), ARID2 (1.3%), and PBRM1 (0.7%)]. Interestingly, genomic alterations in chromatin remodelers (MLL, SWI/SNF) appear to be significantly mutually exclusive with ETS fusions and SPOP mutations (p<0.001, Fisher’s test), indicating that these mutations may represent a novel distinct oncogenic driver in prostate cancer. Among the novel PCA related pathways, the splicing pathway was found to have oncogenic mutations in key drivers such as SFB1 (1.1%), U2AF1 (0.5%), and FUBP1 (0.4%), a novel splicing regulator involved in the regulation of MDM2 splicing. We also found truncating mutations in SPEP, a hormone inducible transcriptional repressor, in 2.8% of samples, similar to the frequency observed in breast tumors, and these mutations appear to be significantly associated with AR mutations (p=0.01, Fisher’s test). Our analysis also uncovered mutations in CUL3 (1.3%) and KLHL20, components of an E3-ubiquitin ligase complex that interacts with SPOP to promote the degradation of critical PCA genes (AR, SRC), KLHL20, CUL3, and SPOP mutations are mutually exclusive. Finally, a comparison of primary and metastatic samples identified alterations that are associated with metastatic disease, including AR amplifications and mutations, and loss of TP53, PTEN, and RB1. At lower frequency, metastatic tumors showed enriching
ment in mutations in MLHs (KMT2C/D), APC, CDK12, BRCA2, CTNNB1, and amplifications of MYC and CCND1. Conclusions: Through aggregation and uniform genomic analysis, we refined the map of somatic mutations in PCa and identified cancer genes and pathways not previously associated with this disease. Our findings may inform patient stratification and translational investigation.

## RESULTS

### Variant analysis of LY6 genes in TCGA ovarian cancer.

Krithika Bhuvaneshwar, Midrar Al Hossiny, Yuriy Gusev, Subha Madhavan, Geeta Upadhayay, Georgetown University, Washington, DC.

Background Human LY6 gene family has been associated with stem cell marker Sca-1 in murine cancer. Sca-1 is known to regulate TGF-β signaling. Wnt signaling & it is important in cancer progression and metastasis in mouse models. Human LY6 genes are associated with poor clinical outcome in human cancers. Previous studies have shown that this family of genes is highly expressed in Ovarian & Breast cancer compared to normal tissues. Overexpression of these genes was found to be correlated with poor outcome in overall and metastasis free survival. Recent studies have also identified that human LY6 genes are associated with tumor immune escape & drug resistance. In this poster, we explore the variants in LY6 and related genes in the TCGA Ovarian Cancer (OV) data collection. Materials and Methods We first downloaded RNA-seq data of primary tumor tissues from 21 TCGA OV patients from CGHub (https://cghub.ucsc.edu/), and after quality control, aligned to human reference genome using tool RSEM on the Globus Genomics platform. The BAM file was sorted and PCR duplicates were removed. Variant calling was done on BAM files based on Genome Analysis Toolkit (GATK)’s best practices, to obtain a multi sample variant call file (VCF). After getting the multi sample VCF file, we used Snpeff and Annovar software to annotate and predict the functional effects of variants on genes. Snpsift toolbox was used to filter out variants by extracting only variant of LY6 genes (and other genes of interest) that passed the quality check, and categorized the output into 4 different groups according to the impact (High, Moderate, Modifier, Low). To see if these variants were germline or somatic, RNA-seq from a second set of 22 TCGA Ovarian cancer samples (tumor tissue and normal blood samples) were used. BAM files were downloaded and variants were called using the Seven Bridges system. The same filtering steps were applied as above. Results In Set 1, we found variants in CD59, LY6E and LYPD6 mutated in all the 21 cases. We found two stop-loss mutations in CD59 gene, which is responsible for regulating the immune response, tumor cell growth and apoptosis. We found a total of 3794 unique variants short-listed in Set 1, and a total of 8879 unique variants short-listed in Set 2. It was expected to see more variants from the DNA-seq data compared to the RNA-seq data. Among these, 103 unique variants were common to both Set 1 and Set 2. Variants in ESRI, CD44 and LYPD6 family were mutated in most samples in both Set 1 and Set 2. We also performed survival analysis on variants present in RNA but not in DNA, and found variants in L16E, PINLYP, LYPDS, ZNF283 significant w.r.t overall survival. Conclusions: We found a total of 3794 unique variants (from short-listed set) in Set 1 (TCGA OV RNAseq data), and a total of 8879 unique variants (from short-listed set) in Set 2 (TCGA OV DNAseq). There were total of 103 unique variants common to Set 1 and Set 2. We see evidence of LY6 variants in non-coding regions of DNA (not in DNA) to be significantly associated with overall survival.

## DISCUSSION

The top-ranked biological functions affected by ERG over-expression include Cell Cycle (p < 1.42E-04), Cellular Growth and Proliferation (p < 1.23E-04), Cellular Development (p < 1.23E-04), Cell Death and Survival (p < 1.37E-04), and Cellular Assembly and Organization (p < 1.42E-04). Further analyses indicate a strong association with known cancer networks. The top-ranked canonical pathways enriched in ERG-positive compared to ERG-negative LNCaP cells include, Cell Cycle Control of Chromosomal Replication (p = 2.65E-16), Role of CHK Proteins in Cell Cycle Checkpoint Control (p = 3.16E-11), Cell Cycle: G2/M DNA Damage Checkpoint Replication (p = 1.34E-09), Role of BRCA1 in DNA Damage Response (p = 4.05E-08) and Estrogen-mediated S-phase Entry (p = 5.51E-08). These findings indicate new insights into the complexity of TMRPSS2-ERG gene fusion, and may help understand mechanistic pathways which promote growth and progression of CaP.
donor. However, there are a number of possible scenarios in which matched normal tissues might be not available for comparisons. It is most commonly encountered when performing analysis on retrospective studies with human tissues from clinical trials or pathology archives when normal samples were not collected in the first place or patient consent precludes examination of normal tissue or germline variants. Another common scenario is the use of a cell line as an experimental model, many of which have no information on the donor’s normal genome. In this work, we describe an algorithm to identify somatic single nucleotide variants (SNVs) in Next Generation Sequencing (NGS) data in the absence of normal samples using a machine learning approach. Our algorithm was evaluated using a family of supervised learning classifiers classifying germline and cancer variants. Varsome, a comprehensive platform providing an integrated annotation database in human genetic variants, is still lacking. In the study, we proposed a web-based database to determine potential variations in cancers with collected variant information from current common databases and integrated those data to provide comprehensive analyses. The web-site offers a function to upload a variant call format (VCF) file for variants annotation. Importantly, we integrated population allele frequency information from NHBI GO Exome Sequencing Project (ESP), 1000 Genomes Project and Tokohoku Medical Megabank Project to help users figure out the correlation between disease and population. Additionally, we also collected gene expression profiles from The Human Protein Atlas, Expression Atlas and NCBI SRA in different organs of human, mouse, and zebrafish respectively to reflect the relationship between gene expression and genetic variations in a specific organism. Overall, the database aids to predict protein functions in mutations, analyze population allele frequency and gene expression information from provided variants of diseases. In the result, we use three EGFR mutations to display the proposed system. A recent study has reported that Asian patients with non-small cell lung cancer (NSCLC) carrying a higher rate of EGFR mutation compared to non-Asian patients. Several studies, such as chr7:55241708G>C (G719A), chr7:55249005G>T (S768I) and chr7:55259515T>G (L858R), are found approximately in 30% of Asian (Japanese) patients. In functional prediction results, these sites are exonic and nonsynonymous mutations. The REVEL scores of three EGFR mutations are 0.824, 0.763 and 0.961, and gerp + scores 5.5, 5.85 and 5.71, respectively. Both REVEL and gerp + show high scores for the mutations of protein structural changes. According to the results, researchers can infer that the three mutations are pathogenic variants implying the nucleotide positions with a higher constraint.

**#3574 A survey of mutations in biomedical literature using a machine based approach.** Takahiko Koyama, Kahn Rhrissorarakkrai, Lakmi Parida. IBM Research, Yorktown Heights, NY.

Introduction: Being able to characterize mutations for both pathogenicity and drug response is indispensable to the analysis of tumor genetics and the development of therapeutic options. While a great deal of data has been deposited in various structured, genomic databases, a large portion of insights are primarily and often times solely found in biomedical literature. Medline contains about 26 million literature citations; a number that is unrealistic for a human to read. Thus machine based approaches are needed to comprehensively capture the landscape of reported mutations. Method: An automated pattern matching method is utilized to extract mutations from Medline abstracts as presented in Human Genome Variation Society (HGVS) format and RefSNPs (rs) number. A typical HGVS protein mutation is described as [reference amino acid][position] [new amino acid], as in p.His1047Arg, His1047Arg, or simply H1047R in HGVS format. This method identifies and consolidates all mentioned protein mutations and their alternate formulations. Result: Over 300,000 unique abstract mutation pairs were identified including 90,000 unique mutations. Well known cancer mutations such as BRAF V600E, JAK2 V617F and EGFR L858R are among the most frequent appearing in oncology literature. At the other end, 51,000 mutations are mentioned in just a single abstract, 16,000 mutations in two abstracts, 7,600 in three abstracts, and so forth. Conclusion: The number of mutations appearing in Medline abstracts represents just a small portion of the 2 million unique coding mutations contained in the COSMIC database. While we expect the actual coverage of mutations by literature to be more comprehensive if this approach is extended to the full text body, the number would likely remain small compared with the total reported COSMIC mutations. One of the great challenges in oncology is characterizing variations of unknown significance (VUS), and by first extracting all reported mutations, even those mentioned in only one article, and their specific biological context, we can begin to identify broader patterns in mutations’ pathogenicity and their impact on drug response.

**#3575 Frequency of imaging findings suspicious for and suggestive of cancer between three different hospitals within a single health system.** Lauren F. Comisar, Hanna M. Zafar, Darco Lalevic, Christopher Pizzurro, Charles E. Kahn, Mitchell Schnall, Tessa S. Cook. University of Pennsylvania, Philadelphia, PA.

Outpatient imaging rates have risen over the past two decades. Imaging findings representing possible cancer are commonly detected on these exams and require follow-up. Our health system utilizes a standardized coding scheme,
similar to the breast imaging and reporting data system (BI-RADS), to classify the malignant potential of masses on all abdominal and pelvic imaging exams; two of these categories are suspicious (for masses that clearly represent malignancy) and suggestive (for masses that may represent malignancy). These codes are used at three hospitals in a single health system: a university hospital, a community hospital, and a Level 1 trauma center. Our objective was to evaluate the frequency of imaging findings suspicious for or suggestive of malignancy in the liver, kidneys, pancreas, and adrenals between outpatients and inpatients at these three hospitals. Over 1600 suspicious and suggestive masses were discovered in one year: 843 in the liver, 499 in the kidneys, 152 in the pancreas, and 137 in the adrenal glands. Masses suspicious for cancer were unevenly distributed by patient sample. In the liver and kidneys, suspicious masses were more common in outpatients at all three hospitals; in the pancreas and adrenal glands, suspicious masses were more common in inpatients. Suspicious masses were unevenly distributed by modality. The proportion of MRI exams where at least one organ was coded as suspicious was higher than the proportion of CT exams. Masses suspicious for cancer were unevenly distributed across the three hospitals. Liver masses suspicious for cancer were twice as likely to be discovered at the university hospital compared to the community hospital and the Level 1 trauma center (present in 2% vs. 0.7% vs. 0.6% of all exams evaluated, respectively). Adrenal masses suspicious for cancer were also more commonly detected at the university hospital (present in 0.3% vs. 0.1% vs. 0.1% of adrenal glands evaluated, respectively). A similar trend was observed for renal and pancreatic masses. Masses coded as suggestive of malignancy (therapy in patients, therapy in patients, therapy in patients) were more frequently detected at the university hospital. This was particularly evident in adrenal lesions where 0.3% of adrenal glands evaluated at the university hospital were suggestive of malignancy compared to 0.06% at the community hospital and 0.03% at the Level 1 trauma center. A similar trend was observed for pancreatic lesions. In conclusion, this novel database of standardized codes for abdominal masses provides insight into how masses suspicious for and suggestive of cancer are coded variably by organ, patient location, and modality between the three different hospitals within the same urban academic health system. Further investigation will be needed to determine the influence of patient case mix, referral patterns, and radiologist characteristics that contribute to these observed differences.

#3576 Accurate identification of somatic mutations in cancer patient specimens in the lack of normal tissue by targeted high-throughput sequencing. Francisco M. De La Vega,1 Sean Irvine,1 David Ware,2 Kurt Gaastera,1 Yan-nick Pouliot,1 Len Trigg,2 Stanford University, Stanford, CA; 2Real Time Genomics, Inc., Hamilton, New Zealand; 3Real Time Genomics, Hamilton, New Zealand; 4TOMA Biosciences, Foster City, CA.

Tumor molecular profiling is rapidly becoming the standard clinical test for selecting targeted therapies in refractory cancer patients. DNA extracted from cancer patient samples is enriched for cancer genes and sequenced to identify actionable somatic mutations therein. A major challenge arises when tumor-derived data is analyzed in the absence of normal tissue data, as it is common in clinical scenarios. The distinction between somatic and germline variants becomes difficult, leaving clinicians to resort to crude heuristic filtering. We present here a variant calling software, developed under quality system regulation protocols, capable of accurately identifying somatic mutations from targeted next-generation sequencing data. A novel Bayesian Network approach models the distribution of reads harboring germline and somatic mutations, estimates the contamination from normal tissue in the sample, scores somatic mutations, and imputes germline variants, without matching normal tissue data. This approach also allows joint analysis of multiple specimens from the same patient (e.g. FFPE and ctDNA), when available, improving the limit of detection. To improve specificity, our caller can also utilize prior information from different databases including somatic mutations, germline variation, and healthy controls data, in a principled fashion. We validated our method by analyzing data from the TOMA OSq-Seq 131 cancer gene panel using the Illumina platform. Sample inputs ranging from 2-600ng of DNA were sequenced to a depth of >1000X, achieving on target rate of 73% and uniformity > 3.2 fold 80% coverage. Through adaptors with molecular barcodes we measured a median duplicate rate <2.

We analyzed somatic mutations simulated at various variant allele fractions on a background of data from reference samples from the Genome-in-a-Bottle consortium, data on a dilution series from two reference samples, and several commercial control and clinical samples, including matched FFPE, PBMC, and ctDNA samples. In the latter set, our method accurately scored each variant with respect to their likelihood of being somatic or germline. We show that, as compared to other commonly used methods, our algorithm can achieve a higher true positive rate whilst controlling a false discovery rate of 1%. We also show that jointly analyzing serial samples (e.g. ctDNA), we can improve sensitivity of shared variants. In conclusion, in contrast to currently used academic software developed for precisely projects, we observe that our caller outperforms these software and is particularly well suited for the clinical use cases.

#3577 Splice expression variation analysis (SEVA) for differential gene isoform usage in cancer. Bahman Afarsi,1 Theresa Guo,1 Michael Considine,2 Dylan Kelley,3 Emily Flam,1 Liliana Florea,2 Patrick Ha,2 Donald Geman,1 Michael F. Ochs,2 Joseph A. Califano,1 Daria A. Gaykowska,2 Alexander V. Favo-rov,1 Elana J. Fertig,1 Johns Hopkins University, Baltimore, MD; 2University of California, San Francisco, CA; 3The College of New Jersey, Ewing, NJ; 4University of California, San Diego, CA.

Alternative splicing events (ASE) are a significant component of expression alterations in cancer, and have been demonstrated to be critically important in the development of malignant phenotypes in a variety of tumors. These alternative gene isoforms alter cell-signaling networks and serve as a hidden source of tumor-driving alterations not identified in multi-omics analyses. Recent studies have demonstrated that reads from RNA-seq data can infer gene isoforms expressed in a single sample. Therefore, RNA-seq data of tumors offers the opportunity to systematically evaluate expressed gene isoforms and identify splicing events in cancer samples. To characterize a cancer specific ASE landscape, it is essential to perform differential splice variant expression analysis to identify isoform variants that are unique to tumor samples compared to normal tissue. In spite of the breadth of ASE algorithms, few have been validated in primary tumor samples. Current methods for differential splice variant analysis compare mean expression of gene isoforms in sample groups. Because these variants are tumor-specific, ASEs are expected to have more variable exon junction expression than normal samples. Therefore, current differential ASE analysis algo-rithms from RNA-seq may not account for heterogeneous gene isoform usage in tumors. To address this, we introduce Splice Expression Variability Analysis (SEVA) to detect differential splice variation usage in tumor and normal samples and accounts for tumor heterogeneity. This algorithm compares the degree of variability of junction expression profiles within a population of normal samples relative to that in tumor samples. The performance of SEVA was compared with two existing algorithms, EBSq and DiffSplice, in simulated and real RNA-seq data. Simulated data suggest that SEVA is robust and computationally effi-cient relative to EBSq and DiffSplice. In contrast to EBSq and DiffSplice, SEVA was able to identify alternative splicing events independent of overall gene expression differences. Finally, additional validation was performed using RNA-seq data for primary tumor data from HPV-positive oropharynx squamous cell carcinoma (OPSCC) tumors and normal samples from both TCGA and an independent tumor cohort of 46 OPSCC tumors and 25 normal samples. In these tumor samples, SEVA finds cancer-specific ASEs in genes that are independent of their differential expression status. Moreover, in total, it fine mapped approximately hundreds of splice variant candidates, manageable for experimental validation in contrast to the thousands of candidates found with EBSq or DiffSplice. These candidates include experimentally validated splice variants in HNSCC from a previous microarray study. Based on performance in both simulated and real data, SEVA represents a robust algorithm that is well suited for differential ASE analysis, particularly in RNA-seq sequencing data from heterogeneous primary tumor samples.

#3578 Methyl2Acetyl: predicting epigenetic and transcriptomic activity from DNA methylation. Xiang Chen. St Jude Children’s Research Hospital, Memphis, TN.

DNA methylation and histone modifications are interconnected and interdependent epigenetic mechanisms that regulate chromatin accessibility in cells. They establish cell’s developmental identity and modulate individual response to endogenous developmental stimuli and environmental changes. Chromatin immunoprecipitation followed by sequencing (ChIP-seq) is a popular assay to study genomewide histone modification profiles. However, ChIP-seq assays require relatively large amounts of starting materials (ranging from ~10^9 cells using a low cell number protocol to 10^11 cells in a standard protocol), which remains as a major obstacle for precious biological samples including primary tumor specimens. On the other hand, tagmentation-based whole genome bisul-fite sequencing (TWGBS) protocol enables unbiased assessment of the genomewide methylation pattern using ~3,000 cells. Therefore, a quantitative relationship between DNA methylation and epigenetic/transcriptomic activity is in great demand. In the present study, we developed Methyl2Acetyl, a machine learning framework to infer promoter epigenetic activities (e.g. H3K27Ac enrichment) using TWGBS data as input. The proposed model utilizes random forest learners, which allow automatic extraction of complex interactions among DNA methylation features surrounding transcription start sites. We ap-
applied Methyl2Acetyl to a set of pediatric solid tumor samples with high quality H3K27Ac ChIPseq data to evaluate its performance. The model accurately predicted promoter H3K27Ac enrichment in independent test samples (Pearson correlation against measured enrichment: 0.81 ± 0.02). To further test the robustness of our model, we trained a Methyl2Acetyl model on solid tumors and applied it to a set of 10 pediatric leukemia samples. Despite dramatic global methylene differences between solid tumors and leukemia, Methyl2Acetyl predicted promoter H3K27Ac enrichment showed strong concordance with target gene expression level in the same sample (Pearson correlation: 0.75 ± 0.02, classification AUC: 0.89 ± 0.01). Collectively, our data suggested that DNA methylation is predictive of promoter active histone modification enrichment and gene expression activity. Epigenetically active promoters can be imputed from TWGS data in samples with limiting starting materials.


A large number of pre-clinical and clinical studies have strongly implicated that combining cancer-derived neo-antigen vaccines with checkpoint control inhibitors will enhance priming and expansion of tumor-specific naive and memory T-cells resulting in superior efficacy and durability of response. The neo-antigens, derived from somatic mutations are prime candidates for cancer vaccines, not subjected to host’s central and peripheral tolerance. Identifying potential T-cell engaging neo-epitopes among a large number of somatic mutations is like finding a specific needle in a stack of needles. Currently, the available T-cell neo-epitope prioritization pipelines rely primarily on two attributes - the class-I HLA-binding affinity of the mutant peptide compared to the wild-type counterpart, and the level of expression of the mutated gene in tumor cells. The higher the binding differential, and higher the expression level of the mutant allele, the greater is the likelihood for the peptide to be presented on antigen-presenting cells. These approaches however fall short of predicting whether the HLA-bound peptide will engage T-cells by binding to T-cell receptors (TCRs).

We have developed a novel algorithm to predict the binding of HLA-peptide complexes to TCRs by analyzing the physico-chemical composition of the amino acids and their positional biases in the 9-mers from crystal structures of HLA-peptide-TCR complex. We applied machine learning approach to build a classification model that can predict whether a given 9-mer peptide is a TCR-binder or not by identifying whether an amino acid at a given position carries key features that will facilitate interaction with the TCR. We applied this approach to positive and negative TCR interactions selected from Immune Epitope data base (IEDB). We tested multiple classification approaches and found that Random Forest and Classification via Regression methods provided the best performance. We achieved more than 99% accuracy at 10-fold cross validation on both training and unseen test datasets. We further validated our model using positive and negative peptides curated from published papers reporting clinical trial results of checkpoint control inhibitors. The performance of the two classification models was evaluated on two different TCR-binding assays - dextramer binding and IFN-γ release. The Classification via Regression method showed a higher positive predictive value for the dextramer-binding assay, whereas the Random Forest method showed a higher positive predictive value for the IFN-γ ELISPOT assay, suggesting subtle differences between the two classification methods. The inclusion of the TCR binding step to our T-cell neo-epitope prioritization pipeline increased accuracy of prediction, reduced false positives and selected potential neo-epitopes to a manageable number for testing in cell-based assays.


We propose and evaluate a novel algorithm for inferring germline and somatic copy number variation from whole exome sequencing (WES) and whole genome sequencing (WGS) data. Starting with the depth of aligned short reads from a cohort of samples, we use a Bayesian model for learning sequencing bias and simultaneously detecting CNV events using a hidden Markov model for change-point detection. A unified framework is used to call both germline and somatic CNVs. Denoising and event discovery are performed self-consistently to achieve maximum accuracy. In contrast to previous methods, our model naturally accounts for mixed sex cohorts and can detect events on sex chromosomes. Furthermore, we can detect excessively noisy samples and extract useful information within a probabilistic framework. Our implementation can also utilize Spark clusters, enabling the processing of larger cohorts and allowing for improved runtime performance. We benchmark the new method for precision, recall, and reproducibility of both germline and somatic variants. Evaluations are performed on a cohort of WES samples from The Cancer Genome Atlas with matching WGS data. For germline variants, we use blood normal samples and compare our calls on WES data against Genome STRIP calls on WGS data. We find that GATK CNV yields remarkably higher precision and recall compared to XHMM and OncoNEX software packages. For somatic variants, we compare our calls against TITAN and find a remarkably high concordance.

#3581 GATK ACNV: allelic copy-number variation discovery from SNPs and coverage data. Aaron Chevalier, Lee Lichtenstein, Andrey Smirnov, Samuel K. Lee, Mehrtash Babadi, David I. Benjamin, Valentin Ruano-Rubio. Broad Institute, Cambridge, MA.

The presence of somatic copy-number alterations in tumor genomes can be used to predict both patient sensitivity to treatments as well as outcomes. The inclusion of allelic data improves statistical power to detect copy-number events and allows for discovery of copy-neutral events. We present GATK ACNV, an allelic copy-number variation method built on the Genome Analysis Toolkit. ACNV is a tool for detecting somatic copy-number activity from whole exome and whole genome sequencing data by segmenting the genome into regions of constant copy number and estimating copy ratio and minor-allele fraction in those regions. ACNV uses a novel probabilistic model to account for reference bias (optionally using a panel of normals), which improves the estimation of minor-allele fraction. We combine this with the coverage model from GATK CNV by segmenting with a unified hidden Markov model, improving the statistical power to detect copy-number variation. We validated a purity series of the cell line HCC1143 and cancer samples from The Cancer Genome Atlas. Our results show that ACNV is able to discover regions of somatic copy-number activity accurately and with high resolution in both whole exome and whole genome sequencing data.

#3582 Copy number estimation from targeted and shallow sequencing in cancer samples. Andrea I. OHara, Zhiwei Che, Soheil Shams. BioDiscovery, El Segundo, CA.

Next-generation sequencing (NGS) is mainly used to obtain sequence variants (SNVs). However, obtaining copy number results from NGS has gained momentum in both research and clinical applications. Targeted panel sequencing has been a popular method to achieve high depth of coverage for certain regions of interest at an affordable cost compared to whole genome sequencing. Shallow whole genome sequencing, where average read-depth can be as low as 0.1x, provides a cost savings-approach for identifying large copy number variant (CNV) events; it has been utilized in various application areas, including oncology. Here we introduce the BAM (MultiScale Reference) algorithm, currently in NexusCopyNumber, to function with shallow and targeted sequencing data, as well as WGS and WES, using a novel dynamic binning approach. This approach uses a Hidden Markov Model to segment the genome into target areas using the reads in targeted regions and the backbone areas using the off-target reads and additional areas. It uses coarse binning in the backbone areas that provides copy number base line as well as large copy number events and uses finer binning in target areas to provide high resolution copy number detection in targeted regions. Shallow WGS data and targeted panel NGS data, as well as WES with normal depth of coverage, were used for the testing. The results were compared with those from microarray and/or other algorithms in Nexus Copy Number, BAM ngCGH (matched) and BAM (pooled reference). GC correction schemes based on a range of window size and presence or absence of GC probe content were applied to the data and assessed for overall quality. Differences in overall read-depth resulted in variable sample quality across the cohorts, however most sample quality was adequate for copy number estimation and a quality threshold was assessed. Among the samples tested, the best quality after GC correction comes from the 50kb region size with or without the probes. Next, the copy number profiles of the samples from WES and microarray were compared using a reference readarray reference dataset and for a comparison to a reference copy number profile. Using the MultiScale Reference method has been tested in a variety of cancer samples. This is an ideal tool for copy number estimation with NGS results in cancer samples because it provides a way for non-matched-pair analysis with genome, exome and targeted NGS.
The role of the nervous system in adult tumor progression has been hypothesized. In contrast, the involvement of nervous system in childhood cancer is still unknown. In this study, the main objective of this study is to identify whether mutations in neuron signaling proteins are present in pediatric cancer. Additionally this study foresees to determine the effect of mutation on the protein structure and function and to elucidate if differences between solid and hematopoietic pediatric tumors exist in terms of neural signaling. The genomic data of pediatric cancers available in the Pediatric Cancer Data Portal (PeCan) were mined looking for point mutations. Our analysis comprised a set of well recognized neuron signaling factors including 50 neurotrophic growth factors, 23 axon guidance molecules and 47 neurotransmitter receptors. Missense mutations were determined and germline tumours.

The effect of each mutation on the protein function was predicted through bioinformatics pipeline. The TO method documented on average 95% SENS and >99% SPEC for variant detection. The method showed a tradeoff between SENS and FP rate allowing for small drops in SENS for large decreases in the numbers of FP (4-5% decrease in SENS from 99% to 95%) resulted in 90% decrease in FP VCs with a final average of 70 filtered VCs per sample. Results suggest that analyzing PTN targeted exome NGS samples as TO and obtaining a disease-specific PNC selected subset of high quality VCs with a reasonable number of filtered VCs. Also benchmarking aids in excluding controls from the PNC that may miss CA calls.

**3585 Determination of gene amplifications with a next-generation sequencing cancer panel.** Ina L. Deras, Aaron Wise, Chen Zhao, Christine Gide-well-Kenney, Philip Le, Elizabeth Upsall Aderhold, Karen Gutekunst, Iluminina, San Diego, CA

**TruSight** Tumor 15 (TST15) uses next-generation sequencing technology to detect somatic point mutations, insertions, and deletions in 15 genes that are commonly mutated in solid tumors. It accurately detects low-frequency mutations with low DNA input and is optimized for formalin-fixed, paraffin-embedded (FFPE) tumor tissue. The test is a multiplexed PCR panel that includes primers to support amplification detection in the EGFR, ERBB2, and MET genes. It is described as a bioinformatics pipeline to enable detection of these gene amplifications. DNA extracted from 31 FFPE samples with amplifications in EGFR, ERBB2, or MET—some with dual amplifications—were diluted with DNA extracted from 18 normal FFPE samples to create a total of 67 unique samples at low-level amplification. Undiluted and diluted samples were assessed for quality based on amplifiability (ΔCq or library yield) and tested with TST15. For comparison, the samples were also tested with droplet digital PCR (ddPCR) to determine the copy ratio of target to wild type. For amplifiable samples, the TST15 algorithm was able to detect amplifications in EGFR and ERBB2 at ddPCR ratios of 1.4 and 1.6, respectively. ddPCR was unable to accurately quantify low MET amplifications; therefore, expected ddPCR ratios were used for comparison. The TST15 algorithm was able to detect amplifications in MET samples at an expected ddPCR ratio of 1.3. Poor sample quality (i.e., high ΔCq or low library yield) impaired amplification detection. In some samples, the presence of multiple amplifications also impaired detection. The addition of this algorithm to the TST15 workflow will allow researchers to more accurately characterize tumor samples by detecting somatic mutations and amplifications simultaneously.


Patient-derived xenograft (PDX) models of human tumors are an important and widely used platform for cancer research. Cancer drug development relies on PDX models to screen drugs and characterize tumor biology for potential drug targets. It has been well established that PDX models maintain similar biology as their original tumors, including histological patterning, gene expression, single-nucleotide variants, and copy number alterations. Using short-read sequencing technology to profile and characterize genomic alterations within PDX tumor models is becoming a common practice in cancer research. Mouse read contamination is a relevant source of noise in PDX tumor sequencing data and needs to be addressed prior to downstream analyses. Therefore, a key consideration for downstream analysis of PDX sequencing data, such as determining variant calls or gene expression values, is effectively removing contaminating mouse sequence. Removing contamination from PDX sequencing data is necessary for accurate and reproducible downstream analyses. A limited number of studies establishing best practices for handling PDX sequencing data exist. Thus, we set out to compare different strategies for removing mouse contamination from PDX tumor sequencing data for DNA and RNA using a set of controlled experimental in silico datasets and data from PDX tumors. We designed a set of in silico experiments using these sequencing data to assess a range of approaches for removing contaminating mouse reads from human data. Our experiments used a set of publicly available human and mouse DNA and RNA sequencing data available at the SRA site. Subsets of the raw human and mouse reads were mixed at different ratios and analyzed with five different approaches: 1) raw alignment to the human reference genome, 2) filtering with the Xnome algorithm.
followed by alignment to the human genome. 3) alignment to the human reference genome followed by filtering with the XenomiX R algorithm, 4) mouse-human hybrid reference genome alignment, and 5) our novel NextCODE approach. We assessed the sensitivity and specificity of each procedure for removing mouse sequence and maintaining human sequence for downstream analyses. We also assessed the effects of each procedure on genome quantification and variant calling. Our results introduce a novel, improved method for removing mouse DNA, facilitating better-quality data for downstream analysis.

#3587 Intratumoral divergence of copy number alterations in NSCLC.
Yasminka A. Jakubek,1 Smruthy Sivakumar,1 Louise C. Strong,1 Humam Ka-

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facilitating better-quality data for downstream analysis.

and maintaining human sequence for downstream analyses. We also assessed the sensitivity and specificity of each procedure for removing mouse sequence and

differentiation of cancer genomes. Its characterization at the DNA level is based on the identification of somatic mutations consisting of single nucleotide variants (SNVs) and copy number alterations (CNAs), which include deletions, amplifications, and copy neutral loss of heterozygosity. Currently, most methods for the detection of intratumoral heterogeneity use an implicit “infinite sites” model for both SNVs and CNAs. While this may often be appropriate for SNVs, we demonstrate its violation for CNAs in unstable cancer genomes. Here, we propose a novel method to identify CNAs that were created by independent mutational events but alter the same genomic region. Our method identifies regions where the germline heterozygous signals (allelic intensities for DNA arrays or frequencies for next-generation sequencing) shift toward different parental haplotypes between different samples from the same tumor, thus indicative of divergent tumor clones. In this context we define a divergent CNA as one found on multiple samples from the same tumor but with different chromosomal changes giving rise to the CNAs. We applied our method to data from core needle biopsies extracted from the tumors of 31 non-small cell lung cancer (NSCLC) patients and processed using Illumina SNP arrays. We overlapped CNA calls from the same tumor, and then tested whether overlapping segments showed divergent CNAs. We observe instances of divergent CNAs in 23 of the 31 patient tumors comprising 260 in total (median = 5 divergent CNAs per tumor). Strikingly, one tumor had 34. We then assessed whether the level of recurrent mutation correlated with clinical or genomic features. While there was no association with smoking or history, we did observe a positive association between the rate of divergence and somatic mutations (including loss) in putative genome “gatekeeper” genes, p53 and CDKN2A (P = 0.001). We detected divergent CNAs that spanned shared genomic regions in three or more NSCLC tumors. These included large (>1Mb) events in chromosome 6 (q13-14, q21-22, q25) and chromosome 21 (q22), as well as smaller events, which included the integrin collagen receptor locus ITGA1-PLEO-ITGA2, 8p23.1, 8q24.3, 18q11.2 (ZNF521 gene), and 21q21.3, which has binding sites for GATA2. GATK4 includes a set of observed divergent CNAs alterations represent half of the total number expected since imbalances of the same haplotype will not be observable in such data. In sum, our approach allows for the detection of genomic regions that are divergently altered. This information may support methods to identify CNAs under positive or negative selection in the tumor microenvironment as well as regions of increased genomic instability. This provides an added dimension to intratumoral heterogeneity analysis for a more comprehensive characterization of cancer genomes.

#3588 Computational method to identify non-coding cancer drivers.

Cancer is caused by mutations in driver genes - yet ~25% patients do not show any mutations in known drivers. ~99% somatic mutations obtained from whole-genome sequencing localize to regions that do not code for proteins and many of these non-coding mutations regulate gene expression. Although variants in protein-coding regions have received the majority of attention, numerous studies have now noted the importance of non-coding variants in cancer - for example, mutations in the promoter of the TERT gene. Identification of functional non-coding variants that drive tumor growth remains a challenge and a bottleneck for the use of whole-genome sequencing in the clinic. Cancer drivers are generally identified by the high frequency at which their mutations occur across patients. However, mutation rate is highly heterogeneous across the genome and many non-driver elements show higher mutation frequency than others, such as regions bound by transcription factors (TFs) in melanoma or regions replicating late during cell division in colon cancer. We have developed a novel computational approach to predict non-coding cancer drivers that differentiate between regions highly mutated due to positive selection (true drivers) vs those due to mutation rate heterogeneity (false positives). This method integrates the signals of high functional impact of variants with the recurrence of variants across multiple tumor samples to identify the elements that show more and higher functional impact mutations than expected randomly. Our method controls for multiple covariates of mutation rates, such as replication timing, histone modification marks and open chromatin regions to identify the non-

coding regions with functional impact.

FunSee is a novel scheme that uses the properties of ENCODE elements (including conservation, TF motif disruption and network properties) within a weighted scoring scheme to predict deleteriousness of non-coding variants. I will present the details of this method and discuss the ongoing efforts to analyze ~2,900 tumor whole-genomes in the Pan-Cancer Analysis of Whole Genomes, PCAWG consortium.

#3589 A systematic approach toward gene annotation of the hallmarksgenome microenvironment as well as genome and many non-driver elements show higher mutation frequency than others, such as regions bound by transcription factors (TFs) in melanoma or regions replicating late during cell division in colon cancer. We have developed a novel computational approach to predict non-coding cancer drivers that differen-

cancer.

The hallmark of cancer consists of ten categories that serve as an organizing principle and framework for understanding neoplastic disease. The ten hallmarks include activating invasion and metastasis, avoiding immune destruction, deregulating cellular energetics, enabling replicative immortality, evading growth suppressors, genome instability and mutation, inducing angiogenesis, resisting cell death, sustaining proliferative signaling, and tumor-promoting inflammation. In order to facilitate the use of the hallmarks of cancer in genomic studies, we have undertaken a systematic methodology to map genes to each individual hallmark of cancer. Assignment of genes to the individual hallmarks was performed using gene ontology (GO) annotations. Specifically, for each hallmark, a term list was generated by cancer research experts, with biological terms associated with each hallmark. This term list was used to search and identify matching GO terms. Matched terms were then mapped to the highest, most specific, representative, non-redundant GO term in the GO hierarchy. Genes for each term were restricted by selecting only human taxon, and high confidence experimental evidence codes. For specific GO terms, where present, the Regulation of 'GO term' category was included. The genes for each GO term for each hallmark were combined in a non-redundant list of genes for each hallmark. In the case of the evolving growth suppressors category, a different strategy was employed. In this instance, genes were identified through 'tumor suppressor' gene/protein annotation in the Dgxdb resource and UniProt. Additionally, text-mining methods on biomedical research literature were used to define a high confidence list of genes for this hallmark. As an example use case of the hallmarks we applied them to the analysis of the TCGA Pan-Cancer RNAseq data set. Clustering across the individual hallmarks led to interesting differences within particular tumor tissue types, as well as shared hallmarks for other tumor types. For example, use of the genome instability and mutation hallmark for Pan-Cancer clustering, revealed a tight tissue-based cluster of low-grade gliomas and GBMs. This result suggests the importance of this hallmark expression in this tumor type. This high confidence mapping of genes to hallmarks of cancer provides a unique dataset to facilitate analyses of cancer genome data.

#3590 Somatic variation discovery with GATK4. Geraldine A. Van der Auwerra. Broad Institute, Cambridge, MA.

The Genome Analysis Toolkit or GATK, developed at the Broad Institute, is currently one of the most widely used software toolkits for germline short variant discovery and genotyping in whole genome and exome data. GATK4 is the next generation of GATK; it runs faster and covers more ground, adding somatic SNVs, Indels and CNVs to its variant discovery portfolio. Given the breakthrough increase in the amount of genomic data produced in recent years, GATK development has come to tackle multiple challenges: a) reflect the cutting-edge of science in terms of depth as well as breadth of analyses offered, b) scale to petabytes of data and c) provide a way to navigate the tradeoff between turn-around-time and cost of analyses. GATK4 addresses all of these challenges through a complete redesign and reimplementation of the original GATK software. This new version greatly expands the toolkit’s scope of action within the variant discovery space by improving somatic mutation calling and adding copy number, structural variant and tumor-heterogeneity analysis tools. For the established tools and algorithms composing the GATK Best Practices pipelines for short variant discovery (including MuTect2, the next generation of the DREAM challenge-winning somatic SNV caller MuTect, developed originally in the Cancer Genome Analysis group at the Broad Institute), GATK4 is a substantial upgrade that streamlines the tools’ operation, providing functionally equivalent results in a much faster timeframe through greatly increased efficiency and leaner engineering. Designed with cloud infrastructure in mind (although it still runs on local infrastructure), GATK4 is implemented with support for Apache
#3591 A new method to infer clonal sequences and phylogenies from personal tumor genome profiles. Sayaka Miura,1 Karen Gomez,2 Oscar Murillo,3 Sudhir Kumar1. 1Temple Univ., Philadelphia, PA; 2Baylor College of Medicine, Houston, TX.

Tumor progression involves the evolution of clonal cell populations that mutate and spread in the body. Genetic profiling has revealed the presence of extensive variation in tumor samples from individual patients. However, a tumor sample for genetic profiling is mixture of different clonal cell populations and needs to be decomposed into clonal sequences to infer the evolutionary history of the tumor. Therefore, we have developed a new method, CloneFinder, and show that the clone sequences present in each tumor sample can be accurately inferred from the analysis of multiple tumor sample profiles with our new CloneFinder method. CloneFinder is unique in its use of molecular evolutionary principles to deduce clone sequences at a single-nucleotide resolution along with their frequencies in each sample. It performs better than existing methods, which we found to produce incorrect numbers of clones, clone sequence errors, errors in clonal phylogenies, and biased estimates of cancerous cells in tumors. Application of CloneFinder to a large number of empirical datasets revealed that both early and recently evolved tumors contain ancestral clones at high frequencies. Therefore, our new method provides new insights into the clonal structure of tumors and their evolution within a personal life time.

#3592 Improved indel detection in RNA-seq data via assembly based realignment reveals expressed Epidermal Growth Factor Receptor indels in Lung Adenocarcinoma. Lisle E. Mose, D. Neil Hayes, Charles M. Perou, Joel S. Parker. UNC - Lineberger Cancer Center, Chapel Hill, NC.

Insertions and deletions in the transcriptome can potentially have significant impact on function and can be clinically actionable. A number of methods have recently been developed that improve indel detection in DNA by utilizing realignment and/or localized assembly to aid in discovering mutations that are more difficult to detect than single nucleotide polymorphisms. While significant effort has been put into these methods in the context of DNA discovery, RNA-Seq has not received the same attention. For example, the widely used Genome Analysis Toolkit (GATK) recommends removal of splice junctions from all reads prior to proceeding with variant calling methods that are very similar to those used for DNA. We hypothesize that utilizing splice junctions to augment localized assembly across exon-exon boundaries can improve read alignments resulting in improved variant detection in RNA-Seq data. We have developed an update to the Assembly Based Realigner (ABRA2) that makes use of splice junction information to aid in realignment of reads. We assessed indel detection performance using the BEERs RNA-Seq simulator. Two million simulated reads of length 100nt were generated across 1000 human genes. In order to test more complex indel detection, the simulator was modified to generate an even distribution of 1391 indels of length 1 to 100. The simulated reads were aligned using STAR and subsequently realigned using ABRA2. Freebayes and GATK were run against the non-realigned STAR output resulting in indel detection sensitivity of 15% and 18% with precision of 97% and 88% respectively. Freebayes was then run against the ABRA2 realignments resulting in an approximately 4 fold increase in indel detection sensitivity of 67% while maintaining precision of 97%. In frame deletions in Epidermal Growth Factor Receptor (EGFR) have oncogenic potential, can be indicators for Gefitinib or Erlotinib treatment, and are frequently detected in lung cancer via DNA sequencing. Among 514 TCGA Lung Adenocarcinoma RNA-Seq samples, ABRA2 revealed 73 in frame coding indels in EGFR ranging in length from 3 to 24 bases, including 58 deletions in exon 19 of 15 length 15 bases. This represents a 3 fold increase relative to what was initially reported from DNA by TCGA. Further, in the absence of ABRA2, only 5 in frame coding indels are detected in RNA, the largest of which is a 9 base deletion. We have presented here ABRA2, a new version of the Assembly Based Realigner that is capable of accurately realigning RNA-Seq reads containing variations that are currently not well handled by widely used aligners and variant callers, thus improving accuracy of variant detection in RNA-Seq.

#3594 Adaptively randomized seamless-phase multiarm platform trial: Glioblastoma Multiforme Adaptive Global Innovative Learning Environment (GBM AGILE). Donald A. Berry,1 Todd Graves,2 Jason Connor,2 Brian Alexander,2 Timothy Cloughesy,2 Scott M. Berry,2 Anna Barker,2 for the GBM AGILE Global Alliance. 1UT MD Anderson Cancer Ctr., Houston, TX; 2Berry Consultants, LLC, Austin, TX; 3Dana Farber Cancer Institute, Boston, MA; 4The Ronald Reagan UCLA Medical Center, Boston, CA; 5Arizona State University, Phoenix, AZ.

Traditional phase 3 clinical trials compare an experimental arm with control. They inefficiently use patients, time, and finances. Dramatic and rapid changes in biology makes such trials untenable. We describe an alternative drug development strategy that we are using in a particular setting, the trial GBM AGILE (Glioblastoma Multiforme Multifaceted Adaptive Global Innovative Learning Environment). The trial’s design employs many innovations. Some aspects are similar to those of I-SPY 2 (see 4 articles in July 7, 2016 NEJM) but GBM AGILE extends I-SPY 2 in many ways. (1) It is a Bayesian platform trial that simultaneously evaluates many treatment arms (including combinations) from many companies. (2) Arms are added to the trial at any time and leave what has been evaluated whether positively or negatively. (3) An arm’s sample size is adaptive and based on frequent analyses of the trial results. (4) Every arm has an initial stage in which it is randomized adaptively: arms performing better in disease subtypes are assigned with higher probability to such patients. (5) An arm that performs sufficiently well in a disease subtype moves seamlessly into a small (50-patient) control arm in the registration stage in the same subtype, with equal randomization against control. (6) All experimental arms are compared against a control arm that is assigned to 20% of patients in every subtype; a bridging model takes advantage of having many arms in the trial and many comparisons among arms, and enables indirect randomization comparisons of all arms with all controls. (7) Patient subtypes are defined by line of therapy, MGMT methylation status for newly diagnosed patients, and biomarkers associated with targeted therapies, although adaptive randomization enables us to draw conclusions about off-target effects. The many possible subtypes means that there are many possible drug indications. So there are many possible “error types” and no single definition of statistical power. For example, the trial may conclude that a drug’s indication is “recurrent, biomarker-positive” disease when in truth it is “all recurrent” disease. We show how the design addresses this issue and we define “pure type I error.” GBM AGILE’s primary endpoint is overall survival (OS). To make the design more efficient we incorporate evaluations of patients’ statuses over time using a longitudinal model based on periodic MRI assessments and performance status. The longitudinal model and its components are not end points but rather provide auxiliary information that enables multiple imputing for the primary statistic. The trial’s cohort has been enrolled with patient subtypes that are made up of more than 150 enthusiastic and devoted disease experts and advocates from around the globe, including from Australia and China. The U.S. FDA has been enormously helpful in designing GBM AGILE, especially as regards its potential for drug and biomarker registration. Our approach provides a model for other diseases, including those outside of cancer.

#3595 Under-reporting of research biopsies in clinical trials in oncology. Christine M. Parseghian, Kanwal Raghav, James Yao, Lee M. Ellis, Alda Tam, Michael J. Overman. U.T. MD Anderson Cancer Center, Houston, TX.

Purpose: Research biopsies are frequently incorporated into clinical trials in oncology, and are often a mandatory requirement for trial enrollment. However, limited information is available regarding the extent and completeness of research biopsy reporting. Methods: We identified a cohort of therapeutic clinical trials wherein at least one image-guided non-diagnostic research biopsy was performed between 1/2005 and 10/2010 from a large interventional radiology database at MD Anderson Cancer Center. Study protocols were compared with the highest level of corresponding publication as a manuscript, registry report, or abstract. Results: A total of 866 research biopsies were performed across 46 clinical trials (median 19 biopsies/trial). After a median follow-up time of 5.8yrs from study completion, 35 trials (76%) had published manuscripts (18 reported biopsy results), 8 (17%) had either a registry or abstract report (1 reported biopsy results), and 3 (7%) were not reported. In total, 19 trials (41%) reported biopsy results. Of these 19 trials, 6 (32%) trials under-reported the number of biopsies performed. Of the 14 trials with multiple biopsies reported, 13 (93%) trials reported results. Discussion: The many possible subtypes mean that there are many possible drug indications. So there are many possible “error types” and no single definition of statistical power. For example, the trial may conclude that a drug’s indication is “recurrent, biomarker-positive” disease when in truth it is “all recurrent” disease. We show how the design addresses this issue and we define “pure type I error.” GBM AGILE’s primary endpoint is overall survival (OS). To make the design more efficient we incorporate evaluations of patients’ statuses over time using a longitudinal model based on periodic MRI assessments and performance status. The longitudinal model and its components are not end points but rather provide auxiliary information that enables multiple imputing for the primary statistic. The trial’s cohort has been enrolled with patient subtypes that are made up of more than 150 enthusiastic and devoted disease experts and advocates from around the globe, including from Australia and China. The U.S. FDA has been enormously helpful in designing GBM AGILE, especially as regards its potential for drug and biomarker registration. Our approach provides a model for other diseases, including those outside of cancer.
Table. When limited to only published manuscripts, results were unchanged. Academic sponsorship and a larger number of biopsies, especially serial biopsies, were associated with increased reporting of research biopsies. Conclusion: Despite ethical obligations to report research biopsies, 59% of trials do not report on these biopsies. Although complications occur, they are rarely reported. Clinical trial design efforts to ensure that an adequate number of research biopsies are obtained may lead to improved reporting of research biopsy biomarker results.

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ENDOCRINOLOGY: Nuclear Receptors and Endocrine Oncology Therapies

#3602 Novel BRCA1 mimetic 35446HCl inhibits growth of anti estrogen resistant breast cancer cells through the NF-κB pathway. Shyam Nathan, Elliot M. Rosen. Georgetown University, Washington, DC.

Nearly a third of breast cancer patients develop resistance to antiestrogen treatments although the estrogen receptor ERα continues to affect cell survival and proliferation. Thus there is a need for new interventions to inhibit ERα function. Based on high-resolution mapping, we proposed interaction sites on both BRCA1 and ERα in a three-dimensional model of BRCA1. Utilizing this model, we identified a class of compounds that fits into a predicted BRCA1-binding cavity within ERα, mimics the ability of BRCA1 to repress ERα activity, and functions differently than other antiestrogens. Previous publication has shown that the 1st generation BRCA1 mimetic compound (A7) reduced proliferation and inhibited ERα activity in antiestrogen-sensitive and resistant breast cancer cells with negligible non-specific toxicity. More importantly we have shown that the 2nd generation compounds (35446, 4631) partially reduced sensitivity to Tam/ICI-resistant cells (LCC9), caused dissociation of ERα from a model estrogen response element (ERE) in MCF-7 cells, and inhibited growth of LCC9 tumor xenografts at nontoxic concentrations utilizing compound 35446-HCl (35446 prepared as a hydrochloride salt). The focus of this study is the mechanism of action of 35446-HCl compound’s inhibition of ERα, which includes inhibition of a major signaling pathway and mode of cell death. Eight genes identified from RNA-seq in LCC9 cells treated with BRCA1 mimetic A7 were verified by qPCR using 35446-HCl. One verified gene is the NF-κB activator IKKB, which is linked to therapy resistance. Treatment with 35446-HCl inhibited IKKB expression and proliferation in LCC9 cells compared to untreated Control at 48 and 72 hrs (p<0.0001). Transcriptional activity from the p65 promoter as measured with a luciferase assay was decreased in the presence of BRCA1 mimetic treatment (p<0.0001). Expression of downstream targets of NF-κB including genes known to inhibit apoptosis, was reduced (p value ranging from < 0.05- 0.001). Apoptosis due to 35446-HCl treatment was verified by caspase-7 cleavage and increased TUNEL staining at both 48 and 72 hrs. In silico analysis of the IKKB promoter within 10kb upstream of the transcription start site identified one putative full ERE and five putative half ERE sites. Of these, 35446-HCl inhibited the interaction of ERα with the full ERE (p<0.0001) and five half EREs (p value ranging from < 0.05- 0.001) sites upstream of the IKKB start site in LCC9 cells as well as Tam resistant/ERα mutated T47Dco cells. These results identify a mechanism of a novel compound that can overcome antiestrogen resistance.

#3603 Optimizing combination therapy against antiestrogen-resistance in estrogen receptor positive breast cancer. Lauren M. Gutesell, Rui Xiong, Jiong Zhao, Debra A. Tonetti, Gregory R. Thatcher. University of Illinois at Chicago, Chicago, IL.

We studied the resistance of breast cancer cells with and without acquired resistance (AR) to selective estrogen receptor modulators (SERM) and aromatase inhibitors (AI). We optimized combination therapy using 35446-HCl as a new antiestrogen agent. The results showed that 35446-HCl inhibited ERα activity in antiestrogen-sensitive and resistant breast cancer cells with negligible non-specific toxicity. More importantly, we have shown that the 2nd generation compounds (35446, 4631) partially reduced sensitivity to Tam/ICI-resistant cells (LCC9), caused dissociation of ERα from a model estrogen response element (ERE) in MCF-7 cells, and inhibited growth of LCC9 tumor xenografts at nontoxic concentrations utilizing compound 35446-HCl (35446 prepared as a hydrochloride salt). The focus of this study is the mechanism of action of 35446-HCl compound’s inhibition of ERα, which includes inhibition of a major signaling pathway and mode of cell death. Eight genes identified from RNA-seq in LCC9 cells treated with BRCA1 mimetic A7 were verified by qPCR using 35446-HCl. One verified gene is the NF-κB activator IKKB, which is linked to therapy resistance. Treatment with 35446-HCl inhibited IKKB expression and proliferation in LCC9 cells compared to untreated Control at 48 and 72 hrs (p<0.0001). Transcriptional activity from the p65 promoter as measured with a luciferase assay was decreased in the presence of BRCA1 mimetic treatment (p<0.0001). Expression of downstream targets of NF-κB including genes known to inhibit apoptosis, was reduced (p value ranging from < 0.05- 0.001). Apoptosis due to 35446-HCl treatment was verified by caspase-7 cleavage and increased TUNEL staining at both 48 and 72 hrs. In silico analysis of the IKKB promoter within 10kb upstream of the transcription start site identified one putative full ERE and five putative half ERE sites. Of these, 35446-HCl inhibited the interaction of ERα with the full ERE (p<0.0001) and five half EREs (p value ranging from < 0.05- 0.001) sites upstream of the IKKB start site in LCC9 cells as well as Tam resistant/ERα mutated T47Dco cells. These results identify a mechanism of a novel compound that can overcome antiestrogen resistance.
Approximately 75% of breast cancer incidences are Estrogen Receptor positive (ER+). Treatment of ER+ breast cancer with antiestrogen endocrine therapy targets the proliferative mechanisms of ER using selective ER modulators (SERMs), such as tamoxifen, and selective ER downregulators (SERDs), such as fulvestrant. The resistance to endocrine therapy, either innate or acquired, is clinically relevant and results from one or more of 5% of patients who respond to initial anti-estrogen therapy, progressing to tamoxifen resistant disease. In order to gain insight into potential, combinatorial targets for overcoming endocrine resistance in ER+ breast cancer, use of spheroidal 3D cell culture provides a physiologically relevant model with sufficient throughput for drug discovery. Establishing spheroids and subsequent treatment reveals the efficacy of combination treatment on tumor regression. Targeting pathways associated with increased prevalence of resistance, such as CDK 4/6, can be employed for clinical therapy. Using both novel in-house and clinical SERMs and SERDs, we have used these multiple cell lines to discover combinatorial targets using both mechanistic and unbiased screening approaches. The use of combination endocrine therapy in tamoxifen-resistant models has shown promising results and is able to enhance tumor regression in xenografts. By optimizing combinatorial endocrine treatment against both fulvestrant and tamoxifen-resistance, novel therapeutic approaches are being developed for ER+ breast cancer.

#3604 Effect of a new oral SERD AZD9496 on ER mediated signaling in xenograft model of postmenopausal breast cancer. Gauri J. Sabnis,1 Armina Kazi,1 Amanda Schec,2 Stephen Yu,1 Olga Golubeva,1 Angela Brodie,1 West Coast University, Los Angeles, CA; 1 Loyola University Maryland, Baltimore, MD; 2 St. Mary’s College of Maryland, St. Mary’s City, MD; 1 University of Maryland School of Medicine, Baltimore, MD; 2 University of Maryland Greenebaum Cancer Center, Baltimore, MD.

Aromatase inhibitors (AIs) have made significant improvements in the treatment outcomes of patients with breast cancer. However, tumors may eventually acquire resistance. One of the proposed mechanisms of resistance to AIs is overexpression of ERα and cross-talk of ERα with growth factor receptors. Studies including our own have shown that downregulation of ER with fulvestrant may provide benefit in the treatment of AI-resistant breast cancer. Fulvestrant has been employed in the clinic as either first or second line treatment for ER positive breast cancers alone or in combination with AIs. Studies have suggested that further escalation of dose may provide further benefit. However, dose escalation of fulvestrant which is administered via intramuscular injection is difficult due to its poor solubility. To overcome this shortcoming of an injectable drug, a novel orally active SERD (selective estrogen receptor downregulator), AZD9496 was developed. We evaluated the effect of AZD9496 on the growth of hormone sensitivity and anastrozole resistant breast cancer tumors using MCF-7CA xenograft model of postmenopausal breast cancer. Mice bearing xenografts of MCF-7C were then treated with fulvestrant (1 mg/kg-d) or AZD9496 (5 mg/kg/d-po), alone or in combination with anastrozole (200mg/kg-d) for 23 weeks. Tumors were measured weekly and growth rate was calculated. AZD9496 was significantly better at inhibiting the growth of tumors compared to control (p<0.001) and anastrozole (p=0.06) while being equally effective as fulvestrant (p>0.99). In the second study, efficacy of AZD9496 was evaluated on an anastrozole resistant MCF-7CA xenografts. Tumors were treated with anastrozole (200mg/d) for 13 weeks. During this time, the tumors initially regressed but eventually began to grow and had doubled in volume. At this time point, they were regrouped to receive second line treatment. Single agent AZD9496 was marginally significant and comparable to continued anastrozole treatment (p=0.07). Nevertheless, second line treatment with AZD9496 was equally effective as fulvestrant (p=0.36). AZD9496 treatment was also equally effective in reducing the expression of ERα protein in MCF-7CA xenografts as fulvestrant. Next, we measured the effect of AZD9496 on the mouse uterus. Uterine weight of mice treated with AZD9496 was not significantly different from mice that were treated with anastrozole (p=0.99). Furthermore, AZD9496 did not decrease the expression of ERα in the uterus, confirming its selectivity for mammary ERα. AZD9496 treatment was also able to reduce intratumoral aromatase activity. However, it was not due to direct inhibition of the enzyme, but due to reduction in ERα mediated signaling. These results suggest that AZD9496 may be a better alternative to fulvestrant due to its oral bioavailability, selectivity for mammary ER, and ability to reduce aromatase activity while being equally effective as fulvestrant.

#3605 The role of androgen receptor in invasive lobular breast carcinoma. Hillary Stires, Rebecca B. Riggins. Georgetown University, Washington, DC.

The problem for women with estrogen receptor α (ER) breast cancer – approximately 70% of all breast cancers – is that while the majority of tumors initially respond to estrogen treatment including Tamoxifen and aromatase inhibitors, they will develop endocrine resistance. A histological subset of breast cancer called invasive lobular carcinoma (ILC) accounts for 15% of all breast cancer cases and approximately 90% of ILC are ER-positive. Despite clinical markers suggesting a better prognosis for ILC compared to the more common invasive ductal carcinoma (IDC), recent data suggests women with ILC develop Tamoxifen resistance at a higher rate than IDC. New strategies for treating hormone refractory tumors are needed that not only overcome anti-estrogen resistance, but also present a more tolerable side effect profile and have fewer dose-limiting toxicities than adjuvant chemotherapy. The majority of ER-positive breast cancers also express androgen receptor (AR) and while AR presence in breast cancer is controversal, increased expression of AR leads to Tamoxifen resistance in IDC cell lines. Since ILC has more AR expression than IDC, the role of AR in ILC was studied by treating cells with the non-aromatizable androgen dihydrotestosterone (DHT) and the synthetic androgen R1881 as well as the anti-androgen Enzalutamide. We have previously began to characterize a Tamoxifen resistant variant of the ILC cell line SUM44PE termed ILC-CTM cells. In both SUM44PE and LCC-Tam cells, AR protein expression increases in response to androgens, an action that is blocked by Enzalutamide. Interestingly, androgens do not promote growth in ILC cells, but Enzalutamide is still able to inhibit growth. These results suggest Enzalutamide may be an effective alternative therapy for women with ILC. Future studies will focus on whether regulation of nuclear receptor co-factors influences differences in AR signaling in ILC.

#3606 RBP2 promotes tamoxifen resistance by altering ER function and activating IGF1R and EGFR/HER2 signaling in histone methylation-dependent and -independent manners in breast cancer. Hee-Joo Choi,1 Taekwon Son,2 Hyung-Yong Kim,1 Kyueng-Whan Min,1 Young-Ha Oh,1 Jeong-Yeon Lee,2 Kuong G.,1 Hanyang Univ., Seoul, Republic of Korea; 2 Seoul National Univ., Seoul, Republic of Korea.

Retinoblastoma binding protein 2 (RBP2, also known as JARID1A), a member of the JARID1 family of histone H3 lysine K4 demethylases, has been considered to have an oncogenic potential in several types of human cancers including breast cancer. Although physical interaction between RBP2 and estrogen receptor (ER), a crucial factor for hormone-dependent breast cancer, has been implied, the role of RBP2 in ER-dependent breast cancer remains largely unknown. Here, we demonstrate that RBP2 is a novel therapeutic target for tamoxifen resistance in ER+ breast cancer as an ER co-regulator and an activator of receptor tyrosine kinase (RTK) signaling. In large cohorts of breast cancer patients including METABRIC, high expression of RBP2 was associated with poor response to tamoxifen therapy in ER+ breast cancer. Consistently, RBP2 induced tamoxifen resistance in ER+ breast cancer both in vitro and in vivo. Using RNA-sequencing analysis, we identified that many RBP2 target genes are overlapped with ER-dependent- and tamoxifen resistance-associated genes. Mechanistically, RBP2 induced not only hyperactivation of ER-dependent transcription but also estrogen agonist activity of tamoxifen-bound ER via the interaction with ER, thus increasing the expression of NRP1, a co-repressor of ER. RBP2 also formed a transcriptional repressive complex with ER, NRP1, and HDAC1 to induce H3K4 demethylation-mediated epigenetic gene silencing of IGBP4 and IGBP5, leading to the activation of IGF1R and its downstream, PI3K/AKT pathway. Furthermore, enhanced AKT phosphorylation by RBP2 was mediated by not only IGF1R but also EGF/HER2 signaling via increasing the stability of EGFR and HER2 proteins in a demethylase activity-independent manner. We further confirmed that combinational treatment with tamoxifen and PI3K inhibitor BKM120 restored tamoxifen sensitivity in RBP2-overexpressing breast cancer. Taken together, these findings suggest that RBP2 mediates tamoxifen resistance in ER+ breast cancer by altering ER activity and activating IGF1R and EGF/HER2 signaling pathways in both a histone methylation-dependent and -independent manner. Therefore, RBP2 may be a promising prognostic marker and therapeutic target for endocrine therapy-resistant breast cancer.

#3607 Metastasis-associated protein 1 negatively regulates expression of androgen receptor in ER-positive breast cancer cells. SeungSu Kim, Seoul National University, Seoul, Republic of Korea.
Androgen receptor (AR) is expressed in more than 80% of ER-positive breast cancer and its expression is correlated with better prognosis in ER-positive breast cancer. However, little is known about the regulation of AR expression in breast cancer. We have previously reported that metastasis-associated protein 1 (MTA1), a cancer-promoting gene that is overexpressed in breast cancer, increases AR expression in breast cancer. Herein, we investigated the role of MTA1 on AR expression in three different ER-positive breast cancer cell lines.

First, small interfering RNA-mediated knockdown of MTA1 increased protein and mRNA expression of AR in MCF7, T47D, and ZR75-1. Overexpression of MTA1 down-regulated expression of AR in these cell lines. Activity of the reporter-encoding AR promoter (−2408 bp + −1126 bp) was decreased in the presence of an AR-dependent cancer cell line, leading to a better understanding of the heterogeneity of this disease and development of therapeutic modalities tailored for each disease subtype. Hormone receptor positive breast cancers, which represent 75% of breast cancer cases, are routinely treated with therapies targeting the estrogen receptor (ER) axis, including aromatase inhibitors or anti-estrogens, either alone or in combination with other agents. Recent studies have shown that the androgen receptor (AR) is widely expressed across all subtypes of breast cancer, with AR detected in 75-95% of ER positive tumors. Mounting preclinical evidence from has demonstrated that AR agonists can suppress AR and ER positive (AR/ER+) breast cancer cell growth. Furthermore, prior to the development of ER/AR-targeted therapy, androgens were used to treat breast cancers with favorable clinical responses. RAD140 is an oral, nonsteroidal, nonaromatizable selective androgen receptor modulator (SARM) with a distinct tissue selectivity profile and mechanism of action. Here we examined the molecular activity of RAD140 in breast cancer cells and evaluated its efficacy using AR/ER+ breast cancer xenograft models. The tissue-specific activity of RAD140 was examined using androgen response element (ARE)-driven reporter assay in AR/ER+/ZR75 breast cancer cells and AR/ LNCaP prostate cancer cells. RAD140 was observed to be a potent AR agonist, comparable to dihydrotestosterone (DHT), in breast cancer cells, but did not induce AR activity in prostate cancer cells, while DHT demonstrated full AR agonist activity. In vivo anti-tumor efficacy of RAD140 was evaluated using AR/ER-positive breast cancer cell line- and patient-derived xenograft models. Ziyang Yu, Suqin He, Jeffrey Brown, Jamal Saeh, Gary Hattersley. Radius Health, Inc., Waltham, MA.

Breast cancer is the most frequently diagnosed malignancy for women in the US with 246,660 new cases predicted in 2016. Histological and molecular characterization of breast tumors has led to a better understanding of the heterogeneity of this disease and development of therapeutic modalities tailored for each disease subtype. Hormone receptor positive breast cancers, which represent 75% of breast cancer cases, are routinely treated with therapies targeting the estrogen receptor (ER) axis, including aromatase inhibitors or anti-estrogens, either alone or in combination with other agents. Recent studies have shown that the androgen receptor (AR) is widely expressed across all subtypes of breast cancer, with AR detected in 75-95% of ER positive tumors. Mounting preclinical evidence from has demonstrated that AR agonists can suppress AR and ER positive (AR/ER+) breast cancer cell growth. Furthermore, prior to the development of ER/AR-targeted therapy, androgens were used to treat breast cancers with favorable clinical responses. RAD140 is an oral, nonsteroidal, nonaromatizable selective androgen receptor modulator (SARM) with a distinct tissue selectivity profile and mechanism of action. Here we examined the molecular activity of RAD140 in breast cancer cells and evaluated its efficacy using AR/ER+ breast cancer xenograft models. The tissue-specific activity of RAD140 was examined using androgen response element (ARE)-driven reporter assay in AR/ER+/ZR75 breast cancer cells and AR/ LNCaP prostate cancer cells. RAD140 was observed to be a potent AR agonist, comparable to dihydrotestosterone (DHT), in breast cancer cells, but did not induce AR activity in prostate cancer cells, while DHT demonstrated full AR agonist activity. In vivo anti-tumor efficacy of RAD140 as monotherapy, compared with fulvestrant, or in combination with a CDK4/6 inhibitor (palbociclib) or mTOR inhibitor (everolimus) was evaluated in AR/ER+ patient derived xenograft (PDX) models and cell line-derived xenograft (CDX) models. Tumor-bearing mice were randomized into treatment groups and received vehicle, RAD140, fulvestrant, palbociclib, everolimus or combinations of RAD140 with palbociclib or everolimus. Tumor volume and growth was measured to evaluate efficacy. At the end of the study, plasma and tumor samples were collected for pharmacokinetic and pharmacodynamic analysis. In AR/ER+ breast cancer PDX models, RAD140 treatment led to substantial tumor growth inhibition that was greater than that seen with fulvestrant. Furthermore, combination of RAD140 with palbociclib or everolimus produced further enhanced anti-tumor efficacy in these models. In summary, RAD140 demonstrated tissue-selective AR agonism with marked anti-tumor activity in AR/ER+ breast cancer CDX and PDX models, together with enhanced anti-tumor activity in combination with a CDK4/6 inhibitor or mTOR inhibitor.

RAD140, a selective androgen receptor modulator, has a differentiated mechanism of action in AR/ER positive breast cancers. Ziyang Yu, Suqin He, Dannie Wang, Jeffrey Brown, Jamal Saeh, Gary Hattersley. Radius Health, Inc., Waltham, MA.

Recent studies have shown that the androgen receptor (AR) is widely expressed across all subtypes of breast cancer, with AR detected in 75-95% of estrogen receptor (ER) positive tumors. Prior to the development of ER-targeted therapy, androgens were used to treat breast cancers with favorable clinical responses. The lack of tissue-selectivity and understanding of mechanism of action of classic androgens led to its declined use in this disease. Selective androgen receptor modulators (SARMs) are a class of molecules developed as tissue selective non-steroidal androgens that modulate AR signaling. However, the effect of SARMs on AR signaling in breast cancer cells is not well understood. RAD140 is an orally available SARM with potent anabolic activity in muscle and bone but a highly attenuated effect in seminal vesicles and prostate. Its efficacy is expected to be increased in breast cancer with AR function. To further our understanding of the mechanism of action of RAD140 in breast cancer cells, we examined the effects of RAD140 on AR and ER signaling pathways. The specificity and selectivity of RAD140 were examined using competitive binding assays for AR, ER, glucocorticoid receptor (GR) and progesterone receptor (PR), and using an AR reporter assay in AR/ER+ ZR-75 breast cancer cells and LNCaP prostate cancer cells. RAD140 was found to bind AR with high affinity and selectivity, with 250-fold target selectivity over the next nuclear receptor PR. The AR reporter assay results demonstrated a potent AR agonist activity of RAD140 in ZR-75 breast cancer cells but not in LNCaP prostate cancer cells, in contrast to the full agonist activity of DHT seen in both cell types. RAD140 treatment led to substantial tumor growth inhibition in AR/ER+ breast cancer xenografts. Samples of RAD140-treated xenografts were analyzed by immunohistochemistry, western blotting and quantitative real-time PCR to evaluate the modulation of AR and ER pathways. It was found that the expression of known AR target genes including KLK2, FKBP5 and the tumor suppressor gene, ZBTB16, were potently induced in RAD140-treated tumors. Furthermore, RAD140 led to substantial suppression of known ER target genes including progesterone receptor (PR), ERα and ERβ1. In conclusion, RAD140 is a potent AR agonist with marked anti-tumor activity in AR/ER+ breast cancer cells with robust activation of AR target genes, and a unique mechanism of action that leads to the suppression of ER signaling, and marked anti-tumor activity.

Synergy between androgen receptor (AR)-targeting agents and PARP inhibitors in breast cancer. Michael A. Gordon, Nicholas D’Amato, Jessica Christenson, Beatrice Babbs, Haihua Gu, Julia Wulfkuhle, Emmanuel Petracon, Anthony Elia, Jennifer K. Richer. Univ. of Colorado Anschutz Medical Campus, Denver, CO; George Mason University, Manassas, VA.

Background: AR is expressed in ~60% of HER2+ and up to 30% of triple negative breast cancer (TNBC) with growing evidence that AR plays a role in BC growth and survival. Next-generation AR-targeting agents including SEV1 (selective CYP17-lyase and AR inhibitor) and ENZA (ENZA inhibitor) have shown promise in preclinical and clinical models of prostate cancer and are currently being evaluated in BC. Approved therapeutics targeting mTOR such as everolimus (EVEROL) and HER2-targeting agents provide significant clinical benefit in some BC patients; however, de novo and acquired resistance remain critical issues that may be partially attributed to compensatory AR activity. Hypothesis: Due to cross-regulation between AR and the HER2/Pi3K/mTOR pathway in BC, targeting AR in combination with approved agents for these pathways may improve responses. Methods: Human BC cell lines HER2+ (BT474, BT474-HR20, SKBR3) and TNBC (MDAMB453, BT494) and an AR+ TNBC mouse mammary carcinoma model were treated with multiple doses of AR-targeting agents SEV1 or ENZA and EVEROL, alone or in combination and assayed over time using Incucyte instrumentation and combination indices calculated using CalcuSyn. Anchorage independence was tested by growth on soft agar. Pathway component genes and proteins were measured. In vivo, NOD/SCID mice with HER2+ trastuzumab-resistant BT474-HR20 xenografts were treated with ENZA, EVEROL, or the combination. Similar in vivo experiments with SEV1 are ongoing. Results: Both SEV1 and ENZA significantly inhibited proliferation of both HER2+ and TNBC cell lines. Both significantly inhibited growth on soft agar in 1 Met-1 cells (p<0.005 for SEV1, p=0.032 for ENZA). Using 9 dose combinations of EVEROL with SEV1 or ENZA, synergy was observed in multiple cell lines in vitro, but only in lines containing activating PIK3CA mutations. DHT induced a cell line-specific increase in pHER2 and/or pHER3 that was attenuated by AR inhibition. EVEROL alone caused an increase in total AR, pHER2, and pHER3, but was strongly inhibited by AR inhibition. In vivo, ENZA inhibited BT474-HR20 xenografts, and combining ENZA with EVEROL decreased tumor viability more than either single agent (p<0.001, repeated measures ANOVA). Similar in vivo studies with SEV1 are planned. Conclusion: Agents targeting AR synergize with EVEROL. Combination therapies targeting AR and the mTOR and HER2/HER3 pathways may provide mechanisms for increased anti-tumoral efficacy of HER2+ and TNBC. A strong inhibitor of anchorage-independent growth than ENZA and will thus be further investigated in combination with approved HER2/Pi3K/mTOR pathway-directed therapeutics in vivo. This work was funded by DOD BCPR - Clinical Translational Award BC120183 W81XWH-13-1-0909 to JKR and 0991 to AE and R01 CA187733 to JKR.
#3611 Proteasome subunit PSMD1 participates in p53 degradation and regulates proliferation of breast cancer cells. Toshiyuki Okumura,1 Kazuhiro Ikeda,1 Wataru Sato,1 Kuniko Horie-Inoue,1 Satoshi Inoue,1 Satoru Takeda,2 Koji Okamoto,1 Saitama Medical University, Saitama Prefecture, Japan; 2University of Colorado, CO.

Breast cancer is the most common cancer among women in developed countries. Generally, 70% of primary breast cancer patients express estrogen receptor, thus endocrine therapy is often efficient for the disease using antiestrogen such as tamoxifen as well as aromatase inhibitor. During long-term therapy, however, a substantial proportion of women recurrent and acquire resistance to endocrine therapy. Understanding of the mechanism underlying acquired endocrine resistance would be a first step toward overcoming hormone-refractory cancer. In this study, we identified candidate genes that contributed to tamoxifen resistance in MCF-7 breast cancer cells by functional screening based on short hairpin RNA (shRNA) library. We found that several candidate genes were overexpressed in breast cancer patients and their abundance was positively associated with poor prognosis of cancer patients in clinical database. Moreover, the genes associated with poor prognosis also showed positive association with shorter survival for patients treated with tamoxifen. Expression levels of these candidate genes were upregulated in hydroxysteramofin (OHT)-resistant MCF-7 cells (OHTR) compared with parental cells. Proliferation of OHTR cells was repressed by siRNAs targeting 26 subunits, non-ATPase 1 (PSMD1) and tetraspanin12 (TSPAN12). Furthermore, knockdown of PSMD1 inhibited cell cycle progression and p53 protein degradation. p21 and 14-3-3-3r, target genes of p53, were upregulated by PSMD1 knockdown. These findings would be used for the development of alternative diagnostic and therapeutic options for endocrine-resistant breast cancers.

#3612 Combination FGF4R and ER-targeted therapy for invasive lobular carcinoma. Kevin Levine,1 Jian Chen,1 Matthew Sikora,2 Nilgun Tasdemir,1 George Tseng,1 Shannon Pahaula,1 Rachel Jankowiak,1 David Dubbs,1 Priscilla McAuliffe,1 Adrian Lee,1 Steffi Oesterreich1.1University of Pittsburgh, Pittsburgh, PA, 2University of Colorado, CO.

Background: Invasive Lobular Carcinoma (ILC) is an understudied subtype of breast cancer. Distinctive properties of ILC include growth patterns, metastatic behavior, receptor status (almost universally estrogen receptor (ER) positive), and survival outcomes (Long-term survival is lower in patients with ER-ILC compared to the other main histological subtype, invasive ductal carcinoma). Our lab recently generated six long-term estrogen deprivation (LTED) models of ILC cells. We performed RNA-Sequencing on these six LTED cell lines and identified differentially expressed genes compared to their parental cells cultured with estrogen. We overlapped these results with a previously published analysis of a tamoxifen-resistant ILC cell line and found that FGF4R is the most consistently overexpressed gene in the setting of acquired resistance to endocrine therapy in ILC cells. Hypothesis FGF4R is an important mediator of resistance to endocrine therapy in ILC. Methods: To study the role of FGF4R in acquired resistance to endocrine therapy, we used siRNA, multiple shRNAs, and specific small molecule inhibition for growth assays of ILC cells. To study the role of FGF4R in resistance to endocrine therapy, we collected 129 well-curated ER-ILC tumor specimens. We performed gene expression analysis on the pre-treatment samples using a custom NanoString panel. Results: FGF4R inhibition decreases parental and LTED ILC cell growth in classic 2D growth conditions, in the setting of ultra-low attachment, and in colony formation assays. Mechanistically, FGF4R and estrogen signaling are antagonistic in parental ILC cells. In our clinical samples, increased expression of FGF4R is predictive of shorter time to distant recurrence. Conclusion: FGF4R may play an important role in both acquired and de novo resistance to endocrine therapy in ILC. Future studies will assess the efficacy of combining FGF4R inhibitors with ER-targeted therapy for patients with ILC.

#3613 Development of chemically modified peptide inhibitor ERAP targeting BIG3-PHB2 complex on hormone-resistant breast tumor. Toyomasu Katagiri, Tetsuro Yoshimaru. Tokushima Univ., Tokushima, Japan.

Approximately 70% of breast cancer cells express estrogen receptor alpha (ERα), and depend on estrogen (E2) for proliferative and metastatic activity. The current endocrine therapies for breast cancer are mainly based on targeting ERα signaling using selective ERα modulators, ERα downregulators, and aromatase inhibitor. However, up to 50% of patients with ERα-positive tumors either initially do not respond or become resistant to these drugs. The precise molecular mechanisms for the endocrine resistance contributes to be an active area of research. Therefore, identifying the factors and pathways responsible for resistance and defining ways to overcome it lead to develop novel molecular-target therapies to endocrine resistance. Recent findings support that the Brefeldin A-inhibited guanine nucleotide-exchange protein 3-prohibitin 2 (BIG3-PHB2) complex plays a crucial role in E2/ERα signaling modulation in these cells. Moreover, specific inhibition of the BIG3-PHB2 interaction using ERα activity-regulator synthetic peptide (ERAP: 165-177 amino acids) derive from a helical BIG3 sequence, a dominant-negative peptide inhibitor leads to the significant anti-tumor effect. However, duration of its effect is very short for clinical use. Here, we report the development of the chemically modified ERAP using stapling methods (stapled ERAP) to improve duration of its anti-tumor effects. The stapled ERAP specifically inhibited the BIG3-PHB2 interaction, thereby exhibiting the long-lasting suppressive activity and their intracellular localization was membrane-permeable polyarginine sequence supposedly through the formation of stable α-helix structure induced by the stapling. Importantly, a combination of stapled ERAP and tamoxifen caused a synergistic inhibitory effect in breast cancer cell growth. Tumor bearing mice treated with every 7 days with stapled ERAP treatment effectively prevented the BIG3-PHB2 interaction as well as daily treatment, leading to the complete regression of E2-dependent tumor in vivo. Our findings suggest that stapled ERAP may be a promising anti-tumor drug to suppress the growth of luminal-type breast cancer in clinical use.

#3614 Antibody validation revises estrogen receptor beta research. Sandra Andersson,1 Matsen Sundberg,2 Nusa Pristoveck,3 Ahmed Ibrahim,4 Phillip Jonsson,5 Bobbala Katona,1 Carl-Magnus Claussou,1 Agata Zieba,2 Margareta Ramstrom,3 Ola Soderberg,1 Cecilia Williams,2 Anna Asplund.1 Uppsala University, Uppsala, Sweden; 2KTH Royal Institute of Technology, Solna, Sweden; 3University of Houston, Houston, TX.

The discovery of estrogen receptor β (ERβ/ESR2) in 1996 was a landmark discovery. Its homology with breast cancer pharmacological target and predictive biomarker estrogen receptor α (ERα/ESR1) raised hopes for improved endocrine therapies. However, after twenty years of intense research the therapeutic promises have not materialized. We here perform a rigorous validation of 13 anti-ERβ antibodies, and demonstrate that the vast majority are unspecific. Using well-characterized controls and a panel of validation methods, we conclude that only the rarely used monoclonal antibody PPZ0506 targets ERβ in a specific manner in immunohistochemistry. We applied this antibody for protein expression profiling in 44 normal and 21 malignant human tissues and found that detection of ERβ protein is limited to testis, ovary, placenta, lymphoid cells, granulosa cells tumors, and a subset of malignant melanoma and thyroid cancers. This expression pattern aligns well with RNA-seq data, but contradicts a multitude of studies. We did not find evidence that normal or cancerous human breast express ERβ protein. Our study highlights how inadequately validated antibodies can lead an exciting and promising field astray.

#3615 Combined targeting of estrogen receptor alpha and nuclear transport pathways remodel metabolic pathways to induce apoptosis and overcome tamoxifen resistance. Eylem Kulkoyluoglu-Cotul,1 Zeynep Madak-Erdogan,1 Yosef Landesman,1 Kinga Wrobel.1 University of Illinois Urbana-Champaign, Urbana, IL, 2Karyopharm Therapeutics Inc., Newton, MA.

There is a critical need for novel therapeutic approaches to re-sensitize recurrent ERα (+) tumors to endocrine therapies. The objective of this study was to elucidate mechanisms of improved effectiveness of combined targeting of ERα and XPO1, a nuclear transport protein in overcoming endocrine resistance. Using Cignal Finder pathway analysis, Seahorse metabolic profiling and GC/MS whole metabolite profiling, we found that combination of 4-OH-Tam and Selinexor (SXR), being evaluated in multiple later stage clinical trials in patients with relapsed and refractory hematological and solid tumor malignancies, inhibited Akt phosphorylation by changing the localization of the kinase. Since we observed dramatic changes in Akt activity we hypothesized that glucose utilization pathways and metabolic profile of breast cancer cells would change in tumors that are XPO1 and SXR-resistant. These cells were more dependent on mitochondria for energy production. Their glucose and fatty acid dependency decreased in the presence of SXR and cells were more dependent on glutamine as the mitochondrial fuel source. We showed that combined targeting of XPO1 and ERα rewire metabolic pathways, increase demand on mitochondria and causes increased production of ROS that would eventually lead to apoptosis. Re-modelling metabolic pathways to regenerate new vulnerabilities in endocrine-resistant tumors is novel, and given the need for better strategies for improving therapy response of relapsed ERα (+) tumors, our findings show great promise for uncovering the role ERα-XPO1 crosstalk plays in reducing cancer recurrences.

ENDOCRINOLOGY: Nuclear Receptors and Endocrine Oncology Therapies

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Breast cancer is a common cancer among women worldwide. Hormone-mediated therapy to treat estrogen receptor alpha (ERα) positive breast cancers include the use of ER antagonist, tamoxifen, aromatase inhibitors and other compounds that degrade ERα. Unlike ERα, ERβ has been shown to have tumor-suppressive function in various cancers, including breast cancer. Recent studies have identified, synthesized, and tested the clinical safety of ERβ-selective agonists. Given the tumor-suppressive properties of ERβ, it may be possible to use these compounds to induce or activate the ERβ and test their role in the chemoprevention and blocking the progression of breast cancer. We have investigated the therapeutic utility of ERβ agonists in the prevention and progression of breast cancer using MMTV-HER2/neu mice transgenic mouse model. MMTV-HER2/neu mice develop premalignant lesions at 4-5 months, and tumors starting at month 7 due to overexpression of the HER2/neu proto-oncogene. MMTV-HER2/neu mice were treated with an ERβ agonist, Lyn500307 examined the prevention and progression of mammary cancers in these mice. When compared to controls, ERβ agonist-treated mice exhibited a significant decrease in the development of neoplastic changes. Differential gene expression analysis revealed a significant change in the expression of a number of genes in response to Lyn500307 treatment. Pathway analysis identified our model for chemokines signaling pathways, particularly TNF, in blocking the development of neoplastic changes resulting from treatment with ERβ agonists. Our studies also show a decrease in the formation of mammary tumors in HER-2/ neu mice with preexisting neoplastic changes when treated with Lyn500307. This study suggests that ERβ agonist treatment may be a valuable therapeutic option for the prevention in women at increased risk of breast cancer and in blocking the progression of hormone receptor positive breast tumors.

A paradigm shift for insulin-like growth factor 1 receptor function in triple negative breast cancers. Alison Obr, Sushil Kumar, Raymond Birge, Teresa Wood. Rutgers University-NJMS, Newark, NJ.

Signaling through the insulin-like growth factor type 1 receptor (IGF-1R) is complex in breast cancers. Early studies reported that expression of the IGF-1R correlated with estrogen receptor (ER) expression and predicted a favorable prognosis. Moreover, loss of IGF-1R has been associated with breast tumor progression into a more undifferentiated phenotype. We have generated and characterized a mouse line containing both MMTV-Wnt1 and MMTV-dnIGF-1R (kinase dead-IGF-1R) transgenes (referred to subsequently as bigenic mice). MMTV-HER2/neu mice developed premalignant lesions at 4–5 months, and tumors starting at month 7 due to overexpression of the HER2/neu proto-oncogene. MMTV-HER2/neu mice were treated with an ERβ agonist, Lyn500307 examined the prevention and progression of mammary cancers in these mice. When compared to controls, ERβ agonist-treated mice exhibited a significant decrease in the development of neoplastic changes. Differential gene expression analysis revealed a significant change in the expression of a number of genes in response to Lyn500307 treatment. Pathway analysis identified our model for chemokines signaling pathways, particularly TNF, in blocking the development of neoplastic changes resulting from treatment with ERβ agonists. Our studies also show a decrease in the formation of mammary tumors in HER-2/neu mice with preexisting neoplastic changes when treated with Lyn500307. This study suggests that ERβ agonist treatment may be a valuable therapeutic option for the prevention in women at increased risk of breast cancer and in blocking the progression of hormone receptor positive breast tumors.

Role of MTDH in estrogen-regulated gene expression. Xiangbing Meng, Yujun Li, Jesus Gonzalez Bosquet, shujie yang, Kristina W. Thiel, Kimberly K. Leslie. Univ. of Iowa College of Medicine, Iowa City, IA.

Disruption of estrogen signaling is widely associated with the development of breast, endometrial, and ovarian cancers. As a multifunctional mediator of carcinogenesis, metadherin (MTDH)/AEG-1 overexpression has been associated with numerous types of cancers, with reported roles in tumor initiation, proliferation, invasion, metastasis and chemoresistance. At the molecular level, MTDH has been shown to interact with proteins that drive tumorigenesis, including NF-κB, PLZF, BCCIP and SND1. Through analysis of the Cancer Genome Atlas (TCGA) datasets for ER-positive endometrial and breast cancers, we found that over 25% of all gene expression correlated with MTDH. Using Affymetrix microarrays, we characterized the differences in gene expression between estrogen-treated parental and MTDH-deficient endometrial and breast cancer cells. We also explored a possible interaction between MTDH and ER by immunoprecipitation and found that MTDH and ER associated in both breast and endometrial cancer cells. We also explored a possible interaction between MTDH and ER by immuno precipitation analysis demonstrated that estrogen stimulation promoted the interaction of MTDH with ER in the nucleus. These data suggest that MTDH and ERα interact in the nucleus with estrogen treatment, and MTDH/ERα co-regulated genes may have functions in drug resistance, metastasis and tumor progression.

RARalpha interactome in breast cancer, the S100 calcium binding protein A3 binds to the retinoid receptor. Mineko Terao. Istituto di Ricerche Farmacologiche Mario Negri, Milano, Italy. All-trans-retinoic acid (ATRA) is used in the treatment of acute promyelocytic leukemia. ATRA is endowed with promising anti-tumor activity in other tumor types, including breast cancer. In breast cancer, the anti-tumor activity of ATRA is mediated by the retinoid nuclear receptor, RARα (EMBO Mol Med 2015 7:950-972). RARα is a ligand-dependent transcriptional factor, which is part of nuclear complexes regulating the expression of target genes. To define the RARα interactome in breast cancer cells we took a proteomic approach. We performed mass-spectrometric analysis of RARα immuno-precipitates from nuclear extracts of an ATRA-sensitive breast cancer cell line over-expressing the receptor. We identified 30 proteins differentially interacting with RARα in the absence/presence of ATRA. Three of these proteins FABP5, HSP27 and S100A3 interact with RARα in the absence of ATRA and their binding to the receptor is dramatically reduced upon addition of the retinoid ligand. Binding of the three proteins to RARα was validated by different types of immuno-precipitation
actions are coordinated is key to understanding how transcription is regulated and will suggest new targets in steroid hormone regulated cancer and in other steroid hormone-related diseases.

#3623 Preclinical efficacy of the selective GR antagonist, CORT125134. Hazel Hunt, Joseph Belanoff, Thad Block, Stacie P. Shepherd. Conceptor, Menlo Park, CA.
The role of activation of the glucocorticoid receptor (GR) in the response of several solid tumor types to chemotherapy has been reported by several groups. Skor et al.1 have demonstrated that the non-selective GR antagonist mifepristone can potentiate the effect of paclitaxel in a mouse xenograft derived from a human triple negative breast cancer (TNBC) cell line. GR activation is thought to play a role in the regulation of pro- and anti-apoptotic genes. Isikbay et al.2 have demonstrated the benefits of combining androgen deprivation therapy with GR antagonism in prostate cancer xenograft model. Signaling via GR appears to be able to compensate for lack of androgen receptor signaling, and to contribute to castrate resistance. CORT125134 is a novel, selective GR antagonist being developed for the treatment of a variety of solid tumors, in combination with an appropriate selective Estrogen Receptor Modulators (SERMs) or aromatase inhibitors. An emerging mechanism of ER\(^+\) BC resistance to endocrine therapy, and consequently disease relapse, has been associated with a set of “hotspot” mutations in and near to helix-12 of the ER\(^+\) ligand binding domain. Selective Estrogen Receptor Degraders/Down-regulators (SERDs) represent an important pharmacological strategy being applied to develop treatments for resistant ER\(^+\) BC. Here, we compare 2 of the most frequent ER\(^+\) hotspot mutations (Y537S and D538G), with ER\(^+\) H9251/WT, and the ability of a set of SERM/SERDs and other ER\(^+\) ligands to bind, antagonize, degrade/stabilize ER\(^+\) and affect cell proliferation. Common themes that emerged included the observation that the concentration of each drug required to bind, antagonize or degrae ER\(^+\) Y537S or ER\(^+\) D538G was typically higher than that required for ER\(^+\) WT, although the extent of the shift varied between drugs and the type of measurement. An unexpected observation was that hydroxy-tamoxifen (a major active metabolite of tamoxifen) stabilized nuclear ER\(^+\) Y537S protein. This represents a potential mechanism that may limit the efficacy of Tamoxifen in treating ER\(^+\) Y537S ER\(^+\) BC.

#3622 Crosstalk between automethylation and phosphorylation tightly regulates G9a and GLP coactivator function with steroid hormone receptors. Coralie Pouillard, Michael Stellaup. University of Southern California, Los angeles, CA.
Hormone-activated steroid receptors (SR) bind to specific regulatory elements on DNA and recruit coregulators which remodel chromatin, regulate assembly of the transcription complex, and regulate transcription of their target genes. As for many coregulators, the lysine methyltransferases G9a and GLP function in both activation and repression of transcription depending on the gene. Indeed, G9a and GLP catalyze methylation of H3K9, a well-known repressive mark, but can also act as coactivators of SR (e.g. estrogen receptor \(\alpha\) and glucocorticoid receptor or GR). G9a and GLP can form homo- and heterodimers via interactions between their C-terminal domains. At the molecular level, we established that GLP, as well as G9a, is methylated on the N terminal domain of the protein, which is the region implicated in coactivator function. This event is induced by recruitment of HP1\(^{\gamma}\), while HP1\(^{\gamma}\) binding to G9a and GLP is inhibited by aurora B-mediated phosphorylation of G9a or GLP adjacent to the methylation site. In transient reporter gene assays, mutations of G9a and GLP methylation sites significantly decreased their coactivator activity with GR. Inversely, inhibition of Aurora kinase B activity increased the reporter gene activation. In addition, inhibition of the kinase activity of Aurora B increased the expression of endogenous GR target genes positively regulated by G9a and GLP, while depletion of HP1\(^{\gamma}\) inhibited their expression. Moreover methylated G9a or GLP can form a ternary complex with HP1\(^{\gamma}\) and GR in cell lines, and HP1\(^{\gamma}\) was recruited to the GR binding sites of the target genes positively regulated by G9a and GLP (but not on GR target genes that do not require G9a or GLP). Interestingly, the subset of GR target genes positively regulated by G9a and GLP contains some genes involved in cellular movement. At the physiological level, we established that G9a and GLP act as coactivators of GR target genes that mediate glucocorticoid repression of cell migration, which accompanies epithelial-to-mesenchymal transition associated with cancer progression. In this study we demonstrated that G9a and GLP methylation is required for their coactivator function with GR, because it facilitates recruitment of HP1\(^{\gamma}\) which functions selectively as a coactivator for genes positively regulated by G9a, GLP and GR. Inversely, G9a and GLP phosphorylation antagonizes these effects. This competition could be involved in the switch to determine whether G9a and GLP function as coactivator or co-repressor on specific target genes. Defining the specific molecular functions of SR coregulators and how their recruitment and

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Experiments. We focused our attention on S100A3, a calcium binding protein whose function is largely unknown. Pull-down and Far-Western experiment indicated that S100A3 binds directly to RAR\(^\alpha\). Binding is specific to this nuclear receptor isotype as similar interactions with S100A3 are not observed with RAR\(\beta\), RAR\(\gamma\) or RXR\(\alpha\). S100A3 over-expression inhibited RAR\(\alpha\) ligand-depen- dent transactivation; suggesting that calcium-binding is a co-repressor. We designed specific shRNAs targeting S100A3 and we are in the process of evaluating their functional effects on ATRA-dependent anti-tumor activity in retinoid sensitive breast cancer cell lines.

#3621 Characterization of the effects of estrogen receptor alpha Y537S and D538G mutations on receptor pharmacology. Steven J. Hartman,1 Tracy Kleinheinz,1 Jonathan White,7 Stephen Daly,7 Ria Goodwin,7 Wei Zhou,1 Jun Liang,1 Xiaojing Wang,1 Daniel F. Ortwine,1 Lori Friedman,1 Martin O’Rourke,1 Ciara Metcalfe,1 Robert A. Blake,1 Genentech, Inc., South San Francisco, CA; 2Charles River Laboratories, Harlow, United Kingdom.
The frontline therapy for estrogen receptor alpha (ER\(\alpha\)) positive Breast Cancer (ER\(^+\) BC) involves various forms of endocrine therapy, consisting of either Selective Estrogen Receptor Modulators (SERMs) or aromatase inhibitors. An emerging mechanism of ER\(^+\) BC resistance to endocrine therapy, and consequently disease relapse, has been associated with a set of “hotspot” mutations in and near to helix-12 of the ER\(^+\) ligand binding domain. Selective Estrogen Receptor Degraders/Down-regulators (SERDs) represent an important pharmacological strategy being applied to develop treatments for resistant ER\(^+\) BC. Here, we compare 2 of the most frequent ER\(^+\) hotspot mutations (Y537S and D538G), with ER\(^+\) H9251/WT, and the ability of a set of SERM/SERDs and other ER\(^+\) ligands to bind, antagonize, degrade/stabilize ER\(^+\) and affect cell proliferation. Common themes that emerged included the observation that the concentration of each drug required to bind, antagonize or degrade ER\(^+\) Y537S or ER\(^+\) D538G was typically higher than that required for ER\(^+\) WT, although the extent of the shift varied between drugs and the type of measurement. An unexpected observation was that hydroxy-tamoxifen (a major active metabolite of tamoxifen) stabilized nuclear ER\(^+\) Y537S protein. This represents a potential mechanism that may limit the efficacy of Tamoxifen in treating ER\(^+\) Y537S ER\(^+\) BC.

ATC has been shown to provide a significant improvement in the efficacy of paclitaxel in a pancreatic cancer model. In a MiaPaca-2 xenograft model, paclitaxel was given i.v. every 4 days to mice either alone or in combination with CORT125134. The combination provided significantly better tumor group inhibition than paclitaxel alone (p<0.0001). These encouraging results in a pancreatic cancer model extend the potential utility of GR antagonism in a variety of solid tumor types. In the TNBC model, alternative dosing regimens have been investigated, and suggest that stress during dosing is a confounding factor in these studies. 1. Skor MN, Wonder E, Kocherginsky M, et al. Glucocorticoid receptor antagonism as a novel therapy for triple negative breast cancer. Clinical Cancer Res. 2013;19(22):6163-6172. 2. Isikbay M, Otto K, Kregel S, et al. Glucocorticoid receptor activity contributes to resistance to androgen-targeted therapy in prostate cancer. Horm Cancer. 2014; 5(2):72-89.

#3624 CAR T cells targeting ICAM-1 trigger strong anti-tumor effects against advanced human thyroid tumors. Irene M. Min, Yогinda Vedvyas, Enda Shevlin, Marján Zaman, Brian Yrwws, Weinib Wang, Susan Park, Mau- reen Moore, Theresa Scognamiglio, Rasa Zarnegar, Thomas J. Fahey, Moonsou M, Jin, Man, Yoon Soo, M.D., New York, NY.
Introduction: Poorly differentiated thyroid cancer and anaplastic thyroid cancer (ATC) are rare yet inherently lethal malignancies with limited treatment options. Many malignant tumors, including papillary thyroid cancer (PTC) and ATC, are associated with increased expression of intercellular adhesion molecule-1 (ICAM-1), providing a rationale for utilizing ICAM-1-targeting agents in the treatment of aggressive types of thyroid cancer. Therefore, we developed a third-generation chimeric antigen receptor (CAR) targeting ICAM-1 to leverage adoptive T cell therapy as a new treatment modality against advanced thy-roid cancer. Methods: We created a firefly luciferase-expressing human ATC model in mice that develops systemic metastases very rapidly. ATC engrafted mice were treated with human peripheral blood T cells modified with a lentivi- rus encoding an ICAM-1 specific CAR (ICAM-1-CAR) to investigate their therapeuetic efficacy. Tumor burden was longitudinally measured by whole body bioluminescence imaging of luciferase-positive tumor cells. Effectortarget as says consisting of ICAM-1-CAR T cells co-cultured with multiple malignant and non-malignant target cells were used to investigate specific target cell death and orchestrating toxicity in vitro using luminescence and flow cytometry. Results: ICAM-1-CAR T cells demonstrated the ability to target and kill ATC cell lines in vitro. Strikingly, despite heterogeneous expression of ICAM-1 in ATC cell lines, addition of cytotoxic CAR T cells induced increased ICAM-1 expression by T cell-derived interferon gamma such that all cell lines became targetable by ICAM-1-CAR T cells. Patient-derived, poorly differentiated PTC cells overexpressed ICAM-1 and were also mostly eliminated by autologous ICAM-1-CAR T cells in vitro. In mice with systemic ATC, a single administrati on of ICAM-1-CAR T cells at a clinical dose mediated significant tumor killing with a 100-fold reduction in primary tumor burden compared to pre-treatment. Reductions in tumor burden persisted for over 80 days and treated mice demon-strated significantly improved survival without toxicity. Conclusion: Our
Thyroid hormone, thyroxine, promotes cell proliferation and β-catenin activation in colorectal cancer. Yee-Shin Lee, 1 Meng-Ti Hsieh, 1 Yu-Tang Chin, 2 Alec Herbergs, 3 Paul J. Davis, 4 Han-Chung Wu, 3 Hung-Yun Lin, 1 + Taipei Medical University and Academia Sinica, Taipei, Taiwan; 2 Wan-Fang Medical Center, Taipei Medical University, Taipei, Taiwan; 3 The Cleveland Clinic, Cleveland, OH; 4 Albany College of Pharmacy and Health Sciences, Albany, NY; 5 Academia Sinica, Taipei, Taiwan.

Backgrounds: Being a crucial hormone regulating growth, metabolism and various physiological processes, the status of thyroid hormone has long been implicated in cancer risks and tumor developments. In the present study, we aimed to look at the role of thyroid hormone, thyroxine (T4), in colorectal cancer cell proliferation and β-catenin activation, which is highly involved in both normal and oncogenic developments of the gut. Materials and Methods: The effects of T4 in colorectal cancer cell lines HCT 116 (APC wild type) and HT-29 (APC mutant) as well as the primary cells derived from colorectal cancer patients were studied. Cell proliferation was evaluated according to cell counting, MTT assay and qRT-PCR. The activation of β-catenin was examined by Western blotting, qRT-PCR and immunoprecipitation. Results: The results showed that T4 increased the cell number of both HCT 116 and HT-29 cells compared to the untreated cells. In both cell lines, T4 induced nuclear β-catenin accumulation and the protein levels of WNT/β-catenin targets Cyclin D1 and c-Myc. The mRNA expression of CTNNB1 was elevated by T4 in HCT 116 cells, but not in HT-29 cells. In APC wild type HCT 116 cells, T4 increased the WNT4 mRNA expression and decreased the association between β-catenin and the WNT-regulated β-catenin destruction complex. Moreover, the cell numbers of T4-treated primary cells were higher compared to the untreated cells while the mRNA expressions of proliferative genes PCNA, CCND1 and c-Myc, and mRNA expressions of CTNNB1 and WNT4 were also observed. Conclusions: T4 promoted cell proliferation and β-catenin activation in colorectal cancer. In the cells with different APC mutation status, the activation of β-catenin was regulated by different mechanisms.

Anti-cancer effect of ulipristal acetate in uterine leiomyosarcoma.

Jeong-Won Lee, Ji-Eun Hong, Young-Jae Cho, Cheul Hun Choi, Duk-Soo Bae, Hye-Kyung Jeon, Jae Ryoung Hwang. Samsung Medical Center, Seoul, Republic of Korea.

Objectives: Uterine leiomyosarcoma (LMS) is a rare uterine malignancy that arises from the smooth muscle of the uterine wall. Although LMS is an aggressive tumor associated with a high risk of recurrence, current radiotherapy and chemotherapy is limited role in the treatment. Therefore, the introduction of a new therapy for LMS of the uterus is urgently needed. Ulipristal acetate (UPA), selective progesterone receptor modifier is used for pre-operative treatment of moderate to severe symptoms of uterine fibroids because UPA-treated fibroids show the smooth muscle of the uterine wall. We examined the anti-cancer effect of UPA in LMS cells, and these findings suggest that PPARG may be a promising therapeutic target for treatment of bladder cancer.

Validation of PPARG and RXRA as drivers of bladder cancer. Jonathan T. Goldstein, 1 Craig Stratchdee, 1 Fujiko Duke, 1 Juliann Shih, 1 Matthew Meyerson, 2 Broad Institute, Cambridge, MA; 3 Dana Farber Cancer Institute, Boston, MA.

A subset of muscle-invasive bladder cancer (BCa) is typified by PPARG overexpression. PPARG regulates a plethora of genes involved in bladder cancer patients, and similarly, 5% of BCa patients possess hotspot mutations in the requisite heterodimer partner of PPARG, RXRA (S427F, S427Y). We used genetic perturbation to study the role of PPARG in bladder cancer. Our results show that overexpression of RXRA and PPARG mutant alleles activate expression of PPARG and PPARA target genes in a ligand-independent manner, and that bladder cancer cell lines dependent on PPARG for viability. These findings suggest that PPARG may be a promising therapeutic target for treatment of bladder cancer.


T-cell recruiting bispecific antibodies and antibody fragments have been used to harness the cytotoxic potential of T cells for cancer treatment. We have adopted a knobs-into-holes antibody format and produced T-cell dependent bispecific antibodies (TDB), as full length, humanized IgG1 antibodies with natural antibody architecture, which allow one arm to target various B cell antigens while the other arm recruits T cells by binding to the CD3ε subunit of the T-cell receptor. One B cell antigen targeted is CD79b, a component of the B cell receptor complex, which has been clinically validated by an anti-CD79b antibody-drug conjugate (ADC) as a safe and effective therapeutic target for B cell malignancies (Leukemia 2015 Pfeiffer et al.). In the present work, we show that anti-CD79b/CD3 TDB is active against lymphoma cells with a wide range of CD79b antigen levels in vitro. In addition, anti-CD79b/CD3 TDB appears to be insensitive to the status of cellular signaling pathways in lymphoma cells as it is active against cell lines that are resistant to the PTK inhibitor ibrutinib or an anti-CD79b ADC with a cleavable tubulin inhibitor as the payload. In vivo, anti-CD79b/CD3 TDB administration inhibited tumor growth in B-cell lymphoma xenograft models and resulted in potent B-cell depletion in the blood and spleens in a humanized murine model. To assess the safety of targeting CD79b with a T-cell recruiting bispecific antibody in non-human primates, a surrogate anti-CD79b/CD3 TDB with comparable in vitro potency was produced with a target arm that recognizes cynomolgus monkey CD79b and the same anti-CD3 arm. In a single dose pharmacokinetic/pharmacodynamics/safety study, anti-cynoCD79b/CD3 TDB administration at 1mg/kg resulted in potent B cell depletion, as well as T cell activation and proliferation, which was assessed by flow cytometry on blood and lymphoid tissue and immunohistochemistry on lymphoid tissue. The pharmacokinetic properties of anti-cynoCD79b/CD3 TDB resembled that of IgG antibodies; though with a faster clearance likely due to CD79b antigen internalization and enhanced binding to CD3 when compared to previously described anti-CD20/CD3 TDB. Transient cytokine release was observed as elevated levels of IL-2, IL-6, IFN-gamma and TNF-alpha were detected within 24 hours following administration. Anti-cynoCD79b/CD3 TDB was well-tolerated in the majority of dosed animals without toxicologically significant findings. Collectively, these preclinical data suggest anti-CD79b/CD3 TDB can be a potential therapeutic agent in B cell malignancies.

Engineering a novel asymmetric head-to-tail 2+1 T-cell bispecific (2+1 TCB) IgG antibody platform with superior T-cell killing compared to 1+1 asymmetric TCBs. Christian Klein, 1 Christiane Neumann, 1 Tanja Fauti, 1 Tina Weinzierl, 1 Anne Freimoser-Grundshober, 1 Inja Waldhauer, 1 Linda Fahrm, 1 Sylvia Herter, 1 Erwin van Puijenbroek, 1 Sara Colombetti, 1 Johannes Sam, 1 Sabine Lang, 1 Sherry Dudal, 1 Wolfgang Schäfer, 1 Jörg T. Regula, 1 Samuel Moser, 1 Oliver Ast, 1 Ralf Hoß, 1 Eckhard Mössner, 1 Peter Brüninger, 1 Marina Bach, 1 Pablo Umana, 1 Roche Pharma Research & Early Development, Schlieren, Switzerland; 2 Roche Pharma Research & Early Development, Basel, Switzerland; 3 Roche Pharma Research & Early Development, Penzberg, Germany.
Penetration of the blood brain barrier (BBB) remains a significant hurdle in the development of biologic therapeutics for CNS-related cancers. Antibody-based molecules typically do not cross the BBB in amounts required for therapeutic efficacy. Receptor-mediated transporters in the luminal membrane of brain capillary endothelium, which normally transport circulating endogenous proteins and macromolecules, are also responsible for the transport of therapeutic antibodies across the BBB.

To overcome this barrier, antibody drugs have been conjugated to small molecules, liposomes, and peptides to allow enhanced delivery across the BBB. Cytotoxic molecules have also been conjugated to antibodies to deliver therapy to CNS tumors. Antibody-drug conjugates (ADCs) are among the most promising classes of novel CNS-directed therapeutics for the treatment of brain tumors. ADCs combine the targeting properties of an antibody with the cytotoxic properties of chemotherapeutics.

The Antibody-Drug Conjugate (ADC) Approach

ADCs consist of an antibody linked to a cytotoxic agent. The antibody portion of an ADC directs the cytotoxic payload to the tumor, ensuring that the drug is preferentially delivered to tumor cells. The cytotoxic payload is designed to be released intracellularly upon internalization of the ADC, resulting in the destruction of the tumor cell.

The key components of an ADC are:

1. **Targeting Antibody**: Determines the specificity and selectivity of the ADC for the target tumor cells.
2. **Cytotoxic Payload**: Responsible for inducing cell death in the tumor cells.
3. **Linker**: Connects the antibody to the cytotoxic payload, ensuring that the payload is released intracellularly upon internalization.

The choice of antibody, cytotoxic agent, and linker is crucial in determining the efficacy and safety profile of an ADC. The antibody should have high affinity and specificity for the target tumor antigen, while the cytotoxic agent should have robust activity against the tumor cell.

One of the most promising approaches for enhancing the delivery of ADCs to the CNS is the use of BiTE® (Bi-specific T-Cell Engager) technology. BiTE® molecules are T-cell directed cytotoxic agents that recruit and activate T-cells to target and kill tumor cells.

**BiTE® Technology**

BiTE® technology involves the use of Bi-specific T-cell engagers (BiTE®) to redirect T-cells to target tumor cells. BiTE® molecules are composed of two single-chain variable fragments (scFv) linked by a flexible linker, with each scFv specific for a different receptor on the tumor cell surface. When the BiTE® binds to both receptors, it triggers the activation of the T-cell, which then杀 damages the tumor cell.

**BiTE® Design Considerations**

- **Selectivity**: The BiTE® must be designed to bind to the tumor cell surface antigens while avoiding activation of normal T-cells or other immune cells.
- **Engagement**: The BiTE® must engage multiple T-cells to ensure complete tumor cell lysis.
- **half-life**: The BiTE® must have a long enough half-life to penetrate the CNS and achieve therapeutic levels.

**Clinical Applications**

BiTE® technology has been used to develop antibodies targeting a variety of tumor antigens, including CD19, CD20, CD30, and DLL3. Preclinical studies have shown that BiTE® molecules can effectively target tumor cells in the CNS, leading to promising results in clinical trials.

**Future Directions**

Ongoing research is focused on improving the efficacy and safety of BiTE® technology, including the development of novel linkers and cytotoxins, as well as the optimization of antibody designs to enhance tumor cell engagement and T-cell activation.

**Conclusion**

BiTE® technology represents a promising approach for the delivery of therapeutics to the CNS, particularly for the treatment of brain tumors. Continued research and development in this field are expected to lead to improved therapeutic options for patients with CNS malignancies.
poses an Fc domain that maintains long half-life, yet lacks binding to Fcy receptors to reduce Fc-mediated effector functions. XmAb18087 stimulated robust RTCC of SSTR2+ cell lines including medulary thyroid carcinoma (TT), lung carcinoma (A549), and CHO cells overexpressing SSTR2, with EC50s of ~1 to 100 ng/ml. XmAb18087 also upregulated CD69 and CD25 activation markers on T-cell specific effector T-cells. SSTR2+ cell lines were not depleted, and because a control bispecific (anti-RSV x CD3) was ineffective. In addition, XmAb18087 (3 mg/kg weekly) reduced tumor burden of an established A549 xenograft in NSG mice engrafted with 10⁷ human PBMC. We next assessed XmAb18087 activity in cynomolgus monkeys. As SSTR2 is not expressed in peripheral blood, target cell depletion cannot be monitored in vivo. In vitro, SSTR2 bispecific antibodies induce effects such as lymphocyte extravasation, cytokine induction, and T-cell activation, which can serve as pharmacologic markers for activity in target organs. XmAb18087 dosed once at 30 or 60 µg/kg rapidly activated peripheral T cells, as quantified by CD69 and CD25 induction (peaking at ~8-12 hr). T cells rapidly extravasated from blood, with a nadir by 8 hr. Cytokines IL6 and TNF were rapidly induced, peaking at ~1-8 hr and returning to baseline by 24 hr. To explore repeat dosing, XmAb18087 was dosed at 1 or 10 µg/kg on Days 0 and 7 in a second study. The first dose of both 1 and 10 µg/kg again stimulated peripheral T-cell activation, extravasation, and cytokine induction. These responses decreased markedly after the second dose, suggesting that SSTR2+ target cells remained depleted for at least 7 days. In summary, these results on human cells, in mice, and in monkeys support clinical evaluation of XmAb18087 as a bispecific interface that targets tumors and peripheral T-cells, with marked cytokine induction and T-cell activation. We demonstrate its activity in peripheral blood and are indicative of T-cell-mediated depletion of SSTR2+ target cells. Importantly, these responses may also serve as useful surrogate markers of NET depletion in clinical trials of XmAb18087.

#3634 A novel tumor-targeted 4-1BB agonist and its combination with T-cell bispecific antibodies: an off-the-shelf cancer immunotherapy alternative to CAR T-cells. Christina Claus, Claudia Ferrara, Sabine Lang, Rosmarie Autschbach, Stefanie Albrecht, Sylvia Herter, Maria Amann, Sandra Richards-Grau, Johannes Sam, Sara Colombetti, Marina Bacac, Christian Klein, Pablo Umana. Roche Innovation Center Zurich, Zurich, Switzerland.

Immune cell costimulation via 4-1BB agonism has shown anti-tumor activity in the clinic and is an important element of next-generation chimeric-antigen-receptor (CAR) adoptive T-cell therapy approaches. However, the clinical development of first-generation, 4-1BB agonistic antibodies has been hampered by significant hepatic toxicity. Activity of such first-generation, 4-1BB agonistic antibodies typically depends on their hyperclustering via Fc-gamma-receptor (FcγR)-binding. Here we describe a next generation, tumor-targeted 4-1BB agonist whose activity is independent of FcγR-binding. The molecule consists of an IgG fusion protein composed of a trimeric, human 4-1BB ligand (4-1BLB), a targeting Fab moiety recognizing fibroblast activation protein (FAP), and a heterodimeric Fc region engineered to be devoid of interactions with FcγRs and C1q. The molecule mediates potent costimulation of CD8+, CD4+ and NK cells, but only in the presence of FAP-expressing cells, such as cancer associated fibroblasts, which are highly prevalent in many solid tumors. This FAP-targeted 4-1BB agonist is significantly more potent and efficacious than first-generation, standard 4-1BB agonistic antibodies when compared side-by-side in preclinical models. We show its activity in a variety of preclinical models including reporter cell assays, assays with primary T- and NK-cells, ex-vivo assays with patient tumor-derived material including cancer cells, stroma cells and tumor-infiltrating lymphocytes, fully immunocompetent murine tumor models (employing a surrogate, murineized molecule targeting murine FAP and carrying murine 4-1BLB), and in human hematopoietic stem cell-humanized mice with human tumor xenografts. We also demonstrate its activity in combination with checkpoint inhibitors and with T-cell redirecting approaches, such as a CEA-CD3 T-cell bispecific antibody. We show that hepatic toxicity of first generation, standard 4-1BB antibodies is dependent on FcγR interactions and the next generation, tumor-targeted 4-1BB agonistic antibody described here is safe and does not induce any hepatotoxicity in preclinical models including non-human primates where it was tested at doses of up to 50 mg/kg and where it showed a long circulatory half-life. Its combination with T-cell bispecific antibodies induces a massive T cell accumulation in the tumor, accompanied with an elevated CD8/Treg ratio, as compared to the respective monotherapies. Therefore, we conclude that tumor-targeted cross-linking NETS and 4-1BLB provides a safe and effective way for the co-stimulation of T cells for cancer immunotherapy and its combination with T-cell bispecific antibodies may provide an alternative, but more convenient, off-the-shelf approach to CAR T-cell therapies. The molecule is scheduled to enter clinical trials soon.

#3635 Improved T Cell activation bioassays to advance the development of bispecific antibodies and engineered T cell immunotherapies. Richard L. Somberg, Pete Stecha, Denise Garvin, Jim Hartnett, Frank Fan, Mei Cong, Jey Cheng. Promega Corp., Madison, WI.

T cells play a central role in cell-mediated immunity and can mediate long-term protection against infections and tumors. However, T-cell therapies are fraught with challenges, including the need for cell proliferation, cytokine induction, and target cell killing. New approaches are needed to improve the development of T-cell immunotherapies. We describe a variety of immunotherapy strategies aimed at inducing, strengthening or engineering T cell responses that have emerged as promising approaches for the treatment of diseases such as cancer and autoimmunity. Current methods used to measure TCR-mediated T cell proliferation and cytokine production rely on primary PBMCs as a source of T cells, which must be stimulated via co-culture with APCs or transduction with lentiviral particles. This process is time-consuming and highly variable due to the variability in their reliance on donor primary cells, complex assay protocols and unqualified assay reagents. As a result, these assays are difficult to establish in quality-controlled drug development settings. To overcome this barrier, we developed two reporter-based bioluminescent T cell activation bioassays that can be used for the development of bispecific antibodies and engineered T cell immunotherapies. The assays consist of Jurkat T cells genetically engineered to express luciferase downstream of either NFAT or IL-2 response elements. The T cell activation bioassays reflect the mechanisms of action of biologics designed to induce TCR and/or CD28-mediated T cell activation, as demonstrated using anti-CD3 and/or anti-CD28 antibodies as well as blinatumomab, a bispecific antibody that simultaneously binds CD3 expressed on T cells and CD19 expressed on malignant B cells. The bioassays are pre-qualified according to ICH guidelines and show assay specificity, precision, accuracy and linearity required for routine use in potency and stability studies. Finally, our data illustrate the use of reporter-based T cell activation bioassays for characterizing and measuring the activity of engineered chimeric antigen receptor T cells.

#3636 Potent antitumor activity of duvortuxizumab, a CD19 × CD3 bispecific T cell receptor chimera, in lymphoma models. Liat Izak1, Dana E. Callen, Maha Elgawly2, Leopoldo Luistro1, Syr Johnson2, Jaime Bald1, A Kate Sasser1, Sriram Balasubramanian1, Janssen Research & Development, LLC, Spring House, PA; 2MacroGenics, Inc., Rockville, MD.

Duvortuxizumab (NJ-64052781 or MG0011) is a CD19 × CD3 DART®, protein designed to engage and redirect CD3+ T-cells to eliminate CD19+ B-cells through T-cell-mediated cytotoxicity. Duvortuxizumab displays potent killing activity in lymphoma cell lines and animal models and is currently in clinical development for the treatment of B-cell malignancies. Here we examined duvortuxizumab activity alone and in combination with standard chemotherapy regimens in preclinical lymphoma models. Duvortuxizumab plus bendamustine increased T-cell mediated cytotoxicity of CD19+ Raji and DOHH2 lymphoma cells in the presence of isolated human pan T-cells (effectortarget 7, twice weekly); benda- mustine was dosed once at 25 mg/kg on day 1. Dosing with bendamustine alone inhibited tumor growth by 79% (p=0.0002) whereas duvortuxizumab alone resulted in complete tumor regression with no signs of relapse for up to 90 days. Dosing with duvortuxizumab plus bendamustine resulted in complete and durable tumor regression and elimination. Analysis of tumor-infiltrating lymphocytes (TILs) 3 days after the start of therapy showed that bendamustine inhibited CD3+ T-cell infiltration and activation whereas duvortuxizumab increased both. The combination showed greater activation of CD4+ and CD8+ T-cells. At 48 days after the last duvortuxizumab dose, TILs had a large percentage of CD3+ CD45RA CCR7+ cells, indicative of effector memory T-cells. This activation was accompanied by higher expression of PD-1 on T-cells and PD-L1 on tumor cells, the latter of which may have been caused by inclusion of prednisone; however, cytotoxicity against tumor cells was maintained. In a patient sample-derived model of diffuse large B-cell lymphoma, duvortuxizumab (twice weekly for 4 consecutive weeks in combination with CHOP (given once) resulted in rapid tumor regression that was more sustained than CHOP treatment alone. In summary, duvortuxizumab-mediated anti-tumor activity as a single agent and in combination with standard chemotherapy in lymphoma preclinical models. Duvortuxizumab-mediated tumor killing and T-cell activation were maintained or increased in the
presence of multiple chemotherapeutics, suggesting the potential clinical utility of combining duvortuxizumab with standard therapies in the treatment of B-cell malignancies.


Introduction: Immunotherapy with the combination of monoclonal antibodies (mAbs) that block PD-1 (nivolumab) and CTLA-4 (ipilimumab) has shown clinical benefit beyond that observed with either mAb alone. We have developed PD-1 x CTLA-4 bispecific proteins aimed at inducing antitumor immunity through simultaneous blockade of both checkpoint molecules. Two proteins, a tetravalent PD-1 x CTLA-4 bispecific DART protein (bivalent for both PD-1 and CTLA-4) and a trivalent PD-1 x CTLA-4 TRIDENT protein (bivalent for PD-1 but monovalent for CTLA-4) were engineered; the TRIDENT protein was designed to promote CTLA-4 blockade through avidity when bound to PD-1 on cells co-expressing both receptors, thus favoring PD-1 over CTLA-4 blockade in cells that do not co-express both molecules. Methods: Proteins were engineered from humanized PD-1 and CTLA-4 mAB sequences and demonstrated favorable biophysical properties. Binding assays were performed by ELISA or flow cytometry. Co-igation of PD-1 and CTLA-4 was assessed in an enzyme-fragment complementation assay (DiscoverX). T-cell activation was tested in reporter cell assays, staphylococcal enterotoxin B-stimulated PBMCs or MLR assays. Results: Both DART and TRIDENT proteins showed equivalent potency in binding immobilized recombinant PD-1 or PD-1 expressing cell lines, inhibition of PD-1 interaction with PD-L1 or PD-L2 as well as reversal of PD-1/PD-L1 mediated T-cell signal inhibition. In all assays, both formats showed activities that were comparable to those of the precursor PD-1 mAb. With respect to CTLA-4, the DART protein showed a minor potency loss in binding to CTLA-4 expressing cells, inhibition of CTLA-4/B7 interaction and reversal of T-cell signal inhibition compared to the precursor mAb. The TRIDENT protein, however, showed substantial lower potency than the DART protein in all CTLA-4 assays, consistent with the monovalent nature of the CTLA-4 arm. Importantly, in cells that co-express both receptors, DART and TRIDENT proteins show comparable co-engagement of PD-1 and CTLA-4, as shown by enzyme-fragment complementation, suggesting that anchoring through PD-1 compensates for the decreased CTLA-4 avidity of the TRIDENT molecule when both target receptors are expressed. Similarly, in T-cell co-activation assays, both DART and TRIDENT proteins showed a comparable increase in IFN-gamma response that recapitulated that observed with the combination of the individual parental mAbs. Conclusion: Both PD-1 x CTLA-4 DART and TRIDENT bispecific molecules block PD-1 and CTLA-4 pathways, with the TRIDENT protein demonstrating a PD-1-biased binding preference, consistent with its design intended to reduce CTLA-4 blockade in the absence of PD-1 co-expression. Both molecules showed comparable T-cell activation activity. Further development of bispecific PD-1 x CTLA-4 molecules for cancer treatment is warranted.


Bispecific T cell engagers (BiTE®) recruit highly cytotoxic host CD3+/CD8+ T cells for destruction of tumor antigen-specific malignant cells. Bearing a defined single-chain antibody fragment (scFv) binding to CD3+ on T cells and a selected scFv binding to tumor-specific antigen, BiTE-based antibodies suffer from a narrow therapeutic window and short t1/2 of requiring careful dose administration and continuous infusion for efficacy. To overcome these limitations, Amunix has developed the ProTIA (Protease Triggered Immune Activators) platform by incorporating its proprietary XTENprotein polymer half-life extension technology into the design of such T cell-dependent bispecific cytotoxic payload (BiTE) molecules. XTEN is a high-capacity, long acting prodrug in which enzyme cleavage of defined length containing the embedded prodrug sequence of XTEN. Insertion of the unique protease site enables selective cleavage of ProTIA by tumor-associated proteases, facilitating localized release of active BCPs in tumor tissues. Amunix’ lead ProTIA molecule, AMX-168, is in development for treatment of epithelial cancer associated ascites. AMX-168 consists of an eEpCAM scFv coupled to an eCD3 scFv that is linked to an XTEN protease sequence of defined length containing the embedded prodrug cleavage site. AMX-168 is shown to release the active eEpCAM-eCD3 BCP by tumor-specific proteases cleaving intact XTEN. Importantly, protease-treated AMX-168 BCP exhibits (1), high specificity for EpCAM and CD3 targets; (2), achieves low picomolar caspase activity in a panel of EpCAM+ human cancer lines in the presence of human PBMC; (3), induces expression of CD25 and CD69 T cell activation markers; and (4) release of key hallmark cytokines within 24 h when incubated with a mixture of EpCAM+ tumor cells and PBMC. The t1/2 of eEpCAM-eCD3 ProTIA in mice is 32 h vs 3.5 h for its BCP counterpart, indicating potential for exposure of the long-acting AMX-168 compared to the BCP-only entity in human colorectal HCT-116 tumor cells + PBMC in NOD/SCID mice. AMX-168 and its BCP-only moiety demonstrated therapeutic efficacy vs vehicle, with NTGI of 97% and 82%, respectively. AMX-168 demonstrated sustained tumor regression compared to the BCP-only moiety, supporting efficient conversion of AMX-168 to the active BCP at tumor site, and improved tumor exposure of the long-acting AMX-168 compared to the BCP-only entity. Safety assessment of eEpCAM-eCD3 ProTIA for uncontrolled cytokine release in the presence of PBMC but not EpCAM+ target cells, demonstrated ProTIA induces significantly less cytokine production compared to the BCP-only moiety. AMX-168 is currently in preclinical development with an expected IND/CTA filing by the end of 2017. Successful proof-of-concept studies of AMX-168 support further development of a broad pipeline of ProTIA therapeutics addressing a spectrum of common and advanced malignancies.

#3639 Therapeutic targeting of tumorigenic EphA2+/EphA3+ brain tumor initiating cells with bi-specific antibody in human glioblastoma. Maleeha Qazi,1 Parvez Vora,1 Chirayu Chokshi,1 Chitra Venugopal,1 Max London,1 Amy Hu,2 Minomi Subapandtiha,1 Mohini Singh,1 Jarrett Adams,1 Jason Moffat,1 Sachdev Sidhu,1 Sheila K. Singh1. 1McMaster University, Hamilton, Ontario, Canada; 2University of Toronto, Ontario, Canada.

Glioblastoma (GBM), the most aggressive primary human brain tumor, carries a dismal prognosis and is increasingly characterized by cellular and genetic intra-tumoral heterogeneity (ITH). Many of the 14 members of the ephrines producing hepatocellular carcinoma receptor (EphR) family and their ephrin ligands are expressed in GBM cells and constitute potential molecular targets for novel therapeutic agents. We hypothesize that multiple members of the EphR family play a critical role in orchestrating the clonal evolution of GBM progression. Individual Eph receptor targeting strategies have shown only modest pre-clinical success, likely because single agent therapy cannot target the degree of ITH in GBM. Using a highly specific human Eph receptor monoclonal antibody (mAb) panel (EphR proﬁler), we identiﬁed several Eph receptors with dysregulated expression in recurrent GBM compared to primary GBM. With our unique chemoradiotherapy-adapted, patient-derived xenograft model of GBM, we identiﬁed EphA2 and EphA3 expression to be upregulated after therapy. Here we show that EphA2 and EphA3 co-expression marks a highly tumorigenic cell population in recurrent GBM with higher in vitro and in vivo self-renewal and proliferation capacity compared to EphA2+/EphA3-. EphA2+/EphA3+ or EphA2-/EphA3- cells. Lentiviral mediated knockdown of EphA3 expression blocks this self-renewal and proliferation capacity in recurrent GBM. Through further characterisation using mass cytometry (CyTOF) assay, we ﬁnd that EphA2 and EphA3 is co-expressed with multiple brain tumor initiating cell (BTIC) markers (CD133, CD15, Bmi1, Sox2, Integrin 66 and FoxG1). Considering the important role of EphA2+/EphA3+ cells in GBM tumorigenesis, we generated a mice model in which mice were engrafted with a human GBM tumor cell line, and this ITH, with selective compounds that inhibit BTIC and Eph receptor activity with minimal off-target effects. Comprehensive Eph receptor proﬁling of individual patient-derived GBM will allow us to develop a therapeutic strategy for each patient’s tumor, employing polytherapy with mAbs against Eph receptors expressed at recurrence.

#3640 Novel humanized anti-Sialyl-Tn, anti-CD3 bispecific antibodies demonstrate tumor and T-cell speciﬁcity for immune activation at the tumor site. David A. Eavarone, Jillian Prendergast, Patricia E. Rao, Jenna Stein, Jeff Behrens, Daniel T. Dransfeld. Siamab Therapeutics, Newton, MA.

Tumor-associated carbohydrate antigens (TACAs) historically have been challenging targets for antibody therapeutics. Sialyl-Tn (STn) is a cancer specific antigen that is expressed on the surface of carcinomas including ovarian, colon, prostate, and pancreatic tumors but is rarely present in normal tissue. STn expression has been linked to innate immune suppression, a cheomoresistant pheno-
notype, metastasis, and poor prognosis. Previous attempts to target this antigen in the clinic with synthetic glycan vaccines proved safe but lacked efficacy. We have developed humanized bispecific antibodies targeting STn and CD3 for T-cell recruitment and activation at the tumor site. These bispecific antibodies were selected for optimal tumor targeting using our glycan microarray that enriches for candidates whose binding is independent of antigen density. STn-selective binding was demonstrated. Current lead candidates exhibited low nanomolar EC50 binding in flow cytometric assays against both STn expressing tumor cells and T cells. Quantification of T-cell activation and T-cell induced tumor killing in vitro provides a basis for the further clinical development of these bispecific antibody candidates.

#3641 EGF/CD16A TandAbs are efficacious NK-cell engagers with favorable biological properties which potentially kill EGFR+ tumors with and without Ras mutation. Michael Kluge, Kristina Ellwanger, Uwe Reusch, Ivica Fucek, Michael Weichel, Torsten Hancke, Stefan Knackmus, Joachim Koch, Martin Treder. Affimed GmbH, Heidelberg, Germany.

Constitutive EGFR activation plays an important role in the pathophysiology of various solid cancers, such as colorectal cancer, non-small cell lung cancer or squamous cell carcinomas of the head and neck. Tyrosine kinase inhibitors (TKI) and monoclonal antibodies (mAbs), which interfere with signal transduction and activation of EGFR, are approved for treatment of such cancers. However, intrinsic or acquired resistance to these treatments has been described for many patients. Natural killer cells (NK-cells) are important effectors of innate immunity and NK-cell engagement has shown evidence of promising safety in patients compared to T-cellengagers. To specifically utilize the cytotoxic potential of NK-cells to eliminate EGFR-expressing tumors, we developed tetravalent bispecific EGF/CD16A TandAbs comprising fully human Fv domains recognizing human and cynomolgus EGFR and CD16A. TandAbs recognizing epitopes in the extracellular domain of EGFR differing from epitopes targeted by other mAbs were characterized. Lead candidate AFM24 shows superior cytotoxicity in terms of ADCC (main mode of action) and reduced inhibition of EGFR-mediated phosphorylation compared to cetuximab. Importantly, inhibition of EGFR-signaling is believed to contribute to skin toxicity caused by therapeutic mAbs and TKI. AFM24’s cytotoxic activity was tested against EGFR+ tumor cell lines including some carrying a Ras mutation, which is a negative prognostic biomarker and renders cells less susceptible to cetuximab or paclitaxel. The cetuximab-resistant CRC cell line HCT-116 or the NSCLC cell line A549 (both with Ras mutations) were efficiently killed with EGFR/CD16A TandAbs in vitro. In vivo data in the HCT-116 model indicate anti-tumor efficacy of AFM24, while no effect of cetuximab was seen. Importantly, AFM24 does not activate NK-cells without target cell binding and does not bind to any other members of the EGFR family. While binding and cytotoxic efficacy of many therapeutic mAbs are impaired by serum IgG, no substantial change in AFM24’s binding affinity to NK-cells was observed in the presence of high concentrations of human IgG. In calcine-release cytotoxicity assays with NK-cells as effectors, we showed that the presence of IgG had only little inhibitory effect on AFM24 efficacy compared to cetuximab. In addition, binding of an anti-CD16 mAb with AFM24 in 1:1 molar ratio in NK-T-cell assays was substantially lower than with cetuximab. Taken together our data demonstrate that AFM24 is a highly potent human antibody displaying favorable biological properties over existing mAbs. This human/cynomolgus cross-reactive agent is currently in preclinical development to treat EGFR+ malignancies and has the potential to exhibit a favorable side effect profile and reduced toxicity to overcome resistance to other targeted anti-EGFR therapeutic agents.

#3642 Tumor-antigen expression-dependent activation of the CD137 co-stimulatory pathway by bispecific DART® proteins. Liqin Liu,1 Chia-Ying K. Lam,2 Yatana Long,3 Lislana Widjaja,1 Yinhuai Yang,1 Kalpana Shah,1 Doug Smith,2 Joanna Pan,2 Syd Johnson,2 Eizo Bonvini,1 Paul Moore1. 1MacroGenics, Rockville, MD; 2MacroGenics, South San Francisco, CA.

Introduction: CD137 (4-1BB) is a co-stimulatory molecule expressed by activated T and NK cells that, upon interaction with its CD137 ligand, further supports cell activation, proliferation and survival. Activation via CD137 holds great promise for cancer immunotherapy; however, current CD137 agonistic interventions are associated with systemic safety concerns. To develop a therapeutically modal that reduces the potential for systemic CD137 effects, we applied the DART® bispecific platform to generate proteins that can induce tumor-antigen dependent T-cell activation. Methods: DART molecules were constructed containing anti-CD137 variable regions together with either anti-HER2 or anti-EphA2 variable regions. DART binding properties were evaluated by ELISA or flow cytometry; signaling responses assessed using a NF-κB luciferase reporter cell line expressing CD137. Co-stimulatory activity was characterized with primary human T cells in the presence or absence of tumor target-antigen-expressing cells. Results: ELISA and flow cytometry analysis demonstrated that both HER2 x CD137 and EphA2 x CD137 DART molecules bind their respective antigens with high affinity. Co-expression of a tumor cell line expressing HER2 or EphA2 revealed tumor-antigen-dependent CD137 pathway activation by HER2 x CD137 and EphA2 x CD137 DART molecules on T-cell responses, co-stimulation T-cell assays were performed. In the presence of the relevant antigen-positive cell line, each respective DART molecule was able to promote T-cell proliferation or tumor cell release in a HER2 or EphA2-dependent manner. No T-cell co-stimulation was observed by either DART molecule in the absence of antigen-expressing tumor cells. Furthermore, the level of tumor antigen-dependent co-stimulation supported by the DART molecules correlates with the level of tumor target expression. Consistent with the preferential induction of CD137 by the CD8 T cell subset, CD137-based DART proteins induced a substantial increase in the fraction of CD8+ central memory and effector memory T cells in the presence of the proper tumor antigen expressing cells. Conclusions: HER2 x CD137 and EphA2 x CD137 DART molecules promote T-cell co-stimulation in a tumor antigen-dependent manner and may provide an opportunity to target the CD137 co-stimulatory pathway for cancer immunotherapy, while limiting systemic T-cell activation and related side effects.

#3643 INCAGN1876, a unique GITR agonist antibody that facilitates GITR oligomerization. Ana M. Gonzalez,1 Mariana L. Manrique,1 Lukasz Swiec,1 Thomas Horn,2 Jeremy Waigt,1 Yuqi Liu,1 Shiwien Lin,1 Dennis Underwood,1 Ekaterina Breous,1 Olivier Leger,1 Volker Seibert,1 Taha Merghoub,1 Roberta Zappasodi,1 Gerd Ritter,1 David Schaeber,1 Kevin N. Heller,1 Kimberli Brill,1 Peggy Schere,1 Gregory Hollis,1 Reid Huber,1 Marc van Dijk,1 Jennifer Bueli,1 Daniel Becht1, Nicholas S. Willems1,2 Agneus Inc, Lexington, MA; 1Agneus Inc, 4-Antibody, Basel, Switzerland; 2Memorial Sloan Kettering Cancer Center, New York, NY; 3The Ludwig Institute for Cancer Research, New York, NY; 4Incyte Corporation, Wilmington, DE.

Glucocorticoid-induced TNFR family related protein (GITR, CD357 or H11001) is a member of the tumor necrosis factor receptor superfamily (TNFRSF). Like other TNFR family members, GITR utilizes multiple oligomerization states to regulate the initiation of downstream signaling during T cell activation by antigen presenting cells (APCs). The formation of receptor superclusters, comprised of two or more trimERIC molecules, has been defined for multiple TNFRs as a means of regulating downstream signal transduction. For co-stimulatory TNFRs, like GITR, CD137 and OX40, signaling outcomes in T cells are primarily mediated via the NF-κB pathway that promotes cell survival and effector cell activities in response to suboptimal T cell receptor (TCR) stimulation. It has been hypothesized that the manipulation of the oligomeric states of co-stimulatory TNFRs using antibodies may have therapeutic utility in enhancing the activity of tumor-reactive T cells, either as single agents or in combination with other immunomodulatory or immune education strategies. Here we describe a structure-based analysis of two functionally distinct classes of anti-human GITR antibodies that stabilize unique conformational states of the receptor. INCAGN1876, a human IgG1 monoclonal anti-GITR antibody, was found to engage a conformational epitope located within a β-turn of the extracellular domain of GITR. This antibody binding site modified the equilibrium of GITR monomer, dimer and trimers to promote receptor oligomerization, resulting in downstream NF-κB signaling. Notably, this mode of INCAGN1876 receptor engagement enabled it to effectively activate the GITR pathway in recently primed T cells. By contrast, a second reference anti-GITR antibody required concomitant TCR co-engagement in order to modulate the GITR pathway. High content confocal analysis was used to evaluate the β-turn in the extracellular domain of GITR and confirm our T cell functional analysis. The ability of INCAGN1876 to engage and effectively activate GITR on recently primed T cells may enable them to overcome suppressive features of the tumor microenvironment. Notably, INCAGN1876 was shown to promote T cell co-stimulation both as a single agent and in combination with other antibodies targeting PD-1, CTLA-4 and OX40. Finally, we observed potent T cell co-stimulatory activity of INCAGN1876 to Fc variants of this antibody with diminished binding to the inhibitory Fcy receptor (FcyR). CD32B. The superiority of an IgG1 antibody in these assays was consistent with the potential to achieve optimal GITR clustering by FcyRs, while maintaining the potential for FcyR-mediated effector cell activity directed toward intra-
moral GTR high regulatory T cells. INCAGNI1876 is currently under evaluation in Phase I/II studies in subjects with advanced metastatic solid tumors (NCT02697591).

**#3644 Combination of OX40L fusion protein with an anti-tumor vaccine reduces lung metastasis and prolongs survival in a murine model of metastatic triple negative breast cancer.** Anthony S. Malamas,2 Scott A. Hammon,3 Jeffrey Schlam,1 James W. Hodge,1 National Cancer Institute, Bethesda, MD,2 MedImmune, Gaithersburg, MD.

OX40 is a costimulatory receptor on activated T-cells that potentiates the proliferation, survival, and memory formation of the CD4+ and CD8+ immune populations upon its interaction with OX40L on antigen presenting cells. The engagement of OX40 on regulatory T-cells (Tregs) with its ligand has also been shown to inhibit their suppressive capabilities. The use of exogenous OX40L agonists for cancer immunotherapy is particularly attractive in combination with cancer vaccines in order to boost the immune activity of antigen specific T-cells and overcome self-tolerance and immunosuppressive mechanisms of the tumor. We investigated the efficacy of this strategy in the 4T1 murine mammary tumor model of metastatic triple-negative breast cancer by combining an OX40L fusion protein (OX40L-FP) with a poxvirus-based cancer vaccine (MVA-Twist-TRICOM) that induces T-cell responses specific to the tumor antigen Twist, a transcription factor highly expressed in lung metastases. OX40L-FP was administered 3 and 6 days after the prime and boost vaccinations to maximize its interaction with OX40 on activated T-cells. As single agents, OX40L-FP and MVA-Twist-TRICOM had minimal effect on the formation of lung metastases in 4T1 tumor bearing mice. However, when combined, the two immunotherapies significantly decreased the number of metastatic colonies per lung, and prolonged survival after surgical resection of the primary tumor. Antibody depletion studies demonstrated that the observed anti-tumor effects were mediated by both CD4+ and CD8+ T-cells. Immune subset analysis revealed that the combination therapy increased the total number of CD4+Foxp3- and CD4+ effector memory T-cells in both the lung and periphery compared to the monotherapy controls. More importantly, the two agents synergistically induced greater CD4+ Twist-specific T-cell responses, as determined by ex vivo proliferation assays using T-cells isolated from the lung and spleen that were stimulated with Twist protein. Although the combination had no added effect on the number of total CD8+ and CD8+ effector memory T-cells in relation to the vaccine monotherapy, tetramer staining revealed a significant increase in CD8+ T-cells that were specific for Twist. We also observed a greater number of CD8+ T-cell memory precursors, as well as a greater number of IFNγ-producing CD8+ T-cells in both the lung and periphery. Tregs isolated from the lung and splenic tissue were found to be significantly less suppressive following the combination therapy, compared to those isolated from mice that received the OX40L-FP and MVA-Twist-TRICOM single agent treatments. This study provides the rationale for pairing OX40 agonists with cancer vaccines to stimulate CD4+ and CD8+ T-cell responses, while inhibiting Treg function, as a way to induce specific immunity and clearance of metastatic tumors.

**#3645 Preclinical characterization of a multi-specific DARPin molecule targeting PD1 and VEGF.** Jennifer Krieg, Schiegg Dieter, Taylor Joanna, Chris- tel Herold, Laurent Juglar, Nicolò Segamonti, Muscha Mueller, Ulrike Fiedler, Michael Tobias Stumpf, Dan Snell, Molecular Partners AG, Schlieren-Zurich, Switzerland.

Background: Combination therapy of immune-modulating drugs with modalities targeting tumor cells directly or the tumor stroma are increasing in clinical investigation and practice. In particular, the combination of immune checkpoint modulators targeting PD1 and molecules targeting the tumor stroma, like anti-VEGF therapeutics, are being utilized in multiple permutations and have shown promising results. We have developed a new class of protein therapeutics DARPin molecules for simultaneous targeting of PD1 and VEGF. The multi-specific PD1 and VEGF targeting molecules were tested for inhibition of PD1 and VEGF mediated functions in cellular functional assays and in syngeneic mouse models. The anti-tumor effect was monitored by caliper measurement and on T-cell infiltration and angiogenesis by immunohis- tochemistry. Results: The multi-specific human PD1-VEGF targeting DARPin molecule inhibits PD1 mediated downregulation of T-cell receptor signaling in a reporter cell assay in the range of the benchmark antibody Nivolumab. Potent picomolar inhibition of VEGF mediated VEGFR2 signaling was also demon-

strated for the molecule. Moreover the molecule showed potent inhibition of PD1 in an MLR assay to a similar level as compared to Nivolumab. A murine surrogate molecule was also produced and showed potent PD1 inhibition in vitro. Syngeneic mouse models demonstrate a strong anti-tumor effect of the surrogate molecule and an increase in the infiltration of T-cells into the tumor phase 1 clinical trial in solid tumors and lymphoma. It binds FcγRIIA with high affinity and demonstrates enhanced immune stimulatory activity compared to its parent dacetuzumab. An SEA version of an anti-mouse CD40 antibody (mouse IgG2a), SEA-1C10, has been generated. As expected, SEA-1C10 bound to mouse FcγRI, the functional homolog of human FcγRIIA in mice, at

**IMMUNOLOGY: BITES Bispecifics and Checkpoints**

**#3646 Characterization of the novel antibody drug conjugate MEN1309 and its target antigen Ly75.** Alessandro Bressan,1 Alessio Fiascarielli,2 Giuseppe Merlino,1 Corrado Carrisi,1 Daniella Bellarosa,1 Rachel Dusek,2 Rahel Awde,2 Sudha Swaminathan,2 Arnima Bish,2 To Uyen T. Do,3 San Lin Lou,2 Dee Aud,2 Jonathan Terrett,2 Keith Wilson,3 Christian Rohlf,4 Monica Binaschi,1 Merarini Ricerche S.p.A., Pomezia (RM), Italy; 2Oxford BioTherapeutics, Inc., San Jose, CA; 3Cytoxms Therapeutics, San Francisco, CA; 4Oxford BioTherapeutics, Ltd., Abingdon, UK, Abingdon, United Kingdom.

Ly75 (CD205, DEC-205) is a C-type lectin receptor involved in antigen uptake and processing, mainly expressed by antigen presenting cells (APC). The short cytoplasmic tail contains motifs for amino acid-based endocytosis, making this receptor an ideal target antigen for an antibody drug conjugate (ADC)-based antitumoral therapy. MEN1309 is a novel fully humanized ADC which binds to Ly75 with high affinity as shown by ELISA and FACS analysis. The antibody is conjugated to a maytansinoid DM4, a potent tubulin inhibitor, through a cleavable linker. The ability of Ly75 to internalize the antibody after binding was determined using an immunofluorescence assay that showed a rapid, efficient, and near complete internalization over a one hour time course. The expression of Ly75 mRNA and protein was investigated in human cancer cell lines derived from different histotypes and revealed high expression in pancreas, bladder, triple negative breast cancer (TNBC) cells and in diffuse large B-cell lymphoma (DLBCL). Indeed, MEN1309 shows a powerful (pM range) in vitro cytotoxic activity on different cancer cell lines expressing Ly75, whereas it exerts a weaker effect on antigen-negative cells. Besides the mechanism of action (MoA) of MEN1309 as an ADC, the putative efficacy of the antibody to drive an ADCc response was investigated through in vitro binding and functional assays. In spite of a high binding affinity of MEN1309 to FcγRIIa, no ADCc response was observed, suggesting that the high internalization rate of the antigen could hamper the triggering of NK responses. Moreover, in order to characterize the functional role of Ly75 in cancer cell lines, its expression was downregulated by siRNA demonstrating an inhibi-

**#3647 Therapeutic activity of effector function-enhanced, non-fucosylated anti-CD40 antibodies in preclinical immune-competent rodent tumor models.** Shyra J. Gardai, Weiping Zeng, Che-Leung Law. Seattle Genetics, Both- ell, WA.

Recent success of immune checkpoint inhibitors (CPIs) in the clinic underscores the role of cancer immune surveillance and the therapeutic potential of targeting immune receptors. Unlike CPIs, many members of the TNF receptor superfamily (TNFRSF), e.g., CD40, 4-1BB, OX-40, and GITR deliver agonistic signals to immune cells that may directly activate antitumor immune responses and, in combination with CPIs, may improve outcomes of active immunothera-

apy. Sugar-Engineered Antibody (SEA)-CD40 is a non-fucosylated, humanized IgG1 anti-human CD40 monoclonal antibody currently being evaluated in a phase 1 clinical trial in solid tumors and lymphoma. It binds FcγRIIA with high affinity and demonstrates enhanced immune stimulatory activity compared to its parent dacetuzumab. An SEA version of an anti-mouse CD40 anti-

body (mouse IgG2a), SEA-1C10, has been generated. As expected, SEA-1C10 bound to mouse FcγRIV, the functional homolog of human FcγRIIA in mice, at
a higher affinity than parent 1C10. Enhanced FcRIV engagement by SEA-1C10 translated into stronger antitumor activity in the CD40 (+) B16F10 melanoma and CD40 (+) A20 lymphoma models. In the A20 model, tumor free mice that survived the initial A20 tumor implantation and SEA-1C10 treatment were rechallenged with live A20 cells. Twenty nine out of thirty one such tumor free mice successfully rechallenged. Importantly, the current study is the first report to describe the targeted insertion of an immunomodulatory agent into a human-mouse chimeric CD40 gene. This allows for incorporation of the extracellular domain of human CD40 while keeping the transmembrane and cytoplasmic domains of mouse CD40. Lineage-restricted expression of the CD40 TG on B cells and monocytes was confirmed. CD40 TG cells responded to SEACD40-mediated agonistic signaling in vitro by secreting CD40 signature cytokines and up-regulating the costimulatory molecule CD80. These experimental systems will further be applied to address clinically important questions including mechanism(s) of anti-tumor effects, biomarkers indicating immune activation, dosing strategies, and timing of combinatorial regimens with CPIs.

#3648 Targeting glypican-2 in neuroblastoma via single domain antibody-based immunotoxins and chimeric antigen receptor T cells. Nan Li,1 Haiying Fu,1 Stephen Hewitt,1 Dimitar Dimitrov,1 Javed Khan,1 Mitchell Ho.1
1National Institutes of Health, Bethesda, MD; 2jilin University, Changchun, China; 3National Institutes of Health, Frederick, MD.

Neuroblastoma is the most common extracranial solid tumor in children. Approximately 45% of patients receiving standard therapy relapse and ultimately succumb to metastatic disease. One of the major challenges in the treatment of neuroblastoma and other pediatric cancers is the lack of effective targets. As such, there is an urgent need for a new therapeutic target. Here we demonstrate that glypican-2 (GPC2) protein is highly expressed in nearly half of neuroblastoma cases. High GPC2 expression has been correlated with poor overall survival when compared to patients with low GPC2 expression. The reduction of GPC2 expression inhibits neuroblastoma cell growth and induces tumor cell apoptosis through downregulation of Wnt/β-catenin signaling. We have discovered a group of human single domain antibodies specific for GPC2 and have used them to make two forms of antibody therapeutics, antibody-toxin conjugates (immunotoxins) and chimeric antigen receptor (CAR) T cells. Treatment with the immunotoxin inhibits proliferation of GPC2-positive neuroblastoma cells in vitro and mouse models. The CAR T cells targeting GPC2 suppress the growth of neuroblastoma xenograft tumors and eradicate disseminated neuroblastomas in mice. Our study establishes GPC2 as a new target of antibody-based cancer therapy and demonstrates that single domain-based antibody therapeutics can be used in the treatment of neuroblastoma.

#3649 Combination of mesothelin-targeted immune-activating fusion protein and anti-PD-L1 augments antitumor immunity and prolongs survival in murine model of ovarian cancer. Yang Zeng, Bingshao Li, Qiyuan Liu, Patrick Reeves, Ann Sluder, Jeffrey Gelfand, Timothy Brauns, Mark Poznansky, Huabin Chen. Massachusetts General Hospital, Boston, MA.

Background and Purpose: Although immunotherapy as an adjuvant to surgery and chemotherapy has been investigated in ovarian cancer (OC) as a means of reducing tumor recurrence and improving survival, there remains a significant unmet need for combinatorial strategies to enhance the antitumor immune response. The purpose of this study was to develop a novel combination immunotherapy for OC, utilizing our novel fusion protein to target and generate a cellular immune response to mesothelin (MSLN) in conjunction with blockade of the PD-1/PD-L1 checkpoint to restore the function of cytotoxic T cells in order to enhance cancer control and prolong survival. Experimental Procedures: Luciferase-expressing ID8 cells were employed to establish an intraperitoneal ovarian tumor model in immunocompetent C57BL/6 mice. The efficacies of the MSLN-targeted immune-activating fusion protein (VIC-008), aPD-L1, and the combination were evaluated. Mice received 4 intraperitoneal (i.p.) treatments of VIC-008 from day 7 post tumor inoculation weekly, and 6 treatments of aPD-L1 i.p. every other day from 4 weeks post inoculation. Tumor growth was monitored by in vivo imaging of luciferase activity. Survival time was calculated as life span from the day of tumor inoculation. In immunological studies, mice were sacrificed 7 weeks after tumor cell inoculation. Immune cells from lymph nodes, ascites and tumors were stained with antibodies against multiple immune cell markers and profiled by flow cytometry. Results: VIC-008, aPD-L1 or combination treatment delayed tumor growth. The combination treatment resulted in the greatest prolongation in survival, followed by aPD-L1 treatment and then control condition. Inactivation treatment groups (P = 0.001) was associated with increased levels of intratumoral CD3+CD8+ T cells (P = 0.0001). The combination treatment also reduced the proportion of CD4+ CD25+ Foxp3+ Treg cells (P = 0.0001) in the lymph nodes. An increased number of CD8+ CD27+ CD44+ memory T cells (P = 0.0134) were observed in ascites in the combination treatment group. CD11b+ CD11c+ dendritic cells were enriched in ascites in VIC-008 treatment (P = 0.001) and aPD-L1 treatment groups (P = 0.001). More CD11c+ CD38+ (M1) (P = 0.0361) and fewer CD206+ CD106+ (M2) (P = 0.0285) macrophages were found in the tumors of the combination treatment group. Conclusion: Our results suggest that, through activating dendritic cells and enhancing antigen presentation and cross-presentation, VIC-008 augments antitumor CD8+ T cell responses and facilitates generation of memory T cells when combined with PD-1/PD-L1 blockade, providing long-term antitumor effects. Our findings demonstrate for the first time a mechanistic rationale for combining VIC-008 and aPD-L1 in treatment of OC in mice, positioning this combination therapy as a potential promising new immunotherapeutic approach for OC.

#3650 Preclinical evaluation of the PARP inhibitor rucaparib in combination with PD-1 and PD-L1 inhibition in a syngeneic BRCA1 mutant ovarian cancer model. Liliane Robillard,1 Minh Nguyen,1 Andrea Loehr,2 Sandra Orsolic,2 Rebecca S. Kristeleit,2 Kevin Lin,3 Mitch Rapooni,1 Thomas G. Hardig,3 Andrew D. Simmons,1 1Covis Oncology, Inc., Boulder, CO; 2Cedars-Sinai, Los Angeles, CA; 3University College London, Cancer Institute, London, United Kingdom.

Background: Rucaparib (CO-338) is an oral small molecule inhibitor of poly(ADP-ribose) polymerase (PARP)-1, PARP-2 and PARP-3 that has shown clinical activity in patients with BRCA1 and BRCA2 mutated advanced ovarian cancer. Monoclonal antibodies against programmed death receptor-1 (PD-1) and programmed death-ligand (PD-L1) have also shown efficacy in advanced ovarian cancer patients. It has been reported that BRCA1 and BRCA2 mutated tumors have a higher mutational load and increased CD8+ T cell infiltration, suggesting that the combination of rucaparib and immune checkpoint inhibition may be complementary. However, PARP inhibition has also been reported to have an immunosuppressive effect in preclinical studies. Methods: Subcutaneous syngeneic models using the BRCA1 wild-type C2Km (P35/-, myc, KrasG12D, Akt-myr) and BRCA1 mutant BrKras (BRCA1/-; P35/-; myc, KrasG12D, Akt-myr) murine ovarian cell lines were developed in the murine FVB/N background. Antibodies targeting PD-1 (RMP1-14) and PD-L1 (10F.9G2) were dosed by intraperitoneal injection at 5-10 mg/kg twice weekly, while rucaparib was administered by oral gavage at 150 mg/kg twice daily. Treatment was initiated at a tumor volume of ~150mm3 (n=15/group). Animals were dosed for 21 days, and tumors were allowed to regrow to day 76. Results: In vitro cytotoxicity assays demonstrated that rucaparib was 155-fold more potent in the BRCA1 deficient BrKras cell line (IC50 = 84 nM) than the isogenic BRCA1 wild-type C2Km cell line (IC50 = 13 μM). An in vivo study using the syngeneic BrKras model was performed in mice treated with: vehicle, rucaparib, PD-1, PD-L1, rucaparib + PD-1, and rucaparib + PD-L1. All monotherapy and combination groups resulted in significant tumor growth inhibition and were followed for survival analysis. The median survival time (MST) and % cures (defined as undetectable growth at Day 76 post-tumor implantation) for vehicle, PD-1, PD-L1, PD-1 and rucaparib monotherapy treated animals was 34 days (0%), 41 days (4%) and 76 days (88%), respectively. The rucaparib + PD-1 and rucaparib + PD-L1 combination groups demonstrated greater efficacy than the monotherapies, with a MST of >76 days (100%) and >76 days (88%), respectively. Dose response and immune profiling studies are ongoing. In vivo data from this study will be presented. Conclusions: The combination of rucaparib with PD-1 and PD-L1 inhibition improved survival in a BRCA1 mutant syngeneic model.
Preclinical examination of the effects of MT-3724, an engineered toxin body targeting CD20, in mantle cell lymphoma. Shengqiang Huang, Taylor Bell, Yang Liu, Hui Guo, Carrie Li, Makhudm Ahmed, Laura Lam, Hui Zhang, Zhihong Chen, Michael L. Wang, Leo Zhao, Krystle Nomie. University of Texas MD Anderson Cancer Center, Houston, TX.

Mantle cell lymphoma (MCL) accounts for 6-8% of all non-Hodgkin lymphoma cases and is a therapeutic challenge. MCL is characterized by the expression of different B-cell markers such as CD-19, CD-20 and BSA/PAX5, and CD-20 is strongly expressed and can be used as a potential target. MT-3724 was developed by Molecular Templates and is an engineered toxin body (ETB) targeting CD-20. MT-3724 binds CD-20 and forces its own internalization. The drug subsequently self-reoutes to the cytosol to enzymatically inactivate and permanently inhibit protein synthesis via ribosome inactivation. By selectively and specifically targeting CD20-positive cells, MT-3724 may decrease cell proliferation and induce apoptosis in MCL. We tested the effects of MT-3724 by in vitro cell proliferation in 3 ibritinib-sensitive cell lines and 5 ibritinib-resistant cell lines (4 primary resistant and 1 acquired resistant). We also measured the levels of apoptotic cells in both ibritinib-sensitive and -resistant cell lines treated with MT-3724 by Annexin V/PI staining. Lastly, we conducted an in vivo efficacy assay of MT-3724 in a MCL PDX model resistant to a wide-range of drugs, including ibritinib. MT-3724 inhibited cell proliferation effectively and efficiently in most ibritinib-sensitive and ibritinib-resistant cell lines in a dose-dependent manner. IC_{50} < 500 ng/ml was characterized as sensitive to MT-3724, and > 500 ng/ml was considered resistant to MT-3724. Resistant cell lines were found in ibritinib-sensitive cell lines, the 3 cell lines (Jeko-1, Mino and Rec-1) were sensitive to MT-3724 with IC_{50} values of 139.1, 309.3 and 457.7 ng/ml, respectively. Regarding the ibritinib-resistant cell lines, 4 cell lines (Maver-1, JVM-13, Jeko-R and Granta-519) were sensitive to MT-3724 with IC_{50} values of 124.6, 155.1, 266.2 and 412.4 ng/ml, respectively, and 1 cell line (Z-138) was resistant to MT-3724 (IC_{50} = 1231 ng/ml). However, no significant differences in IC_{50} values were found between ibritinib-sensitive and -resistant cell lines (p = 0.36). In a time-dependent assay, 300 ng/ml MT-3724 also reduced cell proliferation in 2 ibritinib-sensitive cell lines (Mino and Jeko-1) and 2 ibritinib-resistant cell lines (Jeko-R and Maver-1) over time. Furthermore, MT-3724 also induced cell apoptosis in both ibritinib-sensitive (Jeko-1) and -resistant (Jeko-R and Maver-1) cell lines. Lastly, MT-3724 was administered intraperitoneally for three consecutive weeks in a PDX model resistant to a wide-range of agents. Interestingly, MT-3724 dramatically reduced tumor burden and increased survival (median of 27 days) of the PDX mice. MT-3724 is the first toxin engineered body targeting CD-20 used in MCL, which may be a potential therapeutic candidate for MCL, especially for drug-resistant cases.

Combination activity of LAG3 and PD-1 targeted therapies is significantly enhanced by the addition of phosphatidylinerse targeting antibodies to establish anti-tumor immune response in murine triple negative breast cancer. Michael J. Gray, Jian Gong, Jeff T. Hutchins, Bruce D. Freimark. Peregrine Pharmaceuticals, Tustin, CA.

Previous studies utilizing NanoString immune profile analysis demonstrated that intratumoral levels of LAG3 (lymphocyte activation gene 3) mRNA increased in response to phosphatidylinerse (PS) and PD-1 targeting antibodies in murine triple negative breast cancers (TNBC). This suggests LAG3 acts to attenuate immune system activation during I/O therapies and that PD-1 and LAG3 function cooperatively in suppressing immune system activation. Here we show that adding PS targeting antibodies can further enhance the effectiveness of antibodies targeting LAG3 and/or LAG3-PD-1. We first examined expression of LAG3 and PD-1 in the murine TNBC model E0771 and found that tumor associated T-cells (CD4+ and CD8+) have expression of both markers. Mice implanted with TNBC tumors were next treated with antibodies targeting PS, PD-1, and LAG3 alone and in combination with each other. Interestingly, the addition of PS targeting antibodies not only increased the effectiveness anti-PD-1 effectiveness as previously observed, but also enhanced anti-LAG3 treatment, showing that PS targeting antibodies are capable of augmenting additional I/O therapies. Combination of anti-LAG3 and anti-PD-1 targeting antibodies resulted in a significant decrease in tumor growth with complete tumor regression in 80% of the animals (along with the ability to completely reject secondary TNBC challenge) compared to 0% in the anti-PD-1+LAG3 treatment group. Immunoprofiling showed that the addition of PS targeting antibodies to these checkpoint therapies, including the combination of anti-PD-1+LAG3, resulted in a phenotypic type associated with enhanced immune system activation and immune-surveillance including increased tumor infiltrating lymphocytes (TILs) with upregulation of T-cell associated activation pathways, increased Th1 to Th2 profile, and enhanced antigen presentation processing /presentation mechanisms along with intratumoral (IT) LAG3 associated IgG activity. This data demonstrates that adding PS targeting antibodies to clinically relevant therapies, including PD-1+LAG3, may significantly enhance their ability to activate and redirect the host immune system into recognition and elimination of tumor cells compared to single and combinational treatments that lack PS targeting antibodies.


Background: ERY974 is a humanized IgG4 bispecific T-cell redirecting antibody (TRAB) currently in Phase 1 clinical trial (NCT02748837). ERY974 consists of a common light chain and two different heavy chains that respectively recognize glycopen-3 (GPC3) and CD3. The Fc portion of ERY974 is modified to lose FcγR binding to prevent GPC3-independent Fc-mediated effector function. However, binding activity to FcRn, an important factor in the PK profile of IgG, is maintained. ERY974 simultaneously binds to GPC3 on the cancer cell surface and to CD3 on the T cell surface, and induces TRAB-dependent cellular cytotoxicity mediated by the potent effector function of T cells. ERY974 shows strong antitumor activity against gastric, lung, ovarian, head & neck, and esophageal cancer-derived xenograft tumors in a non-obese diabetic/severe combined immunodeficiency (NOD-SCID) mouse model injected with human T cells. Cancer immunotherapy, as represented by immune checkpoint inhibitors such as PD-1, PD-1-L, and CTLA-4 antibodies, has been recently demonstrating remarkable clinical benefit in various tumor types. However, the number of patients who have survival benefit is limited, and combining cancer immunotherapy with other agents is required to improve the efficacy. Although ERY974 monotherapy is expected to show clinical activity based on the preclinical data, we examined whether further improvement of ERY974-induced efficacy is attained by combination with chemotherapy. Method & Results: We evaluated the combination effect of ERY974 with chemotherapy against xenograft tumors of MKN45 (gastric cancer) or NCI-H446 (lung cancer) either in a NOD-SCID mouse model injected with human T cells or in a humanized non-obese diabetic/shi-SCID/IL-2Rγ-cy7 mouse model with which differentiated human T cells are constitutively supplied. Although ERY974 monotherapy shows only minor antitumor effect against MKN45 and NCI-H446, combination therapy significantly enhanced efficacy over its monotherapy in xenograft model. A Phase 1 clinical study (NCT02694822) is currently ongoing to evaluate the safety and pharmacokinetic (PK)/pharmacodynamic (PD) profile of ERY974 in combination with pazlitaxel or cisplatin in NCI-H446 tumors caused a tumor disappearance without regrowth for a long period. Conclusion: These preclinical data suggest the possibility that the strategy of combining ERY974 with chemotherapy may succeed in increasing the clinical benefit. Now the combination effect is being further investigated to clarify the mechanism.
namic (PD) relationships in patients with advanced solid tumors. AGEN2034 is a human IgG4 antibody that binds selectively to PD-1 with high affinity and potentiates T cell responsiveness via the blockade of PD-L1 and PD-L2 binding to PD-1. Here we evaluated the pharmacologic effect of combining AGEN1884 with AGEN2034, and other molecules targeting the PD-1/PD-L1 pathway, on primary human T cell immune responses. AGEN1884 combined effectively with AGEN2034, and other antibodies targeting the PD/L-PD/L pathway, to promote superior T cell immune responses compared to monotherapies. Consistent with these in vitro findings, the co-administration of AGEN1884 with an anti-PD-1 antibody in cynomolgus monkeys (Macaca fascicularis) induced a dynamic PD effect, including a proliferative T cell response in peripheral blood, as compared to animals receiving antibodies alone. Finally, co-administration of an anti-mouse CTLA-4 antibody together with Agenex’s tumor-specific neo-epitope-based vaccine (AutoSynVax™) in mice induced effective amplification of vaccine-driven T cell responses, compared to animals that received the vaccine alone. These data further exemplify the versatility of harnessing antibody-mediated CTLA-4 blockade to influence apical events involved in T cell priming by antigen presenting cells. Taken together, these in vitro and in vivo findings demonstrate that the combination of AGEN1884 with PD-1 pathway blockade or with neo-epitope-based vaccines has the potential to provide therapeutic activity that is superior to that of either checkpoint- or vaccine-based monotherapies.

**#3655 Biomarker strategy to guide the clinical development of ImmTACTM molecules, a novel class of bispecific T cell engaging biologic drugs.** Cheryl McAlpine,1 David Krige,1 Sandra Herrero-Gonzalez,1 Sarah Franklin,1 Jacob Hurst,1 William Shingler,1 Sanjay Patel,1 Andy Johnson,1 Debbie Parker,1 Christina M. Coughlin,2 Namir J. Hassan,1 Bent K. Jakobsen1,1 ImmunoLoco Ltd., Abingdon, United Kingdom;2 Immunocore Ltd., Philadelphia, PA.

IMCgp100, a first in class immunotherapy, is a T cell redirecting bispecific biologic comprising an affinity enhanced T-cell receptor specific for gp100 and an anti-CD3 scFv. Phase I/IIa data has provided evidence of a favourable safety profile, and durable responses in both cutaneous and uveal melanoma have been observed. To complement the clinical studies we have developed a comprehensive biomarker strategy to aid our understanding of pharmacodynamics, patient response and potential mechanisms of resistance which includes analysis of markers in both the tumour and periphery. The data obtained to date provide evidence of the pharmacodynamic effects of the molecule including chemokine/ cytokine release, in both the tumor and periphery, and lymphocyte infiltration into tumors. In addition, changes in the levels of certain chemokines following the first dose of IMCgp100 were associated with tumor shrinkage. The biomarker strategy we have developed forms the basis for the support of the first dose of IMCgp100 were associated with tumor shrinkage. The biomarker strategy we have developed forms the basis for the support of the first dose of IMCgp100 were associated with tumor shrinkage. The biomarker strategy we have developed forms the basis for the support of the first dose of IMCgp100 were associated with tumor shrinkage. The biomarker strategy we have developed forms the basis for the support of the first dose of IMCgp100 were associated with tumor shrinkage. The biomarker strategy we have developed forms the basis for the support of the first dose of IMCgp100 were associated with tumor shrinkage.


Melanoma is one of the deadliest forms of skin cancer, with a dim prognosis when metastasized, leading to the highest number of skin cancer related deaths. In recent years there has been a focus on the use of checkpoint inhibitor therapies like anti-CTLA-4, anti-PD-1 and anti-PD-L1 to treat melanoma. PD-L1, HVEM and VISTA expression on cancer cells has been shown to promote immune evasion and tumor survival. CTLA-4, when engaged on tumor cells leads to their apoptosis and LIGHT signaling leads to recruitment of T cells and effective tumor clearance. Thus, these molecules do not function in isolation, we need to consider tissue pleiotropy and the expression of co-stimulator and co-inhibitory molecules on tumor tissue. Our laboratory has characterized and established five primary patient derived melanoma cell lines, ME1-2, ME-L-V, 3MM, KFM and GLM-2. In an effort to understand tumor tissue pleiotropy, we conducted a comprehensive expression pattern screening of eight co-inhibitory, 10 co-stimulatory, 32 immune cell markers in human blood, in vitro and ex vivo dendritic cells (DCs) express both PD-1 and PD-L1, and an activation signal via TLR agonists triggers downregulation of PD-1 to empower the T cell stimulatory capability of DCs. In contrast, tolerogenic DCs remain PD-1 positive, correlating to T cell-unresponsiveness. Anti-PD-L1 Ab directly induces maturation of DCs and renders them capable of stimulating T cell proliferation. Similarly, anti-PD-L1 Ab treatment in tumor-bearing mice induces massive infiltration and activation of CD11c+ DCs in the spleen and draining lymph node, indicating that PD-1 is a negative regulator in DCs. Furthermore, ablation of DCs prior to anti-PD-L1 Ab treatment in an established MC38 tumor model in CD11c-DTR mice suggests a crucial role of DCs in mediating response to PD-L1 treatment. In support of the preclinical evidence that DCs are the primary target of anti-PD-L1 Ab, we analyzed RNA-seq data from tumor biopsies at baseline in patients with renal cell carcinoma.

**IMMUNOLOGY: Phosphatidylinerse-targeting antibodies enhance anti-tumor activity of a tumor vaccine in a HPV-induced tumor model.** Genevieve Weir,1 Tara Quinton,2 Jeff T. Hutchins,3 Bruce D. Freimark,3 Mariannne Standford3.1 Immunovaccine, Inc., Halifax, Nova Scotia, Canada;2Peregrine Pharmaceuticals, Inc., Tustin, CA.

Antibodies targeting phosphatidylinse (PS) have been shown to induce anti-tumor responses by induction of tumor-specific T cells. Based on this observation, we evaluated the responses of PS and PD-1 targeting antibody therapy to enhance anti-tumor responses of a HPV16 peptide vaccine formulated in DepoVax™ (DPX) in mice bearing HPV-transformed C3 mouse tumors. The addition of PS-targeting antibody (mch1N11) to DPX/metrnomic cyclophosphamide (mCPA) immunotherapy prolonged survival in comparison to mice receiving an isotype control in combination with DPX/mCPA. When anti-PD-1 was added to mch1N11 + mCPA, there was no increase in survival. The addition of mch1N11 to DPX/mCPA immunotherapy had no effect on tumor growth or survival in the aggressive B16-F10 model. TIL analysis revealed an increase in CD8+ T cells, antigen specific CD8+ T cells and PD-1+ T cells in the tumor with mch1N11 treatment. The expression of surface markers for macrophages (CD68high, F4/80) and dendritic cells (CD11c+) were also increased in the tumors of mice treated with mch1N11. RT-qPCR analysis of the tumor confirmed higher mRNA expression of T cells markers (CD8, Granzyyme B, PD-1) and antigen presenting cell markers (F4/80, CD74) in the spleen, expression of cell surface markers for monocytes (CD11b) and PD-1+ T cells (CD8) were elevated in groups treated with mch1N11 in combination with anti-PD-1. Combined, these findings indicate that in this model, PS-targeting antibodies can enhance the activity of phagocytic cells involved in antigen presentation. We have found that PD-1 expression increases as anti-tumor activity increases, therefore these results also provide an indication that antibodies targeting PS enhance the anti-tumor immune response induced by DPX/mCPA therapy. The observations suggest that PS-targeting antibodies may enhance therapeutic vaccines for the treatment of cancer.

**IMMUNOLOGY: Dendritic Cells as Critical Immune Targets**

**#3658 Dendritic cells dictate the responsiveness of PD-L1 blockade in cancer.** Maud Mayoux,1 Marieke F. Fransen,2 Andreas Roller,3 Ines Matos,1 Vesna Pulko,1 Vaios Karanikas,1 Pablo Umana,1 Christian Klein,1 Ferry A. Ossendorp,1 Wei Xu1.1 Roche Innovation Center Zurich, Schlieren, Switzerland;2Leiden University Medical Center, Leiden, Netherlands;3Roche Innovation Center Basel, Basel, Switzerland.

Recent advances in cancer immunotherapies with PD-1/PD-L1 pathway blockade have transformed the way that cancer is being treated, leading to durable responses and prolonged overall survival. The general thinking is that PD-1/PD-L1 blockade reinvigorates tumor-infiltrating PD-1+ T cells with exhausted phenotypes. However, a mechanistic understanding on why only a subset of patients (10-30%) responds to checkpoint inhibition remains largely unknown, as does the exact immune mechanism of PD-1/PD-L1 blockade. Here, we discovered that PD-L1 blockade mediates anti-tumor immunity via dendritic cells. In human blood, in vitro and ex vivo dendritic cells (DCs) express both PD-1 and PD-L1, and an activation signal via TLR agonists triggers downregulation of PD-1 to empower the T cell stimulatory capability of DCs. In contrast, tolerogenic DCs remain PD-1 positive, correlating to T cell-unresponsiveness. Anti-PD-L1 Ab directly induces maturation of DCs and renders them capable of stimulating T cell proliferation. Similarly, anti-PD-L1 Ab treatment in tumor-bearing mice induces massive infiltration and activation of CD11c+ DCs in the spleen and draining lymph node, indicating that PD-1 is a negative regulator in DCs. Furthermore, ablation of DCs prior to anti-PD-L1 Ab treatment in an established MC38 tumor model in CD11c-DTR mice suggests a crucial role of DCs in mediating response to PD-L1 treatment. In support of the preclinical evidence that DCs are the primary target of anti-PD-L1 Ab, we analyzed RNA-seq data from tumor biopsies at baseline in patients with renal cell carcinoma.
prior to treatment with atezolizumab and found that the abundance of genes related to cross-presenting DC subsets (such as XCR1) correlates with a survival advantage in response to atezolizumab (HR=0.13, median OS is 16.2 months in patients with DC gene score <50% versus NR (not reached) in those >50%). In conclusion, we discovered abundance of tumor-infiltrating DCs as a novel biomarker that predicts treatment response to clinical checkpoint inhibitors. We postulate that checkpoint inhibition directly on tumor-infiltrating DCs likely contributes to amplification of Ag-specific priming of tumor-specific T cells.

#3659 WT1 and PRAME mRNA transfected TLR 7/8-polarized fast DC vaccines in AML patients mount specific immune responses and impact progression free survival. Iris Bigalke,1 Guri Solem,1 Dag Jøsefsen,1 Yngvar Fleisland,1 Kirsti Henningsen,1 Lisbeth Skoge,1 Signe Speitelen,1 Stein Sebøe-Larsen,1 Dolores J. Schendel,1 Gunnar Kvalheim1.2. Oslo University Hospital, Oslo, Norway; 3Medigene Immunotherapies GmbH, Germany.

Patients diagnosed with acute myeloid leukemia (AML) may not be eligible for curative intensive treatment due to co-morbidity factors or age. Here we report results of 5 AML patients in morphological remission after incomplete induction/consolidation chemotherapy treated with dendritic cells (DCs) targeting WT-1 and PRAME. DCs were produced as described previously (Subklewe et al 2014), using a maturation cocktail containing the TLR 7/8 ligand R848. These DCs show a polarized release of IL-12p70 combined with low IL-10 upon stimulation. 2.5 or 6e+6 DCs per antigen were injected intradermally once weekly for 4 weeks (wks), in wk 6 and thereafter at monthly intervals. Blood and bone marrow (BM) samples were collected at regular intervals. Minimal residual disease (MRD) was measured in BM by quantitative PCR of WT-1 and PRAME expression and by morphological examination. Immune responses were assessed by analysis of intracellular interferon gamma expression or proliferation following stimulation with peptides spanning WT-1, PRAME, hTERT and survivin or after stimulation with autologous WT-1 and PRAME DCs. A 57 year old woman with intermediate risk M4 AML was vaccinated over 22 months after chemotherapy. Five weeks after start of vaccination she mounted strong CD8 responses against PRAME combined with an increase in hTERT response, suggesting WT-1 signals in BM showed low positive levels throughout vaccination, but she remains in morphological remission 33 months after end of chemotherapy (Evoc). A 50 year old man with M2 intermediate risk AML, initially not eligible for BM transplantation, showed specific immune responses against WT-1 during 10 months of vaccination. Due to Bell’s Palsy he was treated with cortisone which immediately reduced the vaccine effect, accompanied by increase of blasts in the bone marrow. Following new induction therapy he received BM transplantation and is currently in CR. A 68 year old woman with M1 intermediate risk AML is under vaccination for 24 months. WT-1 signals in BM continue to be slightly elevated without any sign of morphological relapse. CD4 responses and low CD8 responses are detected against WT-1, PRAME and hTERT. A 73 year old woman with M1 good risk AML relapsed after 6 months of DC vaccination without mounting specific immune responses. DC treatment was combined with 5-Azacitidine without any immunological and clinical effects. A 59 year old woman with good risk AML has been vaccinated for 14 months and is in remission for 21 months since Evoc. WT-1 in BM remains slightly elevated whilst the initially positive PRAME signal is negative. Our results show that in 4 out of 5 AML patients fast TLR-polarized DCs mounted vaccine specific responses, 3 patients continued to be in CR after 21, 25 and 33 months respectively, following suboptimal primary chemotherapy. Immune responses are under investigation and will be presented.

#3660 Blocking the DC-HIL receptor reverses the T-cell suppression induced by proliferating melanoid-derived suppressor cells in common cancer types. Masato Kobayashi,1 Jin-Sung Chung,2 Muhammad Beg,1 Yull Arriaga,1 Uditi Verma,1 Kevin Courtney,1 John Mansour,1 Barbara Haley,1 Sadak Han,1 Yutaka Horiiuchi,2 David Harker,1 Purva Gopal,1 Ponncoza D. Cruz,1 Kiyoshi Arizumi1.1. UT Southwestern Medical Center at Dallas, Dallas, TX; 2Faculty of Medicine, Saitama Medical University, Saitama, Japan.

Myeloid-derived suppressor cells (MDSC) are the most potent suppressors of T-cell function, and their exponential proliferation in cancer states counteracts the benefits of immunotherapy given to these patients. Having discovered the T-cell-inhibitory DC-HIL receptor, we showed that DC-HIL is responsible for MDSC’s T-cell suppressive function. We found that melanoma patients (but not healthy controls) harbor in the blood an expanded population of DC-HIL+ MDSC, whose suppressor effects in vitro can be blocked by our 3D4 anti-DC-HIL mAb. To determine whether similar outcomes apply to non-melanoma cancers, we recruited patients with metastases from bladder (BL, n=44), kidney (KI, n=44), melanoma (MEL, n=20), pancreatic (PA, n=17), and prostate (PR, n=9) cancer. FACs analysis of blood samples showed all cancer types (except LU) were associated with elevated blood HLA-DRlowCD14+ MDSC vs. age-matched controls (n=21, median of 0.5%). % DC-HIL+ MDSC in PBMC was also significantly high in all cancer types; median of 2.5% for BL; 1.7% for BR; 3.5% for CO; 3.6% for KI; 0.3% for LU; 1.8% for PA; and 4.8% for PR, vs. 0.04% for healthy controls. MDSC in all cancer types showed high DC-HIL+positivity (20-90% vs. 8% for controls). We assayed T-cell functions and cytokine production in vitro by co-culturing them at varying cell ratios, with co-stimulators. Activity was determined by % suppression in IFN-γ secretion, which was correlated with % DC-HIL+positivity of MDSC. MDSC from KI (n=3) were more suppressive than those from CO and PA, with suppressor activity correlating with DC-HIL-positivity; Pearson’s r for 0.5 for CO (n=13) and 0.73 for PA (n=8). We evaluated the effects of 3D4 anti-DC-HIL mAb on MDSCs of melanoma patients following new induction therapy (EoC). A 50 year old man with M2 intermediate risk AML, initially not eligible for BM transplantation, received BM, which immediately reduced the vaccine effect, accompanied by increase of blasts in the bone marrow. Following new induction therapy he received BM transplantation and is currently in CR. A 59 year old woman with M1 intermediate risk AML is under vaccination for 24 months. WT-1 signals in BM continue to be slightly elevated without any sign of morphological relapse. CD4 responses and low CD8 responses are detected against WT-1, PRAME and hTERT. A 73 year old woman with M1 good risk AML relapsed after 6 months of DC vaccination without mounting specific immune responses. DC treatment was combined with 5-Azacitidine without any immunological and clinical effects. A 59 year old woman with good risk AML has been vaccinated for 14 months and is in remission for 21 months since Evoc. WT-1 in BM remains slightly elevated whilst the initially positive PRAME signal is negative. Our results show that in 4 out of 5 AML patients fast TLR-polarized DCs mounted vaccine specific responses, 3 patients continued to be in CR after 21, 25 and 33 months respectively, following suboptimal primary chemotherapy. Immune responses are under investigation and will be presented.

#3661 Breaking down the barrier restricting infiltration and differentiation of APC in the tumor microenvironment with a first-in-class antibody targeting Semaphorin4D, and rational combination therapies. Elizabeth E. Evans,1 Holm Bussler,1 Crystal Malloy,1 Christine Keily,1 Sebodl Torno,1 Maria Scribner,1 Cathie Foster,1 Alan Howell,1 Stephen R. Comey,1 Leslie Baich,1 Alyssa Knapp,1 John E. Leonard,1 Terrence L. Fisher,1 Siwen Hu-Lieskov,2 Antoni Ribas,3 Ernest S. Smith,1 Maurice Zauner,11. Vaccinex, Rochester, NY; 2UCLA, Los Angeles, CA.

Purpose: We expand mechanistic findings in preclinical studies to demonstrate that antibody blockade of Semaphorin 4D (SEMA4D, CD100) reduces expansion of MDSC and shifts the balance of myeloid cells within the TME to facilitate tumor rejection. Efficacy is further enhanced when combined with various immunotherapies. Design of Phase 1b/2 combination trials of VX15/2503 was completed, and several 1b/2 combination immunotherapy trials are planned. Results: SEMA4D restricts migration of monocytes and promotes expansion of suppressive myeloid cells in vitro. Strong expression of SEMA4D at the invasive margins of actively growing tumors in vivo restricts the infiltration and modulates polarization of leukocytes in the TME. Antibody blockade of SEMA4D facilitates recruitment of activated DCs and T lymphocytes in preclinical models. M-MDSCs were significantly reduced in tumor and blood following treatment. A significant shift towards increased TH1 cytokines (IFNγ, TNFα) and CTL activity was observed in tumor significantly inhibited tumor growth by 50-70%, reduced blood levels of MDSC, and enhanced cytotoxic T lymphocyte response. Our findings support DC-HIL blockade as a potential treatment for metastatic cancers, with high blood levels of DC-HIL+ MDSC as a prognostic marker for the best responders.

IMMUNOLOGY: Dendritic Cells as Critical Immune Targets
IMMUNOLOGY: Dendritic Cells as Critical Immune Targets
1b/2 trials of combination therapy with avelumab in NSCLC patients who
are immunotherapy naïve, and combinations with anti- anti-PD-1 and/or
anti-CTLA-4 in melanoma and HNSCC patients who are refractory to PD1
inhibitors are planned in 2017.

#3662

Activation of CD40 while inhibiting IL6/STAT3 using oncolytic
viruses induces mature DCs with high cytokine production but blocks PDL1
expression. Emma Eriksson,1 Ioanna Milenova,1 Jessica Wenthe,1 Anna Dimberg,1 Rafael Moreno,2 Gustav Ullenhag,1 Ramon Alemany,2 Angelica Loskog1.
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Uppsala University, Uppsala, Sweden; 2IDIBELL, Barcelona, Spain.
The tumor microenvironment (TME) consists of tumor cells and stroma,
including fıbroblasts, blood vessels, immune cells and extracellular matrix. The
TME supports tumor progression, metastasis as well as resistance to cancer
therapeutics. In pancreatic cancer, the TME is dense due to overproduction of
collagen and the tumor is infıltrated with suppressive myeloid cells such as M2
macrophages and myeloid suppressor cells. One key regulator of myeloid cells is
CD40, a receptor expressed on a variety of cell types. CD40/CD40L signaling
results in production of cytokines and chemokines by myeloid cells but also
endothelial and epithelial cells to alert the immune system of immediate danger.
4-1BB is expressed by lymphocytes and dendritic cells (DCs). Stimulation via
4-1BBL drives lymphocyte expansion and regulates memory formation. IL6 signaling leads to STAT3 phosphorylation in myeloid cells and tumor cells leading
to suppressive phenotypes, tumor proliferation, and angiogenesis. Further,
STAT3 signaling enhances production of TGFb, which in turn leads to overexpression of collagen. We have constructed a family of oncolytic adenoviruses
(LOAd) activating the CD40, 4-1BB and/or inhibiting IL6 signaling. The LOAd
viruses (-, 700, 703, 713) were investigated for their capacity to activate human
monocyte-derived DCs as well as their effect on pancreatic tumor cells and
stroma (fıbroblastic stellate cells, endothelial cells) using flow cytometry, MTS
assay and ProSeek Proteomics. The LOAd viruses expressing a trimerized
CD40L, 4-1BBL and/or a scFv IL6R showed effıcient oncolysis of tumor cells but
primary stellate cells were unaffected. However, stellate cells reduced tumorpromoting factors such as FGF5, PlGF, amphiregulin, Gal3, TGFb and collagen
type I. Dendritic cells increased costimulators, cytokines as well as chemokines
but PDL1 was not expressed when IL6/STAT3 was blocked. Infected endothelial
cells upregulated receptors important for lymphocyte transmigration (ICAM,
VCAM and E-Selectin). Taken together, our data demonstrates that it is possible
to utilize oncolytic adenoviruses to spark immune activation at the same time
changing biological processes via STAT3 blockade and/or CD40/4-1BB pathway
activation to reduce factors that promotes tumor progression.

#3663 An anti-CD4 depleting antibody reverses regulatory T-cell-induced

suppression of dendritic cells while preventing nonspecifıc CD4ⴙ T cell responses in tumor-bearing mice. Satoshi Ueha,1 Haru Ogiwara,1 Shoji Yokochi,2 Yoshiro Ishiwata,2 Francis Shand,1 Shohei Hori,3 Kazuhiro Kakimi,4 Satoru Ito,2 Kouji Matsushima1. 1The University of Tokyo, Tokyo, Japan; 2IDAC
Theranostics, Inc, Tokyo, Japan; 3RIKEN, Yokohama, Japan; 4The University of
Tokyo Hospital, Tokyo, Japan.
Administration
of an anti-CD4 depleting antibody induces tumor-specifıc
⫹
CD8 T cell responses in tumor-bearing mice, although the mechanisms underlying this phenomenon remain unclear. CD4⫹ Foxp3⫹ regulatory T cells (Treg)
impair antigen presentation by DCs through CTLA4-mediated down-regulation of CD80/CD86. In the present study, we investigated the effects of an antiCD4 depleting monoclonal antibody (mAb) on DC responses in the B16F10 and
LLC subcutaneous tumor models, and compared these effects with those of Treg
targeting therapy. Administration of anti-CD4 mAb up-regulated CD80/CD86
in tumor-infıltrating DCs, and in migrating-type but not resident-type DC subsets in the tumor-draining lymph node. The upregulation of CD80/CD86 on
these DC populations was correlated with increased proliferation of tumorspecifıc CD8⫹ T cells in the tumor-draining lymph node. Anti-CD4 mAb-induced CD80/CD86 up-regulation and tumor-specifıc CD8⫹ T cell responses
were remarkably higher than that induced by an anti-CD25 depleting mAb or an
anti-CTLA4 blocking mAb, and were equivalent to that induced by diphtheriatoxin-induced selective depletion of Tregs in Foxp3-DTR mice. Importantly, the
activation of CD4⫹ conventional T cells and B cells in the peripheral lymph
nodes that was observed following Treg depletion was not observed following
anti-CD4 mAb administration. In conclusion, anti-CD4 depleting antibody
therapy is an effective way to evoke anti-tumor CD8⫹ T cell responses by reversing regulatory T cell-induced suppression of dendritic cells, and to reduce the
risk of severe autoimmunity in cancer patients.
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#3664 CDX-1402, a dendritic cell targeted fusion protein designed to elicit
immunity to mesothelin and HER2 expressing tumors. Li-Zhen He,1 Jenifer
Widger,1 James Testa,1 Laura Mills-Chen,1 Biwei Zhao,1 Jeff Weidlick,1 Crystal
Sisson,1 Anna Wasiuk,1 Laura Vitale,1 Joel Goldstein,1 Henry Marsh,2 Tibor
Keler,1 Venky Ramakrishna1. 1Celldex Therapeutics, Inc., Hampton, NJ; 2Celldex
Therapeutics, Inc., Needham, MA.
The use of mAbs to target antigens to the endocytic receptor Dec205 on
dendritic cells (DC) is an effective means to elicit helper and cytolytic T cell
responses in the presence of appropriate adjuvants to activate DC. We have
translated this concept to clinical studies using a fully human Dec205-specifıc
mAb genetically altered to include the entire NY-ESO-1 cancer antigen (CDX1401), which when combined with TLR agonists results in effective stimulation
of both cellular and humoral NY-ESO-1-specifıc responses in cancer patients
concept, we developed a new fusion protein in which the Dec205 mAb is engineered to carry two tumor-related antigens in tandem [ECDs of mesothelin
(MSLN) and HER2]. HER2 and MSLN are broadly expressed in selected tumor
types and provide an expanded opportunity for this immunotherapy approach.
We previously generated an anti-mouse DC-targeted HER2 vaccine (␣Dec205HER2) and a MSLN vaccine (␣Dec205-MSLN) and demonstrated in mouse
studies that they were potent in eliciting strong and broad CD4 T cell immunity,
cross priming of CD8 T cells and humoral responses specifıc for HER2 or MSLN
antigens, respectively. In this work we tested the impact of expressing two tumor
antigens in the same construct on the immune responses to each antigen. Mice
of various genetic backgrounds were immunized with equimolar amount of
␣Dec205-HER2 (6.8 ␮g), ␣Dec205-MSLN (5 ␮g) or ␣Dec205-MSLN-HER2
(8.3 ␮g) in combination with poly IC-LC plus anti-CD40 or anti-CD27 as adjuvant. Similar levels of CD4 and CD8 T cell responses upon single or dual antigen
vaccination were observed in the assessment of intracellular cytokines and
IFN␥-ELISPOT after ex vivo stimulation with peptide pools derived from either
HER2 or MSLN. High titers of anti-HER2 and anti-MSLN IgG, including IgG1
and IgG2a, were elicited upon ␣Dec205-MSLN-HER2 vaccination. Both humoral and cellular responses were boosted by multiple dosing of the vaccine.
Based on these data we developed CDX-1402 using our anti-human Dec205
mAb genetically fused to MSLN and HER2. CDX-1402 was shown to effectively
deliver these antigens to human DC in vitro for activation of antigen-specifıc T
cells. In vitro stimulation of healthy volunteer circulating T cells with autologous
DC treated with CDX-1402 plus TLR agonist resulted in T cell cultures that
produced IFN␥ only when presented with CDX-1402-treated DCs or DC loaded
with a select panel of HLA-I/II synthetic peptides derived from either MSLN or
HER2 but not with control peptides. We also observed that T cells sensitized to
CDX-1402 recognize cancer cell lines that express the vaccine antigen and HLA.
Altogether, the data lend support for the use of CDX-1402 as a novel immunotherapeutic for treatment of multiple cancer types expressing MSLN and/or
HER2.
#3665

Tumor Treating Fields (TTFields) plus anti-PD-1 therapy induce
immunogenic cell death resulting in enhanced antitumor effıcacy. Tali Voloshin, Orna Tal-Yitzhaki, Noa Kaynan, Moshe Giladi, Anna Shteingauz, Mijal
Munster, Roni Blat, Yaara Porat, Rosa S. Schneiderman, Shay Cahal, Aviran
Itzhaki, Eilon D. Kirson, Uri Weinberg, Yoram Palti. Novocure, Haifa, Israel.
Tumor Treating Fields (TTFields) are an effective anti-neoplastic treatment
modality delivered via noninvasive application of low intensity, intermediate
frequency, alternating electric fıelds. TTFields is approved for the treatment of
both newly diagnosed and recurrent glioblastoma. TTFields interrupt cancer
cell mitosis by disrupting microtubules and septin fılaments, which play key
roles in mitosis. The mitotic effects of TTFields include abnormal chromosome
segregation and ER stress, which trigger different forms of cell death. We evaluated the in vitro and in vivo effects of TTFields combined with an immune
checkpoint inhibitor (anti-PD1) on immunogenic cell death. Murine Lewis lung
carcinoma (LLC) and ovarian surface epithelial (MOSE) cells were treated with
TTFields using the inovitroTM system. Levels of calreticulin (CRT) on the surface of treated cells and intracellular ATP levels were evaluated using flow cytometry. High mobility group box 1 (HMGB1) secretion was measured using an
ELISA assay. Mice were implanted with LLC cells were treated with TTFields,
anti-PD-1, or a combination of the two modalities. Tumor volume was monitored; flow cytometry analysis was performed for phenotypic characterization of
infıltrating immune cells. TTFields induced elevated cell surface expression of
CRT, decreased intracellular ATP levels, and promoted HMGB1 secretion. In
vivo, the combined treatment of lung tumor-bearing mice with TTFields plus
anti-PD-1 led to a signifıcant decrease in tumor volume compared to anti-PD-1
alone or to the control group. Signifıcant increases in CD45⫹ tumor infıltrating
cells were observed in the TTFields plus anti-PD-1 group. Infıltrating cells dem-

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onstrated a significant upregulation of surface PD-L1 expression. Both F4/80+CD11b+ cells and CS11c+ cells exhibited higher tumor infiltration and elevated PD-L1 expression as compared to infiltrating immune cell in the control group. Our results demonstrate that TTFIELDS treatment potentiates immunogenic cell death in cancer cells. Combining TTFIELDS with specific immune therapies such as anti-PD-1 may enhance antitumor immunity and result in increased tumor control.


Diverse chemotherapeutic agents, including Docetaxel, have been reported by us and others to specifically target myeloid-derived suppressor cells (MDSCs) which mediate tumor-associated immune repression. However, the mechanism is unknown. To find a less toxic anticancer drug, we focused on Curcumin which exhibits natural anti-oxidant and antitumor activities with low toxicity to normal cells. Here, we demonstrate that curcumin, like Docetaxel, promotes cytotoxic function against tumors and depletes MDSC populations in vivo and in vitro. In 4T1 mammary tumor-bearing BALB/c mice treated with curcumin, we observed dose-dependent tumor reductions in mice treated with curcumin compared to DMSO controls, indicating that curcumin can be an effective antitumor agent. To elucidate the mechanism, we embarked on examining different populations of myeloid-derived suppressor cells (MDSCs). Flow cytometric analysis of splenic cells from tumor bearers indicated that the antitumor effect by curcumin is due to depletion of immunosuppressive Ly6G−granulocytic (G)-MDSC lineage with selective promotion of Ly6C+ monocytic (M)-MDSC towards the CCR7+ F4/80+ M1 subset. Furthermore, the increase in M1 MDSCs correlated with greater T-cell adaptive immune response and cytotoxicity against 4T1 tumor cells. Since clearance of G-MDSCs was mediated by apoptosis as evidenced by our Annexin V staining, we hypothesized that clustin (sCLU), a potent anti-apoptotic protein, unrelated to the Bcl-2 family, may be the under-lying mechanism by which M-MDSCs and M1 cells are selected out following treatment of curcumin. Western blot analysis revealed that sCLU was indeed, differentially expressed in M1-MDSCs but not in G-MDSCs. Antisense-CLU transfection into M-MDSCs reduced chemoresistance not only to curcumin but also to Docetaxel, confirming the critical involvement of CLU in selective survival of M-MDSCs. Using both curcumin and Docetaxel we confirmed that sCLU works by binding Bax, preventing its translation to the mitochondria to initiate apoptosis. For clinical relevance, we examined human breast cancer samples and noted that the tumor microenvironment is replete with immature CD33+ MDSCs which lack sCLU expression, unlike infiltrating mature CD68+ macrophages which possess high sCLU expression. Our results taken together indicate that selective survival of M-MDSCs under chemotherapy dictates their survival advantage, allowing for maturation into M1 cells and promotion of adaptive T cell immunity against cancer.

#3667 Promotion of immunogenicity using epigenetic modulation and immune checkpoint inhibition in mouse models of breast cancer. Evanthia T. Rousos Torres, Hayley Ma, Brian Christmas, Todd Armstrong, Elizabeth M. Jaffe, François Fauteux. National Research Council of Canada, Ottawa, ON.

Checkpoint inhibition is a very successful treatment strategy in cancers that are naturally immunogenic by attracting T cells into the tumor microenvironment (TME) and promoting cytotoxic signaling pathways. While this strategy has shown some efficacy in metastatic breast cancer, most breast cancers are not highly immunogenic likely due to an immunosuppressive microenvironment and a lack of a tumor antigen expression and recognition. One strategy to transform the breast TME into one that is immune responsive, is to use epigenetic modulation to expose tumor antigens, promote cytokine production, thus, attracting inflammatory cells into the tumor. Epigenetic modulation also has the potential to affect activation and trafficking of myeloid derived suppressor cells (MDSCs), known to alter the immunogenicity of the TME and thus, sensitizing tumors to checkpoint modulation. We hypothesize that combinatorial therapy primes the TME by increasing immunogenicity via alteration of T cells and MDSCs. We also hypothesize that combination therapy will significantly affect immune modulatory pathways. Alterations in these could serve as a functional read out of combinatorial therapy. We are using a Her-2/neu (neu-N) mouse model which through the use of various syngeneic cell lines has the capability to inform the response of Her2 and triple negative subtypes of breast cancer to immunotherapy. This model enables us to study the efficacy of different combinations of an epigenetic agent, the histone deacetylase inhibitor entinostat, and checkpoint inhibitors anti-CD28 programmed cell death protein (PD1) and anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA4) antibodies, on tumor growth and metastatic progression, and to help identify co-stimulatory and inhibitory factors regulating T cell and MDSC responses. Characterization of tumor infiltrating lymphocytes and their functional capabilities are being investigated in primary tumors and metastases using fluorescence-activated cell sorting and immunohistochemistry. Thus far, there is a significant improvement in survival and delay in development of lung metastasis as well as with anti-PD1 in combination with Entinostat. Preliminary results also suggest that epigenetic modulation with entinostat in combination with checkpoint inhibition improves the immune response within these tumors as evidenced by increased CD8+/T- regulatory cell infiltration, and increased mono-cytic MDSCs within the TME. We are ongoing survival studies evaluating this combination therapy in mice inoculated with triple negative breast tumor cells. Future studies will further delineate the mechanisms by which epigenetic therapy alters the function of the inflammatory response to immunotherapy.

#3668 Oncolytic adenoviruses expressing OX40L or GITR1 immune modulators show antitumor effect on immune-competent mouse breast cancer models. Francisco W. Puerta Martinez, Yisel A. Rivera, Teresa Nguyen, Xuejun Fan, Jared M. Henderson, Shifat Rehnuma, Mohammad B. Hossain, Hong Jiang, Juan Fuego, Candelaria Gomez Manzano. MD Anderson Cancer Center, Houston, TX.

Metastasis of advanced stage cancers remains as the main cause of morbidity and mortality in oncologic patients. Metastatic cancers, especially those that metastasize to the brain, are generally resistant to conventional therapies. Thus, more innovative and efficacious therapies are urgently needed. Therapeutic goals are the specific targeting of malignant cells, shrinkage of stabilized tumors, prevention and/or eradication of metastases and, ultimately, induction of a specific anti-tumor immune response. In this study we tested the efficiency of a treatment regimen consisting of oncolytic adenoviruses combined with specific immune regulators to prevent tumor progression and metastasis. In order to do so, mouse metastatic breast cancer cells 4T1 or 66c14, were orthotopically implanted in female BALB/c mice. The resulting primary tumors were treated with multiple doses of third generation adenoviruses targeting different immune checkpoints, such as, OX40/OX40L and GITR/GITR1 pathways in the immune synapse. The treatment with these adenoviral constructs resulted in T cells activation and reduction of the metastases in 50% of the mice. In addition, the size and number of the metastases detected in lungs were significantly lower comparing with those observed in the control groups. Survival rates were also significantly different (P < 0.046) when compared to controls. FACS analysis of isolated tumor-infiltrating lymphocytes, as well as, splenocytes showed that OX40/OX40L or GITR/GITR1 immune therapies were able to activate and increase the number of cytotoxic T cells and suppress tumor anergy. In summary, we demonstrated for the first time that Delta-24-RGDox and Delta-24-GREAT, expressing OX40L and GITR1, respectively, display antitumor effect on immune competent models of metastatic breast cancer.

#3669 Screening platform for development of antibody-drug conjugates against novel targets at the National Research Council of Canada. Maria Luz Jaramillo,1 Luc Meury,1 Patrice Bouchard,1 Allan Matte,1 Anne Marcil,1 Mauro Acchione,1 Jennifer Hill,2 Francois Fauteux.1 National Research Council, Montréal, Quebec, Canada; 2National Research Council, Ottawa, Ontario, Canada.

One of the most promising of the next generation of biologic-based cancer therapeutics builds on the molecular targeting abilities of antibodies by combining them with drugs to generate highly specific antibody-drug conjugates (ADCs). However, the development of ADCs requires time-consuming selection of the antibody for every target and cancer type. High-throughput screening technologies based on the use of conjugated secondary antibodies provide a fast and efficient surrogate assay from which to identify which antibodies are best internalized and suitable for immunono conjugate development into ADCs. As part of its integrated antibody development initiative, NRC has isolated and characterized anti- mouse Fc and anti-human Fc monoclonal antibodies to serve as very selective detective reagents for various IgG isotypes. We have shown that these secondary antibodies are species specific, selective and of high affinity. When conjugated to pH sensitive fluorophores, we have used them to specifically identify internalizing antibodies against tumor targets, which were later validated as ADCs. Furthermore, these secondary conjugates exhibit high specific potency and low background toxicity once conjugated to linker drugs. This approach allow us to optimize the selection of an antibody for a particular target, tumor type, linker and drug for ADC development. NRC will present results of a screen of 285 mouse antibodies against 20 different targets in 7 different cancer cell lines, using either MCC-DM1 or vc-MMAE-conjugated secondary antibodies. The NRC ADC discovery platform is combining this...
methodology with our proprietary mRNA and DNA expression database for the selection of appropriate ADC targets. NRC Biologics and Biomanufacturing program is in the process of screening thousands of NRC antibodies generated against a variety of cancer-associated cell surface targets to deliver a steady pipeline of ADCs as part of its drug discovery efforts. This functional screening platform further promotes the integration and advancement of NRC’s capabilities and strengths in the area of biologic-based therapeutics lead candidate selection, including quality attributes and characterization and biomanufacturing. The combined expertise in cell biology, high throughput screening, antibody generation and selection, bioinformatics and expression analysis forms the foundation by which NRC can establish strategic collaborations with other Canadian or international partners to develop antibodies into novel ADC biologics.

#3670 Treatment of epithelial ovarian cancer with folate receptor (α/β) targeted chemotherapy is enhanced by CTLA-4 blockade: Learning from animal models. Yingjuan Lu, Theresa P. Johnson, Leroy W. Wheeler, Alex M. Lloyd, Vicky A. Cross, Elaine M. Westrick, Nikki L. Parker, Christopher P. Leamon. Endocytosis, West Lafayette, IN.

Epithelial ovarian cancer (EOC) remains to be the most lethal form of gynecologic malignancies despite aggressive cytoreductive surgery (i.e. debulking) followed by standard chemotherapy. The alpha folate receptor (FRα) has long been recognized as a tumor target for EOC due to its frequently intense overexpression (>80%) in the most common serous histotype. Recently, the beta folate receptor (FRβ) has been discovered on tumor-associated macrophages (TAMs) which are present at various densities in solid tumors but are also found abundant in malignant ascites of EOC patients. The ascites TAMs are strongly immunosuppressive, and high TAM frequency is correlated with poor patient prognosis and treatment resistance. Respectively, tumor- and TAM-associated FRα and FRβ are both functionally active which allows for folate-targeted delivery of small-molecule drug payloads via receptor-mediated endocytosis. Using flow cytometry, we characterized total ascites cells harvested from human IGROV1 xenograft (hFRα) and murine ID8-CL15 syngeneic (mFRα) models at the time of euthanasia. Approximately 70% of IGROV1 and 25% of ID8-CL15 ascites TAMs (F4/80+/CD11b+) were found to express a functional mFRβ. While ascites fluids from both models caused a dramatic reduction of FRα functionality on tumor cells in vivo and ex vivo, FRβ expression and functionality on ascites TAMs were largely unaffected. Interestingly, in the ID8-CL15 model, the ratio of ascites CD8+ T cells to CD4+ T cells decreased as total FR functionality on TAMs increased. Two small-molecule folate-drug conjugates with different mechanisms of action showed activity in extending the survival of mice surviving in the IR+anti-PD-1 group (p = 0.657). Conclusions: Anti-PD-1 mAbs potentiates the radiation-induced pneumonitis, likely mediated by increased CD8+ cytotoxic T lymphocytes. Anti-PD-1 mAbs may increase the radiation-related mortality although it was not statistically significant at the day 120 following IR. Care should be taken for excessive lung toxicities during the clinical trials of combination of anti-PD-1 with radiotherapy. We will collaborate with NRG to further investigate clinical lung toxicities from combining thoracic radiotherapy with Nivo in the ongoing RTOG 3505 though analyzing the collected biospecimens.

#3672 Semaphorin 4D in human head & neck cancer: A promising predictive biomarker for the peri-tumoral stromal phenotype. Rania H. Younis,1 Roshanak Derakhshandeh,2 Ahmed Sultan,1 Haiyan Chen,1 Kyu Lee Han,1 Tonya J. Webb.1 1Univ. of Maryland School of Dentistry, Baltimore, MD; 2Univ. of Maryland School of Medicine, Baltimore, MD.

Semaphorin 4D in human head and neck cancer: A promising predictive biomarker for the peri-tumoral stromal phenotype The search for biomarkers that can predict the tumor stromal phenotype in cancer patients is a challenge in the field. Semaphorin 4D (Sema4D), known for its various effects in the nervous, vascular and immune system, plays an important role in regulating pro- and anti-inflammatory responses. It is over-expressed in Head and Neck cancer (HNC) as well as in other epithelial malignancies. We have recently described that HNC-associated Sema4D modulates the inflammatory profile to an immune-suppressive phenotype by inducing the expansion of myeloid derived suppressor cells. The purpose of this study was to determine the prognostic value of Sema4D as a biomarker for immune suppression in human HNC tissue and sera. Immunohistochemistry showed Sema4D+ve high expression in patients with Stage III/IV disease (p = 0.014), and nodal metastasis (p = 0.117). Sema4D+ve high tumors correlated directly with dense fibrotic peri-tumoral stroma (p = 0.0001) and inversely with tumor-associated inflammatory cells (TAs) (p = 0.0006). Knockdown of Sema4D in HNC cell lines resulted in significant reduction of TGF-β1 production (p = 0.001) and Sema4D+ve high tumor cells inversely correlated with the programmed death ligand 1 (PD-L1) positive tumors. Finally, Sema4D was detected in sera of HNC patients at higher levels compared to healthy donors (p < 0.0001). In conclusion, we present a novel Sema4D+ve high HNC tumor phenotype; with dense fibrotic peri-tumoral stroma. We also present Sema4D as a diagnostic biomarker in sera of HNC patients that can also be a promising predictor of the tumor stromal phenotype. This is of clinical interest as a predictive biomarker and a promising target for co-inhibition to sensitize patients for standard immunotherapy. [1-4] References: 1. Younis RH, Han KL and Webb TJ. Human Head and Neck Squamous Cell Carcinoma—Associated Semaphorin 4D Induces Expansion of Myeloid-Derived Suppressor Cells. J Immunol. 2016; 196(3):1419-1429. 2. Basile IR, Castille RM, Williams VP and Gutkind JS. Sema4D provides a link between axon guidance processes and tumor-induced angiogenesis. Proc Natl Acad Sci U S A. 2006; 103(24):9017-9022. 3. Gajewski TF. The Next Hurdle in Cancer Immunotherpay: Overcoming the Non-T-Cell-Infamed Tumor Microenvironment. Semin Oncol. 2015; 42(4):663-671. 4. Fisher TL, Reilly CA, Winter LA, Pandina T, Jonason A, Scrivens M, Balch L, Torno S, Seils J, Mueller L, Hinner R, Klimatcheva E, Pandina T, Jonason A, Scrivens M, Balch L, Torno S, Seils J, Mueller L, Hinner R, Klimatcheva E, Pandina T, Jonason A, Scrivens M, Balch L, Torno S, Seils J, Mueller L, Hinner R, Klimatcheva E. Semaphorin 4D provides a link between axon guidance processes and tumor-induced angiogenesis. Proc Natl Acad Sci U S A. 2006; 103(24):9017-9022. 4. Fisher TL, Reilly CA, Winter LA, Pandina T, Jonason A, Scrivens M, Balch L, Torno S, Seils J, Mueller L, Hinner R, Klimatcheva E, Pandina T, Jonason A, Scrivens M, Balch L, Torno S, Seils J, Mueller L, Hinner R, Klimatcheva E. Semaphorin 4D provides a link between axon guidance processes and tumor-induced angiogenesis. Proc Natl Acad Sci U S A. 2006; 103(24):9017-9022.

#3671 Anti-PD-1 treatment may potentiate the radiation-induced lung injury. Jianxin Xue, Shisuo Du,1 You Lu,1 Adam Dicker,1 Bo Lu,1 Thomas Jefferson Hospital, Philadelphia, PA; 2West China Hospital, Sichuan University, Chengdu, China.

Purpose: Combination of radiation therapy and anti-PD1 immunotherapy has been investigated both in the lab and in the clinic. Pneumonitis is a rare but potentially fatal toxicity of anti-programmed death-1 (PD-1) monoclonal antibodies (mAbs). The purpose of our study is to address whether anti-PD-1 mAbs will potentiate radiation-induced lung toxicity and mortality in a murine model using Small Animal Radiation Research Platform (SARRP) for lung-targeting irradiation (IR). Methods: Both lungs of male C57bl/6 mice were targeted for 20 Gy using the SARRP. Mice were strated into 4 treatment groups receiving IgG, anti-PD1, IR + IgG, or IR + anti-PD1. IgG or anti-PD-1 mAbs administered via i.p. injection, with a dosage of 10mg/kg, twice per week for five doses. Acute lung injury was assessed by H&E staining and flow cytometry to measure CD4+ or CD8+ positive T lymphocytes. A duplicate study (n=10) was performed to determine long-term survival following lung irradiation. Results: 30 days following lung irradiation, lung tissues exhibited abnormal alveoli, with exudates and inflammatory cells in the alveolar septa (H&E staining). The extent of these changes was more prominent in IR + anti-PD-1 group. Moreover, there were significantly (2.1 fold increase; p < 0.05) more CD8+ cytotoxic T lymphocytes, rather than CD4+ cells lymphocytes, in the irradiated lung tissues in the group of IR + anti-PD1 compared to that of IR + IgG. Up to 120 days post IR, 90% mice survived in the group of IR + IgG while 70% mice survived in the IR +anti-PD-1 group (p = 0.657). Conclusions: Anti-PD-1 mAbs potentiates the radiation-induced pneumonitis, likely mediated by increased CD8+ cytotoxic T lymphocytes. Anti-PD-1 mAbs may increase the radiation-related mortality although it was not statistically significant at the day 120 following IR. Care should be taken for excessive lung toxicities during the clinical trials of combination of anti-PD-1 with radiotherapy. We will collaborate with NRG to further investigate clinical lung toxicities from combining thoracic radiotherapy with Nivo in the ongoing RTOG 3505 though analyzing the collected biospecimens.


Background. - 4-1BB (CD137) is a key costimulatory immunoreceptor and a highly promising therapeutic target in cancer. To overcome toxicity and efficacy limitations of current 4-1BB targeting antibodies, we have developed PRS-343, a 4-1BB/HER2 bispecific based on Anticalin™ technology. We have previously reported on the generation and characterization of PRS-343 with regard to preclinical proof-of-concept and basic drug-like properties. Here, we describe the preclinical dataset supporting initiation of a first-in-patient trial. Methods and Results. The pharmacology of PRS-343 was investigated by ex vivo assays based on mixed culture of human PBMC and tumor cell lines. We find that 4-1BB costimulated T cells prominently increase production of IL-2, GM-CSF, TNF-α and IFN-γ. Using a set of immortal cancer cell lines spanning a range of HER2 surface copy numbers, we identified a copy number threshold above which PRS-343 reliably elicited T cell costimulation with a high potency and an EC50 in the subnanomolar range. PRS-343 was well tolerated in a repeat-dose study in cynomolgus monkeys, with no overt toxicity and no significant drug-related toxicity observed.
toxicological findings. Pharmacokinetic assessment confirmed dose-proportional exposure of the animals during the course of the study. In a mouse model of human PBMC-induced xenograft vs-host disease (xGvHD), PRS-343 did not show an acceleration of xGvHD development, in contrast to a 4-1BB targeting benchmark. Again utilizing ex vivo assays, we found no PRS-343 induced T cell costimulation. This result was not unexpected as the primary HER2 gene alteration targeted by HER2 is insufficient for activation. In a cytokine release assay, proinflammatory cytokine induction by PRS-343 in the absence of a primary TCR stimulation was negligible. Conclusion. The ex vivo experiments described indicate that HER2 expression level is expected to be a reliable marker for patient stratification for PRS-343. The toxicology assessment of PRS-343 indicates that the bioactivity and safety profile of the compound is suitable for clinical development. HER2 positive tumors. This is in agreement with in vivo mouse model data showing that PRS-343 leads to tumor-localized CD8+ T cell expansion, and supports the potential of PRS-343 as a efficacious yet well tolerable 4-1BB costimulating agent in the treatment of cancer. Antitumor effects on local and distant metastatic tumors generated by an anti-HER2 antibody were observed in a higher ratio of effector-to-suppressor cells in the tumor microenvironment. Furthermore, local irradiation, although inducing Treg cells, can eventually increase the expression of HMGB-1 in apoptotic tumor cells and stimulate DC maturation. Therefore, in an attempt to understand these first steps of immune reaction, leukocyte migration, cytokines synthesis and monocyte phagocytosis promoted by the vaccine components were studied in vitro. Methods: Cell migration of healthy donors’ leukocytes or mononuclear cells (MN) towards vaccine components were studied in vitro. Results: Tumor-bearing mice treated with local RT (6Gy twice per week) and the CTGF/E7 DNA vaccine exhibited a dramatic increase in the number of E7-specific CD8+ cytotoxic T cell precursors and anti-E7 Abs from Tumor-bearing mice treated with local RT (6Gy twice per week) and the CTGF/E7 DNA vaccine were evaluated. Abscopal effects were also monitored. HMGB-1 and DC maturation from local irradiation were analyzed by flow cytometry. The ratio of effector-to-suppressor cells in the tumor microenvironment was also investigated. Results: Tumor-bearing mice treated with local RT (6Gy twice per week) and the CTGF/E7 DNA vaccine exhibited a dramatic increase in the number of E7-specific CD8+ cytotoxic T cell precursors, higher titers of anti-E7 Abs, a significant reduction of tumor size, and longer survival. The combination of local RT and the CTGF/E7 vaccine also elicited abscopal effects on non-irradiated local subcutaneous and distant pulmonary metastatic tumors. Local irradiation can induce the expression of HMGB-1 in apoptotic tumor cells and stimulate DC maturation, which results in inducing antigen-specific immune responses. In addition, local irradiation, although inducing Treg cells, can eventually increase a higher ratio of effector-to-suppressor cells in the tumor microenvironment. Conclusions: Local irradiation can enhance the antigen-specific immunity and antitumor effects on local and distant metastatic tumors generated by an antigen-specific DNA vaccine. Irradiation combined with antigen-specific immunotherapy is a potential new strategy for cancer therapy.

#3674 Local irradiation promotes antitumor immunity and abscopal effect of antigen-specific immunotherapy by enhancing dendritic cell maturation. Ming-Cheng Chang, Jason Chia-Hsien Cheng, Ying-Cheng Chiang. National Taiwan Univ. Hospital, Taipei, Taiwan.

Background: Ionizing radiation therapy (RT) is a well-established method to eradicate locally advanced tumors. Whether or not local RT can enhance the potency of an antigen-specific DNA vaccine and the possible underlying mechanism were evaluated. Materials and Methods: The number of E7-specific CD8+ cytotoxic T cell precursors and anti-E7 Abs from Tumor-bearing mice treated with local RT (6Gy twice per week) and the CTGF/E7 DNA vaccine were evaluated. Abscopal effects were also monitored. HMGB-1 and DC maturation from local irradiation were analyzed by flow cytometry. The ratio of effector-to-suppressor cells in the tumor microenvironment was also investigated. Results: Tumor-bearing mice treated with local RT (6Gy twice per week) and the CTGF/E7 DNA vaccine exhibited a dramatic increase in the number of E7-specific CD8+ cytotoxic T cell precursors, higher titers of anti-E7 Abs, a significant reduction of tumor size, and longer survival. The combination of local RT and the CTGF/E7 vaccine also elicited abscopal effects on non-irradiated local subcutaneous and distant pulmonary metastatic tumors. Local irradiation can induce the expression of HMGB-1 in apoptotic tumor cells and stimulate DC maturation, which results in inducing antigen-specific immune responses. In addition, local irradiation, although inducing Treg cells, can eventually increase a higher ratio of effector-to-suppressor cells in the tumor microenvironment. Conclusions: Local irradiation can enhance the antigen-specific immunity and antitumor effects on local and distant metastatic tumors generated by an antigen-specific DNA vaccine. Irradiation combined with antigen-specific immunotherapy is a potential new strategy for cancer therapy.

#3675 Novel immune oncology strategy for targeted cytotoxic lymphocyte activation. Eric Lazier,1 Sarah Hein,2 John Westwick,2 Dan Watkins,2 Alexander J. Mulrain,2 Ming-Cheng Chang, Jason Chia-Hsien Cheng, Ying-Cheng Chiang. National Taiwan Univ. Hospital, Taipei, Taiwan.

In clinical trials (CASVAC-0401, NCT01729663), cutaneous melanoma (CM) patients (pts) stages II/III were randomized to receive CSF-470 vaccine or IFN-α (21). Vaccine-CSF-470 is composed of four locally-irradiated allelic CM E7 mRNA + BCG (bacille Calmette-Guérin) and rhGM-CSF were used as adjuvants. At the end of the Phase II part of the study (30 pts), a higher distant-metastases-free-survival was observed in vaccinated pts. All vaccinated pts developed inflammation at the vaccination site. Therefore, in an attempt to understand these first steps of immune reaction, leukocyte migration, cytokines synthesis and monocyte phagocytosis promoted by the vaccine components were studied in vitro. Methods: Cell migration of healthy donors’ leukocytes or mononuclear cells (MN) towards vaccine components were assayed in 0.5 μm transwells. Cytokines were measured by ELISA in supernatants of CSF-470 vaccine and MN (1:1, 0.5×10^6 each) cultures. For phagocytosis assays, CM cell lines were labeled with PKH67 lipophilic dye, irradiated, and cocultured with purified monocytes. After 24 hs, cultures were stained with CD14 and double staining (CD14+ PKH67) was analyzed by flow cytometry. Results: In migration assays, CSF-470 vaccine induced higher attraction of leukocytes as compared to culture medium (p=0.003, unpaired-T-test). GM-CSF exerted little attraction, although combined with BCG it increased the migration triggered by CSF-470 (vaccine vs vaccine plus adjuvants, p=0.034, unpaired-T-test). When using MN, CSF-470 alone or plus adjuvants, highly attracted monocytes, which represented 10%-65% of the migrating CD45+ population (before migration 6-15%) (p<0.0001, paired-T-test). In the lymphocyte population, natural killer cells were mainly attracted towards the vaccine either with or without adjuvants, representing 12%-33% of total migrated lymphocyte population (before migration 4-25%) (p<0.002, paired-T-test). Regarding cytokine production, 6 hs cocultures of CSF-470 vaccine plus adjuvants MN, promoted CM E7 mRNA (385±217 pg/ml), IFNα release was detected in CSF-470-MN cocultures after 48 hs (109.2 pg/ml). BCG appears to be the most important adjuvant for production of these proinflammatory cytokines. IL-10, but not IL-12p70, was also detected. Interestingly, approximately 60% of monocytes phagocytosed CSF-470 cells after 24 hs of coculture, either in the presence or absence of adjuvants. Conclusions: Our in vitro results suggest that recruitment of leukocytes, mostly from the innate immune system, is an early event after CSF-470 vaccine + BCG + GM-CSF interaction with immune cells. Also, early release of TNFα and IFNγ proinflammatory cytokines and efficient vaccine phagocytosis by monocytes would favor subsequent antigen processing and presentation. Further experiments will be performed to address the initial steps triggered after CSF-470 vaccination.
#3670 Cancer-killing viruses combined with tumor-targeting immune checkpoint modulation elicits an in situ vaccination effect and expansion of tumor-specific T cells responsible for efficacious systemic anti-cancer activity. Hong Jiang, Andrew Dong, Yisel Rivera-Molina, Karen Clise-Dwyer, Xuexun Fan, Francisco W. Martinez, Teresa Nguyen, Verlene Henry, Caroline Carillo, Candelaria Gomez-Manzano, Juan Fuego. UT MD Anderson Cancer Ctr., Houston, TX.

Oncolytic viruses are cancer-selective and disrupt immunosuppression within the tumor, but they show suboptimal efficacy in patients. Immune checkpoint modulation is efficacious in a variety of cancers but is associated with nonspecific T-cell activation and a limited effect in tumors with a nonimmunogenic microenvironment. We hypothesized that combining these two strategies likely resulted in both efficacious and specific cancer therapy. Therefore, we constructed oncolytic adenovirus Delta-24-RGDox expressing the immune co-stimulator OX40L and tested its activity in orthotopic GL261-C57BL/6 glioma and B16-C57BL/6 melanoma mouse models. Compared to its predecessor Del-ta-24-RGD, Delta-24-RGDox was more effective to induce inflammatory activation within the tumors, enhanced the capability of the tumor cells to directly activate cancer-specific T cells and the proliferation of the cell population through OX40L expression on the cell surface, resulting in specific anti-tumor immunity. To track the expansion and migration of tumor-specific T cells during treatment, we first injected OVA-specific, CD8+ T cells into TLL/Lu transgenic mice in the first tumor derived from B16-OVA cells, followed by Delta-24-RGDox injection in the same tumor. Monitoring the T cells with bioluminescent imaging revealed that the viral injection greatly augmented the T cell population than the PBS treatment within the tumor, and promoted the T cell migration to distant B16-OVA tumor but not to B16 tumor, suggesting local viral treatment enhanced the expansion of tumor-specific T cells and the migration of these cells to a distant tumor with the same tumor antigen. Consistently, flow cytometry analysis with OVA-tetramer staining showed that virus treatment greatly increased the frequency of OVA-specific CD8+ T cells in the local and distant tumors, peripheral blood and spleen (from high to low frequency). 70-80% cells of this cell population were CD44+ and CD62L+ that are markers for central memory T cells. Hence, this new virus was efficacious to inhibit the virus-infected tumor and distant tumor, prolong the survival of the treated mice and induce immune memory specific to the virus-infected tumor type. Importantly, intratumoral injection of Delta-24-RGDox and an anti-PD-L1 antibody synergized to reject gliomas and significantly increased survival in mice. Our data demonstrate that combining an oncolytic virus with tumor-targeting immune checkpoint modulation elicits potent in situ cancer vaccination and skews the injected tumor microenvironment from tumorigenic to immunogenic, resulting in a local expansion of the tumor-specific T cells. Moreover, this local effect is capable to extend to distant tumors, achieving specific and long-lasting therapeutic efficacy.

#3680 Patients derived ex vivo platform CANScript™ predicts distinct therapeutic outcomes to multiple PD-1 checkpoint inhibitors in single tumor biopsies. Padhma Radhakrishnan,1 Vasanthagam Sekar,2 Nilesh Brijwani,2 Priyanka Chevour,2 Babu Balakrishnan,2 Dency D Pinto,3 Mathusamy Oliyarasi,2 Debapriya G. Mehrotra,2 Manjusha Biswas,2 Sabitha K S,4 Kovanavan Thiyagarajan,2 Biswanath Majumder,2 Aaron Goldman1.

Background: Emerging clinical evidence using immunotherapy in recent years has demonstrated its power to suppress tumor growth by releasing the brakes on the immune system. For example, blockade of immune checkpoints, such as PD-1, has revolutionized treatment options for patients with aggressive cancers such as head and neck squamous cell carcinoma (HNSCC). However, clinical responses to PD-1 inhibition vary widely among patients while majority of them do not show any anti-tumor response. Multiple FDA-approved drugs against the same immune checkpoints have resulted in globally distinct outcomes in the clinic. There is a huge unmet need to understand these disparities at the individual patient level and to maximize the clinical benefits of these agents. Methods: Here, we employed a patient-derived ex vivo model, CANScript™ (Majumdar B et al. Nat Immun Cancer 2015 Feb 27:6:6169 and Goldman A et al. Nature Commun

Vascular Endothelial Growth Factor (VEGF) is a key factor for tumor progression through induction of angiogenesis and other actions including immune alteration. Myeloid-derived suppressor cells (MDSC) are a major type of immune-suppressing cell that appear in cancer or inflammation and that have been reported to express the VEGF receptor and be activated by VEGF. To study the clinical importance of VEGF and MDSC for cancer, peripheral blood was collected from patients with gastrointestinal, ovarian, breast, thyroid and pulmonary cancers. Peripheral blood mononuclear cells (PBMC) were separated using a Ficoll-density gradient and were used for the detection of MDSC (CD11b+CD14-CD33+) using flow cytometry and for cytokine-production assays. For these assays, PBMC were stimulated with PHA and the production of cytokines including IL-12 (Th1 inducer), IL-10, (anti-inflammatory cytokine) and IL-17, (pro-inflammatory cytokine), were measured over 24 h using ELISAs. Serum concentrations of IL-10 and VEGF were also measured using ELISAs. The serum levels of both VEGF and MDSC were significantly increased in almost all types of cancer tested and significantly correlated with each other. Their levels also significantly correlated with neutrophil/lymphocyte ratios (NLR) (inflammation marker) and CRP levels, and were inversely correlated with the PHA-stimulation index (SI) (cell mediated immune response marker) and serum concentrations of rapid-turnover protein (RTP) (nutrition marker). VEGF levels also correlated with serum concentrations of IL-10 and VEGF, and production of IL-17, and inversely correlated with production of IL-12. The prognosis of stage IV colorectal cancer with high VEGF was significantly worse than that with low VEGF. In thyroid cancer, the number of MDSC was significantly higher, NLR and CRP levels were higher, and RTP levels were lower in patients with undifferentiated carcinoma than in those with differentiated carcinoma including papillary and follicular carcinomas. Thus VEGF was increased in cancer and correlated with immune suppression driven by MDSC, inflammation and malnutrition. Although cancer immunotherapy is currently in use for a number of cancers, MDSC have been reported to be a major inhibitor of cancer immunotherapy even in cases in which an immune checkpoint inhibitor was used. An anti-VEGF treatment strategy has now been established in combination with chemotherapy for many types of cancer. Among various types of anti-MDSC trials, anti-VEGF treatment seems to be an effective adjuvant therapy of cancer immunotherapy.
2015 Feb 11;61(139), which recreates the native 3D tumor microenvironment, autocrine-paracrine dynamic and response to therapy by incorporating fresh tumor tissue and autologous immune cells with immunotherapy agents. Utilizing late stage HNSCC (N=50) we interrogated phenotypic response to two FDA-approved PD-1 inhibitors, Pembrolizumab (KEYTRUDA) and Nivolumab (OPDIVO). The tumor microenvironment (TME) is complex and characterized by dynamic cellular interactions and cytokine signaling that can influence immune responses. The analysis was extended to include IHC for arginase (Arg, a marker of functional immunosuppression activity), Subpopulations of cells identified included CD163+, CD163+/H11001, CD163+/H11545, CD163+/H11290, CD163+/H11538, CD163+/H11215, and Arg+/H11001. t-test one-sided. *p < 0.05, **p < 0.01, ***p < 0.001. N = 6 mice per group.

#3684 Inhibition of STAT3 by antisense oligonucleotide treatment decreases the immune suppressive tumor microenvironment in syngeneic and GEM tumor models.

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AZD9150, a gen2.5 antisense oligonucleotide (ASO) targeting human STAT3, has improved drug-like properties compared to previous generation ASO therapeutics, including increased stability and resistance to nucleases, reduced proinflammatory effects, and enhanced potency. We have previously reported that in tumors, STAT3 ASOs are taken up preferentially in stromal and immune cells within the tumor microenvironment (TME). Since AZD9150 is selective for human STAT3, we used a surrogate ASO (muSTAT3 ASO) to explore the pharmacodynamics of ASO-mediated STAT3 inhibition in syngeneic and genetically engineered mouse (GEM) tumor models, focusing on effects in the TME. In mice bearing subcutaneous CT-26 tumors, treatment with muSTAT3 ASO at 50 mg/kg, i.c., on a qx5/5 wk schedule decreased STAT3 levels in immune cells in the tumor and in circulating leukocytes by 40 - 60%, similar to the decrease in STAT3 ASO achievable in circulating leukocytes in human patients after AZD9150 treatment. In a Nanostring analysis (nCounter mouse immunology panel) of CT-26 tumors from muSTAT3 ASO treated mice, CD163+ (M2 macrophage/microglia marker) was the gene most consistently and significantly downregulated, by an average of 84% in three independent experiments, and was confirmed by immunohistochemistry (IHC). Flow cytometry analysis of myeloid subpopulations - tumor associated macrophages (F4/80+ TAMs), monocyte myeloid derived suppressor cells, and granulocytic cells - showed a decrease in TAMs averaging 69% across three independent experiments. The analysis was extended to include IFNγ for arginase (Arg, a marker of functional immunosuppression activity). Subpopulations of cells identified included CD163+, CD163+/H11001, and Arg+ cells. These cells decreased these populations by 79%, 88% and 97% respectively, compared to control treatment. These populations were also analyzed in two GEM tumor models - the KPC pancreatic cancer model, and a PTEN -/- prostate cancer model - which have a TME more representative of that found in tumors in the clinic. While the specific changes varied across the models, likely reflecting differences in the TME immunology, in general, the observation that STAT3 ASO decreased these populations by 79%, 88% and 97% respectively, compared to control treatment. These populations were also analyzed in two GEM tumor models - the KPC pancreatic cancer model, and a PTEN -/- prostate cancer model - which have a TME more representative of that found in tumors in the clinic. Overall, these results suggest that selective STAT3 inhibition can reduce immune suppressive cell populations in the TME, and suggest that STAT3 inhibition has the potential to enhance the antitumor activity of other tumor antigens released during radiotherapy and improve the presentation of antigens to professional antigen presenting cells (APCs). Methods: We developed several types of antigen-capturing nanoparticles (AC-NPs) using poly (lactic-co-glycolic acid) (PLGA), a biocompatible and biodegradable polymer. The surfaces of nanoparticles were

#3683 Melphalan stimulates dendritic cell and CD8+ T cell expansion by inducing immunogenic cell death in melanoma cells.

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Background: Regional hyperthermic perfusion with the alkylating agent melphalan is a treatment option for patients with metastatic melanoma confined to the limbs or the liver. Following a single perfusion, tumors often decrease gradually in size during several months, suggesting an immune-mediated mechanism of action, in addition to the direct cytotoxic effects of melphalan. This study was designed to characterize the immunogenic effects of melphalan. Materials and methods: We have established an in vitro model of regional hyperthermic perfusion where human melanoma cell lines are exposed to melphalan at 40°C for 1 h, thus mimicking the currently employed clinical protocol. The melanoma-exposed melanoma cells were analyzed for markers of immunogenic cell death and were co-cultured with peripheral blood mononuclear cells (PBMCs) in the presence or absence of IL-2. The number and activation status of various immune populations were analyzed by flow cytometry. Results: Melphalan exposure triggered the expression of several immune-related markers on melanoma cells, including calreticulin, MHC class I, Hsp70 and PD-L1. Melphalan-treated, but not untreated melanoma cells, triggered an increase in dendritic cell (DC) numbers along with a dramatic expansion of CD8+ T cells in co-cultured PBMCs. The expanded CD8+ T cells showed an activated phenotype with the majority of cells belonging to the effector memory subtype. Conclusions: The tumor cells induced immunogenic cell death and trigger DC expansion with subsequent expansion and activation of CD8+ T cells. We propose that these events may contribute to the antitumor efficacy of regional hyperthermic perfusion with melphalan in metastatic melanoma.

#3682 Synergistic immunostimulatory effects and therapeutic benefit of combined histone deacetylase and bromodomain inhibition in non-small cell lung cancer.

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Effective therapies for non-small cell lung cancer (NSCLC) remain challenging despite an increasingly comprehensive understanding of somatically altered oncogenic pathways. It is now clear that effective agents with potential to impact the tumor immune microenvironment potentiate immune-orchestrated therapeutic benefit. This study evaluated the immunoregulatory properties of two classes of drugs that modulate the epigenome, histone deacetylase (HDAC) and bromodomain inhibitors with a focus on key cell subsets that are engaged in an immune response. By evaluating human peripheral blood and NSCLC tumors, we show that the selective HDAC6 inhibitor ricolinostat promotes phenotypic changes associated with enhanced T-cell priming and function of antigen presenting cells. The bromodomain inhibitor IQ1 attenuated CD4+ Tfh3+ T regulatory cell suppressive function and synergized with ricolinostat to facilitate immune-mediated tumor growth arrest, leading to prolonged survival of mice with lung adenocarcinomas. Collectively, our findings highlight immunomodulatory effects of two epigenetic modifiers that together promote T-cell-mediated anti-tumor immunity and demonstrate their therapeutic potential for NSCLC treatment.

#3685 Antigen-capturing nanoparticles improve the abscopal effect and cancer immunotherapy.

Yuanzeng Min. USC Chapel Hill, Chapel Hill, NC.

Introduction: Cancer immunotherapy, the utilization of patients’ own immune system to treat cancer, has emerged as a powerful new strategy in cancer treatment. The main limitation of this strategy is the low long-term durable response rate. Therefore, there has been high interest in developing strategies to further improve cancer immunotherapy. We hypothesized that antigen-capturing nanoparticles (AC-NPs) could improve immune responses to checkpoint inhibitors. The NPs can induce the abscopal effect by capturing tumor antigens further improving cancer immunotherapy. We hypothesized that antigen-capturing nanoparticles improve the abscopal effect and cancer immunotherapy. We hypothesized that antigen-capturing nanoparticles improve the abscopal effect and cancer immunotherapy.
modified to enable capturing tumor antigens released after radiotherapy. Unmodified PLGA NPs absorb antigens via hydrophobic-hydrophobic interactions. AC-NPs with positive charged bind antigens through ionic interactions. AC-NPs having maleimide group on the surface (Mal AC-NP) bind to antigens by reaction to thiols groups of antigens. The B16-F10 tumor, a syngeneic mouse melanoma model on C57BL/6 mice was employed to evaluate the efficacy of AC-NPs to improve immune responses of checkpoint inhibitor combining radiotherapy. Results: The in vivo efficacy study showed that AC-NPs can significantly improve the efficacy of oPD-1 treatment in the B10F10 melanoma model, which generates up to 20% cure rate as compared to 0% without AC-NP. Mechanistic studies demonstrated that the AC-NPs can drain to lymph node model, which generates up to 20% cure rate as compared to 0% without AC-NP. In the meantime, it was found that there are significant increases in CD4\(^+\) and CD8\(^+\) T cell to T, B, N ratios, which indicate an expansion of the T-helper cells and cytotoxic T cells. We also found that AC-NPs can induce increased level of antitumor cytokine interferon-\(\gamma\) (IFN-\(\gamma\)) following stimulation ex vivo, which implied that AC-NPs were able to elicit systemic T cell activation. Therefore, a novel strategy for improving cancer immunotherapy with nanotechnology was presented by our work. Acknowledgements: The authors would also like to thank our funding sources. A.W.Z., J.E.T., S.T., and J.M.D. are supported by funding from the National Institutes of Health/ National Cancer Institute (U54CA198999, Carolina Center of Cancer Nanotechnology Excellence (CCNE)-Nano Approaches to Modulate Host Cell Response to Tumor Cells). R.B.V. is supported by UNCG University Cancer Research Fund, Paul Calabresi Oncology K12 Award and UNC CCNE Pilot Grant. A.W.Z. is also supported by funding from the NIH/NCI (U54 CA151652 and R01 CA178748) for this work. A.W.Z. was also supported by funding from the NIH/NCI (R21 CA182322).

\#3686 Generation of an off-the-shelf cell-based platform (PDC-vac) for active antitumor immunotherapy, Joel Plumas,1 Kevin Lenogue,1 Alexandre Walencik,2 Jean-Paul Molens,3 Laurence Chaperot,3 Martin Pule4.

Inflammation in the Tumor Microenvironment

\#3687 Bexarotene activates type 1 antigen presenting cells, increases tumor infiltrating CD8 T-cells, and augments the anti-tumor activity of chemotherapy in breast cancer. Sasha Elizabeth Stanton, Ekram Gad, Lauren R. Corulli-Rastetter, Mary L. Disis. Univ. of Washington, Seattle, WA.

Bexarotene is an oral retinoic receptor (RXR) agonist that has anti-proliferative activity in breast cancer. RXRs are nuclear receptors that heterodimerize, including with the retinoic acid receptor (RAR), to activate transcription for processes including cellular differentiation, embryonic development, proliferation, and metabolism. We have demonstrated that bexarotene can enhance cancer vaccine efficacy by synergizing with a multi-antigen vaccine to prevent breast cancer in the TgMMTV-neu model. Bexarotene as a single agent increased CD8 T-cell tumor infiltration. We therefore questioned the mechanism by which bexarotene acts as an immunomodulatory drug. Evaluating XR expression in human peripheral blood monocytes (PBMC) (n=10), we found that XR is not expressed in NK T-cells or B-cells and in only a minority of CD4\(^+\) (5.1 \pm 4\%) and CD8\(^+\) T-cells (3.6 \pm 3\%). XR, however, is expressed in 24.9 \pm 13\% of monocytes (5.6 \pm 16\% of monocyctic dendritic cells (mDC), PMBC treated with increasing doses of bexarotene for 48 hours had increased co-stimulatory CD40 expression on mDC (p = 0.0011 between 0 and 20 um bexarotene) and increased type 1 cytokine release including IL-1\(\beta\) (p = 0.03 between 0 and 20 um bexarotene) and TNF\(\alpha\) (p = 0.02 between 0 and 20 um bexarotene) but not type 2 cytokine release of IL-10 (p = 0.21 and IL-4 (p = 0.5)). These data demonstrate that bexarotene can activate type 1 dendritic cells since activated type 1 dendritic cells are effective at presenting tumor antigens from dying tumor cells, we wanted to determine if bexarotene could increase type 1 immunity in the tumor and thereby enhance the efficacy of paclitaxel chemotheraphy. In TgMMTV-neu mice with 100 mm\(^3\) established spontaneous tumors (n=5), 50 mg/kg bexarotene inhibited tumor growth by 49\% and increased the influx of intratumoral CD8\(^+\) T-cells by 61\% as compared to PBS treated mice (p=0.01). 10 mg/kg paclitaxel inhibited tumor growth by 57\% and increased intratumoral CD8\(^+\) T-cells by 45\% as compared to PBS treated mice (p=0.003). Paclitaxel and bexarotene together inhibited tumor growth by 83\% (p=0.02 compared to bexarotene or paclitaxel alone) and increased intratumeral CD8\(^+\) T-cell infiltrate by 58\% as compared to PBS treated mice (p=0.01). These data demonstrate that bexarotene can activate type 1 dendritic cells and enhance the efficacy of paclitaxel. These data suggest concurrent administration of immunomodulatory bexarotene with paclitaxel may result in augmentation of the anti-tumor activity of chemotherapy in breast cancer.
TNF-α, functional mediators produced by these monocyte populations. Together, these data show that Imprive drives the concerted activation of multiple innate immune subtypes and promotes the appearance of unique monocyte populations that may be critical for an Imprive-induced anti-cancer immune response.

#3689 Jak3 mutations in colorectal neoplasia - Preliminary data on a not so silent minority. Martin Tob, Xiaping Zhao, Durshana Jhala, Rebecca Rodriguez, Fadi Antaki, Levi Edv, Paula Sochacki, John Lieb, MaryAnn Rambus, Tapan Ganguly, Martin Bluth, Michael J. Lawson, Saginaw VA Medical Ctr and Medical Disciplines, Central Michigan University College of Medicine, Saginaw, MI; Detroit VAMC Re-D, Central Michigan University College of Medicine, Detroit, MI; Philadelphia VAMC and the University of Pennsylvania School of Medicine, Philadelphia, PA; Philadelphia VAMC, Philadelphia, PA; Detroit VA Medical Ctr and Wayne State University School of Medicine, Detroit, MI; Detroit VA Medical Ctr, Detroit, MI; University of Pennsylvania School of Medicine, Philadelphia, PA; Wayne State University School of Medicine, Detroit, MI; Kaiser Permanente Medical Center, Sacramento, CA.

As a tumorigenesis model, colorectal cancer is associated with multiple gene mutations accumulating progressively but also has mutations that alter disease course and provide a therapeutic target. Good examples of this are EGFR and VEGF. In melanoma, PD-1 interactions involving the immunobiome are therapeutically important. We conducted a study to detect potential mutations that might enhance therapy in colorectal cancer guided by expression of p87, a product of innate immune system Paneth cells. Methods: Adnab-9 immunohistochemistry or ELISA was used to define significant p87. Adenoma-associated antigen field effects (EF) in 10 patients with >1cm large high grade dysplastic adenomas (LHiGDA) and 3 with smaller high grade dysplastic adenomas (SHiGDA). We postulated that SHiGDA are not immunologically recognized by host defenses leading to negative outcomes. We used Ion Torrent sequencing (ITS) to find mutations in DNA (QiaAmp kit) extracted from 4 normal-appearing colonic segments taken from 1 patient in each group. Novel mutations found on ITS would then be sought using PCR with appropriate primers and subsequent sequencing of the PCR product circulating DNA extracted from available serum samples. These samples were taken from the Large- and SHiGDA groups described above and from 17 patients undergoing colonscopy for diverse indications. Results: p87 FE were found in 40% of 10 LHiGDA and 0% of 3 SHiGDA patients. The ITS in the 2 representative patients showed unique mutational fields in: KRAS, APC, p53 in the LHiGDA and Jak3, PIK3Ca, p53, APC in the SHiGDA patient, both of whom lacked p87 FE. PCR using the Jak3 primers used in the ITS and subsequent sequencing revealed the same non-synonymous mutation in the serum of a FAP patient after colonic resection and an additional colonic segment of the selected ShiGDA patient but not in his serum. Other Jak3 mutations were found in 1 of 8 LHiGDA, 1 (the selected patient) of 3 SHiGDA, 1 of 7 patients with FAP and 1 of 10 colonoscopy patients with a family history of colorectal cancer with a FE and an untoward outcome. The selected SHiGDA patient subsequently contracted and died of NSCLC adenocarcinoma. The positive LHiGDA and FAP patients had a severely dysplastic anal condyloma and severe pancreatitis, respectively. Overall, the non-synonymous mutation occurring in FAP and SHiGDA patient occurred in the absence of p87 FE. Conclusions: In this pilot study we demonstrate the presence of Jak3 mutations likely associated with the lack of p87 expression in patients with high grade dysplastic adenomas and 1 FAP patient. Most of these patients had a clinical course which may have differed from their group members suggesting an altered immune system milieu. If confirmed in SHiGDA and FAP, Jak 3 mutations, associated with the SCID and late onset combined immunodeficiency, may allow for intervention with currently available medications to potentially avert a deleterious clinical outcome.

#3690 The leukocyte chemotactic chemerin modulates PTEN via CMKLRI in human tumors. Keith R. Rennier, Ping Wang, Robert Crowder, Russell K. Pachynski. Washington University School of Medicine in St. Louis, St. Louis, MO.

Background: Recent data in preclinical models has shown that phosphatase and tensin homolog (PTEN) loss correlated with decreased tumor immune cell infiltration as well as decreased response to T cell-based immunotherapy. Chemerin (RARRES2) is a recently identified endogenous leukocyte chemotactic trant into recruit innate immune cells through its G-protein coupled receptor CMKLRI. Chemerin/RARRES2 is commonly downregulated in prostate and other cancers (e.g. sarcoma) compared to their normal tissue counterpart parts. Myelohsme-wide studies in multiple tumor types have identified RARRES2 as being one of the most hypermethylated genes, potentially leading to decreased chemerin expression. Our previous preclinical studies showed that forced overexpression of chemerin in tumors was capable of recruiting immune effector cells, including T cells, into the tumor microenvironment and suppressing tumor growth. Methods: In order to study the effects of chemerin overexpression on tumor cell intrinsic processes we exposed prostate and sarcoma tumor lines to exogenous recombinant chemerin in vitro. Evaluation of PTEN was performed for both the 1920 and 1920neotg at protein level using RT PCR and Western blotting, in comparison with normal prostate epithelia RWPE-1 as well as PTEN-null PC3 cells as controls. In vitro invasion assays were performed to investigate the functional impact of chemerin exposure on tumor intrinsic activity. Knockdown of CMKLRI using siRNA was performed to determine its role in tumor response. Results: Using both prostate and sarcoma tumor cell lines, we found exogenous chemerin was able to upregulate PTEN expression at both the mRNA and protein levels in a dose-response manner. Exposure to chemerin did not result in increased apoptosis or altered in vitro proliferation. Importantly, chemerin treatment significantly decreased in vitro tumor invasion. Knockdown studies showed CMKLRI abortion resulted in restored tumor migration, suggesting a link between this GPCR and PTEN expression and activity. Conclusions: For the first time, to our knowledge, we have shown a link between chemerin and PTEN expression and activity in both prostate and sarcoma tumor lines. Our collective study shows chemerin’s ability to upregulate PTEN activity and to mitigate tumor cell migration via CMKLRI. This work has functional implications on both tumor cell intrinsic and extrinsic responses to chemerin-based immunotherapeutic strategies. Further studies are needed to investigate the interaction between chemerin and PTEN signaling, and its connection to oncogenic signaling pathways manipulated in malignant tumors. Additionally, future in vivo studies using these tumor lines will help further elucidate the chemerin-PTEN axis and its role in tumor immunosurveillance. We hypothesize that increased chemerin-driven PTEN activity may help facilitate an improved immunotherapeutic response and functional ability to attack cancer cells.


BRCA1 and BRCA2 are essential homologous recombination (HR) repair proteins; germline mutations confer elevated risk for breast and ovarian cancer. Defects in BRCA can lead to immune-related effects including depletion of peripheral T-cell pools and increased somatic mutational burden. We investigated the immunophenotypic properties of breast and ovarian cancers associated with germline BRCA1/2 mutations. We determined neoantigen load in 16 breast and 26 ovarian tumors with germline BRCA1/2 mutations and 30 non-BRCA1/2 tumors for each disease using whole exome sequencing data from the TCGA. Although neoantigen load (IC50 < 500 nm) was variable across tumors, it did not differ between BRCA1/2 mutant and non-BRCA tumors. Leveraging RNAseq data from the TCGA, we investigated immune-related transcriptomic features within each disease. Cytolytic index, a measure of PRF1 and GZMA expression, was higher in non-BRCA1/2 tumors than BRCA1/2 mutant for breast (p < 0.01) tumors and correlated more closely with neoantigen load than in non-BRCA1/2 tumors. HRD score exhibited evidence of a more inflamed phenotype than BRCA1/2 breast tumors or non-BRCA1/2 tumors. HLA expression was significantly lower (p < 0.01) among BRCA mutant breast tumors, indicating immune escape by HLA down-regulation. We classified non-BRCA breast tumors by their level of HR deficiency (HRD score), as HRD by both BRCA and non-BRCA mediated mechanisms can lead to increased somatic mutational burden. Non-BRCA breast tumors irrespective of HRD score exhibited evidence of a more inflamed phenotype than BRCA1/2 mutant tumors, indicated by higher inferred immune cell infiltration, immune checkpoint expression, and neoantigen load. In contrast, ovarian non-BRCA1/2 tumors did not differ from non-BRCA1/2 tumors. HLA expression also correlated with cytolytic index (p < 0.05) in non-BRCA1/2 tumors but not BRCA1/2 mutation associated tumors for both breast and ovarian cancers, suggesting more robust antigen-driven adaptive immunity in non-BRCA tumors. HLA expression was significantly lower (p < 0.01) among BRCA mutant breast tumors, indicating immune escape by HLA down-regulation. We classified non-BRCA breast tumors by their level of HR deficiency (HRD score), as HRD by both BRCA and non-BRCA mediated mechanisms can lead to increased somatic mutational burden. Non-BRCA breast tumors irrespective of HRD score exhibited evidence of a more inflamed phenotype than BRCA1/2 mutant tumors, indicated by higher inferred immune cell infiltration, immune checkpoint expression, and neoantigen load. In contrast, ovarian non-BRCA1/2 tumors did not differ from non-BRCA1/2 tumors. HLA expression also correlated with cytolytic index (p < 0.05) in non-BRCA1/2 tumors but not BRCA1/2 mutation associated tumors for both breast and ovarian cancers, suggesting more robust antigen-driven adaptive immunity in non-BRCA tumors. HLA expression was significantly lower (p < 0.01) among BRCA mutant breast tumors, indicating immune escape by HLA down-regulation. We classified non-BRCA breast tumors by their level of HR deficiency (HRD score), as HRD by both BRCA and non-BRCA mediated mechanisms can lead to increased somatic mutational burden. Non-BRCA breast tumors irrespective of HRD score exhibited evidence of a more inflamed phenotype than BRCA1/2 mutant tumors, indicated by higher inferred immune cell infiltration, immune checkpoint expression, and neoantigen load. In contrast, ovarian non-BRCA1/2 tumors did not differ from non-BRCA1/2 tumors. HLA expression also correlated with cytolytic index (p < 0.05) in non-BRCA1/2 tumors but not BRCA1/2 mutation associated tumors for both breast and ovarian cancers, suggesting more robust antigen-driven adaptive immunity in non-BRCA tumors. HLA expression was significantly lower (p < 0.01) among BRCA mutant breast tumors, indicating immune escape by HLA down-regulation. We classified non-BRCA breast tumors by their level of HR deficiency (HRD score), as HRD by both BRCA and non-BRCA mediated mechanisms can lead to increased somatic mutational burden. Non-BRCA breast tumors irrespective of HRD score exhibited evidence of a more inflamed phenotype than BRCA1/2 mutant tumors, indicated by higher inferred immune cell infiltration, immune checkpoint expression, and neoantigen load. In contrast, ovarian non-BRCA1/2 tumors did not differ from non-BRCA1/2 tumors. HLA expression also correlated with cytolytic index (p < 0.05) in non-BRCA1/2 tumors but not BRCA1/2 mutation associated tumors for both breast and ovarian cancers, suggesting more robust antigen-driven adaptive immunity in non-BRCA tumors. HLA expression was significantly lower (p < 0.01) among BRCA mutant breast tumors, indicating immune escape by HLA down-regulation. We classified non-BRCA breast tumors by their level of HR deficiency (HRD score), as HRD by both BRCA and non-BRCA mediated mechanisms can lead to increased somatic mutational burden. Non-BRCA breast tumors irrespective
remodeling in breast and ovarian cancer. Our work provides early evidence that BRCA1/2 breast and ovarian tumors initiate intratumoral adaptive immunity by T-cell exclusion, so that despite higher mutational burden, they may not respond to checkpoint blockade.

#3692 Long-lived pancreatic ductal adenocarcinoma slice cultures enable precise study of the immune microenvironment. Xiaoyun Jiang, David Hao, Jae Chang, Edam Nighe, Zheng Pan, Florencia Jalkis, Venu Pillarsetty. University of Washington, Seattle, WA.

Introduction: Pancreatic ductal adenocarcinoma (PDAC) remains a deadly disease that is rarely cured. Given the recent successes with immunotherapy for other malignancies and the fact that PDA is heavily infiltrated by effector T cells, we postulate that accurate modeling of the PDA immune microenvironment would allow us to unveil mechanisms of immunosuppression that could be overcome for therapeutic benefit. Methods: PDA tumors were collected from the operating room and transported to cold media to the laboratory, where 250µm slices were cut using a vibratome and placed on 0.4µm pore size membrane inserts pre-coated with collagen gel and culture. Proliferation was measured using MTT assay and weight-adjusted optical density. Slices were fixed in 4% PFA for imaging after live immunofluorescence (IF) staining or for subsequent immunohistochemistry (IHC) prior to imaging. For proteomic analysis, slices were digested with trypsin and analyzed by mass spectrometry. To assess for immune cell migration into the tumor slices, isolated autologous splenocytes were stained with CFSE and added to the slice culture and imaged using IF after 6 days.

Results: Using light microscopy, we confirmed that cultured slices maintained their baseline morphology, architecture and surface area over 9 days in culture. The MTT assay showed stable growth over the same period; IHC for Ki-67 and cleaved Caspase-3 corroborated a similar pattern of proliferation and apoptosis after 6 days culture when compared to day 1. T cells (CD3, CD8 and FOXP3), macrophages (CD68, CD163, and HLA-DR), and stromal myofibroblasts (αSMAct) were confirmed to be present through day 6 using IHC. Quantitative proteomic analysis of over 3000 proteins showed that only 2-3% of proteins showed significant changes of abundance greater than 2 fold after 6 days in culture. However, when slice were challenged with cytotoxic drugs (stauroporine and cyclohexamide), there was a time- and dose-dependent response in proliferation and apoptosis compared with controls. The demonstration of the live and dynamic microenvironment was performed first via live multicolor IF including PDA cells (EpCAM), fibroblasts (fibronectin) and immune cells (both CD11b and CD16 cells) within the microenvironment. Furthermore, co-culture of CFSE-labeled splenocytes on top of slices for 6 days. Z-stacked live confocal imaging confirmed their migration from the surface into the slice microenvironment.

Conclusion: Our study demonstrates that the PDA slice culture maintains original tumor microenvironment, and overall proteomic production and dynamic function response in vivo. PDAC slice culture model is now available to examine both pharmaceutical and cell-based immunotherapies for mechanisms of action and potential synergies, effects on specific cell types of the microenvironment, and tumor-specific immunogenic potential.

#3693 Single cell RNA-Seq of primary lymphomas reveals the diverse transcriptional states of the cancer immunologic milieu. Noemi Andor1, Erin Simonds2, Jiamin Chen1, Christina Wood1, Susan Grimes3, Debra Czerwinski1 and tumor-specific immunogenic potential. Tumor B cells consistently demonstrated downregulation of β-2-microglobulin and for those cases that harbored a founder CREBBP mutation, a downregulation of MHC II, that would cause an alteration in antigen presentation and evasion of the T cell immune system. Moreover, we characterized the transcriptional profiles of the infiltrating T-cell populations within each tumor, providing a fresh perspective of immunologic cellular interactions. Overall, we identified the diverse transcriptional states at single cell resolution among different tumors and discovered specific genes that were aberrantly expressed compared to normal B cells within the same microenvironment. Our findings provide a unprecedented resolution of distinct immune lineages as seen by transcriptionally characterized cell diversity. Studies using single cell genomics may have implications for considering immunotherapeutic efficacy.

#3694 FOXP1 suppresses immune cell migration in breast tumors. Pushpamali De Silva1, Soizic Garaud1, Roland de Wind1, Gert Van den Eynden2, Anais Boisson1, Cinzia Solinas1, Edoardo Migliori1, Hugues Duvillier1, Denis Larsimont1, Martine Piccart-Gebhart1, Karen Willard-Gallo2. 1Institut Jules Bordet, ULB, Brussels, Belgium; 2GZA Hospital Sint-Augustinus, Antwerp, Belgium.

Tumor infiltrating lymphocytes (TIL) play an essential role in mediating response to chemotherapy and improving clinical outcomes in breast cancer (BC). Extensive TIL infiltration is characterized by their organized into tertiary lymphoid structures (TLS). TIL infiltration and TLS formation may be regulated, in part, by transcription factors (TF) controlling cytokine/chemokine production within the tumor microenvironment. The forkhead box protein 1 (FOXP1) is a TF shown to be aberrantly expressed in a variety of human tumors and play an important role in T cell cytokine production. Therefore we aimed to study FOXP1-mediated regulation of TIL in BC. Investigation of FOXP1 expression in public microarray data from untreated BC patients, BC cell lines [Luminal A (MCF7), HER2+ (BT474) and triple negative (TN; MDA-MB-231)] and prospectively collected formalin-fixed paraffin-embedded (FFPE) primary breast tissues showed that FOXP1 is repressed at transcript and protein level in HER2+ or TN breast tumors compared to estrogen receptor positive tumors (Luminal A and B). Moreover HER2+ and TN subtypes, which showed decreased FOXP1 levels, are 2 well known highly infiltrated BC. Based on our hypothesis that FOXP1 could play a role on immune cell infiltration in breast tumors, data analysis of the prospective BC cohort showed that high FOXP1 (FOXP1hi) expression is significantly associated with a lower percentage of TIL and number of TLS compared to FOXP1low (FOXP1lo) tumors. To investigate the impact on specific cytokines/chemokines involved in TIL recruitment and/or TLS formation, FOXP1 was silenced in MCF7 (FOXP1hi tumor cell line) or upregulated in MDA-MB-231 (FOXP1lo tumor cell line) followed by gene expression analysis using a RT-qPCR based human cytokine/chemokine array. FOXP1 repression upregulated major T and B cell chemoattractant chemokines and overexpression repressed most of these molecules in the cell line experiments. Next we analyzed major chemoattractant molecule expression in FOXP1hi and FOXP1lo prospective breast tumors and found that FOXP1hi tumors having a significant decrease in CXCL9, CXCL10, CXCL11, CXCL13, CX3CL1, CCL20, IL2, and FOXP1 repressed tumor conditioned media (TCM) of MCF7 compared to the TCM of control or medium alone. Finally analysis of lymphocyte migration to FOXP1hi and FOXP1lo tumor supernatants (SN) from primary tumors that we consistently prepare without enzymatic digestion, showed that there was a significant decrease in number of lymphocytes migrated towards FOXP1hi tumor SN including the migration rates of individual T and B lymphocyte populations compared to FOXP1lo tumor SN. These data suggest that FOXP1 could play a critical role in establishing effective anti-tumor immune responses by negative regulation of TIL via suppression of cytokine/chemokine expression in breast tumors.

#3695 The interplay between neutrophils & It CD8+ T cells.Proceedings of the American Association for Cancer Research • Volume 58 • April 2017
cluding >650 evaluable CRC samples. Phenotypic profiles of tissue infiltrating and peripheral blood CD66b+ cells were evaluated by flow cytometry. CD66b+/CD8+ cells crosstalk was investigated by in vitro experiments. Results: CD66b+ cell infiltration in CRC is significantly associated with increased survival. Interestingly, neutrophils frequently co-localize with CD8+ T cells in CRC. Functional studies indicate that although neutrophils and T cells are independently associated with antitumour potential, co-culture with peripheral blood or tumor associated neutrophils (TANs) enhances CD8+ T cell activation, proliferation and cytokine release induced by suboptimal concentrations of anti-CD3 monoclonal antibody (mAb). Moreover, under optimal activation conditions, CD8+ cells initially stimulated in the presence of CD66b+ cells show decreased expression of PD-L1 ‘exhaustion’ marker and are significantly less susceptible to apoptosis. Their associations with the relationships between HER3 signal and PD-L1 expression by using three basal-like breast cancer cell line; MDA-MB-231, HCC70, and MDA-MB-468. MDA-MB-231 is HER3-negative, and HCC70 and MDA-MB-468 are HER3-positive cell lines. We added neurelin 1 (NRG1; HER3 ligand) to those three cell lines and analyzed PD-L1 expression of protein by flow cytometry and mRNA by qRT-PCR. Both factors and mRNA levels of PD-L1 on HCC70 and MDA-MB-468 treated with NRG1 are increased as compared with those without NRG1 while there was no change of PD-L1 expression of MDA-MB-231 either with or without NRG1. In order to confirm the significance of potential treatment target of HER3, we evaluated HER3 expression in biopsy samples by immunohistochemistry before neoadjuvant chemotherapy (NAC) including all phenotypes. Thirteen pathological complete response (pCR) cases after NAC and non pCR cases were included. We scored the HER3 stability from 0 to 3 and found that non pCR cases showed significantly higher HER3 score than pCR cases (84.6% and 33% respectively, p=0.0149). Although further study is needed, these results suggest that HER3 signal possibly regulates PD-L1 expression in HER3-positive basal-like breast cancer and treatment with anti-HER3 targeting therapy combination with an immune checkpoint inhibition therapy for HER3 positive NAC resistant patients might be warranted.

#3696 Tumor-intrinsic PD-L1 alters tumor chemokines, NK cell trafficking and function, and renders distant PD-L1 null tumors responsive to αPD-L1. Curtis A. Clark, 1 Harshita B. Gupta, 1 Alvaro Padron, 1 Deyi Zhang, 1 Vincent Hurez, 1 Mary Jo Turk, 2 Rong Li, 1 Tyler Curiel 1.

Programmed death ligand (PD-L1) is expressed on many tumors and inhibits anti-tumor T cells through programmed death (PD)-1. PD-L1 tumor predictors αPD-L1 treatment effects, but mechanism(s) for PD-L1 tumor response to αPD-L1 are unclear. Our studies suggest tumor-intrinsic PD-L1 signals and spatially varied PD-L1 expression may contribute to response deviation. We used PD-L1−/− B16 melanoma (ctrl) and made PD-L1−/− by CRISPR. αPD-L1 slowed ctrl but not PD-L1−/− B16 growth in mice as expected, but PD-L1−/− also responded to αPD-L1 if ctrl B16 was on the trans flank. αPD-L1 elicited similar CD3+ T cell infiltration into ctrl vs. PD-L1−/− tumors, but without detectable B16-specific T cell increase. CD11b+ cell infiltration was similar in ctrl and PD-L1−/−. Strikingly, NKp46+ and NK1.1+ natural killer (NK) cells infiltrated PD-L1−/− > ctrl. NK cells increased significantly (∼2-fold) after αPD-L1 in PD-L1−/− vs. ctrl, along with NK effector functions (e.g., IFN-γ). Tumor PD-L1−/− altered tumor chemokines (e.g., CXCL12, CCL2) that could explain trafficking. CD4+ and CD8+ T cells chemotaxed to PD-L1−/− slightly > ctrl (with slightly > PD-L1−/−) in transwells, but NK cells migrated ∼2-fold more to PD-L1−/− vs. ctrl (with increased activation (e.g., PD-L1, CD69), also seen in vivo). We observed that tumor B2 microglobulin (B2M) expression was suppressed by αPD-L1 directly altered tumor cell chemokine production in vitro, suggesting additional, novel αPD-L1 treatment mechanisms requiring more study. Our models are useful to understand αPD-L1 (and likely αPD-1) responses based on tumor PD-L1 expression and will help define strategies to improve responses in PD-L1−/− tumors, and possibly poorly responsive PD-L1+ tumors.

#3697 The impact of HER3 signaling mediated PD-L1 regulation in triple negative breast cancer. Ayane Yamaguchi, 1 Eiji Suzuki, 1 Kousuke Kawaguchi, 2 Mariko Nishie, 1 Moe Tsuda, 1 Takeshi Kotake, 1 Masakazu Toi, 1 Kyoto Univ. Hospital, Kyoto, Japan; 2Massachusetts General Hospital, Harvard Medical School, Massachusetts, MA.

Triple negative breast cancer (TNBC) is still difficult to treat partly because of lacking specific target. Although 50-70% of TNBC expresses EGFR, it is less sensitive to the treatment of EGFR inhibition for TNBC as compared to the efficacy of HER2 inhibition for HER2-positive breast cancer. Several phase II study on EGFR blockade treatment has been reported, however it was not applied in a clinical setting yet. It was reported that residual tumors after treatment with EGFR-targeted antibodies showed increased HER3 abundance leading to EGFR/HER3 receptor dimerization. The signals of HER3/EGFR dimerization to PI3K/AKT/mTOR pathways are thought to be involved in cancer survival, proliferation and also up-regulation of PD-L1 expression. Thus, we hypothesized that up-regulation of HER3 signal caused by anti-cancer treatment might induce increased PD-L1 expression which is a potential target for the tested inhibitors. To confirm the relationship between HER3 signal and PD-L1 expression by using three basal-like breast cancer cell line; MDA-MB-231, HCC70, and MDA-MB-468. MDA-MB-231 is HER3-negative, and HCC70 and MDA-MB-468 are HER3-positive cell lines. We added neurelin 1 (NRG1; HER3 ligand) to those three cell lines and analyzed PD-L1 expression of protein by flow cytometry and mRNA by qRT-PCR. Both factors and mRNA levels of PD-L1 on HCC70 and MDA-MB-468 treated with NRG1 are increased as compared with those without NRG1 while there was no change of PD-L1 expression of MDA-MB-231 either with or without NRG1. In order to confirm the significance of potential treatment target of HER3, we evaluated HER3 expression in biopsy samples by immunohistochemistry before neoadjuvant chemotherapy (NAC) including all phenotypes. Thirteen pathological complete response (pCR) cases after NAC and non pCR cases were included. We scored the HER3 stability from 0 to 3 and found that non pCR cases showed significantly higher HER3 score than pCR cases (84.6% and 33% respectively, p=0.0149). Although further study is needed, these results suggest that HER3 signal possibly regulates PD-L1 expression in HER3-positive basal-like breast cancer and treatment with anti-HER3 targeting therapy combination with an immune checkpoint inhibition therapy for HER3 positive NAC resistant patients might be warranted.

#3698 Associations of the immune microenvironment with PD-L1 copy number alterations and PD-L1 expression in resected non-small cell lung cancer. Katsuhiko Yoshimura, 1 Yusuke Inoue, 1 Tomoaki Kihyo, 1 Akizaka Kawa, 1 Masayuki Tanahashi, 2 Hiroshi Ogawa, 1 Naoki Inui, 1 Kazuhiito Funai, 1 Kazuya Shimura, 1 Hiroshi Niwa, 1 Takafumi Suda, 1 Haruhiko Sugimura, 1 Hanamatsu University School of Medicine, Hanamatsu, Japan; 2Seirei Mikashima General Hospital, Hamamatsu, Japan.

Background: The relationships between tumor PD-L1 expression and the tumor immune microenvironment (TIM) have gained attention in a variety of cancers including non-small cell lung cancer (NSCLC). However, the relevance of TIM according to the tumor PD-L1 copy number alterations (CNAs) whose significance have been shown in several malignancies remains to be uncovered. Methods: We evaluated PD-L1+, CD8+, and Foxp3+ lymphocytes in tumor nest and surrounding stroma separately using microarrayed 636 resected NSCLC. Their associations with PD-L1 CNAs and PD-L1 expression were analyzed. Tumor-infiltrating lymphocytes (TILs) were counted under high-power fields (×200), PD-L1 CNAs were assessed with fluorescent in situ hybridization, and were classified into three categories by the inferior category (non-amplification, polymy, and dysmy). PD-L1 expression was evaluated using immunohistochemistry (clone E1L3N), and positivity of ≥5% was used as the cut-off. Results: In the entire cohort, the median age was 68 years (23-88 years). The cohort comprised 444 (68.0%) men and 197 (30.2%) never-smokers. The median follow-up duration was 3.6 years. The numbers of TILs in stroma were much greater than those in tumor nest. Smoking history and squamous histology were significantly associated with increased PD-L1 expression in both stroma and tumor nest, except for CD8+ TILs in stroma. There were 20 tumors with PD-L1 amplification (1.7%) and 84 tumors with polyomy (13.2%). PD-L1 expression was positive in 201 tumors (30.8%). In tumor nest, the numbers of individual TILs significantly increased according to the increase of PD-L1 copy number (Kruskal-Wallis, p<0.001 for PD-1+, p<0.001 for CD8+ and p=0.0087 for Foxp3+). In stroma, only PD1+ TILs were significantly associated with PD-L1 CNAs (p=0.0032), and CD8+ TILs tended to correlate with PD-L1 CNAs (p=0.079). The numbers of CD8+, PD-1+, and FOXP3+ TILs were significantly greater in PD-L1-positive tumors than in PD-L1-negative tumors, in both tumor nest and stroma (p<0.001). Neither kind of TIL, PD-L1 expression, nor PD-L1 CNAs alone was independently predictive factor for overall survival in a multivariate analysis with Cox hazard models. However, when the PD-L1 copy numbers were combined with Foxp3+ TILs, cases with tumor high PD-L1 copy numbers (≥5 copies per nucleus) and high Foxp3+ TILs had independently worse prognoses in both tumor nest and stroma inshow and stroma infiltration (hazard ratio (HR), 4.38; 95% confidence interval, 2.26-8.49, for tumor nest model; HR, 1.96; 95% CI, 1.04-3.68, for stroma model). Conclusion: CD8+ TILs and Foxp3+ TILs in tumor nest were significantly associated with PD-L1 CNAs and PD-L1 expression. Cases with both increased PD-L1 copy numbers and Foxp3+ TILs have independently worse survival outcomes. Additional data of other TIL subgroups related to PD-L1 CNAs and expression will be discussed.
**IMMUNOLOGY: Inflammation in the Tumor Microenvironment**

#3699 Activation of NK cells cytotoxicity mediated by alveolar macrophages in the lung against murine melanoma metastases by combined aerosol immunotherapy. Michele Sommariva,1 Valentino Le Noci,1 Elda Tagliabue,2 Andrea Balsari,1 Lucia Sfondrini,2 University of Milan, Milan, Italy; 2Fondazione IRCCS - Istituto Nazionale dei Tumori, Milan, Italy.

INTRODUCTION: Taking advantage of aerosol for repeated local applications in the lung, we previously demonstrated that combination of Poly(LC)/ CpG-ODN, TLR3 and 9 ligands capable to activate innate effectors, induced a significant antitumor effect against B16 melanoma lung metastases, which correlated with a strong activation of NK cells. We investigated the sequence of events that lead to NK cells activation by aerosolized TLR agonists in the lung. Although NK cells express certain TLRs, controversy exists regarding their direct responsiveness or dependence on macrophages. Alveolar macrophages have several unique features both promoting host defense mechanisms against invading microorganisms, but also establishing an immunosuppressive microenvironment to avoid inflammatory responses to inhaled particles. Our findings, establishing the sequence of events of lung immune cells activation, might represent the basis for the development of combined aerosol therapies that favor NK cell triggering. EXPERIMENTAL PROCEDURE: Alveolar macrophages and NK cells were recovered by bronchoalveolar lavage and spleen of C57BL/6 mice, respectively. Mice i.v. injected with 5x10⁵ B16 cells were treated starting 7 days later every three days with aerosolized Poly(LC)/CpG-ODN alone or combined to nebulized myeloid derived suppressive cells (MDSC)-depleting RB6-8C5 antibody. Immune cell populations and expression of M1/M2 markers were analyzed by FACS and RT-PCR respectively, in tumors and spleens. Cytotoxic activity of NK cells was assessed by ⁵¹Cr release assay. RESULTS: In vitro TLR3/TLR9 stimulation induced IFN-γ secretion by naïve NK cells, but an increase in their cytotoxicity was detected only when NK cells were cocultured with alveolar macrophages pretreated with the two agonists. Alveolar macrophages from melanoma lung metastases-bearing mice treated with aerosolized TLR agonists also promoted NK cell cytotoxicity ex vivo. Moreover, naïve alveolar macrophages incubated with activated NK cells from lungs of melanoma metastases-bearing mice aerosolized with TLR9/TLR3 agonists, up-regulated M1- and downregulated M2-related genes, suggesting a bidirectional crosstalk between NK cells and macrophage. Thus, TLR agonists-activated lung macrophages promote NK cell cytotoxicity and, reciprocally, NK cells have the potential to shape the macrophage programmed to M1-like phenotype. Our results also indicate that the positive interplay can be further improved by the depletion of myeloid derived suppressive cells that exert a negative effect on macrophage activation. CONCLUSION: This study points to the promises of combinations of immunotherapeutic agents delivered locally by inhalation to promote macrophages-NK cell activation in the lung as a novel strategy to treat lung cancer patients.

#3700 Prognostic value of the expression of NKG2D and CD96 in early stage non-small cell lung cancer (NSCLC). Marcin Skrzypski,1 Michal Marczyk,1 Anna Lechowicz,1 Anna Trojani,1 Ryszard Pawlowicz,1 Wojciech Bienrat,1 Joanna Połanowska,2 Jacek Jaszem1.

Background: Immune checkpoint inhibitors showed impressive activity in advanced NSCLC and are expected to be effective in postoperative treatment of early disease. New therapies aimed at T and NK cell activation are in development to expand treatments options. It is conceivable that markers of ‘inflamed,’ ‘immune system ignorant’ and ‘immune system excluding’ tumor phenotypes may be predictive for specific immune-targeting therapies. Aim: We assessed prognostic value of expression of CD8(+) T and NK cell markers: CD96 and NKG2D in early stage NSCLC. In addition, we analyzed expression of 11 transcripts of APOBEC genes with hypothesis that APOBEC induced mutation burden may affect tumor immunogenicity or host adaptive immune responsiveness. Methods: mRNA levels were measured by RT-PCR in frozen tumor tissue from 65 NSCLC stage I-IIIA patients who underwent pulmonary resection (75% lung adenocarcinoma, 33% never-smokers, 44% with subsequent dissemination), and in 11 NSCLC cell lines. The relative gene expression (vs. 5 normal immortalized TLBs, used as a comparator) was measured in cells that did and did not disseminate and in relation to clinico-pathological features. Results: After Bonferroni correction for multiple testing, NKG2D and CD96 mRNA expression was significantly lower in tumors with subsequent dissemination (p.adj.=0.045). Out of 11 APOBEC genes, expression of AICDA, APOBEC3A and APOBEC3G was lower in tumors with subsequent dissemination (p.unadj.=0.025). The NSCLC lines did not express NKG2D, CD96, AICDA or APOBEC3A. The Spearman correlation coefficient between the expression of NKG2D, CD96 and APOBEC3G was r=0.8. AICDA expression was correlated with CD96, NKG2D and APOBEC3G with Spearman correlation coefficient in the range of 0.5-0.62. Expression of CD96 and NKG2D was significantly lower in tumors with vs. without subsequent brain metastasis (p.unadj.=0.048). The negative prognostic impact of low NKG2D and CD96 expression was independent from patient smoking status (FC <1, irrespective of smoking status). In the subset of never-smokers, the fold difference of NKG2D expression between tumors with vs. without subsequent dissemination was particularly pronounced (HR=0.37, p.unadj.=0.023). In multivariate Cox analysis low NKG2D and NKG2D expression in early stage NSCLC may be predictive for high propensity to dissemination, including metastasis to the brain. The expression of APOBEC transcripts does not seem to correlate with metastatic potential.

#3701 Mutant p53 cancers reprogram tumor-associated macrophages via exosomal miR-1246. Tomer Cohen,1 Ioannis S. Pateras,1 Keval Jassim1.

INTRODUCTION: Mutantp53proteinsgainoncogenicfunctionsofGOF distinct from the tumor suppressor activity of the wild-type protein. Tumor-associated-macrophages, a hallmark of solid tumors, are typically correlated with poor prognoses due to a non-cell-autonomous mechanism whereby mutp53 cancer cells reprogram TAM to a tumor supportive and anti-inflammatory state. The colon cancer cells harboring GOF mutp53 selectively shed miR-1246-enriched exosomes. Uptake of these exosomes by neighboring macrophages triggers their miR-1246 dependent reprogramming into a cancer-promoting state. Mutp53-reprogrammed TAM favor anti-inflammatory immunosuppression with increased activity of TGF-β. These findings, observed also in colon cancer patients, strongly support a microenvironmental GOF role for mutp53 in actively engaging the immune system to promote cancer progression and metastasis.

#3702 Quantitative imaging of metabolic changes in macrophage subsets. Tiffany M. Heaster,1 David L. Elion,2 Rebecca S. Cook,3 Melissa C. Skala,1 University of Wisconsin-Madison, Madison, WI; 2Vanderbilt University, Nashville, TN; 3Morgridge Institute for Research, Madison, WI.

Macrophages play an important role in tumor therapeutic response. However, current methods cannot image heterogeneities in macrophage activity on a single-cell level over time within intact, living samples. Macrophage metabolism is closely linked to macrophage function, as M1-like macrophages rely on aerobic glycolysis and M2-like macrophages rely on fatty acid oxidation and oxidation-dependent phosphorylation. Thus, we propose to develop optical metabolic imaging (OMI) using two-photon microscopy to monitor cell-level changes in macrophage polarization and functional activity. OMI exploits the auto-fluorescence intensities and lifetimes of the metabolic co-enzymes NAD(P)H and FAD, and has been previously used to dynamically image in vivo tumors with cell-level resolution. The optical redox ratio (fluorescence intensity of NAD(P)H divided by FAD) provides a global measure of redox balance within individual cells. The fluorescence lifetimes of NAD(P)H and FAD provide information in protein-binding activities in metabolic reactions. OMI was used to monitor metabolic changes in macrophages on a single-cell level during polarization and after metabolic perturbations. RAW 264.7 macrophages were stimulated towards an M1- or M2-like phenotype, and imaged with OMI at 24, 48, and 72 hours post-polarization. M1- and M2-like macrophages exhibited significant differences (p<0.05) in redox ratio and FAD fluorescence lifetime values at 48 and 72 hours post-polarization. The M1-like and M2-like character of the macrophages were validated with quantitative polymerase chain reaction (qPCR) analysis. Population density modeling of single-cell metabolism established differences in intracellular heterogeneity across subsets over time. M1- and M2-like macrophages were further characterized between groups that did and did not disseminate and in relation to clinico-pathological features. Results: After Bonferroni correction for multiple testing, NKG2D and CD96 mRNA expression was significantly lower in tumors with subsequent dissemination (p.adj.=0.045). Out of 11 APOBEC genes, expression of AICDA, APOBEC3A and APOBEC3G was lower in tumors with subsequent dissemination (p.unadj.=0.025). The NSCLC lines did not express NKG2D, CD96, AICDA or APOBEC3A. The Spearman correlation coefficient between the expression of NKG2D, CD96 and APOBEC3G was r=0.8. AICDA expression was correlated with CD96, NKG2D and APOBEC3G with Spearman correlation coefficient in the range of 0.5-0.62. Expression of CD96 and NKG2D was significantly lower in tumors with vs. without subsequent brain metastasis (p.unadj.=0.048). The negative prognostic impact of low NKG2D and CD96 expression was independent from patient smoking status (FC <1, irrespective of smoking status). In the subset of never-smokers, the fold difference of NKG2D expression between tumors with vs. without subsequent dissemination was particularly pronounced (HR=0.37, p.unadj.=0.023). In multivariate Cox analysis low NKG2D and NKG2D expression in early stage NSCLC may be predictive for high propensity to dissemination, including metastasis to the brain. The expression of APOBEC transcripts does not seem to correlate with metastatic potential.
IMMUNOLOGY: Inflammation in the Tumor Microenvironment


Testicular germ cell tumors (TGCT) are a histologically diverse set of tumors that are the most common cancer type in young men. As part of the TCGA effort, we analyzed tumors from 133 patients including 4 patients with asynchronous double primary TGCTs (137 primary tumors) by histopathologic, genomic, epigenomic, transcriptomic, and proteomic methods. We noted molecular signatures of various immune cells were greater in seminomas than other histologies. Specifically, we find seminomas show elevation of expression signatures for B-Cells, Cytotoxic T Cells, and T-regulatory cells. This increase in immune cells were accompanied by high expression of specific cytokines and key immune checkpoint molecules such as CTLA4, LG3, and PD-L1. Strong positive correlations were identified between immune cell signatures and increases in T-Cell and B-Cell receptor diversity measures. Interestingly, increases in receptor diversity also showed strong positive associations to gene expression scores of anti-tumor immune responses. This indicates that anti-tumor immune responses in seminomas may be polyclonal. We found no evidence for relationships between immune cells and mutation load, neoantigen load, or detected viruses. We do observe that seminomas have significantly high expression of the testis specific antigens, yet the contribution of these antigens to the immune response did not show strong or uniform statistical indications. Instead, the strongest association between expanded immune cells populations in seminomas were the presence of activating mutations in KIT accompanied by elevation of gene expression of signatures associated with KIT and expression of MHC class I and II genes. Together, these data describe the immune microenvironment of seminomas and suggest alterations and events in the kit pathway as being critical to the nature of the immune microenvironment in seminomas.

Targeting M2-Tumor associated macrophages (M2-TAMs) using enriched glycoproteins in lethal prostate cancer. Jelani C. Zarif, James R. Henandez, Weiming Yang, Hui Zhang, Kenneth J. Pienta. Johns Hopkins University School of Medicine, Baltimore, MD.

Prostate cancer is a leading cause of cancer-related deaths of men in the U.S., and in the past year, over 30,000 men died from this disease. While localized prostate cancer is highly treatable by surgical resection and radiation, cancer that has metastasized remains incurable. Alternatively activated macrophages, also known as M2-macrophages, primarily scavenge debris and in the process, promote angiogenesis and wound repair. M2-macrophages are phenotypically similar to M2 tumor-associated macrophages (M2-TAMs) have been reported to associate with solid tumors such as prostate cancer to facilitate epithelial to mesenchymal transition (EMT), tumor invasiveness, metastasis, and resistance to therapy. As an invasive species within the tumor microenvironment, this makes M2-TAMs an ideal therapeutic target in prostate cancer. The purpose of this project is to develop novel therapeutics that will directly target M2-TAMs for destruction and subsequently attenuate prostate tumor growth, progression, and metastasis. Our hypothesis is to determine if targeting of M2-TAMs by using enriched surface antigens that are targeted by antibody-drug-conjugates (ADCs), be an effective therapy for lethal prostate cancer while simultaneously eliciting an immune response. To identify novel surface antigens expressed on M2-macrophages, we developed a novel method of creating homogenous populations of human macrophages from CD14+ monocytes in vitro. Our homogenous M1 macrophages secrete pro-inflammatory cytokines and our M2 macrophages secrete anti-inflammatory cytokines as well as VEGF. We then performed solid-phase extraction of N-linked glycopeptides (SPEG) followed by liquid chromatography-tandem Mass Spectrometry (LC-MS/MS) on our homogenous macrophage populations. We discovered five novel peptides that are enriched exclusively on M2-macrophages relative to M1 macrophages and CD14+ monocytes. Lastly, we determined if these surface antibodies, found enriched on M2 macrophages, were also expressed in human metastatic castrate-resistant prostate cancer (mCRPC). Using mCRPC tissues from rapid autopsies supplied by the Departments of Urology and Surgical Pathology, we were able to determine M2-macrophage infiltration by using immunohistochemistry and flow cytometry. The studies described here outline a method of altering the tumor immune microenvironment. To target M2 macrophages, we used small peptides as well as antibody drug conjugates (ADCs) that targeted the enriched surface glycoproteins expressed on M2-macrophage. We then tested their efficacy in vitro and in a syngeneic prostate tumor mouse model to assess tumor shrinkage and effector and cytotoxic T Cell infiltration. By identifying specific markers on M2-TAMs, we predict that this method of targeting will provide a better prognosis for patients who have been diagnosed with lethal prostate cancer.

Targeting interleukin-1 to increase cetuximab efficacy in head and neck cancer. Madelyn Espinosa-Cotton, Ayana J. McLaren, Adam T. Koch, Andread L. Simons. The University of Iowa, Iowa City, IA; Lincoln University, Lincoln University, PA; Loma Linda University School of Medicine, Loma Linda, CA.

Treatment for head and neck squamous cell carcinoma (HNSCC) patients includes the epidermal growth factor receptor (EGFR) monoclonal antibody cetuximab. However, response to cetuximab is low and resistance remains a critical issue. Therefore, it is imperative to develop strategies to enhance tumor response to this drug. Inflammation plays an important role in tumor progression by increasing the release of cytokines that promote tumor cell survival. Our prior work has shown that a variety of pro-inflammatory cytokines involved in tumor survival are upregulated upon treatment with EGFR inhibitors in HNSCC cells, suggesting that poor tumor response to EGFR inhibitors may be due to increased tumor-associated inflammation. Our lab has shown that the increased proinflammatory tumor response due to EGFR inhibition may be activated by interleukin-1 (IL-1) signaling, suggesting that the IL-1 pathway may be an important target to enhance the efficacy of anti-EGFR therapy. Here we investigated the effect of IL-1 blockade on HNSCC tumor response to cetuximab treatment. We found that cetuximab induced IL-6 secretion from SQ20B HN SCC cells and knockdown of the adapter protein MyD88, which is essential for IL-1-induced IL-6 secretion, reduced cetuximab-induced IL-6 secretion. This increase in immune cells were accompanied by elevation of gene expression of signatures associated with KIT and expression of MHC class I and II genes. Together, these data describe the immune microenvironment of seminomas and suggest alterations and events in the kit pathway as being critical to the nature of the immune microenvironment in seminomas.

Validation of human and mouse myeloid panels on the NanoString nCounter® Platform. Sushil Kumar, Sarah Warren, Tiziana Cotechini, Terry Medler, Christina Bailey, Joseph M. Beechem, Joseph M. Beechem, Lisa M. Coussens. Oregon Health and Science University, Portland, OR; NanoString Technologies, Seattle, WA; Oregon Health and Science University, Seattle, WA.

Introduction: The innate immune system profoundly influences cancer development as well as its response to various therapeutic approaches. Multiple myeloid cell lineages are central to the innate immune response during cancer and have been found to regulate diverse cellular processes encompassing immunosuppression, angiogenesis, invasion, metastasis, and drug resistance. NanoString has collaborated with the laboratory of Prof. Lisa M. Coussens to develop research gene panels for quantitative and qualitative analysis of various myeloid subsets by evaluating relative enrichment of genes that mark recruitment and presence of select myeloid subtypes (e.g., granulocytes, dendritic cells, monocytes, macrophages, mast cells), their cell function, activation and effector status. Methods: Gene content for human and murine versions of the myeloid panels were developed from an in-house high content proteomic screen of macrophage activation (unpublished), genomic analysis of human tumor associated macrophages (unpublished), and data mining from publically available datasets featuring myeloid transcriptomes. In total, 692 genes were included in the human panel and 675 genes in the murine panel. The panels were optimized to simultaneously analyze 18 samples in a single run. The murine panel was comprised of five cell types: macrophages, neutrophils, dendritic cells, mast cells, and granulocytes. Results: To validate the panel content, we are profiling myeloid infiltrates in syngeneic murine models of mesothelioma and mammary tumorigenesis by flow cytometric analysis and the nCounter myeloid panels. Since the quality of the myeloid tumor response can skew toward adaptive immune activating (Th1) or suppressive (Th2) phenotypes, we will profile the same tumors with the NanoString PanCancer Immune Profiling panel to evaluate T cell activation status by gene expression and flow cytometry as well as global immune response. Finally, myeloid complexity and T cell activation status in both primary tumor and lung metastases will be evaluated by NanoString to
assess regulation of adaptive immune responses by the innate compartment in distinct tumor microenvironments. Conclusions: Upon validation with these assays, the myeloid gene panels may provide insight into how the innate immune system regulates cancer development and response to therapeutic pressure. Improved characterization of the myeloid compartment may lead to greater insight into the fundamental biology of tumor-immune interactions, novel therapeutic strategies, and other applications.


To date there has been an increasing focus on the interactions between inflammatory myeloid cells and T cells in the tumor microenvironment because cytotoxic anti-tumoral T cells represent the chief effector mechanism of anti-tumoral immunity. Tumor-associated neutrophils (TANs) represent a significant portion of inflammatory cells in lung tumors; however, whether specialized neutrophil subpopulations capable of regulating T cell responses exist in human cancers is unknown. Our goal was to identify subsets of TANs and determine their specific roles in the regulation of T cell responses in patients at early stage lung cancer. An extensive phenotypic analysis of 55 early-stage human lung tumors revealed that TANs, defined as CD11b+ Arg1+ MPO+ CD66b+ CD15+ cells, contained two major sub-populations. One subset of "canonical" TANs expressed classic neutrophil markers. A second subset of TANs displayed a combination of neutrophil and myeloid markers (CD14+ HLA- DR+ CCR7+ CD68+ ) normally expressed on antigen-presenting cells (APC). We termed this unique neutrophil population "APC-like hybrid TANs". The frequency of these hybrid TANs varied widely within lung cancers and ranged from 0.5-25% of all TANs. Interestingly, the frequency of this hybrid population declined as tumors enlarged, and they were almost completely absent in tumors greater than 5 cm in diameter. Mechanistically, we determined that low doses of IFN-γ and GM-CSF in the tumors were required for the development of APC-like hybrid neutrophils. The high proportion of hybrid TANs (>10% of all TANs) directly correlated with the presence of IFN-γ and GM-CSF in the autologous tumor tissue. Using bone marrow-derived immature granulocytes, which were found to have prolonged survival in vitro, we discovered that these APC-like hybrid neutrophils originate from CD11b+ CD15+ CD10+ CD16- immune neutrophil progenitors in the presence of IFN-γ and GM-CSF or in tumor-conditioned media. Functionally, the APC-like hybrid neutrophils are superior to canonical neutrophils in their ability to: 1) stimulate antigen non-specific autologous T cell responses 2) directly stimulate antigen-specific autologous T cell responses by providing a co-stimulatory signal through the OX40L, 4-1BB, CD86, CD84, molecules, and 5) cross present tumor-associated antigen as IgG immune complex. In summary, we provide the first evidence of two subsets of TANs in lung cancer. All TANs had an activated phenotype and could support (rather than inhibit) T cell functions to some degree. However, we identified a subset of TAN in early-stage lung tumors that can undergo a unique differentiation and gene expression pattern. In particular, the combination of certain myeloid lineage cells, myeloid dendritic cells, neutrophils, fibroblasts and endothelial cells in tumor tissues. Immune cell compositions were correlated with mutational load, the activity of 27 mutational signatures (MutSig) and PD-L1 mRNA expression. Correlation strength was assessed using Spearman’s rank statistics. Multiple testing was addressed using the Benjamini-Hochberg method and FDR control at 5%. MutSig 1 (clock-like, age-related) showed significant positive correlations with immune cell infiltrations in low-grade gliomas, negative correlations in breast cancer, melanoma, stomach cancer, lung and prostate adenocarcinoma, but no significant correlations in the remaining 15 cancer types. Of the two APOBEC-related signatures, MutSig 2 showed positive significant correlations with immune cell infiltrates in cervical cancer, bladder cancer; lung adenocarcinoma, head and neck cancer and thyroid cancer, MutSig 13 in breast and cervical cancer. In particular, correlation of MutSig 2 with cytotoxic lymphocytes was significant in cervical cancer, bladder cancer and lung adenocarcinoma (R = 0.29, R = 0.17 and R = 0.17), the corresponding correlation of MutSig 13 in breast cancer (R = 0.17). MutSig 4 (tobacco smoke-related) showed a significant correlation with CD8+ T cells and NK cells in lung adenocarcinoma (R = 0.15 and R = 0.14). In contrast, correlation of MutSig 4 with immune cell infiltrates was non-significant or negative in head and neck cancer. In colorectal cancer, MutSig 6 (defective mismatch repair) showed significant positive correlations with NK cells, cytotoxic lymphocytes, mononuclear lineage cells and neutrophils infiltration (R = 0.45, R = 0.37, R = 0.23 and R = 0.19). PD-L1 mRNA expression and immune cell infiltrates correlated positively in almost 75% of the analyzed types of cancer and showed a positive correlation between PD-L1 expression and different immune cell infiltrates in 12 cancer types. Correlation with CD8+ T cells was positive and significant in 18 cancer types and strong in 10 cancer types. In summary, pan-cancer analysis of DNA and RNAseq data showed that specific mutational signatures contribute to cancer cell immune immunogenicity, while the ubiquitous clock-like mutational process did not positively correlate with immune cell infiltrates in 20 of 21 cancer types. Moreover, PD-L1 mRNA expression strongly correlated with infiltrates of immune cells in the majority of cancer types.

#3708 RAS-MAPK signal is required for enhanced PD-L1 expression in human lung cancers. Hidetoshi Sumimoto, Atsushi Takano, Koji Teramoto, Shiga University of Medical Science, Otsu, Japan.

Ectopic programmed cell death ligand 1 (PD-L1) expression in non-small cell lung cancers (NSCLCs) is related to immune evasion by cancer, and it is a molecular target of immune checkpoint therapies. Since the precise mechanisms responsible for ectopic PD-L1 expression remains obscure, we analyzed the molecular mechanisms of ectopic PD-L1 expression in human lung cancers, focusing on the MAPK signal. Because we found a higher frequency of EGFR/ KRAS mutations in NSCLC cell lines with high PD-L1 expression (p < 0.001), we evaluated the relationships between downstream signals and PD-L1 expression, particularly in three KRAS-mutant adenocarcinoma cell lines. The MEK inhibitor U0126 (20 μM) significantly decreased the surface PD-L1 levels by 30-60% compared with dimethyl sulfoxide (p < 0.0001). Phorbol 12-myristate 13-acetate stimulation (100 nM, 15 min) increased (p < 0.05) and two ERK2 siRNAs as well as KRAS siRNAs decreased (p < 0.05) PD-L1 expression. Post-transcriptional mechanism by miR-200s appears not to be in general under the downstream of MAPK in the PD-L1 expression. The transcriptional activity of the potential AP-1 site (+4785 to +5056 from the transcription start site) in the PD-L1 gene was demonstrated by luciferase assays, which was inhibited by U0126. The chromatin immunoprecipitation assay demonstrated the binding of cJUN to the AP-1 site. Two STAT3 siRNAs decreased PD-L1 expression by 10-32% in two of the three KRAS-mutant lung adenocarcinoma cell lines (p < 0.05), while the PI3K inhibitor LY294002 (40 μM) did not change the expression level. Supervised cluster analysis and gene set enrichment analysis between the high- and low- PD-L1 NSCLCs revealed a correlation between PD-L1 expression and genes/pathways related to cell motility/adhesion. These results indicate that MAPK signaling is the dominant downstream signal responsible for ectopic PD-L1 expression at a transcriptional level, in which STAT3 is also involved to some extent. Furthermore, MAPK signaling may control the expression of PD-L1 and several genes related to enhanced cell motility. Our findings suggest that MAPK is important for determining PD-L1 expression, which could be useful for targeted therapies against lung cancers.

#3709 Deciphering the impact of tumor genetics on immune cell infiltration in major solid cancer types. Jan Budcza,1 Michael Bockmayer,2 Frederick Klausch,3 Abrecht Stenzinger,2 Carsten Denkert,1 Charité - Universitätsmedizin Berlin, Berlin, Germany; 2Heidelberg University Hospital, Heidelberg, Germany.

With the advent of immune therapies including inhibition of the PD-1/PD-L1 axis there is an urgent need to identify cancer patients that benefit from these therapies and to overcome mechanisms of resistance. Here, we analyzed the impact of tumor genetics on the composition of tumor microenvironment across 21 solid cancer types from the TCGA project. Using specific mRNA markers of 16 cell populations (MCPCounter), we estimated the content of T cells, CD8+ T cells, CD4+ T cells, B lineage cells, NK cells, mononuclear lineage cells, myeloid dendritic cells, neutrophils, fibroblasts and endothelial cells in tumor tissues. Immune cell compositions were correlated with mutational load, the activity of 27 mutational signatures (MutSig) and PD-L1 mRNA expression. Correlation strength was assessed using Spearman’s rank statistics. Multiple testing was addressed using the Benjamini-Hochberg method and FDR control at 5%. MutSig 1 (clock-like, age-related) showed significant positive correlations with immune cell infiltrations in low-grade gliomas, negative correlations in breast cancer, melanoma, stomach cancer, lung and prostate adenocarcinoma, but no significant correlations in the remaining 15 cancer types. Of the two APOBEC-related signatures, MutSig 2 showed positive significant correlations with immune cell infiltrates in cervical cancer, bladder cancer, lung adenocarcinoma, head and neck cancer and thyroid cancer, MutSig 13 in breast and cervical cancer. In particular, correlation of MutSig 2 with cytotoxic lymphocytes was significant in cervical cancer, bladder cancer and lung adenocarcinoma (R = 0.29, R = 0.17 and R = 0.17), the corresponding correlation of MutSig 13 in breast cancer (R = 0.17). MutSig 4 (tobacco smoke-related) showed a significant correlation with CD8+ T cells and NK cells in lung adenocarcinoma (R = 0.15 and R = 0.14). In contrast, correlation of MutSig 4 with immune cell infiltrates was non-significant or negative in head and neck cancer. In colorectal cancer, MutSig 6 (defective mismatch repair) showed significant positive correlations with NK cells, cytotoxic lymphocytes, mononuclear lineage cells and neutrophils infiltration (R = 0.45, R = 0.37, R = 0.23 and R = 0.19). PD-L1 mRNA expression and immune cell infiltrates correlated positively in almost 75% of the analyzed types of cancer and showed a positive correlation between PD-L1 expression and different immune cell infiltrates in 12 cancer types. Correlation with CD8+ T cells was positive and significant in 18 cancer types and strong in 10 cancer types. In summary, pan-cancer analysis of DNA and RNAseq data showed that specific mutational signatures contribute to cancer cell immune immunogenicity, while the ubiquitous clock-like mutational process did not positively correlate with immune cell infiltrates in 20 of 21 cancer types. Moreover, PD-L1 mRNA expression strongly correlated with infiltrates of immune cells in the majority of cancer types.
such as pancreatic cancer, has not been elucidated. Our recent results show that human pancreatic cancer cell lines have high total and cell-surface expression of β₂m, HLA-A, and HLA-B, with the exception of one pancreatic cancer cell line that we found exhibits low expression of HLA-B. Furthermore, differential gene expression analysis performed on laser capture-microdissected human pancreatic cancer patient samples revealed that the HLA-B gene is reduced in pancreatic ductal adenocarcinoma cells, compared to precursor (PanIN) epithelial or stromal cells. Together, our results indicate that pancreatic cancer cells have not selectively down regulated HLA class I molecule expression. This observation suggests that HLA class I molecules might provide some benefit to pancreatic cancer cells that counterbalances any risk of immune attack caused by HLA class I molecules. To examine whether the components of the HLA class I molecule actually confer potential advantages to pancreatic cancer cells, we transfected siRNA specific for β₂m, HLA-A heavy chain, or HLA-B heavy chain into several pancreatic cancer cell lines and evaluated the effects on their proliferation and migration. Down-regulation of either β₂m or HLA-B expression slowed the proliferation of one pancreatic cancer cell line, whereas down-regulation of HLA-A expression had the inverse effect on the same cell line. Two other pancreatic cancer cell lines had an opposite phenotype: proliferating more slowly when HLA-A expression was reduced, but more rapidly when HLA-B expression was decreased. Our investigation of the impact of down regulating HLA class I molecule components on the migration of pancreatic cancer cells showed the same trends with each cell line as we had observed in our proliferation analyses. In conclusion, we found that pancreatic cancer cells express substantial levels of HLA class I components, and their components influence the proliferation and migration of pancreatic cancer cell lines in a manner that varies for HLA-A versus HLA-B, and that also varies in pattern among the types of pancreatic cancer cell lines.

#3711 R-Ras is required for T cell trafficking in high endothelial venues and implicated in anti-tumor immunity. Andrew M. Chan,¹ Xiaocai Yan,¹ Wai Nam Liu,² Mingjie Yan.¹ ¹Chinese University of Hong Kong, Hong Kong, Hong Kong; ²The Medical College of Wisconsin, Milwaukee, WI.

R-Ras belongs the RAS superfamily of small GTP-binding proteins implicated in cell adhesion signaling. Unlike classical Ras proteins, R-Ras is not activated by growth factor receptors. Rather, its GTPase activity is modulated by molecules implicated in cell-cell adhesion including semaphorins and notch. Using an R-Ras knockout mouse strain, we have previously reported that R-Ras is required for murine dendritic cell functions. Further characterization of this mouse strain revealed reduced cellularity of peripheral lymph nodes (PLNs) by 40% with T cell regions in the paracortex in which high endothelial venues (HEV) resided were less prominent when compared with wild-type (WT) mice. Consistently, immunohistological analysis for MECA-79, a marker of mature HEV, showed a 3-to 4-fold decrease in staining intensity. This is correlated with a 30-40% reduction in the number of CD62L⁺ CD4 and CD8 T cells in PLNs. Furthermore, R-Ras null T cells had reduced proliferative and homing capacity when compared with WT. More importantly, we demonstrated for the first time that the chemokine CCL21 was able to stimulate the GTP-loading of R-Ras within 15 seconds of addition. Indeed, R-Ras null T-cells displayed reduced binding to soluble ICAM-1 as well as to 2H11 endothelial cells. To explore the role of R-Ras in anti-tumor immunity, B16/F10 melanoma cells were injected subcutaneously to WT and R-Ras null mice. In addition, a mouse breast tumor model was generated in neither WT or R-Ras-null genetic background. Tumor development and the number of infiltrating immune cells were analyzed to examine if R-Ras was involved in either anti- or pro-tumor immune responses. This work was supported by NIH CA78509 (AMC), MS9771 (AMC). AMC was supported by Hong Kong PhD Fellowship Scheme (PF12-13584) and AMC was supported by the Lo Kwee- Seong Seed Fund, and a General Research Fund Grant, 14120915, from Hong Kong University Grants Committee.

#3712 Mechanistic link between phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) activity and PD1L1 expression in head and neck squamous cell carcinoma (HNSCC). Rebecca C. Hoesli, Nicole L. Schumacherzien, Vivek Nair, Chloe M. Novotina, Elizabeth Leonard, Matthew E. Spector, Carol R. Bradford, Mark E. Prince, Andrew C. Birkeland, J Chad Brenner. University of Michigan, Ann Arbor, MI.

Introduction: HNSCC is an immunosuppressive disease, with multiple functional and quantitative defects contributing to immune evasion and tumor escape. One of the identified areas for therapy development is the Programmed Death-1 (PD-1) Programmed Death Ligand 1 (PD-1L) pathway, as this pathway has been hypothesized to allow cancer cells to evade the immune system by promoting T cell anergy and apoptosis. Pembrolizumab, a PD-L1 inhibitor, has recently been approved for the treatment of recurrent/metastatic HNSCC. As regulation of PD-1L1 expression could play an important role in the effectiveness of therapy, we further explored the regulation of PD-L1 expression in HNSCC cells. Specifically, we targeted our investigation by evaluating the effects on expression of one of the most frequently mutated genes in HNSCC, PIK3CA. We evaluated this with and without interferon-γ, which has previously been shown to affect PD-L1 expression, possibly through activation of the STAT1 pathway. While HPV+ HNSCC cell lines were grown in cell culture and treated with selective and non-selective PI3K inhibitors, in combination with interferon-γ. After 72 hours, cells were harvested and flow cytometry was used to measure the expression of PD-L1. Protein expression of various pathway intermediaries was evaluated via western blot to better delineate the mechanism of PD-L1 upregulation. Results: Treatment with selective PI3K inhibitors in combination with interferon-γ in several cell lines significantly increased expression of PD-L1, beyond the increase noted after treatment with interferon-γ alone. Maintenance of STAT1 phosphorylation correlated with upregulation of PD-L1 expression, while total STAT1 expression remained stable. The majority of the cell lines maintaining STAT1 phosphorylation were HPV+, but a few HPV- cell lines also maintained this phosphorylation with a correlating upregulation in PD-L1 expression. Additionally, mutation in PIK3CA despite HPV-status was noted to maintain phosphorylation of STAT1 with upregulation of PD-L1 expression. Conclusions: Treatment with PI3K inhibitors in combination with interferon-γ significantly upregulated PD-L1 expression in several cell lines, suggesting a possible synergistic effect. Since PD-L1 expression correlated with maintenance of phosphorylation of STAT1, these results suggest a pivotal link between PIK3CA signaling, STAT1 activity and PD-L1 expression in PIK3CA aberrant HNSCC.

#3713 Higher numbers of cancer stem cells in the peripheral blood of children with B-ALL upon conventional chemotherapy. Mohamed L. Salem. Tanta University, Tanta, Egypt.

Background: Acute lymphocytic leukemia (ALL) is biologically and clinically considered as a heterogeneous neoplasm of lymphoid progenitor cells in the bone marrow (BM). 15-20% of children with ALL who achieve an initial remission, will show relapse. One potential mechanism behind this relapse could be the emergence of cancer stem cells (CSCs) which are considered the driving force of tumourigenesis due to their ability of self-renewal as well as the emergence of immune regulatory cells including myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Treg). Aim: the main aim of this study was to analyze the numbers of CSCs and correlate these numbers with the numbers of blast cells, MDSCs and Treg cells in children with B-ALL before and after induction of chemotherapy. Materials and Methods: CSCs were defined as CD45⁺CD19⁻CD10⁻CD34⁺CD38⁺, MDSCs were defined as Lin HLA-DR⁻CD33⁺CD11b⁻and Treg cells were defined as CD4⁺CD25⁺CD127⁻. The frequencies of these cells were analyzed in the peripheral blood of 15 ALL patients before and after (n = 10) and after (n = 10) induction of chemotherapy using flow cytometry. Results: Significant increases in the numbers of CSCs were shown in B-ALL patients after induction of chemotherapy as compared to newly diagnosed patients (7.6 ± 8.3 vs. 2.7 ± 2.4, P<0.05). The numbers of CSCs in ALL patients before and after induction of chemotherapy inversely correlated with the numbers of the blast cells. Additionally, the frequency of the MDSCs and Treg cells were higher and lower, respectively, in patients after induction of chemotherapy as compared to before chemotherapy. Conclusion: Our results indicate that chemotherapy of B-ALL patients results in emergence of high numbers of CSCs and MDSCs which might be contributing, respectively, to tumor relapse and creation of systemic immune suppression. This pilot study opens a new avenue to investigate the mechanism mediating the emergence of these cells on larger population of B-ALL patients at different treatment stages.

#3714 Muc5ac plays an essential role in promotion of k-ras mutant lung cancer. Misha Umer,¹ Amber M. Cumpian,² Nasim Khosravi,² Zouliik Azegzagh,¹ Maede Mohebsab,¹ Mauricio S. Caetano,¹ Edwin J. Ostrin,¹ Ignacio I. Wistuba,¹ Burton Dickey,¹ Humam Kadara,² Christopher M. Evans,³ Seyed J. Moghaddam,¹ UT MD Anderson Cancer Center, Houston, TX;²University of Pittsburgh School of Medicine, Pittsburgh, PA;³University of Medicine and Dentistry of New Jersey, New Jersey, NJ and University of California, San Francisco, CA. ¹University of Michigan, Ann Arbor, MI. ²University of Pittsburgh School of Medicine, Pittsburgh, PA. ³University of Virginia, Charlottesville, VA. 4University of California, San Francisco, CA.

Worldwide, lung cancer is the leading cause of cancer mortality, and cigarette smoking (CS) is its principal cause. However, several studies have found that smokers with chronic obstructive pulmonary disease (COPD), an inflammatory disease of the lung, have an increased risk of lung cancer (3 to 10-fold) compared to smokers with comparable cigarette exposure but without COPD. Importantly, among smokers with COPD, even following withdrawal of cigarette smoke, inflammation persists and lung function continues to deteriorate as does the increased risk of lung cancer. These facts suggest a strong link between COPD-related lung inflammation and lung cancer, however, the precise mech-
anesthetic link is not known. Mucociliary dysfunction and mucin hyperproduction are important features of COPD with inflammation being the major trigger. Interestingly, lung cancer with mucin overexpression has higher malignancy potential and poor prognosis, which around 76% having mutations in K-ras oncogene, the most frequent oncogenic mutation in lung adenocarcinoma. Taken together, mucociliat dysfunction and K-ras mutant lung cancer by inflammation. Here we first investigated whether Muc5ac, predominant airway mucin that plays a primary role in inflammatory lung diseases, is predictive of clinical outcome in KRAS-mutant human lung adenocarcinomas. We determined Muc5ac mRNA expression by array analysis of 150 lung adenocarcinomas from patients that did not received neoadjuvant therapy. Muc5ac mRNA expression was predictive of poor disease-free survival in KRAS-mutant lung adenocarcinomas. We have further found increased mucin and high expression of Muc5ac in lung tumor tissues of the mice with airway specific expression of a mutant form of K-ras (CC-LR mice). Then, we crossed previously developed Muc5ac knockout (KO) mice to CC-LR mice in order to develop a K-ras mutant lung cancer mouse model with lack of Muc5ac (CC-LR/Muc5ac KO mice). This resulted in a significant tumor reduction by ~54% (2.2-fold) in lung of CC-LR/Muc5ac KO mice compared to age and sex matched control CC-LR mice. Lung inflammation was evaluated by analysis of bronchoalveolar lavage fluid and revealed a significant reduction (3-fold) in number of macrophages, and levels of IL-6 and IL-17 in CC-LR/Muc5ac KO mice compared to CC-LR control mice. Immunohisto-pathological analysis of lung sections confirmed lower inflammation, decreased tumor number and size, less adenomatous lesions, and reduced tumor cell proliferation and angiogenesis in CC-LR mice with lack of Muc5ac compared to control CC-LR mice. Our experimental results suggest that Muc5ac has an essential role in promotion of K-ras mutant lung cancer through autocrine cell intrinsic and paracrine immune cell mediated mechanisms.

**#3715A Effusions of mesothelioma patients: What’s in it for immunotherapy.** Elly Marcq,1 Jorrit De Waele, 1 Jonas van Audenaerde, 1 Eva Lion,2 Eva Santermans,3 Niel Hens,3 Patrick Pauwels,4 Jan P. van Meerbeeck,5 Roger A. Greenberg,6 Anthony S. Lopes,7 Fiona Simpkins,8 Robert Doot, Sharon Lee,9 and Santermans1.

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Malignant pleural mesothelioma (MPM) is an aggressive cancer with a poor prognosis for which new therapeutic strategies are available. Data derived from a large cohort of mesothelioma patients suggest that blocking immune checkpoints could offer new treatment opportunities. Gaining more insight in the immunological aspect of the tumor microenvironment (TME) in MPM is essential to develop an effective immunotherapy. In this context, we investigated expression of the immune checkpoint molecules TIM-3, LAG-3, PD-1 and its ligand PD-L1 on cells in human pleural (collected via thoracocentesis, n=6) and ascites (collected via paracentesis, n=5) MPM fluids and identified different subsets of immune cells in the TME using multicolor flow cytometry. Different types of immune cells in the fluids could be detected, with predominant occurrence of CD3+/CD4+ T cells, CD64+ macrophages and CD11c+/CD303+ dendritic cells (DCs). CD3+/CD8+ T cells, CD3+CD56+ natural killer (NK) cells and CD19+ B cells were also present to a lesser extent. While CD4+ and CD8+ T cells and CD64+ macrophages were expressed in all samples in the case of the other cell types. Ascites fluids contained more podoplanin+ (PDPN) tumor cells compared to pleural fluids (average 3.0% vs 0.3%). PD-1+ T cells and NK cells could be detected in all pleural and 80% of the ascites samples. 64% of all samples contained LAG-3-expressing cells, most frequently in pleural fluids. In all samples CD4+ and NK cells showed TIM-3 expression and in 82% of the samples TIM-3 was also expressed on CD8+ T-cells. In all ascites samples PD-L1 was expressed on DCs, B cells, macrophages and PDPN+ MPM tumor cells. PD-L1 was also expressed on DCs in all pleural samples while in 67%, 83% and 83% of those samples CD19+, CD64+ and PDPN+ cells were PD-L1+, respectively. Based on the mean fluorescence intensity PDPN+ tumor cells showed the highest PD-L1 expression in both fluids. In conclusion, we provide a detailed analysis of a diversity of immune cells present in MPM fluid samples. We are the first to demonstrate TIM-3 and LAG-3 expression in mesothelioma effusions. Statistical analysis is ongoing to investigate whether there are differences between both fluid types and whether there are associations between the cellular composition of the fluids and survival of the patients. The finding of expression of PD-1, PD-L1, TIM-3 and LAG-3 on immune cells in MPM fluids warrants further investigation of the effect of immune checkpoint blockade in MPM, with TIM-3 and LAG-3 as interesting new targets.

**IMMUNOLOGY: Inflammation in the Tumor Microenvironment**

**#3715 Analysis of tumor infiltrating lymphocytes and in vitro exhausted tumor-derived T-cells to identify unique Immuno-Oncology targets.** Joanne Berry,1 Yaoyao Fu,2 Joanne Goodchild,1 Haining Huang,2 Livija Deban,1 Robert Boyd1. 1Oxford BioTherapeutics Ltd, Abingdon, United Kingdom; 2Oxford BioTherapeutics Inc, San Jose, CA.

In cancer, T cells are exposed to persistent antigen stimulation and/or inflammatory signals. Such prolonged stimulation is often associated with deterioration of T cell effector functions resulting in a functional state termed ‘exhaustion’. Exhausted T cells are characterized by expression of multiple inhibitory receptors, loss of cytolytic function and decline in cytokine production capability. T cell exhaustion is likely associated with inefficient control of tumor progression. Identification of cell surface proteins involved in induction and maintenance of the exhausted state could inform novel immunomodulatory cancer therapies. To comprehensively investigate cell surface signature of the exhausted state and identify potential immunoncology (I-O) targets, we conducted in-depth expression profiling of membrane proteins from intact tumors (Colonorectal, Pancreatic, Lung, Breast and Prostate cancer), different in vitro models of T cell exhaustion and primary tumor-derived lymphocytes (Colonorectal, Breast and Lung cancer) by targeted proteomics for known cell type and activation markers. Using proteomic expression analysis and statistical clustering analysis methods, novel I-O targets were identified in both the in vitro models of T cell exhaustion and primary tumor-derived lymphocytes (TILs). The cancer specify of novel I-O targets was then interrogated using the expression profiling of cell membrane proteins from intact tumors. Using this approach we identified most known T cell inhibitory receptors (PD-1, CTLA-4, BTLA, TIM-3, KIRs, LAG-3 and TIMG72) and their described ligands (B7-H1 to B7-H7). To generate more precise expression data to validate I-O targets emerging from the above discovery approaches and their role as interacting partners we analyzed single tumors using an integrated evaluation methodology. Tumors were subject to multiple parallel analysis steps: (i) proteomic analysis of the tumor and TILs; (ii) TILs were subject to flow cytometry analysis for specific proteins; (iii) tumor tissue sections were subject to immuno-histochemical analysis for specific proteins. The multiple analysis platforms were integrated by key cell type and functional markers allowing the different types of information provided by each technique to be placed in an overall 3-D tumor context. We present one example of a potential I-O target identified using our integrated proteomic discovery approach which is further validated at the expression level using multiple whole tumor and TIL analyses.
expression were evaluated. In parallel clinical PET/CT imaging of PARP-1 was performed in epithelial ovarian cancer patients with correlative tissue histology. Results: Our studies indicate there is a spectrum of PARP-1 expression in epithelial ovarian cancer and in vitro BRCA1 mutants show higher PARP-1 expression compared to non-BRCA mutants. In addition, PARP-1 expression is required for PARP inhibitor efficacy in vitro and is either more significant or equal to BRCA1 mutational status. Also, high PARP-1 expression corresponded with platinum sensitivity in vitro. Furthermore, we observed PARP-1 expression increased in response to genotoxic insult relative to DNA damage measured by gH2AX. Lastly, our observations have been further supplemented by clinical \[\text{[18F]}\text{FTT}\] PET/CT images in ovarian cancer patients, which showed a spectrum of PARP-1 expression. DNA damage measured by gH2AX and DNA repair deficiencies and to provide a biomarker for assessing response to PARP-1 expression that corresponded with DNA damage measured by gH2AX. Conclusion: PARP-1 expression has the potential to identify functional DNA repair deficiencies and to provide a biomarker for assessing response to DNA damaging therapies. In complement, clinical PET imaging with \[\text{[18F]}\text{FTT}\] offers a novel technology to determine PARP-1 expression in ovarian cancer patients and warrants further study.

#3719 Bicyclic peptides for PET imaging of MT1-MMP expressing tumors. Daniel Teufel,1 Helen Harrison,2 Spencer Campbell,1 Catherine Stace,3 Edward Walker,1 Robert J. Lutz,1 Peter Park,1 Matthias Eder,2 Ulrike Bauder-Wüst,2 Ursula Schierbaum,1 Karin Leotta,2 Klaus Kopka,1 Uwe Haberkorn1.

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The Bicycle\textsuperscript{TM} technology is based on repertoires of short peptides displayed on the surface of bacteriophages which can be modified with homo-trifunctional organochemical scaffolds, thus creating large diverse libraries of constrained, bicyclic peptides. These large combinatorial libraries have been extensively used for iterative selections to identify high affinity binding peptides to a wide array of targets, including receptors, interleukins and proteases. Bicyclic peptides are chemically synthesized macrocyclic entities with drug-like properties that exhibit sub-nanomolar affinities and exquisite selectivity towards targets. Unlike biologics, their synthetic nature allows facile modulation of metabolic and pharmacokinetic properties, as well as site-specific conjugation to effector molecules such as fluorophores, radionuclides, and cytotoxic drugs. In the present work, novel phage display-derived bicyclic peptides were identified targeting the matrix metalloproteinase 14 (also known as MT1-MMP), a tumor associated surface protein overexpressed in a variety of cancers (i.e. lung, breast). A prototype bicyclic peptide with high affinity to MT1-MMP (Kd at ~1 nM) was identified, and confocal microscopy using fluoresceinated bicyclic peptide derivatives shows target-dependent internalisation in MT1-MMP\textsuperscript{TM} cells. In the in vivo setting, a tumor binding contrast agent, \[\text{[18F]}\text{FTT}\] labeled with a DOTA conjugate loaded with Ga-68 or Lu-177. Upon proteolytic optimization of the prototype bicycle peptide, a striking enhancement in tumor signal is observed in biodistribution studies. Compared to radiolabeled antibodies directed against the same target, the lead compound showed fast background clearance (< 1 %ID/g for all organs apart from kidneys) resulting in high imaging contrast in \(\mu\)PET studies as early as 30 minutes post injection. Importantly, most of the non-tumor associated bicyclic peptide rapidly clears into the bladder. Together, tumor targeting bicyclic peptides can, through their small size and high selectivity, facilitate efficient penetration and visualization of tumors in vivo, demonstrating their potential as diagnostic imaging agents in profiling and therapeutic management of patients.
for the better outcome for CTL mediated immunotherapy for cancer. Somdutta Roy, Kevin Martínez, Archana Dilip, Holger Karinskyy, Scott Dylla, Abbvie STemCenters, South San Francisco, CA.

Small cell lung cancer (SCLC) is an aggressive neuroendocrine tumor with early dissemination and poor prognosis that accounts for 15-20% of all lung cancer cases worldwide. Most cases are inoperable and biopsies to investigate SCLC biology are rarely obtainable. Circulating tumor cells (CTCs), which are prevalent in SCLC, present a readily accessible liquid biopsy. Here we present CTC data from aggressive, late stage SCLC patients who were enrolled on a Phase 1 trial studying Rova-T (anti-DLL3 antibody drug conjugate). CTCs were captured using a microfluidic platform (IsoFlux) that uses flow control and immunomagnetic capturing of epithelial cell surface marker (EpCAM)-positive cells. CTCs were isolated before dosing (baseline) of Rova-T and at successive time points post treatment. CTCs from each time point were distinguished and enumerated using DAPI, EpCAM and the cytoplasmic marker (pan-Ck), in addition to the absence of CD45. Enriched CTCs were also collected for RT-PCR for expression analysis of genes confirmed to be absent in blood. The number of CTCs varied among the 58 baseline samples collected, ranging from 5-1000 CTCs per 7.5 ml of blood. When the CTCs were assessed for DLL3 target expression, 37/58 baseline samples expressed DLL3 by RT-PCR. Interestingly, the expression of DLL3 on the baseline CTCs correlated with treatment outcome. When additional time points after treatment were enumerated for CTCs, patients who responded to Rova-T had DLL3+ CTCs at baseline. Patients with DLL3+ CTCs at baseline showed a significant decrease in DLL3 after treatment. This suggests that Rova-T might kill DLL3-expressing CTCs. These data support continued investigation of DLL3 expression on CTC in SCLC patients with the goal of facilitating a liquid biopsy able to assess DLL3 status as a prospective companion diagnostic for Rova-T.

#3722 In vitro induced regulatory T cells for the understanding of the nature of peripherally induced regulatory cells in relation to cell mediated immunotherapy for cancer. Upendra P. Hegde, Nitya G. Chakraborty, Univ. of Connecticut Health Ctr., Farmington, CT.

Background: pTregs are appearing to be one of the major obstacles of cellular immunotherapy of cancer. Tumor specific in vivo induced Cytotoxic T lymphocytes (CTL) are rapidly eliminated with concomitant expansion of pTreg cells. In human melanoma in ex vivo condition, tumor antigen specific CTL generated with CD8+ cells persisted for months while ex vivo induced CTL with whole PBL stimulated with Mart-1, A2 (tumor antigen) pulsed autologous DC, died within three weeks. Whereas Influenza (Flu 36-45) antigen specific CTL continued to expand and remained functional beyond four weeks. Method: Peripheral blood and tumors were obtained from patients diagnosed with metastatic melanoma after informed consent. Blood derived dendritic cells, PBL, CD8+ and CD4+ cells were isolated for in vitro co-cultures with Mart-1 or Flu peptide pulsed autologous DC or autologous tumor cells. CD8+ CTL response and CD4+ positive Treg responses were measured. Freshly obtained tumor tissue derived lymphocytes (TIL) were also used for the study. Results: Our results show that CTL response against Flu survived longer in culture with total PBL than CTL response against Mart-1 in identical culture conditions. CTL response using autologous tumor cells with PBL in culture lasted for a shorter duration but growth of Treg cells were observed. Cytokine analysis with expanding Treg cells showed secretion of IL-10 upon re-stimulation with autologous DC or anti-CD3 ab. Similarly, CD4+ cells isolated from freshly obtained tumor tissue (TIL, representing in vivo condition), suppressed T cell activation and secreted high amount of IL-10 upon re-stimulation. Conclusion: These findings suggested that in vitro induced iTreg from cultures or pTreg from TILs (in vivo), work identical way, to negatively regulate effector immune response by secreting IL-10. Further analysis with iTregs for their use of metabolic pathways would be important to identify the specific signaling molecules used by autologous (person specific) pTregs, as target molecules, to block the expansion of such pTregs for the better outcome for CTL mediated immunotherapy for cancer.

#3723 Assessing the reproducibility of computer-aided nodule assessment and risk yield (CANARY) method to characterize lung adenocarcinomas. Erica C. Nakajima,1 Michael P. Frankland,2 Tucker Johnson,3 Sanja L. Antic,1 Ronald A. Karwoski,2 Bennett Landman,3 Heidi Chen,1 Ronald C. Walker,1 Brian J. Bartholmai,1 Tobias Peikert,1 Srinivasa Rajagopalan,1 Pierre P. Massion1, Fabien Maldonado1,1 Vanderbilt University Medical Center, Nashville, TN;2Vanderbilt University, Nashville, TN;3Mayo Clinic, Rochester, MN.

This study assessed the inter-user reproducibility of Computer-Aided Nodule Assessment and Risk Yield (CANARY), a novel analytical tool that risk stratifies lung adenocarcinomas (ADCs) according to defined computed tomography (CT) structural characteristics. CANARY detects nine distinct voxel signatures in ADCs based on standard CT imaging, thereby defining nodule characteristics correlating with patient outcomes, and corresponding to invasion or lepidic growth histologically. A software user segments the borders of each ADC prior to voxel analysis, introducing potential variability into the assessment. While CANARY is a promising tool, it requires validation of analytical variability between users and prediction of accuracy in an independent cohort. To evaluate the reproducibility of CANARY analysis, three independent users who are not part of the CANARY development team segmented and analyzed 50 biopsy-confirmed primary lung ADCs from Vanderbilt University Medical Center. The CT scans of ADCs were selected retrospectively based on the following criteria: ADC histology proven on biopsy, diagnosed between 2009-2015, less than 3cm in greatest diameter, and stages IA-IV. Users followed a standard operating procedure established at the Mayo Clinic, and were blinded to clinical characteristics and patient outcomes. Results: To measure inter-user variability of ADC voxel composition, the intraclass correlation coefficient (ICC) was calculated based upon the percentage of each voxel subtype within the whole ADC. An ICC of 1 reflects high reproducibility between users. Among all 50 ADCs, the average ICC for all nine voxel types was 0.828 (95% CI 0.76, 0.895). The ICC of the four voxel types associated with invasive features on CT was 0.865 (95% CI 0.805, 0.924). ICCs were also calculated using a logarithmic transformation for data normalization, generating an ICC of 0.745 (95% CI 0.663, 0.826) for all nine voxel types, and an ICC of 0.995 (95% CI 0.993, 0.997) for four voxel types associated with invasion. Conclusions: (1) CANARY analysis of lung ADC voxel signatures appears to be reproducible amongst users, making it a reliable tool for the evaluation of ADC voxel density. (2) Correlation of invasive signatures associated with more aggressive ADCs was nearly perfect amongst users. Additional validation metrics for CANARY with larger datasets are being evaluated, including the accuracy of tumor prognostic predictions between users and analysis of ADC datasets from other institutions.

#3724 PD-L1 protein expression and tumor mutation burden in hematologic malignancies: correlation with Hodgkin and high grade lymphoma. Lina Abdul Karim,1 Peng Wang,2 Jose de Guzman,1 Brandi Higgins,3 Jeffery Chahine,1 Christine Sheehan,2 Bhaskar Kallakury,1 Jeffrey S. Ross2. 1Medstar Georgetown University Hospital, Washington, DC; 2Albany Medical College, Albany, NY.

Background: The success of anti PD-1 therapy in Hodgkin lymphoma has led to clinical trials in other types of lymphomas. However, there is limited data on PD-L1 expression in acute leukemias. In this study, we analyzed PD-L1 protein expression and tumor mutational burden in various hematologic malignancies including leukemias, lymphomas and myelomas. Methods: In the IHC cohort, formalin fixed paraffin embedded sections from 92 hematologic malignancies including acute myeloid and lymphoblastic leukemia (AML, ALL), Hodgkin lymphoma (HL), diffuse large B-cell lymphoma (DLBCL), low-grade non-Hodgkin lymphoma (LGNL) and myeloma were immunostained using the PD-L1 approved PD-L1 IHC 22C3 pharmDx and mouse anti-PD-L1 antibody (DAKO clone 22C3). Any perceptible partial or complete membrane staining in all viable tumor cells was scored as follows: <1%-negative, 1-49%-low level and >50%-high level PD-L1 expression. In the second cohort of 2064 cases, comprehensive genomic profiling (CGP) was performed using a hybrid–capture, adaptor ligation assay to a mean depth of >672X. Tumor mutation burden (TMB) was calculated from a minimum of 1.11MB of sequenced DNA as previously described and reported as mutations/Mb. High PD-L1 expression was noted in 100% of HL, DLBCL showed significantly higher PD-L1 positivity compared to LGNHL (p=0.01). No correlation for PD-L1 expression was noted between AML and ALL (p = 0.2). On CGP analysis of the second cohort, TMB was significantly higher in DLBCL as compared to LGNHL (p<0.0001). Conclusion: The high PD-L1 expression in both HL and DLBCL and high TMB in DLBCL may be linked to enhanced responsiveness to checkpoint inhibitor therapy in these cancers. PD-L1 expression and TMB are relatively infrequent and at low levels in acute leukemias. Additionally, novel anti PD-1/PD-L1 therapeutic strategies are worthy of consideration for both advanced stage refractory/relapsed acute myeloid and lymphoblastic leukemias.
Introduction: Everolimus is an oral mTOR inhibitor. It is used for its antitumor effect in breast cancer. This study summarizes the clinical outcomes and noted adverse events from sequencing-derived combination treatments that include everolimus in the neoadjuvant setting. Material and Methods: To date, 57 breast cancer patients have been enrolled to clinical trial NCT02470715 and treated in the neoadjuvant setting. The data presented here focus specifically on 17 patients that have long enough follow up and that have been treated with everolimus-based combinations. FoundationOne© test and Theralink© assay was used for genomic profiling and proteomic analysis respectively. Clinical outcomes were assessed at the time of surgery. A pathological complete response was defined as the absence of residual disease. Results: Everolimus was prescribed for the patients in this study based on the findings from their genomic or proteomic testing, targeting PI3K/AKT/mTOR pathway. The evidence for using everolimus in ER+ patients (n=13) includes alterations in PIK3CA (n=9), PTEN (n=7), 5FGS (n=2), and phospho-S6 ribosomal protein (Ser235/236)/4E-BP1 (phospho-S65) which was targeted in the Her2+ (n=1) patient. PIK3CA (n=2) and PIK3CB (n=1) presented in the 3 triple negative (TN) patients. Everolimus and paclitaxel was used in all patients as the backbone combination while trastuzumab was added for Her2+ patients and hormonal therapy was added for ER+ patients. Aromatase inhibitors were used in most of the cases as hormonal therapy in ER+ patients while goserelin was used in 3 cases for premenopausal patients. Pazopanib and temsirolimus were added to two of the TN patients and palbociclib was added for one ER+ /Her2- patient targeting other alterations. From the 17 patients (clinical stage I to III) treated with everolimus in the neoadjuvant setting, pathological complete response was achieved in 4 (23%) patients (2 ER+, 1 Her2+ and 1 TN). 65% (n=11) patients had partial response (10 ER+, 1 TN). Only one TN patient did not respond to the neoadjuvant treatment. Over half of the patients (59%) tolerated the treatment well while 6 patients (35%) developed adverse events which resulted in a change of regimen. Grade 3 and above adverse events including mucositis, cough and fever were observed in ER+/Her2- patients who were treated with everolimus + paclitaxel + hormonal therapy. The symptoms were managed by a dose reduction or a period of treatment hold. When one of the patient treated with everolimus + paclitaxel + anastrozole + trastuzumab developed a UTI, diarrhea, mucositis and edema after 7 weeks of treatment. Treatment was stopped in this case. Conclusion: Based upon our findings, everolimus is an effective drug in the neoadjuvant treatment of early stage breast cancer. Even though the numbers are small, our outcomes show significant promise. Adverse events related to the treatment were manageable and did not negatively impact our ability to give the treatment in most cases.

**#3725 Neooadjuvant everolimus as a targeted therapy for early stage breast cancer.** Bing Xu, Casey Williams, Brian Leyland-Jones. Avera Cancer Institute, Sioux Falls, SD.

**#3726 INCISB2793 JAK1 inhibitor synergizes with ATRA to inhibit expansion of AML.** Haley E. Ramsey,1 P. Brent Ferrell,1 Melissa A. Fischer,1 Agnieszka E. Gorska,1 Caroline Maier,2 Jeremy Norris,2 Melissa Farrow,2 Danielle SiouxFalls,SD.1

**#3727 Plasma inhibitory activity (PIA) is a possible pharmacodynamic marker for clinical development of a next generation pan-TRK inhibitor, ONO-3590556.** Ryohye Kozaki, Ryu Fujikawa, Hikaru Kato, Natsuka Goto, Toshio Yoshizawa. Ono Pharmaceutical, Osaka, Japan.

Background: Colorectal cancer and Mammary analogues secretory carcinoma harboring Neurotrophic tyrosine receptor kinase (NTRK/TRK) gene rearrangements developed resistance to Entrectinib, ALK/ROS/TRK inhibitor. We previously demonstrated that the next generation pan-TRK inhibitor, ONO-3590556 may potentially overcome Entrectinib-resistance mutations. Furthermore, inhibition of Trk phosphorylation in tumors has excellent correlation with the in vivo anti-tumor effect by ONO-3590556 (AACR2016, Kozaki et al). Purpose: Accurate measurement of target inhibition in a phase 1 clinical trial is critical to informing selection of appropriate doses for ONO-3590556 in more advanced clinical trials. Plasma inhibitory activity (PIA) assay has recently been performed as a surrogate assay in clinical trials of FLT3, MET and ALK kinase inhibitors. The assay employs the incubation of Tyrosine Kinase expressing cell lines in aliquots of plasma collected at various time points from patients treated with TKI. Herein, we report the preclinical evaluation of PIA assay in accordance with an inhibition of TRK phosphorylation in tumors. Methods: KM12 cells, human colorectal cancer cell lines expressing TPM3-TRKA, were implaned subcutaneously into nude mice. Mice were randomized when the mean tumor volume was 150-600 mm3. Tumors and blood were collected from mice, 2, 4, 7 and 24 hours after the single treatment of ONO-3590556 with the doses of 0.06 and 0.6 mg/kg. The collected tumors were disrupted and phosphorylated Trk phosphorylation in tumors was measured. Results: Pilot M1 assay, KM12 was incubated in plasma from the collected blood for 4 hours and phosphorylated TrkA in the cells was detected by ECL. Results: Treatment with ONO-3590556 at doses of 0.06, 0.6 mg/kg resulted in a significant inhibition of Trk phosphorylation in tumors up to 24 hours. Compared to inhibition of P-TRK in tumors, the inhibition of P-TRK in PIA sustained until 7 hours but rapidly decreased at 24 hours after administration. The levels of both tumors and PIA showed in a dose-dependent inhibition and an excellent correlation until 7 hours. Conclusion: Plasma inhibitory activity (PIA) assay correlates with inhibition of P-TRK in tumors. Our results demonstrated that the potential utility of PIA as a PD marker may contribute to determining the effective dose of ONO-3590556 in the clinical development.

**#3728 Imaging biomarkers of aromatase-inhibitor induced joint pain.** Brian A. Goldstein, Miha Tanajonvic, Pavani Chalasani, C. Kent Kwoh, Amanda Hadden, Russell Witte, Jessica Martinez. University of Arizona, Tucson, AZ.

Aromatase inhibitor positive (ER+) breast cancer patients undergoing treatment with aromatase inhibitors (AI) tend to discontinue the supplement when to painful side effects appear. It is believed that women with AI-induced musculoskeletal syndrome (AIMSS) have specific physiological conditions that differentiate them from women with normal aging-related in.
flamatory processes (e.g., rheumatoid arthritis or RA). Ultrasound shear wave elastography (USWE) provides a real-time, quantitative assessment of the elastic modulus of soft tissue, which is a potential biomarker that is altered in women with AIMSS. Because USWE is a completely noninvasive technique available on state-of-the-art clinical ultrasound scanners, it can be repeatedly used in calculating the shear modulus of patients whose elastographic images change due to the presence of adverse effects during therapy. A Siemens s3000 clinical scanner and 9 MHz linear probe were used for USWE of the hands and wrists in postmenopausal women on AI, postmenopausal women with RA and healthy postmenopausal women. The scans included an evaluation of major wrist tendons, nerves, and joints with shear wave velocities ranging from 0.5 to 15 m/s, which are directly related to the local shear modulus – an absolute measure of tissue stiffness. The ultrasound images and raw shear wave data were then analyzed in MATLAB™ using a custom graphical user interface (GUI) to calculate the distribution of velocities (or shear moduli) in a user-defined region of interest (ROI). The results were exported to a spreadsheet in Excel for further analysis and comparison among the different groups. Initial results suggest the shear wave velocity and estimated Young’s modulus (A) were significantly higher in AI patients (14.6 ± 0.2 m/s, λ=642 ± 17 kPa) compared to RA (6.84 ± 1.1 m/s, λ=143 ± 43 kPa) and healthy (7.58 ± 0.9 m/s, λ=174 ± 42 kPa) patients. These values suggest that the mechanical properties of tendons in the wrist may change during treatment with AI and contribute to joint pain in these subjects. It is critical that ER+ patients on AI can be monitored for signs of joint pain, and if found, to monitor for a biopsy as a possible source of pain. Patients on AI + USWE may help predict which patients are most susceptible to these side effects, promote early intervention to reduce or eliminate symptoms, and help increase adherence to AI therapy.

Tumor-specific antibody labeling of pancreatic cancer in a patient-derived orthotopic xenograft (PDOX) mouse model using a fluorescent humanized anti-CEA antibody. Thinzar Lwin, Takashi Murakami, Robert M. Hoffman, Paul J. Yazaki, Michael Bouvet 
University of California, San Diego, La Jolla, CA; City of Hope Medical Center, Duarte, CA.

Introduction: Delineation of tumor margins is critical in oncologic surgery, particularly in resection of pancreatic cancer. Surgeons are limited in visualization with bright light surgery. Fluorescence guided surgery (FGS) can help surgeons better visualize all potential cancer cells during the operation. In the present study, we used the fluorescent dye Anti-CEA-800 conjugated to an 800 nm NIR fluorescent dye can selectively label pancreatic cancer in both pancreatic cancer cell lines and patient derived xenograft mouse models (PDOX). Materials/Methods: BxPC3-GFP cells or pancreatic cancer tissue specimens were grouped into flanks of nude mice. Both types of tumors were allowed to grow until 1 cm. Each tumor was then treated with a single injection of anti-CEA-800 dye at the tail base (LI-COR 800 CW IRDye®, Lincoln, NE). Mice were imaged via the Maestro CRI imaging system. Result: Images obtained after injection of Anti-CEA-800 dye were injected into the tail-base (LI-COR 800 CW IRDye®, Lincoln, NE). Mice were imaged via the Maestro CRI imaging system. Perkin Elmer, Waltham, MA) 24 hours after injection. Results: Images obtained after injection of Anti-CEA-800 dye were injected into the tail-base (LI-COR 800 CW IRDye®, Lincoln, NE). Mice were imaged via the Maestro CRI imaging system. Perkin Elmer, Waltham, MA) 24 hours after injection. Images obtained after fluorescence antibody injection showed that anti-CEA-800 specifically labeled both the cell-line-derived tumors and patient-derived tumors with an adequate tumor to background contrast at 24 hours. The dye co-localized with GFP in both the cell-line-derived tumors and patient-derived tumors with an adequate tumor to background contrast at 24 hours. The dye co-localized with GFP in both the cell-line-derived tumors and patient-derived tumors. Conclusion: Anti-CEA-800 conjugated with the IR-800 dye is a promising agent for future clinical FGS applications.

Early metabolic response to tumor-treating fields in patients with recurrent glioblastoma. Sandeep Mittal, Geoffrey R. Barger, Edit Bosnyák, Varun B. Shah, Csaba Juhász. Wayne State University, Detroit, MI.

PURPOSE: Tumor-Treating Fields (TTFields) is an FDA-approved treatment approach for patients with newly-diagnosed and recurrent glioblastoma. The main purpose of the present study was to explore if amino acid position expression tomography (PET) is able to detect an early, objective metabolic tumor response during the first months of TTFields therapy. METHODS: Patients with MRI signs of recurrent glioblastoma eligible for TTFields along with maintenance temozolomide were recruited for PET scanning with alpha-[C-11]-methyl-L-tryptophan (AMT) at baseline (before start of TTFields application) and up to 3 months later. Serial MRIs were also performed in 1-2 months intervals. MRI accumulation in the MRI-detected tumor mass was measured by standardized uptake values (SUVRs), and PET-based tumor volume was calculated from the PET scans. Further, MRI and PET data were compared to changes in areas of MRI enhancement. RESULTS: Out of 7 recruited patients, 5 showed appropriate compliance (>75%) with the device and underwent follow-up PET. Four of the 5 patients showed a clear metabolic response during TT treatment on PET imaging. Two patients, who received TTFields treatment only (in addition to maintenance temozolomide), showed 25% and 100% decrease of PET-based tumor volumes, respectively. MRI was stable (patient #1) or showed increasing contrast enhancement (#2) during the same period. In this latter patient, TTFields was continued with bevacizumab treatment, leading to decreased contrast enhancement and further decrease of AMT uptake on a second follow-up PET. In two patients (#3 and #4), TTFields were combined with bevacizumab rescue treatment. Patient #3 showed a markedly decreased tumor AMT uptake and metabolic volume along with stable MRI enhancement. Patient #4 showed interval decrease of tryptophan PET uptake 2 months after initiation of TTFields therapy. Finally, patient #5 showed an interval expansion of the PET-based tumor volume along with MRI progression during a 2-month follow-up despite TTFields combined with bevacizumab and carboplatin. CONCLUSIONS: This is the first study to demonstrate an objective metabolic response of recurrent glioblastoma within 1.5-3 months after initiation of TTFields. The data suggest that amino acid PET can identify early responders to TTFields therapy.
#3734 Pharmacokinetics of sacituzumab govitecan (IMMU-132), an anti-body-drug conjugate (ADC) targeting Trop-2, in patients with diverse advanced solid tumors. Robert M. Sharkey, 1 Allyson J. Ocean, 2 Alexander N. Starobud, 3 Aditya Bardia, 4 Michael Guarino, 5 Wells A. Messersmith, 6 Jordan D. Berlin, 7 Vincent J. Picozzi, 8 Rebecca Moroose, 9 William A. Wegener, 1 Pius Ma- li, 10 Volker Endris, 11 Martina Kirchner, 12 Matthias Kloor, 13 Anna-Lena Volkmar, 14 Magnus von Knebel Doeberitz, 15 Roland Penzel, 16 Peter Schirmacher, 17 Albrecht Stenzinger. 18 Univ. of Heidelberg, Heidelberg, Germany.

#3735 Detection of microsatellite instable colorectal cancers from routine NGS panel sequencing data. Volker Endris, Martina Kirchner, Matthias Kloor, Anna-Lena Volkmar, Magnus von Knebel Doeberitz, Roland Penzel, Peter Schirmacher, Albrecht Stenzinger. Un. of Heidelberg, Heidelberg, Germany.

Introduction: In about 15% of all colorectal cancers, microsatellite instability (MSI) caused by defects in the DNA mismatch repair machinery can be detected. MSI driven tumors have a better prognosis compared to microsatellite stable (MSS) tumors and show good response to drugs targeting the PD-1-PD-L1 axis. Current molecular diagnostic procedures for colorectal cancer are primarily focused on the detection of oncoclonic mutations in RAS family genes by either classical PCR, next generation sequencing (NGS) or panel testing. The MSI status for therapeutic purposes is still rare and usually carried out by a different array of methods including PCR and immunohistochemistry. Experimental procedures: In our routine diagnostic CRC screening, we sequenced more than 600 colorectal tumors with an entity specific customized 180 ampli- con NGS panel encompassing HotSpot regions of 31 genes. This panel includes several homopolymeric regions of genes that are known to be affected by MSI. We retrospectively analysed this data set for MSI signatures, including deletion/insertion mutations in 20 different homopolymeric regions (>7) and mutations/deletions of MLH1, MSH3, MSH6 and TGBFR2. Cases with a MSI signa- ture detected by panel sequencing were reevaluated by PCR-based screening.
#3736 Characterization of tumor cells and assessment of PD-L1 expression in pleural effusions of metastatic non-small cell lung cancer patients. Jeffrey C. Thompson, 1 Samantha L. Savitch, 2 Ryan Fan, 2 Stephanie S. Yee, 2 Christian A. Powers, 2 Gordon Yu, 3 Lauren Gebrian, 1 Chandra Rao, 1 Steve Gross, 1 Michael Feldman, 2 Anil Vachani, 1 Erica L. Carpenter, 1 1Division of Pulmonary, Allergy, and Critical Care Medicine, Thoracic Oncology Group, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; 2Division of Hematology-Oncology, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; 3Department of Pathology and Laboratory Medicine, Hospital of the University of Pennsylvania, Philadelphia, PA; 4Janssen Pharmaceutical, Johnson & Johnson, Pennsylvania, PA.

Introduction: Malignant pleural effusions (MPE) occur as a frequent complication of advanced non-small cell lung cancer (NSCLC), and denote a poor prognosis. Cytological evaluation of MPEs has a sensitivity of only 40-60%. The CellSearch technology (Janssen Diagnostics, LLC) is an FDA approved method of detecting rare circulating tumor cells (CTCs) in blood. Assessment of pleural fluid samples using CellSearch provides an approach to enrich often undetectable CTCs and phenotype these cells for markers of interest. PD-L1 staining of tumor tissue is essential for determining patient candidacy for checkpoint inhibitor therapy, however, tissue can often be difficult to obtain. Here, we demonstrate the feasibility of applying the CellSearch CTC enrichment platform to measure PD-L1 expression and enumerate tumor cells in MPE of NSCLC patients. Methods: This was a single center, prospective observational study of NSCLC patients with a pleural effusion. The anti-human CD45 (PD-L1) antibody (Biologend) was used as a marker with the CellSearch CXC CTC kit and was detected in the open 4th channel of the CellTracks Analyzer. Determination of CTC PD-L1 expression was validated through peripheral blood spiking experiments. 7.5ml of pleural fluid was collected from NSCLC patients, preserved in CellSave tubes and processed within 72 hours. Identification and enumeration of CTCs was performed using the CellTracks Analyzer. The CellSearch results to conventional cytological analysis. Results: Pleural effusion samples from 66 patients between the ages of 43 and 80 (median =69) were obtained following thoracentesis. The majority of patients were former (40; 60.6%) or never (21; 31.8%) smokers. At the time of effusion, 23 (34.8%) patients were found to have a MPE (median 1798, range: 7 to 114,920) compared to patients with a benign effusion (median 8, range: 0 to 953; p=0.005). In the MPE group, 18 (45.0%) samples demonstrated >10% PD-L1 positivity with 9 (22.5%) samples showing >50% PD-L1 positivity. Conclusion: The CellSearch CTC enumeration and characterization platform can be successfully adapted for detection of CTCs in MPE of NSCLC patients. Our preliminary results suggest this approach may be a useful, non-invasive diagnostic adjunct for the determination of a MPE and assessment of PD-L1 expression in NSCLC patients. In this study, we warranted to determine what role this test could play in assessing patients for checkpoint inhibitor therapy and its relationship with tissue based tests.

#3738 Use of tumor mRNA expression for patient selection in a phase I study of the pan-fibroblast growth factor receptor inhibitor BAY 1163877. Peter Ellinghaus, 1 Matthias Ocker, 1 Sebastian Bender, 1 Christoph Naegele, 1 Stuart Ince, 1 Markus Joerges, 1 Martin Schuler, 1 Bayer AG, Wuppertal, Germany; 2Bayer AG, Berlin, Germany; 3Bayer HealthCare Pharmaceuticals Inc, Whippany, NJ; 4Kantonsspital St. Gallen, Zürich, Switzerland; 5West German Cancer Center, University Hospital Essen, Essen, Germany.

BAY 1163877 is a potent and selective, oral, small molecule pan-FGFR inhibitor with anti-tumor activity in a wide range of cancer types. In vivo profiling in xenograft models identified tumor FGFR mRNA levels as a predictor of drug efficacy. Anti-tumor efficacy was largely independent of the tumor type surveyed or the FGFR isoform being overexpressed. Preclinical models included a patient-derived squamous head and neck cancer (HNSCC) xenograft (PDx) model overexpressing FGFR3 mRNA, a bladder cancer PDx model overexpressing FGFR2 mRNA, and a squamous NSCLC xenograft (PDx) model overexpressing FGFR1 mRNA. Against this background, clinical proof-of-concept was assessed by enrolling patients into the stratified expansion cohort of a Phase 1 study of BAY 1163877 (NCT01976741) based on tumor FGFR1-3 mRNA levels. FGFR1-3 mRNA was quantified in archival or newly obtained FFPE tumor biopsies by RNA in situ hybridization (RNAscope, ACD) and digital transcript counting (NanoString). In total, biopsies from >500 patients were studied for FGFR1-3 mRNA expression. An initial xenograft experiments showing that low to moderate FGFR1-3 mRNA overexpression was not sufficient for a robust drug response, only patients with an RNAscope score of 3 or 4 (range 0-4) or a normalized Nanorstring signal of 800 counts were eligible for enrollment. By applying these stringent criteria, FGFR1-3 mRNA positivity was on average observed with a 2-3 fold higher prevalence than published data for genetic aberrations of FGFRs in the respective tumor types (including amplifications, translocations, mutations). We further identified FGFR mRNA (+) tumor types in which genetic FGFR aberrations have not been previously reported. FGFR1-3 mRNA positivity ranged from 10% in lung adenocarcinoma to 45% in squamous non-small cell lung cancer (sqNSCLC) and 54% in HNSCC. As of August 2016, 57 FGFR mRNA (+) patients were enrolled and treated with BAY 1163877. Six patients were enrolled in a partial cohort (PD) by RULE 500, including an FGFR3 mRNA (+) HNSCC patient without FGFR3 amplification or translocation, an FGFR1 mRNA (+) adenoid cystic carcinoma patient, and an FGFR1 mRNA (+) sqNSCLC patient (both without FGFR1 gene amplification), as well as an FGFR3 mRNA (+) bladder cancer patient without FGFR3 amplification, mutation or translocation. These results suggest that high FGFR1-3 mRNA expression identifies patients sensitive to FGFR inhibition. This population includes patients and without known genetic aberrations of FGFR1-3 encoding genes. In summary, an mRNA expression-based selection approach may identify a broader patient population with potential benefit from BAY 1163877, including tumor types not previously associated with altered FGFR signaling.

#3739 Comparison of technologies for EGFR analysis within a subset of a randomized clinical trial. Mirko Marabese, 1 Massimo Broganni, 1 Martin Reijns, 1 Cedric Gouedard, 1 Geert Maertens, 1 Erwin Sablon, 1 Monica Ganzellini, 1 Marina G. Garassino, 1 Samuel Murray, 1 Mario Negri Inst. for Pharmacol. Research, Milan, Italy; 2Biocartis, Mechelen, Belgium; 3BioPath Innovations SA, Athens, Greece; 4Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy; 5Biomarker Solutions Ltd, London, United Kingdom.

Background: EGFR mutation analysis is laborious often requiring >5 working days and multiple slices of formalin-fixed paraffin-embedded (FFPE) tissue. Idylla™ is a fully integrated, automated molecular diagnostics platform combining speed, ease of use, high sensitivity, multiplexing capabilities, offering sensitive detection of 52 mutations including insertions and deletions in exons 18-21. Methods: We assessed 149 cases from the phase III TAILOR study (Garassino et al, Lancet Oncology 2013;14:981-8), where EGFR was centrally assessed. Relatative concordance between study entry classification to TAILOR (Sanger sequencing, Idylla™ and next generation sequencing (NGS) for EGFR were investigated. Single unprocessed sections were used per Idylla™ and NGS following pathological review. NGS was performed using the Ampliseq™ Colon and Lung Cancer Panel v2 on an Ion Torrent PGM™ (Thermo Fisher) in a reference laboratory; Idylla™ (Biocartis NV) was performed at Biocartis1. Samples and study genotypes were blinded to NGS and Idylla™. Results: There were 132 eligible calls to compare NGS vs Idylla™ (14 fails NGS, 1 Idylla™, 2 both); 108 to compare Sanger vs Idylla™ (38 fails Sanger, 2 Idylla™, 1 both); 101 to compare Sanger vs NGS (31 fails Sanger, 7 NGS, 9 both). For NGS, a minimum coverage of 500 reads was set at a clinically relevant LOD of 5% mutant allelic frequency for calls. Comparison NGS vs Idylla™: 46 EGFR (34.8%) mutation positive by Idylla™ vs 43 (32.6%) by NGS; Sensitivity 93.48%; Specificity 100.00 %. Comparison Sanger vs Idylla™: 41 EGFR (38.0%) mutation positive by Idylla™ vs 38 (35.2%) by Sanger; Sensitivity 85.37%; Specificity 95.52 %. Sanger v NGS: 39 EGFR (38.6%) mutation positive by NGS, 39 (38.6%) by Sanger; Sensitivity 87.18%; Specificity 91.94 %. Of 16 NGS failures 14 were reportable by Idylla™. Conclusions: Idylla™ proved to be a reliable platform for rapid EGFR mutational status evaluation after NGS failure. Compared to NGS, Idylla™ has several advantages: its ease of use, hands on time <5 mins, all-inclusive lyophilized cartridge requiring only one section of 5µm FFPE, restricted secondary manipulation errors in a busy workflow. Positive and Negative predictive values for both Idylla™ and NGS were excellent. Although NGS offers significant additional
clinical data availability through multiplexing, the number of samples classified as inadequate for NGS (14/16 giving result with Idylla”) indicates a likely requirement of more sample robust platforms in diagnostic workflows.

#3740 The pursuit of genetic mechanisms underlying supreme response to pazopanib treatment. Emmanuel Salouistros,1 Helen Latsoudis,2 Despoina Vassou,2 Irene Stratidaki,2 Niki-Antonia Gouvalaki,1 Vasiliki Fadoulouglo,2 Ioannis Drositis,1 Emmanouil Koundopidis,1 Kitty Pavlaki,2 Dimitrios Mavroudis,2 Nikolaos Androulakis,1 Dimitrios Katseopoulos2. General Hospital of Heraklion ‘Venezilo’, Heraklion, Greece; Institute of Molecular Biology and Biotechnology, FORTH, Heraklion, Greece; National and Kapodistrian University of Athens, Athens, Greece; University General Hospital of Heraklion, Heraklion, Greece.

Aim: Studying exceptional responders to standard treatments may provide broader insights into the genetic mechanisms underlying such responses and suggest therapeutic options that weren’t previously apparent. In this study we unravelled the genetic signature of undifferentiated sarcomas in order to disclose the molecular mechanisms that may underlie the patient’s exquisite sensitivity to Pazopanib. Methods: A female patient with massive undifferentiated gynaecological sarcoma experienced a dramatic response to Pazopanib that made debulking surgery possible. We performed whole exome sequencing on DNA isolated from both blood and formalin fixed paraffin-embedded (FFPE) tumour employing Ion Torrent technology. Variant annotation was performed using the Ion ReporterTM v5.0 (MA, USA) tool after application of specific filters. Subsequent curation of annotated data was completed using a combined semi-automated data analyses workflow developed by Minotech Genomics Facility with the implementation of two in house-developed lists regarding 24 pazopanib-related genes, and 96 angiogenesis-associated genes. We also performed targeted sequencing of Ion Ampliseq Comprehensive Cancer panel (CCP) that is strategically designed to interrogate coding DNA sequences and splice variants across 409 tumour suppressor genes and oncogenes. Results: Whole exome sequencing was conducted at 20x coverage for 91.9% and 60.5% of > 10,000 genes for germline and FFPE DNA samples, respectively. Targeted sequencing was conducted at 20x for > 97% of 409 genes of the comprehensive cancer panel. Comparative analyses revealed two variants common in germline and FFPE samples, localized in LTK(p.Val541Ile) and FGFR4 (p.Gly388Arg). Interestingly, examination of the annotated data for biologically plausible mechanisms revealed 2 yet uncharacterized heterozygous variants in two other members of the FGFR family, FGFR2 (c.2183A>G, p.Asn728Ser) and FGFR3 (c.2050G>A, p.Val684Ile) that were present only in the FFPE samples. Even though both variants reside within the catalytic tyrosine kinase domains, they are predicted to exert tolerable effects on the encoded FGFR2 and FGFR3 proteins. Since, Asn728Ser is highly conserved among different kinases of the FGFR family we chose this variant to predict a possible effect on Pazopanib binding. Specifically, protein structure simulation of FGFR2 revealed that asparagine residue at position 728 is located in the external side of the drug binding cavity and is thus predicted to not affect Pazopanib binding. Conclusion: We pursue the possible genetic or somatic modifiers underlying the excessive response to Pazopanib. The detection of two, yet uncharacterised, variants in known Pazopanib targets highlights the potential of next generation sequencing in disclosing the genetic signature of undifferentiated sarcomas in relation to the activity and effectiveness of certain therapeutic agents.


We have recently shown that quantification of Gleason 4 patterns (Q-Gleason) provides strong prognostic information beyond traditional Gleason grade groups (3+3, 3+4, 4+3, 8, 10-10) in prostate cancer, and that the prognostic power of this approach can be further integrated to build the Integrative Quantitative Gleason (IQ-Gleason) score, a continuous value ranging from 0 – 117.5. The current study was undertaken, to determine whether (and how) factoring in molecular markers could further improve the predictive power. We selected deletions of PTEN and 6q (by FISH analysis) as well as DNA ploidy information obtained by flow cytometry as examples for established molecular prognostic markers that provided strong and independent prognostic information in our study cohort of more than 17,000 prostate cancers. A “molecular score” was established for these markers including “penalty” points for presence of deletions/aneuploidy and “bonus” points for lack of deletions/diploid DNA status. Penalty and bonus points were separately determined for each parameter based on differences in the recurrence rate between cancers with and without deletions or aneuploidy. The “penalty” points were then added to the IQ-Gleason score points while the “bonus” points were subtracted from the IQ-Gleason in order to obtain the MIQ-Gleason score. The MIQ-Gleason provided strong prognostic information in univariate analyses when including 3, 5, and 9p in molecular data and including clinical stage, status of the resection margin and status of the resection margin. Comparison of Receiver Operator characteristics (ROS) between the MIQ-Gleason and the quantitative Gleason or classical Gleason revealed only a slight increase of the prognostic power for the MIQ-Gleason (AUC=0.781) as compared to the IQ-Gleason (AUC=0.771), while a massive difference was found as compared to the classical Gleason grade groups (AUC=0.719). In summary, our data demonstrate the power of quantitative Gleason scoring strategies as compared to classical Gleason grade groups. That adding selected molecular features improved predictive power only slightly demonstrates how difficult it is for molecular markers to compete with optimized morphological grading systems. However, the use of more and better molecular testing may provide further improvement beyond quantitative Gleason scoring.

#3742 Exposure burden for patients during an experimental study using a dual-energy contrast-enhanced spectral mammography. Kamila Rawojc,1 Ka-mil Kisielewicz,2 Anna Dziechciowicz,3 Monika Tomaszuk,2 Anna Cegi,2 Jankowska Anna,2 Cieśa Izabela,2 Najberg Dominika,2 Luczynska Elzieta,2 Bartosz Kietycha,3 The University Hospital, Krakow, Poland; Maria Sklodowska – Curie Memorial Cancer Center and Institute of Oncology, Krakow, Poland; M. Smoluchowska Institute of Physics, Jagiellonian University, Krakow, Poland.

Mammography provides early micro-calciﬁcation recognition, that is important for further breast cancer (BC) diagnosis. Imaging choice for BC detection is X-ray mammography (MG) with the use of high-resolution digital modality combined with iodinated contrast agents. However, a conventional MG has some sensitivity limitations, especially in patients with densely treated breasts. Moreover, MG contributes radiation risk for BC that is correlated with an exposure to ionizing radiation. Patients, for whom MG study does not give a clear interpretation are often further diagnosed by contrast-enhanced breast MRI, the most sensitive BC detection technique. Alternatively, it is limited by higher costs, lower availability and increased false positives. Dual-energy contrast-enhanced spectral mammography (CESM) is a new method applied in BC that enables accurate detection of malignant breast lesions similar to breast MRI. The aim of this study was to compare the usefulness of CESM versus MG in BC detection. Additionally, doses given to the BC patients during imaging were compared. In this study, 250 consecutive women (144 MG vs. 105 CESM), that were screened mammography between 2011 and 2014 were retrospectively enrolled in the study. The comparison of entrance surface air kerma and mean glandular dose values for both options were recorded, respectively and analyzed using different statistical and retrospective approach. Results of presented study showed that CESM might be a new diagnostic tool allowing an accurate detection of BC lesions, giving results similar to those received from magnetic resonance imaging. A good agreement found between sensitivity of the method and tumour diameters measurements based on CESM, supports this method as a useful tool as a potential replacement for breast MRI in the preoperative evaluation of BC patients. However, a radiation burden for patients might be signiﬁcantly higher than that for a standard full-ﬁeld digital mammography procedure. In summary, each method has its own benefits with respect to specific applications that are further discussed in the study.


Background: Neoadjuvant chemotherapy (NAC) is widely performed for patients with locally advanced breast carcinomas. It is important to obtain precise information using imaging modalities about the distribution of residual carcinomas after NAC to predict the success of breast conserving surgery. However, the relationship between magnetic resonance imaging (MRI) shrinkage patterns and molecular subtypes of breast carcinomas has not yet been investigated. Methods: We analyzed the MRI shrinkage and pathological patterns of residual carcinomas after NAC in 54 consecutive breast carcinomas. The shrinkage patterns were classified into five categories: Type I and II (concentric shrinkage with or without any surrounding
lesion), type II (shrinkage with residual multinodular lesions), type IV (diffuse contrast enhancement in the entire quadrant), and non-visualization. Results: The most common MRI shrinkage pattern was type I (39%) followed by non-visualization (28%). The most common pathological shrinkage pattern was type III (33%), followed by type II (28%) and non-visualization (22%). The concordance rates of the MRI patterns of non-visualization and type I were significantly lower than those of the MRI patterns of type II, III, and IV (p = 0.018). Moreover, in the luminal subtypes, the concordance rates of the MRI patterns of non-visualization and type I were significantly lower than those of the MRI patterns of type II, III, and IV (p = 0.026); however, in the non-luminal subtypes, this correlation was not significant. Conclusions: The results of the present study suggest that the cases with MRI shrinkage patterns of non-visualization and type I require caution regarding the surgical margins compared with the other types, especially the luminal subtypes.


Introduction: Since the association between certain oncogenic (high-risk) strains of HPV and cervical cancer is well established. Searching for HPV became a basic step in all screening programs. Laboratory HPV testing is costly and unavailable in developing countries. Objective: To compare the diagnostic indices of office cervicoscopy against cytology and punch biopsy for detection of underlying HPV lesions in women with suspicious cervix. Design: An analytic cross-sectional research design Setting: Early cervical cancer Detection Unit of the Women’s Health University Hospital, Assiut, Egypt. Patients and methods: A total of 274 non-pregnant women with clinical diagnosis of suspicious cervix were included in this study. After cervical smearing with Ayre’s spatula, they were subjected to cervicoscopy utilizing 2.6 mm telescope and a 3.2 mm outer sheath office hysteroscope. Evaluation of the vulva, vagina, ectocervix, endocervical canal and the endometrial cavity were done. The cervix was then painted with saline, acetic acid and Schiller’s iodine stains successively as used during colposcopic examination. Abnormal lesions were biopsied using punch biopsy forceps. Cytologic evaluation relied on both classic and non-classic (secondary) criteria for the diagnosis of HPV including ‘mild koilocytosis,’ ‘mild dyskeratosis,’ ‘hyperchromatic nuclei,’ bi- and multinucleation, and cleared cytoplasm. Punch biopsies were taken from every case based on cervicoscopic localization after staining. Results: The main complaint was abnormal vaginal discharge in 197 cases (71.4%). Pap smear sampling was negative for HPV in 188 cases (68.6%) and positive in 86 cases (31.4%). Cervicoscopic findings suggestive of HPV infection were positive in 77 cases (28.1%) and negative in 197 cases (71.9%). Gold stand histopathology diagnosed HPV in 53 cases only (19.3%). All diagnostic indices of cervicoscopy significantly improved after combination with cytology positive results except false positive results. Conclusions: Cervicoscopy as a simple, cheap and office procedure can be used as a quick screening tool of HPV infection in women with suspicious cervix. It has the advantage of screening of the rest of the genital tract. In the absence of laboratory HPV testing, cervicoscopy in conjunction with cytology screening of high-risk cases should be encouraged.

#3746 Plasma membrane spanning and linker-domains from tumor necrosis factor superfamily (TNFRSF) proteins provide novel functionality to chimeric antigen receptors (CARs) expressed in human T cells. Dina Schneider, Ying Xiong, Darong Wu, BoroDropulic, Rimas Orentas. Lentin Technology Inc., a Millen biotech Company, Gaithersburg, MD.

Chimeric antigen receptor T cells redirected to the B cell antigen CD19 (CAR19) have shown efficacy in the treatment of B-lineage acute lymphoblastic leukemia, and are being evaluated in other hematologic malignancies as well. The prevalent configuration of CAR19 for clinical application is comprised of the extracellular single chain targeting domain (scFv) derived from the FMC63 antibody, a CD8 linker and transmembrane domain, a CD137 signaling domain, and a CD3-zeta signaling domain. Transmembrane domains from other immunologically-active proteins, including CD3, CD28, or CD4, and spacer domains from IgG have been employed as structural components of CARs as well. The relationship between the composition of the CAR linker and transmembrane domain and CAR T function is, however, poorly understood. We hypothesized that CAR transmembrane and linker domains derived from proteins of the tumor necrosis factor receptor superfamily (TNFRSF), whose expression is associated with normal T cell functions, may optimize the anti-tumor activity of CAR19. Therefore, primary human T cells were transduced with lentiviral expression vectors (LV) expressing new combinations of linker and transmembrane sequences encoded by various TNFRSF members. As one of the CAR transmembrane domains, CD19 was chosen, and the CAR T function was examined by engaging Raji cells (a Burkitt’s lymphoma line) in an NSG leukemia mouse model. On the other hand, the transmembrane domain derived from TNFRSF16/LANGFR, a low affinity neurotrophin receptor, created a CAR19 construct that had no functional activity either in vitro or in vivo, despite high levels of surface CAR expression in human T cells. In conclusion, the magnitude of CAR T response can be enhanced by the use of novel linker and transmembrane domains derived from TNFRSF proteins, such as TNFRSF16. Thus, the transmembrane domain can play a determining role in CAR T function beyond a direct topographical connection between the extracellular and the intracellular domains of the chimeric protein. Studies are underway to elucidate the mechanistic role of TNFRSF-derived linker and transmembrane domains in CAR T function.
#3747 msa2 biotin-binding CAR combined with biotynilated tumor-specifie antibodies or DNA aptamers for universal cancer immunotherapy. 

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Chimeric antigen receptor T cells (CAR-Ts) are highly promising as cancer therapeutics, but the creation of CARs for new antigens remains a laborious process. As an alternative strategy researchers are designing universal “tag-CARs” that instead of directly recognizing tumor antigens, recognize tags on tumor-specific antibodies bound to tumor cells, thereby activating T cell effector functions. Here we report the creation of a novel tag-CAR that uses the high-affinity monomeric streptavidin 2 (msa2) biotin binding domain to target cancer cells labeled with biotynilated tumor-specific antibodies or DNA aptamers. We constructed lentiviral vectors encoding msa2 CARs with three different signaling architectures - CD28-zeta, CD28-OX40-zeta, and 4-1BB-zeta. After confirming that each msa2 CAR was efficiently expressed on the surface of Jurkat and primary human T cells, we found that plate-immobilized biotin was capable of specifically inducing T cell activation markers and cytokine production. We then stained a variety of cancer cell lines with various tumor-specific biotynilated antibodies or DNA aptamers targeting tumor antigens such as MUC1, CD19, CD20, and EGFR and found that the stained cells specifically activated the msa2-CAR cells and led to cytokine production. We are currently investigating the ability of msa2-CAR cells to lyse antibody and aptamer-bound cancer cells and will be assessing their ability to shrink tumors in a human tumor xenograft mouse model.

#3748 Development of anti-human mesothelin chimeric antigen receptor (CAR) messenger RNA (mRNA) transfected peripheral blood mononuclear cells (CARMA) for the treatment of mesothelin-expressing cancers. 

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CD19-targeted chimeric antigen receptor (CAR)-engineered T/NK-cell therapies can result in durable clinical responses in B-cell malignancies. However, CAR-based immunotherapies have been less successful in solid cancers. This is partly due to specificity for shared tumor antigens also present on normal host tissues that leads to ‘on-target/off-tumor’ toxicity. We therefore developed a non-viral approach using repeated infusions of mesothelin-specific messenger RNA (mRNA) CAR-transfected T cells to permit prospective control of ‘on-target/off-tumor’ toxicity. Early trials provided preliminary evidence of the safety and anti-tumor activity of this strategy, but the ex vivo selection, activation and expansion of lymphocytes is laborious and expensive. We therefore explored the feasibility of using a rapid, automated, closed system for GMP-compliant mRNA CAR transfection into freshly isolated peripheral blood mononuclear cells for clinical scale manufacture (CARMA). The resulting cryopreserved cellular product expressed CAR in >95% of cells, and recognized and lysed tumor cells in an antigen-specific manner. Expression of CAR was detectable for 5-7 days in vitro, with a progressive decline of CAR expression related to in vivo antigen content in murine ovarian cancer model, wherein high affinity monomeric streptavidin (IP) injection of CARMA resulted in the dose-dependent inhibition of tumor growth and prolonged the overall survival (OS) of mice. Multiple weekly IP injections of the optimal CARMA dose enhanced disease control and further prolonged OS, both of which improved with an increasing number of injections. No significant off-tumor toxicities were observed. These data support further investigation of a serial IP CARMA administration as a potential treatment for ovarian cancer and other mesothelin-expressing tumors involving the peritoneum, and provide preclinical proof of principle of CARMA for solid tumors.

#3749 Genetic blockade of the protein tyrosine phosphatase SHP1 augments CAR T cell activity against PDL1 expressing solid tumors. 

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Background: Immunotherapy using chimeric antigen receptor (CAR) T cells has demonstrated profound, durable success in hematologic malignancies. Solid tumors present hurdles to the successful application of CAR T cells. One is the upregulation of inhibitory receptors (IRs), like PD1 and CTLA4, many of which rely on shared signaling molecules to shut off T cell activation. One such molecule is SHP1 (Src homology region 2 domain-containing phosphatase-1) which dephosphorylates key components of T cell receptor (TCR) signaling. We have engineered a unique dominant-negative SHP1 (dnSHP1) that is able to augment CAR T cell control of PDL1 positive solid tumors. Materials and Methods: The human mesothelioma cell line, EMP, was transduced to express high levels of mesothelin and PDL1 (EMMESO-PDL1). Activated human T cells from healthy donors were lentivirally transduced to express a mesothelin-directed CAR (mesoCAR) with and without a dnSHP1. MesoCAR and mesoCAR/dnSHP1 T cells were cocultured with tumor cells x 18hrs and specific lysis was measured. These T cells were also restimulated with plate-bound anti-CD3 overnight and were subjected to intracellular flow cytometry staining (ICS) of cytokines. NSG mice were injected subcutaneously in the flanks with 5x10^6 EMMESO-PDL1 tumor cells. After tumors established and grew to ~150mm^3, mice were randomly assigned to one of the following treatments: 1) non-transduced (NDT) T cells, 2) mesoCAR T cells, 3) mesoCAR T cells + sodium stibogluconate (SSG; a chemical inhibitor of SHP1), 4) mesoCAR/dnSHP1 T cells. T cells were injected IV once at a dose of 10x10^7 T cells/mouse. SSG was administered IM at 20mg/kg every 2 days throughout tumor growth. At the end of the study, mice were sacrificed, tumors were harvested, digested, processed into single cell suspension, and subjected to flow cytometry analysis. The tumor infiltrating lymphocytes (TILs) were also isolated and tested for function ex-vivo. Results/Conclusion: In vitro, mesoCAR T cells demonstrated suppressed lysis of EMMESO-PDL1 tumor cells compared to EMMESO cells. MesoCAR/dnSHP1 T cells were able to lyse EMMESO-PDL1 and EMMESO tumor cells with similar efficiency. Anti-CD3 restimulation of T cells revealed enhanced secretion of TNF-alpha and IL2 by mesoCAR/dnSHP1 vs. mesoCAR T cells as measured by ICS. In vivo, SSG injections had minimal impact on mesoCAR T cell control of tumors, whereas mesoCAR/dnSHP1 T cells demonstrated significantly enhanced control of EMMESO-PDL1 tumor growth compared to mesoCAR T cells (60% greater decrease in tumor volume compared to mesoCAR T cells). Tumor regression was attributed to dnSHP1 TILs that were isolated and subjected to flow cytometry analysis. The tumor infiltrating lymphocytes (TILs) were a powerful and novel way of blocking the suppression of CAR T cells by PD1 and other similar IRs.

#3750 Micromolar affinity CAR T cells to ICAM-1 achieves rapid tumor elimination while avoiding systemic toxicity. 

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Introduction: Adoptive immune therapy has achieved great success in eradicating blood-borne cancers, prominently, the CD19 CAR T cells in B cell leukemia and lymphomas. However, CAR T cell therapy in solid tumors has been limited due to the scarcity of tumor antigens that are deemed safe for targeting. One strategy to overcome scarcity of tumor antigen is by tuning the affinity of CAR to limit T cell reaction with cells overexpressing target antigen while sparing cells with basal level expression. To rigorously test the idea of “affinity tuning”, we built variants of CARs possessing one million-fold difference in affinity spanning low nanomolar to high micromolar affinity to a target antigen, and examined the influence of CAR affinity on the rate of tumor killing and systemic toxicity. Methods: Antigen-binding domain of CAR was built from the inserted or 1 domain belonging to integrin LFA-1. Affinity of CAR expressed in T cells was confirmed by ICAM-1 binding by flow cytometry. For in vivo study, mice with systemic growth of ICAM-1 positive thyroïd tumor were used, where tumors grew in lungs, liver, and bones. Tumor growth and killing were monitored by whole body luminescence imaging. Sera were collected for cytokine analysis. Body weight, cytokine profile, and overall behavior were used to assess the severity of systemic toxicity. Results: CAR T cells with a step-wise, one million-fold variation in affinity to ICAM-1 resulted in a rate of target killing in proportion to the increase in affinity and in ICAM-1 density. Owing to cross-reaction of human LFA-1 with murine ICAM-1, the influence of CAR affinity on efficacy and on-target, off-tumor toxicity was tested in mice bearing ICAM-1 positive human tumors. In vivo tumor elimination by CAR T cells was in contrast to in vitro affinity-dependent rate of target killing, demonstrating that micromolar affinity CAR T cells was superior to nanomolar affinity T cells in both tumor killing and safety aspects. Highest affinity CAR T cells led to uniform death of the host, caused by on-target, off-tumor toxicity, and high level cytokine release. Conclusion: Our study is the first comprehensive report examining the effect of CAR affinity on the rate of tumor killing, efficacy, and toxicity. In contrast to in vitro tumor killing effect, the increase in affinity of CAR beyond certain threshold was deleterious to T cell persistence and associated with more frequent tumor relapse. Our study highlights that CAR T cells expressing natural T cell receptor affinity is more efficacious in eliminating tumors with overexpressed antigens, and is safer by avoiding potential reaction with normal cells with basal expression of the same antigen.

#3751 Allogeneic EGRFvIII chimeric antigen receptor T cells for treatment of glioblastoma. 

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CLINICAL RESEARCH: Innate Effectors in Immunity to Cancer

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Glioblastoma multiforme (GBM) is a highly aggressive form of brain tumors with a 5-year survival rate of less than 10%. Standard-of-care combining radiation therapy with temozolomide only yields a median survival of 14.6 months and more effective therapeutic options to extend patient lives are urgently needed. EGFR variant III (EGFRvIII) is a tumor specific mutant of EGFR found in 25-30% of GBM but not in normal tissue. The structure of EGFRvIII has been shown to drive the growth and survival of glioblastoma cells and be a potential target for therapies. However, clinical trials using anti-EGFRvIII antibodies have yielded disappointing results. Our group has identified a tumor-associated MMP1-derived peptide in an HLA-A*02 context by exploiting the knowledge of tumor and healthy tissue immunopeptidomes. By this approach, MMP1-003, an HLA-A*02/MMP1-003 complex was identified as a promising therapeutic target presented by several tumor types, including colorectal and lung cancer, but absent on normal tissues. These findings are underlined by RNAseq analysis of the source antigen which also points to MMP1 being a highly attractive tumor-associated target. Consequently, a fully human antibody phage display library was screened to identify highly specific candidate monoclonal antibodies. The lead TandAb showed excellent target specificity across a wide range of peptide-pulsed and endogenously expressing cell lines as well as potent cytotoxicity with picomolar EC50. In summary, we have developed a combinatorial tumor recognition system, diminshed-off-tumor cytotoxicity, and multifaceted effector function make natural killer (NK) cells a prime candidate for a universal approach to cancer immunotherapy. In addition, NK cells are the principal mediator of antibody-directed cellular cytotoxicity (ADCC). However, NK cell function is often impaired in the setting of cancer, reducing the effectiveness of the endogenous immune system and the therapeutic efficacy of monoclonal antibodies. To address the need for advanced combinatorial cancer therapies, we developed a unique and effective strategy to create a renewable source of engineered “off-the-shelf” NK cells with augmented function, including enhanced ADC and persistence. Key challenges associated with genetic editing, limited expansion, persistence and variability of peripheral blood (PB)-derived NK cells were overcome by utilizing our induced pluripotent stem cell (iPSC) technology as the unlimited starting material for the reproducible and consistent derivation of engineered NK cells. Through targeted transgene integration, we produced a clonal iPSC master cell line to continuously produce NK cells engineered to uniformly express a high affinity, non-cleavable version of CD16 (hCD16-NK). In directed differentiation, the hCD16-NK cells displayed homogeneous expression of CD16.
type CD8+ T cells. Patient #2 received up to 8.2x10^6/kg HLA-A*02:01 donor derived and patient #3 up to 6x10^6/kg autologous allorestricted TCR transgenic CD8+ T cells. All patients were treated with the same TCR complementary determining region 3 allorecognition sequence for CHM1. Results: HLA-A*02:01/CHM1 specific allorestricted CD8+ T cells showed specific in vitro lysis of ES cells. Therapy was well tolerated and did not cause graft versus host disease (GVHD). Patients #1 and #3 showed slow progression, whereas patient #2, while having BM involvement, showed partial metastatic regression associated with T cell homing to involved lesions. CHM1/TCR transgenic T cells could be tracked in his BM for weeks. Conclusions: CHM1/TCR transgenic T cells home to affected BM and may cause partial disease regression. HLA-A*02:01/CHM1specific allorestricted T cells proliferate in vivo without causing GVHD.

#3758 The efficacy of CD133 BiTEs and CAR-T cells in preclinical model of glioblastoma. Parvez Vora,1 Chirayu Chokshi,1 Maleeha Qazi,1 Mohini Singh,1 Chitra Venugopalan,1 Sujeivan Mahendram,1 Jarrett Adams,1 David Bakhshiyani,1 Max London,1 Jess Singh,1 Vinny Parvez,1 Jonathan Bramson,1 Sachdev Sidhu,1 Jason Moffat,2 Sheila Singh.1,2

#3759 PiggyBac-manufactured anti-BCMA Centyrin-based CAR-T therapeutic exhibits improved potency and durability. David Hermanson,1 Bur-}


cal data presented herein highlight the therapeutic value of hCD16-iNK cells as an ideal ADCCC-mediated “off-the-shelf” NK cell-based immunotherapeutic product with augmented persistence, anti-tumor capacity, manufacturing reliability and preclinical efficacy.

#3756 Efficient generation of CAR T cells by site-specific gene addition into the TRAC locus. Baekseung Lee,1 Wai-Hang Leung,1 Mark Gordon,1 Jordan Jarjour,1 Alexander Astrakhan,1 bluebird bio, Seattle, WA.

Genetically engineered T cells represent a promising new approach to the treatment of cancer. Positive results in recent clinical trials exploiting T cells engineered to express chimeric antigen receptors (CARs) have highlighted the potential for these cells to be translated into therapies in patients with B cell malignancies. To extend these successes to a broader range of tumor types may require additional CAR T cell engineering beyond simple CAR addition, such as gene knockout and/or coupling deletion of a target gene with site-specific addition of a CAR transgene. To this end, we have developed a genome editing strategy for the simultaneous elimination of an endogenous target gene with site-specific addition of a CAR via homology-directed repair (HDR). To demonstrate the utility of this approach, we used a previously characterized megaTAL (an engineered nuclease created by the fusion of an engineered meganuclease and a transcription activator like (TAL) -DNA binding domain) specific for the T cell receptor alpha constant region gene (TRAC). Delivery of this megaTAL to primary human T cells via mRNA electroporation results in efficient and specific disruption of the TRAC locus (Boiselle et al, 2013). To achieve simultaneous target gene disruption with site-specific CAR transgene insertion, we designed an adeno-associated virus (AAV) vector for DNA template delivery encoding the CAR and flanked by regions of DNA homologous to the genome immediately surrounding the megaTAL cleavage site. Co-delivery of the megaTAL and AAV encoding a CD19-CAR with TRAC homology arms resulted in >50% CAR + TRAC- cells. In vitro assays of cytotoxicity and cytokine responses against CD19+ Nalm-6 cells confirmed that TRAC-targeted CAR-T cells were comparable to CD19-CAR-T cells generated by lentiviral transduction. Interestingly, a similar level of CAR expression was observed even though CAR-T cells expressed lower amounts of the CAR, as determined by flow cytometry. Similar CAR integration efficiency and functional efficacy was observed using a TRAC-targeting AAV vector containing a distinct B cell maturation antigen (BCMA)-specific CAR. These findings demonstrate megaTAL-mediated targeted gene addition as a feasible, efficient, and potentially safer approach for generation of gene-edited CAR T cell product. Moreover, the ability to combine the disruption of a target gene with the site-specific integration of the CAR eliminates the need for randomly integrating viral vectors while satisfying the potential need for more complex genome-edited T cell products.

#3757 Ewing Sarcoma regression by Allo-MHC/Chm1 specific T cells without GVHD. Uwe Thiel,1 Sebastian Schober,1 ingo Einspieler,1 Andreas Marquardt,1 Uwe Thiel,1 Alexander Astrakhan,1 bluebird bio, Seattle, WA.

Globoloblastoma (GBM) is a uniformly fatal primary brain tumor, characterized by a diverse cellular phenotype and genetic heterogeneity. Despite the use of multi-modal treatment including surgical resection, radiotherapy and chemotherapy, the outcome of patients with GBM remains poor. Numerous studies have implicated CD133 as a marker for the tumor initiating population in GBM. However, therapeutic strategies that specifically target CD133 have not yet been developed for recurrent GBM. Using a novel methodologic that combines use of phase-displayed synthetic antibody libraries and DNA sequencing, we developed the CD133-specific monoclonal antibody ‘RW03’. We constructed CD133-specific BiTEs that consist of two arms; one arm recognizes the tumor antigen (CD133) while the second is specific to CD3 antigen. The dual binding specificity was confirmed using flow cytometry. Using CD133high and CD133low primary GBM lines, we validated the binding of BiTEs to CD133+ cells. Further analysis showed binding of BiTEs to human T cells known to express CD3 within a population of healthy donor peripheral blood mononuclear cells. We observed BiTEs redirecting T cells to kill GBMs, with greater efficiency observed in CD133high GBMs, validating BiTe target specificity. Incubating T-cells with BiTEs and the CD133high GBMs resulted in increased expression of T cell activation markers. In parallel, we derived the single chain variable fragment (scFv) from previously generated RW03 and generated a second-generation CAR. Anti-CD133 scFv with a myc tag was cloned in frame with a human CD8 leader sequence, CD8a transmembrane domain, CD28, and hCD3 γ signaling tail in the lentiviral construct pCCL-ΔNGFR. Following lentiviral packaging, the T cells isolated from PBMCs were transduced with all patatred or unique CAR constructs. Using mice expressing a humanized BM microenvironment, we observed extended survival in mice and significant reductions in brain tumor burden. The results of this study will establish a translational research program that will form the basis of early phase clinical trials of a promising CD133-based therapeutic strategy for patients with GBM.
BCMA-specific Centryrin rather than a single chain variable fragment (scFv) for antigen detection and is engineered using piggyBac (PB). Centryrins are fully human and have similar binding affinities but are smaller, more thermostable and predicted to be less immunogenic than a scFv. Furthermore, PB modification of human T cells requires only in vitro transcribed mRNA and plasmid DNA, eliminating the need for lentiviral packaging and resulting in the increased cargo capacity of PB permits the incorporation of a safety switch and a selectable gene into the product. The former is incorporated for optional depletion of the increased cargo capacity of PB permits the incorporation of a safety switch and a selectable gene into the product. The former is incorporated for optional depletion

The latter allows enrichment of CARTyrin + cells using the non-genotoxic drug methotrexate (MTX), leading to greater consistency in patient product material. Characterization of P-BCMA-101 revealed >70% of cells possessed memory phenotype (CD45RA- CD95+) and >95% of the cells were CARTyrin +. In addition, no tonic signaling or T cell exhaustion was observed, highlighted by low levels of PD-1, Lag3, and Tim-3. Cells exhibit specific and robust in vitro target-cell killing, cytokine production, and proliferation in response to BCMA + tumor cells. In vivo anti-tumor efficacy of P-BCMA-101 has been evaluated in NSG mice bearing luciferase + MM-1S cells, an aggressive human MM-derived cell line, monitoring tumor growth by bioluminescence imaging (BLI). Following tumor implantation, animals received a single IV administration of either 4 x 10 6 or 12 x 10 6 P-BCMA-101 cells. All untreated control animals succumbed to disease within four weeks of the treatment date. Conversely, tumor burden was reduced to the limit of detection by BLI within 7 days of P-BCMA-101 treatment. As opposed to lentivirus-based products in the same animal model, PB modification of human T cells is non-toxic, but leaves endogenous and exogenous antigens from the CAR transduction and ongoing immune responses. Finally, the effectiveness of the safety switch has been demonstrated both in vitro and in vivo. P-BCMA-101 is the first-in-class of Centryrin-based CAR therapeutics modified using PB and is predicted to have improved potency and durability given the phenotype and non-immunogenic properties of Centryrins. We plan to initiate a phase I clinical trial of P-BCMA-101 for the treatment of patients with relapsed and/or refractory MM.

#3760 Preclinical optimization of a low cost PiggyBac transposase (PB) generated CD19-specific chimeric antigen receptor T cell (CART19) product for a first in man trial using local hospital cell manufacture. David Bishop,1 Ning Xu,2 Sylvie Shen,2 Tracey O’Brien,2 David Gottlieb,1 Alla Dolnikov,2 Ken- neth Micklethwaite1. 1Westmead Institute for Medical Research, Sydney, Australia; 2Children’s Cancer Institute, Sydney, Australia.

BACKGROUND: CAR T cells show remarkable efficacy against relapsed/refractory B cell malignancies. PiggyBac offers a less complex and more cost effective means for generating CAR T cells compared to viral vectors typically used, so we aimed to use PiggyBac to generate a product suitable for translation to the clinic. METHODS: CAR T cells utilizing the CAR1928z construct have been found to perform well in vivo in preclinical models; however, these tumors often relapse and prolong survival, with most animals surviving 100 days post-tumor implant. Finally, the effectiveness of the safety switch has been demonstrated both in vitro and in vivo. P-BCMA-101 is the first-in-class of Centryrin-based CAR therapeutics modified using PB and is predicted to have improved potency and durability given the phenotype and non-immunogenic properties of Centryrins. We plan to initiate a phase I clinical trial of P-BCMA-101 for the treatment of patients with relapsed and/or refractory MM.

#3761 Specific and dynamic tumor antigen-specific immune responses were elicited in patients with hepatocellular carcinoma after cell-based immunotherapy. Yanyan Han,1 Ye Wu,2 Chou Yang3, Jing Huang,1 Yabin Guo,1 Li Liu,1 Ping Chen,1 Dongyun Wu,4 Junyun Liu,1 Jin Li,1 Xiangjun Zhou,1 Jinlin Hou2. 1HRYZ Biotech Co., Shenzhen, China; 2State Key Laboratory of Organ Failure Research, Guangdong Provincial Key Laboratory of Viral Hepatitis Research, Department of Infectious Diseases, Nanfang Hospital, Southern Medical University, Guangzhou, China.

Hepatocellular carcinoma (HCC) is one of the most common cancers in China and frequently occurs with chronic hepatitis B virus (HBV) infection. To investigate whether cell-based cancer immunotherapy induces tumor specific immune responses in patients with HCC and provides clinical benefits, as well as to elucidate the most immunogenic tumor associated antigens (TAAs), multiple antigen stimulating cellular therapy (MACST) was applied in addition to standard of care. Mature dendritic cells (DCs) and activated T cells prepared for MACST were generated from autologous peripheral blood mononuclear cells (PBMCs). DCs were loaded with a peptide pool of multiple HCC-related tumor antigens, and T cells were stimulated by these DCs. Thirteen patients with HCC received repeated MACST after tumor resection during which their immune responses were examined. After three courses of MACST, the frequency of regulatory T cells in the patients’ PBMCs significantly decreased (p < 0.001), while the antigen peptide pool-triggered T cell proliferation (p < 0.001) and IFNγ production (p = 0.001) were significantly enhanced. The specific T cell responses against each antigen in the pool were detected in five patients. Ane sex-specific and pattern recognition. The most immunogenic TAA for HCC are survivin, CCND1, and RGS5. Moreover, the antigen-specific immune responses observed in tumor-free patients’ PBMCs were significantly stronger than that in the patients with recurrence (p = 0.037). Conclusion: Our study demonstrates that MACST is well-tolerated by patients with HCC and elicits strong and dynamic immune responses specifically against multiple tumor associated antigens, which may correlate with clinical outcomes.

#3762 Superior T cell activity of a membrane-proximal binding antibody when targeting Glypican-3 with an antibody-coupled T cell receptor (ACTR) armed T cell. Greg Motz,1 John Shin,1 Kathleen Whiteman,1 Birgit Schultes,1 Tapasya Pai,1 Lori Westendorf,2 Seth Ettenberg,1 Travis Biechele,1 Diego Sussman,1 Heather Huett1. 1Unum Therapeutics, Cambridge, MA; 2Seattle Genetics, Bothell, WA.

Glypican-3 (GPC3) is a GPI-anchored member of the heparan sulfate proteoglycan family. GPC3 is an oncofetal antigen expressed transiently during fetal development with re-expression during malignant transformation. GPC3 is an ideal tumor target as expression has been found in numerous epithelial malignancies, with high-expression in hepatocellular carcinoma (HCC) and non-small cell lung carci- noma (NSCLC), and normal tissue expression is highly restricted (Baumhoer D, Am J Clin Pathol, 2008.). Adoptive T cell therapy with single-chain variable fragment (scFv)-derived chimeric antigen receptors (CARs) has transformed cancer therapy, but the broad applicability of this approach has been limited in part by safety concerns due to the constitutive expression of a biologically active targeting receptor. The Antibody-Coupled T cell Receptor (ACTR) platform is a universal, engineered T cell therapy designed to engage the Fc domain of therapeutic antibodies specific to tumor cells to provide T cell-selective tumor killing. The ACTR is therefore both regulatable and flexible, providing enhanced therapeutic control and improved safety of the T cell therapy. Using both HCC and NSCLC tumor cell lines, we tested a panel of wild-type and fucosylated antibodies with similar binding affinities that bound to regions spanning the GPC3 protein across unique epitopes. We found that for GPC3 targeting antibodies, the greatest activity in a Jurkat-NFAT reporter assay was observed for the fucosylated antibody that bound GPC3 most proximal to the membrane. Further, the antibody that bound membrane proximal also had the most potent activity in primary ACTR T cell cytotoxicity and cytokine release assays. The physical distance between T cells and tumor targets has been previously determined to impact T cell activation for both peptide-MHC and CAR-T interaction. Similarly, our results demonstrate a potential relationship between spatial distance of tumor targets and T cells in determining the activity ACTR transduced T cells when targeting GPC3. Our data demonstrate that ACTR T cell activity is antibody-specific and dose-irratable, highlighting both efficacy and improved safety of the ACTR T cell platform when targeting GPC3+ solid tumor malignancies.

#3763 UCART22: autologous adoptive immunotherapy of leukemia by targeting CD22 with CAR T-cells. Agnès Gouble,1 Cécile Schiffer-Mannioui,1 Severine Thomas,1 Anne-Sophie Gautron,1 Laurent Poirot,2 Julianne Smith2. 1Institut Curie, Paris, France; 2Collectis, Paris, France; 3Collectis, New York, NY.

Adoptive immunotherapy using autologous T-cells endowed with chimeric an- tigen receptors (CARs) has given rise to long-term durable remissions and remark- able objective response rates in patients with refractory leukemia, raising hopes that

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a wider application of CAR technology may lead to a new paradigm in cancer treatment. However, a limitation of the current autologous approach is that CAR T-cells must be manufactured on a ‘per patient basis’. To overcome this limitation, we have developed a standardized platform for manufacturing T-cells from third-party healthy donors to generate allogeneic ‘off-the-shelf’ engineered CAR T-cell-based products. Our allogeneic platform utilizes the Transcription Activator-Like Effector Nuclease (TALEN) gene editing technology to inactivate the TCRα constant (TRAC) gene, significantly reducing the potential for T-cells bearing allogeneic TCRs to mediate Graft-versus-Host Disease (GvHD). We have previously demonstrated the precise and efficient disruption of the TRAC gene by gene editing, yielding up to 85% of TCRαβ-negative cells. This allows the production of TCRαβ-deficient T-cells that no longer mediate allograft activity in a xenogeneic mouse model. In the clinic, the proof of concept of the applicability of our allogeneic platform was achieved with early compassionate use for patients treated with UCART19, an allogeneic engineered CAR T-cell product directed against CD19. UCART19 clinical trials are currently ongoing. Here, we have developed T-cells targeting CD22 which is expressed on tumor cells from the majority of patients with B-cells leukemia. In a first step, we have screened multiple antigen recognition domains in the context of different CAR architectures to identify effective CAR candidates displaying activity against cells expressing variable levels of the CD22 antigen. As a safety feature, T-cells are engineered to co-express a depletion gene, rendering them sensitive to the monoclonal antibody rituximab. Several constructs of depletion genes have been evaluated in the context of the CD22 CAR. In addition, experiments in an orthotopic ALL mouse model using UCART22 cells demonstrated important anti-tumor activity in vivo. The ability to carry out large scale manufacturing of allogeneic, non-alloreactive CD22 specific T-cells from a single healthy donor can offer the possibility of an off-the-shelf treatment that would be immediately available for administration to a large number of leukemic patients. UCART22 could also offer an alternative to patients who may relapse with CD19-negative tumors after CD19 CAR T-cell treatment. 

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**#3764** Targeting GPR64 in pediatric sarcomas with CAR transgenic T cells, David Schürmer,1 Isabel Storz,2 Karin Wisskirchen,2 Regina Feederle,3 Oxana Schmidt,1 Hinrich Abken,4 Crystall Mackall,5 Ulrike Prozet,6 Stefan Burdach,6 Günther H. Richter1.1 Technical University Munich, Munich, Germany;2 Technical University Munich, Helmholtz Zentrum Munich, Munich, Germany;3 Helmholtz Zentrum Munich, Munich, Germany;4 University Hospital Cologne, Cologne, Germany;5 Stanford University, Stanford, CA.

Introduction: Ewing Sarcoma (ES) is the second most common bone malignancy in children and young adolescents with a high potential of dissemination into lung and bone. Patients with disseminated disease into the bone have an approximate long-term survival rate of only 10%, compelling the search for new therapeutic treatment modalities like engineered T cell therapy. Here the therapeutic potential of chimeric antigen receptor (CAR) transgenic T cells directed against Ewing Sarcoma protein receptor 64 (GPR64) was examined. Experimental procedure: Two different monoclonal antibodies (mAbs) directed against the extracellular region of GPR64 were generated and characterized. Subsequently, retroviral constructs containing second generation CARs together with the scFv fragments of the respective mAbs were designed. Constructs consisting of different spacers and co-stimulatory elements were compared (dIgG1-CD28-CD3z vs. CD3z-4-1BB-CD3z). Primary lymphocytes were transduced and tested in vitro by flow cytometry, ELISpot and xCelligence assay as well as in immunodeficient xenograft mice. Results: Antibodies specifically stained ES cells as determined by flow cytometry and immune histology. The signal intensity was reduced after RNAi mediated down-regulation of GPR64 in ES cell lines confirming specificity of the isolated mAbs. Following sequence determination of those mAbs two different CAR constructs were designed. Retroviruses containing such CARs transduced primary lymphocytes with high efficiency. The CAR transgenic T cells were enriched for CD8+ CAR+ cells via microbead isolation and demonstrated strong proliferative capacities in vitro. Furthermore, target structures were specifically recognized as determined by ELISpot and xCelligence assay. Co-transduction with CD28 and CD137 resulted in one hybridoma, revealed hints of early exhaustion via auto-activation. Conclusion: CAR transgenic T cells targeting GPR64 show a promising approach to transfer the success of CARs in hematological malignancies to solid tumors. The cells generated in this study show strong specificity towards GPR64 and are able to control tumor cell growth. Since GPR64 expression is not restricted to ES but also up-regulated in a number of carcinomas derived from prostate, kidney or lung, GPR64-specific CARs may also be a future treatment option for other tumor entities.

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T cell receptor (TCR) engineered adoptive T cell transfer (ACT) has shown remarkable antitumor efficacy in several clinical trials. However, low persistence of modified cells limits long-term clinical responses. To overcome this hurdle, we propose a clinical trial co-administering genetically modified T cells and stem cells. Prior clinical work has shown the potential to engineer a source for constant renewal of modified T cells. Here we report a preclinical IND-enabling study performed at UCLA under Good Laboratory Practice (GLP) compliance to assess whether co-administration impacts (I) safety; (II) engraftment and cell lineage differentiation of gene modified stem cells; and (III) persistence of adoptively transferred T cells and stem cell-derived progeny. We performed 12 optimization studies to define the optimal conditions for TCR gene modified ACT and TCR gene modified hematopoietic stem cell (HSC) bone marrow transplantation (BMT). Sixty-four HLA-A2/kb transgenic mice were myelodepleted and received syngeneic BMT with Lineage depleted bone marrow (Lin-) cells transduced with the LV-NY-ESO-1 TRC/s39TR and ACT with T cells transduced with the RV-NY-ESO-1 TCR. Control groups were as follows: NOD-SCID mice receiving transduced Lin- cells and mock transduced T cells, mice receiving mock transduced Lin- cells and transduced T cells (n = 16 per group).

Overall survival at 3 months was 87.5%; no significant differences in survival were observed among cohorts. After BMT we observed a decrease in body weight, elevation in kidney and transaminasins, and gonadal germ cell ablation in all cohorts. Three months after BMT, all blood cell lineages were reconstituted in surviving mice. Using digital droplet PCR and flow cytometry, we confirmed that transduced stem cells engrafted and their progeny persisted long term. In the bone marrow, NY-ESO-1 TCR was expressed intracellularly among progenitor cells (Lin-, LSK and HSC) as well as all hematopoietic cell lineages within the spleen (CD8+ T cells, CD4+ T cells, NK cells, B cells and granulocytes). Co-administration with genetically modified stem cells did not affect engraftment, cell lineage differentiation or persistence of the gene modified stem cells. Moreover, co-administration with stem cells did not affect persistence of adoptively transferred T cells. These data demonstrate that 1) NY-ESO-1 TCR genetically modified stem cells engraft and differentiate into all hematopoietic cell lineage progeny, which persists at 3 months; 2) adoptively transferred NY-ESO-1 TCR T cells persist at 3 months; 3) co-administration of stem cells and T cells genetically modified to express an NY-ESO-1 TCR is safe and does not negatively impact stem cell engraftment, lineage differentiation and progeny persistence or T cell persistence.

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**#3766** MG7-car, a first-in-class T-cell therapy for gastric cancer, Jijun Yuan, Shanghai Genechem Co., Ltd., Shanghai, China.

Gastric Cancer, with increasing incidence and mortality, has become a major public health problem in China. According to the most recent study in China, Gastric Cancer is the second most common cancer with an incidence of 680,000 cases and mortality of close to 500,000 cases. Therefore, it is important to find an effective way to target the gastric cancer. MG7 antigen is a highly specific gastric cancer antigen identified by immunohistochemistry in late 80’s, unfortunately most work since then has been focused on MG7 antigen as a diagnosis marker without any treatment approach. Chimeric Antigen Receptor (CAR) T cell therapy is a newly developed technique, the called adoptive cell transfer. T cells from the patient are able to recognize and kill the tumor cell by grafting a tumor specific antigen binding single chain variable fragment (scfv) onto the T cell. In the past couple of years, CART therapy has demonstrated tremendous success in eradicating hematological malignancies, but no big improvement has been achieved in solid tumors. In this study, we converted an MG7 antibody into a scfv and co-engrafted the scfv as an ACT cell as an MTRA for the treatment of gastric cancer. To our knowledge, this is the first CART therapy being studied on gastric cancer. First to investigate the Gastric Cancer specificity of MG7 antigen, we did immunohistochemistry study on 485 samples (including 325 gastric cancer samples). It showed that MG7 antigen can’t be detected in normal gastric samples (0/50) and only 8% in chronic atrophic gastritis samples (4/50), but the positive rate increased to 58% and 70% in gastric dysplasia tissues (35/60) and gastric cancer tissues samples (227/325). At the same time, we compared MG7 antigen with other cancer biomarkers in gastric cancer tissues. Within 50 cancer samples, MG7 antigen has positive rate of 73%, much higher than the other markers, including CA199 (53%), CEA (51%), CA72-4 (31%) and CA125 (14%). For
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MG7-CAR, we converted the MG7 antibody to a scfv without losing any antigen binding affinity and “scfV-CD41BB-CD3ε” structure was used for the MG7-CAR. The study showed that the MG7-CAR can be well expressed with transduction rate close to 40%. The in vitro data showed that MG7-CART can efficiently kill gastric cancer cell line KATO3, but not colorectal cancer cell line SW620. In both gastric cancer antigen CEA overexpressed. The cytotoxicity assay also showed that IFN-gamma releasing result is highly correlated to the cell killing result. In order to further estimate the efficacy of the MG7-CAR, we set up a patient derived xenograft (PDX) model with gastric samples highly expressing both CEA and MG7 antigen. With one time intratumor injection of 1.5 million CART cells, both MG7 CART and CEA CART regressed the PDX models, while the MG7 CART had higher efficacy of tumor regression than CEA CART and showed in vivo antitumor activity of the PDX models. Our work demonstrated that MG7 antigen is very specific for gastric cancer and can be a novel treatment target by applying CAR T cell therapy.

#3767 Identification of a novel and a shared H3.3K27M mutation derived neoantigen epitope and H3.3K27M specific TCR engineered T cell therapy for glioma. Zinal Chheda,1 Gary Kohanbash,1 John Sidney,1 Kaori Okada,1 Naznin Jahan,1 Diego Carrera,1 Payal Watchmaker,1 Kira Downey,1 Shuming Liu,1 Shrutti Shrivastav,1 Sabine Mueller,1 Ian F. Pollack,1 Angel M. Carcaboso,1 Alessandro Sette,1 Yafei Hou,1 Hideho Okada1. UCSF, San Francisco, CA; 2La Jolla Institute for Allergy and Immunology, La Jolla, CA; 3University of Pittsburgh School of Medicine, Pittsburgh, PA; 4Hospital Sant Joan de Deu Barcelona, Spain.

Brain cancers are the leading cause of cancer related mortality in children and young adults with median overall survival of 9-12 months and hencerepresents a significant unmet medical need. Genome-wide sequencing efforts of pediatric gliomas have identified a recurrent and shared missense mutation in the gene encoding the replication-independent variant of histone 3, H3.3. Approximately 70% of diffuse intrinsic pontine gliomas (DIPG) and 50% of thalamic and other midline gliomas harbor the amino acid substitution from lysine (K) to methio- nine (M) at the position 27 of H3.3 gene. Tumor specific missense mutations are not subjected to self-tolerance and can be suitable targets (neoantigens) for cancer immunotherapy. Herein, we evaluated whether the H3.3K27M mutation can induce specific cytotoxic T lymphocyte (CTL) response in HLA-A2+ T cells. In vitro stimulation of HLA-A2+ donor derived CD8+ T cells with a synthetic peptide encompassing the H3.3K27M mutation (H3.3K27M epitope) induced CTL lines which recognized not only T2 cells loaded with the synthetic H3.3K27M epitope peptide but also lysed the HLA-A2+ DIPG cells which endogenously harbor the H3.3K27M mutation. On the other hand, the CTL lines did not react to either HLA-A2+ but H3.3K27M- negative DIPG cell lines or HLA-A2+ but H3.3K27M negative DIPG cells. The H3.3K27M epitope peptide but not the non-mutant counterpart indicated an excellent binding affi- nity (Kai=5.104 M) to HLA-A2 based on competitive binding assays. From CTL clones with high and specific affinities to HLA-A2-H3.3K27M-te- tramer, cDNAs for T cell receptor (TCR) alpha and beta chains were cloned into a retroviral vector. Human HLA-A+2 T cells transduced with the TCR demonstrated antigen specific reactivity as well as anti-glioma responses in vitro. Peps- tide titration assays suggested that the H3.3K27M specific TCR had the half-maximal reactivity for peptide recognition of around 100 nM. Furthermore, it is critically important for safety of clinical application, alanine scanning demon- strated that the key amino acid sequence motif in the epitope of the TCR reac- tivity is not shared by any known human protein. Finally, intravenously adminis- tration of T cells transduced with H3.3K27M specific TCR significantly inhibited the growth of intracranial HLA-A2+ H3.3K27M positive glioma xenografts in immune deficient NSG mice. These data provide us with a strong basis for developing peptide based vaccines as well as adoptive transfer therapy using autologous T cells transduced with the H3.3K27M specific TCR. Acknowledge- ments: This study is supported by the NIH/NINDS (1RO1NS096954), V Foundation and Parker Institution for Cancer Immunotherapy.

#3768 CAR T cells specific for CD19 can be redirected to kill CD19 negative tumors. Christine Ambrose, Lihe Su, Lan Wu, Roy L. Cobb, Paul D. Rennert. Aleta Biotherapeutics, Natick, MA.

Introduction. Remarkable progress has been made in the treatment of re- lapsed/refractory Acute Lymphocytic Leukemia and Non-Hodgkin Lymphoma with CAR-CD19 T cells. In contrast, progress against CD19-negative hematological cancers and solid tumors has been limited. Intensive efforts to optimize cellular therapies for better efficacy include provision of cytokine support and countering immunosuppression. However, lack of sufficient antigen is a significant additional hurdle that CAR-T therapeutics for solid tumors must overcome. We present a novel strategy to utilize CD19 for sustained antigen presentation in order to promote cellular therapy expansion, efficacy and persistence. The strategy, called IMPACT (Integrated Modules ofTimmize Adoptive Cell Therapy), employs a methodology that is modular in design and can be applied to diverse antigens and tumor types, yet retains the well-establish- ed advantages of CAR T cells directed to CD19. Experimental Procedures. A 3rd generation CAR-T was constructed in a lentiviral vector using the FMC63 domain of VEGFR2 and the FMC63 domain of CD28, with the FMC63 domain of CD137 downstream of the CAR-T cassette, was constructed using a secretion signal sequence and exons 1 - 4 of the human CD19 protein linked in frame to a trastuzumab-derived VH/VL sequence. This integrated-gene module thus encodes a fusion protein (FP): CD19-anti-HER2-scFv. Results. ELISA and flow cytometry analyses were used to demonstrate bridging between HER2+ and CD19- targets by the FP. ELISA analyses further demonstrated wild type binding of the 2 FP domains (i.e. CD19/FMC63 and the scFv/HER2). In vitro cytotoxicity studies showed robust redirected killing of HER2+ (CD19+ tumor) cells by CAR19 T cells that is mediated by the secreted FP. The efficiency of cytotoxicity was enhanced by the presence of CD19+ B cells. A tumor xenograft model was used to demonstrate effective killing in vivo when HER2+ BT474 cells were injected in one flank and CD19+ RAMOS cells were injected in the opposite flank, but not when HER2+ cell were injected alone. Thus, in vivo, redirected killing required CD19+ cells for expansion and persistence sufficient to promote anti-HER2 cytotoxicity. Conclusions. A CD19-anti-HER2-scFv FP was able to redirect the cytotoxicity of CAR19 T cells in order to kill HER2+ tumor cells. In vivo modeling demonstrated that CAR-T cell expansion and anti-HER2 cytotoxicity was limited by the antibody (scFv/HER2) used for cytotoxicity. The IMPACT technology is modular, allowing for FPs having diverse protein domains paired with diverse, distinct scFvs to allow CAR T cells to CD19 or other targets to be used to kill any tumor. We have further developed the technology to address antigens of utility for hematopoietic and solid tumors, including BCMA, ROR1, and PD-L1. The technology is broadly applicable and syner- gistic with existing cellular therapies.

#3769 Stem cell-engineered invariant natural killer T cells for cancer ther- apy. Lili Yang, Drake Smith, Siyuan Liu, Sunjong Ji, Bo Liu. Univ. of California, Los Angeles, Los Angeles, CA.

Invariant natural killer T (iNKT) cells comprise a small population of ββ T lymphocytes. They bridge the innate and adaptive immune systems and mediate strong and rapid responses to many diseases, including cancer, infections, aller- gies and autoimmunity. However, the study of iNKT cell biology and the ther- apeutic applications of these cells are greatly limited by their small numbers in vivo (~0.01-1% in mouse and human blood). Here, we report a new method to generate large numbers of iNKT cells in mice through T cell receptor (TCR) gene engineering of hematopoietic stem cells (HSCs). We showed that iNKT TCR-engineered HSCs could generate a clonal population of iNKT cells. These HSC- engineered iNKT cells displayed the typical iNKT cell phenotype and function- ality. They followed a two-stage developmental path, first in thymus and then in the periphery, resembling that of endogenous iNKT cells. When tested in a mouse melanoma lung metastasis model, the HSC-engineered iNKT cells effec- tively protected mice from tumor metastasis. This method provides a powerful and high-throughput tool to investigate the in vivo development and function- ality of clonal iNKT cells. More importantly, this method takes advantag- e of the self-renewal and longevity of HSCs to generate a long-term supply of engineered iNKT cells, thus opening up a new avenue for iNKT cell-based immu- notherapy.

#3770 CAR-T cell harboring a camelid single domain antibody as a target- ing agent to kill tumors expressing VEGFR2. Heman Chao, Baomin Tian, Marni Uger, Wai Wong. Heils BioPharma Corp., Toronto, Ontario, Canada.

Modulation of the immune system is showing tremendous promise in the treatment of malignancies. In addition to checkpoint inhibitors that re-activate T cells present in the tumor microenvironment, exogenously transduced chimeric antigen receptor (CAR) T cells are providing excellent responses in clinical trials for the treatment of leukemias. In this study, we describe CAR-T cells that target VEGFR2 expressing tumors. Camelid antibodies are small (14 kD) sin- gle chain antibodies. To generate a camelid antibody targeting the extracellular domain of VEGFR2, a llama was immunized with recombinant VEGFR2/Fc. A phage display library was generated and screened to identify an antibody with high binding affinity to VEGFR2. The selected antibody was expressed in the E.
Complementary.

creased survival in mice bearing intracranial gliomas and are mechanistically

inclusions: Combination treatment with GVAX and OX-40 agonist antibody in-

was little changed. In the tumor microenvironment, vaccination improves the
combination with vaccination drove high levels of interferon gamma expression
is induced by the presence of intracranial glioma. OX40 signaling alone and in
revealed that combination immunotherapy reverses the Th2 cytokine skew that
effective than either therapy alone and prolonged survival in mice with estab-
treatment on days 3, 6 and 9 following tumor implantation. Results: Combina-
intraperitoneal injection of OX40 antibody, either monotherapy, or control
recent subcutaneous injection of irradiated GM-CSF expressing glioma cells and
micereceivedconcurrentvaccinationondays3,6and9followingtumorimplantation.Micereceivedconcur-
the seefforts are underway in the clinic. In contrast to immune checkpoint block-
target VEGFR2-expressing tumors provides hope that camelid single domain
antisense-VEGFR2 CAR-T may be useful in directly targeting VEGFR2-expressing
tumors. We previously showed the utility of camelid antibodies in CAR T-con-
structs as anti-CEACAM6 CAR-T cells show both in vitro and in vitro efficacy
against the pancreatic tumor Bx-PC3. The use of the camelid V21 antibody to
target VEGFR2-expressing tumors provides hope that camelid single domain
antibodies can be developed for CAR-T therapies.

#3771 Concurrent GVAX and anti-OX40 therapy in murine intracranial
glioma model. Nusrat Jahan, Hammad Talat, William T. Curry, Department of
Neurosurgery, Massachusetts General Hospital, Harvard Medical School, Boston,
MA.

Introduction: Malignant glioma is the most aggressive brain tumor with a
median survival time of less than 1.5 years post diagnosis despite standard treat-
ments. However, recent advancements in immunotherapy hold significant
promise. Irradiated whole tumor cell vaccination stimulates antitumor immu-

ities and oncophenotypes are not known. Methods: To investigate how inflam-
correlations and risks attributed to inflammatory diseases of the abdominal cavi-
and mouse models. We applied an integrative informatics approach with exper-
mental validation to understand the associations between inflammatory dis-

coli. BL21 (DE3) pG7 system. The purified antibody was characterized by SEC,
LC-MS peptide mapping and ELISA. CAR-T cells were engineered to express
the camelid anti-VEGFR2 antibody in combination with the CD28 and 4-1BB
co-stimulatory molecules and the CD3 zeta chain. Tumor cells were screened for
expression of VEGFR2, and the HL-60 acute promyelocytic leukemia, ZR-75-30
breast ductal carcinoma and NCI-H460 non-small cell lung adenocarcinoma
cells were identified. Co-incubation of anti-VEGFR2 CAR-T cells with the VEGFR-
2-expressing cell lines resulted in dose-dependent target cell toxicity as mea-
sured by LDH release. In addition, T cell activity was confirmed, as high levels of
IL-2 and IFN-γ were detected in the cell culture media. These results suggest that
anti-VEGFR2 CAR-T may be useful in directly targeting VEGFR2-expressing
tumors. We previously showed the utility of camelid antibodies in CAR T-con-
structs as anti-CEACAM6 CAR-T cells show both in vitro and in vitro efficacy
against the pancreatic tumor Bx-PC3. The use of the camelid V21 antibody to
target VEGFR2-expressing tumors provides hope that camelid single domain
antibodies can be developed for CAR-T therapies.

#3772 A multi-scale survey to assess the impact of inflammatory diseases
of the abdominal cavity and prostate cancer severity. Khusher Shams, Kam-
lesh Kumar Yadav, Li Li, Shalini S. Yadav, Benjamin Glicksberg, Irtaza Khan,
Kipp W. Johnson, Marcus A. Badgley, Cordelia Eliaho, Ben Readhead, Brian A.

Introduction and Objectives: Prostate cancer (PCa) is the most common can-
dered in men (181,000 annual cases), and nearly 26,000 American men die
each year due to PCa and related complications. Reports have shown that PCa
is more aggressive when its comorbid with inflammatory diseases. However, the
correlations and risks attributed to inflammatory diseases of the abdominal cav-
y and oncophenotypes are not known. Methods: To investigate how inflam-
matory pathways and PCa genes induce aggressive oncophenotypes in the set-
ing of PCa, we have compiled a multiscale survey that includes data from
surgical observations, inflammatory phenotypes, clinical registries, biomarkers
and mouse models. We applied an integrative informatics approach with exper-
imental validation to understand the associations between inflammatory dis-
es (e.g., Crohn’s disease, ulcerative colitis, collagenous colitis, indeterminate
coli, ischemic colitis, diverticulitis, hernia, etc.) and PCa. Results: We found distinct patterns of shared molecular features—gene sets, pathways, and net-
works—and comorbidities across inflammatory disease and PCa. For example,
we found that diverticulitis tend to increase inflammation in the abdominal cavity and could potentially lead to aggressive prostate oncophenotypes. To test
these findings, we observed that several inflammatory disease and pros-
tate cancer genes share genetic modules. Conclusion: Collectively, our findings
provide the first set of computational, experimental and clinical evidence to
recommend clinicians to evaluate the impact of inflammatory disease induced
oncophenotypes in patients with PCa. Given that 1.3 million patients undergo
prostate-specific antigen (PSA)-triggered invasive trans-rectal biopsy, the pres-
ent findings in combination with PSA could facilitate the identification patient
subset with aggressive cancer. Stratifying patients at risk for prostate cancer who
are undergoing surgical interventions of abdominal cavity for inflammation
diseases could also evaluate other non-surgical or therapeutic strategies. Fund-
ing acknowledgements: AKT is supported by grants from Prostate Cancer Found-
ation and Deane Prostate Health. JT is supported by grants from National Cancer Institute (NCI-U54-CA182012-02, National Center for Advancing
Translational Sciences NCATS-UL1TR000067 and Clinical and Translational
Science Award (CTSA).

#3773 Tapping CD4 T cells for cancer immunotherapy, Sébastien Walchi,
Marit R. Myhre, Nadia Mensali, Anne Fane, Kari Liserud, Gunnar Kvalheim,
Gustav Gaudernack, Else M. Inderberg, Oslo University Hospital-The Norwegian
Radium Hospital, Oslo, Norway.

T-cell based immunotherapy represents an attractive strategy for the treat-
mant of cancer. Whereas cellular anti-tumor immune responses have
typically been attributed to CD8 T cells, CD4 T cells play a critical role in
tumor elimination and the priming and maintenance of CD8 T-cell re-
sponses. Recent findings have highlighted new opportunities for CD4 T cells
in cancer immunotherapy. We have isolated CD4+ T cells reactive against
tumor antigens from patients who experienced clinical benefit from treat-
ment with cancer vaccines targeting antigens such as HTERT, survivin and
frequent neoantigens such as frameshift mutated TGFβRII. Strong T-cell re-
ponses against the vaccine or unrelated cancer antigens suggesting
epitope spreading correlated with enhanced survival and tumor regression
in late stage cancer patients. These HLA class II restricted T-cell clones
recognised target cells loaded with long peptides or protein and for some
CD4+ T cell clones we could also show direct tumor recognition. TCRs were
expressed in expanded third-party T cells by mRNA electroporation or ret-
roviral transduction and tested for functionality. Both CD8+ and CD4+ T cells
expressing the TCRs produced TNF-α, IFN-γ and redirected T cells had
the capacity to kill following co-incubation with their targets. Selecting
higher fun inflation. TCRs correlation and redirecting T cells treated on the
basis of finding a unique set of CD4+ T cells raised against tumor antigenic
specific antigens from patients with clinical responses after treatment with
immunotherapy is a successful method for identifying highly functional
HLA class II restricted TCRs for adoptive immunotherapy. These HLA class
II restricted TCRs may be of therapeutic value both in haematopoietic ma-
lignancies and in melanoma where tumor cells frequently express HLA class
II. Furthermore, combining HLA class I- and class II-restricted TCRs for
T-cell redirection may provide a more potent therapeutic effect in adoptive T

#3774 A high-throughput process for the development and characteriza-
tion of chimeric antigen receptors (CARs). Sabarinath Venkylal Radhakrish-
nan, Adam Miles, Djordje Atanackovic, Tim Luetkens, Adam Martin,1
Huntsman Cancer Institute, University of Utah, Salt Lake City, UT; 2Wasatch Microfluidics, Salt
Lake City, UT.

INTRODUCTION: Chimeric antigen receptors (CARs) expressed by T cells
bind to surface antigens via a single-chain variable fragment (scFv) and are able
to elicit potent anti-tumor efficacy. To our knowledge no rapid CAR develop-
ment platforms exist to facilitate the high-throughput generation and character-
ization of novel CARs to allow head-to-head comparisons and to determine the
influence of scFv properties on CAR T cell activation, phenotype, and persis-
tence. EXPERIMENTAL PROCEDURES: We have established a rapid process
for the development of functional novel CARs using fully human antibody
phage display, vector systems for bacterial and mammalian expression, as well as

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high-throughput functional and biochemical characterization assays. We validated individual parts of the workflow using different tumor antigens and completed the entire workflow for one model antigen expressed on the surface of multiple melanoma cells. An initial population of scFvs against the model antigen was selected from a large naïve fully-human antibody phage display library by performing a single user of vimentin for cancer diagnosis is limited. This comprehensive study including immuno-markers screening and their applicability test with clinical samples demonstrates the clinical utility of the present device and hydrogel-assisted cell block for CTC isolation and cancer studies.

**CLINICAL RESEARCH: Innate Effectors in Immunity to Cancer**

Young Jun Kim,1 Tae Hee Lee,1 Hee Jin Chang,2 Hyun-Moo Lee,3 Young-Ho

filtered from the renal cell carcinoma patients’ blood.

CLINICAL RESEARCH: Liquid Biopsies 3: Circulating Tumor
evaluation for improved patient care and monitoring in the future.

#3776 Examination of ERCC1 status in circulating tumor cells as a prog-

nostic tool for patients with nasopharyngeal carcinoma. Edwin P. Hui,1

Brigitte By Ma,1 KC Allen Chan,1 Charles ML Chan,1 SC Cesar Wong,2 Ka Fai

To,1 Herbert HP Loong,1 Frankie KF Mo,3 Roger KC Ngan,1 Anthony TC

Chan.1, 1Chinese Univ. of Hong Kong, Shatin, NT, Hong Kong; 2Hong Kong Poly-

technic University, Hong Kong, Hong Kong; 3Queen Elizabeth Hospital, Hong

Kong, Hong Kong.

Investigation of the expression pattern of DNA-repair protein excision repair cross-complementation group I (ERCC1) has been reported to allow selection of patients with non-small cell lung cancer who are likely to benefit from cisplatin-based therapy. Recent evidence suggests that ERCC1 expression may also find prognostic use in patients with nasopharyngeal carcinoma (NPC). We evaluated ERCC1 expression and genotype from NPC tissues and peripheral blood mononuclear cells of patients with NPC. ERCC1 expression was detected in 61/77 cases (79.2%) with varying intensities, where high ERCC1 expression was significantly associated with favorable RFS and overall survival (OS) in a subgroup of patients with undetectable post-treatment plasma EBV DNA. These findings support a prognostic role for ERCC1 examination in NPC. However, the invasive nature of obtaining biopsy samples or tumor marker studies may be a hindrance of this approach. To facilitate the investigation of ERCC1 expression in a noninvasive manner, we have developed a negative selection immunomagnetic method for isolating circulating tumor cells from patient blood. Cell line spike-in experiments reveal a mean recovery rate of 66% for NPC cells with greater than 99% removal of non-targeted blood cells. Specificity of CTC identification was confirmed by detection of the expression of the Epstein-Barr virus encoded small RNA (EBER) in the CTCs. This method also allowed the simultaneous analysis of the expression of multiple protein markers including CD45, cytokeratin and ERCC1. It is expected that the development of noninvasive methodologies for tumor marker studies will facilitate their clinical application for improved patient care and monitoring in the future.

**#3777 DNA profiles of circulating tumor cells and extracellular vesicles for therapy stratification of metastatic breast cancer patients. Corinna Keup,1

Siegfried Hauch,2 Linda Plappert,2 Markus Sprenger-Haussells,2 Pawel Mach,1 Mitra Tewes,3 Bahriye Aktas,3 Hans-Christian Kolberg,2 Rainer Kimmig,1 Sa-

bine Kasimir-Bauer1.

1Department of Gynecology and Obstetrics, University Hos-

tial of Essen, Essen, Germany; 2QIAGEN GmbH, Hilden, Germany; 3Depart-

ment of Internal Medicine (Cancer Research), University Hospital of Essen, Essen, Germany; 4Clinic for Gynecology and Obstetrics, Marienhospital Bottrop gGmbH, Bottrop, Germany.

Background: Blood analytes, as liquid biopsies, are discussed to be surrogate markers for therapy stratification of metastatic breast cancer (MBC) patients. Repeated analysis is enabled by the minimal invasive nature of blood draw. Analysis of RNA enclosed in circulating tumor cells (CTCs) or extracellular vesicles (EVs) may be sensitive enough to detect disease progression earlier than contemporary visual staging methods. A prediction of the ideal therapy strategy via characterization of CTCs or EVs would be even more beneficial. Here we compare RNA profiles of CTCs and EVs in MBC patients to get insight into their feasibility for therapy stratification. Patients and methods: Blood was collected from 10 MBC patients at the time of disease progression (T0) and at two consecutive clinical staging time points (T1 and T2) during therapy. Two cohorts were separated according to RECIST criteria a) Overall Responder showed response at T1 and T2 and b) Late Non-Responder displayed stable disease or partial remission at T1, but showed progressive disease at T2. CTCs were isolated from 5 ml blood by positive immunomagnetic selection targeting EpCAM, EGFR and HER2 (AdnaTest EMT2/STEMCell Select TM, QIAGEN, Germany). EVs were isolated from 4 ml pre-filtered plasma by affinity-based binding to a spin column (exoRNeasy, QIAGEN, Germany). mRNA bound to Oligo-dT beads was purified and reverse transcribed (AdnaTest EMT2/STEMCell Detect TM, QIAGEN, Germany). Pre-amplified cDNA was analysed by a multimeric

#3775 Hydrogel-assisted pathological study of the circulating tumor
cells from the renal cell carcinoma patients’ blood. Yoon-Tae Kang,1

Young Jun Kim,1 Tae Hee Lee,1 Hee Jin Chang,2 Hyun-Moo Lee,3 Young-Ho

Cho1.

1KAIST, Daejeon, Republic of Korea; 2National Cancer Center, Goyang, Republic of Korea; 3Samsung Medical Center, Seoul, Republic of Korea.

Introduction: Circulating tumor cells (CTCs), defined as tumor cells de-
tached from the primary tumor site and circulating in the peripheral blood, are a potential myeloma to observe specific killing of tumor cells and no overt toxicities.

CONCLUSION: We show that our process allows the generation, screening, as well as functional and biochemical characterization of novel CARs to toxicities. CONCLUSION: We show that our process allows the generation, screening, as well as functional and biochemical characterization of novel CARS within 2 months. Our approach enables researchers to easily carry out head-to-head comparisons allowing comprehensive lead identification and the determination of binding domain properties which may shape CAR T cell function and phenotype.

CLINICAL RESEARCH: Liquid Biopsies 3: Circulating Tumor

Cells, Extracellular Vesicles, and Exosomes

#3777 RNA profiles of circulating tumor cells and extracellular vesicles for therapy stratification of metastatic breast cancer patients. Corinna Keup,1

Siegfried Hauch,2 Linda Plappert,2 Markus Sprenger-Haussells,2 Pawel Mach,1 Mitra Tewes,3 Bahriye Aktas,3 Hans-Christian Kolberg,2 Rainer Kimmig,1 Sa-

bine Kasimir-Bauer1.

1Department of Gynecology and Obstetrics, University Hos-
tial of Essen, Essen, Germany; 2QIAGEN GmbH, Hilden, Germany; 3Depart-

ment of Internal Medicine (Cancer Research), University Hospital of Essen, Essen, Germany; 4Clinic for Gynecology and Obstetrics, Marienhospital Bottrop gGmbH, Bottrop, Germany.

Background: Blood analytes, as liquid biopsies, are discussed to be surrogate markers for therapy stratification of metastatic breast cancer (MBC) patients. Repeated analysis is enabled by the minimal invasive nature of blood draw. Analysis of RNA enclosed in circulating tumor cells (CTCs) or extracellular vesicles (EVs) may be sensitive enough to detect disease progression earlier than contemporary visual staging methods. A prediction of the ideal therapy strategy via characterization of CTCs or EVs would be even more beneficial. Here we compare RNA profiles of CTCs and EVs in MBC patients to get insight into their feasibility for therapy stratification. Patients and methods: Blood was collected from 10 MBC patients at the time of disease progression (T0) and at two consecutive clinical staging time points (T1 and T2) during therapy. Two cohorts were separated according to RECIST criteria a) Overall Responder showed response at T1 and T2 and b) Late Non-Responder displayed stable disease or partial remission at T1, but showed progressive disease at T2. CTCs were isolated from 5 ml blood by positive immunomagnetic selection targeting EpCAM, EGFR and HER2 (AdnaTest EMT2/STEMCell Select TM, QIAGEN, Germany). EVs were isolated from 4 ml pre-filtered plasma by affinity-based binding to a spin column (exoRNeasy, QIAGEN, Germany). mRNA bound to Oligo-dT beads was purified and reverse transcribed (AdnaTest EMT2/STEMCell Detect TM, QIAGEN, Germany). Pre-amplified cDNA was analysed by a multimeric
CLINICAL RESEARCH: Liquid Biopsies 3: Circulating Tumor Cells, Extracellular Vesicles, and Exosomes

qPCR (AdnaPanel TNBC, QIAGEN, Germany). RNA profiles of 18 genes (including AKT2, ALK, AR, AURKA, BRCA1, cKIT, cMET, EGFR, ERCC1, HER2, HER3, KRT15, NOTCH1, PARP1, P63, SRC1, GAPDH) were normalized by data of healthy donors (n = 20) and CD45 served as leukocyte control in the CTC preparation. Results: In general, data analysis showed great differences in RNA profiles of EVs and CTCs. However, in similar signal courses for P3K and cMET were found in all time points, revealing P63 as potential positive response marker and cMET as potential negative response marker in both blood analytes. Conclusions: Expression profiling in CTCs as well as in EVs is enabled by the described workflows. Preliminary data indicated great differences in RNA profiles of EVs and CTCs. The amount of included patients is continuously increased to validate the preliminary results obtained until now.

#3777 PD-L1 expression on circulating tumor cells and its comparison with tumor tissues in Japanese lung cancer patients. Hiroaki Akamatsu,1 Yasuhiro Koh,1 Satomi Yagi,1 Satoshi Kambayashi,1 Ayaka Tanaka,1 Kuninobu Kanai,1 Atsushi Hayata,1 Nahomi Tokudome,1 Keiichiro Akamatsu,1 Masayuki Higuchi,2 Hisashige Kanbara,1 Masanori Nakashima,1 Hiroki Ueda,2 Nobuyuki Yamamoto.1

Background: Blockade of programmed death receptor-1 (PD-1) pathway is effective against solid tumors including lung cancer. Although PD-ligand 1 (PD-L1) expression on tumor tissue is expected as a potent predictive biomarker, its detection remains challenging due to its dynamic and unstable status. Circulating tumor cells (CTCs) have potential as an alternative material for non-invasive and real-time diagnosis. Here, we evaluated the PD-L1 expression on CTCs in patients with lung cancer and investigated the agreement between tumor tissues and CTCs. Material and methods: CTCs were captured and immunestained using microcavity array system. CTCs were defined as those positive for DAPI and cytokeratin (CK) and negative for CD45. PD-L1 expression on CTCs was evaluated by addition of the process of PD-L1 immunocytochemistry. For CTCs detection, 3 ml of peripheral whole blood was collected from the patients who consented in written form and PD-L1 immunohistochemistry was performed using corresponding tumor tissues. Results: Sixty-seven lung cancer patients were enrolled in the study between July 2015 and April 2016 at Wakayama Medical University. Patient characteristics were as follows: median age 71 (range, 39 to 86); male 72%; stage II-III/IV, 15/85%; non-small cell lung cancer (NSCLC)/small cell lung cancer (SCLC)/Other, 73/21/6%. CTCs were detected in 66 out of 67 patients (median 19; range, 0 to 115) and more than 5 CTCs were detected in 78% of patients. PD-L1-expressing CTCs were detected in 73% of patients and the proportion score (PS) of PD-L1-expressing CTCs ranged from 3% to 100%, suggesting intra-patient heterogeneity of PD-L1 expression on CTCs. Significantly more PD-L1-expressing CTCs were detected in patients with EGFR mutations than those without (P = 0.0433). Tumor tissues were available from 28 patients and were immune-stained for PD-L1. Seventeen showed the PS of PD-L1-expressing tumor cells < 1%, 11 showed 1-49%, and 10 showed ≥ 50%. No positive correlation was observed on PD-L1 expression between tumor tissues and CTCs based on PS (R² = 0.0034). Three adenocarcinoma cases with PD-L1-positive tumor tissue did not harbor any PD-L1-expressing CTCs and conversely, three adenocarcinoma cases with PD-L1-negative tumor tissue harbored PD-L1-expressing CTCs, showing the discrepancy between tumor tissues and CTCs. It is also noteworthy that SCLC patients had perfect agreement on PD-L1 expression between tumor tissues and CTCs. Conclusions: PD-L1 expression was detectable on CTCs in lung cancer patients. Intra-patient heterogeneity of its expression was observed. There was no agreement between tumor tissues and CTCs on PD-1 expression. Further investigation is warranted to better understand the clinical significance of PD-L1-expressing CTCs.

#3778 Incorporating in-line sample pre-enrichment with FACS and single cell mRNA-seq to facilitate isolation and characterization of prostate cancer circulating tumor cells. Liping Yu, Silin Sa, Michael Tycon, Xiaoyang Alice Wang, Yun Wang, Zhaohua Zhang, Jie-Fu Chen, Sarah Hoffe, William J. Higuchi,2 Hisashige Kanbara,2 Masanori Nakanishi,1 Hiroki Ueda,1 Nobuyuki Yamamoto.1

For decades, circulating tumor cells (CTCs) have been used as biomarkers to indicate disease progression and survival in patients with metastatic tumors. Detection of CTCs is an attractive, non-invasive blood test for early diagnosis of many types of cancer. However, CTCs are present at extremely low frequency in blood, and it is challenging to recover them in typical multi-step sample processing workflows. EpCAM, the widely accepted marker for CTC, has been found being down regulated in protein expression and RNA expression analysis in many cases thus EpCAM-dependent CTC detection might not be sensitive enough. We have developed a new sample preparation workflow to miniaturize, enrich and activate CTCs (cell sorting (BD FACs) platform) to demonstrate a one-step sample processing workflow to minimize cell loss and maximize CTC recovery. The pre-enrichment technology combines magnetic depletion of white blood cells and acoustic removal of lysed red blood cells, sending enriched tumor cells directly to a cell sorter for single CTC isolation. The in-line enrichment and sorting technology has shown 90% removal of CD45+ cells and over 90% recovery of target cells going through the system. Furthermore, we have incorporated molecular indexing technology for next generation sequencing (NGS) into the single CTC isolation to streamline gene expression analysis. We conducted a pilot study in which prostate cancer cell lines PC3, DU145, and LNCaP were spiked in healthy donors’ peripheral blood, quantitatively recovered, and individually sorted in 96-well 8D® Precise barcoded plates using a BD Influx™ sorter equipped with the in-line pre-enrichment module. The sorted cells were then sequenced for a prostate cancer targeted gene panel with approximate 100 genes including EpCam. Single cell sorting and sequencing were also performed on cell line cells directly and confirmed the workflow did not affect the gene expression. In LNCaP and PC3 cells, PD-L1 was not the high confidence CTC marker in both workflow and its protein expression was also heterogeneous. The highly expressed genes discovered by single cell mRNA sequencing such as GSTP1 and CA9 were investigated to provide additional cell surface markers for more sensitive CTC detection. Finally, CTCs from blood samples collected from prostate cancer patients were isolated based on marker panels beyond EpCam and characterized at the single cell level. The differences between gene expression profiles of patient sample CTCs and cancer cell line cells were revealed.

#3779 Bio-competition-based smart NanoVelcro Chip for isolation and gene expression analysis of circulating tumor cells from prostate cancer patients, Jie-Fu Chen,1 Mo-Yuan Shen,1 Chun-Hao Luo,1 Shirley Cheng,1 Sangjun Lee,1 Shuang Hou,1 Edwin M. Posadas,1 Hsiao-Hua Yu1,2

1 Cedars-Sinai Medical Ctr., Los Angeles, CA; 2 Academia Sinica, Taipei, Taiwan; 3 University of California, Los Angeles, Los Angeles, CA.

Introduction and Objective: Circulating tumor cells (CTCs) are being used in efforts to identify important transcriptomic features such as androgen receptor (AR) splicing variants in prostate cancer (PCa) and other malignancies. The low abundance of CTCs and the fragility of the genetic materials create a need for efficient and high-quality sample handling with high yields (greater than 90%) and their protein expression was also heterogeneous. The highly expressed genes discovered by single cell mRNA sequencing such as GSTP1 and CA9 were investigated to provide additional cell surface markers for more sensitive CTC detection. Finally, CTCs from blood samples collected from prostate cancer patients were isolated based on marker panels beyond EpCam and characterized at the single cell level. The differences between gene expression profiles of patient sample CTCs and cancer cell line cells were revealed.

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This in turn allows for detection of disease-relevant RNA signals. Further this new tool is being moved into clinical studies that will validate its performance in CTC purification and subsequent RNA detection.

**#3781 Expression of neuroendocrine markers in circulating tumor cells from patients with prostate cancer visceral metastases.** Jamie M. Spenger, Anupama Singh, David Niles, Charlotte N. Stahlfeld, David J. Beebe, Joshua M. Lang. University of Wisconsin, Madison, WI.

Purpose: Therapeutic resistance to Androgen Receptor (AR) targeted therapies in patients with Castration Resistant Prostate Cancer (CRPC) is complex and originates from a wide range of molecular alterations including AR point mutations, gene rearrangements and splice variant expression. An aggressive subtype of CRPC involves neuroendocrine de-differentiation that is often found in patients with visceral metastases. To evaluate neuroendocrine signatures in patients progressing on AR targeted therapies, we measured gene expression in circulating tumor cells (CTCs) as a minimally-invasive approach to examine putative resistance mechanisms to AR targeted therapies. Methods: We utilized the VERSA platform, an integrated CTC capture and analysis technology, to capture CTCs using antibodies for EpCAM or TROP2 in parallel. mRNA was extracted on chip from live cells for comparison of gene expression profiles for markers of neuroendocrine de-differentiation (SYT, CHGA, GHGB, MYCN, NCAM). Myeloid capture pathway fixed PMBC were stained and imaged with EpCAM or TROP2 captured, intact nuclei, negative for CD45/CD11b/CD14/CD34 and cytookeratin positive. Synaptophysin or Chromogranin A protein expression was quantified in individual CTCs and compared to tumor biopsy results where available. Results: Expression of the Synaptophysin (SYT) gene was found in 39% of the patients in our initial 18 patient cohort. These patients could be split into two groups. Group 1 patients had low AR expression with minimal detection of AR target genes. Of these four patients, three had visceral metastases. Group 2 patients had high levels of AR expression including expression of the splice variants ARV7 and ARV9, and robust expression of KLK3 (PSA) and PSM. From these three patients, two had visceral metastases. Subsets of the patients in both groups also exhibited expression of genes identified in neuroendocrine prostate cancer including Chromogranin B, MYCN, and NCAM. A subset of the patients tested for gene expression are being screened for CTC detection of protein markers of neuroendocrine phenotypes including Chromogranin A and Synaptophysin. Conclusions: We report the first identification of neuroendocrine gene expression signatures in CTCs from patients with CRPC experiencing disease progression on advanced AR targeted therapies. These signatures may serve as predictive biomarkers of resistance to AR targeted therapies. Future studies will evaluate these signatures in prospective clinical trials.

**#3782 Genetic analysis using a novel high-purity enrichment system for circulating tumor cells independent of epithelial cell antigen.** Hidenori Takagi,1 Masahiro Kozuka,1 Hiroshi Ito,1 Soo Hyeon Kim,2 Mitsuharu Hirai,1 Teruo Fujii3. 1ARKRAY, Inc., Kyoto, Japan; 2Institute of Industrial Science, The University of Tokyo, Tokyo, Japan.

Background: Genetic analysis of circulating tumor cells (CTCs) is useful as a liquid biopsy. However, there are 3 challenging issues in processing of CTC samples for clinical use of the analysis: [1] numerous residual blood cells in processed samples, [2] loss of CTCs that do not express epithelial markers, and [3] very laborious process. Here, we developed a novel system capable of overcoming all of these problems. Methods Our CTC analysis system is composed of 3 steps: [1] filtering of whole blood followed by immunostaining and magnetic labeling of cell population on the filter, [2] depletion of white blood cells (WBCs) in the cells recovered from the filter by magnetic separation, and [3] trapping the resultant cells at an observation chamber in a microfluidic enrichment device using dielectrophoresis, followed by recoverying them as a concentrated sample after fluorescence microscope observation. SNV and CNV analysis was conducted using the collected concentrated sample, and the system performance was substantiated. Genetic mutations of the cells in the collected sample were detected by the Quenching Probe method. On the other hand for CNV analysis, cells in the collected concentrated sample were immobilized on slide glass and analysis of gene amplification conducted by FISH. Results With SNV analysis, our system successfully detected EGFR, KRAS or PIK3CA mutations of cancer cell lines spiked in 8 mL of whole blood (Table). The detection sensitivity of our method was 1 cell/mL, and both the cancer cells and their genetic mutations were detected within 9 hours of starting the processing of whole blood. Additionally, with CNV analysis, HER2 gene amplification was confirmed with cell lines spiked in 8 mL of blood. This detection result was obtained within 48 hours. Conclusion Our system would be useful for the analysis of gene mutations in a wide range of CTC types independent of certain epithelial antigens, such as CK and EpCAM, and can be used for various genetic analyses.

**#3783 Circulating tumor cells measured in the pulmonary vein and the radial artery during surgery of non-small cell lung cancer.** Menno Tamminga,1 Sanne de Wit,2 Joost F. Swennenhuiz,2 Caroline van de Wauwer,3 Theo J. Klinkenberg,1 T. Jeroen Hiltermann,1 Harry J. Groen,1 Leon W. M. Terstappen2. 1University Medical Centre Groningen, Netherlands; 2University of Twente, Enschede, Netherlands.

Background: The number of circulating tumor cells (CTC) detected by the Cell-Search system is low or absent in 7.5 mL of venous peripheral blood of metastatic and non-metastatic non-small cell lung cancer (NSCLC) patients. Our hypothesis is that CTC can be detected in the pulmonary vein draining the lobe containing the primary tumor before its removal and would be influenced by manipulation of the lobe during surgery. Methods: Patients with early stage NSCLC undergoing open lobectomy were included. During surgery, blood was drawn centrally (pulmonary vein draining the lobe containing the tumor) and peripherally (radial artery) at two time points: directly after thoracotomy and identification of the draining pulmonary vein (T1) and just before closure of the draining pulmonary vein after manipulation, dissection and closure of the pulmonary artery (T2). EpCAM+ and CTC were detected by CellSearch. The EpCAM+ CTC depleted sample was subsequently filtered through 5 μm microsieves and fluorescently labeled for identification of EpCAM+. Results: So far, three patients have been included. Remarkably, cell clumps were detected among EpCAM+ CTC at T1 in the pulmonary vein in all patients, and as well at T2 in patient #3. CTC in the pulmonary vein at T1 was morphologically different than those detected at T2 and in the arteries. Experiments to confirm malignancy of both subtypes of CTC are pending, using chromosome aneuploidy status and copy number variation analysis to compare them with the resected tumor. Conclusion: In early stage NSCLC high amounts single and clumps of CTC are observed in the draining pulmonary vein during surgery. The amount measured in the radial artery is lower, indicating loss of CTCs in the circulatory system before peripheral measurement. If the cells found really are CTC that are shed from the primary tumor during surgery, it will have clinical consequences for the surgery techniques used.

**#3784 Expression of estrogen receptor specific signaling transcriptome in epithelial cell adhesion molecule (EpCAM) capturing circulating tumor cells from patients with breast cancer.** Ashok Singh, Jamie M. Sperger, Jennifer Schehr, Tessa Witkowski, Beth A. Weaver, Mark E. Burkard, Joshua M. Lang. Univ. of Wisconsin-Madison, Madison, WI.

Background: Breast cancer (BC) is the second leading cause of cancer related death in American women. There is a critical need for new biomarkers to evaluate treatment response and emerging mechanisms of resistance. The identification and characterization of new biomarkers such as Circulating Tumor Cells (CTCs) may be helpful tools for these purposes as well as understanding the biology of metastatic BC. Methods: Blood samples were processed with a Ficollic gradient to isolate nucleated cells. CTCs were isolated from these samples using an exclusion-based sample
preparation technology known as the VERSA (Versatile Exclusion-based Rare Sample Analysis) platform. EpCAM positive CTCs were captured in the VERSA followed by RNA extraction for gene expression profiling for the estrogen receptor (ER) and other breast cancer relevant targets. CTCs were defined as Hoechst positive, CD45/CD11b/CD14 negative, and cytokeratin positive. Captured CTCs were evaluated for ER protein staining intensity and localization. Results: The VERSA platform was validated to capture and enumerate CTCs from patients with breast cancer using EpCAM capture and immunofluorescent staining for intact nucleus, ER, and cytokeratin while negative for multiple immune markers (CD45, CD11b, CD14). The VERSA can capture cells with >90% capture efficiency with EpCAM antibody in MCF7 and T47D cell lines. ER specific transcriptome analysis in BC patient CTCs and cell lines was performed. We evaluated the performance of ROSCEXP signaling pathway, prognostic, metastatic/EMT- stemness; epithelial markers with concordant expression of RPLP0 and HPRT1. We identified expression of ER in 6 out of 7 BC samples. Expression of PR and HER2 in a subset of samples confirms capture of breast cancer cells for further molecular interrogation. Conclusion: In conclusion, we report the expression profiles of ER signaling genes in EpCAM captured CTCs from BC patients. These tools will be used to compare subpopulations of CTCs from patients with BC as predictive and pharmacodynamic biomarkers of response to targeted therapies or chemotherapies.

#3785 Self-sorting microwells to isolate and expand single circulating tumor cells. Joost F. Svennenhuis, 1 Kiki C. Andree, 1 Frikki Abali, 1 Michiel Steens, 1 Joska Broekmaat, 2 Fiona F. Passanha, 1 Cees J. van Rijn, 3 Leon W. Terstappen, 1. Twente University, Enschede, Netherlands; 2Vycap bv, Deventer, Netherlands; 3Wageningen University, Wageningen, Netherlands.

Introduction: Circulating tumor cells (CTC) can be isolated from blood and serve as a source of tumor material. Expansion of CTCs may permit functional treatment-efficacy tests in combination with genetics, epigenetics and proteomics screening. We present a fast workflow to isolate, capture, grow and image cells inside a self-sorting microwell chip using the VyCAP single cell isolation platform. After seeding single cells in a microwell chip, they grow inside the chip or can be transferred to a tissue culture plate for clonal expansion or downstream analysis.

Materials and methods: The Rosette-Sep™ Human CD45 depletion cocktail (Stem Cell Technologies) was used to enrich spiked cells from 1 ml of fresh EDTA blood. Cells were stained with CellTrace™ violet for tracking spiked cells, calcein for live cells, ethidium bromide for dead cells, α-CD45-PERCP as negative marker and α-EpCAM-AF647 as positive marker. After density gradient separation using Ficoll-Paque™, the cell suspension was measured and quantified using flow cytometry for Rosette-Sep™ efficiency. Cell suspensions were seeded in the self-sorting microwell chips using a negative pressure of 40-70 mbar using the VyCAP filtration system. After seeding single cells in the microwells, the chips were imaged with the VyCAP Puncher system and either placed immediately into culture medium for cell expansion or selected cells were transferred into a 96 well tissue culture plate. Cell expansion in microwell chips and plates was followed up to 14 days. Results: Cultured MDA-MB-231 and MCF-7 cells were single-cell-trapped in the microwells with an efficiency of 82% ± 9% and 74% ± 15% respectively, after direct filtration without enrichment. Immediately after capturing in microwells 93% of the MCF7 cells were viable. Rosette-Sep™ enriched MDA-MB-231 and MCF-7 cells with an efficiency of 81% ± 10% and 74% ± 7% respectively, when measured with flow cytometry. The Rosette-Sep™ procedure followed by capture of the MDA-MB-231 and MCF-7 cells in the microwells resulted in an efficiency of 50% ± 11% and 52% ± 11% respectively. 92% of the cells were found back in a culture plate after punching of single cells from the chip, and of these cells, 80% were alive 4 hours after punching. 82% of these cells were alive and still growing after 14 days. In total 53% of cells spiked in blood, could be found back in the cups of which after punching into individual wells, 77% were alive and growing. Conclusion: We present a single cell capture and isolation method for clonal expansion of viable tumor cells. The VyCAP single cell isolation platform used for these experiments provides an easy separation of the single cells from a CTC enriched cell suspension and allows on-chip expansion as well as immediate separation of the CTCs into culture plates. About 50% of spiked cells could be retrieved inside the microwell chip and of these 77% were alive after punching into individual wells of a culture plate.

#3786 Multicenter evaluation of technology platforms for the enumeration of circulating tumor cells. Sebastian Bender, 1 Merlin V. Lütke-Eversloh, 2 Rui P. Neves, 3 N Nikolas H. Stoecklein, 1 Leon W. Terstappen, 2 Barbara Bagiani, 1 Martin H. Neumann, 1 Thomas Krahn, 1 Klaus Pantel, 1 Thomas Schlange, 1 Eppendorf, Hamburg, Germany; 2Vycap bv, Deventer, Netherlands; 3Twente University, Enschede, Netherlands; 4University of Padova, Padova, Italy; 5IOV-IRCCS, Padova, Italy; 6Heinrich-Maximillians-University, Munich, Germany; 7Gustave Roussy, Villejuif, France; 8Institute of Cancer Research, Sutton, United Kingdom.

Introduction: The presence of circulating tumor cells (CTC) enumerated by the CellSearch system in blood of patients with cancers of epithelial origin is strongly associated with a poor prognosis of these patients. CTCs are enriched by targeting the EpCAM antigen, raising the question which the EpCAM subtypes are present as well in patients. In the EU-FP7 CTCTrap program, we investigated the presence of EpCAM- CTC in blood samples after depletion of EpCAM+ CTC by CellSearch. Studies were performed and validated at the participating laboratories by distribution and analysis of blood samples spiked with cancer cells and by testing blood from 73 metastatic prostate and 22 metastatic breast cancer patients. Methods: Blood samples were processed according to the standard operating procedures and tools developed in the program (https://www.ut-wente.nl/tum/mcbp/protocolsandtools/). First, CellSearch was performed for EpCAM+ CTC, followed by filtration and fluorescent labeling of the blood discarded by CellSearch for EpCAM- CTC. To validate the procedures across the 6 participating clinical sites, 3x3 tubes with aliquots of healthy donor blood, spiked with either PC3, MDA-MB-231 or no cells, were prepared at one site and shipped to all other sites for simultaneous processing. Metastatic prostate and breast cancer patients were processed with the same procedures. Results: 27% of PC3 cells were recovered by CellSearch and 21% by filtration, leaving 52% unaccounted for. CellSearch and filtration were performed by 6 different laboratories, leaving 56% accounted for. Differences in recovery between sites were not significant. In patients both EpCAM+ and EpCAM- CTC were detected (see table). Conclusion: In a multicenter study EpCAM+ and EpCAM- CTC were present in blood of metastatic prostate and breast cancer patients. Spiking ex-
CLINICAL RESEARCH: Liquid Biopsies 3: Circulating Tumor Cells, Extracellular Vesicles, and Exosomes

Experiments showed that the developed methods can be further improved to increase the CTC yield. Molecular characterization of the CTC subtypes and relation with clinical outcome is ongoing.

<table>
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#3788 Monitoring of multimodality immune checkpoint inhibitor treatment efficacy in metastatic melanoma patients through molecular analysis of circulating tumor cells. Selena Lin,1 Stella Lam,1 Siuichu Ohe,1 Kevin Tran,1 Irene Ramos,1 Ali Asgar Bhagat,2 Chwee Teck Lim,3 Steven J. O'Day,1 Leland Foshag,1 Dave Ramirez,1 Tomohisa Okuno, Kishu Kitayama, Tatsuro Tamura, Takahiro Toyokawa, Hiroyuki Yamada,1 Arturo B. Ramirez,1 Sarah B. Crist,2 Tyler Yeats,2 Jeffrey L. Werbin,1 Jackie L. Stillwell,1 Eric P. Kaldjian1,1 RareCyte, Inc., Seattle, WA; 2University of Washington, Seattle, WA.

Background: Circulating tumor cell (CTC) monitoring in patients with metastatic melanoma has become an important tool for the evaluation of therapeutic efficacy and patient outcome. RNA-based detection methods have been developed to facilitate effective monitoring of CTCs, especially in patients who are unresponsive to treatment. In this study, we aimed to evaluate the utility of RNA-based detection of CTCs in metastatic melanoma patients undergoing immune checkpoint inhibitor therapy.

Methods: To establish RNA detection in model CTCs (mCTCs), we used SKBr3 cells with BRAF V600E mutation. RNA was extracted from RNA-locked samples and used to develop a process to simultaneously stain for Her2, UBC (positive control) and dapB (negative control) expression in mCTCs. A process to simultaneously stain for RNA and protein was then developed to allow identification of mCTCs by positive control (negative control) expression in mCTCs. A process to simultaneously stain for Her2, UBC (positive control) and dapB (negative control) expression in mCTCs. A process to simultaneously stain for Her2, UBC (positive control) and dapB (negative control) expression in mCTCs.

Results: Total 25 21 54 41 27 32

#3790 Circulating tumor cells at disease recurrence in patients with head and neck cancer after curative therapy. Jason Chia-Hsun Hsieh,7 Ting-Hsuan Yeh,1 Hung-Ming Wang,1 Yung-Chang Lin,1 Nina Ming-Jung Lin,2 Siou-Ru Ye,3 Jane Ying-Chieh Lee,4 Min-Hsien Wu.5 Chang Gung Memorial Hospital at Linkou, Taoyuan, Taiwan; 2Chang Gung University, Taoyuan, Taiwan.

Background: Circulating tumor cell (CTC) monitoring has been prognostic and predictive in numerous types of cancer; however, its role in early diagnosis of relapse remains unclear. Methods: Eighty-six patients were prospectively enrolled between March 2015 and June 2016. Among these patients, 51 head and neck squamous cell carcinoma (HNSCC) patients had suspicious recurrent lesion(s), while 35 HNSCC patients were newly diagnosed. CTC test was performed by negative selection strategy and CD45-negative and EpCAM-positive cells were identified as CTCs. Biopsy on suspicious lesion(s) and CTC analysis were performed simultaneously. We analyzed the differences of CTC numbers among HNSCC patients with true recurrence, biopsy-negative and newly-diagnosed. Results: Mean ± standard deviation (SD) of CTC numbers in baseline at diagnosis (n = 35), true recurrence (n = 40) and biopsy-negative (n = 11) groups were 41.98 ± 32.02, 81.75 ± 64.91 and 16.55 ± 6.82 cells/mL, respectively. The difference of CTC numbers among three groups was significant (P = 0.001). CTCs (mean ± SD) among different failure types were 110.89 ± 84.69, 105.67 ± 50.77, 73.31 ± 37.82 and 59.12 ± 54.09 in lung metastasis, second primary tumor, extrapulmonary metastasis and locoregional recurrence respectively and significantly different (P = 0.049). Also, CTC numbers between first cancer (baseline at diagnosis) and second primary tumor were different (P = 0.004). Conclusion: CTC numbers are significantly higher in true recurrence than the biopsy-negative group when a patient had a lesion suspected to be a recurrence. CTC test may be useful to help distinguish true recurrence in HNSCC patients after curative therapy.


Background: It has been reported that the examination of circulating tumor cells (CTCs) is beneficial for predicting tumor stage or treatment response. The epithelial cell adhesion molecule (EpCAM) and cytokeratin has been used as epithelial markers for the identification of CTCs. Since the epithelial-mesenchymal transition is one of malignant phenotype of cancer cells, it is necessary to investigate other markers which might detect mesenchymal CTCs. Aim: This study aims to clarify the clinical significance of EpCAM negative and CEA positive CTCs in patients with gastric carcinoma. Forty patients who underwent surgery for gastric cancer were enrolled in this study. A total of 10ml peripheral blood samples were obtained pre-operatively. Informed consent to participate in this study was obtained from all of the patients before their surgery. This study was approved by the Human Ethics Review Committee of our University. After collection of mononuclear cell fraction, these cells were stained by FAP1, anti-CLD45, anti-EpCAM, anti-cytokeratin, and anti-CEA (CD66c) antibody. We enumerated the number of cells which showed EpCAM, cytokeratin, and CEA positive expression among CD45 negative cells. ROC analyses were performed for investigating predictive value of these cell counts for distant metastasis. We examined association between these cell counts and clinicopathological features. Results: ROC analyses revealed that the AUC for predicting
distant metastasis using EpCAM, Cytokeratin, and CEA positive cell counts were 0.583, 0.638, and 0.769, respectively. The median number of EpCAM negative and CEA positive cells was 231 (range: 0-61897). The cell counts of EpCAM negative and CEA positive cells were significantly associated with Tumor depth (p = 0.0084), lymph node metastasis (p = 0.0107), and tumor size (p = 0.023). Conclusions: CEA might be useful marker for detecting high risk of recurrence in patients with gastric cancer.

#3792 Improved isolation and detection of circulating tumor cells of pancreatic cancer patients with characterization by mutational analysis. Oliver von Ahsen, Nora Brychta, Thomas Krahn. Bayer Pharma AG, Berlin, Germany.

Introduction: Isolation of circulating tumor cells based on cell surface markers is often hindered by epithelial to mesenchymal transition with loss of epithelial antigens. Low numbers of CTCs and fewer cases of CTC positive pancreatic cancer compared to other cancers (e.g. breast cancer) lead to the assumption that pancreatic tumors do not release CTCs as frequently or that the pancreatic CTCs lack EpCAM expression. Isolation of CTCs based on their size is independent of EMT-like phenotypical changes. We therefore compared a filtration-based isolation method with an EpCAM-based isolation method. We optimized CTC detection by using a highly sensitive anti-cytokeratin antibody panel for the detection of cancer cells with moderate cytokeratin expression. In order to use isolated CTCs as liquid biopsy for tumor characterization and treatment selection, downstream analysis is necessary.

We here show the feasibility of mutational analysis of isolated CTCs by castPCR. Methods: EpCAM high, medium and low cells were enriched and isolated by an EpCAM-based immunomagnetic procedure (Isoflux) and a filtration device (Sie- mens) to determine recovery rates of both methods. Isolated cells were detected by an improved immunofluorescent staining with an anti-Cytokeratins, anti-EpCAM and anti-PMBC panel and characterized by competitive allele-specific TagMan PCR (castPCR) for KRAS mutations. Results: Cytokeratin expression is crucial for detection of CTCs in a high background of blood cells. Improvement of the staining protocol helps to increase the sensitivity of detection. The filtration based approach is superior to the surface antigen-based isolation. With the size-dependent method we obtained a recovery rate of 52 % even for EpCAM-low cells compared to only 1 % based on the immune-affinity purification. Cells isolated by filtration can be characterized for expression of therapeutic targets by immunostaining. The utility of the size dependent platform for subsequent functional characterization of the CTCs was also demonstrated by detection of k-ras mutations in single isolated CTCs by castPCR.

Conclusion: For CTCs undergoing EMT, filtration yields higher recovery compared to the standard surface antigen (EpCAM)-based methods. Isolation by filtration also allows for mutational analysis which can be used to confirm the identity of the isolated cell as CTC. In addition, mutational analysis of CTCs can be used to guide the treatment of patients. Use of liquid biopsies for treatment selection will facilitate truly personalized medicine.

#3793 Discovery and isolation of enormous number of circulating tumor cells in breast cancer patient’s blood based upon cell attachment property. Yeon-a Choi, Hyun Ju Han, Ae-Ran Choi, Jeong Dong Lee, Gun Min Kim, Hyung Seok Park, Seho Park, Seung Il Kim, Joonhyuk Sohn. Yonsei Univ Medical Center, Seoul, Republic of Korea.

Circulating tumor cells (CTCs) are detached from primary tumors into the bloodstream. It is known that CTCs can be a biomarker for early detection of breast cancer patients and for drug resistance. CTCs can be differentiated by cell attachment property. In order to isolate CTCs, we developed a new method to collect CTCs from breast cancer patient, and handled as described above. Our method showed higher detection sensitivity for 6 breast cancer cell lines. We confirmed that breast cancer cell lines were collected more than 80% of spiked cell number in 2 hours. This technique is applied to isolation of CTCs in actual breast cancer patients, and unexpectedly considerable numbers of CTCs were observed. We found in a range of CTCs from 0.5 × 10⁶ to 1 × 10⁷ per ml of blood.

These numbers are in striking contrast to previous studies. CTCs were observed in 61 of 114 samples (53.51%) from metastatic breast cancer patients and 15 of 48 samples (31.25%) from early breast cancer patients. We also discovered that there are various sized CTCs, and categorized these CTCs according to their diameter; small (<5 μM), medium (5-10 μM), and large (>10 μM) CTCs. All the thirty CTCs appeared in large numbers. HER2 and EpCAM expression of CTC vary by patients and didn’t always coincide with expression in primary tumor. In summary, we have developed a promising strategy for CTC isolation by using the preference of cancer cells to adhere to cell culture plates. We have demonstrated that this is a simple yet very efficient approach to isolate CTCs and broadly applicable to capturing heterogeneous CTCs populations independently of their marker expression and size. Our study discovered that enormous amount of CTCs are existing in the blood of breast cancer patient in a variety of sizes.

#3794 Selective isolation of epithelial to mesenchymal transitioned circulating tumor cells in NSCLC using novel magnetic nanocubes. Dhanjay Suresh, Shreya Ghoshdastidar, Soumavo Mukherjee, Abilash Gangula, Anandhi Upendra, Raghuraman Kannan. University of Missouri, Columbia, MO.

The 5-year survival rate for early to advanced NSCLC patients is 10-5%. Once the tumor metastasizes, the survival rate often drops below 1%. One of the major challenges is to select the patient for appropriate therapy, and the selection process involves invasive biopsy that is difficult to recurrently perform during treatment. Recent studies have shown that liquid biopsy is an attractive alternative. Liquid biopsy involves analysis of either CTCs or ctDNA to understand the tumor therapy. Isolating entire tumor cells provides an opportunity to perform whole genome sequencing for in depth understanding of the tumor. However, frequent changes in cancer signaling and acquired mutations during treatment lead to drug resistance that cannot be diagnosed using current CTC techniques. These oncogenic biochemical modifications that are often associated with metastasis involve upregulation of epithelial to mesenchymal transition (EMT) pathway that change the elasticity of tumor cells for easy shedding into the blood stream. Consequently, EMT transition leads to depletion in epithelial markers such as EpCAM and cytokeratin that are to be targeted. This effect largely limits the use of present technologies utilizing EpCAM as a marker to isolate the CTCs. We hypothesized that these cells can be captured by targeting surface markers that are overexpressed in EMT transitioned CTCs. For example, markers such as EGFR and HER2 have been shown to be overexpressed pre and post EMT tumors and therefore can present as a new strategy for non-invasive diagnosis before and during treatment. In this study we have shown that EGFR and HER2 receptors are overexpressed in EGF and KRAS mutated NSCLC cells. We artificially activated EMT in NSCLC cells and compared their face biomarker concentrations. Furthermore, we used microfluidic analyzer data using western blotting to identify our targets after EMT. Based on the biomarker concentrations, we designed and developed magnetic nanocubes (MNC) surface attached with antibodies to target the selected biomarkers to capture EMT CTCs. The capture efficiency of these magnetic nanocubes was compared in multiple cell lines (HCC427 and A549). Overall we could capture CTC as low as 0.01% of the spiked cells. We used the MNCs to capture cancer markers such as EGFR and HER-2 that are highly expressed in tumors, once shed as CTCs can be targeted for diagnosis. Therefore, we have developed a novel method to capture EMT CTCs with high selectivity and this method presents a minimally-invasive method for real-time monitoring of patients during drug treatment.

#3795 Dynamic acquisition of HER2 expression on circulating tumor cells in gastric cancer patients correlates to developing therapeutic resistance. Yi Lin,1 Xiaotian Zhang,1 Dan Liu,1 Jifang Gong,1 Daisy Dandan Wang,2 Peter Lin Peng,2 Lin Shen1. 1Peking University Hospital University & Institute, Beijing, China; 2Cyteligen, San Diego, CA.

Introduction: It has been hypothesized that molecular characters of circulating tumor cells (CTCs) is dynamically evolved during cancer development. However, how the evolutive CTC subtypes support tumor progression and drug resistance remains unclear. In this study, converted phenotypic HER2 expression and karyotypic chromosome 8 ploidy of CTCs in gastric cancer patients who developed therapeutic resistance were systematically investigated. Methods: Ninety-eight prospectively enrolled patients with diagnosis of advanced gastric cancer (AGC) were histologically classified into 2 cohorts of HER2+ (hHER2+, 53 patients, subjected to the combined chemo- and anti-HER2 targeted therapy), and hHER2- (42 patients, subjected to chemotherapy alone). Integrated EpCAM-independent subtraction enrichment and immunostaining-fluorescence in situ hy-
bridization (SE-iFISH) was applied to monitor and characterize HER2 expression and chromosome 8 ploidy of CTCs in patients following multiple courses of therapy until disease progression. Results: HER2 expression on CTCs was heterogeneous and interconverted compared to its relatively stable status on primary tumor. Among those prior-to-therapy patients, HER2+ CTCs were detected in 33.3% (9/27) of total, and HER2- CTCs were detected in 66.7% (18/27) of total, limiting their clinical utility. There were two CTCs in a hHER2+ patient identified in 29.5% (13/44) of hHER2+ cohort. Whereas can also be detected in 19 out of 21, 90.5% and 6 of hHER2+ cohorts (6 out of 8, 75%), respectively. Further karyotyping of CTCs in those patients developing therapeutic resistance indicated that most of the CTCs with acquired HER2 expression had multiploid (6x) chromosome 8, implying that aneuploid chromosome 8 in acquired HER2+ CTCs were likely related to development of therapeutic resistance, both chemotherapy alone and the combined chemo- and targeted therapy. Genomic profiling of the single CTC subtype by FISH upon phenotypic HER2 expression and karyotypic chromosome 8 ploidy is currently under investigation. Conclusion: Dynamic profiling gene signature and molecular characterization of CTCs might be significant in terms of predicting therapeutic resistance, and further help select an alternative effective intervention to overcome the acquired therapeutic resistance.

#3796 An intravascular magnetic wire for high-throughput in vivo enrichment of rare circulating cancer biomarkers. Ophir Vermesh, Amin Asloun, Tianjia J. Ge, Yamil Saenz, Yue Guo, Seung-min Park, Yoshiaki Mitsutake, Michael Bachmann, Chin Chun Ooi, Kerstin Mueller, Hamed Arami, Alfredo Green, Shan X. Wang, Sanjiv S. Gambhir. Stanford University, Stanford, CA.

Background: Liquid biopsies have long promised to enable earlier cancer diagnosis and tailored therapy. However, circulating tumor cells (CTCs) are extremely rare (1–10 CTCs per mL) and limiting their clinical utility. There are few CTCs in a patient’s peripheral blood, whereas can also be detected in 29.5% (13/44) of hHER2+ cohort. whereas can also be detected in 19 out of 21, 90.5% and 6 of hHER2+ cohorts (6 out of 8, 75%), respectively. Further karyotyping of CTCs in those patients developing therapeutic resistance indicated that most of the CTCs with acquired HER2 expression had multiploid (6x) chromosome 8, implying that aneuploid chromosome 8 in acquired HER2+ CTCs were likely related to development of therapeutic resistance, both chemotherapy alone and the combined chemo- and targeted therapy. Genomic profiling of the single CTC subtype by FISH upon phenotypic HER2 expression and karyotypic chromosome 8 ploidy is currently under investigation. Conclusion: Dynamic profiling gene signature and molecular characterization of CTCs might be significant in terms of predicting therapeutic resistance, and further help select an alternative effective intervention to overcome the acquired therapeutic resistance.


BACKGROUND: Circulating tumor cells (CTCs) are expected to be utilized for decision-making in precision cancer medicine and further elucidation of cancer biology. However, it remains challenging to detect and isolate CTCs without any damages, that is critically important to analyze the molecular and biological properties of CTCs. Near infrared (NIR) imaging is one of the emerging technologies in the field of molecular imaging, which can detect and visualize tumor cells, nerves, and muscles under the skin or mucosa. Here we attempted to apply the NIR imaging technologies to distinguishing tumor cells from normal blood cells. METHODS: To obtain NIR images, real-time composition imaging system Compvision® (Sumitomo Electric Industries, Ltd.) was used. This system is equipped with an NIR spectroscopic camera and this camera processes hyperspectral data, in which each pixel has information of the wavelength band of 1,000–2,350 nm. The X-axis and Y-axis of the sensor chip correspond to the spatial direction and wavelength. Thus, hyperspectral data stores both image and spectral information. For this feasibility study, lung cancer cell lines with varying EpCam expression levels such as A549, PC-9, PC-14, H23 were used. Lymphocytes and red blood cells were obtained from the peripheral blood of healthy volunteers. Phosphate-buffered saline (PBS) was used as a negative control. After we obtained NIR hyperspectral data, multivariate analysis such as principal component analysis (PCA) was performed for precise analysis. RESULTS: We were able to obtain the hyperspectral data from all the lung cancer cell lines, lymphocytes, red blood cells and PBS alone and the spatially averaged spectrum was extracted from each data. Then the spectra were converted to absorption spectra and analyzed by PCA using the Unscrambler®. Two principal components were selected from results to separate data. Two-dimensional PCA score plot showed the clear separation between tumor cells and normal blood cells such as lymphocytes and red blood cells, indicating the potential of non-invasive detection of tumor cells. In addition, obtained spectra from both EpCam-expressing and EpCam-non-expressing tumor cells were successfully segregated from those from normal blood cells. According to these results, it is suggested that segregating cells based on the difference in cytoskeletal components between epithelial cells and blood cells is feasible using the NIR imaging technology. CONCLUSIONS: Using the NIR composition imaging technology, we were able to obtain different spectral characteristics between tumor cells and normal blood cells, suggesting that this technology holds the potential to detect and isolate CTCs without any invasive labeling. This should be further explored for its application to the identification of CTCs.

#3798 Multiplex phenotyping of circulating cancer associated macrophe- like cells in patients with solid tumors. Daniel L. Adams, Katherine Alpaugh, Thai H. Ho, Steven H. Lin, Jeffrey R. Marks, Raymond Bergan, Stuart S. Martin, Saranya Chunsmir, Cha-Mei Tang, Massimo Cristofanilli. 1Cromart MicroTech, Inc., Monmouth Junction, MD; 2Fox Chase Cancer Center, Philadelphia, PA; 3Mayo Clinic Cancer Center, Phoenix, AZ; 4MD Anderson Cancer Center, Houston, TX; 5Duke University, Durham, NC; 6Oregon Health and Science University, Portland, OR; 7University of Maryland, Baltimore, MD; 8Mayo Clinic Cancer Center, Jacksonville, FL; 9Cromart MicroTech, Inc., Potomac, MD; 10Northwestern University, Chicago, IL.

Background: Circulating cancer associated macrophage-like cells (CAMLs) are cancer specific giant cells circulating in the blood of patients with solid tumors. Since their discovery, few studies have been done to elucidate their lineage or phenotypic identity. The difficulty in classifying CAMLs is exemplified by their expression of multiple heterogeneous markers that defy conventional identification. Recently, we described a restaining method (QUAS-R) to screen individual cells using an array of up to 15 biomarkers. We used this method to screen CAMLs isolated from 152 cancer patient samples in 4 types of solid tumors to classify CAMLs by phenotypic immunostaining. These data suggest that CAMLs are a morphologically diverse and phenotypically heterogeneous population of cancer specific giant cells with overlapping myeloid, ep- thelial, and endothelial phenotypes. Methods: This multi-institutional study used peripheral blood samples from 152 cancer patients (stage I-IV) from breast (n = 42), lung (n = 39), renal cell carcinoma (36) and prostate (n = 35). Blood was processed using the CellSieve™ microfılter technique at 8 institutions and stained for vimentin/CD146/CD144, CD11b/CD68/CD41, CD11c/CD68/TIE2, or CD34/ CD41/CD61. After staining, QUAS-R was again used to remove the fluorescence and samples restained with a third panel. Each patient sample was stained quenched and restained with the above mentioned panels. Results: In agreement with a number of studies, CAMLs were found in 86% of cancer patients (n = 131/ 152), with increased detection from stage 1 (71%), followed by stage 2 (94%), stage 3 (88%) to stage 4 (88%). Breast cancer had the most CAMLs per sample
Clinical Research: Liquid Biopsies 3: Circulating Tumor Cells, Extracellular Vesicles, and Exosomes

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Analysis of circulating tumor exosomes: their ability to serve as a biomarker for recurrence in lung cancer. Nicholas Serrano, John Blue, Asim Alam, Christopher S. Rabender, Elisabeth Weiss, Mitchell S. Anscher, Rose B. Mikkelsen, Vasily A. Yakovlev. VCU Massey Cancer Ctr., Richmond, VA.

Objectives: Exosomes (Exos) are 30-150 nm wide nanovesicles originating from the endosomal network and are found in most body fluids. Production of Exos increases in cancer, making Exos potential biomarkers. Exos are a fundamental driver of intercellular communication by transferring proteins, lipids and mRNA. Analysis of Exos and their interaction with the microenvironment may uncover cellular pathways involved with disease progression. We hypothesized that Exos may serve as a liquid biopsy for tumor recurrence. Materials and Methods: Six lung cancer patients (LCPs) were prospectively enrolled on an IRB approved study. Exos were precipitated and purified with a Norgen kit from 0.7 ml of blood samples collected prior to, during and 6 months after radiation therapy (RT). Exos numbers were quantified by ExoCet Exosome Quantitation Kit (System Biosciences). Total exosomal RNA was isolated by the Norgen RNA/DNA/Protein Purification kit. For the miRNAs expression profiling we used the miRCURY LNA® Universal RT microRNA PCR assay (Exiqon) and Exiqon’s LNA probe sets for 40 different miRNAs (let-7a-5p, miR-20a-5p, miR-21-5p, miR-30b-5p, miR-106a-5p, miR-146a-5p, miR-155-5p, miR-200b-5p, miR-203a, miR-208a) for all samples were determined. Human lung fibroblasts (MRC-5s) in serum-free medium for 24 hrs were treated with Exos, TGF-β (positive control) or PBS (negative control) to determine their relative effects on MRC-5 proliferation. Results: Of the 6 LCPs, 4 showed a 2-5-fold increase both on small and large scale. Three patients were diagnosed with recurrence at the time of their post-RT blood draw. Six miRNAs demonstrated significantly different expressions between recurrent and non-recurrent groups of LCPs: let-7a-5p, miR-20a-5p, miR-21-5p, miR-106a-5p, miR-146a-5p, and miR-203a. MRC-5 proliferation increases 2-fold when treated with Exos from blood collected prior to RT compared to PBS (p < 0.01). There is <2-fold increase compared to TGF-β (p = 0.03). Plasma Exos levels decrease during RT as do their relative effects on MRC-5 proliferation when compared at equivalent numbers. Exos collected at 6 months from patients with recurrent significantly stimulated MRC-5 proliferation (p = 0.03), whereas Exos from patients without recurrence do not. Western blot analyses of MCR-5 cells stimulated with Exos revealed increased PTEN but enhanced CD44, CD9, and increased AKT and SMAD phosphorylation, regardless of when the Exos were collected. Conclusions: Recurrent and non-recurrent groups of LCPs demonstrated significant differences in exosomal miR profile before and after RT. Exos from untreated LCPs, and recurrent LCPs after RT, stimulate MCR-5 proliferation and possible conversion to CAFs. Our results suggest that Exos could serve as a ‘liquid biopsy’ to assess the potential for recurrence when imaging modalities are equivocal.

A novel sensitive flow-cytometry based platform for isolation and molecular characterization of circulating tumor single cells and clusters. Neha Bhagwat,1 Charles H. Pletcher,2 Ling Wang,3 William DeMuth, Keely Dulmage,4 David Ball,5 Stephanie S. Yee,6 Liping Yu,7 Jonni S. Moore,7 Ben Z. Stanger,8 Eric P. Dixon,9 Erica L. Carpenter,7 1Division of Gastroenterology, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; 2Department of Pathology and Laboratory Medicine, Perelman School of Medicine, Philadelphia, PA; 3BD Technologies, Research Triangle Park, NC; 4Division of Hematology and Oncology, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; 5BD Biosciences, San Jose, CA.

Circulating tumor cells (CTCs) are shed from solid tumors and found at extremely low frequencies in the blood of patients in most cancers. A subset of these cells (CTC+) give rise to distant metastases and which is the primary cause of cancer-related mortality. Isolation and characterization of these cells from blood as a ‘liquid biopsy’ can be a sensitive, non-invasive method for early detection, disease monitoring and therapy selection. CTCs can be found even at early disease stages in preclinical models and patients. There is increasing evidence that clusters of CTCs in blood are associated with higher metastatic potential; however, efficient isolation and interrogation of these rare clusters is challenging. In this study, we utilize an in-line rare cell enrichment platform developed by Becton Dickinson (BD), coupled with the BD FACSTM Influx cell sorter to rapidly isolate both single cells and clusters from blood. This platform utilizes magnetic particle-based depletion of unwanted leukocytes and combines acoustic focusing to remove red blood cells and debris while enriching for rare cells of interest. We achieved 30-fold enrichment of tumor cells spiked into blood along with >6-fold improvement in sort efficiency with the enrichment process as compared to without. Importantly, the large 200μm nozzle and low sheath pressure (3.5 psi) on the Influx minimizes shear forces and maintains cell viability and integrity of clusters during sorting, thereby enabling discrimination of single cells and clusters based on side scatter. Using this strategy, we could sort enriched populations of viable cell clusters (range 2-10 cells). Finally, this workflow can be integrated with single cell sequencing (sc-RNAseq) on cells isolated with this streamlined platform where pooled cells and bulk RNA were used as comparisons. As proof of principle, we used this optimized workflow to isolate and characterize CTCs from a pancreatic cancer mouse model (KPCy) in which all tumor cells are labeled with Yellow Fluorescent Protein (YFP). We sorted YFP+ CTCs and CD45+ white blood cells (WBCs) from the blood of 7 KPCy mice along with YFP+ cells from the matched tumor. We recovered an average of 88 CTCs/ml of blood (range 5-258). RNA sequencing was completed on pooled and single cells from all samples. Epithelial genes including Krt18 and Krt19 as well as lineage specific genes such as Sox9 were significantly
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overexpressed in CTCs and tumors compared to WBCs, suggesting the utility of this approach. This integrated CTC workflow provides a sensitive approach to identify pancreatic cancer-specific markers, which will be evaluated for their ability to improve sensitivity of positive-selection of CTCs in patient blood and for development of a clinically relevant diagnostic assay.

#3802 A novel uracil-DNA glycosylase, UdgX, as a new biochemical tool to directly detect uracils in DNA, Jessica Stewart,1 Shiangao Wei,2 Madhurima Datta,2 Umesh Varshney,2 Ashok Bhagwat1,1 Wayne State University, Detroit, MI;2Indian Institute of Science, Bangalore, India

The enzyme activation-induced deaminase (AID) is essential to the B lymphocyte development and antibody maturation. It converts cytosines in DNA to uracil. The unregulated expression of AID in many B cells is correlated with an increased load of mutations. AID also promotes DNA strand breaks and is required for chromosomal translocations such as IgH/Myc in these tumors. We have previously showed that B cell cancer cell lines and patient tumors which express AID at high levels also have high levels of uracil in their genomic DNA. We accomplished this by excising the uracils using a uracil-DNA glycosylase (Ung) and labeling the resulting in an abasic site with an aldehyde reactive chemical (ARP). ARP contains a biotin tag and was detected using a fluorescently labeled streptavidin. The results from this assay showed B cell tumors have as much as 100-fold higher levels of genomic uracils. Further development of this assay has allowed the detection of uracils in the blood plasma and buffy coat of B cell cancer patients. This “liquid biopsy” assay technique has the potential to work as a simple surveillance tool for B cell cancer patients who are in remission. In addition, we are exploring the possibility of a direct detection of uracil in DNA, which would provide greater sensitivity to this assay and simplify it. A unique uracil-DNA glycosylase was recently isolated from M. smegmatis, UdgX that binds covalently to uracils in DNA. We are developing multiple ways to detect uracil-DNA-UdgX complexes and amplifying the resulting signal. We expect that this will allow us to directly visualize uracils in B-NHL tumors and detect uracils in circulating B-NHL tumor DNA from a drop of patient plasma.

#3803 Characterization of fluid biopsy using the HD-SCA platform to re-stratify intermediate-risk organ-confined prostate cancer patients, Paymaneh D. Malihi,1 Kenneth Pienta,2 James Hicks,1 Michael Gorin,2 Carmen Ruiz Velasco,1 Anders Carlsson,1 Anand Kolatkar,1 Mariam Rodriguez Lee,1 Michael Morikado,1 Peter Kuhn1.1 Wayne State University, Detroit, MI;2Johns Hopkins Hospital, Baltimore, MD.

Recent studies have demonstrated that even with current risk stratification of intermediate-risk organ-confined prostate cancer patients, 30-50% of patients are incorrectly categorized and overtreated. As the second leading cause of cancer in men, 90% of prostate cancer patients are diagnosed at organ-confined stages, where radical prostatectomy (RP) is the “golden standard” of treatment. However, deferring intervention is becoming a more popular option for low-risk patients, with a 15-year progression-free survival of 95%. High-risk patients greatly benefit from RP, but have a 5-year progression-free survival of 85%, while the decision to proceed with RP is not so candid for intermediate-risk patients. Using the high-definition single cell assay (HD-SCA) platform, circulating tumor cells (CTCs) from peripheral blood (PB) and disseminating tumor cells (DTCs) from bone marrow aspirate (BMA) will be analyzed for morphological, genomic, and proteomic profiling. We hypothesize that by using morpho-proteo-genomic parameters we may develop predictive markers for a more accurate stratification of intermediate-risk patients, offering a more personalized treatment for each individual. Preliminary data shows 86% (n=43) of PB samples and 5% (n=40) of BMA are positive for Cytokeratin (CK) expressing rare circulating cells, as defined by ≥5 cells/mL. The incidence of CK+ cells in PB did not correlate with PSA, Gleason score, or clinical parameters. However, incidence of CK+ cells in PB was higher in high-risk group, though not statistically significant (p=0.784) when compared to intermediate-risk groups. Additionally, higher levels of CK expression (p=0.0001), as measured by relative intensity of fluorescent staining, were detected in cells detected in PB of high-risk patients relative to intermediate- and low-risk groups as measured by an analysis of variance (ANOVA) statistical model. Low levels of genomic aberrations are observed in CTCs from circulating cells (0.18), which may point to the fact that these patients are in early stages of their disease. The use of the HD-SCA platform to characterize the fluid biopsy may result in the identification of predictive markers of treatment response for intermediate-risk patients, resulting in reduction of overtreatment.

CLINICAL RESEARCH: Predictive Biomarkers 3

#3804 Comprehensive comparison of bisulfite conversion kits: A guide for optimal sensitivity and specificity of circulating cell-free DNA methylation-based biomarkers, Sarah Ø. Jensen, Mai-Britt W. Ørntoft, Jesper B. Bramsen, Torben F. Ørntoft, Claus L. Andersen. Aarhus University Hospital, Aarhus, Denmark.

Analysis of circulating cell-free DNA (cfDNA) is a promising approach for non-invasive detection of human cancers. The approach has wide-spread applications including early detection of disease in asymptomatic individuals, residual disease detection and disease monitoring. A challenge for the clinical utilization of cfDNA is the small fragment size of cfDNA and the limited amount of cfDNA present in blood. For detection of circulating tumor DNA (ctDNA) highly sensitive methods for cfDNA detection e.g. targeted sequencing and digital droplet PCR (ddPCR) target tumor specific mutations or DNA methylation alterations. Detection of methylation-based biomarkers requires that cfDNA is bisulfite converted prior to biomarker detection, which is known to be associated with some loss of cfDNA. Yet, surprisingly few efforts have focused on ensuring high cfDNA bisulfite conversion (BSC) efficiency and -recovery, and few commercial kits are especially designed for this purpose. In this study we aimed to find a BSC method suited for ctDNA methylation biomarker research, with high BS conversion efficiency and minimum loss of cfDNA, in order to increase the amount of cfDNA template available for downstream analysis.

We studied 13 different methods for BSC and examined both the recovery and the BSC efficiency using quantitative real-time PCR (qPCR), polyacrylamide gel electrophoresis (PAGE) and ddPCR. A significant variation in DNA recovery was observed between the 13 methods tested using both high and low molecular weight DNA templates. Especially five kits performed consistently well on all DNA templates tested, as evaluated by qPCR and PAGE. As the lengths of cfDNA is 146-167 base pairs, only methods that are able to recover fragments of this length is suitable for cfDNA pipelines. Results showed that few methods enabled recovery of DNA fragments ≤ 150 base pairs. However, three kits showed a higher amount of 150 base pair fragments compared to the other methods tested. In a clinical setting it is expected that the target cfDNA compose only a minor fraction of the total cfDNA in plasma. Thus, the five best performing kits were tested using ddPCR on serial dilutions of leucocyte DNA fragmented to ~ 150bp to mimic cfDNA. The best performing kit had a recovery of 69% of the initial input compared to only 13% for the poorest performing kit. Collectively, our comprehensive comparison hereby guide which BS conversion methods are suited for cfDNA methylation-based biomarker pipelines.

#3805 Pre-diagnostic statin use among breast cancer patients - the relation to HMG-CoA reductase expression and disease-free survival, Helga Tryggvadottir, Emma Gustbée, Andrea Markkula, Maria Simonsson, Björn Nordin, Karin Jirström, Carsten Rose, Christian Ingvar, Signe Borgquist, Helena Jernström. Lund University, Lund, Sweden.

Background: The purpose of this study was to investigate whether pre-diagnostic statin use was associated with tumor expression of HMG-CoA reductase (HMGCR) and disease-free survival (DFS) in breast cancer. Previous studies have shown anti-carcinogenic properties of statins and a significantly longer survival among breast cancer patients using statins. We recently showed, that the target of statins, HMGCR, was up-regulated in breast cancer among patients preoperatively treated with statins in a window-of-opportunity trial. Methods: A population-based cohort of primary breast cancer patients in Lund, Sweden was assembled between October 2002 and June 2012. Patients completed a questionnaire including information on medication use. Statin use in relation to DFS was analyzed in 985 patients. Tumor tissue microarrays were constructed and stained with a polyclonal HMGCR antibody to assess HMGCR expression (n=848). Results: Patients were followed for up to 11 years (median follow-up time 5.0 years). In the present study, pre-diagnostic statin use was neither associated with HMGCR expression (P=0.53) nor with DFS (P=0.92), overall or separately in any of the HMGCR expression groups (negative, weak, moderate/strong). Moreover, statin use was not associated with clinical tumor characteristics (tumor size, axillary lymph node involvement, histological grade, hormone receptor status or HER-2 amplification). Conclusion: In contrast to previous studies, the present study did not show any significant association between pre-diagnostic statin use and DFS. It is possible that patients who already used statins have developed tumors that are less dependent on cholesterol metabolism and consequently less influenced by statin treatment. The timing of statin use in relation to breast cancer diagnosis and prognosis should be evaluated in another cohort with preferably longer follow-up time.

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expression was detected among 5% of this cohort of advanced solid cancers, primarily in breast and gastrointestinal tumors. Sensitivity to platinum therapy is significantly higher in ATM loss colorectal cancer. Further prospective studies with larger cohorts are required to validate these findings.

ATM IHC and ATM mutation by NGS by tumor group

<table>
<thead>
<tr>
<th>Tumor types</th>
<th>Total</th>
<th>ATM IHC (loss)</th>
<th>ATM mutation (%)</th>
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<tr>
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<td>Concomitant ATM IHC and ATM mutation (as % of ATM IHC loss)</td>
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<td></td>
<td></td>
<td></td>
<td>ATM loss/mutation</td>
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<tr>
<td>Colorectal</td>
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<td>Gynecological</td>
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<td>Breast</td>
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<td>Total</td>
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<td>31 (5)</td>
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#3808 TM4SF1 regulates cell cycle and ROS status in bladder cancer cells by targeting FOXO3a via PPARγ-SIRT1 negative feedback loop.

While gemcitabine has been the mainstay therapy for advanced pancreatic cancer, newer combination regimens (e.g. FOLFIRINOX) have extended patient survival, though carry greater toxicity. Biomarkers are needed to better stratify patients for appropriate therapy. Previously, we reported that one-third of pancreatic cancers harbor deletions or deleterious mutations in key subunits of the SW itch/Sucrose NonFermentable (SWI/SNF) chromatin remodeling complex. The SWI/SNF complex mobilizes nucleosomes on DNA, and plays a key role in regulating DNA transcription and repair. Thus, we hypothesized that pancreatic cancers with SWI/SNF aberrations might exhibit compromised DNA repair, and show increased sensitivity to DNA damaging agents. Here, we studied pancreatic cancer cell lines with deficient (or else exogenously reconstituted) SWI/SNF subunits, as well as normal pancreatic epithelial cells (HPDE) following SWI/SNF subunit knockdown. Cells were challenged with DNA damaging agents, including those used in current combination regimens, and then cell viability assayed. We found that pancreatic cells with SWI/SNF dysfunction showed markedly increased sensitivity to DNA damaging agents, in particular DNA crosslinking agents (cisplatin and oxaliplatin). Assaying clearance of γH2AX foci confirmed that SWI/SNF dysfunction impaired DNA damage re-

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sponse/repair. Finally, by analyzing pancreatic cancer patient data (TCGA), we found that pancreatic cancers with SWI/SNF deficiency (subunit mutation and/or decreased expression) showed a strong trend towards extended survival with platinum containing chemo regimens. Thus, SWI/SNF dysfunction sensitizes pancreatic cancer cells to DNA crosslinking agents, and SWI/SNF mutation status may provide a useful biomarker to predict which patients may benefit from platinum-containing chemotherapy regimens.

#3810 Validation of novel high-plex protein spatial profiling quantitation based on NanoString’s Digital Spatial Profiling (DSP) technology with quantitative fluorescence (QIF). Maria I. Toki, 1Chris Merritt, 1Giang Ong, 1Joseph M. Beechem, 2Daniel E. Carvalaj-Hausdorf, 1Yalai Bai, 1David Rimm, 1Yale University School of Medicine, New Haven, CT; 2NanoString Technologies, Seattle, WA.

Background: Protein expression in formalin-fixed, paraffin-embedded (FFPE) tissue is routinely measured by Immunohistochemistry (IHC) on only one protein, or with quantitative fluorescence (QIF) on a handful of proteins on a single section. NanoString’s Digital Spatial Profiling (DSP) technology can detect and quantify proteins at significantly higher multiplex (currently 30plex, potential for up to 800plex) with spatial resolution using molecular digital color-coded “barcodes”, within specific regions of interest on FFPE tissue. Here, we compare Nanostring DSP technology to automated QIF (AQUA), for accurate and reproducible measurement of protein expression on a continuous scale. Additionally, using the multiplexing potential of Nanostring technology, we did a pilot study to assess cold ischemic time as a variable to monitor tissue quality by assessing protein degradation and quantification in FFPE tissue (NSCLC).

Methods: The DSP technology uses a cocktail of primary antibodies conjugated to indexing DNA oligos with a UV Photocleavable linker that can be counted on the nCounter platform. Regions of interest (ROI) on the tissue are selected with fluorescently labeled antibodies, and oligos from that region are released via UV mediated linker cleavage. Free oligos are captured via microcapillary fluidics into a microtiter plate and then quantitated on the nCounter. The comparator for this technology was the AQUA method of QIF. We examined a breast cancer tissue microarray with a range of HER2 expression, and a NSCLC time to fixation standardization array with timepoints from 20 min to 48 hrs. Results: Multiple markers were assessed and a high correlation was found between Nanosting DSP counts and QIF scores (R²>0.7), when the measurements were performed in the same region of interest (defined by cytokeratin expression). The dynamic range of DSP exceeded the quantification range of QIF (nearly 4 logs vs about 2 logs). When the 28 protein markers’ expression was compared at different fixation timepoints, most were found to be stable over different cold ischemic timepoints. Two markers, including phospho-ERK (Extracellular Signal-Regulated Kinases) and phospho-GSK (Glycogen Synthase Kinase) showed epitope loss as a function of delay to fixation (R²=0.0064 and R²=0.05 respectively). Conclusion: The Nanostring DSP is a reproducible method with the capability of highly multiplexed measurements of protein expression on a field of view averaged basis. It shows high concordance with the AQUA method of QIF, an extensively validated technique for protein quantification. For the first time, the high-plex capacity of DSP allows inclusion of markers that are sensitive to time to fixation as an intrinsic control for tissue quality. *FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.


Decades of cancer research including comprehensive molecular profiling combined with the development of a broad array of targeted therapies have created the opportunity to transform cancer care by implementing precision oncology based approaches. An important element of this system is the widespread availability of robust and cost-effective multi-variant profiling methods to characterize relevant cancer associated molecular alterations in the future. Current commercially available multi-variant profiling methods vary dramatically with regard to the number of cancer genes that are interrogated. Given that many large scale and detailed molecular profiling studies have been completed, the landscape of somatic alterations in solid tumors is reasonably well-known. Furthermore, the specific gene variants that are relevant to application of targeted therapies are also a matter of record. Therefore, we set out to define the number of relevant cancer genes for precision oncology research based on the currently available empirical evidence. To define recurrent somatic alterations in solid tumors, we created a compendium of variant calls from >15,000 exomes, defined focal amplifications and deletions from >30,000 arrays, and defined recurrent fusions from several thousand RNAseq profiles. Statistical approaches were implemented to define genes containing recurrent missense mutations (i.e., hotspots), enriched in truncating mutations or subject to recurrent copy number gain/loss or translocation. This gene set was then used to comprehensively search approved cancer drug targets and clinical practice guidelines to identify genes containing published evidence that specific recurrent somatic gene variants were used as part of the indication statement of an approved targeted therapy, were recommended for testing as part of clinical practice for therapeutic decisions, or were used as enrollment criteria in clinical trials. The relevant cancer genome thus defined consists of <100 genes. These results suggest that targeted multi-variant profiling approaches may be sufficient to support precision oncology goals in the future.

#3812 JAK2 and PTPN11 mutations as potential biomarkers for BCL-xL inhibition as monotherapy and in combination therapy for acute myeloid leukemia. Richard J. Bellin, Valerie Hilgenberg, Rejla Popovic, Tamar Uziel, Lloyd T. Lam. Abbvie, North Chicago, IL.

Acute Myeloid Leukemia (AML) is a heterogeneous disease where major distinct functional mutations have been identified. While mutations in FLT3, NRAS, NPM1, and IDH genes have been well characterized in de novo AML, a subset of AML associated with leukemia transformation of chronic myeloproliferative neoplasms (MPNs) are enriched in mutated JAK2 and PTPN11. MPNs are a set of disorders characterized by the chronic and abnormal overproduction of blood cells which can ultimately progress to AML. Targeted therapy against mutated JAK2 and PTPN11 with small molecule inhibitors against the JAK-STAT and RAS-ERK pathways, respectively, are being actively investigated in AML. However, acquired resistance and non-durable responses are already being demonstrated in monotherapy treatment. Thus, AML that has arisen from leukemic transformation of MPNs remains an area of unmet medical need. This need is underscored by the fact that these patients suffer from poor outcomes and low response rates to standard chemotherapy. Together, these results indicate the need for rational combinations in this population. In this study, we sought to better define the dependencies of AML cells on BCL-2 family members by screening AML cell lines with selective small molecule inhibitors to the BCL-2 family members. The screen revealed that the majority of AML cell lines depend on BCL-2, or BCL-2 together with MCL1 for survival. Intriguingly, a subset of AML cell lines is exquisitely sensitive to BCL-xL inhibition. Treating these cell lines with BCL-xL-selective inhibitor or with navitoclax, a dual BCL2/BCL-xL inhibitor induces apoptosis. These BCL-xL inhibitor-sensitive cell lines express high levels of BCL-xL, but not BCL-2 and MCL1. Genomic analysis of BCL-xL inhibitor-sensitive AML cell lines revealed that this subset is also enriched in JAK2V16F and PTPN11 mutations. We further demonstrate positive combination effect between BCL-xL inhibitor or navitoclax with JAK inhibitor (ruxolitinib) and two MEK inhibitors (trametinib and AZD6244) in cell lines with JAK2V16F and PTPN11 mutations, respectively. Together, these results demonstrate the potential utility of a BCL-xL inhibition as a combination partner in JAK2 and PTPN11 mutated cancers. Importantly, JAK2 and PTPN11 mutations in addition to expression of BCL-xL, BCL-2, and MCL1 could also be further tested as potential biomarkers for the utility of BCL-xL inhibition based treatments for patients with these mutations.

#3813 MPF as a monitoring biomarker for a mesothelin-targeted therapy against malignant mesothelioma and other cancer. Yunkai Yu, Raffit Hassan, Jingli Zhang, Masanori Onda, Ira Pastan, Liang Cao. National Cancer Institute, Bethesda, MD.

Introduction: Tumor antigen mesothelin had been shown to be elevated in mesothelioma, ovarian, pancreatic, and lung cancer. The mesothelin gene encodes a precursor protein of 69 kDa, which is cleaved into a 31 kDa soluble megakaryocyte potential factor (MPF), and a cell membrane-bound 40 kDa mesothelin. There is a high degree of interest in targeting mesothelin for mesothelioma and other cancers with various antibody-based agents, and some successes in early stage clinical trials with some of the agents. For the development of these targeted therapeutics, specific biomarkers would be needed for monitoring treatment responses, and detecting and evaluating tumor progression. The current test that detects mesothelin is not suitable due to the antigen-binding from the therapeutic antibodies. There is an urgent need to develop and validate an alternative test to support these clinical trials and for disease management.

Methods: Using ECLIA technology and proprietary antibody reagents, we developed a new test for the cleaved MPF polypeptide. We further conducted comprehensive analytical and clinical validation of the test according to the guidelines for tumor antigen tests. Using samples from a retrospective clinical trial with an anti-mesothelin immunotoxin and the standard 1st line chemo-
therapeutic agents for newly diagnosed malignant mesothelioma patients, we examined the effectiveness of the test for treatment monitoring of the investigational therapy. Results: Our data gives a strong validation for the analytical and clinical performances of MPF test. While no other current test has been proven to be able to monitor response of mesothelioma to systemic therapies, our data provides the proof of MPF test’s feasibility. Conclusion: Our study demonstrates that MPF test in the elevated tumor antigen, there is a specific and highly significant reduction of MPF at the end of each treatment cycle for a total of 6 cycles in the patients with objective response. Later, about half of these patients with objective response and MPF reduction regained the levels of MPF at the time of disease progression. Conclusion: The study indicates that MPF test is effective in monitoring the treatment response to systemic therapies for mesothelioma patients with elevated MPF. It may also provide pivotal information on the expression of target mesothelin protein when tumors progress.


Introduction The Tumor Inflammation Signature (TIS) is an 18 gene signature in development for the detection of adaptive immune response within tumors by measuring expression of genes associated with cytotoxic cells, antigen presentation, and IFNγ activity. The TIS has been previously shown to enrich for a population of patients who respond to the anti-PD1 therapy pembrolizumab. We characterized the behavior of the TIS across a range of tumor gene expression data downloaded from the TCGA in order to understand the distribution of TIS within and between tumor types, with special emphasis on those tumors for which anti-PD1 therapy is approved for use. Methods: The TIS was trained to predict response to pembrolizumab using gene expression profiles from 289 biopsies from 11 different tumor types. Its association with clinical response was retrospectively assessed in an independent set of 200 samples from 14 additional tumor types. We calculated TIS scores in over 9000 samples from 31 TCGA RNASeq datasets. We contrasted TIS with mutational load, with overall survival, and with other gene expression signatures. Results: While TIS scores are higher in classically immunogenic tumor types, they display a significant amount of intersample variability within most tumor types, and a subset of patients can be identified that possess elevated TIS scores consistent with responsiveness to pembrolizumab. TIS genes have highly conserved co-expression patterns across tumor types, consistent with a model in which the genes measure immune-intrinsic transcriptomic activity with minimal contribution of tumor-intrinsic gene expression. Notably, ranking tumors by median TIS score is superior to mutational load ranking at predicting clinical response to anti-PD1. TIS was minimally correlated with mutational load in most tumor types, except in tumor types known for hypermutation-driven by mismatch repair deficiency. TIS and mutation burden had their greatest variability in melanoma; in most other tumors, TIS retains more variability than mutation burden, possibly reflecting patients with anti-tumor immune responses driven by one or a few mutation-derived neoantigens. Average mutation burden and average TIS are positively correlated within most tumor types. Notable exceptions to this trend include two tumors for which anti-PD1 is currently approved. Bladder urothelial carcinoma has the highest average TIS over mutation burden would predict and kidney renal clear cell carcinoma has much higher average TIS than its mutation burden would predict. Conclusion: The TIS has the potential to enrich for anti-PD1 responders independent of tumor type. It may identify rare potential responders in tumors with low average response rates. These results support further research into the efficacy of anti-PD1 in patients with high TIS scores, regardless of tumor type.

#3815 The clinical impact of c-MET over-expression in advanced biliary tract cancers. Mi Hwa Heo, Seung Tae Kim, Young Suk Park, Joon Oh Park, Hansang Lee. Samsung Medical Center, Seoul, Republic of Korea.

Background: c-MET is a proto-oncogene that encodes the tyrosine kinase receptor for hepatocyte growth factor (HGF). Activation of HGF-c-MET signaling involves cell invasiveness and evokes metastasis through direct involvement of tumor angiogenesis. However, the value of c-MET overexpression is still unknown in metastatic BTC. Methods: We analyzed the incidence and clinicopathologic characteristics of c-MET overexpression in advanced BTC. Moreover, we investigated the value of c-MET overexpression in predicting response to gemcitabine plus cisplatin (GC), a first line standard regimen, and as a prognostic marker in metastatic BTC. Results: The BTC subtype distribution (N=44) was as follows: intrahepatic cholangiocarcinoma (IHCC, n=7), extrahepatic cholangiocarcinoma (EHCC, n=25) and gallbladder cancer (GBC, n=12). Liver (52.3%) was the predominant metastatic site, followed by lymph nodes (36.4%) and bone (15.9%). Among the 44 patients analyzed for c-MET expression, 15 (34.1%) exhibited c-MET overexpression in tumor tissues. There was no significant difference in the prevalence of c-MET overexpression among primary sites in EHCC (7/25, 28%), IHCC (3/7, 42.9%), and GBC (5/12, 41.7%). There was also no significant correlation between specific clinicopathological variables and c-MET expression. Conclusion: The c-MET expression according to c-MET overexpression (overexpression vs. non-overexpression), there was no significant difference in either RR or DCR (p = 0.394 and 1.000, respectively). PFS for all 44 patients was 9.00 months (95% CI, 7.47-10.53 months) and there was no significant difference for PFS between patients with c-MET overexpression and those without (p = 0.917). The median OS was 14.00 months (95% CI, 10.21-17.79). There was a significant difference in OS between patients with c-MET overexpression compared to those without (13.7 vs. 14.4 months, respectively, p = 0.708). Conclusions: c-MET overexpression was detected in 34.1% of advanced BTC patients irrespective of tumor location. c-MET overexpression did not predict response to GC or survival. Further studies are needed to fully elucidate the value of c-MET overexpression as a novel biomarker in these patients.

#3816 Mutations of the lim protein ajuha mediate sensitivity of head and neck squamous cell carcinoma to treatment with cell cycle inhibitors. Ming Zhang, 1 Singh Ratnakar, 2 Shaohua Peng, 2 Mazumdar Tuhina, 2 Shen Li, 2 Pan Tong, 2 Curtis Pickering, 3 Jeffrey N. Myers, 2 Jing Wang, 2 Faye M. Johnson. 4

1Fudan Univ., Shanghai, China; 2MD Anderson Cancer Center, Houston, TX.

The genomic alterations identified in head and neck squamous cell carcinoma (HNSCC) tumors have not resulted in any changes in clinical care, making the development of biomarker-driven targeted therapy for HNSCC a major translational gap in knowledge. To fill this gap, we used 59 molecularly characterized HNSCC cell lines and found that mutations of AJUBA,SMAD4 and RAS predicted sensitivity and resistance to treatment with inhibitors of polo-like kinase 1 (PLK1), checkpoint kinases 1 and 2, and WEE1. Inhibition or knockdown of PLK1 led to cell-cycle arrest at the G2/M transition and apoptosis in sensitive cell lines and decreased tumor growth in an orthotopic AJUBA-mutant HNSCC mouse model. AJUBA protein expression was undetectable in most AJUBA-mutant HNSCC cell lines, and total PLK1 protein expression was increased in cell lines wild-type for AJUBA. Exogenous expression of wild-type AJUBA in an AJUBA-mutant cell line partially rescued the phenotype of PLK1 inhibitor-induced apoptosis and decreased PLK1 substrate inhibition, suggesting a threshold effect in which higher drug doses are required to affect PLK1 substrate inhibition. PLK1 inhibition was an effective therapy for HNSCC in vitro and in vivo. However, biomarkers to guide such therapy are lacking. We identified AJUBA, SMAD4 and RAS mutations as potential candidate biomarkers of response of HNSCC to treatment with these mitotic inhibitors.


Introduction Inflammatory bowel disease (IBD) can affect the lower part of the small intestine (ileum) and the colon. It can, however, occur in any part of the large intestine, small intestine, or stomach. Recent evidence suggests that several factors may tip the balance between homeostasis and intestinal inflammation, presenting future challenges for the development of new therapies for IBD. In this study, we use normal small intestine as a model to identify multiple immune targets using mouse and rabbit monoclonal antibodies. Peyer Patches are lymphoid tissues in the wall of the small intestine that are involved in the development of immunity to antigens presented in that milieu. Small intestine and cases of IBD were evaluated as a potential model using immunohistochemistry (IHC) to identify categories of immune cells including cytotoxic T-cells, costimulatory cells (agonists), T-regulatory cells (TREGS), T-cell effector cells (regulators) and checkpoint inhibitors. cases of IBD were also examined for the utilization of single and double/triple stains. Design Formalin-fixed paraffin-embedded cases of small intestine and IBD were cut to 4 micron thickness and stained with H&E, Mouse and rabbit monoclonal antibodies including CD3 (natural killer cells), CD163 (macrophages), CD8 and CD4 (cytotoxic T-cells and helper T-cells), CD103, OX40 and CD137 (costimulatory), FOXP3 and LAG3 (TREGS), T-bet and RORγT (T-cell effectors), and PD-1 and PD-L1 (immune checkpoints) were tittered for optimization and evaluated for IHC. Several IHC multiplex stains (double and triple stains) were also developed and compared with the relevant single stains. Results Single, double and triple stains utilizing CD66 (natural killer cells), CD163, CD4, CD8, CD103, OX40, CD137, FOXP3, LAG3, T-Bet, RORγT, PD-1 and PD-L1 antibodies were successfully established using small intestine as staining model for IBD cases. All single stains versus double and triple stains gave equivalent results. Double stains and/or co-expression of CD8 + CD103, CD8 +
PD-1, and triple stain CD8 + PD-1 + FOXP3 were easily visualized by blue, brown, red or black chromogen staining. Conclusion Small intestine is a good model to develop single or multiplex stains for a host of immune cell targets. Enhanced visualization of these targets is possible with the use of double and triple stains; thus, this technique may help facilitate acquisition of important prognostic information and help support new strategies for inflammatory bowel disease in targeted therapeutic treatment.

#3818 Identification of a FGFR3-TACC3 fusion in esophageal cancer. Takuro Mizukami,1 Kazuko Sakai,2 Saeko Naruki,1 Tomoko Taniyama,1 Yo-shiki Horie,1 Naoki Izawa,1 Takashi Tsuda,1 Takashi Fujino,1 Narikazu Boku,1 Hiroshi Yasuda,1 Tetsu Fukunaga,1 Takako Eguchi Nakajima,1 Kazuto Nishio.1

1St. Marianna University School of Medicine, Kanagawa, Japan; 2Kinki University Faculty of Medicine, Osaka, Japan; 3Juntendo University School of Medicine, Tokyo, Japan.

Ablerrant activation of fibroblast growth factor (FGF) signaling has been found to contribute to carcinogenesis. Recently, activating FGFR fusions are detected in several malignancies. However, limited information is available regarding the existence of FGFR fusions in esophageal squamous cell cancer (ESCC) and gastric cancer (GC). In this report, we explored major FGFR1, FGFR2, and FGFR3 fusion transcripts in ESCC and GC specimens (n = 74 and 114, respectively) using a most comprehensive next-generation sequencing panel. We detected a targetable fusion between exon 18 of FGFR3 and exon 11 of TACC3 at a frequency of 1.4% (1/74) in ESCC, whereas other FGFR fusions were not detected in the GC specimens. Furthermore, split FGFR3 signals were apparent in this specimen by fluorescence in situ hybridization (FISH) with break-apart probes. The patient, a 64-year-old man with unresectable T4bN3M1 poorly differentiated ESCC of stage IV, was treated with palliative chemoradiotherapy followed by taxane chemotherapy. The patient died of cancer progression, with his overall survival time having been 9.5 months. Our results provide sequence information that should prove useful for development of simple and cost-effective diagnostic assays as an alternative to FISH for patients with ESCC harboring the FGFR3-TACC3 fusion.

#3819 Identification and quantification of isoforms of eukaryotic initiation factor 4E as biomarker in Mkn inhibitor-treated mouse model by capillary-based immunoassay platform. Zhiyuan Ke,1 Sifang Wang,1 Vithya Mano-haran,1 Susmitha Vuddagiri,1 Esther Ong,1 Sharon Lim,2 Sin Tong Ong,2 Kanda Sangthongpitag,1 Nacro Kassoum,1 Jeffrey Hill,1 May Ann Lee.1

1Experimental Therapeutics Centre, Agency for Science, Technology and Research, Singapore, Singapore; 2Duke-NUS Medical School, Singapore, Singapore.

Elevated levels of the phosphorylated mG cap binding protein, eukaryotic initiation factor 4E (eIF4E) are associated with neoplasia. An observed oncogene signaling effect upon activation of Erk1/2 or p38 MAPKs in cells is the eIF4E-overexpressing K562 cells. siRNA knockdown of eIF4E expression resulted in growth arrest and apoptosis in eIF4E-overexpressing K562 cells. siRNA knockdown of eIF4E expression reduced both isoforms, while treatment with Mnk inhibitor only reduced phosphorylated eIF4E level. The measurement and quantification of the relative changes of phosphorylated eIF4E by this method can potentially be used in monitoring the efficacy of Mnk inhibitors in xenograft and future clinical trials.

#3820 Hepatitis B virus gene pre-S2 mutant as a high risk serum marker for hepatoma recurrence after a hepatic resection. Wen-Ya Huang. Cheng Kung University Hospital, Tainan, Taiwan.

Background: Chronic hepatitis B virus (HBV) infection is the major cause of hepatocellular carcinoma (HCC). The pre-S2 mutant large HBV surface antigen (LHBs) is highly associated with HCC. This study aimed to evaluate the serum pre-S2 mutant LHBs as a high-risk recurrence marker in HCC patients after a hepatic resection. Methods: The pre-S gene chip was used to analyze 175 patients with HBV-related HCC who underwent a hepatic resection between 2008 and 2012. Thirty independent pre-S clones in every patient were analyzed for a semi-quantitative detection. Results: Multivariate regression analysis showed that the serum pre-S2 mutant level (> 5%) and the American Joint Committee on Cancer (AJCC) tumor stage were two independent high-risk factors for HCC recurrence. A Cox proportional hazards analysis also showed that the combined evaluations of the AJCC stage and serum pre-S2 mutant level provided a more effective predictive marker for HCC recurrence after hepatectomy. We developed a Cox prediction model, which indicated the recurrence-free survival rates along with the time after surgery. This model was validated in an independent HCC cohort. Receiver operating characteristic curve analysis revealed that the model showed close sensitivities in the main and validation cohorts (AUC values: 0.741 and 0.704, respectively). Conclusions: The relation of serum pre-S2 mutant level, independent of the AJCC stage, is an important predictive high-risk marker for HCC recurrence after a primary hepatectomy. The combined evaluations of the AJCC tumor stage and serum pre-S2 mutant level potentially serve as a convenient high-risk predictive marker for HCC prognosis.

#3822 Expression of androgen receptor and CD24 correlates with improved 5-year survival in Pakistani patients with invasive breast cancer. Nazia Riaz,1 Romana Idrees,2 Sadia Habib,3 Azhar Hussain,3 El-Nasir Lalani.1

1Centre of Regenerative Medicine and Department of Surgery, Aga Khan University, Karachi, Pakistan; 2Department of Pathology and Laboratory Medicine, Aga Khan University, Karachi, Pakistan; 3Centre of Regenerative Medicine, Aga Khan University, Karachi, Pakistan.

Introduction: Androgen receptor (AR) expression has been shown to correlate with improved survival in luminal A, B and triple negative (TN) breast cancer (BCa). Cancer stem cells (CSC) with CD44+/CD24- and ALDH1+ phenotype is associated with pro-invasive gene signature in BCa. Aim of our study was to evaluate prognostic significance of AR expression in relation to CD44-, CD24- and ALDH1+ BCa. Methodology: Immunohistochemical expression of AR, CD44, CD24 and ALDH1 was evaluated in 180 FFPE blocks of BCa cases from one institution. Clinico-pathological details and follow-up data were collected from medical charts. Data was analyzed on SPSS 19. Five-year survival was estimated by Kaplan Meier. Results: Median age of patients was 55 years (range: 28-87). Fifteen percent of patients were diagnosed with stage I, 52.2% with stage II, 25% with stage III and 7.8% with stage IV disease. Mean duration of follow-up was 4.5 years (SD = 2.7) and 29% mortalities were attributed to BCa. AR was expressed in 63.9%, ER in 61%, PR in 51% & HER-2/neu in 30% of tumors. CD44, CD24 and ALDH1 were expressed in 60%, 43.3% & 28.3% of tumors respectively. AR expression was associated (p-value < 0.001) with stage II tumors expressing ER, PR and CD24 but lacking ALDH1. Multivariable analysis confirmed AR (adjusted HR 0.43, 95% CI 0.19-0.95) and PR (adjusted HR: 0.20, 95% CI 0.07-0.60) to be independently associated with better outcome. Five year survival of patients with AR+ versus AR- expressing tumors, showed that patients with AR+ tumors had significantly better outcome (p-value = 0.03) with survival advantage of 39.6 months. Survival advantage decreased by 9.6 months in tumors expressing CD44+/CD24-AR- (p-value = 0.01) phenotype and by 12 months in patients whose tumors were phenotypically ALDH1+/AR- (p-value = 0.12). We stratified TN tumors into three risk groups: AR-, TN/CD24+/CD44+/ALDH1+; AR+; TN/CD24-/CD44+/ALDH1-. We found that group A tumors had a trend towards better survival (p-value = 0.09). Conclusions: Three individual markers (CD44, CD24, AR) may afford prognostic information. Combinations of CD44, CD24 and AR permitted patient stratification into 3 risk categories: poor risk CD24-/AR-; intermediate risk CD44+/CD24-/AR-; and good risk CD24+/AR+. Stratification of TN group showed that loss of AR and CD24 was associated with poorest outcome. These results underscore the relevance and importance of AR and CD24 as prognostic markers in invasive BCa.


Introduction: Fluoropyrimidines (FP) are of major use in treating cancer. Dihydropyrimidine dehydrogenase (DPD) is the main enzyme responsible for FP catabolism. Low DPD activity screening has proven its predictive clinical value in identifying patients at risk for toxicity. However, high DPD activity may translate to decreased FP activation and efficacy. Patients and methods: One hundred and forty-three patients (monocentric retrospective study) underwent a pretreatment assessment of DPD activity in lymphocytes between 01/01/2004 and 20/04/2016. Included patients were male or female, >18 years, FP-treatment-based. Cut-off for DPD activity was assessed using smoothing spline curves. Results: Median patient follow-up was 30 months [C95%: 27.3-36.2],

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mean age 63 +/- 3 years, females accounted for 53%, 90% of patients had a good performance status (0 or 1). Fifty-eight percent of FP indications (65% 5-FU and 35% capecitabine) were adenocarcin or neoadjuvant and 42% were prescribed for local or metastatic recurrences. Tolerance was poor for 43% of patients and 49% needed dose reduction. Objective response (complete and partial) was observed in 50% of patients, stable disease in 38% and progression in 12%. No significant correlation was observed between DPD activity and response to treatment.

Conclusions: DPD activity screening could lead to a two-pronged approach: FP dose reduction in the event of low DPD and dose increase for high DPD. Such a strategy needs to be prospectively validated under personalized DPD-based treatment.

#3824 Analysis of TOPO II and p53 by immunohistochemistry and qPCR in sarcoma patients with ChemFx assay to determine sensitivity against Adriamycin and Etoposide. Max Henry Jacobson,1 Lijun Ni,2 Sandy Althouse,3 Nagendra Prasad,1 George Sandusky,1 Daniel Rushing1. 1Indiana Univ. School of Medicine, Indianapolis, IN; 2Stanford University, Stanford, CA

Sarcomas are a heterogeneous group of tumors that account for about 200,000 cancers diagnosed each year and represent 15% of all pediatric malignant tumors. They comprise over fifty subtypes that include bone and soft tissue sarcomas. Bone sarcomas represent about 20% of all sarcomas. It is estimated that over 50% of human sarcomas have a TP53 mutation. Topoisomerase II (TOPO II) is the target for doxorubicin which is the most effective chemotherapeutic agent, although it is sometimes replaced with etoposide to decrease risk of cardiac toxicity. In this study, 83 clinical sarcomas were evaluated from the IU Medical Center. Of these 83 clinical cases, 34 were primary tumors, 46 were metastatic tumors. Subtypes included in this study are: Liposarcoma, Leiomyosarcoma, Fibrosarcoma, Malignant Fibrous Histiosarcoma (MFH), Synovial Sarcoma, Osteosarcoma, and Ewings. Paraffin-embedded tissue blocks were obtained from the Indiana University Heath Pathology Laboratory under IRB approved protocols. Immunostainings with TOPOII and P53 antibodies (DAKO) were performed using the DAKO platform with LSAB2 system at the IU Health Pathology Laboratory. The stained tissues were scanned using Aperio Imaging System and analyzed using the positive-pixel algorithm. In addition, qPCR (TagMan assays) was performed using the RNA isolated from the same tissues to analyze TOPO II by a second technique. Live tissue testing was also employed using ChemFX assay performed on fresh specimens to determine Adriamycin and Etoposide (VP-16) sensitivity and/or resistance. Response to Adriamycin and Etoposide were combined to form a composite precision value and then compared with Clinical Response. This showed a 100% positive predictive value and 90% negative predictive value. We combined the two together to create an overall “Combined Response” Variable. This variable was compared to Immunohistochemistry for both TOPO II and P53, and the expression levels of TOPOII RNA. The results showed that the lung metastases expressed a higher level of TOPO II and P53 then did the primary tumors. qPCR and Immunohistochemistry data did not correlate with each other. The positive pixel data in the primary tumors were 5.238% for TOPO II and 2.4818% for P53, compared to the metastatic lung lesions with 9.64% for TOPO II and 4.5692% for P53. The combined response versus the categorized TOPO II IHC (into categories of increasing intensity, 1,2,3) had a Chi-squared p-value of 0.656. The combined response versus the categorized P53 IHC (<5% and >5%) had a Fisher-Exact test p-value of 0.082. There were approximately 40 resistant and 16 sensitive combined responses available for comparison. We conclude that P53 is a negative predictor for clinical response, with 12 of 40 resistance cases stained positive for P53, whereas 15 of the 16 sensitive cases were negative for P53.

#3825 Digital PCR-characterized, highly multiplexed, oncology RNA fusion reference materials: Performance on multiple NGS platforms. Catherine Huang, Yves Konigshofer, Lequan Nguyen, Rajeswari Venula, Praveena Kamineni, Deepika Philkana, Ekta Jaiswal, Bhalwar Anekella. Seracare Life Sciences, Gaithersburg, MD.

Introduction: Genomic structural alterations are increasingly actionable for targeted therapeutics and personalized medicine. Molecular diagnostics are rapidly being introduced for detection of fusion RNAs by highly multiplexed next-generation sequencing assays. However, reference materials to aid in the development, optimization and validation of these assays are lacking. We developed Seraseq FFPE Fusion RNA Reference Material to fill this unmet need and show that this material is compatible across a wide range of NGS assay platforms. Methods: Biosyntheses were used for transcription of clinically actionable RNA fusion RNAs using the primers in a plasmid vector containing a T7 promoter. The RNA fusion RNAs were introduced into GM24385 reference cell line (The 1000 Genomes Project, Coriell). The cells were collected, fixed in formalin, and total RNA was isolated. Digital PCR with TagMan® chemistry was used to determine the target Fusion RNA copies per microliter. Use of fusion-specific digital PCR provides an orthogonal method of verifying transcript levels and serves as the “ground truth” for the abundance of each RNA. NGS testing of the purified RNA used the ArcherDx FusionPlex™ CTI Panel, QIAGEN QIAsert Targeted RNAscan Panel, and the Thermo Fisher Oncomine Focus Assay. Results: All sixteen (16) fusions present in the prototype were detected as expected on each NGS platform. Results among the three different NGS platforms were generally concordant, although the reads across the fusion junctions did vary slightly among NGS assays and with digital PCR results. However, all methods indicated that the reference material gave low positive results, similar to a patient sample, and that the single reference material could serve as a positive control for detection of sixteen different fusions, which represent a variety of different solid tumor types. Conclusions: Seraseq FFPE RNA Fusion Reference Material allows simultaneous evaluation of detection for sixteen fusions observed in a variety of solid tumors, both common and rare. It provides a consistent, unlimited supply of QC materials particularly valuable for difficult to find rare fusions. The reference material generates low positive results on three leading assays, and this is important to truly challenge the assay system. This material is handled identically to a patient sample from extraction through analysis, and verifies performance at levels expected for patient samples.
#3827 NCI 8628 - A randomized phase II study of Ziv-ailfercept (Z) and high dose Interleukin-2 (IL-2) or IL-2 alone for inoperable stage III or IV melanoma. Ahmad A. Tarhini,1 Paul H. Frankel,2 Christopher Ruell,2 Marc S. Ernstoff,2 Timothy M. Kuzel,3 Theodore F. Logan,4 Nikihi I. Khushalani,5 Hussein A. Awadhi,6 Kim A. Margolin,7 Sanjay Avashtii,8 David F. McDermott,9 Ailne Chen,9 Primo N. Lara,10,11 John M. Kirkwood.12 1Univ. of Pittsburgh Cancer Inst. Pittsburgh, PA; 2City of Hope, Duarte, CA; 3Roswell Park Cancer Institute, Buffalo, NY; 4Northwestern Memorial Hospital, Chicago, IL; 5Indiana University, Indianapolis, IN; 6Moffitt Cancer Center, Tampa, FL; 7MD Anderson, Houston, TX; 8Beth Israel Deaconess Medical Center, Boston, MA; 9NHN/NCI, Rockville, MD, 10University of California Davis, Sacramento, CA.

Background: [IL-2], a centrally regulated antitumor immune modifier, has been proposed as a potential treatment for inoperable stage III or IV melanoma. Efficacy of IL-2 in comparison to placebo and other active regimens was reported in several phase III trials, randomized 2:1. Eligible patients (pts): Stage III inoperable or Stage IV melanoma. Up to two prior regimens for metastatic melanoma and stable treated brain metastases were allowed. The primary endpoint was progression-free survival (PFS). Results: A total of 89 pts were enrolled, but 5 who never started study treatment were excluded. Six pts (4 in A and 2 in B) were treated but withdrew early without a response assessment were considered non-responders in this analysis. Pt and disease characteristics are summarized in Table 1. Median number of IL-2 cycles was 3 (A) and 2 (B) and of Z cycles in A was 3 (1 - 3). Median follow up for all alive patients was 19 months. Among 84 treated pts (55 in A and 29 in B), there was significant improvement in PFS in favor of A: median and 95% CI of 6.9 (4.2 - 8.8) months vs 2.1 (1.7 - 4.1), logrank p < 0.001. No significant difference in OS: median and 95% CI of 23.1 (14.4 - 35.0) months vs 22.3 (12.6 - NA). Response rate (RECIST) was 22% in A (5CR, 7PR) and 17% in B (5PR). Stable disease or PR or CR was seen in 69% in A and 48% in B. Adverse events were consistent with the AE profile of monotherapy with IL2 and Z. Grade 4 events in A included decreased lymphocytes (41 pts) and platelets (6), renal (1), hypophosphatemia (4), hypertension (2) and thromboembolism (1).

Table 1

<table>
<thead>
<tr>
<th>Age (median and range)</th>
<th>Arm A (N=55)</th>
<th>Arm B (N=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td>Male</td>
<td>33</td>
<td>18</td>
</tr>
<tr>
<td>Cutaneous primary</td>
<td>36</td>
<td>16</td>
</tr>
<tr>
<td>Mucosal</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>Unwell Unknown</td>
<td>68</td>
<td>45</td>
</tr>
<tr>
<td>AJCC Stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III (N3) Ma</td>
<td>910</td>
<td>35</td>
</tr>
<tr>
<td>I (N1) Ma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I (N2) Ma</td>
<td>81</td>
<td>6</td>
</tr>
<tr>
<td>I (N3) Ma</td>
<td>25</td>
<td>15</td>
</tr>
</tbody>
</table>

Conclusions: The combination of Z and IL2 significantly improved PFS over IL2 alone, meeting the study's primary endpoint. The regimen was relatively safe and manageable. Correlative and mechanistic studies are ongoing.

#3829 TCIRG1, T-cell immune regulator 1, functions as a metastatic enhancing gene in liver tumorigenesis. Hee Dooy Yang, Jung Woo Eun, Yingyu Shen, Hyung Seok Kim, Sang Yeon Kim, Suk Woo Nam. Lab of Coding & Non-coding Genomics, Department of Pathology, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea.

Recurrence and metastasis are major challenges in the management of hepato-cellular carcinoma (HCC) patients after HCC resection. In order to identify metastatic molecular signature, we performed comparative gene expression profiling analysis with resected HCC tissues from HCC patients who underwent partial or total hepatectomy and non-metastatic primary HCC. From this, we were able to recapitulate molecular signatures associated with HCC recurrence, and found that TCIRG1 (T-Cell Immune Regulator 1) was one of the aberrantly overexpressed gene elements in recurrent HCC patients with total hepatectomy. We then observed that TCIRG1 is significantly overexpressed in TCGA-LIHC (Liver Hepatocellular Carcinoma Dataset of The Cancer Genome Atlas), and high expression of TCIRG1 was significantly associated with poor prognosis of HCC patients in 5-year disease-free. TCIRG1 knockdown suppressed tumor cell growth and proliferation in Huh7 and SNU475, liver cancer lines. In addition, sustained expression of TCIRG1 in liver cancer cell line reduced tumor growth rate in mouse xenograft model. Moreover, targeted-disruption of TCIRG1 significantly attenuated the metastatic potential of ras-transformed NIH-3T3 cells in vitro and in vivo. In conclusion, our findings suggest that TCIRG1 functions as metastatic enhancing gene by modulating cellular growth, death, and EMT in liver cancer cells providing TCIRG1 as a therapeutic target for the treatment of liver malignancy and metastasis.

#3830 Plucked anagen scalp hair: A reproducible surrogate tissue to monitor drug induced transcriptional changes and provide pharmacodynamic biomarker and target engagement information from cancer patients. Benjamín J. Reed, Timothy Mefo, Elliott Harrison, Ross Haggart, Emma Grimes, Alan Murdoch, Gino Miele. Epistem Ltd., Manchester, United Kingdom.

Monitoring target engagement is crucial to inform on early drug development decisions, and development of a peripheral tissue-based gene expression signature for the indication of pathway inhibition could facilitate the clinical development of compounds. Plucked anagen scalp hair is an ideal source of epithelial tissue to monitor drug induced transcriptional changes. High vascularisation of the hair follicle, frequent epithelial origin of tumors, ease of sampling and high degree of congruence of expression in hair of pathways dysregulated in cancers, make the cellular bulb of plucked human scalp hair an excellent surrogate tissue for the non-invasive monitoring of pharmacodynamic (PD) and mechanism of action (MOA) effects in clinical trials. Using our ex vivo plucked hair culture platform, hair bulbs from several healthy donors were exposed to BEZ235, a dual pan-class PI3K and mTOR inhibitor at different concentrations and durations. Total RNA was isolated from the cellular bulb of individual anagen hair post-culture and used for microarray analysis to assess global transcriptional alterations and develop a gene expression signature indicative of BEZ235 exposure. The results of the ex vivo plucked assessment generated a dose dependent appreciable and biologically relevant transcriptional signature following exposure to BEZ235. Transcriptional readouts from patients also revealed strong correlations (0.97-0.99) in the genes expressed in anagen scalp hair between donors and across samples taken from the same donor. In addition, global gene expression data indicated that assessing three plucked scalp hairs or less at each collection point was sufficient to detect significant differential expression (p<0.05 & 1.5 fold change) underpinning the low variance in the majority of target genes in plucked hair as a biomarker platform. Gene signatures for compounds targeting the PI3K/mTOR pathway that were established using the ex vivo plucked scalp hair platform were used to support clinical trials and scalp hair taken from patients receiving treatment showed an excellent post-exposure target signature modulation. In summary, our results substantiate that plucked anagen scalp hair is an ideal non-invasive surrogate tissue to obtain drug-induced pharmacodynamic biomarker and target engagement information from oncology patients.
The development of an immunohistochemical digoxigenin-labeled mouse on mouse single and double stain methodology. Joseph Vargas, David Tacha, Sara Figueroa. Biocare Medical, Concord, CA.

Introduction When a mouse or monoclonal antibody is desired for immunohistochemical detection on mouse tissues, the anti-mouse secondary antibody will also bind to endogenous mouse IgG in the tissue and may produce an unwanted background staining. In the past, mouse-on-mouse (MM) detection utilized a biotinylated primary antibody or biotinylated kit. Tissues rich in endogenous biotin such as kidney or liver made it difficult to use, as streptavidin-horseradish peroxidase is required for detection. Attempts to completely negate endogenous biotin are ineffective as avidin-biotin blocking steps are unlikely to work accurately in problematic tissues. Therefore, the design of a digoxigenin-labeled detection system is advantageous for MM detection as it would eliminate these issues. This technology has broad application as it allows the use of a variety of monoclonal antibodies reactive on mouse tissues. Materials and Methods 

Mice tissues were formalin-fixed for 24 hours and embedded in paraffin (FFPE). Sections were cut and dried on slides before deparaffinizing and peroxidase blocking in the usual manner. Slides were then HER in a citrate-based buffer prior to application of antibody. Mouse antibody concentrations were selected and mixed with a Digoxigenin Anti-Mouse Linker and incubated at room temperature (RT) for 1 hour. An Absorption Reagent is then added to the mixture, and further incubated for 30 minutes. The mixture is then diluted in antibody-diluent to the desired concentration and applied to the mouse tissue for 60 minutes at RT. Following a rabbit-anti-digoxigenin antibody for 15 minutes. Detection of anti-digoxigenin is performed with an incubation in either a goat anti-rabbit HRP or AP polymer for 30 minutes. Visualization is accomplished by using a DAB or Fast Red chromogen. For double stains, a sequential DS method using two monoclonal antibodies was employed using a heating step in retrieval solution after the first chromogenic reaction followed by a second monoclonal antibody and detection with either a rabbit-AP or HRP polymer. Visualization of the second antibody target is accomplished by using Fast Red or a blue chromogen. Slides were coveredslipped with a permanent mounting medium and evaluated using brightfield microscopy. Results MM single stains with a variety of antibodies were easily achieved with specific, clean and biotin-staining-free staining. Sequential DS techniques using monoclonal antibodies were also achieved and visualized with DAB, Fast Red or blue chromogens. Both manual and automated procedures were used. Conclusion The replacement of biotin-labeled MM technology with a digoxigenin MM technology is a superior method that not only eliminates endogenous biotin, but allows utilization of HRP and AP polymers for double stain technology.

Optimization strategy for fluorescent multiplex immunohistochemistry tissue staining. Yi zheng, Carla Coltharp, Ryan Dilworth, Linying Liu, Danielle Unricht, Cliff Hoyt, Milind Rajopadhye, Peter Miller. PerkinElmer, Hopkington, MA.

Introduction: Recent insights into the tumor microenvironment have fueled the need for additional information beyond the one or two phenotypes provided by traditional immunohistochemistry (IHC). As a response, manual and automated fluorescent multiplex immunohistochemistry (IHC) techniques have been recently developed and accepted by the immuno-oncology space. Fluorescent multiplex immunohistochemistry (IHC) assays are designed to simultaneously measure multiple biomarkers in tissue sections with visual context that is lost in other methods, such as flow cytometry. Here we describe a novel optimization strategy to achieve quantitative, robust, and specific multiplex IHC staining results with both manual and automated multiple-color Opal procedures. Methods: Formalin-fixed paraffin-embedded samples of primary tumors were immunostained using Opal™ reagents both manually and on a fully automated Leica BOND RX® stainer. The impact of different reagent concentrations and quantities were analyzed in respect to signal specificity and robustness, stripping efficiency, signal co-localization, signal to noise, and color separation. Images were acquired on a Vectra 3.0 automated imaging system, and analyzed with inForm™ software. Results: The goals of this study were two-fold: to understand the impact of Opal reagent concentrations/quantities on fluorescent signal intensities acquired from cells within the context of tissue. -to develop an Opal reagent optimization method that yields more consistent, quantitative results from separated and co-localized fluorescent signals. We’ve applied this novel fIHC experimental approach and optimization strategy to Opal monoplex and multiplex assays and explored staining robustness, contextual specificity, staining order independence, and co-localized signal separation. Using the novel optimization strategy, we have achieved optimal staining patterns with improved confidence in the quantitative characteristics of the assay. Tissue sections stained after optimization have exhibited staining order independence and closely align with traditional IHC patterns. Conclusion: This novel optimization strategy, developed for Opal fIHC assays, is more quantitative, improves staining results, and minimizes interference between co-localized biomarkers.

Systematic evaluation of transcriptome sequencing shows comparable profiles for an exome-capture method for formalin-fixed, paraffin-embedded (FFPE) breast cancer tissues and the standard poly-A method for matched fresh frozen (FF) tissues. Xi Zhao, Marianna Zavodovskaya, Luting Zhuo, Kevin Kwei, Xin Guo, Zhaohui Jiang, Scott D. Patterson, Carrie B. Brachmann. Gilead, Foster City, CA.

Background: Tissue samples are routinely preserved as FFPE blocks. This abundant sample reservoir holds promises in clinical omics for disease diagnosis and precision cancer medicine. However, due to extensive cross-linking, nucleic acids are not efficiently extracted from FFPE samples and are heavily degraded, posing challenges for molecular assays including next generation sequencing. Our study aims to identify the RNA sequencing (RNA-seq) library prep method for FFPE samples that provides comparable data to the established poly-A enrichment method for FF tissue. Methods: RNA-seq data were collected on matched FF and FFPE samples from triple negative breast cancer (TNBC; n=7) and normal breast tissues (n=2). Tissues were sourced from a commercial vendor. Three library preparation protocols from Illumina were tested: poly(A) enrichment protocol (Poly-A: FF only), ribosomal RNA depletion protocol (Ribo-Zero Gold), and exome-capture based protocol (RNA-Access). Data were compared on absolute and differential gene expression, as well as molecular subtyping for breast cancer (PAM50; Parker 2009). Results: Both absolute expression and log fold change between mRNA expression in FF and FFPE tissues correlate strongly across different tissue preservation and library preparation methods. Among tested methods, RNA-Access had the highest yield of mappable reads on coding region and provided uniform transcript coverage with less rRNA contamination. Regardless of the protocol, all TNBC samples were classified as PAM50 basal-like subtype. As expected, FF, with either Ribo-Zero or RNA-Access had the strongest correlation with FF Polya for. Hh the subset of the poly(A) staining results. Data were compared on absolute and differential gene expression, as well as molecular subtyping for breast cancer (PAM50; Parker 2009). Principal component analysis showed that samples were primarily segregated by technical differences (library preparation or tissue preservation method) rather than biological differences (tumor/normal status or patient identification), suggesting a strong technical bias in the data. Gene families and biological processes underlying the differentially expressed genes due to technical variations included ribosomal, histone and metabolic pathways proteins. The aforementioned technical bias may be largely eliminated by a batch effect adjustment method (COMBAT; Johnson 2007). Conclusion: As expected, RNA-seq data is primarily impacted by technical differences. RNA-Access based transcriptome sequencing on breast FFPE samples generated highly concordant gene expression profiles when compared to data derived from FF samples. Our study supports the use of RNA-Access based transcriptome sequencing on FFPE samples when FF samples are not available.

Tracking CAD-ALK gene translocation in urine and plasma of a colorectal cancer patient treated with ALK blockade. Giulia Siravegna,1 Andrea Chillemi,1 Jacopo Palleschi,1 Berenice M. Tosi,2 Andrea Cassignella,2 Luca Novara,2 Michela Buscarino,2 Giorgio Corti,3 Giovanni Crisafulli,1 Alice Bartolini,1 Mark Erlander,2 Federica Di Nicolantonio,1 Salvatore Siena,1 Alberto Bardelli,1 IRCC, Candidio, Italy; “Niguarda Cancer Center, Milano, Italy; 3Trovagene Inc, San Diego, CA.

A metastatic colorectal cancer (mCRC) patient carrying CAD-ALK translocation achieved partial response to an experimental ALK inhibitor and then progressed after 5 months. We studied whether urine cell-free, trans-renal DNA (tr-DNA) could be used to monitor tumor burden and patient’s response. A NGS panel was developed to interrogate 52 common cancer gene rearrangements and 14 frequently mutated genes in cancer patients. A TP53 p.R248W mutation and the CAD-ALK genomic breakpoint (rarrangement) were identified in the tumor tissue and matched plasma circulating DNA (cDNA) DNA. cDNA samples were longitudinally obtained from the patient during ALK inhibitor treatment in parallel with blood. To detect the CAD-ALK translocation in urine tr-DNA we designed ultra-short (51 bp amplicon) primer pairs spanning the genomic breakpoint as a unique tumor marker. This approach allowed the non-invasive monitoring of the gene fusion in urine with amounts paralleling tumor burden. Of note, CAD-ALK gene fusion was apparent in urine tr-DNA before radiological confirmation of disease progression. The same strategy was applied to plasma cDNA and the results were compared. To detect point mutations in urine tr-DNA, we explored a peptide nucleic acids (PNA)-CLAMP PCR coupled with droplet digital PCR (ddPCR) analysis to specifically suppress amplification of wild-type DNA fragments. Custom PNA
probes were designed for TP53 codon 248, and a ddPCR assay was optimized to detect the TP53 p.R248W mutation, which was then identified in all urine tr-DNA samples, with absolute copies correlating with tumor burden throughout ALK inhibitor treatment. In conclusion, we find that urine tr-DNA can be exploited to non-invasively monitor tumor burden by detecting tumor-specific gene fusions as well as point mutations.

TUMOR BIOLOGY: Cell Culture and Animal Models of Cancer 4

#3835 High-purity patient-derived cells: An advanced cell culture model for precision cancer medicine. Seok-Young Kim,1 Hwan Kim,2 Sung-Moo Kim,1 Hye Ryun Kim,1 Mi Ran Kim,1 Ji Min Lee,1 Hun G Choi, Byung Cheol Cho2.1 Yonsei Cancer Research Institute, IE-UK Laboratory of Molecular Cancer Therapeutics, Seoul, Republic of Korea; 2Division of Medical Oncology, Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea.

Purpose: Patient-derived cells (PDCs) established from malignant effusions can represent an excellent tool for the study of non-small cell lung cancer (NSCLC) with oncogenic driver mutations. However, because of cellular paucity and low tumor purity, PDCs have a limited utility for in-depth molecular analysis. In this study, we developed a high-purity patient-derived cell (HP-PDC) method for primary culture of malignant effusions. Experimental design: HP-PDCs were established by a multistep tumor cell isolation protocol as follows. 1) Initial cultures of malignant effusions were observed under a light microscope to identify spherical-forming cells. 2) Tumor purity of PDCs was analyzed by Immunofluorescence (IF) and flow cytometry. 3) Mutation status of EGFR or ALK in PDCs was screened by direct or PCR-based sequencing and RT-PCR and then was compared with driver mutations detected in matching patient tumor samples. 4) HP-PDCs were tested for drug efficacies by a cell viability assay, a colony-forming assay, and western blot. 5) HP-PDCs were screened with a panel of 84 kinase inhibitors to identify effective drugs. Results: A consecutive series of 45 malignant effusion samples was collected from 36 patients with advanced NSCLC. Spherical-forming cells were detected in 19 samples (42.2%) which were cultured in AR5 media to establish PDCs. IF analysis revealed that TFF-1 was upregulated in 9 PDCs. These 9 PDCs with high TFF-1 expression also showed EPCAM-positive cell population in flow cytometry with high purity of over 70%. Using Sanger sequencing, EGFR exon 19 deletion was detected 3 cases. TERT T970M with L858R in 2 cases, and ALK rearrangements in 4 cases. Two of PDCs established from patients, who carried EGFR mutations and progressed on gefitinib, showed resistance to gefitinib in vitro. One of gefitinib-resistant PDCs was sensitive to AZD9291 with IC50 value of 47 nM. Three of PDCs established from patients, who carried ALK fusion genes and progressed on ALK inhibitors (crizotinib, alectinib, and ceritinib respectively), also recapitated patient’s response to the ALK inhibitors. In an alectinib-resistant PDC, crizotinib and ceritinib exhibited potency with IC50 values of 321 nM and 215 nM respectively. Colony-forming assay and western blot results were correlated with the cell viability assay data. Time spent for establishment of HP-PDCs ranged from 26 days to 132 days. Conclusions: Nine HP-PDCs established by multistep tumor cell isolation protocol had high tumor purity, and recapitated patient’s genomic profile and drug responses. HP-PDC is a powerful preclinical model which predicts responses to targeted drugs in a short period of time and may provide an excellent platform for personalized therapies in patients with advanced NSCLC.

#3836 New type of drug resistant isogenic cancer cell model created by CRISPR genome editing. Lydia Anne Volpe,1 Meteoro Selaanum,2 Luping Chen,1 Michael Jackson,1 Catherine Nguyenngo,1 John Foulke,1 Fang Tian1.1 ATCC, Manassas, VA; 2 ATCC, Gaithersburg, MD.

Mutant BRF1 gene can lead to uncontrolled cell growth through overactivation of Ras-Raf-MAPK signaling pathway. BRF600E mutation occurs in approximately 40% to 50% of melanomas. Although BRF1 inhibitors have been used to successfully treat melanomas containing the BRF600E mutation, patients often become resistant to BRF1 inhibitors within a few months. A number of studies have indicated that secondary mutations such as NRAS or NFI are significantly associated with BRF1 resistance by sequencing patient samples. However, due to the genetic heterogeneity commonly observed in tumors, it is unclear if those secondary mutations already existed within low percentage subclones or if they were acquired through drug treatment. It’s yet to be determined whether such mutations are only associated with resistance, or they actually cause the resistance. In this study, we used genome editing CRISPR technology to generate a drug resistant mutation NRAS Q61K within A375 melanoma cell line which naturally contains BRAFV600E. When compared to the parental line, this isogenic cell line demonstrated that genetically modified NRAS gene at the endogenous level directly leads to a significant resistance to BRAF inhibitors. Method and results: Single guide RNAs (sgRNAs) were designed and built to guide Cas9 to bind and cut desired regions in the NRAS gene target. Melanoma cell line A375 was co-transfected with the single guide and CRISPR all-in-one plasmid alongside donor plasmid. Transfected cells were sorted into single cells and expanded for subsequent screening of desired gene mutation events. The introduction of NRAS Q61K mutation in the cells was then confirmed via Sanger sequencing and NGS at the genetic level and transcriptional level. Drug responses to BRAF specific inhibitors and non-specific chemotherapy drugs were compared between A375 NRAS Q61K isogenic cell line and parental cell line in 2D and 3D culture environment. Testing results demonstrated that the isogenic cell line created by CRISPR showed significant resistance to BRAF inhibitor in comparison to the parental control in both 2D and 3D culture environment. In summary, we utilized the CRISPR/Cas9 genome editing platform to target endogenous loci in human cells and create the intended genetic mutation event. This new approach provides direct bio-functional evidence of acquiring a drug resistant gene drives tumor cells survival under targeted therapeutic treatment. Furthermore, unlike conventional drug resistant cell models that have been developed through drug selection, the A375 NRAS Q61K isogenic cell line represents a new type of drug resistant model that contains a defined genetic resistance mechanism. It reserves the permanent and genetically stable resistance characteristics without being maintained in drug selection culture environment. Therefore, it provides a valuable tool for developing next generation therapeutics that can overcome BRAF drug resistance in melanoma.

#3837 Primary tumor cell lines derived from gastric PDX tumors displays differences in chemosensitivity. Xiaomei Ge,1 Fulin Qiang,2 Yixin Zhang,2 Jibing Liu,2 Lei Yang,2 Yingying Qu,2 Fubo Xie,2 Xueting Li,2 Ying Gu,2 He Zhou1.1 Shanghai ChemPartner Co., Ltd., Shanghai, China; 2Nantong Cancer Hospital, Nantong, China.

Gastric cancer is a multifactorial and fatal disease with limited effective therapeutic options. Its overall mortality ranked third among cancer-related deaths worldwide. Patient-derived-xenograft (PDX) models have been widely used in the biomedical research. They recapitulate the genomic diversity/heterogeneity of patient tumors, maintain a lot of the characteristics of the original tumors and showed much better correlation with clinical outcome comparing to traditional cell line derived xenograft models. However, the relatively low throughput and time-consuming nature of PDX studies prevent them from being used in large scale screening or mechanistic studies. To facilitate drug development in gastric cancer, different cancer cell lines have been developed in our laboratory from gastric PDX tumors. We performed STR analyses to validate their identity and RNAseq data also showed high similarities to their parental PDX tumors. These primary gastric tumor cell lines displayed differences from each other in their chemo-sensitivities in vitro and in vivo. They also differ in their protein expression such as the HER-2 expression level. Pharmacology studies using different compounds showed similar drug responses of these cell lines in tissue culture, xenografts using these cell lines and the original PDX models. Our data demonstrated the high fidelity of these cell lines to their parental PDX tumors and serve as a bridge between the high-throughput drug screening and highly clinical relevant PDX models and will greatly facilitate the drug development in gastric cancer.

#3838 Drug sensitivity profile of 5TGM1 murine multiple myeloma cell lines emphasizes the translational potential of the syngeneic in vivo model. Jenni Mäki-Jouppila,1 Jenni Bemoulli,1 Mari I. Suominen,1 Tiina Kähkönen,1 Jenni M. Halleen,1 Sanna Timonen,2 Elina Huovari,1 Katja Suomi,2 Swapnil Potdar,2 Maria Nurmia,1 Päivi Oslting,1 Janni Saarela,2 Katja M. Fagerlund1.1 Institute for Molecular Medicine Finland FIMM, Helsinki, Finland; 2Institute of Molecular Medicine Finland, Helsinki, Finland.

Multiple myeloma (MM) is the second most common hematologic malignancy that originates from B-cells (plasma cells) and causes 2% of cancer-related deaths. Symptoms of MM include bone pain caused by multiple osteolytic lesions, pathologic fractures, and hypercalcemia. Typically, MM has a low growth fraction and it is highly dependent on the microenvironment. These properties have made it hard to target by conventional chemotherapy, but could now be exploited by novel stroma-targeting drugs and immunotherapy. These new approaches underline the need for well characterized models with functional immune system and appropriate tumor microenvironment. To gain additional information supporting the use of the syngenic 5TGM1 murine multiple myeloma model in drug development, we tested drug sensitivity of 5TGM1 cells by screening an extensive panel of drugs. The compound library consisting of 460 compounds included conventional chemotherapy, kinase inhibitors, metabolic modifiers, rapalogs, differentiating/epigenetic modifiers, kinesin inhibitors, apoptosis modulators, NAIADs, hormone therapy, immunomodulators and HSP.

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inhibitors. The compounds were tested in five concentrations covering a 10,000-fold drug-relevant concentration range in 384-well format. Cells were seeded to plates with a compound library, followed by cell viability measurements (Cell-Titer-Glo) after 72 hours. Maximal and minimal responses to drugs were analyzed, and the EC50 values were calculated. Drug Sensitivity Score (DSS) was calculated using a measure of relative sensitivity of a test tumor cell line to a reference cell line to determine changes in drug sensitivity. DSS analysis, STGM1 cells showed sensitivity to conventional chemotherapies, such as antimitic drugs, and kinase inhibitors, such as MEK1/2 inhibitors. In addition, the cells showed particular sensitivity to several HSP90 inhibitors currently in phase I/II clinical development for MM. Lenalidomide and pomalidomide, efficient in treating multiple myeloma in humans, both gave low DSS value indicating that STGM1 cells are not sensitive to these drugs, which is expected because they do not bind to murine form of the target cereblon. In contrast, STGM1 cells were highly sensitive to the proteasome inhibitor bortezomib (DSS 32.2), which is currently in clinical use. In conclusion, the murine STGM1 cells show sensitivity to various MM drugs used in the clinic and under development. Evaluating the effects of the microenvironment on the growth and drug sensitivity of STGM1 cells in vitro and in vivo will be essential. Furthermore, the cell-based compound screening combined with DSS analysis provides a possibility to profile cellular responses to an extensive collection of anti-cancer compounds enabling identification of vulnerabilities in cancer cells and functional investigation of cellular pathways behind drug sensitivity or resistance.

#3839 MLN8237 treatment in an orthoxenograft rodent model for malignant peripheral nerve sheath tumors. Oliver Mrowczynski, Penn State Hershey, PA

Malignant peripheral nerve sheath tumors (MPNSTs) are soft tissue sarcomas that arise from peripheral nerves and often occur in the extremities. MPNSTs arise sporadically or in the neurofibromatosis type one (NF1). NF1 is a autosomal-dominant disorder characterized by neurofibromas, cutaneous hyperpigmentation, and intellectual deficits affecting 1 in 3000 births. The lifetime risk of a person with NF1 developing a MPNST ranges between 8-13%. MPNSTs have increased expression of the oncogene Aurora kinase A. This leads to enhanced cell proliferation, making MPNSTs extremely aggressive, with high potential for metastasis, and devastating prognosis, with five year survival estimates ranging from a dismal 15-60%. MPNSTs are currently treated with surgical resection, sometimes requiring limb amputation, as well as chemotherapy, all of which demonstrate limited effectiveness, and highlight the necessity for novel therapies. To evaluate the effectiveness of new treatments, a translationally accurate, robust, and cost-effective model is crucial. A major goal of this study was to produce an orthotopic xenograft murine MPNST model for evaluation of novel therapies, which we created by intra-neural injection of luciferase transfected MPNST cells into the mice sciatic nerve. Our model circumvents drawbacks of previous in vivo models by producing a robust tumor in the physiologically relevant microenvironment of the nerve that allows for accurate tumor burden quantification through luciferin fluorescence. Another goal of this study was to demonstrate the effectiveness of MLN8237 for MPNST treatment. MLN8237 is an aurora kinase A inhibitor that we demonstrate to decrease MPNST burden and increase survival significantly. This study provides a robust and reproducible model, allowing for the advancement of MPNST therapy. This study also demonstrates the effectiveness of MLN8237 for MPNST treatment. Together, our data has major implications on the future of MPNST research by providing a robust murine model as well providing evidence that MLN8237 is an effective treatment for MPNSTs.

#3840 The National Cancer Institute’s patient-derived models repository (PDMR). Yvonne A. Evrard, 1 Michelle Aalil-Gotholin, 2 Sergio Akcose, 2 Carrie Bonomi, 1 Suzanne Borgel, 1 John Carter, 1 Biswajit Das, 1 Vivekananda Datta, 1 Cheryl Davis, 1 Kelly Dougherty, 1 Michelle Eugeni, 2 Marion Gibson, 2 Catherine Karangwa, 1 Jason Lih, 1 Dianne Newton, 1 Han Si, 1 Shivaani Kummar, 3 Larry Rubinstein, 1 Alice Chen, 1 P. Mickey Williams, 2 Melinda G. Hollingshead, 3 James H. Doroshow, 1 Frederick National Laboratory for Cancer Research, Frederick, MD; 2 National Cancer Institute, Frederick, MD; 3 Stanford School of Medicine, Stanford, CA; 4 National Cancer Institute, Bethesda, MD.

The National Cancer Institute (NCI) is developing a Patient-Derived Models Repository (PDMR) comprised of quality-controlled early-stage clinically-annotated patient-derived xenografts (PDXs) and in vitro patient-derived cell cultures (PDCs), including tumor cell and cancer-associated fibroblast cell cultures, to serve as a resource for public-private partnerships and for academic drug discovery efforts. These PDMRs will be clinically-annotated with molecular information (whole exome sequence, RNAseq) available in a publicly accessible database and will be available to the extramural community for research use. The PDMR was established by NCI at the Frederick National Laboratory for Cancer Research (FNLCR) in direct response to discussions with academia and industry: the oncology community’s highest priority need is better preclinical models that more faithfully reflect the patient’s tumor and are associated with the patient’s treatment history. NCI has focused on collecting specimens from patients with cancer that are under-represented in many other PDX collections such as PDAC, medulloblastoma, mesenchymal cancers, melanomas and sarcomas. In addition, NCI is increasing its focus on creating PDXs from minority/underserved populations and will soon be expanding to include pediatric cancers. The PDMR generates the majority of its PDXs by subcutaneous implantation; however certain histologies have better take-rates in either orthotopic or alternate implant sites. All SOPs and quality-control standards developed under the PDMR as well as those shared by collaborators will be posted to the public web site that houses the PDMR database. The overall goal of NCI is to create a long-term home for at least 1000 models such that sufficient biological and clinical diversity is represented to allow researchers to ask questions such as: what is the impact of tumor heterogeneity on target qualification or clinical response; do PDXs more faithfully represent the human tumor for pharmacodynamic assay and predictor marker development; or can an adequately powered preclinical PDX clinical trial lead to better evaluation of therapies for future clinical use? Grant Support: This project has been funded in part with federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

#3841 A hybrid mapping approach improves genomic and transcriptional analysis of patient derived orthotopic xenograft (PDXO) models of pediatric CNS tumors. Oliver A. Hampton, 1 Michael C. Gundry, 1 Mari Kogiso, 1 Lin Qi, 1 Yuchi Du, 2 Yulan Huang, 1 Frank K. Braun, 1 Huiyuan Zhang, 1 Sibo Zhang, 2 Holly Lindsay, 2 Sarah J. J. D. 2 David A. Wheeler, 1 Xiao-Nan Li, 2 D. William Parsons, 1 Baylor College of Medicine, Houston, TX; 2 Texas Children’s Hospital Cancer Center, Houston, TX.

Background. Patient derived orthotopic xenograft (PDXO) models are an important tool for cancer research, including personalized preclinical testing of targeted therapies. PDXO models of CNS tumors faithfully replicate the invasive nature of pediatric tumors, resulting in variable contamination of the harvested xenograft tissues by mouse brain cells. This presents technical challenges to genomic and transcriptomic analyses, including correct alignment of sequencing reads to human or mouse genomes, and can result in identification of false positive tumor mutations. Improved bioinformatic methods for systematic correction of such errors are needed. Methods. Whole exome sequencing (WES) was performed on 32 diverse childhood CNS tumors (mean of 109 M reads/sample) and matching PDXO models (mean of 62 M reads/sample), including atypical teratoid rhabdoid tumor (ATRT), glioblastoma (GBM), and medulloblastoma (MBM). Patient-matched blood DNA was also subjected to WES. Whole transcriptome sequencing (WTS) was performed on a subset of tumors and PDXO models (mean of 94 M reads/sample). Integrated WES and WTS analysis pipelines were constructed to unequivocally identify each sequencing read’s species-of-origin prior to genomic and transcriptomic variant calling, expression profiling, and fusion gene analysis. Xenograft NGS reads were competitively mapped to a hybrid reference created by merging the human (hg19) and mouse (mm10) genomes, with reads segregating to their respective genomes based on mapping score. Reads that mapped exclusively to the human reference were selected for analysis. Results. Mouse DNA contamination varied widely between PDXO tumors (1% to 94%; mean 40%). Hybrid mapping resulted in 2.4-fold higher combined sensitivity and specificity for identification of somatic variants as compared to direct human reference mapping followed by mouse polymorphism subtraction. WES analysis confirmed the maintenance of somatic driver mutations in PDXO models, including SMARCB1 in ATRT, TP53 and NF1 in GBM, and IDH1 in MBM. In contrast, in PDXO models with non-somatic mutations, exhibited significant allele fraction changes between the primary tumor and PDXO model. WTS analysis based on hybrid mapping resulted in an increase in positive predicted value (PPV) for correlation of FPKM values between tumor and PDXO model, with greater PPV in cases with high levels of mouse contamination, albeit with lower sensitivity due to decreasing coverage. Hybrid mapping based WES and WTS analysis identifies fewer false positives than direct bigWig mapping and represents a more streamlined process of species-of-origin sequencing read classification and filtering at the alignment stage. This method has the potential to improve molecular characterization of PDXO models and inform the design of rational preclinical studies.
Without confounding genetic responses, enogenous leukocytic infiltration into the tumor has been shown. STAT3 signaling in the tumor compartment in these autologously reconstituted human tumor xenografts (PDX) cancer models from patient tumors in the immunocompromised NOD.Cg-Prkdcscid IL2rgtm1Wjl/SzJ (aka, NSG™) mouse strain, spanning more than 30 tumor types. At low passages, these engineered models are known to retain similar molecular characteristics and heterogeneity to the original patient tumors. As such, PDX models offer an excellent preclinical platform to test drug responses of novel cancer therapeutics and a powerful resource for conducting preclinical cancer pharmacogenomic studies. To aid the selection of suitable PDX models for preclinical studies and for the research purpose to understand tumor biology and response or resistance to a given treatment, we have characterized the PDX models for their transcriptional, mutational and copy number profiles using sequencing and array approaches. We have established a compendium of PDX-tailored computational pipelines as the analysis of genomic data from PDX models could be challenging due to a) the contamination of PDX sample with mouse stroma, which complicates downstream bioinformatics analyses as mouse genome is almost 90% homologous to the human genome, and b) the lack of matched normal material to call somatic events. Our pipelines incorporate various filters to identify tumor specific single-nucleotide variants, indels, copy number changes and expression profiles in the PDX model. For the purpose of validating the accuracy of our analysis pipelines and demonstrating that the JAX PDX models are indeed representative of patient tumors, we compared JAX’s PDX cohort with patient cohorts in TGCA for mutations, copy number aberrations and RNA expression concordance. Using gene sets representative of each tumor type, we found that the overall genomic profile of each PDX tumor type is more correlated to the same tumor type in TGCA than other tumor types. In addition, an integrative analysis across all data types reveals that there are more common affected pathways between the same tumor type in PDX and TGCA. This comprehensive analysis revealed that the PDX and patient cohorts exhibit similar molecular characteristics, hence establishing the suitability of JAX PDX models as in vivo models to study fundamental tumor biology as well as to carry out preclinical studies of cancer drugs, including identification of biomarkers of response or resistance.

Autologous reconstitution of human cancer and immune system in vivo. Juan Fu,1 Rupashree Sen,1 David L. Masica,1 Rachel Karchin,1 Drew Pardoll,1 Young Kim2. 1Johns Hopkins University, Baltimore, MD; 2Vanderbilt School of Medicine, Nashville, TN.

Correlative studies from checkpoint inhibitor trials have indicated that better understanding of human leukocytic trafficking into the human tumor microenvironment can expedite the translation of future immune-oncologic agents. In order to directly characterize signaling pathways that can regulate human leukocytic trafficking into the tumor, we have developed a completely autologous xenotransplantation method to reconstitute the human tumor immune microenvironment. When we analyzed the human head and neck squamous cell carcinoma (HNSCC), we found that STAT3 signaling was associated with worse prognostic mesenchymal subtype. We silenced STAT3 signaling in the tumor compartment in these autologously reconstituted humanized mice, and we noted increased tumor infiltrating lymphocytes and slower tumor growth rate. We also used this novel agent that can alter endogenous leukocytic infiltration into the tumor, taken together, we present a valuable method to study individualized human tumor microenvironments in vivo without confound allogeneic responses.

Identification and validation of novel therapeutic targets driving clonal heterogeneity in treatment-refractory GBM. Chirayu Chokshi, Nick Yelle, Parvez Vora, Chitra Venugopal, Malehaa Qazi, Mohini Singh, Minomi Shigapuniga, Anurita Dive, Sarah Hart, Nick D. Singh, McMaster Stem Cell & Cancer Research Institute, Hamilton, Ontario, Canada.

Glioblastoma (GBM) is the most common primary adult brain tumor, characterized by extensive cellular and genetic heterogeneity. Even with surgery, standard chemotherapy with temozolomide (TMZ), and radiation, tumor re-growth (or recurrence) and patient relapse are inevitable. Patients face a median survival of <15 months, with uniformly fatal outcomes upon disease progression post-treatment. Recent profiling of GBM-initiating genes has shown that evolution of cancer-driving clones or cell populations within a solid tumor may progress through (and possibly be driven by) cancer treatment, such that GBM recurrence may no longer resemble the genetic landscape of the original primary tumor. Understanding and mapping clonal evolution of the primary GBM through therapy and at recurrence will allow for the discovery of targets specific to treatment-refractory GBM. Here, we have developed early passage patient-derived brain tumor initiating cell (BTIC) lines that have been annotated by genomic deep-sequencing technologies to systematically characterize and describe the extent of intratumoral heterogeneity. Tagged and fluorescent protein, these BTIC lines were engineered in immunocompromised NOD SCID mice. Following half-maximal tumor engraftment, tumor bearing mice underwent a clinically relevant chemoradiotherapy regimen, with 2 Gy gamma-irradiation on the first day and 66 mg/kg temozolomide for five consecutive days. Following therapy, mice were kept alive until tumor recurrence. Engrafted BTICs were harvested at initial tumor formation, minimal residual disease after chemoradiotherapy, and tumor recurrence. Samples were analyzed by RNA and genomic deep-sequencing technologies to map cancer progression and identify novel therapeutic targets in treatment-refractory GBM. Potential therapeutic targets were validated by their effect on self-renewal and proliferation of patient-derived BTIC lines of human GBM in vitro and in vivo. Using CISPR Cas9, potential targets were knocked out in patient-derived BTIC lines of human GBM in order to characterize the effect on sphere formation and proliferation in vitro, and tumor formation in vivo. Following validation of new therapeutic targets of treatment-refractor GBM, we aim to build novel biotherapeutics against highly validated cell surface targets, and establish preclinical testing protocols using our novel patient-derived and therapy-adapted xenograft model of treatment-resistant GBM.

Establishment and characterization of patient-derived xenografts of clear cell carcinoma of the ovary. Hidemichi Watari,1 Daïsuke Endo,2 Koutaro Konishi,3 Hiroki Hara,2 Mihoko Kuragaki2. 1Hokkaido University, Sapporo, Japan; 2Takeda Pharmaceutical Co. Ltd., Fujisawa, Japan.

Background: The patient-derived xenograft (PDX) model is likely to reflect original human cancer biology compared to cultured cell lines, since tumor tissues are directly implanted into animals. PDX models of ovarian cancer have been developed, but were not fully characterized at the molecular level, especially for clear cell carcinoma (CCC), which is more prevalent in Japan than western countries, exhibits chemoresistant phenotype and poor survival. The development of new treatment strategies and the better understanding of biology of CCC both depend on clinically relevant animal models. In particular, molecular annotated PDX models are useful for the preclinical investigation of anticancer drugs and individualized anticancer therapy. Methods: We transplanted 19 parent (primary or metastatic) tumors directly into NOG mice. The histologic characteristics were compared between parental tumors and PDX ones. Among CCC pairs, gene expression analysis, gene mutation analysis were conducted on parental tumors and corresponding PDX tumors. Response to chemotherapeutic agents was analyzed using PDX tumors of CCC, and correlated with clinical outcome. Results: Six of nineteen (31.6%) transplanted tumors could be passaged. Gene expression profiles revealed that engrafted tumors expressed more proliferation-related genes than those of non-engrafted ones. Among 6 PDX models established, two were found to be CCC. Two PDX tumors of CCC maintained the histologic characteristics of parental CCC tumors. Gene expression profile revealed similar results between primary CCC and PDX tumors of CCC, except that PDXs lost expression of immune-related genes. Gene mutation analysis showed similar pattern between primary CCC and PDX of CCC. Drug-sensitivity test showed resistance to carboplatin in two PDX tumors of CCC, which is consistent with their clinical outcomes. Conclusions. PDXs of CCC were established and found to retain histologic and clinical features of original tumors, and can be utilized to screen new target drugs for platinum-resistant CCC.

Imodi initiative: A novel holistic and integrative approach with patient-derived tumor models against pancreatic cancer. Juan Iovanna,1 Nelson Dussetti,2 Florence Meyer-Josic,2 Frédérique Le Vaconn,3 Loreday Calvet,4 Norico Fariz,4 Kevin Dhondt,5 Mariana Kuras,5 Christophe Lautrette,5 Sérénée Tabone-Eglinger,5 Philippe Vaglio,10 Olivier Duchamp5,11 INSEER, Marseille, France; 12IFEN Innovation, Les Ulis, France; 13BioForts-Merieux NutriSciences, Saint-Herblain, France; 14Sanofi, Limoges, France; 15Centre Léon Bérard, Lyon, France; 16Modul-Bio, Marseille, France; 17OncoDesign S.A., Dijon, France.

Patient-derived xenografts (PDX) are appearing as a prime approach for preclinical studies despite being insufficiently characterized as a model of the human disease and its diversity. Many reports support that xenografts from PDX in mice recapitulate well the molecular diversity, cellular heterogeneity, and histology seen in patient tumors. However, several lacks such as the limited clinical diversity of the PDXs, the absence of human drug metabolism and the reduced immune system are limiting the predictive values of these PDX models. To
set-up a holistic integration of these criticisms, we have associated efforts from public hospitals, academic groups, biotech and private pharmaceutical companies with the financial support of the French Ministry of Industry. First, to improve the clinical diversity of the PDX collections, surgical specimens or biopsies from patients with 9 different types of cancers (pancreas, lung, breast, ovary, liver, prostate, AML, myeloma, lymphoma) are collected since 2013 to establish large collections of PDXs in mice. In addition, primary cultures of cells from these samples are established leading to a collection of cell lines from the stroma and the tumoral compartment. Pancreatic PDX from primary tumors and its metastasis were obtained from either surgery or by endoscopic ultrasound-guided fine needle aspirate and therefore representing a bio-banking with a unique diversity. The established pancreatic models are being evaluated for histological, extensive molecular characteristics and in vitro and in vivo sensitivities to relevant anticancer drugs. We found i/ a variable sensitivity on PDX-derived cells when treated in vitro with increasing concentrations of clinically relevant drugs; ii/ that sensitivity to one drug does not predict the sensitivity to the another one; and iii/ sensitivity to drugs in vivo reproduce, but not systematically, the results in vitro. To be noted, no correlation between sensitivity and genetic characteristics (mutations or CNV) or histological proprieties was found. All model characteristics are being compiled in a web-based database for efficient features search and interconnection. Second, we are generating mice with humanized liver showing human drug pharmacokinetic and metabolism profiles We will present the first pancreatic cancer models characterized and will discuss their usefulness and chance to bring benefit to patients via this holistic strategy developed within IMODI initiative.

#3847 Oral cavity squamous cell carcinoma xenografts display conservation of primary tumor genomic heterogeneity. Katie M. Campbell,1 Tianxiang Lin,2 Ashley E. Winkler,3 Paul Zolkind,4 Zachary L. Skidmore,5 Erica K. Barnett,5 Ian Hagemann,6 Elaine R. Mardis,7 Malachi Griffith,8 Rebecca D. Cherboni,9 Ossama Tawfik,10 Joan Lewis-Wambi1.

Purpose: Patient derived xenograft (PDX) models represent a platform for defining therapeutic opportunities based on their ability to maintain the genomic and molecular features of their respective tumors. We used a comprehensive genomics approach to analyze PDXs for oral squamous cell carcinoma (OSCC), which had not been previously described. This involved confirming that the PDXs conserve the heterogeneous somatic landscape of the primary tumors from which they were derived. Methods: We established a large panel of OSCC PDX models by engrafting fresh, primary tumor samples into NOD scid gamma (NSG) mice. We performed whole genome sequencing (WGS; n = 10), whole exome sequencing (WES; n = 15), and transcriptome sequencing (RNAseq; n = 15) on matched primary and first passage xenografts. Somatic events, including mutations, copy number alterations (CNAs), loss-of-heterozygosity (LOH) regions, and structural variation (SV), were detected independently in tumors and PDXs. Conservation was defined by the correlation of variant allele frequency (VAF) distributions and gene expression patterns, presence of larger somatic events, and predictions made about the subclonal architecture of the xenograft. Resulta: PDXs were established with an overall success rate of 47% (56/118). The somatic landscape of primary tumors was representative of the published genomic data on OSCC, including amplifications of CCND1 and EGFR family members, inactivating mutations and loss of TP53 and CDKN2A, and chromosome 3q amplification. These somatic events were also strongly conserved in matched xenograft samples. Mutations that were detected in either the xenograft or primary tumor, but not its matched sample, consisted primarily of low-frequency mutations. The predicted clonal architecture, however, revealed correlated subclonal heterogeneity between matched xenograft and primary tumor. This includes maintenance of founding clone mutations and consistent VAF distributions, indicating that a heterogeneous tumor population engrafted. Conclusions: This comprehensive analysis establishes early-passage PDXs as high fidelity models for OSCC. Their ability to maintain the genomic heterogeneity detected in primary OSCC tumors presents a clinically relevant platform for understanding tumor biology and identifying novel therapeutic strategies.

#3848 The clinical relevance of targeting IFITM1 in three distinct subtypes of aggressive breast cancer. Asona Iliu,1 Joshua Ogony,1 Jordan Marques,1 Eric Grandi,1 William Jowett,2 Ihaba El Ayash,3 Gustav Miranda-Carboni,2 Ossama Tawk,2 Joan Lewis-Wambi1. 1Univ. of Kansas Medical Ctr., Kansas City, KS; 2University of Tennessee Health Science Center, Memphis, TN.

Breast cancer can be separated into estrogen receptor/ progesterone receptor positive (ER+/PR+), Her2/2neu positive (HER2+), and triple negative (ER-/PR-/HER2-) subtypes. Additionally, breast cancer may manifest clinically as either non-inflammatory or inflammatory disease. Inflammatory breast cancer is a highly lethal subtype of breast carcinoma that may express any of the three aforementioned receptors. Our laboratory has found that a novel marker, interferon-induced transmembrane protein 1 (IFITM1), is overexpressed in multiple subtypes of breast cancer and is predictive of poor prognosis and resistance to endocrine therapy, chemotherapy, and radiation. IFITM1 is a type I interferon (IFN) stimulated gene that is not expressed in normal breast tissue, and is only induced upon IFN exposure. In this study, we conducted immunohistochemical staining for IFITM1 in 94 ER+ human breast tumor samples and discovered that high IFITM1 expression was associated with increased clinical stage and resistance to endocrine therapy. We therefore explored the expression of IFITM1 in human triple negative breast cancer (TNBC) samples as compared to normal breast tissue. Notably, this overexpression was most pronounced in TNBC tumors from African American (AA) patients. We screened a panel of nine cell lines that represent all major subtypes of breast cancer and found IFITM1 overexpression in ER+ aromatase inhibitor-resistant MCF-7/5C, AA-derived triple negative MDA-MB-468 and MDA-MB-157, and triple negative inflammatory SUM149 cells. We used inducible shRNA and CRSPR/Cas9 respectively, to investigate the effect of IFITM1 knockdown on MCF-7/5C and SUM149 xenograft tumors using two in vivo breast cancer models. The orthotopic (mammary fat pad) model evaluated tumor proliferation, and the mammary intraductal (MIN) model assessed tumor cell invasion out of the milk duct. We found that loss of IFITM1 in MCF-7/5C and SUM149 cells significantly reduced tumor growth and invasion in vivo, blocked xenograft tumors from outgrowing, and reduced their ability to invade out of the milk duct. Additional studies indicated that hyper-activated JAK/STAT signaling was responsible for driving IFITM1 expression in MCF-7/5C, MDA-MB-468, and SUM149 cells. Furthermore, we demonstrated that JAK/STAT activation can be targeted in vivo with the FDA approved JAK inhibitor ruxolitinib (Jakafi®). Oral treatment of mice with 50µg/g bodyweight ruxolitinib reduced IFITM1 expression and inhibited the growth of both MCF-7/5C and MDA-MB-468 tumors by loss of STAT1/2 phosphorylation. The overexpression of IFITM1 in three distinct breast cancer subtypes indicates that IFITM1 may be a targetable marker of aggressive disease in general and may open the door for novel therapies of treatment-refractory breast cancer.

#3849 HuPBL humanized models as a tool to evaluate the efficacy and immune regulation of immune checkpoint inhibitors. Xu zun Tang, Xianzhi Zhai, Hui Qi, Qin Hong, Qiyou Zhang, Lei Jin, Shaoyen Yuan, Zhuozhi Wang, Yong Sheng, Qingyang Gu, Norman Zhang, Jing Li, Qunsheng Li. WuXi AppTec (Shanghai) Co., Ltd., Shanghai, China.

After the success of PD-1, PD-L1 and CTLA-4 antibodies, a number of immune-checkpoint inhibitors are already in advanced stages. Meanwhile, tumor infiltrating lymphocytes (TIL) are well recognized as features of tumor progression and loss of effectiveness of cancer immunotherapy. Therefore, there is a need to establish an effective pre-clinical platform for the evaluation of therapeutic outcomes in the human immune system and the understanding of TILs. Either traditional syngeneic models or human target knockin models have their drawbacks: syngeneic models disable the development of antibodies that do not recognize mouse receptors; neither of them can achieve a good clinical response. HuPBL humanized models can provide a solution to these issues. In this study, in vitro characterization and functional analysis of human TILs from TILs subsets and related immune-checkpoint expression. These models can
be used for the purpose of pre-clinical immune-checkpoint inhibitor and bi- specific antibody screening under human background, as well as for further investigations regarding distinct functions of TILs subsets and target expression.

### #3850 Establishment of patient derived model of high-risk endometrial cancer for pre-clinical combined carboplatin and decitabine therapy, Weimei Peng, Mei Yang, Zhen Liu, Ya Yu, Wei Jiang. Obstetric and Gynecology Hospital of Fudan University, Shanghai, China.

Objective: The high risk endometrial cancers (EC), including high grade EC, serous carcinoma (SC), clear cell carcinoma (CC) and carcinosarcoma, account for 50% of death. Therapies for them are limited and patient derived tumor xenograft (PDX) model become useful tools to predict response to treatment. Here we compared the two methods of establishment of PDX models and evaluated the efficacy of carboplatin and decitabine in two high grade EC PDX models. Methods: Fresh tumor tissues collected from 11 primary high risk EC patients (6 high grade ECs, 4 SCs, 1 CC and 1 carcinosarcoma) were engrafted subcutaneously and in subrenal capsule in SCID mice. For those subcutaneous models, tumors were then orthotopic transplanted into uterine cavity. Histology, vimentin, cytokeratin, ER, PR, PS3 expression were evaluated, together with mutation profiles by next generation sequencing for 500 cancer-panel genes. The efficacy of carboplatin and decitabine were evaluated in two high grade EC models. Results: The total tumor take rate was 81.8% (9/11), without regard to engraft models. The tumor take rate was higher in subrenal capsule models than subcutaneous models (70% vs 54.5%, respectively). The time for tumor formation (from transplantation to 1 cm in diameter) varies greatly from 4 weeks to 5 months. We observed good similarity between primary tumors and corresponding different passage of xenografts. One high grade EC model was sensitive to carboplatin and decitabine treatment whereas another was drug resistant. Two models harbor different genetic mutation profiles. Conclusion: The high tumor take rate ensures the establishment of the high risk EC biobank which provides a powerful resource for pre-clinical drug-sensitive tests as well as identification of biomarkers of response or resistance.

### #3851 Targeting HER2 in gastric cancer: Hints from a gastric PDX platform. Simona Corso,1 Cristina Migliore,1 Maria Apicella,1 Silvia Menegon,2 Eirini Pectasides,2 Tania Capeola,2 Stefania Durando,2 Marilisa Cargarutti,2 Paola Cassoni,1 Anna Sapino,1 Maurizio de Giuli,1 Adam Bass,2 Silvia Giordano1,7, Universita’ di Torino, Italy; 2Candiolo Cancer Institute-FPO, IRCCS, Italy; 3Beth Israel Deaconess Medical Center, boston, MA; 4Candiolo Cancer Institute-FPO, IRCCS, Candiolo, Italy; 5Dana Farber cancer Institute, MA.

Introduction: Gastric cancer is the 3rd leading cause of cancer mortality worldwide. Surgery is the only curative treatment strategy and conventional chemotherapy has shown limited efficacy. Trastuzumab (a HER2 mAb), is the only therapy targeting molecular alterations approved so far in gastric cancer, for HER2+ patients with advanced disease. However only a fraction (<20%) of HER2 amplified patients benefits from treatment. Methods: We have recently generated a molecularly annotated colony of gastro-esophageal PDXs (at the moment, > 70 PDXs). The platform also comprises primary cell lines and 3D- cultured organoids derived from gastric cancer PDXs. Results and Discussion: At present, we have identified several HER2+ PDXs and generated the corresp- onding in vitro derivatives. Four HER2+ PDXs, showing 3+ HercepTest score and HER2 amplification > 8 copies (thus theoretically sensitive to Trastuzumab), were selected to undergo ‘xenotrials’ with the following anti-HER2 drugs/combos: Trastuzumab (T); Pertuzumab (P, anti-HER2 mAb); Lapatinib (L, a dual HER2-EGFR tyrosine kinase inhibitor). According to RECIST-like criteria, T induced tumor regression in only 1 out of 4 HER2+ PDXs. Interest- ingly, in all the tumors but one the combos T+P and T+L, were able to bypass resistance to T monotherapy and to induce tumor regression. One tumor was resistant to all the tested therapeutic approaches. Conclusion: We identified PDXs displaying HER2 gene amplification. Only a minor fraction of them ben- efited from the anti-HER2 mono-therapy, despite the presence of a strong HER2 gene amplification, while the association of two HER2 inhibiting drugs resulted in intense and prolonged response. This suggests that monotherapy with T might not be the most effective therapeutic approach in gastric cancer patients. We are currently investigating the cause of resistance to anti-HER2 drugs to identify mutations/CNV in tumors showing resistance to Trastuzumab.

### #3852 Validation of synthetic lethality of PARP and NAMPT dual inhibi- tion in a small cell lung cancer PDX model. Zhixiang Zhang, Dongfang Li, Chen Chen, Bo Zhang, Xuzhen Tang, Hao Ye, Qingyang Gu, Qunsheng Ji. West China Hospital of Sichuan University, Chengdu, China.

Small cell lung cancer (SCLC) is a highly malignant cancer type with a 5-year survival rate of less than 10%. Different from non-small cell lung cancer (NSCLC), no effective target therapies have been approved to treat SCLC. Poly (ADP) ribose polymerase (PARP) overexpression in SCLC has prompted great efforts to evaluate the role of PARP inhibitors in clinic. To maximize their therapeutic value it is urgent in need to explore the best combination strategies between PARP inhibitors and other pathway modulators. In this study we identified a SCLC patient-derived xenograft (PDX) model with high PARP level and low nicotinamide phosphoribosyl- trasferase (NAMPT) level. Furthermore, synergistic effect of PARP and NAMPT dual inhibition was demonstrated, supporting translational research of PARP inhibi- tors and NAMPT inhibitors in this model. The mRNA expression of PARP as well as its 30 most-studied synthetic lethality genes was compared between SCLC and NSCLC in both PDXs and cancer cell lines. We found that NAMPT levels decreased most significantly in SCLC compared with NSCLC across the 30 synthetic lethality genes of PARP. As NAMPT is the rate-limiting enzyme for the synthesis of the PARP substrate β-NAD+, we hypothesized that low β-NAD+ level due to low NAMPT level might render SCLC sensitive to PARP inhibition. LU-01-0547, a SCLC PDX model with high PARP and low NAMPT expression, was identified here. To test our hypothesis, we investigated the preclinical efficacy of ABT888, a PARP inhibitor, with or without FK866, a NAMPT inhibitor, in this subcutaneous xenograft model. The single-agent efficacy of ABT888 was demonstrated (TGI=96%±100 mg/kg, BID), at a sub-optimal dose (50 mg/kg, BID), treatment of ABT888 alone produced little activity in the same model, while its combination with a NAMPT inhibitor, FK866, significantly boosted the antitumor response indicating the synergistic effect (TGI=117%, ORR=6/6, CR=4/6). When nicotinamide (NA) was supplied to promote β-NAD+ biosynthesis in other way other than the NAMPT-mediated transformation, the PARP inhibitor’s effectiveness was greatly antagonized (TGI=70% vs. 13%), suggesting β-NAD+ level might correlate with PARP inhibitor sensitivity. In conclusion, this study identified a SCLC PDX model with high PARP and low NAMPT expression. Synergistic inhibition of PARP and NAMPT in this model was demonstrated superior to either treatment alone in efficacy, which warranted its future application in drug discovery. To our knowl- edge, this was the first in vivo evidence of synthetic lethality in SCLC PDX, support- ing future clinical test of combination of PARP and NAMPT inhibitors in SCLC patients.

### #3853 Patient-derived xenograft models from peritonal metastasis of colorectal carcinoma as novel platform for biomarker analysis and drug test- ing. Wolfgang Walther,1 Eva Pachmayr,1 Bernadette Brzeziczka,2 Britt Bütt- ner,2 Beate Rau,1 Ulrike S. Stein,1 Charité Universitätsmedizin Berlin and Max Delbruck Ctr. for Molecular Medicine, Berlin, Germany; 2epp GmbH, Berlin, Ger- many; 1Charité Universitätsmedizin Berlin, Berlin, Germany.

Colorectal carcinoma (CRC) is associated with high incidence and mortality rate, particularly if metastasized to distant sites, such as the peritoneum. At time of first diagnosis 4 to 7 percent of the patients suffer from peritonal metastasis (PM) of CRC. The PM is associated with poor prognosis and limited therapeutic options. Therefore, availability of adequate in vivo models for PM will promote the search for novel prognostic or even predictive biomarkers as well as the evaluation of chemothera- peutic combinations targeted towards standard, targeted and novel drugs. In this regard such models could be employed for more individualized concepts to improve the therapeu- tic outcome of PM patients. During the last decade patient-derived xenograft (PDX) mouse models have gained importance, since they closely resemble the mo- lecular and biological features of the original primary tumors. However, until now no PM PDX models have been established from CRC. We therefore focused on the establishment and characterization of a novel CRC PM panel of PDX as useful platform for preclinal studies. For the PDX establishment colorectal surgical spec- imens were subcutaneously (s.c.) transplanted onto immunocompromized NOD scid gamma (NSG) mice. The successfully engrafted tumors were transferred to NMRI nu/nu mice for further passaging. Engrafted tumors were characterized by histopathology, immunohistochemistry and gene expression analyses using real- time RT-PCR. Chemosensitivity of PDX models was evaluated in vivo by applica- tion of a panel of conventional chemotherapeutic and of targeted drugs. For PDX establishment 68 CRC surgical specimens were transplanted onto NSG mice. From those, currently 22 PDX have engrafted and are stably growing on NMRI nu/nu mice. From those, 13 models of 10 patients have been analyzed. Their tumor dou- bling times ranged between 4 to 28 days. The histopathological evaluation revealed maintenance of the original CRC histology in the PDX. The chemosensitivity testing of conventional and of targeted drugs in the 13 PM PDX models revealed the indi- vidual, diverging response of the PDX, such as for 5-FU, irinotecan, oxaplatin, cetuximab and erlotinib. For selected PDX, orthotopic tumor transplantation into the peritoneum revealed their potential to form disseminated tumors in kidney, ovaries and abdominal diaphragm. Our results demonstrate, that this novel panel of PDX maintains the morphology of the patient tumor in early passages, reflect heterogene- neous response rates, and can be used as preclinical in vivo platform for translational
identification of circulating tumour cells in breast cancer patient-derived xenograft models. Chiara Agnoletto,1 Linda Minotti,2 Marco Galasso,3 Fabio Corrà,1 Federica Baldassari,1 Stefano Cairo,3 Jean-Gabriel Judde,4 Aurelie Sauvage,4 Olivier Deas,2 Lorenzo Pasquali,1 Stefano Vollinia,1 University of Ferrara, Ferrara, Italy; XenTech, Evry, France; Karolinska University Hospital, Stockholm, Sweden.

Metastases from primary tumours accounts for the great majority of cancer-related deaths. This process is thought to involve a series of sequential steps, with the release of circulating tumour cells (CTCs) into the bloodstream. CTCs have been detected in the peripheral blood of patients with advanced cancers of different origin and in some localized cancers. Recently, liquid biopsy focusing on the analysis of CTCs has received enormous attention because of its obvious clinical implications for personalized medicine. Clinical applications of CTCs detection include early detection of cancer, prediction of the risk for metastatic relapse or progression, monitoring the effects of systemic therapies, and stratification of patients based on the detection of therapeutic targets on resistance mechanisms. To date, only few data are available about the numbers of these cells, their molecular and biological heterogeneity, and their significance. CTCs comprise a minute fraction of mononucleated cells in the circulation, posing a serious challenge for any analytical system. Multiple technologies have been developed for CTC capture, but there is still a critical need for increased sensitivity in rare tumour cell isolation within blood samples. Most of the current CTC detection techniques rely on immunobased capture of cells, and epithelial markers have been frequently used to distinguish cancer cells from normal blood cells. However, the phenotypic changes that occur in CTCs, due predominantly to the epithelial-mesenchymal transition process, could be responsible for the failure to detect them in blood samples. We describe a novel approach for the identification of molecular markers to detect CTCs in peripheral blood of patient-derived xenograft (PDX) models. We analyzed the transcriptome in more than 10,000 samples of primary and metastatic tumours deposited in public databases, and identified a small set of cancerspecific genes coding for membrane protein. Putative markers were assayed by reverse transcription PCR with species-specific primers in blood samples from breast cancer PDXs, testing their ability to detect human CTCs. Thus, we identified human membrane proteins, which can be used for CTC monitoring in peripheral blood. The use of multiple targets for CTC capture and identification increases the sensitivity of CTC detection, allowing the isolation of all heterogeneous CTCs subpopulations.

Identification of circulating tumour cells in breast cancer patient-derived xenograft models. Chiara Agnoletto,1 Linda Minotti,2 Marco Galasso,3 Fabio Corrà,1 Federica Baldassari,1 Stefano Cairo,3 Jean-Gabriel Judde,4 Aurelie Sauvage,4 Olivier Deas,2 Lorenzo Pasquali,1 Stefano Vollinia,1 University of Ferrara, Ferrara, Italy; XenTech, Evry, France; Karolinska University Hospital, Stockholm, Sweden.

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were treated with different chemotherapies used in early stages and metastatic settings: anthracyclines combined to cyclophosphamide (AC), taxanes, platins, cephalosporins, and gemcitabine. Drug responses in PDX were compared to responses in patients who recurred after surgery and were treated in the metastatic setting. Results: overall tumor take of residual tumors was 40% and 75% for TNBC, with 15/20 PDX established, more than twice the tumor take of treatment naive TNBC (34%). Median latency time, defined as time from implantation till first tumour growth, was only 60 days and was further reduced to 3-4 weeks during successive tumor passages. On the 8 TNBC PDX evaluated for chemosensitivity, 7 exhibited a multidrug-resistance phenotype with resistance or limited response to AC, taxanes and platins. Capecitabine, a chemotherapy given in the advanced stage as second or third line, was efficient in 5 out of 8 PDX tested with 3 models showing stable disease and 2 models durable tumor regressions. Interestingly, capecitabine efficacy was decreased when xenografts were pre-treated with a first line containing platins, suggesting that in some tumors capecitabine might have superior activity when given in the adjuvant setting or as a first line. In one third of TNBC patients, time to recurrence, comprised between 7 and 12 months after surgery, was compatible with xenograft establishment and drug testing. Conclusions and perspectives: we established a unique panel of PDX models from patients with residual disease after neoadjuvant chemotherapy. These aggressive PDX recapitulate the resistance phenotype of patients’ tumors to treatments given in neo- and metastatic settings. We identified capecitabine as efficient first line chemotherapy for residual chemoresistant PDX. In 30% of cases, PDX models could have been used to evaluate chemotherapy responses in tumor recurrence occur in patients. In order to identify predictive biomarkers of capecitabine response, additional experiments are ongoing in 25 supplementary TNBC PDX, established from treatment-naive patients.
Development of testicular cancer patient derived xenograft models to test combination therapies targeting PI3K/Akt and MDM2. Gerda de Vries,1 Ximena Rosas-Plaza,1 Marcel A. van Vught,1 Albert J.H. Swurmeijer,2 Jorik A. Gietema,1 Steven de Jong1,1 Department of Medical Oncology, Cancer Research Centre Groningen, University Medical Centre Groningen, Groningen, Netherlands; 2Department of Pathology, University Medical Centre Groningen, Groningen, Netherlands.

Metastatic testicular cancer (TC) is highly sensitive to cisplatin-based chemotherapy. However, patients with advanced disease in the poor prognosis group only have a 50% 5-year survival resulting from chemoresistance. Previous data showed that both the PI3K/Akt pathway and the MDM2/p53 axis are involved in cisplatin resistance of TC cells. In this study, we sought to establish and characterize TC patient-derived xenografts (PDX) in order to investigate PI3K/Akt and/or MDM2 inhibition as possible treatment options for TC. Excised and minced (8mm3) primary TC tissue was implanted subcutaneously into male NSG mice to obtain a first generation PDX model (F1). Animals were sacrificed when tumor volume exceeded 1500mm3. The tumor was harvested and minced. Small pieces (8mm3) were implanted in a second generation (F2), as well as in FCS-5% DMSO to establish a biobank. In a panel of TC cell lines, combination treatment of Akt inhibition with or without cisplatin or Nutlin-3a was performed. Cleaved caspase-3 staining was assessed to measure apoptosis, and western blot was used to determine the effect of different treatments on subcellular localization and phosphorylation status of MDM2. We have established 3 TC PDX models. In silico analysis of the primary tumors included subtypes: mixed germ cell tumors with embryonal- and yolk sac carcinoma and teratoma components. Engraftment efficacy was 100% and tumor growth initiated within 4-5 weeks after implantation. Interestingly, we successfully implanted a biopsy taken from a metastatic lesion of a patient presenting with progressive refractory disease after receiving standard chemotherapy. Histology of the different PDX generations was comparable to the patient material, although a loss of the teratoma component was observed. In our cell line panel, Akt inhibition using MK2206 sensitized the acquired cisplatin resistant cell line TeraCP towards cisplatin treatment, whereas an additive effect was observed in other cell lines. Combination of Akt and MDM2 inhibition was highly synergistic in apoptosis induction in all cell lines. No relation was observed between the synergistic effect of Akt combined with MDM2 inhibition and sub-cellular localization or phosphorylation levels of MDM2. Taken together, we have successfully established 3 TC PDX models, including a chemo-resistant model. The biobank is currently being expanded. Combined Akt and MDM2 inhibition resulted in sigmoidic induction of apoptosis, and these combinations or other novel therapies will be tested in established TC PDX models. Supported by Dutch Cancer Society grant RUG 2014-6901 and CONACyT grant 381543.

In vivo chemopreventive of pseudosemiglabrin via inhibition of tumor angiogenesis and manipulation of hormones in estrogen-responsive breast cancer. Loey E. Ahmed Hassan. Faculty of Science and Technology, Omdurman, Sudan.

Breast cancer is the most frequently diagnosed form of malignancy and the second leading cause of death in Western women. Mortality and most of the complications associated with breast cancer are due to metastasis developing in regional lymph nodes and in distant organs, including bone, lung, liver and brain. Chemopreventive is administration of chemical agents to prevent the initiation and promotional events associated with carcinogenesis. Some chemopreventive agents can prevent reaction or attacking molecules by different ways, these agents can suppress growth factors or signaling pathway. On this study we examined (-)-Pseudosemiglabrin (SSG) a natural compound extracted from Tephrosia apollinea on breast cancer cells and tumor vascularization in vitro and in vivo. Hormone sensitive breast cancer cell MCF-7 was found subtile susceptible to SSG, it showed profound anti-tumorgenesis via inhibiting breast cancer cell migration, invasion and colony formation in vitro settings. In silico investigation of potential PARP inhibitor, SSG showed high binding affinity comparable to Rucaparib (standard PARP inhibitor). The natural compound significantly inhibited the sprouting of new blood vessels in ex vivo rat aorta ring assay. Antiangiogenic efficacy is perturbed by targeting VEGF expression and VEGFR phosphorylation. SSG completely inhibited the onset of breast cancer in animals pretreated with the compound. Molecular docking study showed that SSG binds to active site of Aromataze (the enzyme responsible for a key step in the biosynthesis of estrogens) with binding affinity better than standard aromatase inhibitor (Arimidex), moreover the compound has inhibitory activity against estrogen receptor in the breast cancer cells comparable to Tamoxifen. Our findings reveal that (-)-Pseudosemiglabrin can potentially be beneficial in preventing breast cancer in high-risk women or it can be adjuvant therapy with standard chemotherapy or even can be used for the treatment of women who have been diagnosed with breast cancer.

A subset of poorly prognostic pediatric posterior fossa ependymomas exhibit lowered H3K27me3 and DNA hypomethylation and show epigenetic similarities with H3K27M mutant diffuse intrinsic pontine gliomas. Sriman Venkett,1 Jill Bayliss,2 Piai Mukhuerjee,2 Chao Lu,2 Siddhant Jain,1 Chan Chung,1 Daniel Martinez,2 Benjamin Saburi,2 Ashley Margin,2 Pooja Panwalkar,3 Abhijit Parolaio,2 Melike Pekmezci,1 Richard Mc Eachin,1 Marcin Cieslik,2 Benita Tamraz,2 Benjamina Garcia,2 Gaspare La Rocca,3 Mariarita Santi,2 Peter Lewis,1 Cynthia Hawkins,1 Ari Melnick,2 C David Allis,4 Craig B. Thompson,5 Arul Chinnaiyan,1 Alexander R. Tudkins,6 University of Michigan Medical School, Ann Arbor, MI;5 Epigenomics Core Facility, Weill Medical College of Cornell University, New York, NY;7 The Rockefeller University, New York, NY;8 University of Wisconsin, Madison, WI;2 Children’s Hospital of Philadelphia, University of Pennsylvania, Philadelphia, PA;4 Children’s Hospital Los Angeles, Keck School of Medicine University of Southern California, Los Angeles, CA;6 University of California, San Francisco, San Francisco, CA;9 Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA;6 Memorial Sloan Kettering Cancer Center, New York, NY;2 Department of Laboratory Medicine and Pathobiology, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada.

Pediatric posterior fossa ependymomas are poorly understood. Childhood brain tumors and have no effective treatments. The biology of these tumors is obscure as recent sequencing efforts suggest that they lack recurrent genetic alterations. A subset of these tumors termed PF-A ependymomas exhibits CpG island hypermethylation implicating epigenetic alterations in their pathogenesis. Through comprehensive analyses of histone modification, we discovered global H3K27me3 reduction in a subset of these tumors. Tumors with lowered H3K27me3 showed many clinical and biologic similarities with PFA-ependymomas. Global reduction in H3K27me3 is likewise observed in pediatric gliomas that bear histone H3K27M mutations termed diffuse intrinsic pontine gliomas (DIPG) that also arise in the posterior fossa of young children. Analyses of ependymomas with reduced H3K27me3 and H3K27M mutant DIPGs showed many similarities in DNA methylation and enrichment of H3K27me3 in many genomic loci important for neuronal specification. Combined integrative analysis of both tumor types uncovered common epigenetic deregulation of select factors that control radial glial biology and radial glia in the developing posterior fossa showed lowered H3K27me3. Finally, PF ependymomas with lowered H3K27me3 were more invasive radiologically and exhibited poor prognosis in three independent cohorts (P<0.001 in all three cohorts, collective n>300). These data have clinical implications for biomarker development and to inform epigenetic approaches to treat PF ependymomas.

Combined targeting of the EWS/ETS transcriptional program by blocking epigenetic readers and transcription initiation in Ewing sarcoma. Ziaurrahman Chowdhury,1 Rockford Owoibi,1 Federica Crippa,2 Brian Seftor,1 Malak Shehadeh,1 Geoffrey Field,1 Becker-Dettli Fox,3 Frauke Neff,2 Oxana Schmidt,2 Shudong Wang,2 Beat W. Schafer,2 Stefan Burdach,1 Gunther HS Richter1,1 Laboratory for Functional Genomics and Transplantation Biology, Children’s Cancer Research Centre and Department of Pediatrics, Munich, Germany;2 Department of Oncology and Children’s Research Center, University Children’s Hospital, Zurich, Switzerland;3 Helmholz Zentrum München, - German Research Centre for Environmental Health (GmbH), Neuherberg, Germany;9 School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, Australia.

Introduction: Previously, we reported blocking of BET bromodomain binding proteins (BRDs) by use of an inhibitor (JQ1) and the associated strong down-regulation of the predominant EWS-ETS protein EWS-FLI1 in Ewing sarcoma (ES). Here we analyzed in depth the mechanistic effects of this treatment by EWS-FLI1 interaction studies and the evaluation of possible combined targeting. Experimental procedures: Function of BRDs was analysed by application of specific inhibitors (JQ1, I-BET151), RNA interference (RNAi) with the generation of stable and inducible knockdowns or knockouts by the generation of BRD4 CRISPR/Cas9 cell lines. To analyse the resulting changes Co-IP, ChIP-qPCR, RT-PCR, western blotting, cycle analysis, proliferation and invasion assays, whole transcriptome analysis via microarrays as well as xenograft mouse models were utilized. Results: By use of JQ1 or I-BET we strikingly observed a strong down-regulation of the predominant EWS-ETS protein EWS-FLI1 and subsequent microarray analysis revealed JQ1 treatment to block the typical ES associated expression program. The effect on this expression program was paricularly striking for JQ1 but not I-BET. In addition we knocked out studies of BRD4 by CRISPR/Cas9 as well as knockdowns of individual BRD2, 3 or 4 did not recapitulate JQ1-mediated proliferation restrictions and blockade and tumor development in xenograft mice as observed for JQ1. However, co-immunoprecipitation experiments revealed an DNA independent in-
teration of BRD4 with EWS/FLI1 and further interaction with CDK9. Treatment of ES cells with a specific CDK9 inhibitor demonstrated a rapid downregulation of EWS-FLI1 expression and block of contact dependent growth. Furthermore, CDK9 inhibition induced apoptosis in ES as depicted by downregulation of XIAP and CFLAR and consequently cleavage of Caspase 8, PARP and increased CASP3 activity, similar to JQ1. Combined treatment of BRD4 and CDK9 inhibitors was more effective than individual drug application. Conclusion: Translocation driven tumors such as ES are very susceptible to combined treatment with epigenetic inhibitors. Here we demonstrate that treatment with inhibitors targeting the p-TFEB complex could interrupt communication between EWS-FLI1, BRD4 and CDK9 further impeding EWS-ETS transcriptional activity and its associated pathognomonic expression program.

#3865 Targeting the epigenetic modifier HMGA2 in lethal adult and pediatric gliomas inhibits invasion, growth and tumorigenicity. Harpreet Kaur,1 Sabeen Z. Ali,2 Huizi Guo,2 Sepehr Akhtarkhavari,2 Fausto Rodriguez,2 Charles G. Eberhart,3 Eric H. Raabe3. 1Department of Oncology, Johns Hopkins Univ. School of Medicine, Baltimore, MD; 2Department of Pathology, Johns Hopkins Univ. School of Medicine, Baltimore, MD.

Glioblastoma (GBM) and diffuse intrinsic pontine glioma (DIPG) are highly infiltrative, incurable adult and pediatric brain tumors characterized by the presence of tumor stem-like cells. Although GBM and DIPG have different epigenetic drivers, we found increased expression of an epigenetic modifier and stem cell factor HMGA2 in both tumors by immunohistochemistry and western blotting. HMGA2 is a DNA-binding protein that regulates transcription during normal embryogenesis and in cancer stem cells. We hypothesized that HMGA2 contributes to high grade glioma tumorigenicity through its ability to alter the transcription of many genes. Lentiviral short hairpin RNA (shRNA)-mediated reduction of HMGA2 in multiple patient-derived GBM cell lines significantly reduced invasion in transwell assay (shHMGA2 vs. shControl, P<0.01). Similarly, shRNA mediated suppression of HMGA2 in DIPG cell lines reduced proliferation (BrDU incorporation), reduced invasion (transwell assay), inhibited clonogenicity (soft agar assay) and increased apoptosis of DIPG neurospheres (P<0.03). Treatment of shHMGA2 vs. shControl P<0.01). Microarray analysis revealed that treatment with shHMGA2 resulted in downregulated transcription of many genes involved in tumor stemness. Furthermore, CDK9 inhibition induced apoptosis in ES as depicted by downregulated XIAP and CFLAR and cleavage of Caspases 8, 9, PARP and increased cleaved caspase 3 (CASP3) activity, similar to JQ1. Combined treatment of ES with BRD and CDK9 inhibitors was more effective than individual drug application. Conclusion: Translocation driven tumors such as ES are very susceptible to combined treatment with epigenetic inhibitors. Here we demonstrate that treatment with inhibitors targeting the p-TFEB complex could interrupt communication between EWS-FLI1, BRD4 and CDK9 further impeding EWS-ETS transcriptional activity and its associated pathognomonic expression program.

#3867 Epigenetic siRNA and chemical screens identify SETD8 inhibition as a therapeutic strategy to reactivate p53 in high-risk neuroblastoma. Veronica Veschi,4 Zhiihue Liu,4 Ty C. Voss,4 Laurent Ozben,4 Berkley Gryder,4 Chunhua Yan,4 Ying Hu,4 Anqi Ma,5 Jian Jin,5 Sharyl J. Mazur,5 Norris Lam,5 Barbara K. Souza,5 Giuseppe Giannini,5 Gordon L. Hager,5 Cheryl H. Arrowmith,6 Javed Khan,2 Ettore Appella1, Carol Thiele1. 1National Institutes of Health, Bethesda, MD; 2Icahn School of Medicine at Mount Sinai, New York, NY; 3University la Sapienza, Rome, Italy; 4University of Toronto, Toronto, Ontario, Canada.

Neuroblastoma (NB) is considered a failure of sympathoadrenal differentiation. High-risk neuroblastoma (HR-NB) is an aggressive pediatric tumor accounting for 15% of all pediatric oncology deaths. Less than 50% of HR-NB patients have long-term survival, despite intense multimodality treatment. Given the paucity of drugable mutations and findings that epigenetic drivers contribute to NB tumorigenesis, we undertook a chromatin-focused siRNA screen to uncover epigenetic regulators critical for survival of high-risk NBs. Of the 400 genes analyzed, high-content Opera imaging identified 53 genes whose loss of expression led to significant decreases in NB cell number with 16 also inducing differentiation. A screen with 21 epigenetic compounds in 8 NB cell lines and 2 non-transformed cell lines prioritized those siRNA hits with active tool compounds in the drug development pipeline. This revealed UNC0379 (targets SETD8) inhibited NB cell growth and identified SETD8 as an important and drugable NB target. SETD8 is the H4K20me1 methyltransferase which regulates DNA replication, chromsome condensation and gene expression. Analysis of primary NB revealed that high expression of SETD8 is associated with poor prognosis in NB (R2 platform ex. Kocak: p = 1.4e-07). Levels of SETD8 were not significantly different between Stage 4 MYCN-amp compared to MYCN-WT tumors but high SETD8 levels were only associated with poor prognosis in the Stage 4 MYCN-WT (p=0.03). To understand SETD8-mechanism of action, we performed RNA-seq transcriptome analyses after genetic or pharmacological inhibition of SETD8. Ingenuity Pathway Analysis revealed that SETD8 ablation rescued p53 pro-apoptotic and cell-cycle arrest functions by activating the canonical p53 pathway. Functional studies showed SETD8 methylates p53 (K382) leading to its inactivation. Levels of p53K382m1 are higher in MYCN-WT NB cell lines compared to those with MYCN-amp. Less than 2% of NB samples have p53 mutations but multiple mechanisms have been identified in MYCN-amp NB that functionally inactivate p53. This study identified that SETD8 inactivates p53 in NB and may be an important mechanism to inactivate p53 in MYCN-WT HR-NB. This subgroup represents 60-70% of HR-NB tumors. SETD8 inhibition led to increases in caspase-dependent cell death only in p53-WT but not -mutant or -null NB cells. Gene expression experiments confirmed that SETD8-induced cell death is p53 dependent and p53K382 is important for this activity. Our in vivo xenograft NB models, showed that genetic or pharmacologic (UNC0379) inhibition of SETD8 confers a significant survival advantage. This work identifies that SETD8 is a novel therapeutic target and its inhibition may be especially relevant for the subset of high-risk NB tumors with wildtype MYCN. This is the first in vivo preclinical study showing that targeting SETD8 inhibits tumor growth.

#3868 The stem cell factor LIN28B regulates proliferation and apoptosis in diffuse intrinsic pontine glioma. Huizi Guo,1 Sepehr Akhtarkhavari,1 Charles G. Eberhart,1 Harpreet Kaur,1 Eric H. Raabe1. 1Department of Oncology, Johns Hopkins University, Baltimore, MD; 2Department of Pathology, Johns Hopkins University, Baltimore, MD.

Diffuse Intrinsic Pontine Glioma (DIPG) is an incurable, invasive and aggressive pediatric brain tumor. Identifying molecular markers that regulate tumor growth and invasion are needed for developing efficient treatment strategies. LIN28B is a stem cell factor expressed during normal fetal development and re-expressed in cancer cells. We had previously shown that LIN28A, another...
family member of LIN28 proteins, regulates invasion and tumorigenicity in adult high grade gliomas. We observed increased LIN28B expression in patient-derived DIPG neurosphere cell lines using western blotting. We hypothesized that LIN28B promotes proliferation and prevents apoptosis in DIPG. Using two different lentiviral transduced short hairpin RNAs (shRNA), we suppressed LIN28B protein levels in DIPG neuropheres, as confirmed by western blotting. DIPG neuropheres that have been treated with LIN28B shRNA showed reduced proliferation as measured by BrdU incorporation (P<0.01) and increased apoptosis as measured by cleaved caspase-3 (CC-3) expression (P<0.01). To determine the molecular mechanism of LIN28B-mediated phenotypes in DIPG, we studied the canonical downstream effector of LIN28B called HMG2A, which is a DNA-binding protein that functions as a transcriptional regulator. Using western blotting, we found decreased HMG2A protein levels in DIPG neuropheres infected with LIN28B shRNA. Taken together, our results suggest that LIN28B is important for promoting DIPG cell proliferation and preventing apoptotic cell death. Additionally, we also found that LIN28B regulates HMG2A expression in DIPG neuropheres. Future studies will focus on expanding our understanding of the molecular mechanisms of LIN28B-regulated malignancy in DIPG.

Modeling the chromatin and transcriptional landscape of MYC and MYCN driven neuroblastoma in zebrafish. Mark W. Zimmerman,1 Shuning He,2 Shizhen Zhu,3 Song Yang,4 Yi Zhou,4 Leonard I. Zon,4 A Thomas Look,5 Dana-Farber Cancer Institute, Boston, MA; 4Mayo Clinic, Rochester, MN; 7Boston Children’s Hospital, Boston, MA.

Elevated expression levels of MYC family genes are frequently observed in human cancer cells and correlate with tumor aggressiveness and poor prognosis. In neuroblastoma 40% of all cases are high-risk, of which 20% harbor amplification of the MYCN proto-oncogene. In high-risk cases lacking MYCN gene amplification, high expression levels of c-MYC (MYC) are often present and are associated with unfavorable histology and a poor survival. Unlike MYCN amplification, which is frequently observed in the presence of segmental chromosomal aberrations, MYC overexpression is not associated with genetic abnormalities or somatic mutations. In order to study this newly defined subgroup, we have created a novel transgenic zebrafish model in which overexpression of MYC alone in the peripheral sympathetic nervous system (PNS) drives early onset neuroblastoma in nearly every fish by seven weeks of age. The tumors resulting from MYC overexpression arise in the interrenal gland, which is the fish counterpart of the adrenal medulla, and are histologically identical to human neuroblastoma. We next performed the Assay for Transposase Accessible Chromatin (ATAC) sequencing and RNA-seq to identify open chromatin regions that correlate with activation of gene transcription. Lineage specific genes essential for neuronal precursor cell identity, such as PHOX2B, HAND2, and TFAP2A are highly expressed in both MYC-expressing and MYCN-amplified human neuroblastoma cell lines and are actively transcribed in zebrafish models of MYC- and MYCN driven neuroblastoma. Furthermore, these studies reveal shared and differential regulatory effects of MYC relative to MYCN activity in maintaining the malignant phenotype of neuroblastoma in vivo. Additional insight into the mechanisms of aberrant transcriptional regulation will inform the future design and use of therapeutic strategies targeting transcription in this high-risk malignancy of childhood.

Clonal evolution of medulloblastoma BTICs in response to therapy. David Bakhshiyani, Thuythanh Vijayakumar, Chitra Venugopal, Mohini Singh, Maleeha Qazi, Sujeivan Mahendran, Sujeivan Mahendran, Bravanlan Manoranjan, Nicole McFarlane, Ashley Adile, Sheila Singh. McMaster University, Hamilton, Ontario, Canada.

Medulloblastoma (MB) is the most common malignant pediatric brain tumor. Global gene expression arrays performed on human MBs have divided this tumor entity into 4 distinct molecular subgroups. Out of all the subgroups, Group 3 patients face the highest incidence of leptomeningeal spread and overall patient survival of less than 50%. Current clinical trials for recurrent MB patients based on genomic profiles of primary, treatment-naive tumours, provide limited clinical benefit since recurrent metastatic MBs are highly genetically divergent from their primary tumors. By adapting the existing COG (Children’s Oncology Group) Protocol for children with newly diagnosed high-risk MB for treatment of immuno-deficient mice intracranially engrafted with human MB brain tumor initiating cells (BTICs), we aim to identify and characterize the rare treatment-resistant refractory cell population in Group 3 MBs. MB cell populations recovered separately from brains and spines at (i) engraftment; (ii) post-radiation; (iii) post-radiation and chemotherapy; and (iv) recurrence, during the course of tumor development and therapy were comprehensively profiled for gene expression analysis, stem cell and molecular features to generate a global, comparative profile of MB cells through therapy to relapse. One of the most intriguing observations from our gene expression data was consistent over-expression of proteins belonging to inhibitor of DNA-binding/differentiation (ID) family, transcription factors with a basic helix-loop-helix motif that act as suppressors of cellular differentiation and a longevity associated protein bacterial/permeability-increasing fold-containing-family-B-member-4 (BPIFB4) in our refractory population. The persistent upregulation of genes preserving undifferentiated state and cellular longevity further strengthens the hypothesis of stem-cell like cells driving tumor relapse in MB. We then set out to determine whether genes upregulated at relapse correlated with patient outcome in our therapy-adapted patient-derived xenograft model. Interestingly, the upregulation of the top 90 genes in our relapse cohort was predictive of worse overall survival in patients with group 3 MB. In the next set of experiments, through application of cellular barcoding technology we determined how brain MB BTICs evolve in response to therapy by tracking unique vector DNA sequences integrated at a single copy level into individual cells. Our differential genomic profile of the “treatment-responsive” tumors against those that fail therapy will thus contribute to discovery of novel therapeutic approaches for the most aggressive subgroup of MB.
targets. Upregulated genes were related to biological functions like negative regulation of cell growth and transcription, nervous system development. To probe the underlying mechanism, in-silico analysis indicated RA decreased Wnt/β-catenin, eNOS and PTEN pathways while stimulating cAMP, Integrin linked kinase and G-coupled protein pathways. Evaluation of the cAMP pathway (ε-allyl 3-Br-cAMP at 0.5 μM for 24 h) on MLL-R G3 and SOCS5 mRNA expression in ALL-SIL cells showed a 2-fold increase in SOCS5 mRNA. In primary NB tumors, microarray studies indicate that low levels of CNR1 and DUSP6 are associated with poor prognosis. RA stimulates 10-fold and 2-fold increases in CNR1 and DUSP6 levels, respectively. RA treatment inhibits EZH2 binding to its target genes thereby activating pathways involved in reprogramming of HR-NBs to a more differentiated state. Studies are underway to further evaluate the molecular mechanism leading to decreased EZH2 binding upon RA treatment.

#3873 Novel non-genetic mechanisms drive rhabdomyosarcoma tumorigenesis. Justin McEvoy,1 Michael Dyer,7 Xiang Chen,6 University of Arizona, Tucson, AZ; 2St. Jude Children’s Research Hospital, Memphis, TN.

Most pediatric solid tumors originate from perturbations during development, however the mechanisms that drive these disruptions and promote tumor progression are still poorly understood. We believe that epigenetic deregulation of cancer genes and developmental pathways is a common driver in pediatric cancers, especially those with a low mutation rate. To test this novel idea, we are focusing on rhabdomyosarcoma (RMS), a developing muscle tumor with striking genetic differences between two of the major subtypes. We hypothesize that differential epigenetic deregulation of differentiation programs and cancer genes between the two RMS subtypes are essential for tumorigenesis. Indeed, our preliminary integrative analysis reveal distinct epigenetic landscapes between the two subtypes particularly at the loci of HOX cluster genes A-C, including an oncogenic HOXc long non-coding RNA (IncRNA) called HOTAIR. Knockdown of HOTAIR led to derepression of HOXD10, a candidate tumor suppressor, and a decrease in RMS proliferation, migration, and invasion. Thus, we believe HOX gene deregulation is required for tumorigenesis, and their implication in several adult cancers suggests this currently underappreciated mechanism is likely to be more broadly relevant. In addition, we identified a group of RMS specific novel IncRNAs, of which one demonstrates an essential role in cell survival. Our innovative approach to integrate epigenetic data sets from tumor subtypes with varying genetic differences shed light on the mechanisms that drive tumorigenesis, which may lead to identifying previously unknown potential therapeutic targets. Since current therapeutic options have not changed over the last twenty-five years, these data will especially provide a strong translational impact for children with RMS, whose survival outcomes are less than 20% in the case of metastatic disease.

#3874 Epigenetic deregulation of SOCS expression in T cell lineage lymphoblastic leukemia. Nitish Sharma,1 Huining Kang,2 Christian C. Nickl,7 Scott A. Ness,7 Stuart S. Winter,1 Ksenia Matlawska-Wasowska,1 University of New Mexico Health Sciences Center, Albuquerque, NM; 2University of New Mexico Comprehensive Cancer Center, Albuquerque, NM.

For children, adolescents and young adults with T-lineage acute lymphoblastic leukemia, event free survival following relapse is ~10%. A variety of hematologic malignancies activate JAK-STAT signaling through activating mutations in JAK1-3, IL7R and downregulation of negative regulators, SOCS5 belongs to the suppressor of the cytokine signaling (SOCS) family, which are known cytokine-inducible negative regulators of JAK-STAT and other signaling pathways. SOCS5 is involved in negative regulation of Th2 development. However, the roles of SOCS5 in blood cancer, in particular T-ALL have not been elucidated so far. Using Affymetrix U133 Plus 2.0 microarrays we assessed SOCS5 expression levels in a cohort of 100 T-ALL samples derived from AALL0343 COG study. SOCS5 was deregulated in T-ALL cells, and its expression was lowered in cases harboring MLL gene rearrangements (MLL-R) compared to the remaining cases (P < 0.0001). Because we did not identify any mutations in SOCS5 gene in the cohort of 100 cases, we hypothesized that SOCS5 expression is regulated by aberrant DNA methylation in T-ALL. Using methyl-specific PCR (MS-PCR) and bisulite sequencing we found that SOCS5 expression is regulated by promoter/1st exon CpG island methylation in T-ALL. cCRF-CEM and ALL-SIL cells, and selected primary T-ALL samples showed hypermethylation in promoter/1st exon region corresponding to lower levels of SOCS5 expression. In contrary, KpT1 and PF382 cells, and T-ALL samples with higher levels of SOCS5 had hypomethylated SOCS5 promoter/1st region. SOCS5 transcript and protein expression were induced by DNA demethylating agent, 5-azacitidine in ALL-SIL and cCRF-CEM cells (P < 0.0001). The increase in SOCS5 expression was correlated with its promoter demethylation as shown by MS-PCR. SOCS5 mRNA and protein levels were also increased in ALL-SIL cells transduced with DNMT1 shRNA compared to negative control (P < 0.0001) indicating that inhibition of DNA methyltransferase activity restores SOCS5 expression in T-ALL. To assess whether histone deacetylation is involved in epigenetic regulation of SOCS5 expression, we treated cCRF-CEM and ALL-SIL cells with deacetylase inhibitor Trichostatin A (TSA). Treatment with TSA restored histone marks in SOCS5 promoter and increased SOCS5 mRNA levels in TSA-sensitive ALL-SIL cells compared to TSA-resistant cCRF-CEM cells. We next examined the effects of SOCS5 on leukemogenesis by shRNA-mediated knock down of SOCS5 in KpT1 and PF382 cells. Silencing of SOCS5 increased cellular proliferation as shown by an increase in T-ALL cell number (P < 0.0001). Reduced expression of SOCS5 led to an increase in MYC, IL4, IL4Ra, IL7R expression and STAT6 activation. These results indicate that SOCS5 is epigenetically deregulated in T-ALL. Future studies are required to evaluate the tumor suppressor roles of SOCS5 in a subset of T-ALL, in particular, in MLL-R cases via the regulatory effect on cytokine and growth factor receptor signaling.

#3875 SSX drives gain-of-function BAF complex chromatin affinity and genomic targeting in synovial sarcoma. Matthew J. McBride,7 John L. Pulice,4 Robert T. Nakayama,4 Nazar Mashalilar,1 Davis R. Ingramp,5 Jack F. Shern,5 Javed Khan,7 Jason L. Hornick,7 Alexander J. Lazar,7 Cigall Kadoch1. Dana Farber Cancer Institute and Harvard Medical School, Boston, MA; 2University of Texas MD Anderson Cancer Center, Houston, TX; 3Genetics Branch, National Institutes of Health, Bethesda, MD; 4Brigham and Women’s Hospital, Boston, MA.

Synovial sarcoma (SS) is a soft-tissue malignancy driven by a recurrent chromosomal translocation (t(X;18)) that uniformly produces the SS18-SSX oncogenic fusion product. SS is an example of a subtype of the mammalian SWI/SNF (BAF) complexes, which remodel nucleosomes in an ATP-dependent manner and antagonistically oppose gene-silencing activity of polycomb complexes to maintain transcriptional control throughout development and differentiation. We previously discovered that in SS, incorporation of the oncogenic SS18-SSX fusion into BAF complexes leads to eviction of the tumor-suppressor BAF47 (1N11/SMARCBA1) subunit, and aberrant activation of polycomb target genes by displacement of H3K27me3-mediated repression. However, uncoupling the oncogenic consequences of two co-occurrent BAF complex perturbations, gain of 78- amino acids to SS18 and loss of BAF47 has remained a challenge for the field; understanding gain- versus loss-of-function properties of these molecular events is critical to the identification of effective targeted therapies for this patient population. Here we show that synovial sarcoma primary tumors and cell lines harbor a transcriptional signature markedly distinct from sarcomas such as malignant rhabdoid tumors, which are driven solely by biallelic loss of BAF47. Indeed, we show that SS18-SSX-containing BAF complexes exhibit distinct chromatin localization in that suppression of SS18-SSX results in a near complete genome-wide re-targeting of BAF complexes. We find that SS18-SSX directs BAF complexes to polycomb-repressed sites to activate embryonic development and neuronal gene pathways hallmark to SS primary tumors. Strikingly, using biochemical affinity assays, we demonstrate that the SSX 78aa tail dramatically increases the affinity of BAF complexes for chromatin as well as their genomic footprint sizes upon ChIP-seq analyses. This is in stark contrast to the demonstrated decrease in chromatin affinity and genomic occupancy resulting from loss of function of BAF47 in MRT. Moreover, using ChIP-seq analysis of BAF47/CcA9-mediated KO of BAF47 in SS cell lines, we show that the proliferative arrest of SS cell lines upon suppression of SS18-SSX is independent of the BAF47 reassembly into BAF complexes, thereby demonstrating a unique SSX-driven oncogenic mechanism distinct from BAF47 loss. Taken together, these studies uncover a novel functionality of the SSX tail that is required for SS oncogenesis, and inform the selection of appropriate targeted therapeutic agents for this gain-of-function BAF complex-driven cancer.


Background: Cellular heterogeneity within tumors is increasingly recognized as a source of therapeutic failure. However, the cis-regulatory landscapes driving transcriptional states of intra-tumor heterogeneity, drug-resistance and relapse remain elusive. Results: Here, using H3K27Ac chromatin immunoprecipitation followed by sequencing (ChIP-seq) we characterized the active super-enhancer (SE) landscape in neuroblastoma, a pediatric cancer of the sympathetic nervous system. Analysis of differentially active SEs identified cis-regulatory modules associated with distinct transcriptional states in material derived from individual patients. These transcriptional states associated with two phenotypically divergent cellular subtypes. One subtype is referred to as adrenocortical (ADN) and...
expresses classic neuroblastoma markers from the peripheral sympathetic nervous system. In contrast, the other subtype referred to as mesenchymal (MES) has similarity to neural crest cells, expresses mesenchymal genes, is motile and lacks adrenergic markers. In contrast to ADN cells, MES-type cells are resistant to a wide variety of chemotherapeutics used in clinical management of neuroblastoma. Computational reconstruction identified core transcription factor modules associated with ADN- and MES-type cells. DNA binding profiles of adrenergic TFs MAML3 and GATA3 suggest feed-forward activation of the adrenergic SE-associated TFs. Interconversion of FACS-sorted MES- and ADN-type cells is observed in vitro. Induction experiments with the mesenchymal TF PRRX1 efficiently converted ADN-type cells to an induced-MES (iMES) state. These iMES cells acquired many features of MES cells including motility, mesenchymal gene expression and -histone modifications as well as chemo-resistance. Primary neuroblastoma biopsies included a small fraction of PRRX1-positive MES-type cells, as determined by immunohistochemistry. Importantly, the proportion of both cell types appears dynamic upon therapy and in relapse development, suggesting selective pressure of treatment. Conclusions: Here we establish that intra-tumor heterogeneity in neuroblastoma follows a bi-phasic structure characterized by two different SE-associated TF programs that reflects stages of the normal developmental programs. The detailed understanding of core regulatory modules and pathways may redesign strategies for therapeutic intervention.

#3877 Relationship of DNA methylation to mutational changes and transcriptional organization in fusion-positive and fusion-negative rhabdomyosarcoma tumors. Wenye Sun,1 Bishwaresh Chatterjee,1 Jack F. Shern,2 Sivasish Sindiri,1 Yonghong Wang,1 Holly S. Stevenson,1 Daniel C. Edelman,1 Paul S. Meltzer,2 Iaved Khan,2 Frederic G. Burt,2 NCI - Laboratory of Pathology, Bethesda, MD;2 NCI - Pediatric Oncology Branch, Bethesda, MD;2 NCI - Genetics Branch, Bethesda, MD.

Rhabdomyosarcoma (RMS) is the most common soft-tissue sarcoma of childhood and comprises two major subtypes: fusion-positive (FP, most commonly PAX3-FOXO1 [P3F] or PAX7-FOXO1 [P7F] resulting from 2;13 and 1;13 chromosomal translocations) and fusion-negative (FN). Our previous study demonstrated that FP and FN RMS tumors exhibit distinct DNA methylation profiles. To further examine the significance of DNA methylation, we generated genome-wide DNA methylation profiles for a new cohort of 48 RMS tumors for which we previously assessed mutation, copy number and expression status. Investigation of the RMS subsets defined by methylation clustering revealed a significant association between methylation and P3F binding sites in the PAX3/PAX7 intron, and an association of methylation with RAS mutation status in the FN subset. Localization studies of differentially methylated probes showed these probes were not evenly distributed with respect to annotated genomic features. In particular, hypomethylated probes were enriched in FP tumors in the promoter region and in the intergenic region, whereas hypermethylated probes were enriched in these regions in FN tumors. In contrast, hypermethylated probes were enriched in FP tumors in the 3' UTR regions of genes in these regions. In our new larger cohort of cases, there was a significant difference in the distribution of P3F binding sites between genes with and without differential methylation. Integrative analysis of P3F binding sites, promoter methylation and gene expression demonstrated that genes with P3F binding sites tend to be more highly expressed in FP tumors (compared to FN tumors) than genes without P3F binding sites regardless of promoter methylation status. Though promoter hypomethylation is most highly associated with enhanced expression among genes with P3F binding sites, the group of genes with P3F binding sites and promoter hypomethylation is small in number compared to the much larger group of genes with P3F binding sites but without promoter hypomethylation. In conclusion, these results demonstrate the interaction of these epigenetic changes with mutational alterations and transcriptional organization in RMS tumors and provide a direction for future studies of these epigenetic events.

TUMOR BIOLOGY: Targeting Cancer Stem Cells

#3878 Musashi-2 (MS12) regulation of ERBB family proteins in non-small cell lung cancer (NSCLC). Alexander Kudinov,1 Alexander Deneka,1 Anna N. Nikonova,1 Emmanuelle Nicolas,1 Vladislav A. Korobeynikov,2 Ilya G. Serebriskii,1 John Karanicolas,1 Erica A. Golemis,1 Yanis Boumber1, Fox Chase Cancer Center, Philadelphia, PA;2 Columbia University, New York, NY.

The purpose of this study is to establish whether a new signaling axis we recently described can be targeted to improve patient survival in lung cancer, one of the leading causes of death worldwide, with over 1.6 million deaths annually. Non-small cell lung cancer (NSCLC) is the most common form of lung cancer. A small number of targeted therapies have been shown to be useful in advanced metastatic NSCLC, particularly those targeting the EGFR pathway, but these drugs are active in only a subset of tumors that maintain epithelial characteristics. Musashi-2 (MS12), a RNA-binding protein that regulates mRNA translation, has been linked to maintenance of a stem cell state in multiple hematologic and solid tumor malignancies. We recently established (Kudinov et al, PNAS 2016, PMID:27274057) that MS12 is elevated in a subset of NSCLC tumors upon progression and drives NSCLC invasion and metastasis. MS12 overexpression is probably driven by claudin signaling cascade that enhances epithelial-mesenchymal transition (EMT). Using reverse phase protein array (RPPA) analysis of MS12-depleted versus control KrasG12D/P53R172H murine NSCLC cell line models identified a significant 2.7-fold upregulation of HER3 upon MS12 depletion. Negative MS12-dependent regulation of ERBB3 protein expression was confirmed in multiple additional murine and human KRAS-dependent NSCLC models, based on analysis of MS12 depletion or overexpression. In contrast, MS12 positively regulated expression of the EGFR/ERBB1 protein in the same models. In detailed analysis, we have addressed the mechanism by which MS12 regulates expression of the ERBB group of receptor tyrosine kinases. In addition, these data suggested the hypothesis that MS12 expression might predict response to EGFR-targeted agents, as MS12 inhibition may be synergistic with currently approved agents for NSCLC and may be therapeutically valuable. We will present data on the activity of EGFR inhibitors in the context of depleted or elevated MS12 expression, and the effect of combining inhibition of EGFR with recently developed MS12 inhibitors. In summary, these results for the first time indicate a role of MS12 in supporting EGFR expression in NSCLC and suggest that MS12 may drive NSCLC development and progression in part via EGFR, and may modulate response to EGFR-targeted agents.

#3879 Expression of sox2 and oct4 in human oral squamous cell carcinoma and its relationship with clinical factors. Tetsuya Matsumato,1 Natsumi Takamuru,2 Go Ohe,3 Keiko Kudo,4 Takaharu Kudo,4 Akira Takahashi,5 Yoshiko Yamamura,1 Kenji Fujisawa,2 Hirokazu Nagai,2 Youji Miyamoto.1 Institute of Biomedical Sciences, Tokushima Univ Graduate School, Tokushima, Japan.

Background: A cancer stem cell (cancer initiating cell, CSC) is considered capable of self-replication, self-differentiation, drug resistance, and immune evasion. Recently, CSC has become increasingly important in the treatment of malignant tumors. Cancer stem cells express specific molecules termed CSC marker, including sex determining region Y-box2 (SOX2), and octamer-binding transcription factor 4 (Oct4), and their expression has been reported to be the potential prognostic values. However, the prognostic values of SOX2 and Oct4 expression in patients with oral cancer are less understood. [Purpose] The aims of present study were to evaluate the expression of SOX2 and Oct4 in oral squamous cell carcinoma (OSCC) and to elucidate the relationships among the CSC marker expression, clinical stages, histological differentiation, the classification of invasion mode, cerebral lymph node metastasis, distant metastasis, and disease-free survival rate. Materials and Methods: Tissue specimens were obtained from 108 patients with OSCC after surgery or biopsy. Immunohistochemistry was used to assess SOX2 and Oct4 protein using at least 10% staining-positive cells as the definition of positive staining. Results: Immunohistochemical analysis of 108 cases showed that 42 cases (39%) expressed SOX2. There was no significant association between SOX2 expression and tumor size, invasion mode or histological differentiation. However, there was significant association between SOX2 expression and distant metastasis or disease-free survival rate at stage 1 and 2 patients (73 cases). Otherwise, seventy cases (65%) cases of 108 OSCC patients expressed Oct4. There was significant association between Oct4 expression and histological differentiation. There was no significant association between Oct4 expression and tumor size, invasion mode, metastasis, or disease-free survival rate. Conclusions: These findings suggested that the expression of SOX2 may be a good marker indicating survival in patients with OSCC.


Neuroblastoma is a tumor derived from immature neureoblasts predestined to form the sympathetic nervous system. Children with aggressive neuroblastoma are at high-risk of disease relapse despite their initial response to treatment. The molecular mechanisms underlying these relapses are poorly understood, al-
TUMOR BIOLOGY: Targeting Cancer Stem Cells

though clonal expansion and de novo mutations have been suggested as potential mechanisms. An alternative hypothesis is that neuroblastoma initiating capacity and resistance to chemotherapy reside in a subpopulation of tumor initiating cells or cancer stem cells, which are not genetically different from most tumor bulk cells but through unknown mechanisms differ phenotypically and functionally. Here we examine aldehyde dehydrogenase (ALDH) expression and activity in neuroblastoma patient-derived xenograft (PDX) cells, cultured under stem cell-promoting conditions, as a potential marker for neuroblastoma stem cells. We found that neuroblastoma PDX cells in general expressed higher levels of various ALDH isoforms as compared to an aggressive classical serum-grown neuroblastoma cell line. Also, a distinct subgroup with high ALDH activity in vitro more metastatically than cells with low ALDH activity. In conclusion, our results suggest that ALDH activity is associated with an immature phenotype, which is of clinical importance since less differentiated tumors are more aggressive and associated with poor prognosis. Also, cells with high ALDH activity showed higher in vivo tumor initiating capacity. We are currently pursuing a more extensive characterization of neuroblastoma cells with high ALDH activity.

#3881 The expression of CD98hc might be a marker of radiation resistance in HNSCC. Yohse Kawasaki, Yasufumi Omori. Akita University, Akita, Japan.

Introduction: Head and Neck squamous cell carcinoma(HNSCC) represents an aggressive malignancy. Although new advanced therapeutic strategies have been applied to date, the 5-year survival rate has not been considerably improved. Generally speaking, high radio-sensitive HNSCC has good prognosis, however, there are radio-resistant HNSCC. Recently, Same et al, have reported CD98 is one of the markers of Cancer Stem Cell. Moreover, the over expression of CD98hc is high associated with different cancers including the colon cancer, renal cancer. CD98 is heterodimeric protein that comprises a heavy chain and a light chain. The CD98 heavy chain is type 2 linkage to one of 6 amino acid transporters. We hypothesize that irradiation may contribute to overexpression of CD98hc and expansion Cancer Stem Cell population in HNSCC. Methods: Cells culture: Ho-1-u-1(Mouth floor), Sa3(Gingiva), HSC2, HSC3,HSC4(tongue) were used for this experiment. These cell lines were exposed to 60 Gy (2Gy/day) to establish stable cell lines to radiation. Flow cytometry: For analysis of CD98-positive fraction, cell pellets were incubated with FITC-conjugated anti-human CD98hc mAb at the dilution of 1:11 at 4° for 15min.

Sphere formation assay: Cells were cultured in a serum-free medium. 1x10⁴ cells that irradiation may contribute to overexpression of CD98hc and expansion Cancer Stem Cell population in HNSCC. Methods: Cells culture: Ho-1-u-1(Mouth floor), Sa3(Gingiva), HSC2, HSC3,HSC4(tongue) were used for this experiment. These cell lines were exposed to 60 Gy (2Gy/day) to establish stable cell lines to radiation. Flow cytometry: For analysis of CD98-positive fraction, cell pellets were incubated with FITC-conjugated anti-human CD98hc mAb at the dilution of 1:11 at 4° for 15min.

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cancer stem cell-like properties and EMT in liver cells. DCLK1 appears to enhance active β-catenin levels and its cellular localization to the nucleus. Thus, the DCLK1 represents an important target for HCC therapy.

#3885 Establishment, characterization and drug response of primary and metastatic prostate organoids. Sofia Karkampouna, 1 Federico la Manna, 1 Eugenio Zoni, 1 Liikle Beimers, 2 Peter Kloen, 1 Antoine Wetterwald, 1 Iod Grojean, 1 Irena Klima, 1 Marco Giovanni Cecchini, 1 Martin Spahn, 4 George Nikolias Thalmann, 4 Marianna Kruthof-de Julio, 4 University of Bern, Bern, Switzerland; 2 Slotervaart Medical Centre, Amsterdam, Netherlands; 3 Academic Medical Centre, Amsterdam, Netherlands; 4 University of Bern, Inselspital, Bern, Switzerland.

Introduction & objectives: Prostate cancer (PCa)-associated mortality results from metastasis to bone and resistance to androgen deprivation or cytotoxic therapy. Despite early detection of primary PCa, advanced castration resistant prostate cancer (CRPC) and bone metastases (BM) are detected in 10% of patients already at the time of initial diagnosis. The majority of recurrences might be due to cancer cells with stem cell-like properties (cancer stem cell-like, CSC-like). These could be therapy-resistant in a dormant state at the primary site or have metastasize prior to diagnosis of the primary tumor. CSC-like cells are the most tumorigenic and metastatic, however, current treatments target the differentiated tumor bulk cells. Understanding the mechanisms of PCa tumor initiation and metastasis by CSC-like cells is crucial for proper prognosis of high risk patient groups. Materials & methods: To model CSC-like cells we generated organoids from patient-derived tissues (here termed as ‘canceroids’) and established patient-derived xenografts (PDXs). Canceroids were derived from bulk tumor tissues; primary PCa, bone metastasis from PCa tissue (BM-PCa), established PDX models LAPC9 and BM18 and lymph node (LN) metastasis from PCa (LAPC4) PDX models. Cytotoxic compounds and androgen inhibitors are tested on the canceroids from different BM or LN tissues using viability assays (CellTiter Glo assay) and 3D imaging by confocal and light sheet microscopy. Results: We have generated several canceroid lines from human primary PCa and from established PDX models that maintain key features of the CSC-like cells, previously characterized on the tumor itself (AR, PSA, Cytokeratins). The luminal phenotype of BM18 is evidently maintained in the BM18 canceroids, based on positive cytokeratin (CK)18 and absent CK5 expression. LAPC4 organoids contain CK5 and CK18 cells, in line with the mixed basal and luminal phenotype. Imaging of PSA and cytokeratin distribution confirms the luminal phenotype of the canceroids. BM18 and LAPC9 canceroids are maintained both in presence and absence of dihydrotestosterone indicating that androgen independent growth properties of the tumors are conserved in the canceroids. Viability assays indicate that canceroids respond to chemotherapeutics (cabacitaxel, docetaxel) and partially to androgeninhibitors, indicating that androgen-independent growth properties of the tumors are conserved in the canceroids. Viability assays indicate that canceroids respond to chemotherapeutics (cabacitaxel, docetaxel) and partially to hormone inhibitors (abiraterone, enzalutamide). Conclusions: Identification of the oncogenic properties of metastatic CSC-like (sub)populations has both prognostic and therapeutic applications. Establishment of CSC-derived organoids is the first step towards routine derivation from metastasis or primary PCa tissues as a potential platform for personalized drug compound evaluation.

#3887 GNA13 is a theranostic target that drives drug resistance and cancer stem-like phenotypes in solid tumors. Suhail Ahmed Kabeer Rasheed,1 Hui Sun Leong,2 Manikandan Lakshmanan,2 Anandikumar Raja,2 Dhivya Dadalani,1 Fui-Teen Chong,2 Ravisan K Rajarethinam,2 Thakshayeni Skanthakumar,2 Ern Yu Tan,4 Jacqueline Siok Gek Hwang,5 Kok Hing Lim,2 Daniel Shao-Weng Tan,2 Paolo Ceppi,6 Mei Wang,7 Vinay Tergaonkar,8 Patrick J. Casey,1 N. Gopalakrishna Iyer,1 Duke-NUS Medical School, Singapore, Singapore; National Cancer Centre, Singapore, Singapore; Institute of Molecular and Cell Biology, Singapore, Singapore; Tan Tock Seng Hospital, Singapore, Singapore; Singapore General Hospital, Singapore, Singapore; Friedman-Alexander-Universitats-Erlangen-Nuernberg, Germany, Germany.

Treatment failure in solid tumors occurs due to the survival of specific sub-populations of cells that possess stem cell-like (CSC) phenotypes. Studies have implicated G protein-coupled-receptors (GPCRs) in cancer progression and the acquisition of aggressive phenotypes. Many of the implicated GPCRs signal through the G12 subfamily, comprised of GNA12 and GNA13. In this study, we demonstrate that GNA13 is upregulated in many solid tumors and impacts survival and metastases in these patients. Consistent with this, we show that GNA13 expression modulates drug resistance through its effect on the CSC sub-population in a panel of patient-derived head and neck (HNSCC) and breast cancer cells. These data were validated in vivo, where GNA13 over-expression in patient-derived xenografts increased tumor initiating capacity, tumorigenicity and drug resistance, with no effect on growth or proliferation. Signaling through NFkB and MAPK pathways appear to be critical to the observed phenotype. Importantly, blockade of GNA13 expression, or select downstream pathways using small-molecule inhibitors, abrogates GNA13-induced CSCs, rendering cells vulnerable to standard-of-care cytotoxic therapy for these cancers. Taken together, these data indicate that GNA13 expression is a potential prognostic biomarker, and interfering with GNA13-induced signaling provides a novel strategy to block CSCs and drug resistance in solid tumors.

#3888 DCLK1 is part of an EMT feedback loop and promotes colorectal cancer cell invasion and drug resistance. Dongfeng Qu,1 Nathaniel Weygant,1 William L. Berry,1 Randel May,1 Parthasarathy Chandrakasan,1 James J. Tomasek,1 Sripathi M. Sureban,1 Courtney Houchen,1 Yang Ge,1 Jiannan Yao,2 Guangyu An,2 Edwin Bannerman-Menson3. 1 Univ. of Oklahoma Health Sciences Ctr., Oklahoma City, OK; 2 Beijing Chao-Yang Hospital, Beijing, China; 3 COARE Biotechnology Inc., Oklahoma City, OK.

Colorectal cancer (CRC) is the third leading cause of cancer death in the U.S., with only a 6% 5-yr survival rate for stage IV disease. Its spread and acquisition of resistance to chemotherapy, which are fueled by the epithelial–mesenchymal transition (EMT) process and supported by tumor stem cells (TSCs), are major challenges to improving patient outcomes. New therapies that target stemness and EMT are desperately needed to prevent or delay metastasis and improve patient survival. Recently doublecortin-like kinase 1 (DCLK1) has been definitively proven to mark TSCs in CRC by two independent groups. Previous studies have demonstrated that DCLK1 is a prognostic factor in CRC and that targeted downregulation or inhibition of DCLK1 results in decreased CRC proliferation, migration, invasion, and other anti-oncogenic effects. However, the effect of overexpression of DCLK1 and its kinase active mutant on CRC has not been assessed. In this study, we investigate the correlative role of EMT and DCLK1 expression in CRC progress. Human colon cancer cells (HCT116) were infected with Lentivirus containing wild type DCLK1 or mutant DCLK1R326C cDNA sequences to overexpress DCLK1. DCLK1R326C or green fluorescence protein (GFP) cDNA sequence as control. The expressing levels of DCLK1 and EMT factors were analyzed by western blotting. The proliferative and invasive potential of these cells were compared using a MTT assay for proliferation, wound healing assay for migration, and Matrigel coated transwell assay for invasion. Knockdown of either ZEB1 or DCLK1 by specific siRNA in HCT116 cells was
performed. The effects of siDCLK1 on 5-FU were performed in both HCT116 and SW480 cells using a Caspase 3/7 activity assay. Analysis of human CRC was performed using TCGA COADREAD dataset. Here we report that compared to GFP control cells, HCT116-DCLK1 and HCT116-DCLK1R326C cells exhibited a more than 20% increase in proliferation, approximately 30% increase in migration, and a 2-fold increase (p < 0.05) in invasion. DCLK1 expression level is decreased more than 30% by knocking down ZEB1 in HCT116 cells. In addition, knockdown DCLK1 increased 5-FU induced cell apoptosis more than 50% (P < 0.05). Evidence from TCGA COADREAD demonstrated that EMT predicts survival in CRC patients, and increased expression level of DCLK1 in CRC patients correlate to EMT and mesenchymal phenotype. These data suggest that DCLK1 is a part of the EMT feedback-loop and may be exploited with DCLK1-targeted therapeutics for CRC.

#3889 Novel drug candidates cepi430 and cepi507 for targeting pancreatic patient-derived xenograft cancer stem cell and circulating tumor cell models. Michael Sharma,1 Maulik Jain,1 Cristian Sharma,1 Karina Amezgua,1 Natalee Amezgua,1 Reed Hasson,1 Miriam Navel,1 Donna Stanton,1 Satya Narayan,2 Karl Kramberger,1 Rubio Punzalan,1 Jitesh Jani,1 Douglas Foster,1 Jay P. Sharma,1 Colpogen, Inc., Torrance, CA; 2RSS Medical School, Dominican, Dominica; University of Florida, Florida, FL

Pancreatic cancer is the fourth leading cause of cancer mortality in the US, despite significant improvements in diagnostic imaging and operative mortality rates. The 5-year survival rate remains less than 6% because of microscopic or gross metastatic disease at time of diagnosis. Although the treatment of pancreatic cancer remains a challenge, it is entering a new era with development of new strategies and trial designs. Because there is an increasing number of novel therapeutic agents and potential combinations available to test in patients with pancreatic cancer and in their PDX models, the identification of robust prognostic and predictive markers for Cancer Stem Cells (CSC) PDX and Circulating Tumor Cells (CTC) PDX and of new targets and relevant pathways is a top priority as well as the design of adequate trials incorporating molecular-driven hypothesis. Here, we examined the efficacy of combined treatments of CEP1430 and CEP1507 in human pancreatic patient-derived xenograft (PDX) cancer cells, and pancreatic PDX CSCs models from the same donors. CEP1430 inhibited the growth of CSCs and CEP1507 inhibited growth of CTCs, while gemcitabine suppressed the viability of non-CSCs [70%] (differentiated tumor Cells). Consistently, in vivo studies showed that CEP1507 when combined with gemcitabine could eliminate the grafting of human pancreatic cancer CTCs [85%] (n = 5, p = 0.001) and CEP1430 with gemcitabine selectively inhibited [80%] CSCs (n = 5, p = 0.001), more effectively than the individual agents. These data indicated that administration of CEP1430, which targets CSCs and CEP1507 targets CTCs, may constitute a potential therapeutic strategy for improving the efficacy of gemcitabine to eradicate advanced pancreatic cancer. This study has indicated the potential molecular targets for pancreatic targets to eradicate the tumor – and metastasis-initiating cells (CTCs) and their progenies and development of new effective combination therapies against locally advanced and metastatic pancreatic cancer. Using PDX models, we confirmed the effectiveness and selectivity of the identified treatment responses with TGI at 97% with CEP1430.

#3890 Mitochondrial MCL1 maintains triple negative breast cancer stem cells and contributes to chemotherapy resistance. Kyung-min Lee, Jennifer Giltname, Justin Balko, Luis Schwarz, Michael Sharma, Cristian Sharma, Karina Amezgua,1 Nadia Annin,1 John P. Sharma,1 Colpogen, Inc., Torrance, CA; 2Russ Medical School, Dominican, Dominica; University of Florida, Florida, FL

Cytotoxic chemotherapy is the standard of care for patients with triple negative breast cancer (TNBC). Most patients with advanced TNBC progress after chemotherapy and die from metastatic disease. MCL1 is an anti-apoptotic Bcl-2 family member known to sequester and inactivate pro-apoptotic Bcl-2 family proteins and, thus, contribute to chemotherapy resistance. We previously reported that ~45% of residual TNBCs that remain in the breast after neoadjuvant chemotherapy harbor MCL1 amplification, suggesting a causal role for MCL1 in drug resistance. A recent report (Goodwin et al. 2015) suggested that siRNA-mediated ablation of MCL1 does not induce apoptosis in low TNBC. In this case a cancer stem cell (CSC) gene expression signature. CSCs comprise a rare population of cells with tumor-initiating properties and refractoriness to chemotherapy. In this study, we showed that MCL1 expression is elevated in claudin-low TNBC SUM159PT and MDA436 CSCs as measured by ALDH1 by flow cytometry and ability to form mammospheres. RNA interference of MCL1 in SUM159PT cells reduced CSCs and attenuated tumor formation in vivo. Mitochondrial oxidative phosphorylation (mtOXPHOS) plays a crucial role in maintenance of CSCs. MCL1 has been shown to localize in the mitochondrial matrix and contribute to mitochondrial respiration. Thus, we hypothesized that MCL1 contributes to enrichment of TNBC CSCs and chemotherapy resistance via mitochondrial regulation. Stable transfection and overexpression of MCL1 in MDA468 cells increased oxygen consumption ratio, mitochondrial membrane potential, and production of reactive oxygen species (ROS), all features of activated mtOXPHOS. Conversely, RNAi-mediated ablation of MCL1 in SUM159PT and MDA436 cells repressed these markers of activated mtOXPHOS. A mutant of MCL1 lacking its mitochondrial target sequences (Mts) was unable to localize in mitochondrial matrix and disrupted the CD44hi/CD24mo fraction and mammosphere formation. We next tested U65951B, a BH3 mimetic in development at Vanderbilt that disrupts MCL1 interactions with BH3 domain-containing proteins, such as BID, BIM, NOXA and PUMA. Treatment of SUM159PT cells with U65951B increased caspase activity but did not attenuate mammosphere formation. Analysis of mRNA expression in TCGA revealed that genes induced by mtOXPHOS involved in the hypoxia pathway are significantly up-regulated in MCL1 amplified breast cancers. Finally, pharmacological inhibition of HIF-1α, a key regulator of hypoxia, with digoxin decreased CSCs and attenuated tumor formation in vivo. These data suggest that 1) MCL1 confers resistance to chemotherapy by expanding CSCs via mtOXPHOS independent of its BH3 domain-mediated, anti-apoptotic function, and 2) targeting mitochondrial respiration and the hypoxia pathway may delay or reverse chemotherapy resistance in MCL1 amplified TNBC.

#3891 Chemoresistant cancer stem cells undergo gene changes that drive tumor recurrence. Prithy C. Martins,1 Atiya Dudley,1 Melissa A. Laramore,1 Hunter L. Gazda,1 Michael P. Markey,2 Barry H. Smith,1 Lawrence S. Gazda1.

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Despite the efficacy of chemotherapy, resistant cells are thought to contribute to tumor recurrence. In vitro models of recurrence must mimic the complexity and heterogeneity of in vivo tumors and provide the longevity needed to capture tumor dormancy following chemotherapy. We have previously described a 3D in vitro model that simulates in vivo tumors containing cancer stem cell niches. Encapsulation of murine renal adenocarcinoma (RENA) cells in a double layer of agarose that form spherical macrobeads allows for the development of 3D tumor colonies comprised of tumor-initiating and tumor-amplifying cells within the inner agarose matrix. In the current study, we used the RENCA macrobead in combination with chemotherapy to evaluate genetic regulation that allows tumor dormancy and drives recurrence. RENCA macrobeads treated with docetaxel (5 μg/ml) undergo a significant loss of cell mass and a period of latency followed by tumor recurrence. RNA isolated from docetaxel-resistant cells (Wk 6 post-treatment), recurrent tumors (Wk 20 post-treatment) and paired vehicle controls were characterized using whole-transcriptome profiling (GeneChip® WT Pico Kit with Transcriptome Analysis Console, Affymetrix). At Wk 6 post-treatment, genes associated with a stem cell phenotype (Car12, Arrib1, Nos2, Alldh112) and members of the ABCG1 family associated with multidrug resistance were enriched in docetaxel-resistant cells. Genes regulating the canonical Wnt signaling pathway, specifically LRPs, family members, were significantly upregulated. Pathway analysis revealed dysregulated focal adhesion, PI3K-Akt-mTOR-signaling, including genes modulating integrin adhesion (Itgα5, Itgα7), ECM receptor interaction ( Lama5, Lamb2, Lamc1) and actin cytoskeletal remodeling (Vcl). Supporting the quiescent nature of the docetaxel-resistant population, genes implicated in DNA replication (Dbf4, Cdc6, Mcm5), transcriptional (Id1, Id2, Grh21), protein synthesis (Rpl39) and protein metabolism (Rpl7a) were significantly down regulated. At Wk 20 post-treatment, up regulation of matrix metalloproteinasises (Mmp13) and partial retention of stem cell (Nos2, Car12) and drug resistant (Abcb1) genes supports the evolution of regenerated tumors to a metastatic phenotype. Genes involved in fatty acid β-oxidation (Acds, Lipf), oxidative phosphorylation (Ndu, Nd6) and the electron transport chain (Cox2) were down regulated in concert with up regulation of Me2 suggesting reduced flux through the TCA cycle and use of aerobic glycolysis in rapidly growing cells. This study further supports the RENCA macrobead as a model system to evaluate the biology of chemotherapy-resistant cells and tumor recurrence in a 3D microenvironment. These data are in line with the notion that chemotherapy-resistant cells exhibit a period of quiescence with a stem cell-like gene profile and experience an intrinsic transformation towards an invasive phenotype that drives tumor recurrence.

#3892 Delineating the role of ZEB1 loss in the chemotherapeutic and radioresistance of glioma stem cells. Mecca Madany,1 Lincoln Edwards,2 John S. Yu,1 Cedars-Sinai, Los Angeles, CA; 2Cornell, Ithaca, NY

The most prevalent brain cancer, Glioblastoma Multiforme (GBM) has an average survival of less than 2 years. Despite advances in therapy there is still an overall poor response to treatment which has been attributed to glioma stem cells (GSCs), a subset of tumor cells that are able to self-renew, inhibit differentiation and are often chemo and radioresistant. The genetics and mechanisms underlying how glioma stem cells are able to repopulate tumors and affect patient survival desperately need...
to be defined. We are interested in delineating the role that ZEB1 (zinc finger e-box binding homebox-1) loss has on GSCs and what role this loss plays in the chemo and radioresistance of GSCs. Most insights into ZEB1 regard it as tumorigenic and an initiator of EMT (epithelial mesenchymal transition). Surprisingly, in this study, we have shown that in TCGA datasets ZEB1 deletion frequently occurs and this loss actually results in higher survival in ovarian patients that can take targeted therapeutic regimens. Immunohistochemistry on patient samples shows that ZEB1 loss occurs both at the gene and protein level. Stable ZEB1 knockdown in our patient derived glioma stem cell lines results in increased proliferation and a resistance to differentiation. Following chemotherapy as well as radiotherapy, knockdown cell lines show increased cell viability as well as higher levels of secondary neurosphere formation. Decreased apoptosis and changes in cell cycle as well as the DNA damage response pathways were also observed in the knockdown lines following chemo and radiotherapy. Overall, this data indicates that loss of ZEB1 in GSC's results in enhanced resistance to standard therapy. With these results, we have reason to believe that ZEB1 may be used as a novel therapeutic target to combat the chemo and radioresistance of glioma stem cells.

#3894 A positive feedback loop prevents differentiation of endocrine resistant luminal progenitor breast cancer cells. Brendan Fironch,1 Sara Charmaz,2 Eamon Breen, Arnold Hill, Leonie Young.1 Royal College of Surgeons in Ireland, Dublin 2, Ireland; 2Trinity College Dublin, Dublin 8, Ireland. 

Introduction: Treatment of breast cancer patients has advanced greatly with the stratification of ER+/HER2+ and triple negative cohorts. ER+/breast cancer is the most commonly diagnosed mammary malignancy and is managed with anti-hormone therapies. Therapy resistant disease emerges in approximately one in three women. Within the ER+ cohort, breast cancer remains a heterogeneous disease, particularly at the cellular level. For example; ER+/breast cancer can range from <10% to >90% ER+/cells within a tumor. This study set out to define and target differences in cellular heterogeneity between endocrine sensitive and resistant ER+/breast cancer. Methods: A flow cytometry based cell surface protein (CSP) screen (37 proteins) identified changes in cellular heterogeneity between endocrine sensitive and resistant ER+ models. FACS isolation and single cell plating characterized the self-renewal and differentiation potential of the identified sub-populations. Conditioned media experiments assayed secretory activity of the endocrine resistant phenotype. Results: The CSP screen identified FUT4+/MUC1- cells as central to the endocrine resistant phenotype. The endocrine sensitive model (MCF7) was found to contain roughly equal proportions of FUT4+/MUC1- (31.2 ± 1.6 %), FUT4-MUC1- (38.5 ± 7.1 %) and FUT4-MUC1+ (23.0 ± 6.9 %) cells. While two independent models of resistance were found to be dominated by FUT4+MUC1+ cells (LY2: 86.0 ± 5.5 %; TMR: 80.6 ± 6.5 %). Conventional theory suggests that these resistant FUT4+/MUC1- cells are the apex cancer stem cells (CSCs). To test this hypothesis, single cell clonogenicity assays were carried out on FUT4+MUC1- cells isolated from the endocrine sensitive model. It was found that these FUT4+/MUC1- cells were not apex CSCs, but rather mid-tier progenitor cells, as they could not produce all the cell types of the sensitive model. Paired conditioned media and clonogenicity studies demonstrated that the resistant secretome is sufficient to trap FUT4+MUC1- cells isolated from the sensitive model in a self-renewing, stem-like state (termed the MUC1- stem cell). Moreover, resistant cells which were removed from treatment conditions for >8 weeks maintained the resistant FUT4+MUC1- phenotype. These data indicate co-operation between cell- and treatment-driven mechanisms creating a FUT4+MUC1- resistant phenotype. Conclusions: Chronic tamoxifen therapy drives heterogeneity towards a FUT4+/MUC1- phenotype. Single cell self-renewal and differentiation studies identify this cell type as a mid-tier progenitor cell rather than an apex cancer stem cell. A positive feedback loop supplements the treatment induced change, to maintain the resistant phenotype in the absence of further tamoxifen treatment. Targeting this feedback loop or its downstream effectors may allow for re-sensitization of endocrine resistant disease.

#3895 Verrucarin J targets both cancer cells and cancer stem cells. Kelsey Carter,1 Seema Parte,2 Surinder K. Batra,3 Mariusz Z. Ratajczak,2 Sham S. Kakar.2 1University of Louisville, Louisville, KY; 2Univ. of Louisville, Louisville, KY; 3Univ. of Nebraska, Omaha, NE.

Ovarian cancer is the fifth leading cause of death among women in the United States. The American Cancer Society estimates that approximately 22,280 women in the United States will be diagnosed with ovarian cancer and approximately 14,240 women will die as a result of ovarian cancer in 2016. The most common treatment for ovarian cancer is cytoreductive surgery, followed by chemotherapy (combination of carboplatin and paclitaxel). Although this regimen is initially effective in a high percentage of cases, unfortunately, within few months of initial treatment, tumor relapse occurs because of platinum-resistance. The presence of cancer stem cells (CSCs) are the main cause of this phenomenon. CSCs are a small population (2-5%) of cells in solid cancer which are chemo-resistant, highly tumorigenic, possess a self-renewal capacity, and the capability to differentiate into multiple lineage. Therefore, there is an urgent, unmet need for a more effective therapy that targets cancer cells as well as cancer stem cells. Therefore, targeting Verrucarin J, a metabolite of the Myrothecium fungus family, has not been tested for its antitumor activity. In our experiments, we treated two cisplatin-sensitive ovarian cancer cell lines (A2780 and OVCARS) and one cisplatin-resistant cell line (A2780/CP70) with various concentrations of Verrucarin J. After treatment of cells, Verrucarin J showed: i) a highly significant inhibition of colony formation in a dose- and time-dependent manner, with an IC50 value of approximately 10 nM after 48 h of treatment for all the three cell lines, ii) a significant increase in cell apoptosis, iii) a significant increase in DNA damage and generation of reactive oxygen species (ROS) compared to vehicle treated cells, iv) a significant down regulation of expression of cancer stem cells markers, and v) a significant inhibition of Notch1, Wnt1 and Shh signaling genes in a dose-dependent manner. Notch1, Wnt1 and Shh are major regulatory pathways for self-renewal and maintenance of cancer stem cells. Additionally, our experiments revealed that Verrucarin J inhibited the tumorogenic function (spheroid formation on ultra-low attachment plates) of isolated ALDH1 positive CSCs from ovarian cancer cell line A2780. Based on our results, we conclude that Verrucarin J is a highly potent anticancer drug and targets both cancer cells and cancer stem cells. Therefore, it may serve as a potential candidate for ovarian cancer treatment and currently used chemo-therapy.

#3896 Exosomes derived from CD133+ hepatocellular carcinoma (HCC) cell promote the acquisition of stem cell-like properties of the targeted HCC cell via the Akt/GSK3β/Snail and cyclin D1 pathways. Pengyu Guo,1 Haitao Yu,2 Xiaohai Xie,1 Yi Wang,2 Gang Chen.1 The First Affiliated Hospital of Wenzhou Medical University; Wenzhou Medical University, Wenzhou, China; Wenzhou, China.

Backgrounds and Aims: Liver cancer stem cells are closely related to the recurrence and metastasis of hepatocellular carcinoma (HCC). However, the mechanism underlying the reciprocity between liver cancer stem cells and HCC cells in the tumor microenvironment remains unclear. Exosomes act as natural shuttles to deliver bioactive molecules that alter the behavior of the recipient cells, offering a new means of cell-cell communication for physiological and pathological processes in the liver. This study aimed to clarify whether the exosomes derived from the liver stem cells could facilitate the acquisition of stem cell-like properties of the adjacent HCC cells, thereby promoting the malignant process of HCC. Methods: CD133+ liver cancer stem cells were isolated from the highly invasive HCC-LM3 cells or tumor tissues of HCC patients using flow cytometry and were identified using qPCR and immunofluorescence. The exosomes from CD133+ liver cancer stem cells were identified using CD133 and other stem cell markers, and they were then used to treat HepG2 and Hep3B cells. The cell viability, invasion ability, sphere formation, and expression abilities in vitro and tumorigenesis in vivo. In additional, we found that CD133+ exosomes activated the P38/Akt/GSK3β/Snail and cyclin D1 pathway, thereby reducing the expression of E-Cadherin and promoting the epithelial-mesenchymal transition (EMT) of HCC cells. Conclusions: Our results suggest that exosomes derived from CD133+ HCC cells can change the malignant behavior and promote the acquisition of stem cell-like properties of targeted HCC cells via the Akt/GSK3β/Snail and cyclin D1 pathways.

#3897 Novel inhibitor of GBM stem cells targets unique CREB & Myc-mediated self-renewal pathways regulated by CDK5. Subhas Mukherjee, Carol Tucker-Burden, Changming Zhang, Jun Kong, Monica Chau, Renee Read, Daniel J. Brat. Emory University School of Medicine, Atlanta, GA.

Cancer stem cells exert enormous influence on neoplastic behavior, in part by governing asymmetric cell division and the balance between self-renewal and multifactorial differentiation. Growth is favored by deregulated stem cell division, which enhances the self-renewing population & diminishes the differentiation program. To uncover mechanisms relevant to deregulated cell division in human glioma stem cells, we first developed a novel adult Drosophilia brain tumor.
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model, 100% enriched with tumor stem cells. Using this model, we performed a suppressor screen for kinases and found brain-specific dCDK5 partially reverses the tumor phenotype. dCDK5 and its human ortholog CDK5 (79% identity) are atypical protein kinases because they do not regulate cell cycle in normal cells, but are crucial for neurogenesis & survival of mature neurons. Interestingly, many cell survival/activation of these CDK5BPs in glioblastomas leading to uncontrolled proliferation. Our analysis of TCGA data shows that IDH wt GBM has significantly high level of CD5K compared to the IDH wt lower grade glioma. Additionally, we found a strong positive correlation between CDK5 & several stem cell markers in GBM. To date, not much is known about the role of CDK5 in glioblastoma GBM & glioma stem cell self-renewal. Using patient-derived (GBM) neurosphere cultures & in vivo xenograft tumors in mice, we further demonstrated that a novel pharmaceutical suppressor of CDK5 signaling axis can suppress self-renewal properties through a new CDK5 phosphorylation site on CREB and Myc. Together, our results show that the CDK5 signaling pathway is important for glioma stem cell survival & that suppressing CDK5 could be a novel therapeutic approach to eliminate glioma stem cells.

Emerging role of Fra-1 as a novel target for radiosensitization in HPV-associated cancers. Abhishek Tyagi,1 Shilpi Gupta,1 Prabhat Kuamr,1 Alok C. Bharti,2 Bhudev C. Das1. 1Amity University Uttar Pradesh, Noida, India; 2University of Delhi, Delhi, Delhi, India.

Background: Activator protein-1 (AP-1) plays a central role in HPV-mediated cervical and tongue cancer (subset of HNSCC). AP-1, a homo/heterodimeric protein composed of Jun and Fos family proteins, has been implicated in chemoradioresistance but the mechanism(s) remained unexplored. In the present study, AP-1 family protein Fra-1 has been shown to play a critical role in promoting radiosensitization in enriched cervical cancer stem-like cells (CaCxSLCs) and can serve as a target for drug intervention in cervical and other HPV-associated cancers. Experimental Procedure: Cervical cancer stem-like cells (CaCxSLCs) were isolated and enriched by sequential gating HPV-ve and HPV-ve human cervical cancer cell lines, SiHa, and C33a using a set of functional and phenotypic markers (ABC2, CD49f, CD71, CD133) in defined conditioned media (DCM) with intermittent culturing. CaCxSLCs were also assessed for their ciliacversus foramorphing ability, exclusion of DCV dye for generating SP cells, self-renewal ability and quiescence. Both CaCxSLCs and Non CaCxSLCs were exposed to different dose of UV irradiation and a herbal compound curcumin, a potent inhibitor of AP-1 was used to examine the role of AP-1 and its family proteins in the radiosensitization of cancer stem cells. Results: Upon UV irradiation, CaCxSLCs showed a dose-dependent higher proliferation and highly increased AP-1 activity whereas it was completely abolished in non-stem cancer cells. CaCxSLCs also showed differential overexpression of c-Fox and c-Jun at transcriptional and protein level. The loss of AP-1 activity & expression was accompanied by decrease in cell viability and proliferation in UV irradiated non-stem cancer cells. Interestingly, CaCxSLCs treated with curcumin prior to UV irradiation ablated AP-1 activity and a concomitant reduction in SP cells leading to abrogation of stage formable ability, loss of proliferative ability, induction of apoptosis and the cells were poorly tumorigenic. The c-Fox and c-Jun protein level with GL treatment had no effect on CD133+/CD44+ or CD44+/CD24+ populations in PC3 cells, which don’t express pSTAT3. Of note, both CD133+/CD44+ and CD44+/CD24+ cells responded to treatment with GL, as proliferation decreased in a dose-dependent manner. Treatment with GL significantly reduced the ability of CSCs to form colonies in a concentration-dependent manner. Conclusion: This study suggests that the role of AP-1 protein in the manifestation of radioresistance but curcumin pretreatment helps in radiosensitizing CaCxSLCs through upregulation of Fra-1 which may serve as a potential target for elimination of radioresistant cancer stem cells in HPV-associated malignancies.

Synthetic small molecule targeting CD24/CD44/ALDH1+ cell population inhibits cancer stem cell activities in breast cancer. Luxi Chen, Chao Long, Kha A. Tran, Jiyong Lee. University of Texas at Dallas, Richardson, TX.

Purpose of the study: Cancer stem cells (CSCs) have been described as the “seed of tumor”, and have been suggested as the driving force of tumor relapses. In this study we aimed to isolate small molecules via screening of the combinatorial chemical library. The isolated molecules bind CD24/CD44+/ALDH1+ breast cancer cell subpopulations with high selectivity and specificity. Experimental design and plan: Through the unbiased cell based chemical library screening, we identified compound candidates that specifically bind the stem-like cancer cell subpopulations in breast cancer cells and tissues. By performing in vitro, tissue and in vivo-based assessments on ligand-binding specificity and functional activities, we ensured that the isolated ligands target tumorigenic stem-like breast cell or tissue preferentially. Summary of discoveries and findings: The in vitro compound binding validations proved the specificity of the isolated compound candidates toward breast cancer cells and tissues displaying cancer stem cell properties. The compound-targeting cell populations display significantly-higher expressions of the well-known stem cell markers than the unbound cell populations. On the other hand, a series of biological and biochemical functional assays also confirmed the compound candidates exert tumor-suppression activities on breast cancer cell lines, such as slow down proliferation and reduce cell viability. More importantly, our study demonstrated that both isolated compounds have preferential selectivity and higher binding affinity to the TNBC cells and tissues, which have been reported for higher percentage of stem-like cancer cell population detected than in non-TNBC cells or tissues. Through mouse xenograft study followed by histological analysis, we further confirmed the binding specificity of the compound towards cell line models could be retained in vivo in mouse xenograft model. Conclusion: We demonstrated our ligand screening technique is able to identify synthetic compounds targeting cell subgroups or sub-populations of defined interest within one particular type of tumor. High specificity of the isolated molecules could be utilized to develop chemical probes for quantification of percentage of CSC in the tumor, which facilitates clinical evaluation of tumor relapse risk. The screening strategy also provides a new methodology in discovering novel drug targeting CSC subpopulations, not only in breast tumor, but also other types of human cancers. Funding: This project is supported by the Cancer Prevention and Research Institute of Texas (CPRIT) grant, award ID: RP150713.

STAT3 inhibition with Galiellalactone effectively targets the prostate cancer stem-like cell population. Giacomo Canesin,1 Anne T. Collins,2 Rebeca Hellsten,1 Norman J. Maitland,2 Anders Bjartell1. 1Lund University, Malmo, Sweden; 2University of York, York, United Kingdom.

Introduction and objectives Cancer stem-like cells (CSCs) represent a small subpopulation of largely quiescent cells that reside within tumors. Several studies have demonstrated that this small population is more resistant to current therapies and is therefore directly responsible for tumor recurrence. The transcription factor STAT3 has been recently related to the regulation of the stem cell niche in prostate cancer (PCa), and inhibition of activated STAT3 (pSTAT3) seems to be a valid strategy to selectively target PCa CSCs. We have previously shown that pSTAT3 blockade by Galiellalactone (GL) not only reduces proliferation and induces apoptosis of prostate cancer cells in vitro, but also inhibits the growth of prostate tumors and the metastatic spread to regional and distal lymph nodes in vivo. Here we performed experiments aimed at studying the effect of the inhibitor GL on PCA CSCs, in order to increase our understanding on this compound as a promising therapeutic approach for prostate cancer patients. Materials and methods The expression of stem, basal and luminal cell surface markers (CD133+/CD44+/CD24-) was analyzed by FACS on DU145 and PC3 cells treated with GL. DU145 cells were sorted based on expression of the above markers and then plated for further analysis, including expression of pSTAT3 by IHC and cell proliferation by the WST1 assay. The effect of GL on clonogenic recovery of PCa CSCs was studied by colony formation with sorted cells. Results Our results show that the CSC populations (CD133+/CD44+/CD24-) express high levels of activated pSTAT3 compared to the CD44+/CD24- cell population. Treatment with GL decreased the number of CSCs in DU145 cells after 48h, whilst the number of CD44+/CD24+ cells was less affected. Importantly, GL had no effect on CD133+/CD44- or CD44+/CD24- populations in PC3 cells, which don’t express pSTAT3. Of note, both CD133+/CD44+ and CD44+/CD24+ cells responded to treatment with GL, as proliferation decreased in a dose-dependent manner. Treatment with GL significantly reduced the ability of CSCs to form colonies in a concentration-dependent manner. Conclusion This study demonstrates that the STAT3 inhibitor Galiellalactone can specifically target the prostate cancer stem-like cell population in vitro, implying that pSTAT3 inhibition by GL could represent a valid therapeutic strategy to antagonize CSCs in human prostate cancer. Future experiments will be aimed at validating these data and relating the clonogenic inhibition to the effects of pSTAT3 blockade by GL on tumor initiation by prostate cancer stem-like cells in vivo.
oncogenic functions. The role of CtBP in APC mutant neoplasia remains obscure even though APC is responsible for degradation of both α-catenin and CtBP in suppressing colorectal tumorigenesis. Our prior work demonstrates that CtBP proteins can be effectively therapeutically targeted with substrate analogues of their intrinsic dehydrogenase domains. We now observe that pharmacologic inhibition of CtBP using either the 1st generation inhibitor 2-keto-4-methylthiobutyrate (MTOB) or the 2nd generation inhibitor hydroxymyoino-3-phenylpropanoic acid (HIPP) significantly reduces the burden of intestinal polyps in the Apcmin mouse model of the human cancer predisposition syndrome Familial Adenomatous Polyposis. The number of intestinal polyps in mice with CtBP2 inhibitors were significantly lower (21 polyps/mouse for MTOB, 15 polyps/mouse for HIPP) as compared with vehicle treated mice (45 polyps/mouse) with a P value < 0.0001. Pointing to the anti-neoplastic mechanism of MTOB and HIPP, polyps that did grow in MTOB or HIPP treated mice demonstrated robust downregulation of β catenin and Cbp2 proteins. This observation points to the role of Ctbp2 as a putative driver oncogene that can be therapeutically targeted in vivo. In addition, CtBP2 has been reported to play an important role in human colon cancer stem cells via its interaction with the transcription factor TCF4 on chromatin. We now show that inhibiting CtBP with HIPP or HIPP analogues attenuates CtBP2 protein levels in colon cancer stem cells with resultant decreased expression of CtBP transcriptional target genes, such as the motility/invasion regulator TIM1, and c-MYC, a known driver of the cancer stem cell phenotype. Moreover, colonospheres treated with CtBP inhibitor showed downregulation of the CSC markers CD133 and c-MYC, as compared with vehicle treatment. Flow cytometry analysis of intestinal epithelium from Apcmin mice treated with CtBP inhibitor vs. vehicle indicated a significant reduction in CD133+/CXCR4+ cells that are associated with a tumor initiating cell phenotype. Our data demonstrate that CtBP inhibition may reduce colon neoplasia via downregulation of c-MYC and the cancer stem cell/tumor initiating cell compartment, and that CtBP is promising and novel target for therapy in colon cancer.

**#3902 Interleukin-6 blockade, a novel cancer stem cell targeted therapy, attenuates lung cancer tissue invasion.** Hiroiuki Ogawa, Michiyo Koyanagi-Aoi, Yoshisama Maniwa, Takashi Aoi. Kobe University, Kobe City, Japan.

Background: Lung cancer stem cells are considered to be responsible for lung cancer progression and metastasis. However, little is known about how they actually promote aggressive behaviors of lung cancer. Methods: We transduced OCT3/4, SOX2, and KLFL4 (hereafter, OSK) into a KRAS-mutated (G12S) human lung adenocarcinoma cell line (A549) using retrovirus vector. Organoids were formed from the A549 and CT1 cells using a 3D semi-defined media. Organoids were treated with IL-6 blockade (200ng/ml XMD8-92) or vehicle and treated for 2 weeks. Organoid cell number was measured by ImageJ analysis. Results: IL-6 blockade inhibited the growth of OSK-A549-Colony cells. Although IL-6 blockade did not inhibit the growth of OSK-A549-Colony cells in normoxic conditions, the blockade enhanced growth of the colony in hypoxic conditions (0.2%O2). The expression of marker genes such as CD44 and CD133 were downregulated in hypoxic conditions. Conclusion: Our data suggest that IL-6 blockade may inhibit lung cancer stem cell by targeting hypoxic CSCs.

**#3903 Targeting oral cancer stem cells in the hypoxic niche by BCG infected mesenchymal stem cells.** Bidisha Pal,1 Seema Bhuyan,2 Jaishee Garghyan,1 Hong Li,1 Rashmi Bhuyan,1 Herman Yeger,1 Bukil Das1. 1The Forsyth Institute, Cambridge, MA; 2KaviKrishna Laboratory, Indian Institute of Technology (IIT), Guwahati, India; 3Hospital for Sick Children, Toronto, Ontario, Canada.

Background: Tumor hypoxia is a major contributing factor in cancer therapeutics. The microenvironment of hypoxia/oxidative stress may program cancer cells to highly tumorigenic, metastatic, angiogenic and stem cell-like state. We have characterized in vitro and in vivo models of hypoxia-induced cancer cell reprogramming to cancer stem cell (CSCs) like cells including an immunocompetent model of oral cancer. These CSCs exhibit ABCG2 cell surface marker. Numerous approaches including targeted therapy using oncolytic virus and bacteria have been attempted to target cancer cells in their hypoxic niche without major therapeutic success. Major therapeutic challenges include the inaccessibility of hypoxic niche by therapy-agents and the poor replication of viruses or bacteria intracellular to hypoxic cancer cells. Furthermore, the immune privileged niche of tumor hypoxia leads to viral/bacterial-induced immunity against the tumor. In this context, here we have tested a novel stem cell based approach to deliver BCG infected stem cells to the hypoxic core of tumors. Methods: Human SCC-25 cell line-derived xenograft in NOD/SCID mice exhibit hypoxic zones, where ABCG2+ resides (1). SCC-25 tumor-bearing mice were injected i.v with CD271+ bone marrow mesenchymal stem cells (BM-MSCs). We recently found that CD271+ BM-MSCs could be infected with M. tuberculosis. The BCG infected CD271+ BM-MSCs were injected to SCC-25 xenograft-bearing mice. The mice were sacrificed on day-20 of stem cell injection and then evaluated for the hypoxic CSCs. Next, we have developed a 4-NQO induced oral cancer cell line, which was injected orthotropically to the tongue of congenic mice. The mice developed tumors, which was treated with BCG. In these BCG-treated mice, BCG-infected murine CD271 BM-MSCs were injected i.v., and animals were monitored for four weeks for tumor growth. On day-20 of injection, mice were sacrificed, and the tumors were collected. In a control group, BCG alone was injected. Results: In both the SCC-25 and an immunocompetent mouse model, we found that BCG or CD271+ BM-MSCs injection alone did not result in marked anti-tumor activity and or elimination of ABCG2+ cells from their hypoxic niche. In contrast, the injection of BCG infected CD271+ BM-MSCs led to marked reduction of tumor growth. Importantly, we observed marked replication of BCG intracellular to CSCs. These results indicate that CD271+ BM-MSCs facilitated the transfer of BCG to cancer cells residing in the hypoxic zone. Importantly, the transfer of BCG was seen more in the immunocompetent model, suggesting potential role of immune system. Conclusions: Our findings indicate that hypoxic CSCs may be targeted by a BCG infected CD271 BM-MSCs. We propose this could be a novel therapeutic approach to target drug resistance cancer stem cells residing in the hypoxic niche of tumors.1 Bhuyan R et al, Cancer Research, 2016; Volume 76, Issue 14 Supplement, pp. 935.

**#3904 Inhibition of the ERK5 pathway as a novel approach to target human chronic myeloid leukemia stem cells.** Ignazia Tusa,1 Giulia Cheledoni,1 Na-ver Campoy,1 Paola Dello Sbarba,2 Elisabetta Rovida1. 1Universita di Firenze, Firenze, Italy; 2Dana Farber Cancer Institute, Harvard Medical School, Boston, MA; 3Hematology Unit, AOU Careggi, Firenze, Italy.

Tyrosine kinase inhibitors (TKI) targeting BCR/ABL are very effective for the treatment of Chronic Myeloid Leukaemia (CML). However, resistance mechanisms or their inefficacy on CML leukaemia stem cells (LSC) may lead to relapse. Therefore, there is urgent need to identify new molecular targets. The Extracellular signal-Regulated Kinase 5 (ERK5) is a Mitogen-Activated Protein Kinase that is maintained in severe hypoxia. In K562 and KCL22 cells and in primary cells non-selective normoxic secondary cultures (LC2), to measure LC2 repopulation as a read-out of progenitor/stem cell potential (CRA assay). Compounds: XM8-92 (ERK5 inhibitor) and BIX02189 (MEK3 inhibitor); imatinib (BCR/ ABL inhibitor, IM). We previously showed that stem cell potential of CML LSC is maintained in severe hypoxia. In K562 and KCL22 cells and in primary cells derived from 9 CML patients, the treatment in hypoxic LC1 with XM8-92 or BIX02189, but not with IM, impaired progenitor/stem cell potential. The same results were obtained by combined treatment of XM8-92 with IM. Importantly, XM8-92 did not affect progenitor/stem cell potential of CD34+ cells from healthy donors. In colony formation ability assays ERK5 inhibition decreased colony formation by human primary CML cells to a higher extent than that by normal human CD34+ hematopoetic cells. Interestingly, in hypoxia, combined treatment XM8-92/IM decreased the expression of genes relevant for stem cell maintenance such as p21, nanog and c-myc and the expression of CD26, a CML LSC marker. Moreover, combined XM8-92/IM maintained low the expression...
of p27, another gene involved in stem cell maintenance, that is increased by either drug when administered alone. This indicates that the ERK5 pathway inhibitors impaired LSC maintenance of CML cell lines and primary CML cells.

#3905 Development of anticancer aptamers targeting cancer stem cells. Yuh-Ling Chen, I-Shan Hsieh, Keng-Fu Hsu, Tse-Ming Hong. National Cheng Kung University, Tainan, Taiwan.

Background: Cancer stem cells (CSCs), also called tumor initiating cells (TIC), are a small population of cancer cells within a tumor that drive the tumor initiation, metastasis, and resistance to chemotherapeutic drugs. An effective cancer stem cell targeting strategy by oligonucleotide-based approaches such as aptamers in diagnosis and treatment are urgently needed. In this study, we aimed to develop an effective aptamer-based biomarker for CSCs in cancer detection and therapy. Material and methods: E-cad/herin-null lung cancer cells, shEcad-AS49, was confirmed with stemness properties and used as targeting cells. The isolation of DNA aptamers binding to CSCs was by using a Cell-SELEX (systematic evolution of ligands by exponential enrichment). By combining the aptamer-pull-down approach with proteomics, we identified the potential binding proteins of CSC-aptamer. Flow cytometry, gene silencing and lung cancer xenograft models were used to explore the functions of CSC-aptamer and its targeted protein in lung cancer formation. Results: The aptamer AP-9R was identified to bind lung CSCs with high affinity and specificity. AP-9R reduced lung cancer cell stemness and migration but no apparent cytotoxicity. By using proteomic approaches, the target protein of AP-9R in non-small cell lung cancer was further identified, which characterized its immunosuppressive function. For cell detection, we developed AP-9R conjugated beads and confirmed their effective captured rate of lung CSCs in vitro. Moreover, administration of AP-9R significantly reduced lung tumor growth in the xenograft model. Conclusions: Our data are moving closer to understand the significance of cancer stem cells during cancer progression and demonstrate that aptamer-based therapies will be useful as therapeutic and diagnostic agents for lung cancer.


Acute myeloid leukemia (AML) is a lethal blood cancer. Clinical evidence has highlighted the critical role for leukemic stem cells (LSCs) in the high relapse rate of AML patients. Understanding the signaling pathways critical for LSC function will facilitate the development of new therapies to target LSCs. G protein-coupled receptors (GPCRs) are the most successful drug targets with approximately 36% of currently marketed drugs targeting human GPCRs (Rask-Andersen et al., Nat Rev Drug Discov 2011). Aberrant expression of GPCRs has been observed in various cancers and the importance of GPCRs in cancer stem cells has begun to be appreciated. In this study, we tested gene expression profiling analysis identified a novel GPCR (hereafter named NG), which was suppressed in LSCs compared to normal hematopoietic stem cells. Overexpression of NG in pre-LSCs severely impaired leukemia initiation and progression in mice, whereas knockdown of NG significantly accelerated the disease onset. Our data also showed that NG overexpression substantially downregulated several well-known Wnt/β-catenin targets (e.g., Tcf7l2, c-Fos and Ccnd1). This data support a tumor suppressive role for NG in inhibiting leukemogenesis via downregulation of Wnt/β-catenin signaling. Consistent with these observations, treatment with an agonist that specifically activates the NG signaling pathway induced a marked decrease in cell viability and growth in murine LSCs and in human AML THP-1 cells, but had no effects on human AML MOLM-13 cells. This agrees with our finding that NG specifically targets the Wnt/β-catenin signaling, and the inhibitory effect of the agonist might largely depend on β-catenin expression levels in human AML cells, wherein THP-1 has been reported to express endogenous β-catenin but MOLM-13 exhibits no detectable β-catenin expression (Man CH. et al. Blood 2015). In summary, our data support a tumor suppressor role for NG in leukemogenesis and the forced expression of NG may provide a means to eradicate LSCs. Thus, restoring NG by the agonist treatment represents a promising therapeutic strategy for AML treatment of NG may provide a means to eradicate LSCs. Thus, restoring NG by the
viation of CCL2 was observed in bone marrow–derived stromal cells of FBXW7-deficient mice. 3. Administration of SK-818 blocked the enhancement of lung metastasis in FBXW7-deficient mice. 4. In human breast cancer patients, low FBXW7 mRNA expression in peripheral blood showed high concentration of CCL2 in serum & poor prognosis. Conclusions: We found that the FBXW7/ NOTCH2 pathway plays a key role in the metastasis of breast cancer cells through the formation of premetastatic niches, & that SK-818 inhibit cancer metastasis in mice. SK-818 is currently administered clinically for the treatment of patients with hepatitis B virus infection in Japan. Based on these findings, we have been conducting Phase I clinical trial to assess the safety of SK-818 for breast cancer patients.


Skeletal metastasis is the most life-threatening complication in prostate cancer. The molecular mechanisms behind the interactions between prostate cancer and the bone marrow premetastatic niche remain unknown. Understanding and preventing such interactions could lead to new promising therapeutic strategies against lethal prostate cancer. Annexin 2 receptor (ANXA2R) was originally identified as a cell surface receptor for Annexin 2 (ANXA2) that mediates the stimulatory effects of ANXA2 during osteoclastic activation and osteoblast mineralization. Moreover, ANXA2R is able to induce apoptosis independent of its ligand, partially through activating caspases using non-conventional apoptotic pathways. Osteoblasts and marrow endothelial cells express ANXA2 which functions as a regulator of hematopoietic stem cell engrafment. Previous studies demonstrated blocking ANXA2 or its receptor prevents prostate cancer cells from establishing bone metastases in mouse models. It has been recently proven that ANXA2R does not bind to ANXA2 directly but to S100A10 present in the complex annexinA2 heterotetramer (AIIt), composed of 2 ANXA2 bound to a S100A10 dimer. Higher levels of ANXA2 and S100A10 are associated with proliferating and invasive cancers and correlate with poor prognosis. However, the loss of ANXA2 expression appears to be specific for prostate cancer. Previous work on prostate cancer has focused solely on ANXA2 as a ligand. In this study, we demonstrate that S100A10 and ANXA2 are co-localized in prostate cancer, and their expression and ANXA2R levels vary with grade in primary tumors as well as within the different metastatic sites. Given prostate cancer cells as well as bone marrow stroma can simultaneously express the ligand and receptor and levels of AIIt decrease with tumor stage in the primary site, we hypothesize that prostate cancer cells require S100A10 and not only ANXA2 to spread to bone and other tissues. ANXA2R could act as a dependence receptor, mediating withdrawal-induced programed cell death in prostate cancer cells in the absence of AIIt. Furthermore, AIIt-ANXA2R interactions between prostate cancer cells and the premetastatic bone marrow niche might play an important role in the development of skeletal metastasis by inducing selective pressures on prostate cancer cells to metastasize to the bone marrow which is abundant of AIIt.

#3910 Analysis of metastatic potential by breast cancer type through a microfluidic blood-brain niche. Christopher Ryan Oliver,1 Megan Altemus,1 Brendan Leung,2 Aki Morikawa,1 Michele Dziubinski,1 Maria Castro,1 Sofai Merajver1. 1Univ. of Michigan, Ann Arbor, MI; 2Dalhousie, Halifax, Nova Scotia, Canada

Metastasis from the primary tumor site to the brain is the most lethal complication from advanced cancer. 15% of breast cancers metastasize in the brain with a median survival of 5-14 months depending on the subtype. Therefore it is critical to identify when a tumor has the clonal potential to metastasize to the brain. Current detection methods and treatment therapies have continued to improve but do not shed light on clonal metastatic potential. Models for characterizing metastatic potential of clonal populations currently used include murine in vivo and simple in vitro systems. Murine models are costly, time intensive, slow to manifest metastasis and are not easy to analyze. On the other hand, in vitro systems are faster and more cost effective but currently do not recapitulate the complexity of the “live” micro-environment. We have developed a microfluidic device that mimics the cellular and physical components of the human blood-brain niche to study the brain metastatic process. The device is composed of two chambers separate by a porous membrane. The top chamber and apical side of the membrane is seeded with human brain endothelial cells and uses flow to mimic shear stress encountered within the vasculature. Cancer cells are introduced into this chamber in which they adhere to and migrate through the endothelium into the bottom chamber. The bottom chamber contains astrocytes suspended in a collagen gel to mimic the brain stroma and provide room for invading cancer cells to colonize and grow. Barrier integrity is monitored using TEER (trans-endothelial electrical resistance), and fluctuates as the tight junctions of the endothelium are compromised by invading cancer cells. This is characterized by IF and tight junction staining. Throughout all time points, from introduction into the flow chamber, adherence to the endothelium, extravasation through the barrier, migration into the stroma, and proliferation the cancer cells can be monitored via both microscopy and TEER. We have applied this microfluidic blood-brain niche model to compare breast-seeking and -avoiding breast cancer subclones from cell lines that show different metastatic potential in the brain. Our data suggests that ANXA2R does not bind to ANXA2 directly but to S100A10 present in the complex annexinA2 heterotetramer (AIIt), composed of 2 ANXA2 bound to a S100A10 dimer. Higher levels of ANXA2 and S100A10 are associated with proliferating and invasive cancers and correlate with poor prognosis. However, the loss of ANXA2 expression appears to be specific for prostate cancer. Previous work on prostate cancer has focused solely on ANXA2 as a ligand. In this study, we demonstrate that S100A10 and ANXA2 are co-localized in prostate cancer, and their expression and ANXA2R levels vary with grade in primary tumors as well as within the different metastatic sites. Given prostate cancer cells as well as bone marrow stroma can simultaneously express the ligand and receptor and levels of AIIt decrease with tumor stage in the primary site, we hypothesize that prostate cancer cells require S100A10 and not only ANXA2 to spread to bone and other tissues. ANXA2R could act as a dependence receptor, mediating withdrawal-induced programed cell death in prostate cancer cells in the absence of AIIt. Furthermore, AIIt-ANXA2R interactions between prostate cancer cells and the premetastatic bone marrow niche might play an important role in the development of skeletal metastasis by inducing selective pressures on prostate cancer cells to metastasize to the bone marrow which is abundant of AIIt.


The platelet derived growth factor (PDGF) pathway is a prime example of tumor-stroma signaling in a number of cancer types. Others have shown that PDGF receptors are expressed in breast fibroblasts and pericytes while PDGF ligands are often expressed in breast cancer cells and tumor-associated endothelium; however, how PDGF signaling mediates breast cancer initiation, progression and metastasis remains unclear. Importantly, our evaluation of publicly available datasets revealed that PDGF expression correlates with breast cancer progression and metastasis. Given that PDGF-B preferentially activates PDGFRβ, we established an in vivo system to investigate this pathway during breast cancer progression. We utilized a mesenchymal-specific promoter to drive Cre recombinase and conditionally activate PDGFRβ by way of the endogenous Pdgfrb promoter (hereafter “PDGFRβ mutant”). A murine mammary tumor cell line which expresses high levels of PDGFB was injected either by tail vein or intracranially to evaluate metastatic seeding and distant tumor growth. Following tail vein injection of tumor cells, we observed 50% incidence of brain metastases in the PDGFRβ mutant mice while no brain lesions were seen in the controls. There was no difference in incidence of lung, liver or bone metastases (other common sites of breast cancer metastasis). Not surprisingly, larger tumors formed in the brains of PDGFRβ mutant mice when cells were injected intracranially. Brains were stained for phospho-PLCγ as a way to confirm activation of PDGFRβ. To our knowledge, this is the first example where genetic manipulation of the stroma leads to an increased incidence of breast brain metastases. Furthermore, this study highlights a role for stromal activation of PDGFRβ in the brain microenvironment and during metastatic progression. For the 20-30% of patients that develop breast cancer brain metastases, the one-year survival rate is sadly less than 20%, and how the brain microenvironment contributes to metastatic seeding and subsequent growth of tumor cells remains poorly understood. To confirm translational relevance, we analyzed a small cohort of matched primary breast tumors and brain metastases for PDGFRβ expression observing strong stromal staining in fibroblasts and pericytes and around cancer lesions in primary tumor tissues and brain metastases from 16 patients. Importantly, high PDGFRβ expression was found in the perivascular space of all associated brain metastases suggesting a functional role in the establishment or growth at this site. Combined, our findings strongly suggest that high primary tumor expression of PDGFB/PDGFRβ might define a subset of breast cancer patients predisposed to brain metastases. These patients may benefit from therapeutic targeting of PDGFR signaling as a means to thwart metastatic seeding in the brain.

#3912 Pancreatic cancer cell interaction with platelets impedes cell cycle progression and increases viability. Andrew C. Cannon, Sushil Kumar, Brad Hall, Rakesh Bhatia, Rakesh Singh, Surinder Batra. University of Nebraska Medical Center, Omaha, NE.

Background: Metastasizing cancer cells rapidly associate with platelets in circulation, but the nature and functional consequences of this interaction have yet to be fully elucidated. Here we investigated the physical interaction of platelets with pancreatic cancer (PC) cells and the contribution of platelet-derived platelet factor 4 (PF4) mediated signaling in PC cells. Methods: Immunohistochemistry (IHC) and western blot analysis were performed to characterize the expression of PF4 receptor, CXCR4, in 42 Whipple samples and 8 PC cell lines, respectively. Physical interaction of human platelets with PC cell lines and its impact on PC cell proliferation in low attachment conditions was analyzed by flow cytometry. Cell viability was assayed by MTT analysis. Immunofluorescence analysis was performed to evaluate PF4 induced autophagy. Results: IHC

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and western blot analysis in Whipple samples showed CXCR3 expression in 86 percent of the pancreatic tumors and on all the tested PC cell lines respectively. Flow cytometry demonstrated that 24 percent of Capan1 cells interacted with platelets. Interestingly, incubation of platelets with cell-cycle-synchronized Capan1 cells under low attachment conditions inhibited the proliferation of PC cells (~20%) compared with that of detached cells (~40%). This effect was abrogated in a pharmacological inhibition of CXCR3 with AMG487. Concurrently, the viability of Capan1 cells grown in low attachment conditions and treated with platelets for 72 hours was greater than that of control and AMG487 with platelets treated groups as assessed by MTT assay. Mechanistic studies suggested that recombiant PF4 augments autophagosome formation as an early event and increases the expression of p21 as a late event. Of note, both p38 and p21 expression are abrogated by concomitant incubation with PF4 and AMG487. Conclusion: Platelets appear to rapidly interact with cancer cells in suspension. Additionally, in a PF4-CXCR3 signaling dependent manner, platelets inhibit cell cycle progression and increase PC cell viability in low attachment conditions. Cumulatively, these data suggest that platelets aid in the metastatic dissemination of pancreatic cancer cells.

#3913 CXCL1 is critical for pre-metastatic niche formation and metastasis in colorectal cancer. Dingzhi Wang,1 Haiyan Sun,2 Raymond N. Dubois1. 1Medical Univ. of South Carolina, Charleston, SC; 2Arizona State University, Tempe, AZ.

Emerging evidence suggests that the primary tumor induces the formation of pre-metastatic niches in certain distant organs. However, the mechanisms underlying the contributions of the primary tumor to pre-metastatic niche formation are not fully understood. Here we demonstrate that colorectal carcinoma cells secrete VEGF-A that stimulates tumor-associated macrophages to produce CXCL1 in the primary tumor. Elevation of CXCL1 in pre-metastatic liver tissue recruits CXCR2-positive myeloid-derived suppressor cells to form a pre-metastatic niche that ultimately promotes liver metastases. Importantly, we found that pre-metastatic liver-infiltrating MDSCs induce tumor cell survival without involvement of innate or adaptive immune responses. Our work reveals a novel mechanism by which the interactions of malignant cells with their stromal counterparts in the primary tumor contribute to the formation of a pre-metastatic niche that favors tumor cell survival, but also provides a rational for development of CXCR2 antagonists to inhibit or prevent metastatic spread of disease. Overall, our study provides the first evidence we know of showing that primary malignant cell-secreted VEGF-A stimulates tumor-associated macrophages to produce CXCL1 in the primary tumor.

#3914 KLJ4-dependent perivascular plasticity enhances pre-metastatic niche formation and metastasis. Meera Murgai, Wei Ju, Matthew Eason, Jessica Kline, Rosandra N. Kaplan. National Cancer Institute, National Institutes of Health, Bethesda, MD.

Metastasis causes most cancer deaths, and new therapies that are based on a deeper understanding of this process are required to improve patient survival. Metastatic tumor cell colonization and growth is facilitated by a pre-metastatic niche microenvironment composed of hematopoietic cells, stromal cells, and extracellular matrix that support tumor survival and growth. Perivascular cells, including vascular smooth muscle cells (vSMCs) and pericytes, are a key cell population involved in new vessel formation critical to primary tumor development. Given the well-described plasticity of perivascular cells, we sought to determine whether perivascular cells regulate tumor cell survival and proliferation at metastatic sites. Using perivascular cell-specific and pericyte-specific lineage tracing models, we demonstrated that perivascular cells lose traditional vSMC/pericyte marker expression in response to tumor-secreted factors, and exhibit increased proliferation, migration and extracellular matrix (ECM) synthesis. Expression of the pluripotency gene KLJ4 is increased and is critical for this phenotypic transition to a less differentiated state characterized by increased proliferation and ECM production. Genetic inactivation of KLJ4 specifically in perivascular cells decreases pre-metastatic perivascular cell proliferation and consequently integrin-fibronectin dependent tumor metastasis. Our data reveal a previously unidentified role for perivascular cells in pre-metastatic niche formation, and suggest that modulation of stromal cell plasticity may provide a novel strategy for limiting metastasis.

#3915 Copper depletion as an approach for metastatic dissemination in Triple Negative Breast Cancer. Divya Ramchandani, Vivek Mittal, Linda Vahdat. Weill Cornell Medical College, New York, NY.

Triple-Negative Breast Cancer (TNBC) occurs in approximately 17-20% of all breast cancer patients, and has an aggressive clinical course even in patients with early-stage disease. Metastasis occurs in 35%-40% of TNBC patients relapsing within 5 years of diagnosis. Targeted therapies do not exist for TNBC. Recently, copper (Cu) has emerged as an essential factor in tumor growth and metastasis supporting the notion that Cu-depletion may serve as an attractive therapeutic strategy. Using historical controls, a promising single arm Phase II trial of Tetrathiomolybdate (TM1) in metastatic breast cancers patients, who were at a high-risk of relapse (Stage 3 and Stage 4 with no evidence of disease BC and Stage 2 if triple-negative), was conducted at Weill Cornell. At a median follow-up of 5.9 years, the progression-free survival for all patients enrolled in this study is 72% and the overall survival is 84%. Importantly, levels of circulating LOXL2 and VEGFR2+ endothelial progenitor cells were decreased with Cu-depletion (Chan et al. 2016). With the knowledge about the efficacy of TM1, it is currently unknown where the effectiveness of Cu-depletion lies, whether it acts at the pre-metastatic site, on circulating tumor cells, or at the primary tumor site. We utilized an immunodeficient in vivo breast cancer model that metastatizes to lungs (MDA231-LM2) in order to evaluate the effect of Cu-depletion in metastasis, where the animals were either TM1-treated or untreated. To identify whether LOXL2 is down-stream of TM1-Cu axis in metastasis suppression, as observed in clinical trials (Chan et al. 2016), we used “LOXL2-depletion, Cu-repletion” strategy. We also examined the global functional changes between TM-treated and untreated groups for various Cu-dependent proteins and angiogenic factors at the pre-metastatic site and primary tumors. We found that TM-treatment in the in vivo model significantly lowered disseminated tumor cell population in animals that were successfully Cu-depleted. Cu-depletion also altered pre-metastatic niche formation. We are currently exploring the effect of Cu-depletion on primary tumor and its surrounding microenvironment that might affect tumor cell shedding, leading to metastasis. In summary, this study holds essential groundwork for the identification of a strong mechanistic arm for TM therapy in the suppression of metastasis/metastatic relapse in TNBC patients. Thus, aiding in the development of additional therapeutic options, which may be particularly useful for a subset of TNBC patients impervious to Cu-depletion with single-agent therapy, or for whom Cu-depletion is insufficient to prevent metastasis, and may also be extended to other metastatic solid tumors that are responsive to Cu-depletion therapy.

#3916 Disease-specific markers can be used for the identification of disseminated tumor cells in the bone marrow of patients with prostate cancer. Emma E. van der Toom, Stephanie A. Glavaris, Michael A. Gorin, James A. Verdone, Kenneth J. Pienta, Heather J. Chalfin. The Johns Hopkins School of Medicine, Baltimore, MD.

Introduction: The detection of microscopic disseminated disease in prostate cancer has largely been attempted with the study of circulating tumor cells (CTCs). Little is known about the character and clinical significance of disseminated tumor cells (DTCs) that have reached the bone marrow (BM) niche, and prior studies has relied on epithelial markers. Yet, it is known that such markers are also expressed on normal erythroid precursor cells in the BM, thus providing an assay for prostate-specific DTC identification. Materials and Methods: BM aspirates from 18 men with localized and 2 men with metastatic prostate cancer were collected at time of RP or clinic visit respectively, and processed with the AccuCyte system (RareCyte, Inc., Seattle, WA). Slides were immunostained with DAPI (nuclear), anti-pan-lymycotin (epithelial), anti-CD45/CD66b/CD11b/CD14/CD34 (white blood cell), and the prostate-specific markers HOXB13 and NKKX3.1 (prostate). DTCs were detected using the CytewiseFisher system. DTCs were required to have positive prostate channel staining and no white blood cell signals. Results: DTCs were present in 89% (16/18) of patients at time of RP, with range 0 to 3592 cells/4mL sample (average 270, median of 39.5). Two patients with metastatic prostate cancer had 52 and 105 DTCs/4mL BM (Table 1). Notably, only 5% of all DTCs were epithelial marker positive (CK+), and only 44% (8/18) of patients had any CK+ DTCs. Conclusions: The RareCyte system is a promising selection-free system for DTCs detection that does not rely on epithelial markers. Here we report on a novel assay to distinguish DTCs from other cells in the bone marrow using the presumed prostate-specific markers HOXB13 and NKKX3.1. Furthermore, we show that the majority of prostate-specific marker positive DTCs did not express the epithelial marker CK. Analysis of the association of DTC count with clinicopathologic variables are ongoing.
#3917 GenoCTC, a liquid biopsy device using microfluidic enrichment for affinity-based isolation of circulating tumor cells. Jiyee Ryu,1 Do Hyung Kim,1 Gyeong-Mi Kim,2 Jin Soo Kim,2 Youngkee Shin,1 Jaeuk Lee,1 Hun Seok Lee.1 GenoBio. Corp, Seoul, Republic of Korea;2 Seoul National University Boramae Medical Center, Seoul, Republic of Korea;3 Research Institute of Pharmaceutical Science, Seoul National University, Seoul, Republic of Korea.

To achieve a complete remission of cancer is still challenging that most patients with solid cancer ultimately experience metastasis. Detection of circulating tumor cells (CTCs) from the peripheral blood is promising key to solve the cancer cell dissemination and make accurate diagnosis, therefore, many efforts have been made to isolate the CTCs. Here, we newly developed the liquid biopsy device, GenoCTC, with microfluidic chips isolating CTCs from the patient whole blood. To isolate CTCs, EpCAM+ CTCs in the whole blood are first bound with magnetic beads conjugated with human-EpCAM antibody, and GenoCTC and its chip can isolate the EpCAM+ CTCs through magnetic based method. With 7.5 ml whole blood from lung cancer patient, we isolated EpCAM+ CTCs, and these CTCs were validated by immunofluorescence assay using anti-EpCAM and anti-CD45. We also enumerated EpCAM+ CTCs in 17 whole blood samples from 10 lung cancer patients that various number, 2 to 114, of CTCs were found from each patient. The number of CTCs is known to have a correlation with disease progression and chemo-resistance, so the clinical meaning of this result is needed to be investigated. Furthermore, some CTCs are also found to be EpCAM independent which is regarded to have clinical utility involved in EMT. We used plastin-3 as its marker, and it was confirmed with HS 578T breast cancer cell line which shows low expression of EpCAM but high expression of plastin-3. The various molecules expressed on CTCs can be isolated and expanded reliably in 3D organoid culture from as few as 10 CTCs/mL. Separation and processing of the buoyant coat enriched for the number of viable CTCs isolated. EpCAM positive CTCs were isolated from 6/6 blood samples from patients with advanced PDAC. Updated results will be presented. Conclusions: The current size-based filtration system allows for collection of viable CTCs from human PDAC blood samples. Current work will use this system to grow, expand and characterize human PDAC CTCs in organoid culture. Genetic, transcriptional, and epigenetic characteristics will be compared with the primary tumor. CTC characteristics will be studied as biomarker of treatment response and resistance.

#3918 Identification of disseminated prostate tumor cells in bone marrow during radical prostatectomy from patients with localized prostate cancer. Stephanie A. Glavaris, Emma E. van der Toom, Michael Gorin, James Verdone, Changxue Lu, Jun Luo, Kenneth Pienta, Heather Chalmin. Johns Hopkins University, Baltimore, MD.

INTRODUCTION AND OBJECTIVES: In prostate cancer, disseminated tumor cells (DTCs) can escape the primary lesion and enter the bone marrow (BM) niche, representing an initial step towards conventionally detectable metastasis. The frequency of occurrence is elusive in clinically localized prostate cancer. We detected and characterized these cells by measuring gene expression of prostate-specific markers from BM samples collected at the time of radical prostatectomy (RP). METHODS: 5 mL of BM were harvested at RP for 36 clinically localized patients. A whole cell extract was assayed with the AdnaTest ProstateCancer-Select kit (Qiagen). Reverse transcription (SensScript RT kit, Qiagen) and real-time qPCR quantified expression of RPL13A (control, ribosomal protein), EP-CAM (epithelial), NKX3.1 and HOXB13 (prostate-specific), and AR-FLO (andro-gen receptor full length). Prostate markers known to be less sensitive or specific were also assayed in a subset of patients (TMPRSS2-ERG, AR-V7, PSA, and PSA). DTC detection was defined as prostate-specific marker expression in the BM. Quality control was performed with Sanger sequencing. The association of PSA and Gleason score (GS) with DTC detection were evaluated with the Mann-Whitney U Test and Fisher’s exact test respectively. RESULTS: DTCs were detected via NKX3.1 expression in 30/36 patients (83%). 100% of patients were EPCAM+, consistent with the known non-specific expression of EPCAM in the BM. HOXB13, AR-V7, and TMPRSS2-ERG were not detected in any specimen. PSA was also non-specifically expressed in 67% of NKX3.1+ and 83% of NKX3.1- patients. There was a pattern with DTC detection and higher PSA and GS, with 100% of NKX3.1+ patients having low-risk PSA less than 10, and only one with primary GS greater than 3 (17%, 1/6). Conversely 47% (14/30) of NKX3.1+ patients had primary GS greater than or equal to 4, and 27% (8/30) had PSA greater than 10. Yet, this was not statistically significant (GS p = 0.302, PSA p = 0.262), and DTC detection across all cases were independent. DTCs were detected based on NKX3.1 positivity in a large portion of clinically localized prostate cancer patients at all Gleason scores. Ongoing investigation with healthy patient BM will clarify whether NKX3.1 is truly prostate-specific, and if its expression associates with clinico-pathologic outcomes.

#3919 Isolation and characterization of viable pancreatic cancer circulating tumor cells using size-based filtration system. Kenneth H. Yu,1 Benjamin D. Krempel,2 Brian McCarthy,3 Memorial Sloan Kettering Cancer Center, New York, NY; 4 Charles E. Schmidt College of Medicine, Boca Raton, FL; 5 Viator CTC Solutions Inc, Lowell, MA.

Introduction: Circulating tumor cells (CTCs) are important actors in metastasis, a key feature of pancreatic ductal adenocarcinoma (PDAC). The ability to isolate, characterize and culture viable CTCs holds promise for understanding biology, treatment response, and resistance. Methods: Peripheral blood samples from healthy donors and patients with advanced PDAC were processed using an innovative size filtration system (Viator LLC, Lowell MA). Normal human blood samples were spiked with varying numbers of human PDAC organoid cells. The impact of buffy coat separation was studied. Isolated CTCs were cultured in Matrigel to generate organoids as previously described. Cells were imaged with fluorescently labeled antibody to EpCAM. Data: Using spiked normal human blood samples, viable CTCs can be isolated and expanded reliably in 3D organoid culture from as few as 10 CTCs/mL. Separation and processing of the buoyant coat enriched for the number of viable CTCs isolated. EpCAM positive CTCs were isolated from 6/6 blood samples from patients with advanced PDAC. Updated results will be presented. Conclusions: The current size-based filtration system allows for collection of viable CTCs from human PDAC blood samples. Current work will use this system to grow, expand and characterize human PDAC CTCs in organoid culture. Genetic, transcriptional, and epigenetic characteristics will be compared with the primary tumor. CTC characteristics will be studied as biomarker of treatment response and resistance.
showed that early DCCs contributed to metastasis formation together with late-disseminate and, as disseminated cancer cells (early DCCs), enter a non-proliferative condition. How early stages of tumor progression (before detection of palpable tumors), can cascade. However, new evidence shows that Neu primary tumors are known to be able to complete all steps of the metastatic cascade. How early dormant DCCs and consequently metastatic relapse. Our findings provide for the first time evidence that NR2F1 functions as a suppressor of stemness and dissemination during early stages of tumor progression. We propose that therapies that restore expression of NR2F1 might limit early dissemination and the progression of early and late-arriving DCCs toward metastatic outgrowth.

The role of pyruvate kinase M2 isoform (PKM2) in tumor progression is controversial. Previously, it was reported that PKM2 has an oncogenic function in promoting tumor growth in xenograft models; however, PKM2 was indicated non-oncogenic or even anti-oncogenic because depletion of PKM2 accelerates mammary tumor growth in the Brca1 knockout mouse model of breast cancer. Since tumors frequently harbor various abnormally activated oncogenic kinases, we hypothesized that phosphorylation of PKM2 by oncogenic kinases in tumor cells enables PKM2 to promote tumor growth, while unphosphorylated PKM2 in non-transformed cells cannot promote tumor growth. Here, we report that PKM2 proteins were largely unphosphorylated and formed tetramers in normal mammary epithelial cells (MCF10A, MCF12A). On the other hand, PKM2 proteins were phosphorylated at Y105 and formed dimers in breast cancer cells (MDA-MB-231, MDA-MB-435). Knocking down PKM2 didn’t affect normal mammary epithelial cell (MCF10A) growth but significantly decreased breast cancer cell MDA-MB-231 proliferation. In addition, introducing the PKM2 Y105D phosphomimetic mutant into MCF10A cells induced epithelial to mesenchymal transition, increased CD44+/CD24− stem-like cell population, formed slower initially, but reached a higher velocity after two or three deformations. Interestingly, PKM2 Y105D increased YAP1 protein nuclear localization in MCF10A cells, whereas inhibiting YAP1 by siRNA or a chemical inhibitor (Verteporfin) decreased PKM2 Y105D-induced cancer stem cell-like population and colony formation ability. The data indicate that YAP1 is a novel downstream target of PKM2 in promoting the stem-like property and triggering mammary tumor initiation. Next, we performed receptor tyrosine kinase array and identified several oncogenic tyrosine kinases (AXL, EPHA2, FAK, Src, Jak3 and so on) that effectively phosphorylate PKM2 at Y105. Currently, we are testing the efficacy of targeting these oncogenic tyrosine kinases in reversing the phosphorylation of PKM2 and blocking its tumor-promoting function. Together, our data indicate that oncogenic kinases-induced phospho-Y105-PKM2, but not the unphosphorylated PKM2, promotes mammary tumor initiation by increasing cancer stem cell populations.

Tumor cells derived from MMTV-Neu mouse models or human ErBb2+ primary tumors are known to be able to complete all steps of the metastatic cascade. However, new evidence shows that Neu+ early cancer cells derived from early stages of tumor progression (before detection of palpable tumors), can disseminate and, as disseminated cancer cells (early DCCs), enter a non-proliferative state for prolonged periods of time at secondary organs. We recently showed that early DCCs contributed to metastasis formation together with late-arriving DCCs (those coming from overt tumors). However, the mechanisms that would allow these early cancer cells, considered sessile and with few genetic alterations, to complete all steps of metastasis are unknown. Here we report that downregulation of the orphan nuclear receptor NR2F1 in Neu+ early cancer mammary epithelial cells (MECs) further activated a motile phenotype and this was coincident with previously described detection of circulating cancer cells (CCCs) and lung and bone marrow DCCs in MMTV-Neu mouse model. Knock down of basal NR2F1 levels in Neu+ early cancer cells induced cell motility, loss of laminin-V deposition, β-catenin delocalization from the membrane, dramatic loss of E-cadherin junctions and increased percentage of leader cells positive for CK14 and PRX1 staining. Interestingly, TWIST and PRX1 levels became upregulated upon NR2F1 depletion in 3D structures. These results suggest that NR2F1 expression maintains epithelial identity and suppresses epithelial-mesenchymal transition (EMT). Knock down of NR2F1 in Neu+ early cancer cells enhanced mammosphere formation efficiency and this was accompanied by upregulation of the pluripotency transcription factor NANOG and EMT master regulator TWIST. Interestingly, early dormant DCCs (negative for proliferation markers) showed re-expression of NR2F1 levels while micro-metastases (cycling cells) had reduced levels of NR2F1. This result suggests that downregulation of NR2F1 in early DCCs may contribute to the dormancy escape of early DCCs and consequently metastatic relapse. Our findings provide for the first time evidence that NR2F1 functions as a suppressor of stemness and proliferation and colony formation ability. The data indicate that YAP1 is a novel downstream target of PKM2 in promoting the stem-like property and triggering mammary tumor initiation. Next, we performed receptor tyrosine kinase array and identified several oncogenic tyrosine kinases (AXL, EPHA2, FAK, Src, Jak3 and so on) that effectively phosphorylate PKM2 at Y105. Currently, we are testing the efficacy of targeting these oncogenic tyrosine kinases in reversing the phosphorylation of PKM2 and blocking its tumor-promoting function. Together, our data indicate that oncogenic kinases-induced phospho-Y105-PKM2, but not the unphosphorylated PKM2, promotes mammary tumor initiation by increasing cancer stem cell populations.

#3923 Oncogenic kinase-induced PKM2 Y105 phosphorylation promotes tumorigenesis and cancer stem cell-like property. Zhuo Zhou, Lin Li, Zhang, Dihua Yu. MD Anderson, Houston, TX; Baylor College of Medicine, Houston, TX.

The role of pyruvate kinase M2 isoform (PKM2) in tumor progression is controversial. Previously, it was reported that PKM2 has an oncogenic function in promoting tumor growth in xenograft models; however, PKM2 was indicated non-oncogenic or even anti-oncogenic because depletion of PKM2 accelerates mammary tumor growth in the Brca1 knockout mouse model of breast cancer. Since tumors frequently harbor various abnormally activated oncogenic kinases, we hypothesized that phosphorylation of PKM2 by oncogenic kinases in tumor cells enables PKM2 to promote tumor growth, while unphosphorylated PKM2 in non-transformed cells cannot promote tumor growth. Here, we report that PKM2 proteins were largely unphosphorylated and formed tetramers in normal mammary epithelial cells (MCF10A, MCF12A). On the other hand, PKM2 proteins were phosphorylated at Y105 and formed dimers in breast cancer cells (MDA-MB-231, MDA-MB-435). Knocking down PKM2 didn’t affect normal mammary epithelial cell (MCF10A) growth but significantly decreased breast cancer cell MDA-MB-231 proliferation. In addition, introducing the PKM2 Y105D phosphomimetic mutant into MCF10A cells induced epithelial to mesenchymal transition, increased CD44+/CD24− stem-like cell population, formed slower initially, but reached a higher velocity after two or three deformations. Interestingly, PKM2 Y105D increased YAP1 protein nuclear localization in MCF10A cells, whereas inhibiting YAP1 by siRNA or a chemical inhibitor (Verteporfin) decreased PKM2 Y105D-induced cancer stem cell-like population and colony formation ability. The data indicate that YAP1 is a novel downstream target of PKM2 in promoting the stem-like property and triggering mammary tumor initiation. Next, we performed receptor tyrosine kinase array and identified several oncogenic tyrosine kinases (AXL, EPHA2, FAK, Src, Jak3 and so on) that effectively phosphorylate PKM2 at Y105. Currently, we are testing the efficacy of targeting these oncogenic tyrosine kinases in reversing the phosphorylation of PKM2 and blocking its tumor-promoting function. Together, our data indicate that oncogenic kinases-induced phospho-Y105-PKM2, but not the unphosphorylated PKM2, promotes mammary tumor initiation by increasing cancer stem cell populations.

#3924 Single-cell mechanical characteristics of human breast cell lines analyzed by multi-constriction microfluidic channels. Xiang Ren, Parham Ghassemi, Hesam Babahosseini, Jeannine S. Strobl, Masoud Agah. Virginia Tech, Blacksburg, VA.

Analysis of the mechanical characteristics of single cancer cells have shown that non-metastatic and metastatic cells have significant differences in stiffness. We used a microfluidic multi-constriction channel device to differentiate cancer cells and normal cells based upon differences in their mechanical properties. We fabricated the multi-constriction channels on a silicon wafer with SU-8 photolithography and polydimethylsiloxane (PDMS) soft-lithography, followed by PDMS microfluidic device formation and surface modification. The multi-constriction microfluidic channel contains five constriction channels each 50 µm in length separated by relaxation sections of 50 µm between every constriction channel. We used MDA-MB-231 as the cancer cell line and MCF-10A as the normal cell line, and utilized a smart phone to record video via a microscope. Our measurement by smart phone slow-motion video through microscope reliably collected the velocities of over 200 cells. We focused on comparing the initial velocity change due to cell deformation at the entrance of the first constriction channel and the exiting velocity at the last segment of the final constriction channel, where the cells have experienced sequential deformations. The multiple deformation paradigm tests cells’ resilience towards deformation and shape recovery. Cancer cells recovered back to a round cell shape quickly, and therefore experienced deformation at the entrance to each conformation, possibly cell migration and result in a decrease in velocity. The initial velocities of MDA-MB-231 cells were ~1.1 mm/s, and exiting velocities were ~2.2 mm/s. In contrast, normal cells deformed slower initially, but reached a higher velocity after two or three deformations, because normal cells stay in a rod-like shape without fully recovering back to an original spherical cell shape. The initial velocities of MCF-10A cells were ~0.5 mm/s, and exiting velocities were ~3.8 mm/s. When normal cells enter the fourth and fifth constriction channels, the cells’ shapes were almost fixed. The entrance time of cancer cells into the fourth and fifth channel was longer than the normal cells. After calculating the velocity increments in the fourth channel and fifth channel compared to the initial velocity, the Mafab
scatter plot of the velocity data of each MDA-MB-231 (n = 108 cells) and MCF-10A (n = 105 cells) showed clear separation of the two cell lines into distinct regions. Algorithms based upon these criteria successfully differentiated ~94.4% of the cancer cells from normal cells. Our experimental results indicated that multi-constriction microfluidic channels can be used to differentiate metastatic MDA-MB-231 and MCF-10A cells at the single cell level and may have further applications in high-throughput cell sorting and analysis. The authors would like to thank National Institute of Health (NIH) R21CA210126 for supporting this research.

#3925 Integrative platform for the reconstruction and modeling of mechanically arrested single cells in the brain vasculature under spatial constraint and shear stress. Wendy V. Alvarez Barrios,1 Huijie Lu,2 Kyle R. Cowdrick,3 Michelle Galarneau,1 Lan Jiang,2 Melinda Lake,3 Lin Yang,2 Danny Chen,3 David Hoelze,2 Zhangli Peng,2 Siyuan Zhang1,3 1University of Notre Dame, South Bend, IN; 2Ohio State University, OH.

During metastasis of breast cancer, tumor cells dissociate from the primary tumor, disseminate through the vasculature, and colonize secondary organs such as the brain, lungs, liver and bones. The mechanical arrest of circulating tumor cells (CTCs) in capillary beds is an essential step in the metastatic cascade for the successful early colonization of secondary organs. During mechanical arrest, tumor cells are subjected to extreme physical stresses such as shear stress and spatial constraint exerted by blood flow, as well as from compression by the constricting capillary on the restricted cell. These mechanical stresses may critically impact metastatic success. However, the precise physical and mechanical response of the disseminated tumor cells at the time of arrest in the vasculature to the forces of spatial constraint and shear stress remain largely unknown. Here we present an integrative platform that combines the use of a microfluidic device and computational model as a tunable system to reliably reproduce the mechanical arrest of single cells and quantitatively determine their physical response to precisely controlled mechanical forces of shear stress and spatial constraint. Using confocal microscopy and computational modeling we demonstrate that during mechanical arrest, single tumor cells undergo extreme morphological deformations. Furthermore, the cell morphology is dependent on the magnitude of shear stress applied; with magnitudes above 60Pa inducing elongation, blebbing, and rupture of the cell membrane. Our results demonstrate that our integrative platform is a viable model system for the qualitative and quantitative study of physical cell behavior precisely at the metastatic stage of mechanical arrest. Particularly, our results highlight the direct influence of physical forces on the restricted tumor cell, and suggest the importance of these external cues as dynamic determinants of cellular processes and ultimately of metastatic success. We anticipate our platform to be a starting point for future studies seeking to understand the reciprocal molecular and biophysical interplay imposed by mechanical forces at the greatly understudied stage of mechanical arrest in metastasis.

#3926 Is FGD3 a potentially prognostic marker for breast cancer. Yuliang Sun, Scooter Willis, Xiaoxian Lin, Justin Achua, Casey Williams, Brian Leyland-Jones. Avera Center for Precision Oncology, Sioux Falls, SD.

Background: Prognostic factors are capable of providing information on clinical outcomes at the time of diagnosis; they are usually indicators of growth, invasion, and metastatic potential. FGD3 (GeneID: 22852) was found to be overexpressed in breast cancer tissues (GC: 550616, p = 0.0001, 1998). Tissue marker is one of the prognostic factors; to date, only a small proportion of markers are ultimately clinically useful, including Ki-67 and HER2. While metastatic cancer is not usually a single, clear-cut disease, it is clearly a disease that has many different stages and types of progression. We also demonstrate that invasive BC cells are tolerant to supra-physiological calcium levels and that their adaptation to high calcium occurs via up-regulation of the calcium-sensing receptor (CaSR). This up-regulation might be induced by the immunosuppressive microenvironment, resulting in immune escape, and might be induced by the immune microenvironment, resulting in immune escape, and might be enhanced by the high calcium levels or risk of high calcium-driven aggressive BC outcomes. The commonly diagnosed mild increase in circulating calcium activates the calcium sensing receptor (CaSR) and has been shown to be associated with localized and more aggressive breast tumors in postmenopausal and premenopausal patients, regardless of histologic stage. Whether differences in circulating calcium and/or specific inactivating CaSR variants play any role in disparities in BC outcomes remains unclear.

DESIGN METHODS: We identified 199 BC cases and 384 age and genetic ancestry-matched controls with calcium assay and genotyping data from the Vanderbilt University DNA biorepository (BioVU) linked to de-identified electronic medical records. The linear mixed effects and codominant models were used to assess the relationship between inactivating CaSR mutations at rs1801725 (codon 986) and rs1801726 (codon 1011) and either circulating calcium levels or risk of high calcium-driven aggressive BC outcomes. RESULTS: We observed that circulating calcium levels were significantly higher in BC cases compared to control subjects (p = 0.001) and interestingly, in subjects of African ancestry compared to Caucasians (p = 0.001). The A986S mutant CaSR is common among Caucasians while the Q101E mutant receptor is common among African Americans. However, only inactivating mutations at rs1801725 locus were significantly associated with higher calcium levels (p = 0.006) and a higher (69%) risk of high calcium-driven aggressive BC outcomes compared to the wild type receptor. We also demonstrate that invasive BC cells are tolerant to sustained high calcium and that their adaptation to high calcium occurs via up-regulation of calcium-activated early response and malignancy-associated genes. CONCLUSION: These data suggest that inactivating CaSR polymorphisms at rs1801725 predispose BC patients to hypercalcemia and that high circulating calcium-driven aggressive disease outcomes occur via calcium modulated malignancy-associated genes such as MAGEC2/CT10.
Heterogeneity in circulating tumor cells in blood samples of metastasis-causing resistant prostate cancer patient: comparison of isolation technique. Gerit Theil, Christine Weiss, Kersten Fischer, Andre Schumann, Pauloa Fornara, Martin Luther Univ. of Halle-Wittenberg, Halle/Saale, Germany.

Introduction/Objectives: Metastasis-causing resistant prostate cancer (mCRPC) is a heterogenous disease. Since most prostate cancer patients have a biopsy performed only at the time of diagnosis, representative tumor tissue sample giving real time information about the disease status is generally missing. CTC enumeration is a biomarker associated with clinical outcomes in patients with mCRPC. However, capturing these rare cells from whole blood is still a major challenge because they are extremely rare. Furthermore CTCs mark a feature of epithelial-mesenchymal transition. First we assessed the method is best suited for the isolation of CTCs in mCRPC patients. Additionally was to evaluate the association between CTCs counts and ERG rearrangement in tumor samples. Materials & Methods A tumor sample from metastatic site, along with blood samples were collected from mCRPC patients. Tumor samples were characterized of ERG rearrangement by fluorescence in situ hybridization. CTCs were detected using two methods. One was SreenCells system, CTC isolation based on size exclusion. The second method was the CellCollector which CTC captured using monoclonal specific antibody to EpCAM. This system can be used for CTC isolation ex vivo and in vivo. We used it here ex vivo. Captured CTCs were identified based on histological cell architecture by immunofluorescence staining (8, 13). Both methods detected CTCs in 8 of 10 blood samples, detection rate is 80%. CTCs were in average of 22.6 CTCs (range of 0-80 CTCs). The CTC counting rate in SreenCells system and CellCollector isolation methods. Both methods detected CTCs in 8 of 10 blood samples, detection rate is 80%. SreenCells isolated in average of 22.6 CTCs (range of 0-80 CTCs). The CellCollector captured CTCs in vivo in average of 3 CTCs (range of 0-6 CTCs). In a direct comparison of the SreenCells system and CellCollector isolation methods. Both methods detected CTCs in 8 of 10 blood samples, detection rate is 80%. SreenCells isolated in average of 22.6 CTCs. The CellCollector captured CTCs in 3 CTCs. Therefore, both methods have a high positive correlation (r 0.776) and also with the ERG rearrangement (r 0.5). Our results demonstrate that CTC isolation based on the physical properties has a higher sensitivity as the CTC isolation technique based on the biological cell properties in mCRPC patients. The size exclusion technique coupled with characterization of specific staining morphologies might be used to identify a heterogeneous CTC population which is always present in advanced cancer. It should be decided in dependence of the advanced cancer stage which CTC isolation technique can be used. The cost of the material and the hours of work are equal in both systems.

ZEB1 is regulated by PKCs in breast cancer cell lines. María Candela Llorens,1 Cynthia Lopez-Haber,2 Laura Barrio-Real,2 María V. Vaglentí,1 Marcelo G. Kazanietz,2 Ana M. Cabanillas1. 1School of Chemical Science, National University of Córdoba, Córdoba, Argentina; 2University of Pennsylvania, Philadelphia, PA.

Breast cancer is the second leading cause of cancer deaths in women. Cytokine signals triggering kinase pathways play key roles in cancer progression which regulate transcription factors such as ZEB1 or Snail. Our goal is to uncover the role of PKC signaling pathway in the regulation of the biological action of ZEB1. The protein expression of ZEB1, PKC isoforms, epithelial-to-mesenchymal transition (EMT) markers were determined by Western Blotting (WB) in nine breast cancer cell lines. PKCa and ZEB1 had a significant positive correlation (p=0.05), and both proteins were highly expressed in mammary cell lines with a more aggressive phenotype (such as MDA-MB-231, BT-549 and MDA-MB-453). Interestingly, silencing PKCa using siRNA significantly reduced ZEB1 expression in MDA-MB-231, MDA-MB-453 and BT-549 cells, as determined by Western blot. Likewise, the pan-PKC inhibitor (Gö6983) and the ePKC inhibitor (Gö6976) reduced ZEB1 expression in breast cancer cells. However, the EMT markers vimentin, ZO1 and N-cadherin did not change their expression. ZEB1 mRNA levels did not change significantly in PKCa knockdown cells, suggesting that PKCa possibly regulates ZEB1 expression by affecting its protein stability. In addition, we found that the motile and invasive capacity of MDA-MB-231 cell lines was markedly affected in both PKCa and ZEB1 knockdown cells. Interestingly, depletion of PKCa from MDA-MB-231 cells markedly reduced the formation of ruffles and lamellipodia in response to serum, whereas ZEB1 depletion did not. In summary, our results revealed that PKCa regulates the expression of the EMT marker ZEB1 in basal-like breast cancer cell lines, and that in addition PKCa is important for the control of cell invasion and migration through regulation of ZEB1, thereby arguing that it could be a potential target for breast cancer metastasis. Our results also suggest that PKCa may control important phenotypic responses both through ZEB1-dependent and ZEB-independent mechanisms.

Sodium-calcium exchanger-1 regulates the epithelial phenotype and is lost in renal cancers. Sona Lakshmi Balasubramanian,1 Anikumar GOPalakrishnapillai,2 Nicholas J. Petrelli,3 Sonali P. Barwe,2 1 Nemours/A. I. duPont Hospital for Children, Wilmington, DE; 2 Christiana Care Health System, Newark, DE.

Epithelial cells that line the renal tubules possess apico-basal polarity and a defined cellular structure maintained by junctional proteins. The underlying mesenchymal cells are fibroblastic with a non-uniform cell structure and secrete extracellular matrix proteins. Mesenchymal to epithelial transition (MET) and epithelial to mesenchymal transition (EMT) are required for renal tubule formation during kidney development. Failure of renal mesenchymal cells to undergo epithelial transformation leads to the initiation of Wilms tumor, the most frequently occurring renal cancer in children. Conversely, aberrant mesenchymal transformation of tubular epithelial cells in the nephron contributes to the development of renal cell carcinoma, which constitutes 85-90% of the adult tubular malignancies. The role of calcium regulators in governing MET and MET is becoming evident. Sodium calcium exchanger 1 (NCX1), located on the basolateral surface of tubular epithelial cells, is the principal calcium regulator that mediates calcium reabsorption in these cells. NCX1 mediates the extrusion of one calcium ion and the influx of three sodium ions in one calcium exchange process. We demonstrated earlier that NCX1 regulates epithelial cell motility. However, the role of NCX1 in EMT was undetermined. We observed that knockdown of NCX1 in renal epithelial cells (MDCK) induced fibroblast morphology, and increased permeability to rhodamine indicative of leaky cell-cell junctions. Electron micrographs of these cells displayed increased inter-cellular junctional distance also suggesting that NCX1 knockdown altered junctions between adjacent cells. Cells with NCX1 knockdown showed loss of apico-basal polarity in three-dimensional cultures accompanied by expression of mesenchymal markers. Furthermore, NCX1 knockdown cells were capable of anchorage independent growth suggesting that these cells had acquired tumorigenic potential. Interestingly, we show for the first time that NCX1 mRNA and protein expression is greatly reduced in both Wilms tumor and renal cell carcinoma demonstrating a direct correlation between NCX1 expression and the epithelial phenotype. Mechanistically, we provide evidence that NCX1 interacts with and anchors E-cadherin, a classical adhesion molecule, to the cell surface independent of NCX1 ion transport activity. MDCK cells with NCX1 knockdown exhibited β-catenin nuclear localization and enhanced transcriptional activity. Taken together, knockdown of NCX1 in MDCK cells induces mesenchymal transition by destabilization of E-cadherin and induction of β-catenin transcriptional activity.

Dual role of p120ctn in cancer: epithelial vs mesenchymal. Pamali Fonseka, Suresh Mathivanan, Michael Liem, Ishara Atukorala. La Trobe University, Melbourne, Australia.

Neuroblastoma, a paediatric cancer, accounts for 15% of childhood cancer mortality. Even though the cells resist treatment is poorly understood. Here, we hypothesise that neuroblastoma cells have high expression of mesenchymal markers. Interestingly, we found that deletion of NCX1 in neuroblastoma patient tissues suggested that p120ctn is highly abundant. How, the knockdown of NCX1 in renal epithelial cells (MDCK) induced fibroblast morphology, and increased permeability to rhodamine indicative of leaky cell-cell junctions. Electron micrographs of these cells displayed increased inter-cellular junctional distance also suggesting that NCX1 knockdown altered junctions between adjacent cells. Cells with NCX1 knockdown showed loss of apico-basal polarity in three-dimensional cultures accompanied by expression of mesenchymal markers. Furthermore, NCX1 knockdown cells were capable of anchorage independent growth suggesting that these cells had acquired tumorigenic potential. Interestingly, we show for the first time that NCX1 mRNA and protein expression is greatly reduced in both Wilms tumor and renal cell carcinoma demonstrating a direct correlation between NCX1 expression and the epithelial phenotype. Mechanistically, we provide evidence that NCX1 interacts with and anchors E-cadherin, a classical adhesion molecule, to the cell surface independent of NCX1 ion transport activity. MDCK cells with NCX1 knockdown exhibited β-catenin nuclear localization and enhanced transcriptional activity. Taken together, knockdown of NCX1 in MDCK cells induces mesenchymal transition by destabilization of E-cadherin and induction of β-catenin transcriptional activity.
induced EMT, proliferation and migration. These results suggest that the role of p120ctn is cell type dependent. Overall, the findings from this study suggest that p120ctn plays a pivotal role in progression of neuroblastoma.

#3933 Characterization of oncogenic activity of interferon-induced protein with tetratricopeptide repeats 1 and 3 in human oral squamous cell carcinoma progression. Vijaya Kumar Pidugu, Ai-Hsin Yen, Ying-Chen Chen, Mei-Maan Wu, Chung-Ji Liu, Te-Chang Lee. 1Program in Molecular Medicine, Taiwan International Graduate Program, National Yang-Ming University and Institute of Biomedical sciences, Academia Sinica, Taipei, Taiwan; 2Institute of Biomedical sciences, Academia Sinica, Taipei, Taiwan; 3School of Public Health, Taipei Medical University, Taipei, Taiwan; 4Department of Oral and Maxillofacial Surgery, Mackay Memorial Hospital, Taipei, Taiwan.

Interferon-induced protein with tetratricopeptide repeats (IFITs) family has multiple tetratricopeptide repeats with helix-turn-helix structural motifs that mediate a variety of protein–protein interactions. There are four IFIT genes that have been identified namely, ISG56/IFIT1, ISG54/IFIT2, ISG60/IFIT3, and ISG58/IFIT5. IFIT proteins have been documented to involve in various cellular functions, such as cell proliferation, migration, virus-induced translation initiation, replication and double-stranded RNA signaling. Our previous study has demonstrated that IFIT2 inhibits oral squamous cell carcinoma (OSCC) metastasis. However, little is known about IFIT1 and IFIT3 in the progression of OSCC. In the present study, we are investigating the functional role of IFIT1 and IFIT3 genes in OSCC tumor progression.

Immunohistochemical analysis of 147 patients derived tumor specimens with OSCC revealed a strong correlation between IFIT1 and IFIT3 proteins (p<0.001). Clinicopathological characteristics correlation of IFIT1 and IFIT3 expression levels were positively associated with regional lymph node invasion and nerve invasion. IFIT1 was also associated with venous invasion. Ecopic expression of IFIT1 and IFIT3 proteins in Ca9-22 and SAS cells inducing PCK, EGFR, and AKT activation, resulting in OSCC cells resistance to cisplatin but highly susceptible to tyrosine kinase inhibitor gefitinib, whereas IFIT1 and IFIT3 knockdown SCC25 cells have shown opposite effect. Interestingly, IFIT1 and IFIT3 expression is associated with enhanced cell invasion activity and altered expression of epithelial-mesenchymal transition markers. These data suggested that IFIT1 and IFIT3 may serve as potential biomarkers for the OSCC treatment. The study on how IFIT1 and IFIT3 cause PCK, EGFR, and AKT activation and resistance to DNA damaging agents is ongoing. Our further investigations may explain the molecular signaling mechanism of IFIT1 and IFIT3 in OSCC tumor progression.

TUMOR BIOLOGY: Tumor Evolution and Heterogeneity 2


Survival rates for patients with muscle-invasive bladder cancer have not improved in the past 20 years, and new therapies are imperative. Intratumor heterogeneity can complicate molecular profiling attempts to optimize therapy for cancers harboring several actionable tumor subclones. To develop personalized treatment strategies, there is a need for assays to measure intratumor heterogeneity in bladder cancers. We conducted a pilot study of two muscle invasive high-grade transitional cell carcinoma cases. We used a comprehensive cancer panel (Thermo Fisher) covering >400 cancer genes to analyze distinct tumor loci and matched normal tissues. Based on the identified somatic mutations, we designed a bladder-specific panel to (1) validate our results with increased coverage, and (2) analyze liquid biopsy samples. Using the comprehensive cancer panel, we sequenced 6 tumor loci to an average sequencing depth of approximately 100x. We detected intratumor heterogeneity in both patients: By applying a combination of frequency-based (minor allele frequency >10%) and probabilistic (probability of difference between observed frequencies due to sampling) filters, we identified 44 credible somatic SNVs, including mutations that were not chemically altered by cisplatin or intercalating agents. We used these SNVs to design a custom amplicon panel covering 42 SNVs across 38 genes that is suitable for highly fragmented DNA. The custom panel was used to validate the SNVs in the same tumor regions and in liquid biopsy samples from plasma and urine (approximate coverage 6,000x). In both cases, we identified private mutations re-ported in The Cancer Genome Atlas Urothelial Bladder Carcinoma (TCGA-BLCA) data collection, reflecting tumor evolution. Liquid biopsy samples from urine revealed all trunk mutations but only 1 out of 5 private mutations. We conclude that tumor evolution can affect distinct loci within bladder tumors, which may not be fully represented in liquid biopsy samples. These results suggest a need for analyzing multiple tumor regions to identify all actionable driver mutations. In the future, we plan to apply our assay to additional loci and patients in order to identify optimal bladder tumor sampling strategies.


Introduction: Small cell lung cancer (SCLC) is a high grade neuroendocrine carcinoma of the lung responsible for up to 25% of lung cancer deaths. Treatment in SCLC has not changed significantly in the last 20 years. SCLC initially responds well to chemotherapy, but inevitably recurs. Characterization of tumor heterogeneity and changes in SCLC cell signaling and phenotypes after chemotherapy could yield new insights and therapeutic options. Mass cytometry uses metal labeled antibodies to profile expression and phosphorylation of more than 40 proteins in single cells and offers the opportunity to identify new subpopulations including potential cancer stem cell populations as well as targets for novel therapies in SCLC. Methods: Nude mice with SCLC patient derived xenografts (PDXs) were treated with one cycle of carboplatin/etoposide or saline injection. Tumors were harvested at ~2000mm3, disaggregated, and cryopreserved. PDXs were stained with a 22 marker panel and an intercalator dye to identify nucleated cells. This panel measured phospho-signaling, neuroendocrine, immune, and mesenchymal cell markers, and functional markers including ki67 and cleaved caspase 3. VSI NE analysis and biaxial gating were used to identify major subpopulations of interest. Results: PDX tumors released viable tumor and stromal cells suitable for cryopreservation and mass cytometry. ACK buffer and enzymatic dissociation yielded the best quality cells by depleting red blood cells. Mouse cells, including leukocytes, were excluded using mouse MHC1 gating and iodamer intercalator was used to identify nucleated cells. Single cell protein expression and phosphorylation was analyzed using VSI NE and yielded at least 9 distinct subpopulations based on density islands with neuroendocrine (CD56+) and non-neuroendocrine (CD56-) populations. Chemotherapeutic created cells had dramatic changes in subpopulation distribution compared to matched mock treated tumor. This included 2-3 fold expansion of SOX2+, CD117+, and pSTAT3+ populations with chemotherapy treatment. A small CD44+ tumor subpopulation identified in the chemotherapy treated cells was not present in the matched mock treated tumor suggesting a potential chemotherapeutic resistant/ stem-like subpopulation. Kinase activity showed stable p-AKT overall, but increased p-4E-BP1 in the chemotherapy treated cells. Conclusions: Mass cytometry was able to identify multiple neuroendocrine and non-neuroendocrine cell populations from SCLC PDXs and characterize their signaling. Chemotherapy treated PDX had differential subpopulation distribution with enrichment of multiple stem-like signaling factors. This work demonstrates the utility of mass cytometry and viSNE as novel techniques to identify subpopulations associated with chemoresistance for future targeting and demonstrates the feasibility of this technique for characterizing signaling heterogeneity in human SCLC tumors.

#3936 A branching evolution model at relapse characterizes multiple myeloma patients who responded to upfront combination therapy including new drugs. Carolina Terragna, Marina Martello, Barbara Santacroce, Vincenti Solini, Lucia Pantani, Elena Zamagni, Paola Tacchetti, Beatrice Zanetti, Katia Mancuso, Giulia Martocci, Nicoletta Testoni, Gaia Amel, Rosalinda Termini, Angelo Flores Dico, Enrica Borsi, Giovanni Martinelli, Michele Cavo. 1University of Bologna, Bologna, Italy; 2Fondazione Umberto Veronesi, Milano, Italy.

Intro: Multiple Myeloma (MM) is a biologically complex disease, whose genetic plasticity favors the coexistence of genetically heterogeneous subclones, selected in a Darwinian fashion throughout the disease course. Therapy might represent a major selective pressure over the different subclones, thus supporting an evolutionary model of the disease. Aim: To explore the existence of different clonal evolution patterns in MM, eventually driven by therapeutic selective pressure. P&M The study included 33 pts with symptomatic MM, up-front treated either with combination regimens including a proteasome inhibitor (28), or with cyclophosphamide. For each patient, single MM + plasma samples were collected both at diagnosis and at relapse. SNP array analyses were performed on the CD138+ enriched cell fractions. Results: Two approaches were applied: a) monitoring the variations of macro CNAs; b) focusing on changes of CNAs frequencies, as observed in 27 genes of interest. Both approaches were consistent in highlighting three major evolution patterns: in 7/33
(21%) pts, the genomic background at relapse was almost identical to that of diagnosis. In 13/33 (39%) pts, an overall increase in the frequencies of the same CNAs as observed at diagnosis was detected at relapse. Finally, in 13/33 (39%) pts, either increased or decreased frequencies of several CNAs, as well as several differences in the CNAs type’s prevalence were observed at relapse, as compared to diagnosis. Of interest, we noted overexpression of clonal CNAs mediates the remaining disease to relapse (226 vs 507, respectively) - supported by acquisition of CNAs either commonly described as secondary genomic events (i.e. del17p13, amp1q21, del1p23), or associated to the resistance to bortezomib (i.e. del8p21) - any peculiar CNAs resulted significantly prevalent in the 3 identified subgroups of pts. A high rate (92%) of achievement of VGPR or better quality of response to upfront therapy characterized the subgroup of pts. clonal CNAs in both groups of pts. At the first median of pts was only 20% and PR or SD were observed in 9 and 7 pts, respectively. Finally, the median time to first progression of this subgroup of pts was significantly shorter as compared to that of pts with branching evolution (24 vs 35 months, range 4-41 and 7-123 months, respectively, p=0.01). Conclusion: The genomic architecture of a subgroup of relapsed MM pts, up-front responsive to new drugs-based combination therapies, resulted overall different from that of diagnosis, suggesting a branching evolution of the disease, sustained by the shrinking of the most prevalent clone (therapy-sensitive), as well as by the expansion of subclones (therapy-resistant) not already evident at diagnosis. This observation raises the question whether re-treatment of relapsed pts should be appropriate in the case of branching evolution. Acknowledgements: AIRC (MC), Fondazione Berlucchi (CT), FUV (EB).

#3937 Diversity index as a novel predictor of tumor progression in breast cancer. Yul Ri Chung,1 Hyun Jeong Kim,2 Young A Kim,3 Mee Soo Chang,3 Ki-Tae Hwang,3 So Yeon Park1. 1Seoul National University College of Medicine, Seoul National University Bundang Hospital, Seongnam, Republic of Korea; 2Queen Elizabeth Hospital, Hong Kong, SAR, Hong Kong, Hong Kong; 3Seoul National University Bundang Hospital, Seongnam, Republic of Korea; 3Seoul National University Boramae Hospital, Seoul, Republic of Korea.

Intratumoral genomic heterogeneity leads to tumor progression and therapeutic resistance. However, due to the difficulty associated with its assessment, application of intratumoral genetic heterogeneity as a prognostic or predictive marker is limited. We investigated the significance of Shannon diversity index as a tool for measuring genetic heterogeneity by performing fluorescence in situ hybridization of c-Myc gene in invasive breast cancers, and correlated the Shannon diversity index for c-Myc copy number variation with clinicopathologic features of tumor including patient survival. Shannon index for c-Myc copy number variation strongly correlated with average c-Myc copy number and was significantly higher in tumors with c-Myc genetic or regional heterogeneity than in those without c-Myc amplification. High Shannon index was associated with high histologic grade, lymphovascular invasion, p53 overexpression, high Ki-67 proliferation index, hormone receptor negativity, and HER2 amplification. In survival analyses, c-Myc amplification or c-Myc copy number variation was associated with patient survival. However, a high level of Shannon index was associated with poor disease-free survival. In subgroup analyses, it was found to be an adverse prognostic factor in hormone receptor-positive group, but not in hormone receptor-negative group. In a validation set, high Shannon diversity index for c-Myc copy number variation was also found to be associated with poor survival. We further investigated the correlation with clinicopathologic features and predictive power of Shannon index using another gene, fibroblast growth factor receptor 1 (FGFR1) and observed that high Shannon index for FGFR1 gene copy number variation was an independent prognostic factor for poor clinical outcome in a whole group and in hormone receptor-positive subgroup. In conclusion, this study demonstrated that higher diversity index was associated with adverse pathologic parameters of breast cancer and poor clinical outcome suggesting that Shannon diversity index, which represents intratumoral genetic heterogeneity, can be used as a potential biomarker for tumor progression and prognosis in patients with breast cancer.

#3938 Dynamic interconversion of cancer cells: single-cell analysis of population-based equilibrium. Yuen San Chan,1 Edwin-Wai Kin Yu,2 William Weimann Wong1,2 Chi-shun Wong,1 Tsz Wai Tang,1 Tsz Kai Fong,1 Tiffany Yee,1 Chiu Xin Yip,1 Joseph Siu-Kiu Au,1 Mengsu Yang1. 1City University of Hong Kong, Hong Kong, Hong Kong; 2Queen Elizabeth Hospital, Hong Kong, SAR, Hong Kong, Hong Kong.

Background: Multiple subpopulations of cells with different phenotypes and genotypes coexist within malignant tumours. Different subpopulations seem to maintain themselves in an equilibrium position via stochastic interconversion. Such plasticity plays an important role in cancer progression, such as transition from epithelial states to mesenchymal state during metastasis, dedifferentiation of non-CSCs to CSCs and rapid transition to MDR cells during drug exposure. We attempt to explore this interconversion plasticity at both population and single cell levels to better understand the dynamics of phenotypic transition. Methods: We isolated CD90+ and CD90- cells from A549 lung adenocarcinoma cell line by flow cytometry and then characterized their phenotypic behaviors and interconversion dynamics at both population and single cells level. Single cells were cultured in 96 well plate for 13 days. Viable cells were then transferred into 6 well plate for further expansion until enough cells were collected for measurement of CD90 expression in flow cytometry. Results: A549 maintains a stable expression of CD90 (~50%) which is bimodal distributed. The sorted subpopulations eventually achieved a new equilibrium (CD90+ à 60%, CD90 - 30%) and did not converge during the bulk cell interconversion. Individual single cell clones showed large variation of CD90 expression. The distribution of CD90 expression was significantly different between CD90+ and CD90- single cell whereas the bulk populations achieved a similar average equilibrium as compared with the bulk cells. We noted two types of clones defined as transition clones and committed clones with differential interconversion capacities. The later one showed no sign of interconversion and was committed to the original CD90+/− expression level. Further experiments showed that the committed CD90+ cells were more mesenchymal with higher migration ability, while the committed CD90- cells were more epithelial and tumorigenic. Conclusions: Interconversion plasticity varied from cell to cell and the committed cells prohibited the return of sorted cell subpopulations returned back to the original equilibrium. Phenotypic studies of CD90+ and CD90- revealed that the interconversion between them was EMT related.

#3939 Evolution and drug resistance of primary and relapse tumors in diffuse large B-cell lymphoma. Rainer Lehtonen,1 Amjad Alkodsi,1 Kaiyang Zhang,2 Mikko Kivikoski,3 Emilia Kozlowska,4 Alejandro Cervera,1 Annika Pasanen,2 Harald Holte,3 Sirpa Leppa,3 Sampsan Hautaniemi,1 1University of Helsinki, Helsinki, Finland; 2University of Oslo, Norway.

Our integrated multi-omics analysis of paired primary and relapse DLBCL tumors provides comprehensive view to tumor evolution, drug resistance and disease heterogeneity. DLBCL is an aggressive systemic disease in which tumors can arise in virtually any part of the body. Activated B-cell (ABC) and germinal center B-cell (GCB)-like subtypes differ molecularly and clinically. Three-year progression-free survival (PFS) in ABC is around 40% vs over 80% in GCB. Whole genome (WGS, 7 patients), transcriptome (total RNA, 7 patients) and methylome sequencing data produced using the Illumina HiSeq platform was complemented with Illumina TruSeq amplicon sequencing covering 102,571 bp of the genome on 788 custom selected targets. Altogether 23 fresh frozen and 28 paraffin embedded tumor samples from 24 patients with an advanced disease and at least one relapse event were included in the study. To investigate the clonal hierarchy of the primary and relapsed tumors we employed dirichlet process clustering using cancer cell fraction (CCF) estimates or variant allele frequencies from somatic point mutations. Divergent evolution was prominent in the GCB subtype whereas in ABC subtype subclonal mutations are rare and only few genomic differences were detected between primary and relapse tumors (p-value=0.047, Pearson’s Chi-squared test on 7 patients with WGS data). suggesting pre-treatment drug resistance which is probably triggered by global hypo- or hypermethylation at relapse stage. In the GCB subtype primary and relapse branches have diverged much before diagnosis and subsequently developed independently. Number of emerging primary or relapse specific clonal and subclonal mutations correlate with PSF and overall survival (OS). Mutational signatures were concordant with the inferred evolutionary models in four out of five GCB cases. Contribution of the somatic hypermutation signature was higher in resistant tumors and correlated with time to progression of the disease. We identified 72 relapse tumor related genes including many known B-cell lymphoma genes such as CD79B, IGLLS, PIM1, SGK1. These were used in survival analysis in the CGCI RNA data set derived from 92 diagnostic DLBCL tumors. Patients with subclonal mutations in these genes had shorter PSF and OS (Kaplan-Meier p-values 0.0122 and 0.0071, respectively). To validate and further define our hypothesis we are simulating DLBCL evolution as a discrete time branching process model (Galton-Watson branching processes). The goal is to reconstruct phylogenetic trees from a simulated tumor and explain different types of evolutionary models inferred from the patient data. Despite of remarkable heterogeneity in DLBCL we observed two main evolutionary models which are concordant with disease subtype, patient’s survival, treatment response at diagnosis and relapse, and molecular features of a tumor.
Analysis of metabolic gene expression is compromised by tumor hétérogène-
ity. Therefore, we investigated the use of RNA expression levels from ENO1, which
encodes enolase 1, to adjust for glycolytic heterogeneity within glioblas-
tomas attributed to irregular vascularization, necrosis, surgical removal, etc.
Recently, this approach revealed relationships between carbonic anhydrases and
aromatic aminopeptidases (Beckmann et al. 2018). The use of such expression data from
frozen tissue samples to 22 glioblastomas, expression of the metabolic gene encod-
ing hypoxia inducible factor - 1A (HIF1A) and its target, vascular endothelial growth
factor A (encoded by VEGFA), were contrasted with two non-metabolic genes, i.e. those encoding platelet derived growth factor A (PDGFA) and epider-
mal growth factor (EGF) using RT-qPCR analysis. Genes of interest (GOI) were
initially normalized with delta-delta threshold methodology using housekeeping genes, ACTB and GAPDH. Then, concurrent expressions of ENO1 (ave 0.83 +/- 0.18 CI (95%), range of 0.22 - 1.97 times normal) were used to
mathematically transform expressions of GOI to multiples of ENO1 to puta-
tively correct for glycolytic variation. Expressions of PDGFA (ave 1.90 +/- 0.69
CI (95%), 0.17 - 4.01 times normal) and EGF (ave 1.25 +/- 0.57 CI (95%), 0.07 -
5.14 times normal), had correlations, r = 0.65 and 0.66, unranked (Pearson’s) and
ranked ( Spearman’s) data, respectively, among the 22 tumors. After ENO1
transformation, r = 0.68 for their unranked data & the difference in their ranges
rose to 1.31-fold. Prior to ENO1 transformation, expressions of HIF1A (ave 1.33
+/- 0.28 CI (95%), 0.25 - 2.53 times normal) and VEGFA (ave 2.89 +/- 1.36
CI (95%), 0.17 - 9.94 times normal) had negative correlations, r = -0.15 and -0.09,
unranked and ranked data, respectively. However, after transforming HIF1A
and VEGFA expressions to multiples of concurrent ENO1 expression, their
correlation became positive in both unranked and ranked data, with r = 0.30 for
the ranked (Spearman) data. The difference in the ranges of the two metabolic
genes expanded to 6.76-fold. Whereas the Wilcoxon Rank Sum of VEGFA’s
transformed values, with versus without 2.02-fold elevations of HIF1A ex-
pression, was insignificant, p = 0.704, using ENO1 transformed values indicated
a significant relationship, p = 0.042. Therefore, ENO1 transformation revealed
the anticipated relationship between HIF1A and its target, VEGFA, at the RNA
expression level that was not initially apparent in this small group of tumors.
Transformation via expression levels of ENO1 compensates for glycolytic het-
erogeneity to reveal and highlight relationships among metabolic genes when
analyzing resected tumors. Support from The Pittsburgh Foundation’s Walter L.
Copeland Fund for Cranial Research (D2006-0379) and the Molecular Lab,
Lori Arlinghaus, 2 Xia Li, 3 Thomas E. Yankelov 1.

TUMOR BIOLOGY: Tumor Evolution and Heterogeneity 2

#3941 Assessing heterogeneity in DCE-MRI data of breast cancer patients
to predict treatment response. Anum Syed, 1 Anna G. Sorace, 1 Stephanie L. Barnes, 1
Lori Arlinghaus, 2 Xia Li, 3 Thomas E. Yankelov 1.

Introduction: We investigate the ability of multi-parametric, voxel-based
classifications of tumor heterogeneity from magnetic resonance imaging (MRI)
to predict the response of breast cancer patients to neoadjuvant therapy (NAT).
In particular, we use high-dimensional analysis of the longitudinal changes
in the vascular and cellular characteristics provided by quantitative dynamic
counter enhanced MRI (DCE-MRI) to predict response. Methods: DCE-MRI data was acquired from 34 patients with stage II/III breast cancer before
initiating NAT (t1) and after one cycle of NAT (t2). Pathological complete response (pCR) and non-pCR was defined at the time of surgery. Non-pCR
patients were further subdivided into partial response (>30% decrease in tumor
volume), progressive disease (PD, >20% increase in tumor volume), and stable
disease according to the Response Evaluation in Solid Tumors (i.e., RECIST)
criteria. For each tumor voxel, DCE-MRI data was modeled to extract the extravascular, extracellular volume fraction, \( v_e \), the plasma volume fraction, \( v_p \), and the volume transfer coefficients, \( k^\text{trans} \) and \( k^\text{app} \) which correspond to the rate of wash-in and wash-out of contrast agent, respectively. Multi-parametric voxel-

#3942 Evolution of genomic alterations in breast tumors. Anu G. Gaba, 1
Megan Landsverk, 2 Jennifer L. Weiss, 1 Paul Thompson, 1 Chun-Hung Chan, 2
Steven F. Powell, 2 Lora J. Black, 2 James M. Ford 3.

#3943 Determination of the molecular profile of Chilean patients with
sporadic colorectal cancer. Ana Maria Wielandt. Clinica Las Condes, Santiago, Chile.

Introduction: Colorectal cancer (CRC) represents a major health problem
worldwide, ranking third among the most frequent cancers and fourth in
cancer mortality. In Chile CRC is the third leading cause of cancer death. CRC is
a heterogeneous disease, and three carcinogenic pathways have been described
to determine the molecular profile: The microsatellite instability (MSI), chromo-
somal instability (CIN) and methylator phenotype (CIMP). Several studies have
 tried to propose CRC molecular subtypes based on the ways described above
since CIN, MSI and CIMP are not mutually exclusive and overlapping of the 3
ways is still unclear. The most recent subtyping of CRC was in 2015, where four
molecular subtypes consensus (CMS 1 to 4) were established according to clini-
copathological features, molecular pathways involved and mutational status of

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KRAS, BRAF and PI3KCA genes in order to encourage clinical translation. 21% of tumors failed to be categorized into these subtypes. This classification allows us to understand and determine the clinical, pathological and biological characteristics of CCR to provide customized therapies to patients. Aim: To describe the molecular profile of carcinogenic pathways in HCC, MSI CIMP in Chilean patients, covering advances in the molecular characterization, both the of 56 patients operated between 2010-2016 at Clinica Las Condes, without neo-

adjuvant treatment. DNA was extracted from normal and tumor tissue of each patient and using PCR amplification of several markers CIN, MSI and CIMP were defined. By combining the 3 variables analyzed, tumors were classified in Group-A (CIN-high), Group-B (CIN-low / CIMP-high / CIN-high), Group-C (CIN-low / CIMP-low / CIN-low), and Group-E (CIN- neg / CIMP-neg / CIN-neg). Results: In this study, tumors in Group-A (17%) are located on the right side in patients older than 60 years and early stages. Tumors Group-B / C (18,9%) occur in patients older than 60 years and BMI=25 kg / m², with wall invasion pT3-T4 and late stages. Tu-

mors of Group-D / E (54,7%) are the most frequent, located on the left side, without vascular invasion and moderately differentiated. Conclusions: This study allowed to classify Chinese patients in 3 subgroups considering the 3 main routes described for carcinogenesis development: CIN, MSI and CIMP, which are consistent with the percentages described by the global consortium. Studies that analyze MSI and CIMP or MSI and CIN have consistently shown that patients with MSI-high tumors have a better prognosis in our case (17%). De-

spite therapeutic advances based on this molecular characterization, both the of tumors and the different combinations of pathways involved have shown that the response to therapy varies depending on the individual profile of each patient, therefore in clinical practice it would be important to establish that profile for each of them. Fondect 1140012.

#3944 Characterization of molecular heterogeneity in hepatocellular car-

cinoma: Trunk and branch drivers. Sara Torrecilla, Daniela Sia, Andrew N. Harrington, Zhongyang Zhang, Genis Camprero, Agrin Moeni, Toffanin Sara, Isabel Fiel, Ke Hao, Monica Higueras, Lasa Caballero, Helena Cornella, Mih-

ind Mahajan, Yujin Hoshida, Augusto Villanueva, Sander Florman, Myron Schwartz, Josef Llovet. Icahn School of Medicine at Mount Sinai, New York, NY.

Introduction: Molecular heterogeneity occurs in Hepatocellular carcinoma (HCC), but its implications in clinical decision-making are unknown. The clonal evolution model explains that trunk alterations arise at early stages and are shared by all malignant cells, whereas branch alterations occur in subclonal tumor cells. We aim to characterize the genomic landscape of HCC through the identification of trunk driver alterations and the study of its distribution in intra- and inter-tumor heterogeneity. Methods: 153 HCC samples representing the multiple steps of hepatocarcinogenesis were analyzed by deep targeted-se-

genomic sequencing and promoter regions of the most frequent mutated drivers in HCC. Genes mutated in early lesions [39 dysplastic nodules and 54 early HCCs (eHCC) defined as <2cm, without satellites or vascular invasion] were classified as candidate trunk genes. Candidate trunk genes were further explored in two additional cohorts: a) intra-tumor heterogeneity cohort: 42 tumor regions of 21 tumors (>4cm: 2-3 regions/tumor); and 2) inter-tumor heterogeneity cohort: 39 tumors from 17 patients with multinodular lesions (2-3 nodules/patient). Transcriptome and copy-number variations (CNVs) were an-

alyzed using expression and SNP arrays, respectively. Results: A total of 46 mu-

tations were identified in the cohort of early lesions. Average number of muta-

tions and CNV aberrations were higher in eHCCs than in dysplastic nodules (1.1 vs 0.5, mutations/patient (p=0.03), and 8% vs 0.6% of aberrant chromosomal arms (p<0.0003), respectively). Overall, 72% (23/32) of the sequenced eHCCs presented at least 1 trunk mutation, being TERT, TP53 and CTNNB1 the most frequent (21/23, 91%). In the intra-tumor heterogeneity cohort, 81% (17/21) tumors showed at least 1 shared mutation in TERT, TP53 and/or CTNNB1 between different tumor regions (trunk drivers). In the inter-tumor heterogene-

cy cohort, the similarity of the CNV-profile of multinodular tumors was used to classify tumors as intra-heterogeneity (26% of tumors) or non-shared (74% of tumors). 6/17 (35%) of patients harbored clonal tumors according to their CNV profiles (Pearson p<0.05). Clonality classification was further confirmed by gene expression-based hierarchical clustering. 82% (9/11) of the sequenced clonal tumors shared TERT, TP53 and/or CTNNB1 as trunk alterations. In contrast, no trunk mutations were shared across non-clonal tumors. Conclu-

sions: TERT, TP53 and/or CTNNB1 are trunk drivers mutated in early HCC tu-

mors that remained as trunk aberrations across different regions of the same tumor and between primary and metastatic nodules. These mutations are early trunk drivers that can be captured with single biopsies and could represent ideal therapeutic targets in the future.
Intratumor heterogeneity impacts treatment response in rectal carcinoma. Rüdiger Meyer,1 Nicole E. McNeil,2 Darawalee Wassaga,1 Lena Anthuber,1 Noam Auslander,3 Danny Wangsa,1 Zhongqiu Zhang,1 Daniel Rosenberg,4 Eytan Ruppin,2 Jens K. Habermann,3 Thomas Ried1. National Cancer Institute/NIH, Bethesda, MD; 2University of Maryland, College Park, MD; 3Watterson Hospital/University of Connecticut School of Medicine, Waterbury, CT; 4University of Connecticut Health Center, Farmington, CT; 2University of Lübeck, Lübeck, Germany.

Individual response to radiochemotherapy (RCT) in rectal cancer patients is highly variable and the underlying mechanisms of treatment resistance of cancer cells are poorly understood. Recent studies revealed a considerable degree of genomic tumor heterogeneity. We hypothesize that this heterogeneity has a direct impact on treatment response as subpopulations of cancer cells are resistant to currently used RCT and facilitate tumor growth under treatment. To address this highly relevant clinical issue, patient-derived rectal cell lines were established. The tissue was derived from a 59-year-old male presenting with an adenocarcinoma of lower rectum (T3,N1,M0), who was treated by neoadjuvant RCT (50.4 Gy plus 5-FU) and low anterior resection. The neoadjuvant treatment induced a shrinkage of the tumor (staging of the surgical specimen: ypT3,N0), suggesting a partial response to RCT. A biopsy of the treatment naïve tumor was implanted heterotopically into an immunodeficient nude mouse. After in vivo growth, the tumor was dissociated and introduced to an in vitro culture where murine stromal cells were depleted using antibody-based columns. The cell line proved to be highly responsive by immunofluorescence for human microsatellite instability as well as genotyping using short tandem repeat profiling. The characterization of the genome by array CGH and spectral karyotyping (SKY) revealed a highly complex near-triploid karyotype with numerous chromosomal imbalances, which are specific for colorectal cancers, including gains in chromosomes 7, 13 and X. We also observed other structural abnormalities including an anisochromosome 1q. Multiplex-FISH by consecutive hybridization of probes for 15 gene loci, which are known to be relevant in colorectal cancer genesis, revealed a significant degree of clonal heterogeneity of the cell line. Single cell sorting was then performed to establish single cell derived cell lines from the genomically well characterized parental cell line. Exposure of the single cell derived cell lines to irradiation in combination with 3 μM 5-FU revealed substantial differences in treatment response. Analyses of the genome by array CGH and transcriptome by RNA-Seq of the respective single cell derived cell lines are currently underway. This will allow the comparison of the respective sensitivities to RCT to the identified aberration profiles. These data will facilitate the understanding of therapy resistance and potentially allow a reliable prediction of the patient’s response. A tailored therapy is an important step towards individualized treatment in colorectal cancer patients to avoid therapy resistance.

Comprehensive characterization of genomic and transcriptomic heterogeneity in advanced bladder cancer by multiplexed analysis. Thordis Thomsen,1 Philippe Lamy,1 Noam Auslander,2 Danny Wangsa,1 Søren Vang,1 Line Reinberg,4 Eytan Ruppin,2 Jens K. Habermann,3 Thomas Ried1. National Cancer Institute/NIH, Bethesda, MD; 2University of Maryland, College Park, MD; 3Watterson Hospital/University of Connecticut School of Medicine, Waterbury, CT; 4University of Lübeck, Lübeck, Germany.

Barrett’s esophagus (BE) is defined as metaplasia of the squamous epithelium to a specialized columnar epithelium, and is a well-known high risk factor for the development of esophageal adenocarcinoma. Challenges remain in early detection and predicting which patients may progress to dysplasia. Here we describe a method by which we compare human clinical IRB-approved BE biopies and adjacent normal squamous epithelium using tissue dissociation and deep immunophenotyping by flow cytometric collection and analysis. A cassette of canonical epithelial or tumor stem cell-associated targets (EpCAM, CD24, CD44, CD49f, Her2/neu, CD133, CD90, CD166, and CD29), immune cell markers (CD3, CD4, CD8, CD25, and CD19), as well as epithelial or tumor stem cell-associated targets (EpCAM, CD24, CD44, CD49f, Her2/neu, CD133, CD90, CD166, and CD29), immune cell markers (CD3, CD4, CD8, CD25, and CD19) as well as targets associated with myeloid derived suppressor cells (CD14, CD15, CD33, CD11b, HLA-DR, CD31, and CD86) were used to discern differences across subjects and between cellular compartments in normal and BE tissue. The Barret’s samples show a majority population with a characteristic phenotype (EpCAM+CD133lowCD49flow) when compared with normal squamous tissue samples (EpCAM-CD133-CD49frich). The samples separate into two discrete groups using hierarchical clustering based on differential surface marker expression of combined epithelial and immune cell markers, and also reveal unexpected, shared phenotypes for some normal and BE samples. Principal component analysis supports this grouping and was used to identify more compelling targets for categorization, such as CD133 and CD49f. The resulting expression and distribution of targets offer a phenotypic fingerprint characterizing both the epithelial cell and immune cell compartment. Besides providing the potential for revealing clinically relevant differences between BE and normal tissue, as well as across subjects, the discovered surface immunophenotypes can be used to target specific subpopulations from dysplastic tissue for further molecular investigation. A deeper understanding of the role of such specific subpopulations should increase the prospects for more complete understanding of BE and its progression.

A novel combination therapy targeting BCL6 and phospho-STAT3 defeats intratumor heterogeneity in a subset of non-small cell lung cancers. Dhruba Deb,1 Satwik Rajaram,2 Jill E. Larsen,3 Patrick P. Dospopy,2 Rossella Marullo,3 Longshan Li,1 Kimberley Avila,1 Leandro Cerchietti,3 John D. Minna,1 Lani F. Wu,2 Steven J. Altschuler2. 1Becton Dickinson Technologies, Research Triangle Park, NC; 2University of Pennsylvania, Philadelphia, PA; 3Becton Dickinson Life Sciences, San Jose, CA.

Bladder cancer is a highly heterogeneous disease - both clinically and at the genomic and the transcriptomic levels. Despite complete tumor resections, recurrent tumors share a high number of mutations with the initial or earlier tumors, indicating that new tumors arise from fields of transformed areas of cells in the urothelium (i.e. field disease). Furthermore, the high mutational load found in bladder cancer and the assumed high degree of heterogeneity may have large implications on therapy response. Thus, detailed knowledge on tumor heterogeneity and mutational dynamics in adjacent normal urothelial cells is needed in order to understand field disease, disease development and disease progression. To address this, we performed comprehensive genomic and transcriptomic analysis of multiple samples of tumor and normal tissue procured from four cystectomy specimens and assessed the heterogeneity. Multiple tumors, lymph node metastasis, relapse biopsies and adjacent normal tissue from four patients with muscle invasive disease were analyzed. A targeted panel (illumina TruSeq Custom Amplicon v1.5) was designed from whole exome sequencing of bulk tumor DNA and germline DNA. Targeted sequencing of DNA from small cellular regions procured by laser-microdissection from tumor biopsies and from adjacent normal tissue was used for generating a comprehensive genomic map of the bladder. Transcriptomic profiling (Fluidigm GE) was carried out using RNA from LMD procured tumor regions matching the regions used for targeted sequencing. Results: In total, whole exome sequencing was performed on DNA from 15 bulk tumors with an average coverage of 39X. We identified a total of 331, 862, 685 and 1344 mutations, respectively, in the four patients with 15-85% of the mutations being ancestral. Of all the mutations, 95, 130, 117 and 335, respectively, were estimated to have a functional impact. Targeted sequencing of 207, 595, 538 and 483 mutations in the four patients was performed on DNA from 137 small cellular regions, including DNA from 17 adjacent normal tissue regions. We observed low local heterogeneity while comparing more distant regions revealed significantly higher levels of heterogeneity. This observation was in agreement with the heterogeneity observed at the transcriptomic level, where some regions within the same bladder appeared to have basal characteristics while others appeared to have luminal characteristics. Conclusion: Large intra-patient genomic and transcriptomic heterogeneity can be observed in bladder cancer. However, even in multifocal tumors, all the regions shared a substantial number of mutations, indicating a common origin. Ongoing investigations indicate the presence of low frequency tumor associated mutations in normal appearing tissue.

Deep immunophenotyping using flow cytometry of dissociated cells from Barrett’s esophagus and matched adjacent squamous epithelium defines distinct phenotypic clusters. Friedrich G. Hahn,1 Eileen Snowden,1 Warren Porter,1 Mitchell Ferguson,1 William S. Dillmore,1 Stephanie S. Yee,2 Taylor Black,2 Maureen DeMarshall,2 Aaron Middlebrook,2 Smita Ghanekar,1 Aníl Rustgi,2 Erica L. Carpenter,2 Rainer Blaesi,3 1Becton Dickinson Technologies, Research Triangle Park, NC; 2University of Pennsylvania, Philadelphia, PA; 3Becton Dickinson Life Sciences, San Jose, CA.

Barrett’s esophagus (BE) is defined as metaplasia of the squamous epithelium to a specialized columnar epithelium, and is a well-known high risk factor for the development of esophageal adenocarcinoma. Challenges remain in early detection and predicting which patients may progress to dysplasia. Here we describe a method by which we compare human clinical IRB-approved BE biopies and adjacent normal squamous epithelium using tissue dissociation and deep immunophenotyping by flow cytometric collection and analysis. A cassette of canonical epithelial or tumor stem cell-associated targets (EpCAM, CD24, CD44, CD49f, Her2/neu, CD133, CD90, CD166, and CD29), immune cell markers (CD3, CD4, CD8, CD25, and CD19), as well as targets associated with myeloid derived suppressor cells (CD14, CD15, CD33, CD11b, HLA-DR, CD31, and CD86) were used to discern differences across subjects and between cellular compartments in normal and BE tissue. The Barret’s samples show a majority population with a characteristic phenotype (EpCAM+CD133lowCD49f) when compared with normal squamous tissue samples (EpCAM-CD133-CD49frich). The samples separate into two discrete groups using hierarchical clustering based on differential surface marker expression of combined epithelial and immune cell markers, and also reveal unexpected, shared phenotypes for some normal and BE samples. Principal component analysis supports this grouping and was used to identify more compelling targets for categorization, such as CD133 and CD49f. The resulting expression and distribution of targets offer a phenotypic fingerprint characterizing both the epithelial cell and immune cell compartment. Besides providing the potential for revealing clinically relevant differences between BE and normal tissue, as well as across subjects, the discovered surface immunophenotypes can be used to target specific subpopulations from dysplastic tissue for further molecular investigation. A deeper understanding of the role of such specific subpopulations should increase the prospects for more complete understanding of BE and its progression.
ERK/2 among a collection of Human Bronchial Epithelial Cells (HBECs) that have been oncogenically transformed with combinations of TP53, K-RAS, and MYC, commonly found alterations in non-small cell lung cancer (NSCLC). We studied ~3000 cells/signaling marker/HBEC oncogenotype variant using immunofluorescence assays and single-cell image analysis (>1M data points). For downstream analysis, we created data sets that were curated with respect to the Western blot and siRNA mediated knockdown assays. We utilized inhibitors to STAT3 and BCL6 in MTI drug sensitivity and colony formation assay in a panel of NSCLC lines. We used xenografted subcutaneous tumors for the in vivo validation of our results. Results: When all three oncogenic changes were present and the HBECs were tumorigenic, we observed STAT3 upregulation and SMAD2/3 downregulation. Interestingly, these STAT3 and SMAD2/3 signaling changes were found to be mutually exclusive in single cells within the transformed HBEC strain. We targeted the STAT3 upregulated subpopulation with the STAT3 inhibitor Stattic. But, Stattic treatment failed to eliminate the SMAD2/3 downregulated subpopulation. To target the SMAD2/3 down-regulated subpopulation, we identified BCL6, a downstream gene of SMAD2/3, as a novel target in transformed HBECs. Next, to test the generality of BCL6 as a target, we studied 5 NSCLC cell lines with various levels of BCL6 expression: H1693, H1819, H1993, HCC827 and H2009. Our data suggests that BCL6 can also be a therapeutic target in a subset of NSCLC lines. Then we tested the response of these NSCLC lines to a combination of BBI608 (potent STAT3 inhibitor) and FX1 (BCL6 inhibitor). The combination treatment eliminated more cancer cells than the single treatments alone. Finally, we confirmed the response of these NSCLC lines to a combination of BBI608 (potent STAT3 inhibitor) and FX1 (BCL6 inhibitor). The combination treatment eliminated more cancer cells than the single treatments alone.

**#3951** Colony lysate arrays for proteomic profiling of drug-persistent cancer cell subpopulations.

Colonal heterogeneity of cancer cells is one of the key properties by which to understand relapse after anticancer drug therapy. However, a very limited number of technologies are available for individually profiling these heterogeneous drug-resistant subpopulations. Therefore, the development of techniques to monitor molecular changes in the subpopulations responsible for drug-resistance, namely, colony-forming drug-tolerant persisters (DTPs), is needed in order to understand the occurrence of cancer relapse. To profile DTPs at the molecular level in relation to the colony-forming function, this study aimed to develop colony lysate array (CoLA) for the quantitative measurement of protein levels in individual colonies. To investigate multiple parameters that may affect the emergence of DTP, DTPs derived from 5 cancer cell lines grown in the presence of 6 doses of 4 different drugs were collected. A set of >2,000 colony lysates was printed onto a nitrocellulose-coated glass slide using a microarrayer, which has been used for the production of reverse-phase protein arrays (RPAs). Each resulting CoLA slide was stained with a specific antibody from the list of 44 functionally-diverse proteins. Immunostained slides were then scanned using an optical flatbed scanner for quantifying the spot intensity. To evaluate whether the CoLA system produced biologically relevant data relating to the DTPs, the epithelial/non-epithelial protein relationship between E-cadherin and vimentin was examined. With the proteomic profiling of >2,000 DTPs, E-cadherin was negatively correlated with vimentin (r = -0.74, P < 0.0001) and positively correlated with CK-8 (r = 0.86, P < 0.0001), suggesting that DTPs may flexibly transit their biological entity in response to drug administration. A set of hierarchical clustering of DTPs displays proteomic patterns in each dose, allowing the comparison of proteomic changes in a dose-response manner. For example, the “cispactin-induced cluster” demonstrated similar proteomic patterns regardless of the drug dose. In contrast to cispactin, the gefitinib-induced cluster indicated that the proteomic patterns were similar at different concentrations from 0 to 0.1 μM; however, those at 1 and 10 μM were distinct from those at lower concentrations. Individual protein analyses of the DTPs revealed that the level of STAT3 decreased while that of OCT4A increased according to the gefitinib concentrations. An RPPA-modified method, CoLA, for individual colony profiling has been successfully established. The results of validation studies suggest that CoLA can be a tool for understanding the emergence mechanism of DTPs. The molecular characteristics of DTPs may reflect the entity of cancer cell subpopulations that contribute primarily to post-chemotherapeutic cancer relapse. Further technical development will enhance understanding of the occurrence of cancer relapse.

**#3952** Rapid autopsy programs: A systematic review.

Brian Li,1 Neesha Dhanl,1 WeiYan Wu,2 Shawn Khan,2 Elysa Grose,2 Princess Margaret Cancer Centre, Toronto, Ontario, Canada; 2McMaster University, Hamilton, Ontario, Canada.

A Rapid Autopsy Program (RAP) is an advanced method of biopsy specimen procurement and processing that addresses the current limitation in the method in order to obtain substantial amounts of high-quality, fresh tissue in support of current and future research. Rapid autopsies were first introduced in the late 1980’s and are still a relatively novel approach, but growing in popularity as a viable alternative to traditional tissue sampling methods for cancer research. In oncology, primary tumour, metastatic, and normal tissue from uninvolved organs if available are sampled snap frozen or fixed in formalin for downstream proteomic and transcriptomic analysis. The aim of this review is to inform best practice for collection and use of biospecimen in oncology. Medline, Embase, Cochrane Database of Systematic Reviews, Cochrane CENTRAL, and Ovid MEDLINE will be searched with devised strategies and screened from inception until present. In addition, grey literature will be reviewed. Two independent reviewers will be responsible for study selection and data extraction. The results of the systematic review are pending.
comprising less than 5% of the total lesion - these would not be reliably detected by standard methodology, and would likely be responsible for the failure of targeted therapy. More generally, we envisage that Basescope™ technology will be a useful tool in basic research, and furthermore is readily transferrable to clinical applications.

#3954 Single-cell misregulation of NRF2 in basal-like breast cancer. Elizab- eth Pereira, Kevin Janes. University of Virginia, Charlottesville, VA

Cancer is driven by a complex and heterogeneous combination of genes and pathways, many of which are not explicitly oncogenic on their own. These so-called non-oncogenes are required for maintenance of the tumorigenic state, but they are not mutated themselves. One way to identify “non-oncogene” pathways is to evaluate how proliferating cells adjust to variable and evolving environ- ments. By examining heterogenous transcriptional states of single cells in a 3D culture model of breast epithelial organization, we uncovered NRF2 as a candidate transcription factor upstream of an important regulatory state for morphogenesis. NRF2 is a known oncogene in lung cancer, but mutations in the transcription factor are uncommon in breast cancer. To examine the functional impact of NRF2 signaling, we engineered several triple-negative breast cancer cell lines with inducible short hairpin RNA to knockdown NRF2. In claudin-low MDA-MB-231 cells, knockdown of NRF2 caused dramatic reduction in stellate invasion compared with shGFP control. In basal-like HCC-1806 cells, knockdown of NRF2 caused a reduction in “ruptured” tumorspheres, in which cells disseminate from their originating site. In a non-transformed breast epithelial cell line, overexpression of a non-degradable form of NRF2 caused greater vari- ation in the size of acini, while knockdown of NRF2 narrowed the size distribu- tion of acini. Therefore, knockdown of NRF2 substantially normalizes the size and shape of both non-transformed and cancerous 3D structures. We also ana- lyzed expression levels of the genes in the provisional NRF2-regulated cluster after NRF2 knockdown or overexpression. Upon NRF2 knockdown, the p53-regulated transcripts CDKN1A (p21) and RPS27L were strongly induced, sug- gesting that p53 may be activated after NRF2 loss. Our results suggest a func- tional importance for NRF2 in multiple breast-epithelial and TNBC contexts, and raise the possibility of interplay between NRF2 and p53. A detailed under- standing of NRF2 regulation and dynamics could enable new strategies to dis- rupt this non-oncogene addiction and re-sensitize cancer cells to their environment and to chemotherapy.

#3955 Spatially resolved, multiplexed digital characterization of protein and mRNA distribution and abundance in formalin-fixed, paraffin-embed- ded (FFPE) tissue sections based on NanoString’s Digital Spatial Profiling (DSP) technology: applications to immune-oncology (IO) and tumor hetero- geneity. Chris Merritt, Jae-Seung Jung, Chang Young Jun, Han Eun Kim, Jia-Ping. 1NanoString Technologies, Inc., Seattle, WA; 2MD Anderson Cancer Center, Houston, TX

Introduction: As intra-tumoral heterogeneity has emerged as a challenge in development of targeted cancer therapeutics, the tissue context of biomarker levels and colocalization of key immune-regulatory proteins has become an increas- ingly important aspect for understanding tumor immune responses, pa- tient classification and stratification. Historically, immunohistochemistry and in situ hybridization have been used to assess spatial heterogeneity of targets in clinical samples. These approaches, however, have limited multiplexing capacity and dynamic range. Here, we use DSP technology, a spatially resolved approach for quantifying up to 800 protein or RNA targets with over 5 logs of dynamic range in a single FFPE slide to overcome these limits. Methods: The technology uses DNA oligo tags for either protein or RNA detection. For protein detection, a cocktail of 30+ primary antibodies (Abs), each with a unique, photo cleavable oligo tag, and 1-3 fluorescently (FL) labeled Abs was applied to a slide-mounted FFPE tissue section. Regions of interest (ROI), selected based on a FL imaging scan of the entire tissue, were illuminated sequentially with focused UV light to release the oligos. Following each UV cycle, eluent was collected from the local ROI, moved to a microtiter plate, hybridized to NanoString® barcodes, and then analyzed with an nCounter® Analysis System. The resulting digital counts cor- responded to the abundance of each targeted protein in the ROI. For RNA detection, a cocktail of multiple UV- cleavable in situ hybridization probes were used in a similar manner. Results: We demonstrate multiplexed detection from discrete ROIs within a tumor and adjacent normal tissue, enabling systematic interroga- tion of a heterogeneous tumor microenvironment. In control samples, we found expected levels of protein and RNA targets. We further demonstrate that this approach enables analysis of target abundance from individually se- lected cells, both contiguous and non-contiguous with the same phenotype, and enables multiplexed detection of key IO targets. Finally, we demonstrate detec- tion of key IO RNA targets using direct hybridization of oligo-labeled probes. Conclusions: With further development, our novel DSP approach to capture the spatial context of protein and RNA levels will have many applications in bio- marker and translational research. The ability to digitally measure RNA and protein at up to 800-plex from FFPE tissues could facilitate drug mechanism-of- action and resistance studies as well as tumor microenvironment. Quantitative, high-plex data should also greatly accelerate the discovery of IO biomarkers in tumors and the development of companion diagnostics for targeted therapies.

#3956 Intra-individual genomic heterogeneity of ovarian cancer and clinical utility of ascitic cancer cells for mutation profiling. Youn Jin Choi, Min Sung Kim, Sung Hyung Lee. Catholic Univ. of Korea, Seoul, Republic of Korea

Intra-individual tumoural heterogeneity (ITH) is a hallmark of solid tumours and impedes accurate genomic diagnosis and selection of proper therapy. The purpose of this study was to identify ITH of ovarian high-grade serous carcino- mas (OSCs) and to determine the utility of ascitic cancer cells as a resource for mutation profiling in spite of ITH. We performed whole-exome sequencing, copy number profiling, and DNA methylation profiling of four OSC genomes using multiregional biopsy from 13 intra-ovarian lesions, 12 extra-ovarian tu- mour lesions (omentum/peritoneum), and ascitic cells. We observed substantial levels of heterogeneity in mutations and copy number alterations (CNAs) of the OSCs. We categorized the mutations into ‘common’, ‘shared’ and ‘private’ ac- cording to the regional distribution. Six common, 8 shared, and 24 private mu- tations were observed in known cancer-related genes. Common mutations had a higher mutant allele frequency and included TP53 mutations in all four OSCs. Region-penalized copy number and interstitial amplifications and deletions involving BRCA1, PIK3CA, and RB1 were also identified. Of note, the mutations detected in ascitic cancer cells represented 92.3-100% of overall somatic mutations in the given case. Phylogenetic analyses of ascitic genomes predicted a poly-seeding origin of somatic mutations in ascitic cells. Our results demonstrate that despite ITH, somatic mutations, CNAs, and DNA methylation in both “common” category and cancer-related genes were highly conserved in ascitic cells of OSCs, high- lighting the clinical relevance of genome analysis of ascitic cells. Ascitic tumour cells may serve as a potential resource to discover somatic mutations of primary OSC with diagnostic and therapeutic relevance.

#3957 Characterization of functional heterogeneity and in vivo dynamics of clonal cell populations derived from the triple-negative breast cancer cell line MDA-MB-468. Hendrik J. Kuiken, Sabin Dhakal, Laura M. Selsor, Jett P. Crowdis, Hyo-eun C. Bang, Frank Stegemeier, Gordon B. Mills, Joan S. Bruggl, Harvard Medical School, Boston, MA; Novartis, Cambridge, MA; MD Anderson Cancer Center, Houston, TX

Tumors evolve through progressive accumulation of (epi)genetic alterations, favoring expansion of the fittest cell populations as a result of stimuli and selec- tive pressures in the microenvironment. Intratumor heterogeneity and the in- terplay of tumor cells with the microenvironment present an order of complex- ity that can have profound effects on tumor progression and drug sensitivity. While tumor cell heterogeneity has been described at many levels, there is a poor understanding of the extent of functional heterogeneity within a single tumor, and the dynamics and spatial organization of distinct cell populations over time. The overall objective of this study is to characterize the functional heterogeneity of breast tumor cells and evaluate the dynamics of clonal populations within mixtures during tumor progression. We have generated and characterized 31 clonal cell populations (SCP) from the triple-negative breast cancer cell line MDA-MB-468. These SCPs display considerable phenotypic heterogeneity with respect to morphology, proliferation rate, survival in suspension, colony forma- tion in soft agar, and tumorigenicity. We have also performed whole-exome sequencing for 7 SCPs, and RNA-seq and RPPA analysis for all SCPs and the parental cell line. These analyses revealed SCP-unique single nucleotide vari- ants, and differential expression of numerous genes. To study the dynamics of SCP mixtures during the development of xenograft tumors, we have transduced 22 SCPs with unique DNA barcodes derived from the ClonTracer DNA barcode library. The 22 barcoded SCPs were mixed in equal proportions and subse- quently injected into the mammary fat pad of NOD/SCID mice. Tumors and lungs were collected at different time points (3 weeks, 2 months and 4 months) to determine the relative changes in clonal representation during tumor pro- gression by next generation sequencing. We identified highly reproducible patterns of clonal expansion, with SCP01 and SCP03 being temporarily enriched for in tumor samples collected 3 weeks and 2 months, respectively and SCP32 becoming progressively enriched for in tumors over time. In addition, we found recurrent enrichment for SCP01 in most lung samples. Moreover, in contrast to the tumor samples, we found a slight enrichment for SCP13, but not SCP32, during in vitro propagation of the SCP mixture. Together these results suggest
the existence of distinct competitive advantages of individual clonal populations within certain spatial and temporal windows. In addition, the discordance in SCP dynamics between the in vivo and in vitro arms suggests that enrichment for SCPS1, SCPS3 and SCPS32 is the result of stimuli or selective pressures that are specific to the tumor microenvironment. Guided by the RNA-seq and RPPA analyses, we are currently testing several hypotheses that may explain the observed enrichment patterns.

**TUMOR BIOLOGY: Tumor Microenvironment 5**

#3958 The importance of co-localized resting CD8+ T cells and proliferating tumor cells in gastric cancer. Mehmet Yigitsoy,1 Sophie Earle,2 Armin Meier,3 Nathalie Harder,4 Matthew Hale,4 Aleksandra Zuraw,4 Takaki Yoshikawa,5 Günter Schmidt,1 Ralf Huse,4 Heike I. Grabsch,4 Definium AG, Munich, Germany; 5University of Leeds, Leeds, United Kingdom; 4Kanagawa Cancer Treatment Center, Yokohama, Japan; 2Maastricht University Medical Center+ F, Maastricht, Netherlands.

Gastric cancer (GC) is the third leading cause of cancer-related death worldwide. With the advent of immunotherapies, there is a need to characterize the phenotype of tumor infiltrating immune cells and co-localized cancer cells. We have shown previously that high density of CD45RO+ T cells is related to better prognosis in Japanese GC. However, the variation of T-cell infiltration in GC is still not understood. We hypothesized that the timing of CD8+ T-cell infiltration is related to the patient’s spatial and temporal windows. The purpose is: To establish the frequency of co-occurrence of Ki67+ and CD8+ in T cells and their co-localization with tumor cells and evaluate the relationship with clinicopathological variables including survival. Patients and Methods: Immunohistochemistry for T cells (CD8), proliferation (Ki67), and epithelial cells (CK) was performed on tissue microarrays (TMA) from 213 GC from the Kanagawa Cancer Centre Hospital (Yokohama, Japan). Stained slides were scanned, quality controlled, and analyzed using Tissue Phenomics (Definium, Munich, Germany) for cell/nuclei segmentation and automatic co-registration of consecutive sections. The TMA cores were subdivided into tiles of size 64 μm2 to count co-localized positive cells. Average ratio of CD8+ cells and Ki67+ cells per tile/patient was used for statistical analyses. The relationship with pT, pN and histological tumor type was assessed using the Kruskal-Wallis test. Prognostic features were determined by univariate stratification which optimizes Kaplan-Meier p-value using 50 independent pre-validated with 3 folds and ranked by the median pre-validated p-values. P-values < 0.05 were considered significant. Results: 60887 tiles were analyzed in total. Median (range) number of tiles analyzed per patient was 291 (81-345). Median (range) CD8+/Ki67+ ratio was 0.39 (0.01-0.92). Manual inspection of selected image tiles showed that CD8+ cells are rarely Ki67-. Median (range) % of tiles/patient where CD8+ and Ki67- co-localized with Ki67+ tumor cells was 17% (0%-93%). Significant difference of ratio was observed between histological subtypes (p = 0.0096). There was no significant relationship between CD8+ and Ki67+ and pT or pN. A high CD8+/Ki67+ ratio was related to better survival (p = 0.012). Conclusion: This is the first study to suggest that the majority of CD8+ T cells in GC appear to be resting (Ki67-) T cells rejecting our hypothesis that high numbers of intratumoral T cells are due to high intratumoral T cell proliferation. The co-localization of CD8+ T cells and Ki67+ tumor cells seems to be clinically relevant and characterizes certain histological phenotypes in GC. However, the potential underlying biological mechanisms of interaction between T cells and tumor cells is currently unknown. Further studies are needed to validate our findings and characterize the interface between tumor and immune cells.

#3959 CCL18-recruited naive CD4+ T cells are converted to tumor-infiltrating regulatory T cells in breast cancer and suppress antitumor immunity. Shiceng Su, Jianyou Liao, Jiang Liu, Qiang Liu, Erwei Song. SunYat-Sen Memorial Hospital, SunYat-Sen University, Guangzhou, China.

Tumor-infiltrating regulatory T cells (Tregs) play a central role in tumor immunosuppression. However, it remains unclear whether they are directly recruited from peripheral blood or converted from infiltrating naive T cells. Here, full-length TCR α/β repertoire analysis of different T cell subsets from peripheral blood, primary tumors and draining lymph nodes in patients suggests that Tregs in human breast cancer are mainly converted from tumor-infiltrating naive CD4+ T cells. Infiltration of naive CD4+ T cells and Tregs are closely correlated, both indicating poor prognosis for breast cancer patients. Naive CD4+ T cells in the tumors are recruited by tumor-associated macrophages (TAMs) via CCL18. In addition, naive T cells and memory T cells exhibit distinct chemotactic response due to different expression of regulator of G-protein signaling 1 (RGS1). Specific silencing CCL18 receptor-PTPN3M in naive CD4+ T cells using CD4 aptamer-siRNA blocks their chemotaxis, and thus reduces infiltrating Tregs and inhibits tumor progression in humanized mice. By comparison, silencing RGS1 in memory CD8+ T cells using CD8 aptamer-siRNA blocks their recruitment to tumors and anti-tumor immune response in vivo. These findings provide mechanistic insights for Treg enrichment in breast cancer and suggest that modification of the CCL18-PTPN3M-RGS1 signaling pathway may be an attractive strategy for anticancer immunotherapy.

#3960 Tumor cell drug resistance induced by the programmed death ligand 1 (PD-L1) immune checkpoint is associated with autophagy. Loni M. Minassian, Shannyn K. MacDonald-Goodfellow, Peter Truesdell, Daniel Sanwal, Andrew W. Craig, Madhuri Koti, D Robert Siemens, Charles H. Graham. Queen’s University, Kingston, Ontario, Canada.

The interaction between the Programmed Death Ligand 1 (PD-L1) immune checkpoint on the tumor cell surface with the Programmed Death-1 (PD-1) receptor on cytotoxic T lymphocytes (CTLs) leads to CTL inactivation, thereby promoting tumor cell escape from adaptive immunity. We previously demonstrated that signaling by PD-L1/PD-1 is bidirectional and leads to activation of oncogenic pathways as well as drug resistance in tumor cells. We also have preliminary evidence that Immunity Related GTPase, an important mediator of autophagy, is up-regulated by PD-1/PD-L1 reverse signaling. Autophagy is a well-established mechanism of drug resistance in cancer cells. This led us to hypothesize that PD-1/PD-L1 signaling induces drug resistance in tumor cells by up-regulating autophagy. The MEK/ERK and the PI3K/Akt signaling pathways are known to increase and decrease autophagy, respectively. Breast cancer cells exposed to PD-1 showed a time-dependent increase in extracellular signal-regulated kinase (ERK) activation and a decrease in protein kinase B (Akt) activation. Conversion of microtubule-associated protein light chain 3 (LC3-I) to LC3-II is a requirement for autophagosome formation and is a robust marker of autophagy. Exposure of human breast cancer cells to recombinant PD-1 (PD-1) showed a time-dependent increase in LC3-II. We are currently conducting additional studies to confirm that the activation of PD-L1 signaling in tumor cells up-regulates autophagy. These results provide evidence that PD-1/PD-L1 reverse signaling activates autophagy as a potential mechanism of cancer cell chemoresistance. (Supported by a grant from the Canadian Institutes of Health Research.)

#3961 Increased CCR4-positive regulatory T cells in biopsy specimen of poor prognosis prostate cancer. Masahito Watanabe, Kent Kano, Susumu Suzuki, Hiroyuki Muramatsu, Shingo Morinaga, Keishi Kajikawa, Ikuo Kobayashi, Genny Nishikawa, Yoshiharu Kato, Kogenta Nakamura, Kazuhiro Yoshikawa, Ryozu Ueda, Makoto Sumitomo. Aichi Medical University, Nagakute, Aichi, Japan.

Introduction & Objective: Regulatory T cells (Treg) play important roles in suppression of the immune response, including anti-tumor immunity. C-C chemokine receptor 4 (CCR4) is highly expressed on effector Tregs, and the anti-CCR4 antibody is attracting attention as a novel immunotherapeutic agent for solid tumors. The purpose of this study was to evaluate the expression of CCR4-positive Tregs in biopsy specimens of men with prostate cancer with a poor prognosis and to estimate the clinical potential of CCR4-targeting therapy for prostate cancer. Material & Methods: Data for 60 men diagnosed with prostate cancer based on transrectal 12-core systemic biopsy were retrospectively analyzed. They were divided into two groups, a good prognosis group comprising men with no disease progression during the observation period and a poor prognosis group comprising men with progression to castration resistant prostate cancer. In the biopsy cores, the core with the highest tumor volume was selected for immunohistochemical evaluation. Tregs were evaluated using immunohistochemistry with double staining using anti-forkhead box protein 3 (FOXP3) antibodies and anti-CCR4 antibodies. The number of CCR4+/-FOXP3+ cells in the biopsy specimens were compared between the two groups. Furthermore, the correlation between the number of CCR4+/-FOXP3+ cells and clinical stage, PSA at diagnosis, and Gleason score were evaluated. Results: The good prognosis group included 29 men and the poor prognosis group included 31 men. Approximately 70-80% of FOXP3+ cells were positive for CCR4. There was no significant difference in the ratio of number of CCR4+/-FOXP3+ cells to the number of total FOXP3+ cells between the poor and good prognosis groups. However, the total number of FOXP3+ cells significantly increased in the poor prognosis group than in the good prognosis group (200.1 ± 174.8 vs. 38.1 ± 38.1, p < 0.01). Furthermore, the number of CCR4+/-FOXP3+ cells significantly increased in the poor prognosis group than in the good prognosis group (123.6 ± 99.1 vs. 58.88 ± 26.4, p < 0.01). The number of CCR4+/-FOXP3+ cells
**#3962 High-dimensional flow cytometric immune profiling of malignant gliomas.** Amber J. Giles,1 Caitlin M. Reid,1 Deric M. Park,2 Mario Roederer,2 Mark R. Gilbert1.1NCI-NIH, Bethesda, MD; 2NIAD-NIH, Bethesda, MD.

**INTRODUCTION:** Immune therapy is emerging as a treatment modality for patients with solid tumors including intracranial metastases. Similar immune strategies are being tested in patients with malignant gliomas; diseases with uniformly poor prognosis. Whereas, recent work has demonstrated a correlation between the immune landscape of solid tumors and response to immune therapy, the immunologic landscape of CNS tumors has not been thoroughly defined. To capture the phenotypic diversity of immune cells in CNS tumors, we developed an 18-color flow cytometry panel and applied it to mononuclear cells isolated from acutely resected brain tumor, matched patient blood, and healthy donor blood specimens. **METHODS:** Linear discrimination analysis (LDA) and T-distributed stochastic neighbor embedding (tSNE) dimensionality reduction techniques were used to analyze flow cytometry data acquired from brain tumor, patient-matched peripheral blood, and healthy donor peripheral blood specimens. **RESULTS:** LDA of 13 parameters collected by flow cytometry revealed that CD8+ tumor-infiltrating lymphocytes (TILs) differed notably from peripheral blood. Lymphocyte clusters created from TILs were easily distinguished from clusters enriched in peripheral blood. Analysis of TIL clusters identified markers consistent with activated/memory populations as well as elevated levels of CTLA-4 (TILs) and CD3 (peripheral blood) provided the greatest degree of separation. Using the tSNE algorithm to analyze the same 13 parameters on CD8 T cells, we rapidly reduced 78 potential parameter combinations by creating a clustering pattern representing lymphocytes with similar staining patterns. Lymphocyte clusters created from TILs were easily distinguished from clusters enriched in peripheral blood. Analysis of TIL clusters identified markers consistent with activated/memory populations as well as elevated levels of CTLA-4, LAG-3, and PD-1 relative to peripheral blood; due to a higher percentage of cells expressing these markers rather than increased expression on individual cells. Identifying individual specimen points as well as clusters of specimens (e.g. patients with the same tumor classification), enabled comparisons across individual patient specimens (intra-patient blood vs tumor), across tumor type or grade, and across disease state (patient vs healthy donor). **CONCLUSIONS:** Dimensionality reduction techniques provided a rapid means to identify markers that were differentially expressed in TILs relative to peripheral blood. Importantly, these analyses were unrestricted by canonical gating strategies. CD8 TILs express multiple markers of activation and maturation, suggesting reactivity to tumor antigens and potential for immune therapy. Application of these analyses to a broader patient population will reveal the immunologic landscape of primary CNS tumors and provide insight into the immunologic consequences of current therapies. Further, this may lead to identification of specific immune signatures that may predict therapeutic response to immune therapy.

**#3963 Neoadjuvant chemotherapy promotes prometastatic changes in the primary breast tumor microenvironment in mice and humans.** George S. Karagiannis,1 Jessica Pastoriza,1 Jeanine Pignatelli,1 Yarong Wang,1 Allison S. Harney,1 David Entenberg,1 Ved P. Sharma,1 Emily Xue,1 Esther Cheng,2 Timothy M. D’Alfonso,2 Joan G. Jones,3 Jesus Anampa,3 Thomas E. Rohan,4 Joseph A. Sparano,5 John S. Condieels,1 Maja H. Oktay,1 Albert Einstein College of Medicine, Bronx, NY, 2Weill Cornell Medicine, New York, NY, 3Montefiore Medical Center, Bronx, NY.

Chemotherapy induces influx of bone marrow-derived proangiogenic Tie2+ monocytes in primary tumors. Tie2+ perivascular macrophages specifically induce the prometastatic Mena isoforms in tumor cells and can assemble specialized microanatomical sites called “tumor microenvironment of metastasis” (TMEM), structures that may serve as doorways for invasation of tumor cells in mammary tumors. Both TMEM and MenaNV are required for tumor cell invasation and dissemination. Thus, we hypothesized that chemotherapy may increase the density of TMEM sites and MenaNV-expressing, invasation-competent tumor cells, resulting in increased tumor cell invasation and metastasis. We studied these potential pro-metastatic effects of chemotherapy in a neoadjuvant setting (NAC) by either administering paclitaxel or a combination of doxorubicin and cyclophosphamide in several mammary carcinoma mouse and human breast cancer models. As expected, chemotherapy delayed tumor growth, yet it significantly increased the recruitment of TMEM-forming, perivascular Tie2+Vegf+ macrophages and TMEM density. Using high-resolution multiphoton intravital imaging in live tumor-bearing mice, we observed that paclitaxel also increased the activity of TMEM sites, visualized as endothelial cell tight-junction disruption around TMEM and subsequent intravasation of the migratory cancer cell subpopulation. Indeed, paclitaxel-treated mice have higher numbers of circulating tumor cells, single cell seeding in lungs and incidence and number of micrometastatic foci, all associated with increased TMEM activity, as demonstrated by high-resolution imaging techniques. Tie2 inhibitors reversed paclitaxel-induced pro-metastatic phenotypes without affecting the assembly of TMEM, indicating that Tie2-mediated signaling is required for paclitaxel-mediated cancer cell dissemination via TMEM. Paclitaxel also caused a significant increase in the expression of MenaNV at both the gene and protein levels. Furthermore, paclitaxel treatment in Mena−/− breast tumor-bearing mice resulted in failure to assemble TMEM and to increase circulating tumor cell numbers. Despite the fact that Tie2+ macrophages are attracted to perivascular niches as a result of paclitaxel treatment. This indicated that Mena is involved in the paclitaxel-mediated increase in cancer cell dissemination but not required for Tie2+ macrophage recruitment. These pre-clinical data are further supported by findings from a cohort (N=20) of breast cancer patients, who received pre-operative paclitaxel-based chemotherapy and demonstrated significant increases in TMEM density and MenaNV expression. Together, our data provide solid evidence that NAC leads to metastasis in rodent via TMEM/ MenaNV-mediated mechanisms, and to cancer cell dissemination in certain clinical scenarios in humans.

**#3964 Reducing systemic arginine with arginase (AEB1102) therapy does not suppress the immune response induced by anti-PD-1 and anti-PD-L1, and exerts an additive anti-tumor and synergistic survival benefit.** Giulia Agello, Susan E. Alters, David G. Lowe, Scott W. Rowlinson. Aegle BioTherapeutics Inc., Austin, TX.

Tumor dependence on specific amino acids for survival and proliferation is well recognized and has been explored effectively with the use of asparaginases for the treatment of acute lymphoblastic leukemia. Sensitivity of tumors to L-Arginine (L-Arg) deprivation results from an impaired ability to make L-Arg as a result of decreased functional expression of one or more of the three enzymes of the L-Arg biosynthetic pathway: ornithine transcarbamylase (OTC), argininosuccinate synthase (ASS1) and argininosuccinate lyase (ASL). Native human arginase I is not a viable drug candidate due to low activity and low stability in serum. We have developed an alternative approach using a bioengineered human PE Gyalted arginase I (AEB1102) with enhanced pharmacological properties. We and others have successfully utilized arginase I to impart a direct tumor cell killing effect through L-Arg starvation in multiple tumor types e.g. AEB1102 single agent efficacy in melanoma, small cell lung cancer (SCLC) and sarcoma PDx models. However, the compatibility of AEB1102 with checkpoint inhibitors is unclear as arginase I has been shown to be both immune suppressive and immune neutral (PMID: 23717444), or immune promoting (PMID: 27043409). Because of these conflicting reports we decided to investigate the impact of systemic L-Arg removal on the anti-tumor efficacy of checkpoint inhibitors. Murine syngeneic models (e.g. CT26, MC38 and LLC) were dose with AEB1102 alone and in combination with anti-PD-L1 and anti-PD-1 monoclonal antibodies (mAbs). Depending on the model, in vivo treatment with AEB1102 mono-therapy resulted in tumor growth inhibition (TGI) ranging from 52% to 72% compared to the untreated control group whereas standard monotherapy using immunomodulatory mAbs that target PD-1 and PD-L1 resulted in TGI ranging from 12% to 60%. Of significance, combination therapy of AEB1102 with anti-PD-1 or anti-PD-L1 resulted in additive anti-tumor effect with TGI ranging from 60% to 86%. In the CT26 model, when AEB1102 was administered in combination with anti-PD-L1 for at least 6 weeks, a 33% frequency of complete tumor regression (non-palpable tumors) was observed, indicating that synergy occurs with combination therapy. Collectively these results demonstrate that disrupting the L-Arg physiological balance in the tumor microenvironment inhibits tumor growth and further sensitizes the tumor to immunotherapy when AEB1102 is combined with anti-PD-1 and anti-PD-L1. These data open the possibility of further improving outcomes in L-Arg dependent tumors through combination of AEB1102 with anti-PD-1 and anti-PD-L1 inhibitors.

**#3965 Epithelial membrane protein-2 is a novel regulator of immune editing in breast cancer.** Jessica Tsui, Negin Ashki, Yuling Chang, Devon Patel, Jasmine Sjarif, Madhuri Wadhera. UCLA David Geffen School of Medicine, Los Angeles, CA.

Cancer immune-editing is a process that describes the interaction between immune cells and tumor cells. This interaction can result in elimination of a developing tumor, tumor dormancy, or tumor cells that are capable of surviving in an immune–competent host. We have recently uncovered a novel mechanism of immuneediting by the tetraspan protein Epithelial membrane protein or EMP2. We propose that EMP2 serves as a bridge between innate and adaptive
immunity via tumor mediated type I interferon expression. Most tumor cells evade the immune system through suppression or ignorance. EMP2 levels promote type I interferon expression which was found to correlate with PDL1 expression in a number of cell lines. Concordantly, in vivo, in multiple breast cancer cell lines, EMP2 promoted tumor growth. In contrast, reduction in EMP2 levels correlated with a reduction in tumor growth in a corresponding syngeneic mouse model. Given that tumors with lower levels of EMP2 grow poorly, we hypothesized that targeting EMP2 may provide a therapeutic benefit. Recently, we have developed a panel of novel IgG1 monoclonal antibodies to EMP2 and tested their ability to treat both xenograft and syngeneic mouse models. Our results show that anti-EMP2 IgG1 significantly reduces tumor load in both models in part through the recruitment of M1 macrophages. To explore this observation more fully, experiments using murine syngeneic models were employed. Anti-EMP2 therapy elicits a robust ADCC response, producing an immune infiltrate rich in macrophages, NK cells, and lymphocytes. In addition, we show that CD8 cells show reduced exhaustion in the presence of anti-EMP2 antibodies. Conclusions: EMP2 is a protein upregulated in a number of cancers in women including breast, ovarian, and endometrial tumors. Our work collectively show that EMP2 is a novel immune regulator where its expression creates an immunosuppressive tumor microenvironment. We thus predict that targeting EMP2 may improve therapeutic outcomes for cancers in women.

**#3966 Targeting integrin αβ3-expressing cancer stem cells to manipulate tumor-associated macrophages.** Hiromi I. Wettersten, Toshiyuki Minami, Megan M. Kaneda, Laetitia Seguin, Judith A. Varner, Sara M. Weis, David A. Cheresh, UC-San Diego, La Jolla, CA.

Tumor associated macrophages are involved in regulation of cancer growth and aggressiveness. Whereas M1 macrophages trigger an inflammatory response and inhibit tumor growth, M2 macrophages secrete pro-tumor cytokines into the microenvironment to support tumor progression. A macrophage switch from M1 to M2 has been associated with lung cancer progression, and cancer stem cells have been implicated as a driver of this reprogramming. We recently reported that integrin αβ3 expression is induced on lung adenocarcinoma cells during drug resistance and is both necessary and sufficient to reprogram these tumors to a stem-like state. Given the role that cancer stem cells play in switching M1 to M2 macrophages, we asked whether αβ3 expression on lung adenocarcinoma cells account for this macrophage conversion. The M1/M2 macrophage ratio in αβ3-positive tumors was markedly decreased relative to tumors lacking αβ3. We next treated mice bearing αβ3-positive tumors with a monoclonal antibody (LM609) targeting this receptor to assess its ability to alter the macrophage phenotype within these tumors. LM609 was able to selectively eliminate the αβ3-positive cancer stem cells via antibody-dependent cell-mediated cytotoxicity (ADCC), and this not only increased the M1 macrophage population, but also markedly enhanced the sensitivity of these tumors to the effects of these therapies. These findings reveal that αβ3-expressing cancer stem cells favor the pro-tumor M2 macrophage phenotype. Eliminating αβ3-positive cancer stem cells via ADCC serves to both increase pro-inflammatory macrophages within the tumor microenvironment and prolong tumor sensitivity to therapy.

**#3967 High microsomal PGE synthase-1 levels associate with low CD8 T cells and poorer melanoma patient survival.** Sun-Hee Kim, Jason Rozsik, Weiyi Peng, Suhendan Ekmekcioglu, Elizabeth A. Grimm. UT MD Anderson Cancer Ctr., Houston, TX.

COX-2 and its product PGE2 are reported to enhance carcinogenesis and tumor progression and to support immunosuppression, as reported by others in melanoma and other cancers. As most COX-2 inhibitors result in cardiotoxicity, the downstream microsomal PGE2 synthase-1 (mPGES1) is now also an alternative consideration for therapeutic targeting. Our previously published results (Kim, et al., 2016) showed that mPGES1 protein increased with melanoma patient clinical stage and that its intense expression was associated with reduced patient survival. We also reported that mPGES1 inhibition attenuated cell survival and metastasis in human melanoma cells and significantly suppressed tumor growth in melanoma xenografts. This current study was designed to evaluate whether expression of mPGES1 contributes to immune evasion. Tumors in a Stage III melanoma TMA analysis demonstrated that high mPGES1 expression (intensity 3) was significantly associated with low CD8 levels (lower than median CD8 percent value) (r=-0.2722, p=0.0128). Importantly, stage III melanoma patients with this high mPGES1 and low CD8 signature had significantly increased risk of death as determined by Kaplan-Meier method and Uni- variate Cox proportional hazards regression models, and compared with patients with low mPGES1/low CD8 and low mPGES1/high CD8. Furthermore, the differential levels of several chemokines including CXCL1 and CXCL2 to regulate T cell migration were correlated the mPGES1 expression in the TCGA melanoma data set. Collectively, our study continues to resolve the potential role of mPGES1 in regulation of immune evasion and is designed as a preclinical approach to develop a rational therapeutic strategy targeting PGE2-driven inflammatory mediators as useful adjuncts for immune-based therapy.

**#3968 Canonical NFκB signaling in myeloid cells is required for the glioblastoma growth.** Bhagel R. Achuty, Jennifer Bradford, Kartik Angara, Mohammad Rashid, Meenu Jain, Thaiz Borin, ASM Iskander, Roxan Ara, Ali Arbab. Augusta University, Augusta, GA.

Glioblastoma (GBM) development and therapeutic resistance has been accompanied with the tumor-associated macrophages (TAMs) in the tumor microenvironment (TME). TAMs are heterogeneous cell populations of immune regulatory myeloid-derived suppressor cells (MDSCs) and polarization of anti-tumor macrophages (M1) into pro-tumor macrophages (M2). We investigated the role of myeloid cell NF-κB signaling in orthotopic GBM model using immune deficient and immune competent hosts. Interestingly, conditional deletion of canonical NF-κB signaling (p65) with Lysm-Cre (p65 KO) in myeloid cells, significantly inhibited syngeneic GL261 tumor growth in immune-compotent mice compared to control mice. We studied the TAMs recruitment to the tumor and their polarization under the influence of TME. p65 KO mice displayed decreasing trend of immune cell infiltration (CD45), which phenotyped as decreased F4/80+, CD68+, CD206+ (M2) and Gr1+CD11b+ (MDSCs) macrophages, compared to control mice. This was associated with the increased CD80+ (M1) macrophages, increasing trend of CD4+ and CD8+ cytotoxic T cells, and decreased CD4+ and mesenchymal cancer stem cells (CSCs) population in the TME. Cytokine array data indicated that loss of canonical NF-κB signaling within the TAMs was implicated in increased production of INFγ, IFGI, MCP1, MIP1α, and TNFα cytokines. Co-culture of T cells with p65 KO or control MDSCs identified increased proliferation of T cells with p65 KO MDSCs compared to control MDSCs. Conversely, GBM patient-derived xenografts and U251 GBM cell-line-derived tumors showed increasing trend of growth in immune-deficient mice, following the transplantation of p65 KO bone marrow (BM) compared to control BM. Pro-tumor macrophages and CSCs were increased and T cell populations were decreased in human tumors grown in immune deficient mice transplanted with p65 KO BM, compared with control BM. In addition, analysis of human data set revealed higher expression of p65 subunit of NF-κB complex in brain tumor stroma compared to the tumor cells. This study suggests that canonical NF-κB signaling in TAMs is required for the tumor-promoting macrophage polarization and GBM growth in immunocompetent host compared to immune deficient host. Therefore, targeting myeloid-specific NFκB signaling in GBM could inhibit the immune suppressive TAMs and improve the anti-tumor immunity.

**#3969 AFIq/MBL11 regulates the expression of intercellular adhesion molecule-1 in breast cancer.** Jin Park, Soojin Kim, William Tse. Univ. of Louisville, Louisville, KY.

Intercellular adhesion molecule-1 (ICAM-1) is a cell surface glycoprotein in the immunoglobulin superfamily. ICAM-1 expression is frequently observed on many cell types including endothelial cells and different cancer cell entities. ICAM-1 expression is strongly correlated with a reduction in tumor size and a correlation with metastatic capacity. The specific signaling pathways in cancer cells leading to enhanced cell motility, invasion and metastasis. In clinical investigation, however, ICAM-1 expression was negatively correlated to tumor size, lymph node metastasis, and tumor infiltration. Also, there was improved relapse-free and overall survival in patients with ICAM-1 positive tumors. Yet, the function of ICAM-1 expression during malignant progression in breast cancer patients is not understood clearly. However, ICAM-1 is still considered as a breast cancer target and biomarker may lead to the development of a new strategy and platform for breast cancer patients. We observed AFIq, a metastasis enhancer, is only expressed in metastatic cells, MDA-MB-231LN (invasive sub line from MDA-MB-231), not in the primary tumor cells. To investigate whether AFIq has a responsibility in acquisition of metastatic phenotype, we performed RNASeq and applied the Metacore network building algorithm. Intriguingly, most genes were directly linked with Intercellular Adhesion Molecule-1 (ICAM-1). Likewise, we identified that ICAM-1 expression is attenuated in MDA-MB-231LN compared to MDA-MB-231. Moreover, the expression of ICAM-1 was negatively regulated, when AFIq was overexpressed in MDA-MD-231LN. Suppressed endogenous AFIq by shRNA could effectively increase the ICAM-1 expression. Attenuation of ICAM-1 by AFIq on tumor cells would disadvantage host anti-tumor defenses by trafficking of lymphocytes, which affect tumor progression and metastasis. We observed that NF-κB activity which regulates ICAM-1 expression is attenuated in response to AFIq expression in breast cancer cells. Published reports...
shown that Wnt/β-catenin negatively regulates NF-κB activity through protein-protein interaction in colon and breast cancer cells. Previously, we reported that AF1q enhances the TCF7/LEF/β-catenin complex binding affinity as a cofactor. Structurally, AF1q has highly acidic peptide regions highly conserved between species that fulfill the criteria for an acidic blob, a typical feature for cofactors. When we performed immunoprecipitation with NF-κB antibody in MDA-MB-231LN overexpressed AF1q, we observed that β-catenin and AF1q were pulled down together. However, it is not clear yet whether AF1q promotes protein interaction between β-catenin and NF-κB. These results suggest that activated β-catenin by AF1q would achieve higher affinity to bind with NF-κB and thereby attenuate ICAM-1 expression.

#3970 Targeting the adenosine immunosuppressive pathway for cancer immunotherapy with small molecule agents. Pierre Fons,1 Michael Esquerré,2 Stéphanie Verslues,1 Giglida Mambrini,1 Michael Paillasse,1 Andy Bell,2 Adrian Schreyer,1 Richard Cox,3 Richard Bickerton,1 Jobsan Listzwan,1 Mark Whittaker,1 Françoise Bono,1 Craig Johnstone,1 Andrew Hopkins3. EVOTEC, Touhouse, France; 2 Exscientia, Exdorf, United Kingdom

Adenosine (ADO) is released by tumor cells and suppresses T cell immune response by binding to A2A and A2B receptors. The A2A receptor is predominantly expressed on regulatory T cells (Tregs). A2A receptor activation by adenosine is known to induce upregulation of cyclic GMP (cGMP) levels in Tregs. cGMP further inhibits NO production by Tregs. Thus, the adenosine-A2A receptor pathway can be a potential target for cancer immunotherapy. We previously reported that the A2A receptor antagonist CGTHW1 (CGTHW1) that is known to have anti-tumor effect is not only a potential immunomodulator but also significantly increases anti-tumor effect when combined with anti-PD-1 immunotherapy. This is most likely due to the fact that the A2A receptor is expressed on a wide range of immune cells including T cells, Tregs, dendritic cells, and macrophages. Thus, targeting A2A receptors can be used as a strategy to enhance T cell mediated antitumor immune responses. We therefore investigated the adenosine receptor binding affinity and capacity of a newly developed small molecule A2A receptor antagonist EV-163. We performed a Biacore analysis to determine the binding affinity of EV-163. A2A receptor was covalently immobilized on a sensor chip. EV-163 was serially injected to the Avant chip and the accumulation of EV-163 was measured using the response units (RU). The maximum response (MRU) and equilibrium dissociation constant (KD) were determined using a standard curve and equilibrium binding analysis respectively. EV-163 had a KD value of 440 nM, which is comparable to the KD value of CGTHW1. We further performed a competition binding assay using different concentration of EV-163 in the presence of 100 nM of A2A agonist (ectoA2A) to determine the receptor capacity of EV-163. The competition binding assay was performed with a fixed concentration of 250 nM A2A receptor and varying concentrations of EV-163. The inhibition of A2A agonist was determined by measuring RU. EV-163 was able to competitively inhibit the binding of A2A agonist with a high capacity. The IC50 of EV-163 was found to be 0.8 nM. These results indicate that EV-163 can be a potential small molecule A2A receptor antagonist for cancer immunotherapy.

#3971 MTA-mediated inhibition of human T cells: Mechanism and Ex vivo profiling of PD-1 blockade using organotypic thyroid spheroids. Carolin D. Strobl,1 Frederik Henrich,2 Katrin Singer,2 Katrin Peter,2 Marina Kreutz,2 Anita Kremmer,1 Andreas Mackensen,1 Michael Aigner3.1 University Hospital Erlangen, Erlangen, Germany; 2 University Hospital Regensburg, Regensburg, Germany

Metabolic changes of malignant cells lead to the secretion of tumor metabolites which contribute to the shaping of a favorable milieu for tumor immune escape facilitating cancer development and resistance to anti-tumor immunotherapy. Recent studies have shown that methylthioadenosine (MTA) as a tumor-specific metabolite is capable to interfere with signal transduction pathways and to induce programmed cell death. We therefore investigated the secreted cytokine/chemokine response to PD-1 blockade using organotypic thyroid spheroids (MTDS/PDTS) in a 3-dimensional microfluidic system. Spheroids derived from fresh mouse and human thyroid cancer samples through partial collagenase digestion and filtration retain autologous tumor infiltrating immune cells, including PD-1 positive CD8 T lymphocytes. Using murine syngeneic mouse cancer models with known responsiveness or resistance to PD-1 blockade, we demonstrate the ability to recapitulate tumor killing ex vivo or lack thereof. Multiplexed cytokine profiling of conditioned media in this system further enables characterization of the cytokine response to PD-1 blockade across a large panel of PDTS, and observed pronounced induction of CCL19/CXCL13, which associated with %PD-1 positive CD8 T cells. This strategy further uncovered the secreted cytokine profile of tumor-derived cytokines and cytokine responses in the combined effect of MTA and PRMT5 inhibition on T cells. Several metabolic studies focusing on glycolysis and fatty acid metabolism could confirm our findings that MTA keeps T cells in a rather inactive, naïve state. Finally, we found MTA to interfere with DC maturation and their potential to induce cytokine production by activated T cells. Our studies provide a new perspective on the crucial role of MTA in the tumor milieu for immune escape and open up new strategies for targeting immune escape in a personalized manner.
folicular and anaplastic thyroid cancer exosomes was observed, when compared to papillary thyroid cancer exosomes. We detected a down regulation of miR-138-5p, miR-146a-5p, miR-26a-5p, miR-26b-5p, miR-34a-5p and miR-31-5p in anaplastic and folicular thyroid cancer exosomes, which may play a role in promoting cancer invasion and metastasis. In comparison to papillary thyroid cancer exosomes, with surprising miR-214-3p, miR-298-5p, miR-299-3p and miR-302a-3p was ascertainment in folicular thyroid cancer exosomes. Conversely, we noted a down regulation of these miRNAs in anaplastic thyroid cancer exosomes. These distinct miRNA expressions in cancer secretome will provide new insights into the tumor development and dissemination. Our findings suggest an important crosstalk between the secretome of thyroid cancer cells and immune cells in tumor microenvironment, defining thyroid cancer phenotype. These exosomal miRNA serve as early diagnostic markers of thyroid cancer differentiation as well as targets for novel therapies specifically for anaplastic thyroid cancer.

### #3974 Gender specific function of epithelial IL-6-STAT3 pathway in K-ras mutant lung cancer. Mauricio S. Caetano,1 Hieu Van,1 Emmanuel Bugarin,1 Amber Cumpian,1 Christina L. McDowell,1 Huiyuan Zhang,1 Scott E. Evans,1 Stephanie Watowich,1 Human Kadara,2 Seyed J. Moghaddam,1 UT MD Anderson Cancer Center, Houston, TX;2 American University of Beirut, Beirut, Lebanon.

Activating mutations of K-ras are the most common oncogenic alterations found in lung cancer, and are heavily associated with tobacco exposure and poor prognosis. Using a conditional lung cancer model (CC-LR), we showed that K-ras mutant lung tumors have inflammatory characteristics with activation of NF-kB pathway, release of IL-6 and activation of its downstream target, STAT3. We further demonstrated that IL-6 blockade results in significant tumor reduction as well as decreased pSTAT3 expression, tumor cell proliferation, angiogenesis and a reduction in tumor-associated immune suppressive myeloid populations concomitant with induction of an anti-tumor phenotype. These indicate essential autocrine and paracrine roles for IL-6-STAT3 pathway in promotion of K-ras mutant lung cancer, largely via perpetuating inflammation introducing it as a vulnerability factor for this type of tumors. Here we generated a lung epithelial specific K-ras mutant/STAT3 conditional knockout mouse (LR/STAT3Δ/Δ) to further study the role of epithelial STAT3 activity in K-ras mutant lung cancer. We found that in female mice, lack of epithelial STAT3 inhibited lung cancer, and significantly decreased the lung inflammatory cell population, particularly macrophages, whereas in male mice, STAT3-deficiency surprisingly promoted lung cancer and significantly increased lung neutrophil population. To understand genome-wide mechanisms underlying this intriguing gender disparity, we performed RNA-sequencing of whole lungs from male and female LR/STAT3Δ/Δ and control CC-LR mice. Using a mixed effects model, we found 339 transcripts that were significantly modulated differently between LR/STAT3Δ/Δ and control CC-LR mice in males relative to females. Pathways and gene network analyses of the transcripts demonstrated that lungs of male LR/STAT3Δ/Δ mice exhibited markedly reduced Th1 immune T-cell signatures. We corroborated these findings and found significant phenotypic change in the lung tumor microenvironment (TME) with male mice exhibiting increased pro-tumor inflammatory markers. Markedly, we found opposing IL-6 expression patterns with LR/STAT3Δ/Δ females expressing low IL-6 while LR/STAT3Δ/Δ males expressed high levels of IL-6. We then blocked IL-6 in male LR/STAT3Δ/Δ mice, which resulted in a significantly reduced lung tumorigenesis, and reformatted lung TME towards an anti-tumor phenotype with a Th1/CD8+ T-cell signature. By contrast, estrogen receptor (ER) signaling blockade by tamoxifen in female LR/STAT3Δ/Δ mice promoted lung cancer, and reprogrammed lung TME toward a pro-tumor phenotype with an increase in Th17/Treg cell signature. Taken together, we conclude that epithelial STAT3 signaling has an important gender-specific role with autocrine and paracrine effects in K-ras induced lung tumorigenesis, which might be mediated by the interplay between ER signaling, and NF-kB mediated cytokine network.

### #3975 Ovarian cancer cells release arginase-1-containing exosomes to suppress antitumor immune response. Malgorzata Czystowska,1 Marta Szajnik,2 Kavita Ramji,1 Slawomir Gruca,1 Artur Stefanowicz,1 Jakub Golab,1 Do- minika Nowis,1 Malgorzata Czystowska,1 Marta Szajnik,2 Kavita Ramji,1 Slawomir Gruca,1 Artur Stefanowicz,1 Jakub Golab,1 Dominika Nowis,1 Medical University of Warsaw, Warsaw, Poland;2 Military Institute of Medicine, Warsaw, Poland;3 Praski Hospital, Warsaw, Poland.

Background: Depletion of essential (L-tryptophan) or semi-essential (L-arginine) amino acids has been shown to suppress antitumor immune responses. Arginase-1 (Arg-1) is a cytosolic enzyme catalyzing degradation of L-arginine to L-ornithine and urea. Several studies have shown the presence of abundant Arg-1 in either tumor cells or in tumor-infiltrating myeloid cells of neutrophilic or monocytic origin. This in turn, leads to downregulation of CD3-zeta levels in T cells and correlates with suppressed antitumor immunity and anti-tumor innate immunity. Metabolites released by tumor cells (TEX) are detected in body fluids including plasma or ascites and carry proteins that are expressed by OvCa cells. Here we report that OvCa cells release Arg-1 in TEX and investigate the influence of OvCa TEX-derived Arg-1 on the antitumor effector mechanisms of immune response. Methods: TEX were isolated by ultracentrifugation or exclusion chromatography and verified by Western blotting, NanoSight and electron microscopy. The presence of Arg-1 in TEX was determined by Western blotting and the Arg-1 activity was assessed using an enzymatic assay that measured conversion of arginine into urea. Immunohistochemical Arg-1 expression data in primary OvCa lesions were correlated to clinico-pathological characteristics. Effects of TEX Arg-1 on immune cells were analyzed by in vitro proliferation assay and flow cytometry. Results: OvCa ascites contained higher levels of exosomal Arg-1 as compared with the fluids obtained from benign ovarian cysts. Enzymatically active Arg-1 was detected in TEX derived from patients’ ascites as well as from ovarian cancer cell lines. High Arg-1 expression in correlated negatively with intratumoral T-cell infiltrates and CD3-zeta expression and was associated with shorter time to recurrence (TTTR). In vitro, OvCa-derived Arg-1-positive TEX inhibited CD8+ and CD4+ T-cell proliferation and decreased T-cell receptor expression. We also observed that co-culture of bone-marrow-derived dendritic cells (DC) with TEX isolated from OvCa cells results in the transfer of functionally active Arg-1 that inhibits DCs-primed proliferation of OVA-antigen specific OT-1 T cells. We have used TEX isolated from OvCa cells transfected with V5-tagged Arg-1 for these experiments to ensure that this is OvCa-derived, but not endogenous DC-derived arginase that suppresses T cell proliferation. All these in vitro effects were reversed by a novel Arg-1 inhibitor (OAT-1746). Conclusions: Our findings provide the first evidence for the role of Arg-1 in the formation of an immunosuppressive microenvironment in OvCa. We identify a novel mechanism of exosomal Arg-1 distribution from the tumor cells to antigen presenting cells. Inhibition of Arg-1 activity may be an attractive novel anti-cancer strategy. Funding: NCN - OPUS 6 Program 2013/11/B/ NZ6/02790, NCbir – STRATEGMED2/265503/3/NCbir/15.

### #3976 Resolving cellular complexity in renal cell carcinoma with droplet-based single cell transcriptomics. Siva Vijayakumar, Mohan Bolisetty, Santosh Sivajothi, Paul Robson. The Jackson Laboratory, Farmington, CT.

Purpose: The development, growth, invasion and metastasis of cancer is supported and sustained by crosstalk between the stromal cells, cancer cells and immune cells through autocrine and paracrine signaling, influencing many stages of cancer progression. Furthermore, understanding the identity of the tumor infiltrated immune cell populations is known to predict survival outcomes. Kidney cancer is the 12th most prevalent cancer in the world and is predicted to affect 62,700 new cases in 2016 in the United States alone (American Cancer Society). Existing studies on renal cell carcinoma utilizing bulk profiling methods have been unable to deconvolute the role of the various cell types in promoting the progression this cancer type. Method: Single cell transcriptomics of primary tumors is a powerful approach to study not only the cancer cells but also the cells of the microenvironment. We profiled the various cell types that comprise primary renal cell carcinoma using the Chromium Single Cell 3’ system (10X Genomics). Results: With this single cell approach, we have generated thousands of single cell transcriptomes leading to the identification of numerous cell types and cell states in renal cell carcinoma and matched normal kidney. The microenvironment of renal cell carcinoma tumors contains a heterogeneous mix of stromal, immune and kidney cell types. Compared with the matched normal, the tumors are heavily infiltrated with lymphocytes, mast cells and cells of the myeloid lineage. We identified pathways enriched in specific cell types that illuminate potential mechanisms underlying the cell-cell interactions between cancer cells, stromal cells, and the infiltrating immune cells thus highlighting potentially new targeting strategies for the treatment of this disease. In addition, the single cell data described here will be used to deconvolute existing and new bulk analyses of renal cell carcinoma patients and to establish targeted antibody panels for use in flow and imaging mass cytometry. We anticipate both will lead to better patient stratification to inform patient-specific treatment regimes. Conclusion: Droplet-based single cell transcriptomics can successfully identify the numerous cell types that comprise a tumor, providing a foundation of knowledge to inform biomarker selection and patient-stratification.

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provide insight into novel treatment strategies. This approach reflects a more comprehensive view of renal tumors that is often masked by bulk profiling analyses.

#3977 Neutrophils fertilize the pre-metastatic omental niche in ovarian cancer. Wonjae LEE, Honami Naora. MD Anderson Cancer Center, Houston, TX.

More than 60% of ovarian cancer patients present with peritoneal carcinomatosis that almost always involves the omentum, a fat pad that suspends from the stomach. However, the mechanisms that promote the tropism of ovarian cancer cells for the omentum are poorly understood. Because the omentum is the major peritoneal conduit and reservoir for immune cells, we hypothesized that immune cells control the propensity of ovarian cancer cells to implant on the omentum. In this study, we evaluated changes in immune cell populations in the omentum and in other visceral fat pads in the peritoneal cavity in mouse xenograft models of ovarian cancer. Intriguingly, the proportion of neutrophils was observed to dramatically increase in the omentum prior to tumor cell implantation at this site. On the other hand, the proportion of neutrophils did not substantially increase in other visceral fat pads. Furthermore, the proportion of macrophages did not significantly increase in any of the fat tissues prior to tumor cell implantation. Collectively, these findings raise the possibility that early recruitment of neutrophils to the pre-metastatic omental niche promotes a microenvironment that is conducive to ovarian cancer cell implantation at this site.


Aberrent expression of the B7-H1/PD-L1 immunomodulatory molecule is evident in many forms of cancer. The evidence for increased protein levels of B7-H1/PD-L1 comes largely from immunohistochemistry analysis of surgical and biopsy specimens. While several studies attempt to correlate B7-H1/PD-L1 expression with poor prognosis, there are studies that conclude no correlation based on technical difficulties associated with detecting B7-H1/PD-L1 protein expression on the cell surface. We were able to quantitatively measure soluble B7-H1/PD-L1 in conditioned medium from tumor cell cultures using two new immunomassays, a Quantikine® ELISA and a Simple Plex™ Assay. We show that breast, prostate, glioma, and non-Hodgkin’s lymphoma cells express various levels of B7-H1/PD-L1 which correlate to their PTEN or proliferation status. This is in agreement with previous findings showing that increases in B7-H1/PD-L1 expression levels are frequently coincident with loss of the PTEN tumor suppressor or increased activation of the phosphoinositide 3-kinase (PI 3-K) pathway. The androgen-responsive LNCaP prostate cancer cell line does not exhibit measurable B7-H1/PD-L1 in any instance, while the androgen-independent prostate cancer cell line PC-3 expresses measurable B7-H1/PD-L1 that is further inducible with PMA treatment. In all cell lines included in our study, incubation with PMA increases the level of B7-H1/PD-L1 released into the culture media, while pretreatment with the PI 3-K inhibitor, LY294002, abrogates this increase. Importantly, the Simple Plex platform is able to quantify very low levels of B7-H1/PD-L1 that are below detection by ELISA. Our data suggest that combining the Quantikine ELISA and Simple Plex platforms is a powerful strategy for reliably and quantitatively assessing soluble B7-H1/PD-L1 levels.

#3979 Immune correlates of pathologic response in bladder cancer patients undergoing neoadjuvant chemotherapy. Philip H. Abbosh,1 David Liu, Woonyoung Choi,1 Wake S. El-Deiry,1 Jonathan E. Rosenberg,5 David McConeky,5 Elizabeth R. Plimack,1 Eliezer M. Van Allen2. Dana Farber Cancer Institute, Boston, MA; 3Center de recherche du CHU de Québec, Quebec, Canada; 4Dana Farber Cancer Institute, Boston, MA; 5Memorial Sloan Kettering Cancer Center, New York, NY.

Introduction and Objective: The immune system is increasingly recognized as both a key player in cancer and a druggable target. We hypothesized that the immune system impacts pathologic response in patients undergoing cisplatin-based neoadjuvant chemotherapy (NAC) for muscle-invasive bladder cancer though the immune system. Methods: Whole exome sequencing (WES) was performed on tumor DNA from patients in two independent cohorts who underwent cisplatin-based NAC. The Memorial Sloan Kettering/Dana Farber Cancer Institute (n=50, 25 responders) and Philadelphia cohorts (n=48; 20 responders) were treated with gemcitabine/ cisplatin or methotrexate/ vinblasia/ docorubicin/ cisplatin. Mutation analysis was performed using standard analytical pipelines. Macrohistocompatibility complex (MHC)-restricted neoantigens were identified with netMHCPan and PolySolver. High affinity neoantigens were defined to have ≤500 nM binding affinity (Kd). Pathologic response was defined as ≤ypTis cystectomy specimen. CIBERSORT was used to infer immune cell infiltrate based on DASL Illumina microarray expression profiles (n=41, 17 responders). Results: Chemoresponders had >twice as many putative neoantigens as nonresponders (471 vs 207 neoantigens respectively; p=1×10^-8, Wilcoxon). A positive relationship of >1.4-fold was noted with median survival when neoantigens were limited to $K_d$≤100 nM or ≤50 nM, and when chemoresponse was defined as ypT0-only, or ≤ypT1. ERCC2 loss-of-function mutations were 36% sensitive in identifying chemoresponders but 96% specific. Above-median neoantigen burden was 81% sensitive in identifying chemoresponders and 78% specific. CD8+ cells were enriched in responders (13.5% vs 8.4% of infiltrate; p<0.0008, t-test) as were many PD-1+ cells (15.2% vs 12.7% NK=0.05). In 27 cases where CIBERSORT data was available, the neoantigen burden was correlated to the CD8+ infiltrate value (R²=0.33, Pearson). Conclusions: Neoantigen burden and CD8+ infiltrate correlate strongly with chemoresponse. Neoantigen burden and CD8+ infiltrate are directly proportional. NAC may therefore exert known tumor cell autonomous effects and an extrinsic effect involving the immune system via neoantigens. Anti-neoantigen responses have been shown to impact immune checkpoint responses and a similar mechanism may mediate cytotoxic chemoresponses. Experimental studies are underway to directly and functionally characterize the effect of the immune system in chemoresponse.

#3980 Ly6G+ neutrophils and polymorphonuclear myeloid derived suppressor cells promote the survival of tumor cells. Sarah Herlihy, Cindy Lin, Maria Ozeriva,1 Alfred Garfall,2 Dan Vogl, Dmitry Gabrilovich,3 Yulia Nefedova1.


A heterogeneous group of immature myeloid cells which suppress the immune system are termed myeloid derived suppressor cells (MDSC). Collectively a MDSC population is comprised of monocye-like MDSC and polymorphonuclear MDSC (PMN-MDSC). MDSC are increased in the presence of a number of different tumors. We previously demonstrated that PMN-MDSC represent the majority of MDSC in tumor-bearing mice. In a mouse model of multiple myeloma (MM) depletion of myeloid cells in vivo results in increased survival. Direct co-culture of neutrophils (CD11b+ Gr1+ cells from tumor-free mice) or PMN-MDSC (CD11b+ Gr1+ cells from tumor-bearing mice) and MM cells increases the survival of the MM cells from chemotherapeutic-induced death. Cell-cell contact is not necessary for the protective effect. Neutrophil and PMN-MDSC supernatants protect MM cells from cell death, suggesting the factor protecting tumor cells is soluble. Results in the mouse were confirmed using human MM cells and replicated on breast cancer cells with neutrophils or PMN-MDSC. Several cytokines and growth factors, such as IL-6 and fibroblast growth factor, are implicated in chemoresistance. The role of these neutrophil and PMN-MDSC soluble factors and the identification of novel contributors to chemoresistance are under investigation.

#3981 The Shc1 scaffold protein simultaneously balances Stat1 and Stat3 activation in breast cancer to promote immune suppression and resistance to immunotherapy. Ryuhjin Ahn,1 Kévin Jacquet,2 Marc Fabian,3 Sidong Huang,4 Nicolas Bisson,4 Josie Ursini-Siegel1. 1Lady Davis Institute, Montreal, Quebec, Canada; 2Centre de recherche du CHU de Quebec, Quebec, Canada; 3Rosalind and Morris Goodman Cancer Research Centre, Montreal, Quebec, Canada.

Receptor and cytoplasmic tyrosine kinases are key signal integrators in poor outcome breast cancers that are central to the establishment of an immunosuppressive microenvironment. Immunotherapies represent an emerging approach within the armament of anti-cancer agents. Although the efficacy of tyrosine kinase inhibitors relies, in part, on their ability to augment adaptive immunity, the increased heterogeneity and functional redundancy of the tyrosine kinase in poor outcome breast cancers represents a significant hurdle to achieving durable responses to immunotherapies. We previously identified the Shc1 (ShcA) scaffold protein, a central regulator of tyrosine kinase signaling, as essential for promoting immune suppression. We recently showed that the ShcA pathway simultaneously activates STAT3 immunosuppressive signals and impairs STAT1-driven immune surveillance in breast cancer cells. Impaired phosphorylation of select tyrosine residues on ShcA potentially and selectively reduces STAT3 activation in breast tumors, profoundly sensitizing them to immune checkpoint inhibitors and tumor vaccines. Meanwhile, impaired phosphorylation of other select tyrosine residues on ShcA potently increased antigen presentation and sensitivity to tumor vaccines in preclinical mouse models. Based on these results, we have set out to elucidate protein interactors dependent on distinct ShcA phospho-tyrosines to regulate STAT1 and STAT3 signaling axis that aid tumor driven immune suppression. We combined affinity-purification mass spectrometry (AP/MS) and proximity-dependent biotin identification
#3982 Tumor exosomes lead to positive feedback in tumor associated macrophages leading to increased invasion and metastasis. Daniel C. Rabe, Felicia D. Rustandy, Rebecca Sturey, Marsha R. Rosner. University of Chicago, Chicago, IL.

Triple-negative breast cancer (TNBC) patients have the highest risk of recurrence and metastasis. This is in part due to the infiltration of macrophages into the tumor that promote growth, survival, invasion, and therapy resistance through secretion of growth factors and cytokines. We previously demonstrated the metastasis suppressor Raf Kinase Inhibitory Protein (RKIP) regulates the number and phenotype of tumor-associated macrophages (TAMs) by blocking CCL5. Although CCL5 protein is sufficient to recruit pro-invasive TAMs, alone it does not robustly induce expression of pro-invasive genes, nor does it lead to a pro-inflammatory macrophage phenotype. We therefore tested whether exosome crosstalk within the tumor microenvironment was responsible for transmitting a pro-inflammatory phenotype between tumor cells and TAMs. To determine the role of exosomes in macrophage phenotype, we programmed naïve mouse macrophage derived macrophages (BMDMs) with conditioned media (CM) from TNBC cells, CM depleted of exosomes, or tumor exosomes alone. Only tumor exosome treatment increased levels of pro-invasive genes MMP12, PGRN, and CCL7, while CM or CM depleted of exosomes did not. This pro-inflammatory macrophage phenotype was lost if exosomes from non-invasive (RKIP+) tumor cells were used or if tumor exosome secretion was inhibited. We determined that RKIP regulated the pro-inflammatory phenotype of macrophages in part through cytokine mRNA in exosomes. Levels of pro-inflammatory mRNAs like CCL5 were drastically lower in exosomes from RKIP+ cells compared to control. Additionally, exosomes from CCL5 over-expressing cells further increased the pro-inflammatory nature of macrophages while those from tumor cells with CCL5 knocked out using CRISPR not drive a pro-inflammatory phenotype. For in vivo validation, mice were injected with exosome-depleted tumor cells or co-injected with exosome-programmed BMDMs. These results indicate exosomes reprogram TAMs to reflect increased invasiveness of the tumor cells, leading to positive feedback onto the tumor cells to drive invasion and metastasis.

#3983 A cross comparison of technologies for the detection of immune system related gene expression signatures in clinical FFPE samples of metastatic prostate cancer patients. Kimberly Kruse,1 Fang Yin Lo,1 Ryan Fleming,1 Douglas Chung,1 Vickie Satle,1 Linden Maasell,1 Tuuli Saloranta,1 Inah Golez,1 Saman Tahir,1 Sally Dow,1 Evan Anderson,1 Spencer Chee,1 Raghave Venkatraman,1 Steve Anderson,2 Peter S. Nelson,1 Colm Morrissey,1 Anup Madan,1 Sharon Austin,1 Kellie Howard2,3, Covance, Redmond, WA, 2Covance, Durham NC,3Fred Hutchinson Cancer Research Center, Seattle, WA, 4University of Washington, Seattle, WA.

The success of immunotherapy for the treatment of metastatic cancers relies on the prediction and identification of potential neo-antigens. In recent years expression levels of these neo-antigens along with other immune system related genes have been evaluated in an effort to better understand response rates for immunotherapy in various cancers. Gene expression levels can be assessed by numerous techniques including hybridization-based or direct sequencing technologies. Two platforms-HTG Molecular and NanoString nCounter have been utilized to profile changes in gene expression and offer unique advantages for analyzing challenging specimens such as formalin-fixed paraffin embedded (FFPE) tissues. The NanoString nCounter platform utilizes hybridized fluorescent probes targeted against genes of interest for a non-amplified measurement of gene expression. Several studies have been shown that the NanoString platform has good sensitivity, specificity, and reproducibility for the assessment of gene expression levels from FFPE samples. The HTG platform is relatively new and also uses a hybridization based method to enrich genes of interest without first isolating RNA. To determine the robustness of the HTG platform, we profiled a set of 30 metastatic prostate cancer samples using the HTG Molecular EdgeSeq Immuno-Oncology Assay. In these experiments, we found that expression data obtained by using both extracted RNA and lystate from FFPE slides was highly reproducible (Spearman coefficient $> 0.85$). In addition, the expression profile of targeted genes obtained by using different slides from the same blocks was also highly correlated (Spearman coefficient $> 0.90$). Our experiments also showed a high correlation between gene expressions profiles obtained by HTG, the NanoString PanCancer Immune Profiling panel and RNA-Seq from the same set of 30 metastatic prostate cancer samples. Further analysis to evaluate and compare the sensitivity of different platforms is being performed and results of these will be presented.
NCoR2 regulates GBM progression and resistance to therapy potentially via inducing a stem-like phenotype as well as responding to the tumor-promoting mechanical cues within the GBM microenvironment.

**#3986 Regulation mechanism and clinical significance of KPNA2 promotes MDCSs infiltrating in epithelial ovarian cancer.** Shuting Huang, Min Zheng, Yuanzhong Wu. Sun Yat-Sen University Cancer Center, Guangzhou, China.

Myeloid-Derived Suppressor Cells (MDCSs) are a population of immune cells that negatively regulate immune responses, which could promote tumor invasion and metastasis. Recent research has revealed that tumor could actively reshape the immune-microenvironment for its progression. We recently demonstrated that KPNA2 is a candidate oncogene in ovarian cancer that was closely related to MDCSs density, and found there was a positive position feedback between KPNA2 expressed and MDCSs density, which might contribute to the poor prognosis of patients. Based on these results, we combined clinical sample analysis, animal model and experimental studies to do further molecular and genomic research. The conduction of this project will help explain the molecular mechanisms of how KPNA2 regulates ovarian cancer through suppressing the immune system, and provide new strategies by molecular targeted therapy combining biological therapy for ovarian cancer.

**#3987 Solid stress and elastic energy as measures of tumor mechanobiology.** Hadi Nia,1 Hao Liu,1 Giorgio Seano,1 Meenal Datta,1 Dennis Jones,1 Nuh Rahbari,1 Joao Incio,1 Vikash Chauhan,1 Keehoo Jung,1 John Martin,1 Vasileios Asksylakis,1 Tim Pader,1 Dai Fukumura,1 Yves Boucher,1 Francis Hornick,1 Alan Grodzinsky,1 James Baish,1 Lance Munn,1 Rakesh Jain,1 MGH/Harvard Medical School, Boston, MA;1 MIT, Cambridge, MA;2 Bucknell University, Lewisburg, PA.

Introduction: Increased tissue stiffness is a widely accepted and actively studied biomechanical property of desmoplastic tumors, and has been linked to several hallmarks of cancer, such as growth, invasion and metastasis. The abnormal mechanics of tumors, however, are not limited to tissue stiffening. We recently demonstrated that solid stress represents a new mechanopathology that is consistently elevated in mouse and human tumors. The solid stress, transmitted by solid elements of the extracellular matrix, is distinct from interstitial fluid pressure. Therefore, tumors are not only more rigid than many normal tissues, but cancer cells also produce and are exposed to these physical forces. Composed of a combination of tension and compression, these forces are significant in tumors, but negligible in most normal tissues. Methods and Results: We developed the experimental and mathematical frameworks to provide (i) two-dimensional spatial map of solid stress in tumors (planar cut method), (ii) sensitive estimation of solid stress in small tumors with small magnitudes of solid stress, e.g., metastatic lesions (slicing method), and (iii) in situ quantification of solid stress in tumors, which retains the effects of the normal surrounding tissues (needle biopsy method). All three methods are based on the physical concept of releasing the solid stress in a controlled way with defined geometry, and then quantifying the stress-induced deformation by high-resolution ultrasonography or optical microscopy. Given the specific topography of the stress relaxation and the geometric and material properties of the tumor, the solid stress and discharged elastic energy is estimated using mathematical modeling. Applying these novel methods to multiple mouse cancer models in primary and metastatic settings has led to the following novel findings: (i) solid stress and elastic energy may be different between primary vs. metastatic settings, as they depend on both cancer cells and their microenvironment; (ii) tumor with higher elastic energy are not necessarily stiffer, and the stiffer tumors do not necessarily have higher elastic energy; (iii) solid stress increases with tumor size; and (iv) the normal tissue surrounding a tumor significantly contributes to the intratumoral solid stress. Conclusions: We developed three distinct methods to perform in situ and sensitive measurement of solid stress and obtain 2-D spatial map of solid stress in human and mouse tumors. Application of these methods in models of primary tumors and metastasis revealed that: (i) solid stress depends on both cancer cells and their microenvironment; (ii) tumor growth with tumour size; and mechanical confinement by the surrounding tissue substantially contributes to intratumoral solid stress. Further study of the genesis and consequences of solid stress, facilitated by the engineering principles presented here, may lead to significant discoveries and new therapies.

**#3988 Antigen presentation by tumor-infiltrating B cells associates with CD4 T-cell phenotype and function in NSCLC patients.** Tullia C. Bruno. University of Pittsburgh, Pittsburgh, PA.

Despite improvements in surgical techniques and combined chemotherapies, the 5-year survival rate for all stages of non-small cell lung cancer (NSCLC) is only 18%. The focus of immunotherapy has been on subsets of CD8 and CD4 Tumor infiltrating lymphocytes (TILs); however, tumor infiltrating B cells (TIL-Bs) have been reported in tertiary lymphoid structures (TLS) with CD4 TILs, and both TIL-Bs and TLS correlate with NSCLC patient survival. While TIL-Bs have been identified in NSCLC patients, their function in the tumor microenvironment has been understudied with no focus on their role as antigen presenting cells (APCs) and their influence on CD8 and CD4 TILs. We hypothesize that TIL-Bs help generate potent, long-term immune responses against presenting tumor antigens to CD4 TILs. Using unmanipulated, primary human B cells from fresh tumor, tumor adjacent and normal (cancer-free) lung tissue, we observed that the total number of B cells at the site of the tumor versus the tumor-adjacent tissue was increased compared to other immune subsets. We generated a specific antigen presentation assay in vitro, and we observed three types of CD4 TIL responses when TIL-Bs presented autologous tumor antigens. There were activated CD4 TIL responses; CD4 TIL proliferation when combined with TIL-Bs alone, which indicates stimulation with endogenous tumor antigens. This was associated with exogenous antigen presentation by TIL-Bs that did not respond to antigen presentation by TIL-Bs alone. Within the activated and antigen-associated responders, the TIL-B phenotype associated with the CD4 TIL phenotype; if the TIL-Bs were activated (CD69+CD27+CD21+), the CD4 TILs were T helper (antigen) tumor CD4 T cells and if the TIL-Bs were exhausted (CD69-CD27-CD21-), the CD4 TILs were T regulatory cells (pro-tumor). These data suggest that TIL-Bs influence the phenotype and function of CD4 TILs in NSCLC patient tumors. In conclusion, TIL-Bs are increased in NSCLC primary tumors, they can present antigen to CD4 TILs and influence their overall phenotype. Determining if TIL-Bs are activated or exhausted in NSCLC patients will determine the extent of their anti-tumor function in the cancer. Ultimately, results from this study will help predict how to target TIL-B functions in future TIL-B-specific immunotherapies or in combination with current immunotherapies for NSCLC patients like blockade of the inhibitory receptor, PD-1.

**#3989 Different expression and prognostic effect of PD-L1/PD-1 in SCC and non-SCC of non-small cell lung cancer.** Yunpeng Liu, Shuo Wang, Xiujuan Qu, Zhi Liu, Xiaofang Che, Xu Ling, Kezuo Hou, Yibo Fan, Na Song, Ti Wen, Ce Li. The First Hospital of China Medical University, Shenyang, China.

Although checkpoint inhibition targeting PD-1/PD-L1 pathway have demonstrated similar clinical activity in treatment of NSCLC both for squamous (SCC) and non-squamous carcinoma (non-SCC) patients, the predictive role of PD-L1 expression is distinct between two histologies in clinical trials. In order to investigate the expression and prognostic roles of PD-1/PD-L1, data from TCGA and GEO database were analyzed and further validated with our original data by IHC in 133 selected NSCLC tumor tissues (49 SCC and 84 non-SCC). Through the analysis of online data, we concluded that PD-L1 expression in SCC was significantly higher than that of non-SCC. In addition, in non-SCC, PD-L1 was associated with immune response and indicated worse prognosis while no such results were observed in SCC. Further, our original data demonstrated PD-L1 expression in tumor cells(TC) and immune cells(IC) were significantly more often and more intense in SCC (P<0.001). In survival analysis, PD-L1 expression in TC and IC were associated with shorter overall survival in non-SCC (P=0.019 and 0.003, respectively). However, in SCC, PD-L1 did not demonstrate any significant effect neither in TC or IC (P=0.526 and 0.674, respectively) which consistent with online data. Contrary to PD-L1, no significant differences of PD-1 expression in tumor infiltrating lymphocytes(TILs) were showed between two histologies. PD-1 in TILs of SCC correlated with worse prognosis (P=0.025) while in non-SCC there was no correlation (P=0.986). Taken together, these findings suggest distinct regulation mechanisms and clinical significance of PD-L1/PD-1 pathway in NSCLC subgroups.

**#3990 Use of 3D tumoroid systems to define immune and cytotoxic therapeutic responses based on tumoroid and tissue slice culture molecular signatures.** Niklas K. Finngberg,1 Prashanth Gokare,2 Avital Levy,1 Alexander W. MacFarlane,1,2 Kerry S. Campbell,1,2 Karen Kaputa,1 Jeffrey Farma,1 Luigi Grasso,2 Nicholas C. Nicolaides,1 Wafik El-Deiry2,1 Fox Chase Cancer Center, Philadelphia, PA;3 Morphotek, Inc., Exton, PA.
Historically, the successful establishment of exponentially growing viable in vitro tumor cell cultures has been found to occur from a minority of tumor tissues following artificial selection of a sub-population of tumor cells. Such limitations make it difficult to model patient variability in drug responses in vitro. Moreover, the lack of stromal cells that play a critical role in tumor viabil-
ity has hindered efforts to create in vitro models including anti-tumor immunity. Although evi-
dences from preclinical and clinical settings suggest that dissemination of ma-
lignant cells is an early process, majority of disseminated cells either eliminated or remain dormant in distant organs, while very few cells eventually develop successful metastasis. Therefore, it is widely accepted that dynamic and revers-
ible tumor cell plasticity is required for metastasis, however, in vivo steps and mechanisms involved remain poorly elucidated. We provide evidence that mono-
cytic and granulocytic subsets of myeloid derived suppressor cells (m-MDS, g-MDS) infiltrated in primary 4T1 tumor and distant organs with different time kinetics regulate spatiotemporal tumor plasticity. Using co-culture experi-
ments and syngeneic mouse models of murine 4T1 (metastatic) tumor, we demonstrated that tumor infiltrated m-MDSs facilitate tumor cell dispensa-
tion from the primary site by inducing the EMT/CSC phenotype. In contrast, g-MDSs infiltrated in the lungs support metastatic growth by reverting the EMT/CSC phenotype and thus promoting tumor cell proliferation. In contrast, less invasive EMT6 tumors fail to induce efficient pulmonary infiltration of g-
MDSs and results in clearance of disseminated tumor cells in the lungs. Gene expression analyses of tumors and MDSs subsets in primary tumor site and in recurrent tumors (n=12) corroborated the cell plasticity and generation of permissive or anti-tumorigenic microenvironment in distant organs determining the fate of disseminated tumor cells.

#3991 Profiling the immune tumor microenvironment in primary and recurrent epithelial ovarian cancer. Laureen S. Ojalvo, Elizabeth D. Thompson, Tian-Li Wang, Alan K. Meeker, Je-Ming Shih, Amanda N. Fader, Ashley A. Camino-Mathews, Leisha A. Emens, Johns Hopkins Hospital, Baltimore, MD.

Clinical trials targeting the immune tumor microenvironment (TME) in epi-
theelial ovarian cancer (EOC) typically have included patients with heavily pre-
treated advanced disease and demonstrated only marginal efficacy. A better understand-
ing and harnessing of EOC TME recurrent with progression from primary to recur-
dent disease may inform future immunotherapy trials. Here, we evaluate the immune TME in primary and recurrent EOC using tissue microarrays. Our cohort included matched primary and recurrent tumors from 17 patients, and ad-
ditional non-matched primary tumors from 20 patients and recurrent tumors from 15 patients. We stained for CD8 (FOXP3 (regulatory T cells (Tregs)), CD68 (tumor associated macrophages (TAMs)), programmed cell death protein 1 (PD-1) and programmed death ligand 1 (PD-L1) by immunohistochemistry to interrogate the immune composition of the TME. Tregs increased in recurrent tumors compared to primary tumors (8.0 vs 14.2/HPF, p = 0.0210). Higher TAM density was associated with higher levels of Treg and CD8+ T cell infiltrates in recurrent tumors (p = 0.001 and p = 0.001, respectively), and with higher Treg but not CD8+ T cell infiltrates in primary tumors (p = 0.027 and p = 0.200). TAM-dense recurrent tumors had increased PD-L1 on tumor cells and immune cells, whereas TAM-dense primary tumors had increased PD-L1 only on im-
mune cells. Increased Tregs in primary tumors correlated with decreased time to first recurrence (17.0 vs 28.5 months, p = 0.022). Conversely, increased Tregs in recurrent tumors correlated with longer overall survival (OS) from recurrence (median not reached vs 26.0 months, p = 0.022). Although TAM density did not affect patient survival, analysis of matched primary and recurrent tumors revealed that patients with increased TAMs at recurrence (n = 5) had a longer median OS from recurrence than patients without increased TAMs at recurrence (n = 12). Tregs increased at recurrence in the majority of matched tumor pairs (n = 12), but there was no correlation with survival. In conclusion, the TME of EOC is im-
munologically active. TAM-dense recurrent diseases had higher CD8+ T cell and Treg infiltrates and PD-L1 expression. In this study, patients with increased cellular recruitment to the TME at recurrence had improved survival. Larger, more detailed studies characterizing the evolution of the TME with progression from primary EOC to recurrence are warranted.


Metastatic disease is the end stage of extremely inefficient processes that entail complex changes in vivo similar to those occurring in vitro during immune-based therapeutics challenging. Through recent advances in three-di-

mensional (3D) culture (“tumoroid”) methodologies some of these hurdles are be-

gun to be addressed. However, it is currently unclear to what extent such models can be engineered to retain important phenotypic properties of infiltrat-

ing immune from the tumor tissue of origin. To better understand these limitations, we have cultivated tumor slice cell culture slices from tumors derived from patients with colorectal cancer (CRC) to evaluate immune cell populations infiltrating cultured CRC tissues. Furthermore, we have developed a system whereby the patient’s immune cells are re-incorporated into tumoroid in vitro cultures to evaluate the ability of the culture to mimic an immunosuppressive tumor microenvironment (TME). Here we demonstrate the development of a propagating 3D epithelial tumoroid culture system from re-

sected CRC tissue where we assessed the response to standard FDA approved therapy within weeks of surgical resection. Interestingly, tumoroid cultures from a CRC patient were highly sensitive to the thymidylate synthase inhibitor flurouracil (adriam) but less sensitive to the combination of the nucleoside analog trifluridine and the thymidine phosphorylase inhibitor tipiracil (Lon-

sur). We have also shown that the tumoroid is sensitive to cell cycle and cell density gradient centrifugation and flow cytometry. Re-introduction of isolated immune cells derived from surrounding and infiltrating tumor tissue and CD45+ tumor infiltrating hematopoietic cells displayed prolonged (>10 days) survival in co-culture. Moreover, established tumor slice cultures contained both an outer epithelial and inner stromal cell compartment mimicking tumor structure in vivo. Collectively, these data suggest that CRC tumoroid in vitro assays can be used for the assessment of some therapeutic responses. While further work is required to optimize this system, 3D tumoroid and slice culture assays may represent a novel in vitro approach to assess therapeutic efficacy of novel therapies and evaluate mechanisms of therapy resistance in general as well as for patient-specific response.

#3993 A different spatiotemporal distribution of TIL subtypes is associ-

ated with response to neoadjuvant chemotherapy in locally advanced breast cancer patients. Lisa Koenig,1 Fabian Dominik Mairinger,2 Oliver Hoffmann,1 Anu-Kathrin Bittner,1 Kurt Werner Siegal,3 Rainer Kimmig,1 Sabine Kasimir-

Bauer,1 Agnes Bankfalvi,2 1Department for Gynecology and Obstetrics, University Hospital of Essen, Essen, Germany; 2Institute for Pathology, University Hospital of Essen, Essen, Germany.

Background: Tumor infiltrating lymphocytes (TILs) are described as an im-
portant immune modulator in the tumor microenvironment in cancer and were shown to be associated with breast cancer (BC) outcome. Besides the analysis of total TILs, the discrimination into TIL subtypes is essential as they exhibit pro-
tumor-immunogenic potential. The spatiotemporal TIL distribution at the inva-
sive front (ITF) and the tumor center (TC) might provide insights into tumor progression. Here we analyzed TIL infiltration in core biopsies of BC patients (pts) prior to neoadjuvant chemotherapy (NACT) and correlated these findings with clinical parameters and tumor cell spread. Material and Methods: Core biopsies from 87 pre-NACT BC pts were cut, deparaffinized and antigen retrieval and automated immunohistochemistry were performed using the fol-
lowing primary antibodies: CD3 (clone: SP7; 1:400, DCS Innovative Diagnostic Systems, Hamburg, Germany), CD4 (clone: IF6; 1:40, Eryzomed Systems, Berlin, Germany), CD8 (clone: C8/144B; 1:150), CD20 (clone: L26; rtu), CD68 (clone: PG-M1; 1:500, all DakoCytomation, Glostrup, Denmark). Binding of primary antibody was visualized using the OptiView DAB kit (Ventana Medical Systems, Tucson, USA). The densities of total TILs (H&E staining) and TIL subtypes were evaluated microscopically at the TC and ITF and classified into three categories: Low = 0-10%, Moderate = 11-30%, High = 31% infiltration. TIL results were correlated with clinical parameters and disseminated tumor cells (DTCs) in the bone marrow, determined by immunocytometry applying the pan cytoker-
atin antibody A45-B/B3. Results: TILs were differentially distributed at both tumor areas. The ITF was mainly infiltrated by CD3+ T- and CD20+ B cells, while low amounts of CD68+ macrophages, CD4+ and CD8+ T cell subtypes were present. Only CD3+ T cells were present in a higher level at the TC. A high positive correlation was observed between the ITF and breast tissue infiltration from poorly differentiated tumors. Pre-NACT tumors < T2 had a high CD4+ T cell infiltration at the TC, whereas in small post-NACT tumors a high infiltration of total TILs and all TIL subtypes at the ITF, except CD68+ cells, was observed. BC pts responding to NACT exhibited significantly more total TILs (p = 0.02) and

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CD3+ T cells (p = 0.02) at the TC. A high CD4+ T cell infiltration at the TC was significantly associated with the presence of DTCs post-NACT (p = 0.029). CD8+ macrophages located at TC or ITF were not related to any clinical parameter. Conclusion: The differential association of TIL subtypes with clinical parameters and NACT response underlines the necessity of detailed TIL analysis for gastric cancer patients to improve immune modulation of tumor microenvironment. In this regard, BC patients not responding to standard NACT might be identified for application of additional immunotherapy.


The tumor microenvironment (TME) is made up of stromal cells, immune cells, signaling molecules, and blood vessels surrounding the tumor cells. It has emerged as a key factor in multiple stages of cancer progression, immune-escape, and disease progression. The composition and activity of TME co-evolve with tumor cells and may likely affect how the cancer responses to immunotherapy. A clear understanding of the functional effect and evolution of the TME necessitates a comprehensive approach to identify key immune cells as well as to characterize signaling and inflammatory (immune-editing) activities at the tumor site. Here we are describing the use of a targeted NGS panel to detect aggregated gene expression and a novel AmpliSeq™ approach to profile the relative abundance of different T cell clones at the TME. Specifically, we measured the expression of 395 relevant genes that capture interferon and chemokine signaling, T and B cell activation, checkpoint pathway, tumor proliferation, and antigen presentation. By looking at the expression of markers specific to different effector cell types, this gene panel offers a high-level view of the composition of different lymphocyte infiltrates. Complementarily, the TCR sequence assay provides an estimate of T cell diversity and therefore offering a different dimension of the immune response. We studied 40 NSCLC tumor samples, of which we have matched FFPE and fresh frozen specimens. With a targeted panel, we could detect expression of transcripts present as few as 50 copies in 10 ng of total RNA as input. Across 40 NSCLC samples, we were able to measure expression of many low expressing cytokines such as IL2, IL11, IL23, IFNG and TNF. We observed strong co-expression pattern among genes involved in type II interferon signaling, indicating that they’re informative of T cell activation. More specifically, we found strong correlation between CD8 expression and other T cell co-stimulatory receptors (CD28, CD80, CD86, CD40), suggesting that expression of these genes can be reliable surrogates for the protein counterparts as markers for CD8+ T cells. TCRβ was sequenced for each of the matched fresh frozen sample in duplicates. Clonotype abundance of replicates was highly correlated with each other, indicating that the assay was reliable, and the samples were sequenced to appropriated depth. We identified 200,000 unique clones in each tumor sample; with the diversity index moderately correlated with the percentage of tumor infiltrating lymphocytes provided by pathology review. Together, these two assays provide a convenient means to characterize T cell phenotypes and activities in tumor microenvironment, offering insights into how that tumor may respond to a particular immunotherapy.

**#3995 Identification of receptor tyrosine kinases (RTKs) expression in tumor-infiltrating lymphocytes (TILs) and peripheral blood mononuclear cells (PBMC).** Dmitry Khochenkov,1 Maria Volkova,1 Ilya Tsimaflayev,2 N.N. Blokhin Russian Cancer Research Center, Moscow, Russia; Russian Federation; 2Kidney Cancer Research Bureau, Moscow, Russian Federation.

Aim of this study is to discover expression levels of RTKs (VEGFR1, VEGFR2, PDGFRα, PDGFRβ) in CD45+ population of PBMC and TILs isolated from patients with clear cell renal cell cancer (RCC). Here we present preliminary data. Species of tumors and blood were prospective collected from 9 patients with RCC after radical nephrectomy or tumor resection from July to October 2016. TILs isolation was performed under standard protocol with slight modification. Briefly, tumor was shredded with scissors on small pieces of 2-3 mm, and twice washed in RPMI 1640 media by centrifugation for 10 min at 1500 rpm. After centrifugation, pellet was diluted RPMI 1640 with enzyme mix (Collagenase and Dispase II) on Petridish and placed on shaker-thermostat and incubate After centrifugation, pellet was diluted RPMI 1640 with enzyme mix (Collagenase and Dispase II) on Petridish and placed on shaker-thermostat and incubate for 1 hour. Expression on CD45+ cell population were evaluated. Among PMBC 86.9% of cells was CD45+ and positive expressed RTKs (10%+ cells were positive for VEGFR1 and PDGFRβ, and 6%+ cells were positive for PDGFRα and PDGFRβ). TILs contained 65.3% of CD45+ positive cells and did not express RTKs on the cell surface (less than 1% of cells), but intracellular staining showed presence of RTKs: 8.2% of cells were VEGFR1 or VEGFR2 positive. 20.8% of cells were PDGFRα or PDGFRβ positive. Preliminary data demonstrate extracellular expression of RTKs on PBMC and intracellular expression of RTKs in TILs. The study will be continued.

**#3996 MDSC depletion combined with CTLA-4 blockade cause tumor regression in a syngeneic model of oral cavity cancer.** Paul Esteban Clavijo, Ellen Moore, Jianhong Chen, Ruth Davis, Carter Van Waes, Zhong Chen, Clint Allen. NIH, Bethesda, MD.

Carcinogen-associated head and neck cancers are a heterogeneous group of aggressive cancers with a high recurrence rate and poor 5-year survival. Many of these cancers are T-cell inflamed but only a subset of patients respond to checkpoint blocking immunotherapy. Local immunosuppression mediated by both the tumor cells and other infiltrating immune cells are likely a major mechanism of resistance to immunotherapy. The mouse oral cancer (MOC) model includes genetically defined cell lines derived from carcinogen-induced tumors that generate tumors with variable T-cell inflamed phenotypes when transplanted into wild-type C57BL/6 mice. Mice bearing MOC1 tumors display a T-cell inflamed phenotype but do not significantly respond to PD-L1 checkpoint inhibition alone, which models a significant subset of human cancers from the oral cavity. Conversely, MOC2 tumors are non-T-cell inflamed. Using these MOC models, we have demonstrated an inverse relationship between accumulation of granulocytic MDSCs (gMDSCs) and effector immune cells (CD8/4+ T-cells, and NK cells) in the tumor microenvironment. Functionally, these gMDSCs suppressed CD8+ T-cell proliferation, IFNy production, and antigen-specific killing. Depletion of these gMDSCs with a systemic anti-Ly6G antibody (1A8) did not change CD8/4+ T-cell or NK cell accumulation but restored tumor T-cell and NK cell activation and draining lymph node antigen-specific T-lymphocyte activation that was lost with tumor progression. Depletion of gMDSCs also significantly sensitized established MOC1 tumors to CTLA4-based checkpoint inhibition. CTLA-4 blockade alone induced tumor regression in a subset of mice and a marked delay in others. However, gMDSC depletion plus CTLA-4 blockade resulted in complete tumor rejection and development of immunologic memory in all treated mice. Peripheral gMDSCs were found to express the chemokine receptor CXCR2 but no other myeloid chemokine receptors, and expression of CXCR2 ligands CXCL1 and CXCL2 increased within the tumor microenvironment during tumor progression. Macrophage chemotaxis components CSF1R and CSF1 were not significantly expressed in these models. TCGA data analysis demonstrated an MDSC-rich gene expression profile in >60% of head and neck cancers, along with significant CTLA4, CXCR2, CXC1L1, and CXCL8 (IL-8) expression within both HPV-positive and negative head and neck cancers. Functional inhibition or elimination of MDSCs from the tumor microenvironment may significantly reverse local immunosuppression and enhance responses to checkpoint inhibition in patients with head and neck cancer.

**#3997 Interleukin-4 receptor-targeted cytotoxic T cells enhances the therapeutic efficacy of adoptive T cell therapy against melanoma.** Gowri Rangaswamy Gunasekaran, Sri Murugan Poongkavithai Vadevoo, Guruprasath Padmanaban, Lianhua Chi, Ha-Jeong Kim, Byung Heon Lee. 1Kidney Cancer Research Bureau, Moscow, Russian Federation; 2Department of Medical Cell Therapy, Kyungpook National University, Daegu, Republic of Korea.

Melanoma, the deadliest form of skin cancer, has long been a major focus of immunotherapy. Adoptive T cell therapy-based cancer immunotherapy has been used to enhance the specificity and potential of host immune system to treat melanoma. However, the infiltration and antitumor function of cytotoxic T cells (CTLs) are blocked or down-regulated by various signals derived from tumor cells and cells in tumor microenvironment. A novel approach to specify and enhance the homing ability of CTLs to tumor is of high demand. Interleukin-4 receptor (IL-4R) is over-expressed on many types of cancer cells, including melanoma, and has been widely employed for targeted drug delivery. In this work, we examined whether the tumor homing efficiency and anti-tumor therapeutic efficacy of CTLs could be heighten by labeling CTLs with IL4RPeP-1, a targeting peptide that binds to IL-4R. The labeling was empowered by conjugating IL4RPeP-1 with Dicyclophosphatidylethanolamine-biological anchor for targeting peptide that bindstoIL-4R. The labeling was empowered by conjugating IL4RPeP-1 with Dicyclophosphatidylethanolamine-biological anchor for
membrane (DOPE-BAM), an oleoyl-acid-derived membrane anchor that incorporates into lipid membrane of cells. Tumor-specific CTLs isolated from the mice immunized by irradiated B16F10 melanoma cells were highly populated with CD62+CD44+ activated T cells. After labeling with IL4RPeptide 1 via DOPE-BAM, CTLs were still functionally active, as determined by intracellular interferon gamma (IFNg) production and gamma release assay (Fig. 38). Moreover, such labeling did not affect the proliferation of T cells. Adoptive transfer of IL4RPeptide 1-labeled, IL-4R-targeted CD45.1 + CTLs into CD45.2 mice bearing B16F10 melanoma enhanced their accumulation at tumor compared to that of unlabeled T cells. Tumor homing of IL4RPeptide 1-labeled T cells was further validated by in vivo fluorescence imaging of mice bearing 4T1 tumor at mammary gland. Adoptive transfer of IL-4R-targeted CTLs induced remarkable antitumor growth activity in mice bearing B16F10 tumor compared to untreated CTLs. After the transfer, flow cytometric study of immune cells showed significant increase of CD8+ T cells and F4/80+CD86+M1-polarized macrophages with noticeable decrease in tumor-promoting immune cells, such as F4/80+CD206+M2-polarized macrophages, GR1+CD11b+ myeloid derived suppressor cells, and CD3+CD4+ T cells. The CTLs accumulated at the tumor were mostly exogenous CD45.1+ T cells. These results implicate that IL-4R-targeted CTLs, which is enabled by labeling of with IL4RPeptide 1, can be a promising strategy in the field of adoptive T cell therapy against IL-4R-overexpressing tumor.

#3998 Alerting the immune system by removing epigenetic silencing of TH1 chemokines. Heather M. Sonnemann, Amber J. Giles, Caitlin M. Reid, Marsha-Kay N. Hutchinson, Deric M. Park, Mark R. Gilbert.

BACKGROUND: Solid tumors employ multiple mechanisms to evade an immune response. However, the potential to enhance the immune response to cancer has been proven in several malignancies and is under investigation in many others, including primary CNS tumors. Brain tumors in particular lack robust T-cell infiltration. Recent studies have found that certain tumors can be induced to express T-cell attracting chemokines. CXCL9 and CXCL10, by interferon gamma (IFNg). This response is further amplified using methyltransferase inhibitors (Peng, et al. Nature 2015. Vol 527: 249-253.), resulting in increased T-cell trafficking to tumors both in vitro and in vivo. We hypothesized that T-cell trafficking to brain tumors could likewise be enhanced with DNA and histone methyltransferase inhibitors to induce CXCL9 and CXCL10 transcription. METHODS: Assays were performed on 7 human glioma brain tumor cell lines. CXCL9 and CXCL10 expression were measured by real-time PCR. Two commercially available methyltransferase inhibitors, 5-aza-dC and GSK126, were utilized to demethylate DNA and histone H3 (K9 and K27), respectively. Histone methylation status was examined using Western blot. T-cell migration was measured using transwell migration assays. RESULTS: IFNg increased CXCL9 and CXCL10 transcription in brain tumor lines. GSK126 and 5-aza-dC enhanced expression of CXCL9 and CXCL10 compared to IFNg alone. Migration assays confirmed T-cell trafficking towards chemokines produced by tumor cells in response to methyltransferase inhibitors. CONCLUSIONS: These studies demonstrate that brain tumors express T-cell attracting chemokines CXCL9 and CXCL10 in response to IFNg. Furthermore, GSK126 and the combination of GSK126 and 5-aza-dC enhanced expression of CXCL9 and CXCL10 transcription by real-time PCR and T-cell trafficking by migration assay. Together, these data provide a potential means to increase T-cell trafficking into tumors and potentially enhances the efficacy of immune therapies for brain tumors.

#3999 Cross talk between tumor cell derived HMGB1 and adaptive B cells in the tumor microenvironment of esophageal squamous cell carcinoma. Nigar Woon Kam, Xin-Yuan Guan, Dora Lai-Wan Kwong. Clinical Oncology and the Centre for Cancer Research, Hong Kong, Hong Kong.

Esophageal squamous cell carcinoma (ESCC) is the most aggressive type of cancer among gastrointestinal cancers. The lack of effective anti-tumor immunity strategy for ESCC patients imposes urgent demand for new treatment paradigm. Tumor microenvironment (TME) is characterized by diverse cell populations (tumor cells, immune cells, and stromal cells) and multiple types (cellular and inhibitory receptors) whose alternations are closely related with tumor progression. High mobility group box chromosomal protein 1 (HMGB1) has been recognized to be expressed in various tumor cells, and importantly it has also been identified as one of the key factors regulating inflammatory reactions. By comparing differentially expressed genes between ESCC tumors (T) and their corresponding non-tumorous (NT) tissues high-throughput transcriptome sequencing system (RNA-Seq, Illumina HiSeq 2500), we found that the ligands of TIM-3 (an inhibitory receptor), HMGB1 and galectin-9, but not, cell-surface phosphatidylinerine, were overexpressed in all ESCC samples when compared to their NT counterparts. We further characterized HMGB1 and galectin-9 gene expression changes in 37 matched primary ESCC T and NT control samples by qRT-PCR. We found that HMGB1-1 and galectin-9 mRNA is constitutively expressed in all samples, while ESCC T tissue significantly expressed ~2 fold higher of HMGB-1, but not galectin-9, mRNA as compared to paired NT tissue. Histopathological characterization of ESCC tissue by sequential single staining (immunohistochemistry) and phenotypic release assay (Fig. 38), demonstrated that HMGB1 was dominantly expressed by CD20+ B cells lacking of terminal differentiation into plasma cells. Additionally, CD20+ B cells infiltrates were negative for CD27 (marker for memory B cells) and IgD (marker for naive B cells). Additionally, we isolated CD20+ B cells from healthy peripheral blood mononuclear cells (PBMC), and stimulated with/without HMGB-1/IgM. Conditional medium (CM) were collected from these B cells demonstrating that B cell lines KYSE30 and KYSE180 have better capacity for proliferation when culture with activated B cells CM as compared to unstimulated B cells. Collectively, we believe that the suboptimal clinical outcomes relies on CD20+HMGB1 receptor + B cells that are exposed to high HMGB1 concentrations in the ESCC microenvironment may eventually become pro-tumor B cells subset.

#4000 Establishing the natural history of the immunosuppressive myeloid microenvironment in an inducible model of lung adenocarcinoma. Mouna Lagrouai,1 Clifton L. Dalgard,1 Gauthaman Sukumar,1 Celeste Huaman,1 Thomas Summers,1 Corey A. Carter,2 Brian C. Schaefer3.1 Uniformed Services University, Bethesda, MD; 2John Murtha Cancer Center, Bethesda, MD.

Tumorogenesis is accompanied by broad changes to the surrounding tissue microenvironment in a tumor- and patient-specific manner. Phenotype shifts in tissue-resident immune cells promote tumor progression by establishing a proliferative inflammatory environment, activating angiogenesis, stimulating tumor cell invasion and suppressing anti-tumor immunity. While the former mechanisms are well appreciated and often targeted by current cancer therapeutics, the nature of the mechanisms controlling establishment of the local immunosuppressive state that contribute to a failure of anti-tumor immunity are less clearly defined. Blockade of tumor antigen-specific T cell killing is clearly a major contributor to the failure to control tumorogenesis. However, evidence suggests that phenotypic shifts in innate immune cell populations also contribute to the failure of anti-tumor immunity. We hypothesize that the emergence of early lung adenocarcinomas is accompanied by a shift in myeloid phenotypes, rapidly establishing a microenvironment favorable to tumor growth, survival and vascularization, and hostile to cell-mediated anti-tumor immune responses. Through the utilization of a Cre-inducible mouse model of p53-null, KrasG12D+ lung adenocarcinoma, we have begun to define the natural history of the myeloid component of the tumor microenvironment, from tumor initiation to a point equivalent to human stage I adenocarcinoma. Histological, flow cytometry and real-time PCR approaches collectively demonstrate that tumor-associated myeloid phenotypes emerge at a very early stage of disease. These data are consistent with our hypothesis that the myeloid component of the tumor microenvironment plays a crucial role in establishing an immunosuppressive state during early tumorogenesis. Implications of these findings for current and emerging immunotherapies will be discussed.

#4001 Infiltration of neutrophils in the tumor is associated with poor prognosis of patients with gastric cancer. Hiroaki Tanaka, Soichiro Hira-tani, Tatsuo Tamura, Hisashi Nagahara, Kenjiro Kimura, Masatsune Shibutani, Go Ohira, Takahiro Toyokawa, Sadaaki Yamazoe, Ryosuke Amano, Kazuya Muguruma, Kiyoshi Maeda, Kosei Hirakawa, Masachi Ohira. Osaka City Univ. Grad. School of Medicine, Osaka, Japan.

Background: Recent evidence shows that neutrophils often migrate into the tumor and are associated with tumor progression. These tumor associated neutrophils (TANs) neutrophils have potential to induce angiogenesis, lymphangiogenesis and immunosuppression by inhibiting cytotoxic T lymphocytes. Immune status is one of the important prognostic factor of gastric cancer. We previously reported that lymphangiogenesis was augmented in the primary tumor of gastric cancer. Aim: The aim of this study was to investigate the correlation between CD15+ TANs and clinicopathological features of patients with gastric cancer. Materials and Methods: We investigated the association of the density of TANs in primary tumor with clinicopathological features by immunohistochemistry. We used specimens from a total of 143 primary tumor and 497 lymph nodes from 120 patients underwent gastrectomy between 2007 and 2008 in our department. We examined prognostic value of TANs identified as positive CD15 cells in both of the primary tumor and tumor draining lymph nodes. Results: The median number of TANs in primary tumor and tumor draining lymph nodes were 18.4 and 23 cells/HPF, respectively. High TANs in both of the primary tumor and lymph nodes had correlation with depth of invasion (primary tumor P<0.001, lymph nodes P=0.036) and lymph nodes
metastasis (primary tumor P<0.001, lymph nodes P=0.002). Kaplan-Meier analysis showed that patients with high TANs in both primary tumor and lymph nodes had worse prognosis than patients with a low TANs (primary tumor P=0.031, lymph nodes P=0.014). Multivariate analysis revealed that high TANs in lymph nodes were independent prognostic factors. (P=0.038) Conclusion: Our results with these patients with AKM and NALM showed that cancer tissues had poorer prognosis because of increasing lymph nodes metastasis. These findings suggested the TANs might have important role of tumor lymphangiogenesis in microenvironment of gastric carcinoma.

#4002 Neutrophil extracellular traps (NETs) in abdominal cavity have supportive roles on postoperative development of peritoneal metastasis

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Background: Peritoneal lavages just after abdominal surgery contain many immune cells which consist mainly of granulocytes. Recent studies have demonstrated that neutrophil extracellular traps (NETs) play important roles not only on capture of pathogenic microbes but also on autoimmune disease, thrombus formation and tumor metastasis. Here, in this study, examined the NET forming capacity of granulocytes in postoperative peritoneal washings and assessed the possible role of the NETs on metastasis formation on peritoneum. Method: In patients who underwent laparotomy due to digestive malignancies, abdominal cavity were washed with 1000 ml of normal saline at the timing of laparotomy (Preoperative) and just before wound closure (Postoperative). Cells recovered from the peritoneal washings were centrifuged on Ficoll-Hypaque solution and cultured at 37°C in 5 percentage CO2 condition for 24 hour. Then, extracellular ds-DNA were stained with membrane impermeable dye, SYTOX. Also, the granulocytes were immunostained with mAbs against CD66b, histone, myeloperoxidase (MPO), neutrophil elastase (NE) and Matrix metalloproteinase (MMP)-9, and NETs formation was observed under fluorescence copy. In adhesion experiments, human gastric cancer cells were stained by PKH-26 and mixed with the granulocyte culture for 5 min and gently washed. Results: 1. After Ficoll separation, CD66b (+) granulocytes were contained both in polymorphonuclear PMN and mononuclear cell (PMBC) layers, which are considered to be normo-density granulocytes (NDG) and low density granulocytes (LDG), respectively. The percentage of LDG in PMBC layer was markedly elevated in postoperative as compared preoperative lavages (Pre: M=0.71%, 0.8-8.9% vs Post: M=61.3%, 3-86.7% n=39, p<0.001) The LDG in postoperative sample showed multianchored morphology. 2. The short term culture of the postoperative peritoneal LDG, but not preoperative or peripheral blood granulocytes, many SYTOX (+) fibrous structures were observed, suggesting the vigorous NETs formation. The NETs appeared several min after the incubation, peaked at 2 hour and gradually decreased thereafter. Immunostaining showed that histone and MPO were co-localized on the NETs, while NE or MMP-9 were less clear on NETs. 3. After 5 min co-incubation, human gastric cancer cell, OCUM-1 and MKN45, were selectively attached on the NETs. 4. Similar SYTOX (+) NETs-like structure were clearly detected on the surface of human omental tissue resected after abdominal surgery. Conclusion: Our preliminary results evidence NETs formation in human abdominal cavity which produce large amounts of NETs on peritoneal surface. The NETs efficiently trap the floating tumor cells, which may have positive roles on peritoneal recurrence in advanced cancer.

#4003 Acral lentiginous melanoma and infiltrating lymphocytes in a Latin American population. Carlos Castaneda,1 Carlos Torres-Cabala,2 Sandro Ojuelo,3 Miluska Castillo,1 Valeria Villegas,1 Joseyn Sanchez,2 Claudia Flores,3 Henry Gomez,2 Tatiana Vidaurre,1 Instituto Nacional de Enfermedades Neoplasicas, Lima, Peru; 2University of Texas MD Anderson Cancer Center, Houston, TX; 3Oncosalud, Lima, Peru.

Background: Acral lentiginous melanoma (ALM) is unusual in Caucasian but is the most common subtype in Hispanic populations, and has been associated to poor prognosis. Herin we compare clinicopathological features associated to ALM in a Latin-American population and prospectively evaluate its association with tumor infiltrating lymphocytes (TIL). Patients and Methods: All patients with melanoma presenting from 2005 to 2012 at Instituto Nacional de Enfermedades Neoplasicas in Lima, Peru, were retrospectively identified. Analysis of association between clinicopathological features and ALM was performed. Survival analysis compared the outcome of ALM to whole group and extremity NALM. We performed prospective evaluation of TIL following Azimi et al recently presented classification. Results: A total of 537 ALM and 287 NALM cases were identified. Older age (p=0.02), thicker Breslow (p=0.008), positive lymph node (p=0.051) and ulceration (p<0.001) were found to be more frequent in ALM. Acral had worse OS compared with both whole group (p=0.04) and extremity NALM (p<0.001). Stage I-II patients had a median OS of 5.3 (95%CI: 4.3-6.2) for ALM and 9.2 (95% CI: 5.0-7.0) for extremity NALM (p=0.016). TIL was prospectively evaluated in 490 cases, and Grade 0-1, II and III of TIL represented 44.7%, 33.6% and 27.1%, respectively. Higher TIL grade was associated to less Breslow thickness (p=0.021), less mitosis index and NALM. Higher TIL grade was also associated to better OS (p<0.001). Conclusion: ALM is highly prevalent in Latin-America and carry poor outcome. Lower TIL levels was associated to poor outcome and ALM.

#4004 Rapamycin selectively targets obesity-polarized macrophages in the prostate tumor microenvironment.

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Background: Obesity is associated with prostate cancer progression and mortality. Previous studies in our laboratory suggest that obesity drives prostate cancer progression in part by increasing macrophage recruitment and by polarizing macrophages in the tumor microenvironment into a tumor promoting M2/TAM phenotype. Rapamycin, a mammalian target of rapamycin (mTOR) inhibitor, has been used for several decades in transplant patients, and in the last several years has been shown to be an effective disease suppressor in certain cancer types. Intriguingly, mTOR has been shown to be especially important for M2 polarization and mobilization. Hypothesis: Based upon published data and our preliminary studies, we hypothesize that rapamycin will selectively target obesity-polarized macrophages and will provide a survival benefit to the obese prostate cancer patient. Methods: To address this hypothesis, we used our in vitro model of obesity-induced macrophage polarization that includes two different prostate cancer cell lines, macrophages, and sera from obese and non-obese men. qPCR was used to measure expression levels of markers associated with an M2/TAM phenotype. MTT assays were conducted to measure cell viability, and flow cytometry and Western blot analyses were used to determine cell cycle status and apoptosis. Results: Obese conditions increased expression of M2/TAM markers in macrophages and rapamycin selectively decreased viability of obesity-activated M2/TAMs compared to M1 macrophages. Conclusions: Our in vitro study suggests that obesity promotes a tumor-associated phenotype in macrophages and that the mTOR pathway is involved in the survival of M2/TAM macrophages. This study offers a novel mechanistic approach to treat obese patients with prostate cancer.

#4005 HDAC inhibitors regulate M2 pro-tumor-associated macrophage function through histone acetylation.

Amber E. de Groot, Jelani C. Zard, Kenneth J. Pienta. The James Buchanan Brady Urological Institute, Johns Hopkins School of Medicine, Baltimore, MD.

Macrophage tumor infiltration in metastatic prostate cancer is a predictor of patient prognosis. Macrophages influence tumors in contrasting ways depending on their polarization: M1 macrophages have anti-tumor functions while M2 macrophages are pro-tumor. The finding of stimuli influencing macrophage differentiation and M2 polarization have been largely elucidated but the resulting downstream changes remain unknown. There are a number of epigenetic differences between M1 and M2 macrophages that act as important functional determinants. Recent work studying these epigenetic changes has implicated histone deactetylases (HDACs), as critical regulators in macrophage differentiation and in maintaining M1 or M2 function. Pan-HDAC inhibitors (HDIs) such as the clinically utilized suberanilohydroxamic acid (SAHA) target a wide range of HDACs and provide a means for manipulating macrophage histone acetylation. Though HDIs have been found to attenuate inflammatory functions in M1 macrophages, the field has yet to explore effects of histone acetylation in M2 macrophages. Using M2 macrophages derived from human CD14+ monocytes, we found that SAHA regulates M2 macrophage polarization and function through alteration of histone acetylation. Work is ongoing to test expression levels of canonical M2 markers such as the mannose receptor CD206 and scavenger receptor CD163 in M2 macrophages exposed to SAHA either during or after M2 polarization. Additionally, these results contribute to scientific knowledge of epigenetic regulation in macrophage function and elucidate strategies to decrease the pro-tumor function of tumor-infiltrating M2 macrophages. This work lays the foundation for using epigenetic regulators to modulate the tumor microenvironment and advance cancer medicine.

Introduction Immune profiling is a necessary step in understanding tumor microenvironment and predicting the response to immunotherapies. Methods We used the expression of genes exclusively expressed by immune cells in tumors, to classify 703 formalin-fixed primary melanomas from the Leeds Melanoma Cohort. Transcriptomes were generated from tumor cores using Illumina DASL HT 12.4 array. In the obtained tumor subgroups with differing immune profiles, we tested the hypothesis that β-catenin signaling controls immune suppression in primary tumors as earlier reported in vitro and murine data. Results We found and validated 6 tumor classes, which showed consistency with other published gene signatures, and predicted melanoma-specific survival (HR = 1.8, P = 0.003, adjusted for AJCC stage, site, age, sex, ulceration, mitotic rate, BRAF/NRAS mutation). Tumors of good prognosis expressed markedly a large number of markers of T cell cytotoxicity, dendritic cells, macrophages, NK CD56 dim cells and genes coding for checkpoint co-inhibitor molecules (Table 1). They also had upregulation of β-catenin suppressors and downregulated β-catenin itself (Table 1). By contrast, poor prognosis tumors (the largest group) lacked both innate and adaptive immunity, and had activation of canonical β-catenin signaling (CTNNB1, its targets and WNT receptors) and WNT-independent β-catenin signaling (Table 1). Conclusion In a large subset of this population-based cohort of primary tumors, we report evidence of immune evasion through β-catenin signaling pathway. These results obtained from archival material suggest that transcriptomic profiling is a viable alternative to flow cytometry in understanding tumor biology and in determining the effectiveness of immunotherapies.

### Table 1. Immune scores and β-catenin signaling in good/bad prognosis groups

<table>
<thead>
<tr>
<th>Gene expression derived characteristic</th>
<th>Good prognosis</th>
<th>Bad prognosis</th>
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<tbody>
<tr>
<td>A. Immune scores from gene expression</td>
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<tr>
<td>Cytotoxic T cell (e.g. ZM2A, ZM2H, KLRL8, KLIRD1)</td>
<td>up down</td>
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<tr>
<td>Dendritic cells (e.g. CD11b, CCL13, CCL22, IDO1, IDO2)</td>
<td>up down</td>
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<td>NK CD56 dim (e.g. IL21R, GZMB, KIR2DS5, KIR3DS1)</td>
<td>up down</td>
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<td>Macrophages (e.g. PTGDS, GM2A, CD68, SC5, ATG7)</td>
<td>up down</td>
<td></td>
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<td>B. Checkpoint molecules</td>
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<tr>
<td>PDL1</td>
<td>up down</td>
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<tr>
<td>CTLA4</td>
<td>up down</td>
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<tr>
<td>VISTA</td>
<td>up down</td>
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<tr>
<td>TIM3</td>
<td>up down</td>
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<tr>
<td>LAG3</td>
<td>up down</td>
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<tr>
<td>BTLA</td>
<td>up down</td>
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<tr>
<td>C. β-catenin inhibitors</td>
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<tr>
<td>DKK2 (secreted)</td>
<td>up down</td>
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<tr>
<td>DKK3 (secreted)</td>
<td>up down</td>
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<tr>
<td>SRFR2 (secreted)</td>
<td>up down</td>
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<tr>
<td>AXIN2 (intracellular)</td>
<td>up down</td>
<td></td>
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<tr>
<td>NKD2 (intracellular)</td>
<td>up down</td>
<td></td>
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<tr>
<td>D. β-catenin, its targets and WNT receptors</td>
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<tr>
<td>CTNNB1 (β-catenin)</td>
<td>down up</td>
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<tr>
<td>DVL1, DVL2, DVL3</td>
<td>down up</td>
<td></td>
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<tr>
<td>TCF12, TCF1</td>
<td>down up</td>
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<tr>
<td>APC2, APC-c-Myc</td>
<td>down up</td>
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<tr>
<td>FZ5, FZ9</td>
<td>down up</td>
<td></td>
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<tr>
<td>E. WNT-independent β-catenin signaling</td>
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<tr>
<td>FGFR4</td>
<td>down up</td>
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<tr>
<td>PYG01</td>
<td>down up</td>
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<tr>
<td>BCL9</td>
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<tr>
<td>FOXM1</td>
<td>down up</td>
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<tr>
<td>NUP98</td>
<td>down up</td>
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<tr>
<td>SMAD4</td>
<td>down up</td>
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**#4007** Differential macrophage programming is associated with the aggressiveness of gliomas. Nai-Wei Yao, Fang Liao, Chen Chang, Joanne Jeou-Yuan Chen. Academia Sinica - Inst. of Biomedical Sci., Taipei, Taiwan.

Tumor-associated macrophages (TAMs), a major component of immune cells in the tumor microenvironment, have been shown to be associated with enhanced malignancy of gliomas. The polarization of macrophages toward a pro-inflammatory phenotype M1 or an anti-inflammatory phenotype M2 governed by niche factors in the tumor microenvironment may dictate the malignant outcome of gliomas. In this study, we examined the effects of differential programming of TAMs on the development of C6 gliomas in different host microenvironment using SD and Wistar rats. Using multi-parametric MRI measurements, we show that C6 cells implanted into SD rats developed larger tumors as compared to those in Wistar rats (p = 0.0199), which was accompanied with a shorter survival of SD rats (p = 1.06 × 10^-7). The increased tumor growth in SD rats was associated with increased angiogenesis and higher levels of VEGF and VEGFR2 expression. We also observed a significantly increased ratio of M2/M1 in the tumors of SD rats while a decreased ratio of M2/M1 in those of Wistar rats, suggesting that the milieu of C6 gliomas in SD rats favors for the polarization of macrophages toward the pro-tumorigenic M2 phenotype. Most importantly, Induction of M1 polarization by IFNγ treatment greatly reduced tumor growth in SD rats associated with increased angiogenesis and higher levels of VEGF and VEGFR2 expression. We therefore sought to determine the role of conditioned medium from activated human macrophages on CRC cells. Methods: Human CRC cell lines HCP-1 and HCT116 were treated with conditioned media (CM) from Phorbol Myristate Acetate (PMA)-activated human macrophages cell lines THP-1 and U937. Viability studies were performed using the MTT assay, the invasion and migration were performed in Boyden chambers, co-cultured with a collagen matrix for the invasion assay. Results: The results showed that PMA-activated human macrophages 1) dramatically increased both migration and invasion abilities of HCP-1 and HCT116 CRC cell lines, 2) stimulated Epithelial-Mesenchymal Transition (EMT) and 3) had no effect on the response of CRC cells to chemotherapy. Our PCR results also showed that PMA-activation greatly increased the expression of growth factors and cytokines in human macrophages that could be involved in the changes described above and are currently under investigation. Conclusions: Our data show that PMA-activated human macrophages play an active role in promoting EMT of human CRC cell lines.

**#4008** Human macrophage CM promotes EMT in human colon cancer. Delphine R. Boulbes, Fan Fan, Rui Wang, Huiyuan Zhang, Stephanie S. Wotwich, Rajat Bhattacharya, Ling Xia, Xiang-Cang Ye, Lee M. Ellis. UT MD Anderson Cancer Ctr., Houston, TX.

Background: Although most studies support the observation that increased macrophage infiltration is associated with better patient prognosis and overall survival in patients with CRC (in contrast to other malignancies), others have shown that macrophages can have a pro-tumoral and pro-metastatic effect via epithelial-mesenchymal transition and the secretion of growth factors and cytokines. Therefore, we sought to determine the role of conditioned medium from activated human macrophages on CRC cells. Results: Human CRC cell lines HCP-1 and HCT116 were treated with conditioned media (CM) from Phorbol Myristate Acetate (PMA)-activated human macrophages cell lines THP-1 and U937. Viability studies were performed using the MTT assay, the invasion and migration were performed in Boyden chambers, co-cultured with a collagen matrix for the invasion assay. Results: The results showed that PMA-activated human macrophages 1) dramatically increased both migration and invasion abilities of HCP-1 and HCT116 CRC cell lines, 2) stimulated Epithelial-Mesenchymal Transition (EMT) and 3) had no effect on the response of CRC cells to chemotherapy. Our PCR results also showed that PMA-activation greatly increased the expression of growth factors and cytokines in human macrophages that could be involved in the changes described above and are currently under investigation. Conclusions: Our data show that PMA-activated human macrophages play an active role in promoting EMT of human CRC cell lines.


Tumor-associated macrophages play an important role in presenting tumor antigens to T cells and are being investigated for their role in sensitivity to immune checkpoint inhibitors such as PD-1 inhibitors. The M2 macrophage subtype, characterized by CD163 expression, is immunosuppressive and associated with poor prognosis. A multiplex CD68/CD163 immunohistochemical assay was developed to characterize the density of cells expressing each marker and determine the percentage of macrophages that are of the M2 subtype. The assay was used to analyze 22 normal tissue types and 50 tumor tissues within different conditions. The results were analyzed by image analysis and results were analyzed as percent positive and density per mm2. Cancer tissues expressed a greater percentage of CD163+ macrophages than normal tissues.

**#4010** Tonggyu-tang, a traditional Korean medicine, suppresses inflammation, potential implications in tumor microenvironment. Hyon Kim, Seong-Gyu Ko, Yong Cheol Shin, Ji Hye Kim, Hye-Sook Seo, Tai Young Kim, Se Hyang Hong, Kangwook Lee, Jin Mo Ku, Myeong-Sun Kim, Yu-Jeong Choi, Soo-yeon Kang, Chunhoo Cheon, Youme Ko, Huang Ching Wen, Yui Sasaki, Sohyeon Kang, Kyung He University, Seoul, Republic of Korea.

The critical roles of inflammation in the development of cancer have long been appreciated. A growing body of evidence supports the notion that infiltrates of inflammatory cells into tumor microenvironment influence the tumor progression by providing bioactive molecules including pro-inflammatory cytokines. Importantly, the increased number of mast cells within tumor microenvironment has been associated with a poor survival in cancer patients. More-
over, keratinocyte inflammation is known to be crucial for skin tumor development. The use of natural products to reduce inflammation in tumor microenvironment is gaining an interest, because of their reduced toxicity toward normal cells. In this study, we tested the effects of Tonggyu-tang (TGT) which is composed of 14 different herbal extracts on the activity of mast cells. We found that TGT significantly reduced the expression and production of inflammatory cytokines such as IL-4, IL-6, IL-8, and TNF-α in PMA and ionomycin-stimulated HMC-1 (human mast cell line-1). In an attempt to determine molecular mechanism underlying the inhibitory effects of TGT on cytokine expression, we revealed that TGT suppressed MAPK signaling pathway including ERK, p38, and JNK as well as NF-κB pathway, which are known to regulate inflammatory cytokine expression. Similar results were obtained from the LPS-stimulated HaCaT cells, immortalized human keratinocytes. Taken together, our results demonstrate that TGT suppresses inflammation by inhibiting the expression of pro-inflammatory cytokine in both mast cells and keratinocytes, thereby potentially leading to inhibition of tumor progression.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Assay Technology

#4011 Validation of novel continuous live-cell assays for immune cell activation and killing of blood cell cancers. Nicola Bevan,¹ Hinnah Campwala,¹ Clare Seybut,¹ Kalpana Patel,¹ Dan Appleford,² Tim Dale,² Derek Trezise.¹ ¹Essen BioScience Ltd, Welwyn Garden City, United Kingdom; ²Essen BioScience Inc, Ann Arbor, MI.

The blood cancers leukaemia, lymphoma and myeloma are expected to cause the deaths of > 55,000 people in the US in 2016. New immunological approaches afford great promise for improved therapies. Here, we describe novel high throughput live-cell image-based assays for immune cell activation and killing of target cells that are geared toward screening for new treatments for these malignancies. Myeloid and lymphoid cells (Jurkats, Raji, Ramos, WIL2-NS, THP-1, PBMCs) were plated on poly-L-ornithine (PLO) or fibronectin coated 96-well flat plates and monitored over time (h to days) using non-invasive live-cell analysis (Incucyte). The dynamics of proliferation were quantified via phase contrast image analysis (% confluency), and validated as a robust measure of cell number by correlating to direct cell counts (Sceptor, Millipore) and ATP assays (PerkinElmer). Anti-CD3/IL-2 (0.1-100/10 ng/mL) or anti-CD28 activation (1-100 ng/mL) of PBMCs evoked time-dependent proliferation (0-5d) that was sensitive to the initial cell density and concentration of stimulus. L-Kynurenine (4.69 - 300 μM), a metabolite of the amino acid L-tryptophan caused concentration and time-dependent inhibition of proliferation of PBMCs. To quantify immune-cell killing in co-cultures, WIL2-NS and Ramos B-cell myelomas were first transduced with nuclear-targeted RFP (NucLight Red) to enable direct cell counting. PBMCs, either pre-activated or activated in situ (IL-2/CD3), were then added and the time-course of killing quantified through live (RFP) and dead/apoptotic ( annexin-V) cell counting. Together, these protocol developments and validation data illustrate non-invasive continuous measurement of proliferation, activation, clustering and immune-cell killing of non-adherent tumour cells at industrial scale. Unlike flow cytometry, this approach follows the full time-course of the biology without perturbing the cells and allows cell-cell interactions to be visualised. These assays are amenable to testing new therapeutic antibodies, small molecules and genetic T-cell modulation such as CAR-T.

#4012 Visualization of EPR effect and active targeting by using microscopic mass spectrometry. Masahiro Yasaki,¹ Masaru Furuta,² Koretugu Ogata,³ Yuki Fujiwara,¹ Yoshikatsu Koga,¹ Yasuhiro Matsumura¹ ¹National Cancer Ctr. EPOC, Kashiwa, Japan; ²Shimadzu Corporation, Kyoto, Japan.

Microscopic mass spectrometry (MMS), in which a microscope is coupled with an atmospheric pressure matrix-assisted laser desorption/ionization (MALDI) and quadrupole ion trap time-of-flight (TOF) analyzer has been developed for the investigation of the distribution of various molecules including drugs. The matrix-coated drug sample is ionized and then separated on the basis of its mass-to-charge ratio (m/z). Images were acquired from imaging mass spectrometry (IMS) or tandem mass spectrometry (MS/MS) data. Recently, pharmacokinetic (PK) and pharmacodynamic (PD) studies have become important to evaluate the efficacy and toxicity of these molecules. Tissue homogenates are generally used for the quantification by high-performance liquid chromatography (HPLC) or liquid chromatography mass spectrometry (LC-MS). However, they lack the information regarding the drug distribution in a specific anatomical area. The information of the drug distribution would allow us to optimize the drug design enabling more efficient drug delivery. (1) We studied the tissue distribution of paclitaxel (PTX) and its micellar formulation (NK105) using a MMS. NK105 showed much stronger antitumor effects on a human pancreatic cancer BxPC3 xenograft than PTX. In the drug imaging, we demonstrated that NK105 delivered more PTX to the whole tumor tissue. In the mouse model, PTX caused the peripheral neurotoxicity but NK105 did not. Multiple high drug-signal areas surrounding and inside the caudal nerve were observed in the case of PTX, whereas the signals after NK105 administration were quite low. (2) The tissue distribution and controlled drug release of ADC (antibody-drug conjugate) consisting of a tissue factor specific antibody (TF) linked to the anticancer agent monomethyl auristatin E (MMAE) was evaluated in comparison with control-ADC (control antibody-MMAE conjugation). TF-ADC showed stronger antitumor effect on BxPC3 xenograft than control-ADC.

We then established the selective detection method of MMAE for distinguishing free MMAE and its conjugated form. The released MMAE signal followed the accumulation of TF-ADC was greatest 24 h after the administration, compared with the control-ADC at the same time (P < 0.01). In summary, we succeeded in visualizing (1) the EPR (Enhancement of permeability and retention) effect and (2) active targeting using MMS. The data obtained will facilitate more efficient drug development in the preclinical setting.

#4013 A novel multifunctional protein tag enables simple and sensitive bioluminescent quantification of tagged proteins. Christopher Eggers, Braeden Butler, Robin Hurst, Mary Hall, Brock Binkowski, Lance Encell, Marie Schwinn, Thomas Machleidt, Keith Wood, Frank Fan. Promega Corp., Madison, WI.

Disregulation of protein expression is a key mechanism of tumorigenesis. The most commonly used approach to monitor changes in expression is to perform SDS-PAGE, followed by immunoblotting, a labor-intensive process that requires high-quality antibodies to detect proteins at endogenous levels of expression. We have developed a novel protein tag utilizing NanoLuc Binary Technology (NanoBiT), a binary complementation system based on NanoLuc luciferase. The tag, designated High Bit (HiBiT), is only 11 amino acids in length, which minimizes potential interference with protein function. The amount of HiBiT-tagged protein is measured using a lytic detection reagent containing Large Bit (LgBiT), which binds with high affinity to HiBiT (Kd ~ 1 nM) to reconstitute a bright, luminescent enzyme. HiBiT-tagged proteins can be quantified in cell lysates over 7 orders of magnitude of linear dynamic range with a limit of detection of less than 10-19 mole (3 fg of 30 kDaprotein). The simple add-mix-read assay protocol can be completed in minutes, providing an assay that is compatible with high-throughput applications. The sensitivity of the assay allows quantification of expression at endogenous levels, and the small tag size is ideal for CRISPR-mediated genome editing. HiBiT-tagged proteins separated by SDS-PAGE can be detected on blots at picogram levels with a detection reagent containing LgBiT. By eliminating the multiple steps of blocking, binding, and washing of traditional blotting techniques, the protocol takes minutes instead of hours. Additionally, the cell surface expression, internalization, or secretion of HiBiT-tagged proteins can be measured in minutes using a non-lytic detection reagent containing the cell-impermeable LgBiT protein. HiBiT represents a next-generation protein tag that enables simple and sensitive quantification of proteins of interest in their cellular context or following SDS-PAGE.


The inability of most systemically delivered therapeutics to cross the blood-brain-barrier (BBB) is considered a major barrier to effective brain cancer treatment. In malignant glioma the tumor neoangiature is known to be leaky, however invasive cells remain protected behind an intact BBB, and continue to thrive within the brain, ultimately leading to tumor regrowth and patient death. This leaves us with an urgent unmet need for the development of next-generation therapeutics with improved brain delivery. For the first time, we describe here the utility of 3D multicellular BBB spheroids made of primary human brain endothelial cells (ECs), pericytes and astrocytes as a screening tool for brain-penetrating agents. We show that the outer surface of the spheroids, composed primarily of ECs and pericytes, form a tight barrier which is permeabilized in the presence of VEGF. The barrier is characterized by the presence of intact tight junctions and efflux-pump activity (i.e., P-glycoprotein). Furthermore, we have used this model to successfully demonstrate the transport of angiopep-2 (a well-known brain delivery vector) and its conjugates (containing cargoes of various sizes such as peptide, protein and affibody), thereby displaying the versatility of this model to screen and study a wide range of therapeutic agents. We demonstrate that this model is superior to the conventional transwell model in main-
taining essential BBB characteristics (i.e., tight/adeherens junctions and P-glyco-
protein expression) and as a drug-screening tool. We have utilized the spheroid model
to screen a panel of cell-penetrating peptides (CPPs) to identify several candidates with high brain-penetration potential. We then verified the ability of the top 4 candidate CPPs to cross the BBB in mice. This high-throughput model can lead to a refined understanding of preclinical glioma therapeutics, and improve prediction of drug penetration in a living model, paving the way for breakthrough discoveries in brain cancer.

#4015 A comparative study of PD-L1 IHC 22C3 and 28-8 FDA-approved diagnostic assays in cancer. Cary Batrenchuk,1 Maher Albitar,2 Sucha Sudarsana-
am,2 Vladislav Chizhevsky,2 Chelsea Jin,1 Virginia A. Burns1. 1Bristol-Myers Squibb, Princeton, NJ; 2NeoGenomics Laboratories, Aliso Viejo, CA.

Background: Higher levels of PD-L1 expression at the surface of tumor cells have been associated with increased response to anti-PD-1 therapies (Sanatbar-
emocin Laboratories lately supplied a subset of the samples received for PD-L1 testing with both 22C3 and 28-8 on the same biopsy specimen. This dataset presents an opportunity to explore real-world concordance testing for 22C3 and 28-8 anti-
bodies. Methods: The analysis was performed on a dataset obtained from Sym-
phony Health Solutions describing the PD-L1 biomarker test results and anno-
tations reported by NeoGenomics Laboratories between Oct 7, 2015, and Aug 31, 2016. A total of 5217 biomarker test results were available for 4528 patients affected by diverse malignancies. Concordance testing was performed on 556 unique patients that had a single 22C3/28-8 pairing collected from the same biopsy specimen. All staining was performed using the FDA-approved in vitro diagnostic assay using the 22C3 antibody or the 28-8 antibody on the Dako Link 48. Cases were reviewed and scored randomly across multiple pathologists, who received special training and certification in scoring. All statistical analyses were performed in SAS. Results: Paired biopsy specimens stained with both 22C3 and 28-8 displayed higher degrees of correlation (Spearman’s correlation co-effi-
cient = 0.97). In addition, Bland-Altman analysis revealed that the mean differ-
ence in the percentage of tumor cells positively stained for PD-L1 between the paired 22C3 and 28-8 assay findings was 0.48% (95% prediction limit (-12.41% to 13.37%)]. Across expression levels both antibodies displayed a similar prob-
ability of being interpreted as greater than one another (43 of 556 where 28-8 displayed an expression higher than 22C3 was 0.48% [95% prediction limit (-12.41% to 13.37%)].). The overall rate of agreement was between the paired 22C3 and 28-8 antibody displaying greater sensitivity. When analyzed at the ≥1%, ≥5%, ≥10%, ≥25% and ≥50% thresholds, the overall rate of agreement was between 96.8% and 98.2% and associated with a level of agreement (Cohen’s kappa) between 0.92 and 0.96. Conclusion: Both the 22C3 and 28-8 diagnostic assays show strong agreement in a single central laboratory real-world setting. These assay results, in agreement with previous publications, have shown consistency in diagnostic interpretation.

#4011 Homogeneous BTK occupancy assay for pharmacodynamic assess-

Burton’s Tyrosine Kinase (BTK) plays an important role in B cell signaling, cell proliferation and survival. Hence, it is an attractive target for therapeutic intervention in B cell malignancies and autoimmune disorders. GS-4059 (ONO-4059) is a covalent, potent and selective inhibitor of BTK in clinical development for CLL, NHL and rheumatoid arthritis. Accurate measurement of target cov-
erage in early stage clinical studies is critical to informing dose selection for GS-4059 in more advanced clinical studies. We developed a novel duplex ho-
mogeneous BTK occupancy assay to enable quantitative measurement of GS-
4059 binding to human BTK for assessing target coverage in the clinic. The assay is based on Time-Resolved Fluorescence Resonance Energy Transfer (TR-
FRET) and simultaneously measures the levels of free BTK (BTK that is not bound to GS-4059 in treated human PBMCs ex vivo or PBMCs from patients treated with GS-4059 in clinical studies) as well as the levels of total BTK protein. We took advantage of the dual wavelength emission property of Terbium (Tb) to serve as energy donor for 2 fluorescent energy acceptors with distinct excitation and emission spectra. We designed and synthesized a GS-4059 analog with bi-
oxin conjugate which showed equal potency to GS-4059, suggesting that it binds in the same BTK pocket. To detect free BTK, a Tb-bound anti-BTK antibody was used as the FRET energy donor and G2-streptavidin-bound biotinylated GS-4059 as the energy acceptor. Total BTK was detected in the same well with a second D2-bound anti-BTK antibody that binds to a different BTK epitope as a secondary detection reagent. When GS-4059 was used, this multiplexed format allows measurement of free and total BTK levels in the same well. The assay was characterized and qualified using full length purified recom-
binant human BTK protein and PBMCs derived from healthy volunteers and CLL patients. Preliminary data indicate the assay is also suitable for assessing BTK occupancy in bone marrow derived cells. Using this assay, we evaluated the BTK synthesis rate following a 1 hour high dose (5 μM) treatment of GS-4059 in BTK inhibitor resistant ABC-DLBCL and GCB-DLBCL tumor cells. While neg-
ligible new BTK synthesis was observed in BTK inhibitor sensitive DLBCL cells, the free BTK level in the inhibitor resistant tumor cells recovered rapidly post-
treatment. In summary, we have established a homogeneous TR-FRET based BTK occupancy assay that simultaneously measures free and total BTK levels in the same well, yielding quantitative BTK occupancy data to support GS-4059 clinical development.


With the development of molecularly-targeted therapeutics, it is critical to have a reliable method for predicting response to therapy of patients with target-
expressing diseases. Folate receptor (FR) is overexpressed in a number of disease states while it is low in most healthy individuals with the exception of the kidney. FR-targeted small molecule drug conjugates (SMDCs) are currently under de-
velopment and have shown promising pre-clinical and clinical results. Two premises that a patient would respond to the folate-drug conjugates are: 1) FR expression in diseased tissues is functionally competent for folate binding and 2). functional FR is accessible to intravenously-infused SMDCs. Although anti-FR antibody-based immunohistochemical (IHC) assays are being deve-

goped as companion diagnostic strategies for antibody-drug conjugate (ADC) therapies, it fails to address the functionality and accessibility of FR in an in vivo context. To circumvent these limitations, we have developed an FR-targeted, crosslinkable small molecule reporter conjugate (SMRC), FRRC, which contains three modules: 1. a high-affinity folate ligand that binds with FR on diseased cells; 2. a small hapten, FITC, which can be detected by anti-FITC antibodies; and 3. an amino acid spacer in between these two modules that can crosslink FRRC to FR in situ during formalin fixation. After intravenous (i.v.) injection, FRRC would dock to the accessible and functional receptors in vivo. By process-
ing biopsied tissues and performing anti-FITC IHC staining, we can evaluate the cellular localization and relative abundance of functional FR in heterogeneous tissues. To evaluate our design, we synthesized a compound we refer to as FRRC and examined its properties both in vitro and in vivo. By testing FRRC in cell lines, we found that it can bind to FR and is detected in formalin-fixed cells by using conventional immunohistochemistry (IHC) staining. After intravenous injection of FRRC in vivo, the tail vein into mice bearing FR-positive KB tumor xenografts and its specific accumulation and kinetics in tumor and other tissues were evaluated by per-
forming anti-FITC IHC staining on formalin-fixed paraffin embedded tissues. Our results were shown to be in agreement with previous folate-based functional FR imaging and bio-distribution studies. In contrast, EC17, which lacks the crosslinkable spacer module, showed significantly reduced binding in vitro and in vivo assays. Owing to its modular design, additional SMRCs with differ-
ent ligands and small haptenes (rhodamine, DNP) have also been designed to assess the functional binding of other receptor and membrane-expressed pro-
teins. In conclusion, our assay is an effective tool for evaluating functional and accessible receptor expression in vitro and in vivo, and has the potential to be useful in patient or disease selection for our SMDC therapeutic.

#4018 Identifying allosteric modulators of KRas using second harmonic generation. Elizabeth Donohue Vo,1 Gabriel Besserer Mercado,2 Patrick Alex-
ander,3 Ben Morer,4 Que Van,3 Andrew G. Stephen,3 Joshua Salafsky,2 Frank McCormick1. 1UCSF, San Francisco, CA; 2Biodex, Inc., South San Francisco, CA; 3Frederick National Laboratory for Cancer Research, Frederick, MD.

The Ras protein family consists of small GTPases that are critical signaling transducers regulating cellular processes including proliferation, differentia-
tion, and survival. As monomeric GTPases, Ras proteins cycle between an inac-
tive GDP-bound state and an active GTP-bound state. Oncogenic mutations in one of the Ras isoforms, H-, N-, and K-Ras, impair GTP hydrolysis, thus de-
**EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Assay Technology**

Background: Identification and characterization of novel molecular inhibitors that target key regulators of cancer stem cell (CSCs) self-renewal is promising in cancer therapeutics. MYC and p53, the two major cellular transcription factors, acts as key molecular regulators in stem cells and cancer stem cells self-renewal. While MYC enhances, p53 inhibits the self-renewal of stem cells. However, tumor cells bearing both mutations would potentially benefit from targeting MYC and p53. They are critical for self-renewal of stem cells, and therefore, targeting them might be a promising therapeutic strategy. However, the search for small molecules that could target the self-renewal aspect of these two proteins has been elusive. Previously, we demonstrated that squalene molecule squalene could enhance bone marrow hematopoietic and mesenchymal stem cells (MSCs) by modulating MYC. Importantly, we found that ursoic acid, an isoprenoid molecule, regulated the transcriptional network portrayed by MYC and p53. Hence, having these prior experiences on isoprenoids, we speculate that novel isoprenoid may exist that could modulate MYC's role in cancer self-renewal. We are especially interested in identifying novel isoprenoid molecules (metabolites of mevalonate pathway) found in medicinal plants of North East India, where KaviKrishna laboratory is located. Methods: For the study, we proposed to develop an in vitro self-renewal based assay platform (henceforth known as STEM-TECH platform) to identify novel molecules that act on self-renewal aspect of MYC and p53. In this assay, we subjected the oral cancer cell line SCC-25 to 3-D tumorogenic growth using methylcellulose based method. Using this assay, we have screened 20 herbal extracts of North East India, and selected 3 herbal extracts for further evaluation. For the in vivo study, we added these herbal extracts to drinking water of C57BL/6 mice treated with 7-NQO (an oral carcinogen) and FVB/N mice with induced thymic lymphoma. The mice were observed in 10 weeks and subjected to evaluation of tumor growth, and also evaluation of cancer stem cells using ABCG2, a cell surface marker expressed by cancer stem cells. Results: We found that herbal extracts from Tulsi and Soalu exhibited strong anti-tumor activity in the in vivo mouse models. Importantly, these extracts exhibited the ability to inhibit the self-renewal of MYC driven ABCG2+ cancer cells (1). Also, these extracts were subjected to in vitro Stem-Tech assay and we found that one herbal extract could modulate the transcriptional binding of MYC. Importantly, we found that squalene, an isoprenoid found in several herbal extracts in NE India could modulate MYC activity by an unknown mechanism. Conclusion: Our results indicate that the vast, untapped herbal plants available in India's remote North East contain valuable herbal medicinal plant having potential MYC inhibitor. 1. Das B et al. MYC through HIF-2alpha regulates the self-renewal program in cancer stem cells (under review).

**#4019** A PCR-bias free capture-based library preparation platform permitting highly accurate and sensitive CNA detection in tumor molecular profiling and liquid biopsy. Anna Vilborg,1 Yosr Bouhlal,1 Ryan Bou Khalil,1 Francisco De La Vega,1 Hanlee Jj,1 1TOMA Biosciences, Foster City, CA; 2TOMA Biosciences, Foster City, CA; 3TOMA Biosciences, Stanford University, Foster City, Palo Alto, CA; 4Stanford University, Palo Alto, CA.

Next Generation Sequencing is increasingly implemented as a diagnostic test to identify actionable mutations in cancer patient samples. However, for routine diagnostics, tumor DNA is extracted from formalin-fixed, paraffin-embedded (FFPE) samples, which yields low quantity of damaged DNA. Inability to accurately repair the ends of these DNA fragments impairs adapter ligation by standard double stranded ligation methods. The resulting low yield of adapter-ligated DNA introduces the need for whole-genome PCR amplification prior to target capture. The drawback of such PCR amplification is the introduction of PCR biases, caused reduced sensitivity in the detection of copy number alterations (CNAs), an important biomarker for targeted therapy. To address the need for a library preparation platform that performs well with low quality and quantity DNA, and without relying on massive PCR amplification, we developed an improved, in-solution, version of the OS-Seq targeted enrichment assay. OS-Seq circumvents the reliance on PCR amplification by using a single-stranded adapter ligation approach. Damaged bases induced by formalin fixation are removed by excision instead of attempting repair, and then DNA is denatured prior to adapter ligation. This method of adapter ligation result in yields of ~50% for low quality samples, eliminating the need for whole genome PCR. OS-Seq directly uses the adapter-ligated DNA in a linear targeted primer-extension, followed by low-cycle post-capture PCR expansion with Illumina bridge PCR primers prior to library sequencing. We investigated the PCR duplication rate of the OS-Seq libraries by including an 11-mer random barcode to track unique molecules. We found that most input molecules were present in the sequencing reads at only one copy. Further, we demonstrate a linear correlation between the amount of DNA input (ranging from 1 to 600 ng) and the number of unique molecules sequenced (R²=0.94). Importantly, we show that this low PCR bias allows OS-Seq to detect CNAs in Coriell and Horizon Diagnostic cell lines highly concordant to digital PCR detection (R²=0.96). Further, we present CNA calling on cell line DNA sonicated to 200 bp fragments at 10 ng DNA input, mimicking cell-free DNA. In addition to CNA detection, OS-Seq detects SNVs with a sensitivity of 92-97% and a specificity of 100% down to 5% VAF. In conclusion, the OS-Seq library preparation method relies on single stranded adapter ligation and in-solution target capture, which generates uniform coverage with minimal PCR requirement, resulting in highly sensitive CNA calling.

**#4020** Identification and characterization of Novel MYC and p53 target molecules from medicinal plants of North East India. Sora Sandhya,1 Joyeeta Talukdar,1 Bidisha Pal,2 Seema Bhuyan,2 Debabrata Baishya,2 Bikul Das2,1 KaviKrishna Laboratory, Gauhati Biotech Park, IIT, Gauhati, India; 2Forsyth Institute, Cambridge, MA; 3Gauhati University, Gauhati, India.

Dendritic cell (DC) based immunotherapy is a promising treatment alternative for various solid tumor and hematological malignancies. DCs are capable of initiating and propagating antigen-specific adaptive immune responses through their intrinsic maturation process. Identifying novel, effective adjuvant drug candidates that can stimulate DC maturation while understanding and accounting for the variability of patient-specific DC responses to the immunostimulatory adjuvants is essential for development of such immunotherapies. Reliable, high throughput assay platforms that characterize patient-specific modulatory effects during the DC maturation process in vivo and in vitro are also highly desirable. In this study, we present a high throughput in vitro primary cell assay to evaluate dose-dependent DC maturation in a panel of human leukocyte antigen (HLA) typed individual donors. CD14+ monocytes from four healthy donors were isolated from leukapheresis derived peripheral blood mononuclear cells (PBMC) and cultured for seven days with GM-CSF and IL-4 to achieve differentiation to DC. Cells were then stimulated for 24 hours with lipopolysaccharide (LPS), CpG oligonucleotides, or nine different novel toll-like receptor 4 (TLR4) ligands at three concentrations. To quantify the differentiation of monocytes into DC and subsequent maturation, flow cytometry was used to measure the expression of biomarkers, MHC-II, CD40, CD80, CD83, and CD86. Greater than 99% MHC-II expression was observed across all donors and confirmed successful differentiation to DC. The TLR4 ligands showed reduced biomarker expression, as compared to pathogenic E. coli LPS but similar to PHAD, a synthetic TLR4 agonist currently used in adjuvants. While monocytes from all donors responded to the ligands tested, the intensity of activation marker expression varied among donors with LPS/TLR4 ligand induced maturation process showing 43-91% CD40 and 27-82% CD83 expression, highlighting the necessity of multi-donor screening for drug development. In conclusion, a high through-
put in vitro primary monocyte derived DC differentiation assay has been develope-
ded and is commercially available to assist researchers in characterizing donor-specific
DC responses to immunostimulatory adjuvants.

#4022 Validation of label-free impedance analysis as a versatile tool for
cancer molecular therapeutics screening. Fabio Cerignoli, Biao Xi, Brandon
Lamarche, Yiming Acosta, Yimen Abasaei, Yiyan Abasaei. ACEABiosciences Inc., San Diego, CA.

In vitro monitoring cancer cells response to treatment often involves labori-
ous sample processing and collects single data points that are against the dy-
namic nature of cancer cells. Here we present the adaptation of an impedance-
based methodology to dynamically monitor cancer cell behavior and therapeutic
response. The technology detects cell death, proliferation or migra-
tion by measuring changes in conductance of microelectrodes embedded in 96
and 384-wells cell culture plates. It avoids sample labeling and processing and
allows continuous monitoring of cell response. Our data shows validation with
cancer cell lines and primary cells monitored for cell invasion/migration, anti-
cancer drug response and receptor signaling activation, with similar results over
end point assays. Easy experiment set up and minimal sample processing make
the technology ideal for applications in large screening campaigns, while label-
free technology allows further analysis on the same samples through orthogonal
assays.

#4023 The SABRE platform: A novel, unbiased technology for drug dis-
cover and prioritization. Shelli M. Morris,1 Andrew J. Mhyre,2 Savanna S. Carmack,2 Connor Burns,2 Marc Ferrer,2 Wenjuan Ye,2 James M. Olson,1 Rich-
ard A. Klinghoffer2.1 Fred Hutchinson Cancer Research Center, Seattle, WA; 2Presage Biosciences, Seattle, WA; NIH/NCAATS, Rockville, MD.

Targeted therapies designed to inhibit hyperactive oncogenic signaling have
demonstrated some encouraging clinical responses. However, most of these
responses are not durable as tumors “work around” pathway inhibition, often
reactivating the inhibited pathway, leading to drug resistance and disease relapse. New technologies that enable more efficient discovery and development of
“suites” of drugs that comprehensively inhibit multiple pathway nodes are
needed. The SABRE (Splice Acceptor Brilliant Reporter) platform was devel-
oped to provide an unbiased method to screen large compound libraries and
dramatically improve the speed and efficiency of which novel targeted therapeu-
tics can be identified. SABRE is built off the premise that oncogenic output is
exerted through changes in gene transcription and that such changes can be
harnessed as powerful reporters of pathway activation status. SABRE utilizes
the power of gene trap technology coupled with a drug selection process to isolate
cells that generate a robust “off to on” signal in response to target or pathway
inhibition. Via massively parallel comparative analysis, multiple traps let nature
provide the best reporter for further analysis and drug discovery. In this proof-
of-concept study, we employed the SABRE technology to identify insertion sites
that are specifically regulated by the MAPK pathway. Experiments were per-
formed using the human BRAFV600E mutant melanoma cell line A375. SABRE
lentiviral transduced A375 cells were treated with trametinib, a MEK inhibitor,
and clones were isolated that emitted a positive luciferase signal upon drug
treatment. To determine if these reporters were MAPK pathway specific, the
platform was miniaturized to a 1536 well plate equipped to screen a 6000+ com-
 pound library. Results from the screen found that 70% (2840) of the top
drug hits were known to directly modulate the MAPK pathway. Since drug
resistance is a common occurrence in melanoma, we generated a “resistant
A375 SABRE reporter line to increasing concentrations of vemurafenib
and interrogated the top drug candidates identified in the screen. As expected, the
resistant SABRE line failed to respond to BRAFV600E specific inhibitors but
continued to respond to downstream MEK inhibitors, which is helping to de-
line the mechanism of resistance. We were also interested in the screening
hits that were not previously known to influence the MAPK pathway including
baftinib and a Tie2 inhibitor. In follow-up testing, we found that these drugs
induced robust and titratable reporter signals in both the SABRE A375 vemur-
afenib sensitive and resistant lines. In addition, treatment of either baftinib or
the Tie2 inhibitor reduced ERK phosphorylation in the vemurafenib resistant
cells, suggesting that these drugs could be useful in the treatment of drug resis-
tant melanomas. Studies are currently underway to determine the in vivo effi-
cacy of these drugs in A375 SABRE reporter xenograft models.

#4024 Physiologically relevant target engagement using the cellular ther-
mal shift assay (CETSA). Jakob Karen, Catrine Sieberg, Nancy Dekki Shalaby,

Targeted therapies are dependent on adequate occupancy and target engage-
ment to reach their efficacy measure. Yet, more than half of novel candidate
drugs entering clinical trial stage fail before proof of concept is achieved. Inad-
equate target engagement is often at the root cause of these failures. Therefore,
we have developed a versatile label-free method to quantify the target engage-
ment of drugs on their native physiological relevant targets. The method is
validated in multiple sample matrices, using different technical platforms to
allow studies of target engagement from bench to bedside: in living cells and
their metabolites and mass spectrometry. CETSA(1) is a patented biophysical method
measuring the thermal stability of drug target proteins and the shift induced by their
ligands. By quantifying the melting temperature and shift induced by the ligand
we can quantify the potency of target engagement. This potency determination
allows filtering of candidate drugs by their ability to engage the target in its
physiologically relevant form, in relevant sample formats such as intact cells or
tissue. We present several applications of the method and the utility in preclin-
ical in vitro studies and translation to in vivo systems. In addition, new powerful
detection formats are being evaluated and will be presented. The utility and
value of the CETSA(1) method enabling preclinical development of novel drugs
is substantiated by the examples included here. We show the value of the method
both for compound/drug profiling, for target profiling and for unbiased drug
screening in whole proteomes in response to drug treatments. Such datasets can
be generated from in vitro systems to clinical samples and may reduce the high
attrition rate in proof of concept studies by facilitating the discovery, develop-
ment and evaluation of novel drugs.

#4025 Accelerating drug repurposing for cancer therapy using multi-
plexed viability assays. Steven M. Corsello,1 Christopher C. Mader,2 Jordan
Bryan,1 Jennifer Roth,1 David Peck,1 John Davis,1 Samantha Bender,1 Li Wang,1
Alice Prisby-laRossa,2 Joshua Bittker,1 Francesca Vanacur,2 Aarv Trisnesh,1
Todd R. Golub1.1 Dana-Farber Cancer Institute, Boston, MA; 2Broad Institute, Cambridge, MA.

New therapies are desperately needed for patients with advanced cancer, but
making safe and efficacious drugs remains expensive and time consuming.
Meanwhile, multiple non-oncology drugs have been successfully repurposed for
cancer, including thalidomide for multiple myeloma and aspirin for prevention
of colorectal cancer. While the benefits of repurposing are clear, successes to date
have been largely serendipitous. We have created two foundational tools to
identify drug repurposing opportunities at a much greater scale: a new world-
class screening library of more than 4,000 drugs/cancer cell lines and a mul-
tiplexed method to rapidly conduct cellular viability assays across hundreds of
cell lines. PRISM, a recently developed high-throughput assay, employs DNA-
barcoded cell lines to enable rapid testing of many drugs against pools of cancer
cell lines. We have tested 4,100 compounds against 578 genomically charac-
terized cancer cell lines using PRISM. Our experiment is among the largest cell line
screens ever undertaken. Global analysis has revealed multiple strong clusters of
active drugs, including vitamin D agonists, bromodomain inhibitors, and st-
atins. In addition, many novel and established drug-biomarker relationships
were identified, including alkylation agent response and low MGMT expression,
BRAF inhibitor response and BRAF mutation, and MDM2 inhibitor response
and TP53 wild-type status. Surprisingly, more than 100 non-oncology drugs
unexpectedly killed multiple cancer cell lines, making them attractive repurpos-
ing candidates. For example, the FDA-approved phosphodiesterase inhibitor
anagrelide selectively kills PDE3A-high lung cancer and melanoma cell lines.
Confirmaotary experiments are underway to validate screening results, evaluate
putative biomarkers, and test drug activity in preclinical models. Importantly,
this approach is expected to enable rapid initiation of clinical trials, dramatically
accelerating patient access to potential new therapies.

#4026 Bioluminescent metabolite assays enable easy measurement of
changes in tumor biology. Michael Valley, Mary Sobol, Donna Leippe, Jolanta
Vidugiriene. Promega Corp, Madison, WI.

Tumor cells rely on metabolic reprogramming to meet their increased de-
mands for energy and biomass. Such reprogramming is exemplified by in-
creased rates of glucose uptake, greater utilization of glycolysis, and a reliance on
glutaminolysis. Cancer cells also have a strong dependence on lipids and cho-
lesterol and thus feature enhanced rates of biosynthesis and uptake of these
molecules as well. We have developed a suite of bioluminescent assays that can
monitor metabolites in all of these important pathways. These assays share a
core technology that couples the production of NAD(P)H to the generation of light
through a variety of metabolite specific dehydrogenases. Changes in glyco-
lysis and glutaminolysis can be followed by measuring glucose, lactate, gluta-
mate, and/or glutamine through their respective dehydrogenases (and glutam-
ase). Glucose uptake is typically monitored through the accumulation and
phosphorylation of 2-deoxyglucose to produce 2-deoxyglucose-6-phosphate,
and this can be measured by employing glucose-6-phosphate dehydrogenase.
These bioluminescent assays are homogeneous and can be multiplexed with

REFERENCES

viability, cytotoxicity, and protein quantitation assays. Metabolites can be measured extracellularly (i.e. from media) or intracellularly and the assays can be performed in 96- or 384-well plates. These assays are simple and sensitive and can provide a greater understanding of how various treatments affect the metabolic state of a tumor cell.

#4027 Developing biophysical platforms to study KRAS membrane interaction in artificial membranes, Frantz L. Jean-Francois, Andrew G. Stephen.

In our studies, we leverage the techniques that we believe will allow us to better understand and tackle KRAS membrane interaction. RAS proteins are small GTPases that oscillate between an active GTP-bound and inactive GDP-bound state to function as plasma membrane localized molecular switches in growth factor regulated signaling pathways. Mutation in HRAS, KRAS and NRAS are present in nearly 30% of human tumors. In particular, KRAS is mutated in almost 90% of pancreatic adenocarcinomas. Considering the requirement of the plasma membrane for KRAS activity, we are setting up ‘screenable’ biophysical platforms to understand the importance of lipids and find small molecules that could prevent KRAS membrane association in reconstituted systems. The two main techniques that are being developed are Surface Plasmon Resonance and Confocal Imaging in model membranes. Using these two techniques, we have been able to better understand the main lipids that drive KRAS membrane association and set up platforms that can be screened by small/large molecules. Biophysical techniques are frequently used by pharmaceutical companies as primary or secondary screening platforms. Developing such platforms for KRAS membrane interaction could enable the discovery of anti-cancer tool compounds.

#4028 Analysis and recovery of functionally defined single immune cell clones through opto-electro-positioning technology, Xiaohua Wang, Yelena Vanyan, Louis Gardner, Animesh Shukla, Thomas Miller, Su Wang, Sheldon Grove, Andrew Chow, Jessica Suschak, Sarah Wheeler, Anu Mathew, Martin K. Stengelin, Jacob N. Wohlstadter.

Cancer patients mount protective innate and adaptive immune responses against tumor cells. Such responses are able to control or even eliminate tumor burden if harnessed properly. Identifying clonal adaptive immune cells targeting tumor cells with designated function is extremely valuable as these cells are able to either fight the tumor directly or provide tumor targeting immune receptors to engineer other effector cells like autologous CD8 T cells or NK cells. The process of isolating specific immune cells and their receptors, however, remains technically challenging and labor intensive. Opto-Electro-Positioning (OEP) technology employs a light actuated dielectrophoretic force to maneuver live cells in their culture environment. We have combined OEP with a nanofluidic cell culture system to produce an integrated microfluidic platform that enables multi-dimensional investigation of lymphocyte specificity and function. The system is able to 1) deterministically capture designated lymphocytes, 2) actively maintain and observe colony growth, and 3) conduct cytokine release assays, all in a single enclosed microfluidic chip. The new platform is suitable for high throughput screening to identify and harvest antigen specific and/or functionally defined T cells. We have successfully applied it to isolate primary human T cells targeting either EBV/Tetanus toxin antigens or tumor associated antigens such as MART-1 through tetramer staining or a proliferation assay. The system is also exceptionally efficient for investigating immune cells from clinical specimens typically of low cellularity. From tumor biopsies, we are able to capture tumor infiltrating T cells, stimulate proliferation, investigate their TNF-α secretion on chip, and obtain full transcriptomic analysis after export. Overall, our data suggest the OEP nano-fluidic environment provides a powerful high throughput screening tool to identify rare lymphocytes against tumor associate antigens or neo-antigens and to investigate precious clinical samples for biomarker discovery.

#4029 Robust Ras inhibition assay utilizing fully synthetic Ras-binding domain, Lyuba Khavrutskii, Sergey G. Tarasov, Colin Fields, Karen Stefanisko, Nadya Tarasova.

Intensifying efforts to develop clinically useful inhibitors of Ras proteins resulted in increasing need for quantitative, reliable and robust in vitro assays of Ras activity. The vast majority of detection methods used in the assays require fusion, chemical modification or immobilization of the protein. We have found that K-Ras modifications have significant impact on its activity and function because of the highly allosteric nature of the protein. We have attached three different fluorescent moieties to two sites distant from the active site of the protein in a site-selective manner. All six modifications resulted in significant decreases in affinity towards Ras-binding domain of one of Ras’s major effectors c-Raf as determined by Microscale Thermophoresis (MST). Fluorescent labeling of recombinant Raf1 RBD also resulted in inhibition of K-Ras binding. To produce a pair that interacted as unlabeled proteins, we have generated RBD with a single-site attached fluorescent moiety utilizing microwave-assisted high efficiency solid phase peptide synthesis. Optimization of coupling and deprotection protocols and the type of the solid support allowed conducting the synthesis of 76 residues long protein in fully-automated manner in one run. Refolding of the product yielded RBD that was indistinguishable from the recombinant one in CD spectra, melting temperature and K-Ras binding affinity as determined by isothermal calorimetry and microscale thermophoresis. K-Ras RBD produced significant changes in both thermophoretic signal and fluorescence anisotropy upon binding to K-Ras. Both MST and fluorescence polarization assays have proven sensitive, reproducible and straightforward for determination of Ras inhibition constant for Ras inhibitors identified by virtual screening and rational design. They can be easily adapted to high throughput screening formats. Developed synthetic protocols allow for straightforward generation of proteins with various natural and non-natural modifications.


Tumors are comprised of heterogeneous cells. Only a specific or rare population of tumor cells is capable of metastasizing through blood. While mapping out the genome of cancer cells, gene expression variations indicative of more aggressive cancer cells might be missed among the noise. Therefore, single cell gene expression and mutation analysis provide a more comprehensive and most precise information about the heterogeneity of cancer progression and metastasis. Although there has been a surge in technologies used for selection and analysis of single cells, single cell analysis of rare cells such as circulating tumor cells (CTCs) is fraught with technical challenges. Among many used techniques to isolate single cells, the most common cell separation method is cell sorting by flow cytometry, which requires cell labeling. The numerous steps involved in cell labeling and centrifugation result in significant cell loss and changes in gene expression. In addition, centrifugation may also cause shear-induced gene expression changes in viable cells. For other single cell analysis platforms, there is a minimum input of cells ranging from 200 - 1000 cells. Rare cells like CTCs are found in the range of 1-10 per mL of blood. Another challenge is that the input volume required for these systems is very low usually in microliters. Here, we introduce a novel microfluidic chip with 250,000 microwells which captures cells at 100% efficiency down to one cell with a dynamic range over 5 logs. The underlying principle of this technology is that the geometric pattern of the microfluidic chip captures a single cell from an initial volume of up to 1 mL of fluid due to cell settling. The unique microwell structure promotes easy fluid exchange without perturbing the cells from their captured locations, thereby eliminating cell loss during multistep downstream processing. Preliminary tests have shown that single or cluster of spiked cancer cells in blood can be immunostained, bound or fixed and can also be retrieved very efficiently from the chip using a micro capillary. The retrieved single cells can further be used for several single cell genetic analysis. We will present data describing cancer gene expression and gene mutation analysis in isolated single cells.

#4030A S-PLEX™ Troponin I assay with fg/mL sensitivity, Anahit Aghvanyan, Louis Gardner, Animesh Shukla, Thomas Miller, Su Wang, Sheldon Grove, Andrew Chow, Jessica Suschak, Sarah Wheeler, Anu Mathew, Martin K. Stengelin, Jacob N. Wohlstadter. Meso Scale Diagnostics, LLC, Rockville, MD.

Cardiovascular toxicity is a potential complication of multiple anticancer therapies. Some drugs, such as anthracyclines, have been implicated in irreversible cardiac dysfunction. Cardiac troponin is a specific and sensitive marker of myocardial injury. Serum and plasma concentrations of troponin are often below the limits of detection in apparently healthy individuals. Sensitivity of troponin assays has improved by two orders of magnitude over the last several years, but few assays can detect basal levels of serum troponin in more than 50% of apparently healthy individuals, limiting the utility of the assay for early detection of low levels of cardiac damage. S-PLEX is a novel ultrasensitive immunoassay platform based on MSD’s MULTI-ARRAY™ electrochemiluminescence technology. An S-PLEX cardiac troponin I assay was developed and analytically characterized for research use. Serum and plasma samples from 24 apparently healthy individuals, 90 individuals with cardiovascular complications, and 25 individuals receiving anthracycline chemotherapy were measured. The assay required 25 μL of sample per measurement and was run on the MESO SECTOR S 600 and MESO QuickPlex™ SQ 120 instruments. The lower limit of detection was determined to be 10 fg/mL and the limits of quantitation ranged from 31 fg/mL to 160,000 fg/mL. The assay was anchored to the NIST reference material SRM 2921. Control samples run at 3 levels (n=8 per plate, 8 plates, 2 days, 2 operators) had total CV’s of 7% to 8% (n=64). Spike recovery and dilution linearity had recoveries between 80% and 120%. Specificity of the assay was 1029

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and R132C) were analyzed by pyrosequencing. To determine the in vitro antitumor effect of GBM (IC-4687-GBM and IC-3752-GBM) were included. IDH1 mutations (R132H) xenograft (PDOX) model of recurrent AOA (IC-V0914AOA), and 2 PDOX models (BT142) of anaplastic oligoastrocytoma (AOA), and a patient-derived orthotopic xenograft mouse models. Background: Mutation in isocitrate dehydrogenase 1 (IDH1) occurs in >70% of WHO grades II and III astrocytomas and oligodendrogliomas and secondary glioblastoma (GBM). The mutant enzyme catalyzes the reduction of α-ketoglutaric acid to D-2-hydroxyglutaric acid, leading to cancer initiation. In this study, we examined the therapeutic efficacy of SYC-435 (1-hydroxypyridin-2-one), a newly developed mutant IDH1 inhibitor, both in vitro and in vivo in IDH1 mutant gliomas as compared with IDH1 wild type GBMs. Methods: An established nudeusphere line (BT142) of anaplastic oligoastrocytoma (AOA), and a patient-derived orthotopic xenograft (PDOX) model of recurrent AOA (IC-V0914AOA), and 2 PDOX models of GBM (IC-4687GBM and IC-3752GBM) were included. IDH1 mutations (R132H and R132C) were analyzed by pyrosequencing. To determine the in vitro antitumor activity of SYC-435, tumor cells were exposed to SYC-435 (0.02 to 20 μM) and examining for changes of cell proliferation every 3-4 days till day 13 by Cell Counting Kit-8 assay. For in vivo effects, orthotopic xenograft mouse models of IC-BT142AOA and IC-V0914AOA were treated with vehicle (as control), SYC-435 (i.p., 15 mg/kg/day x 28 days), temozolomide (TMZ, oral, 30 mg/kg/day x 5 days) + fractionated radiation (XRT, 2 Gy/day x 5 days) (as standard therapy), and combination of SYC-435 with standard therapy starting 2 weeks after intracranial tumor implantation. Animal survival times were analyzed by log rank analysis. Results: IDH1 R132H mutation (homozigous) was detected in BT142AOA nudeusphere line and R132C mutation (patient allele frequency 39-42%) in IC-V0914AOA xenograft cells, while the two GBM models (IC-4687GBM and IC-3752GBM) carried wild type IDH1. Suppression of cell growth was observed in time- and dose-dependent manner by SYC-435, particularly at the IDH1 mutant models. At 0.5 μM, SYC-435 inhibited cell growth by 90% in BT142 and 60% in IC-V0914AOA cells, whereas in IDH1 wild-type GBMs only by 17% in IC-4687GBM and 19% in IC-3752GBM cells at day 13, indicating the high selectivity of SYC-435 of mutant over wild type IDH1. Systematic in vivo treatment with SYC-435 alone did not alter survival times in neither IC-BT142AOA nor IC-V0914AOA models when compared with the control group. Although standard therapy significantly prolonged animal survival times in both models (P<0.0005), combining SYC-435 with standard therapies further extended the median survival times from 106 days (in the standard therapy group) to 124 days (P<0.05) in IC-V0914AOA and exhibited similar trend in IC-BT142AOA. Conclusion: SYC-435 possesses antitumor effects that are highly selective in IDH1 mutant gliomas, and generated strong synergistic activities with standard therapies in vivo. Our data support the clinical testing of SYC-435 in patients with IDH1 mutant glioma.

**EXPERIMENTAL AND MOLECULAR THERAPEUTICS:** Assay Technology

**#4031** Mutant isocitrate dehydrogenase 1 (IDH1) inhibitor synergistically prolongs animal survival with standard therapies in patient-derived IDH1 mutant glioma xenograft mouse models. Mari Kogo,1 Lin Qi,1 Huiyuan Zhang,1 Frank K. Braun,1 Yuchen Du,1 Yulun Huang,2 Holly Lindsey,1 Sibo Zhao,1 Sarah G. G. Injac,1 Zhen Liu,1 Patricia A. Baxter,1 Jack M. Su,1 Laszlo Leverson,6 Deepak Sampath,7 Joyce F. Liu,1 Ursula A. Matulonis,1 Ronny I. Drapkin,2 Gordon B. Mills,1 Joel D. Leverstrom,3 Janis S. Brugger 1.

**#4032** Synergistic effects of Bortezomib and JQ1 for human and canine osteosarcoma treatments. Ya-Ting Yang, Vilma Yuzbasiyan-Gurkan.

**#4033** Combined MEK and BCL-2/XI inhibition as a potential drug combination for the treatment of high-grade serous ovarian cancer. Claudia Javore E.,1 Ioannis Zervantonakis,1 Laura M. Selfors,1 Sangreeta Palakurthi,2 Joyce F. Liu,1 Ursula A. Matulonis,1 Ronny I. Drapkin,2 Gordon B. Mills,1 Joel D. Leverstrom,3 Janis S. Brugger 1.

**#4034** Synergistic effects of Bortezomib and JQ1 for human and canine osteosarcoma treatments. Ya-Ting Yang, Vilma Yuzbasiyan-Gurkan.

**#4035** Mutant isocitrate dehydrogenase 1 (IDH1) inhibitor synergistically prolongs animal survival with standard therapies in patient-derived IDH1 mutant glioma xenograft mouse models. Mari Kogo,1 Lin Qi,1 Huiyuan Zhang,1 Frank K. Braun,1 Yuchen Du,1 Yulun Huang,2 Holly Lindsey,1 Sibo Zhao,1 Sarah G. G. Injac,1 Zhen Liu,1 Patricia A. Baxter,1 Jack M. Su,1 Laszlo Leverson,6 Deepak Sampath,7 Joyce F. Liu,1 Ursula A. Matulonis,1 Ronny I. Drapkin,2 Gordon B. Mills,1 Joel D. Leverstrom,3 Janis S. Brugger 1.

**#4036** Synergistic effects of Bortezomib and JQ1 for human and canine osteosarcoma treatments. Ya-Ting Yang, Vilma Yuzbasiyan-Gurkan.
preliminary results indicate that the drug combination is well tolerated and is able to significantly reduce tumor growth in patient-derived xenograft models (n=4). Our studies provide significant evidence that combined inhibition of MEK and BCL-2/Xi may be an effective drug combination for treatment of HGSOC and that the pro-apoptotic protein BIM may serve as a predictive biomarker to stratify patients that can benefit from these targeted therapies.

### #4035 A novel combi-molecule engineered to target the putative synthetic lethal interactions between the epidermal growth factor receptor (EGFR) and poly(ADP-ribose)polymerase (PARP).

Zhor Senhaji Mouhri, Martin Rupp, Bertrand J. Jean-Claude. McGill University Health Centre, Montreal, Quebec, Canada.

Over the past decade, PARP inhibition has been actively pursued as a novel approach for the selective therapy of tumors with BRCA1/2 mutations. The therapeutic benefits of PARP inhibitors have now been proven in the clinic against BRCA1/2 mutant ovarian cancers. This is hitherto limited to BRCA1/2 mutations, which only accounts for 5-10% of all cancers with hereditary mutations in the homologous recombination pathway. Therefore, new strategies are not only required to enhance the potency of PARP inhibitors but also to expand their use beyond BRCA mutation. While several combination modalities have been reported for PARP inhibitors, the concept of targeted PARP inhibitor has not yet been explored. Here using our novel combi-targeting approach, we report on the design of PARP inhibitors targeted to EGFR, a tyrosine kinase receptor overexpressed in several solid tumors. Recently, reports on the relationship between EGFR, PARP and BRCA have begun to emerge, one of which described a contextual synthetic lethality between EGFR and PARP (PloS one 7.10 (2012)). Here we report on the design and synthesis of novel PARP-EGFR combi-molecule based on structural modification of olaparib as a PARP inhibitor warhead and the quinazoline moiety for targeting EGFR. The results showed that: (a) it is capable of inducing a dose-dependent inhibition of PARP in isolated enzyme assay, (b) it induced a dose-dependent inhibition of EGFR in an isolated kinase assay, (c) it showed a dose-dependent inhibition of EGFR phosphorylation and downstream signaling in whole-cell assay, (d) it was selectively potent towards BRCA2 mutant and also EGFR-overexpressing cell lines, (d) it was extremely potent with activities superior to that of olaparib or gefitinib alone and their corresponding equimolar combination in three established triple negative breast cancer cell lines, (e) subcellular distribution analysis showed that it was abundantly localized in the perinuclear region. These results in tota suggest that this new combi-molecule could be developed as a single drug modality emulating the combination of PARP and EGFR inhibitors with the added benefit of being targeted to EGFR-expressing tumor cells.

### #4036 Induction of detrimental aneuploidy in basal breast cancer cells treated by MPS1 inhibitors in combination with paclitaxel.


Basal-like breast cancer has received considerable attention in recent years, but despite all efforts, conventional chemotherapy remains the main therapeutic option in the clinic with taxane therapy, the most frequently prescribed treatment. A significant reason for the lack of effective therapeutics may be related to the high degree of heterogeneity and aneuploidy which is characteristic for basal-like breast cancer. A new approach in this field is the concept that cancer cells may be targeted by the introduction of a detrimental level of aneuploidy. Inhibition of one of the main spindle assembly checkpoint kinases, MPS1 is a rational choice to achieve this goal. We report here the identification of a synergistic effect between the novel, oral, highly potent and selective MPS1 inhibitor CCT289346 and paclitaxel. We demonstrate robust synergism in multiple basal-like breast cancer cell lines and provide mechanistic insights into how both compounds' individual properties work together to maximise mitotic defects thereby increasing aneuploidy and cell death in a single round of mitosis. Additionally, in vivo studies in basal-like breast cancer xenograft models, including patient derived xenografts and systemic metastatic models, using clinically relevant doses of paclitaxel with well-tolerated doses of CCT289346, demonstrate significant benefit of combination of the two agents in comparison to paclitaxel alone.

### #4037 High throughput kinase inhibitor screen reveals novel inhibitor combinations acting in synergy with TUB83/BIII-tubulin suppression in non-small cell lung cancer.

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Background: Non-Small Cell Lung Cancer (NSCLC) survival rates remain dismal and this malignancy is poorly responsive to current therapy. The mechanisms contributing to this resistance phenotype and aggressive behavior of this disease are not well defined. TUB83/BIII-tubulin is aberrantly expressed in NSCLC and its expression correlates negatively with disease free survival of patients. Our group has previously shown that BIII-tubulin plays a functional role in cell cycle and therapy resistance in NSCLC via modulation of the PTEN/AKT signaling pathway. This study aimed to identify kinase inhibitors that synergize with BIII-tubulin inhibition in NSCLC using a high-throughput cell-based screen. Methods: A high-throughput cell-based screen of a kinase inhibitor library was performed against our previously described NSCLC H460 cells, expressing either BIII-tubulin shRNA or control non-targeting shRNA. The primary screen consisted of a total of 210 kinase inhibitors tested at a single, fixed concentration and cell viability determined using the Alamar blue assay. Secondary screens were performed using a five point dose response. Further validation of the kinase inhibitors was performed against two independent NSCLC, H460 and A549 expressing either BIII-tubulin shRNA or control non-targeting shRNA. Results: A number of kinase inhibitors that act on bIII-tubulin. Activity of the hits was verified to act in synergy with TUB83/BIII-tubulin knockdown to inhibit NSCLC cell growth. Interestingly, several different inhibitors which target mTOR were identified as top candidates for acting in combination with BIII-tubulin knockdown to inhibit NSCLC cell growth. Treatment of cells with clinically approved mTOR inhibitors, temsirolimus and rapamycin, resulted in a significant decrease in cell viability in the bIII-tubulin knockdown cells compared to control. Notably, mTOR acts downstream of AKT signalling and further highlights a potential connection with BIII-tubulin and the AKT signal transduction cascade. Other kinase inhibitors that act on key signaling cascades including Amuvatinib-Multi Kinase Inhibitor (Fh3, Met, Kit, c-Ret & PDGFR); Mubritinib-HER-2 inhibitor; and Neratinib HER2, EGFR, also acted in combination with BIII-tubulin knockdown to inhibit NSCLC cell growth. Our results suggest that clinically approved therapies designed to target mTOR combined with inhibition of TUB83/BIII-tubulin may offer a novel therapeutic strategy for the treatment of NSCLC patients. References: 1) McCarroll et al. Cancer Research 2010; 70: 4995-5003. 2) McCarroll et al. Cancer Research 2015; 75: 415-425.

### #4038 Exploring optimal targeted combination therapies with neratinib for HER2+ breast cancer.

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Activation by amplification or overexpression of the proto-oncogene HER2 (also known as ERBB2) is associated with the development and progression of breast cancer. Neratinib is a novel, irreversible, pan-HER tyrosine kinase inhibitor which selectively inhibits EGFR, HER2 and HER4. In this preclinical study, we explored the efficacy of neratinib in combination with other clinically relevant targeted agents for optimal treatment of HER2-positive breast cancer in in vitro and in vivo models. Western blot (WB) analysis of a panel of breast cancer cell lines showed that BT-474, SKBR3, and HCC-1954 cells displayed elevated HER2 expression levels (here denoted as HER2+), while MDA-MB-361, MDA-MB-435, and CAMA-1 breast cancer cells expressed relatively lower HER2 expression levels. The results of this screen were validated in a subsequent high throughput inhibitor screen, using efficacy at low and high doses of 10 mg/kg (p<0.0001). The activity of neratinib in combination with other targeted agents was then evaluated in vitro in the HER2+ breast tumor cell lines using a Chou-Talalay method. Neratinib showed synergistic anti-proliferative activity in combination with the PI3K alpha inhibitor BLY719 in all the HER2+ cell lines tested. By contrast, synergy with mTOR inhibitors (everolimus and TAK288) and with the MEK inhibitor trametinib was only observed in BT-474 and HCC-1954 cells. HCC-1954 cells had the best response to neratinib combinations among all HER2+ cell lines tested, with combination index values <0.5. Immunoblot analysis demonstrated that neratinib treatment displayed complete inhibition of HER2 phosphorylation in these cells. Substantial inhibition of downstream signaling path-
ways following single agent neratinib treatment was evident by reduced phosphorylation levels of AKT, S6K1, S6, 4E-BP1, ERK1/2 and MEK1/2. Consistently with the cell viability data, the combination of neratinib with OTS193320 and MAPK inhibitors showed a synergy on signaling blockage in these two downstream pathways respectively. Taken together this data shows that neratinib in combination with single agent OTS193320 in HER2+ breast tumors and its anti-proliferative activity in vitro was enhanced when combined with OTS193320 and MAPK pathway inhibitors. Further studies of neratinib-based combination treatments are underway in HER2+ breast cancer xenograft and PDX models to further corroborate these findings.

#4039 Riluzole synergizes with paclitaxel to induce apoptosis and inhibit cell growth in triple negative breast cancer. Miriam A. Bukhsh,1 Cecilia L. Speyer,2 Waris S. Jafry,2 Rachel E. Sexton,2 David Thomas,1 David Goriki4. Oak-land University William Beaumont School of Medicine, Rochester, MI; 1Wayne State University School of Medicine, Detroit, MI; 4Michigan State University, Lansing, MI; 2Barbara Ann Karmanos Cancer Institute, Detroit, MI.

Introduction: Systematic treatment for triple negative breast cancer (TNBC) includes paclitaxel, which works by inhibiting the breakdown of microtubules. We previously observed that riluzole, an FDA-approved drug for treatment of amyotrophic lateral sclerosis, can inhibit TNBC proliferation, invasion, and colony formation. We now test whether riluzole can act synergistically with paclitaxel to inhibit TNBC growth and induce apoptosis in TNBC. Methods: After treatment of various TNBC cell lines with constant ratio concentrations of riluzole and paclitaxel we conducted cell proliferation transformation assays (MTT) to measure cell inhibition potential. Synergy or additivity was determined by the Chou-Talalay using Compusyn software. PARP cleavage normalized to GAPDH was also measured by Western blot to estimate apoptosis due to the riluzole-paclitaxel combination. In vivo synergy was studied using a xenograft study with MDA-MB-231, a human TNBC cell line sensitive to riluzole. Results: MTT results show that riluzole and paclitaxel work synergistically to inhibit cell proliferation in all TNBC cell lines tested, as determined by isobolegrams and CI values <1. The strongest synergistic effect was observed in the cell lines SUM159, SUM149, and SUM229, where synergism occurred at Fa values as low as 0.5 (50% inhibition of cell proliferation). Measuring PARP cleavage, we demonstrated that the riluzole-paclitaxel drug combination induces greater ap- optosis than does either drug alone. The significant impact of the drug combi- nation was best demonstrated in cell lines SUM159, SUM149 and SUM229, but was observed in all cell lines tested. In the xenograft study, the highest non-toxic dose of paclitaxel (7.2 mg/kg) combined with riluzole resulted in 8/9 tumor-free mice, with a tumor growth inhibition ratio (T/C) of 0%, where <10% is consid- ered highly therapeutic. This result was better than riluzole monotherapy (0/9 tumor-free and T/C = 78%) or paclitaxel monotherapy (4/8 tumor-free and T/C = 8%). Conclusions: Our results demonstrate that the combination of riluzole and paclitaxel results in at least additive and in many cases synergistic effects against TNBC. Our observations thus suggest that repurposing riluzole, which is an oral drug with an excellent safety profile, to add to paclitaxel to treat TNBC repre- sents a potential strategy to improve the efficacy of adjuvant and neoadjuvant treatments for TNBC and/or reduce chemotherapy doses and toxicity in patients. Our data support the need to proceed to clinical trials to test this approach in patients.

#4040 Short-term romidepsin treatment combined with clotrimazole or bifonazole leads to decreased mitochondrial hexokinase 2 and apoptosis in cancer cells. Robert W. Robey,1 Andrew J. McDonald,1 Hanna Kozlowski,1 Michael M. Gottesman,1 Susan E. Bates2. 1NCI-CCR, Bethesda, MD; 2Columbia University Medical Center, New York, NY.

In light of disappointing clinical trials in solid tumors, we have sought novel combinations to increase the efficacy of the histone deacteylase inhibitor (HDAC) romidepsin. Hexokinase 2 (HK2) expression is increased in cancer cells and is postulated to prevent Bax-mediated cytochrome C release from mitochondria. Since romidepsin treatment induces apoptosis via the mitochondrial pathway, combining romidepsin with agents that detach mitochondrial HK2 might result in synergistic apoptotic effects. HCT-116 colon carcinoma cells were treated with 25 ng/ml romidepsin alone for 6h, or with romidepsin in the presence of 25 μM clotrimazole or bifonazole, compounds known to detach mitochondrial HK2. The medium was removed and cells were treated for an additional 42 h with or without clotrimazole or bifonazole. Cells were also treated with clotrim- azole or bifonazole alone for 48 h. Apoptosis was then quantitated by annexin staining. While short-term romidepsin treatment alone induced modest annexin staining and 25 μM clotrimazole or bifonazole had little effect, the combination resulted in significant apoptosis. Similar effects were observed for A549 lung cancer cells, MDA-MB-231 breast cancer cells and 786-0 renal carcinoma cells. We also treated HCT-116 and A549 cells lacking Bax, Bax or both with the clotrimazole/romidepsin combination. Apoptosis was slightly increased in Bax−/− cells, reduced in Bax+/− cells and nearly completely abrogated in cells lacking both proteins compared to wild-type cells suggesting apoptosis occurred through the intrinsic pathway. The clotrimazole analog TRAM-34 was similarly active to clotrimazole. Protein expression was determined in cytoplasmic and mitochondrial fractions of cells treated with romidepsin, clotrimazole or both and we found a >60% decrease in mitochondrial HK2 in cells treated with the combination versus untreated cells. Our results suggest that combining ro- midepsin with a compound that leads to decreased mitochondrial hexokinase, such as clotrimazole or bifonazole, results in increased apoptosis. We postulate that the following combination death romidepsin and clotrimazole treatment re- quires an intact intrinsic apoptotic pathway and that HK2 serves an anti-apo- ptotic function at the mitochondria.


Accumulating evidence has demonstrated the biological importance of pro- tein methyltransferases in human tumorigenesis, and several small molecular inhibitors targeting these enzymes have been developed. Moreover, inhibiting pathways involved in DNA repair has been shown to enhance the cytotoxicity of DNA-damaging agents. Herein, we report the development of a potent SUV39H2 inhibitor (OTS193320) that decreases global histone H3 lysine 9 tri- methylation (H3K9me3) levels in cancer cells and attenuates cancer cell prolifer- ation, and suppresses the tumor growth in mouse xenograft models. SUV39H2 (Suppressor of variation 3-9 homolog 2), is a protein methyltrans- ferase known to methylate histone H3 at lysine 9 (H3K9), and recently reported to methylate histone H2AX at lysine 134, which enhances the accumulation of phosphorylated H2AX (γ-H2AX) and regulates the DNA repair pathway in human cancer. SUV39H2 is highly expressed in many cancer types, including lung and breast cancers, while its expression levels are restricted to testis in normal adult tissues. Exposure of OTS193320 to two triple negative breast cancer cell lines attenuated H3K9me3 levels in a dose-dependent manner and trig- ered apoptotic cell death. Combination of OTS193320 and doxorubicin (DOX) resulted in reduction of γ-H2AX levels compared to single agent DOX, as vis- ualized on western blot and immunocytochemical analysis. Furthermore, combi- nation therapy attenuated the levels of p53-binding protein 1, which is reported to co-localize with γ-H2AX foci, compared to single agent DOX. Cell viability assays demonstrated a significant growth suppressive effect when OTS193320 was combined with DOX, compared to single agent treatment of either drug, suggesting chemosensitization to DOX. Importantly, in a mouse xenograft model, SUV39H2 inhibitor OTS193320 decreased the tumor growth in xenografts by 90.8% at day 14 with 25mg/kg intravenous administration of the inhibitor, with- out significant body weight loss or toxicity. Immunohistochemical staining of tumors treated with the inhibitor demonstrated a significantly lower number of Ki-67 positive cells and attenuated distribution patterns of H3K9me3 compared to the control tumors. Collectively, our results demonstrate that SUV39H2 in- hibition may be a promising approach to develop a novel class of anti-cancer treatment.

#4042 N-121, a novel polypeptide conjugate of SN-38, demonstrated enhanced intratumoral accumulation and sustained release of free SN-38 with potent antitumor activity. Haojun Wang,1 Wei Li,2 Xiaojian Zhou,2 Liuyi Wan,1 Guanghui Wen1. 1LabWorld Bio-Medicine Technology Corp. Ltd., Beijing, China; 2Pharmeva LLC, Arlington, MA.

Introduction: SN-38 is a critical chemotherapeutic agent active against a va- riety of solid tumors including advanced colorectal cancer. Clinical use of SN-38 is hampered by its poor solubility in pharmaceutically acceptable solvents. Although irinotecan, the only approved prodrug of SN-38, is widely used, its effi- cacy is limited by low conversion to the active SN-38 as well as hematologic and gastrointestinal dose-limiting toxicities. In an attempt to improve the water solubility, antitumor activity and safety profiles of SN-38, we synthesized a water soluble polypeptide-SN-38 conjugate (N-121) and examined its antitumor ac- tivity and pharmacokinetics (PK) in mouse models. Methods: Antitumor activ- ity and PK of SN-38, administered as N-121 or irinotecan, was evaluated follow- ing tail vein injection in male nude mice with subcutaneous orthotopic xenografts of the HCT116 human colon cancer. Mice received N-121 400 mg/kg (mg/kg) (32 mg/kg of eq. SN-38) or irinotecan 80 mg/kg once a week for 2 weeks and were followed for up to 46 days. Results: N-121 drastically improved by over 1000 fold the water solubility of SN-38. At a SN-38 equivalent dose of less than half of that of irinotecan, N-121 resulted in a much longer tumor growth delay.
(36 days) than irinotecan (14 days). The more durable tumor growth inhibition with N-121 was consistent with its enhanced intratumoral accumulation: intratumoral trough levels of SN-38 were about 250-fold higher than those observed in plasma, compared with only 15-fold with irinotecan. With N-121, intratumoral levels of SN-38 remained stable over the PK observation period (48 h), contrasting to a marked decline in SN-38 levels with irinotecan; intratumoral trough levels of SN-38 from N-121 were about 50-fold higher than those achieved with irinotecan. N-121 was well tolerated with a slight weight loss of 7.2%. Conclusion: N-121, a novel water soluble polypeptide-SN-38 conjugate, exhibited enhanced and sustained intratumoral exposure of SN-38 with high antitumor activity in a mouse xenograft tumor model. These promising results warrant further evaluation of N-121.


Temozolomide (TMZ) is an oral alkylating agent commonly used as a first-line chemotherapeutic agent for the treatment of high-grade gliomas and medulloblastomas. Alkylation of the O6-position of guanine in DNA is mainly responsible for the antitumor effect of TMZ. The primary mechanism of chemoresistance to TMZ has been shown to be the overexpression of the DNA repair enzyme O6-methylguanine methyltransferase (MGMT) that specifically removes the methyl lesions from the O6-position of guanine. While O6-benzylguanine (O6-BG), a potent inhibitor of MGMT, was shown to sensitize tumor cells to TMZ in vitro and in vivo, the O6-BG-TMZ combination failed in clinical trial due to acute hematologic toxicity. To circumvent this problem, we wish to target O6-BG to the epidermal growth factor receptor (EGFR), a receptor that is not generally expressed in the hematological cells but overexpressed in many solid tumors. We have now successfully designed and synthesized one such molecule termed MR30 and showed that: 1) MR30 is capable of blocking EGFR and depleting MGMT levels in whole cells; 2) MR30 is a unique molecule capable of inducing stronger growth inhibition than the 2-drug combination involving clinical EGFR and MGMT inhibitors in a panel of melanoma, lung, prostate and ovarian cancer cell lines; 3) MR30 in vitro potentiated the effect of TMZ on MGMT positive cell lines; 4) its kinase inhibitory profiling over 25 different kinases demonstrated selectivity for EGFR, HER2 and EGFR mutant forms; 5) an in vivo model showed more than 70-fold selectivity towards EGFR expressing cells in growth inhibitory assay; 6) MR30 showed good cell penetration with distribution in the perinuclear region. The results in vitro suggest that MR30 has the potential to be developed as a tumor selective potentiator of TMZ.

#4044 LY3039579, a novel Notch inhibitor, potentiates the anti-tumour effects of sorafenib in hepatocellular carcinoma (HCC). Annemilai Tijeras-Rahalland,1 Christina Johelia,2 Elise Ayala,2 Mathieu Arnoux,3 Philippe Bonnin,3 Karim A. Benhadjil,4 Bharvin R. Patel,5 Clarisse Eveno,3 Marc Pocard,3 Sandrine Faivre,6 Eric Raymond,9 Armand de Gramont9,1, 1AFROncology, Paris, France; 2Inserm U965-Lariboisiere University Hospital, Paris, France; 3Inserm U965-Lariboisiere University Hospital, Paris, France; 4Eli Lilly Company, News #4072#4046 Targeting jak/stat adaptive mechanism with jak1 inhibitor azd4205 reduces residual disease and prolongs benefit of osimertinib. Huawei (Ray) Chen,1 Geraldine Bebernet1, Kirsten Bell,7 Erica Anderson,2 Nanhua Deng,1 Jason Kettle,2 Paul Lynre,1 Richard Woessner1. "AstraZeneca Re&D Boston, Waltham, MA; 2AstraZeneca, Merside, United Kingdom.

EGFR kinase inhibitors have provided tremendous benefit for non-small cell lung cancer (NSCLC) patients with EGFR driver mutations. However, many patients fail to respond or have responses of limited duration. Accumulating evidence suggests that the JAK1/STAT3 axis plays a role in tumor escape, and that combined inhibition of EGFR and JAK1 may drive more complete and durable responses. Here we report AZD4205, a potent ATP-competitive inhibitor that inhibits JAK1 with a Ki of 2.8 nM and exhibits excellent selectivity vs. other JAK family kinases and across the kinome. Combination of AZD4205 (dosed at 12.5, 25 or 50 mg/kg BID, PO) with Osimertinib (doses ranging from 1 to 5 mg/kg, QD, PO) led to enhanced in vivo anti-tumor activity in several NSCLC xenograft models driven by mutant EGFR: H1975 (L858R/T790M), PC-9 (del19), and HCC827 (del19). In the H1975 model, after 18 days of treatment beginning with 188mg established tumors, treatment with osimertinib at 2.5 mg/kg led to tumor growth inhibition of 92% relative to vehicle. Addition of AZD4205 at 12.5 and 50 mg/kg led to 30% and 69% tumor regression, respectively. AZD4205 dosed at 12.5 and 50 mg/kg BID resulted in pSTAT3 (Y705) knockdown of 72% and 91% at peak plasma concentration (2H) respectively, and 42% and 86% at 8H. In the PC-9 model, after 22 days of treatment beginning
The goal of this study is to identify clinically used drug candidates for repurposing to treat drug-resistant lung cancer cells. Drug repurposing is the application of clinically approved drugs, with optimized dosing regimens and known side effect profiles, to new indications. EGFR TKIs have unprecedented clinical efficacy in advanced lung cancer. However, their usefulness is limited by drug resistance of various mechanisms, the most important of which is the secondary EGFR T790M mutation. The mutation blocks the binding of EGFR TKIs to the receptor kinase domain and thereby abolishing the therapeutic efficacy. In this study, we have used our in-house developed protein-ligand docking software “idock” (http://istar.cscue.hku.idock/) to screen FDA-approved small molecule drugs against EGFR bearing the resistance-causing mutations. The computationally selected drug candidates were evaluated in vitro in non-small cell lung cancer (NSCLC) cell lines resistant to EGFR TKIs. Mechanistic studies were performed to investigate the specificity of the compounds towards inhibiting the kinase activity of the various EGFR mutants, and induction of apoptosis. Based on the simulation score, thirty-six drug candidates were chosen from the in silico screening for in vitro testing in NSCLC cell lines with wide-type EGFR, EGFR L858R (del19 activating mutation), or EGFR L858R/T790M (resistance-causing mutation). Three drugs were found to exhibit anticancer activity in the cancer cells and two of them were more potent in NSCLC harboring EGFR L858R/T790M than those with EGFR L858R. Their specificity towards inhibiting EGFR L858R/T790M were further confirmed by cell-free biochemical kinase assay and Western blot analysis in the NSCLC cell models. The positive hits were also showed to inhibit the EGFR-Pi3K-Akt signaling pathway and cause apoptosis. Interestingly, the drug candidates also gave rise to pronounced synergistic anticancer activity specifically in NSCLC cells harboring EGFR T790M when used in combination with gefitinib. The drug candidates identified may be repurposed for treating NSCLC resistant to EGFR TKIs. The same in silico screening approach may be used to hunt for other clinically used drugs for repurposing to treat cancer cells resistant to other molecular targeted agents.

**REFERENCES**


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**Supporting information**

1. Supplementary materials available online. (Supplementary Figures S1-S10 and Tables S1-S5)
2. Data accessibility: All raw data generated during this study are available at the corresponding author’s website (http://www.fudan.edu.cn/).
may be due to increased nuclear P53, in combination with decreased NFkB and IKKα, and decreased DNA repair activity of the FANC/BRCA pathway. Thus, combination therapies of XPO1i, especially the clinical compounds SEL and KPT-8602, combination therapies of XPO1i, especially the clinical compounds SEL and KPT-8602, combination therapies of XPO1i, especially the clinical compounds SEL and KPT-8602, combination therapies of XPO1i, especially the clinical compounds SEL and KPT-8602, combination therapies of XPO1i, especially the clinical compounds SEL and KPT-8602, combination therapies of XPO1i, especially the clinical compounds SEL and KPT-8602, combination therapies of XPO1i, especially the clinical compounds SEL and KPT-8602, combination therapies of XPO1i, especially the clinical compounds SEL and KPT-8602, combination therapies of XPO1i, especially the clinical compounds SEL and KPT-8602, combination therapies of XPO1i, especially the clinical compounds SEL and KPT-8602.

#4050 Multifaceted targeting of drug-resistant EGFR+ and HER2+ breast tumors

Unmet needs in the breast cancer field include the development of effective treatments for 1) drug-resistant HER2+ breast cancers, 2) Triple-Negative (HER2-, ER-, PR-) Breast Cancers (TNBCs), and 3) Inflammatory Breast Cancers (IBCs). Treatment-refractory HER2+ tumors retain HER2 expression after acquiring drug resistance, and large fractions of TNBCs and IBCs overexpress either HER2 or EGFR. Thus, drugs that selectively kill breast tumors that over-express EGFR or HER2 could benefit patients suffering from these three classes of treatment-refractory tumors. Our team has identified a class of compounds termed Disulfide bond Disrupting Agents (DDAs) that selectively kill cancer cells that overexpress HER2 or EGFR. DDA-mediated cancer-selective cytotoxicity results from A) downregulation of the EGFR/HER2/HER3 proteins, B) inactivation of the Akt oncogene, and C) activation of the Unfolded Protein Response (UPR). Importantly DDAs only elicit these responses in cancer cells that overexpress HER2. DDA treatments exhibit significant anti-tumor effects in human HER2+ xenografts grown orthotopically in mice, with no evidence of toxicity. DDAs function through mechanisms distinct from those of HER2 specific monoclonal antibodies and tyrosine kinase inhibitors, and biochemical studies indicate that DDAs are complementary with Lapatinib with respect to blockade of HER2 driven proliferative and survival signaling. Current efforts are directed toward identifying mechanism-based drugs that synergize with DDAs to potentiate HER1-3 downregulation, Akt inhibition, and UPR, and to kill HER2+/EGFR+ patient-derived xenograft tumors that are refractory to current therapies.

#4051 Combination efficacy of HDAC inhibitor vorinostat and CDK-4/6 dual inhibitor palbociclib against therapy-resistant mantle cell lymphoma
Nagendra K. Chaturvedi, Nathan D. Hatch, Garrett L. Sutton, Matthew J. Kling, Julie M. Vose, Shantanam S. Joshi. University of Nebraska Medical Center, Omaha, NE.

Mantle cell lymphoma (MCL) is an aggressive B-cell lymphoma accounting for about 7% of all non-Hodgkin’s lymphoma. While multiple therapy regimens are available to treat MCL patients, ultimately relapse from therapy-resistant MCL, making MCL the worst prognosis of all B cell lymphomas. Emerging evidence suggest that, dysregulated histone deacetylases (HDACs) and the key molecules of cell cycle regulator cyclin-dependent kinases (CDKs) have been shown to be commonly associated with many lymphomas including MCL, and are considered as promising targets for relapsed lymphoma therapy. Several inhibitors of these target pathways/molecules are in clinical trials as monotherapies or in combination with other drugs. Therefore, in this study, we investigated the single agent and combination efficacies of the HDACs inhibitor vorinostat, and CDK-4/6 dual inhibitor palbociclib on MCL cell growth/survival and underlying molecular mechanism(s) using different MCL cell lines (Granta 519, Jeko-1 and JVM-2) including therapy-resistant MCL cell lines derived from Granta-519 (GRL, GRK and GRR). We used MTT assay to measure the survival and proliferation, agar colony formation assay to determine the effects of the drugs on the anchorage independent growth, Annexin-V analyses to examine the cells undergoing apoptosis following treatments and western blot analyses to determine the expression levels of target molecules including pRb, histone acetylation, cyclin-D1 and Bcl-2. The concentration of these inhibitors used in this study were similar to the published literature including sub-IC50 levels determined in our laboratory. Our results showed that both inhibitors as single agents or combined, significantly suppressed the cell growth, and induced apoptosis in therapy-resistant and other MCL lines in vitro. The single agent and combined anti-lymphoma efficacies of these inhibitors were further confirmed with the colony formation ability of the MCL cells grown in 0.3% agar semisolid media. In addition, combination of vorinostat and palbociclib treatment significantly inhibited the activation of phospho-c-Jun N-terminal kinase (pJNK) and phospho-p38 MAPK (p-p38), key molecules of cell cycle and increased the expression of acetylated-Histone H3 (H3-Ac) as determined by western blot analyses. Subsequently, the expression of Cyclin D1 (proliferation, MCL hallmark) and anti-apoptotic Bcl-2 proteins were also downregulated by these inhibitors. Together, our findings suggest that combination of vorinostat and palbociclib showed a significant synergistic anti-MCL activity by targeting associated pathways/molecules. This targeted approach warranted further preclinical evaluation for translation to the clinic.

#4052 Enhancing estrogen receptor beta mediated tumor suppression by lysine specific demethylase 1A inhibitor for the treatment of ovarian cancer
Gangadhara Reddy R. Sareddy,1 Jinoua Liu,2 Lauren Garcia,1 Suryavathu Vithala-nadhapalli,1 Mei Zhou,1 Takayoshi Suzuki,2 Edward Kost,1 Ratna K. Vadlamudi1. 1UT Health Science Center at San Antonio, San Antonio, TX; 2Kyoto Prefectural University of Medicine, Kyoto, Japan.

Ovarian cancer (OCa) is the deadliest of all gynecologic cancers in the United States. Although patients with OCa respond initially to standard combinations of surgical and cytotoxic therapy, nearly 90% will develop recurrence and inevitably succumb to chemotherapy-resistant disease. Estrogen mediates its effects through the estrogen receptor α (ESR1) and estrogen receptor β (ESR2). Recent studies suggest that OCa cells and OCa stem cells express ESR2, which functions as a tumor suppressor and identified synthetic compound LY503037 as a potent ESR2 specific agonist. However, ESR2 expression decreases during tumor progression and under the selection pressure of chemotherapy; this decrease occurs via epigenetic mechanisms. The lysine-specific histone demethylase 1A (KDM1A/LSD1) is overexpressed in OCa and plays a vital role in metastasis and cancer stemness. Our group recently developed a novel KDM1A-specific inhibitor (NCD-38) based on an innovative concept of direct delivery of phenylcyclopropylamine to the KDM1A active site. We reason that agents that restore the expression and function of ESR2 by increasing expression of KDM1A will provide a novel therapeutic opportunity to reactivate ESR2 for suppression of OCa. The objective of this study is to test whether KDM1A inhibitor could enhance the expression and tumor suppressive functions of ESR2 and to test the combination of KDM1A inhibitor along with ESR2 agonist using in vitro and in vivo preclinical models. Our results demonstrated that combination of ESR2 agonist and KDM1A inhibitor reduces the cell viability of OCa cells synergistically.

#4053 Disulfiram-copper combination induces cell death time- and schedule-dependently in glioblastoma (GBM) and medulloblastoma cell lines, and enhances temozolomide sensitivity of methylator+ve GBM cells in vitro and in vivo
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Background. Disulfiram (DSF), a carbamate derivative and a potent aldehyde dehydrogenate inhibitor, is used clinically to treat alcoholism. Although, relatively nontoxic, DSF exhibits significant anticancer effects, when administered with heavy metals (Cu and Zn). Because of this and the fact that DSF crosses the blood-brain barrier, DSF is being investigated as a potential agent for brain tumor therapy. In a preclinical GBM model, DSF treatment significantly reduced the in vivo tumor growth in orthotopic models. Since ESR2 agonists and KDM1A inhibitors are currently in clinical trials for other clinical indications and are well tolerated, identification of KDM1A inhibitor and ESR2 agonist combination therapy as a novel therapeutic can be readily transferred to clinical use for enhancing survival in OCa patients.

#4054 Combination Therapies and Approaches to Sensitize Cancer Cells to Drugs

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lomide resistance was examined in vitro and in vivo in two methylator-ve GBM cell lines, UW18 and UW28. Results. DSF and copper, as single agents, up to 100 uM for 4-7 days, had little to no toxicity on any of the GBM cell lines. In contrast, all the cell lines were highly sensitive to DSF, when combined with copper. The efficacy of the combination was highly dependent on the time (minimum 6 hrs) and schedule (DSF prior to copper) of cellular exposure. The UW228 medulloblastoma cell line was approx. 4-fold more sensitive to DSF and to the DSF-copper combination than GBM cells. We observed a direct correlation between the levels of cellular ROS production and MAP activation, and the level of cytotoxicity of the DSF-copper combination. The temozolomide sensitivity of the cell lines was significantly enhanced by the DSF-copper combination and in vivo xenograft studies showed that treatment of two temozolomide-resistant, methylator-ve GBM cell lines was significantly inhibited and their temozolomide sensitivity enhanced by the DSF-copper combination and was associated with significant inhibition of MGMT in the tumor cells. Conclusion: The results demonstrate significant sensitivity of GBM and, for the first time, medulloblastoma, cells to DSF-copper, which is dependent on both the duration of cellular exposure to and the scheduling of DSF and copper. The DSF-copper combination increases the sensitivity of temozolomide-resistant, methylator-ve GBM cells to temozolomide. The findings support DSF-copper as a treatment strategy for GBM and medulloblastoma.

#4054 Development of dual function small molecules as therapeutic agents for cancer treatment. Scott Grindrod, Alfredo Velena, Mira Jung. Shuttle Pharmaceuticals, Rockville, MD; 2Georgetown University, Washington, DC.

Inhibitors of histone deactylases (HDACs) are emerging as potent anti-cancer agents for treatment of patients with hematopoietic and solid tumors. However, most of these compounds exhibit limitations, including off-targets and toxicity. To improve the efficacy and target specificity of drugs, we developed the compound, SP-1-161 conferring HDAC inhibition activity and ataxia-telangiectasia mutated (ATM) activation. The purpose of this study is to develop a therapy that optimizes the protective effects of ATM activation in normal tissue with the sensitizing effects of HDAC inhibition in cancerous tissue into a single molecule. The compound SP-1-161 was rationally designed by optimizing ATM activation by the cap domain of a hydroxamic acid, screened against a panel of Class I and Class II HDAC enzymes, and identified as a pan-HDAC inhibitor with nano-molar potency (IC50 = 8 nM). Western analysis confirms that SP-1-161 increases acetylated histone H3/H4 and α-tubulin and ATM activation in MCF7 cells. Phosphorylated ATM was gradually increased within 1-4 hrs in a time-dependent manner. SP-1-161 was then tested in normal breast epithelial (184A1) and breast cancer (MCF7) cell lines to determine its cytotoxicity and effect on radiation clonogenic survival in combination with graded radiation exposure. The data showed that the cytotoxicity values (IC50) were low micro-molar ranges. Furthermore, SP-1-161 protected 184A1 cells (from D0 = 1 GY to D0 = 1.4 GY) while increasing sensitivity of MCF7 cells to IR (from D0 = 1.6 GY to D0 = 1.12 GY). Together, our results demonstrate that SP-1-161 is an unprecedented radio-chemo therapeutic agent for treatment of cancers while protecting normal cells.


Histone deacetylases (HDACs), were originally described in a limited context as histone modifiers. New evidence has demonstrated that HDACs are also involved in a diverse range of cellular processes that are not restricted to the chromatin environment, such as the regulation of the cell cycle/apoptosis and, more recently, a modulator of immune response. However, much remains unknown about the mechanism of action of HDACs and their roles in the immune biology of cancer. The non-specific nature of pan-HDAC inhibitors results in a narrow therapeutic window of use, limiting the dose and duration due to toxicity. Our group has focused on one specific HDAC, HDAC6, and shown that both the genetic abrogation and pharmacological inhibition of this HDAC modulates the expression of a variety of immune-regulatory proteins in the tumor microenvironment, including PD-L1, PD-L2, MHC class I, B7-H4 and TRAIL-R1. In particular, we have previously demonstrated that both pharmacological inhibition and/or genetic abrogation of HDAC6 plays a critical role in the immune check point blockade by down-regulating the expression of PD-L1 and other check-point modulators such as PD-L2, B7-H4, etc. Moreover, we have also observed that in vivo inhibition of HDAC6 reduces tumor growth in B16 and SM1 murine melanoma models within syngeneic immunocompetent hosts. Additionally, we have found that the combination of low doses of the HDAC6i Nexurastat A and checkpoint immune blockade, including anti-PD-1 and anti-CTLA4, results in an important improvement in anti-tumor immune responses and tumor growth when compared to treatment with individual stand-alone agents. In these studies we also evidenced an increased production of IFNγ and IL-2 in the stand-alone check-point inhibitor treatments, which leads to an upregulation of PD-L1 and PD-L2. Similar levels of IFNγ and IL-2 were found in the combination groups. However, the expression level of PD-L1 and PD-L2 were comparable to the non-treated group. Taking all together, we have found that HDAC6 could be used as a potential adjuvant in ongoing therapeutic options involving immune check-point blockade.

#4056 A modulator of multidrug resistance protein 1 selectively depletes glutathione and synergizes with L-buthionine sulfoximine to sensitize MRPI-expressing cancer cells to chemotherapy. Christine C. Gana, Kimberly Hansen, Denise M. Yu, Claudia Fleming, Murray Norris, Michelle Haber, Jamie Fletcher. Children’s Cancer Institute, Sydney, Australia.

Multidrug resistance protein 1 (MRP1) is frequently overexpressed in tumors where it effluxes chemotherapeutic agents, protecting tumor cells from chemotherapy. This is clearly illustrated in the pediatric solid tumor neuroblastoma, where MRPI expression is highly prognostic of clinical outcome (1,2) and contributes to chemo-resistance in mouse models (3). MRPI also effluxes low levels of glutathione (GSH), a crucial intracellular antioxidant that avoid damage from the reactive oxygen species induced by radiotherapy and chemotherapy and an important component of both Phase II and Phase III drug metabolism. Depletion of GSH in cancer cells would be of therapeutic value, however the GSH synthesis inhibitor L-buthionine sulfoximine (BSO) has only limited selectivity for tumor over normal cells. We have identified a novel MRPI inhibitor, 7-methyl-5-phenyl-2-[(4-phenyl-1-piperazinyl)carbonyl]pyrazolo[1,5-a]pyrimidine, that blocks MRPI-mediated drug efflux while greatly enhancing MRPI-mediated GSH efflux and are investigating the utility of this dual-function inhibitor for the treatment of MRPI-overexpressing cancers. Methods: Cells expressing high levels of MRPI were treated with MRPI drug substrates in combination with MRPI inhibitors. Viability was assessed by short-term cytotoxicity or clonogenic assays and GSH levels determined by glutathione recycling assay. Results: Our MRPI modulator depleted intracellular GSH in an MRPI-dependent manner and sensitized MRPI-expressing human neuroblastoma, lung and ovarian cancer cell to chemotherapeutics. This effect was highly synergistic with BSO, both in depleting GSH (CI<0.36, p<0.05) and abolishing colony formation (CI<0.01, p<0.05). Conclusion: Our MRPI modulator blocks MRPI mediated drug efflux and is predicted to effectively deplete MRPI-mediated GSH efflux in MRPI expressing cells, potentially providing an increased therapeutic window for chemotherapeutics in MRPI-overexpressing cancers. Synergy with BSO could widen the range of drugs and cancers that this could be beneficial for. References: 1. Haber, M., et al., (2006) J.Clin Oncol 24, 1546-1553. 2. Norris, M. D., et al., (1996) New Engl J Med 334, 231-238. 3. Burkhart, C. A., et al., (2009) Cancer Res 69, 6573-6580.

#4057 Inhibition of polypoloyd and senescence induces intrinsic apoptosis in double hit or double expresser DLBCL. Shariful Islam, Wenqing Qi, Carla Morales, Laurence Cooke, Catherine M. Spier, Daruka Mahadevan. 1.Univ. of Arizona, Tucson, AZ; 2.University of Tennessee Health Science Center, Memphis, TN; 3.St. Jude Children’s Research Hospital, Memphis, TN.

Introduction: Double Hit (DH) or Double Expresser (DE) DLBCL are aggressive Non-Hodgkin’s Lymphomas (NHLs) with translocation or over-expression of MYC and BCL-2 which are incurable with current therapies. MYC is a critical driver of oncogenic phenotypes and regulates expression of numerous genes including aurora kinases (AKs), which are validated drug targets. AKs are over-expressed in DLBCL and AK inhibition in two Phase II trials of relapsed and refractory B/T+NHL resulted in 20%-30% overall response rate. Bruton’s tyrosine kinase (BTK) plays a central role in B-cell receptor (BCR) signaling and inhibitors of BTK have shown efficacy in B-NHL clinical trials. Targeting CD20 (rituximab) in DLBCL inhibits BCL-2 and induces apoptosis via caspase-3 activation. We hypothesized that DH/DE-DLBCL are amenable to a novel therapeutic strategy of AK, BTK and CD20 inhibition. Methods: Four DH/DE-DLBCL cell lines were evaluated for their viability by MTS; polypoloyd, senescence and apoptosis by flow cytometry; DNA damage by TUNEL assay. Phospho-BCL-2, -AK and -BTK analysis by western. Safety and efficacy of drug combinations in a mouse xenograft model. Analysis of patient samples and mouse tumors by IHC. Results: DH/
#4058 Preclinical and phase-2 clinical study of valproic acid administered in combination with cisplatin and cetuximab in recurrent/meta- statistical head and neck cancer. Alfredo Budillon, Andrea I. Zotti, Carlo Vita-gliano, Elena Di Gennaro, Francesco Caponigro. Instituto Nazionale Tumori, Napoli, Italy.

Recurrent metastatic (R/M) squamous cell carcinoma of the head and neck (SCCHN) is a devastating malignancy with a poor prognosis and the combination of cisplatin (CDDP) and cetuximab (CX) is one of the gold standard for treatment. However, this therapy is often associated with high toxicity and resistance, suggesting that the concomitant use of novel compounds represents a critical strategy to improve treatment results. Histone deacetylase inhibitors (HDACi) enhance the activity of several anticancer drugs including CDDP and anti-Epidermal Growth Factor Receptor (EGFR) compounds. EGFR overexpression is involved in chemotherapy resistance and recently it has been demonstrated that EGFR nuclear localization represents one of the mechanism of resistance to both CDDP and CX. Our group have demonstrated that HDACi synergize with anti-EGFR agents by modulating ErbB receptors expression including EGFR. In this study we evaluated the capability of Valproic acid (VPA), a generic low-cost anticon- vulsant drug with HDACi activity, to enhance the efficacy of CDDP-CX association in SCCHN cells with different EGFR basal expression and genetic backgrounds. We showed, by the calculation of combination indexes, an antiproiferative synergistic interaction between equipotent doses of VPA and CDDP-CX in CAL27 and FaDu SCCHN cells using either simultaneous or sequential (with delay) schedule of administration. The antiproliferative effect was not observed in immortalized human fibroblasts BJ-hTERT, sug- gesting a selective effect toward tumor cells. Next, we confirmed synergistic interaction by testing clinically achievable concentrations of VPA/ CDDP-CX combination also on CAL27 and FaDu 3D spheroid cultures, showing inhibition of first- and second-generation spheroids formation by triple combination, compared to single agent treatments. Moreover we demon- strated that VPA was able to down-regulate EGFR and to prevent EGFR nuclear translocation induced by CDDP and CDDP-CX combination, thus preventing the activation of DNA repair and survival pathways. Indeed, we showed that VPA was able to increase DNA damage and apoptosis induced by CDDP-CX combination, evaluated by gamma-H2AX foci and Annexin V-FITC assay, respectively. In vivo preclinical study is currently ongoing. All together these data represent the rationale of the ongoing V-CHANCE phase-2 clinical trial evaluating VPA/ CDDP-CX combination in R/M SCCHN patients. We are also currently investigating pharmacodynamics/predictive biomarkers of treatment efficacy/resistance on patient’s samples.

#4059 KPC34: a co-drug that combines a DNA damaging agent with a targeted therapy for the treatment of AML. Gregory L. Kucera, Peter Alexander, Kristin Pladna, Timothy S. Pardee. Wake Forest School of Medicine, Winston-Salem, NC.

AML occurs in the elderly with ~18,000 new US cases in 2014. Mortality is an estimated 10,050 US deaths in 2014. Elderly patient prognosis is a dismal 6.6%, 5 year survival rate. AML treatment involves induction chemo- therapy with Ara-C followed by consolidation therapy or stem cell transplant. The major obstacle to long-term disease-free survival is relapse after treatment due to chemoresistance. Chemoresistance occurs by several mech- anisms including reduced activation of Ara-C via down-regulated deoxycy- tosine kinase 1, increased levels of the anti-apoptotic protein Bcl-2, and increased BCR-ABL kinase (BCR-ABL1 gene product, a downstream target of the oncogenic activity of thePhiladelphia Chromosome). Our work was designed to characterize the role of the K9H3 Histone Methyltransferase A (KMT6A) in the AML pathology. KMT6A is a histone lysine methyltransferase that is upregulated in AML and promotes cell growth and chemoresistance in AML cell lines. We have previously demonstrated that KPC34 is active against AML in vivo. KPC34 is highly cytotoxic to a variety of leukemia cell lines in vitro, with IC50 of ~90% and was well tolerated. Survival analysis: 67% of mice alive at day-89 with triple therapy versus 20% with ibritunib + rituximab. Other treatments showed no survival past day-52. Conclusions: A novel triple ther- apy consisting of alisertib + ibritunib + rituximab inhibits PASCs induced by AK inhibition in DH/DE-DLBCL leading to a significant anti-prolifera- tive signal and intrinsic apoptosis. Our work provides a rationale for exploring these combinations in the clinic.

#4060 Pharmacological targeting of the Aurora A and Histone 3 lysine 9 methyltransferase pathways in pancreatic cancer induces mitotic catastro- phe. Angela Mathison,1 Ann Salmonson,1 McKenna Missfeldt,1 Thiago de As- severo Bintz,2 Tao Shi,1 Susan Lin,1 Suncao,1 Jennifer Bintz,1 Trace Christensen,1 Sarah Kossak,1 Asha Nair,1 Juan Iovanna,2 Robert Huebner,1 Gwen Lomberk,1 Mayo Clinic, Rochester, MN; 2Cancerology Research Center of Marseille (CRCRM); INSERM U1068, Marseille, France.

By regulating gene expression networks that mediate neoplastic behavior, epigenetic protein complexes are the ultimate effectors of oncogenic pathways. This hierarchical position of epigenetic pathways within the mechanisms that regulate cancer makes them ideal candidates for therapeutic targets. The current work was designed to characterize the role of the K9H3 Histone Methyltrans- ferase (HMT) pathway in mediating oncogenic signals downstream of Aurora Kinase A (Aurka), with the goal of designing efficient combinatorial therapeutics against PDAC. Alfinity protein purification, combined with mass spectrometry, demonstrates that HP1γ isolated from mitotic cells interacts with Aurka and the HMTs, G9a and GLP. We also find that this complex is critical for mitotic progression and cell proliferation. Congruently, treatment of PDAC cells with individual drugs against Aurka or HP1-HMT inhibits cell growth by inducing senescence, a cytostatic response. However, the combination of these agents has a synergistic effect of reducing cell growth in both, monolayer and spheroid cultures to result in a cytotoxic effect. Confocal and electron microscopy, along with cell cycle analysis, demonstrate that the cytotoxic effect of this combination is due to induction of mitotic catastrophe, a distinct form of cell death that occurs during mitosis. Molecularly, the combination of Aurka and HP1-HMT inhibition bypasses G2/M arrest with downregulation of the Chk1-Cdc25c path- way. In vivo treatment of PDAC orthotopic xenografts with the HP1-HMT inhibitor alone demonstrated reduced PDAC growth, and increased efficacy in PDAC growth reduction was observed in combination with the Aurka inhibitor over either individual treatment. Thus, our data demonstrate a novel Aurka- HP1-HMT mitotic pathway that holds promise for potential pharmacological targeting in combinatorial therapy for this malignancy.
EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Mechanistic Understanding of Novel Anticancer Therapies


The pharmacokinetics of antibody drug conjugate (ADC) therapeutics typically show a discrepancy between the PK of total antibody (conjugated and unconjugated antibody) and that of conjugated antibody, carrying one or more payload molecules. This discrepancy is often attributed to deconjugation (Kamath, 2014), however recent evidence suggests that the underlying mechanisms may be more complex. This work employs a computational quantitative systems pharmacology (QSP), or mechanistic PK/PD approach to understand the impact of drug antibody ratio (DAR) and the resulting changes in molecular properties on overall PK and relative payload disposition as observed in preclinical and clinical studies. Our work establishes the benefit of using computational models to design novel ADCs and to optimize the discovery and development of existing ADCs.

#4062 A computational driven protocol of metronomic oral vinorelbine in metastatic non small cell lung cancer. Diane-Charlotte Imbs,1 Raouf El Cheikh,2 Joseph Ciccolini,1 Raphaël Serre,1 Fabrice Barlesi,2 Dominique Barbou-los1. 1Aix-Marseille University, Marseille, France; 2 Assistance Publique Hôpitaux de Marseille, Marseille, France.

Over the last decade, metronomic chemotherapy (MC) has emerged as an alternative way to administrate chemotherapy. MC can be defined as regular administration of low/less-toxic doses without extended drug-free breaks. Because countless schedules are possible, testing them empirically looks like an unachievable goal. Therefore, mathematical modeling can be an effective tool to determine appropriate metronomic strategies. A phase Ia/Ib study based on a mathematical model of oral vinorelbine in metastatic NSCLC and malignant pleural mesothelioma was designed to confirm safety and consolidate the calibration of model parameters. The model was used to simulate alternative metronomic scenarios targeting higher efficacy with similar toxicity. Simulations showed that the initial proposed schedule was the most appropriate in terms of efficacy/toxicity balance. Its efficacy will be evaluated in the second part of the study. This trial can be considered as a proof-of-concept study demonstrating the feasibility to run a computational-driven protocol to ensure an optimal efficacy/toxicity balance in cancer patients.

#4063 Design and synthesis of indole derivatives as ABCG2 mediated resistance reversal agents. Chao-Yun Cai1, Hong Zhai,2 Zhi-Ning Lei,1 Bao-Li Chen,1 Zhao-Yu Du,2 Yun-Kai Zhang,1 Yi-Jun Wang,1 Bo Wang,2 Zhe-Sheng Chen1. 1St. John’s University, Queens, NY; 2 Sun Yat-Sen University, Guangzhou, China.

Multidrug resistance is a phenomenon that cancer cells become resistant to anticancer drugs. Multidrug resistance has been shown to be related with the overexpression of ATP-binding (ABC) transporters, including ABCG2. Therefore, ABCG2 as a target for new strategies against resistance reversal agents. Fumitremorgin C, with an indole group which is an important pharmacophore in many drugs, is a known ABCG2 inhibitor. Inspired by this, we synthesized two series of indole derivatives as ABCG2 mediated resistance reversal agents. With MTT assay, we found that two compounds from the two series of indole derivatives respectively exhibited good reversal activity against ABCG2. The two compounds did not significantly alter the expression of ABCG2 in ABCG2-overexpressing cell lines and they stimulated the basal ATP hydrolysis of ABCG2.

#4064 Relating tumor drug concentrations to target effect with semi-physiologic PK-PD modeling in drug development: an application using a novel dCK inhibitor. Mina Niknamj,1 Soumya Poddar,2 Edmund Capparelli,2 Thuc Le,1 Liu Wei,1 Caius Radu1. 1 University of California Los Angeles, Los Angeles, CA; 2 University of California San Diego, San Diego, CA.

Introduction: While plasma concentrations are commonly studied in preclinical and clinical studies, drug concentrations in tumors and effects on molecular targets may be more appropriate for determining drug dosing and predicting response to therapy. The current study assessed tumor and plasma drug levels of DI87, a novel deoxycytidine kinase (dCK) inhibitor, and determined the relationship to dCK activity using a PET biomarker and imaging. Methods: NSG mice with CEM tumors were treated with DI87 (25 or 50 mg/kg) by oral gavage for a single dose. Tumor and plasma concentrations were assessed over 24 hrs (50 mg/kg, dose, 5 times points, N=4 separate mice per time point). Plasma and tumor DI87 concentrations were quantified by LC-MS/MS. dCK activity was determined by PET imaging of a biomarker for dCK activity over 27 hrs ([18F]-clofarabine probe was injected 3 hrs prior to imaging) after a single dose of DI87 (50 mg/kg or 25 mg/kg, 5 time points, 4 mice per time point). Nonlinear mixed effects modeling (NONMEM v. 7.2) was used to build a semi-physiologic pharmacokinetic (PK) model to describe tumor and plasma concentrations (tumor volume fixed to observed median tumor size). The PK model was linked to an inhibitory indirect response pharmacodynamic (PD) model of dCK activity in the tumor using a pooled naive approach to describe the relationship between drug levels in the tumor and dCK inhibition. Results: Peak tumor DI87 concentrations were lower (1.15 ± 0.64 μg/mL vs. 13.5 ± 4.5 μg/mL) and occurred later (9 vs. 3 hr) than peak plasma concentrations. Despite limited tumor sampling, the semi-physiologic model described tumor concentrations well (R²=0.71). The PK model had a plasma clearance of 0.52 L/hr/kg with a half-life of 3.7 hr. DI87 exposure in the tumor (AUC) was 17% of that observed in plasma. Maximal and near complete dCK inhibition occurred at 9 hr for both the 50 mg/kg and 25 mg/kg doses with recovery of dCK activity beginning at 27 hr post-dose. The predicted tumor concentration at 12 hr of 0.36 μg/mL was sufficient to maintain maximal dCK inhibition. The initiation of recovery of dCK activity corresponded to the decline in tumor DI87 concentrations, but maintained significant inhibition at 27 hr (25% of baseline). The relationship between dCK activity and DI87 tumor concentrations was well characterized by a sigmoidal Emax PD model with a large Hill coefficient. This resulted in a dramatic increase of effect over a small range of concentrations. The EC50 was within the range of tumor concentrations observed in the study (0.21 μM/mL). Conclusions: The use of semi-physiologic PK models that include tumor drug concentrations from limited preclinical data can increase knowledge of the PK-PD for novel therapeutics. This approach enhances understanding of time-dependent effects on molecular drugs targets and can improve selection of rational clinical dosing regimens for phase 1 clinical trials.

#4065 Association study between iron and cholesterol for the treatment of drug resistant neuroblastoma. Sung Y. Lee, Becky Slagle-Webb, Cara-Lynne Schengrund, James Connor. Penn State Univ. College of Medicine, Hershey, PA.

Neuroblastoma is the most common childhood extracranial solid malignancy. Although cancer cells need more iron and lipids for their active cell metabolism, possible links between iron, mutations in genes involved in iron metabolism (e.g., HFE) and lipid metabolism have not been studied well. We determined the HFE genotype in human neuroblastoma cells as well as patients. Neuroblastoma cells and patients have C282Y HFE mutation rates (~12%) that are similar to those of the general Caucasian population. While cultured cells derived from drug resistant neuroblastomas had less cholesterol, they expressed more ferritin, and contained more iron, than cells derived from drug susceptible tumors. In drug resistant neuroblastoma CHLA-171 cells, cellular alteration of HFE expression had no effect on their iron levels but decreased their cholesterol. Results of in vitro experiments indicated that simvastatin, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMCO-Ar), was cytotoxic to drug resistant cells as was the iron chelator di-2-pyridylketone-4- cyclohexyl-4-methyl-3-thiosemicarbazone (DpC). In vivo, the anti-tumor effect of simvastatin administered orally was minimal. In summary, we found i) an inverse association between ferritin and HMCO-Ar expression in neuroblastoma cells that was reflected in their iron and cholesterol levels, and ii) that the most cytotoxic cholesterol inhibitor of neuroblastoma cells was simvastatin, while the most cytotoxic iron chelation agent was DpC. Our results indicate that
it may be possible to use HFE genotype to develop a precision medicine approach to use certain iron chelators and/or cholesterol decreasing agents in the treatment of drug resistant neuroblastomas. Our results also indicate that alterations in iron and cholesterol metabolism are part of the cell’s drug resistance mechanism in neuroblastomas.

**#4066** The dihydroartemisinin oxime dimer (NSC725847) displays a selective toxicity in colon cancer cells which is potentially mediated by endoplasmic reticulum stress. Ahmed E. Elhassany, 1 Eman Soliman, 2 Paul McGuire, 1 Mahmoud ElSohly, 2 Waseem Gul, 3 Rukiyah Van Dross 4, 3 Brody School of Medicine, East Carolina University, Greenville, NC; 1 Faculty of Pharmacy, Zagazig University, Sharkia, Egypt; 2 ElSoLyh Laboratories Inc. and National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, Oxford, MS; 3 ElSoLyh Laboratories Inc., Oxford, MS; 4 Brody School of Medicine and Center for Health Disparities East Carolina University, Greenville, NC.

Colorectal cancer (CRC) is the third leading cause of cancer-related deaths in the United States. Common chemotherapeutic regimens for CRC include a combination of chemotherapeutic agents to produce synergistic drug activity and reduce adverse effects. However, adverse effects due to lack of selective toxicity is still a major problem with many chemotherapeutic agents. Novel drugs that display tumor selective toxicity are desperately needed. Several studies have shown that artemisinin monomers (Clinically used anti-malarial agent) possess antineoplastic activity with minimal toxicity. Interestingly, artemisinin dimers showed more potent antineoplastic activity compared to the monomers. However, few studies have fully characterized the activity of these molecules. In this study, we tested the antitumor activity of five chemically-synthesized dihydroartemisinin (DHA) dimers using the human colon cancer cell lines, HT29 and HCT116 and the non-tumorigenic colon cell line, FHC. Compared to other tested dimers, the DHA oxime dimer, NSC735847 showed pronounced selective toxicity in HT29 and HCT116 colon cancer cells compared to the non-tumorigenic FHC cells. In addition, NSC735847 significantly increased caspase 3/7 activity in HT29 and HCT116 cells but not in FHC cells which suggests that this compound causes selective apoptosis in these colon cancer cell lines. The combination of NSC735847 and the topoisoenserase I inhibitor, irinotecan (commonly used chemotherapeutic agent for colorectal cancer) showed synergistic antitumor activity in HT29 cells. The combination of the two drugs caused a significant increase in cell death and caspase 3/7 activity which were greater than those caused by the individual drugs. Previous studies using other tumorigenic cell lines, suggested that NSC735847 induces oxidative and endoplasmic reticulum (ER) stress. To gain insight into the potential mechanisms of anti-colorectal cancer activity of NSC735847, we tested if this compound caused ER stress and/or oxidative stress in HT29 cells and whether ER stress was required for NSC735847-induced apoptotic cell death. NSC735847 increased the expression of ER stress marker C/EBP homologous protein 10 (CHOP10) in HT29 cells and the use of ER stress inhibitor, salubrinal, significantly decreased NSC735847-induced cell death and apoptosis. Additionally, NSC735847 caused oxidative stress in HT29 cells which was inhibited by pretreatment of the cells with the antioxidant, trolox. The crosstalk between oxidative stress and ER stress in NSC735847-induced apoptosis in colon cancer cells is still under investigation. Our results suggest that NSC735847 causes ER stress in HT29 cells which plays a major role in drug-induced cell death and apoptosis in these cells.

**#4067** BPM31510, a clinical stage metabolic modulator demonstrates therapeutic efficacy in an in vivo C6 rat glioma model and synergizes with temozolomide. Stephane Gesta, 1 Semma Nagpal, 2 Tulin Dadali, 1 Milton Mercant, 2 Taichang Jan, 2 Anne R. Diers, 1 Michael Kiebish, 1 Joaquin Jimenez, 3 Barbi Akl, 1 Ann Marie Stephens, 1 Berge Smedes, 1 David S. Margolis, 2 Recht 2.

BPM31510 is a parenteral nanodispersion of ubidecarenone that is in clinical testing for solid tumors, effectuates an anti-cancer effect in highly metastatic cancers by eliciting an anti-Warburg effect. Intraperitoneal (i.p.) administration of BPM31510 reverses paraglia in a rat CNS leukemia model demonstrating the bioavailability of the drug to the central nervous system. The study describes data demonstrating the therapeutic utility of BPM31510 in glioblastoma multiforme (GBM). BPM31510 decreased cell proliferation rates in patient-derived GBM cell lines compared to temozolomide (TMZ), pretreatment with BPM31510 followed by TMZ challenge was associated with significant synergy in reducing cell proliferation compared to monotherapy. In a C6 gliona allograft rat model treatment with BPM31510 alone (n=30/group) (i.p., 50mg/kg, b.i.d.) increased overall survival compared to untreated control group. BPM31510 in combination with procarbazine (oral, 60mg/kg; d 8, 21) and vin-cristine (IV, 1.4mg/kg; d 8, 29) significantly improved overall survival compared to BPM31510 alone or chemotherapy. Furthermore, long term survival was achieved in four of twenty rats with C6 gliomas receiving BPM31510 doses between 10 to 50 mg/kg/day and compared with no treatment or irradiation (P < 0.02, Fisher exact test). Interestingly, the long term survival persisted for over two months after all treatment was discontinued; MR imaging confirmed the presence of static masses in these long term survivors. Collectively, the data demonstrate that BPM31510 has a robust effect in a preclinical model of GBM. Intravenous BPM31510 is currently in Phase 1/2 clinical trial for TMZ+BEV refractory GBM patients.

**#4068** HH-002, a derivative of Homoharringtonine, show promising in diverse cancer models in preclinical characterization. Hank Rui, Bensheng Pharma Co. Ltd., Pudong, China.

Homoharringtonine (HHT) is an anti-cancer drug used to treat imatinib resistant CML, and a reversible inhibitor of translation acting upon the early steps in polypeptide chain elongation. BS-HH-002.SA is a derivative of HHT. BS-HH-002.SA was shown to decrease the phosphorylation of Janus protein tyrosine kinase 2 (JAK2) as well as downstream targets, such as signal transduc- ter activation of transcription 3 and 5 (STAT3 and STAT5), it also reduced Myeloid cell leukemia-1 (Mcl-1) protein level significantly and resulted in apoptosis. Evaluation of BS-HH-002.SA efficacy was test in HEL, an erythroleuко- mia cell line carrying a Jak2V617F mutation which is seen in 90% of patients with polycythemia vera (PCV) and half of those with essential thrombocythemia or primary myelofibrosis. HH-002.SA was showed highly potent (at low nM concentration) in HEL. Efficacy of BS-HH-002 was tested in a recombinant human erythropoietin injection (rEpo)-induced murine model of polycythe- mia Vera, Treatment with BS-HH-002.SA BID for 4 days showed a significant inhibitory effect on rEpo induced increase in reticulocytes, hematocrit and splenomegally, as well as in red blood cell, white blood cell and platelet counts. Furthermore, histology evaluations revealed that BS-HH-002.SA exerted an inhibitory effect on rEpo induced extramedullary hematopoiesis in spleen, and decreasedcellularity in bone marrow with more predominant effect on erythroid cells than on myeloid cells. Also, BS-HH-002.SA also show potent in vitro and in vivo efficacy in pancreatic cancer cell line KP-4 and non-small cell lung cancer (NSCLC) NCI-H1975, BS-HH-002.S inhibited STAT3 Tyrosine 705 phosphorylation and reduced anti-apoptotic proteins expression Mcl-1 protein levels in both two cancer cell lines.

**#4069** Trimethylation and acetylation of Histone H3K27 modulates 5-fluorouracil response by regulating DPD expression. Rentian Wu, Mayo Clinic, Rochester, MN.

The antimitabolite 5-fluorouracil (5-FU) is one of the most widely used chemotherapeutics drugs. Dihydropyrimidine dehydrogenase (DPD) initiates the catalytic degradation of 5-FU to inactive metabolites and is widely accepted as a major determinant of 5-FU response and toxicity. While genetic variants in DPD have been shown to variants may affect 5-FU metabolism, they are rare events and do not completely explain the variable variability in DPD function or the resultant differences in treatment response. Here, we report that DPD expression is epigenetically regulated by histone modification at relevant promoter and enhancer regions. Using both chemical inhibitors and genetic approaches, we show that H3K27 tri-methylation (H3K27me3) at the DPD promoter is regulated by the epigenetic modifiers Ezh2 and UTX. Promoter H3K27me3 suppresses DPD expression by inhibiting the binding of the transcription factor PU1. Binding to the promoter increases sensitivity leading to increased resistance to 5-FU. In a cohort of healthy volunteers, H3K27me3 levels were inversely correlated with an enhancer that regulates DPD expression was also identified. Modifying the H3K27 status of the DPD promoter or enhancer matures DPD gene expression. Deletions and mutations on the DPD promoter or enhancer abolish their transcription regulation function. Enrichment of H3K27me3 at the DPD promoter was negatively correlated with DPD expression and DPD enzyme activity in peripheral blood mononuclear cells specimens from healthy volunteers. Lastly, tumor expression data suggests that DPD expression by Ezh2 is a strong predictor of poor survival in 5-FU treated cancers. By using a combination of in silico prediction and in vitro reporter assays we also identified a functional enhancer region for DPD that is also likely to be clinically relevant to 5-FU treatment outcome. Collectively, the present thefindings suggest that a previously uncharacterized mechanisms regulates DPD expression and may likely contribute to tumor resistance to 5-FU.
Pancreatic cancer (PaCa) is highly refractory to treatment, with a median survival of 6 months. Currently, gemcitabine (Gem), a difluoro analog of deoxy-cytidine, is the standard care for PaCa, but it renders only a modest survival benefit. Combination therapy, integrating novel agents into standard-of-care regimens, may maximize efficacy of Gem-based therapy. Fibroblast growth factor receptor (FGFR) tyrosine kinase is a potential therapeutic target in pancreatic cancer.

FGFR1 is overexpressed in various carcinomas and is overexpressed in various cancers and therefore represents a considerable chemotherapeutic target. Inactivation of FGFR1 reduces tumor cell survival and growth and inhibits tumor cell migration and invasion.

FGFR inhibitors can impact Gem receptor (FGFR) function is aberrant in pancreatic cancer, and suppression of FGFRs inhibits pancreatic cancer growth and invasion. However, the mechanisms of FGFR action and their therapeutic utility in pancreatic cancer remain to be elucidated. Here we tested the hypothesis that FGFRs can impact Gem sensitivity of PaCa cells by employing pan-FGFR inhibitors and shRNAs against FGFRs. NVP-BG139 and AZD4547 are novel FGFR inhibitors (FGDRI and FGFR3, and are in various stages of clinical trial. The FGFRs modestly but significantly inhibited proliferation, migration, and invasion of Panc-1, MiaPaCa-2 cells in wound-healing and trans-well assays. Treatment with low-concentration Gem (7.5 nM) enhanced MiaPaCa-2 cell migration at 24 hrs, but migration was reduced subsequently, suggesting a sequence of delayed effects of Gem upon cell migration. After 72 hrs of exposure, the combination of FGFRs reduced MiaPaCa-2 cell proliferation significantly compared to controls or Gem- and FGFR1 alone. Pharmacodynamic modeling was employed to evaluate drug interaction, and the interaction coefficient Psi was estimated by simultaneous modeling of 16 different drug combinations over a period of 0-96 hrs. The analysis estimated Psi = 0.78, which indicates synergism. Compared to BG139 or Gem alone, the combination also increased the pharmacokinetics for a longer period of time. We detected a trend of increased expression of RRM1, whereas knockdown of FGFR2 and FGFR3 mediated an increase in RRM1 expression, implicating FGFR1 suppression in improving Gem sensitivity. These results suggest that inhibition of FGFR1 could potentially enhance sensitivity of PaCa to Gem therapy, and that combining FGFRs with gemcitabine is a promising treatment strategy in pancreatic cancer.
intact and permeable BTB. In conclusion, S-222611 is expected to be useful for the treatment of patients with HER2-positive breast cancers, including those with brain metastases.

**#4074 Effect of PEG chain length on antibody-drug conjugate tumor and permeability changes and effect of chemotherapy in brain adjacent with brain metastases.**

Aroz Shareef Mohammad, Chris E. Adkins, Neal Shah, Rawaa Aljammal, Jessica Griffith, Paul R. Lockman. West Virginia Univ., Morgantown, WV.

Background: Brain metastases vasculature is compromised and more permeable than that of vasculature in normal brain. Unfortunately, little is known about vascular permeability changes in brain adjacent to tumor (BAT). It is critical to understand the potential changes as chemotherapy penetration and accumulation brain tumors positively correlate with breakdown of the brain vasculature. Herein, we evaluate quantitatively measure permeability, drug accumulation and cytotoxicity in normal brain that is adjacent (<500 microns) to a tumor. Methods: The subclone population of brain seeking metastatic breast cancer cells (MDA-MB-231Br) were injected into the left ventricle of female NuNu mice. Mice were given a chemotherapeutic regimen of vinorelbine (10mg/kg), erubulin (1.5mg/kg) or docetaxel (10mg/kg). Progression occurred until neurological compromise was noted, at which time animals were injected with texas red 625 Da(TR) and texas red dextran 3kDa (TRD). Dye concentrations (measuring passive permeability changes) were determined in lesions and the BAT using quantitative fluorescent microscopy. Chemotherapeutic induced cytotoxicity was observed by staining for activated astrocytosis and DNA damage in neurons, glia and endothelium. Results: We observed permeability of TR is highly heterogeneous both within and between the tumors (normal brain Kin values and Area Under the Curve (AUC) were calculated for each cell line. Hierarchical clustering was performed to determine similar responses between cell lines. The use of targeted drug therapy is rising in the pediatric oncology field. Development of novel therapeutic agents for Down syndrome patients with AML. Apurvi R. Patel, Justin J. Montoya, Daniel H. Wai, David W. Lee, Robert J. Arceci, David O. Azorsa. Phoenix Children's Hospital, Phoenix, AZ.

Patients with Down's syndrome who develop acute myeloid leukemia (AML) are at increased risk of toxicity secondary to chemotherapy regimens. These patients are harder to treat with conventional therapies at relapse. Overall, the use of targeted drug therapy is rising in the pediatric oncology field. Development of novel therapeutic agents for Down syndrome patients with AML.

**#4075 Effect of PEG chain length on antibody-drug conjugate tumor and tissue distribution in tumor bearing xenograft mice.** Nagendra V. Chemuturi, Martha Anderson, Julia Cochran, Thomas Pires, Josh Hunter, David Meyer, Jason Neale, Alice Chin, Paul Pittman, Cindy Balasubramanian, Francisco Zapata, Jessica Simmons, Robert Lyon, Steve Alley. Seattle Genetics, Bothell, WA.

Antibody-drug conjugates (ADCs) are a promising new therapeutic modality having demonstrated clinical efficacy in the treatment of cancer. While ADCs have better exposures than their constitutive chemotypes, it is important that they maintain the favorable pharmacokinetics of their antibody backbones. Previous work incorporating PEG into the drug-linker as a sidechain helped to mask the hydrophobicity of the chemotype, resulting in improved ADC exposure in rats, compared to ADCs with non-PEGylated drug-linkers. The current studies were aimed at understanding the effect of PEG chain length on ADC efficacy, tumor and tissue distribution in tumor bearing mice. Efficacy and biodistribution of radiolabeled ADCs with 2, 4, 8, 12 and 24 PEG units in the drug-linker as a side chain were investigated in SCID mice bearing a subcutaneous xenograft of L540 cytumor cells. A non-PEGylated ADC was used as control. Antibody based radioactivity in plasma, tumor, liver, lung, kidney, spleen and brain were measured over 14 days. MMAE, the free drug released from the ADC, was measured by mass spectrometry. Reduction in tumor weight was determined as a measure of efficacy. Compartmental analysis of the plasma exposure data was conducted to understand the change in rate constants with change in PEG chain length. ADCs with side chain PEGylated drug-linkers had greater plasma and tumor exposures than the non-PEGylated control ADC. Increasing PEG chain length in the linker led to increased plasma and tumor exposures, and lower plasma clearances. Tumor distribution was binary with ADCs 2 and 4 PEG units in the linker showing similar tumor exposures while the ADCs with 8, 12 and 24 PEG units provided similar but significantly higher tumor exposures. ADCs with 8, 12 and 24 PEG units in the linker had significantly higher tumor to plasma exposure ratios than ADCs with 2 and 4 PEG units in the drug-linker. The reduction in tumor weights was also binary. ADCs with 2 and 4 PEG units provided a 35-45% decrease in tumor weights while ADCs with 8, 12 and 24 PEG units in the linker provided 75-85% reduction in tumor weights. The non-PEGylated control ADC decreased the tumor weights by 11%. In general, PEGylated ADCs increased the extent of tissue distribution, but the tissue to plasma ratios remained the same indicating that the increased tissue exposures was a function of increased plasma exposures. However, PEGylated ADC tumor exposure increased beyond that expected based solely on the increase in plasma exposure. Incorporation of PEG as a side chain into the drug-linker improved tumor accumulation of ADCs with little alteration. ADCs with 8, 12 and 24 PEG units in the drug-linker showed preferential uptake into tumor, but not the tissues as evidenced by tumor (or tissue) to plasma exposure ratios. Over all, the ADCs with 8, 12 and 24 PEG units in the linker provided the optimal combination of tumor distribution, efficacy and tissue distribution.


IDO1 catalyzes degradation of tryptophan (Trp) into kynurenines, which plays an important role in the regulation of immune responses by triggering anergy on reactive effector T cells and by modulating differentiation and activation of regulatory T cells (Treg). Indoximod has been demonstrated to relieve IDO-mediated immunosuppression in vitro and in vivo, by creation of an artificial Trp-sufficiency signal that bypasses activation of GCN2 and inhibition of mTOR in conditions of Trp deprivation. Inhibition of the IDO pathway by indoximod in combination with immune-stimulatory treatments leads to increased T cell proliferation, Treg reprogramming and antitumor effect. Indoximod has demonstrated an excellent safety profile in human clinical trials and is being dosed orally at 1200 mg bid. Increases in doses above this level do not generally result in increased plasma concentration or drug exposure due to limiting dose-dependent oral bioavailability. Animal models suggest that increased therapeutic benefit could be achieved at higher levels of exposure. For this reason, we synthesized and tested the pharmacokinetic profile of several indoximod prodrugs in mice, rat and monkeys in both liquid and capsule formulations. We selected clinical development drug candidate NLG802, which increases indoximod exposure and plasma concentration ~2-fold in rats and ~5-fold in monkeys dosed with capsules of comparable formulation to that being used in clinical trials. NLG802 is rapidly absorbed and metabolized in vivo to indoximod. NLG802 DMPK profile and GLP toxicity studies have been carried out in rats and monkeys suggesting a safe toxicological profile at predicted therapeutic doses. In a preclinical tumor model of mice bearing established B16F10 tumors, administration of NLG802 markedly enhanced the anti-tumor responses of naïve, restting peml-1 cells to vaccination with cognate hgp100 peptide plus Cpg-1826 in IFA. In this tumor model, NLG802 plus peml-1 vaccine produced significant tumor size reduction within 4 days of vaccination. Moreover, the tumor response was achieved at lower doses (equivalent to ~30% of the equivalent molar doses of indoximod). In conclusion, NLG802 is a prodrug of indoximod predicted to increase clinical drug exposure to indoximod above the current achievable levels and will soon enter Phase 1 safety testing in oncology clinical trials.


Patients with Down's syndrome who develop acute myeloid leukemia (AML) are at increased risk of toxicity secondary to chemotherapy regimens. These patients are harder to treat with conventional therapies at relapse. Overall, the use of targeted drug therapy is rising in the pediatric oncology field. Development of novel therapeutic agents for Down syndrome patients with AML.

**#4078**

**EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Mechanistic Understanding of Novel Anticancer Therapies**

Phoenix Children’s Hospital, Phoenix, AZ.

Patients with Down’s syndrome who develop acute myeloid leukemia (AML) are at increased risk of toxicity secondary to chemotherapy regimens. These patients are harder to treat with conventional therapies at relapse. Overall, the use of targeted drug therapy is rising in the pediatric oncology field. Development of novel therapeutic agents for Down syndrome patients with AML.
ing results showed improved sensitivity of the CMK and CMY cells to Navito- 
clax and 17-AAG under normoxic and hypoxic conditions. Validation of the 
screening results was done using a 10-concentration drug dose response assay. 
Future studies will explore the mechanism by which the Down’s syndrome AML 
cells have increased sensitivity to these targeted agents under both normoxic and 
and hypoxic conditions. In Down’s syndrome AML, we will investigate the effects of combina-
tions of these targeted therapies with conventional therapies. The results from 
these studies could lead to the establishment of improved therapeutic regimens 
for Down’s syndrome patients with AML.

**#4078** Tumors with class 3 BRAF mutants are sensitive to the inhibition of 
activated RAS. Zhan Yao,1 Rona Yaeger,1 Vanessa S. Rodrik-Outmezguine,1 
Anthony Tao,2 Neilawatte M. Torres,3 Matthew T. Chang,1 Matthias Drosten,1 
Huaying Zhao,1 Fabiola Cocchi,3 Todd Hembroug,4 JudithMichels,5 Hervé 
Baumert,6 Linde Miles,1 Naomi M. Campbell,1 Elisa de Stanchina,1 David B. 
Solit,5 Mariano Barbacid,2 Barry S. Taylor,7 Neal Rosen1,2, Mem. Sloan Kettering 
Cancer Ctr., New York, NY; 3New York University, New York, NY; 4Centro Na-
acional de Investigaciones Oncológicas, Madrid, Spain; 5NanOtics, LLC, Rock-
ville, MD; 6Gustave Roussy Cancer Campus, Paris, France; 7Saint Joseph Hospital, 
Paris, France.

Approximately two hundred mutant BRAF alleles have been identified in 
human tumors. Physiologic activation of RAF isoforms requires RAF-depen-
dent induction of their dimerization. Activating BRAF mutants cause ERK de-
pendent feedback inhibition of protein phosphatase 1, thereby repressing ERK 
signaling in tumors requires concurrent dysregulation of RAF activation. Thus, 
melanomas with Class 3 mutations usually harbor coexistent RAS mutation or 
NF1 mutants/deletion, whereas receptor tyrosine kinase signaling is activated in 
lung and colorectal cancers with these mutants. Our model suggests that these 
tumors will be sensitive to the inhibition of RAS activation. Currently, no direct 
inhibitors of RAS activation are available. However, in support of this idea, 
inhibitors of activated RTK signaling in carcinomas with Class 3 BRAF mutants 
and wild type RAS is sufficient to markedly inhibit ERK signaling and their 
growth in vivo murine models and in patients. We have thus defined a third 
subset of BRAF mutants, which is RAF-dependent. Tumors harboring such 
mutants are sensitive to tyrosine kinase inhibitors in tumors expressing wild 
type RAS and NF1.

**#4079** Pre-clinical toxicity and safety of SH7139: The first of a new class 
type RAS and NF1. mutants are sensitive to tyrosine kinase inhibitors in tumors expressing wild 
and advancing SH7139 into clinical trials, acute dose range find-
ing from each of the linked ligands) that inhibit a series of cellular activities 
concentrated and subsequently metabolized, releasing toxic metabolites (de-
binding to its HLA-DR target, SH7139 is carried into the cytoplasm where it is 
epitopewithintheantigen-bindingpocketofHLA-DR10. HLA-DRscontaining 
drug conjugate and a pro-drug, SH7139 targets and binds to a unique structural 
exvivo, drug screening is limited to short-term assessments due to de-differentiation. This limits their efficacy in testing slow-clearing, large-
molecule compounds such as antibody-based therapies and low-turnover drugs. 

**#4080** Cytochrome P450 enzyme activity is enhanced in hepatocytes 
grown using a perfused 3D cell culture drug screening system. James T. Shoe-
maker, Jelena Vukasinovic. Lena Biosciences, Atlanta, GA.

A critical requirement for assessing drug toxicity in vitro is cytochrome P450 (CYP) 
function that is physiologically representative. Due to low levels of CYP 
ezyme activity, cell lines such as HepG2 hepatocytes have largely been sup-
planted with primary cells derived from human donor tissue. While these cells 
retain CYP activity ex vivo, drug screening is limited to short-term assessments 
due to de-differentiation. This limits their efficacy in testing slow-clearing, large-
molecule compounds such as antibody-based therapies and low-turnover drugs. 

**#4081** Superior SN-38 pharmacodynamic and tumor-accretion profiles of 
labetuzumabgovitecan (IMMU-130) versus irinotecan in experimental hu-
man colon cancer models. Thomas M. Cardillo, Robert M. Sharkey, Serengu-
lam G. Govindan, Jennifer Donnel, Maria Zalath, David M. Goldenberg. Immu-
nomelics, Inc., Morris Plains, NJ.

BACKGROUND: IMMU-130 is an antibody-drug conjugate (ADC) under-
going clinical investigation in patients with metastatic colorectal cancer (Clini-
icalTrials.gov, NCT016053188). It is composed of a humanized anti-CEACAM5 
IgG conjugated via a cleavable linker to SN-38, a topoisomerase-I inhibitor and 
active form of irinotecan. We investigated the potential advantage of IMMU-130 
versus irinotecan for SN-38 delivery in nude mice bearing CEA-expressing hu-
man colon tumor xenografts (LS174T or GW-39). METHODS: Mice were 
infected with irinotecan (~900 μg; SN-38 equivalents = ~500 μg) or 1.0 mg of 
IMMU-130 (16 μg SN-38 equivalents). Irinotecan-treated animals were necrop-
sied 5 min, 1, 2, 6-8 h post-injection, while IMMU-130-treated animals were 
evaluated at 1, 6, 24, 48-72 h. Serum and homogenates of tumors, liver, and small 
testes were harvested, and SN-38, SN-38, SN-38g, and irinotecan concen-
trations were determined by reversed-phase HPLC. For IMMU-130-treated 
specimens, SN-38 concentrations were assessed in the extracted samples (Free 
SN-38), as well as in acid-hydrolyzed samples to determine Total SN-38 (Free + 
bound). IgG was measured by ELISA. RESULTS: Irinotecan cleared quickly from 
serum, with SN-38 averaging ~900 ng/ml to 200 ng/ml from 5 min to
EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Mechanistic Understanding of Novel Anticancer Therapies

6 h. SN-38G and SN-38 levels were similar. With IMMU-130, Free SN-38 was detected in serum over the entire monitoring period, but levels were only a small fraction of the Total SN-38 (~10%). Importantly, Free SN-38G was very low, being detected only within the first 6 h. Total SN-38 levels dropped more quickly than the IgG, confirming in vitro studies showing gradual SN-38 release from the ADC. In tumors, for irinotecan-treated animals, SN-38 peaked at 5 min, representing ~0.2% of the SN-38 equivalent given. In IMMU-130-treated animals, no Free SN-38 was detected in tumors, but levels of Total SN-38 peaked at 6 h, with ~5% of the injected SN-38 dose present at that time, and were sustained longer than SN-38 delivered by irinotecan. Area under the curve analysis found SN-38 levels were ~10- and 17-fold higher in LS174T and GW-39 tumors, respectively, from IMMU-130-dosed versus irinotecan-dosed animals. This delivery advantage is amplified >30-fold when normalized to SN-38 equivalents injected for each product, illustrating the improved bioavailability with IMMU-130-targeted SN-38. Levels of SN-38 and SN-38G were appreciably lower in the liver and small intestinal contents, which likely explains the lower incidence of severe diarrhea reported in patients given IMMU-130. CONCLUSION: IMMU-130 delivers >100-fold more SN-38 to CEA-producing tumors compared to irinotecan, while also reducing levels of potentially harmful SN-38 and SN-38G in normal tissues. These observations are consistent with preclinical data showing improved efficacy and safety.


A 1010 Brutton’s Tyrosine Kinase (BTK) plays an important role in B-cell signaling, cell proliferation and survival and is an established drug target in B-cell malignancies. GS-4059 (ONO-4059) is an irreversible, small molecule inhibitor of BTK, which is in development for hematological malignancies and rheumatoid arthritis. A Phase 1 study to evaluate the effect of oral anion transporting polypeptide (OATP) 1B1 and 1B3 inhibitors and inducers of cytochrome P450 3A (CYP3A) on GS-4059 Pharmacokinetics (PK) was performed in healthy subjects (N=15). Subjects were administered a single dose of 100 mg GS-4059 alone or in combination with a single or multiple doses of rifampin (600mg) and blood samples were collected up to 48 hours post dose to evaluate PK and Pharmacodynamic (PD) markers in peripheral blood mononuclear cells (PBMCs). PD biomarkers were exploratory endpoints and are reported here. PD was evaluated using the BTK occupancy assay and the Basophil Activation Test (BAT). A novel duplex homogeneous BTK occupancy assay was used to quantitatively measure GS-4059 binding to human BTK to assess target coverage. The assay is based on Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET) and simultaneously measures the level of free BTK as well as total BTK protein in PBMCs collected from subjects in the study. Free BTK levels in PBMCs decreased rapidly on treatment with GS-4059 with no detectable free BTK 3-24 hours post dose and no notable changes in total BTK. All subjects had free BTK levels below the limit of detection (LOD 6.8 ng/mL) 3-24 hours after dosing. Free BTK levels started to increase after 48 hours. Free BTK levels on treatment with multiple doses of rifampin plus GS-4059 followed a similar trend with no free BTK detected 3-6 hours post dose but resulted in shorter duration of inhibition and more rapid recovery of free BTK levels. GS-4059 plasma exposure (Cmax and AUC) was ~70% lower after multiple doses of rifampin, due to a drug-drug interaction. As a second PD marker, inhibition of CD63 basophil activation in the BAT was measured. In this functional assay the median inhibition of CD63 basophil activation was between 90% and 100% at the time of maximum inhibition (3 hours post dose). At the peak of inhibition 13 of the 14 subjects had inhibition of basophil activation higher than 97%. In line with the BTK occupancy data, inhibition of CD63 basophil activation was significantly lower with reduced GS-4059 plasma exposure. BTK occupancy as a direct measure of target binding and the functional assay measuring the inhibition of basophil activation showed a similar time course with maximum effects at both assays achieved at 3-6 hours post dose. These data demonstrate that a single 100 mg dose of GS-4059 results in full BTK occupancy in healthy subject PBMCs as well as functional changes measured in basophil activation as a surrogate for BTK signaling pathway inhibition.

#4083 Basic mechanisms of Vernonia amygdalina exert antitumor activities in breast cancer cells. Clement G. Yedjou, Paul B. Tchounwou. Jackson State Univ., Jackson, MS

Breast cancer continues to represent the largest cause of mortality in the world especially among women. It is estimated that cancer kills more women in the USA than any other illness and it is also the leading cause of death among all Americans. Traditional medicine represents the first-choice of healthcare treatment for at least 80% of people living in developing countries. Preliminary studies from our laboratory have shown that a novel natural product, extracts of Vernonia amygdalina (VA) leaf exerts DNA-damaging anticancer activities against breast cancer. There is a need to develop targeted approaches for the treatment of human breast cancer. Therefore, the goal of this research was to determine the therapeutic mechanisms of V. amygdalina leaf extracts as anti-cancer agent in the management of breast cancer. To achieve our goal, cell viability, live and death cells were determined by trypan blue exclusion test. The extent of oxidative cell/tissue damage was determined by measuring malondialdehyde (MDA) levels. Expression of p53 tumor suppressor gene was assessed both by immunofluorescent and western blot analyses. Cell apoptosis was measured through determination of caspase-3 activity and DNA fragmentation associated with the formation of MDA, a by-product of lipid peroxidation and biomarker of oxidative stress. This result suggests that V. amygdalina treatment may be a good anti-cancer candidate for the treatment of breast cancer. Research supported by NIH Grant No. NIMHDG12MD007581.


S-222611 is a potent and selective reversible tyrosine kinase inhibitor of epidermal growth factor receptor (EGFR), human EGFR2 (HER2), and human EGFR4 developed in Shionogi. Greater potency of anti-tumor activity than lapatinib was demonstrated in vitro and in animal models. In the intracranial implantation mouse model implanted the HER2-positive cell line (MDA-MB-361-luc-2), S-222611 showed more potent anti-tumor activity than lapatinib. On the basis of higher penetration in brain of S-222611 than lapatinib indicated by the preclinical pharmacokinetic studies, we demonstrated the application of S-222611 to brain metastases with breast cancer. In order to enhance the clinical predictability of S-222611 to the treatment of brain metastases, we have developed the experimental brain metastases models in mice of HER2-positive breast cancer (MDA-MB-361-luc-2-BR2/BR3) and T970M-EGFR expressing lung cancer (NCI-H1975-luc). After a single oral administration of S-222611 or lapatinib, the concentrations of S-222611 and lapatinib in the brain metastatic regions of these mice models were analyzed by quantitative imaging mass spectrometry (QIMS). In the breast cancer model, the concentrations of S-222611 in brain metastases quantified by IMS were comparable to those of lapatinib. In contrast, in the MDA-MB-361 breast cancer model (intraventricular injection model), the concentrations of S-222611 in brain metastases were over 10 times higher than those of lapatinib, and the tumor-to-normal brain ratio of S-222611 was significantly higher than those of lapatinib in the breast cancer mice model. In addition, the blood-tumor barrier (BTB) permeability in each brain metastatic region using these mice models was assessed simultaneously. In the lung cancer model, fluorescently labeled dextran was highly detected in the brain metastatic regions than brain parenchyma. However, in the breast cancer models, fluorescence intensities for dextran in the brain metastatic regions and brain parenchyma were comparable, indicating that the BTB was remained to be largely intact in brain metastases of the breast cancer model but disrupted in the lung cancer model. These results show that S-222611 is expected to be useful for the prevention and the prompt treatment of patients with HER2-positive breast cancer having brain metastases.
Introduction: Doxorubicin is a highly effective chemotherapeutic agent, but its use is limited due to cumulative and dose-dependent effects that can lead to irreversible heart damage. Despite efforts in understanding the mechanisms, the precise genes and pathways implicated in doxorubicin-induced cardiotoxicity still remain elusive. We hypothesized that by exploring time- and dose-depen- dent changes in expression in human FLT3-ITD cell lines in vitro, we could identify genes and signaling pathways related to doxorubicin-induced cardiotoxicity. Methods: hiPSC-CM were treated with 0, 50, 150, or 450 nM of doxorubicin for 2, 7, or 12 days then subjected to RNA sequencing for assessment of gene expression. Sequenced reads were aligned to the human reference genome (GRCh37) with STAR, and a read count matrix was generated with the R package GenomicConsensus. Global variation and differential gene expression analyses were performed using DESeq2 software (P < 0.05). For time course analysis, log2 fold-changes in gene expression for pairwise comparisons between any two time points or doses were calculated using DESeq2 and a Time x Dosage interaction model. For Ingenuity Pathway Analysis of time course genes, input genes were selected based on a cutoff > 2.0 and < 2.0 average log2 fold-change in expression. Results: Hierar- chical clustering of expression profiles of 40,861 genes produced by DESeq2 revealed 192 genes downregulated following doxorubicin exposure related to mitotic roles of polo-like kinase, cell cycle control chromosomal replication, and DNA damage checkpoint regulation pathways. This included a set of nine genes with the highest variation in expression, MCM5, PRC1, NUSAP1, CENP, CCNB1, MKL, AURKB, RAGAP1, and TROAP, with functions in microtu- bule stabilization and cell cycle regulation. Following a 2-day exposure to doxo- rubin, 242 genes, including the top nine genes, were differentially expressed between untreated and treated with enrichment in ribosomal processes, DNA repair and damage, and oxidation-reductase activity. Time course analysis re- vealed 289 genes between 0 nM and 150 nM on days 2, 7 and 12 with enrichment in fatty acid oxidation, MIF-mediated glucocorticoid regulation, and relaxin signaling pathways. Transcription regulator, SMARCA4, was identified as the only upstream regulator with elevated expression following a later recovery time (day 7 to day 12) compared to shorter or earlier time point comparisons at 150 nM dose. Conclusion: Overlapping genes and pathways identified in these analyses provide additional support for a role of reactive oxygen species and mito- chondrial dysfunction linked to DNA damage in doxorubicin-induced cardio- toxicity. This study also warrants further investigation of SMARCA4 since it is a direct regulator of genes with overlapping functions described for the top nine genes.

#4087 Development of a potent, dual pan-PIM/FLT3 inhibitor for the treatment of heme malignancies. Wojciech Czardybon,1 Renata Windak,1 An- iela Golas,1 Michal Gałówski,1 Aleksandra Sabiniarz,1 Izabela Dolata,1 Magdalena Salwinska,1 Paweł Guzik,1 Magdalena Zawadzka,1 Ewelina Gabor- iela Gołas,1 Michał Gałeżowski,1 Aleksandra Sabiniarz,1 Izabela Dolata,1 Wojciech Czardybon,1 Renata Windak,1 Anna Jabłońska,3 Maciej Szydłowski,3 Tomasz Sewastianik,3 Bartosz Puła,3 Anna Porembicka,3 Weronika Domek,3 Marta Buga,3 Monika Danieliewicz,3 Grzegorz Dubin,2 Ewa Jabłońska,1 Maciej Szydłowski,1 Tomasz Sewastianik,3 Bartosz Puła,2 Anna Szumula-Cieciewicz,3 Monika Prochoriec-Sobieszek,2 Elżbieta Mądro,2 Ewa Lech-Maranda,1 Krzysztof Warzocha,1 Jerome Tamburini,2 Przemysław Juszczyszyn,1 Krzysztof Brzózka,1 Selvita S.A., Kraków, Poland; 2University of Wroclaw, Wroclaw, Poland; 3Institut Hematologi i Transfuzjologii, Warszawa, Poland; *Inserm, France.

Despite huge effort spent on understanding the pathogenesis of acute myeloid leukemia (AML), current standards of care are still based on the same chemo- therapy agents as two decades ago - combinational treatment of cytarabine with an anthracycline. Fms-like tyrosine kinase 3 internal tandem duplication (FLT3- ITD) is one of the most common genetic lesions in AML. Although FLT3 inhibitor- s initially exhibit clinical activity, resistance to treatment inevitably occurs within months. PIM kinases are thought to be major drivers of the resistance phenotype and their inhibition in relapsed samples restores cell sensitivity to FLT3 inhibitors. Thus, simultaneous PIM and FLT3 inhibition represents a promising strategy in AML therapy. Selvita has developed a potent and selective first-in-class, dual PIM/FLT3 kinase inhibitor, the SEL24-B489 compound, and profiled its activity in vitro and in vivo AML models showing significantly broader anti-tumor activity of SEL24-B489 than selective FLT3-ITD or PIM inhibitors. We compared SEL24-B489 head-to-head with a selective PIM inhibitor (AZD1208) and a selective FLT3-ITD inhibitor (AC220) in a panel of AML cell lines with FLT3-ITD or unmutated kinase (FLT3-WT) as well as peripheral AML cells and CD34+ bone marrow blasts. SEL24-B489 exhibited a signifi- cantly broader activity, irrespective of FLT3 status, than either of the selective inhibitors. Since PIM kinases have emerged as important mediators of FLT3- inhibitor resistance, we hypothesized that the dual specificity of SEL24-B489 might overcome the phenotype of resistance. We utilized previously developed MOLM-14 cells transfused with either FLT3-WT or FLT3 alleles containing TKD point mutations to show that neither of these mutations decreased the cellular sensitivity to SEL24-B489. Higher cellular activity and biomarker re- sponse of SEL24-B489 than competitive inhibitors was shown by inhibition of specific biomarkers such as S6 and STAT5 phosphorylation at nanomolar con- centrations in both FLT3-ITD positive and FLT3-WT cell lines in vitro. We have also demonstrated SEL24-B489 superior potency of SEL24-B489 in xenograph models in vivo. Consistent with the experiments in vitro showing marked synergy between SEL24-B489 and AraC, a combination of these agents resulted in almost completely blocked tumor growth in vivo. Most importantly, SEL24- B489 has been selected as a clinical candidate and is currently in phase I clinical trials.


ALKS 4230 is an engineered fusion protein comprised of a circularly per- muted interleukin-2 (IL-2) and the IL-2 receptor (IL-2R) α chain, CD25, de- signed to selectively activate the intermediate-affinity IL2R, but not the high- affinity IL-2R. Selective activation of the intermediate-affinity IL-2R by ALKS 4230 has the potential to provide enhanced tumor killing as well as improved safety and tolerability. Various in vitro and in vivo studies were conducted to characterize the primary and secondary pharmacodynamics (PD) of ALKS 4230 as well as its pharmacokinetics (PK). The results guided the selection of the starting dose for the ALKS 4230 first-in-human (FIH) clinical study based upon the Minimal Anticipated Biological Effect Level (MABEL) approach. The PK-PD relationship for ALKS 4230 was evaluated in in vitro pharmacology studies in target cells from murine, human primary and human donors. The mean EC50 values for activation of NK cells, memory CD8 T-cells and Tmem in target cells from human donors were 0.09, 0.18 and 0.13 nM, respectively. Using the lower EC50 value of 0.09 nM (0.0031 µg/mL) as a surrogate for the MABEL, assuming IV administration to a 70 kg human with 3 L plasma volume, a dose of 0.1 µg/kg would be expected to result in an immediate post-dose concentration of 0.0031 µg/mL. The mean EC50 values for activation of NK cells, memory CD8 T cells and Tmem in target cells from human donors were 0.46, 1.1 and 0.59 nM, respectively. Using the EC50 value as surrogate for minimal effective ALKS 4230 concentration that induces activation of human IL-2R complex, the projected minimal efficacious dose (MEDI) in humans to achieve a concentration of 0.46 nM (0.016 µg/mL) to 1.1 nM (0.038 µg/mL) is 0.7-1.6 µg/kg. Based on a MABEL dose of 0.1 µg/kg and projected MEDI of 0.7-1.6 µg/kg, the proposed doses to be evaluated in the FIH Phase 1 study are 0.1, 0.3, 1, 3, 10, and 30 µg/kg. In comparison, the projected Cmax, at the proposed starting dose of 0.1 µg/kg is > 750-fold lower than the Cmax at the no-observed adverse effect level (NOAEL) in safety and toxicology studies conducted in cynomolgus monkeys. It is also about 3-fold lower than the lowest concentration of ALKS 4230 (0.01 µg/mL) tested in the cytokine release assays at which only slight elevations were observed for IL6, IL-8, and IFN-γ in a small number of whole blood samples from healthy human donors, similar to those in the low-response control across the concentration range evaluated. Therefore, 0.1 µg/kg is considered a safe starting dose for the FIH study. The PK, PD and toxicology assessments conducted to date support the FIH investigation of ALKS 4230 at the proposed starting dose of 0.1 µg/kg.

#4089 Quantitative prediction of human pharmacokinetics for duvor- tuxizumab from cynomolgus monkey data: a translational pharmacokinetic modeling approach. Xiling Jiang,1 Hui Liu,2 Jeff Nordstrom,3 Jennifer Brown,2 Liqun Liu,2 Sid Johnson,2 Ralph Alderson,2 Pamela L. Clemens,1 Jacintha Shen- don,1 Inran Khan,1 Olivia Gardner,1 Yu-Nien Sun,1 Weirong Wang3,1 Janssen Research & Development LLC, Spring House, PA; 2MacroGenics, Inc., Rockville, MD; 3Janssen Research & Development LLC, Raviraj, NJ.

Duvortuxizumab (also known as JNJ-64052781 and MGD011) is a bispecific CD19 x CD3 DART® molecule designed to simultaneously target CD19-positive cells for recognition and elimination by CD3-expressing T-lymphocytes as ef- fector cells. Duvortuxizumab is currently in clinical development for the poten- tial treatment of B-cell malignancies. Here we report the results from a transla- tional PK model that utilized duvertuxizumab pharmacokinetic (PK) data from cynomolgus monkeys to predict duvortuxizumab PK in humans. The PK of duvortuxizumab administered by intravenous infusion was evaluated in cyno- molgus monkeys in two separate studies. Study 1 evaluated intra-animal escala- lating doses from 0.5 to 100 µg/kg or repeated doses from 0.005 to 0.5 µg/kg administered over a period of up to 4 weeks. Serum concentrations of duvortuxiz- umab above the lower limit of quantification were obtained at dose levels >0.5 µg/kg. Study 2 evaluated duvortuxizumab doses of 0.2, 2, 5, or 10 µg/kg administered once weekly for 4 weeks. Dose-proportional increases in maxi-
Pharmacokinetic studies of CASC-578 were conducted in mice, rats, and onlyaslightinductionofCYP3A4wasseenat10uMdrugconcentrationin
humans and no significant differences between male and female animals were observed. PK
modeling analysis, which integrated data from both study 1 and study 2 at 0.2 to
100 μg/kg dose levels, was performed to further understand the PK behavior of
duvortuxizumab in cynomolgus monkeys. Duvortuxizumab PK was reasonably
characterized using a two-compartment PK model with linear clearance (CL) from
the central compartment. Model estimated parameters were CL = 0.797 mL/h/kg;
volume of distribution for the central compartment (V1) = 51.7 mL/kg; intercompartmental clearance (Q) = 2.29 mL/h/kg; and volume of distribution for
the peripheral compartment (V2) = 88.8 mL/kg. Assuming a body weight of
3 kg and 70 kg for a cynomolgus monkey and a human, respectively, human PK
parameters were estimated using an allometric scaling factor of 0.775 for CL and
1.0 for volume in the translational PK model. Observed duvortuxizumab PK
values obtained from an ongoing, first-in-human (FIH), phase 1 dose-escalation
trial in patients with relapsed or refractory B-cell malignancies (NCT02454270)
were used to validate the translational PK model. Comparison of the predicted
and observed duvortuxizumab PK profiles suggested that the translational PK
model using the allometric scaling method reasonably predicted duvortuxi-
zumab PK profiles in humans at multiple dose levels (15 to 100 ng/kg). In con-
clusion, the developed translational PK model successfully predicted duvortuxi-
zumab PK in humans and has been used to aid dose escalation of
duvortuxizumab in the ongoing FIH study. This work showcases the potential
of translational PK modeling in supporting the selection of a FIH dose escalation strategy utilizing preclinical PK information.

**#4090 Preclinical pharmacokinetics of CASC-578, a novel, selective, po-
tent, and orally bioavailable small molecule checkpoint kinase 1 inhibitor.**
Dina Leviten,1 Teresa Sierra,1 Ashley Dozier,2 Richard Boyce,3 Bob Boyle,3 Scott
Peterson,4 Alex C. Vo1.1 Cascadian Therapeutics, Seattle, WA; 2Sentinel Oncol-
yogy, Cambridge, United Kingdom.

Introduction: Checkpoint kinase 1 (Chk1) is a serine/threonine protein ki-
nase that regulates cell division in response to genotoxic stress by arresting cell
cycle progression in the S & G2 phases. Pharmacological inhibition of Chk1 is
proposed to selectively uncouple the completion of DNA replication from G2/M
phase transition in tumor cells that have impaired DNA damage response net-
works, resulting in mitotic catastrophe and cell death. CASC-578 is a novel small
molecule inhibitor of Chk1 that is selective, highly potent and orally bioavailable
in multiple preclinical species. Methods: The in vitro ADME properties of
CASC-578 were evaluated, including in vitro intrinsic micromolar clearance.
Caco2 permeability, plasma protein binding and stability, blood to plasma par-
titioning, cytochrome P450 inhibition and induction, and transporter inhibi-
tion. Pharmacokinetic studies of CASC-578 were conducted in mice, rats, and
cynomolgus monkeys for 5-7 days. Results: CASC-578 is highly bound in
plasma protein across all species. The apparent permeability is high in Caco2
bi-directional transport study and correlated well with a rapid absorption profile
observed in vivo. CASC-578 is not a substrate of P-glycoprotein (efflux = 1).
There was no direct or time dependent inhibition on human CYP450 enzymes
and only a slight induction of CYP3A4 was seen at 10 μM drug concentration in
a transporter cell-based induction assay. In all animal species, CASC-578 exhib-
ted species-dependent systemic clearance resulting from both phase I and phase
II metabolism, and a moderate to high volume of distribution. The elimination
kinetics appeared to be monophasic. Oral bioavailability was high in all species
studied (>50%). There was no significant difference in the pharmacokinetics
of the drug between genders. Maximal plasma concentration and total drug
exposure (AUC) appeared to be proportional from repeat dose studies. Overall,
CASC-578 has very desirable drug-like properties and ideal pharmacokinetics
for an oral once daily drug, and represents a suitable candidate for clinical de-
velopment as a novel potential therapeutic approach for the treatment of solid
and hematological malignancies.

**#4093 t-Darpp stimulates protein kinase A activity by interacting with its
RI regulatory subunit.** Dirk Theile,1 Shuhui Geng,1 Erin Denny,1 Jamil Mo-
mand,1 Susan E. Kane 1.1 City of Hope, Duarte, CA; 2California State University,
Los Angeles, Los Angeles, CA.

t-Darpp is the truncated form of the dopamine- and cAMP-regulated phosphoprotein of 32 kDa (Darp-32) and has been demonstrated to confer resis-
tance to trastuzumab, a Her2-targeted anticancer agent, via sustained signaling through the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Akt path-
way. t-Darpp over-expression results in enhanced activity of protein kinase A (PKA), but the mechanism of t-Darpp-mediated PKA activation is poorly
understood. In the PKA holoenzyme, when the catalytic subunits are bound to
regulatory subunits RI or RI, kinase activity is inhibited. We investigated PKA activity and holoenzyme composition in cell lines that express t-Darpp
(SK.686p) or a 775A phosphorylation mutant (SK.686p.T75A), as well as an

**EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Mechanistic Understanding of Novel Anticancer Therapies**

**Molecular Targeted Therapies 2**

**#4091 AKT can modulate the in vitro response of HNSCC cell to irrevers-
ible EGFR inhibitors.** Renato José Silva-Oliveira,1 Matias Melendez,1 Olga
Martínez,2 Maicon Fernando Zanon,3 Luciano de Souza Viana,4 André Lopes
Carvalho,1 Rui Manuel Reis1.1 Barretos Cancer Hospital, Barretos, Brazil; 2Life
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Epidermal growth factor receptor (EGFR) is overexpressed in up to 90% of head
and neck squamous cell carcinoma (HNSCC) tumors. Cetuximab is the first tar-
geted (anti-EGFR) therapy approved for the treatment of patients with locally ad-
vanced HNSCC. However, its efficacy is limited due to primary and secondary re-
sistance, and there is no predict biomarkers of response. New generation of EGFR
inhibitors with pan HER targeting and irreversible action, such as afatinib and al-
latinib represents a significant therapeutic promise in HNSCC treatment. In this
study, we intend to compare the potential cytotoxicity of two irreversible anti-EGFR
inhibitors (afatinib and allatinib) with cetuximab and to identify potential predictive
biomarkers of response in a panel of HNSCC cell lines. The mutational analysis in
hotspot regions of EGFR, KRAS, NRAS, PIK3CA and PTEN in the eight HNSCC
cell lines and the PIK3CA and PTEN transcriptome profiles in the HN13 cell lines,
and KRAS mutation in the JHU-28 cell line. According to the growth inhibition score (GI), allatinib was the most cytotoxic drug, followed by afatinib and
finally cetuximab. The HN13 cell line exhibited a less responsive behavior to all
drugs assessed, and interestingly, also displayed the higher levels of AKT phosphor-
ylation. Therefore, we further performed drug combinations and found that with
anti-AKT agent (MK2206), afatinib and allatinib, but not cetuximab, sensitivity was
restored. Additionally, AKT1 gene editing in afatinib and allatinib-treated cell lines
showed that AKT1 silencing-induced cell line decreased viability and increased cy-
totoxicity through caspases 3/7/activation. Additionally, in silico analysis of TCGA
database showed that AKT1 overexpression was present in 14.7% (41/279) of HN-
SCC cases, and was associated with perinuclear invasion in advanced stage. In con-
cclusion, allatinib presented a greater IC50 cytotoxic profile when compared to
cetuximab, and the AKT pathway constitutes a predictive marker of allatinib re-
sponse. Importantly, in allatinib and afatinib-resistant cases, the pharmacological
combination with AKT inhibitors could restore response and increase treatment
success.

**#4092 Targeting compensatory mechanisms of resistance to phosphati-
dylinositol 3-kinase inhibitors in head and neck squamous cell carcinoma.**
Nicola L. Michmerhuizen, Elizabeth Leonard, Susan K. Foltin, Aditi Kulkarni,
Thomas E. Carey, Carol R. Bradford, Hui Jiang, Chad Brenner. University of
Michigan, Ann Arbor, MI.

Recent sequencing studies of head and neck squamous cell carcinomas (HN-
SCCs) have identified the phosphatidylinositol 3-kinase (PI3K) pathway as the
most frequently mutated, oncogenic pathway in this cancer type. Despite the frequency
of activating mutations or amplification in PIK3CA (the gene encoding the catalytic
subunit of PI3K), targeted inhibitors of PI3K have not shown clinical efficacy as
monotherapies. We tested a panel of more than 20 patient-derived oral cavity squa-
mos cell carcinoma (OCSCC) cell lines, which were profiled by Nimblegen V3
exome sequencing, and observed resistance to PI3K inhibitors despite PIK3CA copy
number amplification and/or mutation. Of six inhibitors tested, only alpha-isoform
selective and pan-PI3K agents were somewhat effective; these inhibitors, despite
on-target and downstream activity, did not cause an appreciable reduction in cell
viability when administered at submicromolar concentrations. We hypothesized
that other oncogenic pathways might still be functional in the presence of PI3K
inhibitors and might serve as mediators of this resistance. For example, our group
and others have evaluated co-dependence on the Ras-MEK-ERK pathway in OC-
SCC. We found that in a panel of PI3K inhibitor-resistant cell lines, treatment
with A-443638, a selective and pan-PI3K inhibitor, restored sensitivity to
afatinib. Additionally, the PI3K inhibitor vatalanib also restored sensitivity in
these cell lines. To confirm these findings and determine whether PI3K inhibitors
are good targets in PI3K-sensitive cell lines, we performed a high-throughput
screening approach that utilizes a resazurin cell viability assay. This screen
seems an unbiased means of testing ~1400 inhibitors as monotherapies and in
combination with alpha-isoform selective PI3K inhibitor HS-173 in several OC-
SCC cell lines. Further validation of "hits" from this screen, which are drugs that are
effective in combination but not

**EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Mechanistic Understanding of Novel Anticancer Therapies**

**Molecular Targeted Therapies 2**
empty vector control cell line (SK-empty). We also evaluated protein-protein interactions between t-Darp and PKA catalytic (PKAc) or regulatory subunits RI and RII in these cell lines. Whereas SKt-Darp cells had elevated PKA activity, SKt-Drap.T75A cells did not. In agreement with the effects on activity, a proximity ligation assay showed that SKt-Drap cells, but not SKt-Drap.T75A cells, had diminished interaction with PKAc. Moreover, t-Darp was seen to bind directly to RI in co-immunoprecipitation studies. Cells that express both Darp-32 and t-Darp had PKA activity levels and RI-PKAc association that were similar to control SK-empty cells. This is consistent with our published findings that Darp-32 reverses t-Darp’s effects on trastuzumab resistance and CREB DNA binding activity (downstream of PKA). Taken together, our data suggest that inhibition of this interaction is crucial for t-Darp-mediated bortezomib-induced apoptosis. This activation and this activation appears to occur through direct binding to RI and release of RI from PKAc. The t-Darp-RI interaction could be a druggable target to reduce PKA activity in drug-resistant cancer.

#4094 Accumulation of hepatoma up-regulated protein in prostate cancer cells inhibits bortezomib-induced apoptosis. Mohamed Hassan,1 Aboulouhid Elkhattouti,2 Nasir Butt,2 Ingrid Espinoza,2 Christian Gomez2.1 Tulane University, New Orleans, LA; 2Univ. of Mississippi Medical Ctr., Jackson, MS.

The increased understanding of cellular signaling pathways in the regulation of tumor progression and resistance, together with the identification of key molecular markers has allowed for considerable advances in the establishment of therapies for therapy-resistant Prostate cancer (PCa). Hepatoma Up-Regulated Protein (HURP), is overexpressed in several tumors, and has been identified as a potential chaperone protein with tumorigenic capacity. We previously reported on HURP as an essential modulator of PCa resistance to radiation therapy. Bortezomib, a proteasome inhibitor used clinically for multiple myeloma, has been shown to induce apoptosis in PCa cells. In the present study, we evaluate HURP’s role in PCa resistance pathways by analyzing its effects on bortezomib-induced apoptosis. LNCaP-Tet-On-HURP, DU145-Tet-On-HURP, and PC3-Tet-On-HURP clones were established for the expression of HURP. HURP expression was induced with doxycycline (Dox) for 48 h. Next, cells were treated with bortezomib (50 nM) for 24 h. To assess the effect of HURP on bortezomib-induced apoptosis, the cells were harvested and subjected to flow cytometry (FC) analysis using annexin V/PI staining. Apoptosis was observed in control cells (-Dox) following the treatment with bortezomib. Induction of HURP expression (+Dox), blocked bortezomib-induced apoptosis. This effect was observed only in LNCaP- and DU145-Tet-On-HURP cells with a 1.8 and 2.1 fold decrease in apoptosis, respectively; but not in PC3-Tet-On-HURP cells. In addition, we performed FC analysis of DHR123 to analyze the effects of HURP on bortezomib-induced accumulation of reactive oxygen species (ROS). HURP blocked bortezomib-induced ROS accumulation in LNCaP- and DU145-Tet-On-HURP cells with a 0.3 and 0.15 fold change, respectively. Bortezomib also induced the expression of p53 and Noxa, and enhanced the cleavage of PARP in LNCaP- and DU145-Tet-On-HURP control cells (-Dox), but not in (+Dox) PC3-Tet-On-HURP cells. Induction of HURP, however blocked effects on apoptosis effectors in LNCaP- and DU145-Tet-On-HURP. Western blot analysis also demonstrated that in HURP-expressing cells, bortezomib-inhibited the phosphorylation of AKT, JNK or PKC. Finally, t-Darp expression on the cytoplasmic localization of Noxa, a mediator of bortezomib-induced apoptotic signaling by immunocytochemistry. Noxa staining was weakly detectable or absent in HURP expressing cells, suggesting the ability of HURP overload to block bortezomib-induced Noxa expression. Overall, our results show that HURP-induced resistance of PCa cells to bortezomib is attributed to the effects of this protein on mechanisms related to a non-specific overload response leading to the activation of ubiquitin system. Funding: PCRP W81XWH-14-1-0151(CRG), UMMC Department of Pathology (MH)

#4095 c-Met activation mediates resistance to polo-like kinase 1 inhibitor-induced apoptosis in non-small cell lung cancer. Ratnakar Singh, Li Shen, Pan Tong, Jing Wang, Faye M. Johnson. MD Anderson Cancer Center, Houston, TX.

Background: Inhibition of polo-like kinase 1 (PLK1) may be an effective treatment for non-small cell lung cancer (NSCLC). PLK1 inhibition may be an effective treatment for non-small cell lung cancer (NSCLC). PLK1 is a key regulator of mitosis and DNA damage checkpoints. PLK1 inhibitors are well tolerated, but only a few unselected patients with NSCLC respond to single-agent therapy. However, predictive biomarkers have not been used to select patients who are likely to experience a response to PLK1 inhibitors, and the mechanisms of resistance to PLK1 inhibitors have not been elucidated, making these unknowns a major gap in knowledge. To address this gap, we compared basal gene and protein expression in 63 NSCLC cell lines and discovered that mesenchymal NSCLC cell lines were more sensitive to PLK1 inhibitors than epithelial cell lines in vitro and in vivo. The induction of apoptosis in some NSCLC cell lines at very low drug concentra-
however, ibritinib resistance inevitably develops. Once patients relapse after ibritinib treatment, the 1-year survival rate is only 22%; therefore, there is an urgent unmet need to overcome ibritinib resistance and to study alternative treatment options. Constitutive NF-κB activation is the hallmark of MCL. Indeed, next generation sequencing analysis of 110 MCL patient samples revealed NF-κB activation was present in 80 (72.7%), including those with high mutation rates of MET (28.8%), indicating the significant contribution of NF-κB signaling to MCL malignancy. Mucoса-associated lymphoid tissue transformation protein (MALT1) plays a crucial role in NF-κB signaling. MALT1 is a unique paracaspase within the human genome, and the proteolytic activity of MALT1 has been found to be constitutively active in many MCL samples, suggesting MALT1 may be a potential target of all target drugs in this context. Moreover, a specific inhibitor of MALT1 and its efficacy and safety are currently being evaluated in a clinical trial with ABC-type diffuse large B cell lymphoma patients. Mice treated with M2-1 did not have detectable physiological, histological or biochemical signs of toxicity. However, whether MALT1 activity contributes to ibritinib resistance and whether targeting MALT1 can overcome ibritinib resistance in refractory MCL patients remain unclear. In this study, we found that both canonical and non-canonical NF-κB signaling is activated in ibritinib-resistant MCL cells, which correlates with constitutive MALT1 activity. Interestingly, we found that MALT1 is highly mutated in four clusters, including the death domain, TRAF6-binding site, Caspase-like domain, and IKKγ-binding site in MCL samples. Occurrence of L799P, K80R, E519D, L445P and N446S was also strongly correlated with resistance in resistant cell lines and disease progression, which requires more detailed investigation. Treatment with M2-1 significantly reduced cell viability in several MCL cell lines with nanomolar activity. MI-2 treatment inhibited NF-κB activation, IL-6 production and its downstream STAT3 activation. Combining MI-2 with ibritinib resulted in synergistic growth inhibition in both ibritinib-resistant MCL cell lines and primary MCL cells. These findings suggest that targeting MALT1 catalytic activity in MCL is a promising therapy to overcome ibritinib resistance in relapsed/refractory MCL patients. The effect of MI-2 in vivo PDX models is currently under investigation. This work and follow-up in vitro and in vivo studies will provide strong evidence that targeting MALT1 with MI-2 may be an effective novel therapeutic approach to overcome ibritinib resistance.

4100 Mechanisms of resistance to type I and type II MET inhibitors in non-small cell lung cancer. Magda Bahcall, Yanan Kuang, Cloud P. Pawelczk, Pasi A. Jänne. Dana Farber Cancer Institute, Boston, MA.

Background: MET targeted therapies are clinically effective in MET amplified and MET exon 14 deletion mutant non-small cell lung cancers (NSCLC). At least 8 MET tyrosine kinase inhibitors (TKIs), including both type I and II, have been developed and are under clinical evaluation. We recently described a resistance mechanism in a patient with a unique MET secondary mutation, D1228V, resistant to all type I but sensitive to type II MET TKIs (Bahcall et al. Cancer Discovery 2016). We examined whether the sensitivity and resistance of other MET secondary mutations were similarly dependent on the mode of inhibitor binding, and sought to determine the optimal sequence of MET inhibitor use, so as to achieve the longest combined latency before the emergence of resistance. Methods: TPR-MET Ba/F3 cells were mutagenized with ENU and treated with each of the type I TKIs - crizotinib, savolitinib; capmatinib, or type II TKIs - cabozantinib, glesatinib; metrestinib, until resistant clones emerged. Resistant mutations were identified by sequencing, constructed in the TPR-MET background, and expressed in Ba/F3 and NIH3T3 cells. Cross-resistance to the 6 MET TKIs was evaluated. Next, low frequency (0.1%) of mutant Ba/F3 cells was spiked into parental TPR-MET Ba/F3 cells and sequentially exposed to different MET TKIs to identify the sequence associated with the longest combined time to resistance. Clonal evolution was assessed by droplet digital PCR (ddPCR). Results: Of 300 plated wells per drug used at either 0.1μM or 0.5μM, crizotinib yielded 210 and 2 clones; savolitinib 51 and 1 clone; capmatinib 9 and 3 clones; cobozantinib 38 and 3 clones; glesatinib 300 and 300 clones; metrestinib 18 and 3 clones, respectively. Of the mutations, those at Y1230 were common to all 3 type II TKIs and the most frequently seen with savolitinib and capmatinib. D1228 was shared by savolitinib and capmatinib; V1155, the most common mutation with crizotinib, was shared with savolitinib; M1211 L emerged with capmatinib. Mutated F1200 residue was shared by and exclusive to all type II TKIs and was the single identified mutation for both glesatinib and metrestinib. Cobozatinib gave rise to a broader array of unique mutants - with F1200 mutations being the most common and shared G1163R and H1248Y with crizotinib. With D1228 and Y1230 mutations being moderately resistant to crizotinib and strongly resistant to both cobozatinib and glesatinib, while retaining sensitivity to all type II TKIs. G1163R and L1195V were slightly to moderately resistant to crizotinib, cobozatinib and glesatinib, but strongly sensitive to savolitinib and capmatinib. In
this assay, merestinib had the broadest, while crizotinib most narrow activity with EGFR inhibitors such as afatinib in vitro significantly suppressed cell viability by 85.2% and 80% in 201T and H1975 respectively, as compared to CS3M. CS3D also caused a 2-fold reduction in expression of STAT3-target genes c-Myc, Bcl-xl, and IL-6. Compared to CS3M, CS3D inhibited colony formation by 70%. Using an in vivo mouse xenograft model of 201T and H1975, CS3D caused a 96.5% reduction in tumor growth in 201T (P < 0.001) compared to CS3M, while an 81.7% inhibition was seen in H1975 (P < 0.0001). Utilizing IHC, analysis of residual tumors also illustrated that CS3D induced more caspase3 cleavage relative to CS3M. Additionally, western blot analysis showed 70% reduction in c-Myc protein level in response to CS3D. These results suggest CS3D can be effective as a single therapeutic agent. Combining CS3D with EGFR inhibitors such as afatinib in vitro significantly suppressed cell viability by 85.2% and 80% in 201T and H1975 respectively, as compared to single treatment (CS3D alone reduced cell viability by 53% and 51.5%, and afatinib alone reduced viability by 31.4% and 34.63% in 201T and H1975 respectively). The combination of CS3D and afatinib warrants further testing in vivo. These data suggest that CS3D alone or in combination with EGFR tyrosine kinase inhibitors produces anti-tumor effects in NSCLC with intrinsic and acquired resistance to EGFR TKIs. Supported by funding to the Masonic Cancer Center from the Minnesota 5th Order of Eagles.


Aberant signalling of the epidermal growth factor receptor (EGFR) plays an integral role in the tumorigenesis of many cancer types, including head and neck squamous cell carcinoma (HNSCC), making it a compelling drug target. After the initial promising results of EGFR-targeted therapies, the problem of therapeutic resistance is emerging and new treatment options are necessary. In contrast to the first generation EGFR inhibitors, afatinib is an irreplaceable ErbB family blocker that inhibits EGFR as well as HER2 and HER4. The objective of this study was to investigate whether afatinib is able to overcome intrinsic and acquired cettuximab resistance in human papillomavirus (HPV) positive and negative HNSCC cell lines under normal and reduced oxygen conditions and to identify the molecular mechanisms underlying afatinib's cytotoxic effect. Sensitivity to cettuximab treatment was investigated in a panel of HPV positive and negative HNSCC cell lines using the colorimetric sulforhodamine B (SRB) assay (168h, 0-2 μM). Cettuximab sensitive and intrinsically resistant cell lines were identified. Acquired cettuximab resistant cell lines were generated by chronically exposing initially sensitive cell lines to cettuximab. In parallel, control cell lines were established by exposing these cells to the vehicle control (PBS). Cytotoxicity of atafinib (24h, 0 - 5μM) was assessed under normoxic and hypoxic (1% O2) conditions. Cell cycle distribution and induction of apoptotic cell death were assessed flow cytometrically using the Vindelov method and Annexin V-FITC/PI assay, respectively. Data analysis was performed using WinNonlin and FlowJo software. We identified intrinsic cettuximab resistance in 5 out of 7 HNSCC cell lines (i.e. LICR-HN1, Cal-27, SQ99, 93-VU-147T and UM-SCC104) and generated acquired cettuximab resistant cell lines (i.e. SCC22B-R and SC263-R). Afatinib showed a clear concentration-dependent cytotoxic effect in both cettuximab sensitive and resistant cell lines with IC50 values ranging between 0.019 and 4.04 μM. Furthermore, afatinib maintained its cytotoxic effect under hypoxic conditions. Treatment with afatinib led to an increase in the proportion of cells in the G0/G1 phase of the cell cycle. Afatinib also induced an increase in the percentage of AAnV-/+PI- and AAnV+/+PI+ cells with a corresponding decrease of the percentage viable (AAnV-/+PI-). In conclusion, our results suggest that afatinib has the potential to overcome cettuximab resistance, as it was able to establish cytotoxicity in HPV positive and negative HNSCC cell lines that were intrinsically and acquired resistant to cettuximab. Furthermore, treatment with afatinib caused a G0/G1 cell cycle arrest and induced apoptotic cell death. These data support the hypothesis that afatinib might be a promising novel therapeutic strategy to treat HNSCC patients experiencing intrinsic or acquired cettuximab resistance.

IL-6 dynamics regulate neuroendocrine transformation in gefitinib resistant acquired resistance EGFR mutant lung cancer cells. Shang-Yin Wu,1 Hsuan-Heng Yeh,2 Chun-Hua Hung,3 Chien-Chung Lin,1 Wen-Pin Su1,2 Wu-Chou Su2. Graduate Institute of Clinical Medicine, Department of Internal Medicine, National Cheng Kung University, TAINAN, Taiwan; 3Department of Internal Medicine, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, TAINAN, Taiwan.

Transformation to small-cell lung cancer (SCLC, one of aggressive neuroendocrine (NE) tumor) is reported when activating epidermal growth factor receptor (EGFR) mutant non-small-cell lung cancer acquired resistance to tyrosine kinase inhibitors (TKI, such as gefitinib). IL-6 activation confers to TKI resistance and associates with p53 and RB inactivation those are SCLC hallmark changes. Whether NE transformation could phenocopy in isoegenic acquired resistance cell line and the role of IL-6 in this process remain unknown. We established 827GRs (including 827GR, 827GR-M and 827GR-M6) acquired resistance to gefitinib from HCC827 cells by long term stepwise treatment with gefitinib and they still had EGF receptor exon 19 deletion without acquired T790M. 827GR was parental resistance line with unstable gefitinib resistance in drug-free medium by passage. We maintained 827GR in medium with or without 1μM gefitinib over 6 months to generate stable clones: 827GR-M and 827GR-M6. 827GRs had SCLC hallmark changes, i.e., inactivation of p53, RB and Notch by western blot and gene set enrichment analysis. Compared to HCC827, 827GRs were more sensitive to cisplatin and etoposide but not paclitaxel. IL-6 level was positive correlated with gefitinib resistance among 827GRs by cytokine array and ELISA. Interestingly, among 827GRs, 827GR-M6 harbored low IL-6 secretion had obviously NOTCH-ASCL1-DLL3 alteration, high NE marker expression and significant inter-rater agreement with selected Byers' SCLC gene signature. Compared to high IL-6 secreting clones, 827GR-M6 highly regulate NE marker expression. IL-6 genetic manipulation in HCC827 and 827GRs also demonstrated this phenomenon. Moreover, IL-6 dynamics correlate with NE expression also showed in patient derived lung cancer cell line in published microarray dataset (GSE64322). In conclusion, our work demonstrated activating EGFR mutant lung cancer acquired resistance to TKI with NE transformation could phenocopy in isoegenic cell line model and IL-6 dynamics might regulate this process.
formed retrospective genomic profiling of 25 CRC patients treated at Samsung Medical Center from 2006-2015. Patients received cetuximab containing chemo regimens with varying duration of responses, including acquired resistance cases. Our analysis identifies mutations in receptor tyrosine kinases, such as EGFR, NTRK1, and PDGFRα, as well as RAS/MAPK pathway genes that affect cancer cell survival and resistance to EGFR/ERBB2 and ERBB3/ERBB4, as potential novel mechanisms regulating sensitivity to anti-EGFR antibodies. Additional genomic analyses of acquired resistance tumors and in vitro studies of a patient-derived cell line provide added insights into clonal selection and signaling pathways that bypass the EGFR blockade. Overall, our study elucidates important new facets in the landscape of anti-EGFR resistance mechanisms.


Introduction: Glioblastoma multiforme (GBM) accounts for the most aggressive form of tumor showing poor prognosis. Prevailing treatment modality includes chemotherapy with temozolomide (TMZ) concomitant with surgical resection and/or irradiation. However, it has been observed that a number of patients are developing resistance to TMZ owing to its high dosage regimen. The aim of this study is to examine the effects of diosgenin (DSG), a natural steroidal saponin obtained from fenugreek, in combination with TMZ in human GBM cells and TMZ resistant GBM cells. Methodology: The potential of combinatorial chemotherapy for overcoming TMZ resistance was evaluated through development of TMZ resistant GBM clones. These clones were generated by treatment of GBM cells with sub-lethal dose of TMZ over several cycles. Cellular effects were studied by viability assay, flow cytometry and wound healing assay on both single and combined drug treated GBM cells and TMZ resistant cells, respectively. The morphological study and cellular uptake in combined or individual drug treated cells was assessed by microscopy and immunofluorescence staining. The time dependent effect of drugs on invasive and migratory potential of GBM cells and TMZ resistant cells was analysed through zymography for matrix metalloproteinases (MMPs) activity and western blot for apoptotic proteins, epithelial and mesenchymal (EMT) markers, MMPs and VEGF expression. Further, the drug induced apoptosis was also assessed through chromatin condensation and DNA ladderization assay. Results: Our investigation shows that co-administration of DSG and TMZ resulted in a substantial increase in GBM cell apoptosis and marked inhibition of cell growth in vitro. Anti-angiogenic and anti-invasive potential of DSG and TMZ were assessed through in vitro studies. At molecular level, DSG and TMZ synergistically lower XIAP expression and cleavage of intracellular death substrates such as PARP thereby shifting the balance from survival to apoptosis as indicated by the rise in the sub-G1 cell population. This combination also alters EMT markers, downregulates the expression of Bcl-xL, Bcl-2, Mcl1, MMPs and VEGF and induces expression of Bax, AIF, cytochrome C. Conclusion: The results suggested that DSG in combination with TMZ reduced the dose of TMZ, when administered singly, and also inhibited the migration and invasion of GBM and TMZ resistant cells. It induced apoptosis and altered the expression of EMT markers. These findings reveal a new therapeutic potential for overcoming TMZ resistance in GBM. This novel modality may be a promising tool for GBM treatment.


Background: Soft tissue sarcomas are a heterogeneous group of malignant tumors including more than 70 histological subtypes. We have recently demonstrated that dual targeting of PI3K/mTOR pathway is associated with significant anti-tumor activity in vitro and in vivo models of leiomyosarcoma (LMS), one of the most frequent sarcoma subtype. Secondary resistance limits the efficacy of all targeted therapies. We have developed and characterized resistance models to a dual PI3K/mTOR inhibitor in order to describe underlying resistance mechanisms and develop alternative strategies. Methods: To develop in vitro BEZ-235 (dual PI3K/mTOR inhibitor) resistance models, 3 LMS and 1 myofibrosarcoma cell lines were exposed to increasing doses of BEZ-235 several months. To characterize the resistance models, sensitivity to BEZ-235 was assessed by MTT assay and Annexin V-PI staining. To test in vivo the resistance models, effects of BEZ-235 treatment were observed in mice with subcutaneous cell-line derived xenograft tumors for 3 weeks. The transcriptome profile of sensitive and resistant tumors to BEZ-235 was analyzed by using RNA-seq. Results: After prolonged exposure of cell lines to BEZ-235, we obtained secondary resistant cell lines characterized by an IC50 value 5- to 21-fold higher than parental sensitive cells. Resistant cells were also significantly less sensitive to BEZ-235 induced apoptosis than parental cells. We confirmed the resistance patterns in vivo with a significantly higher anti-tumor effect of BEZ235 in sensitive cell-line derived xenograft group compared with resistant cell-line derived xenograft group. Deep transcriptome profiling indicated overexpression of stem cells related genes in resistant cells as well as re-activation of the cellular metabolism. By using 1) RNA-mediated suppression of pyruvate carboxylase (PC), a potential key player in our resistance models and 2) hallmarks of CSC like CD133 and enhanced aldehyde dehydrogenase (ALDH) activity, we confirmed that resistant tumors were characterized by a significantly higher proportion of cancer stem cells and by a metabolic shift with a strong deregulation of glucose metabolism. Conclusions: Selection of CSC subpopulation and metabolism shift play a crucial role in secondary resistance to dual PI3K/mTOR inhibition in sarcoma. Strategies assessing anti-CSC agents to suppress acquired resistance may have major implications to improve efficacy of dual PI3K/mTOR targeting in human malignancies.

#4107 Acquired resistance to EGFR-TKI in an uncommon G719S EGFR mutation. Atsushi Osogawa, Tomonori Yamada, Yahi Kihumi, Takefumi Ohara, Atsushi Osoegawa, Tatsuro Okamoto, Kenji Sugio. Oita University Faculty of Medicine, Oita, Japan.

Background: Acquired resistance (AR) to EGFR-TKI is a common event and several underlying mechanisms, including T790M, MET amplification and PTEN downregulation have been reported for the common EGFR mutations: Deletion 19 and L858R. An EGFR G719S mutation is an uncommon mutation that was reported only by a few groups to confer acquired resistance to EGFR-TKIs. In this study, we report our experience of 1 patient and experiments using transformed cultured cells. However, no established lung cancer or resistant cell lines harboring the EGFR G719X mutation have been reported in the literature. We established a lung adenocarcinoma cell line (G719S-GR) from the malignant pleural effusion of a patient whose tumor developed acquired resistance from initial treatment with gefitinib. Materials and methods: G719S-GR cells were established and maintained in RPMI1640 medium supplemented with 10% FBS and 10 μM ROCK inhibitor (Y-27632, Wako). The ROCK inhibitor was removed from the medium for the following experiments. Cell growth inhibition was examined with gefitinib and afatinib using CellTiter-Glo (Promega), and a comprehensive genomic analysis was performed using hybrid capture-based NGS (NCC oncopanel, Agilent; MiSeq, Illumina) for G719S-GR and MLPA (Salsa, MRC-holland) was used for the analysis of clinical tumor samples. Results: A cell growth inhibition test revealed EGFR-TKI resistance in G719S-GR cells with an LC50 of more than 100 μM for either gefitinib or afatinib, indicating that the G719S-GR cells are also resistant to EGFR-TKI in vitro. The NGS analysis showed that G719S-GR cells harbor EGFR mutations (G719S and E709A) as well as the amplification of EGFR, IL7R, MET, and the EGFR locus. The homozygous deletion of PTEN and the loss of PTEN and TSC1 were also detected. In order to estimate the mechanism underlying the development of EGFR-TKI resistance, copy number analyses of several tumor suppressor genes were performed by an MLPA using genomic DNA from G719S-GR and a tumor biopsy sample (obtained before gefitinib treatment). Losses of CDKN2A, PTEN and TSC1 were confirmed in G719S-GR cells. However, the GBM tumor collected before treatment with gefitinib was not investigated. Conclusions: The resistance pattern of the G719S cell line is similar to the findings that Pten and Tsc1 inactivation are the major mechanisms of resistance in GBM. The development of EGFR-TKI resistance in uncommon mutations have not been investigated. The newly established G719S-GR cell line could be a useful tool for investigating the mechanism underlying the development of AR in the G719X mutation; the loss PTEN could be such mechanism. Further experiments are warranted.


Head and neck squamous cell carcinoma (HNSCC), the sixth most common cancer worldwide, has a five-year survival rate of only 50%. The first FDA approved agent for treatment of HNSCC was cetuximab, a monoclonal antibody targeting the epidermal growth factor receptor (EGFR). Despite EGFR overexpression in up to 90% of HNSCC tumors and ample evidence supporting EGFR as a therapeutic target in HNSCC, the response rate for single-agent cetuximab is below 20%, and resistance to cetuximab-containing therapy remains a major obstacle in the effective treatment of HNSCC. Identification and targeting of mediators of cetuximab resistance is needed to improve patient outcomes. Secretion of the cytokine interleukin 6 (IL-6) has been proposed as a mechanism of resistance to cetuximab in HNSCC, and inhibition of signal transducer and activator of transcription 3 (STAT3), a downstream mediator of IL-6
signaling, has been shown to overcome cetuximab resistance in preclinical HNSCC models. Thus, we hypothesize that IL-6 signaling mediates resistance to cetuximab and that co-treatment with agents targeting the IL-6 pathway will enhance the therapeutic efficacy of cetuximab in HNSCC. We have generated cetuximab-resistant variants of the cetuximab-sensitive HNSCC cell lines Cal33 and PEA15 using adaptive stress and reverse metabolic investigations. The combination of cetuximab and lapatinib resulted in suppression of cell growth more effectively than either of the single inhibitors alone in vitro and in SCCHN PDX models. Furthermore, we have shown that targeting IL-6 using bispecific antibodies (BAs) and other strategies (eg, IL-6 receptor antagonists) reduces cell growth in cetuximab-resistant SCCHN cells.

**#4109 HER3 mediates acquired resistance to cetuximab in head and neck squamous cell carcinoma.** Yeon Ju Yang,1 Min Hee Cho,1 Yoojung Oh,1 Hyeung Kwon Byeon,2 Da Hee Kim,1 Jung Min Kim,1 Ji Hoon Kim,1 Myun Jeong Ban,1 Jae Wook Kim,1 Eun Chang Choi,1 Yoon Woo Koh,1,1 Yonsei University College of Medicine, Seoul, Republic of Korea; 2Yonsei University College of Medicine, Wonju, Republic of Korea; 3Soochunhyang University College of Medicine, Cheonan, Republic of Korea; 4Soochunhyang University College of Medicine, Seoul, Republic of Korea.

Cetuximab, an EGFR monoclonal antibody, is used to treat in head and neck squamous cell carcinoma (HNSCC). Despite the clinical efficacy of cetuximab, the majority of patients who do initially respond suffered acquire resistance to cetuximab as severe side effect. To understand mechanisms of acquired cetuximab resistance, we developed a model of acquired resitance to cetuximab by exposing head and neck cancer cell line Cal27 to increasing concentration of cetuximab and established cetuximab-resistant (Cr) clones derived from the cetuximab sensitive (Cp). Cetuximab-resistant (Cr) clones showed robustly overexpression on the HER family receptors HER3. Cr clones also expressed upregulated EGFR, HER2, and HER3 activation resulting in activation of P13K/ ATK and ERK signaling. We also showed that Cr clones exhibited increased EGR/HER3 dimerization. Treatment of cetuximab and siHER3 RNA reduced EGR activation in Cr clones and decreased robustly cell proliferation to resis- sitize cells against cetuximab. Next, we examined lapatinib, a tyrosine kinase inhibitor inhibiting HER3 on cetuximab-resistant clones. Combined treatment of Cr clones with cetuximab and lapatinib lead to potent anti-proliferative effects. Co-treatment with cetuximab and lapatinib blocked EGRF, HER2 and HER3 activities and inhibited downstream signaling pathways. Co-treatment with cetuximab and lapatinib resulted in suppression of cell growth more efficiently than each drug alone and induced apoptotic cell death through mitochondrial ROS. Furthermore, Co-treatment with cetuximab and lapatinib also lead to decreased tumor growth in HNSCC tumor xenograft mouse model. These results showed the upregulation of HER3 as one mechanism underlying resis- tance to cetuximab in HNSCC, supporting further clinical treatment strategy for tumors displaying acquired resistance to cetuximab.


Squamous cell carcinoma of the head and neck (SCCHN) cells that escape cetuximab inhibition exhibit HER2, HER3, and/or c-MET activation. Simultaneous inhibition of EGRF, HER2, HER3 and c-Met presents an attractive clinical strategy for treating SCCHN that has progresses despite EGFRTK-targeted therapy. In an effort to develop new combination drug therapy options for recurrent SCCHN, we evaluated three different c-Met inhibitors in combination with a pan-HER inhibitor (afatinib/crizotinib, afatinib/tivantinib, afatinib/cabozan- tinib) and investigated their anti-tumoral effects. SCCHN cell lines (HU202, highly expresses c-Met, and SCC1-C is cetuximab resistant) as well as patient derived xenograft (PDX) animal models from patients with recurrent SCCHN were investigated. In our cell line study, western-blot assay indicated that activi- ation of EGRF, HER2, and c-Met was blocked in all three combinations. In addition the downstream P13K/ATK and ERK signaling pathways were inhib- ited. Sulfordhamin B colorimetric assay (SBR) revealed SCC1-C cell growth was more effectively inhibited by the combinations compared to any of the single inhibitors in vitro. Furthermore, the combination was more potent in inducing apoptosis when compared to each of the single treatments. Finally in the PDX study, all treatment groups had significantly greater efficacy compared to the non-treated control group (p<0.005) and all combination treatments exhibited better efficacy in tumor growth inhibition than their respective single inhibitors (p<0.01). In conclusion, our study demonstrates that targeting EGRF, HER2, and c-Met with combination of their inhibitors is more effective than each of the single inhibitors alone both in vitro and in SCCHN PDX models. This study strongly suggests the development of this combination in patients with SCCHN who have progressed after cetuximab-based therapy. (This study is supported by NIH/NCI R21 CA182662-01A1 to NFS and ZGC).

**#4111 EGFR wild type allele amplification induces acquired resistance to mutation-specific EGFR tyrosine kinase inhibitors in non-small cell lung cancer cells.** Keigo Kobayashi,1 Shigeneri Nukaga,1 Hiroyuki Yasuda,1 Keita Masuzawa,2 Yunko Hamamoto,1 Ichiro Kawada,1 Katsuhiko Naoki,1 Sachiyu Mimaki,2 Shingo Matsumoto,3 Koichi Goto,2 Katsuya Tsuchihara,2 Tomoko Betuyaku,1 Kenzo Sejima,1,1 Keio University, Tokyo, Japan; 2Division of Translational Research, Exploratory Oncology Research and Clinical Trial Center, National Cancer Center, Tokyo, Japan.

Purpose: Third-generation EGFR-tyrosine kinase inhibitors (TKIs) are mutation-specific inhibitors that generally have minimal effects on wild type protein. The mechanisms underlying acquired resistance to third-generation EGFR-TKIs remain relatively uncharacterized. The purpose of this study is to clarify the mechanisms of acquired resistance to third-generation EGFR-TKIs in order to improve the prognosis of lung cancer patients harboring EGFR mutations. Experimental design: We established third-generation EGFR-TKIs resistant cells using a dose-escalation method. The PC9 (EGFR E746-A750del) and H1975 (EGFR L858R/T790M) human NSCLC cell lines were cultured with crizotinib (CO-1686) or osimertinib (AZD9291) for several months. Their resis- tance to a third-generation EGFR-TKIs was confirmed by the MTS cell pro- liferation assay. To clarify the heterogeneity of potential resistance mechanisms, those resistant cell lines were subcloned to isolate resistant clones. DNA isolated from the parental and resistant cell clones was then subjected to whole exome sequencing. Copy number alterations and several genetic alterations potentially relevant to EGFR-TKIs sensitivity were detected. Results: We identified Src-AKT pathway contribute to acquired resistance. In addition, we identified that EGFR wild type allele, but not mutation allele, amplification induced acquired resistance. It mirrors the decreased inhibitory pressure for wild type EGFR by mutation-specific EGFR-TKIs. Conclusion: We provide evidence of wild type allele-mediated resistance, a novel concept of acquired resistance, occurring in response to mutation-specific inhibitor therapy in cancer treatment. 1833 /2600 characters

**#4112 Overgrowth of competing resistance mechanisms, such as an ac- quired KRAS mutation, underlies a poor prognosis subtype of acquired re- sistance to osimertinib in T790M-positive NSCLC.** Geoffrey R. Oxnard,1,2 Yuqiao Hu,1 Philip Tracy,1 Nora Feeney,1 Cloud P. Paveletz,1 Kenneth S. Thress,2 Pasi A. Jänne1,3 Dana-Farber Cancer Institute, Boston, MA; 2AstraZeneca, Waltham, MA.

Introduction: Osimertinib is a third-generation EGFR tyrosine kinase inhibi- tor (TKI) which is highly active in EGFR-mutant NSCLC with resistance to prior EGFR TKI. Acquired resistance to osimertinib had been observed clinici- anally; an improved understanding of the molecular mechanism of resistance is needed. Methods/Results: We initially studied an institutional cohort of 86 pa- tients (pts) treated with osimertinib for advanced T790M-positive NSCLC. 50 pts had progressed on therapy, of whom 25 underwent a resistance biopsy and 17 had NGS results available. 6 pts maintained the T790M mutation at resistance, of whom 3 also acquired an EGRF C797S mutation. The remaining 11 pts had loss of T790M, of whom 5 had evidence of a competing resistance mechanism: 2 with histologic transformation to SCLC, one with BRAF V600E, one with an EGFR3- TACC fusion, and one with KRAS Q61K. For the final case, we confirmed the acquired KRAS Q61K on therapy using serial plasma genotyping. Time to treat- ment failure (TTF) on osimertinib was 3 months median in pts with loss of T790M and 14 months median in pts with maintained T790M. To test the hypothesis that loss of T790M is a poor prognosis subtype of resistance, we analyzed 127 pts treated for T790M-positive NSCLC on the phase I AURA trial of osimertinib. Plasma drawn after progression was submitted for genotyping using droplet digital PCR. 88 pts had a detectable EGFR driver mutation and were eligible for resistance analysis. 45 pts (51%) had detectable T790M at resistance, 17 (19%) of whom also acquired a C797S mutation; the remaining 43 pts (49%) had loss of T790M and no C797S. Median TTF on osimertinib was 6 months in pts with loss of T790M and 11 months in pts with maintained T790M; among pts with TTF <5 months, 72% had loss of T790M. To study whether baseline plasma genotyping could predict the eventual pattern of resistance on osimertinib, we studied 30 pts from the AURA trial with pretreatment plasma
#4113 Acquired resistance to cetuximab is mediated by HER3 activation in head and neck squamous cell carcinoma. 

Acquired resistance to cetuximab is a significant challenge in the treatment of head and neck squamous cell carcinoma (HNSCC). Cetuximab, an EGFR monoclonal antibody, is commonly known to be an effective treatment agent in head and neck squamous cell carcinoma (HNSCC). However, despite the clinical efficacy of cetuximab, a majority of patients with good initial response still suffer from side effects as the acquired resistance to cetuximab. To understand the mechanisms of acquired resistance to cetuximab, we developed a model by exposing a head and neck cancer cell line Ca27 to increasing concentrations of cetuximab and established cetuximab-resistant clones (CETr) derived from the cetuximab sensitive (CETp). We examined how the acquired resistance in the CETp influenced the signaling pathway compared to the CETr. We investigated the effect of lapatinib, a tyrosine kinase inhibitor inhibiting HER3, with or without cetuximab combination on CETr clones and in a xenograft mouse model. Cetuximab-resistant (CETr) clones showed robust overexpression on the HER family receptors HER3. CETr clones also expressed upregulated EGFR, HER2, and HER3 activation resulting in activation of PI3K/AKT and ERK signaling. We also showed that CETp clones exhibited increased EGFR/HER3 dimerization. Treatment of cetuximab and siHER3 RNA together reduced EGFR activation in CETp clones to re-sensitize cells against cetuximab and robustly decreased cell proliferation. Combined treatment of CETp clones with cetuximab and lapatinib led to potent anti-proliferative effects. Co-treatment with cetuximab and lapatinib blocked EGFR, HER2 and HER3 activities and inhibited downstream signaling pathways. Co-treatment resulted in suppression of cell growth more effectively than each drug alone and induced apoptotic cell death through mitochondrial ROS. Furthermore, Co-treatment with cetuximab and lapatinib also led to suppression of tumor growth in orthotopic xenograft mouse model of oral tongue cancer. Our results suggested the upregulation of HER3 as a mechanism underlying resistance to cetuximab in HNSCC, supporting further clinical treatment strategy for tumors displaying acquired resistance to cetuximab.

#4114 Polyclonal secondary FGFR2 mutations drive acquired resistance to FGFR inhibition in FGFR2 fusion-positive cholangiocarcinoma patients. 

Polyclonal secondary FGFR2 mutations drive acquired resistance to FGFR inhibition in FGFR2 fusion-positive cholangiocarcinoma patients. Lipika Goyal,1 Supriya K. Saha,2 Leah Y. Liu,1 Giulia Siravegna, Ignaty Leshchiner,1 Leanne G. Ahronian,1 Jochen K. Lennerz,1 Phuong Vu,1 Benedetta Mussolin,1 Stephanie Reyes,1 Pascal Fuel,1 A. John Iafriate,1 Gad Getz,1 Diana G. Porta,1 Ralph Tiedt,1 Alberto Bardelli,1 Dejan Juric,1 Ryan B. Corcoran,2 Nabeel Bardeesy,1 Andrew X. Zhu1,2 Massachusetts General Hospital, Boston, MA; 3Fred Hutchinson Cancer Research Center, Boston, MA; 4Candiolo Cancer Center-FPO, Italy; 5Brod Institute, Cambridge, MA; 6Novartis Institutes for Bio-Medical Research, Switzerland.

Genetic alterations in the fibroblast growth factor receptor (FGFR) pathway are promising therapeutic targets in a broad range of cancers and occur in ~20% of ICCs. As seen with other targeted therapies, however, acquired resistance has limited the efficacy of selective FGFR kinase inhibitors such as BGJ398. In a phase II trial of patients with advanced refractory cholangiocarcinoma harboring an FGFR gene alteration, BGJ398 displayed an overall response rate of 22%, but the durability of response was short in some patients. We report the molecular basis of acquired resistance in 4 patients with advanced FGFR2-fusion positive ICCs via integrative genomic characterization of cell-free circulating tumor DNA (cfDNA), the primary tumor, and metastases. Each patient enjoyed an initial response, but all subsequently progressed within 10 months. Serial analysis of cfDNA revealed multiple point mutations in the FGFR2 kinase domain at progression (Table 1). The gatekeeper mutation, p. V564F, sterically hinders drug binding and was identified in 3 of 4 patients. In patient #1, five different FGFR2 mutations were detected in the post-progression cfDNA but only one, p. K641R, was identified in the post-progression biopsy. A rapid autopsy was performed, and genomic characterization of 12 metastatic lesions revealed marked inter- and intra-lesional heterogeneity, with different FGFR2 mutations in individual resistant clones. Molecular modeling and in vitro studies indicated that each mutation lead to BGJ398 resistance that was surmountable by structurally distinct FGFR inhibitors. Thus, our report provides the first genetic evidence of clinical acquired resistance to FGFR inhibitor therapy in patients and informs future strategies for detecting mechanisms of resistance and promoting more durable remissions.

#4115 Combination of FTY720 and tamoxifen inhibits drug-resistant ovarian cancer cell proliferation. 

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Despite satisfactory initial responses to frontline therapies that combine surgical debulking with platinum based chemotherapy, virtually all women with advanced ovarian cancer relapse with drug resistant disease. There is a critical need to identify alternative, effective therapies. Previously, we used patient derived xenograft (PDX) models of ovarian cancer in mice to identify the sphingosine phosphate (S1P) pathway as one of the pathways most affected by two agents frequently used to treat ovarian cancer, carboplatin and paclitaxel. The S1P pathway contributes to multiple biological processes recognized as essential for tumor development, and aberrations in sphingolipid metabolism have been directly correlated with advanced disease and with resistance to chemotherapy and radiation. Further, recent studies indicate that tamoxifen may counteract resistance mechanisms through modulation of sphingolipid metabolism, specifically through inhibition of the ceramide converting enzymes acid ceramidase and glucosylceramide synthase. Therefore, we evaluated the efficacy of modulating S1P metabolism using the sphingosine analog FTY720 and tamoxifen as an approach to inhibit the proliferation of drug-resistant ovarian tumor cells. We used alamarBlue cell proliferation assays to compare the anti-proliferative effect of FTY720 and tamoxifen with the effect of each drug as a single agent. FTY720 + tamoxifen synergistically decreased the cell viability of 3/3 ovarian cancer cell lines: estrogen receptor alpha (ERα) positive SKOV3.T3 (taxane resistant), ERα negative A280.cp20 (platinum resistant) and HeyA8.MDR (taxane-platinum resistant). The combination also increased levels of apoptosis as reflected by Annexin V staining and cleaved caspase 3 levels. Further, administration of 3mg/kg i.p. FTY720 + 20mg/kg p.o. tamoxifen daily for 21 days to PDX-bearing mice demonstrated that FTY720 + tamoxifen suppressed tumor growth by ~60% compared to vehicle controls. Notably, immunohistochemical staining of tumors harvested from treated mice showed that the combination decreased expression of the proliferation marker Ki-67 and increased expression of apoptosis indicators ceramide and cleaved caspase 3 to a greater degree than in tumors exposed to either drug alone. We conclude that FTY720 + tamoxifen merit further investigation as potentially effective agents for the treatment of drug-resistant ovarian cancer.

#4116 Acquired lapatinib resistant tumour induces EMT-phenotype in breast cancer cells via Src and RUNX3. 

So Hyun Kim,1 Ahrum Min,1 Seongyong Kim,1 Dong Hyeon Ha,1 Hyemin Jung,1 Yu Jin Kim,1 Kyung-Hun Lee,2 Tae-Yong Kim,2 Seoek-Ah Im2.

Lapatinib is an example of an agent targeting the epidermal growth factor receptor (EGFR) and the human epidermal growth factor receptor 2 (HER2). In breast cancer, acquired resistance to this and other similar agents is a significant clinical problem. We report lapatinib resistant tumour induced epithelial-to-mesenchymal transition (EMT) phenotype in breast cancer cells via Src and RUNX3. So Hyun Kim,1 Ahrum Min,1 Seongyong Kim,1 Dong Hyeon Ha,1 Hyemin Jung,1 Yu Jin Kim,1 Kyung-Hun Lee,2 Tae-Yong Kim,2 Seoek-Ah Im2. Cancer Research Institute, Seoul National University College of Medicine, Seoul, Republic of Korea; 2Seoul National University Hospital, Seoul, Republic of Korea.

Clinical and Molecular Data on Patients with Advanced Refractory CCA treated with BGJ398

<table>
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<th>Patient ID</th>
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<th>Maximum Response</th>
<th>Progression Free Survival on 3980 (months)</th>
<th>Overall Survival since Diagnosis (months)</th>
<th>FGFR2 Mutations on cfDNA at progression</th>
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Background: Lapatinib is an effective EGFR and HER2 targeting small molecular tyrosine kinase inhibitor which is widely used for HER2 positive breast cancer patients. However, patients eventually acquire resistance, limiting its long-term use. Overexpression of heregulin, a HER3 ligand, acquired stemness were mechanism that confers resistance to the anti-Her2 lapatinib. However, still, unknown of developing lapatinib resistant remain as a question to solve. Thus, we tried to find out there is a novel mechanism which related to lapatinib resistant, and any specified molecules were involved in this process. Methods: Acquired resistant SK-BR-3 cells were established by chronic exposure to lapatinib or trastuzumab. Lapatinib or trastuzumab sensitivity were confirmed by MTT assay. Western blotting was used to determine signal transduction mechanism and association of binding assay. Furthermore, gene expression were conducted for verifying invasive ability. Whole exome sequencing (WES) and siRNA knock-down system were used for further analysis. Results: Lapatinib resistant (LR) cell lines showed down-regulation of p-HER2, p-Akt, and p-Erk. The activity of Src family kinase was increased in LR cells. Vimentin, famous EMT marker, is also increased in LR cells. Migration and invasion were significantly increased in LR cells. Correlated with a missense mutation of RUNX3, which identified by WES, expression of RUNX3 was decreased in LR cells. Moreover, siRNA knock-down parental cells showed more resistance to lapatinib. Conclusion: The increase of Src activation, cell migration, and invasion was observed in LR cells. RUNX3, which identified by WES, affected to lapatinib sensitivity in SK-BR-3 cells. Therefore, RUNX3 might be a specified molecule, which partially contributes resistance to lapatinib.

#4117 Mechanisms of acquired resistance to c-Src kinase inhibitor in triple negative breast cancer. Rabia A. Gilani, Eric J. Lachacz, Sameer Phadke, Li Wei Bao, Xu Cheng, Evelyn M. Jiagge, Matthew B. Soellner, Sofia D. Merajver. Univ. of Michigan, Ann Arbor, MI.

Triple negative breast cancers (TNBCs) are especially lethal due to their high metastatic potential and propensity to recur. TNBCs comprise the only subset of breast cancer for which there are no FDA-approved targeted therapies. We have developed a mechanistically novel dual c-Src/p38 inhibitor, UM-164, that has potent in vivo anti-TNBC activity. However, single-agent targeted therapies are prone to resistance and rational drug combinations can overcome or prevent resistance. We developed TNBC cell lines that are resistant to UM-164. Significant morphology and cell signaling changes occurred in the drug-resistant cell lines. Most crucial is the observation that in drug-resistant cell lines the expression of c-Src and/or p38 does not return in the presence of UM-164, suggesting that resistance is not occurring via constitutive activation enabling mutations of c-Src and/or p38 kinases. However, compared to their drug-sensitive counterparts, the UM-164-resistant cells have significantly hyperactivated MEK1/2 and ERK1/2 signaling when treated with UM-164. Thus, we tested combinations of UM-164 + MEK1/2 inhibitors in several TNBC cell lines and patient derived xenografts. We found that trametinib (MeKi1), a FDA-approved inhibitor of MEK1/2) is synergistic (Chou-Talay CI at ED90 = 0.4. CI < 1 denotes synergy) with UM-164 to inhibit the growth of UM-164-resistant MDA-MB-231 and SUM-149 cell lines. These results support our hypothesis that activation of MEK/ERK signaling can act as an escape mechanism that leads to resistance to UM-164. Future research into rational combinations of targeted therapies delivered in data driven schedules may hold the key to improving survival in TNBC.

#4118 The EMT transcription factor TWIST1 mediates resistance to EGFR inhibitors in EGF-mutant non-small cell lung cancer. Zachary A. Yochum,1 Haiyun Wang,2 Jessica A. Cades,1 Sushel Khetarpal,1 Eric H. Huang,2 Phouc T. Tran,2 Timothy F. Burns,1 University of Pittsburgh School of Medicine, Pittsburgh, PA; Johns Hopkins University School of Medicine, Baltimore, MD.

Recent advances in the treatment of non-small cell lung cancer (NSCLC) stem from the paradigm shift of classifying patients into subtypes based upon the presence of distinct molecular drivers. Subsets of patients, such as those with EGFR mutations and ALK translocations, have dramatic responses in their tumors to tyrosine kinase inhibitors (TKIs) that specifically inhibit these oncogenic drivers. While mechanisms that lead to resistance to TKIs therapeutic resistance is inevitable. For EGFR-mutant NSCLC, there are multiple described mechanisms of resistance to EGFR TKIs, including epithelial-mesenchymal transition (EMT). Previous studies have implicated the AXL kinase and ZEB1, an EMT transcription factor (EMT-TF), in EMT-mediated EGFR TKI resistance. We have previously demonstrated that the EMT-TF, TWIST1, is required for oncogene-driven NSCLC tumorigenesis, including those tumors with EGFR mutations. In this study, we investigated the role of TWIST1 in EMT-mediated resistance to EGFR TKIs. We have demonstrated that genetic or pharmacologic inhibition of TWIST1 resulted in growth inhibition in a panel of EGF-mutant NSCLC cell lines and apoptosis in a subset of these lines. Interestingly, TWIST1 overexpression in EGFR-mutant NSCLC cell lines led to EGFR TKI resistance. Conversely, knockdown of TWIST1 in an erlotinib resistant EGFR-mutant NSCLC cell line restored erlotinib sensitivity. We found that TWIST1 mediates resistance to EGFR TKIs through suppression of apoptosis possibly through decreasing the expression of the pro-apoptotic Bcl-2 member, BCL2L11 (BIM). We observed that TWIST1 knockdown increased BIM levels, while TWIST1 overexpression inhibited the growth of BIM+ cells. Furthermore, TWIST1-mediated resistance was overcome by treatment with the BCI-2/BCL-XL inhibitor, ABT-737. Knockdown of BIM recapitulated the resistance seen following TWIST1 overexpression, suggesting that TWIST1 suppression of BIM is a mechanism through which TWIST1 leads to EGFR TKI resistance. To explore the role of TWIST1 in modulating EGFR inhibitor sensitivity in vivo, we used an inducible EGFR transgenic mouse model, CCSP-rTA/ERGFL858R(CE), which expresses ERGFL858R in the lung and a EGFR-mutant/Twist1 transgenic model, CCSP-rTA/ERGFL858R/Twist1- tetO- luc (CET), which expresses both EGFRFL858R in the lung, the CET mice had a significantly increased tumor burden, decreased apoptosis and a decreased overall survival compared to CE mice following erlotinib treatment. In summary, we found that TWIST1 overexpression leads to EGFR TKI resistance by suppressing EGFR TKI-induced apoptosis through suppressing BIM expression. Future studies aim to establish the mechanisms of TWIST1 suppression of BIM expression and determine if our TWIST1 inhibitor, harmine, is effective in overcoming EMT-mediated resistance.

#4119 The development of EGFR resistant mutation, T790M, in lung adenocarcinoma is acquired through a specific cytosine deamination mechanism. Khaled Hassan, 1 Najwa El Kadi, 1 April Davis, 2 Gregory Kalemkerian, 1 Luo Wang, 1 Hasan Korkaya 2. University of Michigan, Ann Arbor, MI; 1Georgia Cancer Center, Augusta, GA.

EGFR resistant mutation, T790M, in lung adenocarcinoma is acquired through a specific cytosine deamination mechanism. Background: Epidermal growth factor receptor (EGFR) activation mutations occur in 15% of lung adenocarcinomas. This leads to constitutive activation of EGFR, which triggers multiple downstream survival and proliferation pathways. Currently, EGFR tyrosine kinase inhibitors (TKIs) are first line therapy for stage IV non-small cell lung cancer (NSCLC) patients with EGFR mutations. Despite initial significant response to TKIs, most tumors develop resistance. The main mechanism of resistance detected in 50-60% of cases is a cytosine to thymine (C>T) single nucleotide transition mutation at position 2369. This causes a threonine to methionine amino acid change at position 790 (i.e. T790M). Our data suggests that the C>T mutation is an acquired event secondary to cytosine deamination by Activation Induced Cytosine Deamination enzyme (AICDA). Results: Single cell clones of lung adenocarcinoma cell line, PC9, were treated with EGFR TKI. At baseline, these clones have EGFR exon 19 deletion but no evidence of T790M mutation by digital droplet PCR (ddPCR). However, after treatment with a serial increasing dose of EGFR TKI, T790M mutation was detected by ddPCR. Assessing whether cytosine deamination enzymes were altered by this treatment, a significant increase in AICDA expression was seen. Furthermore, a recombinant AICDA protein could deaminate cytosine at position 2369 in vitro. In addition, using mass spectrometry and methylation specific primers, we determined that cytosine at position 2369 is in fact methylated. This further supports our hypothesis since 5-methylcytosine is deaminated into thymine directly. Since in germinal center B-lymphocytes, AICDA is activated through a non-canonical NFkB mechanism, we assessed NFkB pathway in PC9 cell line. RelB and p52 expression were significantly increased after TKI treatment. In addition direct interaction between RelB and AICDA promoter was confirmed by ChIP Assay. These findings were also seen in a mouse PC9 xenograft model. Daily oral gavage of EGFR TKI caused significant increase in the expression of RelB as well as AICDA. Adding NFkB inhibitor twice weekly inhibited the expression of RelB and AICDA. Finally, knocking down AICDA by shRNA prevented the development of T790M mutation in PC9 cell lines after TKI exposure. Conclusion: Our data suggest that the T790M mutation could be actively acquired after TKI treatment through a cytosine deamination process by AICDA. This would have significant implications for treatment with targeted therapy. In fact, Imatinib resistance in CML and GIST tumors have a similar C>T single nucleotide transition mutation.

#4120 Acquisition of chemoresistance to mTORC1 inhibition due to activation of the GSK-3 β/4E-BP1 pathway might predict poor prognosis of mRCC patients. Hiromi Ito,1 Akira Nagaoaka,1 Norihiko Tsuchiya,1 Yamagata University, Yamagata, Japan; 2Niigata graduate school of medical and dental science, Niigata, Japan.

1The acquisition of chemoresistance to mTORC1 inhibition due to activation of the GSK-3 β/4E-BP1 pathway might predict poor prognosis of mRCC patients. Hiromi Ito,1 Akira Nagaoaka,1 Norihiko Tsuchiya,1 Yamagata University, Yamagata, Japan; 2Niigata graduate school of medical and dental science, Niigata, Japan.

Purpose: PI3K-pathway activation is the second most common genetic abnormality occurring in head and neck squamous cell carcinomas (HNSCC). Mutation or amplification of PIK3CA, the gene coding for the p110alpha subunit of PI3K, occur in 34%-56% of HNSCC and there is evidence that targeting PI3Ka in these tumors can be radiosensitizing. Small molecule kinase inhibitors of PI3Kalpha are showing promising activity. However, the use of this molecule is limited by its high systemic toxicity and severe side effects. In this study we aimed to apply novel nanotechnology for targeted drug delivery via the cell adhesion molecule P-selectin, which was previously shown to be overexpressed in tumor microvasculature. Furthermore, P-selectin is up regulated following radiation therapy (RT), which could enhance drug delivery using targeted nanoparticles. Experimental design: We explored the efficacy and specificity of targeted delivery of BYL719, a potent alpha-specific PI3K inhibitor, using nanoparticles that selectively target P-selectin present in the tumor microvasculature in PIK3CA-mutated HNSCC. We compared the antitumor effects of nanoparticle delivery versus standard oral gavage with free drug in PIK3CA mutated HNSCC cell line- and patient-derived xenografts (PDxAs). In addition, we compared blood glucose and insulin levels between standard BYL719 administration and nanoparticle delivery to test different treatment modalities. Furthermore, we sought to evaluate the radiosensitizing properties of BYL719 when combined with fractionated RT of 5X4Gy. Results: P-selectin targeted delivery of 50 mg/kg/week BYL719 resulted in a significant tumor growth delay, which was comparable to the standard drug administration of 350 mg/kg/week (50 mg/kg/day). While blood glucose and insulin levels were spiking after standard oral gavage of BYL719, these parameters were virtually unchanged upon nanoparticle administration of the drug. The radiosensitizing abilities of low-dose nanoparticle-linked BYL719 were comparable to the drug concentrations used in standard daily BYL719 administration. When concomitantly with fractionated RT, both methods showed robust enhancement of radiotherapy response in all the models tested resulting in durable control of tumor growth. Conclusions: P-selectin targeted delivery of a PI3K inhibitor in in vivo models yielded enhanced radiosensitization by the inhibition of the PI3K/Akt/mTOR pathway without affecting glucose or insulin levels. The magnitude of pathway inhibition was sufficient to radiosensitize several HNSCC animal models. This novel targeting strategy could be translated to the clinic to treat patients with PIK3CA activated and radioresistant HNSCC tumors sparing most of the systemic adverse effects of PI3K inhibition.


A major focus in immuno-oncology research is finding new immuno-oncology targets, including those that alter the character and frequency of T-cell-mediated anti-tumour responses. Screens using CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-Cas9-mediated genome editing seem well placed to identify new targets. However, although CRISPR-Cas9 gene editing works well in primary T cells using electroporation, use of a lentivirus one vector system has proved challenging in primary T cells. We have used several different approaches to identify the most useful method for transduction of primary human T cells with CRISPR components. Electroporation of sgRNAs and mRNA encoding Cas9 into proliferating T cells achieves transduction with high efficiency, but this is less effective for primary T cells. We have used CRISPR-Cas9 mediated gene editing in T cell lines to assess the gene targeting rates of around 37% for gene knockout. Thus, primary T cells are amenable to CRISPR-Cas9 gene editing, and the capacity to rapidly modify loci enables generation of primary T cell models suitable for comprehending the function of modified receptor-ligand pairs involved in an immune checkpoint response. Our pooled sgRNA-Cas9 screens in cancer cell lines have used our limited library of libraries, which include genes such as CD3. However, we have not achieved sufficient coverage to include many of the key regulators of T cell function. CRISPR-Cas9 technology might be useful for this purpose. This work marks a significant step toward understanding the full potential of CRISPR-Cas9 gene editing in primary T cells.
tion in myeloid derived suppressor cells (MDSCs). We are using an siRNA approach in these cells, which are generated by PBMC co-culture with cancer cell lines for 7 days, or by culture in the presence of recombinant GM-CSF and IL-6 for 7 days. Our initial data indicate that these MDSCs can effectively suppress autologous, as well as allogeneic, CD8 T cell proliferation mediated by anti-CD3 and anti-CD28 stimulation, suggesting that siRNA lipid delivery is effective in MDSCs. We will use our druggable genome plus arrayed siRNA library to identify targets that when knocked down inhibit the capacity of MDSCs to suppress T cell proliferation. We anticipate that these data will be useful in identifying new targets that are involved in regulating an immune response to tumour development and progression.

#4124 POPDC1 inhibits breast cancer cell migration and proliferation, and is regulated by EGFR (ErbB1) and CAMP. Johanna N. Amunjela, Steven J. Tucker. Univ. of Aberdeen, Aberdeen, United Kingdom.

Despite progress in the development of new treatments, breast cancer remains the second leading cause of global cancer morbidity and mortality (1). Identification and validation of effective therapeutic targets is thus essential for the development of better breast cancer treatments and improved prognoses. This project seeks to develop POPDC proteins as potential therapeutic targets for inhibiting breast cancer migration and proliferation. POPDC1 (also known as blood vesel epiderial substance or BVE5), is a cAMP-binding transmembrane protein encoded by the POPDC1 gene. Loss or suppression of POPDC1 has been correlated with enhanced aggressive cancer cell behaviour (2-6). Suppression of POPDC1 promotes cell migration and invasion in hepatocellular carcinoma and tumourigenesis in colorectal cancer (2,4,5,7). Furthermore, we have previously shown that loss of POPDC1 promotes cell proliferation and migration in breast cancer and glioblastoma cells (3,8). Using a cAMP-agarose pulldown assay, we confirmed that POPDC1 interact with, and is upregulated by cAMP in luminal A (MCF7) and triple-negative (MDA231) breast cancer cells. Furthermore, immunocytochemistry and Western blotting has shown that POPDC1 expression is abrogated in MCF7 and MDA231 breast cancer cells compared to normal breast cells (HMePc). To confirm that POPDC1 negatively regulates migration and proliferation in breast cancer cells, we conducted knock-in experiments where overexpression of POPDC1 significantly inhibited cell migration and proliferation in MCF7 and MDA231 cells. Interestingly, epidermal growth factor receptor (EGFR/ErbB1) signalling negatively regulates POPDC1 expression in MCF7 and MDA231 cells and reduces the capacity of POPDC1 to inhibit cell migration. Epidermal growth factor reversed POPDC1-mediated inhibition of cell migration, an effect blocked by the AG 1478 (an EGFR inhibitor), suggesting EGFR-dependent modulation of POPDC1 activity. Furthermore, we have established for the first time using human breast tissue sections that Her2- status correlates with low POPDC1 expression while Her2+ status correlates with high POPDC1 expression. This suggests further investigation between EGFR and POPDC1 and that any links between these molecules diverges at the level of the different EGFRs. Taken together, this strong dataset shows that POPDC1 plays a major role in breast cancer tumourigenesis that is at least in part, modulated by EGFR and cAMP signalling pathways. Indeed, these seem to demonstrate differential control of POPDC1 expression. This interplay and the fact that POPDC1 expression, which is associated with a downregulation of the MUC1 and CIN85 complex in breast cancer, seems to be associated with HER2 status in breast cancer. We are currently investigating the MUC1/CIN85-dependent signaling pathway with a particular focus on the ability of CIN85 to modulate MUC1 glycosylation by controlling its plasma membrane-Golgi trafficking. Ongoing experiments in an in vivo mouse model will confirm the ability of these drug compounds to decrease tumor growth and metastasis and validate CIN85/MUC1 complex as a viable therapeutic target and support development and testing of more potent antagonists.

#4125 Comparison between knockdown of Pim-1, Pim-2 and Pim-3 identifies Pim-2 as the most relevant Pim oncogene in hepatocellular carcinoma. Ulrike Weihrach, Pia Kärrz, Achim M. Aigner. Univ. of Leipzig, Leipzg, Germany.

Introduction: Pim-Kinases (Pim - proviral integration site for moloney murine leukemia virus; Pim-1, -2, -3) are a family of serine/threonine kinases with anti-proliferative (low dose, 0.05-0.3 M) effects in all three cell lines, including p53 mutant Huh-7 and p53 null Hep3B cells. The same cell lines were strongly resistant to Nutlin-3a. Gene expression analyses were conducted with cells exposed to NSC207895 to examine effects of MDM4 inhibition on p53 targets Bax, Puma, and p21. In SK-Hep1 cells, NSC207895 treatment led to 2.5-fold overexpression of Bax, 27.4-fold of Puma, and 23.1-fold of p21. Huh-7 showed 11.8-fold upregulation of Puma and 12.4-fold of p21, and Hep3B exhibited a very strong 58.4-fold increase in p21 gene expression with the same treatment. Interestingly, Hep3B showed 3.9-fold overexpression of p63 and 1.99-fold of p73 gene expression with NSC207895 exposure, suggesting novel interactions between MDM4 and p63/p73 in the absence of p53. Conclusions: Inhibition of MDM4 shows efficacy in in vitro models of HCC through upregulation of p63, p73, and p53 to activate p53 tumor suppressive signaling. Thus, targeting MDM4 may be a viable option for all HCC patients, no matter the p53 mutation status.
**EXPERIMENTAL AND MOLECULAR THERAPEUTICS: New Targets and New Drugs**

**#4128** Mechanism of action of G-quadruplex forming oligonucleotide homologous to the telomere overhang in melanoma. Gagan Chhabra, Luke Woodby, Anita Sanjali, Mark Fakes, Marko Ivanovic, Pooja Vinay, Zachary Schrank, Benjamin E. Ramirez, Neelu Puriri. 1Univ. of Illinois at Chicago, Rockford, IL; 2Univ. of Illinois at Chicago, Chicago, IL

T-oligo, a guanine-rich oligonucleotide (GRO) homologous to the 3’ telomeric overhang of telomeres, elicits potent DNA-damage responses (DDRs) in cancer cells. However, the detailed molecular mechanism of action of T-oligo in cancer cells is largely unknown. Recent studies suggest that GROs can form G-quadruplexes (G4) which are stabilized by the hydrogen-bonding of guanine residues. This study aims to examine the G4-forming capabilities of T-oligo in vitro and to investigate the molecular mechanism of G4 single-stranded (SS) T-oligo G4-T-oligo induced DDRs in melanoma cells (MM-AN). G4-formation by T-oligo was confirmed using the SS-T-oligo and G4-T-oligo on a polyacrylamide gel under non-denaturing conditions. NMR studies for T-oligo in KCl confirmed that T-oligo forms G4 structures. Immunofluorescence studies conducted with an anti-G-quadruplex antibody (BG4), a G4 detecting antibody, showed 88.4% co-localization of T-oligo and BG4 in the nuclei of melanoma cells confirming the ability of T-oligo to form G-quadruplexes inside melanoma cells. While G4-T-oligo was found more stable in nuclease degradation assay by DNase I, it had a decreased anti-proliferative effects compared to SS-T-oligo. However, G4-T-oligo had similar cellular uptake as SS-T-oligo. Further, two telomere complex proteins TRF2 and POT1 which are mainly found at the telomere ends were found to be upregulated (2.0 fold) by T-oligo suggesting TRF2 and POT1 mediated telomere overhang dissociation. We also found that T-oligo can co-localize with telomere binding proteins TRF2 and POT1 by 88.4±4.5% (n=12) and 84.5±8.8% (n=10) respectively. Western blot analysis results also showed upregulation of both p-JNK and total JNK by 4.0- and 2.0-fold respectively. To further confirm the involvement of p-JNK in T-oligo mediated apoptosis we used a specific JNK inhibitor SP600125. Western blot analysis showed that T-oligo mediated upregulation of p-JNK was reversed in presence of SP600125. Results from an MTT assay showed a 73.8% decrease in cell viability after T-oligo treatment alone; however, cell viability was decreased to 45.8%, and 25.3% when SP600125 was present at concentrations of 10 µM, and 12 µM respectively, in comparison to diluent. T-oligo also inhibited mRNA expression of hTERT, a catalytic subunit of telomerase by 50%. We further investigated the effect of the JNK inhibitor SP600125 on hTERT expression and found that treatment with SP600125 in presence of T-oligo partially reversed the downregulation of hTERT. We found a 16% decrease in hTERT expression in comparison to 50% reduction by T-oligo treatment alone. In conclusion, these studies demonstrate that T-oligo can form G-quadruplexes and the anti-proliferative mechanism of T-oligo may be mediated through POT1 and TRF2 as well as via JNK-activation inducing hTERT-inhibition in melanoma cells.

**#4129** Novel oncogenic BRAF fusions and impact on targeted therapies. Sonia C. Dolfı, Ann Silk, Bhavna Paratala, Whitney Petrosky, Srilatha Simhadri, Rutger Cancer Inst. of New Jersey, New Brunswick, NJ

BRAF mutations are driver events in a number of cancers including thyroid cancer and melanoma. The most common, BRAF V600E, alters normal BRAF protein activity in the mitogen-activated protein kinase (MAPK) pathway by constitutively activating BRAF and inducing proliferative signaling and tumor growth. Small molecule tyrosine kinase inhibitors targeting tumors with the V600E mutation have been evaluated in clinical trials and are now approved for melanoma. While BRAF missense mutations have been extensively characterized for oncogenic potential and actionability in genomically-guided therapy, BRAF gene fusions have been underappreciated for not only their functional role in cancer but also in differential drug response. More recently, data suggest that alternative approaches may be needed for treatment of patients with BRAF fusion-containing tumors. We have identified two novel BRAF fusions in tumors from patients with papillary thyroid cancer and melanoma. Both fusions result in an in-frame fusion of a novel gene partner at the 5’ end of the fusion, an intact BRAF reading frame at the 3’ end, and loss of the BRAF auto-inhibitory domain. We hypothesized that these novel BRAF fusions act as oncogenic drivers, and the mechanism of BRAF activation differs from that caused by V600E mutations and may be fusion partner-specific. These fusions have been engineered in the laboratory and tested for tumorigenic potential and functional activity. BRAF fusion expression in non-transformed cells induces colony formation similar to the V600E mutation indicating tumorigenic potential. These BRAF fusions also constitutively activate the MAPK pathway in the absence of stimulation as demonstrated by phosphorylated ERK and MEK proteins. Additionally, BRAF fusion-expressing cells form tumors in vivo similarly to the BRAF V600E-expressing cells. These tumors are highly proliferative as demonstrated by strong Ki67 immunohistochemical staining and display MAPK pathway activation as evidenced by phosphorylated ERK. BRAF fusion-expressing cells have differential sensitivity to MAPK pathway inhibitors compared to cells with the V600E mutation as measured by reduced MAPK signaling. Inhibition of the MAPK pathway is relevant in targeting BRAF fusion-containing cells but not always the same paradigm at point mutations. Collectively, our data suggest that BRAF fusions are functional and represent novel therapeutic targets, but may need an alternative approach as compared to tumors with BRAF missense mutations.

**#4130** Characterization of URS1 as a prognostic biomarker and therapeutic target for lung cancer. Atsushi Takano, Yohei Miyagi, Yataro Daigo. 1Institute of Medical Science, The University of Tokyo, Tokyo, Japan; 2Kanagawa Cancer Center Research Institute, Yokohama, Japan; 3Shiga University of Medical Science, Otsu, Japan

Lung cancer is the most frequent cause of cancer deaths worldwide and its overall prognosis remains poor. Therefore, next generation of biomarkers and therapeutic strategies for lung cancer are eagerly awaited. We selected genes that were overexpressed in the majority of lung cancer using our gene expression profile database. During this process, we identified URS1 (Up-regulated in lung cancer) (1). As a candidate, immunohistochemical analysis showed that URS1 expression was observed in the majority of non-small cell lung cancer (NSCLC) patients (P = 0.0013 by log rank test). Multivariate analysis revealed highest level of URS1 expression was an independent prognostic factor for NSCLC patients. Moreover, reduction of URS1 by siRNA or treatment with URS1 inhibitor significantly suppressed lung cancer cell growth probably through cell cycle arrest at G2/M. Exposure of cancer cells to URS1 inhibitor significantly suppressed These data suggest that URS1 is a possible prognostic biomarker and therapeutic target for lung cancer.


Triple negative breast cancer (TNBC) - defined as estrogen receptor- (ER), progesterone receptor-(PR), and human epidermal growth factor receptor 2-(HER2) negative - is a highly aggressive form of breast cancer prevalent in African-American (AA) women. Because ER- and HER2-targeted therapies are ineffective in TNBC, current treatment strategies rely on cytotoxic chemotherapy. There is a tremendous need for new approaches that can identify effective therapies with less toxicity for this devastating form of breast cancer. Nuclear receptors are highly druggable targets. Classical examples include ligand-activated receptors like ER, but orphan nuclear receptors are also emerging as targets for cancer therapy. We recently published that increased mRNA expression of estrogen-related receptor beta (ERRβ, gene symbol ESRRB) correlates with better recurrence- and distant metastasis-free survival in women with TNBC, and that a small molecule agonist ligand for ERRβ (DY131, DY) has growth inhibitory and anti-metastatic activity in TNBC cell lines of multiple molecular subtypes.

The goal of our current work is to validate ERRβ as a novel therapeutic target for TNBC in two distinct ways. First, we sought to determine whether ERRβ is correlated with survival outcome specifically in African-American (AA) women. Analysis of The Cancer Genome Atlas (TCGA) Breast Cancer RNAseq data shows that low ERRβ mRNA expression is specifically associated with poor overall survival in AA women with TNBC. This association is not observed in AA women with ER- or HER2-positive disease. We further find that while ERRβ mRNA expression is decreased in tumor vs. normal breast tissue regardless of race, this decrease is more pronounced in AA women. Ongoing studies in an independent cohort of AA and white women will determine whether this is due, in part, to lower copy number changes at the ERRβ locus (chromosome 1q42.43). Second, we sought to determine whether ERRβ expression could predict response to current treatment strategies commonly used in breast cancer, particularly TNBC. Using multiple combined datasets, we find that high ERRβ expression is associated with improved recurrence-free survival (RFS) in women with TNBC who received adjuvant or neoadjuvant therapy with anthracyclines (e.g. doxorubicin), and that ERβ ligand ERβ836,181 significantly suppressed ERβ activity and its downstream signaling in TNBC. These data indicate that ERβ ligand ERβ836,181 is a suitable candidate for clinical trials in TNBC.

**#4132** Roles of the acetyltransferases CBP/p300 in breast cancer. Iqbal Muhmud, Guimei Tian, Daiqing Liao. University of Florida College of Medicine, Gainesville, FL

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Experimental and Molecular Therapeutics: New Targets and New Drugs

Intrinsic drug resistance renders certain breast cancer (BC) patients unresponsive to standard-of-care therapies. For example, ~50% of patients with estrogen receptor-positive (ER+) tumors do not respond to endocrine therapies (ETs, e.g., tamoxifen). Acquired drug resistance is another cause for treatment failure and deadly relapse. Therefore, new strategies that tackle both intrinsic and acquired resistance are needed for improving clinical outcome for BC patients. ER-alpha, the defining factor for ER+ tumors, is an estrogen-activated transcription factor (TF) that drives tumor growth and progression. It critically depends on the coactivators CBP/p300 for gene expression underpinning tumor biology of ER+ subtypes that account for ~75% of all BC cases. Recent advances revealed that another hormone receptor, the androgen receptor (AR), is expressed in the majority of ER+ BC. As a result, AR-positive prolactinomas induced by fetal alcohol exposures. Pregnant Fischer 344 rats were fed between gestational days 7 and 21 with a liquid diet containing alcohol, pair-fed with isocaloric liquid diet, or fed ad libitum with rat chow. At 60 days of age, female offspring rats were ovariectomized and received a subcutaneous estradiol implant. These rats were sacrificed at 3 months after the estradiol implants. Pituitary tumors were collected and pituitary tumor samples were prepared and maintained in cultures. Alcohol-fed pituitary tumorspheres, but not control-fed pituitary tumorspheres, expressed DPPA4 and a panel of genes related to multipotility (OCT4, NANOG, KLF4, and CD133) and showed increased invasiveness and marked growth in cultures and in mice xenografts. The RNA sequence analyses identified selective expression of DPPA4 in the pituitary of fetal alcohol-exposed rats but not in control BCs as well as in a subset of ER-BCs. Evidence indicated that AR signaling is implicated in ET resistance. For the HER2-enriched tumors, high levels of AR expression correlate with poor prognosis. Similarly for ER-alpha, CBP/p300 act as key coactivators for AR-mediated gene expression. Additionally, other oncogenic TFs (e.g., beta-catenin/TCF and STAT3) also depend on CBP/p300 for gene activation. Importantly, these pathways are active in all BC subtypes, regardless of the hormone receptor status. CBP/p300 overexpression and heightenened acetylation activity are detected in clinical BC samples and correlate with disease progression. Thus, CBP/p300 represent attractive targets for developing novel therapies broadly applicable for treating all major BC subtypes. We have discovered a novel class of highly potent CBP/p300 inhibitors. Our previous studies indicated that this class of compounds is highly effective to suppress tumor growth in vivo using a xenograft mouse model. We will present genomic features of CBP/p300 in different breast cancer subtypes and experimental data underlying potential mechanisms by which CBP/p300 regulate oncogenic phenotypes, as well as effects of pharmacologic inhibition of CBP/p300 on sensitivity of breast cancer cells to standard anticancer therapeutics.

Amrubin (AMR) is a synthetic cytotoxic agent that is currently in clinical trials for the treatment of various types of cancer. It targets the tumor microenvironment, particularly the stromal components, and inhibits tumor growth and metastasis. Amrubin has shown to inhibit tumor cell proliferation, migration, and invasion in vitro and in vivo. It has been found to be effective in a variety of cancer models, including breast, lung, and prostate cancer. The mechanism of action of Amrubin involves the inhibition of the PI3K/AKT/mTOR signaling pathway, which is known to play a critical role in tumor growth and survival. Additionally, Amrubin has been shown to induce apoptosis and enhance the effectiveness of chemotherapeutic agents through the modulation of their targets.

Inactivation/loss of PTPRS promotes ERK activation which sensitizes colorectal cancer cells to ERK inhibitors. Thomas B. Davis,1 Mingli Yang,1 Michael J. Schell,2 Heimang Wang,3 Le Ma,1 W. Jack Pledger,1 Timothy J. Yeatman1,4 Gibbs Cancer Ctr. and Research Inst., Spartanburg, SC; Moffitt Cancer Center & Research Institute, Tampa, FL.

The RAS pathway is a driver of many cancers, and has been targeted for the development of a variety of therapeutic inhibitors of BRAF, MEK, and more recently ERK. One of challenging issues is to predict response to targeted therapies. Here we report identification of PTPRS, a receptor-type protein tyrosine phosphatase, as a regulator/biomarker of ERK activation and inhibitor response in colorectal cancer. To identify the genes with high frequency mutations that might be predictive of RAS pathway dependency (‘addiction’), we used a gene expression signature score to stratify 468 colorectal cancer tumors that also underwent targeted exome sequencing for 1321 cancer-associated genes. We found that, when the masking effects caused by mutant KRAS, BRAF and NRAS was iteratively removed, mutant PTPRS was strongly correlated with RAS pathway activation, with a mutation rate of 10.4% (22 out of 211 remaining tumors). This led us to inactivate PTPRS in vitro using a specific peptide inhibitor, siRNA or CRISPR approaches in various colorectal cancer cell lines. Inhibition/loss of PTPRS significantly elevated phosphorylation of ERK and AKT and substantially sensitized cancer cells to the treatment of ERK inhibitors, which was enhanced by an AKT inhibitor. Surprisingly, PTPRS did not modulate the activity of EGFR and the ERK kinase, MEK. Our data indicate that PTPRS negatively regulates ERK signaling downstream of the EGFR/RAS/RAF/MEK pathway in a mechanism independent of AKT and DUSP6. As a result, inactivation/loss of PTPRS promotes ERK activation and ‘addiction’, thereby contributing to increased sensitivity to ERK inhibitors.


Endometrial cancer is the most common gynaecological malignancy and its incidence is increasing. Tissue renin angiotensin systems (RAS) are known to stimulate angiogenesis, cell proliferation and migration. All of these actions potentiate cancer growth and spread. We have previously demonstrated that endometrioid endometrial cancers express both prorenin and prorenin receptor

The Prolactin-secreting pituitary tumors (prolactinomas) are the most common pituitary tumors in humans. Majority of prolactinomas are adenomas and benign and slow growing, but in some cases, they are locally aggressive and invasive. In a rat animal model we found that fetal alcohol exposed female rats develop aggressive prolactinomas following estrogen administration. Several studies have suggested that human prolactinomas seem to arise because many tumor and embryonic stem cell markers such as OCT4, SOX2, Nanog, and Klf4 are expressed in malignant tumor. The developmental pluripotency associated 4 (DPPA4) gene has an important role in self-renewal and pluripotency in embryonic stem cells. It is re-expressed in several malignant tumors and is identified as a new pluripotency-related oncogene. We studied with DPPA4 expresses and function in aggressive human prolactinomas induced by fetal alcohol exposures. Pregnant Fischer 344 rats were fed between gestational days 7 and 21 with a liquid diet containing alcohol, pair-fed with isocaloric liquid diet, or fed ad libitum with rat chow. At 60 days of age, female offspring rats were ovariectomized and received a subcutaneous estradiol implant. These rats were sacrificed at 3 months after the estradiol implants. Pituitary tumors were collected and pituitary tumor samples were prepared and maintained in cultures. Alcohol-fed pituitary tumorspheres, but not control-fed pituitary tumorspheres, expressed DPPA4 and a panel of genes related to multipotility (OCT4, NANOG, KLF4, and CD133) and showed increased invasiveness and marked growth in cultures and in mice xenografts. The RNA sequence analyses identified selective expression of DPPA4 in the pituitary of fetal alcohol-exposed rats but not in control BCs as well as in a subset of ER-BCs. Evidence indicated that AR signaling is implicated in ET resistance. For the HER2-enriched tumors, high levels of AR expression correlate with poor prognosis. Similarly for ER-alpha, CBP/p300 act as key coactivators for AR-mediated gene expression. Additionally, other oncogenic TFs (e.g., beta-catenin/TCF and STAT3) also depend on CBP/p300 for gene activation. Importantly, these pathways are active in all BC subtypes, regardless of the hormone receptor status. CBP/p300 overexpression and heightenened acetylation activity are detected in clinical BC samples and correlate with disease progression. Thus, CBP/p300 represent attractive targets for developing novel therapies broadly applicable for treating all major BC subtypes. We have discovered a novel class of highly potent CBP/p300 inhibitors. Our previous studies indicated that this class of compounds is highly effective to suppress tumor growth in vivo using a xenograft mouse model. We will present genomic features of CBP/p300 in different breast cancer subtypes and experimental data underlying potential mechanisms by which CBP/p300 regulate oncogenic phenotypes, as well as effects of pharmacologic inhibition of CBP/p300 on sensitivity of breast cancer cells to standard anticancer therapeutics.

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Endometrial cancer is the most common gynaecological malignancy and its incidence is increasing. Tissue renin angiotensin systems (RAS) are known to stimulate angiogenesis, cell proliferation and migration. All of these actions potentiate cancer growth and spread. We have previously demonstrated that endometrioid endometrial cancers express both prorenin and prorenin receptor
Lymphoma cell viability by inhibiting LUBAC and NF-KB activity. Therapeutic agents for the treatment of ABC-DLBCL. Tides were far more potent than any of the previously generated compounds that inhibited the NF-KB canonical pathway in ABC-DLBCL cell lines. Many of these new peptides diminished cell viability, disrupted LUBAC complex formation and inhibited the NF-KB pathway. In conclusion, new approaches for the treatment of cancer through their potential to restore anti-tumor immunity and to synergize with existing therapies. As part of our investigations into novel small molecule inhibitors of IDO1, we screened a number of human and murine tumor lines for expression of tryptophan dioxygenases. All of the human and murine tumor lines that we tested were found to be tryptophan dioxygenases unless induced with IFN-gamma. Our screen included testing 50 primary human melanoma lines developed from tumor samples obtained from New Zealand Melanoma patients (NZMel lines) for expression of IDO1 and IDO2. After culture with IFN-gamma, 84% of the NZMel lines were IDO1+ and IDO2+. 4% were IDO1+ only; 2% were IDO2+ only; whilst 10% did not express either IDO1 or IDO2. Tryptophan dioxygenase (TDO) was not detected in any of the NZMel lines before or after IFN-gamma exposure. To overcome the need for IFN-gamma induction for our cell based assays of IDO1 inhibitory activity of our novel compounds, we transfected the wild-type murine Lewis Lung carcinoma line (LLTC) to constitutively express murine IDO1 (LLTC-mIDO1) or human IDO1 (LLTC-hIDO1), and used these engineered lines to assay for potential species selectivity of our inhibitors. IDO1 expression in the lines was consistent and stable, but when LLTC-hIDO1 cells were implanted subcutaneously into syngeneic mice for in vivo testing of inhibitors, we found considerable heterogeneity in IDO1 expression between tumors. Approx. 10% of tumors were IDO1 positive, 10% were IDO1 negative and the remaining 80% were IDO1 negative for IDO1 expression by Western blot analysis. More intriguingly, when fragments of an IDO1+ or an IDO1- donor tumor were implanted into new recipients, the same degree of intermittent heterogeneity in IDO1 expression was seen in the subsequent generation of tumors. In contrast, subcutaneous tumors developing from murine GL261 glioma cells similarly engineered to express IDO1 (GL261-hIDO1) or to be non-expressors of IDO1 (GL261-hiIDO1), and were used for subsequent in vivo studies of intratumoral IDO1 expression on its effects on immune cell infiltration and tumor growth. GL261-hiIDO1 tumors had a significantly faster growth rate than the wild-type tumors, and 24 days after implantation, had reached a mean tumor volume of 3600 mm³ compared to a mean tumor volume of 500 mm³ of wild-type tumors. Analysis of the immune cell infiltrates, showed that 14-day GL261 wild-type tumors had a 3-fold higher percentage of CD3+ T cells than that in GL261-hiIDO1 tumors. CD8+ cells made up 55% and 25% of the CD3+ cells in wild-type and GL261-hiIDO1 tumors, respectively. Percentage of FoxP3+ (Treg) cells was higher in GL261-hiIDO1 tumors compared to that in wild-type tumors.

#4139 Role of zinc transporters in prostate cancer and a potential association with racial disparity. Chandra K. Singh,1 Kareem M. Malas,1 Caitlin Tydrc,2 Khalid A. Azkowitz,3 Najib Ahmed,4 University of Wisconsin-Madison, Madison, WI; 2Medical College of Wisconsin-Milwaukee, Milwaukee, WI. Optimal intracellular zinc concentration is essential for many cellular functions as it serves as a catalytic and/or structural cofactor for a variety of proteins. Although a number of proteins are tangled in regulating cellular zinc homeostasis, the most important are two protein families of zinc transporters, 14 members of solute carrier family 30 (SLC30A) and 10 members of solute carrier family 39 (SLC38A). These two families are known to transport zinc into- and out-of the cytoplasm, respectively. Prostate cells accumulate a high amount of zinc to sustain a metabolic condition unique to the prostate which is characterized by a truncation of the Krebs cycle and production of high amounts of citrate. Zinc depletion has frequently been noted in the progression of prostate cancer (PCa). Whether zinc transporters are a steering cause of zinc depletion in PCa development and progression and are key determinants in the racial disparity in PCa is not well studied. In this study, we determined the connection of zinc transporters (SLC39A1-14 and SLC30A1-10) in PCa, in the perspective of racial health disparity in human PCa samples taken from African-American (AA) and European-American (EA) patients and compared them with respective adjacent benign samples. We also assessed the mRNA level of zinc transporters in normal prostate epithelial cells (NPEC) and RWPE1 and among PCa cells derived from AA (MDA PCa 2b, E006AA-PAR, E006AA-H) and EA patients (DU145, PC3, 22Rv1, LNCaP, C4-2B). In addition, we performed a dataset analysis of the Oncomine database for differential expression profile of zinc transporters in PCa versus normal prostate. We found that SLC39A3 mRNA levels were differentially expressed in PCa with a significant downregulation of SLC39A1, SLC39A9, SLC39A11, SLC39A13 and SLC39A14, and upregulation of SLC39A3, SLC39A5, SLC39A6 and SLC39A8. Further, SLC30A3 showed a significant downregulation of SLC30A5 and SLC30A6 and upregulation of SLC30A1, SLC30A9 and SLC30A10, in PCa. Further, compared to EA samples, the AA PCa showed an increased trend of SLC39A5, SLC39A6 and SLC39A9. In addition, compared to AA samples, the EA PCa showed a trend of increasing SLC30A1 and SLC30A9 and decreasing SLC39A10. These data provide evidence that the zinc transporters may be linked to racial disparity of PCa in AA versus EA. Moreover, Ingenuity Pathway Analysis revealed regulatory interactions between zinc transporters and tumor suppressor/promoter genes known to be modulated in PCa. These genes are HOXB13, ELAVL1, DIRAS3, ALFP, CSF2, CCL4, INSR, AKT, IL6, TGFBP1, CHI3L1, EGFR and HNF4A. Overall, our study provides interesting data about transcription profiles of zinc transporters and their interaction with tumor suppressor and promoter genes in PCa, which may offer novel strategies for the management of PCa by pharmacologically modulating zinc transporters.

#4140 Identification of a selective MLKP2/KIF20A inhibitor with high in vivo antitumor activity. Yves Collette,1 Stephanie Misery-Leunki,2 Catherine Guillou,1 Denis Carniato,2 Bernard Pau,1 Bruno Goud,1 Norbert Vey,1 France; 3ICSN-CNRS, Gif-sur-Yvette, France; 4DC2A Consulting, Paris, France; 5BPCONSEIL, Saint-Gély-du-Fesc, France; 6BIOKINESIS, Paris, France. Mitotic kinases are essential regulators of cancer cell replication and migration. The mitotic kinase MLKP2/KIF20A, a member of the kinase-6 family, plays an
essential role during cytokinesis and was identified as a potential new target for cancer chemotherapy.1 We have previously identified Paprotrain, a new synthetic compound, as the first selective MKLP2 inhibitor.2 Recently, we obtained Paprotrain analogues with higher potency on MKLP2.3 Herein we describe the identification and characterization of BKS0349, a new potent analogue of Paprotrain. BKS0349 shows promising preclinical activity against human cancers and has shown an even more restricted specificity profile when tested on a large set of kinases. In-vitro this compound is highly cytotoxic on a wide panel of human cancer cell lines (IC50 ranged 10-70 nM, which corresponds to 1000 fold improvement of paprotrain potency) while no toxicity is observed on human normal cells such as peripheral blood mononuclear cells (PBMC) and primary hepatocytes (IC50 >90fold). BKS0349 is also well tolerated in short-term administrations (200 mg/kg, twice a week for 4 weeks). In xenografted nude mice, in-vivo treatment with BKS0349 compound demonstrates a high antitumor activity against various human cancer cell models, either sensitive or resistant to some standard-of-care treatments. In addition, human cancer cells treated in vitro as well as in-vivo with BKS0349 compound display Golgi scattering and a mitotic arrest leading to cell death, as hallmarks of BKS0349 mode of action. These findings show that BKS0349 is a potential new target for cancer chemotherapy and BKS0349 is a good candidate to be developed for cancer treatment.1 Paclitaxel targets FOXM1 to regulate KIF20A in mitotic catastrophe and breast cancer paclitaxel resistance. P. Khongkow et al. Oncogene, 2015, 1-13.2 Relocation of Aurora B and Survivin from centromeres to the central spindle impaired by a kinesin-specific MKLP-2 inhibitor. S. Tcherniuk et al. Angew. Chem. Int. Ed., 2010, 49:8228-8231.3 New MKLP-2 inhibitors in the paprotrain series: design, synthesis and biological evaluations. C. Labrière et al. Bioorg and Med Chem, 2016, 24:731-734.

#4141 Phosphoproteome networks display consistent hyperactive kinase activity in pancreatic cancer: evidence for new therapeutic options. Tessa Y. Le Largé,1 Maarten F. Bijlsma,1 Bissame El Hassouni,1 Nicolla Funel,1 Nicole C. van Gielen,1 Helene Damhofer,1 Jaco C. Knol,1 Sander R. Piersma,1 Thang Y. Pham,1 Henk M. Verheul1, Hanneke W. van Laarhoven,1 Geert Kazemier,1 Etta Giovanny Batista1, Connie R. Timosenko1,2 VU university Medical Center, Amsterdam, Netherlands;4 Academic Medical Center, Amsterdam, Netherlands;3 University of Pisa, Italy.

Introduction Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal disease due to its aggressive nature. Patients typically present with distant metastases, at which point cytotoxic agents can extend life expectancy by several months at most. Large-scale phosphoproteomics complements our knowledge obtained from genomics and transcriptomics as it provides information on which proteins and kinases are phosphorylated, thereby implicating pathways that are activated. This approach in cancer research may lead to improved patient selection for treatment with tyrosine kinase inhibitors (TKI). This study is the first to employ phosphotyrosine-based phosphoproteomics on three different preclinical PDAC models as well as patient tumor tissues to understand the aggressive nature of this disease and identify new drug targets. Approach We performed phosphoproteomics on a panel of 11 PDAC cell lines, 7 primary cell cultures, 10 patient-derived xenografts (PDX) and 16 fresh frozen human tumor tissues. Tyrosine phosphopeptides were enriched via immunoprecipitation and phosphopeptides were analyzed by high-resolution nano- LC mass spectrometry. Results Using phosphotyrosine-based phosphoproteomics, we identified a total of 1723 tyrosine phosphorylated proteins and 138 phosphorylated kinases, representing 27% of the kinome. The reproducibility of our workflow was very high, with Pearson correlation coefficients of r = 0.937 for technical replicates of cell lines and r = 0.876 for biological replicates of tumors. In our cell line panels, multiple kinases were commonly highly phosphorylated (e.g. PTK2, EPHA2, EGFR and MET). Functional testing of PTK2 by using TKI defactinib in primary cell lines with high phosphorylation resulted in inhibition of proliferation and migration in vitro. Inhibition of EPHA2 by shRNAs resulted in reduced proliferation in vitro. To validate the relevance of these candidate target proteins in vivo, the tyrosine phosphoproteome of PDXs and human tumors was analyzed. In these studies, cancer kinase activity analysis based on kinase phosphorylation levels and kinase-substrate networks validated these common active nodes in the majority of these tumors. Conclusion Our extensive tyrosine phosphoproteome analysis spanning a wide range of PDAC models revealed high phosphorylation levels of multiple kinases. Interestingly, the phosphorylated kinase profiles of tumors and cell lines did not show as much heterogeneity as expected, taken into account the existence of biological subtypes in PDAC identified through genomics and transcriptomics. The aggressive biology of this disease may be correlated with the consistent activation of multiple pathways, some of which we have shown to be targetable in vitro. This study prompts further validation and prognostic evaluation of the identified active kinases to improve treatment of PDAC.

#4142 Regulation of tumor suppressor decorin by APE1 in head and neck squamous cell carcinoma. Christina A. Wicker,1 Timothy L. Scott,1 Rangawamy Suganya,2 Susan M. Arnold,1 Yolanda M. Brill,3 Craig M. Horbinski,3 Dana Napier,1 Joseph Valentino,1 Mahesh R. Kubritomi,1 Guoqiang Yu,1 Tadahide Izumi1, University of Kentucky, Lexington, KY; 2Houston Methodist Research Institute, Houston, TX; 3Northwestern University, Chicago, IL

Oral cancers have a high rate of recurrence, and a tendency to develop treatment resistance. From 2009 to 2013, Kentucky had a disproportionately higher incidence of invasive oral cancers and resultant higher mortality compared to the national average (SEER & NPCR, Kentucky Cancer Registry). Apurinic/Apyrimidinic endonuclease (APE1) is a key protein in DNA repair, and an oncogene in NMIBC expressing wild-type APE1 and extremely low levels of APE1 showed an eight-fold reduction of tumor suppressor decorin (DCN), with wild-type APE1. Decorin is a known tumor suppressor and extracellular matrix protein, which can suppress tumor proliferation, migration and angiogenesis. Tumors with decreased decorin are associated with increased mortality. These studies aim to elucidate APE1’s role in decorin regulation in oral cancer and identify biomarkers, which may aid in diagnosis, treatment, or predicting prognosis. Immunohistochemistry studies of head and neck squamous cell carcinoma from Kentuckians were analyzed aided by APE1’s high resolution scanning capabilities, and software. Analysis showed a significant decrease in decorin in both tumor and carcinoma in situ compared to benign tissue. Whereas, APE1 was significantly increased in tumors and carcinoma in situ compared to normal APE1 and decorin total protein in carcinoma in situ. This data supports that increased APE1 may deleteriously decorin. Superoxide Dismutase 3, which is important in detoxifying extracellular ROS, was also significantly decreased in tumor and in carcinoma in situ. This along with previous research showing increased ROS in the tumor microenvironment and APE1 activation by ROS may explain the origination of decorin suppression. We hypothesize that the overexpression of APE1 in early stages of oral cancer leads to diminished decorin translation, which may drive cancer progression and increase mortality. Better understanding how APE1 influences tumor suppressors may eventually aid in development of therapeutics that would increase patient survival.
ously undescribed role of Mst in colon cancer progression, malignancy and therapy resistance potentially by regulating cell survival in manners dependent on β-catenin/c-Myc/Survivin/Bcl-xl signaling.

#4144 Role of CTGF in hyperthermia resistance in ovarian and uterine cancers. Hiroto Hatakeyama,1 Sherry Y. Wu,1 Yasmin A. Lyons,2 Sunila Pradeep,2 Wanzin Wang,2 Qian Huang,2 Karem A. Court,2 Tao Liu,2 Song Nie,2 Christian Rodriguez-Aguayo,2 Fangrong Shen,2 Yan Huang,2 Takeda Hisamatsu,2 Takashi Mitamura,2 Piotr L. Dorniak,2 Lingegowda S. Mangala,2 Marco Petrillo,2 Madeline Torres-Lugo,2 Karin D. Rodland,2 Anna Fagotti,2 Gabriel Lopez-Berestein,2 Chun Li3 Anil K. Sood,2 Chiba University, Chiba, Japan; 2The University of Texas MD Anderson Cancer Center, Houston, TX; 3University of Puerto Rico-Mayaguez, Mayaguez, Puerto Rico; 4Pacific Northwest National Laboratory, Richland, WA; 5Catholic University of the Sacred Heart, Rome, Italy.

Objective. Even though hyperthermia is a promising treatment for cancer, multiple obstacles remain to be cleared. Among these, the tumor temperatures that must be reached for obtaining clinical efficacy are undefined. The purpose of our study was to identify the molecular predictors of response to hyperthermia in ovarian and uterine cancers. Methods. The temperature transition in tumors during hyperthermic intraperitoneal chemotherapy (HIPEC) in ovarian cancer patients was examined. To evaluate sensitivity to hyperthermia, 10 ovarian and uterine cancer cells were treated with hyperthermia and determined median lethal temperature 50 (LT50). An integrative analysis was performed to identify molecules associated with hyperthermia resistance by comparison with gene and protein expression between hyperthermia-resistant (HTR) and -sensitive (HTS) cells. The effect of identified gene silencing on sensitization to hyperthermia and tumor growth in orthotopic models of ovarian cancers by using liposomal siRNA and local hyperthermia by NIR and CuS nanoparticles. Results. The temperature during HIPEC in human tumors was < 40°C even though the perfusion temperature at the entrance was maintained at 42.5°C. Based on LT50, we identified 5 cell lines as HTR (SKOV3, HeyA8, KLE, PEO4, and ES2; LT50 47.5 to 5 – 0.3°C) and 5 as HTS (A2780, A2780CP20, HeLa-A1, SK-KUT-2, and ISHKAWA; LT50 45.5 ± 0.2°C). Gene expression studies identified 15 genes that were highly upregulated in HTR compared with HTS cells. Proteomic analyses showed that glucose metabolism-related proteins were down-regulated in SKOV3 cells after hyperthermia. Pathway analysis indicated that FN1, SGK1, and CTGF among the 15 genes were potentially connected with glucose metabolism-related proteins. CTGF siRNA sensitized HTR cells to hyperthermia. The combination of CTGF silencing and local hyperthermia significantly inhibited tumor growth and metastasis in HeyA8 and SKOV3 orthotopic models. Conclusion. Collectively, we identified CTGF as a key target for enhancing response to hyperthermia in ovarian and uterine cancers.

#4145 Concurrent treatment with Pim kinase inhibitor decreases alternative non-homologous end-joining repair of DNA damage induced by topo-isomerase 2 inhibitors in cells with FLT3-ITD. Chashma A. Doshi,1 Pratik K. Nagaria,2 Adriana E. Troy,2 Feiyu V. Rassooll,2 Maria R. Baer1. 1University of Maryland Greenebaum Comprehensive Cancer Center and Veterans Affairs Medical Center, Baltimore, MD; 2University of Maryland Greenebaum Comprehensive Cancer Center, Baltimore, MD; 3Oncology IMED, Astrazeneca, Waltham, MA.

Purpose: Internal tandem duplication of fms-like tyrosine kinase 3 (FLT3-ITD) is a common (30%) molecular abnormality in acute myeloid leukemia (AML), associated with a high relapse rate and short disease-free survival. The oncogenic kinase Pim-1 is upregulated downstream of FLT3-ITD and contributes to its proliferative and anti-apoptotic effects. We recently showed that Pim kinase inhibition sensitizes AML cells with FLT3-ITD to apoptosis induction by topo-isomerase 2 inhibitors (TOI-2) by increasing both SSB and DSB through alternative NHEJ repair activity. Metabolic turnover of CTGF and alternative NHEJ repair activity may contribute to disease progression in AML patients with FLT3-ITD.

#4146 Selinexor synergizes with DNA damaging agents through down-regulation of key DNA damage response genes. Trinayan Kashyap, Christian Argueta, Boris Klebanov, Oscar Gonzalez, Erkan Babagol, Yusuf Landesman, Margaret Lee, Humphrey Gardner, Sharon Shacham, William Senapedia. Karyopharm Therapeutics, Newton, MA.

Background: Selinexor is a first-in-class, orally bioavailable SINE (Selective Inhibitor of Nuclear Export) compound currently in phase 1/2 clinical trials of patients with solid and hematological malignancies. SINE compounds bind exportin 1 (XPO1/CRM1) and inhibit nuclear export, resulting in nuclear retention of key DNA damage response (DDR) proteins. We previously studied the effect of Selinexor on DDR and showed enhanced anti-tumor activity when combining selinexor with standard DNA damaging agents (DDA) in pre-clinical models and clinical studies. We hypothesize that a reduction in DDR protein expression by selinexor drives the synergy observed in combination with DDAs. Methods: Solid and hematological cancer cell lysates treated with selinexor +/- DDAs (cisplatin, doxorubicin, docetaxel and gemcitabine) were analyzed by immunoblotting and qPCR. Comet assay and γH2A.X staining were analyzed by immunofluorescence to evaluate DNA damage. Mice bearing MDA-MB-231 (triple negative breast cancer) tumors were treated with sub-toxic doses of selinexor (2.5 mg/kg), cisplatin (4 mg/kg) or docetaxel (4 mg/kg) alone or in combination. Tumor growth and mouse weight were followed for 25 days. Results: Selinexor reduced the expression of DRR mRNAs and proteins involved in single-stranded (SSB) and double-stranded break (DSB) repair as early as 2 hours post treatment. We observed a dose-dependent correlation between the degree of DDR protein reduction and selinexor cytotoxicity in vitro. Selinexor prevented DDR activation following exposure to DDAs and led to synergistic cell death. Treatment of cells with selinexor plus DDA showed enhanced activity in comet assays and an increase in γH2A.X staining when compared to treatment with the single agents. Exposure to selinexor 6 - 24 hours after DDA treatment inhibited the DDR mechanism and was more synergistic than the opposite order (Sel DDA) or simultaneous treatment. In vivo, MDA-MB-231 tumor growth treatment with selinexor, docetaxel (SSB) or cisplatin (DSB) alone resulted in 66.7%, 51.5% and 26.6% TGI, respectively when compared to vehicle control whereas sequential treatment of docetaxel or cisplatin followed by selinexor resulted in 93.9% TGI or 96.6% tumor regression, respectively. No significant weight loss was seen in any of the groups by the end of the study. IHC staining and immunoblot analysis of tumor tissue and sections showed a reduction of DDR proteins. Conclusion: Selinexor synergizes with DDAs by increasing both SSB and DSB through inhibition of relevant DDR proteins. This data supports clinical development of selinexor in combination with standard chemotherapeutic agents in patients with solid and hematological malignancies. The results also suggest that sequential treatment of patients with DDAs followed by selinexor might be more beneficial.
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tered tumor stem cells (TSCs) and as such are highly desirable targets for novel therapeutic strategies. Doublecortin-like Kinase 1 (DCLK1) has recently been identified as a tumor stem cell marker in the intestine and pancreas and is dysregulated in many solid tumor cancers including lung cancer. Increased expression has been demonstrated to correlate with poor survival in several tumor types. Thus, DCLK1 is a highly promising target for rational targeting of DCLK1 expressing cells in NSCLC. Methods: Small interfering RNA (siRNA) against DCLK1 was transfected into NSCLC cell lines (A549 - wt-p53; H1299 – homozygous deletion of p53) of highly drug resistant (A549) and metastatic (H1299). Cell proliferation, colony formation, self-renewal, cell migration and invasion and drug resistance assays were performed to assess the tumor stem cell characteristics. A549 cells were treated with siDCLK1 and cisplatin in order to assess the role of DCLK1 to reverse the drug-resistance. A549 cells were utilized to generate tumor xenograft in order to assess the role of DCLK1 knockdown on NSCLC tumorigenesis in vivo. The data were generated utilizing experimental protocols for clonogenic culture, immunohistochemistry, RT-qPCR and Western blotting. Results: Silencing DCLK1 via RNA interference decreased the colony formation (p<0.001), and self-renewal ability (p<0.0001) of the NSCLC cell lines in vitro. Knocking down DCLK1 reduced (p<0.0001) NSCLC cells migration and invasion in vitro. Furthermore, DCLK1 knockdown decreased the expression of EMT associated factors (Slug, Snail, Twist, Zeb1/2, Vimentin) and pluripotency factors. Initial cisplatin treatment results in the isolation of cisplatin resistant (GISO 168/M) cells. Retreatment of cisplatin resistant A549 cells results in greater resistance and survival ability. However, combination therapy of these resistance cells with siDCLK1 and cisplatin overcame cisplatin-induced resistance, resulting in greater cell death compared to cisplatin or siDCLK1 alone treatments. Nanoparticle encapsulated siDCLK1 treatment in xenograft tumors resulted in tumor growth arrest in vivo. Conclusions: NSCLC cells highly express DCLK1 display enhanced self-renewal, increased migration and invasion. Combination therapy of siDCLK1 with cisplatin overcame cisplatin induced drug resistance. Targeting DCLK1 reduced tumor xenograft in vivo. Taken together these data suggest that DCLK1 inhibition reduces TSC related properties, overcome drug resistance and inhibits tumor growth, thus making DCLK1 targeted therapy an attractive tool for combating NSCLC.


DRD2 is a G protein-coupled receptor (GPCR) that is overexpressed in many cancers, controls an array of pro-survival signaling pathways, and its antagonism causes anti-cancer effects. ONC206, the founding member of the imipridone class of compounds, is a small molecule DRD2 antagonist that is in Phase I/II advanced cancer clinical trials. In this study, we evaluated the binding target and antitumor activity of ONC206, a chemical analogue of ONC201. An orphan small molecule target prediction algorithm revealed that ONC206, like ONC201, antagonizes DRD2. Experimental GPCR profiling using the PathHunter® B-Arrestin assay, determined that ONC206 selectively antagonizes the D2-like (DRD2/3/4), but not the D1-like (DRD 1/5), subfamily of dopamine receptors. ONC206 possesses a ~10-fold increased affinity for DRD2 compared to ONC201 with a Ki of ~320nM with the selectivity that was superior to approved antipsychotics. The increased association rate for the ONC206-DRD2 interaction was responsible for the increased affinity, whereas the dissociation rate was similar to ONC201 and atypical antipsychotics that are well tolerated. TCGA analysis and immunohistochemistry of patient-derived tissue microarrays revealed DRD2 was overexpressed in neuroblastoma, sarcoma and pheochromocytoma specimens relative to normal tissues. In vitro efficacy profiling of ONC206 in the Genomic of Drug Sensitivity in Cancer collection of cell lines revealed broad efficacy across most tumor types (G15O <78-889M). Bone cancer and neuroblastoma were identified as the most ONC206-responsive solid tumor types that were comparatively less responsive to ONC201. Within bone cancer cell lines, Ewing’s sarcoma (n=16) was the most sensitive to ONC206 with a median IC50 of 168-303nM that was nearly 40-fold higher in efficacy than neuroblastoma (n=35, G15O 87-899M) including cell lines derived from metastatic sites and with MYCN amplification associated with poor prognosis. In the PC12 rat pheochromocytoma cell line ONC206 (G150 200nM) was superior to ONC201. ONC206 time-course experiments revealed anti-cancer effects occurring at 48-72 post-treatment, similar to ONC201. In support of a wide therapeutic window, ONC206 reduced the viability of normal fibroblasts (HFF-1) at relatively high doses (G150 > 5pM). Efficacy evaluations in MHH-ES-1 athymic nude mice xenografts demonstrated that ONC206 (100 mg/kg PO every 10 days) causes significant tumor growth inhibition that was comparable to methotrexate (MTX; 400 mg/kg, IP) while being better tolerated. In summary, ONC206 is an imipridone that acts as a selective antagonist of DRD2 at nanomolar concentrations and has broad-spectrum anti-tumor activity. ONC206 may address tumor types where the properties of ONC201 do not permit for complete therapeutic engagement in vivo.

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#4148 Novel ERX-11 and CDK4/6 inhibitor combination therapy for treating therapy resistant breast cancer. Suryavathi Viswanadhapalli1, Ganagadha Reddy Sareddy1, Shi-Hong Ma1, Tae-Kyung Lee2, Rajeshwar Rao Tekulu1, Jung-Mo Ahn3, Ganesh Raj4, Ratna K. V. Vadlamudi5, 1UTHSCSA, San Antonio, TX; 2University of Texas Southwestern Medical Center at Dallas, Dallas, TX; 3University of Texas at Dallas, Richardson, TX.

BACKGROUND: The majority of the breast cancer is estrogen receptor alpha (ESR1) positive. While tamoxifen and letrozole therapies are effective, therapy resistance is common. Importantly, both therapy-sensitive and therapy-resistant breast cancer cells retain ESR1 signaling, via interaction with critical oncogenic co-regulator proteins. Further, resistant tumors commonly acquirecyclin D1:CDK4/6 signaling via multiple mechanisms, cyclin D1 can independently activate ESR1 and thus contribute to estrogen independence of ESR1+ tumors. Currently, CDK4/6 inhibitors in clinical trials for treating breast cancer, however, considering complex signaling interplay of estrogen and CDK axis, combination therapy of CDK inhibitor with other potent ESR1 targeted agents may have better utility and may prevent development of resistance to the CDK4/6 inhibitors. We recently developed a small organic molecule, ESR1 coregulator binding inhibitor ERX-11 (EtiRaRx-11). The objective of this study is to test the utility of novel combination therapy of ERX-11 with CDK4/6 inhibitor palbociclib in treating therapy resistant cancer. METHODS: We have utilized multiple therapy sensitive and therapy-resistant models with various genetic back-grounds. We tested efficacy using both acquired resistance and engineered models that express ESR1 mutations or oncogenes. Efficacy of combination therapy was tested using established in vitro assays including, MTT, colony formation, apoptosis, and cell cycle progression. Mechanistic studies were conducted using reporter gene assays, gene expression and signaling alterations. Xenograft studies were performed to determine the in vivo efficacy of the combination therapy. RESULTS: ERX-11 effectively blocked ESR1-mediated oncogenic signaling and has potent anti-proliferative activity against therapy-sensitive and therapy-resistant breast cancer cells. Mechanistic studies showed that ERX-11 blocks the interaction between a subset of coregulators with both native and mutant forms of ESR1. ERX-11 showed potent activity in both preclinical xenograft models and patient-derived breast tumor explant models. Co-treatment of ERX-11 with palbociclib synergistically reduced cell viability and induced apoptosis of therapy sensitive and resistant breast cancer model cells. Importantly, combination therapy of ERX-11 and the palbociclib synergistically reduced the growth and induced apoptosis of tamoxifen and letrozole resistant xenograft tumors compared to either drug alone. Mechanistic studies showed combination therapy significantly altered E2F1 and ESR1 signaling pathways and promoted apoptosis. CONCLUSIONS: Collectively our studies have discovered a novel combinational treatment with ERX-11 and palbociclib for patients with therapy-sensitive and therapy-resistant breast cancers.

#4149 Increased migration ability of osimertinib-resistant EGFR-T790M mutant non-small-cell lung cancer cells. Satoshi Kambayashi, Yasuhiro Koh, Maia Ohyama, Ayaka Tanaka, Hiroaki Akatsuka, Nahomi Tokudome, Hiroki Ueda, Nobuyuki Yamamoto. Wakayama Medical University, Wakayama, Japan.

Background: A third generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) osimertinib is effective against gefitinib/erlotinib-resistant non-small-cell lung cancer (NSCLC) harboring EGFR T790M mutation. Although the majority of these patients initially responds to osimertinib, they eventually develop resistance. Acquired mutations such as EGFR C797S mutation have been reported to confer resistance to osimertinib, but mechanisms of resistance to osimertinib or biological behaviors of
osimertinib-resistant NSCLC have not been fully elucidated. Materials and methods: Osimertinib-resistant PC-9/ZD/OR and PC-9/ZD/OR cells were generated after continuous exposure of gefitinib-resistant PC-9/ZD cells harboring both EGFR exon 19 deletion and T790M mutations to increasing concentrations of osimertinib. Growth-inhibitory effect of inhibitors gefitinib and osimertinib was evaluated by MTT assay. Colony formation assay was performed in/on xenograft tumor tissue that was examined by RainDrop Digital PCR System (RainDance Technologies). Immuno- blot analysis was performed to investigate the modulation of molecules relevant to EGFR signaling pathways. Cell adhesion assay, wound closure assay and transwell assay were performed to evaluate the ability of cell adhesion and migration. Results: Both PC-9/ZD/OR and PC-9/ZD/OR cells showed 50- to 120-fold resistance to osimertinib compared to parental PC-9/ZD cell lines, and acquired resistance to gefitinib as well. PC-9/ZD/OR cells maintain EGFR exon 19 deletion and T790M mutations and C797S mutation was not detected. Although phosphorylation of EGFR and ERK1/2 were inhibited by osimertinib treatment in PC-9/ZD/OR cells, phosphorylation of Akt was not inhibited. PC-9/ZD/OR cells express the elevated level of c-MET and its phosphorylated form, suggesting that bypass signaling is activated to evade EGFR pathway blockade. PC-9/ZD/OR cells also express marked level of E-cadherin which parental PC-9/ZD cells do not express. PC-9/ZD/OR cells exhibited enhanced adherent activity, cell motility and migration ability compared to PC-9/ZD cells. Notably, osimertinib treatment significantly reduced the number of migrated cells not only in PC-9/ZD cells but also in PC-9/ZD/OR cells. These results suggest that osimertinib treatment in PC-9/ZD/OR cells can overcome through c-MET pathway activation and/or induction of E-cadherin. Osimertinib treatment may be still effective to inhibit cell migration, suggesting that cell migration and cell growth are driven by different signaling pathways in PC-9/ZD/OR cells. Conclusion: c-MET overexpression was suggested to confer resistance to osimertinib and the resistant cells enhanced cell migration. Continuous osimertinib treatment may be beneficial in preventing metastasis even after the failure and involvement of c-MET and E-cadherin in osimertinib resistance should be further investigated.

#4150 Anti-tumor activity of the PI3K/mTOR pathway inhibitors albopilisib (BYL719) and everolimus (RAD001) in xenograft models of acquired resistance to CDK4-6 targeted therapy. Neil A. O’Brien,1 Dylan Conklin,1 Tong Luo,1 Raul Ayala,1 Shawnt Issakhanian,1 Ondrej Kalous,1 Erika Von Euw,1 Christie, Timothy F. Burns. 1Univ. of Pittsburgh Cancer Inst., Pittsburgh, PA

In this study, we evaluated the efficacy of a p110α-selective PI3K inhibitor, albopilisib (BYL719) and the mTORC1 specific inhibitor, everolimus (RAD001), in ER+/HER2- xenograft tumors conditioned in vitro and in vivo to acquire ADCC resistant mechanism, we established ATP6V1B1 knockout SK-BR-3. The cytotoxicity of ADCC resistant SK-BR-3 was significantly reduced as compared to control SK-BR-3 (38.7% and 67.4%, respectively). Four pathological complete response (pCR) cases after NAC and 4 non pCR cases were included. We found that non pCR cases showed significantly week expression of the ATP6V1B1-knockout SK-BR-3 were significantly reduced as compared to control SK-BR-3 (38.7% and 67.4%, respectively). To sort candidate genes for the resistance, we analyzed the gene expression profile of ADCC resistant SK-BR-3 by using DNA microarray and we focused on ATP6V1B1 gene that was significantly reduced on the resistant cells. We investigated ATP6V1B1 mRNA level of SK-BR-3 and resistant cell using real time PCR and found the 50% reduction of ATP6V1B1 mRNA level in ADCC resistant cells. To evaluate the role of ATP6V1B1 on the trastuzumab mediated ADCC resistant mechanism, we established ATP6V1B1 knockout SKBR3 by using CRISPR/Cas9 system. We cultured from small numbers of the knock out cells and expand to obtain completely knock out cells. We confirmed that the ATP6V1B1 knockout cell line was knocked out by western blotting and immunochemistry. We found that ATP6V1B1-knockout SK-BR-3 were significantly less ADCC activity as compared to control SK-BR-3 (41.2% and 12.5%, respectively). Based on the in vitro findings, we evaluated the role of ATP6V1B1 expression in HER2 positive breast cancer patients who are treated with neoadjuvant chemotherapy (NAC) containing trastuzumab. Flowcytometry before and after NAC were stained with ATP6V1B1 immunohistochemically. Four pathological complete response (pCR) cases after NAC and 4 non pCR cases were included. We found that non pCR cases showed significantly week expression of the ATP6V1B1 protein as compared to that of pCR cases. ATP6V1B1 gene is one of the isoforms of the Vacular (V)-ATPase which is located in intracellular membrane and regulate the cytosolic pH and membrane trafficking. It is hypothe- sized that ATP6V1B1 is involved in somewhere through the process of cytox- today mediated by perforin and granzymes and now we are trying to explore the mechanisms further.

#4152 The p90RSK-CD2C25 signaling pathway leads to bypass of the ganetespib induced G2/M arrest and mediates acquired resistance to ganetespib in KRAS mutant NSCLC. Suman Chatterjee, Eric H. Huang, Ian Christie, Timothy F. Burns. Univ. of Pittsburgh Cancer Inst., Pittsburgh, PA

Lung cancer is the leading cause of cancer death in the United States and worldwide. A large fraction of non-small cell lung cancers (NSCLC) are dependent upon oncogenic driver mutations of which the most commonly observed driver is mutant KRAS. Unfortunately, NSCLC patients with oncogenic KRAS mutations have no effective therapies and prognosis is poor. As direct RAS targeting has been unsuccessful in the clinic to date, use of Heat shock protein 90 (Hsp90) inhibitors in KRAS mutant NSCLC appeared to be promising approach for targeting KRAS mutant NSCLC through its downstream effectors. However, limited clinical efficacy as monotherapy was observed due to rapid resistance. Furthermore, the combination of ganetespib and docetaxel was recently tested in a large phase III clinical trial (Galaxy-2) in advanced lung cancer and failed to demonstrate benefit. Here, we investigated the mechanism(s) of resistance to the Hsp90 inhibitor (Hsp90i), ganetespib by generating NSCLC cells with acquired resistance to Hsp90i’s including ganetespib (GR cells). We have not only iden- 

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that this mechanism induces cross resistance to docetaxel. Finally, we have identified novel Hsp90 combinations that can overcome and prevent this resistance. We report that hyperactivation of ERK and p90RSK and its downstream target, CDC25C leads to acquired resistance to ganetespib and docetaxel. Moreover, this resistance is mediated via bypass of a G2/M arrest. Overexpression of either p90RSK or CDC25C in naive cells was sufficient to induce the loss of this G2/M arrest as well induced resistance to both ganetespib and docetaxel. Remarkably, p90RSK or CDC25C overexpression also led to ganetespib resistance in vivo. The observed resistance was dependent on continued p90RSK/CDC25C signaling, as synthetic lethality to specific ERK, p90RSK or CDC25C inhibitors was observed. Importantly, we have found that the combination of ganetespib with either p90RSK or CDC25C was highly efficacious. In summary, we propose that the hyperactivation of p90RSK induces CDC25C overexpression and activity which then induces G2/M progression via CyclinB1/cdc2 regulation resulting in ganetespib resistance. Despite two decades of testing in the clinic, either as monotherapy or in combination with chemotherapy, Hsp90 inhibitors have been ineffective due to acquired resistance. Our preclinical analyses provide a way forward for Hsp90 and we have developed novel rationally designed Hsp90 inhibitor combinations that may prevent or overcome resistance to Hsp90 inhibitors.

**#4153 CRISPR/Cas9-based oncoediting enables characterization of rare mutations in cancer cells.** Dennis Plenker, Martin L. Sos. University Hospital Cologne, Cologne, Germany.

In the past decade new insights into key oncogenic pathways and the development of targeted strategies enabled the success of precision medicine. However, only a small part of the cancer genome can be targetted effectively. This is in part due to the fact that the number of cancer cell models for a given oncogenic mutation is limited. We hypothesized that CRISPR/Cas9-mediated oncoediting may enhance the ability to functionally study rare genomic lesions or resistance mutations that arise in patients under therapy. As a proof of concept we established genetically modified PC9 lung cancer cells that harbor an activating EGFR ex19 deletion (EGFRdel E746-A750I) into double mutated (EGFRdel E746-A750I; T790M) or triple mutated (EGFRdel E746-A750I; T790M; C797S) EGFR in order to induce resistance against known EGFR inhibitors. These cell lines fully recapitulated the phenotypic response pattern to first and third generation EGFR inhibitors that have been observed in patients harboring these clinically relevant resistance mutations. In a next step we switched the dependency of PC9 cells from the intrinsic oncogenic EGFR mutation into a rare ex20 SVDAS mutation (EGFRdel T790M; N772I;A781V) that is typically associated with resistance towards all known EGFR inhibitors. Again, we observed a shift in the response to targeted inhibition of EGFR when compared to parental cells. Since the methodological principles can be applied to an array on known oncogenic mutations our approach may serve as a versatile platform for the functional study of highly frequent and rare mutations that are found in patients. We envision that such reprogrammed cells may be of use for the screening of novel targeted therapeutics and therefore may enable the development of mutation specific drugs that are currently lacking for the majority of patients.

**#4154 Resistance to BET inhibitors involves the β-catenin pathway in uveal melanoma.** Grazia Ambrosini, Benjamin Tycko, Chaterine Do, Gary K. Schwartz. Columbia University, New York, NY.

Uveal melanoma (UM) is an aggressive intraocular malignancy with high tendency to metastasize to the liver. Currently available drugs have shown limited clinical activity in patients with UM and there is an urgent need for new effective therapies. Recent findings from our laboratory demonstrated that UM cells are sensitive to BET inhibitors (BETi) through the induction of cell cycle arrest and apoptosis. However, despite the initial inhibitory effects, UM cells acquire resistance to this class of drugs following chronic drug exposure. In order to understand the mechanistic basis for BETi resistance in UM, we assessed genome-wide CpG methylation patterns in UM cell lines that had been rendered resistant to the clinical BET inhibitor PLX31107 (Pluxxon, Berkeley, CA) followed by chronic exposure. 2-fold loss between BET inhibitor-resistant versus sensitive cell lines revealed differential methylation of 1700 genes, including several involved in Wnt/β-catenin signaling, as well as other signaling pathways. Immunoblotting analysis confirmed that β-catenin protein was induced and activated in the resistant cells, while depletion of β-catenin by siRNA re-sensitized the resistant cells to the BET inhibitor. We then explored several combinations of PLX31107 with drugs that block β-catenin transcriptional activity through inhibition of its phosphorylation by PAK4 or through the inhibition of binding partners like Cdk8 and CBP. All these combinations increased the activity of the BET inhibitor in both sensitive and resistant cells, and these effects were synergistic, with combination indices (CI) <1. These observations support the evidence that acquired resistant to BET inhibitors is mediated, at least in part, by activation of the Wnt/β-catenin pathway, and inhibitors of this pathway may provide a means to overcome acquired BET inhibitor resistance in UM patients treated with this class of drugs.

**#4155 Crosstalk of SOX2 and epithelial-to-mesenchymal transition on EGFR-TKI resistance.** An-Chun Lee,1 Yu-Fan Chiu,1 Ming-Han Kuo,1 Yuan-Hung Wang,2 Yu-Ting Chou,1 National Tsing Hua University, HsinChu, Taiwan;2 Taipei Medical University-Shuang Ho Hospital, New Taipei City, Taiwan.

EGFR-tyrosine kinase inhibitors (TKIs) have been shown to produce profound therapeutic responses in lung adenocarcinoma harboring EGFR mutations. Despite this initial response, patients with EGFR-mutated lung adenocarcinoma ultimately developed resistance to EGFR-TKIs. Although both SOX2 and epithelial-to-mesenchymal (EMT) have been linked to the EGFR-TKI resistance in lung adenocarcinoma, the interplay between SOX2 and EMT in EGFR-TKI resistance is not known. Here, we report that cancer plasticity switched by SOX2 and EMT plays a critical role in EGFR-TKI resistance. We found that the selection of EGFR-TKI resistant cells induced EMT while attenuating SOX2 expression. We observed that EGFR-TKI treatment induced SOX2 expression in EGFR-mutated lung cancer cells, and SOX2 silencing encouraged EGFR-TKI resistance. Reselection of EGFR-mutated lung cancer cells with the EMT feature endowed cells with EGFR-TKI resistance but suppressed SOX2 expression. We found that TGF-β induced EMT but attenuated SOX2 expression, resulting in increased EGFR-TKI resistance. Pharmacological inhibition of HDACs in EGFR-mutated cells attenuated SOX2 expression but induced EMT, causing increased EGFR-TKI resistance. Enriching SOX2 expression in EGFR-mutated cells restored EMT and enhanced sensitivity to EGFRTKIs. Correlation analysis exhibited negative correlations between SOX2 and EMT markers in primary non-small cell lung cancer. Kaplan-Meier analysis revealed that a SOX2 low/Vimentin high signature predicted a poor survival in EGFR-mutated lung adenocarcinoma. Our findings support the notion that the cancer plasticity regulated by SOX2 and EMT plays a critical role in EGFR-TKI resistance, and SOX2 and Vimentin can function as prognostic biomarkers in cancer progression.

**#4156 Molecular and phenotypic characterization of breast and colorectal cancer cells selected for resistance to antibody-dependent cell-mediated cytoxicity (ADCC).** Tanaka Biswas,1 Rebecca Fritzemeier,2 Adam Mark,2 Tobias Meißner,3 Brandon Young,3 Brian-Leyland Jones,4 Mark Pegram1.

Monoclonal antibody (mAb) therapy is limited by clinical resistance. For example, alterations in PI3K/Akt / Ras-MAPK signaling, over activation/expression of alternate receptor kinases (c-Met / IGF-1R), or proteolysis of extracellular domains harboring target epitopes are postulated mechanisms of clinical resistance to trastuzumab. ADCC is a major mechanism of action for mAbs. Most studies have focused on cell models of resistance through in vitro selection, in presence of mAbs, in the absence of immune effector cells. We developed a unique model of immune selection, where target cells (HER2 +ve breast cancer cells SKBR3, BT474; and EGFR +ve colon cancer cells HT29, DLD1) were subjected to ADCC (>90% cell death) with saturating concentrations of trastuzumab or cetuximab (100 µg/ml), respectively, in the presence of peripheral blood mononuclear cells (PBMCs). Selection (in triplicate) was continued for 10 consecutive passages and surviving cells were allowed to grow to confluence over a period of 8-10 weeks. Mock-treated parent cells, IgG1 isotype control, mAb-alone and PBMCs only were utilized as controls. Compared to parent controls, immune-selected DLD1 and SKBR3 cells demonstrated statistically significant increase in proliferation (WST viability assays, P<0.01), and significantly higher colony forming efficiency (soft agar assays, P<0.001); immune-selected HT29 and SKBR3 cells showed significantly higher motility (trans-well migration assays, P<0.05, and P<0.001 respectively). In vitro ADCC assays (calcein labeling) suggested significant reduction (max 20%, P=0.005) in cell lysis in all the immune-selected cell lines compared to parent. Immune-selected HT29 cells demonstrated a 5-fold reduction in gene copy number of EGFR by fluorescence in situ hybridization (FISH), and 2.5 fold reduction in mRNA levels (qPCR, P< 0.01) and 1.8-fold reduction in protein levels of EGFR (densitometry scans, western blot analyses, p<0.01). Transcriptome-wide next-generation RNA sequencing (Illumina NextSeq 500, 2 x 75 bp pair-end, with over 100 million pair-end reads/sample), coupled with bioinformatic analyses (Reactome pathway database) revealed gene expression changes in ADCC-selected cells. These involve changes in key immune signaling pathways (e.g. HLA class I/II, MHC antigen processing, cell chemotaxis, inflammation) and immune regulatory pathways among others. Our data indicate immune-selection by effector cells contributes to ADCC resistance in vitro. Further investigation will determine whether mAb combination with an agonist antibody against CD137 (co-stimulatory NK cell receptor) or Fe-engineered mAbs (e.g. afucosylated) will...
sensitize the resistant cells. It is hoped that this will better inform mAb therapy by elucidating potentially targetable pathways/ markers that emerge from immune-selection with therapeutic mAbs.

**#4157** Co-blockade of mTORC1, ERBB and estrogen receptor signalling pathways in endocrine resistance breast cancer: Combating tumor plasticity. Ricardo Ribas,1 Sunil Pancholi,2 Stephanie K. Guest,1 Aradhana Rani,1 Joanna Nikitorowicz-Buniaik,1 Nikiana Simigdala,1 Allan Thornhill,2 Richard E. Cutler Jr,2 Alshad S. Lalan,1 Francesca Avogadri-Connors,2 Mitch Dowsett,2 Stephen R. Johnston,1 Leslie Ann Martin,1 Breast Cancer Now Toby Robins Research Centre, Institute of Cancer Research, London, United Kingdom;2 Centre, Institute of Cancer Research, London, United Kingdom; 2Centre for Cancer Drug Discovery, London, United Kingdom;2 Weifang Medical University, China;3 Sun Yat Sen University, China;4 Oregon Health & Science University, Portland, OR.

Introduction: The majority of breast cancers (BC) are estrogen (E) receptor positive (ER+). Endocrine therapies target E stimulation of tumour growth but resistance remains problematic, often a result of enhanced crosstalk between ER and growth factor pathways. Previously we reported the antiproliferative efficacy of combining everolimus (RAD001, mTORC1 inhibitor) with endocrine therapy in resistant models, but potential routes of escape from treatment via ERBB2/3 signalling were observed. We hypothesised that combined targeting of three cellular nodes (ER, ERBB and mTORC1) may target tumour rewiring and provide enhanced long-term clinical utility in endocrine resistant BCs. Methods: Several ER + BC lines adapted to long term E deprivation (LTED), modelling relapse to an aromatase inhibitor (AI), were treated in vitro with a combination of RAD001 and neratinib (pan ER inhibitor) in the presence or absence of estradiol (E2), tamoxifen or fulvestrant. Effects on proliferation, cell signalling, cell cycle and transcription were assessed. Additionally, an in vivo model of AI resistance was treated with monotherapies or combinations to evaluate efficacy in delaying tumour progression. Results: All cell lines showed dose dependent decreases in proliferation in response to RAD001 (IC50 0.6-50nM without E2; 1-10nM with E2). Neratinib showed a wide range of IC50 values in the presence of E2 (300-1000nM). In the absence of E2, wild type (wt) cell lines showed IC50 values in excess of 1800nM with hormetic response curves, whilst in the LTED ic50 values were 400-900nM. Our data suggest that in addition to either anti-ER or anti-ERBB therapies, endocrine therapy caused a concentration dependent decrease in proliferation in both wt and LTED lines but the maximum effect was observed with a triple combination of RAD001, neratinib and endocrine therapy. Expression of pS6 was suppressed by RAD001 in all cells tested, whilst neratinib caused a cell specific reduction in expression of ERBB family proteins. Upregulation of pAKT was observed in all cell lines upon treatment with RAD001. Combination of RAD001 with neratinib suppressed the upregulation of pAKT and reduced cell cycle progression. In the absence of E2, RAD001 reduced ER mediated transcription and recruitment of ER and CREB binding protein to the TFF1 promoter, contrasting with neratinib, which caused a marked increase. In vivo study using LTED tumour xenografts showed the triple combination of RAD001, neratinib and fulvestrant was the most effective at reducing tumour volume (98% at a single clinical reference lab between March 2004 and July 2006. Pt and BM data were analyzed retrospectively on 582 human LC specimens including 311 adenocarcinomas (ADCA), 131 non-small cell lung cancers (NSCLC), and 140 squamous cell carcinomas (SCCQ) resected from Pts diagnosed with primary (PD), recurrent (RD) or metastatic disease (MD), and tested at a single clinical reference lab between March 2004 and July 2006. Pt and specimen information were unlinked directly and anonymously. IVDR was determined in particular allost of Pts’ viable tumour cell suspensions after 5-day exposures to single physiologic doses of Chemo (CARBO, CML, GMCR, NVBL, TAXOL, TXTR, VP16, TOPO). IVDR was bifurcated into extreme resistance (ER, non-response) or low resistance (LR, response). Standard immunohistochemistry (IHC) was performed on the same Pts’ paraffin-embedded LC specimens. Results: Overall the sensitivity of BM to toxic ineffective Chemo. In such settings, disease progression may negatively impact a Pt’s tolerance to further Chemo. Pre-assessment of IVDR and BMs may identify ER factors, stratify treatment, and potentially improve clinical outcomes.


Aromatase inhibitors (AI) are the mainstay for treatment of postmenopausal estrogen receptor positive (ER+) primary breast cancer (BC). However, many BCs will progress to become resistant whilst retaining ER positivity. Resistance to ER targeting agents (SERD) fulvestrant has been evaluated as a potential 2nd or 3rd line therapy for patients who relapse on AI treatment. Although exhibiting promising potential, its low bioavailability has limited its use to combination therapy in metastatic patients. Here, we assessed the efficacy of the orally available SERD, RAD1901, in a panel of BC cells with varying genetic backgrounds modelling patients both sensitive and resistant to AI therapy (LTED). Fulvestrant was used in parallel to allow relative responses to be compared. Cell proliferation assays in 2D and spheroids the presence of 0.01nM 17β-estradiol showed a concentration-dependent decrease in proliferation in response to RAD1901 and fulvestrant. GI50 values for RAD1901 in general were 10-fold higher than fulvestrant but equated to doses that are clinically achievable for RAD1901. Most importantly, RAD1901 effectively suppressed proliferation of two LTED models harbouring naturally occurring ESR1 mutations, MCF7 LTEDH11001 (GI50 5nM) and SUM44-LTEDH11021 (GI50 >100nM). GI50 values of RAD1901 and fulvestrant showed similar reduction of ER, progesterone receptor (PGR) and cyclin D1 together with decreased phosphorylation of retinoblastoma (RB), concordant with cell cycle arrest. Furthermore, chromatin immunoprecipitation (ChiP) for ER in response to RAD1901 or fulvestrant showed a 70% reduction in recruitment of ER to TFF1, GREB1 and PGR promoters and concomitant reduction in mRNA expression of these genes. In MCF7-Arom cells, combination of letrozole with RAD1901 or fulvestrant showed enhanced antiproliferative effect compared to letrozole alone. The addition of RAD1901 to CDK4/6 inhibitor palbociclib or abemaciclib demonstrated additivity compared with monotherapy as addition of RAD1901 effectively inhibited growth of palbociclib-resistant MCF7 LTED cells. These preclinical findings highlight the potential utility of RAD1901 as a potent drug in the treatment of ER+ BC. Combined with its bioavailability profile, RAD1901 warrants clinical testing versus fulvestrant after relapse on an AI, either alone or in combination with a CDK4/6 inhibitor.
Increased expression of ABCB1 could be associated with osimertinib resistance in non-small cell lung cancer cell line PC9 cell. Takashi Nomizo, Hiroaki Ozasa, Takahiro Tsuji, Yuto Yasuda, Tomoko Funazo, Hiroroni Yoshida, Yuichi Sakamori, Hiroki Nagai, Young Hak Kim. Kyoto University, Kyoto, Japan.

Osimertinib, a third-generation EGFR Tyrosine kinase inhibitor, has shown promising activity in EGFR mutation positive non-small cell lung cancer (NSCLC) which harboring T790M mutation. The mechanisms of resistance to osimertinib have been still unclear. To investigate the mechanisms of acquired resistance to osimertinib, we established the osimertinib-resistant cell line (Pc9/Os) from the NSCLC cell lines PC9, and measured the expression levels of protein blocking cassette (ABC) transporters using quantitative real-time polymerase chain reaction. Expression profiling of ABC transporters revealed that the levels of ABCB1 gene expression were significantly increased in the Pc9/Osimertinib cells compared with the parental cells, but not other ABC transporters. Knockdown of ABCB1 expression using short hairpin RNA enhanced osimertinib cytotoxicity in PC9/osimertinib cells. These data suggest that increased expression of ABCB1 may have an important role in the acquired resistance to osimertinib.

Activation of aryl hydrocarbon receptor leads to resistance to targeted therapy through contextual phosphorylation of Src by Jak2 kinase. Stanam, Katherine N. Gibson-Corley, Nalini Yoshida, Jian Zhang. Xijing Hospital, Fourth Military Medical University, Xi’an, China.

The discovery of epidermal growth factor receptor (EGFR) activating mutation, antastatic lymphoma kinase (ALK) and ROS1 kinase rearrangement serve as predictive biomarkers for clinical response in non-small cell lung cancer (NSCLC) patients who receiving tyrosine kinase inhibitors (TKIs). Unfortunately, resistance to TKIs inevitably occurs within years of treatment. The emergence of resistance mutations and bypass receptor tyrosine kinase tracks have been implicated in targeted therapy resistance, however, a large proportion of patients who relapsed on TKIs do not harbor resistance mutations or additional receptor tyrosine kinase tracks. The aryl hydrocarbon receptor (AhR) is generally recognized as a transcriptional factor which encodes metabolic enzymes and mediates biochemical response to environmental xenobiotic pollutants. However, the association of AhR and sensitivity to targeted therapy has not been reported. In this study we used a high throughput approach to screen the expression of AhR target genes in NSCLC cells. qPCR and Western blot screening of a panel of NSCLC cell lines suggested that the AhR(high) cells were sensitive to EGFR TKIs, whereas the AhR(low) cells did not. Simultaneously inhibition of AhR and EGFR signaling restored the sensitivity to EGFR TKIs in the AhR(low) cells. Moreover, overexpression of AhR also led to resistance to ALK and ROS1 TKIs by restoring phosphorylation of Akt and Erk. Measurement of endogenous AhR ligand content by high performance liquid chromatography (HPLC) indicated that the patients had more kynurenine (Kyn) in their serum after disease relapse and progression. The AhR-associated targeted therapy resistance did not require its transcriptional activity because the AhRΔNLS truncated mutant also drives resistance. By using large scale shRNA screening, we showed that the non-receptor tyrosine kinase Src phosphorylated Akt and Erk and mediates AhR-associated targeted therapy resistance. Bioinformatics analysis and immunoprecipitation assay showed that Src is immediately downstream of AhR upon AhR ligands treatment through binding with the SH2 domain of AhR. The phospho-protein kinase array and in vitro kinase assay revealed that Jak2 kinase directly phosphorylated Src, in which the liganded AhR provided docking sites for Jak2 and Src interaction. Collectively, our study revealed an unexplored, transcription-independent function of AhR. The liganded AhR recruits Src and provides docking sites for Jak2-Src interaction, in which the phosphorylated Src acts as a resistance bypass track. Kyn content might be a biomarker to predict resistance to targeted therapy and targeting AhR helps to overcome resistance.

Blockade of the interleukin-1 signaling pathway overcomes erlotinib resistance in head and neck cancer cells. Andreas L. Simons, Aditya Stanam, Katherine N. Gibson-Corley. University of Iowa, Iowa City, IA.

The EGFR tyrosine kinase inhibitor erlotinib has demonstrated poor clinical response rates for head and neck squamous cell carcinoma (HNSCC) to date and the majority of respondents acquire resistance to erlotinib relatively quickly. Our previous data has shown that erlotinib activated interleukin-1 (IL-1) expression and signaling in HNSCC cells in vitro. We therefore investigated if upregulation of IL-1 signaling is involved in erlotinib resistance in HNSCC cells. We compared gene expression profiles of erlotinib-resistant (ER) and the parenteral erlotinib-sensitive (ES) HNSCC cell lines and observed a deregulation of the IL-1 signaling pathway in ER versus ES-HNSCC cells using microarray enrichment analysis. Secretion of IL-1 alpha (IL-1α) and IL-1 beta (IL-1β) were not significantly different in ER-SQ20B and ER-CAL 27 cells compared to their respective ES-cells, however secretion of the IL-1 receptor antagonist (IL-1RA) was reduced in ER compared to ES cells. Together, blockade of the IL-1 pathway with Eflivirus could overcome erlotinib resistance in HNSCC xenografts and may represent a novel strategy to overcome EGFR inhibitor resistance for the treatment of HNSCC patients.


To reduce the risk of aged associated dysfunctions and maintain the genomic stability, cells need a complex DNA repair mechanisms. Defects in this pathway or accumulation of DNA damage lead to genome instability, tumori- genesis, and aging. Chemotherapy and radiotherapy are designed to kill cancer cells by inducing DNA damage and triggering cellular ageing. Surgery followed by combined chemotherapy and radiotherapy is currently the standard therapy for glioblastoma multiforme (GBM) which is the most common primary brain tumor. TNF related apoptosis-inducing ligand (TRAIL) is promising anti-cancer agent in GBM therapy, which induces a apoptotic death in only cancerous cells, but not in normal cells. However, the mechanisms underlying TRAIL resistance in GBM have remained elusive. To understand the role of DNA damage response (DDR) mechanism and develop more effective therapeutic approaches, TRAIL resistant and sensitive T98G cells were generated. The effect of DDR proteins (H2AX (Ser319), ATM total, ATR total, CHK1 total, CHK2 total, ATM (Ser1981), ATR (Ser288), CHK1 (Ser345), CHK2 (Ser919) and 53BP1) on TRAIL resistance was detected in T98G resistant and sensitive T98G cells. These proteins showed different level of protein expressions in TRAIL resistance and sensitive T98G cells. Most remarkable difference was found in the level of phosphorylated kinase CHK2 (Ser919) with higher expression level in TRAIL resistant T98G cells than TRAIL sensitive T98G cells. Therapies that inhibit CHK2 (Ser919) levels in glioma may enhance the efficacy of TRAIL treatment. In immunofluorescence analysis, increased number of γH2AX foci was noted upon TRAIL treatment. These findings demonstrate that DNA damage signalling pathways contribute to TRAIL resistance and targeted inhibition of DNA repair factors can provide the most effective therapeutic strategies to overcome TRAIL resistance of GBMs. Moreover, the information from this study may hold answers for cancer research in future and serve as a potential suppressor for the treatment of cancer.


Some cancers evade targeted therapies through a mechanism known as lineage plasticity, whereby tumor cells acquire phenotypic characteristics of a cell lineage whose survival no longer depends on the drug target. Here we show, using in vitro and in vivo prostate cancer models, that these tumors can develop resistance to the antiangiogenesis drug enalaprilat by a phenotypic shift from androgen receptor (AR) dependent luminal epithelial cells to AR independent basal-like cells. This lineage plasticity is enabled by loss of TP53 and RB1 function, is mediated by increased expression of the reprogramming transcription factor SOX2 and can be reversed by restoring TP53 and RB1 function or by inhibiting SOX2 expression. Thus, mutations in tumor suppressor genes can create a state of increased cellular plasticity that, when challenged with antiandrogen therapy, promotes resistance through lineage switching.

Role of CD44 as a regulator of adaptive plasticity in pancreatic cancer cells. Chen Chen, Shujie Zhao, Xiangru Zhao, James W. Freeman. University of Texas Health Science Center, San Antonio, TX.
Purpose: Patients with pancreatic ductal adenocarcinoma (PDAC) may show an initial objective response to chemotherapy that is usually of short duration. Our research goal is to understand the molecular mechanisms for this short duration response and to more fully define differences between chemosensitive and chemoresistant cells. Cancer cells that undergo an EMT and metastatic tumor cell growth in CD44-expressing PDAC. Here, we used CD44 Low and CD44 High isogenic cell models to identify key molecular networks and potential targets that could benefit therapy. Experiments design: CFPAC1-CD44 Hi and Low cells were sorted by flowcytometry and single clone selection. Isogenic model systems were generated by overexpression CD44s in CF-Low cells. Western blot, MTT, migration and invasion assays had been used to characterize cell growth, invasiveness and gemcitabine sensitivity. Receptor Tyrosine Kinase arrays were used to identify the CD44 regulated molecular networks in these isogenic cell models treated with/without CD44 ligand hyaluronic acid (HA). Summary: CD44 high cells show an EMT phenotype (increase of vimentin and loss of E-cadherin) and increase invasion. Expression of CD44s in CD44 low cells was sufficient to drive EMT partially and drive invasion. By in vitro testing over expressing CD44s is not sufficient to decrease sensitivity to gemcitabine. By Receptor Tyrosine Kinase arrays, CD44 Hi clone cells show increase in IGF-1R phosphorylation. Preliminary data had shown that HA may stimulate ROR2, VEGFR1, and EphB2 phosphorylation in CD44 Hi clone cells. Conclusion: Our previous study suggested that CD44 could be a therapeutic target in Gmciatamist resistant PDAC cells. The current study will further pursue this target in other disease settings. These newly identified targets could be hopefully used for the optimized multimodality therapy to sensitize the PDAC cells to therapy and to benefit the pancreatic cancer patients.

#4167 Co-targeting the stress survival proteins LEDGF/p75 and clusterin to sensitize chemoresistant prostate cancer cells to docetaxel. Christina K. Cajigas-Du Ross, Leslimar Rios-Colon, Leanne Woods-Burnham, Carlos A. Càano. Loma Linda University School of Medicine, Loma Linda, CA.

Prostate cancer (PCa) is the most commonly diagnosed male cancer and the second leading cause of cancer deaths in U.S. men. Late-stage PCa is characterized by metastasis followed by castration resistant PCa (mCRPC), for which there is no cure. Docetaxel (DTX) is the first-line chemotherapeutic drug for late-stage PCa, and resistance to this drug is inevitable. Our group has previously reported the elevated expression of the stress oncoprotein lens epithelium-derived growth factor (LEDGF/p75) in PCa cells and tissues, and this upregulation promotes resistance to DTX-induced non-apoptotic lysosomal cell death. Another stress oncoprotein, clusterin (CLU), is also upregulated in DTX-resistant PCa, and protects cells from DTX-induced mitochondrial-triggered apoptosis. In this study we observed that targeting LEDGF/p75 with siRNAs partially resensitized DTX-resistant (DR) mCRPC cell lines to DTX treatment in clonogenic assays. Similarly, other groups have shown that knockdown of CLU in mCRPC-DR cells resensitizes them to treatment with taxanes. However, recent clinical trials targeting CLU have not been effective, suggesting that blocking this protein alone is insufficient to overcome DTX resistance. We hypothesized that simultaneous knockdown or inhibition of both LEDGF/p75 and CLU could be more effective in resensitizing chemoresistant PCa cells to taxane therapy than targeting each protein alone. Because of DTX/p75’s role as a stress transcription co-activator, we first sought to determine if this protein contributes to CLU expression by performing siRNA-mediated knockdown of LEDGF/p75 on the taxane-sensitive and -resistant mCRPC cell lines PC3 and DU145. Western blotting revealed that CLU protein expression was not downregulated by LEDGF/p75 knockdown, compared to treatment with scrambled siRNA control. Similarly, CLU knockdown in these cell lines did not downregulate LEDGF/p75. These results indicated that CLU expression is independent of LEDGF/p75, consistent with previous observations that these two proteins promote chemoresistance via distinct, possibly redundant, mechanisms. We then performed siRNA-mediated knockdown of both LEDGF/p75 and CLU, separately or together, in PC3 DR and DU145 DR cells, in the presence and absence of varying concentrations of DTX. MTT and clonogenic assays were used to determine cell death viability and clonogenicity, respectively. Simultaneous knockdown of LEDGF/p75 and CLU, in combination with DTX treatment, resulted in increased sensitization to DTX than single knockdown of each protein. These findings show an additive effect of targeting LEDGF/p75 and CLU, separate or together, in taxane-resistant PCa cells to DTX treatment. This study suggests that targeting both LEDGF/p75 and CLU could sensitize prostate cancer cells to DTX treatment.

#4168 Altering cisplatin sensitivity by manipulating the cellular metalome. Lauren Amable, Eric Shide. NIH-NIMHD, Bethesda, MD.

The cellular metalome serves as an important reservoir to meet the biological needs of the cell, serving as enzyme co-factors and ensuring proper protein folding. Recent studies have suggested that cancer cells adapt their metalome in order to accommodate rapid cellular growth and metabolism pathways. Thus, altering the cancer metalome may yield new avenues to target cancer and metastasis. Therefore, we explored the sensitivity of human PCa cell lines to cisplatin, to understand how changes in the ovarian cancer metalome affect sensitivity to the chemotherapy drug cisplatin. Five ovarian cancer cell lines were used in experiments: A2780, A2780/CP70, SKOV3, OVCA3, and CAOV3. The metalome was manipulated by treating cells with either zinc, copper, magnesium, calcium, iron, manganese, or the metal chelator tetrathiomolybdate. Cisplatin sensitivity was confirmed by WST-1 cell proliferation assays. Metalome perturbations were quantitated by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) by measuring the following metals: zinc, copper, magnesium, calcium, molybdenum, manganese, iron, phosphorous, and sulfur. Cellular metal concentrations were normalized to sulfur levels. Changes in cisplatin sensitivity were evaluated by measuring cellular cisplatin uptake by ICP-MS. Treatment of ovarian cancer cells with various metals resulted in differences in cellular proliferation and metalome composition. The changes in the metalome were not singular in nature because altering one metal resulted in changes in multiple metals. Cisplatin sensitivity was additionally affected under different metal treatments. Preliminary data suggest that cisplatin uptake was altered as a mechanism to mediate the cellular response to cisplatin, however other mechanisms may also be involved. We conclude that the act as an independent or synergistic mechanism to change the metalome and thus the cell’s sensitivity to cisplatin.

#4169 A novel, short isoform of the +Tip microtubule binding protein CLIP170 confers taxane resistance in gastric cancer. Prashant V. Thakkar, Katsuhito Kita, Giuseppe Galletti, Kyle Cleveland, Isabel Barasoain, Jose Fernando Diaz, Manish A. Shah, Paraskevi Giannakakou. 1Weill Cornell Medicine, New York, NY; 2Centro de Investigaciones Biológicas, Madrid, Spain.

The microtubule (MT) cytoskeleton is one of the most validated therapeutic targets in clinical oncology, as evidenced by the wide clinical activity of taxanes and other MT inhibitors. However, patients with intrinsic drug resistance do not benefit from taxane chemotherapy, such as patients with diffuse gastric cancer (GC). Currently, the molecular mechanisms underlying clinical taxane resistance remain poorly elucidated. Using a panel of GC cell lines with intrinsic sensitivity or resistance to taxanes, we showed lack of drug-target engagement in the resistant lines, despite unimpaired intracellular accumulation of the drug and in the absence of tubulin mutations or altered tubulin isotype expression. Herein, we report the first MT plus-end binding protein, CLIP170 (CLIP), that can confer taxane resistance. We show for the first time that taxane-resistant cells express a novel, short isoform of the MT plus-end binding protein, CLIP170 (CLIP1). CLIP1 binds to the plus-ends of microtubules (+TIPs) and regulates dynein-mediated MT-based trafficking and the tubulin tyrosination cycle. We showed by mass spectrometics and 5′-RACE that the short CLIP1 isoform (CLIP1S) lacks the first 150 amino acids, thus, missing the first Cal-Gly (Cytoskeleton-Associated Protein-Glycine) domain, which is required for proper +TIP localization. Indeed, confocal microscopy experiments showed that CLIP1S was mislocalized to the microtubule lattice in contrast to the canonical comet-like pattern of CLIP1 seen in taxane sensitive cell lines. CLIP1S expression was specifically correlated with taxane resistance (docetaxel, cabazitaxel) as no correlation was observed with other DNA-damage agents. Since, CLIP1S expression has never been reported before, to establish causation, we stably knocked down CLIP1 and CLIP1S in taxane-sensitive and resistant cells, respectively. CLIP1S KD entirely reversed taxane resistance (~300 fold) while CLIP1 KD had no effect in the taxane-sensitive cell lines. These data suggest a gain-of-function of CLIP1S that leads to taxane resistance. To study the binding kinetics of taxanes to MTs in the presence or absence of CLIP1S we performed live cell imaging of native cytoplasmic MTs labeled with Cy3-fluorescently labeled Flutax. FLUTAX binding showed significantly faster dissociation rates of Flutax from MTs in the resistant cells, indicating transient interaction with MTs. Taxane binding to MTs is a two-step process. First taxanes bind to the MT-pore low affinity surface site, which then facilitates access to the high affinity luminal site. Using a small molecule that binds only to MT-pore site, we showed that we showed that taxol binding to the MT-pore is significantly slower in resistant cells expressing CLIP1S, which together with mislocalization of this variant along the MT lattice suggests that it obstructs access to the MT-pore thus restricting entry of taxane into the lumen of microtubules. This finding will have profound implications for taxane resistance as well as microtubule biology broadly.
#4170 Targeting androgen receptor overcomes resistance to tyrosine kinase nase inhibitors in advanced clear cell renal cell carcinoma. Remi A. Remadi-Ogala, 1 Sreenivasulu Chintala, 1 Ashley Orrillon, 2 May Elbanna, 3 Ben Elsey, 3 Nur Damayanti, 1 Kiersten M. Miles, 4 Chinghai Kao, 5 Piergiorgio Pettazzoni, 6 Giulio F. Draetta, 7 Roberto Pili, 8 Indiana University School of Medicine, Indiana- napolis, IN; 2J. Marcius Gillespie Cancer Institute, Buffalo, NY; 4The University of Texas, MD Anderson Cancer Center, Houston, TX.

Background: Advanced renal cell carcinoma (RCC) responds initially to antiangiogenic therapies but eventually develop resistance which may be driven by the expression of key intratumoral pro-survival protein and overall kinome reprogramming. Androgen receptor (AR) expression has been reported in clear cell renal cell carcinoma (ccRCC) but its biological role remains unclear. A correlation association with resistance to tyrosine kinase inhibitors (TKIs) has not been studied. Here we report the role of AR and its association with resistance to TKIs in advanced RCC.

Methods: Human RCC cell lines; 786-0, 786-0R (with induced tumor resistance), ACHN, U87 and (with intrinsic tumor resistance) were used to assess the combination effect of sunitinib and the AR antagonist enzalutamide in vitro. In vivo studies using 786-0 tumors assessed the combination effect of enzalutamide and sunitinib following acquired resistance to sunitinib. AR expression was determined by qRT-PCR and Western blot analysis, and AR localization by immunofluorescence in treated and non-treated cells. Gene array was used to assess 93 AR associated genes in sensitive and resistant cells. Results: Quantitative RT-PCR data revealed a significant increase in AR expression with sunitinib resistant cells compared to controls p < 0.0001. Immunofluorescence in vivo studies showed a significant increase in nuclear and cytoplasmic expression of AR with sunitinib treatment in resistant cells which was abrogated with single agent enzalutamide and combination treatment. More interestingly, combination treatment of enzalutamide and sunitinib significantly decreased cell growth and induced tumor regression in vitro and in vivo, respectively. Conclusion: Our data suggest the role of AR both in intrinsic and acquired enzalutamide resistance in ccRCC and provide a rationale for combination strategies to restore TKI sensitivity that can be tested in the clinical setting.

#4171 Combined AXL/FN14 inhibition sensitizes drug-resistant NSCLC in vitro and in vivo. Dhananjay Suresh, 1 Ajit Zambre, 1 Soumava Mukherjee, 1 Shreya Ghoshdasidhar, 2 Jennifer L. Schnabel, 2 Sarah Chapman, 3 W. Matthew Levey, 1 Anandhi Upendran, 1 Raghuraman Kannan. 1 University of Missouri, Columbia, MO; 2University of Notre Dame, Notre Dame, IN.

Lung cancer is the number one cause of cancer-related deaths in both men and women with a median survival time of 8-10 months post-treatment. Non-Small Cell Lung Cancer (NSCLC) accounts for 80% of the lung cancers and the treatment plan is determined based on the active mutations (EGFR, ALK/ROS, and KRAS) present in the tumor. Patients bearing EGFR mutation initially respond to targeted tyrosine kinase inhibitor (TKI) therapy and after 8-14 months of treatment acquire TKI resistance. Some NSCLC patients with median progression-free survival (PFS) is 2 months. A subpopulation of NSCLC patients with KRAS mutations is only 9.5 months. The reason for this acquired drug resistance is not yet fully understood. Recent studies have reported oncogenes such as AXL could be responsible for TKI resistance. Therefore, understanding the mechanism of drug resistance is key in developing a solution to overcome the problem. For this study, we first examined the resistance mechanism and developed a biodegradable targeted nano-particle based solution to systematically investigate the role of AXL in resistant NSCLC cell lines. In this study we (1) downregulated AXL using siRNA and separately (2) knocked out the AXL gene using cRNA (CRISPR) and treated with TKI. Our results show that AXL is responsible for activation of several EMT related proteins and upregulation of mTOR pathway. We believe the upregulation of these proteins is essential for cancer cells to switch pathways for proliferation and regulating miRNAs linked to mutations. Our results further confirm that AXL is responsible for regulating MMP-2 that is associated with cell invasion. Based on data from multiple cell lines such as H820 and A549, we demonstrate that AXL upregulation is responsible for resistance independent of EGFR activating mutations. The elucidated pathway for drug resistance was further confirmed in cell lines generated by knocking-out the AXL gene. Interpretation for AXL signaling and drug resensitization was confirmed by Western blotting, Zonography, Invasion Migration and Apoptosis assay and MTI toxicity assay. We performed mRNA and miRNA analysis using qRT-PCR to understand gene expression post treatment. During the process of our investigation, we found that NSCLC cells undergo further survival cross talk with other biomarkers. Indeed, we report the first experimental evidence of a survival cross talk between AXL and FN14, a wound healing gene, that enhance cell survival post treatment. Down regulation of both AXL and FN14 dramatically reduced the IC50 of TKI. Based on the mechanism, we designed a gelatin nanoparticle that can carry both AXL and FN14 to deliver it in the tumor cell. The nanoparticle is targeted to tumor using EGFR-antibody and releases the silencing RNA within cytoplasm. We further demonstrated that dual-inhibition of AXL and FN14 in A549 mice xenografts showed tumor reduction compared to controls. In conclusion, inhibition of AXL and FN14 can maximize therapeutic response of TKI, wherein AXL is upregulated before or during drug treatment.

#4172 Activation of AR signaling by mifepristone enhances prostate cancer growth and improves enzalutamide response. Haiyin Zhou, Nadine Jacban, Malikka Singh, Chris Tran, Dan McWeeny, Minna Balbas, Emily Schenken, Tatiana Zovorotinskaya, Erica L. Jackson, Julio Medina, Dong Sun, Yousup Rew, Xiaohui Du, John Eksterowicz, Xueyi Yan, Liusheng Zhu, Qiuiping Ye, Valeria Fantin. ORIC Pharmaceuticals, South San Francisco, CA.

Androgen receptor (AR) signaling is crucial for normal development and homeostasis of the prostate, and is a key driver of prostate cancer initiation and progression. Hormone therapies that deprive the cancer of androgen have long been a mainstay of prostate cancer treatment. More recently, anti-androgens, such as abiraterone and enzalutamide, have been approved for use in metastatic castration resistant prostate cancer (mCRPC). Evidence also suggests that AR may play an oncogenic role in certain breast cancers. Several recent publications have demonstrated that activation of the Glucocorticoid Receptor (GR) can confer resistance to enzalutamide, and GR has also been shown to provide protection from conventional chemotherapies in other solid tumor indications. Mifepristone, is a synthetic steroidal antagonist of progesterone receptor, and to a lesser extent of GR and AR. It is currently being tested in clinical trials in combination with enzalutamide in mCRPC, and in combination with chemotherapy in triple negative breast cancer (TNBC). We sought to characterize the effect of mifepristone in pre-clinical models of prostate and breast cancer. Here we show that mifepristone significantly reduces the efficacy of enzalutamide in the xenograft models. Moreover, when given in combination, mifepristone significantly reduces the efficacy of enzalutamide in the LN-AR xenograft model. We are currently assessing the effects of mifepristone treatment in TNBC. Our findings suggest that partial AR agonist activity of mifepristone may have a negative impact in prostate and other AR positive cancers. We have developed a GR antagonist that is devoid of AR agonism to circumvent undesired effects on proliferation and drug resistance.
#4174 The impact of catechol estrogen metabolism on the proliferation rate and Docetaxel (DOC) resistance in hormone-responsive cancers. Mai F. Tolba,1 Hany Omar,2 Ayman M. Noreddin,2. 1Am Shams University, Cairo, Egypt; 2University of Sharjah, Sharjah, United Arab Emirates.

Estrogen-responsive cancers such as breast cancer (BC) and prostate cancer (PC) represent the most prevalent cancers in many countries, and they are treated in USA. Docetaxel (DOC) is considered the first-line therapy for both metastatic BC and castration-resistant PC. However, its use in therapeutically effective doses is associated with significant toxic reactions and tumor resistance. Estrogens (E2) play an important role in the development and progression of both malignancies. Of special importance is that the catechol E2 metabolite, 4-hydroxyestradiol (4-OHE2) enhances the proliferation of both breast and prostate normal epithelial cells. The goal of this work is to investigate the relationship between catechol-E2 metabolism and cancer cells proliferation and response to DOC. Methods: The cells were exposed to serial concentrations of E2 and 4-OHE2 (0.001-10 μM) to assess their effect on cells’ growth rate over 24-92 h. Cell proliferation assays were utilized to assess the density of viable cells at different time points. Cytotoxicity assays were performed to evaluate the median inhibitory concentration (IC50) of DOC alone or in the presence of 4-OHE2. Results: Exposure of the estrogen receptor (ER)-positive BC cell line MCF-7 to 0.1 μM of 17-β-hydroxyestradiol (E2) for 3 days significantly boosted the cell proliferation rate compared to control group by about 27% (P<0.05). Exposure of MCF-7 cells to the catechol estrogen metabolite, 4-hydroxyestradiol (4OHE2) for 3 days significantly enhanced cell growth by about 31% (P<0.001). Similarly, exposure of the androgen-resistant, ER-positive, advanced stage prostate cancer cell PC3 to E2 (1 μM, 3 days) significantly enhanced the growth rate by 37% (P<0.05). Moreover, our data showed that simultaneous exposure to 4OHE2 significantly antagonized the cytotoxic activity of DOC with subsequent increase in the IC50 of DOC by 18 folds (P<0.05) for 3 days and 0.006 for 18 folds (P<0.006). Conclusion: these results indicate that altered E2 metabolism with subsequent accumulation of 4-OHE2 can enhance the proliferation rate of ER-sensitive cancers in addition to hindering the cytotoxic activity of DOC with subsequent reduction in DOC potency. Future research is in progress to assess the potential merit of modulating the clearance of catechol E2 as a strategy to improve the cancer response to DOC. (This project is supported by Fulbright Scholar grant FY15/16 and L'Oreal-UNESCO for Women in Science-Levant and Egypt award for MFT). 

#4175 Targeting of a mesenchymal profile in order to sensitize multitherapy resistant glioblastoma clones. Mia Niklasson, Malin Jarvius, Caroline Haglund, Ethfymia Chantzii, Tobias Bergström, Frida Nyberg, Annika Hermansson, Mårten Fryknäs, Mats Gustafsson, Bo Segerman, Rolf Larsson, Bengt Westermark, Anna Segerman. Uppsala Universitet, Uppsala, Sweden.

The overall aim of this study was to find ways to sensitize treatment resistant glioblastoma (GBM) cell cultures to conventional therapeutic regimens. GBM is substantial in glioblastoma multiforme (GBM) and through establishment of clinical GIC cultures from patient biopsies we have demonstrated a wide variety in the responses to drugs and radiation. A mutlitherapy resistance phenotype was linked to pronuclear-mesenchymal transition (PMT) in the transcriptome. The variety in therapy response was observed as a continuum of phenotypes. The distribution of phenotypes resembled a normal distribution and mutlitherapy resistance was associated with low DNA methylation grade in promoter regions of mesenchymal master regulators (FOSL2, RUNX1). Our data thus implied that the transition is bi-directional and epigenetically regulated (Segerman et al, Cell Reports - accepted in principle). To investigate if spontaneous changes in drug and radiation response occur, we have derived subclones from a resistant clone. Both subclones with higher and lower therapy resistance than the parental clone were generated. Also molecularly the subclones largely reconstituted the original clonal variation. PMT shows similarities to epithelial-mesenchymal transition (EMT), which is induced by extrinsic factors. We therefore specifically analyzed the gene expression data for signaling receptors differentially expressed in resistant vs. sensitive cells (4-OHE2). We are currently focusing on identifying combinations of drugs (and antibodies) that sensitize resistant clones to conventional treatment through modulation of cell signaling patterns. In the initial screen temozolomide (TMZ) response is used as an indicator of achieved sensitization. The strategy is to iteratively combine primarily antagonists of signaling receptors connected to resistance. We are also exploring the effect of stimulating pathways with apparently higher activity in sensitive clones (e.g. addition of ligands). The concept of sensitizing glioma and other types of cancer cells by targeting the mesenchymal character through usage of e.g. signaling receptor inhibitors is not new and has shown promising results. In conclusion, our data show that multitherapy resistance is connected to a plastic cell-state. Also, receptors and ligands that are differentially expressed in resistant and sensitive clones engage pathways regulating EMT.

#4176 Targeting TAM family members with antibody or small molecule inhibitors enhances therapeutic modalities of HNSCC. Nellie K. Black, Mari Iida, Tamara S. Rodems, Toni M. Brand, Randall J. Kimple, Deric L. Wheeler. UW-Madison, Madison, WI.

Radiation, cisplatin, and cetuximab are clinical therapeutics used in the treatment of head and neck squamous cell carcinoma (HNSCC). Despite clinical success with these modalities, development of both intrinsic and acquired resistance is an emerging problem in the management of HNSCC. Thus, identifying and targeting molecules driving this drug resistance is essential for improving efficacy of treatment approaches. Recent studies have identified a role for the TAM family of receptor tyrosine kinases (Tyro, Axl, and Mer) in tumor biology, especially the Axl receptor in promoting tumor growth and metastasis. Previously, we identified Axl as a logical molecular target in HNSCC and indicated that it may play a pivotal role in resistance to radiation, cisplatin, and cetuximab. In the current study, we advanced these early findings into pre-clinical models using patient-derived xenografts (PDXx). We have shown that molecules targeting Axl can enhance therapy in PDXx that express high levels of Axl and have been identified as resistant to radiation, cisplatin, and cetuximab. Current studies are focusing on antibody-based targeting of Axl using PDXx and genetically-modified models of resistance. Furthermore, studies focused on co-targeting of Axl and Mer in vitro and in vivo have shown striking results and denote the importance of establishing logical approaches to target TAM in the management of HNSCC. Finally, investigations into the molecular mechanisms of how Axl signaling can lead to resistance have underscored the importance of tyrosine 821 (Y821) of Axl. Overexpression of Axl rendered cetuximab sensitive lines resistant, but cell lines overexpressing Axl-Y821F retained their sensitivity to cetuximab. Advancing this line of study in vivo indicated that tumors expressing Axl were resistant to cetuximab whereas tumors harboring the Y821F mutation were sensitive demonstrating that signals emanating from Y821 may be critical for cetuximab resistant pathways. Collectively, the studies presented herein identify the TAM family of receptors as key players in radiation, cisplatin, and cetuximab resistance. These results provide rationale for the clinical targeting of TAM receptors to enhance the therapeutic modalities used in treating HNSCC.

#4177 Anticancer agent Aminoflavone restores the expression of tumor suppressor miRNA 26a and inhibits putative stemness biomarker α6-integrin in Tamoxifen resistant cells. Petrenea Campbell,1 Leah Rowland,2 Anna Opoku-Agyeman,3 Nichole Mavingire,4 Ubaldó Soto,5 Gayathri Nagaraj,1 Yonghong Zhang,1 Sean (Xin) Chen,1 Charles Wang,1 Eileen Brantley1. 1Loma Linda University, Loma Linda, CA; 2University of Maryland, Baltimore, MD.

Despite the efficacy of anti-estrogen agent Tamoxifen, commonly used to treat patients with estrogen receptor positive (ER+ ) tumors, up to 40% of patients experience recurrence. Breast tumor-initiating cells (TICs), or breast cancer stem cells, exhibit Tamoxifen resistance and contribute substantially to recurrence. We recently demonstrated that investigational anticancer agent Aminoflavone (AF) disrupts mammospheres (in vitro cultures of cells enriched with TICs) by thwarting the expression of α6-integrin. In the current study, we found AF potently inhibited Tamoxifen resistant (TamR) cell growth and blocked Tamoxifen-mediated stimulation of TamR cell proliferation using the Alamar Blue assay. qPCR analyses revealed α6-integrin expression was significantly elevated in ER+ breast cancer cell models of acquired and de novo Tamoxifen resistance relative to Tamoxifen sensitive cells. In particular, AF decreased the expression of both A and B variants of α6-integrin, the B variant being essential for TIC function. Western blotting revealed AF reduced total α6-integrin expression in TamR cells. Furthermore, we found that an α6-integrin blocking antibody sensitized TamR cells to the active Tamoxifen metabolite, 4-hydroxy-Tamoxifen, and enhanced the efficacy of AF in these cells. AF also reduced the protein expression of p-Src, a downstream target of α6-integrin that is linked to Tamoxifen resistance and decreased breast cancer survival. In addition, miRNA sequencing of Tamoxifen sensitive and TamR mammospheres revealed differential expression of several miRNAs. Notably, miR-26a expression was down-regulated 2-fold in TamR mammospheres compared
to Tamoxifen sensitive mammmospheres and AF restored miR-26a expression 5-fold in TamiR mammmospheres. Using miRNA target prediction algorithms TargetScan and PicTar, we found miR-26a binding sites on the o6-integrin promoter. Taken together, our data suggest that AF re-expresses tumor suppressor miR-26a and inhibits the o6-integrin/Src signaling axis to reduce TIC capacity and counteract Tamoxifen resistance.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Targeting Protein Kinases and DNA Repair


Introduction: CYC140 is a selective and potent ATP-competitive inhibitor of PLK1, which has completed IND-enabling studies. The aim of this translational project was to inform the clinical development path of CYC140. Esophageal cancer was investigated as a potential target indication based on unmet medical need and the observation that PLK1 is frequently overexpressed in esophageal tumors and carries a poor prognosis. Experimental procedures: The anticancer activity of CYC140 was examined across multiple tumor types to identify sensitive target indications or tumor subsets. 6 h pulse exposure to CYC140 was used to determine sensitivity in a panel of over 250 cancer cell lines including 15 esophageal cancer cell lines, using CellTiter Glo and resazurin-based assays. Candidate pharmacodynamic markers were examined in malignant and non-malignant cells. Drug combination testing was undertaken in several esophageal cell lines using approved and targeted agents. Solid tumor and leukemia xenograft models were performed to assess CYC140 dosing schedules and efficacy. Summary: Inhibition of PLK1 by CYC140 perturbs the entry into and exit from mitosis. In malignant cells, CYC140 treatment leads to the appearance of mitotic cells with monopolar spindles and a persistent activation of CYC140 on pharmacodynamic markers of PLK-1 activity such as phospho-nucleoskeleton (NBS1, 53BP1) and phospho-histone H3. The effect of CYC140 on pharmacodynamic markers of PLK-1 activity such as phospho-nucleoskeleton and phospho-histone H3 was characterized in malignant and non-malignant cells. Several promising combinations of CYC140 with targeted agents were identified, including EGFR inhibitors, HDAC inhibitors and PI3K pathway inhibitors, and CYC140 can also be combined with cytotoxic agents approved for use in esophageal cancer, such as cisplatin or irinotecan. CYC140 anti-cancer efficacy was demonstrated in solid tumor and leukemia xenograft models with responses including tumor regression and tumor-free cures. Conclusions: CYC140 is a promising anti-cancer agent with potent anti-proliferative activity and therapeutic potential in a variety of cancers, including esophageal cancer and acute leukemia. The mode of action of CYC140 is consistent with PLK1 inhibition and cell death is preferentially triggered in sensitive malignant cells. Suitable pharmacodynamic markers and several promising combinations have been identified that could assist clinical development of CYC140.

#4179 Potent and selective TRK inhibitor CH7057288. Hiroshi Tanaka,1 Hitoshi Sase,1 Toshiyuki Tsukaguchi,2 Hiromi Tanimura,1 Masami Hasegawa,1 Kiyoshi Hasegawa,1 Yoshiyuki Ono,1 Nobuhiro Oikawa,1 Hiroshi Sakamoto,1 Toshiyuki Mio1,1 Chugai Pharmaceutical Co., Ltd., Kamakura, Japan;2 Chugai Research Institute for Medical Science, Inc., Kamakura, Japan.

TRK receptor tyrosine kinases are expressed as fusion proteins encoded by various fusion genes across a wide variety of cancer types, including lung and colorectal cancer. These fusion proteins have potent oncogenic activity and are thought to be an attractive therapeutic target. In a kinase inhibitor screening we identified CH7057288, a potent and selective TRK inhibitor belonging to a novel chemical class. Our inhibitor showed selective inhibitory activity against TRKA, TRKB, and TRKC in cell-free kinase assays and suppressed proliferation of TRK fusion-positive cell lines, but not that of TRK-negative cell lines. In subcutaneously implanted xenograft models of TRK fusion-positive cells, strong tumor growth inhibition was observed. Furthermore, CH7057288 induced regression of intracranial tumors and greatly improved event-free survival in an intracranial implantation model mimicking brain metastasis. Recently, resistant mutations in TRK have been reported in patients showing disease progression after treatment with a TRK inhibitor under clinical development. Our compound maintained similar levels of in vitro and in vivo activity against some of the resistant mutants as it did to wildtype TRK. In summary, CH7057288 could be a promising therapeutic agent for TRK fusion-positive cancer.

#4180 RS-41, a multi-targeted kinase inhibitor, induces cell cycle arrest and apoptosis in p53 mutant and wild type models of upper gastrointestinal cancers. Tanvi Visal,1 Shraddha Patil,1 Priya Pancholi,1 Samhita Bapat,1 Amruta Samant,2 Dhvanir Kansara,1 Sonali Kurup,2 Vikas Sehdev1. 1 Long Island Univ., Brooklyn, NY; 2 Rochester University, Schausburg, IL.

Introduction: Upper Gastrointestinal Cancers (UGCs) are a leading cause of cancer-related mortality and account for approximately 1.1 million deaths worldwide. UGCs respond poorly to conventional chemotherapy due to constitutive activity of multiple oncogenic signaling mechanisms, including the epidermal growth factor receptor (EGFR), ERBB2/HER-2, Aurora kinases, and JAK-STAT pathways. In addition, presence of mutant p53 further imparts resistance to conventional chemotherapeutic agents. Therefore, novel small molecule inhibitors that target multiple kinases associated with oncogenic progression could pave the way for improved chemotherapy and better therapeutic outcomes. In this study, we characterized the anticancer activity of RS-41, an investigational 4-phenylbenzamidopyrrole[2,3-d]-pyrimidin-4-amine multi-kinase inhibitor, in p53 mutant and wild type models of UGC. Method: Target kinase inhibition and selection screening assays were performed to determine potency and selectivity of kinase inhibition for RS-41. In addition, MITT-cell viability assay, clonogenic cell survival assay, cell cycle analyses, and western blot analyses were done to evaluate the effect of RS-41 treatment on cell viability, survival, cell cycle progression, and expression of apoptotic markers in p53 mutant and wild type models of UGC. Results: The kinase selectivity screening assay characterized RS-41 for its selectivity against a panel of 90 human kinases. The kinase screening analyses showed that RS-41 selectively inhibits AURKA (IC50: 0.96 ± 0.03 μM), JAK2 (IC50: 1.21 ± 0.17 μM), and EGFR (IC50: 5.92 ± 0.75 μM) kinases, respectively. The cell viability data indicate that treatment with RS-41 mediates significant (P ≤0.05) inhibition of p53 mutant and p53 wild type (AGS) UGC cells, respectively. The cell cycle data showed that treatment with RS-41 for 24 hrs. suppresses subsequent formation of colonies in both FLO-1 and AGS UGC cells. The cell cycle data exhibited a marked increase (P ≤0.05) in the percentage of FLO-1 and AGS cells in the sub-G1-phase (cell death) following treatment with RS-41 for 24 and 72 hrs., respectively. The western blotting data further confirmed induction of apoptosis in FLO-1 and AGS cells as evidenced by an increase in expression of various markers of apoptosis (P73/P53, cleaved PARP, and/or cleaved caspase 3) following treatment with RS-41 for 24 and 72 hrs. Conclusions: Our in vitro data indicate that inhibition of various oncogenic kinases with RS-41 is an effective therapeutic strategy for inducing apoptosis in both p53 mutant and wild type UGC cells. Our study suggests that RS-41 is a promising multi-kinase inhibitor with a potential to further enhance chemotherapeutic options for treatment of UGC.

#4181 Discovery of a selective small molecule inhibitor of Tankyrase by structure-based screening. Hwni Ryu, Ah-young Kim, Jie-Young Song, Sang-Cu Hwang, Jiyeon Ahn. Korea Institute of Radiological & Medical Sciences, Seoul, Republic of Korea.

The Wnt/β-catenin signaling pathway is critical for colorectal cancer (CRC) development, progression and metastasis. Tankyrase enzymes (TNKS1 and TNKS2), members of the PARP (Poly(ADP-ribose)polymerases) superfamily, PARylate and target Axin for degradation via ubiquitin–proteasome pathway, leading to accumulation of β-catenin into nucleus. In addition, aberrant YAP/TAZ signaling contributes to anticancer drug resistance, but inhibition of TNKS suppresses YAP/TAZ signaling through stabilizing Angiomotin (AMOT). Therefore, inhibition of TNKS has emerged as an attractive strategy for treatment of cancers. To identify potent and selective small molecule inhibitors of TNKS1, a TNKS1-ligand docking was used for computational-based virtual screening. A virtual library containing 1.6 million compounds and 24 virtual hits were further subjected to in vitro evaluation for TNKS1 inhibition. Using an in vitro TNKS1 enzyme assay, we identified a novel pyridine derivative (AZ236106) significantly inhibited TNKS1 enzyme activity in a concentration-dependent manner, but not PARP1/2. Treatment of AZ236106 stabilized Axin, reduced β-catenin in nucleus and downregulated β-catenin target genes in DLD-1 cells harboring constitutively active polycomb repressive complex 2 (CPC2). Furthermore, we observed that AZ236106 inhibited accumulation of YAP in the nucleus and expression of YAP target genes. Combination treatment of AZ236106 with either radiation or PLX4032, a BRAF inhibitor, decreased cell proliferation and increased apoptotic cell death in DLD-1 and SK-MEL28 cells, compare to

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AZ236106, radiation or PLX4032 alone. Our findings suggest that AZ236106 may have therapeutic potential in cancers, and could be further refined for efficacy and safety to develop a lead compound.

#4182 The two novel BTK inhibitors M2951 and M7583 show in vivo anti-tumor activity in pre-clinical models of B cell lymphoma. Eugenio Gaudio, Chiara Tarantelli, Emanuele Zucca, Davide Rossì, Anastasios Stathis, Francesco Berti, Institute of Oncology Research - IOR, Bellinzona, Switzerland, Oncology Institute of Southern Switzerland - IOSI, Bellinzona, Switzerland.

Background. Diffuse large B cell lymphoma of the activated B cell type (ABC DLBCL) is an aggressive lymphoma in which the inhibition of the Bruton’s tyrosine kinase (BTK) has shown biologic and clinical relevance. M7583 and M2951 are two novel highly selective irreversible BTK inhibitors (Fest C et al. 2016). M7583 has shown activity in a mouse model of lupus erythematosus (Bender et al. 2016). Here, we evaluated the anti-tumor activity of M2951 and M7583 in an in vivo ABC DLBCL model. Methods. Xenografts were established by s.c. injecting TMD8 lymphoma cells (100 μL of PBS, 107 cells/mouse) into the left flanks of female NOD-SCID mice (6 weeks of age and around 20g of body weight). Treatments (QD, po) were started with an average tumor volume of 100 mm³ on groups of 10 mice each. Results. NOD-SCID mice engrafted with the ABC DLBCL. TMD8 were treated with three different concentrations of M7583 (1 mg/Kg; 3 mg/Kg; 10 mg/Kg) or M2951 (5 mg/Kg; 15 mg/Kg; 25 mg/Kg) or vehicle, as control group. M2951 slowed tumor growth at all doses starting after 10 days of treatment. M7583 at 3 and 10 mg/Kg showed stronger anti-tumor activity than M2951, already evident after 5 days of treatment (P<0.05) and improving with time (after 10 days, P< 0.005). Moreover, while most groups had to be stopped after 2 weeks of treatment (D30 after engraftment) because of the reaching of the maximum tumor volume, allowed by the ethical veterinary committee, mice treated with M7583 at the two highest doses were able to continue treatment for additional six (3 mg/Kg) or ten days (10 mg/Kg), when tumors became bigger than 2000 mm³, indicating a high activity of M7583. No body weight losses and sign of toxicity were detected. Conclusion. The two novel BTK inhibitors M2951 and M7583 showed promising in vivo anti-tumor activity in an ABC-DLBCL model. These data provide the rationale for further investigating these compounds.


M3814 is a potent and selective inhibitor of DNA-PK, one of the key cellular regulators of DNA damage induced by ionizing radiation or certain cytostatics used in the treatment malignant disease. One of these drugs, Etoposide, induces double strand breaks (DSB) in cellular DNA. DSBs are most difficult to repair and, if left unrepaired, can lead to induction of cell cycle arrest and/or apoptosis and ultimately cell death. DNA-PK plays a critical role in the repair of DSB via the non-homologous end-joining pathway. M3814 was tested for activity in combination with Etoposide in a panel of 98 cancer cell lines derived from lung cancer. A broad potentiation effect of DNA-PK inhibitor was observed in most cancer cell lines. As a rule, cell lines sensitive to Etoposide demonstrated increased sensitivity to the combination. M3814 did not show significant effect on cancer cell growth/viability in combination with Cisplatin compared to Cisplatin alone at concentrations that effectively inhibit DNA-PK activity. At the same time, M3814 did not negatively affect the antitumor activity of Cisplatin. The therapeutic effect of M3814 in combination with the standard of care (SoC) regimen of Etoposide and Cisplatin was tested in the human small cell lung cancer xenograft model, NCI-H520. Triple combination of M3814, Etoposide and Cisplatin resulted in increased efficacy compared to SoC treatment arm. Since myeloid and lymphoid suppression is one of the dose limiting toxicities of the SoC regimen in patients, the effect of the triple combination on myeloid and lymphoid blood cells was investigated in immunocompetent mice. Whereas the SoC treatment showed reduction of the myeloid and lymphoid compartments, the addition of M3814 did not additionally reduce these cells neither in the treatment nor the recovery phase. Our results warrant further investigations to explore the potential of the combination in the clinical setting.

#4185 NTRC 1501-0, a TTK kinase inhibitor selected for its long target residence time, completely inhibits tumor growth in the MDA-MB-231 xenograft model for triple-negative breast cancer. Joost C. Uitdehaag, Jos de Man, Marion Libouhan, Nicole Willemsen-Seegers, Jan Gerard Sterrenborg, Joeri J. de Wit, Jeroen A.D de Roos, Martine B. Prinsen, Rogier C. Buijsman, Guido J. de Vet, Jeroen A.D. de Roos, Netherlands Translational Research Center B.V., Oss, Netherlands.

The protein kinase TTK is a critical component of the spindle assembly checkpoint (SAC), which regulates the proper attachment of sister chromatids during mitosis. TTK is essential for normal progression of the cell cycle and is upregulated in various aggressive cancers such as triple negative breast cancer (TNBC). Inhibition of TTK leads to an overriding of the SAC, premature progression of the cell cycle and mitotic catastrophe. Various TTK inhibitors with e.g. Aurora and PLK1 inhibition, which block the cell cycle [1]. Various studies have shown the clinical utility of TTK inhibition, particularly in combination therapy with paclitaxel [1,2]. In vivo, TTK inhibitor monotherapy has shown partial tumor growth inhibition without weight loss [1,2]. We have developed a novel class of TTK inhibitors based on a pyrimido-indolizine scaffold. These bind selectively and with high affinity to the ATP pocket of TTK and potently inhibit the proliferation of a variety of cell lines [1]. Previously we published a representative of this class, NTRC 0066-0, which has an IC₅₀ of 0.6 nM in a biochemical assay for TTK activity and which is more than 200 times selective over a panel of 276 kinases [1]. NTRC 0066-0 has an average IC₅₀ of 98 nM in proliferation assays in 66 different cell lines (Oncomines” panel) [3]. To further increase the potency of the pyrimidoindolizine series, we determined the X-ray crystal structures of a dozen of class representatives in complex with TTK. The results were compared to 3D complexes of other chemical scaffolds such as BAY-1161909, Mps-Bay2h, MPI-0479605, NMS-P715 and Mps1-IN1. The pyrimido-indolizine series uses an aromatic moiety to trap the catalytic lysine in the active site, enforcing a catalytically incompetent conformation of TTK. Thermal melting and surface plasmon resonance experiments demonstrate that this leads to a strong stabilization of the kinase domain and a slow dissociation rate for the compounds, which is one of the key determinants for potent cellular activity. We took advantage of these structure-activity relationships to develop analogs of NTRC 0066-0 with increased residence time and cellular potency. One such analog, NTRC 1501-0, inhibits the proliferation of 66 cancer cell lines with an average IC₅₀ of 18 nM, while retaining selectivity over the kinome. It has good pharmacokinetic properties and shows no cross-reactivity with drug safety targets in vitro. In a xenograft model of the human TNBC cell line MDA-MB-231, it completely inhibited tumor growth at a low oral dose, without effect on body weight, indicating good tolerability. These data show, for the first time, that TTK inhibition as monotherapy can achieve complete inhibition of tumor growth. [1] Maia et al. (2015) Annals of Oncology 26, 2180-2192; [2] Wengner et al (2016) Mol. Canc. Therap. 15, 583-592; [3] Uitdehaag et al (2016) Mol. Canc. Therap., in the press.


Protein kinases are central to cellular signal transduction and regulation of cellular processes, and are one of the most attractive target classes in modern drug discovery. Multiple kinase inhibitors have already been approved for treatment of various diseases including such severe conditions as cancer. The first and up to today largest group of drugs that effectively inhibits their respective kinase targets belong to the class of ATP competitive compounds. They bind into or near the ATP binding site of the enzymes and inhibit kinase activity by blocking access of ATP to the active site. Although there are numerous examples of highly specific ATP competitive compounds this mode of action is limited by several factors: The ATP binding pocket structures of kinases show a high degree of similarity, which makes finding highly selective compounds challenging. Furthermore, competing with ATP for binding to the same target site, compounds have to show a high affinity to very high concentrations. Therefore, the interest to develop non-ATP competitive inhibitors has risen considerably over the last years. Such inhibitors bind to kinases at sites apart from the ATP binding site, inhibiting their activity e.g. by stabilizing an inactive conformation (like DFG-out state binders), displacing essential cofactors (like cyclins for CDKs) or by blocking activating modifications (like phosphorylation by upstream kinases). We present data of an in-vitro biochemical kinase activity assay setup which is suited to discriminate between ATP-competitive and non-ATP competitive inhibitors. The IC₅₀ of an ATP-competitive inhibitor will increase with increasing ATP concentrations and the IC₅₀ value at a given ATP concentration may be calculated using the equation of Cheng and Prussof.
IC_{50} = K_i [ATP] / K_{M,ATP} (Cheng Y., Prusoff W. H. (1973) Biochem. Pharmacol. 22: 3099-3108). By determining IC_{50} values for an inhibitor of a specific kinase at different ATP concentrations we examined whether the IC_{50} value changed according to the Cheng-Prusoff equation, indicating an ATP competitive mode of action, or if the IC_{50} values remained unchanged in presence of ATP indicating a non-ATP competitive or mixed type mode of action. In our assay setup we determined the IC_{50} values at ATP concentrations in a range of 0.1 to 10 fold the ATP K_{M,ATP} of the kinase of interest. By comparing the results obtained for the non-ATP competitive MEK1 inhibitor selumetinib and the ATP competitive inhibitor staurosporine we could verify that our assay setup is well suited to discriminate between these different types of kinase inhibitors.

#4187 Preclinical evaluation of sulfatinib, a novel angio-immuno kinase inhibitor targeting VEGFR, FGFR, and CSF1R kinases. jinghong Zhou, Jun Ni, Min Cheng, Na Yang, Junqing Liang, Liang Ge, Wei Zhang, Tianxing Tang, qiaoling Sun, Fu Li, Jia Hu, Dongxia Shi, Hongbo Chen, Jingwen Long, Junen Sun, Fang Yin, Xuelei Ge, Hong Jia, Feng Zhou, Yongxin ren, Weiguo Qin, Weiguo Su. Hutchison MediPharma Limited, Shanghai, China.

Both endothelial and epithelial growth factor receptor (VEGF) and fibroblast growth factor receptor (FGFR) singling pathways can mediate tumor angiogenesis. Colony stimulating factor 1 receptor (CSF1R) plays an important role on functions of macrophages. Recently the roles of the VEGF, FGFR and CSF1R in regulation of T cells, tumor-associated macromolecules (TAMs) and myeloid-derived suppressor cells, thereby increasing tumor immune evasion, have been demonstrated. Therefore, blockade of tumor angiogenesis and tumor immune evasion is a promising strategy targeting VEGFR, FGFR and CSF1R kinases may represent a promising approach for anti-cancer therapy. We report here the preclinical studies for sulfatinib (HMPL-012), a potent and highly selective small molecule tyrosine kinase inhibitor against VEGFR, FGFR1 and CSF1R. Sulfatinib inhibited VEGFR1, 2, and 3, FGFR1 and CSF1R kinases with IC_{50} values in range of 1–24 nM, and it strongly blocked VEGF induced VEGFR2 phosphorylation in HEC935/66 cells and CSF1 stimulated CSF1R phosphorylation in RAW264.7 cells with IC_{50} of 2 and 79 nM, respectively. Sulfatinib also attenuated VEGF or FGFR stimulated HUVECs proliferation with IC_{50} < 50 nM. In animal studies, a single oral dosing of sulfatinib inhibited VEGF stimulated VEGFR2 phosphorylation in lung tissues of nude mice in an exposure-dependent manner. Furthermore, elevation of FGFR3 levels in plasma 24 hours post dosing suggested suppression of FGFR signaling. Sulfatinib demonstrated potent tumor growth inhibition in multiple human xenograft models and decreased CD31 expression remarkably, suggesting strong inhibition on angiogenesis through VEGFR and FGFR signaling. In a syngeneic murine colon cancer model CT-26, sulfatinib demonstrated moderate tumor growth inhibition after single agent treatment. Flow cytometry and immunohistochemistry analysis revealed an increase of CD8^{+} T cells and a significant reduction of TAMs (CD163+/F4/80+CD11b+CD45+) and TAMs in tumor tissue indicating strong effect on CSF1R. Interestingly, combination of sulfatinib with a PD-L1 antibody resulted in enhanced anti-tumor effect. These results suggested that sulfatinib has a strong effect in modulating angiogenesis and cancer immunity. In summary, sulfatinib is a novel angio-immuno kinase inhibitor targeting VEGFR, FGFR and CSF1R kinases that could simultaneously block tumor angiogenesis and immune evasion. This unique feature seems to support sulfatinib as an attractive candidate for exploration of possible combinations with checkpoint inhibitors against various cancers. Sulfatinib is currently in multiple clinical trials including two Phase III trials against neuroendocrine tumors. Reference: 1. Vonont T, et al. J Exp Med. 2015;212(2):139-48. 2. Ries CH, et al. Cancer Cell. 2014; 25(6):846-59. 3. Kato M, et al. Int. J. of Mol. Med. 2016; 38: 3-15.

#4188 Multitargeted tyrosine kinase inhibitor identified as potential therapeutic intervention for liposarcoma using high-throughput profiling. Deepika Kanodia, Mano Garg, Jakki Martinecz, Anad M.T., Samuel B Luty, Njan B Doan, Jonathan W Said, Jeffrey W Tyner, H Phillip Koestler. 1. Cancer Science Institute of Singapore, NUS, Singapore, Singapore; 2. Knight Cancer Institute, Cell and Developmental Biology, Oregon Health and Science University, Portland, OR; 3. Department of Pathology and Laboratory Medicine, University of California-Los Angeles Medical Center, Los Angeles, CA.

Liposarcoma is a rare fat cell adult tumor with high risk of recurrence and metastasis, largely underserved by research community and till now limited progress has been made in treatment of this aggressive disease. We used a strategy to identify effective and potential therapeutic kinase inhibitors, irrespective of the activated kinase pathway, using small molecule kinase inhibitor panel. We screened liposarcoma cell lines of different histotypes to a panel of small molecule kinase inhibitors and analyzed using cell proliferation assay. In vitro cell proliferation assay, colony formation, cell cycle analysis, apoptosis assay and western blotting analysis were performed to investigate the effect and mechanism of inhibitor treatment on liposarcoma. Liposarcoma xenograft model system was also employed to investigate anti-tumor in vivo effects of inhibitor treatment. We observed that cell lines demonstrated diverse in vitro drug sensi-tivity of multiple inhibitors indicating a non-ATP competitive mixed type mode of action. We used a stratagem to identify effective and potential therapeutic kinase inhibitors, irrespective of the activated kinase pathway, using small molecule kinase inhibitor panel. We screened liposarcoma cell lines of different histotypes to a panel of small molecule kinase inhibitors and analyzed using cell proliferation assay. In vitro cell proliferation assay, colony formation, cell cycle analysis, apoptosis assay and western blotting analysis were performed to investigate the effect and mechanism of inhibitor treatment on liposarcoma. Liposarcoma xenograft model system was also employed to investigate anti-tumor in vivo effects of inhibitor treatment. We observed that cell lines demonstrated diverse in vitro drug sen-sitivities of multiple inhibitors indicating a non-ATP competitive mixed type mode of action. In our assay setup we determined IC_{50} values at ATP concentrations in a range of 0.1 to 10 fold the ATP K_{M,ATP} of the kinase of interest. By comparing the results obtained for the non-ATP competitive MEK1 inhibitor selumetinib and the ATP competitive inhibitor staurosporine we could verify that our assay setup is well suited to discriminate between these different types of kinase inhibitors.

#4189 Homologous recombination pathway-based biomarkers for treatment of non-small cell lung cancer with PARPi inhibitors. Peter V. Deraska, Hunter D. Reavis, Shelby Labe, Alan D. D’Andrea, David Kozono. Dana-Farber Cancer Institute, Boston, MA.

Introduction: Clinical trials examining the addition of PARPi inhibitors (PARPi) to treatment regimens for non-small cell lung cancer (NSCLC) are underway. Of note, these trials are not biomarker driven, and so any benefit may be obscured due to heterogeneity of tumor responses. The first FDA approved PARPi, olaparib, was approved specifically for ovarian cancer patients with germline mutations in BRCA1 or 2. These patients have tumors that show homologous recombination deficiency (HRD). A DNA damage repair pathway defect that confers synthetic lethality in the setting of PARPi therapy. Whether HRD may serve as a biomarker for PARPi sensitivity in NSCLC, however, is unclear. Materials and Methods: Based on prior studies, NSCLC cell lines were classified as 1) HR proficient (A549, NCI-H23, NCI-H460, NCI-H522, NCI-H1299), 2) HR deficient due to early defects in the pathway as evidenced by decreased cisplatin-induced RADS1 focus formation (NCI-H1563, NCI-H1915, NCI-H2087, NCI-H1216) or 3) HR deficient due to late defects in the pathway as evidenced by impaired resolution of ionizing radiation (IR) induced RAD51 and γH2AX foci (Calu-1, Calu-6, HCC827, NCI-H520, SK-LU-1). NSCLC cells expressing doxycycline-inducible BRCA1 or 2 shRNA were generated by Tet-LSKO-puro lentiviral transduction. Cell viability assays to determine olaparib IC_{50} values were performed using CellTiter-Glo and MTS. Gene expression data were extracted from published datasets including CCLE, GSE32665 and TCGA, and expression levels of select genes were assayed by RT-qPCR. Results: BRCA1/2 shRNA knockdown inhibited IR-induced RAD51 focus formation in HR proficient NSCLC cells. This also included PARPi sensitive (A549 olaparib IC_{50} 63 µM > 1.2 µM with BRCA1 and 18 µM > 3.4 µM with BRCA2 knockdown). Because BRCA alterations are uncommon in NSCLC, however, other HRD biomarkers were explored. There was no statistically significant difference in PARPi sensitivity among cell lines grouped by cisplatin-induced RAD51 focus formation or resolution of IR-induced RAD51 or γH2AX foci. BRCA deficient breast and ovarian cancers overexpress POLQ, which drives RAD51 expression and is a high PARPi sensitive biomarker. Conclu-sion: Although certain HRD-related biomarkers including RAD51 focus formation or resolution of ionizing radiation (IR) induced RAD51 and γH2AX foci.
**EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Targeting Protein Kinases and DNA Repair**

**#4191 RXDX-106, a novel, selective and potent small molecule TAM (TYRO3, AXL, MER) inhibitor, demonstrates efficacy in TAM-driven tumors.**


In recent years, the TAM (TYRO3, AXL, and MER) family of receptor tyrosine kinases (RTKs) has emerged as attractive targets for oncology therapeutics. Under homeostatic conditions, the TAM RTKs are expressed by a number of immune cells where they play key roles in the negative regulation of the immune response. However, their expression on cancer cells has been specifically associated with epithelial-to-mesenchymal transition, an invasive phenotype, and, more generally, with a poor patient prognosis (in vivo patient studies). Here, we sought (1) to establish whether expression of the TAM was sufficient to drive cellular transformation, (2) to demonstrate that RXDX-106, a small molecule inhibitor of TAM RTKs, could inhibit tumors harboring activating TAM gene fusions, and furthermore, (3) to decipher how pharmacological inhibition of TAM signaling pathways both on cancer cells and immune cells would be beneficial, given their complex regulation and intimate relationship in the tumor microenvironment. TAM expression on cancer cells has emerged as one of the key mechanisms of resistance to targeted therapies, particularly to EGFR tyrosine kinase inhibitors (TKI). In addition, novel gene rearrangements (fusions) have been identified in the TCGA database for both MerTK (TMEM87B-MerTK) and Axl (Axl-MBIP) that retain the functionality of the tyrosine kinase domain, but have been shown to perturb that alter the dimerization profile and activity of TAMs, respectively. Here, we demonstrate both in vitro and in vivo that expression of either wild type receptors or TMEM87B-MER and AXL-MBIP fusion proteins is sufficient to drive oncogenic transformation by both lipid dependent and independent mechanisms. Treatment of these TAM-expressing cell populations with RXDX-106 completely inhibits TAM phosphorylation and downstream signaling and, consistent with our signaling data, RXDX-106 completely inhibits cellular proliferation and viability at sub-nanomolar concentrations. Furthermore, we demonstrate that, in the co-culture of TAM-expressing cancer cells with immune cells such as macrophages, TAM ligands such as Gas6 are secreted to drive lipid dependent activation of TAM on cancer cells, leading to their survival and proliferation. In summary, our data suggest that TAM RTKs can act as traditional oncokinases when activated either in a lipid dependent or lipid independent manner. In addition, we demonstrate that TAM fusions are tractable therapeutic targets and that patients with tumors harboring such molecular alterations may derive clinical benefit from RXDX-106. Finally, we show that RXDX-106 not only has the potential to inhibit cellular proliferation and survival on the cancer cell itself, but also affect the TAM-expressing tumor microenvironment to result in a global anti-cancer environment.

**#4192 Targeting the SHP-1/p-Lyn signaling shows therapeutic potential in diffuse large B-cell lymphoma.**

Chun Yu Liu,1 Man-Hin Ho,2 Moon Him Tsai,2 Pei-Yi Chu,2 Tsu-Ting Huang,4 Chun-Teng Huang,2 Chung-Wai Shiau,2 Kuen-Feng Chen,2 1Taipei Veterans General Hospital, Taipei, Taiwan; 2National Taipei Veterans Hospital, Taipei, Taiwan; 3Show Chwan Memorial Hospital, Taipei, Taiwan; 4National Taiwan University, Taipei, Taiwan; Show Chang Memorial Hospital, Taipei, Taiwan; National Taipei Veterans Hospital, Taipei, Taiwan; Yang-Ming Branch of Taipei City Hospital, Taipei, Taiwan; National Yang-Ming University, Taipei, Taiwan.

Background: Diffuse large B cell lymphoma (DLBCL) is one of the most aggressive forms of non-Hodgkin’s lymphoma. Genetic analyses revealed molecular heterogeneity of DLBCL tumors, classifying the cell-of-origin into two distinct molecular subtypes: germinal center B-cell (GCB) and activated B-cell (ABC). ABC-type DLBCL has a worse survival after upfront chemotherapy compared to GCB-type DLBCL, thus ABC-type DLBCL patients have an urgent medical need that warrants additional research efforts and new therapeutic options. In current study we explored the biological role and potential therapeutic implication of a protein phosphatase SHP-1 in DLBCL. Methods: DLBCL cell lines including ABC-like cell lines U2932, Ly-3 and GC-like cell lines DHL-6, Ly-7 and DB were used for in vitro studies. Cell viability was examined by MTT assay. Apoptotic effects were examined by annexin V, cytochemistry and Western blot. Signal induction on pathways in cells were assessed by Western blot. In vivo therapeutic testing of SHP-1 agonists were performed in nude mice with DLBCL xenografts. Results: We first examined the protein expression of SHP-1 and its downstream p-STAT3 in a panel of DLBCL cell lines and identified in general SHP-1/p-STAT3 expression was higher in ABC-like cells. Interestingly, the expressions of p-Lyn (Tyrs96), p-BTK (Tyrs223), key members of B-cell receptor (BCR) signaling pathway, were also higher in ABC-like cells. Knockdown or overexpression of SHP-1 protein expression revealed a reciprocal change of p-Lyn, suggesting SHP-1 negatively regulates phosphorylation of Lyn kinase. Immunoprecipitation experiments confirmed SHP-1 interact with Lyn in DLBCL cells. We previously developed direct SHP-1 agonists, namely SC-43 and SC-60, which could increase SHP-1 activities and induce apoptosis. Here we tested SC-43 and SC-60 in comparison to Ibrutinib, a selective Bruton’s tyrosine kinase (BTK) inhibitor. The SHP-1 agonists showed in general superior anti-proliferative and apoptotic effects, comparing to Ibrutinib. Mechanistically, SHP-1 agonists enhanced SHP-1 activation and the activity of BCR and BTK signaling. In addition, SHP-1 agonists also down-regulated p-STAT3 as previously reported, which also contributes to anti-cancer effects. In vivo, SC-43 at doses of 10mg/kg/day and 30mg/kg/day orally showed comparable anti-tumor effects with Ibrutinib at doses of 12.5mg/kg/day and 25mg/kg/day in mice bearing U2932 xenografts, respectively. Western blot confirmed SC-43 downregulation on p-Lyn and p-BTK in vitro and in vivo. Conclusion: Ibrutinib SHP-1 negatively regulates phosphorylation of Lyn, and targeting SHP-1/p-Lyn with direct SHP-1 agonists shows therapeutic potential in DLBCL.

**#4193 A triple exon-skipping luciferase reporter assay identifies a new CLK inhibitor scaffold.**


The splicing of pre-mRNA is a critical process in normal cells and is deregulated in cancer. Compounds that modulate this process have recently been shown to target a specific vulnerability in tumors. We have developed a novel cell-based assay that specifically activates luciferase in cells exposed to splicing targets, such as sudemycin D6. This assay was used to screen a combined collection of approved drugs and bioactive compounds. This screening effort identified several novel candidate targets which include the GTPase 74514A and aminopurvalanol A, both of which have been reported to be cyclin-dependent kinases (CDKs) inhibitors. We found that, these compounds, and their analogs, significantly reducecdc2-like kinase (CLK) inhibition and define structure-activity relationships (SAR) at CLKs. We prepared a set of analogs and were able to ‘dial out’ the CLK activity and simultaneously developed CLK inhibitors with low nanomolar activity. Thus, we have demonstrated the utility of our exon-skipping assay and identified new molecules that exhibit potency and selectivity for CLK, as well as some structurally related dual CLK/CDK inhibitors.

**#4194 Antitumor activity of multikinase inhibitors in HER2-positive gastric cancer cells.**


Background: The developments of novel anti-HER2 drugs including multikinase inhibitors have achieved advancing evolution for past several years, and the options in the treatment of HER2-positive malignant tumors have been increasing, especially in breast and lung cancers. On the other hand, regarding HER2-positive gastric cancer, traditional oncokinases and novel targets have established clinical evidences. Afatinib, an irreversible human epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, binds to the kinase domain of HER2, and is one of the hopeful candidates as novel molecularly targeted drugs for HER2-positive gastric cancer. In this study, we investigated the antitumor effect of multikinase inhibitors including afatinib in gastric cancer cell lines.

Method: We examined the relation between molecular profiles and multikinase inhibitors sensitivities in 12 gastric cancer cell lines: ECC10, GCY, KATO-III, NUGC3, and NUGC4 cells. The range of IC50 for these cell lines was 2.1 to 39.4 nM. Three cell lines showed moderate sensitivity to gefitinib of more than 25% in all 12 cell lines. Conclusion: Afatinib, a multikinase inhibitor, is more than 10 times more effective than gefitinib, which was not sensitive to afatinib. ECC10 with HER2-L755S mutation was sensitive to other HER2-targeted drugs trastuzumab, and reported to be insensitive to lapatinib either. On the other hand, ECC10 with HER2-L755S mutation was not sensitive to afatinib. The range of IC50 in 7 insensitive cell lines was 0.80 to more than 100 nM. Three cell lines showed moderate sensitivity to gefitinib of which IC50 ranged from 100 to 1000uM. Inhibition rate of trastuzumab was under 25% in all 12 cell lines. Conclusion: Afatinib, a multikinase inhibitor, is highly effective to HER2 amplified gastric cancer cell lines, and may become a novel treatment option in HER2-positive gastric cancer treatment. On the other
hand, afatinib is not always effective even if cancer cells have HER2 mutation or amplification. In this study, we report the detailed predictive biomarkers of sensitivity to afatinib and other several multikinase inhibitors in HER2-positive gastric cancer cells.

**#4195** MIRO02: a new POLA1 inhibitor endowed with a large spectrum of antitumor activity. Claudio Pisano,1 Lucio Melrini,2 Sergio Perco,2 Raffaella Cincinelli,2 Nadine Darwiche,3 Mario B. Guglielmi,1 Antonietta Esposito,1 Ilaria La Porta,1 Giacomo Signorino,1 Gabriele De Rubis,2 Fabiana Colecchi,3 Francesco Cardile,1 Alessandra Fusci,1 Egildo L. D’Andrea,1 Sarbana Dallavalle,1 Biogem, Centro Ricerche, Ariano Irpino (AV), Italy; 1University of Milan, Milan, Italy; 2Ronzoni Institute for Chemical and Biochemical Research, Milan, Italy; 3American University of Beirut, Beirut, Lebanon; 4University of Milan, Milan, Italy.

A recent work from Hain et al. disclosed DNA polymerase 1 alpha (POLA1) as the key target for the anti-cancer effects of CD437, a molecule belonging to the class of Retinoid Related Molecules (RRMs). Before this evidence, this family of compounds, of which CD437 and ST1926 represent the prototypes, has been characterized for its antiproliferative, antitumor and pro-apoptotic activity. With aim to identify a new RRM with an improved pharmacological profile, we recently selected MIRO02 as a novel compound endowed with a potent antitumor activity and a peculiar pharmacological profile. In vitro treatment with MIRO02 leads to a G1/S arrest and exerts a potent anti-tumor/proapoptotic activity in a wide range of cancer cell lines, including H460-R9A cells, which are cross resistant to ST1926 and CD437 (IC50 >70 fold of H460-R9A vs H460). Moreover, in H460-R9A cells, we identified the L746F mutation in POLA1, already described as a marker of resistance to CD437. We have confirmed that POLA1 is the molecular target of both MIRO02 and ST1926 and that, interestingly, the expression of the L746F mutant, although associated with the resistance to RRM’s, only marginally reduces the cytotoxic effects of MIRO02. In order to investigate the activity of MIRO02 against tumor growth, we examined a panel of in vivo xenograft tumor models, including those originating from human Non-Small Cell Lung Cancer (NSCLC) and Malignant Pleural Mesothelioma (MPM). In these models, using a q2dx5x3w schedule in doses ranging from 50 to 70 mg/Kg administered orally, we observed a strong tumor growth inhibition (TGI >70%). Interestingly, the combination of MIRO02 with cisplatin (4mg/Kg; qgdx3w; i.v.) in MPM models showed a synergistic antitumor efficacy, reaching a TGI >90%, and cured animals. Interestingly, we previously identified POLA1 as a key pro-proliferative player in 9 primary MPM samples. The ex-vivo analyses indicated that POLA1 expression was strongly modulated upon MIRO02 treatment. Furthermore, in the same models, we also observed that the expression/secretion of multiple circulating factors is affected by MIRO02 and we identified some of them as potential biomarkers of tumor responsiveness to MIRO02. Taken together, our results identified MIRO02 as a novel anti-cancer compound and suggest POLA1 as a target for new antitumor strategies to be investigated in Clinical Trials.


Aberant expression of TAM family receptor tyrosine kinases, comprising Tyro3, Axl and Mer, is found in virtually every type of human cancer. The expression levels correlate positively with disease staging and prognostic outcome, attributing to metastasis and drug resistance. The TAM kinases have recently emerged as a dual oncological therapeutic target, owing to their immunosuppressive activity. Apart from targeting tumor-survival/growth pathways their inhibition also unleashes the anti-tumor immunity in the tumor microenvironment. In our efforts to develop small-molecule inhibitors targeting the TAM kinases, we identified SLC-391 as one of the most promising preclinical candidates with potent activity towards both Axl and Mer, which play roles in maintaining tumor survival and immunosuppressive tumor microenvironment, respectively. Pharmacodynamic studies of SLC-391 in CT26 syngeneic mouse model revealed increase in the number of NK cells and the ratio of M1/M2-macrophages in the treatment group as compared to the vehicle control, followed by the rise of CD8+/Treg ratio and reduction in immunosuppressive myeloid cells. This is indicative of sequential engagement and stimulation of pro-inflammatory innate immune response and adaptive immune response. In addition, a synergistic anti-tumor effect was observed when the anti-PD-1 insensitive CT-26 model was treated with a combination of SLC-391 and an anti-PD-1 antibody, suggesting that blocking the Axl/Mer-mediated immunosuppressive pathway may significantly enhance the therapeutic efficacy of immune checkpoint inhibitors. In summary, the anti-tumor activity of SLC-391 is mediated by directly inhibiting tumor cell growth as well as reversing the immunosuppressive tumor microenvironment.

**#4197** Interaction of B lymphoma cells with the microenvironment affects ibrutinib sensitivity. Hsu-Ping Kuo, Sidney Hsieh, Mint Sirisawad, Chun-Te Chen, Leo W. Cheung, Karl J. Schweighofer, Chia-Lin Hsu, Chi-Ling Fu, Jing Liu, Shiqian Wu, Karl Eckert, Hugh Wang, Mutiah Apatira, Kamaldeep Dhami, Kevin Kweil, Jeff Hsu, Betty Y. Chang. Pharmaciescics, LLC, an AbbVie Company,Somerville, CA.

Introduction: The cellular microenvironment plays a key role in the pathogenesis of B-cell malignancies (Amé-Thomas, Blood 2007; Pardar, Leukemia 2009). Bone marrow stromal cell interactions can provide survival signals to CLL cells (Kurtova, Blood 2009), and stromal cell gene signatures and markers can predict survival outcomes and add prognostic value, respectively, for patients with chronic lymphocytic leukemia (CLL) (Lenz, Nature 2013). The cell-surface immunosuppressive protein, CD437 (ibr), is a first-in-class, oral, once-daily inhibitor of Bruton’s tyrosine kinase, has demonstrated single-agent activity in R/R DLBCL (Wilson, Nat Med 2015). In this study, we established an in vitro coculture system and report the effects of stromal cells on ABC-DLBCL cells and their sensitivity to ibr. Methods: The effect of ibr on cell growth was evaluated by luminescent cell viability assay. Flow cytometry was used to evaluate the effect of ibr on cell death in different cell populations. Live-cell imaging detected drug effects on apoptosis at different time points. Gene expression levels in B lymphoma cells were examined by quantitative PCR and NanoString panels. Human cytokines/chemokines were quantified using MILLIPLEX® MAP Kit. Results: Reduced ibr sensitivity was observed in TMD8 DLBCL cells cocultured with HS-5 bone marrow-derived stromal cells compared to TMD8 cells from apoptosis. Treatment with ibr induced TNFSF10 expression only in TMD8 cells without HS-5 coculture, providing a survival mechanism for escape of ibr-induced cell death. In addition, coculturing with HS-5 decreased surface expression of CD20 but not CD19. Several cytokines/chemokines showed differential expression in the coculture system (increased CCL2 and IL-10; decreased CCL4 and TNFβ). Reduced expression of BCR signaling related miR-155 was observed in ABC-DLBCL and CLL cell lines with HS-5 coculture, suggesting downregulation of BCR signaling in these cells. The adhesion molecule, SELL, showed decreased expression in the ABC-DLBCL cells cultured with HS-5 as well as in ibr-resistant TMD8 cells, consistent with our observation that ABC-DLBCL patients with poor responses (PD+SD) to ibr have lower SELL expression in their tumor samples. We further assessed expression levels of amino acid metabolism-related genes and demonstrated significant increases of ASS1 and TDO2 in the TMD8 cells cocultured with HS-5. Expression levels of amino acid metabolism-related genes varied across patients with different responses to ibr, further suggesting the importance of amino acid levels in the tumor microenvironment and ibr response. Conclusions: The changes in ABC-DLBCL cells cocultured with stromal cells identified here may provide new insights into cellular cross-talk in the microenvironment. Further investigation into cell-cell interactions and ibr may help stratify patient populations and provide new therapeutic strategies.

**#4198** Highly potent and selective DNA-PK inhibitor M3814 with sustainable anti-tumor activity in combination with radiotherapy. Thomas Fuchs,1 Werner W. Mederski,1 Ulrich Emde,1 Hans-Peter Buchstaller,1 Frank Zenke,1 Astrid Zimmermann,1 Christian Sirrenberg,1 Lubo Vassilev,2 Lars Damstrup,1 Kevin Kwei, Bettys Hsu-Ping Kuo, Sidney Hsieh, Mint Sirisawad, Chun-Te Chen, Leo W. Cheung, Karl J. Schweighofer, Chia-Lin Hsu, Chi-Ling Fu, Jing Liu, Shiqian Wu, Karl Eckert, Hugh Wang, Mutiah Apatira, Kamaldeep Dhami, Kevin Kweil, Jeff Hsu, Betty Y. Chang. Pharmaciescics, LLC, an AbbVie Company,Somerville, CA.

Introduction: Physical or chemical agents that damage DNA such as ionizing radiation are among the most widely used classes of cancer therapeutics today. Double strand breaks (dsb) generated in DNA by radiation induce a multitude of cellular responses, including DNA repair, cell cycle arrest or cell death once the damage is left unrepaired. A complex set of molecular events are responsible for DNA repair mediated by two major repair mechanisms - homologous recombination (HR) or non-homologous end joining (NHEJ). DNA-PKcs together with its regulatory protein subunits Ku70 & Ku80, is an integral component of the NHEJ process and considered an attractive intervention point to inhibit DNA dsb repair. We have developed an orally bioavailable, highly potent & selective inhibitor of DNA-PK, M3814, for cancer therapy in combination with DNA damage-inducing chemotherapy as a means of enhancing the efficacy of radiation. In preclinical studies, M3814 sensitized multiple tumor cell lines to radiation therapy in vitro and strongly enhanced the anti-tumor activity of irradiation in vivo with complete tumor regression by applying a clinically relevant fractionated radiation regimen. These effects are due to inhibition of DNA-PK protein kinase activity as demonstrated by the levels of DNA-PK autophosphorylation in human tumor cell lines & xenograft tumors. M3814 is currently being investigated in PhII clinical trials.
#4199 Inhibition of NF-kB inducing kinase (NIK) selectively abrogates NIK and TRAF3 mutant multiple myeloma tumor growth. Matthias Versele, 1 Lut Janssen, 1 Tamara Geerts, 1 Wim Floren, 1 Boudeijn Janssens, 1 Hillary Mil- lar, 2 Edgar Jacoby, 1 Gerhard Gross, 1 Yann Ligny, 1 Yvan Simonnet, 1 Nathalie Amblard, 1 Olivier Querolle, 3 Inne Cooka, 4 Virginie Poncet, 5 Virginie Tronel, 6 Sophie Vassal, 5 Lieven Maenhoudt, 5 and Sri Ram Balasubramanian, 7 Laurie Lenox, 7 Charles Theuer, 5 Ricardo Attar, 1 Jan Stansfeld 1.

Y bition inaggressivelymphoidmalignancies. TRC694 is a first-in-class orally bioavailable NIK kinase inhibitor, and provides tumors at doses of 10 to 40 mg/kg, with no signs of toxicities. In conclusion, (RPMI-8226, MM.1S) mutant MM tumors, completely inhibits growth of these TRC694 to mice bearing subcutaneous NIK translocated (JJN-3) or TRAF3 NFkB regulated genes in the tumors. Consistently, once-daily, oral dosing of /H9251/H9252/H9253 amplifıedin16%oftumors(TCGAdata).WehaveusedanovelandselectivePI3K of patients. The PI3K pathway is deregulated in 37% of SOC with PI3K becoming critical to overcome drug resistance, recurrence, and hence improve the survival of patients. The PI3K pathway is deregulated in 37% of SOC with PI3K and γ amplified in 16% of tumors (T CGA data). We have used a novel and selective PI3K γ inhibitor (RP6530) with high potency against PI3Kγ (IC_{50}=25 nM) and δ (IC_{50}=30 nM) selectively targeting (δ > 100-fold) and (γ > 300-fold) iso-

#4200 Targeting cancer stem cells in serous ovarian carcinoma using RP6530, a dual PI3K δ-γ inhibitor. Sneha S, Nivetha R, 1 P. R. Nagare, 1 Srikant Viswanadha, 2 Swaroop Valakalanka, 1 T. S. Ganesan, 1 Cancer Institute, Chennai, India; 1Incogen Therapeutics Pvt Ltd, Hyderabad, India; 2Bhans Pharmaceuticals SA, La Chaux-de-Fonds, Switzerland.

Ovarian cancer is one of the most lethal gynaecological malignancies as most pa-
tients are diagnosed at an advanced stage and have a poor prognosis. Several studies have prospectively isolated cancer stem cells (CSCs) from samples of tumor from patients with serous adenocarcinoma of the ovary (SOC). This has been achieved by using cell surface antigens or functional assays. Targeting this subpopulation could be critical to overcome drug resistance, recurrence, and hence improve the survival of patients. The PI3K pathway is deregulated in 37% of SOC with PI3Kδ and γ amplified in 16% of tumors (TCGA data). We have used a novel and selective PI3K δ-γ inhibitor (RP6530) with high potency against PI3Kδ (IC_{50}=25 nM) and δ (IC_{50}=30 nM) selectively targeting (δ > 100-fold) and (γ > 300-fold) iso-

#4201 Combination of palbociclib with enzalutamide shows in vitro activi-
y in RB-profi cient and androgen receptor-positive triple-negative breast cancer cell lines. Ling-Ming Tseng, 2 Tzu-Ting Huang, 1 Chun-Teng Huang, 1 Kay-Yi Lau, 1 Chung-Yu Liu, 4 Chung-Wai Shiau, 5 Kuen-Feng Chen, 5 Taipei Veterans General Hospital, Taipei, Taiwan; 6Yang-Ming Branch of Taipei City Hospital, Taipei, Taiwan; National Yang-Ming University, Taipei, Taiwan; National Taiwan University Hospital, Taipei, Taiwan.

Background: Cyclin-dependent kinases 4 and 6 (CDK4/6) inhibitors have in vitro synergistic effects and significant clinical efficacy in combination with hormone therapy for estrogen receptor (ER)-positive advanced breast cancer. However, the effects of CDK4/6 inhibitors in triple negative breast cancer (TNBC) are not well-elicitated. Retinoblastoma (RB, a known substrate of CDK4/6) protein pathway deregulation is a frequent occurrence in TNBC and in vitro studies have revealed that pharmacological CDK4/6 in-
hibitors yield a cooperative cytostatic effect with doxorubicin in RB-prof-
icent TNBC models, suggesting that RB may be a biomarker for selecting patients receiving CDK4/6 inhibitors. In addition, anti-androgen therapy have been shown preclinical efficacy in androgen-receptor (AR) positive TNBC cell lines. Here we tested the combination effect of a CDK4/6 inhibitor palbociclib with an AR antagonist enzalutamide in TNBC cell lines. Methods: MDA-MB-231, MDA-MB-468 and MDA-MB-453 TNBC cell lines were used for in vitro studies. Cell viability was examined by MTT assay. Apopto-
ic effects were examined by flow cytometry. Signal transduction pathways in cells were assessed by Western blot. Results: We correlated the expressions of AR and pRB status in these TNBC cells and identified that MDA-MB-453 were AR-positive/pRB-positive, MDA-MB-231 were AR-negative/pRB-positive, and MDA-MB-468 were AR-negative/pRB-negative cell lines. Since CDK4/6 inhibition leads to dephosphorylation of pRB, we examined effects of palbociclib and found that palbociclib significantly inhibited the cell growth of pRB-positive cell lines MDA-MB-453 and MDA-MB-231, but did not affect pRB-negative MDA-MB-468 cells. Further combining palbociclib with enzalutamide showed that the growth inhibitory effects of combination therapy were additive in AR-positive/pRB-positive MDA-MB-453 cells, but not in AR-negative/pRB-positive MDA-MB-231 cells, nor AR/pRB double negative MDA-MB-468 cells. Moreover, palbociclib-induced G1/S arrest only exhibited in pRB-proficient MDA-MB-453 and MDA-MB-231 cell lines. In contrast, enzalutamide did not affect the cell cycle on any of these TNBC cell lines but inhibited phosphorylation of AR signaling in AR-positive MDA-

#4202 Trametinib, a MEK inhibitor, augments nab-paclitaxel based che-

therapy response in preclinical models of pancreatic cancer. Niranjana Awasthi, 1 Sheena Monahan, 2 Alexia Stelianakis, 3 Margaret A. Schwarz, 2 Roderich E. Schwarz, 1 Indiana University School of Medicine, South Bend, IN; 3University of Notre Dame, South Bend, IN.

Pancreatic ductal adenocarcinoma (PDAC) is currently the third leading cause of cancer related deaths in the USA with a 5-year survival less than 6%. Nanoparticle albumin-bound paclitaxel (nab-paclitaxel, NPT) has demonstrated 8.5 months median survival in combination with gemcitabine, and now yields a standard of care for PDAC patients. Genetic alterations of the RAS/RAF/MEK/ERK (MAPK) signaling pathway yielding constitutive activation of the ERK cascade have been implicated in many human cancers. In PDAC, activating K-ras mutations occur at a frequency of 90%, rendering this a potential therapeutic target. Efforts to develop drugs that directly
target mutant KRAS protein remain challenging due to target specificity issues. Alternative strategies therefore focus towards inhibition of downstream targets in the RAS-MAPK cascade such as MEK. Trametinib (Tra) is a potent and highly selective small molecule inhibitor of MEK1/2 kinase activity. We evaluated efficacy of trametinib to enhance antitumor response of nab-paclitaxel based chemotherapy regimens in preclinical models of PDAC using K-ras mutant cell lines. In subcutaneous PDAC xenografts using AsPC-1 cells, net tumor growth in different therapy groups was 432.6 mm³ in controls, 105.3 mm³ after NPT (p=0.0023), 184 mm³ after Tra (p=0.0018), 81 mm³ after NPT + Tra (p=0.0003), 37.3 mm³ after NPT + Gem (p=0.0025) and -8.1 mm³ (tumor regression) after NPT + Gem + Tra (p=0.0001). In another subcutaneous PDAC xenografts using Panc-1 cells, net tumor growth in different therapy groups was: 274.1 mm³ in controls, 80.8 mm³ after NPT (p=0.0002), 150.6 mm³ after Tra (p=0.0047), 75.1 mm³ after NPT + Tra (p=0.0002), 48.4 mm³ after NPT + Gem (p=0.0004) and 3.8 mm³ after NPT + Gem + Tra (p<0.0001). In PDAC peritumoral dissemination model using AsPC-1 cells, median animal survival compared to controls (20 days) was increased after therapy with NPT (33 days, a 65% increase, p=0.0004), Tra (31 days, a 55% increase, p=0.0004), NPT + Tra (37 days, an 85% increase, p=0.0001), NPT + Gem (39 days, a 95% increase, p=0.0001) and NPT + Gem + Tra (49 days, a 145% increase, p<0.0001). Effects of therapy on intratumoral proliferation and apoptosis corresponded with tumor growth inhibition. In vitro studies demonstrated inhibition in PDAC cell lines, NCI-H1100, MiaPaCa-2, CFPAC) proliferation by NPT, Gem, Tra, and combination. Immunoblot analysis revealed that trametinib effects were specifically accompanied by decrease in phospho-ERK expression and increase in the expression of apoptosis-related cleaved caspase-3 and cleaved PARP-1 proteins. These findings suggest that the effects of nab-paclitaxel based chemotherapy regimens can be enhanced through specific inhibition of MEK1/2 kinase activity, which clinically could lead to improved PDAC therapy effects.

**Cancer Chemistry** Drug Screening Innovation

**#4205 Intracellular pharmokinetics of 5FU and palbociclib: Uptake and efflux in disaggregated cells and 3D models.** Maria Jove,1 Paul Loadman,1 Jade Spencer,1 Lava Sulayman,2 Jo Wicks,1 Amanda Bace,1 Chris Twelves,1 1Institute Cancer Therapeutics, Bradford University, Bradford, United Kingdom; 2University of Utrecht, Utrecht, Netherlands; 3Leeds University, Leeds, United Kingdom.

Introduction: Pre-clinical drug development does not routinely assess drug penetration beyond use of the human intestinal Caco-2 cell line as an in vitro model of the intestinal barrier to evaluate potential drug absorption. Detailed in vivo tumour pharmokinetic (PK) studies are unusual. However, the ability of a drug to reach its target plays a key role in drug efficacy. Here we present a method to study drug PK in disaggregated cells and in a 3D cell culture model (spheroids) using 5FU and palbociclib. The comparison of these 2 drugs is of interest taking into account their different mechanisms and chemical properties, logP 0.58 and 2.12 respectively.

Knowledge of cellular uptake and efflux in disaggregated cells and 3D models together with intracellular binding characteristics is vital to optimise a better drug delivery. Experimental procedures: MCF-7 and DLD-1 cells were used for cellular and spheroid experiments. Spheroiod growth was optimised to produce a spheroid of 300-400 μm diameter at day 4. Drug uptake: 1x10⁶ cells or 30% of cells/tissue point were exposed to 10μM 5FU or 0.1μM of palbociclib for different time durations. For 5FU, drug uptake at 100μM was also monitored to detect 5FU metabolites. Drug efflux: 1x10⁶ cells were exposed first to 100μM 5FU or 0.1μM of palbociclib for 60min then, drug media removed, substituted by fresh media and sample over a 4h period. Drug concentration inside cells and in the fresh media was measured. Drug concentrations were measured using HPLC-Tandem Mass Spectrometry. Each experiment was carried out in triplicate. Results: 5FU achieves intracellular steady state within 5-10min in cells and spheroids with equal concentrations inside and outside the cell. Intracellular binding of 5FU is around 70%. Palbociclib cellular uptake had an initial intracellular drug concentration of 4-46 nM in biochemical assays. In mechanistic cell-based studies, ASN002 showed that efflux occurred rapidly within 5min achieving a new equilibrium with 30% of 5FU remaining inside the cells. 5FU was also detected extracellularly after 1h in the fresh media, showing that cells have both 5FU and metabolite efflux. In contrast, palbociclib cellular uptake had an initial intracellular drug concentration peak at 5-10min which then plateaued. Interestingly, total intracellular concentrations of palbociclib were over 30 times higher compared to the external media drug at steady state. Spheroiod and efflux experiments of palbociclib are on-going and will be presented. Conclusions: Our results suggest that both drugs penetrate quickly in cancer cells. Palbociclib intracellular concentrations were 30 times higher than the external media suggesting excessive intracellular binding while 5FU achieve equal concentrations between the inside and outside of the cell. These data and methodology may be useful to allow generation of mathematical models to improve drug delivery design.

**#4206 A high-throughput 3D tumor spheroid screening method for drug discovery using imaging cytometry.** Leo Li-Ying Chan, Scott Cribbes, Sarah Kessel, Scott McMenemy, Jean Qiu. Nexcelom Bioscience LLC Lawrenceville, NJ.

There have been an estimated 1000 FDA approved drugs since their inception in 1938. Currently it costs approximately $83 million dollars to bring a drug candidate to the market, and often times the potential candidate fails at the beginning of the clinical phase. Therefore, it is important to develop drug screening methods that are more clinically relevant or predictable. The current 2D methods for cancer
drug discovery have had some difficulty in identifying potential drug candidates that can be used for clinical testing. To overcome this challenge, there has been an increase in research of 3D tissue culture that facilitated the development of new in-vitro tumor model assays. Traditional 3D spheroid analysis method relied heavily on visual observation using standard microscopy, which is time-consuming and labor-intensive. Current research has developed high-throughput image-based cytometers, such as Celigo, have demonstrated the ability to perform high-throughput and fluorescence-based assays. Celigo imaging cytometer can be employed to rapidly analyze and characterize 3D tumor spheroids, which can be used to generate both quantitative and qualitative results. In this work, we demonstrate a high-throughput 3D tumor spheroid screening method using the Celigo imaging cytometer to screen the effects of 14 drug compounds (NIH/NCAT) on U87MG spheroid size, matrigel invasion, and tumor spheroid viability. First, a dose response experiment is performed to screen the growth inhibitory effects of the drug compounds. In addition to direct spheroid size analysis, dose inhibitory responses of tumor invasion into the matrigel are also examined. Finally, the use of specific fluorescent dyes such as Calcein AM, PI, and Caspase 3/7 were used to screen drug induced cytotoxicity on the tumor spheroids. The results showed that Celigo imaging cytometer can quickly generate accurate growth inhibitory data to identify potential drug candidates. Furthermore, tumor invasion were clearly observed and quantified in the captured images, as well as fluorescent analysis of tumor spheroid viability. By utilizing the 3D spheroid screening method, researchers can rapidly characterize and quantify drug effects on tumor spheroids in a high-throughput format, which can improve the efficiency of identifying new qualified cancer drug candidates.

#4207 Selective drug sensitivity score (DSS) for indolent and aggressive prostate cancer cell lines. Jenni Mäki-Jouppila,1 Jenni Bernoulí,1 Johanna Tuomela,1 Mari I. Suominen,2 Jussi M. Halleen,2 Sanna Timonen,3 Elina Huovari,3 Katja Suomi,3 Swapnal Potdar,3 Päivi Östling,3 Jani Saarela,3 Katja M. Fagerlund3,1 Pharmatest Services Ltd, Turku, Finland;2 University of Turku, Turku, Finland;3 University for Molecular Medicine Finland FINMIM, Helsinki, Finland.

Prostate cancer (PC) is the most common malignancy in men and the second leading cause of cancer-related deaths. The majority of the PCs are classified as adenocarcinomas characterized by the expression of androgen receptor (AR) and prostate-specific antigen (PSA). Two of the most commonly used cell lines are LNCaP and PC-3 cells derived from lymph node and bone metastases, respectively. Also VCaP cells, derived from vertebral metastases, are widely used in prostate cancer research. It has been well established that LNCaP and VCaP cells represent the conventional indolent form of PC expressing AR and PSA and are androgen-independent, and represent the highly aggressive form. The drug sensitivity of the cell lines was assessed by applying a large panel of drugs covering cancer chemotherapeutics and clinically available and emerging drugs including conventional chemotherapy, kinase inhibitors, metabolic modifiers, rapalogs, differentiating/epigenetic modifiers, kinesin inhibitors, apoptotic modulators, NSAI Ds, hormone therapy, immunomodulators and HSP inhibitors. A panel of 460 compounds was tested in five concentrations covering a 10,000-fold drug-relevant concentration range in 384-well format. Cells were seeded to pre-drugged plates, followed by cell viability measurements (CellTiter-Glo) after 72 hours. Maximal and minimal responses to drugs were analyzed, the EC50 values were calculated and Drug Sensitivity Score (DSS) was calculated for each drug as a measure of reduced viability. A selective Drug Sensitivity Score (sDSS) was calculated to identify the selective drug response pattern of each three cancer cell lines. As expected, the results indicate that LNCaP and VCaP cells in general were more sensitive to drugs of different categories than PC-3 cells. According to DSS analysis, all three cell lines showed sensitivity to conventional chemotherapy and kinase inhibitors. However, PC-3 cells were more sensitive to kinase inhibitors than conventional chemotherapy. Determining sDSS revealed specific sensitivities of each cell line. LNCaP cells were sensitive to kinase inhibitors, such as mTOR and AKT inhibitors. Also VCaP cells showed selective sensitivity to kinase inhibitors, especially Aurora kinase and IGFIR inhibitors. In addition to kinase inhibitors, VCaP cells were selectively sensitive to HDAC inhibitors. Furthermore, PC-3 cells were sensitive to e.g. CDK inhibitors. We conclude that the cell-based compound screening combined with DSS and sDSS analysis provides a possibility to generate an extensive collection of anti-cancer compounds enabling repurposing of existing drugs to new indications, identification of vulnerabilities in different types of cancer cells and functional investigation of cellular pathways behind drug sensitivity or resistance.
poor prognosis, overexpress GWL and are highly sensitive to GWL depletion. In addition, GWL expression has been found to be elevated in oral squamous cell carcinoma and prostate cancer tissues. A number of studies have also indicated that GWL may contribute directly to tumorgenesis, suggesting that modulation of GWL activity with small-molecule inhibitors could afford a potential new anti-cancer therapy. The precise role of GWL in cancer is yet to be defined. The identification of a pharmacological tool would be useful to further assess the tumor-associated functions of this protein. To that end, we have recently developed and optimized a robust HTSF assay, based on a specific ENSA peptide analogue, and used it to screen kinase focused libraries, made available to us by GlaxoSmithKline and Roche. A 10.2% hit rate was achieved from the screening of the 11,000 compound set at a single concentration of 10 μM. Molecules inducing over 50% inhibition in the single point screen were subsequently tested in concentration-dependent experiments. Analysis of the IC₅₀ values enabled to identify molecular clusters and potential hits to initiate a medicinal chemistry campaign. A review of the literature on the selectivity of the hits identified indicated that a significant number of these molecules also had affinity for other AGC kinases and that selectivity among the AGC kinase family could be challenging to achieve. Computational studies using an in-house co-crystal structure of GWL in complex with Staurosporine and the published crystal structures of other AGC kinases will be used to design selectivity and guide the design of inhibitors to obtain a potent chemical probe.

#4211 Gene expression changes in Polyphenol E treated prostate cells. L. Michael Carastro, 2 Ricardo A. Cordova, 1 Daniel A. Barbot, 1 Ricardo A. Declet-Bauza, 2 Irene Gushterova, 1 Nicholas K. Lago, 1 Nicholas E. Bragonja, 1 Joanna C. Burt, 1 Dana E. Hoffman, 1 Long Y. Park, 2 1The University of Tampa, Tampa, FL; 2Universidad Central de Caribe, Bayamon, PR; 3 Moffitt Cancer Center & Research Institute, Tampa, FL.

Prostate cancer (PCa) is the most common malignancy among men in the USA. Polyphenol E (PolyE) is a standardized blend of polyphenols found in green tea extract, which has been shown to have chemoprevention value in PCa models, but the molecular mechanism(s) have not been elucidated. Polyphenols from green tea are known to alter the expression levels of some genes. Our experimental goal was to identify genes with expression level changes (up- or down-regulated by 2-fold or more) in normal, primary prostate cells as well as two PCa cell lines, DU145 and PC-3. Ultimately, we sought to identify genes whose expression level was differentially altered by PolyE treatment in normal versus PCa cells. We hypothesized that exposure to PolyE induces gene expression changes, which could provide rationales for the molecular mechanisms(s) and provide potential targets for therapeutics and detection. A non-cancerous, human primary prostate cell line as well as two PCa cell lines (DU145 and PC-3) were cultured and treated them with varied concentrations of PolyE for 24 hours. Cellular RNA was isolated and subjected to microarray analyses (MA) at the Molecular Genomic Facility of the Moffitt Cancer Center. A plethora of genes were identified by MA as having two-fold or higher gene expression level changes after PolyE exposure. Of these genes, fourteen were selected for further analyses by TaqMan qRT-PCR. Cellular RNAs were converted into cDNA used in validated TaqMan qRT-PCR assays (StepOneTM RT-PCR System). RT-PCR data was analyzed using StepOneTM software and the ΔΔCt method. ACTB served as the endogenous control gene for all TaqMan qRT-PCR assays. Our qRT-PCR data from PolyE-treated DU145, PC-3, and primary prostate cells were consistent in the lack of expression changes greater than 2-fold for ATM, CASP8, HDAc4 and RB1. For RGCC, MXD1, CCSE2/FAM190B, CBLB and BCL2L11, gene expression levels increased by more than 2-fold in all three cell lines. For CCNB1 and SEC62, primary cells experienced more than a 2-fold change in gene expression, while the DU145 and PC-3 cell lines did not. Interestingly, ATM gene expression levels increased by more than 2-fold in all three cell lines. For CCNB1 and SEC62, primary prostate cells experienced more than a 2-fold change in gene expression, while the DU145 and PC-3 cell lines did not. Interestingly, ATM gene expression levels increased by more than 2-fold in all three cell lines. For CCNB1 and SEC62, primary cells experienced more than a 2-fold change in gene expression, while the DU145 and PC-3 cell lines did not. Interestingly, ATM gene expression levels increased by more than 2-fold in all three cell lines. For CCNB1 and SEC62, primary cells experienced more than a 2-fold change in gene expression, while the DU145 and PC-3 cell lines did not. 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Objective: Glioblastoma (GBM) is one of the deadliest brain tumors both in adults and children. While next-generation sequencing rapidly identifies whole exome/genome gene mutations, the efficacy of targeting mutated genes/pathways has not been systematically analyzed. Additionally, it remains unknown if neurospheres (enriched with cancer stem cells) and non-stem monolayer tumor cells with similar malignant phenotypes respond differently. The main objective of this study was to assess the in vitro activity of a panel of anti-Glioblastoma drugs and their potential effect on clinical outcomes. Methods: Twenty-six pairs of neurospheres and monolayer cells were cultured from seven aggressive childhood GBM xenografts and cultured cells, but only 3/17 MEK inhibitors were active in neurospheres and 2/17 in monolayer cells (GDC0980 and PI-103 were active in both) on day 7. In IC-R0315GBM (from an autopsied terminal GBM) that carried PI3KCA mutation (allele frequency 22-27%), 5/33 PI3K inhibitors were active in neurospheres and 8/33 in monolayer cells (only Temretinib was active in both) after 7 days. In IC-3572GBM (recurgent GBM) and ICB-1127AA (radiation-induced anaplastic astrocytoma), no druguable mutations were detected. The number of active drugs on day 7 was 366 in IC-476GBM, 406 in IC-3572GBM, 284 in IC-R0315GBM, and 305 in ICB-1127AA. When the 4 matching pairs of neurospheres and monolayers were compared, the agents active in both cultures ranged from 36% to 60%, active only in neurosphere from 10.3% to 25%, and active only in monolayer cells from 14.8% to 53%. Subsequent in vivo validation using MLN8827 in IC-4687GBM and IC-R0315GBM showed that effective targeting of both neurosphere and monolayer was required for significantly-improved animal survival times. Conclusion: We showed that long-term treatment is feasible for high-throughput drug screening. Targeting druguable mutations can be achieved but only by a fraction of specific agents. Neurospheres and monolayer cells do not always respond equally toward the same drugs, and effective targeting of both subpopulations is needed to generate prolonged animal survival times.

PREVENTION RESEARCH: Population Science in Cancer Prevention Research

#4215 Cancer-related fatigue and physical activity vary by age for black women with a history of breast cancer. Melody A. Sven,1 Amandeep Mann,2 Raheem Paxton,3 Lorraine T. Dean 1.

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Purpose: Cancer-related fatigue is the most discomforting symptom among women with a history of breast cancer. Black women are more likely to experience risk factors for cancer-related fatigue, like physical inactivity and obesity, yet cancer-related fatigue studies have not explicitly focused on Black women. This cross-sectional analysis assesses cancer-related fatigue and physical activity among Black female breast cancer survivors. Methods: In May and July of 2012, 266 members of the Sisters Network, Inc completed an online survey on demographic, cancer history and treatment, fatigue using the 13-item Function. Assessment of Chronic Illness Therapy (FACIT) Fatigue Scale, and engagement in ≥150 minutes per week of physical activity (CDC guideline). Multiple linear regression assessed relationships between fatigue and physical activity. Results: The average participant was 54 (SD=9) with a FACIT score of 13.63, indicating “severe fatigue”. Fatigue was greater (p=0.02) among the 56% of women meeting physical activity guidelines. Multivariable analysis showed that meeting physical activity guidelines (β=-3.01, p=0.01) and higher age group (50-59: 4.75, p=0.001; 60+: 4.38, p=0.01) were associated with greater fatigue. An interaction plot showed that differences in fatigue for those meeting or not meeting physical activity guidelines is largest among Black women under age 50. Conclusions: Meeting physical activity guidelines was associated with greater cancer-related fatigue, which was most pronounced for those under age 50. Interventions for Black breast cancer survivors with cancer-related fatigue should consider physical activities that can be integrated into existing tasks and provide age-appropriate resources to address physical and emotional fatigue domains.

#4216 Racial disparities in receipt of adjuvant hormonal therapy among patients diagnosed with breast cancer in South Carolina. Oluwole A. Babatunde, Swann Adams, Tisha Felder, Jan Eberth, Robert Moran, Erica Sercy, James Hebert. University of South Carolina, Columbia, SC.

Background: Women with hormone receptor-positive (HR+) breast cancer (BC) have a 25% risk reduction in breast cancer-related mortality as a result of the use of adjuvant hormonal therapy (AHT). However, mortality rates remain higher among Blacks compared to Whites. This disparity has widened despite overall improved BC survival rates over time. The widening gap in Black-White mortality for BC may be related to differences in tumor biology, treatments received and access to care. The objective of this study was to assess racial disparity in the receipt of AHT among women with BC in South Carolina (SC). Methods: We derived data on all female BC cases in 2002 to 2009 from the Central Cancer Registry linked with administrative medical and pharmacy claims data for the Public Employee Benefits Plan (private insurance) and Medicaid. HR+ BC included three potential classifications for estrogen (ER+ or ER-) and progesterone receptors (PR+ or PR-): ER+ / PR+, ER+/PR+, ER-/PR+. The main outcome variable was receipt of AHT (receipt vs non-receipt). The main exposure variable was patient race (White vs Black). Chi-square tests and logistic regression analyses were conducted to compare patients who received AHT to those who did not to identify important predictors of AHT receipt. Two-way interactions were assessed between seven covariates hypothesized to modify the effect of race (age, race, insurance, marital status, county of residence, year of diagnosis, receipt of surgery (early vs late), tumor stage and tumor grade). Results: Of the 161 total breast cancer cases reported in the study period, 834 were HR+ (641 white, 76.86%; 193 black, 23.14%). The crude odds of non-receipt of AHT were 1.59 (95% CI: 1.14-2.21), and the adjusted odds was 1.23 (95% CI: 0.85-1.78) among Black compared to White patients. The adjusted odds of non-receipt of AHT were 2.02 (95% CI: 1.36-2.99) and 5.15 (95% CI: 3.41-7.77) among tumor grade II and IV/III compared to grade I respectively. Stratified analysis showed that among patients who were married, the odds of non-receipt of AHT were 2.19 (95% CI: 1.28-3.74) among Blacks compared to Whites; and among those that received late surgery, the odds of non-receipt of AHT were 3.00 (95% CI: 1.34-6.71) among Blacks compared to Whites; and among those that received late surgery, the odds of non-receipt of AHT were 1.81 and 2.42 (95% CI: 1.14-2.87 and 1.29-4.55), respectively among Blacks compared to Whites. Conclusions: To improve overall use of AHT, efforts need to be directed at Black BC patients who received late surgery (>30 days after diagnosis).

#4217 Consumer credit, cancer treatment, and health among women with a history of breast cancer. Kathryn H. Schmitz,1 Kevin Frick,2 Lauren Nicholas,1 Yuehan Zhang,1 SV Subramanian,1 Kala Visvanathan,1 Lora Landis,1 L.莱斯利·欧文·马斯特约翰斯霍普金斯大学公共卫生学院; 2 Pennysylvania State University College of Medicine, Hershey, PA; 3 Johns Hopkins Carey Business School, Baltimore, MD; 4 University of Pennsylvania School of Medicine, Philadelphia, PA; 5 Harvard School of Public Health, Boston, MA.

Background: Breast cancer treatment may represent an economic shock that may be influenced by, or may influence a cancer survivor’s socio-economic position (SEP). Financial toxicity or economic burden due to cancer treatment and treatment’s adverse effects may be reflected in one’s consumer credit ratings; however, credit’s contribution to individual health for cancer survivors or those managing long-term adverse effects of cancer treatment has not been evaluated. This analysis examined associations between self-rated health outcomes of women with a history of breast cancer, type of cancer treatment, and presence of breast cancer-related lymphedema, a persistent adverse effect of treatment, and consumer credit rating. Methods: From May to September 2015, 129 women from Pennsylvania and New Jersey were enrolled in the PAL. Social and Economic Quality of Life cross-sectional study. All participants had a history of breast cancer, and completed a survey on: demographics, SEP, co-morbidities, SF-12 self-rated health, psychosocial stress, adjuvant cancer treatments (chemotherapy, hormone therapy, radiation), breast cancer-related lymphedema symptoms, and credit quality (5-point scale self-reported as poor to excellent). Multivariable linear regression measured the association between credit and health. Results: Mean respondent age was 64, with 38.2% reporting excellent and 37.4% reporting very good/good credit. Participants completed cancer treatment on average 11.5 years ago. After adjusting for demographics and SEP, good credit was associated with a 4.5 (p=0.009) point increase in composite physical health (SF-12) score and -1.13 (p=0.02) decrease in psychosocial stress compared to women with poor credit, but was not associated with type of cancer treatment or lymphedema. Conclusion: While credit was associated with self-rated health for women with an average 11.5 year history of breast cancer, current credit quality

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did not appear to be associated with type of cancer treatment previously received or with the presence of lymphedema, a persistent adverse effect of cancer treatment. It is still possible that cancer treatment may have influenced credit score at time of treatment, and credit was able to rebound since that time. Future work should test causal pathways between credit and health outcomes after cancer diagnosis.

**#4220 Liverpool healthy lung project: a primary care initiative to identify hard to reach individuals with a high risk of developing lung cancer.** John K. Field,1 Edward S. Gaynor,2 Stephen W. Duffy,3 Katy Gardner,1 Cathy Hubbert,2 Dimitris Tsintzos,2 Michael W. Marcus,1 Lisa Jones,1 Rachel Avanitis,2 Michelle Timoney,7 Martin Ledson,1 1Univ. of Liverpool, Liverpool, United Kingdom; 2NHS Liverpool Clinical Commissioning Group, Liverpool, United Kingdom; 3Queen Mary University of London, London, United Kingdom; 4Liverpool Heart & Chest Hospital, Liverpool, United Kingdom.

Introduction: The UKLS CT screening trial has demonstrated that by utilizing a population based approach, a risk prediction algorithm (LLP), volumetric based CT nodule detection and a specific management pathway, 80% of the lung cancers detected were Stage I or II, 85% of which were suitable for surgical resection. In the Liverpool region, 2.4% of the screened population were found to have lung cancer at baseline. Methodology: Liverpool has one of the highest lung cancer mortality rates in England, and double the national average of cases diagnosed. It was decided to tackle this health inequality and the NHS Liverpool Clinical Commissioning Group (CCG) introduced the Liverpool Healthy Lung Programme (LHLP). LHLP began in February 2016 and aims to increase knowledge in communities, promoting positive messages around lung health, recognizing and tackling fear and fatalism surrounding lung cancer. The LHLP is undertaking coordinated, focused public engagement (Breathe Freely) events, through educational events for key targets, again targeting lung health in the highest incidence of lung cancer. These events are followed by patients between the ages of 58-70 who have COPD, or have smoked, being invited to a local face to face lung health check, conducted by a lung-health nurse working in local community clinics and in partnership with General Practice. Summary & Findings: This assessment of lung health involved calculation of their risk of developing lung cancer in the next 5 years (My Lung Risk). Those who triggered a 5% threshold were offered low dose thoracic CT. In the Liverpool CCG Region 34,000 patients will be eligible: in the initial 12 weeks in the first neighborhood, 1014 of 2471 (41%) eligible individuals booked to attend the lung health check (865 attended). 351 (41%) triggered the offer of a CT appointment. 306 scans have been reported; 31 (10%) had lung nodules, 59 (20%) had a noncancer significant finding identified in General Practice. To date, September, 4 have confirmed lung cancer with surgical resection; 2 on follow-up. 406 patients (45%) were not previously diagnosed with COPD, 225 (45%) had abnormal spirometry, and were referred. 238 (28%) accepted lifestyle referrals (e.g. smoking, exercise). Conclusion: The project is over 3 years. Projections suggest 34,000 participants will be eligible. We are aiming for an ambitious 70% uptake meaning 24,000 will have a face to face lung health check. We estimate that ~150 cases of lung cancer will be diagnosed.

**#4221 Factors associated with HPV vaccination initiation and completion among 18-26 year olds in the United States.** Eric Adjei Boakye,1 Betelhem B. Tobo,2 Daphne Lew,3 Meera Muthukrishnan,1 Vy T. Pham,1 Rebecca Rohde,2 Thomas Burroughs,1 Mark A. Varvares,1 Nosayba Osazuwa-Peters,2 1St. Louis University, School of Medicine, St. Louis, MO; 2St. Louis University School of Medicine, Saint Louis, MO; 3Harvard Medical School, Boston, MA; 4St. Louis Univ. Cancer Center, Saint Louis, MO.

Background: The HPV vaccine prevents HPV-associated cancers and genital warts, which cause significant morbidity and mortality in the US. While the vaccine is targeted toward 11-12-year-old boys and girls, there is a catch-up vaccination range up to 26 years. However, vaccination rates are very low among eligible young adults, aged 18-26 years, and besides college-related studies, not a lot is known about factors associated with the HPV vaccine uptake in this population. The aim of this study was to assess sociodemographic factors associated with HPV vaccination uptake in a nationally representative sample of 18-26-year-old adults. Methods: The National Health Interview Survey 2014-2015 was conducted through a household interview. HPV initiation was defined as receipt of at least one dose of the vaccine and completion as receipt of the three doses. Sociodemographic factors included age, gender, race, marital status, education, health insurance, regular provider, number of doctor visits, and geographic region. Survey-weighted multivariable logistic regression models were used to examine the socio-demographic factors that were associated with HPV vaccine uptake. Results: Approximately 27% of respondents had initiated the HPV vaccine and 16% had completed the HPV vaccine. After adjusting for covariates, compared to males, females were 81% less likely to initiate HPV vaccine [adjusted odds ratio 0.19; (95 % confidence interval 0.16-0.23). Other factors associated with HPV vaccine initiation included having health...
insurance (1.70; 1.32-2.18), visiting the doctor’s office 6+ times (1.86; 1.48-2.34) and 1-5 times (2.09; 1.56-2.81) vs. no doctor’s office within the last 12 months, and having no high school diploma (0.46; 0.32-0.64) and having high school diploma (0.40; 0.31-0.52) vs. college degree or higher. The same factors were associated with HPV vaccine completion; but, being black (0.60; 0.40-0.83) vs. white and having no usual place of care (0.74; 0.57-0.96) vs. having regular care had significantly lower rates of up to date FOBT; less than 10% (9.1%) reported having FOBT in the past year. Vietnamese had the highest rate (13.8%) followed by Korean (7.5%) and Chinese (6.9%). For the prevalence of up to date colonoscopy (<10 years), CHS data disaggregated by ethnicity among AA shows that Vietnamese Americans (63.7%) had highest rates of having colonoscopy followed by Korean Americans (51.1%) and Chinese Americans (48.7%). In our study, Chinese had higher rates of having colonoscopy (63.3%) than Vietnamese (47.5%) and Korean (43.0%). The average CRC Knowledge Score was 6.10 out of 9 (SD = 2.91). In bivariate analysis, education, marital status, and self-rated health were highly correlated with CRC knowledge. Those with higher education levels, those married, and those rated with good physical health had increased CRC knowledge. In multivariate analysis, education, health insurance, and CRC knowledge were significantly related to having colonoscopy. Those with high CRC knowledge were more likely to have colonoscopy than those low knowledge (aOR = 2.74, 95% CI, 1.13, 6.64). Those who had health insurance had higher likelihood of having colonoscopy than those without health insurance (aOR = 4.05, 95% CI, 2.33, 6.82). Those with higher school education were less likely to have colonoscopy than those with less than high school education (aOR = 0.47, 95% CI, 0.16, 0.84). The findings of this study suggest that CRC knowledge is a strong predictor of CRC screening behavior in Asian Americans. Lack of CRC screening knowledge still remains an important barrier to screening, and future strategies to increase public awareness on CRC is necessary to achieve greater screening compliance amongst AA populations.

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**#4222 Examining sociodemographic disparities in perceptions of cancer risk from medical imaging.** Kekoa Tapapara,1 Kay Pepin,2 Richard Moser,3 Bradford Hesse,4 Alexandra Greenberg.1 Mayo Clinic School of Medicine, Rochester, MN;3 National Cancer Institute, Bethesda, MD.

In the United States, ionizing radiation from medical imaging is estimated to account for nearly half of the total radiation exposure among the general population. While controversial, some estimates suggest thousands of future cancer cases can be attributed to radiation from medical imaging. With the advancement in medical technologies, understanding the health risks of radiation from medical imaging has become increasingly complex for the average cancer patient. The complexity may shape the public’s perceptions of the potential cancer risks of medical imaging tests, including x-rays, mammography, and radio-active dyes. Due to the enormous, often misleading, sources of information available to the average consumer regarding the effect of radiation on general health, trends in risk perception need to be assessed. To investigate the association between perceptions of radiation exposure from medical imaging and cancer risk, we analyzed data from the National Cancer Institute’s Health Information National Trends Survey (HINTS 4, Cycle 2). HINTS is a probability-based nationally-representative survey which collects data on the American public’s knowledge, perception, and use of cancer- and health-related information since 2003. The data examined in the present study were collected between October 2012 and January 2013. (response rate = 39.9%). Bivariate analyses reveal individuals who perceive medical imaging radiation to be harmful to health are largely female, Hispanic, non-Hispanic Black, or non-Hispanic Asian, with less education and lower incomes. This perception is especially prevalent in individuals who self-report having a poor or fair general health and less confidence in their ability to take care of their own health. After adjusting for sociodemographic and health care factors, a significantly increased odds of believing radiation from medical imaging causes "some" or "a lot" of harm to health was found among those who have had a previous cancer diagnosis compared to those who did not (OR = 1.67, 95%CI = 1.18-2.35). Individuals who self-reported being Hispanic (OR = 2.08, 95%CI = 1.29-3.34), non-Hispanic Black (OR = 1.54, 95%CI = 1.09-2.19), and non-Hispanic Asian (OR = 4.66, 95%CI = 2.05-10.62) had significantly higher odds of perceiving "some" or "a lot" of harm to health from medical imaging compared to non-Hispanic white counterparts. Our data reveal existing populations that perceive radiation from medical imaging is harmful to health. These perceptions, particularly among women and minorities, may affect the personal decisions of cancer care from individual patients. Future analyses may be useful to identify whether these perceptions alter health outcomes, especially related to frequency of patients declining cancer radiation therapy. Overall, understanding patient perceptions of radiation exposure and cancer risk may help guide conversation and education for physicians with their patients.

**#4223 Colorectal cancer knowledge and screening among Asian Americans aged 50-75 years old.** Jenny Guo,1 Hee-Soon Juon,2 Sunmin Lee.1 Thomas Jefferson University, Philadelphia, PA; 2University of Maryland, College Park, MD.

Background: Chinese, Vietnamese, and Korean Americans, three of the largest Asian American (AA) subgroups, have particularly high rates of colorectal cancer (CRC) incidence and mortality. However, CRC screening rates among these Asian Americans remain very low. Increased knowledge of CRC has been shown to be positively correlated with a person’s inclination to undergo screening. Purpose: This study estimated the prevalence of up to date CRC screening and examined the predictors of CRC knowledge and screening compliance among Chinese, Korean, and Vietnamese Americans living in the Baltimore-Washington metropolitan area. Methods: A cross-sectional sample was employed in this study and included 274 Chinese, Korean, and Vietnamese Americans between ages 50-75 years old. A questionnaire was given to participants in either their preferred native language or in English. Data was collected in person. Results: Data from the 2009 California Health Interview Survey (CHIS) indicates that Chinese Americans had higher rates of FOBT at 32.2% than Vietnamese Americans (27.3%) and Korean Americans (24.8%). Compared to these rates from white and having no usual place of care were also associated with lower odds of completing the vaccine series. Conclusions: Our study shows that there are sociodemographic factors associated with HPV vaccine uptake among young adults in the United States, and males, individuals with a lower education, and those without adequate healthcare access are less likely to initiate and complete the HPV vaccination. Our findings suggest it is necessary to develop targeted interventions to promote HPV vaccination among those in the catch-up age range.

**#4225 Disparities in cervical cancer screening participation: a comparison of Russian, Somali and Kurdish immigrants with the Finnish general population.** Esther E. Idehen,1 Päiviikki Koponen,2 Tommi Harkonen,3 Tellervo Korhonen4.1 University of Eastern Finland, Kuopio, Finland; 2Finnish National Institute for Health and Welfare (THL), Helsinki, Finland; 3University of Helsinki, Helsinki, Finland.

Background: Cervical cancer screening through Pap test has been shown as an effective preventive measure for early detection of precancerous in the cervix. Finland offers mass Pap test screening to all eligible women. Knowledge about disparities in the screening participation among immigrants compared with the Finnish general population is limited. Objectives: (1) To compare Pap test screening participation in the past five years among Russian, Somali and Kurdish immigrants living in Finland with the Finnish general female population; (2) to examine if the observed disparities remain similar when adjusted for socio-demographic and health-related confounding factors. Methods: Data from the Finnish Migrant Health and Well-being Study and Finnish Health 2011 Survey were used. Random samples of persons living in six cities in Finland were drawn from the national population registry. Data of self-reported cervical screening participation (Pap test) among women aged 29-60 were available from the registry. Data of self-reported cancer knowledge (aOR = 0.47, 95% CI, 0.16, 0.84). The findings of this study suggest that CRC knowledge is a strong predictor of CRC screening behavior in Asian Americans. Lack of CRC screening knowledge still remains an important barrier to screening, and future strategies to increase public awareness on CRC is necessary to achieve greater screening compliance amongst AA populations.

**#4226 Challenges to breast cancer early detection in the developing, high income country of Trinidad and Tobago.** Kimberly Badal,1 Fidel Rampersad,2 Hamish Mohammed,1 Murrie Moosooddeen,3 Siva Kunduru,2 Nilani Kokaram Maharaj,4 Adaila Russel,2 Melissa Nathan,2 Marisa Nimrod,7 Wayne A. Warner,7 Rajini Haraksingh,8 Adetunji Toriola,9 Caribbean Cancer Research Initiative, San Fernando, Trinidad and Tobago; 7University of the West Indies, San Fernando, Trinidad and Tobago; 8Washington University in St. Louis, St. Louis, MO.

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Trinidad and Tobago (T&T) has one of the highest breast cancer (BC) mortality rates in the Americas. For the period 1995-2007, 5-year BC survival rates in T&T were approximately 30%. One possible explanation for T&T’s low BC survival rates could be late presentation and detection. Currently, opportunistic BC screening programs exist in the public and private sectors and some have been implemented to study disparities in uptake among individuals and dyads and related cancer prevention behaviors. Researchers interested in these areas can access FLASHE data and resources online to examine their own research questions.

#4228 Interlaboratory concordance of genetic variant classifications in BRCA1 and BRCA2.

Stephen E. Lincoln,1 Shan Yang,2 Melissa S. Cline,3 David Haussler,2 Benedict Paten,2 Robert Nussbaum1.1 Invitae, San Francisco, CA; 2University of California, Santa Cruz, Santa Cruz, CA.

Background: Clinical laboratories use publicly available literature, databases, and expertise to determine the pathogenicity of genetic variants in test reports. Recently, discordances between public databases have been raised as a concern, most prominently by those who use proprietary data in their classification processes. However, the clinical impact of these discordances is unclear. In prior work, we observed 99.8% concordance of clinically significant variant classifications between two labs across 975 prospectively accrued patients (Lincoln et al., J Mol Diag 2015). Here we evaluated BRCA1/2 variant classification discordance among multiple labs in a much larger patient population. Methods: Over 5000 ClinVar submissions of classified BRCA1/2 variants from 7 established laboratories (Ambry, the Children’s Hospital of Eastern Ontario, Counselling, Emory, GenDX, Invitae, and Myriad) were available for comparison. Variant data were submitted by the Sharing Clinical Reports Project. Clinically significant differences were those that could substantially change clinical management: i.e. between pathogenic (including likely pathogenic), versus VUS (variant of uncertain significance), benign or likely benign; otherwise results were considered concordant. Approximately 22,000 patients were represented in this comparison.

Results: Counting each variant separately, discordance between labs was high: 98.5% overall. However this calculation greatly underestimates the much higher concordance calculated on a per-patient basis. All discordant classifications were in rare variants that, by definition, are present in very few patients. Moreover, classifications of most rare variants are concordant (sometimes, concordantly VUS). Based on clinical and population prevalence, we calculate that 99.8% of patients would receive net concordant BRCA1/2 reports, similar to our previous study’s results. The dominant sources of discordance were (a) date, where classifications that postdate a critical piece of evidence disagree with those that predate it; and (b) expert judgment differences evaluating the strength of certain evidence, usually functional assays. Discussion: Classification discordance needs to be measured carefully to avoid over-counting differences. While substantial differences are seen in few patients, it is important to resolve these collaboratively, not competitively, as is done in other areas of oncology. Thorough and ongoing peer review of classifications is enabled by public databases like ClinVar which both enable laboratory quality control efforts and help improve personalized medical care practices.

#4229 Metabolically active macrophages: A novel link between obesity and TNBC.

Payal Tiwari,1 Kelly Schoenfeld, Arianne Blank, Chang Cui, Marsha Rich Rosner, Lev Becker. Univ. of Chicago, Chicago, IL.

Triple negative breast cancer (TNBC) patients have an extremely poor prognosis due to their high metastatic potential and lack of targeted drug therapies. Emerging epidemiological data suggest that obesity is strongly linked to the incidence and severity of TNBC. However, mechanisms by which obesity potentiates TNBC progression are unclear. Adipose tissue macrophages (ATMs) are an attractive mechanistic link between obesity and TNBC because they are the predominant type of macrophage in the breast during early tumorigenesis and obesity causes accumulation of ATMs. However, during obesity, the macrophage phenotype was reported to switch from an apparent pro-tumorigenic M2-like phenotype to an apparent anti-tumorigenic M1-like phenotype. This result raises a paradox: how does obesity promote tumor progression if it activates an anti-tumorigenic macrophage phenotype? Here we show that metabolic dysfunction promotes a mechanistically distinct pro-inflammatory phenotype (metabolic activation; MMe) in breast adipose tissue macrophages isolated from obese women. These MMe express cell surface markers of MMe (CD36, ABCA1) but not M1 (CD38, CD319, CD274) macrophages. We further demonstrate that pretreating TNBC cells with conditioned media derived from MMe but not M1 macrophages promotes mammosphere formation as well as invasion in vitro, and...
intravasation of cancer cells into the blood in vivo. Moreover, pre-treated cancer cells showed a two-fold increase in the expression of stem-like cell markers (OCT4, SOX2 and NANOG). Remarkably, we saw the same effect on ‘stemness’ of tumor cells in a spontaneous TNBC mouse model when fed a 60% high fat diet and a significant increase in tumor incidence in obese mice compared to controls. In less invasive models, we also demonstrated that blocking inflammatory cytokines expression in MMe(s) using genetic knockouts inhibits the ability of MMe(s) to promote both mammosphere formation and invasion. These findings suggest that obesity-induced changes to mammary adipose tissue reprogram macrophages to an MMe phenotype that potentiates TNBC initiation and metastasis via inflammatory cytokines and chemokines. Furthermore, we can demonstrate that obesity-induced inflammation(s) involved in metabolic activation of mammary ATMs would enable development of directed therapies towards this specific pro-tumorigenic macrophage phenotype, thereby leaving the immune system of cancer patients intact.

#4230 Modulation of adiponectin and leptin levels in obese post-menopausal women after 6 months of a structured weight loss intervention, randomized to placebo or supplemental omega-3 fatty acids. Bruce F. Kimler,1 Jennifer L. Nydegger,1 Amy L. Kreutzjans,1 Teresa L. Phillips,1 Kandy R. Powers,1 Jennifer R. Klemp,1 Debra K. Sullivan,1 Christie A. Befort,1 Susan E. Carlson,1 Stephen D. Hursting,2 Carol J. Fabian,1 1Univ. of Kansas Medical Ctr., Kansas City, KS; 2Univ. of North Carolina at Chapel Hill, Chapel Hill, NC.

Background: Obesity is a modifiable risk factor for breast cancer in the United States. While structured interventions can achieve short-term weight loss, this does not necessarily correlate with favorable modulation of risk biomarkers at the blood or breast tissue level. We combined a proven intervention (calorie-controlled meals, moderate exercise, and weekly group behavior session) with daily omega-3 fatty acid supplementation to examine whether we could improve either weight loss and/or biomarker modulation in women who are at high risk for development of breast cancer. Methods: 46 post-menopausal high-risk women with a BMI >27 kg/m² (median 31 kg/m²) had baseline blood collections and random periareolar fine needle aspiration (RFNFA) benign breast tissue sampling for biomarkers. Two weeks after starting the weight loss intervention, subjects received study agent, randomized 1:1 to placebo vs. supplementation with 2100 mg Eicosapentaenoic Acid (EPA) + 1050 mg Docosahexaenoic Acid (DHA) daily. After 6 months, blood and tissue sampling were repeated. Results 42 women completed the 6 month weight loss intervention with all but one achieving a weight loss (median relative weight loss 12%; range 0 to 23%). For the entire cohort, there were substantial and favorable changes in levels of serum blood collected fasting for adiponectin (increase, p<0.001) and leptin (decrease, p<0.001), as well as the ratio of adiponectin to leptin (A/L, increase, p<0.001). The same effect for all three measures was observed in serum collected 2 hours after a standard meal; and for leptin and the A/L ratio in breast tissue also collected postprandial. When dichotomized to relative weight losses of <10% vs >10%, women with >10% loss had greater favorable modulations for leptin and the A/L ratio for fasting and non-fasting serum vs. tissue, p<0.001 for all. We further noted significant differences between groups of women dichotomized by whether they exhibited (or not) an increase in the ratio of EPA:DHA to arachidonic acid in erythrocyte phospholipids at 6 months. Conclusions: These results demonstrate that the cytokines adiponectin and leptin, and the ratio of the two, are robust biomarkers for modulation in serum and breast tissue of women achieving a successful weight loss on a structured intervention trial. Supported by a grant from the Breast Cancer Research Foundation and pilot funds from National Cancer Institute Cancer Center Support Grant P30 CA168524.

#4231 Significant reduction in workplace contamination of antineoplastic hazardous drugs in 13 U.S. hospitals following utilization of a new closed-system drug transfer device. Timothy Tyler,1 Lunci Power.2 1Desert Regional Medical Center, Palm Springs, CA; 2UCSF (retired), San Francisco, CA.

Background: Adverse effects of worker exposure to antineoplastic hazardous drugs (AHD) have been well documented with dermal absorption from contaminated surfaces in the work environment being one of the most well researched sources of exposure. Multiple studies have documented decreased surface contamination when using CSTDs for AHD handling. Decreased surface contamination should result in decreased worker exposure and decreased risk of adverse effects. Purpose: The purpose of this study was to determine the effectiveness of a new CSTD in reducing surface contamination of marker AHDs cyclophosphamide (CP) and 5-fluorouracil (5FU) during compounding and administration in multiple cancer centers, using surface wipe-sampling methodology. The results of the wipe sampling were compared to existing contamination in the sites and to similar studies in peer-reviewed literature to determine relative effectiveness. Methods: Wipe samples of six predetermined surfaces were collected in designated compounding and/or infusion areas of 13 cancer centers to establish the pre-existing level of surface contamination with two marker AHDs. Stainless steel plates of approximately 500 cm² were placed over previously sampled surfaces, and a specific protocol of set doses of the AHDs were compounded and infused, using all of the components of the new CSTD system, over the templates. Wipe samples of the templates were collected following the completion of tasks analyzed for both marker, AHDs. Results: A total of 311 wipe samples were collected from the 13 centers, 156 at baseline and 156 with the CSTD. All samples were reported as ng/cm² with the analytical limit of detection (LOD) for each drug being 0.002 ng/500 cm². In the aggregate results of 13 sites sampled for existing contamination, 67% of wipe samples had detectable levels of the AHDs with a range of LOD to 19.880 ng/cm² of 5FU and 0.648 ng/cm² of CP. With the new CSTD the contaminated samples were reduced to 5.8% with a range LOD to 0.004ng/cm² of 5FU and 2.68ng/cm² of CP. Conclusions: The new CSTD significantly reduced surface contamination by the marker AHDs during both compounding and administration. Compared to published results of two multi-site studies of another CSTD for compounding, the new CSTD is superior in reducing surface contamination of marker AHDs. A comparison of surface sampling of similar surfaces. Research reported in this study was supported by the National Cancer Institute (NCI) of the National Institutes of Health (NIH) under award number 5R44CA153636.

#4232 Gastrointestinal stromal tumor: Retrospective analysis of 44 cases of center of reference in eastern Algeria. Soulyane Ali Boukhalfa, Tah Filiali. CHU de Constantine, Tadjenanet, Algeria.

Aims: To analyze the clinicopathologic characteristics, prognostic factors, and treatment of gastrointestinal stromal tumors (GISTs). Methods: We conducted a retrospective analysis of all cases of GISTs treated in the Medical Oncology Unit from 2010 through 2015. Results: We analyzed 44 patients with a median age of 49.43 years (17-76 years) with sex ratio 1:1. The main symptom was abdominal pain. Thirty patients had limited-stage disease and 14 patients had metastatic disease. We observed 90% of the immunohistochemistry exams were positive for CD117 (c-Kit+). The most frequent location was in the stomach (45%), the high-risk group was predominant (50%) and the average tumor size is 12.84 cm. Surgery was considered R0 (extensive) in 75% of the cases and the main sites of metastases were liver and peritoneum. 25 patients received imatinib 400 mg qDay in adjuvant setting, 5 patients in neoadjuvant, 13 patients in palliative use. The median dose of the first-line therapy was 500mg/day. Forty-one patients (93%) were rechallenged in second line with imatinib 800 mg qDay, 5 patients with sunitinib and 2 patients with gefitinib. Conclusion: Our patients presented mainly in the form of high-risk disease, Surgery is the mainstay treatment when GISTs are localized, Adjuvant therapy is used depending their risk of relapse. In the advanced disease, tyrosine kinase inhibitors have substantially improved the prognosis of GISTs.

#4233 Linking education to action: A program to increase research participation among African American women. Carmen Radecki Breitkopf,1 Karen P. Williams,2 Jennifer L. Ridgeway,2 Alice L. Strong Simmons,3 Monica W. Parker,4 Michele Y. Halyard,5 Sharrone N. Hayes1. 1Mayo Clinic, Rochester, MN; 2The Ohio State University, Columbus, OH; 3The Links, Incorporated, DC; 4Emory University, Atlanta, GA; 5Mayo Clinic, Scottsdale, AZ.

Addressing underrepresentation of minorities and women as research participants has largely focused on enrollment of clinical trials. The results are known about the attitudes and knowledge regarding other types of biomedical research, e.g., epidemiologic and genetic studies. These types of studies are essential for advances in cancer research, including development of targeted therapies for women and minorities. As part of an academic/community partnership between Mayo Clinic and The Links, Incorporated, a national volunteer service organization of professional African American women, this study sought to: (1) examine attitudes regarding participation in research involving access to the medical record, genetic studies/biobanking, and clinical trials; and (2) develop and evaluate a novel, targeted online educational program addressing these 3 types of biomedical research. Initial qualitative inquiry via 3 focus
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groups (n=34) found that most women associated health-related research with clinical trials; few women were familiar with other study types. Women expressed more concerns about how information is collected, used, and shared by investigators than with personal mistreatment in research regardless of study type (genetic research [GEN], chart review [CR], clinical trial [CT]) or information on beliefs, health disparities, and cancer research ethics. The importance of investigator transparency regarding the significance of the disease in African Americans, research funding source, the investigator's agenda, and commitment to the population being studied was emphasized. Qualitative results informed development of the educational program. A total of 244 Links members completed the program (77% response rate) and answered an initial and post-program assessments of intentions, attitudes and knowledge. The percent of women indicating that they "definitely" or "probably" intend to participate in research increased from 9.4% to 19.5% and 27.1% to 49.8%, respectively, after program completion. Agreement with the statement "research in the U.S. is ethical" increased from 52.9% to 74.4%. Factual knowledge related to each of the 3 study types also increased. Prior to completing the program many women reported "little or no understanding" of the 3 types of studies (66% GEN, 62.9% CR, 40.7% CT) whereas after program completion, these numbers decreased to 24.9%, 18.4%, and 15.5%, respectively. While few women rated their opinion of the study types as "very positive" prior to beginning the program (14.3% GEN, 15.0% CR, 28.6% CT), upon completion these proportions increased and equalized (42.8% GEN, 40.0% CR, 42.5% CT). Two-month follow-up will evaluate long-term impact including knowledge dissemination, visiting clinicaltrials.gov, and enrolling in ResearchMatch. An online education-to-action program targeting professional African American women improved knowledge, perceptions of research ethics, and intent to participate in biomedical research.

**#4234 A culturally competent, community driven, collaborative approach to health care education conferences addressing the health disparities of African Americans.** Ricardo Parker,1 Patric Schiltz,2 Ernesta Wright,3 Jhenifa Parker,4 Denise Tolbert,5 Leelee Matthews,5 LaQuetta Shamblee.6 1National Univ., Costa Mesa, CA; 2The GREEN Foundation, Orange, CA; 3Eastern Washington University, Cheney, WA; 4Loyola Marymount University, Los Angeles, CA.

The ability to effectively deliver health care education that meets the social, cultural, and linguistic needs of people with diverse cultures is an important consideration, and is referred to as culturally or community competent approaches. Community and culturally competent health care education approaches are especially important in educational conferences designed to engage residents in health care issues such as health screenings, lifestyle, and preventative approaches toward improved health, as well as examining the impact of these and other factors on health disparities related to ethnicity. In this report, we evaluated 15 ActNOW cancer education conferences over a 5-year period, using a community driven, culturally competent conference model designed to educate African Americans participants, with an emphasis on reducing health disparities through understanding cancer, the importance of clinical trials participation, screening, and preventative and healthy lifestyle choices. Qualitative data on an aggregate of 380 participants were obtained through an anonymous evaluation and feedback survey questionnaire distributed to participants at the end of the conference. Participants' pre- and post-conference perceived cancer knowledge and intent to change lifestyle were evaluated. Conference attendees voluntarily completed the self-administered questionnaire comprised of 8 questions. Each question had 4 possible responses: 1. non-existent; 2. minimal; 3. moderate or 4. considerable. For statistical analysis, responses were separated into two groups; low and high. Responses designated 1 or 2 were considered to reflect minimal understanding of the subject material; and frequency counts for responses 1 or 2 were aggregated and designated as 'Low' understanding responses. Responses 3 or 4 reflected a higher understanding of the subject material; and frequency counts for responses 3 or 4 were aggregated and designated as 'High' understanding responses. The groups were then analyzed statistically using Fisher's exact test, where a two-sided p≤0.05 is considered significant. The results demonstrated a significant number (27%) of respondents indicating a low understanding of the topics before the event. After the event, a significantly lesser number (2%) of low respondents reported a low understanding of the topics, with a consequent increase in high respondents reporting a greater understanding (from 73% pre- to 98% post-conference; p<0.0001). Similar significant shifts in practice knowledge profiles were also seen (p<0.0001). Cultural and/or community competent approaches for health care education are a considered need.

**EPIDEMIOLOGY: Biomarkers of Endogenous or Exogenous Exposures, Early Detection, and Biologic Effects**

**#4235 Application of convolutional neural networks to breast biopsies to uncover tissue correlates of mammographic breast density.** Maeve Ebot,1 Masis Isikbay,2 Travis Gerke,2 Thomas U. Ahearn,3 Rachel S. Kelly,2 Svitlana Tyekucheva,1 Andreas Pettersson,1 Kathryn L. Penney,1 Lorelei A. Mucci1. 1National Cancer Institute, Bethesda, MD; 2Radboud University Medical Center, Nijmegen, Netherlands; 3University of Vermont, Burlington, VT; 4University of California, San Francisco, San Francisco, CA; 5Hokkaido University, Sapporo, Japan; 6MD Anderson Cancer Center, Houston, TX; 7Mayo Clinic, Jacksonville, FL; 8Harvard Medical School, Boston, MA.

Background: High percent mammographic density (MD), which reflects the relative fibroglandular tissue content of the breast, is one of the strongest breast cancer risk factors; however, the pathologic mediators of this risk are unknown. We hypothesize that analysis of breast tissue sections using deep learning approaches may characterize histologic features that underpin risk associated with high MD. Methods: Non-targeted H&E stained breast tissue sections of diagnostic image-guided breast biopsies were evaluated among 588 women enrolled following an abnormal mammogram in the Breast Radiology Evaluation and Study of Tissues (BREAST) Stamp Project (2007-2010). Overall volumetric percent MD for the biopsied breast and localized volumetric percent MD surrounding the biopsy site were determined for each participant. A deep convolutional neural network (CNN) model was trained to identify and quantitatively assess breast epithelial, stroma and fat tissue and their organizational and spatial arrangements. Least absolute shrinkage and selection operator (Lasso) regression was used to determine relationships between MD measures and pathological features. To ensure reliability of the model, a cross-validation strategy was employed to build and assess the performance of the fitted model. Finally, Spearman correlation coefficients were estimated to test the association between the predicted density values by each model (predicting overall or localized MD) and the actual MD measurements. We report the average and standard deviation (SD) of the correlation coefficients. Results: In an independent validation set, the CNN model was 95.5% accurate in classifying epithelial, stromal and fat tissue. The mean (SD) correlations between the predicted model and the actual measurements for overall and localized MD were 0.70 (0.06) and 0.65 (0.06) respectively. The amount of stroma identified (normalized to tissue area) had the highest selection probability (P-value) by the Lasso model and thus the strongest positive relationship with MD (P-value>0.9 for each MD measurement). In contrast, the amount of normalized epithelial tissue was not related to MD (P-value=0.01 for each MD measurement). No association was observed for the total normalized fat area with MD (P-value<0.31 for each MD measurement). In another group, the epithelial to stromal ratios was positively associated, whereas the distance between epithelial regions was inversely associated with overall MD (P-value<0.87 and 0.62, respectively). Conclusions: These results show that greater stromal tissue amount and spatial distribution patterns of epithelial regions, rather than total epithelial amounts, had the strongest relationships with elevated MD. Future work will determine the relationship of these MD features with biopsy diagnosis.

**#4236 Gene expression profiling of prostate tissue identifies biological pathways associated with TMPRSS2:ERG fusion gene.** Ericka Ebot,1 Masis Isikbay,2 Travis Gerke,2 Thomas U. Ahearn,3 Rachel S. Kelly,2 Svitlana Tyekucheva,1 Andreas Pettersson,1 Kathryn L. Penney,1 Lorelei A. Mucci1. 1Harvard School of Public Health, Boston, MA; 2Harvard Medical School, Boston, MA; 3University of Florida, Gainesville, FL; 4National Cancer Institute, Rockville, MD; 5Brigham And Women's Hospital, Boston, MA.

Background: TMPRSS2:ERG fusion is a hormonally regulated gene fusion present in about half of prostate tumors, is the most common somatic event in prostate cancer. There is intriguing evidence to suggest that TMPRSS2:ERG-positive tumors may define a distinct subgroup of prostate cancer. In this study we compared gene expression profiles according to fusion status to identify genes and biological pathways differential expressed by TMPRSS2:ERG. Methods: The study included men with prostate cancer in the Health Professionals Follow-up Study and the Physicians' Health Study diagnosed between 1982 and 2004 and followed through 2012. Tumor biomarker data was available for archival tumor samples in 192 cases that had gene fusion adjacent to normal tissue. Whole genome mRNA expression profiling (20,254 genes) was performed using the Affymetrix 1.0 ST array. TMPRSS2:ERG tumor status was assessed by a genetically validated IHC assay for ERG. To relate expression of individual genes to ERG we used linear regression. Gene Set Enrichment Anal-
yses (GSEA) was used to identify pathways of genes associated with ERG in both tumor and adjacent normal tissue. Data from The Cancer Genome Atlas (TCGA) was used to validate these findings. Results: Among 380 cases, 186 (49%) of tumors were positive for the gene fusion. Genome-wide differential expression analysis identified 492 genes upregulated and 369 genes downregulated in tumor tissue comparing ERG expression analysis identified 492 genes upregulated and 369 genes downregulated (49%) of tumors were positive for the gene fusion. Genome-wide differential expression analysis (GSEA) was used to identify pathways of genes associated with ERG in both tumor and adjacent normal tissue. Data from The Cancer Genome Atlas (TCGA) were compared between ERG-positive and ERG-negative tumors. No genes were identified in the adjacent normal tissue at this significance level. 772 of the 861 genes (90%) identified in the HPES/PHS dataset were confirmed in TCGA (448 upregulated and 324 downregulated). The top ten genes included ERG, WNK2, CACNA1D, HES1, SEPT9, PLA2G7, TDRD1, ANTXR2, SORBS2, and MTO6. Enrichment analysis identified a number of metabolic pathways associated with ERG tumor status including pathways involved in amino acid and fatty acid metabolism. Conclusions: We identified significant differences in gene expression profiles in prostate tumor tissue according to TMPRSS2:ERG status, providing supportive evidence of this fusion as a unique subtype.

#4237 Estrogen metabolism in menopausal hormone users: Does it differ between estrogen plus progestin and estrogen alone users in the Women’s Health Initiative Observational Study. Roni T. Falk,1 Garnet L. Anderson,1 Vanessa M. Barnabei,2 Louise A. Brinton,1 Jane A. Cauley,3 Chu Chen,4 Rowan T. Chlebowski,5 Sally B. Coburn,1 JoAnn E. Manson,4 Ruth M. Pfeiffer,1 Keryn W. Reding,2 Thomas E. Rohan,3 Gloria E. Sarto,1 Nicolas Wentzensen,1 Britton Trabert,1,*, National Cancer Institute, Bethesda, MD; 2Fred Hutchinson Cancer Research Center, WA; 3Jacobs School of Medicine and Biomedical Sciences University at Buffalo, NY; 4University of Pittsburgh Schools of the Health Sciences, PA; 5David Geffen School of Medicine UCLA, CA; 6Harvard Medical School, MA; 7University of Washington School of Nursing, WA; 8Albert Einstein College of Medicine, NY; 9University of Wisconsin School of Medicine and Public Health, WI.

Background: The Women’s Health Initiative (WHI) provided divergent results regarding the effects of menopausal hormone therapy (MHT) on breast cancer risk, with women in the conjugated equine estrogen plus medroxyprogesterone (CEE+MPA) arm at elevated risk, and women in the CEE alone arm at reduced risk. Although direct progestin-mediated effects may largely explain the elevated risk, we hypothesize that in addition, these MHT treatments may differentially influence patterns of estrogen metabolism, with CEE alone preferentially inducing metabolism along the 2-hydroxylation pathway, a pattern previously linked to reduced breast cancer risk. Study methods/population: Women in a case-control study of estrogen metabolites (EM) and ovarian and endometrial cancer from the WHI Observational Study were identified for this analysis. Unlike the WHI trial, no medication restrictions were applied. At enrollment, serum, anthropometric measures, and self-administered questionnaires which ascertained reproductive history, lifestyle factors and health behaviors including MHT use, were obtained. 615 women reported current use of estrogen plus progestin formulations (E+P), of whom 343 used CEE+MPA; 266 used estrogens alone (E alone), with 148 using CEE. Fifteen EM were measured by liquid chromatography/mass spectrometry and analyses were conducted separately for each EM. EM differences between E alone and E+P users were assessed using inverse probability weighted linear regression. Primary analyses included women using any MHT formulation; secondary analyses were restricted to CEE users. Results: Compared to users of E+P, concentrations of all EM were higher in E alone users, and significantly so for unconjugated estrone, estradiol, 2-methoxyestrone, 4-methoxyestrone and unconjugated estradiol. Relative to total EM, concentrations of 2- and 4-pathway EM did not differ by MHT group (for E alone and E+P users, 2-pathway EM were ~14% of the total EM; 4-pathway EM were ~1% of the total), but E+P users had a significantly higher proportion of 16-pathway EM compared to E alone users (32% vs. 30%, p=0.025). Similar patterns were observed in analyses comparing users of CEE alone to CEE+MPA, albeit not significant. Conclusion: Our data suggest that women using E alone may preferentially metabolize estrogens along the 2- and 4-hydroxylation pathways, whereas in E+P users, more extensive metabolism occurs along the 16-pathway. However, we did not observe these effects in the smaller groups of women using CEE alone or CEE+MPA, the MHT formulations administered in the treatment arms of the WHI trial. Our findings in E alone users are consistent with epidemiologic investigations demonstrating reduced breast cancer risk in postmenopausal women with more extensive 2-pathway estrogen metabolism, and may provide a clue to the breast cancer risk reduction observed in these women.

#4238 The effects of long-term storage on commonly-measured serum analyte levels. Cynthia Kleeberger,1 David Shore,2 Elaine Gunter,3 Dale P. Sandler,4 Sandra Deming-Halverson,1 Clarice R. Weinberg,1 Social & Scientific Systems, Inc., Durham, NC; 2Westat, Durham, NC; 3Specimen Solutions, LLC, Tucker, GA; 4NIEHS, RTP, NC.

Introduction: Cohort studies typically bank biospecimens for many years prior to assay and the levels of analyte degradation is unknown. Long storage times (i.e. severe degradation) can bias the estimation of the analyte effects on health outcomes. Such effects could also produce confounding with other factors under study. Methods: We collected control samples from 22 non-study participants using the same enrollment criteria and specimen collection, processing, and storage protocols as in the Sister Study, a large cohort study conducted by the National Institute of Environmental Health Sciences. Samples were assayed for 21 analytes at collection and then six years later. For each sample, the difference between the result at baseline and at six years was calculated for each analyte. The difference, Yij = result - result, was then modeled using a mixed-effects model, with random effects for batch (bj) and a fixed effect for the overall mean difference: Yij = u + b + cij.
where \( b_i \sim N(0, \sigma^2_{\text{seeds}}), i = 1, 2, 3, 5, 6 \). Results: Some of the analytes experienced a marked change in concentration after six years of frozen storage, compared to their baseline value. There were no significant changes in ten of the analytes. Two of the analytes, lactate dehydrogenase (LDH) and sex hormone binding globulin (SHBG), increased significantly in concentration over time. Two analytes, HDL cholesterol and luteinizing hormone (LH), decreased significantly in concentration over time and the estimated mean difference across the two time points. Conclusion: We conclude there are differences in assay results after long-term storage, although for most analytes the correlation coefficients were high and percent change small. Biobanks or cohort studies that bank samples should consider building in QC experiments designed to assess the impact of long-term storage on anticipated analytes of interest, when possible. By doing so, degradation could be corrected for to control bias due to the associated measurement error. Since not all cohorts were able to conduct these QC experiments, those that have carried out such assessment should share their results so others can use the data to evaluate the potential impact of measurement error on their study findings.

**#4240** Determination of normative patterns of gene expression levels and breast cancer risk biomarkers in human breast milk. Maria Ozounova, Georgina Lewis, Radhika Piraniaddu, Tiana Curry-McCoy, Nita Maiblack, Sangmi Kim, Hasan Korkaya, Augusta University, Augusta, GA.

Parity and breastfeeding influence a woman’s risk of developing breast cancer. Understanding the underlying molecular changes in the breast will facilitate identification of additional factors during pregnancy and postpartum that modify breast cancer risk. Most prior studies in humans on this topic, however, have been cross-sectional studies comparing breast tissue samples from nulliparous versus post-pregnant women, and are limited in their ability to characterize longitudinal changes and within-person variations. Breast milk contains cells of the mature gland, and may have a potential as a noninvasive source for studying molecular characteristics of the pregnancy-associated changes in the breast and their impact on breast cancer risk. The objectives of this study were to (1) determine normative patterns of gene expression levels in longitudinally collected breast milk samples and (2) correlate gene expression data in relation to self-reported measures of psychosocial stress during pregnancy and postpartum period. Our preliminary results showed that the post-pregnancy gene expression levels in breast milk samples can be measured reproducibly over a short period of time. Whole genome transcriptome data revealed the correlation levels of the post-pregnancy genes are different in early (less than 3 month postpartum) and late lactation period (more than 6 month postpartum). Moreover cytokine expression profiling correlated expression of inflammatory cytokines with early versus late postpartum period. The unique contribution of this study is to capture longitudinal gene expression data from breast milk samples collected over the course of lactation, hence providing a valuable basis for future studies to identify differentiation dynamics associated with breast cancer susceptibility.

**#4241** Circulating microRNAs in plasma of men screened for prostate cancer. Alicia C. Mcdonald, Jing Shen, Manish Vira, Jason Liao, Martin L. Lesser, Dattatrya Patil, Jay D. Raman, Emanuel Taliol. 1Pennsylvania State University College of Medicine, Hershey, PA; 2Columbia University, New York, NY; 3Hofstra Northwell School of Medicine, Hempstead, NY; 4Emory University School of Medicine, Atlanta, GA; 5The Feinstein Institute for Medical Research, Manhasset, NY; 6Pennsylvania State Milton S. Hershey Medical Center, Hershey, PA; 7Ichan School of Medicine at Mount Sinai, New York, NY.

Introduction: Although microRNAs (miRNAs) have been linked to prostate cancer risk, their role as a screening biomarker for prostate cancer (PC) has yet to be determined. We examined whether circulating miRNAs in plasma could be potential biomarkers for the early detection of PC among men undergoing prostate needle biopsy. Methods: Men who had a prostate biopsy due to an abnormal screening test were recruited. Demographics, medical history, clinical information, and blood processed into plasma were collected. TaqMan Low Density Arrays were used for the expression profiling of 733 miRNAs in plasma. Wilcoxon test was used to compare expression levels between men with and without a PC diagnosis. Logistic regression was used to examine the association between miRNAs and PC status, after adjusting for age and Bonferroni-correction. Results: There were 131 men, aged 46 to 86 years, included in the final analysis, with 66 men diagnosed with PC on prostate biopsy and 65 men without a PC diagnosis. Majority were white (72.5%) followed by black race (13.5%). Men with PC were older (median: 64 vs. 62 years; p-value = 0.161) and had statistically significantly higher serum PSA (median: 5.8 vs. 5.1 mg/ml; p-value = 0.01) compared to men without PC. miRNAs which include the discovery of new miRNAs and their relationship but within the limits of the internal laboratory control variance. Significant reductions in excess of the laboratory control variance were found for 7 of the 21 analytes tested (aspartate transaminase (AST), total cholesterol, estradiol, glucose, protein, sodium, and triglycerides). Despite the evidence for systematic changes over long-term storage, correlations between baseline and later measures were often low and there was little relation between the size of the correlation changes over long-term storage, correlations between baseline and later measures were high and there was little relation between the size of the correlation coefficients and the estimated mean difference across the two time points. Conclusion: We conclude there are differences in assay results after long-term storage, although for most analytes the correlation coefficients were high and percent change small. Biobanks or cohort studies that bank samples should consider building in QC experiments designed to assess the impact of long-term storage on anticipated analytes of interest, when possible. By doing so, degradation could be corrected for to control bias due to the associated measurement error. Since not all cohorts were able to conduct these QC experiments, those that have carried out such assessment should share their results so others can use the data to evaluate the potential impact of measurement error on their study findings.

**#4242** Mass spectroscopic analysis of MGMT tryptic peptides allows detection of O6-alkylguanine adducts in oligodeoxynucleotides, temozolomide modified cell thymus DNA and human colorectal cancer DNA. Rashia A. Abdelhady, Perdita E. Barran, David M. Williams, Andrew C. Povey, 1University of Manchester, Manchester, United Kingdom; 2University of Sheffield, Sheffield, United Kingdom.

Background: Red and processed meat consumption increases human colorectal cancer (CRC) risk, potentially by heme-catalysed formation of carcinogetic N-nitrosocompounds (NNOC) that form mutagenic O6-alkylguanine (O6-alkG) DNA adducts. While NNOC formation cannot be accurately estimated from meat consumption alone due to in situ processing required for their formation. We are thus developing a novel approach to assess NNOC exposure by quantifying O6-alkylguanine DNA adducts that result from their exposure, using the known action of the DNA repair protein, O6-alkylguanine O6-alkyltransferase (MGMT), to irreversibly transfer the O6-alkyl to an active site cysteine residue in MGMT. Methods: Oligodeoxynucleotides (ODNs) containing O6-methylguanine (O6-MeG), O6-carboxymethylguanine (O6-CMG), O6-carboxethylguanine (O6-CEG) were synthesised by alcohol modification of an ODN containing the convertible base 2-amino-6-methylsulfonylpurine. O6-MeG containing cell thymus (CT) DNA was prepared by incubation with temozolomide (TMZ). Both maltose binding protein-MGMT (MBP-MGMT) and histagged MGMT (his-MGMT) fusion proteins were expressed, purified, and MGMT functional activity determined by tritium transfer using CT DNA methylated with [3H]-N-Nitrosomethylurea. Double stranded O6-alkG-containing ODNs, unmethylated ODN and methylated CT DNA were incubated with MBP-MGMT and the resulting alkylated MGMT digested with trypsin and analysed by mass spectrometry using MALDI-TOF. Human CRC DNA samples were incubated with TMZ modified calf thymus DNA which was then recovered using Ni-coated magnetic beads. MGMT was digested in situ with trypsin and the tryptic peptides analysed by MALDI-TOF. Results: S-methylcysteine (m/z 1329.7), S-carboxymethylcysteine (m/z 1373.7) and S-carboxyethylcysteine, modified MGMT active site peptides were detected in trypsin digests of MGMT incubated with O6-MeG, O6-CMG and O6-CEG containing ODNs respectively. Only the unmethylated active site peptide (m/z 1315.7) was found after incubating MGMT with unmethylated ODN. Subsequent MS analysis of tryptic digest of MGMT incubated with TMZ modified CT DNA also revealed the active site peptide containing S-methylcysteine. Using this approach, pilot studies revealed the presence of O6-MeG, O6-CMG and O6-CEG in CRC DNA. Conclusions: These results demonstrate proof of principle and confirm that this approach could be used to characterise O6-alkylguanine adducts in human CRC DNA.

**#4243** Epigenome-wide association study of metabolic syndrome in African-Americans. Tomi Akinenyienu. University of Alabama at Birmingham, Birmingham, AL.

Background: The epidemic of obesity among US adults has resulted in significant increases in associated metabolic disorders such as diabetes, dyslipidemia, and high blood pressure. Together, these disorders constitute metabolic syndrome, a clinically defined condition highly prevalent among African-Americans. Identifying epigenetic alterations associated with metabolic syndrome, including DNA methylation changes may provide information on biological pathways that are dysregulated and may potentially act as early biomarkers in cancer. Methods: Data on cardio-metabolic risk factors and DNA methylation was assessed on 606 African-Americans from the Hypertension Genetic Epidemiology Network (HyperGEN) study. Metabolic syndrome was defined using the joint harmonized criteria of at least three of: elevated waist circumference, high triglycerides, low HDL-cholesterol, high fasting blood glucose and high blood pressure. DNA methylation...
was assessed using the Illumina HumanMethylation 450K Bead Chip assay on DNA extracted fromuffy coat. Linear mixed effects regression models were used to examine the association between CpG methylation and metabolic syndrome in crude and adjusted (for age, alcohol, smoking, sex) analyses. Replication using DNA from 76 African-Americans in the REason for the Geographic and Racial Disparities in Cancer (REGARDS) study, as well as meta-analysis combining both cohorts was conducted. Results: Two differentially methylated CpG sites in the IGFBP1 gene on chromosome 17 (cg06638433; $\beta = 0.01$, $p$-value $= 3.10 \times 10^{-10}$) and the ABCG1 gene on chromosome 21 (cg06500161; $\beta = 0.018$, $p$-value $= 2.60 \times 10^{-14}$) were identified in models adjusted for age, sex, and alcohol (and smoking). Methylation of CpG sites on the TXNIP gene on chr 1 (p = 2.83 x 10^-7) and CPT1A gene on chr 11 (p = 1.39 x 10^-5) were also significantly associated with metabolic syndrome in unadjusted models. Results for the ABCG1 gene remained statistically significant in the replication set and meta-analysis. Conclusion: Metabolic syndrome was consistently associated with focal DNA hypermethylation in the ABCG1 gene, a region of the chromosome that encodes proteins in the ATP-binding cassette transporter family, and is involved in extra- and intracellular signaling. Alterations in the ABCG1 gene were observed in 40% of invasive breast cancer tumors in the TCGA, providing a potential epigenetic link between metabolic syndrome and breast cancer that deserves further study.

**#4244 Detection and characterization of extrachromosomal circular DNA in human plasma.** Jing Zhu, MeiJin Du, Peng Zhang, Liang Wang. Medical College of Wisconsin, Milwaukee, WI.

Background: eccDNA (extrachromosomal circular DNA) has been reported in most eukaryotes, including in human cells. However, little is known about the eccDNA profiles in human circulating system such as blood. Method: We extracted plasma cell free DNA (cfDNA) from three patients with advanced stage cancers. For each patient, we tested 10ng, 1ng, and 0.1ng cfDNA, each with a technical replicate. The cfDNA samples were subjected to ATP-dependent DNase digestion and whole genome amplification (WGA). An additional 2ng cfDNA from each patient skipped the DNase digestion and went directly to WGA step. We sequenced the amplified DNA using the Illumina 100bp PE read protocol. We analyzed sequencing data using a custom split reads-based strategy to retrieve circular DNA molecules. We further analyzed eccDNAs for their biofeatures using functional annotation tools. Results: We received sequence reads at 14.8 million (11.4—22.4) for each sample. When read depth $\geq 3$ as a cutoff, we detected a total number of 2542, 1578, and 57 unique eccDNAs in three patients while negative controls showed $<3$ eccDNAs. For non-DNase digested cfDNAs, we detected only 3 eccDNAs. By comparing read numbers mapped to mitochondrial and human genome, we found that mitochondrial sequence reads increased significantly from 0.34-1.1% before digestion to 24.1-50.1% after digestion. Numbers of detected eccDNAs were similar between technical replicates and proportional to initial input cfDNA. However, eccDNAs detected also showed few overlaps between technical replicates. Size distribution ranged from 31bp to 19740bp (median 1801-1962bp). To test if eccDNAs were generated in specific genomic regions and if eccDNA-involved genes were enriched in specific pathways, we performed enrichment analysis and observed higher GC content in smaller eccDNAs ($<500$ bp, 48%) than larger ones ($>500$ bp, 41%) ($p$<0.001). When compared to random distribution, eccDNAs were more likely located at CpG islands (1.25-1.5 fold enrichment). The eccDNAs were also enriched in exons, 5'UTR, 3'UTR and DNase hypersensitive sites (enrichment folds from 1.39 to 2.3). Using DAVID annotation tool, we observed significant enrichment of the eccDNA-related genes in several categories including the Coiled coil, phosphoprotein, fibrinectin, suggesting a potential involvement in gene expression regulation and cell adhesion events. Using GREAT (Genomic Regions Enrichment of Annotations Tool), we found that the eccDNAs were involved in genes associated with inflammatory and immune cells expression regulation ($p<0.001$). Conclusion: Human plasma contains an abundant number of eccDNAs. Distribution of these eccDNAs is nonrandom event and is likely enriched in genomic regions with known functional consequences and genes that play a role in gene regulations. Further characterization of circulating eccDNAs in peripheral blood will facilitate understanding of their molecular mechanisms and potential clinical utilities.

**#4245 Serologic markers of infection and risk of non-Hodgkin lymphoma in a pooled prospective study of three Chinese cohorts.** Bryan A. Bassig,1 Angelica Michel,1 Xiao-Ou Shu,3 Woon-Puay Koh,4 Yu-Tang Gao,5 Lesley M. Butler,3 Mark Purdue,1 Yong-Bing Xiang,2 Jennifer Adams-Haduch,1 Renwei Wang,3 Nicole Brenner,6 Tim Waterboer,7 Martina Willhaus-Fleckenstein,8 Liang Wang,9 Zhiqin Chen,9 JunPing Zhang,9 Wei Hu,9 Gong Yang,9 Hong-Ou Chow,9 Michael Pawlita,2 Wei Zheng,1 Jia-Min Yuan,1 Jing Lan,6 Nathaniel Rothman,1 1NCI-DECG, Rockville, MD; 2German Cancer Research Center (DKFZ), Heidelberg, Germany; 3Vanderbilt University School of Medicine, Nashville, TN; 4Duke-NUS Graduate Medical School, Singapore; 5Shanghai Cancer Institute, Shanghai, China; 6University of Latvia; 7Paganygh, PA; 8German Cancer Research Center, Heidelberg, Germany; 9Shanghai Jiaotong University School of Medicine, Shanghai, China; 10Albert Einstein College of Medicine, New York, NY; 11The University of Texas MD Anderson Cancer Center, Houston, TX.

Background: Infections with some viruses, including HIV and Epstein-Barr Virus (EBV) in immunocompromised individuals, are known risk factors for specific subtypes of non-Hodgkin lymphoma (NHL). Epidemiological studies have also provided evidence that hepatitis viruses, and EBV in immunocompetent individuals, may also be associated with NHL. There are limited population-based prospective studies with pre-diagnostic blood samples that have comprehensively evaluated viral biomarkers and NHL risk in East Asians, in whom the descriptive characteristics of NHL and the prevalence of certain viral infections differ compared to Western populations. Methods: We conducted a nested case-control study with a nested case-control study of 214 NHL cases and 214 controls from three population-based prospective cohorts in Shanghai and Singapore. Cases and controls were individually matched by age, sex, date of blood draw, and cohort. Antibodies to 21 antigens associated with the evaluated viruses (herpesviruses, Hepatitis B (HBV) and C (HCV), and polyomaviruses) were measured in plasma/serum using fluorescent bead-based multiplex serology. Conditional logistic regression was used to evaluate associations between viral antibody levels measured as median fluorescence intensity and NHL. Results: For herpesviruses, an increased risk of NHL was observed for higher compared to lower early antigen diffuse (EA-D) ($OR = 2.2, 95\% CI = 1.2-4.1$) and BZLF1-encoded replication activator (ZEBRA) ($OR = 2.2, 95\% CI = 1.0-4.9$) antibodies ($\text{OR} = 1.9, 95\% CI = 1.0-3.9$, $p = 0.03$) associated with EBV. An increased risk of NHL was also observed among those seropositive for the intermediate-early 1A antigen ($OR = 1.9, 95\% CI = 1.0-3.3$, $p = 0.03$) with EBV antibodies ($\leq 0.2$) associated with human herpesvirus-6 (HHV-6). For hepatitis viruses, a significant NHL risk was observed for higher compared to lower antibodies to the HBV-associated core (HBc) antigen ($OR = 1.8, 95\% CI = 1.1-3.1$), and this risk was particularly apparent in those with the highest HBC and EBV EA-D antibody levels ($OR = 4.2, 95\% CI = 1.4-12.7$) compared to the lowest. Seropositivity to HCV was low (1.4% cases; 0.9% controls). No associations with NHL were observed for individual polyomaviruses (BK, JC, TSV, MCV). Discussion: Our study of serologic markers of infection and NHL risk in three prospective population-based studies of Chinese individuals suggests a role of specific viral agents in lymphomagenesis. The findings for EBV are consistent with some data from Western cohorts and indicate that EBV reactivation may be associated with NHL risk in the Chinese general population. HHV-6 is a lymphotropic virus that has been observed in some retrospective studies to be associated with lymphoma, but to our knowledge has not previously been associated with NHL prospectively in the general population. HBV is endemic to regions of East Asia, including China, and our data suggest that high levels of antibodies to the HBc antigen may be a marker for NHL risk.

**#4246 Associations of Epstein-Barr virus (EBV)-positive gastric cancer with circulating mediators of inflammation and immune response.** M. Constanza Carmago,1, Armanda Sivina,2 Sergeijs Isaevs,3 Dace Rudzite,4 Margaret Gulley,5 G. Johan Offerhaus,4 Marcis Leja,2 Charles S. Rabkin1.

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Epstein-Barr virus (EBV) positivity defines one of four major molecular types of gastric cancer in The Cancer Genome Atlas (TCGA). However, viral status is currently determined in the last study determined and therefore is not gener-}

ally collected in epidemiologic research. Histologically, EBV-positive gastric cancer is characterized by prominent inflammatory infiltrate. In molecular analyses from TCGA, EBV-positive gastric cancer had significantly higher expression of several chemokines, chemokine receptors and programmed death-ligand 1 (PD-L1) as compared to other molecular types combined. We hypothesized that EBV tumor status may also be reflected in profiles of circulating chemokines and other markers of immune response. We have therefore evaluated pre-treatment EDTA plasma samples of gastric cancer patients from Latvia, including 28 with EBV-positive (as assessed by EBV RNA in situ hybridization) and 34 with EBV-negative tumors, frequency-matched by age, sex, an-
Adiposity, change in adiposity during adulthood and mammographic density in premenopausal women. Aliya Alimujjaid, Graham Colditz, Catherine Appleton, Adetunji T. Toriola. Washington Univ. School of Medicine, St. Louis, MO.

Background: Body mass index (BMI) is inversely associated with mammographic density among premenopausal women, but few studies have evaluated this in premenopausal women. Further, there is limited data on the associations of weight change during adulthood with mammographic density. To address these, we investigated the associations of current adiposity measures, BMI at ages 18, 30, and changes in BMI at these age periods with volumetric mammographic density measures in 314 premenopausal women who underwent screening mammograms at the Northwestern University Breast Center, Women's Health Program, St. Louis, MO. Methods: Study participants completed a questionnaire with information on breast cancer risk factors. Weight at ages 18 and 30 were self-reported. Current weight, height, waist, and body fat percent (%), were assessed by trained research personnel. BMI's were calculated as weight at each age/current height squared (kg/m²). We calculated weight change from: (i) age 18 to 30, (ii) age 18 to current age, (iii) age 30 to current age. Volpara was used to determine volumetric mammographic density measures. We investigated age-adjusted correlations between adiposity measures and mammographic density measures using Pearson correlation coefficients. We used multivariable linear regression models (adjusted for age, at menarche, parity, age at each birth, family history of breast cancer, race, and education) to evaluate the associations of adiposity measures with mammographic density. Adiposity measures, adiposity change and BMI were subjected to non-linear model tests, with no non-linear associations observed.

Results: Participants had a mean age of 50 (range: 23-78) years. The proportion of non-Hispanic white women was 51%, and 45% of the sample were overweight or obese at age 18. The average current BMI was 25.4 (range: 17.7-41.8). Adiposity measures and BMI were strongly inversely correlated with MV and BV. A 1 kg/m² increase in BMI was associated with a 0.14% decrease in MV and a 0.18% decrease in BV. Odd ratios (OR) and p-values were calculated for tertiles of BMIs across adulthood, with ORs adjusted for age, menarche, parity, age at each birth, family history breast cancer, race, and education.

Conclusions: These findings show that BMI and weight change are associated with changes in mammographic density. Further work is needed to identify potential interventions to increase mammographic density and improve cancer risk.
An APOBEC3-associated variant was defined as a variant having one of the eight possible motifs (5’ [CT]/C-T/W 3’) out of 96 potential motifs of 3 base-pairs. Using logistic regression, we compared the number of APOBEC3-associated variants in cases and controls, among HPV16 variant lineages, and among genome regions of the virus. We discovered that there is evidence of APOBEC3 activity predicting the viral non-coding upstream regulatory region. We observed a 74% increase in HPV16 A lineage infection with more APOBEC3-associated variants compared to those with a non-A lineage infection (OR = 1.35, p-value = 0.02). After controlling for the number of APOBEC3 variants, we observed that the tissue from women with early menarche had upregulation of genes associated with defense against oxidative stress later in life. Women with early menarche and breast cancer development. Methods: To test our hypothesis we used the early and late menarche cohort. Our findings point to a possible interplay between telomere and mitochondrial dysfunction, and epigenetics in carcinogenesis. Additional studies should also examine these associations in larger cohorts with greater racial/ethnic, gender, and socioeconomic diversity to validate these findings.

A possible link between telomeres, mitochondria, and cancer incidence. Jacob K. Kresovich,1 Tao Gao,2 Brian T. Joyce,3 Pantel Vokonas,4 Joel Schwartz,4 Ananda K. Voinov,5 Minho Shong,6 Jiri Zavadil,4 Arthur P. Grollman 1.

Background: PGC1A and PGC1B encode transcriptional factors that regulate mitochondrial biogenesis and have been implicated in the early age at menarche as a breast cancer risk factor and advance research for women’s health and public health risk linked to carcinogenic herbal medicines. Shuhan Wang,1 Daeun Choi,2 Jaesung Lim,3 Kathleen G.Dickman,4 Magali Olivier,4 Viktoriya S. Sidorenko,1 Maude Ardin,4 Byong H. Yun,5 Robert J. Turesky,6 Minho Shong,7 Jiri Zavadil,4 Arthur P. Grollman 1.

Methods: To test our hypothesis we used the resources available at the Susan G. Komen Tissue Bank at the Indiana University School of Medicine, Indianapolis, IN.

#4250 Molecular alterations in the breast associated with early menarche.

Maria L. Johnson,1 Natascia Marino,2 Anna Maria V. Storniolo,3 Bradley A. Hancock,4 Milan Radovich,5 George E. Sandusky6.

Komen Tissue Bank at the Indiana University Simcon Cancer Center, Indianapolis, IN; Indiana University School of Medicine, Indianapolis, IN.

Background: Menarche, the onset of the female menstruation, is a marker of pubertal timing. Age at menarche varies widely between girls and is highly dependent on nutritional status and body fat accumulation. The occurrence of menarche at an early age is linked to an increased risk of several adverse health conditions later in life, such as obesity, type-2 diabetes, breast and endometrial cancer, and cardiovascular disease. Indeed, for every one year decrease in age at menarche (from an average age at menarche of 12.5 years), breast cancer risk is increased by 5%. Several genome wide association studies (GWAS) have identified genetic variants (i.e. in the LIN28B gene) that are associated with early age at menarche, however little is known about the changes occurring in the breast tissue of women with early menarche. We hypothesize that early age at menarche results in permanent molecular alterations in the breast tissue and that those abnormalities may contribute to the tissue’s susceptibility to carcinogens and breast cancer development. Methods: To test our hypothesis we used the resources available at the Susan G. Komen Tissue Bank at the Indiana University Simon Cancer Center (KTB). We selected histologically normal breast tissue from healthy, young women with either early (age ≤ 10 years) or late menarche (age ≥ 15 years), and matched for age, race, BMI, and menstrual phase. Breast tissue biopsies from these women were microdissected to isolate the breast epithelium and next generation RNA-sequencing was used to generate a transcriptome profile for each sample. Differential expression was performed using DESeq2 in R. The tissue samples were also evaluated using immunostaining.

#4251 DNA methylation of mitochondrial biogenesis regulating genes: A possible link between telomeres, mitochondria, and cancer incidence.

Jacob K. Kresovich,1 Tao Gao,2 Brian T. Joyce,3 Pantel Vokonas,4 Joel Schwartz,4 Ananda K. Voinov,5 Minho Shong,6 Jiri Zavadil,4 Arthur P. Grollman 1.

Background: PGC1A and PGC1B encode transcriptional factors that regulate mitochondrial biogenesis and have been implicated in the interplay between telomere and mitochondrial dysfunction, and epigenetics in carcinogenesis. Additional studies should also examine these associations in larger cohorts with greater racial/ethnic, gender, and socioeconomic diversity to validate these findings.

Results/Conclusions: Preliminary data show significant differences when comparing the transcriptome profiles of the microdissected breast epithelium from the early and late menarche cohort. Specifically, we observed that the tissue from women with early menarche had upregulation of genes associated with defense against oxidative stress and/or infectious bacteria (lactoferrin [LTF], ceruloplasmin [CP]), cell adhesion, (ITGα1, ITGα2, ITGα3, ITGα4, ITGβ2), immune response (CARD9, LAIR1), and had downregulation of ubiquitination pathways (USP40, AMFR) and lipoprotein metabolism (OSBPL1A, LIPH, PIGN). Immunohistochemical evaluation of markers of oxidative stress (LTF, CP), cell proliferation (Ki67), and immune infiltrates (CD45, CD20, CD8, CD68) is underway. Together, this information will give us the opportunity to better understand early age at menarche as a breast cancer risk factor and advance research for women’s health.

#4252 New molecular evidence associating exposure to aristolochic acid with urothelial cancers in South Korean patients: Implications for global public health risk linked to carcinogenic herbal medicines.

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Animal studies have shown that loss of PGC1A protects against cancer as a possible link between telomeres, mitochondria, and cancer incidence. Further evaluation is underway.
adduct analysis, can address exposure to AA in extended UTUC case series, thereby assisting in the design of preventive measures against this global public health funding. IARC: SW is Recipient of research support from the Drs. Martin & Dorothy Spatz Charitable Foundation; Henry and Marsha Lauder grant to KGD, VSS, APG.

#4255 Associations of coffee consumption and caffeine intake with mammographic breast density. Lusine Yaghjian, 1 Graham Colditz, 2 Bernard Rosner, 3 Aleksandra Gasparova, 1 Rulla Tamimi, 4 Univ. of Florida, Gainesville, FL; 5Washington University in St. Louis, MO; 6Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

Purpose: Previous studies suggest that coffee consumption and caffeine intake may be associated with reduced breast cancer risk which may result from stimulating effect of coffee on estrogen metabolism enzymes and subsequent changes in bioavailable estrogen. We investigated the association of coffee consumption and caffeine intake with mammographic breast density by woman’s menopausal status and, in postmenopausal women, by hormone therapy (HT) Method: This study included 4,130 cancer-free women within the Nurses’ Health Study and Nurses’ Health Study II cohorts. Percent breast density (PD), absolute dense and non-dense areas were measured from digitized film mammograms using a computer-assisted thresholding technique; all measures were square-root transformed for the analysis to improve normality. Diet was assessed with a semi-quantitative food frequency questionnaire, average cumulative coffee/caffeine consumption was calculated using data available from all the questionnaires preceding the mammogram date. Information regarding breast cancer risk factors was obtained from baseline or biennial questionnaires closest to the mammogram date. We used generalized linear regression to quantify associations between exposures and breast density measures, adjusted for potential confounders. Associations were adjusted separately for regular coffee, decaffeinated coffee, total coffee, and energy-adjusted caffeine intake. Results: In multivariable analyses, decaffeinated coffee was positively associated with PD in premenopausal women (2 cups/day: β = 0.23, p-trend = 0.03). In postmenopausal women, decaffeinated and total coffee were inversely associated with PD (decaffeinated coffee 2 cups/day: β = −0.24, p-trend = 0.04; total coffee 4 cups/day: β = −0.17). These associations appeared to be driven by the inverse associations of these exposures with absolute dense area. Among current HT users (n = 980), regular coffee and caffeine intake were inversely associated with PD (regular coffee 4 cups/day: β = −0.29, p-trend < 0.01; caffeine 4th vs. 1st quartile: β = −0.32, p-trend < 0.01). Among past users (n = 430), decaffeinated coffee was inversely associated with PD (2 cups/day β = −0.70, p-trend = 0.02). Conclusions: Our findings suggest that among current HT users, regular coffee and caffeine intake are inversely associated with mammographic percent breast density.

#4256 Impact of diesel engine exhaust exposure on the airway transcriptome. Eduard I. Drizik, 1 Seena Cybergett, 2 Roel Vermeulen, 3 Yuxin Meng, 3 Debra Silverman, 4 Nathaniel Rothman, 4 Marc Lenburg, 4 Danzhi Ren, 4 Huawei Duan, 5 Yong Niu, 5 Jun Xu, 5 Wei Fu, 5 Kees Mielisefiet, 6 Baosen Zhou, 7 YuFang Yang, 7 Meng Ye, 7 Xiaowei Jia, 7 Tao Meng, 7 Ping Bin, 7 Yuxin Zheng, 7 Debra Silverman, 4 Nathaniel Rothman, 4 Avrum Spira, 7 Qin Lan, 7 Boston University, Boston, MA; 8Utrecht University, Utrecht, Netherlands; 9Chinese Center for Disease Control and Prevention, Beijing, China; 10National Cancer Institute, Rockville, MD; 11Chao Yang Center for Disease Control and Prevention, Chao Yang, China; 12Hong Kong University, Hong Kong, Hong Kong.

Rationale: Recent epidemiological studies show that Diesel Engine Exhaust (DEE) exposure is associated with lung cancer, however the mechanism by which this occurs is not well understood. The goal of this study was to assess the transcriptomic alterations in the nasal epithelium of DEE exposed workers from factories where diesel engines are utilized. Methods: Nasal epithelium brushings were obtained from 41 subjects who work in a factory with DEE exposure, and 38 comparable control subjects who work in factories without any DEE exposure. The median Elemental Carbon (EC) levels of exposed individuals was 60.7 µg/m³, with a range of 17.2–105.4 µg/m³, respectively. RNA was isolated from nasal epithelial cells, and profiled for gene expression using Affymetrix microarrays. Linear modeling was used to detect differential expression between DEE exposure and controls. Pathway enrichment in differentially expressed genes was assessed using GO Biological Process and KEGG terms via EnrichR. Results: We found 234 genes that were differentially expressed between samples derived from DEE exposed participants versus controls at FDR < 0.25. Within this set of genes, we observed a higher expression of genes involved in oxidative stress, and in cell proliferation, cellular transcription, and regulation of apoptosis. In addition, we found that genes involved in ion transport, such as CFTR, were expressed at lower levels in DEE exposed samples. Conclusions: Chronic DEE exposure associates with changes in the airway transcriptome,
with increased stress response as a major effect of DEE exposure. The transcriptomic alterations we identified may help provide insight into the underlying mechanisms of DEE carcinogenicity.

#4257 Dietary nutrient intake, ethnicity, and epigenetic silencing of lung cancer genes detected in sputum in New Mexican smokers. Shuguang Leng, Maria Picchi,1 Piotr Filipczak,2 Frank Gilliland,3 Steven A. Belinsky.1 Lovelace Respiratory Research Inst., Albuquerque, NM; 4University of Southern California, CA.

Promoter hypermethylation of lung cancer (LC) genes detected in sputum assesses field carcinization and predicts LC risk. Hispanic smokers have greater risk for methylation than non-Hispanic Whites (NHW). We aimed to identify novel dietary nutrients affecting methylation of LC genes in sputum and determine the degree of ethnic disparity explained by diet. A cross-sectional population-based study was conducted with dietary intake assessed using a validated Harvard food frequency questionnaire in 327 Hispanics and 1502 NHWs from the Lovelace Smokers cohort (LSC). Twelve LC genes with diverse cellular functions whose concomitant methylation strongly predicted LC risk were studied. A global association was identified between dietary intake and gene methylation (Fperm=0.003). Seventeen nutrient measurements were associated with methylation with magnitude greater than seen for folate and these nutrients together accounted for 36% of the variance in methylation. Six protective nutrients were identified and their insufficient intake in Hispanics explained approximately 42% of ethnic disparity in methylation. Functional validation of protective nutrients that also explained ethnic disparity was provided by showing an effect of a red wine supplement on LC methylation in a mouse model. We conclude that understanding of the biological mechanisms underlying the observed epidemiologic associations are needed to guide the development of intervention strategies and the most effective public health messages for breast cancer prevention.

#4259 The relation of childhood diet to the timing of puberty in boys: Results from the Longitudinal Studies of Child Health and Development. Alya Alimujigaj,1 Graham A. Colditz,2 Catherine S. Berkey,3 Siobhan Sutcliffe.1 Washington University School of Medicine, Saint Louis, MO; 2Harvard Medical School, Boston, MA.

Background: Although accumulating evidence from different disciplines supports an early-life contribution to prostate cancer (PCa) risk, few studies have focused on this life stage. One major reason for this dearth of research is the decades-long span of time between early-life exposures and PCa onset, making traditional epidemiologic study designs challenging. To overcome this challenge, we took advantage of several well-supported or recently observed PCa risk factors - age at peak height velocity (APHV), height at age 13, and adult height - to shorten the time required to study early-life exposures and to improve exposure classification by using these risk factors as markers of future PCa risk. We focused our analysis on childhood diet because it influenced APHV in a similar study of girls, and because of its strongly suspected role in PCa development. Methods: We analyzed data from the Longitudinal Studies of Child Health and Development, which followed 67 Caucasion boys from Boston from birth through adolescence in the 1930–40s. At each biannual (or annual) visit, study staff measured participants’ height and weight, and assessed their diet by dietary history interviews. We used these data to estimate age- and energy-adjusted nutrient intake, diet scores (combining information on fat and protein intake), and information on vegetable protein from ages 1-10 into a healthy diet score), age-specific height-2-scores, body mass index, peak height velocity (PHV), and APHV. We investigated crude associations between dietary and anthropometric measures by Pearson correlation coefficients, and multivariable-adjusted associations by linear regression. Results: Childhood consumption of a Western diet (as measured by our fat-animal protein score) was positively associated with height at age 13 (beta coefficient β=0.08, P<0.001) and adult height (β=0.05, P=0.013), and inversely associated with APHV (β=−0.06, P=0.014). In contrast, childhood consumption of a healthy diet (as measured by our vegetable protein score) was inversely associated with height at age 13 (β=−0.09, P=0.008). No notable associations were observed for diet and PHV. After controlling for height at ages 1-2 as a marker of genetic growth potential, only the association between Western diet and height at age 13 remained significant (β=0.08, P<0.001). Our findings suggest that consumption of a Western diet during childhood (1-10 years of age) is associated with greater height at age 13, a possible marker of both earlier onset of puberty and greater height. As this measure was recently associated with later PCa risk/mortality in a large Danish cohort study, our findings may point towards an early dietary contribution to PCa risk. Future studies should explore this possible association further for its potential to inform primary PCa prevention strategies.

#4258 Influence of lifestyle factors on adipocyte size in human breast tissue. Kristin L. Campbell,1 Nagarajan Kannan,2 Sarah E. Neil-Sztramko,3 Connie J. Eaves,4 Jonathan P. Little,5 Itona Ciszmadia,6 David Zhu,7 Sarah Sayyari,1 Kelcey Bland,1 James D. Johnson.1 Univ. of British Columbia, Vancouver, British Columbia, Canada; 2Mayo Clinic, Rochester, MN; 3McMaster University, Hamilton, Ontario, Canada; 4Terry Fox Laboratory, Vancouver, British Columbia, Canada; 5Univ. of British Columbia, Kelowna, British Columbia, Canada; 6University of Calgary, Calgary, Alberta, Canada.

Background: Accumulating epidemiological studies positively associate healthy body mass index (BMI) and higher physical activity with lower risk of postmenopausal breast cancer. Many of the proposed biomarkers underlying these associations, such as insulin, inflammatory markers, steroid hormones, and adipokines, are produced or regulated by adipose tissue. However, the biological impact of lifestyle factors at the level of the breast tissue, particularly adipose tissue in the breast, is unclear and may play a role in the etiology of breast cancer. Using a cross-sectional approach, we examined the impact of demographic and lifestyle factors on histological features of breast adipose tissue.

Results from the Longitudinal Studies of Child Health and Development. Kristin L. Campbell, 1Nagarajan Kannan, 2Sarah E. Neil-Sztramko, 3Connie J. Eaves, 4Jonathan P. Little, 5Itona Ciszmadia, 6David Zhu, 7Sarah Sayyari, 1Kelcey Bland, 1James D. Johnson. 1Univ. of British Columbia, Vancouver, British Columbia, Canada; 2Mayo Clinic, Rochester, MN; 3McMaster University, Hamilton, Ontario, Canada; 4Terry Fox Laboratory, Vancouver, British Columbia, Canada; 5Univ. of British Columbia, Kelowna, British Columbia, Canada; 6University of Calgary, Calgary, Alberta, Canada.

Background: Genetic alterations are considered to be accumulated in normal tissues at extremely low levels by exposure to various carcinogenic factors, and the degree of accumulated alterations, namely mutation burden, is likely to be associated with prostate cancer (PCa) risk. Few studies have focused on this life stage. One major reason for this dearth of research is the decades-long span of time between early-life exposures and PCa onset, making traditional epidemiologic study designs challenging. To overcome this challenge, we took advantage of several well-supported or recently observed PCa risk factors - age at peak height velocity (APHV), height at age 13, and adult height - to shorten the time required to study early-life exposures and to improve exposure classification by using these risk factors as markers of future PCa risk. We focused our analysis on childhood diet because it influenced APHV in a similar study of girls, and because of its strongly suspected role in PCa development. Methods: We analyzed data from the Longitudinal Studies of Child Health and Development, which followed 67 Caucasian boys from Boston from birth through adolescence in the 1930–40s. At each biannual (or annual) visit, study staff measured participants’ height and weight, and assessed their diet by dietary history interviews. We used these data to estimate age- and energy-adjusted nutrient intake, diet scores (combining information on fat and protein intake), and information on vegetable protein from ages 1-10 into a healthy diet score), age-specific height-2-scores, body mass index, peak height velocity (PHV), and APHV. We investigated crude associations between dietary and anthropometric measures by Pearson correlation coefficients, and multivariable-adjusted associations by linear regression. Results: Childhood consumption of a Western diet (as measured by our fat-animal protein score) was positively associated with height at age 13 (beta coefficient β=0.08, P<0.001) and adult height (β=0.05, P=0.013), and inversely associated with APHV (β=−0.06, P=0.014). In contrast, childhood consumption of a healthy diet (as measured by our vegetable protein score) was inversely associated with height at age 13 (β=−0.09, P=0.008). No notable associations were observed for diet and PHV. After controlling for height at ages 1-2 as a marker of genetic growth potential, only the association between Western diet and height at age 13 remained significant (β=0.08, P<0.001). Our findings suggest that consumption of a Western diet during childhood (1-10 years of age) is associated with greater height at age 13, a possible marker of both earlier onset of puberty and greater height. As this measure was recently associated with later PCa risk/mortality in a large Danish cohort study, our findings may point towards an early dietary contribution to PCa risk. Future studies should explore this possible association further for its potential to inform primary PCa prevention strategies.

#4260 Increased mutation burden in normal-appearing lung tissues with high cancer risk. Emi Kubo,1 Hideyuki Takeshima,2 Noriko Motoi,2 Toshikazu Ushijima1. 1National Cancer Center Research Institute, Tokyo, Japan; 2National Cancer Center Hospital, Tokyo, Japan.

Background: Genetic alterations are considered to be accumulated in normal tissues at extremely low levels by exposure to various carcinogenic factors, and the degree of accumulated alterations, namely mutation burden, is likely to be associated with cancer risk. In lung tissues, smoking is a well-known carcinogenic factor, and is considered to be involved in accumulation of mutation burden. However, due to the limitation of detection methods of such extremely low frequency mutations, the presence of mutation burden in normal human lung tissues has been unclear. To overcome this limitation, we recently developed a new method to detect mutations with extremely low frequency by sequencing 100 DNA molecules using a next generation sequencer[Yamashita, submitted]. Aim: We aimed to reveal 1) the presence of mutation burden in normal lung tissues, and 2) its association with cancer risk. Methods and results: The presence of somatic mutations was analyzed in 55 cancer-related genes (totally 15,724 bp) in normal lung tissues (n = 11) obtained from background tissues of lung metastasis of cancer patients, without smoking history, other than those with lung cancers (“entirely normal lung tissues”) and non-cancerous lung tissues (n = 11) of lung cancer patients with smoking history (“smoking-exposed normal tissues”). The mutation frequency in smoking-exposed normal tissues (2.7 ± 0.8×10−7/bases) was significantly higher than that in entirely normal
tissues (1.8 ± 0.5×10^7/bases) (p= 0.0189). C to T transition was most frequent in both smoking-exposed normal tissues (60% of detected base substitutions) and entirely normal tissues (61%), but the fractions were not different between the two groups. In contrast, C to A transversion, the signature mutation of smoking, was more frequent in smoking-exposed normal tissues (4%) than in entirely normal tissues (0.8%). In some malignant tissues, higher levels of somatic mutations occurred at the C to A transversion at a loci of SN (`OR = 3.75% (95% CI = 0.54-26.06%)). Conclusion: It was shown, for the first time, that somatic mutations were accumulated in normal lung tissues with high cancer risk, possibly by exposure to tobacco smoking.

**#4261 Cancer in childhood and molecular epidemiology - The KIKME case-control study.** Manuela Marron,1 Sebastian Zahnreh,2 Olesya Sinizny,1 Heinz Schmidberger,2 Moritz Hess,3 Patricia Sadre Dodras,1 Iris Abebeckwinkel-4 Thomas Hinkel,4 Steffen Rapp,4 Anne Ebersberger,1 Christian Grad,2 Eva Holzhäuser,1 Lukas Eckhard,5 Dirk Proschek,5 Maria Blettner,3 Peter Kaatsch,6

**#4262 Smoking impacts endogenous estradiol and testosterone levels in young healthy women.** Carolina Ellberg, Håkan Olsson, Helena Jernstrom. *Lund University, Lund, Sweden.*

**#4263 Inflammatory gene expression differences among prostate cancer patients exposed to the World Trade Center aftermath.** Dana Hashim, Yixuan Gong, Matthew Galsky, Charles Baker, Paolo Boffetta, Michael J. Donovan, Emanuela Taioli, William K. Oh. *Mt. Sinai Icahn School of Medicine, New York, NY.*

**EPIDEMIOLOGY: Biomarkers of Endogenous or Exogenous Exposures, Early Detection, and Biologic Effects**

**Epidemiology**

**Background:** The World Trade Center (WTC) collapse occurred on September 11, 2001. Among those working or living near the WTC, a new cancer syndrome (WTC cancer) has emerged. This cancer is characterized by the presence of a high number of tumors in the pleura, thorax, and lymph nodes. The etiology of WTC cancer remains uncertain, and the role of environmental factors in its development is still under investigation.

**Methods:** A case-control study with 600 participants was conducted, including 300 cases with WTC cancer and 300 controls without WTC cancer. The study was nested within the New York City Health Department's Health Insurance Plan (HIP) database. The primary outcome was the presence of WTC cancer. Participants were matched by age, sex, and race.

**Results:** The risk of WTC cancer was significantly higher among individuals who worked at the WTC or lived in the immediate vicinity during the attack compared to those who did not. The risk was also higher among individuals who had respiratory symptoms or were exposed to fine particulate matter. The risk was lower among individuals who had received radiation therapy or chemotherapy.

**Conclusion:** The study provides evidence for the role of environmental factors in the development of WTC cancer. Further research is needed to identify the specific environmental factors that contribute to this cancer syndrome.
endogenous E2 and total T levels in current OC users and non-users was thus observed. Therefore, history of OC use needs to be incorporated in studies on associations between smoking and breast cancer risk.

#4264 Smoking exposure and quantitative levels of estrogen-receptor expression in ER+ breast tumors. Ebonee N. Butler, John A. Baron, Jeanette T. Bensen, Mengjie Chen, Kathleen Conway, Andrew F. Ollhan, Melissa Troester, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Introduction: Smoking is a suspected risk factor for breast cancer and has been linked to increased risk of estrogen-receptor positive (ER+) disease in some epidemiologic studies. Cigarette smoke has also been shown to have antieigenetic effects, leading to a potentially contradictory hypothesis that smoking would lead to decreased ER expression driven by smoking-induced DNA damage. ER status and breast tumors with respect to ER may mask quantitative associations between smoking and ER expression. Methods: Using data from the Carolina Breast Cancer Study (CBCS), we examined relationships between smoking and quantitative levels of ER expression from tumors of 1,297 women with ER+ disease (i.e., > 10% of tumor cells ER+ by immunohistochemical analysis). We used unweighted logistic regression to estimate associations between categorical measures of smoking and quartiles of ER protein or ER$^*$ mRNA expression, represented by odds ratios (OR) and 95% confidence intervals (95%CI). We also examined associations between smoking and quantitative ER levels among women with ER+ breast tumors, stratified by menopausal status. Multivariate regression models include adjustment for age, race, stage, grade, and tumor size. Results: Quantitative ER$^*$ was positively associated with any history of smoking (i.e., current at time of diagnosis or former smoking) (OR=1.96 and 95% CI: 1.24 to 3.10). In addition, smoking more than 1 pack of cigarettes per day was associated with high ER$^*$ expression (OR=3.19 and 95% CI: 1.29 to 7.91) as smoking duration of > 20 years (OR=2.08 and 95% CI: 1.16 to 3.74). Among former smokers, we observed higher ER$^*$ expression among those who quit 5–10 years prior to diagnosis. The magnitudes of association between smoking and quantitative ER expression were similar for both pre- and post-menopausal women, but were not reflected in similar association on the protein level. Conclusions: Among women with ER+ breast cancer, smoking dose and duration was positively associated with elevated ER$^*$ mRNA levels, regardless of menopausal status. Absence of this association for protein suggests that ER$^*$ RNA merits further consideration, but may suggest that ER$^*$ RNA more sensitively captures biological differences than ER protein expression.

EPIDEMIOLOGY: Familial and Hereditary Cancers

#4265 Risks of familial breast cancer associated with known and proposed breast cancer susceptibility genes. Kara N. Maxwell, 1 Thomas Paul Stulik, 1, 2 Jena M. Lallyquist, 3 Joseph Viji, 4 Susan L. Neuhausen, 1 Steven N. Hart, 1 Vi gnesh Ravichandran, 5 Tinu Thomas, 4 Ann Maria, 1 Kasimtian A. Schrader, 6 Raymond Moore, 3 Chunling Hu, 3 Brad Wubbenhorst, 1 Brandon M. Wenz, 1 Jenna M. Lilyquist, 3 Joseph Vijai, 4 Susan L. Neuhausen, 2 Steven N. Hart, 3 Vijalette Delong, 1 John Hopper, 6 Katherine L. Nathanson, 1 Fergus J. Couch, 3 Jeffrey N. Weitzel 2, John L. Hopper, 1 Mark A. Jenkins, 1 Roger L. Milne, 2 Graham G. Giles, 15 Dallas R. English, 1 Finlay A. Macrae, 5 Amanda B. Spurde, 1 Ingrid M. Winship, 6 Christophe Rosty 1, 1 University of Melbourne, Parkville, Australia; 2 Cancer Council Victoria, St. Kilda, Australia; 3Queenland Institute of Medical Research Berghofer, Herston, Australia; 4University of Melbourne, Carlton, Australia; 5Royal Melbourne Hospital, Parkville, Australia.

Background: Tumour mismatch repair (MMR) deficiency, determined by immunohistochemical (IHC) loss of MMR protein expression, is used diagnostically to identify individuals with Lynch syndrome. A high proportion of colorectal cancers (CRCs) and endometrial cancers (ECs) that demonstrate tumor MMR-deficiency are categorised as having “Lynch-like syndrome” due to the absence of MSH6 or MLH1 methylation and germline MMR gene mutations after standard screening approaches. The aim of this study was to investigate somatic causes of tumor MMR-deficiency in patients with Lynch-like syndrome. Methods: Population-based participants with incident MMR-deficient colorectal (n=193; ACCFR and MCCS) or endometrial cancer (n=197; ANECs and MCCS) were categorised as either Lynch syndrome, MLH1 methylated or Lynch-like after screening for germline MMR gene mutations and for tumor MLH1 gene promoter hypermethylation. Lynch-like tumors were tested for somatic MMR gene mutations (point mutations and loss of heterozygosity) using AmpliSeq Ion Proton custom capture sequencing and for MSH2 or MSH6 gene promoter methylation. Overall survival for molecularly defined subgroups of Lynch-like CRCs were compared to Lynch syndrome related CRCs using cox regression models to estimate hazard ratios (HR) and 95% confidence intervals adjusting for age at CRC diagnosis, sex, AJC stage and grade. Results: Lynch-like tumors comprised 32% (63/193) and 23% (45/197) of the MMR-deficient CRCs and ECs, respectively compared with 27% and 15% for Lynch syndrome and 41% and 62% for MLH1 methylated CRCs and ECs, respectively. Two somatic mutations were identified in the MMR gene indicated by the pattern of MSH6 and MLH1 methylation and germline MMR gene mutations after standard screening approaches. The proportion of tumors with double somatic alterations was highest for both CRC and EC tumors showing MSH2 deficiency (40% and 64.3%). The mean age at diagnosis for the Lynch-like CRCs with double somatic mutations was 49.7 ± 15.8years which was not significantly different from the Lynch syndrome CRCs (n=52; 45.4 ± 11.3years; p=0.2) but was younger than the Lynch-like CRCs with different classification of the MLH1 mutation (n=83; 70 ± 8.9years;p=0.0001). The adjusted HR for double somatic Lynch-like CRCs was 2.58 (95% CI, 0.77-8.67) compared with Lynch syndrome CRCs (p=0.01). No evidence of tumor MSH2 or MSH6 gene promoter methylation was identified in either (5% MSH2-deficient or MSH6-deficient Lynch-like CRCs or ECs tested (n=34 and n=12, respectively). Conclusions: Double somatic mutations in the MMR genes represent a significant proportion of the unexplained Lynch-like MMR-deficient subtype for both population-based CRC and EC. Triaging strategies used to identify Lynch syndrome for both CRC and EC should include tumor testing for somatic mutations in the MMR genes.

#4267 Association of polygenic risk scores and family history with the risk of chronic lymphocytic leukemia (CLL). Geffen Kleinstern, 1 Silvia de Sanjose, 1 Nicola Camp, 1 Claire M. Vajdic, 1 Timothy G. Call, 2 Dennis Robinson, 3 Neil E. Kay, 4 Julie Cunningham, 4 Yolanda Benavente, 5 Alain Monereau, 1 John Spinelli, 1 James R. Cerhan, 4 Susan L. Slager 1, Mayo Clinic, Rochester, MN; 2Hospital de Llobregat, Barcelona, Spain; 3University of Utah, Salt Lake City, UT; 4University of NSW, Sydney, Australia; 5Sorbonne Paris Cité Cen tre, Paris, France; 6BC Cancer Research Center, Vancouver, British Columbia, Canada.

Background: Thirty-four single nucleotide polymorphisms (SNPs) are associated with CLL risk to-date. Moreover, family history (FH) of hematological malignancy has been consistently found to be associated with CLL at this time. Larger cancer control studies are needed to fully evaluate the cancer risks associated with moderate penetrance and proposed breast cancer susceptibility genes.

Case-control analysis demonstrated significant associations with FBC for ATM, PALB2, and TP53 mutations (OR>3.0, p<10^-4), BARD1 mutations (OR=3.2, p=0.012), and CHEK2 truncating mutations (OR=1.6, p=0.041). Our results therefore demonstrate that only approximately 4% of BRCA1/2 negative FBC patients have mutations in genes definitively associated with breast cancer at this time. Large case-control studies are needed to fully evaluate the breast cancer risks associated with moderate penetrance and proposed breast cancer susceptibility genes.

EPIDEMIOLOGY: Biomarkers of Endogenous or Exogenous Exposures, Early Detection, and Biologic Effects

#4268 Smoking exposure and quantitative levels of estrogen-receptor expression in ER+ breast tumors. Ebonee N. Butler, John A. Baron, Jeanette T. Bensen, Mengjie Chen, Kathleen Conway, Andrew F. Ollhan, Melissa Troester, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Introduction: Smoking is a suspected risk factor for breast cancer and has been linked to increased risk of estrogen-receptor positive (ER+) disease in some epidemiologic studies. Cigarette smoke has also been shown to have antieigenetic effects, leading to a potentially contradictory hypothesis that smoking would lead to decreased ER expression driven by smoking-induced DNA damage. ER status and breast tumors with respect to ER may mask quantitative associations between smoking and ER expression. Methods: Using data from the Carolina Breast Cancer Study (CBCS), we examined relationships between smoking and quantitative levels of ER expression from tumors of 1,297 women with ER+ disease (i.e., > 10% of tumor cells ER+ by immunohistochemical analysis). We used unweighted logistic regression to estimate associations between categorical measures of smoking and quartiles of ER protein or ER$^*$ mRNA expression, represented by odds ratios (OR) and 95% confidence intervals (95%CI). We also examined associations between smoking and quantitative ER levels among women with ER+ breast tumors, stratified by menopausal status. Multivariate regression models include adjustment for age, race, stage, grade, and tumor size. Results: Quantitative ER$^*$ was positively associated with any history of smoking (i.e., current at time of diagnosis or former smoking) (OR=1.96 and 95% CI: 1.24 to 3.10). In addition, smoking more than 1 pack of cigarettes per day was associated with high ER$^*$ expression (OR=3.19 and 95% CI: 1.29 to 7.91) as smoking duration of > 20 years (OR=2.08 and 95% CI: 1.16 to 3.74). Among former smokers, we observed higher ER$^*$ expression among those who quit 5–10 years prior to diagnosis. The magnitudes of association between smoking and quantitative ER expression were similar for both pre- and post-menopausal women, but were not reflected in similar association on the protein level. Conclusions: Among women with ER+ breast cancer, smoking dose and duration was positively associated with elevated ER$^*$ mRNA levels, regardless of menopausal status. Absence of this association for protein suggests that ER$^*$ RNA merits further consideration, but may suggest that ER$^*$ RNA more sensitively captures biological differences than ER protein expression.
not been an evaluation of the interactive effects among genetic factors and FH with CLL risk. Methods: We pooled data from 8 CLL case-control studies within the InterLymph Consortium (1499 CLL cases and 2601 controls). We computed a polygenic risk score (PRS), a weighted average of the number of risk alleles across the 34 SNPs, with the weights being the log of the previously reported odds ratio for the SNP. We categorized the PRS by tertiles using the cutoff points based on the distribution of all InterLymph controls (N=8228). Self-reported FH data was available for 60% of cases and 73% of controls. FH was defined as any hematological malignancy in one or more first-degree relative. Logistic regression was used to estimate ORs and 95% confidence intervals (CIs) adjusted for age, sex, socioeconomic status and study. Results: The median age at diagnosis in FH cases and controls was 67 years and median interval between diagnosis and controls was 67% were male in CLL cases and 57% in controls. As expected, FH was associated with CLL risk (OR= 2.14, CI = 1.60-2.86). The median PRS in the cases was 0.40 and in the controls was -0.36 with the frequency of CLL cases in the upper PRS quintile as 48% while in the lowest quintile only 6%. The PRS was strongly associated with CLL risk (OR= 2.90, CI = 2.35-3.56 for upper versus middle quintile). When jointly modeling FH with PRS, a significant interaction was observed (P =0.03). When stratifying by FH, the upper quintile of the PRS had an 11.8-fold (CI = 3.97-34.8) increased risk relative to those in the middle quintile in the FH+ strata, while a 3.11-fold (CI = 2.35-4.10) increased risk was observed in the FH- strata. Conclusions: Our data suggest that the PRS has a strong association with CLL risk and this association varies with FH status. A two-point linkage analysis between the disease and each marker was performed using TwoPoint-QLinkage. A regional-based linkage analysis was performed using MERLIN. We identified five loci that corresponded to a gene or a portion of a gene. Two-point linkage analyses were then performed on the risk of CLL for those markers using TwoPoint-QLinkage. The final signal on 14q13 was NAP153, a transcription factor that is a tumor suppressor in brain tumors. It should be noted that all previous evidence linking these genes to cancer was based on somatic mutations; this is the first time any of these genes has been shown to be significantly linked to germline disease risk in a family-based study. We plan to perform targeted sequencing on the linked regions to elucidate the exact causal variant.

#4269 Risk of different cancers among first-degree relatives of pancreatic cancer patients and impact of probands’ germline mutation on sibling cancer risk. Samuel O. Antwi,1 Sarah E. Fagan,2 Kari G. Chaffee,3 William R. Bamlet,3 McWilliams R. Robert,4 Ann L. Öberg,5 Gloria M. Petersen,1 *Epidemiology, Mayo Clinic, Rochester, MN;1 Epidemiology, Tulane University, New Orleans, LA;1 Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN;2 Medical Oncology, Mayo Clinic, Rochester, MN.

Background: There have been varying reports on risk of different cancers, other than pancreatic cancer (PC), among first-degree relatives (FDRs) of PC patients. Because the pattern and scope of aggregation of PC with other malignancies in families of PC patients are not entirely clear, we investigated risk of 15 common malignancies among FDRs of unselected PC probands. Methods: The study included 17,181 FDRs with more than 336,000 person-years at risk. The FDRs were identified through sequentially enrolled PC probands (n = 2,305) in the Mayo Clinic prospective pancreatic cancer patient registry from 2000-2016. Data on family history of cancer were provided by the probands at the time of enrollment in structured risk factor questionnaires. Standardized incidence ratios (SIRs) and 95% confidence intervals (CIs) were calculated by comparing cases of each cancer type observed among the FDRs with those expected using data from the Surveillance Epidemiology and End Results Program (SEER). Stratified analyses were performed among the FDRs of probands on whether the related proband tested positive for a mutation in ATM, BRCA1, BRCA2, CDKN2A, CHEK2, MUTHY, BNN, PALB2, or PMS2; or tested negative for mutation in a total of 25 sequenced cancer susceptibility genes. Results: Compared to the SEER reference population, risk of PC was two-fold higher than expected among the FDRs (SIR = 2.04, 95% CI: 1.78-2.31) and 12-fold higher than expected among FDRs with FH of PC in at least two blood relatives (SIR = 11.99, 95% CI: 10.48-13.64). Siblings of mutation-positive probands had higher risk of PC (SIR = 13.57, 95% CI: 6.19-25.76) than siblings of mutation-negative probands (SIR = 8.91, 95% CI: 6.73-11.57). For other cancer types, primary liver cancer was elevated among female FDRs (SIR = 2.10, 95% CI: 1.34-3.12), whereas breast (SIR = 3.16, 95% CI: 1.63-5.32) and ovarian (SIR = 6.61, 95% CI: 1.33-19.31) cancers were elevated only among siblings of the mutation-positive probands. There also were suggestions of lower than expected risk of other malignancies, such as bladder, colorectal and prostate cancers, among the FDRs as compared with the SEER population. Conclusions: These findings confirm familial aggregation of PC with breast and ovarian cancers, and further suggest a potential aggregation of PC and primary liver cancer among female FDRs of PC probands. The elevated risks of breast cancer and ovarian cancer among siblings of the mutation-positive probands suggests a strong influence of genetic susceptibility shared with the related proband, possibly due to an inherited mutation in BRCA1, BRCA2, or PALB2. These findings lend support to genetic counseling and targeted screening of certain cancers in high-risk families.

#4270 Family history in first degree relatives and risk of gastric cancer in the alpha-tocopherol, beta-carotene cancer prevention study. Min'kong Song, Maria Constanza Camargo, Stephanie J. Weinstein, Demetrius Albanes, Charles S. Raskin. National Cancer Institute, Bethesda, MD.

Background: Studies of the familial associations of gastric cancer risk have largely been limited to case-control approaches which are susceptible to recall bias and cannot distinguish temporality. Furthermore, associations with family history could be explained by shared exposure to Helicobacter pylori infection, the most important environmental risk factor for gastric cancer. Notably, the risks of early onset disease and diffuse-type tumors are thought to have a different genetic component. We therefore examined in a prospective study the associations of gastric cancer risk with history of gastric cancer in first degree relatives, controlling for H. pylori infection. Methods: We selected 605 incident gastric cancer cases and 4,129 controls with known H. pylori serology status from the Finnish Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study among male smokers aged 50-69 at baseline. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated using logistic regression with adjustment for H. pylori and other potential confounders. All statistical tests were two-sided. Results: Family history of gastric cancer was associated with increased risk (OR = 1.84, 95% CI = 1.32-2.58). The association appeared stronger with gastric cancer in siblings (OR = 2.87, 95% CI = 1.64-5.03) than with gastric cancer in parents (OR = 1.53, 95% CI = 1.05-2.24). Distinguishing cases by anatomic subtype, the family history association was significant for noncardia gastric cancer (n = 305; OR =2.15, 95% CI =1.50-3.07), but not for cardia cancer
Population-wide vs. carrier-probability-based BRCA1/2 mutation testing in the Washington Ashkenazi Study. Ana F. Best, Margaret A. Tucker, Hormuzd A. Katki. National Cancer Institute, Division of Cancer Epidemiology and Genetics, Bethesda, MD.

Background: Currently, women are referred for BRCA1/2 mutation testing if their family history of breast and ovarian cancer indicates they have sufficient probability of carrying mutations. In contrast, calls have intensified for population-wide testing for BRCA1/2 founder mutations among Ashkenazi Jews, due to relatively high mutation prevalence (2.5%) and decreasing genotyping costs. However, population-wide testing would strain genetic counseling resources and incur costs from testing mostly mutation-negative women. We compared the performance of population-wide vs. carrier-probability-based BRCA1/2 testing in the community-based Washington Ashkenazi Study (WAS). Methods: BRCA1/2 carrier-probabilities based on reported family histories were calculated using BRCAPRO for 4589 probands (102 BRCA1/2 mutation-carriers) in WAS. For each carrier-probability threshold, we compared the percent of mutations found (sensitivity) vs. the percent of women requiring mutation testing, overall and by proband age. At example carrier-probability thresholds, undetected and detected carriers’ attributes were compared using nonparametric tests. Results: A 1.2% carrier-probability threshold identified 80% of BRCA1/2 mutations in the 35% of women with highest carrier-probability. For women under age 40, 94% of BRCA1/2 mutations were identified in the 58% of women with highest carrier-probability. Compared to identified mutation-carriers, missed mutation-carriers had no personal cancer history, no affected first- and second-degree relatives (p<0.0001), more unaffected first-degree relatives (p=0.0002), and were older (p=0.012; median age=53). Conclusion: Carrier-probability-based BRCA1/2 mutation testing identified large differences in mutation-carriers and would have avoided testing for many mutation-negative women. Missed mutation-carriers were older and cancer-free and would gain minimal benefit from risk-reducing interventions. A cost-effective carrier-probability threshold should acceptably tradeoff identifying BRCA1/2 mutation-carriers before they get cancer while testing as few women as possible.

Mutations in BRCA1 and BRCA2 and associated with hereditary pancreatic cancer. Sahar Nissim, 1 Ignaty Lechchin, 1 Joseph D. Mancias, 1 Matthew B. Greenblatt, 2 Ophelia Maertens, 1 Christopher A. Cassa, 1 Jill A. Rosenfeld, 1 Andrew G. Cox, 1 John Hedgepeth, 1 Julia Wücherpfennig, 1 Andrew J. Kim, 1 Jake E. Henderson, 1 Patrick Gonyo, 1 Anthony Brandt, 1 Ellen Lorimer, 1 Bethany Unger, 1 Jeremy W. Prokop, 1 Jeremy W. Heidel, 2 Xiao-Xu Yamagami, 1 Nobuyuki Susumu, 1 Hitoshi Tsuda, 3 Daisuke Aoki1. 1 Brigham and Women’s Hospital, Boston, MA; 2 Broad Institute of MIT and Harvard, Cambridge, MA; 3Graduate School of Biomedical Sciences, Saitama, Japan

Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest solid cancers with limited treatment options despite intensive research efforts. Familial predisposition to PDAC is thought to occur in ~10% of cases, but causative genes have not been identified in most of these families. Uncovering the genetic basis for PDAC susceptibility has immediate prognostic implications for families and can provide precious mechanistic clues to PDAC pathogenesis. Here, we perform whole-genome sequence analysis in a family with high incidence of PDAC and identify a germline nonsense mutation in the member of RAS oncogene family-3 (RAB3) gene that has never before been directly associated with hereditary cancer. The truncated mutant allele (RAB3_p.Ser36*) co-segregates with cancer occurrence. To evaluate the contribution of the RAB3 mutant allele in hereditary cancer, we generated a Rab3 heterozygous mutant zebrafish and found increased susceptibility to cancer formation in two independent cancer models. Complementary unbiased approaches implicate RAB3 in LFS pathway regulation. RNA-Seq and genome-set enrichment analysis of juvenile rab3 mutants reveals a KRAS upregulation signature. Furthermore, affinity-purification mass-spectrometry for proteins associated with RAB3 or RAB3_p.Ser36* identifies RAP1 GTPase-GDP Dissociation Inhibitor 1 (RAP1GDS1, SmgGDS), a chaperone that regulates prenylation of RAS GTPases. Indeed, in vitro studies demonstrate that RAB3_p.Ser36* accelerates KRAS prenylation, and this impact is lost in the absence of H/N/KRAS proteins. Whereas heterozygous rab3 mutant zebrafish exhibit abnormal craniofacial, skeletal, and growth defects consistent with human RASopathies, and these defects are partially rescued with the MEK inhibitor trametinib. Our findings support a gain-of-function rather than a null function typically associated with premature protein truncations. The discovered causative RAB3 germline mutation provides new diagnostic opportunities for genetic testing in other cancer families and uncovers an alternative mechanism for dysregulated RAS signaling in development and cancer.

Variant reclassifications in hereditary cancer genetics and their implications for clinical care. Thomas P. Slavin, 1 Stacy W. Gray, 1 Lily R. Van Tongeren, 1 Ilana Solomon, 1 Christina Rybak, 1 Bita Nehoray, 1 Lili Kuzmich, 1 Mariana Niell-Swiller, 1 Kathleen R. Blazer, 1 Kai Yang, 1 Julie Culver, 1 Sharon Sand, 1 Danièle Castillo, 1 Josef Herzog, 1 Jeffrey N. Weitzel 1. 1 City of Hope, Duarte, CA; 2 University of Southern California Norris Comprehensive Cancer Center, Los Angeles, CA.

BACKGROUND: Clinicians who provide genetic cancer risk assessment (GCRA) are dependent on laboratory reporting of germline results to inform cancer screening and treatment recommendations. Efforts to enhance variant classification and harmonization, such as ClinVar, will lead to an increase in the number of variant reclassifications. As the rate of variant reclassification on care is unknown, we evaluated the frequency and clinical impact of variant reclassification on individuals seen for GCRA. METHODS: We retrospectively evaluated data on 7,356 participants enrolled through the Clinical Cancer Genomics Community Research Network (CCGCRN) at City of Hope and Olive View Medical Center from September 1996- October 2016. RESULTS: 4,969 commercial genetic tests yielded a total of 1,610 variants of any category, of which 181 unique variants in 20 genes were reclassified. BRCA1 and BRCA2 (BRCA) and mismatch repair genes comprised 73.5% and 5.5% of the genes reclassified. Compared to initially reported variants, reclassified variants were older (p=0.0001), more unaffected first-degree relatives (p=0.0002), and were older (p=0.012; median age=53). Conclusion: Variant reclassification may change clinical care. Thirteen variants carried by 15 individuals were upgraded from a variant of uncertain significance (VUS) to likely pathogenic or pathogenic (10 BRCA, 3 MLH1 or MSH2). These reclassifications prompted additional prophylactic surgical interventions (i.e., bilateral salpingo-oophorectomy), specialist referrals, and surveillance recommendations for at-risk patients and family members. Three variants (NRB p.Arg215Trp, PSEN p.Ala79Thr, and MET c.1200+2T>G) were downgraded from likely pathogenic or pathogenic to VUS. Prior to downgrade to VUS, 2 cases had unnecessary surveillance procedures. CONCLUSIONS: Since many genetic variants will be reclassified over time, it is critical that laboratories deliver prompt notification of reclassifications, and that providers involved in GCRA discuss the possibility of variant reclassification with patients and family members and collect patient/proxy information during informed consent so that re-contact is possible. Given the non-trivial effort required for variant reclassification and patient/participant re-contact, system-level interventions are needed to facilitate genomic reinterpretation and the return of results to individuals over time.

The contribution of deleterious germ-line mutations of susceptibility genes to ovarian, fallopian tube, and peritoneal cancers in Japanese. Akira Hirasaawa, 1 Issei Imoto, 1 Takuya Naruto, 1 Tomoko Akahane, 1 Wataru Yamagami, 1 Nobuyuki Susumu, 1 Hitoshi Tsuda, 1 Daikusu Aoki 1. 1 Keio University School of Medicine, Tokyo, Japan; 2Graduate School of Biomedical Sciences, Tokushima University, Tokushima, Japan; 3National Defense Medical College, Saitama, Japan.

Different ethnic groups present specific morphological features in ovarian cancer (OC). High-grade serous OC is more frequently found in Caucasian women, in contrast clear cell OC is more frequently found in East Asian women. Although inherited mutations of BRCA1 or BRCA2 (BRCA1/2) and other genes are known to predispose to OC, the contributions of these inherited mutations on disease burden is not well characterized in Japanese OC patients. Using a comprehensive genetic testing panel, the aim of our study was to identify the prevalence of pathogenic germ-line mutations of candidate genes associated with OC. 1093
with genetic predisposition to OC in Japanese patients with ovarian, fallopian tube, or peritoneal cancer. Samples from 236 individuals with unselected ovarian, fallopian tube, or peritoneal cancer, which were obtained from the Keio Women’s Health Biobank (KWB) from the School of Medicine at Keio University (Tokyo, Japan), were used for this study. Germ-line DNA was enriched using the SureSelect XT Target Enrichment System (Agilent Technologies) designed for 75 or 79 genes as a custom OC panel, followed by sequencing using MiSeq (Illumina). Detected variants, including point mutations, small indels, and a gross deletion, were classified according to the American College of Medical Genetics and Genomics (ACMG) recommendations. Furthermore, BRCA1/2 variants were interpreted using resources from Myriad Genetic Laboratories. Forty-two (17.8%) OC patients had germ-line mutations of cancer predisposition genes. BRCA1/2 pathological germ-line mutations were found in 27 cases (11.4%) while six cases (2.5%) had a mutation in a mismatch repair gene (MLH1, MSH2, MSH6, or PMS2). Furthermore, ten cases (4.2%) had germ-line mutations in the RAD51D, ATM, MRE11A, FANC, or GABRA2 genes. Of patients with a BRCA1/2 mutation (n=27), we found that the most common histological subtype was high-grade serous OC, but we also found two cases with clear cell carcinoma. Of 42 cases with an inherited mutation, we found that 25% (6/24) had no family history of cancer, and 78% (31/41) were under 60 years old at diagnosis. We found that approximately 18% of unselected Japanese patients with ovarian, fallopian tube, or peritoneal cancer were associated with inherited mutations of cancer-predisposing genes. Our study demonstrates that comprehensive genetic testing is informative for all women with fallopian tube and peritoneal cancers to establish personalized clinical management and genetic counseling, irrespective of the patient’s histological subtypes, ages or family histories.

**#4275 Pathogenic germline variants in Mexican patients with hereditary breast and ovarian cancer syndrome.** Felipe Vaca-Paniagua, Rosalía Quezada-Urban, Clara Díaz-Velásquez, Rina Gitler, Gabriela Torres-Mejía, Luis Ignacio Terrazas, Patricia Rojo-Castillo, Max Sirota-Toporek, Andrea Figueroa-Morales, Oscarilyhistories.

neticcounseling, irrespective of the patient’s histological subtypes, ages or family histories.

- **#4276 Oral contraceptive use and breast cancer risk: a cohort study of BRCA1 and BRCA2 mutation carriers.** Liesa H. Schrijver, Hakon Olsson, Antonis Antoniou, Roger Milne, Kelly Phillips, Nadine Andrieu, Douglas Easton, David Goldgar, Christophe Engel, Karin Kast, Marie-Jose Blom, Thea Moolt, John Hopper, Floor Van Leeuwen, Mary-Beth Terry, Matti Perheentupa, IBCCS, BCER, Netherlands Cancer Institute, Amsterdam, the Netherlands; Lund University, Lund, Sweden; University of Cambridge, Cambridge, United Kingdom; University of Melbourne, Cancer Council Victoria, Melbourne, Australia; University of Melbourne, Peter MacCallum Cancer Centre, Melbourne, Australia; Institute Curie, PSL Research University, INSERM, Paris, France; University of Utah Salt Lake City, UT; University of Leipzig, Germany; Greywater and the Netherlands Cancer Institute, University Hospital Carl Gustav Carus, Dresden, Germany; University of Melbourne, Melbourne, Australia; Columbia University, New York, NY.

Background: BRCA1 and BRCA2 mutation carriers are at high risk of breast and ovarian cancer. Oral contraceptive preparations (OCPs) may reduce ovarian cancer risk, but its effect on breast cancer risk remains unclear. Methods: Combined data from three cohorts of 5705 BRCA1 and 3521 BRCA2 mutation carriers (IBCCS, BCER and kConFab) were analyzed using age-dependent Cox regression models stratified for study and birth cohort. We conducted the first prospective analyses on this topic. Our additional main retrospective analyses were left-censored 5 years preceding date of baseline questionnaire to control for survival bias. The full-cohort retrospective analyses, without left-censoring, was performed using Cox proportional hazard model. Results were considered most valid, while retrospective analyses were most powerful. Results: For BRCA1 mutation carriers we found no association between ever OCP use and risk of breast cancer in the prospective analyses (HR=1.08, 95% CI 0.75-1.56), but 23% and 27% increased risks for ever OCP use in the left-censored and full retrospective analyses, respectively. Prospectively, an increasing trend for longer duration of use, especially before first full-term pregnancy (FFTP) was found (left-censored analyses: &lt5 years HR 1.05 (95% CI 0.84-1.33), 5-9.9 years HR 1.15 (95% CI 0.92-1.44), &gt10 years HR 1.41 (95% CI 1.10-1.81), p-trend=0.001 for duration of use before FFTP). For BRCA2 mutation carriers we found a positive association between each OCP use and risk of breast cancer prospectively (HR=1.73, 95% CI 1.03-2.97), but retrospectively findings were inconsistent (HR=1.06, 95% CI 0.85-1.33 and HR=1.52, 95% CI 1.28-1.81 for the left-censored and full analyses, respectively). Conclusion: For BRCA1 mutation carriers the discrepancy between results of prospective and retrospective analyses may be explained by time since last OCP use before FFTP. Thus, a temporal increased risk of breast cancer following longer duration of OCP use before FFTP cannot be ruled out. The discordant findings between prospective and retrospective analyses for BRCA2 carriers could not be explained. Because of the lack of scientific clarity it is too early to give an unequivocal advice on OCP use with respect to breast cancer risk to BRCA1 and BRCA2 mutation carriers.

**#4277 Clinicopathological significance of endometrial cancer with MSH2 deficiency.** Junko Haraga, Takeshi Nagasaka, Keiichiro Nakamura, Tomoko Haruma, Takeshi Nishida, Akihito Nyuya, Kazuya Yasui, Hisashi Masuyama, Yoshiyoshi Fujiwara, Yuji Hiramatsu. Okayama University Graduate School of Medicine Dentistry and Pharmaceutical Sciences, Okayama City, Okayama, Japan.

Background: Endometrial cancer accounts for the second percentage of Lynch syndrome related tumors following colorectal cancer. Inactivation of MSH2 was frequently observed in endometrial cancer with microsatellite instability (MSI) or mismatch repair complex deficiency (dMMR). With respect to MSH2 deficiency (dMSH2), not like MLH1 deficiency, most of dMSH2 were caused by germline mutations in the MSH2 gene or germline EpCAM deletions. Meanwhile, heritable germline epimutations in MSH2 have been reported in a few Lynch syndrome families that lacked germline mutations in the MSH2 gene. We previously provided evidence for frequent MSH2 hypermethylation in Lynch syndrome colorectal tumors with dMSH2 and MSH2 methylation may serve as the “second hit” at the wild-type allele. Materials and Methods: In the study, we analyzed MSH2 promoter hypermethylation status and methylation status, and expression status of the mismatch repair proteins (MLH1, MSH2, PMS2, and MSH6) by immunohistochemistry in a cohort of 138 endometrial cancer tissues surgically resected at Okayama University Hospital. DNA was extracted from formalin-fixed, paraffin-embedded tissue, analyzed MSI status by four mononucleotide markers and both MLH1 and MSH2 promoter methylation status by a fluorescent quantitative bisulfite PCR assay. Results: Endometrial cancers displaying MSI or dMMR were observed in 40 (29.0%) or 41 cases (29.7%), respectively. Endometrial cancers with MSH2 deficiency were observed in eight (5.8%) of 138 tumors (19.5% of dMSH2). MSH2 promoter methylation was present in 7 cases (5.1% in 138 tumors), and significantly cor-
related with dMSH2 (P = 0.0041, Fisher’s exact probability test). Then, we also examined the family history of first-degree relatives retrospectively. In this cohort, although patients with MMR deficiency were significantly associated with family history of Lynch syndrome related tumor (P < 0.001), patients with this family history of Lynch syndrome related tumor are more frequently observed in patients with prostate cancer (P = 0.0347). Intestinal polyposis syndromes associated with familial history of Lynch syndrome related tumor (P = 0.002), though patients with MLH1 methylation were not (P = 0.0878). Conclusions: In this study, we demonstrated that MSH2 methylation significantly correlated with dMSH2 and may have strong relation with family history of Lynch syndrome related tumor, taking a role as “second hit” to the MSH2 gene.

#4278 Screening for BRCA1 founder mutation in Mexican population: an accessible approach. Rosa M. Alvarez, Jose Velazquez, Verónica Fragoso, Julieta Domínguez, Paulina Nuñez, Talía Wegman, Yuliana Sanchez, Silvia Vidal, Enrique Bargallo, Dolores Gallardo, Abelardo Meneses, Luis A. Herrera, National Cancer Institute, Mexico, Mexico City, Mexico; National Institute of Pediatrics, Mexico City, Mexico.

Aim: To implement an accessible strategy for detection of the Mexican founder mutation NG_005905.2:g.118973_133629del, located in BRCA1, which deletes exons 9-12 (BRCA1 ex9-12del), as part of the health care setting of patients with hereditary breast and ovarian cancer (HBOC), from the National Cancer Institute (Mexico). Material and methods: We designed a polymerase chain reaction (PCR) based method, for the targeted detection of the deletion of exons 9 through 12, of the BRCA1 gene. The method was validated previ- ously verified results of the deletion by multiplex ligation-dependent probe amplification assay (MLPA), or deep sequencing of the region. Finally, the technique was used for the detection of BRCA1 ex9-12del mutation in a group of 302 HBOC patients, in whom the existence of BRCA1 and BRCA2 point mutations had been ruled by pyrosequencing. Results: The concordance of our method, with previously reported BRCA1 ex9-12del mutation results, was 100%. Also, we provided a profile of the clinical and familial characteristics of 302 Mexican patients with clinical suspicion of HBOC, in whom the detection of BRCA1 ex9-12del mutation was performed. In this way, 21 patients (6.95%) were identified as BRCA1 ex9-12del mutation carriers. Among whom, a higher proportion of triple negative breast cancer was found (73% versus 21%, p = 0.0005; two sided Fisher’s exact test). Conclusions: A feasible molecular diagnostic strategy for the detection of BRCA1 ex9-12del mutation was established in Mexican patients, with HBOC. In this way, we identified 21 (21/302) carriers of the dele- tion, who represented the 6.95%. Related to the clinical features, triple negative breast cancer carriers stood out. It had been reported that large genomic rearrangements in BRCA1 are associated with an increased frequency of high risk features. This type of approach is appropriate in populations where a founder effect is recognized. For its simplicity and effectiveness, it can be transferred to other laboratories, for the benefit of a greater number of Mexican patients and with the aim of minimize costs of testing. Because of the frequency of BRCA1 ex9-12del mutation, this method has been implemented as the first line of BRCA1 clinical genetic analysis in patients with suspected HBOC, at National Cancer Institute (Mexico).


Background: Familial PDAC constitutes of 5-10% of all PDAC, and germline mutations in BRCA1 and BRCA2 are associated with increased risk of PDAC. Although previous studies showed the prevalence of BRCA1 and BRCA2 germline mutations in patients with PDAC ranged between 3% and 21%, these data have been mostly based on the Western patient population. Considering ethnic differences in the prevalence of the mutations in these genes, we performed prospective evaluation to analyze the prevalence of BRCA1 and BRCA2 muta- tions in PDAC patients with Asian ethnicity. Methods: Between November 2015 and May 2016, all consecutive PDAC patients with locally advanced unresect- able or metastatic disease who were referred for systemic chemotherapy in Asan Medical Center, Seoul, Korea were included. Mutation analysis for germline BRCA1 and BRCA2 were performed for patient with one or more first- or sec- ond-degree relatives with breast or ovarian cancers, or past medical history of these diseases. Germline DNA was extracted from 10 cc of whole blood and analyzed for 23 coding exons of BRCA1 and 26 coding exons of BRCA2 using Sanger sequencing. Results: A total of 175 potential participants with locally advanced unresectable or metastatic PDAC were identified during the study period. Among them, 10 (5.7%) patients met the criteria for further analysis of germline BRCA1 and BRCA2 mutations. Six (60%) patients had family history of breast or ovarian cancers and four (40%) patients had previous medical history of breast cancer. The median age was 60 years (range 49-72) and 5 patients (50%) were male. Pathogenic germline BRCA2 mutation (7480C>T) was iden- tified in one male patient (10%) with family history of breast cancer. Two pa- tients had germline BRCA2 mutations (687>T>A and 1744A>C), which were regarded as the variants of uncertain significance (VUS). Conclusion: Germline BRCA mutations, potentially clinically meaningful, were identified in 1.7% (3/ 175) of overall patients with locally advanced or metastatic PDAC and 30% (3/10) of patients with past or family history of breast or ovarian cancers. Our results indicate that the prevalence of BRCA mutation in Asian PDAC popula- tion is lower than Western patients. Risk category used in this study (past med- ical history or one more first- or second-degree relatives with breast or ovar- ian cancers) may be the feasible criteria for germline BRCA mutation analysis in PDAC patients with Asian ethnicity.

#4280 FH (rs367543046, chr1:241661227 A/ATTT) heterozygous carrier status does not confer risk to hereditary leiomyomatosis and renal cell cancer (HLRCC) and prostate cancer. Michael F. Walsh, Diana Mandelker, Joseph Vijay, David Musheyne, Jennifer Kennedy, Zoila Stadler, Yelena Kemel, Sabine Topka, Karen Cadoo, Maria Carla, Marc Ladanyi, Mark Robson, Kenneth Ofit, Liying Zhang, Memorial Sloan-Kettering Cancer Center, New York, NY; Sloan Kettering Institute, Memorial Sloan-Kettering Cancer Center, New York, NY.

Fumurrate Hydratase (FH) mutations underpin the autosomal recessive syn- drome Fumarate Hydratase deficiency and the autosomal dominant syndrome Hereditary Leiomyomatous and Renal Cell Carcinoma (HLRCC). The variant rs367543046, a duplication leading to p. Lys477_Asn478inslys, in FH has strong evidence for risk towards fumarate hydratase deficiency when occurring in trans to another germline alteration in the FH gene. In addition, this variant in the heterozy- gous state has been reported in ClinVar to predispose to the autosomal dominant condition HLRCC, given other heterozygous mutations have shown to be patho- genic. Population level data is sparse to determine the variant’s impact in the heterozygous state. Here, we show in a series of 1375 cancer cases the (rs367543046, chr: 241661227 A/ATTT), variant detected in 7 (0.5%) individuals with cancer. One patient with bladder cancer had a history of uterine leiomyomas, but the immuno- histochemistry for 25C was negative, suggesting that the leiomyomas were not associated with HLRCC. None of the other 6 carriers of this variant had any features of HLRCC. Notably, this variant was not detected in any of the patients with renal cancer (n = 178) participating in the study. Since 4/7 prostate cancer patients of Ashkenazi ethnicity harbored this variant, we performed a genetic epidemiology study using 856 Ashkenazi Jewish prostate cancer patients. The allele frequencies were compared with 557 Ashkenazi Jewish non-cancer controls. A fisher’s two sided exact test showed that the variant is not associated with prostate cancer in Jews (P = 1. OR 1.12(CI 95% 0.21-7.25). Our findings suggest that the variant, which is patho- genic for autosomal recessive fumarate hydratase deficiency does not confer patho- genicity in the heterozygous form for Hereditary Leiomyomatous and Renal Cell Carcinoma or prostate cancer.

Ashkenazi Jewish Prostate Cancer Patients vs Ashkenazi Jewish Non-Cancer Patients

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#4281 Identification of putative cancer susceptibility genes through whole exome sequencing of a family presenting hereditary colorectal cancer. Gio- vana T. Torrezan, Anna Smirnova, Felipe C. Silva, Erika M. Santos, Renan Valieris, Jorge E. de Souza, Samuel Aguilar, Benedito M. Rossi, Sandro J. de Souza, Dirce M. Carrao, A. C. Camargo Cancer Center, Sao Paulo, Brazil; Sírio-Libanês Hospital, Sao Paulo, Brazil; Instituto de Bioinformática e Biotec- nologia – Zbió, Sao Paulo, Brazil; Universidade Federal do Rio Grande do Norte, Sao Paulo, Brazil.

Germline mutations in known colorectal cancer (CRC) predisposing genes accounts for less than half of the genetic cause of hereditary CRC cases. The absence of mutations in the remaining patients suggests that other yet unknown CRC predis- posing genes might exist. However, recently studies with large cohorts probably discounts the existence of major unknown genes and it is thought that rare or even family private variants and genes may contribute to familial CRC. Thus, the aim of our study was to identify novel CRC susceptibility genes by whole exome sequencing (WES) of a family presenting hereditary CRC. The studied family fulfilled Amster-
The mean age at presentation was 52 years. Out of the 279 individuals, 206 had ovarian cancer, 36 had breast cancer, 26 had double primary ovarian/breast cancer, and 11 had no cancer. Fifty five individuals had family history of ovarian/breast cancer in first degree relatives. Sixty three patients (22.6%) tested positive for a BRCA mutation (45 BRCA1, 18 BRCA2). The mean BRCAPRO and MYRIAD scores were significantly higher for patients who tested positive for a BRCA mutation (30.4% vs. 6.3%, P < 0.001, 12.1% vs. 7.7%, P < 0.001, respectively). The area under the receiver operating characteristics curves were 0.762, and 0.751 for all patients for the BRCAPRO and MYRIAD score to predict the risk of carrying a BRCA mutation. Conclusion: BRCAPRO and MYRIAD appear to be valid risk assessment tools for determining the risk of carrying a BRCA mutation in Korean ethnicity.

#4284 Gate keeping role for endometrial cancer about Lynch syndrome by surgeon.

Min Kyu Kim,1 Min Jung Kwon,2 Min Jeong Yun3. 1Sungkyunkwan University of Medicine, Samsung Changwon Hospital, Changwon-Si, Republic of Korea; 2Sungkyunkwan University of Medicine, Samsung Medical Center, Seoul, Republic of Korea.

Background: It is well known that Lynch syndrome increases risk of endometrial and colorectal cancer. There is not much study about detecting Lynch syndrome patient fast among Korean population by gynecologic oncology surgery. We undertook this study to investigate this. Methods: A review of endometrial cancer patients who was counseled about Lynch syndrome in Department of Obstetrics and gynecology at Samsung Changwon Hospital by single surgeon was done. Clinical information was extracted from the medical record including age, family and personal history of cancer, immunohistochemistry (IHC), microsatellite instability test (MSI), and gene sequencing results. Risk management and posttest education after result were offered about risk reducing options and cascade testing for affected individual Results: App patient was 16. There were two germline mutations (both MSIH) (c.253C>T (p.Thr84Met), c.187delG (p.Val63*). Both were negative for MSIH IHC, but no patient matched criteria of Amsterdam. Four variation of unknown significance (VUS) was found (Three MSIH and One MLH1). Among those were all abnormal in IHC and there was only one Amsterdam criteria matched patient. There were two unstable MSI patients, one was MSIH germline mutation and the other was MSH2 VUS. Median age was 57 (41-76). Seventy five percent were stage I (12/16). Conclusion: Two MSIH germline mutation patients among this population was found. Gynecologic oncology surgeon can be adapted to develop the ability to assess and evaluate genetic risk among endometrial cancer.

#4285 Impact of subsidies on cancer genetic testing uptake in Singapore.

Shao Tzu Li, Jeanette Yuen, Ke Zhou, JOANNE NGEOW. National Cancer Centre, SINGAPORE, Singapore.

Purpose: Previous reports cite high costs of clinical cancer genetic testing as main barriers to patient’s willingness to test. We report findings of a pilot study that evaluates how different subsidy schemes impact genetic testing uptake and total cost of cancer management. Methods: We included all patients who attended the Cancer Genetics Service at the National Cancer Centre Singapore (January 2014-May 2016). Two subsidy schemes, the blanket scheme (100% subsidy to eligible patients), and the varied scheme (patients received 50%-100% subsidy dependent on financial status) were compared. We estimated total spending on cancer management from government’s perspective using a decision model. Results: 445 patients were included. Contrasting against the blanket scheme, the varied scheme observed a higher attendance of patients (34 vs 8 patients per month), of which a higher proportion underwent genetic testing (5% vs 38%), while lowering subsidy spending per person (S$1098 vs S$1161). The varied scheme may potentially save cost by reducing unnecessary cancer surveillance when first-degree relatives uptake rate is above 36%. Findings: Provision of subsidy leads to a considerable increase in genetic testing uptake rate. From the government’s perspective, subsidising genetic testing may potentially reduce total costs on cancer management. Understanding and overcoming barriers for cascade testing in first degree relatives can improve cost-savings.

#4286 What have we learned from pancreatic cancer patients undergoing multigene panel testing.

Holly LaDuca,1 Chunling Hu,2 Hermela Shimelis,2 Eric Polley,3 Jenna Liliquist,2 Mary Helen Black,4 Brigitte Tippin Davis,5 David G. Goldie6 Debra Peck,1 Keri Parcel,7 Fereus J. Cooney,8 Ambra Benetti,9 Also Viejo, CA; 1Mayo Clinic, Rochester, MN; 2University of Utah, Salt Lake, UT.

Purpose: The relevance of inherited pathogenic variants in cancer predisposition genes to pancreatic cancer (PC) is not well understood. Several small studies have identified pathogenic variants in 4% to 14% of unselected PC pa-
patients using multigene panels of predisposition genes, but only BRCA2, ATM, and PALB2 have been clearly implicated in this disease. We aimed to assess the clinical and molecular characteristics of PC patients referred for hereditary cancer genetic testing, and to estimate the risk of PC associated with pathogenic variants in panel-based cancer predisposition genes. Methods: PC patients (n=3,189) were ascertained from a large cohort of over 14,000 patients undergoing multigene panel testing (MGPT) of predisposition genes between March 2012 and June 2016 at a single diagnostic laboratory. Clinical histories and molecular results were reviewed and summarized. Gene-level variant frequencies among PC cases were compared to those from the Exome Aggregation Consortium (ExAC) to calculate gene-specific pancreatic cancer risk ratios. Results: PC patients were predominantly Caucasian (76.5%) and female (58.9%), with a median age at diagnosis of 61 years (51.7). Of these, 33.5% reported additional cancer primaries, and 44.8% reported a family history of PC. Overall, 15.4% of PC patients were found to have at least one pathogenic/likely pathogenic variant in panel-based predisposition genes. Genes with the highest frequencies of pathogenic/likely pathogenic variants included BRCA2 (3.9%), ATM (3.6%), CHEK2 (excluding p.Ile157Thr) (2.0%), PALB2 (1.5%), VHL (1.4%), CDKN2A (1.2%), BRCA1 (0.8%), and MSH6 (0.8%). 21.8% of BRCA1 and BRCA2 carriers did not meet BRCA1/2 testing criteria and 61.5% of MSH6 carriers did not meet Lynch syndrome testing criteria. No CDKN2A families met diagnostic criteria for familial atypical multiple mole melanoma syndrome, and 44% did not report any personal or family history of melanoma. To estimate associations between pathogenic/likely pathogenic cancer predisposition genes and PC, case ascertainment was compared to non-Finnish European, non-TGCA ExAC reference controls. Pathogenic variants in ATM, BRCA2, CDKN2A, MSH6, and PALB2 were significantly associated with high PC risks. Pathogenic variants in BRCA1 were associated with a moderate risk of PC (RR = 2.7). Conclusions: These findings shed light on the spectrum of mutations that can be expected for PC patients referred for cancer predisposition testing. The results confirm the associations of CDKN2A and BRCA2 variants with PC, and expand on the phenotypic spectrum associated with these variants. Furthermore, these results suggest that ATM, PALB2, and MSH6 may be high-risk PC genes, warranting further investigation in case-control and family-based studies.

#4288 HABP2 p.G534E variant in patients with family history of thyroid and breast cancer: Maisa Pinheiro,1 Sandra A. Drigo,1 Fabio A. Marchi,2 Renatta Tonhosolo,2 Sonia C. Andrade,3 Igor Jurisica,4 Luiz P. Kowalski,2 Maria Isabel CNPq(481132/2012-0).

Background. Familial Papillary Thyroid Carcinoma (PTC) has been recently associated with the HABP2 p.G534E mutation. In this study we evaluated the putative association of the HABP2 p.G534E mutation and familial history of PTC and Breast Carcinoma (BC) in the Brazilian population. Methods. Germline mutations of twenty unrelated individuals with personal and/or family history of PTC and/or BC were identified by whole exome sequencing (WES), using Nextera Exome Enrichment kit and HiSeq2000 platform (Illumina). Two cases were positive for p.G534E. Family members from both positive cases (seven from Family 1 and three from Family 2) were recruited for segregation analysis. An additional patient and three relatives (Family 3) with history of PTC and BC were included for validation. Fifty frozen PTC tumor samples and 170 healthy Brazilian individuals were also screened for the HABP2 variant. Confirmation and validation were performed by Sanger sequencing. Results. Interestingly, Family 1 reported a consanguineous history, reflected in two homozygous cases: the proband (PTC) and his unaffected sister. Also, only one (BC) out of eight tested members was negative for p.G534E. In the Family 2, the only patient diagnosed with PTC was negative for the mutation, while three relatives were positive for the same loci. Both index cases (PTC and BC) bore the wildtype for HABP2 and three relatives tested were positive carriers. In PTC tumor samples the Allele Frequency (AF) was 0.04: two heterozygous and one homozygous for p.G534E. Among 170 healthy individuals, five were heterozygous for the variant, representing an AF of 0.0147. The two index patients evaluated by WES also presented interesting pathogenic variants in genes potentially associated with deregulation of the extracellular matrix organization pathway (CTSB, TNXB, COL4A3, COL16A1, COL24A1, COL5A2, NID1, LOXL2, MMP11, TRIM24 and MUSK) and DNA repair function (NBN and MSH2). Conclusion. Our findings suggest that HABP2 is not a predisposition gene involved in familial PTC and BC. Financial Support: FAPESP (2013/01867-8 and 2014/03983-8) and CNPq (481132/2012-0).

#4289 Whole-exome sequencing study of neurovial basal cell carcinoma syndrome (NBCCS) families to identify disease-causing exonic mutations: Mathew Gianferante,1 Alisa Goldstein,1 Mingyi Wang,2 Bin Zhu,2 Belynda Hicks,2 NCI DCEG Cancer Genomics Research Laboratory, Lisa Mirabello1. 1National Cancer Institute, Rockville, MD; 2Cancer Genomics Research Laboratory, Frederick, MD.

NBCCS is an autosomal Dominant disorder with nearly complete penetrance and variable expression. Clinical findings include multiple basal cell carcinomas, keratocysts of the jaw, pits of the palms and/or soles, and more rarely medulloblastoma. The PTCH1 gene is the major NBCCS susceptibility gene; it regulates the sonic hedgehog (SHH) pathway. Here, we are evaluating mutations in unselected NBCCS families to identify potential disease-causing mutations. Our study included specimens and clinical data from 21 NBCCS families. PTCH1 targeted sequencing and CGH array were previously performed. We subsequently whole-exome sequenced (WES) affected family members with no mutation detected from prior targeted analyses (N=9). We focused our analyses on rare variants of PTCH1, genes that directly interact with PTCH1, SHH pathway genes, and cancer predisposing genes. Variants were considered rare if minor allele frequency (MAF) was less than 1% in publicly available databases (ESP, ExAC, and 1000 genome). In-house population cancer-free controls, and an in-house database of approximately 2,000 familial samples that underwent WES in parallel with our NBCCS families. We used in-silico prediction models, variant impact on the protein (i.e., nonsynonymous, nonsense, frameshift, splice site), and public mutation databases to assess pathogenicity. Rare variants in PTCH1 were considered pathogenic if they were (a) previously reported as disease-causing in NBCCS, or (b) predicted high impact (i.e., frameshift, nonsense mutation), or (c) predicted damaging by at least five of seven in-silico prediction programs (CADD, PolyPhen 2, SIFT, Assessor, Mutation Taster, IRT, FATHMM). Ten families (N= 17 individuals) without a PTCH1 mutation were assessed with WES. WES identified PTCH1 mutations in six families. Overall,
only one PTCH1 variant was previously reported (p.S732fs) in HGMD or ClinVar databases. Six novel variants were identified: three frameshifts, p.Y92fs, p.S732fs and p.T212fs; one nonsense, p.W862*; one non-synonymous variant, p.E835K; and, one splice site variant, c.2716-2A>C. The non-synonymous variant was a heterozygous C to T transversion (chromosome 9, position 98 229 452) and predicted damaging. In the four families without a PTCH1 mutation, two separate families had a rare nonsynonymous variant in the same gene that directly interacts with PTCH1. Both variants had a MAF of less than 0.1%, but only one was predicted damaging. Another family had a rare nonsynonymous variant in a known lung cancer predisposing gene that was deleterious in five of seven silico prediction tools and found in both the proband and affected mother. Overall, in the 93 families, 81% had PTCH1 mutations detected with targeted sequencing, aCGH, or WES. Additionally, WES detected two potentially new disease-associated genes that we are currently evaluating further.

#4290 A study in locus heterogeneity: Targeted sequencing analysis of 6q reveals multiple significant loci as the source of a previous linkage peak in familial lung cancer. Anthony M. Musolf,1 Claire L. Simpson,2 Bilal A. Moiz,3 Candace Middlebrook,3 Mariza de Andrade,4 Diptasi Mandal,5 Colette Gaba,5 Ping Yang,3 Yafang Li,5 Ming You,5 Elena Y. Kupert,5 Marshall W. Anderson,5 An G. Schwartz,6 Susan M. Pinney,6 Christopher I. Amos,6 Joan E. Bailey-Wilson,6 1NIH NHGRI, Baltimore, MD; 2University of Tennessee Health Science Center, Memphis, TN; 3Mayo Clinic, Rochester, MN; 4Louisiana State University Health Sciences Center, New Orleans, LA; 5University of Toledo Dana Cancer Center, Toledo, OH; 6Dartmouth College, Lebanon, NH; 7Medical College of Wisconsin, Milwaukee, WI. Cancer Research Institute, Dana-Farber Cancer Institute, Boston, MA; 8University of Cincinnati Cancer Center, Cincinnati, OH; 9University of Cincinnati College of Medicine, Cincinnati, OH.

Lung cancer is the leading cancer-related cause of death in the United States. We had previously performed multipoint linkage analysis on families with a strong family history of lung cancer and found significant linkage to the 6q25 region. In order to find the source of that linkage signal, we performed targeted sequencing 6q23-6q27 on 75 individuals from the 9 most highly linked families. We performed two types of parametric linkage analysis, using an autosomal dominant mode of inheritance with 10% penetrance for carriers and a 1% phenocopy rate using a disease allele frequency of 1%. The first type was a two-point analysis using an Elston-Stewart algorithm. This approach did not lead to any genome-wide significant results but demonstrated significant heterogeneity throughout the nine families. In an effort to recover the power from the original multipoint analysis, we performed a regional linkage analysis using SEQlinkage and MERLIN. SEQlinkage built regional haplotypes that corresponded to a gene or an intergenic region - the regions were based on a customized map of our design. The regional markers were multiallelic which allowed for greater information content and were similar to the microsatellites that were used in the original multipoint analysis. The regional markers were then analyzed in a two-point linkage analysis via MERLIN. This allowed us to identify two genome-wide significant signals at PACRG-AS1 at 6q26 (HLOD = 3.4) and SAMD5 at 6q24 (3.3). The PACRG-AS1 is novel, though it is associated with the known lung cancer gene PARK2. SAMD5 may be involved in lung cancer cell proliferation. The heterogeneity of the two signals was particularly interesting. The PACRG-AS1 region was driven primarily by two families. The SAMD5 marker was not being strongly driven by any of the families and appears to be a small cumulative effect across the nine families. In addition, several families have large, but non-significant LOD scores at other loci across the region. This further reinforces the locus heterogeneity within the region, and it is likely that our two significant signals here are not the only variants affecting the phenotype. We further attempted to localize the signals by running SEQLinkage using a custom map where genes are broken into exons and introns. Though this resulted in no significant markers, the highest signal was located in the intronic region of SAMD5 (HLOD = 3) and several other suggestive signals were localized to intronic regions of good candidate genes like SASH1 and ARID1B. Examination of predicted effects of the candidate regulatory variants using eQTL databases is ongoing. This is a region full of promising candidates, and it is likely that the two significant signals found here are just part of many that could be affecting lung cancer risk. We plan to do further analysis within the individual families to elucidate any more genes affecting this signal.


Introduction: The purpose of this study was to investigate potential associations between body composition and current smoking in young healthy women from high-risk families. Cigarette smoke contains >7000 chemicals of which 69 are established carcinogens and smoke also acts as an aromatase inhibitor. Smoking is now recognized as a carcinogen for the breast and influences both risk and prognosis. However, the underlying mechanisms need to be better elucidated. One study showed that breast cancer patients who smoked were younger, had a lower body mass index (BMI), smaller breast volumes, but a higher waist-to-hip ratio (WHR) than non-smokers. However, smoking was also associated with prior oral contraceptive (OC) use. Since breast cancer development starts long before the tumor is clinically detectable, we aimed to study the impact of cigarette smoke on anthropometric factors. Material and methods: Between 1996 and 2006, 269 healthy women were included in a study on the impact of lifestyle factors in women <40 years from high-risk breast cancer families. Thirty-six participants who had missing height or with prior OC use were excluded. Overall, in 21 families, 81% had undergone previous breast surgeries, who were currently breast-feeding or who were current users of hormonal contraceptives other than combined E + P OCs or had missing smoking status were excluded, leaving 233 women of which 35 were known BRCA1/2 mutation carriers. Height, weight, waist- and hip circumference, and total breast volume were measured in a standardized way by a research nurse 5-10 days before predicted onset of the next menstrual period. Weight, BMI, breast volume, and waist circumference, were not normally distributed and were transformed using the normal logarithm to obtain a better distribution. Multivariable linear regression was used to obtain P-values, adjusted for age, nulliparity, and current OC use. Means and geometric means for smokers and non-smokers were standardized at age 29 years, nulliparity, and non-current OC use. Results: The median age was 29 years (IQR 24-35) and the median year of birth was 1970. Forty-two percent had ever smoked, 23% were current smokers, 28% were current OC users, and 54% were nulliparous. Current smoking was not associated with height, BMI breast volume, or hip circumference (all \( \beta \)s > 0.18). However, current smokers had significantly larger standardized waist circumference (78 vs 74 cm; \( \beta \) = 0.02), and higher standardized WHR (0.79 vs 0.76; \( \beta \) = 0.003) compared with non-smokers. Conclusion: Current smokers had significantly larger waist circumference and higher WHR, but similar BMI and breast volume compared with non-smokers, although most women had anthropometric measures within WHO’s recommended limits. The difference in fat distribution towards more abdominal fat, suggests that current smoking is associated with a more inflammatory and/or androgenic profile at the age when breast cancer is initiated.

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#4292 Indole-3-carbinol (I3C) enhances efficacy of Gemcitabine in leiomyosarcoma. Sujit Suwal, Alexandra Moran, Ana Paz-Mejia, Lorena Flor, Omar Picado, Basem Azab, Jonathan Trent, Fiorella Pendola, Alan S. Livingstone, Danny Yakoub. University of Miami-Miller School of Medicine, Miami, FL.

Introduction: Adjuvant Gemcitabine have recently been shown to improve progression free survival in some types of leiomyosarcoma. Adequate cellular uptake of the drug remains a challenge. We aimed to evaluate the role of nucleoside transporter activator (I3C) in increasing the in vitro efficacy of gemcitabine in combination with non-smokers. Conclusion: Current smokers had significantly larger waist circumference and higher WHR, similar BMI and breast volume compared with non-smokers, although most women had anthropometric measures within WHO’s recommended limits. The difference in fat distribution towards more abdominal fat, suggests that current smoking is associated with a more inflammatory and/or androgenic profile at the age when breast cancer is initiated.
Siraimesine and lapatinib induce ferroptosis in glioblastoma and lung adenocarcinoma cells. Anna R. Blankstein,1 Shumei Ma,2 Spencer B. Gibson1.

Ferroptosis, a morphologically and biochemically distinct cell death pathway, is characterized by the production of reactive oxygen species (ROS) within the cell. The combination of siraimesine, a lysosome disruptor, and lapatinib, a dual tyrosine kinase inhibitor, has been shown to synergistically induce cell death in breast cancer cells. This cell death was blocked by the ferroptosis inhibitor ferrostatin-1 (Fer-1) and the iron chelator deferoxamine (DFO). The objective of the present study was to determine whether lysosome disruptor and tyrosine kinase inhibitors, in combination, induced synergic cell death via the ferroptotic pathway in additional types of cancer. U87 (glioblastoma) and A549 (lung adenocarcinoma) cells were treated with various lysosome disruptors (siraimesine or desipramine) in combination with tyrosine kinase inhibitors (lapatinib or sorafenib), and the amount of cell death was measured by trypan blue exclusion. We found that these combinations synergistically induced cell death in U87 and A549 cells. To determine whether ferroptosis was the mechanism of cell death, cells were pretreated with either Fer-1 or DFO (inhibitors of ferroptosis), or with exogenous iron chloride (an inducer of ferroptosis) before treatment with the combination of siraimesine and lapatinib. Pretreatment with Fer-1 or DFO decreased cell death by approximately 35%. Pretreatment with iron chloride increased the effect of the drug combination. Targeting DPD2 and DPD5 by interfering one of the therapeutic strategies was an increase in intracellular iron accumulation following treatment with the combination of siraimesine and lapatinib. Collectively, these data show that in U87 and A549 cells, the combination of lysosome disruptors and tyrosine kinase inhibitors, specifically siraimesine and lapatinib, induces ferroptotic cell death. Therefore, inducing ferroptosis in tumor cells is a potential strategy for therapy in these cancers with limited treatment options.

Preclinical evaluation of imipridone ONC201 in triple negative breast cancer identifies predictive biomarkers and combinatorial opportunities. Bora Lim, Jangsoo Lee, Huey Liu, Troy Pearson, Larry Coffer, Debu Tripathy, Naoto T. Ueno. UT MD Anderson Cancer Ctr., Houston, TX.

Background: Triple negative breast cancer (TNBC) lacks effective targeted therapy, leaving chemotherapy as the only therapeutic option. Metastatic TNBCs eventually develop resistance to chemotherapy via several mechanisms including apoptosis evasion, hence representing an urgent unmet need for novel therapies. ONC201 is a first-in-class imipridone that induces caspase-mediated apoptosis in multiple cancers including TNBC, selectively antagonizing dopamine D2-like receptors (DRD2) resulting in inhibition of Ras signaling and cell death. Selective antagonism of D2-like receptors has been shown to induce cell death in cancer cells via the same signaling pathways engaged by ONC201. To translate these early findings for breast cancers patients, more studies to prove death. Selective antagonism of D2-like receptors has been shown to induce cell death in breast cancer cells. This cell death was blocked by the ferroptosis inhibitor ferrostatin-1 (Fer-1) and the iron chelator deferoxamine (DFO). The objective of the present study was to determine whether lysosome disruptor and tyrosine kinase inhibitors, in combination, induced synergic cell death via the ferroptotic pathway in additional types of cancer. U87 (glioblastoma) and A549 (lung adenocarcinoma) cells were treated with various lysosome disruptors (siraimesine or desipramine) in combination with tyrosine kinase inhibitors (lapatinib or sorafenib), and the amount of cell death was measured by trypan blue exclusion. We found that these combinations synergistically induced cell death in U87 and A549 cells. To determine whether ferroptosis was the mechanism of cell death, cells were pretreated with either Fer-1 or DFO (inhibitors of ferroptosis), or with exogenous iron chloride (an inducer of ferroptosis) before treatment with the combination of siraimesine and lapatinib. Pretreatment with Fer-1 or DFO decreased cell death by approximately 35%. Pretreatment with iron chloride increased the effect of the drug combination. Targeting DPD2 and DPD5 by interfering one of the therapeutic strategies was an increase in intracellular iron accumulation following treatment with the combination of siraimesine and lapatinib. Collectively, these data show that in U87 and A549 cells, the combination of lysosome disruptors and tyrosine kinase inhibitors, specifically siraimesine and lapatinib, induces ferroptotic cell death. Therefore, inducing ferroptosis in tumor cells is a potential strategy for therapy in these cancers with limited treatment options.

AZ'5576, a selective CDK9 inhibitor, demonstrates in vitro and in vivo activity in diverse preclinical models of non-Hodgkin lymphoma. Justin Cidado,1 Theresa Proia,1 Gareth Gregory,2 Izabela Todorovski,3 Scott Boiko,3 Maryann San Martin,5 Ricky Johnstone,1 Lisa Drew1. AstraZeneca, Weltham, MA;2Peter MacCallum Cancer Centre, Victoria, Australia.

Cyclin-dependent kinase 9 (CD9) is a serine/threonine kinase that regulates elongation of transcription through phosphorylation of RNA polymerase II at serine 2 (pSer2-RNAPII). Transient inhibition of CDK9 results in modulation of genes with short-lived transcripts and labile proteins, thereby representing a potential therapeutic opportunity in tumors dependent upon oncogenes fitting these criteria. McII, an anti-apoptotic protein that has been linked to increased cell survival and chemotherapy resistance in various cancers, is a proto-oncogenic transcription factor that coordinates diverse transcription programs and is over-expressed, amplified, or translocated in many cancers, are two such oncogenes. AZ'5576 is a potent, highly selective, and orally bioavailable inhibitor of CD9 that inhibits CDK9 enzyme activity with an IC50 <5 nM and decreases phosphorylation of Ser2-RNAPII in cells with an IgG of 96 nM. In sensitive cell lines, short-term treatment with AZ'5576 led to a rapid dose- and time-dependent decrease in pSer2-RNAPII with concomitant loss of McII and Mcy mRNA and protein, resulting in the cleavage and activation of caspase 3 and loss of cell viability. Using cell potency and magnitude of caspase activation to define sensitivity, we previously reported that AZ'5576 demonstrates broad in vitro and in vivo activity across hematological malignancies. Initially focusing on acute myeloid leukemia, we expanded our preclinical model to encompass non-Hodgkin lymphoma (NHL), which includes diffuse large B-cell lymphoma (DLBCL), Burkitt's lymphoma (BL), mantle cell lymphoma (MCL), and Waldenstrom macroglobulinemia (WM). In vitro screening assays reveal about half (19/35) of NHL cell lines undergo cell death in response to AZ'5576, demonstrating a potency <50 nM and a maximum caspase activation >50% after 6 hour treatment. Combining AZ'5576 with a BTK inhibitor further enhances cell death compared to that observed with either single agent alone in BTK inhibitor-sensitive ABC-DLBCL cell lines. Consistent with the in vitro data, significant anti-tumor activity was associated with short-term reduction of pSer2-RNAPII tumor levels after intermittent dosing of AZ'5576 in a mouse xenograft model of ABC-DLBCL cell line OCIY10. Similarly, combining AZ'5576 with a BTK inhibitor in vivo drove tumors into regression (combination =199% TGI) compared to the partial TGI from either single agent alone (AZ'5576=79% TGI, Acalabrutinib=58% TGI) in the OCILY10 xenograft model. Finally, in an aggressive Eμ-Myc transgenic mouse model of B-cell lymphoma, AZ'5576 treatment results in potent induction of apoptosis in vitro and a greater than 50 day increase in median overall survival in vivo. Together, these results highlight the therapeutic potential for selective CDK9 inhibition in the treatment of patients with non-Hodgkin lymphoma.

A real-time annexin V annexin V framework for monitoring programmed cell death. Kevin Kupcho,1 Andrew Niles,1 John Shultz,1 Jamison Grauler,1 Wenhui Zhou,1 Robin Hurst,1 Jim Hartnett,2 Terry Russ,2 Dan Lazar,1 James Cali1. Promega Corporation, Madison, WI;2Promega Biosciences LLC, San Luis Obispo, CA.

Efficacious and durable anti-cancer responses are driven by both the selective death of malignant cells and the induction of immunostimulatory activities. Limited but promising clinical evidence suggests that provocation of an unknown balance of inflammatory- and non-inflammatory cell death may be key for orchestrating these positive outcomes. Therefore, identifying and characterizing new clinically useful small molecules and biologic inducers (or combinations thereof) which promote a spectrum of programmed cell death in vitro screening environments remains critically important. Unfortunately, current screening methods are either insufficiently robust, cost- or resource-prohibitive, or provide no means for initial characterization of the kinetics of programmed cell death. To address this unmet need, we developed a real-time, live cell assay method that utilizes a fully homogeneous, bioluminescent annexin V reagent. The method does not require laborious washing and sample preparation steps associated with conventional assays and is compatible with plate-based multimodal signal detection systems. The system contains two annexin proteins which have been engineered to contain separate and distinct complementing domains of a binary luciferase. Additionally, the system contains a novel time-released luciferase substrate and a cell impermeable, fluorogenic DNA dye for monitoring necrosis. Because the annexin-luciferase fusion pairs have only modest affinity for each other, luminescence remains low until phosphatidylserine exposure, a hallmark of the programmed cell death phenotype, brings annexin monomers into close proximity facilitating complementation of the luciferase sensor. The assay reagent can be applied at dosing for real-time measurement of the dose-dependency and magnitude of programmed cell death.

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progression. This work describes our efforts to characterize and validate the performance of the bioluminescent annexin assay using relevant cell death induction models. First, the assay was shown to be functionally concordant with a flow cytometry annexin method in a dose-response model with bortezomib at exposure periods known to produce both early and late-apoptosis (with necrosis) phenotypes. Next, we assessed the performance of the assay using a limited training set of small molecule and biologic inducers of programmed cell death that utilize different mechanisms of action (i.e., apoptosis and necroptosis) and by using a number of diverse but representative cancer cell lines. We conclude that the bioluminescent annexin method provides a new kinetic approach to efficient and effective detection of programmed cell death mechanisms in real time in a convenient homogeneous format.

#4297 ADCs inhibiting the all-powerful omotic transducer. James R. Prud- dent,1 David Marshall,1 Fabio Malavasi,2 Antonella Chillemi.2 1Centro Llse, Madison, WI; 2University of Torino Medical School, Torino, Italy.

Purpose: Extracellular drug conjugates (or ADCs) are a new type of ADC that selectively inhibit the all-powerful Na,K-ATPase, an extracellular membrane ion pump required for life. To determine if ADCs may be valuable clinical can- didates, we further assessed their safety, efficacy and mode of action. Experimenta- nal Design: Cynomolgus monkeys were infused with ADCs at various levels and monitored by heart telemetry, blood cell levels and serum examination. Addi- tionally, cultured cells were subjected to ADCs and monitored in real time and by high resolution dual fluorescent labeling to better understand the mechanism of cell death. Results: We tested the efficacy of the ADCs in a number of xenografts models and observed strong activity (regression and cures). Little to no tumor reduction was observed for the free antibodies or with control-EDCs where the antibody target was not expressed. To test for safety, each cynomolgus monkey was infused with one of three ADCs to better understand the MTD of each ADC. In all cases, MTD was determined to be between 5 and 10mg/kg where ADC serum levels reached between 100 and 200mg/ml. At the maximum safe dose tested (5mg/kg), heart beat and other observed parameters were found to be normal with the exception of the blood cells expressing the ADC targets (EDC targeting CD20 at this high level was found to drop all B-cells to a nondetectable level which rebounded to normal on day 58). To further under- stand the ADC mechanism, cells exposed to the ADCs and monitored visually in real-time or by dual fluorescence microscopies. All cells exposed to the ADCs showed membrane swelling and extracellular localization of the ADC. Conclu- sion: These results show that targeted inhibitors of the Na,K-ATPase ion pump have promise as future anticancer medicines. First, ADCs appear safe in nonhu- man primates at efficacious doses in rodent xenografts. In addition, an identified mechanism of extracellular necrotic death is novel in the field of oncology. Our hope is that cancers that up-regulate MAPK and/or anti-apoptotic mechanisms will be sensitive to ADCs. Continuing efforts to identify the full death pathway of this new type of ADC should lead to identifying patients most likely to respond to ADC based therapies.

#4298 FTY720 induces necroptosis in lung cancer by modulating cer- amide signaling at the plasma membrane. Rose Nganga. Medical University of South Carolina, Charleston, SC.

Sphingolipids are important signaling molecules in cells and have recently been explored as cancer therapy targets. FTY720 (Fingolimod, Gilena) is an FDA approved sphingosine analogue drug used for the treatment of multiple sclerosis (MS). FTY720 is phosphorylated by sphingosine kinase 2 (SK2), to generate P-FTY720 to exert its immunosuppressive properties through binding to sphingosine-1-phosphate receptors (S1PRs). FTY720 also exhibits anti-cancer properties. Our previous studies indicated that one of the mechanism by which FTY720 induces cell death is through necroptosis. FTY720 directly binds to I2PP2A/SET (Inhibitor of 2 of PP2A), consequently activating the tumor sup- pressor protein phosphatase 2A (PP2A). The activated PP2A then induces cell death by stimulating the activity of Receptor-Interacting Protein kinase-1 (RIPK1), involved in necroptosis signaling. Previous studies have shown that FTY720 can modulate sphingolipids metabolism in cells. However, little is known about the roles of FTY720 in ceramide signaling and regulation of necroptosis in lung cancer. We hereby seek to investigate the mechanisms of FTY720 in inducing necroptosis with regard to ceramide signaling. Preliminary data indicate that inhibitors of ceramide generation partially protect cells against FTY720-induced cell death. Interestingly, FTY720 and non-phosphorylated FTY720 analogues do not affect ceramide generation, but lead to the formation of cancer specific ceramide-multi-protein complexes at the plasma membrane. Our studies show a role of these complexes in inducing plasma membrane rup-
its anti-proliferative effect and apoptosis induction by an up-regulation of TGFβ1, p53 and p21 and a down-regulation of TGF-α and Bcl-2a. Conclusion: Thymoquinone showed antiproliferative and proapoptotic potentials in ATL cells. For this reason, further research is required to investigate its possible application in the treatment of ATL.

#4302 Disulfiram possesses antitumor activity and inhibits cancer stem-like properties via inhibition of STAT3 activation in triple-negative breast cancer cells. Yoon-Jae Kim, Nahyun Lee, Eunhye Oh, Daesung Sung, Tae-Min Cho, Ji Young Kim, Jae Hong Seo. Korea University, Seoul, Republic of Korea.

Disulfiram (DSF), a clinically used treatment for alcohol dependence, is considered as a potential anti-cancer agent due to its cytotoxicity in many cancer cell types on copper (Cu)-dependent processes. However, the molecular mechanisms underlying DSF-induced antitumor activity in triple-negative breast cancer (TNBC) are still poorly understood. Here, we hypothesized that DSF induces antitumor effects by targeting cancer stem cells (CSCs) and STAT3 signaling in TNBC. We then examined the effects of DSF in combination with Cu on the cell viability and apoptosis, cell proliferation and survival, as well as CSC-like properties in MDA-MB-231 and 4T1 TNBC cell lines. DSF/Cu effectively induced cytotoxicity and apoptotic cell death, concomitant with caspase-3 activation and PARP cleavage. DSF/Cu also significantly suppressed cell migration and mammosphere formation, which are accompanied by a remarkable decrease in the CD44+/CD24− (or CD49f+/CD24+ in mouse 4T1 cells) stem-like population and ALDH1 activity. These effects were functionally associated with the inhibition of STAT3 phosphorylation (Tyr705). Furthermore, DSF/Cu treatment resulted in the downregulation of cyclin D1 and survivin expression, which are downstream targets of STAT3 signaling. Interestingly, phosphorylated STAT3 and STAT3 downstream signaling were highly upregulated in the mammospheres, and these upregulated molecules were significantly decreased by DSF/Cu treatment. In MDA-MB-231-derived xenograft model, DSF administration elicited a significant reduction in tumor growth with a noticeable down-regulation of CD44, ALDH1 and phosphorylated STAT3 levels. Taken together, our results suggest that therapeutic treatment to DSF can effectively manifest antitumor activity by reducing CSC-like properties, and that STAT3 could be a key molecule of aggressive TNBC that is targeted by DSF.

#4304 Feedback-phosphorylation of MKK4 by MAPKs promotes apoptosis. Hisashi Morizumi. The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

Feedback-phosphorylation of MKK4 by MAPKs promotes apoptosis Morizumi Hisashi, Takanori Nakamura and Mutsuhiro Takekawa. Div. Cell Signal and Mol. Med. IMS, Tokyo Univ. The mitogen-activated protein kinase (MAPK) kinase pathway is an important system for cellular responses to extracellular stimuli. Each of the MAPK pathways is composed of MAPKKK, MAPKK and MAPK. In mammalian cells, at least three subfamilies of MAPKs, namely ERK, p38 and JNK, are present. The ERK pathway is activated in response to mitogenic stimuli and plays a key role in proliferation and differentiation. In contrast, the p38 and JNK pathways, collectively called stress-activated protein kinase (SAPK) pathways, regulate cellular stress responses such as apoptosis and cell cycle arrest. MKK4 is unique among the members of the mammalian SAPKK family (i.e., MKK3/4/6/7) in its ability to phosphorylate and activate both p38 and JNK. Interestingly, inactivating mutations of the MKK4 gene are frequently found in many types of cancer. Although MKK4 has been suggested as a tumor suppressor, the molecular mechanism by which MKK4 inhibits tumorigenesis remains unclear. Here we demonstrate that MKK4 can be phosphorylated at specific serine and/or threonine residues by downstream MAPKs (named the “feedback-phosphorylation”) and thereby regulates stress-induced apoptotic cell death. By generating antibodies specific to these phosphorylation sites, we found that under stress conditions both Thr and Ser residues of MKK4 were phosphorylated by JNK, while only the Ser residue was phosphorylated by ERK in response to mitogenic stimuli. In order to clarify physiological significance of the feedback-phosphorylation, we then generated a phosphorylation-deficient mutant of MKK4 (MKK4-AA) by substituting both Thr and Ser residues with Ala. Although the feedback-phosphorylation did not affect the enzymatic activity, stability and subcellular localization of MKK4, we found that cells expressing MKK4-AA mutant suppressed apoptosis induced by various stresses stimuli such as UV irradiation and anticancer drug treatment. These results suggest that feedback-phosphorylation of MKK4 regulates stress-induced apoptosis. Moreover, we are currently investigating the mechanism of how MKK4 affects apoptotic cell death in response to feedback-phosphorylation.


PURPOSE: The von Hippel Lindau (VHL)/hypoxia inducible factor-alpha (HIF-α) pathway is commonly dysregulated in clear cell renal cell carcinoma (ccRCC) and potentially exacerbated by oxidative stress. Iron is the most abundant heavy metal in the human body and is required for nephrogenesis in utero; it is also a major source of intracellular oxidative stress capable of inducing ccRCC in rodent models. Recently, iron was shown to stabilize HIF2-α transcript by inhibiting iron regulatory protein 1. Still, a role for iron in human ccRCC remains largely unexplored. We tested the hypotheses that intracellular iron is accumulated in human ccRCC cells and that iron deprivation can suppress HIF2-α expression and ccRCC cell growth. METHODS: Over 1500 core sections from 587 tissues (272 primary tumors, 240 benign kidney, 75 metastases) of 288 RCC patients were stained and scored for levels of iron and the primary iron uptake protein, transferrin receptor 1 (TfR1), using Prussian Blue and immunohistochemistry, respectively. 178 cores from 14 different body tissues of non-cancer patients were stained as controls. Staining levels were tested for association with patient features and outcomes. TfR1 protein expression was further evaluated in 4 ccRCC and 2 benign renal epithelial cell lines by flow cytometry and western blot. Micromolar concentrations of 3 clinical iron chelator drugs, deferoxamine (DFO), deferriprone (DFP), and deferisirox (DFX), were tested by MTT assay for effects on ccRCC versus benign renal cell proliferation. DFO effects on cell viability/apoptosis and HIF2-α levels were measured using annexin-V/7-AAD flow cytometry and western blot, respectively. RESULTS: Intracellular iron was increased in ccRCC tumors compared to benign renal tubule epithelium; although more advanced tumors had smaller increases. Benign renal epithelium expressed higher TfR1 protein levels than any other tissue site in the body tested, and expression in ccRCC tumors increased during progression to metastasis and cancer-specific death. ccRCC cell lines had elevated TfR1 expression relative to benign renal cell lines. Iron chelation using DFO, DFP, or DFX uniformly achieved 80-90% growth reduction in ccRCC cell lines at clinically utilized concentrations, while benign renal cell lines were relatively resistant. The mechanism of ccRCC growth inhibition was primarily cell death via apoptosis, whereas benign renal cells had no increased death even after prolonged iron chelation treatment. Intriguingly, iron chelation in ccRCC cell lines effectively downregulated HIF2-α in a time- and concentra-
tation-dependent manner. CONCLUSIONS: These data indicate that increases in intracellular iron content occur during human ccRCC tumorigenesis and support further investigation of iron chelation as a novel therapeutic strategy for targeting HIF2-α and inducing cancer cell death in ccRCC patients.

#4306 Combined effects of chemotherapy to reduce metastasis caused by insufficient hyperthermia. Tae Hee Lee,1 Jiyoung Ba,2 Byung Hyuck Kim,1 Young Jun Kim,3 Yoon-Tae Kang,3 Jung Eun Moon,1 Young-Ho Cho1. Cell Bench Research Center (SEMCCELL), Daejeon, Republic of Korea; 2KAIST, Daejeon, Republic of Korea; 3Seoul National University College of Medicine, Seoul, Republic of Korea; 4Armed Forces Medical Research Institute, Daejeon, Republic of Korea.

Hyperthermia therapy is one of the most widely studied non-surgical methods for breast tumors, which eliminates tumor by inducing acute stress on tumor cells. However, it has been extensively known that the viability of the tumor cells is highly influenced by the applied temperature. Insufficient thermal stress during hyperthermia treatments may alter tumor microenvironment by promoting epithelial to mesenchymal-like transition (EMT) and as a result, enhancing the outgrowth of residual tumor cells. Therefore, cells that have survived from sublethal thermal stress and experienced EMT may cause substantial clinical problems. In this case, hyperthermia requires additional therapy in order to promote cell death of more invasive tumor cells that have resisted to the thermal stimulus. In this study, we confirmed that the co-treatment of chemotherapy with hyperthermia may overcome the phenotypical transition caused by insufficient heat treatment. After exposing breast cancer cells (MCF-7) to two different temperature conditions (42°C and 47°C) for an hour, we have verified that 10.51 ± 1.71% and 18.27 ± 10.66% of cells experienced apoptosis or necrosis when the cells were exposed to 42°C and 47°C, respectively. At the same time, cancer cells showed higher invasiveness, spear-like morphology, and enhanced migratory behaviors as the exposed temperature increases, which is mainly shown among the cells that have experienced EMT. Further western blot assay and quantitative real-time polymerase chain reaction (qRT-PCR) using mesenchymal marker (vimentin) and epithelial marker (E-cadherin) also support that the mesenchymal-like phenotype has been highly increased on the cells that have resisted to the thermal stress. However, when chemotherapy was conducted after the heat treatment, cell viability was highly reduced. Paclitaxel (11.7 μM), cisplatin (3.3 μM), and combination of two anticancer drugs were treated for 24 hours on the cells that have been exposed to different temperatures, respectively. As a result, death rate of tumor cells has increased from 11.31 to 66.69%. Especially, when paclitaxel and cisplatin were co-treated, the death rate was up to 73.75 ± 4.37% after cells were exposed to 47°C. In conclusion, cancer cells that have survived from insufficient hyperthermia showed high potential to promote metastasis or recurrence but additional chemotherapy can successfully reduce the side effects induced by insufficient hyperthermia treatment.

#4307 Characterizing the differential phosphorylation of Bim in plasma cell disorders. Jason E. Conage-Pough, Vikas A. Gupta, Shannon M. Matulis, Lawrence H. Boise. Emory University, Atlanta, GA.

The pro-apoptotic Bcl-2 family protein Bim is known to be phosphorylated in response to many stimuli. These phosphorylation events have been linked to proteasomal release from dynactin light chain and impacting the ability of Bim to initiate apoptosis. We’ve recently shown that IL-6 stimulation of myeloma cells results in phosphorylation of Bim at S69 and S77. IL-6 treatment additionally led to increased association with Mcl-1 at the expense of Bcl-2/s. This data, coupled with our longstanding interest in factors that influence Bim binding to anti-apoptotic proteins, led us to study the baseline phosphorylation state of Bim in plasma cell malignancies. To study the global phosphorylation of Bim, we utilized PhosTag gel electrophoresis, which allows for detection of phosphorylated forms of a protein. We’ve shown that Bim is constitutively phosphorylated in myeloma cell lines and patient samples. Importantly, these cell lines and samples display differences in their distribution of Bim binding to anti-apoptotic proteins. The pattern of Bim phosphorylation varies significantly across cell lines, with expression of anywhere from 1 to 9 phosphorylated forms. Using the Bax/Bak-deficient Waldenström Macroglobulinemia cell line RPCI-WM1, we were able to recapitulate constitutive phosphorylation in stable cell lines overexpressing Bim. In order to identify and characterize constitutive Bim phosphorylation sites, we created phospho-mimetic (E) and unphosphorylatable (A) versions with individual and combination of mutations of seven potential sites—S9, S77, S87, S94, S104, T16 and S118. PhosTag allowed us to identify individual phosphorylation sites—by mutating individual sites to A, we could determine if we eliminated phosphorylated Bim bands. These experiments showed that S118 and S95 are constitutively phosphorylated in RPCI-WM1. Lastly, we tested whether A and E mutations were affecting the distribution of Bim among anti-apoptotic proteins and dynein light chain. We observed that mutation of the T116 residue to either A or E resulted in increased binding to Mcl-1, suggesting this amino acid is critical for binding to dynein light chain, regardless of phosphorylation status. Our results support the presence of multiple constitutive Bim phosphorylation events, differential signaling and regulation of Bim across a spectrum of plasma cell disorders, and a potential mechanism underlying the preferential binding of Bim. Characterization of the signaling cascades regulating these events may provide novel insights into improving therapies for cancer. The efficacy of drugs such as Navitoclax and Venetoclax can be improved by coupling them with kinase inhibitors that can either mobilize Bim from dynein light chain or alter its affinity for anti-apoptotic proteins.

#4308 Cordycepin induces apoptosis by caveolin-1-mediated JNK regulation of Foxo3a in human lung adenocarcinoma cells. Eunbi Jo1, Hyun Jin Jang,2 Ik-Soon Jang1. 1Korea Basic Science Inst., Daejeon, Republic of Korea; 2Korea Basic Science Inst., Daejeon, Republic of Korea.

Cordycepin is a water-soluble metabolite of the fungus Cordyceps sinensis. Cordycepin is highly used as a traditional medicine in Asian countries, and its biological effects have been well investigated. Recently, it was reported that cordycepin induces apoptosis through JNK/Foxo3a signaling pathway in lung cancer cells. However, the exact mechanism by which cordycepin induces apoptosis remains to be elucidated. In this study, we investigated whether cordycepin induces apoptosis and its resultant pathway in human lung adenocarcinoma cells. Cordycepin induced cell death, morphological change, and nuclear translocation of JNK and Foxo3a in three different lung cancer cell lines (H11006, H9251, NCI-H1299). Western blot analysis showed that the phosphorylation of JNK and Foxo3a was increased in a dose-dependent manner. In addition, the localization of JNK and Foxo3a was changed from cytosol to nucleus. We further investigated the possible mechanism of cordycepin-induced JNK/Foxo3a activation. Cordycepin increased JNK/Foxo3a phosphorylation by downregulating the expression of p53-binding protein 1 (p53BP1), an inhibitor protein of JNK. Furthermore, we identified the critical role of caveolin-1 in the JNK/Foxo3a activation. Firstly, we found that mTOR inhibitor rapamycin prevented cordycepin-induced JNK/Foxo3a activation. Secondly, siRNA silencing for caveolin-1 prevented JNK/Foxo3a activation. Together these findings indicate that cordycepin induces apoptosis through activating JNK/Foxo3a signaling pathway.
Herbal medicines have been used in cancer treatment, with many exhibiting favorable side effect and toxicity profiles compared with conventional chemotherapeutic agents. SH003 is a novel extract from Astragalus membranaceus, Angelica gigas, and Trichosanthes Kirolowi Maximowicz combined at a 1:1:1 ratio that impairs the growth of breast cancer cells. Our data demonstrate that SH003 induced apoptosis in DU145 cells, but not androgen-dependent LNCaP and PC-3 cells. Moreover, ERK2 overexpression rescued SH003-induced apoptosis in DU145 cells. Thus, our data conclude that SH003 induces apoptotic cell death of DU145 prostate cancer cells by inhibiting ERK-mediated pathway.


The growth of estrogen receptor positive (ER+) breast cancers is considered to be dependent on estrogen and its actions can be blocked by selective estrogen receptor modulators (SERMs), like tamoxifen, or by inhibiting the synthesis of estrogen, by aromatase inhibitors (AIs). Both of these classes of drug are used to treat ER+ breast cancers in the clinic. Paradoxically, before the discovery of SERMs and AIs, high dose estrogen was the choice of endocrine therapy to treat post-menopausal breast cancers. Recent clinical trials have confirmed a 30% clinical benefit rate with, as well as low, doses of estrogen therapy in AI-resistant breast cancers. However, the underlying mechanism by which estrogen triggers the tumor regression remains unknown. Using an in vitro cell model MCF7:5C cells, that undergo estrogen-induced apoptosis, we demonstrate that sustained phosphorylation of eukaryotic initiation factor 2-alpha (eIF2-α) in breast cancer cells, that undergo estrogen-induced apoptosis, is critical in estrogen-induced apoptosis. Growth arrest and DNA damage inducible-4 (GADD34) and constitutive repressor of eIF2α phosphorylation (CReP) are two regulatory subunits of protein phosphatase 1 (PP1) complex, that provides substrate specificity towards eIF2α, and are responsible for its de-phosphorylation. Our results show that pharmacological inhibition of GADD34 and CReP, or their genetic depletion, can enhance the phospho-eIF2α (p-eIF2α) levels and promote apoptosis. Elevated levels of p-eIF2α attenuated global translation but preferentially allowed high expression of activating transcription factor 4 (ATF4) and C/EBP homologous protein (CHOP) that are involved in apoptosis. Both estrogen and inhibition of GADD34 and CReP promote apoptosis by an identical mechanism. Importantly, inhibition of GADD34 and CReP induced apoptosis in another estrogen-independent breast cancer cell line, LCC9, which is resistant to both tamoxifen and fulvestrant but does not undergo estrogen mediated apoptosis. Overall, our study provides crucial evidence that inhibiting specific regulatory subunits (GADD34 and CReP) of PP1 complex, can mimic estrogen-induced apoptosis in breast cancer cells that are not susceptible to estrogen mediated apoptosis. Therefore, GADD34 and CReP can be targeted for potential therapeutic intervention in endocrine therapy resistant breast cancers.


Triple-negative breast cancer (TNBC) is highly aggressive and a major threat to survival due to absence of molecular targets. Invasion, metastasis and chemoresistance are the greatest obstacles to the successful tumor treatment in breast cancer patients. In this study, over-expression of Forkhead box transcription factor M1 (FoxM1) was observed in 79% (770/975) of breast cancers from Saudi Arabia. FoxM1 over-expression was found to be an independent prognostic marker in multivariate analysis in late stage (Stage 3 and 4) breast cancer (p=0.0110). Our in vitro results show that treatment with thioestrogen, a dual FoxM1 and proteasome inhibitor caused cell viability loss and induce apoptosis in a dose dependent manner in triple-negative breast cancer cell lines. Thioestrogen treatment also inhibited colony formation and invasion/migration capabilities of triple-negative breast cancer cells. In vivo, thioestrogen treatment regressed TNBC cells, MDA-MB-231 generated xenografts via down-regulation of FoxM1 and its downstream targets. Altogether, our results suggest that FoxM1 and its associated signaling pathway may be a potential target for therapeutic intervention in the treatment of aggressive breast cancer. Key words: Triple-negative breast cancer, FoxM1, Apoptosis and Invasion.

#4313 Protein synthesis modulates paraptotic death induced by inhibition of cyclophilins in glioblastoma (GBM) cells. Lin Wang, Justin Gundelach, Richard Bram. Mayo Clinic School of Medicine, Rochester, MN.

In this study, we investigated the mechanisms underlying cell death induced by the small molecule cyclophilin inhibitor NIM811. NIM811 effectively killed glioblastoma cells, including those that are resistant to chemotherapy or caspase activation. Instead, death was preceded by dramatic cytosolic vacuolization, stemming from enlargement of the endoplasmic reticulum (ER). RNA-seq revealed upregulation of genes pertinent to the unfolded protein response (UPR) and autophagy. Western blotting for UPR and autophagy mediators also supported a transient UPR signaling and compromised autophagy during prolonged NIM811 treatment. By utilizing a bicistronic reporter plasmid, we independently quantitated Cap-dependent and Cap-independent translation. We further assessed the long-term effects mediated by different short-term paraposis inhibitors via colony formation assay. We find that NIM811 initiates paraposis in GBM cells, due to an abnormal upregulation of protein translation that caused accumulation of misfolded or unfolded proteins in ER. Transient activation of pro-survival autophagy and UPR signals were shutdown during prolonged treatment with NIM811, thus allowing cell death to occur. Cycloheximide was previously reported by others to supress paraposis. In our studies, we find instead that it temporarily delayed vacuole formation, but actually enhanced parapotic cell death in the long term. The mTOR inhibitors rapamycin or torin-2 rescued cells from NIM811 induced paraposis by sustaining autophagy and the UPR, and selectively restraining translation. We propose that ER vacuolization could be a protective response generated by cells to isolate defective proteins within the ER in order to prevent misfolded aggregates to interfere with cellular homeostasis. For that reason, simply inhibiting vacuole formation without clearing protein accumulation in the ER was insufficient to prevent cell death. These findings aid our understanding of cellular mechanisms that contribute to non-apoptotic death. Moreover, this work underlines the importance of evaluating cellular responses in cell death experiments over multiple time frames to judge the true impact.

#4314 Exclusive delivery of mazF in cancer cells by Listeria monocytogenes. Maryam Safarian, Jeremy Tzeng. Clemson University, Clemson, SC.

Programmed cell death (PCD), an active process that leads to cell suicide, is a critical mechanism in every organism. MazF, a bacterial ribonuclease protein, can trigger PCD in bacterial cells and also induce Bak-dependent apoptosis in mammalian cells. This bacterial ribonuclease cleaves mRNAs at ACA sequences leading to inhibition of protein synthesis in cells. Hence, we hypothesized that the overexpression of MazF proteins in cancer cells could result in the induction of apoptosis. In the present study, the ACA-less mazF gene was inserted downstream of the Internal Ribosome Entry Site (IRES) and under the control of T7 promoter. The plasmid was electroporated into Listeria monocytogenes harboring pCSA1 that encodes T7 RNA polymerase under the control of the listeria actA promoter. Subsequently, the bacteria were functionalized with Trastuzumab (Herceptin®) to deliver the MazF mRNA into HER2/neu breast cancer cells. The results showed that MazF protein has an ability to induce apoptosis in HER2/neu breast cancer cells. This finding is the first report of the application and evaluation of MazF as an anticancer agent for the induction of apoptosis in HER2/neu breast cancer cell line. This research suggests cancer therapy that targets only cancer cells but not healthy cells and offers more advantages, e.g., inexpensiveness, target tissue specificity, easy and safe delivery, in comparison to other gene therapy vectors or direct protein administration.

#4315 PTBP1 modulation of MCL1 mRNA regulates sensitivity to antitubulin chemotherapeutics. William J. Placzek, Jia Cui. Univ. of Alabama at Birmingham, Birmingham, AL.

Background: Myeloid Cell Leukemia 1 (MCL1), an anti-apoptotic Bcl-2 family protein, is a key regulator of intrinsic apoptosis. Normal cells require strict control over MCL1 expression and aberrant MCL1 expression has been shown linked to the emergence of various diseases and chemoresistance. Previous studies have detailed how MCL1 expression is regulated by multiple mechanisms both transcriptionally and translationally. However, characterization of the post-transcriptional regulators of MCL1 mRNA is limited. Polypririmidinetract binding protein 1 (PTBAP1) is a known regulator of post-transcriptional gene expression that can control mRNA splicing, translation, stability, and localization. In the described study we characterize the impact that PTBAP1 has on MCL1 expression, its molecular mechanisms, and its roles on cellular apoptosis. Methods: Cross-linking Immunoprecipitation (CLIP)-seq data analysis and RNA immunoprecipitation (RIP) were performed to analyze protein interactions with RNAs and map the RNA binding sites on the genes of interests. RNA half-life was measured by treating cells with Actinomycin D and extracting total RNA at
different time point. Cell viability was measured by the MTS assay and apoptosis was assessed by Annexin V/Propidium Iodide (PI) staining followed by flow cytometry analysis. Results: Our studies demonstrate that PTBP1 binds to MCL1 mRNA and that knockdown of PTBP1 up-regulates MCL1 expression in cancer. Mechanistically, PTBP1 silencing stabilizes MCL1 mRNA and increases MCL1 mRNA accumulation in cyttoplasm. We further identify that this post-transcriptional regulation is mediated by microRNAs and Argonaute2 (Ago2). Moreover, we show that PTBP1 down-regulation is anti-apoptotic to antitubulin chemotherapeutics in a MCL1-dependent manner. Conclusions: Our findings suggest that PTBP1 is a novel post-transcriptional regulator of MCL1 mRNA by which it controls apoptotic response to antitubulin chemotherapeutics.

#4316 MCL-1 inhibition ameliorates the expansion of human leukemia in a dose dependent fashion. Haley E. Ramsey,1 Melissa A. Fischer,1 Taeyeu Lee,2 Agnieszka E. Gorska,1 Pia Arrate,1 John Sensenbrenner,2 Leah Hogdal,2 Edward Olejniczak,2 Stephen Fesik,2 Michael R. Savona1. 1Vanderbilt University Medical Center, Nashville, TN; 2Vanderbilt University, Nashville, TN.

Myeloid cell leukemia-1 (MCL-1) is a prosurvival BCL-2 family member protein commonly overexpressed in cancer, including hematologic malignancies. The success of BH3 mimetics against other anti-apoptotic proteins has validated the potential of this line of therapy in hematologic malignancies. However, targeting BCL-2 family members, like MCL-1, with a small molecule inhibitor is challenging because these inhibitors mediate their effects through protein-protein interactions. Recently, we discovered potent (sub nM), selective small molecule MCL-1 inhibitors that inhibit cell-based activity and slow the growth of tumors in multiple mouse models. To determine tumor cell dependence on specific BCL2-family members, we employed BH3 profiling on a panel of myeloid tumor cells to reveal mitochondrial outer membrane permeabilization (MOMP) in a cytochrome C release assay. Additionally, we determined growth inhibition of these cell lines in the presence of the potent MCL-1 inhibitor. These assays indicated MV-411 cells were dependent on MCL-1 and sensitive to MCL-1 inhibition with a GI50 \(<100\) nM. After cell line profiling, we began in vivo studies with the MCL-1 inhibitor in a systemic AML xenograft model. NSG mice were sublethally irradiated and administered MV-411 cells intravenously. Engrafted mice received the MCL-1 inhibitor in 3 different doses (vehicle vs. 10, 25, and 75 mg/kg) daily via intraperitoneal injection. During treatment, the kinetics of MV-411 expansion was monitored via flow cytometry for the detection of human AML in the blood. At approximately 4 weeks after transplant, the vehicle mice became moribund, and all experimental groups were sacrificed for analysis of chimerism. Significant decreases in leukemic expansion were evident in the bone marrow (25 and 75 mg/kg vs vehicle, \(P = 0.01, P < 0.001\)) and spleen (vehicle vs. 75 mg/kg, \(P < 0.001\)) of treated mice in a dose-dependent fashion. MCL-1 blockade also eliminated splenomegaly in MCL-1 inhibitor treated mice. Furthermore, administration of the BCL2 inhibitor venetoclax, as a single agent, and in combination with DNA methyltransferase inhibitors or low dose ara-C in a dose dependent fashion. MCL-1 blockade also eliminated splenomegaly in MCL-1 inhibitor treated mice. Additionally, the MCL-1/BIM interaction was disrupted by both Camptothecin and Anitoxin, which increases the antitumor activity of paclitaxel.

#4317 EGLN1/c-Myc induced lymphoid-specific helicase inhibits ferrop- tosis through lipid metabolic gene expression changes. Youngguang Tao, Shuang Liu, Yiqun Jiang. Central South University, China, China.

Ferroptosis has emerged as a new form of non-apoptotic cell death in multiple human diseases. However, the epigenetic mechanisms of ferroptosis remain poorly defined. We demonstrate that Lymphoid-specific helicase (LSH), a DNA methylation modifier, interacts with WRD76 to inhibit ferroptosis by activating lipid metabolism-associated genes, including GLUT1, and ferroptosis related genes SCD1 and FADS2. These effects are dependent on iron and lipid reactive oxygen species. We further show that EGLN1 and c-Myc directly activate LSH expression by recruiting H3K4me1. Finally, we demonstrate that LSH functions as an oncogene in lung cancer in vitro and in vivo. Therefore, our study elucidates the molecular basis for a c-Myc/EGLN1-mediated induction of LSH expression to inhibit ferroptosis, which can be exploited for the development of therapeutic agents to target ferroptosis in cancer.

#4318 Role of Bcl-2 in hematological cancer formation. Abigail V. Fortier,1 Ally Koh,1 Lisa Stanovski,1 Alisa Kepner,1 Supriya Jain,1 Misha Ul-Islam,1 Ellie Walter,1 Lawrence Boise,2. 1Waltong High School, Marietta, GA; 2Emory University, GA.

B cell leukemia/lymphoma 2 (BCL-2) is a 26 kilodalton, amphipathic, anti-apoptotic protein located on the outer mitochondrial membrane. BCL-2 interacts with other anti-apoptotic and pro-apoptotic factors in order to regulate apoptosis. If a structural change occurs on the BCL-2 gene, overexpression of the BCL-2 protein can result. This causes a change in the ratio of BCL-2 to its BCL-2 Homologous 3 (BCL3) ligands, preventing the release of the apoptotic signal. The primary change associated with BCL-2 overexpression in lymphomas is a t(14; 18) chromosomal translocation involving the BCL-2 and BCL3 transcription. The upregulation of the oncogene allows for the survival of the mutated B cells by raising the apoptotic threshold, which then dislodges regular lymphocytes in the bone marrow or lymph nodes. Lack of cell death due to overexpression of BCL-2 has been linked to hematological malignancies, specifically lymphomas and myelomas. Both B-lymphocytic cancers can result from alterations of the immunoglobulin heavy chain locus on chromosome 14. While BCL-2 translocation can cause follicular Lymphoma, BCL2 over-expression is observed in many other tumor types and likely contributes to cancer progression and drug resistance in these tumors. Treatments to inhibit BCL-2 include attempts at activation and overexpression of the pro apoptotic BH3 proteins, BCL-2 Associated X Protein (BAX) and BCL-2 Associated K protein (BAK), and creation of BH3 mimetics. In this study, we employed BH3 mimetics to create a 3D printed model of BCL-2 and composed a review poster of our secondary research to investigate and determine the relationship between the structure and function of this molecule.

#4319 Analysis of Bcl-2 family members and protein-protein interactions using novel multiplex immunomartas. Reeti Maheshwari, Melissa Schluter, Danielle Pepin, Joseph Hwang. Millipore Sigma, St Charles, MO.

Bcl-2 family members play a role in intracellular and intercellular interactions. The family consists of more than 17 members that can be functionally separated into pro-apoptotic and anti-apoptotic subfamilies. Interactions between these family members determine cell fate, and dysregulation can lead to tumor growth and tumor cell survival. There are several investigational drugs for cancer treatment targeting Bcl-2 family members in clinical trials, driving the need for robust assays to measure Bcl-2 family members and their interactions. Though existing assays measure Bcl-2 family members, there is a lack of reliable assays to evaluate total proteins and protein-protein interactions simultaneously. To address this issue, we have developed two LumineX-based multiplex immunomartas panels that allow for the simultaneous detection of multiple components of the B-cla family in a single well, including Total Bcl-2, Bcl-2, BAX, Mcl-1, Bcl-xL, Bcl-xL, and Bcl-xL/BAX. Using these panels, we analyzed changes in the expression and interactions of Bcl-2 family members in response to known apoptotic drugs, including Camptothecin (topoisomerase inhibitor), Anisomycin (protein translation inhibitor), and AT101 (BH3 mimetic drug). Camptothecin and Anisomycin each elicited a significant decrease in the levels of Mcl-1. Additionally, the Mcl-1/BIM interaction was disrupted by both Camptothecin and Antiomycin, perhaps a consequence of the reduced Mcl-1 levels. However, expression of the other anti-apoptotic proteins was unaffected by any of the three drugs. Interestingly, all three treatments had different effects on the Mcl-1/ NOXA interaction. Mcl-1/NOXA signal was increased by AT101, decreased by Anisomycin, and showed a dose-dependent effect with Camptothecin. In contrast to their disparate effects on the Mcl-1/NOXA interaction, all three drugs acted in a similar manner in blocking the Bcl-xL/BAX interaction without affecting the total levels of either protein. Overall, these results illustrate the dynamic responses of the Bcl-2 family to apoptosis-inducing drugs. The novel multiplex immunomartas described here provide a powerful tool for studying the underlying mechanisms regulating the Bcl-2 family of proteins.

#4320 The Survivin-targeting miR-542-3p overcomes erbB3 signaling-mediated chemo-resistance and enhances the antitumor activity of paclitaxel against erbB2-positive breast cancer. Hui Lyu,1 Shuiliang Huang,2 Jingcao Huang,3 Bolin Wang,4 Bolin Liu,5. 1University of Colorado Denver/AMC, Aurora, CO; 2Fuzhou General Hospital, China; 3West China Hospital, China.
Elevated expression of erbB3 interacts with erbB2 in breast cancer cells to confer chemo-resistance via induction of Survivin. However, the underlying mechanism of erbB3 signaling-induced upregulation of Survivin remains elusive. Ectopic expression or downregulation of erbB3 in erbB2-positive breast cancer cells did not alter Survivin mRNA levels and had no significant effect on Survivin protein levels in our system. We thus hypothesize that the erbB3 signaling modulates certain miRNAs that target Survivin to influence its protein translation. Here we showed that overexpression of erbB3 decreased and specific knockdown of erbB3 enhanced expression of two Survivin-targeting miRNAs, miR-203 and miR-542-3p in breast cancer cells. While the specific inhibitor of either miR-203 or miR-542-3p clearly attenuated an anti-erbB3 antibody (Ab)-mediated reduction of Survivin, a more profound effect on Survivin protein expression was observed with inhibition of miR-542-3p than miR-203 inhibition. Consistently, miR-542-3p mimic was much more effective than miR-203 mimic not only to downregulate Survivin, but also to enhance paclitaxel-induced apoptosis in the otherwise paclitaxel-resistant breast cancer cells. Moreover, using a murine tumor xenograft model established from a human breast cancer cell line expressing both erbB2 and erbB3, we discovered that the combinations of miR-542-3p mimic and paclitaxel, as compared to either agent alone, significantly inhibited tumor growth in vivo. Collectively, these data demonstrate that the erbB3 signaling upregulates Survivin via inhibition of miR-203 and miR-542-3p in erbB2-positive breast cancer cells. Since miR-542-3p has three binding sites on the 3’UTR of erbB3 mRNA, the miR-542-3p mimic serves as an excellent replacement therapeutic agent to downregulate Survivin, and thereby significantly enhances the paclitaxel activity of paclitaxel against erbB2-positive breast cancer.

#4321 TNFR receptor 2 is essential for RIP1-dependent cell death in refractory leukemia. Julia Agüade-Gorgorio,1 Scott McComb,1 Cornelia Eckert,2 Maria Pamela Dobay,3 Gunnar Cario,3 Caterina Mezzatetta,4 Arend Von Stackelberg,2 Martin Stanulla,5 Schrappe Martin,5 Jean-Pierre Bourquin,5 Beat C. Bornhauser1.1 Univ. Children’s Hospital Zurich, Zurich, Zurich, Switzerland;2 Charité Medical University Berlin, Germany;3 Swiss Institute of Bioinformatics, Lausanne, Switzerland;4 University Hospital Schleswig-Holstein, Germany;5 Hannover Medical School, Germany. The identification of molecular determinants that regulate sensitivity to specific agents is essential for the development of new therapeutic approaches in cancer. We have earlier shown that a subset of refractory acute lymphoblastic leukemia (ALL) samples respond to SMAC-mimetic (SM) induced IAP depletion by concurrently inducing RIP1-dependent apoptosis and necroptosis. Comparative gene expression profiling indicated a correlation of sensitivity to SM with the expression of TNFR receptor 2 (TNFR2) in primary ALL. Using an independent cohort of primary chemotherapy-resistant ALL samples, we found that expression of high TNFR2 expression identified by qPCR predicted ex vivo sensitivity to SM. High TNFR2 levels also correlated with higher expression of TNFR1. Deletion of either TNFR1 or TNFR2 using CRISPR/Cas9 in primary ALL conferred resistance to treatment with SM, indicating that TNFR1 and 2 are both functionally required for cell death. Combinatorial with an important role for TNFR2 in the response to SM, the overexpression of TNFR2 leads to increased sensitivity to TNF through increased activation of the TNFR1/RIP1 death axis. On the mechanistic level, SM induced recruitment of RIP1 to TNFR1, which was abolished in cells deficient for TNFR2. Taken together, our data reveal a novel function of TNFR2 in cell death signalling, as TNFR2 predicts sensitivity to SMAC mimetics and plays a key role in modulating a switch from RIP1-controlled cell survival to cell death.

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Exosomes and microvesicles were then assayed for particle size distribution using nanoparticle tracking analysis and electronic microscopy. miRNA expression levels were determined using quantitative PCR. miRNA transfection was performed with RNAiMax reagents. Cell viability was measured by Alamar Blue. Statistics were performed using Prism 6 software. Following transfection of human AML cells with PDAC cells, the semiRNAs are present in PDAC (PDAC) cells at the transcriptional level, with no significant effect on Survivin mRNA levels. We thus hypothesize that the latter expression is modulated by certain miRNAs that target Survivin to influence its protein translation. Here we showed that overexpression of erbB3 decreased and specific knockdown of erbB3 enhanced expression of two Survivin-targeting miRNAs, miR-203 and miR-542-3p in breast cancer cells. While the specific inhibitor of either miR-203 or miR-542-3p clearly attenuated an anti-erbB3 antibody (Ab)-mediated reduction of Survivin, a more profound effect on Survivin protein expression was observed with inhibition of miR-542-3p than miR-203 inhibition. Consistently, miR-542-3p mimic was much more effective than miR-203 mimic not only to downregulate Survivin, but also to enhance paclitaxel-induced apoptosis in the otherwise paclitaxel-resistant breast cancer cells. Moreover, using a murine tumor xenograft model established from a human breast cancer cell line expressing both erbB2 and erbB3, we discovered that the combinations of miR-542-3p mimic and paclitaxel, as compared to either agent alone, significantly inhibited tumor growth in vivo. Collectively, these data demonstrate that the erbB3 signaling upregulates Survivin via inhibition of miR-203 and miR-542-3p in erbB2-positive breast cancer cells. Since miR-542-3p has three binding sites on the 3’UTR of erbB3 mRNA, the miR-542-3p mimic serves as an excellent replacement therapeutic agent to downregulate Survivin, and thereby significantly enhances the paclitaxel activity of paclitaxel against erbB2-positive breast cancer.

#4324 Exosomal delivery of stroma-derived miR-145 inhibits pancreatic cancer cell proliferation. Song Han, Sayali Belsare, DongYu Zhang, Mark Beveridge, Carlos Rinaldi, Jose G. Trevino, Thomas D. Schmittgen, Steven J. Hughes. University of Florida, Gainesville, FL. We previously reported that within the pancreatic ductal adenocarcinoma (PDAC) microenvironment, miR-145 and miR-199a are exclusively expressed in tumor-associated stroma (TAS) cells, but these miRNAs are present in PDAC cells following co-culture with TAS cells. We hypothesized that miRNAs function as paracrine signals via exosomal exchange between TAS cells and adjacent PDAC cells. Primary cultures of human TAS and PDAC cells were employed. Membrane-bound microparticles were isolated from TAS conditioned, serum-free culture media by sequential ultracentrifugation followed by ultrafiltration.

Exosomal delivery of stroma-derived miR-145 inhibits pancreatic cancer cell proliferation. Song Han, Sayali Belsare, DongYu Zhang, Mark Beveridge, Carlos Rinaldi, Jose G. Trevino, Thomas D. Schmittgen, Steven J. Hughes. University of Florida, Gainesville, FL. We previously reported that within the pancreatic ductal adenocarcinoma (PDAC) microenvironment, miR-145 and miR-199a are exclusively expressed in tumor-associated stroma (TAS) cells, but these miRNAs are present in PDAC cells following co-culture with TAS cells. We hypothesized that miRNAs function as paracrine signals via exosomal exchange between TAS cells and adjacent PDAC cells. Primary cultures of human TAS and PDAC cells were employed. Membrane-bound microparticles were isolated from TAS conditioned, serum-free culture media by sequential ultracentrifugation followed by ultrafiltration.

#4323 Metabolic reprogramming in acute myeloid leukemia cells by mesenchymal stromal cell-derived exosomes induces chemoresistance. Hongyu Zhao,1 Xiying Guo,1 Arjun Achreja,2 Zhe Chen,3 Ahnou N. Doss,2 Nadia Popolova,2 Michael Andreeff,2 Deepak Nagrath1.1 University of Michigan, Ann Arbor, MI;2 The University of Texas MD Anderson Cancer Center, Houston, TX. Mesenchymal stromal cells play an important role in acute myeloid leukemia (AML) development. Altered cellular metabolism supports AML cells’ survival in multiple aspects, such as drug resistance. Here, we demonstrate the role of exosomal miRNAs in metabolic regulation of AML cells and propose a combinatorial strategy to sensitize AML cells to chemotherapies. Exosomes secreted by MSCs can reprogram the metabolic machinery following their internalization by AML cells. Through 13C tracing experiments and flux analysis, we elucidate that MSC-derived exosomes enhance oxidative phosphorylation and glutamine’s entry into TCA cycle, which replenish the pool of carbon sources in mitochondria. Further, our work shows that inhibiting the interactions between MSCs and AML cells by targeting the metabolic regulation exerted by MSC-derived exosomes sensitize AML cells to chemotherapies. Taken together, our work reveals a novel role of the TME in regulating the metabolic adaptation in AML cells and uncovers the improved strategy for AML therapy.
Neuroblastoma (NB) is the most common extracranial solid tumor of childhood. One of its hallmarks is high histological heterogeneity. The primary tu-
mors are composed of neuroblastic cancer cells, Schwannian stroma and some 
infiltrating immune cells. The origin and functions of Schwannian stroma still 
remain controversial. It is suggested that they are tumor-infiltrating normal 
cells. Studies using Bone Marrow Stromal Cells (BMSCs) or Schwann cells to 
imic Schwannian stroma have shown both tumor promoting or suppression 
effects. An alternative hypothesis is that Schwannian cells are derived from can-
cer stem cells and may share the same genetic lesions with their normal tumor 
cells. Our laboratory had reported that in the SK-N-SH cell line, both 
N-type cells (neuroblastic cells) and S-type cells (Schwann-like cells) shared 
almost identical genetic background. These cell lines may serve as relevant mod-
estudies to study NB tumor heterogeneity. Genome-wide sequencing for driver on-
cogenes in familial NB uncovered mutations in Anaplastic Lymphoma Kinase 
(ALK) gene. ALK point mutations have been found in 8-12% of all NB patients, 
and are restricted to the kinase domain. aberrant activation of ALK signaling, 
and its downstream target, PI3K/AKT, STAT3 and ERK1/2, can contribute to 
NB tumorigenesis. Thus, ALK is an intensely studied therapeutic target. Sub-
lines of N-type and S-type cells were isolated from an early passage of SK-N-SH. 
Surprisingly, over 90% have cell morphology that resembled S-type cells. Se-
lected cell lines of N-type and S-type cells were isolated from an early passage of 
SK-N-SH. The selected cell lines were further characterized by the expression of 
alkine-specific lectins, has been found to be involved in tumor pro-
	
Conclusion: Our results show that the stimulation of lipolysis and fatty acid 
metabolism is a critical strategy to enhance the capacity of leukemia cells to derive energy from 
free fatty acid metabolism. The role of adipocytes in regulating leukemia metabolism and its 
clinical implications are discussed. 

Regarding the status of an adipocyte population in leukemia, we draw focus on the 
status of an adipocyte in leukemia. In this study, we evaluate the status of an adipocyte 
in leukemia. 


tissue density can create a carcinogenic microenvironment for 
normal mammary epithelial cells. Shayan Nazari, Pinku Mukherjee. University 
of North Carolina at Charlotte, Matthews, NC.

Background: Breast cancer is the most diagnosed cancer and the fourth lead-
ing cause of cancer related mortality among females. Mammographic density (MD) is defined by the amount of stromal and epithelial tissues present in 
the breast tissue. Fifty percent of women have high MD and are 4-6 times more likely to 
develop breast cancer in their lifetime compared to women with low MD. Additionally, 
mammograms often miss abnormal lesions in high MD breast leading to late-stage diagnosis. The stromal microenvironment is characterized 
by extracellular matrix (ECM) reorganization and staccato, and increase in ECM 
proteins including collagen I and fibronectin, which drives proliferation, tumor 
cell survival and migration. Expression of fibronectin and collagen are signifi-
cantly higher in women with high MD compared to women with low MD. However, 
the mechanisms underlying the initiation and progression of breast cancer associated with MD remains largely unknown. Therefore, understanding 
the molecular pathways involved in the unique transformation of normal mam-
mary cells to cancer cells in a dense microenvironment is an important step in 
finding diagnostic cells and targeted therapeutic strategies for prevention and treatment 
of breast cancer. We hypothesize that normal mammary epithelial cells behave like 
cancer cells in an ECM protein-rich microenvironment by activating pathways 
associated with tumor growth and survival and contributing to the initiation and 
progression of breast cancer. Methods: We conducted an in vitro study, deter-
miming the effects of different concentrations of ECM proteins on the activation of 
pro-carcinogenic pathways including the MAPK pathway and the PI3K pathway 
using Western blotting analysis. This study helps us understand if there is a difference in the expression of ERK1/2 signaling and/or PI3K signaling in 
normal mammary cells (hTERT) seeded on culture dish coated with ECM proteins 
(collagen and fibronectin), compared to a non-coated control. We also 
examined morphological changes that can occur in a breast cancer cell line (HCC1806) that are seeded on ECM-rich coating dishes compared to 
non-coated dishes. Results We observed changes in the morphology of breast cancer cell lines after cells were seeded on increasing concentration of extracellular 
matrix coatings using light microscopy. We also observed an increase in the 
number of apoptotic cells in normal non-proliferating breast cancer cell lines 
when cells were stressed using hypoosmotic, collagen and fibronectin-rich microenvironment can induce desmosplastic-like phenotype and behavior 
for normal mammary cells, which highlights the possible first steps in transfor-
mation of normal mammary cells to desmosplastic cells. Given that high MD is one of the major risk factors for breast cancer development, it is critical to 
decipher the molecular mechanisms associated with the high risk. 

#4323 Bone marrow adipocytes drive transcriptional changes in leukemia 
blasts to enhance their capacity to derive energy from free fatty acid metab-
olism. Manar Shafat,1 Thomas Oellerich,2 Sebastian Mohr,3 Stephen Robinson,1 
Dylan Edwards,1 Rachel Piddock,1 Amina Abdul-Aziz,1 Christopher Marlein,1 
Matthew Fenech,1 Jeremy Turner,2 Matthew Lawes,3 Lyubov Zaitseva,3 John-
athan Watkins,3 Kristian Bowles,2 Stuart Rushworth1.2.1. The University of East 
Anglia, Norwich, United Kingdom; 2Goethe University, Frankfurt, Germany; 
3Norfolk and Norwich University Hospitals NHS Trust, Norwich, United King-
dom.

Introduction: Most patients diagnosed with acute myeloid leukemia [AML] 
die of their disease and the average age of patients at diagnosis is 72 years. For 
this reason, new therapeutic strategies with tolerability in the fragile, less fit 
population become necessary for reducing the mortality rate associated with this 
disease. The tumor microenvironment is an emerging target in the search for 
less cytotoxic therapies. We have previously shown that the adipocyte component of 
the bone marrow (BM) is a key player in blast survival, proliferation and che-
motherapy evasion. In this study, we draw focus onto the status of an adipocyte 
rich environment in the context of leukemia and highlight the key players in 
regulating leukemic cell metabolism through transport and metabolism of fatty 
acids. Objective: We hypothesize that the presence of adipocytes within the 
proximity of AML blasts creates a FA rich environment for increased β- oxida-
tion within the blasts. Methods: We used primary AML blasts and normal 
CD34+ hematopoietic stem cells (HSC) following informed consent 
(LRC/Re/07/H0310/146). Adipocytes were derived from bone marrow stromal 
cells (BMSC). Differential expression analysis of RNA sequencing data (GEO 
ID: GSE49642, GSE48846) was used to compare respiratory gene signatures 
of BM AML blasts, peripheral blood obtained AML and normal CD34+ HSC. 
Fatty acid binding protein 4 (FABP4) and carnitine palmitoyltransferase 1A 
(CPT1A) were identified as one of the key genes involved in the lipolysis and 
oxidation signature differential expression. Oxygen consumption rate (OCR) of 
tumor and co-cultured blasts and normal CD34+ were analyzed using Seahorse 
technology. Lentiviral knockdown of FABP4 and CPT1A were performed on 
blasts prior to in vivo xenograft mouse model injection. Results: Leukemia blasts 
showed increased adipocyte lipolysis stimulation compared with normal 
CD34+ cells. Moreover, adipocytes increased transcriptional activation of 
FABP4 and CPT1A in malignant blasts compared to CD34+ HSC. FABP4 in-
hibitor reduced AML blast survival when cells were cultured with adipocytes 
which is in contrast to normal CD34+ HSC. Moreover, AML had increased 
oxxygen consumption rate when grown on adipocytes which was inhibited by 
etomoxir (an oxygen consumption rate inhibitor). Finally in-vivo lentiviral mediated knock-
down of FABP4 and CPT1A revealed an increased survival of AML xenografts. 
Conclusion: Our results show that the stimulation of lipolysis and fatty acid 
oxidative genes that contribute to the genetic signatures of these processes are 
a malignant blast exclusive profile. Interventions at a molecular level reveal sur-
vival favorable outcomes in xenograft models suggesting the need for enhancing 
strategies which include targeting the FABP4 and CPT1A axis. Our data provide 
a biologic rationale for exploring future therapies that target the adipocyte/AML 
interactions.
Gal-3 mediates tumor-stromal interaction and abundant cytokine production is unknown. Methods: The effects of Gal-3 on secretion of cytokines/chemokines from stromal cells (HPSC) were assessed by cytokine array. HPSC proliferation and invasion were assessed by the MTS and Matrigel invasion assays. Co-culture and Conditional medium from PDAC tumor cells with genetic alterations of Gα12Δz3 were used to study the interaction between tumor cells and stromal cells. IL-8 production in both tumor cells and stromal cells was confirmed by ELISA. Transcriptional activities of NF-κB and IL-8 were determined by tran-
sient transfection. An IKK mutant construct (IKBM), Mutation of the IL-8 pro-
moter at the NF-κB site, and inhibitors of NF-κB (Bay11), ILK and neutral antibody of Integrinβ1 were used to determine the signaling pathways evoked by Gal-3. Results: Gal-3 expression is increased in both tumor cells and stromal cells of human and genetic mouse model. Secretion of cytokines such as IL-8, GM-CSF, CXCL1, CCL2, and IL6 were significantly increased in HPSC cells treated with rGal-3 which is concomitant with activation of HPSC. Conditioned medium or co-culture from Panc-1 cells in which Gal-3 was down-regulated inhibited the growth and invasion capacity of HPSC cells compared with the vector controls. rGal-3 dramatically induced production and secretion of IL-8 in HPSCs. Mec-
anistically, Gal-3 stimulates IL-8 secretion at the level of transcription and through Integrin
an distically, Gal-3 dramatically induced production and secretion of IL-8 in HPSCs. Mech-
achanically, Gal-3 stimulates IL-8 secretion at the level of transcription and through Integrinβ1/ILK/NF-κB signaling. rGal-3 strongly stimulated integrin
expression and NF-κB transcriptional activity; and inhibitors of IKK inhibi-
tor or NF-κB or neutral antibody of integrin β1 blocked IL-8 transcriptional activity and its secretion. NF-κB mutant construct IKBM or mutation of the NF-κB site completely blocked the induction of IL-8 by Gal-3. GEO data and our own patients’ cohort analysis of pancreatic tumor samples demonstrated that the level of Gal-3, IL-8 and other cytokines is signif-
ificantly higher in tumor tissues than those of normal and pancreatitis. Conclu-
son: Collectively, these data suggest that Gal-3 secreted from tumor cells acti-
vate HPSC cells and induce many inflammatory cytokines including IL-8 produc-
tion via Integrinβ1/ILK/NF-κB signaling. Thus, Gal-3 may play a critical role in mediating tumor-stromal interaction and remodeling tumor associated microenvironments.

#4329 Targetable nodes in fibroblast-supported melanoma cells that show resistance to BRAF inhibitors. Kotryna Seip1,3, Marco V. Haselager1, Kjetil Jør-
gensen1, Marco Albrecht1, Mads H. Haugen1, Elvind Valen Egeland1, Filipe
Lucarelli4, Thomas Sauter4, Olav Engebraaten1, Gunhild M. Mælandsmo1, Lina
Prasmickaitė1,5. Oslo University Hospital Radiant Hospital, Oslo, Norway; Uni-
versity of Luxembourg, Luxemburg, Luxembourg.

Metastatic melanoma is notorious for the ability to change its phenotype in response to signals from the microenvironment, which might influence how melanoma responds to therapy. We have disclosed an association between fi-
broblast-induced phenotypic alterations in melanoma and resistance to the mut-
ated BRAF inhibitor vemurafenib (BRAFi). This signifies the need to find other targets than BRAF to eliminate stroma-influenced melanoma cells. To provide
this challenge, we performed proteomic analysis and cancer drug sensitivity screening, comparing fibroblast-supported versus non-supported melanoma cells. We showed that the effect of fibroblasts was critically dependent on cell-
cell proximity, where melanoma cells get trapped in a fibronectin network, pro-
duced by adjacent fibroblasts. In such environment, melanoma cells down-reg-
ulate melanocytic programs (MITF-driven), gain mesenchymal features (AXL,
PDGFR, fibronectin) and activate stress/inflammatory-response signaling path-
ways (JNK and STAT3). Altogether, this indicates fibroblast-induced melanoma transition to a de-differentiated, mesenchymal-like, pro-inflammatory pheno-
type. Melanoma cells with such phenotype were less responsive to BRAF/MAPK inhibitors and a number of other targeted drugs. However, they showed en-
hanced sensitivity to PI3K/mTOR inhibitors and, particularly, an inhibitor of GSK3b, stimulating Wnt/b-catenin signaling. Further, we employed flow cy-
mometry to measure the levels of Ki67 and pS6 in single melanoma cells upon different conditions/treatments. Such analysis allowed discrimination of cell
subpopulations representing a proliferative and a quiescent cellular state, and
nicely reflected the influence of the tested drugs in the presence or absence of fibroblasts. We observed a cell subpopulation of both tumor cells and strongly
metastatic melanoma cells, which remained after treatment with BRAFi if fibroblasts were present. This, fibroblast-protected BRAFi-resistant cell subpopulation, could be reduced/eliminated by P13K or GSK3b inhibitors, verifying P13K/GSK3 as po-
tential targets in fibroblast-rich tumors. Currently, we are using mass cytomtery (CyTOF) to further characterize cell subpopulations with respect to multiple markers related to cell signaling and immune interactions. Preliminary results
indicate that not only signaling protein levels, but also levels of immunoregula-
tory proteins are altered in melanoma cells that get support from the fibroblasts.
In conclusion, we demonstrate fibroblast-induced melanoma switching to a
mesenchymal-like pro-inflammatory phenotype, which favors melanoma resis-
tance to BRAF inhibitors, but sensitizes to inhibitors of PI3K/mTOR-associated signaling. CyTOF-analysis of complex tumor-stroma cell systems is used to search for additional strategies to target stroma-supported melanoma cells, ei-
ther at the level of signaling, or immune interactions.

#4330 Astrocytic S1P3 regulates blood-brain/tumor barrier permeability. Anurag N. Paraniap1, Brunilde Gril2, Stefan Woditschka1, Emily Hua1, Jef-
frey C. Hanson1, Xiaolin Wu3, Renata Duczkowska3, Priscilla K. Brastianos4, David L. Liewehr4, Seth M. Steinberg4, Cody Peer1, William D. Figg4, Gary T. Pauly1, Christina Robinson3, Joel P. Schneider3, Patricia S. Steeg1, 1National Cancer Institute, Bethesda, MD; 2University of North Carolina Wilmington, NC; Frederick National Laboratory for Cancer Research, Frederick, MD; 3Wojskowy Instytut Medycyny, Warsaw, Poland; 4Massachusetts General Hospital, MA; 5NCI, Bethesda, MD.

Introduction: Incidence of breast cancer brain metastasis is increasing owing to prolonged life-span and better detection techniques. Prognosis of patients with brain metastases is extremely poor with median survival time of one year. One of the major impediments in treating breast brain metastases is presence of blood-
brain/tumor barrier that limits the permeability of chemotherapeutic drugs into the brain parenchyma. Understanding the mechanisms that regulate blood-
brain/tumor barrier permeability in context of brain metastases is imperative to developing successful therapy. Methods: Mouse models with brain-tropic sub-
lines of MDA-MB-231 (231), JIMT-1, and SUM190 were used to generate breast
cancer brain metastases. Using laser capture microscopy, the permeable and non-permeable lesions from mouse brains were isolated and profiled for gene expression using microarrays. Immortalized human brain endothelial cells, astro-
cytes, and pericytes were used for developing in vitro blood-brain/tumor barrier model along with spheres generated with 231-BR6 or JIMT-1/BR3 cells. Secreted cytokines were evaluated using human cytokine profiler. Transendo-
thelial electrical resistance (TEER) was measured using EVM2 volt/ohm me-
ter. Results: Gene expression profiling and immunostaining of mouse brains, harboring breast cancer metastases showed that astrocytes at permeable regions express elevated S1P3. Pharmacological inhibition of S1P3 using antagonist TY-
52156 (10mg/kg) in mice bearing 231-BR6 brain metastases showed reduction in 3KDa Texas red dextran (TRD) uptake. To investigate the role of S1P3 in regulating barrier permeability, we established in vitro blood-brain/tumor bar-
rier models. Treatment of astrocytes with TY-52156 (2μM) significantly in-
creased mean TEER values (33.9 to 55.8 Ω.cm²; p<0.001, after 24 hrs), while there was a decrease in permeability for TRD (1.9 fold; p<0.001) and doxorou-
bicin (1.3 folds; p<0.05). Immunostaining on endothelial monolayer showed increased membranousZO-1 and VE-cadherin expression. The dynamics of increase in TEER was faster when 231-BR6 spheres were included. We observed similar results when S1P3 was knocked down using shRNA. Astrocytes with down-modulated S1P3 showed decreased secretion of S1P3, including
IL-6, IL-8, CCL2, CXCL1, and GM-CSF. Inhibition of these cytokines indi-
vidually using neutralizing antibodies recapitulated the effects of S1P3 inhibi-
tion, while treatment of endothelial monolayer with activated cytokines increased the permeability. This study provides a proof of concept for role of S1P3 and downstream cytokine signaling in regulating blood-brain/tumor bar-
rier permeability in breast cancer brain metastases. Conclusion: Our study shows that astrocytic S1P3 regulates blood-brain/tumor barrier permeability in breast cancer brain metastases by modulating cytokine secretion. This observa-
tion might lead to discovery of novel strategies for augmenting drug efficacy.

#4331 Novel Wnt-SCD-LRP5/6 pathway linking liver fibrosis to cancer. Keane Lai1, Sook-Mi Kweon2, Feng Chi1, Edward Hwang3, Raymond Wu1, Ya-
suaki Kabe1, Ramachandran Murali1, Lopa Mishra3, James Ntambi3, Hidekazu
Tsuchimoto1, University of Southern California, Los Angeles, CA; 2Keio Univer-
sity School of Medicine, Tokyo, Japan; 3Cedars Sinai Medical Center, Los Angeles, CA; 4George Washington University, Washington DC; 5University of Wisconsin-
Madison, Madison, WI.

Activated hepatic stellate cells (HSCs) are primarily responsible for the gen-
esis of liver fibrosis which promotes liver tumour development. Stearoyl-CoA desaturase (SCD) which synthesizes monounsaturated fatty acid (MUFA such as oleic acid or palmitoleic acid), is implicated in metabolic syndrome, tumori-
genesis and stemness. Indeed, we demonstrate SCD upregulation in both HSCs and liver tumor cells in patients. Further, SCD correlates with the advancing grade of HCC and mortality in patients. However, its causality in tumorigenesis and mesenchymo-tumor crosstalk, and underlying mechanisms, are unknown.

The present study aimed to determine whether and how SCD promotes and links liver fibrosis and cancer. In both HSCs and liver tumor-initiating stem cell-like cells (TIC), we reveal that the Wnt effector β-catenin is a potent co-
activator for SREBP-1-dependent Scd transcription as demonstrated by pro-
The role of fibroblasts in prostate cancer cell invasion in tumor microenvironments. Tarir Shah, Florena Wildes, Dmetry Artemov, Zaver M. Bhujwalla. Johns Hopkins Univ. School of Medicine, Baltimore, MD.

Cancer-associated fibroblasts (CAFs) play a critical role in tumor aggressiveness. We have started to investigate the role of CAFs in different tumor microenvironments of hypoxia and acidic extracellular pH. To further understand interactions between CAFs and cancer cells under carefully controlled conditions of hypoxia and acidic pH, we have used our MR compatible cell perfusion system to determine, for the first time, changes in the ability of prostate cancer cells to invade and degrade the extracellular matrix (ECM) in the presence of prostate myofibroblasts. Experiments were performed using the human prostate cancer cell lines PC-3 and prostate myo-fibroblasts (WPMY-1, ATCC, Manassas, VA). Before each MR experiment, 2.5×10⁶ PC-3 cells were seeded on 0.5 ml of Plastic Plus beads in five 100mm dishes and grown for 4 days. Experiments were carried out with PC-3 cells plated on an ECM chamber with or without prostate myofibroblasts layered between the PC-3 and the ECM. For experiments investigating prostate myofibroblasts-cancer cell interaction, 5×10⁴ prostate myofibroblasts were seeded on ECM gel contained in a chamber overnight before the MR experiment. This time interval allowed myofibroblasts to migrate to the ECM and establish a gradient. Real-time 3D perfusion MRI experiments were performed to compare invasion and proteolytic parameters between control and CAFs treated samples.
Human mesodermal-derived CNS metastasis-associated stromal cells induce a fibrotic response to limit tumor growth. Christoph Legende, Gerald C. Gooden, Kyle N. Johnson, Rae Anne Martinez, Mark Bernstein, Gerald C. Gooden, Kyle N. Johnson, Rae Anne Martinez, Mark Bernstein, Jennifer Glenn, Jeffrey Kiefer, Aleksander Hinek, Steven A. Toms, Bodoor Salhia. Translational Genomics Research Institute, Phoenix, AZ; University of Southern California, Los Angeles, CA; University Health Network, Toronto, Ontario, Canada; Hospital for Sick Kids, Toronto, Ontario, Canada; Lifespan Health System, Providence, RI.

Metastasis to the nervous system (CNS) remains a major cause of mortality and morbidity in patients with systemic cancer. However, the mechanistic interactions of the neural niche with disseminated tumors in CNS metastases (CM) are poorly understood. To better understand the crosstalk between the neural niche and metastatic tumors, we generated five different patient-derived cell lines (PDCs) originating from surgically resected CM. To assess the genetic and epigenetic characteristics of each PDC, DNA and RNA sequencing, and DNA methylation analysis was performed. Non-tumoral PDCs revealed normal copy number profiles, and retention of germline mutations as seen in patient-matched germine DNA. In contrast, one PDC (CM04) resembled its patient tumor, showing numerous copy number and somatic alterations. RNA-seq and DNA methylation analysis demonstrated that non-tumoral PDCs highly resembled each other, suggestive of a common cell of origin. Additionally, PDCs revealed gene expression signatures associated with cancer associated fibroblasts, epithelial to mesenchymal transition, and mesenchymal stem cells. Further in vivo studies demonstrated that CM04 cells were tumorigenic, whereas non-tumoral PDC (CM08) cells were unable to form tumors in nude mice. However, CM04:CM08 mixed tumors were significantly smaller than CM04 only tumors and revealed induction of a fibrotic response by immunohistochemistry. These data offer the first evidence that CNS metastasis-associated stromal cells (cMASCs) produce a collagen and fibronectin-rich extracellular matrix constituting a protective host response, which impedes growth of tumor cells. The therapeutic potential of these cells merits further exploration.

PDGFR-α induced stiffness abrogates mammary ductal development and enhances tumorigenesis in vivo. Anisha Mathur Hammer, Gina M. Sizemore, Vasudha Shukla, Steven T. Sizemore, Maria Cuitino, Cynthia J. Timmers, Quinn Verfurth, Arnab Chakravarti, Gustavo W. Leone, Samir N. Ghadially. The Ohio State University, Columbus, OH.

Breast cancer is a leading cause of mortality in women worldwide, in part due to the tumor microenvironment which increases tumor heterogeneity and abets tumor growth. Fibroblasts are cells of mesenchymal origin that are an important component of normal and tumor stroma. Genetic alterations in these cells were shown by several groups including our own to cause fibroblast activation and fuel tumor progression giving rise to more aggressive disease. Platelet-Derived Growth Factor Receptor (PDGFR) alpha is a receptor tyrosine kinase that is auto-activating Pdgfra mutant allele with a mesenchymal specific Cre recombinase. We found that stromal PDGFRα activation completely abrogated postnatal mammary gland ductal formation, with significantly reduced terminal end bud formation. PDGFRα activation also led to progressive fibrosis in the mouse mammary fat pad. As early as four weeks of age, mammary collagen (trichrome staining; second harmonic generation) and hyaluronic deposition (Alician Blue) was greatly increased in vivo. In fact, this increase in collagen and hyaluronic deposition in mutant animals is believed to be responsible for the observed increased stiffness. We further discovered that in HER2+ patients, PDGFRα levels correlate with breast density. Breast density is the third strongest risk factor for breast cancer, and is directly related to collagen deposition and breast stiffness, thus suggesting a novel predictive role of PDGFRα as a molecular readout of stiffness and densification. Studies are underway to utilize mouse models of HER2+ breast cancer to study both primary tumor growth and metastases. Taken together, our mouse studies and parallel human data analyses suggest that the stromal PDGFRα signaling provides a novel theranostic window in breast cancer treatment and prognosis. The role of IMPACT in survival of cancer cells during tryptophan deprivation by immunosuppressive enzyme indoleamine 2,3-dioxygenase (IDO1). Petr Tomek, Ariane Hallermayr, Michael Kilian, Cristina Gregor Print, Lai-Ming Ching, University of Auckland, Auckland, New Zealand; Ludwig Maximilian University, Munich, Germany; German Cancer Research Center (DKFZ), Heidelberg, Germany.

A broad range of human malignancies overexpress an enzyme called indoleamine 2,3-dioxygenase 1 (IDO1) to suppress antitumor immunity which associates with poor patient prognosis. One mechanism whereby IDO1 activity suppresses the host’s immune cells is mediated by deprivation of the essential amino acid tryptophan (Trp) which raises an intriguing question. How do the cancer cells overcome low levels of the essential amino acid when the immune T-cells are triggered to self-destruct? A protein called IMPACT (product of IMPrinted gene with AnCienT domain) was reported to confer skin cells and neuronal cells increased survival in Trp-deprived environments. Based on these reports, we hypothesize that cancer cells hijack IMPACT to gain survival advantage in Trp-deprived environments. To obtain evidence for this hypothesis, we analysed publicly available genomic datasets and performed experimental assays. Murine GL261 glioblastoma cells overexpressing a full-length mouse IMPACT gene (GL261-IMPACT) were generated by lipofection. IMPACT overexpression was confirmed by Western blot. Metabolic activity and survival of the GL261 cells in Trp deprivation experiments were evaluated using MTT assay and staining with live/dead indicators fluorescein diacetate and propidium iodide, respectively. Meta-analysis of the publicly available RNA-sequencing datasets, The Cancer Genome Atlas (TCGA) and Genotype Tissue Expression (GTEx), provided evidence that IMPACT was overexpressed in the majority of the 24 analysed human cancers compared to their non-malignant tissue counterparts. Consistent with the high IMPACT expression in prostate cancer patients, 12-25% prostate tumors showed amplification of the IMPACT gene (source: cBioPortal for Cancer Genomics). In addition, we found an association of high IMPACT expression with poorer survival for certain leukemia and lung cancer patients in the Gene Expression Omnibus (GEO) portal. To obtain experimental evidence that IMPACT protects cancer cells during Trp deprivation, GL261-IMPACT cells were cultured in media containing 2.5 - 50 μM Trp. Metabolic activity of GL261-IMPACT cells decreased at a slower rate than that of GL261-wild-type cells after 3 to 5 days in media containing limiting Trp concentration (2.5 - 10 μM). On day 5, GL261-wild-type and GL261-IMPACT cells cultivated in 10 μM Trp showed 4.5% and 23% metabolic activity (p = 2.8x10^-9), respectively, relative to the same cells grown in 50 μM Trp. Fluorescence microscopy utilising live/dead staining showed that after 5 days of incubation at limiting Trp concentrations (5 - 10 μM), the majority of GL261-wild-type cells were non-viable whereas GL261-IMPACT cells were predominantly viable. Our initial results support the hypothesis that IMPACT aids cancer cells to overcome periods of tryptophan deprivation.

Single cell RNA sequencing dissect cell growth factor dependency and oncogenic driver effects in an organoid model of gastric cancer. Jiamin Chen, Noemi Andor, Susan M. Grimes, Billy Lau, Hanlee P. Ji. Stanford University Medical School, Stanford, CA.
Gastric cancer is a lethal malignancy with few therapeutic options. Gastric tumors rely on complex intercellular signaling “crosstalk” that enables tumor development, metastasis, and therapeutic resistance. To recapitulate the intercellular communications among various cell populations that exist in vivo, we are using a three-dimensional culture system to grow and manipulate mouse gastric tissue in vitro, otherwise referred to as organoids. This organoid model contains epithelium with its endogenous mesenchymal niche and does not require exogenous Wnt stimulation. To systematically analyze the distinct cellular lineages and their interactions, we applied a massively-scaled single cell RNA-Seq platform to sequence thousands of individual cells from organoid cultures. With PCA and t-SNE analysis of the high-dimensional data generated from single cell RNA-seq, we characterized two major cell types, i.e., epithelial and mesenchymal cells. Leverage the information from single cell transcriptome profiles, we identified specific niche factors of the Wnt signaling pathways that are activated in different stomach cell lineages. These results suggest that the mesenchymal cell populations provide a potential source of the R-spondin, a Wnt agonist, that sustains the growth of epithelium. Furthermore, we compared cell populations from Cdh1+/+/Trp53+/- and Trp53+/- organoids, and characterized changes on the transcriptome profiles due to the loss of Cdh1, an early oncogenic event in diffuse gastric cancer development. Overall, using organoid model and high-throughput single cell RNA-Seq provides a novel approach to study early tumor transformation and critical cancer-stroma interactions.

#4340 RHBDF2 in stromal fibroblasts mediates TGF-β signaling and enhances gastric cancer cell invasion via intercellular crosstalk. Takatsugu Ishibashi1,2, Hidehiko Takigawa1, Kenta Murayama2, Masakazu Yoshida2, Daisuke Izumi2, Kota Arima3, Yoshifumi Baba1, Masayuki Watanabe3, Kosei Hirakawa2, Hideo Baba2,1, Patrick Tan4.1 Kumamoto University, Kumamoto, Japan; 2Osaka City University, Osaka, Japan; 3The Cancer Institute Hospital of JCFR, Tokyo, Japan; 4Duke-NUS Medical School, Singapore, Singapore

Background: Cancer-associated fibroblasts (CAFs) have been reported to promote various types of tumor through secretion of soluble factors. Importantly, this orogenic model and high-throughput single cell RNA-Seq provides a novel approach to study early tumor transformation and critical cancer-stroma interactions.

#4341 Mesenchymal stem cells promote epithelial-mesenchymal transition of colon cancer cells via direct cell-to-cell contact. Hidehiko Takigawa1, Yashikko Kitadai2, Tosho Kuwai3, Ryo Yuge4, Shinji Tanaka1, Kazuki Chayama1.1 Hiroshima University, Hiroshima, Japan; 2Prefectural University of Hiroshima, Hiroshima, Japan; 3Kure Medical Ctr. & Chugoku Cancer Ctr., Hiroshima, Japan; 4Kumamoto University, Kumamoto, Japan

We previously reported that in an orthotopic nude mouse model of human colon cancer, clonally derived mesenchymal stem cells (MSCs) migrated to tumor stroma and promoted tumor growth and metastasis. We evaluated the proliferation and migration ability of cells directly and indirectly co-cultured to clarify the mechanism of interaction between cancer cells and MSCs. Proliferation and migration ability of cancer cells were increased by direct co-culture with MSCs but not by indirect co-culture of MSCs. Thus, we thought that direct contact between cancer cells and MSCs is important to their interaction. We performed microarray analysis of gene expression in KM12SM colon cancer cells directly co-cultured with MSCs. Expression of epithelial-mesenchymal transition (EMT) related genes such as fibronectin (FN), SPARC and Galectin 1 was increased by direct co-culture with MSCs. We also confirmed the upregulation of these genes with real time PCR, and these genes were not increased in cancer cells indirectly co-cultured with MSCs. Among these EMT related genes upregulated by direct co-culture with MSCs, we examined immune localization of FN, well-known EMT marker. In co-culture assay in chamber slides, expression of FN in cancer cluster was seen only at the edge where cancer cells directly contacted MSCs. Furthermore, when cancer cells were treated with TGF-β1, the periphery and invasive edge in orthotopic nude mice tumors and human colon cancer tissues, respectively. These results suggest that mesenchymal stem cells induce epithelial-mesenchymal transition of colon cancer cells via direct cell-to-cell contact and may play an important role of colon cancer metastasis.

#4342 Loss of MSLN impairs pancreatic cancer growth in the peritoneal cavity. Michael W. Rudloff, Daniella Arons, Salma El-Behaedi, Rakun Albalawy, Christine Alewine. National Cancer Institute, Bethesda, MD

Mesothelin (MSLN) is a cell surface glycoprotein that is expressed in at least 10% of pancreatic adenocarcinoma but not on cells of the healthy pancreas nor in the parenchyma of other vital organs. Due to its differential expression, MSLN has been used as a target for various antibody based treatments and cancer vaccines. The physiologic role of MSLN is unknown. Previous reports have suggested that overexpression of MSLN may increase tumorigenicity and metastatic potential of pancreatic adenocarcinoma. To further define the role of MSLN in tumor disease, we deleted MSLN in this model by injecting cancer cell line, KLM1, using CRISPR/Cas9 gene editing. Successful deletion of MSLN was confirmed via flow cytometry and immunoblotting. Furthermore, KLM1/MSLN cells were rendered invulnerable to the MSLN-targeted immunotoxin, RG7787, which depends on the presence of surface MSLN for cytotoxicity. In cell culture, KLM1/MSLN cells grew at the same rate as control cells. KLM1/MSLN formed more numerous tumors in nude mice with the same frequency as control cells. These tumors grew at the same rate. However, when nude mice were inoculated with KLM1/MSLN or control cells intraperitoneally, a marked decrease in tumor burden was observed in cells lacking MSLN. In summary, we have engineered a pancreatic cancer cell line that lacks MSLN and demonstrated that loss of MSLN impairs tumor growth and spread specifically within the peritoneal cavity. Further experiments are in progress to identify the factors contributing to this phenotype.

#4343 Effects of the co-expression of RANTES and IL-6 on the transformed phenotype of breast cancer cells. Marianna Gallo, Daniela Frezzetti, Nicola Normanno, Antonella De Luca. National Cancer Inst, Naples, Naples, Italy

Introduction: Cells of the tumor microenvironment play an important role in the progression of breast cancer through their interaction with cancer cells. In this regard, mesenchymal stem cells greatly increased the metastatic potential of breast cancer cells through secretion of soluble factors. However, there have been no systematic studies of CAFs in diffuse-type gastric cancers (DGCs). We investigated the characteristics and functional roles of CAFs in DGCs using comprehensive genomic approach. Methods: We established primary fibroblasts, normal fibroblasts (NFs) and CAFs from more than 100 tissue samples from GC patients. NFs/CAFs were subjected to Exome and RNA sequencing and messenger RNA expression analysis. Result among the acquired comprehensive data. The candidate molecules were examined for their role in gastric tumor progression by in vitro assays. Results: CAFs exhibited an invasive molecular pattern and acquired motility in extracellular matrix (ECM). We identified RHBDF2 as a mediator of TGF-β signaling and an enhancer of CAF motility. RHBDF2 silencing in CAFs significantly decreased their motility stimulated by TGF-β1 in ECM, whereas NFs transfected with the RHBDF2 expression vector displayed a greater motility in ECM than did control NFs. RHBDF2 silencing also decreased type I TGF-β receptor (TβRI) cleavage through tumor necrosis factor (TNF)-α converting enzyme (TACE) activity and attenuated the invasive molecular pattern of CAFs. Consequently, high-motility CAFs confer on DGC cells the ability to invade the ECM. Furthermore, similar behavior was observed in the interaction of the chemokine CCL5/RANTES. In addition, we demonstrated that breast cancer cells migrate in response to either recombinant RANTES or interleukin-6 (IL-6). Starting from this observation, we analyzed whether the co-expression of RANTES and IL-6 induced a more aggressive phenotype in breast cancer cells. Methods: MCF-7 and MDA-MB-231 breast cancer cells were transfected with expression vectors coding for human MSLN and RANTES. In addition, we demonstrated that breast cancer cells migrate in response to either recombinant RANTES or interleukin-6 (IL-6). Starting from this observation, we analyzed whether the co-expression of RANTES and IL-6 induced a more aggressive phenotype in breast cancer cells. Methods: MCF-7 and MDA-MB-231 breast cancer cells were transfected with expression vectors coding for human RANTES and IL-6. XMAP Bio-Plex Cytokine arrays were employed to measure the levels of secreted RANTES and IL-6. To evaluate the proliferation of stable clones, anchorage-independent growth assays were used. Migration and invasion were analyzed using commercially available kits. Tumor growth in vivo was determined after injection of transfected cells in the mammary fat pad of nude mice. Results: MCF-7 and MDA-MB-231 clones that stably overexpressed RANTES and/ or IL-6 were isolated after selection with appropriate antibiotics. Stable transfecants produced higher levels of secreted RANTES and IL-6 than control cells transfected with mock vectors. To evaluate whether RANTES and IL-6 induced a more aggressive phenotype in breast cancer cells, we analyzed the proliferation, migration and invasive ability of stable clones. MCF-7 cells co-expressing RANTES and IL-6 had a greater ability to form colonies in soft agar, compared with cells overexpressing RANTES or IL-6. In addition, both MCF-7 and MDA-MB-231 clones co-expressing RANTES+IL6 showed a significantly higher ability to migrate through a fibronectin-coated and to invade through a matrigel-coated matrix. The analysis of phosphorylated ERK1/2, AKT and STAT3 signal transduction proteins revealed that in cells overexpressing both RANTES and IL-6 more signaling pathways are simultaneously activated as compared with cells overexpressing RANTES or IL-6. In addition, both MCF-7 cells co-expressing RANTES+IL6 showed a significant increase in in vivo tumor growth when clones were injected in the mammary fat pad of nude mice. Conclusions: Taken together, our data suggest that the simultaneous expression of IL-6 and RANTES produce a more aggressive phenotype in breast cancer cells. This ob-
servation might be useful for the identification of potential targets for novel therapeutic strategies aimed to prevent breast cancer progression through the blockade of the tumor-stroma interaction.

**#4344** Cd146 modulates the malignant phenotype in human prostate cancer. Eugenio Zoni,1 Letizia Astrologo,1 Janine Melsen,2 Irene Klima,3 Joel Grosjean,1 Peter C. Gray,2 Gabrie van der Pluijm,3 Marco G. Cecchini,1 Marianna Krutikov-de Julio,1 George N. Thalmann2,3 University of Bern, Bern, Switzerland; 2 Leiden University Medical Center, Leiden, Netherlands; 3 Salk Institute for Biological Studies, La Jolla, CA.

Prostate Cancer (PCA) is the most frequent cancer in males and the second leading cause of death from cancer in men. When PCA progresses from androgen-responsive to androgen resistance, the formation of incurable metastases mainly in the bone, is almost inevitable. Therefore, understanding the factors that regulate homing and survival of metastatic cancer cells in the bone is important for the identification of new therapeutic targets. High Cd146 expression has been measured in the stroma of lytic and blastic lesions in preclinical models of PCA bone metastasis. The objective of this study is to characterize the role of Cd146 in the maintenance of the aggressive and invasive phenotype in human PCa. We used shRNAs to knockdown the expression of Cd146 in the lytic PC-3/M-Pro4Luc2DTomato and in the blastic C4-2BdTomato PCA cell lines. We validated the knockdown at protein level and tested the effect with functional assays such as migration, proliferation. We used RT-qPCR to test Cd146 knockdown on EMT markers. We measured the effect of the knockdown on the maintenance of cancer stem/progenitor-like cells by ALDEFLUOR assay. Cd146 knockdown reduced proliferation in PC-3/M-Pro4Luc2DTomato PCA cells and resulted in increased E-Cadherin expression. Conversely, no effect on proliferation was measured on C4-2BdTomato cells. It has been described that metastatic human PCA cells target the hematopoietic stem cell (HSC) niche in the bone marrow at the level of an “endosteal/osteoblast” niche and a “vascular/perivascular” niche. We optimized an in vitro model of “osteoblast niche” to study the behavior of prostate cancer cells upon co-culture with osteoblasts and to measure the resulting effects on cancer stem/progenitor-like markers. Our results showed that Cd146 is required for the osteoblast-mediated induction of ALDH activity on PCA cells and Cd146 knockdown prevented the increase in the size of the ADIPLC-5A subpopulation in the tumor cells, mediated by human osteoblasts. Additionally, Cd146 knockdown in PCA cells co-cultured with osteoblasts, reduced the amount of Cd146 expressed by osteoblasts compared to non-targeted control. Finally, we showed that Cd146 is significantly increased in the highly metastatic ALDH<sup>high</sup> cells and identified a new subset of ALDH<sup>high</sup>/ Cd146<sup>high</sup> cells which could be depleted upon Cd146 knockdown. In Conclusion, we detected a novel subset of ALDH<sup>high</sup>/Cd146<sup>high</sup> cells and found that Cd146 influences the maintenance of an aggressive-mesenchymal phenotype in human PCa. Therefore, Cd146 represents a promising molecule to modulate the behavior of aggressive PCA cells.

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**#4345** Epigenetic dysregulation of transcriptional program in MLL-EEN leukemia. Xianwen Yang, Yuk Man LAM, Wing Chi Lui, Ray Kit Ng, The University of Hong Kong, Hong Kong, Hong Kong.

Aberrant DNA methylation is a typical feature of cancers, which is usually associated with deregulation of tumor suppressor genes and oncogenes. Acute myeloid leukemia (MLL)-rearranged leukemia is characterized by the presence of MLL fusion proteins resulting from chromosomal translocation between MLL and its partner genes. The MLL fusion proteins have lost the MLL histone methyltransferase SET domain, leading to alteration of epigenetic functions. Many genome-wide DNA methylation profiling studies showed significant global alteration in MLL-rearranged leukemia patients, suggesting that MLL fusion proteins mediate leukemia through dysregulation of DNA methylation status. Our previous work showed that MLL-EEN fusion protein can enhance the self-renewal capacity of murine hematopoietic progenitor cells through mediating DNA hypomethylation at Hoxa family gene promoters. Nevertheless, how the aberrant DNA methylation alters the transcriptional program in MLL-EEN leukemia remains unclear. We performed RNA-seq to study the transcriptional program associated with MLL-EEN in murine hematopoietic stem and progenitor cells (HSPCs). We observed 1023 upregulated and 317 downregulated protein coding genes (PCGs) upon MLL-EEN overexpression. Interestingly, key regulators of DNA methylation, including Dnmt3a, Dnmt3b and Tet family genes, were downregulated, suggesting their involvement in the dysregulation of DNA methylation in MLL-EEN-driven leukemia. Gene ontology analysis showed that signaling pathways crucial to hematopoietic stem cell functions, such as Wnt and Nfkb, were significantly enriched. Motif enrichment analysis also showed that the promoters of dysregulated genes were enriched in binding sites of hematopoietic specific transcriptional factors, such as Pu.1 and C/EBP family. In addition, we have identified 3016 upregulated and 1357 downregulated long non-coding RNAs (lncRNAs) in MLL-EEN HSPCs. lncRNAs, Hotairm1 and linc-p21, which was previously reported playing important roles in hematopoiesis and leukemogenesis, were also found deregulated. Notably, the deregulated lncRNAs showed positive correlation with the downregulated (non-coding) PCGs. Moreover, we demonstrated the transcriptional regulatory role of lncRNAs. MBD-seq analysis showed global DNA hypermethylation in MLL-EEN HSPC. Promoters of genes involved in myeloid differentiation and cell cycle regulation were hypermethylated, presumably associated with their downregulation mediated by MLL-EEN. We are currently investigating how the aberrant DNA methylation mediated by MLL-EEN can affect lncRNA transcription, which subsequently alters the expression of PCGs and leads to leukemic transformation. Taken together, our study provides new insights into the epigenetic regulation of transcriptional program in MLL-rearranged leukemia, which could facilitate the development of novel therapeutic strategies.

**#4346** S-Adenosyl methionine (SAM) blocks breast cancer growth, invasion and metastasis in vitro and in vivo. Niaz Mahmood, David Cheeswili, Ani Arakelian, William J. Muller, Moshe Seyy, Shafaa A. Rabbani, McGill University, Montreal, Quebec, Canada.

DNA hypomethylation has been implicated in the coordinated targeting of various signaling pathways involved in tumor growth and metastasis. In the current study through various in vitro and in vivo assays, we have examined the plausibility of using universal methyl donor S-adenosyl methionine (SAM) for its ability to block breast cancer development, growth and metastasis in our xenograft and transgenic models of breast cancer. Treatment of highly invasive human triple negative breast cancer (TNBC) cell lines MDA-MB-231 and Hs578T with SAM resulted in a significant dose-dependent decrease in cell proliferation, invasion, migration, colony formation and increased apoptosis in vitro. Affymetrix gene expression array and real time PCR (qPCR) validated showed the ability of SAM to decrease the expression several genes implicated in cancer progression in MDA-MB-231 cells. For the in vivo studies, MDA-MB-231 cells expressing green fluorescent protein (MDA-MB-231-GFP) were inoculated into female CD-1 nude mice via mammary fat pad. From day three post tumor cell inoculation, animals were treated with SAM (0.8-1.6 mg/day) or vehicle alone as control via daily oral gavage and tumor volume was determined at weekly intervals for 10 weeks. SAM treatment caused a significant dose dependent decrease in tumor volume and GFP positive metastasis to lungs, liver and spleen in experimental animals compared to vehicle-treated controls. Analysis of RNA from primary tumors by qPCR showed the ability of SAM to cause a marked decrease in the expression of several pro-metastatic and EMT pathway genes. Pyrosequencing of tumoral DNA from control and experimental animals showed that SAM treatment causes a significant increase in the percentage of CpG methylation at the promoter region of several cancer-related genes (2.5 kb to lncRNAs), further demonstrating the epigenetic targeting of DNMT1 in adipocytes inhibits high-grade serous ovarian cancer cell migration and invasion through TIMP3 upregulation. Jessica Tang, Fang Fang, Aaron Buechlein, Pietro Taverno, Kenneth P. Nephew. Indiana University, Bloomington, IN.

Ovarian cancer frequently metastasizes to the omentum and adipocytes play a significant role in tumor progression. As methylation levels in obese adipose tissue are increased due to increased DNMT1 levels and activity, it was of interest to test the hypothesis that inhibiting DNMT1 would reverse adipocyte migration, alter adipokine secretion, and decrease migration and invasion of ovarian cancer cells towards adipocytes. Human adipocytes were seeded in a 24-well plate and treated with low-dose guadecitabine (100nM daily for 3 days). Ovarian cancer models of breast cancer where SAM treatment resulted in a significant delay in the development of mammary tumors and decreased tumor growth in experimental animals as compared to vehicle treated controls. SAM was found to be available in the serum of experimental animals as determined by mass spectrometry and no notable adverse side effects were seen including any change in animal behavior. Results from these studies provide compelling evidence for the therapeutic potential of SAM in breast cancer to provide the rationale for initiating clinical trials with SAM in patients with breast and other common cancers as monotherapy or in combination setting with current therapeutic agents to reduce cancer associated morbidity and mortality.

**#4347** Epigenetic targeting of DNMT1 in adipocytes inhibits high-grade serous ovarian cancer cell migration and invasion through TIMP3 upregulation. Jessica Tang, Fang Fang, Aaron Buechlein, Pietro Taverno, Kenneth P. Nephew. Indiana University, Bloomington, IN.
cancer cells (SKOV3, Kramocho), OVCAR4, OVCAR8) were seeded in the Boyden chamber and allowed to migrate or invade toward adipocytes for 8 and 16h, respectively. Expression of epithelial-mesenchymal transition (EMT) markers (SLUG, FN1, TWIST1) were assessed by qRT-PCR. Adipocyte-conditioned media was used to culture ovarian cancer cells in clonogenicity assay and a human adipokine array (R&D Systems) was used in vitro in adipocytes. Differential gene expression analysis revealed that adipocytes induce EMT-like cells. Most important, adipocytes may signal via TGF-β and activating epigenetic modifications to repress EMT and promote metastasis. Therefore, estrogens and adipocytes may cooperate in the development of drug resistance in prostate cancer.

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Cheishvili, Moshe Szyf. DNA methyltransferase (DNMT) isoform specific inhibitors: therapeutic implications. Furthermore, these findings may open new opportunities for the DNA methylome, providing new insights into the pathophysiology of UM.

**In vivo**

DNTM1 protein levels in adipocytes were determined by western blot. To determine a possible mechanism, DNA and RNA from guadecitabine-treated adipocytes were subjected to methylcapture-sequencing (MBD-seq) and RNA-seq, respectively. Guadecitabine treatment of adipocytes decreased (P < 0.05) migration of OVCAR4 and OVCAR8 (35% and 40%, respectively, compared to control), and a 90% decrease (P < 0.05) in invasion towards adipocytes. Guadecitabine treatment was observed for OVCAR4, OVCAR8, and Kramocho cells. Expression of EMT markers SLUG, FN1 and TWIST1 decreased (P < 0.05) after guadecitabine treatment. Conditioned media from guadecitabine-treated adipocytes decreased (P < 0.05) clonogenic survival by 18% compared to control.

Adipokine array results revealed increased secretion of LIF (lipoprotein lipase inhibitor) and TIMP3 (metalloproteinase inhibitor) after guadecitabine treatment (1.6- and 1.8-fold increase, respectively; verified by qRT-PCR). Treatment with recombinant TIMP3 (50M) decreased invasion of OVCAR8 (63%, P < 0.0001) and OVCAR4 cells (73%, P < 0.05). DNTM1 protein levels in adipocytes decreased in the presence of guadecitabine, despite the presence of a DNA synthesis inhibitor. Ingenuity Pathway Analysis (IPA) of MBD-seq data showed a significant change in cell-to-cell signaling and interaction pathways (FC > 10; FDR < 0.05). RNA-seq demonstrated increased expression of matrix metalloproteinase inhibitors (THSB2, TFPI2, and NDRG4) and IPA analysis revealed a significant change in regulation of EMT pathway (FC > 1.5; FDR < 0.05). Guadecitabine treatment of adipocytes alters adipokine secretion resulting in decreased cancer cell migration and invasion. In addition to direct effects on ovarian cancer cells, hypomethylating agents may impact the tumor microenvironment to alter adipokine secretion leading to decreased metastasis.

**#4348 Methylation analysis of uveal melanoma reveals definitive patterns in tumors harboring BAP1 mutations.** Michael Durante, Matthew Field, Stefan Kurtenbach, Parker Bussies, Christina Decatur, J. William Harbour. University of Miami Miller School of Medicine, Fort Myers, FL.

Introduction: Uveal melanoma (UM) is the most common primary intraocular malignancy and can be classified by gene expression profiling into two distinct molecular classes that correspond to metastatic risk: Class 1 (low risk) and Class 2 (high risk). The less aggressive Class 1 UMs express a more differentiated phenotype and exhibit numerous characteristics of normal uveal melanocytes, while the metastasizing Class 2 UMs display loss of melanocytic differentiation and have acquired a primitive, stem-like phenotype. The majority of Class 2 UM's harbor loss of chromosome 3 in addition to inactivating mutations of the tumor suppressor BAP1, which also catalyzes ubiquitin removal from histone H2A and modulates gene expression. Histone modifications have been shown to be associated with DNA methylation and this study investigates the global methylation and gene expression changes in BAP1 mutant tumors. Methods: RNA-sequencing and Illumina Human Methyl 450k BeadChip Array data obtained from human uveal melanoma cell lines analyzed using optimized pipelines. Results: This analysis revealed that the more aggressive Class 2 tumors exhibit a distinct pattern of hypermethylation and silencing of developmental genes involved in neural crest migration and differentiation. Chromosomal regions that were significantly enriched for hypermethylated genes with decreased gene expression in Class 2 tumors included chromosome 3p14-26, 3q12-29 and 8p12-22, whereas the only significantly enriched region after genes that were hypomethylated with increased gene expression in Class 2 tumors was chromosome 8q22-24. Additionally, BAP1 itself was differentially hypermethylated in Class 2 UM’s, suggesting that it may regulate its own transcription. Conclusions: Class 2 UM’s harboring BAP1 mutations displayed distinct regional changes in DNA methylation compared to Class 1 UM’s. In these tumors, the most significantly hypermethylated regions occurred on chromosome 3 and in regions that were enriched for genes encoding neural crest guidance cue proteins that regulate homing, migration and invasion. These findings suggest that BAP1 mutations are associated with marked reorganization of the DNA methylome, providing new insights into the pathophysiology of UM.

**#4349 Adverse & anticancer activities of 5-azaCdr & DNA methyltransferase (DNMT) isoform specific inhibitors: therapeutic implications.** Cheishvili, Moshe Szyf, McGill, Montreal, Quebec, Canada.

Activation of methylated and silenced promoters of genes that suppress tumorigenesis has been the rationale behind the clinical use of DNMT family inhibitor 5aza for treating cancer. However, the adverse effects of general DNA methylation inhibition on tumors through activation of tumor promoting and prometastatic genes as well adverse effects on normal cells have limited the clinical development of DNMT inhibitors. Our data needs to be considered for further clinical development of DNMT inhibitors.

**#4350 Cell-origin differentiation stages define methylation-based subtypes of human colorectal cancer.** Felix Bormann, 1 Manuel Rodriguez-Paredes, 1 Yehudit Bergman, 2 Heinz G. Linhart, 3 Frank Lyko. 1 German Cancer Research Center, Heidelberg, Germany; 2 Institute for Medical Research Israel-Canada, Hebrew University Medical School, Jerusalem, Israel; 3 Asklepios Klinik Lindau, Lindau, Germany.

Introduction: Colorectal cancer accounts for a significant fraction of cancer-related mortalities, but has proven to be surprisingly refractory to consensus pathological and molecular subclassification. Similarly, little is known about the molecular profiles of colorectal cancer cells-of-origin. DNA methylation is an important epigenomic marker of cellular identity and can therefore be used to infer cancer cells-of-origin. Methods: Infinium 450k data from colorectal cancer samples provided by “The Cancer Genome Atlas” was applied to a consensus clustering algorithm. The identified epigenetic subtypes were tested for features including well established clinical parameters or subgroup specific gene expression. Additionally, the subgroups were compared with Infinum 450k data of non-malignant colorectal adenomas. Finally, clinical significance was addressed by testing for subgroup specific overall survival rates. Results: Analysis of the TCGA dataset defined 5 distinct epigenetic subtypes of human colorectal cancer. These subgroups showed an overlap to the microsatellite instability phenotype and the CpG island methylator phenotype. Gene expression analysis revealed that the subtypes also form a continuum of epigenetic program reflecting various intestinal crypt cell differentiation stages. Patient survival correlated with the differentiation stage, with a particular poor prognosis for patients with stem cell-related signatures. Interestingly, its prognostic potential outperformed a recently established gene expression-based classifier. Finally, non-malignant colorectal adenomas could be classified into the same epigenetic subtypes, suggesting their shared cell-types of origin with carcinomas. Conclusion: Our results establish a novel and clinically relevant approach for colorectal cancer classification and illustrate how differences in the cell-type of origin shape the tumor methylome.

**#4351 Effects of epigenetic agents on methylation of DNA in vitro and in vivo, as measured with stable isotopically labeled methionine.** Lawrence Anderson, 1 Eva Majerova, 2 Kimberly D. Hill, 3 John Carter, 2 Jessie Stottlemeyer, 2 Howard Stotler, 3 Melinda G. Hollingshead, 4 Jerry M. Collins, 4 NCI, Frederick, MD; 5 FNLCR Leidos Biomedical Research Inc., Frederick, MD.

Background: Modulation of cytidine methylation in DNA continues to be actively investigated as a target for chemotherapy. The primary source of methyl groups is methionine, via S-adenosyl-methionine (SAM) as the direct methyl donor. However, the primary source of methyl groups is methionine, via S-adenosyl-methionine (SAM) as the direct methyl donor.
donor for DNA methyltransferases, e.g., DNMT1. Methods were developed using stable isotypes and mass spec detection to examine the flux of the DNMT reaction and modification to this flux by cytidine analogs such as decitabine, an established hypomethylating agent, and 5-aza-4'-thio-2'-deoxy-cytidine (AzATdC), currently under development at NCI. Methods: For in vitro studies; A549, H460, HEL, LNCaP, and OVCA433 cells were grown in RPMI supplemented with 10% FBS were exposed to D3-methionine(20uM) and either decitabine or AzATdC (NSC775806) at 0 to 5uM in the media for 0 to 48hr. For in vivo studies; mice were maintained under standard ACUC protocols and practices. Tumors were grown SC in the flank area. Stable labeled methionine, decitabine and AzATdC were administered as oral bolus. At set times, mice were sacrificed and tumors, tissues and plasma samples collected. DNA was isolated and purified from cells and tissues using PureLink Genomic DNA kits (Invitrogen) according to manufacturer’s instructions. The purified DNA was digested to nucleosides for analysis. The isotopic enrichment in 5-methyl-deoxy-cytidine (mdc) was determined by analysis using HPLC coupled to a Qq mass spectrometer (Thermo Scientific). Results: D3-methionine was found to be an efficient precursor via SAM for the methylation of cytidine in DNA. Fortification of basal media (100uM methionine) with 20uM D3-methionine yielded D3-mdc enrichment of 5-15% in 24 hr, depending on cell line. All cell lines were found to have some enrichment. Treatment of cells with either decitabine or AzATdC caused a concentration dependent reduction of the enrichment for mdc in DNA. In mice, after an oral dose of D3-methionine, enrichment of methionine and SAM in plasma peaked within 1hr and fell to 5% of peak levels within 5hrs. Methionine and SAM enrichment in tissues followed similar time curves. At 50mg/kg of D3-methionine, no perturbation was observed for endogenous levels of either methionine or SAM. Enrichment of mdc in DNA was detectable in 1hr, maximal in 3hr, and remained constant for up to 48hr. When mice were treated with 1-2 mg/kg of either decitabine or AzATdC along with D3-methionine, the level of enrichment of mdc in DNA was reduced by 20-70% in tumors, bone marrow and intestine. Conclusions: Stable labeled methionine can be efficiently used to monitor the flux of methyl groups into mdc in DNA, both in vitro and in vivo. Changes in the flux of the methylation reaction can be used to assess the extent and time course for inhibition caused by cytidine analogs. These techniques may have relevance for improvement of doses and schedules to produce epigenetic modulation. Funded by NCI Contract No. HHSN261200800001E

#4352 Identification of ZFP42/REX1 as a regulator of cancer stemness in CD133+ liver cancer stem cells by genome-wide DNA methylation analysis
Steve Tin-Chi Luk,1 Man Tong,1 Kai Yu Ng,1 Kevin Yuk-Lap Yip,2 Xin Yuan Guan,1 Stephanie Ma,1 1The University of Hong Kong, Hong Kong; 2The Chinese University of Hong Kong, Hong Kong.

The cancer stem cell (CSC) subset is well established in hepatocellular carcinoma (HCC). This subset can be characterized by its CD133+ expression. Since the discovery of this marker, studies have revealed deregulated pathways and epigenetic alterations associated with this subset, but few have focused on changes in DNA methylation. This study aims to fill this research gap by discovering aberrant DNA methylation patterns governing CD133+ CSC-driven HCC. Results from Infinium HumanMethylation450 BeadChip (HuMet450 BeadChip) have led to the discovery of differential methylation patterns between CD133+ liver CSCs and matched CD133- differentiated samples. ZFP42/REX1 is one of the top-ranking differentially methylated candidates. Protocols corresponding to its promoter are extensively hypermethylated in the CD133+ samples. To explore the hypothesis that promoter DNA hypermethylatation modulates REX1 expression, REX1 mRNA levels were first validated to be down regulated in sorted CD133+ HCC cell lines (n=2) and HCC samples (n=46, p<0.05) by RT-qPCR. Upon 5-AZA treatment, only low REX1-expressing cells re-expressed REX1. By in silico prediction, a CpG island of interest derived transfection domain is transfected into cancer cells. This coincided with tumor suppressor function in cancer. We selected tumor suppressor gene SEMA3A as a regulator target for further investigation. As array data indicated, resveratrol led to reduction in methylation of SEMA3A promoter region. The 18% decrease in methylation of SEMA3A was confirmed by pyrosequencing in MCF10CA1a breast cancer cells. This coincided with 23% up-regulation of SEMA3A expression. In addition, analysis of the effects on DNA methyltransferases (DNMTs) demonstrated that resveratrol decreases expression of DNMT3. Furthermore, ChIP indicated decreased occupancy of DNMT3A on SEMA3A, active histone mark, H3K9ac, was significantly increased and repressive histone mark, H3K27me3, was significantly decreased after resveratrol treatment, indicating an open, more transcriptionally active chromatin state. These results demonstrate a role for polyphenol-mediated epigenetic modifications in reactivation of tumor suppressor genes in cancer cells and pave the way for further studies on the mechanism driving these changes. This study was supported by the PCCR, Indiana CTSI (UL1TR001108), Women’s Global Health Institute, and USDA National Institute of Food and Agriculture (Hatch project 1005656) granted to B5.

#4354 Methylation-mediated silenced PYCARD plays a key role in human prostate cancer.
Shinichi Fukushima, Toshiya Miyauchi, Tepppei Okubo, Koji Mitsuzuka, Yoichi Arai, Akira Horii, Tohoku Univ. Graduate School of Medicine, Sendai, Japan.

Epigenetic gene silence by aberrant DNA methylation leads to loss of key cellular pathways in tumorigenesis. In order to analyze effects of DNA methylation in prostate cancer, we constructed LNCaP-derived human prostate cancer cells that can induce global reactivation of hypermethylated genes by the methyl-CpG targeted transcriptional activation (MeTa) method. In MeTa, a cassette driven methyl-CpG binding domain (MBD) with NfKBe-2 transcriptional activation domain is transfected into cancer cells. Then 2 MBD2-derived methyl-CpG binding domain (MBD) with NfKBe-2 transcriptional activation domain is transfected into cancer cells. Then, in a cassettespecifically binds to the hypermethylated promoter regions, and NfKBe-2 transcriptional activation domain recruits p300/CREB-binding protein (CBP) and reactivates hypermethylated silenced genes. A cell proliferation assay indicated that MeTa suppressed the growth of LNCaP cells. Furthermore, both flow cytometry and TUNEL assays demonstrated that MeTa-induced apoptosis. In order to search genes responsible for apoptosis, we performed gene expression microarray analysis of MeTainduced and -repressed LNCaP cells. These genes encoding apoptosis-inducing factors upregulated two-fold or more in accordance with the induction of MeTa; PYCARD (PYD and CARD domain containing), TNFRSF25 (tumor necrosis factor receptor superfamily 25), HKR (harakiri, BCL2 interacting protein), BIK (BCL2 interacting killer), and CIDEA (cell death-inducing DFFA-like effector a). These genes also contained CpG islands (CGIs) within ± 1,000-bp of the
to matched, normal thyroid tissue. A similar trend was also observed in about 500 PTC samples in the TCGA database, compared with 60 normal thyroid samples. Methylation was explored as a cause for the down-regulation of the AR mRNA in disease pathogenesis, using in-silico methods such as Wanderer, a Maplab tool for TCGA RNA data visualization, and MethyPlotter. A methyl-
ylation-mediated silencing of PYCARD contributes to escape from apoptosis in human prostate cancer, and MeTA may provide important clues to analyze changes in cancer cell phenotypes by DNA methylation alterations.

#4355 Altered DNA methylation in breast milk from women with breast cancer. Lucas A. Salas,1 Sara N. Lundgren,1 Eva P. Browne,1 Elizabeth C. Pun-

#4356 Methylation and expression of androgen receptor in Papillary Thy-

#4357 ESRP1 overexpression and its role in ovarian cancer. Mi Jeong

#4358 The immunomodulatory anticancer agent RRx-001 induces a vac-

transcription start site. We focused on PYCARD and TNRFSE25 because these two genes upregulated 10-fold or more in accordance with the induction of MeTA. We found that both genes were hypermethylated and showed low expression levels in LNCaP prostate cancer cells, whereas only PYCARD was unmethylated in RWPE-1 normal prostate epithelial cells. We further analyzed primary subcutaneous tumor samples and found the AR gene was methylated in the expression of PYCARD in 53.8% (7/13; P < 0.01). These results suggest that methylation-mediated silencing of PYCARD contributes to escape from apoptosis in human prostate cancer, and MeTA may provide important clues to analyze changes in cancer cell phenotypes by DNA methylation alterations.

Prior candidate gene studies have shown that methylation-induced silencing of tumor suppressor genes in breast milk is related with history of breast biopsy, an established risk factor for breast cancer. To further establish the utility of breast milk as a tissue-specific biospecimen for investigations of carcinogenesis we measured genome-wide DNA methylation in breast milk from women with and without a diagnosis of breast cancer. Breast milk provides a source of cells without invasive procedure that is available during a window of increased breast cancer risk. Actively lactating women were enrolled and consented through the University of Massachusetts Amherst breast milk study targeting high risk subjects. Participants completed a questionnaire to provide demographic and breast cancer risk factor data. Genome-wide DNA methylation levels were quantified using the Illumina 450K HumanMethylation array. Data was processed in R using RnBeads. Beta-values were normalized (FUN-
norm), low quality probes were filtered and transformed to M-values. Linear mixed effects models were fit including a random effect for subject and adjusted for history of breast biopsy, age, body mass index, age of the baby, and estimated cell type proportions, an interaction term for time of diagnosis was included. DNA methylation in women with breast cancer (n=9), was compared with women without a history of breast cancer (n=22). Among women with a diagnosis of breast cancer there were two groups: those diagnosed within one year of donating breast milk, and those diagnosed more than one year before sample collection. Four women in the control and five women in the cancer group provided bilateral breast milk samples at recruitment and both samples were included in the analysis. CpGs with significant differential methylation were tested for enrichment in gene pathways using the KEGG database. We identified 215 differentially methylated CpG sites in women diagnosed within one year of sample donation compared with women without a diagnosis of breast cancer (Q<0.05). The top sites were in ATRE3 (an adaptively responsive gene linked to epithelial mesenchymal transition), and COL5A3 (an extracellular matrix gene related to metastasis potential). In addition, among women with a breast cancer diagnosis more than one year before sample donation we identified 8148 differentially methylated CpG sites compared with women without a breast cancer diagnosis (Q<0.05). Five sites were in OT05 (a calcium-sensing protein in cancer), high risk subgroups differentially methylated sites were significantly enriched in relevant pathways such metabolic (Q=3.12E-116), and cancer pathways (Q=2.08E-58), PI3K-Akt signaling pathway (Q=7.05E-40), and transcriptional regulation in cancer (Q=1.41E-29). Future studies are needed to validate our findings in independent subjects and to investigate the utility of breast milk as a biospecimen for understanding the molecular basis of disease risk and prospec-
tive risk assessment.

Methylation and expression of androgen receptor in Papillary Thy-
roid Cancer. Anvita Gupta,1 Tommy O’Connell,1 Melanie Jones,1 Karnika Singh,1 Monica Schwarz,1 JK Rasamny,1 Dorota Halicka,1 Jianguang Li,1 Codrin Iacob,1 Nina Suslina,1 Stimson Schantz,2 Edward Shin,1 Zbigniew Darzynkiewicz,2 Raj Tiwari,1 Jan Gielebien.1 1New York Medical College, Valhalla, NY; 2New York Eye and Ear Infirmary, New York, NY.

Papillary Thyroid Cancer (PTC) accounts for the vast majority of thyroid cancers and comprises more than 90% of neoplasms in the endocrine system. In the last 30 years, the incidence and prevalence of PTC has dramatically risen in developed countries, with a three-fold higher incidence in women than in men. With an overall five-year survival rate of 98.1%, early stage PTC has a favorable prognosis. However, PTC exhibits increased aggressiveness with poor prognosis in men diagnosed with the disease. These striking observations led us to explore the role of androgen and androgen receptor (AR) in this disease. We found an approximately 70% decrease in median AR RNA expression (p<0.0001) in 24 PTC patient tissue samples (from New York Eye and Ear Infirmary), compared

The immunomodulatory anticancer agent RRx-001 induces a vac-
cine-like interferon response through epigenetic induction of viral mimicry. Susan J. Knox. Stanford University School of Medicine, Stanford, CA.

RRx-001, sourced from the aerospace industry and currently in phase II clinical trials, is a novel anti-cancer agent that mediates immunomodulatory effects, either directly through polarization of tumor associated macrophages or indi-
rectly through vascular normalization and increased T-lymphocyte infiltration. With multiple additional mechanisms of action including upregulation of oxid-
ative stress, depletion of GSH and NADPH, anti-angiogenesis and epigenetic modulation, RRx-001 is being studied as a single chemotherapeutic agent to sensitize tumors to prior therapy and to prime tumors to respond to radiation, chemotherapy and immunotherapy in combination therapy studies. In this study, we identified another mechanism, viral mimicry, which refers to the ‘un-
veiling’ of epigenetically silenced viral genes that provoke an immune response and may contribute to the anti-cancer activity of RRx-001. Specifically, RRx-001 inhibited the growth of human colon cancer cells (HCT 116) and decreased levels of the DNA methyltransferases Dnmt1 and Dnmt3a in a time and dose-dependent manner. Treatment of HCT 116 cells with 0.5 μM RRx-001 for 24 hours significantly increased transcripts of inter-
feron (IFN) responsive genes and this induction was sustained for up to 4 weeks after transient exposure to RRx-001. ELISA assays showed that RRx-001 in-
creased secretion of type I and III IFNs by HCT 116 cells, and these IFNs were confirmed to be bioactive. Transcription of endogenous retroviruses was in-
duced by RRx-001 through demethylation of the promoter of endogenous ret-
roviruses as determined by methylation-specific polymerase chain reaction and combined bisulfite restriction analysis. Immunofluorescence staining with J2 antibody confirmed induction of double-stranded RNA. In conclusion, transient exposure of HCT 116 cells to low-dose RRX-001 induced transcription of silenced retroviral genes present in the cancer cell DNA with subsequent synthesis of this “pseudo-pathogenic” stimulus, mimicking an antiviral defense. RRX-001-mediated IFN induction may have the potential to improve the efficacy of immunotherapies as well as radiotherapy, standard chemotherapy and molecularly targeted agents when used in combination.


There is no doubt that testing for disease specific methylation changes can guide disease risk assessment, facilitate detection of the disease, support prognostication and personalization of treatment as well as guide post treatment patient care (1). Nevertheless, the use of methylation biomarkers in standard patient care is still marginal. Difficulties in implementation of methylation biomarker testing in standard in-vitro diagnostics are mainly attributed to challenges in establishment of the systematic approach allowing for efficient discovery and subsequent validation of clinical utility of potential disease specific methylation biomarkers. We have combined state-of-the-art genome wide methylation screening technologies (including: newest Illumina Methylation-EPIC 850K BeadChip) enabling methylation biomarker discovery with the cost and time efficient locus specific technologies to streamline the development, validation and implementation of methylation biomarkers for clinical disease management. With the use of technologies that in an affordable fashion enable screening for genome wide methylation changes in a substantial number of clinical samples, we were able to discover clinically relevant disease specific methylation signatures e.g. in CLL. The high technical complexity of the genome wide screen technologies prevents them for being straightforward applicable in diagnostic settings. Thus we used techniques such as Methylation-Sensitive High Resolution Melting (MS-HRM) (2) to develop assays fulfilling the requirements for diagnostic applications. Subsequently, we evaluated the clinical relevance of the most promising biomarker candidates in chronic lymphocytic leukemia, breast and lung cancers, using these assays (3, 4).


**#4360 Epigenetic alteration at HBV integrants is associated with methylation at-flanking human genome.** Yoshiyuki Yamamoto, Fumio Itoh, Hiroyuki Yamamoto, Fumio Itoh, St. Marianna Univ. School of Medicine, Kawasaki, Japan.

Integration of DNA viruses into the human genome plays an important role in various types of tumors, including hepatitis B virus (HBV)-related hepatocellular carcinoma. However, the molecular details and clinical impact of HBV integration on either human or HBV epigenomes are unknown. Here, we show that methylation of the integrated HBV DNA is related to the methylation status of the flanking human genome. We developed a next-generation sequencing-based method for structural methylation analysis of integrated viral genomes (denoted G-NaVI). This method is a novel approach that enables enrichment of viral fragments for sequencing using unique baits based on the sequence of the HBV genome. We detected integrated HBV sequences in the genome of the PLC/PRF/5 cell line and found variable levels of methylation within the integrated HBV genomes. Allele-specific methylation analysis revealed that the HBV genome often became significantly methylated when integrated into highly methylated host sites. After integration into unmethylated human genome regions such as promoters, however, the HBV DNA remains unmethylated and may eventually play an important role in tumorigenesis. The observed dynamic changes in DNA methylation of the host and viral genomes may functionally affect the biological behavior of HBV. These findings may impact public health given that millions of people worldwide are carriers of HBV. We also believe our assay will be a powerful tool to increase our understanding of the various types of DNA virus-associated tumorigenesis.

**#4361 An integrated genome-wide methylation analysis with gene expression in normal breast tissues identifies differentially methylated CpG loci associated with obesity.** Daniel Y. Weng, Min-Ae Song, Theodore M. Brasky, Catalin Marian, Renny S. Lan, Adana A. Llanos, Scott L. Spear, Bhaskar V. Kallakury, Jo L. Freudenheim, Peter G. Shields.

**#4362 Coordinated regulation of several microRNAs in lung cancer patients.** Irene Ferrer, Ana Salinas, Angela Marrugal, Jon Zugazagoitia, Amancio Carnero, Luis Paz-Ares, Sonia Molina-Pinelo.

**#4363 Bisulfite DNA sequencing analyses to detect methylation patterns in the p73 gene promoter in prostate cancer cell lines.** Nicholas E. Bragance, L. Michael Carastro, Johannes J. Schabot, Jong Y. Park.

**Acknowledgement:** We thank National Cancer Institute for funding this study. Background: Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancers. NSCLCs are mainly classified adenocarcinoma and squamous cell carcinoma. It seems more and more obvious that specific molecular analyses are necessary to elucidate the complexity of clinically relevant phenotypes which determine the lung carcinogenesis. This includes the role of microRNAs (miRNAs) in the disease, involved in the complexity of gene expression regulation. Different modulator mechanisms are involved in the biogenesis and function of miRNAs, one of them is DNA methylation. Alterations in these mechanisms may contribute to the tumor genesis and progression. The purpose of this study was to assess miRNA methylation patterns in patients with NSCLC to study the potential of coordinate regulation of miRNAs as a relevant mechanism involved in this pathogenesis. Materials and Methods: DNA methylation of gene clusters was analyzed by Illumina 70 subjects. The samples were divided into two cohorts. A first cohort constituted from 47 patients who had undergone surgical resection for clinical early stage NSCLC. A second group from 23 subjects was used as health cohort. DNA was extracted using the QIAamp DNA Mini Kit. For each assay, 500 ng of DNA was treated with sodium bisulfite using EZ DNA Methylation “Kit and cleaned with ZR-96 DNA Clean-up Kit”, before standard Illumina amplification, hybridization, and imaging steps. Methylation data were processed using the RnBeads R package. Results: We identified two clusters clearly correlated by methylation in lung cancer, involving 49 miRNAs differentially hypomethylated in tumor samples compared to control tissue. Some of these miRNAs have been implicated in several pathways in cancer. Conclusions: Our results strongly imply hypomethylation of two miRNA clusters, which represent key targets in unravelling of the mechanism of lung tumorigenesis.
rather reversible modifications, e.g., CpG methylation. The CpG islands in tumor suppressor gene promoters are often hypermethylated, thus down regulating their expression. The p73 gene is a member of the p53 tumor suppressor family of proteins, and like p53, has a bifurcated promoter. The p73 P1 promoter transcribes a full-length mRNA, which then translates into the truncated pro-apoptotic p73. The promoter is therefore a good candidate for demethylating agents. Methods. Hypermethylated DLD-1 human colorectal cancer cells were cultured for three days and treated daily with demethylating agents 5-azacytidine (AZA) or RRX-001 across a dose range (0.5 μM to 5 μM). Standard tests of population doubling (RPD) and cytotoxicity (trypan blue) were performed across the dose range for each compound. Treated cells were subjected to a 500 μm gel-insert scratch migration assay and video microscopy was used to record time-to-convergence (TC). Methylation specific PCR was performed on harvested cells across the dose range and compared to controls for eight genes associated with CIMP (SOCS1, MINT-1, hMLH, NEUROG1, THBD, HAND1, ADAMTS1, IGFBP3). Results. 5-azacytidine and RRX-001 both demonstrated >50% reduction in RPD at a dose of 2 μM, with a linear dose-dependent retardation of population growth from 0.1 μM to 1.0 μM. Trypan blue test also demonstrated linear increase in cytotoxicity for both agents up to 1 μM. Mean TC was 38.6hrs +/- 2.4hrs (1SD) for control, solvent control (DMSO), and 0.5μM AZA & RRX-001. Mean TC for 1.0μM and 2.0μM AZA was 52.0hrs +/- 3.75hrs, respectively (p<.0001). Mean TC for 1.0μM and 2.0μM RRX-001 was 57.0hrs +/- 2.0hrs and 96.0hrs +/- 5.0hrs, respectively (p<.0001). DLD-1 cells were treated with a concentration range of 0.5μM to 5μM of 5-azacytidine and RRX-001 and observed over 8 Cpg islands across the dose spectrum. hMLH, SOCS, and IGFBP3 were resistant to demethylation for both compounds at all doses. MINT, HAND, and THBD were partially demethylated by both agents, but ADAMTS1 was demethylated by AZA only. In each of the genes that demonstrated CpG island demethylation the demethylation was incomplete, with strong methylated bands present despite an additional demethylated band also being demonstrated. RRX-001 demonstrated a dose-dependent demethylation of NEUROG1 and THBD as integrated density of demethylated bands decreased against increasing dose of drug. Conclusions. This study demonstrates the demethylation ability of both agents against the methylene of 5 out of 8 genes associated with CIMP, which is a known to be associated with EMVI; an independent risk factor for poor prognosis in rectal cancers. The biological and demethylating effects occur at doses below those that may be considered cytotoxic, suggesting that demethylation itself is reducible the rate of cell division and migration of cancer cells. Further work is required to quantify the effects of both agents on the methylene of DLD-1 cells, and to explore the mechanisms of action of both compounds.

#4364 Establishment and validation of a breast cancer panel using targeted next generation bisulfite sequencing (INGBS). Matthew L. Poulton,1 Ryan Drennan,2 Andrew Miller,3 Andrew Miller,3 Ann Meyer,3 Garth H. Rauscher,7 Liying Yan1,2 EpigenDx, Inc., Hopkinson, MA;2University of Illinois at Chicago, Chicago, IL.

Aberrant DNA methylation (e.g., global reduction in DNA methylation and differential methylation of promoter regions) is a hallmark of cancer and may function in various ways to influence transcription. We developed a panel of bisulfite specific methylation sequencing assays in order to assess the methylation status of large number of genes using pyrosequencing. By combining these pyrosequencing assays into a targeted NextGen bisulfite sequencing panel, we can screen both a large number of genes and a large number of samples simultaneously. We first assembled the 40 pyrosequencing assays and sensitivity and reproducibility were analyzed on pilot DNA samples isolated from FFPE tissue. Assays were specifically designed to work in highly fragmented and cross-linked DNA from formalin-fixed, clinical samples. Initial pyrosequencing on the pilot samples showed that 24 of the 40 assays gave quality validation and sample results. Reasons that some assays were not successful may be due to the A/T or G/C content within the amplicon, PCR Bias, low amplification efficiency, or strong cross-linking from fixation within the assay to name a few. The initial 40 assays were next assembled in a combination of 7 multiplex PCRs in which gradient PCRs were performed and analyzed for optimal multiplex amplification using a bioanalyzer. Final assay multiplexing combinations were established and PCR bias testing was performed by INGSB on a sample set high of low methylated DNA mixed at different ratios. Assays with an R-square of less than 0.9 between the expected and generated methylation from this mixing were removed, leaving us with 21 validated assays in our panel (9 of 24 assays that were previously validated in pyrosequencing and 12 of 19 assays that could not be validated in pyrosequencing). In the present study we looked at DNA methylation changes in breast cancer development using paired adjacent normal and in situ tissue from a 30 patients with breast cancer as well as 11 various normal tissues other than breast, and 12 cell lines for a total of 83 samples in a panel of 21 genes. The results of the INBS were compared with the pyrosequencing results. This process of panel development, where small amounts of bisulfite treated DNA can be amplified in a target specific manner, prior to sequencing, makes investigating a large number of samples on a large panel of genes possible in a small amount of time. We observed a high correlation between the pyrosequencing and INGSB sequencing results, and we show that there is several genes that are either hypermethylated or hypomethylated in tumor tissue when compared to adjacent normal tissue.

#4365 Epigenetic and biological effects of 5-azacytidine and RRX-001 on DLD-1 colorectal cancer cell lines. Rory Kokeelaar, Huw Jones, John Beynon, Dean Harris, Gareth Jenkins. ABMU / Swansea University, Swansea, United Kingdom.

Introduction. Extramural vascular invasion (EMVI) in rectal tumours is an important risk factor for disease recurrence and death. Recent studies have demonstrated CpG island hypermethylation phenotype (CIMP) to be an independent risk factor for developing EMVI. This study investigates the cellular processes linking hypermethylation with EMVI by an in vitro model of rectal cancer, and assess the biological and epigenetic effects of known and putative demethylating agents. Methods. Hypermethylated DLD-1 human colorectal cancer cells were cultured for three days and treated daily with demethylating agents 5-azacytidine (AZA) or RRX-001 across a dose range (0.5 μM to 5 μM). Standard tests of population doubling (RPD) and cytotoxicity (trypan blue) were performed across the dose range for each compound. Treated cells were subjected to a 500 μm gel-insert scratch migration assay and video microscopy was used to record time-to-convergence (TC). Methylation specific PCR was performed on harvested cells across the dose range and compared to controls for eight genes associated with CIMP (SOCS1, MINT-1, hMLH, NEUROG1, THBD, HAND1, ADAMTS1, IGFBP3). Results. 5-azacytidine and RRX-001 both demonstrated >50% reduction in RPD at a dose of 2 μM, with a linear dose-dependent retardation of population growth from 0.1 μM to 1.0 μM. Trypan blue test also demonstrated linear increase in cytotoxicity for both agents up to 1 μM. Mean TC was 38.6hrs +/- 2.4hrs (1SD) for control, solvent control (DMSO), and 0.5μM AZA & RRX-001. Mean TC for 1.0μM and 2.0μM AZA was 52.0hrs +/- 3.75hrs, respectively (p<.0001). Mean TC for 1.0μM and 2.0μM RRX-001 was 57.0hrs +/- 2.0hrs and 96.0hrs +/- 5.0hrs, respectively (p<.0001). DLD-1 cells were treated with a concentration range of 0.5μM to 5μM of 5-azacytidine and RRX-001 and observed across the dose spectrum. hMLH, SOCS, and IGFBP3 were resistant to demethylation for both compounds at all doses. MINT, HAND, and THBD were partially demethylated by both agents, but ADAMTS1 was demethylated by AZA only. In each of the genes that demonstrated CpG island demethylation the demethylation was incomplete, with strong methylated bands present despite an additional demethylated band also being demonstrated. RRX-001 demonstrated a dose-dependent demethylation of NEUROG1 and THBD as integrated density of demethylated bands decreased against increasing dose of drug. Conclusions. This study demonstrates the demethylation ability of both agents against the methylene of 5 out of 8 genes associated with CIMP, which is a known to be associated with EMVI; an independent risk factor for poor prognosis in rectal cancers. The biological and demethylating effects occur at doses below those that may be considered cytotoxic, suggesting that demethylation itself is reducible the rate of cell division and migration of cancer cells. Further work is required to quantify the effects of both agents on the methylene of DLD-1 cells, and to explore the mechanisms of action of both compounds.
whereas positive correlation was observed in CHG and CHH1 context. mRNA and serum protein expressions of IFNγ, IL4, IL6, IL10, IL2, TNFα were not correlated with promoter methylation status of MGMT and RUNX3 genes. Smoking habit was associated with IFNγ, IL6 and TNFα for mRNA expression and IL2 for protein expression. Alcohol consumption was statistically associated with TNFα for mRNA expression and IL10 for protein expression. Betel nut consumption was significantly correlated with IL4 and IL6 for mRNA expression and IFNγ, IL4, IL6, TNFα were with protein expression. Tobacco consumption was correlated with only protein expression of IL2 and IL6. Smoked food consumption was statistically associated with IFNγ, IL4, TNFα for mRNA expression and IL6 and IL10 for protein expression. Conclusions: Promoter methylation of MGMT was correlated with mRNA expression but RUNX3 was not. Both RUNX3 and MGMT promoter methylation was not found to be associated with alteration of cytokine expression whereas they were associated with different life style factors in EC cases from North-East India.

**#4367** LOX expression, LINE 1 DNA methylation and prognosis in oesophageal cancer. Rebecca T. Kalikale, Yoshihumi Baba, Yuki Kitano, Kensuke Yamamura, Keisuke Miyake, Kenichi Nakamura, Hideo Baba. Kumamoto University, Kumamoto, Japan.

Background: In cancer cells, DNA methylation may be altered in two principle ways; global DNA hypomethylation and site-specific CpG island promoter hypermethylation. Since Long interspersed element-1 (LINE-1 or L1) constitutes a substantial portion (approximately 17%) of the human genome, the extent of LINE-1 methylation is regarded as a surrogate marker of global DNA methylation. In previous studies, we demonstrated that LINE-1 hypermethylation was strongly associated with a poor prognosis in oesophageal cancer, supporting its potential role as a prognostic marker. Lysyl oxidase (LOX) is an extracellular matrix remodelling enzyme and its upregulation has been associated with poor prognosis in various cancers since it has been reported to promote malignancy. We conducted this study to examine the correlations between LOX expression, LINE-1 methylation and the clinical pathological features in patients with oesophageal cancer. Methods: We examined LOX expression in 8 oesophageal cancer cell lines by quantitative reverse-transcriptase polymerase chain reaction (RT-PCR) and Western blotting. The expression levels of LOX in 150 oesophageal cancer tissues and adjacent normal mucosa was measured by RT-PCR and immunostaining. Results: Our Gene Expression Microarray revealed that LINE-1 hypomethylated oesophageal tumours presented higher expression level of LOX compared with LINE-1 hypermethylated tumours. Similar results were observed in the comprehensive analyses using public array data of oesophageal cancer cell lines. In cell lines, LOX mRNA was expressed at a high level in TE-6, TE-11, TE-14 and at a low level in TE-1, TE-4 and KYSE 30 and similar expression patterns for catalytically active LOX were observed in Western blot analysis. In immunohistochemical study, LOX was predominantly identified in the cytoplasm of oesophageal cancer cells. LOX expression was significantly associated with LINE-1 methylation levels (P < 0.034). LOX expression was significantly associated with invasion depth, tumour stage and venous invasion. Overall and cancer-specific survival rates of high LOX expression group were significantly lower than those of low LOX expression group (P = 0.012 and 0.008 respectively). In multivariate analysis high LOX protein expression was an independent prognostic factor in oesophageal cancer. Conclusions: The expression of LOX is up-regulated in oesophageal cancer cell lines and resected tumour specimens and its controlled epigenetically. Our findings suggest that LOX can serve as a useful prognostic biomarker in oesophageal cancer patients and might be potential therapeutic target.

**#4368** The clinicopathological significance of glutathione S-transferase P1 hypermethylation in breast cancer, a meta-analysis and literature review. Mingli Liu. Morehouse School of Medicine, Atlanta, GA.

Background: Glutathione S-transferase P1 (GSTP1) has been reported to function as a tumor suppressor gene in various types of human cancers including breast cancer (BC). However, the association and clinicopathological significance between GSTP1 hypermethylation and BC remains unclear. The purpose of this study is to conduct a meta-analysis and literature review to investigate clinicopathological significance of GSTP1 methylation in BC. Method: A detailed literature search was made in PubMed, EMBASE, Web of Science and Google Scholar databases. The data were extracted and assessed by two reviewers independently. Odds Ratios (OR) with 95% confidence corresponding intervals (CIs) were calculated. A total of twenty four relevant articles were available for meta-analysis. Results: The frequency of GSTP1 hypermethylation was significantly increased in BC than in benign breast disease, the pooled OR was 7.09, p < 0.00001. The rate of GSTP1 hypermethylation was significantly different between early stage I/II and later stage III/IV, OR was 2.05, p < 0.0001. In addition, the rate of GSTP1 hypermethylation was also significantly different regarding the node involvement status. The rate of GSTP1 hypermethylation in node positive BC was higher than that in node negative BC, OR was 1.77, p = 0.006. However, GSTP1 hypermethylation was not significantly associated with ER, PR and HER2 status. Conclusion: In summary, our meta-analysis indicated that the frequency of GSTP1 hypermethylation was higher in BC than in benign breast disease. The rate of GSTP1 hypermethylation in late stages of BC was higher than that in early stages. Our data suggested that GSTP1 hypermethylation could be a risk factor and a diagnostic biomarker of BC carcinogenesis. GSTP1 hypermethylation may have a role in the progression of BC. GSTP1 is a potential drug target for development of demethylation treatment for patients with BC.

**MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Genomic Landscape of Head and Neck and GI Cancers**

**#4370** ASNS and other significant mutations in head and neck squamous cell carcinomas: mutational landscape in a South Asian population. Sadiq Rehmani,1 Sana Abdul Razzaq,2 Sarah Madhani,1 M Kamran Azim,2 Rashida Ahmed,3 Mumtaz Khan,3 Kusloom Ghias3. 1Mount Sinai St. Luke’s Hospital, Icahn School of Medicine at Mount Sinai, New York, NY; 2University of Karachi, Karachi, Pakistan; 3Aga Khan University, Karachi, Pakistan.

Head and neck cancers are the sixth most common cancer type globally and contributing significantly to the burden of disease in South Asia. Specifically in Pakistan, they are the most commonly diagnosed cancer in males and the second most common in females. The increasing burden of head and neck squamous cell carcinoma (HNSCC) in the region along with a unique set of risk factors merited a deeper investigation of the disease at the genomic level. Whole exome sequencing of HPV-negative HNSCC samples and matched normal genomic DNA (n = 7) was performed. Smoking, oral tobacco use and alcohol were identified as among the most commonly affected pathways in all the samples. This data is the first of its kind from the Pakistani population. The results of this study can guide a better mechanistic understanding of HNSCC in the population, ultimately contributing new, rational therapeutic targets for the treatment of the disease.

**#4371** Genomic characterization of oropharyngeal squamous cell carcinoma in Korean population. Jee soo Chae,1 Weon Seo Park,2 Dongwan Hong,2 Jong-II Kim,3 Yuh-Seog Jung3. 1Seoul National University, Seoul, Republic of Korea; 3National Cancer Center, Goyang, Republic of Korea.

Objective: Oropharyngeal squamous cell carcinoma (OPSCC) is a subtype of head and neck squamous cell carcinoma (HNSCC). Environmental risk factors such as tobacco, alcohol, and especially, human papillomavirus (HPV) are known to be associated with HNSCC. Also, large-scale sequencing studies identified several HNSCC-associated genes such as TP53, EGFR and PIK3CA. However, still the initiation and progression of OPSCC and mutational profiles of OPSCC in Korean population are unclear. Methods: Frozen tumor mucosa, tumor tissues and blood were collected from nine OPSCC patients; 6 HPV positive and 3 negative. Adjacent non-tumor tissues were obtained about 1 cm apart from the tumor tissue and were histologically confirmed as non-neoplastic. We then performed whole exome sequencing with Agilent SureSelect Human All Exon Kit v4+ UTR, yielding average on-target coverage of 100X. Results: First, we compared somatic alterations of adjacent non-tumor and tumor tissue to understand the genomic status of non-tumor mucosa. Comparison of genome-wide copy number alterations (CNA) between non-tumor and OPSCCs showed that majority of somatic CNA (SCNA) was occurred after tumor progression. Also, non-tumor mucosa rarely harbored genomic changes, showing average nonsilent mutations rate of 0.07 per Mb. In contrast, OPSCC showed 1.0 mutation per Mb with few shared mutations with non-tumor. These results indicate occurrence of new distinct subclones after tumor progression. Second, somatic mutations and CNA analysis of 9 OPSCC identified two distinct groups divided by the HPV status of tumor. HPV (+) tumors showed lower mutation burden than HPV(-) tumors (0.63 and 1.73 mutations per Mb, respectively). And TP53
and CDKN2A mutations were detected exclusively in HPV(-) group, accounting for 66.6% of HPV(-) tumors. Furthermore, HPV(-) tumors showed greater degree of chromosomal alterations compared to HPV(+) tumors. Conclusions: Although there are many studies to understand the relationships between pre-malignant and malignant tissue, there is a lack of understanding for genetic changes function on non-epithelial mucosa. Our data show that genomic picture of normal crypt mucosa rarely changes and tumor develops in distinct sub- clones. This would provide a glimpse into the fundamental cellular changes of non-tumor cells of tonsillar crypts and oral cavity. Also, we provide a mutational landscape in HNSCC of Korean population. Although our OPSCC showed recurrent mutations and copy number alterations of genes related to RTK/Pi3K- AKT/mTOR signaling pathways, similar as western, mutation burden in tumors was relatively lower, and somatic mutations on well-known HNSCC-associated genes such as PIK3CA, FAT1 and HRAS weren’t detected. Further investigation and molecular experiments are required to understand the mechanism of carcinogenesis and to discover early driver mutations of HNSCC.

#4374 Chromosome 3q22-29 amplification is linked to increased expression of multiple genes in key pathways deregulated in head and neck squamous cell cancers and cell lines. Hui Cheng,1 Xiping Yang,1 Han Si,1 Anthony Saleh,1 Jamie Coupar,1 Robert L. Ferris,1 Wendell G. Yarbrough,2 Mark E. Prince,3 Thomas E. Carey,4 Carter Van Waes,1 Zhong Chen1. National Institutes of Health, Bethesda, MD; 2University of Pittsburgh Cancer Institute, Pittsburgh, PA; 3Yale School of Medicine, New Haven, CT; 4University of Michigan, Ann Arbor, MI.

As part of the Cancer Genome Atlas (TCGA) Network, our comprehensive genomic analysis of 279 head and neck squamous cell carcinomas (HNSCCs) found frequent chromosomal copy number variation (CNV) and mutations of potential biologic and therapeutic importance. This underscored an urgent need to identify cell line models that harbor genomic alterations representative of HNSCC. We performed whole exome DNA and transcriptome RNA sequencing on 15 human papillomavirus HPV(-) and 11 HPV(+) HNSCC cell lines. HNSCC lines harbored chromosome gains (3q, 5p, 7q, 8q, 11q) and losses (3p, 5q, 8p, 18q), consistent with those found in HNSCC tumors by TCGA and previous karyotype studies. Integrative genome-wide analysis of CNV with gene expression uncovered over 1500 genes that display significant correlation between CNV and gene expression in both TCGA tumors and cell lines. Ingenuity Pathway Analysis revealed multiple genes that converge on key pathways and functions deregulated in HNSCC, including PI3K/AKT/mTOR, NF-kB, RAS/ MAPK, TP53, death receptor signaling, inflammation, and differentiation. Intriguingly, 103 genes displaying significant amplification and increased expression were predominantly located on chromosome 3q22-29. These genes encode components involved in the PI3K/AKT/mTOR, Hippo, TGF-beta and Wnt/beta-catenin pathways, cell cycle, translational and post-translational regulation, and mitochondrial biosynthesis. Fisher’s exact test and survival analysis showed significant co-occurrence and worse overall survival of 3q26.3 amplification and TP53 mutation in HNSCC patients from TCGA datasets; 3q26.3 encompasses 53 genes including PIK3CA, PLD1, AXL, CDK4 and SOX2. HNSCC cell lines also harbor common mutations found in TCGA, such as TP53, FAT1 and NOTCH1, and novel and rare tumor suppressor genes, such as MYH9. Our findings suggest that these cell lines could serve as models for mechanistic studies and pharmacologic screening, and investigation of genomic and expression alterations as potential biomarkers for precision diagnosis and prognosis of HNSCC.

#3179 Network analysis of karyotype study data identifies distinct genomic signatures of Barrett's esophageal adenocarcinoma (EAC). Dunfa Peng,1 Yan Guo,2 Heidi Chen,2 Jun Eul Hwang,2 Kyung Hee University, Seoul, Republic of Korea; 3Chonnam National University, Gwangju, Republic of Korea.

Purpose: Numerous studies have indicated that Sex-determining region Y-box 2 (SOX2) is involved in many squamous cell carcinomas. However, the role of SOX2 in head and neck squamous cell carcinoma (HNSCC) remains unclear. Therefore, we investigated whether activation of SOX2 is significantly associated with prognosis in HNSCC. Methods: Gene expression signature reflecting SOX2 activation was identified in HNSCC cohort, and patients were stratified into two groups according to this signature: SOX2-high group or SOX2-low group. Validation of the signature was sought in two independent patient groups. The association between the signature and prognosis of patients was assessed. Results: The SOX2-low group was associated with poor prognosis for HNSCC in three independent patient cohorts. In a multivariate analysis, the impact of SOX2 signature on overall survival (OS) was independent of other clinical variables [hazard ratio (HR), 1.45; 95% confidence interval (CI), 1.09 - 1.92; P=0.01]. In patients who received radiotherapy (RT), SOX2-low group had significantly poor OS than those in SOX2-high group. Conclusions: SOX2-low signature is associated with poor prognosis in patients with HNSCC and could be used to predict patients who would benefit from RT.

#4375 Complex interactions between genomic and epigenomic alterations in esophageal adenocarcinoma. Dunfa Peng,1 Yan Guo,2 Heidi Chen,2 Jun Eul Hwang,2 Kyung Hee University, Seoul, Republic of Korea; 3Chonnam National University, Gwangju, Republic of Korea.

Backround: The incidence of Barrett’s-related esophageal adenocarcinoma (EAC) is rapidly rising in the USA and the Western countries. However, the interaction between genomic and epigenomic alterations in regulating gene expression networks in EAC is poorly understood. Results and Discussion: We carried out an integrative analytical approach to identify DNA copy number, promoter DNA methylation and gene expression in a panel of esophageal adenocarcinomas, using chip-based technologies. Our analysis displayed significant differences between EAC and normal samples in DNA copy numbers, gene expression, and DNA methylation profiles. Our results identified remarkable incidence of DNA copy number (CN) alterations showing that 90% of probes had a copy number aberration (gain or loss) in at least one tumor, suggesting a vast chromosomal instability in EAC. Integrative analysis indicated that altered expression of 1755 genes was associated with changes in CN or methylation. We found that expression alterations in 84 genes (56 overexpressed and 28 down regulated) were associated with changes in both CN and methylation. These data suggest a strong interaction between genetic and epigenic events to modulate gene expression in EAC. Of note, we detected a prominent K-RAS signature in EAC. Network analysis predicted activation of several important transcription factor networks in EAC, including beta-catenin, MYB, TWIST1, SOX7, GATA3 and GATA6. 56 genes were overexpressed in EAC with simultaneous promoter
hypo-methylation and gene amplification, including CDH17 and GATA6. Not-
tably, several genes associated with inflammation such as COX2, IL8 and IL23R
were over-expressed and hypomethylated in EAC, a reaffirmation of the under-
lying inflammatory process in Barrett’s tumorigenesis and a suggestion of an
epigenetic regulation of these genes. Conclusions: In summary, our integrative
analysis suggests that epigenetic alterations in EAC demonstrates a complex
interaction between molecular mechanisms in EAC and provides several novel
insights for future studies.

#4376 Next-generation sequencing of gastric cancer samples from Rw-
da: a feasibility study. Mary D. Chamberlin, Francine B. De Abreu, Torrey L.
Gallagher. Geisel School of Medicine at Dartmouth, Lebanon, NH.
Background: Gastric cancer is associated with high morbidity and mortality
around the world. In developing countries, patients often present with advanced
disease creating a challenge for effective treatment. Next generation sequencing
is identifying mutations in more than 50% of gastric cancers in developed coun-
tries. Chronic infections and other geographic differences may lead to variations
in somatic mutations that could lead to different approaches to treatment. In this
pilot study we sought to determine if an overseas collaboration between the
University of Rwanda College of Medicine and Health Sciences and the Geisel
School of Medicine at Dartmouth was feasible and effective at determining mol-
ecular profiles of gastric cancer. Methods: Patients referred for endoscopy at
University Teaching Hospital of Kigali, Rwanda were consented to participate
prior to the procedure. Biopsy tissues were processed for routine pathologic
assessment. Those determined to be gastric adenocarcinoma were included. Formal-
alin-fixed paraffin embedded tissues, H&E stained slides and tissue blocks
were transported from Rwanda to New Hampshire, USA by visiting professors
participating in the Human Resources for Health Program between May 2015 and
July 2016. DNA and RNA from 39 samples were extracted using at least 7 FFPE
unstained slides according to AllPrep RNA/DNA FFPE Kit Protocol. Library
preparation was performed using 5 ng DNA using KilpaLLAMA MampSTM Lung and Colon Hot Spot Panel. Library quantification was per-
formed using Qubit, and samples with at least 4 nM were normalized, pooled
and sequenced on the v3 cartridge on the Illumina’s MiSeq system. For data
analysis, FASTq files were uploaded to Pillar Biosciences, where sequence align-
ment, annotation, and variant classification were performed. Results: Eighteen samples had insufficient tissue for nucleic acid extraction. Of the 39 samples
processed for sequencing, 17 had low library quantification due to low DNA
concentration or poor quality. Mutations were detected in 9/22 samples (41%).
One sample contained 3 mutations while the majority of mutated cases had 1-2
mutations identified. A total of 12 mutations were identified: TP53 (4), SMAD4 (2), ERBB4 (2) (S341L and D228N), PTEN (2) (K267RfsTer9 and C136Y), PRW7 (1) and KRAS (1). Conclusions: Overseas collaboration for next gen-
eration sequencing of gastric cancer samples is feasible. Quality control is a com-
mon challenge and may improve with larger sized biopsies or tissue blocks.
Frequency of mutations is lower than expected. ERBB4 mutations identified are
not known to be pathogenic. The PTEN mutations observed are considered
pathogenic and this may be a feasible target for therapy. A fusion panel and
amplification profiling may be more effective in identifying potential targets for
therapeutic trials. Limitations: Small sample size. Panel limited to mutations.

#4377 EBV associated gastric cancer in Korean. Woochan Lee. Seoul Na-
tional University, Seoul, Republic of Korea.
Gastric cancer was the third leading cause of death worldwide and most prev-
alent malignant cancer in Korea, comprising 20.8% of all cancers. The Cancer
Genome Atlas (TCGA) divided gastric cancer to four subgroups; EBV (Epstein-
Barr Virus infected, 9%), MSI (Microsatellite instability, 22%), GS (Genomically
stable, 20%), CIN (Chromosomal instability, 50%). We sequenced transcrip-
tomes of 11 gastric cancers associated with EBV infection (EBVaGC) tissues, 11
gastric cancers not associated with EBV infection (EBVnGC) tissues and 22
matched normal gastric mucosa for find unique features of Korean EBVaGC.
We compared RNA expression, EBV gene expression, germline mutations, and
somatic mutation between EBVaGC and EBVnGC. We found IKKBE gene, which
is taking an essential role in regulating antiviral signaling pathways, ex-
pressed higher on EBVaGC tumor than EBVnGC tumor (p-value = 1.73 x 10^-10).
While most EBV genes appeared to be similar, BMRF1,2 genes made distinct
patterns compared to previous TCGA report in expression of EBV which inte-
grated into human cells. The BMRF-2 gene is associated with cell-to-cell spread
of EBV, which is major infection mechanism of EBV. We found novel germline
mutations on PAPPC3, PGA3, and C4A,B in almost every samples, which are can-
didate for Korean specific mutations.

#4378 Gastric cancer organoid culture shows preserved genomic stability
in long-term passage. Sarah Siu Kuen Yue, Helen Hoi Ning Yan, Ho Cheong
Siu, Siu Lun Ho, Wai Yin Tsui, Desy Chan, Annie Shuk Yee Chan, Bernard Ch
Hang Lee, Anthony Kin Wang Chan, Suei Yi Leung. Department of Pathology,
The University of Hong Kong, Hong Kong.
In recent years, techniques, normal epithelial and tumor cells can be directly cultured from clinical specimens in vitro and expanded long-term with a very high success rate. Thus, this constitutes a good
platform for various mechanistic functional studies and clinical applications. A
previous karyotyping and gene expression profiling study has indicated that
organoid culture of normal epithelial cells can be propagated for long periods of
time without any genomic alterations. However, data on the genomic stability
of tumor organoids in long-term culture are not yet available. We have successfully
established organoid cultures derived from either primary tumor or lymph node
metastasis from 4 gastric cancer patients. For each sample, we performed long-
term culture for at least 6 months. DNA was extracted from blood, as well as the
early and late passes of these organoids, and submitted for whole-exome se-
quencing (WES), achieving a mean coverage approach across 90X. Somatic muta-
tions detected in early versus late passage gastric cancer organoids were com-
pared. Furthermore, we examined the organoids for copy number variations
based on the coverage and loss of heterozygosity (LOH) information derived
from exome sequencing. We detected between 80 to 228 somatic mutations in
the 4 early passage organoids, in which over 84-99% of them were retained
after 6 months of culture. We detected 18 new 31 and 38 new mutations in the 4
long-term organoids, respectively. The one case with a substantial large number
of new mutations (n=98) appeared to have emerged from a subclone of TP53
wild-type cells in a TP53 mutant tumor. Notably, the C=T mutation was the most
dominant mutation spectrum in this case, constituting 48%. Despite the
presence of frequent long segment LOH and chromosomal aberrations in early
passage organoids, indicating the presence of chromosomal instability, these
patterns of aberrations were stably maintained in long-term passage. Overall,
with the current organoid culture protocol, tumor genomes are mostly stably
maintained with the accumulation of only a small number of new mutations.
Our results indicate that this is a reliable and stable in vitro cell culture model
for various cancer-related and clinical studies.

#4379 Somatic exomic landscape of small intestinal adenocarcinomas.
Ulrika A. Hanninen,1 Riku Katainen,1 Jiri Hamberg,1 Jukka-Pekka Mecklin,2
Linda Forsström,1 Esa Pitkänen,1 Netta Mäkinen,1 Lauri A. Aaltonen3. Ge-
nome-Scale Biology Research Program, Research Programs Unit, University of
Helsinki & Department of Medical and Clinical Genetics, Medicum, University of
Helsinki, Finland, 2Department of Surgery and Education & Science, Jyväskylä
Central Hospital and University of Eastern Finland, Jyväskylä, Finland,
3Gastroenterology, Charles University Hospital, Prague, Czech Republic.
Small intestinal adenocarcinoma is a rare cancer with a high mortality rate. The
small intestine makes up less than 1% of the gastrointestinal tract, how-
never, small intestinal tumors constitute less than 5% of gastrointestinal tumors.
Of small intestinal tumors, around one third are aggressive adenocarcinomas
with poor outcome (5-year survival rate is approximately 30%). Reasons for
their rarity are still unclear. Predisposing factors include Crohn’s disease,eliac
disease, hereditary genetic syndromes such as familial adenomatous polyposis
(FAP) and hereditary nonpolyposis colorectal cancer (HNPCC), and dia-
tary factors. Due to the low incidence of small intestinal adenocarcinomas
(SIAs), the number of genetic studies remains insufficient. Since there are lim-
ited data available to guide the therapeutic decisions, our aim is to characterize
the somatic mutational landscape of SIAs in a population-based material using
exome sequencing. We collected information on all patients diagnosed with
SIA in Finland between the years 2006-2011 utilizing the Finnish Cancer Regis-
try. This registry maintains a nation-wide database on all cancer cases diagnosed
in Finland since 1953. From these we selected all cases with 1) confirmed small
intestinal primary tumor, 2) available tumor material, and 3) the tumor content of
at least 50%. In order to focus solely on small intestinal tumors, we excluded
tumors of the papillary region since they might also have arisen from the pan-
tes or the biliary tract. Altogether 107 tumors representing all three parts of the
small intestine were selected for exome sequencing. The tumor data was filtered
against the ExAC and SISU databases to remove germline variations. In addi-
tion, Sanger sequencing of the corresponding normal tissue will be used to verify
the somatic origin of the most interesting observed mutations. The data analysis
is ongoing. The average number of mutations in a tumor was 358 (range 40
- 1666). Preliminary findings show that 10-20% of those tumors representing all
three parts of the small intestine were selected for exome sequencing. The most
frequent mutations, map against the current organoid culture protocol, tumor genomes are mostly stably
maintained with the accumulation of only a small number of new mutations. Our results indicate that this is a reliable and stable in vitro cell culture model for various cancer-related and clinical studies.

#4380 Molecular and cellular biology / Genetics: Genomic landscape of head and neck and gi cancers

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between the discovered mutations and patients’ survival time. This comprehensive characterization of the molecular basis of SIAs will provide better insight on how these tumors arise and possibly how they should be treated.


We performed an integrated clinical and bioinformatic analysis of colorectal cancers (CRCs) genotyped at our institution from 4/2014–7/2016 to comprehensively characterize genomic alterations in metastatic CRC (mCRC). We analyzed 1008 samples (474 primaries, 534 metastases) from 985 mCRC patients and 128 early stage CRCs sequenced with MSK-IMPACT, a hybridization capture next generation sequencing assay. Metastatic CRCs were divided into 3 groups by mutation burden and MSiSensor algorithm score: microsatellite stable (MSS) (n=939; 95%), microsatellite-high (MS-H) (n=41; 4%), and ultra-mutated (n=5; 1%). Early stage CRC were enriched for MSI-H due to clinical selection and were 53% MSS, 44% MS-H, and 4% ultra-mutated. Ultra-mutated tumors exhibited >100 mutations and harbored hotspot mutations in POLE in 8 cases and a potential novel POLE alteration in 1 case. We evaluated the frequency of oncogenic alterations in MS-H and MSS mCRCs. The frequency of the resistance biomarkers KRAS and NRAS did not vary between MS-H and MSS mCRCs (46% vs 41%, p=0.6). Potentially actionable alterations were enriched in MSI-H tumors (78% vs 33%, p<0.001). Metastases did not have more actionable alterations than primary tumors. We classified clinically relevant targets using the OncoKB classification as level 2B (FDA-approved target in another disease type), 3A (target with compelling early clinical evidence in CRC), and 3B (target with compelling early clinical evidence in another disease type). Twelve percent (109/939) of MSS mCRCs had a level 2B target: BRAF V600E (5%), ERBB2 amplification (AMP) (4%), MET AMP (1%), BRCA1/BRCA2 alteration (1%), TSC1/TSC2 mutation (1%), EGFR mutation (<1%), RET fusion (<1%); there was significant enrichment of BRAF V600E (24%) and BRCA1/BRCA2 alterations (29%) in MSI-H versus MSS mCRC (p<0.001). NTRK fusions, the main 3A alteration identified, occurred in 7% of MSI-H and <1% MSS CRC (p<0.001). Level 3B alterations at ≥ 1% in MSS CRC included PIK3CA (15%), NRAS(3%), AKT1 (1%), MAP2K1 (1%), and ERBB2 (1%) mutations and FGFR1 AMP (2%). PIK3CA and PTCH1 mutations were both enriched in MSI-H versus MSS mCRC (32% vs 1%, p<0.01; 27%, v <1%, p<0.001, respectively). Analysis of mutation frequencies in 3 MSS CRC disease states - early stage resected primary (The Cancer Genome Atlas, TCGA), primary site of mCRC, and metastatic site - found significant depletion of IDH2 MT mutations in metastases. We also found significant and progressive enrichment of TP53 alterations (58% TCGA, 73% primaries of mCRC, 79% metastases) and BRAF mutations (4% TCGA, 9% primaries of mCRC, 10% metastases) in advanced disease, suggesting a role of these genes in aggressive disease. One third of the BRAF mutations in our cohorts were not V600 but known to be oncogenic. In this large cohort of mCRC subjects, significantly more patients harbored unique mutations in MSI-H and MSS CRC have a similar frequency of resistance biomarkers, MSI-H CRC more commonly harbor actionable alterations.

#4381 The mobile genome of colorectal cancer: Characterization of retrotransposon insertions in 202 colorectal cancer whole genomes. Tatiana Ca-juo, Paivi Sulo, Riku Katainen, Ulrika Hänninen, Tomas Tanskanen, Johanna Kondelin, Jiri Hamberg, Niko Välälä, Kimmo Palin, Outi Kilpiövaara, Esa Pitkänen, Lauri A. Aaltonen. Genome-Scale Biology Research Program, Research Programs Unit and Department of Medical and Clinical Genetics, Medicinum, University of Helsinki, Helsinki, Finland.

The aim of this study is to characterize the landscape of retrotransposon insertions in 202 colorectal cancer whole genomes. We performed an integrative analysis of colorectal cancer patients using next-generation sequencing approaches. We found that retrotransposon insertions can contribute to genomic variation, which may be associated with the development of colorectal cancer. Furthermore, these insertions may be associated with the resistance to chemotherapy and the risk of recurrence. Our study highlights the importance of integrating molecular and clinical data to better understand the biology of colorectal cancer.

#4382 Genomic events associated with metastasis of colorectal cancer by whole-genome sequencing. Yi Pan, Joanna Tong, Johnny Kwan, Kwok Wai Lo, Anthony Chan, Ka Fai To. Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong, Hong Kong.

Background: The diversity of the genomic landscape in colorectal cancer (CRC) is understood to be due to the accumulation of mutations in primary tumors and metastatic spread of cancer cells to other organs. Genetic alterations in primary tumors may be different from those in metastatic tumors, suggesting a heterogeneity in the genetic landscape of CRC. The aim of this study was to investigate the genetic alterations associated with metastatic spread of CRC.

Methods: We performed whole-genome sequencing of 257 CRC patients, including 100 patients with primary tumors and 157 patients with metastatic tumors. We used a hybridization capture approach to enrich for exonic regions and performed sequencing on the Illumina HiSeq 2000 platform with 100 base-pairs paired-end reads. Each sample was sequenced to a minimum of 40x median coverage. To detect retrotransposon insertions we utilized TraFiC (Transposome Finder in Cancer). To identify retrotransposon-mediated translocations that were not identifiable using TraFiC, we utilized DELLY and the European database of L1-HS retrotransposon insertions in humans (euL1db).

Results: We identified a total of 5085 somatic insertions. 2% of these insertions were located in exons, whereas 45% were in introns. We identified several genes with recurrent insertions, some of these loci being known fragile sites. However, we identified 13 known cancer genes with two or more insertions. Furthermore, we identified two patients with insertions in exon 16 of APC, suggesting that these insertions could be initiating tumorigenic events. To conclude, we found retrotransposon insertions in the clinical setting and further suggest that these insertions could be contributing to metastasis of CRC.

#4383 Novel fusion transcripts in colorectal cancer patients revealed by next-generation RNA sequencing. Yury Choi,1 Chae Hwa Kwon,1 Hee Ji Park,2 Yeo-Jin Won,3 Do Youn Park.1,2 Pusan National University, Busan, Republic of Korea;3 Pusan National University Hospital, Busan, Republic of Korea.

Background: Colorectal cancer (CRC) is the second most common cancer in the world, with an estimated 9 million new cases and 1.7 million deaths. The genetic landscape of CRC is complex, with mutations in various genes leading to the development of CRC and its progression. The identification of novel fusion transcripts in CRC can provide new insights into the molecular mechanisms underlying CRC development and progression.

Methods: We performed next-generation RNA sequencing on 257 CRC patients, including 100 patients with primary tumors and 157 patients with metastatic tumors. We used a hybridization capture approach to enrich for exonic regions and performed sequencing on the Illumina HiSeq 2000 platform with 100 base-pairs paired-end reads. Each sample was sequenced to a minimum of 40x median coverage. To detect retrotransposon insertions we utilized TraFiC (Transposome Finder in Cancer). To identify retrotransposon-mediated translocations that were not identifiable using TraFiC, we utilized DELLY and the European database of L1-HS retrotransposon insertions in humans (euL1db).

Results: We identified a total of 5085 somatic insertions. 2% of these insertions were located in exons, whereas 45% were in introns. We identified several genes with recurrent insertions, some of these loci being known fragile sites. However, we identified 13 known cancer genes with two or more insertions. Furthermore, we identified two patients with insertions in exon 16 of APC, suggesting that these insertions could be initiating tumorigenic events. To conclude, we found retrotransposon insertions in the clinical setting and further suggest that these insertions could be contributing to metastasis of CRC.
quencing and were not found in another cohort. Overexpression of these fusion genes, except B4GALT1-BAG1, increased proliferation of CRC cells. In addition, overexpression of NAGLU-IKZF3 enhanced migration of CRC cells. These results reveal novel fusion transcripts and their tumorigenic effects in CRC. Ultimately, it could illuminate a genomic-driven strategy to develop personalized medicine. Key words: fusion transcript, colorectal cancer, RNA-seq.

#4384 Targeted sequencing reveals distinct and rare pathogenic variants in Caucasians with colorectal cancer. Pooneh Mokarram,1 Sudhir Varma,2 Hamed Azimi,3 Hasti Olumi,1 Ali reza Safarpour,1 Michael Nickerson,4 Hassan Brim,1 Hassan Ashktorab1, Shiraz University, Shiraz, Islamic Republic of Iran; 2Hihura, Washington, MD; 3Howard Univ., Washington, DC; 4NIH, Bethesda, MD.

PURPOSE: Next-generation sequencing (NGS) is currently used to establish mutational profiles in many multigene diseases such as colorectal cancer (CRC) which is on the rise in many parts of the developing world including in the Middle East. Little is known about its genetic hallmarks in these populations. AIM: To identify variants in 15 CRC-associated genes in patients of Iranian descent. METHODS: CRC specimens from 63 patients were used to establish the variants’ profile on an Ion Torrent platform by targeted exon sequencing. To rule out technical artifacts, the variants were validated in 13 of these samples using an Illumina NGS platform. Validated variants were annotated and compared to variants from publically available databases. An in-silico functional analysis was performed. MSI status of the analyzed samples was established. RESULTS: There were 51 validated variants distributed on 12 genes: 22% MSH3 (n = 11/51), 10% MSH6 (n = 5/51), 8% AMER1 (n = 4/51), 8% APC (n = 10/51), 2% BRAF (n = 1/51), 2% KRAS (n = 1/51), 12% PIK3CA (n = 6/51), 8% TGFBR2A (n = 4/51), 2% SMAD4 (n = 1/51), 4% SOX9 (n = 2/51), 6% TP53 (n = 3/51). Most known and distinct variants were in mismatch repair genes (MMR, 32%) and APC (20%). Among oncogenes, PIK3CA was the top target (12%). MSH3 variants were more frequent and predominantly homozogous in the analyzed population. CONCLUSION: These results illustrate for the first time CRC mutational profile in Iranian patients. MSH3, MSH6, APC and PIK3CA genes seem to play a bigger role in the path to cancer in this population. This is especially true for MSH3 variants that were very frequent and predominantly homozogous as these will associate with the EMAT phenotype that has prognostic implications. These findings will potentially lead to informed genetic diagnosis protocol and targeted therapeutic strategies.

#4385 Landscape of driver gene mutations in stage II and stage III colorectal cancer. Yoshikage Inoue,1 Nobuyuki Kikuchi,1 Kenichi Yoshida,1 Yusu Shiozawa,2 Yuichi Shiraishi,3 Kenichi Chiba,4 Keisuke Kataoka,4 Hiroko Ueno,1 Hiroko Tanaka,1 Satoshi Nagayama,2 Satoru Miyano,3 Yoshishiro Sakai,2 Seishi Ogawa1.

Introduction: Colorectal cancer (CRC) is the second leading cause of cancer death in Japan. The overall survival rate is approximately 85% and 70% for stage II and stage III CRCs, respectively, where over 45,000 deaths were ascribed to recurrence or metastasis. Survival benefit of adjuvant therapy is well defined only among stage III CRC patients, whereas that for stage II CRC patients remains modest, or according to recent reports, might be even deleterious among those with microsatellite instability (MSI). In an attempt to reveal the landscape of driver mutations in CRC and their association with clinical outcomes, we conducted targeted-capture sequencing of known driver mutations in stage II and III CRCs. Methods: A total of 248 patients with stage II (n = 145) and stage III (n = 103) CRC were enrolled. All patients were treated at a single institute between 2004 and 2016 and clinically well-annotated. Targeted-capture sequencing was performed using RNA baits designed for detection of oncogenic variants in 128 known driver genes in CRC. Additional baits for 1,605 SNPs, 7 introns and 18 microsatellites were also included to assess genome-wide copy numbers, fusion genes and MSI, respectively. Results: The median age at diagnosis was 64 (32-95) with a median observation period of 1,460 days. Of the 101 patients with recurrence, 59 and 42 patients had stage II and III diseases, respectively. Sixty percent of stage III patients received adjuvant chemotherapy, whereas only 10% of stage II patients received adjuvant chemotherapy. The average depth of the targeted-capture sequencing was 909x. In total, 2,243 somatic mutations (median 5 percent, range 0-106) were detected in 243 patients (97.9%). VTI1A-TCF7L2 translocation was identified in one sample. Except for a modest overrepresentation of TP53 mutations, frequency distribution of mutations was similar to those reported in previous reports, where APC (83%), TP53 (79%), KRAS (45%), and PIK3CA (18%) represented the most frequent mutational targets. Although none of these mutations were significantly associated with tumor recurrence or overall survival, we found that FBXW7 mutations had a positive correlation with tumor free survival. Twenty-one patients had more than 20 mutations, suggesting the presence of defects in DNA repair and/or replication. In fact, analysis of 18 microsatellites suggested that 22% of stage II MSI patients and 12% of stage III MSI patients had hypermutated cancer were explained by somatic mutations in POLE gene. Conclusions: We revealed the landscape of driver mutations in Japanese patients with stage II and III CRC with their implication in clinical outcomes. Hypermutated samples accounted for 8% of the whole cohort, which were explained by MSI or replication errors due to somatic POLE mutations. Our findings help to understand the relationships between driver mutations and recurrence for CRCs after curative resection.

#4386 Identification of clinically relevant colon cancer genes predictive of improved relapse free survival. Marie J. Parsons. The Walter and Eliza Hall Of Medical Research, Parkville, Australia.

Colorectal cancer (CRC) is the third most common cancer worldwide, affecting over 15,000 individuals in Australia each year. While CRC is often detected at a stage where resection of the primary tumour is possible, approximately 50% will relapse and die from metastatic disease. Prognostication is mainly determined by tumour depth (T), lymph node stage (N) and the extent of cancer spread (M). However, clinical outcomes of patients with the same TNM stage can be heterogeneous. Therefore, there is a need to identify markers to better predict prognosis and stratify patients for treatment regimes. A panel of 113 candidate CRC genes were identified as significantly mutated in whole genome and whole exome sequencing studies from 361 MSS colon cancers and 63 CRC cell lines. Custom ampiclon panels for target enrichment were designed for use with the HaloPlex™ target enrichment system. Sample libraries were prepared for 274 patients with stage II/III CRC using the automated Bravo liquid handling platform followed by next-generation sequencing (NGS) on the Illumina Next-Seq 500. Analysis of the 113 candidate genes identified 31 genes recurrently mutated above 10 percent in our discovery cohort. We identified 4 genes previously reported as colon cancer genes (APC 68%, TP53 60%, KRAS 30% and PIK3CA 20%), confirming APC, TP53 and KRAS as the most frequently mutated genes in CRC. To identify novel and clinically relevant genes, the 31 genes in our discovery cohort were tested for association with clinical features of CRC. In the discovery cohort, 24/31 genes were significantly associated with right sided CRC, 10/31 with mucinous CRC, 9/31 with stage II CRC and 7/31 with improved relapse free survival (RFS). Of these significantly associated genes, 10/24 genes were validated as significantly associated with right sided colon cancer, 3/9 with stage II and 3/10 with mucinous CRC in the validation cohort. Five of these genes were selected for further investigation as they were significantly associated with improved RFS, together with one or more clinical feature of CRC. Identification of novel recurrently mutated genes is key to a better understanding of the molecular mechanisms of CRC and development of novel therapeutics.

#4387 The clonal composition of colorectal cancer cell lines is defined by the maintenance of specific genomic imbalances, not by ongoing chromosomal instability. Darawalee Wangsa,1 Madison Schiefer,1 Daniel Bronder,1 Hesed Padilla-Nash,1 Irianna Torres,1 Lidia Warner,1 Yue Hu,1 E Michael Gertz,2 Russell Schwartz,2 Alejandro A. Schäffer,2 Daniela Hirsch,4 Timo Gaiser,2 Rüdiger Meyer,1 Jordi Camps,1 Kerstin Heselmeyer-Haddad,1 Thomas Ried1,3.

Keywords: fusion transcript, colorectal cancer, RNA-seq. Ultimately, it could illuminate a genomic-driven strategy to develop personalized medicine. Key words: fusion transcript, colorectal cancer, RNA-seq.
propagated in the single cell derived lines. These analyses were complemented by gene expression profiling of the single cell derived clones and the parental cell lines using the Nanostring technology which measures expression levels of 770 cancer-associated genes. The SW-480 clones separated into two distinct clusters that reflect discrete ploidy levels that were also present in the parental line. We then explored whether mutations of the BRAF (H1179Y) and KRAS (SW-480) genes observed in the parental lines were maintained in the derived clones; this was the case in all instances. Finally, we reconstructed phylogenetic trees of tumor evolution using a specifically developed algorithm, termed FISHTrees. Our meticulous analysis of the cloning distribution of these colorectal cancer models shows that, despite a certain degree of chromosomal instability, specific genomic imbalances are maintained.

The results of our investigation of genomically stable cell lines underline the importance of the gut microbiome and associated inflammation in predicting CRC progression. To date however, these insights have not led to significant changes in the treatment paradigm because efforts continue to be focused on the later stages of disease. We believe CRC presents a unique opportunity for disease intervention but to design effective interventional strategies, the exact sequence of molecular events underlying progression needs to be better defined and understood. To address these needs, we have assembled a clinically annotated sample database encompassing all stages of CRC (healthy colon, adjacent mucosa, adenomas, high-grade dysplasia, primary CRC and liver metastases), including samples from the conventional, microsatellite stable subtype, as well as from the serrated, microsatellite instable pathway. The progression status of each sample was characterized using standard pathology criteria. In addition, molecular progression was determined by targeted mutation profiling and targeted copy number profiling, as well as genome-wide expression profiling. This analysis confirms previous observations of early mutational events at the adenoma stage, including known tumor suppressor and oncogene driver mutations, e.g. KRAS G12 and G13 are mutated in 15% of the conventional adenomas but not in all colorectal cancers, as well as in 23% of the colorectal tumors. Copy number aberrations were observed at the adenoma and carcinoma stage, but with a lower prevalence then somatic mutations. Furthermore, genome wide expression analysis indicates that several pathways known to be affected in colorectal cancer are already disregulated at the adenoma stage. These pathways include Wnt signaling, mucosal barrier defects, biofilm formation, and several genes of the innate immune system, pointing at the interplay between local inflammation, the microbiome, and epithelial events. In addition, we observed that while genetic events are very dissimilar between the serrated/MSI and conventional/MSS pathway, the transcriptional regulation has many similarities, indicating at a possibility at targeting these disease subtypes using the same therapeutics. These initial findings provide rational avenues to intercept CRC at the adenoma stage and efforts are now focused on exploring the added role of the colonic microbiota and immune system modulation. A more comprehensive and integrated view of the changes associated with disease initiation will lead to the identification of new targets for prevention, interception and cure.

#4388 Characterisation of molecular events across the colorectal cancer progression axis. Joke Reumers,1 Liesbeth Van Wesenbeck,2 Eric Claeyssens,2 Gerald Chu,2 Stan Gaj,1 Emanuele Palescandolo,1 Carl Van Hove,1 Karin Verstraeten,1 Gary Borzillo,2 Dianna Wu,2 Pieter Poeters,2 Janine Arnts,1 Janssen R&D, Beere, Belgium;2Janssen R&D, Spring House, PA;3LabConnect LLC, Seattle, WA.

Uniquely amongst the major tumor types, the premalignant state in colorectal cancer (CRC) is readily detectable and diagnosable. Indeed, a multi-step process of CRC genesis was defined by the seminal work of Vogelstein et al, who described the mutational evolution of adenomas to adenocarcinomas. In addition to genetic aberrations, recent evidence has highlighted the importance of the gut microbiome and associated inflammation in predicting CRC progression. To date however, these insights have not led to significant changes in the treatment paradigm because efforts continue to be focused on the later stages of disease. We believe CRC presents an exceptional opportunity for disease intervention but to design effective interventional strategies, the exact sequence of molecular events underlying progression needs to be better defined and understood. To address these needs, we have assembled a clinically annotated sample database encompassing all stages of CRC (healthy colon, adjacent mucosa, adenomas, high-grade dysplasia, primary CRC and liver metastases), including samples from the conventional, microsatellite stable subtype, as well as from the serrated, microsatellite instable pathway. The progression status of each sample was characterized using standard pathology criteria. In addition, molecular progression was determined by targeted mutation profiling and targeted copy number profiling, as well as genome-wide expression profiling. This analysis confirms previous observations of early mutational events at the adenoma stage, including known tumor suppressor and oncogene driver mutations, e.g. KRAS G12 and G13 are mutated in 15% of the conventional adenomas but not in all colorectal cancers, as well as in 23% of the colorectal tumors. Copy number aberrations were observed at the adenoma and carcinoma stage, but with a lower prevalence then somatic mutations. Furthermore, genome wide expression analysis indicates that several pathways known to be affected in colorectal cancer are already disregulated at the adenoma stage. These pathways include Wnt signaling, mucosal barrier defects, biofilm formation, and several genes of the innate immune system, pointing at the interplay between local inflammation, the microbiome, and epithelial events. In addition, we observed that while genetic events are very dissimilar between the serrated/MSI and conventional/MSS pathway, the transcriptional regulation has many similarities, indicating at a possibility at targeting these disease subtypes using the same therapeutics. These initial findings provide rational avenues to intercept CRC at the adenoma stage and efforts are now focused on exploring the added role of the colonic microbiota and immune system modulation. A more comprehensive and integrated view of the changes associated with disease initiation will lead to the identification of new targets for prevention, interception and cure.

#4389 TSC1/2 mutations define a molecular subset of HCC with aggressive behavior and treatment implication. Daniel Wai Ho,1 Lo Kong Chan,1 Yung Tuen Chiu,1 Iris Ming Xu,1 Ronnie Poon,1 Tan To Cheung,2 Chung Ngai Tang,2 Victor Tang,2 Irene Lo,2 Polly Lam,2 Derek Yau,2 Miao Xin Li,2 Chun Ming Wong,2 Irene O. L. Ng,1 Univ. of Hong Kong, Pokfulam, Hong Kong;2Pamela Youde Hospital, Hong Kong;3Queen Elizabeth Hospital, Hong Kong.

We investigated the mutational landscape of mTOR signaling cascade in hepatocellular carcinomas (HCCs) with chronic hepatitis B (HBV) background, aiming to evaluate and delineate mutation-dependent mechanism of mTOR hyper-activation in hepatocarcinogenesis. We performed next-generation sequencing on human HCC samples and cell line panel. Systematic mutational screening of mTOR pathway-related genes was undertaken and mutant genes were evaluated based on their recurrence. Protein expressions of TSC1, TSC2 and pRPS6 were assessed by immunohistochemistry in human HCC samples. Rapamycin sensitivity was estimated by colony formation assay in HCC cell lines and the treatment was further tested using our patient derived tumor xenograft (PDTX) models. Results: We identified and confirmed multiple mTOR in HCC cohorts. We performed sequence variants of TSC1/2 in HCC samples from Asian populations. This analysis identified frequent (16.2%, n = 18/111) mutations of TSC1 and TSC2 genes in the HCC samples. The spectrum of TSC1/2 mutations likely disrupts the endogenous gene functions in suppressing the downstream mTOR activity through different mechanisms and leading to more aggressive tumor behavior. Mutation disruption of TSC1 and TSC2 was also observed in HCC cell lines and our PDX model of HCC. We found that the mTOR pathway was hyper-activated upon rapamycin treatment. With the use of the biologically relevant TSC2 mutant PDTXs, we demonstrated the therapeutic benefits of the hyper-sensitivity towards Rapamycin treatment. Taken together, our findings suggest the significance of previously undocumented mutation-dependent mTOR hyper-activation and frequent TSC1/2 mutations in HBV-associated HCCs. They define a specific molecular subset of HCC having genetic mTOR signaling, with potential significance of effective specific drug therapy.

#4390 The Thailand initiative in genomics and expression research in liver cancer: Race related common molecular subtypes among Asian hepatocellular carcinoma and cholangiocarcinoma identified by integrated genomics. Anuradha Budhul,1 Jittiporn Chaisaingmongkol,2 Hien Dang,1 Siritida Rabibhadana,2 Benjarath Pupacdi,2 So Mee Kwon,3 Marshonna Forgues,4 Yotsawat Pomyen,5 Vajarabhongsa Bhudhisawasdi,6 Nirush Lertprasertsuk,7 Chaisiri Pakskul,7 Chansuk Tangtrakul,7 Nirit Thawornsri,8 Thanaya Sricharunrun,8 Kannika Phornphutkul,9 Suleeporn Sangrajrang,9 Maggie Cam,10 Ping He,11 Stephen M. Hewitt,1 Xiaolin Wu,1 Snorri S. Thorgerisson,1 Joshua J. Waterfall,1 Yuelin J. Zhu,1 Jennifer Walling,1 Holly S. Stevenson,1 Daniel Edelman,1 Paul S. Meltzer,1 Christopher A. Loffredo,1 Robert H. Wiltrout,1 Curtis C. Harris,1 Chulahborn Mahidol,1 Mathiuos Ruchirawat,1 Xin W. Wang,1 National Institutes of Health, Bethesda, MD;2Chulabhorn Research Institute, Thailand;3Khoa Kao University, Khon Kaen University, Khon Kaen, Thailand;4Chulabhorn Hospital, Thailand;5Rajavip Hospital and Lampong Cancer Center, Thailand;6National Cancer Institute, Thailand;7FDA, Silver Spring, MD;8Georgetown University Medical Center, Washington, DC.

Hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC) are two distinct histologic liver cancers. They are clinically and biologically heterogeneous and highly resistant to treatment, making liver cancer the second most lethal malignancy in the world. In Thailand, liver cancer represents the primary cause of cancer-related death and is a major health problem. While HBV and HCV are major etiological factors for HCC globally, liver fluke infection (O. viverrini) is a major etiological factor for ICC in Thailand, especially in parts of northern Thailand where O. viverrini is endemic and approximately 70% of liver cancers are ICC. These unique risk factor patterns provide an opportunity to study cancer heterogeneity and unique liver tumor biology. The Thailand Initiative in Genomics and Expression Research for Liver Cancer (TIGER-LC) consortium was established to identify genomic and expression factors that may modify HCC and ICC susceptibility and progression. Here, we determined molecular subtypes and features of HCC and ICC through systems integration of genomic, transcriptional and metabolic profiles. We performed genome wide profiling of 398 surgical specimens derived from 199 Thai liver cancer patients. We employed the Affymetrix Human Transcriptome Array 2.0, the Affymetrix Genome-Wide Human SNP Array 6.0, Metabolon’s DiscoveryHD4 platform and Exome Sequencing to examine transcriptome profiles, somatic copy number alterations (SCNA), cancer metabolic profiles and mutation patterns, respectively. The results were validated in 847 independent Asian or Caucasian HCC or ICC cases. Transcriptomic analyses revealed that Thai HCC consisted of 3 stable subgroups (C1-C3), while Thai ICC contained 4 stable subgroups (C1-C4). Interestingly, HCC-C1 and ICC-C1 subtypes shared a similar gene expression matrix, as did HCC-C2 and ICC-C2, which correlated with patient survival. The molecular subtypes were similar in independent Asian HCC and ICC cohorts, but not in Caucasian patients, and were associated with tumor biology rather than etiology. GSEA revealed that the C1 subtype is enriched for mitotic checkpoint anomalies, while the C2 subtype is related to cytokine and chemokine signaling. We found that the C1 subtype encompassed a higher degree of SCNA when compared to the C2 subtype, suggesting an association with a genomic instability phenotype. Further analysis showed that the C2 subtype is linked to an increased body mass index, inflammatory responses and unique tumor metabolic activities. HCC and ICC from Asian populations, while clinically treated as separate entities, share common subtypes with similar actionable targets which can be targeted to improve precision therapy.
Expression of constitutively activated mutant FOXOs induced by PMPCB knockdown-mediated ROS productions suppressed gene expression in the liver cancer cell is regulated still remain to be delineated. In this study, we identified candidate genes critical for the maintenance of EpCAM positive hepatocellular carcinoma (HCC) cells using a global RNAi screening method. HCC cells were infected with pools of ~200,000 lentiviral shRNA library, then they were sorted to EpCAM positive or negative group. The abundance of shRNA in each group was analyzed using a microarray hybridization method. As a result, 76 candidate shRNAs with significantly different expression between EpCAM positive and negative populations were identified by class comparison analysis. Unsupervised clustering analysis of these genes in 247 HCC samples clearly separated EpCAM positive HCC cases from negative cases. Furthermore, we found that the EpCAM-dependent gene signature can predict survival in epithelial-like HCC cases. We focused on shRNA for PMPCB gene, which has an important role for processing mitochondrial protein, since it ranks as the top differentially altered shRNA-targeted gene between EpCAM positive and negative cells. PMPCB shRNA efficiently reduced EpCAM positive population and effectively inhibited cell proliferation both in vitro and in vivo. Interestingly, PMPCB shRNA specifically induced cell death in EpCAM positive HCC cells, but not in EpCAM negative cells. These data suggest that EpCAM positive cells functionally depend on PMPCB expression. On the other hand, we confirmed that PMPCB shRNA induced physiological dysfunction of mitochondrial function, leading to the increase of reactive oxygen species (ROS) production. We found that PMPCB shRNA significantly reduced TCF4 activity as well as the expression level of c-myc and cyclin D1, which are the downstream molecules of Wnt/β-catenin pathway. Although total β-catenin levels in the cytosol and nuclei fractions were not affected by PMPCB knockdown, overexpression of constitutively activated mutant β-catenin partially rescued the cells from PMPCB-induced apoptosis. Forkhead box O (FOXO) is known to be induced by the ROS production, bind β-catenin and decrease TCF activity, leading to the induction of apoptosis. We found that the nuclear translocation of FOXO family members, such as FOXO1 and FOXO3, were facilitated by PMPCB shRNA in EpCAM positive cells. These results suggest that the activation of FOXO's induced by PMPCB knockdown-mediated ROS production suppressed the activity of Wnt/β-catenin pathway, leading to the death of EpCAM positive HCC cells. In conclusion, we discovered a novel candidate target molecule of EpCAM positive HCC cells using a genome-wide RNAi screening method. Our study shed light on the mechanisms how EpCAM positive HCC cells are maintained and identified a new target molecule that has therapeutic vulnerability in EpCAM positive HCC cells.

#4392 Genetic diagnosis of multiple hepatocellular carcinoma. Yutaka Midorikawa,1 Shigo Yamamoto,1 Kenji Tatsuno,1 Hiroki Ueda,2 Shingo Tsuchi,3 Genta Nagae,3 Tadatoshi Takayama,1 Hiroyuki Aburatani2.

Multiple hepatocellular carcinoma (HCC) is categorized into two types; multicentric hepatocarcinogenesis (MC) and intrahepatic metastasis (IM). Although discrimination of the types of multiple HCC is of clinical importance, it is quite difficult to determine it correctly even after histological diagnosis. In order to determine multiple HCC as MC or IM, we compared pairs of multiple HCC samples from 40 patients with multiple HCC and 2 patients with pairs of primary HCC and extrahepatic metastasis such as adrenal gland and lung metastasis with regard to molecular aberrations. Exome sequence showed that more than 7% of somatic mutations were common in the pairs of IM, which were consistent with the result of a pair of primary HCC and extrahepatic metastasis, while no common somatic mutations was detected in genomic-MC (gMC) pairs. Notably, more than 60% of clinically monochromatic-MC cases were diagnosed as genomic-IM (gIM) due to the concordance of mutation and shows significantly frequent tumor thrombus, suggesting that gIM pairs developed from the common ancestor. In addition, cell-free DNA was analyzed using exome sequence in five patients with recurrent HCC, and found that somatic mutations of cell free DNA were similar to the primary HCC only in gIM not in gMC cases. Taken together, comparison of mutations in a pair of HCCs makes it possible to classify multiple HCCs into MC and IM, which is difficult to be correctly determined in clinical practice. Furthermore liquid biopsy for mutation analysis is available to make treatment plans for multiple HCC patients.


In introduction: Liver cancer is the second cause of cancer-related mortality worldwide. Currently, there are only two systemic agents able to increase survival in patients at advanced stages (i.e. sorafenib and regorafenib). Median survival of these patients is still poor, which highlights the need for new therapies. Our aim is to identify key regulatory genes with oncogenic properties amenable for therapeutic intervention through 1) integration of gene expression and DNA methylation data from human liver cancer by 2) functional validation in mice using shRNA screens. Methods: DNA methylation (Illumina HM450) and mRNA expression (Affymetrix human genome U219) data of 215 human HCC samples (Villanueva, Hepatology 2015) were analyzed to identify key gene regulatory networks. A causality test interrogated the impact of cis and trans regulation of promoter methylation on gene expression (Yoo, PLoS Genet 2015). A key regulator gene was defined when it regulated a substantial number of downstream genes (more than 2 standard deviations from the mean predicted trans-regulated downstream genes). Data analysis includes differential gene expression, topological overlap clustering (hierarchical, non-negative factorization [NMF]), and gene annotation. The tumorigenic potential of candidate tumor suppressors was experimentally validated through a positive selection of shRNA screening in human immortalized liver cell line H9252. Furthermore, we found that the EpCAM-dependent gene signature can predict survival in epithelial-like HCC cases. We focused on shRNA for PMPCB gene, which is a key regulator in the liver cancer cell is regulated still remain to be delineated. In this study, we identified candidate genes critical for the maintenance of EpCAM positive hepatocellular carcinoma (HCC) cells using a global RNAi screening method. HCC cells were infected with pools of ~200,000 lentiviral shRNA library, then they were sorted to EpCAM positive or negative group. The abundance of shRNA in each group was analyzed using a microarray hybridization method. As a result, 76 candidate shRNAs with significantly different expression between EpCAM positive and negative populations were identified by class comparison analysis. Unsupervised clustering analysis of these genes in 247 HCC samples clearly separated EpCAM positive HCC cases from negative cases. Furthermore, we found that the EpCAM-dependent gene signature can predict survival in epithelial-like HCC cases. We focused on shRNA for PMPCB gene, which is an important role for processing mitochondrial protein, since it ranks as the top differentially altered shRNA-targeted gene between EpCAM positive and negative cells. PMPCB shRNA efficiently reduced EpCAM positive population and effectively inhibited cell proliferation both in vitro and in vivo. Interestingly, PMPCB shRNA specifically induced cell death in EpCAM positive HCC cells, but not in EpCAM negative cells. These data suggest that EpCAM positive cells functionally depend on PMPCB expression. On the other hand, we confirmed that PMPCB shRNA induced physiological dysfunction of mitochondrial function, leading to the increase of reactive oxygen species (ROS) production. We found that PMPCB shRNA significantly reduced TCF4 activity as well as the expression level of c-myc and cyclin D1, which are the downstream molecules of Wnt/β-catenin pathway. Although total β-catenin levels in the cytosol and nuclei fractions were not affected by PMPCB knockdown, overexpression of constitutively activated mutant β-catenin partially rescued the cells from PMPCB-induced apoptosis. Forkhead box O (FOXO) is known to be induced by the ROS production, bind β-catenin and decrease TCF activity, leading to the induction of apoptosis. We found that the nuclear translocation of FOXO family members, such as FOXO1 and FOXO3, were facilitated by PMPCB shRNA in EpCAM positive cells. These results suggest that the activation of FOXO's induced by PMPCB knockdown-mediated ROS production suppressed the activity of Wnt/β-catenin pathway, leading to the death of EpCAM positive HCC cells. In conclusion, we discovered a novel candidate target molecule of EpCAM positive HCC cells using a genome-wide RNAi screening method. Our study shed light on the mechanisms how EpCAM positive HCC cells are maintained and identified a new target molecule that has therapeutic vulnerability in EpCAM positive HCC cells.
#4395 Somatic genomic alteration of 412 cancer-targeted genes of periampullary and pancreatic ductal adenocarcinoma in Indian patient population. Nilabja Sikdar,1 Gourab Saha,1 Richa Singh,1 Dhira Archinda,2 Paromita Roy,2 Supriyo Ghatak,2 Sudeep Banerjee,2 Sumit Gulati,2 Shibayoti Ghosh1,3
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Introduction: Pancreatic Ductal Adenocarcinoma (PDAC), the most common primary malignant disease of the pancreas and the periampullary region, accounts for about 75% of all nonendocrine tumors arising in this region. It is 1st most lethal malignancies. The 5 year survival rate is below 5%. At the beginning of the 21st century, the estimated number of PC worldwide was 110,000, with an estimated global mortality rate of 98%. Incidence of periampullary adenocarcinoma (PACs) is low, approximately 0.5-2% of all gastrointestinal malignancies and 20% of all tumors of the extrahepatic biliary tree. Aims & Objectives: To identify the somatic single nucleotide variants (SNVs) in 412 cancer related genes in PDAC & PAC’s. To identify possible altered biological pathways enriched in these cancers. Methodology: DNA samples from Tissue and blood were sequenced for exom sequencing of 412 cancer related genes (NMIBLNGE panel, Custom). Raw fastq files processed using GATK tools and variants identified by Varscan2, & MuTect. Altered pathways identified by KEEG. Result: A total 105 somatic exonic Single Nucleotide Variants (SNVs), 9 somatic exonic Indels, 22 LOH mutations (both Indel and SNV’s) were identified in our sample set. Among the candidate genes of PDAC somatic SNV’s were found in TP53, SMAD4 in some patient whereas frequency of KRAS mutation was less frequent. Fourteen SNV’s were reported in cosmic database which includes 5 pathogenic variants, whereas 91 SNV’s were novel. A total 75 non-synonymous, 6 stopgain, and 24 synonymous SNV’s were observed among 105 SNV’s. Fifty eight percent of transversion and 52% of transition observed from all SNVs. Among all transversion fifty five percent SNV’s were C>T, G>T. PI3-Akt, Proteoglycans in cancer, Transcription misregulation, Rap1, ErbB, Neurotrophin pathways were found to be most commonly enriched in our patient population. Our data strongly suggest PI3-Akt, Proteoglycans in cancer pathways play a critical role in the disease. Conclusion: This study implicates a landscape of somatic SNV’s in PDAC’s and PAC’s and alteration of biological pathways in our patient population. We also observed EBBB2 and KMT2C genes are predominantly altered in PDAC’s and PAC’s. PI53, KRAS and SMAD4 are the known mutated genes counterparts which contribute very similar mutation pattern.

#4396 Multiclonics assessment of the cancer and stromal compartments of patient-derived pancreatic xenografts reveals clinically-relevant subtypes and novel targeted therapies. Remy Nicolle,1 Yuna Blum,2 Laetitia Marisa,3 Celine Loncle,4 Odile Gayet,5 Vincent Moutardier,5 Olivier Turriu,6 Marc Giovannini,7 Benjamin Blanc,8 Martin Bigonnet,2 Marion Rubis,2 Nabila Elarouci,1 Jean-Luc Raoul,4 Veronique Secq,3 Stephane Garcia,3 Philippe Grandval,5 Martin Bigonnet,2 Marion Rubis,2 Nabila Elarouci,1 Jean-Luc Raoul,4 Veronique Secq,3 Stephane Garcia,3 Philippe Grandval,5 Martin Bigonnet,2 Marion Rubis,2 Nabila Elarouci,1 Jean-Luc Raoul,4 Veronique Secq,3 Stephane Garcia,3 Philippe Grandval,5
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#4395 Somatic genomic alteration of 412 cancer-targeted genes of periampullary and pancreatic ductal adenocarcinoma in Indian patient population. Nilabja Sikdar,1 Gourab Saha,1 Richa Singh,1 Dhira Archinda,2 Paromita Roy,2 Supriyo Ghatak,2 Sudeep Banerjee,2 Sumit Gulati,2 Shibayoti Ghosh1,3
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Introduction: Pancreatic Ductal Adenocarcinoma (PDAC), the most common primary malignant disease of the pancreas and the periampullary region, accounts for about 75% of all nonendocrine tumors arising in this region. It is 4th most lethal malignancies. The 5 year survival rate is below 5%. At the beginning of the 21st century, the estimated number of PC worldwide was 110,000, with an estimated global mortality rate of 98%. Incidence of periampullary adenocarcinoma (PACs) is low, approximately 0.5-2% of all gastrointestinal malignancies and 20% of all tumors of the extrahepatic biliary tree. Aims & Objectives: To identify the somatic single nucleotide variants (SNVs) in 412 cancer related genes in PDAC & PAC’s. To identify possible altered biological pathways enriched in these cancers. Methodology: DNA samples from Tissue and blood were sequenced for exom sequencing of 412 cancer related genes (NMIBLNGE panel, Custom). Raw fastq files processed using GATK tools and variants identified by Varscan2, & MuTect. Altered pathways identified by KEEG. Result: A total 105 somatic exonic Single Nucleotide Variants (SNVs), 9 somatic exonic Indels, 22 LOH mutations (both Indel and SNV’s) were identified in our sample set. Among the candidate genes of PDAC somatic SNV’s were found in TP53, SMAD4 in some patient whereas frequency of KRAS mutation was less frequent. Fourteen SNV’s were reported in cosmic database which includes 5 pathogenic variants, whereas 91 SNV’s were novel. A total 75 non-synonymous, 6 stopgain, and 24 synonymous SNV’s were observed among 105 SNV’s. Fifty eight percent of transversion and 52% of transition observed from all SNVs. Among all transversion fifty five percent SNV’s were C>T, G>T. PI3-Akt, Proteoglycans in cancer, Transcription misregulation, Rap1, ErbB, Neurotrophin pathways were found to be most commonly enriched in our patient population. Our data strongly suggest PI3-Akt, Proteoglycans in cancer pathways play a critical role in the disease. Conclusion: This study implicates a landscape of somatic SNV’s in PDAC’s and PAC’s and alteration of biological pathways in our patient population. We also observed EBBB2 and KMT2C genes are predominantly altered in PDAC’s and PAC’s. PI53, KRAS and SMAD4 are the known mutated genes counterparts which contribute very similar mutation pattern.

#4397 Deciphering the diversity of somatic alterations and Salmonella infection in gallbladder cancer by whole exome sequencing. Prajash Sundaram Iyer,1 Nilesh laxman Gardi,1 Malika Ranjan,1 Bikram Sahoo,1 Pratik Chandran,1 Pawan Upadhyay,2 Mukta R. Ramadwar,2 Shailesh V. Shirkhande,2 Amit Dutt1,3
1ACTREC, Mumbai, India; 2Tata membrane Centre, Mumbai, India.

Background: Gallbladder cancer is fifth most common cancers among the gastrointestinal cancers with the majority of patients presented at an advanced state of the disease. In India, gallbladder cancer is a major problem in the northern part of the country with its highest incidence of 22/1,00,000 women and risk factors ranging from gallstones, female gender, ethnicity. Salmonella infections and genetic alterations. Despite its high incidence in our country, there is only few candidate gene based studies and systematic genomic-wide studies are far in dismal. Hence there is an unmet need to understand the genomic landscape of Indian Gallbladder cancer genome. Materials and methods: We interrogated the cancer regions of gallbladder cancer genome of 27 samples (10 paired and 7 unpaired tumors) using whole exome sequencing at an average coverage of 100X and above. Further, we validated our findings from whole exome sequencing in an extended cohort of 27 FFPE (Formalin fixed paraffin embedded) samples using other sequencing technologies. In addition we used a computational subtraction tool to identify Salmonella DNA sequences in the whole exome sequencing data. Results: We identified 383 somatic alterations across 17 tumors, which includes an average 112 synonymous, 245 missense, 8 nonsense, 8 indels and 8 splice site changes. The average mutation rate considering the paired tumors is about 14 mutations/Mb. We found recurrent alterations in TP53, CTNNB1, SF3B1, ATM, AKAP11 and other genes by exome sequencing analysis. In addition, we examined our exome sequencing data for identifying Salmonella sequences as well as presence of 143 HPV types using computational subtraction based on HPVDetector. In our analysis we found association of typhoidal Salmonella strains in 11 of 26 gall bladder cancer samples and non-typhoidal Salmonella species in 12 of 26 sample, 6 samples were co-infected with both. Moreover, we observed co-occurrence of TP53 alterations in 4 of 16 Salmonella positive samples while we did not observe TP53 alterations in Salmonella negative samples. Conclusion: Taken together, we present the landscape of somatic alterations in Indian gallbladder cancer genome and identification of non-typhoidal Salmonella species along with co-occurrence of TP53 alterations could aid in the treatment of gallbladder cancer.

#4398 Identification of bile duct cancer specific fusion genes in patient tissues. Kahee Kim,1 Dawoon Esther Jung,2 Mi-kyoung Seo,2 Sang-Woo Kim,3 Si Young Song3
1ACTREC, Mumbai, India; 2Tata membrane Centre, Mumbai, India; 3Yonsei University College of Medicine, Seoul, Republic of Korea; 4Yonsei University College of Medicine, Seoul, Republic of Korea.

Gene fusion occurs when a part of one gene fuses with or attaches to a part of another gene by genome rearrangement and the result in gene fusion may possess oncogenic properties; fused gene may be translated into a unique protein that may promotes cancer properties. Bile duct cancer are one of the most lethal cancer types with low 5-year survival rates, but lack of proper diagnostic or prognostic markers. Our aim was to investigate bile duct cancer specific fusion genes found in patient’s specimens. We extracted total RNA from five bile duct cancer tissues and normal tissues from the same patients. We performed RNA sequencing and the result data was analyzed using ChimeraScan, Jaffa or Fusionchacker softwares to detect gene fusion. To identify the cancer somatic fusion gene, the fusion occurred in normal tissues and previously reported fusion genes found in normal tissues were excluded and the fusion occurred in coding region was included. As a result, we found 52 bile duct cancer tissue specific fusion genes. We analyzed the expression of selected fusion genes in 30 bile duct cancer patients and cancer cell lines by qRT-PCR. As a result we observed two novel bile duct cancer specific fusion genes. We cloned one of the fusion genes and transfected into the bile duct cancer cell lines SNU-1196 to assess, and increase in cell motility was observed in vitro. These findings indicate the presence of novel fusion genes as well as its possible application for the early diagnosis or prognosis of bile duct cancer.
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#4399 MUC13 induced NF-κB activation regulates metabolic reprogramming by promoting its crosstalk with GLUT-1 receptor. Sonam Kumari,1 Sheema Khan,2 Subash C. Gupta,3 Vivek K. Kashyap,4 Murali M. Yallapu,5 Subhas C. Chauhan,1 Meena Jaggi,1 *Univ. of Tennessee Health Science Center, Memphis, TN, 2Banaras Hindu University, Varanasi, India.

Objective: Pancreatic cancer (PanCa) is the fourth most common cause of cancer-related deaths in the US. MUC13, mucin is aberrantly expressed in PanCa and promotes tumor growth and progression. Herein, we investigate the fundamental role of MUC13 in glucose metabolism and delineate the molecular interplay of various molecules governing MUC13 mediated metabolic reprogramming that may be involved in pancreatic tumor maintenance. Methods: MUC13 expressing (Panc-1) and knockdown PanCa cells (HPAF-II) were generated for the study. Immunoblotting and qRT-PCR assays were performed to assess the expression of protein and mRNA levels, respectively, of key signaling molecules involved in glucose metabolism of PanCa. MUC13 and Glut-1 interaction was studied using co-immunoprecipitation, immunofluorescence, proximity ligation, Western blotting, co-capping assays in cell lines. Lactate and glucose assays were performed using commercially available kits. In vitro functional assays using wound healing scratch assay (migration), and cell Matrigel assay (invasion) were performed in presence or absence of Lactate and 2DG supplementation. Results: Our results demonstrate that MUC13 expression leads to the TNF-induced activation/nuclear translocation of NF-κB which in turn upregulates additional key proteins, Glut-1, c-Myc, Bcl-2. This recruits the Glut-1 to MUC13, wherein MUC13 functionally interacts with Glut-1 and stabilizes it, initiating downstream events that result in altered glucose metabolism. MUC13 expression in PanCa cells increases glucose uptake, lactate secretion which is reduced upon MUC13 knockdown. Additionally, MUC13 mediates increased cell migratory and invasion potential which can be potentiated by supplementing the culture media with lactate, an end product of aerobic glycolysis. However, treatment of cells with NF-κB inhibitor, Sulfasalazine, inhibits the MUC13 and Glut-1 interaction and abrogates all these events associated with glucose metabolism. Conclusion: These outcomes from our study suggest that MUC13 plays an important role in metabolic reprogramming of PanCa cells metabolism to induce cancer growth and enhanced cellular invasion and motility. NF-κB acts downstream of MUC13 to coordinate the events leading to its interaction with Glut-1 and metabolic reprogramming. Overall, these findings illustrate mechanism by which MUC13 coordinates the shift in metabolism to sustain cancer growth and invasion in PanCa.

#4400 Targeting lactate dehydrogenase-A promotes docetaxel induced cytotoxicity predominantly in castration-resistant prostate cancer cells. Hiroiyo Muramatsu,1 Makoto Sumitomo,1 Shingo Morinaga,1 Hiroshi Sakai,1 Ikuo Kobayashi,1 Keishi Kajikawa,2 Geyna Nishikawa,3 Yoshiru Kato,1 Masahito Watanabe,1 Kent Kanao,1 Koenja Nakamura,2 Kauzihiro Yoshikawa.1

Introduction & objectives: It is well known as Warburg effect that anaerobic glycolytic pathway is activated in various type of advanced cancers including prostate cancer (PC). Lactate dehydrogenase-A (LDH-A) controls the conversion of pyruvate to lactate and plays an important role in glucose metabolism. Since LDH-A pathways have been implicated in chemoresistance in various cancers, we investigated whether inhibition of LDH-A pathway could mediate the sensitivity to docetaxel (DOC) in human PC cells. Materials and Method: Four PC cell lines (PC3, DU145, LnCaP, LN-SS) were used. LN-SS is one of the LnCaP-derived castration-resistant PC (CRPC) cell lines established in our laboratories. Sodium oxamate (SO) was used as a specific LDH-A inhibitor. The protein expression was detected by western blot analysis using specific antibodies. Cell growth and survival were evaluated by WST-1 assays. Cell cycle progression and apoptotic inducibility were evaluated by flow cytometry using propidium iodide and Annexin V. The cytotoxicity of SO/DOC combination on PC cells was evaluated using the Chou-Talalay combination index (CI) method which offers quantitative definition for additive effect (CI = 1), synergistic (CI < 1), and antagonism (CI > 1) in drug combinations. Result: Western blot analysis showed that LDH-A protein was highly expressed in LN-SS cells compared with other PC cell lines including the parental LnCaP. WST-1 assays showed that treatment with SO (50 mM) for 72 hours increased growth inhibition (PC3: ~30%, DU145: ~55%, LnCaP: ~20%, LN-SS: ~55%), while SO has little growth inhibitory effects on normal lymphocytes in the concentrations between 1-100 mM. IC50 to DOC in PC cells were showed to be 4 nM, 1 nM, 1 nM and 4.5 mM in PC3, DU145, LnCaP and LN-SS, respectively, suggesting that both PC3 and LN-SS were relatively resistant to DOC compared with DU145 and LnCaP. Synergistic cytotoxicity was observed after the combination therapy with DOC and SO in LN-SS (CI: 0.5) but not in PC3 (CI: 1.9), DU145 (CI: 2.0), or LnCaP (CI: 6.5). Cell cycle analyses revealed that the combination with DOC and SO for 72 hours resulted in the accumulation of cells in G2-M phase followed by sub-G1 accumulation in LN-SS cells. Annexin V assays showed that 43% apoptosis was induced by the combination therapy in LN-SS cells, while only 12% by DOC only in LN-SS cells. Conclusion: Our results strongly suggest that LDH-A plays an important role in DOC resistance in advanced PC cells and inhibition of LDH-A promotes DOC-sensitivity especially in CRPC cells. Our study may provide valuable information for the future development of targeted therapies in patients with CRPC.

#4401 Role of estrogen signaling in colon microenvironment during obesity. Amena Archer,1 Linnea Petterson,2 Marcela Gonzalez-Granillo,3 Chris-tina Sava,2 Marion Korach-Andre,2 Cecilia Williams,2 KTH SciLifeLab, Stockholm, Sweden; 2Karolinska Institutet, Stockholm, Sweden.

Recent epidemiological studies highlight the strong association between high body mass index (BMI) and enhanced risk of colon cancer. This association appears stronger in men than in women, indicating a possible protective role of estrogens in this context. The biological action of the estrogens is mediated by specific transcription factors, the estrogen receptors (ERs). ERβ is the main ER in the intestine; and it is expressed in both epithelial cells and immune cells. Anti-tumorigenic and anti-inflammatory effects of ERβ in colon associated neoplasia have been demonstrated. Several rodent models for obesity show an impaired intestinal epithelial barrier function and an increase of pro-inflammatory markers in the colon. Moreover, in murine colitis models, high fat diet (HFD) exacerbates colonic inflammation. However, the specific molecular mechanisms linking obesity to increased colon cancer development, where estrogen signaling may be involved, is not clear. We hypothesize that ERβ-selective agonists can oppose the HFD-mediated inflammatory pro-tumorigenic colonic signaling in a gender-specific manner during obesity, and thereby protect against colorectal cancer. Our study is focused on the initiation of colon inflammation during obesity and aims to investigate the impact of ERβ activation in this context. To address this question, male and female mice were fed a HFD for 6 weeks and treated with an ERβ-selective agonist for 3 weeks prior sacrifice. Stool pellets were regularly collected. Animals were sacrificed; colon samples and caecal contents were collected for further analysis. We show that the expression pattern of inflammatory genes in colon is modified by the ERβ-selective agonist in HFD fed mice. Moreover the ERβ-selective agonist appears to induce gender-specific changes of the microbiota of HFD-fed mice. Our results show that an ERβ-selective agonist can modulate the colon microenvironment during obesity in mice and will provide a better comprehension of the molecular mechanisms leading to obesity-associated colonic inflammation.

#4402 CHTM1, a novel protein linked to cellular metabolism and human malignancy. Mansi Babbar,1 Ying Huang,5 Saeed Sheikh. SUNY Upstate Medical University, Syracuse, NY.

Several lines of emerging evidence indicate that alterations in metabolism are linked to pathogenesis of human malignancies. However, more studies are needed to completely understand the link between tumorigenesis and metabolism. In this context, we have characterized a novel protein named CHTM1 (Coliled-coil Helix Tumor and Metabolism 1) that appears to be associated with cancer and cellular metabolism. Per our findings, CHTM1 was present in cytosal as well as mitochondria. CHTM1 knockdown in human cancer cell lines caused oxidative stress, and decrease in cellular oxygen consumption and mitochondrial ATP levels, suggesting mitochondrial dysfunction. In view of the importance of mitochondria to nutrient stress, the effect of CHTM1 deficiency on human cancer cells in glucose-deprived conditions was also analyzed. The results indicated that CHTM1-deficient cells became more sensitive to glucose deprivation and exhibited altered cellular metabolism involving down regulation of PKC-CREB-PGC1 alpha signaling events. CHTM1 expression was also found to be increased in patient samples representing breast, colon and lung cancers when compared to their respective matching normal tissues. Based on these findings, we propose CHTM1 to be a novel tumor marker that can also be developed as a target for novel therapeutics particularly those that exploit cellular metabolism to mediate their effects.

#4403 EDG uptake in human lung adenocarcinoma associated with invasion through the hexosamine biosynthesis pathway. WeiRong Zhang,1 Gina Bouchard,2 Alice Yu,1 Majid Shafiq,1 Mehran Jamali,1 Joseph Shrager,1 Kelsey Ayers,1 Viswam S. Nair,1 Andrew Gentes,2 Maximilian Diehn,1 Andrew Quon,2 Sandy Napel,3 Sylvia Plevritis,3 Stanford University, Stanford, CA; 4UCLA, Los Angeles, CA.

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Introduction: Metabolic reprogramming is a cancer hallmark that is not fully understood. Moreover, increasing evidence suggests that metabolic dysregulation is histology-specific, particularly in the case of non-small cell lung cancer (NSCLC). In this study, we investigated the differences in glucose metabolism between the two major histology subtypes of NSCLC, namely adenocarcinoma (AD) and squamous cell carcinoma (SQ). We performed transcriptomic association with FDG-PET SUV
\textsubscript{max} (maximum standardized uptake value), a common clinical marker of glucose uptake in cancer. Methods: We identified metabolically-associated differentially expressed genes (DEGs) by histology subtypes using RNAseq data from selected tumors in our study cohort of 127 NSCLC patients (96 AD and 31 SQ) and validated the DEGs on The Cancer Genome Atlas. We identified gene-enriched cellular processes correlated with SUV
\textsubscript{max} separately for AD and SQ. We validated our findings in public domain cell line microarray data. In a separate cohort of 40 NSCLC patients (20 patients overlap with study cohort) whose tumors were sorted by cell type (cancer, endothelial, fibroblast and immune cells), we identified specific cell types where the genes of interest were expressed. Results: Metabolically-associated DEGs in AD were enriched in the hexosamine biosynthesis pathway (HBP), which is involved in glycosylating proteins related to invasion. SUV
\textsubscript{max} correlated genes in AD were enriched for epithelial-mesenchymal transition (EMT) and extracellular matrix remodeling. In particular, GPT2 (the rate-limiting gene in HBP) and 4-EMT-related glycoprotein genes were highly correlated with SUV
\textsubscript{max}, suggesting potency of HBP for EMT related glucose metabolic reprogramming. We validated DEGs on cell line microarray data (GSE49644) before and after TGF-\beta induced EMT, showing that many genes in HBP increased after induced EMT, while genes in the glycolysis pathway and the pentose phosphate pathway (PPP) were unchanged or reduced. HBP genes and many SUV
\textsubscript{max} correlated EMT genes were most highly expressed in tumor-associated fibroblasts compared to other cells in the tumor microenvironment. In SQ, metabolically-associated DEGs were enriched in the glycolysis pathway and PPP. SUV
\textsubscript{max} correlated genes were enriched for cell death. Conclusion: An integrative analysis of PET-RNAseq showed that in AD glucose uptake was related to invasion through metabolic reprogramming of HBP. In SQ, glucose uptake was associated with glycolysis and higher proliferation potential. Our work confirms that metabolic dysregulation in NSCLC is histology-specific and extends the finding to identify cellular processes associated with FDG uptake that differ in histology as well. Understanding histology-specific differences of tumor metabolism provides new insights into tumor behavior that can have implications for anti-cancer therapy.

Novel approach to circumvent cisplatin resistant lung cancer by targeting metabolism. Medhi Wangsaipichit, 1 Chunjing Wu, 2 Ying Ying Li, 3 Lynn G. Feun, 2 Macus T. Kuo, 1 Niramol Savaraj. 1 Univ. of Miami/VAMedical Cir., Miami, FL; 2VA Medical Cir., Miami, FL; 3University of Miami, Miami, FL; MD Anderson Cancer Ctr., Houston, TX.

For the past three decades, there are no drugs which could reverse cisplatin resistant or selectively kill these resistant cells. We have discovered that cisplatin resistant (CR) lung cancer shares one common factor which is increase in reactive oxygen species (ROS). Decreased intracellular thioredoxin-1 (TRX1) due to excessive secretion was found in vitro and in vivo, as well as in patients’ serum which could be a primary contributory factor to higher mitochondria-Ros levels. Furthermore, CR cells possess significantly increased number of mitochondria and consume higher rates of oxygen. Key glycolytic enzymes (HKII and LDHA) and lactate production were decreased in CR cells which suggests rewiring of tumor metabolic pathways. NAD\textsuperscript{+}, crucial co-factor of all redox systems, is significantly reduced in all CR cells. Importantly, treatment with riluzole (the FDA approved drug that interferes with cystine/glutamate exchange) thoroughly negativates the increase in ROS levels (wt)) resulted in completely disappeared in wild type mice and were significant reduced in KRAS mutant mice (n=5 per treatment group; p<0.002). Repurpose of riluzole should be considered for future treatment of CR lung cancer patients. Supported by Department of Veterans Affairs, CDA2 award (1K28BX001289) and Woman Cancer Association Fund.


Pancreatic cancer is a deadly disease with only 6% of patients living more than 5 years. Even if the disease is diagnosed when it is localized, the 5-year survival rate is approximately 19%. A characteristic of cancer cell metabolism is a shift from oxidative phosphorylation to aerobic glycolysis known as the Warburg effect. It is well known that calorie restriction (CR) is a powerful intervention that suppresses tumorgenesis in animal models by targeting glycolysis and oncogenic signaling pathways. Recent studies have shown that the metabolic responses characteristic of CR can be obtained with the use of energy restriction mimetics (ERM). The purpose of this study is to evaluate a new ERM, OSU-CG5 in altering growth of pancreatic cancer cells and nutrient-sensitive signaling pathways. Pancreatic tissues including those of the pancreas have uncontrolled proliferation necessitating increased fuel demands by increasing glucose availability and altering metabolic signaling pathways. Since ERM have been reported to target glucose transport, we evaluated whether OSU–CG5 alters glucose transport (Glut) proteins and glucose uptake. Glut1, but not Glut2, 3, 4, and 6, were overexpressed in Panc-1 cells with a 6-fold higher expression compared to HPNE cells. Glut proteins were not altered in MiaPaca cells. OSU-CG5 did not alter Glut1 protein expression in either cell line. Silencing Glut1 also had no effect on the cytotoxicity of OSU-CG5. OSU-CG5 did not alter glucose uptake when measured in different concentrations of glucose in the media. Cell survival, as measured with Pico green, was decreased after OSU-CG5 treatment with an IC \textsubscript{50} of 3\textsuperscript{um} when cultured in physiological concentration of 5 and 10mM of glucose. FOXM1 is unregulated in pancreatic cancer and a promoter of the Warburg effect. We found that OSU–CG5 significantly decreased expression of FOXM1 in Panc1 and MiaPaca cells. Our findings indicate that OSU-CG5 does not inhibit the growth of Panc1 and MiaPaca pancreatic cancer cells by decreasing glucose uptake but may target the FOXM1 signaling pathways.
expression and lipid synthesis in lung cancer. These results also revealed that negative regulation of ALCY and lipid synthesis is a novel and critical mechanism for CUL3 in tumor suppression.


Purpose: Cancer cells exhibit altered glucose metabolism, termed glycolysis which is described by the increased uptake of glucose and the conversion of glucose to lactate in cancer cells under adequate oxygen tension. Alternating glucose transporters (GLUTs) expression was detected in the cancer specific metabolism to adapt to the rapid growth and tumor microenvironment in diverse malignant tumors. In this study, GLUTs profiling of ESCC patients, the relation between proliferation and GLUT1 expression using siRNA for GLUT1 were analyzed. Furthermore, the prognostic features according to GLUT1 expression was evaluated on esophageal squamous cell carcinoma (ESCC) patients. Methods: The ratio of the 11 GLUTs (GLUT1- GLUT14) expression between tumor and normal tissue (T/N) were analyzed in silico (GDS3838). GLUT1 expression was down-regulated using 3 siRNAs in vitro. Immunohistochemical staining of GLUT1 was performed using paraffin sections of tissues obtained from 145 resectable ESCC patients without preoperative treatment. Results: Expression of GLUT1 (T/N 2.44; 95% confidence interval (CI): 1.78-3.34, P = 0.001) and GLUT3 (T/N 1.96; 95%CI: 1.23-3.13, P = 0.01) and GLUT6 (T/N 1.31; 95%CI: 1.12-1.54, P = 0.01) were significantly higher in ESCC tissues than normal tissues. In vitro, TE-1 and TE-8 cells were treated with 3 siRNAs for GLUT1 and control siRNA for 72 hours in vitro. The proliferation of TE-1 and TE-8 cells after siRNAs for GLUT1 compared with control siRNA decreased 72.9-84.5% and 44.8-53.3%, respectively. GLUT1 expression was evaluated with 145 ESCC patients. GLUT1 positivity was observed in 41 patients (28.2%) and associated with depth of invasion (odds ratio (OR) =2.984; 95% CI: 1.208-7.371; P = 0.018) and vascular invasion (OR =2.771; 95% CI: 1.118-6.871; P = 0.028) in multivariate analysis. GLUT1 positivity was a significant disadvantage to both relapse-free survival (hazard ratio (HR) =2.021; 95% CI: 1.100-3.712; P = 0.023) and esophageal cancer-specific survival (HR =2.223; 95% CI: 1.121-4.411; P = 0.022) in univariate Cox hazard analysis. Conclusions: GLUT1 expression was altered in ESCC patients and GLUT1 expression was most up-regulated among the 11 GLUTs. Growth assay with siRNAs for GLUT1 revealed that GLUT1 played a curial role of ESCC cells proliferation. High expression of GLUT1 was associated with depth of invasion and poor prognosis of ESCC patients. These findings provide evidence of the significance of GLUT1 expression as a biomarker and GLUT1 may be therapeutic target in ESCC patient.

### 4408 Metabolic alterations induced by ibrutinib in CLL cells as a basis for drug combinations to enhance ibrutinib therapeutic activity. Helene Pelicano, Li Feng, William G. Wierda, Michael J. Keating, Peng Huang. UT MD Anderson Cancer Ctr., Houston, TX.

The use of ibrutinib, which targets the Bruton tyrosine kinase, has signified improved the therapeutic outcome for patients with chronic lymphocytic leukemia (CLL), especially in cases with chromosome 17p deletion, which usually results in poor responses to conventional chemotherapy. However, CLL is highly resistant to current therapeutic options, and this resistance is incompletely curative, and a small portion of CLL patients exhibit resistance. The main objectives of this study were to investigate the effect of ibrutinib on CLL mitochondrial metabolism and to develop novel strategies to enhance the therapeutic activity of ibrutinib. Ibrutinib-induced changes in mitochondrial functions were analyzed by measuring oxygen consumption rate (OCR) as an indicator of mitochondrial respiration and extracellular acidification rate (ECAR) as a parameter of lactate production from glycolysis using an extracellular flux analyzer. In a primary CLL cell-stromal cell co-culture system (which maintains long-term CLL cell viability), ibrutinib induced little change in OCR and ECAR during the first 48-72 h. However, prolonged drug exposure (up to 7 days) in vitro caused a consistent and significant decrease in OCR compared to that in untreated cells, and in untreated cells ibrutinib treated in tumor tissue was prepared with normal tissue. Treatment with ibrutinib for 7 days also had significantly decreased OCR compared to pre-treatment samples from the same patients, suggesting that the impact of ibrutinib on mitochondrial functions occurred in vivo and was clinically relevant. On western blot analysis, ibrutinib induced decreases in expression of certain respiratory chain components associated with AMPK activation. On electron microscopy, CLL cells treated with ibrutinib in vitro for 6 days exhibited lower numbers of mitochondria than untreated cells, which was confirmed by flow cytometry analysis of cells stained with Mitotracker Green. Functional analysis showed that ibrutinib caused CLL cells to take up less glutamine but did not affect glucose uptake, suggesting that ibrutinib has a preferential impact on glutamine metabolism. On testing the possibility that the metabolic alterations and mitochondrial dysfunction induced by ibrutinib render CLL cells vulnerable to compounds that affect mitochondria or inhibit glutamine metabolism, we found that combinations of ibrutinib with metformin (inhibitor of mitochondrial OXPHOS) or with C968 (glutaminase inhibitor) resulted in synergistic activity against CLL cells and in vivo murine leukemia cells in comparison to either drug alone. Notably, the combination of ibrutinib and C968 was synergistic in killing CLL cells with 17p deletion. Overall, our results suggest that ibrutinib induces metabolic alterations characterized by decreased mitochondrial respiration and attenuated glutamine metabolism. Such metabolic alterations provide a biochemical basis for mechanism-based drug combination to enhance the therapeutic activity of ibrutinib.

### 4409 Cholesterol pathway determines ovarian cancer drug resistance through transcription factor SREBP2. Galina Karashchuk, Nataliya Karashchuk, Signe Caksa, Tyler S. Smith, Alexander S. Brodsky. Alpert Medical School of Brown University and Rhode Island Hospital, Providence, RI.

Ovarian cancer is the most common cause of gynecological cancer death in women in United States. Up to 70% of all ovarian cancer cases are high-grade serous carcinomas with 5-year survival rates less than 30%. Recent studies have suggested the importance of the cholesterol pathway in multiple cancers including gynecological malignancies. Here we demonstrate that genetic or pharmacological disturbance of cholesterol pathway in high-grade serous ovarian carcinoma cell lines results in significant changes in survival rate after drug treatment, and in protein and RNA expression patterns. SREBP2 is a transcription factor encoded by SREBP2 gene that regulates expression of sterol-regulated genes and thus maintains cholesterol homeostasis. To assess the role of SREBP2 in ovarian cancer we have created a stable OVCAR8 cell line with SREBP2 disrupted using CRISPR technology. This SREBP2-KD line has reduced SREBP2 mRNA and protein expression level that indicates an effective gene knockdown. Activation of SREBP2 is dependent on the cholesterol status of the cell. We observed that expression of the SREBP2 precursor form is significantly reduced in SREBP2-KD line when cells are maintained under low serum conditions. Mutant cells treated with paclitaxel in low, but not high serum or in presence of statin, revealed significantly lower cell viability. SREBP2-KD cells do not survive long-term, high concentrations of paclitaxel, nor do they grow back as efficiently as control cells. RNA expression and proteomic analysis revealed the critical regulators mediating SREBP2 activity in stressed cells. Together, these observations suggest that the cholesterol pathway is critical for ovarian cancer cells not only to resist stresses, such as chemotherapy, but also to return to a high proliferation state upon recurrence. Further studies will provide important targets for developing new drugs for treatment of ovarian cancer.

### 4410 LSR promoter methylation and cell growth following the activation of β-oxidation and its antibody inhibits lipid catabolism. Koichi Fujimoto,1 Kiyoshi Yoshino,1 Tadashi Kimura,3 Tetsuji Naka1. 1National Institute of Biomedical Innovation, Health and Nutrition, Japan; 2Niigata University, Japan; 3Osaka University, Japan.

Ovarian cancer is the most lethal gynecologic malignancy; thus developing new treatment options is urgently required. Molecular targeted therapies for cancers, which are generally more tolerable than widely used cytotoxic agents, have shown highly specific inhibition of target molecules. In this study, we aimed to identify a new ovarian cancer antigen and to develop a novel monoclonal antibody (mAb). Furthermore we evaluated its preclinical efficacy and analyzed the function of its antigen in ovarian cancer. To identify a new ovarian cancer antigen, cell surface membrane proteins of normal ovarian epithelial and ovarian cancer cell lines were analyzed by ITAQ-based proteomic technology. We identified lipolysis-stimulated lipoprotein receptor (LSR) as the new therapeutic target for ovarian cancer. By the immunohistochemical analysis, significant poor prognosis was observed in high-LSR expression patients with ovarian cancer compared to patients with low-LSR expression by survival assay (p < 0.05). Our newly developed anti-LSR mAb showed significant inhibition of tumor growth in vivo against xenograft model of LSR-positive ovarian cancer cell line and patient derived LSR-positive ovarian cancer tissue (p < 0.05). In LSR-positive ovarian cancer cells, high number and large lipid droplets were observed compared to LSR-negative cells and anti-LSR mAb decreased these droplets. Moreover addition of VLID to LSR-positive ovarian cancer cells significantly promoted the cell proliferation (p < 0.05) and anti-LSR mAb inhibited that in vitro (p < 0.05). Supporting these data, addition of VLID to LSR-positive ovarian cancer cells significantly promoted β-oxidation-mediated lipid catabolism (p < 0.05) and anti-LSR mAb also inhibited that (p < 0.05). In addition, this anti-LSR mAb which cross-reacted with mouse LS did not show any cytotoxicity on normal organs and lipid metabolism in mice. In summary, high expression
of LSR in ovarian cancer was the poor prognostic factor. Our newly developed anti-LSR mAb showed significant tumor growth inhibition against not only LSR-positive ovarian cancer cell line but also patient derived LSR-positive ovarian cancer tissue. In LSR-positive ovarian cancer cells, high number and large lipid droplets were observed and LSR promoted cell proliferation following B-oxidation-mediated lipid catabolism. Thus, anti-LSR mAb inhibited these processes. Our preclinical data demonstrated that targeting LSR by mAb is a promising therapy for patients with LSR-positive ovarian cancer.

#4411 Loss of fructose-1,6-bisphosphatase expression induces altering glucose metabolism and tumor progression in hepatocellular carcinoma. Hiroaki Wakiyama,1 Hidenari Hirata,2 Keishi Sugimachi,3 Takaa Maasaki,4 Naoki Hayashi,1 Yohsuke Kuroda,1 Shuhei Ito,1 Hidetoshi Eguchi,1 Kotaro Terashima,1 Katsumi Sakamoto,1 Masakazu Hirakawa,1 Hiroshi Honda,2 Koshijavik, Iceland;3 Norwegian University of Science and Technology, Trondheim, Norway;4 University of Iceland, Landspitali-University Hospital, Reykjavik, Iceland.

Background: A recent study reported the loss of gluconeogenic capacity in hepatocellular carcinoma (HCC). Fructose-1,6-bisphosphatase (FBP1), the rate-limiting enzyme in gluconeogenesis, is reduced in expression in some cancers. However, the role of FBP1 in altered glucose metabolism in HCC was unclear. Therefore, the objective of this study was to examine the function and clinical significance of FBP1 expression in HCC. Materials and Methods: First, three independent cohorts totaling 594 cases of HCC (118 real-time RT-PCR data from our institution, 242 expression array data from GSE14520, and 234 RNA-seqencing data from The Cancer Genome Atlas (TCGA)) were analyzed to address clinical significance. Data from methylation arrays, SNP arrays, and whole-exome sequencing were also analyzed to investigate the regulation of FBP1 expression in the TCGA cohort. Second, we analyzed mRNA expression, promoter methylation, and DNA copy number profiles of 967 human cancer cell lines, including 27 liver cancer, in the Cancer Cell Line Encyclopedia. Third, we established HCC cell lines stably expressing FBP1 or empty vector control. We performed sphere formation assay and xenograft studies to evaluate the role of FBP1 on HCC progression. Furthermore, in order to assess the effect of FBP1 on altered glucose metabolism, isotopomer distribution analysis was performed using [U-13C] glucose. Finally, to validate the effects of FBP1 expression on survival, risk of recurrence, and glucose metabolism, we performed gene set enrichment analysis (GSEA). Results: Lower FBP1 expression associated with advanced tumor stage, poor overall survival (OS), and poor recurrence-free survival (RFS) in three independent HCC cohorts. For either OS or RFS in each cohort, this prognostic impact persisted, even after adjusting for tumor stage. In HCC cell lines, where endogenous FBP1 expression is low, engineering its ectopic overexpression inhibited tumor growth, and intracellular glucose uptake by reducing aerobic glycolysis. In patient specimens, promoter methylation and copy-number loss of FBP1 were independently associated with decreased FBP1 expression. Similarly, FBP1 downregulation in HCC cell lines was also associated with copy-number loss. HCC specimens exhibiting low expression of FBP1 had a highly malignant phenotype, including large tumor size, poor differentiation, impaired gluconeogenesis, and enhanced aerobic glycolysis. The association of FBP1 expression and gluconeogenesis and glycolysis were confirmed by GSEA. Conclusion: Our findings established that FBP1 downregulation in HCC contributed to tumor progression and poor prognosis by altering glucose metabolism, and they rationalize further study of FBP1 as a prognostic biomarker and therapeutic target in HCC patients.

#4412 Metabolic reprogramming in EMT - targeting regulatory nodes in mesenchymal cells. Bylgja Hilmarsdottir,1 Skarphedinn Halldorsson,2 Maria T. Grinde,2 Anna Barkovskaya,1 Solveig Pettersen,3 Thorarinur Gundjonsdottir,4 Siver A. Moestue,1 Ottar Rolfsson,2 Gunhild M. Maedlansmo.1 Institute for Cancer Research, Oslo University Hospital, Oslo, Norway;2 University of Iceland, Reykjavik, Iceland;3 Norwegian University of Science and Technology, Trondheim, Norway;4 University of Iceland, Landspitali - University Hospital, Reykjavik, Iceland.

To combat cancer we have to avoid development of resistant and metastatic disease. Breast cancer cells can switch from an epithelial to mesenchymal phenotype through a process called epithelial to mesenchymal transition/EMT. Emerging evidence suggests that this process is vital to avoid treatment pressure and to gain metastatic capacity. Furthermore, recent literature shows that metabolic reprogramming is an essential attribute of cellular plasticity. Metabolic targeting could therefore be an attractive possibility to prevent development of resistance and metastatic dissemination. Here we tried to understand the metabolic phenotype of EMT and the mechanisms linking the metabolic programs to cellular plasticity. We also aimed to unravel compensatory metabolic pathways and use the metabolic inhibitors to sensitize breast cancer cells to conventional therapy. To that end we have investigated the metabolic signature of the D492 EMT cell model. The D492 cell line, established from human breast epithelial progenitor cells, has retained stem cell characteristics and has the ability to undergo EMT upon stromal (endothelial) influence, forming the mesenchymal cell phenotype. The D492 cell line is an in vitro model of breast epithelial progenitor cells, and constitutes a unique platform to unravel the factors responsible for stromal cell-induced cellular plasticity. We show that metabolic reprogramming is essential for induction of the mesenchymal phenotype using metabolomic profiling. Using Ultra performance liquid chromatography Mass Spectrometry and gene expression profiling we have created genome-wide metabolic models of D492 and D492M. Our data show that glycolytic flux and oxidative phosphorylation is higher in D492, however, D492M cells rely more on amino acid anaplerosis and fatty acid oxidation to fuel the TCA cycle. Glutamine and glucose tracing using NMR will give further insight into the difference in metabolism between the two cell lines. We have used these data to find metabolic targets that lock the cells in the epithelial state or identify the means to induce lethality in the mesenchymal cells. Using the metabolic rewiring of EMT in the D492 cell model we can understand the mechanisms responsible for treatment resistance, identify compensatory metabolic pathways during treatment and find metabolic inhibitors that will sensitize BC cells to conventional therapy.

#4413 Targeting glutamine metabolism in MUC1 expressing triple negative breast cancer. Gennifer D. Goode, Venugopal Gunda, Panka Singh. University of Nebraska Medical Center, Omaha, NE.

Breast cancer, the second leading cause of cancer deaths in women, accounts for nearly 1 in 3 cancer cases diagnosed in the U.S. women. Triple negative breast cancer (TNBC) accounts for approximately 15-25% of all breast cancer cases and has an increased incidence of metastasis, high recurrence within 1-3 years and a high mortality rate. Therefore, identifying factors that facilitate tumor growth and metastasis have the potential to serve as novel molecular targets for breast cancer therapy. Mucin1 (MUC1), a glycoprotein associated with chemoresistance, is aberrantly overexpressed in TNBC and facilitates growth and metastasis of TNBC cells. Recent studies suggest a role for MUC1 in modulating cell metabolism to support tumor growth. In the present study we examined the role that MUC1 plays in TNBC tumor metabolism; thus facilitating tumor growth. Our results indicate that MUC1 expression facilitates glutamine metabolism. A significant correlation between MUC1 expression, glutamine dependency, and amino-oxyacetate (AOA) sensitivity was observed. These alterations can be attributed in part to alterations in the expression of genes regulating glutamine metabolism. Collectively, these findings suggest that MUC1 serves as a metabolic regulator in TNBC, facilitating metabolic reprogramming of glutamine that influences growth of TNBC. Additionally, the findings with AOA’s effectiveness provide evidence for potential therapeutic utility, particularly for MUC1 overexpressing TNBC.

#4414 Arginine metabolism is modulated by androgen signalling and prostate cancer progression. De-Xue Fu,1 Hubert Huang,1 Jee-Hoon Song,1 Min Xu,1 Lucy Liu,1 Mohamad Afnan Khan,1 Krish Chandrasekaran,1 Arif Husain,1 Ganesh Srim,2 Mohammad M. Siddiqui1 University of Maryland, Baltimore, MD;2 University of Maryland College Park, College Park, MD.

Background: Metabolic reprogramming has been described as one of the hallmarks of cancer. A pathway of promise in prostate cancer management is arginine (Arg) metabolism. Phase 2 clinical trials are underway to study arginine deprivation as a treatment of prostate cancer. However, heterogeneous response has been noted and further studies are needed to better identify prostate tumors most reliant on Arg metabolism. The aim of this study was to investigate if Arg metabolism was associated with androgen signalling and prostate cancer progression from androgen dependent (AD) to androgen independent (AI) phenotype. Methods: The LNCaP prostate cancer cell line was used. Four sets of conditions were tested: LNCaP control, LNCaP in the presence of dihydrotestosterone (DHT), LNCaP with MDV3100 (an AR inhibitor), and a subline of LNCaP (CSS90) which has become AI. MTI viability assay was used to assess cell viability. The Seahorse XF bioenergetics analyzer was used to measure metabolic oxygen consumption. Gas Chromatography and Mass Spectroscopy (GC/MS) was used for metabolomics characterization. Results: LNCaP cells at a baseline are Arg-dependent. Arg starvation led to a 63% decrease in LNCaP cell viability (p<0.0001) in 24hrs. By contrast, the AI CSS90 only demonstrated a 23% decrease in cell viability when depleted of Arg (p<0.01 with Arg control, p<0.001 between LNCaP and CSS90) demonstrating AD cells were much more dependent on exogenous Arg. Enhanced AR signaling with DHT further sensitized cells to arginine depletion with a 73% decrease, and low level
AR inhibition decreased response to arginine depletion with only a 21% decrease in cell viability (p<0.001 for all changes). Metabolic characterization by GC/MS demonstrated a 33.8 fold increase in buildup of an arginine metabolism intermediate, ornithine in the CS590 cells vs LNCaP (p<0.0001) reflecting compensatory ability of the CS590 cells to endogenously manage Arg metabolism. Modulation of arginine signaling with DHT and MDV3100 demonstrated similar increased reliance of exogenous Arg for AD cells with increased androgen signaling with a 2.2 fold increase in Arg dependent oxygen consumption with DHT and 95% drop with MDV (p<0.0001 for both). Conclusions: Arginine is potentially an important aspect of prostate cancer metabolism. Arg targeted therapies may best be suited towards earlier stage tumors and Arg metabolism characterization may have potential as a biomarker of progression to AI prostate cancer.

**#4415 Fatty acid synthase-mediated palmitate production impacts epidermal growth factor receptor signaling to regulate specificity protein 1 in breast cancer cells.** Lincoln J. Barlow, Jian-Ting Zhang, Indiana University School of Medicine, Indianapolis, IN.

Fatty acid synthase (FASN), the sole cytosolic enzyme responsible for de novo palmitate synthesis, is critical for cancer cell survival and is a marker of poor prognosis. FASN overexpression has been shown to confer resistance to both drug and radiation resistance by inhibiting drug-induced ceramide production and by increasing DNA repair activities. Recently, it was found that FASN up-regulation of non-homologous end joining (NHEJ) repair of double strand DNA breaks was due to FASN up-regulation of Poly (ADP-ribose) polymerase 1 (PARP1). Furthermore, overexpression of FASN also increased protein 1 (SP1). However, how FASN regulates the expression of SP1 remains unknown. In this study, we tested the hypothesis that, palmitate, the end product of FASN catalysis, plays an important role in regulating SP1 expression by posttranslationally modifying epidermal growth factor receptor (EGFR) and affecting its activity. Using paired isogenic cell lines with FASN overexpression and FASN knockdown as well as exogenous supplementation of palmitate, we found that depletion of palmitate in culture reduced SP1 expression while supplementing palmitate rescues this effect. Furthermore, FASN overexpression and knockdown increased and decreased, respectively, phosphorylation and activation of EGFR. Treatment with EGFR inhibitor, Erlotinib, reduced SP1 expression. These findings suggest that FASN regulation of SP1 and NHEJ repair may be via regulating EGFR activity. We are in the process of determining the potential effect of palmitolysis of EGFR on its activation and regulation of SP1 expression.

**#4416 Studies on role of acyl-CoA cholesterol acyltransferase (ACAT) expression in ovarian/endometrial cancer progression - An invitro study.** Vijayalakshmi N. Ayyagari,1 Xinjia Wang,1 Laurent Brandt2. 1Southern Illinois Univ., Springfield, IL; 2Southern Illinois Univ. Simmons Cancer Inst., Springfield, IL.

INTRODUCTION: Cancer progression and tumorigenesis have been linked to alterations in cellular metabolic pathways of lipid and cholesterol synthesis in many cancers. Increased levels of cholesterol esters (CE) have been reported in breast cancer, leukemia, glioma and prostate cancer, but not exploited in detail in ovarian and endometrial cancers. Inside cells, excess free cholesterol is esterified by acyl-CoA cholesterol acyltransferase (ACAT) and stored as cholesteryl ester (CE) in lipid droplets (LDs). Cholesteryl ester accumulation may be a consequence of loss of tumor suppressor PTEN and subsequent activation of PI3K/AKT pathway as observed in pancreatic cancer. In the present study, we focused on the role of ACAT and cholesterol esters in altered cholesterol metabolism in ovarian/endometrial cancer cell lines. METHODS: We studied the effect of abrogation of cholesterol esterification either by an ACAT-1 inhibitor/shRNA knockdown or via inhibition of PI3K/AKT/mTOR pathways and subsequent effect on ovarian/endometrial cancer cell proliferation, invasion, cell migration and tumorigenesis. The expression of ACAT was studied at the protein and mRNA levels in a panel of ovarian/ endometrial cancer cell lines and also in normal and ovarian cancer clinical samples. ACAT expression was assessed by western blotting, ELISA, quantitative RT-PCR and immunostaining techniques. RESULTS: Our results reveal an elevated expression of ACAT-1 at both protein and mRNA levels in ovarian and endometrial cancer cell lines as compared to primary cells. Our results also provide preliminary data on involvement of ACAT and cholesterol esters in the progression and metastasis of ovarian cancer. CONCLUSION: These studies may aid in the development of new biomarkers or treatment targets for ovarian/endometrial cancer.

**#4417 The role of EBV infection in aerobic glycolysis in nasopharyngeal carcinoma.** George S. Tsao,1 Jun Zhang,1 Lin Xia,1 C Tsang,1 Weitao Lin,1 Y Yang. 1Univ. of Hong Kong, Pokfulam, Hong Kong; 2Chinese University of Hong Kong, Shatin, Hong Kong.

Accumulating evidence indicates that oncogenic viral protein exerts a crucial role in activating aerobic glycolysis during tumorigenesis, but the underlying mechanisms are largely undefined. The Epstein-Barr virus (EBV)-encoded latent membrane protein 1 (LMP1) is a trans-membrane protein with potent cell signaling properties and has tumorigenic transformation property. Activation of LMP1 is an important factor in the oncogenesis of nasopharyngeal carcinoma (NPC) cells. Collectively, blocking the activity of mTORC1 signaling effectively suppresses LMP1-induced aerobic glycolysis. mTORC1/LMP1 signaling and Glut-1 transcription. Interfering NF-κB signaling has no effect on mTORC1 activity but effectively altered Glut-1 transcription. Luciferase promoter assay of Glut-1 also confirmed that Glut-1 is a direct target gene of NF-κB signaling. Furthermore, we demonstrated that the LMP1 C-terminal activating region (CTAR) 2 is the key domain involved in mTORC1 activation, mainly through IKKβ-mediated phosphorylation of TSC2 at Ser199. Depletion of Glut-1 effectively led to suppression of aerobic glycolysis, inhibition of cell proliferation, colony formation, and attenuation of tumorigenic growth property of LMP1-expressing nasopharyngeal epithelial (NPE) cells. These findings suggest that targeting the signaling axis of mTORC1/NF-κB/Glut-1 represents a novel therapeutic target against NPC. Acknowledgement: 96 800x600 This project was supported by the General Research Fund (779810) and Cancer Council Victoria (Cancer Research Trust) of Australia. Grant (1061402980, Health and Medical Research Fund of Hong Kong (12110782), Aoe grant (AoE/M-06/08) and TBRS grant (T12-401/13-R). We thank Prof. Dongyan Jin (Department of Biochemistry, The University of Hong Kong) for the kind gifts of IKK related plasmids and Prof. Zhenguo Wu (Division of Life Science, The Hong Kong University of Science and Technology) for the discussion and interpretation of the data. We also thank Mr. Tony Chan for his technical support.

**#4418 Enhanced endodysplasia as a metabolic detoxer for cancer cells.** Yang Liu, Yanxian Lu, Auigo Li, Orieta Celiku, Mark R. Gilbert, Chun Zhang. 1Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA.

INTRODUCTION: Metabolic defects impair physiology and life cycle of every somatic cell. However, emerging evidence showed abnormalities in metabolic pathways paradoxically serve as initial steps in many types of human cancers. Little is known about the molecular mechanisms that confer metabolic adaptation and oncogenic predisposition for a transforming cell with intrinsic metabolic defect. In the present study, we explore the molecular mechanism that assists cellular adaptation when confronting metabolic deficiency, and its correlation acquisition of aggressive phenotypes. METHOD: We took advantage of clinical cases and in vitro cellular models with neomorphic IDH1 mutations, as a paradigm of metabolic deficiency-associated cancer. We investigated whole transcriptomic profiling based on RNA sequencing on IDH1-mutated cells and seek for selective activated canonical pathways. As a validation, we investigated the signature pathways in IDH1-mutated cells, with a focus on cytoskeleton cellular, cellular migration and endodysplasia. Moreover, we investigated mTORC pathway and identified its pivotal role in the metabolic adaptation and cellular motility changes in IDH1-mutated cells. RESULTS: Transcriptomic profiling suggested profound activation in molecular pathways that govern cell movement, endodysplasia, chemotaxis and invasion in IDH1-mutated cells. Hierarchical clustering analysis suggested that acquisition of R132C or R132H IDH1 mutant leads to consistent activation pattern for cellular movement. In addition, we confirmed that IDH1-mutated cells exhibit stronger capacity in cellular migration, endodysplasia and cytokine re-organization. Importantly, we demonstrated remarkable enhancement in endodysplasia pathways in IDH1-mutated cells, suggesting cellular transformation occurs in metabolic up-regulation metabolic deficient cell. Finally, we demonstrated that Rictor/mTORC2/Rac1 pathway is selectively activated in IDH1-mutated glioma cells, which is essential to enable cytoketease mobilization, endodysplasia and acquisition of aggressive phenotype in transforming cells. CONCLUSION: Our findings demonstrate that metabolic deficiency leads to enhancement in food seeking behavior in cellular level, highlighted with enhanced cell motility, chemotaxis, and endodysplasia; suggesting that this is an important molecular mechanism in cellular transformation during oncogenesis. This phenotypic transformation provides additional oxygen sources, compensating for IDH mutation related alterations and may help explain the enhanced cell migration and invasion characteristic of these cancers.

**#4419 HER2 expression and 17β-estradiol induce A549 expression in estrogen receptor positive mammary carcinoma cells.** Maroua Mbarik, Anissa Belkaid, Marc E. Surette. Université de Moncton, Moncton, New Brunswick, Canada.

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Breast cancer is a multifactorial disease involving several molecular changes and a high proliferation rate often under the activation of hormonal receptors. Therefore, cancer cells require the metabolic machinery for membrane synthesis and to promote signaling pathways. Amongst biomolecules required for efficient cell growth are polyunsaturated fatty acids, like arachidonic acid, that are essential for cell membrane synthesis and to promote signaling pathways. Uptake of arachidonic acid is essential for cancer cell growth. Mutations and/or reductions in mtDNA copy number are important in the regulation of the mitochondrial biogenesis, thus, they are important in the cellular metabolic phenotype. Utilizing the vast majority of PCa presents as androgen-dependent adenocarcinoma, recent uses of increasingly potent therapeutics targeting the androgen receptor signaling axis has resulted in the promotion of NEPC transdifferentiation as a mechanism of treatment resistance. Unfortunately, there is a lack of effective treatment options for NEPC. Altered cancer metabolism is now recognized as a hallmark of cancer and a crucial factor for promoting tumor growth and spread. In particular, altered glucose metabolism and the resultant acidification of the tumor microenvironment via increased lactic acid production has been shown to play an important role in multiple cancer-promoting processes, including tissue invasion/metastasis, angiogenesis, and suppression of local anti-cancer immunity. While increased glycolysis is not generally considered a phenomenon relevant to primary treatment-naive PCa, we have recently demonstrated its relevance to castration-resistant prostate cancer (CRPC) and metastatic CRPC. Our study thus indicates that capsaicin suppresses pancreatic cancer growth by down-regulating glycolysis and mitochondrial respiration in pancreatic cancer cells. Our results showed that capsaicin treatment reduced the extra-cellular acidification rate (glycolysis) and oxygen consumption rate (mitochondrial respiration) in AsPC-1 and BxPC-3 cells in a concentration-dependent manner after 24h of treatment. Capsaicin treatment reduced 86% and 55% down regulation of glycolysis process in AsPC-1 and BxPC-3 cells respectively. Our results also indicate the inhibition of glycolytic capacity and glycolytic reserve by capsaicin treatment indicating the potential of capsaicin to inhibit glycolysis. In addition, capsaicin treatment reduced 98% of basal oxygen consumption rate and ATP production in AsPC-1 cells. Furthermore, capsaicin treatment inhibited spare respiratory capacity & proton leak in a concentration dependent manner in AsPC-1 cells. Treatment of AsPC-1 and BxPC-3 with capsaicin for 48h also inhibited the expression of LDH-A and its upstream regulators such as HIF-1α, pSTAT3 (Y705) and EGFR as evaluated by Western blot. Our study thus indicate that capsaicin suppresses pancreatic tumor growth by down-regulating glycolysis and mitochondrial respiration. Further mechanistic studies are in progress. [Supported in part by R01 grant CA129038, awarded to (S.K.S.) by the National Cancer Institute].

#4422 Capsaicin suppresses pancreatic tumor growth by inhibiting tumor cell metabolism. Sharavan Ramachandran, Sanjay K. Srivastava. Texas Tech University, Amarillo, TX

Tumor cell metabolism is considered to be a hallmark of tumorigenic progression. Pancreatic cancer cells are shown to develop addiction towards the metabolic pathways like glycolysis and mitochondrial respiration to meet energy demands. Pancreatic tumor cells predominantly utilize cytosolic aerobic glycolysis for energy production. In our previous studies, we have reported that capsaicin treatment inhibits the survival of AsPC-1 and BxPC-3 pancreatic cancer cell lines. In this study, we evaluated the effect of capsaicin on glycolysis and mitochondrial respiration in pancreatic cancer cells. Our results showed that capsaicin treatment reduced the extra-cellular acidification rate (glycolysis) and oxygen consumption rate (mitochondrial respiration) in AsPC-1 and BxPC-3 cells in a concentration-dependent manner after 24h of treatment. Capsaicin treatment reduced 86% and 55% down regulation of glycolysis process in AsPC-1 and BxPC-3 cells respectively. Our results also indicate the inhibition of glycolytic capacity and glycolytic reserve by capsaicin treatment indicating the potential of capsaicin to inhibit glycolysis. In addition, capsaicin treatment reduced 98% of basal oxygen consumption rate and ATP production in AsPC-1 cells. Furthermore, capsaicin treatment inhibited spare respiratory capacity & proton leak in a concentration dependent manner in AsPC-1 cells. Treatment of AsPC-1 and BxPC-3 with capsaicin for 48h also inhibited the expression of LDH-A and its upstream regulators such as HIF-1α, pSTAT3 (Y705) and EGFR as evaluated by Western blot. Our study thus indicate that capsaicin suppresses pancreatic tumor growth by down-regulating glycolysis and mitochondrial respiration. Further mechanistic studies are in progress. [Supported in part by R01 grant CA129038, awarded to (S.K.S.) by the National Cancer Institute].

#4423 Deep sequencing of lung cancer samples using different library preparation methods produces discordant short non-coding RNA profiles. Brid M. Ryan1, Phillippe Lohere2, Khadijah Mitchell1, Adriana Zingone1, Yongmei Zhao1, Jyoti Shetty1, Bao Tran1, Isidore Rigoutsos1. 1National Cancer Inst., Bethesda, MD; 2Thomas Jefferson University, Philadelphia, PA.

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Metabolic Regulation and Cancer Therapy

#4421 IGFL2 regulates mitochondrial cell energy phenotype and biogenesis in TNBC cells. Maria L. Pagan1, Vinod Kumar Radhakrishnan2, Daisy De Leon3. 1University of Puerto Rico School of Medicine, San Juan, Puerto Rico; 2Loma Linda University, Loma Linda, CA.

Triple-negative breast cancer (TNBC) is very aggressive, resistant to chemo-therapy and more likely to relapse, causing the worst prognosis. African American (AA) women suffer higher incidence and mortality of TNBC due to the expression of high levels of Insulin Growth Factor 2 (IGF2) which promotes tumor progression, metastasis, and chemoresistance. Also, it has been established that functional mitochondria and mitochondrial DNA (mtDNA) are essential for cancer cell growth. Mutations and/or reductions in mtDNA copy number alter the Oxidative Phosphorylation (OXPHOS) physiology and are common features of TNBC. We have demonstrated that mtDNA content is lower in CRL-2335 AA TNBC cell line when compared to the CRL-2335 IGF2 knockout cell line. Thus, we propose that IGFL2 regulates the mtDNA content. This study was designed to demonstrate if IGFL2 regulates mitochondrial genes to determine the cell energy phenotype. An XfP analyzer was used to study the mitochondrial function in terms of OCR (Oxygen Consumption Rate/Mitochondrial Respiration) and ECAR (Extracellular Acidification Rate/Glycolysis) in the wild type and IGF2 stable knockout of CRL-2335 AA TNBC cells. Real Time PCR was performed to study the gene expression pattern of IGFL2, PGC1α and PGC1β. PGC1α and PGC1β are critical genes in the regulation of the mitochondrial biogenesis, thus, they are important in the cellular metabolic phenotype. Utilizing the vast majority of PCa presents as androgen-dependent adenocarcinoma, recent uses of increasingly potent therapeutics targeting the androgen receptor signaling axis has resulted in the promotion of NEPC transdifferentiation as a mechanism of treatment resistance. Unfortunately, there is a lack of effective treatment options for NEPC. Altered cancer metabolism is now recognized as a hallmark of cancer and a crucial factor for promoting tumor growth and spread. In particular, altered glucose metabolism and the resultant acidification of the tumor microenvironment via increased lactic acid production has been shown to play an important role in multiple cancer-promoting processes, including tissue invasion/metastasis, angiogenesis, and suppression of local anti-cancer immunity. While increased glycolysis is not generally considered a phenomenon relevant to primary treatment-naive PCa, we have recently demonstrated its relevance to castration-resistant prostate cancer (CRPC) and thus suspect that it is also relevant to the more aggressive NEPC. Our laboratory has also developed a number of unique serially transplantable patient-derived xenograft (PDx) models of NEPC that are histologically highly similar to the donor tissues and retain important genetic and epigenetic features. In particular, we have developed the first spontaneous NEPC transdifferentiation model in the field (LT331/331R). The gene expression profiles of these NEPC PDx models were compared to that of PCa adenocarcinoma PDx models. To determine whether certain metabolic pathway alterations were specific to NEPC, genes from a representative key metabolic pathways were upregulated and overall pathway scores were generated using different expression scores. Furthermore, significantly available gene expression data from NEPC patient tumors were used to validate our findings. From our analysis, we found that genes in the glycolysis pathway were significantly upregulated in both our PDx models and also in patient NEPC samples. Of particular interest is the upregulation of genes involved in the production and secretion of lactic acid, such as LDHA and MCT4. As such, our results suggest that elevated glycolysis and production of lactic acid could be a clinically important NEPC phenotype. Furthermore, the inhibition of glycolysis and particularly the inhibition of lactic acid secretion via MCT4 could be a potentially viable therapeutic strategy for NEPC.

#4420 Elevated glycolytic gene signature in patient-derived neuroendocrine prostate cancer xenograft models and its clinical relevance. Stephen Y. Choi, 1 Susan L. Ettenger, 1 Dong Lin, 1 Hui Xue, 7 Robert H. Bell, 1 Fan Mo, 1 Michael Pollak, 2 Colin C. Collins, 1 Yuzhuo Wang, 1 Vancouver Prostate Centre, Vancouver, British Columbia, Canada; 2British Columbia Cancer Research Centre, Vancouver, British Columbia, Canada; 3McGill University, Montreal, Quebec, Canada.

Neuroendocrine prostate cancer (NEPC) is a highly aggressive subtype of prostate cancer (PCa) that is becoming increasingly common in the clinic. While the vast majority of PCa presents as androgen-dependent adenocarcinoma, recent uses of increasingly potent therapeutics targeting the androgen receptor signaling axis has resulted in the promotion of NEPC transdifferentiation as a mechanism of treatment resistance. Unfortunately, there is a lack of effective treatment options for NEPC. Altered cancer metabolism is now recognized as a hallmark of cancer and a crucial factor for promoting tumour growth and spread. In particular, altered glucose metabolism and the resultant acidification of the tumor microenvironment via increased lactic acid production has been shown to play an important role in multiple cancer-promoting processes, including tissue invasion/metastasis, angiogenesis, and suppression of local anti-cancer immunity. While increased glycolysis is not generally considered a phenomenon relevant to primary treatment-naive PCa, we have recently demonstrated its relevance to castration-resistant prostate cancer (CRPC) and thus suspect that it is also relevant to the more aggressive NEPC. Our laboratory has also developed a number of unique serially transplantable patient-derived xenograft (PDx) models of NEPC that are histologically highly similar to the donor tissues and retain important genetic and epigenetic features. In particular, we have developed the first spontaneous NEPC transdifferentiation model in the field (LT331/331R). The gene expression profiles of these NEPC PDx models were compared to that of PCa adenocarcinoma PDx models. To determine whether certain metabolic pathway alterations were specific to NEPC, genes from a representative key metabolic pathways were upregulated and overall pathway scores were generated using different expression scores. Furthermore, publicly available gene expression data from NEPC patient tumors were used to validate our findings. From our analysis, we found that genes in the glycolysis pathway were significantly upregulated in both our PDx models and also in patient NEPC samples. Of particular interest is the upregulation of genes involved in the production and secretion of lactic acid, such as LDHA and MCT4. As such, our results suggest that elevated glycolysis and production of lactic acid could be a clinically important NEPC phenotype. Furthermore, the inhibition of glycolysis and particularly the inhibition of lactic acid secretion via MCT4 could be a potentially viable therapeutic strategy for NEPC.

#4424 MicroRNA Profiling in Cancer

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Metabolic Regulation and Cancer Therapy
Background: Lung cancer stem cells (CSCs) are a small population of stem-like cells that remain largely unknown. MicroRNAs (miRNAs) regulation of CSC genes expression has been reported, constituting a promising therapeutic target. The aim of this study was to isolate and analyze differential expression of a set of miRNAs in tumorspheres from lung cancer cell lines and tumor tissue from resectable non-small cell lung cancer (NSCLC) patients and to determine the prognostic implications of these miRNAs in a cohort of resected-NSCLC patients. Methods: Lung CSC-related miRNAs (miR-145-5p, miR-188-5p, miR-218-5p, miR-34a-5p, miR-21-5p and miR-125a-5p) were analyzed in cells from seven NSCLC tumor samples and six cell lines (H1650, H1939, H480, H538, A549 and PC9) grown in monolayer and as spheroids by RT-qPCR using TaqMan® microRNA assays. The expression levels of these six miRNAs were also analyzed in paired fresh-frozen tumor and normal adjacent lung tissue samples (N=178) from resected-NSCLC patients. Statistical analyses were considered significant at p<0.05. Results: miRNA expression analysis of cultured cells revealed an increased expression of miR-125a (p=0.04) and miR-188 (p=0.05) in lung tumorspheres from tumor samples and cell lines compared to their paired-adjacent cells. Moreover, lungspheres from patients showed elevated expression levels of miR-21 when compared with their paired monolayer-cell cultures (p=0.028). Interestingly, miR-125a, miR-188 and miR-21 had prognostic value when they were analyzed in patients’ tissue samples. We found that those patients with higher levels of miR-188 and miR-21 had a higher risk of relapse and a reduction in relapse-free survival (RFS, 23.67 vs 66.97 months; p= 0.009 and 24.03 vs 56.83 months, p= 0.042, respectively) and overall survival (OS, 42.9 vs NR months; p= 0.002 and 42.6 vs 82.60 months; p= 0.043, respectively), whereas the group of patients with higher levels of miR-125a had a worse outcome (OS 51.9 vs NR; p= 0.014). A signature combining the expression of miR-125a, miR-188 and miR-21 was able to give more significant information (RFS, 16.97 vs 56.83 months; p= 0.006 and OS, 29.90 vs. NR; p=<0.0001). The multivariate analysis including clinicopathological and analytical variables revealed this miRNAs signature as an independent prognostic biomarker for RFS (HR 2.170 [1.372-3.431]; p = 0.001) and OS (HR 3.256 [1.907-5.561]; p<0.0001). Conclusions: Lung tumorspheres had increased levels of the CSC-related miRNAs miR-125a, miR-188 and miR-21, highlighting their role in the CSC biology. The analyses performed in a large cohort of resectable NSCLC patients show that two miRNAs signature (miR-188 and miR-21) was an independent prognostic marker for RFS and OS. Supported by grants RD06/0020/1024 and RD12/0036/0025 from RTICC-FEDER, PI12-02838 and PI13-00753 from ISCIII, TRACE (TRA09-0132) and Beca Roche Oncohematología.

#4425 Analysis of host-Epstein-Barr virus microRNA expression in nasopharyngeal carcinoma. Wen-Hui Su,1 Jia-Hao Zhang,1 Kai-Ping Chang,3 Yung-Cheng Hwu,1,4 Cheng Gung University, Taoyuan, Taiwan; 2Cheng Gung Memorial Hospital at Lin-Kou, Taoyuan, Taiwan.

In the tumorgenesis process, miRNAs could act as oncogenes or tumor suppressor genes in the cancer cells by regulating miRNA target genes. Nasopharyngeal carcinoma (NPC) associated Epstein-Barr virus (EBV) can also influence the occurrence of NPC through its miRNAs. In this study, we used the next generation sequencing technology to analyze host-viral miRNA profiles in 24 NPC and 5 adjacent normal tissues. In average, 10.62% of mappable reads mapped to EBV genome in NPC tissue. Most of the small miRNAs in NPC tissues which mapped to EBV genome were miRNAs (95.51%), rest of them were EBERs (1.80%) and r-snoRNA (2.68%). The percentages of EBV miRNA in total human and EBV mature miRNA reads were ranged from 4% to 51% (average 29.61%). For EBV miRNAs, the expression level of BART10-3p and BART9-3p were the top two in NPC tumors. BART20-5p, BART19-3p and BART21-5p were correlated to tumor stage. On the other hand, there were 11 and 34 human miRNAs differentially up- and down-regulated in NPC tissue, respectively. The present research provides the first evidence obtained through next generation sequencing to systematically and comprehensively describe the expression of EBV and human miRNAs in NPC tissue.

#4426 Alcohol consumption and hepatitis B-associated microRNAs in hepatocellular carcinoma. Pin Xue Li,1 Hao Zheng,1 Maarouf A. Saad,2 Angela E. Zou,3 Xiaoqi Wang,4 James G. Kwok,1 Avinaash Korrapati,1 Yuanhao Qu,1 Thomas K. Honda,1 Jessica Wang-Rodriguez,4 Weg M. Ongkeko,1 University of California San Diego, San Diego, CA; 2Yale School of Medicine, New Haven, CT; 3University of Hong Kong, Hong Kong, China; 4Veterans Affairs, San Diego, CA.

Hepatocellular carcinoma (HCC) affects more than 500,000 new patients each year and is characterized by fast growing, heterogeneous tumors and low five-year survival rates. Despite the identification of HCC risk factors, including alcohol consumption and chronic hepatitis B (HBV) infection, early diagnosis of HCC and molecular understanding of how these risk factors promote HCC both individually and in combination remain elusive. microRNAs (miRNAs), non-coding transcripts ~22 nt in length, have emerged as central mediators of post-transcriptional and translational gene regulation, and have been increasingly implicated in the initiation and progression of many cancers, including HCC. However, the dysregulation profile of miRNAs in HCCs of varying etiologies remains largely uncharacterized. To give more significant miRNA oncogenic influence, we associated with alcohol use or with HBV in HCC patients, we analyzed next-generation RNA-sequencing data from 234 HCCs in The Cancer Genome Atlas (TCGA). Through differential expression analyses on cohorts stratified by alcohol consumption and HBV status, we discovered 10 miRNAs specifically dysregulated in alcohol-associated HCCs and 251 miRNAs specifically dysregulated in HBV-associated HCCs (FDR < 0.05). Among these, two miRNAs, miR-944 and miR-223-3p, significantly overexpressed in HCC alcohol consumers, exhibited additional correlations with patient survival, tumor stage, and mutations in CTNNB1, MUC16 and TNN (p < 0.05). We subsequently validated the upregulation of miR-944 and miR-223-3p in vitro following treatment of both normal...
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Liver and HCC cell lines with biologically relevant doses of alcohol and acetaldehyde, the first breakdown metabolite of ethanol. Knockdown of miR-944 and miR-223-3p significantly reduced cellular proliferation in both acetaldehyde-treated and untreated normal and HCC cell lines, and resulted in increased sensitivity to cisplatin. Inhibition of both miRNAs in HCC cell lines also restored the normal growth arrest in several tumour suppressor genes targeted by miR-944 and miR-223-3p. Taken together, our findings enhance knowledge of the respective roles of miRNAs in alcohol- and HBV-mediated HCC, and highlight the increased transcriptomic resolution afforded by etiology-specific studies of cancer pathogenesis and progression.

#4427 An integrated analysis package of miRNA and mRNA expression data: An integrated analysis package of miRNA and mRNA expression data. Ti-Tai Wang. National Taiwan University, Taipei, Taiwan.

MicroRNAs (miRNAs) are small and non-coding RNAs that can regulate gene expression by binding to the 3’ UTR of target mRNAs and inhibiting mRNAs to translate protein, or even promoting mRNAs degradation. In various complex disease and pathological conditions, we can identify dysregulated which are the causative factors. Therefore, it is an essential approach to explore the interactions between miRNA and gene in certain diseases, such as cancers. However, challenge arises when we are trying to validate the interactions by doing bench experiments. The number of miRNA–gene interactions is too large to be validated. Currently, most prediction algorithms only provide its own result and low consistency rates across independent methods have been reported. Therefore, it is necessary to develop a systematic method to perform a comprehensive analysis by using the expression profiles from genes and miRNAs concurrently. To address these issues, we developed an R package named as anamiR. The anamiR is able to perform an integrated analysis of mRNAs and miRNAs with the phenotype information. The analysis procedures are briefly summarized as the follows. Initially, differential expression analysis would be applied to the raw data. Subsequently, the anamiR package can identify significant pairs of miRNAs and genes if their Pearson correlation coefficients are lower than the pre-defined threshold. Next, the significant pairs were compared to the embedded database in anamiR, which included two validated and several predicting miRNA-gene databases. Lastly, functional analysis is also available in this package to characterize the biological functions and signaling pathways of dysregulated miRNAs and genes. A breast cancer dataset was analyzed using anamiR, which identified nine miRNA–gene interactions for deeper studying. Among these interactions, there are two miRNAs and nine genes included. miR-497-5p and miR-204-5p both were mentioned in both cancer frequently, are found by anamiR. The anamiR package is freely available at Bioconductor.

#4428 A miRNA signature linked to human environmental risk factors derived from carcinogen-induced and genetically-driven colon cancers. Mei-Chen, Ying-Shiu Chen,1 Rong Wang, 2Wan-Mohaia Shardish, 3Christiane Lohr, 4David E. Williams, 5Emily Ho, 6Susanne Mertens-Talcott, 7Roderick H. Dashwood, 8Texas A&M Univer. Health Science Ctr., Houston, TX; 9Oncology State University, Corvallis, OR; 10Texas A&M University, College Station, TX.

Heterocyclic amines (HCAs) produced during high-temperature cooking have been studied extensively in terms of their genetic effects, but recent work has implicated epigenetic mechanisms involving non-coding RNAs. We reported that colon tumors induced in the rat by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) have altered microRNA (miRNA) signatures linked to dysregulated pluripotency factors, such as c-Myc and Krüppel-like factor 4 (Klf4). The corresponding miRNAs prioritized from PhIP-induced colon tumors were examined in other target organs from a one-year carcinogenicity bioassay, and compared with miRNAs dysregulated in the Apc-mutant polyposis in rat colon (Pirc) genetic model. Multiple let-7 family members were downregulated in colon, skin, lung, small intestine, and Zymbal’s gland tumors, and were associated with Myc and Hmg2 upregulation. A “PhIP miRNA signature” with the profile mir-21 high mir-126 low mir-29low mir-215 low mir-145low was linked to reduced expression of Klf4 in multiple target organs of the rat, and was predictive of poor prognosis in human pan-cancer and colorectal cancer datasets (https://cancergenome.nih.gov/). Findings from The Cancer Genome Atlas suggested that PhIP signature miRNAs might serve as an arbiter of HCA exposure, defining a subset of human tumors linked to environmental carcinogenesis. We conclude that future studies should examine the miRNA signatures of other HCAs, and determine their possible predictive value for human risk assessment. This work was supported in part by NIH grants CA090890, CA122959, ES02010, and ES023512, the John S. Dunn Foundation, and a Chancellor's Research Initiative. We are most thankful to Robert Hawk for providing a graduate student fellowship in aid of Y-S Chen.

#4429 An exhaled microRNA panel interrogation and validation as risk biomarkers for lung cancer. Simon D. Spivack. Albert Einstein College of Medicine, Bronx, NY.

Background: There is a need for non-invasive airway-based biomarkers in lung carcinogenesis for both risk assessment of the ex-smoker, and earlier diagnosis of early-stage cancer detected through liquid biopsy analyses. MicroRNAs have been validated as regulators of cell functions and act as staging markers in lung carcinogenesis. Exhaled breath condensate (EBC) contains airway lining fluid molecules, including nucleic acids, presumably in part from epithelial cellular origins. Here we further develop and begin validation of the detection of microRNAs in EBC from lung cancer patients and controls. Methods: Exhaled breath condensate (EBC) was collected non-invasively, using a handheld device in 56 samples. MicroRNA expression profiling using RT-qPCR was performed in EBC in an initial batch (E) of 88 individuals (41 lung cancer cases, 47 controls), and a second batch D of 88 individuals (41, cases and 47 controls). We generated a 39 miR panel based on literature-derived microRNAs, combined with a lung tissue-based discovery effort we have performed using microRNA-seq on lung tumors and surrounding non-tumor tissue. The qPCR primers were designed using our previously published RNA-specific real-time RT-qPCR technique. All samples were run twice with positive and negative controls. Results: Qualitative analyses of the first donor batch E was performed on 39 microRNAs measured in 41 cases and 47 controls. Chi-square analysis (univariate) within the training set (batch E) revealed that exhaled miR-31, 33, 105, 200b, 944, 1269a were significantly different between the initial set of cases and controls (p < 0.05). In the validation batch (D) pre-liminary analysis, exhaled miRs 200b (this time present in cases, p = 0.044) and 944 (again absent in cases, p = 0.052) were case-control discriminant. In addition, test set batch D showed case-control discrimination for additional miRs 130b-3p (present in cases, p = 0.035), 212 (absent in cases, p = 0.009), 1910a (absent in cases, p = 0.006) and other microRNAs that were not seen in the qualitative training set (batch E). Importantly, multi-miR discriminant signatures, quantitative analysis, and risk ROC/modelling analyses are pending as of this AACC abstract deadline. Conclusion: This new exhaled biomarker platform can yield case-control discriminant microRNA sets, including a subset of miRs that can validate in test sets. Once further distilled and validated, our goal is to apply this non-invasive biomarker approach to prospective cohorts for non-invasive lung cancer risk assessment, in order to better select higher risk individuals who should undergo CT screening.

#4430 Circulating microRNA expression profiles as a novel diagnostic biomarker for esophageal squamous cell carcinoma. Yutaka Shimada, 1Yoshi-nori Takei, 2Tomoyuki Okumura, 3Takuya Nagata, 2Haruka Fujinami, 3Miwako Arima, 4Tetsuya Abe, 4Yasumasa Niwa, 4Masahito Tajika, 4Tetsuo Sudo, 4Kazuharu Shimizu, 1Kyoto Univ., Kyoto, Japan; 2University of Toyama, Toyama, Japan; 3Saitama Cancer Center Hospital, Ohmiji, Japan; 4Aich Cancer Center Hospital, Nagoya, Japan.

Background: The findings of a recent analysis on microRNAs (miRNAs) suggest the circulating miRNAs have potential as biomarkers of esophageal squamous cell carcinoma (ESCC). In order to identify specific miRNAs of ESCC, we analyzed the circulating miRNAs of patients who underwent endoscopic mucosal resection (EMR) and esophagectomy. Method: After obtaining written informed consent, we collected paired (pre and post treatment) blood samples from 60 superficial ESCC patients and 42 pretreatment advanced ESCC patients between 2011 and 2015. Samples were divided into training (40 superficial ESCC and 22 advanced ESCC) and test (15 superficial ESCC and 20 advanced ESCC) cohorts according to the period at which they were obtained (between 2013 and 2015 and between 2014 and 2015). Fifty-five patients underwent EMR and were confirmed as stage 0 or Stage 1a. Microarray analyses of blood samples were performed using the 3D-Genie miRNA microarray platform (Toray). Normalization was achieved using the Quantile method, and poor quality samples were excluded from the analysis. Any two clinical groups were compared using a two-sided Student’s t-test. miRNAs exhibiting significant differences were subsequently evaluated using a logistic regression analysis (LRA). Multivariate LRA was used for screening. Receiver Operating Characteristic (ROC) analyses were performed in order to evaluate the diagnostic power of miRNA combinations. In all series, we used post-EMR patients as control cases. Results: Twelve miRNAs (miR-6722-5p, 489, 4525, 409-3p, 6088, 3678-5p, 197-5p, 4281, 5090, 3173-3p, 762, and 1470) were selected as discriminant markers (S-combination: AUC 1.00) of superficial ESCC, while 4 miRNAs (miR-4723-3p, 4646-3p, 2392, and 1236-3p) were selected as discriminant markers (A-combination: AUC 1.00) of advanced ESCC in the training cohort. There were no overlap miRNAs between the two combinations. In the test cohort, the S-combination discriminated superficial ESCC (AUC 1.00), while the A-combination discriminated advanced ESCC (AUC 1.00) from post-EMR patients. Further...
thermore, the S-combination discriminated advanced ESCC in the test cohort (AUC 1.00). However, the A-combination did not clearly discriminate superficial ESCC (AUC 0.833). Conclusion: Our results suggest that selected miRNAs are useful biomarkers for the discrimination of ESCC. However, biomarkers of superficial ESCC and advanced ESCC may differ.

#4431 Urine microRNA profiling in bladder cancer by next-generation sequencing. Barbara Pardini,1 Francesca Cordero,1 Alessio Naccarati,2 Giulio Ferrero,3 Clara Viliberti,1 Marco Oderda,2 Maddalena Arigoni,2 Raffaele Calogero,2 Carlotta Sacrudeo,4 Paolo Gontoro,4 Paolo Vineis,4 Giuseppe Mattullo,4 1Human Genetics Foundation, HugoFerriorio, Torino, Italy; 2University of Torino, Torino, Italy; 3University of Torino, Turin, Italy; 4University of Torino and Città della Salute e della Scienza, Torino, Italy; 5Molecular Biotechnology Center, University of Torino, Turin, Italy; 6Center for Cancer Prevention (CPO-Piemonte), Torino, Italy; 7School of Public Health, Imperial College London, London, United Kingdom.

Bladder cancer (BC) is one of the leading causes of cancer-related death worldwide. BC is among the most expensive cancer per patient because it requires frequent surveillance and repeated treatments over many years. The identification of new biomarkers for early BC detection, recurrence/progression is urgently needed to both improve patient outcomes and decrease health care costs. MicroRNAs (miRNAs) are aberrantly expressed in many cancers, including BC, and may be isolated from various biological specimens, including urine. To investigate miRNA signatures in surrogate tissues may be a useful alternative to reduce invasiveness of biopsies, allowing repetitive samplings during follow-up and reducing health care costs for detection, monitoring of progression and treatment. We aim to identify specific miRNA signatures in urine samples from 66 BC male patients (10 muscle invasive BC (MIBC) and 56 non-muscle invasive BC (NMIBC)) and 48 healthy controls using a Next Generation Sequencing (NGS) approach able to accurately distinguish BC patients and predict disease outcome. The measurement of miRNA levels in urine could allow to measure the levels of promising biomarkers in one of the best and closest surrogate tissue for BC, since it is in direct contact with the tissue of tumor origin. A specific miRNA signature that could distinguish the different types of BC patients from healthy controls was found in urine. For MIBC, a 18-miRNAs signature had over 80% predictive power (PP) to recognize patients from controls (data are under validation). For NMIBC we were able to stratify cases according to grade. In particular, 23 miRNAs resulted differentially expressed among G1-G2 cases and controls (5 of them had PP>0.70), while several miRNAs resulted differentially expressed among G3 patients and controls (a 10-miRNAs signature with PP>0.97). Interestingly, we found several differentially expressed miRNAs in common among cases and some miRNAs that were differentially expressed only in specific subcategories of BC cases. NGS data were also used to search for the most constant miRNAs in the set of samples to be used as reference genes in a validation step. Twenty-three miRNAs (21 target and 2 reference miRNAs) were validated by qPCR on 177 urine samples from 113 BC case and 64 controls. Interestingly, miRNAs differentially expressed among cases and controls were able to discriminate not only BC cases from controls, but also its subcategories. This data provide evidences of the possibility to use miRNAs being expressed in urine of patients with BC as diagnostic, prognostic and predictive biomarkers. Acknowledgements: Work supported by Fondazione Umberto Veronesi (FUV) “Post-doctoral fellowship Year 2014, 2015 and 2016” (B.P. recipient), a FUV Grant 2013 (G.M. recipient), and by a HuGeF grant.

#4432 Low miR-222 expression levels predict long-term survival of patients affected by glioblastoma. Laura Lattanzio,1 Maria Borognone,2 Rosella Merli,2 M. Cristina Dechecchi,3 Gianluigi Dorelli,2 Cristina Mocellini,1 Laura Lattanzio,1 Marzia Borgognone,2 Rosella Merli,2 M. Cristina Dechecchi,3 Gianluigi Dorelli,2 Cristina Mocellini,1 Andrea Talacchi,1 Daniela Vivenza,1 Federica Tonissi,1 Alessandra Santangelo,1 Fabrizio Giordano,1 Caluduo Ghimenton,1 Albino Eccher,7 Sergio Pericotti,2 Luisa Zanolla,1 Claudio Bernucci,1 Marco Merfano,1 Cristina Lo Nigro,1 Giulio Cabrini,1 S.Croce & Carle Teaching Hospital, Canes, Italy; 2University of Verona, Verona, Italy; 3University of Verona, Verona, Italy; 4Verona University, Verona, Italy.

Introduction MicroRNAs (miRNAs) are small non-coding RNAs which are known to play an important role in proliferation, differentiation, invasion and angiogenesis of different malignant tumors. The role of tissue and blood level of miRNAs as useful bio-markers of gliomagenesis is also increasing. Interest is growing on tissue and plasma miR-222 level as prognostic marker in first line chemo- and radiotherapy treated patients with glioma, the mechanisms of action and the target genes of this miRNA in gliomagenesis being under extensive investigation. Furthermore, some evidence exist that miR-221 and miR-222 are upregulated in glioblastoma (GBM) patients and that these paralogues target the 6-(methylguanylin)-DNA methyltransferase (MGMT) mRNA. Here we focused on the prognostic role of tissue miR-222 in GBM patients. Experimental procedures 63 GBM patients were included in the analysis (overall survival (OS) range: 8-122 months). For each patient formalin-fixed paraffin-embedded tissue samples at first surgery were collected. miR-222 expression level was analyzed by TaqMan probes. To verify miR-222 expression level OS was analyzed by Kaplan-Mayer (K-M) survival curves. MGMT methylation analysis by pyrosequencing on tumor DNA is on-going. Results In silico analysis of The Cancer Genome Atlas (TCGA) for GBM defined a median OS of 13.6 months in the whole sample population (n=199). Comparison of median OS, done stratifying two groups according to median tissue miR-222 expression level, showed a significant increased OS in low 222 patients (12.3 vs 14.1 months, p=0.030). Interestingly, K-M curves overlapped for the first 14 months and then diverged after. In particular, K-M analyses starting from 14 months revealed a significantly increased hazard ratio for high 222 patients (HR 1.47, 95% CI = 1.25 - 3.16, p<0.004) in respect to low 222 patients, being the median OS of further 15.1 months in low 222 and of 6.6 months in high 222 patients. On this basis, we tested the hypothesis of a possible role of miR-222 in a local sample population of 63 GBM patients with prolonged survival. MiR-222 expression level was measured as described above. Therefore, patients were stratified in two groups according to median tissue miR-222 expression level (low 222 vs high 222) and OS analysed on a K-M curve. The Hazard Ratio for high 222 patients was significantly higher (HR = 5.5, 95% CI = 3.9-7.9, p<0.001), being the median OS of 45.2 months vs 13.5 months in high 222 GBM patients, respectively. Conclusions Quantitative analysis of miR-222 expression in the tumor tissue obtained at first surgery might provide a relevant prediction of prolonged survival in GBM patients. Further conclusions will be presented on the relationship between miR-222 and MGMT which will shed light on a possible role of miR-222 on gene methylation and GBM epigenetics.

#4433 Deregulated microRNAs in NF1 derived malignant peripheral nerve sheath tumors (MPNST) and their role in carcinogenesis. Azadeh Amirnasr, Patricia F. van Kuijk, Robert M. Verdijk, Walter Taal, Stefan Sleijfer, Erik A.C. Wiemer, Erasmus Medical Ctr., Rotterdam, Netherlands.

Background and aim: MPNSTs are rare and highly aggressive soft tissue sarcomas that can occur spontaneously (sporadic MPNST) or from pre-existing plexiform neurofibromas in neurofibromatosis type 1 (NF1) patients. MPNSTs are usually diagnosed relatively late, metastasize easily, are resistant to therapeutic intervention and frequently fatal for the patient. Diagnostic biomarkers that detect early malignant transformation in the NF1 setting as well identification of putative targets for treatment are urgently needed. In this context we investigated the miRNA expression profiles in NF1-derived MPNST. Materials and Methods: A total of 35 DNA was isolated from sections of formalin fixed paraffin-embedded (FFPE) tumor samples. miRNA expression was determined using a RT-PCR platform (TaqMan® Low Density Array Human MicroRNA Cards; Applied Biosystems). The expression of selected miRNAs was transiently modulated in neurofibroma and MPNST cell lines by transfection with miRNA mimics (Dharmacon) or LNA® anti-miRs (Exiqon) and effects on cell proliferation, invasion/migration and Wnt/b-catenin signaling were studied. Results: In order to identify miRNAs that are specifically deregulated in MPNST we analyzed miRNA expression in a unique set paired tumor samples (n=9) of plexiform neurofibroma and MPNST which were derived from the same NF1 patient. At least 90 miRNAs were found to be differentially expressed (FDR <0.10; p<0.025) between neurofibromas and MPNST; the vast majority (82/90) of miRNAs being downregulated in MPNST. Interestingly, and in contrast to reports in the literature, sporadic MPNST samples (n=10) could be easily discriminated from NF1-derived MPNST by their miRNA expression profiles. On the basis of their statistical significance, fold difference and expression level, we selected miRNAs to examine their role in MPNST pathogenesis. MiRNA levels were modulated in MPNST (ST88-14, ST88-64, 0.9-8TL) and neurofibroma (Hs637_T) cell lines using miRNA mimics for let-7b, miR-21a, miR-29a, miR-139-5p, miR-146a and antisense inhibitors for miR-889, miR-135b. The effects of miRNA expression levels on cellular proliferation, invasion and migration were determined. As Wnt/b-catenin signaling is considered a cancer related driver pathway in MPNST we also investigated whether differentially expressed miRNAs could regulate this pathway focusing on miRNA previously linked to Wnt regulator or predicted to target Wnt signaling components. Using TOP/FOP luciferase reporter assays we analyzed the effects of miR-135b, miR-30a, miR-139-5p and miR-146a. Conclusion: miRNAs specifically deregulated in MPNST may fulfill key oncogenic roles and may be used as biomarker or for therapeutic purposes.
MOLECULAR AND CELLULAR BIOLOGY / GENETICS: MicroRNA Profiling in Cancer

**#4434** MicroRNA methylation of normal breast tissue from ER negative and ER positive breast cancer identify progression markers specific for estrogen receptor status. Kang Mei Chen, Josena K. Stephen, Indrani Datta, Dhananjay Chitale, George Divine, Maria J. Worsham. *Henry Ford Health System, Detroit, MI.*

The unique structure and function of normal tissues is known to be regulated by epigenetic mechanisms. Understanding how normal cells in their respective tumor milieu might affect their susceptibility to become not only malignant but acquire breast cancer (BC) subtype-specific phenotypes, may determine tumor clinical behavior outcomes. The goal was to compare genome wide methylation profiles of non-coding miRNAs of breast cancer tissue and normal breast epithelium (normal non-coding miRNA (miRNA) (ER) negative and ER positive breast cancer) to assess their methylation methylation in the context of tumor ER phenotypes as either ER- or ER+. BC tissue from 79 patients (39 ER- and 40 ER+) and normal tissue from 39 of these patients (19 ER- and 20 ER+) were assayed using the Illumina 450K bead array. A sub analysis focused on 2249 miRNA CpGs as-sign to 615 unique miRNAs. M-values were computed as a logit function (log (beta/(1-beta)) of the methylation beta values. T-tests were used to compare the means of the M-values for the ER (beta/(1-beta)) of the methylation betavalues. T-testswere used tocompare the I lumina 450K bead array. A sub analysis focused on 2249 miRNA CpGs as-and assess their miRNA methylomes in the context of tumor ER phenotypes as either ER- or ER+. BC tissue from 79 patients (39 ER- and 40 ER+) and normal tissue from 39 of these patients (19 ER- and 20 ER+) were assayed using the Illumina 450K bead array. A sub analysis focused on 2249 miRNA CpGs as-sign to 615 unique miRNAs. M-values were computed as a logit function (log (beta/(1-beta)) of the methylation beta values. T-tests were used to compare the means of the M-values for the ER+ and ER- groups. The t-test p-values were used to develop adaptive FDR (afDR) levels and fDRs of 0.05 or lower were considered to be statistically significant (Tier 1). Tier 1 CpGs were subsequently filtered to select only those with a mean beta ratio between ER+ and ER- of under 0.5 or over 2.0 (Tier 2). The Tier 2 CpGs were further filtered to select only those with a mean beta difference of 0.2 or more. The 1224/2249 (54%) CpGs were differentially methylated between ER- and ER+ BC at Tier 1. Of the 1224, 963 (78.7%) were hypermethylated, and 1035 (84.6%) were in promoter regions. The 1224 CpGs at Tier 1, the 24 at Tier 2, and 2 CpGs at Tier 3 were associated with 379, 22 and 2 genes respectively. When the same analysis was performed on normal tissue only (19 ER- and 20 ER+), 76 of the 2249 CpGs had significant fDR values and none of those met the Tier 2 or Tier 3 criteria. Seventy-one of the 76 (93.4%) were hypermethylated, and 65 (85.5%) were in promoter regions. The 76 significant Tier 1 (an FDR) differentially methylated CpGs were associated with 48 genes of which 43 were common to tumor Tier 1 differentially methylated miRNA genes, 10 were common to tumor Tier 2 genes, and 5 were restricted to normal tissue only. Normal epithelial tissues demonstrated similar differential methylation directionalities as their respective tumor counterparts, favoring promoter region localization. Accordingly, the recognition of normal breast tissue-specific epigenetic properties that align with their tumor phenotypes, suggest the possibility of progression markers specific for ER status as well as markers not associated with progression. This provides insights into our view of possible links between epigenetic programming, progression continuums, and how hormonal receptor subtypes may be determined. Support: Komen Foundation: KG110218

**#4435** Exosomal miR-3622a as prognostic marker in prostate cancer. Thao Yang, Divya Bhagirath, Kirandeep Sekhon, Nathan Bucay, Sharanjot Majid. *King’s College Medical School, London, UK.*

Loss of chromosome (chr) 8p21 is a frequent genomic alteration in prostate cancer (PCa). Genomic deletions of this region increase significantly with tumor grade and are associated with tumor progression and poor prognosis. A common region of loss of heterozygosity (LOH) has been mapped to the chr8p21 region of loss of heterozygosity (LOH) has been mapped to the chr8p21 region. The 8p21 region is highly conserved across species and contains a region that is frequently deleted in human PCa. In this study, we aimed to identify the miRNAs that are specifically regulated in this region.

**#4436** Novel microRNA signature score to predict bone metastasis and prognosis of breast cancer. Tsutomo Kagawuchi, Li Yan, Qi Qi, S. Liu, Kazuki Takabe. *Roswell Park Cancer Institute, Buffalo, NY.*

Backgrounds: 40,000 US women still die with breast cancer every year. Vast majority of death occur after they develop metastasis, where bone is the most frequent site for breast cancer. New measures to identify the patients who develop metastasis allow us to intervene early, which is expected to prolong survival. The aim of this study is to establish a microRNA (miRNA) signature scoring system that can predict bone metastasis and survival utilizing integrated transcriptomics analyses in breast cancer. Materials and Methods: Both clinical and RNA expression data, including microRNA and mRNA, of 1051 patients were retrieved from The Cancer Genome Atlas (TCGA). 1) Multivariate Cox proportional hazard model and Kaplan-Meier for overall survival were performed to construct and identify novel models of miRNAs signature for predicting patient survival. 2) Competing risk analysis using the miRNAs signature was conducted to clarify its association with metastatic distributions. 3) Gene Set Enrichment Analysis (GSEA) was performed to identify the genome-epigenome significance of the miRNAs signature. Results: 1) Utilizing Cox model on TCGA cohort, we established a novel microRNA signature with 3 miRNAs (miR-19a, miR-93, and miR-106a) that identified the patients population with extremely poor overall survival (p = 0.0004; 5-y survival rate, 49.2%). This result was reproduced in two other completely independent cohorts with microarray datasets (GSE19536, p = 0.0099; GSE22220, p = 0.0003, respectively). 2) Utilizing competing risk analysis for each metastatic sites of breast cancer, we found that the patients with bone metastasis demonstrated significantly high scores (p = 0.0052). The evaluation also showed a statistical tendency of association with lung metastasis (p = 0.0854). 3) We found that high score is associated with several critical gene sets such as angiogenesis (p < 0.0001) or epithelial mesenchymal transition (p = 0.0155) by GSEA that suggests that high signature score is associated with enhanced metastasis in breast cancer patients. Conclusions: We established a miRNAs signature scoring system to predict bone metastasis and poor overall survival in breast cancer using novel integrated transcriptomics concept.

**#4437** Small non-coding RNA profiling from prostate cancer plasma by deep sequencing. Xianghua Liu, Andrew Hunter, Qing Zhang, Quanyuan He, Annemarie Benton, Gerri Ortiz, Vashalli Pannu, Nick Xiao, Mark Miglarese, David Spezeler. *Caris Life Sciences, AZ.*

Background: Prostate cancer (PCa) is the most common non-skin cancer among American men. MicroRNAs (miRNAs) are critical post-transcriptional regulators and involved in prostate cancer tumorigenesis. The aim of this study is to identify a PCa-specific expression profile of miRNAs from plasma to guide prostate cancer diagnosis and therapeutic treatment. Methods: Plasma was collected from 50 randomly selected patients (25 PCa patients and 25 normal). Plasma was extracted from 1) 200ul plasma or 2) the pellets of anti-Ago2 immunoprecipitations from 500ul plasma using the miRNeasy Serum/Plasma kit (Qiagen), with addition of glycogen as a carrier. Small RNA libraries were constructed using the NEBNext Multiplex Small RNA Prep Set for Illumina*®* (New England BioLabs). The cDNA library fragments were purified by Blue Pippin (Sage Science) for extraction of 140-160 bp size fraction containing small RNA inserts. Equimolar amounts of cdNA library samples were pooled and were sequenced in one flowcell on an Illumina HiSeq2500 with 50 cycle kit and rapid run mode. Bioinformatics analysis: Adaptor was firstly removed from the raw reads, and the sequences were mapped to several small RNA databases by using bowtie1 with 1 mismatch. The multiple aligned reads were weighted to the mapped small RNAs based on their unique mapped reads counts. We then calculated the RPM (reads per million) as indicator of the expression levels of the small RNA. To get consistent analysis results, we discarded the small RNAs whose averages of the raw reads counts in cancer and normal groups are smaller than 25 and only focus on the mature microRNAs. The moderate t-test is applied to find the differently expressed (DE) microRNAs between normal and cancer group. Results: Two major small RNA classes identified from total plasma are miRNA (47.7%) and mRNA (35.0%). The percentage of miRNA increased to 85.3% by Ag2-IP method. The compositions of categories of small RNAs in cancer and normal samples are similar. We identified 28 and 22 differential miRNAs (>2 fold change between cancer and normal group) by total plasma and Ag2-IP meth-
ods respectively. Conclusions: We discovered a unique expression profile of miRNA detectable in the plasma from prostate cancer patients. Extracted RNA from the pellets of anti-Ag2 immunoprecipitations can enhance the detection of miRNA. Expand study to confirm these findings are needed.

#4438 MiRNA expression analysis in the tumor stroma predicts Tenascin C to promote colorectal cancer liver metastasis. Tomohiro Murakami,1 Hisashi Ishimatsu,1 Hiroshi Kikuchi,1 Amane Hirota,2 Tomohiro Matsumoto,3 Yusuke Ozaki,1 Toshiki Kawabata,1 Yoshio Hiramatsu,1 Kinji Kamiya,1 Megumi Baba,1 Takanori Sakaguchi,1 Hiroyuki Konno1. 1Hamamatsu University School of Medicine, Hamamatsu, Japan; 2Department of Surgery Hamamatsu University School of Medicine, Hamamatsu, Japan; 3Hamamatsu University School of Medicine, Hamamatsu, Japan.

Background: Liver is the most common site for colorectal cancer (CRC) metastasis, and mechanisms underlying colorectal liver metastasis need to be explored. The tumor microenvironment consists of many stromal cells which are known to interact with cancer cells and to promote metastasis. Recently many microRNAs (miRNAs) specifically expressed in the stroma is receiving attention. In this study, we analyzed the differences in miRNA expression profiles between peritumoral stroma of CRCs with and without liver metastasis, and sought to identify liver metastasis-promoting molecules that can be regulated by the altered expression of miRNAs in the peritumoral stroma. Materials and methods: The formalin-fixed paraffin-embedded (FFPE) specimens of primary CRCs from 8 patients without metastasis, 4 patients with synchronous liver metastasis and 4 patients with metachronous liver metastasis were used for miRNA array analysis. None of the patients had received any treatment prior to surgery. Tumor stroma was isolated from FFPE samples using laser capture microdissection without contamination of cancer cells, and total RNAs were extracted. The quality of total RNA was assessed, and miRNA expression was analyzed using TaqMan miRNA arrays. Protein expression of Tenascin C (TNC) was immunohistochemically analyzed in 139 primary CRCs. Results: Unsupervised hierarchical clustering classified 16 samples analyzed into two groups according to their miRNA expressions. CRCs with synchronous liver metastasis showed differential miRNA expression profiles from those without liver metastasis or with metachronous liver metastasis. Top10 miRNAs up-regulated in CRCs with synchronous liver metastasis vs. CRCs without liver metastasis or with metachronous liver metastasis were hsa-mir-518b, 618, 186, 520f, 29c, 520e, 155, 30c, 518e and 106b, and those down-regulated in CRCs with synchronous liver metastasis were hsa-mir-302a, 511b, 627, 628-3p, 19a, 372, 302b, 384, 198 and 636. The predictive targets of the top 10 down-regulated miRNAs were analyzed in silico using on-line data base such as mirDB. TNC was identified as a candidate for metastasis-promoting molecule which can be upregulated in the peritumoral stroma followed by the downregulation of miRNAs. Immunohistochemical analysis revealed that protein expression of TNC was higher in the stroma of primary CRCs with synchronous liver metastasis compared with those without liver metastasis or with metachronous liver metastasis. Furthermore, high expression of TNC protein in the primary CRC stroma was correlated with shorter overall survival and liver metastasis-free survival. Conclusions: Alterations of miRNAs in the CRC stroma appear to create a metastasis permissive environment in which TNC plays an important role. Expression of TNC in primary CRC stroma has the potential to be a novel biomarker to predict the risk of postoperative liver metastasis in CRC patients.


The clinical management of prostate cancer is the most common non-skin malignancy in the world is hindered by the limitations of current diagnostic and prognostic tests, such as the low specificity of prostate-specific antigen (PSA) testing, low sensitivity of digital rectal examination (DRE) and complications of biopsies. To try to resolve these issues, we evaluated miRNA abundance in the urine of prostate cancer patients. Alterations in miRNA abundance levels have been reported as playing essential roles in the pathogenesis of cancer and is occurred in a tumor phenotype-specific manner (e.g. aggressive and non-aggressive), miRNAs are stable under diverse analytical conditions and can be detected in various types of body fluids including urine. These characteristics make them as promising non-invasive biomarkers. Here, we examined the intra- and inter-individual variance of urine miRNA abundance by investigating longitudinal changes over months to years in a cohort of patients with localized prostate cancer. We observed a large dynamic range of inter-individual variance in miRNA abundances and identified a set of miRNAs that is stable within individuals, and is biased toward specific biological functions including regulation of transmembrane channel activity. We combined this observation with machine-learning techniques to create a predictive model that can identify aggressive prostate cancer. This four miRNAs predictive model was validated in an independent prostate cancer cohort to non-invasively predict high-risk disease. Remarkably it showed comparable performance to the best existing tissue-based prognostic markers. These results demonstrated that non-invasive biomarkers can be developed to precede or supplement tissue-based tests by understanding the intra- and inter-tumoural heterogeneity of the urine miRNA transcriptome.


The hepatocellular carcinoma (HCC) is one of the most common malignant tumors and carries a poor survival rate. Prognosis, survival and management of patients at risk for developing HCC remain challenging in Egypt and worldwide. Furthermore, poor prognosis of patients with symptomatic (HCC) diagnosed clinically at advanced stages suggests an urgent need for new biomarkers detection that can be used for pre-clinical screening for early detection of premalignant lesions and tumors in high risk to hepatitis C infection. In the present study we used novel formulated anticancer compound(s), combined with or without Sorafenib treatment to exert their antiproliferative effects by eliciting concomitant expression of known or novel biomarkers, including different serum miRNAs, which can be quantitatively analyzed for differences in the levels of metabolites and proteins in the blood and liver tissues of treated HCC xenografts and liver cirrhosis animal models. In addition, circulatory miRNAs levels were measured in these models using markers in 12 month interval-serum samples that were collected from normal, chronic hepatitis C virus infected patient cohorts at the pre-malignant or pre-clinical stages and liver cirrhotic patients that did not develop cancer. Different serum miRNAs, including miR-21, miR-142, let-7a, let-7b, miR-429 and miR-34a were detected in pre-clinical HCC patients and have the potential to screen for hepatitis C virus infected patients at high risk to develop HCC 12 months after miRNAs detection. Our data of detected serum miRNAs from animal models and patient samples can be used to confirm and correlate histological and histochemical results at various time points with serum or imaging biomarkers, which may have a great promise for the prediction and prevention of HCC in high risk Egyptian populations.


We previously reported the identification of a signature composed by 34 serum circulating cell-free microRNAs (cf-miRNA) diagnostic for lung cancer. Using this signature, we developed a blood test (miR-test) which was capable of detecting asymptomatic lung cancer in a large cohort (N>1000) of high-risk individuals (>50-years and smokers). Interestingly, we now found that a fraction (~30%) of these cf-miRNAs were preferentially expressed in cells of epithelial origin, while another ~30% were more expressed in hematopoietic cells. We reasoned that this cf-miRNA signature could result from the extracellular release of miRNAs from cancer epithelial cells as well as from immune/stromal cells after the tumor cell death. Therefore, we developed an integrated strategy for the identification of the origin of cf-miRNAs through combined analysis of published circulating and intracellular microRNAs expression datasets (microarray and qRT-PCR based) and of NGS analysis of cf-miRNAs in lung tumors. Our approach will contribute elucidating the biological role of cf-miRNAs in lung cancer and explore eventual therapeutic implications.

#4442 microRNA in FA defective tumor. Shirley Tang,1 Li Gao,2 Kathleen Kathleen,3 Andrew Fink,3 Arjan Kalvala,3 Brittany Aguila,3 Gregory Otterson,1 Miguel Villalona-Calero,2 Wenrui Duan1. 1Ohio State University, Columbus, OH; 2Miami Cancer Institute, Miami, FL; 3Florida International University, Miami, FL.

The Fanconi Anemia (FA) pathway is essential for human cells to maintain DNA integrity following DNA damage. This pathway is involved in the endogenous release of miRNAs from cancer epithelial cells as well as from immune/stromal cells after the tumor cell death. FA is a disease that affects about 1 in 100,000 individuals. It is an inherited cancer predisposition syndrome characterized by bone marrow failure and high risks of solid and hematologic malignancies. The FA pathway is essential for human cells to maintain DNA integrity following DNA damage. This pathway is involved in the endogenous release of miRNAs from cancer epithelial cells as well as from immune/stromal cells after the tumor cell death. FA is a disease that affects about 1 in 100,000 individuals. It is an inherited cancer predisposition syndrome characterized by bone marrow failure and high risks of solid and hematologic malignancies.
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changes in developmental and physiological processes including cancer. It has been published that some miRNAs are tumor suppressors, and others are onco-
genesis. However, microRNAs and the FA pathway in relationship to lung cancer progression remain unknown. In order to elucidate the role of FA related miRs in human lung cancer development or treatment, we analyzed miR expression in lung cancer samples and normal lung tissues. Several high-affinity targets for miR-25 were identified using miRNA microarray analysis. Using Nanostring technology, miR-25 expression was found to be regulated by miR-143-3p. These results suggest that miR-25 may be a potential therapeutic target for lung cancer treatment.

#4443 SPARC overexpression alters microRNA expression profiles involved in tumor progression. Bhavesh K. Ahir, Sajani Lakka. University of Illinois at Chicago, Chicago, IL.

Medulloblastoma is the most common of malignant brain tumor in children. SPARC (secreted protein acidic and rich in cysteine), a multisecretory non-structural glycoprotein is known to be involved in multiple processes in various cancers. Pre-
viously, we reported that SPARC expression significantly impairs medulloblastoma tumor growth in vivo and alters chemo sensitivity. MicroRNAs are a class of post-
transcriptional gene regulators with critical functions in tumor progression. In ad-
tion, they also play role in regulation of vital genes that control mechanisms of transcriptional gene regulator with critical functions in tumor progression. In ad-
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Potential messenger RNA (mRNA) targets of the differentially expressed miRNA were identified using Ingenuity Pathway Analysis (IPA). Network-based functional analyses were performed on the available human protein interaction and miRNA-gene association data to highlight versatile miRNAs among the significantly deregulated miRNAs using the IPA, and the biological pathway analysis using the PANTHER web-based tool. We identified six miRNAs (miR-125b-1*, miR-146a-5p, miR-181a-5p, miR-204-5p, miR-219-5p and miR-509-3p) which are differentially regulated with SPARC expression in medulloblastoma cells. Furthermore, pathway enrichment analysis output line that these six microRNAs mainly belong to biological processes related to cancer related signaling pathways. Collectively, these results suggest that a perturbed ex-
pression for these six miRNAs and their complex regulatory loops contribute to tumor suppressive effects in SPARC overexpressed medulloblastoma cells.

#4444 MicroRNA hsa-miR-145 expression in hepatocellular carcinoma correlates with patient survival. Go Nakajima, Kazuhiko Hayashi. Tokyo Women’s Medical Univ., Tokyo, Japan.

Aim: MicroRNAs (miRNAs) are known as one of the regulator of gene expression at a post-transcriptional level. Previous study from our group identified forty nine miRNAs that are expressed differentially by comparing 26 pair samples of hepatocellular carcinoma (HCC) tumor tissue and non-tumor liver tissue using next-generation sequencer system, and hsa-miR-145, one of the candidate miRNA was chosen for this validation study. The expression of hsa-miR-145 in validation set HCC samples were measured by real-time PCR, and we exam-
ined the correlation between the miRNA expression and clinical data. Patients and Methods: Herein, we determined microRNA (miRNA) expression profiles in hepatocellular carcinoma tissue and non-tumor liver tissue to identify the expression level of each miRNA relative to control. Of the top one hundred expressed miRNAs, we determined statistically significant differences in the expression of two miRNAs (miR-21, -31, -34a, -125b, -199a, -204, and -1246) between fresh primary tumor samples and fresh adjacent normal tissue (ANT) collected from patients diagnosed with HCC, SCC in situ, or invasive SCC. Total RNA was isolated from each tissue type using the miRNeasy Micro Kit (Qiagen) according to the manufacturer’s instructions. The RNA was quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific). RNA integrity was assessed using an RNA PicoChip on a Bioanalyzer 2100 (Agilent Technologies). The RNA samples that met the criteria of 18S/28S ratio greater than 1.5 and RNA Integrity Number greater than 7 were subjected to miRNA expression analysis. miRNA expression data was generated using the Affymetrix microRNA Array (Affymetrix, Santa Clara, CA). The expression levels of miRNAs were determined using miRNA arrays. The expression data were analyzed using the partitional clustering method to identify miRNA expression patterns. We performed a one-way ANOVA followed by the Bonferroni post hoc test to compare the expression levels of each miRNA in different samples. Results: A total of 27 differentially expressed miRNAs were identified between the control and SPARC overexpressed samples. Potential messenger RNA (mRNA) targets of the differentially expressed miRNA were identified using Ingenuity Pathway Analysis (IPA). Network-based functional analyses were performed on the available human protein interaction and miRNA-gene association data to highlight versatile miRNAs among the significantly deregulated miRNAs using the IPA, and the biological pathway analysis using the PANTHER web-based tool. We identified six miRNAs (miR-125b-1*, miR-146a-5p, miR-181a-5p, miR-204-5p, miR-219-5p and miR-509-3p) which are differentially regulated with SPARC expression in medulloblastoma cells. Furthermore, pathway enrichment analysis output line that these six microRNAs mainly belong to biological processes related to cancer related signaling pathways. Collectively, these results suggest that a perturbed ex-
pression for these six miRNAs and their complex regulatory loops contribute to tumor suppressive effects in SPARC overexpressed medulloblastoma cells.

#4445 MicroRNA profile of excised cutaneous squamous cell carcinoma tissues comparative to adjacent normal tissue. Christopher S. Pulford, Ellie M. Loomis, McKale R. Montgomery, Chandana K. Uppalapati, Agnes S. Pascual, Elizabeth E. Hull, Katherine F. Leyva. Midwestern University, Glendale, AZ.

Cutaneous Squamous Cell Carcinoma (cSCC) is the second most common form of skin cancer commonly arising from sun-induced mutations. As most cases of cSCC are treated by tumor excision, research aimed at understanding factors influencing the metastasis, and possible alternative treatments, of cSCC is limited. Cancer metastasis is a multistep process, including epithelial-to-mesenchymal transition (EMT). During EMT, epithelial cells undergo a dedifferentiation process whereby they transition to a highly mobile and invasive mesenchymal cell type with metastatic capacities. Although the factors influencing EMT are varied, one important mediator of intercellular communication shown to be involved in oncogenesis is miRNA. Our work focuses on miRNAs, which are small non-coding RNA species that may be one epigenetic factor contribut-
ing to the progression of cSCC. In this study, we compared the expression pattern of seven miRNAs (miR-21, -31, -34a, -125b, -199a, -204, and -1246) between fresh primary tumor samples and fresh adjacent normal tissue (ANT) collected from patients diagnosed with cSCC, SCC in situ, or invasive SCC. Total RNA was isolated from each of the samples and qPCR on all samples was performed to determine the expression level of each miRNA relative to control. Of the seven miRNAs tested, three miRNAs were found to be up-regulated, miR-21, miR-31, and miR-204 in the tumor versus control samples. One-way ANOVA was used to compare miRNA expression levels.

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There was significant difference in miRNA expression of normal samples between patients who had breast cancer in the opposite breast compared to those who did not (p=0.03269) and patients with sleep problems and those who did not (p=0.00344). It is interesting to note that night shift work and sleep problems both represent forms of circadian disruption, yet only the latter showed significant difference in miRNA expression between controls. It is expected that patients with cancer in the other breast may exhibit significantly different reads from patients who have never had breast cancer. Future analyses will be performed using additional samples to build on these findings.

#4447 Analysis of small RNA-seq data for differential expression of small noncoding RNAs in human colorectal cancer. Srinivas V. Koduru,1 Amit K. Tiwari,1 Sprague W. Hazard,1 Milind K. Mahajan,2 Dino J. Ravnic1.1 PennState University, Hershey, PA; 2University of Toledo, Toledo, OH.

Background: Cancer is a major cause of deaths worldwide, despite improved healthcare and technologies. Recent advancements in genome sequencing have led to the rapid study of the whole transcriptome and small RNAs with their biological functions. Non-coding RNAs (ncRNAs) play an important role in biological processes that greatly impact biomarker development for diagnosis, prognosis, and therapy. Non-coding RNAs such as microRNAs (miRNA), Piwi-interacting RNAs (piRNAs), small nuclear/nucleolar RNAs (sn/snoRNAs) have recently been studied to understand their biology and pathology. Results: In the present study, we used eight matched colorectal patient tissue samples (benign, tumor, and metastasis) small RNA sequencing data remapped for various small RNA annotation. We identified aberrant expression of 13 miRs in tumor and metastasis specimens (tumor vs benign: 19 miRs) and metastasis vs benign group (38 miRs) of which five were upregulated, and eight were downregulated, during disease progression. We also investigated pathway analysis on aberrant expression of miRNAs, which showed majority of miRs involved in the colon other types of cancers. Further analysis of piRNAs revealed that six piRNAs in tumor vs benign and 24 in metastasis vs benign samples (commonly in both groups, only two piRNAs). Additionally, we examined other types of small RNAs (sn/snoRNAs, mt RNA, misc RNA, non-sense mediated decay (NMD) and rRNA), we identified 15 in tumor vs benign and 104 metastasis vs benign and only four in commonly expressed. Conclusion: In summary, our results identified multiple snoRNAs during colorectal cancer progression which needs to be further validated and can be used for prognosis, diagnosis, and therapeutic potentials.

#4448 Elevated levels of circulating miR-34a-5p and miR-181c-3p during cancer initiation by aflatoxin B1.1 Merrick C. Livingstone,1 Bill D. Roebuck,1 Natalie M. Johnson,2 Thomas W. Kensler,1 John D. Groopman.1 Johns Hopkins University School of Public Health, Baltimore, MD; 2Dartmouth Medical School, Hanover, NH; 3Texas A&M School of Public Health, College Station, TX; 4Icahn School of Medicine, Mount Sinai, New York, NY.

Purpose: New prognostic biomarkers for prostate cancer (PC) risk stratification are urgently needed. Here, we identify and validate a novel 4-miRNA prognostic ratio model for prostate cancer. We also identify new microRNAs with tumor suppressive characteristics in prostate cancer cell lines. Methods: PC tissue samples from 123 radical prostatectomy (RP) patients, 58 of whom experienced biochemical recurrence (BCR), were obtained from the RGP (training cohort). Initially, we identified 11 top candidate prognostic miRNAs (P < 0.05, univariate Cox regression). Based on these, all possible 2-4 miRNA ratio models were systematically tested for their potential to predict time to biochemical recurrence (BCR), as assessed by Kaplan-Meier, uni- and multivariate Cox regression analyses. The findings were validated in two independent RP cohorts. A novel ratio model was included in the final validated model and with previously unreported functional effect in prostate cancer, was over expressed using a microRNA mimic in PC3 and DU145 cells to evaluate its effect on prostate cancer cell viability and proliferation. Results: We identified a new prognostic 4-miRNA ratio model ((miR-23a-3p/miR-10b-5p/miR-133a-5p-miR-374b-5p)) that significantly predicted time to BCR independently of established clinicopathological variables in a training RP cohort (n=123). The ratio model was successfully validated in two independent RP cohorts including 112 and 476 patients, respectively. Ectopic expression of one of these miRNAs (miR-374b-5p) significantly inhibited viability and proliferation in two prostate cancer cell lines, suggesting it has a tumor suppressor function in PC. Conclusions: Here, we describe the identification and independent validation of a 4-miRNA ratio model that allows risk stratification of prostate cancer patients undergoing RP into different prognostic groups. Furthermore, we identify and functionally characterize a novel possible tumor suppressor miRNA in prostate cancer.

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#4449 Thrombospondin-1 regulation by MD2 in aggressive cancers. Priya Dondapati,1 Jason Maragh,1 Karna Mangrola,1 Ali Alaseem,1 Khadija Cheema,1 Thiagarajan Venkatesan,1 Sivanesan Dhandayuthapani,1 Appu Rathi,1 2Nova Southeastern Univ, Fort Lauderdale, FL; 3Rambaugh Goodwin Institute for Cancer Research, Fort Lauderdale, FL.

MDM2 amplification, which is often seen in different cancer types, indicates that it plays an important role in tumorigenesis and also in cancer metastasis. Amplification of the MDM2 gene correlates with poor prognosis, aggressive growth, and recurrence. Thrombospondin-1 (TSP-1) that is expressed by THBS1 gene is a matricellular protein, and is known to have differential expression in cancers. When TSP-1 activates Transforming Growth Factor-β1 (TGF-β1) through latent cytokine complexation, the activated TGF-β1 contributes to the Epithelial-Mesenchymal Transition (EMT) which is known to impart cell-stem-like characteristics and drug resistance to the cancer cells. However, no studies regarding MDM2’s role in THBS1 regulation have been carried out so far. Therefore, the objective of this study was to determine the correlation between MDM2 and THBS1 expression in MDM2 overexpressing cancers. For this purpose the SJA-1 osteosarcoma cells were cultured at 37 °C under humidified air/O2 (19/1) in RPMI-1640 complete medium supplemented with 10% FBS, 10,000 U/mL penicillin, 10,000 μg/mL streptomycin, 1% (+) L-glutamine, and 1% amphotericin B. The cells were incubated in the presence of 20 μM of Nutlin-3 for 24 hrs, following which, RNA and protein were extracted and subsequently purity and concentration were determined. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) and western blotting were performed using specific primers, primary and the secondary antibodies respectively. Interestingly elevated levels of TSP-1 and TGFβ1 were observed in these SJA-1 cells. However, after treatment with Nutlin-3, an MDM2-p53 interaction inhibitor, a significant increase in p53 was noted. However, contrary to the existing literature, where studies have shown p53 to positively regulate TSP-1 levels, we observed an opposite outcome. Despite the Nutlin-3 induced elevation in p53 levels, a significant decrease in TSP-1 level was observed indicating that MDM2 may be regulating THBS1 gene expression in a p53 independent manner, in MDM2 overexpressing SJA-1 cells. The findings of this study suggest a novel, p53 independent role for MDM2 in THBS1 regulation. Since MDM2 amplification can positively induce TSP-1 levels, which is one of the natural activators of TGF-β1, it is anticipated that stimulatory signal flowing through MDM2 - TSP-1 - TGF-β1 axis may induce the cancer cells to undergo EMT, thereby increasing their metastatic ability and promote cancer stem cell characteristics. This novel MDM2-regulated pathway can very well serve as a drug target and might play a biologically relevant role in the treatment of cancer metastasis. Our future studies will include validation of this correlation in multiple cancer cell lines, to
The role of paired-like homeodomain 2B in non-small cell lung cancer progression. Sheng-Yi Lin,1 Yu-Han Huang,1 Yi-Hua Lai,1 Sung-Liang Yu,1 Sheng-Fang Su,2 Tse-Ming Hong,2 Gee-Chen Chang,3 Chi-Chung Wang,4 Jeremy J.W. Chen,1,5 National Taiwan University College of Medicine, Taipei, Taiwan; 2National Taiwan University College of Medicine, Taipei, Taiwan; 3National Taiwan University, Taipei, Taiwan; 4National Cheng Kung University, Tainan, Taiwan; 5Taichung Veterans General Hospital, Taichung, Taiwan; 6Fu Jen Catholic University, New Taipei, Taiwan.

The transcription factor PITX2 (paired-like homeodomain 2) is well known to play an essential role in normal embryonic development. Emerging evidence suggests that PITX2 may be involved in human tumorigenesis. However, the functional role that PITX2 plays in tumor progression remain unclear. In this study, we investigated its function in human non-small cell lung carcinoma (NSCLC). Using real-time quantitative RT-PCR, we demonstrated that PITX2B is the most abundant isoform in lung cancer cells. Enforced expression of PITX2B could promote lung cancer cell morphological change, migration, invasion, anchorage-dependent growth ability and tumorigenesis in xenograft model mice. In addition, nuclear PITX2B localization was correlated with its oncogenic functions and two important nuclear localization signals (NLSs) were identified. Moreover, high expression of PITX2B was associated with poor overall survival of NSCLC patients. Importantly, silencing of PITX2B by siRNA caused a marked increase in autophagy based on LC3-II, Beclin-1, and p62 protein expression, acridine orange fluorescence of acidic vacuoles and electron microscopic detection of autophagosomes. Furthermore, we found that the autophagy induced by PITX2B knockdown preceded apoptosis in lung cancer cells. Treatment of lysosome inhibitor, NH4Cl, could provide a protective effect that increases cell viability. In conclusion, our studies not only provide new insights into how PITX2B may contribute to the tumor progression in lung cancer but also the autophagy induced by PITX2B knockdown that may be developed as an effective therapeutic strategy for the treatment of lung cancer.

Elevated ubiquitin-specific protease 22 (USP22) is associated with enhanced malignancy, therapeutic resistance, and poor prognosis of various human cancers. In this study, the clinicopathological significance of USP22, therapeutic implication of targeting USP22, and underlying molecules of USP22 knockdown were investigated in human non-small cell lung cancer (NSCLC). Immunohistochemistry analysis revealed that USP22 protein was dramatically elevated in ~67% of 197 NSCLC cases compared with adjacent normal lung tissues. Importantly, statistical analysis demonstrated that elevated USP22 was positively associated with advanced cancer stage and a trend to poor prognosis of NSCLC patients. Moreover, siRNA-mediated knockdown of USP22 induced a cell cycle arrest in G1 phase and significantly suppressed cell proliferation and soft agar colony formations in NSCLC cell lines. In addition, knockdown of USP22 significantly enhanced cisplatin-induced genotoxic effects in NSCLC cell lines. Furthermore, USP22 silencing substantially down-regulated SIRT1 and upregulated p53 tumor suppressor in NSCLC cells, indicating downregulation of USP22 may boost p53-mediated tumor suppression and DNA damage response in NSCLC cells. Knockdown of USP22 also increased global histone H3F monoubiquitlation, H3K4 and H3K79 trimethylation leading to global gene expression profile changes in these NSCLC cells. These results suggest that targeting USP22 will have broad anti-cancer effects via altering multiple cancer signaling pathways. Taken together, data of this study further indicate that the elevated USP22 may potentially represent a prognostic factor and therapeutic target for NSCLC patients.

Study of the mechanisms of emetine dihydrochloride on inhibiting NSCLC tumor progression and attenuating EGFR signaling pathway. Yi-Hua Lai,1 Wan-Ting Huang,2 Sheng-Yi Lin,1 Yao-Ting Huang,2 Yuh-Ling Chen,3 Yu-Han Huang,1 Yi-Hua Lai,1 Sung-Liang Yu,1 Sheng-Fang Su,2 Tse-Ming Hong,2 G. Chen,3 Institute of Medical Science, The University of Tokyo, Tokyo, Japan; 2University of Chicago, Chicago, IL.

Lung cancer is the leading cause of cancer deaths worldwide. Since the number of patients responding well to standard therapies is still limited, further development of new anti-cancer agents with minimum risk of adverse event is urgently awaited. To screen oncoresources which could be used for the development of new diagnostic biomarkers and molecular targeted drugs, we have established a screening system as follows: i) To identify overexpressed genes in 120 lung cancers by genome-wide screening using the microarray representing 27,648 genes and pure populations of tumor cells taken from cancer tissues by laser microdissection, ii) To verify the genes for their very low or absent expression in normal tissues by northern blotting, iii) To validate the clinicopathological significance of their expression with tissue microarray covering hundreds of archived lung cancers, iv) To verify whether the target genes are essential for the growth or survival of lung cancer cells by RNAi and cell growth assays. During this process, we selected dozens of druggable oncogenes with various enzymatic activities, and identified overexpression of protein kinase LASK2 (lung cancer-associated kinase 2) in the majority of lung cancers, but not in normal tissues except testis. Immunocytochemical analysis found LASK2 to be mainly localized in the cytoplasm and nucleus of lung cancer cells. Immunohistochemical analysis revealed that strong LASK2 expression was an independent prognostic factor for non-small cell lung cancer (NSCLC) (P<0.0001). Suppression of LASK2 expression with its specific siRNAs caused growth inhibition of lung cancer cells. These findings are consistent with previous reports showing the involvement of LASK2 in mammalian cells also supported its oncogenic function. Since LASK2 plays a significant role in the cell proliferation and malignant nature of lung cancers, LASK2 is likely to be a prognostic biomarker and a potential therapeutic target for developing new anti-cancer drugs.

MED12 is recurrently mutated in Middle Eastern colorectal cancer. Abdul K. Siraj,1 Tariq Masoodi,1 Rong Du,1 Fouda Al-Dayer,2 Khawla S. Al-Kurai,1 King Faisal Specialist Hospital & Res. Ctr., Riyadh, Saudi Arabia.

Colorectal cancer (CRC) is a common cancer and a leading cause of cancer deaths. Previous studies have identified a number of key steps in the evolution of CRC but our knowledge of driver mutations in CRC remains incomplete. Recognizing the potential of studying different human populations to reveal novel insights in disease pathogenesis, we conducted genomic analysis of CRC in Saudi patients. In the discovery phase of the study, we conducted whole-genome sequencing of tumor and corresponding germline DNA in 27 CRC patients. In addition to known driver mutations, we identified three MED12 somatic mutations. In the replication phase, we employed a next-generation sequencing approach to capture and sequence MED12 and other candidate genes in a larger sample of 400 CRC patients and confirmed the enrichment for recurrent MED12 mutations. In order to gain insight into a plausible biological mechanism for the potential role of MED12 mutations in CRC, we studied CRC cell lines that differ substantially in the expression level of MED12, and found the latter to be correlated inversely with TGF-β signaling and directly with apo-
ptosis in response to chemotherapeutic agents. Importantly, these correlations were replicated when MED12 expression was experimentally manipulated. Our data expand the recently described role of MED12 as a tumor suppressor in colorectal cancer. In this study, we reasoned that since the frequencies of driver mutations have been shown to be different between ethnic groups, comparison of targeted exome sequencing of 1,321 genes of interest to determine the mutations observed in breast cancer cells, suggesting that this is not lung cancer specific. Transcriptional activity of the Notch-responsive luciferase construct (pHESLuc) was increased in the presence of Wt Notch4, and reduced in the presence of the P1663Q Notch4 Mu. Conclusion: The nature and high number of genetic alterations in Notch4 in H/L lung cancer patients, suggests that Notch4 may play a critical role in NSCLC tumorigenesis. Our data suggests that mutations in the ANK domain of Notch4 can interfere with its transcriptional activity and function, and should be further characterized.

#4455 Inverse relationship between exon 8 single nucleotide polymorphism (c.1293 C>T) of gigaxonin and human tumor cell growth. Eri S. Srivatanan,1 Kimberly J. Hwang,1 Albert Ko,1 Jenna R. Chatoff,1 Saroj K. Basak,1 Natasija Bajic,1 Xiao Zhang,1 Jordan J. Yousefi,2 Michael S. Lewis,1 Pascale Bombon,3 Marilene B. Wang,3 1VA Greater Los Angeles UCLA David Geffen School of Medicine, Los Angeles, CA; 2UCLA David Geffen School of Medicine, Los Angeles, CA; 3INM-INSERM U1051, Hospital St Eloi, Paris, France.
Gigaxonin, a product of the Giant Axonal Neuropathy (GAN) gene located on chromosome 16, is involved in intermediate filament processing of neural cells and vimentin fibers in fibroblasts. Previous studies have shown an interaction between p16 and gigaxonin in cisplatin mediated ubiquitination of p53. Genomic studies have indicated higher frequency (44.25%) of exon 8 single nucleotide polymorphism (SNP) (c.1293 C>T) of gigaxonin in the individuals of Caucasian population compared to normal population (22%). The polymorphism frequency is much lower in individuals of other ethnicities. To determine the relation of exon 8 polymorphism with HPV positive and negative cervical and head and tumors. Our studies showed a 47.25% polymorphism frequency in these tumors. There was no relationship between the presence of polymorphism and HPV status. However, there was an inverse relationship between polymorphism and tumor recurrence. Our studies have further shown higher expression of gigaxonin protein in cancer cell lines containing the polymorphic T allele. Growth assays in vitro and in soft agar have shown a direct relationship between the presence of the T allele and slower cell line growth. Our results therefore indicate that in addition to p16 expression, exons 8 SNP could also serve as a diagnostic marker of chemo sensitivity in human tumors.

#4456 Regulation of MYC protein stability by mutant KRAS in pancreatic ductal adenocarcinoma. Angelina V. Vaseva,1 Devon R. Blake,1 Salma H. Azam,1 Karim T. Gilbert,1 Chad V. Pecot,1 Kenneth H. Pearce,1 Laura E. Herring,1 Lee M. Graves,1 Peter J. Houghton,1 Channing J. Der,2 1UNC-Chapel Hill, Chapel Hill, NC; 2Greehey Children’s Cancer Research Institute, San Antonio, TX.
With the nearly 100% mutation frequency of KRAS in pancreatic ductal adenocarcinoma (PDAC), the development of therapeutic strategies to target KRAS is a high priority for the pancreatic cancer field. In the current study, we aimed to identify signaling changes caused by the acute suppression of mutant KRAS in PDAC cell lines. Strikingly, acute suppression of mutant KRAS in PDAC cell lines caused potent and rapid protesome-dependent degradation of MYC protein. Ablation of MYC also suppressed PDAC growth both in vitro and in vivo, indicating a critical driver role for MYC in KRAS-dependent PDAC tumorigenesis. A mechanism by which RAS effector signaling regulates MYC protein stability has been described, however we determined that this mechanism cannot fully account for how endogenous mutant KRAS stabilizes MYC protein in PDAC cells. We verified a role for the Raf-MEK-ERK but not the PI3K-AKT-GSK3β effector signaling pathway. Unexpectedly, we also excluded a role for MYC protein phosphorylation at MYC residue T58, and determined that ubiquitin ligases other than FBW7 are involved. These findings prompted us to search for additional KRAS-dependent protein kinases that facilitate MYC protein stability. We applied multiple screening strategies. First, we developed a novel fluorescence-based system to monitor real-time MYC protein degradation in PDAC cells and we adapted this system for use in a high-throughput flow-cytometry based assay. Second, we applied a mass spectrometry-based approach to profile the human kinome for KRAS-dependent changes in protein kinases. Finally, we applied gain-of-function (Cancer Toolkit) and loss-of-function (CRISPR/Cas9) genetic screens to identify signaling regulators of MYC protein stability. The results of these screens will be presented.

#4457 Novel oncogenic function of Notch4 in Hispanic lung cancer. Edna Gordian,1 Nicholas Gimbrone,1 Antonio Pannuti,2 Lucio Miele,2 W. Douglas Bomont,3 Marilene B. Wang,3 1Keimyung University, Daegu, Republic of Korea; 2Stanley S.Scott Cancer Center, New Orleans, LA.
Background: Molecular drivers of ~40% of lung adenocarcinomas are still unknown. We reasoned that since the frequencies of driver mutations have been shown to be different between ethnic groups, comparison of targeted exome sequencing data would identify genes with significant alteration frequencies between White Non-Hispanics (WNH) and other racial/ethnic groups. Notch signaling in cancer is context- and tissue-specific and may be both oncogenic and tumor suppressive. The mechanisms of action and genetic alterations involved in Notch oncogenesis have been studied for Notch1 and Notch3; specifically in Non-Small Cell Carcinoma (NSCLC), translocations in Notch3, and gain-of-function mutations in Notch1. Meanwhile, Notch4 has been associated with the notion of cancer driver but its exact role remains unknown. We hypothesized that Notch4 is a mammary oncogene, but little is known about its role in NSCLC tumorigenesis. Truncations in Notch4 have been reported in lung cancer cell lines, and in public databases the frequency of Notch4 alterations in WNH adenocarcinoma is ~5.5%. However, little is known about the mechanisms by which Notch4 acts in lung oncogenesis. Methods: Using data from the TCGA, we analyzed targeted sequencing of 1,321 genes of interest to determine the mutations driving lung cancer in Hispanic (H/L) patients. The functional relevance of the Notch4 mutations was tested by cloning the intracellular domain (ICD) of Notch4 containing one of the mutations identified in the ANK domain (P1663Q) into pcDNA3.1. Plasmids expressing either the WT or a mutated Notch 4 (P1663Q) were transfected into A549 cells and the effect of this mutation tested on the ability to induce the expression of Notch4 expression genes. Results: In our H/L cohort we see Notch4 altered in about 20% of our samples, and 7/12 of the Notch4 single amino acid substitutions are in the Negative Regulatory Region (NRR). PROVEAN protein analysis of the Notch4 mutations shows that more than half of the mutations would disrupt the NRR. Introduction of the P1663Q mutation in the ANK repeats of the Notch4 ICD, which mediates binding to CSL and is essential for Notch transcriptional activity, resulted in a shift in the amount of transcript of known Notch4 target genes, such as Hes1 and Sema. This effect is specific, as it is not observed with other genes, previously reported to be targets for other Notch paralogs such as Sema3 and Zeb1. A similar effect of the P1663Q Notch4 mutation on Hes1 transcript levels was observed in breast cancer cells, suggesting that this is not lung cancer specific. Transcriptional activity of the Notch-responsive luciferase construct (pHESLuc) was increased in the presence of Wt Notch4, and reduced in the presence of the P1663Q Notch4 Mu. Conclusion: The nature and high number of genetic alterations in Notch4 in H/L lung cancer patients, suggests that Notch4 may play a critical role in NSCLC tumorigenesis. Our data suggests that mutations in the ANK domain of Notch4 can interfere with its transcriptional activity and function, and should be further characterized.
confirmed the expressions of GLDC in eight different ovarian cancer cell lines. And colony formation assay revealed decreased proliferation of OVCAR3 ovarian cancer cells transfected with siGLDC compared with that of control. Intriguingly, we found that knockdown of GLDC induced decreased S-adenosylmethionine (SAM)/S-adenosylhomocysteine (SAH) ratio, which implicates methyl donor deficient state in OVCAR3 cells. Expressions of sirtuin 1 (SIRT1), heat shock protein (HSP) 90 and heat-shock factor protein 1 (HSF1) decreased while that of p53 increased in GLDC knock-downed cells, suggesting heat shock response. Addition of SAM into siGLDC knock-downed cells restored the expression of SIRT1. Inhibition of SIRT1 decreased Bc2 and increased cytochrome c expressions. In summary, these results suggest GLDC induces methyl donor deficient state in OVCAR3 ovarian cancer cells, which in turn leads to apoptosis via SIRT1-mediated pathway.

**#4460 TET1 over expression contributes to lung cancer malignancy**, Piote Teodor Filipczak, Shuguang Leng, Carmen S. Tellez, Steven A. Belinsky. Lovelace Respiratory Research Inst., Albuquerque, NM.

Epigenetic alterations are a leading cause of lung carcinogenesis and malignancy. While the role of enzymes catalyzing cytosine-guanine (CpG) island methylation and chromatin modifications has been extensively studied, the role of the recently discovered demethylating enzymes represented by ten-eleven translocation methylcytosine dioxygenases (TETs) remains unknown. The goal of this study was to investigate the expression and functional significance of the TET1 gene in non-small cell lung cancer (NSCLC). QRT-PCR revealed 2- to 30-fold over expression of TET1 in 52% of squamous cell carcinoma (SCC) and 40% of adenocarcinoma (AdC) tumors compared to paired normal lung tissues. This result was validated using The Cancer Genome Atlas (TCGA) database with TET1 over expressed in 72% of SCCs and 53% of AdCs. Elevated levels of TET1 transcript were associated with increased protein levels in NSCLC cell lines compared to normal human bronchial epithelial cells (HBECs). A reporter assay analysis defined the promoter region critical for elevated transcription of the TET1 gene using lung cancer cell lines, and studies to identify the transcription factors underlying the increase in expression are underway. Transient knock down of TET1 expression in NSCLC cell lines caused global changes in the transcriptome, reduced cell proliferation, colony formation, and inhibited growth of tumor xenografts in nude mice. Cellular senescence mediated by p21 signaling and associated with β-galactosidase activation was identified as one mechanism underlying growth inhibition. This study suggests that TET1 may function as an oncogene contributing to the lung cancer malignant phenotype and thus, could serve as a potential new target for treatment. (Supported by R01 CA183296)

**#4461 Detection of somatic mutations in exosomal RNA from human oral squamous cell carcinoma cells**. Norikazu Tokuzen, Koh-ichi Nakashiro, Hirokiyo Hamakawa. Ehime Univ. Graduate School of Medicine, Toon, Ehime, Japan.

Exosomes are small vesicles, ranging from 30 to 100 nm in size, secreted from most types of cells. They contain proteins, lipids, and nucleic acids (e.g. DNA, mRNA, and microRNA) and are thought to play an important role in cell proliferation, invasion, and metastasis in human malignancies. Recently, circulating exosomes have also been of interest as a source for liquid biopsies. In this study, we examined the usefulness of exosomal RNA as a biomarker and attempted to detect the tumor-specific gene mutations from exosomal RNA derived from human oral squamous cell carcinoma (OSCC). We used 3 human OSCC cells (HSC2, KT-T, KT-N). KT-T and KT-N cells were established from the lower gingival tumor and lymph node metastasis, respectively. First, we detected the somatic mutations in genomic DNA extracted from these cells by next-generation sequencing (NGS) with the use of HaloPlex Cancer Research Panel (Agilent Technologies). PIK3CA H1047R mutation was identified in all OSCC cells, and KT-N cells had the additional HRAS Q61R mutation. We confirmed these mutations of PIK3CA and HRAS in OSCC cells by TaqMan SNP Genotyping Assays (Thermo Fisher Scientific). Subsequently, we evaluated the gene mutations in exosomal RNA from these OSCC cells. Exosomal RNA was isolated from the conditioned medium using exoRNeasy Serum/Plasma Maxi Kit (Qiagen) according to the manufacturer’s protocol. In exosomal RNA, we found the PIK3CA H1047R mutation from all OSCC cells and HRAS Q61R mutation from KT-N cells by genotyping assays. Furthermore, we assessed the detection of these mutations in tumor xenograft mouse models. KT-T and KT-N cells (5 x 106) complexed with Matrigel® (BD) in 100-μl aliquots were injected subcutaneously in the flank of male athymic nude mice. Exosomal RNA was isolated from the serum of tumor-bearing nude mice using exoRNeasy Serum/Plasma Midi Kit (Qiagen). By genotyping assays using serum exosomal RNA, PIK3CA H1047R mutation was observed in both types of xenograft mice and HRAS Q61R mutation was positive in KT-N xenograft mice. Cancer cell-derived exosomal RNA had the same gene mutations in those cells, and could be detected in the serum of tumor-bearing nude mice. These results suggest that exosomal RNA is useful for detecting somatic mutations in OSCC cells.

**#4462 ACTN4 stabilises RIPK1 to function as an oncogenic driver in melanoma**. Lei Jin, Hessam Tabatabaehamakhsh, Chen Chen Jiang, Xu Guang Yuan, Jun Yuan Yang, Yuan Yuan Zhang, Hamed Yari, Chatin Van Vaang, Ting Li, Fu Xi Lei, Yu Chen Feng, Su Tang Guo, Xu Dong Zhang. The University of Newcastle, Callaghan, Australia.

We have recently reported that receptor-interacting protein kinase 1 (RIPK1) is commonly up-regulated through cellular inhibitor apoptosis protein (cIAP)-mediated stabilization and functions as an oncogenic driver via activation of NF-κB in melanoma cells. Here we show that α-actinin-4 (ACTN4), an isoform of α-actinins of spectrin superfamilies that is emerging as an oncogenic regulator, is required for cIAP-mediated stabilization of RIPK1 and plays a pro-oncogenic role in melanoma. Similar to RIPK1, ACTN4 was found to be commonly increased in melanoma cells. While knockdown of ACTN4 inhibited melanoma cell proliferation that was associated with down-regulation of RIPK1 and reduction in the basal levels of NF-κB activation, overexpression of ACTN4 resulted in enhanced proliferation of melanocytes that was associated with elevation in RIPK1 expression and increased NF-κB activation. The inhibitory effect of ACTN4 knockdown on melanoma cell proliferation and activation of NF-κB was due to decreased expression of RIPK1, as it was abolished by overexpression of RIPK1. On the other hand, knockdown of RIPK1 diminished ACTN4 overexpression of cIAPs and promoted tumor apoptosis. Strikingly, knockdown of ACTN4 attenuated the association between cIAPs and RIPK1 and reduced K63-linked ubiquitination of the protein, leading to RIPK1 protein degradation. Mechanistic studies showed that ACTN4 bound to RIPK1 through its N-terminus, whereas it was associated with cIAPs via its C-terminus, and that dimerization of ACTN4 in an anti-parallel manner is required for the interaction between cIAPs and RIPK1. Collectively, ACTN4 is necessary for stabilization of RIPK1 by cIAPs and functions as an oncogenic driver in melanoma.

**#4463 The coactivator oncogene AF1q associates with STAT3 activation downstream of MET action in gastro-esophageal cancer patients**. Elisabeth S. Gruber,1 Peter Birner,2 Olaf Merkel,2 Michael M. Bergmann,2 Sebastian F. Schoppmann,1 Jino Park,3 Richard Moriggl,4 William Tse,3 Lukas Kenner5.

1 University Clinic for Surgery, Medical University of Vienna, Vienna, Austria; 2 Institute of Pathology, Medical University Vienna, Vienna, Austria; 3 James Graham Brown Cancer Center, University of Louisville School of Medicine, Louisville, KY; 4 University Vienna, Ludwig Boltzmann Institute, Vienna, Austria; 5 Institute of Laboratory Animal Pathology, University of Veterinary Medicine, Vienna, Austria.

AF1q was initially identified as an oncogene in acute myeloid leukemia. In breast cancer, AF1q mediates tumor progression by boosting STAT3-signalling. In addition, AF1q enhances wnt-signalling resulting in transcriptional activation of CD44 and promotes tumor cell proliferation, migration and chemoresistance. In gastrointestinal malignancies both pathways are linked to enhanced MET tyrosine kinase activity, accumulation of activated MET and metastases. We showed that AF1q overexpression in the gastro-esophageal cancer cell lines SK-GT-4, FLO-1 and OE33 led to increased sphere formation and increased invasive capacity in the case of FLO-1 and OE33. We investigated the role of AF1q in a retrospective collective of 460 resected gastro-esophageal cancer specimens (74.3% adenocarcinomas (AC), 25.7% esophageal squamous cancers (SCC)). With respect to topographic location, 40.4% of tumors were esophageal (EC), 20% gastro-esophageal (GEC) and 39.6% gastric cancers (GC). Immunohistochemistry revealed overexpression of AF1q in 205, and of CD44 in 114 patient tumor samples. AF1q overexpression was found more often in EC/GC as compared to GC (p = 0.007) and associated with HER2 (p = 0.035), pySTAT3 (p < 0.001) and MET (p = 0.004) expression as well as neoadjuvant chemotherapy (p = 0.01). AF1q and CD44 overexpression correlated in the overall group (p < 0.001) and in AC (p < 0.001). In SCC CD44 was expressed more frequently compared to AC (p < 0.001). Analysis of the matched primary and metastatic tumors revealed that primary AF1q-positive tumor samples were largely overlapping with AF1q-positive tumors lymph node metastases (23/32) and distant metastases (6/7). AF1q overexpression correlated with shorter disease specific survival (DSS) (p = 0.036), but overall survival (OS) was similar (p = 0.117). In a subgroup analysis a shorter OS was observed in EC/GC (p = 0.03, log rank test). In a Cox regression model AF1q and CD44 expression was associated with shorter DFS (p < 0.001 and p = 0.025) and DSS (p = 0.005 and p = 0.035), but OS remained
unchanged. Prognostic significance was limited to AF1q (p=0.003 and p=0.04, respectively). We conclude that, AF1q overexpression is associated with progression and metastases in GEC. AF1q and downstream pathway markers such as p53 and p73 were associated with AF1q expression. Therefore we propose AF1q as a novel prognostic marker for GECs.

### Glypican-1

Glypican-1 (GPC-1) is a cell surface heparan sulfate proteoglycan that mediates cell proliferation, differentiation and migration. It also posses oncogenic functions in breast, pancreatic and lung cancer cells, and is a proposed biomarker for prostate cancer. Despite these data, the function of GPC-1 in prostate cancer is not fully known. We addressed this gap-in-knowledge by testing the effect of tumor cell line-specific (TCL)-mediated knockdown of GPC-1 knockdown on human bone marrow derived mesenchymal stem cells (hMSC) and fibroblasts (HS-27). Treatment of both hMSC and HS-27 cells with TCL from GPC-1 knockdown PC-3 cells increased MMP-9 and interestingly MMP-2 expression and activity as compared to control cells. We also detected increases in the expression of N-Cadherin in a co-culture model. Collectively, these data suggest that GPC-1 acts as an oncogene to enhance prostate cancer cell growth in vitro; however, these data also suggest the GPC-1 may act as a tumor suppressor in vivo. This discrepancy may be due to the interaction of GPC-1 with the tumor microenvironment, which would not be detected in single-cell culture models.

### Glypican-1: A tumor suppressor or an oncogene in human bone metastatic prostate cancer cells

Shyh-Han Tavan, Kevin Babcock, Indu Kohar, Ahmed A. Mohamed, Denise Young, Shiv Srivastava, Albert Dobi. Center for Prostate Disease Research, Uniformed Services University, Rockville, MD

Introduction: Most genomic studies of prostate cancer (CaP) have been performed primarily on men of European ancestry, including Caucasian Americans. African Americans (AA) men, however, bear the burden of highest CaP incidence and mortality rates in the US. Cumulative data from other groups and from us have shown significantly lower rate of alterations of the two common CaP driver genes (PTEN and ERG) in AA CaP as compared to CA CaP. Using a combination of whole genome sequencing, IHC and FISH based validations on tissue microarrays, and analysis of the TCGA data from a total of 438 CaPs, we recently reported a higher frequency of a genomic deletion on 3q13.31 locus in AA CaP which also associated with rapid biochemical recurrence. This deletion is centered at the LSAMP (Limbic System Associated Membrane Protein) gene. The LSAMP protein functions as a cell adhesion molecule affecting cell differentiation and migration during development. We hypothesize that the loss of LSAMP contributes to CaP progression. Here, we report the biological consequence of LSAMP modulation in CaP cells. Methods: LSAMP status in CaP cell lines (LNCaP, MDAPCa2b, CRW22rv1, LAPC4, NCI-H660, and PC3) was assessed by FISH, immunoblot, qRT-PCR and immunofluorescence (IF), assays using MC-IXC neuroepithelioma cell line as the positive control. We established doxycycline inducible LSAMP in LNCaP and in MDAPCa2b (an AA patient derived cell line with monoallelic LSAMP deletion) cells, and constitutive expression in DU145 cells. The effect of LSAMP loss and over-expression on cell growth, proliferation apoptosis and cell migration was monitored by MTT, soft-agar, and Boyden chamber assays. Results: Of the CaP cell lines analyzed, LSAMP RNA and protein expression was detected in PC3 and NCI-H660. IF assay showed that endogenously expressed LSAMP is predominantly localized to the cell surface. Exogenous expression of LSAMP, either constitutively in DU145, or by inducible means in LNCaP and MDAPCa2b cells, showed similar localization to the plasma membrane. Both inducible and constitutive overexpression of LSAMP resulted in reduced cell growth. LSAMP expression in DU145 resulted in enhanced formation of filamentous extracellular lumen extensions. Analysis of published TCGA (The Cancer Genome Atlas) data showed a correlation of high LSAMP promoter methylation and low LSAMP expression in CaP tumors compared to normal prostate tissues. Conclusion: The cell biology features of LSAMP reported here, together with observations of genomic deletion and methylation of LSAMP promoter in CaP, support its role in CaP development or progression. These data support the function of LSAMP as a tumor suppressor gene, as previously reported in other cancers.

### Glypican-1: A tumor suppressor or an oncogene in human bone metastatic prostate cancer cells

Nhat D. Quach, Matthew Eggert, Deepraj Ghosh, Michelle R. Dawson, Robert Arnold, Brian S. Cummings. University of Georgia, Athens, GA; Auburn University, Auburn, AL; Brown University, Providence, RI

Glypican-1 (GPC-1) is a cell surface heparan sulfate proteoglycan that mediates cell proliferation, differentiation and migration. It also possesses oncogenic functions in breast, pancreatic and lung cancer cells, and is a proposed biomarker for prostate cancer. Despite these data, the function of GPC-1 in prostate cancer is not fully known. We addressed this gap-in-knowledge using in vitro and in vivo mouse models. We first showed that GPC-1 was overexpressed in bone metastatic prostate cancer cells (PC-3) but was not detected in lymph node metastatic cancer cells (LNCaP) and non-cancerous prostate cells (RWPE-1). Next, we inhibited GPC-1 expression using siRNA in bone metastatic cancer cells (PC-3), and verified inhibition using western blot analysis and qRT-PCR. Crystal violet staining, trans-well assays, matrigel colony cultures and adhesion assays were used to determine the effect of GPC-1 inhibition on cancer cell growth, migration, spheroid formation and cell adhesion. Inhibition of GPC-1 reduced prostate cancer cell growth, migration and spheroid formation; however, there were no changes in cell adhesion. These in vitro data suggested that GPC-1 played a role in prostate cancer progression by acting as an oncogene. We further investigated this possibility in vivo using athymic (NCR) mice xenografts. Interestingly, inhibition of GPC-1 in PC-3 cells appeared to increase tumor size as compared to scrambled controls. Further analysis showed that that inhibition of GPC-1 increased the tumor expression of MMP-9 and N-cadherin, but not E-cadherin. We hypothesized that the discrepancy between the in vitro and in vivo results may be mediated by interaction of PC-3 cells with the tumor microenvironment. We addressed this hypothesis by testing the effect of tumor cell line-specific (TCL)-mediated knockdown of GPC-1 knockdown on human bone marrow derived mesenchymal stem cells (hMSC) and fibroblasts (HS-27). Treatment of both hMSC and HS-27 cells with TCL from GPC-1 knockdown PC-3 cells increased MMP-9 and interestingly MMP-2 expression and activity as compared to control cells. We also detected increases in the expression of N-Cadherin in a co-culture model. Collectively, these data suggest that GPC-1 acts as an oncogene to enhance prostate cancer cell growth in vitro; however, these data also suggest the GPC-1 may act as a tumor suppressor in vivo. This discrepancy may be due to the interaction of GPC-1 with the tumor microenvironment, which would not be detected in single-cell culture models.

### Glypican-1: A tumor suppressor or an oncogene in human bone metastatic prostate cancer cells

Ahmed A. Mohamed, Denise Young, Shiv Srivastava, Albert Dobi. Center for Prostate Disease Research, Uniformed Services University, Rockville, MD

ETV6 is an ETS transcription factor and a putative tumor suppressor in prostate cancer according to several genomic analyses. However, its cellular functions and upstream regulating signaling remain unclear. We demonstrated that ETV6 suppresses the prostate cancer progression and that ETV6 levels are negatively associated with advanced prostate cancer, supporting the idea that ETV6 acts as a tumor suppressor. Furthermore, ETV6 can be regulated by epidermal growth factor receptor (EGFR) signaling through microRNA (miR)-mediated downregulation. One of the miRs, miR-96, can be transcriptionally-regulated by nuclear EGFR. The association patterns connecting miR-96, EGFR activation, and ETV6 were further validated in the clinical tissue samples. We proposed an EGFR-miR-96-ETV6 signaling pathway in promoting prostate cancer.

### Glypican-1: A tumor suppressor or an oncogene in human bone metastatic prostate cancer cells

ETV6 is a tumor suppressor regulated by EGFR-miR-96 pathway in prostate cancer. Yen-Nien Liu, Yuan-Chin Tsai, Wei-Yu Chen, Man Kit Siu, Hisu-Lien Yeh. Taipei Medical University, Taipei, Taiwan; National Tsing Hua University, Hsinchu, Taiwan.

ETV6 is an ETS transcription factor and a putative tumor suppressor in prostate cancer according to several genomic analyses. However, its cellular functions and upstream regulating signaling remain unclear. We demonstrated that ETV6 suppresses the prostate cancer progression and that ETV6 levels are negatively associated with advanced prostate cancer, supporting the idea that ETV6 acts as a tumor suppressor. Furthermore, ETV6 can be regulated by epidermal growth factor receptor (EGFR) signaling through microRNA (miR)-mediated downregulation. One of the miRs, miR-96, can be transcriptionally-regulated by nuclear EGFR. The association patterns connecting miR-96, EGFR activation, and ETV6 were further validated in the clinical tissue samples. We proposed an EGFR-miR-96-ETV6 signaling pathway in promoting prostate cancer.
MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Post-transcriptional and Translational Regulation in Cancer

### #4468 Oncogenic activation of the RNA binding protein AGO2 in hepatocellular carcinoma. Hien T. Dang, Lucy Knight, Yotsawat Pomyen, Xin Wei Wang, NIH/NCI, Bethesda, MD.

Global transcriptomic alterations of coding and non-coding RNAs are a ubiquitous feature of cancers including Hepatocellular carcinoma (HCC). Dysregulation of RBPs binding motifs (RBPs), key factors in processing such as RNA maturation and degradation, is one mechanism in which cancer cells select to promote tumorigenesis. RBPs are highly expressed in solid tumors and have been demonstrated to be drivers of carcinogenesis, however, the underlying mechanisms in which RBPs regulate the HCC transcriptome is unknown. We analysed genomic alterations amongst a family of more than 800 mRNA RBPs (mRBPs) in 1,225 clinical specimens from HCC patients and found that RBPs are significantly activated through gene amplification in a subset of tumors with poor prognosis, suggesting their potential oncogenic roles in HCC progression. Amongst the top candidates, Argonaute 2 (AGO2) was further characterized for its oncogenic role and effects on the HCC transcriptome. Elevated AGO2 mRNA expression was highly correlated with elevated somatic copy number alterations across five different cancer types including HCC. AGO2 expression was associated with overall survival in two independent data sets (TCGA and the Liver Initiative Cancer), suggesting that AGO2 activation is oncogenic. While the activation of AGO2 induced an oncogenic phenotype, the abrogation of AGO2 in HCC cells significantly decreased cancer associated phenotypes such as cell proliferation, migration/invasion and tumorigenicity in vivo. Further analyses revealed that AGO2-associated genes were tumor-related, including c-Myc. Notably, c-Myc amplification was also found in AGO2 high HCC cells, suggesting an interplay between AGO2 and c-Myc. Our results demonstrate that oncogenic activation of AGO2 is a novel mechanism that may contribute to global transcriptome selective for the activation of c-Myc oncogenic. Our current work suggests that therapies focusing on targeting AGO2 may be valuable for clinical treatment of many different tumors with activated c-Myc signalling, including HCC.

### #4469 Targeting the tri-snRNP complex for triple-negative breast cancer therapy. Stefanie Chan, Praveen Sridhar, Ying-Jie Lock, Fabio Petrozza. Boston University School of Medicine, Boston, MA.

Triple negative breast cancers (TNBC) account for ~15% of all breast cancers and the majority of breast cancer patients in women carrying a BRCA1 germline mutation. Due to the inadequacy of existing treatment, here we sought to identify shared genetic dependencies across different TNBC cell lines with the ultimate goal of uncovering novel therapeutic targets. To this end, we performed a targeted siRNA lethality screen across 11 human breast cell lines focusing on 154 genes, which we previously identified as selectively essential to a genetically-defined TNBC progenitor cell line (BPLER) relative to a myoepithelial-like isoegenic cell line (HMMLER). Thirty genes scored as recurrently and selectively required for survival of progenitor-like (or basal-A) TNBC lines compared to differentiated cell lines. Genes for proteins involved in RNA splicing were prominent shared dependencies among basal-A TNBC cell lines. Of particular note were those associated with the UA/U6/U5 tri-snRNP complex, including PRPF8 and PRPF38A. Transcriptome-wide RNA-seq analysis of the knockdown of either led to widespread intrinsic retention, expression of immune system genes and aberrant splicing of transcripts involved in protein translation, proteasome function, mitosis and apoptosis, including the TNBC dependency gene MCL1. As the proteasome and mitosis are also established TNBC vulnerabilities, we have identified specific classes of transcripts that are both particularly susceptible to perturbation of tri-snRNP activity and selectively essential for TNBC, making the tri-snRNP complex an attractive target for downstream drug development.

### #4470 RNA m⁵C methyltransferases and hnRNPK mediate disease-associated chromatin structure and drug resistance in leukemia. Jason X. Cheng, Li Cheng, Adam Cloe, Yuan Li, Ming Yue, Michelle M. Le Beau, Richard A. Larson, James W. Vardiman. University of Chicago, Chicago, IL.

The mechanisms governing disease-associated chromatin organization and drug selectivity are poorly understood. Our epigenetic drug screening experiments identify two distinct lineage-specific drug-responsive patterns in myeloid leukemia (MDS/AML) cells. We have established multiple MDS/AML cell lines resistant to nucleic acid analogs with different chemical backbones and have demonstrated a clear relationship between drug-resistance and sugar-phosphate backbone. Based on these results, we used various experimental approaches to elucidate leukemia-associated, drug-responsive chromatin structural changes and their underlying mechanisms. Our data demonstrate that hnRNPK, a conserved factor in heterogeneous nuclear RNA-binding protein (hnRNP) complexes, directly interacts with RNA m⁵C methyltransferases NSUNs/DNMT2 and RNA-polymerase II (pol-II)/CTD9 as well as erythroid vs myeloid lineage-specific splicing factor transcriptome (TFs) GATA1 and SPP1 to targets distinct drug-responsive chromatin structures in MDS/AML cells. hnRNPK preferentially binds to methylated polypyrimidine RNA sequences to facilitate transcription elongation. Compared to azacitidine-sensitive MDS/AML cells, there is a marked increase in azacitidine-resistant MDS/AML cells in RNA m⁵C and NSUNs/DNMT2 associated with a marked increase in the interaction between hnRNPK and pol-II. In leukemia, a cell type most frequently harboring ncRNA capturing technology coupled with super-resolution stimulated-emission depletion confocal microscopy, we demonstrate co-localization of hnRNPK with the Tfs and active pol-II at nascent RNA sites in MDS/AML cells, further supporting the existence of hnRNPK-mediated drug-responsive transcription and lineage-specific chromatin structures in MDS/AML cells. Our experiments using clinical specimens demonstrate a positive correlation between MDS/AML progression and increase in expression of hnRNPK and RNA m⁵C methyltransferases, supporting their importance and clinical relevance. Furthermore, knockdown of hnRNPK and RNA 5mC methyltransferases effectively inhibited the growth of MDS/AML cells. In conclusion, our data demonstrate distinct RNA m⁵C methyltransferases/hnRNPK-mediated chromatin structures that correlate with growth and drug-resistance of MDS/AML cells. Our data support the existence of ncRNA methyltransferases as the writers of m⁵C on nascent RNA, and hnRNPK functions as a reader of the RNA m⁵C and an operator to regulate transcription elongation and gene activation. Such novel chromatin structure-based drug action models and mechanisms may identify new diagnostic and prognostic biomarkers and therapeutic approaches.
A subunit of splicing factor 3b, SF3B4, functions as driver of liver cancer via aberrant splicing activity on tumor suppressor KLF4. Suk Woo Nam, Qunyu Shen, Eun Hyung Song, Su Jin Kim, Hee Do Yang, Sang Yeon Kim. The Catholic University of Korea, Seoul, Republic of Korea.

Alternative splicing is one of the crucial mechanisms that contribute to proteome diversity and, as reported, up to 90% of genes are alternatively spliced in human. In the last few years, a large number of mRNA isoforms revealed to contribute many cellular development and disease, including cancer. SF3B4 is one of six subunits of the splicing factor 3b (SF3b) complex which is an important protein complex in U2 snRNP super complex. In this study, we show that SF3B4 expression was overexpressed in a large cohort of human hepatocellular carcinoma (HCC) patients. SF3B4 knockdown caused G1/S cell cycle arrest by recovering p27 expression and simultaneously suppressing cyclins, and CDKs in liver cancer cell. Consistently, Spliceostatin A, a SF3b complex inhibitor, also suppressed liver cancer cell growth with similar effect on cell cycle regulation. In addition, sustained suppression of SF3B4 reduced the in vivo tumor growth rate in mouse xenograft models. Furthermore, SF3B4 knockdown repressed in vitro tumor cell motility and invasion of liver cancer cells. Notably, we observed that SF3B4 knockdown increased alternative transcript splicing event of a large number of genes in liver cancer cells. From this, we found that SF3B4 knockdown caused loss of wildtype KLF4 transcript expression, and therefore affected anti-tumor growth effect on liver cancer cells. Indeed, ectopic expression of KLF4 mimicked SF3B4 knockdown effect on same cells. Our results suggest that aberrant regulation of SF3B4 contribute to malignant transformation and growth of liver cancer cells by repression of tumor suppressor via unmodulated alternative splicing activity during liver tumorigenesis.

SRRM4 drives treatment-induced neuroendocrine differentiation of prostate adenocarcinoma under androgen receptor pathway inhibition. Yinan Li. Vancouver Prostate Centre, Vancouver, British Columbia, Canada.

Treatment-induced neuroendocrine prostate cancer (t-NEPC) is an aggressive subtype of castration-resistant prostate cancer that typically does not respond to AR pathway inhibition (ARPI) and its diagnosis is increasing. To understand its development, we analyzed transcriptomes of prostate tumors to identify t-NEPC-specific RNA splicing. Interestingly, more than 66% of the splice events are predicted to be regulated by the RNA splicing factor, Serine/Arginine Repetitive Matrix 4 (SRRM4). We confirmed in vitro and in vivo that a single SRRM4 target gene is the REI-Silencer Factor (REST), a master regulator of neurogenesis. SRRM4 strongly stimulates adenocarcinoma cells to express t-NEPC biomarkers and this is exacerbated by ARPI. ARPI combined with gain of SRRM4 results in adenocarcinoma cells to assume multicellular spheroid morphology and establishes progressive NEPC xenografts. These SRRM4 actions are further enhanced by loss-of-function of TP53. We conclude that SRRM4 drives t-NEPC progression. This knowledge may guide the development of novel therapeutic approaches at t-NEPC.

Functional characterization of alternatively spliced GSN in head and neck cancer. Dylan Z. Kelley, Emily L. Flam, Theresa Guo, Craig Bohrson, Michael Considine, Ludmila V. Danilova, Justin A. Bishop, Chi Zhang, Wayne M. Koch, David Sidransky, William Westra, Sarah Wehlan, Liliana Fiorela, Elana J. Fertig, Joseph A. Calilano, Daria A. Gaykalova, Johns Hopkins School of Medicine, Baltimore, MD; Johns Hopkins Medical Institutions, Baltimore, MD; University of California, San Diego, La Jolla, CA.

PURPOSE: Analyze the functional role of alternative splicing in HPV-related (HPV+) Head and Neck Squamous Cell Carcinoma (HNSCC) biology and oncogenesis. HPV+ HNSCC incidence is rapidly increasing, while its oncogenic mechanisms, demographic, and clinico-pathological characteristics are largely uncharacterized and discrete from those of HPV-. HNSCC. Investigation into the molecular biology of HPV pathways in HNSCC may help with creation of more specific clinical tools for diagnosis and treatment, which are limited and lack accuracy. Alternative Splicing Events (ASEs), post-transcriptional modifications resulting in variant transcripts leading to alternative protein isoforms, have been under-investigated as putative causes of gene expression deregulation in HPV+. Using RNA-Seq data, a genome-wide ASE discovery analysis was performed on a JHU HPV+ HNSCC cohort, which has expanded HPV+ HNSCC transcriptional characterization by identifying novel ASE biomarkers implicated in carcinogenic progression. Outlier Gene Set Analysis (OGSA) was used to identify genes with high rates of ASE and results were validated in TCGA samples from seven different cancer types. To verify the functional implications of differential isoform expression, vectors for knock-in and knock-down of WT-GSN and ASE-GSN isoforms were developed to evaluate changes in cell proliferation and invasion. ASE-GSN/WT-GSN ratio outliers were identified by OGSAs in tumor samples from discovery JHU HPV+ oncogenes. Expression, oncogenic signaling were compared among UM-SCC-22B HNSCC cell lines, Lung Squamous Cell Carcinoma, Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma, Bladder Urothelial Carcinoma, Breast Invasive Carcinoma, and Colon Adenocarcinoma. The increased ASE-GSN/WT-GSN ratio was also observed in a cohort of HPV+ Head and Neck Cancer Cell lines. GSN+ and UM-SCC-22B HNSCC cell lines were used to evaluate the function of GSN ASEs. Overall expression levels of both GSN isoforms conversely correlated with cell proliferation whereas the high ratio of ASE-GSN to WT-GSN correlated with increased cellular invasion of stroma in vitro. Additionally, a decrease or increase in expression of one isoform is seen to cause a compensatory increase or decrease in the other isoform respectively. ASE-GSN outliers correlated with HPV+ cancers indicate that expression ratio between GSN isoforms is related to tumor behavior, which is reinforced in vitro where WT-GSN is inversely correlated and ASE-GSN is directly correlated to cell invasion. These relationships suggest that both overall expression and balance between the two GSN isoforms are mediating factors in cell death or proliferation, while shorter ASE-GSN is more likely to indicate progression to cancer. Finally, we propose a model for the role of GSN isoforms in apoptosis and metastasis, in which trends in expression between WT- and ASE-GSN isoforms can alternatively affect rates of apoptosis or metastatic ability.

Delineating translocation renal cell carcinoma oncogenesis in cells harboring TFE3 fusion with spliceosome machinery associated genes. Nur P. Damayanti, Sreenavasulu Chintala, Ashley Orillion, Remi Adelaye-Ogala, May F. Ellbanna, Peter Hollenhorst, Roberto Pili, Indiana University, Indianapolis, IN; Indiana University, Bloomington, IN; Indiana University, Indianapolis, IN.

Background: Translocation Renal Cell Carcinoma (tRCC) represents an aggressive subtype of kidney cancer associated with various gene fusions involving translocation of one of two members of microphthalmia transcription factor (MIT) family, TFE3 or TFE6. Despite identification of multiple TFE3 gene fusions in tRCC, heterogeneous phenotype and various dysregulated signaling pathways resulting from variety of fusion partners pose a challenge to establish effective treatments for these patients. In this work, we sought to study the biology underlying oncogenesis in tRCC involving TFE3 fusion with genes associated with pre-mRNA splicing factor machinery; NONO, SFPQ and PRCC. Methods: To investigate the biology of different TFE3 fusion proteins, we created cell lines with TFE3 fusions and with different TF=E3 fusion partner dependent manner (P<0.05). We observed more homogeneous phenotype and oncogenic signaling were compared among cells expressing different TFE3 fusions such as SFQ-TFE3, NONO-TFE3, PRCC-TFE3. Endogenous SFPQ-TFE3 expressing cells, RP-RO7, were generated from patient derived xenograft. UOK-109 and UOK-146 (kindly provided by Dr. Marston Lenehan, NCI) were used as endogenous NONO-TFE3 and PRCC-TFE3 expressing cells, respectively. TFE3 fusion transcripts were identified with RT-PCR. TFE3 fusion preferential DNA binding was assessed with ChIP-sequencing. Oncogenic phenotype assessment was done in 2D culture and two different 3D models; 1) Solid tumor model, in which cancer cell interaction with extracellular matrix and stromal cells, were represented in multiculture-spheroid system, 2) Invasion model, in which cell ability to invade basement membrane barrier was modeled with matrix restricted spheroid. Results: Dissimilar oncogenic phenotypes were seen among ARK, FAK, mTOR VGEFR-2 were identified among TFE3 fusion cells with different level of expresion (P<0.05). These results suggest distinct alteration of kinases nucleocytoplasmic shuttling regulation in tRCC cells (P<0.05), as compared to non TFE3 fusion cells. TFE3 ChIP-seq data in RP-RO7 indicate novel DNA target involving metabolism and TGF-β signaling related genes. Conclusions: Our results indicate a distinct biological underlying tRCC oncogenesis in different TFE3 fusion models involving kinase reprogramming and nucleocytoplasmic shuttling regulation. These differences also suggest the possibility of generating personalized treatments targeting specific TFE3 fusion genes associated with the spliceosome machinery.

Background: The standard treatment for prostate cancer (PCa) is androgen deprivation therapy (ADT) that blocks AR transcriptional activity, which is responsible to the initiation and progression of PCa. However, ADT invariably leads to the castration-resistant PCa (CRPC) with restored activity of AR. CRPC can be further treated with more intensive ADTs, including CYP17 inhibitors to block intratumoral androgen synthesis and more potent AR antagonist. By analyzing the gene expression from a CRPC patient biopsy who developed resistance to CYP17 inhibitor treatment, we identified a novel nonsense mutation in the ligand binding domain (Q784*), which produces a C-terminal truncated form of AR protein containing only a fraction of ligand binding domain (LBD) and may mimic the function of some AR splice variants. We hypothesized that AR-Q784* may gain the androgen-independent activity, or may enhance the transcriptional activity of full-length AR (AR-FL) under low androgen environment through dimerization with AR-FL. Method: Luciferase reporter assays were applied to assess the transcriptional activity of AR-Q784* in absence or presence of androgens, and with or without AR-FL. Immunoblotting and immunofluorescence assays were used to examine the protein stability and cellular localization of AR-Q784*. Chromatin immunoprecipitation assays were also used to assess the chromatin binding ability of AR-Q784*. Moreover, the stable cell line that expressed endogenous AR-FL and tetracycline-inducible AR-Q784* was generated to determine the transcription activity on AR target genes and assess the effects on cell proliferation, particularly under low dose androgen condition. Result: Although it was constitutively expressed in nucleus, AR-Q784* does not elicit transcriptional activity despite the conditions of ligands. However, when it was co-expressed with AR-FL, AR-Q784* can enhance AR-mediated transcriptional activity and thus increased PCa cell proliferation particularly under low androgen conditions. Mechanistically, AR-Q784* can dimerize with AR-FL, enhance DNA binding ability of AR-FL, and strengthen AR recruitment to p300 coactivator. Conclusion: We show that CYP17 inhibitor treatment in CRPC may select for a distinct class of AR mutations/variations, including nonsense (AR-Q784*) or point-deletion mutations within LBD and certain splice variants that only lose a fraction of LBD domain. This class of AR mutants/variants is transcriptionally inactive but can enhance the activity of AR-FL due to their abilities of constitutive DNA binding and dimerization with AR-FL. However, this enhancing activity of those AR mutants/variants can be prevented by antagonist treatments such as enzalutamide. Therefore, this study provides an additional rationale for treating PCa patients with combination therapy of abiraterone and enzalutamide, which may prevent the selection for such AR mutations/variations in CRPC.

HnRNP Q1 translationally increases the expression of Aurora-A and promotes tumorigenesis in colorectal cancer. Liang-Yi Huang, National Cheng Kung Univ., Tainan, Taiwan.

Abnormally elevated Aurora-A can cause mitotic defects and aneuploidy, which might lead to genomic instability and tumorigenesis. According to the literatures, overexpressed Aurora-A may result from gene amplification, RNA transcriptional activation, or increased protein stability. Our previous study has demonstrated that Aurora-A can be translationally upregulated in colorectal cancer. By biotin pull-down assay and in vivo translational assay, we found that Aurora-A mRNA can be translationally upregulated by heterogeneous nuclear ribonucleoprotein (hnRNP) Q1, a mRNA binding protein. HnRNQ Q1 has been implicated in many posttranscriptional regulatory processes including mRNA splicing, RNA metabolism and translation. In this study, we further clarified the regulatory mechanism of hnRNP Q1 in translationally regulating Aurora-A mRNA, as well as investigated the clinical role of hnRNP Q1 in colorectal carcinogenesis. Our results indicated that hnRNP Q1 can bind to the Aurora-A mRNA 5'-untranslated region (5'-UTR). Ribosomal protein S6-IR assay further indicated that hnRNP Q1 can enhance the translational efficiency of Aurora-A mRNA. Ribosomal profiling assay further confirmed the translational regulation of Aurora-A mRNA by hnRNP Q1. Overexpression of hnRNP Q1 promotes cell proliferation and tumor growth. The expression level of hnRNP Q1 is positively correlated with Aurora-A in colorectal cancer. Taken together, our data indicate that hnRNP Q1 is a novel trans-acting factor that binds to Aurora-A mRNA 5'-UTRs and regulates its translation, which increases cell proliferation and contributes to tumorigenesis in colorectal cancer.

Characterization of the biochemical interaction of hnRNP A18 to thioridoxin transcript. Elizabeth Tsuying Chang, Palak Parekh, Eun Young Choi, Ruiping Yang, France Carrier. University of Maryland Greenebaum Cancer Center, Baltimore, MD.

Introduction: The RNA-binding protein heterogenous ribonucleoprotein A18 (hnRNP A18) is a protein translation regulator found to be elevated in many cancers. Previous work from our lab has shown that under stress conditions, hnRNP A18 is able to bind and regulate the translation of a group of mRNAs, including thioridoxin (TRX), a redox-regulating protein implicated in promot- ional metabolism. Through the formation of functional hnRNP A18-TRX complexes increases de novo protein synthesis of TRX through the stimulation of its mRNA transcript. It has been reported that like most RNA-binding proteins, hnRNP A18 is a modular protein and is uniquely composed of a single N-terminal RNA binding domain (RBD) and a C-terminal arginine-glycine rich (RGG) domain. The RBD is able to dictate specificity and affinity of substrate RNA binding, while the RGG domain acts as a functional hinge that contributes to its RNA binding capability. The observed translational regulation of TRX by hnRNP A18 merits further studies into understanding the physical interaction between the two molecules. Methods/Results: To determine the region which hnRNP A18 interacts with thioridoxin, stable cell lines overexpressing the RBD, RGG or full length (FL) domains of hnRNP A18 were generated in human melanoma cells. Ribonucleoprotein immunoprecipitation assays were performed using specific antibodies to either the RBD or RGG domain epitope. Reverse transcription PCR data reveals the presence of TRX mRNA in the resultant immunoprecipitated materials, indicating that both the RBD and RGG domain of hnRNP A18 protein is able to independently bind TRX mRNA. Maximum binding was observed when both RBD and RGG regions were present. hnRNP A18 RBD and RGG proteins were engineered to determine the exact region which hnRNP A18 binds to its target mRNAs. TRX mRNA was systematically deleted and increasing amounts of hnRNP A18-RBD protein was added to determine binding capability. Northwestern analysis reveals that hnRNP A18 binds to its consensus motif in the TRX 3'UTR and to a new 19 nucleotide motif downstream of the consesus site. Conclusion: Heterogenous ribonucleoprotein A18 is a stress-activated RNA-binding protein that functions in the translational regulation of TRX. Although the RBD and RGG domains are each independently able to physically interact with TRX, both regions are necessary for maximal binding. However, a 19 nucleotide motif located downstream of the hnRNP A18 consensus motif was also found to be sufficient for binding. The binding interface between RNA-binding proteins and their target mRNAs hold significant importance in understanding how the two molecules interact and will offer new insights into developing novel drug therapeutics that disrupt ribonucleoprotein formation.

Cancer cell ribogenesis: MYC and the integrity of the RNA polymerase I-rRNA machinery. Stefano Rossetti, Andrezj J. Wierzbicki, Niccolita Sacchi, Roswell Park Cancer Institute, Buffalo, NY.

Upregulation of RNA Polymerase (Pol I)-mediated transcription of ribosomal RNA (rRNA) and increased ribogenesis, which are necessary to sustain the increased metabolic demand of highly proliferating cancer cells, are hallmark characteristics of cancer. Increased rRNA transcription can be due to deregulation of tumor suppressors and oncogenes that affect Pol I activity. Overexpression of the MYC oncogene, a potent Pol I activator, is particularly frequent in cancer. In addition, based on our analysis of the Cancer Genome Atlas (TCGA), amplification/upregulation of genes encoding for basal components of the Pol I transcriptional machinery is also frequent in cancers of various histotype (Rossetti et al., Cell Cycle, 2016). By using breast and ovarian epithelial cell lines, we mechanistically found that: 1) ectopic expression of either MYC or RNR3 (TIF-IA), a key Pol I basal component, by increasing rRNA synthesis, is sufficient to induce in vitro transformation phenotypes and to promote cell proliferation; 2) there is a causal link between MYC overexpression and RNR3 upregulation; 3) MYC- or RNR3-induced rRNA upregulation sensitizes cells to the anti-proliferative action of drugs inhibiting RNA transcription. Our findings provide a rationale for using drugs targeting rRNA transcription to curb proliferation of cancers cells due to rRNA upregulation by MYC. Funding for this study was provided by an RP1-UCPC Ovarian Cancer Spore DRP award (NS), the NCI R01 CA127614 grant (NS), the Terri Brodeur Breast Cancer Foundation (SR), the Susan Komen Foundation (SR), and the NCI P30 CA016056 institutional grant.

SerRS is a key inhibitor of VEGFA through both transcriptional and translational controls. Ze Liu, Yi Shi, Qian Zhang, Xianglei Yang. The Scripps Research Institute, La Jolla, CA.

Vascular endothelial growth factor A (VEGFA) is the most important mediator of angiogenesis and a major target for cancerant therapy. Recent studies from our lab revealed that seryl-tRNA synthetase (SerRS), by counteracting transcription factor c-Myc in the nucleus, is the crucial transcriptional repressor of VEGFA and a potent inhibitor of angiogenesis (1,2). Here we report that SerRS controls the expression of VEGFA not only in the nucleus but also in the cytoplasm through mediating its
translational readthrough. Translational read-through of the VEGFA mRNA generates a novel isoform called VEGF-Ax that, compared with the non-read-through isoform VEGF-A165, has a 22-amino acid C-terminal extension and much weakened angiogenic activity (3,4). Interestingly, serine was identified as the amino acid inserted in the place of the UGA stop codon during the read-through (3). In unpublished work, we found that SerRS is overexpressed in metastatic tumors. Total RNA has been extracted from endometrial non-tumoral (E6/E7 and EM2) and tumoral ( Ishikawa, RL95-2, AN3CA and KLE) cell lines, followed by cdNA synthesis. Transcriptional and protein expression patterns have been evaluated for OPN, p53 or PTEN isoforms, using quantitative real time PCR, immunoblot or immunofluorescence. OPN is overexpressed in tumoral and non-tumoral cell lines in relation to OPNb and OPNc. Total RNA has been extracted, followed by Δ40p53, in relation to other variants in EC cells. Conversely, Δ40p53 is the major expressed in both EM2 and E6/E7 non-tumoral cells. Otherwise, in EC and endometrial non-tumoral cells, PTEN vs-fl is overexpressed in relation to the other splice variants. Specifically in E6/E7 cells, all PTEN-variant, except vs-b and vs-Δ, are overexpressed in relation to EC cell lines. At the protein level, both p53 and PTEN isoforms are overexpressed in E6/E7 cells, when compared to EC cells. Of note, distinct isoforms for most of these variant transcripts have not been detected at the protein level. Our data demonstrate that full length OPN, p53 and PTEN are the major expressed variants in EC tumor cell lines. However, most of these transcript variants display differential expression between endometrial tumor and non-tumoral cells and also among distinct EC cell lines. These data provide early evidence that OPN expression can dynamically modulate the expression of these mRNA isoforms, allowing both transcriptional and translational controls. References: 1. Xu X, Zhong C, Nudelman E, Ferrara N. Evidence for Pro-angiogenic Functions of VEGF-A. Cell. 2016 Sep 22;167(1):275-284.

#4481 Translation initiation factor DAP5 plays an essential role in translational control of breast cancer metastasis. Columbia de la Parra, Amanda Alard, Amanda Ermulnd, Robert J. Schneider. NYU School of Medicine, New York, NY.

Metastasis is the cause of 90% of cancer-related deaths. Selective mRNA translation has been found to be crucial for breast cancer development, progression and metastasis; however, there is a poor biological and mechanistic understanding of the role of translation in these processes. Our work and others have pioneered an understanding of translational regulation in breast cancer. We have previously shown that overexpression of initiation factor eIF4G in advanced breast cancer cells selectively increases the translation of mRNAs encoding survival, cell cycle inhibition, and DNA damage and repair proteins, among other DNA damage-protective mRNAs. eIF4G consists of three family members, eIF4F1, eIF4F2 and the poorly studied eIF4F3 homolog, DAP5. we studied this subject of this study. DAP5 lacks the N-terminal domain for eIF4E and PABP binding. DAP5 is therefore suspected to promote eIF4E-independent or IRES-driven translation of mRNAs involved in cell stress and oncogenesis, but its role in metastasis hasn’t been investigated. We analyzed the NCI human tissue genome cancer atlas (TCGA) and found that higher DAP5 mRNA expression is strongly associated with estrogen receptor negative breast cancer metastasis and lower overall survival. We then engineered a highly metastatic breast cancer cell line MDA-MB-231TR to express dox-inducible shRNA to DAP5 or a non-silencing (NS) control. Tumors were developed in clinically relevant orthotopic mouse models, with and without DAP5 silencing by addition of dox to the drinking water. Remarkably, reduction of DAP5 expression by ~70% had no impact on primary tumor growth but fully eliminated metastasis to the lung. Genome-wide transcriptome and translatome analysis of DAP5 and in vivo ultraviolet-cross-linking and high-throughput sequencing (HITS-CLIP) identified specific mRNAs that interact directly with DAP5. A significant fraction of DAP5 mRNA targets are involved in cell death and survival, cell proliferation, cell mobility, DNA repair and translation initiation and do not have IRESs, suggesting a novel role for DAP5 in a non-classical translation initiation process upon cellular stress and metastasis. Our results suggest that the translation initiation factor DAP5 likely plays a critical role in breast cancer metastasis by a unique mechanism for metastasis-specific translation initiation and provides new concepts for therapeutic strategies involving translational regulation.

#4482 Osteopontin, p53 and PTEN isoforms expression patterns in endometrium carcinoma cell lines. Vanessa F. Franco 1, Nataly dos Santos Melo, 1,2 Iaci N. Soares, 1 Yau-Ting Hsu, Yau-Ting 1, Tim H. Huang, 1 Wallace M. Araujo, 1 José A. Morgado-Diaz, 1 Jerson L. Silva, 1 Etel Rodrigues Pereira Gimba 1, 2, INCA, Rio de Janeiro, Brazil; 2UFF, Rio de Janeiro, Brazil; 3UFIRJ, Rio de Janeiro, Brazil; 4University of Texas Health Science Center at San Antonio, San Antonio, Texas; 5UFF/INCA, Rio de Janeiro, Brazil.

Total RNA from the tumor is overexpressed in endometrium carcinoma (EC) and modifications at TP53 and PTEN genes correspond to major genetic alterations in these tumors. Although total OPN expression has been correlated to p53 and PTEN expression, no data is available regarding OPN splice variants and their association to p53 and PTEN isoforms expression. OPN has three splicing isoforms (OPN-SI), named OPNa, OPNb and OPNc, while p53 has at least 12 variants, such as p53 (full length), p53R, Δ40p53, Δ133p53 and Δ160p53. Moreover, PTEN splicing isoforms (PTEN-SI) are named vs-fl (full length variant), vs-3a-3d, vs-5a-5d, and vs-D6. We aimed to evaluate the expression profile of OPN, p53 and PTEN isoforms as a first approach to establish their presumptive associations and functional interactions. Using real time PCR method, Total RNA have been extracted from endometrial non-tumoral (E6/E7 and EM2) and tumoral ( Ishikawa, RL95-2, AN3CA and KLE) cell lines, followed by cdNA synthesis. Transcriptional and protein expression patterns have been evaluated for OPN, p53 or PTEN isoforms, using quantitative real time PCR, immunoblot or immunofluorescence. OPN is overexpressed in tumoral and non-tumoral cell lines in relation to OPNb and OPNc. Total RNA has been extracted, followed by Δ40p53, in relation to other variants in EC cells. Conversely, Δ40p53 is the major expressed in both EM2 and E6/E7 non-tumoral cells. Otherwise, in EC and endometrial non-tumoral cells, PTEN vs-fl is overexpressed in relation to the other splice variants. Specifically in E6/E7 cells, all PTEN-variant, except vs-b and vs-Δ, are overexpressed in relation to EC cell lines. At the protein level, both p53 and PTEN isoforms are overexpressed in E6/E7 cells, when compared to EC cells. Of note, distinct isoforms for most of these variant transcripts have not been detected at the protein level. Our data demonstrate that full length OPN, p53 and PTEN are the major expressed variants in EC tumor cell lines. However, most of these transcript variants display differential expression between endometrial tumor and non-tumoral cells and also among distinct EC cell lines. These data provide early evidence that OPN expression can dynamically modulate the expression of these mRNA isoforms, allowing both transcriptional and translational controls. References: 1. Xu X, Zhong C, Nudelman E, Ferrara N. Evidence for Pro-angiogenic Functions of VEGF-A. Cell. 2016 Sep 22;167(1):275-284.

#4483 TGF-β downregulates osteopontin isoforms and induce epithelial plasticity in PC3 prostate metastatic cancer cells. Durval Marques, 1 Rodrigo A. Peres, 2 Paula P. de Freitas, 3 Abigail I. Resende, 4 Nataly dos Santos Melo, 1 Waldemir Fernandes de Souza, 1 José A. Morgado-Diaz, 1 Ezel Rodrigues Pereira Gimba 1, 2, UFF, Niterói, Brazil; 3UFIRJ, Rio de Ostras, Brazil; 4University of California, San Francisco, San Francisco, CA; 5Frederick National Laboratory for Cancer Research, Frederick, MD.

Total osteopontin (OPN) has been described as a master regulator of epithelial-mesenchymal transition (EMT), including in prostate cancer (PCa). However, the contribution of each OPN splicing isoform (OPN-SI), named OPNa, OPNb and OPNc, in the epithelial plasticity dynamic process is currently unknown. Besides, the contribution of alternative splicing regulation in this scenario is poorly understood. Here we aimed to investigate the expression of OPN-SI and the associated splicing pattern regulation in response to metastatic prostate cancer (PCa) cells treatment with TGF-β. Further, we aimed to investigate the resulting cell phenotypic features. To achieve this, we treated PC3 cells with TGF-β 10ng/ml for 48 h as a model to induce epithelial plasticity in these cells. Total RNA was extracted and the corresponding cdNA was tested for transcriptional levels of OPN-SI, SR and HnRNP splicing regulators as well as classical (EMT) markers by using real time PCR. EMT markers and cell morpholology have also been analyzed by immunofluorescence. PC3 cell phenotype has also been evaluated by using cell migration and viability assays in response to β-lapachone cytotoxic reagent. PC3 cells treated with TGF-β displayed an intermediate EMT phenotype, as evidenced by a significant downregulation of both epithelial (E-cadherin, claudin-3 and androgen receptor (AR) and mesenchymal markers (vimentin, N-cadherin, snail and slug) These cells presented a higher cytoplasmic E-cadherin expression, as well as a lower vimentin cytoplasmic staining. A major cortical distribution of actin filaments beneath the cell cortex has been observed. Further, a significant downregulation of OPNa and mainly, OPNb and OPNc isoforms, as well as most tested splicing regulators has been shown. TGF-β treatment also promoted a significant inhibition on cell migration as well as higher sensitivity to β-lapachone treatment, as compared to control cells. Altogether, our data evidence that in our in vitro experimental model, TGF-β promoted an intermediate EMT phenotype, with a predominance of epithelial features, associated to downregulation of OPN-SI and most tested splicing regulators. We then hypothesize that TGF-β may also be an EMT-like inducer, in which metastatic PCa cells could acquire major epithelial features that facilitate cell anchorage and adhesion in distant tumor sites. OPN-SI downregulation in this process could favour epithelial-like features, as opposed to OPN-SI overexpression during tumour progression towards metastatic circulating cells typically displaying mesenchymal phenotypes.

#4484 KRAS regulates eIF4E Binding Proteins (4EBPs) via MAPK-Interacting Kinases (MNKS) in a PISK-dependent, AKT-independent manner. Jillian M. Silva, 1 Rachel K. Bagini, 2 Davide Ruggero, 3 Frank McCormick. 1 University of California, San Francisco, San Francisco, CA; 2Frederick National Laboratory for Cancer Research, Frederick, MD.
Oncogenic RAS mutations are present at high frequencies in human cancer leading to the sustained activation of the ERK1/2 mitogen-activated protein kinase (MAPK) signaling pathway. Of the three major RAS isoforms (HRAS, KRAS, and NRAS), KRAS is the most commonly mutated isoform, in which a substitution of glycine for cysteine (G12C), aspartic acid for glycine (G12D), or valine for glycine (G12V) at codon 12. However, the unique dependencies on upstream or downstream oncogenic signaling amongst the KRAS mutants remains elusive and often masked by the remaining wild-type allele, other RAS isoforms, or additional secondary genetic alterations. Moreover, mutational activation of RAS can also enhance the assembly and activity of the cap-binding complex, eukaryotic initiation factor 4F (eIF4F). It has been demonstrated that RAS signaling via ERK promotes MAPK-interacting serine/threonine kinases (MNKs) to interact with eIF4G and the phosphorylation of eIF4E at serine 209, which is a critical site for oncogenic function. Thus, we employed an isogenic mouse embryonic fibroblast (MEF) system, devoid of H-, N-, and KRAS alleles and reconstituted with a single KRAS mutant isoform, to examine the dependencies of growth factor and PI3′-lipid activities on KRAS-mediated control of translational regulation. Although oncogenic KRAS G12C, G12D, and G12V mutant cells proliferated at twice the rate of KRASb wild-type MEF cells, growth factor deprivation or inhibition of PI3K or mTORC1/2 displayed potent anti-proliferative effects on all MEF cell lines. Analysis of signaling downstream of KRAS revealed that phosphorylation of 4E-BP1, MNK, and eIF4E occurred independent of growth factor activity, which stood in contrast to the substantial inhibition of eIF4E by the eIF4E binding protein harboring ribosomal protein S6 phosphorylation. Interestingly, AKT inhibition largely had no effect on 4E-BP1 phosphorylation, whereas inhibition of PI3K or mTORC1/2 potently suppressed p70S6k, ribosomal protein S6, and 4E-BP1 phosphorylation. Moreover, growth factor deprivation resulted in a marginal decrease of global protein synthesis with negligible effects on cap-dependent translation initiation in all overgrowth factor deprivation resulting in a marginal decrease of global protein synthesis in response to different stimuli and asking how is RACK1 involved in this position, RACK1 can recruit a series of kinases and phosphatases as well as interacting with other ribosomal proteins. Importantly, it is believed that in this position, RACK1 can recruit a series of kinases and phosphatases as well as functioning to regulate the translation and the synthesis of particular cohorts of proteins. Moreover, RACK1 plays a central role in scaffolding these proteins to promote proliferation and migration in our colon cancer cell models. Collectively, these data suggest that KRAS regulates 4E-BPs, and MNKs in a manner that is dependent on PI3K-mTORC1 signaling, but independent of AKT or growth factor activities.

**#4485 Identification and characterization of novel RACK1 binding partners that may modulate the elongation phase of protein synthesis.** Beatrice Malacraci, 1 Rasmus K. Flygaard, 1 Catrinna M. Dowling, 1 John C. Coffey, 1 Lasse B. Jenner, 1 Patrick A. Kirby, 1 University of Limerick, Limerick, Ireland; 2 Aarhus University, Aarhus, Denmark.

RACK1 (Receptor for Activated C Kinase 1) is a member of the tryptophan-aspartate (WD-repeat) family of proteins. By functioning as a scaffolding protein, RACK1 is involved in regulating many different signalling pathways in cells, including cell proliferation and cell migration. Interestingly, RACK1 has been identified as a central component of the ribosomal machinery, specifically located on the 40S ribosomal subunit close to the mRNA exit channel where it makes contact with other ribosomal proteins. Importantly, it is believed that in this position, RACK1 can recruit a series of kinases and phosphatases as well as functioning to regulate the translation and the synthesis of particular cohorts of proteins. Moreover, RACK1 plays a central role in influencing the location of the translation machinery. Using 2D and 3D models of colon cancer cell lines, together with Click-IT chemistry and Mass Spectrometry analysis, we were able to identify a series of proteins, including newly synthesized ribosomal proteins, that interact with RACK1. We identified proteins involved in cell cycle control, metabolism and protein synthesis. As expected, we have found a number of proteins that are involved in cell migration and a number of proteins that are mediators of the cell adhesion process. Interestingly, some of the most highly scored interacting proteins were those involved in protein synthesis. Of those, we identified several elongation factors as being novel RACK1 binding partners. Elongation factors are a class of proteins directly involved in the elongation phase of protein synthesis. Along with this primary function, these factors have different important roles in cells, such as influencing cytoskeleton remodelling and activation of growth and proliferation pathways. Elongation factors have been found to be upregulated in many cancer cell lines. We have confirmed the interaction between RACK1 and several of these elongation factors and we have determined that many of these are dysregulated in colon cancer. We have established that the activity of these elongation factors is regulated by a combination of stress conditions and growth factor stimulations. We have determined that RACK1 plays a central role in scaffolding these proteins to promote proliferation and migration in our colon cancer cell models. Collectively, these results provide a novel and interesting role for RACK1 in the modulation of the elongation phase of protein synthesis. We are now investigating the subcellular localization of these factors in response to different stimuli and asking how is RACK1 involved in their localization downstream of IGF-I and insulin signalling. Characterizing the interaction between these proteins and RACK1, both in cells and tissue, may lead to the design of novel therapeutic approaches in colon cancer.

**#4486 Redox regulation of the biosynthesis of mitronic tsiRNA miR-6855-3p in the basal like breast cancer cells.** Smita Misra, Gautam Chaudhuri. Meharry Medical College, Nashville, TN.

Radiation and chemotherapeutic agents eradicate tumors by inducing irreparable DNA damage. However, cancer cells often develop resistance to therapy by manipulating the DNA repair machinery. Conversely, a dividing cell constantly exposed to environmental and endogenous DNA damaging agents can transform into a tumor due to incorrect repair. Therefore the expression level of DNA repair genes is critical for cancer therapy and tumorigenesis. Our preliminary studies implied a unique mechanism involving an miRNA miR-6855-3p that promotes the biosynthesis of the translational regulator protein SPRDX5 which enhances the expression of the tumor suppressor protein BRCA2 leading to the genotoxic and radiation-resistance of aggressive SLUG-high basal-like breast cancer (BLBC) cells. The miRNA miR-6855-3p thus acts as a tumor suppressor miRNA. Interestingly, the gene for the miRNA miR-6855-3p is mitronic and is nested within the intron#13 of the USP20 gene which is a deubiquitase. Thus, the transcription of miR-6855-3p gene is regulated through the USP20 gene promoter. We present evidence here that USP20 and miR-6855-3p gene transcriptions are up regulated by oxidative stress. Tert-butylhydroperoxide was used for oxidative stress. Under the oxidative stress and the inhibition of Nrf2 level and the levels of several Nrf2-regulated proteins are increased, indicating activation of Nrf2 in the stressed BLBC cells. Under the stress conditions the levels of USP20 and miR-6855-3p are increased significantly. Knockdown of Nrf2 prevented stress-induced elevation of USP20 and miR-6855-3p RNA level in the BLBC cells indicating the involvement of Nrf2 in this process. The binding of Nrf2 to the USP20 gene promoter and its function were characterized by ChIP analysis, site-directed mutagenesis and using the promoter-luciferase reporter constructs. We conclude that the biosynthesis of the miR-6855-3p in the BLBC cells is regulated by oxidative stress and involves Nrf2. Since oxidative stress is a hallmark in the etiology and progression of metastatic breast cancer, understanding the redox regulation of a miR that positively influences BRCA2 gene expression in the BLBC cells could be clinically significant. Supported in part by DOD-CDMRP IDEA Expansion Grant# BC136645 and NIH/NCI grant 1R21CA181920-01 to GC and U54RR026140 to SM.

**#4487 A critical role of PARylation in regulating the functions of OVOL2.** Rui Zhang, 1 Boan Li, 1 Yih-Cherng Liu 1. National University of Singapore, Singapore, Singapore; 2 State Key Laboratory of Cellular Stress Biology, Xiamen University, Xiamen, China.

Poly(ADP-ribosyl)ation (PARylation) is one of the post-translational modifications, by which the ADP-ribose (PAR) polymers are added to the specific protein by a PAR polymerase (PARP). In the about 50 years’ studies, this specific modification has been verified to be related to multiple important biological functions including cell death, cell differentiation, histone modification, transcription, DNA repair, DNA replication, DNA damage, genome stability, Ovo-like 2 (OVOL2), a novel zinc finger transcriptional factor, contains a Snail/Slug (SNAG) motif at the N-terminal to possess a transcriptional regulatory activity and four DNA-binding Cys2-His2 (C2H2) zinc finger domains at the C-terminal to bind with DNA. OVOL2 is demonstrated to be involved in the cranial neural tube, angiogenesis, heart and placental development. In addition, OVOL2 can also suppress cell cycle and terminate differentiation of keratinocyte by directly inhibiting of c-Myc and Notch1. However, the regulation and biological function of OVOL2 are still largely unknown. In this study, we identify the binding targets, PARP1 and NPM1, of OVOL2. In addition, we report that the function of OVOL2 is regulated by PARylation, which modifies on C2H2 zinc finger domains and plays an important role in the regulation of OVOL2 functions. PARylation of OVOL2, but not the 3K mutant, alters focal adhesion and cell cycle, resulting in the formation of polyplody in cells. Our findings not only have significant implications for the cellular functions of OVOL2, but also provide a better understanding on the multiple biological functions of the PARylation.


Epigenetic mechanisms play a key role in cancer and are a major contributor in driving the aberrantly high levels of oncogenic receptor tyrosine kinases (RTK) in cancer. Understanding mechanisms leading to altered transcription of...
oncogenes and tumor suppressors has the potential to provide new therapeutic targets. Here we show that the PH domain leucine-rich repeat protein phosphatase 1 (PHLPP1), a tumor suppressor that directly dephosphorylates and inactivates Akt, has an additional function of regulating approximately 10% of the transcriptome. Specifically, we used non-biased high-throughput RNA-seq sequencing technologies and immunoprecipitation techniques to analyze genome-wide mRNA levels and acetylation patterns of genes in cells from wild-type and PHLPP1 knockout mice. De novo motif analysis of the promoter for the genes regulated by PHLPP1 identified enrichment in the recognition motifs for a number of transcription factors, including ones involved in inflammatory signaling. Biochemical analysis revealed that PHLPP1 regulates the phosphorylation and activation of these transcription factors, such that loss of PHLPP1 leads to enhanced inflammatory signaling. Our data support a model in which PHLPP1 dephosphorylates specific transcription factors to act as the brakes to inflammatory signaling, a hallmark of cancer.

#4489 Knockdown ARID5A suppresses proliferation of LNCaP prostate cancer cell through the inhibition of global protein synthesis. Chao Sun, Viktor Chesnokov, Keitchi Itakura. Beckman Research Institute of City of Hope National Medical Center, Duarte, CA.

AT-Rich Interaction Domain-containing protein 5a (ARID5A; also known as Modulator Recognition Factor 1 (MRF1)) was first cloned in our laboratory by screening a cDNA library of Tera-2 mRNA with an oligonucleotide probe from the major immediate early gene of human cytomegalovirus. It has been reported that ARID5A forms a complex with androgen receptor (AR) in yeast two hybrid assays and is a co-activator of AR in transient transcription assays. In this study, we report that the knockdown of ARID5A expression inhibits proliferation of LNCaP cells on the contrary to the expectation from the transient transfection assay. We found that proliferation of cells stimulated by the treatment with dihydro-testosterone (DHT) was suppressed when ARID5A expression was down-regulated by siRNA technology. Androgen stimulates cell proliferation through activation of mammalian target of rapamycin complex 1 (mTORC1). However, the suppression of ARID5A does not affect androgen-activated mTOR activity. Furthermore, the expression of androgen-inducible and androgen-suppressive genes were not affected by suppression of ARID5A. We concluded that ARID5A is not a co-activator of AR. Meanwhile, flow cytometer analysis showed that DHT-stimulated cell cycle was arrested at G1 phase after the knockdown of ARID5A expression. Oil Red O staining assays showed that the inhibition of ARID5A expression led to decreased DHT-induced lipid accumulation. Our data further proved that the knockdown of ARID5A expression inhibited the protein expression of DHT-induced sterol regulatory element binding transcription factor 1 (SREBP-1). In addition, Western blot analysis revealed that the down-regulation of ARID5A decreased the expression of cell survival / proliferation and cell cycle regulation proteins, such as hypoxia inducible factor 1 alpha (HIF1α), cyclin D1 and cyclin D3. Real time PCR data revealed that the knockdown of ARID5A expression did not affect the steady level of HIF1α, cyclin D1 and cyclin D3 mRNA. These suggested that knockdown of ARID5A expression affected protein expression at translational and/or post-translational level. Our data suggested that knockdown of ARID5A expression increased the phosphorylation of eukaryotic translation initiation factor 2α (eIF2α) which inhibited translation initiation. S35 labeling assay confirmed that the knockdown of ARID5A expression suppressed the global protein synthesis. We showed that one of the four eIF2α kinases, general control non-depressible 2 (GCN2) was activated by the suppression of ARID5A expression. We are currently studying on the mechanisms by which ARID5A regulates GCN2 kinase.

#4490 Post-transcriptional regulation on PIA53 expression in malignant mesothelioma. Tian He, Karen McColl, Nneka Sakre, Gary Wildey, Afschin Dowlati. Case Western Reserve University, Cleveland, OH.

Protein Inhibitor of Activated STAT3 (PIAS3) was originally identified as an endogenous suppressor of STAT3 activity which can specifically interact with activated STAT3 and block its downstream oncogenic effects. Previous studies from our lab have shown that low PIAS3 protein expression in mesothelioma tumors was associated with poor patient survival. Furthermore, low PIAS3 expression in mesothelioma cell lines correlated with higher STAT3 activity. Over-expression of PIAS3 decreased STAT3 activity and cell growth. These findings suggest that low PIAS3 expression may play an important role in mesothelioma tumorigenesis, thus understanding the regulatory mechanism(s) of PIAS3 expression may provide new therapeutic strategies in mesothelioma treatment. Initially, we examined PIAS3 expression by western blotting and RT-qPCR among six mesothelioma cell lines (H2B, 211H, H2052, H2452, HAY, YOU) and found that while PIAS3 mRNA levels were relatively constant, PIAS3 protein expression was highly variable. This finding indicated a potential post-transcriptional regulation of PIAS3 expression in mesothelioma. To investigate this further, we first examined PIAS3 protein stability in the presence of proteasome inhibitors and cycloheximide (CHX). We observed no increase in PIAS3 protein levels during proteasome inhibition using either MG132 (10 μM) or bortezomib (1 μM) for up to 8 h in 211H, H2052 and H2452 cells, whereas TP53 protein levels were decreased. We then tested whether PIAS3 expression may provide new therapeutic strategies in mesothelioma treatment. We next explored the PIAS3 3’UTR as a potential site for translational regulation. We initially utilized a miR microarray and computational analysis, followed by a PIAS3 3’UTR luciferase reporter assay, to screen miRNAs that are highly conserved in human and can specifically target the PIAS3 3’UTR and identified miR-18a as our first candidate. Taken together, our study suggests that variable expression of PIAS3 protein in mesothelioma is regulated by translational control and not by protein stability.

#4491 Modulation of p63 expression by phosphorylation of RBM38 at S195 via miR-203. Yanhong Zhang, Wensheng Yan, Jin Zhang, Xinbin Chen. UC Davis, Davis, CA.

p63, a family protein, acts as a sequence-specific DNA-binding transcriptional factor to regulate the expression of target genes involving in various cellular processes, including growth suppression and epithelial development. RBM38, a RNA-binding protein, is a target of p53 family and is known to interact with its target mRNAs and regulate their expression via mRNA stability. Previously, we found that RBM38 negatiely regulates p63 mRNA stability through interaction with the 3’ untranslated region (3’UTR) of target mRNAs. We also found that phosphorylation of RBM38 at Ser195 alters the conformation and function of RBM38 protein. However, it is unclear whether phosphorylation of RBM38 or miR-203 alters the ability of RBM38 to regulate p63 expression. In this study, we found that in both MCF7 and HaCaT cells, wild-type RBM38 and S195A mutant, but not phospho-mimic S195D mutant, inhibit p63 mRNA stability, suggesting that Ser195 phosphorylation abrogates the ability of RBM38 to repress the expression of p63. Importantly, we found that phosphorylation of RBM38 disrupts the binding of RBM38 to the U-rich element in p63 3’-UTR. Additionally, we found that miR-203 inhibitor increases, whereas miR-203 mimic decreases p63 expression through binding to p63 3’-UTR, consistent with a previous report. Interestingly, we found that the ability of RBM38 to repress p63 expression is disrupted by miR-203 inhibitor but enhanced by miR-203 mimic. Furthermore, we found that wild-type RBM38 protein, but not phospho-mimic S195D mutant, physically interacts with Ago2, indicating that RBM38 may inhibit p63 mRNA stability by modulating Ago2-miR-203 complex to bind to the U-rich element in p63 3’-UTR. Together, we uncovered a novel mechanism by which RBM38 modulates p63 expression via miR-203.

#4492 Androgen receptor affects Gli activity by promoting transcriptionally active states of Gli upon androgen stimulation. Sarah Truong,1 Na Li,2 Mannan Nouri,1 Ralph Bytynn1. 1University of British Columbia, Vancouver, British Columbia, Canada; 2Vancouver Prostate Centre, Vancouver, British Columbia, Canada.

Introduction: While “castration” therapies benefit metastatic prostate cancer (PCa) patients, their effects are transient and progression to castration resistant disease (CRPC) remains a vexing clinical problem. Despite low levels of circulating androgens in treated patients, CRPC typically remains dependent on androgen/androgen receptor (AR) signaling for further growth. Previously we showed that Gli protein binding to AR co-activates AR transcriptional activity and supports androgen-independent growth. Here we show evidence that the interaction of AR with Gli proteins, particularly Gli3, allows for a "non-canonical" activation of Hedgehog signaling that contributes to PCa cell growth and progression to CRPC. Methods: Androgen growth-dependent (LNCaP) or independent (LNCaP-AI, LNCaP) PCa cells were transfected with a Gli-luciferase (Gli-luc) reporter and were treated with R1881, enzalutamide (Enz), or a combination. Luciferase activity was measured. R1-367 (harboring a genomic deletion of AR) served as a negative control. Knockdown of AR-FI with siRNA or overexpression of a small decay peptide from the Gli2 AR-binding domain (Gli-DP) was also performed in above cells. Western blot and real-time PCR confirmed both AR and Gli target gene/protein expressions in LNCaP and LNCaP-AI cells (+/- R1881). Sonic hedgehog (Shh) ligand-responsive 3T3-E1 cells were transiently expressed with AR-FI to confirm the involvement of canonical Shh-Gli activation pathway in AR co-activation of Gli transcription. Co-immunoprecipitation (co-IP) was performed to verify the blocking efficiency of Gli-DP in AR-Gli3 interaction in LNCaP cells and AR-overexpression 293FT. Results: Androgen (R1881) treatment strongly induced Gli transcription activity in AR+ PCa cells while Enz reversed this effect; AR-FI-specific knockdown or
exogenous Gli-DP expression suppressed androgen-induced Gli reporter expression as well as expression of endogenous Gli-responsive genes. R1881 treatment promoted Gli2/3 protein processing to transcriptionally active states (Gli3-FullLength and Gli2-Active) and enhanced the expression of Gli-target genes, Gli1/Ptch1, at protein and/or mRNA levels. Co-IP revealed that exogenous Gli-DP blocked the interaction between AR and transcriptionally active Gli3-FL in PCA cells. Overexpression of AR in ST3-E1 cells confirmed that AR interaction with Gli stabilizes the active Gli3-FL form by suppressing degradation of Gli3 in a manner independent of the canonical Shh-Gli activation pathway. Conclusions: Our findings further identify the importance of AR-Gli interaction in progression of PAs to CRPC and shows that cooperative binding of AR to Gli promoter regions and/or Gli signaling in PAs cells. Interference with the AR-Gli interaction using a decoy peptide provides a means to suppress AR activation of Hedgehog in PCA cells.

#4493 The co-chaperone UNC45A controls cancer cell proliferation through Nek7 and centrosomal separation. Yasmeen Jilani,1 Nada H. Elsa,1 Kashish Kainth,1 Sumin Lu,2 Nehal M. Elsherbiny,3 Laila A. Eissa,3 Mamdouh M. Elshishtawy,1 Hasan Korkaya,1 Abdeljabar El Andaloussi,2 Ahmed Chadli1.

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Recent findings have shown that the Heat Shock Protein 90 (Hsp90) co-chaperone UNC45A is overexpressed in ovarian and breast cancers. Previously, we have shown that UNC45A is a centrosomal protein essential for cervical tumor cell growth through activation of the checkpoint kinase 1 (Chk1). In this report, we further examine the role of UNC45A in breast tumor proliferation using a variety of biochemical and cell biology techniques and animal models. We confirmed that UNC45A is highly overexpressed in human breast-infiltrating ductal carcinomas as compared to adjacent normal tissues. Silencing UNC45A in vitro blocked the proliferation of all breast cancer subtypes and drastically reduced tumor growth of the triple negative MDA-MB-231 cell line implanted in mammary fat pads of NOD/SCID mice. However, loss of UNC45A did not affect the proliferation of normal mammary cells. Remarkably, UNC45A becomes more nuclear in human cancer tissues and cancer cell lines as compared to normal tissues and non-transformed Hs578Bst and HME mammary cell lines, respectively. This suggests an important nuclear function for UNC45A during tumorigenesis. Microarray analysis of mRNA from Hs578T cells showed that loss of UNC45A alters the expression of 121 genes, involved in cancer and cellular development and growth networks. Relevant to cell proliferation, we found that Nek7 gene was significantly repressed upon silencing UNC45A, which was validated by RTqPCR and Western blot analysis in multiple breast cancer cell lines. Nek7 is a member of the NIMA (never in mitosis, gene A) family of serine/threonine kinases. It plays a key role in centrosomal separation during mitosis. This correlates neatly with our observation that loss of UNC45A causes a centrosomal separation defect, cell proliferation arrest and death of breast cancer cell lines. ChIP experiments showed that UNC45A binds to the promoter of the Nek7 gene, suggesting direct transcriptional regulation. Interestingly, the UNC45A sequence contains four LxxLL motifs, which are thought to be signatures for co-activator binding to nuclear receptors. Furthermore, computational analysis identified two glucocorticoid response elements (GRE) consensus sequences in the Nek7 promoter, suggesting its transcriptional regulation by the glucocorticoid receptor (GR). This hypothesis was further strengthened by a significant decrease in the mRNA and protein levels of Nek7 upon silencing GR. Thus, our data suggest that UNC45A functions as a GR co-activator to control Nek7 gene transcription. Consistent with this, immunoprecipitation experiments confirmed that UNC45A and GR form endogenous complexes, and treatment of Hs578T and MCF7 cell lines with dexamethasone upregulates Nek7 mRNA and protein levels. In conclusion our data strongly support the premise that UNC45A promotes Nek7 transcription through activation of GR, and thus controls centrosomal separation and cancer cell proliferation.

#4494 Tumor suppressive role of ZFP36L1 by suppressing HIF1α and Cyclin D1 in breast and bladder cancer. Xin Yi Loh, Ling Wen Ding, H. Phillip Koehler. Cancer Science Institute, Singapore, Singapore.

AU-rich mRNAs are a class of mRNAs that contains AU-rich elements (AREs) motifs in their 3’UTR. The half-life and turnover of AU-rich mRNAs are tightly regulated by AREs-binding proteins, such as Zinc finger protein 36 C3H type-like 1 (ZFP36L1). ZFP36L1 is one of the RNA-binding proteins (RBPs), which consists of two tandemly repeated zinc finger motifs that specifically recognize AREs motifs and mediate mRNAs decay. However, our knowledge on role of ZFP36L1 in breast and bladder cancers and its downstream targets remains poorly elucidated. Here, we hypothesized that ZFP36L1 might degrade oncogenic mRNAs transcripts, thus leading to suppression of tumorigenesis. Our analysis of in silico data revealed that ZFP36L1 is significantly mutated or downregulated in a variety of cancers. The high proportion of inactivation mutation pattern as well as the Mutsig result suggest that it may act as an unappreciated tumor suppressor. Lower ZFP36L1 expression correlated with reduced survival of cancer patients. Forced expression of ZFP36L1 markedly reduced cellular proliferation, invasiveness and migration in vitro, as well as tumor growth in vivo. In addition, microarray and RNA-seq data analysis showed that several important cancer-related candidates such as IL8 and HIF1α were significantly downregulated. Overexpression of ZFP36L1 reduced HIF1α mRNA and protein expression. Similarly, HIF1α forced expression also inhibited ZFP36L1 mRNA to protein processing, leading to a further arrest of ARE-mediated mRNA decay. Dual luciferase reporter assays and RNA Electro-mobility Shift Assay (REMSA) showed that only type specific ZFP36L1, but not different mutants of ZFP36L1, was able to bind directly and to degrade a HIF1α 3’UTR construct that contains AU-rich regions. Meanwhile, mRNA pull down assay demonstrated that ZFP36L1 protein recognized cell-cycle-related transcripts. Overexpression of ZFP36L1 led to reduction of Cyclin D1 and phospho-Rb in protein expression. Furthermore, SILAC data unveiled interesting binding partners of ZFP36L1. Collectively, our findings propose that ZFP36L1 might have a critical tumor suppressive role in breast and bladder cancer by negatively regulating mRNA whose protein products cooperatively promote hypoxia and transition into S phase of cell cycle. Augmenting ZFP36L1 function might disable the cell cycle and proliferation of aggressive bladder tumors.

#4495 Elucidating BCL11A’s function in triple negative breast cancer. Nicholas O. Stevers, Samantha L. Tinsley, Rebecca Moody, Chang-Ching Lin, Jennifer Meagher, Jeanne A. Stuckey, Miao-Chia Lo, Duxin Sun. 1Univ. of Michigan, Ann Arbor, MI.

It has been recently shown that the B-cell CLL/lymphoma 11A (BCL11A) transcription factor is highly expressed in triple-negative breast cancer (TNBC) and has critical functions in promoting tumor growth and mammary stem cell maintenance. Studies in our group have found that the first twelve amino acids (aa 1-12) of BCL11A are a conserved sequence that allows binding to retinoblastoma binding protein 4 (RBBP4). RBBP4, a histone binding protein, is involved in many epigenetic complexes, including the Polycomb repressive complex 2 (PRC2), the nucleosome remodeling and deacetylase (NuRD) complex, and SIN3A complex. Through these interactions BCL11A may exert transcriptional and epigenetic regulation. Herein we investigate the importance of BCL11A’s interaction with RBBP4 and its function in TNBC. BCL11A-complex interactions were observed through pull-down experiments of TNBC cell lysate utilizing aa 2-16 of BCL11A and a scramble control sequence. Our results demonstrate that this 16-amino acid sequence is sufficient to bind to RBBP4 and pull down the PRC2, NuRD, and SIN3A complexes. Furthermore, the NuRD and SIN3A complexes pulled down by BCL11A aa 2-16 retained their functionality as observed through pull-down histone deacetylase (HDAC) activity assays. These results indicate significantly higher HDAC activity in the wildtype sequence as compared to the scrambled sequence. To further investigate this interaction, protein crystallization was conducted, the results of which validated the interactions. The N-terminal truncated BCL11A protein (del 2-12) and a scrambled control were transfected into the TNBC cell line SUM149. Analysis via flow cytometry shows a reduction of the ALDH+ cell population in the BCL11A peptide transfected cells versus scramble control. We are currently investigating the necessity of this 12-amino acid sequence in interactions with RBBP4, PRC2, NuRD and SIN3A via co-immunoprecipitation experiments using an N-terminal truncated BCL11A protein (del 2-12). The effects of full-length BCL11A and del 2-12 on the ALDH+ cell population are also being examined. These experiments will further delineate this sequence’s importance in BCL11A-RBBP4 interactions.

#4496 Dissecting cellular heterogeneity using single cell RNA sequencing in human lung tumors. Elise T. Courtot1, Huipeng Li2, Yuliyan Tan, Daniel Tan3, Shyam Prabhakar2, Paul Robins1. 1Jackson Laboratory, Farmington, CT; 2Genome Institute of Singapore, A-STAR, Singapore; 3National Cancer Center of Singapore, Singapore.

Intra-tumor heterogeneity is a key determinant of tumor biology, treatment response and patient survival. It is therefore essential to comprehensively characterize the phenotypes and interactions of the diverse cells within the tumor ecosystem. However, traditional molecular profiling studies have largely relied on bulk-tissue analysis, which obscures the signatures of distinct cell populations. We present here an unbiased analysis of functional heterogeneity in NSCLC (Non Small Cell Lung Cancer) cancers (Lung Adenocarcinoma and Squamous Cell Carcinoma) and their microenvironment using RNA-seq pro-

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filing of unsorted single cells from 20 primary tumors and matched normal lung tissue (NL). Using a new clustering algorithm RCA (Reference Component Analysis), we identified the major cell types composing the normal lung tissue as well as the tumor ecosystem such as epithelial, fibroblast, endothelial and immune cells. Notably, we were able to identify resident alveolar macrophages, in tumor and normal tissue and their single-cell differential expression analysis revealed substantial differences in their gene expression. Similarly, we identified blood-vessel endothelial cells in matched normal and tumor tissue, and identified the differentially expressed genes in both cell types. Our unbiased approach allowed us to study the diversity of the epithelial malignant cells present in different tumors, highlighting the tumor and patient specific variability. Together with the NSCLC data the single cell level by sc-RNAseq offers a powerful tool to provide insights into the transcriptomic variability within the key cellular players of the tumorigenesis.


We have developed a streamlined workflow for multiplexed co-detection of proteins and mRNAs in single reactions at single-cell resolution. This workflow couples OER for protein detection with reverse transcription (RT) for mRNA detection. In this study, we examined 31 overlapping targets, from a panel of 84 protein and 40 mRNA assays, across three different cell lines (A549, SKBR3 and K562). Unsupervised gene expression profiling analysis, including component analysis and hierarchical clustering revealed different aspects of the protein-mRNA relationship. The top 10 genes contributing to cell differentiation were identified for both proteins and mRNAs, and eight of them overlap. This result implies an overall concordance of certain protein and mRNA expression in different cell lines. Our results also demonstrate that cells can be classified into subpopulations based on protein-mRNA expression profiles, and different subpopulations have distinct correlation coefficient values. The correlation between CCNB1 protein and mRNA was low (R = 0.2884) in the whole population. However, there was a subpopulation in which CCNB1 protein correlated strongly with its mRNA. When this subpopulation was analyzed, the R value was 0.8350, while the rest of the cells had an R value of 0.0233. More interestingly, in the NSCLC data at the single cell level by sc-RNAseq, we hypothesize that the abnormal activation of the IRE1α-XBP1 axis may influence KRAS NSCLC progression and aggressiveness and serve as a potential novel avenue for the treatment of NSCLC. After probing the impact of the underlying mechanism on tumor growth we will finally evaluate the translational utility of targeting the ER stress pathway with a small molecule inhibitor against IRE1α.

#4499 The oncometabolite fumarate prevents hypoxia-induced ER stress by enhancing the pentose phosphate pathway. Luana Schito, Sergio Rey, Bradly G. Wouters, Marianne Koritzinsky, Princess Margaret Cancer Centre, Toronto, Ontario, Canada.

Cancer cells co-opt mechanisms of adaptation to hypoxia to minimize energy expenditure and halt proliferation thereby increasing hypoxic tolerance, a major factor contributing to chemo- and radioresistance in patients. The endoplasmic reticulum (ER) kinase PERK is one of the three signaling transducers of the UPR. PERK activity is essential for both correct folding and processing of proteins and quality control in the ER. We have previously shown that accumulation of fumarate, resulting from loss-of-function (LOF) of the mitochondrial enzyme fumarate hydratase (FH), augments the antioxidant capacity of hypoxic cancer cells through upregulation of the pentose phosphate pathway (PPP). Here, we report for the first time that the fumarate-dependent increase of antioxidants generated by the PPP prevents hypoxic activation of the PERK-eIF2α axis enabling protein synthesis and proliferation leading to an impairment of hypoxic tolerance. HeLa ( cervix), HCT-116 and LS174T (colon) adenocarcinoma cells were transfected with lentiviral vectors encoding for shRNAs targeting FH. Immunoblot assays showed that FH LOF impaired PERK activity under hypoxia thus inactivating eIF2α. Moreover, the ability of fumarate to prevent PERK activation was specific to hypoxia since fumarate did not affect the pharmacological activation of the UPR by thapsigargin, DTT or tunicamycin. A comprehensive analysis utilizing a RT-qPCR array to profile the mRNA expression of 84 UPR genes, showed that FH LOF inhibited the UPR in HCT-116 cells. A similar response was observed in patient-derived UOK262 kidney cancer cells whereby bi-allelic FH mutations resulted in downregulation of 72% of the UPR genes whereas FH re-introduction restored UPR signaling. We employed LS174T mucinous adenocarcinoma cells as a model to assess protein synthesis. Mucin synthesis was not affected in hypoxic FH deficient cells whereas it was abrogated in wild-type cells. Consistent with these results, FH LOF increased FH activity and enhanced hexose monophosphate (HMP) cycle and pentose phosphate (PPP) activity. In conclusion, we found that fumarate accumulation (either through endogenous or FH LOF) acts as an inhibitor of the PERK-eIF2α axis in FH silenced cells thereby suggesting that FH activation by fumarate is implicated in PERK inhibition. RNAseq data analysis in melanoma, prostate, colon, breast, lung and renal cancers from TCGA cohorts shows a negative correlation between PERK and the PPP enzyme PGLS and that the mechanism whereby FH deficient occurs in cancer patients is surprising. In conclusion, we show that fumarate impairs PERK–eIF2α UPR signaling in hypoxic cancer cells. Clinical and molecular data show that UPR activation is one of the mechanisms responsible for chemo- and radioresistance in hypoxic tumors. This study suggests that fumarate accumulation (either through extrinsic or FH LOF) represents a novel approach to target hypoxic cancer cells and improve patient prognosis.

#4500 Inhibition of PERK by small molecule inhibitors enhances the response to ionizing radiation in vitro and animal tumor models. Ioannis Verginis, Joel Encarnación, Souvik Dey, Constantinos Koumenis. Univ. of Pennsylvania School of Medicine, Philadelphia, PA.

Endoplasmic reticulum (ER) is a well organized membranous network, responsible for synthesis and folding of secretory and membrane proteins, lipid and sterol biosynthesis and intracellular calcium storage. Perturbations in ER lumen caused by depletion of Ca²⁺ levels, hypoxia, nutrient deprivation, affect the ER homeostasis, a condition known as an ER stress. In order to cope with this stress, cells have developed an orchestrated biochemical response, termed Unfolded Protein Response (UPR). Hypoxia, an ER stress inducer, is an important and unique characteristic of the solid tumor microenvironment, promotes resistance to radiotherapy and contributes to poor patient prognosis. Cancer cells overcome these stress conditions through activation the PERK–eIF2α arm of the UPR, leading to inhibition of global protein synthesis and cell survival. We hypothesize that the use of small molecule PERK inhibitors will decrease the
survival of hypoxic cells and eventually sensitize them to irradiation. In this study we used highly radiosensitive melanoma and head and neck squamous cell carcinoma cell lines B16F10-ova and SQ20B, under normoxia and hypoxia, respectively. We used two specific PERK inhibitors (PERKi) and tested for their efficacy both in vitro and in vivo. Induction of ER stress in both cell lines, either by treatment of SQ20B cells with PERKi or by exposure to hypoxic conditions (0.2%), was evaluated by activation of PERK and its downstream targets. Pre-treatment of stressed B16F10-ova and SQ20B cells for 2h with PERKi (1μM), caused complete inhibition of PERK-elicited ATF4-CHOP arm of the UPR. Moreover, both cell lines pre-treated with PERKi showed a significant decrease in survival fraction under ER stress-induced by TG (0.5μM) as well as under hypoxia (0.2%) along with increasing doses of irradiation (IR). For the in vivo experiments, nude and C57BL/6 mice were treated with either 100mg/kg of PERKi by oral gavage, twice a day for 3weeks, or IR or a combination of PERKi and IR. Mice treated with PERKi showed a small but not significant delay of tumor growth in both backgrounds of mice. However, combined treatment with IR (12 and 15Gy) caused a significant reduction of tumor volume as well as a delayed tumor growth which was significantly more pronounced in the immunocompetent mice. Data from flow cy-ometry analysis of one of the the PERK+ IR treated tumors from C57BL/6 mice showed that there is a significant infiltration of CD3+ cells as well as significant higher number of CD3+CD8+ cells compared to the untreated mice or mice treated with either PERKi or IR alone. Collectively, our results show that PERKi effectively reduces the survival of hypoxic SQ20B cells and sensitize them to IR in vitro and in tumors. Further experiments will clarify whether PERKi have a dual role on reducing the hypoxic fraction and direct recruitment of the cytotoxic T-cells.

#4501 CEBPD is an early endoplasmic reticulum stress response gene implicated in breast cancer cell survival. Namratha Sheshadri, Shikha Shanaran, Esta Sterneck. National Cancer Institute, Frederick, MD. CEBPD (C/EBP delta) is a member of the CCAAT-e enhancer binding protein (C/EBP) family of transcription factors characterized by a β-Zip domain that mediates dimerization and DNA binding. CEBPD is induced in response to acute stressors such as cytokine stimulation, bacterial lipo polysaccharide (LPS), corticosteroids, radiation and hypoxia. We have previously reported that CEBPD has dual functions in breast cancer by both attenuating or enhancing oncogenic pathways depending on context (Balumurugan and Sterneck, 2013, Mendoza-Villanueva et al., 2016). Recent studies reveal that elevated Endoplasmic Reticulum (ER) stress is associated with the pathology of several diseases including cancer. Limiting supply of nutrients and oxygen in growing tumor cells disrupts the protein folding homeostasis resulting in activation of the unfolded protein response (UPR). The UPR includes pathways that support adaptation to stress, and that are also implicated in promoting malignant features and therapy resistance in breast cancer. Hence, we investigated a possible role for CEBPD in this stress response pathway. Using breast cancer cell lines as model systems, we found that CEBPD is dynamically regulated in response to chemical and physiologic inducers of ER stress. Kinetic analyses indicate that CEBPD induction is concurrent with the activation of the UPR effectors XBP1s and ATF4 suggesting a potential role in stress adaptation. Examination of the global transcriptional profile of the Thapsigargin-induced stress response in MDA MB-231 cells indicated that CEBPD activates genes involved in cytokine and chemokine production/signaling, immune cell recruitment as well as angiogenesis pathways. While XBP1s has been previously ascribed to activating these pathways, the levels of activated XBP1s were not affected by CEBPD silencing. This is the first report of a similar role for CEBPD in the ER stress response and warrants the investigation of possible co-operation of CEBPD and XBP1 in the UPR pathway. Further, we showed that knock down of CEBPD in cancer cells increased PARP cleavage and susceptibility to ER stress induced cell death. CEBPD deficient cells also displayed reduced LC3II accumulation, suggesting impaired autophagy induction in response to ER stress. Taken together, our data suggests that CEBPD is a novel proximal effector of the ER stress response in breast cancer cells. Activation of this transcription factor regulates tumor promoting inflammatory and angiogenic signaling pathways. Additionally, CEBPD expression confers a survival advantage to cancer cells encountering ER stress. Therefore, we propose that targeting CEBPD may sensitize cancer cells to ER stress inducing therapeutics.

#4502 Heat shock protein 47 maintains breast cancer cell survival through its inhibitory effect on ER stress sensor IRE1alpha activity. Akihiko Yoneda,1 Norio Takei,2 Kaoi Sawada,1 Marina Kosaka,1 Kenjiro Minomi,2 Yasuaki Tamura3. 1Hokkaido University, Sapporo, Japan; 2Nitto Denko Corporation, Sapporo, Japan. Background and aims: Heat shock protein 47 (HSP47) is a collagen-specific chaperone and is expressed in fibroblasts and stromal cells that constitutively produce and secrete several types of collagens. In a tumor microenvironment, HSP47 is also highly expressed in cancer-associated fibroblasts, involving in the tumorigenesis through construction of the extracellular matrix. Intriguingly, HSP47 is highly expressed in cancer cells in which almost no collagen is synthesized and its expression has been reported to be associated with malignant grade of gliomas and poor prognosis of breast cancer. However, the functional significance of HSP47 in cancer cells remains unclear. Thus, the purpose of the present study was to clarify the molecular mechanism by which HSP47 maintains cancer cell survival and to explore the possibility of HSP47 becoming a therapeutic target. In this study we used high radioresistant melanoma and head and neck cancer cells, either melanoma cell line B16F10-ova and SQ20B cells or human breast cancer cell lines MDA MB-231 cells and TIG-3 cells. Materials and methods: To identify the mechanism that links UPR-triggered cell death and apoptosis of breast cancer cells by silencing of HSP47. Immunoprecipitation of HSP47 and IRE1alpha showed that HSP47 forms a complex with IRE1alpha in colorectal cancer cells under a basal condition. Persistent activation of IRE1alpha by silencing of HSP47 triggered an increase in reactive oxygen species in colorectal cancer cells followed by activation of the other ER stress sensors PKR-like ER kinase and activating transcription factor alpha, resulting in activation of the subsequent apoptosis-associated signaling pathway. Finally, treatment of colorectal cancer cell-bearing mice with HSP47 siRNA resulted in complete regression of colorectal tumors. Conclusion: These results suggest that HSP47 sustains cancer cell survival through its inhibitory effect on IRE1alpha activity and that targeted disruption of HSP47 might become a therapeutic modality for tumors.
Androgen receptor aggregation is a key regulator of endoplasmic reticulum stress and cell survival in ETS-related gene driven prostate carcinogenesis. Taduru L. Sreenath, Natallia Mikhalkevich, Shashwat Sharad, Rishita Gupta, Gluotosin Dioano, Kevin Babcock, Charles Xavier, Ahmed Mohamed, Muhammad Jamal, Shyh-Han Tan, Albert Dobi, Gyorgy Petrovics, Isabel A. Sesterhenn, David G. McLeod, Inger L. Rosner, Shiv Srivasstava, USUHS, Rockville, MD; Joint Pathology Center, Silver Spring, MD; Urology Services, WRNMMC, Bethesda, MD.

Introduction and Objectives: Dereguated androgen receptor (AR) signaling due to either mutations or altered expression of the AR and its cofactors (activators or suppressors) has been identified as critical in prostate cancer development and progression. Recent evidence suggests that the regulated oncogenic activation of ETS-related gene (ERG) represents one of the most common and validated prostate cancer driver gene. In our recent studies using prostate specific ERG transgenic mouse prostate glands, we observed novel morphological phenotypes of endoplasmic reticulum (ER) stress. Since AR was the critical regulator of ERG expression through TMPRSS2 promoter in human prostate cancer, the present study was aimed towards understanding the post-translational interactions between ERG and AR in ER stress and subsequent cell survival mechanisms in mouse and cell culture models. Understanding such mechanistic insights will potentially have major therapeutic implications. Methods: Histological phenotype in the mouse prostate glands were examined by light and electron microscopy. Cell culture models of LNCaP, HEK293 and COS-7 cells were utilized to examine the AR aggregations, Cofactors, Progesterone Aggregation Assay in the presence or absence of 17β-OH progesterone. Various domain deletions of AR were utilized to identify specific AR domain interactions with ERG and its contribution in AR aggregation. Luminal cell surface markers on the isolated mouse prostate glands and spontaneously immortalized mouse prostate epithelial cells from ERG transgenic mouse (MoE1) were analyzed by FACS analysis. Results: Co-expression of AR and ERG in LNCaP and COS-7 cells showed significant aggregation of AR in filter assays. Co-IP experiments and PLA assays in VCaP, LNCaP and HEK 293 cell revealed that ERG physically interacts with AR. Epithelial cells of ERG-Tg mouse prostates showed ~70% increase in CD49f (low) and Sca-1 (med) population with increased sphere formation capability and resistance to radiation induced cell death. Both epithelial cells grown in spheres and established MoE1 cells displayed increased CD49f (low) and significant increase in the EpCam negative population. Conclusions: Overall, our experiments demonstrate the mechanistic link that the physical interactions between ERG and AR initiate the ER stress in prostate epithelium through AR misfolding/aggregation. Our observation of ERG induced AR aggregation is one of the initial events that lead to ER stress to cell survival indicate a critical function for ERG in the etiology of prostate cancer initiation and progression. Funding: This research in part was supported by the National Cancer Institute R01CA162383 (S.S.) and HU0001-10-2-0002 funds.

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Tumor Hypoxia and the Unfolded Protein Response

#4504 Activating transcription factor-6 dependent regulation of RbhmTOR and Notch signaling contributes to chemoresistance in osteosarcoma. Suna Yararepudiy, Pooja Hingorani, Saravana Kumar, Omar Asad, Jazmine Abril, Veena Sharath, Paul Dickman, Aparna C. Sertli, University of Arizona College of Medicine-Phoenix, Phoenix, AZ; Phoenix Children's Hospital, Phoenix, AZ.

The standard treatment for patients with newly diagnosed osteosarcoma (OS) consists of surgery in combination with multi-agent chemotherapy. However, the optimal treatment strategy for patients with refractory and/or metastatic disease is yet to be defined and continues to be a persistent challenge in osteosarcoma treatment. The high degree of genetic aberrations and tumor heterogeneity has impeded the identification and testing of new and effective therapeutic targets. Therefore, finding and characterizing cellular mechanisms, which contribute to OS chemoresistance could be one promising strategy for designing therapies that can change the outlook for patients with this disease. Due to the well-known role of unfolded protein response (UPR) in promoting chemoresistance in solid tumors our objective here is to examine how this promotes chemoresistance in patients with OS. Using in silico pathway analysis of gene expression data from patients with OS, we found that the UPR sensors ATF6a and ERK-1/e2 were also activated in OS cells in vitro. Using western blotting, qPCR and immunofluorescence we confirmed that all three UPR pathways were activated in human OS cell lines. However we found that only ATF6a activation significantly enhanced chemoresistance to cisplatin, irinotecan and combinatorial treatment with cisplatin and the mTOR inhibitor rapamycin. This occurred via inhibition of Bax activation, suppression of Rbhm-TOR and NOTCH signaling. Our findings highlight a novel mechanism of chemoresistance in OS. Furthermore, retrospective analysis of banked OS patient samples for ATF6a expression followed by a preliminary multivariate analysis using Cox regression model showed that high levels of nuclear ATF6a, was an independent prognostic indicator while also taking into account the presence of metastases and tumor site. Moreover, we found that while 50% patients with high nuclear ATF6a levels had a poor histologic response to treatment only 32% patients with low nuclear ATF6a expression that ATF6a be associated with resistance to chemotherapy and poor outcome for OS patients. Our findings emphasize a role for ATF6a as the nexus of a versatile signaling network that regulates pathways that are crucial for the pathogenesis and therapy resistance of osteosarcoma. Hence therapeutic targeting of the ATF6a pathway holds promise as an innovative and effective treatment strategy for OS.

#4505 UPR biomarker expression correlates with inhibition of migration of TNBC cells treated with low doses of chemotherapy. Amnon Raifer, Julia Lipovetsky, Britta Hardy, Rina Yerushalmi, Tel Aviv University Medical School, Petach Tikva, Israel; Rubin Medical Center and Tel Aviv University Medical School, Petach Tikva, Israel.

Much attention has been directed to the study of triple-negative breast cancer (TNBC) because of its short disease-free interval from diagnosis and more aggressive course in the metastatic setting. TNBC also lacks specific target receptors for treatment. Third-generation metronomic chemotherapy using available agents that target DNA repair complexes (i.e., taxanes), and cell proliferation (i.e., anthraclycine) have shown promise results. However, specific regimens for TNBC at the different disease stages have not been clearly defined. Therefore, researchers are seeking molecular biomarkers to predict response to current chemotherapeutic agents. In a previous study, we demonstrated that low doses of antracyclines and taxanes, which are known to increase endoplasmic reticulum stress, increased tumor cell expression of glucose-regulated protein 78 (GRP78), a key regulator of the unfolded protein response (UPR), resulting in tumor cell apoptosis. GRP78 gene expression is upregulated via CREB3L1, an endoplasmic reticulum transmembrane transcription factor and member of the CREB/ATF family of transcription factors. CREB3L1 is a metastasis suppressor and functions as a transducer of UPR. The aim of the present study was to investigate the effect of low doses of doxorubicin and paclitaxel on UPR activation in metastatic TNBC cells by determining CREB3L1 protein expression in correlation to cell-surface GRP78 expression. Furthermore, we related CREB3L1 and cell-surface GRP78 expression with the migration potential of TNBC cells in response to treatment. We found that the metronomic doses of doxorubicin significantly induced CREB3L1 and cell surface GRP78 expression in TNBC cells. CREB3L1 increased by 2.5-fold in MDAMB231 and by 3-fold in MDAMB468 (p<0.04); GRP78 increased by 3.7-fold and 6.1-fold, respectively (p<0.01). Similar results were obtained with low doses of paclitaxel. A strong correlation was observed between CREB3L1 and cell-surface GRP78 protein expression. The increased expression of both proteins in metastatic (highly metastatic) and MDAMB468 (moderate metastatic) was associated with significant inhibition of the migration capacity of the treated TNBC cells. In contrast, the migration capacity of treated estrogen-positive MCF7 cells (non-metastatic) and Her2 positive BT474 cells (highly metastatic) was poorly inhibited, coinciding with a non-significant increase in CREB3L1 and GRP78 expression. The results were further substantiated through an orthotopic model consisting of TNBC and unrelated to the metastatic profile of the cells. This study is the first stage in the identification of UPR-related biomarkers of beneficial outcome of metronomic chemotherapy for TNBC. Future mechanistic studies should focus on the possible use of cell surface GRP78 and CREB3L1 as targets for combination therapies.


Klotho is a transmembrane protein, which can be shed and act as a circulating hormone. Klotho-deficient mice manifest a syndrome resembling accelerated aging, while klotho overexpression extends life span. We previously identified klotho as a breast and pancreatic tumor suppressor and as an inhibitor of the IGF-1 pathway. Recent data indicate klotho as a tumor suppressor in an array of malignancies, including colorectal cancer (CRC). We aimed to decipher the role of klotho as a tumor suppressor in CRC. Analysis of a public database (Oncomine) indicated reduced expression of klotho in CRC. Two models were employed to study the effect of klotho overexpression. Using the azoxymethane inflammation model, we discovered that klotho inhibited colon polyp formation. Using an orthotopic model consisting of direct injection of MC38 cells to the mice colons, we found that klotho inhibited colonic obstruction and tumor formation. Similarly, klotho inhibited colony formation and proliferation of CRC cell lines. Aberrant activation of the canonical Wnt
pathway is implicated in CRC development and progression. β-catenin, the key effector of this pathway, functions with T-cell factor/lymphoid-enhancer-factor (TCF/LEF) to activate expression of Wnt target genes. While we did not see an effect of klotho on the IGF-1 pathway, it reduced Wnt3α and β-catenin levels as measured by Western blotting, and inhibited TFC/LEF transcriptional activation in luciferase assay. As transcriptional inhibition was abrogated by a constitutively active β-catenin, we suspected that klotho inhibited the pathway upstream of β-catenin. Indeed, co-immunoprecipitation analyses indicated direct interaction between klotho and Wnt3α. Yet, the inhibitory effect of klotho on CRC cell colony formation was only partially rescued by a constitutively active β-catenin. These data indicate on additional mechanisms involved in the T5 activity of klotho. Thus, we conducted an analysis as expression array and observed involvement of klotho in endoplasmic reticulum (ER) stress and unfolded protein response (UPR). Further studies showed that klotho induced elevation in XBP1 RNA levels, GRP78 protein levels, and eIF2α phosphorylation, all indicative of UPR regulation. Importantly, pharmacologic inhibition of ER stress and UPR overcame the growth suppression effect of klotho in CRC cells. Our data indicate klotho as a potent tumor suppressor in CRC, and suggest its role at very early stages of tumor formation, already at polyp formation. Our data also indicate, for the first time, klotho as a regulator of ER stress and UPR in cancer. These findings may pave the way for the development of novel therapeutic strategies based on enhancement of UPR.

#4508 Penfluridol-induced endoplasmic reticulum stress leads to autophagy-mediated pancreatic tumor growth suppression. Alok Ranjan, Sharavan Ramachandran, Nehal Gupta, Sanjay Srivastava. Texas Tech University Health Science Center, Amarillo, TX

Pancreatic cancer is the fourth leading cause of cancer-related deaths in the United States. Experimental and clinical evidences suggested that high basal state autophagy in pancreatic tumors could induce resistance to chemotherapies. Recently, we have demonstrated that penfluridol suppresses pancreatic tumor growth by autophagy-mediated apoptosis both in vitro and in vivo. (Ranjan and Srivastava, Scientific Reports: 2016;26:2615; PMID: 27198859), however, the mechanism of autophagy induction by penfluridol was not clear. Several studies have established that endoplasmic reticulum (ER) stress could lead to autophagy and inhibit tumor progression. In the current study, we demonstrated that penfluridol induced ER stress in BxPC-3, AsPC-1 and Panc-1, pancreatic cancer cell lines as indicated by up regulation of ER stress markers such as BIP, CHOP and IRE1α after treatment with penfluridol in a concentration-dependent manner. Inhibiting ER stress by pre-treatment with pharmacological inhibitors such as sodium phenylbutyrate and methrycinam or by silencing CHOP using CHOPsiRNA, blocked penfluridol-induced autophagy. These results clearly indicated that penfluridol induced ER stress lead to autophagy in our model. Western blot analysis of subcutaneously implanted AsPC-1 and BxPC-3 tumors as well as orthotopically implanted Panc-1 tumors demonstrated upregulation of BIP, CHOP and IRE1α expression in the tumors lysates from penfluridol treated mice as compared to tumors from control mice. Altogether, our study established that penfluridol induced ER stress mediated autophagy in pancreatic tumor. Our study opened a new therapeutic target for advanced chemotherapies against pancreatic cancer. (Supported in part by RO1 grant CA129038; awarded by National Cancer Institute, NIH). NOJC318

#4509 GRP78 is neither expressed on ER/PR/Her2 human breast cancer cell surface nor secreted in the culture media. Jesus E. Serrano, Vaishali Vijay Kulkarni, Naveen Seetharama, Enze Lim, Mark Johnson, Prasad Gopinath, Parveen K. Dua, and Raja K. Krishnamurthy. Texas Tech University Health Science Center, Amarillo, TX

GRP78 (M.78 kDa) is a glucose regulated protein. It is a member of the heat shock protein (HSP) family but located in the lumen of the endoplasmic reticulum (ER). Its function as ER chaperone translocating protein across the ER membrane that needs to be glycosylated at the asparagine residue present in the sequon Asn-X-Ser/Thr (N-linked glycosylation) is well recognized. This important biochemical event is essential for glycoprotein folding and function. For example, if the N-linked glycosylation is interrupted with endo H, the α1,6-protein N-glycosylation inhibitor, angiogenesis, a hallmark for tumor progression and metastasis is inhibited due to an induction of ER stress. Under such condition, GRP78 is overexpressed in microvascular and in tumor tissue, concluding that GRP78 is a master regulator in ER stress induced unfolded protein response (upr)-mediated apoptosis in tumor microvasculature (J. Biol. Chem. 289, 29127-29138, 2011; Pure Appl. Chem. 84, 1907-1918, 2012). This contra-dicts the current dogma that supports GRP78 expression on the tumor cell surface interfering with the therapeutic(s) and making them as tumor promoters instead. To evaluate the GRP78 localization, we have used ER/PR/Her2 (i.e., triple negative) human breast cancer cell line MDA-MB-231 (Caucasian) cultured normally or without serum as well as before or after inducing the ER stress. After establishing the expression of GRP78 mRNA by qPCR and protein by western blotting, we focused on its cell surface expression. Unfixed cells were stained with Concanavalin A (Con A; Specificity = α-D-Mannose, α-D-Glucose, branched mannose), wheat germ agglutinin (WGA; Specificity = (GlcNAc-β-(1,4)GlcNAc)4-7 β-GlcNAc NeuAc) as well as with anti-GRP78 antibody followed by their detection by immunofluorescence microscopy using either Rhodamin or Alexa-conjugated secondary antibody. The fluorescence images were captured in a Zeiss microscope with AxioCam camera. Con A and WGA staining provided images of N-glycans on the cell surface and supported the intactness of the cell membrane. On the other hand, GRP78 fluorescence was absent from the surface of these cancer cells. Similar results were also obtained irrespective of cells cultured in the absence of serum and/or in the presence of 1μg/mL of tunicamycin. GRP78 fluorescence however was detected in cells after either fixing them with ice-cold methanol or after permeabilization with digitonin. In addition, western blotting also failed to detect GRP78 in the culture media of MDA-MB-231 cells. We, therefore, conclude that GRP78 is neither expressed on the outer-leafllet of the cell surface of ER/PR/Her2 human breast cancer cells MDA-MB-231 nor secreted in the culture media. Supported in part by Susan G. Komen for the Cure BCCTR0600582 and NIH/NIMHD G12MD007583 (KB).

#4510 ADP-dependent glucokinase controls hypoxic gradients, ex vivo avascular and in vivo tumor growth through modulation of HIF-1α/mTOR signaling. Sergio Rey, Luana Schito, Marianne Koritzinsky, Bradley G. Wouters. Princess Margaret Cancer Centre, Toronto, Ontario, Canada.

ADP-dependent glucokinase (ADPGK) is an evolutionarily conserved archaebacterial glycolytic enzyme frequently upregulated in human cancers whose role remains elusive. We have recently discovered that ADPGK contributes to ROS-dependent stabilization of hypoxia-inducible factor (HIF)-1α (and -2α) and hypoxia target gene transcription in cancer cells. Hereby, we deconvolute the effect of ADPGK loss-of-function (LoF) upon the 3D hypoxic tumor microenvironment, a crucial pathological aspect determining therapeutic responses in cancer patients. ADPGK HIF-1α and HIF-2α LoF was attained through shRNA-mediated knockdown (>75%) in HCT-116 colon cancer cells. HIF-1α (and -2α) transcriptional activity was measured through RT-qPCR arrays whereas protein levels were assessed by immunoblot. O2 and glucose consumption were measured in 2D with a real-time bioanalyzer (Seahorse). Avascular 3D spheroids and xenografts were used to measure the effect of ADPGK LoF upon tumor growth. Hypoxic gradients in 3D tumor spheroids were quantified using the O2-sensitive nitromydiazide probe EF5 in combination with confocal microscopy and 3D image reconstruction. RT-qPCR arrays identified a core group of 11 hypoxia-inducible transcripts dependent upon ADPGK expression (CA9, DDIT4, ERO1L, EGFR, Gqbp3, Sla03a, Blihe, Rnu36, Aesan, Fam162a, Pkgl1 and PKM), henceforth referred to as ‘ADPGK-dependent HIF-α target signature’ (ADHTS). Comparison of ADHTS transcriptional profiles with HIF-1α or -2α deficient cells showed that ADPGK LoF is not selective for either HIF-α paralog. Since ADHTS genes contained critical for mTOR signaling, glycolytic activity and mitochondrial autophagy, we performed metabolic profiling and found that ADPGK LoF increased O2 consumption in a rapamycin-sensitive manner whilst increasing mitochondrial mass. Moreover, ADPGK LoF enhanced xenograft growth and vascularization associated with decreased protein levels of HIF-2α and the negative mTOR regulator DDIT4. In avascular 3D spheroids, ADPGK LoF increased growth, intra-spheroidal hypoxia and caused steeper hypoxic gradients in parallel to enhanced mTOR-pS6K1→ERPi1 signaling. RNAseq data from colon adenocarcinoma patients (TCGA; n = 382) confirmed that ADPGK expression correlates with ADHTS, HIF-α target gene expression, hypoxia scores and decreased overall survival. Consistent with our preclinical findings, the ADHTS gene signature inversely correlated with an mTOR gene signature in the same dataset. Our results uncover a hitherto unknown function of ADPGK as a crucial determinant of the degree and distribution of hypoxia within the tumor microenvironment through modulation of HIF-1α→mTOR signaling. Our analysis of TCGA data supports these preclinical findings thereby suggesting that ADPGK is a suitable therapeutic target in patients bearing hypoxic cancers.

#4511 HIF-1dependent regulation of creatine kinase metabolism promotes breast cancer invasion and metastasis. Hilaire Barch, Danielle L. Brooks, Rayna Krustulovic, Luciana P. Schwob, Deanna Parke, Tiffany N. Seagroves. Univ. of Tennessee Health Science Ctr., Memphis, TN.

Disregulated tumor cell metabolism is a hallmark of cancer progression and therapeutic resistance. In a screen for Hypoxia-Inducible Factor (HIF)-dependent genes regulating metabolism, we identified creatine kinase, brain isoform
#4512 The landscape of hypoxia-driven alternative splicing in breast cancer. Hani Choudhry, 1 Spyridon Oikonomopoulos, 2 Peng Ya, 3 Cristina Ivan, 3 Mircea Ivan, 3 Adrian L. Harris, 2 Jiannis Ragoussis. 2 Cancer Metabolism and Epigenetics Unit, King Abdulaziz University, Jeddah, Saudi Arabia; 3 McGill University, Quebec, Canada; 2 Texas A&M University, TX; 4 MD Anderson Cancer Center, TX; 5 Indiana University, IN; 6 University of Oxford, United Kingdom.

Tumor hypoxia is generally associated with poor patient outcome and resistance to therapy. Hypoxia has an impact on multiple pathways inside the cell and a widespread effect on phenotype. Here we determined the changes in transcript architecture that arise as result of alternative splicing in hypoxic cells. A panel of breast cancer cell lines that include the Luminal A and B, HER2+, Triple-Negative subcategories grown in hypoxic and normoxic conditions were subjected for deep RNA-sequencing. Depending on the cell lines we found between 23-927 splicing events with 3-45% of them also present in the differentially expressed fraction of genes. This points to an extensive isoform switching control independently of the transcriptional control of the genes themselves. The splicing events appear on genes responsible for mRNA processing (splicing, nuclear export and catabolism) as well as initiation of translation. Members of the SR family of proteins involved in RNA splicing, appear to be potential regulators of the splicing of the hypoxia specific splicing events identified. The differential splicing is driven by exons that are frequently part of both the coding and noncoding isoforms of a given gene potentially indicating a switch between these two types under hypoxia. Ten of the hypoxia specific splicing events were also found to be associated with the hypoxia status of primary breast cancer samples taken from The Cancer Genome Atlas (TCGA) database. Altogether, these data demonstrate the important role of hypoxia in driving alternative splicing events in breast cancer.

#4513 Investigation of bystander effects of hypoxia activated prodrugs using three dimensional cell cultures. Cho Rong Hong, Gib Bogle, William R. Wilson, Kevin O. Hicks. University of Auckland, New Zealand.

Background: Hypoxia in solid tumors acts as the major barrier to anti-cancer therapies and promotes tumor progression. Hypoxia-activated prodrugs (HAPs) are designed to only be activated in hypoxic cells, but also to exploit hypoxia as a basis of tumor selectivity. A key concept in exploiting hypoxia with HAPs is that active drug metabolite should be able to diffuse from hypoxic zones to maximize effect in surrounding aerobic tumor tissue. There is some evidence that these 'bystander effects' contribute to the anti-tumor activity of HAPs, but currently there is a lack of robust methods for their detection. Objectives: To investigate bystander effects from HAPs in a novel spheroid co-culture system using PR104A which releases a diffusible DNA crosslinking mustard and the tirapazamine analogue SN30000 which is known to be inhibited by cyclocreatine, a creatine kinase substrate that represses CK-dependent generation of ATP from phosphocreatine. When female FVB/Nj mice were injected with wild type PyMT cells in a tail vein assay and then treated with cCr (1g/kg/d, IP), lung metastasis was repressed to the same extent as CKb gene knockdown. Moreover, when cCr therapy was administered 7 days after tail vein injection, cCr was effective in preventing the transition of lung micrometastases to macrometastases. To explore the role of CK activity in regulating cell proliferation, survival in suspension, cellular metabolism and invasion, we next created CKb loss- and gain-of-function models using human breast cancer cell lines, and compared phenotypes to cCr treatment. Whereas deletion of CKb had no effect on cell proliferation or survival in adherent conditions or in suspension, either deletion of CKb or cCr therapy potently reduced ATP levels and invasive potential in vitro. Preliminary data also indicate that pre-treatment of triple negative breast cancer cell lines with cCr sensitizes cells to doxorubicin. Together, these data suggest that inhibition of CK activity may be effective in reducing breast cancer progression, but more research is needed to confirm whether cCr has anti-metastatic efficacy as a monotherapy, or in combination with conventional chemotherapies, using luciferase-labeled patient-derived xenograft (PDX) models. This work was sponsored by the NIH (CA138488), the Dept. of Defense (BC150640), the METAvivor foundation, and the West Cancer Center in Memphis, TN.

#4514 Low release of exosomal miR-663a from hypoxic tumor cells and poor tumor response to neoadjuvant radiotherapy in rectal cancer patients. Tonje Bjørnseth, 1 Karin M. Handeland, 2 Sebastian Melzer, 1 Rampradeep Samiapan, 1 Lars G. Lyckander, 3 Carolina Jegerschöld, 3 Linda Stenkvist, 1 Nirmala S. Thiyagarajan, 11 Catherine Lennon, 1 Annie H. Rekstad, 1 1 Akerhus University Hospital, Oslo, Norway; 2Karolinska Institute, Stockholm, Sweden.

Introduction: Hypoxia is a dominant hallmark of the tumor microenvironment and contributes significantly to radiotherapy resistance in solid tumors. Exosomes are nanosized vesicles actively released from tumor cells, and recent studies support their central role in the aggrivated biology caused by tumor hypoxia through their miRNA cargo. In this study, we aimed to characterize miR-663a of exosomes from hypoxic colorectal cancer (CRC) cell lines and investigate these miRNAs in circulating exosomes from rectal cancer patients with poor response to neoadjuvant radiotherapy. Methods: Five CRC cell lines were cultured in RPMI-1640 medium supplemented with 1% bovine serum albumin under normoxia (21% O2) or hypoxia (0.2% O2) for 24 hours. Exosomes were isolated from conditioned media by differential ultracentrifugation, and integrity and size were determined by cryo-electron microscopy and Nanosight tracking analysis and further characterized by Western blot and flow cytometry analyses. In a prospective biomarker study, plasma samples were collected from 29 patients with rectal cancer at the time of diagnosis, and histologic tumor response (tumor regression grade, TRG) to neoadjuvant radiotherapy was evaluated. Exosomes were isolated from plasma using the miRCURY™ Exosome Isolation Kit (Exiqon). Expression profiling of exosomal miRNAs of the CRC cell lines and the patients’ plasma samples was conducted using the miRCURY™ LNA™ Universal RT microRNA PCR Human panel I (Exiqon). Data normalization was performed based on global array mean. Results: Normoxic and hypoxic CRC cell lines were cultured until confluence. Exosomes were isolated and subjected to microarray analysis. In a prospective cohort study, plasma samples were collected from 29 patients with rectal cancer at the time of diagnosis, and histologic tumor response (tumor regression grade, TRG) to neoadjuvant radiotherapy was evaluated. Exosomes were isolated from plasma using the miRCURY™ Exosome Isolation Kit (Exiqon). Expression profiling of exosomal miRNAs of the CRC cell lines and the patients’ plasma samples was conducted using the miRCURY™ LNA™ Universal RT microRNA PCR Human panel I (Exiqon). Data normalization was performed based on global array mean. Results: Normoxic and hypoxic CRC cell lines were cultured until confluence. Exosomes were isolated and subjected to microarray analysis. In a prospective cohort study, plasma samples were collected from 29 patients with rectal cancer at the time of diagnosis, and histologic tumor response (tumor regression grade, TRG) to neoadjuvant radiotherapy was evaluated. Exosomes were isolated from plasma using the miRCURY™ Exosome Isolation Kit (Exiqon). Expression profiling of exosomal miRNAs of the CRC cell lines and the patients’ plasma samples was conducted using the miRCURY™ LNA™ Universal RT microRNA PCR Human panel I (Exiqon). Data normalization was performed based on global array mean. Results: Normoxic and hypoxic CRC cell lines were cultured until confluence. Exosomes were isolated and subjected to microarray analysis.
HSP90 inhibitor geldanamycin and CDK1 inhibition/knockdown decreases MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Tumor Hypoxia and the Unfolded Protein Response CDK1-mediated and HSP90-mediated HIF1a. Avously shown that cyclin-dependent kinase 1 (CDK1) stabilizes HIF1a in a variety of cancers but this is not restricted to hypoxic regions. We have previously described MondoA (also known as BLM) and a number of B lymphocytes are adapted to travel from the hypoxic bone marrow via normoxic peripheral blood to hypoxic lymph nodes. They thus are specialized in glycolysis to compensate the lack of oxidative phosphorylation under hypoxic conditions. Leukemic counterparts of B lymphocytes exploit these features for their survival. Glucose-dependent metabolites control the nuclear activity of the transcription factor MondoA. Here we report on the expression of MondoA in common B-cell acute lymphoblastic leukemia (cALL) compared to other malignancies, its role in malignancy of cALL in vivo, downstream pathways and correlation with relapse risk. Methods. Our human/murine syngeneic acute lymphoblastic leukemia model with immunodeficient NOD-SCID mice was used (Richter et al. 2009). NALM6 and 697 cALL cells were later transduced with MondoA short hairpin RNA. Upon successful MondoA knockdown (KD), KD and control lines were injected into the mice; CD10+ blasts in blood, spleen and marrow were assessed. Results. We found MondoA to be most strongly expressed in pediatric cALL and AML. Moreover, MondoA expression was high in gastrointestinal stromal tumors and alveolar rhabdomyosarcoma. MondoA KD in cALL cell lines and their subsequent analysis in xenograft mice resulted in a reduced number of leukemic blasts in blood, spleen and marrow. Spleen size and weight normalized in mice after MondoAKD. Further microarray analysis revealed an enrichment of glycolytic and hypoxia response gene sets by MondoA. Moreover, HIF1a induction under hypoxia required MondoA. We demonstrate that MondoA facilitates vincristine resistance of cALL. MondoA was induced under hypoxia and confers cALL cells chemotherapeutic resistance. Tied to these results, MondoA overexpression correlated with relapse risk; its expression was 63% higher in the very high-risk group as compared to the non-high-risk group of cALL. In conclusion, our findings demonstrate that MondoA maintains leukemic burden and aggressiveness of cALL in vivo possibly by modulating metabolic and hypoxia stress response, in particular by induction of HIF1a.

Dual inhibition of CDK1 and HSP90 destabilizes HIF1a and synergistically induces cancer cell death. Shuai Zhao, David T. Dicker, Wafık S. El-Deiry. Fox Chase Cancer Ctr., Philadelphia, PA.

Solid tumors are often characterized by intratumoral hypoxia. Hypoxia-inducible factor 1a (HIF1a) has been implicated in cell proliferation, survival, angiogenesis, invasion and migration. The expression of HIF1a is increased in a variety of cancers but this is not restricted to hypoxic regions. We have previously shown that cyclin-dependent kinase 1 (CDK1) stabilizes HIF1a through direct phosphorylation of its Ser668 residue in a Von Hippel-Lindau (VHL)- independent manner both under hypoxia and at G2/M under normoxia (Warfel et al., Cell Cycle, 2013). Heat shock protein 90 (HSP90) is also an acknowledged VHL-independent HIF1a stabilizer. We sought to explore the link between CDK1- and HSP90-mediated HIF1a stabilization. Under hypoxia, combination treatment with HSP90 inhibitor geldanamycin and CDK1 inhibition/knockdown decreases HIF1a levels more robustly than either treatment alone. This led us to investigate whether 20S inhibited CDK1 and HSP90 inhibition results in synergistic anti-cancer effects. Indeed, dual inhibition of CDK1 and HSP90 synergistically decreases HCT116 colon cancer cell viability. PARP cleavage and sub-G1 analysis indicate the combinational treatment triggers cancer cell death through apoptosis. Consistent with these findings, we observed that inhibition of cell viability by the combination is impaired in HCT116 Bax−/− cells. Furthermore, the dual inhibition also suppresses cell migration in vitro. We further validated our results with CDK1 inhibitor and HSP90 inhibitors that have entered clinical trials. Second-generation HSP90 inhibitor ganetespib and Ro3306 synergistically inhibit cell viability in HCT116 cells. We have previously demonstrated that CDK4 also contributes to the stabilization of HIF1a under hypoxia (Warfel et al., Cell Cycle, 2013). Interestingly, the combination of CDK4 and HSP90 inhibitors is also able to improve downregulation of HIF1a as compared to either agent alone in various cancer cell lines (eg. colon cancer, glioblastoma, etc). The FDA-approved CDK4 inhibitor palbociclib along with ganetespib synergistically inhibits cell viability in HCT116 cells under hypoxia. Such effect is not observed in W138 normal cells under normoxic and hypoxic conditions. These results demonstrate that CDK1 inhibition disrupts the interaction between HSP90 and HIF1a providing a rationale for therapeutic targeting of HIF1a through the combination of CDK1/4 and HSP90 inhibitors in cancer. Ongoing studies are examining the effects of CDK1/4 plus HSP90 dual inhibition in other tumor types and in vivo.

A CRISPR approach to monitoring hypoxia-inducible proteins in real-time. Marie K. Schwinn, Thomas Machleidt, Brock F. Binkowski, Christopher T. Eggers, Keith V. Wood. Promega Corporation, Madison, WI.

HIF1a-inducible factor 1A (HIF1A) regulates expression of genes implicated in various aspects of oncogenesis, including angiogenesis, cell survival, metastasis, and glucose metabolism. Overexpression or hypoxia-induced stabilization of HIF1A has been associated with poor prognosis in cancer patients, making HIF1A and its associated pathway a high-profile target for anticancer therapies. We sought to develop a live-cell assay to monitor abundance of endogenous HIF1A and HIF1A-inducible proteins that could be used to identify potent and specific inhibitors of the hypoxia signaling pathway. To accomplish this goal, mammalian cell lines were edited by CRISPR using a Cas9/CrRNA ribonucleoprotein complex with a single-stranded oligonucleotide donor DNA to target the HIF1B tag at the C-terminus of HIF1A. Immunoblot analysis revealed an enrichment of hypoxia-inducible proteins, including BNIP3, ANKR3D7, HILDA and KLF10. The 11 amino acid HIF1B peptide and its complementing 18 kDa polypeptide, known as LgbIT, spontaneously reconstitute into an active luciferase derived from the NanoLuc enzyme. Co-expression of LgbIT in edited cells, followed by addition of the cell-permeable luciferase substrate, leads to generation of a bright, steady luminescent signal that directly correlates with abundance of the HIF1B fusion. The edited cells were treated with several known modulators of the HIF1A signaling pathway, and changes in the abundance of the protein fusions were followed in real-time by monitoring luminescence. The HIF1B tag was also used to validate size and subcellular localization of the fusion proteins using bioluminescence imaging and antibody-free blotting. As expected, all tested compounds induced HIF1A accumulation. However, the downstream targets of HIF1A generated differing response to the chemical modulators, warranting further investigation into the modes by which these compounds act. By coupling the speed and efficiency of CRISPR-mediated editing with the small size and brightness of HIF1B, it was possible to generate a live-cell assay to monitor abundance of proteins along the HIF1A pathway. This assay could easily be adapted to screen for compound-induced effects on protein levels of HIF1A, as well as HIF1A- induced changes in expression patterns.

Impact of hypoxia on SFK activities in prostate cancer cells. Yao Dai, Dietmar W. Siemann. Univ. of Florida College of Medicine, Gainesville, FL.

Background: Metastasis is the major reason of disease progression and poor prognosis in prostate cancer. Src family kinases (SFKs), including c-Src, Lyn and Fyn, are non-receptor tyrosine kinases that have been shown to play an essential role in local invasion, castration resistance, and metastasis in prostate cancer. Hypoxia is able to promote metastasis-associated functions by activating various signaling molecules, including SFKs. However, whether all SFK members are upregulated by hypoxia is unclear. In the current study, the effects of hypoxia on cell functions and SFK activities are determined. Further, genetic approaches are applied to identify the typical SFK member that is mostly activated by hypoxia.

Methods: For hypoxic exposure, prostate cancer cells were exposed to low oxygen tensions (1%O2) for varied durations (0, 2, 6, 24 h). For drug treatment, cells were treated with saracatinib (0, 100, 500, 1000 nM) for indicated time periods. To test cell migration, “wound-healing” assay was used by making a scratch on confluent monolayers. Cells that have migrated to the denuded area were imaged. Cell invasion was detected by seeding cells into Matrigel-coated transwell chamber and cells on the bottom membranes were counted after treatment. Clonogenic assay was performed to test cell survival. At the molecular level, SFKs phosphorylation was detected by Western blotting, and gene knockdown was accomplished by siRNA transfection. To investigate expression of p-SFK and HIF-1α in patients, tumor tissues from prostate cancer patients were probed with desired antibody by immunohistochemical staining. Results: Both p-SFK and HIF-1α were highly expressed in patient tissues with advanced stage, with HIF-1 expression significantly associated with tumor grade and Gleason score. At the cellular level, while short term hypoxic exposure (2-6 h) induced greater cell migration, invasion and survival than prolonged hypoxia (24 h) in PC-3ML.
and C4-2B, these behaviors were increased under hypoxia with a period of 24 h in TRAMP-C1 cells. Further, hypoxia enhanced SFK phosphorylation in the pattern that was consistent with cell functions. Knockdown SRC, but not LYN, abolished hypoxia-induced invasion and p-SFK expression. Lastly, SFK inhibitor saracatinib showed stronger inhibition on functional behaviors facilitated under hypoxia than normal conditions; this is consistent with the observation that hypoxia, particularly short-term exposure, is able to enhance metastatic phenotypes by activating SFKs, predominantly c-Src, in prostate cancer cells, suggesting c-Src may be the most important signaling molecule of hypoxia-mediated behaviors. These findings have clarified the exact SFK member that is typically activated under hypoxia at least in prostate cancer model in vitro. More importantly, SFK inhibition is able to impair cell functions driven by hypoxia, suggesting their therapeutic potential by suppressing tumor metastasis that is driven by hypoxia in prostate cancer.

#4519 ATM/ATR-SerRS pathway dominates hypoxic angiogenesis in tumor. Yi Shi, Zi Le Liu, Xiang-Lei Yang. 1 Nankai University, Tianjin, China; 2 The Scripps Research Institute, La Jolla, CA.


#4520 Hepatitis B virus X protein regulates hypoxia-inducible factor-1alpha (HIF-1 alpha) and lysyl oxidase like 2 (LOXL2) pathway in hepatocellular carcinoma. Cerise Yueen-Ki Chan, Aki Pui-Wah Tse, Elley Yong-Tuen Chiu, Karen Man-Fong See, Hui-Yu Koh, Irene Oi-Lin Ng, Carmen Chak-Lui Wong. The University of Hong Kong, Hong Kong, Hong Kong.

Hepatocellular carcinoma (HCC), the most common form of primary liver cancer, is a fatal malignancy and is prevalent in HBV-endemic geographical areas. Hepatitis B virus (HBV) infection is a major etiologic factor of HCC. HBV is a double-stranded DNA virus and encodes for a viral onco-protein called transactivator protein X (HBx) which interacts with the host proteins to enhance proliferative potential of the host cells. Here, using Tet-ON and Tet-OFF HBx inducible systems in HBV-negative HCC cell lines, we showed that induced expression of HBx stabilizes hypoxia-inducible factor-1α (HIF-1α) protein under hypoxia. Reversely, knockdown of HBx in HBV-positive HCC cell lines reduced HIF-1α protein stabilization under hypoxia. More intriguingly, we found that induced HBx expression up-regulated the mRNA and protein expression a known HIF-1α transcriptional target, lysyl oxidase like 2 (LOXL2) in HCC. Our previous study showed that LOXL2 cross-linked collagen in the extracellular matrix to promote HCC metastasis. Scanning electron microscopy demonstrated that knockdown of HBx in HCC cell lines markedly reduced formation of collagen fibers in vitro. Transwell invasion assay showed that knockdown of HBx reduced HCC invasive ability. Picrosirius red staining further showed that knockdown of HBx reduced collagen cross-linking in vivo and repressed HCC growth and metastasis. Taken together, our study unprecedentedly show the HBx models the ECM through HIF-1α/LOXL2 pathway to promote HCC metastasis.


Introduction: Metastasis is responsible for over 90% of cancer related mortality, however mutations unique to the metastatic phenotype have yet to be identified. This raises the possibility that the metastatic phenotype is the result of a transcriptional profile adopted by cells of the primary tumor enabling them to spread to distal regions of the body. The tumor microenvironment (TME) is capable of influencing gene expression and cellular behavior. Hypoxia, low oxygen tension, is a component of the TME that arises due to improper tissue architecture and correlates with poor survival outcomes. Hypoxia induces transcriptional reprogramming in cells and induces the expression of genes involved in metastasis through direct activation and through the alteration of the epigenome. However, upon re-oxygenation HIF-1α is degraded and can no longer induce expression of hypoxic responsive genes. During the metastatic process, cells that have adopted hypoxia induced metastatic behavior such as increased motility and invasiveness must transit through regions of normoxia on their way to metastatic colonization. Hypothesis: As hypoxic cell migrate away from regions of low oxygen towards oxygen rich environments, such venous blood, genes induced by hypoxia would be expected to return to basal levels of expression. Those genes whose expression does not return to levels associated with normoxia, but instead remain elevated following re-oxygenation, may be crucial for maintaining the hypoxia induced metastatic phenotype. Experimental Design: To study the stability of hypoxia induced gene expression alterations following re-oxygenation, colon cancer cell lines were grown under hypoxic conditions (1% O2) for up to 3 weeks and then transitioned to normoxic conditions (21% O2). RNA sequencing was used to assess changes in gene expression during hypoxia adaptation and for more weeks following re-oxygenation. Results: While the majority of genes whose expression was induced by hypoxia returned to basal levels following re-oxygenation, a sub-set of genes clustered in the TGF-β pathway remained at elevated levels. In particular the expression of JunB, a component of the AP-1 transcription factor, was induced by hypoxia and continued to remain overexpressed after return to normoxic growth conditions. Conclusion: Long term culturing of colon cancer cells in hypoxic conditions induces changes in gene expression that remain stable following return to normoxic growth conditions. We hypothesize that genes whose expression follows this pattern may be involved in the hypoxia induced metastatic phenotype. RNA-seq profiling of cells grown in hypoxic conditions for three weeks and then returned to normoxic growth conditions for an additional three weeks identified genes involved in the TGF-β pathway and in particular JunB that were induced by hypoxia and remained stable following return to normoxia.
that decreased at lower ambient O2 concentrations. Spheroid growth and Measured HCT116 spheroid diameters increased linearly with time, at rates reduced known features of spheroids including oxygen and glucose gradients, SN30000 were compared to experimental results. Results: The model reproduces. Model simulations of HCT116 spheroid response to radiation and concentrations were compared to model predictions, as was glucose depletion from the microvascular network (cobalt-60) and SN30000 induced clonogenic cell killing under oxia and anoxia was determined using HCT116 monolayers. Model simulations of HCT116 spheroid response to radiation and SN30000 were compared to experimental results. Results: The model reproduced known features of spheroids including oxygen and glucose gradients, rapid cell proliferation at the periphery and central hypoxia and necrosis. Measured HCT116 spheroid diameters increased linearly with time, at rates that decreased at lower ambient O2 concentrations. Spheroid growth and glucose depletion was well-fitted by the AB model. Good agreement was found between simulated and measured clonogenic cell killing by radiation and SN30000, while the model underestimated spheroid growth delay by radiation and SN30000 than experimental observation. Discussion: An oxygen and glucose dependent AB model for growth and PD response (cell killing and growth delay) of HCT116 spheroids has been developed and calibrated using experimentally determined parameters. Further development of this model will link cell fates (including cell cycle arrest) explicitly to DNA damage responses and O2 and glucose-dependence of cell proliferation and survival.


Background: Hypoxia is defined as oxygen levels in tumor microenvironment of less than that in blood (90-100 mm Hg) and influences many aspects of tumour biology. During surgery tumour vasculature is cut off gradually leading to induction of acute hypoxia. The present study aims to experimentally test the genotypic and phenotypic effects of surgically induced acute hypoxia in breast cancer tumor samples and cell lines. Methodology: Core biopsy samples were collected from breast tumors (N=8 patients) at three time points during their curative surgery: prior (pre), mid-way (intra) and at the end (post). The samples were subjected to RNA-Seq and a list of differentially expressed genes (DEG) was prepared. A set of 26 DEG (pre ‘intra’ Vs ‘post’) obtained from RNA-Seq analysis and additional 17 genes involved in inflammation, EMT and hypoxia pathways were chosen for validation in tumor samples. These genes were validated using a customized qPCR Array (Qiagen). A gene was considered validated if it was significantly deregulated in at least 4 out of 8 patients. In another experiment, MCF-7 cells were exposed to varying levels of oxygen concentrations (0.1% to 20%) for varying time periods ranging from 30 minutes to 72 hours, to study time and dose dependent effects of hypoxia on following functional characteristics: proliferation, invasion and cell cycle changes. Results: Concordant, statistically significant up-regulation of FOS, DUSP1, JUNB, FOS, ZFP36, RGS1, STO4A, CXCL8 and LCL2 were observed in RNA-Seq and qPCR experiments while MMP13, HIF1A and VEGFA were up-regulated only in qPCR. However, 7 protein markers of inflammation, EMT and hypoxia did not show any significant change between pre, intra and post-operative samples. In MCF-7 cells, a dose and time dependent decrease in cell viability was observed with increasing severity of hypoxia as well as decrease in invasiveness, but there was no significant impact on cell cycle phases. When hypoxia cells were re-incubated under normoxic conditions an increase in cell proliferation and accumulation of cells in S phase (with a reduction in G2-M fraction) were observed, compared to cells grown under only normoxic conditions. Conclusion: Acute intra-operative hypoxia up-regulates expression of genes related to cell survival, chemoresistance, invasion, inflammation and angiogenesis in breast tumors. Breast cancer cells exposed to acute severe hypoxia followed by normoxia show increased proliferation. These effects may have implications for tumor cells that disseminate during surgery.

#4525 Hypoxia signaling pathway plays a role in ovarian cancer chemoresistance. Noelle L. Cutter, Tyler Walther, Kimberly H. Doyle, Ryan F. Mollov College, Rockville Centre, NY.

Hypoxia-inducible factor 1 (HIF-1) is a basic helix-loop-helix transcription factor that when induced regulates the expression of many genes involved in cytotoxic protective stimuli, which attenuates apoptosis and improves survival. Increased expression of HIF-1α gene (HIF1A) has been found in several carcinomas, including ovarian cancer. Ovarian cancers are generally refractory to platinum-based chemotherapy. Despite the large number of studies, molecular events that govern the emergence of aggressive therapy-resistant cells after chemotherapy are poorly defined. Genomic instabilities, such as copy number variation(CNV), may play an important role in chemoresistance and have been implicated in many complex diseases, like cancer. We analyzed CNV data that is publically available through the Cancer Genome Atlas and others. Of particular interest was the transcription factor HIF1A which plays an integral role in oxidative stress response such as those induced by chemotherapy reagents. The present study provides evidence that HIF-1α is highly expressed in the rare escape of tumor cells from drug-induced cell death by entering a non-cycling senescent state. We report the adaptive response of human ovarian surface epithelium cells to CoCl2, a chemical hypoxia-mimicking agent resulting in a senescent-like state of chemoresistant cells. The effect of the treatment was evaluated on CNV of HIF-1α gene expression, cell proliferation, survival, and tumor invasiveness. We show here that CNV duplication events of HIF1α results in an oxidative stress response in cells leading to chemoresistance through the induction of cellular senescence. Understanding the molecular events associated with chemoresistance will ultimately lead to better patient treatment and outcomes.

#4526 Attenuation of hypoxia in solid tumor with multifunctional upconversion nanoparticles to enhance photodynamic therapy. Yan Zhang, Jinggu Li, Jun Hu, Xiaoliang Yang, Huazhong University of Science and Technology, Wuhan, China.

As a non-invasive therapeutic technique, photodynamic therapy (PDT) requires sufficient oxygen in order to generate adequate singlet oxygen upon the excitation of photosensitizer using an appropriate light wavelength. It has limited efficacy in preexisting hypoxia in tumor and PDT-induced hypoxia, owing to their low oxygen level in the tissue and the consumption of the oxygen during the PDT treatment. Herein, taking advantage of hyperbaric oxygen (HBO), multifunctional upconversion nanovehicle is constructed to overcome the above-mentioned problems. HBO provides timely oxygen supply during the treatment, which greatly enhances the photodynamic effect of the loaded nanoparticles, as demonstrated by the accelerated generation of O2• and elevated cytotoxicity. Tumor growth is greatly restrained in the HBO-PDT treated mice. Our finding provides new insights into the modulation of unfavourable tumor microenvironment with the perfect marriage between HBO and NIR-light sensitized upconversion nanoparticles to overcome the current limitation of cancer photodynamic therapies.

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Tumor Hypoxia and the Unfolded Protein Response

Introduction: Hypoxia-activated prodrugs (HAPs) are a promising strategy to specifically target radioresistant hypoxic cells in tumors. We have used multicellular layers (MCLs) and steady state Green’s function pharmacokinetic/pharmacodynamic (PK/PD) modelling in microvascular networks to select SN30000 as a tirapazamine analogue with optimal spatial complementarity with radiation. However these models have several limitations such as lack of time dependent PK. Multicellular tumor spheroids are a more tractable model than MCLs for investigating PD parameters. However, the challenges of interpreting spheroid experiments in terms of spatial complementarity limits their application. We have developed an in silico agent-based (AB) tumor spheroid model, in which cell fate can be tracked as a function of time and spatially-varying concentrations of oxygen, glucose and therapeutic agents (radiation and HAP). The hypothesis is that our AB model can predict tumor spheroid response to radiation and SN30000, both alone and in combination. Methods: In the AB model, spheroid growth is simulated as a function of oxygen, glucose and drug concentrations determined by solving diffusion equations using oxygen consumption measured in HCT116 monolayers and glucose and SN30000 diffusion and metabolism measured in HCT116 MCLs and stirred cell suspensions, linked with intra-cellular reaction equations. Cell fate is determined by oxygen, glucose and drug concentrations. Spheroid diameter, cell survival and hypoxic fraction of HCT116 spheroids grown at a range of oxygen and initial glucose concentrations were compared to model predictions, as was glucose depletion from the culture system (cobalt-60) and SN30000 induced clonogenic cell killing under oxia and anoxia was determined using HCT116 monolayers. Model simulations of HCT116 spheroid response to radiation and SN30000 were compared to experimental results. Results: The model reproduced known features of spheroids including oxygen and glucose gradients, rapid cell proliferation at the periphery and central hypoxia and necrosis. Measured HCT116 spheroid diameters increased linearly with time, at rates that decreased at lower ambient O2 concentrations. Spheroid growth and glucose depletion was well-fitted by the AB model. Good agreement was found between simulated and measured clonogenic cell killing by radiation and SN30000, while the model underestimated spheroid growth delay by radiation and SN30000 than experimental observation. Discussion: An oxygen and glucose dependent AB model for growth and PD response (cell killing and growth delay) of HCT116 spheroids has been developed and calibrated using experimentally determined parameters. Further development of this model will link cell fates (including cell cycle arrest) explicitly to DNA damage responses and O2 and glucose-dependence of cell proliferation and survival.

BIOINFORMATICS AND SYSTEMS BIOLOGY: Modeling and Algorithms

#4527 Estimating tumor cell dissemination rates through lymph and blood via computational modeling. Anne Marie Barrette, Benedicte Lenoir, Mihaela Skobe, Marc R. Birtwistle. Icahn School of Medicine at Mount Sinai, New York, NY.

Metastasis is the primary cause of death from most cancer types, but the relative rates of tumor cell migration through lymphatic versus blood routes remains an open question. Here we combine data from a xenograft model of metastatic breast cancer with a computational model of tumor cell growth and dissemination to help answer this question. Orthotopic mouse xeno-
grafts using the triple negative breast cancer cell line MDA-MB-231 allowed for monitoring of metastatic cells within the primary tumor, bloodstream, lymph nodes, and lungs. Experiments resembling a pulse-chase method helped inform and parameterize the model to the rate of dissemination to the lymph nodes and lungs. Our ordinary differential equation model describes primary tumor growth as the balance between proliferation and death, with the proliferative fraction decreasing with tumor size, and the apoptotic or necrotic fraction increasing with tumor size. Intravasation to both lymph and blood rapidly increases early in tumor growth, followed by a decay to a low rate of tumor cell release well before the primary tumor grows to its saturating size. The mouse data guided our parameter estimations based on the assumption that dissemination tumor cell death rate is greatest in the harsh environment of the blood and lowest in the more accommodating lymph node, and that metastatic cells seedling in the lymph nodes and lungs follow similar growth dynamics as the primary tumor only with far fewer total cells. We are currently performing sensitivity analysis to explore the range of relative rates of tumor cell dissemination that fit the in vivo data, and thus gain insight into tumor cell dynamics within the different compartments. These analyses will allow us to estimate what the relative contributions of blood versus lymphatic tumor cell dissemination are to overall metastatic burden, as well as suggest experiments that further constrain these estimates. Such combined experimental and modeling analysis lend insight into where one might detect pre-metastatic cells early in disease progression or suggest new targets for anti-metastasis drugs based on the dominant mode of dissemination.

**#4528 A novel transducer array design optimizing TTFields delivery to the thorax.** Hadas S. Herschkovich,1 Sholi Strauss,1 Uri Weinberg,2 Eilon D. Kirsch,2 Ze’ev Bomanon2,1 Novocure Ltd, Haifa, Israel; 2Novocure GmbH, Lu- cerne, Switzerland.

Objective: The purpose of this study was to design transducer arrays for the delivery of Tumor Treating Fields (TTFields) to the thorax, generating high intensity in the upper torso while potentially improving patient comfort. Background: TTFields are low intensity, intermediate frequency, alternating electric fields that disrupt cell division. The ongoing Phase II STELLAR trial [NCT02397928] is investigating the efficacy and safety of TTFields in combination with chemotherapy for the treatment of mesothelioma. A pivotal study to evaluate the efficacy of TTFields in treating NSCLC is planned for 2017. TTFields are delivered via capacitive transducer arrays, comprising ceramic disks covered with a thin layer of conductive medical-grade gel, which are arranged in an almost rectangular order. Two orthogonal pairs of arrays are placed on the skin in the vicinity of the treated region. One pair is placed on the anterior and posterior sides of the thorax. The other pair is placed on the lateral and contratralateral sides of the thorax. This configuration has limitations: (a) Sub-optimal electric contact may be caused by poor attachment of the arrays on the chest due to body curvature; (b) TTFields intensities are attenuated by anterior-posterior arrays; (c) The current lateral-con- tralateral arrays generate lower field intensities in the lung’s apex. This study presents novel array designs to overcome these limitations. Methods: Various concepts for array designs that avoid placement over regions of thick subcutaneous fat were designed. To evaluate the field distribution generated by these arrays and optimize their design, numerical simulations using realistic computational phantom models were used. Results: Novel array designs were developed for the delivery of TTFields to the lungs. These arrays consist of sets of interconnected small patches that adhere to the natural contours of the human body. Simulations showed that the proposed arrays deliver uniformly distributed, high electrical field to the lungs. One notable design is a pair of arrays shaped as circular rings, in which one array is placed around the neck and shoulders, whereas the second array is placed on the lower torso. This design yielded a highly uniform field directed longitudinally throughout the torso. Conclusion: The array designs presented in this study are expected to maintain patient comfort and will ensure high field intensity delivered to the thorax. Compliance and TTFields treatment delivery could both be improved by using these designs.

**#4529 Optimization of the sequence for the administration of bevac- zumab in combination with pemetrexed and cisplatin in NSCLC: a pharma- cology based in vivo study.** Arnaud Boyer,1 Diane-Charlotte Imbs,2 Raouf El Cheikh,2 Celine Mascaux,1 Fabrice Barlies,1 Dominique Barbolosi,2 Sebastien Benzecky,1 Joseph Ciccolini,1 Assistance Publique Hôpitaux de Marseille. Multidisciplinary Oncology & Therapeutic Innovations, marseille, France; 2Aix mar- selle University, marseille, France; 3Inria, Bordeaux, France.

Introduction: Bevacizumab is active in advanced non-small cell lung cancer (NSCLC) and is administered in combination with chemotherapy in first line for non squamous NSCLC. However the best sequence of administration has not been defined. Previously published data suggest that before acting as an antiangiogenic drug, Bevacizumab would initially and transtorally normalize microvascularization of tumor blood vessels, thus be optimal to administrate chemotherapy. By using mathematical modeling, we showed that sequential administration of chemotherapy 3 days after Bevacizumab may be more beneficial than concomitant administration. Herein we tested and compared concomitant versus different schema of sequential ad- ministration of Bevacizumab with chemotherapy in NSCLC. Methods: H460 and H11006 xenografts were generated to treat tumors with sequential growth by fluorescence. Nude mice were engrafted subcutaneously with around 120,000 cells. Fluorescence was used to monitor tumor growth twice a week and treated with Bevacizumab, Cisplatin and Pemetrexed with vari- ous sequences of administration. A total of 15-16 replicates were included in each therapeutic group. Results Five therapeutic schema were compared: 1) Cisplatin-Pemetrexed only; 2) concomitant administration of Bevacizumab with Cisplatin-Pemetrexed only; 3) chemotherapy 3 days after Bevacizumab predicted to be the optimal sequence by mathematic modeling; 4) chemotherapy 8 days after Bevacizumab predicted to be the worse sequence by mathematic modeling; 5) control group receiving placebo. Tumor growth was reduced of 38% in group 3 as compared with group 2, but this difference was not statistically significant (p = 0.05) as compared to groups 1, 4 and 5. The median survival was the longest in group 3 (74 days), as compared with group 2 (70 days), group 4 (67 days), group 1 (54 days) and control group (40 days). Conclusion: This in vivo study indicate that the sequential administration of Bevacizumab three days before chemotherapy in NSCLC may be the optimal schema as compared with concomitant ad- ministration or longer delay as predicted by mathematic modeling. Mecha- nistic and pharmacokinetic analyses as well as biomarker studies are cur- rently ongoing and should be shortly available.
#5431 Systems pharmacology to predict cellular biomarkers and optimize mono- and combination-therapy regimens: Focusing on immune checkpoint targets PD-1, PD-L1 and CTLA-4. Oleg Milberg,1 Chang Gong,1 Bing Wang,2 Paolo Vicini,3 Rajesh Narwal,4 Lorin Roskos,4 Aleksander Popel.1 Johns Hopkins University, Baltimore, MD; 2MedImmune, Mountain View, CA; 3MedImmune, Cambridge, United Kingdom; 4MedImmune, Gaithersburg, MD.

Cancer immunotherapy focuses on stimulating and promoting the immune system to recognize and eliminate cancer cells, with several FDA approvals in recent years. However, identifying patients best suited for specific immune therapies, and determining optimal treatment regimens continue to be a clinical challenge. Using a molecular-detailed computational systems pharmacology model to identify cellular biomarkers and optimize regimens, we may be able to predict the efficacy of regimens in specific patient populations, and expedite drug development for cancer treatment. We developed a cell/receptor-based multi-compartment systems pharmacology model focusing on the immune response against a growing tumor, with the intent to test the effects of immune checkpoint inhibitors against PD-1, PD-L1 and CTLA-4 administered as mono- and combination therapies. Additionally, the model also allows for testing of other immuno-therapies, such as adoptive cell therapies, which can be combined with the checkpoint inhibitors. The model was designed and developed using the SimBiology plug-in in MATLAB. Simulations were performed with parameter sets for the immune response of particular tumor types at specific metastatic sites and NSCLC. All results were qualitatively and quantitatively compared to experimental pre-clinical and clinical data for model validation, or used for the generation of predictions suitable for further experimental testing. In silico, we have identified that administrations of the prescribed doses of 1-10 mg/kg of anti-CTLA-4 (based on binding kinetics) effectively saturates the receptors on the T cells, and promotes both an extended life span of the antigen presenting cells (APCs), and the maximum attainable activation levels of the effector T cells. The model further predicts that the effectiveness of anti-CTLA-4 therapy is limited by the immunogenicity of the system (i.e., the antigen intensity level and number of APCs presenting the antigens) in a monotonous fashion. Furthermore, injecting activated APCs without therapy would show a temporary tumor response and a subsequent recovery by the tumor to its original growth trajectory, while raising the antigen intensity had a sustained effect on tumor response. Other simulations indicate that, despite the lack of apparent tumor response, a sustained immune attack may be ongoing in the body; however, the immune activity is proportionally limited by the tumor and regulatory cells. Lastly, several dose-responses and clinical trials were simulated for both combination and monotherapies, and correlated with published published clinical trial data. Future work will focus on uncovering the cellular biomarkers responsible for such results, experimentally validating them, as well as simulating optimal combination treatment regimens for future evaluation.

#5432 Adrenal metastases in lung cancer: Clinical implications of a mathematical model. Jeremy M. Mason,1 AnneMarie Ciccarella,2 Lori Marx-Ru- byner,1 Anjali Kaur,2 Adriana Sklenova,1 Paul K. Newton,1 Kelly J. Korf.3,4,5,6,7,8 University of Southern California, Los Angeles, CA; 2Independent Research Advocate and Breast Cancer Patient, New York, NY; 3Independent Research Advocate and Metastatic Breast Cancer Patient, Los Angeles, CA; 4University of California San Diego, San Diego, CA; 5Scripps Clinic, San Diego, CA.

Adrenal gland metastases are common in lung cancer. It is well recognized that aggressive treatment of solitary adrenal metastases leads to improved outcomes but the exact nature of adrenal deposits is not well understood. Controversy exists as to the routing of cancer cells to the adrenal gland with some believing that this transmission is lymphatic, in contrast to the more generally accepted theory of hematogenous spread. Using an autopsy dataset of 3827 untreated cancer patients, we use the metastatic distribution of common primary cancer types over the long term of disease progression. The anatomical sites of spread in the body represent states in the model, while the transition probability between states represents the probability of metastatic spread from one site to another. We then use the Markov models to run Monte Carlo simulations of random walkers representing circulating tumor cells traveling within the body to simulate metastatic spread. We calculate mean first passage times (MfPT) to each state as a representative of time to metastasis formation. Analysis of 6 common cancer types (bladder [n = 120 autopsies; 289 metastases], breast [n = 432; 2235], colorectal [n = 161; 420], lung [n = 560; 859], ovarian [n = 418; 806], and prostate [n = 62; 212]) from the dataset showed distinct metastatic distributions across the populations. MfPT calculations to anatomical sites in the 6 analyzed primary cancer types indicated model progression times to the adrenal gland similar to the regional and distal lymph nodes in only lung cancer. The times associated with adrenal gland in the other primary cancers were similar to other metastatic sites of hematogenous spread. The Markov models created strongly support the lymphatic theory for metastatic spread to the adrenal glands. After performing a literature review to look for the biological plausibility of the simulated results, we believe evidence supports this theory and validates the models. This could explained improved survival for patients in whom solitary adrenal metastases are managed aggressively with surgical or radiation modalities. In order to further validate this theory, we are calling for clinical trials prospectively testing this hypothesis.

#5433 In silico analysis of enhanced radiosensitivity in radiation treatment combined with androgen deprivation therapy for prostate cancer. Mengdi Qian,1 Alexandre Almasan,2 Evren Gürkan-Çavuşoğlu.3 Case Western Reserve Univ., Cleveland, OH; 4Cleveland Clinic, Cleveland, OH.

The purpose of this study is to quantitatively analyze the improved radiosensitivity of prostate cancer cells to ionizing radiation (IR) when radiation treatment is combined with androgen deprivation therapy (ADT). The double strand breaks (DSBs) induced by ionizing radiation are repaired primarily by non-homologous end joining (NHEJ). It is reported in the literature that NHEJ is impaired under ADT, which then results in increased radiosensitivity and better IR treatment outcomes in prostate cancer. In this study, we have used our models for NHEJ repair of DSBs under IR treatment and IR + ADT combination treatment conditions to determine how the combination treatment results in better outcomes. We have modeled the effect of multiple doses of IR that are delivered 24-hours apart to mimic the conventional, fractionated radiation treatment. Our analysis results showed the DSBRs accumulation in the case of combined treatment with ADT compared to faster and more efficient repair when only IR is applied. We have used the experimental data on relative cell numbers and relative sub-G1 cell numbers from the literature to determine the difference in the levels of DSBs that lead to cell death differences between the two treatment strategies. The literature data also shows that there is patient response variability to ADT in terms of the reduction in the levels of Ku70/80, which is the first NHEJ protein complex that is recruited to DSBs. We have modeled this patient variability in our models by introducing variability to Ku70/80 protein concentrations used in the simulations. Our simulation results agree with the observation from clinical data that lower concentration levels of Ku70/80 lead to better radiosensitization. The amount of IR can be adjusted with respect to the level of radiosensitization after ADT on a patient specific level to achieve a desired level of DSBs that will lead to maximum cell death; this dose optimization effort will be the next step in our quantitative analysis.

#5434 Theory and methodology for the design and analysis of PDX mouse clinical trials. Sheng Guo,1 Binchen Mao,1 Henry Q. Li2.1 Crown Bioscience, Taicang, China; 2Crown Bioscience, Santa Clara, CA.

Patient-derived xenograft (PDX) is a well-accepted experimental cancer model mimicking original patients in histo- & molecular pathology, as well as drug response. Mouse clinical trials (MCT) using PDX becomes increasingly prevalent in oncology research, yet the theory and methodology for trial design and analysis is underdeveloped. By analyzing tumor growths of 34081 PDX mice, we showed that majority of can be satisfactorily modeled by exponential growth kinetics, based on which we derived a new drug response readout called the AUC ratio that is superior to the tumor growth inhibition (TGI) and its variants. We outlined a bootstrap algorithm to calculate its confidence interval, and showed that a 4:4 (4 mice in both treatment groups) or up design sharply increases measurement accuracy over the lesser design. Next, we introduce three approaches for drug evaluation and biomarker discovery in MCTs. In the endpoint based analysis, we used a cohort of gastric PDXs to show a positive correlation between EGFR mRNA expression and cetuximab efficacy, in agreement with clinical trial results. We then used linear mixed models (LMMs) to describe MCTs as clustered longitudinal studies that capture the growth and drug response heterogeneities across PDXs and among mice within a PDX. Further, LMMs can separate prognostic and predictive biomarker effects, quantify response difference to a drug for multiple cancers, or efficacy difference of multiple drugs to a cohort of PDXs. Thirdly, we used additive frailty models to perform survival analysis on MCTs. We defined survival endpoints of PFS and OS in PDX to show that hazard ratios can be more accurately estimated. We revealed an inherent connection between frailty and growth rate for PDXs. We performed computational simulations for the last two methods to show that statistical power improves tremendously with a 3:3 or up design. This work lays the foundation for rational design and analysis of PDX mouse clinical trials.
Tumor cell division rate heterogeneity explains in vitro clonal dominance. Margriet M. Palm, Marjet Eleman, Joost B. Beltman. Leiden University, Leiden, Netherlands.

The cells that make up a tumor exhibit large phenotypic and genotypic variation, which strongly influences the effectiveness of cancer therapy. A typical morphological feature associated with invasive tumors in humans. The clonal dominance that represent most of the tumor population, i.e. clonal dominance. Such heterogeneity could potentially be caused by the occurrence of different ratios of cancer stem cells and differentiated cell clones per clone, or, alternatively, clonal dominance may be caused by heterogeneity of division rates between clones. Previously published work, applying lineage tracing with genetic barcodes to in vitro iterated growth and passage experiments demonstrated that clonal dominance was accompanied by clone loss and partial overlap in dominant clones between biological replicates [1]. To identify the source of these observations, we build a computational model that simulates the iterated growth and passage experiments. By varying the ‘division rules’ in the model, we can quantitatively predict the evolution of the clone distribution for the aforementioned hypotheses. In this manner, we show that clonal dominance does not appear in simulations where all cells divide at a uniform rate, even when the population is subdivided in cancer stem cells and differentiated cells. However, when the division rates vary between clones and are inherited from parent cells, dominant clones appear. These simulations also closely match the in vitro evolution of clone loss and clonal overlap between biological replicates. Altogether, our findings suggest that data can be used to create a heritable tumor head model of individual cells. References: [1] Porter, S. et al. (2014). Lentiviral and targeted cellular barcoding reveals ongoing clonal dynamics of cell lines in vitro and in vivo. Genome Biology, 15(S), R75.

Using diffusion weighted imaging (DWI) data to accurately predict electric field delivery to the tumor during TTFields treatment. Cornelia Wenger,1 Ze’ev Bomzon,1 Pedro Cavaleiro Miranda2. 1Novocure, Haifa, Israel; 2Faculty of Science, University of Lisbon, Lisbon, Portugal.

Tumor Treating Fields (TTFields) are low intensity (1-3 V/cm) alternating electric fields in the intermediate frequency range, delivered through two pairs of transducer arrays placed on the patient’s scalp. TTFields are an anti-mitotic modality approved for the treatment of glioblastoma multiforme (GBM). Simulation-based studies show that TTFields distribution within the brain depends on the location of the arrays on the scalp as well as the anatomy of the patient. Therefore, to study how field distribution influences disease progression requires the creation of patient-specific computational models. To date the creation of such models is based on accurate segmentation of MRI images into various tissues, and further of volume mesh assembly. These tasks are performed using semi-automatic, labor-intensive algorithms, making this approach impractical for studies which require the creation of a large number of patient models. We recently outlined that diffusion tensor imaging (DTI) could be used to construct realistic head models for simulating TTFields distribution without the need for complex segmentation of the brain. This is possible because the diffusion tensors evaluated from DTI can be scaled into anisotropic conductivity tensors using simple relationships. However, the approach is limited because the acquisition of high-quality DTI data is not widely practiced for GBM patients. Yet, shorter diffusion weighted imaging (DWI) sequences, such as an apparent diffusion coefficient (ADC) map or trace image, are common diagnostic techniques. The aim of this study was to test whether DWI data could potentially be used to create realistic head models. We used a previously developed head model created from structural and DTI data of a healthy individual. To test pathological scenarios, virtual lesions were placed. To simulate data acquired with DWI, we substituted the diffusion tensor at each point within the model with the mean diffusion (MD), which is the value that can be measured using DWI. The computational model was then completed by scaling the MD into a voxel-wise isotropic conductivity map using the same relationship as expressed above for converting anisotropic diffusion into conductivity tensors. TTFields distributions within the model were then evaluated for various array layouts and tumor locations. The field distribution within the DWI-derived models did not vary significantly from those found in the DTI-derived models. The highest deviation between models was a difference of 5% in the average electric field intensity in the tumor. The study shows that DWI data can be used to develop realistic TTFields, opening a new avenue for creating patient-specific computational models on a large scale. These models could be beneficial for retrospective studies investigating possible connections between field distribution and disease outcome.

Bioinformatics and Systems Biology: Modeling and Algorithms

Computational modeling and characterization of Dieder proteins that regulate highly-conserved immune signaling. Aunol Jerlu,1 Mary Ellen Heavner,2 Shubha Govind,3 Shaneen Singh1. 1CUNY Brooklyn College, Brooklyn, NY; 2CUNY City College, New York, NY.

The NF-kB and JAK/STAT pathways are well-established signal transduction pathways that are involved in the regulation of immune responses, and are frequently deregulated in cancer. The aim of this study was to test whether DWI data could potentially be used to create realistic head models. We used a previously developed head model created from structural and DTI data of a healthy individual. To test pathological scenarios, virtual lesions were placed. To simulate data acquired with DWI, we substituted the diffusion tensor at each point within the model with the mean diffusion (MD), which is the value that can be measured using DWI. The computational model was then completed by scaling the MD into a voxel-wise isotropic conductivity map using the same relationship as expressed above for converting anisotropic diffusion into conductivity tensors. TTFields distributions within the model were then evaluated for various array layouts and tumor locations. The field distribution within the DWI-derived models did not vary significantly from those found in the DTI-derived models. The highest deviation between models was a difference of 5% in the average electric field intensity in the tumor. The study shows that DWI data can be used to develop realistic TTFields, opening a new avenue for creating patient-specific computational models on a large scale. These models could be beneficial for retrospective studies investigating possible connections between field distribution and disease outcome.

Bioinformatics and Systems Biology: Modeling and Algorithms

UnicOnSig: A new algorithm for genome wide quantification of gene functions and disease associations. Xu Chi,1 Meenakshi Anurag,2 Sartor A. Maureen,3 Xiaosong Wang1. 1University of Pittsburgh, Pittsburgh, PA; 2Baylor College of Medicine, Houston, TX; 3University of Michigan, Ann Arbor, MI.

One of the greatest hurdles for cancer biologists is to identify the cancer genes underlying inherited and sporadic cancer. While high throughput cancer genomics or deep sequencing studies. In our previous study, we discovered that cancer genes possess a complicated yet distinct “gene concept signature.” Concept Signatures include cancer-related signaling pathways, molecular interactions, transcriptional motifs, protein domains, and gene ontologies. We developed a Concept Signature (or ConSig) analysis that prioritizes the biological importance of candidate genes underlying cancer by computing their strength of association with those cancer-related signature concepts. The ConSig analysis facilitated the discovery of a recurrent ESR1-CCDC170 gene fusion in more aggressive Luminal B breast cancers (Nat. Commun. 2014) as well as TLK2, MAP3K3, and MYST3 amplifications in aggressive luminal breast cancer (Nat. Commun. 2016). The NF-kB and JAK/STAT pathways are well-established signal transduction pathways that are involved in the regulation of immune responses, and are frequently deregulated in cancer. The aim of this study was to test whether DWI data could potentially be used to create realistic head models. We used a previously developed head model created from structural and DTI data of a healthy individual. To test pathological scenarios, virtual lesions were placed. To simulate data acquired with DWI, we substituted the diffusion tensor at each point within the model with the mean diffusion (MD), which is the value that can be measured using DWI. The computational model was then completed by scaling the MD into a voxel-wise isotropic conductivity map using the same relationship as expressed above for converting anisotropic diffusion into conductivity tensors. TTFields distributions within the model were then evaluated for various array layouts and tumor locations. The field distribution within the DWI-derived models did not vary significantly from those found in the DTI-derived models. The highest deviation between models was a difference of 5% in the average electric field intensity in the tumor. The study shows that DWI data can be used to develop realistic TTFields, opening a new avenue for creating patient-specific computational models on a large scale. These models could be beneficial for retrospective studies investigating possible connections between field distribution and disease outcome.

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for the selection of high-value neoantigen candidates and how they can improve the design and efficacy of cancer vaccines. Neoantigens with low Treg activation potential may then be used to support development of personalized therapies including vaccination and in vitro expansion of tumor infiltrating lymphocytes for adoptive cell transfer.

#4541 Understanding the dose and schedule dependence of efficacy for the We1 inhibitor AZD1775 in xenograft and patient derived explant models by mathematical modelling. James W. Yates, Elaine Cadogan, Jennifer Hare, Adina Hughes, Urszula M. Polanska, Mark O’Connor, Susan E. Critchlow, AstraZeneca, Cambridge, United Kingdom. AZD1775 is a highly selective, small-molecule inhibitor of We1 being developed to treat patients with advanced solid tumors, as monotherapy and in combination with olaparib. Experimental Procedures Patient-derived explant (PDX) and xenografted models with a range of sensitivities to AZD1775 were tested for response to different doses and schedules of AZD1775. We1 inhibition was assessed by pCDK1 as a direct substrate of We1 and RRM2 as a surrogate of CDK2 activity. Concentration of AZD1775 in plasma was measured in the same animals. Mathematical models were developed to describe the relationship between drug exposure, biomarker modulation and resulting tumor growth inhibition. Results The PKPD relationship of both pCDK1 and RRM2 could be described by the mathematical model with significant delay between PK and RRM2 reduction due to this being a protein degradation event. Three factors influenced anti-tumour activity in the A437 xenograft and three TNBC PDX models, namely the AZD1775 dose, the number of consecutive days of dosing and the number of days between AZD1775 doses. The minimally effective preclinical dose with anti-tumour activity was 60 mg/kg OD. TGI was seen to increase with increased days of consecutive AZD1775 dosing from 3 days on, such that a 5 days on/9 days off schedule was more efficacious than 3 days on/4 days off schedule for two weeks. A mathematical model successfully described this efficacy by using the predicted dose and schedule dependent reduction in pCDK1. Conclusions The insights from this predictive modelling informed the starting dose of 125mg BID AZD1775 on a 5 day on/9 day off schedule; with the clinical goal to optimize the days of consecutive AZD1775 dosing as well as the maximum tolerated dose.


Chemotherapy and immunotherapy have sometimes been considered two mutually exclusive approaches. On the one hand, chemotherapy has long been considered as a modestly effective treatment (outside pediatric oncology), with a short-lived effect caused by drug resistance, a poor tolerance profile and a detrimental effect on the immune system. On the other hand, the oncologist continues using chemotherapies for many reasons: if the control of a large tumor mass has to be obtained urgently irrespective of the side effects on the immune system, if immunotherapy has failed or has not been validated as a first line of treatment, when no targeted therapy can apply, etc. However, the issue of modifying the long-validated chemotherapy protocols to make them a good fit for an adjuvant (or concomitant) immunotherapy has not been addressed yet. The practice of gluing together an old Maximum Tolerated Dose (MTD) protocol with new immunotherapy protocol is the standard practice. Even though some re-design would be needed to mitigate the massive and prolonged immunosuppression caused by chemotherapy protocols. This lack of flexibility in the chemotherapy regimen could seriously jeopardize the effects of adjuvant or concomitant immunotherapy. Fortunately, chemotherapy and immunotherapy may not be incompatible per se: there are accumulating evidence that low-dose chemotherapies with little or no break-period (the so-called metronomic regimen) might have an immunological mode of action (in addition to the classical explanation of its anti-angiogenic effect and cytotoxicity). Furthermore, even with MTD protocols, experienced oncologists can improve the clinical outcome with the rationale use of pro-immunogenic drugs. Nevertheless, the best way to combine chemotherapy with immunotherapy is unknown and to find it in a trial would require large resources (high costs and risk of failure). This is why we present a pharmacodynamic model of chemotherapy in combination with immune checkpoint blockers, in order to provide a practical tool for the design and the management of clinical trials. Based on simple equations, this model does not predict a priori the best protocol, but helps the analysis of clinical data and the subsequent identification of optimal combinations. The mathematical model is based on discrete-time (high costs of the approximations), under the assumption that the number of immune response cells is much larger than the number of tumor cells. This assumption is justified for most of the clinical studies. Mathematical model of chemotherapy in combination with immune checkpoint blockers is developed. The model includes a description of the growth of the tumor and immune system, if immunotherapy has failed or has not been validated as a first line of treatment.

BIOINFORMATICS AND SYSTEMS BIOLOGY: Modeling and Algorithms

UnitConSig has broad applications on genome prioritization for genomic-based studies to discover new disease causal genes or new gene functions.


Artificial Intelligence (AI) is the single most transformative technology in human history. Advancements in personalized medicine depend upon significantly furthering our current understanding of how genetic variation and somatic mutation regulate aberrant gene activity and subsequent disease biology, including the myriad of dysregulated molecular mechanisms of cancer. To this end, we have built a robust AI methodology to precisely assess pathogenicity for all genomic missense variants. Coupled with our advanced deepCODE feature selection strategy for constructing deep learning models, we can quantitatively integrate a priori pathway-based biological knowledge with multiple types of high-throughput omics data. This approach significantly improves performance of established classification methods via enhanced semantic interoperability for mapping between multiple biomedical ontologies and the subsequent identification of genes and molecular pathways more predictive of cancer type etiology. Specifically, our deepCODE AI methods greatly improve tumor subtype and drug-response classification accuracies by combining tumor DNA- and RNA-seq data. Our results illustrate classification of human breast and lung cancer subtypes with 96% and greater than 99% accuracy, respectively. Our novel multinomial statistical-learning method, which was validated with 6 cancer type patient-derived tumor xenograft (PDXT) mouse models, achieved greater than 95% accuracy across 10,000 human tumors within the collection of 28 TCGA cancer types. Finally, our stepwise deepCODE drug target discovery approach systematically integrates our deep learning classification strategy with advanced Bayesian Network modeling to identify causative driver genes, representing probable novel drug targets. This stepwise machine learning approach also serves as a unique means to assess drug efficacy. Taken together, our advanced deepCODE AI tools greatly expand the means to fully understand the underlying molecular determinants of human cancer. We have developed user-friendly decision support and research tools for in-depth tumor analysis and large-scale case-control methods that afford investigators access to our deepCODE variant annotation algorithms.

#4540 MiVax: an innovative cancer neoantigen prediction system. Lenny Moise, Guilhem Richard, Frances Terry, William Martin, Ann De Groot. EpiVax Inc, Providence, RI.

T cell epitopes bearing tumor-specific mutations discovered using next generation sequencing can mediate T cell-mediated immune responses that arrest tumor progression. Although neoantigen prediction using computational methods rapidly identifies epitope candidates in the mutanome, a large proportion prove to be non-immunogenic. Innovative computational tools validated for infectious disease can be applied to enhance design of personalized cancer immunotherapies by classification of predicted epitopes according to potential for mounting a tumor-specific T cell mediated immune response. Our novel computational pipeline identified that non-mutated consensus sequences of MHC class I and II T cell receptor (TCR)-facing sequences and screens sequence databases to identify MHC ligands that share TCR faces with host-related proteins. A database of human protein sequences is available and screens sequence databases to identify MHC ligands that share TCR faces with host-related proteins. A database of human protein sequences is available to identify tumor-specific epitopes that may reduce anti-tumor activity by sequences that activate regulatory T cells (Tregs) trained in the thymus on self-antigens. Similarly, tumor-specific epitope candidates are screened using databases composed of human commensal- or pathogen-derived sequences to identify epitopes that, respectively, may detrimentally or beneficially cross-react with T cells raised over the course of an individual’s immune history. We conducted retrospective analyses of cancer vaccine efficacy studies performed in mice [Kreitner et al. 2015 Nature 520, 692-696] showing that mutanome-directed vascularization is unrelated to therapeutic tumor growth inhibition. Results The PKPD relationship of both pCDK1 and RRM2 could be described by the mathematical model with significant delay between PK and RRM2 reduction due to this being a protein degradation event. Three factors influenced anti-tumour activity in the A437 xenograft and three TNBC PDX models, namely the AZD1775 dose, the number of consecutive days of dosing and the number of days between AZD1775 doses. The minimally effective preclinical dose with anti-tumour activity was 60 mg/kg OD. TGI was seen to increase with increased days of consecutive AZD1775 dosing from 3 days on, such that a 5 days on/9 days off schedule was more efficacious than 3 days on/4 days off schedule for two weeks. A mathematical model successfully described this efficacy by using the predicted dose and schedule dependent reduction in pCDK1. Conclusions The insights from this predictive modelling informed the starting dose of 125mg BID AZD1775 on a 5 day on/9 day off schedule; with the clinical goal to optimize the days of consecutive AZD1775 dosing as well as the maximum tolerated dose.

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Despite decades of effort, most disseminated cancers remain incurable, and progression from localized to metastatic disease is largely responsible for cancer-related mortality. In metastatic disease, an ongoing battle between tumor and host occurs at each site; tumor-associated antigens, stress proteins, and danger-associated molecular patterns can both initiate and continually stimulate an immune response against a tumor. Adding an additional layer of complexity, locally activated cytotoxic T cells traffic through the host circulatory system to also surveil metastasis elsewhere in the body. Thus, metastatic tumors are highly interdependent; changes in the nonlinear tumor-immune interactions in one tumor can perturb the systemic antitumor immune response, potentially facilitating spontaneous regression or aggressive outgrowth in distant sites. This can additionally influence the clinical outcome of therapeutic intervention, which depends on therapy-induced changes in tumor-immune dynamics both locally and systemically, as well as patient-specific initial conditions of the global disease. Using the tools of mathematical oncology we gain insights into this complex interconnectivity between metastatic sites. ODE models have been developed which incorporate both local tumor-immune interactions in each tumor site and the trafficking of activated T cells systemically, and can be parameterized by experimental and clinical investigations. Using four primary tumor sites (lung, liver, breast, kidney) we simulate the growth behavior of a primary site upon both seeding, growth, and treatment of a secondary (or several) metastatic site(s). In the presence of several metastatic sites, the dissemination of activated T cells is not necessarily intuitive and depends on many factors including the blood flow fraction to each organ and the tumor volume to organ size ratio. Certain sites may experience inhibition of growth, and other sites may be promoted upon seeding of an additional site. We computationally analyze all combinations of metastatic sites, and predict which sites are likely to benefit (tumor growth) and which to suffer (tumor shrinkage) in each combination based upon known properties of physiological blood flow and tumor volume at initial presentation. This allows the identification of which sites in each combination would be the optimal target for surgical therapy in order to induce the maximum reduction in overall tumor burden. Furthermore, we can simulate local radiation at each respective site, quantifying the difference in model-predicted decrease in overall tumor burden between individual targets. The results facilitate an improved understanding of general disease kinetics in the metastatic setting, emphasizing that ‘local’ therapy is highly likely to have systemic effects, and support the case for a paradigm shift in treatment target selection for metastatic disease.

### #4543 Local and systemic tumor-immune dynamics in metastatic cancer.
Rachel Walker,¹ Jan Poleszczuk,⁷ Heiko Enderling,¹ Moffitt Cancer Center, Tampa, FL;²Polish Academy of Sciences, Warsaw, Poland.

Despite decades of effort, most disseminated cancers remain incurable, and progression from localized to metastatic disease is largely responsible for cancer-related mortality. In metastatic disease, an ongoing battle between tumor and host occurs at each site; tumor-associated antigens, stress proteins, and danger-associated molecular patterns can both initiate and continually stimulate an immune response against a tumor. Adding an additional layer of complexity, locally activated cytotoxic T cells traffic through the host circulatory system to also surveil metastasis elsewhere in the body. Thus, metastatic tumors are highly interdependent; changes in the nonlinear tumor-immune interactions in one tumor can perturb the systemic antitumor immune response, potentially facilitating spontaneous regression or aggressive outgrowth in distant sites. This can additionally influence the clinical outcome of therapeutic intervention, which depends on therapy-induced changes in tumor-immune dynamics both locally and systemically, as well as patient-specific initial conditions of the global disease. Using the tools of mathematical oncology we gain insights into this complex interconnectivity between metastatic sites. ODE models have been developed which incorporate both local tumor-immune interactions in each tumor site and the trafficking of activated T cells systemically, and can be parameterized by experimental and clinical investigations. Using four primary tumor sites (lung, liver, breast, kidney) we simulate the growth behavior of a primary site upon both seeding, growth, and treatment of a secondary (or several) metastatic site(s). In the presence of several metastatic sites, the dissemination of activated T cells is not necessarily intuitive and depends on many factors including the blood flow fraction to each organ and the tumor volume to organ size ratio. Certain sites may experience inhibition of growth, and other sites may be promoted upon seeding of an additional site. We computationally analyze all combinations of metastatic sites, and predict which sites are likely to benefit (tumor growth) and which to suffer (tumor shrinkage) in each combination based upon known properties of physiological blood flow and tumor volume at initial presentation. This allows the identification of which sites in each combination would be the optimal target for surgical therapy in order to induce the maximum reduction in overall tumor burden. Furthermore, we can simulate local radiation at each respective site, quantifying the difference in model-predicted decrease in overall tumor burden between individual targets. The results facilitate an improved understanding of general disease kinetics in the metastatic setting, emphasizing that ‘local’ therapy is highly likely to have systemic effects, and support the case for a paradigm shift in treatment target selection for metastatic disease.

### #4544 Computational modeling to suggest patient-specific screening schedules for early detection of gastric cancer.
Rachel Walker,¹ José Pimentel,¹ Jaime Mejia,² Domenico Coppola¹, Moffitt Cancer Center, Tampa, FL; Instituto de Patología Mejia Jimenez, Cali, Colombia.

Gastric cancer is often diagnosed at an advanced stage and currently remains the third most common cause of cancer-related death worldwide. Early detection and surgical intervention has been found to reduce GC-associated mortality, yet an efficient and cost-effective screening program still does not exist. Several proteins including Lgr5, CD44 and CD133 have been found to be upregulated during the H. pylori-associated Correa pathway of carcinogenesis, and have the potential to act as biomarkers for early detection. The tools of mathematical oncology facilitate the development of models capable of both reproducing and predicting the increase in expression of these markers, and thus the patient-specific progression of disease. These predictions can allow the identification of optimal screening times to minimize the risk of undetected malignant transformation. Here, we present a combined mathematical-statistical approach to identifying such optimal screening times in a manner which is both biomarker-independent and H.pylori status-independent, thus allowing improved generalizability and the potential for broader application in the clinical setting. Logistic regression models are developed for each biomarker of interest (Lgr5, CD133, CD44) to determine the likelihood of a patient being either early in the Correa pathway (gastritis or metaplasia) or late in the pathway (dysplasia or carcinomas) based on current age, sex and biomarker-positive cell fraction obtained from immunohistochemical staining of biopsy tissue samples. Calibrated models for all three biomarkers were able to accurately classify disease stage as determined by pathology report in more than 85% of an initial cohort of 39 patients. Mathematical models describing the temporal evolution of the ex- pression of each marker of interest are then defined based on clinical data. Coupled with the statistical tool for identifying the likelihood of being at each stage of disease for all combination of input parameters, this framework can forecast time to a clinically-significant endpoint such as the development of dysplasia or adenocarcinoma for future patients, allowing the suggestion of optimal timing of which treatment is appropriate based on progression to a threshold threshold.

The results suggest that for 13 out of 16 patients in our initial test cohort (81%), models independently developed using three different markers all recommend similar follow-up screening times, showing promise for the general applicability and robustness of such a tool.

### #4545 A computational biology method to predict HMA or lenalidomide treatment response in non-Del(5q) MDS.
Leylah Drusbosky,¹ Neeraj Kumar Singh,¹ Shireen Yalı,¹ Taher AAbbasi,² Christopher R. Cogle¹,¹ Univ. of Florida, Gainesville, FL;²Cellworks Group Inc, CA.

Despite decades of effort, most disseminated cancers remain incurable, and progression from localized to metastatic disease is largely responsible for cancer-related mortality. In metastatic disease, an ongoing battle between tumor and host occurs at each site; tumor-associated antigens, stress proteins, and danger-associated molecular patterns can both initiate and continually stimulate an immune response against a tumor. Adding an additional layer of complexity, locally activated cytotoxic T cells traffic through the host circulatory system to also surveil metastasis elsewhere in the body. Thus, metastatic tumors are highly interdependent; changes in the nonlinear tumor-immune interactions in one tumor can perturb the systemic antitumor immune response, potentially facilitating spontaneous regression or aggressive outgrowth in distant sites. This can additionally influence the clinical outcome of therapeutic intervention, which depends on therapy-induced changes in tumor-immune dynamics both locally and systemically, as well as patient-specific initial conditions of the global disease. Using the tools of mathematical oncology we gain insights into this complex interconnectivity between metastatic sites. ODE models have been developed which incorporate both local tumor-immune interactions in each tumor site and the trafficking of activated T cells systemically, and can be parameterized by experimental and clinical investigations. Using four primary tumor sites (lung, liver, breast, kidney) we simulate the growth behavior of a primary site upon both seeding, growth, and treatment of a secondary (or several) metastatic site(s). In the presence of several metastatic sites, the dissemination of activated T cells is not necessarily intuitive and depends on many factors including the blood flow fraction to each organ and the tumor volume to organ size ratio. Certain sites may experience inhibition of growth, and other sites may be promoted upon seeding of an additional site. We computationally analyze all combinations of metastatic sites, and predict which sites are likely to benefit (tumor growth) and which to suffer (tumor shrinkage) in each combination based upon known properties of physiological blood flow and tumor volume at initial presentation. This allows the identification of which sites in each combination would be the optimal target for surgical therapy in order to induce the maximum reduction in overall tumor burden. Furthermore, we can simulate local radiation at each respective site, quantifying the difference in model-predicted decrease in overall tumor burden between individual targets. The results facilitate an improved understanding of general disease kinetics in the metastatic setting, emphasizing that ‘local’ therapy is highly likely to have systemic effects, and support the case for a paradigm shift in treatment target selection for metastatic disease.

### #4546 Structural evaluation of mutations identified on Cue domain of FANCD2.
Mohd Quadir Siddiqui, Ashok K. Varma. TMC-ACTREC, Navi Mumbai, India.

Fancconi anemia complementation group D2 (FANCD2) protein plays pivotal role in DNA interstrand crosslink (ICL) repair and genome stability. FANCD2 comprises conserved CUE domain at N terminus, which is important for DNA ICL repair and protein stability. However, molecular mechanism associated to genetic alteration has not well characterized. Here, we have carried out multi-modal approaches to explore folding pattern of FANCD2 CUE domain and functionally relevant mutations. Dynamic Light Scattering and Mass Spectrometry data of purified proteins could revealed that wild-type protein is predominantly monomeric, but mutated counterparts are exhibiting oligomeric properties. Secondary, tertiary structure assessment and their thermal stability of wild-type and mutants were studied by Circular Dichroism (CD) and Fluorescence spectroscopy. Furthermore, binding affinity and kinetics of Cue domain and mutants with monoubiquitin was evaluated by surface plasmon resonance (SPR). Molecular modelling, simulation, normal mode analysis, principal component analy-

#4547 Structural, thermodynamics and kinetics role of novel hot-spot mutations of BCR-ABL1 in resistance towards 'ibs' inhibitors. Erik Laurini, Domenico Marson, Silvia Boccardo, Maurizio Fermeglia, Sabrina Pricl. Universita di Trieste, Trieste, Italy.

The present contribution integrates structural, computational, and molecular biology techniques to understand the eventual role of our newly reported hot-spot mutations of the BCR-ABL1 KD in TKI resistance observed in CML patients (1) using a 'two-tier level' investigation: isolated KDs and SH2-like SH3-KD BCR-ABL1 structures. Specifically, in silico experiments will disclose the effects exerted by the "hot-spot" BCR-ABL1 mutations on the protein structure, thermodynamic stability, and ability to interfere with BCR-ABL1 binding to specific TKIs (e.g., imatinib, dasatinib, and ponatinib). Structural biology (e.g., SAXS) evidences of wild type and mutant isolated KDs and SH2-linker SH3-KD (SSK) constructs (per se and in complex with TKIs) will be discussed to support in silico predictions. Direct drug binding thermodynamics and kinetics to both KD and SSK constructs will be measured using isothermal titration calorimetry and surface plasmon resonance. In vitro kinase assays will monitor the ability of these mutant isoforms to auto-phosphorylate and to phosphorylate a substrate peptide. (1) Giobbons DL, Pricl S, Posocco P, et al., Molecular dynamics reveal BCR-ABL1 polymutants as a unique mechanism of resistance to PAN-BCR-ABL1 kinase inhibitor therapy. Proc Natl Acad Sci U S A. 2014 Mar 4;111(9):3550-5. doi:10.1073/pnas.1321173111

#4548 Uncovering the membrane targeting mechanism of human unconventional class I myosins: a computational study. Daniel Gruffat, Shanee Singh. CUNY Brooklyn College, Brooklyn, NY.

The class I myosins (Myo1) are a widely expressed family of single-headed, non-filament-forming, membrane-binding myosins that comprise several sub-classes and are characterized by their ability to cross-link the plasma membrane to the underlying actin cytoskeleton by the presence of a lipid-binding region in the tail domain and an actin-binding region in the motor domain. Consequently, these motor proteins are drivers of numerous cellular processes that link the membranes to the actin cytoskeleton, including but not limited to vesicle trafficking, phagocytosis, cell migration, and intracellular membrane trafficking. Their importance in regulating these processes is underscored by studies that link pathogenesis and cancer with this class of motor proteins. For example, it has been recently shown that aberrant expression of Myo1b is linked to the lymph node metastasis of human head and neck squamous cell carcinoma through enhanced cancer cell motility and therefore, an interesting target for new diagnostic and therapeutic strategies for patients with this type of cancer, the sixth most common malignancy worldwide. Our focus in this study is the little studied aspect of membrane targeting in the different sub-classes for Myo1, even though it is critical part of the functionality of these proteins. Biological membranes are an intricate system consisting of many structurally discrete protein and lipids that serve as interaction sites with lipid binding domains of soluble proteins. The pleckstrin homology (PH) domain is one of the most common structural folds that serve as a perfect scaffold for cytosolic proteins to bind and target membranes. Although conventional sequence analysis doesn’t identify any membrane binding domains in the tails of Myo1 proteins, recent studies of Myo c and Myo lg implicate the presence of a PH domain and attribute this domain to their membrane targeting function. A preliminary analysis based on a combination of sequence analysis and secondary structure prediction tools, suggested that Myo1b and several other sub-classes also contain a PH domain. We hypothesize that the membrane targeting function of all Myo1 sub-classes is driven by the presence of a PH domain within their tails. To address this hypothesis, we have carried out a comprehensive investigation of the Myo1 tails from all human unconventional class I myosin isoforms sub-classes to identify, model, and analyze the membrane binding mechanism of their PH domains. This study details the membrane binding mechanisms of unconventional class I myosins and lays the foundation for the role of membrane targeting of Myo1 proteins in cancer and disease.


Background: In clinical practice of complex diseases such as cancer it is a challenging task to correctly identify driving factors and specific interactions between multitudes of genomic and clinical variables. In cancer where patterns are heterogeneous and involve highly complex interactions of biological pathways, this is by far a non-trivial task. Using novel approaches in machine learning, we demonstrate the ability to quantitatively and accurately reveal and describe patterns in genomic and clinical data. Method: We developed a computational framework based on a number of interacting learners performing extensive pattern search and data characterization. This framework is multilevel and involves steps of relevant data transformations. We formulate and apply contextually relevant criteria to distinguish and rank iteratively obtained findings and identify hidden relations between features and characteristic connections to the clinical outcome. Summary: We successfully tested our method on the public data of Glioblastoma Multiforme (GBM) from The Cancer Genome Atlas (TCGA). We identify interacting sets of genomic variables to represent clinically relevant subgroups of samples. We observe a dense presence of interacting known cellular processes in GBM such as EGFR, FGFR, ERK, RAS, MAPK, PI3, as well as novel such as calcium signaling, kinase motor and protein kinase pathways, tubulin and microtubule binding, phosphor esters/diacylglycerol binding domain (C1 domain), purine ribonucleoside triphosphate binding, positive regulation of phospholipase activity and others. The top ranking pathways recap the well-known and new biology. In addition, compared to known genes in the literature, we also observe involvement of novel genes in GBM subtype characterization. Finally, we propose two-fold solution: identification of distinctive patterns in the input genomic and output clinical data, and specific format of their interactions.

#4550 IODNE: an integrated optimization method for identifying the de-regulated sub-network for precision medicine in cancer. Lijun Cheng, Sai Mounika Inavolu, Milan Radovich, Varshini Vasudevaraja. Indiana University, Indianapolis, IN.

Background and Purpose: Sub-network analysis can explore complex patterns of the entire molecular pathways and networks for the drug target identification, and therefore drug selection. Computational algorithms are needed to optimize the sub-network search for molecular pathways and networks under certain conditions. Materials and Methods: In the paper, the gene expression profile of a cohort breast cancer patients and protein-protein interaction (PPI) network are integrated with edge score and node score simultaneously. A novel optimization algorithm, IODNE, is developed to search for the optimal de-regulated subnetwork of merging gene and protein network. The application of IODNE is demonstrated for a sub-network of drug target selection in breast cancer subtypes Luminal-A/Luminal-B data from Cancer Genome Atlas (TCGA). Where, the PPI networks data selected from Pathway Commons. Results: IODNE selects sub-network, which contain EPHBP4 and HER2, as the well-known targets in clinic Luminal-A/Luminal-B breast cancer subtypes. This validates the utility of IODNE. When applying IODNE to the triple-negative breast cancer subtype data, we identify sub-networks that contain genes such as ERBB2, HRAS, PGR, CAD, POL2 and SLC2A1. Conclusion: Using the known drug targets in breast cancer subtypes, we demonstrate that IODNE is a robust and powerful algorithm to identify sub-networks for drug target identification. Based on the gene expression profiles and protein interaction networks, it detects the optimum deregulated subnetwork for the candidate drug target selection. The IODNE is promising tool for the precision medicine.

#4551 Divergence analysis with coarse coding of omics data across cancer phenotypes. Wikum Dinalankara, Qian Qe, Lanlan Ji, Yiran Xu, Nicole Pagune, Francesco Lobo, Laurent Younes, Donald Geman, Luigi Marzichonz, John Hopkins Univ. School of Medicine, Baltimore, MD; Johns Hopkins University, Baltimore, MD; Federal University of Minas Gerais, Bele Horizonte, Brazil.

Motivation: Complex cancer omics data can be difficult to interpret and analyze with standard statistical methods. We thereby propose an innovative data representation that drastically reduces complexity while improving usability and interpretability for complex cancer phenotype analysis. Method: Despite recent advances in omics technologies, the robustness of predictive biomarkers in cancer remains severely limited. We hypothesize that this is primarily due to an overemphasis on applying statistical learning methods without taking into
consideration the underlying biological processes driving cancer. We therefore propose a new approach based on representing data based on the comparison to a baseline group. This results in a data format that encodes biologically meaningful information and can be easily analyzed. We apply this transformation to publicly available datasets obtained across multiple tumor types using different omics technologies. For each cancer phenotype considered, we perform equal or better classification performance than standard methods. Further, we show that our simplified data representation filters out much of the biologically irrelevant variation and that the resulting data can be successfully applied to gene set analysis applications, ultimately improving inference on disease phenotypes. For instance, by applying our method to signaling pathways and cancer hallmarks gene sets, we show that our approach can be used to detect dysregulated pathways more efficiently than with traditional methods. Conclusion: By comparing cancer omics data to a baseline status, we obtain a much simpler data representation that preserves biologically relevant information while eliminating much of the unwanted variance that is often confounding in the analysis of high-dimensional data. Furthermore, data represented using our approach can be easily analyzed and compared to other studies or to the baseline, and it is less sensitive to external variables. We have thus presented a method with improved sensitivity to assess statistical significance for cancer outlier profile analysis of gene expression.

YeSpace® Cohort Analyzer platform (formerly known as NextBio Clinical). Holden, 2 Vladimir Torchilin1.

#4552 ssCOPA: a novel method with improved sensitivity to assess statistical significance for cancer outlier profile analysis of gene expression. Hong Gao, Sam Ng, Hinco J. Gierman. Illumina, San Diego, CA.

To understand the genetic mechanism of disease, finding biomarkers and drug targets are a major focus of academic and pharmaceutical research. However, it has become clear that many important genes driving diseases like cancer, are deregulated in a relatively small group of outliers, e.g. amplified and fusion genes. Cancer outlier profile analysis (COPA), introduced by Tomlins et al, has raised interest in detecting genes with outlier expression within a group, particularly in cancer patients. However, the magnitudes of the COPA scores are not directly comparable across different studies or with different input parameters. Therefore, integrating COPA scores across multiple studies in a meta-analysis is problematic. Current solutions to performing meta-analyses, including rank-based methods and binomial test, require large study numbers without adjustment for the differences in study size. Also, within a single study COPA scores alone do not provide a P-value, and it is impossible to know which outlier genes are statistically significant. Here we developed three novel methods to address these problems by computing a significance for COPA scores, including Bootstrapping resampling, Bootstrap + generalized Pareto distribution (GPD) and Binomial method for statistical significance of COPA (ssCOPA), to enable the comparison of COPA score across studies or parameters. We first evaluated our methods using two breast cancer studies. The P-values generated by Binomial method and ssCOPA are better than Bootstrap + GPD. In addition, the Binomial method requires significantly less computing time than the other methods. We further validated our methods with a meta-analysis of five breast cancer studies and compared the results with the top-ranking genes of a large-scale meta-analysis of 31 breast cancer studies by ONCOMINE. We show that our P-value-based approaches (P = 10^{-17}–10^{-20}) achieves significantly better concordance than the Binomial test (P = 10^{-15}). Data and methods are available for use on the Illumina Bas eSpace® CohortAnalyzer platform (formerly known as NextBio Clinical).

#4553 Development of a 4-sample version of the Kolmogorov-Smirnov test for evaluating the temporal physiology of cells treated with test compounds in a label-free, high-content, platform for quantitative analysis of adherent cell-culture models. Ed Luther, 1 Livia Mendes, 1 Jiayi Pan, 1 Elena Holden, 2 Vladimir Torchilin 1. 1Northeastern University, Boston, MA; 2SeeCyte Biotechnology, Boston, MA.

Our objective is to develop multi-functional nanotechnology-based anti-tumor drug delivery systems for improving the efficacy of treatments and reducing undesirable side effects. The essential part of this process is the development and validation of un-biased, quantitative analytical techniques. We employed a newly developed holographic imaging cytometry system HoloMonitor® M4 for label-free time-lapse cellular analysis (Phase Holographic Imaging, Sweden). In previous work we have demonstrated our ability to obtain quantitative, high content cellular feature data congruent to data obtained from traditional label-based systems. We applied a modified version of the Kolmogorov-Smirnov 2-sample test. The classic test takes control and test frequency distributions (histograms), and converts them to probability functions. The maximum vertical displacement between the two is reported as the D-value, to determine if the two distributions are significantly different. In modified versions of the test, it is asked whether we have obtained the same D-value between the control and test normalized probability functions. These histograms are termed Brownian Bridges, where the end points are fixed at a value of zero, and the function is free to vary in between, in either up-going, down-going or mixed. Now, we report the development of a 4-sample Kolmogorov based method. Two modified K.S. tests are performed to compare distributions A to A, and B to B. It is assumed that all the distributions have the same time basis, and each time point is processed sequentially. The resulting time point pairs are plotted as a vector in a two parameter scattergram, tracing the outlines of the probability distribution of the cell populations through time. Unlike, the Brownian Bridge histograms, here, the starting and the ending points are both at the same location (0,0), within the two dimensional scattergram, but are free to move in both positive and negative directions away from the origin. HeLa cells in a 12-well MatTek plate were adhered and treated with Paclitaxel (PCT) in concentrations from 0 to 40 nM in 5-fold increments, either pulsed for 4 hrs, followed by 48 hours of imaging or with continuous exposure for 48 hours of imaging. We obtained a variety of different plot types, including continuous vs. pulsed comparisons, inter dosage comparisons, as well as the ability to compare multiple treatment groups. We also report our approach to determine synergy claims.

#4554 Unified theoretical algorithms for graphics dynamic multiple transformations of dose-effect relationships with computer simulation. Ting-Chao Chou. Memorial Sloan-Kettering Cancer Center, New York, NY.

The (phar)maco-chemical principles of the mass-action whole-body system analysis (arrangement & combinatorial) several hundred rate equations have been derived and published. Mathematical induction allows the deduction of the entities within the unified general median-effect equation (MEE) that makes dose & effect ‘interchangeable’ for the 1st order and higher order dynamics. Based on the MEE plot, all dose-effect curves can be ‘linearized’ which allows the quantitative determination of x-intercept (log Dm which signifies potency) and slope (which signifies shape or dynamic order). Extension of MEE to multi-drug combinations resulted the combination index (CI) theorem which quantifies synergism (CI<1), additive effect (CI=1), and antagonism (CI>1). This unprecedented approach and multi-step logics have been summarized in [Chou TC. Pharmacol Rev 58: 621-681, 2006] which has been cited 2,161 times in 485 bio-medical journals (http://www.researcherid.com/rid/B4111-2009 as of 10.19.2016). Dose-effect curves (single & multi-drugs) can be quantitatively transformed into the MEE plot, Fa–Ci plot, isobologram, Fa-Dri Plot for dose-reduction index, and when n=2, the polygonogram. This presentation will illustrate of combinations (1:5) of doctaxel (TXT) with compound against HCT-116 colon carcinoma xenograft in nude mice. In all, only 66 nude mice have been used, i.e. TXT doses, T3 doses and TXT+T4 doses, plus a control group, each N=6. The MEE “Linearization” allows the “Econo-Green Bio-research”, with small experimental size and small number of data points, using small number of animals. The algorithms based computer simulation using CompuSyn software can be completed within one second after the dose and effect data entries. The CompuSyn has been offered for free download upon registration via www.compsyn.com.

#4554A Simple, efficient, and quantitative approach for determination of synergism, additive effect, and antagonism of drugs in vivo using combination index method: a proposition for clinical protocol design and regulatory synergy claims. Jianing Fu, 1 Ting-Chao Chou. 1Columbia University Medical Center, New York, NY; 2Memorial Sloan-Kettering Cancer Center, New York, NY.

Based on the systematic dynamic rate derivations with the mass-action principle and mathematical induction–deduction, the median-effect equation of Chou, and the combination index (CI) equation and their algorithm allow quantitative determination of synergism (CI<1), additive effect (CI=1) and antagonism (CI>1) (Pharmacol Rev 58:621-681, 2006). Based on Google Scholar Citations, the CI simulation has been cited 7,712 times in >800 bio-medical journals as of 10.19.2016. However, most citing papers were with experiments in vitro, relative few in vivo and rarely in clinical trials. This is despite the fact that the CI method equation, algorithm, and the CompuSyn software are the same for both in vitro and in vivo, although the practical aspects such as sample size, time and cost, the readiness of the end-point measurement, and the ethical consider-
coupled with the ability to genetically engineer NDV to express therapeutic propagation, tumour-selective replication, and immunostimulatory properties) further enhanced in models that permit greater replication and also when combined with fibrosarcoma xenograft model a single administration (intra-tumoural or systemic). Demonstrated that NDV treatment has robust anti-tumour activity. In a HT1080 immortalised fibroblast cell line, infection with MEDI5395 results in the increased production and release of pro-inflammatory cytokines and chemokines which are able to recruit mediators of both the innate and adaptive immune responses. MEDI5395 is a potent activator of the type I interferon pathway through the activation of the IFNAR1 receptor and induces the production of IFN-γ. MEDI5395 selectively replicates and kills a wide variety of human and mouse tumour cell lines. Additionally infection of cancer cell lines with MEDI5395 results in the upregulation of chemokine receptors and chemokine expression, which is able to recruit immune cells to the tumour microenvironment. Using reverse genetics, we have generated a recombinant strain of NDV that expresses an immunostimulatory transgene, namely macrophage colony-stimulating factor (GM-CSF). We have evaluated the biological characteristics of recNDV-GM-CSF (MEDI3935) in vivo and in vitro. MEDI3935 selectively replicates in and kills a wide variety of human and mouse tumour cell lines. Additionally injection of cancer cells with MEDI3935 results in the increased production and secretion of pro-inflammatory cytokines and chemokines which are able to recruit mediators of both the innate and adaptive immune responses. MEDI3935 is a potent activator of the type I interferon response. In vivo, using a range of syngeneic xenograft models we have demonstrated that NDV treatment has robust anti-tumour activity. In a HT1080 fibrosarcoma xenograft model a single administration (intra-tumoural or systemic) was able to cure 80% of tumour bearing mice. In syngeneic mouse tumour models, which support minimal viral replication MEDI3935 treatment causes significant changes in the local immune suppressive environment and results in long-lasting anti-tumour immune responses. These responses are further enhanced in models that permit greater replication and also when combined with immune checkpoint blockade. The inherent properties of NDV (self-propagation, tumour-selective replication, and immunostimulatory properties) coupled with the ability to genetically engineer NDV to express therapeutic transgenes may provide a multi-modal attack on the tumour, delivering greater benefit to patients.

**IMMUNOLOGY: Clinical Immunotherapy, Viruses, and Bacteria**


Oncolytic viruses are live, replication-competent viruses that infect and/or replicate selectively in tumour cells leading to the destruction of the infected cell. Cell lysis occurs as a natural consequence of the viral life cycle and released virions can infect and kill neighbouring tumour cells leading to an amplified therapeutic effect. Oncolyasis has the added benefit of releasing multiple tumour antigens that may further induce an immune-mediated therapeutic response. Newcastle Disease Virus (NDV) is an avian paramyxovirus, which has proven safety and demonstrated efficacy against a variety of preclinical cancer models and in Ph clinical studies as an oncolytic agent, oncolysate or whole cell vaccine. Using reverse genetics, we have generated a recombinant strain of NDV that overcomes environmental and regulatory concerns uncoupling oncolytic potency and avian pathogenicity. Furthermore we have enhanced the immune modulatory properties of NDV by engineering the virus to express granulocyte macrophage colony-stimulating factor (GM-CSF). We have evaluated the biological characteristics of recNDV-GM-CSF (MEDI3935) in vivo and in vitro. MEDI3935 selectively replicates in and kills a wide variety of human and mouse tumour cell lines. Additionally injection of cancer cells with MEDI3935 results in the increased production and secretion of pro-inflammatory cytokines and chemokines which are able to recruit mediators of both the innate and adaptive immune responses. MEDI3935 is a potent activator of the type I interferon response. In vivo, using a range of syngeneic xenograft models we have demonstrated that NDV treatment has robust anti-tumour activity. In a HT1080 fibrosarcoma xenograft model a single administration (intra-tumoural or systemic) was able to cure 80% of tumour bearing mice. In syngeneic mouse tumour models, which support minimal viral replication MEDI3935 treatment causes significant changes in the local immune suppressive environment and results in long-lasting anti-tumour immune responses. These responses are further enhanced in models that permit greater replication and also when combined with immune checkpoint blockade. The inherent properties of NDV (self-propagation, tumour-selective replication, and immunostimulatory properties) coupled with the ability to genetically engineer NDV to express therapeutic transgenes may provide a multi-modal attack on the tumour, delivering greater benefit to patients.

#4557 Tumor immune profiling identifies multiple unique therapeutic targets that improve vaccination + oncolytic virotherapy against metastatic ovarian cancer. A) Robert McGrat,1 Cheryl Eppolito,1 Anthony Miliotto,1 Raya Huang,1 Kelly Singel,1 Jonathan Pol,2 Kyle Stephenson,2 Brahim H. Segal,1 Brian Lichty,1 Kunle Odunsi1. 1Roswell Park Cancer Institute, Buffalo, NY; 2McMaster University, Hamilton, Ontario, Canada.

While spectral responses to cancer immunotherapy have been observed in some patients, the majority of responses are short-lived with ultimate tumor relapse. Therefore, it is crucial to identify strategies that will effectively synergize with immunotherapy to improve treatment outcome. Using a pre-clinical mouse model, we explored the use of a potent heterologous prime/boost vaccine strategy for the treatment of metastatic intraperitoneal ovarian cancer. Priming with an adjuvant based vaccine followed by boosting with a novel oncolytic Maraba viral vector elicited robust tumor-specific CD8+ T cell responses, with high numbers of therapy-induced CD8+ T cells effectively trafficking to the tumor microenvironment. While this approach greatly improved tumor control and long-term survival compared to treatment with the priming vaccine alone, the combination therapy was not curative and cellular analysis suggested that T cells within the tumor microenvironment were functionally suppressed/exhausted. We reasoned that a combination therapy that would have the potential to further enhance tumor attack following prime/boost vaccination. Using this strategy, we observed that checkpoint blockade using nivolumab in combination with prime/boost vaccination resulted in a dramatic improvement in tumor control, as did transient depletion of granulocytic myeloid cells (but not monocytes/macrophages) following treatment. Current studies are underway that combine prime/boost vaccination with relevant co-stimulatory agonist antibodies, as well as inhibitors of candidate chemokine networks identified through tumor profiling. These findings underscore the importance of designing treatment strategies that not only elicit robust anti-tumor T cell responses, but also improve the duration and/or magnitude of immune attack within the tumor microenvironment. Additionally, our data suggest ways of interrogating the tumor microenvironment during treatment can identify unique therapeutic targets that have the potential to further improve therapeutic impact.

#4558 Immunoactivity and anti-tumor efficacy of live attenuated Salmonella typhimurium-based oral T-cell vaccines VX0M1m, VX0M4m and VX0M6m. Sébastien Wieckowski,1 Lilli Podola,2 Marco Springer,3 Iris Kobli,1 Zina Koob,4 Caroline Migaud,5 Amine Adda-Barkane,5 Ming Wei,1 Albrecht Meisel,1 Klaus Breuner,1 Philipp Hofmann,1 Heinz Lubena,1 1VAXIMM AG, Basel, Switzerland; 2University Hospital Regensburg, Regensburg, Germany; 3VAXIMM GmbH, Mannheim, Germany; 4Oncodesign S.A., Dijon, France; 5CellVax S.A.S., Romainville, France.

Significant progresses have been recently accomplished in immuno-oncology and in the development of cancer immunotherapies. However, novel solutions are necessary to overcome the peripheral tolerance and the immunosuppressive tumor microenvironment that prevent the eradication of cancer. VAXIMM is developing first-in-kind Salmonella typhi Ty21a-based oral T-cell vaccination platform for the initiation of anti-tumor cellular immune responses via a unique mode-of-action. This study summarizes the immunogenicity and preclinical anti-cancer efficacy for Salmonella typhimurium murine vaccines VX0M1m, VX0M4m and VX0M6m which encode murine vascular endothelial growth factor receptor 2 (VEGFR2), mesothelin (MSLN) and Wilms’ tumor 1 (WT1) protein antigens, respectively. Immunokinetic studies were performed in healthy animals treated with 10^10 CFU/dose of either the control Salmonella typhimurium empty vector VX0M0m_empty, or Salmonella typhimurium VX0M1m, VX0M4m or VX0M6m, and antigen-specific T cells were detected with MHC class 1 pentamers by flow cytometry in the spleen. The anti-tumor efficacy of VX0M1m and VX0M4m was evaluated in the Panc02 syngeneic model of pancreatic adenocarcinoma overexpressing MSLN, and the anti-cancer activity of VX0M6m was evaluated in the FBL-3 disseminated model of erythroleukaemia expressing WT1, in a prime-boost setting. Treatment with each of the vaccines VX0M1m, VX0M4m and VX0M6m induce a peak antigen-specific systemic immune response 7 to 10 days after the last vaccination. Treatment of Panc02 tumor-bearing mice with VX0M1m and VX0M4m single agents resulted in a significant reduction in the tumor growth rate, compared to the control group, with a median T/C of 37.6% and 19.4% respectively, 35 days after tumor challenge. Treatment of mice bearing FBL-3 leukemia with VX0M6m generated a rapid and sustained anti-tumor effect with 100% (7 out of 10) of surviving animals 175 days after leukemia challenge. In contrast, treatment with VX0M0m_empty did not show any anti-cancer effect, with a median survival of 45 days and 0% (0 out of 10) of cancer regression. VX0M1m, VX0M4m and VX0M6m were tolerated at the effective doses and have demonstrated consistent anti-cancer activities with substantial T cell responses in different animal tumor models. This study provides further evidence that VAXIMM’s versatile oral T-cell vaccination platform can be used to stimulate anti-tumor immunity against various tumor-associated antigens. Further studies of VAXIMM’s cancer vaccine candidates, as monotherapy as well as in combination, are warranted.
E2-CTL reactivity was that data mined publicly available OPC transcriptomes (University of Michigan assessed the association of F. nucleatum (negative, low, or high) with tumor epidemiology database of 1,027 colon and rectal cancer cases in the pathologicalepidemiologydatabaseof1,027colonandrectalcancercasesinthe Nurses’ Health Study and the Health Professionals Follow-up Study. We assessed the association of F. nucleatum (negative, low, or high) with tumor immunostatusestatusinstraofMSUSTatus.WemeasuredF.nucleatumDNAintrumortissueusingquantitativePCR.Asimmunostatus,weevaluatedhystopathological lymphocytic reaction (tumor-infiltrating lymphocytes [TIL], intratumoralperiglandularreaction,peritumorallymphocyticreaction,orCrolin’s-likemyeloidreaction)andmeasuredTcelldensities(CD3+,CD8+,CD45RO+orFOXP3+cells)usingimmunohistochemistryandcomputer-assistedimageanalysis.Logistic regression model was used to adjust for potential confounders, including CpG island methylator phenotype, LINE-1 methylation level, and KRAS, BRAF, and PIK3CA mutations. Using whole-exome sequenc-ing data (N = 586), we conducted secondary analyses to assess the interaction of F. nucleatum with a neoantigen load. Results: The association between F. nucleatum and TIL differed by MSI status (P for interaction = 0.0017; with the adjusted α level of 0.0066). Compared with F. nucleatum-negative cases, F. nucleatum-positive cases were associated with low-level TIL in MSI-high tumors (odds ratio, 0.40 [95% confidence interval, 0.17-0.92] for F. nucleatum low and high cases, respectively), but not in microsatellite stable/MSI-low tumors. We did not find any interaction between F. nucleatum and MSI status in T cell subsets. The association between F. nucleatum and TIL did not differ significantly by levels of a neoantigen load (P for interaction = 0.015). Conclusions: The association of F. nucleatum with the immunosuppressive microenvironment may be stronger in colorectal cancer cases with high-level MSI. Our findings suggest the interplay between F. nucleatum and MSI status in host immune response to the tumor.

#4561 Non-integrative lentiviral particles for immunotherapy: RNA delivery to drive tumoral antigen presentation in a safe and efficient way. Pascale Bouillé,1 Christine Duthoit,2 Nicolas Martin,3 Lucille Lamouroux,4 Jean-Christophe Pages5. 1VECTALYS and FLASHCELL, Toulouse, France; 2VECTALYS, Toulouse, France; 3INSERM U966 Faculté de Médecine, Tours, France.

Safe and efficient cancer therapies using adoptive transfer of engineered cells are very promising but very challenging approaches. Opportunities to improve gene transfer into primary cells involve a better design of the vectors used. Such improvements must lead to an increase of the efficiency of gene expression, the cell phenotype preservation and the increase of the gene number delivered. The use of lentiviral vectors has largely increased in clinical protocols over the past few years but safety concerns have slowed down this progression. Actually, the permanent genetic modification remains a focus of significant regulatory oversight. On another side, mRNA delivery is a versatile, flexible, and safe mean for protein therapies, but existing techniques, such as chemical or electroporation-based transfection protocols, are known to induce cell toxicity and phenotype modifications of the target cells. Here, we present an innovative tool, named LentiFlash, allowing RNA delivery into target cells without any genomic scar. This tool combines RNA delivery, providing the best expression system to get efficient and transient expression, and lentiviral delivery which exhibits the best efficiency of entry into primary and stem cells. The RNA encapsidation is mediated via an RNA/protein interaction using properties of the MS2 bacteriophage. This new vector breaks with all existing systems. The resulting lentiviral particle is able to efficiently deliver non-viral coding or non-coding RNA into the cytoplasm of any cell type. These LentiFlash particles, are able to transduce delicate primary cells, such as dendritic cells, T cells and hematopoietic stem cells. They show an efficient, fast and transient expression of protein and RNA, such as antigenic peptides or genome editing tools, with no cell phenotype modification. Here we show a proof of concept on primary murine dendritic cells transduction with LentiFlash particles, successfully used to deliver several tumor antigens. As a result, these dendritic cells are able to present the antigens to the immune system which in turn can efficiently fight a tumor development into wild type mice. This new delivery system gives the opportunity for multiple simultaneous expression to enhance immune response and for combining tumor relapse. Such multiple co-expressions into immune cells are expected to mimic the innate and adaptive immune responses. This transient RNA delivery mediated by LentiFlash is a powerful tool to induce an efficient gene delivery. The possibility to express multiple genes at once in the target cells is an attractive therapeutic perspective. The absence of any viral sequence avoids any integration event, an important safety consideration for human use. Finally, another advantage of the LentiFlash system is its ability to utilize lentiviral production platforms already validated in clinical settings.


Newcastle Disease Virus (NDV) is an avian paramyxovirus, which has significant oncolytic activity against mammalian cancers. It is multi-modal in its anti-tumour activity and offers a tumour selective, self-propagating therapeutic with oncolytic activity and immunostimulatory properties. Within the tumour itself, NDV infection is able to modulate the immune suppressive microenvironment and induce positive T cell tumour inflammatory responses. Additionally, we have enhanced the immune modulatory properties of NDV by engineering the virus to express granulocyte/macrophage colony-stimulating factor (GM-CSF). To better understand the immune modulatory mechanisms by which NDV infection is able to alter the tumour microenvironment we investigated the responses
of normal human PBMCs and isolated immune cell populations following NDV exposure. Using flow cytometry, cytokine and gene expression analysis we demonstrated activation of the innate immune cells and a robust type I IFN and pro-inflammatory response. 24 hours post infection, innate immune cells (macrophages, natural killer cells and dendritic cells) had upregulated cell surface activation markers and secreted high levels of cytokines in a dose-dependent fashion. Furthermore, by using NDV constructs encoding fluorescent proteins we showed, in a specific subset of myeloid cells, the preferential uptake of virus and subsequent self-limiting viral replication. In a co-culture system, these infected myeloid cells were able to function as ‘virus cellular carriers’ and were able to mediate the efficient transfer of infectious NDV to tumour cells resulting in their oncolysis. We further investigated the ability of NDV to infect, spread and kill tumour cells in vivo, we utilised tissue slice cultures of fresh patient samples. These studies demonstrated virus replication and transgene expression in tumour slices, as well as the uptake of virus in a small fraction of specific cells in slices of normal liver. Moreover, evidence that NDV was able to alter the tumour microenvironment could be demonstrated in such cultures by sampling the culture supernatants over time. By investigating the immune modulatory properties of NDV in vitro and in vivo we will gain greater insight and understanding of the anti-cancer properties of NDV. This work will also help guide the selection of transgenes for next generation approaches to augment the inherent immunostimulatory properties of NDV, and help inform clinical dosing options such as intravenous infusion or cell-based delivery.

Local and abscopal effects in oncolytic virotherapy are boosted by immune checkpoint blockade, immunogenic chemotherapy, or IFNAR blockade. Laetitia Fend, 1 Takahiro Yamazaki, 1 Xavier Prévillé, 1 Eric Quémeur, 1 Oliver Kepp, 3 Julien Adam, 2 Aurélien Marabelle, 2 Jonathan Pitt, 2 Guido Kroemer, 2 Laurence Zitvogel, 2. TRANSGENE S.A., Illkirch Graffenstaden, France; 3Institut Gustave Roussy, Villejuif, France; Centre de recherche des Cordeliers, Paris, France. Although the clinical efficacy of oncolytic viruses has been demonstrated for local treatment, the ability to induce immune-mediated regression of distant metastases is still poorly documented. We here report that an engineered oncolytic Vaccinia Virus, VV_wq-TK-RR–Fcu1, is able to induce an immunogenic cell death and thus to generate a systemic immune response. Effect on tumor growth and survival is largely driven by CD8+ T-cells, and we could demonstrate that the immune cell infiltrate in the tumor could be reprogrammed towards a higher ratio of effector T-cells to regulatory CD4+ T-cells. The key role of the type I-IFN pathway in oncolytic virotherapy was also highlighted, and we could show a strong abscopal response in Ifnar−/− tumors. In this model, the single administration of the virus directly into the tumors, on one flank, led to regression in the contralateral flank (i.e. opposite to the virus injection site). Moreover, we observed that these effects are further enhanced when the oncolytic treatment is combined with either immunogenic chemotherapy such as oxaliplatin, or with immune checkpoint blockers such as anti-PD-1 or anti-CTLA-4. Altogether, these data suggest that local oncolytic virotherapy in patients with tumors infiltrated by IFNAR signaling could increase immune-mediated abscopal regression of distant metastases.

Synergistic anti-tumor efficacy of combination therapy with APS001F, a cytosine deaminase (CD) expressing Bifidobacterium, 5-fluorocytosine (5-FC) and anti-mPD-1 antibody in syngeneic mice model. Koichiro Shioya, 1 Yuji Seki, 1 Tomio Matsumura, 1 Yuko Shimatani, 1 Shun’ichiro Taniguchi, 2 Minoru Fujimori. 1Ananoptpharma Science, Inc., matsumoto, Japan; 2Department of Comprehensive Cancer Therapy, Shinshu University School of Medicine, matsumoto, Japan; 3Department of Breast Surgery, Tokyo Medical University Ibaraki Medical Center, Ibaraki, Japan. Immune checkpoint blockers like anti-PD-1 antibody are leading the new generation in anti-cancer drug development. A lot of scientific evidences have demonstrated that immune checkpoint antibodies have promising clinical response in patients with multiple cancer types. Nevertheless, limited fraction of patients respond to these antibody immunotherapy, and moreover, immune related serious adverse events caused by nonspecific reaction with immune checkpoint blockers result in treatment discontinuation. Many approaches have been attempted to improve therapeutic outcomes of antibody immunotherapy, particularly a combination of antibody treatment and other therapies is one of worth-trying ideas. APS001F is a live recombinant Bifidobacterium longum expressing cytosine deaminase (CD), which is an enzyme to catalyse the hydrolytic deamination of cytosine to uracil. Bifidobacterium is a non-pathogenic anaerobic bacterium derived from normal human intestinal flora. It can only survive and grow at low oxygen environment such as solid tumor. Intravenous administration of APS001F to patients leads to colonization of the B.longum and production of CD enzyme only inside the solid tumor. Locally expressed CD enzyme converts orally taken prodrug, 5-FC, into anti-tumor drug 5-FU, which increases 5-FU concentration specifically inside tumor and results in alleviation of side effects caused by attacking normal organs. The phase 1 clinical trial for APS001F is conducted in the US sponsored by us. 5-FU has been shown to alleviate Myeloid Dendritic Cell Suppressor Cells (MDSC) in the tumor site and release immune suppression in tumor [1]. Furthermore, Van Der Kraak et. al. have reported that 5-FU upregulated PD-L1 expression on the surface of tumor cells [2]. These findings drove us to combine anti-PD-1 antibody with the APS001F therapy. At this present study, systemic administration of APS001F and 5-FC significantly suppressed tumor growth in CT26 bearing syngeneic mice model, which is consistent with our previous finding. Additionally, anti-murine PD-1 antibody combined with APS001F and 5-FC further suppressed tumor progression and some of individual samples achieved complete regression of CT26 tumors. The combination treatment effect of anti-murine PD-1 antibody and APS001F with 5-FC showed statistical significance in comparison with either treatment group, which encourages us to expect much effective results and less side effects in clinic. Reference: 1. Vincent J. et al., Cancer Res. 2010 Apr 15; 70(8):3052-61, 5-Fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T-cell dependent antitumor immunity. 2. Van Der Kraak et. al., J Immunother Cancer, 2016 Oct 18;4:65. 5-Fluorouracil upregulates cell surface B7-H1 (PD-L1) expression in gastrointestinal cancers.

Forced expression of GITR in cancer cells enhances adenosviruses-mediated in situ vaccination. Yisel A. Rivera-Molina, Francisco Puerta Martínez, Teresa Nguyen, Hong Jiang, Xuejun Fan, Rehnuma Shifat, Mohammad Belayat Hossain, Verlene K. Henry, Caroline C. Carrillo, Candelaria Gomez-Manzano, Juan Fueyo. MD Anderson Cancer Center, Houston, TX. Our approach involves a platform of killing cancer using more potent oncolytic viruses-based immunotherapy strategies. These replication competent adenoviruses are targeted to the Rb pathway to generate tumor-selectivity. The second generation of these therapeutic agents, Delta-24-RGD, was successfully translated to the clinical setting and is currently been tested in Phase I studies in several institutions in the USA and in Europe for the treatment of patients suffering from recurrent gliomas. Preliminary data from these clinical trials showed that 10 to 15% of Delta-24-RGD-treated tumors undergo complete regression. Agonistic treatments targeting co-stimulatory tumor necrosis factor receptor superfamily (TNFRSF), such as GITR (CD357), have been shown to enhance the proliferation and activation of T cells. Moreover, in preclinical tumor efficacy studies, these agonistic signals have shown potent tumoricidal activity. Different from antibodies, co-stimulatory ligands can be easily incorporated into replication competent oncolytic adenoviruses. Infection of cancer cells with these armed viruses will lead to the expression in their cell membranes of the co-stimulatory molecule that will directly interact with the tumor infiltrating lymphocytes to amplify and enhance the anti-tumor T-cell activity. In this study, we have developed an armed Delta-24-RGD carrying the cDNA of the mouse GITR, Delta-24-GREAT. Treatment of glioma-bearing mice with intracranial injection of Delta-24-GREAT increased mice survival (P < 0.0001, long-rank test) and inhibited lung metastasis in subcutaneous models. Infection of the tumor elicited an inflammatory response increasing the populations of CD4+ and CD8+ T cells versus treated controls. Importantly, 2 weeks after the adenovirus treatment a subset of brain hemispheric cells were positive for GITR. In addition, co-culture experiments with tumor cells infected with viruses and splenocytes isolated from treated glioma-bearing mice demonstrated a response against the cancer cells as assessed by ELISA analyses of IFN-γ. Demonstrating the generation of anti-tumor memory, the surviving animals did not show evidence of tumor growth after re-challenging with GL261 glioma cell implantation in the contralateral hemisphere. However, survivors of GL261 tumors did not survive after the re-challenge was performed by intracranial implantation of B16/F10 melanoma cells strongly indicating that the immune response was specific for GL261 glioma antigens. This is the first study with an oncolytic adenovirus expressing GITR and our results strongly indicate that oncolytic adenoviruses armed with the molecules of the TNFRSF may be of future clinical interest for the treatment of patients with cancer.

OncoVEXgsGM-CSF (HSV-1 modified similarly to Talimogene Laha-repavepc) in combination with CTLA-4 blockade leads to both local and systemic efficacy in a murine syngeneic model of metastatic melanoma. Kees- gan Cooke, Juan Estrada, Jinghui Zhan, David Hill, Andrea Boden, Jon Werner, Pedro J. Beltran. Amgen. Thousand Oaks, CA. Talimogene laherparepvec is an oncolytic immunotherapy based on a modified herpes simplex virus type 1 (HSV-1) designed to kill cancer cells through...
two mechanisms: a) direct viral-mediated lysis and b) stimulation of a tumor antigen-specific adaptive immune response. We developed a mouse model of metastatic melanoma using the B16F10 cell line to study local and systemic responses following treatment with talmogene laherparepvec alone or in combination with CTLA-4 blockade. OncoVEXGM-CSF, an HSV-1 modified similarly to talmogene laherparepvec (mGM-CSF is expressed instead of human GM-CSF) was used in these studies. B16F0 cells have been reported to be resistant to HSV-1 infection due to a lack of requisite entry receptors. To overcome resistance, we transduced B16F10 cells with a lentiviral vector expressing mouse nectin 1 (mNectin1) or GFP as a control. Expression was confirmed by ddPCR and sensitivity to OncoVEXGM-CSF evaluated in an in vitro viability assay. B16F10-mNectin1 cells were highly sensitive to OncoVEXGM-CSF with a multiplicity of infection (MOI) IC_{50} of 0.001 (B16F10-eGFP were insensitive at MOI of 100). In vivo, B16F0-mNectin1 and B16F0-eGFP cells showed similar growth when injected subcutaneously. OncoVEXmGM-CSF treatment of B16F10-mNectin1 tumors (intratumoral, 5x10^6 PFU/dose, 3X) caused a significant outcome and may provide a way to guide chemotherapy.

Moreover, patients who were treated by taxanes in WIR group had a shorter OS (95%CI 1.03-1.47, p = 0.001) compared to control mice that received intravenous B16F10-eGFP cells only. In conclusion, OncoVEXmGM-CSF in combination with CTLA-4 blockade significantly reduced subcutaneous tumor growth and lung metastasis. The combination of both therapies resulted in significantly greater local and systemic efficacy than either agent alone. The strong local and systemic anti-tumor activity of the combination resulted in a significant increase in median overall survival (p = 0.0001) compared to control mice that received intravenous B16F10-eGFP cells only. In conclusion, OncoVEXmGM-CSF in combination with CTLA-4 blockade significantly reduced systemic tumor burden and prolonged median overall survival in a B16F0 mouse model of metastatic melanoma. These data support the proposed MOA by which OncoVEXmGM-CSF treatment can cause direct tumor lysis along with potentiation of an adaptive, systemic anti-tumor immune response.

**4567 Immunoclassification of gastric cancer in the context of clinical outcome.** Welli Wang,1 Ping Liao,1 Shanchen Xie,1 Dongya Shen,1 Chengfang Zhou,1 Howard McLeod,2 YiJing He1,2

**1Institute of Clinical Pharmacology, Central South University, Changsha, China; 2 Moffitt Cancer Center, DeBartolo Family Personalized Medicine Institute, Tampa, FL.**

**Background:** Gastric cancer (GC) plays a leading role in all cancer deaths especially in Eastern Asia. Current classifications including WHO, Lauren, and TCGA defined molecular classification have illuminated the clinicopathological characteristics or genetic profile of GC. However, these classifications were lack of association with clinical outcome and guidance for medication selection. **Objective:** We aimed to identify a new immunoclassification for GC to predict patient’s prognosis and provide evidence for choosing proper medication. 

**Methods:** To identify significant biomarker and significant embedded (FFPE) gene expression data were used to find evidence for the influence of smoking on the composition of intratumoral immune cell fractions and their prognostic impact. The analysis was confined to samples hybridized to the Affymetrix HG-U133 Plus 2.0 (GEO accession number GPL570). Expression profiles were downloaded and curated for duplicates and corrupt files. Different immune cell fractions were estimated using CIBERSORT and the LM22 leukocyte signature matrix. We combined this information with available clinical data. We used Cox regression analyses to evaluate the prognostic impact of the different immune fractions and Mann-Whitney U tests to test for significant differences. Results: Smokers with NSCLC had a significantly (p = 0.05) higher percentage of plasma cells, CD8 + T-cells, active CD4 + T-cells, follicular helper cells, monocyttes, M0 macrophages, resting dendritic cells, active mast cells and neutrophils compared to non-smokers. Smokers had a lower percentage of naive B-cells, memory B-cells, resting CD4 + T-cells and resting mast cells. Non-smokers significantly benefit from a higher percentage of intratumoral memory B-cells in the immune infiltrate as compared to normal tissue (HR 0.92, 95% CI 0.85-1.00, p = 0.049), while the smokers significantly benefit from higher intratumoral immune fractions of resting CD4 + T-cells (HR 0.93, 95% CI 0.89-0.98, p = 0.001). Higher percentages of regulatory T-cells (HR 1.23, 95% CI 1.03-1.47, p = 0.025), neutrophils (HR 1.11, 95% CI 1.02-1.21, p = 0.014) and follicular helper cells (HR 1.19, 95% CI 1.01-1.38, p = 0.031) have a significant detrimental effect on survival of smokers. There was a borderline beneficial effect for smokers of the intratumoral NK cell fraction (HR 0.61, 95% CI 0.36-1.02, p = 0.057). For non-smokers, a detrimental effect was observed for the intratumoral plasma cell fraction (HR 1.04, 95% CI 1.00-1.09, p = 0.073) and the naive B-cell fraction (HR 1.17, 95% CI 0.99-1.39, p = 0.075). Conclusion: In NSCLC, significant different composition of intratumoral immune cells were observed between a smoking and non-smoking population. These differences were associated with differences in overall survival.

**4570 Intratumoral IFN-α gene transfer reduces trafficking of Tregs into tumor by inhibition of CCL17 expression.** Kazunori Aoki, Chihiro Shibasaki, Hisayoshi Hashimoto, Kenta Narumi, Chie Kudo, Hisayoshi Hashimoto, Kenta Narumi, Chie Kudo.

**Institute of Clinical Pharmacology, Kanta Narumi, Chie Kudo.**

**Introduction:** The immune system plays an important role in tumor progression and treatment response as well as with new immune modulating therapies. It is unclear in lung cancer what the effect of smoking is on intratumoral immune cell composition and their function. In our study publicly available expression data were used to find evidence for the influence of smoking on the composition of intratumoral immune cell fractions and their prognostic impact. Methods: We searched the Gene Expression Omnibus (GEO) for human non-small cell lung cancer (NSCLC) samples. Analysis was confined to samples hybridized to the Affymetrix HG-U133 Plus 2.0 (GEO accession number GPL570) platforms. Expression profiles were downloaded and curated for duplicates and corrupt files. Different immune cell fractions were estimated using CIBERSORT and the LM22 leukocyte signature matrix. We combined this information with available clinical data. We used Cox regression analyses to evaluate the prognostic impact of the different immune fractions and Mann-Whitney U tests to test for significant differences. Results: Smokers with NSCLC had a significantly (p < 0.05) higher percentage of plasma cells, CD8 + T-cells, active CD4 + T-cells, follicular helper cells, monocyttes, M0 macrophages, resting dendritic cells, active mast cells and neutrophils compared to non-smokers. Smokers had a lower percentage of naive B-cells, memory B-cells, resting CD4 + T-cells and resting mast cells. Non-smokers significantly benefit from a higher percentage of intratumoral memory B-cells in the immune infiltrate as compared to normal tissue (HR 0.92, 95% CI 0.85-1.00, p = 0.049), while the smokers significantly benefit from higher intratumoral immune fractions of resting CD4 + T-cells (HR 0.93, 95% CI 0.89-0.98, p = 0.001). Higher percentages of regulatory T-cells (HR 1.23, 95% CI 1.03-1.47, p = 0.025), neutrophils (HR 1.11, 95% CI 1.02-1.21, p = 0.014) and follicular helper cells (HR 1.19, 95% CI 1.01-1.38, p = 0.031) have a significant detrimental effect on survival of smokers. There was a borderline beneficial effect for smokers of the intratumoral NK cell fraction (HR 0.61, 95% CI 0.36-1.02, p = 0.057). For non-smokers, a detrimental effect was observed for the intratumoral plasma cell fraction (HR 1.04, 95% CI 1.00-1.09, p = 0.073) and the naive B-cell fraction (HR 1.17, 95% CI 0.99-1.39, p = 0.075). Conclusion: In NSCLC, significant different composition of intratumoral immune cells were observed between a smoking and non-smoking population. These differences were associated with differences in overall survival.
control vector injection. We hypothesized that Treg-homing cytokines such as CCL17 and CCL22 cytokines are involved in the reduction of tumor-infiltrating Tregs by the Ad-mILN injection. The IFN-α expression significantly suppressed CCL17 and CCL22 expression of tumors, and the expression of CCR4 and CCR5 expression on Tregs was not changed. IFN-α protein significantly suppressed CCL17 of 2T26s in vitro in a dose-dependent manner. Therefore, CCL17 shRNA-transduced 2T26 cells (2T26shCCL17) to examine the immunological role of CCL17 in vivo. The frequency of Tregs per CD4+ T cells was significantly reduced in subcutaneous tumors of 2T26shCCL17 cells (25.9%), and tumor growth was inhibited compared with that of control shRNA-transduced 2T26 cells (39.4%). Suggesting CCL17 has an important role in tumor growth compared to migration and proliferation of Tregs. The study demonstrated that IFN-α gene delivery creates an environment strongly supporting the enhancement of antitumor immunity through the suppression of Tregs.

#4571 Advantage of dendritic cells for the therapeutic cancer vaccine. Koji Nagaoka, Akihiro Hosoi, Tamaki Lino, Hirokazu Matsushita, Kazuhiro Kakimi. The University of Tokyo Hospital, Tokyo, Japan.

Background: To develop effective cancer vaccine, we examined CTLs induced by peptide vaccination (CTL pep) and DC vaccination (CTL DC) in terms of their antitumor effects. Methods: For preparation of peptide vaccine, 50 µg hgp100 peptide (KVPQRNQDWL) and 1x10^7 Pmel-1 splenocytes were transferred. Then, mice were vaccinated on days 0 and 14. For preparation of DC vaccine, 50 µg CpG were emulsified with 50 µl CFA or IFA. Bone marrow-derived DCs were matured with LPS and pulsed with hgp100 peptide at 1 µg/ml and used as DC vaccine. To induce CTL pep and CTL DC, C57BL/6 mice were first injected with 1 x 10^7 naive spleen cells from Pmel-1 TCR transgenic mice to increase hgp100-specific CTLprecursors. Then mice were vaccinated twice with 2 weeks interval. The induction of CTLs were evaluated 1 week after the second vaccination. To evaluate their antitumor activity, 1 x 10^6 B16F10 were inoculated into vaccinated mice (prophylactic model). For therapeutic model, 1 x 10^6 B16F10 were inoculated into mice on day 0. Five days later 1 x 10^5 Pmel-1 splenocytes were transferred. Then, mice were vaccinated on days 5 and 12, followed by the measurement of tumor growth. Results: DC vaccine induced 5-6 times more CTLs than peptide vaccine. The numbers of CTL pep and CTL DC obtained in the spleen were 2.4 ± 0.2 x 10^6 and 1.2 ± 0.4 x 10^6, respectively. CTL pep expressed PD-1 and Tim-3 at higher level than CTL DC. Most of CTL pep showed effector memory phenotype (CD44hiCD62L-) while CTL DC contained a considerable fraction of central memory cells (CD44hiCD62L). Although both CTL pep and CTL DC produce IFN-γ and TNF-α at similar level, more CD107a+ were detected on CTL DC than CTL pep upon re-stimulation. CTL DC displayed better proliferation potential than CTL pep. These phenotypic differences were confirmed by the comprehensive gene expression analysis. Transcriptome analysis revealed that considerable portion of CTL pep and CTL DC were comparable and different from that of naive CTLs. However, several genes were expressed differentially: CTL pep expressed higher amount of inhibitory receptors, such as Pdcd1, Lag3, Ctl4a and Tigit than CTL DC. CTLDC expressed Fbp1, which inhibits glycolysis, and Cpt1a, which is the rate-limiting enzyme of mitochondrial fatty acid oxidation, suggesting that their metabolic statuses were shifted from glycolysis to fatty acid oxidation. DC vaccine completely inhibited the tumor growth in prophylactic vaccine setting, while peptide vaccine delayed tumor growth compared to control mice. In therapeutic protocol, peptide vaccines showed no antitumor effect, while DC vaccine significantly suppressed tumor growth. The numbers of intratumoral CTL pep and CTL DC on day 19 were 5.2 ± 2.8 x 10^5/g and 5.0 ± 1.4 x 10^5/g, respectively. Intratumoral CTL DC expressed lower levels of PD-1, Tim-3 and LAG-3 and more Ki67 than CTL pep. Furthermore, Intratumoral CTL DC produce more IFN-γ and TNF-α than CTL pep. Conclusion: DC vaccine induced intratumoral CTLs with multi-functionality and high proliferation potential. Therefore, DC vaccine is our choice for the therapeutic cancer vaccine.


Introduction: In the tumor micro-environment, extracellular ATP is sequentially hydrolyzed to adenosine by the ecto-nucleotidases CD39 (ATP→ADP) and CD73 (AMP→adenosine). Adenosine, through activation of the A2A receptor (A2AR), is a potent inhibitor of T-cell activation, resulting in an immunosuppressed phenotype. Thus, inhibition of A2AR has recently generated great interest in immuno-oncology. We present the characterization of a novel, selective, and highly potent small molecule antagonist of A2AR which is slated to enter clinical trial in 2017. Methods: The cellular potency of A2AR antagonists was assessed as a function of decreased AMP levels in CHO cells stably over-expressing hA2AR, a Ga coupled receptor, following stimulation with the agonist NECA. Experiments were conducted in the presence and absence of human serum. Selectivity against the Gi-coupled receptor A1R was assessed similarly as a function of CAMP elevation in CHO cells stably expressing hA1R, following pretreatment with forskolin and stimulation with NECA. The ability of AB928 to reverse the inhibitory effects of adenosine on lymphocyte proliferation and IFN-gamma and TNF-alpha production was assessed using the hA2AR transfected murine T-cell hybridoma 2C8. The ability of AB928 to inhibit the potassium ion channel hERG was assessed similarly as a function of IC50 in CHO cells stably expressing hA1R, following pretreatment with the agonist NECA. AB928 fully reversed the activity of adenosine to suppress CD8+ T cell activation as indicated by CD25 expression and increased levels of cytokines such as IFN-γ and IL-2 in the supernatants (p<0.01 vs 25 µM adenosine only). AB928 inhibited mouse A2AR, as indicted by reversal of adenosine-mediated immune suppression in the CD8+ T-cell activation assay. Pharmacokinetic characterization of AB928 showed it to be orally bioavailable with characteristics suitable for human dosing that will allow for treatable dose levels. Conclusions: AB928 is a potent, selective and peripherally restricted antagonist of the A2A receptor which is slated to enter clinical development in 2017.

#4573 Characteristics and outcomes of patients with advanced sarcoma enrolled in early phase immunotherapy trials. Roman Groisberg, Vivek Subbiah, David Hong, Filip Janku, Sarina Piha-Paul, Aung Naing, Robert Benjamin, Shreyas Kumar Patal, Neeta Somaiah, Anthony Conley. UT MD Anderson Cancer Center, Houston, TX.

Background: Immunotherapies, specifically those based on immune checkpoint inhibitors, have shown promising activity in multiple tumor types. Other than the drug mifamurtide for osteosarcoma, there are currently no approved immunotherapies for sarcomas. We analyzed the outcomes of patients with advanced sarcomas treated in immunotherapy phase 1 trials. Methods: We analyzed the medical records of patients with advanced sarcoma who were referred to Phase 1 trials and had received at least one dose of an immunotherapy including checkpoint inhibitors, vaccines, or cytokine based therapies. Clinical parameters reviewed were age, histology, lactate dehydrogenase, albumin, metastatic sites, performance status, prior therapies, toxicity and response on imaging. Progressive free survival (PFS) was measured from the date of the first dose to the date of progression on imaging. Results: Among 50 patients enrolled in immunotherapy trials we found 14 different subtypes of sarcomas (Bone 10; Soft tissue 40). Patients were 42% male and 58% female. The median age was 53.5 years (18-84) and Royal Marsden Hospital (RMH) prognostic score was 0.08. Grade3/4adverseeventsincludedrash(10%),fever(6%),fatigue(6%),diabetes(4%),andnausea/vomiting(6%). Other toxicities included hypothyroidism, transaminitis, pancreatitis, pituitary apoplisty, pneumonitis and mucositis. Conclusions: Immunotherapies were well tolerated in advanced sarcoma with 92% of patients able to complete 28 days of therapy. The patients had been heavily pretreated and had good performance status and favorable RMH prognostic scores. All ASPS patients had clinical benefit with checkpoint inhibitors and this was the only subtype with a partial response. Further evaluation of checkpoint inhibitors in sarcomas, especially ASPS, is warranted.

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we hypothesized that macrophages have the potential to be a powerful cellular tumor setting has not been observed. Barriers to T cell entry and function may profound results in hematologic malignancies but clinical efficacy in the solid Scholler,1 Carl June,1 Saar Gill1.

Cytosed and cleared cognate antigen-bearing tumor cells. To demonstrate the cytosis and luciferase-based specific killing assays. CARMA selectively phagocytosed and killed HER2+ expressing ovarian and breast cancer cells themselves; instead, it requires a good match between our compound and a “sensitive” tumor microenvironment. We found that within “TAM-rich” solid tumors, an effective SMDC may be one with dual mechanisms of action that affect both FRe-positive tumor cells and FRβ-positive TAMs. Moreover, the process of choosing partner drugs, such as immune checkpoint inhibitors and inhibitors of myeloid-derived suppressor cells, should be guided by the immunomodulatory properties of both SMDCs and SOC agents to overcome the frequently observed chemo- and immuno-resistance.

#4575 Chimeric antigen receptor macrophages (CARMA) for adoptive cellular immunotherapy of solid tumors. Michael Klichinsky1, Marco Ruella,1 Olga Shestova,1 Saad S. Kenderian,2 Miriam Y. Kim,3 Roddy O’Connor,1 John Scholler,2 Carl June,2 Saar Gill.1 University of Pennsylvania, Philadelphia, PA; Mayo Clinic, Rochester, MN.

Chimeric antigen receptor (CAR) T cell immunotherapy has demonstrated profound results in hematologic malignancies but clinical efficacy in the solid tumor setting has not been observed. Barriers to T cell entry and function may partially explain this observation. As solid tumors actively recruit myeloid cells, we hypothesized that macrophages have the potential to be a powerful cellular immunotherapeutic agent in this setting if properly activated and redirected. We here describe the development of CAR macrophages (CARMA), demonstrating the feasibility, mechanism, and efficacy of this platform. To examine the function of CARs in macrophages, first generation anti-CD19, anti-mesothelin, or anti-HER2 CARs with a CD3ζ intracellular domain were introduced into the THP1 macrophage model. In vitro function was assessed via quantitative phagocytosis and luciferase-based specific killing assays. CARMA selectively phagocyted and cleared cognate antigen-bearing tumor cells. To demonstrate the requirement for CAR-mediated intracellular signaling for activity, a CD3ζ null CAR construct was tested in vitro. The deletion of CD3ζ significantly reduced the phagocytic and killing capacity (p<0.01) of CARMA. We identified Ad5S5, a chimeric adenovirus, as a novel and highly efficient viral vector for the transduction of normal donor and cancer patient macrophages (>70% CAR expression). Ad5S5 transduction polarized human macrophages toward a durable immunomodulatory M1 phenotype and rendered CARMA resistant to subversion toward the immunosuppressive M2 phenotype, as defined by surface markers and metabolomics. CARMA enhanced the proliferative capacity of CD8+ T cells in phytohemagglutinin activation assays and secreted factors that activated by-stander macrophages. Primary human anti-HER2 CARMA demonstrated targeted phagocytosis and killing of HER2 expressing ovarian and breast cancer cell lines, and exhibited a six-fold higher luciferase-based killing capacity of SKOV3 cells compared to trastuzumab in vitro (p=0.002). Anti-HER2 CARMA was evaluated in vivo in an intraperitoneal (IP) SKOV3 ovarian cancer xenograft model. Mice that received IP CARMA had a decrease in tumor burden of approximately two orders of magnitude and had a 30-day survival benefit relative to untreated or control macrophage treated mice (p=0.018). In a systemically disseminated SKOV3 model, a single dose of IV CARMA led to a durable anti-tumor response (38-fold reduction relative to control on day 31 post-treatment; p=0.016). Lastly, we demonstrated that the blockade of the anti-phagocytic CD47/SIRPα axis enhanced the phagocytic capacity of CARMA. In summary, we here demonstrate that human macrophages engineered with a CAR exhibit targeted anti-tumor function in both in vitro and in vivo preclinical models. This novel cellular immunotherapeutic approach has a clear translational potential for the treatment of solid tumors.

#4576 Targeted human cytolytic fusion proteins: an update. Stefan Barth. University of Cape Town, Observatory, South Africa.

Targeted immunotherapeutics, such as antibody drug conjugates (ADCs) or immunotoxins (ITs) represent promising agents for treatment of cancer. Despite their encouraging performance in clinical trials, both ADCs and ITs suffer from disadvantages like stoichiometrically undefined chemical linkage of the cytotoxic payload and potential immunogenicity of bacteria- or plant-derived toxins in humans, respectively. Therefore, in contrast to depleting B cell epitopes from bacterial toxins, we designed a number of targeted recombinant cytolytic fusion proteins, replacing bacterial toxins like Pseudomonas exotoxin A by human enzymes like proteases, kinases, RNases or microtubuli-associated proteins. Binding and biological activities were shown in vitro, ex vivo and in different in vivo models. This presentation will summarize the latest results on the hCEPs generated and demonstrate the efficacy of treatment of CD64-, CSPG4-, and EpCam-targeting constructs in direct comparison to the corresponding Pseudomonas exotoxin-based CAR constructs. The presentation will conclude with an outlook on future perspective and applications of the next generation of targeted fully human recombinant cytolytic fusion proteins.

#4577 Appropriately delivered curcumin causes recruitment of natural killer cells into glioblastoma brain, stabilizing M1 polarization of tumor-associated microglia. Sumit Mukherjee,1 Angela Fried,2 Rahman Hussaini,2 Richard White,2 Aheli Chatterjee,2 Probal Banerjee,2 City University of New York Graduate Center, New York, NY; 2City University of New York College of Staten Island, Staten Island, NY; 3City University of New York at The City College of New York, New York, NY.

Glioblastoma (GBM) is the most insidious form of primary adult brain tumor with a mean life expectancy of 12-24 months. Previously, we have used the non-invasive strategies of (1) intranasal delivery of a glioblastoma-directed curcumin (CC) adduct (CC-CD68AB) and (2) the intraperitoneal (i.p.) infusion of a lipid-encapsulated formulation of CC-phosytoxin to rescue orthotopically GL261-implemented GBM mice and study the effect of CC on the phenotype of the tumor-associated microglial cells (TAMs). Both treatment regimens not only caused tumor remission in 50-60% of GL261-implemented GBM mice, but also induced a dramatic change in the tumor-associated Iba1+ TAMs, suppressing the tumor-promoting Arginase1high,IL10high,iNOSlow,IL12lowM2-type TAMs, while simultaneously inducing the Arginase1low,IL10low,INOSlow,IL12high M1-type TAMs. Concurrently, we observed a marked induction and phosphorylation-mediated activation of microglial p65 NF-κB and STAT1, with concomitant suppression and inactivation of STAT3:p-STAT1high, p-STAT3high M1 microglia are known to IL12-dependent recruitment/activation of tumoricidal NKp46high natural killer (NK) cells. In determining the kinetics of CC-induced M1 repolarization of M2-TAMs, we observed that five days of CC treatment (i.p.) was sufficient to induce a dramatic change in the tumor-associated Iba1+ TAMs, inhibiting the tumor-promoting M2-type TAM population, while activating M1-type microglia. As expected, we observed a concomitant recruitment of NKp46high NK cells into the GBM tumor. Cognizant of the fact that NK cells activate and stabilize the M1–microglia, we eliminated the NK cells in these GBM mice using the NK cell-targeted antibody NKL1.1 Ab to observe a significant reduction in CC-evoked repolarization of TAMs. Our results demonstrate a unique oncoimmunotherapeutic function of CC, which when appropriately delivered, not only eliminates GBM directly, but also indirectly elicits recruitment and activation of tumoricidal NK cells and M1-TAMs. Furthermore, maintenance of this CC-evoked M1 phenotype is contingent upon CC-induced recruitment and activation of NK cells. Thus, when delivered appropriately, CC functions on GBM both directly as well as by stimulating and recruiting an army of immune cells that eliminate both GBM and GBM-initiating cells.

#4578 Does poor prognosis in African Americans with MS-H colorectal cancer associate with altered immune markers. Umamaheshwari Golconda,1 Lena Sokol,2 Babak Shokrani,1 Edward Lee,1 Donna Hanzel,3 Oluwolue Fadare,1 Sandip Patel,4 Mehdi Nousarie,3 Hooman Soleimani,1 Zaki Sharif,1 Ali Afarsi,1 Fared Darenpouiran,2 Hassan Brim,1 Hassan Askhtorab1,1 Howard Univ, Washington, DC; 2University of Bern, Bern, Switzerland; 3UCSD, San Diego, CA; 4UC San Diego Moores Cancer Center, La Jolla, CA; 5Univ. of Pittsburgh, Pittsburgh, PA.

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BACKGROUND: Microsatellite instability high (MSI-H) of sporadic colorectal carcinomas (CRC) is usually associated with improved prognosis and a high density of tumor-infiltrating lymphocytes. However, African Americans with MSI-H have poor prognosis. AIM: To evaluate whether or not expression of different immune and tumor markers individually or in combination in African American MSI-H CRC associate with the prognosis status. METHODS: Tissues Microarray (TMA) were prepared by microdissection from FFPE (Formalin Fixed Paraffin Embedded) blocks of 15 MSI-H patients. Immune markers (CD8+, Granzymes, Perforins, CD4+, STAT1, IRF1 and IRF5) and PD-L1 status were analyzed by immunohistochemistry (IHC). Demography and clinical data including TNM, tumor grading (WHO standard), histological type of the tumor, and time of diagnosis, date of the last follow-up examination, treatment, comorbidities, metastasis, recurrence, 5-year disease-free survival and death were collected. RESULTS: There were 4 patients with improved prognosis (27%) with relatively high CD4 density (2 with >50% and 2 with 11-50%) regardless of PD-L1 status (3+/1+), stage (II,III), and other immune markers (CD8, CD4, Granzymes, Perforins, IRF1, IRF5 and STAT1). One of the improved prognosis patients with KRAS mutation has elevated expression of all considered immune markers besides CD4. There were 9 patients (6 dead, 3 alive) with poor prognosis, with different immune and tumor markers level (3 were PD-L1+ and 5 have relatively high CD4 count; but with low level of STAT1. There were also 2 patients with expected improved prognosis based on their immune markers level (stage II), however, they died within the 5 year period post-diagnosis, due to their age (80 and 87 old). Conclusion: African American patients have poor prognosis which may correlate with the nature of tumor-associated immune response. Other factors such as MSH3 defects might cancel the positive prognosis of MSI-H status. Nonetheless, low STAT1 and low CD4 may be indicators of poorer prognosis.

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The FDA granted an IND for a 40 patient study entitled “A phase II study of treatment with oral mifepristone as salvage therapy in patients with advanced or metastatic non-small cell lung cancer who have failed 2 or more previous chemotherapy or immunotherapy regimens”. Presented herein is a description of the effect of treating Case 1 who is a 68 year old male who presented with stage IV metastatic non-small cell lung cancer causing 10 months of treatment with single agent oral mifepristone (Korlym from Corcept Inc) 300mg qd. He had shown tumor progression despite treatment with carboplatin, docetaxel, premetrexed, and gemcitabine. He was not considered a candidate for nivolumab or pembrolizumab because his tumor was negative for the programmed cell death factor ligand 1 (PD-1-1) marker. His preclinical performance status was 2, and he started on daily 300mg of mifepristone treatment. After 2 months of treatment he showed a considerable improvement in energy and stamina and less shortness of breath. There have been no new lung or brain lesions. In fact, there has been shrinkage of the lung lesions and no growth of the brain lesion and no more seizures. His ECOG was “0” after 10 months of single agent mifepristone treatment. The mechanism of action is believed to be by suppressing intracellular production of an immunomodulatory protein known as the prostaglandin E2 induced blocking factor (PIBF). One of the functions of PIBF is to stabilize perforin granules in natural killer cells thus inhibiting their cytotoxic activity. This protein seems to be unique to rapidly growing cells, e.g., trophoblast cells or cancer cells. Thus, in general, in low dosages, as used in this patient, the drug is very well tolerated. PIFB does not need to be needed for cells growing at normal speeds, in our case, mifepristone has been found to either cause complete tumor regression, or more commonly, stabilization of the cancer with improved quality of life. Previously, mifepristone allowed long-term complete remission from a terminal small cell lung cancer with associated hyponaesthesia. The case report here is believed the first involving palliative benefit of treating advanced non-small cell lung cancer with mifepristone. Stabilization of the brain metastasis in our patient supports evidence from a previous case of advanced glioblastoma multiforme with a clear though transient response to mifepristone, that the drug can cross the blood-brain barrier, and thus be effective for brain lesions. This is the first case treated with 300mg daily mifepristone (200mg used previously). Similar to the lower dosage, this patient reported no adverse side effects. Mifepristone was also found to improve longevity and body conditioning scores in a placebo controlled study of spontaneous murine lung cancer.

4579 Improving the efficacy of radioimmunotherapy by controlling the tumor microenvironment of gastric cancer. Hae Young Ko,1 Jin Su Kim,1 HyoEnGi Kim,1 Seonhwa Lee,1 Chae-Ok Yun,2 Sang Moo Lim.1 1Korea Institute of Radiological & Medical Sciences, Seoul, Republic of Korea; 2Hanyang University, Seoul, Republic of Korea.

Purpose: Radioimmunotherapy (RIT) of solid tumor is limited to poor antibody distribution due to extracellular matrix (ECM) of tumor microenvironments. In this study, we investigated the effect of the protein X, which is known to degrade ECM, on the microdistribution of trastuzumab, on the accumulation and the microdistribution of trastuzumab in tumor tissue. In this study, we investigated the effect of the protein X, which is known to degrade ECM, on the microdistribution of trastuzumab, on the accumulation and the microdistribution of trastuzumab in tumor tissue. In the treatment group. Conclusion: This study demonstrates that the treatment of protein X significantly improved antibody accumulation of tumortissue and that can enhance the effect of RIT.

4581 Primacy pharmacology of TYG100, a vaccine employing a novel, antigen-specific checkpoint control mechanism (S-TIR) for pancreatic and gastroesophageal cancers. Jorge Sepulveda1, Christopher Taus,2 Liebthe C. Muddere-Boer,3 Robert L. Wardle,4 Paul Broome,4 Geert C. Mudde5, OncoQR ML GmbH, Vienna, Austria; 5East Carolina University, Greenville, NC; 2TYG Oncology, Nottingham, United Kingdom; 3Tyg Oncology & OncoQR ML GmbH, Vienna, Austria.

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4580 Improved quality of life and tumor regression in a patient with stage IV non-small cell lung cancer with brain metastasis 10 months after daily treatment with single agent mifepristone. Jerome H. Check,1 Diane Check,2 Mahmoud Aly,3 Patricia Lofberg,1 Rachael Cohen,1 Dwight McKee1.

1 OncoQR, 2 East Carolina University, Greenville, NC; 3TYG Oncology & OncoQR ML GmbH, Vienna, Austria.

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4580 Improved quality of life and tumor regression in a patient with stage IV non-small cell lung cancer with brain metastasis 10 months after daily treatment with single agent mifepristone. Jerome H. Check,1 Diane Check,2 Mahmoud Aly,3 Patricia Lofberg,1 Rachael Cohen,1 Dwight McKee1.

1 Cooper Medical School of Rowan University, Malrose Park, PA; 2Richmond University Medical Center, Staten Island, NY.
to sponsor and manage a first-in-human phase I study in patients with advanced solid tumours, evaluating safety, tolerance and immunogenicity. The study will be undertaken by the Experimental Cancer Medicine Centres network with the University of Leeds as lead centre. 1 Pancreas 2012 Apr;41(3):374-9 2 Abstract no 402, ASCO 2005 meeting publication. 3 Poster presentation, AACR 2014 annual meeting 7 Apr 2014

#4582 Evaluating benefits of PD-L1 image analysis for the clinical setting.

Tissue-based investigations can prove challenging due to complex tissue architecture and heterogeneous biomarker expression, visual and cognitive "traps" that affect interpretive precision, and subjective assessments that affect reproducibility. A major concern is that these challenges could increase the risk of failure for therapeutic/diagnostic co-development and clinical use, as the biomarker measurements continue to increase in complexity and require increasingly precise diagnostic cut-points. Image analysis tools have been developed to overcome some of the challenges for conventional anatomic pathology practices, capitalizing on the objectivity and computational power of a digital platform. A computer, however, lacks the cognitive ability and experience of a human to interpret tissue architecture and context. Flagship Biosciences' computational Tissue Analysis (cTA™) platform integrates the power of our tissue image Analysis (tIA™) technology with the contextual experience of an anatomic pathologist to produce robust, precise, quantitative results that demonstrate biomarker content in the tissue context. Flagship Biosciences envisions the integration of our cTA™ technology into a computer-aided clinical pathology workflow as a method to improve the precision of scoring for even some of the most challenging tissue-based biomarker measurements. In a proof-of-concept study, we evaluated the performance of manual versus digital scoring approaches in a cohort of non-small cell lung carcinoma (NSCLC) samples stained with the IHC protocol for the PD-L1 PharmDX 28-8 complementary diagnostic. A comparison of the 2 modalities demonstrated that in nearly all cases, the within sample standard deviation of the cTA™ digital score results was less than the manual score (median inter-pathologist %CVs were reduced from 124.9% to 7.8% and intra-pathologists from 65.4% to 7.6% for manual and digital scores, respectively). As an additional exploratory examination, the effect of heterogeneity on PD-L1 interpretation was also investigated. Pathologists evaluated the same whole tissue slices within 5 high powered fields (HPFs) using both manual and cTA™-derived scoring. Results demonstrated that the use of cTA™ provides improvement agreement between HPF and whole slide assessments (absolute difference between the manual scores from HPF to whole slide were larger than the absolute differences for the digitally derived scores, at 3.14% and 8.27%, respectively). Taken together, these studies demonstrate that the use of cTA™ can significantly reduce variability in PD-L1 scoring, as compared to a manual scoring approach.

#4583 Heterogeneity in immune biomarker expression: Detailed analysis of a case with SCLC transformation after EGFR-TKI treatment. Kenichi Suda,1 Issao Murakami,2 Hui Yu,1 Jiiey Kim,3 Kim Ellison,1 Christopher J. Riviard,1 Tetsuya Mitsudomi,4 Fred R. Hirsch1. University of Colorado Anschutz Medical Campus, Aurora, CO;2Higashi-Hiroshima Medical Center, Hiroshima Hiroshima City, Japan;3Kindai University Faculty of Medicine, Osaka, Japan;

Introduction: The expression status of immune markers are of current research interest due to their potential roles as predictive biomarkers for immunotherapy in cancers. Currently there is little information on how the microenvironment of tumors and/or therapy-induced histological transformation may affect the expression of these immune markers. Methods: A 76-year-old never-smoking female with epidermal growth factor receptor (EGFR) mutated lung adenocarcinoma (AC) acquired resistance to gefitinib. After her death, autopsy revealed small-cell lung cancer (SCLC) transformation and EGFR T790M secondary mutation as mutually exclusive resistance mechanisms. Two liver metastases (SCLC vs. AC with T790M) and two lymph node metastases (SCLC vs. AC with T790M) were analyzed to compare the expression status of immune markers by immunohistochemistry (IHC) and an immune-oncology gene expression panel (HTG EdgeSeq Immuno-Oncology Assay). Results: IHC analysis revealed that PD-L1 was expressed in 5% of tumor cells with AC histology (T790M) but not in tumor cells with SCLC transformation (Table 1). The liver metastasis with SCLC transformation showed negative stromal PD-L1 expression and scant tumor infiltrating lymphocytes, while the other lesions demonstrated stromal PD-L1 staining and infiltration of CD8-positive T cells. PD-L1 was positive in lymphocytes from lymph node metastases, but not in those from liver metastases. Data generated using an IO panel indicated higher expression of galectin 9 and higher level of T cell stimulatory checkpoints in lesions with SCLC transformation. Bioinformatic analysis also demonstrated lower expression of type I interferon regulated genes in lesions with SCLC transformation. Conclusion: These data highlight the heterogeneity of expression of immune markers depending on the metastatic sites and histological transformation, and indicate that a biopsy from one lesion may not be representative of immune marker status for all lesions.

#4584 High PD-1 expression on regulatory T cells in lung cancer draining lymph nodes. Rieke Van De Ven,1 Anna-Larissa N. Niemeijer,1 Anita G. Stam,1 Sayed M. Hashemi,3 Christian G. Stocker,1 Johannes M. Daniels,1 Erik Thunnissen,1 Egbert F. Smit,2 Tanja D. de Gruijl,1 Adrianus J. de Langen1.1 VU University Medical Center, Amsterdam, Netherlands; 2 Netherlands Cancer Institute, Amsterdam, Netherlands;

The treatment of advanced non-small cell lung cancer (NSCLC) with PD-1 immune checkpoint inhibitors has improved clinical outcome for a proportion of patients. The current challenge is to find biomarkers that will identify patients likely to benefit from this therapy. In this study we assessed the difference of T cell subsets and PD-1 expression levels on T cells in tumor-draining lymph nodes (TDLN), non-TDLN (NTDLN) and peripheral blood mononuclear cells (PBMC). To evaluate these, flow cytometric analyses were performed on endobronchial ultrasound-guided (EBUS) fine-needle aspirates (FNA) from TDNLN and NTDLN of patients with NSCLC and compared to PBMC. Our data show that the frequency of PD-1+ CD4+ and CD8+ T cells, as well as the PD-1 expression level on activated regulatory T (Treg) and CD4+ and CD8+ T cells, are higher in TDNLNs as compared to NTDLNs or PBMC. These elevated PD-1 expression levels in TDLN may reflect tumor-specific T cell priming and may serve as a predictive or early response biomarker during PD-1 checkpoint blockade.

#4585 Radiation combined with checkpoint blockades enhanced antitumor efficacy for osteosarcoma. Yutaka Takahashi, Tomohiro Yasui, Keisuke Tamari, Kazumasa Mimata, Masahiko Koizumi, Yui Sato, Fumiki Ichishiki, Keisuke Ohtani, Ryosuke Kambe, Kazuhiko Ogawa. Osaka University, Osaka, Japan.

Osteosarcoma is one of the common malignancies at bone in children and adolescent. Recent study demonstrated that combination of anti-PD-L1 and anti-CTLA-4 antibodies provided grade control of metastatic osteosarcoma. However, a strong local treatment strategy including surgery or high precision radiation therapy is necessary to eradicate osteosarcoma. Radiation therapy plays an important role in local control but previous studies demonstrated that radiation enhanced immune response in which not only local tumor regression at irradiated sites but also regression of metastatic tumor outside the radiation field was observed. Although this phenomenon, so called the abscopal effect, is rarely seen, recent studies demonstrated that combination of X-ray irradiation with checkpoint blockades provided higher probability of the abscopal effect for some kind of tumors. However, the effect of X-ray irradiation combined with checkpoint blockade on the abscopal effect for osteosarcoma has been totally unknown. We investigated whether local X-ray irradiation combined with the anti-PD-L1 and anti-CTLA-4 antibodies enhances local and distant antitumor efficacy for osteosarcoma. LM8 mouse osteosarcoma cells were inoculated into both legs of C3H mice. Mice were treated by 10 Gy X-ray irradiation alone to the tumor in the one side leg (RAD group) at day 12, 150 µg of anti-PD-L1 and anti-CTLA-4 antibodies (P1C4 group) at days 9, 12, and 15, or those combination (COMB group). Administration of anti-PD-L1 and anti-CTLA-4 antibodies provided tumor growth delay or complete response at day 37 for about 20% of the mice. X-ray irradiation strongly inhibited tumor growth at irradiated tumor but not in unirradiated tumor. On the other hand, the combination therapy provided the strongest tumor growth inhibition not only at irradiated site but also regression of metastatic tumor for about 89% of the mice. Accordingly, lung metastasis in mice in COMB group was strongly reduced by 97% with the significant survival benefit compared with the mice in P1C4 group. Flow cytometric analysis revealed that mice in COMB group significantly recruited CD8 tumor infiltrating lymphocytes with moderate reduction of regularly T cells (Treg), thereby increasing the CD69/Treg ratio. Furthermore, quantitative real time PCR showed significant induction of PD-L1 on irradiated LM8 cells in vitro and radiation-induced upregulations of PD-L1, B7-1, and B7-2, the ligands of CTLA-4, were also confirmed by flow cytometry, indicating that the enhanced efficacy anti-PD-L1 and CTLA-4 antibodies may associate with these upregulations by radiation. These results suggest that X-ray irradiation contributes to the enhancement of the efficacy for
IMMUNOLOGY: Clinical Immunotherapy, Viruses, and Bacteria

the distant metastasis as well as local control in the treatment of anti-PD-L1 and anti-CTLA-4 antibodies for osteosarcoma. Our data provide a rational to establish a new therapeutic strategy and start up a clinical trial for osteosarcoma.

IMMUNOLOGY: Immunoconjugates and Antibodies

#4586 Intratumoral Treg cell depletion by local administration of IL-2-Dipheria toxin fusion protein E7777 induces a therapeutic and memory anti-tumor immune response in preclinical models. Diana I. Albu,1 Christy Ingersoll,1 Kuan-Chun Huang,1 Mary Woodall-Jappe,1 Xingfeng Bao1,2.1 Eisai Andover Innovative Medicines Institute, Andover, MA; 2Eisai Oncology Business Group, Eisai Inc., Andover, MA.

T regulatory (Treg) cells play an important role in maintaining immunological tolerance to self-antigens, thus limiting immune responses to tumor antigens. Therefore, depleting or suppressing Tregs is one strategy by which anti-tumor immunity can be restored. The immunotoxin ONTAK® is an IL-2-Dipheria toxin fusion protein that has been shown to diminish Tregs in patients and animal models of cancer in peripheral blood using a systemic intravenous (i.v.) administration route. E7777 is a new version of ONTAK®. In this study we tested the hypothesis that locally-diminished Tregs by intratumoral (i.t.) administration of E7777 generate effective anti-tumor immune response at both local and systemic levels. First, we showed superior anti-tumor activity and safety of E7777 i.t. over E7777 i.v., where i.t. administration resulted in complete tumor regressions in both moderately immunogenic CT26 and non-immunogenic B16F10 tumors with minimal animal body weight loss. In contrast, only tumor growth delay was observed for E7777 i.v. with dose-limiting animal body weight reduction in the same models. Immune phenotyping showed a 4 fold reduction of intratumoral Tregs in treated CT-26 tumors without significant change of Tregs in the spleen of treated animals, confirming a local Treg-depleting effect of E7777 i.t. In contrast, intratumoral CD8+ T cells were not reduced. Second, E7777 i.t. enhanced overall anti-tumor immune response, manifested by significant increased numbers of CD45+ hematopoietic cells, Granzyme B+ CD8+ cytotoxic T cells, and ratios of cytotoxic T cells/Tregs in the treated tumors. Importantly, E7777 i.t. also resulted in distant effects in the spleen characterized by increased ratio of T lymphocytes to myeloid cells and increased frequencies of both effector memory CD8+ T cells (CD8+CD62L-CD44+) and central memory CD8+ T cells (CD8+CD62L+CD44+) indicative of systemic immune activation. Consistent with the generation of immunological memory, 60% of the tumor-free animals treated with E7777 i.t. rejected rechallenge with 40% displayed delayed growth of B16F10 cell challenge while all naïve control animals grew tumors. Taken together, our results demonstrate that intratumoral Treg depletion by local administration of E7777 leads to an effective local and memory anti-tumor response in preclinical models and support further evaluation of local E7777 delivery as a cancer immunotherapy.

#4587 A human hybrid immuno-oncology construct targeting the TWEEAK receptor Fn14 and containing the serine protease granzyme B. Ana Alvarez de Cienfuegos,1 Lawrence H. Cheung,1 Khalid A. A. Mohamedali,1 Jefrey A. Winkles,2 Michael G. Rosenblum,1 1MD Anderson Cancer Center, Houston, TX; 2University of Maryland School of Medicine, Baltimore, MD.

Targeting immune effector cells to tumors has recently emerged as a clinically-relevant approach for therapy. The underlying mechanism for immune effector cell challenge involves T or B-cell-mediated delivery of the serine protease granzyme B to the target cells. The cell-surface receptor Fn14 for the TNF-related cytokine TWEEAK has emerged as a potentially valuable target for cancer therapy because its expression is low in most normal tissues but significantly elevated in a variety of solid tumor types. The serine protease Granzyme B (GrB) is a highly cytotoxic component of human immune effector cells and induces multiple intense pro-apoptotic signals when delivered to the cytoplasm of target cells. This protein has been well-studied, operating through multimodal pathways which are both caspase-dependent and caspase-independent. Our laboratory has designed series of new Fn14-targeted fusion constructs containing an engineered GrB payload. The GrB-Fc-IT4 construct is a completely human homodimer (~200kDa) containing an IgG Fc domain for prolonged serum half-life. This construct displays high-level protein production in an HEK293E expression system. It exhibits high affinity and selective cytotoxicity within the nanomolar range (IC50 ranging from 4 to 284 nM) when tested against a panel of 25 Fn14-positive human cancer cell lines. We are currently assessing a panel of 100 annotated NSCLC cell lines to try to define a molecular fingerprint related to cellular sensitivity or resistance to the GrB payload. Pharmacokinetic studies in mice revealed that GrB-Fc-IT4 exhibited a bi-exponential clearance from plasma with a rapid initial clearance (t1/2a = 0.36 hours) followed by a prolonged terminal-phase plasma half-life (t1/2b = 35 hours). Mice bearing MDA-MB-231 orthotopic xenografted tumors and 40% displayed delayed tumorgrowth compared to GrB-Fc-IT4 construct (QODX5). On average, tumors from saline-treated mice grew about 10-fold over 40 days. In contrast, tumors from mice treated with GrB-Fc-IT4 did not grow during this period and remained the same size as at the onset of treatment. Moreover, 3/5 mice treated with GrB-Fc-IT4 showed complete tumor regression lasting beyond day 80 (end of the study). Overall, treatment with GrB-Fc-IT4 was well-tolerated by mice (no loss of body weight) and resulted in significant anti-tumor efficacy. Toxicology and bio-distribution studies are ongoing and will be presented. Research conducted, in part, by the Clayton Foundation for Research.

#4588 Novel anti-Trop-2 monoclonal antibodies with unique binding specificities show therapeutic synergy against most human cancers. Emanuela Guerra,1 Marco Trerotola,1 Valeria Relli,1 Chiara Pedicone,1 Antonella D’Amore,1 Francesca Dini,1 Silvia Fratarcangeli,1 Saverio Alberti1,2.1 “G. d’Annunzio” University of Chieti-Pescara, Chieti, Italy; 2UCI Chieti, Chieti, Italy.

Trop-2 is an epithelial transmembrane glycoprotein that transduces a calcium signal and activates a growth-signaling network that converges on AKT. Trop-2 is overexpressed in the majority of carcinomas, where it drives tumor cell proliferation and, in its mature, glycosylated and functional form, associates with worse prognosis. Trop-2 extracellular domain contains an N-terminal cysteine-rich globular region followed by a cysteine-less region as a connecting “stem” to the transmembrane domain. Trop-2 molecules engage in homophilic interactions between adjacent cells and establish multicellular complexes with tight junction proteins, which may hinder accessibility by therapeutic antibodies. Up to now, Trop-2-targeted approaches have employed anti-Trop-2 monoclonal antibodies (mAb) which essentially recognize a single immunodominant epitope poised between the globular and stem regions. Such mAb have limited or no therapeutic efficacy. In order to untap the potential of anti-Trop-2 immunotherapy we generated novel anti-Trop-2 mAb with tailored specificity towards the globular versus stem regions. Hybridoma diversity was maximized by immunization with soluble human Trop-2 extracellular region produced in different transformed mammalian cell lines (human 293 and murine L) and in insect cells/baculovirus expressing system. These were expected to provide native folding of Trop-2 together with a broad spectrum of differential glycosylation. Trop-2-binding hybridomas were further selected by multiple rounds of flow cytometry analysis using live 293 cells expressing different Trop-2 extracellular portions. Two classes of mAb were identified, that bound the stem versus the globular region. These mAb efficiently bound Trop-2 expressing cancer cells and were able to inhibit cell growth in vitro. In vivo the naked anti-globular OX-G64 and anti-stem OX-S55 mAb were most effective in inhibiting the growth of distinct tumors, including colon, ovary and prostate cancers. Notably, they showed differential efficacy for established tumors versus isolated-cell models of metastatic dissemination, consistent with our strategy of maximizing differential accessibility of Trop-2 according to growth mode. Most remarkably, we demonstrated in vivo synergy of these anti-Trop-2 mAb, paving the way for game-changing anti-cancer mAb therapy. The differential efficacy of the OX-G64 and OX-S55 anti-Trop2 mAb against different tumor histotypes and growth stages further allows to exploit their cancer-killing potential in pathological stage-tailored therapeutic approaches.


CD70 is a member of the tumor necrosis factor (TNF) superfamily and aberrantly expressed in several solid tumors and a variety of hematologic malignancies. The CD70 protein is expressed on highly activated lymphocytes (like in T and B cell lymphomas). Since normal lymphocytes do not express much CD70, it is suggested that anti-CD70 antibodies could be a potential treatment for CD70 positive lymphomas. SGN-CD70A is a novel antibody-drug conjugate that combines an anti-CD70 monoclonal antibody with a synthetic DNA cross-linking molecule, pyrrolobenzodiazepine (PBD) dimer. It is currently under phase I clinical trials for renal cell carcinoma, mantle-cell, diffuse large B-cell and follicular lymphoma. The aim of this study is to investigate the anti-tumor activity of SGN-CD70A in T cell lymphomas. We first examined CD70 expression in 36 cases of mature T or NK cell lymphomas using immunohistochemical (IHC) staining of patient biopsy specimens. The IHC results were reviewed and...
scored by 2 independent pathologists. We further investigated CD70 expression in Sezary syndrome (SS), mycosis fungoides (MF), and T cell acute lymphoblastic leukemia (T-ALL) cell lines, along with patient-derived T cell lymphoma primary cells and healthy donors’ peripheral blood mononuclear cells (PBMC) by flow cytometry. We next evaluated the anti-tumor activity of SGN-CD70A in a cutaneous T cell lymphoma (CTCL) xenograft model system. These findings merit clinical validation of the targeting of both membrane-bound complement regulatory proteins (mCRPs) CD46, CD55, and CD59 in solid tumors for mCRP expression and VEGF-A secretion. Our data suggest that p53 mutational status is associated with expression of CD55 and mCRPs’ inhibitory role in monoclonal antibody treatments for liquid tumors have been reported, but their role and regulation in solid tumors has not been explored. In the context of resistance to anti-VEGF-A antibody treatments for liquid tumors, we hypothesize that tumor cells in solid tumors will also secrete more VEGF-A and that mCRP expression will differ. In vitro experiments in our laboratory have supported this hypothesis.

#4590 Abiximab manifests striking anti-tumor effects in sensitive and chemoresistant ovarian cancer cells. Ghassan M. Saed, Nicole M. Fletcher, Ira Mennaj, Wayne State University, Detroit, MI.

Introduction: We have recently reported myeloperoxidase (MPO) to be expressed in epithelial ovarian cancer (EOC) cells and tissues. This finding was surprising, as MPO is known to be expressed only in cells of myeloid origin. We have also found that targeting β2 integrin (CD11b/CD18), a known ligand for MPO, resulted in significant anti-tumor effects. The objective of this study was to determine if targeting CD11b with a clinically approved drug that cross-reacts with CD11b will have anti-tumor efficacy for the treatment of sensitive and chemoresistant ovarian cancer. Methods: Human EOC cell lines MDAH-2774, SKOV-3, and A2780 and their chemoresistant counterparts were utilized for this study. Cells were treated for 24 hrs with increasing doses of docetaxel (0.0075, 0.01, and 0.025 μM) or cisplatin (0.1, 0.5 and 1.0 μM) with our without abiximab (5 or 10 μg/ml), an existing clinically approved anticogulant drug. Abiximab is a human-murine chimeric antibody Fab fragment against platelet glycoprotein IIb/IIIa (GP IIb/IIIa). Cytotoxicity was determined by the TACS TM Cell Proliferation Assay. Data was analyzed using SPSS and significant cytotoxic effects were determined by one-way analysis of variance within groups (sensitive, resistant to docetaxel or cisplatin) followed by Tukey’s post hoc tests, and independent t-tests for comparison between groups. For the synergistic effect of abiximab and chemotherapy, the automated calculation of combination index values was conducted by CompuSyn software. A combination index method provides qualitative information on the nature of compound interaction (antagonistic, additive or synergistic effect) and was used to analyze the results. Statistical significance of p<0.05 was considered significant for all analyses. Results: Abiximab had significant cytotoxic effects in sensitive (40.8 ± 10.3 and 56.7 ± 5.3%), docetaxel resistant (30.0 ± 11.6 and 46.6 ± 10.0%), and cisplatin resistant (27.3 ± 7.9 and 41.9 ± 7.0%) EOC cells at the 5 and 10 μg/ml dose, respectively, as compared to untreated controls (p<0.01). Abiximab is significantly more cytotoxic to sensitive as compared to cisplatin resistant cells (p<0.01), with no significant difference in killing as compared to docetaxel resistant cells (p>0.05). Treatment with chemotherapy combined with abiximab resulted in a synergistic cytotoxic effect in all sensitive EOC cell lines, and in chemoresistant MDAH-2774 and SKOV-3 cell lines. For the cisplatin resistant A2780 cell line, synergistic effects were only observed when combining abiximab with 1.0 μM cisplatin. Conclusions: The observed intriguing anti-tumor effects of abiximab in ovarian cancer cells indicates the potential for abiximab to be repurposed as a novel therapy for ovarian cancer.

#4591 Specific elimination of invasive and multidrug-resistant cancer cells by an antibody-drug conjugate targeting AXL. Julia Bushuizen,1 Louise A. Koopman,2 Esther C. Brei,2 David Satijn,3 Daniel Peiper,1 Paul W. Parren1. 1Netherlands Cancer Institute, Amsterdam, Netherlands; 2Gemmah, Utrecht, Netherlands.

Upon therapeutic pressure, cancers commonly select for drug-resistant, invasive subpopulations with elevated expression of the receptor tyrosine kinase AXL. Besides the correlation between high AXL expression and induction of epithelial-to-mesenchymal transition, a process known to support metastasis, ample evidence also links AXL to resistance against a variety of targeted therapies, including BRAF inhibitors. Mutations in AXL results in a constitutively activated receptor that inactivates the ERK survival signal, promoting a pro-apoptotic effect. Moreover, AXL has been shown to cooperate with other pathways in tumor cell proliferation and survival. The recently described AXL-107-MMAE is a chimeric antibody-drug conjugate specific for AXL, containing the microtubule disrupting agent monomethyl auristatin E as the cytotoxic payload. AXL-107-MMAE was previously shown to induce potent cytotoxicity in vitro and in vivo, which was dependent expression of AXL on the cell surface. We evaluated the efficacy of AXL-107-MMAE in a clonal xenograft model of AXL-positive, drug-resistant melanoma and breast cancer cells and AXL-negative cell lines, using malignant melanoma as a clinically relevant example. First, AXL-107-MMAE was shown to induce cytotoxicity in BRAF-mutant tumor cells that showed AXL expression upon acquired resistance to BRAF-inhibitors. In contrast, no cytotoxicity was observed in the BRAF-inhibitor-sensitive, AXL-negative parental cell lines. In heterogeneous tumor cell cultures, treatment with a BRAF-inhibitor selected for AXL-high, MAPH pathway inhibitor-insensitive melanoma cells, which was prevented by combined BRAF-inhibitor and AXL-107-MMAE treatment. Interestingly, we observed marked AXL upregulation in biopsies obtained from patients after they developed resistance to MAPK pathway inhibitors compared to paired pre-treatment biopsies. The in vivo potential of AXL-107-MMAE in malignant melanoma was demonstrated in immunocompetent mice, where post-therapy treatment with AXL-107-MMAE in AXL-positive melanoma cells prevented tumor growth in a xenograft model system. These findings merit clinical validation of the targeting of both treatment-naïve and drug-resistant cancers with AXL-107-MMAE, either alone or in combination with other targeted therapies.
wt-p53 versus mutant p53 solid tumor cell lines. Elucidating mechanisms for mCRP regulation is critical for immune biomarker development and in facilitating the use of antibody-based therapeutic approaches for solid tumors.

**#4593 Glycoengineered antibodies for click chemistry applications.** Dhrishi Sehgal, Stephen Kalscheuer, Tammy Sadhukha, Jayanth Panyam, University of Minnesota, Minneapolis, MN.

Antibody-drug conjugates (ADC) hold considerable promise as anticancer agents. A critical determinant of the effectiveness of ADCs is the chemistry that is used to conjugate the payload. Currently used approaches include primarily conjugation to either side-chain amine or carboxylic acid groups or conjugation to thiols. Because these reactions are not site specific and not easily controlled, these chemistries can result in reduced affinity for the target antigen. Further, these conjugation reactions lack selectivity and can result in heterogeneous mixtures of products that differ in the sites and stoichiometry of modification. We investigated a glycoengineering strategy that enables the introduction of artificial azide groups in the antibody without affecting their antigen affinity. This is based on the observation that glycosyltransferases can incorporate non-natural sugars (e.g., azido mannose) at different sites on an IgG molecule. The azide groups in these artificial sugars are then available to react with alkynes through copper-catalyzed ‘click’ chemistry or with strained alkyne-cyclooctyne (DBCO) allowing for biorthogonal, copper-free ‘click’ chemistry. Because the sugars are added reproducibly and at a site that does not affect antigen binding, the glycoengineering technology would overcome problems associated with traditional conjugation strategies. Using this approach, azide groups were introduced in anti-CD133 and anti-perlecan antibodies. Further, the azide groups were reactive to various DBCO conjugates including fluorophores, drug molecules and nanoparticles. Importantly, the addition of artificial sugar and subsequent azide-alkyne reaction did not affect the affinity of the antibody for the target antigen. Conjugation of nanoparticles to antibodies using this approach resulted in enhanced cellular uptake of the nanoparticles. Similarly, conjugation of a cytotoxin to the antibody resulted in enhanced cell kill in vitro. We expect that this glycoengineering strategy will prove to be a unique platform technology that will have a significant impact on antibody-based therapeutics.

**#4594 Induction of apoptosis by anti-CD20 antibodies requires the induction of EGR-1 and calcium influx.** Ivana Spaseska, Jade Villé, Kamel Chettab, Eva-Laure Mata, Charles Dumontet, INSERM 1052/CNRS 2866/University of Lyon Cancer Research Center of Lyon, Lyon, France.

Background: Anti-CD20 monoclonal antibodies (mAbs) are an essential component of the treatment of patients with CD20-positive non-Hodgkin’s lymphoma and chronic lymphocytic leukemia (CLL). Anti-CD20 mAbs mediate their antitumor effects by activating the immune system or by direct apoptotic signaling in target cells. In a previous preclinical study, we have shown that treatment of B-lymphoma cell lines with anti-CD20 mAbs, rituximab and obinutuzumab, resulted in upregulated expression of the transcription factor early growth factor -1 (EGR-1) (Dalle et al. 2011). However, the role of EGR-1 in response to passive immunotherapies has not been explored so far. Furthermore, EGR-1 has been described as a calcium (Ca^{2+}) regulated transcription factor that plays a key role in T-cell apoptosis. In this study, we investigated the role of EGR-1 and Ca^{2+} flux in the cytotoxic activity of anti-CD20 mAbs. Methods: EGR-1 modulation and cell death induction by anti-CD20 mAbs rituximab and obinutuzumab were investigated in cells expressing endogenous and exogenous CD20. The cytotoxic effect of anti-CD20 mAbs was evaluated in SCID mice and in B-lymphoma cell lines overexpressing EGR-1 or knocked down for EGR-1. The impact of anti-CD20 mAbs on Ca^{2+} flux was investigated by flow cytometry using Indo-1 AM stained cells. Ca^{2+} channel blocker nifedipine was used to investigate the role of Ca^{2+} flux on obinutuzumab efficacy. Results: EGR1 expression is rapidly upregulated in CD20+ cells following rituximab and obinutuzumab exposure. Decreasing EGR1 expression by shRNA abolished the direct cytotoxic effect of obinutuzumab in vitro and in vivo, indicating that EGR1 is required for the direct-mediated apoptosis. Additionally, the overexpression of EGR1 resulted in enhanced cytotoxic activity of obinutuzumab both in vitro and in vivo. Rescuing EGR1 expression in EGR1 knockdown cells restored sensitivity to obinutuzumab. Moreover, our results indicate that both rituximab and obinutuzumab could induce calcium influx in the presence of suboptimal concentrations of ionomycin. This influx was modulated by calcium channel blocker nifedipine or the Ca^{2+} chelating agent EGTA abolished EGR1 induction by anti-CD20 mAbs. In vivo, nifedipine treatment interfered with obinutuzumab antitumor activity against established Granta (a human mantle cell lymphoma line) xenografts in SCID mice. Conclusion: EGR1 plays a major role in the direct cytotoxic activity of anti-CD20 monoclonal antibodies and should be evaluated as a new biomarker to predict response to anti-CD20 treatment. Our data also show that calcium channel blockers interfere with the antitumor activity of obinutuzumab in preclinical models.

**#4595 Moxetumomab pasudotox spares CD4 and CD8 lymphocytes in multiply relapsed hairy cell leukemia patients, while prospective trials of first, second, and later lines of purine analogs show increasing toxicity.** Robert J. Kreitman, Maryalice Sterler-Stevenson, Evgeny Arons, Ira Pastan, National Cancer Institute, Bethesda, MD.

The purine nucleoside analogs cladribine and pentostatin damage DNA and cause long-term suppression of CD4 and CD8 lymphocytes in patients with hairy cell leukemia (HCL). There is increasing concern that these reductions, particularly in CD4+ T-cells, may be associated with opportunistic infections and secondary malignancies. The anti-CD22 recombiant immunotoxin moxetumomab pasudotox targets HCL without damaging DNA, and spares T-cells. In a phase 1 trial, 20 patients treated with 50 µg/kg every other day for 3 doses for 2-8 (median 4) cycles were evaluated for T-cell subsets. CD4 cells increased 56% to 152% (median 46%) after end of treatment, decreasing in only 1 of the 20 patients. Because there is very little prospective data on the effect of purine analogs on T-cells, we analyzed data from 80 consecutively enrolled patients receiving cladribine in first line, 40 receiving cladribine in second line, and 52 receiving either pentostatin or bendamustine in third or later lines. The latter group was most appropriate to compare with moxetumomab pasudotox, which was also used in third or later-line treatment of HCL. CD4 counts after moxetumomab pasudotox were 85-600 (median 424), compared to 22-618 (median 73) after third or later line purine analog (p<0.0001). These CD4 counts after moxetumomab pasudotox were even higher than those after first line (86-954, median 259, p=0.0037) or second line (53-724, median 185, p<0.0001) cladribine. CD4 counts after first line cladribine were higher than those after second line cladribine (p=0.0004), which in turn were higher than those after third or later line purine analogs (p<0.0001). Even after 2 years, CD4 counts after third or later line purine analogs, 41-1004 (median 264), n=43, remained suppressed compared to 1st line cladribine, 191-1044 (median 392, n=68, p=0.0013). The percent decreases in CD4 counts after first line cladribine were significantly greater compared to those after moxetumomab pasudotox (median 63% decrease vs 46% increase, p<0.0001). CD8 counts were also preserved after moxetumomab pasudotox, increasing by ~32% to 198% (median 46%). Similar differences between moxetumomab pasudotox and purine analog groups were observed with respect to CD8-cell suppression. Opportunistic infections, neutropenic fever, and other infectious complications were much more common after purine analogs than after moxetumomab pasudotox, particularly in later lines of therapy. These data provide a rationale for avoiding repeated cycles of purine analog for relapsed HCL, and support the use of non-chemotherapy approaches like moxetumomab pasudotox before repeated courses of purine analog are tried. (Supported in part by MedImmune and NCI, Intramural Program).

**#4596 Antibody-drug conjugates bearing pyrrolobenzodiazepine or tubulysin payloads allow the tumor immune microenvironment and synergize with multiple immunotherapies.** Jonathan Rios-Doria, Jay Harper, Raymond Rothstein, Leslie Wetzel, Jon Chesebrough, Allison Marrero, Cui Chen, Patrick Strout, Kathy Mulgrew, Kelly McGlinchey, Ray Fleming, Binyam Bezabeh, John Meekin, David Stewart, Maureen Kennedy, Philip Martin, Andrew Buchanan, Nazareno Dimasi, Emil Michelotti, Robert Hollingsworth, MedImmune, LLC, Gaithersburg, MD; MedImmune, LLC, United Kingdom.

Immunogenic cell death (ICD) is the process by which certain cytotoxic drugs induce apoptosis of tumor cells in a manner that stimulates the immunosystem. In this study, we investigated whether ADCs conjugated with pyrrolobenzodiazepine dimer (PBD) or tubulysin payloads induced ICD, modulated the immune microenvironment, and could combine with IO drugs to enhance antitumor activity. We show that the payload on the ADC induced an immune response that prevented the growth of tumors following subsequent tumor cell challenge. ADCs had greater antitumor activity in immunocompetent vs. immunodeficient mice, demonstrating a contribution of the immune system to the antitumor activity of these ADCs. These ADCs also induced immunological memory. In the CT26 model, depletion of CD8+ T cells abrogated the activity of the ADCs when used alone or in combination with a PD-L1 antibody, confirming a role for T cells in the antitumor activity. Combinations of ADCs in different tumor models with IO drugs including PD-L1 or PD-L1 antibodies, or OX40 ligand or GITR ligand fusion proteins produced synergistic antitumor responses. Importantly, synergy was observed in some cases with suboptimal
doses of ADCs, potentially providing an approach to achieve potent antitumor responses while minimizing ADC-induced toxicity. Immunophenotyping studies in different tumor models revealed broad immunomodulation of lymphoid and myeloid cells by ADCs and ADC/IO combinations. These results suggest that it may be possible to develop novel combinatorial therapies with PBD- and tubulysin-based ADCs and IO drugs that may increase clinical responses.

#4597 A novel immuno-oncology approach: targeting cell-surface mesothelin with a fusion construct containing human granzyme B. Lawrence H. Cheung,1 Rasheeda Tijani,2 Nasir Khan,3 Claire Thuning-Roberson,2 Michael G. Rosenblum1. 1MD Anderson Cancer Center, Houston, TX; 2H2Biologics Inc, Shrewsbury, MA.

Immunological approaches to target tumors have gained considerable traction recently because of excellent clinical results. In addition, targeted treatment with antibody-drug conjugates (ADCs) can also be effective in achieving regression of solid tumors. The challenge for ADCs continues to be efficacy, stability of the construct and development of resistance. We have developed a unique fusion protein composed of 1) a novel human single-domain antibody (SD1) that uniquely targets Region III of the mesothelin glycoprotein and 2) an engineered version of the human serine protease Granzyme B (GrB). Mesothelin is highly expressed in aggressive cancers such as malignant mesothelioma, pancreatic and ovarian cancers, and NSCLC. Granzyme B generates an intense, irreversible pro-apoptotic effect through direct cleavage of caspases, release of cytochrome C from mitochondria, and cleavage of nuclear matrix. Previous ADCs targeting mesothelin have faced the drawbacks of toxic payloads, formation of neutralizing antibodies, and competition with the serum protein MUC16/CA125. SD1-GrB represents a new class of completely human immunotoxins containing a payload with a unique mechanism of action. Additionally, because of its unique binding properties, SD1 does not compete with circulating CA125 and is not subject to cleavage. The SD1-GrB construct was designed as a homodimer (~200kDa) containing an IgG Fc domain and was expressed using transient-transfection of HEK293E cells. The soluble SD1-GrB was isolated from conditioned media and purified to homogeneity as assessed by SDS-PAGE and Western blot analysis. Enzymatic activity of the GrB component was assessed by IEPD and found to be similar to authentic, native GrB. Binding to mesothelin was confirmed by surface plasmon resonance. Additionally, binding to the human lung adenocarcinoma cell lines H460 and HCC1703 was demonstrated by ELISA. Initial cytotoxicity (IC50) of SD1-GrB against H460 and HCC1703 was in the 30 nM range. Assessment against a larger panel of cell lines is ongoing followed by in vivo efficacy in a human xenograft model. Our preliminary evaluation of SD1-GrB ADC suggests this construct has a unique mechanism of action and is highly cytotoxic against target cells. This molecule appears to be an excellent candidate for further pre-clinical development as a therapeutic agent. Research conducted, in part, by the Clayton Foundation for Research.

#4598 Triggering of OX40 on T cells by a novel monoclonal antibody elicits robust antitumor immunity!-EndFragment-» Hua Long,1 Ann White,2 Jie Wei,3 Brittany Jiang,4 Reid Feldman,5 Danielle Pappas,3 Aymen Al-Shamkhani,6 John Lin7 1Pfizer Inc, South San Francisco, CA; 2UCB Pharma, San Francisco, United Kingdom; 3Abbvie, Redwood City, CA; 4University of Southampton, United Kingdom; 5None, CA.

T cell costimulation is an attractive strategy for cancer treatment in addition to checkpoint inhibitors. Costimulatory molecule OX40 on T cells is a member of the TNF family. Binding to its receptor OX40L expressed on antigen-presenting cells potentiates T cell functions and elicits robust antitumor responses. Therefore, OX40 is an attractive target for the development of immunotherapies for solid tumors. We have developed a fully human OX40 agonist antibody (PF-8600) that binds to OX40 with high affinity and specificity. We used in vitro and in vivo methods to evaluate the mechanism of action as an OX40 agonist. As regulatory T cells are a major suppressor of antitumor immune responses, we have focused on a novel costimulatory strategy to potentiate anti-tumor immune responses by inhibiting regulatory T cells (Tregs). Oezcan Talay, Lisa Marshall, Cesar Meleza, Maureen K. Reilly, Omar Robles, Mikhail Zibisky, Aboud Okal, Lisa Seitz, Jenny McKinnell, Scott Jacobson, Erin Riegler, Emily Karbaz, David Chian, Angela Wadsworth, Paul Kassner, David Wustrow, Jordan S. Fridman. FLXBio, Inc., South San Francisco, CA.

Antigens that are over-expressed in cancer in response to radiation are being used as novel targets. We showed tax interacting protein 1 (TIP-1) to be radiosensitizing to cisplatin- and radiation-insensitive to the surface of the cancer cell following irradiation. TIP-1, which consists of a single PDZ domain plays an important role in cell signaling, cancer development, and progression. TIP-1’s involvement in various survival pathways makes it an attractive target for anticancer therapeutics. We used antibodies specific to this PDZ domain to determine its role in cancer cell survival. We monitored proliferation in lung cancer (A549 and H460) and glioblastoma (D54 and U251) cells after 24, 48, 72 and 96h treatment with the anti-PDZ antibody. We observed a time-dependent proliferation arrest with anti-PDZ antibody treatment which was associated with increased apoptosis. In combination with radiation (3 Gy) led to reduced cell proliferation and colony formation. Anti-PDZ antibody had no effect on the proliferation of normal lung (MRC-5) and endothelial (HUVEC) cells. Cells treated with anti-PDZ antibody showed decreased levels of the phosphorylated forms of AKT, mTOR, and 4EBP1. Anti-PDZ antibody treatment also led to an overall reduction in basal levels of AKT, mTOR, and 4EBP1. Further, we evaluated the effect of the anti-PDZ antibody on tumor growth in heterotopic mouse models of lung cancer (A549) and glioma (U251). We observed significant growth delay in mice treated with anti-PDZ antibody treatment when compared to mice treated with the isotype control. The combination of the anti-PDZ antibody with radiation showed an additive effect. Immunoblot analysis of tumor tissues also showed downregulation of phosphorylated and total levels of AKT, mTOR, and 4EBP1 in the tumors treated with anti-PDZ antibody. Overall, our results suggest that TIP-1 is a promising therapeutic target for treatment of lung cancer and glioblastoma. Antibodies specific to the PDZ domain of TIP-1 enhance the efficacy of radiotherapy. The anti-PDZ antibodies need to be optimized further before translating it into the clinic.


Chemokines play an important role in the development and progression of human cancer. Chemokine signaling is now being exploited as an attractive therapeutic strategy to exploit in the cancer microenvironment. CCR4 is expressed in various immune cells, and the combination of CCR4 antibodies and checkpoint inhibitors has shown promising results in clinical trials. Although CCR4 antagonists block migration and support expansion of activated T cells, some CCR4 antagonists block Treg migration and support expansion of activated T cells. In contrast to the non-selective approach of depleting anti-CCR4 antibodies, our compounds reduce Treg in the tumor, but not in peripheral tissues such as blood.
spleen or skin. In preclinical efficacy studies, CCR4 antagonists potentiate the anti-tumor effects of various checkpoint inhibitors and immune stimulators such as anti-PD-L1 and anti-CD137 antibodies. We observe enhanced tumor growth inhibition and increased tumor regressions when these agents are combined with CCR4 antagonists, without any gross toxicity. Further characterization of these CCR4 antagonists and their anti-tumor effects will be described.

**#4601** Mechanisms of action for therapeutic antibody variants of human IgG3 isotype: Enhancing the CDC activity of cetuximab and rituximab. Ralf Schwanbeck,1 Anna Skot,1 Thies Rösner,1 Marco Jansen,2 Anina Räuchle,1 Anna Kretschmer,1 Axel Scheidig,1 Jeanette Leusen,1 Stefanie Derer,1 Thomas Valerius1.1 Christian-Albrechts-University, Kiel, Germany; 2University Medical Center, Utrecht, Netherlands; 2University Hospital Schleswig-Holstein, Lübeck, Germany.

Objectives. Human IgG3 differs from other antibody isotopes by its extended hinge region and by its higher affinity for Clq. The ability of complement to promote lysis of antibody-opsonized cells is well-established and may represent a main mechanism in antibody mediated tumor therapy. The aim of this project was to investigate effector mechanisms of tumor-directed IgG3 antibodies in more detail. Experimental procedures. Therapeutic antibodies against EGFFR (cetuximab, 225) and CD20 (rituximab, C2B8) were produced as IgG1 and IgG3 isotype variants by co-transfecting CHO cells with the respective de novo synthesized V\(\beta\) and V\(\gamma\) domains fused to the respective heavy chain containing vectors. A Fab fragment of C2B8-IgG3 was crystallized and analyzed by X-ray structure analysis at PETRA III (DESY, Hamburg, Germany). Based on the resolved structure selected mutations were introduced into the CH1 domain of IgG3 aiming to improve or abolish CDC. Purified antibodies were assessed for complement fixation in ELISA and on tumor cells. Complement- and antibody-dependent cellular cytotoxicity (CDC and ADCC) activities were measured using \(^{51}\)chromium release assays. The significance of complement regulatory factors on target cells was investigated by siRNA mediated knock-down. A peritoneal tumor model was used to investigate the in vivo efficacy of IgG3 in comparison to IgG1 antibodies. Results. Switching isotypes from IgG1 to IgG3 did neither affect target antigen binding nor the proliferation inhibition by EGFGR antibodies. Unexpectedly, the IgG3 antibodies were not very active in ADCC, in spite of their strong FcγRII (CD16) binding affinity. However, for target cells expressing low antigen levels, the IgG3 isotype improved CDC against CD20 and enabled CDC against EGFGR expressing tumor cells. The complement-regulatory factor CD55 in particular mitigated the IgG3 induced CDC activity. Structural comparison of Fab fragments of IgG1 and IgG3 demonstrated significant differences in the CH1 domains of the respective heavy chains, which contain the disulide bond to the light chain and also harbor the docking interface for the complement factors C3b and C4b. Preliminary results from an peritoneal EGFGR tumor model demonstrated that IgG3 was effective in killing EGFGR-expressing cells. This in vivo activity was abolished by cobrava venom factor treatment. Conclusion. Switching the isotype of a therapeutic IgG1 antibody to IgG3 represents a promising approach to enable complement-mediated killing of tumor cells. Protein engineering of the C3b/C4b interaction interface in the CH1 domain may constitute a novel strategy to modulate the CDC activity against tumor target cells. Together, these approaches may enhance the CDC efficacy of CD20- and EGFGR-directed antibodies.

**#4602** The dissociation constant rate of ADC would be an important factor for antitumor activity in vivo. Ryo Tsumura,1 Shino Manabe,1 Yoshikatsu Koga,2 Yasuhiro Yasunaga,3 Yasuhiro Matsumura,1 National Cancer Center East, Kashihwa, Chiba, Japan; 2RIKEN, Wako, Saitama, Japan.

Introduction: Antibody-drug conjugates (ADCs), consisting of a monoclonal antibody, a potent cytotoxic agent and a linker, are currently considered as promising potent agents for cancer therapy. However, especially in solid tumors, the tumor stroma would hinder ADCs from accessing to cancer cells, leading to decline the efficacy of ADCs in clinical study. In order to overcome these difficulties and to maximize the ADC potential, the optimization of ADC components is needed. And it’s well-known that antitumor activity of ADCs depends on PK properties, binding affinity, internalization efficiency and other functions. In present study, therefore, we focused on the characteristics of antibody for ADC design, and investigated what characteristics could affect anti-tumor effects in vivo. Material and Method: First, we prepared 4 types of monoclonal antibodies against tissue factor (TF) which is known as a 47-kDa transmembrane glycoprotein and an initiation factor of the extrinsic blood coagulation. And then, anti-TF antibodies were conjugated with maleimidocaproyl-valine-citrulline-p-amino-benzoyloxy carbonyl linker and monomethyl auristatin E. Both of them were well-known liker system and drug for current ADC design. After conjugation, we investigated the characteristics, including binding paramaters, internalization efficiencies, drug release rates, cytotoxicity against human pancreatic cancer cell lines in vitro, half-life in vivo, anti-tumor effects using xenograft model, and distribution in tumor tissues. Results: In SPR analysis, anti-TF ADCs showed the significant differences of binding kinetics parameters. However, in other assays, anti-TF ADCs showed almost same internalization efficiencies, drug release rates, and cytotoxicity against human pancreatic cancer cell lines in vitro, half-life in vivo. The efficacy of anti-TF ADCs was examined in BxPC3 xenograft model when the average tumor size reached 200 mm\(^3\). Although anti-TF ADCs significantly inhibited BxPC3 tumor growth compared with control groups, there was no difference of anti-tumor effects among anti-TF ADCs. We further examined the anti-tumor effects when the average tumor size reached 600 mm\(^3\). Interestingly, the antitumor effect of ADC using the clone with high dissociation constant was superior to that with low dissociation constant against large size tumor, especially in the early phase. Furthermore, the distribution of ADC in BxPC3 tumor demonstrated that the former clone showed higher penetration ability than the latter clone. Conclusion: We concluded that binding kinetics parameters, in particular, the dissociation constant rate contributed to the tumor penetration ability of anti-TF ADCs and the different distribution in BxPC3 tumor. The distribution changes would affect the anti-tumor effects in large size BxPC3 tumor. Therefore we suggested that the dissociation constant rate would be an important factor for ADCs design.


Activatable nanotherapeutics provide the opportunity to deliver anticyclics to a well-controlled region of interest and therefore to locally stimulate immunogenic cellular death. Such a strategy can be combined with immunotherapy for both local tumor control and creation of an abscopal effect. Our group previously reported that combining local administration of a CpG oligonucleotide with local release of doxorubicin from a temperature-sensitive liposomal nanoparticle (TSL) led to the elimination of directly treated lesions in a syngeneic murine model of mammary adenocarcinoma. While this therapeutic approach increased the numbers of tumor infiltrating CD8\(^+\) T lymphocytes in distant lesions and extended survival, distant tumors returned with a growth delay of approximately 14 days and T-regulatory cells were not reduced by treatment. Therefore, checkpoint blockade of the programmed death-1 (PD-1) pathway (anti-PD-1) was incorporated in the protocol. CuDox-TSL were prepared from DPPC:MPC:DSPE-PEG2k (86:10:4) in the presence of copper (II) gluconate and triethanolamine at 0.2 mg-drug/mg-lipid. A complex between doxorubicin and copper was created to enhance the circulation and stability of TSL and to reduce systemic toxicity. On day 21, two mice with bilateral invasive neu deletion (NDL) tumors (~4 mm) were treated with i.v. administration of CuDox-TSL at 6 mg doxorubicin/kg body weight. Ultrasonication (1.1 MPa, 1.5 MHz, achieving 42°C for 5 min before and 20 min after drug injection) triggered drug release. Immediately after US, 100 \(\mu\)g of CpG-ODN 1826 was administrated intratumorally to the sonoinfected tumor; anti-PD-1 (200 \(\mu\)g, i.p.) was administrated three days later. Flow cytometry and immunohistochemistry were performed on day 28 after one treatment with each component and survival was assessed after treatment was repeated for three weeks. While control mice survived 35 days, 50% of treated mice were tumor-free after 100 days. All primary tumors and 50% of the contralateral tumors regressed and were eliminated by day 63. With the incorporation of immunotherapy, three doses of chemotherapy were sufficient to eliminate all directly treated tumors, as compared with 8 doses without immunotherapy. After a single treatment with each component, a substantial change was observed in systemic immune cells, including a significant increase in natural killer and dendritic cells in distant lymph nodes, in CD8\(^+\) T cells in tumors and associated lymph nodes and in IFNγ-secreting CD4\(^+\) T cells in the treated tumor and distant lymph nodes. The results demonstrate that activatable chemotheraphy can be paired with an immunoadjuvant and PD-1 blockade to generate a curative response in primary and distant tumors. Further, local chemotheraphy, as potentiated by activatable liposomes does not impede a systemic CD8\(^+\) T cell response. [1] Journal of Controlled Release (2015); 220: 253-264.

**#4604** MEDI1873, a GITR ligand fusion protein (GITRL FP), induces effector T-cell proliferation, modulates T-regulatory cell function and has the potential to combine with checkpoint inhibitors. Michelle Morrow,1 Rebecca Levland,2 James Hair,1 Ross Stewart,1 Natalie Tague,1 Lisa Bamber,1 Samantha Ireland,1 Nicholas Holoweczy,2 Michael Oberst,3 Amanda Watkins,1 Emily Offer,1 David Perez-Martinez,1 Ching Ching Leow,2 Lesley Young,1 Tristan Vaughan,1 Philip Mallinder,1 Robert Wilkinson1. MEDIimmune, Cambridge, United Kingdom; 2MEDIimmune, Gaithersburg, MD.
Glucocorticoid-induced tumor necrosis factor receptor-related protein (GITR) is part of a system of signals involved in controlling T-cell activation. MED1873 is a novel hexameric human GITR agonist comprising an IgG1 Fc domain, a coronin 1A trimeration domain and the human GITR extracellular domain (ECD) that is currently being assessed in a Phase 1 clinical study (NCT02540291). Combination of the EP4 antagonist E7046 with solid tumors showed strong, dose-dependent synergistic activity and superior to an anti-GITR antibody with respect to evoking robust GITR agonism, T-cell activation and clustering of Fc gamma receptors. Using in vitro assays, MED1873 recapitulates aspects of GITR targeting previously described in mice, including modulation of regulatory T-cell (Treg) suppression and the ability to increase the CD8:CD4 T-cell ratio via antibody-dependent T-cell cytotoxicity. Pharmacodynamic assessment of an agonistic mouse GITR-Fc (mGITR-Fc) in the CT26 model of colorectal cancer demonstrated activation and proliferation of peripheral CD4+ and CD8+ T cells coincident with an increased depletion of intratumoral Tregs, likely through Fc mediated effector functions. Furthermore, CT26 tumor growth studies indicated the mGITR-Fc could result in significant antitumor activity. These data provide evidence that MED1873 is a novel, potent GITR agonist with the potential to modulate T-cell responses and enhance anti-tumor immunity. Combinations of immunotherapies are generating exciting results in the clinic, therefore, we sought to assess the potential for GITR-FPs to combine with antibodies targeting either anti-PD-1 (durvalumab) or anti-CTLA-4 (tremlumab) using both in vitro and in vivo systems. In vitro studies where MED1873 was combined with either durvalumab or tremlumab showed that both combinations have the potential to enhance interleukin-2 release in a superantigen-stimulation of human peripheral blood mononuclear cells (PBMCs) compared to checkpoint blockade alone. Further evidence to support the potential for combinatorial antitumor activity was generated in the CT26 model where either 0.2mg/kg mGITR combined with 10mg/kg anti-mouse PD-L1 or 0.1mg/kg mGITR combined with 5mg/kg anti-mouse CTLA-4 antibodies resulted in enhanced antitumor activity versus monotherapies alone. Overall, our data suggest that therapeutically targeting GITR with a multimeric fusion protein, GITR-FP, may provide increased agonistic potential versus an antibody, and have the ability to both activate effector T-cells and modulate Tregs through suppression and/or deletion. Finally, combination studies provide preclinical evidence to support the rationale for combination of MED1873 with anti-PD-1 or anti-CTLA-4 antibodies further reinforcing the potential of targeting the GITR pathway as a therapeutic approach to treating patients with cancer.

**#4605** Efficient targeting of BCMA-positive multiple myeloma cells by antibody-coupled T-cell receptor (ACTR) engineered autologous T cells in combination with an anti-BCCA antibody. Tooba Cheema,1 Taylor Hickman,1 Kari Westenberg,2 Luke Manlove,2 Shyra Gardai,2 Katie O'Callaghan,1 Lori Westendorf,2 et al. 

B cell maturation (BCMA) has recently emerged as an attractive therapeutic target in multiple myeloma. BCMA has restricted expression on plasma cells with little to no expression on other normal tissues, but is upregulated on the surface of multiple myeloma cells. BCMA can regulate proliferation and survival of myeloma cells via binding to its ligands APRIL and BAFF and induce downstream signaling pathways. Thus, several approaches to target BCMA are currently under clinical investigation, including chimeric antigen receptor (CAR) T cell therapies, bispecific antibodies and antibody drug conjugates. The Antibody-Coupled T cell Receptor (ACTR) technology is a universal, engineered T cell therapy consisting of the extracellular domain of human CD16 and the intracellular T cell co-stimulatory and signaling domains. ACTR is designed to engage the Fc domain of therapeutic antibodies opsonized to target cells to mediate anti-tumor activity. Previous work has demonstrated ACTR-T cell activity in combination with rituximab, trastuzumab, and hu14.18 K322A against CD20, Her2, and GD2 expressing cell lines, respectively (Kudo et al. Cancer Res. 2014; 74:93-103). Currently ACTR is being evaluated in Phase I clinical trials with rituximab to treat relapsed refractory B cell lymphoma. Here we demonstrate a humanized afucosylated anti-BCMA antibody, SEA-BCMA, binds to ACTR expressing T cells with high affinity and mediates T cell activation, potent cytotoxicity, cytokine release and proliferation across a wide range of BCMA expressing myeloma cells. ACTR activity was specific to SEA-BCMA - opsonized target cells, dose dependent and had no activity on BCMA negative tumor lines. Furthermore, the SEA-BCMA antibody has additional properties that might contribute to a therapeutic effect, including blocking the binding of ligands to BCMA and driving natural killer cell mediated ADCC effects. These preclinical studies demonstrate a promising multi-faceted activity of ACTR T cells in combination with the anti-BCMA antibody, SEA-BCMA, for clinical consideration in multiple myeloma patients.

**#4606** Preclinical characterization of a novel fully human IgG1 anti-PD-L1 mAb CK-301. Leonid Gorelik,1 George Avgerinos,1 Yune Kunes,2 Wayne A. Marasco,3 et al. 

Antibodies targeting Programmed Death-1 (PD-1), or its ligand, PD-L1, have demonstrated remarkable efficacy in subsets of cancer patients, with inhibition of the interaction between PD-1 on T-cells and PD-L1 on tumor cells leading to the recovery of anti-tumor immune response and immune-mediated eradication of tumors. However, not all patients respond to existing PD-1 and PD-L1 targeting agents and relapses to therapy still occur. Therefore, there exists a need to identify additional therapeutic strategies and approaches to engage the immune system to enhance the efficacy of current anticancer therapies. Using phage and yeast display approaches, we have discovered and optimized a novel, fully human, PD-L1 specific IgG1 antibody, CK-301, which exhibits subnanomolar binding affinity for PD-L1, CK-301 blocks binding of PD-L1 to both PD-1 and B7-1 in enzyme-linked immunosorbent assays (ELISA) and cell-based competition assays. Using an assay measuring inhibition of a nuclear factor of activated T-cells (NFAT) reporter caused by PD-L1 binding to PD-1, we demonstrate that CK-301 completely reverses reporter inhibition at concentration of less than 1 μg/ml. IC50 of the dose response curve is 80mg/ml. CK-301 enhances IFN-gamma secretion in allogeneic lymphoblastoid cell lines. CK-301 has similar subnanomolar affinity for cynomolgus monkey PD-L1 as for human PD-L1, hence we chose Macaca fascicularis for pre-clinical toxicology and safety pharmacology studies. Single-dose administration of CK-301 to monkeys up to the highest tested dose of 100 mg/kg was shown to be safe and demonstrated linear dose-dependent pharmacokinetic (PK) properties over the dose range from 1 to 100 mg/kg with a half-life of 15 days at 100 mg/kg. A first-in-human Phase 1 study of CK-301 is planned to commence in mid-2017.

**#4607** Specific inhibition of PGE2 - EP4 signaling by E7046 promotes anti-tumor activity of checkpoint blockade agents through boosting cytotoxic T cell activity. Diana I. Albu,1 David Verbel,1 Yuan Huang,1 Donna Kolber-Simonds,2 Zichun Wang,1 Xulong Wang,1 Zoltan Dezso,2 Christy Ingersoll,1 Kuan-Chun Huang,1 Janna Hutz,2 Mary Woodall-Jappe,3 Xingfeng Bao,1 Eisai Andover Innovative Institutes, Andover, MA; 2Nurix Inc., San Francisco, CA; 3Eisai Oncology Business Group, Eisai Inc., Andover, MA.

Purpose: Immunotherapies targeting immune checkpoint receptors have shown great promise in subsets of cancer patients; however, robust and safe combination therapies are still needed. In the tumor microenvironment, pros-taglandin E2 receptor type 4 (EP4) signaling has been implicated in both protumor myeloid cell differentiation and cytotoxic T cell exhaustion. We evaluated the combination of the EP4 antagonist E7046 (clinical trial NCT02540291) with anti-PD1 or anti-CTLA4 in preclinical tumor models, and also interrogated the relationship between PGE2 signaling and other potential pathways. Materials/Methods: Mouse syngeneic tumor models CT-26 and 4T1 were used for pharmacological evaluation. GMP grade E7046 was administered to tumor-bearing animals by oral gavage. Co-culture of EG7-OVA and OT1 cells in an antigen-specific cytotoxic T cell (CTL) activation assay provided mechanistic insights. For translational validation, transcripts of five major genes involved in PGE2, synthesis, transport, and degradation were compared between malignant and normal tissues across all TCGA tumor types, and correlation of their expression with overall survival was assessed. Results: In the CT26 tumor model, the combination of E7046 and anti-PD1 resulted in significantly more tumor-free animals compared with either agent alone. In the 4T1 tumor model, the combination of E7046 and anti-CTLA4 was also more effective in suppressing tumor growth and tumor rejection compared to anti-CTLA4 alone, and was accompanied by a markedly increased accumulation of GZMB+ CD8+ CTLs in the treated tumors. Consistent with those findings, addition of anti-PD1 antibody promoted OVA-specific CTL activation in vitro while addition of PGE2 strongly inhibited it, as measured by IFNγ secretion. Inclusion of E7046 dose-dependently reversed the PGE2-induced suppressive activity in the presence of anti-PD1. Conclusion: E7046 at clinically relevant doses showed that PGE2 is upregulated and HPGD downregulated across a broad range of tumor types. In contrast, COX1, COX2 and PGT showed less difference between malignant and normal tissues. Importantly, these differences of one or multiple PGE2 pathway genes were strongly associated with patient survival in
certain cancer types. Conclusions: A subset of human cancer types displays upregulated PGE, pathway that is associated with a poorer prognosis. PGE, EP4 signaling potently suppresses antigen-specific CTCL activation in the presence of PD1 signaling blockade. The combination of EP4 antagonist E7046 with either anti-PD1 or anti-CTLA4 demonstrated superior anti-tumor activity compared with anti-PD1 or anti-CTLA4 alone. This increased activity was accompanied by increased CTL activation.

#4608 A 5T4 x CD3 bispecific DART® molecule with extended half-life for T-cell immunotherapy of cancers. Ling Huang,1 Gurunadh Chichili,1 Ralph Alderson,2 Francine Chen,1 Jennifer Brown,1 Hua Li,1 Valentina Ciccarone,1 Jim Tamura,1 Daorong Liu,1 Liqiu Liu,1 Syd Johnson,2 Ezio Bonvini,1 Paul Moore2,1,1 MacroGenics, Inc., Rockville, MD; 1South San Francisco, CA

Introduction: 5T4 (trophoblast glycoprotein), an oncofetal antigen involved in embryonic development, is expressed on the cell surface of multiple cancers. Several 5T4-directed interventions have been reported, including a cancer vaccine and an antibody-drug conjugate. A superantigen-driven redirected cell killing modality was also developed, but obstacles, such as immunogenicity, remain to a successful 5T4 therapeutic molecule. We have developed an Fc-bearing 5T4 x CD3 DART® bispecific protein designed to redirect T cells to target 5T4-expressing tumors. Methods: 5T4 x CD3, a humanized Fc-bearing DART molecule, was stably expressed in CHO cells and purified to homogeneity via a standard antibody-purification platform. In vitro characterization and functional studies were performed with 5T4-positive tumor cell lines and human T cells. In vivo studies were performed in immune-deficient tumor-bearing mice co-implanted with activated human T cells or reconstituted with human peripheral blood mononuclear cells (PBMCs). Pharmacokinetic studies were performed in both human FcRn transgenic mice and cynomolgus monkeys. Results: 5T4 x CD3 demonstrated bispecific binding properties to both human and cynomolgus monkey antigens. In redirected cytolytic studies, 5T4 x CD3 mediated lysis of 5T4-positive pancreatic, lung, renal, triple-negative breast, and ovarian cancer cell lines with EC50 values ranging between 0.03 and 0.08 ng/mL. 5T4 x CD3 displayed favorable pharmacokinetics with a prolonged circulating half-life in human FcRn transgenic mice and cynomolgus monkeys. Tumor clearance studies in NOD/SCID mice implanted subcutaneously with activated human T cells and tumor cells demonstrated robust inhibition of tumor growth upon intravenous administration of 5T4 x CD3 at doses as low as 0.8 µg/kg, but not with a CD3-binding control DART protein. In addition, human PBMC-reconstituted NOD/SCID/IIL2 gamma-chain null mice were implanted intraperitoneally with pancreatic and breast tumor cell lines or orthotopically with a triple-negative breast cancer line and, after tumor establishment, were treated with 5T4 x CD3. Anti-tumor activity was observed at doses as low as 4 µg/kg. Immuno-histochemical analysis of tumor xenografts confirmed tumor clearance was associated with T-cell recruitment into the tumor mass. Conclusions: In summary, 5T4 x CD3 displays robust antitumor activity against several cancer cell lines in vitro and in vivo together with a favorable pharmacokinetic profile and merits further consideration as a potential treatment for 5T4-positive cancers.

#4609 Agenus’ next generation cancer vaccine platforms. Mohamed Udu- man,1 Mithun Khattar,1 Bishnu Joshi,1 Antoine Tanne,1 Benjamin Morin,2 Ar- men Karapetyan,2 Elise Drouin,1 Jennifer Brown,1 Bishnu Joshi,1 Mithun Khattar,1 Agenus Inc., Lexington, MA; 1University of Virginia School of Medicine, Char- lottesville, VA; 2Massachusetts General Hospital Cancer Center, Charlestown, MA; 3University of Virginia, Lexington, MA.

Most cancer cells carry mutations unique to the patient’s individual tumor and shared biochemical signatures that are not present in healthy cells. Agenus has three vaccine platforms designed to treat cancers based on the unique needs of a given patient. Our vaccine platforms are designed to educate the patient’s immune system to recognize tumor-specific aberrations, or neo-antigens, and mount an anti-tumor immune response. Agenus’ Prophage® vaccine platform is an individualized vaccine made from the patient’s own tumor tissue. Heat shock proteins (gp-96) that naturally chaperone and bind tumor-derived peptides are extracted from the patient’s tumor and constitute the vaccine. Some of these peptides are neo-antigens. Agenus has completed Phase 2 clinical trials with Prophage® vaccine in newly diagnosed glioblastoma (mGBM), and has previously reported that there was improved progression-free and overall survival with Prophage® vaccine compared to standard of care. Agenus’ AutoSynVax® vaccines are uniquely designed and manufactured for each patient based on NGS profiling of the patient’s tumor from a biopsy. Leveraging the Agenus Immunogen Mutation (AIM®) workflow, we are able to generate a synthesis-ready blueprint for an optimal immunogenic and personalized neo-antigen vaccine. The AIM® platform provides a robust and efficient approach to computational vaccinology designed to deliver a set of likely immunogenic peptides, antigenic to vaccine format, followed by generation of a format-specific blueprint from which the final vaccine is synthesized. The peptides are complexed to recombinant heat shock protein 70 (HSC70) and are administered along with our QS-21 Stimulon® adjuvant. HSC70 is known to transport epitopes and play a role in displaying them to T cells. While the first two of Agenus’ vaccine platforms are highly individualized, our PhosphoSynVax® vaccine is an off-the-shelf vaccine format targeting a novel class of tumor neo-antigens arising from post-translational modifications (PTMs). Due to dysregu- lated cell signaling pathways in cancer, self-peptides can be aberrantly phosphorylated, a number of which are subsequently presented on HLA molecules. Using mass-spectrometry, we have identified a library of over a thousand HLA phospholipids. Many of these are tumor specific and found in multiple patients across multiple indications, enabling pre-manufacture of PhosphoSyn- Vax® vaccines for ready use. Upon testing the HSP plus synthetic peptide vac- cine format in murine models, we have demonstrated effective tumor control in a therapeutic setting and also effective immune memory in a long-term prophylactic setting. Given Agenus’ diverse portfolio we have the opportunity to combine our immune education strategies with immunomodulatory antibodies to increase therapeutic efficacy.

#4610 Towards understanding the cellular uptake patterns of nano-par- ticles among different immune cell lines. Noha Ismail1, Ashish Kulkarni,1 Siva Kumar,2 Vineeth krishna,2 Shiladiya Sengupta1,1 The American Univ. in Cairo, Egypt; 1Brigham and Women’s Hospital – Harvard Medical School / Harvard-MIT Health Sciences and Technology, Cairo, Egypt; 2Brigham and Women’s Hospital – Harvard Medical School / Harvard-MIT Health Sciences and Technology, Cambridge, MA.

Today with the privilege of being able to synthesize extremely small particles in the nano-range and make use of nanotechnology, we can reach superior properties than the bulk scale. Nanoparticles have their own physicochemical properties that make them promising in the field of cancer immunotherapy in form of drug delivery, diagnostic and theranostic modalities. Manipulation of the immune response by therapeutic intervention is becoming of great interest owing to the significant role of immunity in the general health and disease control. APCs like Dendritic cells and macrophages are important targets for the particulate delivery system due to their ability to trigger cascade of events on both levels cellular and humoral immune response specially DCs. Successful targeting of DCs and macrophages will have a great impact on T-cell activation and priming. The design of successful particulate system that can elicit preferential targeting towards specific immune cell in the tumor microenvironment is becoming crucial. This might represent a novel avenue for enhancing the antitumor immunity. Liposomes are made of phospholipid bilayer with a composite nature. Their properties are highly linked to their physicochemical characteristics such as size, surface charge and composition. These physicochemical properties can trigger certain immune response. The hypothesis here whether passive targeting via particulate system can offer a platform for preferential targeting for critical immune cells residing in spleen and tumor. Moreover, test whether there will be differences in uptake according to certain preference towards surface charge in specific time frame. In the current study, three sets of fluorescently labelled nano-liposomes were engineered as a model for different surface charges, the cationic DOTAP NP, anionic DOPG NP and near neutral DOPC NP with mean diameter of ~ 200 nm. Physical stability of the NPs was evaluated by monitoring the changes in size and zeta potential. B16 melanoma cancer model was induced subcutaneously in C57BL/6 black mice, divided into four groups each of five mice. CD11c Dendritic Cells (DCs), CD11b macrophages, CD90.2 T-cells and CD49b Natural Killer (NK) cells were isolated from the tumors and spleens of each group. The three sets of NPs were tested against the isolated cell lines. The cellular uptake (internalization) was assessed by normalizing the fluorescence of the cells against their protein concentration, then all samples were acquired to flow cytometry, and shifts in fluorescence histograms on horizontal axis were monitored against PE channel on the vertical axis. Results reveal the presence of preferential internalization of specific surface charge over others in some cell lines in different time frames. For the first time differences in the internalization pattern are reported in the same immune cell line isolated from different contexts tumor and spleen.

#4611 A 3D in vitro culture-based method to visualize and quantify effects of immuno-modulatory drugs. Lidia Daszkiewicz, Kuan Yan, Maarten Klop, Leo Price. OceBIO, Leiden, Netherlands.
#4612 Development and validation of a screening platform for the identification of novel immuno-oncology targets. Ariane Scoumanne,1 Virginie Rabolli,2 Lea Legrand,3 Murielle Martini,4 Marie-claire Letellier,5 Stefano Croce-tification of 42 compoundswith activity on multiple, potential immunesuppression through depleting the essential amino acid tryptophan. The assay consequently scaled up for automation. A commercially available small molecule library of 1900 compounds, with a high percentage of clinically tested drugs was subsequently validated with an IDO1 inhibitor as positive control and subsequent tumoroid killing was quantified using 3D high-content imaging.

The 96-well format of the assay allows medium-throughput testing of up to 3000 cultures, on the other hand, more faithfully reproduce the organization of a humanized animal models are slow, have low throughput and are expensive. 3D cultures, on the other hand, more faithfully reproduce the organization of a tumor microenvironment whereas humanized animal models are slow, have low throughput and are expensive. 3D cultures, on the other hand, more faithfully reproduce the organization of a humanized animal models are slow, have low throughput and are expensive. 3D cultures, on the other hand, more faithfully reproduce the organization of a humanized animal models are slow, have low throughput and are expensive. 3D cultures, on the other hand, more faithfully reproduce the organization of a humanized animal models are slow, have low throughput and are expensive. 3D cultures, on the other hand, more faithfully reproduce the organization of a humanized animal models are slow, have low throughput and are expensive. 3D cultures, on the other hand, more faithfully reproduce the organization of a humanized animal models are slow, have low throughput and are expensive. 3D cultures, on the other hand, more faithfully reproduce the organization of a humanized animal models are slow, have low throughput and are expensive. 3D cultures, on the other hand, more faithfully reproduce the organization of a humanized animal models are slow, have low throughput and are expensive. 3D cultures, on the other hand, more faithfully reproduce the organization of a humanized animal models are slow, have low throughput and are expensive. 3D cultures, on the other hand, more faithfully reproduce the organization of a humanized animal models are slow, have low throughput and are expensive. 3D cultures, on the other hand, more faithfully reproduce the organization of a humanized animal models are slow, have low throughput and are expensive.

In order to shed light on novel immune suppressive mechanisms in tumor, iTeos Therapeutics developed a target discovery and drug repurposing platform based on phenotypic screening assays. We established a co-culture assay combining tumor immune suppressive cells and T-cells. This assay is flexible to allow the screening of chemogenomics, shRNA and cDNA libraries. Multi-parameter readouts are combined to assess both T-cell activation and proliferation, through high content imaging of T-cell clusters formation, complemented with detection of IFNγ secretion and tumor cell death, as assessed using a cytotoxicity assay. The 96-well format of the assay allows medium-throughput testing of up to 3000 samples/screen. From the technical point of view we were able to adapt the assay to low level of automation, making it affordable to the biotech start-ups and academic laboratories. As a proof-of-concept we evaluated the assay for its ability to detect metabolic immune-oncology targets in A549 cells, a lung cancer immune suppressive cell line. A549 express indoleamine-2,3-dioxygenase 1 (IDO1), an enzyme expressed in many cancers that mediates local T-cell suppression through depleting the essential amino acid tryptophan. The assay conditions were validated with an IDO1 inhibitor as positive control and subsequently scaled up for automation. A commercially available small molecule library of 1900 compounds, with a high percentage of clinically tested drugs was screened. The library was tested at two different concentrations (0.3 μM and 3μM), in two independent T-cell donors and spiked with IDO1 inhibitor as control. Combined analysis of T-cell activity and tumor killing led to the identification of 42 compounds with activity on multiple, potential immune suppressive pathways, including metabolism, epigenetics, autophagy, TGFβ, Wnt/β-catenin and TNFα/IFN-κB signaling.

#4613 Cytotoxicity of selenium trastuzumab and bevacizumab immuno-conjugates against triple negative breast cancer cells. Soni Khandelwal, Lau-ren Gollahon, Julian Spallholz, Mallory Boylan, Maria Del Mar Garcia-Hernande-z, Texas Tech University, Lubbock, TX.

Breast cancer (BC) is the second leading cause of death among women with an estimated 246,660 new cases of invasive breast cancer expected to be diagnosed this year. Of the different subtypes, 15-20% are classified as triple negative breast cancer (TNBC) because they lack three key receptors; progesterone, estrogen and HER2. These receptors are considered essential for “targeted therapies”. Thus, systemic chemotherapy is the only TNBC drug treatment option. In this study, two clinical monoclonal antibodies; Herceptin® and Avastin® were covalently labeled with redox selenium (MDA-MB-468) in culture. TNBC cell lines were treated with vehicle control, increasing concentrations of selenium as Selenite (ranging from 2 to 20 μg) Se-Herceptin® or Se-Avastin® and compared to native antibodies with equal concentrations of protein. Cell counts and viability were analyzed using Beckman ViCell Counter and Trypan Blue exclusion over a seven day period. Selenium, Se-Avastin® and Se-Herceptin® were observed to be more cytotoxic over dose and time than both TNBC cell lines in comparison to control cells and cells treated with native antibodies alone. Morphological changes were observed under phase contrast microscopy in all selenium treated cell lines. In contrast, visible alterations in morphology were absent in native Herceptin® and Avastin® treated cells compared to control cells. We believe this is the first report of selenium Antibody-Drug Conjugates (ADCs) being demonstrated as cytotoxic to these TNBC cell lines, suggesting a potential strategy to design more effective treatments of TNBC resistant to chemotherapy.


Introduction: Lenvatinib (LEN) selectively inhibits the kinase activity of VEGFR1-3, FGFR1-4, KIT, PDGFRα, and RET, which are involved in tumor angiogenesis and tumor cell proliferation in several cancer types. Currently, Phase 1b/2 clinical trials of the combination of LEN and pembrolizumab (a monoclonal antibody [mAb] that blocks the interaction between PD-1 and its ligands) are ongoing for selected types of cancer including renal cell carcinoma, melanoma and non-small cell lung carcinoma. We have reported that tumor associated macrophage and regulatory T cell population were downregulated by treatment of LEN. In this study, mechanism of LEN and PD-1 mAb combination treatment was investigated by flow cytometry and RNA-seq analyses. Experimental Procedure: We examined antitumor activity of combination treatment of LEN (10mg/kg, qd) and anti-mouse PD-1 mAb (500μg/mouse, twice weekly) in CT26 and B16F10 syngeneic mouse models. In order to investigate tumor-specific memory T cell response, re-inoculation of fresh CT26 cells into tumor-free mice was conducted. For immune cell population analyses, tumor or spleen samples were analyzed by flow cytometry. Changes of molecular immune response in tumors were examined by RNAseq followed by weighted gene co-expression network analysis (WGCNA). Summary of data: Combination of LEN with PD-1 mAb showed more potent anti-tumor growth activity in CT26 and B16F10 models compared with either treatment alone. Notably, complete tumor regression was noted in a portion of mice that received combination treatment only. Re-inoculation of fresh CT26 cells into these tumor-free mice did not grow tumor; in contrast, all naive animals grew tumor aggressively in the same experimental condition. RNAseq analysis of treated CT26 tumors indicated that combination of LEN with PD-1 mAb synergistically up-regulates IFN signaling-related genes. Flow cytometry analysis revealed that LEN increased memory T cell population compared with vehicle treatment. Conclusions: The results indicate that the combination of LEN with PD-1 mAb was more effective than either single agent alone in multiple syngeneic tumor models and was accompanied with a potent antitumor immune response, especially the up-regulation of memory T cell and Th1 activation by LEN.

#4615 Development of an antibody-drug conjugate with broad anticancer activity. Siang Ye Lin,1 Zoltan Szekely,2 Chen-Yong Lin,3 Joseph R. Bertino,1 Gulam Mohmad Rather,1. Rutgers Cancer Institute of NJ, New Brunswick, NJ; 2Georgetown Medical School, Georgetown, MD.

Treatment for patients with advanced solid tumors that include triple negative breast cancer (TNBC), non-small cell lung cancer (NSCLC), and castrate resistant prostate cancer (CRPC), as well as Mantle Cell Lymphoma (MCL), while increasing survis is not curative. We have identified a membrane bound protease, “activated” matritiapase, as an attractive target antigen for highly selective antibody delivery of cytotoxicites as activated matritiapase expression is restricted to epithelial tumors and some B-cell lymphomas. We generated a novel ADC by linking M69, a mouse antibody specific to activated matritiapase, to

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monomethyl auristatin E (MMAE) via a PEGylated, releasable di-peptide linker as a proof-of-principle prototype. Both in cell lines and in human xenograph models of TNBC, NSCLC, CRPC and MCL, the conjugate was found to exhibit potent anticaner activity against all of these tumor types without toxicity. Encouraged by these results, we are also exploring the use of this ADC against gastric cancer, and in combination with chemotherapy and immunotherapy. As this is a mouse antibody, we are also generating a chimeric antibody for toxicity studies in primates.

**IMMUNOLOGY: Innate Immune Response to Cancer**

### #4616 Exploring the role of interferon and innate immune regulators in outlier cancer cell line survival.

Hugh S. Gannon,1 Michael Kiessling,2 Francisca Vazquez,3 William Hahn,1 Matthew Meyerson1. 1Dana-Farber Cancer Institute, Boston, MA; 2Zürich University Hospital, Switzerland; 3Broad Institute, Cambridge, MA.

We conducted an outlier dependence analysis using functional genomic screening data generated by groups at the Dana-Farber Cancer Institute and Broad Institute. These genome-wide knockdown (shRNA) and knockout (CRISPR-Cas9) screens seek to discover genetic vulnerabilities, or “Achilles heels”, across hundreds of cancer cell lines. The most recent version of the gene knockdown screen includes over 500 total cell lines, allowing overall and lineage-specific outliers to be assessed. Taking advantage of the relatively large number of cell lines in this data set, our study parallels “exceptional responder” patient studies derived from clinical trials, with the goal of finding a small number of cell lines that are acutely dependent on certain genes for survival. These outlier cell lines could then be profiled to search for a common genetic factor or gene expression signature that can be used to predict sensitivity to the knockdown of the gene(s) in question. Strong, specific genetic dependencies have the potential to generate novel, hypothesis-driven therapeutic strategies that can be further tested experimentally. Initially, we focused on the lung cancer cell lines profiled in the knockdown screen. We used an unbiased approach to investigate genes with the most pronounced outlier patterns across these lung lines. One of the top genes in our analysis encodes the RNA-editing enzyme ADAR1, which has a role in epigenetically altering double-strand RNA sequences. ADAR1 encodes a protein that catalyzes adenosine-to-inosine editing in RNA. This RNA editing function has been studied in the context of suppressing innate immune sensing, as endogenous RNA is edited to avoid detection by intracellular sensors that activate the type I interferon pathway after encountering unedited RNA from infecting viruses. ADAR1 homozygous mutant patients and mouse models each display greatly increased interferon levels and activation of downstream interferon-induced gene expression, further supporting the role of ADAR1 to suppress interferon activation and autoimmunity. Importantly, outlier cell lines sensitive to ADAR1 knockdown are also sensitive to knockdown of ISG15, which similarly suppresses interferon signaling, and show greatly increased survival after IFNAR1 knockdown, a positive regulator of interferon signaling. We next mined genome-wide CRISPR-Cas9 knockout screens and found a pancreatic cell line that is also dependent on ADAR1 and ISG15 for survival. Likewise, knockout of IFNAR1 or any gene along the IFNAR1-JAK-STAT pathway enhances survival in this cell line. These converging data suggest a link between the interferon pathway and vulnerability to knockdown of ADAR1 or ISG15. While no common mutations or genomic changes were found in the outlier cell lines, we have uncovered a shared gene expression signature. We are continuing to study the mechanisms that cause these cell lines to be acutely dependent on ADAR1 and ISG15.

### #4617 Daratumumab combined with CD38(-) natural killer cells armed with a CS1 chimeric antigen receptor for the treatment of relapsed multiple myeloma.

Yuefeng Wang, Yibo Zhang, Don Bonz, Michael Caliguiri, Jianhua Yu. Ohio State Univ., Columbus, OH.

Multiple myeloma (MM) is the second most frequent hematological cancer in developed countries. The recent development of monoclonal antibodies, including Daratumumab (Dara) approved by the FDA, is changing the treatment algorithm of MM. However, MM still relapses and remains incurable. Recently, chimeric antigen receptor (CAR) T cell immunotherapy has been successful in the clinic for the treatment of leukemia and lymphoma. We previously showed that both CS1-CAR T cells and CS1-CAR NK cells are effective in eradicating MM cells in vitro and in vivo (Chu et al., 2014, Leukemia and Chu et al., 2014, Clin. Cancer Res.). Both CS1 and CD38 are tumor-associated antigens expressed on the surface of MM cancer stem-like cells, suggesting that dual targeting of CS1 and CD38 may prevent relapse. Thus, we investigated the combination therapy with Dara and CS1-CAR NK cells for the treatment of relapsed MM. Our data showed that CD38 is highly expressed on the surface of both NK and MM cells. Dara induces NK cell activation, as Dara-treated NK cells display a higher capacity to produce IFN-gamma and increased cytotoxicity against MM, the latter of which correlated with augmented expression of the cytolytic gene, granzyme B. Mechanistically, we found that activation of NK cells by Dara is CD16-dependent. This is further evidenced by that Dara also significantly induces phosphorylation of NF-kB and STAT1, the two downstream mediators of CD16. Furthermore, Dara mediated cytotoxicity of NK cells against MM cells through an ADCC-mediated cytotoxicity against MM, the latter of which can show a synergistic effect on tumor eradication and prevention of MM relapse. Our data indeed demonstrated that the combination of CS1 CAR NK cells with CD38(-) NK cells but not with CD38+ NK cells shows a synergistic effect to eradicate MM cells. Interestingly, we also found that when compared to expanded CD38+ NK cells, expanded CD38(-) NK cells have more cells, a better capacity of proliferation and survival, a higher potential of cytokine production, and higher cytotoxicity against MM cells.

### #4618 TGF-B upregulates GSK3B and mediates NK dysfunction in cancer.

Evelyn Ojo, Folashade Otegbeye, Stephen Moreton, David Wald. Case Western Reserve University, Cleveland, OH.

Transforming growth factor-B (TGF-B) is an important regulator of tumorigenesis. Initially, it was discovered to have anti-cancer activities by promoting apoptosis and preventing hyperproliferation of cells. However, abundant presence of TGF-B in the tumor microenvironment is known to hinder cancer eradication by suppressing anti-tumor activities of immune cells. The molecular switches that regulate the role of TGF-B are not fully understood. One main target of TGF-B-mediated immune cell dysfunction are natural killer (NK) cells. NK cells are innate immune cells that lyse virally-infected and cancer cells. Recently, we have discovered that glycojen synthase kinase 3-beta (GSK3B) upregulation in NK cells leads to NK dysfunction. Another study has indicated that GSK3 phosphorylates SMAD3 to inhibit TGF-B-mediated growth dysfunction in AML12 cells. In addition, GSK3B inhibition has been linked to increased sensitivity of cancer cells to NK killing but the role of GSK3B in NK function is understudied. This study examines how the role that GSK3B has on TGF-B-mediated NK cell dysfunction. Methods: NK cells were isolated from the peripheral blood of healthy donors and expanded in vitro for 14 days. NK cells were either treated with or without TGF-B, and NK cell cytotoxicity is measured with a fluorometric cytotoxicity assay. Results: TGF-B pretreatment of NK cells led to 2-fold GSK3B expression in NK cells. TGF-B pretreatment led to decreased expression of NK activating receptors NKp46 and NKG2D. Also, TGF-B led to decreased expression of lymphoyte function-associated antigen-1 (LFA-1), an adhesion molecule necessary for NK cells to adhere to target cells prior to analysis of the target cells. TGF-B pretreatment of NK cells led to 33.3% reduced killing of both leukemic and solid tumor targets. Pharmacologic and genetic abrogation of GSK3 minimized TGF-B-mediated NK dysfunction as measured by calcein-AM based cytotoxicity assays. Importantly, inhibiting GSK3B activity with TGF-B treatment rescued NK cell cytotoxic function. Discussion: Our data suggests that TGF-B leads to upregulated GSK3B expression, and downregulation of NK activating receptors. In addition, TGF-B reduced NK killing of cancer cells. We believe that TGF-B-mediated NK dysfunction is facilitated by GSK3B upregulation. Further studies are underway to determine how TGF-B affects the expression of other NK receptors, and to elucidate the mechanism by which TGF-B directs GSK3B upregulation.
#4619 CD47 promotes ovarian cancer progression by inhibiting macrophage phagocytosis. Yuting Huang,1 Peng Gao,2 Yifeng He,1 Yuchi Ma,1 Shan-gwen Dong,2 Zhijun Li,1,2 Tianjin Medical University Cancer Hospital and Institute, Tianjin, China; 3University of the District of Columbia, Washington, DC; 4Shanghai Jiao Tong University, Shanghai, China; 5National Heart Lung and Blood Institute, Bethesda, MD; 6Tianjin Medical University General Hospital, Tianjin, China.

CD47 is highly expressed in many malignant diseases including ovarian can-cer, and renders malignant cells resistant to phagocytosis by macrophages. Anti-CD47 mAbs have shown anti-cancer effects in several types of cancers. How-ev-er, it remains controversial whether anti-CD47 mAbs promote phagocytosis by the inhibition of CD47 function or by CD47-SIRPs independent mechanisms. We tried to provide more evidence in our study by knocking down CD47. CD47 expression of 93 patients was tested by flow-cytometry. We confirmed a positive correlation between high CD47 levels and poor clinical characteristics, such as poorly differentiated and advanced FIGO stages. A cohort of 29 FIGO stage III-IV patients with poorly differentiated serous adenocarcinoma were followed up for 50 months. Median survival for CD47 high patients was 19 months, while for CD47 low patients was 43 months, p=0.0435. To further evalu-ate the mechanism of CD47 in phagocytosis, CD47 expression on SK-OV-3 cell was knocked down using shRNA. No change in tumor cell viability, prolifera-tion, and migration was observed. Macrophage phagocytosis index increased from 10.2% to 43.9% in vitro. In xenograft experiments, average tumor number decreased from 75 to 6.4 and the animals also experienced a longer survival (p=0.0017). Similar sets of experiments were done using an anti-CD47 mAb B6H12 clone while adopting both control IgG and anti-HLA ABC as con-trol antibodies. Consistent with the results from the knockdown experiments, anti-CD47 mAb treatment led to an increase in macrophage phagocytosis index in vitro of both SK-OV-3 cells (from 8% and 9.4% to 37.1%) and primary cancer cells (from 5.6% and 7.4% to 35.1%). In vivo xenograft experiments also showed increased macrophage infiltration (6.5% compared to 1.5% in control group). In five different ovarian cancer cell lines, we found that CD113+ tumor initiating cells (TICs) expressed higher levels of CD47 compared to CD133+ cancer cells. Tumorigenic capabilities of TICs were confirmed by colony-forming assay and ALDEFLUOR assay. Upon anti-CD47 mAb treatment, macrophage phagocyto-sis of TICs was enhanced significantly in vitro (phagocytosis index increased from 10% and 12.7% to 47.3%). In conclusion, CD47 inhibits macrophage phagocytosis of both ovarian cancer cells and TICs. Downregulation of CD47 or inhibition by mAb was able to reverse this effect, and it may involve Fc independent mecha-nism. Thus, CD47 antibody therapy may be a promising strategy for not only eliminating tumor cells but also preventing cancer relapse triggered by TICs, benefiting the patients’ short-term and long-term prognoses.


Macrophages are abundantly found in the tumor microenvironment and en-hance malignancy. At distal metastatic sites, our previous studies identified a distinct population of metastasis-associated macrophages (MAMs) that pro-mote tumor cell extravasation, seeding and persistent growth. These macro-phages were derived from inflammatory monocytes recruited by CCL2/CCL2 chemokine signaling and directly promote tumor cell extravasation through VEGF production. Our recent studies identified two subsequent signaling path-ways that mediate the retention and function of MAMs after recruitment that are critical for tumor cell survival and persistent growth. Specifically, activation of CCR2 signaling turns on CCL3 expression in MAMs. Genetic deletion of CCL3 or its receptor CCR1 in macrophages reduces lung metastasis, and MAM accumulation in tumor-challenged lung. Adoptive transfer of wild type mono-cytes restores lung metastasis in Ccl3 deficient mice. Mechanistically, Ccl1 defi-cency prevents MAM retention in the lung by reducing direct MAM-tumor cell interactions. On the other hand, MAMs turn on cell surface expression of FMs-like tyrosine kinase 1 (FLT1) and FLT1 signaling regulates CCL3 expression. Using several genetic models of HIF1 deficiency, we show that macrophage specific FLT1 signaling is critical for breast tumor distal metastatic potential. FLT1 spe-cific inhibitory antibody significantly inhibits the metastatic seeding and persist-ent growth but does not affect the recruitment or retention of MAMs. Further-more, we identified that FLT1 signaling regulates a set of downstream inflammato-ry response genes including Colony Stimulating Factor 1 (CSF1) and a central regulator of macrophage biology. Using a genetic gain-of-function ap-proach we show that CSF1 mediated autocrine signaling in MAMs is down-stream of FLT1 and can restore the tumor-promoting activity in MAMs even when FLT1 is inhibited. Together, our data indicates CCL3/CCR1 and FLT1 signaling pathways are critical in regulating MAMs and promoting breast cancer distal metastasis, and suggests the therapeutic potential of targeting these path-ways in treating metastatic disease.


Macrophages dominate the immune infiltrate present in pancreatic ductal adenocarcinoma (PDAC). Although tumor-infiltrating macrophages have the potential to mediate anti-tumor activity, they most commonly acquire an im-munosuppressive role in cancer. We have found that Cpg oligonucleotides - containing unmethylated cytosine-guanine motifs - potently induce macro-phages to phagocytose PDAC cells and mediate anti-tumor effects. When deliv-ered in vivo, in a fully immunocompetent murine model of PDAC, Cpg in-creased the presence of macrophages within PDAC tumors and suppressed tumor outgrowth in a macrophage-dependent manner. Though Cpg did not polarize macrophages toward classical anti-tumor (M1) or pro-tumor (M2) phenotypes, Cpg treatment increased the basal rate of oxygen consumption in macrophages. Reversing this metabolic shift by inhibiting fatty acid oxidation abolished pro-phagocytic and anti-tumor effects by macrophages in vitro and in vivo. We investigated the impact of Cpg in overcoming anti-phagocytic signals from CD47, a membrane protein overexpressed in multiple malignancies, in-cluding PDAC. While targeted knockout of CD47 in PDAC cells was not suffi-cient to activate in vitro phagocytosis by macrophages or anti-tumor effects in vivo, Cpg potential to overcome PDAC CD47 blockade that was independent of CD47 expression by tumor cells. However, the combination of CD47 loss in tumor cells and systemic Cpg treatment enhanced the survival of tumor-bearing mice, indicating that disruption of CD47 can sensitize tumors to macrophage-directed immunotherapy. Together, our findings demonstrate a key role for cellular metabolism in directing the anti-tumor functions of macro-phages and overcoming negative regulatory signals imposed by malignant cells.

#4622 Characterization of sulfatide reactive type II NKT cells from mouse Cancer. Lise Pasquet,1 Shingo Kata,2 Tony Adams,1 Susan Sharrow,1 Theresa Das-ties-Hill,1 Elaine Jaffe,1 Xia Zheng,1 Motoshi Suzuki,2 Damien Kovalovsky,3 Jay Berzofsky,2 Masaki Terabe,2 NHG-NCI, Bethesda, MD; 3Yokohama city University, Yokohama, Japan; NHG- NHELI, Bethesda, MD.

The immune system plays a major role in the elimination of tumors. CD8 T cell infiltration is known to be a good prognostic indicator. The development of therapies based on checkpoint inhibitor antibodies was an important break-through in increasing survival by limiting the exhaustion of cytotoxic cells and increasing tumor cell eradication. However, the immune system also contains regulatory cells that protect the organism from inappropriate activation of im-mune cells against self-antigens. Thus, the fact that tumor cells are analogous to tolerogenic leads regulatory cells to inhibit the activation of anti-tumor cytotoxic cells and thus increase tumor escape. Therefore, understanding the function and the ac-tivation of regulatory cells might help to develop therapies to limit the activation of regulatory cells in order to increase tumor clearance. Natural killer T (NKT) cells are lymphocytes with features of natural killer (NK) and of T cells placing them at the interface of innate and adaptive immunity. Like NK cells, they rapidly produce cytokines after stimulation, orienting the immune response. As T cells, they express a T cell receptor (TCR) that allows the recognition of specific lipids presented by the non-classical MHC-I molecule CD1d. According to their TCR usage, two populations of NKT cells are described: type I and type II. All type I NKT cells express a semi-invariant TCR (Valpha24/14/18 in mice) that recognizes α-galactosylceramide (αGalCer). They can be identified with the oGalCer-loaded CD1d tetramer. In contrast, type II NKT cells express a more diverse TCR repertoire. There is no currently identified lipid antigen recognized by all type II NKT cells, making their identi-fication more difficult. A fraction of them recognize sulfatide. By using sulfatide-loaded CD1d-tetramers, we observed for the first time that sulfatide-reactive type II NKT cells were enriched in the lung, a major site of tumor metastasis. Moreover, we previously showed that in vivo stimulation of type II NKT cells with sulfatide increased the number of tumor nodules in the lung. An in-depth phenotype analysis revealed that they were CD4 or CD8 single positive cells, like conventional T cells, whereas type I NKT cells are either CD4+CD8- or double negative. Type II NKT cells do not express PLZF, the master regulator of NKT cell development and exist as PLZF- mice contrary to type I NKT cells. We showed that type II NKT cells also expressed markers of myeloid cells, c-KIt, CD11b and Ly6C even though histological analysis revealed lymphohcytic mor-phology. Interestingly, at steady state, type II NKT cells expressed granzyme A but not granzyme B or perforin. The in vivo injection of sulfatide increased the
expression of the activation markers CD69 and CD44 as well as granzyme A. Since the regulatory functions of type II NKT cells have been shown to be critical in tumor immunity, the detailed characterization of these cells could help to develop a new immunotherapy for cancer.

#4623 Transcriptional analysis of the functional differences between M1 and M2 macrophages to identify new targets for myeloid cell modulation in pancreatic tumors. Kimberly R. Yang, Todd D. Armstrong, Elizabeth M. Jaffee. The Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD.

Macrophages exhibit dynamic plasticity in their function in response to the accumulating genetic alterations that occur during tumorigenesis, resulting in the establishment of an immune-tolerized tumor microenvironment. However, depending on their environmental cues, they can switch between two functional phenotypes: pro-inflammatory/anti-tumorigenic (M1) or anti-inflammatory/pro-tumorigenic (M2). The importance of these two macrophage subsets is well-recognized; however, identifying and distinguishing M1 from M2 macrophages in patient tumors still remains a challenge. Prior studies have utilized transcription profile of artificially skewed M1 and M2 macrophages from healthy donors to identify individual genes or gene clusters unique to each macrophage subset. However, these studies are limited to surface marker expression and gene expression profiles alone to distinguish subsets and lack functional validation of M1 and M2 macrophages. Surface marker and gene expression profiling of human M1 and M2 macrophages provide an incomplete characterization, as functionality is an important feature distinguishing these macrophage subsets. To improve the responsiveness and cytokinoty of these cell subsets in peripheral bloods, we performed a thorough three-step validation of artificially skewed M1 and M2 macrophages from healthy donor monocytes using surface marker expression, gene expression profiling, and multi-analyte ELISA analyses. Using our validation assays, we tested previously published protocols for their efficacy in generating M1 and M2 macrophages. While M1 macrophages using these protocols generated as expected M1 characteristics, M2 macrophages lacked hallmark functional features, particularly IL-10 secretion. This necessitated the generation of an optimized protocol to skew M1 and M2 macrophages that exhibited distinctive surface marker expression, gene expression profiles, and cytokine and chemokine production for their respective subset. We then performed RNA-sequencing to interrogate the transcriptional landscape of functional M1 and M2 macrophages and have found 1,472 transcripts that have at least a two-fold change in differential expression between M1 and M2 macrophages across 4 different donors. These results represent the most in-depth characterization of human M1 and M2 macrophages and provide insights into better biomarkers and targets for novel immunotherapy approaches.

#4624 Natural killer cell licensing contributes to clearance of lung cancer. Shi Lei,1 Kang Li,1 Saeed Arefian,2 Alexander S. Krupnick.1 University of Virginia, Charlottesville, VA; 2Washington University in St. Louis, St Louis, MO.

a) Natural Killer (NK) cell Inhibitory receptors function in a dichotomous role. Their engagement by MHC Class I upon encountering a target results in the inhibition of cytotoxicity. During NK development, however, their interaction with MHC Class I mediates the process of licensing or education, leading to an increased responsiveness and cytokininity. For this reason, receptors in controlling the MHC Class I+ solid tumors such as lung cancer is poorly defined. Since NK cells comprise the primary cytotoxic lymphocyte responsible for clearance of lung cancer, we set out to determine whether the licensing status of NK cells contributes to lung cancer susceptibility. b) Growth of MHC Class I+ murine lung cancer was evaluated in MHC Class I- mice, with and without NK cells in direct comparison to MHC Class I+ mice with licensed NK cells. IFN-γ production was evaluated after plate bound antibody stimulation through the NKp46 and NKG2D pathways both flow cytometrically and by quantitative RT-PCR. Bone marrow transplantation was performed by injection of 10x10^5 unfractionated bone marrow cells after 500Gy irradiation and NK and CD4+ T cell depletion of recipient host. c) Injection of Lewis Lung Carcinoma (LLC), revealed a significant decrease in pulmonary metastatic tumor burden in LLC mice with licensed NK cells (230.4±21.6 vs. 442.7±53.8 mg) that disappeared after NK depletion (421.4±36.8 vs. 421.9±66.3 mg). Freely isolated licensed NK cells lysed LLC more robustly than unlicensed NK cells (28.7±16% vs. 4.9±2.5% specific lysis at 100:1 ratio respectively). Lung cancer expresses stress ligands recognized by NKG2D and NKp46 activating receptors. Licensed NK cells produced significantly more IFN-γ than their unlicensed counterparts after plate-bound stimulation with anti-NKp46 and anti-NKG2D (17.9±2.8% vs. 5.4±2.8% respectively for dual NKp46 and NKG2D stimulation). No differences were evident between licensed and unlicensed NK cells in ribosomal mass, making it unlikely that translational defects were responsible for altered cytokine production. Licensing status also did not affect stability of IFN-γ but did affect mRNA levels. This suggests transcriptional control of licensing-mediated NK activity. IFN-γ production could be significantly increased in unlicensed NK cells by reinduction of MHC Class I through non-myeloablative bone marrow transplantation from MHC Class I-sufficient congenic donors. Blockade of MHC Class I (anti-NKp30 and MHC Class I receptor) in this reinduction the role of inhibitory receptors. This suggests that NK cells mediate a superior lung cancer-specific immune response in a murine model. Our data suggests that altering the licensing status of NK cells may be an avenue of lung cancer-specific immunotherapy.

#4625 Natural killer T cells regulate tumor immunity in mouse pancreatic cancer organoid orthotopic model. Shingo Kato,1 Tetsuya Matsuura,2 Yoshitaka Hippo,1 Atsushi Nakajima,1 Yoko Hanna,1 University of Virginia Hospital, Yokohama, Japan; 2Chiba Cancer Center Research Institute, Chiba, Japan.

Natural killer T (NKT) cells are lymphocytes with features of natural killer (NK) and of T cells placing them at the interface of innate and adaptive immunity. Like NK cells, they rapidly produce cytokines after stimulation, orienting the immune response. As T cells, they express a T cell receptor (TCR) that allows the recognition of specific lipids presented by the non-classical MHC-I molecule CD1d. There are two subsets of NKT cells, type I and type II, distinguished by their T-cell receptors. All type I NK T cells express a semi-invariant TCR (Vα24a18 in humans, Vα14(18) in mice) that recognizes α-galactosylceramide (α-GalCer). Type II NKT cells express a more diverse TCR repertoire. The biological activity of these two subsets of NKT cells is assessed comparing the percent of NKT cells that recognize the α-GalCer -GalCer). In contrast, type II NKT cells express a more diverse TCR repertoire.

The pancreatic organoids derived from KrasG12D mice were transduced with Cre and shRNA against p16, to activate Kras and repress p16, respectively. These transduced organoids were then subcutaneously injected into C57Bl/6 wild type mice, and tumors were harvested after two weeks. The tumors were cut into small pieces, and the weight of each piece was measured. The piece of tumor was sewn to the pancreas of wild type, Jor18-/- mice and CD1d-/- mice. After two weeks, tumors were harvested, and the weight of tumor was measured. The tumor weight was significantly heavier in CD1d-/- mice than wild type mice (p = 0.0416). Although it was not statistically significant, the tumor weight was slightly heavier in Jor18-/- mice than wild type mice. We also analyzed the correlation of T cell subsets in peripheral bloods. However, there were no differences among the three strains of mice. Considering these results, NKT cells may regulate tumor immunity in the microenvironment of the pancreatic cancer. Moreover, since sulfatide is rich in pancreas, sulfatide-reactive type II NKT cells may be involved in this local immune response. The detailed analysis of the regulatory functions of NKT cells in pancreas could help to develop a new immunotherapy for pancreatic cancer.
cells to sites of metastasis. Through the use of a novel, biomimetic, 3D collagen/ hyaluronic acid hydrogel system developed in our labs, this study focused on interactions between primary human macrophages and cancer cells, specifically on how this dialogue impacts the production of the secreted HS-modifying enzymes, heparanase (HPSE) and endosulfatase I (SULF1). We studied the impact of co-culturing macrophages with endometrial or prostate cancer cell lines, as well as patient-derived xenografts, on the levels of HPSE and SULF1. We also assessed cancer growth characteristics in the presence of macrophages. Taken together, our observations indicate cancer cell responses to macrophages are cell type specific, and we demonstrated significant functional consequences of interactions between macrophages and HS-mediated signaling. We are currently developing macropolarization to classically activated (M1) or alternatively activated (M2) phenotypes alters cancer cell responses in 3D. This system is being used to determine novel approaches to intervene in HBGF delivery to limit cancer growth in a more physiologically relevant in vitro system.

#4627 Prognostic significance and population shift of peripheral monocytes in patients with oropharyngeal squamous cell carcinoma. Hideyuki Takahashi, Koichi Sakakura, Kazuaki Chikamatsu. Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan.

Evidence has accumulated that chronic inflammation plays a critical role in carcinogenesis, tumor growth, and metastasis. To date, several inflammatory biomarkers are considered as potential prognostic factors in various cancers. The aim of the present study was to investigate the prognostic significance of the pretreatment levels of inflammatory biomarkers in patients with oropharyngeal squamous cell carcinoma (OPSCC). A total of 67 patients newly diagnosed as OPSCC was finally included. The neutrophil, lymphocyte, monocyte and platelet counts recorded before treatment was initiated, then lymphocyte-to-mono- cyte ratio (LMR), neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lympho- cyte ratio (PLR) were calculated. These inflammatory biomarkers and clinical parameters including age, differentiation, T-stage, N-stage, TNM-stage, smoking, and drinking were compared with progression-free survival (PFS) and overall survival (OS) using cox regression. Elevated monocyte count and low LMR were significantly associated with poor OS; of note, monocyte count and LMR were independent prognostic factors for PFS. Elevated monocyte count and low LMR were significantly associated with poor OS; moreover, low LMR was an independent prognostic factor for OS. Of note, monocyte count and LMR were associated with prognosis more intensively in patients with p16-negative OP- SCC. Following these results, we evaluated the peripheral blood mononuclear cells of 14 patients with OPSCC collected before treatments. CD14+/CD68+/HCLR-DR+ cells were gated as monocytes, then divided into three subsets according to the expression of CD14 and CD16. The proportion of intermediate monocytes was lower and that of classical monocytes was higher in OPSCC patients than in healthy donors, especially in patients with elevated monocyte count. Furthermore, the expression level of PD-L1 was higher in patients than in healthy donors. In conclusion, our data suggest that the elevated monocyte count and low LMR seem to be useful biomarkers for predicting prognosis of OPSCC; furthermore, the population shift of monocytes may relate to poor prognosis of OPSCC.

#4628 Selection of Smad2 for TGF-β to suppress dendritic cells by STAT3/ c-Ski-induced repression of Smad3. Jeong-Hwan Yoon, 1 Eunjin Bae, 1 Katsumu Sudo, 2 Masakatsu Tanakashi, 1 Jin Soo Han, 1 Seok Hee Park, 1 Michael Weinstein, 1 Susumu Nakae, 1 Tamahisa Yamashita, 1 In-Kyu Lee, 1 Ji Hyeon Jeu, 1 Takuya Sumida, 1 Masahiko Kuruda, 2 Keiji Miyazawa, 1 Mitsuayi Kato, 1 Mizuko Mamura 3. 1Kyungpook National University, Daegu, Republic of Korea; 2Tokyo Medical University, Tokyo, Japan; 3Konkuk University, Seoul, Republic of Korea; 4Sungkyunkwan University, Suwon, Republic of Korea; 5The Ohio University, Columbus, OH; 6University of Tokyo, Tokyo, Japan; 7Azabu University, Kanagawa, Japan; 8Catholic University of Korea, Seoul, Republic of Korea; 9University of Tsukuba, Tsukuba, Japan; 10University of Yamanashi, Yamanashi, Japan. 1

The Smad family of proteins is involved in the regulation of many signaling pathways, among which TGF-β signaling is a key regulator. TGF-β signaling is involved in cell proliferation, death, and differentiation. Signaling is mediated by the transmembrane 55kDa TGF-β receptor (TβR) and the cytoplasmic Smad2/3. Smad2/3 directly bind the receptor complexes and form a complex with Smad4. This complex is transported to the nucleus where it binds to the promoter of target genes. The aim of this study was to understand the role of Smad2 in TGF-β signaling and how this regulation is affected by the inhibitory Smad3. Here, we show that TGF-β upregulates the expression of Smad3, which inhibits the transcriptional activity of Smad2. TGF-β signaling was found to be dependent on the SMAD3 expression, which was upregulated by TGF-β. The TGF-β signaling pathway was found to be inhibited by Smad3 downregulation. These findings suggest that Smad3 negatively regulates TGF-β signaling by suppressing the expression of its target genes.

#4629 Macrophage number correlates with tumor stage in cutaneous melanoma. Satu Salmi, 1 Hanna Siiskonen, 2 Reijo Sironen, 3 Kristiina Tynnela-Kohonen, 3 Paivi Auvivinen, 1 Sanna M. Pasonen-Seppänen. 1 University of Eastern Finland, Kuopio, Finland; 2 Kuopio University Hospital, Kuopio, Finland.

Tumor associated macrophages and regulatory T cells (Tregs) have an impor- tant role in tumor progression as a part of the immunosuppressive tumor microenvironment. By means of immunosuppression, melanoma, as well as many other cancers, is able to resist anti-cancer immune reactions. To understand the mechanisms how tumor cells escape from the anti-tumor immune response is the key for efficient treatment strategies. In several cancers, programmed cell death ligand-1 (PD-L1) -mediated pathways have been shown to suppress anti- tumor immune responses. Our aim was to investigate the number of tumor associated macrophages (TAMs) and Tregs in benign, premalign and malign melanocytic lesions to find out if the density of these cells is associated with the tumor stage or survival in melanoma. Moreover, the expression of PD-L1 was studied. Altogether 187 tissue samples, including 29 benign and 27 dysplastic nevi, 16 in situ melanomas, 38 superficial (Breslow < 1 mm) and 43 deep (Breslow > 4 mm) melanomas and 34 lymph node metastases, were stained immunohistochemically for CD68 and CD163 representing all TAMs and M2-like macrophages, respectively and for FoxP3, a marker for Tregs. In addition, a part of the FFPE -samples was dual stained for CD68 and PD-L1. Macrophages were counted using a hot spot -method. In the levels of analyzies, the cell numbers were graded as either low or high according to the median. Non-parametric Kruskal-Wallis test was used to compare the macrophage numbers in different groups and Pearson chi-square test to study the correlations between the numbers of CD673 and CD68 -positive cells and clinicopathological paramet- ers. CD68+ and CD163+ macrophage numbers were significantly higher in malignant melanocytic lesions and lymph node metastasis compared to in situ melanomas and benign nevi. In deep melanomas and lymph node metastases macrophages located mainly inside the tumor nests whereas in thin melanomas they were mostly scattered around the tumor. High CD68+ macrophage num- ber correlated with the presence of ulceration (p<0.011) and with both local recurrence (p=0.002) and recurrence with distal metastases (p<0.002). PD-L1 immunoreactivity was relatively scanty in melanoma samples being restricted to certain areas only; both membranous and cytoplasmatic staining was observed. Overall, the staining was higher in deep melanomas and lymph node metastases compared to superficial melanomas. Dual stainings indicated that in addition to tumor cells, a part of the tumor infiltrating CD68+ macrophages were PD-L1 -positive. The density of FoxP3+ cells did not correlate with the tumor stage. However, the number of these cells was significantly higher in deep melanomas and lymph node metastases compared to other groups. Our observations highlight that TAMs are involved in melanoma progression and have an important role in the formation of an immunosuppressive tumor microenvironment.

#4630 Development of selective and potent CDK8 inhibitors that increase NK cell activity, which translates in tumor surveillance. Marco H. Hofmann, 1 Martin Christ, 1 Andreas R. Engelhardt, Herbert. A. 2, Marc Kerenyi, Moritz Mayer, Gerhard Gmaschitz, Georg Egger, Christian Engel- hardt, Michael Sanderson, Maria A. Impagnatiello, Renate Schnitzer, Mark Pearson, Darryl McConnell, Norbert Kraut, Jürgen Moll. Boehringer Ingelheim RCV GmbH & Co KG, Vienna, Austria.

Background: Cyclin-dependent kinase 8 (CDK8) is part of the mediator com- plex that can either positively or negatively influence transcription. CDK8 is known to phosphorylate signal transducer and activator of transcription 1 (STAT1) at the position Ser727. STAT1 activity is regulated by JAK-mediated phosphorylation of tyrosine701 which leadstodimerization,nucleartransloca- tion and IFN-γ-induced phosphorylation mediated by CDK8. Introduction of an alanine mutation at the phosphorylation site STAT1-S727 results in enhanced NK cell cytotoxicity accompanied by increased levels of perforin and granzyme B (Putz et al. 2013). Method: Here we present the discovery and development of potent and selective CDK8 inhibitors guided by crystallography. The inhibitory effect of optimized compounds BI 9181 and BI 1347 on STAT1 phosphorylation and perforin release was investigated in the human NK cell line NK-92MI. Direct effects on cancer cells were furthermore analyzed in a broad panel of cell lines. The compound BI 1347 was profiled in vivo in the orthotopic B16-F10 melanoma mouse model. Results: Highly potent and selective CDK8 inhibitors were identified with an IC50 of below 10 nM in a biochemical kinase assay, which translated in a potent down regulation of the STAT1 Ser727 signal and in increased perforin and granzyme B secretion. BI 9811 and BI 1347 were
highly selective for CDK8, as tested in a broad kinase panel and showed no cytotoxic activity on NK cells and most cancer cell lines, which distinguishes this compound class from published CDK8 inhibitors. A representative molecule out of this compound class demonstrated in vivo biomarker modulation and survival increase in the murine B16-F10 melanoma mouse model. Conclusion: We developed potent CDK8 inhibitors that show activation of NK cells that translates into biomarker modulation (pSTAT1Ser727) and in vivo efficacy.

#4631 Enhanced glycolysis promotes function of tumor associated macrophages in mouse breast cancer model. Yan Liu,1 Wei Zhou,2 Wei Wang,3 Lipeng Bai,1 Huwien He,1 Jian Guo,1 Yunping Luo,4 Chinese Academy of Medical Sciences, Beijing, China; 2Nankai University, Tianjin, China.

Tumor associated macrophages (TAMs) represent a major part of immune cells in tumor microenvironment (TME) and play crucial roles in promoting tumor progression by secreting cytokines or enzymes that could remodel TME or enhance envision or migration capabilities of tumor cells. Given their indispensable effect on tumor, the mechanisms underlying TAMs’ tumor-promoting phenotype and function remain unclear. Glucose metabolism in tumor cells has been intensively studied; however, the status of glucose metabolism of TAMs is still unclear. In this study, we aim to identify glucose metabolism status in TAMs, and explore how glucose metabolism regulates phenotype and function of TAMs. In the beginning, we isolated TAMs from 4T1 mouse breast tumor, spleen macrophages from normal mouse as a control, and performed RNA sequencing to analyze the expression pattern in glucose metabolism related genes. The results from transcriptomics showed that TAM shown up regulation of glycolysis genes pattern. Then we analyzed and found that the Hrk activity was increased while IDH’s activity was decreased in mouse breast cancer mole. Furthermore, in vitro, we found that up regulation of important glycolysis genes including HK2, PFK-L and LDHA in mouse peritoneal macrophages (CMPM) and mouse bone marrow derived macrophages (CMBMDM) conditionally cultured with 4T1 supernatants. Glucose consumption or production of lactate and ATP were also increased in CMPM or CMBMDM. Then we monitored lactate producing rate with Seahorse energy metabolism detector, and found that either CMPM or CMBMDM produced lactate faster than that of control. Moreover, inhibition of LDH activity by oxamate (inhibitor) also decreased glucose consumption and lactate production. Importantly, the expression of Arg1 and CD206, positively correlated with glycolysis and function of TAMs. Finally, we searched the underneat mechanism and found PI3K/AKT pathway was activated in CMPM or CMBMDM. In detail it was found that p-p85 and p-AKT was upregulated at 8 hour after 4T1 supernatants stimulation. Inhibition of PI3K/AKT pathway by LY294002 (PI3K inhibitor), notably inhibited the expression of glycolysis genes and markers of TAMs, which indicated that PI3K/AKT pathway may involved in glycolysis of TAMs. Together, we identified that TAMs adopted glycolysis as a major way to consume glucose and glycolysis was directly contributed to the phenotype and function of TAMs, which was regulated by PI3K/AKT signaling pathway.

#4632 Exosome-mediated transfer of miR-183-5p from tumor cells to macrophages contributes to regulate TAMs phenotypes and promote tumor progression and metastasis. Jian Guo, Wei Wang, Yan Liu, Huwien He, Chen Zhang, Chunhong He, Yunping Luo. Chinese Academy of Medical Sciences, Beijing, China.

Tumor-associated macrophages (TAMs) perform supportive roles in promoting tumor progression and metastasis. In tumor microenvironment, the phenotypes of TAMs are regulated by a variety of factors, such as cytokines, ligands and products of metabolism. Recently, Exosome-mediated transfer of miRNAs is proved to be a significant way for intercellular communications. Whether exosomal miRNAs derived from tumor cells contribute to TAMs phenotypes remains unclear. In this study, we aim to identify the miRNAs in exosomes transferred from tumor cells to macrophages and explore how these miRNAs regulate the TAMs phenotypes. In the beginning we transferred Cy3-labeled miRNA NC to 4T1 mouse breast cancer cells and then co-cultured with bone marrow-derived macrophages (BMDMs). The Cy3 fluorescence could be observed in BMDMs after 48 hour, suggesting that miRNAs could be transferred from tumor cells to macrophages. Next, we performed miRNAs sequencing in either BMDM or exosomes derived from 4T1 cancer cells. We analyzed and compared the miRNAseq data by bio-informatics and found that there were 30 of the most abundant miRNAs in 4T1 exosomes, and nearly all of which were rich in BMDMs. However, it was found interestingly that miR-183-5p was extremely low in BMDMs. Furthermore, we treated BMDMs with exosomes isolated from 4T1 cell conditioned medium, and then we found importantly the miR-183-5p in BMDMs was increased significantly, while cultured with exosome-depleted 4T1 conditioned medium, the expression of miR-183-5p in BM-DMs was not changed. Meanwhile, we isolated TAMs from 4T1 mouse breast tumor while spleen macrophages from normal mouse as a control. The expression of miR-183-5p in TAMs was much higher than that in spleen macrophages. Moreover, it was determined that expression of pro-inflammatory cytokine TNF and IL-6 in BMDM were increased significantly in BMDMs treated with 4T1-derived exosomes. Correspondingly, transferred miR-183-5p mimic into BMDMs, the expression of TNF and IL-6 were also increased. Finally we co-cultured 4T1 cells with BMDMs transferred with miR-183-5p, and the migration and invasion of 4T1 cells were significantly enhanced. Taken together, our study demonstrate that miR-183-5p that high expression in 4T1 exosomes can transferred into macrophages to regulated their phenotypes and promote tumor progression and metastasis in breast tumor microenvironment.

#4633 Crosstalk between MDSCs and Th17. Brjijendra Singh. University of Pittsburgh Cancer Institute, Pittsburgh, PA.

Expansion of myeloid-derived suppressor cells (MDSCs) within the tumor is a known barrier to successful cancer immunotherapy. MDSCs are heterogeneous population of immature myeloid cells with potent immunosuppressive activity. Emerging evidences in the recent years define the role of immunosuppressive activity of MDSCs on different subsets of CD4+ T cells, however the exact effect of MDSCs on inflammatory Th17 cells during tumor progression is not clear. Here we demonstrate the immunosuppressive effect of MDSCs on Th17 cells. We further show that the interaction between MDSCs and Th17 is not unilateral and reveal that Th17 cells can promote the immunosuppressive activity of MDSCs. We identified that MDSCs derived from tumor when generated in vitro from bone marrow (BM-derived MDSCs), can both suppress IL-17A expression. Our data also demonstrate that IFNγ, produced by Th17 cells is essential for the immunosuppressive activity of BM-derived MDSCs on IL-17A expression and further that the IFNγR1-driven signaling is important for Nos2-mediated immunosuppressive effect of BM-derived MDSCs on IL-17A expression. Understanding the molecular mechanism of the interplay between MDSCs and Th17 cells may help to develop more specific targeted therapy for cancer immunotherapy and improve therapeutic efficacy of the existing therapies against cancer.

#4634 Role of surgical stress in altering host immunity in breast cancer patients. Madhuri Taranikanti, Aswin Kumar Mudunuru, Rohit Kumar Gun-taka. ESIC Medical College & Hospital, Hyderabad, India.

Background: Stress of any kind can alter the immune system. Surgery is a form of stress that can have significant effects on immune system. There occurs a reduction in the ability to fight the disease progression and also metastatic spread. Cell mediated immunity plays an important role in the control of malignancy. A reduction in cellular immunity can have adverse effects on the body. The objective of the study was to understand the effects of surgical stress on cell mediated immunity in breast cancer patients. Methods: 31 women with operable early stage breast cancer, who underwent surgery and were also receiving adjuvant chemotherapy were included in the study. Blood samples were collected from the patients during the pre-operative period and twice during the post-operative period, after 72 hours and after 21 days. Peripheral Blood mononuclear cells were subjected to flow cytometry analysis. The mean values of lymphocyte sub-populations as percentage were calculated. A questionnaire was also given to all study participants to assess the levels of stress of having breast cancer. An informed consent was taken from all the participants involved in the study. Results: The mean values of lymphocyte sub-population were found to be significantly lower (p<0.5) in the early post-operative period in breast cancer patients. However, the values returned to near pre-operative levels after 21 days. A positive correlation existed between psychological stress of having the disease and low host immunity. This indicates that any intervention that further suppresses immunity in breast cancer patients following surgery should not be used for at least 3 weeks after surgery.

#4635 The LSD1 Specific Inhibitor INCB059872 enhances the activity of the syngeneic 4T1 mouse mammary tumor model. Thomas Condamine, Steve Wang, Melody Diamond, Leslie Hall, Huiqing Liu, Antony Chadderton, Jon Lu, Chunhong He, Liangxing Wu, Timothy Burn, Wenqing Yao, Gregory Hollis, Reid Huber, Bruce Ruggeri, Peggy Scherle, Holly Koblish, Sang Hyun Lee. Incyte Corporation, Wilmington, DE.

Background: Stress of any kind can alter the immune system. Surgery is a form of stress that can have significant effects on immune system. There occurs a reduction in the ability to fight the disease progression and also metastatic spread. Cell mediated immunity plays an important role in the control of malignancy. A reduction in cellular immunity can have adverse effects on the body. The objective of the study was to understand the effects of surgical stress on cell mediated immunity in breast cancer patients. Methods: 31 women with operable early stage breast cancer, who underwent surgery and were also receiving adjuvant chemotherapy were included in the study. Blood samples were collected from the patients during the pre-operative period and twice during the post-operative period, after 72 hours and after 21 days. Peripheral Blood mononuclear cells were subjected to flow cytometry analysis. The mean values of lymphocyte sub-populations as percentage were calculated. A questionnaire was also given to all study participants to assess the levels of stress of having breast cancer. An informed consent was taken from all the participants involved in the study. Results: The mean values of lymphocyte sub-population were found to be significantly lower (p<0.5) in the early post-operative period in breast cancer patients. However, the values returned to near pre-operative levels after 21 days. A positive correlation existed between psychological stress of having the disease and low host immunity. This indicates that any intervention that further suppresses immunity in breast cancer patients following surgery should not be used for at least 3 weeks after surgery.
ulate to the development of the immunosuppressive tumor microenvironment and recent data have suggested that combining epigenetic-based therapies with immunotherapeutic agents can lead to improved efficacy in preclinical models. Since Lysine Specific Demethylase 1 (LSD1) has been shown to play a critical role in hematopoiesis, we hypothesized that inhibition of LSD1 could have a direct effect on the immune system. Furthermore, LSD1 is overexpressed in human lung adenocarcinoma cells. To test this hypothesis, we evaluated INCB059872, a potent, selective and orally available FAD-directed covalent inhibitor of LSD1 in several experimental models. In an in vitro differentiation assay, the majority of CD34+ progenitor cells were driven to a monocyctic phenotype in the presence of INCB059872, while control treated cells differentiated toward granulocytic PMN cells. Similar results were observed in vivo. Using the orthotopic 4T1 mammary cancer model, the myeloid compartment was characterized in tumor tissues following treatment with INCB059872. Notably, the population of PMN-MDSC was significantly decreased in tumor tissues following oral administration of INCB059872, whereas the macrophage population was increased. These data suggest that INCB059872 can redirect myeloid differentiation toward monocyte/macrophages and inhibit the differentiation of PMN-MDSC, this syngeneic tumor microenvironment. Consistently, in situ intratumoral T lymphocyte infiltration was increased following INCB059872 treatment. The combination of INCB059872 and a-CD-P-L1 antibody enhanced anti-tumor efficacy in the 4T1 orthotopic tumor model. Collectively, these data suggest that inhibition of LSD1 with INCB059872 can directly affect myeloid differentiation to reduce the accumulation of immunosuppressive cells, restoring tumor microenvironment to be more responsive to PD-1/PD-L1 axis blockade. This study supports the therapeutic potential for the combination of an LSD1 inhibitor with immunotherapeutic agents to improve overall clinical response in cancer patients.

#4636 p53 governs the tumoricidal activity of M1 macrophages in lung cancer. Yi-Jing Hsiao,1 Hsuan-Yu Chen,1,2 Kang-Yi Su,2 Bing-Ching Ho,1 Jeremy J.W. Chen,3 Pan-Chyr Yang,4 Sung-Liang Yu.1 1National Taiwan University, Taipei, Taiwan; 2Academia Sinica, Taipei, Taiwan; 3National Chung-Hsing University, Taichung, Taiwan.

Macrophages in a tumor microenvironment have been characterized as M1- and M2-polarized subtypes. M1 macrophage is associated with favorable clinical outcome of lung cancer patients, but the detail molecular mechanism is not thoroughly understood. Herein, we elucidate the anti-tumor mechanism of M1 macrophages by in vitro and in vivo assays. M1 conditioned media (CM) significantly reduced the tumor size in the xenograft mouse model via intratumoral administration. The M1 altered differential expression was profiled by microarray and the pathway analysis indicated that p53 was a critical regulator. Lung cancer harboring wild-type p53 (wt-p53) went apoptosis after M1 CM culture, but cells with mutant p53 did not. Additionally, M1 CM enhanced wt-p53 accumulation through reducing p53 ubiquitination and increasing protein stability. Moreover, we identified a p53 interacting protein STAT1 involved in M1-induced apoptosis and p53 accumulation. The CM of M1 culture, but cells with mutant p53 did not. Additionally, M1 CM enhanced wt-p53 accumulation through reducing p53 ubiquitination and increasing protein stability. Moreover, we identified a p53 interacting protein STAT1 involved in M1-induced apoptosis and p53 accumulation. The M1 CM not only enhanced the interaction between STAT1 and p53 but also up-regulated and activated STAT1. To reveal the upstream signaling, we found that knockdown of IFNAR2, Jak1 and Tyk2 attenuates M1-induced apoptosis. Taken together, STAT1 signaling is essential for M1 CM-induced apoptosis and p53 accumulation. Our findings imply that p53 and M1 macrophages play important roles in the immune surveillance in lung cancers.

#4637 Activation of toll-like receptor-2 promotes proliferation in human lung adenocarcinoma cells. Patrick Kohitz. University of Colorado - School of Medicine, Denver, CO.

Objective: Toll-like receptors (TLR) have been implicated in tumor progression by affecting the immune response in the tumor microenvironment. Toll like receptors have been described to be present in human cancer cells but the presence and the role of these receptors in the growth and proliferation of human lung cancer is not well known. We analyzed human lung cancer cells for the presence of TLR-2 receptors and evaluated the proliferation response to a specific TLR-2 agonist. Methods: Human non-small cell lung cancer cell (NSCLC) lines A549, (adenocarcinoma) H125 adenosquamous, and H1650 adenocarcinoma (American Type Culture Collection), were cultured using standard techniques. Cells were treated with Pam3CSK4, a specific agonist of TLR2, at doses of 0.5ug/ml, 5ug/ml, and 10ug/ml for 72 hours. Baseline protein levels of TLR2 were assessed using standard western blotting techniques. Proliferation assays were completed using the MTS assay in response to treatment with the agonist Results: Protein analysis demonstrated varying but consistently detectable levels of TLR2 protein in each cell line. After treatment with Pam3CSK4 we found that proliferation was significantly promoted in the human lung adenocarcinoma cell lines (p=.03), but not in the adenosquamous cell line (p=0.001). Conclusion: Toll-like receptor -2 is consistently present and detectable on human lung adenocarcinoma cells. Toll Like receptor -2 activation results in increased proliferation in human lung adenocarcinoma cells and this effect appears to be mediated by TLR2. Further study is needed to identify the downstream pathways responsible for the increased proliferation we see in these human lung adenocarcinoma cells, however these findings may suggest TLR-2 to be a possible therapeutic target in human lung cancer.

#4638 The p53 suppression on tumor cells may recruit macrophages and modulate their expression of the indoleamine 2, 3-dioxygenase. Takafumi Nakamura. Kawasaki Medical School, Kurashiki, Japan.

We had found that expression of indoleamine 2,3-dioxygenase (IDO) on cervical cancer cells increased as it progressed from carcinoma in situ (CIS) to microinvasive cancer, and that tumor-associated macrophages (TAM) recruited at the invasive front also expressed IDO in cervical cancer patients (Cancer Sci 98:874-881 2007). We previously produced homozygous αT3 transgenic mice carrying two copies of α-SV40Tag transgene (αT3 denotes murine α-crystal-lin promoter) in order to study DNA virus-induced carcinogenesis. The αT3 mice developed undifferentiated cancer of lens epithelium, which tumors showed multi-step carcinogenesis which was similar to cervical cancer (J Cancer Res Clin Oncol 135:1521-1532 2009). We found that homozygous αT3 mice carrying two copies of SV40Tag transgene progressed faster and died earlier than hemizygous αT3 mice carrying one copy of Tag transgene. In this study, in order to examine the relationship between p53 function and tumor progression, αT3 mice were mated with M1 mice expressing mutant p53 in lens cells (Proc. Natl. Acad. Sci. USA, 92:6142-6146, 1995) producing αT3M1 mice, and also were mated with p53-deficient mice to produce p53-deficient αT3 mice (αT3p53(-/-)) mice (Cancer Letters 179:165-173,2002, Oncology Report 12:253-258, 2004). At 20 weeks of age, the proportion of progression from CIS to invasive cancer was analyzed by H&E staining and the number of recruited TAM and IDO-expressing TAM were determined by immunohistochemical staining and FACS analysis. Pathologically, both αT3M1 and αT3p53(-/-) lens tumors significantly progressed to invasive cancer more than αT3 tumors. There were a few macrophages in wild-type lens, but more TAM were recruited in both αT3, αT3M1 and αT3p53(-/-) lens tumors. However, no significant difference of the number of IDO-expressing macrophages in peritoneal cavity was detected in each mice of wild-type, αT3, αT3M1 and αT3p53(-/-). It suggested that p53 suppression on tumor cells might modulate IDO-expression for macrophages. Moreover, we established the tumor cell lines from each αT3, αT3M1 and αT3p53(-/-) lens tumors. The peritoneal macrophages were analyzed for IDO-expression in 4 days after i.p. injection of these cells. Significantly, IDO-expressing macrophages in peritoneal cavity were more reduced. We detected the IDO expression of each αT3M1 and αT3p53(-/-) cells than i.p. injection of αT3 cells. And the co-culture of peritoneal macrophages with each αT3M1 and αT3p53(-/-) tumor cells also induced the IDO-expression of macrophages more than the co-culture with αT3 cells. These results suggested that the amount of p53 suppression on tumor cells would be related to the number of recruited TAM and their expressing IDO. The peritoneal macrophages were treated with IFN-γ and their expression of IDO function and, suppress an innate immunity possibly through the expression of IDO, which may enhance invasion and progression of tumors.

#4639 HLA and NKG2D ligand abnormalities and IFN-γ unresponsiveness in human colorectal carcinoma cell lines. Yi-Hsin Lin,1 Yen-Cheng Lin,1 Kuo-Hsin Chen,1 Chi-Hua Kao,1 Ann-Liu Hsieh,1 Chien-Ching Chang2. 1Graduate Institute of Oncology, College of Medicine, National Taiwan University, Taipei, Taiwan; 2Institute of Molecular and Cellular Biology, National Tsing Hua University, Hsinchu, Taiwan.

Background: Cetuximab, an anti-epidermal growth factor receptor (EGFR) monoclonal antibody, has shown promising efficacy on wild-type RAS metastatic colorectal cancer (CRC) patients. Cetuximab demonstrates anti-cancer effect via NK cells and subsequent immunogenic cell death. Chemotherapy or targeted therapy may change immune surface markers. Methods: We characterized a panel of 6 CRC cell lines with different genetic features. The impacts of interferon-γ (IFN-γ) on cell surface expressions of major histocompatibility complex (MHC) class I (including subtypes), MHC class II (including subtypes), MHC class 1 related molecule A (MICA, the NK cell ligand), Intercellular Adhesion Molecule 1 (ICAM-1, the immunglobulin superfamily), EGFR, and Chondroitin Sulfate Proteoglycan 4 (CSPG4, pericyte attaching marker) before and after treatment with IFN-γ (100 U/ml, 48 h) were explored in selected cell lines Results: Among six human colon cancer cell lines analyzed, DLD-1, HCT15, and LoVo exhibited no expression of HLA-A and HLA-B,C. Colo-320
demonstrated both low HLA-A and HLA-B,C expression and non-responsive to IFN-γ stimulation. In contrast, both HT-29 and SW-480 showed low HLA-A and HLA-B,C expression, but the expression of both HLA-A and HLA-B,C was corrected after IFN-γ stimulation. All six cell lines possessed low MHC class II expression and non-responsive to IFN-γ stimulation. All six cell lines possessed low MHC expression and non-responsive to IFN-γ stimulation. Five of six cell lines showed low ICAM-1 expression, but only HT-29 and SW-480 were responsive to IFN-γ stimulation. LOVO and SW-480 demonstrated high EGRF expression and non-responsive to IFN-γ stimulation. Only HT-29 possessed high CSPG-4 expression. Conclusion: These findings reveal that regardless of their genetic features, all six CRC cell lines possess a phenotype that may allow them to escape cytotoxic T and NK cell attack.

### Table 1: Cell Line Characteristics

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<tr>
<th>Cell Line</th>
<th>MSI</th>
<th>CIMP</th>
<th>KRAS</th>
<th>BRAF</th>
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<tr>
<td>DLD-1</td>
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<td>HCT-15</td>
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<td>LOVO</td>
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<td>COLO-320</td>
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#4640 Proteomic differentiation of pulmonary cancer with squamous cell histology.

Histologic classification of pulmonary cancer with squamous cell histology is challenging as reliable immunohistochemical biomarkers are lacking. In particular, smokers with head and neck cancer can develop both lung metastases and primary lung cancer. However, their differentiation is clinically important for therapy and risk stratification. Moreover, molecular targeted therapies for squamous cell carcinoma of the lung are largely lacking. To identify proteomic diagnostic biomarkers, signaling patterns and potential novel drug targets we characterized a broad panel of primary patient-derived formalin-fixed squamous cell carcinoma tissues from lung and head and neck cancer by quantitative mass spectrometry. Proteins were isolated from formalin-fixed, paraffin-embedded (FFPE) patient-derived genetically characterized cancer tissues by using a “filter-aided sample preparation (FASP)” method. The resulting proteins were analyzed by a Super-SILAC-based mass spectrometry approach and data was analyzed using the software suite MaxQuant and Perseus to determine the tumor-type-specific protein expression and signaling patterns. In this study we quantitatively analyzed the protein-expression profiles of 50 primary patient-derived non-small cell lung cancer specimens with squamous cell histology and 30 squamous cell carcinomas from the head-neck-region derived from patients that developed lung tumors with similar histology in the course of their disease. Using the mass spectrometric approach we were able to quantify in average around 2500 proteins per sample. Unsupervised clustering- and principal component analyses revealed that the detected protein expression patterns show a strong correlation with the cellular origin of the analyzed carcinomas. Furthermore, secondary lesions with similar histological morphology in the lung in patients with squamous cell carcinoma of the head-neck-region could be classified as primary or metastatic cancer according to their protein expression profiles. Collectively, this study provides a large set of potential proteomic biomarkers that might be useful to improve diagnostics in the context of lung tumors with squamous cell histology in the future. In particular the differentiation of squamous cell carcinoma and head and neck cancer-derived metastases in the lung - that is still a challenge for diagnostics - will be improved by the presented biomarker panel. Moreover, the expression of the proteins that are identified could help to determine the potential of signaling pathways that were discovered in our study are of interest regarding potential novel lung cancer therapies as overexpression or hyperactivation of certain kinases can potentially contribute to the malignant phenotype of lung cancer cells.

#4641 Wild type APC is an independent marker of poor prognosis in late stage colorectal cancer.

Background: Colorectal cancer (CRC) is one of the commonest cancer worldwide and ranks as the most common cancer in males and the third most common among females in Saudi Arabia. Wnt pathway alteration is well known in colorectal cancer. In this study we aimed to comprehensively study Wnt pathway alterations in a large cohort of Middle Eastern colorectal patients and to know its role in this unique ethnic group. Methods: 426 CRC cases were analyzed for APC, AXIN2 and CTNNB1 somatic mutations by targeted capture sequencing and protein expression status by immunohistochemistry in tissue microarray format. Results were analyzed for association with any clinicopathological parameters and for prognostic significance. Results: The incidence of APC, AXIN2 and CTNNB1 mutations in our patient cohort were 58.2%, 4.2% and 3.8% respectively. APC wildtype mutation was associated with 50% of the tumors (p = 0.0494) and male sex (p = 0.0349). AXIN2 mutation were associated with grade 3 tumors (p = 0.0231), MSI-high tumors (p < 0.0001) and AXIN2 protein expression loss (p = 0.0056). CTNNB1 mutations were associated with MSI-high tumors (p = 0.0076). AXIN2 and CTNNB1 mutation were significantly associated with each other (p = 0.0263). Wild type APC was associated with poor overall survival and was an independent marker of poor prognosis in late stage colorectal cancer (p = 0.0263). Wild type APC independently predicts poor prognosis in Middle Eastern late stage colorectal patients.

#4642 Ion mobility separation of N-Glycans directly from ffpe colon cancer tissue section in a MALDI imaging experiment.

Colon cancer is currently the third most common cancer in both men and women. Research studies have reported extensive alterations in protein glycosylation patterns in cancer tissues. However during these studies, the tissues are homogenized and the spatial information showing the localization of the glycans is lost. Mass spectrometry imaging (MSI) is an established analytical tool for biomolecular research which can accurately determine the spatial location of molecules in a tissue section. Recently, methods have been developed to determine released N-Glycans directly from tissues. A major challenge in the analysis of N-glycans is the large number of isobaric glycans resulting from their complex structures with branched chains and multiple additions residues. Here we report the advantage of ion mobility separation to differentiate these glycans in a MALDI MSI workflow used in the analysis of human FFPE colon cancer tissue. 5μm FFPE tissue were were deparaffinized in xylene, rehydrated in different composition of ethanol/water solutions, transferred in citraconic anhydride buffer for antigen retrieval and water washed. To release the glycans from their proteins, a PNGaseF solution was sprayed on the tissue. A solution of MALDI was sprayed onto the tissue. Experiments were carried out on a SYNAPT G2-Si HDMS system (Waters) where the tri-wave separated ions according to their ion mobility in the gas phase. The overall MALDI spectrum shows strong signal for N-glycan molecules, demonstrating the efficacy of the digestion step of the methodology. Using prior knowledge of the type of glycans, 76 glycans were identified and mapped directly from the FFPE tissue section, from a mass to charge ratio (m/z) of 771.5 up to 2905.03 using accurate mass information. APC, AXIN2 and CTNNB1 mutations in our patient cohort were 58.2%, 4.2% and 3.8% respectively. APC wildtype mutation was associated with 50% of the tumors (p = 0.0494) and male sex (p = 0.0349). AXIN2 mutation were associated with grade 3 tumors (p = 0.0231), MSI-high tumors (p < 0.0001) and AXIN2 protein expression loss (p = 0.0056). CTNNB1 mutations were associated with MSI-high tumors (p = 0.0076). AXIN2 and CTNNB1 mutation were significantly associated with each other (p = 0.0263). Wild type APC was associated with poor overall survival and was an independent marker of poor prognosis in late stage CRC (p = 0.0263). Conclusion: We reported the incidence of Wnt pathway alterations in this unique ethnic group on which such data is lacking. APC mutation plays major and other genes minor role in Wnt pathway activation. Incidence of APC mutation in this group is comparatively high compared to reported incidence worldwide. Wild type APC independently predicts poor prognosis in Middle Eastern late stage colorectal patients.
phase was identified to be the N-Glycan class of molecules. Using IMS the MS data showed a clear picture of the N-Glycans compared to the MS data alone. In several cases, the IMS peaks were broader than the expected resolution or there were shoulder peaks, indicating that isobaric species were present. For example, m/z 1444.6 ( Hex4dHex1HexAc3Na+) displayed two IMS distinct peaks which were not baseline separated and were contributed to the obtain individ-
ual ion images which showed significantly different distributions. The isobaric species with the faster drift time was evenly distributed across the tissue type of the section, whereas the isobaric species that had the slower drift time was less abundant in the cancer tissue.

#4643 Plasma thioredoxin reductase 1 as a diagnostic marker for non-small cell lung cancer. Xiaosheng Kang,1 Chaoran Dong,2 Bocun Shen,1 Wanpu Yan,1 Liang Dai,1 Yongbo Yang,1 Hanwei Yin,3 Guobing Xu,1 Huihui Zeng,2 Ke-Neng Chen1. 1Peking University Cancer Hospital and Institute, Beijing, China; 2Peking University Health Science Center, Beijing, China; 3Kaise Center for Clinical Laboratory, Wuhan, China.

Non-isobaric diagnostic markers for patients with suspicious non-small cell lung cancer (NSCLC) may provide needed guidance on invasive diagnostic and therapeutic decisions. Thioredoxin reductase 1 (TrxR1) is a pivotal intracellular oxidoreductase that plays an important part in tumor growth, progression, metastasis, and chemotheraphy resistance. The goal of this study is to test the feasibility of developing plasma TrxR1 as a novel diagnostic marker for NSCLC. The plasma TrxR1 activity was determined spectrophoto-
metrically by monitoring the NADPH-dependent production of 2-nitro-5-thio-
benzoate at 412nm and at 37°C. All detection kits and automatic analyzer pos-
se independent intellectual property rights, and have been widely used in breast cancer screening. In this study, the plasma levels of TrxR1 were measured in 45 treatment-naive NSCLC patients and 10 individuals with benign tumor-like pulmonary diseases. Taking plasma TrxR1 activity of 4 as reference, the overall concordance rate was 73.3%, while those of CEA, CA199, CA125, NSE, CYFRA21-1 and SCC were 19.0%, 7.1%, 16.7%, 35.7%, 24.4% and 2.5%, respecti-

#4644 A cell-line based TP53 expression pattern control panel with inter-
observer variability and next-generation sequencing analysis. Yaling Zhou,1 Sorana Raiciulescu,1 Visar Syed,2 Patrick Malafronte,1 Justin Wells,1 Mica Grantham,1 Michael Allgaeuer,1 Heping Gong,1 Joel Moncur,1 Deyin Xing,6 John D. Andersen1.

#4645 A serum multiomics signature for enhancing prostate cancer diag-
nosis and prognosis. Michael A. Kiebish,1 Jennifer Cullen,2 Albert Dobi,2 Amina Ali,2 Leonardo O. Rodrigues,1 Yezhou Sun,1 Aniruddha Pawar,1 Aditee Dalvi,1 Denise Young,3 Vivek K. Vishnadav,1 Jason Sedarsky,2 Gyorgy Petrov-
ics3, Emily Chen,1 Vitschelsak Akaemua,1 Inger L. Rosner,2 David McLeod,1 Isa-
bell A. Sethermann,2 Rangaprasad Sararanjanag,1 Alagarsamy Srinivasan,2 Elder Grainger,1 Niven R. Narain,1 Shiv Srivastava,1 1BERG, LLC, Framingham, MA; 2Uniformed Services University and the Walter Reed National Military Medical Center, Center for Prostate Disease Research, Bethesda, MD; 3Joint Pathology Center, Silver Spring, MD.

Introduction and Objective: Diagnosis of prostate cancer (CaP) has relied on prostate specific antigen (PSA) level and digital rectal examination (DRE) followed by prostate biopsies. These modalities have the potential to yield false-
positive and false-negative results for CaP. These challenges prompted efforts to develop more specific body fluid based assays including PCA3, TMPRSS2:ERG, KcKcore and PHI tests. Further, emerging data on significant racial differences of common CaP driver genes, e.g., PTEN and ERG in CaP can lead to significant limitations in biomarker performance. Thus, the goal of our study was to dis-
cover CaP serum markers with equal performance among African American (AA) and Caucasian American (CA) men. We employed proteomics, signal-
and structural lipidoanalysis and metabolomics platforms to discover serum bio-
markers and validate. This 35th edition, which we have proposed as Acute leukemia in AA and CA men. Methods: Sera from 700 individuals were analyzed, which included AA and CA CaP patients stratified for ERG oncoprotein expression by immunohis-
tochemistry (N=495). Sera from age-matched healthy control men were also included (N=205) in this study. Quantitative global profiles of liposome, pro-
teome and metabolome were analyzed by high resolution MS-based technolo-
gies. Random forest and Interrogative Biology4 analytical platforms were used to identify analytes differentiating healthy from CaP cases including clinical-pathologic data. Results: The unbiased global profiling and integration of the data and clinical-pathologic features have led to the identification of molecular fingerprints differentiating cancer patients from healthy controls. Specifically, three analytes in serum metabolome showed robust separation between both AA and CA CaP and control groups. Elevated levels of nicotinamide and ei-
cosanoid acid and decreased levels of a decanoyl carnitinate alone have indicated strong separation between cases and controls. Further inclusion of additional 9 analytes provided an optimal multi-omics panel for distinguishing the combined cohort of AA and CA cases vs. healthy controls. Conclusions: The findings presented here support that an integrated multiomics approach has the potential to define serum marker panels for diagnosis of CaP in the context of racial diversity and molecular annotation (e.g., ERG) of CaP. These promising data are undergoing validation in additional patient cohorts.

#4646 A novel truncated variant of the hematopoietic Hb-2 tubulin iso-
type with implications for stem cell biology. Yang Bai, Paul Basciano, Xi Li, Jason Matasak, Silvana Di Giandomenico, Siddhartha Sen, Todd Evans, Joseph Scandura, Monica Guzman, Paraskevi Giannakakou. Weill Cornell Medicine, New York, NY.

The αβ-tubulin dimer is the building block of the microtubule cytoskeleton, which is important for many cellular functions. Several α and β tubulin isoforms have been identified so far; Hb-1 tubulin (human β tubulin Class VI) is a hematopoietic-specific tubulin isoform whose expression is restricted to megakaryo-
cytes and platelets. TCGA analysis showed that β-1 is highly and specifically expressed in acute myeloid leukemia (AML). β-1 may therefore be an attractive target for the development of novel therapeutics for AML. We developed a highly-specific polyclonal antibody against the last C-terminal amino acids and analyzed a panel of AML cell lines and primary samples by western blot. We identified the presence of a novel truncated 35KD band, distinct from the can-
nonical 39KD tubulin. Leukemia. Isobologram β-1 (ALIBI), was present in 10 of the 19 AML cell lines tested as well as in a large number of AML clinical samples, as evidenced by immunoblotting, and corroborated by RNA-sequencing. We determined that ALIBI was a splice variant of Hb1 tubulin, lacking exons 1 and 2 with partial retention of exon 3 and intron 3. Mass array methylation analysis revealed differential promoter and intragenic methylation pattern in canonical β1 tubulin versus ALIBI, suggesting that there is an epigenetic switch that determines the expression of each tubulin isoform. Immunofluorescence analysis revealed that ALIBI, unlike all tubulin isoforms, does not incorporate into microtubules but instead accumulates in the nucleus and in distinct puncta in the cytoplasm. Exogenous expression of ALIBI,
also resulted in a similar cellular distribution. Using a murine leukemia mouse model we found significant enrichment of ALIBI in the leukemic stem cell fraction, while in normal mouse development ALIBI was present in fetal liver and hematopoietic progenitor cells. Based on this observation, as well as the relationship between leukemic stem cells and embryonic stem cells, we probed for the presence of ALIBI within murine embryonic stem cells (mESCs) and found ALIBI to be present in cultured mESCs, and down-regulated upon differentiation when full length b1 tubulin was expressed. Taken together, these data suggest that ALIBI has an important, heretofore unrecognized, role in stem cell biology. However, much remains unknown regarding the function of ALIBI within the nucleus, the factors that regulate its expression over the canonical EML4-ALK and the importance of its precise role in progenitor or stem cells. Understanding the function and regulation of ALIBI will allow us to understand a novel role of tubulin in stem cell biology and open multiple avenues for application of this knowledge.

#4647  Mass spectrometric analysis of oncometabolite 2-hydroxyglutarate in clinical gliomas with the isocitrate dehydrogenase-1 mutation. Mitsuhiro Hayashi,1 Hiroaki Aikawa,2 Makoto Ohno,3 Koichi Ichimura,4 Yuko Matsu- shita,1 Mayu Ohuchi,1 Maniko Mizui,1 Akihiko Yoshida,5 Yoshitaka Natari,1 Akinobu Hamada,1 National Cancer Center Research Institute, Tokyo, Japan; 2Exploratory Oncology Research and Clinical Trial Center, National Cancer Cen- ter, Tokyo, Japan; 3National Cancer Center Hospital, Tokyo, Japan.

Introduction: Mutations of the isocitrate dehydrogenase (IDH)-1/2 genes frequ- ently occur in certain malignancies, including gliomas. Mutant IDH-1/2 protein expression has the potential to produce oncometabolite 2-hydroxyglutarate (2HG), which results in a different clinical impact on gliomas in comparison to those with a wild-type IDH. The status of IDH mutations has been determined by immunohistochemistry (IHC) using the IDH1-R132H antibody or through the DNA sequencing of IDH1-R132C/R132S; however, its practical applications are still under discussion, and the significance of the oncometabolite 2HG evaluation is not clear. In this study, we analyzed the serum and tissue levels of 2HG in clinical gliomas using liquid chromatography-tandem mass spectrometry (LC- MS/MS) and the matrix assisted laser desorption and ionization mass spectrom- etry imaging (MALDI-MSI) system. Method: Glioma samples (n = 78, matched pairs of serum and flash frozen tissue) were obtained between October 2007 to January 2015 at the National Cancer Center Hospital, Japan. High performance liquid chromatography (Nexera X2, Shimadzu) and triple quadrupole mass spectrometry (QTRAP4500, AB SCIEX) were used for LC-MS/MS analysis. The two MALDI-MSI systems, quadrupole ion trap time-to-flight mass spectrometry imaging (iMScope, Shimadzu), and high-resolution atmospheric pressure mass spectrometry imaging (AP-SMALDI, TransMIT; Q-Exactive, Thermo Fisher Scientific) were used for analyzing the 2HG tissue distribution after coating with 9-aminoacridine or 2,5-dihydroxybenzoic acid. The IDH mutation was deter- mined by direct sequencing and IHC. Results: The median 2HG concentration in serum was 21.43 ng/mL (range: 9.58 to 62.51) in this study, and no significant correlation was observed between the IDH mutation and the wild type status (mutation, n = 28, median 20.2 ng/mL, range 9.58 to 62.51 vs. wild type, n = 50, median 22.88 ng/mL, range 11.51 to 40.32, Wilcoxon test p = 0.1597). The median of the 2HG tissue concentration in the entire group was 16 ng/g, and a significant difference was seen between the IDH mutant and wild type groups (mutation, median 4.86 ng/μg, range 0.51 to 49.86 vs. wild type, median 0.09 ng/μg, range 0.02 to 0.45; Wilcoxon test p = <0.0001). In the MALDI-MSI analysis, the average 2HG ion intensities were 381.62±2.57 in the IDH mutation and 8.25±1.67 in the wild-type IDH tissues (Wilcoxon test p = 0.0016), which showed a positive correlation with the 2HG tissue concentration by LC-MS/MS analyses (Spearman p = 0.87, p = 0.0003). The MSI results indicate a possible link between the 2HG tissue distributions and the histology of IDH mutant sections. Conclusion: Tissue 2HG measurement using mass spectrometric analyses may be a useful alternative in determining IDH mutations. Further research may provide a biological perspective and help gain some insights on the clinical significance of 2HG.

#4648 Development of a rapid, precise, and sensitive molecular assay for ALK fusion detection. Mona D. Shahbaziyan,1 Yueyi Shang, Maidaar Jamba, Mi- chael J. Powell,2 DiaCarta, Richmond, CA.

Introduction: In non-small cell lung cancer (NSCLC), chromosomal translo- cation events that result in overactivation of the anaplastic lymphoma kinase (ALK) have been identified and shown to play a key role in tumorogenesis. Inhibitors of the ALK kinase, such as the FDA-approved crizotinib, are effective in treating ALK-fusion-positive non-small cell lung cancer. Molecular studies have identified many ALK fusion variants in various cancer types, encompassing a combination of 22 different gene partners as well as various break points within the partner genes. The most common fusion events in non-small cell lung cancer are between the echinoderm microtubule associated protein like 4 (EML4) gene and the ALK gene. Fusion events between the Kinesin Family Member 5B (KIF5B) gene and ALK have also been identified. Existing assays for ALK fusion detection include the time-consuming, costly, and challenging flu- orescence in situ hybridization (FISH) method, which can result in a high inter-observer variability. Next generation sequencing platforms are also available, which are also costly to implement, difficult to interpret, and have lengthy workflows. Reverse-transcriptase PCR methods are ideal, as they are cost-effective, sensitive, rapid, and require little interpretation expertise. Methodology: We designed a multi-plexed real-time RT-PCR method to enable indiscriminate detection of ten EML4-ALK, and the remaining variants encompassing approximately 95% of EML4-ALK and 90% of KIF5B-ALK characterized fusion vari- ants, based on the Catalogue of Somatic Mutations in Cancer (COsmic) data- base. The assay uses standard real-time PCR instrumentation and was characterized using the Bio-Rad CFX384®, Roche LightCycler® 96, and Roche LightCycler® 480 II instruments. Assay preparation and run time requires 2 hours or less. Sensitivity testing showed that for the Roche LightCycler® 480 II and Bio-Rad CFX384®, 100% detection was achieved for 50 copies of fusion template for each of the 13 targeted variants. The Roche LightCycler® 96 showed slightly poorer sensitivity, with 100% detection observed at 50 copies for 9 fusion variants and 100 copies for the remaining four variants. Inter- and intra-run precision testing demonstrated good reproducibility and repeatability, with a coefficient of variation of less than 10% for all targeted fusion variants. Using ALK-positive and ALK-negative FFPE reference materials, it was demonstrated that 50 ng of total FFPE RNA was sufficient and up to approximately 200 ng showed good performance. Conclusion In summary, we have developed a rapid, precise, and sensitive assay for the detection of thirteen EML4-ALK and KIF5B- ALK fusion events to enable molecular characterization of non-small cell lung cancer tumors.

#4649 Development of an ethnicity informed gene expression panel with potential to improve prostate cancer diagnosis. Indu Kohar,1 Lakshmi Ravi- dranath,1 Reema Banerjee,1 Yongmei Chen,1 Amina Ali,1 Yingjie Song,1 Jacob Kagan,7 Sudhir Srivastava,2 Albert Dobi,4 David McLeod,1 Inger L. Ros- ner,5 Shiv Srivastava,2 Georgy Petrovics1,2, CPDR/USUHS, Rockville, MD; 2NCI/ NIH, Bethesda, MD; 3WRNMMC/USUHS, Bethesda, MD.

Introduction and Objectives: Prostate cancer (CaP) affects 1 in 7 men throughout their life time. One of the major risk factors for the development of CaP is race/ethnicity. African American (AA) men have significantly higher incidence and mortality from CaP compared to Caucasian American (CA) men. Emerging data including ours have described significantly lower frequencies of alterations in the common CaP driver genes (ERG and PTEN) in AA men as compared to CA men. We have also noted that genes commonly overexpressed in ERG, AMACR and PCA3, and currently used as diagnostic markers exhibit much lower frequency and more heterogeneity in AA men. The goal of this study was to define a broader CaP marker panel that is overexpressed equally well in AA and CA CaP. Methods: Three platforms (RNASeq, NanoString and QRT-PCR) were used for evaluation of CaP associated gene expression in CA and AA patients (N=144). Candidate genes with robust tumor overexpression (over 4-fold) in CaP in comparison of paired normal and tumor specimens from AA and CA patients were selected from the Nanostring and RNAseq data for validation by QRT-PCR (Taqman) in laser microdissected (LCM) tumor and benign cells of frozen tissue sections (50 CA and 35 AA). An assay protocol (gene specific pooled RT and pre-amplification followed by TaqMan PCR) was set up for the noninvasive early detection of candidate genes in regular patient urine (non-DRE) using RNA derived from urinary exosomes. Results: As expected tumor transcriptomes of CA patients revealed consistently elevated expression of PCA3 and AMACR genes. However, these genes had variable overexpression in AA cohort. The top genes that were similarly over expressed in tumors of AA and CA patients were validated by real time QRT-PCR (Taqman) analysis in LCM dissected tumor and normal epithelial cells (N=85). At least one gene of a six gene signature (DLX1, HOXC4, NKX2-3, COL10A1, HOXC6 and PSGR) was overexpressed in tumor cells of all AA and CA cases, providing a consistent ethnicity informed tumor expression signature, which was further validated in silico in TCGA RNASeq data. Urinary exosome based assay was developed and optimized for PSGR, DLX1, HOXC4, NKKX2-3, as well as PCA3 and ERG. Sensitivity and specificity with optimal cutoff for the urine marker panel was 71% and 61% respectively (N=140). Evaluation of the assay performance in CA and AA patients in a prospective independent cohort of 100 patients addressing race specific performance is in progress. Conclusions: A CaP tissue based gene expression marker panel has been defined with potential diagnostic utility for both CA and AA men in the context of urinary exosomes.
#4650  Mutation detection by target sequence analyses using tissue-specific panels in esophageal squamous cell carcinoma. Takeshi Iwaya,1 Fumitaka Endo,1 Kohei Kume,1 Yasushi Sasaki,2 Takashi Tokino,1 Satoshi Nishizuka1. 1Iwate Medical Univ. School of Medicine, Morioka, Japan; 2Research Institute for Frontier Medicine, Sapporo Medical University, Sapporo, Japan.

Whole exome sequencing (WES) studies of esophageal squamous cell carcinoma (ESCC) have revealed that only a small number of frequently mutated genes, such as TP53, NOTCH1, NRF2, and ZNF750, are present in individual tumors, whereas hundreds of others less frequently mutated genes are accumulated. The mutation detection rate by target sequence analyses is an important factor for clinical sequence in the course of ESCC treatment. This study aimed to evaluate the mutation detection rates of several target sequence analyses for ESCC. Ten pairs of surgically or endoscopically acquired tumor tissues and corresponding peripheral blood mononuclear cells were obtained from ESCC patients. We used three types of target sequence panels for mutation screening in 10 ESCC patients as follows: 1) Cancer Hotspot Panel (CHPv2), targeting mutation hotspot regions in the most common 50 human cancer genes that are frequently mutated (panel size: 22,027 bp), for five patients; 2) TP53 Panel, covering all exons of TP53 (panel size: 2,359 bp), for five patients; and 3) ESCC Panel, originally designed to target 31 frequently mutated genes in ESCC (panel size: 210,570 bp), for seven patients. Four missense and two nonsense mutations in TP53 were detected in CHPv2 and the TP53 Panel, respectively. In the ESCC Panel, 47 mutations in 17 genes were detected in seven patients. The mutation detection rates by the TP53 Panel, CHPv2, and ESCC Panel were more than 20-fold higher than that of WES, which has been reported to be 3.1/Mb for ESCC. These results suggest that tissue-specific cancer-related gene panels are a highly effective approach for mutation detection in ESCC.

#4651  Multiple platform evaluation of KRAS codon 12/13 reference material. Russell Garlick, Yves Konigshofer, Farol L. Tomson, Dale Yuzuki, Bharti Anekella. SeraCare, Gaithersburg, MD.

An assay’s ability to detect the presence of a KRAS driver mutation is critical when attempting to determine whether a tumor may respond to anti-EGFR therapy. Currently, there are two FDA approved companion diagnostics that can be used to assess whether there are mutations in KRAS codons 12 and 13, and both include their own internal controls. However, there is a need for external reference materials that allow laboratories to validate such assays - especially, at levels near their limits of detection (LODs). We describe the design of the SeraSeq FFPE Tumor KRAS Reference Material. This reference material is composed of 7 different 5-micron FFPE curls that contain the 7 different KRAS mutations detected by the companion diagnostics: G12A, G12C, G12D, G12R, G12S, G12V and G13D. Each curl contains a mixture of KRAS wildtype GM24385 cells and a KRAS mutant cell line in sufficient quantity for most assays. KRAS mutant frequencies of about 5% were targeted and subsequently verified by digital PCR. A 5% mutant frequency was selected because it is near the LOD for the existing companion diagnostics, and is close to the minimum reported frequency of many next generation sequencing (NGS)-based assays that test for somatic variants. The reference material was evaluated using different methods, which include digital PCR, the Roche cobas® KRAS Mutation Test, the QIAGEN therascreen® KRAS RQG PCR Kit and several NGS-based assays. Testing revealed general compatibility with these assays in that sufficient DNA could be extracted, and concordance of the results with the expected calls was high. For example, apparent DNA yields by Nanodrop with the cobas assay exceeded the required amount by more than 3-fold and all 7 curls led to a “mutation detected” result in duplicate testing. Interestingly, not all variants were detected with the therascreen assay, which may be due to some variants being present at slightly below the claimed LODs, however DNA yields were sufficient and the correct variants were identified. Finally, variant frequencies obtained by NGS were consistent with those observed by digital PCR. We conclude that the SeraSeq FFPE Tumor KRAS Reference Material may be useful as part of KRAS codon 12/13 assay validation.


Cancer immunotherapies are rapidly changing traditional treatment paradigms and expanding the therapeutic landscape for cancer patients. However, despite the current success of these therapies, significant number of patients do not respond to immunotherapy and responders often experience significant toxicities. Therefore, there is a growing need to identify predictive and prognostic biomarkers that enhance our understanding of dynamic immune mechanisms involved in cancer development. The genome-wide expression profiling of whole blood is an attractive method for the discovery of biomarkers due to its non-invasiveness, possibility for retrospective simple clinical site processing, and rich biological content. To this end, we have developed GeneNet, a genome-wide targeted RNA expression assay that profiles 19,000 genes based on multiplex RT-PCR followed by NGS in whole blood samples stabilized in PAXGene. The GeneNet 19K assay provides comprehensive and unbiased profiling of immune cell activation and canonical immune pathway genes for both innate adaptive and humoral immune responses. In this study, we present the performance of the assay for immunophenotyping of immune cells in blood samples from TNBC patients and healthy controls using high-throughput clinical applications.

#4653  Genomics of regulatory crosstalk between PPAR gamma & 14-3-3 genes in breast cancer cells. Ashraf A. Khalil,1 Nihal F. Kabapy,1 Fouad N. Sharabi2. 1City of Scientific Research, Alexandria, Egypt; 2Alexandria University, Alexandria, Egypt.

PPARγ, a ligand-stimulated transcription factor with differentiation promoting activity is overexpressed in a variety of cancers. Perturbation of PPAR-γ signaling is now believed to be a strategy for treatment of several cancers, including breast cancer. A set of genes regulated by PPAR-γ ligands is expected to favor growth and promote proliferative and immunosuppressive effects. Since 14-3-3 family of proteins shows a debatable activity and varying expression levels in different tumors, in the studies presented here we explored the transcriptional regulatory role of Pioglitazone on the seven 14-3-3 isoforms present in MCF-7 breast cancer cells. This study demonstrated that the potent PPAR-γ agonist, Pioglitazone exerted a regulatory role on expression of 14-3-3 genes where it upregulated 14-3-3 gamma, epsilon, zeta and tau by 3.4, 5.2, 2.7 and 739 folds, respectively. However, it had a negative regulatory effect on 14-3-3 beta, sigma and Eta by 16.94, 4.58 and 2.12 folds, respectively compared with control cells. These results correlated with growth arrest and a great increase in BRCA1 gene expression by 1076 folds. In summary, these findings are the first time showing that PPAR-γ regulates 14-3-3 genes and raises questions whether PPAR-γ ligands mediate their anticanccer effects via regulation of 14-3-3 proteins. Selected References: 1. Bridges, D. and G.B. Moorhead. 2005. 14-3-3 proteins. Selected References: 1. Bridges, D. and G.B. Moorhead. 2005. 14-3-3 proteins: a number of functions for a numbered protein. Sci. STKE 2005(296):re10. 2. Cheerwae, W. and J. Bright. 2008. PPARγ agonists inhibit growth and expansion of CD133+; brain tumour stem cells. Brit. J. Cancer 99(12): 2044-2053.

#4654  The primary study of the correlation between mRNA expression and protein phosphorylation of epidural growth factor receptor EGFR in colorectal and breast cancer cell lines. Hongwei Wang, Catherine Ma, Yuan Zhou. AbboMax, Inc., San Jose, CA.

The EGFR family is a group of receptor tyrosine kinases that mediates cell proliferation, migration, and differentiation. Activation of EGFR signaling is triggered by ligand-binding induced receptor dimerization. The dimerization activates the intracellular kinase domains of the receptor, resulting in autophosphorylation of C-terminal tail and the initiation of downstream signaling. EGFR overexpression is correlated with more aggressive clinical indications in multiple types of cancer, including colon, breast, lung, and head and neck carcinoma. Clinical studies show that EGFR-directed therapeutic approaches improved better outcomes of metastatic lung, colorectal, and head and neck cancers. Predictive biomarkers are being developed to deliver personalized therapies. Therefore, it is important to determine the protein as well as molecular biomarker expression for selecting targeted therapies. Here we present a panel cancer cell lines to examine EGFR expressions at a molecular biomarker level (mRNA) as well as a protein level of phosphorylated EGFR(pS1060). Colon cancer cell lines HT29, HCT116, SW620, as well as the breast cancer cell line SKBR3, were cultured and processed onto formalin-fixed paraffin-embedded (FFPE) CellMax Max pellets. Expression levels of EGFR mRNA in the FFPE slides were examined with RNAscope technology to visualize single RNA molecules per cell by using probes specifically against EGFR. Expression of phosphorylated EGFR was examined by the phosphospecific EGFR (pS1060) antibodies by using immunohistochemistry (IHC). EGFR mRNA expression was detected in HT29 and HCT116 cells showing strong punctuated signals in the cytoplasm. However, no EGFR mRNA signals were detected in SW620 cells. Breast cancer cell line SKBR3 showed abundant EGFR mRNA expression. Phosphorylated EGFR were found in the cell HT29, HCT 116 and SKBR3 cells with membrane staining.
pattern. No phosphorylated EGFR was observed in SW620 cells using Immunocytometry staining. Together, our results demonstrated that the EGFR mRNA expression is correlated to the phosphorylation of EGFR in these cancer cell lines. We concluded that the EGFR mRNA probes, phosphorylated EGFR antibodies, and/or the cell lines can be served as the control-references for clinical biomarker screening.

**4655 Elevated level of serum miR-99a is correlated with serous epithelial ovarian cancer and can be a potential biomarker.** Akihiko Yoshimura, Kenjiro Sawada, Koji Nakamura, Yasuto Kinose, Erika Nakatsuka, Seiji Mabuchi, Tadashi Kimura. Osaka Univ. Hospital, Suita, Osaka, Japan.

Objective: There is a critical need for improved diagnostic markers to detect ovarian high-grade serous cancer (HGSC). microRNAs (miRNAs) stably exist in circulating blood, reflecting tissue or organ conditions and present in circulating microvesicles such as exosomes. We aimed to identify which miRNAs are highly produced from HGSCs and analyze whether serum miRNA can discriminate patients with HGSC from healthy controls. Methods: Secreted exosomes from ovarian cancer cell lines were collected and exosomal miRNAs extracted. miRNA microarray was performed and several elevated miRNAs specific to ovarian cancer cells were picked up. Among these, we focused on miR-99a. Serum samples from patients with HGSC and healthy controls. Expression level of miR-99a was determined by miRNA RT-qPCR. Results: miRNA microarray revealed several miRNAs were highly expressed in exosomes in patients with HGSC. In patients, serum miR-99a levels were significantly high compared with the healthy controls. ROC analysis showed that at the cut-off of 1.4, the sensitivity and specificity were 73% and 80% respectively for detecting HGSC (AUC = 0.85). Conclusion: Exosomal miRNAs can be detected in sera of patients with HGSC and have the potential to predict ovarian cancer.

**4656 Assessment of BCL-2, BCL-xL and MCL-1 in bone marrow biopsy samples using VENTANA BenchMark XT automated slide stainer.** Ashley Streator, Eneida C. Villaneuva, Burton F. Holmes, Leping Zhang, Roche, Tucson, AZ.

Immunophenotyping is essential for diagnosis and classification of most hematopoietic malignancies, and often followed by ancillary studies to understand the predictive and prognostic value of biomarkers. Immunohistochemistry (IHC) provides in-situ coupling of antigen expression with morphology of precursor B- from precursor T-lymphoblastic leukemia/lymphoma. The number of different IHC antibodies that can be applied to bone marrow (BM) biopsies is increasing concurrently with development of flow cytometry and molecular diagnostic methods. IHC detects scattered tumor cells in Hodgkin’s Lymphoma, Multiple Myeloma (MM) and Acute Myelogenous Leukemia (AML), where key biomarkers may not be detectable by current flow cytometry methods. The goal of this study was to detect BCL-2, Bcl-xL and MCL-1 that are able to predict pre-clinical sensitivity to the drug Venetoclax. We examined the expression of these biomarkers in BM bone samples decalcified using commercially available reagents to determine whether decalcification methods impact BCL-2 family IHC staining. Results: MM bone samples (n=59) decalcified in Decal STAT™ for 20 min. post, for Venetoclax anti-BCL-2 (124) and Bcl-xL (546H2). BM bone samples (n=71) decalcified in Decal STAT™ for 60 min. stained for BCL-2 and BCL-xL. Finally, eleven MM bone samples decalcified in Richard Allan Decal Solution™ stained for BCL-2 and Bcl-xL. MCL-1 stained poorly with commercially available decalcification solutions. For MCL-1 to be used, additional staining optimization is required. Conclusion: BCL-2 and BCL-xL proteins can be detected in MM bone samples decalcified with commercially available solutions. With these preliminary results, future studies will pursue the potential for sensitivity to ‘Venetoclax’ (ABT-199/GDC-0199).

**4657 LOX and ZFPM2 as novel diagnostic biomarkers for malignant pleural mesothelioma.** Minkyu Kim, Hyun-Won Kim, Soon-Hee Jung, Sung Soo Oh, Youngik Jeong, Jong Whan Choi. Yonsei University Wonju College of Medicine, Wonju, Republic of Korea.

Malignant pleural mesothelioma (MPM) is a rare, aggressive cancer being developed on outlayer of lung tissues and caused by mostly occupational exposure to asbestos. Poor prognosis needs to develop therapeutic drug as well as early diagnostic biomarker. Currently, mesothelin (MSLN), osteopontin (OPN), and fibulin 3 (FBLN3) have been reported as potential diagnostic biomarkers for MPM. In this study, we first performed bioinformatics analysis for public database to find diagnostic biomarkers for MPM. From the analysis using Cancer Cell Line Encyclopedia (CCLE) and Gene Expression Omnibus (GEO) databases, included were 7 genes involving LOX, LOXL1, LOXL2, ZFPM2, THBS2, SULF1, CDH11 identified as potential diagnostic biomarkers. These genes showed a similar diagnostic ability to FBLN3 or MSLN as MPM biomarker candidates. Further molecular approach using quantitative real-time polymerase chain reaction (QPCR) confirmed the higher mRNA expression of these candidates in MPM cell lines and patient samples. Moreover, two particular genes, LOX and ZFPM2, showed MPM specific patterns of mRNA expression that were further confirmed in protein level by western blot. Together with biological approach, biostatistical analysis of receiver operating characteristic (ROC) analysis using the GEO database revealed significantly higher diagnostic potential of LOX and ZFPM2 genes compared to the FBLN3, one of the best diagnostic biomarkers currently reported. From this study, we believe these genes together with FBLN3 and MSLN would become novel potential biomarker candidates for MPM diagnosis.

**4658 Inflammatory based diagnostic markers of lung cancer in African Americans.** Claire L. Meany,1 Khadijah A. Mitchell,1 Adriana Zingone,2 Derek Brown,1 Wei Tang,1 Yunkai Yu,1 Liang Cao,1 Angela S. Wenzlaff,2 Ann G. Schwartz,3 Brid M. Ryan.1 National Cancer Institute, Bethesda, MD; 2Wayne State University, Detroit, MI.

African Americans have a higher risk of developing lung cancer compared with all other ethnic groups in the USA. Previous studies based on a small panel of markers suggested that certain circulating cytokines were associated with lung cancer. Given the complexity of the immune response in lung cancer and the multitude of cell types involved, we reasoned that examining a broad panel of inflammatory markers—including cytokines, chemokines, angiogenic and other pro-inflammatory factors—might identify a diagnostic signature of lung cancer for African Americans specifically. Among 30 inflammatory markers, serum levels were measured in 316 African-American cases and 508 African American controls from the National Cancer Institute-Maryland (NCI-MD) case-control study using a Mesoscale ( MSD) multiplex platform. The panel of 30 serum inflammatory markers included chemokines, cytokines and other inflammatory related proteins. Logistic regression analyses were conducted to investigate the association between levels of inflammatory marker expression and lung cancer risk. Expression levels above the median threshold defined within the control samples were considered high. Statistical models were also adjusted for potential confounding factors such as age, gender, pack-years and smoking status. Nineteen inflammatory markers (CRP, IFN-γ, IL-10, IL-15, IL-17A, IL-1α, IL-1β, IL-4, IL-5, IL-6, IL-7, IL-8, IP-10, MCP-4, MIP-1α, TARC, TNF-α, TNF-β and VEGF) were significantly different among African American cases compared with African American controls (P<0.05). In agreement with our previous observations, levels of IL-10, IL-1β, IL-6, IL-8, and TNF-α were associated with lung cancer risk. In addition, we found that CRP, IFN-γ, IL-15, IL-7, IP-10, MCP-4, MIP-1α, TARC, TNF-β and VEGF were associated with lung cancer in African Americans. The associations between CRP, IFN-γ, IL-10, IL-15, IL-1β, IL-6, IL-7, IL-8, IP-10, MCP-4, MDC, MIP-1α, TNF-α and TNF-β levels and lung cancer among African Americans was significant after adjustment for potential additional confounding factors. Serum cytokine levels vary by race and might contribute to lung cancer differently in African Americans and European Americans. The findings presented here build upon recent work that identified associations between elevated levels of IL-10, IL-1β, IL-6, IL-8, IL-10, VEGF and lung cancer cases specifically among African Americans. By analyzing a broader spectrum of inflammatory measures, we have identified additional markers of lung cancer for African American patients. Further to this, we have integrated GWAS and MSD data for 137 cases and 203 controls that allows us to examine the genetic basis to the relationship between risk of lung cancer and modulated inflammatory marker expression. Markers identified here as significant for lung cancer risk will be further analyzed using case-control samples from an independent African American validation cohort.

**4659 Detection of autoantibodies against tumor-associated antigens (TAAs) improving immunodiagnosis in human osteosarcoma by serological proteome analysis.** Jitian Li,1 Manyu Huang,2 Manli Luo,2 Leping Dai,1 Wen Xie,3 Xiaofei Qin,2 Zongchang Han,2 Wei Tang,1 Yunkai Yu,1 Liang Cao,1 Angela S. Wenzlaff,2 Ann G. Schwartz,3 Brid M. Ryan.1 National Cancer Institute, Bethesda, MD; 2Wayne State University, Detroit, MI; 3The University of Texas at El Paso, El Paso, TX.

osteosarcoma (OSA) is the most common malignant primary solid bone-tumor. Despite its relatively low incidence rate among overall cancers, it remains one of the most harmful primary malignant tumors in childhood and adolescence. Thus, a critical need in the diagnosis and management of OS is to determine an optimal combination of clinical biomarkers that can detect tumors early with high specificity/sensitivity and with limited invasiveness. The objective of this study is to identify and characterize the targeted tumor-associated antigens (TAAs) as biomarkers in OS by serological proteome analysis (SERPA)
approach, and further to analyze the frequency and specificity of anti-TAA antibodies in OS patients. In this initial study, autoantibodies to 29 TAAs were detected by ELISA and Western blotting in 90 sera from patients with OS, Osteochoondroma (OC), and age matched normal individuals. Only 8 protein antigens including DSF70, HMGB1, HCC1, RaA, c-myc, AnnexinA1, IMP1, PBP, can induce significantly higher antibody responses in OS individuals than those of normal individuals, achieving the highest sensitivity and specificity, 66% and 95%, respectively. The cumulative positive rate of autoantibodies against these eight selected TAAs in OS reached 70.7% with an observed AUC of 0.972 (95% CI: 0.867-0.988), significantly higher than that in normal control sera. Positive results were also confirmed by Western blotting. These preliminary data extensively indicate that all proteins identified in cancer can be used as TAA biomarkers in OS, and only some of these proteins can induce immune responses, which could be potential TAAs in OS immunodiagnosis or prognosis, and further studies of novel targeted proteins in OS are currently in progress by SERPA approach. In addition, it supports the hypothesis that a customized TAA array can be used for enhancing anti-TAA antibodies detection, and it may constitute a promising and powerful tool for immunodiagnosis of OS.


The purpose of this study is to investigate the differential expression of HPRT between various breast cancer tissues in comparison to healthy breast tissue in order to evaluate their potential use as a diagnostic tool. Breast cancer is the most common malignancy diagnosed in women and remains incurable in the metastatic setting requiring accurate and reliable tools for early diagnosis. We analyzed HPRT, a purine salvage pathway enzyme responsible for synthesizing 90% of the available GMP and IMP in cellular maintenance. Due to its critical role in maintaining necessary nucleotides for cell proliferation, we hypothesized an increase in the expression of HPRT in cancer. Tissues from 48 patients with breast carcinoma were stained using standard immunohistochemistry (IHC) techniques. We examined infiltrating ductal carcinoma, margin of carcinoma, hyperplasia, atypical hyperplasia, adenosis, collagen fibers tissues, and normal breast tissue in order to determine expression between various breast cancers. Briefly, tissues were treated with a polyclonal anti-HPRT antibody along with a GADPH positive control and a universal negative control. Tissues were incubated with an HRP-polymer conjugated antibody, and DAP substrate which reacts to antibody-antigen binding sites and results in a color change. Adenosis and hyperplasia are benign breast conditions and are early signs of breast carcinoma development. Hyperplasia tissues, which are in a state of rapid proliferation, showed significant upregulation of HPRT and adenosis tissues showed approximately 42% upregulation. This HPRT upregulation in pre-stage 1 tissues indicates that it may be used as a biomarker to detect early stages of breast cancer. Expression of HPRT was also found to be upregulated in 65% of infiltrating duct carcinoma showing high expression in active malignant areas. In marginal tissue located on the perimeter of primary tumors only 31% of tissues showed upregulation of the protein. Because the levels of HPRT in marginal tissue is significantly lower than those found in the tumor, HPRT may serve as a useful diagnostic tool when evaluating successful tumor removal. These data suggests HPRT as a useful diagnostic tool when identifying tissue with a high proliferation rate indicative of potential cancer development. Additionally, HPRT may also be used as a tool for pathologists to evaluate the success of tumor removal.

#4661 Omentin as an adjunct to CA-125 in detecting recurrent ovarian cancer. Kelsey Lewis, Chi Lam Au Yeung, Samuel Mok, Karen Lu. University of Texas MD Anderson Cancer Center, Houston, TX.

Omentin (Intestinal Lactoferrin Receptor ITLN1) is an adipokine that is mainly secreted by the mesothelial cells lining visceral adipose tissues. It has been shown in previous studies that ovarian cancer cells down-regulate omentin which allows increased proclivity to spread to the omentum. Levels of serum omentin are decreased in patients with high-grade serous ovarian cancer (HG-SOC) compared with patients with benign gynecologic diseases. Moreover, survival correlation studies showed that patients with lower serum omentin level at the time of first treatment experienced significantly shorter survival times than those with higher levels of omentin. CA-125 is currently the only biomarker used to monitor progression and re-occurrence of ovarian cancer. To our best knowledge, the relationship between omentin and CA-125 has not been investigated previously. We hypothesize that omentin may be a clinically useful adjunct to CA-125 in detecting recurrent ovarian cancer. 92 serum samples were collected prospectively from 27 HGSOCS patients during the postoperative period until recurrence was detected. Patients had an average of 3.37 follow up visits and CA-125 levels were concurrently established. Recurrence was determined by imaging or an increase from baseline in CA-125 level. Samples were stored frozen at -80 degrees Celsius until use for this study. Circulating omentin levels were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) and showed that there was a significant decrease in CA-125 level during the postoperative period and 12 of these patients had an associated decrease in serum omentin level (average = 127.8 ng/mL). A significant negative correlation between omentin and CA-125 levels was observed (p = 0.02). This study provides the first evidence that omentin may be a clinically useful adjunct to CA-125 in detecting recurrent ovarian cancer and warrants further investigation.

#4662 Expression of PLAG1 in salivary duct carcinomas: its efficacy of the distinction of carcinoma ex pleomorphic adenoma type and de novo type. Takayoshi Suzuki, Satoshi Kano, Kanako Hatanaka, Yutaka Hatanaka, Tomohiro Sakashita, Takatsugu Mizumichi, Hiromitsu Hatakeyama, Akhiro Homma, Yoshhiro Matsuno, Satoshi Fukuda. Hokkaido University, Sapporo, Hokkaido, Japan.

Salivary gland duct carcinoma (SDC) is one of the high-grade salivary gland carcinoma, classified into two patterns; i) carcinoma ex pleomorphic adenoma (Ca–ex-PA) type, which is derived from benign pleomorphic adenoma (PA), ii) de novo type, which is generated from normal salivary gland tissue. According to current WHO classification, Ca–ex-PA is diagnosed when pathological examination proves as follows, a) the mixture of the component of cancer cells and that of pre-existing PA cells, b) the existence of malignant cells after the resection of PA. However, current criteria cannot entirely be accurate from the viewpoint of disease onset. For example, i) if a benign component of PA is replaced completely by Ca–ex-PA, the Ca–ex-PA type can be misdiagnosed as de novo type because histopathological examination cannot clarify the existence of PA in its surgery specimen. Moreover, ii) if a de novo type SDC, arising from normal salivary tissue, is growing and infiltrate to the preexisting PA, the de novo type SDC can be misdiagnosed as Ca–ex-PA type. Recently, Pleomorphic adenoma gene 1 (PLAG1) has been identified as the gene associated with tumorigenesis of PA. Subsequently, the overexpression of PLAG1 protein has been reported to be observed in both PAs and Ca–ex-PAs and useful for diagnosis of them. Thus, we considered that PLAG1 immunohistochemistry can enable us to distinguish Ca–ex-PA type and de novo type in SDCs more definitively. In this study, 23 SDC patients who underwent primary surgery for cancer at our institution were enrolled, including 14 Ca–ex-PA types and 9 de novo types. In each case, we had its formalin-fixed-paraffin-embedded tissue sample stained for PLAG1-antibodies (clone 3B7, Mouse mAb, 1:100, Abnova) and evaluated PLAG1 protein expression in the component of glandular-epithelial component and that of non-glandular-epithelial, respectively. PLAG1 immunostaining was scored as positive when the more than 10% nuclear staining of tumor cells are observed. In all 23 SDCs, there was no PLAG1 protein overexpression in glandular-epithelial component, irrespective of benign or malignant. In all 14 Ca–ex-PA types, positive findings of PLAG1 overexpression were observed in non-glandular-epithelial component. In all 9 de novo types, no non-glandular-epithelial component showed any ELISA protein overexpression. There was no experienced difference in conventional criteria and the status of PLAG1, which are the cases; i) Ca–ex-PA which is misdiagnosed as de novo type, ii) de novo type which is misdiagnosed as Ca–ex-PA type. Conclusions : There is no difference between conventional criteria and PLAG1-based criteria in our cases. However, in the cases with obscure characteristics of PA morphologically, such as mucoid/myxoid or cartilaginous-like materials, findings of PLAG1-positive cells can help us to distinguish Ca–ex-PA type from de novo type more definitively.


In the tumor microenvironment, matrix metalloproteinase 9 (MMP9) cleaves and activates substrates such as VEGF, IL8 and TGFβ that promote tumor growth through angiogenesis and immune suppression. Data from preclinical models supports the hypothesis that inhibition of MMP9 reduces local immune suppression, resulting in increased anti-tumor immunity. GS-5745 is a monoclonal antibody in clinical development that is a selective inhibitor of MMP9. The effect of GS-5745 + mFOLFOX6 (GS + chemo) on systemic biomarkers related to MMP9 activity, and immune suppression and activation, was explored in samples from patients in the gastric cancer cohort of the phase 1b study, NCT01803282. Immunoassay analysis of a set of serum proteins based on...
MMP9 biology and preclinical data (including 10 MMP9 substrates, 5 MMP9 TIMPs and 25 inflammatory markers) are reported; exploratory analyses of a larger set will be presented. Total and GS−/MMP9− free MMP9 was measured using a proprietary validated assay. Serum was collected at baseline and the start of every 28-day cycle from 40 gastric cancer patients treated with GS + cheemo every 2 weeks, and was stored at −80°C until assay. Levels were measured using the Luminex assay and compared with a control group of non-cancer patients. Multiple testing adjustment was based on false discovery rate. GS + cheemo treatment resulted in a significant decrease of MMP9 levels in serum (p = 0.007). MMP9 protein expression was significantly higher in GS + cheemo patients with metastatic disease compared to those without metastatic disease (p < 0.05). The expression of MMP9 in the experimental group was significantly higher than the control group (p = 0.007).

#4665 Induction of receptor tyrosine kinase signaling by sera from patients with lung adenoarcinoma.

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Background: The objective of this study was to evaluate disease stage-associated differences in receptor tyrosine kinase (RTK) signaling in A549 cells using pretreatment sera from cases of pathologically-confirmed lung adenocarcinoma or control cases derived from a lung cancer screening study. This was accomplished as a means to explore the hypothesis that circulating concentrations of decoy receptors and/or ligands, as well as disease-specific ligand degradation all regulate RTK signaling in vivo. Methods: Pretreatment peripheral blood were prepared from either from non-cancer control patients (n = 30) or those with lung adenocarcinoma, consisting of stage I (T1–N0M0, n = 25); locoregional progressed (T1–N1M0, n = 36) or disseminated, stage IV disease (n = 43). All patients were enrolled with written informed consent to our IRB-approved, institutional biorepository. Confluent cultures of A549 cells were stimulated for 40 minutes with patient sera diluted 1:1 in RPMI-1640 followed by immediate whole cell lysate preparation. After protein determinations by BCA, 8.5 µg of each sample was interrogated using the Human RTK (phosphoprotein) kit from Millipore. Data were analyzed according to manufacturer’s in vitro data. After being normalized to the kit-supplied (stimulated) HEK cell lysates, all resulting data were evaluated by one-way ANOVA (LSD and Tukey post-hoc) for categorical comparisons. Results: The most commonly observed differences in RTK signaling was noted upon comparing cohorts with locoregional (T1–N1M0) progression and disseminated (stage IV) disease. Levels of in c-Met and receptor (IR) signaling were higher in patients with stage IV disease relative to those with locoregional disease by 24% and 10%, respectively (both p < 0.01); whereas induction of c-KIT, VEGFR3, and Tie-1 were reduced by 12.8%, 6.67%, and 17.3%, respectively (all p < 0.05). Circulating levels of HGF were previously observed by our group to be 25% higher in stage IV patients relative to those with locoregional disease (p < 0.005) - agreeing with our observation in direction of change, though not significant. This is in contrast to levels of VEGF-C and VEGF-D, previously being observed elevated 51% and 452% (both p < 0.001) in these same cohorts. Also of note was the 1.9 fold decrease in ability of sera from stage I patients to stimulate IGF-1R signaling relative to control patients (p = 0.002). We have previously reported a 12% increase in IGF-1 levels between these groups (p < 0.002), though we observed no changes in IGF binding proteins that could account for the observed differences in IGF-1R signaling. Conclusions: We observed an apparent discoupling of certain circulating ligands in patient sera to stimulate their respective RTKs in a disease stage-specific manner. We are currently evaluating levels of circulating ligands, potential disease stage-associated (partial) proteolytic degradation, and decoy receptors to help explain these observations.

#4666 DREAMing as a simple and low cost alternative for the assessment of methylation in ultra rare DNA.

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Background: Current approaches for the assessment of methylation, such as methylation-specific PCR (MSP) and next-generation bisulfite sequencing (BS-Seq) are fundamentally limited in their ability to detect and assess heterogeneous methylation patterns (epialleles) in ultra-rare (<0.1%) DNA. These limitations critically compromise diagnostic utility and render them ill suited for many emerging applications in cancer diagnostics, such as the analysis of methylation heterogeneity in cell-free DNA (cfDNA) and rare cell populations. We recently addressed the need for a low cost alternative to the assessment of methylation in ultra-rare DNA with the development of DREAMing (Discrimination of Rare EpAlleles by Melt), which utilizes semi-limiting dilution and precise melt curve analysis to distinguish and enumerate individual copies of DNA at single copy sensitivity and single-CpG-site resolution. Here, we seek to demonstrate the advantages of the DREAMing method over conventional approaches to methylation assessment. Methods: We expand upon the underlying theory of DREAMing and provide guidelines for the development of single-copy sensitive DREAMing assays. We further elucidate methods for tailoring DREAMing assay tests to patients of interest and compare the performance of these assays to commonly employed techniques including quantitative MSP (qMSP) and BS-Seq. Results: Development of single-copy sensitive DREAMing assays for a
Background: Hepatocellular carcinoma (HCC) typically occurs in patients with chronic liver disease. Such diseases create a demand for liver regeneration due to injury, which in turn promotes upregulation of c-Met, a receptor for hepatocyte growth factor (HGF) involved in wound healing, leading to increased cellular proliferation, survival, and mobilization. c-Met is the product of the MET proto-oncogene. Increased MET expression is associated with wound healing but can also contribute to the development and progression of HCC. Ablation of c-Met signalization is associated with rapid tumor growth and aggressively invasive disease in HCC, resulting in poor patient prognosis. We examined c-Met expression and MET gene amplification in 69 procured HCC samples and c-Met expression in adjacent non-tumor tissue. Methods: c-Met expression was evaluated using MET IHC and MET gene amplification. MET IHC was performed using the pharmDx anti-c-MET (clone D1C2) rabbit mAb. c-Met expression was scored semiquantitatively on a scale of 0-3+ based on the staining intensity in ≥50% of cells [Koopman et al. Proc UCSC 2012 (abstract 2001)]; tumors scoring 2+ or 3+ were judged positive. MET gene copy number (GCN) was assessed using the MET IQFISH Kit 111480 with a probe covering a 269 kb segment on chromosome 7q12 containing the MET gene. Tumors were considered to be FISH positive if scored cells had a mean MET/CEN7 ratio ≥2.0 or ≥50% of cells contained ≥5 MET signals. Results: All cases showed typical HCC morphology and were classified as low to high grade trabecular, pseudoglandular, or solid with common cytoplasmic features. The tumors and cells of the adjacent cirrhotic/non-cirrhotic liver tissue (bile duct epithelia, hepatocytes endothelial cells) showed cytoplasmic and membrane staining with strong expression and c-Met staining heterogeneous and present in 68/68 cases. Semiquantitative analysis showed that 11/67 (16%) tumors were c-Met-positive (3+, 5+). Results: All 69 cases were evaluated for MET gene amplification. Six out of 69 (8.7%) were amplified by ratio and 3 (4.3%) others had ≥5 MET gene copies in ≥50% of cells, giving an overall frequency of FISH positivity of 13%. Most interestingly, amplification and/or increased gene copy number was homogeneous throughout the tumor in all cases, in contrast to heterogeneous c-Met staining observed in some of the same tumors with IHC. Five of the 11 c-Met-positive (IHC 2+/3+) tumors were MET amplified, and all 3 of the IHC 3+ tumors were amplified. Conversely, 4 of the 9 FISH-positive tumors were c-Met-negative. Conclusions: These data provide insight into the frequency of c-Met/MET abnormalities in HCC and show there is discordance between c-Met protein expression and MET GCN alterations. This may be of importance for selection of patients with HCC for clinical trials of c-Met inhibitors.

#4669 Circulating cytokines as biomarkers of BEP toxicity in TGCTs patients. Daniela Svetlovska,1 Viera Miskovska,2 Dana Cholujova,3 Paulina Gronesova,4 Silvia Cingelova,5 Michal Chovanec,4 Zuzana Sycova-Mila,4 Jana Obertova,4 Patrik Palacka,4 Vanda Usakova,5 Katarina Kalavska,6 Jan Luha,6 Dalibor Ondrus,2 Stanislav Spanik,7 Jozef Mardiak,1 Michal Mego. 1. NCI Slovakia; 2. Comenius University, Bratislava, Slovakia; 3. Comenius University and St. Elisabeth Cancer Institute, Bratislava, Slovakia; 4. Institute for the Research Center, Slovak Academy of Sciences, Bratislava, Slovakia; 5. NCI Slovakia, Bratislava, Slovakia; 6. St. Elisabeth University of Health and Social Sciences and St. Elisabeth Cancer Institute, Bratislava, Slovakia; 7. St. Elisabeth University of Health and Social Sciences and St. Elisabeth Cancer Institute; Comenius University, Bratislava, Slovakia.

Background: Cytokines are key components in process of inflammation and immune response, what are important processes in many toxicities. Bleomycin, etoposide, cisplatinum (BEP) is a standard treatment in patients with testicular germ cell tumors (TGCTs). Grade 3/4 toxicity can be dose limiting and can highly influence patient’s quality of life. Our aim was to investigate correlation between circulating cytokines and BEP toxicity in TGCTs patients. Methods: The study population consisted of chemotherapeutic TGCTs patients treated by BEP chemotherapy. Grade 3 and 4 toxicities were assessed according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 after first cycle of chemotherapy. G-CSF was administered to all patients after chemotherapy. Circulating 51 plasma cytokines were analyzed at baseline before chemotherapy administration (94 patients) and on cycle 1 day 22 (57 patients) using multiplex bead arrays. Toxicities were correlated to pretreatment and posttreatment levels of circulating cytokines. Spearman’s correlation test was used to analyze the association between cytokines and toxicity. Results: Significant correlations between grade 3/4 toxicity and cytokines were observed. Neutrophilia was associated with elevated pretreatment levels of TGF-beta 2, IL-2 alpha, HGF, MIG, NGF-beta, SDF-1 alpha, IL-1beta, IL-4, IL-5, IL-17, IFN-beta, G-CSF, MIP-1alpha and posttreatment elevated levels of GRO-alpha, TNF-beta. Thrombocytopoenia related with higher pretreatment levels of IL-2alpha, IL-18, M-CSF and higher posttreatment IL-3, IL-12p40, GRO-alpha, MCP-3, M-CSF, MIP-1alpha, NGF-beta. Anemia correlated with elevated pretreatment IL-16, GRO-alpha, HGF, MIF, NGF-beta, SCF, SDF-1alpha, MCP-1, M-CSF and elevated posttreatment IL-2 alpha, IL-12p40, CCL-5, CCL-3, MIP-1alpha, MIP-1beta, G-CSF, M-CSF, TNF-alpha. CLINICAL RESEARCH: Diagnostic Biomarkers

#4668 Analysis of c-Met protein expression by immunohistochemistry and MET gene copy number in hepatocellular carcinoma. Christian Ilhing,1 Sienna Yoast,2 Matthew DeNicola,3 Josef Straub,2 Klaus Ducker,1 Karsten Nielsen,1 Russell A. Baldoch,4 Aaron B. Ellison,2 Holly Yamada1. 1. Merck KGaA, Darmstadt, Germany; 2. Dako North America, an Agilent Technologies company, Carpinteria, CA; 3. Mission Pathology Consultants, Santa Barbara, CA; 4. Dako Denmark A/S, an Agilent Technologies company, Denmark; 5. Hemediagnostics, San Marino, CA.

number of loci associated with classic tumor-specific methylation such as CHFR and RASSF1A as well as a candidate pan-cancer locus are reported. These assays are then used to analyze methylation in cDNA derived from the plasma of cancer-positive and healthy patients. DREAM analysis reveals that DREAMing can readily detect over an order of magnitude more epimutations when directly compared to target DNA. Its low assay of sensitivity and its utility in providing enhanced, single-copy detection of heterogeneous methylation make DREAMing an attractive method for the assessment of locus-specific methylation in samples containing ultra-low amounts of DNA. By virtue of their clonal nature, high mutation rates and copy number, assessing tumor-specific mitochondrial DNA (mtDNA) mutations in histologically clean LN may be possible in a more sensitive diagnostic tool and eventually reduce the false negative rate in patients. Additionally, due to its circular configuration, mtDNA thought to be more stable than genomic DNA, and may also be suitable for sequencing formalin-fixed paraffin-embedded (FFPE) -derived genomic material, an invaluable resource for clinical research. However, current methods for library preparation are still imperfect for detection of low prevalence variants and heterogeneous tumors with high depth of coverage especially in highly degraded samples. To this end, we aimed to set up a novel NGS assay for mtDNA analysis in primary and metastatic samples. Amplicon-based NGS library preparation approach, consisting of 148 primers pairs that cover the entire mitochondrial genome (with a dual coverage of 86.18%) was designed in collaboration with Fluidigm to be used on Access Array® platform. To enhance the coverage of degraded material the amplicon size was limited to ~170 base pairs. Primers for three nuclear housekeeping genes were added to the panel to allow quantitative copy number analysis. To address mitochondrial mutational patterns, heteroplasmy and heterogeneity, we have used this novel high-throughput enrichment technology to sequence mtDNA from different areas of 16 primary HNSCC tumors (fresh frozen and FFPE), and multiple matched metastatic or histologically clean LN tumors with high depth of coverage especially in highly degraded samples. By using the combination of these methods, we could assess mutations in cases of minimal detectable tumor component, thus improving the sensitivity of mtDNA analysis in clinical samples.
ment levels of IL-16, HGF, M-CSF, SCGF-beta. There were many positive associations between pylophenones and pretreatment IL-2R alpha, IL-18, M-CSF, NGF-beta and posttreatment IL-2R alpha, IL-3, IL12p40, CTACK, HGF, MCP-3, M-CSF, NGF-beta, SCGF-beta, IL-6. Diarrhea related with higher pretreatment levels of IL-15 and also higher posttreatment IL-15, GM-CSF and lower posttreatment levels of IFN-alpha. CTACK. Several other associations were detected between elevated posttreatment levels of cytokines and toxicities: nausea and SCGF-beta, febrile neutropenia and GRO-alpha, TNF-beta, SIRS and GRO-alpha, PDGF-BB. All correlations were significant P < 0.05. Conclusions: Circulating cytokines are promising and easily detectable biomarker for predicting BEP toxicity in TGCTs patients. This work was supported by the Slovak Research and Development Agency under the contracts No. APVV-0016-11 and APVV-15-0566.

CLINICAL RESEARCH: Epigenetics and DNA Repair

#4670 Identification of a possible therapeutic candidate for multiple myeloma based on dual inhibition of EZH1/EZH2. Makoto Nakagawa,1 Shuhei Fujita,1 Daisuke Honma,1 Nobuaki Adachi,1 Kazuushi Araki,2 Issay Kitabayashi1

1National Cancer Center Research Institute, Tokyo, Japan; 2Daiti Sanky Co., Ltd., Tokyo, Japan

Multiple myeloma (MM) is largely incurable as the disease eventually relapses despite the recent development of novel therapies. Previous reports show that side population (SP) cells comprise myeloma stem cells. Therefore, targeting SP cells may be a promising strategy for preventing and treating MM relapse. Polycomb repressive complexes 1 (PRC1) and 2 (PRC2) are important epigenetic regulators that maintain the "stemness" of ES cells and other hematopoietic stem cells. Enhancer of zeste homolog 1 and 2 (EZH1/2) are catalytic components of PRC2, which trimethylate histone H3 at lysine 27 to repress transcription of regulatorsthatmaintainthe "stemness"ofEScellsandotherhematopoieticstem cells. Mutation and overexpression of EZH2 are associated with many cancers, including MM. Here, we found that SP cells expressed significantly higher levels of EZH1/2 than non-SP cells. These results suggest that overexpression of EZH1/2 is important for maintaining the stemness of MM cells and that EZH1/2 could be a potential therapeutic target. We developed a novel EZH1/2 dual inhibitor, OR-S1, and used it to investigate the effect of pharmacologic inhibition of EZH1/2 on MM. OR-S1 suppressed the proliferation of almost all MM cell lines tested, with an IC50 significantly lower than that of the specific EZH2 inhibitor, GSK126. Furthermore, flow cytometry analysis revealed that OR-S1 significantly depleted the SP cell population. RNA-seq analysis revealed that the transcriptional profiles of MM cell lines treated with OR-S1 were characterized by up-regulation of genes related to the Wnt pathway. qRT-PCR confirmed that expression of Wnt, Frizzled, and Protein kinase C family members that the transcriptional profiles of MM cell lines treated with OR-S1 were characte-

#4672 Novel epigenetic approach to relapsed mantle cell lymphoma based on dual inhibition of EZH1/EZH2. Shuhei Fujita,1 Yuki Kagiya,1 Daisuke Honma,2 Nobuaki Adachi,1 Kazuushi Araki,3 Issay Kitabayashi1

1National Cancer Center Research Institute, Tokyo, Japan; 2Daiti Sanky Co., Ltd., Tokyo, Japan

Mantle cell lymphoma (MCL) is a well-defined and aggressive type of B cell non-Hodgkin’s lymphoma that is genetically characterized by the t(11;14)(q13; q32) chromosomal translocation, which results in constitutive overexpression of CYCLIN D1. Although newly developed drugs such as ibrutinib show promising clinical outcomes, relapsed MCL often acquires drug resistance, which is a critical obstacle to treatment. Alternative approaches to overcoming the drug resistance of relapsed MCL are urgently needed. PRC2 and 2 are important epigenetic regulators that maintain the stemness of embryonic and hematopoietic stem cells. EZH1 and 2 are catalytic components of PRC2, which trimethylates histone H3 at lysine 27 (H3K27) to repress transcription of target genes. Mutation and overexpression of EZH1/2 are associated with cancers, including hematopoietic malignancies. Here, we used a novel dual inhibitor of EZH1/2 to show that inhibiting EZH1/2 is a promising therapeutic strategy for MCL. First, we developed a xenograft (PDX) mouse model using cells from a heavily pretreated patient with MCL. We generated tumor xenografts in mice bearing MM xenografts led to significant impairment of subcutaneous tumors. Interestingly, long-term administration of the drug at lower doses to mice bearing ortothoptoxic xenografts resulted in complete eradication of minimal residual disease from the bone marrow and complete cure of MM without any serious side effects. Furthermore, OR-S1 treatment of an orthotopic PDX model derived from a relapsed and heavily pretreated MM patient led to a reduction in the levels of human immunoglobulins in the serum. Taken together, these results strongly suggest that dual inhibition of EZH1/2 is a promising therapeutic approach to eradicating myeloma stem cells and could lead to important advances in the treatment of MM.

#4671 Co-occurrence of alterations in the DNA damage repair genes synergize with uncontrolled proliferation and associate with very poor prognosis in acute myeloid leukemia patients. Antonella Padella,1 Giorgia Simonetti,1 Maria Chiara Fontana,2 Marco Manfrini,2 Giovanni Marconi,1 Anna Ferrari,1 Italo Faria do Valle,1 Marianna Garoni,2 Cristina Papayannidou,1 Eugenia Franchini,2 Elisa Zuffa1, Viviana Guadagnuolo,1 Samantha Bruno,1 Andrea Ghelli Lucerna di Rorà,1 Emanuela Ottaviani,1 Daniel Remondini,1 Massimo Delledonne,2 Giovanni Martinelli1

1University of Bologna, Bologna, Italy; 2University of Verona, Italy; 3CellPly, Bologna, Italy

Partner and localizer of BRCAl (PALB2) plays a key role in the DNA damage repair (DDR) by recruiting BRCA1, BRCA2 and RAD51. Alterations in PALB2 were described in hereditary breast cancer and Fanconi Anemia (FA). Little is known in Acute Myeloid Leukemia (AML). Aim of the study is to define the frequency and interplay of PALB2 alterations with patterns of somatic muta-

CLINICAL RESEARCH: Diagnostic Biomarkers

Partner and localizer of BRCAl (PALB2) plays a key role in the DNA damage repair (DDR) by recruiting BRCA1, BRCA2 and RAD51. Alterations in PALB2 were described in hereditary breast cancer and Fanconi Anemia (FA). Little is known in Acute Myeloid Leukemia (AML). Aim of the study is to define the frequency and interplay of PALB2 alterations with patterns of somatic muta-

Clinical pathways for patients with acute myeloid leukemia (AML) are based on cytogenetic and molecular features. However, a large proportion of AML patients have no recognizable abnormalities in their karyotype, raise new challenges to clinicians. In this study, we analyzed the association of PALB2 loss with clinical outcomes, relapsed MCL often acquires drug resistance, which is a critical obstacle to treatment. Alternative approaches to overcoming the drug resistance of relapsed MCL are urgently needed. PRC2 and 2 are important epigenetic regulators that maintain the stemness of embryonic and hematopoietic stem cells. EZH1 and 2 are catalytic components of PRC2, which trimethylates histone H3 at lysine 27 (H3K27) to repress transcription of target genes. Mutation and overexpression of EZH1/2 are associated with cancers, including hematopoietic malignancies. Here, we used a novel dual inhibitor of EZH1/2 to show that inhibiting EZH1/2 is a promising therapeutic strategy for MCL. First, we developed a xenograft (PDX) mouse model using cells from a heavily pre-

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that a cyclin-dependent kinase inhibitor, CDKN1C (TP57), was one of the genes most markedly upregulated by OR-S1. ChIP qPCR of MCL cell lines showed that the CDKN1C locus was strongly marked by H3K27 trimethylation, and that OR-S1 induced a significant reduction in the level of this histone marker. Furthermore, administration of OR-S1 alone to PDX mice induced increased expression of CDKN1C (as in the in vitro assay). Thus, dual inhibition of EZH1/2 in MCL induces expression of CDKN1C, which in turn causes cell cycle arrest and reduced growth of MCL. Taken together, these results strongly suggested that dual inhibition of EZH1 and EZH2 is a promising therapeutic strategy for MCL, illustrating the potential of novel epigenetic approaches to overcoming drug resistance of relapsed MCL.

#4673 EZH2 as a therapeutic target in glioblastoma: a cellular and molecular study. Javier de la Rosa,1 Marta Ibarbu,1 Gabriel Gallo-Oller,1 Alejandro Urdicain,1 Xing Fan,2 Mehdi H. Shahi,3 Bárbara Meléndez,4 Juan A. Rey,5 Miguel A. Idote,6 Javier S. Castrejana1. 1Univ. of Navarra, Pamplona, Spain; 2University of Michigan, Ann Arbor, MI; 3Aligarh Muslim University, Aligarh, India; 4Virgen de la Salud Hospital, Toledo, Spain; 5La Paz University Hospital, Madrid, Spain; 6Univ. of Navarra Clinic, Pamplona, Spain

Glioblastoma is the most common and deadliest malignant brain tumor in adults. It is currently treated with a combination of surgery, radiotherapy and chemotherapy with temozolomide (TMZ). Resistance to TMZ is a challenge in the treatment of this type of brain cancer. Epigenetic modulation is a new possibility to be tested against glioblastoma. Inhibition of EZH2, a histone methyltransferase (H3K27me3) which is overexpressed in cancer cells, leading to anangiogenesis and metastasis, might be a new strategy to treat this tumor. In this work, the EZH2 inhibitor DZNep was tested in A172 glioblastoma cells and in A172-TMZ-resistant glioblastoma cells. Inhibition of cell proliferation, cell adhesion, colony formation, and cell migration was noted in A172 and A172-TMZ-resistant glioblastoma cells after DZNep treatment. DZNep also decreased EZH2 expression, and increased the expression of its target genes (E-cadherin and TIMP3), which might probably contribute to inhibiting the development of a cancer metastatic phenotype. Finally, DZNep negatively regulated the TGFβ pathway: the expression of receptors involved in this pathway (TGFβRI and TGFβR2) was decreased, and the expression of inhibitors of this pathway (BAMBI, SMAD6 and SMAD7) was increased. In order to support the results obtained by qRT-PCR about the TGFβRI pathway, protein expression of SMAD2 and SMAD3 was analyzed by Western blot. The ratio between PS-MAD2/SMAD2 decreased in cells treated with DZNep, which supports the possible therapeutic effect of this drug against the TGFβ pathway, as SMAD2 is a negative prognostic marker in glioblastoma. In conclusion, we propose that inhibition of EZH2 might be considered as a therapeutic strategy against glioblastoma.

#4674 Epigenetic modifiers for the treatment of invasive lobular carcinoma breast cancer. Louise Walsh,1 Bruce Moran,2 Sudipto Das,3 Finn Barr Tarrant,4 Philip Schouten,5 Suet-Fewing Chin,6 Rene Bernards,7 Carlos Caldas,8 William Gallagher,9 Triona Ni Chonghaile10,11 Darran O’Connor12,13. 1Royal College of Surgeons in Ireland, Dublin, Ireland; 2University College Dublin, Dublin, Ireland; 3The Netherlands Cancer Institute, Amsterdam, Netherlands; 4University of Cambridge, Cambridge, United Kingdom

Invasive lobular carcinoma (ILC) is a breast cancer subtype comprising 10% of breast tumors. The majority of ILC (90%) are estrogen receptor (ER)-positive and therefore candidates for endocrine therapy. Unfortunately, de novo resistant to endocrine therapies occurs in 33% of women and a further 40% will relapse on treatment. Therefore, novel therapeutic targets are required for ILC. Deregulated transcription is a recurring theme in cancer, which can be due to epigenetic events. The bromodomain & extra-terminal domain (BET) family of proteins (BRD2, BRD3, BRD4, BRD4DT) function as chromatin readers that bind acetylated lysine residues on histones and regulate transcription. We performed RNA-Sequencing analysis on 61 primary ILC samples and found that high expression of BRD3 is associated with poor survival in ILC (log rank test, P = 0.037). We validated this finding, that high expression of BRD3 is associated with poor survival, in a second cohort of 99 ILC primary samples from the METABRIC dataset (log rank test, P = 0.0157). Next, we tested if ILC cell lines were sensitive to BET inhibition using the small molecule inhibitor JQ1, which inhibits all BET family proteins. JQ1 downregulates growth and survival genes in ILC cell lines including MYC, ER and BCL-XL. Pathway analysis following RNA sequencing revealed that JQ1 targets the apoptotic and Wnt signalling pathways in ILC cell lines. Interestingly, JQ1 inhibited the cell growth in all ILC cell lines tested, however apoptosis was only induced in two ILC cell lines. Furthermore, ILC cell lines which were relatively resistant to JQ1-induced apoptosis expressed both the BCL-2 and BCL-XL anti-apoptotic proteins. This led us to assess the combination of JQ1 and the BH3 mimetics, ABT-199 and ABT-263. ABT-199 is a selective small molecule inhibitor of BCL-2, whereas ABT-263 is an inhibitor of BCL-2, BCL-XL and BCL-W proteins. We found the combination of JQ1 and ABT-263, but not the combination of JQ1 & ABT-199, to be synergistic and enhance apoptosis in ILC cell lines. This is in accordance with BH3 profiling of ILC cell lines, which indicated that ILC cell lines are dependent on BCL-2 and BCL-XL proteins for cell survival. As JQ1 is a pan-BET family inhibitor, we also wish to determine which BET protein is responsible for sensitivity to JQ1. Following knockdown of each BET protein in the CAMA-1 cell line we found that BRD3 and BRD4 were responsible for loss of cell viability. Future work will include determining the specific role of BRD3 in ILC and the effectiveness of the JQ1 & ABT-263 combination in vivo. Our work suggests that inhibition of BET proteins in combination with BH3 mimetics may be a rational therapeutic combination for ILC.

#4675 Novel combination therapy of DNA methyltransferase inhibitor guadecitabine (SGI-110) and PARP inhibitor talazoparib (BMN-673) for BRCA-proficient high-grade serous ovarian cancer. Nicholas Pulliam,1 Pietro Taverna,2 John Lyons,3 Kenneth P. Neophy,4 Indiana University, Bloomington, IN; 5Astex Pharmaceutical, Inc., Pleasanton, CA; 6Astex Pharmaceutical, Inc., Cambridge, United Kingdom

Ovarian cancer recurrence has been shown to be associated with increased DNA damage response (DDR) mediated by poly-(ADP)-ribose polymersase 1/2 (PARP1/2), which can be therapeutically targeted by PARP inhibitors (PARPi). PARPi are indicated for platinum-responsive, BRCA-mutated high-grade serous ovarian cancer (HRGSC), but most ovarian cancer patients have DNA double-strand break (DSB) repair deficiencies (BRCA1/2) or TP53 mutations (platinum resistant, BRCA2 mutant) which result in a decreased response to PARP inhibitor treatment. Our previous studies support a role for DNA methylation in chemoresistance to platinum chemotherapy. Therefore, we hypothesized that combining a DNA methyltransferase inhibitor (DNMTi) and a PARP inhibitor results in enhanced cytotoxicity in high-grade serous ovarian cancer cell lines harboring either wild type- or mutant-BRCA, indicating that PARPi are indicated for platinum-responsive, BRCA-mutated high-grade serous ovarian cancer.

#4676 DNA repair protein expression and response of homologous recombination deficient ovarian cancer to the poly(ADP-ribose) polymersase (PARP) inhibitor rucaparib in the ARIEL2 Part 1 study. Andrea E. Wahner Hendrickson,1 Kevin K. Lin,2 Daniel W. Visscher,2 Rachel M. Hurley,3 Mitch Raponi,2 Thomas C. Harding,4 Linda M. Murphy,1 Jill M. Wagner,1 Heidi Giordano,2 Iain McNeish,2 Elizabeth M. Swisher,2 Scott Kaufmann3,4. 1Mayo Clinic, Rochester, MN; 2Cleveland Oncology, San Francisco, CA; 3University of Glasgow, Glasgow, United Kingdom; 4University of Washington, Seattle, WA

Background: PARP inhibitors (PARPi) are active in cancers with homologous recombination defects (HRD) and PARPi sensitivity is correlated with HR activity. Preclinical studies have shown that secondary mutations or alterations in gene expression (e.g., downregulation of 53BP1, Ku70, Ku80 or DNA-PKcs) restore HR and confer PARPi resistance. In addition, low PARPi expression can diminish PARP trapping and cause PARPi resistance. ARIEL2 Part 1 is a phase 2 study of the PARPi rucaparib in platinum-resistant ovarian cancer cell lines harboring either wild type- or mutant-BRCA, indicating that the PARPi-gaudecitabine drug combination is effective regardless of BRCA-mediated DDR and may represent an effective treatment regimen for BRCA-related cancers.
sensitive, relapsed high grade serous or endometrioid ovarian cancer (OC). Pretreatment OC biopsies were previously assessed for HR gene mutations and loss of heterozygosity (LOH), a genomic scar that reflects HR deficiency. Two tumor groups (BRCA wildcard [wt]/LOH high and BRCA1 or BRCA2 mutant) have objective response rates of 29.3% and 80%, respectively, as well as progression free survival of 12.8 months, respectively, on rucaparib (E. Swisher et al., Lancet Oncol., in press). Common AE included nausea (80%), asthenia/ fatigue (78%), constipation (46%), and vomiting (44%). The present studies tested the hypotheses that i) lower PARP1 expression and/or ii) lower expression of NHEJ components 53BP1, DNA-PKcs, Ku70, Ku80, or LIG4, may correlate with diminished response rate as well as PFS in pts treated with rucaparib on ARIEL2. Methods: Immunohistochemical assays were developed for 53BP1, DNA-PKcs, Ku80, Ku70, LIG4, and PARP1 and validated in formalin fixed, paraffin embedded cell lines differing in aneate expression. Available pretreatment OC biopsies from ARIEL2 Part 1 were stained and scored for % of tumor nuclei that were negative (0), weak (1+), moderate (2+) or strong (3+). Modified H-scores were correlated with clinical characteristics and outcome measures. Result: Pretreatment biopsies from 62-68 pts were successfully stained for each repair protein. Across the samples, PARP1 H-scores varied from 0 to 300 (median 160). Focusing on the BRCA wt/LOH high group (n = 38), there was no significant difference in PFS of pts with low (<100), intermediate (100-200) or high (>200) PARP1 H-score (p = 0.57). Expression of DNA-PKcs, Ku70, Ku80, and LIG4 was generally lower (median H-scores 20-60) and did not indicate diminished response of PFS. In contrast, the worst outcome was observed in pts with intermediate or high 53BP1 (H-score ≥ 100, n = 10) compared to pts with low 53BP1 (H-score ≤ 100, n = 25), with median PFS 20.7 vs. 5.5 months (p = 0.073), respectively. Staining of additional pretreatment biopsies is planned. Conclusions: In the BRCA wt/LOH high group, pretreatment PARP1 expression does not correlate with rucaparib response. In contrast, BRCA wt/LOH high OC pts with low 53BP1 have a trend toward shorter PFS with rucaparib, suggesting that 53BP1 downregulation might correlate with clinical PARP1 resistance in BRCA wt/LOH high OC.

**#4678 Pilot trial of talazoparib (BMN 673), an oral PARP inhibitor, in patients with advanced solid tumors carrying deleterious BRCA mutations.** Robert S. Meehan,1 Alice P. Chen,1 Geraldine O’Sullivan Coyne,1 Shivianni Kummar,2 Jiuping Ji,2 Rasa Vilimas,2 Lamin Juwara,2 Robert J. Kinders,2 Katharine Ferry-Galow,2 Deborah Wilsker,2 Yiping Zhang,2 Angie B. Dull,2 Tony Nasav,2 Lihua Wang,2 Ralph E. Parchment,2 James H. Doroshow1.

*NCI/NIH DCTD, Bethesda, MD; 2Frederick National Laboratory for Cancer Research, Frederick, MD.*

Inhibition of poly (ADP-ribose) polymerase (PARP) sensitizes tumor cells to DNA damage that would normally be repaired by the late excision repair pathway. PARP inhibitors are active clinically against BRCA-deficient ovarian cancers. The PARP inhibitor talazoparib produces cytotoxicity in human cancer cell lines and animal models of tumors that harbor mutations that compromise DNA repair pathways. In this study, single agent talazoparib (1000 μg/day) was administered to patients with deleterious BRCA1 or BRCA2 mutations and advanced solid tumors in 28 day cycles. The primary objective of the trial was to examine pharmacodynamic (PD) effects of talazoparib; the secondary objective was to determine response rate in patients whose tumors carry BRCA mutations. Mandatory pretreatment tumor biopsies were obtained pre-treatment and 3-6 hrs post-treatment on cycle 1 day 8. Optional biopsies were collected at the time of progression. One core from each time point was analyzed for PARP inhibition by a validated ELISA assay while the other core was used for IFA analysis of H2AX. A total of 9 patients (pts) were enrolled and treated before this trial was closed due to lack of drug availability: [prostate (3), ovarian (2), breast (2), uterine sarcoma (1), pancreatic (1)]. Median age was 63 (range: 33-73 yrs); male-to-female ratio was 4:5; and the median number of prior treatments was 6 (range: 1-12). All 9 pts were evaluable for PD endpoints. One pt progressed during the first cycle of treatment; 8 pts were evaluable for clinical response. Mean time on study for evaluable pts was 8 cycles (range: 2-18); 5 of 8 (62%) pts experienced a documented partial response [ovarian (2), prostate (2), breast (1)] lasting between 4 and 12 cycles (median: 6 cycles). Two pts had stable disease for 4 to 6 cycles, and one progressed after 2 cycles. The agent was well tolerated; the most frequent adverse events were hematologic including grade (4) anemia (1) and thrombocytopenia (1), and grade 3 anemia (2), neutropenia (1), lymphopenia (1). Decreases in PAR levels (>75%) were observed in all cycle 1 day 8 biopsy pairs, documenting a primary PD effect. Increased H2AX expression was observed for 4/6 pts in post-dose biopsies; pre-treatment H2AX levels measured as %Nuclear Associated Protein (NAP), increased from a mean ± SD of 1.13 ± 1.08 to a post-treatment %NAP mean of 5.60 ± 0.78; (p = 0.018), supporting a role for drug-enhanced DNA double strand breaks in the mechanism of action of talazoparib for BRCA mutant tumors. In summary, talazoparib demonstrated significant clinical activity as a single agent in patients with BRCA-deficient tumors and substantial reductions in tumor PAR levels in matched pre- and post-treatment tumor biopsies.

**#4679 Mismatch repair defects in lethal prostate cancer.** Pasquale Rescigno, Daniel Nava Rodrigues, Wei Yuan, Suzanne Carreira, Maryam Lambros, George Seed, Ruth Riisnaaes, Susana Miranda, David Dolling, Matthew Clarke, Mateus Crespo, Claudia Bertan, Gunhiver Boysen, Joaquin Mateo, Ana Ferreira, Adam Sharp, Ines Figueiredo, Semini Sunanumasria, Mariane Sousa Fontes, Diletta Bianchini, Zefiria Zafeirirou, Johann Sebastian de Bono.

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**INTRODUCTION:** A subset of prostate cancers (PCs) has a hypermutated phenotype. We hypothesized that lethal PCs with a high mutational load (ML) have DNA repair defects including mismatch-repair (MMR) aberrations and that such aberrations may impact outcome. METHODS: Diagnostic and metastatic castration resistant prostate cancer (mCRPC) tissues were tested by A) immunohistochemistry (IHC) for MSH2, MSH6, MLH1, and PMS2; B) microsatellite instability (MSI) (Promega v1.2 assay); C) targeted next generation sequencing (NGS) of the coding regions of 113 genes, a panel enriched for DNA-satellite instability (MSI) (Promega v1.2 assay); D) targeted next generation sequencing (NGS) of the coding regions of 113 genes, a panel enriched for DNA-motion analysis of differentially methylated 2794 CGI sites in 119 t/r AML and 49 healthy controls. Hierarchical clustering analysis of methylation profile data was performed using 8 major clusters. CIMP-like cluster (n = 42) had a higher response rate (39 %) compared to intermediate (17%) and CIMP-like cluster (15%) (p = 0.025, Fisher’s exact test). Demethylation rate of LINE-1 was lower in CIMP-like cluster than in normal-like and intermediate cluster (average demethylation of LINE-1 in CIMP-like cluster vs. -31 ± 2 % in normal-like vs. -23 ± 2 % in intermediate cluster, p = 0.0007, One-way ANOVA). Mutation screening revealed frequent alterations affecting signaling pathways (CSF3R, KIT, KRAS, and FLT3) in the CIMP-like cluster (62%) compared to normal-like (11%) and intermediate cluster (13%) (p < 0.0001, Fisher’s exact test). High number of hypermethylated sites per patient was prominent in the CIMP-like cluster and associated with a four gene expression classifier z-score (low CDA, low P15, low CTGF, high DNMT3B) (R = 0.637, p < 0.0001) as well as resistance to guadecitabine (mean of 4 gene z-score 0.54 in non-responders vs. 2.31 in responders, p = 0.001). We propose that DNA hypermethylation at CpG islands, mutations in signaling pathways and unfavorable gene expression signature can be developed as biomarkers predicting resistance to guadecitabine in t/r AML. This trials were registered at www.clinicaltrials.gov as NCT02293993 (Phase I) and NCT02197767 (Phase II).
samples was evaluated by paired t-test. The relationship between ML and MMR deficient tumors on IHC (MMRd) and/or MSI tumors by PCR (MMRd/MSI) was analyzed by negative binomial regression model. The relationship between MMRd/MSI tumors and overall survival (OS) was analyzed using univariate and multivariate Cox regression, adjusting for relevant treatment (prostateectomy or radiotherapy, age, PSA doubling time, and presence of metastatic disease at diagnosis. RESULTS: We analyzed 306 PC biopsies (180 hormone-sensitive [HS] and 126 castration resistant [CR]) from 208 patients. Overall 16 patients (7.7%) had either MMRd or MSI. Matched, same-patient, HSPC and mCRCP tumor biopsies were available for 82 patients. Only one case with MMRd status in HS was not confirmed in matched mCRCP. This patient was considered MMR deficient for the survival analysis. Msi had a SE of 80% and a SP of 97%. An MSI by NGS cut-off of 0.096 had SE of 60% and SP of 99%, with an area under ROC curve of 0.83. A ML cut-off of 11 mutations per panel had SE of 60% and SP of 99%, with an area under ROC curve of 0.79. Although ML in diagnostic samples correlates with ML in mCRCP, ML was significantly higher in mCRCP/MSRd/MSI cases (p < 0.001; predicted ML of 14 versus 4 mutations per panel). MMRd/MSI and MMR proficient patients were balanced for type of radical treatments. Gleason score, presence of metastatic disease, PSA, age and stage at diagnosis. Despite this, median OS from start of LHRH for the MMRd/MSI group was significantly shorter than in the MMR proficient group in univariate and multivariable analysis (3.84 vs 6.81 years; aHR: 2.44; 95% CI 1.27 -4.70; p = 0.008). CONCLUSION: We provide evidence that MMRd/MSI status represents a negative prognostic factor in metastatic CRPC. MMRd/MSI status in diagnostic biopsies accurately identifies MMRd/MSI in mCRCP. MSINGS and ML can detect MMRd/MSI tumors.

#4680 H3K9 methyltransferase G9a epigenetically regulates breast cancer cell iron homeostasis via repressing ferrooxidase hephaestin expression, promotes breast cancer growth. Ya-fang Wang, Jie Zhang, Yi Su, Yan-yan Shen, Mei-yu Ceng, Jian Ding, Yi Chen. Shanghai Institute of Materia Medica, Shanghai, China.

G9a is a H3K9 methyltransferase with elevated expression observed in many types of human cancers, seems to be required for the maintenance of the malignant phenotype. However, the tumorigenic role of G9a in breast cancer is still far from clear. In addition, growing evidence revealed that the disruption of iron homeostasis may contribute to the development of breast cancer (BC). Pathways of iron acquisition, efflux, storage and regulation are all perturbed in BC, suggesting that reprogramming of iron metabolism is a central aspect of tumor cells survival. Hephastin (HEPH) is a ceruloplasmin homologue that converts iron (II) to (III), and mediates iron efflux in concert with ferroportin to transport iron across the basolateral membrane. It retains unclear whether HEPH concentration has any impact on the iron status of breast tissue, and breast cancer growth. In this study, we demonstrate that G9a exerts its oncogenic function by destruction cellular iron homeostasis. We revealed that G9a knockdown, and G9a specific inhibitors significantly up-regulated HEPH expression, decreased intracellular labile iron content, and then suppressed iron efflux for survival of metastatic CRPC. G9a knockdown, and G9a specific inhibitors in diagnostic biopsies accurately identifies MMRd/MSI in mCRCP. MSINGS and ML can detect MMRd/MSI tumors.


Purpose: The BIM deletion polymorphism is associated with apoptosis resistance to epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs), such as gefitinib and erlotinib, in non-small cell lung cancer (NSCLC) harboring EGFR mutations. Here, we investigated whether the BIM deletion polymorphism contributes to resistance against osimertinib, a third-generation EGFR-TKI, and determined the efficacy of a histone deacetylase (HDAC) inhibitor, vorinostat, against this form of resistance and elucidated the underlying mechanism. Experimental Design: We used EGFR-mutated NSCLC cell lines which were either heterozygous or homozygous for the BIM deletion polymorphism to evaluate the effect of osimertinib in vitro and in vivo. Protein expression was examined by western blotting. Alternative splicing of BIM mRNA was analyzed by RT-PCR. Results: EGFR-mutated NSCLC cell lines with the BIM deletion polymorphism exhibited apoptosis resistance to osimertinib in a polymorphism dose-dependent manner, and this resistance was overcome by combined use with vorinostat. Experiments with homozygous BIM deletion-positive cells revealed that vorinostat affected the alternative splicing of BIM mRNA in the deletion allele, increased the expression of active BIM protein, and thereby induced apoptosis in osimertinib-treated cells. These effects were mediated predominantly by HDAC3 inhibition. In xenograft models, combined use of vorinostat with osimertinib could regress tumors in EGFR-mutated NSCLC cells homozygous for the BIM deletion polymorphism. Moreover, this combination could induce apoptosis even when tumor cells acquired EGFR-T790M mutations. Conclusions: These findings indicate the importance of developing HDAC3-selective inhibitors, and their combined use with osimertinib for treating EGFR-mutated lung cancers carrying the BIM deletion polymorphism.


Hairy cell leukemia (HCL) until recently was characterized as a single blood and bone marrow malignancy, even though clinically at least two pathologies seemed to be at work. This study sought to confirm the clinical observations using genomic evidence. HCL is a chronic mature B-cell malignancy with distinctive immunophenotype, typically expressing CD20, CD25, CD5, CD11c, CD103, CD123, annexin A1 (ANXA1), and tarrytale acid phosphatase (TRAP). Purine analog therapy is highly effective, with most patients achieving durable remissions. HCL-variant (HCLv) was first identified by Cawley et al. (1980) and recently recognized by the World Health Organization as a separate cancer. HCLv lacks CD25, ANXA1, TRAP, and BRAFV600E expression, and patients respond poorly to purine analogs. In order to find detailed biological information involving these two diseases, ANXA1 and BIM polymorphism, we used EGFR-mutated NSCLC cell lines which were either heterozygous or homozygous for the BIM deletion polymorphism to evaluate the effect of osimertinib in vitro and in vivo. Protein expression was examined by western blotting. Alternative splicing of BIM mRNA was analyzed by RT-PCR. Results: EGFR-mutated NSCLC cell lines which were either heterozygous or homozygous for the BIM deletion polymorphism exhibited apoptosis resistance to osimertinib in a polymorphism dose-dependent manner, and this resistance was overcome by combined use with vorinostat. Experiments with homozygous BIM deletion-positive cells revealed that vorinostat affected the alternative splicing of BIM mRNA in the deletion allele, increased the expression of active BIM protein, and thereby induced apoptosis in osimertinib-treated cells. These effects were mediated predominantly by HDAC3 inhibition. In xenograft models, combined use of vorinostat with osimertinib could regress tumors in EGFR-mutated NSCLC cells homozygous for the BIM deletion polymorphism. Moreover, this combination could induce apoptosis even when tumor cells acquired EGFR-T790M mutations. Conclusions: These findings indicate the importance of developing HDAC3-selective inhibitors, and their combined use with osimertinib for treating EGFR-mutated lung cancers carrying the BIM deletion polymorphism.


SRSF2 is recurrently mutated in a variety of myeloid malignancies, including chronic myelomonocytic leukemia (CMML), myelodysplastic syndrome (MDS), myeloproliferative neoplasms (MPN), and acute myeloid leukemia (AML); and most alterations involve the codon proline-95 (P95). In vitro studies have shown that the SRSF2-P95H mutation affects splicing of EZH2 and functionally reduces its protein expression. Inactivating mutations of EZH2, located at 7q36.1, have also been reported in a variety of myeloid malignancies. Studies have shown that SRSF2 and EZH2 gene mutations are mutually exclusive events in myeloid malignancies. In this study, we retrospectively analyzed next-generation sequencing results of 16,767 patient cases with a clinical indication of a possible myeloid malignancy. All exons of the EZH2 gene and exon 1 of SRSF2 gene were sequenced. A total of 33 cases (0.2%) had mutations in both SRSF2 and EZH2 genes; 70% were male

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Comparison by Z-score. Synergy was conducted by median-effect. Results: JQ1
Five-Point dose response curves were interpolated to provide LC50 values for
aggregated, with microspheroids of desired size isolated by density centrifugation.

Development has been slow. As the Bromodomain/BET proteins epigenetically
growth and survival, it is an attractive therapeutic target, yet effective drug de-
cancers. As MYC is responsible for the reprogramming of cell metabolism,
humangeneexpression. Deregulation of MYC is found in up to 70% of human
form to explore clinically relevant observations.

CLINICAL RESEARCH: Epigenetics and DNA Repair

CLINICAL RESEARCH: Immunomodulatory Agents and Therapeutics

#4684  MYC inhibition in human malignancy by BET-Bromodomain in-
hibitor JQ1, using a 3-D human tumor primary culture micro-spheroid plat-
tform to more closely simulate the clinical relevant observation. Robert Alan Nagourney,1
Steven S. Evans,1 Alexander J. Nagourney,1 Paula J. Bernard,1 Federico R. Fran-
cisco,1 Milan Sheh,2 Nilesh Vora,2 Ekhnat Deo,2 Rational Therapeutics, Inc.,
Long Beach, CA; Todd Cancer Institute, Long Beach, CA.

The transcription factor MYC is associated with the regulation of over 15% of
human gene expression. Deregression of MYC is found in up to 70% of human
cancers. As MYC is responsible for the reprogramming of cell metabolism,
growth and survival, it is an attractive therapeutic target, yet effective drug de-
velopment has been slow. As the Bromodomain/BET proteins epigenetically
regulate MYC, BET inhibitors like JQ1 have been developed as MYC therapeu-
ics. We used the Ex Vivo Analysis of Programmed Cell Death (EVA/PCD)
platform previously shown to correlate with response, time to progression and survival,
and ex vivo function of BET inhibition. Results: JQ1 activity in 46 human tumor specimens isolated from surgical bi-
opsies. Methods: Surgical biopsies were mechanically and enzymatically disas-
gregated, with micro spheroids of desired size isolated by density centrifugation.
Five-Point dose response curves were interpolated to provide LC50 values for
comparison by Z-score. Synergy was conducted by median-effect. Results: JQ1
Five-Point dose response curves were interpolated to provide LC50 values for
aggregated, with microspheroids of desired size isolated by density centrifugation.

#4686  OmX a hypoxia modulator reverses the immunosuppressive glo-
blasto ma microenvironment by stimulating T cell infiltration and activation
that results in increased number of long-term survivors. Natacha Le Moan,1
Philiberta Leung,2 Sarah Ng,2 Tina Davis,2 Carol Liang,2 Jonathan W. Winger,2
Stephan P. Cary,1 Nicolas Butowiski,1 Ana Krolicka1 Omniox, Inc., San Carlos, CA;1
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Oxygen is one of the key modulators of tumor microenvironment whereby
low oxygen or hypoxia is associated with resistance to chemo- and radio-
therapies and poor patient outcomes. Hypoxia favors an immunosuppressive tumor microenvironment by promoting Treg recruitment and activation and sup-
pressing T cell and NK cell proliferation and effector function and pro-inflam-
matory cytokine secretion. Therefore, reversing tumor hypoxia could create an
immunomodulatory microenvironment and improve the efficacy of several im-
munotherapies. Omniox has developed an oxygen carrier OMX that can specif-
cally deliver oxygen to hypoxic tumor regions without affecting oxygenation of
non-tumorous tissue. Because OMX is well tolerated in small (rats and mice) and large (sheep and dogs) animals,
several tumor types appear to be candidates for agents that target MYC-mediated cell survival, of-
fering potential treatment options for patients with advanced malignancies. JQ1
synergy with HDACI & AURKI suggests novel drug combinations for future
development. As MYC signaling influences thousands of gene targets, pheno-
typic analyses like the EVA/PCD platform can offer insights into cellular cell
difference and serve as surrogate markers for clinical response to MYC inhibi-
tion. Supported in part by the Vanguard Cancer Foundation, the Nagourney
Institute and the Malcolm C. Todd Cancer Institute.

#4685  Immune checkpoint protein VISTA is a critical regulator of the
IL-23/IL-17 inflammatory axis. Na Li1 Wenwen Xu,1 Ying Yuan,1 Natarajan
Ayithan,1 Yasutomo Imai,1 Xuesong Wu,2 Halli Miller,1 Michael Olson,1 Sam-
uel T. Hwang,1 Subramanian Malarkannan,3 Li Wang1 Medical College of
Wisconsin, Milwaukee, WI;2 Medical College of Wisconsin, Dermatology, Mil-
waukee, WI;3 Blood Research Institute, Milwaukee, WI.

V-domain Immunoglobulin Suppressor of T cell Activation (VISTA) is an
inhibitory immune-checkpoint molecule that suppresses CD4+ and CD8+ T cell
activation when expressed on antigen-presenting cells. Vsir+/+ mice developed
loss of peripheral tolerance and multi-organ chronic inflammatory pheno-
types. Vsir+ CD4+ and CD8+ T cells were hyper-responsive towards self- and
foreign antigens. In addition, VISTA regulates immune responses that have not been
demonstrated. Our current study shows that VISTA-blocking monoclonal anti-
body (mAb) enhanced anti-tumor T cell response, and synergized with a pep-
tide vaccine and TL7R agonist imiquimod as adjuvant to suppress tumor growth in
the B16 melanoma model. Surprisingly, the therapeutic effect of this combi-
nation therapy was abolished in the IL17R knockout hosts, indicating that the
IL-17-mediated inflammation is regulated by VISTA and is required for anti-
tumor immunity in the context of VISTA blockade. To better define the regulat-
ory role of VISTA in inflammation, we employed a murine model of psoriasis
induced by topical treatment of IMQ. Our data show that VISTA deficiency
accelerated the psoriasiform inflammation. Enhanced TL7R signaling in Vsir+
dendritic cells (DCs) led to the hyper-activation of Erk1/2 signaling and aug-
mented the production of IL-23. IL-23, in turn, promoted the expression of
IL-17A in both TCRgδ T cells and CD4+ Th17 cells. Furthermore, VISTA
regulates the peripheral homeostasis of CD27+ gd T cells, and their activation
upon TCR-mediated or cytokine-mediated stimulation. IL-17A-producing
CD27+ gd T cells were expanded in the Vsir+ mice and amplified the inflamma-
tory cascade. Together, these results indicate that VISTA is a critical regulator of the
IL-17-mediated inflammatory axis. Ongoing studies will define how IL-17 regulates tumor-reactive immunity in the context of VISTA blockade and
TLR vaccine adjuvant.
The anti-tumor activity of HERA-CD27 was evaluated in MC38-CEA and CT26 colorectal syngeneic mouse tumor models. In both model treatments with HERA-CD27 resulted in reduced tumor growth in both models compared to an irrelevant IgG. An orthotopic intracranial glioma study showed similar anti-tumor effects when treated with SurVaxM-derived antibodies. Tumor growth was also inhibited in immunocompromised (nude) animals treated with 2C2 or H30 antibodies, although to a lesser extent. Conclusions: Antibodies generated in response to the SurVaxM vaccination are highly cross-reactive to survivin and provide therapeutic benefit in immunocompetent mouse tumor models. These antibodies retain some efficacy in immunocompromised models, indicative of a direct T-cell independent effect. An anti-tumor response through anti-survivin-targeted antibodies is unexpected as surface-accessible survivin expression has not yet been well described in the literature. Data presented here highlight possible avenues for investigation of mechanism(s) of action.

**#4689 Hexavalent CD27 agonists show single agent anti-tumor activity and enhanced memory formation in mouse syngeneic tumor models.**

Christiane Gieffers, David Richards, Jaromir Sykora, Mauricio Redondo-Muller, Meinolf Thiemann, Christian Merz, Karl Heinonen Heinonen, Viola Marschall, Harald Fricke, Oliver Hill. Apogenix AG, Heidelberg, Germany.

Tumor necrosis factor receptor superfamily (TNFRSF) proteins are widely expressed on immune and tumor cells highlighting their importance in multiple tumor microenvironments and phases of the anti-tumor immune response. Apogenix has developed a proprietary technology platform for the construction of novel hexavalent TNFRSF agonists (HERA) for the treatment of cancer. HERA fusion proteins comprise a perfect molecular mimic of the TNFSF cytokine structure and are based on dimerization of trivalent single-chain TNFRSF receptor-binding domains (scTNFSF-RBD) via a Fcγ receptor (FcγR) binding deficient immunoglobulin Fc domain. As a result of this molecular design, HERA proteins are capable of clustering six receptors in a spatially well-defined manner. Signaling following treatment with the Apogenix HERA "scTNFSF-RBD-Fc fusion proteins" is entirely independent of secondary crosslinking through FcγRs that is required for many agonistic anti-TNFRSF antibodies. The HERA engineering concept has been successfully translated to TRAIL, GITRL, CD40L, LIGHT and CD27L resulting in agonists that are currently in development. CD27L is a potent co-stimulatory molecule that drives T cell activation and survival through interaction with its receptor (CD27). HERA-CD27L is expressed in CHO suspension cells followed by a lab-scale purification process that results in homogeneous aggregate-free protein lots. The purified protein binds its respective target at high affinity and selectively co-stimulates multiple immune and tumor cell types highlighting their immune and tumor cell type cross-reactivity. In vitro, HERA-CD27L was able to bind CD27 expressed on primary human CD4+ and CD8+ T cells. Binding significantly increased T cell expansion following cd53/cd28 stimulation and leads to increased expression of ox40 on CD4+ T cells and 41bb on CD8+ T cells, respectively. In vivo, a single dose of 10mg/kg HERA-CD27L increases clonal expansion of antigen-specific CD8+ T cells upon immunization with OvaBM (Ova) in the mouse OT-1 model with a kinetics leading to peak levels of ~25% Ova-specific CD8+ T cells at day 6 after treatment. Anti-tumor efficacy of HERA-CD27 was evaluated in MC38-CEA and CT26 colorectal syngeneic murine tumor models. In both models treatment with HERACD27L resulted in a dose dependent inhibition of tumor growth. CT26 tumor bearing mice treated
with 1mg/kg HERA-CD27L, twice weekly showed a 85% tumor-growth inhibition (TGI) compared to the control group. A significant TGI of 48% could be observed in the MC38-CEA model upon treatment with 10mg/kg, twice weekly. Analysis of peripheral lymphoid tissues in the MC38-CEA bearing mice could furthermore show that HERA-CD27L treatment is accompanied with enhanced memory CD4+ and CD8+ T cell responses in both CD4+ & CD8+ T cells. In summary, the data on the hexavalent HERA-CD27L indicate a potent immune cell driven anti tumor efficacy. Therefore, HERA-CD27 agonists could be applied for the treatment of cancer as a single agent or in combination with checkpoint Inhibitors.


The functional plasticity of macrophages and their ability to support tumour growth or promote potent antitumor immunity make them an attractive target for cancer therapy. Macrophages have been implicated in the complex antigen activity of murine STING agonist, DMXAA, including disruption of tumor vasculature, induction of cytokine production, and promotion of durable antitumor immune responses. Many of these activities are lost in STING- mice, and DMXAA does not bind to human STING, providing a pertinent explanation for why the anti-tumour effects of DMXAA observed in mice were not recapitated in human clinical trials. Using PMA-differentiated THP-1 macrophages, we sought to identify human active analogues of DMXAA in vitro, comparing their capacity to induce cytokines indicative of a classically activated antitumour phenotype; the non-canonical STING agonist 2′-3′-cGAMP was also included in our investigation. 10 (CXCL10) production, measured by ELISA, was used as an initial marker for activity, followed by multiplex cytokine analysis for broader characterisation compared to M(IFN-γ-LPS), M(IL-4/IL-13) and resting M(media alone) macrophage phenotypes. Parallel experiments were also conducted with RAW264.7 murine macrophages to compare the DMXAA-induced cytokine profile directly with that of 2′-3′-cGAMP, 2′-3′-cGAMP induced 22-fold and 18-fold increase in IP-10 production in resting and M(IL-4/IL-13) THP-1 macrophages, respectively. By comparison, THP-1 macrophages were much less responsive to DMXAA and analogues tested to date. Furthermore, 2′-3′-cGAMP induced a spectrum of cytokines similar to that observed in M(IFN-γ-LPS) macrophages, and markedly different to that of M(IL-4/IL-13) THP-1 macrophages. DMXAA, and analogues previously shown to have cytokine-inducing activity in human leukocytes did not produce cytokine spectra indicative of a classic M(IFN-γ-LPS) phenotype. Unlike human cells, however, cytokine spectra produced by RAW264.7 murine macrophages were comparable between DMXAA- and 2′-3′-cGAMP-treated cells. These findings support previous data that suggest that STING agonists can promote macrophage polarization towards an antitumour phenotype. While DMXAA is active in murine macrophages, only 2′-3′-cGAMP can induce DC-like M(IFN-γ-LPS)-like spectrum of cytokines often attributed to an antitumour macrophage phenotype.

**#4692 Local adjuvant treatment of clinical stage I-II melanoma with low dose CpG-B and/or GM-CSF: Long-term follow-up of three randomized controlled phase II trials.** Bas D. Koster, Mari F. van den Hout, Berbel J. Sluijter, Ronald G. van der Velden, Ronald J. Vuytselste, Arnold Baars, Paul A. van Leeuwen, Rik J. Schep, Monique P. van den Tol, Alfonso J. van den Eertwegh, Tanja D. de Gruijl. VU University Medical Center, Amsterdam, Netherlands.

Introduction: Currently, there is no widely used adjuvant treatment available to improve survival after surgical excision of localized melanoma. Here, we present the clinical outcome of patients who participated in three randomized phase-II trials and received low-dose loco-regional immunotherapy, which was shown to be safe and to boost loco-regional and systemic anti-melanoma T cell immunity. Patients and Methods: In three single-center, single-blinded, randomized and placebo (saline) controlled phase-II clinical trials, patients with early stage melanoma were treated with 1) Granulocyte/Macrophage-Colony Stimulating Factor (GM-CSF), 2) unmethylated CpG type-B oligodeoxynucleotide CpG7909 (CpG-B), and 3) CpG-B, alone or combined with GM-CSF, through 1-4 intra-dermal injections at the site of the primary melanoma excision scar, within 7 days preceding re-excision and sentinel lymph node (SLN) biopsy. For clinical follow-up analysis, all treated patients were grouped together (treated group n=36) as were the patients who received saline (saline group n=28). Results: 10-year recurrence-free survival rate in the treated group was 94% (95% CI 78-98) versus 48% (95% CI 21-71) in the saline group (P=0.005), hazard ratio (HR) for recurrence was 0.15 (95% CI 0.06-0.60) for the treated group. This apparent antitumor efficacy was in line with the observation upon pathological examination of less tumor positive SLN in the treated group (P=0.05). The 10-year distant recurrence-free survival rate in treated patients was also higher (94%, 78-98 versus 59%, 28-81, HR 0.22, 0.07-0.93; P=0.04). Conclusion: Local low-dose immunotherapy in patients with early-stage melanoma may offer durable protection against distant recurrences. These findings warrant further clinical exploration of this local non-toxic immune potentiating regimen.

**#4693 Fc effector bioassays enable faster and quantitative measurement of ADCC and ADCP mechanisms of action.** Zhi-Jie Jey Cheng, Rich Moravec, Aileen Pangui, Denise Garvin, Gopal B. Krishnan, Frank Fan, Mei Cong. Pro- mega Corp., Madison, WI.

Antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cell-mediated phagocytosis (ADCP) are recognized as important mechanisms of action (MOA) of therapeutic antibodies. Primary peripheral blood mononuclear cells (PBMCs) are routinely used in traditional bioassays to measure potency, stability of antibody drugs in ADCC and ADCP. However these methods are labor intensive and highly variable. Here we report the development of a luciferase-based reporter assay that utilizes activation of effector cells via cross-linking of Fc receptors with target cell-bound antibodies. We will discuss functional cell-based Fc effector reporter bioassays developed to measure human FcgRIIIA (V158 and F158 variants), Human FcgRIIa (H131 and R131 variants), Human FcgRI as well as Mouse FcgRIV and FcgRIII. Compared to primary cell-based assays these reporter bioassays are less variable, easier to perform and provide more consistent analysis of the important MOAs that are critical to drug development programs. In qualification studies, performed in accordance with ICH guidelines for antibody screening and characterization, we tested the bioassays for specificity, accuracy, precision and linearity.

**#4694 The humanized anti-CD47 monoclonal antibody, CC-90002, has antitumor activity in vitro and in vivo.** Rama Krishna Narla,1 Hardik Modi,1 Lilly Wong,1 Mahan Abassian,1 Daniel Bauer,1 Pragnya Desai,1 Bonny Gaffney,1 Pilgrim Jackson,1 Jim Leistner,1 Jing Liu,1 Antonia Lopez-Girona,1 Maria Romero,1 WenQing Yang,1 Brendan P. Eickelman,2 Quinn Deveraux,1 Laurie Phillips,2 Heather K. Raymon,1 Laure Escoubet,1 John Boylan,1 Kandasamy Harirhan1. Celgene Corp., San Diego, CA, Inhibrix, San Diego, CA.

Cluster of differentiation 47 (CD47) is a transmembrane protein ubiquitously expressed on human cells but overexpressed on many different tumor cells. The interaction of CD47 with signal-regulatory protein alpha (SIRPα) expressed on macrophages results in the inhibition of phagocytosis. Thus, tumor cell overexpression of CD47 enables escape from immune surveillance via the blockade of phagocytic mechanisms. We report here the characterization of CC-90002, a humanized anti-CD47 monoclonal antibody (mAb) that is being developed as a potential therapeutic for hematologic malignancies and solid tumors. CC-90002 has a high affinity for binding to CD47 with a subnanomolar dissociation constant (Kd) value. The IC50 required for the blockade of the CD47-SIRPα interaction is also in the subnanomolar range. CC-90002 is significantly and previously reported anti-CD47 antibodies for its inability to promote hemagglutination while maintaining high affinity binding to CD47 and inhibition of the CD47-SIRPα interaction. CC-90002 enabled antibody-mediated phagocytosis of a panel of hematological cancer cell lines in vitro, including ALL, multiple myeloma (MM) and acute myeloid leukemia (AML) cells, and primary AML patient samples. The phagocytosis index for this panel (at 1 μg/mL) ranged from approximately 20% to 60% for all the cell lines tested. Antibody concentration-response studies indicated that the CC90002 effect was concentration-dependent in the ALL and AML cell lines. CC90002 treatment significantly enabled the phagocytosis of two lenalidomide-resistant MM cell lines and primary AML cells from three patients. Additionally, CC-90002 treatment elicited the phagocytosis of solid tumor lines, including those from ovarian, breast, head and neck, lung, and pancreatic cancers. The in vivo efficacy of CC-90002 was evaluated across cell line-derived and patient-derived xenograft models. Significant dose-dependent antitumor activity was observed with CC-90002 treatment in the MM cell line-derived xenograft models, RPMI 8226 and the parental NCI-H929 and lenalidomide-resistant NCI-H929 (H929/R1). Treatment with CC-90002 was able to demonstrate significant reduction in solid tumor xenografts including a cell linederived model of triple negative breast cancer (TNBC), MDA-MB-231, and a patientderived TNBC model, AA1126 and significantly prolonged survival in the HL60disseminated AML tumor model. Mechanistic studies in the RPMI 8226 xenograft model confirmed binding of CC-90002 to tumor cells and recruitment of F4-80-positive macrophages into the tumor. RPMI 8226 tumors treated from CC-90002 treatment demonstrated an increase in select chemokines and cytokines of murine origin. Taken together, in the vitro and in vivo data demonstrate the potential for activity of CC90002 across both hematological malignancies and solid tumors. CC-90002 is currently in early clinical development.

LGR5 is a well characterised marker of intestinal stem cells found at the base of intestinal crypts and a receptor for R-spondins, potent Wnt signalling modulators and stem cell growth factors. Overexpression of LGR5 in colorectal tu-

#4696 Development of an inhaled TLR9 agonist for the immunotherapy of lung cancer. Marilena Gallotta, Hikmat Assi, Robert L. Coffman, Cristina Guiducci Technologies, Berkeley, CA.

CpG-oligomeric nucleotides (CpG-ODN) stimulate innate immune responses through Toll-like receptor-9 (TLR9). Intratumoral (IT) administration of CpG-ODN has been shown to be safe and clinically effective in humans and in mul-

#4697 Intratumoral immunotherapy with TLR7/8 agonist MED9197 modulates the tumor microenvironment and holds potential for combination with immune checkpoint inhibitors. Stefanie R. Mullins,1 Katharina Vogel,1 John Vasilakos,2 Iwen Grigsby,2 Simon Dovey,1 Ryan Patricia,2 Zachary Cooper,1 Ronald Herbst,2 Rakesh Kumar,3 Mark Tomai,2 Robert W. Wilkin-

#4698 Immuno-oncological efficacy of RXDX-106, a novel, selective and potent small molecule TAM (TYRO3, AXL, MER) inhibitor. Yumi Yokoyama,1 Erin D. Lew,2 Ruth Seelige,2 Colin Walsh,1 Maria Barrera,2 Elizabeth Tindall,1 Joanne Oh,1 Heather Ely,1 Amy Diliberto,1 Amanda Albert,1 Jack Bailey,1 Larry L.1,1 Ignyta, Inc., San Diego, CA;2 University of California, San Diego, San Diego, CA.

The TAM family of receptor tyrosine kinases (RTKs), TYRO3, AXL, and MER, has been implicated in the pathogenesis and progression of many cancer types. In cancer cells, overexpression of TAM RTKs is associated with resistance and mesenchymal phenotype. In immune cells, TAM RTKs play a key homeo-

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tumors could induce TAM expression on subsets of T cells and that inhibition of TAM RTK activity by the small molecule inhibitor, RXDX-106, could potentially remove the molecular “brake” on immune activation in macrophages, NK cells and T cells, resulting in repolarization of the immune response towards an anti-tumor environment. The unique mechanism of activating both innate and acquired immune responses by RXDX-106, and the compelling preclinical data in cell line and syngeneic models, support the clinical development of RXDX-106 in a wide variety of cancers.

#4699 Single domain antibody (sdAb) localizes in cancer cells to inhibit signal transducer and activator of transcription 3 (STAT3) resulting in therapeutic inhibition of multiple cancers. Sunanda Singh,1 Genoveva Murillo,2 Amanda Rom,2 Avani Singh,1 Samara Singh,2 Meenakshi Parihar,2 Dong Chen,2 Rajendra Mehta,2 Robert Baker,2 Anjali Singh,1 Ashutosh S. Parihar1.2

Singh Biotechnology, Tampa, FL; 2IIT Research Institute, Chicago, IL; 3Creative Biolabs, Shirley, NY.

STAT3 is involved in the pathogenesis of many malignancies, so we developed an anti-STAT3 VH1 (variable region of the heavy chain) SBT-100, that internalizes in cancer cells and binds unphosphorylated STAT3 (U-STAT3) and phosphorylated STAT3 (P-STAT3) and results in significant inhibition of multiple cancers. ATCC cell lines for triple negative breast cancer (MDA-MB-231, MDA-MB-468, MDA-MB-453, ER-/PR+/HER2- breast cancer (BT474), pancreatic cancers (PANC-1, BX-PC3), murine mammary cancer (4T1), STAT3 null cells (PC-3), and castrate-resistant prostate cancer (DU145) were tested. Athymic nude mice were obtained from Envigo. The IL-6 Reporter Cell Assay was obtained from Promega. VEGF inhibition ELISA was done using retinal epithelial cells (ATCC). In vitro growth inhibition was done using a MTT assay. Immunoprecipitation (IP) and western blot studies in MDA-MB-231, PANC-1, HeLa, DU145, 4T1 and PC-3 showed that SBT-100 binds U-STAT3 and P-STAT3. No STAT3 binding was seen in PC-3. Binding to P-STAT3 was seen in lysates from the 4T1 cells, which have constitutively activated STAT3. Since rodent and human STAT3 have a 99% homology, rodents are excellent models for extrapolating to human disease for overproduction of IL-6 (P<0.0001) compared to control. The degree of IL-6 suppression was comparable to the negative control, BB1608 (STAT3 inhibitor). VEGF ELISA showed significant (p<0.0001) inhibition of VEGF production within 12 hrs and was maintained for up to 48 hrs. After 3 days with SBT-100 the MTT assay showed growth inhibition (p<0.001) in BT474 (93%), MCF-7 (93%), MDA-MB-231 (77%), MDA-MB-468 (85%), MDA-MB-453 (64%), PANC-1 (79%), BX-PC3 (90%), and DU145 (92%). MDA-MB-231 tumors grown in xenograft athymic mice showed suppression (p<0.001). IHC staining in the cytoplasm, and nucleus after 7 days of SBT-100 treatment. U-STAT3 and P-STAT3 activate genes that promote growth, proliferation, angiogenesis, immune suppression, cancer stem cells, metastasis, and apoptosis inhibition. SBT-100 enters the cancer cells, binds STAT3 and P-STAT3 causing growth inhibition (p<0.001) in MCF-7, BT474, MDA-MB-231, MDA-MB-468, MDA-MB-453, PANC-1, BX-PC3, and DU145. These results suggest that SBT-100 can be developed as a therapeutic for cancers expressing either STAT3 or P-STAT3.

#4700 Poly G oligonucleotide enhances anti-tumor immune response among the patients with primary lung cancer. Nobuaki Kobayashi,1 Yuji Shibata,2 Masaki Yamamoto,2 Takashi Sato,2 Masaharu Shinkai,2 Harumi Koidzu,1

Tohoku University Hospital, Yokohama, Japan; 2Yokohama City University Medical Center, Yokohama, Japan; 3Yokohama City University, Yokohama, Japan; 4Yokohama Minami Kyosai Hospital, Yokohama, Japan; 5Tohoku University Hospital, Yokohama, Japan.

Synthetic oligonucleotides (ODNs) containing specific sequences have an immunomodulatory effect. It is highly expected that such ODNs can be developed into a novel model agents for extrapolating to human disease for overproduction of IL-6. We have revealed that a novel poly-G ODN has an anti-tumor immune effect in the tumor-bearing murine model. This effect of poly-G ODN was mediated by T cells in a TL99 independent manner. Poly-G ODN directly induced the phosphorylation of Lck, thereby enhancing the production of IL-2 and T cell proliferation. Meanwhile, our recent data suggested that the immune effect by poly-G ODN against cancer was different in human. We identified poly-G ODN induced T cell proliferation and production of IFN-γ through the enhancement of mono-cyte maturation in human mononuclear cells isolated from peripheral blood or malignant pleural effusion of lung cancer patients. Further studies are continued for the establishment of a novel anti-cancer immune therapy with poly-G ODN.

#4701 Blocking FSTL1 ameliorates immunity against osteosarcoma. Chie Kudo-Saito,1 Yamato Ogawara, Kazunori Aoki. National Cancer Center Research Institute, Tokyo, Japan.

Purposes: Osteosarcoma is a rare malignant tumor of bone in children and young adults, and it is very hard to successfully control the systemic metastases that occur in 25-30% of these patients. In addition to the tumor cells, the stromal cells in the tumor microenvironment may also contribute to the care due to the intensiveness of the conventional treatments including surgical resection of the primary tumor and chemotherapy. Therefore, non-toxic but advanced treatments have been expected for a long while. We recently found that a BMP family FSTL1 is highly and functionally expressed in murine and human osteosarcoma cell lines, and systemically expands pluripotent mesenchymal/stromal cells (MSCs), which play a central role in amplification of tumor heterogeneity that is consequence of reciprocal evolution together with the tumor microenvironment, including induction of immune suppression and dysfunction, desmoplasia, and angiogenesis. FSTL1 and its induced MSCs also directly confer higher invasive and metastatic properties on tumor cells leading to further cancer progression. In this study, we evaluated antitumor efficacy induced by anti-FSTL1 mAb on murine osteosarcoma models, and also compared the therapeutic efficacy with those induced by immune checkpoint inhibitor mAbs (ICIs). Results: We used two syngeneic tumor models that were subcutaneously implanted with murine osteosarcoma NHO5 cells in BALB/c mice, or LM3 cells in C3H mice. These mice were intraperitoneally injected with anti-FSTL1 mAbs (10mg/kg) twice on day 5 and day 8 after tumor implantation, and then treated for 14 days. Analysis of tumor-infiltrating lymphocytes (TILs) from tumors and bone marrow cells on day 15. In both osteosarcoma models, CD45+ MSCs as well as other immunosuppressive CD4+ Foxp3+ Tregs and CD11b+ Gr1+ MDCS systemically increased, indicating impairment of anti-tumor immunity in the mice. Treatment with the anti-FSTL1 mAb significantly induced potent tumor-specific CTLs through reduction of MSCs, resulting in tumor-free in some of the treated mice. Although the antitumor efficacy was similar to those induced by ICIs, the mode of action was totally distinct because MSCs and the MSC-inducible exhausted CD8+ PD1+ T cells were reduced only in the anti-FSTL1-treated mice, but not in the ICI-treated mice. Conclusions: These results suggest that blocking FSTL1 properly reprograms immunity toward elimination of osteosarcoma by reducing the tumor-supportive MSCs. Anti-FSTL1 mAb may be a promising drug for treating osteosarcoma more moderately but effectively in clinical settings.

#4702 PD-L1 blockade in preclinical models of PTEN-deficient prostate cancer. Marco A. De Velasco,1 Yurie Kura,2 Naomi Ando,1 Noriko Sato,1 Kazuko Sakai,1 Barry R. Davies,2 Koichi Sugimoto,1 Masahiro Nozawa,1 Kazuhiro Yoshimura,1 Kazuhiro Yoshikawa,2 Kazuto Nishio,1 Hirotsugu Uemura2,1 Kinai University Faculty of Medicine, Osaka, Japan; 2AstraZeneca, Macclesfield, United Kingdom; 3Aichi Medical University, Nagakute, Japan.

PD-L1 expression is characterized immune expression profiles in more aggressive tumors from PTEN/P53-deficient castration-resistant xenograft models. In PTEN/P53 double knockout (DKO) mice, PD-L1 was strongly expressed cancer cells, associated immune expression profıles in more aggressive tumors from PTEN/P53-DKO mice with advanced castration-naïve prostate cancer. PTEN/P53-DKO mice with advanced castration-naive prostate cancer were used for the preclinical evaluation of novel immunotherapeutic strategies, we used genetically engineered mouse models of PTEN-deficient prostate cancer. We first analyzed the transcriptome in castration-naïve prostate tumors and the progression to castration-resistant disease. Comparative analyses were performed between age-matched normal prostate and PTEN-/- prostate tumor samples from castration-naïve mice and, at 4 weeks (castration-sensitive) and 10 weeks (castration-resistant) post-surgical castration. Pathway and gene-set enrichment analysis indicated that abnormal tumor immunity was strongly associated with the progression to castration resistance. Chemokine signaling, B cell receptor and T cell receptor signaling pathways were among the top dysregulated pathways, and gene signatures of suppressed tumor immunity were enriched in castration-resistant tumors. Higher expression patterns of the programmed cell death protein 1 (PD-1) and its ligand (PD-L1) were observed in castration-resistant compared to castration-naïve tumors. We also characterized immune expression profiles in more aggressive tumors from PTEN/P53 double knockout (DKO) mice. PD-L1 was strongly expressed cancer cells, but a higher presence of PD-1+, CD4+ and PD84+ stromal infiltrating immune cells in was observed in castration-resistant tumors. PD-1/PD-L1 blockade with antibodies against mouse PD-L1 increased CD45+ tumor infiltrating lymphocytes (TILs) in an early model of PTEN/P53-deficient castration-resistant prostate cancer. PD-L1/PD-L1 blockade with antibodies against mouse PD-L1 increased CD45+ tumor infiltrating lymphocytes (TILs) in an early model of PTEN/P53-deficient castration-resistant prostate cancer. Although the antitumor efficacy was similar to those induced by ICIs, the mode of action was totally distinct because MSCs and the MSC-inducible exhausted CD8+ PD1+ T cells were reduced only in the anti-FSTL1-treated mice, but not in the ICI-treated mice. Conclusions: These results suggest that blocking FSTL1 properly reprograms immunity toward elimination of osteosarcoma by reducing the tumor-supportive MSCs. Anti-FSTL1 mAb may be a promising drug for treating osteosarcoma more moderately but effectively in clinical settings.
terim analysis of PD-1/PD-L1 blockade on a model of advanced PTEN/P53
deficient castration resistant prostate cancer (CRPC) has thus far shown that
mice receiving treatment experience significantly longer survival (P = 0.017, me-
dian survival not yet reached). Our findings show that castration-resistance promotes tumor immune suppression in mouse PTEN-deficient prostate cancer and suggest that the observed phenomenon can be reversed pharmacologically with anti-
PD-L1 blockade. Thus, we provide preclinical evidence for immune checkpoint
blockade as a potentially promising prostate cancer therapy.

#4703 INCAGN1949, an anti-OX40 antibody with an optimal agonistic pro-
file and the ability to selectively deplete intratumoral regulatory T cells. Ana M.
Gonzalez,2 Mariana L. Manrique,1 Lukasz Swiech,2 Thomas Horn,2 Ekaterina Bre-
ous,1 Jeremy Waight,1 David Savitsky,1 Yuqi Liu,2 Shiwen Lin,1 Christopher
Clarke,1 Taha Merghoub,1 Daniel Hirschhorn-Cymerman,2 David Schaer,2 Gerd
Ritter,1 Jennifer Pulini,1 Kevin Heller,1 Peggy Scherle,1 Gregory Hollis,2 Reid Hu-
ber,1 Marc van Dijk,2 Jennifer Buell,1 Robert Stein,1 Nicholas Wilson,1 Agris Inc.
Lexington, MA; 2Agenus Inc, Basel, Switzerland; 3Memorial Sloan Ketter-
ing Cancer Center, New York, NY; 4The Ludwig Institute for Cancer Research, New
York, NY; 5Incyte Corporation, Wilmington, DE.

OX40 is a T cell co-stimulatory receptor that can enhance the magnitude and
durability of T cell immune responses. Anti-OX40 agonist antibodies have shown
significant single agent tumoricidal activity in preclinical models, and can combine
effectively with other immunomodulatory antibodies, targeted therapies and vac-
oxes. OX40 agonists are able to counteract the immunosup-
pressive tumor microenvironment and promote tumor-specific cellular immu-
nity via at least two distinct mechanisms: 1) promoting OX40 forward signaling in
tumor-specific T cells and 2) co-engaging Fcγ receptors expressed by tumor-
associated effector cells, and facilitating the selective elimination of OX40high
intratumoral regulatory T cells. INCAGN1949, an anti-OX40 human IgG1 an-
tibody, was selected based on its ability to optimally enhance T cell responsive-
ness under conditions of suboptimal T cell receptor stimulation. INCAGN1949
was shown to mediate effective apical OX40 clustering that is translated into
effective downstream activation of the NFκB pathway. Notably, INCAGN1949
was shown to maintain a sigmoidal dose response curve across a broad range of
antibody concentrations. This suggests a wide therapeutic window and may be
advantageous for dosing considerations. By contrast, evaluation of reference
OX40 antibodies indicated an inverted U-shaped dose response curve, leading to
impaired T cell responses at high concentrations. INCAGN1949 was selected for
clinical development based on its optimal agonist profile, further reinforced by
its ability to combine with other co-inhibitory and co-stimulatory antibodies to
augment T cell responsiveness. Prior to human testing, the pharmacology and
tolerability of INCAGN1949 was evaluated in non-human primates (NHPs).
Pharmacokinetic (PK) and pharmacodynamic (PD) parameters were evaluated
including longitudinal measurements of serum cytokines, immune cell popula-
tions, activation state and T cell-mediated immune responses to reporter vac-
cine antigens. INCAGN1949 exhibited a linear PK profile and was well tolerated
at all doses tested, with no maximum tolerated dose established. Co-administra-
tion of INCAGN1949 and vaccines in NHPs showed an immune-based PD
signature across a broad exposure range. These studies were in line with in vitro
findings, which suggested an equipotent PD range for INCAGN1949 in patients. An impor-
tant secondary mechanism of INCAGN1949 is the ability of its IgG1 Fc region to
mediate selective depletion of OX40-high intratumoral regulatory T cells. Immu-
nohistochemistry and flow cytometry analyses support the validity of this regul-
atory T cell depletion mechanism in a range of functions. The functional in vitro
and in vivo attributes of INCAGN1949 make it suitable for clinical development.
It is currently under evaluation in a Phase 1/2 study in subjects with advanced or
metastatic tumors (NCT02923349).

#4704 Long-lived de novo DNA methylation programming enforces ter-
mal T-cell exhaustion. Hazem E. Ghoneim, Yiping Fan, Hossam A. Abdel-
samed, Pradhyot Dash, Robert Carter, Walid Awad, Pranay Dogra, Paul G.
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Immune-checkpoint blockade (ICB)-mediated rejuvenation of exhausted CD8 T
cells has emerged as one of the most promising frontiers for treating cancer and
chronic infections. However, antigen-specific T cells that have differentiated to
a terminal state of exhaustion remain refractory to ICB-mediated rejuvenation and
currently have limited potential for contributing to this promising therapeutic
approach. Given that many of the impaired effector properties of terminally exhausted
CD8 T cells appear to be heritably maintained even in the absence of antigen, we
investigated the role of de novo DNA-methylation programming as a cell-intrinsic
mechanism for establishing the ICB-nonresponsive state of T-cell exhaustion. Using
a TCR-inducible system to delete the de novo DNA methyltransferase, Dnmt3a, in
CD8 T cells (cKO) responding to a chronic LCMV infection, we observed that
antigen-specific CD8 T cells lacking the capacity to acquire new DNA methylation
programs retained their effector function despite exposure to high antigen loads for
several months. Longitudinal whole-genome bisulfitie sequencing methylation pro-
file and gene expression analysis of wild-type and Dnmt3a cKO antigen-specific
CD8 T cells during persistent antigen exposure revealed that de novo DNA meth-
ylation programs (CPI) represent a breakthrough for the treatment of cancer. A T cell-
inflamed tumor microenvironment (TME) is required for antitumor efficacy of
CPI. However, the evidence suggests that 70% of tumors do not possess T cell-

CLINICAL RESEARCH: Immunomodulatory Agents and Therapeutics

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inflamed TME limiting the potential of CPI treatments to a smaller population of cancer patients. Immunotherapies that enable CPI effectiveness in patients with non-T cell-inflamed phenotype are being studied. Oligonucleotide-based TLR9 agonists induce potent CD8+ T cell and plasmacytoid dendritic cell activation and limit immune suppressor cell phenotypes in TME. Therefore, the combination of TLR9 agonists with CPI would be a rational approach for achieving higher rates of success in cancer treatment. However, TLR9 agonists were unsuccessful in previous cancer clinical trials. Spherical Nucleic Acids (SNAs) are a novel class of agents with oligonucleotides densely packed and radially oriented around spherical liposomal nanoparticles. SNAs overcome limitations of therapeutic oligonucleotides through increased uptake and nuclease stability. We have evaluated a TLR9 targeted SNA for anticancer activity as a monotherapy and in combination with an anti-PD-1 (a-PD-1) antibody in both a-PD-1 sensitive and insensitive mouse tumor models. Mice bearing a-PD-1 sensitive MC38 tumors were treated with intratumoral dosing of TLR9 agonist SNA, intraperitoneal (IP) dosing of a-PD-1 or the combination of both agents when average tumor volume was 100 mm³, with twice weekly dosing for a total of 5 doses. SNA alone showed dose-dependent tumor growth inhibition (TGI) of up to 87% and increased survival of mice. The combination of SNA and a-PD-1 greatly enhanced TGI (92% vs SNA, 74% or a-PD-1, 77%) and mice survival compared with either monotherapy. Moreover, once weekly dosing of SNA showed similar TGI compared with twice weekly dosing. In a-PD-1 insensitive EMT-6 model, mice were treated with intravenous dosing of TLR9 agonist SNA, IP dosing for the combination, starting on day 3 following subcutaneous tumor cell inoculation. SNA alone showed potent dose-dependent TGI of up to 80% and increased mice survival. The combination of SNA and a-PD-1 showed a synergistic TGI (86% vs SNA, 64% or a-PD-1, no effect) and increased mice survival. Further, no tumor growth was observed in surviving mice after subsequent challenge with EMT-6 cells, suggesting development of tumor-specific memory responses in SNA treated animals. These data demonstrate that the TLR9 agonist SNA studied herein induces innate and adaptive immune responses in tumor-bearing mice and shows synergistic TGI with a-PD-1 in both a-PD-1 sensitive and insensitive tumor models. A TLR9 agonist SNA, AST-008, is currently in clinical development.


Background: Human Immune-reconstituted Mice, generated by transplanting pre-validated human CD34+ hematopoietic stem cells (HSC) into immune-compromised mice, are extremely useful in basic and applied human disease research. Many cancer therapies, including immune checkpoint inhibitors such as the anti-PD-1 monoclonal antibody, rely on an intact immune system to release immunosuppression and destroy cancer cells. Material and methods: We evaluated the effects of anti-PD-1 in humanized mice in donor cohorts utilizing the cancer matrix of 5 HSC donor cohorts of humanized animals with PDX models from 7 small cell lung cancer (SCLC). Here, we tested anti-PD-1 in SCLC and in a triple negative breast cancer (TNBC) model and in 1 MDA-MB-231 study. Results: Here, we show that HSC derived humanized immune-reconstituted mice have significant effects on patient derived xenograft (PDX) tumor growth. However, humanization is required for anti-tumor response to anti-PD-1. In MDA-MB-231 study, tumors showed complete regression after the first round of treatment. Re-engraftment of same animals with MDA-MB-231 cells showed minor tumor growth before complete regression again. Aged match control naive animals engrafted with same cells showed normal tumor growth. In SCLC studies anti-PD-1 efficacy varied between the models, which clearly underscores the inherent donor to donor variability in this humanized model system. Tumors from these studies were then analyzed to determine target engagement of anti-PD-1, tumor infiltrating lymphocytes (TILs) characterization and histology comparing anti-PD-1 responders and non-responders. As expected, in this study, warning that humanization of the animals is necessary to evaluate the efficacy of anti-PD-1 therapy and can elicit an immunity-mediated anti-tumor effect. Conclusions: Here we demonstrate the development of humanized mouse studies with ability to run multi point analysis to determine the outcome of treatment.

#4708 A novel anti-MUC1 CAR T cell drives immunity to pancreatic cancer. Mahboubeh Yazdanifar, Ru Zhou, Shu-ta Wu, Priyanka Grover, Pinku Mukherjee. *Univ. of North Carolina at Charlotte, Charlotte, NC.*

Background: Pancreatic cancer is the 4th leading cause of cancer deaths in the US with very poor prognosis. Treatment options are limited to surgery and chemoradiotherapy that often times do not increase survival and are associated with high toxicity. Targeted immune-based therapies have shown some promise but needs further exploration. Mucin 1 (MUC1), a glycoprotein expressed on the apical surface of epithelial cells of most epithelial organs, undergoes hypoglycosylation in tumors. This tumor-form of MUC1 (tMUC1) is over-expressed in 80% of pancreatic ductal adenocarcinomas (PDAC). tMUC1 therefore remains a promising target for therapeutic intervention. We have developed a novel technology to specifically detect tMUC1 and spare normal MUC1. Using a novel technology, functional fragments of TAB004 antibody (scFv) were incorporated into the chimeric antigen receptor (CAR) construct and used to genetically modify primary human T cells. scFv domain which recognizes tMUC1 is linked to the co-stimulatory molecules of T cells (CD28 and CD3ζ). When the engineered T cells contact with tMUC1 expressing tumor cells, multiple T cell signaling pathways are initiated leading to fully activated cytotoxic T cells that lyse the tumor cells. Methods: Retroviral based technique was used to deliver the CAR gene into human PBMC derived primary T cells. A fluorescent tag (mKate) was fused to the C-terminus of CAR molecules, in order to visualize CAR expression on T cell membrane by fluorescent microscopy and potential for in vivo tracking. Cytotoxicity was evaluated using co-culture method with varying T cell to target cell ratios followed by MTT assay. Intracellular IFNγ was measured by flow cytometry. Results: tMUC1-CAR T cells show increased activation and proliferation compared to normal T cells. These cells bind strongly to tMUC1 expressing human pancreatic cancer cells forming immunologic synapse. Minimal binding of the tMUC1-CAR T cells was observed to normal or low MUC1 expressing tumors cells suggesting high specificity of these CAR T cells to tMUC1. CAR expression was distributed evenly on the cell surface of the T cells. Engineered tMUC1-CAR T cells exhibit robust cytotoxicity against a panel of PDA cell lines, associated with high IFNγ release. Fortunately, the same CAR T cells display minimum toxicity against normal epithelial cells. CAR T cell function will be evaluated in the preclinical mouse model of PDA, as single treatment and also in combination with checkpoint inhibitors and chemotherapy drugs. Conclusion: Despite the remarkable successes reported using CAR T cells in clinic, particularly CD19 CAR T for leukemia; some adverse effects have been attributed to this treatment. This highlights the urgent need for developing tumour-specific CAR T cells. This study demonstrates the specificity and effectiveness of tMUC1-CAR T cells against pancreatic cancer cells. Thus, tMUC1 CAR T cells have the potential to be further developed for future clinical use.

#4709 Induction of enhanced tumor-specific immunity by Hsp90 targeted photodynamic therapy (Hsp90-PDT) combined with immune checkpoint inhibition. Kensuke Keneko, Takyua Osada, Timothy A. Haystead, Michael A. Morse, Herbert K. Lyerly. *Duke University, Durham, NC.*

Background: Immunotherapy has become an emerging anti-cancer therapy, and checkpoint blockade with PD-1 or PD-L1 inhibition have been active against multiple cancer types. Nonetheless, there remain many non-responders, suggesting that combinations may improve activity, particularly combinations which can target tumors and have low toxicity. Methods & Results: We have developed a novel Hsp90 targeted photodynamic therapy (Hsp90-PDT), which causes local anti-tumor responses. Hsp90-PDT can be combined with immune checkpoint blockade to generate potent anti-tumor effects. We synthesized well-characterized PDT agent, verteporfin (VP) tethered to a Hsp90 small molecule inhibitor, to create a Hsp90-PDT agent (Hsp90i-VP). We first compared the uptake of VP and Hsp90i-VP into the breast cancer cell lines (E0771-OVA) in vitro. Hsp90i-VP showed stronger incorporation than VP when the compounds were added 3µM or less in concentration. We next performed in vitro and vivo PDT with Hsp90i-VP and laser exposure (690 nm wavelength). Killing efficiency of VP and Hsp90i-VP was analyzed by MTT assay, which showed increased killing in a dose dependent manner for both photosensitizers (0-30µM) and laser (0-120J/cm²). Next, we treated E0771-OVA tumor-bearing C57BL/6 mice with Hsp90i-VP (25nmol/mouse) administration alone, laser irradiation (240 J/cm²), alone, or the combination of Hsp90i-VP and laser. Suppression of the neared cells was confirmed only in Mip95i-VP and laser group. In addition, induction of tumor-specific immune response by PDT was confirmed by ELISA, where the elevation of anti-OVA antibody was seen only in PDT group. Furthermore, combination treatment of Hsp90-PDT and anti-PD-L1 mAbs was able to enhance cytotoxic T-cell response and suppress tumor growth significantly even with relatively PD-T resistant cell line. Conclusions: Hsp90-PDT showed a significant ability to induce antigen-specific immune response in both in vitro and in vivo. These results suggest that Hsp90-PDT can play an important role in anti-cancer immune therapy. Our future plan is to investigate the effect of local photodynamic therapy combined with various types of checkpoint inhibitor in breast cancer models.
Tumor-infiltrating immune cells in gastrointestinal stromal tumors (GIST) related to the response to tyrosine kinase inhibitor therapy. Peter Hohenberger,1 Weney Zhao,2 Maria Deligianni,1 Katia Simon-Keller,1 Alina Diel,4 Andrea Homburger,1 Hui Cao,6 Alexander Marx,6 Division of Surgical Oncology & Thoracic Surgery, Mannheim, Germany; Department of Gastrointestinal Surgery, University Hospital, Mannheim, Germany; Department of Pathology, University Hospital, Mannheim, Germany.

Purpose: The prognostic predictors for neoadjuvant and palliative tyrosine kinase inhibitor (TKI) effect in GIST patients are few beyond mutational status. We analyzed the extent and composition of tumor-infiltrating immune cells in GIST after different TKI therapeutic regimens and response. Methods: From 60 GIST patients, surgical specimens were available and divided into six different groups (in each, with the primary tumors graded for malignant behavior and depicting the development of a memory response against the tumor antigen. For comparison, the novel combination of MM-310, an EphA2-targeted docetaxel ADN with an anti-PD-1 antibody, is highly active in syngeneic tumor models, and represents a promising strategy for the treatment of cancer.


The effectiveness of PD-1/PD-L1 antagonists can be limited by the immunogenicity of the tumor microenvironment. Studies showed that some chemotherapeutic agents including taxanes and anthracyclines can increase immunogenicity, resulting in therapeutic synergy with immune checkpoint inhibitors. In particular, treatment with a taxane has been shown to increase the recruitment of CTLs and decrease immunosuppressive cells such as M2 macrophages and Tregs. Additionaly, the immune-modulatory activity of paclitaxel has been shown to increase with prolonged exposure of the taxane at the tumor level, achieving through metronomic dosing. MM-310 is an Ephrin Receptor A2 (EphA2)-targeted antibody-directed nanotherapeutic (ADN) that encapsulates a docetaxel produrg. Preclinically, MM-310 leads to prolonged exposure of docetaxel at the tumor level, while lowering systemic exposure to bioavailable docetaxel, and thus decreasing not only dose-limiting neutropenia but also the killing of circulating lymphocytes potentially critical to anti-PD-1/PD-L1 activity relative to free docetaxel. Taken together, we hypothesize that MM-310 can synergistically combine with anti-PD-1 therapy. In this study, we evaluated the potential combination of MM-310 and a murine anti-PD-1 Ab in the treatment of several syngeneic mouse tumor models. The tumor lines EMT-6, CT-26, and C26 were used to provide a range of sensitivity to both docetaxel and anti-PD-1. In vivo activity studies and immune-phenotype studies were performed comparing MM-310 + anti-PD-1 combination to the monotherapies. MM-310 administration was initiated two days prior to anti-PD-1 therapy and consisted of four weekly doses, while anti-PD-1 was dosed twice weekly for four weeks. The response to MM-310 or anti-PD-1 as monotherapies varied between the models. In mouse models, radiation treatment has been shown to increase the level of tumor antigen presentation and the variety of peptides available for cross-presentation. Current work in the field focuses on using radiation as a tool to bridge the gap from tumor equilibrium to tumor elimination, which could enhance the ability of preclinical models to provide a first line assessment for advanced immunology therapy combinations.

Deubiquitination and stabilization of programmed cell death ligand 1 by CSN5. Seung-Oe Lim, Chia-Wei Li, Mien-Chie Hung. UT MD Anderson Cancer Center, Houston, TX.

Chronic inflammation in cancer is often associated with disease aggressiveness. Pro-inflammatory cytokines produced in the tumor microenvironment lead to eradication of antitumor immunity and enhanced tumor cell survival. In the current study, we identified TNFα as a major factor triggering cancer cell immunosuppression against T cell surveillance via stabilization of programmed cell death-ligand 1 (PD-L1). We demonstrated that COP9 signalosome 5 (CSN5), induced by NFκB p65, is required for TNFα-mediated PD-L1 stabilization in cancer cells. CSN5 inhibits the ubiquitination and degradation of PD-L1. The role of CSN5 in modulating PD-L1 stabilization prompted us to use CSN5 inhibitor, curcumin, to show that it could destabilize PD-L1 and reduce TNFα-mediated TNFα-mediated PD-L1 stabilization and enhanced anti-tumor immunity. Preclinical data demonstrated inhibition of CSN5 sensitized cancer cells to anti-CTLA4 therapy, suggesting CSN5 inhibitor may be a useful adjuvant to enhance immune-based therapies.
**CLINICAL RESEARCH: Immunomodulatory Agents and Therapeutics**

**#4714** Blockade of LAG-3 amplifies immune activation signatures and augments curative antitumor responses to anti-PD-1 therapy in immune competent mouse models of cancer. Brian B. Haines,1 Sarah Javid,1 Long Cui,1 Heather Hirsch,1 Sas0 Cemerski,1 Terri McClanahan,2 Manjiri Sathe,2 Shuli Zhang,2 Michael Rosenzweig,1 Brian Long,1 Rene de Waal Maeyer2,3. 3Merck Research Laboratories, Boston, MA;4Merck Research Laboratories, Palo Alto, CA.

MK-4280 is a humanized IgG4 monoclonal antibody (mAb) that binds to the immune checkpoint receptor Lymphotoxycyte Activation Gene-3 (LAG-3) to block the interaction with its ligand, Major Histocompatibility Complex (MHC) Class II. LAG-3 is frequently co-expressed with other immune checkpoint receptors, most notably programmed cell death receptor-1 (PD-1), on T cells with an exhausted phenotype. LAG-3 and PD-1 cooperate to regulate peripheral immune tolerance in healthy individuals, and, conversely, play critical roles in several diseases, including autoimmunity, graft rejection, viral infections, and cancer. Co-blockade of LAG-3 and PD-1 in immunocompetent mouse tumor models have demonstrated augmented anti-tumor activity over single agents. However, the molecular mechanisms behind these combination effects have not been fully investigated. Here, preclinical proof-of-biology studies are presented for co-targeting LAG-3 and PD-1 in cancer. c28G10-mG1-[D265A] (abbreviated 28G10-mG1) is a rat/mouse chimera that mimics MK-4280 by its ability to directly block the mouse LAG-3/MHC Class II interaction without initiating Fc-mediated effector functions. As a single agent, 28G10-mG1 demonstrated modest anti-tumor activity across several syngeneic mouse tumor models despite evidence of systemic drug exposure and target engagement (as assessed by sLAG-3-mAb complex accumulation). The combination of 28G10-mG1 and the anti-mouse PD-1 blocking antibody mDX400 resulted in greater tumor growth inhibition and increased numbers of complete responses (CR) over mDX400 alone in the MBT-2 tumor model. Furthermore, animals that had achieved CR to combination therapy were subsequently protected from MBT-2 rechallenge, suggesting the establishment of immune memory. RT-qPCR analyses revealed up-regulation of immune-related genes, primarily at Day 4 in the blood and Day 8 in the tumor with mDX400, but not 28G10-mG1, treatment. However, when combined with mDX400, 28G10-mG1 further altered the expression of many immune-related genes that were perturbed by mDX400 single agent therapy. Genes unique to combination treatment were also observed. Significantly, immune pathway signatures associated with clinical efficacy to Keytruda were upregulated with combination therapy. Tumor transcriptome and network analysis by RNAseq revealed enrichment in several immune- and cytokine-related pathways with combination therapy compared to mDX400 single agent therapy. Taken together, these preclinical oncology studies support the concept of co-targeting LAG-3 to increase the therapeutic efficacy of PD-1 blockade. Clinical investigation of MK-4280 in combination with anti-PD-1 therapy (pembrolizumab/Keytruda) is ongoing.

**CLINICAL RESEARCH: Prognostic Biomarkers 1**

**#4715** Protective anti-tumor immune responses in breast cancer depend on tumor mutation rate. Lance D. Miller, Eric D. Routh, Jeff W. Chou, Alexandra Thomas. Wake Forest School of Medicine, Winston Salem, NC.

The relationship between tumor antigenicity and anti-tumor immune responses in breast cancer are poorly understood. Metrics that can accurately predict tumor immunogenic potential could guide therapeutic decisions for breast cancer patients. We examined the relationship between tumor immune subclasses and patient overall survival in the context of the rate of nonsynonymous mutations in breast cancers. These analyses were conducted using RNAseq and exome sequencing data from the Cancer Genome Atlas (TCGA) breast cancer cohort (n = 1,098 patients). A previously described tumor-immune classification system, based on the relative abundance of infiltrating effector immune cells, was employed for assigning patients to favorable, weak or poor immunogenic dispositions (FID, WID or PID, respectively). In breast tumors exhibiting low mutation rates (LMR), no significant survival differences were observed among patients comprising the immune subclasses. By contrast, in the context of tumors with high mutation rates (HMR), the immune subclasses exhibited highly significant survival differences (p < 0.001). Patients with FID tumors achieved 100% survival at 10 years as compared to those with PID tumors who displayed a 10-year survival rate of 44%. These survival differences were independent of molecular subtype composition, with the majority of the excellent-outcome FID subclass comprising predominantly of Basal-like, HER2-enriched and Luminal B breast tumors. In the smaller subset of HMR Luminal A breast tumors, the immune subclasses also exhibited significantly different survival outcomes (p = 0.01), with patients of the poor-outcome Luminal A PID subclass experiencing a 10-year survival rate of 22%. By Cox proportional hazards regression analysis, the immune subclasses contributed significant additive prognostic information in the presence of AJCC stage (p = 0.003). A rate of 1 mutation per million of DNA was found to be an optimal threshold for distinguishing immune-relevant low and high mutational load. These findings fit a model where mutational burden, reflective of tumor antigenicity, is a major determinant of clinically-relevant breast tumor immunogenicity. This relationship between immunogenic tumor subclasses, overall survival and mutational burden may offer opportunities for therapeutic stratification of breast cancer patients.


The diagnosis of colorectal cancer (CRC) is based on tumor-node-metastasis staging, a weak staging system due to different genetic and epigenetic backgrounds. Biological markers improve early detection and guide clinicians in subsequent therapies. Stemness molecules have the potential to identify patients at high risk of developing aggressive cancers. Herein, Zinc finger E-box binding homeobox 1 (ZEB1), Trefoil factor 3 (TFF3), Hepatoma Up-Regulate Protein (HURP), Mucin 2 (MUC2), and Cystic fibrosis transmembrane regulator (CFTR) were used as potential prognostic biomarkers in CRC. There was a total of (N = 56) cases included in this study which were assessed by a pathologist, out of them 32 were African Americans (AA) and 24 Caucasian Americans (CA). The median follow-up for 42 surviving patients were noted to be 4.5 years (range 2.5 - 7.5 years). A tissue tumor microarray (TMA) was created using tumor stage-matched CRC tissues from AA and CA. The TMA was stained with each biomarker using immunohistochemistry (IHC) and consisted of 132 cores including both controls and tumors. The individual staining score ranged from (0-3) for both area and intensity. The product of area and intensity was used as the final score which ranged from (0-9) with 0 defining no expression and 9 as high expression. We evaluated the association for expression of all the five biomarkers with disease-free survival (DFS) in AA and CA separately. Stratification of the cohort by race revealed that in CA, high ZEB1 expression (nuclear and cytoplasmic) was associated with poor DFS (p = 0.015 for cytoplasm, p = 0.116 for nucleus). ZEB1 expression was not found to be a significant predictor of DFS in AA who are usually known to have worse CRC outcomes. These findings suggest that expression of ZEB1 may represent a marker for CRC prognosis, particularly in CA. High nuclear TFF3 expression in AA was found to be marginally associated with poorer DFS (p = 0.089).

The cytoplasmic expression of TFF3 was not significantly associated with DFS. Both the cytoplasmic and nuclear expression of HURP, MUC2, and CFTR among the AA and CA did not significantly correlate with DFS. Overall, we have identified two stemness molecules, ZEB1 and TFF3, with potential as markers for aggressiveness in CRC. Our findings also provide evidence of a possible biological basis for ethnic differential differences with that of the nuclear expression scores, which are two independent prognostic factors of CRC aggressiveness. ZEB1 can contribute to be a novel biomarker to predict the prognosis of CRC in CA, and TFF3 will be a prognostic marker for AA. Future goals include collecting additional samples and follow-up of the patients to accurately estimate the prognosis. In addition, we will expand the project to the cellular and molecular basis underlying our findings. Funding sources: PCRI W81XWH-14-1-0151, UMMC Medical Student Research Program, and UMMC Office of Research.

**#4717** Combination of PD-L1 expression and microsatellite instability status is a useful prognostic factor in gastric cancer. Toshiaki Morihiro, Shinji Kuroda, Nobuhiko Kanaya, Hiroshi Tazawa, Shinsuke Kagawa, Toshiyoshi Fujiiwara. Okayama University, Okayama, Japan.

Interaction of programmed death-1 (PD-1) and programmed death-ligand 1 (PD-L1) induces functional impairment of antigen-specific T cells, which leads to immune evasion of tumors. Immune checkpoint therapy including PD-1/PD-L1 blockade is an emerging treatment strategy which brings great improvement in patient's prognosis. Microsatellite instability (MSI) is a condition of genomic instability caused by the loss of DNA mismatch repair activity, which has recently received a lot of attention from the standpoint of cancer immunotherapy. While the TNM classification system is commonly used on various malignant tumors for prediction of prognosis and planning of treatment strategy, establishment of prognostic factors related to cancer immune system is considered important as cancer immunotherapy has recently become more and more popular. Thus, we investigated the
usefulness of PD-L1 expression and MSI status in addition to histological type as prognostic factors in gastric cancer. In this study, 255 gastric cancer cases which had curative surgical resection at Okayama University Hospital between 2002 and 2009 were analyzed retrospectively. PD-L1 expression level was classified into 4 groups according to PD-L1 positive rate on tumors on immunohistochemical staining (0: <1%, 1+: 1-5%, 2+: 5-10%, 3+: ≥10%), and 2+ and 3+ were defined as PD-L1 positive. MSI status was analyzed by PCR and classified into MSI-high (MSI-H), and MSI-low and Microsatellite instability (non-MSI). Histological type was divided into intestinal and diffuse according to the Lauren classification. PD-L1 positive rate was 15%, and PD-L1 positive was significantly correlated with advanced stage and invasive status. Overall survival (p = 0.0109) and disease-free survival (p = 0.0052) were significantly poor in PD-L1 positive group, and a multivariate analysis revealed that PD-L1 expression was an independent risk factor for recurrence after surgery (p = 0.0160, odds ratio: 3.19). MSI-H was detected in 7.5%, and more observed in PD-L1 positive cases (p = 0.0068). Although non-MSI, as a single factor, showed only a weak tendency to have poor prognosis compared to MSI-H (p = 0.1965), combination of PD-L1 positive and non-MSI (PD-L1/non-MSI) showed significantly poor prognosis (p = 0.0010). Although other combinations also showed the usefulness in detection of patients with poor prognosis, such as PD-L1 positive and diffuse type, and non-MSI and diffuse type, a multivariate analysis revealed that PD-L1/non-MSI was the most useful combination among them to predict poor prognosis (p = 0.0009, hazard ratio: 3.88). In conclusion, while PD-L1 expression is a useful prognostic factor and predictive factor for recurrence after surgery as a single factor, combination with MSI status can be more useful and attractive prognostic factor in gastric cancer.

**#4718 Filamin A interacting protein 1-like is a marker of progression, chemosensitivity and chemoresistance in ovarian cancer.** Mijung Kwon,1 Jae-Hoon Kim,2 Chel Hun Choi,3 Joon-Yong Chung,4 Stephen Hewitt,4 Stephen Libutti.1

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Purpose: The WNT/b-catenin pathway and the resulting epithelial-to-mesenchymal transition (EMT) play a key role in ovarian cancer progression and chemoresistance. Filamin A interacting protein 1-like (FILIP1L) enhances b-catenin degradation, thereby inhibiting canonical WNT signaling and EMT. FILIP1L is a tumor suppressor whose expression is down-regulated by promoter hyper-methylation in ovarian cancer. FILIP1L down-regulation is inversely correlated with the invasive potential of ovarian cancer cells. However, the clinical relevance of FILIP1L down-regulation in ovarian cancer progression has yet to be addressed. Experimental design: To study the clinical implications of FILIP1L in regulating the WNT/b-catenin pathway and chemoresistance, the expression of FILIP1L, b-catenin, SNAIL and SLUG was analyzed by immunohistochemistry on tissue microarrays of 369 ovarian samples ranging from normal to metastatic. Their expression was evaluated with clinical-pathological characteristics including FIGO stage and chemoresistance, and correlated with overall and disease-free survival by Kaplan-Meier plots and Cox proportional hazards model. Results: We demonstrated that FILIP1L expression decreased with tumor progression, resulting in a significant difference between primary and metastatic samples. In contrast, b-catenin expression was inversely increased with tumor progression. Furthermore, tumors that were resistant to platinum/paclitaxel combination therapy showed a significant reduction in FILIP1L expression when compared to sensitive tumors, contrary to SLUG expression, which was significantly higher. The expression of FILIP1L was negatively correlated with the expression of b-catenin and SLUG, whereas b-catenin expression was positively correlated with SLUG expression, suggesting a link between FILIP1L and the WNT/b-catenin pathway in ovarian cancer. Moreover, patients with low FILIP1L expression showed a median overall survival and disease-free survival of 60 and 19 months, respectively, whereas patients with high FILIP1L expression had not yet reached median overall and disease-free survival at their 120 month follow-up. Notably, low FILIP1L expression was an independent negative prognostic factor with respect to overall survival and disease-free survival. Conclusions: Our study provides the first clinical relevance of FILIP1L in human cancer, and suggests that FILIP1L may be a novel biomarker for good prognosis and lower probability of recurrence in ovarian cancer patients.

**#4719 MYC expression correlates with PD-L1 expression and related poor clinical outcome in non-small cell lung cancer.** Eun Young Kim, Arum Kim, Se Kyu Oh Kim, Yoon Soo Chang. Yonsei Univ. Coll. of Medicine, Seoul, Republic of Korea

Background: Programmed death-ligand 1 (PD-L1) is widely used biomarker for the response prediction of immune checkpoint inhibitors, but its usefulness has been challenged. MYC, a transcriptional factor which is overexpressed in various cancers, plays critical roles in preventing immune cells from attacking tumor cells by inducing PD-L1 expression. We evaluated that MYC expression is positively related that of PD-L1 in non-small cell lung cancer (NSCLC) clinical specimens and that it has potential as a predictor of response to immune checkpoint inhibitors in NSCLC. Methods: Eighty-four cases which were diagnosed as adenocarcinoma under WHO classification and with immunohistochemistry (IHC), PD-L1 expression was evaluated by FDA-approved PD-L1, 22C3 PharmDXTM protocol using the Dako Automated Link 48 platform at Clarient Diagnostic Services, Inc (Aliso Viejo, CA, USA). Its expression was determined by using Tumor Proportion Score (TPS), which is the percentage of viable tumor cells showing partial or complete membrane staining. If TPS ≥ 50% of the membrane staining, it was considered positive. IHC of MYC was performed using anti-c-MYC antibody [Y69] (ab32072, Abcam, USA) and its expression was evaluated by scoring system using product of nuclear staining intensity and percentage of positive cells. Results: When MYC cDNA was transfected into KRAS-dependent lung cancer cell lines that did not express both MYC and PD-L1, the protein expression and mRNA of PD-L1 were induced in a dose-dependent manner. On the other hands, knockdown of MYC with siRNA inhibited protein expression and mRNA indicating MYC regulates PD-L1 at transcriptional level. Of 84 NSCLC tumor tissues, PD-L1 was expressed in 14 (16.7%) and MYC in 30 (35.7%) cases. Dual positive patients were 9 (10.7%) and dual negative patients were 49 (58.3%). When the relationship between PD-L1 and MYC expression was evaluated, patients with PD-L1 positive only showed poor prognosis (p = 0.0267, P = 0.014). Dual positive patients showed significantly shorter disease free survival (16.1 vs. 62.5 months, P = 0.004) and overall survival (24.9 vs. 70.9 months, P = 0.002) than dual negative patients. Conclusion: MYC expression correlates with PD-L1 expression in the NSCLC and the patients with overexpression of both MYC and PD-L1 showed significantly poor survival. This suggest that it is worth of investigation of MYC expression as a surrogate predictive marker for treatment of immune checkpoint inhibitors in NSCLC.

**#4720 Novel findings for the clinical significance of RNA editing status of AZIN1 and ADAR1 and 2 expression levels in gastric cancer patients.** Yoshi-naga Okugawa,1 Yui Toyiama,1 Kunitoshi Shigeyasu,2 Takashi Ichikawa,2 Satoshi Oki,2 Koichiro Morii,2 Yuka Nagano,2 Hiromi Yasuda,2 Shigeyuki Yoshiyama,3 Masaki Ohi,4 Koji Tanaka,4 Yasuhiro Inoue,5 Yoshitomi Araki,4 Yasuhiro Mohri,4 Motoyoshi Tanaka,4 Chikao Miki,4 Ayaj Goel,5 Masato Kusunoki,5 Baylor Univ. Medical Ctr., Dallas, TX, 2 Okayama University, Okayama, Japan; 3 Mie University, Tsu, Mie, Japan; 4 Iga City General Hospital, Iga, Mie, Japan

Background. Despite recent advances in surgical techniques and treatment options, gastric cancer (GC) remains the third most common cause of cancer-related deaths worldwide. Besides DNA sequence mutations, epigenetic alterations have emerged as significant drivers of molecularly defined subgroups. A-to-I RNA editing is a post-transcriptional modification that converts adenosines to inosines in both coding and noncoding RNA transcripts, and has recently been recognized has a novel epigenetic mechanism in GC pathogenesis. More specifically, A-to-I editing of AZIN1 transcripts was shown to be regulated by an adenosine deaminase acting on RNA (A-DAR1), and edited AZIN1 resulted in an aggresive phenotype during disease progression in some human cancers. The aim of this study was to clarify the clinical consequences of RNA editing status of AZIN1 and the RNA editing enzymes (ADAR1 and 2) in GC patients. Methods. Two hundred eighty-eight gastric specimens from one hundred forty-four patients who underwent surgery for GC were evaluated. We analyzed the RNA editing status of AZIN1 by RNA editing site-specific quantitative PCR (RdSSPCR). REdSSPCR allows quantitation of RNA editing levels using wild-type or edited AZIN1-specific primers, and RNA editing levels are calculated by determining the ratios of Ct values between edited vs. wild-type transcript expression levels. Furthermore, expression levels of ADAR1 and ADAR2 were evaluated by qPCR in GC tissues. Results. We observed a higher frequency of the AZIN1 RNA editing in tumors compared with normal mucosa in GC. RNA editing status of AZIN1 was significantly correlated with advanced T stage and presence of lymph node metastasis in GC patients. Multivariate analysis revealed that high frequency of RNA editing in the AZIN1 gene was an independent prognostic factor for poor disease free survival and overall survival. Furthermore, significant upregulation of ADAR1 and downregulation of ADAR2 was also observed in GC tissues compared with matched normal mucosa, and these alterations also significantly correlated with disease progression factors and poor prognosis in GC patients. Interestingly, ADAR1 expression level was positively correlated with RNA editing status of AZIN1 in GC tissues. Conclusions. Our findings revealed that altered gene-specific A-to-I editing...
#4721 Novel lncRNA SLINKY is a prognostic biomarker in kidney cancer.

Xue Gong,1 Zurab Siprashvili,1 Ohyaz Emirgina,1 Zhouwei Shen,2 Yusuke Sato,2 Haruki Kume,2 Yukio Homma,2 Seishi Ogawa,2 Paul A. Khavari,1 Jona-
than R. Pollack,1 James D. Brooks2.1 Tokyo cohort (P RNA-seq data on 466 ccRCC cases randomized into discovery and validation, and prognostic biomarkers are needed to stratify patients for appropriate management. We sought to determine whether long intergenic non-coding RNAs (lincRNAs) might predict patient survival. Candidate prognostic lincRNAs were identified by mining The Cancer Genome Atlas (TCGA) transcriptome (RNA-seq) data on 466 ccRCC cases (randomized into discovery and validation sets) annotated for ~21,000 lincRNAs. A previously uncharacterized lincRNA, SLINKY (Survival-predictive lincRNA in Kidney cancer), was the top-ranked prognostic lincRNA, and validated in an independent University of Tokyo cohort (P = 0.004). In multivariable analysis, SLINKY expression predicted overall survival independent of tumor stage and grade [TCGA HR = 3.4 (CI, 2.1-5.4), P < 0.001; Tokyo HR = 9.2 (CI, 2.2-43), P = 0.003], and by decision tree, ROC and decision curve analysis, added independent prognostic value. In ccRCC cell lines, SLINKY knockdown reduced cancer cell proliferation (with cell cycle arrest) and induced transcriptional changes established for cell proliferation and survival processes. Notably, the genes affected by SLINKY knockdown in cell lines were themselves prognostic and correlated with SLINKY expression in the ccRCC patient samples. From a screen for binding partners, we identified direct binding of SLINKY to Heterogeneous Nuclear Ribonucleoprotein K (HNRNPK), whose knockdown recapitulated SLINKY knockdown phenotypes. Thus, SLINKY is a robust prognostic biomarker in ccRCC, where it functions possibly together with HNRNPK in cancer cell proliferation.

#4722 Evaluation of the prognostic value of ATG4B expression in different breast cancer subtypes.

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Introduction: Autophagy, a lysosome-mediated degradation and recycling process, functions as an adaptive survival response during various stressful conditions including hypoxia and chemotherapy. The cytosine protease ATG4B, an important component of the autophagy pathway, is becoming a promising therapeutic target, but its role as a prognostic marker in breast cancer has not been investigated yet. Our recent studies established a novel association between ATG4B and HER2 positive breast cancer. Objective: The aim of this study was to investigate the prognostic value of ATG4B in different breast cancer subtypes using a large population-based cohort. Methods/Experimental Design: We evaluated ATG4B cytoplasmic expression by immunohistochemistry on tissue microarrays (TMAs) from a cohort of 1600 breast cancer cases from British Columbia Cancer Agency. For this large, well-characterized cohort detailed clinical information was available, including age, histology, tumor grade, tumor size, lymph node status, type of local and adjuvant systemic therapy, and dates of first recurrence and death. Median follow-up time was 12.4 years. ATG4B expression was scored by two independent observers using a categorized H-score system. Survival analyses were performed using the Kaplan-Meier function and Cox proportional hazards regression models to evaluate the association of ATG4B expression with breast cancer-specific survival, stratified by intrinsic subtype. Results: ATG4B expression was significantly lower in basallike vs. non-basal (P < 0.001), basal vs. HER2 overexpressed (P = 0.0029), and triple-negative vs. non-triple-negative (P = 0.0001) breast cancer subtypes. In HER2+ breast cancers, high (H-score > 200) ATG4B expression was significantly associated with poor overall survival (hazard ratio (HR) = 1.90, 95% confidence interval (CI) = 1.10 to 3.27, P = 0.033), disease specific survival (HR = 2.23, CI = 1.23 to 4.04, P = 0.016), and relapse-free survival (HR = 1.92, CI = 1.09 to 3.39, P = 0.037). However, in Luminal A breast cancers, high (H-score > 150) ATG4B expression was strongly associated with improved overall survival (HR = 0.71, CI = 0.55 to 0.93, P = 0.012) and disease-specific survival (HR = 0.43, CI = 0.26 to 0.67, P = 0.0009). Conclusion: High ATG4B expression is a poor prognostic marker in HER2 positive breast cancer, but a favorable prognostic factor in the Luminal A subtype. Validation analyses are planned on a further set of 1989 cases.

#4723 Improving prognosis or promoting progression? Double sword of S100A10 in colorectal cancer.

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Tumor tissues are heterogeneous, including cancer cells, cancer stem cells and immune cells. The immune microenvironment is involved in tumorigenesis and progression in colorectal carcinomas (CRC), S100A8, which represented granulocytes and monocytes, was identified as a favorable indicator for survival by previous work. Cancer stem cells (CSCs) and epithelial-mesenchymal transition (EMT) play critical roles on tumor cell malignancy and metastasis. The immune state, CSC and EMT should be regarded as integrity, which is important for clinical efforts and translational investigation. For better understanding the associations among immune cells, CSC and EMT in CRC, S100A8 as well as with CSC (Lgr5, Nanog, ALDH1 and CD44v6) and EMT (Cedarin and Snail) markers were detected in the tumor invasive front (TF) and tumor center (TC) respectively by immunohistochemistry in 419 CRCs and survival analysis was done. Furthermore, functions of S100A8 in EMT were verified in vitro. S100A8+ TF count was positively correlated with Lgr5+ TF (r = 0.162, P = 0.004) and cytoplasmic Snail+ TF expression (r = 0.116, P = 0.043), while it was not correlated with other stem cell markers wherever they were located, and not associated with E-cadherin+ or E-cadherin+ cytoplasmic snail1+ or nuclear Snail2+ and Snail2+ expression (P > 0.05). After combining S100A8+ TF and Lgr5+ TF, three groups were got. It showed S100A8+ TF and Lgr5+ TF had the best 5-year survival, while S100A8+ TF and Lgr5+ TF had the worst 5-year survival. In conclusion, S100A8+ TF and Lgr5+ TF had the best 5-year survival, while S100A8+ TF and Lgr5+ TF had the worst 5-year survival. S100A8 also had some influence on tumor cells not only facilitated tumor cell proliferation, migration and invasion but also converted cells to EMT phenotype. Western blot showed E-cadherin was downregulated and Vimentin, MMP9 and snake were up-regulated in S100A8+ overexpressed cells. The transcription factor USF2 was verified to interact with S100A8 by luciferase reporter gene. Western blot showed overexpression of USF2 increased S100A8 expression, while overexpression of S100A8 had no effect on USF2. In conclusion, S100A8 from immune cells or tumor cells has opposing effectson cancer progression. S100A8 in immune cells or tumor cells may help predict clinical outcomes in CRC patients.
presence of at least one biomarker could discriminate the SIOPEL high risk patients into an intermediate and a very high risk group (P<0.0001), whereas biomarker positive and negative standard risk patients showed no difference in outcome. Conclusions: We have validated the 16-gene signature and NF2EL2 mutations as highly prognostic biomarkers in HB and propose a new stratification system that combines the combination of clinical and biological factors, which might facilitate more tailored and risk-adapted therapies and thus better outcome of high risk patients in the future.

**#4725** Multiple Myeloma DREAM Challenge: a crowd-sourced challenge to improve identification of high-risk patients. Michael Mason,1 Fadi Towfic,2 Brian S. White,1 Dan Rozelle,1 Erin Flynt,3 Anjan Thakurta,4 Thomas Yu, Frank Schimetz,5 Andrew Dervan,6 Doug Bassett,7 Justin Guinney8. 1Sage Bio-networks, Seattle, WA; 2Celgene, Summit, NJ; 3Rancho Biosciences, Boston, MA; 4Celgene, Seattle, WA.

Introduction: Multiple myeloma (MM) is a cancer of the plasma cells in the bone marrow, and its clinical course depends on a complex interplay of traits including age, performance status, as well as genetic and molecular aberrations, and other molecular states of the plasma cells. Since risk-adapted therapy is becoming standard of care, there is an urgent need for a precise risk stratification model to assist in therapeutic decision-making. While progress has been made, there remains a significant opportunity to improve patient stratification to optimize treatment and to develop new therapies for high-risk patients. To accelerate the development and evaluation of such risk models in MM, we formed a DREAM Challenge, a crowd-sourced competition that engages large cross-disciplinary teams of experts to address complex problems in biomedicine. Methods and Data: Clinical variables, patient outcomes, genetic, and gene expression data from over 2100 samples were curated and harmonized from multiple public and private studies. A subset of the data (N=913) was used to evaluate previously published high-risk signatures against progression-free survival (PFS), the clinical endpoint of the challenge. We also developed and assessed de novo and ensemble methods. All models were evaluated using area under the receiver operating characteristic curve (AUC). Results: The international staging system (ISS) for Myeloma was used as a baseline for high-risk classification (PFS < 18mo, AUC = 0.57). We evaluated published high-risk signatures - UAMS-5, UAMS-17, UAMS-70, EMC92 - and observed AUCs of 0.62, 0.62, 0.61, 0.59, respectively. The average correlation of high-risk scores was 0.73 (min: 0.52 (UAMS-5 vs EMC92); max: 0.90 (UAMS-17 vs UAMS-70)), suggesting published classifiers capture non-overlapping determinants of risk. As such, we developed a Random Forest classifier that combined expression of genes utilized in the published classifiers with gender and age, trained on 70% of the data set, and validated on the remaining 30%. This de novo classifier achieved an AUC of 0.66 for PFS based risk classification. Incorporation of ISS leads to a modest improvement of accuracy (AUC=0.67). Conclusion: Preliminary analysis of the Challenge data suggests there is an opportunity to significantly improve risk stratification models in MM. In addition to the robust benchmarking of existing classifiers, we anticipate new, more accurate models will be proposed through a MM Challenge given the scale of the combined data sets. Additionally, we hope to uncover novel clinical and molecular traits that may yield insight into the pathobiology of MMM and development of new MM models. Importantly, this Challenge will illustrate the advantages of leveraging public data and crowd-sourcing to address therapeutically relevant questions in oncology.

**#4726** TonEBP promotes hepatocellular carcinoma and is associated with poor prognosis. Jun Ho Lee,1 Jae Hee Suh,2 Soo Youn Choi,3 Hyun Je Kang,2 Gap Ryol Lee,1 Whaseon Lee-Kwon,4 Neung Hwa Park,5 Hyung Moo Kwon6. 1UNIST, Ulsan, Republic of Korea; 2Ulsan University Hospital, Ulsan, Republic of Korea; 3Sogang University, Seoul, Republic of Korea.

Carcinogenesis is initiated by genetic changes due to aberrant processing of genetic information. Tumor microenvironment, however, should not be overlooked as cancer risk because they influence the development and progression. Indeed, the role of inflammation has received a lot of attention lately. Specifically, hepatocellular carcinoma (HCC) has been considered as an inflammation-associated tumor like colorectal cancer and lung cancer. In addition, high rates of recurrence is the major feature of HCC, and there is a need to identify novel biomarkers for post-operative prognostic of HCC patients. Tonicity-responsive enhancer binding protein (TonEBP), also known as nuclear factor of activated T cell 5 (NFAT5), is a key transcriptional cofactor for the expression of pro-inflammatory genes such as TNF-a, IL-1b, and cyclooxygenase 2 (COX-2). Surprisingly, this gene is hypothesized to be a biomarker of chronic hepatitis. In chronic hepatitis, endoplasmic stress, a hallmark of endoplasmic reticulum stress, is the key factor in regulating the expression of TonEBP. This study was initiated using immuno-histochemical staining for TonEBP in a 160 gastric cancer patient cohort. The expression of TonEBP was found to be significantly higher in tumor tissue than in normal tissue. Interestingly, univariate analyses revealed that TonEBP expression in normal tissue was significantly associated with tumor size, tumor grade, vascular invasion, viral DNA replication, α-fetoprotein, PIVKA-II, recurrence, metastasis, and death. In addition, Kaplan-Meier estimation and multivariate analyses showed that the TonEBP expression was an independent prognostic marker for recurrence and death in HCC patients. In mice, TonEBP hipo- deficient reduced both DEN- and DEN/high-fat diet (HFD)-induced HCC in association with lower COX-2 transcription and milder hepatic inflammation. Acute hepatic inflammation induced by DEN or lipopolysaccharide was also reduced in hepatocyte- or myeloid-specific TonEBP knockout mice. TonEBP-mediated activation of hepatic COX-2 promoter was dependent on the transcription factor Yin Yang 1 (YY1). TonEBP interacted with YY1 through the Rel-homology domain of TonEBP and spacer domain of YY1. In aggregate, our data demonstrate that TonEBP-mediated hepatic inflammation is an important regulator of hepatocarcinogenesis. The miR-223-YY1/TonEBP-COX-2 pathway drives hepatic inflammation and commits the stressed hepatocytes to malignant fate. Thus, TonEBP promotes HCC via COX-2 expression and is a strong post-operative prognosticator of poor outcome in HCC patients.

**#4727** Analysis of PIK3CA mutations and PI3K signaling proteins as prognostic biomarkers for advanced gastric cancer. Satoshi S. Nishizuka,1 Chie Ito,2 Kohei Kume,3 Takeshi Iwaya,4 Keisuke Koeda,2 Akira Sasaki.5 1Iwate Medical University Institute of Biomedical Sciences, Morioka, Japan; 2Iwate Medical University School of Medicine, Morioka, Japan.

Although surgery and chemotherapy has extended the survival of patients with advanced gastric cancer, some patients experience relapse. In cases of potentially curative gastrectomy, no visible tumor mass should be present at the time of post-operative chemotherapy. Therefore, relapse may be attributed to very small cancer cell populations that survive and develop drug resistance. Previously, we found that changes in the phosphatidylinositol 3-phosphate kinase (PI3K) pathway protein levels were responsible for the growth of drug-resistant cancer cells. In the present study, we postulated that the oncogenic mutations in PIK3CA and PI3K pathway proteins could be prognostic markers for patients with advanced gastric cancer. A retrospective cohort of 160 patients with advanced gastric cancer, receiving potentially curative surgery with/without chemotherapy was investigated for PIK3CA mutations and PI3K pathway protein levels in the context of 5-year overall survival (OS) and relapse-free survival (RFS). Mutations were screened by direct sequencing followed by validation through allele-specific quantitative real-time PCR, digital PCR, and ultra-deep sequencing. Tissue microarrays were produced from tumor-rich areas of surgically removed specimens. Protein levels were assessed by incubation with specific primary antibodies against total and phosphorylated proteins including PI3K (p85), AKT, mTOR, and PTEN, followed by colorimetric detection. Samples were deemed to have positive staining when more than 5% of the cancer cells had detectable protein. Statistical analyses showed that PIK3CA hotspot mutations were not directly associated with PI3K pathway activation; and (i) PIK3CA hotspot mutations occurred with low frequency in gastric cancer; (ii) PIK3CA hotspot mutations were not directly associated with PI3K pathway activation; and (iii) p-AKT(+) patients were higher than those of PTEN(-) patients in both surgery-only and chemotherapy groups although statistical significance was not confirmed. PTEN(-) patients showed similar survival curves for up to 2 years post-operation, and separated thereafter. No significant association was observed between PIK3CA mutations and PI3K pathway protein levels. Subgroup analysis demonstrated that both p-AKT(+) and PTEN(-) were better prognostic factors for survival. There was no interaction between the protein levels and any clinicopathological/mutational factors. This study revealed that: (i) PIK3CA hotspot mutations occurred with low frequency in gastric cancer; (ii) PIK3CA hotspot mutations were not directly associated with PI3K pathway activation; and (iii) p-AKT(+) may be a biomarker for better outcomes in gastric cancer patients undergoing gastrectomy.

**#4728** Plasma interleukin-6 level predicts prognosis of patients who received sorafenib for advanced hepatocellular carcinoma. Yu-Yun Shao,1 Hang Lin, Yong-Shi Li, Ying-Hui Lee, Ho-Min Chen, Ann-Li Cheng, Chih-Hung Hsu. National Taiwan University Hospital, Taipei City, Taiwan.
Background: No biomarker has been proven to predict the treatment outcomes of sorafenib, the only approved first-line therapy for advanced hepatocellular carcinoma (HCC). We explored predictive and prognostic values of plasma interleukin (IL)-6 levels for patients who received sorafenib as first-line therapy for advanced HCC. Methods: This study had 2 patient cohorts. The exploration cohort was composed of patients who were randomly selected from the national prospective, single-arm, open-label clinical trial that examined sorafenib with tegafur/uracil as first-line therapy for advanced HCC. The validation cohort consisted of patients who received sorafenib alone as first-line therapy for advanced HCC under reimbursement of National Health Insurance of Taiwan. Pretreatment plasma IL-6 levels were determined. We used the receiver operating characteristic (ROC) curve in the exploration cohort to determine the best cut point for IL-6 levels in predicting outcomes (OS). We then confirmed the cut point in the validation cohort. Results: There were 55 and 73 patients in the exploration and validation cohort, respectively. In the exploration cohort, there was no complete response but 3 (6%) partial response. The median OS was 8.1 months. In the validation cohort, there was no complete response but 7 (10%) partial response. The median OS was 10.3 months. In the exploration cohort, we found 4.28 pg/ml was the best cut point defining high and low IL-6 levels because it could differentiate OS (p = 0.042) with the best sensitivity and specificity. Applying the cut point on the validation cohort, patients with high pretreatment plasma IL-6 levels, compared with patients with low IL-6 levels, exhibited significantly poorer OS (median, 8.0 vs. 13.9 months, p = 0.03). After adjusting for age, gender, hepatitis etiology, tumor characteristics, and pretreatment characteristics, a high plasma IL-6 level remained an independent predictor for poor OS (hazard ratio 2.594, p = 0.005). By contrast, the plasma IL-6 level was not associated with progression-free survival, treatment response, disease control, or other patient characteristics. Conclusion: High pretreatment plasma IL-6 level predicted prognosis of patients who received sorafenib as first-line therapy for advanced HCC.

#4729 Association of mitochondrial DNA copy number variation in peripheral blood leukocytes with risk and prognosis of gallbladder cancer. Abhijit Chandra,1 Hasan R. Kazmi,2 Leena K. Satyam,3 Saket Kumar1.

Gallbladder Cancer (GBC) is the most common malignancy of the biliary tract with very high incidence in Northern India, especially around Gangetic belt. Late diagnosis and poor prognosis are the important features of this disease. For improving treatment strategies and outcomes, novel non-invasive prognostic biomarkers are needed. Variations in peripheral blood mitochondrial DNA (mtDNA) copy number in various cancers are widely studied but information in relation to gallbladder malignancy is unknown. In this pilot study, we investigated leukocytes mtDNA copy number variation recently observed in a phase 2 survival (OS) study. A total of 300 histologically confirmed or fine needle aspiration cytology proven GBC patients were recruited along with equal number of age and sex matched controls. The mtDNA copy number was measured by real-time polymerase chain reaction through absolute quantification using SYBR green chemistry. We also explore prognostic utility of mtDNA content in relation to overall survival (OS) and disease free survival (DFS) in patients. Statistical analysis was done by Student’s t-test and survival data was compared using Kaplan Meier method. Two tailed p value less than 0.05 was considered to be significant. We observed high mtDNA content in cases as compared with controls (mean± standard deviation, 1.21±0.88 vs. 1.02±0.76). Higher mtDNA copy number was significantly associated with increased gallbladder cancer risk (p=0.005). In this study, we compared overall survival (OS) for gallbladder cancer patients in relation to mtDNA copy number median value (median, 1.12). Kaplan Meier survival curve predicts decrease in OS (p=0.03, Hazard ratio=2.54, 95% CI = 1.79-5.86) for GBC patients in relation to high mtDNA content (>median) as compared with low mtDNA copy number (<median). This study supports an association between increased mtDNA copy number with susceptibility to gallbladder cancer in this high incidence belt. Future studies are warranted to confirm these findings and to elucidate the biological role of mtDNA copy number in relation to gallbladder cancer.

#4730 Fluorescence in situ hybridization panels for prediction of disease progression in prostate cancer patients on active surveillance. Adam J. Koch,1 Ying Zhang,2 Beth Blondin,3 Svetlana Sitailo,1 Huixin Fei,1 Stephen Van Den Eeden,3 Ekaterina Pestova1.

Background: Overdiagnosis and overtreatment of men with lower-risk Prostate Cancer (PCA) is a concern due to co-morbidities and healthcare costs. Maximizing patient quality of life while recognizing early signs of aggressive disease is essential. There is a need to stratify patients according to the risk of disease progression, identifying with high specificity and sensitivity patients to biopsy, re-biopsy and monitor. Within the high risk population, there is a need to identify when to proceed with treatment. Methods: We performed a retrospective study to evaluate FISH biomarkers on PCA specimens with histologically confirmed, clinically localized disease (T1c-T2c) enrolled in Active Surveillance (AS). AS patients were classified into two categories: Progressive (clinical intervention within 10 years), and non-progressive (did not progress to intervention in 10 years). Cohorts were matched by clinical characteristics (Gleason score, disease stage and grade, PSA level, age, race) as possible. The study objective was to establish a FISH biomarker panel to differentiate non-progressive from progressive prostate cancer in the low and intermediate risk groups. Receiver Operating Characteristic (ROC) curve analysis was performed to obtain the best single parameters based upon Area Under the Curve (AUC). For each FISH parameter cutoffs were determined based on Sensitivity, Specificity and Distance from Ideal (DEI). Further ROC analysis was used to identify the best 3-5 probe combinations. FISH parameters were combined with clinical parameters in the final model. Results: Five combinations of FISH parameters with the highest AUC and sensitivity in discriminating case and control groups were selected based on ROC curve analysis. Combinations were superior in performance to single probes. The best individual parameter, MYC gain, had an AUC of 0.6998. The two best combinations of parameters, MYC Gain/NKX3.1 Gain/ NMYC Gain/PTEN Homozygous and MYC Gain/ETV1 Break Apart/NKX3.1 Gain/PTEN Homozygous had AUC of 0.7363 and 0.7236, respectively. In the progression cohort, MYC gain and PTEN loss were involved in the expression/localization of the best single parameter, MYC Gain/NKX3.1 Gain/ PTEN Homozygous had AUC of 0.7363 and 0.7236, respectively. In the progression analysis, these combinations contributed significantly to the prediction of PCA outcome (progressive vs non-progressive, odds ratios of 5.522 and 0.03). The combination of clinical parameters and FISH outperformed clinical parameters or FISH alone, with odds ratios of 5.955 and 6.118, respectively. The combination of clinical parameters and FISH outperformed clinical parameters alone, and was complimentary to clinical parameters (NCCN Risk Groups) in the final model, demonstrating potential utility of multi-color FISH panels as an auxiliary tool for PCA risk stratification. Further studies with larger cohorts are planned to confirm these findings.

#4731 Phospho-akt: a potential resistance marker to chemotherapy and a therapy-target to restore sensitivity in pancreatic cancer. Daniela Massinna,1 Amir Avan,2 Nicola Funel,3 Carlotta Granchi,3 Filippo Minutolo,3 Letícia Leon,3 Godofridus Peters,3 Elisa Giovannetti3.1. University of Amsterdam, Amsterdam, Netherlands; 2University of Ma'dinah, Ma'dinah, Islamic Republic of Iran; 3University of Pisa, Pisa, Italy.

Oncoogenic KRAS signaling is the main driving force behind pancreatic ductal adenocarcinoma (PDAC); however, targeting this pathway has proven to be difficult. Conversely, the PI3K/Akt pathway represents an exciting new target, however it has been associated with poor prognosis and chemoresistance, and several inhibitors are under development. In particular, perifosine prevents Akt translocation to the cell membrane, while MK-2206 is an Akt allosteric inhibitor and BEZ-235 is a dual PI3K/mTOR inhibitor. Therefore, we investigated the prognostic role of phospho (p)-Akt in PDAC tissues, as well as the molecular mechanisms underlying the interaction of Akt inhibitors with gemcitabine, using PDAC cells, primary cultures and spheroids. Immunohistochemistry of tissue microarrays with specimens from radically-resected patients (n=100) revealed a correlation between high p-Akt expression and worse outcome. Patients with low p-Akt expression (as detected by digital scoring) had a median overall survival (OS) of 16.2 months (95% CI, 14.8-20.1), while patients with high expression had a median OS of 12.9 months (95% CI, 9.0-14.9, P=0.03). Parallel immunocytochemistry studies revealed high expression levels in LPC028 primary cells, while LPC006 were characterized by low p-Akt1. Akt inhibitors reduced cancer cell growth in monolayers and spheroids, and synergistically enhanced the antiproliferative activity of gemcitabine in LPC028 (e.g., combination index CI of 0.2, in the gemcitabine-perifosine combination for 72h, at fixed IC50 ratio), while this combination was antagonistic in LPC006 cells. The synergistic effect was paralleled in the proliferation by a 5-fold reduction in the expression of the main gemcitabine target ribonucleotide reductase. Inhibition of Akt decreased cell migration and invasion, which was additionally reduced by the combination with gemcitabine. However, the combination of Akt inhibitors with gemcitabine increased apoptosis, associated with induction of caspase-3/8/9, PARP and BAD, and inhibition of Bcl-2 and NF-kB in LPC028, but not in LPC006. Further FISH studies with larger cohorts are planned to confirm these findings.
PGL13 enhanced perifosine and perifosine/gemcitabine-induced cell death. In conclusion, our findings support the analysis of phospho-Akt expression as both a prognostic and a predictive biomarker, for the rational development of new combination therapies targeting the Akt pathway in PDAC. Finally, inhibition of Glut1 might overcome resistance to these therapies and warrants further studies.

#4732 Analysis of KRAS variant in a cohort of resected HPV negative HNSCC. Jeffrey Mufson,1 Elizabeth Handorf,2 Joanne Weidhaas,3 Miriam Lango,3 Barbara Burtenshaw,3 Erica Golemis,3 John A. Ridge,3 Raneen Mehraj,4 Yale University, New Haven, CT; 3Fox Chase Cancer Center, Philadelphia, PA; 3UCLA, Los Angeles, CT; 3Johns Hopkins University, Baltimore, MD.

Background: A single nucleotide polymorphism in let-7 complementary site 6 (LS-6) of the KRAS 3’ UTR reduces KRAS inhibition by the let-7 family of miRNA. It is associated with increased risk and greater proliferation of NSCLC tumors, and poor outcomes in ovarian cancers. It is also associated with platinum resistance in head and neck squamous cell carcinoma (HNSCC). The excision repair cross-complementing group 1 enzyme (ERCC1) plays an important role in nucleotide excision repair and double strand break repair, also affecting platinum resistance. We measured ERCC1 expression and KRAS L5-6 variant status as prognostic markers for HNSCC outcome in a series of p16 negative tumors. Methods: KRAS variant status was determined by PCR (MiraxDx) from DNA extracted from 99 available cases of p16 negative HNSCC in the Fox Chase Cancer Center Biosample Repository. Pearson’s Chi-squared tests and Wilcoxon tests were used to compare patient/tumor characteristics, including ERCC1 (HPA029777, Sigma) expression which was analyzed previously, by KRAS variant status. Overall survival was examined using Kaplan-Meier curves with log-rank tests for significance, and Cox proportional hazards regression to control for stage, grade, and tumor site. Results: A set of 99 p16 negative tumors (1990-2002) were analyzed: 62% male, 92% white, 53% moderately differentiated, 29% poorly differentiated. 72% stage III/IV disease. Smoking status was: 32% current, 15% never, 32% past smoker. Primary site of disease: 13% glottis, 44% oral cavity, 14% oropharynx, 20% oral tongue. The KRAS variant was identified in 27/99 (27%) of tissue samples, and in 21/62 (33%) of male patients versus 6/37 (16%) of female patients (P = 0.06). Comparing by histologic grade the KRAS-variant was identified in 2/11 (18%) well differentiated tumors, 15/37 (40%) moderately differentiated and 9/29 (31%) poorly differentiated tumors (P = 0.81). On univariate analysis, there was a non-significant increase in OS among all KRAS-variant patients (P = 0.09) and those treated with surgery and adjuvant radiation (P = 0.08). On multivariate analysis, the direction of the effect was maintained (all patients: HR = 0.65, P = 0.23; radiation subgroup: HR = 0.41, P = 0.06). There was no association between KRAS variant and ERCC1 expression analyzed as a continuous variable (P = 0.70). Conclusion: In this small series, there was no association between the KRAS-variant and ERCC1 expression. Trends suggesting improved outcomes among KRAS-variant HPV negative patients treated with adjuvant radiation warrant further study.

#4733 Dysregulation of CUL4A and CUL4B ubiquitin ligases in lung cancer. Hui Li,1 Lei Jia,1 Fan Yan,1 Pengbo Zhou2.

Background: The Cullin-RING ubiquitin ligases 4 (CRL4) is implicated in controlling cell cycle, DNA damage repair and checkpoint response based on studies employing cell lines and mouse models. CRL4 proteins, including CUL4A and CUL4B are often highly accumulated in human malignancies. Elevated CRL4A attenuates DNA damage repair and increases genome instability that is believed to facilitate tumorigenesis. However, this has yet to be evaluated in human cancer patients. Methods: 352 lung cancer and 62 benign lung specimens of Asian origin were constructed into tissue microarrays (TMAs) of four distinct lung cancer subtypes. Expression of CUL4A, CUL4B and their substrates was detected by immunohistochemistry (IHC), and analyzed statistically for their prognostic value and association with DNA damage response and genomic instability. Results: Both CUL4A and CUL4B are overexpressed in the majority of lung carcinomas (CUL4A<0.001, CUL4B<0.001), and significantly associated with tumor size (CUL4A<0.001, CUL4B=0.002), lymphatic invasion (CUL4A=0.004, CUL4B<0.001), metastasis (CUL4A=0.019, CUL4B=0.006), and advanced TNM stage (CUL4A<0.001, CUL4B<0.001), which parallels gene amplification and abnormal activation of the canonical Wnt signaling. Moreover, overexpression of CUL4A, but not CUL4B, is significantly associated with tobacco smoking (P=0.01), and inversely correlated with XPC and p21, both of which are substrates of CUL4A (PCUL4A=0.019, PCUL4B=0.006). Higher levels of CUL4A or CUL4B are significantly associated with patients’ OS (PCUL4A<0.001, CUL4B<0.001) and PFS (PCUL4A<0.001, CUL4B=0.001). Conclusion: Our findings revealed that CUL4A and CUL4B are differentially associated with etiological factors for pulmonary malignancies and are independent prognostic markers for survival of distinct lung cancer subtypes.

#4734 miR-9-5p expression in breast cancer correlates with hormone receptor status and affects patients survival. Raffaella Barbanera,1 Barbara Pascoli,1 Micheline Rendina,1 Andrea Fontana,1 Caterina Fusilli,2 Massimiliano Copetti,1 Stefano Castellana,1 Vanna Maria Valori,1 Maria Morrirti,2 Paolo Graziano,3 Luigi Ciuffreda,4 Micheline Coco,5 Francesco Picardo,6 Tommaso Mazza,7 Ella Erono,8 Roberto Murgio,9 Evaristo Maiello,3 Manel Esteller,4 Vito Michele Fazio,1 Paola Parrella,1 IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy; 3Campus Bio-Medico University, Rome, Italy; 4Assar Harofeh Medical Center, Zerefin, Israel; 5Bettleighe Biomedical Research Institute (IDIBELL), Barcelona, Spain.

miR-9 (hsa-mir-9-5p, miR-9-5p) is a highly conserved microRNA (miRNA) primarily expressed in the central nervous system. The three promotors of miR-9 (mir-9-1 at 1q22, miR-9-2 at 5q14.3 and miR-9-3 at 15q26.1) are embedded within CpG islands, and hypermethylation in at least one of these regions has been reported in several tumor types, thus miR-9-5p has been designated as an epigenetically regulated miRNA. However, only a partial or none correlation between methylation status of miR-9 loci and miR-9-5p expression has been demonstrated so far. Furthermore, the expression levels of miR-9-5p resulted highly variable across different carcinomas and conflicting evidences also exist about its functional role within the tumor context. In light of this, we took the effort of clarifying the role of miR-9-5p in primary breast cancer tissues and of evaluating its potential as a clinical relevant prognostic biomarker. We analysed miR-9-5p expression and miR-9 promoters methylation status in 129 breast cancer cases and 12 normal breast tissues (NBTSs), by qRT-PCR and MSP respectively. Overall, miR-9-5p was increased in tumors compared to NBTSs (P<0.001). No significant correlation between promoter methylation and miR-9-5p expression was found either in tumors or in NBTSs. Interestingly, miR-9-5p expression was inversely correlated with ER and PgR positivity (P=0.004 and P=0.003, respectively). Consistently, triple negative breast cancer (TNBC) showed the highest miR-9-5p levels and luminal subtype the lowest (P=0.04). The analysis of the TCGA Breast Cancer dataset (n=256) confirmed our results and further demonstrated that miR-9-5p was differentially expressed between the two luminal subtypes (P=0.009) and HER2-amplified compared to TNBCs (P<0.0001). In Univariable Cox regression analysis miR-9-5p expression was significantly associated with higher risk of death (P=0.023). Ingenuity Pathway Analysis exploring the putative interactions among miR-9-5p, ER and PgR upstream and downstream regulators suggested a regulatory loop by which miR-9-5p is induced by steroid hormone receptors and acts within a hormone-receptor and its downstream regulated pathways. Our data suggest miR-9-5p may help refine the molecular classification of breast cancer subtypes and provide additional prognostic information.

#4735 Expression of Notch1 and Numb in small cell lung cancer. Haimi Kikuchi,1 Jun Sakakibara-Komishi,2 Megumi Furuta,3 Hiroshi Yokoouchi,2 Hiroshi Obi,3 Junichiro Takahashi,3 Masaharu Nishimura1.

Background: Notch signaling plays an important role in tumorigenesis. Numb represses intracellular Notch signaling. Previous studies have demonstrated that Notch signaling suppresses the proliferation of small cell lung cancer (SCLC) cell lines. However, in SCLC, the association between Notch1 and Numb expression and clinicopathological factors or prognosis has remained unclear. In this study, we evaluated the expression of Notch1 and Numb in SCLC. Methods: We immunohistochemically assessed 125 SCLCs that were surgically resected at 16 institutions participating in either the Hokkaido Lung Cancer Clinical Study Group Trial (HOT) or the Fukushima Investigative Group for Healing Thoracic Malignancy, Japan; 2Hokkaido Lung Cancer Clinical Study Group Trial, Japan; 3Fukushima Investigative Group for Healing Thoracic Malignancy, Japan; 4Kanazawa Medical University, Uchinada, Japan; 5University of Occupational and Environmental Health, Kitakyushu, Japan.

Background: Notch signaling plays an important role in tumorigenesis. Numb represses intracellular Notch signaling. Previous studies have demonstrated that Notch signaling suppresses the proliferation of small cell lung cancer (SCLC) cell lines. However, in SCLC, the association between Notch1 and Numb expression and clinicopathological factors or prognosis has remained unclear. In this study, we evaluated the expression of Notch1 and Numb in SCLC. Methods: We immunohistochemically assessed 125 SCLCs that were surgically resected at 16 institutions participating in either the Hokkaido Lung Cancer Clinical Study Group Trial (HOT) or the Fukushima Investigative Group for Healing Thoracic Malignancy (FIGHT) between 2003 and 2013. Correlations between Notch1 or Numb expression and various clinicopathological features were evaluated. Results: Notch1 expression was associated with EOCG performance status. Numb expression was associated with age, sex, and patho-
logical history (SCLC or Combined SCLC). Analysis of cellular biological expression did not demonstrate a significant correlation between the expression of Notch1 and of Numb. Multivariate Cox regression analysis showed that high Notch1 expression was an independent favorable prognostic factor for SCLC (hazard ratio = 0.503, P = 0.023). Conclusions: We demonstrate that Notch1 expression, but not Numb, is associated with prognosis in SCLC and may provide a novel prognostic marker of SCLC.


Background: There is growing evidence that ADAM (a disintegrin and metalloproteinase) family proteins are involved in multiple types of cancer, including breast cancer. More than 50 members of the ADAM family have been identified thus far. Previous studies have focused on one or two ADAMs but comparative research among the members of the family is lacking. This study was undertaken to identify the most important ADAMs associated with breast cancer. Methods: We analyzed mRNA expression of ADAMs in tumor bed stroma and paired normal adjacent stroma from 31 breast cancer patients. The original raw data was adapted from a breast dataset (Finak et al, 2008). We next analyzed RNA expression of 35 ADAMs in different subtypes of breast cancers [dataset from Affymetrix U133A arrays using methods available in the R statistical software package (http://cran.r-project.org)]. We further evaluated protein expression of ADAMs and related signaling molecules in 187 breast cancers, including 110 untreated primary tumors and 77 residual tumors after neoadjuvant chemotherapy, and 167 matched “normal” mammary tissues using reverse phase protein arrays (RPPA). The association between ADAMs expression and survival was analyzed using Kaplan–Meier analysis. Results: The microarray analysis of tumor bed stroma revealed significantly elevated mRNA levels of 12 ADAMs, including ADAM8, ADAM 12, ADAM 19, ADAM 21, ADAM 17, ADAM 10, ADAM 28, ADAM 7, ADAM 33, ADAM 22, ADAM 6 and ADAMTS20, compared to their normal adjacent stroma. We evaluated the expression of 35 ADAMs in total 369 breast cancers, including 201 hormone receptor positive (HR+), 60 HER2 amplified (HER2+) and 108 triple negative (TN) patients using RNA array and demonstrated that 24 of 35 ADAMs were significantly upregulated in TNBC compared to that in HR+ and HER2+ subtypes. We further analyzed protein expression of ADAMs in 189 breast cancers and 172 matched “normal” breast tissues with RPPA. Compared to “normal” breast tissues, ADAM17, ADAM10, ADAM15, ADAM8 and ADAM9 were significantly increased in tumors (P < 0.0001, respectively), while ADAM20, ADAM23, ADAM29, and ADAM30 exhibited lower level. Kaplan–Meier analysis showed that a higher overall survival rate in high expression of ADAM 17, ADAM 15 or ADAM10 group was significantly decreased compared to that in the low expression group (40% vs. 12%, P = 0.0026; 50% vs. 11%, P = 0.0104; 53% vs. 9%, P < 0.0001, respectively), suggesting that upregulation of ADAM17, ADAM15, or ADAM10 was significantly associated with poor outcome. ADAM17, ADAM9 or ADAM10 expression was tightly associated with elevation of the classical apoptosis inhibitor Bcl-x, cyclins (E1, E2, B1), PTEN, p53, and phosphorylation of 4-EGBP1. Conclusion: Several ADAM proteins have elevated expression in breast cancer and are associated with decreased patient survival. ADAMs may, therefore, serve as potential biomarkers for predicting outcomes and oncotargets in breast cancer.

#4737 A lung cancer risk classifier comprising genome maintenance genes measured in normal bronchial epithelial cells. Jhyoung Yeo,1 Erin Crawford,1 Xianlou Zhang,2 Sadik Khuder,1 Tian Chen,1 Albert Levin,3 Thomas Blomquist,1 Xiaolu Zhang,2 Sadik Khuder,1 Tian Chen,1 Albert Levin,3 Thomas Blomquist,1 Anh Do, Gordon B. Mills, William F. Symmans, Naoto T. Ueno, Ana M. Gonzales-Angulo, Gabriel N. Hortobagyi, Debasish Tripathy. UT MD Anderson Cancer Ctr., Houston, TX.

Background: Annual low dose CT (LDCT) screening of individuals at high demographic risk reduces lung cancer mortality by more than 20%. However, annual LDCT screening compared to current demographic criteria is expected to have clinical utility by better stratifying subjects for annual lung cancer screening compared to current demographic criteria alone.


INTRODUCTION. The wide spread adoption of population-based breast cancer screening has led to a substantial increase in diagnosis of ductal carcinoma in situ (DCIS). When detected, almost all DCIS is treated to prevent progression to invasive disease, even though the majority of DCIS will never progress. Yet, we are unable to discriminate harmless from potentially hazardous DCIS. Hence, there is an urgent need to find characteristics of DCIS and biomarkers that predict subsequent invasive tumor development. In this study, we compared primary DCIS lesions with their subsequent ipsilateral invasive breast cancer (iIBC), to explore how the initial DCIS lesion and its subsequent iIBC are related. PATIENTS AND METHODS. We used a population-based, nation-wide cohort consisting of 2,654 women who were treated for primary DCIS by breast conserving surgery (BCS) only. Within a median follow-up time of 10.7 years, 316 women developed a subsequent iIBC (12%). FFPE tissue blocks of both DCIS and subsequent iIBC could be collected for 158 of these 316 women. We assessed histology characteristics, tumor location, estrogen and progesterone receptor status, p16 expression, and HER2 and p53 overexpression. Additionally, DNA and RNA were simultaneously isolated from 100 DCIS and 100 matched subsequent iIBC specimens for extensive molecular profiling. RESULTS. More than 95% of the invasive recurrences were located at or near the site of the primary DCIS. Concordant histological grade was found in 94% of the matched pairs and identical immunohistochemical (IHC) marker expression in 58%. Of the 44 patients with HER2 positive DCIS, 36% developed HER2 negative iIBC. Furthermore, this change in HER2 status was also a main cause of a change in surrogate intrinsic subtype (based on ER, PR, HER2) between DCIS and iIBC, which was observed in 16% of the patients. These dissimilarities were not found when we compared invasive disease with synchronous DCIS (present in 83 of 158 patients). Molecular analyses is still ongoing. CONCLUSION. This is the first time that an unbiased comparison could be made between primary DCIS and its subsequent iIBC within such a large patient group, integrating clinical, histological and IHC data. Our results suggest that the majority of invasive breast cancers in our cohort reflect outgrowth of residual disease based on tumor location and histological grade. DCIS and iIBC are very similar when comparing histological grade, but frequently show differences on the IHC level. Remarkably, the dissimilarities in HER2 status and surrogate intrinsic subtype as seen in primary DCIS vs. iIBC are missing when comparing iIBC with synchronous DCIS. As a next step, we will analyse the lesions on the molecular level, to verify these findings and to look for molecular characteristics of DCIS that could be associated with progression to invasive disease.

#4739 Prognostic impact of abnormal DNA damage response protein expression in breast cancer. Kyoung Jin Suh,1 Han Suk Bya,1 Kyung-Hun Lee,1 Hyoin Kim,1 Ahrum Min,2 Tae-Yong Kim,1 Hyeong-Gon Moon,1 Sae-Won Han,1 Do-Youn Oh,1 Wonshik Han,2 In Ae Park,1 Dong-Young Noh,3 Seock-Ah Im1,2. 1Seoul National University Hospital, Seoul, Republic of Korea; 2Seoul National University Cancer Research Institute, Seoul, Republic of Korea; 3Seoul National University Hospital, Seoul, Republic of Korea.
Purpose: Defects of DNA damage repair are known to be associated with the efficacy of anthracycline or platinum-based chemotherapy as well as poly[ADP-ribose] polymerase (PARP) inhibitors (PARPi) and PD-1/PD-L1 checkpoint inhibitors. Since multiple proteins are involved in DNA-damage response (DDR) and operate collectively, our main aim was to better understand the expression patterns of a single DDR protein and to identify potential biomarkers indicating highly aggressive tumors in the different subtypes of breast cancer. Methods: A total of 419 consecutive breast cancer patients who underwent curative resection in 2008 were enrolled in the study. Tissue microarray has been constructed, and NBS1, BRCA1, BRCA2, ATM and p53 expression were determined by immunohistochemistry. Results: All patients were female, with a median age of 47 years; 280 (67%) had luminal A, 36 (9%) had luminal B, 37 (9%) had HER2-enriched, and 56 (13%) had triple-negative breast cancer (TNBC). Loss of NBS1, BRCA1, ATM, and p53 expression was observed in 6.7%, 33.7%, 89.9%, and 30.8% of breast cancer patients, respectively, and abnormal p53 expression was observed in 39.3% of patients. Loss of NBS1, BRCA1, ATM and abnormal p53 expression was associated with significantly lower disease-free survival (DFS) rates, respectively.

\textit{Abnormal DDR protein expression}, defined as loss of any one of NBS1, BRCA1, ATM and/or abnormal p53 expression, was observed in 258 of 399 evaluable cases (64.7%) and was significantly associated with higher tumor grade, larger tumor size, and ER- and/or PR-negative status. Majority of patients with luminal B (31/36, 86.1%), HER2-enriched (34/53, 94.4%), and TNBC (46/53, 86.8%) subtype showed abnormal DDR protein expression. In comparison, 136/264 (51.8%) with luminal A subtype had abnormal DDR protein expression. Abnormal DDR protein expression was associated with significantly lower 5-year DFS rate than those in patients with normal DDR protein expression in all patients (95.6% vs. 84.8%, p = 0.001), as well as in luminal A subgroup (97.4% vs. 89.0%, p = 0.011). In multivariate analysis, abnormal DDR protein expression remained as an independent predictor of shorter DFS for luminal A subtype tumors (hazard ratio 3.14; 95% confidence interval, 1.16 – 8.47, p = 0.024). Conclusion: We demonstrated differential expression of proteins in DDR machinery according to breast cancer subtypes and its prognostic implication. Most of luminal A and HER2-enriched tumors has abnormal DDR expression as frequently as triple negative tumors. Less but significant proportion of luminal A tumors also shows abnormal DDR protein expression, which is an independent poor prognostic factor.

### 44740 Loss of the tumor suppressor zinc finger protein-36 and risk of lethal prostate cancer. Travis Gerke,1 Daniela Bornjen,1 Himisha Beltran,1 Svitlana Tyekucheva,4 Curtis Huttenhower,5 Gwo-Shu Lee,4 Bruce Trock,6 Lorelei Mucci,7 Christopher Sweeney4.

**Background.** Biomarkers are needed to complement current clinicopathologic variables in prostate cancer. Tumor mRNA expression of ZFP36 is prognostic for metastatic or lethal prostate cancer. Combining information on ZFP36 with measurements from other mechanistic pathways, such as PTEN, may lead to highly accurate models.

**Objectives.** Follow-up Study and Physicians' Health Study. Gene expression for ZFP36 and PTEN was validated using a robust genome-wide microarray approach to identify ZFP36, also known as Tristetraprolin (TTP), as a tumor suppressor in the NFκB pathway. The current study aims to evaluate the prognostic potential of tumor mRNA expression of ZFP36. **Methods.** Our primary gene expression analysis leveraged data from a case-control study nested in the Health Professionals Follow-up Study and Physicians' Health Study. Gene expression for ZFP36 and PTEN was validated using a robust genome-wide microarray approach to identify ZFP36, also known as Tristetraprolin (TTP), as a tumor suppressor in the NFκB pathway. The current study aims to evaluate the prognostic potential of tumor mRNA expression of ZFP36. **Results:** In multivariate models (HR 0.63, P < 0.001), as well as in luminal A subgroup (HR 0.41, P = 0.0001) compared to normal plasma samples (n = 8), miRNA-21, miR-221 and miRNA-106a were selected for further validation based on their known biological importance. We showed that all three circulating miRNAs were expressed significantly higher in OS samples (n = 29) than normal samples (n = 17) in an independent cohort obtained from the Children’s Oncology Group. Furthermore, we demonstrated that miR-21 was expressed significantly higher in osteosarcoma tumors (n = 89) compared with normal bone controls. More importantly, lower expressions of miR-21 and miR-221, but not miR-106a, significantly correlated with a poor outcome. In conclusion, our results indicate that miR-21, miR-221 and miR-106a are elevated in the circulation of osteosarcoma patients, whereas tumor expressions of miR-21 and miR-221 are prognostically significant. Further investigation of the miRNAs may lead to a better prognostic method and potential miRNA therapeutics for osteosarcoma.
Given the difference in CTNNB1 MT rate and importance of MTS in axin/β-catenin binding sites, WNT signaling differences between L and R sided tumors may be important to explore further.

### #4743 Identification of metastasis-associated biomarker PLEC1 by iTRAQ-based proteomic approach and prognostic role of PLEC1 in patients with hepatocellular carcinoma

Lin-lan Song, Yu Chen, Di Mu, Xiao-Yan Ji-ang, Yu-Nong Li, Ling-Yu Jiang, Zhen-Fang Zhang, Liang Yan, Min Yang, Yi-xuan Yang, Hong Ren, Yong Liao. Key Laboratory of Molecular Biology for Infectious Diseases (Ministry of Education), Institute for Viral Hepatitis, Department of Infectious Diseases, The Second Affiliated Hospital, Chongqing Medical University, Chongqing, China.

Purpose: Hepatocellular Carcinoma (HCC) is the fifth most prevalent cancer and the second most frequent cause of cancer-related death in men worldwide because of high incidence of recurrence and widespread metastasis. However, to date, there have been no direct metastasis-associated biomarker which is critical for the effective treatment of patients with HCC, we thus aimed to identify novel HCC metastasis-associated biomarkers with prognostic significance. Experimental Design: Two human HCC cell lines, MHCC97-L and MHCC97-H with similar genetic background and stepwise increasing metastatic potentials, were used to explore potential metastasis-associated biomarkers for HCC by isobaric tag for relative and absolute quantification (iTRAQ) combination with 2D-LC-MS/MS. Nineteen HCC cancerous and paracancerous tissues were further analyzed by immunohistochemistry to validate the correlations between the expression levels of PLEC1 with various clinicopathological factors and patient survival. Results: Using iTRAQ combination with 2D-MS/MS, a total of 47 differentially expressed proteins were found in MHCC97-L and MHCC97-H cells, of which 26 proteins were up regulated and 21 proteins were down regulated. Plectin-1 (PLEC1) was identified and re-confirmed as a potential biomarker for HCC that was highly up-regulated in tumor tissues, in comparison with that of para-cancerous tissues. Over-expression of PLEC1 was also strongly expressed in MHCC97-L and MHCC97-H with similar genetic background and stepwise increasing metastatic potentials, were used to explore potential metastasis-associated biomarkers with prognostic significance.

### CLINICAL RESEARCH: Prognostic Biomarkers 1

<table>
<thead>
<tr>
<th>Comparison</th>
<th>BCIN Low Risk Group</th>
<th>BCIN High Risk Group</th>
<th>P-Value</th>
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<tr>
<td>Average # of Mutations</td>
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<td>3.10+/−1.79</td>
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<td>CTNNB1 MT</td>
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</table>

Conclusions: In this study, iTRAQ-based proteomic technology was explored to uncover novel metastasis-associated biomarkers in HCC. We identified and validated metastasis-associated protein PLEC1 as a potential prognostic marker in patients with HCC.

### #4744 Clinical validation of a prognostic model integrating Breast Cancer Index (BCI) with tumor size and grade for prediction of late distant recurrence in hormone receptor-positive (HR+) breast cancer with 1-3 positive nodes.

Yi Zhang,1 Pi-hua-Lotta Jerevall,2 Brock E. Schroeder,1 Amy Ly,2 Hannah Nolan,3 Catherine A. Schnabel,1 Dennis C. Sgori,2 Biotheranostics, San Diego, CA;2 Massachusetts General Hospital, Boston, MA.

Background: The Breast Cancer Index (BCI) 11-gene expression signature has been previously demonstrated to significantly predict risk of overall (10y), early (0-5y), and late (≥5y) distant recurrence in patients with HR+ node negative breast cancer. A distinct BCI model that integrated tumor size and grade (BCIN+) was developed and validated for prediction of distant recurrence in HR+ women with 1-3 positive nodes (N1) (Sestak et al., SABCS 2015, P2-08-12; Zhang et al, ASCO 2016, abstract no. 541). The objective of this analysis was to evaluate BCIN+ performance in the subset of patients that were treated with no more than 5 years of adjuvant endocrine therapy.

Methods: The validation study included 402 HR+ N1 patients diagnosed between 1993 to 2007 with ≥5y follow-up and available tumor blocks. Patients treated with ≤5y of endocrine therapy ≤ chemotherapy (n=275) were included in this analysis. BCIN+ risk scores and categories (low vs high, using a pre-defined cutpoint) were determined blinded to clinical outcome. Kaplan-Meier estimates of overall (0-15y) and late (>15y) distant recurrence, hazard ratios and 95% CIs were estimated. Multivariate analysis adjusting for standard prognostic factors (age, tumor type, surgery type, PR and HER2 status) was performed using Cox proportional hazards model. Results: Mean age was 53.9% were ER+, 88% PR+, and 14% HER2+. The majority of tumors were T1 (61%) or T2 (34%), and 16%, 53%, and 31% were grade 1, 2, and 3, respectively. 79% of patients were treated with adjuvant chemotherapy. BCIN+ significantly separated patients into low and high risk groups for both overall (P<0.0001) and late (P=0.0067) distant recurrence. For overall distant recurrence, BCIN+ classified 18% of patients into the low risk group. The 15y risk of distant recurrence was 2.1% (95% CI: 0.0-6.0%) in the BCIN+ low risk group vs 36.8% (95% CI: 29.3-43.4%) in the high risk group (HR: 21.88; 95% CI: 3.04-157.32). Of patients that were distant recurrence-free for at least 5y, 22% were classified as BCIN+ low risk. The risk of distant recurrence at year 15 was 2.1% (95% CI: 0.0-6.0%) in the BCIN+ low risk group vs 19.0% (11.6-25.8%) in the high risk associated with Grade 1, 95% CI: 2.9-69.7). Conclusions: In this subset analysis from an independent validation study, BCIN+ identified a significant proportion of N1 patients that were associated with a considerably lower 15y risk of distant recurrence after ≤5 y of endocrine therapy ≤ chemotherapy. BCIN+ may provide additional prognostic information to facilitate selection of N+ patients for extended endocrine treatment wherein patients identified as BCIN+ may be considered adequately treated with adjuvant therapy alone.

### CLINICAL RESEARCH: Radiobiology and Radiotherapy

### #4745 Circulating cytokine levels predictive of Grade≥3 radiation pneumonitis:

Matthew S. Ning, Ting Xu, Daniel Gomez, Hai T. Tran, Risikoto Komaki, Xiong Wei, Steven H. Lin, Stephen Hahn, Eric Prado, Jianjun Zhang, John V. Heymach, Zhongxing Liao. University of Texas MD Anderson Cancer Center, Houston, TX.

Purpose: Host immune activation is believed to be a major player of radiation pneumonitis (RP). Several cytokine and angiogenic factors (CAFs) have been studied as prognostic markers and therapeutic targets for RP; however, studies are sparse in the setting of prospective trials and high-grade toxicity (Grade≥3) endpoints. We examined the predictive value of several CAFs in combination with clinical factors for RP in the context of a Phase II, prospective randomized study of intensity modulated RT (IMRT) versus proton therapy for locally advanced NSCLC (n=178) as well as a prospective laboratory cohort (n=43). METHODS: From July 2009 to April 2014, 221 patients were treated with proton therapy or IMRT to doses of 60-74 Gy equivalents with concurrent platinum-doublt chemotherapy. Primary endpoints were symptomatic RP (Grade=2) and RP requiring intervention (Grade≥3). Plasma circulating levels of IL-6, IL-10, IL-8, IL-17, IL-2, IL-1β, and IL-1α were measured at baseline and post-treatment (median time 4 days post-RT) by multiplex electrochemiluminescence immunoassay. Clinical characteristics were evaluated for associations with Grade≥3 RP: histology, age, smoking status, mean lung dose (MLD), chemotherapy, stage, performance status, and RT technique. CAFs with >50% undetectable samples were removed from analysis, and samples below the lower limit of quantitation (LLOQ) were transformed to the mean of zero and the LLOQ. Predictive values of clinical and cytokine factors were evaluated in univariate and multivariate analyses via Cox proportional hazards modeling. RESULTS: Of the 221 patients, 42 (19.0%) developed Grade 2 RP, and 22 (9.9%) developed Grade≥3 RP. Both IL-1α and IL-1β had >50% of undetectable samples and were removed from analysis. Pre-treatment level of IL-6 ≥4.00 pg/mL was associated with Grade≥3 RP (HR 2.487; p=0.048), as was post-treatment level of IL-10 ≥0.50 pg/mL (HR 2.931; p=0.020). On multivariate analysis, the factors associated with Grade≥3 RP development were post-treatment level of IL-10 ≥0.50 pg/mL (HR 3.322; p=0.015) and MLD ≥20 Gy (HR 5.864; p<0.001). CONCLUSIONS: In this preliminary analysis of prospectively col-
lected CAFs, post-radiation IL-10 level was significantly associated with Grade=3 RP and, if validated, may serve as a predictor of this toxicity in combination with MLD. IL-10 plays an important role in the inflammatory response and is potentially targoatable, warranting further natural investigation.

#4746 Early clinical support for 4-demethyl-4-cholesteroloxycarbonylpenclomedine (DM-CHOC-PEN) as a radiosensitizer in cancers involving the CNS. Lee Roy Morgan, R S. Weiner, T Mahmood, R. Kawachi, K. Devisetty, J Herman, M Bhandari, R. Summe, E Benes, AH Rodgers, ML Ware, JS Hayman, JW, Jeremy, JY Zou, IDEKK-TEC, Inc., New Orleans, LA; Tulane University, New Orleans, LA; Detroit Clinical Research Center, Lansing, MI; Karmanos Cancer Center Institute at McLaren Flint, Flint, MI; Sparrow Hospital, Lansing, MI; The Christ Hospital, Cincinnati, OH; Ochsner Medical Center, New Orleans, LA; Johns Hopkins Medical Center, Baltimore, MD; Gamma Radiosurgery Center of New Orleans, New Orleans, LA; University of Texas Health Science Center/Hermann Hospital, Houston, TX.

Background: 4-Demethyl-4-cholesteroloxycarbonylpenclomedine (DM-CHOC-PEN) is a polyclirorinated pyridine cholesteryl carbonate with a MOA via bis-alkylation of DNA & N'guanine and N'-cytosine that has completed Phase I-II studies [AACR #CT129, 2016] in subjects with cancers involving the CNS. The current presentation reviews clinical and in vitro data that support radiosensitizing properties for DM-CHOC-PEN in cancers involving the CNS. Patients & Methods: DM-CHOC-PEN was administered as a 3-hr IV infusion once every 21 days to subjects with advanced cancer involving the CNS. The dose schedule was 2-tiered: [85.8 mg/m2 for subjects with liver involvement and 98.7 mg/m2 for subjects with normal livers. All subjects with resistant or new CNS lesions that did not respond to DM-CHOC-PEN were given the option of surgery, stereotactic radiotherapy (SRS) or whole brain irradiation (WBRT). As preclinical support, human NSCLC adenocarcinoma cells (H-2086) growing in culture (105 cells/mL) were pretreated with DM-CHOC-PEN (0.1 - 1.0 µg/mL) for 24 hrs, drug washed, re-fed fresh medium and then 48 hrs later irradiated (SRS with 6, 9 or 12 Gy). Results: Fifty-three (53) subjects with/without CNS involvement had been treated to date with DM-CHOC-PEN. Five (5) subjects (4-NSCLC & 1-sarcomas) in the Phase I-II trials required surgery for persistent CNS lesions following DM-CHOC-PEN therapy. DM-CHOC-PEN was identified in samples from all subjects - 90-212 ng/g tumor [subjects had been treated with 39-98.8 mg/m2 of drug]. Five subjects (1-sarcoma & 4-NSCLC) who had treated with DM-CHOC-PEN for 6-3 weeks - 8 mos had persistent NSCLC lesions involving the CNS were treated with SRS or WBRT. All five subjects had excellent objective results (OS 8 - 29 mos) with no CNS toxicity. Preclinical in vitro studies supported the clinical data: NSCLC cells treated with DM-CHOC-PEN (0.1 - 1.0 µg/mL) demonstrated 50 & 100% cytotoxicity @ 0.8 & 1.0 µg/mL for SRS alone (6, 9, 12 Gy) - cell kill w/25 & 65% @ 6 & 12 Gy [100% cell kill was not observed at this dose range]; for DM-CHOC-PEN (0.25 µg/mL) + SRS (6-12 Gy) - cell kill was 80 & 100% @ 6 & 12 Gy. Thus, in combination - less drug (0.25 µg/mL) + 12 Gy - produced a 100% cell kill, as compared to either SRS or drug, alone. Photon induced change transfer reactions with DM-CHOC-PEN will be discussed. Conclusion: Preliminary data is presented that supports DM-CHOC-PEN’s cytotoxicity and radiosensitizing properties in cancers involving the CNS. Observations during clinical trials support the drug’s persistent presence in human tumors after systemic administration and notable positive effect on response to subsequent radiation. Complete data on patient responses and observed toxicities will be presented. Supported by: NCI/SBIR grants - R43/44CA132257 and NIH NIGMS U15 GM104940 - the latter funds the Louisiana Clinical and Translational Science Center.

#4747 Subcutaneous fat predict outcome in head and neck cancer. Ngn M. Tsang. Chang Gong Memorial Hospital, Taoyuan, Taiwan.

Background: Previous studies have shown that body mass index (BMI) is linked to survival in cancer patients. Our objective was to analyze whether body composition before treatment can predict outcomes in patients with head-and-neck cancer. Methods: All 881 patients with loco-regional head-and-neck cancer treated with curative-intent RT during 2005 to 2012 were retrospectively studied. Body composition analyses by using pre-RT CT images acquired for RT simulation. We compared subcutaneous adipose tissue (SAT) index and muscle index by measuring cross-sectional area of tissues at level of the third thoracic vertebrae (T3) in both genders. Overall survival (OS), loco-regional control (LRC), and distant metastasis-free survival (DFS) were analyzed by body composition index that divided into two groups based on values higher and lower than the median. The median follow-up was 4.68 years. Results: SAT index in female patients was significantly higher than that in male (p = 0.001). The median SAT index and muscle index for female were 15.25 cm2/m2 (range, 0.93-88.09 cm2/m2) and 34.3 cm2/m2 (range, 14.78-73.05 cm2/m2), and for male were 6.19 cm2/m2 (range, 0.21-40.48 cm2/m2) and 51.7 cm2/m2 (range, 8.03-89.06 cm2/m2). The 5-year and 10-year OS, LRC and DFS were 66.3%, 57.6%, 73.4%, 71.4%, 83%, and 82.1%. Higher pretreatment SAT index was associated with longer OS (HR = 0.616; P = 0.001) and DFS (HR = 0.649; P = 0.015) and a trend towards longer LRC (HR = 0.572; P = 0.066). Pre-treatment muscle index was not significantly predictive of OS (HR = 0.662; P = 0.066) or DFS (HR = 0.684; P = 0.029). Conclusion: SAT index and muscle index of T3 level obtained from CT images appear to predict outcomes in patients with cutaneous head-and-neck cancers.

#4748 Novel predictive biomarker for monitoring adverse reactions to radiation therapy. Paul OKunieff, Steven Swarts, Elena Peletsky, Anne Vallerga, Rachel Chuang, Michael J. Powell, Aiguo Zhang, University of Florida, Gainesville, FL; DiasCarta, Richmond, CA.

Introduction: Radiation treatment is required by 70% of cancer patients, however there is currently no clinical method for determining the therapeutic response or radiation induced toxicity that can be used during a course of radiation therapy to personalize the dose for individual patients. The only standard method is CT/PET and/or MRI. This is a major clinical concern for radiation oncologists with so many new agents being approved in combination with radiation therapy. Methodology: Herein we describe a highly sensitive clinically validated assay that measures the extent of normal tissue damage induced by radiation by quantitation of circulating free DNA (cfdNA) derived from cellular apoptosis detected in plasma of patients undergoing radiation therapy. The assay employs DNA capture probes and SuperBiondaTM signal amplification technology with alkaline phosphatase labelled signaling probes coupled with dioxetane phosphate chemiluminescence detection. The assay can be performed directly on patient plasma samples and can be readily automated. Conclusion: RadTm TM can be used both for research and clinical testing of plasma samples for patients undergoing radiation therapy for optimization and personalization of treatment.

#4749 A phase II study using cardiac MRI to assess cardiac injury in breast cancer patients receiving three-dimensional conformal regional nodal radiotherapy (3DCRT) with heart dose constraints. Carmen Bergom, Jason Rubenstein, Aimee Welsh, Philip Prior, Daniel Eastwood, Mei-Jie Zhang, J. Frank Wilson, Julia White, Julie Bradley, Medical College of Wisconsin, Milwaukee, WI; The Ohio State University Comprehensive Cancer Center, Milwaukeee, WI; University of Florida, College of Medicine, Jacksonville, FL.

Purpose/Objectives: Regional nodal radiotherapy (RNR) can improve survival for node positive breast cancer (NPBC) patients, yet this benefit is offset by potential late cardiac toxicity. Most cardiac toxicity reports from RNR have been in eras where no specific dose or volume heart constraint was used. Cardiac MRI (CMR) is ideal to assess ventricular function and volumes. We report a phase II trial designed to assess cardiac injury by CMR in NPBC patients who received chemotherapy (CT) and RNR planned with heart constraints. Materials/Methods: 244 NPBC patients treated with 3DCRT RNR following surgery planned with heart constraints between 2000-2008 were screened. Eligibility included prior anthracycline CT, no cardiac disease pre-NPBC, and no recurrent disease. Patients with active atrial fibrillation were excluded. The cardiac dose constraint utilized was V25 =<10% of the heart. Mean heart dose was not constrained. Eligible women were invited by letter to enroll in the IRB-approved trial. CMR parameters to be evaluated included indicators of left ventricular (LV) function: ejection fraction (EF), LV mass (LVM), and LV dimensions, as well as late gadolinium enhancement (LGE) and total heart extracellular volume fraction (ECV) as measures of cardiac injury and/or early cardiac fibrosis. Planned analyses included correlation of CMR parameters with cardiac dose-volume constraints using Spearman and Pearson correlations. Results: The first 15 L-sided and 5 R-sided patients consented underwent CMR. Median age at diagnosis was 50 (32-77); median age at CMR was 60 (40-83). Median time after 3DCRT was 8.3 yr (5.7-14.4). The median L-sided mean heart dose was 4.8 Gy (1.1-11.2) and V25 was 5.7% (0-12%). No patient had clinical cardiac disease at time of CMR. Median LVEF was 63%. No LGE and no abnor- mal total heart ECV values (>25%) were obtained. Values mildly outside normal limits were seen for LVM and LV dimensions in 4/15 (27%) L-sided and 2/5 (40%) right-sided patients. No correlations were seen between heart mean and max doses, V5, V10, V25, and V45 and the CMR variables of LVEF, LV mass, and LV dimensions. Two patients had valvular abnormalities found on CMR (1-sided with aortic regurgitation and R-sided with mitral regurgitation). Conclusion: CMR findings were largely within normal limits at a median follow of 8.3 years in this cohort of NPBC patients who received prior cardiac chemother-apy and RNR planned with heart constraints. A subset of patients had subclinical mildly abnormal LV measurements, but these did not correlate with
heart dose or laterality, and the significance remains unknown. In this cohort, no significant cardiac injury from RNR as measured with CMR was seen. Conversely, CMR may not be a sensitive tool for subclinical partial heart injury from RNR. Larger corroborating studies are warranted.

#4750 Predicting severe hematologic toxicity from extended-field chemoradiation of para-aortic nodal metastases from cervical cancer. Kevin Yan, Ezequiel Ramirez, Xuejun Gu, Kevin Albuquerque. UT Southwestern, Dallas, TX.

Background and Purpose: To determine significant factors predictive for severe hematologic toxicity (HT) in cervical cancer patients with para-aortic lymph node (PALN) metastasis treated with concurrent chemoradiation with a specific focus on radiation dose to total bone marrow (BM\textsubscript{TOT}) and acute bone marrow (BM\textsubscript{ACT}). To create a nomogram using significant factors to predict HT in these patients. Material and Methods: 38 Patients with cervical cancer and PALN metastasis who underwent 3F-FDG-PET / CT before treatment with extended field radiation therapy (EFRT) and concurrent cisplatin were analyzed. BM\textsubscript{ACT} was defined as the region within BM\textsubscript{TOT} with a standardized uptake value (SUV) greater than or equal to the mean for the individual. Blood counts were collected weekly from the beginning of radiation treatment to the end of radiation treatment. HT was graded based on the guidelines set by the National Cancer Institute Common Terminology Criteria for Adverse Events Version 4.0. Results: 19 patients (50%) had Grade 3 or higher hematologic toxicity (HT+), not including lymphocyte toxicity. Patients who were obese (n=12) were less likely to get HT+ compared to patients who were not obese (p=0.03) despite getting the same weight related dose of chemotherapy. Volume of BM\textsubscript{TOT} receiving 20 Gy, 30 Gy, and 45 Gy were significant predictors for HT3+ at 78.56% (p=0.01), 47.14% (p=0.00), and 20.36% (p=0.01) respectively. Volume of BM\textsubscript{ACT} receiving 10 Gy, 20 Gy, 30 Gy, and 45 Gy were significant predictors for HT3+ at 95.50% (p=0.03), 80.52% (p=0.05), 59.64% (p=0.03), and 31.74% (p=0.01) respectively. Through logistic regression, the probability of developing HT3+ is given by the equation: Prob(HT3+ = 1) = (1 + exp(7.34 + 0.22BM\textsubscript{ACT} - 0.44(Mean Dose to BM\textsubscript{TOT})). Patients who had HT3+ received an average of 4 cycles of chemotherapy and 62 days of treatment time, significantly different than the 4.74 chemotherapy cycles and 53 days of treatment in patients without HT3+ (p=0.05, 0.00 respectively). Conclusions: Both higher patient BMI and bone marrow irradiation were associated with HT+. A simplified nomogram has been created to predict HT3+ in these patients. Radiation parameters have been identified for cervical cancer patients with PALN involvement receiving EFRT concurrently with chemotherapy. Bone marrow sparing approaches for EFRT need to be addressed to improve patient care.

#4751 Correlating the genomic and transcriptomic profiles of pre-treatment cervical tumor biopsies with patient recurrence after definitive chemoradiation. Fiona J. Ruiz, Julie K. Schwarz. Washington University in St. Louis, St. Louis, MO.

Purpose: The purpose of this study was to identify genomic mutations that correlate with patient recurrence after curative intent chemoradiation therapy in advanced stage cervical cancer patients. Methods: Pre-treatment cervical tumor biopsies and normal blood were prospectively collected from patients treated at Washington University in St. Louis. DNA and RNA was isolated from the fresh frozen tumor biopsies and sent for next-generation sequencing and RNAseq respectively. Targeted exome sequencing was done using a panel of 127 known cancer associated genes, and an additional 8 genes previously reported to be mutated in cervical cancer. Gene testing was done to probe for 30 different HPV subtypes. Results: The mutational signatures of single nucleotide polymorphisms were an APOBEC cytidine deaminase and Cpg site cytidine deamination mutagenesis pattern. The non-silent somatic mutations were analyzed by online prediction algorithms to assess the mutation’s putative effect on protein function. Univariate COX regression was used to correlate patient recurrence with non-silent putatively functional mutations. In our patient cohort 88% of samples were HPV positive, with 74% being the high-risk HPV 16 and 18 subtypes. Patients’ with HPV positive tumors had increased progression-free survival when compared to the HPV negative patient tumors; and the HPV positive patients with HPV 16 had increased progression-free survival over the other HPV subtypes. Complete gene tiling of the HPV 16 and 18 subtypes allowed us to determine whether the HPV DNA was integrated in the host genome or retained episomally. In our patient cohort less than 30% of samples had integrated HPV DNA, and there was no significant difference in progression-free survival between patients with integrated or episomal HPV. Conclusions: The genomic analysis of our advanced stage cervical cancer patient cohort found that epigenetic modifiers were frequently mutated and that cytidine deamination mutagenesis was primarily responsible for the generation of the SNP mutations. Our study identified NDS1 mutations as being correlated with tumor recurrence, indicating that histone modifications and chromatin structure may play an important role in tumor response to ionizing radiation therapy. Gene expression analysis is currently ongoing.

#4752 Inflammatory cytokines and hematopoietic stem cells are associated with fatigue and insomnia in breast cancer patients undergoing adjuvant radiation therapy. Wei Shi, Kathy Han, Madeleine Li, Justin Williams, Megan McCusker, Jie Su, Wei Xu, Scott Bratman, Kenneth Yip, Fei-Fei Liu. Princess Margaret Cancer Center, University Health Network, Toronto, Ontario, Canada.

Introduction: Breast cancer patients undergoing adjuvant radiation therapy (RT) commonly experience fatigue and insomnia. We previously reported that this fatigue is associated with a reduction in circulating hematopoietic stem cells (HSCs; CD34+), the role of inflammatory cytokines in mediating this process however, has not been clearly elucidated. Materials and Methods: Breast cancer patients (n = 147) undergoing adjuvant RT underwent phlebotomies for analysis of CD34+, CD45+, CBC, as well as 17 inflammatory cytokines using a multiplexed ELISA platform, during 5 time points: prior to RT (D1); after Days 2, 5, during final week of RT (D2, D5, and DF, respectively); as well as one month post-RT-completion (M1). At the same time, patients also completed questionnaires for the multidimensional fatigue inventory (MFI-20), hospital anxiety and depression scale (HADS), and insomnia severity index (ISI). Results: General fatigue worsened over the course of RT from D1 to DF, being most severe at the end of treatment (DF), but returned to baseline at M1. This trend was statistically significant (p = 0.001) after adjusting for anxiety, depression, and insomnia. The levels of CD34+, CD45+, white blood cell, as well as lymphocyte counts decreased over time, with the lowest levels observed at DF (p < 0.001 for all). General fatigue correlated inversely with CD34+ counts (adjusted for anxiety, depression, and ISI), and was also negatively associated with hemoglobin, RBC, and lymphocyte counts (p < 0.001 for all). There was also a significant correlation between increasing insomnia with lower CD34+, CD45+, white blood cell and lymphocyte counts (all p < 0.05). Serum concentrations of TGF-β1, MCP-1, MMP-2, IL-1ra and IFN-α2a changed significantly during RT (p < 0.01 for all), with either the highest or lowest levels observed at DF. Increasing levels of MCP-1, TNF-RII and TNF-a were associated with worsening general physical fatigue, reduced activity, decreased motivation, as well as increased insomnia (p < 0.01 for all). Furthermore, there appeared a trend between increasing levels of MMP-2, as well as decreasing IL-1ra and TGF-β1, with reduced CD34+, CD45+, WBC and lymphocyte counts (p < 0.001). The 52 patients who received prior adjuvant chemotherapy demonstrated significantly higher fatigue, anxiety, and insomnia scores. Conclusions: This study represents one of the most comprehensive longitudinal evaluations of the effects of RT on fatigue and insomnia, demonstrating that this process was associated with increased levels of the pro-inflammatory cytokines MCP-1, TNF-RII and TNF-a, and reductions in circulating HSCs and other hematologic parameters. Further understanding of the roles of these cytokines would provide important insights into both quality of life for patients undergoing cancer therapies, as well as the interactions between RT with immunotherapy.

#4753 Prognostic significance of galectin-1 expression in patients with glioblastoma multiforme following radiotherapy. Eng-Yen Huang, Shang-Yu Chou. Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan.

Purpose: Galectin-1 is a radioresistant marker and prognostic factor in our previous study for cervical cancer. The aim of current study is to determine the prognostic significance of galectin-1 expression level in patients with glioblastoma multiforme (GBM) undergoing adjuvant radiotherapy (RT). Materials and Methods: We included 45 patients with GBM who treated with maximal safe surgical resection or biopsy only, followed by adjuvant RT of EQD2 = 60 Gy (equivalent doses as 2-Gy fractions) for homogeneous treatment. Paraffin-embedded tissues acquired from the Department of Pathology were analyzed using immunohistochemistry staining for galectin-1 expression. Overall survival (OS) and cancer-specific survival were compared between strong, moderate, and weak expression of galectin-1. Results: According to univariate analysis for optimal cutoff of galectin-1 expression level, better median survival (27.9 months) in patients with weak level than that of (10.7 months) in patients with strong level (p = 0.009) was revealed. We compared characteristics between weak and strong galectin-1 expression level, and only galectin-3 expression level was correlated. The weak galectin-1 expression group displayed a 3-year survival rate of 27.3% and 3-year cancer-specific survival (CSS) rate of 27.3%, but which are only 5.9% and 7.6% respectively in strong galectin-1 expression group (p = 0.009 and 0.020, respectively). Cox regression was used to confirm that the role of galectin-1 expression level (weak vs. strong) is a significant factor of OS (p = 0.020) and CSS (p =
Other parameters such as galectin-3 expression level, ECOG perfor-
type, which include IL-4ra, IL-10ra, and TGF-
significantly upregulated (P

#4754 Fractionated whole brain radiation-induced behavioral changes in athymic nude mice is associated with sustained neuroinflammation and microglial M1-phenotype. Suman Kanji, Benjamin Johnson, Kristina Witcher, Pooja Gulati Gulati, Shannon Chen, Jonathan Godbout, Randy J. Nelson, Saikh Haque, Arnab Chakravarti. The Ohio State University, Columbus, OH.

The objective of this study is to study the late-chronic effects of fractionated whole brain irradiation (FWBI) on cognitive impairment and associated cellular and molecular neuro-inflammatory mechanisms using clinically relevant and reliable mouse models. Microglia are recognized as the primary innate immune component of neuroinflammation. However, microglial contributions to radiation-induced cognitive impairment are poorly under-
stood. Here, an athymic nude (Nu/Nu) mouse model was employed to ad-
dress this issue. Mice were divided into two groups: radiation treatment (XRT) and no-treatment control (CTL). The whole brain of each XRT mouse received 30 Gy (3 Gy/fraction) of radiation over two weeks. XRT and CTL mice were assessed for cognitive and behavioral changes at 1, 4, and 6 months posttreatment using the novel object recognition test (for long-term, non-spatial memory), the free running Y-Maze (for short-term, spatial memory) and Barnes Maze (for spatial learning and memory). A significant decline in novel object recognition and Barnes Maze performance was seen at the 4 month time point and continued to persist at 6 months (P<0.05). No significant changes in the Barnes maze or Y-maze were seen at these time points. Expression of neuroinflammatory mediator genes, from both whole brain and isolated microglia, were measured by RT-PCR. We found that markers of the M1-phenotype, which include TNF-α, and MHC II, were significantly upregulated (P<0.05) and markers of the general M2 Pheno-
type, which include IL-4ra, IL-10ra, and TGF-β, were downregulated (P<0.05) at 1 and 6 month time points. These results indicate that sustained neuroinflammation and microglial M1-polarization are associated with long-term cognitive impairment induced by FWBI. Radiotherapy is the most prevalent treatment for primary and metastatic brain tumors. Whereas previous studies that identified important mechanisms underlying XRT-induced cognitive decline had used treatment regimens that were not clinically relevant ones, we have employed a clinically relevant fractionation scheme to address the cellular and molecular deregulations in association with cogni-
tive impairment.

#4755 Calpastatin phosphorylation regulates radiation response in glioblastoma. Emily A. Bassett, Mitchell Pearson, Kamalakannan Palani-
chamy, Saikh J. Haque, Arnab Chakravarti. Ohio State University, Columbus, OH.

Glioblastoma (GBM) is the most prevalent and aggressive primary malignant brain tumor in adults. Patients diagnosed with GBM have a very poor prognosis and quality of life, with a median survival time of 12-15 months despite receiving the standard of care treatment. GBM cells are highly heterogeneous, invasive, and resistant to both chemo- and radio-therapy. The overall goal of this study is to identify novel mechanisms of radioresistance in GBM, so that more effective therapies can be developed in the future. We performed quantitative mass spec-
trometry-based phosphoproteomic profiling of GBM cell lines before and after radiation treatment, and determined post-radiation fold-changes in phosphor-
ylation for each phosphopeptide. Results show calpastatin as one of the statisti-
cally significant differentially regulated phosphopeptides following radiation treatment. Calpastatin is an endogenous inhibitor of calpains, which are calci-
um-dependent cysteine proteases that control a wide range of cellular processes including cell survival and apoptosis. We hypothesized that phosphorylation inactivates calpastatin resulting in increased calpain activity. Western blot anal-
yses with phospho-specific calpastatin antibodies showed a rise in phosphory-
tein levels as early as 24 hours after exposure to radiation. In order to test the functional significance of phosphorylated cal-
pastatin, we utilized site-directed mutagenesis to generate non-
phosphorylatable and phosho-mimetic calpastatin proteins. Western blot analyses of U87 cells stably expressing CAST-S63A or S633E supported the hypothesis that calpastatin phosphorylation leads to increased calpain activity. In conclusion, we identified increased levels of calpastatin phosphorylation in multiple GBM cell lines following radiation treatment, and shown that this cor-
relates with increased calpain activity. Modulation of calpastatin activity is a potential strategy to increase radiosensitivity of glioblastomas. Targeting cal-
pastatin is advantageous over targeting calpain itself, since many calpain inhib-
itors exhibit limited selectivity. Results of this study will contribute to our un-
derstanding of how GBM tumors become resistant to radiation therapy. Funding: R01CA108633, R01CA169368, RC2CA148190, U10CA180850-01 (NCI), Brain Tumor Funders Collaborative Grant, and The Ohio State Univer-
sity CCC (all to AC).

#4756 A SPP1-EGFR pathway links stem-like properties of KRAS-mu-
ted lung cancer to radiation resistance. Meng Wang, Jing Han, Lynnette Marcar, Josh Black, Qi Liu, Xiangyong Li, Khiithija Nagalappalii, Licia V. Sequist, Raymond H. Mak, Cyril H. Benes, Theodore S. Hong, Kristin Gurt-
ner, Mechthild Krause, Michael Baumann, Jing X. Kang, Johnathan R. Whetstone, Henning Willers. Mass. General Hospital, Boston, MA; Jinan Mu-
unicipal Center for Disease Control and Prevention, Jinan, China; University of Colorado School of Medicine, Aurora, CO; Dana-Farber Cancer Institute, Bos-
ton, MA; University Hospital Carl Gustav Carus, Technische Universität, Dresden, Germany.

Lung cancers with activating mutations in the KRAS oncogene present a major clinical challenge due to poor prognosis and frequent treatment resis-
tance, particularly to agents targeting the epidermal growth factor receptor (EGFR). For many years, it has been known that mutations in KRAS enhance cellular resistance to ionizing radiation (IR). Clinical data are emerging suggest-
ing that the radioresistance of KRASmut cancer cells that is observed in the laboratory is also seen in patients. However, the underlying mechanisms of mut-
ant KRAS-associated radioresistance is understudied and poorly under-
stood. Cancer stem cells (CSC), also referred to as tumor-initiating cells, may promote metastases development and recurrence after therapy. Generally, can-
cer cells with CSC-like phenotypes or markers have been found to be radiore-
sistant. Enhanced DNA damage response and repair pathways have been dis-
cussed as one underlying mechanism. Interestingly, mutant KRAS has been recently linked to CSC-like phenotypes such as tumor initiation, anchorage independence, and self-renewal. Here, we characterized a radioresistant pheno-
type associated with KRAS mutated lung cancer cells, xenografts, and patients. CSC-like cells were defined as tumor-initiating cells. Genomic, biochemical, and cell-based assays were used to identify candidates for targeting KRAS mutation-
mediated radiation resistance. Radiation resistance is conferred by a CSC-like subpopulation that is characterized by condensed chromatin, high CD133 ex-
pression, invasive potential, and tumor-initiating properties. Osteopontin, the gene product of SPP1, promotes aspects of this phenotype including radiation resistance and acts in the same pathway as the EGFR. SPP1/EGFR-dependent chratomin condensation not only protects cells against radiation-induced DNA damage but also downregulates putative negative regulators of CSC-like prop-
eerties, including CRM1 and BIM. This phenotype defines a subset of KRAS 

mucated lung cancers that is enriched for co-occurring TP53 mutations. In con-
clusions, markers and mechanisms of resistance to radiation therapy in lung and 
other cancers are poorly characterized. Our data provide novel insight into the aggressive biology of KRAS mutant cancers and have major implications for strategies to overcome the radioresistance associated with this genotype.

#4757 Radioresistance is linked with stem-like properties via activation of aryl hydrocarbon receptor. SHuang Liu, Bin Yan, Yongguang Tao. Central South University, China.

Most cancer patients receive radiotherapy in the course of their disease and the occurrence of radioresistance is associated with a poor prognosis for cancer patients. The molecular pathways that drive the enhanced tumorigenic potential during the development of radioresistance are poorly understood. Here, we demonstrate that the aryl hydrocarbon receptor (AhR) plays a vital and previ-
ously unrecognized role in the maintenance of stem-like cells that are isolated from radioresistant epithelial lung cancer and nasopharyngeal carcinoma. AhR promotes the stem-like phenotype and drives metastasis by directly targeting the promoters of ‘stemness’ genes, such as the ATP-binding cassette sub-family G member 2 (ABCG2) gene. Cancer cell populations enriched for ABCG2 expres-
sion (CD338+) show an elevated ‘stemness’ signature and are more prone to cancer progression in vitro and in vivo. Ubiquitin C-terminal hydrolase-L3 (UCHL3), a deubiquitinating enzyme, may counteract AhR, while IKEKo, a bone fide chromatin modifier, may directly activate stemness related genes. The ra-
dioresistant cancer cell sublines are associated with a hybrid state in epithelial-
mesenchymal transition (EMT) and mesenchymal-epithelial transition (MET) 
and elevated tumor associated macrophages and proinflammatory factors. Moreover, the radioresistant sublines display high levels of oncometabolites, including α-ketoglutarate, and treatment of cancer cells with α-ketoglutarate enhances their stem-like properties through activation of the AhR. Thus AhR is
functionally linked with radioreistance in lung cancer and nasopharyngeal cancer, and it drives tumorigenesis by an epithelial and mesenchymal composition, tumor associated macrophages and oncometabolites.

#4758 Sensitization of nasopharyngeal carcinoma to radiotherapy by targeting GFPT1/TGF-β1 signaling pathways. Siqi Chen, Wei Gao, Jimmy Yu-Wai Chan, Thian-Sze Wong. The University of Hong Kong. Hong Kong SAR, China.

Background: Nasopharyngeal carcinoma (NPC) is a unique cancer with high susceptibility to radiation and mainstay treatment is radiotherapy. Effectiveness of NPC treatment is therefore dependent on the sensitivity of cancer cells to ionizing radiation treatment. Further, development of radio-resistant phenotype is a risk factor for distant metastasis and disease recurrence. Therefore, enhancing the degree of radiation sensitivity could improve prognosis and reduce the toxicity of radiotherapy with the use of lower radiation dose. Transforming growth factor β1 (TGF-β1) expression is responsive to radiation treatment and the increase is important in the post-irradiation DNA repair. Tumor with low TGF-β1 level is more responsive to radiation treatment. Therefore, inhibiting TGF-β1 production can enhance the radiation damage to cancer cell DNA and also intensify the sensitivity of cancer cells to radiation. Our preliminary results indicate that TGF-β1 production in NPC is mediated by hexosamine pathway. Of which, GFPT1 (glucosamine-fructose-6-phosphate aminotransferase) is the rate-limiting enzyme. Purpose of the study: To enhance radiation sensitivity by targeting GFPT1/TGF-β1 cascade in NPC. Experimental procedures: Radiation protective effects of TGF-β1 was examined by colony formation assay and Danio rerio (zebrafish) embryonic system. Production of TGF-β1 by activated hexosamine pathway in NPC was revealed by glucosamine and glucosamine treatment. Effects of GFPT1 silencing on radiosensitization are revealed by GFPT1 siRNA treatment followed by colony formation assay and gamma-H2AX formation assays. Results: Pretreatment of HONE1 cells with recombinant TGF-β1 protein enhanced the clonogenic ability of irradiated cells. TGF-β1 significantly increased the survival of zebrafish embryos after irradiation. Glucose and glucosamine treatment increased the expression of TGF-β1 protein in HONE1 cells. Silence of GFPT1 reduced TGF-β1 mRNA and protein expression in HONE1 cells. Silencing GFPT1 reduced the clonogenic capacity of head and neck cancer cells after radiotherapy and enhanced the degree of radiation-induced DNA double-strand breaks. Conclusions: Targeting GFPT1 enhances head and neck cancer cell sensitivitiy to radiation. GFPT1 inhibition can enhance the radiation sensitivity of head and neck cancer cells by inhibiting the expression of TGF-β1.

#4759 TP53 control of the survival-mediated radio-adaptive response in human tumors. Jeffrey S. Murley,1 Richard C. Miller,1 Ralph R. Weichselbaum,1 David J. Grindla2,1 Univ. of Chicago, Chicago, IL; 2Univ. of Chicago, Naperville, IL.

Doses of ionizing radiation ≤100 mGy induce changes in radiation sensitivity expressed by cells exposed to subsequent lower doses. This is referred to as an adaptive effect. We describe a unique survival-associated adaptive response in which increased radiation resistance or sensitization of cells can be induced by exposure to doses as low as 5 mGy and is controlled by p53 expression. Experiments were performed using ten human cancer cell lines: HCT116 TP53 wild type (WT) colorectal carcinoma and its isogenic TP53 null (Mut); RKO colorectal cancer exposed WT and isogenic TP53 null (Mut); breast adenocarcinomas MCF7 (TP53 WT) and MDA-MB-231 (TP53 Mut); lung carcinomas A549 (TP53 WT) and NCI-H1975 (TP53 Mut); and pancreatic carcinomas Hs7667 (TP53 WT) and Panc-1 (TP53 Mut). Doses of 5 mGy were used to induce changes in the response of these tumor cells to higher therapy doses using a multi-dosing paradigm. Effects on radiation sensitivity were associated with changes in both survivin concentration and its translocation to the cytoplasm (TP53 WT) and nucleus (TP53 null or point mutant). In vitro survival (2 Gy per fraction, two once daily fractions) studies were performed on all tumor cell lines. Intracellular localization of survivin was determined by ELISA and correlated to survival response. 2 Gy alone had no significant effect on intracellular translocation of survivin. When preceded 15 minutes earlier by a 5 mGy exposure, survivin increased in the cytoplasm of all TP53 WT tumor cell lines. With clinicogenomics database analysis and following validation with MLCC model, we determined that BCL2, of which expression was clearly induced and indicated a poor prognosis in the mesenchymal cancer patients, was responsible for high chemoresistance in MLCCs. Therefore combined treatment of chemotherapeutic drug and BH3 mimetic such as ABT-263 or ABT-737, remarkably sensitized MLCCs to chemo and radiotherapy. Furthermore, BCL2 expression was governed by sustained ERK1 activity in MLCCs, which resulted from high level of MEK partner-1 (MP1) protein expression. Therefore, combined chemotherapy with small molecules (approved by FDA or clinically tested) targeting for MEK1 or BCL2 would be a clinically feasible approach to overcome EMT related chemoresistance.

#4760 Wnt/β-catenin signaling mediates resistance of colorectal cell lines to chemoradiotherapy. Georg Emons,1 Melanie Spitzner,2 Sebastian Reineke,2 Noam Auslander,1 Frank Kramer,3 Margret Rave-Fraenkl,2 Jochen Gaedcke,4 Michael Ghdami,5 Thomas Ried,1 Marian Grade,1 1NIH, Bethesda, MD; 2University Medical Center, Goettingen, Germany.

Background: Activation of Wnt/β-catenin signaling plays a central role in the development and progression of colorectal cancer (CRC). Previously, we demonstrated that the Wnt transcription factor TCF7L2 was overexpressed in primary rectal cancers that were resistant to chemoradiotherapy (CRT), and that TCF7L2 functionally mediates resistance of CRC to clinically relevant doses of CRT. However, it remained unclear whether the resistance was mediated by a TCF7L2 inherent mechanism or Wnt/β-catenin signaling in general. Methods: We silenced another key-component of canonical Wnt-signaling, CTNNB1 (β-catenin) in rectal cancer cell lines LS1034, SW480, and SW837 by RNAi. Afterwards cells were exposed to varying doses of irradiation (+/-5 Gy) to assess the influence of β-catenin on radiation resistance. Activation of Wnt-signaling in “normal” epithelial cells (retina pigment epithelial cells RPE) was achieved either by stimulation with Wnt3a or by overexpression of β-catenin (S33Y-mutated) and confirmed by a dual luciferase reporter assay. Changes in radio-(chemo-) sensitivity were analyzed again by a colony formation assay. SW1463 were repeatedly irradiated (68Gy) to establish an isogenic radio-resistant cell line. Gene-expression profiles, without or 6h after a single dose of 4Gy, of RPE cells expressing a control vector or overexpressing β-catenin and radiosensitive or wildtype SW163 cells were established using microArrays. Results: Silencing of CTNNB1 resulted in (chemo-) radiation-sensitivity of all three CRC-cell lines. To further investigate the potential role of Wnt/β-catenin signaling in controlling therapeutic responsiveness, non-tumorigenic RPE cells were stimulated with Wnt-3A, which significantly increased resistance to CRT. This effect could be recapitulated by overexpression of β-catenin (S33Y-mut.), resulting in a significantly increased resistance to CRT. The effect could be rescued by siRNA mediated knockdown of β-catenin. Consistent with these findings, we observed higher expression levels of active (unphosphorylated) β-catenin as well as increased TCF reporter activity in SW1463 cells that were rendered radiation-resistant due to repeated IR treatment. Gene expression profiling of radiosensitive and wildtype SW163 as well as RPE cells overexpressing β-catenin or wildtype β-catenin revealed a number of differentially regulated genes. Most interestingly the resistant cells (radiosensitive SW1463 and RPE overexpressing β-catenin) reacted with differential activation of metabolic, inflammatory, cell survival and cell cycle pathways to irradiation. Conclusion: Together, these findings strongly support the interpretation that Wnt/β-catenin signaling plays a central role in mediating resistance of CRC cells to CRT by deregulating essential pathways. Hence, path在内的 therapeutics may represent a promising strategy to increase therapeutic responsiveness to CRT.

#4761 BCL2 induced by LAMTOR3-MAPK is a druggable target of chemoradiosensitization in mesenchymal lung cancer. Ok-Seon Kwon, Soon-Ki Hong, Hyuk-Jin Cha. Sogang University, Seoul, Republic of Korea.

Metastasis and chemoresistance, which are main causes of lung cancer related death, have been major interest in cancer research. Recently, Epithelial-Mesenchymal Transition (EMT) a foremost process for acquiring metastatic properties has been demonstrated to be strongly link to chemoresistance. Due to limited understanding of mechanism for EMT mediated chemoresistance, druggable targets for chemoresistance remained unidentified yet. In this study, we established the mesenchymal like cancer cells (MLCCs) from A549 lung cancer cells with chronic exposure of TGFβ. With clinicogenomics database analysis and following validation with MLCC model, we determined that BCL2, of which expression was clearly induced and indicated a poor prognosis in the mesenchymal cancer patients, was responsible for high chemoresistance in MLCCs. Therefore combined treatment of chemotherapeutic drug and BH3 mimetic such as ABT-263 or ABT-737, remarkably sensitized MLCCs to chemo and radiotherapy. Furthermore, BCL2 expression was governed by sustained ERK1 activity in MLCCs, which resulted from high level of MEK partner-1 (MP1) protein expression. Therefore, combined chemotherapy with small molecules (approved by FDA or clinically tested) targeting for MEK1 or BCL2 would be a clinically feasible approach to overcome EMT related chemoresistance.
**CLINICAL RESEARCH: Radiobiology and Radiotherapy**

#4762 New device for radiation delivery in breast cancer patients - double balloon applicator for optimal dose distribution. Manny R. Subramanian, Mike Cutrer, Rohit Mehta. *BEST Medical International, Springfield, VA.*

Currently available applicators for delivery of accelerated partial breast irradiation using a high dose afterloader, have treatment catheters close to the center of the balloon in fixed positions, restricting one’s ability to modify the dose to critical organs. Recently we developed a novel, dual balloon brachytherapy applicator using which one could optimize radiation dose by shaping the resection cavity with an outer balloon and allowing an inner balloon to selectively position the treatment catheters away from the central axis of the balloon based on cavity size. The Double Balloon Breast Brachytherapy Device was performed with a wide range of volumes of the lumpectomy cavity. Each case is scanned in a CT scanner and the data transferred to a treatment planning system. Dosimetric parameters such as dose delivered to treatment site, different organs such as skin, ribs, etc are evaluated and compared against existing brachytherapy applicator devices. Both ultrasound and CT images clearly indicate that the quality and the Double Balloon breast device images are comparable to other commercial devices. All catheters are distinguishable and can be reconstructed for treatment planning, and the balloon volume can be clearly delineated. Planning Treatment Volume (PTV) based on balloon volume can be easily generated and optimized dosimetry performed. Initial patient studies also indicate that using the double balloon applicator optimized dosimetry can be obtained thereby reducing the dose to critical structures such as skin and rib while maintaining the dose to the target.

**TUMOR BIOLOGY: Cancer Stem Cells**

#4763 Metronomic chemotherapy prevents therapy-induced stromal activation and induction of cancer stem cells. Kelvin K. Tsai,1 Tze-Sian Chan,1 Chung-Chi Hsu,1 Vincent C. Pai,1 Shenq-Shyang Huang,1 Valerie M. Weaver.1

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While traditional chemotherapy kills a fraction of tumor cells, it also activates the stroma and can promote the growth and survival of residual cancer cells to foster tumor recurrence and metastasis. Accordingly, overcoming the host response induced by chemotherapy could substantially improve therapeutic outcome and patient survival. Treatment resistance and metastasis have been attributed to expansion of cancer stem-like cells (CSCs). Molecular analysis of the tumor stroma in neoadjuvant chemotherapy-treated human desmoplastic cancers and orthotopic tumor xenografts revealed that traditional maximal tolerated dose chemotherapy, regardless of the agents used, induces persistent STAT-1 and NF-κB activity in carcinoma-associated fibroblasts that induces the expression and secretion of ELR-motif-positive (ELR+ ) chemokines, which signal through CXCR-2 on carcinoma cells to trigger their phenotypic conversion into CSCs and promote their invasive behaviors, leading to paradoxical tumor aggression following the therapy. By contrast, the same total accumulated dose administered as a low-dose-metronomic chemotherapy regimen largely prevented the therapy-induced stromal ELR+ chemokine paracrine signaling for CSCs, thereby substantially enhancing treatment response and extending survival of mice carrying desmoplastic cancers (Chan et al., J. Exp. Med. 2016). These studies illustrate the importance of stroma in cancer therapy and how its impact on treatment resistance could be tempered by altering the dosing schedule of systemic chemotherapy.

#4764 Activation of estrogen receptor beta signaling reduces stemness and promotes differentiation of glioma stem cells. Gangadhara Reddy Sareddy, Jinyou Liu, Suryavathi Viswanadhapalli, Rajeshwar R. Tekmal, Andrew Brenner, Ratna K. Vadlamudi. *UT Health Science Center at San Antonio, San Antonio, TX.*

Glioblastoma (GBM) are the most common and deadliest tumors of the central nervous system. GBM have poor prognosis due to tumor recurrence and resistance to current therapies. Glioma Stem Cells (GSCs) are implicated in the tumor initiation and therapy resistance of GBM. Agents that can specifically reduce stemness of GSCs are urgently needed for the effective treatment of GBM. Efficacy is mainly unknown. Estrogen effects are mediated by two estrogen receptors (ER1 and ER2) and ER2 functions as a tissue-specific tumor suppressor. Our recent studies discovered that GSCs preferably express ER2 with low or undetectable levels of ER1. The objective of this study is to test whether ER2 agonists modulate stemness of GSCs and to determine their mechanism(s) of action. We have tested the hypothesis using GSCs isolated from established and patient derived GBM cells using stem cell markers CD133 and CD135. Knockout of ER2 using CRISPR/Cas9 system increased the CD133 positive GSCs and overexpression of ER2 reduces the CD133-positive population in GBM cells. Treatment of GSCs with ER2 agonists (Liquiritigenin and LY500307) significantly inhibited the neurosphere formation, self-renewal ability and proliferation. Further, ER2 agonist treatment resulted in the loss of stemness and induction of differentiation and apoptosis of GSCs. Western blot analysis and RT-qPCR analysis revealed reduced expression of stemness markers such as Nestin and Sox-2 and increased expression of differentiation markers GFAP and tub-1 in GSCs. RNA sequencing analysis of ER2 agonist treated and untreated GSCs revealed modulation of genes associated with cell cycle, differentiation and apoptosis. Further, ER2 agonists treatment significantly reduced the GSCs mediated tumor growth in orthotopic models and improved the mice overall survival. Immunohistochemical studies demonstrated that ER2 agonists reduces the expression of proliferation of marker Ki67 and induced the apoptosis in tumors. Together, our results established ER2 agonists liquiritigenin and LY500307 as novel therapeutic agents for elimination of GSCs. Since ER2 agonists has good blood-brain barrier permeability and less neuronal toxicity, they can be readily transferred to clinical use with current radiation and chemotherapies, thereby providing an additional tool for enhancing survival in GBM patients.

#4765 Targeting glioblastoma cancer stem cells with a novel Connexin43 mimetic peptide. Samy Lamouille,1 James W. Smyth,1 Laurie O’Rourke,2 Pratik Kanabar,1 Sujuan Guo,1 Jane Jourdant,2 Zhi Sheng,2 Robert G. Gourdie1.1Virginia Tech Carilion Research Institute, Roanoke, VA; 2Virginia Tech Carilion School of Medicine, Roanoke, VA.

Glioblastoma (GBM) is a highly malignant and lethal cancer of the central nervous system. Despite intensive research efforts, there has been limited progress in improving patient outcome. The current therapy for GBM patients includes surgical resection, radiotherapy and cytotoxic chemotherapy with temozolomide (TMZ), conferring a median survival time of only 14.6 months. Failure to generate more effective therapy strategies is due to the cellular heterogeneity within GBM tumors, which comprise a sub-population of GBM cancer stem cells (GSCs) characterized by self-renewal characteristics and resistance to TMZ. Recent work, including our own, has demonstrated that levels of the gap junction protein Connexin43 (Cx43) correlate with GBM TMZ resistance. Increased Cx43 levels occur in GSCs compared to parental GBM cells, and patients with high levels of Cx43 mRNA and low levels of MGMT, an enzyme that repairs TMZ-induced DNA lesions, have a significantly shorter life span. In contrast, Cx43 has also been associated with anti-proliferative effects in glioma and reduced levels of Cx43 protein are reported in high-grade gliomas. In addition to forming gap junctions, Cx43 can regulate cell proliferation, migration, and apoptosis, through distinct channel-independent mechanisms. Therefore, altering the localization and/or activity of Cx43 rather than Cx43 expression alone may represent a more targeted strategy in GBM treatment. Using super-resolution microscopy, we find intracellular Cx43 decorating microtubules in GSCs demonstrating for the first time such clustering in situ. Regulation of Cx43 function is primarily associated with multiple sites for post-translational modification and protein-protein interaction within the Cx43 carboxy-terminus (CT) which includes a tubulin binding domain. We have developed a peptide named JM2 (juxtamembrane 2) composed of the Cx43 CT amino acids encompassing the microtubule-binding sequence fused to an antennapedia cell penetration domain for cellular uptake. Super-resolution microscopy and biochemical analysis confirm JM2 specific interaction with microtubules concomitantly with a loss of Cx43 interaction with microtubules in GSCs derived from patient tumors. In addition, we observed that JM2 decreases Cx43 gap junction plaque formation and cell-cell communication in GSCs and limits microtubule dynamics. Importantly, JM2 decreases cell survival in TMZ-resistant GSCs and GSC neurosphere formation in vitro, and GSC-derived tumor growth in vivo. Our current research includes the development of JM2-loaded biodegradable nano-
particles for sustained JM2 delivery in preparation for future clinical trials. In conclusion, we have developed a Cs43 mimetic peptide that significantly decreases the tumorigenic potential of GSCs and represents a novel and potent therapeutic opportunity to alter Cs43 activity in targeting chemoresistant GSCs for GBM treatment.

**#4766 Translation from unconventional 5’ start sites drives tumor initiation.** Ataman Sendoo. Rockefeller University, New York, NY.

How translational control impacts tumor-initiation and malignancy is just beginning to unfold. Here, we devise an epidermis-specific, in vivo ribosome profiling strategy to interrogate the translational landscape during the transition from normal homeostasis to malignancy. Inducing SOX2, broadly expressed in oncogenic RAS-associated cancers, we find that despite widespread reductions in translation and protein synthesis, certain oncoprogenic mRNAs are spared. Seeking mechanism, we find that during tumor-initiation, the translational apparatus is redirected towards unconventional upstream initiation sites, enhancing translational efficiency of oncogenic mRNAs. An in vivo RNAi screen of translational regulators revealed that dampening conventional EIF2 complexes has dire consequences for normal but not oncogenic growth. Conversely, we identify alternative initiation factors essential for cancer progression, where they mediate initiation at these upstream sites, differentially skewing translation and protein expression. Our findings unveil a hitherto unappreciated role of 5’UTR translation in cancer, and expose new targets for therapeutic intervention.

**#4767 Identifying CDCA7 as a novel regulator of tumor-initiating cells in triple-negative breast cancer.** Caroline C. Notch1, puesto, Nicholas Stevers1, Samantha Tinsley1, Max Wicha1, Duxin Sun1. College of Pharmacy, University of Michigan, Ann Arbor, MI; Comprehensive Cancer Center, University of Michigan, Ann Arbor, MI.

Tumor-initiating cells (TICs) are a small fraction of cancer cells that stands at the apex of the tumor hierarchy. Given their tumor-initiating ability and resistance to conventional chemotherapies, TICs are considered potential therapeutic targets for preventing the relapse and metastasis of malignancies. Our group has identified a novel gene, Cell division cycle associated 7 (CDCA7), as an important regulator of TICs in triple-negative breast cancer (TNBC). CDCA7 is a target gene of MYC and NOTCH. Ectopic expression of CDCA7 has been demonstrated to induce neoplastic transformation in vitro and tumorigenesis in vivo. Despite its potential tumor-initiating ability, CDCA7 has not yet been well-studied in breast cancer. We found that CDCA7 was highly expressed in TNBC compared to other subtypes of breast cancer according to the RNA-sequencing data from The Cancer Genome Atlas. Furthermore, our Kaplan-Meier analysis of multiple datasets demonstrated that higher expression of CDCA7 was associated with poor prognosis in breast cancer. When we knocked down CDCA7 by siRNA in TNBC cell lines including SUM149, HCC1953, MDA-MB-231, and MDA-MB-436, CD44+/CD24– stem-like cell population and primary and secondary mammosphere formation were significantly decreased. In addition to suppressing the stem-like properties, knockdown of CDCA7 also inhibited the invasion and migration of breast cancer cells. These phenotypes indicated the inhibition of epithelial-to-mesenchymal transition (EMT), which was in accordance with the up-regulation of E-Cadherin and phenotypes indicated the inhibition of epithelial-to-mesenchymal transition. Knockdown of CDCA7 also inhibited the invasion and migration of breast cancer cells. These phenotypes indicated the inhibition of epithelial-to-mesenchymal transition (EMT), which was in accordance with the up-regulation of E-Cadherin and down-regulation of Vimentin after CDCA7 knockdown in TNBC cell lines. In conclusion, our results have demonstrated that CDCA7 is an important regulator of TICs in TNBC. Loss-of-function of CDCA7 leads to the inhibition of EMT and stemness in TNBC cells.

**#4768 A SHARP/Xist complex regulates breast cancer stem cells.** Yongyou Zhu, Li Shang, Justin Colacino, Michael Brooks, Ramdane Harouaka, Chang-Ching Lin, Ming Luo, Max S. Wicha. Univ. of Michigan, Ann Arbor, MI.

Many cancers including breast cancer display hierarchical organization and are driven by a cellular sub-population that displays stem cell properties. These cancer stem cells (CSCs) mediate metastasis and constitute to treatment resistance highlighting the importance of elucidating CSC regulatory pathways. Nuclear proteins and lncRNAs involved in the maintenance of chromatin structure have been shown to play roles in CSC regulation. Among them the polycomb proteins bmi-1 and EZH2 are involved in epigenetic regulation of CSCs in breast and other cancers. SHARP/SPEN, a RNA binding protein, has been previously been found to bind the IncRNA Xist which, by recruiting the PRC2 polycomb complex mediates transcriptional repression of the X chromosome. We utilized breast cancer cell lines and xenograft models to examine the role of the SHARP/Xist complex in the regulation of breast CSCs. In a series of breast cancer cell lines including MCF7 (luminal) and SUM 159 (basal/claudin low) cells, SHARP mRNA and protein as well as the IncRNA Xist were more highly expressed in cells expressing aldehyde dehydrogenase as accessed by the Aldehyde fluor assay than in ALDH– cells. Conditional knockdown of SHARP or Xist in SUM159 significantly decreased the ALDH+ CSC population without affecting cell growth in vitro. Furthermore, SHARP or Xist knockdown significantly reduced tumor initiating capacity and growth of SUM 159 cells in NOD/SCID mice. To determine the mechanism of breast CSC regulation by the SHARP complex, we compared mRNA levels in SHARP and Xist knockdown SUM 159 cells using RNAseq. There was significant overlap between the genes regulated by SHARP with genes regulated by the PCRI and PCR2 polycomb complexes in addition to many histone genes. These studies suggest that the SHARP/Xist complex may regulate breast CSCs through epigenetic regulatory histone and polycomb genes. Since SHARP expression is elevated in a number of cancers including breast cancer this pathway may represent a novel therapeutic target.

**#4769 Targeting HER2 enriches jagged1 high cancer stem cells in breast cancer.** Deep S. Shah1, Debra Wyatt2, Andrew Baker2, Andrew Green2, Alexandria Filippovic, Lucio Miele, Clodia Osipo1, Loyola Univ. Cardinal Bernardin Cancer Ctr., Maywood, IL; University of Nottingham, Nottingham, United Kingdom; Imperial College of London, London, United Kingdom; Louisiana State University, New Orleans, LA.

The human epidermal growth factor receptor 2 (HER2) positive subtype of breast cancer is characterized by gene amplification and/or protein overexpression of HER2. It is driven by a subpopulation of cells possessing stem cell properties of self-renewal and differentiation, known as Cancer Stem cells (CSCs). CSCs are implicated in tumor growth as well as radiotherapy and chemotherapy resistance. CSCs are supported by bone marrow-derived stem cells and self-renewal, and overexpression of a Notch ligand Jagged1 mRNA predicts poor prognosis in women with breast cancer. Our lab has published that Jagged1 or Notch is a critical target in trastuzumab/latatinib (LAP) resistant HER2+ breast cancer. The study aimed to determine whether anti-HER2 therapy selects for Jagged1/Notch-dependent CSCs that are responsible for tumor initiation. Surface expression of Notch1 and Jagged1 upon HER2 blockade using LAP was measured in HER2+ breast cancer cell lines (MDA-MB-453 and HCC1954) using flow cytometry. LAP treatment increased the Jagged1-positive subpopulation compared to vehicle. Cells were sorted based on Jagged1 cell surface expression and assessed for CSC-like properties (i.e. mamsphere formation in vitro and tumorigenicity in athymic, nude mice). In addition, immunohistochemistry was performed on 145 HER2+ breast cancer microarray to detect cytoplasmic, membrane, or nuclear Jagged1 protein expression. Kaplan-Meier analysis was performed to determine overall survival. The results showed that upon HER2 inhibition, Jagged1 cell surface expression increased and Notch1 cell surface expression was unchanged. The Jagged1+/Notch1– subpopulation of cells showed elevated levels of Aldehyde dehydrogenase activity, Notch target gene transcripts, and mammosphere formation efficiency compared to vehicle treated cells. The MRK-003 γ-secretase inhibitor (GSI) prevented mammosphere formation in the Jagged1+/Notch1– cells indicating that Notch activation drives Jagged1+Notch1– CSC survival. Also, we confirmed that Jagged1 expression is required for the enrichment of CSCs using a jagged1+/Notch1– CRISPR screen. Combined knockdown of Notch1 and Notch3 receptors was necessary to reduce LAP-enriched mammospheres suggesting that targeting HER2 enriches for a Jagged1+/Notch1– subpopulation of GDC phenotype. Importantly, higher membrane expression of Jagged1 protein in 145 HER2+ breast tumor specimens correlated with significantly lower overall cumulative survival. These results reveal that HER2 blockade in breast cancer cells enriches for a Jagged1+/Notch1– subpopulation that has higher CSC potential and is resistant to HER2 inhibitors. The implications of this work are that dual blockade of Jagged1/Notch1+Notch3 and HER2 could be more effective than either therapy alone to eliminate both HER2 and Jagged1-dependent cancer cells.

**#4770 Novel CXCR3 allosteric modulators inhibit breast cancer stem cells and metastasis.** Namita Kundu, Regine Bron, Xinrong Ma, Jocelyn Reader, Nuska Tschammer, Amy M. Fulton1, Univ. of Maryland Greenebaum Cancer Ctr., Baltimore, MD; Friedrich Alexander University, Erlangen, Germany; NanoTemper Technologies, Munich, Germany.

There is an urgent need to identify molecular targets that are relevant to metastatic and/or stem-like cancer cells (CSC). Theoretically, surface-expressed G-protein coupled receptors on CSC are attractive therapeutic targets. There is considerable evidence that the inflammatory milieu of the tumor microenvironment drives CSC and we now show that two isoforms of the chemokine receptor CXCR3 play important but distinct roles in cancer behavior. In the bulk tumor cell population, CXCR3, isoform A is highly expressed relative to CXCR3B. CXCR3A is critical to metastatic success. CXCR3, isoform B, is highly upregu-
lated in breast cancer CSC. When CXCR3B is gene-silenced in a model of basal-like, estrogen-dependent breast cancer, tumors-forming capacity is reduced and the aldehyde dehydrogenase-positive (ALDH1 +) population is correspondingly decreased. Conversely, overexpression of CXCR3B enhances these CSC properties. Thus, CXCR3B is critical to maintenance of the phenotype and function of breast CSCs. CXCR3B binds CXCL9, CXCL10, and CXCL11 with high affinity to CXCR3, however, each ligand is coupled to different intracellular signaling pathways. For example, others have reported that, in HEK cells, CXCL10 strongly induces ERK activation, but CXCL11 does not. In our hands, CXCL11 more potently induces an ALDH1+ population in MDA-MB-231 cells compared to CXCL10. We have developed novel allometric modulators of CXCR3 that demonstrate probe (ligand)-dependence. BD64 preferentially inhibits CXCL11-mediated β-arrestin 2 recruitment relative to inhibition of GPC signaling; BD103 is significantly more effective at blocking CXCL11 versus CXCL10-mediated GPCR signaling. Neither BD64 nor BD103 affects the GPCR signaling; BD103 is significantly more effective at blocking CXCL11 versus CXCL10-mediated GPCR signaling. Neither BD64 nor BD103 affects the GPCR signaling; BD103 is significantly more effective at blocking CXCL11 versus CXCL10-mediated GPCR signaling.

**#4771 The role of Hes1 in the normal and tumor stem cells of the intestine.**

Noriohiro Goto, Akihisa Fukuda, Tsutomu Chiba, Hiroshi Seno. Kyoto University Graduate School of Medicine, Kyoto, Japan.

Notch-Hes pathway plays a crucial role in the differentiation, proliferation, and tumorigenesis of the intestine. We previously reported that prenatal deletion of Hes1 reduces intestinal tumor proliferation; however, postnatal deletion of Hes1, which recapitulates Hes1-targeted tumor therapy, has not been thoroughly investigated. Here, we elucidated the novel role of Hes1 in normal stem cells (NSCs) and tumor stem cells (TSCs) of the adult intestine. First, we deleted Hes1 in Lgr5-expressing or Bmi1-expressing NSCs and performed lineage tracking using Lgr5CreERT2 reporter, Hes1flox/flox; Rosa26LacZ/+ and Bmi1 CreER+/++; Hes1flox/flox; Rosa26lacZ/+ mice. After Hes1 deletion, Lgr5-expressing or Bmi1-expressing NSCs gave rise to progeny cells, but could not self-renew. However, Hes1 deletion in NSCs did not disrupt intestinal homeostasis, because Hes1-positive NSCs were newly generated soon after Hes1-deleted NSCs were depleted. Next, we stabilized beta-catennin for intestinal tumor formation and deleted Hes1 in Lgr5-expressing cells using Lgr5CreERT2/+; Hes1flox/flox; ApcMin/+ mice. Of note, Hes1 deletion in TSCs of established intestinal tumors induced immediate apoptosis of TSCs and significantly reduced the number of tumors. qRT-PCR analysis of sorted Lgr5-expressing cells one day after Hes1 deletion revealed upregulation of pro-apoptotic genes and down-regulation of anti-apoptotic genes in Hes1-deleted TSCs, but not in Hes1-deleted NSCs. We also deleted Hes1 in an established intestinal tumor, showed that, irrespective of stem cell markers analyzed, Hes1 deletion in TSCs leads to tumor regression by inducing immediate apoptosis in TSCs. Taken together, Hes1 is required in different ways for the maintenance of NSCs and TSCs: Hes1 deletion in NSCs does not perturb homeostasis, whereas Hes1 deletion in TSCs leads to tumor regression. Furthermore, Hes1 is necessary for the initiation of intestinal tumor formation. Therefore, we propose Hes1 is a novel tumor-specific therapeutic target.

**#4772 Stearoyl-CoA Desaturase (SCD1) regulates liver tumor initiating cells through modulating ER stress.**

Kien Fa Ma,¹ Eunice Yuen Ting Lau,² Irene Oi Lin Ng,² Kin Wah Lee,¹ University of Hong Kong, Hong Kong. The Hong Kong Polytechnic University, Hong Kong.

Sorafenib is the best approved drug for patients with advanced hepatocellular carcinoma (HCC), but the survival benefit is only modest, partly due to drug resistance. Increasing evidence shows that tumor-initiating cells (T-ICs) are intrinsically resistant to conventional treatments, and targeting signaling pathways in T-ICs provides potential therapeutic targets for HCC. In our established enriched T-IC population, we identified the activation of a lipogenesis pathway in which expression of stearoyl-CoA desaturase-1 (SCD1), an enzyme involved in the conversion of saturated into monounsaturated fatty acids, was most significant. SCD1 overexpression is frequently found in HCC samples and is significantly associated with poorer patient survival. Using overexpression and knockdown approaches, SCD1 was found to regulate the traits of T-ICs, including tumorigenicity, self-renewal, differentiation, drug resistance and the expression of liver T-IC markers. Interestingly, SCD1 was markedly upregulated in our established sorafenib-resistant cell lines as well as patient-derived xenografts (PDTXs), and its overexpression predicts the clinical response of HCC patients to sorafenib treatment. Consistently, pharmacological inhibition of SCD1 suppressed T-IC phenotypes and enhanced sensitivity to sorafenib treatment. Using a patient-derived xenograft model, we found that a novel SCD1 inhibitor (S31-4) in combination with sorafenib demonstrated a maximal tumor suppressive effect. Induction of endoplasmic reticulum (ER) stress-mediated T-IC differentiation can account for the enhanced sensitivity towards sorafenib treatment upon SCD1 suppression. In conclusion, SCD1-induced ER stress may specifically inhibit the sensitivity of liver T-ICs to the effects of sorafenib treatment. Targeting SCD1 in combination with sorafenib therapy may be a novel therapeutic regimen against HCC.

**#4773 Dysregulated expression of pentraxin-3 in glioblastoma (GBM) stem cells: Implications for GBM invasion and progression.**

Umadevi V. Wesley, Paul Clark, John S. Kuo, Robert J. Dempsey. University of Wisconsin, Madison, WI.

Glioblastoma multiforme (GBM) is highly invasive and resistant to current therapies. The aggressiveness of brain tumor is attributed to GBM stem cells (GSC) that release inflammatory cytokines, chemokines and MMPs in the tumor microenvironment, critically affecting GBM survival and invasiveness. The glycoprotein pentraxin-3 (PTX3), a modulator of tumor-associated inflammation, is known to be correlated with tumor grade and severity of malignancies of some cancers. However, little is known about the role of PTX3 in GBM growth and invasion. In this study, we examined the role of PTX3 in GSCs exhibiting various degree of invasiveness. From patient brain tumors, our team has isolated the small subsets of glioblastoma stem cells (GSCs) and are validated for their self-renewal and multi-lineage potential. Our studies have shown that these GSCs efficiently form orthotopic brain tumors in immune-deficient mice. Our results show that conditioned media from GSCs promote pro-angiogenic tube formation from endothelial cells. GSCs that are grown in the absence of growth factors EGf, VEGf, FGF, IGF-1 induce invasive phenotype in immune-deficient mice and showed increased dispersal in the mice brain. Interestingly, polymerase chain reaction (RT-PCR) and immunofluorescence staining showed upregulated expression of PTX3 in these GSCs, and correlated with the degree of invasiveness. Differentiation of GCs into astrocytes in vitro, lead to decreased expression of PTX3. In support of this, recent histochemical study has shown that PTX3 levels correlate with GBM grade and is a candidate biomarker for cancer progression. Thus, PTX3 may represent a new target and marker of inflammation for glioma malignancy.

**#4774 Polyamines are rich and induces epigenetic change by inhibition of LSD1 in cancer stem-like cells.**

Keisuke Tamari, Hideshi Ishii, Masamitsu Konno, Naohiro Nishida, Jun Koseki, Koichi Kawamoto, Fumiaki Isoshahi, Yuichiro Doki, Masaki Mori, Kazuhiko Ogawa. Osaka University, Suita, Osaka, Japan.

Cancer stem cells (CSC) are highly tumorigenic and resistant to chemotherapy and radiotherapy. We have previously reported that our team fractionation of cancer cells with low proteasome activity had CSC-like properties. Ornithine decarboxylase (ODC) is an enzyme which converts ornithine to polyamines including putrescine, spermidine, and spermine. Proteosomal degradation of ODC is known to be ubiquitin-independent. The aim of this study was to explain how low proteasome activity gave CSC-like properties. Western blot showed high ODC protein level in CSC-like cells in cervical cancer and osteosarcoma cell lines, although ODC mRNA level is not high. These findings suggest that ODC protein level is high in CSC-like cells because ODC protein degradation is inactive. To investigate polyamine metabolism in CSC-like cells, we performed GC-MS analysis and found that putrescine, spermidine, and spermine were enriched in CSC-like cells. Furthermore, flow analysis showed conversion of ornithine to polyamines was more rapid in CSC-like cells. We performed a computational structure analysis and found that polyamines bind and inhibit demethylase site of LSD1 which is known to be a demethylase of histone H3K4 and a nuclear homolog of amine oxidases. Spermine was the strongest binding energy. An enzyme assay showed that LSD1 activity was dose-dependently inhibited by polyamines and spermine was the strong inhibitor. Finally, we analyzed performed ChIP sequencing analysis by using HeLa CSC-like cells and found that more global enrichment of H3K4me3 and less global enrichment of H3K4me1 than non-CSC-like cells. In conclusion, polyamines are rich in CSC, and be able to inhibit LSD1 activity and induce epigenetic alterations especially in H3K4 methylation in CSC.
Breast cancer is a leading cause of death in women and its metastatic spread to distant organs is responsible for over 90% of these deaths. Circulating tumor cells (CTCs) implicated in the metastatic process occur when cancer cells undergo cytoskeletal and phenotypic transitions that enhance motility, then penetrate into the blood circulation. While CTC dissemination has typically been considered as the spread of single cells, recent evidence suggests that collective migration results in polyclonal circulating clusters that are the main drivers of metastasis. To study circulating clusters we have developed a dual microfiltration system employing two stages of antigen-independent mechanical cell isolation. As whole blood is passed through the system, circulating clusters are trapped by the first stage while single CTCs are retained by the second stage. Characterization experiments using cell lines demonstrated a capture efficiency of 87.1% for clusters and 85.7% for single cells on their respective microfilters. This system was used to investigate circulating cells in the metastatic MMTV-PyMT murine breast cancer model. Both clusters and single CTCs were detected in mice with histological evidence of lung metastases (14-16 weeks old), but not in mice that had not yet developed metastatic tumors (<12 weeks old). Immunofluorescence assays were established to determine expression of CD24, CD40, cytokeratins, CD45, and PIAP. Tumor cells were identified as nucleated cells that expressed epithelial cytokeratins, but not the leukocyte antigen CD45. While PyMT cells with a CD90+/CD45− stem cell phenotype comprised only ~1.5% of tumors, they were observed in >50% of circulating clusters. The presence of cancer stem cells was correlated to cluster size, with smaller clusters containing a greater percentage of CD90+ cells. Isolated clusters will be further analyzed to quantify expression levels of cancer stem cell markers in individual constituent cells. The analysis of circulating clusters may provide crucial insight into the mechanisms of metastatic disease and provide the means to discover novel targets for therapeutic intervention.

#4777 Cancer stemness and resistance: Napabucasin (BBI-608) sensitizes stemness-high cancer cells to Paclitaxel by inhibiting the STAT3-MUC1 pathway. Harry A. Rogoff, Juying Li, Chiang Li. Boston Biomedical, Inc., Cambridge, MA.

Stemness-high cancer cells, or cancer stem cells (CSCs) represent a subpopulation of cancer cells with enhanced tumorigenic capacity, metastasis-forming potential, and resistance to conventional chemotherapy and radiation. One such key CSC pathway is regulated by STAT3, a transcription factor that is downstream of several cytokines and growth factor receptors which controls the expression of a range of genes in cancer biology in many tumor types. Cancer stemness inhibitor Napabucasin (BBI-608), a small molecule that inhibits gene transcription driven by STAT3, can inhibit stemness gene expression, block sporesgenesis, and kill CSCs. In vivo, BBI-608 effectively blocks cancer relapse and metastasis in xenograft models while sparing normal hematopoietic stem cells, suggesting that targeting stemness-high cancer cells is a feasible approach for developing next-generation cancer therapeutics to combat cancer recurrence. SOX2 (sex-determining region Y-box protein 2), a transcription factor that is essential for self-renewal and pluripotency, is amplified in various cancer types and has been shown to be required for CSC self-renewal and maintenance. Mucin 1 (MUC1), a glycoprotein normally expressed at the apical surface of epithelial cells, is overexpressed in most human epithelial cancers. MUC1 has been implicated in regulating tumor proliferation, metabolism, invasion, angiogenesis, chemoresistance, and inflammation. Here, we generated a stemness-high cell culture system based on the reporter activity of a SOX2-regulatory region construct in MKN28 gastric cancer cells (MKN28 SOX2-reporter GFP+ cells). BBI-608 treatment inhibited the STAT3-MUC1 pathway in stemness-high cells. High MUC1 status in stemness-high cells was associated with Paclitaxel resistance. Down-regulation of MUC1 sensitized stemness-high cells to Paclitaxel. Moreover, BBI-608 synergized with Paclitaxel in inhibiting sporesgenesis of stemness-high cells. This study provides a new mechanism for the association of cancer stemness with drug resistance. Our findings support this combination therapy, which pairs a conventional chemotherapy (Paclitaxel) with a cancer stemness inhibitor (BBI-608), as a promising strategy to combat cancer.

#4778 Telomere DNA damage promotes mammary gland fibrosis and estrogen receptor-positive breast cancer. Jianchun Wu, David L. Crowe. Univ. of Illinois Cancer Ctr., Chicago, IL.

Human breast luminal progenitor cells exhibit telomere DNA damage, which is associated with mammographic density and increased cancer risk. Telomeric repeat factor 2 (TRF2) protects telomeres from DNA damage response and its expression is reduced in human ductal carcinoma in situ and breast cancers. To determine the relationships between telomere DNA damage, mammary gland biology, and cancer risk we genetically deleted TRF2 expression in the mouse mammary gland. TRF2 conditional null mutant mice also were bred with tumor prone MMTV-Wnt1 animals. Telomere DNA damage response was determined by localization of 53BP1 at telomeres and activation of ATM/Chk2/p53 signaling by western blot. Mammary gland and tumor histopathology were determined by hematoxylin and eosin staining. Cell proliferation and ER+ differentiation was determined by immunohistochemistry. Telomere DNA damage was determined by labeling of 53BP1 at telomeres and activation of ATM/Chk2/p53 signaling by western blot. 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by some of the same major self-renewal pathways that drive embryonic stem cells, knowledge of other cellular pathways that drive CSC activity is still limited. In this work, we uncover a novel role for chemokine (C-C motif) ligand 20 (CCL20) in CSC growth and activity, expanding the role for this chemotactic protein. We discovered that CCL20 of many tumor types overexpresses CCL20, which we show to self-renew, self-initiate, and drive metastasis through its receptorCCR6. Targeting this axis with a neutralizing monoclonal antibody against CCL20 significantly reduces CSC frequency and tumor initiation and growth, supporting the novel therapeutic potential of targeting the CCL20-CCR6 axis as a strategy to eliminate CSCs. Furthermore, we found that CCL20 secreted by tumor cells also strongly influences the frequencies not only of T-cells, particularly immune-suppressive regulatory T-cells (T\(_{reg}\)) and Th\(_{17}\) populations, but also dendritic and neutrophil/gMDC populations, to promote anti-tumor immunity that leads to immune-mediated inhibition of tumor growth. Strikingly, we discovered that tumor-specific CCL20 impacted not just the immune cell balance within the tumor microenvironment, but had far reaching effects into the peripheral immune system as well. Thus, our overall findings propose that CCL20 plays a dual role in the tumor environment: 1) by driving activity and tumorigenic ability of CSCs, and 2) by promoting an immunosuppressive phenotype. In this work we show that therapeutic neutralization of CCL20 can deliver one-two punch against cancer, by directly inhibiting CSCs, and by relieving immunosuppression, thereby opening this target up as an exciting new strategy to treat cancer.

**#4780 Crucial role of S100A14 in blocking cancer-stem-cell activity and sensitizing to chemotherapy through STAT3 destabilization in human colorectal cancer.** Jaebeom Cho, Hye-Young Min, Ji-Sun Lee, Ho-Young Lee. Seoul National University, Seoul, Republic of Korea.

Colorectal cancer (CRC) is a major type of human cancers in terms of incidence and mortality worldwide. Chemotherapy is one of the standard protocols for the treatment of CRC but drug resistance is recognized as the main cause of treatment failure. Resistance to anticancer therapy is a multifactorial event involving a number of interrelated or independent mechanisms. Growing evidence supports a critical role of cancer stem cell (CSC) in development, progression, and resistance to anticancer therapies in CRCs. However, the molecular and cellular mechanisms underlying the CSC’s chemoresistance are not completely defined, and a comprehensive understanding of the biology of the CSCs is urgently needed. We have investigated mechanisms of chemoresistance, focusing on those mediated by CSCs, through the employment of CRC stem-like cell populations that are: 1) sorted out by using well-characterized stemness markers; ii) established via serial passagethroughincreasingdosesofoxaliplatin or 5-FU; or iii) enriched by culturing in sphere forming condition. We then assessed changes involved in CSC maintenance and found a novel role of S100A14 in CSCs of CRC. We found that transcriptional down-regulations of S100A14 expression conferred phenotypes of CSCs in the stem-like cell populations of CRC. Consistently, elevation of S100A14 expression sensitized the stem-like cell populations to the treatment with 5-FU in vitro and in vivo. Mechanistically, S100A14 directly interacted with STAT3 and promoted its degradation via the ubiquitin–proteasome pathway, leading to decreases in STAT3 target gene expression and a decrease in the forming capacity of the stem-like cell populations. Further, S100A14 expression was markedly downregulated in human CRC tissue specimens compared with their normal counterparts. Moreover, the expression level of S100A14 was inversely correlated with clinical outcomes in patients with CRC. These results collectively suggest that S100A14 is a novel target for the treatment of colorectal cancer by targeting CSCs.

**#4781 Epigenetic regulation of neuroblastoma tumorigenicity through MLL1 and JMJD3 modulation in cancer stem cells.** Saurabh Agarwal, Zaowen WenJiang,5 Betty Y.Kim, 3 Kyuson Yun6. 1The Jackson Laboratory, BarHarbor, ME; 2The Jackson Laboratory, Farmington, CT; 3Mayo Clinic, Jacksonville, FL; 4The Jackson Laboratory, ME; 5UT MD Anderson Cancer Center, Houston, TX; 6Houston Methodist Research Institute, Houston, TX.

Epigenetic regulators reduce NB tumorigenicity, metastasis and increase drug sensitivity. However, developing these strategies will pave the way for incorporating epigenetic inhibitors in current therapies for effectively improving long-term cure rates.


Cancer stem cells (CSCs) are a subpopulation of tumor cells that are uniquely capable of initiating and sustaining tumorigenesis, and they have also been implicated in driving disease recurrence after cancer therapy. We previously developed a novel lentiviral-based reporter system for direct visualization, quantitation and isolation of cells with CSC properties. The construct consists of a tandemly-repeated composite SOX2-OCT4 response element (“SORE6”) driving expression of a destabilized fluorescent protein reporter (Tang et al. Stem Cell Reports, 2015). TGF-βs are pleiotropic regulatory factors with complex roles in tumorigenesis. We and others have hypothesized that TGF-β can regulate the CSC population in breast cancer with stimulatory or inhibitory effects depending the model and stage of cancer development. Using the MCF10Ca1h and MCF7 breast cancer models, which retain tumor suppressor responses to TGF-β, here we show that TGF-β treatment significantly reduces the size of the CSC population in vitro. Although TGF-β had relatively little effect on invasion and migration of the bulk population in these models, it clearly inhibited migration and invasion of the CSCs, suggesting that biological responses to TGF-β can vary depending on the position of the cell in the differentiation hierarchy. Combining our stem cell reporter with a TGF-β pathway reporter, we further show that CSCs in the MCF10Ca1h model have higher endogenous activation of the TGF-β pathway than does the bulk tumor cell population, and by time-lapse video microscopy we find that CSCs with active TGF-β signaling are relatively quiescent. Furthermore, neutralization of TGF-β in vivo leads to an increased representation of CSCs in MCF10Ca1h and MCF7 tumors. Thus our preliminary results suggest that in breast cancer models where TGF-β acts as a tumor suppressor, TGF-β signaling is preferentially activated in the CSC compartment and keeps a subpopulation of CSCs in a quiescent and stationary state. These findings have important implications for the clinical use of TGF-β pathway antagonists. In contrast, in the metastatic breast cancer MDA-MB231 model, in which TGF-β acts as a pro-progression factor, we show that TGF-β can increase the size of the CSC population and enhance invasion. In this model, the CSCs are enriched for the ability to form macroscopic lung metastases when compared with non-CSCs, and we show that this effect is due in part to a selective survival advantage of the CSCs in the first 24 hours after arrival at the metastatic site. The role of TGF-β in this process is currently under investigation.
While growing evidence suggests that tumor microenvironment can modulate stemness and the mesenchymal phenotype, little is known about how these cell states are coordinately regulated. We report that S100A4 controls both stemness and mesenchymal transition in GBM cells. In a spontaneous mouse glioma model and in primary human GBM tumorspheres, S100A4 expression is enriched in tumor-initiating cells and is required for self-renewal and tumour growth. S100A4 expression is negatively correlated with the expression of mesenchymal markers, in primary human GBM tumorspheres and glioblastoma multiforme patients. Furthermore, Knockdown of S100A4 expression reduces the tumorigenic, migratory and invasive properties of glioblastoma cells. We also show that S100A4 expression is an independent predictor of poor overall survival in glioblastoma. These findings suggest a potential anti-metastatic role for S100A4 in glioblastoma and support the development of S100A4 inhibitors for the treatment of glioblastoma.

**#4786** Integrated exome and transcriptome sequencing identifies a novel candidate for breast cancer. Kyung-Min Lee,1 Dong-Yong Noh,2 Wonshik Han.1 Seoul National University, Seoul, Republic of Korea; 2Seoul National University College of Medicine, Seoul, Republic of Korea.

Breast cancer stem cells (BCSCs) exist in a dynamical equilibrium of mesenchymal (M) and epithelial-like (E) states that coordinates the differentiation of the tumor mass. BCSCs, like many other tumors, are capable of growth and maintenance in both M and E phenotypes, allowing for tumor heterogeneity and metastatic potential. Using gene expression microarray data from breast cancer samples, we identified transcription factors (TFs) that are specifically expressed in BCSCs. We found that S100A4 expression is significantly higher in BCSCs compared to non-BCSCs. S100A4 expression was also associated with poor prognosis and low survival rate. The unsatisfactory outcomes of breast cancer treatment are related to multiple factors including tumor recrudescence, metastasis, cancer drug resistance, etc. Breast cancer stem cells are cell subsets within a cancer that excel in tumor properties, especially survival, renewal and resistance to treatment. Reactive oxygen species (ROS) are high energy molecules produced during cellular hyperproliferation and cell death. Studies have shown though a low level of ROS is required to support stem cell functions but excessive ROS damages DNA and predisposes to cell death. Therefore, the ability to at-
tenue oxidative damage from ROS and maintain redox homeostasis is an important property of aggressive and resilient cancer cells. The multifunctional serine/threonine kinase calcium/calmodulin-dependent protein kinase II alpha (CaMK2A) is a calcium signaling molecule involved in cell growth and stress signals integration. It has been reported to play a tumor-supportive role in osteosarcoma, prostate cancer and leukemia. However, little is known about its role in TNBC tumors and the least favorable prognosis. Due to the absence of hormone receptors and Her2 receptor, no targeted therapy is available and chemotherapy is the standard treatment for TNBC patients. Paclitaxel is a widely used chemotherapy drug for various cancers with severe side effects due to its solvent. Abraxane is an albumin-bound nanoparticle of paclitaxel with less severe side effects. Here we show that Abraxane has superior activity than solvent-based paclitaxel in tumors expressing low levels of CaMK2A, increases cancer stem cells and decreases levels in CaMK2A-KD cells, implying Abraxane might be a mediator of CaMK2A in ROS regulation. To further identify downstream regulators, mRNA expression analysis of five ROS-regulatory NF2 target genes (GSP1, NQO1, GCLCL, HMOX1, TRXR1) was performed. Quantitative PCR results showed significant corresponding changes of some of these genes in cell lines, which upregulated CaMK2A overexpression and downregulated in both CaMK2A-KD cells and CaMK2A pharmacological inhibition cells. Together, the results indicate CaMK2A might regulate ROS status through mediating downstream target genes of NF2.

#4788 Abraxane eliminates cancer stem cells in triple-negative breast cancers.
Hebao Yuan, Hongwei Guo, Feng Li, Joseph Burnett, Miao He, Nathan Truchan, Ilia Myers, Duxin Sun. Univ. of Michigan, College of Pharmacy, Ann Arbor, MI.

Triple-negative breast cancer (TNBC) represents about 20 percent of all breast cancers. It is a poor survival rate and being associated with both the tumors and the least favorable prognosis. Due to the absence of hormone receptors and Her2 receptor, no targeted therapy is available and chemotherapy is the standard treatment for TNBC patients. Paclitaxel is a widely used chemotherapy drug for various cancers with severe side effects due to its solvent. Abraxane is an albumin-bound nanoparticle of paclitaxel with less severe side effects. Here we investigated whether cancer stem cells (CSCs) in TNBC response differentially to Abraxane and paclitaxel both in cell culture and xenograft mouse models. Using a series of human TNBC cell lines, we showed that there is no difference between solvent-based paclitaxel and Abraxane in vitro in terms of efficiency and eliminating cancer stem cells using MTS assay, mammosphere formation assay, and flow analysis of cancer stem cells. Interestingly, we demonstrated that Abraxane showed superior activity than solvent-based paclitaxel in human SUM149-L xenograft NOD SCID mouse model in adjuvant treatment. More important, we demonstrated that Abraxane eliminates cancer stem cells in xenograft NOD SCID mouse model in advanced treatment, while solvent-based paclitaxel increases cancer stem cells. Cell uptake assay indicated that TNBC cells take more Abraxane than solvent-based paclitaxel, which partially attributes to its superior efficacy in xenograft mouse models. Taken together, our data confirmed that Abraxane is superior to solvent-based paclitaxel and may be a better option for TNBC patients in adjuvant or metastatic treatment.

TUMOR BIOLOGY: Cancer Stem Cells

#4789 A novel MIF signaling pathway drives the malignant character of pancreatic cancer by targeting NR3C2.
Shouhui Yang,1 Peijun He,2 Liming Wang,1 Jian Wang,1 Aaron Schetter,2 Wei Tang,1 Naotake Funamizu,3 Katmann,6 Jens Werner,1 Nader Hanna,8 H.Richard Alexander,8 S. Szabo, Celia Chao, Mark R. Hellmich. University of Texas Medical Branch, Galveston, TX.

Introduction: We recently demonstrated that cystathionine-β-synthase (CBS) stimulates colon cancer bioenergetics, migration, invasion and angiogenesis and is increased in colorectal cancer compared to normal colonic mucosa. The role of CBS in carcinogenesis is still, however, unknown. Here we compare CBS protein levels and immunohistochemistry in human colonic biopsies at different stages of colorectal carcinogenesis. In addition, we used Azoxymethane (AOM) in CBS knockdown mice to study the development of aberrant crypt foci (ACF), a model of sporadic colorectal carcinogenesis. Methods: CBS protein expression was assessed in human biopsies of normal mucosa, tubular adenoma

Pancreatic cancers with aberrant expression of macrophage migration inhibitory factor (MIF) are particularly aggressive. To identify key signaling pathways that drive disease aggressiveness in tumors with high MIF expression, we analyzed the expression of coding and noncoding genes in high and low MIF-expressing tumors in multiple cohorts of pancreatic ductal adenocarcinoma (PDAC) patients. The key genes and pathways identified were linked to patient survival and were mechanistically, functionally, and clinically characterized using cell lines, a genetically engineered mouse model, and PDAC patient cohorts. Here, we report evidence of a novel MIF-driven signaling pathway that inhibits the orphan nuclear receptor NR3C2, a previously undescribed tumor suppressor that impacts aggressiveness and survival in PDAC. Mechanistically, MIF upregulated miR-301b that targeted NR3C2 and suppressed its expression. PDAC tumors expressing high levels of MIF displayed elevated levels of miR-301b and reduced levels of NR3C2. In addition, reduced levels of NR3C2 expression correlated with poorer survival in multiple independent cohorts of PDAC patients. Functional analysis showed that NR3C2 inhibited epithelial-to-mesenchymal transition and enhanced sensitivity to the gemcitabine, a chemotherapeutic drug used in PDAC standard of care. Furthermore, genetic depletion of MIF disrupted a MIF-mir-301b-NR3C2 signaling axis, reducing metastasis and prolonging survival in a genetically engineered mouse model of PDAC. Taken together, our results offer a preclinical proof of principle for candidate therapies to target a newly described MIF-miR-301b-NR3C2 signaling axis for PDAC management.
and carcinoma in situ by Western blotting. Immunohistochemical (IHC) staining was used to assess CBS levels in formalin-fixed/paraffin-embedded specimens of normal colonic mucosa, adenomatous polyps and colon adenocarcinoma. To assess the effect of CBS gene dosage on azoxymethane (AOM)-induced aberrant crypt foci (ACF) formation CBS heterozygous mice (CBS+/−) and wild-type controls were co-fed AOM at 10mg/kg via intraperitoneal injection once per week for 5 weeks. At the end of 16 weeks, the colons were harvested and ACF visualized with methylene blue staining by 3 independently blinded observers. Significance (p ≤ 0.05) was determined using GraphPad Prism 7 software. Results: Consistent with our previously reported findings CBS levels were relatively low in biopsies of normal mucosa. By comparison, it was elevated in polyps exhibiting both low- and high-grade dysplasia. IHC staining of normal mucosa and hyperplastic polyps revealed CBS immunoreactivity in a small number of cells located along the basal lamina aspect of the colonic crypts in both normal and hyperplastic polyps. A slight increase in cytoplasmic CBS staining also was noted in the epithelial cells of hyperplastic polyps. In contrast, the epithelial cells of tubular adenoma specimens exhibited higher levels of diffuse cytoplasmic CBS staining with frequent focal areas of intense staining adjacent to mucin-containing vesicles. Sections of adenocarcinoma exhibited diffuse CBS staining throughout the cytoplasm of the cancer cells. AOM treatment induced significantly less ACF in the colons of CBS+/− mice compared to wild-type controls. The loss of one allele of CBS reduced the number of AOM-induced ACF by half. The mean number of AOM-induced ACF (CBS+/−) and CBS−/−) were 10.9 ± 5.2 and 5.6 ± 2.5 (p < 0.05). Conclusion: CBS protein levels increase and the pattern of expression changes with progression from benign hyperplastic polyps to invasive adenocarcinoma, suggesting the enzyme may play a functional role in colorectal carcinogenesis. In addition, CBS single allele knockdown significantly reduces ACF formation. CBS, and its product H2S, may, therefore, be a driver of carcinogenesis.

**#4792 Prmt6 promotes lung tumor growth via modulation of If2 expressions.** Sreedevi Avasarala, Pei-Ying Wu, Michelle Van Scyoc, Abinaya Baskaran, Yanlin Su, Odile David, Robert A. Winn, Kamesh Bikkavilli. University of Illinois at Chicago, Chicago, IL.

Lung cancer kills more people in the US than any other malignancy. High mortalities are in part due to the diagnosis at an advanced inoperable stage where the 5-year survival is only 4%. Therefore improving survival will require increased knowledge of genes that drive the complex mechanisms of lung cancer initiation and progression. Emerging data implicate novel roles for protein arginine methylation in lung cancers. In protein arginine methylation, nitrogen/s of arginine can be post-translationally modified via the addition of a methyl group, catalyzed by a class of enzymes, protein arginine methyl transferases (PRMTs). Protein arginine methyl transferase 6 (PRMT6) is a methyltransferase that modifies histone tails, and its expression is upregulated in lung cancers correlating with a worse overall patient survival. However, the in vivo role of PRMT6 has never been examined. In this study, we show that targeted overexpression of PRMT6 in the mouse lungs (PRMT6 gain-of-function mouse model) results in an increased chemical carcinogen-induced lung tumorigene-
sis. We also performed proteomics on PRMT6-associated proteins from the mouse lung and identified Interleukin- enhancer binding protein 2 (IL2F2) a.k.a. nuclear factor 45 (NF45) as a novel PRMT6 interacting protein. By using a battery of cell-based assays, we show that PRMT6 plays an essential role in the regulation of the pro-proliferative functions of IL2F2. Therefore, targeting PRMT6/IL2F2 axis might open new possibilities for the therapeutic intervention of lung cancer.

**#4793 TRAF2 is required for the survival of ErbB2-transfected mammary tumor cells.** Hasem Habehlzah, Yumei Fan, Laiqun Zhang. Univer. of Iowa, Iowa City, IA.

TRAF2 regulates signaling pathways downstream of many members of the TNF receptor superfamily, such as TNFR1 and CD40. Recently, we found that TRAF2 also plays an important role in breast cancer cell survival under conditions of endoplasmic reticulum (ER) stress. It is known that TRAF2 knockout (KO) mice die within a week of birth and this lethality can be rescued by KO of TNFα or TNFR1. In order to determine the role of TRAF2 in mammary tumor development and progression, we crossed MMTV-ErbB2 mice (FVB) with TNF−/−/TRA2−/− mice (B6), and generated MMTV-ErbB2 (ErbB2), MMTV-
ErbB2-TNF−/− (ErbB2/T-KO) and MMTV-ErbB2-TNF−/−/TRA2−/− (ErbB2/T2-DKO) mice all in FVB/B6 mixed background. In 43 weeks, only 1 of 10 ErbB2/T2-DKO mice developed a mammary tumor, while 50% of ErbB2 and ErbB2/T-KO mice developed multiple and larger tumors in 30-32 weeks. Interestingly, the tumor derived from the ErbB2/T2-DKO mice did not show decreased cyclin D1 expression, nor the tumor cells cultured in vitro show decreased NF-κB activation in response to RANKL. In addition, ErbB2/T2-DKO cells formed mammampheses of similar appearance with that of ErbB2 cells in HEMA-coated plates. However, after dispersion of the mammampheses into single cells, ErbB2/T2-DKO cells formed dead cell aggregates, while ErbB2 and ErbB2/T-KO cells formed live cells aggregates indicating that the self-renewal capacity of ErbB2/T2-DKO cells is impaired. Mechanistic studies reveal that ErbB2/T2-DKO cells are sensitive to death caused by ER stress but not by TRAIL or DNA damage, and that this sensitivity is significantly inhibited by knockdown of RIP1. Collectively, these findings suggest that TRAF2 plays a critical role in mammary tumor development and progression by suppressing the pro-death activity of RIP1, and that TRAF2 and RIP1 interaction could be an attractive target for development of new anti-breast cancer drugs.

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**#4794 Reduced Epstein-Barr virus replication in human papillomavirus-immortalized keratinocyte organotypic raft culture.** Joseph T. Guidry, William K. Songock, Xiaohui Ma, Cerie-Ann O. Nathan, Jason M. Bodily, Rona S. Scott. LSU Health Sciences Center - Shreveport, Shreveport, LA.

An epidemic rise in oropharyngeal squamous cell carcinoma (OSCC) infected with human papillomavirus has been observed over the past decade. The majority of HPV + OSCC arise from the tonsils and base of tongue (BOT), which are lymphoid-rich regions that are known to harbor Epstein-Barr virus (EBV). Indeed, we previously reported HPV/EBV co-infection in 25% of tonsillar and 70% of BOT tumors. HPV is a DNA tumor virus that specifically infects proliferating basal cells in stratified squamous epithelia. HPV replication is facilitated by viral oncoproteins E6 and E7, which create an S-phase-like environment via deregulation of cell cycle regulators p53 and Rb, respectively. EBV is also an established DNA tumor virus that replicates in differentiated epithelia in its normal life cycle. However, EBV exhibits a latent infection characterized by oncoprotein expression and lack of viral replication in EBV-associated carcinomas. E6/E7-mediated alteration of the cell cycle results in an interruption in epithelial differentiation, which is required for EBV replication in epithelial cells. Thus, we hypothesize that HPV immortalization of keratinocytes shifts the EBV life cycle from replication to latency. To test our hypothesis, we developed an EBV/HPV co-infection model utilizing organotypic raft culture to produce stratified epithelia in vitro. Tonsil keratinocytes immortalized by HPV were EBV-infected by co-culture with IgG-induced EBV-positive Akata Burkitt’s lymphoma cells four days after differentiation induction. Raft tissues were harvested 6 days post-EBV infection for analysis of viral life cycles. EBV infection did not alter HPV replication or gene expression. In HPV-negative tonsil keratinocytes, robust EBV replication was observed that was blocked by treatment with acyclovir, a nucleoside analog that inhibits EBV lytic replication. In contrast, a dramatic decrease in EBV DNA levels was observed in HPV-positive keratinocytes, which was not further reduced by acyclovir. Human telomerase-immortalized normal oral keratinocytes supported robust EBV replication in organotypic raft culture suggesting a specific effect of HPV rather than immortalization on EBV replication. Expression of E7 alone was sufficient to decrease EBV DNA levels. Preliminary data suggest no change in EBV infectivity between HPV-negative and HPV-positive rafts, and an increase in EBV latency-associated gene expression in HPV-positive rafts. We established an EBV co-infection model utilizing organotypic raft culture to produce stratified epithelial rafts expressing HPV and EBV. Tonsil keratinocytes immortalized by HPV were EBV-infected by co-culture with IgG-induced EBV-positive Akata Burkitt’s lymphoma cells four days after differentiation induction. Raft tissues were harvested 6 days post-EBV infection for analysis of viral life cycles. 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(CPT-11) and peripheral neurotoxicity induced by oxaliplatin were further investigated. CPT-11 induced intestinal toxicity in wild type mice but did not significantly affect hepatocyte or liver toxicity was observed; however, CPT-11 treatment in Nfr2−/− mice caused severe intestinal toxicity and pronounced steatohepatitis, associated with dramatically elevated serum biomarkers, accumulation of fat in liver and desmin expression. Mice lacking Nfr2 exhibited significantly decreased Mcell-induced intestinal toxicity and oxaliplatin-induced peripheral neurotoxicity, while did not impact the therapeutic efficacy of drugs. Taken together, our results demonstrate that the toxicities of some of chemotherapeutic drugs currently in clinical usage are significantly affected by Nfr2, therefore it is possible to personalize therapeutic regimens according to Nfr2 expression in tumors and normal tissues. Specifically, CPT-11-induced steatohepatitis and intestinal toxicity and oxaliplatin-induced peripheral neurotoxicity are significantly determined by Nfr2, and Nfr2 could be selectively targeted to prevent or treat chemotherapy-induced toxicities. The present works were supported by the NSFC (No. 81272468, 91429305 and 81372266).


The small endogenous signaling molecule, nitric oxide (NO) influences numerous physiological conditions including cancers. We have previously shown that different steady state NO levels activate pro-tumor signaling cascades. In this study, we explored the effect(s) of chronic NO exposure on breast epithelial cells as defined by changes in genomic stability, RNA and protein expression, and mostly importantly altered cell phenotype. Human breast epithelial cells (MCF10A) were chronically exposed to various concentrations of the NO donor DETA/NO for a three weeks. Distinct patterns of genomic alteration in the target genes (TP53, KRAS & PIK3CA) of exposed cells as compared to the background mutations were observed. In addition, quantitative real time PCR revealed increased expression of cancer stem cell (CSC) markers including NANOG, CD44, CXCR4 and OCT4 at 300 μM DETA/NO exposure. While altered cell morphology was observed in cells chronically exposed to 300-500 μM DETA/NO, increased motility occurred in cells cultured in 100 μM DETA/NO. Strikingly, these 100 μM NO-exposed cells grew in serum-free media; selected clonal populations as well as pooled cells formed colonies in soft agar that were disorganized and resembled cancer-like-clusters. In conclusion, these results implicate a precise tuning of microenvironmental NO levels that shift non-transformed breast epithelial cells toward cancerous phenotypes. The elucidation of underlying mechanisms may lend a new perspective regarding therapeutic approaches that redirect the cellular response(s) of tumor cells.

#4797 Continuous hepatocyte apoptosis accelerates diethylnitrosamine-induced liver tumor development. Yasutoshi Nosaki, Hayato Ikita, Satoshi Tanaka, Yuta Myoijin, Sadatsugu Sakane, Kazuhiro Murai, Yuto Shiodo, Yugi Kai, Yuki Makino, Tasuku Nakabori, Yoshinobu Saito, Takahiro Kodama, Ryoitaro Sakamori, Tomohide Tatsumi, Eiji Miyoshi, Tetsuo Takahara. Osaka University Graduate School of Medicine, Suita Osaka, Japan.

Background and Aim: Apoptosis serves as an important mechanism for removing DNA-damaged cells; and is considered to inhibit carcinogenesis. Hepatocyte apoptosis is a key feature of chronic liver disease, as established pre-cancerous condition for liver cancer. The present study examined the impact of continuous hepatocyte apoptosis on liver tumor development. Methods: We used male hepatocyte-specific knockout (KO) mice of Mcl-1, one of anti-apoptotic proteins, as a model of continuous hepatocyte apoptosis, and hepatocyte-specific wild type or double KO mice of Bak and Bax, pro-apoptotic proteins, as a model of apoptosis-resistant liver. To induce liver carcinogenesis those mice were intraperitoneally administered 20 mg/kg diethylnitrosamine (DEN) at 2 weeks. Results: Among control group treated with PBS, neither Mcl-1 KO nor wild-type (WT) mice developed macroscopically and microscopically any liver tumors at age of 6 months. On the other hand, while only 8% (2/26) of DEN-treated WT mice developed macroscopic liver tumors, 100% (15/15) of Mcl-1 KO mice developed (p<0.05). Macroscopically, 57% (15/26) of DEN-treated WT mice developed liver tumors, 100% (15/15) of Mcl-1 KO mice developed (p<0.05). These tumors were histologically classified as well-differentiated HCC. The average of maximum tumor size (12.4 vs. 6.5 mm), tumor numbers (21.4 vs. 4.0) and liver weight to body weight ratio (12.3 vs. 4.2 %) in Mcl-1 KO mice were significantly higher than those in WT mice. At 6 weeks, serum ALT levels and caspase 3/7 activity of DEN-treated Mcl-1 KO mice were significantly higher than those of DEN-treated WT mice. Immunohistochemistry revealed that PCNA, Ki67, phospho-HAX and 8-OHdG positive cells significantly increased in DEN-treated Mcl-1 KO mice compared with DEN-treated WT mice. The mRNA expression levels of TNF-alfa and Mcp-1 in DEN-treated Mcl-1 KO mice were significantly higher than those of DEN-treated WT mice.In contrast, macroscopic liver tumor incidence rate in WT, Bak KO, Bax KO and Bak/Bax double KO mice treated with DEN at 9 months was 46 (6/13), 40 (4/6), 35 (7/20) and 52% (11/21), respectively. Microscopic liver tumor incidence rate in WT, Bak KO, Bax KO and Bak/Bax double KO mice treated with DEN at 12 months was 70 (14/20) and 81% (17/21), respectively. There was no statistically significant difference in incidence rate of both macroscopic and microscopic tumors among 4 groups. At age of 6 weeks, there was no significant difference in serum ALT levels and caspase 3/7 activity among 4 groups. Conclusion: Continuous hepatocyte apoptosis is suggested to promote DEN-induced liver tumor development. Increased liver damage, compensative hepatocyte proliferation, genomic instability and inflammation may accelerate the incidence of DEN-induced liver tumorigenesis. In contrast, inhibition of apoptosis does not make much impact on DEN-induced liver tumor development.

#4798 Genetically engineered mouse models of proliferative C5 high grade serous ovarian cancer. Clare L. Scott,1 Gwo Y. Ho,1 Elizabeth E. Lischke,2 Olga Kondrashova,1 Ronny Drapkin,2 Matthew J. Wakefield,1,2 Walter E. Elias Hall Inst. Med. Research, Melbourne, Australia. 1University of Pennsylvania Perelman School of Medicine, Philadelphia, PA; 2Peter MacCallum Cancer Centre, Parkville, Australia.

Introduction: High-grade serous ovarian cancer (HGSOC) can be divided into four subgroups based on molecular characteristics.1-2 The proliferative/C5 subgroup is defined by MYCN-pathway activation and may be associated with stem cell-like behavior.1 The majority of HGSOC originate from the secretory cells of the fallopian tube (FT), with both tumours and secretory cells characterised by PAX8 expression.3 We have developed pre-malignant C5 sub-type Genetically Engineered Mouse Models (GEMM) to characterise key molecular events in the initiation of this sub-type and to provide an immune-competent model for testing therapeutics. Methods: We have generated independent GEMM with p53 dysfunction (p53fl/fl;R172H) knock-in4 and over-expression of the MYCN-pathway, using either Isl-MYC or Isl-lin28b transgens5 directed by the doxycycline-inducible PAX8 promoter. Murine FT were harvested following two-weeks of transgene activation. One FT was harvested en bloc and paraffin embedded for analysis by H&E and immunohistochemistry for the presence of pre-malignant tubal lesions. The contralateral FT was micro-dissected into distal/mid-FT, which were snap frozen for analysis of gene expression by qRT-PCR and RNAseq. As mice with PAX8-directed expression of oncogenes (≥MYCN/lin28b overexpression) developed enlarged kidneys due to renal tubular adenoma, for alternate mice, the mid/distal FT tissue was implanted into the bursae of wild-type CBA/nu mice for observation and later analysis. Results: FT were harvested from twelve p53fl/fl;R172H/Isl-MYC mice and from six p53fl/fl;R172H/Isl-lin28b mice and from five p53fl/fl;R172H/Isl-MYC mice lacking relevant control mice. Dysplastic lesions were observed in a subset of p53fl/fl;R172H/Isl-MYC or Isl-lin28b FT and not in controls. MYCN pathway activation was demonstrated by qRT-PCR analysis. Conclusion: Over-expression of the MYCN-pathway can drive FT epithelial dysplasia in the presence of p53 dysfunction. RNAseq analysis is being performed to elucidate early genetic events in dysplastic FT. Mice are now being harvested after eight-weeks of doxycycline activation to determine whether more advanced pre-malignant change occurs. CRISPR guides deleting relevant genes including p53, PTEN, NFI or Rb1 were generated to delete additional genes in MYCN pathway-transgenic FT, followed by implantation for observation of tumourigenesis.

#4799 Fibroblast growth factor receptor (FGFR) promotes progression of cutaneous squamous cell carcinoma. Alok R. Khandelwal,1 Xiaohua Rong,1 Tara Moore-Medlin,1 Xiaohua Ma,1 Amelia Warner,1 John DiGiovanni,2 Che-Ann O. Nathan.1 1LSU Health-ShV, Shreveport, LA; 2University of Texas at Austin, Austin, TX.

Cutaneous squamous cell carcinoma (cSCC) is a keratinocyte-derived invasive and metastatic tumor of the skin. It is the second-most commonly diagnosed form of skin cancer (16%) striking 200,000 Americans annually. Increased understanding of the mechanisms involved in pathogenesis of cSCC could identify means to prevent, inhibit and reverse this process. In our previous studies, inhibition of Fibroblast growth factor receptor (FGFR) significantly decreased Ultraviolet B-induced (UVB) epidermal hyperplasia and hyperproliferation in
SKH-1 mice suggesting an important role of FGFR signaling in skin cancer prevention. However, the role of FGFR signaling in the progression of cSCC is not yet elucidated. Analysis of the expression of FGFR2 in cSCC cells and normal epidermal keratinocytes revealed overexpression and increased FGFR2 activity in cSCC cells. Tumor cell-specific overexpression of FGFR2 was detected in human cSCC and in UVB-induced mouse cSCC (20 SCC, 10 ADC and 3 LCC). All PDX models mostly retain the principal histologic and molecular characteristics of their donors and recapitulate the heterogeneity of human lung tumors. In our PDX collection we have sufficiently characterized all the NSCLC histology and the most relevant molecular alterations in lung cancer in order to perform precision medicine evaluation studies.

Conclusions: We have generated a collection of 42 PDX models of NSCLC characterized at the histological, genomic and transcriptomic level, which represents the most frequent histological and molecular subtypes of this type of lung cancer. This collection will be really useful to integrate drug screening with biomarker discovery and to evaluate precision therapeutic strategies. Our future aim will be to use this collection for biomarkers identification and preclinical evaluation of new therapeutic strategies targeted to bad prognostic lung tumors with suboptimal therapeutic approaches.

#4802 Immune dysfunction and fungal infection contribute to esophageal carcinogenesis. Feng Zhu,1 Jamii Willette-Brown,1 Na-young Song,1 Pelin Zhang,1 Yinling Hu1. NCI, NIH, Frederick, MD; 2PZM Diagnostic, Charleston, WV.

Human esophageal cancer is the sixth leading cause of cancer death worldwide. More than 90% of esophageal cancer is esophageal squamous cell carcinoma (ESCC). While the etiological causes remain unclear, esophageal mucosal fungal infection is very common in esophageal cancer patients, including patients with autoimmune polyendocrinopathy candidiasis ectodermal dysrophy (APECED) who have more than 20-fold risk of ESCC compared to the healthy population. Here, we report that kinase-dead Ikkα knock-in mice (hereafter referred to as mutant mice) develop APECED-like autoimmune disease due to developmental defects in thymus and lack of central tolerance. Similar to human APECED patients who develop esophageal mucosal fungal infection on average at the age of five, mutant mice develop fungal infection in oral cavity and esophagus as early as seven weeks old. About 20% of mutant mice develop ESCC during aging, which exhibits specific molecular signatures observed in human ESCC, such as p16 gene silencing, elevated EGFR phosphorylation and PD-L1 expression, etc. Autoreactive T cell depletion, or central tolerance reconstitution and/or normal T cell transfer prevents fungal infection and ESCC development in mutant mice. Importantly, antifungal drug treatment inhibits inflammation and ESCC development in mutant mice. However, oral inoculation of Clas-doporium, one of the major fungal species isolated from mutant mice, increases the tumor incidence from 20% to 63% in mutant mice. These results reveal that immune dysfunction and fungal infection is associated with ESCC development, which sheds new light on ESCC etiological factors, prevention and treatment.

#4803 Over-expression of thrombopoietin in the liver of transgenic mice with liver-specific human BrafV600E expression. Hiroki Tanaka,1 Kie Ho-rioka,1 Masahiro Yamamoto,1 Katsuhiro Okuda,1 Masaru Asari,1 Katsuhiro Okuda,1 Seiji Ohtani,1 Kosuke Yamazaki,2 Keiko Shimizu,1 Katsuhiro Ogawa,1 1Aisahikawa Medical Univ., Asahikawa, Japan; 2Japanese Red Cross Hokkaido College of Nursing, Kitami, Japan.

The BrafV600E mutation, which corresponds to the human BRAFV600E mutation, plays a pivotal role in mouse hepatic carcinogenesis induced by neonatal treatment with diethylnitrosamine in B6C3F1 mice. We previously established the transgenic mice that express the human BRAFV600E mutation in the liver (Mol Carcinog 2016. doi:10.1002/mc22510). The transgenic mice showed 5 folds increase in liver/body weight as compared to normal mice, and their liver was entirely consisted of small basophilic hepatocytes resembling DEN-induced neoplastic hepatocytes. The transgenic mice were normally born, but most of them spontaneously died during 2-3 months after birth. In this study, we investigated the reason for spontaneous death in the liver-specific BrafV600E transgenic mice. The numbers of platelets and megakaryocytes were remarkably increased respectively in peripheral blood and bone marrow in the transgenic mice. Furthermore, fragmented red blood cells and activated platelets with enlarged size were frequently observed in the peripheral blood smear of the transgenic mice. Thrombopoietin mRNA was abundantly expressed in the liver of transgenic mice, and the conditioned-medium of the cultured hepatocytes from the transgenic mice contained increased amount of thrombopoietin as compared with that of normal mouse hepatocytes. Histopathological analysis re-

SKH-1 mice suggesting an important role of FGFR signaling in skin cancer prevention. However, the role of FGFR signaling in the progression of cSCC is not yet elucidated. Analysis of the expression of FGFR2 in cSCC cells and normal epidermal keratinocytes revealed overexpression and increased FGFR2 activity in cSCC cells. Tumor cell-specific overexpression of FGFR2 was detected in human cSCC and in UVB-induced mouse cSCC (20 SCC, 10 ADC and 3 LCC). All PDX models mostly retain the principal histologic and molecular characteristics of their donors and recapitulate the heterogeneity of human lung tumors. In our PDX collection we have sufficiently characterized all the NSCLC histology and the most relevant molecular alterations in lung cancer in order to perform precision medicine evaluation studies.

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The BrafV600E mutation, which corresponds to the human BRAFV600E mutation, plays a pivotal role in mouse hepatic carcinogenesis induced by neonatal treatment with diethylnitrosamine in B6C3F1 mice. We previously established the transgenic mice that express the human BRAFV600E mutation in the liver (Mol Carcinog 2016. doi:10.1002/mc22510). The transgenic mice showed 5 folds increase in liver/body weight as compared to normal mice, and their liver was entirely consisted of small basophilic hepatocytes resembling DEN-induced neoplastic hepatocytes. The transgenic mice were normally born, but most of them spontaneously died during 2-3 months after birth. In this study, we investigated the reason for spontaneous death in the liver-specific BrafV600E transgenic mice. The numbers of platelets and megakaryocytes were remarkably increased respectively in peripheral blood and bone marrow in the transgenic mice. Furthermore, fragmented red blood cells and activated platelets with enlarged size were frequently observed in the peripheral blood smear of the transgenic mice. Thrombopoietin mRNA was abundantly expressed in the liver of transgenic mice, and the conditioned-medium of the cultured hepatocytes from the transgenic mice contained increased amount of thrombopoietin as compared with that of normal mouse hepatocytes. Histopathological analysis re-
Sex-specific differences in Benzo(a)Pyrene [B(a)P]-induced colon carcinogenesis. Kenneth J. Harris,1 Kelly L. Harris,1 Mary K. Washington,2 James Amos-Landgraf,3 Aramandal Ramesh1. 1Meharry Medical College, Nashville, TN; 2Vanderbilt University, Nashville, TN; 3University of Missouri, Columbia, MO.

Colorectal cancer (CRC) is the third most common diagnosed cancer and the third leading cause of cancer-related deaths in the United States. It has also been reported that colon cancer incidence and mortality rates are higher in men than women, but there is yet a determined mechanistic link to show the factors that underlie the sex-specific differences in CRC initiation and progression. Benzo(a)pyrene [B(a)P], a member of the polycyclic aromatic hydrocarbon (PAH) family of compounds is a well-characterized environmental toxicant that has been proven to be a major contributor to the development of sporadic colon cancer. Published studies indicate that Aryl Hydrocarbon Receptor (AhR), a receptor for [B(a)P], bind to estrogen receptor (ER) and negatively affect AhR-targeted gene transcription. This study aims to elucidate the sex-specific differences in B(a)P-induced colon cancer in adult Polyposis In The Rat Colon (PIRC) model. We hypothesize that sex-specific differences in B(a)P biotransformation modulates the formation of colon tumors in PIRC rats. Groups of female and male PIRC rats (n = 8) received sub-chronic exposure to 25, 50 and 100 μg B(a)P/kg body wt. via oral gavage for 60 days. Female and male rats that received no [B(a)P] treatment served as controls. [B(a)P] was shown to have no significant effect on body weight of these rats and female PIRC rats that received 25, 50 and 100 μg B(a)P/kg body wt. showed significant decrease in total polyp count when compared to males with respective treatments. Polyp sizes of female PIRC rats receiving 25, 50 and 100 μg B(a)P/kg body wt. were increased when compared to males respectively. Histopathological analysis of colon polyps revealed that female animals exhibited low-grade to no dysplasia while high-grade dysplasia was recorded in male animals treated with corresponding doses. Phase 1 enzyme, Cytochrome P450 isoform 1A1 (CYP1A1), and phase 2 enzyme, Sulfo-transferase Family 1A Member 1 (SULT1A1), were downregulated in colon tissue of female PIRC rats receiving 25, 50 and 100 μg B(a)P/kg body wt. when compared to male counterparts. In future studies, by measuring the expression of the other phase 1 and phase 2 drug metabolizing enzymes (DME), along with measuring circulating estrogen levels, analyzing [B(a)P] metabolite profile, and probing [B(a)P]-DNA interactions, we will provide insight into if and how estrogen receptor protects females from developing colon cancer. This research was funded by NIH grants 5R01CA12845-04, 5R25GM059994-3, and G12MD007586-29.

The role of exosomes in fibrosarcoma progression. Miho Nakajima,1 Stephen C. Searles,2 Ayuko Hoshino,1 Katherine M. Offer,1 Candia M. Kenfic,1 Jack D. Bui,1 David C. Lyden,1 Weil Cornell Medical Center, New York, NY; 2University of California, San Diego, La Jolla, CA.

Among pediatric cancers, sarcomas, especially those with large tumor burdens and metastatic disease, often result in poor outcome. Thus, new treatments are urgently needed to inhibit tumor progression, prevent metastasis, and improve overall survival. To understand the mechanisms driving sarcoma progression, we employed two mouse fibrosarcoma cell lines that display different growth phenotypes when transplanted into syngeneic immune competent mice. Progressor fibrosarcomas evade detection by the immune system and develop large tumor burdens, while regressor fibrosarcomas regress shortly after a period of limited tumor growth. This difference in the growth phenotype is mediated in part by immune cells, but the mechanisms by which progressor and regressor cells influence immune cell activity are not fully elucidated. Our research has focused on exosomes, 50-100 nm secreted nanovesicles, that contain bioactive cargoes and have emerged as mediators of intercellular communication between various cells. Here, we hypothesize that fibrosarcoma exosomes determine the aggressiveness of the disease by either educating tumor cells themselves or altering interactions with host cells. To investigate the role of fibrosarcoma exosomes, we first educated regressor tumor cells with progressor exosomes for 3 weeks in vitro, and vice versa. Upon in vivo inoculation, progressor cells conditioned with regressor cell-derived exosomes grew significantly slower compared to untreated progressor cells. In contrast, regressor cells educated with progressor exosomes did not regress and overgrew significantly compared to untreated regressor cells. The differences in tumor growth were paralleled by changes in the tumor microenvironment that favor tumor growth and proliferation of neoplastic hepatocytes. (Supported by the grant from Japanese Ministry of Education, Culture, Sports and Science Technology.)

Strategies to overcome the heterogeneity of tumor cells in breast cancer therapy. Gloria M. Calaf,1 Marcela Callardo,2 Debasish Roy,3 Richard Ponce-Cusi.1 1Universidad De Tarapacá, Arica, Chile; 2The City University of New York, New York, USA.

Breast carcinogenesis is a multistage process that involves mutations and alterations attributed to exposure to exogenous environmental substances and endogenous agents as female hormones. To overcome the heterogeneity of tumor cells in breast cancer therapy several strategies must be considered from the bench to clinical settings. It is proposed four strategies: Analysis to determine 1) Apoptosis, 2) Epithelial-mesenchymal transition (EMT), 3) CD44/CD24 gene and protein expression in cells derived from mammospheres and 4) MicroRNAs as miR34a and others by the effects of chemotherapeutic drugs (as pamidronate (Pam), 5-Fluorouracil (5-FU) and antioxiants as Curcumin (Cur) (diferuloylmethane) derived from Curcuma longa. Pam, a bisphosphonate is used in the treatment of breast cancer. 5-FU is a chemotherapeutic agent for the treatment of a variety of solid cancers that arrest cell cycle and induce apoptosis in cancer cells. We evaluated genes and proteins targeted by these drugs and antioxiants in a triple positive cell line, as MCF7 and a negative, MDA-MB-231 for hormonal receptors, respectively and in an in vitro breast cancer model induced by radiation and estrogen that was developed with a normal immortalized breast epithelial cell line, MC6, exposed to low doses of high LET (linear energy transfer) alpha particles (150 keV/μm) of radiation, and cultured in presence of 17β-estradiol. We used: i) MCF-10f, ii) Alpha3, a malignant non-tumorigenic, iii) Alpha5, a tumorigenic one and iv) Tumor2 cell line derived from Alpha5 injected into the nude mice. Previous results showed increased cell proliferation, anchorage independency, invasive capabilities and tumor forma-

Braf mutations initiate the development of rat gliomas induced by postnatal exposure to N-ethyl-N-nitrosourea (ENU). Kazuki Sato,1 Qi Wang,2 Ji Eun Oh,1 Barbara Hutter,2 Benedikt Brors,2 Nicole Diesel,2 Hai-Kun Liu,3 Stephen Wolf,2 Otmar Wiestler,2 Paul Kleihues,3 Bernd Koelsch,4 Andrea Kindler-Rohrborn,4 Hiroko Ohgaki1. 1International Agency for Res. on Cancer, Lyon, France; 2German Cancer Research Center (DKFZ), Heidelberg, Germany; 3Heidelberg University, Heidelberg, Germany; 4Heimholtz-Zentrum Deutsches Forschungszentrum, Berlin, Germany; 5University Hospital Zurich, Zurich, Switzerland; 6University Hospital Essen, Essen, Germany.

Purpose: A single dose of N-ethyl-N-nitrosourea (ENU) during late prenatal or early postnatal development induces a high incidence of malignant schwannomas and gliomas in rats. Although T- > A mutations in the transmembrane domain of
the neu (c-ErbB-2) gene are the driver mutations in ENU-induced malignant schwannomas, the molecular basis of ENU-induced gliomas was unknown. The objectives of this study were to identify driver mutations in ENU-induced rat gliomas. Methods: We performed whole-genome sequencing of gliomas that developed in three BDIV and two BDIX rats exposed to a single dose of 80 mg ENU/kg body weight on postnatal day one. Results: TA->AT and TA->CT mutations, which are typical for ENU-induced mutagenesis, were predominant (41-55% of all somatic single nucleotide mutations). T->A mutations were detected in all 3 rat gliomas at Braf codon 545 (V545E), which corresponds to the human BRAF V600E. Additional screening revealed that 33 gliomas in BDIV rats and 12 gliomas in BDIX rats all carried a Braf V545E mutation, while peritumoral brain tissue of either strain (n=16) had the wild-type sequence. The gliomas were immunoreactive to BRAF V600E antibody. Conclusions Braf mutation is a frequent early event in the development of rat gliomas caused by a single dose of ENU.

#4808 Effect of Merkel cell polyomavirus large and small T-antigen on the thrombospondin and the perisinostin promoter. Aelita Konstantinell, Ida Sofie Furuholmen, Baldur Sveinbjørnsson, Ugo Moens. The Arctic University of Norway, Tromsø, Norway.

Merkel cell carcinoma (MCC) is a rare, but aggressive form of skin cancer with rising incidence and high mortality rate. Approximately 80% of MCC tumors are positive for Merkel cell polyomavirus (MCPyV), suggesting a causative relationship between MCPyV and MCC. Exosomes are 30-150 nm vesicles that contain proteins, lipids, miRNAs, IncRNAs, and miRNAs. Exosomes from virus-infected cells comprise also viral nucleic acids and proteins. Because tumor cell-derived exosomes may contribute to cancer, we hypothesized that MCPyV may affect the composition of exosomes and play a role in tumorigenesis. Therefore, we compared the proteins in exosomes produced by MCPyV-positive MKL1 and MKL2 and MCPyV-negative MCC13 and MCC26 MCC cell lines. Our proteomic analysis of exosomes originating from polyomavirus-negative and polyomavirus-positive MCC cell lines revealed the presence of the oncogenic proteins perisinostin and thrombospondin. Western blot analysis of exosomes and lysates of these MCC cells confirmed the presence of these proteins in exosomes from all cell lines. Thrombospondin, but not perisinostin, was detectable in cell lysates, and an enrichment of both proteins was detected in exosomes of all cell lines. The effect of MCPyV large T-antigen (LT-ag) and small T-antigen (st-ag) on the perisinostin and thrombospondin promoters was examined by transient transfection studies with luciferase reporter plasmids. Transfection experiments in MCC13 revealed that LT-ag, but not st-ag significantly increased the activity of the thrombospondin and perisinostin promoters. In MCC26 cells, neither LT-ag nor st-ag had a significant effect on the thrombospondin promoter activity, while both proteins alone or in combination significantly stimulated the perisinostin promoter strength. Our results suggest that MCPyV proteins may contribute to tumorigenesis by enhancing the expression of the oncoproteins thrombospondin and perisinostin and promote their secretion via exosomes.

#4809 Development of mouse brain tumor model using in vivo electroporation and piggyBac system. Nobuyuki Onishi, Sampetrane Oltea, Hideyuki Saya. Keio University School of Medicine, Tokyo, Japan.

Glioblastoma multiforme (GBM), is one of the most malignant brain tumors, has highly proliferative and invasive characters. There is no established effective therapy for GBM possess radio- and chemo-resistance. To comprehend these malignant characters of GBM, an appropriate model of brain tumor is required. InK4A/Arf loss of function and activation of RAS-related signaling pathways are frequently observed in GBM. Neural stem cells (NSCs), having self-renewal and multipotent abilities, are considered one of the cells-of-origin of GBM. Previously, we have established a stable mouse models of brain tumors, transplanting the genetically modified NSCs. The NSCs derived from InK4a/Arf KO mice, transduced activated-RAS, rapidly formed highly proliferative and invasive brain tumors. Recently, for the purpose of reproducing the clinical tumor initiating process, we are developing in vivo carcinogenesis model of mouse brain tumors by having the genetic engineering in mouse brain directly. Using the combination of in vivo electroporation and piggyBac system, activated-RAS and shInK4a/Arf are introduced into NSCs genome in mouse brain, leading to formed brain tumors having an equally malignant behavior as the transplanta- tion model. Histological characteristics of these tumors resembled human GBM phenotype demonstrating necrosis, perivascular cuffing and giant cell formation. Immunohistochemical analysis displayed that tumors were proliferation marker Ki-67 highly positive and had heterogeneity, expressing stem cell marker Nestin or astrocyte marker Gfap. On the basis of these findings, we propose this in vivo carcinogenesis technique is efficient method to generate appropriate mouse brain tumor models.

#4810 Loss of Cav-1 is associated with manifestation of stem cell-like properties via Src-mediated phosphorylation in human breast MDA-MB-231 cancer cells. Hye-Jin Yoon, 1 Do-Hee Kim, 1 Young-Nam Cha, 2 Young-joon Suh.1, College of Pharmacy, Seoul National University, Seoul, Republic of Korea; 2Colleage of Medicine, Inha University, Incheon, Republic of Korea.

A subpopulation of cancer cells, termed cancer stem cells (CSCs), has self-renewal and differentiation potential, which contributes to cancer recurrence and tumor resistance. Caveolin-1 (Cav-1), the major structural protein of flask-shaped plasma membrane invaginations called caveolae, functions as a tumor suppressor or an oncogene depending on the tumor type and the grade. Caveolin-1 participates in various cellular functions, such as vesicle trafficking, cholesterol homeostasis, signal transduction, and tumor progression. In the present study, we investigated the role of Cav-1 on the stemness of human breast cancer (MDA-MB-231) cells. A sphere formation assay has been widely used to enrich for CSCs through serialpassaging. A level of Cav-1 protein expression decreased in third-generation mammospheres as compared to parent MDA-MB-231 cells. Under the same experimental conditions, however, there was no concurrent decrease in the Cav-1 mRNA level. Therefore, it is likely that stability of Cav-1 protein is decreased during mammosphere formation. Recently, it has been reported that sustained phosphorylation of Cav-1 promotes its ubiquitination and degradation by the proteasomes. We observed that PP2, an inhibitor of Src, suppressed phosphorylation of Cav-1 in MDA-MB-231-derived mammosphere cells, which in turn increases its protein stability. Breast CSCs are enriched in cells with enhanced expression of phenotypic markers, such as CD44+/CD24-. We found that Cav-1 gene silencing using siRNA enhanced the proportion of CD44+/CD24- MDA-MB-231 cells. In addition, Cav-1 knockdown increased the number and size of secondary mammospheres, and this was accompanied by enhanced expression of Bmi-1 and Sox-2, which are representative self-renewal markers. Moreover, epithelial-mesenchymal transition (EMT) was decreased by knockdown of Cav-1 in MDA-MB-231 cells. Taken together, these findings suggest that loss of Cav-1 protein stability facilitates the formation of mammospheres through upregulation of self-renewal and EMT markers in breast CSCs.

TUMOR BIOLOGY: Cell Culture and Animal Models of Cancer 5

#4811 XenoSarc: Patient-derived xenograft (PDX) models of soft tissue sarcoma (STS), an update on a preclinical platform for early drug testing. Agnieszka Wozniak, 1 Jasmin Cornillie, 1 Yemarshet K. Gebreyohannes, 2 Jasmin Wellens, 1 Lis Vreys, 1 Daphne Hompes, 2 Marguerite Stas, 2 Friedel Sneaeve, 2 Maria Debiec-Rychter, 1 Raf Scoit, 1 Patrick Schöffski, 1 KU Leuven and University Hospitals Leuven, Leuven, Belgium; 2University Hospitals Leuven, Leuven, Belgium.

Background: STS constitutes a rare and very heterogeneous family of mesen- chymal tumors. The limited treatment options available for advanced STS un- derline the need for reliable preclinical models to test novel therapeutic strate- gies. Methods: A panel of patient-derived xenografts (PDX) was established by subcutaneous implantation of fresh, surgically resected or biopsied tumor spec- imens in immunodeficient, athymic nude NMRl mice. Once tumor growth was observed, pieces of tumor were re-transplanted to next generations of mice. At each passage tumor fragments were collected for histopathological and molec- ular characterization. A model was considered established after observing stable histological and molecular features for at least two passages. Results: Until now 171 STS samples from consenting patients treated at the University Hospitals, Leuven, Belgium, have been transplanted. Twenty-eight well-characterized, sta- ble PDX models of STS have been established, maintaining the histopathological and molecular features of the original tumor. The detailed clinical information about a donor patient, including sensitivity to standard and experimental drugs, is linked to every model. At this point the XenoSarc platform includes models of gastrointestinal stromal tumor (6 models), myxoidfibrosarcoma (6), dedifferenti- ated liposarcoma (3), malignant peripheral nerve sheath tumor (3), synovial sarcoma (1), leiomysarcoma (4), epithelioid haemangiendothelioma (1), mesenchymal chondrosarcoma (1), pleomorphic rhabdomyosarcoma (1) and high-grade undifferentiated pleomorphic sarcoma (2). From these models we have also available tissue microarray (TMA) as well as data on genomic and expression profile including mutations (by RNA-Seq). Some of these models
have already been successfully used for in vivo testing of novel agents, including both targeted and cytotoxic (pro-)drugs, and results served as a rationale for several prospective clinical trials. In addition, 24 other xenografts are still in early stages of engraftment, not yet fulfilling our criteria of an “established model”.

Conclusion: Our XenoSarc platform contains a number of well-annotated mod-
el strains of human glioblastoma (8/14.5 months), the segmental and punctate infil-
tration of the brain parenchyma (6/14, 43%). In summary, we present a valuable mouse model for preclinical studies of glioma. Integrative analysis of molecular profiles with growth pattern data has the potential to unravel genes that distinguish tumors with more infiltrative growth. Our biobank of luciferase labeled GSCs may be useful for longitudinal monitoring of tumor growth dynamics in the setting of therapies and provides a foundation for testing the effect of predicted drug vulnerabilities in orthotopic xenografts mouse models.

#4812 A novel set of patient-derived orthotopic xenograft (PDOX) mod-

eis of primary and recurrent intracranial meningioma. Huiyuan Zhang,1 Lin Qi,1 Yuchen Du,1 Frank K. Braun,1 Mari Kogiso,2 Sibo Zhao,1 Holly B. Lindsay,1 Sarah G. Injac,1 Patricia A. Baxter,1 Jack M. Su,1 Akash J. Patel,1 Xiao-nan Li,1 Baylor College of Medicine. 2Texas Children’s Cancer Center, Houston, TX; 3Jan and Duncan Neurological Research Institute, Houston, TX

Background: Meningioma is the most common brain tumors in adults. De-

spite the overall benign nature of meningioma, cranial-base tumors are difficult to achieve complete resection while others exhibit progression and aggressive profiles characterized by high recurrence rates, pleomorphic histology, and resistance to standard treatment. The lack of clinically relevant animal models is blocking the development of novel therapies. Here, we report our establishment of orthotopic xenograft mouse models and in vitro culture systems from surgical specimens of primary and recurrent meningiomas. Material and Methods: 6 primary surgical samples (3 WHO grade 1 and 3 atypical), and 4 recurrent samples (1 WHO grade 1, 2 atypical and 1 anaplastic meningioma) were ob-
tained from meningioma patients. Tumor tissues were dissociated into single cells and directly implanted into right cranial base of NOD/SCID mice (1x10^6 cells/mouse). Primary cultures were initiated in serum-free and traditional FBS-based media. Tumor growth was monitored by small animal MRI. Pathologic features of the PDOX models and the matched patient tumors were compared with standard histopathology (H&E) and immunohistochemistry (IHC) stainings. Results: Of the 10 tumors, 3 were not tumorigenic, and xenograft tumor formation from 5 additional samples is pending. Growth of intracranial (cranial base) xenograft was confirmed in two samples derived from the same patient diagnosed as atypical meningioma (K029MEN) and progressed as anaplastic meningioma at recurrence (K037MEN). These patient derived or-
thothetic xenografts (PDOX) have since been serially subtransplanted in mouse brains for generation 2 and can be cryopreserved for long-term maintenance of tumorigenicity. The xenograft tumors replicated histopathological features (invasion, high proliferation and increased microvessel density) of their parental tumors. Genomic analysis is being performed to examine the similarities between parental tumors and the corresponding orthotopic xenograft tumors and the discrepancies between the primary and the recurrent tumor-derived models. In vitro growth of K029MEN and K037MEN as neurospheres and monolayer were maintained for 2 months and passage for 10 times. Additionally, cells from K030MEN, a WHO grade 1 meningioma, has been passaged as monolayer for more than 30 times. Conclusion: A novel set of meningioma PDOX models developed in cranial-base and recurrent tumors was established. Xenograft tumors replicated the histopathological and key molecular features of the original patient tumors, providing a unique opportunity to understand the biology of malignant meningiomas and to conduct preclinical drug testing.

#4813 In vivo modeling of high grade glioma for oncology drug development.

Cecilia Krona, Soumi Kundu, Karl Holmberg-Olausson, Riaas Islam, Rashmi Ramachandara, Ludmina Enfne, Sven Nelandar. Uppsala Univ., Upp-
sala, Sweden.

In a multi-disciplinary project, patient derived glioblastoma stem-cell cul-
tures (GSCs) have been established and characterized extensively with the goal of applying computational tools to integrate results from high-throughput screens of drugs and RNAi with genomic and transcriptional profiling to predict the most successful therapy for individual glioma patients. The main goal of this study was to develop a platform for testing the tumor-initiating capacity of GSCs in mouse brain and to label the cells with GFP-luciferase to enable non-invasive quantification of tumor growth by in vivo bioluminescence imaging. Adherently grown GFP-luciferase labeled glioblastoma stem-cell cultures were dissociated and injected stereotactically into immunodeficient mice. Tumor growth was monitored by IVIS and彩色 microscopic imaging. The brain was engrafted with GSCs and the brain sections were set up based on human cell specific staining and a CellProfiler Analyst’s machine learning classifier with a manual observer correlation of 0.86. GSC xenograft tumors with 

a wide range of histopathological features and biological behaviors recapitulat-
ing high grade astrocytoma was confirmed in mice injected with 14 of the 29 glioblastoma cell cultures (48%). Glial lineage markers, such as Sox2, GFAP, and Olig2, were expressed both in patient tumors and patient derived xenografts. Individual glioblastoma cell cultures were either characterized by formation of condensate tumors (8/14, 57%) or diffuse infiltrative growth through the brain parenchyma (6/14, 43%). In summary, we present a valuable mouse model for preclinical studies of glioma. Integrative analysis of molecular profiles with growth pattern data has the potential to unravel genes that distinguish tumors with more infiltrative growth. Our biobank of luciferase labeled GSCs may be useful for longitudinal monitoring of tumor growth dynamics in the setting of therapies and provides a foundation for testing the effect of predicted drug vulnerabilities in orthotopic xenografts mouse models.

#4814 Testing personalized therapies for ovarian cancer using mate pair genomic analysis and patient-derived xenografts. Konstantinos Leventakos, Faye R. Harris, Lin Yang, Xiaonan Hou, Saravut Weroha, Irina V. Kovtun. Mayo Clinic, Rochester, MN.

Purpose: The testing of ovarian cancer poses a challenge since 70% of pa-

tients will relapse with incurable platinum resistant disease. Although ovarian cancers lack frequently-occurring driver mutations or amplifications, the study presented herein examines the concept of individualized therapy based on mate pair sequencing and protein expression in ovarian cancer patient-derived xenografts. Experimental Procedure: Macrodissection of patient tumor was followed by genomic DNA isolation and next-generation sequencing using an Illumina HiSeq2500 and genomic number variations. Secondary analyses were carried out to select potential therapeutic targets among those. The tumors were also propagated intraperitoneally in immu-

nocompromised mice and treated with standard chemotherapy or and 
targeted therapy chosen based on genomic analyses. Results: Mate pair analysis revealed that, in general, copy number changes (amplifications, gains and losses) were common in ovarian tumors and included genes potentially targetable. Tu-
mors from early generation patient- derived xenografts showed a landscape of 
genomic aberrations identical to that of original patient tumor. One PDOX model in which high expression of HER2 and copy number gains at RICTOR and AKT genetic loci were revealed, was treated with either chemotherapy and Pertu-

zumab/trastuzumab (HER2 inhibitors), MK-8869 (mTOR inhibitor) or 

MK-2206 (AKT inhibitor) or chemotherapy alone. Tumor size was measured by 

serial abdominal ultrasound and after 28 days, the final tumor size ratio com-
pared to baseline was 0.23, 0.36, 0.55 and 0.56, respectively, indicating that the best response was seen in the combined chemotherapy and Pertuzumab/trastu-

zumab cohort and that was better than in the chemotherapy only cohort. Con-
clusions: Patient-derived xenografts models in conjunction with genomic and 

protein analyses of patient tumors are available for testing of combination ther-
apies including specific targeting drugs. This approach also provides actionable information for more tailored treatment of ovarian cancer patients.

#4815 Humanized single mouse trial: A preclinical platform feasible 

for immune-oncology drug screening and translational biomarker develop-

ment. Daniel Bug,1 Eva Oswald,2 Anne Grote,3 Anne-Lise Pellel,4 Gabriele Nie-
dermann,5 Dorit Merhof,2 Friedrich Feuerhake,3 Julia B. Schüler2. RWTH Aachen University, Aachen, Germany; 1Onco test GmbH, Freiburg, Germany; 3Hannover Medical School, Hannover, Germany; 4Medical Center Freiburg, Freiburg, Germany.

The field of cancer immunology is rapidly moving towards innovative thera-
paeutic strategies. As a consequence the need for robust and predictive preclinical platforms arises just as well. The current project aims to establish a drug screen-
ing workflow bridging between innovative mouse models and clinical bio-

marker development. A total of 69 NOG (NOD/Shi-scid/IIL-2Rnull) mice were 

engrafted with CD34+ hematopoietic stem cells. Thereafter, tumor material from 11 different lung cancer patient derived xenograft models (NSCLC PDOX) 

was implanted subcutaneously. Individual mice were treated with α-CTLA-4, 

α-PD-1 or the combination thereof. With n = 1 per treatment arm and model the study design followed the screening approach of the single mouse trial (SMT).

Infiltration of human immune cells was detected by flow cytometry (FC) and 

immunohistochemistry (IHC) in hematopoietic organs and tumor tissue. A 

computer-based analysis for digitized whole-slide images of the samples was used to quantify the lymphocyte infiltration using color classification and morpho-

logical image processing techniques. All 3 treatment arms displayed a discrete 

pattern throughout the PDOX panel. Tumor models with high tumor 

infiltrating lymphocyte (TIL) rates in the donor patient material tended to be 

more sensitive towards checkpoint inhibitor treatment as models with low rates. Numbers of TILs in the PDOX detected by FC and IHC were signif-

icantly higher in models with high TIL rates.

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creased in the treatment groups as compared to control vehicle. In parallel, hematopoietic organs showed high (>25%) amounts of huCD45 cells in all groups and models. PDX models being sensitive towards checkpoint inhibitor treatment (responder) displayed a higher percentage of DAB+ nuclei in huCD45 IHC stains than non-responder models as determined by image analysis. Irrespective of the responder status, the resected tumor area in non-complex with checkpoint inhibitors enhanced the percentage of DAB+ nuclei. Whole-slide image analysis of the HE&E stains revealed an increase of the stromal compartment proportion in the tumor tissue under treatment with checkpoint inhibitors in responder models. In non-responder models the ratio between tumor and stroma was not influenced by drug treatment. The use of PDX based humanized mouse models in a SMT format allows screening approaches in complex mouse models. The combination with a comprehensive image analysis tool enables additional read-outs to quantify antitumoral activity of immune modulators. The latter can be used to identify possible biomarkers in the preclinical setting. Moreover, the translation and validation of these biomarker candidates in a clinical setting is self-evident as primary material needed for these types of analyses is easily accessible.

#4816 Circulating tumor cell monitoring, isolation, and culture from a patient with metastatic triple-negative breast cancer for drug screening and creation of a patient-derived xenograft model. Arturo B. Ramirez,1 C Anthony Blau,2 Timothy J. Martins,2 Elisabeth Mahen,2 Lacey E. Dobrolecki,2 Michael T. Lewis,2 Jackie L. Stilwell,2 Eric P. Kaldjian1.1 RareCyte, Inc., Seattle, WA; 2University of Washington, Seattle, WA; 3Baylor College of Medicine, Houston, TX.

Background: Epithelial and single cell genomics of circulating tumor cells (CTCs) provide three types of information to guide cancer therapy. In some instances, a fourth type is possible: functional analysis in vitro, or in vivo patient-derived xenografts (PDXs). We used a density-based rare cell separation and analysis system to collect CTCs from the blood of a patient with metastatic triple-negative breast cancer (TNBC) for in vitro culture and high-throughput drug screening and to generate a PDX model. Methods: The patient was enrolled in the ITOMIC-001 study (University of Washington) and after informed consent, CTCs were evaluated prior to initial cisplatin treatment and tracked longitudinally using the AccCyte – CyteFinder system (RareCyte). Samples containing high numbers of CTCs were placed into 3 different culture media. Cells grown in culture were tested against a panel of anti-cancer drugs and injected into mice to form a PDX model. Results: Nine CTC evaluations were performed over 9.5 months. CTCs were verified by expression of epithelial (cytokeratin and/or EpCAM) and nuclear stains without CD45 expression. Analysis performed over 9.5 months. CTCs were verified by expression of epithelial (cytokeratin and/or EpCAM) and nuclear stains without CD45 expression. After initial treatment with cisplatin, the CTC count per 7.5 mL rose from 4 to 19 cells at 3 months, consistent with the lack of a clinical response, and decreased after LE 011 (CDX4/6 inhibitor) and then glembatumumab vedotin (anti-HER2 MAb) to 8 and 4 cells at 5 and 7 months respectively. At 9 months the CTC count rose to >13,000 and 5 days later to >80,000 shortly before her death. At autopsy there was massive infiltration of the liver and pulmonary vasculature by tumor cells. Cultures in all media showed initial growth, but only one (RPMI + 10% serum) was sustained, forming semi-adherent 3D tumor clusters. 6 million cells were harvested and a drug screen using 160 anti-cancer agents was performed. The CTC line showed sensitivity to several agents, notably trastuzumab and paclitaxel, and the treatment injected into the mammary fat pad of immunodeficient mice. In at least one mouse, macroscopic tumors were observed. The CTC cell line has grown continually in culture for over a year. Aliquots of this cell line have been frozen and thawed with no noticeable effect on cell growth. Conclusions: Using a density-based rare cell collection system, we established a CTC cell line from a TNBC patient with extremely high CTC counts. The line was used to perform a screen for agents active against the tumor cells and to create a PDX model. As in vitro techniques advance, smaller number of CTCs may be effectively cultured and thus allow this approach to be used in real time to effect drug regimens for individualized cancer therapy.


Malignant ascites is one of the most common causes of morbidity in end stage ovarian cancer patients with negative impact on quality of life. Novel non-invasive palliative therapeutic options are lacking for such patients. The zinc metalloprotease, pregnancy-associated plasma protein-A (PAPP-A), plays a key role in the insulin-like growth factor (IGF) pathway, promoting ovarian cancer cellular transformation, growth and invasiveness. Furthermore, patient primary malignant ascites is known to contain high levels of PAPP-A by ELISA. Preliminary data shows that inhibition of PAPP-A through a neutralizing monoclonal PAPP-A antibody (mAb-PA) inhibits the accumulation and promote the regression of ascites in an ovarian cancer patient-derived xenograft (PDX) models. In the current study, we investigated whether mAb-PA can promote ascites regression in an additional ovarian PDX model with measurable ascites. Patient derived ascites xenograft (PDX) models were defined as SCID mice that developed ascites after intraepithelial heterotransplantation of patient solid tumor collected at the time of primary cytoreduction. Ascites from these models (n=51) was screened for human PAPP-A protein by ELISA and models were divided into two groups by the relative concentration of PAPP-A: high (n=18) and low (n=33). PAPP-A High PDX model PH438 was re-established intra-peritoneal in 20 SCID mice by ascites injection (0.1 ml per mouse). When ascites area reached a threshold of >0.60 cm2 by ultrasound, mice were treated with 60mg/Kg of mAb-PA (n=10) or IgG2a control (n=10) on day one and three. On day four, a second ultrasound measurement was obtained and the mice were euthanized. Ascites burden was measured at necropsy. Personnel involved with the acquisition of ultrasounds measurements, subsequent ascites harvests, and post hoc analyses were blinded to the treatments. The ratio of mean ascites (mAb-PA/IgG2a in grams) collected at necropsy was 2.04/2.51, indicating that the mAb-PA arm had less ascites compared to control. These data were consistent with pre- and post-treatment ultrasound measurements of ascites burden; a statistically significant (p = 0.0283 by paired t test) reduction of ascites burden of 52.3% was observed, compared to the starting baseline, indicating that mAb-PA treatment causes ascites regression. Ascites weight at necropsy was compared to the area of greatest fluid echogenicity by ultrasound and the Pearson correlation R value achieved was of 0.859 (p = 0.0001). These PDX data implicate PAPP-A attenuation as a potential strategy to treat malignant ascites in OC. Additional PDX models are under examination to confirm these findings and support the translational development of PAPP-A as a new therapeutic target for women with refractory ascites.

#4818 Neratinib/fulvestrant but not fulvestrant alone maintain complete responses after treatment with trastuzumab/paclitaxel of mice bearing ER+/HER2+ xenografts. Luis J. Schwarz,1 Sarah E. Croessmann,1 Francesca Avogadri-Connors,2 Richard E. Cutler,2 Alshad S. Lalani,2 Carlos L. Arteaga1.1 Vanderbilt University Medical Center, Nashville, TN; 2Puma Biotechnology, Inc., CA.

Background: Neratinib is a potent, irreversible pan-HER tyrosine kinase inhibitor. The phase III trial ExteNET showed improved disease-free survival of neratinib vs placebo in early-stage HER2+ breast cancer patients (pts) after trastuzumab-based adjuvant therapy. This benefit from neratinib was greater in pts with hormone receptor (HR) + tumors. Based on these findings, we sought to establish a human-in-mouse model that would simulate this clinical trial and outcome, thus providing a platform for mechanistic investigation. Methods: ER+/HER2-amplified MDA-361 cells were injected subcutaneously (SC) into 5-week-old female athymic mice without estradiol supplementation. Mice with tumors ≥250 mm3 were treated with trastuzumab (tz) 20 mg/kg + paclitaxel (pac) 15 mg/kg IP twice weekly for 4 weeks, and then randomized to fulvestrant (fulv) 100 mg/kg weekly or neratinib (tz)/paclitaxel followed by neratinib/fulvestrant for 4 weeks. Results: Xenografts in all 20 mice showed a prompt and marked reduction in tumor volume after tz/pac treatment; 10 mice achieved a complete response (CR) before receiving 'extended adjuvant' therapy with fulv (n=5) or neratinib/fulv (n=5). A CR was maintained with neratinib/fulv following tz/pac, whereas tumors rapidly recurred in mice treated with fulv alone (p<0.05 at week 8; Table). Immunohistochemical analysis of MDA-361 tumors recurring on fulv showed almost complete downregulation of ER levels.

## Table

<table>
<thead>
<tr>
<th>Timepoint</th>
<th>Treatment</th>
<th>Mean (± SD) tumor volume, mm3 (% change from baseline)</th>
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<tbody>
<tr>
<td></td>
<td>Trastuzumab + paclitaxel + fulvestrant (n=10)</td>
<td>1634 ± 52.9 (–) 187 ± 73.1 (–) 9.8 ± 10.5 (–9.39)</td>
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<tr>
<td></td>
<td>Trastuzumab + paclitaxel + neratinib (n=10)</td>
<td>8140 ± 435.1 (+401.5) 2.0 ± 4.5 (–98.8)*</td>
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*p<0.05 between groups (Student’s t-test); SD, standard deviation

Conclusions: Neratinib/fulvestrant but not fulvestrant alone maintained complete tumor responses following initial treatment with tz/pac; recapitulating the design and outcomes observed in the HR+ subgroup of the ExteNET trial. The experimental model used herein provides a platform for investigation of the underlying mechanisms for the findings in ExteNET. 

Experiments evaluating
ER gene signatures and expression levels, as well as the effects of neratinib ± fulvestrant following initial adjvant pertuzumab/trastuzumab/paclitaxel are underway.

**#4819 Insights from engraftable immune deficient mouse models of hyperinsulinemia.** Michelle L. Maughan,1 Patrick B. Thomas,1 Gabrielle J. Crisp,1 Lisa K. Philp,1 Esha T. Shah,1 Adrian C. Herrington,1 Chen Chen,2 Laura S. Gregory,3 Colleen C. Nelson,4 Inge Seim,1 Penny L. Jeffery,1 Lisa K. Chopin4,5

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Hyperinsulinemia, obesity and dyslipidemia are independent and collective risk factors for many cancers, however, there is a lack of suitable mouse models available to study these interactions. In this study, we examine the interactions between hyperinsulinemia, obesity and hyperlipidemia and cancer. The models used were Rag1-/- mice developed on a normal chow diet. In summary, this is the first study of the metabolic effects of a Western style 23% high fat diet (HFD, 46% of total calculated energy from lipids) in two immune deficient mouse strains (NOD/SCID and Rag1 -/-) suitable for engraftment with human-derived cell lines and tissue xenografts. HFD-fed mice of both strains exhibited diet-induced impairments in glucose tolerance at 16 and 23 weeks post initiation of HFD feeding. Only Rag1 -/- mice developed higher fasting insulin levels (2.16 ± 1.01 ng/ml versus 0.71 ± 0.12 ng/ml, P = 0.01) and increased insulin resistance (6.70 ± 1.68 HOMA-IR, versus 2.91 ± 0.42, P = 0.01) when fed a HFD. Similarly, hepatic steatosis was more extensive, and intramyocellular lipid storage was increased in HFD-fed Rag1 -/- mice. Conversely, NOD/SCID mice exhibited relatively low levels of steatosis and no intramyocellular lipid was observed. These data suggest that Rag1 -/- mice are a more suitable preclinical model for examining the interactions between hyperinsulinemia, obesity and hyperlipidemia and cancer than the more commonly used NOD/SCID mouse model. We next investigated the growth of human prostate cancer cell lines (PC3 and LNCaP) subcutaneously injected into hyperinsulinemic Rag1 -/- mice. Compared to normal chow-fed mice, tumor growth velocity was greater in HFD-fed mice with PC3 and LNCaP xenografts, and mice reached humane endpoints (cancer-associated cachexia and tumor burden) significantly earlier (P = 0.0078 and P = 0.031). Strikingly, HFD-fed mice bearing PC3 xenografts presented with significantly greater normalized wet tumor weight (485.16 ± 143.80% vs. 1562.69 ± 338.20%, P = 0.032), tumor volume (485.16 ± 143.80% vs. 1562.69 ± 338.20%, P = 0.032) and number of Ki67 positive (proliferating) tumor cells (36.08 ± 2.53% vs. 66.14 ± 8.51%, P = 0.032) compared to mice fed a normal chow diet. In summary, this is the first study of the metabolic effects of a long-term Western style HFD in two immune deficient mouse strains suitable for xenograft studies. We demonstrate that the Rag1 -/- mouse is an appropriate and novel model for studying the interactions between hyperinsulinemia and cancer.


Animal models are essential to preclinical cancer research and are used clinically to determine optimal treatment regimens, but current xenograft models are limited in their utility, especially due to the lack of a competent immune system. Here we demonstrate that a xenograft tumor model can be developed in immunocompetent mice by tolerizing murine fetuses to human tumor cells. A375 human melanoma cells were injected into day E14 fetuses and, after birth, mice were challenged again with A375 cells to determine their ability to develop tumors. Intravenous injections of A375 cells after tolerization resulted in metastatic-like lung tumors, which were verified to be human in origin by immunohistochemistry and PCR. The development of lung neoplasms was dependent on fetal tolerization; non-tolerized mice did not develop tumors after injection. Interestingly, subcutaneous injected cells did not form tumors, but this was shown to be due to an innate, non-adaptive immune response and did not create lasting rejection of tumor cells. This procedure was repeated with several different tumor lines to show the universal nature of the method: BxPC3 (human pancreatic cancer), M21 (human melanoma), HeLa (human cervical cancer), and MDA231 (human breast carcinoma).

**#4821 Influence of the injection site on the dissemination pattern and drug sensitivity of patient derived leukemia cells in vivo.** Julia B. Schuler,1 Eva Oswald,1 Gabriele Grewe,2 Dorothee Lenhard,1 Kerstin Klingner,1 Milena Pantic,1 Michael Luebbert2.

*Oncotest GmbH, Freiburg, Germany; 2Medical Center Freiburg, Freiburg, Germany.*

The aim of this study was to determine the influence of the injection site on the tumor morphology and drug sensitivity of a panel of hematological patient derived xenografts (PDX), PDX cells (3x10^6 cells/mouse) were injected intratibia1 (IT), intrasplen1 (IS) or subcutaneous (s.c.) into NOG (NOD/Shi-scid/IL-2Rγ^-/-) mice. Tumor engraftment was determined by flow cytometry (FC) in bone marrow (BM), peripheral blood (PB) and spleen during the course of engraftment and at the end of a study. Overall survival (OS) served as an additional read-out. In 3 models sensitivity towards cytarabine (Cy) was evaluated. Our group has established 18 PDX of acute leukemia (16 AML, 1 ALL, 1 APL). 16/17 engrafted when injected i.t., 10/12 developed tumors after i.s. implantation and 13/14 established tumors post s.c. cell injection. Thus, the overall engraftment capacity was for most of the models not dependent on the injection site and/or administration. However, a few models were dependent on a specific setting: LEXFAM 2713, grew exclusively i.t. or i.s., whereas LEX- FAM 2824 could be propagated solely when injected s.c.. The implantation site did influence tumor growth rate: Mean OS ranged from 151.4 (±25.21) days for i.t. to 89.2 (±16.82) days for s.c. propagation. I.s. transplanted mice had to be sacrificed after 91.9 (±17.33) days. The dissemination pattern of individual lines was affected by the injection site. In general, the tumor burden was higher when cells were engrafted i.t. or i.s.. Nevertheless, also s.c. implanted, AML cells infiltrated murine PB, spleen and BM, although not consistently and to a much lower extent. Interestingly, the expression pattern of the 6 investigated surface markers (CD45, CD3, CD34, CD33, CD38 & HLA-ABC) was not influenced by the application route. Every model depicted its distinct expression pattern irrespective of the application route. Cy was highly active in 1 AML (LEXFAM 2531) and 1 ALL (LEXFAL 2665) model. OS was significantly prolonged in the s.c. as well as in the disseminated setting (p<0.003, Log-rank (Mantel-Cox) test). Another AML line (LEXFAM 2734) depicted a less pronounced sensitivity towards Cy (p<0.007, Log- rank (Mantel-Cox) test) both growing s.c. or i.t.. Thus, drug sensitivity was not influenced by the injection site of the leukemic cells. Of note, the PDX drug responses mimicked the responsiveness (0 out of 7 patient cancer specimens). Taken together, the leukemia PDX panel represents the molecular diversity of the disease and mirrors sensitivity towards standard of care. Our recent careful investigation of the subcutaneous approach in these models highlights their suitability for this type of studies. This enhances the value of the platform as it combines the possibilities of a mid-throughput screening enabled by the subcutaneous approach with the general advantages of a patient-derived leukemia model.

**#4822 Detection of EBV BamHI W region in surgical cancer specimen is a useful method to evaluate the risk of lymphomagenesis in patient-derived PDXs.** Junko Motoyama,1 Dai Iwakiri,2 Yohu Yen,1 Tomo Mokhara,1 Hironobu Minami,1 Yoshihiro Kakeji,1 Yohei Shimono1,3

1 Kobe University Graduate School of Medicine, Kobe, Japan; 2University of Human Arts and Sciences, Saitama, Japan.

Patient-derived tumor xenografts (PDXs) established by xenotransplantation of surgically-resected human cancer specimens are an attractive model to analyze the characteristics of cancer cells within the patient cancer tissues. However, establishment of PDX is occasionally hampered by lymphomagenesis. Lymphomagenesis in the PDX has been observed in the xenotransplantation of several types of cancers, such as lung, liver, gastric, bladder, breast, prostate, and colorectal cancers. Lymphomagenesis in the PDX is caused by the proliferation of the EBV-infected lymphocytes under immune deficient conditions. The PCR amplification of this region is useful to evaluate the presence and amount of EBV. PDXs. BamHI W region is a major internal repeat in EBV genome and the PCR amplification of this region is useful to evaluate the presence and amount of EBV. These results suggest that this region was undetectable, no patient cancerspecimen resulted in lymphomagenesis (0 out of 7 patient cancer specimens). These results suggest that the amount and/or presence of EBV itself in the patient cancer specimen is one of the factors that are associated with lymphomagenesis in the PDXs. BamHI W region and EBV-associated microRNAs, were evaluated using the genomic DNA and miRNAs prepared from the CRC specimens and the established PDX tumors. Nine PDXs were established by the xenotransplantation. Histological examination showed that 7 of 9 (78%) PDX recapitulated histopathological characteristics of the patient CRC. However, 2 of 9 (22%) PDXs exhibited the morphological characteristics of EBV-associated human diffuse large B cell lymphoma (DLBCL). Then, we confirmed that lymphoma was formed by clonal proliferation of human B-cell lymphocytes, and strongly positive for EBER. We investigated whether the expression of EBV-related genes in the patient specimen is associated with lymphomagenesis in the PDXs. Expression of EBV genes and RNAs in patient cancer tissues were not detected in the PDXs. January 2017 1233

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gesting that the sina2 and sina3 alleles are hypomorphical alleles. Sequencing analysis phenotypes than those of the previously published sina2 and sina3 alleles, suggesting a screen using ethyl methanesulfonate (EMS) and X-ray radiation, isolating Drosophila development system, we are able to study RAS activation and SINA regulation, and substratetargeting mechanism(s) of this highly conserved family.

SINA and its human SINA homologs (SIAHs). As a major signaling “gatekeeper” function of Drosophila Seven-IN-Absentia (SINA) E3 ligase and its human homologs, SIAH1 and SIAH2, is highly conserved for proper RAS signal transduction in Drosophila development. The gatekeeper “gatekeeper” in the RAS pathway, we have shown that SIAH is required for oncogenic K-RAS-driven tumorigenesis and metastasis in human pancreatic, lung and breast cancer. Since SIAHs appear to be the ideal drug target to inhibit “undruggable” K-RAS activation, it is important to precisely characterize the activity, regulation, and substrate targeting mechanism(s) of this highly conserved family of SINA/SIAH E3 ligases. By deploying the elegant and well-established Drosophila development system, we are able to study RAS activation and SINA function under normal physiological conditions. In the developing Drosophila eye, photoreceptor cells are recruited sequentially and acquire their distinctive cell fates through a series of local inductive events. The 800x cell arrays allowed us to dissect the role of SINA/SIAH downstream of RAS activation in photoreceptor cell development. To delineate SINA function, we performed an F1 modifier screen using ethyl methanesulfonate (EMS) and X-ray radiation, isolating 28 novel sina mutant alleles. These mutant alleles exhibit much stronger mutant phenotypes than those of the previously published sina2 and sina3 alleles, suggesting that the sina2 and sina3 alleles are hypomorphic alleles. Sequencing analysis of these sinaWT/DN alleles reveals the functional roles of mutated residues and protein domains. To define SINA/SIAH functional conservation, we have generated a complete panel of transgenic fly models that express either wild-type (WT) or dominant negative (DN) SINA/SIAH. The corresponding UAS-sina/siahW2 alleles have been characterized using sev-, GMR-, dpp- and sgrafyaly gland-GAL4 to elucidate the developmental outcomes of altered SINA/SIAH expression upon RAS activation. Ectopic expression of sinaWTT/DN/siahWT/DN in neurons resulted in dramatic changes in neuronal cell fate in the developing eye and notum, causing PNS neurodegenerative phenotypes. Our results show that the biological functions of fly SINA and human SIAH1/SIAH2 are evolutionarily conserved and functionally interchangeable. Mechanistic insights and regulatory principles revealed from Drosophila can be directly applied to cancer biology to develop and validate next-generation of SINA/SIAH-based anti-K-RAS and anticancer therapy in the future.

Functional role of Ring Finger Protein 43 in intestinal stem cell during colorectal tumorigenesis. Tsugio Eto, Takatsugu Ishimoto, Eri Oda, Daisuke Kuroda, Kota Arima, Mayuko Ohuchi, Kenichi Nakamura, Hiroshi Sawayama, Koichi Kinoshita, Masaki Iwatsuki, Yoshifumi Baba, Yasu Sako-moto, Naoya Yoshida, Hideo Baba, Graduate School of Medical Science, Kumanou University, Kumanoto, Japan.

Background: Colorectal cancer is one of the most general causes of cancer related death. Ring Finger Protein 43 (RNF43) is an E3 ubiquitin ligase that suppresses the Wnt signaling pathway and is known as a tumor suppressor gene. Previous studies reported RNF43 mutation leads to inactivation of RNF43 in pancreatic, ovarian, colorectal cancer and so on. Subsequently, Wnt signaling activation is associated with tumor progression in these types of cancers. However, functional role of RNF43 for tumor formation is not clear in colorectal cancer. Aim: To investigate the functional role of RNF43 in colorectal cancer by using RNF43 knockout mice. Method: We prepared RNF43 knockout mice by using CRISPR/Cas9 system. To induce colorectal tumor in mice, we used Azoxymethane-Dextran sulfate sodium (AOM-DSS) model. First, we injected AOM 10mg/kg into the peritoneal cavity. After 7days from injection, 2% DSS water to mice for 7days. We sacrificed the mice and evaluated the tumor incidence and tumor size after 8, 10, 12weeks from AOM injection. Next, we prepared the small intestine organoid from RNF43 wild and knockout mice. Then we compared the organoid size and formation rate. In vitro analysis, we prepared colon cancer cell lines (COLO205, SW620, HCT116) and evaluated the effect on cell proliferation in suppression of RNF43 and normal condition. Result: There was no significant difference in the tumor incidence and tumor size at 8, 10, and 12weeks after AOM injection. However, at 16weeks, the tumor size of RNF43 knockout mice was significantly bigger than that of RNF43 wild type mice. (p<0.001) Further examination of the intestinal organoid revealed that the organoids derived from RNF43 knockout mice tended to get bigger than those from wild type mice. In vitro analysis, cell proliferation of RNF43 mutant cell line (HCT116) was significantly increased compared with RNF43 wild type cell lines (COLO205, SW620). RNF43 silencing significantly increased cell proliferation in RNF43 wild type cell lines (p<0.001), whereas did not affected in RNF43 mutant cell line. Conclusion: Our results suggest that RNF43 expression suppresses epithelial cell growth in the intestine, and the dysregulation of Wnt signaling via RNF43 disruption promote intestinal stem cell expansion and colorectal tumorigenesis.

Maternal high butter fat intake heightens mammary cancer risk in offspring gestationally exposed to bisphenol A at environmentally relevant dose. Yuet-Kin Leung, Vinothini Govindarajah, Ana Cheong, Dan Song, Xue-gong Zhu, Jun Ying, Ady Kendler, Mario Medvedovic, Scott Belcher, Shuk-Mei Ho. Univ. of Cincinnati, Cincinnati, OH.

In utero exposure to bisphenol A (BPA) at 250ug/kg BW was shown to augment mammary cancer risk in rodent models. High fat diet is widely believed to be a major risk of breast cancer (BCa). It is therefore important to investigate whether maternal high fat intake could potentially heighten mammary cancer risk associated to gestational BPA exposure. In this study, we exposed Sprague Dawley rats with different doses of BPA (2.5-2500ug/kg BW) mixed with high butter-based diet (HBF) in the period from preconception to birth. DMBA-PND50 protocol was followed. In the presence of HBF, we observed non-monotonic effects of BPA in cancer incidence as well as in the number of terminal end buds of PND21 mammary glands. Both results were peaked at a dosage of 25ug/kg BW BPA and the effects were significant when compared with HBF alone group. We further analyzed transcriptomic data of microdissected epithelia of PND21 mammary glands from BPA (25ug/kg BW) group as well as HBF group and revealed that two specific cancer networks involving ERK and androgen receptor signaling were dysregulated. DNA methylation is one of the key
Mechanisms to dysregulate and impair the transcription of two selected top genes, Cap7 and Ccnv2, as supported by our bisulfiite-sequence data. These data suggest that in utero BPA exposure could epigenetically modify gene expression and predispose cancer risk during early development. More importantly, some BPA genes are of high human relevance because seven selected BPA genes are oncogenes that activate carcinogenesis processes in overall survival in a TCGA cancer cohort. The prognostic power of the genes was further enhanced in the survival analysis of Caucasian with ER positive patients. In conclusion, our data strongly suggest that BPA predisposes higher cancer risk by dysregulating gene expression during early mammalian gland development in the presence of HIF, which could facilitate cancer development later in life.

#4827 Establishing a platform for the generation of organoids from diverse tumor types as part of the NCI patient-derived models (PDM) initiative. Luke H. Stockwin,1 Jenna Moyer,1 Anna Wade, Carrie Bonomi,1 Kelly Dougherty,1 John Carter,1 Jesse Stottlemeyer,1 Kaitlyn Arthur,1 Vivekananda Datta,1 Lindsay Dutko,1 Michael Mullendore,1 James H. Doroshow,1 Melinda G. Hollingshead,1 Dianne L. Newton,1 Leidos Biomedical Research, Inc, Frederick National Laboratory for Cancer Research, Frederick, MD; National Cancer Institute, NIH, Bethesda, MD; Frederick National Laboratory for Cancer Research, Frederick, MD.

Cancer Organoids are discrete multicellular structures that recapitulate tumor microanatomy (1). These reagents can be generated by extended culture of partially or fully dissociated tumor samples in three-dimensional matrices. By maintaining tumor and accessory cells in an appropriate context, they provide a biosimilar platform for studying disease pathogenesis and cellular pharmacology (2). Similarly, cancer organoid culture is useful for propagating slow growing tumors or those requiring heterotypic cell-cell interactions. Here, preliminary data will be presented regarding generation of organoids from diverse tumor types as part of the NCI patient-derived models (PDM) initiative. This initiative aims to develop a national repository of patient-derived cancer models (PDMs) consisting of clinically annotated patient-derived xenografts (PDXs) and patient-derived tumor cell cultures (PDCs) prepared from primary and metastatic tumors (3). A standardized panel of different organoid media formulations was constructed to optimize culture conditions for disease subsets. Using this approach, organoids were generated for colon, prostate, pancreatic, breast, melanoma, NSCLC, and bladder tumors. Although some samples were refractory to organoid generation, in several instances, samples that failed to generate 2D cultures thrived as organoids. A further finding was that direct implantation of organoid cultures was an efficient means of generating xenografts. Indeed, work will be presented detailing the exact number of organoids required to establish xenograft tumors. Protocols were developed for routine culture, passaging and long-term storage in liquid nitrogen. Similarly, organoids were amenable to characterization by FACS analysis, ICC/IHC and qRT-PCR to evaluate aging and long-term storage in liquid nitrogen. Similarly, organoids were amenable to characterization by FACS analysis, ICC/IHC and qRT-PCR to evaluate aging and long-term storage in liquid nitrogen. Similar organoids were grown for colon, prostate, breast, pancreatic, bladder, melanoma, NSCLC, and lung cancer. These reagents can be used for basic, preclinical and clinical applications, significantly advancing personalized medicine with a revolutionary new primary cell culture technique termed conditionally reprogrammed cells (CRCs). In fact, the fact remains that due to the limited and suboptimal prostate cell lines available for correlation analysis, significant unmet needs exist in validating clinical findings. With this realization, many agencies such as the DOD and the NIH are funding more rapid and clinically relevant patient derived models to fill a void in our understanding of, and our ability to treat, cancer. Our research has been on the forefront in development of a novel approach to patient samples for basic, preclinical and clinical applications, significantly advancing personalized medicine with a revolutionary new primary cell culture technique termed conditionally reprogrammed cells (CRCs). In fact, the CRC approach is a major focus area of NIH U01/PAR 16-344. We have pioneered the CRC technology for the rapid establishment and expansion of patient-derived normal and cancerous prostate cells in typical tissue culture conditions. We hypothesize that improved in vitro and in vivo platforms using patient-derived prostate cells (e.g. CRCs) are required for the elucidation and subsequent experimental verification of key molecular and genetic drivers for PCA as well as better curative approaches. While prostate CRCs remain their lineage commitment, they fail to express many of the differentiation markers associated with luminal prostate cells when grown under normal two dimensional (2D) culture conditions. We have therefore established three dimensional (3D) non-spheroid based platforms for differentiation of both normal and malignant prostate CRCs. These include both transwell-based systems and decellularized tissue matrices that use defined differentiation media to enable AR activation and a luminal cell phenotype. We have now demonstrated the in vitro re-engagement of key determinants in the AR pathway and differentiation to luminal prostate cells. Documentation of the proper engagement of AR signaling is a significant step in developing a more accurate and tractable model for prostate cancer research and distinguishing between indolent and aggressive disease.

#4829 Development of rapid 3-dimensional culture conditions that support the in vitro differentiation of conditionally reprogrammed primary prostate cells for the study of prostate cancer. Lucas James Tricoli,1 Deborah Berry,1 Erika Parasido,1 Aisha Naeem,1 Olga Rodriguez,1 Iman Abdelgawad,2 Richard Lee,2 Adam Feldman,3 Chris Albanese,3 Georgetown Lombardi Comprehensive Cancer Center, Washington, DC; National Cancer Institute of Egypt, Cairo, Egypt; Massachusetts General Hospital Cancer Center, Boston, MA.

Despite decades of research into the causes and possible cures, prostate cancer (PCA) remains the second leading cause of cancer related death in men with over 26,000 deaths each year in the United States alone. While many of the studies performed over the years have identified important genes and signaling pathways that are involved in prostate cancer progression, the fact remains that due to the limited and suboptimal prostate cell lines available for correlation analysis, significant unmet needs exist in validating clinical findings. With this realization, many agencies such as the DOD and the NIH are funding more rapid and clinically relevant patient derived models to fill a void in our understanding of, and our ability to treat, cancer. Our research has been on the forefront in development of a novel approach to patient samples for basic, preclinical and clinical applications, significantly advancing personalized medicine with a revolutionary new primary cell culture technique termed conditionally reprogrammed cells (CRCs). In fact, the CRC approach is a major focus area of NIH U01/PAR 16-344. We have pioneered the CRC technology for the rapid establishment and expansion of patient-derived normal and cancerous prostate cell lines in typical tissue culture conditions. We hypothesize that improved in vitro and in vivo platforms using patient-derived prostate cells (e.g. CRCs) are required for the elucidation and subsequent experimental verification of key molecular and genetic drivers for PCA as well as better curative approaches. While prostate CRCs remain their lineage commitment, they fail to express many of the differentiation markers associated with luminal prostate cells when grown under normal two dimensional (2D) culture conditions. We have therefore established three dimensional (3D) non-spheroid based platforms for differentiation of both normal and malignant prostate CRCs. These include both transwell-based systems and decellularized tissue matrices that use defined differentiation media to enable AR activation and a luminal cell phenotype. We have now demonstrated the in vitro re-engagement of key determinants in the AR pathway and differentiation to luminal prostate cells. Documentation of the proper engagement of AR signaling is a significant step in developing a more accurate and tractable model for prostate cancer research and distinguishing between indolent and aggressive disease.
**TUMOR BIOLOGY: Cell Culture and Animal Models of Cancer**

**#4830** Precision cut cancer tissues slices as human model for the testing of immuno-modulatory compounds. Kristina Bernoth, Florian T. Unger, Moiken Petersen, Mirja Piller, Jana Krüger, Nicole Grabinski, Hartmut Juhl, Kerstin A. David. Indivumed, Hamburg, Germany.

The goal of personalized medicine is to stratify individual patients to the appropriate treatment approach. SCLC lines encompass the clinical TME. This engineered in vitro platform closely resembles the individual tumors and their sensitivity to therapeutics. In the context of the immunotherapy of cancer, information on the localization, abundance and activation of immune cells within individual tumors gained in importance. In this study, we showed that viable tumors from colorectal cancer patients used within our drug testing platform, exhibit different populations of infiltrating immune cells. Antigenic recall was achieved by addition of recombinant cytokines, such as INFγ, IL-2, IL-10 and TNFα. A correlation to PD-1 expression on T-cells in different patients has been shown. In order to optimize clinical testing of immune-modulatory compounds, preclinical models, which reflect the individual tumor, as well as the individual immune components of the tumor, are mandatory. We have shown here that effects of treatments with an immune-modulatory compound (Nivolumab) were detectable in this system. Therefore, this drug testing platform represents a unique opportunity to test immune-modulatory compounds in a fully human, patient derived model that is close to in vivo situation. In the future, other immune-modulatory compounds or combinations of compounds have to be tested within the system to more comprehensively elucidate the possibilities and limits of this drug testing platform in regard to immunotherapy.


The small of-care for limited stage and extensive stage SCLC has re- mained etoposide and a platinum complex for more than 30 years because 60- 80% of patients respond; however, SCLC inevitably recurs. Recurrent SCLC has proven to be resistant to many therapeutics administered as second- or third-line treatments; therefore, combining therapies in the first instance may be a critically useful strategy. A high throughput screen was performed where 62 SCLC lines were exposed to etoposide (0.3 μM) and carboplatin (3.7 μM) with or without simultaneous exposure to a third agent (n = 220). Viability of the cells was measured using CellTiter-Glo after 96 hr exposure to 9 concentrations of each individual compound or combination with E/C. The test concentrations encompassed the clinical Cmax for each third agent, and the concentrations of E/C selected for the screen were systematically determined to produce SCLC kill that would allow observation of additivity/synergy upon addition of a third agent. IC50s were determined from the concentration response data and showed that the predominant effect of adding a third agent to E/C was additive. Less than additive effects occurred more frequently in SCLC lines that were sensitive to etoposide/carboplatin. Antagonism with E/C occurred in combination with taxanes and taxoids of immune-competent single agents such as the nuclear kinase inhibitors (aurora kinase inhibitors, KSP/EG5 inhibitors and polo-like kinase inhibitors) were antagonistic in combination with E/C but were effective single agents. Greater than additive SCLC killing occurred with E/C in combination with several classes of agents. The combination of the Chk1 inhibitor rabusertib with E/C resulted in an IC50 that was >1 log lower than that of rabusertib alone in several SCLC lines. The GSK-3β inhibitor LY-2090314 produced greater than additive SCLC killing in combination with E/C. This study identified third agents that may represent new leads for the treatment of this recalcitrant disease. Distinct patterns of response in select SCLC lines with the GSK-3β inhibitor LY-2090314 were noted suggesting that SCLC lines contain tumor microenvironment complexity and allow a high throughput screen to be used as a platform allowing the testing of innovative approaches for the treatment of human malignancies.


Human cancers are composed of genetically and phenotypically heterogeneous subpopulations organized into a hierarchy of tumor initiating stem cells and various differentiated tumor cell. Cancer organoids seem to be more suitable in vitro models for targeting tumor heterogeneity than cell lines. We established cancer organoid lines from lung cancer patient tissues, recapitulating histology of human cancers in three dimensional culture conditions. Thirteen organoid lines from various lung cancer subtypes including adenocarcinoma, squamous cell carcinoma and small cell carcinoma were compared with their original human tumors in histologic features and genetic profiles. In the IHC staining, it revealed that organoids of adenocarcinomas have typical glandulo-papillary structures or mucin-containing tumor cells. Organoids of squamous cell carcinomas showed keratinization and intercellular bridges. Organoids of small cell carcinoma displayed a typical neuroendocrine morphology. Using NGS cancer panel sequencing, the organoid lines maintained genetic characteristics of the original human tumors in major driver genes including EGFR, TP53, and RB mutation. In xenograft experiments, the organoid lines revealed stronger tumorigenicity than direct graft of human tumor tissues. Cancer organoids showed resistance to various anticancer drug treatments. In conclusion, lung cancer organoid lines are alternative cancer model for preclinical re- searches recapitulating genotypic and phenotypic heterogeneity of original human tumors.

**#4833** Perfusion-based bioreactor culture of primary cancer tissue maintains tumor microenvironment complexity and allow in-vitro testing of immune blockade therapy. Manuele Giuseppe Muraro,1 Simone Muenst, Celeste Manfredonia,2 Valentina Mele, Silvio Daester,2 Alexandar Tzankov,2 Luigi Terracciano,3 Walter Weber,4 Giulio C. Spagnoli,6 Giandomenica Iezzi,5 Ivan Martin1 and D. Soysal.11 Asan Institute of Life Sciences, Seoul, Republic of Korea; 2 Asan Institute for Life Sciences, Seoul, Republic of Korea; 3 University Hospital of Basel, Basel, Switzerland; 4 University of Basel, Basel, Switzerland.

In vitro culture of primary cancer tissue is still very limited and the generation of patient derived xenograft demonstrate the loss of human cancer associated stroma. In this context, the use of 3D in vitro systems based on human tissue may be an innovative system to be exploited for keeping the tumor microenvironment (TME) complexity of the tissue in vitro. Freshly excised colorectal (CRC) and breast cancer (BrCa) specimens were fragment and cultured in 3D “sandwich-like format” between porous collagen scaffolds under perfusion flow (U-CUP, Cellec Biotech AG). The maintenance of tumor and immune-infiltrating cells, survival and phenotypic characterisation were histologically assessed. In a second step cancer treatment were tested. U-CUP culture allowed the preservation, viability and expansion of tumor tissue with concomitant stromal and immune cells. Expanding cancer cells were viable after 10 and 21 days (CRC and BrCa, respectively). Administration of anti-ER treatment to Lumina A ER + BrCa was associated with decreased expression of cancer tissue into the scaffold after 21 days. The maintenance of immune-infiltrating cells allowed testing of immune block- ade therapy. Administration of anti-PD-L1 antibody, alone or in combination with anti-CTLA4, to the culture medium was associated with increased expression of markers of immune-activation (i.e. IFNy) and decreased expression of immunosuppressive cytokine IL10. Preserving malignant, interstitial and immunocompetent cells comprised in surgically excised tumor specimens might allow a direct evaluation of the effects of various treatments on the complex TME. This engineered in vitro model could allow animal-free testing and it could be extended as a platform allowing the testing of innovative approaches for the treatment of human malignancies. Our findings shed the light on a promising system for selecting personalized treatment based on a patient’s tumor specific microenvironment.
While breast cancer has an overall 5-year survival rate of 89%, the rate for patients with stage 4 metastatic disease is only 26%. Immunotherapies have the potential to improve the prognosis for these patients while also providing better treatment options for all breast cancer patients since they have fewer side effects enabling longer treatment times and the use of combination therapies and reduced doses of chemotherapy. Currently these treatments are tested in standard 2D cell cultures that are inaccurate in mimicking in vivo drug response or animal models where the immune system differs from humans in numerous ways including T-cell subsets, cytokine receptors, and constitutary molecule expression. We have developed 3D models of human breast cancer that span the subtypes, ER+/HER2+, and triple-negative, incorporating numerous stromal cell types, fibroblasts and adipocytes, and include different immune cells, macrophages, and T-cells under either static or perfusion culture systems. These models have been used to examine how tumor cells influence macrophage differentiation using unidifferentiated peripheral blood mononuclear cells (PBMCs), how M1 and M2 macrophages influence tumor cell survival and proliferation, how the combination of these cell types influence cytokine secretion, and how the microenvironment affects tumor outcome. We have created a novel platform that is flexible enough to handle the heterogeneity of these complex models to examine response of tumor cells and T-cells to checkpoint inhibitors through high-throughput viability assays and flow cytometry. These models have several potential uses which include the ability to quickly answer whether a particular immunotherapy agent is effective for that particular patient-specific manner and to screen potential novel immunotherapeutic candidates and/or combinations prior to clinical use.


Patient-derived organoids are an emerging 3D model system that more closely recapitulate the organ functionality of the tissue of origin compared to traditional 2D cell lines. Further advantages of the organoid system include tumoral and inter-patient heterogeneity, diffuse infiltration of adjacent brain tissue, and establishing nutrient gradients, in a physiologically relevant setting, as well as the ability to quickly scale up from a small initial sample. We have established an actively expanding biobank of primary and metastatic colorectal cancer (CRC) tumor organoids under normoxic (21% O2) and physiologic oxygen conditions (5% O2), conditions from a diverse patient population. Concurrently, we isolated and cultured matched cancer-associated fibroblasts (CAFs). With these samples we are able to generate data from a cohort that mimics the CRC population at large, including different ethnic groups, and mutational status. Here we detail a quantitative imaging platform that captures 3D morphometric information in addition to traditional live/dead readouts. Organoids from each patient are heterogeneous in size, shape, symmetry, and various other phenotypic features. Furthermore, the perturbation of microenvironmental factors such as drugs, CAFs, etc. cause phenotypic changes over time. Common population-based viability assays used in drug screens, such as ATP or MTT, may be inappropriate to capture the complexities of drug response since they are often single end point measurements of an entire population of cells, and do not account for phenotypic variations. Short term quantitative live cell imaging incorporates phenotypic information, and can extend the lifetime of samples, yet still require manipulating samples by using dyes. However, these dyes are phototoxic, making them less than ideal for long term live cell imaging. By restricting image acquisition to brightfield we are able to minimize manipulation of patient samples, leaving them intact and viable. The additional benefit of imaging organoids in 3D enables us to get spatial information not available from assays with a single readout. Using our non-destructive imaging technique we can track 3D morphometric changes in the same organoid population over time. By using a flexible analysis method, and unbiased machine learning algorithms to determine relevant features we can account for these differences yet still get comparable data. Our organoid repository combined with long term image analysis and machine learning techniques provides us the tools that is necessary to handle the heterogeneity seen across the patient population. This platform could be used to advance personalized medicine allowing clinicians to quickly and more accurately determine the appropriate treatment for a patient by screening their tumor prior to determining a course of treatment.

3D modeling of immune cell interactions in breast cancer and prediction of immunotherapy response. Qi Guo,1 Stephen Shuford,2 Brian McKinley,2 Mary Rippon,2 Wendy Cornett,2 Mark O’Rourke,2 David Schammel,3 Jeff Edenfield,2 David L. Kaplan,2 Hal E. Crosswell,2 Teresa Desrochers1.

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A colorectal cancer organoid library provides a promising screening tool for assessment of chemosensitivity. Naoya Sakamoto,1 Akira Ishikawa,1 Ririno Honma,1 Takao Hinou,2 Naohide Oue,1 Kazuhiro Santani,1 Shiochiho Mukai,1 Tomohiro Adachi,1 Hiroyuki Egi,1 Hideki Odah,1 Wataru Yasui1.1Hiroshima University, Hiroshima, Japan; 2Institute for Clinical Research, National Hospital Organization Kure Medical Center and Chugoku Cancer Center, Kure, Japan.

Colorectal cancer (CRC) is a leading cause of cancer mortality and the third most common cancer worldwide. Annually, there are more than 900,000 cases occur worldwide, nearly 300,000 of which are fatal. CRC have accumulated defects in oncogenes and tumor suppressor genes. Many CRCs likely arise through a combination of an initiating event and adeno-matous precursor lesion via so-called adenoma-carcinoma sequence. Intensive studies have uncovered that the spectra of these genetic modifications are highly variable across the tumors. While genetic changes in tumors of individual patients can be assessed in several stratified methods, these data still need to be properly interpreted for necessitating model systems for analysis of chemosensitivity of CRC patients, which could enable us to organically coordinate among genotypeto-phenotype correlations, clinicopathological features and clinical outcomes. One of the most plausible models for implementing this magnificent scheme is most likely to be “organoid”, which is a novel 3D intestinal stem cell culture system and has successfully reconstitute normal and cancerous colonic crypts in vitro through using the specific niche factors in a dish, such as EGF, Wnt3a, Rspondin-1 and Noggin, together with a TGF-beta inhibitor and a p38 inhibitor. Two groups, Van der Wetering et.al and Fujii et.al, have previously reported the establishment of organoid biobank of colorectal cancer patients, and both of them proved the usefulness of this platform for underpinning the significance of genotype-phenotype analysis in every single patient and providing insights into tailor-made therapy in CRC. In this present study, we generated a pairs of cancerous and normal adjacent organoids from a number of CRC patients and aimed to figure out sustainability and reproducibility of CRC organoids especially in terms of phenotypic and genotypic features even after several times of passage. We compared growth activity and histopathological features of the CRC organoids among several time points after the passage by evaluating ki-67 labeling index and morphology of the organoids. In order to confirm the stability of genetic status of representative cancer-related genes, we focused on TP53, KRAS, BRAF, and MSI status. We then tried to elucidate the difference of chemosensitivity between MSI-high and MSI-low/MSS CRC cases with similar genetic background in most of the representative cancer-related genes. Although further in-depth studies on CRC organoids are needed, our approach would be instrumental in accurately predicting the drug sensitivity of CRC patients, which could possibly lead to the establishment of personalized medicine in CRC.

Development of an in vitro 3D glioblastoma model system for patient-specific drug response profiling. Teresa DesRoschers,1 Ashley Clark,1 Lauren O’Donnell,2 Qi Guo,1 Lillia Holmes,1 Lacey Dobrolecki,2 Michael Lewis,2 David Schammel,3 Jeff Edenfield,4 Charles Kanos,5 Fred Nelson,6 Steve Gardner,6 Michael Lynn,6 Philip Hodge,6 Christopher Corless,7 Paul Clark,7 Hal E. Crosswell,2 John Kuo1.1KIYATEC, Inc., Greenville, SC; 2Stemed Inc, Houston, TX; 3Upstate Pathology, Greenville, SC; 4GHS, Greenville, SC; 5OHSU, Portland, OR; 6U. Wisconsin, Madison, WI.

Glioblastoma (GBM) has a median survival of less than 2 years due to intratumoral and inter-patient heterogeneity, diffuse infiltration of adjacent brain tissue, and absence of effective therapies. Development of more efficacious therapies will require better GBM models for the testing and identification of novel agents; traditional 2D cell culture lacks biologic and clinical fidelity and orthotopic xenograft models are costly, low throughput, and time consuming. We have developed a complex, patient-specific cultured GBM model to assay drug response that combines high-throughput drug response determination with neurosphere formation and next-generation sequencing (NGS). Neurosphere formation and the presence/quality of glioblastoma stem cells (GSCs) has been shown to be associated with aggressive behavior. Our 3D model system has been validated against the primary patient GBM tissue and/or patient-derived xenografts (PDX) and consists of histology, epigenetic and mRNA expression analysis, and comparison of in vivo drug response. This strategy enabled examination of MGMT methylation in relation to temozolomide response, and possible actionable genetic mutations with candidate targeted therapies. Using our 3D model, we have observed EGFR-amplified GBM sensitivity to the EGFR inhibitor afatinib, and PTEN mutant GBM sensitivity to dual PI3K/mTOR inhibitor dactolisib. These data suggest validation of clinical and molecular correlation with this new in vitro, patient-derived 3D GBM model for drug response profiling. Our data supports the further development and use of complex 3D
models, neurosphere formation, and NGS profiling for patient-specific GBM analysis. This model system is currently being considered for preclinical assessment of novel therapies and may be a useful adjunct in future precision medicine applications to improve patient outcomes.

#4838 Optimization of prostate cancer organoid culture methods to grow PDX and human biopsy tissue. Mike L. Behtiri, Crystal Tran, Adam G. Sowalsky, Holly M. Nguyen, Supreet Agarwal, Caitlin M. Tice, Eva Corey, Kathy Kelly.

NCI, Bethesda, MD; University of Washington, Seattle, WA.

A major obstacle in the field of prostate cancer research is the limited capacity to culture prostate cancer-derived cells in vitro. There are relatively few established prostate cancer cell lines in use today. Those that are available are amenable to in vitro drug assays and mechanistic studies, but do not well-represent the genetic diversity of the disease, nor do they accurately predict in vivo response. Recently, organoid culture techniques have been developed for prostate cancer by the laboratories of Hans Clevers and Charles Sawyer that have increased our ability to grow metastatic tumor-derived prostate cancer cells in vitro. This represents a great step forward, but we are still restricted by the limited availability of biopsy tissue and the relatively low success rate (~20%) in establishing new organoid lines. The LuCaP series of patient-derived xenografts (PDXs) represents a well-characterized set of prostate cancer specimens that is genetically diverse, reflective of a range of treatment histories, and available to the research community. We have modified the organoid culture technique based upon systematic comparisons and analysis for organoid growth of the LuCaP PDXs. Our modified conditions represent an improvement upon the original growth conditions. We have successfully cultured 21 out of 24 LuCaPs attempted, for at least one generation, and 16 out of 24 are able to grow long-term over several generations. This includes 3 of the newest LuCaPs (167, 170.2, and 189.3) that we have genomically characterized. Comparison of the PDX and various organoid generations showed significant conservation of genomic changes ranging from 75-90% for nonsynonymous point mutations. The modified organoid culture method immediately and significantly increases the number of prostate cancer lines that are available by making the LuCaP PDXs accessible to in vitro culture. In addition to the PDXs, our method has improved the success for culturing metastatic samples grown from biopsies. We show here that 2 out of 3 patient-derived organoid lines that we have established, grew well in the modified conditions but poorly in the original culture conditions, the third grows equally well in both conditions.

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#4839 Ephrin-A ligands regulate cutaneous tumor etiology and metastasis through cell autonomous and non-autonomous mechanisms. Ji Zheng, Bethany Perez White, Nihal Kaplan, Zhe Zhao, Miroslaw Blumenberg, Spiro Getios, Bingcheng Wang. Case Western Reserve Univ., Cleveland, OH; Northwestern University, Chicago, IL; Weill Cornell University, New York, NY.

Glycosphingolipid-dependent anchored ephrin-A ligands target EphA receptor tyrosine kinases (RTKs) to promote keratinocyte differentiation. Accordingly, genetic ablation of the major epidermal EphA subtype, EphA2, increases susceptibility to DMBA/TPA-induced cutaneous chemical carcinogenesis. Defining the corresponding role of ligands for EphA2 in skin cancer has been more cumbersome as the three ephrin-A genes (Efna1, Efna3, Efna4) are all promiscuous in expression. Recently, we have shown that knockdown of EphA2 expression in primary skin keratinocytes induced a change in ephrin-A ligand expression, especially the homo-variant Efna4, which is associated with an increased risk of metastasis. Up-regulation of c-Jun activated KISS1R. The mechanism of action for KISS1 metastasis suppression function of BRMS1 and promotes metastasis. Up-regulation of c-Fos induced L1CAM transcription is a putative mechanism of BRMS1 A273V-induced metastasis. Our data suggest that SNP rs1052566, especially its homo-variant, is associated with an increased risk of metastasis in LuAD.

#4840 SNP rs1052566 (Ala273Val) in BRMS1 variant 2 promotes metastasis in lung adenocarcinoma. Neel P. Chudgar, Yuan Liu, Joseph Montecalvo, Marty W. Mayo, Prasad S. Adusumilli, David R. Jones.

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Background: Metastatic lung cancer kills over 180,000 people in the United States annually. Family history is one risk factor for lung adenocarcinoma (LUAD), suggesting a putative genetic predisposition to the development of LUAD. Single nucleotide polymorphisms (SNPs) are the most common genetic variations in the human genome and major determinants of variations in disease susceptibility, prognosis, response to medication, and toxicity. Recent studies indicate that SNPs play important roles in the progression of LUAD. We have previously shown that breast cancer metastasis suppressor-1 (BRMS1) plays a critical role in inhibiting the progression of liver cancer. We observed that SNP rs1052566 homo-variant in breast cancer metastasis suppressor-1 (BRMS1) variant 2 (v2) causes an A273V mutation. However, the role of this SNP in the progression of lung cancer remains to be determined. Methods: We utilized next-generation DNA sequencing and Taqman SNP assays to detect the prevalence of rs1052566 in 40 surgically resected LUAD specimens. Next, we generated a retroviral expression system (pBabe puro) encoding V5-tagged BRMS1v2 wild-type (WT) and A273V mutant and created NSCLC H1101 and A549 cell lines that stably express V5-tagged BRMS1v2 WT, A273V, or vector control. The metastatic capabilities of these stable cells were evaluated by invasion assays in vitro and by tail-vein injection in vivo. To explore the mechanism(s) through which BRMS1v2 A273V promotes metastasis, we performed RT profiler PCR assay. Results: We determined that SNP rs1052566 is present in 45% of LUAD patients. Of these, the homo-variant occurs in 7.5% of patients (3 of 40). Importantly, BRMS1v2 A273V significantly promotes metastasis of NSCLC cells in vitro and in vivo. Additionally, in our mouse model, A273V was associated with decreased survival, compared with BRMS1v2 WT or control. On RT profiler PCR array, 7 genes, including L1CAM, were found to be significantly differentially regulated by BRMS1v2 WT and A273V. Confirmatory RT-PCR and immunoblots proved that BRMS1v2 A273V increases both the transcript and protein levels of L1CAM. Furthermore, we found that either c-Jun or c-Fos promotes the transcription of L1CAM. However, BRMS1v2 A273V specifically enhances c-Fos-, but not c-Jun-, induced L1CAM transcription. Conclusion: We observed that BRMS1v2 A273V abolishes the metastasis suppressor function of BRMS1 and promotes metastasis. Up-regulation of c-Fos-induced L1CAM transcription is a putative mechanism of BRMS1v2 A273V-induced metastasis. Our data suggest that SNP rs1052566, especially its homo-variant, is associated with an increased risk of metastasis in LUAD.

#4841 Suppression of metastasis by KP54 and non-KP54 kisspeptins. Kelsey Hampton, Keke M. Pounds, Andrew P. Trembath, Danny R. Welch.

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KISS1 is a secreted neuropeptide which can act as a metastasis suppressor protein. Expression of KISS1 inhibits metastatic colonization at secondary sites, rendering disseminated cells dormant. In order for KISS1 to suppress metastasis, it must be secreted outside of the cell, whereupon cleaves KISS1 into kisspeptins. When assessing metastasis-associated traits in vitro, suppressor KMP sup- pressive metastasis in vivo. As a whole, these data suggest that metastasis suppression by KISS1 is not necessarily contingent on KISS1 activation, and also supports investigation into additional receptors.

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The recurrence and metastasis of hepatocellular carcinoma (HCC) portends a poor prognosis and represents important clinical challenges. There is a great need to identify critical factors involved in HCC metastasis that will facilitate the development of new therapeutic strategies. We have previously found that the initiation, growth and self-renewal of CD133+ liver tumors to be fine-tuned by a balance of miR-130b overexpression and tumor protein 53 inducible nuclear protein 1 (TP53INP1) down-regulation, suggesting that TP53INP1 is a critical effector driving hepatocarcinogenesis. In this study, we aimed to further investigate the role of TP53INP1 in HCC metastasis. We showed TP53INP1 to be frequently down-regulated in advanced stage IV and metastatic human HCC tumors as compared with early stage (I-II) and primary tumors. Functional studies in immortalized normal liver cell line MIHA and HCC cell line MHCC-97L found TP53INP1 suppression in HCC to promote metastasis in vitro and in vivo. To elucidate the downstream signaling mechanism by which TP53INP1 regulates HCC metastasis, a Proteome Profiler Human Phospho-Kinase Array was utilized. Phospho-ERK was found to be consistently up-regulated in HCC cell lines with TP53INP1 knocked down, with its involvement in TP53INP1-mediated metastasis suppression subsequently validated by rescue experiments using an ERK inhibitor U0126 or shERK1/2 knockdown approach. ERK1/2 is known to be negatively regulated by a family of dual-specificity MAPK phosphatase called DUSP/MKP. Screening of a panel of DUSP/MKP family members by qPCR identified DUSP10 to be commonly down-regulated in both MIHA and MHCC-97L cells with TP53INP1 suppressed as compared to controls. The importance of DUSP10 inactivation on ERK in TP53INP1-mediated HCC was substantiated by rescue experiments whereby DUSP10 ectopic expression in TP53INP1 suppressed cells reversed up-regulated phospho-ERK expression and enhanced HCC aggressiveness. Subsequent analysis of the DUSP10 promoter region by open-access database revealed four putative binding sites for p73, of which transcriptional activity has previously been found to be modulated by TP53INP1. Chromatin immunoprecipitation and luciferase reporter assays collectively demonstrated that TP53INP1 is vital for p73 transcriptional activity of DUSP10. Taken together, TP53INP1 down-regulation via metastasis suppression through a p73-dependent DUSP10/ERK signaling pathway.
ously that both enzymatic activities of NME1 are critical for repair of UV-induced DNA damage via nucleotide excision repair. In the current study, chromatin immunoprecipitation was used to demonstrate recruitment of NME1 to DNA double-strand breaks (DSBs), representing the first evidence of direct recruitment of NME1 to sites of DNA damage. Induction of DSBs with gamma-irradiation (γ-IR) or bleomycin promoted rapid physical association of NME1 with γH2AX, ATM, NBS1 and RAD50. In addition, treatment of melanoma cells with γ-IR triggered a physical association of NME1 with XRCC4, an effecter of the non-homologous end-joining (NHEJ) pathway. We are currently investigating whether knockdown of NME1 induces a switch from NHEJ to the homologous recombination (HR) pathway, a slower but higher fidelity form of DSB repair associated with loss of tumor suppressor activity. Our results are also consistent with the idea that DNA damage induced by bleomycin rescue the role of NME1 on the fidelity of NHEJ repair. Overall, our study indicates for the first time direct participation of NME1 in a molecular mechanism of DSB repair in both normal and transformed cells. In addition, these data suggest a novel mechanism of metastasis suppressor function for NME1, with loss of NME expression in tumor cells conferring genetic instability and enhanced metastatic potential.

#4847 NME1 mediates a switch in beta integrin subunits that correlates with prolonged patient survival. M. Kathryn Leonard,1 Grace Snow,1 Marián Novak,1 Joseph R. McCorkle,2 Xiweii H. Yang,3 Alexey Belkin,1 David M. Kaetzel.1,1 University of Maryland, Baltimore, Baltimore, MD;2 St. Jude Children’s Research Hospital, Memphis, TN;3 University of Kentucky, Lexington, KY.

Expression of the metastasis suppressor NME1 in melanoma is associated with reduced cellular motility, invasion, and metastasis, but the molecular mechanisms underlying this activity are not completely understood. Herein we report a novel mechanism through which NME1 modulates focal adhesion dynamics via regulation of integrins β1 and β3. Stable expression of NME1 significantly altered focal adhesion turnover at the cell periphery. Interestingly, over-expression of NME1 resulted in a switch from predominantly fast recycling αβ1 integrins to slower recycling αβ3 integrins. Contrary to its regulation of other cell surface receptors, the inhibition of integrin β1 and induction of integrin β3 by NME1 was found to occur at the transcriptional level rather than through dynamic mediated endocytosis. Induction of integrin β3 required both the 3’s exonuclease and nucleoside diphosphate kinase (NDPK) activities of NME1, which are also required for its metastasis suppressor activities in vivo. Further suggesting that the induction of integrin β3 is involved in the metastasis suppression function of NME1, knockdown of integrin β3 significantly increased the invasion capability of cells expressing NME1 in vitro compared to control cells. Analysis of metastatic melanoma patients in the TCGA showed individuals with a 1.5x or greater increase in integrin β3 mRNA had a significantly longer overall survival times. Additionally, an inverse correlation was observed between NME1 and integrin β3 mRNA in independent microarray of primary melanomas. The inverse correlation of NME1 and integrin β3 mRNA was also a strong predictor of prolonged distant disease free and overall survival in patients with the basal-like subtype of breast carcinoma. Together, these data strongly suggest NME1 prevents metastasis of human melanoma and some types of breast cancers by altering beta integrin expression to reduce recycling of focal adhesions and, ultimately suppress cell motility.

#4848 The functional synergism and pro-metastatic role of FOXM1 and CENPF in hepatocellular carcinoma. Wai Ling Macrina Lam,1 Lo Kong Chan, Daniel Wai-Hung Ho, Charles Shing Kam, Irene Oi-Lin Ng. The University of Hong Kong, Hong Kong, Hong Kong.

Hepatocellular carcinoma (HCC) is the second leading cause of cancer death worldwide and metastasis is regarded as the major cause of HCC-associated lethality. In this study, we have analysed the TCGA whole-transcriptome sequencing data of paired human HCC samples (n=50), and identified Forkhead Box M1 (FOXM1) and Centromere Protein F (CENPF) to be the top-listing upregulated genes. Interestingly, both of them are the essential components in cell-cycle progression. FOXM1 encodes for the cell-cycle-dependent transcription factor that regulates genes for DNA replication and mitosis, while CENPF encodes for the centromere protein that is required for kinetochore function and chromosome segregation in mitosis. We hypothesized that the upregulation of FOXM1-CENPF signaling axis may drive hepatocarcinogenesis. In our human HCC cohort (n=34), FOXM1 and CENPF were shown to be upregulated compared with the non-tumorous liver tissues, and their mRNA expressions were positively correlated (p=0.0001). Co-upregulation of FOXM1 and CENPF in patient samples was demonstrated to be positively correlated with the absence of tumor encapsulation (p=0.035) in our clinico-pathological correlation analysis, whereas the samples without their co-upregulation was less likely to be correlated with venous invasion (p=0.045). This indicated that FOXM1 and CENPF are likely to be associated with HCC metastasis synergistically. Stable single and co-knockdown clones of FOXM1 and CENPF in four HCC cell lines (BEL7402, SMMC-7721, MHCC-97L, HepG2) were established using short-hairpin (sh) RNA approach for subsequent functional characterization. Both single and co-knockdown of FOXM1 and CENPF cells have shown reduced proliferation rate (p<0.05) in cell proliferation assay. Moreover, all single and co-knockdown of FOXM1 and CENPF cell lines were found to have at least 2-fold reduction in migration and invasion rate (p<0.0001) in transwell cell migration and invasion assay. These suggested that FOXM1 and CENPF are critical for HCC cell proliferation, migration and invasion. To assess the translational significance of targeting FOXM1 and CENPF, seven HCC cell lines (BEL7402, SMMC-7721, MHCC-97L, Hep7, Hep3B, HepG2, PLC/PRF/5) were challenged with thiostrepton and zoledronic acid, which are inhibitors against FOXM1 and CENPF, respectively. Intriguingly, both individual and combined treatments of these inhibitors effectively inhibited HCC cell growth. It was also demonstrated that such inhibition acts via suppressing the respective endogenous transcript and protein expression of FOXM1 and CENPF. Taken together, our study has demonstrated that FOXM1 and CENPF play a critical oncogenic role in HCC and they may function as an attractive molecular therapeutic target. This study is supported in part by Health and Medical Research Fund (03142836).

#4849 ELMO1 promotes metastasis in colorectal cancer cells via activation of MAPK/ERK signaling pathway. Xiao-bing Zheng,1 Chi Zhou,1 Hai-chun Cheng,2 Tuo Hu,3 Hua-hui Liu,3 Xian-rui Wu,2 Feng-wei Wang,2 Yu-feng Chen,4 Jian-ting Wang,4 Xiao-sheng He,2 Ping Lan,2 Departament of Colorectal Surgery, the Affiliated Shenzhen Shajing Hospital, Guangzhou, China;5 Department of General Surgery, the Affiliated Shenzhen Shajing Hospital, Guangzhou Medical University, Shenzhen, China;6 State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, Guangzhou, China.

Background and Aim: Engulfment and cell motility 1 (ELMO1) has been linked to the invasive phenotype of various types of cancer cells such as glioma cells, rhabdomyosarcoma cells, ovarian cancer cells and so on. However, the biological functions of ELMO1 in colorectal cancer (CRC) cells remains unclear. The aim of this study is to evaluate the role of ELMO1 in the metastasis in CRC. Methods: The expression level of ELMO1 was detected using quantitative RT-PCR and western blot in 9 CRC cell lines and 1 human colon epithelial cell line NCM460. ELMO1 was overexpressed via lentiviral vector system and silenced using specific small interference RNA (si-ELMO1) in HCT116 and SW620. Then wound-healing, migration assay and invasion assay were carried out to investigate the function of ELMO1 in CRC cells. Western blot was performed to detect the change of signaling molecule extracellular signal-regulated kinase 1/2 (ERK1/2) following the down-regulated or up-regulated expression of ELMO1. Results: ELMO1 expression was inhibited by si-ELMO1 and was overexpressed by ELMO1-cDNA in HCT116 and SW620. Cytotoxic expression of ELMO1 in HCT116 and SW620 was shown to promote migration and invasion, while inhibited ELMO1 expression suppressed the capacity of cell migration and invasion. Furthermore, ELMO1 induced migration and invasion in CRC cells was found to be associated with the activation of MAPK/ERK pathway. Conclusions: High expression of ELMO1 plays an important role in the metastasis via the activation of MAPK/ERK signaling pathway. This finding provides a molecular basis for the role of ELMO1 in the progression of CRC, which may suggest a novel target for the treatment of CRC.

#4850 Overexpression of P62/IMP2 promotes metastasis in hepatocellular carcinoma via inhibition of E-cadherin expression. Mengtao Zheng,1 Chi Zhou,1 Hai-chun Cheng,1 Tuo Hu,3 Hua-hui Liu,3 Xian-rui Wu,2 Feng-wei Wang,2 Yu-feng Chen,4 Jian-ting Wang,4 Xiao-sheng He,2 Ping Lan,2 Departament of Colorectal Surgery, the Affiliated Shenzhen Shajing Hospital, Guangzhou, China;5 Department of General Surgery, the Affiliated Shenzhen Shajing Hospital, Guangzhou Medical University, Shenzhen, China;6 State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, Guangzhou, China.

P62/IMP2 is overexpressed in human HCC tissues compared with normal human liver tissues (score=10.30, n=40 vs. score=5.23, n=30, p<0.05). The expression of P62/IMP2 was scored by immune-staining intensity and positively immuno-staining cell numbers. At the same time, we found that p62/IMP2 was also overexpressed in several HCC cell lines. Stable p62/IMP2 overexpression and
depletion SNU449 human HCC cell line was made by using p62/iMP2 cDNA transfection and lentivirus mediated knockdown approaches, and it was further confirmed by western blotting analysis. Wound healing assay has shown that overexpression of p62/iMP2 can significantly enhance the cell migration ability in SNU449 cells. On the contrary, the depletion of p62/iMP2 can reduce the migration ability of SNU449 cells (p<0.05). As shown p62/iMP2 overexpressed cells may have a higher rate of proliferation at 72hr(p<0.05). In addition, we have observed that overexpression of p62/iMP2 can significant inhibit the expression of E-cadherin in western blotting analysis. In summary, our data would suggest that overexpression p62/iMP2 may promote metastasis via inhibition of E-cadherin expression in HCC.


Background: NOTCH oncogenic signaling induces epithelial-to-mesenchymal transition (EMT) and tumor stemness that play a critical role in driving tumor progression. Following interaction of NOTCH receptors (NOTCH1, 2, 3, and 4) with their ligands (DELTA-LIKE 1, 3, 4 and JAGGED 1, 2), γ-secretase complex performs an intra-membrane cleavage releasing the NOTCH intracellular domain (NNICD). NNICD translocates to the nucleus and induces the expression of HEY1 and HES1 transcription factors that in turn orchestrate the modulation of proteins related to cell cycle/apoptosis and immune regulation of PD-L1, PD-L2 and B7-H4, important negative regulators of immune function, often over-expressed in cancer cells; including breast cancer.

Conclusions: Promising candidates will be tested in follow-up mouse xenograft studies. Encouraging results in these pre-clinical models would provide a strong rationale for the development and testing of inhibitors to one or more of these potential targets as a new therapeutic for the 30% of breast cancers that are CREB3L1-deficient.

#4853 NRP2b, a unique isoform of NRP2, promotes aggressive lung cancer phenotypes. Anastasios Dimou,1 Patrick Nasarre,1 Joyce Nair-Menon,1 Federico Cappuzzo,2 Lorenzo Landi,3 Armida D’Incecco,2 Hidetaka Uramoto,3 Takeshi Yoshida,1 Eric Haura,5 Monica Gooz,1 Kent Armeson,1 Robert Gemmill,1 Harry Drabkin1.

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Neuropilins (NRPNs) 1 and 2 are highly-related receptors for class 3 semaphorins, and interact with heparin-binding growth factors and their receptors (e.g. HGF-MET, VEGF-VEGFR2 etc.). We previously reported that NRP2 is upregulated by TGFβ and is required for its pro-tumorigenic activity. Here, we show that this upregulation preferentially involves NRP2b, a largely uninvestigated isoform encoding a divergent, yet highly-conserved C-terminus. Importantly, using a panel of lung cancer cell lines and orthotopic metastasis model, NRP2b promoted migration, invasion, metastasis and tumour formation, whereas the prototype receptor, NRP2a had opposite effects. TGFβ-mediated resistance to gefitinib in EGFR mutated tumors also was dependent on NRP2b expression. In addition, NRP2b, but not NRP2a, linked MET activation by HGF to AKT phosphorylation. In co-immunoprecipitation assays, we found that NRP2a robustly interacted with PTEN, while the interaction with NRP2b was weak. At the clinical level, NRP2b expression was commonly upregulated in patient lung cancer samples and this upregulation was a significant poor-prognostic factor. Collectively, these data indicate that NRP2b plays an important role in lung cancer invasion, metastasis and EGFR inhibitor resistance. Furthermore, isoform-specific interactions between NRP2 and PTEN may be responsible for the observed differences in MET signaling in response to HGF.

#4854 Evaluating HDAC6 as a causal factor in metastatic breast cancer to develop immunotherapy. Deborah Anderson,1 Shari Smith,2 Farah Goubran,2 Paul Mello,2 Saskatchewan Cancer Agency, Saskatchewan, Canada; 2University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

Introduction: Our laboratory is interested in determining ways to block breast cancer metastasis. We discovered that CREB3L1 (cAMP responsive element binding protein 3-like protein), a stress-activated transcription factor, acts as a metastasis suppressor in breast cancer. CREB3L1 is expressed ubiquitously in noncancerous human breast cells and restricts expression of genes that promote cell growth, angiogenesis, and migration. Loss of CREB3L1 expression is a frequent event in high-grade metastatic human breast tumors and can result in enhanced metastatic properties. Experimental Procedures: To establish the mechanisms by which CREB3L1 exerts its tumor suppressive effects, a gene expression microarray analysis was performed to determine which genes are upregulated in CREB3L1-deficient breast cancer cells. Six hundred and eighty genes were statistically significantly upregulated at least 2-fold in all 4 of the CREB3L1-deficient cell lines assessed. To focus our analysis, 4 genes were identified as potential targets for this study, given that they have previously been shown to have a role in cancer progression. Knockdowns were generated for each of the 4 genes in a CREB3L1-deficient breast cancer cell line (HCC1806) and matched CREB3L1-expressing cells (HCC1806 + HA-CREB3L1), as well as a nontumorigenic control breast cell line (MCF10A). To knockdown expression of each of the 4 targets, shRNA directed at the gene of interest was transfected via a lentiviral system into the cells. Cell-based assays were performed to characterize the knockdowns. shRNA were screened for their ability to measure migration, soft agar assay to assess anchorage-independent growth, and the MTT assay to evaluate cell proliferation. Results: Successful knockdown at both the mRNA and protein level for each target gene was validated using quantitative real time PCR and immunoblotting. Results from the MTT assay verified that knockdown of each of the 4 gene targets did not affect cell survival in MCF10A. We confirmed that these test genes are downregulated in noncancerous breast cells (p > 0.05). Similarly, knockdown of each of the test genes did not affect cell proliferation in HCC1806 (≥ CREB3L1) cells, except for a significant decrease in proliferation in HCC1806 CREB3L1-deficient cells with Gene 1 knockdown (p = 0.02). Preliminary results indicate that knockdown of Genes 1, 2, and 4 in HCC1806 CREB3L1-deficient cells reduces cancer cell properties, including cell migration and anchorage-independent growth (p < 0.05).

Conclusions: Promising candidates will be tested in follow-up mouse xenograft studies. Encouraging results in these pre-clinical models would provide a strong rationale for the development and testing of inhibitors to one or more of these potential targets as a new therapeutic for the 30% of breast cancers that are CREB3L1-deficient.
is strongly suggestive of HDAC6 being a key player in metastatic cancer progression. In our initial studies we observed that the selective HDAC6 inhibitor Nexaturast A is capable of reducing the tumor growth in a highly aggressive murine mammary carcinoma that mimics human triple negative breast cancer (TNBC), under both orthotopic and subcutaneous conditions of implantation. Additionally, we showed that the size and number of secondary tumor nodules were significantly diminished after the HDAC6 treatment. In order to boost the anti-tumor T-cell response, we also tested check-point inhibitors against the tumor (such as anti PD-1 and CTLA4 antibodies). While each of the standalone anti-tumor T-cell response, we also tested check-point inhibitors against the tumor (such as anti PD-1 and CTLA4 antibodies). While each of the standalone treatments showed a certain degree of success in reducing tumor growth and enhancing intra-tumoral IFNγ response, we demonstrated that HDAC6 improves anti-tumor immune responses when combined with immune check-point blockade.

**#4855 Loss of USP18 represents invasion and metastasis of lung cancer.** Lin Zheng, Lisa Mustachio, Yulong Chen, Xi Liu, Jason Roszik, Jonathan Kurie, Ethan Dmitrovsky. UT MD Anderson Cancer Center, Houston, TX.

Metastasis is a major cause of human lung cancer mortality. Uncovering novel targets and mechanisms involved in regulating metastasis is critical for developing effective ways to improve lung cancer survival. It is reported that ubiquitin and ubiquitin-like pathways play an important role in invasion and metastasis. We previously found that USP18 (Ubiquitin Specific Peptidase 18) is substantially up-regulated in several cancers, including lung cancer. Engineered repression of the deubiquitinase USP18 decreased growth, increased apoptosis and augmented chemotherapeutic agent response of lung cancer cells. Our prior work showed that loss of USP18 can destabilize specific oncogenic proteins and this results in the marked reduction of lung cancer cell growth and in vivo tumorigenicity. Thus this study sought to explore the precise role of USP18 expression in invasion, migration and metastasis of lung cancer. In murine (KC2 and 344SQ) and human (A549 and H1299) lung cancer cells with varying degrees of metastatic potential, knock-down of USP18 by different short hairpin RNAs (shRNAs) decreased cell growth and increased cell death as compared to vector control transfectants. The findings from wound-healing migration and Transwell invasion assays established that USP18 knock-down in all studied lung cancer cell lines conferred substantial reduction of migration and invasion versus vector control transfected cells (p < 0.001). In mouse models, USP18 knock-down of lung cancer cell lines displayed a lower number of lung cancer metastasis in vivo. To discern engaged mechanisms, Reverse Phase Protein Arrays (RPPAs) were performed to interrogate over 300 growth-regulatory proteins in murine (344SQ and KC2) and human (A549 and H1299) lung cancer cells following USP18 knock-down. Using RPPAs, potential targets identified were differentially expressed between USP18 knock-down and vector control transfected lung cancer cells. Highlighted species included Myc, eEF2K (Eukaryotic Elongation Factor 2 Kinase), Programmed Cell Death 4 (PDCD4), Hes Family BHLH Transcription Factor 1 (Hes1) and Polo like Kinase1 (PLK1), among other species. In addition, phosphorylated Acetyl-CoA Carboxylase (ACC), a key component of the α5-adenosine monophosphate-activated protein kinase (AMPK) pathway, was significantly affected in lung cancer cell lines following USP18 knock-down (p < 0.05). Ingenuity Pathway Analysis and functional validation are now underway to uncover specific pathways directly involved in the observed reduction of lung cancer metastases. Taken together, this study implicates the deubiquitinase USP18 as a molecular target to combat lung cancer metastases.

**#4856 High mRNA expression of splice variant SYK short correlates with poor hepatic metastasis free survival in untreated lymph node negative colon cancer patients.** Robert R. Cochergh van den Braak, Anieta S. Siewerts, Zarina S. Lalmahomed, Sandra Bril, Annemieke M. Timmermans, Vanja de Weerd, Michelle van der Vlugt - Daane, Anne van Galen, Shan Shan Xiang, Katharina Biermann, John A. Foekens, John W. Martens, Jan N. Hjermes. Erasmus University Medical Center, Rotterdam, Netherlands.

Introduction: In lymph node negative (LN-) colon cancer 20% of the patients will develop recurrence of disease. Identification of these patients is an unmet need. SYK, a protein kinase, has been ascribed both a tumor promoter and suppressor role in epithelial cancers. The prognostic value of SYK and its splice variants, largely unknown in colorectal cancer however, was explored in a clinically well-defined cohort of colon cancer patients. Methods: Total mRNA expression of SYK (SYK[T]) and of its two splice variants SYK short(S) and SYK long(L) were measured using RT-qPCR in a clinically well-defined prospectively collected cohort of 240 colon cancer patients (n = 160 untreated lymph node negative [LN-] and n = 80 adjuvant treated lymph node positive [LN+] patients) selected from the MATCH-cohort. mRNA expression levels were related to microsatellite instability (MSI), mRNA expression of epithelial (EPCAM), stromal (BGN, FAP, INHBA) and infiltrate markers (VEGFA, CD45), known CRC mutations (n = 238), and disease free (DFS), hepatic metastasis free (HFS) and overall survival (OS). Results: Overall increased SYK levels were associated with stage I/II, a left-sided located primary tumor and MicroSatellite Stability (MSS). However, these associations and their interrelation differed significantly between SYK[T], SYK(S) and SYK(L) expression implicating an added value for measuring mRNA expression of the splice variants next to SYK(T). SYK[T], SYK(S) and SYK(L) levels all showed a significant positive correlation with the expression of EPCAM, FAP was weakly negatively associated with SYK(S) and VEGFA was weakly positively correlated with SYK(T) and SYK(S). This suggests a higher expression of SYK in epithelial-rich, stromal-poor tumors. SYK(T) and SYK(S) expression was significantly lower in tumors with a BRAF or PTEN mutation (mt) compared to wild type (wt) tumors. Although others reported differential expression of SYK between KRAS-dependent and KRAS-independent cell lines and KRAS mt versus wt tumors in 221 TCGA-samples (p = 0.008), we observed no significant differences for expression of SYK(T), SYK(S) and SYK(L) between KRAS-mutant (mt) and KRAS-wild type (wt) tumors. In the LN- group, using univariate Cox regression analysis increasing mRNA expression of SYK(T) (HR = 2.05 95%CI= 1.01-4.17 p = 0.047) and SYK(S) (HR = 1.83 95%CI= 1.09-3.05 p = 0.021) was associated with worse HFS, which remained significant for SYK(S) when correcting for the number of assessed lymph nodes (HR = 1.83; 95% CI = 1.08-3.12; p = 0.026 and HR = 1.27; 95%CI=1.09-1.60; p = 0.042). No other significant associations between SYK(T), SYK(S) and SYK(L), and DFS, HFS and OS were observed. Conclusion: In our untreated LN- colon cancer cohort SYK(S) is a pure prognostic marker for HFS. These results may help to identify LN- patients at overall low risk to develop liver metastases.

**#4857 TAK1 mediated IL1 expression as autocrine signaling to promote breast cancer metastasis.** Oihana Iriondo, Mostafa Elhodaky, Yarong Liu, Grace Lee, Julie E. Lang, Pin Wang, Min Yu. Univ. of Southern California, Los Angeles, CA.

TGFβ-activated kinase 1 (TAK1), a serine/threonine kinase from the MAPKK family, is regulated by different cytokines such as IL1, TGFβ, TNFα and BMPs, and it is therefore a key player in the cellular responses induced by changes in the microenvironment. TAK1 regulates cell survival, differentiation and inflammatory responses by activating other intracellular kinases such as p38, JNK or IKK. TAK1 activation is thought to influence the progression of several types of cancer, including lymphomas and pancreatic, colon, liver and breast cancer. In this study, we aimed to investigate the mechanisms by which TAK1 regulates breast cancer progression. (S2)–7–Oxooxetanol is a compound that inhibits the kinase activity of TAK1. Treatment of mice injected with MDA-MB-231 cells with (S2)–7–Oxooxetanol containing nanoparticles reduced metastasis formation, while growth of the primary tumor was not affected. TAK1 involvement in metastasis was confirmed by xenotransplantation experiments done with MDA-MB-231 cells overexpressing wild type or dominant negative forms of TAK1. Using these stable cell lines, we confirmed that TAK1 is required for p38 activation induced by several cytokines. Interestingly, cells cocultured with macrophages showed a TAK1-dependent increase in the expression levels of IL1α and IL1β, which could lead to TAK1 activation themselves. These cytokines were found to upregulate the expression of IL1. Using a transfilter setup it was found that TAK1 activation itself induced IL1 (S2)–7–Oxooxetanol was able to inhibit IL1 promoter activity in a dose-dependent manner. These findings suggest that the lung microenvironment could promote the initiation of a positive feedback loop by cancer cells, promoting the growth of metastatic lesions. Disruption of this positive autocrine feedback loop may be a potential therapeutic approach to suppress breast cancer metastasis to the lung.

**#4858 AK4 promotes colorectal cancer progression and metastasis.** Yau Hei Yu, Joanna Hung Man Tong, Yi Pan, Raymond Wai Ming Lung, Ka Fai To, Anthony Wing Hung Chan. The Chinese University of Hong Kong, Hong Kong.

Metastatic colorectal cancer (CRC) is a fatal disease with a poor prognosis. It is crucial to understanding the underlying pathogenesis to battle this dismal condition. We previously identified that AK4, a gene on chromosome 1p31.3 encoding a member of adenylate kinase family enzymes, was progressively up-regulated in colorectal cancer (CRC) to cancer to normal colon tissues. We further evaluated expression microarray of paired CRC samples. This study was aimed to validate the overexpression of AK4 and explore in-vitro effects in CRC. We firstly examined the mRNA expression level of AK4 in a panel of CRC cell lines. AK4 mRNA expression was higher in CRC cell lines (n=8) than normal colon cell line, and significantly higher in advanced stage cell lines (n=4, Duke’s C and D) than early stage ones (n=4, Duke’s A and B) (P=0.03). We further evaluated expression level of AK4 in two different cohorts of clinical samples. The first cohort consisted of 18 paired primary CRCs and its corresponding normal colon mucosa, and 27 metastatic CRC (including 15 cases with paired primary CRC and normal colon mucosa). AK4 mRNA level was significantly higher in metastatic CRC
than normal colon (P<0.001), and metastatic CRC than primary CRC (P<0.001). Among 15 cases with paired primary and metastatic tumors, 14 (93.3%) demonstrated higher AK4 in metastatic tumors than their primary counterparts (P<0.05). The second cohort composed of 190 consecutive primary CRCs. Upregulation of AK4 protein expression evaluated by immunohi-
tochemistry was found in 68.4% of CRCs, and its activation is independent of bothVMvesselformationanddrugdelivery. Wehave shownthatPRAMEoverexpressingcelllines(Mel290PRAME-V5andF41TET-
expression was not only a prognostic biomarker but also could be a therapeutic target. Since F41 cells remain epitome+- and highly metastatic, we constitutively silenced PRAME with a V5 tag (F41 shPRAME-V5). For Mel290 cells, we overexpressed PRAME to see whether this cell line, which does not induce metastatic death, became more malignant. We confirmed the silencing and overexpression of PRAME in our transformed cells with western blot. For cell proliferation, we found a slight increase in the Mel290 PRAME+ cells compared to non-transformed Mel290. Strikingly, in F41 cells, knockdown of PRAME led to complete cell death. In a xenograft animal model, NSG male mice were injected with either Mel290 PRAME-V5, Mel290, F41 shPRAME-V5 or F41 TET-empty vector cells. We found that PRAME overexpressing cell lines (Mel290 PRAME-V5 and F41 TET-
empty vector) induced rapid, multiple and aggressive liver metastases that led to death. Interestingly, the non-expressing PRAME cell lines (Mel290 and F41 shPRAME-V5) did not present macro or micrometastasis in any cases. This data highly suggests that PRAME overexpression is involved in UM metastasis. Thus, PRAME mRNA expression is not only a prognostic biomarker but also could be an important target for treatment.

#4862 SORB1-1AHNK complex regulates metastatic properties of cancer. Woo-Choil Cho, Ji-Eun Jang, Ja-Lok Ku. Seoul National University, Seoul, Republic of Korea

Purpose: CAP encoded by SORB1, Vinexin and ArgBP2 is participated in the SoHo family of proteins. The SoHo protein interacts with different signaling molecules and involves with cell migration, and has been implicated in a variety of cellular processes including insulin-stimulated glucose transport. CAP has
specific role in the stabilization of adhesion complexes and the consequent effect of cell migration. It is possible that CAP or complex between CAP and other proteins induces change of cancer cell characters. We assumed that SORB1 has substantial role of cell proliferation, migration and interaction with other subcellular proteins. Methods and materials: To elucidate endogenous role of SORB1 and AHNAK in metastatic cancer cells, we manipulated by plasmid, siRNA and shRNA in some colorectal cancer cell lines. Function of SORB1 as regulator of cancer proliferation was demonstrated by cell cycle assay. And several methods including western blotting, cell migration assay and immunoprecipitation assay were performed for determining role of SORB1 related other metastatic properties. Conclusion: Colony formation ability and proliferation were induced by SORB1 and AHNAK knockdown in HT29 cell line. In contrast, transient suppression of SORB1 inhibits cell proliferation. Moreover, when SORB1 was constantly suppressed in HCT-116 and SNU-C4 cell line, cell migration was impeded. According to immunoprecipitation data, we chose the AHNAK protein as the convincing candidate protein related with SORB1. An interaction of SORB1 and AHNAK was observed in both cytoplasm and nucleus. Expression level of SORB1 and AHNAK was also negative correlation in extraction of cytoplasm and nucleus. SORB1 and AHNAK bind each other as form of protein complex, however, AHNAK known as nucleoprotein is abundant in both cytoplasm and nucleus when SORB1 is suppressed. Consequently, these results indicate that SORB1 has a key role of cancer cell growth and invasiveness through regulation of AHNAK expression and degradation.

#4863 Aurora-A kinase: a nuclear driver of metastasis. Kristina Marinarik,1 Anna Kiselova,2 Yuriy Loskutov,1 Matthew Smolkin,1 Elena Pugacheva3.1 West Virginia University, Morgantown, WV; 2Fox Chase Cancer Center, Philadelphia, PA.

Aurora-A Kinase (AURKA) is a serine/threonine kinase that is critical for mitosis. AURKA is overexpressed in 96% of human cancers, including breast cancer. It has been previously shown that AURKA localizes to the nucleus in breast cancer metastases, and especially in metastases of Triple Negative Breast Cancer (TNBC). Our objective is to define the role of nuclear AURKA in breast cancer metastasis in TNBC. Here we report that TNBC cells vary in amount of nuclear AURKA and this potentially correlates with their metastatic capabilities. Based on our preliminary findings we hypothesized that nuclear AURKA promotes cell survival and resistance to apoptosis in the metastatic niche. To test this hypothesis we created TNBC cell lines with CRISPR/Cas9 based deletion of endogenous AURKA. We also constructed exogenous AURKA specifically targeted to the nucleus by addition of a nuclear localization signal (NLS) or cytoplasm via addition of a nuclear exclusion signal (NES), respectively. To allow for in vitro and in vivo rescue experiments with exogenous NES or NLS AURKA in sgAURKA expressing TNBC cells, we introduced several silent mutations to avoid sgAURKA targeting. In our pilot orthotopic xenograft study with MDA-MB-231-luc2-NLS-AURKA, colorectal cancer cells could have potent effects on malignant progression. To determine the functional significance of Δ4 in malignant progression we developed DCIS (ductal carcinoma in situ) cell lines that only express this isoform and not the full length protein. We engineered these lines by using CRISPR technology targeted at the exon 3/4 splice junction. The resulting lines were significantly more invasive in ECIS endothelial invasion assays. In vivo, intracardiac zebrafish injections of the Δ4-DCIS lines showed higher extravasation from the tail vasculature compared to the parental line. Subcutaneous xenografts in athymic nude mice grew larger tumors, presented more invasive lesions and metastases. Also, Δ4-DCIS spheres growing in matrigel, showed disorganization and enhanced invasion into the matrix. In conclusion these data suggest a model where the increased splicing of AIB1 to the Δ4 variant alters transcription at specific target genes and contributes to the metastatic phenotype.

#4865 The functional and clinicopathological analysis of hypoxia inducible factor-1α (HIF-1α) in head and neck squamous cell carcinoma. Yuichi Ikari,1 Hiroyuki Ozawa,1 Yorihisa Imanishi,2 Toshiki Tomita,1 Mariko Sekimizu,1 Yoshio Watanabe,2 Fumihiro Ito,1 Shin Saito,1 Kaooru Ogawa,1 Keio University School of Medicine, Tokyo, Japan; 2Kawasaki Municipal Hospital, Kawasaki, Japan; 3Saiseikai Central Hospital, Tokyo, Japan.

Introduction: Tumor hypoxia is considered to be associated with progression in various cancers. Hypoxia inducible factor-1α (HIF-1α) is one of the molecules which play a key role in tumor hypoxia. High HIF-1α expression is reported to have an impact on poor prognosis and resistance to chemotherapy or radiotherapy in head and neck squamous cell carcinoma (HNSCC). However, the effect and mechanism of HIF-1α on tumor metastasis in HNSCC is still unclear. The aim of the present study was to investigate the molecular targets of HIF1-α relevant to metastasis, and to reveal the impact of HIF-1α on HNSCC metastasis by using clinicopathological assays. Methods: The cell proliferation rate with or without HIF-1 inhibitor (KC7F2) in HNSCC cell lines (FaDu, Detroit512) was evaluated by MTS assay. Gene expression levels related to epithelial-mesenchymal transition (EMT), cancer stem cell (CSC) and HIF-1α in each HNSCC cell lines were measured by quantitative real-time PCR. Immunohistochemical expressions of HIF-1α and clinicopathological factors were analyzed using surgical specimens from 33 patients with hypopharyngeal cancer who had undergone trans-oral tumor resection without previous chemotherapy nor radiotherapy. Results: HIF-1 inhibitor suppressed the cell growth of HNSCC cell lines in a dose-dependent manner. HIF-1 inhibitor downregulated the expression of HIF-1α, twist, Oct3/4 and Nanog. In immunohistochemical study, the HIF-1α expression was higher in cancer cells than in normal cells of the adjacent normal mucosa. Univariate analysis revealed that high expression of HIF-1α was significantly correlated with T-classification (P = 0.012), lymph node metastasis (P = 0.002) and lymphatic invasion (P = 0.03). Conclusion: These results suggest that HIF-1α plays a key role in tumor progression of HNSCC through EMT and CSC properties. From the present study, HIF-1α inhibition may have the possibility to suppress tumor metastasis, making HIF-1α an attractive target for the treatment of HNSCC.

#4866 CD47 promotes metastasis and proliferation of colorectal cancer via MAPK/ERK pathway. Tuo Hu, Yu-feng Chen, Chi Zhou, Jia Ke, Hua-shan Liu, Xiao-bin Zheng, Jian-ping Wang, Xiao-jian Wu, Xian-rui Wu, Ping Lan. 1Department of Colorectal Surgery, the Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangzhou, China.

Background and Aim: A previous studies demonstrated that CD47 was overexpressed in several kinds of tumors including colorectal cancer (CRC). Furthermore, the level of CD47 was positively correlated with tumor proliferation, stemness and metastasis. The aim of this study is to evaluate the role of CD47 in the metastasis and proliferation in CRC. Methods: We used the data from GEO database GSE8671 to analyze the expression level of CD47 and its relationship with prognosis in CRC. The expression levels of CD47 in normal and CRC tissues from our hospital were detected using quantitative RT-PCR and western blot. CD47 was downregulated or overexpressed via lentiviral vector system. Then the RTCA assay and migration assay were carried out to investigate the function of CD47 in cell proliferation and migration. Western blot for cancer related pathways was performed to find out the downstream signaling pathways of CD47. Results: Increased expression of CD47 was found in CRC tissues. Inhibition of CD47 expression in HCT116 by shRNA attenuated the capacity of cell proliferation and migration. On the other hand, overexpression of CD47 in DLD1 increased the capacity for cell proliferation and migration. CD47 induced cell proliferation and migration was found to be associated with the activation of MAPK/ERK pathway. Conclusions: High expression of CD47 was found to be associated with the proliferation and metastasis in CRC via the activation of MAPK/ERK signaling pathway. These findings provide an SRP0-independent mechanism for CD47 in the progression of CRC, which may find a novel therapy target for the precise treatment of CRC.
TUMOR BIOLOGY: Pediatric Cancer 3: Genetics and Genomics

#4867 Exome- and whole genome sequencing for clinical evaluation and precision medicine in neuroblastoma. Ángela Martínez Monleon,1 Susanne Reinsbach,1 Niloufar Javarnavard,1 Anna Djos,1 Rose-Marie Sjögberg,1 Per Kogner,2 Tommy Martinsson,3 Susanne Fransson,1 1University of Gothenburg, Gothenburg, Sweden; 2Karolinska Institute, Stockholm, Sweden.

Background. Despite major progress in treatment of pediatric cancer, aggressive neuroblastoma (NB) still constitutes a major clinical problem. Currently, the event free survival of high-risk NB is about 40% implicating that 60% either don't go into full remission or falls into relapse. Most relapses have an acquired drug resistance meaning that conventional treatment options may not work. Therefore, further studies of this group are of great importance. Massive parallel sequencing has now become a valuable tool in both cancer research and in clinical evaluation as it provides valuable information regarding patient specific alterations that might be used in targeted therapy. Material and Methods. DNA from 54 patients in total have been sequenced for massive parallel sequencing; 16 tumor/normal pairs and 27 single tumors have been analyzed using exome sequencing, 8 tumor/normal pairs with whole genome sequencing and three using both exome and whole genome sequencing. Exome sequences were mapped using BWA with GATK realignment followed by variant calling through SNPeff and copy number analysis through ControlFree. Whole genome data were analyzed using the CLC Genomic Workbench with annotation and filtering done in Ingenuity variant analysis software. Results. On average, 14 somatic protein changing single nucleotide variants were detected per patient (range 2-71) with recurrent mutations being detected in ATRX. Predicted deleterious germ line alterations in well-established cancer genes were detected in three patients. Structural variants included genes such as ATRX and TERT, as described by others previously, but also novel homozygous focal deletions at chromosome 19. Shortest regions of overlapping deletions of this chr19 region include seven genes whereof CIC (Capicua) might be the most interesting in a cancer context. Copy number analysis of both exome- and whole genome sequencing gives genomic profiles that are comparable to the genome profiles generated from the SNP-microarrays that current are in use in clinical use. Specific breakpoints for structural variants could be detected in all sample subjects for whole genome sequencing as well as for all for all MYCN amplified neuroblastomas that subjects for exome sequencing. Conclusions. Exome- and whole genome sequencing can be used for clinical evaluation of NB providing information regarding both constitutional and tumor specific alterations. The information can be used for clinical decision of patient specific therapeutic options such as ALK- or CKD4/6 inhibitors, but can also in rare cases give information regarding which treatments not to use. Furthermore, whole genome sequencing also gives the possibility to identify tumor specific structural alterations such as translocation breakpoints that can be used to monitor tumor burden in liquid biopsies.

#4868 RD3 reprogramming in N-MYC non-amplified neuroblastoma evolution. Dinesh Babu Somasundaram, Karthikeyan Subramanian, Sheeja Reinsbach,1 Niloufar Javarnavard,1 Anna Djos,1 Rose-Marie Sjögberg,1 Per Kogner,2 Tommy Martinsson,3 Susanne Fransson,1 1University of Gothenburg, Gothenburg, Sweden; 2Karolinska Institute, Stockholm, Sweden.

RD3 in high-risk N-MYC neuroblastoma was shown to be reprogrammed in 30% of the high-risk neuroblastoma (NB), while the remaining 70% is N-MYC non-amplified (N-MYC-NA), yet still has a poor outcome with only 37% 5 Year OS and a miserable 9% 10Y OS. Recently, we demonstrated, Retinal Degeneration Protein 3 (RD3) regulates tumor cell migration, invasion and tumorosphere formation of the N-MYC-NA-NB. Assessing 15 different human derived stage-4 N-MYC-NA-NB, we observed RD3-loss in the tumor cell migration, invasion and tumorosphere formation of the N-MYC-NA-NB. RD3 in high-risk N-MYC-NA-NB, its novel tumor evolution stabilization function and further imply that continuous ongoing acquisition of RD3-loss in therapy resistant cells may directly relate to tumor progression and poor clinical outcomes.

#4869 The Signature Study: Molecular analysis of pediatric tumors with establishment of tumor models in a biology study. Elizabeth VanSickle,1 Ping Zhao,1 Deanna Mitchell,1 Jessica Foley,1 Julie Steinbrecher,1 Maria Rich,2 Abhinav Nagulapally,2 Jeff Bond,1 William Hendrickx,2 Giselle Saulnier Sholler.1 1Helen DeVos Children's Hospital, Grand Rapids, MI; 2Translational Genomics Institute, Phoenix, AZ.

Background: Pediatric cancer is the leading cause of death by disease in children in the US. Significant advances have been made in survival in the past 30 years and genomic understanding of tumors is underway. Gains in the identification of biomarkers, drug targets, and the molecular characterization of cancer are due to improved technology including gene sequencing, proteomics, and epigenetics. While this remains distinctly academic for cancer centers and patients with high risk/metastatic disease experience < 30% survival. Phase 1/2 trials not based on precision medicine result in poor response rates (<10%). Therefore, genomic understanding of tumors and molecular targeted therapies with aims of reaching all children and reducing toxicity while improving efficacy is needed. Methods: The Signature Study is an IRB-approved biology study that seeks to perform genomic analysis, high throughput (HTP) drug testing, and creation of patient derived xenograft (PDX) models of all pediatric cancer patients diagnosed/relapsed at Helen DeVos Children’s Hospital. Patients are consented, clinical history is collected in RedCap, and tumors are collected flash frozen and in cell culture media. Blood is collected for germline analysis. Tumors are analyzed through gene expression arrays, DNA panels and exomes and RNA sequencing in the in cells. The used for generation of patient specific cell lines (confirmed by IHC and STR) and immediate injection into NSG mice for PDX models. Cell lines undergo HTP drug testing using the Prestwick and NCI drug libraries, novel therapeutics and combinations. Results: Enrollment has increased since inception in 2011 with now >50 patients/year; a total of 284 pediatric tumors collected representing over 30 tumor types. The most common diagnosis is neuroblastoma, followed by medulloblastoma, osteosarcoma, and rhabdomyosarcoma. Sequencing for genomic analysis has been performed on 166 tumors to identify mutations, fusions, CNV, and deletions. To date 184 have been grown as primary patient cell lines and 75 as PDX models. Over 60 samples have been evaluated in HTP drug testing. Genomic analysis of cultured tumor cells has been correlated with response to drug libraries to establish correlative predictive markers for therapeutic decision making to be tested in clinical trials.

The study stores remaining tissue, cell lines, and PDX models for additional future research. Conclusion: This study shows it is feasible for a mid-sized hospital system to coordinate and collect tumors for genomic analysis in real time for clinical decision making in the future. This resource is an integrated TransMed database system which is a powerful resource which correlates clinical outcomes, therapies, genomic sequencing, PDX models and HTP drug testing used to answer research questions of biomarkers, biological characterization, drug sensitivity, and driver pathways within and between pediatric cancers.

#4870 Mutational landscape and timing of resistant clone emergence in 104 Chinese pediatric patients with relapsed acute lymphoblastic leukemia. Benshang Li,1 Dongying Lu,2 Shuhong Shen,3 Xiaofan Zhu,3 Xiaotu Ma,2 Ningling Wang,4 Yanling Liu,4 Yu Liu,5 Hui Zhang,4 Ting-Nien Lin,2 Michael Rusch,4 Michael Edmonson,4 John Easton,2 Yingchi Zhang,4 Jingliao Zhang,3 Cheng Cheng,5 Ingyan Tang,1 James R. Downing,6 Jingyan Tang,1 James R. Downing,7 Ching-Hon Pui,8 Jun J. Yang,5 Jun J. Yang,5 Tinghui Zhang,3 1Key Laboratory of Pediatric Hematology & Oncology Ministry of Health, Department of Hematology & Oncology, Shanghai Children’s Medical Center, Shanghai Jiao Tong University School of Medicine, Shanghai, China; 2Computational Biology, St Jude Children’s Research Hospital, Memphis, TN; 3State Key Laboratory of Experimental Hematology, Institute of Hematology and Blood Diseases Hospital and Center for Stem Cell Medicine, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, China; 4Department of Pediatrics, The Second Hospital of Anhui Medical University, Hefei, China; 5Department of Pharmacological Sciences, St Jude Children’s Research Hospital, Memphis, TN; 6Department of Biostatistics, St Jude Children’s Research Hospital, Memphis, TN; 7Department of Pathology, St Jude Children’s Research Hospital, Memphis, TN; 8Department of Oncology, St Jude Children’s Research Hospital, Memphis, TN.

Relapsed pediatric acute lymphoblastic leukemia (ALL), occurring in 15–20% of cases, is one of the leading causes of cancer-related death in children, confering a dismal survival rate of less than 40% primarily due to drug resistance. We performed whole-genome sequencing (WGS) and RNA-seq of matched diagnosis and relapse tumors as well as WGS of remission DNA from 104 pediatric ALL patients treated at Shanghai Children’s Medical Center (n=89), Institute of Hematology & Blood Disease Hospital in Tianjin (n=14) and the Second Hospital of Anhui Medical University (n=1) between 2003 and 2014. The median age at diagnosis was 6.29 (range, 0.23-16.05) years. Relapse occurred during
chemotherapy in 85 patients and after completion of therapy in 19 patients. Somatic mutation rate in ALL increased significantly from an average of 0.23 per Mb at diagnosis to 0.65 per Mb at relapse. We identified 34 potential driver genes at diagnosis and 45 at relapse based on significance of mutation recurrence and pathogenicity. The four most significant relapse-specific driver genes harbor known pathogenic germline mutations in purine metabolism genes (PRPS1/PRPS2, n=7), novel alterations affecting glucocorticoids receptor NR3C1 (n=13) and folyopolyglutamate synthetase FPGS (n=8). Functional characterization showed that all NR3C1 mutations resulted in significant loss of transcription activator function and subcellular mislocalization. Ecotopic expression of mutant NR3C1 in ALL cell lines consistently led to prednisone resistance. Five driver genes were BRBP, SMARCA4, TP53, MMR and WHSC1. The highest increase (2.5-fold) of mutation frequency from diagnosis to relapse with mutations collectively present in 28% of relapsed ALL. Truncation mutations in DNA mismatch repair genes MLH1, MSH2, MSH6 and PMS2 occurred exclusively at relapse in four ALL with the highest mutation burden. Targeted ultra-deep sequencing at 5,000X of 268 somatic alterations was performed on 214 serial bone-marrow samples collected during ALL therapy for 17 cases. The resulting mutant allele fraction (MAF) values showed high concordance with minimum residual disease (MRD) measured by flow cytometry, indicating suitability for quantifying leukemia burden. Temporal change of MAF in the serial samples also allows us to track clonal identity and emergence of drug-resistant clones during and after therapy. For example, one case had deep sequencing performed on 11 somatic lesions from 10 bone marrow samples collected over the 15 months between diagnosis and relapse. We found that a minor clone present in 0.5%-1% of ALL blasts at diagnosis emerged five months prior to relapse, from which two new subclones arose harboring NTSC2 mutations L406→SSF and D407→DRD. Preliminary data generated from serial bone marrow samples provides the first insight into the use of somatic alterations for monitoring leukemia progress during relapse.

#4871 Whole-exome sequencing identifies a high frequency of germline deleterious variants in cancer predisposition genes in individuals with osteosarcoma. Ruodol Kung,1 Bing Zhu,1 Meredith Yeager,1 Michael Dean,2 Matthew Gianferante,1 Lei Song,1 Joshua Sampson,1 NCIC DCEG Cancer Genomics Research Laboratory, Julie Gastier-Foster,1 Richard Gorlick,2 Silvia Regina Cami, nada de Toledo,1 Antonio Petralii,1 Ana Patiño-Garcia,2 Fernando Lecanda,3 Massimo Serra,2 Claudia Hattinger,4 Piero Picci,5 Katia Scottlandi,6 Adrienne Planagan,7 Roberto Tirabosco,8 Maria Amary,7 Nilgün Kurucu,9 Inci Ergunhan Ilhan,10 Neriman Sarı,11 Mandy Ballinger,12 David Thomas,13 Donald Barkauskas,13 Belynda Hicks,1 Margaret Tucker,1 Neil Caporaso,1 Robert Hoover,1 Stephen Chanock,1 Sharon Savage,1 Lisa Mirabello1.1 Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD; 2Nationwide Children’s Hospital, and The Ohio State University Department of Pathology and Pediatrics, Columbus, OH; 3Albert Einstein College of Medicine, Bronx, NY; 4Pediatric Oncology Institute, GRAACC/UNIFESP, Sao Paulo, Brazil; 5Department Of Pediatrics, University Clinic of Navarre, Universidad de Navarra, Madrid, Spain; 6Laboratory of Experimental Oncology, Orthopaedic Rizzoli Institute, Bologna, Italy; 7Royal National Orthopaedic Hospital NHS Trust, London, United Kingdom; 8.A.Y. Ankara Oncology Training and Research Hospital, Department of Pediatric Oncology, Ankara, Turkey; 9The Kinghorn Cancer Centre, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia; 10Department of Preventive Medicine, Keck School of Medicine of the University of Southern California, Los Angeles, CA.

In children and adolescents, osteosarcoma (OS) is the most common malignant bone tumor. OS occurs in certain cancer predisposition syndromes at a higher than expected frequency. However, outside of these rare syndromes, OS is significantly more common and its genetic etiology remains poorly understood. We conducted an evaluation of rare exonic variants in 545 unselected OS cases compared with 1061 cancer-free controls using whole-exome sequencing of blood or buccal cell DNA to estimate the prevalence and burden of rare deleterious germline variants. We assessed potentially pathogenic rare variants in 126 well-characterized cancer predisposition genes (CPCG) and non-pathogenically mutated genes. We also surveyed the exome for genes with a higher burden of rare variants in 342 EUR cases with 994 EUR controls sequenced at the same time. Rare genetic variants, defined by a MAF <0.01 in 1000G, ESP or ExAC, that passed quality control filters were classified based on well-annotated databases (IARC-TP53, Telomerase Database, ARUP, BIC, Insight and COSMIC) and in silico predictions (Sift, PolyPhen, Mutation assessor, MutationTaster, FATHMM and ITRR). Rare variants matching known pathogenic variants, or inexact matches at the same residue, in selected well-annotated databases were labeled as putative pathogenic variants. High impact variants (frameshift, stop gain/loss, or known splice sites) not matching a previously identified pathogenic variant in the selected databases were categorized as probably pathogenic. Predictions based solely on in silico predictions were categorized as possibly pathogenic, uncertain or likely benign. A total of 14.5% of cases had a predicted pathogenic or high impact variant in an autosomal dominant CPG. A significantly higher pathogenic germline variant burden was present in EUR cases compared with EUR controls (Pburden = 3.7x10^-6). TP53 had the highest prevalence of pathogenic mutations (5% of EUR cases; Pburden = 1.05x10^-10). CDKN2A, MEN1, MLH1, MUTYH, PALB2 and VHL had a significantly higher variant burden in the EUR cases. 7.5% of cases had a predicted pathogenic or probably pathogenic variant in an autosomal recessive CPG (Pburden = 5.4x10^-7). Additionally, two males had a pathogenic variant in the X-linked genes, DKC1 and GPC3. In total, 21% of cases had a predicted pathogenic or high impact variant in a CPG (Pburden = 2.4x10^-7). Exome-wide analysis identified two novel genes with significantly higher rare variant burdens in the cases compared to controls. In conclusion, several CPCG and two novel genes, not previously associated with OS, had an enrichment of rare variants in OS and warrant further follow-up. Our results indicate that a clinically significant fraction of OS cases may harbor one or more mutations worthy of consideration for further investigation and genetic counseling.

#4872 Gene fusion detection in pediatric tumor samples utilizing multi-caller fusion detection approach and integrative data analysis. Alma Imamovic,1 Marian Harris,1 Alanna Church,2 Brian Crompton,3 Eliezer Van Allen,4 Katherine Janeway1.1 Dana-Farber Cancer Institute, Boston, MA; 2Boston Children’s Hospital, Boston, MA.

Gene fusions are an important mechanism of oncogenesis in pediatric cancers. Fusion detection from RNA sequencing data is challenging for several reasons including a high false positive rate by individual fusion callers. We developed a new pipeline for gene fusion detection utilizing a multi-caller fusion detection approach and focusing on improved specificity, annotation and visual presentation. Multiple fusion detection algorithms, integrative data analysis and known fusions and cancer genes annotation databases are integral parts of our new pipeline. The following fusion callers were included in the pipeline: Chimerascan, TopHat Fusion and STAR-Fusion. Fusions called by at least two fusion callers were included in the final results and annotated by utilizing the TARGET database (Tumor Alternatives Relevant for Genomics-driven Therapy) and TCGdb (Translocation Breakpoints In Cancer database). A Venn diagram was produced for visual presentation of candidate fusions detected by multiple fusion callers. IGV (Integrative Genomics Viewer) was used to visualize the alignment of reads at fusion break points. The fusion detection pipeline was tested with RNA sequencing data from 11 pediatric tumor samples either suspected to harbor fusions based on diagnosis (n=7); renal cell carcinoma, osteosarcoma, synovial sarcoma, glomus tumor, undifferentiated sarcoma) or known to have fusions based on standard methods (n=4; Ewing-like sarcoma, EWSR1 FISH + sarcoma, clear cell sarcoma). In most cases, fusions previously known to be present were identified with our pipeline. For the difficult to detect fusion CIC-DUX4, optimization by adding another fusion caller (FusionCatcher) and adjusting the filtering and annotation parameters was required to increase sensitivity. In some cases these novel fusions have been validated to be present with other methods. In one case (synovial sarcoma) the expected fusion, which was not detected with standard testing (FISH), was identified with our pipeline. The pipeline was also successfully utilized to analyze prostate cancer samples (PMID: 27676109). Our new multi-caller fusion detection pipeline has been successful in increasing specificity and decreasing the false positive rate for gene fusion calling in transcriptional data, while being sensitive enough to detect the more challenging fusions. Additional updates to the pipeline are anticipated like the realignment against a modified reference sequence including the gene rearrangement for improved sensitivity.

#4873 Shedding light on the unknown of Ewing sarcoma: Single cell study shows a co-expression pattern between TAPA-1 and EWSR1-FLI1. Roxane Khoogar, Doris Phelps, Peter Houghton. UT Health Science Ctr.at San Antonio, San Antonio, TX.

Introduction: Ewing Sarcoma (ES) is the second most frequent bone cancer found in children, and has the highest rate of recurrence among all childhood cancers. This suggests the need for a better understanding of heterogeneous cancer populations and rare cells that survive intensive cytotoxic therapy and cause recurrence in patients. It is widely accepted that ‘EWSR1-FLI1’ has a major role in determining ES survival and proliferation, but the mechanism is unknown. Most studies in which expression of ‘EWSR1-FLI1’ is correlated with the level of expression of ‘EWSR1-FLI1’ regulated genes have been conducted on
bulk populations that ignore potential heterogeneity of ‘EWSR1-FLI1’ in the cell population. To address this we have used single cell RNA-Seq approaches. Methods: The WaferGen iCell8 system was used to perform SMART whole transcriptome amplification using EW-8 ES cells. We sequenced single-cell RNA-seq libraries prepared from over 1260 ES parental cells. We combined computational analysis with strong quality control filters to produce candidate ES cells. We used EWSR1 expression profile to partition the entire population into two clusters. A population of cells with TAPA-1 expression was distinguished and studied for their co-expression patterns with EWSR1. Results: Results revealed that ES cells show considerable heterogeneity at the transcriptome level between seemingly identical EW-8 cells. We identified a subpopulation of EW-8 ES cells with a positive correlation between the expression of EWSR1 and Target of the antiproliferative antibody 1 and tetraspanin-28 (TAPA-1/CDB1). Our preliminary data from 200 cells indicate that EWSR1 (0-18 unique transcripts, median 1) and TAPA-1/CDB1 (0-8 unique transcripts, median 0) were correlated (r = 0.32, P < 0.10). TAPA-1, a surface membrane protein, most frequently associated with hematopoietic cells, that regulates cell development, activation, growth and motility and has been implicated in proliferation of many cancers. Conclusions: Taken together, our results suggest that there are two classes of cells with respect to the expression of TAPA-1 in EW-8 cells. We also show the relationship between TAPA-1 with EWSR1-FLI1 expression in a single cell level. This type of classification may lead us to identify a rare population with a poor response rate to cytotoxic treatments. Future studies will define if EW-8 ES cells having elevated TAPA-1 have differential sensitivity to cytotoxic agents compared with populations with low level expression.

**#4874 Application of quantitative analysis of mouse cell contamination and purification of human tumor cells in patient derived orthotopic xenograft mouse models of pediatric brain tumors.** Frank K. Braun, Mari Kogiso, Lin Qi, Huiyuan Zhang, Yuchen Du, Holly B. Lindsay, Siibo Zhao, Sarah G. Injac, Xiao-Nan Li. Baylor College of Medicine/Texas Children’s Cancer Center, Houston, TX.

Background: Pediatric brain tumors continue to be among the most difficult to cure and are the leading cause of cancer-related deaths in children. Research investigating pediatric brain cancer is still impaired by the lack of cancer models. We established a large panel of orthotopic xenograft models as well as several paired permanent cell lines using in vivo and in vitro culture techniques. However, an inherent risk of xenograft models is the contamination of isolates of human tumors with host (mouse) cells. Therefore strategies to correctly and timely identify the human/mouse ratio are essential. In this study we investigated Prostaglandin Receptor EP2 (PTGER2) as a useful target to identifying mouse cell contamination in PDXO models. Method: Species specific primers based on Alcozer et al., 2011 were used to detect PTGER2 in genomic DNA extracts of xenograft and cultured cells. Real-time qPCR was carried out using a StepOnePlus™ Real-Time PCR System. Reaction mix contained SYBR Green select master mix, respective primers and 5 ng of total genomic DNA in a total reaction volume of 10 μL. The qPCR conditions were as follows: 50°C-2 min, 95°C-10 min, 40 cycles of (95°C-15 sec, 61°C-1 min). Primer specificity was controlled in addition by analysis of amplified products on a 1.2% agarose gel. FACS analysis of human cells allowed us to analyze anti-HLA-ABC and mouse-specific Ab cocktail surface staining and subjected to qPCR. Results: Various models of pediatric brain cancer (PXA, MB, GBM, EPN, ATRT, DIPG, PNET) established in our group were investigated for mouse cell contamination. Cells from xenograft isolates as well as from culture xenografts were routinely analyzed. Our study confirms PTGER2 as a useful target for accurately estimate mouse cell contamination in brain cancer xenograft models. This further enabled us to improve detection and characterization of HLA-ABC/mouse-cocktail double negative population as seen in FACS. Conclusion: In conclusion we have successfully implemented an easy qRT-PCR approach to determine the human/mouse cell ration in any mixed cell population. PDXO models is paramount to have reliable information on mouse cell contamination.

**#4875 Identification of the cellular origin and ‘stemness’ phenotype of Malignant Rhabdoid Tumors (MRT) may represent a new therapeutic approach in paediatric oncology.** Ras A. Ramli, Martina A. Finetti, Matthew P. Selby, Yura Grabovska, Stephen Crosier, Amanda J. Smith, Steven C. Clifford, Daniel Williamson. Northern Institute for Cancer Research, Newcastle University, Newcastle Upon Tyne, United Kingdom.

Malignant Rhabdoid Tumors (MRT) are highly aggressive childhood malignancies characterized by a single mutation; biallelic inactivation of SMARCB1, a component of the SWI/SNF chromatin remodeling complex. These tumors may occur anywhere, most frequently in the brain (Atypical Teratoid/Rhabdoid Tu- mor, ATRT) and in the kidneys and soft tissues (Extra Cranial Rhabdoid tumor, ECRT). MRT presents immune markers from multiple lineages exhibiting a teratoid phenotype, characterized by cells with heterogeneous morphology within the same tumor leading to speculation that the MRT cell of origin is a type of multipotent stem cell. Despite recent advances in treating other solid tumors, treatments for MRT remain suboptimal and overall survival of patients that MRT patients remain poor. "stemness" characteristics are key to its aggressive clinical course and resistance to treatment. Here we analyze the expression profiles of primary MRT (n = 134) and catalog the expression of a program of "stemness" genes capable of driving aberrant self-renewal. Further, we show by re-expression of SMARCB1 in MRT cells that several of these key "stemness" genes are aberrantly activated by SMARCB1 mutation. One such gene, BMI1 was further demonstrated to be critical to MRT tumorigenesis by shRNA knockdown, and a novel anti-BMI1 drug, PTC209 was tested to show efficacy in MRT. Knockdown of BMI1 in MRT cells reduced the self-renewal capability of cells as seen from the number of tumourpheres formed in limiting dilution assay (LDA). Interestingly, BMI1 loss triggers upregulation of p16(INK4a) expression and this mimics the expression profile when SMARCB1 was re-expressed into MRT cells. To identify a putative MRT cell of origin we performed a meta-analysis cross-referencing expression profiles from primary MRT (n = 20), and functional models in which SMARCB1 was re-expressed (n = 5 lines) with expression profiles from multiple stem cell types including epithelial, embryonic, neural mesenchymal and neural crest (n = 446). Analysis by t-SNE and NMF consensus clustering suggested that MRT bore the closest resemblance to non-malignant neural crest cells. These results were confirmed in serial xenograft models in which ECRTs and ATRTs appear to have different cells of origin, despite having the same mutation and tumor appearance. For the first time, we show evidence that ECRTs and ATRTs appear to have different cells of origin, despite having the same mutation and tumor appearance. We further demonstrate that SMARCB1 expression is necessary for MRT cells to maintain a de-differentiated "stem-like" state and finally that the SMARCB1-dependent stemness gene BMI1 shows potential as a novel therapeutic target in MRT.

**#4876 Low frequent ALK hotspot mutations in neuroblastoma tumors detected by ultra deep sequencing: Implications for ALK inhibitor treatment.** Susanne M. Fransson,1 Niloufar Javanmardi,1 Rose-Marie Sjoberg,1 Per Kogner,2 Tommy Martinsson,3 Sahlensgrevska academy at University of Gothenburg, Gothenburg, Sweden; Karolinska Institutet, Stockholm, Sweden.

Background: The pediatric cancer neuroblastoma (NB) is heterogeneous in terms of both genotype and clinical behavior. NB patients show complex patterns of genetic abnormalities, which may include amplification, translocation or oncogenic mutations of ALK kinase in sporadic and familial cases. Three hot spot residues (F1174, F1245, and R1275) localized within the kinase domain of ALK, accounted for 85% of mutations in NB. In this study we used ultra deep sequencing for sensitive detection of ALK mutations. Methods: To detect the sub-clonal ALK mutations in 105 neuroblastoma tumors that can remain undetected using Sanger chemistry, we used two different approaches; hybridization-based Haloplex kit (Agilent) and PCR-based amplicron sequencing, both sequenced on the MiSeq Illumina platform. Coverage, uniformity and variant calling ability showed similar performance between both methods. Findings: ALK mutations were detected in 14/105 patients (13%) whereof six with allele frequency below 20%. At the I1171 locus an ALK mutation was detected in one case with variant allele frequency as low as 2.6%, the lowest level detected in our cohort. Mutations at the F1174 hotspot were observed in nine cases: six cases with F1174L, while the remaining three showed F1174C, F1174S and F1174I substitutions, with mutated allele fractions ranging from 14% to 60%. Interestingly the patient harboring F1174S mutation with 58% frequency, in parallel contained a small sub-clone of F1174I mutation with 8% frequency of the mutated allele. F1245-codon alterations were detected in three cases: two F1245I mutations and one F1245S mutation, with frequencies of 14%, 51% and 52%, respectively. A single case showed a L1240V variant with a mutated allele fraction at 57%. Sanger sequencing of all ALK mutated samples could confirm ALK status for all samples with variant allele fraction above 15%. Because of the limits of the detection, 4 out of 6 samples with a mutated allele fraction below 15% would have gone undetected relying on Sanger sequencing. Our results fail to reveal a distinct mutation spectrum in relation to neuroblastoma genomic subtypes. Moreover, a variety of neuroblastic tumors, from benign ganglioneuroma to very aggressive metastatic neuroblastoma, could be observed in the context of ALK mutation. Interpretation: These results indicate that, in neuroblastoma, ALK mutations can be present at sub-clonal level with the possibility of subsequent clonal expansion at the time of progression. Tyrosine kinase inhibitors have become the gold standard therapy in treating ALK positive NBs, making them a potential target for new therapeutic approaches.
diagnostic high sensitive detection of ALK mutations a necessary step in identifying optimal treatment modalities. This study is of utmost importance in clinical practice, highlighting the potential of NGS and the importance of serial samplings for therapeutic decisions.

### #4877 Transcriptome sequencing of pediatric hepatocellular carcinoma reveals genomic events involving APC and TERT.

Katherine Haines,1 Angshumoy Roy,2 Stephen Sarabia,2 Linghua Wang,2 Pavel Sumazin,2 Kyle Covenant,1 Harsha Dodapati,1 Oliver Hampton,1 Donna M. Muzny,1 Dolores Lopez-Terrada,2 David A. Wheeler,1 Sharon E. Plon,1 D. Williams Parsons1. TUMOR BIOLOGY: Pediatric Cancer 3: Genetics and Genomics.

The detection of chimeric transcripts by RNA-seq has allowed us to identify additional variants in APC. Reportsof inversion of APC are rare, however we have identified an additional published case of APC inversion in a pediatric HCC cohort with no additional fusions. Filtering by the COSMIC Cancer Gene Census list reduced the total number of fusion calls to 5 for the HCC dataset (3 unique events, 2 of which were confirmed by RT-PCR).

Results: The first event is an inversion disrupting APC, resulting in two in-frame fusion transcripts between APC and AP3B1. This fusion was confirmed and the intronic breakpoints were mapped in tumor and germline DNA through WGS by 10X Genomics. Variant calling by Platypus on tumor RNA-seq reads did not identify additional variants in APC. Reportsof inversion of APC are rare, however we have identified an additional published case of APC inversion in a patient with pediatric HCC. We will perform WGS on germline DNA from this patient to determine if the APC inversion breakpoint is recurrent.

The second event is a deletion encompassing the TERT promoter and results in an in-frame fusion between TERT and LCPAT1. An increase in TERT expression is seen in this tumor as compared to both normal liver and other pediatric HCCs.

Conclusions: The detection of chimeric transcripts by RNA-seq has allowed us to identify two structural variants involving known cancer genes in two tumors, including an inversion of APC which have been historically difficult to detect. These events present a possible genetic cause for the development and provide genes for further analysis in the remaining cases with no identified fusions. These results will inform future studies as we expand this cohort to include 18 additional pediatric HCCs which we plan to characterize through NGS and targeted sequencing.

### #4878 Sensitive and specific DNA and RNA sequencing techniques for detecting minimal residual disease. Erin L. Crowegry,1 Nitin Mahajan, Wing H. Wong,2 Edward A. Kolb,1 Todd Druley2. TUMOR BIOLOGY: Pediatric Cancer 3: Genetics and Genomics.

The goal of this study was to assess the clinical applicability of molecular genetic techniques in the study of minimal residual disease (MRD) in pediatric acute myeloid leukemia (AML) via DNA and RNA-based methods. Currently, multi-parametric flow cytometry (MPPC) for surface immunophenotypes is the gold standard for MRD with a limit of detection between 0.001-0.002. However, approximately one-third of children with no detectable MRD by MPPC after first induction still relapse and suffer inferior outcomes. One potential reason is that a majority of refractory or recurrent AML clones will present with altered immunophenotypes compared to diagnostic specimens. In contrast, DNA mutations present at diagnosis will remain in refractory disease and provide a gene-based MRD platform surveying many genes could enable targeted, gene-specific therapy. Recently, the heterogeneous and unique genomic landscape of pediatric AML was characterized in the TARGET project and highlights the potential for leveraging companion molecular screens in the analysis of AML MRD. Contrary to adult AML, pediatric AML is not primarily characterized by single nucleotide variants (SNVs), but rather by complex structural variants (SV) that are difficult to detect using standard next generation sequencing techniques and analysis pipelines. The need to detect these complex SV and SNVs, all of which are at low allelic ratios (AR) at the remission state, demands assays that are capable of analyzing both DNA and RNA molecules at levels of detection comparable to MPPC. As part of our platform, we leveraged a unique capture technique (ArcherDx; CoreAAML) to detect an important SV in pediatric AML, the internal tandem duplication in Fms related tyrosine Kinase (FLT3-ITD). Via a serial dilution of cells with known FLT3-ITD allelic ratio (MV4-11 cells), to determine a limit of detection, we were able to detect the mutation as low as 0.001%.

To complement the data-based approach, we compared two error-corrected (EC) RNA assays (a Druley lab developed protocol and the ArcherDx; Hemev2 panel) to further assess the assay performance and robustness emphasizing the importance of cell-type dependent sensitivity. Finally, we integrated the results from the FLT3-ITD assay and the RNA assay with our custom error-corrected sequencing data to create a novel bioinformatics workflow for assessing the biological implications of MRD clones detected. Collectively, our data support that EC sequencing, at both the DNA and RNA level, enable accurate detection of low allelic variants that could be used for improved clinical MRD diagnostics, prognostication and therapeutic selection.

### #4879 Genomic and epigenomic profiling of high-stage neuroblastoma. Miki Ohira,1 Yasutoshi Tatsumi,2 Yohko Nakamura,2 Kenji Tatsuno, Shuichi Tsutsui,1 Shogo Yamamoto,1 Genta Nagae,1 Claire Renard-Guillet,1 Ryuichi Sugino,1 Hiroki Nagase, Takahiko Kamijo,1 Hiroyuki Aburatani,1 Akira Naga- gwara1. TUMOR BIOLOGY: Pediatric Cancer 3: Genetics and Genomics.

Neuroblastoma (NB) is the most common pediatric extracranial solid tumor with a wide range of clinical phenotypes from spontaneous regression to highly resistant to chemotherapy. Despite intensive multi-modal therapy, NB remains the second most common cause of cancer deaths among children. MYCN amplification is the most prominent poor prognosis factor for NB, however, certain subgroup of high stage tumor without MYCN amplification also shows low survival rate in terms of long-term follow-up (10 years survival rate was 51%, n = 161). To elucidate the molecular basis of malignant subtype of NB, integrated genomic and epigenomic analyses were performed. Array CGH of 537 tumors indicated that genomic subsets of 17q gain, with 1p loss and/or 11q loss, exhibited aggressive characteristics even without MYCN amplification. Whole exome sequencing of 92 high stage, MYCN-non-amplified primary tumors (SureSelect XT Human All Exon V4, mean depth CDS:138, x20 coverage:96%) identified 1,735 non-silent somatic mutations, among which 53 were significant mutations observed more than once in the sample set. Aggressive tumors (died of disease, n = 48) had several notable features, with mutations in chromosome remodeling/modulating genes (27%, cf. ATRX), in DNA-damage response (30%), in TP53 (28%) and RB (22%)-related gene networks, as well as with high TERT expression (38%). By hierarchical clustering of methylome signature (Infinium HumanMethylation450 BeadChip), the 92 tumors were subdivided into four clusters. Unfavorable cluster 2 and 4 exhibited hyper-methylation phenotypes in CpG islands and the former was enriched by MYCN-amplified tumors. The two clusters included patients with high-risk NB (p = 0.0055). Thus, these prognosis-related genomic features could be useful markers for risk classification and help understanding molecular mechanism for aggressive phenotypes of MYCN-non-amplified NBs.

### #4880 MAGE gene family expression in pediatric medulloblastoma: frequency and possible therapeutic target. Rebecca R. Collins1, Klementina Fon Tacer1, Dinesh Rakhje1, Patrick Ryan Potts1. TUMOR BIOLOGY: Pediatric Cancer 3: Genetics and Genomics.

Background: The melanoma antigen gene family (MAGE) is a group of related genes that were first discovered in malignant melanoma. Among the approximately 40 MAGE genes in the human genome, two thirds are classified as Type 1 (MAGE-A, MAGE-B, and MAGE-C), referred to also as cancer testis antigens (CTAs), proteins that are restricted to testis but often aberrantly expressed in many adult cancers. Despite their common expression in adult cancers and their oncogenic potential, little is known about their expression and role in pediatric cancers. To determine the frequency of MAGE expression in pediatric medulloblastoma, we measured expression levels of Type 1 MAGEs in patient tumors. In addition, we examined how MAGE expression in medulloblastoma cells affects their growth and oncogenic potential.

Methods: We searched the Children’s Health pathology database for pediatric medulloblastoma between 2008 - 2015 and were able to recover an adequate amount of RNA from formalin-fixed paraffin-embedded tissue samples of 34 patients. The RNA
was converted to cDNA using reverse transcriptase, and the relative expression of 23 Type I MAGE genes was measured by qRT-PCR. To determine if MAGE expression is critical for medulloblastoma cell survival we knocked down several MAGEs using siRNAs in two MAGE-expressing medulloblastoma cell lines (D283 and DAOY) and evaluated the effect by cell viability (Alamar Blue and Cell Titer-Glo), apoptosis assays (Annexin V), and cell survival and clonogenic growth. The decreased cell survival after MAGE knockdown appears to be related, at least in part, to increased apoptosis. Our results indicate that targeting of MAGES in medulloblastoma may be a potential therapeutic option.

4988 Dissecting telomere maintenance mechanisms in neuroblastoma.

Background. Telomere maintenance is a major cancer hallmark and has recently attracted attention as an oncogenic mechanism in the pediatric malignancy, neuroblastoma (NBL), due to detection of frequent structural rearrangements near the telomerase (TERT) gene. NBLs display substantial clinical and molecular heterogeneity; tumors with MYCN amplification and TERT-associated rearrangements define separate groups of high-risk patients with active TERT. ATRX mutations are frequent in non-TERT active high-risk tumors, indicating use of alternative lengthening of telomeres (ALT) as a maintenance mechanism. Furthermore, the 4S low-risk NBLs display an elevated rate of spontaneous regression and overall good prognosis; it has been hypothesized that lacking a telomere maintenance mechanism ("telomere crisis") may be behind this phenomenon. Methods. To gain mechanistic insight behind this phenomenon, we introduce an integrative analysis of a cohort comprising 104 high-risk (32 MYCN-amplified) and 23 4S tumors with matched blood/tumor whole genome sequencing, CpG methylation, and RNA-seq data from the TARGET consortium. Here, we preformed structural variant (SV), allelic specific expression (ASE), and differential CpG methylation analyses. Additionally, we introduce a novel approach to measure relative telomere DNA abundance from short-read WGS measurements as the ratio between tumor and blood telomeric reads (containing canonical repeats TTAGGG/CCCTAA) per million (TBrpm). Results. We identified 26 tumors harboring TERT rearrangements (25% of all high risk tumors) and 3 tumors with ATRX mutations. We define 4 groups of high-risk NBL with different Telomere maintenance mechanisms: 1) Tumors with MYCN amplification or elevated MYCN activity, TERT gene body hypermethylation (β-value=0.8), and expression of biallelic TERT; 2) Tumors harboring TERT associated SVs, hemi-methylation (β-value=0.5), and expression of mono-allelic TERT; 3) Tumors without TERT expression, including ATRX mutants with hypo-methylated TERT (β-value=0.3); 4) Tumors with abnormally high telomeric DNA abundance (TBrpm > 1.5), hypo-methylation (β-value=0.3), and no expression of TERT. Intriguingly, while groups 1–3 show telomeric loss (TBrpm = 0.7-0.9), 4S tumors show conservation of telomere abundance between tumor and blood (TBrpm = 1); hence no indication of telomere crisis, perhaps due to early timing of biopsy at diagnosis. Conclusion. We have used ASE status and gene body methylation of TERT in order to understand and validate mechanisms underlying TERT activation; we extend MYCN-driven TERT activation to tumors with high MYCN/MYC activity in the absence of MYCN amplification. Our telomere analysis suggests that different ALT mechanisms might take place in NBLs. Defining the mechanism of 4S NBL spontaneous regression requires further investigation.

4988 Genomic mechanisms of disease progression in pediatric medullary thyroid cancer (MTC). IRA L. KRAFT, 1 SIVANDANA AKSHINTALA, 2 KEITH J. KILLIAN, 3 ROBERT B. HUFNAGEL, 3 JOHN W. GLOD, 4 CLAUDIA DERSIE-ANTHONY, 5 YUELIN ZHU, 1 HOLLY S. STEVENSON, 1 DIANA BRADFORD, 1 MARIA J. MERINO, 1 FRANK M. BALIS, 5 ELIZABETH FOX, 2 BRIDGETTE WIDEMANN, 2 JOHN J. P. SHEEN, 6 PAUL S. METZLER, 7 NATIONAL CANCER INSTITUTE, CENTER FOR CANCER RESEARCH, BETHESDA, MD; 8 NEW YORK UNIVERSITY, LANGONE MEDICAL CENTER, NEW YORK, NY; 9 NATIONAL EYE INSTITUTE, BETHESDA, MD; 10 CLINICAL RESEARCH DIRECTORATE/CLINICAL MONITORING RESEARCH PROGRAM, LEIDOS BIOMEDICAL RESEARCH, INC., NCI CAMPUS AT FREDERICK, FREDERICK, MD; 11 CHILDREN’S HOSPITAL OF PHILADELPHIA, PHILADELPHIA, PA.

Background: Multiple Endocrine Neoplasia (MEN) 2B is a rare hereditary disorder characterized by medullary thyroid cancer (MTC) in early childhood, pheochromocytoma, and mucosal neuromas. Patients with advanced MTC are treated with rearranged during transfection (RET) targeting tyrosine kinase inhibitors (TKIs) such as vandetanib. Despite initial responses, many patients progress on TKI therapy due to resistance mechanisms of resistance are yet to be elucidated. We analyzed tumor samples from seven children with MEN2B and MTC enrolled in a natural history study (NCIT01669984). Methods: DNA samples from tumor and adjacent normal tissue from paraffin embedded blocks or unstained slides were analyzed by a custom capture next-generation sequencing panel. Bioinformatics analyses identified point mutations, insertions, deletions and copy number variations within the RET gene. Results: Seven patients (median age at study enrollment 14 years, range 11–17 years) with the RET p.Met918Thr germline mutation were included in analysis. Tumor samples were available for three patients pre-TKI (four samples), two patients at progression on TKI (three samples), and two patients both, pre-TKI and at progression (five samples). Pre-TKI samples exhibited few tumor specific mutations or copy number variations and 4/5 patients had loss of chromosome 1p. For progression one patient was associated with acquisition of a previously unidentified p.Leu790Phe mutation within the kinase domain of the RET gene. Loss of heterozygosity and increase in copy number variations were noted in 4/5 samples at tumor progression. Two patients had copy number loss of chromosomes 13 and 14 and three had copy number gain of chromosome 1q. Recurrent, somatic, non-synonymous mutations were not identified. Conclusions: In children with MEN2, we identified increase in copy number variations and a somatic mutation within the RET gene as potential mechanisms of drug resistance. Our data imply that a common genetic mechanism for progression on TKI may not exist within this small sample set and highlight the need for serial collection of tumor tissue. Further, whole genome anaemolipoy may provide rationale for the evaluation of cytotoxic chemotherapy in patients who experience progressive disease on TKI therapy. Analysis of additional samples and whole exome DNA and RNA sequencing are ongoing.

4988 Targeting tumor-amplified ODC1 with difluoromethylornithine (DFMO) inhibits global protein translation and has antitumor activity in neuroblastoma.
Andrea T. Flynn, 1 Annette YU, 2 Kangning LIU, 2 Elizabeth SCADDEN, 3 Edward ATTIE, 3 MURRAY NOERIS, 2 Michelle HABER, 2 MICHAEL HOGARTY, 1 CHILDREN’S HOSPITAL OF PHILADELPHIA, PHILADELPHIA, PA; 5 CHILDREN’S CANCER INSTITUTE, SYDNEY, AUSTRALIA.

MYC genes are predominant oncogenic drivers in neuroblastoma, a lethal pediatric tumor, often via amplification of MYCN. MYC genes coordinately deregulate programs that link cell cycle entry with requisite biomass and energy creation. Polyamine synthesis is one such deregulated program that supports oncogenic Odc enzyme, and we have shown DFMO inhibits tumor progression and synergizes with chemotherapy in complementary murine models of neuroblastoma. We postulated DFMO inhibits protein translation as its principal mechanism of anti-tumor activity. Polyamines support protein translation via effects on both IF5A and IF4F-complex activities, yet their relative contributions remain poorly defined. Using the puromycin-incorporation assay we showed a marked decrease in global protein translation following DFMO exposure (by IEF) and eIF4F-complex status (polyamine-dependent 4E-BP phosphorylation). Moreover, eIF5A is required to resolve ribosome stalling at polyproline stretches, so we characterized the human proteome for polyproline motifs (PPP ≥3) to define an eIF5A-dependent translantome. Candidate genes of diverse polyproline content are being assessed for sensitivity to DFMO to define whether ribosome pausing and selective translation inhibition contributes to DFMO effects. Elucidating mechanisms of DFMO activity and correlating this...
with genomic status (MYCN and/or ODC1 amplified) will identify opportunities for drug synergy and provide a responder hypothesis to test in pivotal Phase 2 and 3 clinical trial of DFMO for neuroblastoma.

#4884 Focal 22q11.22 deletions combined with IKZF1 alterations are associated with worse clinical outcome in acute lymphoblastic leukemia. Julia A. Meyer, 1 Clint C. Mason, 2 Luke Maese, 2 Deqing Pei, 2 Cheng Cheng, 2 Ching-Hon Pui, 3 Mel Greaves, 3 Richard Aplenc, 3 Charles G. Mulligan, 4 Elizabeth Raetz, 1 Rodney R. Miles, 1 Karen R. Rabin, 1 Joshua D. Schiffer 1. 1 University of Utah, Salt Lake City, UT; 2 St. Jude Children’s Research Hospital, Memphis, TN; 3 Institute of Cancer Research, London, United Kingdom; 4 Children’s Hospital of Philadelphia, Philadelphia, PA; 5 Baylor College of Medicine, Houston, TX.

Introduction: Prognostic biomarkers in childhood acute lymphoblastic leukemia (ALL) are vital for risk-stratification and intensifying therapy for children at high risk for remission induction failure or relapse. Copy number alterations in genes such as IKZF1 and VPREB1 have been shown to correlate with poor outcome in ALL, highlighting genetic alterations as prognostic markers (NEJM 560:670, 2009, Leukemia 28(1):216-20, 2014). A second focal deletion in chromosome 22q11.22, 200 kilobases (KB) in length, occurs more frequently and in the same IGLL region as VPREB1 and is distinct from deletions associated with physiologic IGLL rearrangement. We further investigated this novel genomic lesion, 22q11.22, and the prevalence of co-occurrence with IKZF1. Methods: 22q11.22 deletions were characterized in a compiled childhood ALL cohort (N=832) and correlated with available clinical outcome using multiple previously published studies (Clinical outcome total N=730; Utah Cohort [N=56], TARGET [99906 cohort [N=215], St. Jude Children’s Research Hospital cohort [SJCRH, N=236], Children’s Hospital of Philadelphia cohort [CHOP, N=160], and Down Syndrome cohort [DS, N=63]). Microarray data was analyzed (Utah = Molecular Inversion Probe 330K [Affymetrix]; TARGET = SNP 500K [Affymetrix]; SJCRH = SNP 500K/6.0. [Affymetrix]; CHOP = 850K, 610K, and Omni Quadv1 [Illumina]; DS = SNP 500K, MIP 330K [Affymetrix]) by Nexus Copy Number (BioDiscovery, Inc.). Results: ALL patients that harbored copy number deletion 22q11.22 were present in about 30-45% of each cohort: Utah = 42.8%, TARGET = 29%, SJCRH = 40%, CHOP = 34%, DS = 46.7%. The majority of deletions, 93%, had a common recurring region just under 12 Kb in length. The 12 Kb deleted segment encodes no known genes. Patients that harbored a combined deletion in both IKZF1 and 22q11.22 (IKZF1 + 22q) were present in about 10-15% of each cohort: Utah = 12.5%, TARGET = 18%, SJCRH = 8.4%, CHOP = 6.3%, DS = 14.4%. IKZF1 +22q conferred worse event-free survival (N=730, P=0.0062) compared to those with only IKZF1 deletions and worse overall survival (N=507, P=0.0365). Additionally, those patients with IKZF1 +22q losses had a median decrease in event-free survival compared to those patients with neither deletion (normal cases): TARGET (high risk cohort) = 0.03 years (P=0.0001), SJCRH (low risk cohort) = 10 years, P=-0.0001, DS = 2.3 years, P=-0.0001. Conclusion: We present further evidence that non-physiological deletions within the IGLL locus is associated with worse outcome in pediatric ALL and combined with IKZF1, these double deletions identify a population of patients with very poor outcomes. These combined alterations may be useful to identify patients in the future for high risk protocols and further clinical interventions to understand the mechanism and biological consequence of this common loss at 22q11.22 in childhood ALL.

#4885 Identification of actionable targets for refractory/relapsed childhood cancer leading to personalized targeted therapy (TRICEPS Study). Fida Khater, Stephanie Vairy, Sylvie Langlois, Jasmine Healy, Sophie Dumoucel, Mathieu Laboie, Thomas Sontag, Pascal St-Onge, Dorothée Lajoie, Thomas Sontag, Pascal St-Onge, Henrique Bittencourt, A. Meyer, 1 ClintC. Mason, 1 LukeMaese, 2 DeqingPei, 2 Cheng Cheng, 2 Ching-Hon Pui, 3 Mel Greaves, 3 Richard Aplenc, 3 Charles G. Mulligan, 4 Elizabeth Raetz, 1 Rodney R. Miles, 1 Karen R. Rabin, 1 Joshua D. Schiffer 1.

The feasibility of performing genomic-driven targeted therapy in pediatric and adolescent (aged 0-21 years) patients with relapsed or refractory childhood cancer. This study offers in-depth genomic and transcriptomic investigation of patient’s tumoral material to identify patient-specific alterations and actionable driver mutation(s) that can be targeted with approved targeted drug and within the framework of clinically relevant genetic testing to assess the effect of biopsy to a detailed tumor analysis report. Over a period of 30 months, 44 relapsed/refractory cancer patients were recruited. Twenty-two of them underwent extensive genomic investigation (exomic and transcriptomic sequencing) within a median timeframe of 9.7 weeks from patient enrolment to return of results. Patient screen failures occurred due to benign/neurotic tumor biopsies or low tumor purity resulting in suboptimal DNA/RNA quantity or quality for genomic analysis. In all 22 patients, we have identified clinically relevant genomic alterations (SNVs, indels, fusions, CNAs) and relapse-specific mutations influencing patient management and providing options for personalized interventions. We assessed the functional impact of some of these cancer-specific alterations. This was the case of a novel relapse-specific rearrangement, identified on relapsed childhood ETP-ALL, and leading to asparagine synthetase (ASNS) up-regulation through a promoter exchange. The expression of this fusion was associated with reduced apoptosis following l-asparaginase treatment. This study shows that PPT based on next generation sequencing technology is a powerful approach that could be implemented in the clinic within a foreseeable future to guide treatment of hard-to-treat childhood cancers and to further improve patient care and outcomes.

#4886 The BRIP1 DNA helicase is a 17q dosage sensitive cooperative driver in neuroblastoma. Suzanne Vanhauwaert, 1 Kaat Durinc, 2 Els Janssens, 1 Givani Dewyn, 1 Bram De Wilde, 1 Genevieve Laureys, 1 Daniel Carter, 2 Chengu Belamy, 3 Katelyn De Preter, 1 Christophe Vanneste, 4 Frank Speelman, 3 Ghent University, Ghent, Belgium; 2 Children’s Cancer Institute, Sydney, Australia.

Neuroblastoma (NB) is an aggressive pediatric tumor arising from sympathic neuronal progenitors. NBs have a low mutation burden while copy number alterations are highly recurrent: MYCN amplification is present in half of high risk tumors often accompanied by 1p deletions while MYCN non-amplified aggressive NB frequently exhibit 1q deletions. Remarkably, both high risk groups show almost invariably chromosome 17q gain and we also reported that the mouse syntenic chromosome 11 region was gained during MYCN driven tumor formation. We and others have shown that mutations on 17q act as cooperative drivers during NB development. Using an integrated bioinformatics analysis, we identified several candidate drivers implicated in DNA repair including BRIP1, also known as FANCJ and located on 17q23.2. BRIP1 acts as a DNA helicase in unwinding of stable G-quadruplex (G4) structures in single stranded DNA during replication ensuring timely progression through S-phase. We show that BRIP1 knock down causes increased replicative stress in MYCN overexpressing NB cells as evidenced by increased RPA32 levels and reduced replication fork velocity. Overexpression of BRIP1 in dbMYCN-eGFP transgenic zebrash induced accelerated tumor formation supporting its role as cooperative driver gene. Gene expression profiling after BRIP1 knock down confirmed enrichment for gene sets implicated in DNA replication and repair and are indicative for perturbation of G4 enriched genes. We also identified further additional 17q dosage sensitive genes implicated in replication fork dynamics including BRCA1, BRCA2, ME1 and TOP2A. We propose that 17q gain acts as an amplifier for expression of multiple genes implicated in control of replicative stress and replication fork dynamics. Finally, we explored whether this replicative stress resistance phenotype could represent a novel therapeutic vulnerability for NB cells or other MYCN driven tumor entities. To this end, we tested several compounds for synergetic interaction with G4 stabilizing ligands such as TMPyP4, pyrdoxatin and BRACO-19 with promising results. In conclusion, we identified BRIP1 as 17q cooperative driver in NB tumors. Relief from G4 induced replication fork stalling in rapidly dividing tumor cells. Further, NB cells exhibit replicative stress resistance through upregulation of multiple critical regulators of replication fork dynamics, offering a new venue for therapeutic interventions.

#4887 Comprehensive molecular analysis of pediatric thalamic tumors. Heloisa H. Moser, 1 Suzanne Yoon, 2 Madhuri Kambhampati, 1 Sridevi Yadavalli, 1 Angela J. Waanders, 2 Adam Resnick, 2 Roger J. Packer, 1 Javad Nazarian 1.

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Childhood thalamic tumors are relatively rare cancers, accounting for 5% of all pediatric brain tumors and categorized as midline gliomas such as diffuse intrinsic pontine gliomas (DIPG). We and others have shown that mutations in
genes encoding for histone 3.3 (H3F3A), histone 3.2 (HIST2H3C) and histone 3.1 (HIST1H3B) along with their obligate partner mutations are the major driver mutations in DIPGs. Where recent studies have identified major histone partner mutations associated with DIPGs, more research is required to provide a clear landscape of genomic aberrations associated with thalamic tumors. We hypothesize that comprehensive whole-genome analysis may provide insights into the mechanism of action of the PGBD5 transposase. The PGBD5 protein is a member of the transposase superfamily of proteins, which are involved in the rearrangement of DNA sequences. This suggests that PGBD5 may play a role in the rearrangement of DNA sequences, potentially leading to the development of tumors.

The PGBD5 gene is overexpressed in the majority of rhabdoid tumors, a lethal childhood cancer, suggesting a potential role in the development of these tumors. The overexpression of PGBD5 in rhabdoid tumors is associated with a high frequency of somatic site-specific DNA rearrangements, which may contribute to the development of these tumors. The identification of PGBD5 as a potential oncogene highlights the importance of further research to understand its role in the development of rhabdoid tumors.

The study also highlights the importance of comprehensive whole-genome sequencing (WGS) for understanding the molecular landscape of tumors. The WGS approach is a powerful tool for identifying the genetic alterations associated with the development of tumors, and it can provide insights into the mechanism of action of the identified genes. The WGS approach can also identify potential therapeutic targets, which can be used to develop targeted therapies for the treatment of tumors.

Overall, the study highlights the importance of comprehensive whole-genome analysis for understanding the molecular landscape of tumors, and it provides insights into the mechanism of action of the identified genes. The identification of PGBD5 as a potential oncogene highlights the importance of further research to understand its role in the development of rhabdoid tumors. The WGS approach is a powerful tool for identifying the genetic alterations associated with the development of tumors, and it can provide insights into the mechanism of action of the identified genes. The WGS approach can also identify potential therapeutic targets, which can be used to develop targeted therapies for the treatment of tumors.
expression information into the clinical interpretation of pediatric cancer genomic data. We underscore the importance of releasing the data to the community immediately following generation, so that they may benefit new patients.

#4891 Identification of recurrent high-affinity MHC class I restricted neo-epitopes in neuroblastoma using ProTECT. Arjun A. Rao, Jugmohit Toor, Sarvind Tripathi, Jacob Pfeil, Nikolaos Sgourakis, Sohe Salama, David Haussler. UC Santa Cruz, Santa Cruz, CA.

Introduction: T-cells are trained to differentiate between cell-surface MHC-displayed peptide sequences from self- and non-self proteins and act on the latter. The numerous mutations often associated with cancers can occur in coding regions of the genome and modify the sequence of wild-type proteins, potentially creating targets for immunotherapies. We have developed an analysis pipeline ProTECT (Prediction of T-cell Epitopes for Cancer Therapy) to identify and rank neo-epitopes in terms of immunogenicity. Running ProTECT on a set of Neuroblastomas patients predicted hotspot mutations that bind well to high-frequency MHC alleles – combinations that would potentially benefit a large subset of NBL patients. Methods: ProTECT accepts paired tumor and normal DNA sequencing fastq files, and tumor RNA sequencing fastqs. Mutations are called using a panel of callers and, are annotated [5] to identify coding mutations. Prediction of self-MHC mutated peptides is carried out [6] and the final binding predictions are ranked using an in-house algorithm. Summary: Running ProTECT on 6 Neuroblastoma samples (NBL) from the TARGET (Therapeutically Applicable Research to Generate Effective Treatments) project revealed 2 well-known hotspot mutations in NBL (NRAS Q61K and ALK R1275Q) that bind to common MHC alleles (A*01:01 and B*15:01 respectively). We also found 2 closely related mutations in ALK F1174L and F1174I that are predicted to C*04:01 and C*07:02. We carried out in-vitro refolding and crystal- lization assays [7] for the five highest-ranking mutant NARS and ALK R1275Q predictions. Properly conformed MHC trimers were verified by a monodisperse peak after anion exchange chromatography. SDS gel electrophoresis and Mass-spect confirmed bound peptide for 4/5 tested predictions and 3 of these were used to set up hanging-drop crystallization trials in various conditions. Positive hits were obtained for one (AQDIYRASYY::HLA-B*15:01) and the structure was obtained at 1.7A. The structure suggested the binding of the 10-mer (AQDIYRASYY) to the MHC and this was shown to bind better than the 9-mer using Differential Scanning Fluorimetry [9]. We will run ProTECT on the remaining 100 + TARGET NBL trios, and on relevant cohorts within The Cancer Genome Atlas (TCGA). We aim to reveal clinically relevant hotspot-mutation/MHC pairs. Conclusion: We have described a pipeline for identification and ranking of therapeutically relevant neo-epitopes. We have predicted potential targets for NBL that have been validated in-vitro. References: 1. Cibulskis, K. et al. Nat Biotech 31, 213-219 (2013) 2. Radenbaugh, A. J. et al. PloS ONE 9, e111516 (2014) 3. Larson, D. E. et al. Bioinformatics 28 (3) (2012) 4. Saunders, C. T. et al. Bioinformatics 28 (14) (2012) 5. Cingolani, P. et al. Fly 8, 90-92 (2012) 6. Vita, R. et al. Nucl. Acids Res. (2014) 7. Garboczi, D. N. et al. PNAS 89 (8) (1992) 9. Lance M. Helfman et al. J Immh Meth 432:95-101 (2016) TUMOR BIOLOGY: Therapeutic Intervention of Cancer and Metastasis

#4892 MOSPD2, a newly characterized protein, promotes breast cancer metastasis. Itzhak Mendel, Yaniv Salem, Niva Yacov, Osrrat Propheta-Meiran, Eyal Breuer, Zvi Toren, Ohad regional Bioinformatics, Tel-Aviv University, Tel-Aviv, Israel.

Introduction: We have previously described motile sperm domain-containing protein 2 (MOSPD2), a protein which up until recently no function was ascribed to, as a key regulator of monocytemigration in-vitro. In this study, the role of MOSPD2 in promoting breast cancer migration and metastasis in-vitro and in-vivo was assessed. Experimental procedure: The prevalence of MOSPD2 was evaluated by IHC in tissue microarray layered with cores, representing different stages of invasiveness in breast cancer. MOSPD2 abundance was scored according to the staining intensity on a scale from 0 to 3. MOSPD2 expression was silenced in MDA-231 breast cancer cell line using lentiviral particles for sh-RNA or CRISPR-CAS9 (CRISPR). Cells were then tested by a trans-well assay for migration towards EGF in vitro as well as for lung dissemination following orthotopic or systemic inoculation in vivo. For mechanism studies, EGF-induced signaling events were analyzed. Results: Within normal adjacent tissue (NAT), 82% of the samples had no staining and the remaining 18% percent displayed an intensity of 1. In the tissue cores with in-situ carcinoma pathology, 79% of the samples had no staining intensity while the remaining 21% only scored 1 or 2. However, analysis of invasive and metastatic cancer demonstrated higher frequency in score of 2 and increased staining intensity up to score of 3 compared with NAT and in-situ carcinoma. Thus, the percent of combined scores 2 and 3 for invasive lobular carcinoma, invasive ductal carcinoma and metastatic invasive ductal carcinoma were 63%, 77% and 81%, respectively. MOSPD2 silenced cells, either by siRNA or by CRISPR, were severely impaired in their ability to migrate in-vitro towards EGF. Following EGF activation of CRISPR-Control MDA-231 cells, all tested proteins down-stream to the EGF pathway, including AKT and p38, became phosphorylated. However, in CRISPR-MOSPD2 silenced cells, phosphorylation of all tested proteins excluding p38 were markedly inhibited. Moreover, activation with IGF of CRISPR-MOSPD2 MDA-231 cells did not hinder AKT phosphorylation. In vivo, SCID mice injected orthotopically or systemically with CRISPR-MOSPD2 silenced MDA-231 cells displayed significantly reduced number of metastases in the lungs relatively to mice injected with CRISPR-Control treated cells. Conclusions: We suggest that MOSPD2 promotes metastasis of breast cancer cells and is a potential target for the treatment of metastatic breast cancer.

#4893 AKT1 signaling negatively regulates invasion and metastasis of non-small cell lung cancer cells carrying mutations in KRAS or EGFR. Guanghua Rao,1 Mariaelena Pierobon,2 In-Kyu Kim,1 Wei-Hsun Hsu,1 Jianghong Deng,2 Yong-Wha Moon,1 Emanuel F. Petricoin,2 Yu-Wen Zhang,1 Yisong Wang,1 Giuseppe Giacone1,2. 1George town Univ. Medical Ctr., Washington, DC; 2George Mason University, Institute for Advanced Biomedical Research, Manassas, VA.

Inhibition of AKT1 signaling driven by the phosphoinositide-3 kinase (PI3K) and AKT/protein kinase B (PKB) pathway plays an important role in cancer development and progression, and thus this pathway is an attractive therapeutic target. Although many different inhibitors targeting either PI3K or AKT have been developed, the therapeutic benefit of these inhibitors in the clinic remains elusive, in part because of the perplexing role of AKT signaling in the regulation of cell motility, invasion and metastasis. AKT activation is known to promote migration, invasion and metastasis; however under certain circumstances and different cellular systems, it also exhibits an inhibitory activity on these cellular processes and the underlying cause of these conflicting results is unclear. Through analysis of the Reverse Phase Protein Array (RPPA) performed on a series of non-small cell lung cancer (NSCLC) brain metastasis models generated via intracardiac injection in mice, we identified AKT1 (one of three AKT family members) as a critical negative regulator of A549 tumor metastasis. AKT1 tyrosine phosphorylation (but not AKT2 or AKT3) inversely correlated with the metastatic potential of A549 cells which carry a KRAS mutation. Using AKT1 siRNAs, we demonstrated that inhibition of AKT1 significantly enhanced migration and invasion of KRAS- or EGFR-mutant NSCLC cells, but not wild type KRAS and EGFR cells. Similarly, the AKT inhibitor MK-2206 also enhanced the migration and invasion of KRAS- or EGFR-mutant NSCLC cells in vitro, and promoted metastasis of KRAS-mutant A549 cells in vivo. The PI3K-AKT pathway is downstream of both EGFR and KRAS, whose constitutive activation caused by mutations likely alter the signal output of PI3K-AKT pathway and thus the activity on cell invasiveness. Therefore, our results provide a sound explanation that under certain tumor microenvironmental background (e.g. KRAS or EGFR mutations) the AKT signaling may switch from a promoter to an inhibitor of cell migration, invasion and metastasis. This observation may have strong clinical implications for the development of inhibitors of this pathway for cancer treatment. Furthermore, we found that AKT1 blockade induced phosphorylation of Myristoylated alanine-rich C-kinase substrate (MARKCS) and elevation of p38 were markedly inhibited. Moreover, activation with IGF of CRISPR-MARKCS-silenced cells, either by sh-RNA or by CRISPR, were severely inhibited. In contrast, activation of AKT by AKT1 siRNA knockdown abrogated the activities of migration and invasion induced by AKT1 inhibition. This study unravels an anti-metastatic role of AKT1 in NSCLC cells with KRAS or EGFR mutation, and provides mechanistic insights for the understanding of the potentially contrasting roles of AKT signaling in the regulation of cell invasiveness.

#4894 Identification of small molecules for the prevention and treatment of colon cancer. Deeksha Pal,1 Becca Von Baby,2 Balaji Chandrasekaran,1 Arun Sharma,2 Chandell Damodaran.1 1University of Louisville, Louisville, KY; 2Penn State University, PA.

Metastatic colon cancer or colorectal cancer (CRC) is associated with poor survival rate which is less than 5% due to chemo resistance. We previously showed that overexpression of AKT and Notch1 are key players for progression of metastasis of colon cancer cells. Witherin-A (WA) is a potent herbal molecule that inhibited CRC growth by down regulating AKT and Notch1 signaling in both in vitro and in vivo colon cancer models. We found that WA binds to

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AKT with a predicted binding affinity of -10.6 kcal/mol and induced its activation. So, we designed and developed several analogs of WA, that exhibits a stronger AKT binding (binding energy higher than -10 kcal/mole) and more potent than the parental WA. UL16 is one such compound which induced the growth of colon cancer cells (HCT-116 and SW-620; IC50, 300nM) at nanomolar concentration. Western blot analysis revealed that UL16 inhibits AKT/NF-κB in Re12- signaling axis in colon cancer cells. In addition, UL16 concurrently inhibited Notch1 signaling pathway in colon cancer cells. Then we systematically investigated the anti-migratory and anti-invasive effect of UL16 by cell migration and invasion assays. Inhibition of both migration and invasion of colon cancer cells corresponds with downregulation of mesenchymal markers of Sna1, Slug and β-catenin expression. We also examined the in vivo efficacy of UL16 in ApMin+/– mouse model. Treatment of ApMin+/– with UL16 (0.5 mg/kg/IP/ twice a week) over 12 weeks significantly reduced the number of intestinal polyps (distal 84%; Middle 63% and Proximal 2%) as compared to vehicle treated mice. In addition, we also observed 50% reduction in number of colon tumors in UL16 treated mice. Our ongoing immunohistochemistry studies may confirm the in vitro mechanistic results. In conclusion, our studies suggest that UL16 inhibits both AKT and Notch1 signaling and suppresses intestinal polyp formation in ApMin+/– mice. Therefore UL16 could be a viable therapeutic agent for treating patients with metastatic colon cancer.

#4895 CT20p as a therapeutic for lung cancer with elevated chaperoning containing TCP1 (CCT) expression levels. Ana C. Carr, 1 Amy Sm. Khaled, 2 Rania Bassouani, 3 Annette R. Khaled. 1University of Central Florida, Orlando, FL 2Orlando VA Medical Center, Orlando, Florida, FL

Lung cancer is the leading cause of cancer related fatalities worldwide and although there is a substantial effort in the development of improved, more specific, and less debilitating therapies, very little progress has been made accomplishing this goal. We propose the use of CT20p, a therapeutic peptide developed by our laboratory, as novel therapy for lung cancer. We have previously demonstrated that CT20p has tumor-specific cytotoxicity in breast cancer cells and it induces tumor regression in xenograft models of breast cancer. We have also determined Chaperonin containing T-complex (CCT) to be the intra-cellular target of CT20p and that susceptibility to CT20p correlates with its expression levels, with tumor cells expressing high levels of CCT being the most susceptible. CCT is a large macromolecular complex composed of 8 subunits (CCTα, CCTβ, CCTγ, CCTδ, CCTε, CCTζ, CCTθ, CCTη). Immunoblot probing against four relevant CCT subunits (CCTβ, CCTγ, CCTδ, CCTε) was used to determine their relative expression level in five small cell lung cancer (SCLC) cell lines when compared to normal lung cells. We determined that the expression levels of the four probed CCT subunits varied amongst the five SCLC cell lines, however, the four CCT subunits were always expressed at higher levels when compared to normal lung cells. Immunohistochemistry staining for CCTβ was used on tissue microarray (TMA) of lung cancer progression. Results were interpreted on a scale of 1 to 4 (with 4 being the strongest staining). We determined that CCTβ levels increase with cancer severity and identified a significant correlation between CCTβ levels and cancer progression in both small cell lung carcinoma (SCLC) and Squamous cell lung carcinoma (SqCLC). Using The Cancer Genome Atlas (TCGA) database, we found that SMAD4 (transcriptional mediator of TGF-beta pathway) induced TIMP3 expression levels and cancer progression in both small and non-small cell lung carcinomas. Immunohistochemical staining of CCTβ levels in clinical lung cancer cases range from 10% to 43%, however, only CCT4 gene amplification in SqCLC cases correlated with decreased survival. Altogether, these results suggest that CT20p has the potential for being a therapeutic for multiple types of lung cancer due to increased target expression.

#4896 Role of CDK8 in colon cancer hepatic metastasis. Jiaxin Liang, Mengqian Chen, Mythreya Karthikeyan, M. Marjorette Pena, Daniel Hughes, Vimala Kaza, Chang-Uk Lim, Eugenie Broude, Igor B. Roninson. University of South Carolina, Columbia, SC.

About half of all colon cancer patients will develop liver metastases and the 5-year survival for these patients is less than 13%, making colon cancer the second most lethal cancer worldwide. Cyclin dependent kinase 8 (CDK8), which regulates cell cycle progression, has been identified as an oncogene amplified in many colon cancers. CDK8 acts as a positive mediator of oncogenic transcription pathways regulated by Wnt/β-catenin and TGF-beta, both of which are strongly associated with tumor metastasis. In a colon cancer liver metastasis model based on splenic injection of CT26 murine colon carcinoma cells, treatment with Senexin B, a highly selective small-molecule inhibitor of CDK8 and its paralogue CDK9, strongly inhibited metastatic growth in the liver and prolonged the survival of mice with hepatic metastases. In contrast to the effect on liver metastasis, CDK8 inhibition had little or no effect on cell growth in culture or at primary tumor sites. Hepatic metastasis was inhibited to the same extent when Senexin B was administered starting from the time of tumor injection or only after a later part of the study, suggesting that the drug affected metastatic growth in the liver rather than just initial colonization. Liver metastasis was similarly decreased by CDK8 knockdown in CT26 cells, suggesting that the anti-metastatic activity of Senexin B was due at least in part to its effect on tumor cells. Transcription profiling indicated that CDK8 inhibition by Senexin B was associated with downregulation of several genes associated with cell cycle progression and upregulation of genes associated with cell death. CDK8 inhibition also blocked TGF-beta or Wnt3a-stimulated transwell migration of CT26 cells in vitro. Knockdown of beta-catenin in CT26 cells also increased the expression of MMP7 and MMP10, consistent with the idea that CDK8 and β-catenin act in a redundant manner in promoting tumor progression. Thus, CDK8 inhibition decreased the primary tumor growth in the spleen and metastatic growth in the liver, whereas SMAD4 knockdown, like that of CDK8, selectively inhibited liver metastasis. Senexin B also suppressed hepatic metastasis after splenic injection of human HCT116 colon carcinoma cells. In summary, our data identified CDK8 as a key transcriptional regulator of colon cancer metastatic growth in the liver, interacting with TGF-beta and Wnt/ beta-catenin pathways and regulating the expression of Mmp7 and Timp3. CDK8/19 inhibitors, which are now entering clinical trials, may be effective for the treatment of hepatic metastasis of colon cancer.

#4897 Circadian/melanatonin disruption by dim light at night drives human epithelial breast cancer to a metastatic phenotype. Steven M. Hill, Shulin Xiang, Robert T. Dauchy, Melissa Wren-Dail, Murali Anbalagan, Brian Rowan, Tripp Frasch, David E. Blask. Tulane Univ. School of Medicine, New Orleans, LA.

Epithelial cancers demonstrate 24-hour (circadian) rhythms that are disrupted by exposure to dim light at night (dLAN). This study examined if dLAN-induced circadian/melatonin (MLT) disruption by exposure to dim light at night (dLAN) results in constitutive phospho-activation of ERK1/2, CREB, NF-κB, and STAT3 in breast tumor xenografts. CDK8/19, which were expressed in tumors from rats housed in dLAN, was found to be upregulated in tumors from rats housed in dLAN compared to those from rats housed in LD. CDK8 knockdown decreased the primary tumor growth in the spleen and metastatic growth in the liver, whereas SMAD4 knockdown, like that of CDK8, selectively inhibited liver metastasis. Senexin B also suppressed hepatic metastasis after splenic injection of human HCT116 colon carcinoma cells. In summary, our data identified CDK8 as a key transcriptional regulator of colon cancer metastatic growth in the liver, interacting with TGF-beta and Wnt/ beta-catenin pathways and regulating the expression of Mmp7 and Timp3. CDK8/19 inhibitors, which are now entering clinical trials, may be effective for the treatment of hepatic metastasis of colon cancer.

#4898 eIF4E represses the surface expression of migrating AML cells through regulating Hyaluronic Acid synthesis & CD44 expression. Hiba Zahredine, 1 Biljana Krajcic-Culjovic, 2 Valbona Catal, 3 Mark Lauer, 2 Lucy Skrabanek, 4 Nahibal Khan, 5 Ronald Mudura, 6 Leandro Cerchietti, 3 Vincent Hascal, 5 Craig Jordan. 1Institute for Research in Immunology and Cancer, University of Montreal, Montreal, Quebec, Canada; 2Cleveland Clinic, Cleveland, OH; 3Cornell University, New York, NY; 4University of Denver, Colorado, CO; 5University of Denver, Denver, CO.

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Cancer patients with disrupted 24-hour (circadian) rhythms are reported to have poorer survival as compared to those with normal rhythms. Severe alterations in circadian rhythms predict an increased risk of death in patients with colorectal and breast cancer, suggesting that circadian disruption may impact tumor progression and metastasis. We recently reported that circadian/melanatonin (MLT) disruption by exposure to dim light at night (dLAN) resulted in constitutive phospho-activation of ERK1/2, CREB, NF-κB, and STAT3 in breast tumor xenografts. Included in the panel of targets tested were CDK8/19, which were expressed in tumors from rats housed in dLAN, was found to be upregulated in tumors from rats housed in dLAN compared to those from rats housed in LD. CDK8 knockdown decreased the primary tumor growth in the spleen and metastatic growth in the liver, whereas SMAD4 knockdown, like that of CDK8, selectively inhibited liver metastasis. Senexin B also suppressed hepatic metastasis after splenic injection of human HCT116 colon carcinoma cells. In summary, our data identified CDK8 as a key transcriptional regulator of colon cancer metastatic growth in the liver, interacting with TGF-beta and Wnt/ beta-catenin pathways and regulating the expression of Mmp7 and Timp3. CDK8/19 inhibitors, which are now entering clinical trials, may be effective for the treatment of hepatic metastasis of colon cancer.
Formation of cell surface protrusions has long been associated with tumor cell migration, metastasis, and bone marrow homing & retention. It was shown that increased expression of endogenous or exogenous hyaluronic acid synthesizing enzyme 3 (HAS3) induces growth of microvillus-like cell surface protrusions. Some of the biological signals triggered by HA are dependent on its recognition by the cell surface CD44. In osteosarcoma cell lines with metastatic potential, the activity of the eukaryotic translation initiation factor 4E (eIF4E) increases formation of cell surface protrusions resembling those produced by HAS3 upregulation. eIF4E regulates mRNA export & translation through binding to the 5′ cap of mRNA in the nucleus & the cytoplasm, respectively. Strikingly, analysis of our two complimentary screens (immunoprecipitation of mRNAs bound to eIF4E in the nucleus & mRNA export associated with surface CD44) of eIF4E that we carried out to identify candidate eIF4E mRNA export targets, showed that nearly all the enzymes involved in HA synthesis as well as CD44.

Here, we hypothesize that eIF4E regulates HA synthesis & CD44 overexpression to reshape the surface of cancer cells producing HA-rich microvillus-like pseudopods. Following validation of the positive hits, we used immunofluorescence staining with biotinylated HA binding protein (HABP) to determine the effect of eIF4E expression on protrusion formation. Our data revealed that eIF4E overexpression increased formation of HA-rich cell surface protrusions & was correlated with increased invasiveness of eIF4E overexpressing cells in vitro & matrigel assays, compared to respective controls. In mice, confocal microscopy indicated that protrusions positively stained for CD44.

To reshaping the surface of cancer cells producing HA-rich microvillus-like pseudopods, we directly measured HA using confocal microscopy. We also tested eIF4E expression on protrusion formation. Our data revealed that eIF4E overexpression increased formation of HA-rich cell surface protrusions & was correlated with increased invasiveness of eIF4E overexpressing cells in vitro & matrigel assays, compared to respective controls. In mice, confocal microscopy indicated that protrusions positively stained for CD44.

Eukaryotic translation initiation factor 4E (eIF4E) is a key regulator of mRNA translation in eukaryotic cells. eIF4E binds to the 5′ cap of mRNA and targets it for ribosome binding, allowing translation to proceed. It plays a crucial role in cell growth, proliferation, and survival.

Cysteamine is a small molecule inhibitor of matrix metalloproteinases (MMPs), which are enzymes that break down extracellular matrix. Cysteamine has been shown to suppress tumor metastasis in various models, including those for breast cancer, prostate cancer, and ovarian cancer. It appears to work by inhibiting the activity of MMPs, which are known to play a significant role in tumor invasion and metastasis. This is particularly important for patients with breast cancer, as it is the second leading cause of cancer-related death for patients in this age group. Current therapy for breast cancer is limited, and there is a significant need for novel therapies that can improve survival and quality of life for patients.

Therefore, the authors of the study measured cysteamine suppresses tumor metastasis by inhibiting activity of matrix metalloproteinases without inducing toxicity in mouse models of human ovarian cancer. Akiko Suzuki, Rukmini Bhardwaj, Pamela Leland, Bharat H. Joshi, Raj K. Puri. FDA-CBER, Silver Spring, MD.

Previously, we demonstrated that cysteamine, a small molecule inhibitor of matrix metalloproteinases (MMPs), inhibited the migration and invasion of LM-2-MDA-MB-231 in experimental metastasis in vivo in mouse models of human pancreatic and ovarian cancer. We have also shown that subcutaneous cysteamine administration improved the survival of mice with orthotopically transplanted pancreatic tumors. Herein, we examined the effect of cysteamine on total MMP activity, MMP isoforms and activity by substrate gel zymography in tumors obtained from orthotopic mouse model of human ovarian cancer. We also investigated any gastrointestinal and general toxicity in vital organs of mice treated with the highest dose of cysteamine (250 mg/kg). We developed orthotopic tumors by injecting two human ovarian cancer cell lines in the ovary of female athymic nude mice. After 4 days of tumor implant, the mice were treated with different doses of cysteamine and followed for 5 weeks. Total MMP activity by fluorogenic substrate, MMP-2, 9 and 14 by ELISA and MMP activity by substrate gel zymography were measured in tumor lysates and compared with untreated tumors. We performed H&E staining in vital organs such as heart, lung, liver, kidney, spleen and brain for evaluating general toxicity of cysteamine. In addition, we examined stomach and duodenum from cysteamine treated and untreated mice if they developed any ulcerations. In two orthotopic murine models of human ovarian cancer, consistent with our previous observations, tumor metastasis was significantly decreased compared to controls in cysteamine treated mice. Similarly, total MMP activity was also significantly decreased in primary tumors treated with cysteamine in a dose dependent manner (P<0.05). Zymographic MMP activity was also decreased significantly in cysteamine treated tumors compared to control rebororating with total MMP activity (P≤0.05). Interestingly, ELISA did not show any change. MMP expression in cysteamine treated and untreated tumors was not affected. Furthermore, we did not observe any symptoms of general toxicity in animals treated at the highest dose of cysteamine and major vital organs and gastrointestinal organs from treated animals showed no evidence of histological changes as evidenced by H&E staining. In conclusion, our results suggest that cysteamine is a potent inhibitor of MMP activity without affecting expression. Cysteamine has the potential to inhibit any gastro-intestinal or vital organ toxicity and may be useful therapeutic agent for ovarian cancer either alone or in combination therapy with known anti-cancer agents.

Urokinase-derived peptide UP-7 effectively inhibits metastatic growth of breast cancer through suppression of FAK activation. Young Ae Joe,Hyun-Kyung Kim, Purevjagai Naidasunare, Seung Woo Lee, Rae-Kwon Kim, Su-Jae Lee, Suk Keun Lee, Yong-Ki Hong. Cardiothoracic University of Korea, Seoul, Republic of Korea; 2Hanyang University, Seoul, Republic of Korea; 3Kangnung National University, Kangnung, Republic of Korea.

The urokinase kringle domain of urokinase-type plasminogen activator (UK) has been shown to inhibit angiogenesis in vitro and in vivo and suppress tumor growth in vivo. To avoid limitations in application due to mass production of recombinant proteins, we dissected UK1 sequences to seven peptides based on structure and amino acid characteristics, and examined the anti-angiogenic activities for the constructed peptides. UP-7 peptide derived from β-sheets region of UK1 was the most potent inhibitor of proliferation and migration of endothelial cells (ECs) in vitro, and it also inhibited in vivo angiogenesis in the mouse matrigel plug assay. Such anti-angiogenic activities were not exerted by the scrambled peptide. At molecular level, UP-7 inhibited the VEGF or bFGF-induced phosphorylation of FAK and ERK1/2 and suppressed formation of capillary-like networks in vitro. In a lung cancer animal model xenografted with UP-7-non-sensitive NCI H460, systemic treatment of UP-7 effectively suppressed tumor growth in vivo through inhibition of angiogenesis. Importantly, breast cancer cells such as MDA-MB231 were sensitive to UP-7 in proliferation differently from other cancer cells. It inhibited the migration and invasion of LM-2-MDA-MB231 and suppressed the phosphorylation of FAK. Accordingly, UP-7 potently inhibited lung metastatic growth of LM2-MDA-MB-231 in experimental metastasis model. Taken together, these results suggest that novel UK1-derived peptide, UP-7 can be effectively used for treatment of the metastatic growth of breast cancer. (NRF-2012R1A1A2007175, NRF-2012R1A5A2051476).

Celecoxib fixed-dose combination: antitumor activity on growth and metastasis. Cynthia Lee, Osmond J. D’Cruz, Kevin Ng, Vuong Trieu. Autotelic Inc, Costa Mesa, CA; 2Marina Biotech, Agoura Hills, CA.

The role of COX-2 in the regulation of the tumor microenvironment is well established. COX-2 is overexpressed in many human cancers and is involved in multiple processes such as angiogenesis, invasion, and metastasis. Celecoxib is a COX-2 specific inhibitor that has been shown to exhibit antitumor activity in various cancer models. In this study, the antitumor activity of celecoxib fixed-dose combination was evaluated in vivo and in vitro.

In vivo, the antitumor effect of celecoxib combination was assessed in a murine breast cancer model. Tumor growth was significantly inhibited in mice treated with the combination compared to control groups. Similarly, in vitro, the combination inhibited proliferation, migration, and invasion of cancer cells. These results suggest that celecoxib combination may have potential as an antitumor agent.
Background: Breast cancer and melanoma are associated with a high rate of lymphatic and hematogenous metastasis which results in high rate of cancer mortality. Inflammation and hypertension (HTN) have recently emerged as causal factors for tumor progression and anti-hypertensive agents have been shown to reduce inflammation and suppress tumor growth and metastasis. Lisinopril (LIS), an inhibitor of angiotensin-1 converting enzyme (ACE); ii) Losartan medoxomil (OLM), an angiotensin II receptor blocker (ARB); and iii) Hydrochlorothiazide (HCTZ), a thiazide diuretic along with Celecoxib (CEL), a selective COX-2 inhibitor, CEL, LIS, OLM, and HCTZ were evaluated either alone or in combination for tumor growth suppression and metastatic spread in an orthotopic xenograft model of triple-negative inflammatory breast cancer/SUM149 and subcutaneous xenograft models of melanoma/MDA-MB-435, glioblastoma/UB87-MG, and triple-negative SUM159. Luciferase-tagged SUM149 and MDA-MB-435 cell lines were used to determine the incidence and the burden of locoregional and systemic spread. Mice were monitored twice a week for 9-16 weeks for percent weight loss, tumor volume and survival outcome. Metastatic tumor burden was assessed by measuring tumor volumes and the survival rate of less than 5%. Cell-free DNA (cfDNA) has been well-validated as a biomarker that correlates with tumor burden. More recently, a role for cfDNA in facilitating tumor progression has also been identified. The latter occurs through activation of nucleic acid sensing receptors such as toll-like receptors (TLRs) in both tumor cells and the host environment, which can upregulate pro-metastatic signaling pathways. In addition, tumor-derived extracellular vesicles such as microparticles and exosomes have also been implicated in promoting metastasis by activating pro-invasive pathways in tumor cells and pre-conditioning secondary sites for metastatic establishment. Our laboratory has previously shown that cationic polymers can scavenge negatively charged nucleic acids like cfDNA and abrogate aberrant inflammation in disease models of autoimmunity and infection. Herein, we investigated the ability of the cationic polymer, PAMAM-G3, to inhibit cfDNA as well as anionic tumor-derived extracellular vesicles using in vitro models of pancreatic cancer cell line invasion. We also evaluated the effect of PAMAM-G3 treatment in a bioluminescent syngeneic murine model of pancreatic cancer metastasis. PAMAM-G3 significantly inhibited in vitro invasion of pancreatic cancer cell lines in response to both cfDNA and tumor-derived extracellular vesicles. Moreover, biweekly intraperitoneal treatment with PAMAM-G3 (20 mg/kg) starting 48 hours after pancreatic tumor cell implantation led to a significant reduction in liver metastasis without affecting primary tumor growth. Thus, cationic polymers such as PAMAM-G3 may represent a novel class of therapeutics to combat pancreatic cancer metastasis.

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#4904 GLUT4 exhibits a non-canonical role of regulating lung cancer metastasis. Changyong Wei,1 Abhinav Achreja,1 Jessica Konen,1 Gabriel Sica,1 Melissa Gilbert-Ross,1 Deepak Nagrath,2 Adam Marcus,1 Malu Shannugam1. 1Emory University, Atlanta, GA; 2Rice University, Houston, TX.

Lung cancer continues to be fatal, in part due to the inability to prevent and treat metastasis. Current treatment which is targeted on control of primary tumor is not sufficient to sustain all the metastatic proliferation and importantly, tumor invasion. Among the SL2CA family of facilitative glucose transporters, GLUT1 is largely attributed to be responsible for increased glucose uptake of cancer cells. GLUT1 is however responsible for glucose transport across the blood-brain barrier, expressed in many normal cell types and therefore a less desirable therapeutic target. We previously reported that multiple therapeutic modalities including chemotherapeutics, localizing agents and combinations of insulin-responsive glucose transporter, GLUT4. In this study we investigated a role for GLUT4 in lung cancer. To interrogate contributions of GLUT1 and GLUT4 in proliferation, invasion and migration we generated H1299 and A549 GLUT 1 or GLUT4 knockdowns. Knockdown (KD) of GLUT4 did not inhibit proliferation but suppressed migration and invasion assessed through scratch and Boyden chamber assays, respectively. On the contrary, knockdown of GLUT1 reduced proliferation of these lines. Treatment of H1299 and A549 with our newly developed GLUT4-selective inhibitors also reduced invasion, phenocopying the effects observed with GLUT4 KD. GLUT4 inhibition also reduced H1299 invasion in a spheroid model. We utilized H1299 cells to isolate highly invasive less proliferative “leader cells” and less invasive but highly proliferative “follower cells”. H1299 leader cells more sensitive to mitochondrial inhibitors compared to follower cells. High glucose levels and high glutamine consumption in leader cells resulted in enhanced glycolysis/OXPHOS was further supported by evaluation of glucose uptake/oxygen consumption. Isotope tracer and bioenergetics analyses further support altered nutrient dependencies of leader and follower cells. Lastly, we found that GLUT4 is expressed in patient lung adenocarcinoma specimens including more aggressive micropapillary lung adenocarcinoma. Examination of collective invasion packs in patient samples showed high GLUT4 expression in a subset of more proliferative “follower” cells. These results suggest that in lung cancer population a subset of more invasive cells are reliant on GLUT4 with reduced GLUT1 expression while more proliferative cells rely on high GLUT1 expression, making GLUT4 a promising candidate for targeting metastasis in lung cancer.

#4905 Metastasis promoting long non-coding RNA, FEZF1-AS1, is an important therapeutic target for Ewing sarcoma. Sheetal S. Mitra,1, Anirban P. Mitra,2,3 Jon O. Nagy,1 Jonathan D. Buckley,1 Timothy J. Triche,1 1Children’s Hospital Los Angeles, Los Angeles, CA; 2Children’s Hospital Los Angeles, Los Angeles, CA; 3NanoValent Pharmaceuticals, MT.

Ewing sarcoma (ES) is a universally metastatic tumor of bone and soft tissue. Five-year survival in patients with metastases at diagnosis is about 20% despite intense treatment strategies. 85% of these tumors harbor a chromosomal translocation resulting in a fusion gene, EWS-FLI1. EWS-FLI1 imparts an oncogenic phenotype by modifying critical genes in signaling pathways, thus allowing for uninhibited cell growth, invasion, and metastasis while evading differentiation. EWS-FLI1 is thus an ideal therapeutic candidate, but targeting this fusion protein has proven difficult. In an attempt to better understand ES biology and identify candidate therapeutic targets downstream of EWS-FLI1, we profiled RNA transcription in over 140 ES cases and compared the data to other pediatric cancers. Among the most highly expressed genes that were downregulated as a result of lncRNA knockdowns. FEZF1-AS1 expression was specific for ES and was increased more than a hundred fold in ES when compared to other pediatric tumors. Chromatin immunoprecipitation (ChIP) studies along with luciferase promoter assays showed that EWS-FLI1 induces FEZF1-AS1 expression by binding to promoter GGAA repeats. shRNA-mediated knockdown of FEZF1-AS1 did not affect cell growth or survival. Instead, metastatic potential was markedly reduced, based on Matrigel assays. Conversely, ES cells transfected to overexpress FEZF1-AS1 engrafted better in mice and metastasized faster than control cells. Expression profiling of lncRNA-knockdown cells showed that the lncRNA upregulated genes were involved in cell movement and cell organization. ChIP-seq documented significant reduction in H3K4me3 and the H3K27ac histone marks in the promoters of genes that were downregulated as a result of lncRNA-knockdown. In order to document FEZF1-AS1 function, we delivered FEZF1-AS1-specific antisense oligonucleotide (ASO) and GFP-ASO in anti-CD99-targeted-nanoparticles to ES cells. An efficient uptake of nanoparticles and lncRNA ASO delivery to ES cells led to a 70%

Despite extremely high morbidity and mortality of >90% from metastasis, it remains one of the most challenging process for targeted therapeutic interventions. Identification and characterization of chemical and bioactive compounds from medicinal plants targeting the cell-signaling pathways of Cancer Stem Cells (CSCs), tumor cells and their microenvironment that abet metastasis is of great clinical significance. Hence, MCF-7; a breast cancer cell line, was treated with fractionated alkaloidal extracts from Rhazya stricta with previously documented anti-cancer activity in lung and pancreatic cancer cell lines. RNA-seq analysis was performed for samples treated with compounds that actively inhibited cell migration in scratch assay to further substantiate their anti-metastatic role at the cellular and molecular level. Genomic data corroborated the presence of anti-metastatic compounds that actively down-regulated genes involved in metastasis (CD6), cell migration (KIF23, DLGAP5), in-vivo and in-vitro invasion of freshly isolated biopsy samples from breast cancer patients. For proof-of-concept studies, in mouse models of breast cancer, 21 days post-treatment with extracts, we observed a significant reduction in tumor burden. The study demonstrates the potential of Rhazya stricta extract for the development of new anti-metastatic drugs and provides a framework for further preclinical and clinical development.

#4908 Neuruplin-1 is up-regulated in the adaptive response of prostate tumors to androgen targeted therapies and is prognostic of metastatic progression and patient mortality. Marianna Volpert,1 Brian Tse,2 Elka Ratther,1 Nataly Stylianos,1 Mannan Nouri,1 Melanie Lehman,1 Stephen McPherson,1 Mani Roshan-Monir2, Mandeep Takhar,2 Nicholas Erho,3 Mohamed Atef,4 Ela David,4 Robert Jenkins,4 Asha Ross,1 Jeffrey Karmazin,1 Robert Den,2 Ladan Fazi,1 Martin Gleave,3 Elizabeth Williams,1 Paul Rennie,1 Ralph Buttyn,1 Pamela Russell,1 Colleen Nelson,1 Brett Hollier1. 1Queensland University of Technology, Brisbane, Australia; 2Translational Research Institute, Brisbane, Australia; 3University of British Columbia, Vancouver, British Columbia, Canada; 4GenomeDx Biosciences, Vancouver, British Columbia, Canada; 5Medical Clinic, Rochester, NY; 6Surgical and Medical Institutions, Baltimore, MD; 7Thomas Jefferson University, Philadelphia, PA.

Aims: Androgen-targeted therapies (ATTs) are the mainstream treatment for metastatic prostate cancer (PCa). However, ATTs promote adaptation of tumor cells and lead to castration resistant disease (CRPC). We have recently identified the cell surface receptor, Neuruplin-1 (NR1P1) as increased during EMT and in CRPC. However, the role of NR1P1 in the prostate epithelium is poorly understood. This study aims to determine whether the inhibition of NR1P1 will be a feasible therapeutic strategy for blocking PCa metastasis and therapy resistance. Methods: qPCR and western blotting were used to assess NR1P1 expression in PCa cell lines. NR1P1 expression in CRPC was assessed using a murine LNCaP xenograft model of castration. NR1P1 was knocked down with shRNA 1 and 2 in human and murine CRPC cell lines, respectively. Results: PCa cells were microinjected into the zebrafish yolk sac and metastatic dissemination was imaged 5 days later. NR1P1 expression in radical prostatectomy (RP) samples from Mayo Clinic (545 patients) and Johns Hopkins Medical Institutions (JHMI; 188 patients) cohorts was quantified by Affymetrix exon arrays and multivariable analysis performed. Box scratch migration and invasion assays were performed with the Wound maker” tool and IncuCyte™ FLR imaging systems. Results: NR1P1 levels were elevated in human CRPC xenografts, metastatic and castrate resistant clinical PCa samples (p < 0.0001), and PCa cell lines. NR1P1 suppression significantly reduced metastasis of human xenografts in zebrafish and the migratory and invasive behaviour of metastatic PCa cells (p = 0.0002). Multivariable analysis identified NR1P1 as a significant independent prognostic indicator of metastasis and prostate cancer specific mortality in two large clinical cohorts (Mayo Clinic and JHMI; p = 0.008/0.048 and 0.013/0.034 respectively). We show that NR1P1 knockdown promotes E-Cadherin expression and loss of vimentin in mesenchymal PCa cells. Conclusion: These results will provide the preclinical data necessary to rationalise the use of anti-NR1P1 directed adjuvant therapies for clinical use in PCa patients receiving ATTs, and will pave the way for larger scale preclinical and clinical trials in the PCa setting.

#4909 Androgen targeted therapy induces ZEB1 expression and is associated with progression of androgen loss and therapy resistance. Katrina G. Sweeney,1 Nataly Stylianos,1 Gregor Tezv,1 Atef heel Taherianfard,1 Katrina Pirlo,1 Akanksha Upadhyaya,1 Elka Ratther,1 Melanie Lehman,1 Martin Gleave,1 Jennifer Gunter,1 Elizabeth D. Williams,1 Colleen C. Nelson,1 Brett Hollier1. 1Australian Prostate Cancer Research Centre-Queensland, Institute of Health and Biomedical Innovation, Queensland University of Technology, Translational Research Institute, Brisbane, Australia; 2Vancouver Prostate Centre, British Columbia, Canada.

Reactivation of the embryonic developmental pathway, epithelial-to-mesenchymal transition (EMT) is associated with prostate cancer (PCa) metastasis and therapy resistance. Recent evidence has demonstrated EMT is stimulated following androgen targeted therapy (ATT). In the present study we investigated the role of EMT transcription factor ZEB1 in ATT-driven EMT, PCa metastasis and drug resistance. Immunohistochemistry (IHC) staining of ZEB1 on tissue microarrays of primary tumors from clinical samples demonstrated ZEB1 expression correlated with increased tumor aggressiveness and Gleason score. Upon stratification of patient data (n = 198), high ZEB1 protein expression correlated with a shorter time to biochemical recurrence (BCR). Furthermore, IHC staining of primary tumors from 148 treatment-naïve patients with and without metastasis demonstrated high ZEB1 levels and correlated to a reduced time to metastasis. To delineate the role of ZEB1 as a molecular driver of latent prostate cancer (PCa) metastasis and resistance to therapy, we performed a comprehensive analysis of ZEB1 expression in PCa cell lines and in clinical specimens. Our results demonstrate that ZEB1 expression is up-regulated in CRPC and is associated with increased tumor aggressiveness and Gleason score. These findings provide a potential therapeutic target for patients with CRPC and highlight the importance of developing new therapeutic strategies for the treatment of CRPC.
Interestingly, in models where ZEB1 expression was elevated this was accompanied with repression of classical androgen-receptor genes suggesting ZEB1 is able to regulate the AR transcriptional pathway. We also investigated the role of ZEB1 in cancer invasion and chemoresistance. An inducible model of ZEB1 overexpression in LNCaP cells produced a robust EMT upon ZEB1 expression and was accompanied by invasion induced by an invasive phenotype in 3D cultures. ZEB1 overexpression also conferred resistance to docetaxel (IC_{50} of 8.17±2.45 mM in ZEB1-expressing cells vs. 3.35±0.23 mM in control cells) potentially as a result of a reduced apoptotic cell death response mediated by ZEB1. At suboptimal doses of docetaxel, the percentage of apoptotic cells (annexin-V+/propidium iodide-) decreased 4-fold when ZEB1 was expressed compared with control cells and was accompanied by a reduction in PARP cleavage consistent with decreased caspase activity. In summary, we provide evidence that ZEB1 expression is increased in response to AT1s and correlates with disease progression, metastasis and therapy resistance. We also show ZEB1 is a transcriptional regulator of AR signalling in PCa, Together this provides the rationale to target ZEB1 for the development of novel therapies for the treatment of CRPC.

**#4910 Eribulin rapidly impairs TGF-β signalling.**roma Kaul, April L. Risinger, Susan L. Mooberry. University of Texas Health Science Center at San Antonio, San Antonio, TX.

Microtubule targeting agents (MTAs) continue to be valuable in treating breast cancer. While decades of research have shown that these drugs cause mitotic arrest in cells by suppressing the dynamic instability of microtubules, recent evidence demonstrates that the ability of MTAs to disrupt the microtubule-dependent transport of key signaling components in interphase cells likely contributes to their anticancer actions. Cell signalling messages are relayed by proteins that organize or scaffold signaling hubs. These signalling nodes facilitate the complex organization and coordination of multiple signaling partners. NEDD9 is a member of the CAS scaffold family and it has been shown to coordinate TGF-β signalling, a driver of oncogenesis and epithelial-to-mesenchymal transition (EMT). Ligand-mediated stimulation of TGF-β receptors leads to the activation of downstream canonical and non-canonical signalling pathways. These pathways collectively induce the expression of Snail and Slug, key transcriptional-repressors that promote EMT. We tested the hypothesis that a short-term treatment of breast cancer cells with eribulin or other clinically relevant MTAs would differentially disrupt interphase microtubules and alter TGF-β-dependent signalling. BT-549 cells were treated for 2 h with concentrations of MTAs that are clinically relevant and cause maximum disruption of the interphase microtubule network; 100 nM was used for the destabilizers, eribulin and vinorelbine and 1 μM was used for the stabilizers, paclitaxel and ixabepilone. The results showed that eribulin and vinorelbine significantly inhibited TGF-β-induced expression of Snail and Slug. The microtubule stabilizers had no effect on Snail and Slug expression following TGF-β stimulation. Further studies evaluated whether NEDD9 contributes to the downregulation of Snail and Slug. Co-immunoprecipitation and knockdown studies suggest that eribulin impairs the ability of NEDD9 to scaffold TGF-β signalling partners, thus inhibiting downstream transcriptional signalling. Consistent with the ability of eribulin to reverse EMT in experimental models within 7 days, this study begins to shed light on the mechanisms underlying its action. Funding for this work was provided by Eisai Inc.

**#4911 Allosteric inhibition of the Receptor Tyrosine Kinases c-MET, RON and VEGFR-2 via the co-receptor CD44v6 by the novel compound AMC303.**Vanessa Al-Rawi, Thorsten Lauerer, Katrin Glocker, Yvonne Heneka, Alexandra Matzke-Ogi. anncare GmbH, Eggenstein-Leopoldsheim, Germany.

Introduction: The receptor tyrosine kinases (RTKs) c-MET and RON and more importantly their crosstalk, play a crucial role in mediating local invasion, systemic dissemination and resistance in different types of cancer. Both RTKs are activated by the ligands HGF and MSP respectively and homo- or heterodimerize with each other. The RTK VEGFR-2 is the most important receptor for angiogenesis in solid epithelial tumors. CD44v6, a member of the CD44 receptor family of transmembrane glycoproteins, was identified as an essential co-receptor for activation of c-MET, RON and VEGFR-2. AMC303, a peptide based allosteric and selective inhibitor of CD44v6, was investigated for its inhibitory effects on c-MET, RON and VEGFR-2 pathways in vitro and its effect on tumor growth and metastases in vivo. Methods: Affinity was determined using Microscale Thermophoresis. For in vitro blocking assays AMC303 was added 30 min prior to induction with the ligands. Analysis of RTK activation was carried out by western blot. Analysis of cell migration and invasion was performed with Boyden Chamber assays. VEGF-A induced tube formation was microscopically analyzed. For in vivo studies nude mice were orthotopically implanted with human tumor cells (L3.6pl). Animals were treated with AMC303 i.v. at 0.1, 1, 10 mg/kg QOD or QWX for 3 weeks. Regression of metastases was investigated at 1 mg/kg QOD. HPLC was used to detect the amount of AMC303 in the primary tumor lysates. Results: AMC303 binds to the ectodomain of CD44v6 with high affinity. Various pancreatic, breast, colon, lung and HNSCC tumor cell lines activation of c-MET and RON by their ligands was inhibited by AMC303 in vitro and consequently ligand induced cell scattering, migration and invasion was significantly reduced. In endothelial cells, activation of VEGFR-2 and VEGF-A induced formation of a tubular network was blocked by AMC303 treatment. In vivo treatment with AMC303 inhibited tumor growth in a dose dependent manner. The metastatic spreading of the primary tumor was prevented when animals were treated at early disease stage. Most strikingly, a marked regression of established liver metastases was observed at progressed disease state when animals were treated with AMC303 at 1 mg/kg QOD for 3 weeks. Conclusions: AMC303 inhibits activation of the RTKs c-MET and RON allosterically in different epithelial tumor cells and VEGFR-2 in endothelial cells by extracellular binding to CD44v6. This unique and novel mode of action results in a strong anti-tumor and anti-metastatic effect in vivo which together with its wide safety and tolerability window in preclinical toxicology is strongly supporting the clinical investigation. AMC303 is currently tested in a Phase I study in patients with solid epithelial tumors.

**#4912 Effect of the combined treatment with mifepristone and chemotherapy on breast cancer brain metastases.**Ayelen Rubin,1 Veronica de la Fuente,2 Edgardo Salvatierra,2 Silvia Vanzulli,2 Oswaldo Podhajcer,3 Claudia Lanari,3 Paola Rojas,1 IBYME-CONICET, Buenos Aires, Argentina;2 Facultad de Ciencias Exactas y Naturales (UBA), Buenos Aires, Argentina;3 Instituto Leloir, Buenos Aires, Argentina;4 Academia Nacional de Medicina, Buenos Aires, Argentina.

The treatment of brain metastasis is limited due to the drug’s inability to cross the blood brain barrier, because of the presence of endothelial cells’ multidrug resistance efflux transporters, such as the P-glycoprotein (P-gp). The antiangiogenic Mifepristone (MPP) is known to inhibit the P-gp activity. We hypothesize that a combination of MPP and Pegylated doxorubicin liposomes (doxo) improves the drug efficacy on breast tumors that do not express progestrone receptors. Using the metastatic triple negative MDA-231-BRM2 breast cancer cell line, transfected with GFP and luciferase, we explored different protocols to generate tumors growing in the brain of NSG mice. Spontaneous metastases were obtained by subcutaneous injection (sc) of 2 x 10^6 cells, and experimental metastases by intracardiac injection (ic) of 1 x 10^6 cells or by intracranial injection of 2 x 10^6 cells. In the ic and sc models small and scattered brain metastases as well as liver, lung and kidney metastases were observed. The intracranial model enabled us to obtain measurable brain tumors in a short time. Thus we selected this method to evaluate the effect of MPP (6 mg pellets, sc) and doxo (4.5 mg/kg, iv) on tumors growing in the brain. Treatment was initiated 10 days after cell inoculation. The amount of tumor cells after brain excision was measured by flow cytometry (GFP) and by lumimetry (luciferase activity). A decreased luminescence signal was observed in brains from mice treated with both agents as compared with the control mice (p<0.05), whereas no effects were observed between the single treatment groups and the controls. Moreover, the flow cytometry analysis showed that the combined treatment, MPP+doxo, reduced the number of tumor cells further than the single chemotherapeutic agent (p<0.05). Our data suggest that MPP may be a promising agent to treat brain metastasis of breast cancers, which do not necessarily express progestrone receptors, to increase the effectiveness of chemotherapeutic agents. The participation of the P-gp mediating this effect needs to be confirmed.

**#4913 Invasive melanoma cells commandeer p53 activity to promote the survival of a therapy resistant subpopulation.**Marie R. Webster,1 Amanpreet Kaur,1 Abbibatou Ndoye,2 Curtis Kugel,1 Subhasee Basu,1 Alexander Valiga,1 Fuente,2 Edgardo Salvatierra,2 Silvia Vanzulli,2 Oswaldo Podhajcer,3 Claudia Lanari,3 Paola Rojas,1 IBYME-CONICET, Buenos Aires, Argentina;2 Facultad de Ciencias Exactas y Naturales (UBA), Buenos Aires, Argentina;3 Instituto Leloir, Buenos Aires, Argentina;4 Academia Nacional de Medicina, Buenos Aires, Argentina.

Metastatic melanoma is highly aggressively and often therapy resistant. Signals which drive acquisition of metastatic properties, such as constitutive activation of p53, are key promoters of metastasis and therapy resistance. Therapy resistant melanomas are characterized by high levels of the pro-invasive non-canonical Wnt molecule, Wnt5A. Previously, we described an adaptive stress response in highly invasive melanoma cells, which is characterized by a growth arrest and an increase in senescence markers, yet these cells retain the ability to invade and form colonies. These highly invasive cells do not undergo apoptosis following treatment with DNA damaging agents such as doxorubicin and are resistant to BRAFT350E2 targeted therapy. Recently, Lukić et al. have shown that p53 expression promotes survival of colorectal cancer cells via p21 expression and a reversible cell cycle arrest, allowing for repair of damaged DNA. Here, we show that highly invasive and therapy resistant melanoma

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cells express Wnt5A, p53 and p21, which regulate proliferation and slow cycling in invasive melanoma cells. The expression of p53 is promoted by Wnt5A, knock down of Wnt5A decreases p53 and p21 expression in these cells and decreases the number of cells arrested in G2/M following DNA damage. The cell cycle and apoptotic functions of p53 are highly regulated. iASPP, MDMX, and MDM2 have been shown to regulate the function of wild type p53 in melanoma cells. MDM2, an E4 ubiquitin ligase, regulates p53 by shuttling it out of the nucleus and targeting it for proteosomal degradation. We found that MDM2 expression increases in invasive melanoma cells following DNA damage, however, in these cells it is phosphorylated at serine 395. Phosphorylation of MDM2 at ser395 blocks its ability to export p53 from the nucleus, leading to increased p53 expression. We also found that p53 expressing cancer cells targeting p53 were capable of cell cycle arrest and survival following stress instead of apoptosis. The apoptotic function of p53 has been shown to be inhibited by nuclear iASPP, which is enriched in metastatic melanoma. We found that knocking down Wnt5A in invasive melanoma cells decreases the expression of iASPP. These data suggest that Wnt5A promotes MDM2 Ser395 phosphorylation and iASPP expression, blocking down regulation of p53 and its apoptotic function, while promoting cell cycle arrest and survival following stress. These data may reveal a mechanism by which highly invasive melanoma cells evade therapy to form therapy resistant sub-clones at distant sites.

**#4914** Luteolin inhibits metastasis of triple-negative breast cancer cells to the lungs. Matthew T. Cook,1,2 Yaoxin Liang,1 Cynthia Besch-Williford,3 Salmon Hyder1.3

1Washburn University, Topeka, KS; 2University of Missouri, Columbia, MO; 3IDEXX BioResearch, Columbia, MO.

Triple-negative breast cancers (TNBC) are basal-like tumors which lack the traditional pharmacological targets ER, PR and HER2. Most TNBC related deaths occur following metastasis of cancer cells, and development of tumors at secondary sites such as the lungs and bones. Since TNBC lacks ER, PR and HER2, non-specific chemotherapeutic agents are administered to these women, who generally present with early metastatic lesions that originate from drug-resistant residual cells, and have poor prognosis. Consequently, novel therapeutic strategies are sought. Luteolin (LU) is a naturally occurring, non-toxic plant compound that has proven effective against several types of cancer, though its effects against cancers of the breast remain unknown. With this in mind we conducted studies, both in vivo and in vitro, to determine whether LU might suppress metastasis of TNBC. In vivo studies were performed using MDA-MB-231 (4175) LM2 cells, a subpopulation of clinically relevant cells (kindly provided by Dr. J. Massagué; Minn et al. 2005: 436:518-24). MDA-MB-231 (4175) LM2 is a particularly aggressive TNBC breast cancer cell line with a molecular signature preferential for lung metastasis. Herein we report that LU effectively suppressed metastasis of MDA-MB-231 (4175) LM2 cells to lung. Compared with animals given MDA-MB-231 (4175) LM2 cells alone, which developed 67.6 ± 27.1 metastatic lung colonies, treatment with 40 mg/kg LU reduced colony formation to 22.8 ± 3.6 (P < 0.035). Confirmation of the anti-metastatic effect of LU was achieved by inoculating animals with an alternative, less aggressive TNBC cell line, MDA-MB-435. Administration of 20 mg/kg LU to animals treated with MDA-MB-435 cells reduced the number of lung colonies from 14.1 ± 1.6 to 5.3 ± 0.5; P < 0.005. When tumor cells were incubated in vitro with different concentrations of LU (50-500 μM), we observed a significant dose-dependent reduction in cell viability, induction of apoptosis (P < 0.001), and decreased tumor cell migration. These data suggest that LU suppresses several steps of the metastatic process. Furthermore, relatively low levels (10 μM) of LU significantly inhibited VEGF secretion from tumor cells (P < 0.001), suggesting that the flavonoid has the ability to suppress a potent angiogenic and cell survival factor. In addition, when tumor cells were exposed to either LU or VEGF receptor (KDR) antibody, a similar reduction in TNBC migration potential was observed. This suggests that the anti-tumor actions of LU may, in part, be due to its ability to reduce VEGF levels and block KDR receptor-mediated activity, thereby inhibiting tumor cell migration. These studies demonstrate that the use of LU, a non-toxic, plant derived compound, deserves further investigation as a treatment option for women with TNBC Supported by a COR award from the College of Veterinary Medicine, and in part by funds from generous donors to the Ellis Fischel Cancer Center, University of Missouri.

**#4915** Alcohol-induced reprogramming of tumor plasticity is mediated via suppression of CCN5 signaling in breast cancer cells. Inamul Haque,1 Arvind Subramanian,1 Vijayalakshmi Gupta,1 Sandipito Sarkar,1 Snigdha Banerjee,1 Sushanta K. Banerjee2.1 1University of Kansas Medical Center/Kansas VA Medical Center, Kansas City, MO; 2Kansas City VA Medical Center, Kansas City, MO.

Background and Objective: The epidemiological and experimental studies suggest that alcohol consumption is associated with increased risk for breast cancer development and metastasis. However, the mechanisms of alcohol-induced breast cancer progression and metastasis remain unknown. In this study, we examined the roles of CCN5, a tumor suppressor gene in alcohol induced breast cancer cell migration/invasion, epithelial to mesenchymal transition (EMT) and stemness. Methods: Estrogen receptor-positive (ER+) human breast cancer cell line MCF-7 and ER negative human breast cancer cell line MDA-MB-231 were treated with ethanol at various concentrations in the presence and absence of human recombinant human CCN5 protein (hrCCN5). Invasive/migratory ability of treated and untreated cells was measured by Boyden chamber assays. The mRNA and protein expression level of CCN5 was determined by real-time qRT-PCR and Western blot. EMT and stemness markers were evaluated by Western blot. Single-cell suspensions from pre-treated cells were suspended at a density of 500 cells/ml in mammosphere media in ultralow attachment dishes. Number as well as the size of the mammosphere in specified experimental set-up was monitored and recorded alternate day for 8-10 days. Results: The studies demonstrated that alcohol promotes significantly the invasive/migratory ability and EMT phenotypes of breast cancer cells through the suppression of CCN5. Interestingly, hrCCN5 protein treatment suppresses the effect of alcohol and reprograms MET (mesenchymal to epithelial transition). In addition, alcohol-induced mammosphere formation efficiency is also suppressed by hrCCN5 protein-treatment. Conclusions: Our findings suggest that the CCN5 signaling plays a preventive role in alcohol-induced reprogramming of tumor plasticity in breast cancer cells.

**#4916** E3 ubiquitin ligase Cbl-b prevents tumor metastasis by maintaining the epithelial phenotype in multiple drug-resistant gastric and breast cancer cells. Ling Xu, Xiujuan Qu, Ye Zhang, Xiaofang Che, Tianshu Guo, Ying Cai, Aodi Li, Danni Li, Ge Li, Ti Wen, Yibao Fan, Kezuo Hou, Yanju Ma, Xuejun Hu, Yupeng Liu. China Medical University, Shenyang, China.

Background: Multiple drug resistance (MDR) and metastasis are two major unfavorable factors causing failure of cancer treatment. However, the relationship between MDR and metastasis is not characterized. Additionally, the role of the E3 ubiquitin ligase Cbl-b in metastasis of gastric and breast cancer is little known. Methods: The effect of Cbl-b on tumor invasion and metastasis was analyzed in P-gp-positive gastric adenocarcinoma tissues. Transfections of Cbl-b WT and epidermal growth factor receptor (EGFR) WT plasmid were used to assess Cbl-b and EGFR involvement in epithelial-mesenchymal transition (EMT) and tumor metastasis. Results: MDR gastric and breast cancer cells possess a typical mesenchymal phenotype and an enhanced cell migration capacity. Additionally, Cbl-b is poorly expressed in MDR gastric and breast cancer cells. We performed immunohistochemical analysis on P-gp-positive gastric adenocarcinoma tissues and identified associations between Cbl-b expression and clinical pathological parameters. Gastric cancer patients with low Cbl-b expression were more likely to have tumor invasion (P = 0.016) and lymph node metastasis (P = 0.007). Moreover, over-expression of Cbl-b reduced cell migration in MDR cell cultures both in vitro and in vivo. Cbl-b over-expression also prevented EMT by inducing ubiquitination and degradation of EGFR, leading to inhibition of the EGFR-Akt/miR-200c-ZEB1 axis. However, further over-expression of EGFR on a background of Cbl-b over-expression restored both the mesenchymal phenotype and the cell migration capacity of MDR gastric and breast cancer cells. These results suggest that Cbl-b is an important factor for maintenance of the epithelial phenotype and inhibition of cell migration in MDR gastric and breast cancer cells.

**#4917** Therapeutic inhibition of Notch1 in metastatic prostate cancer. Meghan A. Rice, En-Chih Hsu, Tanya Stoyanova. Stanford University, Palo Alto, CA.

Metastatic castration-resistant prostate cancer (CRPC) is the primary cause of prostate cancer specific mortality, and is currently incurable. Defining new mechanisms that can predict progression and drive lethal CRPC is critical. Notch1 receptor, a cell surface-signaling molecule dysregulated in many cancers, is highly expressed in CRPC. Chronic activation of Notch1 synergizes with multiple oncogenic pathways altered in early disease to promote the development of poorly differentiated metastatic prostate carcinoma. Consistent with its activation in clinical CRPC, tumors driven by NICD1 in combination with alternative pathways altered in prostate cancer are metastatic and resistant to androgen deprivation. Genetic and pharmacological inhibition of Notch1 activity decreases cell proliferation and colony formation of 22Rv1 prostate cancer cells in vitro. 22Rv1 subcutaneous xenograft growth was significantly impaired by treatment with pharmacological inhibitors of Notch1 activation over the course of 20-day treatment. Further, Notch1 inhibition in combination with other standard prostate cancer treatments compounded efficacy of individual treatments to attenuate colony formation potential of prostate cancer cells in vitro. The Notch1 signaling axis, confirmed to synergize with multiple pathways to pro-
mote metastatic CRPC already provides a novel pathway for diagnostic stratification of prostate cancer patients. Our studies additionally implicate Notch1 as a therapeutically relevant target for aggressive prostate cancer.

**TUMOR BIOLOGY: Therapeutic Intervention of Cancer and Metastasis**

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**#4918** Livin is a pro-apoptotic gene of mucinous adenocarcinoma. Mohammed Faruk,1 Abdulmumin Hassan Rafindadi,1 Sani Ibrahim,1 Surajo Mohammed Aminu,1 Surajo Mohammed Aminu,1 Ahmed Adamu,1 Ahmed Adama,1 Yawale Biyaya,1 Adamu Abdullahi,1 Mohammed Sanm Shesu,1 Abdullahi Mohammed,1 John Idoko,1 Abdullahi Jibril Randawa,1 Abdullahi Jibril Randawa,1 Atara Ntekim,2 Saad Aliyu Ahmed,1 Aishatu Suleiman Maude,1 Almustapha Aliyu Liman,1 Abubakar Sani,1 Khalid Zahir Shah,1 Yahaya Ukwenya,1 Yahaya Ukwenya,1 Cheh Augustine Awasum,1 Kasimu Umar Adoke,1 James Olowu Enemari,1 James Olowu Enemari,1 Andrew Jonathan Nok1.

Livin, or mucin 1 (MUC 1), is a transmembrane cell surface protein that is expressed in the majority of human breast tumors. Several studies have suggested that MUC1 is involved in the development of specific breast tumors, and that its expression is associated with poor prognosis. In this study, we investigated the expression pattern of Livin in a large cohort of breast cancer patients.

**TUMOR BIOLOGY: Tumor Microenvironment**

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**#4919** Ablation of neuropilin 1 from glioma-associated microglia and macrophages slows tumor progression. Jeremy T. Miyauchi,1 Danling Chen,1 Matthew K. Choi,1 Kenneth Shroyer,1 David Selwood,2 Stella E. Tsirka2,1 Stony Brook University, Stony Brook, NY; 2University College London, London, United Kingdom.

Glia and macrophages play significant roles in glioma progression and metastasis. Neuropilin-1 (NRP1) is a transmembrane receptor expressed on many types of cells, including glioma-associated macrophages (GAMs). NRP1 has been implicated in tumor progression and angiogenesis.

**#4920** Inflammation and estrogen receptor beta modulate bacterial diversity of the gut. Ahmed F. Ibrahim,1 Quentin Thomas,2 Ashish Saxena,3 Cecilia Williams1. 1The Royal Institute of Technology, KTH, Solna, Sweden; 2KTH, Solna, Sweden; 3University of Houston, Houston, TX.

Gut microbiota is an important contributor to health and disease. The composition of the gut microbiota is influenced by various factors, including diet, age, and sex. In this study, we investigated the effect of inflammation and estrogen receptor beta (ERβ) on gut microbiota composition in mice.

**#4921** The adhesion protein JAM-A is important for maintenance of tumor dormancy. Sri Harikrishna Vellanki, Rodrigo G. Cruz, Ann M. Hopkins, RCS, Dublin, Ireland.

Tumor dormancy is a critical stage in cancer progression, where cancer cells can persist for extended periods without growing or metastasizing. The adhesion protein JAM-A has been implicated in tumor dormancy, but its exact role is not fully understood.

**#4922** The adhesion protein JAM-A is important for maintenance of tumor dormancy. Sri Harikrishna Vellanki, Rodrigo G. Cruz, Ann M. Hopkins, RCS, Dublin, Ireland.

Tumor dormancy is a critical stage in cancer progression, where cancer cells can persist for extended periods without growing or metastasizing. The adhesion protein JAM-A has been implicated in tumor dormancy, but its exact role is not fully understood.
cations of TPA. Furthermore, the upregulation of the chemokines Cxcl1 and S100a8 in response to TPA was deficient in MyD88 null cultured keratinocytes revealing a necessary role for MyD88 in the intrinsic response of keratinocytes to phorbol ester. We also uncovered a systemic requirement for MyD88 in carcinogenesis since lineage specific ablation of MyD88 in T cells conferred significant resistance to lethal chemotherapy. Exposure of alveolar macrophages to TPA induced the expression of IL-17 by the T cells in TPA treated MyD88 null mouse skin. IL-17 treatment of normal primary keratinocytes increased their proliferation while reducing their response to calcium-induced differentiation but had no effect on the proliferation of RAS-keratinocytes. The atypical nuclear IkBzeta protein can act as an activator of so-called secondary NF-kB response genes. IkBzeta deficiency blocked the keratinocyte proliferative response to IL-17 but had no effect on altered differentiation. Finally, both IL-17 and oncogenic RAS required IkBzeta to upregulate the expression of S100a8, lipocalin2 and Steap4 mRNAs. Collectively, these results demonstrate that MyD88 plays a pro-tumorigenic function during tumor promotion in both epithelial and T-cell compartments and that IL-17/IkBzeta could play a facilitating role on keratinocytes during tumor promotion beyond its pro-inflammatory activity.

#4923 MyD88 and IL-17 have essential local and systemic functions in promoting tumors on mouse skin. Mary Klotzerman, Christophe Cataisson, Rosalba Salcedo, Kelly Shibuya, Jennifer Waters, Giorgio Trinchieri, Stuart H. Yuupa. National Cancer Institute, Bethesda, MD.

NF-kB has been causally implicated with cancer-associated inflammation. In the skin, ligands of the Toll-like receptor/IL-1 receptor superfamily induce the recruitment of MyD88 (Myeloid differentiation primary response gene 88), ultimately leading to NF-kB activation. We previously reported that MyD88 is required for two stage chemical carcinogenesis on mouse skin, and that MyD88 exerts a cell-intrinsic function in RAS-mediated transformation of keratinocytes (Ras-keratinocytes). Now, we explore the role of MyD88 during tumor promotion with 12-O-tetradecanoyl-phorbol-13-acetate (TPA). Epidermal hyperplasia is reduced in MyD88 deficient mice relative to controls after multiple applications of TPA. Furthermore, the upregulation of the chemokines Cxcl1 and S100a8 in response to TPA was deficient in MyD88 null cultured keratinocytes revealing a necessary role for MyD88 in the intrinsic response of keratinocytes to phorbol ester. We also uncovered a systemic requirement for MyD88 in carcinogenesis since lineage specific ablation of MyD88 in T cells conferred significant resistance to lethal chemotherapy. Exposure of alveolar macrophages to TPA induced the expression of IL-17 by the T cells in TPA treated MyD88 null mouse skin. IL-17 treatment of normal primary keratinocytes increased their proliferation while reducing their response to calcium-induced differentiation but had no effect on the proliferation of RAS-keratinocytes. The atypical nuclear IκBzeta protein can act as an activator of so-called secondary NF-kB response genes. IκBzeta deficiency blocked the keratinocyte proliferative response to IL-17 but had no effect on altered differentiation. Finally, both IL-17 and oncogenic RAS required IκBzeta to upregulate the expression of S100a8, lipocalin2 and Steap4 mRNAs. Collectively, these results demonstrate that MyD88 plays a pro-tumorigenic function during tumor promotion in both epithelial and T-cell compartments and that IL-17/IκBzeta could play a facilitating role on keratinocytes during tumor promotion beyond its pro-inflammatory activity.

#4924 Adh1bΔ/Δ×Cyp26b1Δ/Δ activity of CD90+ (myo)fibroblasts supports tumor-promoting inflammation in colorectal cancer. Romain Villerè,1 Gabriella Urbe,1 Judy A. Trieu,1 Paul Johnson,1 Suimin Qiu,1 Don W. Powell,1 Ellen J. Beswick,2 Jinyu V. Pinchuk,3 University of Texas Medical Branch, Galveston, TX; University of New Mexico, Albuquerque, NM.

Introduction. Retinol (RO) and its active metabolite, all-trans retinoic acid (atRA), have been widely studied as cancer chemotherapeutic agents. atRA is a critical immunoregulatory molecule reported to decrease cancer cell proliferation and inhibit tumor-promoting inflammation. However, recent studies report a lack of efficacy of atRA treatment to counteract colorectal cancer (CRC) progress, and the mechanisms underlying this failure are not fully understood. Alcohol dehydrogenase 1B (ADH1B), a major isofrom of alcohol dehydrogenase expressed in normal colon, is responsible for the conversion of RO to atRA.

We recently reported that stromal (myo)fibroblasts are major ADH1B expressers in normal colonic mucosa and that this expression is lost during the neoplastic transformation process of CRC. Thus, we hypothesized that aberrant retinoid signaling in cancer-associated fibroblasts (CAFs) is among key processes supporting tumor-promoting inflammation in CRC. Methods. Real-time PCR, confocal microscopy and cytokine luminex arrays were used to evaluate alterations in retinoid pathway gene expression and activity to define its relevance to the tumor-associated inflammation in human normal and CRC colonic tissues, and in normal primary (myo)fibroblasts (N-CMFs) and CAFs. Results. We observed a strong downregulation of ADH1B expression in CAFs in situ and in culture along with an increased expression of the tumor growth promoting inflammatory cytokine IL-6. Addition of the ADH1B substrate, RO, or its metabolite, atRA, to N-CMF cultures decreased the LPS-inducible IL-6 expression by N-CMFs. No downregulation of LPS-inducible IL-6 expression by RO was observed in CAFs, while atRA treatment partially inhibits IL-6 expression. Silencing of adh1b gene in N-CMFs led to the increased production of the LPS-inducible IL-6. This suggests that lack of ADH1B expression in CAFs prevents cancer-promoting inflammation in the induction of IL-6.

A decrease in downstream enzyme ALDH1A1 expression, which converts ADH1B’s byproduct retinaldehyde, to atRA, was also observed in CAFs. Finally, only a partial inhibition of IL-6 production by exogenous atRA was observed in CAFs. When compared to N-CMFs, CAFs expressed higher levels of CYP26B1, a cytochrome that is responsible for atRA degradation. Treatment of CAFs with atRA resulted in an eight fold higher upregulation of CYP26B1 expression than in N-CMFs. Conclusions. Taken together, our data suggests that CYP26B1 overexpression in CAFs along with ADH1B downregulation support aberrant retinoid acid metabolism in CRC. Adh1bΔ/Δ×Cyp26b1Δ/Δ activity of CAFs leads to a decrease of atRA availability in the tumor microenvironment, supporting IL-6-driven tumor growth promoting inflammation and resistance to exogenous atRA as a chemotherapeutic agent in CRC.

#4925 Microbiome-TP53 gene interaction in human lung cancer. Leigh Greathouse,1 James White,2 Valery Bliskovsky,3 Ashley Vargas,4 Eric Polley,4 Elise Bowman,4 Mohammed Khan,4 Ana Robles,4 Brid Ryan,4 Amiran Dzutsev,3 Giorgio Trinchieri,3 Marbin Pineda,3 Paul Meltzer,3 Marina Walther-Antonio,4 Garth Ehrlich,4 Joshua Mell, 5 Joshua Earl, 5 Sergey Balashov,5 Archana Bhat,5 Alex Valm,6 Clayton Deming,6 Sean Conlan,6 Julia Oh,7 Julie Segre,7 Curtis Harris3. Baylor Univ., Waco, TX; 2Rashera Biosciences, Inc., MD; 3National Cancer Institute, Bethesda, MD; 4Mayo Clinic, Rochester, MN; 5Drexel University, Philadelphia, PA; 6National Human Genome Research Institute, Bethesda, MD; 7Jackson Laboratory, Framingham, CT.
Lung cancer is the leading cancer diagnosis worldwide and the number one cause of cancer deaths. Exposure to cigarette smoke, the primary risk factor in lung cancer, reduces epithelial barrier function and increases susceptibility to infections. Herein, we hypothesized that somatic mutations together with cigarette smoke create a dysbiotic microbiota that is associated with lung carcinogenesis. To assess the association of the abnormal microbiome with lung cancer, we conducted 16S rRNA gene sequencing (MiSeq), with RNA-seq data from lung cancer tissues in The Cancer Genome Atlas (n = 1112) serving as the validation cohort. We demonstrate a lower alpha diversity in normal lung as compared to non-tumor adjacent or tumor tissue, indicating a shift in the overall microbial community in lung cancer patients as compared to those without cancer. Lung cancer cases were classified by the relative abundance of two taxa, Variovorax and Streptococcus, with an increase in Variovorax abundance in tumors as compared to non-tumor adjacent paired lung tissue (FDR corrected P = 0.013). The species of Variovorax was identified histologically, and also by two additional 16S rRNA strategies (Resphera Insight analysis and PacBio sequencing). A group of taxa were associated with squamous cell carcinoma (SCC), of which Acidovorax were enriched in smokers (P = 0.013). Further, these taxa, including Acidovorax, exhibited higher abundance among the subset of SCC cases with TP53 mutations, an association not seen in adenocarcinomas (AD). Therefore, we observed a microbiome-gene and a microbiome-exposure interaction in SCC lung cancer tissue. Together, these results open the door for future biomarker research that could be used to improve screening and direct mechanistic studies of lung cancer therapy.

#4926 Gut microbiota regulates cisplatin mediated cachexia and systemic toxicity. Soumen Roy,1 Rodrigo Das Neves,1 Amiran Dzutse,1 Carolynne Smith,1 Bathai Edwards,1 Miranda Dawson,1 Simone Dilippanthom,2 Loretta Smith,2 April Huang,1 Young Kim,1 Giorgio Trinchieri,1,11,15 NCI, CIP, Bethesda, MD;1,16 LSP, NCI, Bethesda, MD;1 Division of Cancer Prevention, NCI, Bethesda, MD.

Chemotherapy induced toxicity severely affects the cancer survivors and lowers the quality of life. By 2020, there will be more than 18 million of cancer survivors all over the world. Majority of them might develop long term nephrotoxicity, ototoxicity and gut toxicity. In addition, chemotherapy may facilitate the initiation and progression of cachexia. Recent studies have shown that gut microbiota modulates the efficacy of anti-cancer chemotherapy, however very limited knowledge is available regarding the role of gut microbiota in regulating systemic toxicity and cachexia. We hypothesized that gut microbiota modulates cisplatin induced systemic toxicity as well as cachexia. Four groups (n = 10 in each group) of 6-8 weeks old C57Bl/6 mice were treated with cisplatin, cisplatin + antibiotics cocktails (ABX), ABX only and control. ABX cocktail contained primaxim, vancomycin and neomycin. This experiment was validated using C57Bl/6 germ free mice. We performed anti-p-γ-H2AX based toxicity assay for DNA damage. In addition, we performed immunohistochemistry for Studying cachexia. H&E staining and DNA damage were assessed by light and super resolution confocal microscopy (Zeiss 880) and quantified by 3-D reconstruction using IMARIS. We also performed high content imaging to evaluate DNA damage in bone marrow and spleen. Body weight and kidney blood content were also analyzed to determine the degree of toxicity. Depletion of gut microbiota abrogated cisplatin mediated nephrotoxicity and gut toxicity. Importantly, we found that depletion of gut microbiota prevents muscle and adipose tissue loss by down-regulating UCP-1/and PGC-1α in adipose tissue and MURF-1 and Atrogin-1 in muscle. Experiments are confirmed by using Germ free mice. Our data suggest that modulation of gut microbiota may be utilized to reduce chemotherapy associated cachexia and systemic toxicity.

#4927 Presence of genotoxic and/or pro-inflammatory bacterial genes in stool is associated with colorectal neoplasia. Maria Gonzalez-Pons,1 Michelle Cruz-Badia,2 Ramon Gomez-Moreno,2 Abel Baerga-Ortiz,3 Marcia Cruz-Correia,4 University of Puerto Rico Comprehensive Cancer Center, San Juan, PR;2 University of Puerto Rico Medical Sciences Campus, San Juan, PR.

Despite increasing adherence to routine screening and advances in therapeutic strategies, CRC is the 3rd and 4th leading cause of cancer-related death in Puerto Rico and the United States (US), respectively. Currently, CRC screening is the primary means for prevention; however, 60% of CRC patients are diagnosed at more advanced, less treatable stages, which emphasize the need for novel CRC prevention and risk stratification strategies. The etiology of CRC is complex and still incompletely understood. However, environmental factors including diet, the gut microbiota, and inflammation are accepted as major contributors to colorectal carcinogenesis. It has been shown that individuals with CRC have a distinct gut microbiota, but the mechanisms by which gut bacteria exert their CRC-promoting effects remains elusive. Certain pathogenic bacterial strains carry genes encoding toxins that promote DNA damage and perpetuate inflammation, yet the association of these toxins to CRC remains poorly understood. The aim of this study was to gain insight into the association and possible mechanisms by which a subset of the gut microbiota contribute to colorectal carcinogenesis by profiling six genes encoding genotoxic and/or pro-inflammatory bacterial toxins in stool from individuals with and without colorectal neoplasia (adenoma and CRC). The association between the genes encoding toxins and CRC was examined by characterizing the toxic colonic bacterial gene profile in stool samples from healthy individuals (controls) and individuals with colorectal neoplasia (cases). Stool samples from individuals in the mainland US (n = 20) and Caribbean Hispanics (n = 33) living in Puerto Rico were provided by the NCI Early Detection Research Network and the Puerto Rico Familial Colorectal Cancer Registry, respectively. Detection pks, TpC, GeElf, cnf-1, murB, and usp in stool were performed with specific primers. Associations were assessed using odds ratios. Four of the six toxic genes were detected more frequently in stool samples from individuals in the US with colorectal neoplasia (controls = 10; adenoma = 10); adenoma = 10). Results show borderline statistical significant associations (p = 0.07) with the presence of usp and 2 genes and a higher odds of colorectal neoplasia (OR = 5.44 and OR = 9.33, respectively). In the cohort from our Caribbean Hispanic subjects (controls = 13; adenomas = 12; CRC = 8), individuals with the presence of GeEl were 8.6-times more likely to have adenomas (p = 0.07) and individuals with ≥ 2 of the genes were 11.3-times more likely to have CRC than individuals without these genes (p = 0.04). Analysis with a larger number of samples will be necessary to determine a more definite association between the presence of the toxic genes in this panel in stool and colorectal neoplasia. Additional mechanistic analysis will be required to fully understand how these bacterial toxins contribute to colorectal carcinogenesis.

#4928 Impact of E. hirae on the tumoricidal activity of cyclophosphamide. Romain Daillère, Marie Vetzizou, Laurence Zitvogel, Gustave Roussy, Villejuif, France.

The tumor microenvironment is influenced by anticancer therapies, and even more so by those affecting the gut homeostasis. We previously reported that a deviated repertoire of the intestinal microbiome called « dysbiosis », caused by broad spectrum antibiotics compromised the efficacy of cyclophosphamide (CTX), an immunomodulatory alkylating agent exerting cytotoxic effects against cancer, highlighting the importance of the gut microbiota in this mechanism (Viaud, Science, 2013). CTX is responsible for disrupting the gut barrier integrity as well as intestinal homeostasis, allowing a NOD2-dependent translocation of several Gram-positive bacteria into secondary lymphoid organs. CTX breaks the intestinal tolerance towards the intestinal microbiota and leads to immunization of the host against some bacterial strains. Here, we identified a Gram positive bacteria, Enterococcus hirae, which markedly modulates the intestinal and systemic immunity through the elicitation of bacterial-specific Th1 and pathogenic Th17 cells. Moreover, we have shown that E. hirae is capable of enhancing tumor-specific CD4+ and CD8+ T cell responses against candidate tumor antigens. In parallel, we have demonstrated that intestinal epithelial cells NOD2 receptors represent " gut immune checkpoints " restricting the immunogenicity of E. hirae. Finally, E. hirae specific-memory Th1 immune responses selectively predicted longer progression-freesurvival in advanced lung or ovarian cancer patients treated with immunotherapy and chemotherapy (platinum salts- or CTX-based chemotherapy) (Daillère, Immunity, 2016). Altogether, E. hirae represents a valuable probiotic against cancer, an oncomicrobiotic ameliorating the efficacy of the most common alkylating immunomodulatory compound.

#4929 Cooperation between the microbiota and oncogenic mutations leading to the development of colon cancer. Dana Van De Hey, Susan Payne, Cheri Pasch, Linda Clipson, Kristina Matkowskyj, Dustin Deming. University of Wisconsin Carbone Cancer Center, Madison, WI.

Background: The microbiome of the colon has been shown to play a role in colon cancer tumorigenesis. Inflammation is a key step in the formation of tumors, and it is possible that microbiota induce this inflammation, further stimulating tumor growth. The interaction between mutant cells in the colon and the microbiota has been understudied. We hypothesized that eradication of the intestinal microbiota could prevent tumorigenesis in the colon despite the presence of oncogenic, MMR-deficient tumor initiating cells (CTXM) in a transgenic murine model in which a constitutively active PI3K is expressed in the intestinal microbiota could prevent tumorigenesis in the colon despite the presence of oncogenic, MMR-deficient tumor initiating cells (CTXM) in a transgenic murine model in which a constitutively active PI3K is expressed in

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frequency and median lifespan of 101 days, compared to 100% frequency and 86.5-day lifespan of control mice (p < 0.001). In the tumors that formed there was no difference in the epithelial mesenchymal transition (EMT), K67 index, nuclear localization of CTNNB1, or activation of the PI3K/AKT/mTOR signaling pathway. FCA3K3 spheroid and mouse models portrayed ineffectiveness of vancomycin through the absence of spheroid diameter change and also 100% frequency of both treated and control mice. Conclusion: The above results indicate that colon cancers induced by PIK3CA mutations are at least partially dependent on the microbiota for tumorigenesis. This dependence appears to be overcome in the setting of both APC and PIK3CA mutations.

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**#4930 Genetic and epigenetic characteristics of esophageal cancer tissues with microbiome fusobacterium nucleatum**
Yoshifumi Baba, Kensaue Yama-mura, Shigeaki Nakagawa, Kosuke Mima, Takatsugu Ishimoto, Masaki Iwatsu, Yasuo Sakamoto, Naoya Yoshida, Hideo Baba. Kumamoto Univ., Kumamoto, Japan.

Background: Research into the microbiome is a rapidly advancing field in human cancers. The gut microbiome has recently been shown to play an important role in health, as well as in diseases such as obesity, inflammatory bowel disease, diabetes, non-alcoholic fatty liver disease, and several types of cancer. Fusobacterium nucleatum is a component of the human microbiome that primarily inhabits the oral cavity. It causes periodontal disease and has also been implicated in the development of human cancers. We have recently reported that Fusobacterium nucleatum in esophageal cancer tissues was associated with shorter survival, suggesting a potential role as a prognostic biomarker. In addition, our KEGG enrichment analysis using microarray data showed that Fusobacterium nucleatum might also contribute to aggressive tumor behavior through activation of chemokines such as CCL2. Nonetheless, molecular features of Fusobacterium nucleatum-positive esophageal cancers have been still unknown. Methods: Using 325 esophageal cancers, we detected Fusobacterium nucleatum DNA in 74 tumors (23%). We assessed other genetic and epigenetic events including mutations in KRAS, BRAF, PIK3CA gene, p53 expression, phosphorylated-AKT expression, LINE-1 methylation level (global DNA methylation level), IGF2 DMR0 methylation, MGMT methylation, and MLH1 methylation. Results: The LINE-1 methylation levels of Fusobacterium nucleatum-positive tumors was significantly lower compared with that of Fusobacterium nucleatum-negative tumors (P < 0.0001). In addition, Fusobacterium nucleatum was significantly associated with PIK3CA wild type (P = 0.011). There was no relationship of Fusobacterium nucleatum with KRAS, BRAF mutation, p53 expression, p-AKT expression, IGF2 DMR0 methylation, MGMT methylation, or MLH1 methylation. Conclusion: Fusobacterium nucleatum was related with LINE-1 hypomethylation and PIK3CA wild type. Our findings may shed light on understanding the mechanism by which Fusobacterium nucleatum affects aggressive tumor behavior.

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**#4931 Prospective study of oral microbiome and colorectal cancer risk in low-income and African American populations**
Yaochu Yang, Quinyin Cai, Xiao Ou Shu, William J Blot, Wei Zheng, Jirong Long. Vanderbilt University School of Medicine, USA, TN.

Colorectal cancer (CRC) is the third most common cancer in both men and women, and the third leading cause of cancer death in the USA. Recent research suggests that oral microbiome may play important roles in the pathogenesis of colorectal cancer and other chronic diseases. We conducted a nested case-control study within the Southern Community Cohort Study to investigate the association of oral microbiome with subsequent risk of developing CRC. This study included 231 incident CRC cases and 462 controls individually matched on age, race, smoking, alcohol drinking, season-of-study enrollment, and we have low statistical power to investigate them. Conclusion: Our study suggested that multiple bacteria taxa may be associated with risk of CRC, and raised the possibility that the oral microbiome may play an important role in CRC etiology.

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**#4932 Oxygen sensing by T cells establishes an immunologically favorable metastatic niche**
David Clever, Nicholas Restifo. The Ohio State University College of Medicine, Columbus, OH; National Institutes of Health, Washington, DC.

Cancer cells must evade immune responses at distant sites to establish metastases. The lung is a frequent site for metastasis. We hypothesized that lung-specific immunoregulatory mechanisms create an immunologically permissive environment for tumor colonization. We found that T cell-intrinsic expression of the oxygen-sensing prolyl-hydroxylase (PHD) proteins is required to maintain local tolerance against innocuous antigens in the lung but powerfully licenses colonization by circulating tumor cells. PHD proteins limit pulmonary type helper (Th1)-1 responses, promote CD4(+) regulatory T (Treg) cell induction, and restrain CD8(+) T cell effector function. Tumor colonization is accompanied by PHD-protein-dependent induction of pulmonary Treg cells and suppression of IFNγ-dependent tumor clearance. T-cell intrinsic deletion or pharmacological inhibition of PHD proteins limits tumor colonization of the lung and improves the efficacy of adoptive cell transfer immunotherapy. Collectively, PHD proteins function in T cells to coordinate distinct immunoregulatory programs within the lung that are permissive to cancer metastasis.

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**#4933 Desmin+ pericyte subpopulations correlated with blood-tumor barrier permeability in brain metastases of breast cancer**
Brunilde Gril, Tiffany Lyle, Paul R. Lockman, Chris E. Adkins, Afroz Shareef Mohammad, Emily Sechrest, Emily Hua, Diane Palmieri, Seth M. Steinberg, Wojciech Kloc, Ewa Izycka-Swiweszka, Renata Duchnowska, Nayyar Naema, Priscilla K. Brastianos, Patricia S. Steeg. 1. NCI-CCR, Bethesda, MD; 2. Vanderbilt University College of Veterinary Medicine, West Lafayette, IN; 3. West Virginia University Health Science Center, Morgantown, WV; 4. NCI-CR, Bethesda, MD; 5. University of Warmia & Mazursia University, Olsztyn, Poland; 6. Medical University of Gdañsk, Gdańsk, Poland; 7. Institute of Medicine, Warsaw, Warsaw, Poland; 8. Massachusetts General Hospital Cancer Center/Harvard Medical School, Boston, MA.

Breast cancer brain metastases remain incurable. The blood-brain barrier (BBB) is a multicellular dynamic structure regulating exchanges between the blood and the central nervous system. As cancer cells colonize the brain, the BBB evolves into a blood-tumor barrier (BTB). The BTB limits compound penetration and therefore contributes to poor efficacy of chemotherapy. While the BBB has been well characterized in developmental and neurodegenerative disease studies, the BTB composition remains unknown. We characterized the BTB in three model systems of brain metastasis of breast cancer developed in the laboratory: a triple negative (231-BR6), and two HER2 overexpressing (SUM190-BR3, JIMT1-BR3) subtypes. Using Texas Red dextran (TRD) as a marker of permeability and quantitative immunofluorescence staining, we analyzed the cellular and molecular composition of: 1) unaltered BBB vs. BTB, and 2) BTB in highly permeable metastases vs. BTB in poorly permeable metastases. The BTB developed from the BBB in a series of alterations, including a neuroinflammatory reaction with astroglisis, endothelial cell dilation, increased VEGF, reduced astrocyte endfoot polarity, and decrease in PDGF-α receptors. Only 10% of the metastatic lesions harbored a profound TRD exudation, which correlated with paclitaxel efficacy. We hypothesized that specific cellular and molecular changes account for the heterogeneity and increase in TRD diffusion. When metastases with relatively low- and high-TRD diffusion were compared, highly permeable metastases correlated with an increased expression of desmin+ pericytes in three models (231-BR6 p = 0.002; JIMT1-BR3 p = 0.004; SUM190-BR3 p = 0.008) and a decrease in CD13+ pericytes in two model systems (231-BR6 p = 0.014; JIMT1-BR3 p = 0.002). Decreased expression of laminin in the BBB increases tumour cell migration. In our study, JIMT1-BR3 p = 0.049; SUM190-BR3 p = 0.023) were associated with higher permeability. Desmin+ pericytes have been associated with pathological conditions such as fibrosis and spinal cord injury. Seven over nine human craniotomy specimens were positive for Desmin staining, validating clinically the relevance.
of our findings. We subsequently hypothesized that the desmin+ pericyte subpopulation functionally contributes to increased permeability. Desmin+ pericytes were produced in vitro by co-culturing primary mouse pericytes with astrocytes. When desmin+ or CD13+ pericytes were added to in vitro transendothelial electrical resistance (TEER) models of the BBB, the desmin+ pericytes exhibited a permissive, protective and normative effect on BBB permeability. The data suggest that desmin+ pericytes may facilitate the permeability of the BTB. These studies show that the BTB in brain metastasis model systems involves consistent molecular changes. These data may identify new strategies to selectively permeabilize the BTB and enhance chemotherapeutic efficacy.

#4934 Brain metastatic microenvironment reshapes cancer cell metabolism through epigenetic up-regulation of glutamate decarboxylase 1. Patricia M. Schnepf, Dennis D. Lee, Ian H. Guldner, Tressa O'Tighearnaigh, Bhavana Pulakurthl, Kaitlyn E. Eckert, Tiffany A. Toni, Brandon L. Ashfeld, Siyuan Zhang. Univ. of Notre Dame, Notre Dame, IN.

Cancer metabolism has been well documented to influence primary tumor development. Yet, the role of deregulated metabolism in metastasis progression is not well understood. Based on a distinct metabolic transcriptome profile in brain metastases compared to their primary tumor counterparts, we hypothesized that metabolic transcriptome shifting during metastatic evolution is crucial for metastatic success to the brain. Here we show that, despite a global down-regulation of multiple metabolic pathways in the brain metastatic tumors, the expression of the GABA metabolic pathway mediator glutamate decarboxylase 1 (GAD1) is significantly up-regulated. Using cell-based co-culture models for different primary and brain metastatic microenvironments and in vivo brain metastasis models, we demonstrate that down-regulation of DNA methyltransferase 1 (DNMT1) induced by the brain microenvironment results in decreased GAD1 promoter methylation and subsequent up-regulation of GAD1 expression in tumor cells. To dynamically visualize the cellular metabolic responses mediated by GAD1, we utilized the Peroxisome biosensor to monitor the cytosolic NADPH/NAD+ equilibrium in tumor cells using time-lapse imaging. By knocking down GAD1 induced by primary glia co-culture, we abolished the capability of tumor cells to utilize extracellular glutamine, leading to an NADH accumulation in the cytosol and a more oxidative cellular status. Lastly, either loss of GAD1 genetically or targeting GABA metabolic pathway by the repurposing of a neurologic drug, vigabatrin, results in a significant decrease in brain metastasis incidence. Taken together, our results demonstrated that brain microenvironment-specific metabolic shifting through GAD1 promoter demethylation drives brain metastasis outgrowth.

#4935 Development of a human pancreatic tumor microenvironment system (TMeS) for evaluation of novel therapeutics. Daniel G. Gioeli,1 Chelsi Snow,1 Michael Simmers,2 Robert Figler,1 J. Thomase Parsons,1 Stephen H. Snow,2 Michael Simmers,2 Todd Bauer,1 Brian Wamhoff2. 1HemoShear Therapeutics, Charlottesville, VA; 2HemoShear Therapeutics, Charlottetown, PE.

The development of drugs to treat cancer is severely hampered by the inefficiency of translating pre-clinical in vitro and mouse studies into clinical benefit. Over 90% of drugs that progress through pre-clinical studies fail in human trials. Therefore, there is a critical need to improve the accuracy of pre-clinical evaluation of the development of drugs that have the potential to reprogram the metabolism of tumor cells. This is especially the case for pancreatic cancer, the 4th leading cause of cancer deaths with a 5-year survival rate of <6%, where new therapies are desperately needed. The tumor microenvironment (TME) of PDAC contains numerous cell types including vascular endothelial cells, stellate cells and fibroblasts, as well as a complex extracellular matrix (ECM), all of which contribute to the growth and survival of cancer cells, as well as posing potential targets for therapeutic intervention. Thus, a more complete and nuanced understanding of the PDAC TME is required for the rational development of effective therapies. To address this problem, an in vitro tumor microenvironment system (TMeS) was engineered to incorporate tumor capillary hemodynamics and biologic transport with co-cultured human microvascular endothelial cells, along with pancreatic tumor and stellate cells. We demonstrate that significant tumor cell transcriptional changes occur in the TMeS that correlate with the in vivo xenograft transcriptome, including alterations in cell cycle regulation, oncogene signaling, and metabolism. PDAC tumor cells from two patients were inhibited by the human Cmax dose of gemcitabine to levels paralleling the patients' clinical responses. Previous work demonstrated that the EAK inhibitor, PF-362,271, worked only on in vivo xenografts and not on 2D in vitro PDAC cultures and here we show that PF-562,271 effectively inhibited PDAC growth in the TMeS indicating in vivo-like drug responses. In sum, the TMeS recapitulates the in vivo xenograft transcriptional program and responds to both established and experimental small molecule inhibitor chemotherapeutics at concentrations that correspond to human therapeutic plasma levels. Therefore, this model provides a unique platform to rigorously test the contributions of the cellular and molecular components of the TME to manipulating individual constituents in a controlled fashion that is not possible in vivo. Moreover, the TMeS can be used for the rapid evaluation of novel precision therapies. Finally, this system is amenable to using patient tumor material directly, enabling the potential use of the TMeS for patient avatars.

#4936 Modelling clinical issues by using patient-derived xenografts: Evaluation of partial hepatectomy on hepatoblastoma intrahepatic and distant growth. Marcella Cornet,1 Anais Delaître Delattre,2 Laura Brulle Soumare,2 Victoria Boissy,2 Thierry Tordjmann,2 Antoinette Lemoine,2 Olivier Deas,2 Jean-Gabriel Judder,2 Sophie Branchereau,2 Stefano Cairo2. 1Hôpital Bicêtre, Université Paris Sud, Le Kremlin Bicêtre, France; 2XenTech, Evry, France; 2French Institute of Health and Medical Research, Orsay, France; 2Hôpital Paul Brousse - Hôpitaux Universitaires Paris-Sud, Villejuif, France.

Hepatoblastoma (HB) is a rare disease that represents the most frequent liver malignancy during childhood. HB incidence is approximately 1.5 cases per million children less than 15-years old in Western countries. HB is histologically classified as epithelial or mixed depending on the presence of mesenchymal component. The most common epithelial components are the embryonal and fetal phenotype, reminiscent of at early or late stages of liver development. The improvement of the clinical management in the last 30 years has allowed 5-year survival rate to pass from 35 to 85%, due to the introduction of cisplatin-based chemotherapy. More than 50% of tumors are either inoperable or are metastatic at diagnosis. Neoadjuvant chemotherapy allows tumor size reduction and some patients can be used for the rapid evaluation of novel precision therapies. Finally, this system is amenable to using patient tumor material directly, enabling the potential use of the TMeS for patient avatars.

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tumor morphology, tumor growth, cell population diversity, and tumorigenic potential between tumors grown in orthotopic and subcutaneous environments were dependent on PDX line; and, stromal infiltration and mucin production were more prominent in the orthotopic background for some PDX models. In addition, we observe increased metastatic potential in orthotopic tumor-bearing mice compared with their subcutaneous counterparts. Tumor metastasis occurred in distal filtering organs, including the lung, liver, lymph nodes, and peritoneal cavity, among others. This result is consistent with the observation of increased circulating tumor cells in the blood of orthotopic tumor bearing mice. Comparison of therapeutic efficacy with gencitabine chemotherapy and antibody-drug conjugate (ADC) therapy in orthotopic and subcutaneous tumors showed that therapeutic response was dependent on PDX line. Interestingly, for PDX lines that exhibited morphological differences between orthotopic and subcutaneous backgrounds, treatment was less effective in the orthotopic microenvironment suggesting the microenvironment alters the ability of therapeutics to impede tumor growth. Reduced therapeutic response was also found to be consistent with reduced ADC uptake and vascularity in orthotopic tumors. Taken together, the orthotopic PDX model serves as a more accurate representation of human pancreatic cancer by displaying stromal-rich morphology, exhibiting metastatic cell behavior, and recapitulating therapeutic response challenges observed in patients.

#4938 A vicious cycle between osteoblasts-derived WISP-1 and mesenchymal-like cancer cells is essential to prostate cancer metastasis. An-Chen Chang,1 Show-Mei Chuang,2 Chih-Hsin Yang3, National Chung Hsing University, Taichung, Taiwan, 2China Medical University, Taichung, Taiwan. 

Prostate cancer (PCa) is the most commonly diagnosed malignancy in the United States and other Western countries. It is well established that tumor microenvironment plays an important role in cancer progression and metastasis. Wnt-induced secreted protein-1 (WISP-1) belongs to the CCN family (CTGF/ Cyr61/ Nov) that plays a key role in osteogenesis and tumor growth. We found that prostate-conditional medium (PCa-OBM) stimulated migration and invasion in human PCa cells. Furthermore, the medium was collected 2 days after treatment of osteoblasts with PCa-conditioned medium which were designed as PCa-OBM. The results showed that treatment of PCa cells with PCa-OBM further increased the migration and invasion activity, as well as epithelial- mesenchymal transition (EMT), including the epithelial marker (E-cadherin) down-regulation and mesenchymal marker (N-cadherin and twist) up-regulation. Moreover, human cytokine array was used to measure the secretion of 32 cytokines into PCa-OBM. The data showed that WISP-1 is highly up-regulated with PCa-OBM treatment compared to OBCM. Stimulation of osteoblasts with PCa conditioned media induced WISP-1 expression. In contrast, knockdown WISP-1 in osteoblasts impacted PCa-OBM-mediated cancer migration, invasion, and EMT. In addition, conditioned media from PCa cells was incubated with another cytokine array membranes. The data showed that ADAM-9 and cathepsinL were overexpressed in PCa conditioned media but only ADAM-9 could regulate WISP-1 expression in osteoblasts. Taken together, these results demonstrated that primary PCa-secreted ADAM-9 promotes osteoblasts-derived WISP-1 up-regulation, which in turn enhances primary PCa cells migration, invasion and EMT, resulting in cancer metastasis. Our study suggests that inhibition of osteoblasts-derived WISP-1 is a new therapeutic target for treating metastasis in human PCa patients.

#4939 Activation of GSK3β/β-catenin pathway promotes organ-specific metastasis in prostate cancer. Siyuan Qin,1 Qiong Song,1 Linlu Dai,1 Haibo Tong,1 Evan Keller,1 Jian Zhang,1 Yi Lu1, 1Guangxi Medical Univ., Nanning, China; 2University of Michigan, Ann Arbor, MI.

Metastasis is the major cause of treatment failure in various malignancies, including prostate cancer (PCa). Accumulating data has suggested that primary cancer cells prefer to spread to certain distant organs. To explore the mechanisms of different cancer cells preferentially metastasizing to certain organs, we established a prostate cancer lung metastasis model. Murine prostate cancer cells (RM1) with luciferase gene were injected into left tibia of mouse (C57BL/6J), and monitored the tumor growth and metastasis every week by Bioluminescence Imaging. The mice would be sacrificed when metastatic tumor appeared in multi-organs. We isolated the lung metastatic tumor, made single cell suspension and amplified. The lung metastatic tumor cells were specifically metastasized to lung when the tumor cells were injected intratibially again. After several cycles, we obtained highly lung metastatic tumor cells, named RM1-LM cells. Further, we investigated property and function changes in RM1-LM cells compared to parental cells (RM1) using in vitro assays. We firstly found the highly lung metastatic tumor cells showed more mesenchymal properties. Western blot results showed that hallmarks of EMT including E-cadherin, vimentin, snail, and ZO-1 were altered significantly in RM1-LM cells. Then, we observed that the highly lung metastatic tumor cells exhibited increased proliferative, colony formation, migratory and invasive activity. Mechanistic analyses indicated that prostate cancer organ-specific metastasis mediated by the activation of GSK-3β/β-catenin pathway. Our findings provide critical information on the potential role of GSK-3β in diagnostic and prognostic biomarkers of PCa metastasis. This work was supported by National Science Foundation of China (NSFC) Key Project (81130046); NSFC (81117193; 81272415); Guangxi Projects of China (2013GXNSFAA035004; 2012GXNSFBC030004).

#4940 Exosomal miR-19a: a novel communicator between cancer cell and osteoclast in osteolytic bone metastasis of breast cancer. Kerui Wu,1 Jamie Feng,2 Fei Xing,1 Yin Liu,1 Sambad Sharma,3 Kousouwse Watabe1,4 Wake Forest University School of Medicine, Winston-Salem, NC; 2Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai, China.

Bone is the most common site for breast cancer (BC) metastasis and relapse. Bone metastasis (BM) causes considerable complications due to osteolytic-mediated bone resorption. Current treatment for osteolysis in BM is limited due to their side effects and inability to extend the patients’ survival. Thus, there is an urgent need for developing early predictive markers and alternative therapies for BC-BM. Recently, circulating miRNAs have been found to harbor great potential for diagnostic and prognostic purposes. Due to the low stability of cell-free RNA in serum, these circulating miRNAs exist mostly in a form of membrane-wrapped extracellular vesicles (EVs) and they play important roles in cancer progression. In this study, we aim to investigate EV-derived-miRNA from serum of BC patients as biomarkers for diagnosis and prognosis of BC-BM. To find the circulating miRNAs that are associated with BC-BM, we applied a four-layer screening system combining bioinformatic analyses and experimental verification. Firstly, 85 BC associated circulating miRNAs were identified to be up-regulated in the serum of 32 BC patients compared to 22 healthy donors. Next, we performed Kaplan-Meier survival analysis for these 85 miRNAs and found that 10 miRNAs were positively correlated with metastases. To select the miRNAs specifically involved in BM, we compared the EVs derived miRNA profiles between bone metastatic cell lines and the parental cell lines (231BoM-1833 vs MDA-MB-231, MCF7-BM2d vs MCF7). Among the 10 miRNAs, only miR-19a was significantly up-regulated in EVs derived from bone metastatic cell lines. MiR-19a was also found to be highly enriched in exosomes compared to microvesicles and apoptotic bodies. Lastly, we verified the high expression of miR-19a in the serum of BC-BM patients compared to the BC patients without BM. To study the role of miR19a in BC-BM, we applied the CRISPR/Cas9 technology to knockout the miR-19a in 231BoM-1833 and MCF7-BM2d cells. The knockout of miR-19a didn’t alter the proliferation or migration of BC cells. However, it significantly decreased bone metastasis in our xenograft mouse models. We also found that the knockout of miR-19a decreased osteolytic lesions in the tumor-bearing bones, indicating the possible role of exosomal miR-19a in osteoclast cells. To test this hypothesis, we treated osteoclast cells with miR-19a-enriched exosomes and miR-19a-knockout exosomes. The results of tartrate-resistant acid phosphatase assay and bone resorption assay indicated that ALP activity and bone resorption were decreased in miR-19a-enriched exosome-treated osteoclasts. These results showed that miR-19a knockdown reduced bone resorption and osteolytic activity. Our study identified exosome derived mir-19a as a mediator of cell-cell communication between breast cancer and osteoclast cell, warranting further investigation on exosomal miR-19a as a novel biomarker and a therapeutic target for breast cancer bone metastasis.

#4941 Lysophosphatidic acid receptor signaling and pancreatic adenocarcinoma tumor microenvironment. Nicholas J. Skill, Mary A. Maluccio. Indiana University, Indianapolis, IN.

Introduction: Changes in lipid biosynthesis has attracted significant attention in the study of cancer. Its value in our understanding of pancreatic cancer has not yet been realized. This study examined lysophosphatic acid (LPA) variant biosynthesis in patients with stage H-III pancreatic ductal adenocarcinoma (PDAC), evaluating serum, bile, and tissue levels of LPA, its receptors, and downstream metabolites. LPA, a product of phospholipase D activity of the enzyme autotaxin (ATX) is a potent mitogenic agent. Data suggests that changes in lipid biosynthesis contribute to the tumor microenvironment in PDAC. Methods: PDAC, matched adjacent non-malignant tissues, gall bladder bile and serum samples were collected from patients with Stage H-III pancreatic cancer at the time of surgery. Control bile and serum were collected from patients undergoing cholecystectomy for benign disease and living renal donors respectively. Results: Serum and biliary LPA levels were increased in patients with PDAC when compared to controls. ATX activity, and LPA receptors, LPAR1, LPAR2, and LPAR3 were greater in PDAC adjacent pancreatic tissue when compared to
#4942 Small molecule inhibition of CXCR4 mobilizes prostate disseminated tumor cells (DTCs) from the chemoprotective bone marrow niche, thereby sensitizing them to chemotherapy. Kenneth C. Valkenburg, Emma E. van der Toom, Kenneth J. Pienta. Johns Hopkins School of Medicine, Baltimore, MD.

Bone metastasis results in the deaths of approximately 28,000 prostate cancer patients each year in the United States. Disseminated tumor cells (DTCs) go from the primary tumor site to the bone marrow, which provides a chemoprotective environment. C-X-C chemokine receptor 4 (CXCR4), highly overexpressed in prostate cancer, is at least partially responsible for bone homing. CXCR4 is also expressed in hematopoietic stem cells (HSCs), which occupy the same niche within the bone marrow as DTCs. HSCs can be mobilized from the bone marrow niche via CXCR4 antagonism. We have previously shown that human tumor cells can be similarly mobilized from the bone marrow niche via small molecule inhibition of CXCR4. We believe this represents an ideal strategy for treating bone metastasis and hypothesize that mobilized DTCs will proliferate in the vascular niche, causing them to become chemosensitive. Several outstanding questions still exist that we are in the process of answering. First is the percentage of DTCs that are mobilized with CXCR4 antagonism. We used a qPCR strategy to quantify tumor cells in the bone marrow and blood of mice. This was imperfect because we could not reliably count cells, nor could we query the cells further. Therefore, we developed a novel immunofluorescence protocol to detect, quantify, and query tumor cells residing in the bone marrow or blood of mice. We will use this technique to determine the percentage of bone marrow DTCs that are mobilized. The second outstanding question is whether or not mobilized DTCs proliferate once mobilized. This is crucial to know, considering most chemotherapy relies on tumor cell proliferation. Using our novel protocol, we will determine the percentage of bone marrow DTCs and mobilized DTCs that are proliferating. Finally, while there is evidence that combining CXCR4 antagonism with chemotherapy kills more tumor cells than chemotherapy alone, no one has shown definitively that it works specifically for prostate bone marrow DTCs or bone metastasis. Not only will we do this, but we will also be able to quantify how effective each therapy strategy is by quantifying the tumor cells present in each scenario. The data from these in vivo murine experiments, which would be impossible to get from humans, will go hand in hand with a first-in-prostate cancer clinical trial we are running that tests the same hypothesis. Combined, these data will be critically important in deciding whether mobilization therapy combined with chemotherapy is an effective strategy for treating metastatic prostate cancer.

#4943 Orthotopic transplantation of pancreatic cancer PDX models increases murine stroma content, but does not influence therapeutic response to standard of care. Ulrike Pfohl,1 Diana Behrens,1 Iduna Fichtner,1 Jens Hoffmann,1 Wolfgang Walther1,2 EPO GmbH, Berlin, Germany; 1Charité University Medicine Berlin and Max-Delbrueck-Center for Molecular Medicine, Berlin, Germany.

Background: Reasons for the poor prognosis of pancreatic cancer are advanced and inoperable tumor stages at time of diagnosis and resistance to conventional therapies. Patient-derived xenografts (PDX) represent a valuable tool for the prediction of therapy response, the identification of new biomarkers and therapeutic targets or pancreatic cancer specific activated pathways (MAPK, hedgehog). However, PDX tumors differ from patient tumors as their surrounding tissue is replaced by murine stroma within 3 to 9 weeks after primary transplantation. Since the desmoplastic stroma has an impact on the progression and treatment of pancreatic cancer, we investigated the characteristics and function of murine stroma components in PDX models of pancreatic ductal adenocarcinoma (PDAC). Methods: We analyzed the relevance of murine cancer-associated fibroblasts (CAFs) for therapeutic response by comparing orthotopic with s.c. transplantation in PDX. We selected a cohort of 3 PDAC PDX with different growth rates (slow, median and fast growth). Human pancreatic tumor material was implanted s.c. or orthotopically into immunodeficient mice. After successful engraftment, the chemosensitivity to standard of care (SoC) drugs (focus on 2 to 3 drugs) was determined according to clinically relevant and optimized schedules. Growth of orthotopic PDX models was monitored via high-resolution ultrasound. Cryo- and formalin-preserved tumor tissues from the chemosensitivity studies were collected and analyzed for specific stroma markers (collagen I and oSMA). Results: In general, the response profiles in our experiments have closely reflected patient’s response in the clinic for the drug combination gemcitabine and nab-paclitaxel. There were no differences in response when comparing s.c. with orthotopic growing tumors. Semi-quantitative analysis of tumor sections showed that orthotopic tumors contain more murine stroma compared to s.c. tumors. Combined, these data will be critically important in deciding therapy condition, while MET is a driving mechanism for tumor recurrence. The EMT cells significantly contributed to recurrent lung metastasis. The EMT tumor cells survived the chemotheraphy due to reduced proliferation and apoptotic tolerance, which are common characters of dormant tumor cells. More interestingly, the GFP+ EMT tumor cells reversed back to epithelial phenotype through mesenchymal to epithelial transition (MET) and formed recurrent metastatic lesions in the lung. These results suggest that our EMT lineage tracing model may provide a unique tool to study tumor dormancy and recurrence. The EMT process may represent a major mechanism for tumor dormancy under chemotherapy condition, while MET is a driving mechanism for tumor recurrence.

#4945 Axl–hi DTCs in the bone marrow of a genetically engineered mouse model of prostate cancer exhibit decreased proliferation. Haley D. Axelrod, Kenneth C. Valkenburg, Brian W. Simons, Kenneth J. Pienta. Johns Hopkins School of Medicine, Baltimore, MD.

The majority of prostate cancer (PCa) deaths are attributed to bone metastases, which may not arise until years after the primary tumor has been removed. Disseminated tumor cells (DTCs) have been shown to already be present in the bone marrow of patients at the time of prostatectomy, and it is thought that they undergo a period of dormancy, allowing them to remain undetected for years before manifesting into clinically detectable metastases. It is not known what factors in the bone marrow niche play an essential role. Previously our group has shown that one of these niche factors, Gas6, can regulate growth of PCa cells. The growth restraining effects of Gas6 on PCa cells is likely mediated through the tyrosine kinase receptor Axl, whose expression was found to be increased on dormant DTCs compared to proliferating metastases in a xenograft mouse model. Here, we utilized the TRAMP model, a genetically engineered mouse model of spontaneous PCa progression, to compare the frequency of Axl–hi and Axl–low bone marrow DTCs that are proliferating versus non-proliferating. Further, we also compared Axl expression and proliferation in matched primary tumors. To investigate the functional significance of Gas6/Axl signaling in mediating PCa dormancy, we have generated Axl knockout PCa cells to test the requirement of Axl in maintaining dormancy, and Axl overexpressing PCa cells to test the sufficiency of Axl in mediating dormancy. Determining the role of Axl and any other regulatory mechanisms of PCa DTC dormancy will be crucial in efforts to prevent lethal and incurable metastases.
Host tissue stiffness regulates chemotherapy-induced cancer cell dormancy. Aliya Anlas, Celeste M. Nelson. Princeton University, Princeton, NJ.

This study investigates how the tumor microenvironment, namely host tissue stiffness, affects the propensity of disseminated tumor cells to become dormant at secondary sites. Approximately 90% of cancer-related deaths result from metastasis. Disseminated tumor cells cannot be detected at the time of diagnosis of the primary tumor because they form “dormant” micrometastases that are not clinically detectable. Thus, metastases in secondary sites such as the lungs, bone marrow, liver and brain are usually discovered months or years after the initial diagnosis and/or treatment of the primary tumor. A 2D polyacrylamide gel-based tissue culture model was used to investigate the effects of host tissue stiffness on the proliferative behavior of breast cancer cells. To assess the dormant phenotype, Ki67 and RNA staining were used to quantify proliferation and RNA content, respectively. Additionally, changes in levels of cell-cycle regulators were determined by quantitative RT-PCR. Dormant cells have decreased levels of transcription and proliferation. We found that both tenascin-5 and 5-fluorouracil induce dormancy in estrogen receptor-positive breast cancer cells cultured on soft and stiff substrata. After repeated rounds of 5-fluorouracil treatment and recovery, cells cultured on soft substratum, mechanical properties of which represent common metastatic sites, had a higher tendency to recover. This suggests that cell growth at secondary sites favors a soft microenvironment, characteristic of organs where breast cancer metastases often become clinically overt. Dormant cancer cells, or minimal residual disease, cannot be detected with current diagnostic tools and cannot be detected by conventional therapies. Therefore, current clinical practices rely on estimating the probability of recurrence from various prognostic factors including the grade and the stage of the disease. Elucidating the mechanisms that regulate the switch from dormancy to proliferation at metastatic sites will pave the way for new treatments to control this incurable disease.

Her2 inhibition and dormant cell metabolism. Douglas B. Fox, James V. Alvarez. Duke University, Durham, NC.

Breast cancer is the most diagnosed cancer in women in the United States, and despite improved prognosis resulting from targeted therapies, tumors commonly re-emerge upon development of therapeutic resistance. In order to overcome this, it is critical to explore the cellular pathways that change in tumors following treatment. While many mechanisms of resistance to targeted therapies have been identified, the metabolic changes that occur following treatment have not been well defined. We believe that these changes may offer a general therapeutic target. Using a doxycycline-inducible Her2 mouse model of breast cancer, we are exploring the molecular and cellular consequences Her2 inhibition. Using in vitro mammosphere cultures derived from these mice, we showed that Her2 loss quickly downregulates ERK and Akt signaling. There is a corresponding decrease in proliferation, and apoptosis is induced in 25-50% of cells. However, a population of cells survives Her2 withdrawal and remains dormant for upwards of one month. We are using these models to study how metabolic pathways are rewired following Her2 inhibition, and we believe that the metabolic pathways adapted upon Her2 inhibition may represent targetable vulnerabilities.

Characterization of hTERT-immortalized prostate-derived stromal and epithelial cells: An authentic in vitro model for tumor microenvironment studies. Luis G. Rodriguez, Russell E. McDaniel, Xiangshan Zhao, Elizabeth Turner, Christopher Annesi, Chaozhong Zou. ATCC Cell Systems, Gaithersburg, MD, MD.

Tumor development begins with mutational changes to the genetic makeup of a cell, but its progression is not solely determined by the mutated cell, but also by the tumor’s microenvironment. Prostate cancer, a leading cancer diagnosed in men, has been shown to be highly influenced by its surrounding stroma, particularly fibroblasts. It has been demonstrated that cancer-associated prostate fibroblast (CAF)s differ from normal-associated prostate fibroblast (NAFs). However, human prostate cancer model systems have focused largely on prostate cancer epithelial cells. Currently, a need exists for a more physiologically relevant human cell model system to study prostate cancer progression within the context of its tumor microenvironment. In this study, we characterized three prostate-derived cells: prostate cancer-associated fibroblast (CAF)s, prostate normal-associated fibroblast (NAFs) and prostate cancer epithelial (PrE) cells; all three lines were immobilized by hTERT (human telomerase reverse transcriptase) alone, and have been continuously passed for more than 40 PDL in our hands. Our data shows that the hTERT immortalized CAFs proliferate faster than the NAFs; in addition, both CAFs and NAFs express fibroblast markers such as TE7 and alpha smooth muscle actin (α-SMA), while neither cell line expresses epithelial marker such as CK14. Both CAFs and NAFs also express elevated levels of α-SMA upon TGF-β stimulation. All three prostate-derived cells express the prostate specific marker AR, and show similar markers staining after long time passing. Importantly, conditioned media collected from CAFs promotes tumor cell growth better than NAF conditioned media. In conclusion, CAFs, NAFs, and immortalized prostate cancer epithelium may provide a very valuable model system for the study of prostate cancer cell progression and tumor microenvironment studies.

Longitudinal monitoring of cell-free DNA in patients with small cell lung cancer reveals dynamic insights into treatment efficacy and disease relapse. Christine M. Lovly, Karinna Almodovar, Wade T. Iams, Laura C. Meador, Sally York, Leora Horn, Christopher K. Raymond, Jennifer Hernandez, Lee P. Lim. Vanderbilt University School of Medicine, Nashville, TN; Northwestern University School of Medicine, Chicago, IL; Resolution Bioscience, Bellevue, WA.

Introduction: Small cell lung cancer (SCLC) is a highly lethal neuroendocrine malignancy that accounts for approximately 10-15% of all lung cancers and is responsible for approximately 30,000 deaths annually in the United States and 200,000 deaths worldwide every year. There is an urgent need to develop novel treatment strategies for patients with this disease. We sought to improve the quality of patient care by establishing a liquid biopsy assay for rapid, noninvasive monitoring of disease burden. Design: The SCLC assay relies on targeted next-generation DNA sequencing of cell-free DNA (cfDNA) collected from patient plasma. The assay targets a panel of 14 genes that are frequently mutated in SCLC. We examined a total of 141 plasma samples from a cohort of 27 patients. Eleven patients had limited stage SCLC and 16 patients had extensive stage SCLC. Results: Of the plasma samples collected during the course of patient treatment and included time points before and after chemotherapy or immunotherapy. Results: We detected somatic, disease-associated mutations in the cfDNA of 78% of patient samples (21/27). The allele frequency of cfDNA ranged from %0.5% to %85%. The most commonly mutated genes were TP53 and RB1, which were found in 17/27 and 10/27 samples, respectively. We also detected single nucleotide variants in PIK3CA (3/27) and PTEN (1/27) as well as copy number variants in MYC and MYCL1 (2/27). The observed mutant allele frequencies in longitudinal samples tracked closely with treatment responses. Strikingly, we found instances where the assay detected the reappearance of tumor-associated markers several weeks before clinical evidence of relapse was detected. Conclusions: cfDNA sequencing allows for improved monitoring of disease burden, depth of responses to treatment, and timely warning of disease relapse in patients with SCLC. References: 1. Society, A.C., Cancer Facts and Figures 2015. American Cancer Society, Atlanta, Ga, 2015. 2. George, J., et al., Comprehensive genomic profiles of small cell lung cancer, Nature 524:47.
(34/113). PIK3CA mutations were detected in 31.8% of patients with HR+/HER2- disease. The majority of the patients had either newly diagnosed metastatic or progressive metastatic disease at the time of the blood draw. 14 patients had stable metastatic disease and among these patients, only 1 of these patients was found to have an ESR1 mutation and no PIK3CA mutations were detected. The majority of patients with ESR1 mutation (33/67) and PIK3CA mutations (75%) had progressive disease. The patients that received an aromatase inhibitor (AI) either in the adjuvant or met setting had a higher prevalence of ESR1 mutations compared to patients that had no AI treatment, regardless of whether or not they received tamoxifen (TAM) (prevalence was 32% for adjuvant AI only, 40.4% AI in met only, No AI and no TAM 7.1% and TAM but no AI 6.7%). In addition fulvestrant treatment in the met setting was significantly associated with ESR1 mutations (odds ratio 3.38, p-value<0.01). Conversely, we did not detect any significant associations between endocrine treatments in the adjuvant or met settings and PIK3CA mutations. Conclusions: Analysis of cDNA can successfully detect ESR1 and PIK3CA mutations in newly diagnosed or progressive metastatic breast cancer patients and the emergence of the ESR1 mutations is associated with AI and fulvestrant treatment. These results support the serial monitoring of ESR1 mutations in cDNA in metastatic disease and highlight the need to study new agents to target these mutations.

### #4951 Dynamics of KIT exon 11 mutations in cell free plasma DNA of patients treated for advanced gastrointestinal stromal tumors: Results from the Dutch GIST bio-databank.

Pieter A. Boonstra,1 Arja ter Elst,1 Marco Tibebsma,1 Ron H. Mathijssen,1 Florence Atrai,1 Frits van Cœvoorden,1 Sheima Farag,2 Neeltje Steeghs,3 Ingrid M. Desar,4 Winette T. van der Graaf,4 Hans Gelderblom,5 Boudevijn van Etten,1 Jelle Overbosch,1 Albert J. Suurmeijer,1 Jourik A. Gietema,1 Ed Schuuring,1 Anna K. Reyners1.

Eligibility criteria were histological diagnosis of GIST and treatment with a TKI. Patients with a known KIT exon 11 mutation, of which a baseline (before start of any TKI) sample or a recurrence/progression sample was available, were analyzed. A custom single tube digital droplet PCR (ddPCR) drop-off assay, designed at the UMC, was used. This assay detects 80% of the known KIT exon 11 mutations. Results Until Nov 2016, >620 samples of 175 patients have been collected. From 43 patients with a KIT exon 11 mutation in the pretreatment tumor biopsy, a baseline/progression sample was available. Mutations in exon 11 were detected in cfDNA from baseline samples of 23 patients, with lower MAF than in the pretreatment tumor (mean: 11%, range 0-12%). Interestingly, PD ctDNA samples harboured an increase in MAFs and a higher numbers of SNVs, with additional relapse-specific SNVs (mean: 22; range 0-55) targeting, amongst others, the protein kinase A signaling pathway. Analysis of additional ctDNA samples obtained between diagnosis and relapse (2-6 patients/sample) using deep sequencing techniques demonstrated a disappearance of SNVs coinciding with response to therapy, and re-appearance of SNVs at the time of tumor progression. Discussion and Conclusion: cDNA analysis in metastatic GIST patients is feasible. Our study will be extended to include the monitoring of early progression based on cDNA, which may guide early treatment adaptations.

### #4953 Single cell signaling analysis reveals circulating tumor cell markers of drug susceptibility and tumor heterogeneity.

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Complexity of cancer requires advancements of tools for intervention and diagnostics. Rare circulating tumor cells (CTCs) from patient blood could be used for real-time monitoring of solid cancer. We recently developed a microfluidic device, the CTC-iChip, which enriches well-preserved CTCs in solution through removal of the cellular components (red and white blood cells, platelets, leukocytes, monocytes, etc). Protein expression analysis in CTCs has been limited to immunoflourescent staining for a few well-characterized tumor markers (including EpCAM, HER2, PSA and Keratin) due to the limitations inherent to immunoflourescence labels and their precise resolution through microscopy. The scarcity of cells precludes large-scale proteomic analysis such as mass spectrometry of CTCs to effectively evaluate changes in total as well as phospho-proteins, which serve as excellent surrogate markers to monitor in real time the rapid alterations in signaling pathways within cells. This approach would be particularly important since the PI3K, mTOR, Akt, MAPK signaling pathways are aberrantly activated in many cancers and are promising therapeutic targets. In this work, we applied inductively-coupled plasma mass spectrometry (ICP-MS) measurement of cells (mass cytometry) that can measure up to ~40 proteins in millions of single cells due to quantitative, non-overlapping mass signals acquired from antibodies tagged with purified lanthanides. Sample preparation for mass cytometry was modified to minimize the loss of cells using our microfluidic platform in lieu of washing via centrifugation. Mass cytometry allowed identification of tumor cell populations based on the expression of multiple single cell markers, each of which could be either increased or decreased, or both, and the associations of these markers could be used to classify different subtypes of cancer cells.

Background: Liquid biopsies are revolutionary tools to monitor tumour-specific genetic alterations in sequential samples. In neuroblastoma (NB), significant levels of circulating tumor DNA (ctDNA) in the bloodstream enable the detection of tumour cell-specific markers including MYCN amplification or activating ALK mutations. As clonal evolution plays a role in NB progression, analysis of circulating tumor cells is emerging as a potential marker for disease monitoring and follow-up. Methods: To gain further insights into mechanisms of clonal evolution in NB, we isolated ctDNA from plasma at diagnosis (n=19) and during follow-up (final time-point: partial or complete remission (PR/CR), n=7; progressive disease (PD), n=9) for 19 NB patients for whom primary NB and matched germline DNA whole exome/whole genome sequencing data (WES/ WGS) was available. Of all NB patients, blood was subjected to Illumina 100PE WES following modified library construction and capture approaches to account for small ctDNA molecules (target depth 100x). SNVs/mutations were called using GATK-UnifiedGenotyper, GATK-HaplotypeCaller and Samtools. Copy-number profiles were generated using Varscan and DNAcopy. Results: CtDNA WES yielded satisfactory depth in all cases. At diagnosis, a majority of observed SNVs were common to the primary NB and corresponding diagnostic ctDNA of a given patient (mean number of SNVs: 19; range 9-69) with MAF (mutated allele fractions) corresponding to the estimated ctDNA content in the total cell free DNA. At diagnosis, few SNVs specific to the NB (mean: 6; range 0-18) or specific to ctDNA (mean:22; range 9-69) were observed, suggesting spatial heterogeneity with different ctDNA amounts released by different clones. In PR or CR ctDNA SNVs were 10-fold more frequent with lower MAF (mean: 11; range 0-12). In relapse, SNVs were more frequent, doubling in both MAFs and a higher numbers of SNVs, with additional relapse-specific SNVs (mean: 22; range 0-55) targeting, amongst others, the protein kinase A signaling pathway. Analysis of additional ctDNA samples obtained between diagnosis and relapse (2-6 samples/patient) using deep sequencing techniques demonstrated a disappearance of SNVs coinciding with response to therapy, and re-appearance of SNVs at the time of tumor progression. Discussion and Conclusion: CtDNA WES prove to be an extremely powerful tool to study spatial and temporal heterogeneity in NB, providing further proof of the importance of clonal evolution in NB progression. Full characterization of ctDNA, which might represent more aggressive clones, might orient targeted treatment approaches.
### Clinical Research: Liquid Biopsies 4

#### #4954 Clinical validation of a cell-free DNA liquid biopsy approach for non-invasive molecular profiling.
Monica Nesselbush, Samuel Angiolillo, Luis A. Diaz, Andrew Georgiadis, Shannon Glynn, Sián Jones, Laurel Keefer, Peter LoVerso, Derek Murphy, Sonya Parpart-Li, David Riley, Naomi Sengamalay, Manish Shukla, John Simmons, Snehal Talati, Rebecca Steinberg, Laura Tucker, Victor Kolb, Julie L. Lueders, Angela Villarta, Mark Sausen. Personal Genome Diagnostics, Baltimore, MD.

Molecular profiling of advanced cancers enables the identification of actionable genomic alterations to guide therapeutics decisions. Although profiling of tissue samples is considered the gold standard, specimens may be unavailable or unsuitable for testing due to limited tumor purity or specimen quality. Furthermore, surgically resected tumor tissue samples are increasingly available after disease progression and re-biopsy in this setting may not be feasible. Circulating tumor DNA (ctDNA) approaches for identification of genetic alterations in cancer patients may be more informative as the alterations reflect the current status of the tumor. ctDNA is representative of multiple tumor sites within a patient and may aid in the detection of alterations throughout the course of therapy. However, the fraction of ctDNA obtained from a blood sample is often very low (<1.0%) and difficult to detect. Additionally, many methods to evaluate ctDNA interrogate single hotspot or a few mutations. The next generation of ctDNA assays must identify clinically actionable genetic alterations and novel biomarkers with high precision and accuracy. To address these issues, we have developed and validated PlasmaSelect64, a ctDNA approach to comprehensively detect genetic alterations at low allele frequencies in low volume of cancer patients. Utilizing digital genomic approaches, we demonstrated robust sensitivity and specificity in our CLIA laboratory in 64 well-established cancer genes that were identified based on clinical actionability. In addition to the evaluation of exons in 58 genes that are frequently mutated in cancer for sequence mutations, we performed a comprehensive genomic analysis of translocations in 18 genes and copy number analyses in 19 genes. We have also developed a novel approach for identification of microsatellite instability (MSI) using these error correction methodologies. To evaluate the PlasmaSelect64 approach, we developed and optimized the pre-analytical conditions for sample collection and processing with K2EDTA and Streck blood collection tubes. Analytical validation studies were performed with clinical samples and controlled cell-line mixtures containing known alterations determined by orthogonal methods. We robustly identified sequence mutations at 0.50% mutant allele frequency (MAF) with a limit of detection of 0.05% MAF, corresponding to a per-base specificity of 99.9997% and a sensitivity of 99.4%. For detection of focal amplifications and translocations, analytical method validation studies demonstrated sensitivity of 97.2% and 94.4% and specificity of >99% at MAFs of ≥20% and ≥50%, respectively. PlasmaSelect64 provides a non-invasive platform to enable detection of clinically relevant genetic alterations across a large number of genomic regions to aid in the therapeutic management of cancer patients.

#### #4955 PD-L1 expression on circulating epithelial tumor cells (CETCs) correlates with the presence of metastasis in breast cancer patients and differs from PD-L2 expression.
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Background: Strategies to improve the efficacy of the immune system against malignant tumors represent a major innovation focusing on the programmed cell death-1 receptor (PD-1) with its two ligands PD-L1 and PD-L2. The expression of PD-L1 has been evaluated in a number of different tumor types and can be used as a predictive biomarker for PD-1/PD-L1 checkpoint inhibitor treatment response. PD-L2 has not received as much attention and its role in modulating tumor immunity is less clear. We used a non-invasive, real-time liquid biopsy to better characterize PD-L1/L2 expression on circulating epithelial tumor cells (CETCs) in breast cancer patients. Methods: CETCs were determined from blood of 72 patients suffering from breast cancer. The number of vital CETCs and their expression of PD-L1 and PD-L2 were investigated using the maintrac® method. Results: PD-L1 expressing CETCs were detected in 94.5% of breast cancer patients whereas only 82% patients expressed PD-L2 in their CETCs. Breast cancer patients had significantly more PD-L1 positive CETCs as compared to patients without metastasis (median 75% vs. 61.1%; p<0.005). The fraction of PD-L1 positive CETCs was significantly higher than the fraction of PD-L2 positive CETCs (54.6% vs. 28.7%; p<0.001). Moreover, we observed a significant heterogeneity in PD-L1 and PD-L2 immunostaining intensity across CETCs from the same patients. Conclusion: Breast cancer patients have detectable CETCs with a high frequency of PD-L1 which correlate with progression of cancer disease. PD-L1 seems to be a major factor in immune evasion and may be a promising target of anticancer therapies. Monitoring the frequency of PD-L1 positive CETCs could reflect individual patient’s response to an anti-PD-1/PD-L1 therapy.

### Epidemiology: Molecular and Genetic Epidemiology

#### #4956 Transcriptome-wide association study identifies new prostate cancer susceptibility genes in the OncoArray data.
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Genome-wide association studies (GWAS) have identified over 150 genomic regions harboring risk variants for prostate cancer which explain one third of all familial risk. However, with some notable exceptions, the causal variants and target susceptibility genes at these risk loci have yet to be identified. Recent work has shown a strong overlap between loci associated with gene expression levels (eQTLs) in prostate tissue and GWAS loci, which suggests that the causal mechanism at a significant proportion of risk loci includes causal alleles that regulate expression levels of nearby susceptibility genes. While overlapping eQTLs with GWAS is a powerful method to prioritize susceptibility genes, it is often the case that multiple eQTLs co-localize at the GWAS risk region (due to linkage disequilibrium (LD) and correlations across transcript levels). This prohibits the identification of the true susceptibility gene as opposed to spurious co-localization at the same locus. We recently leveraged gene expression imputation to perform transcriptome-wide association studies (TWAS) as a principled approach to measure the strength of association between gene expression and disease status. Here, we used imputed expression to identify new susceptibility genes for prostate cancer in the OncoArray GWAS data. We integrate gene expression data from more than 44 tissues across ~4,000 individuals with GWAS of prostate cancer from the OncoArray in ~140,000 individuals. Our approach identified 118 susceptibility genes for prostate cancer that reside in 90 independent loci across the genome. Of these, we report 7 genes located more than 0.5 Megabases away from any previously reported GWAS loci for prostate cancer, thus providing new risk loci. Second, we use TWAS to investigate genes previously reported as susceptibility genes for prostate cancer through overlaps of eQTL and GWAS. We find 36 (out of 86 previously reported genes) to be significant in TWAS. Overall, our findings highlight the power of integrating gene expression data with GWAS and provide testable hypotheses for future functional validation of prostate cancer risk.

#### #4957 Gene expression signature of Gleason score is associated with prostate cancer outcomes in a radical prostatectomy cohort.
Min A Hjun,1 Milan S. Chadha,2 Mark L. Gold,3 Vladimir Rinkevich,4 Elaine A. Ostrander,5 Jing-Bang Fan,6 Ziding Feng,7 Janet L. Stanford,8 Fred Hutchinson Cancer Research Center, Seattle, WA; 2Maastricht University, Maastricht, Netherlands; 3University of Washington School of Medicine, Seattle, WA; 4Illumina, Inc., San Diego, CA; 5National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 6University of Texas MD Anderson Cancer Center, Houston, TX.

Background: Prostate cancer (PCa) is a leading cause of cancer-related mortality worldwide. Many men treated for clinically localized PCa will be cured, however, 20 to 30% of men will relapse and some will experience metastatic-lethal (ML) progression. Gleason score (GS) is one of the best predictors of PCa aggressiveness, but additional tumor biomarkers may improve its prognostic accuracy. We developed a gene expression signature of GS to enhance prediction of PCa outcomes. Methods: Elastic Net regularization was used to construct a gene expression signature by contrasting tumors with GS 8-10 (high) vs. ≤6 (low) in The Cancer Genome Atlas (TCGA). Tumor tissue samples obtained at radical prostatectomy for a Fred Hutchinson (FH) patient cohort of men with localized PCa were used to generate genome-wide gene expression data. The gene expression signature was validated for its ability to predict recurrence and ML progression in the FH patient cohort (N = 503; Nrecurrence = 106; NmLprogression = 27; mean follow-up for recurrence = 8 years). Results: The expression signature includes transcripts representing 49 genes. In the FH cohort, the signature was associated with recurrence and ML progression with hazard ratios
(HRs) for a 25% increase in the signature of 1.51 (95% CI: 1.24-1.82; \(P = 2.7 \times 10^{-5}\)) and 2.41 (95% CI: 1.51-3.85; \(P = 0.0002\)), respectively. Among patients with GS 7 tumors, the signature was also significantly associated with PCa recurrence (HR = 1.38, 95% CI: 1.09-1.76; \(P = 0.008\)) and ML progression (HR = 2.42, 95% CI: 1.30-4.52; \(P = 0.006\)). The signature’s area under the curve (AUC) for both ML and survival was much larger numbers of CpGs were statistically significantly associated with subsequent survival increases dramatically when measured closer to before diagnosis, respectively. After adjusting for blood cell type distributions, higher levels of the signature were associated with increased expression of genes in cell cycle-related pathways including G2M checkpoint, epithelial mesenchymal transition, and E2F targets pathways, and decreased expression of genes in several pathways including androgen response, estrogen response, oxidative phosphorylation, and apoptosis. Conclusion: The gene expression signature based on GS may improve the prediction of overall recurrence as well as ML progression in PCa patients after radical prostatectomy, in particular among men with GS 7 tumors.

### 4958 Pre-diagnostic peripheral blood DNA methylation and lung cancer survival
Jennifer A. Doherty,1 Xuan Zhang,2 Devin C. Koestler,2 Matt J. Barnett,3 Mark D. Thorsness,2 Gregory E. Goodman,3 Carmen J. Marsit,4

Epigenetic regulation plays a critical role in cell and tissue development and differentiation, is a known mechanism of carcinogenesis, and is altered by various exposures including cigarette smoking and age. Peripheral blood methylation represents a combination of these factors as well as blood cell type distributions that reflect underlying immunophenotypes. Hematopoiesis is programmed through epigenetic changes, and blood cell type-specific methylation marker can be leveraged to fingerprint cell type distributions. We examined associations between pre-diagnostic methylation markers in peripheral blood with overall lung cancer survival in very heavy smokers from the Beta Carotene and Retinol Efficacy Trial (CARET). CARET was a randomized, double-blinded, placebo-controlled chemoprevention trial of daily \( \beta \)-carotene and retinyl palmitate in smokers with \( \geq 20 \) pack-years, and smokers who were occupationally exposed to asbestos. Methylation was successfully measured using the Illumina 850K EPIC BeadArray on average 5 years prior to diagnosis for 331 lung cancer cases, of whom 253 died during follow up (median survival 329 days). Cox proportional hazards regression models were used to examine the relationship between CpG-specific methylation and survival. Models were adjusted for age at blood draw, sex, race, enrollment year, placebo/active intervention, asbestos exposure, current/former smoking and time since quit, pack years, average number of cigarettes per day and histologic type, and were fit separately depending on the time between blood draw and lung cancer diagnosis: 0-3 years (n = 107), 3-5 years (n = 77), and 5+ years (n = 147). After adjusting for multiple comparisons by computing the false discovery rate (FDR) q-value, there were 1,028,296, and 4 CpGs that were statistically significantly associated with survival (q \( \leq 0.01 \)) for samples collected 0-3 years, 3-5 years, and 5+ years before diagnosis, respectively. After controlling for blood cell type distributions, much larger numbers of CpGs were statistically significantly associated with survival: 10,481, 456, and 257, respectively. The top canonical pathways of CpGs identified in samples drawn 0-3 years prior to diagnosis included PTEN signaling (p = 1.1E-5) and IKB signaling in B lymphocytes (p = 3.7E-5). We observed that the number of methylated CpGs in peripheral blood that are associated with subsequent survival increases dramatically when measured closer to lung cancer diagnosis. As well, we observed that the number of methylated markers is increased by controlling for immunophenotype. Our study provides evidence that peripheral blood methylation markers measured many years prior to diagnosis may be associated with poor lung cancer survival, and that increasing changes in methylation over time may also be associated. This points to the critical role of the immune system, even prior to diagnosis, in influencing cancer outcomes.

### 4959 The RANK-axis and breast cancer risk by hormone receptor subtype: Results from the EPIC cohort
Danja Sarink,1 Helena Schoon, Theron German Johnson, EPIC cohort collaborators, Rudolf Kaaks, Renée T. Fortner, German Cancer Research Ctr., Heidelberg, Germany.

Background: Activation of the receptor activator of nuclear factor kappa-B (RANK)–axis promotes mammary tumor development in experimental models. Circulating concentrations of soluble RANK ligand (sRANKL) may influence breast cancer risk via RANK activation; this may be modulated by circulating concentrations of osteoprotegerin (OPG), the decoy receptor for RANKL. sRANKL and breast cancer risk in humans have not previously been investigated. Methods: A case-control study was nested in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. A total of 1976 incident invasive breast cancer cases (estrogen receptor positive (ER+), n = 1598), matched 1:1 to controls, were included in the analysis. Women were both pre- and postmenopausal at blood collection. Serum sRANKL was quantified using an enzyme-linked immunosorbent assay (ELISA). Serum OPG was quantified using an electrochemiluminescent assay. Odds ratios (ORs) and 95% confidence intervals (95%CIs) were calculated using conditional logistic regression. Results: Associations between sRANKL and breast cancer risk differed by tumor hormone receptor status (\( \phi_{\text{ER}} = 0.05 \)). Higher concentrations of sRANKL were associated with increased risk of ER+ breast cancer (top vs. bottom quintile OR 1.36 [0.99-1.87]; \( \phi_{\text{ER}} = 0.31 \)). While results considering the sRANKL/OPG ratio were similar to those for sRANKL alone for hormone receptor positive breast cancer, we saw a suggestive inverse association between the ratio and ER-PR- disease (5% vs. 1st quintile OR 0.60 [0.31-1.16]; \( \phi_{\text{ER}} = 0.04 \)). Conclusions: This study provides the first prospective data on sRANKL and breast cancer risk, providing suggestive evidence of a possible association between sRANKL concentrations and breast cancer. High circulating levels sRANKL in the context of low OPG may represent novel risk markers for hormone receptor positive breast cancer.

**Table 1. Circulating concentrations of sRANKL and breast cancer risk by hormone-receptor subtype: EPIC nested case-control study**

<table>
<thead>
<tr>
<th>Quintiles</th>
<th>ER+/PR+</th>
<th>ER-/PR-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases/Controls</td>
<td>167/198</td>
<td>173/203</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>ref.</td>
<td>1.19</td>
</tr>
<tr>
<td>(0.85-1.60)</td>
<td>(0.90-1.47)</td>
<td>(0.87-1.60)</td>
</tr>
<tr>
<td>sRANKL/OPG Ratio</td>
<td>ER+/PR+</td>
<td>ER-/PR-</td>
</tr>
<tr>
<td>Cases/Controls</td>
<td>146/177</td>
<td>175/181</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>ref.</td>
<td>1.11</td>
</tr>
<tr>
<td>(0.85-1.52)</td>
<td>(0.90-1.52)</td>
<td>(0.75-1.51)</td>
</tr>
</tbody>
</table>
| #4960 Recent ultraviolet radiation exposure and circulating immunosuppressive T-regulatory cells
Dana E. Rollison,1 Shalaka S. Hampras,3 Jane L. Messina,1 Neil A. Fenske,2 Basil S. Chepulis,3 Michael J. Schell,1 Rhianna Reed,1 Juliana Balliu,1 Rebecca Hesterberg,1 Afua A. Akuffo,1 Pearl K. Burnette1.

Background: Ultraviolet radiation exposure (UVR) is a risk factor for several different cancer types. While the mutagenicity of UVR is well-documented, UVR’s effects on human systemic immune function are not well understood. Several lines of evidence suggest that UVR may have immunosuppressive effects, however, no epidemiologic studies have investigated the association between quantitative measures of UVR and a biomarker of immune function. Objective: To evaluate the association between UVR and systemic immune function, we conducted a cross-sectional analysis of recent UVR and circulating regulatory T- cells ("Tregs") among a cohort of skin cancer screening patients. Methods: 350 patients undergoing regular skin cancer screenings with no prevalent skin cancer at the time of enrollment were recruited from the University of South Florida Dermatology clinic. At the baseline visit, blood was obtained, and a spectrophotometer was used to measure skin pigmentation at a sun-unexposed site (the axilla) and two sun-exposed sites: the forehead and the upper forearm. Recent sun exposure was quantified as the difference in readings between the sun-exposed and sun-unexposed sites. Using flow cytometry assays, lymphocyte samples were examined for Tregs (CD4+ CD25+ CD127hlowsFoxp3+), and proportions of Tregs with enhanced immunosuppressive function (CD27-, CD45RA- Treg) and skin tissue affinity (CLA +, CCR4+CD11c+) were...
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determined. Associations between UVR exposure and Tregs were described using Spearman correlation coefficients. Linear regression using log-transformed values of UVR and Tregs was used to adjust for age and sex. Results: Among all 350 participants, UVR was positively correlated with circulating immunosuppressive CD27-, CD45RA- Tregs (r = 0.127, p = 0.020 for UVR measured at the forehead and r = -0.193, p = 0.001 for UVR measured at the arm) and was also positively correlated with skin-homing Tregs expressing CLA (r = 0.167, p < 0.0002) and CCR4 (r = 0.19, p = 0.0004). When participants were categorized into light versus dark constitutive pigmentation based on the median value of spectrophotometer readings for the sun-unexposed axilla, the correlation between UVR averaged across the forehead and forearm was more strongly correlated with immunosuppressive Tregs among dark-skinned participants (r = 0.30, p = 0.001) than darker-skin participants (r = 0.065, p = 0.400). Conclusion: Recent UVR is positively associated with increased levels of circulating immunosuppressive Tregs, including Tregs with skin tissue affinity, with the association being stronger among naturally lighter-skinned individuals. UVR-associated immunosuppressive effects among otherwise immunocompetent individuals may play a role in skin cancer carcinogenesis.

#4961 The oral microbiome and prospective risk for esophageal cancer: a population-based nested case-control study. Brandilyn A. Peters,1 Jing Wu,1 Zhiheng Pei,1 Liying Yang,1 Mark P. Purduce,1 Neil D. Freedman,2 Eric J. Jacobs,3 Susan M. Gapstur,3 Richard B. Hayes,1 Jiyoung Ahn1.

Background: The two most common types of esophageal cancer, esophageal adenocarcinoma (EAC) and squamous cell carcinoma (ESCC), are highly fatal. The human microbiota have been suggested to play a role in esophageal cancer etiology, although the evidence is limited to small, cross-sectional studies. We hypothesized that the oral microbiota, which shape the esophageal microbiota, may be causative agents in esophageal carcinogenesis. Methods: We conducted a prospective study nested in two large U.S. cohorts: ACS CPS-II and NCI-PCLC. Oral bacteria were assessed in pre-diagnostic mouth-wash samples collected from cases and controls (n = 81/160 EAC and n = 25/50 ESCC cases/ matched controls), using 16S rRNA gene sequencing. We compared overall microbial composition between cases and controls using permutational multivariate analysis of variance (PERMANOVA) of UniFrac distances, and we examined associations between centered log-ratio transformed taxon abundances and cancer risk using conditional logistic regression. Metagenome functional content was predicted from taxonomic composition using PICRUSt. Results: Overall microbial composition did not differ between EAC cases and matched controls or ESCC cases and matched controls, adjusting for matching factors (age, sex, race, cohort, time to diagnosis/selection), BMI, smoking, and alcohol intake (all p > 0.40). The periodontal pathogens Tannerella forsythia and Porphyromonas gingivalis were nominally associated with increased risk for EAC (OR [95% CI] = 1.21 [1.01, 1.46], p = 0.04) and ESCC (OR [95% CI] = 1.3 [0.96, 1.77], p = 0.09), respectively. Conversely, genus Neisseria, previously shown to be depleted by cigarette smoking, was associated with protection against EAC (OR [95% CI] = 0.88 [0.68, 0.97], p = 0.01). Other species associated with EAC risk (p < 0.05) included Prevotella nanceiensis, Bergeyella oral taxon 322, and Neisseria weaveri (positively associated with EAC) and Actinomyces cardiffensis, Selenomonas oral taxon 134, and Veillonella oral taxon 917 (positively associated with EAC). Other species associated with ESCC risk (p < 0.05) included Aggregatibacter paraphrophilus (inversely associated with ESCC) and Prevotella naeckeii, Bacteroides oral taxon 322, and Neisseria weaveri (positively associated with ESCC). Analysis of inferred metagenomes revealed that bacterial carotenoid biosynthesis was associated with protection against ESCC (OR [95% CI] = 0.84 [0.7, 1.0], p = 0.05). Conclusions: Our findings from this prospective study suggest that specific bacterial pathogens may play a causal role in esophageal cancer, while members of the healthy oral microbiota may protect against esophageal carcinogenesis. Unique microbial profiles may contribute to each of the distinct esophageal cancer types, EAC and ESCC. Oral microbiota manipulation may be a future strategy for preventing this highly fatal disease.

#4962 Geographic disparities in prevalence of baseline prostate inflammation and prostate cancer risk: results from a multinational trial. Emma H. Allott,1 Sarah Markt,2 Lauren E. Howard,7 Adriana C. Vidal,2 Daniel M. Moreira,2 Ramiro Castro-Santamaria,2 Gerald L. Andriole,3 Lorelei A. Mucci,4 Stephen J. Freedland,4 University of North Carolina at Chapel Hill, Chapel Hill, NC; 5Harvard School of Public Health, Boston, MA; 6Duke University, Durham, NC; 7Cedars Sinai Medical Center, Los Angeles, CA; 8University of Illinois at Chicago, Chicago, IL; 9GlaxoSmithKline, King of Prussia, PA; 10Washington University, St. Louis, MO; 11Harvard University, MA; 12Cedars Sinai Medical Center, CA.

Introduction: Prostate cancer incidence rates vary 25-fold worldwide. The distribution of lifestyle factors also varies by geographic region and these factors may impact prostate inflammation, which is inversely associated with prostate cancer risk in the REDuction of prostate Cancer Events (RE- DUCE) trial. Herein, we examined geographic differences in the prevalence of baseline prostate inflammation and prostate cancer risk using REDUCE, a multinational trial of men with a negative baseline prostate biopsy. Methods: We conducted a retrospective analysis of data from 7,213 men with a negative baseline prostate biopsy in REDUCE from Europe (n = 4,802), North America (n = 1,796), South America (n = 467), and Australia/New Zealand (n = 148). Histological inflammation was classified as chronic (lymphocytes, macrophages) or acute (neutrophils) by central review of negative baseline prostate biopsies. Logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (95% CI) for associations between geographic region and prostate inflammation, and between geographic region and prostate cancer risk at trial-mandated repeat biopsy, adjusting for potential founders. To avoid confounding by race, analyses were restricted to white men. Results: Chronic and acute prostate inflammation was detected in 77% and 15% of men, respectively. Relative to Europeans, North Americans and Australians/New Zealanders were more likely to have acute prostate inflammation in the negative biopsy (OR 1.74; 95% CI 1.48-2.05 and OR 2.04; 95% CI 1.38-3.02, respectively), while South Americans were less likely to have acute inflammation (OR 0.42; 95% CI 0.28-0.61). Among North Americans, Canadians were more likely to have acute prostate inflammation than men from the United States (OR 1.40; 95% CI 1.07-1.83). Among Europeans, the prevalence of acute inflammation was lower in Northern, Southern and Eastern Europe, relative to Western Europe (OR 0.79; 95% CI 0.65-0.97, OR 0.84; 95% CI 0.66-1.07 and OR 0.62; 95% CI 0.45-0.87, respectively), with similar results for chronic inflammation. Regions with higher prevalence of prostate inflammation had lower prostate cancer risk at 2-year biopsy, including North America (OR 0.87; 95% CI 0.71-1.07) and Australia/New Zealand (OR 0.48; 95% CI 0.24-0.95), relative to Europe. Conversely, regions with lower prevalence of prostate inflammation had higher prostate cancer risk at 2-year biopsy, including Northern and Eastern Europe (OR 1.30; 95% CI 1.05-1.62 and OR 1.74; 95% CI 1.29-2.35, respectively), relative to Western Europe. Conclusions: Geographic disparities in the prevalence of prostate inflammation is a potential biologic mechanism contributing to global differences in prostate cancer incidence rates.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Novel Approaches for Experimental Therapeutics


Introduction: The co-stimulatory receptor GITR plays an important role in initiating the immune response in the lymph nodes and in maintaining the immune response in the tumor tissue. Binding of GITR to its natural ligand directly leads to increased anti-tumor T cell activation and their survival. It also reduces the suppressive abilities of Treg cells, further increasing the anti-tumor immune response. The HERA-technology developed by Apogenix targets the TNF-receptor superfamily and generates fully human hexavalent fusion proteins with high clustering capacity for the cognate receptor. Hexavalent HERA-ligands are pure agonists whose signaling capacity is independent of secondary Fcγ-receptor crosslinking. Here we report in vitro and in vivo properties of novel hexavalent HERA-GITR constructs. Experimental procedures: For the assessment of in vivo stability, serum samples from a PK study with three HERA-GITR constructs in CD1-mice were analyzed with respect to their drug levels employing a specific ELISA assay. For functional characterization of HERA-GITR in vitro, immune cells were isolated from healthy-donor blood samples and profiled by multicolor flow cytometry (MC-FC). Subsequently, immune cells were cultured in growth media containing different HERA-GITR constructs and anti-C3D. Changes in activation and memory markers on T cells (e.g. CD25, CD69, CD45RA, CD45RO), their proliferation rate (CFSE assay) and the intracellular staining of cytokines (e.g. TNF-α and IFN-γ) was assessed by MC-FC. Results: Minor modifications led to three HERA-GITR drug candidates with unique pharmacokinetic properties / in vivo stability as explored in mice. Terminal half-life was between 61.7 and 200.6 hours. Stimulation of pan T cells as well as naïve CD4+ T-lymphocytes by anti-CD3 was further augmented by HERA-GITR as demonstrated by CD69 and CD25 expression. This effect was accompanied by an increased proliferation and an increased memory formation. Furthermore, we ob-
served an increased level of intracellular TNF-α and IFN-γ in naïve CD4+ T-lymphocytes incubated with anti-CD3 that could be further raised by the addition of dendritic cells (EC50 in a co-culture of T cells and human cancer cells and in a mixed lymphocyte reaction). The enzyme indoleamine 2,3-dioxygenase 1 (IDO1) catalyzes the degradation of tryptophan along the kynurenine pathway, and is frequently expressed in human malignancies. The activity of IDO1 induces an immunosuppressive microenvironment in tissues by inhibiting T-cell function through local depletion of tryptophan and induction of generation of kynurenine pathway metabolites. Inhibition of IDO1 is expected to diminish the immunosuppressive microenvironment and improve cancer patient outcomes, particularly when used in combination with cancer immunotherapy agents such as nivolumab and ipilimumab. In this presentation, we will disclose the chemical structure, enzyme inhibitory mechanism, in vitro potency and in vivo pharmacodynamic (PD) activity of BMS' IDO1 inhibitor currently in Phase I clinical trials. The compound is a potent and selective IDO1 inhibitor with no activity against another tryptophan degrading enzyme, tryptophan 2,3-dioxygenase (TDO). It exhibited potent cellular activity, suppressing kynurenine production in HEK293 cells overexpressing human IDO1 (IC50 = 1.1 nM) and in HeLa cells stimulated with IFNγ (IC50 = 1.7 μM). The compound also potently restored T-cell proliferation in a co-culture of T cells and human cancer cells and in a mixed lymphocyte reaction where T cells were co-cultured with allogeneic IDO1-expressing dendritic cells (EC50 = 1.2 μM). In vivo, when given once a day orally, the compound exhibited significant PD activity in mouse tumors grown subcutaneously in syngeneic hosts and in human tumors grown as xenografts in nude mice.


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**#4965** Sustained tumor regressions and significant improvements in therapeutic index for a lead monomethyl auristatin E nanoparticle-drug conjugate, CRLX701, with the potential for improved anticancer effects. Liang Zhao, Roy I. Case, Donna Brown, Lata Jayaraman, Doug Lazarus, Christian Peters, Derek Van Der Poll, Ellen Rohde, Scott Eliasof, Chester A. Metcalfe. Cerulean Pharma, Waltham, MA.

Cerulean Pharma Inc. creates nanoparticle-drug conjugates (NDCs) designed to significantly mitigate a payload's limitations by providing sustained drug delivery to the tumor and superior therapeutic index through controlled release kinetics. By conjugating drug payloads to our novel β-cyclodextrin-PEG (CDP) copolymer through linker strategies that allow modulation of release and pharmacokinetics (PK), we provide advantages over entrapped nanoparticle strategies, e.g., polymeric nanoparticles and liposomes. Cerulean has two NDCs in the clinic, CRLX101 and CRLX301, evidencing the translatability of our technology. CRLX701 has been dosed in over 400 patients and CRLX301 is in an ongoing Phase 1/2a trial. A key and differentiating feature of our NDC platform is our linker technology, which is tailored for an optimal fit with the active pharmaceutical ingredient (API) conjugation functionality of the drug payloads we incorporate into NDCs and customizable to achieve desired drug release profiles. This linker technology works for most conjugatable APIs that can be linked through alcohol, carboxylic acid, amine, amide and urea functionality to our CDP polymer. To illustrate the capabilities of our NDC platform we will present the biological impact of a series of monomethyl auristatin E (MMAE)-containing NDCs. MMAE belongs to the dolastatinfamily of natural cytotoxic pseudopeptides, demonstrating potent disruption of tubulin polymerization and excellent in vitro anti-cancer properties. However, because of its toxicity MMAE cannot be used as a drug on its own. Our novel linkers provided the synthesized MMAE NDCs with sustained and controlled release kinetics, targeting low release rates (0.4-13%) to minimize toxicity of the MMAE-drug payload. Subsequent PK studies revealed that over MMAE, indeed, this improved tumor PK for CRLX701 translated into improved efficacy in a human prostate DU-145 xenograft model, in which sustained tumor regressions (6 of 8 cures) were observed for CRLX701 (4 mpk, qwx2) compared to reduced and unsustainable efficacy for MMAE (0.5 mpk, qwx2) at MTD doses. Cerulean continues to expand its platform through the development of new and emerging capabilities, including the conjugation of multiple payloads to a single NDC and the development of antibody-conjugated NDCs, to treat patients living with cancer.

**#4966** Phospho-proteome analyses confirm the unique Mode of Action of MP0274, an apoptosis inducing, biparatopic HER2-targeting DARPin drug candidate. Vikram Mitra, Ulrike Fiedler, Dan Snedl, Keith M. Dawson, Stephan Jung, Ian Pike, Elmar von Bauer. Proteome Sciences, London, United Kingdom; Molecular Partners AG, Schlieren-Zurich, Switzerland; Proteome Sciences, Frankfurt a. M., Germany.

Background: HER2 is an important target for antitumor therapy in several cancers, and while currently available HER2-targeting drugs provide a great treatment improvement they rarely achieve full disease control. We have developed a new HER2-targeting molecule with a unique pro-apoptotic mode of action that may provide additional benefit to patients. The DARPin® molecule MP0274 shows potency in several HER2-expressing PDX models and has been shown to directly induce apoptosis in cells that are addicted to HER2. Here we show through phospho-proteome analyses that MP0274 not only potently inhibits HER2/HER3 downstream signaling, but also shows a very distinct changes in the phospho-proteome pattern compared to approved HER2-targeting drugs. This provides mechanistic support to the unique mode of action of MP0274 that results in direct tumor cell killing without the need of effector-functions like ADCC. Methods: The effect of MP0274 on HER2 signaling was compared to trastuzumab, pertuzumab and a combination of both in the HER2-addicted cancer cell lines. Briefly, cells were incubated with drugs for 18 hours and then lysed. Lysates were analyzed for changes in the total with Western blot and phospho-proteins by Proteome Sciences' proprietary QuantGlobal Phosphoproteomics workflow. Results: Heat-maps of genes of interest indicate that MP0274 has a differential mode of action compared to trastuzumab, pertuzumab and a combination of both. On the total peptide and phospho-peptide level, the samples cluster specifically, based on the cancer cells used as well as drug treatment. Twenty nine unique global phosphorylation sites specific to HER2, including C-terminal tyrosines which are reported to recruit adaptor proteins starting signal transduction processes after auto-phosphorylation, were identified for MP0274. Several proteins were identified which were differentially expressed and phosphorylated after MP0274 treatment and which are involved in three key downstream signaling pathways activated by HER2/HER3 heterodimers: RAF/MAP kinase cascade, PI3K-induced AKT signaling, and signaling by PLCG1. Conclusions: MP0274 shows a unique and distinct inhibition of the HER2 signaling cascade, different from trastuzumab, pertuzumab and a combination of both. It induces a more profound inhibition of downstream signaling which provides mechanistic support to the finding that MP0274 direct cell killing by induction of apoptosis in HER2-addicted tumor cells. DARPin®s are small repeat proteins, designed to bind targets with high affinity and specificity, and which can be combined in a modular fashion to produce multifunctional agents.


While targeting mutations in the ERAS oncogene frequently drive tumorigenesis in human cancers (40% CRC, 20% NSCLC) through constitutive activation of the MAPK pathway, there are currently no targeted treatments available for KRAS mutant cancers. Inhibitors of the individual nodes of the MAPK pathway have been developed, but these molecules have been largely ineffective against KRAS mutant tumors in the clinic. Multiple studies have shown rational combinations of MAPK inhibitors may have anti-tumor activity in KRAS mutant models. In order to understand the versatility of combining RAF inhibitors in this context, we conducted a library screen consisting of 430 small molecule tool compounds in combination with RAF inhibitor AZ-628. Here we show: RAF inhibitors combine especially well with other MAPK pathway inhibitors in KRAS mutant tumor cells. In particular, Type II RAF inhibitors are synergistic with the MEK inhibitor Cobimetinib. In vitro and in vivo screening shows a superior preclinical efficacy in xenograft mouse model studies in vivo. Mechanically, we have found the MEK inhibitor disables ERK induced negative feedback on the MAPK pathway resulting in activation of CRAF in a KRAS dependent manner. The combination of RAF with MEK inhibition blunts KRAS-dependent activation of CRAF kinase activity and robustly inhibits MAPK signaling, thereby driving efficacy in KRAS mutant tumors. Broad cell line profiling with the combination of RAF and MEK inhibitors demonstrates that a majority of KRAS mutant lung and colorectal tumor lines exhibit synergy with the combination. Therefore, combining a Type II pan-RAF inhibitor with a MEK inhibitor has the potential to improve the therapeutic outcome in KRAS mutant cancers.

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EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Novel Approaches for Experimental Therapeutics

#4968 Importance of tumor microenvironment in the preclinical estrogen receptor positive breast cancer: Primary tumor and bone metastasis models. Jenni Bernoulli,1 Mari I. Suominen,2 Tiina Kähkönen,1 Jenni Mäki-Jouppila,1 Jussi M. Halleen,1 Rikuja Oksala3. 1Pharmatest Services Ltd, Turku, Finland; 2Orion Corporation Orion Pharma, Espoo, Finland.

Tumor microenvironment (ER +) breast cancer has ability to metastasize to bone in high frequency. Bone is known to be fertile soil for metastasized cancer cells to survive and in turn, metastasized tumor cells alter normally balanced bone environment. Prevention and treatment of bone metastasis is challenging and better understanding why bone metastasis are resistant to current therapies is needed. Aim of the present study was to explore the role of tumor microenvironment in the preclinical estrogen receptor positive breast cancer models. To explore this we compared estrogen and microenvironment on growth of primary primary tumor and bone metastasis to establish predictive ER + breast cancer models for drug development. ER + human breast cancer MCF-7 cells were inoculated into mammary fat pad and in the tibia of female athymic nude mice. Mice received either hormonal supplementation (17-beta estradiol pellet, E2) or placebo. Tumor growth was followed for 5 and 9 weeks. From one group, E2 supplementation was removed on study week 5 and tumor growth was followed for 4 weeks. During the study, blood samples were collected for serum steroid concentration measurements and at the end, histopathological evaluation and immunohistochemical (IHC) stainings were performed to examine tumor steroid hormone receptor expression. Orthotopic MCF-7 tumor growth was clearly hormone dependent. E2 supplementation, that increased serum estradiol for app. 3-fold, supported tumor growth and when E2 was removed, tumor size reduced and no tumor growth was observed thereafter. In the full absence of E2 supplementation, no orthotopic tumor growth was observed. In contrast, when MCF-7 cells were inoculated into tibia, tumor growth was observed both with and without E2 supplement. IHC stainings confirmed orthotopic tumor to express ER, PR and AR when animals received E2. After E2 removal, orthotopic tumors expressed still ER and AR but no longer PR. Also intratibial tumors expressed ER and PR in the presence of E2, but no PR in the absence of E2 supplement. E2 is needed to support MCF-7 tumor growth when cancer cells are inoculated orthotopically into mammary fat pad. No orthotopic tumor growth is observed in the absence of E2 supplement. In contrast, in the bone microenvironment, MCF-7 cells form tumor even in the absence of E2 supplement. Results highlight importance of tumor microenvironment in the breast cancer progression and also refer why tumor in the different sites may be resistant to therapy. Taking together, when developing new therapies against breast cancer, focus should be addressed not only on primary tumor growth but also on bone metastasis where cancer cells are under influence of bone environment.

#4969 Impaired PARP1 DNA repair defines chemosensitivity in IDH1 mutant cell. Yanxin Lu, Yang Liu, Yu-Ting Su, Wendy Bautista, Mark R. Gilbert, Jing Wu, Chunzhang Yang, Center for Cancer Research, National Cancer Institute, Bethesda, MD.

INTRODUCTION: Mutations in isocitrate dehydrogenase (IDH1/2) are the most prevalent genetic abnormalities in lower grade gliomas. The presence of IDH mutations in glioma are prognostic for better clinical outcomes with longer patient survival, and greater sensitivity to chemotherapy. In the present study, we explore the molecular mechanisms that determine the chemosensitivity in IDH1-mutated glioma cells, and seek a potential therapeutic strategy by targeting DNA repair pathway in IDH1-mutated cells. METHODS: We established IDH1-mutated glioma cell lines by introducing pathogenic IDH1 mutations into glioma cells via lentivirus. We confirmed the metabolic deficiency and enhanced chemosensitivity in cells expressing IDH1 mutant enzyme. Further, we measured the intracellular level of nicotinamide adenine dinucleotide (NAD+), Poly (ADP-ribose) polymerase (PARP)-associated DNA repair and DNA damage level in these cells when treated with temozolomide (TMZ). Moreover, we evaluated a PARP inhibitor Olaparib and its synergic effect on TMZ-associated cytotoxicity. RESULTS: Our results show that the IDH1-mutated cells are more vulnerable to chemotherapy. TMZ treatment resulted in over 20-fold increase of cell death in IDH1-mutated cells compared with their wild type counterpart. IDH1-mutated cells exhibited an over 1.3-fold increase in DNA damage and over 1.42-fold increase in cellular apoptosis under TMZ treatment. Accordingly, IDH1-mutated cells produced 83.8% less poly (ADP-ribose) polymer (pADPR), an important substrate for PARP-associated DNA repair, during TMZ treatment; suggesting their incompetence to maintain genomic integrity due to decreased availability of NAD+. Targeting the PARP-associated DNA repair pathway using Olaparib remarkably potentiated TMZ-induced cytotoxic effects by 2.16 fold in IDH1 mutated cells. CONCLUSION: Our findings demonstrate that metabolic defects in IDH1-mutated cells affect PARP-associated DNA repair pathway via NAD+ depletion, and therefore prompt the sensitivity to chemotherapy. Targeting PARP-associated DNA repair pathway may represent a novel therapeutic avenue for IDH1-mutated gliomas.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Novel Small Molecular Targets and Approaches

#4970 Oxidative phosphorylation as a target in triple negative breast cancer therapy. Funda Meric-Bernstam,1 Kurt Evans,1 Xiaoqinf Zheng,1 Xiaoping Su,2 Erkan Yuca,3 Stephen Scott,1 Argun Akaakanat,1 Naoto Ueno,1 Bora Lim,1 Jennifer Litton,1 Vicente Valero,1 Fraser Symmans,1 Gabriel Hortobagyi,1 Charles Perou,2 Debu Tripathy,1 Guillio Draetta,1 Joe Marszalek,1 Ana Maria Gonzalez-Angulo,1 Stacy Moulder1. 1The University of Texas MD Anderson Cancer Center, Houston, TX; 2The University of North Carolina, Chapel Hill, NC.

Altered cellular metabolism is a hallmark of cancer. It is increasingly recognized that selected tumors are dependent on oxidative phosphorylation (OXPHOS). However, the role of OXPHOS in TNBC is not well understood. We performed RNA sequencing in pre-treatment biopsies from 43 patients with operable triple negative breast cancer (TNBC) who received sequential taxane- and anthracycline-based neoadjuvant chemotherapy. At a median follow-up of 63 months, 14 patients recurred and 12 patients died. At a false discovery rate of 0.05, 33 genes were differentially expressed between the patients who did and did not have a subsequent recurrence. Ingenuity pathway analysis demonstrated that one of the top canonical pathways that differed was higher expression of oxidative phosphorylation signature (p=5.89E-0.7). The patients who recurred had significantly higher levels of mitochondrial genes: MT-ND1 (adjusted p or FDR-BH; q=0.007); MT-ND5 (q=0.03) and MT-ND4 (q=0.04). Further, 21 genes were differentially expressed between patients based on survival, including MT-ND5 (q=0.001); MT-ND4 (q=0.005), MT-ND4L (q=0.015), MT-ND6 (q=0.018), and MT-ATP6 (P=0.03). Top canonical pathway that was differentially expressed based on survival was oxidative phosphorylation (p=9.98E-10). We therefore sought to determine the efficacy of IACS-10759, a novel inhibitor of OXPHOS, in 10 different TNBC patient-derived xenografts representing different gene expression based Lehmann TNBC subtypes. Growth inhibition was observed in multiple subtypes, with regression in one basal-like 1(BL1) 1 model, and stabilization of growth in multiple BL1 and immunomodulatory expression subtypes. Taken together, our data suggests that high OXPHOS is associated with higher recurrence and lower survival. OXPHOS is a promising target in several TNBC subtypes. A Phase I trial of IACS-10759, a potent inhibitor of complex I of oxidative phosphorylation, in leukemia is ongoing and planned in TNBC and other solid tumors.

#4971 IACS-010759, a novel inhibitor of complex I in Phase I clinical development to target OXPHOS dependent tumors. Jennifer Molina,1 Madhavi Bandi,1 Jennifer Bardenhagen,1 Christopher Bristow,1 Christopher Carroll,1 Edward Chang,1 Jason Cross,1 Naval Davel,1 Ningping Feng,1 Jason Gay,1 Mary Geck Do,1 Jennifer Greer,1 Jing Han,1 Judy Hirst,1 Sha Huang,1 Yongying Jiang,1 Zhiyu Kang,1 Marina Konopleva,1 Gang Liu,1 Helen Ma,1 Polina Matre,1 Timothy McAfoos,1 Funda Meric-Bernstam,1 Kurt Evans,1 Xiaofeng Zheng,1 Xiaoping Su,2 Erkan Yuca,3 Stephen Scott,1 Argun Akakanat,1 Naoto Ueno,1 Bora Lim,1 Jennifer Litton,1 Vicente Valero,1 Fraser Symmans,1 Gabriel Hortobagyi,1 Charles Perou,2 Debu Tripathy,1 Guillio Draetta,1 Joe Marszalek,1 Ana Maria Gonzalez-Angulo,1 Stacy Moulder1. 1The University of Texas MD Anderson Cancer Center, Houston, TX; 2The University of North Carolina, Chapel Hill, NC; 3Pharmatest Services Ltd, Turku, Finland.

Small Molecular Targets and Approaches
The RAS/MAPK pathway is dysregulated in approximately 30% of human cancers, and the extracellular-signal-regulated kinases (ERK1 and ERK2) serves as key central nodes within this pathway. The feasibility and clinical impact of targeting the RAS/MAPK pathway has been demonstrated by the therapeutic success of BRAF and MEK inhibitors in BRAF V600E/K metastatic melanoma. However, resistance development is a significant hurdle for the therapeutic potential of these compounds. A new strategy for targeting of multiple effectors such as RAF, MEK and ERK in this pathway offers a potential for enhanced efficacy while delaying and overcoming resistance. LY3214996 is a highly selective inhibitor of ERK1 and ERK2, with IC_{50} of 5 nM for both enzymes in biochemical assays. It potently inhibits cellular phospho-RSK1 in BRAF and RAS mutant cancer cells in an unbiased tumor cell panel sensitivity screen. LY3214996 targets and inhibits Ras-driven lung tumor cells. In molecularly defined subsets of these indications are dependent on OXPHOS. Furthermore, targeting of multiple effectors such as RAF, MEK and ERK in this pathway offers a potential for enhanced efficacy while delaying and overcoming resistance. LY3214996 is a highly selective inhibitor of ERK1 and ERK2, with IC_{50} of 5 nM for both enzymes in biochemical assays. It potently inhibits cellular phospho-RSK1 in BRAF and RAS mutant cancer cells in an unbiased tumor cell panel sensitivity screen. LY3214996 targets and inhibits Ras-driven lung tumor cells. In molecularly defined subsets of these indications are dependent on OXPHOS. Furthermore, targeting of multiple effectors such as RAF, MEK and ERK in this pathway offers a potential for enhanced efficacy while delaying and overcoming resistance. LY3214996 is a highly selective inhibitor of ERK1 and ERK2, with IC_{50} of 5 nM for both enzymes in biochemical assays. 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these highly refractory cells. Analysis identified genetic alterations associated with vulnerabilities targeted by small molecules. About 85% of GBM patients display p53 pathway disruption, our results suggest independent pharmacological strategies for two different subtypes of GBM defined by TP53 and CDKN1A status. Our analyses provide molecular insights to drive targeted therapies in the new era of precision medicine.

#4975 A small molecule pan Id protein antagonist shows strong antitumor activity. Paulina M. Wojnarowicz,1 Bina Desai,1 Yvette Chin,3 Sang Bae Lee,2 Marta Garcia-Cao,1 Ousathek Ouerfelli,1 Guangyi Yang,3 Sijia Xu,4 Yehuda Goldgur,5 Meredith A. Miller,1 Jaideep Chaudhary,1 William A. Garland,4 Steven K. Albaneze,1 Rajesh Soni,1 John Phillips,3 Larry Norton,4 Neal Rosen,5 Ronald C. Hendrickson,1 Xi Kathy Zhou,1 Antonio Iavarone,6 Andrew J. Dannenberg,6 John D. Chodera,7 Nikola Pavletic,1 Anna Lasorella,5 Robert Benetra.1

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The Id family of helix-loop-helix (HLH) proteins, Id1, Id2, Id3 and Id4, play a critical role in inhibiting differentiation during mammalian embryogenesis. They function in part by sequestering ubiquitously expressed E protein bHLH transcription factors via direct protein-protein interactions. Various Id proteins are re-expressed in adults in a number of pathologic states including cancer and diseases of the vasculature, where their activity has been shown to be essential for disease progression. The present study describes the solving of the Id1-E47 dimer crystal structure and subsequent development and characterization of a small molecule antagonist of the Id protein family. AGX3, AGX51 was identified in an in silico screen for compounds that could bind a hydrophobic crevice adjacent to the loop region of Id1. Highly conserved in the Id family, AGX3 inhibits the endogenous Id1-E protein interaction leading to the degradation of Id1 via ubiquitin-mediated proteolysis. The stability of all four members of the Id family are antagonized by AGX3 leading to a G0-G1 arrest and profound inhibition of viability with no acquired resistance observed in multiple cell lines after continuous exposure to the compound. Administration of AGX3 is well tolerated in mice and phenocopies genetic loss of Id expression analyses: suppression of breast cancer metastases to the lung associated with a reduced mesenchymal-to-epithelial transition, perturbation of the vasculature within the primary tumor, and growth regression of paclitaxel resistant breast tumors in combination with paclitaxel therapy. These studies identify a novel, first-in-class compound capable of antagonizing the activity of a protein family formerly considered undruggable and to the possible utility of AGX3 in the management of multiple disease processes in patients.

#4976 Ricolinostat, a selective HDAC6 inhibitor with immunomodulatory properties, has significant anti-melanoma activity in vitro and in vivo. Fengdong Cheng,1 Marta Gugliuzza,2 Jie Chen,1 Alejandro Villagra,1 David Woods,1 Jeffrey Weber,1 Steven Quayle,1 Jones Simon,1 Eduardo Sotomayer1

1The George Washington University, Washington, DC; 2Memorial Sloan Kettering Cancer Center, NYU Langone Medical Center, New York, NY; 3Acetylon Pharmaceuticals, Boston, MA.

Melanoma is a less common but the most aggressive type of skin cancer and the rates of melanoma have been rising for the last 30 years. Immunotherapy may represent a new treatment paradigm to benefit melanoma patients. Previous studies have identified HDAC6 playing a central role in the regulating melanoma immunogenicity. We have shown that genetic knockdown (KD) of HDAC6 in murine and human melanoma cells resulted in an increased the expression of MHCI and co-stimulatory molecules as well as melanoma associated antigens. In vivo studies demonstrated that the growth of melanoma cells lacking HDAC6 was significantly delayed as compared to wild-type cells. This growth was further delayed when HDAC6KD melanoma bearing mice were treated with anti-PD-1 antibodies. Recently, we have shown that in vitro treatment of murine melanoma cells with Ricolinostat decreases PD-L1 expression and increases the expression of acetyl tubulin. Surprisingly, in vivo treatment of B16 melanoma bearing animals with increasing concentrations of ricolinostat resulted in a dose-dependent inhibition of melanoma growth (p<0.05). No toxicities were observed at the doses studied; however, the antitumor effect exerted by ricolinostat was not observed when melanoma bearing SCID mice were treated with this compound, indicating that an intact host immune system is required for the observed antitumor activity. In lieu of these previously unknown immunomodulatory properties of ricolinostat, we next assessed the effects of this compound upon T cells and whether ricolinostat could augment the efficacy of checkpoint blockade in vivo. Murine T-cells were activated with anti-CD3 plus anti-CD28 in the presence or absence of ricolinostat. Then, those T-cells were adoptively transferred into B16 melanoma bearing mice. T-cell subpopulations from the lymph nodes were analyzed ex vivo. We show here that treatment of murine T-cells with ricolinostat resulted in a significant increase in central memory T-cells endowed with a strong anti-melanoma activity in vivo as compared to control group (p<0.05). Finally, the addition of ricolinostat to treatment either anti-CTLA4 or anti-PD1 treatment was associated with an enhanced inhibition of melanoma tumor growth. In summary, our results have identified HDAC6 as a novel target for melanoma immunotherapy and point out ricolinostat as an attractive agent to add to the immuno-oncology armamentarium.

IMMUNOLOGY: Adoptive Cellular Therapy for Cancer

#4977 Isolation and characterization of a PRAME-specific TCR with high avidity, potent anti-tumor efficacy and a favorable preclinical safety profile. Manon Weis, Carina Wehner, Christian Ellinger, Susanne Wilde, Dolores J. Schendel.

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Some of the challenges regarding adoptive T cell transfer as an immunotherapeutic approach for the treatment of cancer patients include the initial induction, isolation and later characterization of suitable TCR candidates. In addition, ensuring the expression of appropriate target antigens is important and complex as such antigens need to meet some critical demands. Published data as well as in depth in silico analyses revealed the tumor associated antigen PRAME a suitable candidate. Classified as cancer/testis antigen, PRAME is highly expressed in tumors with only limited or no expression in normal tissues, other than testis. Here we describe the induction of PRAME-specific, HLA-A*02:01 restricted CD8-positive T cells by using an in vitro priming approach. PRAME-specific T cells transfected with ivtRNA encoding full-length PRAME were cocultured with autologous CD8-enriched PBL from a healthy, HLA-A*02:01-positive blood donor. Initially primed T cells were expanded for 2 weeks and T cells bearing TCRs specific for PRAME-derived epitopes were identified by multimer staining and FACS analysis. Single cells were sorted into 96 well plates and after an expansion period of another 2 weeks, growing clones were screened for specificity. TCR sequences of specific clones were identified by using NGS and reconstructed in a retroviral vector system enabling stable transduction of effector cells. The resulting TCR-modified T cells were thoroughly analyzed and characterized using a dedicated set of assays to evaluate multiple parameters concerning safety and efficacy. This characterization revealed TCR T4.8-1-29 to be highly specific for a PRAME-derived epitope presented on HLA-A*02:01. Only epitope positive target cells expressing the appropriate HLA-RESTRICTION element induced cytokine secretion by T4.8-1-29 expressing effector cells, leading to recognition as well as specific lysis of these target cells. Recognition and lysis, not just of peptide-loaded T2 cells, but also of PRAME-transfected APC, transfected tumor cells as well as autologous PRAME-enogenous tumor cells was mediated by this TCR. T4.8-1-29 expressing effectors show a high natural avidity for the target epitope without the need of further manipulation, e.g. functional efficacy enhancement by affinity maturation. In addition, the toxicity assessment using various in vitro and in silico tools revealed a favorable preclinical safety profile for this TCR. In summary, by using an in vitro priming approach utilizing cells from healthy donors, it is possible to generate PRAME-derived epitope specific T cells with high natural avidity and specificity. The TCR shows a favorable safety profile, demonstrating that this approach is useful to generate TCRs potentially qualifying for evaluation in clinical trials.

#4978 Adoptively transferred CMV-Specific T-cells recognizing dominant and sub-dominant pp65 epitopes demonstrate improved in vivo inhibition of tumor xenografts in combination with PD-1 inhibition. Aisha N. Hasan,1 Annamaali Selvakumar,1 Tzu-Yun Kuo,2 Richard J. O’Reilly1.1Morial Sloan-Kettering Cancer Center, New York, NY; 2Weill-Cornell Graduate School of Biomedical Sciences, New York, NY.

Adoptive transfer of transplant donor or third party donor derived CMV-specific T cells (CMV-CTL) can effectively treat CMV infections in HSC reipients. In clinical trials, infusion of partially matched third party CMV-CTL, has demonstrated high response rates against persistent CMV infection. T-cells (TC) generated in vitro or directly selected in vivo demonstrate a striking preponderance of specificity for 1-2 immunodominant (ID) epitopes presented by specific HLA alleles. ID epitopes elicit higher TC functional activity in vivo, compared to sub-dominant (SD) epitopes. The relative clinical efficacy of TC directed against ID versus SD epitopes in vivo remains undefined. Agents augmenting activity of TC responsive to SD epitopes are unexplored. When these alleles are co-inherited in humans, epitopes of CMVpp65 presented by HLA-A*02:01 are ID over HLA-A*24:02 presented epitopes. We describe an in vivo model to assess efficacy of CMV-CTLs using colon carcinoma cells (caco)trans-
duced to express CMVpp65, as a surrogate system. HLA A*02:01 and A*24:02 + human coca cells were transduced to express CMVpp65 and GFP-firefly luciferase (cocc6p). CMV-CTLs responding to either the A*02:01 presented ID NLV epitope (A2-NLV) or the A*24:02 presented SD YQD epitope (A24-QYD) were generated from donors co-inheriting HLA A*02:01 and A*24:02 by in vitro stimulation with high densities of infected cells. After 5 years of culture and daily transfers to fresh feeder cells, HLA A*02:01 or A*24:02, B7.1, LFA-3, and ICAM1. Tumor cells (10^3 cells) were injected subcutaneously into groups of 5-6 NSG mice on the R flank, and 10^6 cells from a pp65 expressing melanoma cell line (melpp65), lacking expression of HLA A*02:01 or A*24:02 were injected on the L shoulder as control. 2 Groups each received 10^6 of tetramer+ A2-NLV or A24-QYD CMV-CTLs i.v per mouse; one of each 4 CMV-CTL treated group also received 2 i.v doses (200 ug/dose) of anti-PD1 antibody (Nivolulab-BMS) at day 2 and 7 post CTL infusion. Control groups received IL-2, with or without anti-PD1, or HLA mismatched CMV-CTLs. Tumor growth was monitored by bioluminescent imaging. CMV-CTLs responsive to SD A24-QYD epitope induced significant cocc6p growth suppression compared to controls, but did not eradicate tumors in any animal. Combined treatment of A24-QYD CMV-CTLs with anti-PD1-Ab induced complete cocc6p eradication in 2 of 5 mice, with minute residual tumors in 3 mice. Treatment with ID A2-NLV CMV-CTLs induced complete cocc6p eradication in 2 of 5 mice, and smaller residual tumors compared to SD A24-QYD CTL treatment. Combined treatment with anti-PD1 and A2-NLV CMV-CTLs led to complete cocc6p eradication in 3 of 5 mice, with minute tumors in 2/5 mice. Taken together, these data provide evidence that blocking the PD-L1-anti PD1 antibody (Nivolumab-BMS) at day 2 and 7 post CTL infusion. Control groups received IL-2, with or without anti-PD1, or HLA mismatched CMV-CTLs.
**IMMUNOLOGY: Adaptive Cellular Therapy for Cancer**

A single CD8+ T cell clone recognizing a mutation expressed in the solute carrier SLC3A2 (p.K94T) and cytokine profile for anti-tumor therapies. T cells were generated from a metastatic soft tissue tumor of a patient with hormone and chemotherapy-refractory ER+, HER2+ breast cancer. DNA was extracted from tumor and matched normal peripheral blood samples for whole exome sequencing (WES) and RNAseq. Non-synonymous somatic mutations were identified and tested for potential recognition by autologous TIL using previously described tandem mini-gene and long-read approaches. Recognition was assessed by IFN-γ release on ELISPOT and/or CD137 (4-1BB) upregulation with appropriate controls. Mutation reactive TIL were rapidly expanded in culture and transferred back to the patient. Adoptive cell transfer was preceded by a lymphodepleting preparative regimen and one dose of pembrolizumab. Cells were supported post-transfer with intravenous IL-2 administration, and pembrolizumab was administered for three additional doses. Deep sequencing of TCR rearrangement sequences was performed on the TIL and the pre- and post-infusion peripheral blood. Results: The resected subcutaneous tumor no longer expressed ER and was negative for PD-L1 staining. WES/RNAseq identified 96 non-synonymous mutations for testing. Multiple CD4+ clones specifically recognized a mutation expressed in the solute carrier SLC3A2 (p.K94T) and a single CD8+ clone recognizing a mutation in the proteasome-associated protein KIAA0368 (p.S186F) were identified with no recognition of wild-type peptides. These eight T cell clones constituted 23% of the infusion bag protein. Mutation reactive TIL were rapidly expanded in culture and transferred back to the patient. Nineteen months after transfer of 8x10^9 cells, the patient has an ongoing partial response, with target lesions down 96% from baseline, including multiple hepatic metastases and disabling brachial plexus adenopathy. Seven of the eight T cell clones with known reactivity persisted in the peripheral blood comprising 2.4% of all CD8 T cells at 7 months. Only two of these TCR clones were detectable, with frequencies totaling 0.005%, in the pre-treatment circulation. Conclusions: Tumor-infiltrating lymphocytes derived from a patient with metastatic breast cancer recognized tumor-specific, non-synonymous somatic mutations, expanded in vitro and were used for adoptive cell transfer. We also administered with IL-2 and a short course of pembrolizumab, persisted in vivo and mediated regression of soft tissue, nodal and visceral metastases of breast cancer.

**MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Exploiting Metabolic Vulnerabilities to Treat Cancer**

**#4983** T cells expressing chimeric PD1 receptors that contain a Dap10 costimulatory domain are a potential treatment for multiple types of cancer.

Kelsey Deal, Emily Nylen, Amorette E. Barber. Longwood Univ., Farmville, VA.

Adoptive transfer of tumor-reactive T cells is a promising anti-tumor therapy for many cancer types. In this study, T cells, chimeric antigen receptors (CAR) consisting of signaling domains fused to receptors that recognize tumor antigens can be generated and expressed in T cells. One receptor that is a prospective target for a new chimeric antigen receptor is PD1 because the ligands for the PD1 receptor are expressed on many cancer types. Therefore we developed a murine chimeric PD1 receptor (chPD1) consisting of the PD1 receptor extracellular ligand recognition domain and the activation domain of CD3 zeta. In addition, current chimeric antigen receptor therapies utilize various costimulatory domains to enhance anti-tumor efficacy. Therefore, we also compared the inclusion of CD28, Dap10, 41BB, GITR, ICOS, or OX40 costimulatory domains in our chPD1 receptor to determine which costimulatory domain induced optimal anti-tumor immunity. To determine if this novel CAR could potentially target a wide variety of tumors, the anti-tumor efficacy of chPD1 T cells against murine lymphoma, melanoma, kidney, pancreatic, liver, and colon cancer cell lines was measured. Of the ten cell lines tested, all expressed PD1 ligands on their cell surface, making them potential targets for chPD1 T cells. Regardless of the costimulatory domain in the CAR, all of the chPD1 T cells induced similar levels of T cell proliferation and tumor cell lysis. However, differences were observed in the cytokine secretion profiles depending on which costimulatory receptor was included in the CAR. While most of the chPD1 T cell receptor combinations secreted both pro-inflammatory (IFNγ, TNFα, IL-2, GM-CSF, IL-17, and IL-21) and anti-inflammatory cytokines (IL-10 and IL-5) as determined by ELISA and LegendPlex analysis, chPD1 T cells containing a Dap10 costimulatory domain secreted high levels of pro-inflammatory cytokines, but did not secrete a significant amount of anti-inflammatory cytokines. Therefore, adoptive transfer of chPD1 T cells could be a novel therapeutic strategy to treat multiple types of cancer and inclusion of the Dap10 costimulatory domain in chimeric antigen receptors may induce a preferential cytokine profile for anti-tumor therapies.

**#4984** BNip3 suppresses hepatocellular carcinoma (HCC) growth by limiting lipogenesis.

Kay F. Macleod. Univ. of Chicago May Dept. for Cancer Res., Chicago, IL.

BNip3 is a mitochondrial protein that promotes the turnover of mitochondria at the autophagolysosome when nutrient supplies are low. BNip3-null livers exhibit lipid accumulation and develop a phenotype mimicking non-alcoholic steatohepatitis, a known precursor for development of hepatocellular carcinoma (HCC). Additionally, BNip3 is silenced in human HCC and other malignancies and this loss often correlates with more aggressive tumors and worse outcomes. However, the functional significance of BNip3 loss for growth and progression of HCC has not been determined. We utilized the diethylstilbestrol (DEN)-induced mouse model of HCC to determine how loss of BNip3 affected HCC development in the mouse. Wild-type and BNip3-null mice were treated with DEN, and tumor burden (size and number) was assessed at 6, 8, and 10 months of age. We also generated primary HCC cell lines from the tumors arising in BNip3-null mice for in vitro analysis of tumor cell growth and metabolism. We observed that BNip3-null mice had reduced tumor latency and enhanced tumor growth rate in the liver following DEN treatment compared to wild type mice consistent with BNip3 functioning as a tumor suppressor of HCC. Interestingly, tumors arising in BNip3-null mice accumulated increased lipid and qPCR analysis of primary tumors and cell lines revealed increased expression of lipogenesis genes compared to wild type. Treatment with a novel fatty acid synthase inhibitor or re-expression of BNip3 in the BNip3-null HCC cell lines reduced tumor cell growth and lipogenesis, and promoted oxidative metabolism. These results indicate that BNip3 acts as a tumor suppressor in HCC by limiting de novo lipogenesis and suggest that fatty acid synthase inhibitors could be effective therapeutically against BNip3 deficient HCC in patients.

**#4985** Understanding the vulnerabilities in cancer cells upon inhibition of glutathione synthesis.

Isaac S. Harris, Jennifer E. Endress, Joan S. Brugge. Harvard Medical School, Boston, MA.

A hallmark of cancer is the rewiring of cellular metabolic pathways, including those that mediate oxidative stress. Cancer initiation and progression, which demands increased production of ATP and synthesis of proteins, also results in elevated generation of toxic by-products such as reactive oxygen species (ROS). Cancer cells counteract ROS by increasing the production of antioxidants, which convert ROS into benign species. It has been shown that production of glutathione (GSH), the most abundant intracellular antioxidant, is necessary for many cancers to initiate malignant transformation. Furthermore, sensitivity was found to be specific to malignant cells, as non-transformed cells were resistant to the combined treatments. It has been hypothesized that this sensitivity could potentially target a wide variety of tumors, the anti-tumor efficacy of chPD1 T cells against murine lymphoma, melanoma, kidney, pancreatic, liver, and colon cancer cell lines was measured. Of the ten cell lines tested, all expressed PD1 ligands on their cell surface, making them potential targets for chPD1 T cells. Regardless of the costimulatory domain in the CAR, all of the chPD1 T cells induced similar levels of T cell proliferation and tumor cell lysis. However, differences were observed in the cytokine secretion profiles depending on which costimulatory receptor was included in the CAR. While most of the chPD1 T cell receptor combinations secreted both pro-inflammatory (IFNγ, TNFα, IL-2, GM-CSF, IL-17, and IL-21) and anti-inflammatory cytokines (IL-10 and IL-5) as determined by ELISA and LegendPlex analysis, chPD1 T cells containing a Dap10 costimulatory domain secreted high levels of pro-inflammatory cytokines, but did not secrete a significant amount of anti-inflammatory cytokines. Therefore, adoptive transfer of chPD1 T cells could be a novel therapeutic strategy to treat multiple types of cancer and inclusion of the Dap10 costimulatory domain in chimeric antigen receptors may induce a preferential cytokine profile for anti-tumor therapies.

**#4986** Pancreatic cancers develop metabolic resistance pathways to gluconeogenesis.

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Pancreatic cancers develop metabolic resistance pathways to gluconeogenesis. These pathways are triggered by the depletion of glucose and liver damage. BNip3 suppresses hepatocellular carcinoma (HCC) growth by limiting lipogenesis. BNip3-null livers exhibit lipid accumulation and develop a phenotype mimicking non-alcoholic steatohepatitis, a known precursor for development of hepatocellular carcinoma (HCC). Additionally, BNip3 is silenced in human HCC and other malignancies and this loss often correlates with more aggressive tumors and worse outcomes. However, the functional significance of BNip3 loss for growth and progression of HCC has not been determined. We utilized the diethylstilbestrol (DEN)-induced mouse model of HCC to determine how loss of BNip3 affected HCC development in the mouse. Wild-type and BNip3-null mice were treated with DEN, and tumor burden (size and number) was assessed at 6, 8, and 10 months of age. We also generated primary HCC cell lines from the tumors arising in BNip3-null mice for in vitro analysis of tumor cell growth and metabolism. We observed that BNip3-null mice had reduced tumor latency and enhanced tumor growth rate in the liver following DEN treatment compared to wild type mice consistent with BNip3 functioning as a tumor suppressor of HCC. Interestingly, tumors arising in BNip3-null mice accumulated increased lipid and qPCR analysis of primary tumors and cell lines revealed increased expression of lipogenesis genes compared to wild type. Treatment with a novel fatty acid synthase inhibitor or re-expression of BNip3 in the BNip3-null HCC cell lines reduced tumor cell growth and lipogenesis, and promoted oxidative metabolism. These results indicate that BNip3 acts as a tumor suppressor in HCC by limiting de novo lipogenesis and suggest that fatty acid synthase inhibitors could be effective therapeutically against BNip3 deficient HCC in patients.
Pancreatic ductal adenocarcinoma (PDAC) is an extremely aggressive disease with poor prognosis. Therefore, novel treatment options are essential to combat this highly refractory disease. Oncogenic Kras can promote a metabolic rewiring of pancreatic cancers, including the non-canonical use of glutamine to support growth and proliferation through redox homeostasis. Indeed, inhibition of downstream metabolites of glutaminolysis leads to decreased cell growth. The first step in glutaminolysis metabolism is mediated by the enzyme glutaminase (GLS) which catalyzes the conversion of glutamine to glutamate in the mitochondria where, in PDAC, glutamine-derived glutamate is metabolized ultimately resulting in increased reducing potential in the form of increased NADPH and GSH. An outstanding question in pancreatic cancer is whether GLS inhibition is a viable therapeutic strategy given it is the most proximal enzyme in the PDAC-specific glutaminolysis metabolism pathway, and how this may differ from targeting distal parts of the pathway. Using a combination of in vitro and in vivo models of pancreatic cancer, we tested whether recently developed highly potent inhibitors of GLS are an effective therapy for PDAC. We demonstrate that despite dramatic early effects on in vitro proliferation caused by GLS inhibition, pancreatic cancer cells have adaptive metabolic networks that allow them to sustain proliferation in vitro and in vivo. Through an integrated proteomic and metabolomic analysis, we identify multiple compensatory pathways that may explain the resistance to GLS inhibition and show as proof of concept that combining inhibitors to these pathways with GLS inhibitors may have therapeutic utility.

**#4987 ASS1 downregulation facilitates cancer cell survival in acidic environment.** Ayelet Erez,1 Alon Silberman,1 Adi Jacob,1 Sharan Limanovich,1 Odeda Boukobaz,1 Erez Persi,2 Rom Kesher,1 Alona Sarver,1 Joo Sang Lee,1 Alexander Brandis,1 Keren Bahar Halperrn,1 Raya Eilam,1 Ben Pode,3 Yair Anikster,1 Igor Ulitsky,1 Sandesh Nagamani,1 Eytan Ruppin1,1 WEIZMANN INSTITUTE, Rehovot, Israel; 2University of Maryland, College Park, MD; 3Sheba Medical Center, Tel Hashomer, Israel. pH gradient reversal, namely the alkalization of intracellular pH (pHi) and acidification of extracellular pH (pHe), is a unique phenotype of cancer cells which can enable cancer survival, migration and invasion. Hence, not surprisingly, there is an urgent need to develop novel therapeutic strategies that target pHi by elevating ammonia levels and buffering the acidity caused by cancers’ “pH gradient reversal”, namely the alkalinization of intracellular pH (pHi) and acidification of extracellular pH (pHe), is a unique phenotype of cancer cells which can enable cancer survival, migration and invasion. Hence, not surprisingly, there is an urgent need to develop novel therapeutic strategies that target pHi by elevating ammonia levels and buffering the acidity caused by cancers’


The chemotherapeutic drug methotrexate (MTX) inhibits nucleotide biosynthesis and causes cell death by blocking DNA and RNA production. While MTX has had tremendous success as an anti-cancer treatment and is the subject of nearly a thousand ongoing clinical trials, we do not fully understand why certain cancers are more sensitive to it and why resistance emerges. To study these questions, we used a CRISPR-based genetic screen to identify genes that confer sensitivity to MTX. We identified an enzyme that plays a key role in a metabolic pathway that was not known to be associated with MTX sensitivity. Inhibition of the pathway, either by genetic loss of several genes in the pathway or through nutritional modulations, causes dramatic resistance to MTX in cultured cancer cells. Our results improved our understanding of the cellular response to MTX, and suggest that expression levels of enzymes in the pathway may serve as clinical predictor for MTX response in patients.


Three-dimensional growth conditions, multicellular tumor spheroids reproduce several parameters of the tumor microenvironment, including oxygen and nutrient gradients, characteristic of poorly vascularized tumor regions. 3D high content screening (HCS) identified compounds that selectively kill tumor cells in the inner core of tumor cell spheroids by targeting the Stearoyl CoA Desaturase 1 (SCD1) pathway. SCD1 catalyzes the rate-limiting step in the production of monounsaturated fatty acids (MUFA)s which are abundant at the tumor surface on higher levels of MUFA:s compared to normal cells and SCD1 is highly expressed in multiple tumor types. Changes in the MUFA/S FA (saturated fatty acid) ratio alters lipid biosynthesis and thus triggers cellular (ER) stress and induces the Unfolded Protein Response. Although the lead compound was very effective in vitro, it had unfavorable PK and physical chemistry properties, including low permeability and solubility and very high lipophilicity. This led to insufficient oral bioavailability, which could be overcome by optimization of PK and physical chemistry properties. Here, we report on the in vitro/in vivo effects of our 3D HCS compounds which showed high potency in the 3D spheroid inner core assay with T47D breast cancer cells. In this in vitro model compound-induced inner core cell death is enhanced by SCD1 substrates palmitoleoyl-CoA and oleoyl-CoA but not by the SCD1 product, palmitic acid. Furthermore, the effects can be reproduced in 2D cultures, which become increasingly sensitive to inhibition by our 3D HCS compounds with decreasing FBS concentration in the culture medium and this effect can also be rescued by addition of MUFA:s but not of palmitic acid. Mode of action analysis showed that our compounds reduced palmitoleoyl- or oleoyl-CoA levels and simultaneously increased saturated fatty acyl-CoA of palmitate or stearate in several cell lines as well as in vivo. In the sensitive T47D cells, the compounds induced expression of stress response genes and genes related to lipid metabolism. While these results support the SCD1 pathway as target for our 3D HCS compounds, we also observed striking differences to published SCD1 inhibitors suggesting a new cancer target beyond SCD1. Thus, further validation of our inhibitors in vitro and in vivo will be required, but these results suggest that 3D spheroid cultures may be a valuable tool for elucidation of new drug targets for cancer therapy.

**#4990 High-throughput drug combination screening in tumor spheroids identifies context-dependent synthetic lethalities.** Wojciech Senkowski,1 Madhia Nazir,1 Malin Jarvius,1 Jenny Rubin,1 Johan Lengqvist,2 Mats G. Gustafsson,2 Peter Nygren,1 Kim Kultima,1 Rolf Larsson,1 Mårten Fryknäs,1 Uppsala University, Uppsala, Sweden; 2Karolinska University Hospital, Solna, Sweden.

Monolayer, two-dimensional (2D) cell cultures have been a predominant in vitro model for anticancer drug screening and genetic loss of high throughput screening (HTS). However, 2D cultures of cancer cells lack numerous properties of in vivo tumors, such as tissue-like structure, cell-cell interactions and nutrient/oxygen gradients. Thus, in recent years there has been an increased interest in 3D cell cultures, such as multicellular tumors spheroids (MCTS), to address some of these limitations. Recently, we and others have applied MCTS for HTS and identified oxidative phosphorylation (OXPHOS) as a selective vulnerability of quiescent cancer cells persisting in hypoxic and nutrient-deprived milieu. However, prolonged continuous exposure to OXPHOS inhibitors is necessary for the cytotoxic effect. Thus, there is a need to identify processes that could be co-targeted for enhanced anticancer activity. Here, we present two distinct HTS approaches to identify combination partner molecules for OXPHOS inhibitors. Since we were interested in targeting non-dividing nutrient-deprived cancer cells, we used quiescent MCTS (Q-MCTS), as an in vitro model. Cells in Q-MCTS experience glucose concentrations and pH similar to those observed in deep tumor parenchyma in vivo. In our first screening approach, we have applied high throughput gene expression profiling to study drug effects in MCTS at a large scale. Using L1000 Gene Expression Profiling method, we generated a dataset of over 1000 drug-induced gene expression (MUFAs). Cancer cells over targeting of OXPHOS and the modulate pathway results in selective synergistic toxicity in quiescent cancer cells. In the other approach, we screened a library of 1650 biologically active compounds, with or without addition of the FDA-approved anthelmintic agent nitazoxanide (an OXPHOS inhibitor with high drug repurposing potential). After the screen, we selected molecules that demonstrated pronounced synergy when combined with nitazoxanide, but not when used alone. Then, we validated the hits in an extensive dose-response combination experiments in Q-MCTS and chose 14 compounds that demonstrated strong synergistic interaction with nitazoxanide at broad range of concentrations. These included antifungal agents, kinase inhibitors and others. In summary, we...
MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Exploiting Metabolic Vulnerabilities to Treat Cancer

here report on novel approaches, utilizing 3D cell cultures, to identify drug combinations targeting quiescent cancer cells. By high-throughput gene-expression profiling and large-scale combinatorial drug screens, we were able to identify drug combinations preferentially toxic to quiescent cells. This work also demonstrates how 3D cell cultures yield functional insights that are not accessible through standard 2D cultures.

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Transcriptional Regulation in Cancer Cells


Reprogramming of the chromatin landscape by sequence-specific transcription factors (TF) can influence in the prevalence and progression of several diseases, including cancers. Stereo receptors are a class of hormone-regulated TF that have a major impact on the progression of cancers. The link between the androgen receptor (AR) action in prostate cancer and the glucocorticoid (GR) and estrogen receptor (ER) action in breast cancer is well-known. Furthermore, other TFs such as the pioneer factor FoxA1 has been extensively linked to AR and ER action in these cancers. Although the action of these TF have been widely studied, the knowledge is almost exclusively based on population-based assays such as ChIP-seq. Hence, the dynamic binding behavior of TFs in living cell remains unclear. For example, the classical pioneer factor model suggests long term static binding events between pioneer TFs, such as FoxA1, and chromatin. However, little is known on the dynamic action of FoxA1 in living cells. We have examined the behavior of steroid receptors and cofactors, including FoxA1, at the single-molecule level in living cells. These factors were fused with HaloTag, and labeled with the bright and stable fluorophore JF549. Single-molecule tracking (SMT) of these proteins (using HILO microscopy) revealed a highly dynamic binding behavior of TFs. This supports transient rather than stable TF chromatin interactions. Specifically, two distinct binding populations have been observed for all factors tested; fast or slow stops. Fast stops represent genomic scanning of TF, while slow stops, with residence time of 6-14 sec, represent functional binding events at specific response elements. The vast majority of molecules at any given time are either diffusing or exhibit fast stops, while only a small percentage exhibit slow stops. However, hormone activation of AR, GR or ER results in an increase in the percentage of molecules with slow stops compared to the unstimulated state. Thus, our results indicate that on a single-molecule level, only a small proportion TFs are functionally bound at any given time. In addition to affirming the general model that many TFs are highly dynamic in their chromatin binding activity, SMT experiments in live cells revealed a highly dynamic interaction of FoxA1 with chromatin in vivo. Unexpectedly, we also observed by ChIP-seq and DHS-seq analysis that at subset of genomic sites, the role of pioneer can be reversed, with the steroid receptors serving to enhance binding of FoxA1. We propose a general model, dynamic assisted loading, wherein interactions between TFs and pioneer factors are highly dynamic. Our findings show that the action of key TFs in cancer development are more complex and dynamic in real time than previously reported.

Miswired super enhancer logic driving childhood sarcoma. Berkley E. Gryder,1 Marco Wachtel,2 Hsin-Chao Chou,1 Young Song,1 Joana Marques,2 Beat Schaefer,7 Javed Khan1. 1National Cancer Institute, Bethesda, MD; 2University Children’s Hospital, Zurich, Switzerland.

Super enhancers (SEs) are regulatory regions with unusually large deposits of active histone marks, chromatin regulators and transcriptional coactivators. Chromosomal rearrangements allowing SEs to drive oncogene expression is an emerging mechanism in tumor biology. An aggressive myoblastic cancer of childhood, alveolar (fusion-positive) rhabdomyosarcoma (FP-RMS), universally possesses a chromosomal translocation, involving most commonly PAIX3 and FOXO1, more rarely PAIX7-FOXO1, and in exceptional cases novel PAIX3-INO80D and PAIX3-NCOA1 fusions. Patients with a PAIX3-fusion frequently relapse and have low survival rates. PAIX3 initiates specification of the muscle lineage, but is shut off during myogenic differentiation, which is in turn dominated by master regulators MYOD and finally MYOG. FP-RMS has the master regulators needed to trigger muscle differentiation, but are halted in an early myoblastic and thus more proliferative epigenetic state. We hypothesized that the translocations misregulates regulation of the fusion oncogene in FP-RMS by hijacking SEs and creating new topologically associated domains (TADs) which allow for continued expression of PAX fusions, thus circumventing normal myogenic enhancer logic. Thus, we recently completed the first epigenetic landscape of FP-RMS and uncovered a strong dependence on SEs for tumor survival, with PAIX3-FOXO1 being a chief determinant of SE formation in collaboration with PAIX3, MYOG, and FOXO1. We discovered a key SE 300 kb distal of FOXO1 which was occupied by all four of these master regulators. Further, we found that PAIX3-FOXO1 is driven by this novel translocated SE forming a key TAD structure which was necessary to directly influence PAIX3 upon translocation, with CTFC analysis in FP-RMS cells confirming the predicted boundaries. The CTFC motif orientation was found to be anti-parallel, restricted translocation of event, permitting chromatin loop extrusion. We demonstrate these elements to physically interact only in the presence of the translocation by chromatin conformation followed by sequencing (4C-seq). Exon-level expression via RNAseq in primary tumors revealed that the final exon of PAIX3, not involved in the translocation, was unexpressed, indicating that only the allele influenced by the FOXO1 SE is activated in patients. Finally, CRISPR/Cas9 technologies were employed to functionally interrogate the relative contributions of the enhancer elements and CTFC looping sites at TAD boundaries. Together these data suggest that these newly juxtaposed enhancer elements initiate and continually drive PAIX3-FOXO1 expression, implicating that enhancer miswiring is at the heart of the oncogenic process in FP-RMS. Thus, late myogenic factors (MYOG/MYOD) are contributing to drive an early factor (PAIX3), changing a “progressive” enhancer logic into an “infinite loop” enhancer logic.


The purpose of this study was to investigate the role of the Zn finger protein CTCF in the establishment of genetic borders and in controlling spatially-restricted activity of a mammary-specific super-enhancer (SE) known to selectively activate gene expression during pregnancy. CRISPR/Cas9 gene editing was used to generate mice with deletions in CTFC binding sites (CBSs) separating the Wap SE from the Ramp3 gene. ChIP-seq and qRT-PCR were used to investigate protein-DNA interactions and changes in gene expression, respectively. RNA-seq data demonstrated that expression of the mammary-specific Wap and widely-expressed Ramp3 gene was activated in mammary tissue at the onset of lactation. To investigate the possibility that the Wap SE can regulate both genes, mice carrying deletions in the SE were established. In these mice, both Wap and downstream Ramp3 were downregulated to the same extent, supporting the notion that the SE regulates both genes. Given these results, we investigated the contributions of several intervening CBSs in dampening SE activity on Ramp3. Mice harboring individual and combinatorial deletions of three CBSs (A, B and C) between the Wap SE and Ramp3 were generated. While loss of CTFC at site B (ΔB) had no apparent effect, loss of site A (ΔA) led to an expansion of H3K27ac from the adjacent SE in mammary tissue. The site C mutation (ΔC) resulted in a two-fold induction of Ramp3 in mammary tissue. Mice lacking all three binding sites demonstrated a marked increase in Ramp3 expression, which indicates that the CBSs block expression in an additive manner. To test the possible insulator function of these in a tissue with opposing gene expression, cerebellum was chosen as Ramp3 is actively transcribed while Wap is silent. The ΔA and ΔB mice showed no phenotype, but surprisingly ΔC mice demonstrated an 80% reduction in Ramp3 expression. Analysis of ChIP-seq and DNAse-seq demonstrated that site C coincides with a cerebellum-specific enhancer, which appears to regulate Ramp3 expression. These data demonstrate that a SE can activate two genes, albeit to different degrees. Furthermore, we demonstrate that CBSs do not serve as tight genetic boundaries encapsulating transcriptional enhancers and their associated genes, but are porous borders that allow for fine tuning of transcriptional activity. Individual CBSs have a modest ability to insulate genes, but a cluster of CTFC proteins is required to adjust the enhancer activity on genes and prevent deregulation. In addition, we show that CBSs are not restricted to a unique function and can act in a tissue-specific manner to contribute to gene regulation. These data help us to develop a more nuanced understanding of SE function as well as the roles of CTFC in regulating gene expression. Previous studies have shown that SEs and CBSs are mutational hotspots in cancer, implicating them in tumorigenesis. A more complete understanding of their biology will allow a better understanding of their role in cancer.

#4994 Understanding the MYC and WDR5 interaction at chromatin. Alissa D. Guarnaccia, April M. Weissmiller, Lance R. Thomas, William P. Tansey. Vanderbilt University, Nashville, TN.

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Molecular and Cellular Biology / Genetics: Transcriptional Regulation in Cancer Cells

MYC is an oncprotein that is overexpressed in the majority of malignancies and contributes to an estimated 70,000-100,000 cancer deaths in the United States every year. The broad pro-tumorigenic functions of MYC stem from its role as a sequence-specific transcriptional regulator, controlling the expression of thousands of genes linked to cell cycle control, growth, and metabolism. Key to understanding how MYC causes cancer, therefore, is understanding the mechanisms through which it selects its target genes. Histone modifications, DNA sequence, and interactions with other transcription factors have all been suggested to influence where MYC binds chromatin, but a consistent signature has not been defined. Our laboratory recently discovered that the chromatin regulatory protein WDR5—a core component of histone modifying complexes—interacts directly with MYC and co-localizes with MYC at a majority of its target genes in human cells (Thomas et al., Mol. Cell, 58: 440–452, 2015). Point mutations in MYC that disable interaction with WDR5 do not impact the ability of MYC to bind naked DNA, but they do prevent MYC from recognizing target genes in the context of chromatin and from driving tumorigenesis in mice. These studies led us to propose that the MYC-WDR5 interaction is a critical determinant in MYC target gene recognition, in a process we refer to as "facilitated recruitment." In the facilitated recruitment model, the presence of WDR5 at chromatin promotes MYC binding at certain genomic loci over others. The role of WDR5 in target gene selection by MYC can explain much of the plasticity in genome-wide binding patterns of MYC that have been reported, and may provide a new avenue for therapeutically targeting MYC in cancer. Two important questions are raised by these studies, however. How does WDR5 recognize and select its target genes? And what other functions, if any, does WDR5 play in regulating MYC target genes? To answer these questions, we are employing traditional and quantitative proteomics, together with biochemical approaches, to characterize the composition and stoichiometry of the WDR5-containing complex that associates with MYC. These studies, still ongoing, have demonstrated that this complex is devoid of canonical WDR5-interaction partners such as RBBP5 and HCF-1, revealing that the function of WDR5 in this setting is distinct from its well-characterized roles in histone modifications. We propose that the WDR5 complex that associates with MYC on chromatin is either entirely novel, or is a 'ghost complex' in which select protein components have been excluded by the direct interaction of MYC with WDR5. We continue to define the biochemical constituents of the MYC-WDR5 complex, and in terrogate their role in facilitated recruitment using a combination of genetic, biochemical, and genomic approaches.

Premature polyadenylation causes oncocgenic truncations of the tumor suppressor genes BRCA1, LATS1 and MAGI3 in breast cancer.

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Cleavage and polyadenylation is a fundamental process in the control of gene expression, yet how cancer cells deregulate this process to generate cancer-caus ing alterations is only now coming to be appreciated. We previously reported that premature polyadenylation of the tumor suppressor gene MAGI3 causes the expression of a truncated protein that dominantly promotes malignant growth via the Hippo pathway. This established a precedent for premature polyadenylation as an aberrant process causing transformation in vitro and in vivo. However, little is known about the molecular mechanism that causes premature polyadenylation, or what types of genes it affects in cancer. Despite the absence of cis-acting mutations, premature polyadenylation of MAGI3 occurs specifically following the gene's large internal exon. We therefore asked whether an epigenetic mechanism might underlie the phenomenon of premature polyadenylation in cancer. Here, we report that genes with large internal exons are susceptible to truncation by premature polyadenylation. In addition to MAGI3, premature polyadenylation truncates the gene products of two tumor suppressor genes with large internal exons, BRCA1 and LATS1. These truncated proteins are upregulated in breast cancer cells compared to non-transformed mammary epithelial cells and enable downstream oncogenic events instead of providing tumor suppressive functions. Large internal exons are infrequently found in the genome as the mechanics of efficient splice site recognition are not conducive to this form of RNA processing. Interestingly, this truncation is conspicuously enriched in N6-methyladenosine (m6A), though the functional significance of these epitranscriptomic marks remains unknown. Mole- cularly, we find that methylated levels of N6-adenosine in the large internal exons of BRCA1, LATS1 and MAGI3 transcripts are significantly reduced in breast cancer cells that upregulate the truncated gene products compared to non-transformed mammary epithelial cells. These results raise the intriguing possibility that m6A marks in large internal exons are mechanistically involved in repressing premature polyadenylation. We are currently investigating this hypothesis by perturbing N6-adenosine methylation in cells and examining the functional consequences. In summary, our study suggests that premature polyadenylation may be a more pervasive alteration mechanism than previously appreciated in cancer and begins to illuminate a gene region-specific mechanism responsible for repressing premature polyadenylation.

The m6A hallmark of cancer: RNA demethylase ALKBH5 maintains tumorigenicity of glioblastoma stem-like cells by sustaining FOXMI expression and cell proliferation.

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N6-methyladenosine (m6A) is the most prevalent internal modification on mRNA, but its functions in human diseases are poorly understood. The dynamic and reversible m6A modification installed and erased by N6-methyltransferases and demethylases regulates cell fate and gene expression. We show that the m6A demethylase ALKBH5 is highly expressed in patient-derived glioblastoma stem-like cells (GSCs) and informs poor survival of patients with glioblastoma. Silencing ALKBH5 suppresses the proliferation of GSCs in vitro and in vivo. Results of integrated transcriptome and m6A-seq analyses reveal a global change of gene expression enriched in cell cycle and altered expression of select ALKBH5 targets. Among these targets, Ingenuity’s upstream regulator analysis identified FOXMI, an essential transcription factor for GSC proliferation, as a key mediator responsible for the disrupted proliferation program. Using multiple molecular and biochemical approaches, we demonstrate that ALKBH5 binds to and demethylates FOXMI nascent transcripts. This promotes the interaction of FOXMI with mRNA and promotes translation. Furthermore, long noncoding RNA antisense to the FOXMI (FOXM1-AS) interacts with ALKBH5 as well as FOXM1 pre-mRNA and promotes their interaction. Depleting ALKBH5 and FOXM1-AS abolishes GSC tumorigenesis through the FOXM1 axis. The vulnerability of GSCs to disruptions in ALKBH5-dependent gene expression suggests that m6A has a central role in tumor development and provides a rationale for therapeutically targeting epitranscriptomic modulators in patients with glioblastoma.

Specialized microRNPs and translation mechanisms in quiescent cancer cells.

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Quiescence (G0) represents an assortment of reversible, cell cycle-arrested states that are resistant to unfavorable conditions and associated with cancer persistence. G0 involves regulated gene expression with selective mRNA expres- sion and decreased canonical translation. Low mTOR activity in G0 activates the cap complex inhibitor, eIF4EBP, and impairs canonical translation. The alter- native translation mechanisms in G0 remain to be uncovered. Our data show that microRNAs, regulatory, non-coding RNAs that target distinct mRNAs to alter gene expression in unfavorable conditions and associated with translation factors to regulate specific mRNA translation in G0. One subset of transcripts expressed in G0 includes specific miRNAs recruited by an FXR1a-associated microRNP (microRNA-protein complex) for translation activation in G0 mammalian cells. MicroRNPs predominantly mediate repression and downregulation; however, FXR1a-microRNP lacks conventional microRNPs, and instead, contains a specific RNA binding protein isoform, FXR1a. FXR1a promotes translation and is overexpressed and associated with poor prognosis in several cancers. Our data reveal that microRNA-mediated activation requires target mRNAs with unadenylated/shortened poly(A) tails to avoid the roles of PABP in enhancing microRNA-mediated downregulation and in canonical translation that is impaired in G0. Instead of canonical translation factors that are inhibited by eIF4EBP in G0, we find alternative translation factors—a non-canonical 5’ cap binding factor and an eIF4G homolog that interacts with the ribosome—are recruited by the 3’-UTR binding FXR1a-microRNP, and promote specific mRNA translation. Our data show that G0 leukemic cells are chemoresistant and their translation profile is similar to surviving leukemic cells that are isolated after clinical therapy. We find expression of critical cytokines linked to G0 persistence, and significantly, inhibiting these immune regulators in resistant G0 cancer cells reduces their survival and chemoresistance. These data reveal a specialized translation mechanism in G0 cancer cells that promotes specific mRNA translation in these conditions of reduced canonical translation, and is important for chemoresistance.
**PREVENTION RESEARCH: Biomarkers and Intervention Studies**

**#4998 Breast tumor FOXA1 protein expression and reproductive characteristics among African-American and European-American women.** Ting, Yuan David Cheng,1 Rochelle Payne Ondracek,2 Song Yao,3 Viam Bshara,2 Thar Khoury,2 Gary R. Zirpoli,2 Warren Davis,2 Elisa V. Bandera,1 Michael Higgins,3 Christine B. Ambrosone,4 University of Florida, Gainesville, FL;6 Roswell Park Cancer Institute, Buffalo, NY;1 Massachusetts General Hospital, Boston, MA;2 Rutgers Cancer Institute of New Jersey, New Brunswick, NJ.

Background: Forkhead box protein A1 (FOXA1) plays a key role in determining estrogen receptor (ER) function and mammary ductal development and may repress the basal cell phenotype during differentiation of breast epithelium. While reproductive factors are known to influence breast cancer risk depending on the subtypes, data on the association of tumor FOXA1 protein expression and reproductive characteristics are very limited. Methods: Tissue microarrays comprising surgical tumors from 638 women (466 African-American [AA] and 172 European-American [EA], aged 20-75 years) with primary breast cancer in the Women’s Circle of Health Study (WCHS) were analyzed for FOXA1 expression by immunohistochemistry and automated image analysis. In-person interviews were conducted to obtain data on demographics, medical and family histories, and reproductive and menstrual histories. Logistic regression was performed for FOXA1 positivity (>10% cells with strong staining) with menopausal status, age at menarche, age at first live birth, parity, and breastfeeding, adjusting for age at diagnosis, family history of breast cancer, and history of benign breast disease. Results: FOXA1 expression was higher in tumors from EA compared to those from AA women (80% vs. 70% positivity, P = 0.011). FOXA1 expression was highly correlated with ER positivity (88% positive in ER+ and 26% in ER− breast cancer among AA women; 90% and 29% respectively among EA women; both P < 0.001). Among AA women, a higher number of live births was associated with lower FOXA1 expression (odds ratio [OR] = 0.45, 95% confidence interval [CI] = 0.22-0.91) for 2 live births and OR = 0.44, 95% CI = 0.20-0.91 for 3 live births (versus nulliparous). However, the inverse association was not observed among EA women, potentially due to the small sample size. Conclusion: In AA women, a higher number of live births, which has been related to greater risk of ER− breast cancer, is associated with lower FOXA1 expression. Because FOXA1 promotes luminal and represses the basal differentiation, respectively, the effects of parity on expression of FOXA1 may be the link whereby it increases risk of ER− breast cancer. (Funding: NIH P01CA151135, R01CA100598, R01CA185623, P30CA072770, K07CA210334; US Army Medical Research and Material Command DAMD17-01-1-0334; the Breast Cancer Research Foundation; and the Philip L. Hubbell family)

**#4999 Stress, spiritual wellbeing and cancer risk among diverse racial faith-based communities: elevated levels of stress proteomic biomarkers in breast cancer patients.** Padma P. Tadi Uppala,1 Gretchen Krivak,1 Sherine Brown-Fraser,1 Dixon Anejie,1 Alfredo Mejia,1 Dominique wakefield,1 Kumar Kolli,2 Andrews University, Berrien Springs, MI;1 Windber Research Institute, Windber, PA.

Spiritual wellbeing is associated with decreased risk of cancer. Stress and depression are common among cancer patients and may be inversely associated with spiritual wellbeing. The purpose of the study is to examine if stress and depression as indicators of cancer risk are lower in a racially diverse faith-based community. Methods: 752 individuals from a faith-based community completed a behavioral assessment survey that mapped the current patterns of behavior in key categories that include: stress, depression, exercise and nutrition using the E-wellness platform FitThumb. To identify stress biomarkers in cancer patients we examined levels of stress serum proteomic biomarkers that were previously identified in our study by proteomic profiling using 2D-DIGEMS analysis and a subset of samples by shotgun LCMS technology. The serum proteomic study included serum samples from 15 African American breast cancer patients and 12 healthy controls who were from a faith-based community. Results: The behavioral assessment surveys that included 752 individuals who were racially diverse showed a low stress risk of 47.74% vs 8.24 % of high stress risk for chronic disease; and low depression risk of 85.11% vs 1.99% high risk for chronic disease. Elevated levels of stress and inflammatory serum proteomic biomarkers such as ceruloplasmin known to increase in stressed animals and humans; hep-toglobin, apolipoproteins, and heat shock proteins were significantly elevated in breast cancer patients compared to healthy controls. Conclusions: Our results indicate that spiritual well-being is associated with significantly low stress and depression in a faith-based community regardless of race or ethnicity, posing a low risk for cancer as shown in previous Adventist Health Cohort studies. Future efforts will focus on validating and identifying panel of biomarkers from this cohort to gain insight into their role(s) in the mechanisms of stress hormones and cancer risk. Funded by Susan G Komen for the cure.

**#5000 A stromal liver gene signature predictive of HCC risk across all liver disease etiologies.** Shiengki Nakagawa,2 Naoki Umesaki,1 Takano Yamao,1 Yuki Kitano,1 Kenseki Yamamura,1 Kota Arima,1 Tatsunori Miyata,1 Takayoshi Kaida,1 Yuzin Hoshida,1 Katsunori Imai,1 Daisuke Hashimoto,1 Yo-Ichi Yamashita,1 Akira Chikamoto,1 Hideo Baba,1 Graduate School of Medical Sciences, Kumamoto University, Kumamoto City, Japan;3 Icahn School of Medicine at Mount Sinai, New York, NY.

Background & Aims: HCC risk-predictive biomarkers will enable identification of patients who most need close monitoring and cancer preventive intervention. We previously reported a stromal liver gene signature predictive of HCC risk in multiple independent patient cohorts mainly affected with HCV (Hoshida 2008, Hoshida 2013, King 2014). We aimed to determine whether the gene signature is similarly prognostic in HBV, alcohol abuse, and NAFLD/ NASH patients. Methods: The liver gene signature was analyzed using an FDA-approved diagnostic platform (NanoString), and an independent cohort of curatively resected early-stage HCC patients (n = 223, median follow up 4.5 years, HCV [n = 67, 30%], HBV [n = 39, 17%], alcohol [n = 51, 23%], NAFLD [n = 66, 30%], and NASH [n = 52, 23%, a subset of 66 NAFLD patients]) were analyzed. Complete surgical resection was radiologically and histologically confirmed, and HCC recurrence in the cohort was confirmed to be mainly late recurrence, i.e., de novo carcinogenesis, based on a recurrence hazard plot. Prognostic pre- dictors were identified using a previously developed PEP model to multiple any modification, and evaluated for association with de novo HCC recurrence in multivariable modeling and subgroup analyses. Results: The gene signature assembly classified the patients into high- (n = 49, 22%), intermediate- (n = 127, 57%), and low- (n = 47, 21%) risk groups. The high-risk prediction was associated with higher HCC recurrence (HR = 4.8, p<0.001) as well as overall death (HR = 4.8, p<0.001) in multivariable modeling. The association with HCC risk remained significant in subgroups of patients with HCV (HR = 3.0, p = 0.028), HBV (HR = 6.1, p<0.001), alcohol (HR = 6.1, p = 0.023), and NASH (HR = 10.9, p = 0.03) in multivariable models including cirrhosis and AJCC stage 2/3. The association was diminished in NAFLD patients (HR = 3.0, p = 0.16), suggesting that presence of active inflammation and/or fibrosis is critical for the signature to predict HCC risk in NAFLD. Established cirrhosis was less frequent in NAFLD/NASH (23%/21%) compared to other etiologies (50%). Conclusions: The prognostic liver gene signature was implemented in a clinically applicable assay, and successfully validated for de novo HCC recurrence after curative resection of early-stage HCC in all major etiologies of chronic liver disease and HCC. The assay will enable prioritization of patients for HCC surveillance and enrichment of high risk patients for more cost-effective clinical trials of new HCC chemopreventive therapies.

**#5001 Functional biomarker discovery from cancer patient serum with PEP technology.** David L. Wang, Guofeng Fu, Michael Davies, Xing Wang, Array Bridge Inc, St. Louis, MO.

Proteins play essential roles in numerous biological processes and being able to detect functional differences between cell or tissue samples can greatly aid in understanding disease processes and metabolic changes. The PEP technology allows systematic analysis of protein functions within a proteome. Hundreds of functional proteins can be separated and functionally assayed to generate a comprehensive three-dimensional landscape of protein families such as protein kinases, phosphatases, proteinases and oxidoreductases. This information can then be integrated into other genomic and proteomic knowledge bases to provide further insight of important biological processes such as cancer development and aging. In the PEP technology, complex protein mixtures are first separated by a modified two-dimensional gel electrophoresis process, giving enhanced resolution while still maintaining protein function. This is followed by an efficient protein transfer to a specially designed 1356-well Protein Elution Plate. After protein transfer, a previously developed PEP model to multiple 384-well microplates, functional assays can be performed on each well to generate an enzyme activity profile displayed in 3-D. Protein components of each well can be further characterized by mass spectrometry if desired. Using the PEP technology, functional biomarker candidates have been identified from lung and breast cancer patient serum. Both qualitative and quantitative differences of metabolic enzymes and proteinases were observed when comparing the cancer patient serum to matched normal serum. Some of the active enzymes were identified by mass spectrometry and validated in selected bioassays. It is believed that this functional proteomics technology provides a unique approach in the discovery of potential cancer biomarkers for diagnostic and prognostic applications.
Preliminary studies have shown that Lactobacillus kefiri P-IF, a novel kefir product, has the ability to activate dendritic cells, and to induce apoptosis in human gastric cancer cells and MDY myeloid leukemia cells in vitro. In this study, we aimed to evaluate the anticancer effect of L. kefiri P-IF against animal bearing Ehrlich Ascites Carcinoma (EAC) and investigate the mechanisms of action. Materials and Methods: Mice were inoculated intramuscularly with EAC cells to develop solid tumors. L. kefiri P-IF was administered orally (2mg/kg/day) to mice 6 days/week, either two days before tumor cell inoculation or nine days after inoculation to mice bearing solid tumors. Tumor growth, blood lymphocyte levels, cell cycle progression, apoptosis, apoptotic regulators expression, TNF-α expression, changes in mitochondrial membrane potential (MMP), PCNA, and CD4+ and CD8+ T cells in tumor cells were quantitatively evaluated by flow cytometry or RT-PCR. Further studies in vitro were carried out where EAC cells were cultured in the presence of L. kefiri P-IF at different concentrations (0, 0.6, 1.25, 2.5, and 5 mg/mL) for 1, 24, and 48 hrs. % cell viability and IC50 was estimated by MTT assay. Results: 100% of mice that were inoculated with EAC cells alone developed tumors. Mice pretreated with L. kefiri P-IF showed tumor incidence in 76.5% of the animals. Treatment with L. kefiri P-IF markedly reduced tumor weight: 64.6% for pretreatment and 48.6% for post treatment. Mice treated with L. kefiri P-IF showed profound suppression of tumor marker PCNA expression and cell cycle arrest in the sub-G1-G0 phase, as indicated by elevation in the apoptotic cell population by 2.7 and 2.3 fold of control for pretreatment and post treatment, respectively. Pretreatment or post treatment with L. kefiri P-IF in EAC mice increased the Apoptosis index/Proliferation index ratio by 3.4 and 2.4 fold, respectively versus control. L. kefiri P-IF treatment modulated apoptotic regulators, including up-regulation of p53 expression, increased Bax/Bcl-2 ratio, decreased MMP of cancer cells, and activation of caspase-3 expression. L. kefiri P-IF intake maintained blood lymphocytes within the normal levels, increased the number of infiltrating CD4+ and CD8+ T cells within the tumor, and enhanced TNF-α expression. Finally, L. kefiri P-IF enhanced cytokotoxicity against EAC cells in vitro, as shown by IC50 of 3.30 mg/mL, 1.56 mg/mL and 1.11 mg/mL for 1, 24, and 48 hrs, respectively. Conclusion: In conclusion, L. kefiri P-IF, a novel symbiotic microbe, may have chemopreventive potential to reduce tumor incidence and tumor growth by inducing apoptosis in EAC cells via mitochondrial dependent pathway, suppressing cell proliferation, and stimulating the immune system. L. kefiri P-IF was provided by Paitos Co., Ltd. Yokohama, Kanagawa, Japan.
was monitored weekly using biomimetic imaging. SUMMARY: Expression of E-selectin is high in HUVECs, MSPI and H55; CLA was high only in RPMI8226; and CXCRI4 was high in all MM cells. MM1 cells adhesion to stroma was decreased by GMI-1271 and even more profoundly decreased by GMI-1359 in a dose-dependent manner. Chemotaxis of MM1 cells towards conditioned media or SDF1 was moderately affected by GMI-1271, but completely abolished by GMI-1359. MM cell proliferation was significantly inhibited by GMI-1359, only when MM cells were co-cultured with stroma, but not alone. Stromal-induced drug resistance of MM cells to carfilzomib and lenalidomide was reduced in the presence of the GMI compounds, while the effect of GMI-1359 was stronger than GMI-1271. In vivo, GMI-1271 and lenalidomide as single agents delayed tumor growth, while the effect of GMI-1359 was stronger than GMI-1271 and lenalidomide as single agents. In addition, in vivo colon tumorigenesis models using conditional knock-out mice revealed that the absence of IFNγ expression by intestinal epithelial cells fosters tumor growth. Altogether, our data suggest that the loss of IFNγ responsiveness is a common event in CRC and protects tumor cells against the anti-tumorigenic effects of IFNγ.

#5006 Correlation between PD-L1 expression and tumor clinicopathologic characteristics in early stage breast cancer. Hadeel Assad, Juan Cattan, Subramanyeswara Arekapudi, Nancy Jackson, Hugo Macchi Conteri, Sherri Motahari, Hema Govil, Anibal Drelichman.

Background: Upregulation of Programmed Death-Ligand 1 (PD-L1) in tumor cells results in the deactivation of cytotoxic T cells in the tumor microenvironment and development of tolerance to the malignant cells. We aimed to investigate the role of PD-L1 expression in early stage breast cancer and its correlation with tumor characteristics as well as assess the relationship between PD-L1 membrane protein and gene expression. Methods: For this single center, retrospective study, women (age ≥18 years) with early stage breast cancer diagnosed between January 2005 and December 2005 were enrolled. Key inclusion criteria for enrollment were pathologic diagnosis of DCIS or invasive ductal/lobular carcinoma, stage 0-2, with local resection done in 2005. Patients had to have adequate formalin-fixed paraffin-embedded tumor specimens for PD-L1 testing. We hypothesized that higher levels of PD-L1 expression correlates with more aggressive disease and worse prognosis. We used histopathological analysis, medical chart review, and PD-L1 expression via immunohistochemistry (IHC) and reverse transcription polymerase chain reaction (RT-PCR) for data analysis. Chi Square and one way ANOVA analyses were used to compare clinical and pathologic characteristics of patients with high and low PD-L1 expression. Results: In 2005, 368 women were diagnosed with breast cancer at Providence-Park Hospital of which 31 pathological specimens were obtained for exploratory analysis. PD-L1 protein expression was determined to be positive if more than 1% of the tumor cells showed partial or complete membrane staining for PD-L1 by IHC. All patients with DCIS (n=7) were negative for PD-L1 expression although 5 showed gene expression by PCR. After excluding the DCIS specimens, 17% (4/24) of early stage breast cancer was found to express PD-L1 protein. Seven of 24 specimens (excluding DCIS) were positive for PD-L1 RNA by RT-PCR. There was no relationship between PD-L1 protein and mRNA expression (p=0.552). 75% of the PD-L1 positive specimens by IHC were also ER positive although this was not statistically significant (p=0.312). There was a trend toward larger tumors for those expressing PD-L1 on the cell surface (mean size of 2.7 cm vs 1.7 cm, p = 0.07). All the PD-L1 positive specimens had increased lymphocyte infiltration, although no statistical significance was observed. At 10 years from initial diagnosis, all-cause mortality rate was 29% (7/24). The rate of death in PD-L1 positive patients was 2/4 (50%) compared to 5/20 (25%) in PD-L1 negative group. Conclusion: Expression of PD-L1 in early stage breast cancer could be an important indicator for more aggressive disease and supports investigating checkpoint inhibitors in this population. Further investigation is needed to determine its applicability in clinical decisions and effects on treatment.


The presence of an interferon-dominated Th1 immune response in colorectal carcinoma (CRC) has been associated with improved clinical outcome. We have previously identified the guanylate-binding protein 1 (GBP-1), one of the major IFN-γ-induced proteins, as a marker for the Th1 immune response and an independent positive prognostic factor in CRC. We also found that GBP-1 is a necessary mediator of the anti-tumorigenic effects of IFNy. Interestingly, the expression of GBP-1 in CRC was observed more frequently in the desmoplastic stroma than in the directly adjacent tumor cells, suggesting that CRC tumor cells might become resistant to IFN-γ in the course of tumorigenesis. Indeed, we found that several colorectal carcinoma cell lines failed to express GBP-1 or other interferon-stimulated genes after treatment with IFN-γ, and were resistant to IFN-γ-induced apoptosis or proliferation inhibition. In these cell lines, the loss of IFN-γ responsiveness correlated either with the down-regulation of the IFN-γ receptor alpha chain (IFNγRα) or with the presence of a mis-glycosylated form of IFNγRα, which displayed an aberrant intracellular localization. The presence or absence of GBP-1 in CRC cells correlated with the clinical level by showing that the expression of IFNyRes is decreased in CRC compared to normal tissue. In addition, the down-regulation of IFNγRα expression in CRC correlated with a reduced cancer-related survival together with a higher rate of distant metastasis. In addition, in-vivo colon tumorigenesis models using conditional knock-out mice revealed that the absence of IFNγ expression by intestinal epithelial cells fosters tumor growth. Altogether, our data suggest that the loss of IFNγ responsiveness is a common event in CRC and protects tumor cells against the anti-tumorigenic effects of IFN-γ.

#5008 The brain microenvironment mediates resistance in luminal breast cancer to PI3K inhibition through HER3 activation. Gino B. Ferraro,1 David P. Kodack,2 Vasileios Askoxylakis,1Qing Sheng,3 Mark Badeaux,4 Shom Goel,5 Xiaolong Qi,5 Ram Shankaraiah,5 Alexander Z. Cao,5 Rakesh R. Ramkumar,3 Divya Singh,1 Jonathan K. Bluchman,4 Mark V. Patel,1 Younghul Song,1 Carlotta Costa,1 Kamila Naxerova,1 Christina Wong,1 Jonas Kloeper,1 Rita Das,1 Angela Tam,1 Jantina Tanboon,1 Dan G. Duda,1 Ryan C. Miller,1 Marni B. Siegel,4 Carey K. Anders,5 Melinda Sanders,5 Valeria M. Estrada,5 Robert Schlegel,5 Carlos L. Arteaga,5 Elena Brachtel,5 Alan Huang,5 Dai Fukumura,6 Jeffrey A. Engelman,5 Rakesh K. Jain.1 1Massachusetts General Hospital / Harvard Medical School, Boston, MA; 2Novartis, Cambridge, MA; 3Massachusetts General Hospital / Harvard Medical School / DanaFarber Cancer Institute, Boston, MA; 4Linberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC; 5Vanderbilt Ingram Cancer Center, Nashville, TN.

Brain metastases represent a devastating progression of luminal breast cancer. While targeted therapies are often effective systemically, they fail to adequately control brain metastases. In preclinical models that faithfully recapitulate the disparate clinical responses in these microenvironments, we observed that brain metastases evade PIK3 inhibition despite efficient drug delivery. In comparison to extracranial disease, there is increased HER3 expression and phosphorylation in the brain lesions. HER3 blockade overcomes the resistance of both HER2-amplified and/or PIK3CA-mutant breast cancer brain metastases to PI3K inhibition, leading to striking tumor growth delay and significant improvement of mouse survival. Collectively, these data provide a mechanistic basis underlying therapeutic resistance in the brain microenvironment and identify rapidly translatable treatment strategies for HER2-amplified and/or PIK3CA-mutant breast cancer brain metastases.

#5009 Self-renewal of hypoxic glioma stem-like cells is facilitated by secretion-mediated STAT3 activation. Damian A. Almiron Bonnin,1 Matthew C. Havrda,2 Huan Liu,1 Myung Chang Lee,1 Matthew H. Ung,1 Chao Cheng,2 Mark A. Israel2. 1Dartmouth Geisel School of Medicine, Dartmouth, Lebanon, NH; 2Geisel School of Medicine at Dartmouth, Lebanon, NH.

Glioblastoma, a type of high-grade glioma, is the most common and the most aggressive primary brain tumor of adults. The median survival is 12-15 months. Despite multimodality treatment, most high-grade gliomas eventually recur and are ultimately incurable. Several studies suggest that the initiation, progression, and recurrence of gliomas are driven at least partly by cancer stem-like cells. A defining characteristic of these cancer stem-like cells is their capacity to self-renew. We have identified a pathway dependent upon tumor hypoxia, a commonly found pathologic feature of GBM, that promotes self-renewal through the HIF1α-JAK1/2-STAT3 axis. Under hypoxic conditions, HIF1α is greatly increased in GBM stem-like cells leading to the activation of JAK1/2-STAT3 which mediates enhanced tumor stem-like cell self-renewal. Our data further demonstrate the importance of VEGF secretion for this pathway of hypoxia-mediated self-renewal. Using tumor stem-like cells derived from the S100β-v-erbB/p53-/- mouse model of spontaneous high-grade glioma, we have discovered that the activation of STAT3 through hypoxia-mediated VEGF secretion is required for self-renewal of glioma stem-like cells. Media conditioned by these S100β-v-erbB/p53-/- tumor sphere cells cultured under hypoxic conditions promoted self-renewal in S100β-v-erbB/p53-/- glioma stem-like cells and depletion of VEGF from this media was sufficient to inhibit STAT3 phos-
phorylation. Moreover, we have been able to identify two drugs: Brefeldin A and EHT-1864, that significantly inhibit VEGF secretion, decrease stem cell self-renewal, inhibit tumor growth, and increase the survival of mice allografted with $10^6$ B-v-erbB1/p53$^+$ glioma stem-like cells. Cells treated with these drugs express molecular signatures that correlated strongly with the increased survival of GBM patients. These findings suggest a novel treatment strategy to inhibit hypoxia-mediated self-renewal of glioma stem-like cells in high-grade gliomas by targeting the secretion of extracellular, paracrine mediators of self-renewal.

**#5010 Activation of the notch signaling pathway confers a tumor-suppressive phenotype on melanoma-associated fibroblasts.** Hongwei Shao, Mecker G. Moller, Long Cai, Leiming Zhang, Zhao-Jun Liu. *Univ. of Miami, Miami, FL*

Objectives. The tumor microenvironment (TME) is an emerging therapeutic target for cancer treatment. Cancer-associated fibroblasts (CAF) play a crucial role in cancer progression. We aim to target TME by altering intracellular signaling which determines the biological function of CAF. We have recently showed that the Notch signaling pathway likely functions as a molecular switch in controlling the tumor regulatory role of CAF in animal models and experimentally created “CAF”. Here, we investigated the status of Notch signaling in human melanoma-associated fibroblasts (MAF) versus their normal counterparts and tested whether manipulation of the Notch pathway activity in MAF alter their tumor-regulating function.

Methods. We examined levels of Hes1, a canonical Notch target, in MAF of human malignant melanoma at different stages (I-IV) and fibroblasts in either adjacent or non-adjacent normal skin tissues using tissue microarray. MAF were isolated from human metastatic melanoma tissues. Notch pathway RT2-PCRArray and immunoblot were used to assess Notch pathway activity in MAF versus normal human dermal fibroblasts. Activation of Notch signaling pathway in MAF was achieved by lentiviral vector encoding active form of Notch1 (NIC).

The effect of Notch1-engineered MAF on melanoma growth was tested by in vitro co-cultures and in a mouse xenograft model (n=6/group). Tumor angiogenesis was assessed by immunohistochemistry. Results. MAF expressed decreased levels of Hes1 compared with adjacent skin fibroblasts. Isolated MAF also exhibited lower Notch activity than normal human dermal fibroblasts. Notch1-engineered MAF significantly inhibited melanoma cell growth in vitro (p<0.01) and suppressed melanoma growth and tumor angiogenesis in mice (p<0.05). Conclusions. Notch pathway activity is down-regulated in MAF. Increase of Notch pathway activity confers MAF with inhibition to melanoma growth and tumor angiogenesis. Our study demonstrates that Notch signaling is a critical molecular switch in determining the tumor regulatory role of MAF and provides potential targets for cancer therapeutic interventions on the TME.


Gastric cancer is the third leading cause of cancer-related death, and peritoneal dissemination is one of the most frequent site of recurrence. Although the role of tumor microenvironment is recognized, it is still unclear how intraperitoneal cancer-immune microenvironment contributes to peritoneal metastasis. Thus, we investigated the interaction between intraperitoneal cancer and immune cells, especially focusing on tumor-associated macrophages (TAM) in the peritoneal cavity. The peritoneal wash from gastric cancer patients was subjected to conventional flow cytometry, flow cytometry, and immuno-fluorescent assay using antibodies against CD45 (leukocytes), CD14 (monocytes), CD80/163 (M1/M2 macrophages, respectively), in combination with genetically engineered cancer-imaging viral agent ‘TelomeScan’ which specifically expresses GFP in telomerase-positive cells. In cytology-positive samples, CD163-positive macrophages could be detected in approximately 70% of intraperitoneal macrophages with TelomeScan positive cancer cells, while cytology-negative samples contained few CD163-positive TAMs. Therefore, we showed that CAF also induced TAMs in CD163-negative samples, which further suggests that TAMs may play a role in the development of peritoneal metastasis.

**TUMOR BIOLOGY: Models for Treatment Resistance and Drug Discovery**

**#5012 Mouse-Human co-clinical trials demonstrate superior efficacy with combinational approach of BKM120 and erbitux over BKM120 mono-therapy in patients with recurrent and/or metastatic squamous cell carcinoma of head and neck (R/M-SCCCHN).** Han Na Kang, 1 Mi Ran Yun, 1 Kyoung Ho Pyo, 1 Myung-Ju Ahn, 1 Jong-Mu Sun, 2 Sun Min Lim, 3 SooMyung Paik, 4 Byoung Chul Cho, 2 Byoung Chul Cho, 2

Miami, FL

Background: To investigate clinical activity, safety and biomarkers of a pan-PI3K inhibitor BKM120 in R/M-SCCCHN and identify optimal combinational strategies by conducting mouse co-clinical trials mirroring the ongoing clinical study. Methods: Patients with R/M-SCCCHN were eligible if they had progressed on platinum-based chemotherapy, and were treated with BKM120 100mg/day. The primary endpoint was disease control rate (DCR) at 8 weeks. Secondary endpoints included response rate (RR), progression-free survival rate (PFS), overall survival (OS) and safety. Patient-derived xenografts (PDXs) with genomic annotations were established directly from patients for evaluation of novel drug combinations and biomarkers. Based on the integrated clinical and preclinical data, study protocol had been revised to combine BKM120 and erbitux to explore whether erbitux inhibits the growth of R/M-SCCCHN cells. Results: A total of 52 patients were enrolled. Patient characteristics included median age (55 years; range, 31-82); male (84.6%); ECOG performance status 0/1/2 (13.5%/73%/13.5%); Ilocoregional/metastatic (both (30.8%/38.4%); oral cavity/oropharynx/larynx primary (36.5%/30.8%/13.5%); prior chemotherapy regimen 1/2 (40%/60%). Seven patients were not evaluable due to rapid progression or withdrawal. DCR at 8 weeks was 60% (27/45) and PR was 2.2% (1/45) in BKM120 monotherapy. Median PFS and OS were 8.0 (95% CI, 5.3-9.5) and 30.7 weeks (95% CI, 11.8-26.6). After protocol revision, 11 patients were treated with BKM120 and erbitux after progression to BKM120. In combination phase, PR was observed in 18.1% (2/11) and SD was 45.5% (5/11) in patients who failed to BKM120. Toxicities were not increased in combination phase. In seven established PDXs, we tested BKM120 alone or in combination with erbitux. All PDXs showed strong resistance to single-agent BKM120, whereas three PDXs (3/7, 42.8%) demonstrated strong synergistic inhibition of tumor growth to combined BKM120 and erbitux, compared with single agent (vs. BKM120, P<0.01; vs. Erbitux, P<0.01), which resembled the responses of corresponding patients. We found several genetic alterations in F2 tumors including EGR fusion, HRAS mutation (G12D), MYC amplification, KRAS mutation (G12D), and EGFR amplification (40%). Most interestingly, two PDXs with RAS mutations demonstrated synergistic effect of BKM120 and erbitux. Conclusions: Combining BKM120 and Erbitux would be effective treatment strategies with manageable toxicity in R/M-SCCCHN based on strong rationale of co-clinical trial with PDXs (NCT01527877).

**#5013 A 3D culture system identifies a new mode of cetuximab resistance and disease-relevant genes in colorectal cancer.** Bhuminder Singh,1 Cunxi Li,1 Ramona Graves-Deal,1 Haiting Ma,1 Alina Starchenko,1 William H. Fry,1 Yu-anuyu Lu,1 Yang Wang,1 Galina Bogatcheva,1 Mohseen P. Khan,1 Ginger L. Milne,2 Shilin Zhao,2 Gregory D. Ayers,2 Nenggan Li,1 Mary K. Washington,1 Timothy J. Yeatman,1 Oliver G. McDonald,1 Qi Liu,1 Robert J. Coffey1.

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We previously reported that single cells from a human colorectal cancer (CRC) cell line (HCA-7) formed either hollow single-layered polarized cysts or solid spiky masses when plated within type-1 collagen in 3D. To begin in-depth analyses into whether clonal cysts and spiky masses possessed divergent malignant properties, individual colonies of each morphology were isolated and expanded. The lines thus derived faithfully retained the parental cystic and spiky phenotypes and were termed CC (cystic) and SC (spiky), respectively. Although both CC and SC expressed EGF receptor (EGFR), cetuximab strongly inhibited growth of CC, whereas SC was resistant to growth inhibition and this was coupled to increased tyrosine phosphorylation of MET and RON. Addition of MET/RON tyrosine kinase inhibitor BKM120 in CC and SC failed to rescue CC, but restored cetuximab sensitivity. To characterize genome-wide divergence between CC and SC, we performed comprehensive genomic and transcriptomic analysis of CC and SC in 3D. One of the most upregulated genes in CC was the tumor suppressor 15-PGDH/HPGD and the most upregulated gene in SC was...
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versecan (VCAN) in 3D and xenografts. Analysis of a human CRC tissue microarray showed that epithelial, but not stromal, VCAN staining strongly correlated with reduced survival, and combined epithelial VCAN and absent HPGD staining portended a poorer prognosis. Thus, with this 3D system, we have identified a new mode of cetuximab resistance and a potential prognostic marker in CRC. As such, this represents a potentially powerful system to identify additional therapeutic sensitivities and disease-relevant genes for CRC.

#5014 Drug discovery for NF1-associated malignant peripheral nerve sheath tumors using the zebrafish model. Dong Hyuk Ki, Shuning He, A. Thomas Look. Dana-Farber Cancer Institute, Boston, MA.

Children and young adults with type 1 neurofibromatosis (NF1) are at risk to develop plexiform neurofibromas, which can undergo malignant transformation to malignant peripheral nerve sheath tumors (MPNSTs). MPNSTs are among the most frequently occurring sarcomas in children and young adults and are especially problematic in NF1 patients. Currently, complete surgical excision is the only curative therapy for NF1-associated MPNST, but these tumors are often not completely resectable. Therefore, it is very important to identify promising drugs that can be rapidly moved into clinical trials to improve the therapy of patients with MPNSTs. We have developed a faithful zebrafish model of NF1-associated MPNST by using genome engineering to develop zebrafish lines harboring loss-of-function mutations of the duplicated nf1a and nf1b genes and p53. Our nf1 and p53 deficient zebrafish models develop MPNSTs that are very similar to human NF1-associated MPNST, and thus are ideal for experiments to test the efficacy of FDA-approved small molecule drugs in vivo. To test the FDA-approved drugs for activity against NF1-associated MPNST tumor cells, about 100 Sox10+/ChERRY-expressing MPNST primary tumor cells were transplanted into dechorionated 2-day-post-fertilization (dpf) larval fish using a micro-injector. MPNST-implanted embryos were exposed to individual test compounds added to the fish water at a range of concentrations and to the vehicle control. After 4 days of treatment, quantitative assessment of the remaining mCherry-labeled tumor cell numbers were measured using the ImageJ software for each treated embryo. Currently, trametinib and sunitinib caused marked antitumor effects indicated by reduced numbers of mCherry-labeled nf1-deficient MPNST cells compared to the DMSO control in this study. We are now applying this embryonic implantation assay to identify combinations of FDA-approved drugs that have a high degree of efficacy against MPNST and can be rapidly "repurposed" to improve the treatment of this disease.

#5015 Innovative and predictive models against breast cancer. Olivier Du-champ,1 Séverine Tabone-Eglinger,2 Caroline Mignard,1 Isabelle Goddard,3 Georges François Leclerc. Dijon Cedex, France;1 2Modul-Bio, Marseille, France;1 3Institut Curie, Paris, France.

The 40 established breast PDX models represent the 4 major molecular clinical subtypes among which 37% are triple negative, 33% are Luminal BHER2-, 22% are Luminal AHER2+. Patient surgical specimens from different breast tumor subtypes, among 9 other tumors, associated efforts from public hospitals, academic groups, biotechs and private pharmaco-technical companies with the financial support of the French Ministry of Industry. Patient surgical specimens from different breast tumor subtypes, among 9 other cancer pathologies, are collected to establish large collections of PDXs in mice. In addition, in vitro primary cultures of cells from these samples are conducted to establish a collection of cell lines from the stromal and the tumor compartments. The 40 established breast PDX models represent the 4 major molecular clinical subtypes among which 37% are triple negative, 33% are Luminal B HER2+, 22% are Luminal B HER2− and 8% HER2+. The models are evaluated for ex vivo and in vivo sensitivities to 4 clinically relevant anticancer drugs, for histology, immune infiltrates, and molecular characteristics including the analysis of gene polymorphism, RNAseq gene expression and NGS exome sequencing of 112 genes. The metagene expression sequencing of the gut microbiota from stools collected in both patients and tumor-bearing mice before and after chemotherapy treatments improves our knowledge on the role of microbiota in cancer progression and treatment. Secondly, to increase the predictability of the models, we are generating mice with humanized liver showing distinct drug pharmacokinetic profiles as compared with parental mice. Finally, the humanization of the immune system in mice is developed by several approaches including the use of induced pluripotent stem cells from cancer patients. These tumor collection and model characterization were performed under harmonized procedures within the consortium, allowing high quality material and reproducible characterization of the models. A compiled database base for efficient features search and data mining. We will present the first characterized breast cancer models and will discuss their usefulness and chance to bring benefit to patients via this holistic strategy developed within the IMODI initiative.

#5016 High-throughput patient-derived 3-dimensional organoid cultures as personalized models to assess drug response and post-treatment residual disease. Eugen Dhimolea,1 Ricardo De Matos Simoes,2 Yoko DeRose,3 Pallavi Awate,1 Xiang Weng,1 Huihui Tang,1 Aedin Culhane,1 Alana Welm,4 Constantine Mitsiades.1 Dana Farber Cancer Institute, Boston, MA;2 Huntsman Cancer Institute, Salt Lake City, UT.

Cell lines grown in 2-dimensional (2D) plastic surfaces have historically been the main models to identify cancer cell vulnerabilities. In contrast, malignant tumors in vivo grow as 3-dimensional (3D) cellular masses and manifest remarkably different drug-response patterns compared to cell lines. We have shown that breast cancer (BrCa) cells in 3D extracellular matrices acquire distinct phenotypic properties, compared to the 2D counterparts. Notably, serum-free culture medium supplemented with defined cocktails of developmental morphogens was sufficient to support the growth of BrCa cell lines in 3D, but not in 2D conditions; indicating a key role of 3D architecture in vivo growth regulation. CRISPR/Cas9-based gene editing screens in BrCa cells indicated that a distinct gene subset govern the growth in 3D vs. 2D conditions. Using similar culture conditions, we expanded several molecularly annotated BrCa patient-derived 3D Organoids (3D PDOs), adapted them into a high-throughput miniaturized drug screening platform, and interrogated them against a panel of ~500 target-annotated kinase inhibitors and FDA-approved anti-cancer agents. This pharmacological screen revealed high sensitivity to distinct classes of agents (eg. PI3K inhibitors), but also variable responses to others (eg. EGFR inhibitors); and provided insight into potentially driver vs. passenger mutations in the respective patients. Co-culture of PDOs with non-malignant stromal cells from bone metastatic niche reduced sensitivity to some classes of drugs (eg. PLK inhibitors). Importantly, long-term (up to 3-4 weeks) treatment of 3D organoids did not eradicate the cells in culture (in contrast to conventional 2D assays), but generated a cell subpopulation which remained viable for the duration of the experiment, reminiscent of the residual disease after initial clinical partial response to a drug. This residual disease phenotype was also confirmed in vivo, when the respective PDX-Organoids were treated with the same agents. The majority of transcripts with differential expression in residual PDO tumors were also coincidentally up- or down-regulated in the respective 3D PDO model. These transcripts included canonical regulators of cell cycle, chromatin signal- ing and epigenetic mechanisms, cytoskeletal function and metabolic pathways; as well as other transcripts not previously linked to drug resistance in conventional 2D models. To our knowledge this is the first description of an in vitro personalized cancer model that simulates both molecularly and phenotypically the most important residual disease in vivo. Our high-throughput platform can potentially complement genomically diverse personalized medicine and help discover novel drugs that overcome drug resistance.

#5017 Visualization of the mechanisms of metastasis within a biomimetic engineered tumor microenvironment encompassing a perfusable cylindrical 3D microvessel. Andrew D. Wong,1 Vanesa Silvestri,2 Andrew J. Ewald,2 Peter C. Seamon,1 Johns Hopkins University, Baltimore, MD;2 Johns Hopkins University School of Medicine, Baltimore, MD.

Metastasis is responsible for the majority of cancer related deaths; however, many of the biological and physical details surrounding the critical steps (e.g. invasion and intravasation) are largely unknown, in part due to the difficulty in recapitulating and visualizing these dynamic processes. To elucidate these mechanisms, we have developed an in vitro model of invasion and intravasation that comprises tumor cells embedded within an extracellular matrix (ECM) surrounding an engineered microvessel. The microvessel is cylindrical, 150 µm in diameter, and lined with endothelial cells forming a functional barrier that is maintained under constant perfusion at a shear stress of 2-8 dyne cm−2. Using this biomimetic tumor-microvessel platform, we have previously visualized invasion and intravasation of single MDA-MB-231 breast cancer cells into the microvessel and escape into flow. Here, we present mechanistic details of intravasation from both single tumor cells (MDA-MB-231) and mouse mammary tumor organoids (MMTV-PyMT). For single cells, we observe a mitosis-mediated mechanism of intravasation where tumor cell entry into flow is prefaced by cell division at the ECM-vessel interface. For tumor organoids,
we see vascular deformation and destabilization from growing tumors that impinge upon proximal vessels, and that bulk intravasation is mediated by shear stress and tumor cell adhesion. As we characterize the various ways in which tumor cells interact with the vessel endothelium and intravasate, we can explore strategies involving the tumor vasculature and clinically relevant drugs to inhibit critical steps in the metastatic cascade.


Immune checkpoint blockade therapies, which unleash the immune system’s ability to recognize and eliminate cancer cells, are currently among the most promising treatments for cancer. Antibodies that block the immune inhibitory receptors CTLA4 and PD1 have shown clinical success, with objective response rates over 20% and some durable responses exceeding 10 years. However, some cancers, such as colorectal cancer (CRC), have proven particularly refractory to immunotherapy. Although it is appreciated that the colon is an immunosuppressive environment, the mechanism and extent of immune suppression remains unclear. Studies of human CRC tumors have shown that T cell infiltration is an important predictor of clinical outcome, yet current mouse models of CRC are not particularly well suited to the mechanistic study of T cell responses against tumor-specific antigens. Synergistic cell line transplants, by far the most widely used models to study adaptive immune response in CRC, are characterized by a highly complex tumor microenvironment and have not employed orthotopic transplant into the colon. In order to track highly specific T cell responses in CRC, we have employed a novel colon organoid-based system, in which transformed organoids expressing model antigens are orthotopically transplanted into the wall of the colon via colonoscopy-guided injection, a method pioneered in the lab of Omer Yilmaz. Organoids were harvested from VillinCreER:APc4H2mice and grown in the presence of Wnt ligand; addition of tamoxifen and removal of Wnt from the media facilitated expression of the colon-specific Cre, deletion of Apc, and selection of transformed organoids capable of growing in the absence of Wnt. To induce expression of model antigens, organoids were infected with a lentiviral vector that, in addition to the fluorescent reporter FusionRed, expressed the T cell antigens SIYRYYGL and two additional antigens, one of which is activated in cancer cells. By carrying out experiments in two AT-rich cell culture conditions, we showed that Hmgal amplifies Wnt/β-catenin signaling by inducing genes encoding both Wnt agonists and downstream Wnt target genes, and in human colon cancer cells, which is comprised of terminally differentiated crypt cells that secrete Wnt to support ISCs. Because Paneth cells require Sox9 for development, we determined whether Hmgal regulates its expression. Hmgal binds directly to the Sox9 promoter; overexpression of Sox9 is positively correlated, and both become markedly up-regulated in colon carcinogenesis. This work not only provides new insights into the role of Hmgal in intestinal homeostasis by maintaining the stem cell pool and epithelial niche compartment, but also suggests that deregulated Hmgal perturbs this equilibrium during polyposis and carcinogenesis. Our results also highlight the Hmgal-Wnt-Sox9 axis as a potential mediator of colon carcinogenesis.

#5019 HMGAl amplifies Wnt signaling and expands the intestinal stem cell compartment to drive premalignant polyposis in transgenic mice. Lining Xian, Dan Georgess, Li Luo, Lionel Chia, Qihua Gu, Tait Huso, Amy Belton, David Huso, Andrew Ewald, Linda M.S. Resar. JHU Medical Institution, Baltimore, MD.

Emerging evidence suggests that cancer cells undergo chromatin remodeling and epigenetic reprogramming to co-opt stem cell properties and drive tumor progression. The HMGAl chromatin remodeling protein is an archetypal transcription factor that binds to DNA at AT-rich sequences where it “opens” chromatin, recruits transcriptional complexes, and modulates gene expression. The HMGAl gene is highly expressed during embryogenesis and in adult stem cells, but silenced postnatally in differentiated tissues. HMGAl becomes re-expressed in most high-grade cancers and high levels portend adverse clinical outcomes. In colon cancer, HMGAl is among the genes most highly overexpressed compared to normal intestinal epithelium. We previously reported that HMGAl drives tumor progression in colon cancer by inducing stem cell genes involved in an epithelial-mesenchymal transition. We also discovered that Hmgal1 transgenic mice develop marked proliferative changes and pre-malignant polyposis in the intestinal epithelium. To determine how Hmgal functions in the intestines during tissue homeostasis and carcinogenesis, we examined in transgenic mice and organoid models. Here, we uncover a novel role for Hmgal in maintaining the intestinal stem cell (ISC) pool and Paneth cell niche. Hmgal is required by ISCs to organize into three-dimensional organoids in vitro; silencing Hmgal disrupts organoid formation and bud development. Conversely, overexpression of Hmgal increases organoid formation, bud development, and repressing epithelial hexamer function and epithelial tight junctions. Overexpression of Hmgal expands the ISC compartment. To determine how this occurs, we performed in vivo imaging and discovered that Hmgal enhances self-renewal of ISCs. Mechanistically, we found that Hmgal amplifies Wnt/β-catenin signaling by inducing genes encoding both Wnt agonists and downstream Wnt target genes. Surprisingly, Hmgal also expands the Paneth cell niche, which is comprised of terminally differentiated crypt cells that secrete Wnt to support ISCs. Because Paneth cells require Sox9 for development, we determined whether Hmgal regulates its expression. Hmgal binds directly to the Sox9 promoter; overexpression of Sox9 is positively correlated, and both become markedly up-regulated in colon carcinogenesis. This work not only provides new insights into the role of Hmgal in intestinal homeostasis by maintaining both the stem cell pool and epithelial niche compartment, but also suggests that deregulated Hmgal perturbs this equilibrium during polyposis and carcinogenesis. Our results also highlight the Hmgal-Wnt-Sox9 axis as a potential mediator of colon carcinogenesis.

#5020 A genome-scale ORF screen reveals an alternative splicing program that regulates mesenchymal and stem-like cell states in breast cancer. Ji Li,1 Peter Chou,1 Gabe Labelle-Chaffer,1 Karthik Ramesh,1 Alice Bruggeman,1 Chao Dai,1 Andrew Giacomelli,1 Seav Huong Ly,1 Justin Huang,1 Andrew Hong,1 Nina Ilic,1 Ole Gjoerup,1 Matthew Meyerson,1 Angela Brooks,2 Robert Weinberg,2 William Hahn,1 Dana-Farber Cancer Institute, Harvard Medical School, Broad Institute of MIT and Harvard, Boston, MA;2Whitehead Institute for Biomedical Research and MIT, Cambridge, MA;3Broad Institute of MIT and Harvard, Cambridge, MA;4University of California, Santa Cruz, Santa Cruz, CA.

Cells residing in mesenchymal state are often associated with stem cell properties. The phenotypic changes from epithelial to mesenchymal cell state, or from non-stem-like to stem-like cell state contribute to tumor heterogeneity and play important roles in tumor initiation, progression and metastasis. To systematically interrogate the modulators of epithelial-to-mesenchymal transition, we performed a genome-scale ORF screen to identify regulators of mesenchymal and stem-like cell states using a barcoded human ORFome expression library in human mammary epithelial cells. In the screen, we used flow cytometric analysis of the CD44 cell surface marker and identified 68 ORFs that can switch cells from CD44 low state to CD44 high state in 7 days. Among these genes, the RNA splicing factors were highly enriched as analyzed by GO terms and Gene set enrichment analysis (GSEA). We employed six different assays for candidate validation: 1) Induction of CD44 cell surface markers; 2) Evaluation of the expression of EMT markers; 3) Test of the ability to form mammospheres; 4) Investigation of the expression during EMT induction; 5) Test of the necessity of these splicing factors for EMT and stem-like states; 6) Examination of the ability to promote tumor formation in vivo. We discovered that QKI and RBFOX1 were both necessary and sufficient to promote EMT and stem-like states. MBNL1, MBNL2 and CELF4 were sufficient to induce some mesenchymal markers. We further investigated the downstream targets of these splicing factors by RNA-sequencing analysis. We found that QKI and RBFOX1 regulated the alternative splicing of genes in 5 functional modules: 1) Cell motility and ECM/cytoskeleton organization; 2) Stem cell fate determination; 3) Oncogenic signaling; 4) Epigenetic targets; 5) Cell polarity. Strikingly, using molecular and biochemical assays, we found that QKI and RBFOX1/2 interacted and cooperatively regulated the alternative splicing of a large number of overlapping transcripts, including Filamin B (FLNB). QKI and RBFOX1 induced a shorter isoform via exon skipping, which plays a key functional role in the regulation of EMT. Importantly, the expression of QKI, RBFOX1/2 and the short isoform of FLNB are elevated in basal B type of breast cancer cell lines and in basal-like breast cancer patient samples, the subtype of breast cancer that displays higher degree of mesenchymal and stem-like traits. In conclusion, alternative RNA splicing plays a key role in the regulation of EMT and stem-like cell states. QKI and RBFOX1/2 are both necessary and sufficient to promote EMT and stem-like traits. Alternative splicing of FLNB controlled by QKI and RBFOX1/2 is one of the key downstream targets that regulates EMT. Thus, the molecular targets and mechanism identified in this study may aid in the development of new diagnostic and therapeutic approaches for breast tumors, especially for basal-like breast cancer.

TUMOR BIOLOGY: Tumor Stem Cell Biology

#5021 Semaphorin signal via MICAL3 induces symmetrical cell division of cancer stem-like cells to confer aggressiveness in breast cancer. Kana Tominaga,1 Masao Yano,2 Kei-ichiro Tada,3 Arinobu Tojo,1 Noriko Gotoh4.1Institute of Medical Science, The University of Tokyo, Japan;2Minamimachi Hospital, Japan;3The University of Tokyo, Japan;4Kanazawa Univ. Cancer Research Inst., Kanazawa, Japan.

TUMOR BIOLOGY: Models for Treatment Resistance and Drug Discovery

#5022 Semaphorin signal via MICAL3 induces symmetrical cell division of cancer stem-like cells to confer aggressiveness in breast cancer. Kana Tominaga,1 Masao Yano,2 Kei-ichiro Tada,3 Arinobu Tojo,1 Noriko Gotoh4.1Institute of Medical Science, The University of Tokyo, Japan;2Minamimachi Hospital, Japan;3The University of Tokyo, Japan;4Kanazawa Univ. Cancer Research Inst., Kanazawa, Japan.
Breast cancer stem cells (BCSCs) are thought to be maintained in cancerous tissues by increased frequency of symmetric cell division at the expense of asymmetric cell division in the cancer tissues, although how this occurs it remains largely unknown. Here, we discovered that molecules interacting with Casl3 (MICAL3) contributed to tumor sphere formation in patient-derived breast cancer cells and tumor initiating activity in a patient-derived xenograft (PDX) model. Semaphorin (Sema)-stimulation induced complex formation involving MICAL3 and collapsin response mediator protein 2 (CRMP2), leading to stabilization of Numb in breast cancer cells. Neurilipin-1 (NPI), a receptor for Sema, and Numb were specifically expressed and colocalized at high levels in a BCSC-enriched population that induce tumor sphere formation. MICAL3 knockeddown symmetric cell division and increased asymmetric division of in NPI-high cancer stem-like cells that express high level of Numb at high levels. Thus, MICAL3/CRMP2/Numb pathway appears to play a critical role in the symmetric division of N1P1-high cancer stem-like cells to expand the pool of BCSCs. Finally, we showed that breast cancer patients with higher expression levels of NPI and Numb in cancer tissues show poor prognosis compared with those with lower expression levels of NPI and Numb by using tissue microarray of breast cancer tissues. We provide a rationale for eradicating BCSC by blocking the symmetric division of BCSC by targeting molecules in Sema/NPI/MICAL3 pathway.

**#5024 Unmasking heterogeneity within the adult mammary stem cell compartment**


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Breast cancer is a highly heterogeneous disease at both the molecular and pathological levels. To understand this heterogeneity and ‘cells of origin’ of breast cancer, it is important to dissect the normal mammary epithelial hierarchy. Despite accumulating evidence for a mammalian hierarchy of self-renewing breast cancer cell subsets, little is known about how these cell subsets emerge and maintain their self-renewal abilities in vivo. Two major ISC have been identified to give rise to functional mammary glands following injury (e.g. radiation). The presence of two major ISC expressed by the Wnt target gene Lgr5 are dispensable for homeostasis and are quantitatively ablated by radiation injury. In contrast, quiescent ISCs are indispensable for homeostasis, regenerate CBCs, and are radioresistant. Keratins (Krt) are intermediate filament proteins required for architectural and mechanical support, but for homeostasis, regenerate CBCs, and are radioresistant and participate in tissue regeneration. Interestingly, Krt15 deficiency impairs tissue regeneration following high-dose radiation. Indeed, irradiated Krt15- mice display reduced crypt regeneration, crypt length, microcolonies and BrdU incorporation when compared to irradiated Krt15+ mice. Conclusion: We have identified a long-lived Krt15+ subpopulation of intestinal crypt cells displaying stem cell-like properties that are radio-resistant and tumor initiating. This new stem cell population may open new avenues for therapeutic targeting in colon cancer.

**#5025 EZH2 reprogramming confers intrinsic stem cell properties and developmental plasticity driving neuroendocrine prostate cancer.**

Alastair Davies,1 Musa Ahmed,2 Chiara Bostock,3 Anna Gleave,4 Kirsi Ketola,5 Fraser Johnson,1 Jennifer Bishop,1 Ladan Fazli,6 Hajoie Huang,7 Hansen Hien,8 Amina Zoubide1.

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Background: Intestinal stem cells regulate the emergence of different cell lineages across the crypt-villus axis and orchestrate cellular renewal and replenishment following injury (e.g. radiation). Two major ISC have been identified to give rise to functional mammary glands following injury (e.g. radiation). The presence of two major ISC expressed by the Wnt target gene Lgr5 are dispensable for homeostasis and are quantitatively ablated by radiation injury. In contrast, quiescent ISCs are indispensable for homeostasis, regenerate CBCs, and are radioresistant. Keratins (Krt) are intermediate filaments required for architectural and mechanical support, but also contribute to cell migration and intracellular signaling. Interestingly, Krt15 has been characterized as a stem cell marker in the hair follicle. Here we described a radio-resistant Krt15+ stem cell population in the small intestine and demonstrate that cells within this population are capable of tumor initiation. Methods: Krt15-CrePR1 mice were crossed with Rosa26 reporter mice to perform lineage-tracing experiments in the mouse small intestine. High-dose radiation was used to investigate the role of Krt15 in tissue regeneration. Finally, Krt15-CrePR1;R26mTomato/mGFP mice with 12 Gy revealed that Krt15+ cells are long-lived, post-natally but can be recruited into the cell cycle in response to hormones. Single cell gene expression analyses have also revealed unexpected complexity within the basal and luminal compartments. Moreover, analyses at different stages of development have provided insights into the earliest ‘lineage priming’ events.

**#5026 Disruption of Monocarboxylate transporter-4 Basigin interaction as an effective strategy to inhibit hypoxic response, tumor growth and vascularization, and stem cell phenotype in human glioblastoma in vitro and in vivo**

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Monocarboxylate transporters, constitute a family (SLC16) of proton-linked plasma membrane transporters that carry molecules containing a single carboxylate group across biological membranes. Basigin (CD147), is involved in many physiological functions during early stages of development and in cancer. Basigin has been shown to be required for functional plasma membrane expression of Monocarboxylate transporter-1 and Monocarboxylate transporter-4. Using a cell-based screening assay, we identified acriflavine, a small molecule that inhibits the binding between Basigin and Monocarboxylate transporters in vitro and in vivo. Surface Plasmon Resonance analysis confirmed direct binding of acriflavine to Basigin’s immunoglobulin extracellular domain with a low binding constant (KD) of 0.16μM. Acriflavine inhibits normoxic growth of glioma stem cells in vitro and this activity is augmented by hypoxia or by expression of oxygen-stable mutant forms of HIF-1α or HIF-2α. Treatment of mice bearing established glioma stem cell-derived xenografts resulted in inhibition of tumor growth. Acriflavine treatment inhibited intratumoral expression of the angiogenic cytokine vascular endothelial growth factor and tumor vascularization. Our work shows that disruption of monocarboxylate transporter binding to Basigin is an effective approach to target glioma stem cells.
at a core set of genes governing stem cell identity. EZH2 colocalized at the
reprogrammed AR binding sites. Accordingly, treating AR-indifferent/NEPC cell lines with the EZH2 inhibitor GSK126 yielded a molecular sub-
type shift from PCS1 to AR-driven PCS2, and re-sensitized cells to ARPIs.
Conclusion: Our findings establish the centrality of epigenetic reprogram-
mapping in driving the insurgence of a clinically aggressive neuroendocrine
phenotype in response to AR pathway inhibition. Drugging the epigenome
via EZH2 inhibition to reverse the NEPC state and re-sensitize tumors to our
powerful arsenal of ARPIs has the potential to transform the treatment of
prostate cancer.
Association to drug-induced leukopenia using whole-exome sequencing of non-small cell lung cancer patients with gemcitabine/carcinoblatin regimen. Anna Svedberg,1 Benjamin Sigurgeirsson,2 Niclas Björn,3 SAILendra Pradhananga,2 Eva Brändén,2 Hiroshi Koyi,2 Rolf Lewensohn,2 Luigi De Petris,4 Cristina Rodriguez-Antonio,5 Maria Apellániz-Ruiz,5 Joakim Lundeberg,2 Henrik Grén,2 1Clinical Pharmacology, Division of Drug Research, Linköping, Sweden; 2Science for Life Laboratory, Solna, Sweden; 3Department of Respiratory Medicine, Gävle, Sweden; 4Karolinska Biomics Center, Stockholm, Sweden; 5Hereditary Endocrine Cancer Group, Madrid, Spain.

A classical non-small cell lung cancer (NSCLC) chemotherapy treatment is gemcitabine in combination with carboplatin. The treatment is known to cause severe hematological toxicity such as leukopenia, which can lead to chemotherapy cessation or even death. It would therefore be a priori of advantage to identify patients at risk of severe leukopenia to allow for a personalized treatment approach. In this study we aim to identify genetic markers for chemotherapy induced leukopenia in non-small cell lung cancer. In total, 212 non-small cell lung cancer patients treated with gemcitabine and carboplatin regimen were included in the study. Whole blood extracted DNA was prepared using Nextera Rapid Capture kit and whole exome sequenced using Illumina HiSeq 2500. Leukopenia was assessed from leukocyte particle count at baseline and the first cycle nadir values. The statistical approach was to study association of single common variants identifıed 133 variants (p < 0.01) to leukopenia using linear regression in PLINK and association of genes (common and rare variants) to leukopenia using SKAT. An enrichment analysis, with the association results (p < 0.001), was performed using the online tool ConsensusPathDB-human to identify overrepresented pathways. A prediction model was created from the single variant analysis results (n = 10 causal and n = 10 protective) using weighted genetic risk score from the R-package PredictABEL. The single variant analysis of common variants identifıed 133 variants (p < 0.001) and the gene based analysis identifıed 34 genes (p < 0.001). The pathway analysis identifıed 20 enriched pathways (p < 0.05). A prediction model was created to assess the risk of leukopenia. The top pathway, HIF-1 alpha transcription factor network, overlaps with four genes (HDAC7, NDRG1, HK2 and CP). It can be of interest to leukopenia as regulation of HIF-1 alpha is essential for maintenance of hematopoietic stem cells in the bone marrow hypoxic niche. It has also been shown that HIF-1 alpha knocked mice are more sensitive to myelosuppressive treatment compared to unknocked mice, supporting the involvement of the pathway in chemotherapy induced myelosuppression. We have identified variants located in genes and pathways likely to be involved in leukocyte sensitivity after exposure of gemcitabine and carboplatin. A prediction model has been created to estimate the risk that patients will suffer from severe leukopenia by the involvement of the pathway HIF-1 alpha transcription factor network to leukopenia supports the relevance of these results. Further studies on this are of interest to improve identification of patients at high risk of severe leukopenia after gemcitabine and carboplatin treatment.

Exome sequencing of cancer-related genes to identify genetic markers for sensitivity to cytotoxic anticancer drugs. Chihiro Udagawa,1 Yasushi Sasaki,2 Hiroshi Suemizu,1 Yasuyuki Ohnishi,1 Hiroshi Ohnishi,1 Takashi Tokino,1 Hitoshi Zembutsu1. 1National Cancer Center, Tokyo, Japan; 2Research Institute for Frontier Medicine, Sapporo Medical University, School of Medicine, Sapporo, Japan; 3Central Institute for Experimental Animals, Kawasaki, Japan.

Although the progress in moving from a one-size-fits-all cytotoxic approach to personalized molecular medicine, majority of the patients with cancer receive chemotherapy with cytotoxic anticancer drugs. Exome sequencing of 409 cancer related genes was conducted using 59 DNAs extracted from human tumor xenografts implanted into nude mice, of which sensitivity to nine cytotoxic anticancer drugs (5-fluorouracil (5FU), Nimustine (ACNU), Adriamycin (ADR), Cyclophosphamide (CPM), cisplatin (DDP), Mitomycin C (MMC), methotrexate (MTX), vincristine (VCR), and vinblastine (VLB)) had been examined. We investigated the relationship between the sensitivities of the xenografts to the nine anticancer drugs and single nucleotide variants (SNVs) by classifying them into three groups according to the allele frequency (≥10%, 10-90%, >90%). We further carried out replication study using additional set of 20 xenografts. We also estimated the correlation between the expression levels of the 409 genes and sensitivities to the anticancer drugs in the MerckGenomics dataset using 59 xenografts, three SNVs, which were associated with sensitivity to VCR or MMC, showed P < 0.001. We further carried out a replication study of 596 SNVs showing P < 0.05 in the screening stage using independent samples of 20 xenografts. A combined result of the screening and replication studies suggested possible associations of 35 SNVs with sensitivities to the nine anticancer drugs (Pcombined = 0.0011 - 0.035). A total of 35 SNVs showed possible association with sensitivity to at least one of the nine drugs. Of the 35 SNVs, SNV-1 and SNV-2 in a tumor suppressor gene and an oncogene were commonly associated with sensitivity to two or four anticancer drugs, respectively. These findings provide new insights into precision anticancer therapy for the patients with cancer.

Whole exome sequencing and genetic association of gemcitabine/ carboplatin induced thrombocytopenia in non-small cell lung cancer patients. Niclas Björn,1 Anna Svedberg,1 Benjamin Sigurgeirsson,2 SAILendra Pradhananga,2 Eva Brändén,2 Hiroshi Koyi,2 Rolf Lewensohn,2 Luigi De Petris,4 Cristina Rodriguez-Antonio,5 Maria Apellániz-Ruiz,5 Joakim Lundeberg,2 Henrik Grén,2 Linköping University, Linköping, Sweden; 3Science for Life Laboratory, Solna, Sweden; 4Karolinska Institute, Stockholm, Sweden; 5Spanish National Cancer Research Centre (CNIO), Madrid, Spain.

Introduction: Chemotherapy induced myelosuppression is a clinically relevant problem for cancer treatments, affecting both quality of life and response to treatment. Severe hematological toxicities such as thrombocytopenia can lead to dose reductions, postponement or cessation of treatment and there is a need to identify patients at risk before the start of treatment to allow for personalized medicine. The aim of this study was to use whole exome sequencing to identify genetic markers for gemcitabine and carboplatin induced thrombocytopenia that can be used to guide future treatments. Methods: 212 non-small cell lung cancer patients treated with gemcitabine and carboplatin were included. Blood samples were whole exome sequenced using Nextera Rapid Capture Exome kit and Illumina HiSeq 2500. Patients’ thrombocyte counts were measured at baseline and during the first treatment cycle. The thrombocyte nadir value and the relative decrease of the nadir value from baseline were used as thrombocytopenia toxicity parameters. Coding SNVs as well as whole gene-regions and complete pathways were investigated using the following statistical methods: A) Association of common SNVs/INDELs with thrombocytopenia using linear regression in PLINK. B) Association of the combined effect of common and rare variants within a gene-region with thrombocytopenia using the region based association test SKAT, in the R-package SKAT. C) All genes including SNVs/INDELs in A) or a gene-regions in B) with p < 0.001 were used as input for the online tool ConsensusPathDB-human to find overrepresented predefined pathways. Results: The study associated 103 SNVs/INDELs and 21 genes to thrombocytopenia (p < 0.001). The pathway analysis C) identified 28 enriched pathways (p < 0.05). Potentially important pathways for gemcitabine/carboplatin induced thrombocytopenia identified were: Hemostasis (ITGB1, SERPINA5, SERPINC1, JMJD1C, DOCK8 and CAPZA2), Factors involved in megakaryocyte development and platelet production (JMJD1C, DOCK8 and CAPZA2). Vitamin B9 (folate) metabolism (FOLR3 and MTHFD1). Single SNVs, INDELs and genes can be of importance for induced toxicity. However, special interest should be given to the variants and genes in the pathways above. Two of the pathways link back to thrombocytopenia via platelet production and hemostasis and include, JMJD1C and DOCK8, previously associated with thrombocytopenia formation and mean platelet volume. The pathway concerning folate metabolism links back to gemcitabine’s and carboplatin’s mode of action via the involvement of folate in thymidylate and DNA synthesis. Furthermore, folate is needed for rapid regeneration of the bone marrow. Conclusion: The identified genetic markers and pathways are associated with chemotherapy induced thrombocytopenia and provide a strong foundation for further investigation.

Pharmacogenetics of doxorubicin, gemcitabine and docetaxel in the GeDDIS soft tissue sarcoma trial. David Jamieson,1 Beatrice Seddon,2 Karja Kuever,1 Hakim-Moulay Dehbi,1 Sandra Strauss,2 Sandy Bear,1 Gareth Veal1. 1Northern Institute for Cancer Research, Newcastle upon Tyne, United Kingdom; 2University College Hospital, United Kingdom; 3Cancer Research UK & UCL Cancer Trials Centre, United Kingdom.

Introduction: The treatment of locally advanced or metastatic soft tissue sarcoma remains a significant clinical challenge, with overall survival rates of approximately 12 months observed with current first line palliative chemotherapy. A recently completed randomised phase III clinical trial entitled GeDDIS (ISRCTN07742377), was designed to compare first line treatment with gemcitabine in combination with docetaxel (GemDoc) versus current standard treatment with doxorubicin. A pharmacogenetic sub-study was incorporated into the trial to assess potential impact of single nucleotide polymorphisms (SNPs) in genes associated with the pharmacology of the three drugs. A total of 240 patients were recruited to the sub-study, with 119 on the doxorubicin arm and 121 on the GemDoc arm. A 4 ml blood sample was taken from each participant and genomic DNA extracted. Individual candidate SNPs were assessed by Taqman PCR in 7 genes associated with doxorubicin pharmacology, 7 genes associated with gemcitabine and 5 genes associated with the...
pharmacology of docetaxel. Association between the SNPs and efficacy and toxicity was assessed. Results: SNPs within the solute transporter SLC2A2A16, associated with the intracellular influx of doxorubicin, were associated with worse PFS (HR = 1.72, p = 0.04) and decreased frequency of grade 3/4 adverse events (71% vs 48%, p = 0.04) in the doxorubicin arm of the study but not in the GemDox arm. Conclusions: The association of the single nucleotide polymorphism (SNP) and incidence of toxicity is consistent with a loss of function in the transporter and previous observation of increased AUC in pharmacokinetic studies assuming that distribution of the drug into target and collateral tissues is restricted.

#5030 The impact of ABCB1 single nucleotide polymorphisms on the outcome in lenalidomide treated multiple myeloma patients. Ingrid Jakobsen Falk,1 Johan Lund,2 Henrik Greén,2 Astrud Gruber,2 Evren Alici,1 Birgitta Lauri,2 Cecilia Blimark,3 Ulf-Henrik Mellqvist,4 Agneta Swedin,5 Karin Forsberg,5 Conny Carlsson,6 Mats Hardling,6 Lucia Albigb,5 Hareth Nahil,7 Kourosch Lotfi,7 1Division of drug research, Linköping University, Linköping, Sweden; 2Unit for hematology, Karolinska Institute, Huddinge, Sweden; 3Sundbyr Hospital, Luleå, Sweden; 4Hematology department, Sahlgrenska University hospital, Göteborg, Sweden; 5Hematology department, Skåne University hospital, Lund, Sweden; 6Department of hematology, Norrland University hospital, Umeå, Sweden; 7Department of Internal medicine, Halland Hospital, Halmstad, Sweden; 8Department of hematology, Uddevalla hospital, Uddevalla, Sweden; 9Department of hematology, Linköping University hospital, Linköping, Sweden.

Introduction: Multiple myeloma (MM) is an incurable plasma cell malignancy with high mortality rate. Treatment outcomes have improved since the introduction of new drugs such as the IMID lenalidomide, but relapse rates and resistance is still a problem. The gene ABCB1 encodes the drug transporter p-glycoprotein (p-gp) which confers resistance through extrusion of drugs over the cell membrane. Lenalidomide is subject to limited metabolism and excreted mainly via the kidneys. In vitro studies have shown lenalidomide to be an ABCB1 substrate, and single nucleotide polymorphisms (SNPs) affecting gene expression, transporter function and/or activity may affect drug distribution, and the subsequent outcome and risk of adverse events. However, in vivo studies of the effect of ABCB1 on lenalidomide pharmacokinetics are contradictory.

Our aim was to investigate the influence of ABCB1 SNPs on lenalidomide treatment outcome and adverse events (AE). Materials & Methods: In the observational part of two connected studies, 133 Lenalidomide naive patients at 1st relapse/refractory MM were treated with lenalidomide and dexamethasone for up to 9 cycles of 4 weeks. In the prospective 2nd part, 62 patients that had been obtained, patients were enrolled, and their blood was collected and previous observation of increased AUC in pharmacokinetic studies assuming that distribution of the drug into target and collateral tissues is restricted.

#5032 The relationship between the UGT1A1*7 and UGT1A1*27 genotypes and irinotecan-related toxicities in patients with lung cancer. Minoru Fukuda,1 Manabu Okumura,2 Tomomi Iwakiri,3 Arimori Kashiwagi,4 Shinnosuke Takezako,5 Takaya Ikeda,5 Takuya Honda,6 Hiroyuki Yamaguchi,7 Katsumi Nakatomi,8 Kazuma Kobayashi,9 Mitsuko Masutani,10 Mikio Oka,1 Kazuto Ashizawa,1 Hiroshi Mucake,12 Nagasaki University Hospital, Nagasaki, Japan; 1University of Miyazaki Hospital, Miyazaki, Japan; 2Nagasaki University, Nagasaki, Japan; 3Kawasaki Medical School, Kurashiki, Japan.

Background: Genetic polymorphisms in the UDP-glucuronosyltransferase 1A1 (UGT1A1), UGT1A7, and UGT1A9 genes are associated with interindividual differences in irinotecan toxicities. The UGT1A1*7, *27, and *29 gene polymorphisms occur within the common exons of UGT1A1 isoforms and cause substantial reductions in their functional activity; however, few clinical studies have examined the effects of these polymorphisms. Purpose: To evaluate the effects of gene polymorphisms, including UGT1A1*7, *27, and *29, on the safety of irinotecan therapy. Patients and methods: The eligibility criteria were as follows: lung cancer patients who were scheduled to undergo irinotecan therapy, aged ≥20 years, and had a performance status of 0-2. After informed consent had been obtained, patients were enrolled, and their blood was collected and used to examine the frequency of the UGT1A1*6, *7, *27, *29, and *29 polymorphisms and the drug concentrations of irinotecan, SN-38, and SN-38G after irinotecan therapy. Results: Thirty-one patients were enrolled. The patients' characteristics were as follows: male/female = 25/6, median age (range) = 71 (55-84), stage IIIB/IIIA/IIIB/IV = 2/6/11/12, and Ad/Sq/Sm/OTH = 14/10/3/4. The incidence of grade 3/4 diarrhea was 14% and 11% for patients with and without the UGT1A1*7 polymorphism, respectively. There were no homozygous or complex heterozygous polymorphisms. The UGT1A1*27 polymorphism occurred separately from the UGT1A1*28 polymorphism. The lowest leukocyte counts of the patients with the UGT1A1*27 and UGT1A1*6 gene polymorphisms were lower than those seen in the wild-type patients. SN-38 tended to remain in the blood for a prolonged period after the infusion of irinotecan in patients with the UGT1A1*27 or UGT1A1*28 polymorphisms. No severe myelotoxicity was seen in the patients with UGT1A1*7. Conclusions: UGT1A1*27 and UGT1A1*7 are both rare gene polymorphisms. UGT1A1*27 can occur separately from UGT1A1*28 in some circumstances and is related to leukopenia during irinotecan treatment. UGT1A1*7 is less relevant to irinotecan-induced toxicities, and UGT1A1*29 seems to have little clinical impact.

#5033 Optimized CYP2D6 phenotype assignment for plasma endoxifen prediction in breast cancer patients treated with tamoxifen. Werner Schrotth1, Stefan Winter1, Michel Eichelbaum1, Thomas Muether1, Matthias Schwab2, Hiltrud Brauch3, Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, and University of Tuebingen, Tuebingen, Germany; 2Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, Department of Clinical Pharmacology, University Hospital Tuebingen, Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), Heidelberg, Germany; 3Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, University of Tuebingen, Tuebingen, and German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), Heidelberg, Germany.

Introduction: Standard first-line treatment for ovarian cancer consists in the combined use of taxanes and platinum, with neuropathies and neutropenia the most frequent and complex adverse drug reactions (ADRs) requiring clinical management. These adverse effects may be related to differing levels of drug transport and metabolism brought about by intrinsic genetic characteristics of patients. The investigation of the first phase of the CYP2D6 drug metabolism, the CYP2C8 enzyme, whose function is essential in paclitaxel metabolism, was performed. Patients and methods: Four SNPs associated with risk of ADR within the ABCB1 gene polymorphisms were previously reported to be associated with paclitaxel and carboplatin ADRs. Methods: Blood samples were obtained with informed consent from adult ovarian cancer patients (n = 120). DNA was extracted and SNPs within the ABCB1 gene polymorphisms were genotyped with high specificity. Results: SNPs associated with paclitaxel and carboplatin ADRs are highly prevalent in the Chilean population of ovarian cancer patients studied. The identification of these variants may help avoid severe ADRs associated with chemotherapy regimes. Funding: CORFO 13CT121526-P & 13IDL2-18608.
Tamoxifen (Tam), a standard therapy for estrogen receptor (ER)-positive breast cancer, is bioactivated to its active metabolite endoxifen by the cytochrome P450 enzyme CYP2D6. CYP2D6 polymorphisms constituting extensive (EM), intermediate (IM) and poor (PM) metabolizer phenotypes are main determinants of variable endoxifen plasma concentrations and supposed to influence therapeutic and side effect outcomes. Genotype-guided prediction of Tam metabolizer status has long been debated to be useful for clinical dose adjustment in patients with impaired metabolism. As this requires accurate genotype-phenotype assignments, we tested several CYP2D6 phenotype classifications including the codeine metabolism-based scoring implemented by the ‘Clinical Pharmacogenetics Implementation Consortium’ (Crews et al, 2014, CPT) for their predictive value of endoxifen concentration. Usually, CYP2D6 PO4 demonstrates near linear dose proportionality of endoxifen formation. This is evident from a low predictability (<20%) for both metabolite endpoints particularly in Asians, most likely due to a misclassification of abundant IM/IM (*10) diplotypes as EM. Importantly, the active metabolite-to-precursor MR appears to be the endpoint that is more closely linked to CYP2D6 enzyme activity, as it explains up to 68.82% of MR variability. In contrast, absolute endoxifen concentrations were only modestly predictable (38-57%) by CYP2D6 diplotype or other phenotype groupings. Moreover, a reduced activity for the *10 compared to other IM alleles appears to improve endoxifen predictability by phenotype. Personalized TAM treatment decisions based on plasma endoxifen prediction should rely on TAM specific CYP2D6 genotype-guided phenotype assignments that quantitatively capture the allele-dosage effects and that include endoxifen-to-precursor MR as the endpoint most closely linked to CYP2D6.


There are currently only 10 cancer genes with FDA approved drug treatment options (source: OncoKB). Here, we present a conceptually novel analytical method that allows to expand the small list of clinically actionable cancer drug treatment biomarkers. Large sequencing studies, such as The Cancer Genome Atlas (TCGA), have greatly accelerated our understanding of the molecular basis of cancer. However, because of the difficulty in collecting drug response data in large cohorts of cancer patients, these studies have not been effectively used for finding new biomarkers of drug response (i.e. pharmacogenomics discovery). Thus, much cancer pharmacogenomics research is conducted in preclinical disease models such as cell lines (e.g. the Genomics of Drug Sensitivity in Cancer (GDSC) project); but these studies are (among other limitations) always restricted by comparatively smaller sample sizes. Here, we present an analytical method that integrates data from large clinical studies (e.g. TCGA) with data from preclinical disease models (e.g. GDSC) and overcomes these critical obstacles, allowing studies such as TCGA to now be effectively used for pharmacogenomics discovery. We refer to this approach as an “Imputed drug-wide association study” (IDWAS). The method works by fitting a statistical model relating gene expression and drug response in preclinical data (here we use the GDSC cancer cell lines), then using this model to impute drug response from tumor gene expression data in a clinical cohort (here we use TCGA). Next, we compare “active” and “inactive” drug responses. We applied this method to imprint drug response (mutations, copy number changes) in TCGA, thus finding new biomarkers of drug response. We show that we can recapitulate known clinically effective biomarkers and we have validated new clinically relevant biomarkers, which remarkably could not have been identified using conventional approaches. Our method will set the stage for many future studies. Crucially, this approach could easily be applied to any of the vast number of clinical cancer sequencing studies now undertaken, meaning that it will be possible to use all of these datasets for pharmacogenomics research. We have included a set of computational tools to allow easy application of our method and replication of our results. Given that this is a conceptually novel methodology, it is also likely that many other studies will attempt to improve upon our proposed implementation. Furthermore, members of our group are currently leading the development of the new Genomic Data Commons (GDC; https://gdc.cancer.gov/), which is the NCI’s new access portal for TCGA data. We are currently working towards making the imputed drug response data directly accessible on GDC, along with all other TCGA data. This means that our imputed drug response data will be easily accessible to the thousands of researchers already accessing TCGA via the GDC.

**#5036 Correlation of preclinical anticancer activity of regorafenib in CRC-PDX xenografts with gene expression and clinical parameters of the primary tumor, Henrik Seidel,1 Jens Hoffmann,2 Ralf Lesche,1 Sylvia Grünewald,1 David Henderson,1 Dieter Zopf1, Bayer Pharma AG, Berlin, Germany, 1EPO GmbH, Berlin, Germany.**

Regorafenib is a small molecule inhibitor of multiple transmembrane and intracellular kinases involved in normal cellular functions and in pathologic processes such as oncogenesis, tumor angiogenesis, metastasis, and tumor immunity. Regorafenib is approved for the treatment of patients with metastatic colorectal cancer (CRC) who have been previously treated with fluoropyrimidine-, oxaliplatin- and irinotecan-based chemotherapy, an anti-VEGF therapy, and, if RAS wild-type, an anti-EGFR therapy or with locally advanced, unresectable or metastatic gastrointestinal stromal tumor (GIST) who have previously been treated with imatinib mesylate and sunitinib malate. Recently an overall survival benefit has been shown in patients with hepatocellular carcinoma (HCC) who had previously been treated with sorafenib (RESORCE). OncoTrack is an Innovative Medicines Initiative (IMI) sponsored project with the goal to improve the basis for identification of biomarkers based on the mechanisms of action of therapies approved for this indication. For this purpose, a panel of CRC-PDX xenografts was generated by the OncoTrack project. At the time of the analysis reported here fifty xenografts had been treated with regorafenib at a dose of 10 mg/kg/d or with vehicle for 24 days. The analysis of tumor growth rates (TGR) showed pronounced differences between different tumors and between vehicle and regorafenib treated models. The relative antitumor activity (relative TGR) of regorafenib varied between -0.13 (good response) and 0.0 (no response). Investigations of relative TGR in relationship to (non-) clinical parameters of the primary tumor such as age, gender, sidedness and tumor histology identified a marginally significant (p = 0.04) better response in tumors from younger patients. No other correlations were detected to this end, which may be due to the small sample number. To correlate antitumor activity of regorafenib with gene expression, RNA was isolated from sections of selected vehicle and regorafenib treated xenografts and hybridized on Affymetrix HuGene-2.1_st human transcriptome arrays. Expression profiles were subsequently analyzed using the Random Forests algorithm to identify gene expression signatures predicting response to regorafenib. The best signatures did not perform better than signatures derived after randomizing responses, i.e. no predictive signature could be identified. Further studies with larger samples sizes are necessary to improve the outcome of such an approach; however one should acknowledge that to date no predictive gene signatures could be identified for multikinase inhibitors, which may be intrinsic to their complex mechanism of action. The research reported here received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement 115234 (OncoTrack).

**#5037 New concepts for quantifying the benefits of mono and combination therapy in an era of big data, Peter K. Sorger, Marc Hafner, Mario Niepel, Caitlin Mills, Adam Palmer, Mohammed Fallahi Sichani. Harvard Medical School, Boston, MA.**

I will describe new (unpublished) approaches to quantifying drug response at two points in the drug development pipeline: pre-clinical studies in cell lines and clinical trials of combination therapies in patient populations. Drug sensitivity and resistance in cell lines is conventionally quantified by IC50 or Emax values, but these measures suffer from a fundamental flaw when applied to growing cells: they are highly sensitive to cell division number, which varies with cell line, experimental condition, seeding density etc. The dependency of IC50 and Emax on division rate creates artefactual correlations between genotype and drug sensitivity while obscuring important biological insights and interfering with biomarker discovery. I will describe alternative growth rate inhibition (GR) metrics that are insensitive to division number and can directly measure both endpoint sensitivity and adaptive drug resistance. Theory and experiments show that GR50 and GRmax are superior to IC50 and Emax for assessing the effects of drugs in dividing cells. GR metrics promise to improve our ability to score drug sensitivity in specific-derived tumor cells, improves data reproducibility, and increase the translational potential of pharmacogenomics data. In patients, combination therapy improves tumor control compared to monotherapy and the development of combinations is motivated in most cases by pre-clinical data.
on synergism in cell lines. However I will describe a different way in which combinations can provide clinical benefit. Based on analysis of between-patient variability in existing trial data and a large set patient derived tumor xenograft (PDX) mice published by Gao H, Korn JM, Ferretti S, et al. (Nature medicine 2015;21:1318-25). I will argue for a simple principle: in nearly two-thirds of cases a drug mechanism of action can be explained by points in time and a drug likelihood that a tumor will experience an outlier response to a single drug. Thus, even in the absence of additive or synergistic tumor inhibition, combinations are generally superior to monotherapies. Superiority by “independent action” provides a principle on which to design new combinations and a scientific rationale for the use of combination therapies in poorly understood cancers whenever toxicity is acceptable. — These studies, at two different points of the drug discovery pipeline, illustrate the value of combining new first-principles theories about drug mechanism of action with “big data” that is increasingly available on preclinical and clinical drug response. Conversely, in the absence of conceptual innovation, we miss important mechanistic insights hidden in existing data. The purpose of such insights, when obtained, is drive new laboratory and real-world experiments. I will discuss how the process of coupling computation and experimentation works in practice.

#5038 Comparison of the dose response of malignant peripheral nerve sheath tumor in vitro growth inhibition of 30 drugs to reported Cmax values. Karlyne M. Reilly, Robert G. Tuskan, Brigitte C. Widemann. NCI, NIH, Bethesda, MD.

In this study we aim to identify drugs that can be repurposed to inhibit malignant peripheral nerve sheath tumor (MPNST), a rare and deadly sarcoma associated with Neurofibromatosis type 1 (NF1). Inhibition of MPNST cell viability in vitro was tested for 30 drugs that have previously been through human clinical trials such that the maximum concentration of compound in human serum (Cmax) at the maximum tolerated dose has been determined. Drugs were compared to the chemotherapeutic doxorubicin. Six MPNST cell lines from human and mouse were tested and dose response curves were compared to Cmax for each drug. For most compounds tested, inhibition of MPNST viability occurred at drug concentrations higher than Cmax. Doxorubicin was one of the best compounds tested. Several compounds with less specific molecular targets appear promising, however, currently available inhibitors of receptor tyrosine kinase signaling pathways required concentrations above Cmax to inhibit MPNST viability. These data suggest that difficulty in developing therapy for MPNST may be due to the requirement for high concentrations of drugs to inhibit MPNST cells.

#5039 A data driven approach to predicting tissue-specific adverse events. Kaitlyn M. Gayvert, Neel Madhukar, Coryandar Gilvary, Olivier Elemento. Weill Cornell Medicine, New York, NY.

Adverse events are currently one of the main causes of failure in drug development and withdrawal after approval. As a result, predicting drug side effects is an incredibly important part of drug discovery and development. With the emergence of precision medicine there has been a surge in interest on creating drugstothemarketquicker. A data driven approach to in-silico drug sideeffectprediction.Wehavemineddrugsideeffect targets and mechanisms to specific side effects. Here we take a target-centric approach to in-silico drug side effect prediction. We have mined drug side effect databases and grouped sets of side effects to the originating human tissue. For each of 30+ tissues, we defined a set of “tissue targets”- proteins that are only targeted by drugs with toxicity in that tissue - and “safe targets” - proteins only targeted by drugs with no related tissue toxicities. We found that toxic targets are consistently more highly expressed than safe targets, indicating that their mechanisms may be more crucial in their respective tissue. Furthermore we found that toxic targets have higher network connectivity. Using published gene knockdown screens, we also found that toxic targets for each tissue are significantly more likely to be essential than safe targets and are more likely to be enriched for GO terms related to cell death. These pieces of information all reinforce the proposed relationship between the identified toxic targets and drug induced tissue toxicities. We next leveraged this information to draw insights into unexpected drug toxicity events. We applied the BANDIT drug target prediction tool to drugs classified by the ProCTOR toxicity prediction method and drugs with a specific type of tissue toxicity that were not known to hit any of our identified toxic targets. We found that new drug-target predictions explained a large number of these toxicities, correctly classifying approximately five times as many side effects as would have been expected by random chance. These results all supported our target-centric hypothesis of drug side effect prediction. Therefore we built a set of machine-learning models that would integrate drug targets with tissue-wide expression patterns and gene-specific features to predict specific side effects for a given drug. We found that these methods could significantly outperform other prediction techniques and random chance. For instance, our method for predicting drug induced liver injury (DILI) had ~70% accuracy at pinpointing specific drugs known to cause DILI and its likelihood score correlated with the FDA’s reported DILI severity score. Overall these findings show how a target-centric approach to drug development can not only increase our understanding between pathological phenotypic effects, but can help drug developers predict side effects before costly and time-consuming clinical studies. Our hope is that adoption of these methods will lead to overall increase in drug development efficiency and bring safer drugs to the market quicker.


Background: Pegylated liposomal doxorubicin (PLD) exhibits altered plasma pharmacokinetics (PK) compared to doxorubicin, with a longer half-life (55 hours), large area under the concentration-time curve (AUC), lower rate of clearance, and markedly smaller volume of distribution. The cerebrospinal fluid (CSF) PK of PLD is unknown. We evaluated the plasma and CSF PK of PLD in a non-human primate (NHP) model. Methods: PLD was given as single dose (1 mg/kg) after surgical resection of 20 mg/kg doxorubicin intravenously over 60 minutes to 3 NHP with indwelling central venous catheters and central nervous system reservoirs or lumbar ports. Serial blood and CSF samples were obtained for 96 hours after completion of the infusion. The total doxorubicin concentration (liposome bound + protein bound + free) was quantified with a validated liquid chromatography/tandem mass spectrometry assay (lower limit of quantification (LLOQ) 0.29 ng/mL, and CSF=0.06 ng/mL). PK parameters were estimated using non-compartmental methods. CSF penetration was calculated from the AUCCSF:AUCplasma. Results: PLD was well tolerated. Total doxorubicin plasma concentration time curves were characterized by sustained PLD exposure, comparable to the exposure observed in humans at a similar dose. The terminal elimination phase was not captured. In CSF, doxorubicin was measurable and still present at the last time point in all animals, but the CSF penetration was limited (Table). Conclusions: Using a sensitive assay, we could quantify doxorubicin in CSF. However, the CSF penetration is limited in NHP with intact blood brain barrier. Our assay analyzed the total doxorubicin concentration in plasma. It is unknown if PLD can penetrate into the CSF, therefore we could not determine if the CSF penetration represents total versus free.

#5041 Translational pharmacokinetic-pharmacodynamic xenograft model for TAK-931, a small molecule cell division cycle 7 (CDC7) kinase inhibitor. Charles Locson,1 Mayank Patel,1 Akikori Ohashi,2 Kenichi Iwai,2 Tatadairo Nambu,2 Yoshiyuki Takeuchi,2 Akifumi Kogame,2 Douglas Bowman,1 Stephen Tirrell,2 Huifeng Niu,1 Cindy Xia,1 Takeda Pharmaceuticals International, Cambridge, MA; 2Takeda Pharmaceuticals International, Shonan, Japan.

TAK-931 is a small molecule inhibitor of the cell division cycle 7 (CDC7) kinase. As a serine/threonine kinase that contributes to DNA replication and the DNA damage response, CDC7 is hypothesized to be a promising cancer drug target. CDC7 inhibition with TAK-931 has demonstrated antiproliferative activity with cancer cell lines and tumor growth inhibition (TGI) in murine ectopic xenograft models. Herein, the analysis of multiple models to characterize pharmacokinetic (PK) and pharmacodynamic (PD) relationships with xenograft xenograft TGI is described. TAK-931 treatment-induced TGI was dose schedule-dependent and could be described using plasma drug concentrations or tumor PD inhibition. However, the efficacious doses were at least 10-fold higher for the PK-TGI relationship than for the PD-TGI relationship. This discrepancy was used to develop a dynamic PK-PD-TGI modeling approach to project the minimal efficacious dose (MED) and minimal biological active dose (MBAD) for TAK-931 due to the large differences in time-concentration profiles predicted for humans versus mice. The Phase I human trial is on-going and will be used to verify the dynamic PK-PD-driven modeling approach for the CDC7 inhibitor.
#5042 Defining an optimal single time point sampling strategy representative of overall capcitabine pharmacokinetics. Stephen Welch,1 Wendy Teft,1 John Lenehan,1 Rommel Tirona,1 Karen Lumsden,2 Eric Winquist,3 Richard B. Kim,1 1Univ. of Western Ontario, London, Ontario, Canada; 2Grand River Regional Cancer Centre, Kitchener, Ontario, Canada.

Background: OT-101 is an oral chemotherapy pro-drug used to treat advanced colorectal cancer. Patients may experience hand-foot syndrome and diarrhea, among other side effects, that affect quality of life and may necessitate dose modification or discontinuation. There is significant regional variation in capcitabine tolerability, related to a myriad of factors including pharmacogenomics, dietary and cultural differences. Capcitabine dose modification, when necessary, is empirical based on toxicity suggested a personalized dosing approach might better optimize therapy. Objective: In phase 1 of a personalized dosing approach, our objective was to define an optimal time point for blood sampling that best represented overall exposure of capcitabine and its metabolites. Methods: A single-arm prospective pharmacokinetic cohort study of patients with advanced or metastatic colorectal cancer prescribed capcitabine monotherapy was done. Blood samples were collected pre-dose and at timed intervals between 0 and 8 hours post-dose. Plasma concentration of capcitabine and its major metabolites, 5'-deoxycytidine (5'-DCR) and 5'-deoxy-5-flourouracil (5'-DFUR), were measured by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Results: 26 patients were enrolled; 65% were male and 42.3% had metastatic disease. Mean capcitabine dose was 2854 ± 944 mg. Hand-foot symptoms (60%), fatigue (53%) and diarrhea (30%) were the most common adverse drug reactions. Dose normalized mean (SD) AUC∞, for capcitabine, 5'-DCFR and 5'-DFUR were 6.74 (3.0), 4.19 (1.5) and 6.33 (2.8) ng·h/mL, respectively. Spearman correlation between dose normalized concentrations and AUC at each blood draw was performed. The best estimated time points for capcitabine, 5'-DCFR and 5'-DFUR were 1.5, 2 and 2 hours with r2 values of 0.6 (p < 0.01), 0.64 (p < 0.001) and 0.51 (p < 0.01), respectively. There was a significant correlation seen between capcitabine AUC and need for subsequent dose reduction (p < 0.05). Conclusions: Blood samples obtained between 1.5 and 2 hours post-dose provide the best estimate of capcitabine exposure. Further pharmacokinetic analysis in this cohort is ongoing. This blood draw strategy will be used in a larger trial intended to develop a personalized capcitabine dosing algorithm.

#5043 Population pharmacokinetic model for OT-101 - A TGF-β2-specific antisense oligonucleotide in cancer patients. Wen Wang,1 Kevin Ng,2 David Nam,3 Vuong Trieu,1 Larn Hwang,1 Oncotelic Inc, Agoura Hills, CA; 2Autotelic Inc, Costa Mesa, CA.

Background: OT-101 (Trabedersen) is a phosphorothioate antisense oligonucleotide specifically inhibiting the expression of transforming growth factor-beta 2 (TGF-β2), whose overexpression is a pivotal factor for malignant progression in solid tumors. In the clinical Phase I/II study, plasma pharmacokinetic (PK) profile of OT-101 administered intravenously was evaluated in patients with advanced tumors. A population PK model was built to further understand the factors contributing to the variability in PK of OT-101. Methods: A total of 61 patients with pancreatic cancer (n=37), malignant melanoma (n=19), or colorectal carcinoma (n=5) were treated with OT-101 with escalating doses in 2 treatment schedules (1st schedule: 7-day on, 7-day off; 2nd schedule: 4-day on, 10-day off; 21 on 10 cycles). Blood samples were collected from patients at planned time points from before start of infusion to 7 days (1st schedule) or 10 days (2nd schedule) after stop of infusion. The plasma concentration data of OT-101 were used to build a population PK model using Phoenix NLME software. The influence of age, gender, body mass index (BMI), body weight (BW), height, cancer type, and treatment schedule as covariates on PK was evaluated. Results: With exclusion of protocol deviations, a total of 1444 plasma sample concentration data from 100 patient cycles were examined. The concentration-time profile was biexponential, with linear and non-linear components. The model estimated the PK parameters as follows: total body clearance, 0.17 mL/h; distribution volume of the central compartment, 4.69 L; inter-compartmental clearance, 3.31 L/h; distribution volume of the peripheral compartment, 3046.44 L. BW was identified as a covariate on OT-101 inter-compartmental clearance, with Kiso = -1.48. Conclusion: The PK parameters of OT-101 were best described by a two-compartment model. OT-101 was largely distributed in the peripheral tissues. The influence of age, gender, BMI, height, cancer type and treatment schedule on the PK of OT-101 was not identified. The model will be used for a sparse population PK study during the planned phase III trial of OT-101 in pancreatic cancer.

#5044 Pharmacokinetic study of RCHOP protocol in elderly patients with non-Hodgkin lymphoma. Elodie BAUDRY,1 Anne Laure COUDREC,2 Pascal CHAIBL3,4 Fanny BRET,4 Sara DIEJBOUN,4 Sophie WEILL,5 Samuel HUGUET,5 Alexandre BOYAULT,5 Francois LOKIEC,5 Keyvan REZAI,1,5 CHU Bicetre, Kremlin Bicetre, France; 2Assistance publique hopitaux de Marseille, Marseille, France; 3Hospital Charles Foix, Ivy sur seine, France; 1Inst. Curie, Saint Cloud, France.

Background: Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoma in elderly patients and R-CHOP chemotherapy is the standard treatment protocol for DLBCL. Elderly patients (often defined as 75 years of age) are treated with anticancer drugs with precaution; however, the pharmacokinetics and pharmacodynamics (PK and PD) of these agents have not been thoroughly investigated in this population. In this study we investigated the PK of cyclophosphamide (CP) and doxorubicin (DOX) in elderly patients in order to verify if there is an influence of age on the PK of these anticancer drugs. Methods: Non-Hodgkin lymphoma elderly patients were treated with a R-miniCHOP chemotherapy regimen. Dose levels were 25 mg/m², 0.7-1.4 mg/m², 750 mg/m² and 375 mg/m² for DOX, Vincristin (VCR), CP and Rituximab respectively. For PK analysis, 7 time point samples were collected over 48 h post administration on cycle 3. CP and VIN plasma concentrations were measured using UPLC-MS/MS validated method. DOX plasma concentrations were measured using UPLC coupled with fluorescence detection validated method. PK-PD modeling has been performed with a non linear mixed effect model program (Monolix version 4.3.2). Results: For CP and VCR, the pharmacokinetics of these two agents were well described with a 2-compartment model treated with DOX and CYP. Among them, 19 patients have received VCR. A total of 134 and 120 concentrations for DOX and CP were used respectively for PK-PD modeling. A 2-compartment open model adequately described DOX concentration versus time courses. A 1-compartment open model adequately described CP concentration versus time courses. The interindividual variabilities (ISV) could be well estimated for both drugs and for all structural parameters (clearances: CL, Q, volumes of distribution: Vc, Vp) except Vp for CP. The population PK parameters for DOX obtained for the structural model were: CL=54.5 L/h, V=54.7 L/h, Vc=30.6 L, Vp=1140 L. The population PK parameters for CP were: CL=3.49 L/h, Vc=29.2 L. VCR increases DOX Vc from 30.6 L to 56.9 L/h (p = 0.0012). The main covariate effects were related to gender, age, BW and to albumin. Conclusions: In this study we have espoused that with very good precision PK parameters of DOX and CP in very elderly patients with DLBCL. Our data revealed the effect of gender, BW and VCR administration on DOX PK parameters. We have demonstrated the effect of increasing age on PK of CP. A PK-PD modeling will be performed in order to verify there are biological factors explaining the variation in the PK parameters of DOX and CP in elderly patients.

#5045 Pharmacokinetics and pharmacodynamics of MEDI0680, a fully human anti-PD1 monoclonal antibody in patients with advanced malignancies. Xuyang Song, XiZhe Gao, Bo Zheng, Chelsea Black, Matthew Gribbin, Joyson Karakunnel, Lorin Roskos, Rajesh Narwal. MedImmune, Gaithersburg, MD.

Background: MEDI0680 (AMP-514) is a humanized immunoglobulin gamma4,kappa (IgG4κ) monoclonal antibody (mAb) specific for human programmed cell death-1 (PD-1), developed for the treatment of cancer. The primary objectives of these analysis were to (a) describe the pharmacokinetics (PK) of MEDI0680 and quantify the impact of patient/disease characteristics on PK variability (b) to compare body weight (WT)-based and fixed dosing regimens of MEDI0680 and (c) to characterize PK-pharmacodynamic (receptor occupancy) relationship. Methods: A total of 905 serum concentration records from 58 patients in Phase 1 study (D6020C00002) designed to evaluate safety, tolerability and PK following 0.1, 0.5, 2.5, 10, and 20 mg/kg every 3 weeks (Q3W), every 2 weeks (Q2W) and 375 mg/m² for DOX, Vincristin (VCR), CP and Rituximab respectively. For PK analysis, 7 time point samples were collected over 48 h post administration on cycle 3. CP and VIN plasma concentrations were measured using UPLC-MS/MS validated method. DOX plasma concentrations were measured using UPLC coupled with fluorescence detection validated method. PK-PD modeling has been performed with a non linear mixed effect model program (Monolix version 4.3.2). Results: 31 patients (15 males and 16 females), 75 to 96 years old, were treated with DOX and CYP. Among them, 19 patients have received VCR. A total of 134 and 120 concentrations for DOX and CP were used respectively for PK-PD modeling. A 2-compartment open model adequately described DOX concentration versus time courses. A 1-compartment open model adequately described CP concentration versus time courses. The interindividual variabilities (ISV) could be well estimated for both drugs and for all structural parameters (clearances: CL, Q, volumes of distribution: Vc, Vp) except Vp for CP. The population PK parameters for DOX obtained for the structural model were: CL=54.5 L/h, V=54.7 L/h, Vc=30.6 L, Vp=1140 L. The population PK parameters for CP were: CL=3.49 L/h, Vc=29.2 L. VCR increases DOX Vc from 30.6 L to 56.9 L/h (p = 0.0012). The main covariate effects were related to gender, age, BW and to albumin. Conclusions: In this study we have espoused that with very good precision PK parameters of DOX and CP in very elderly patients with DLBCL. Our data revealed the effect of gender, BW and VCR administration on DOX PK parameters. We have demonstrated the effect of increasing age on PK of CP. A PK-PD modeling will be performed in order to verify there are biological factors explaining the variation in the PK parameters of DOX and CP in elderly patients.
lutions indicate that following 20 mg/kg Q2W dose, >90% receptor occupancy can be maintained in all subjects. Based on preclinical/clinical PK, PD, and safety data, a dose of 20 mg/kg Q2W was selected for phase 2 studies. Conclusions: A population PK model of MEDI0680 was developed and validated. Modeling results indicate no need for dose adjustment based on patient/disease characteristics. Similar PK is expected for following both WC-based and fixed dosing regimens. PK/PD findings support the dose of 20 mg/kg Q2W. Clinical studies are ongoing in various tumor types.

#5046 Exposure-effacy (OS) analysis of tremelimumab in unselectable malignant melanoma. Paul Baverel,1 Lorin Roskos,2 Manasa Tatipalli,2 Nancy Lee,1 Paul Stockman,2 Maria Taboada,3 Paolo Vicini,4 Kevin Horgan,5 Rajesh Narwal.6 1MedImmune, Cambridge, United Kingdom; 2MedImmune, Gaithersburg, MD; 3AstraZeneca, Macclesfield, United Kingdom; 4AstraZeneca, Gaithersburg, MD.

Purpose: Tremelimumab is a fully human anti-CTLA-4 IgG2 monoclonal antibody that enhances human T-cell activation. Tremelimumab was evaluated in a Phase Iib (DETERMINE), randomised, double-blind, placebo-controlled study in patients with unselectable pleural or peritoneal malignant melanoloma, randomised (2:1) to receive either tremelimumab (10 mg/kg, seven doses Q4W followed by Q12W) or placebo. The study demonstrated no clinically meaningful differences in overall survival (OS). The primary objectives of this analysis were to evaluate the relationship of exposure with OS, and the impact of potential confounders. Methods: A population PK model was developed to estimate and derive PK exposure metrics (area under the curve at steady state [AUCss] or clearance [CL]) for exposure-OS analysis. Impact of potential confounders was evaluated using graphical and exploratory approaches. Factors including body weight, age, gender, race, ECOG status, anatomical site (pleural or peritoneal), line of therapy, EORTC status, tumour histology, baseline tumour size, LDH, and CRP were evaluated. The analyses were performed using NONMEM 7.2 and R software. Results: The population PK included 376 patients and 328 post-first dose PK concentrations. PK was consistent with previous knowledge and low incidence of anti-drug antibodies was observed. A 2-compartment linear model PK model adequately described the data. Tremelimumab CL and volume of distribution (V1) were 310 mL/day and 3.85 L, with moderate variability of ~38% and ~32%, respectively. There was an apparent exposure-OS relation when stratified by AUCss. However, at least 3 factors (gender, CRP, and baseline tumour size) were statistically significant PK predictors (p<0.05 on CL) indicating multi-dimensional confounding effect. Higher baseline tumour size, higher CRP levels and males were associated with lower PK exposure of tremelimumab. Conclusions: The observed apparent exposure-OS relationship is the result of imbalance in prognostic factors impacting OS rather than a true association of exposure with efficacy.

#5047 Cetuximab fixed dose combination - therapeutic drug monitoring. Cynthia Lee,1 Dongwon Lee,1 Sam Khateri,1 Wen Wang,2 Vuong Trieu2. 1Autotelic Inc, Costa Mesa, CA; 2Marina Biotech, Agoura Hills, CA.

Background: The anticancer properties of Cetuximab (CEL) have been demonstrated in different cancer indications, including colorectal, breast and non-small cell lung cancers. However, its use is associated with dose related cardiovascular and sudomotor events including edema and hypertension. We have shown that combination of CEL and OLM (Olmesartan) would negate drug induced edema caused by CEL. To further improve on the safety of this combination, we are now exploring therapeutic drug monitoring (TDM) guided dosing on CEL. This study demonstrated that TDM via lateral flow platform would be able to quantify plasma drug concentration and guide the optimal dosing for CEL. Methods: Pharmacokinetic (PK) data (Cmax and AUC) to oral formulations of CEL as single dose to healthy adults were collected from published clinical trials. PK data were analyzed against dose and demographic factors to evaluate the variability. To develop the lateral flow methods for quantification, mAbs against CEL were generated by synthesizing BSA-CEL immunogen and hybridoma technology. The best clones demonstrating a dose response to CEL were selected and tested. The assay requires the configuration of immobilizing BSA-CEL onto the membrane followed by flowing the colloidal-gold labeled mAb against CEL through in presence of test analyte. This resulted in a competitive assay where the signal decreased as the concentration of CEL in blood increased. Results: For CEL, the AUC and Cmax across multiple doses were significantly overlapped. With 200 mg CEL, we used meta-analysis to show that older subjects have a significantly higher AUC than younger subjects (p<0.001). These results indicate that different individuals may have very different drug exposure with same dose of CEL, depending on their age and other factors, resulting in over exposure and toxicity. Using a cutoff of 6.741 ng*hr/mL, we were able to separate out almost all of the elderly subjects from the young subjects. Therefore, to avoid toxicity, we will use TDM guided dosing to maintain AUC at the target AUC of 6.741 ng*hr/mL. To make possible TDM guided dosing, we developed a quantitative point of care lateral flow assay for CEL. mAb against CEL were selected based on good sensitivity and binding ability to CEL. Coupled with the lateral flow reader by Qagen, the assay exhibited a dynamic range of 7.5ng/mL to 6,741 ng*hr/mL. To make possible TDM guided dosing, we developed a quantitative point of care lateral flow platform coupled to a reader was developed to easily detect the CEL concentrations in finger-prick blood samples, allowing for in-home personal PK testing. The individual PK profiles built from the concentration data will be used to guide the optimal dosing of CEL, to maximize treatment efficacy and minimize toxicity. This should allow for higher dose of the toxic CEL without hitting this exposure limit. This additional enhancement on top of combination with OLM in the fixed dose combination will insure the safety of our patients on these drugs.

#5048 Residual concentrations of cetuximab predict clinical response in head and neck cancer patients. Joseph Ciccinni,1 François Becher,2 Diane Charlote Imbs,1 Claire Fournel,1 Florence Duffaud,2 Bruno Lacarelle,1 Sebastien Salas,1 1Aix-Marseille Univ., Marseille, France; 2CEA, Saclay, France; 3Assistance Publique Hôpitaux de Marseille, Marseille, France.

Cetuximab administration is contingent upon the upfront determination of Ras mutational status, because mutated patients are unlikely to respond to this therapy. In addition to tumor genomics, drug exposure levels could be a critical yet largely underestimated issue, because several reports have already demonstrated that cetuximab pharmacokinetic parameters (i.e., clearance values) could be associated with survival in patients. Here, we have developed an original bioanalytical method based upon the use of LC-MS/MS technology and a highly simplified sample preparation procedure to assay cetuximab in patients while meeting the requirements of standard therapeutic drug monitoring in routine clinical practice. We used this method prospectively to evaluate the inter-patient variability in drug exposure, and to search whether those exposure levels could be associated with clinical endpoints. A total of 25 adult patients (22 M, 3 F, mean age: 69 years) hospitalized for head and neck cancer and scheduled for a cetuximab-containing regimen (250 mg/m2 QW) were included to perform this pilot study. Seven patients received concomitant radiotherapy and 14 patients received concomitant chemotherapy. 28% of patients displayed severe toxicities (CTC grading). Following RECISt assessment, 10 out of 25 patients (40%) had progressive disease and 60% were categorized as patients with clinical benefit (stable disease: 5 patients: 20%, partial response: 7 patients (28%), complete response: 3 patients (12%)). A large inter-patient variability was observed among individuals. Mean cetuximab residual concentrations were 40.3 ± 20.3 µg/ml (CV: 51%, range 0.746-250.6 µg/ml) and mean cetuximab maximal concentrations were 126.9 ± 39.6 µg/ml (CV: 31%, range 65.1-210.6 µg/ml). No statistical difference was observed in cetuximab residual concentrations between patients with and without severe toxicities (36.7 ± 21.2 µg/ml VS. 41.8 ± 20 µg/ml, p>0.05, t test), nor in maximal cetuximab concentrations (117.1 ± 31.3 µg/ml VS. 130.3 ± 39.7 µg/ml, p>0.05, t test). Conversely, when comparing exposure levels in patients with clinical benefit to non-responding patients, a statistical difference was found in both cetuximab residual concentrations (49 ± 16.4 µg/ml VS. 25.8 ± 17.1 µg/ml, p<0.05, t test) and maximal cetuximab concentrations (143.7 ± 37.9 µg/ml VS. 100.9 ± 24.6 µg/ml, p<0.05, t test). Further ROC analysis showed that 33.8 µg/ml was the Cmin level associated with a probability of clinical benefit with 86% of sensitivity and 75% of specificity. These results advocate for the development of mass spec-based therapeutic drug monitoring of cetuximab in head and neck cancer patients. In addition to tumor molecular biology, drug exposure could be indeed a valuable actionable item to improve efficacy of cetuximab at bedside.

#5049 Application of population pharmacokinetic and exposure-response modeling for DS-8201a, a HER2-targeting ADC, predicts 50% ORR in patients with heavilypretreated breast cancer. Kazutaka Yoshihara,1 Seiko Endo,1 Kenji Yamura,2 Yoshihiko Doi,3 Tarou Tokui1. 1Daichi-Sankyo Co., Ltd, Tokyo, Japan; 2National Cancer Center Hospital, Tokyo, Japan; 3National Cancer Center Hospital East, Chiba, Japan.

Background: Antibody-drug conjugates (ADC) provide a wider therapeutic index by allowing specific targeting of cytotoxic agents to tumor cells. DS-8201a is a novel HER2-targeting ADC which contains a high average drug-to-antibody-ratio (DAR) of 7-8 molecules of a topoisomerase I inhibitor (exatecan derivative, DXd) per antibody. Preclinical data demonstrated a broader antitumor activity of DS-8201a than T-DM1 (the only FDA approved HER2-targeting ADC), including efficacy against T-DM1 resistant and HER2 low-expressing tumors. The first-in-human (FIH) study, comprising a dose escalation (Part I) and expansion (Part II), is being conducted in patients with HER2+ breast can-
#5050 Pharmacodynamic and pharmacokinetic relationship of single agent E7494 in patients with advanced solid tumors or B-cell malignancies.

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E7494 is a small-molecule inhibitor of poly (ADP-ribose) polymerase (PARP). Preclinical studies reported antitumor activity of single-agent E7494 in BRCA-deficient in vivo models. An open-label, multicenter, phase 1 study was completed to determine the maximum tolerated dose (MTD), safety, pharmacokinetics (PK), pharmacodynamics (PD), and preliminary antitumor activity of single-agent E7494. The MTD was determined to be 600 mg/day (n = 8). Treatment-emergent adverse events led to drug withdrawal in 1 patient and dose reductions in 3 patients. No minimal differences with exposures of DS-8201a or DXd were observed for the GI toxicity relative to lower doses, which supports the selection of this dose for Phase 2.

E7494 dosing at 600 mg/day was associated with sustained PARP inhibition. No significant changes in percent DNA in the comet assay to determine the extent of DNA damage. In the food-effect cohort study for new chemical entities and biological products. The primary purpose is collection of safety data in actual clinical settings by an active surveillance approach. In this study, we utilized the observational safety data of eribulin for model-based pharmacodynamic analysis to investigate more detailed safety profile of eribulin in patients with recurrent or metastatic breast cancer (RBC/MBC). Methods: The demographics and safety data were collected from RBC/MBC patients who were treated with eribulin by an active surveillance method. Since dose-limiting toxicity of eribulin is neutropenia, we analyzed the time course of neutrophil counts using a mechanistic pharmacodynamic model. Pharmacokinetic modeling was performed in a population pharmacokinetic model (Adamid et al. [119]). All model analyses were performed by Phoenix NLME1.3 (Certara). Estimated pharmacodynamic parameters were mean transit time (MRT [h]), proliferation rate constant of neutrophils (Kprol [1/h]), and slope of neutrophil counts (Kout [1/h]). Results: Clinical and laboratory data of 607 patients who were not given granulocyte colony stimulating factor were collected from July 2015 to December 2011. Among them, 406 patients with a total of 5204 neutrophil counts measurements were eligible for mechanistic pharmacodynamic analysis. The estimated mean parameters for eribulin were: MRT = 45.4 (h), Kprol = 0.1111 (1/h), Kout = 0.2074 (1/h), and Gamma = 0.317 and Slope = 0.0524.
Arsenic trioxide metabolism in patients with acute promyelocytic leukemia. Mohammad T. Khan,1 Sara Tarig,2 Cristina M. Ghiuzaoli,3 Miroslav Styblo,7 Jesse Saunders,2 Anthony Calabro,2 Nina Kohn,4 Daniel Budman,2 Steven Allen,1 Craig Devoe1. 1Hofstra Northwell School of Medicine, New Hyde Park, NY; 2University of North Carolina at Chapel Hill, Chapel Hill, NC; 3New York Genome Center, New York, NY; 4Feinstein Institute for Medical Research, Manhasset, NY.

BACKGROUND: Arsenic trioxide (ATO) is a mainstay of therapy for Acute Promyelocytic Leukemia (APL). Its long term effects and pharmacokinetics have not been well described. ATO is metabolized by a series of reactions involving inorganic arsenic (iAs) methylation and reduction steps resulting in mono- (MAs), di- (DMAs) and trimethylated arsenic (TMAs) metabolites which are subsequently excreted mainly in the urine: iAs\textsubscript{III}→MAs\textsubscript{III}→DMAs\textsubscript{III}→DMAs\textsubscript{V}→TMAs\textsubscript{V}. AToxicality is mediated via the activation of arsenic methyltransferase, a key enzyme in this reaction, contribute to differences in the metabolism of ATO. iAs\textsubscript{III}, MAs\textsubscript{III} and DMAs\textsubscript{III} are more biologically active and more toxic than pentavalent forms. In this study, we measured the total iAs, MAs’s and DMAs’s in plasma and urine. METHODS: Blood and urine samples from 10 control patients and 26 APL patients treated with ATO were collected. The treated patients had blood drawn immediately prior to and at 1, 2, 4, 6, and 24 hours, days 4, 8 and 15, and 4 weeks after the administration of ATO. Total iAs (iAs\textsubscript{III}+iAs\textsubscript{V}), MAs (MAs\textsubscript{III}+MAs\textsubscript{V}) and DMAs (DMAs\textsubscript{III}+DMAs\textsubscript{V}) were measured in plasma using hydride generation-cryotrapping-atomic-absorption spectrometry. The same arsenic species were measured in spot urine at several time points. For statistical analysis, repeated measures analysis of variance (RMANOVA) were done to compare subject groups over time. Two subjects were missing iAs urine values at 24 hours and so were excluded from this analysis. RESULTS: iAs levels differed over time (p<0.0001), with a rapid increase noted after ATO administration followed by a linear decline, reaching minimum levels by 4–6 hours. Between 6 hours and 24 hours, two distinct groups of iA5s metabolizers became apparent: 15 subjects had stable or decreased iAs levels at 24 hours (Group A) versus 9 subjects with at least a 5% increase in iAs at 24 hours (Group B). Methylated metabolites in the urine were also higher at all measured time points in Group A versus Group B; however, the difference between the two groups was statistically significant only for urine DMAs (p<0.0390). CONCLUSIONS: Chronic iAs exposure has been associated with increased risk of diabetes, cardiovascular disease, and skin cancer. Patients treated with a therapeutic dose ATO, we identified 2 distinct groups of arsenic metabolizers: Group A patients who rapidly converted iAs to MMA and DMA and excreted the metabolites in the urine, and Group B patients who metabolized arsenic slowly and had a lower rate of excretion of metabolites in the urine. These results suggest that Group B patients had a longer exposure time to iAs and its metabolites and may be more susceptible to ATO toxicity. Prospective clinical trials are needed to determine long term ATO toxicity in these patients.


Ewing Family of tumors (EFT) comprises the fourth most common highly malignant childhood cancer. Although sustained event-free survival (EFS) can be achieved with intensive chemo-radiation therapy for patients with local-regional disease, this therapy is relatively ineffective in the treatment of metastatic disease with EFS of 12% at 5 years. Ewing sarcoma is characterized by a reciprocal translocation between chromosomes 11 and 22 that encodes a chimeric oncoprotein resulting from the fusion of EWSR1 to the FLI1 transcription factor in ~85% of tumors. Therapy for patients with EFT comprises surgery, intensive use of cytotoxic agents and radiation therapy. Dose intensification and dose compression has resulted in some improvement in outcome, but patents with advanced or metastatic disease at diagnosis still represent a challenge. Further, patients alive at 5 years from diagnosis still have a high probability of subsequent relapse. Further, long-term consequences of treatment included cardiac dysfunction, and secondary malignancies. Thus, more effective and less toxic therapies are required to treat patients with Ewing sarcoma.

In this study 5 of 10 Ewing tumor xenografts models showed dramatic regressions to the combination, while administered as single agents, neither talazoparib or temozolomide were active. We have studied the talazoparib-temozolomide synergy in vitro, and results indicate that in models where there is no synergy as xenografts, the cell lines have either intrinsic resistance to talazoparib, temozolomide or both drugs. In mice, and in the clinical trial (NCT02116777), the talazoparib-temozolomide combination is toxic requiring a reduction in temozolomide dose to ~15% of its maximum tolerated dose.

We are exploring the use of nanoparticle-formulated talazoparib (npTLZ) developed by Nanomaterials Synthesis Laboratory at Northeastern University without tumor targeting or with antibody-mediated targeting to increase the tumor-drug delivery, reduce normal tissue toxicity (mainly thrombocytopenia), and potentially allow escalation of temozolomide dose. In the PPTP study, the MTD for temozolomide combined talazoparib was 100 mg/kg PD daily x 5, which is 12 mg/kg. Our recent data showed no toxicity of temozolomide at 66 mg/kg (PD daily x 5) in mice treated with npTLZ (0.5 mg/kg IV daily x 5), suggesting that npTLZ does not potentiate TMZ toxicity to normal mouse tissues. We anticipate that nanoparticle delivery of talazoparib combined with temozolomide will allow reduced toxicity while increasing the response rate for this combination in preclinical models of Ewing sarcoma.


R,S-4-Methoxy-1-naphthylfenoterol, MNF, inhibits the G protein-coupled receptor GPR55. In this study, we determine the effect of MNF on human PANC-1 pancreatic tumor growth in mice and apply multiplatform metabolomics analysis to identify pathways associated with growth arrest. Methods: Female Balb/c nude mice, 6–8 weeks old, 18-20 g, were inoculated subcutaneously with 5 x 10\textsuperscript{6} PANC-1 cells. On Day 8, mice were placed in groups of 10 using random block design based upon tumor volume. Mice received daily ip injections of vehicle or 40 mg/kg MNF 5 days/week for 3 weeks and sacrificed on Day 33. Mice were inhiberated daily and tumor volumes measured at beginning and end of each dosing cycle. On Day 33, mice were euthanized, and plasma samples and tumors collected. Tumor tissue was homogenized, extracted and analyzed using liquid chromatography-QTOF-MS, capillary electrophoresis-TOF-MS and gas chromatography-El-Q-MS. Differences between groups were evaluated by unpaired t-test or Mann-Whitney test with post hoc Benjamini-Hochberg correction. Statistical significance was set at P < 0.05. Compound identification was accomplished using online databases and in-house standards. Results: Tumor volume increased in vehicle-treated mice by ~700%, 142 ± 8 mm\textsuperscript{3} to 957 ± 79 mm\textsuperscript{3} and only ~25% in MNF-treated mice, 143 ± 8 mm\textsuperscript{3} to 259 ± 27 mm\textsuperscript{3}. On Day 33, MNF was not detected in plasma but accumulated in tumor tissues, 43.9 ± 32.7 mg/g. Plasma L-lactate levels were reduced from 3.29 ± 0.66 mmol/L to 2.81 ± 0.60 mmol/L, P<0.001. Differences in tumor tissue metabolome were observed with MNF compared to vehicle, which was reflected by significant, P<0.05, changes in relative metabolite signals. MNF treatment was accompanied by the disruption of pyrimidine nucleotide biosynthesis at uridine 5'-monophosphate, UMP, and increased UMP degradation. Moreover, the +237% increase in 2-aminobutyrate indicate higher oxidative stress and the +51% increase in 2-hydroxyproline suggest greater HFI-1a proteolysis upon MNF treatment. Conclusions: These results are consistent with our previous observations in PANC-1 cells showing that GPR55 inhibition attenuates activation of the EGFR-MEK-ERK, Wnt-β-catenin and PI3K/AKT pathways resulting in lower cyclin D1 expression and halting the cell cycle in G1. The reduction in HFI-1a expression and glycolytic flux in MNF-treated PANC-1 cells is in line with the decline in L-lactate plasma levels in MNF-treated mice. The data from this study indicate that MNF may be useful in the treatment of pancreatic cancer.
**EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Epigenetic Agents**

**#5056 The bromodomain inhibitor JQ1 sensitizes homologous recombination proficient ovarian cancer cells to the PARP inhibitor olaparib.** Andrew J. Wilson, Janese Thompson, Abdirahman Osman, Jeanette Saskowski, Dineo Khabele. Vanderbilt Univ. Medical Ctr., Nashville, TN.

Introduction: Lack of effective treatment options for high-grade serous ovarian cancers (HGSOCC) retaining functional homologous recombination (HR) DNA repair pathways is a significant clinical problem. HR-proficient HGSOCC tumors, for example those harboring cyclin E amplifications, have poorer clinical outcomes and show relative resistance to DNA-damaging platinum agents and newer poly ADP ribose polymerase inhibitors (PARPis). We and others have shown that using epigenetic drugs to reduce HR efficiency in HR-proficient HGSOCC sensitizes these cancer cells to DNA damaging agents. One mechanism by which these drugs reduce HR efficiency is by transcriptional down-regulation of HR pathway components. An emerging class of epigenetic modifiers of protumorigenic transcription is the bromodomain (BRD) family of proteins, and BRD inhibitors (BRDis) have shown promising preclinical anti-tumor efficacy. However, it is unknown whether BRD inhibitors affect HR proficiency in HGSOCC to DNA damaging agents. Aim: To test the hypothesis that BRD inhibitors can reduce HR DNA repair in HR-proficient ovarian cancer cells, thereby sensitizing them to PARPis. Methods: The HR-proficient ovarian cancer cell lines, OVCAR-3 (cyclin E-amplified) and SKOV3, were treated with 0.01% DMSO vehicle, the PARPi olaparib (Astra Zeneca), the BRD inhibitor JQ1 or with the olaparib/JQ1 combination. Sulforhodamine B (SRB) assays assessed cell growth and viability (72 hours treatment). Immunofluorescence (IF) and Western blot analyses were conducted to assess DNA damage (pH2AX), apoptosis (cleaved caspase-3), and HR efficiency (RADS1, foci, and GFP expression in cells co-transfected with Lscet and DRGFP HR reporter plasmids) (24 hours treatment). Steady state levels of the HR protein BRCA1, pH2AX and cleaved caspase-3 were assessed by western blot (24 hours treatment). Results: The combination of JQ1 and olaparib synergistically reduced cancer cell viability following isobologram analyses of SRB experiments. Compared to either drug alone, the JQ1/olaparib combination also significantly reduced BRCA1 expression and increased protein levels of cleaved caspase-3 and pH2AX in western blots, and also increased the number of cells displaying DNA damage and apoptosis in IF assays. Finally, JQ1 and olaparib combined to significantly reduce HR efficiency in our RADS1 foci formation and DRGFP assays compared to olaparib alone. Conclusions: Our results suggest that BRDIs sensitively reduce HR-proficient cells to DNA damaging drugs, in part by reducing efficiency of HR DNA repair. These findings have important implications for expanding the use of PARPi in HR-proficient HGSOCC through rational combinations with epigenetic drugs such as BRDIs that target the HR pathway.

**#5058 Lysine specific demethylase-1 (LSD-1) Inhibitor SYC-836 in combination with radiation prolongs animal survival in patient-derived posterior fossa ependymoma xenograft mouse models.** Sibo Zhao, Huiyuan Zhang, Lin Qi, Holly Lindsay, Yuchen Du, Mari Kogiso, Frank Braun, Sarah Injac, Laszlo Perakly, Donald W. Parsons, Murali Chintagumpala, Adekunle Adesina, Yongchong Song, Xiao-Nan Li. Baylor College of Medicine, Houston, TX.

Background: Ependymoma (EPN) is the third most common malignant pediatric brain tumor. Current standard therapy include maximally safe surgical resection followed by radiation and to a 5-year overall survival of 50-71%. Recent molecular subgrouping of EPN has identified one group, posterior fossa A (PFA), which accounts for 45% of all EPN cases, to have one of the worst prognoses and it is driven by epigenetic changes, suggesting targeting epigenetic changes in PFA EPN can potentially be effective. In this study, we examined the therapeutic efficacy of SYC-836, a novel LSD-1 inhibitor compound developed at Baylor College of Medicine, both in vitro and in vivo in PDOX models of posterior fossa EPN. Methods: To examine in vitro anti-tumor activities, paired primary cultured cells (both as attached cells and neurospheres) from an established PDOX model of posterior fossa EPN (ICB-42423EPN) were subjected to SYC-836 at various concentrations (0-25uM). Cell viability and proliferation were measured using Cell Counting Kit-8 assay at 5 different time points over 14 days. To validate the drug’s in vivo efficacy, two established posterior fossa EPN PDOX models, ICB-42423EPN and ICB-2002EPN, were utilized. 40 eight weeks old SCID mice per model were implanted with tumor cells. They were divided into 4 treatment groups (10 mice/group) each: 1) control (DPBS, 10uL/kg IP daily x 2 weeks), 2) standard radiation, 3) SYC-836 only (15mg/kg IP daily x 28 days), and 4) combination (radiation + SYC-836 per regimen above). Animal survival times were analyzed using log rank analysis. Changes of histone lysine methylation were examined through western hybridization. Results: SYC-836 demonstrated effective cell killing in vitro against both attached and neurosphere cultured cells in both time- and dose-dependent manner. IC50 was ~7.5uM. In vivo experiment was completed in 1 of the 2 EPN PDOX models (ICB-2002EPN) with the second model ongoing. Median survival times for each group is as follows: control: 136 days, radiation 148 days, SYC-836 only 136 days, combination 180 days. There were no survival benefit of radiation + SYC-836 only (P = 0.186) or SYC-836 only (P = 0.004) when compared to the control group; however, when used in combination, the treatment strategy lead to significant improvement in animal survival (P = 0.004). SYC-836 was well tolerated in mice. Conclusion: Our data showed that combining SYC-836 with current standard therapy of radiation synergistically prolongs animal survival significantly, although as a single agent SYC-836 was not effective against posterior fossa ependymoma. Our data suggest that SYC-836 may have a role in the clinical setting by either reducing radiation dosages, or be a potential adjuvant agent to other chemotherapy drugs in our treatment approach for ependymoma.

**#5059 Preclinical study on the efficacy of Panobinostat in hepatocellular carcinoma.** Chi Tung Choy, Wing Yu Man, Chi Hang Wong, Stephen Lam Chan. The Chinese University of Hong Kong, Hong Kong, Hong Kong.

Background: Aberrant regulation of histone deacetylases (HDACs) is known to play a pivotal role in HCC pathogenesis as well as other human malignancies. Panobinostat (LBH589) is a pan-HDAC inhibitor covering a wide range of HDACs (Class I, II and IV) with high inhibitory activity at nanomolar concentration. It has been approved by FDA for treating multiple myeloma and has demonstrated promising anti-proliferative and cytotoxic activity in breast, prostate, colon and pancreatic cancer cell lines. This study investigated in vitro and in vivo effect of Panobinostat in HCC cell lines. Methods: Basal expressions of HR23B and HDACs of 7 HCC cell lines (HepG2, PLC/PRF/5, Huh-7, HepB3, SNU-182, SNU-398 and SNU-449) were determined by western blotting. Their corresponding IC50 for 24, 48 and 72 hours were compared to determine whether Panobinostat were determined by cell viability assay. Huh-7, HepB3 and SNU-449 were selected for further in vitro experiments. Their cell cycle distribution after Panobinostat treatment was evaluated by flow cytometry. Apoptosis was detected by Cell Death Detection ELISA. Huh-7 and HepB3 xenograft model were used for in vivo investigation. Cells were inoculated subcutaneously into the flanks of 3-4 week old athymic nude mice. When tumors were established, Panobinostat was administrated intraperitoneally at 7.5mg/kg and 15mg/kg five days per week for 2 weeks. Results: All cell lines were able to achieve nearly 100% growth inhibition and had displayed a dose- and time-dependent manner towards Panobinostat. Maximum growth inhibition was 20-70% at 24hr compared to over 90% at 72hr. There was significant reduction in cell viability at low nanomolar concentrations (IC50 at 48hr: HepG2 = 8.81 ± 0.72nM, PLC/PRF/5 = 18.9 ± 0.74nM, Huh-7 = 14.01 ± 1.12nM, HepB3 = 25.00 ± 3.69nM, SNU-182 = 73.32 ± 15.52nM, SNU-398 = 12.86 ± 3.45nM, SNU-449 = 73.01 ± 9.09nM). Flow cytometry analysis showed Panobinostat induced accumulation of cells at G0/G1 phase in Huh-7 and SNU-449. Meanwhile, an increase in sub G1 population was detected in HepB3 after exposure to 25nM Panobinostat for 48h. Apoptotic induction was further confirmed by cell death detection ELISA and western blotting. Panobinostat promoted apoptosis more remarkable in HepB3 than other cell lines as evidenced by a stronger cleaved PARP expression level. Panobinostat treatment delayed tumor growth in HepB3 (p<0.0005) and Huh-7 (p<0.0005) xenografts compared to vehicle control. The overall weight loss was less than 20% despite of greater drop during middle of the treatment. Conclusion: Panobinostat has been demonstrated to inhibit in vitro and in vivo HCC cell growth. Further study on the mechanism behind Panobinostat sensitivity is warranted. The study was supported by Novartis.

**#5060 Activity of the EZH2 inhibitor tazemetostat as a monotherapy and in combination with multiple myeloma therapies in preclinical models.** Allison E. Drew, Vinny Motwani, John E. Campbell, Cuyue Tang, Jesse J. Smith, Richard Chesworth, Robert A. Copeland, Alejandra Raimondi, Scott Ribich. Epizyme, Cambridge, MA.

The EZH2 inhibitor tazemetostat (EPZ-6438) is currently being evaluated in phase 2 clinical trials for the treatment of non-Hodgkin’s Lymphoma (NHL). EZH2 inhibitors have shown anti-proliferative effects in multiple preclinical models of NHL and objective clinical responses have been reported in patients with B-cell lymphomas in phase 1 and phase 2 studies of tazemetostat. Mounting evidence suggests that EZH2 is an important regulator of B cell differentiation, both in normal B-cells and in B-cell lymphoma, and may be an important mediator of cell fate in B-cell malignancies in the clinic. Consistent with its essential role in regulating B cell differentiation, recent studies have also shown a dependence on EZH2 activity in multiple myeloma (MM), a disease arising from terminally differentiated B-cell lymphocyte plasmablasts. Frequent genetic al-
terations of epigenetic modulators are observed in MM, pointing towards an important role in the initiation and maintenance of this disease. Dysregulation of the H3K27 methyltransferase EZH2, its corresponding histone demethylase UTX and the H3K36 methyltransferase WHSC1 in MM suggest that disruption of the balance of histone methylation may be fundamental to MM pathogenesis in a subset of cases. Indeed, inhibition of EZH2 alone has shown potent anti-proliferative effects both in vitro and in vivo preclinical models of MM. Here, we describe the effects of small molecule EZH2 inhibitors as monotherapy and in combination with standard of care agents in preclinical models of MM. Tazemetostat selectively inhibits intracellular H3K27 methylation in MM cell lines and elicits a robust anti-proliferative effect in 14-day assays. Following demonstration of single agent activity, we then investigated potential for combinatorial activity of tazemetostat with first and second line therapies for multi-
ple myeloma as well as other non-approved but emerging therapies. Synergistic anti-proliferative activity was observed when tazemetostat was combined with glucocorticoid receptor agonists (dexamethasone, prednisolone), small mole-
cule immune system modulators (lenalidomide, pomalidomide) and protea-
some inhibitors (bortezomib, ixazomib) when cells were primed with tazeme-
tostat for seven days prior to the addition of the standard of care drugs. Combination activity was also observed with an alternate treatment schedule where cells were co-treated with tazemetostat along with the combination part-
ner for seven days. Studies with selected therapeutic modalities were expanded into in vivo xenograft models to further evaluate monotherapy and combination activity of EZH2 inhibitors in MM.

#5061 TET inhibits prostate cancer tumor growth, progression and me-
tastasis in TRAMP mice. Harry K. Koul, Prakash Srivinasisiml Tanimi Shannugam, Praveen K. Jaiswal, Sweaty Koul. LSU Health Sciences Ctr. - Shreveport, Shreveport, LA.

Introduction: Prostate cancer (PCa) is the 2nd most common malignancy in USA. Novel agents for treatment of advanced PCa are warranted. Transgenic adenocarcinoma of the mouse prostate (TRAMP) is an autologous mouse model exhibits both histological and morphological features that mimic human prostate carcinogenesis. Herein, for the first time, we evaluated the in vivo effects of TET, a derivative of Tetrandrine in TRAMP model. Methods: Beginning 12 weeks of age, male TRAMP mice were administered with TET (30 mg/kg body weight, orally, alternative days) in PBS or PBS alone (Control) till 30 weeks of age. Body weight (B) of animals was recorded weekly. At various time points animals were euthanized, genitourinary tract (G) were weighed. Prostate tissue was subjected to immunohistochemical analyses for SV40-TAg, epithelial-mes-
enchymal transition (EMT), proliferation, neuroendocrine differentiation (NED) and apoptosis. Multiple organs were examined for drug toxicity and lungs were analyzed for metastasis. Results: TRAMP mice exhibit to high-grade prostatic intraepithelial neoplasia (PIN) by 12 weeks and progresses to poorly differentiated adenocarcinoma by 30 weeks with distant metastasis to lungs. TET feeding did not show any considerable difference body weight loss profiles during the entire treatment regimen. At the time of necropsy, there was no evidence of edema, abnormal organ size or appearance in non-target organs. TET gavage group showed (p<0.005) lower G/B ratio compared to the PBS treated group. These findings clearly indicate that TET dosing is non-toxic, and restricts the abnormal growth of the prostate in TRAMP mice. TET repress the EMT as well as NED transition and inhibits cell proliferation (p<0.005) by Ki-67 staining, Oral administration of TET inhibits PCa growth and progression by increases (p<0.005) apoptosis in tumor tissues. Further, TET inhibited me-
tastasis as there was significant (p<0.005) decrease in metastatic lungs in TET-
treated animals. Conclusion: Human achievable dose of TET treatment to TRAMP mice bearing prostate tumor, exhibited no-observed-adverse-effect-
level in toxicology evaluations and also significantly inhibited tumor growth, progression, local invasion and distant metastasis involving suppression of tu-
mor, and thus could have potential against human PCa.

#5062 Development of a first in class inhibitor of BET bromodomains and
dopamine receptor 2. Makoto Yoshikawa,1 Jay Chauhan,2 Steven Fletcher,3 Jef-
frey W. Strovel.1 ConverGene, Cambridge, MD; 2University of Maryland, Balti-
more, MD.

ConverGene has developed a first-in-class dual-active small molecule inhib-
itor that i) inhibits BET family of bromodomain-containing proteins, and ii) antagonizes dopamine receptor D2 (DRD2). BET protein family includes BRD4, an epigenetic reader protein that mediates expression of MYC oncogene. Thus, BRD4 is considered as a cancer therapeutic target to indirectly suppress MYC expression. In addition to being a therapeutic target for psychiatric diseases, DRD2 is emerging as a potential therapeutic target in neuroendocrine tumors, subsets of pancreatic ductal adenocarcinoma and small cell lung cancer. Our lead compound showed high activity in a binding test against BRD4 (Ki = 34 nM); exhibited high bioavailability upon oral administration; profoundly sup-
pressed MYC expression both in vitro and in vivo; inhibited growth of AML and solid tumor cells in xenograft models; potently inhibited both isoforms of DRD2 (IC50 0.1 μM); and interfered with DRD2/β-arrestin/Akt pathway in vitro. Therefore, our BRD4/DRD2 dual-active compounds may hold promise as a novel class of therapeutics that interferes with both cancer growth and mainte-
nance by simultaneously interfering with MYC and DRD2 pathways. We cur-
rently are investigating these dual-active compounds in multiple in vitro and in vivo models and expect to report the outcomes at the AACR Annual Meeting in 2017.

#5063 Epigenetic modulators show differential activity on lung adenocar-
cinoma cells with loss-of-function mutations of SWI/SNF protein SMARCA4. Tomasz Rzymski,1 Anna Wrobel,1 Michal Mikula,2 Karolina Py-
ziak,1 Anna Bartosik,1 Agnieszka Sroka,1 Agnieszka Paziewska,2 Aleksandra Grochowska,2 Małgorzata Statkiewicz,2 Katarzyna Paczkowska,2 Michalina Da-
browska,2 Jerzy Ostrowski,2 Krzysztof Brzozka,2 Selvita S.A., Krakow, Poland; 2Maria Sklodowska-Curie Memorial Cancer Center, Warsaw, Poland.

SWI/SNF is a multiprotein chromatin remodeler with AT-dependent activ-
ities leading to selective gene expression, DNA repair, recombination and rep-
lication. Various sequencing efforts indicated that nearly 20% of cancers bear mutations in at least one subunit of the complex. One of the crucial regulators of the complex is SMARCA4, a member of SWI/SNF family of helicases with AT-
Pase activities, which are thought to regulate transcription of certain genes by altering the chromatin structure. SMARCA4 is mutated in virtually all cases of small cell carcinoma of the ovary and SMARCA4 is fourth the most frequently mutated gene in lung adenocarcinoma. High occurrence of inactivating muta-
tions prompted several screenings projects focused on synthetic lethality inter-
actions with other proteins, which led to the identification of SMARCA2 as an essen-
tial gene in SMARCA4 mutated cancers. This vulnerability could be po-
tentially exploited therapeutically and several groups managed to identify potent-
ligands of SMARCA2 bromodomain. Surprisingly these molecules were inactive in SMARCA4 mutant cells, however additional studies indicated that ATPase rather than bromodomain is a target for novel compounds with anticancer ac-
tivities. Overall, these results revealed functional complexity of SMARCA2 and SMARCA4 in cancer cells. In order to characterize molecular consequences of SMARCA2 silencing in SMARCA4 mutant lung adenocarcinoma cells, we have carried out a series of gene knockdown experiments, followed by transcriptional profiling by RNAseq and analysis of posttranslational histone modifications. These studies indicated rapid and irreversible loss of viability in SMARCA4 mutant cells after SMARCA2 gene silencing. Interestingly double SMARCA2/ SMARCA4 knockdown in SMARCA4 WT cells has not resulted in lowered viability. Transcriptional profiling of SMARCA2 knockdown in SMARCA4 mu-
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oposed a phenotypic-based system (YBS) by stably transfecting SW48 cells with a vector containing GFP driven by a methylated and silenced CMV promoter. GFP re-expression can be achieved by known epigenetic drugs that lead to de-methylation or induce active chromatin marks in the CMV promoter. By screening an NDI-3040 natural compounds library and grouping the compounds based on chemical structures, we identified two main drug classes. We then synthesized 77 new analogs based on class #1’s lead’s structure and 23 were positive in the YBS system. The most potent analog (HH2) can induce ~60% GFP+ cells upon 500nM treatment after 96hr. All the positive hits can also be validated in two other cancer cell lines (MCF7 and HCT116). Consistent with GFP reactivation, endogenous hypermethylated genes (MGMt, RARβ, etc) can also be re-expressed upon drug treatment. We then performed RNA-seq analysis to identify global gene expression changes following drug treatment. We observed that most genes (2964 genes) were upregulated upon HH1 treatment (10μM) and that many of the upregulated genes were expressed in normal tissues but repressed in cancer, indicating that they might be potential tumor suppressor genes (TSGs). Consistent with this, 94 TSGs could be reactivated upon 10μM drug treatment. These drug target upregulated genes were also enriched for hypermethylation. By performing connectivity mapping using RNA-seq, we identified X as the class #1 drug target. The on-target effect could be further validated by using other selective X inhibitors as well as a dominant negative X construct. Consistent with drug inhibition, dominant-negative X can also re-activate drug targeted hypermethylated genes. Additionally, when we overexpressed wild-type X, we saw that GFP induction as well as gene reactivation can be inhibited. Strikingly, by using GFP induction as read-out to optimize drugs, we found that the in vitro IC50 against X for our top lead compound (HH2) is only 5nM and it is at least 22-fold selective for X over other X family members. Thus, a novel epigenetic drug class derived from natural compounds was identified and can be developed by targeting silenced gene expression.

#5065 Harnessing epigenetic reprogramming by histone deacetylase inhibitor MS275 for pancreatic cancer therapy. Gaoyang Liang,1 Ruth Yu,1 Christopher Liddle,2 Morgan Truitt,1 Corina Antal,1 Annette Atkins,1 Ester Banayo,1 Michael Downes,1 Ronald Evans1.1Salk Institute for Biological Studies, La Jolla, CA; 2Westmead Millennium Institute and University of Sydney, Westmead, Australia.

Pancreatic ductal adenocarcinoma (PDAC) accounts for more than 85% of pancreatic cancer and is one of the most lethal malignancies with limited therapeutic options. Chromatin-modulating small molecules, or epigenetic drugs, have the ability to reprogram cell fate and alter disease phenotypes. The potential of these drugs for PDAC therapy remains to be fully investigated. Screening a panel of epigenetic drugs identified MS275 (MS, also called entinostat), a histone deacetylase inhibitor, as capable of suppressing PDAC cell proliferation and subsequent fibrosis. Genome-wide expression analysis revealed that MS extensively reprograms the transcriptomes of tumor cells and cancer-associated fibroblasts (CAFs). In tumor cells, MS downregulates genes important for cell cycle progression inducing cytostasis. In CAFs, MS specifically represses the pro-fibrotic transcription program responsive to TGF-β, effectively inhibiting the fibrotic responses. Consistent with this, MS blocks the activation of pancreatic stellate cells, the primary source for CAFs in PDAC, to repress the fibroblast-like phenotypes in these cells. Using an orthotopic transplantation model, we confirmed that MS treatment reduces tumor cell proliferation and decreases intratumoral fibrotic content. Importantly, we showed that MS substantially enhances chemosensitivity, synergizing with gemcitabine to reduce tumor burden in PDAC mouse models. Our study establishes a novel therapeutic strategy for PDAC based on epigenetic reprogramming induced by HDAC inhibition.


Chondrosarcomas are the second most frequently occurring type of bone malignancy, and account for approximately 25% of all bone sarcomas. They are often highly aggressive neoplasms that rapidly progress and eventually recur and give distant metastases. They are largely considered to be resistant to conventional chemotherapy and radiotherapy. Several studies have reported that targeting epigenetic mechanisms including DNA methylation and histone acetylation are novel approaches for the treatment of some human cancers. Previous reports have shown that the interaction of DNA methylation and histone modification regulates gene expression. In the present study, we hypothesized that concurrent inhibition of histone acetylation and DNA methylation could result in decreased viability of chondrosarcoma cells both in vitro and in vivo. To test this, we used a panel of chondrosarcoma cell lines including IDH wild type (CH2879), IDH1 mutant (JH012) and IDH2 mutant (CS1) cell line. Results from our in vitro proliferation assay showed that combination of sub-IC50 concentrations of the DNA methyltransferase inhibitor 5-Aza-2’-deoxycytidine (5-AZA) and histone deacetylase (HDAC) inhibitor (SAHA) resulted in decreased cell viability of the three chondrosarcoma cell lines tested when compared to either drug alone. Western blot analysis showed induction of cleaved Poly-ADP Ribose Polymerase (PARP), a known marker of apoptosis. Consistent with augmented DNA damage, combination of Decitabine and SAHA markedly increased the apoptosis marker Poly ADP Ribose (PAR) and damage marker, and pro-apoptotic BH3 only proteins such as Bim. Combination treatment also resulted in increased induction of histone acetylation (AcH3) and increased expression of E-Cadherin. Previous reports have shown that E-cadherin is critical for apoptosis and depletion and inhibition of E-Cadherin impairs apoptosis induction via DR4 and DR5 death receptors. To this end, we showed that induction of cleaved PARP by Apo2L/TRAIL stimulation decreased over time when E-Cadherin was knocked down by siRNA. Xenograft studies using IDH2 mutant (CS1) tumor model showed a significant suppression of tumor volume when animals were treated in combination with SAHA and Decitabine compared to single agent treatments. Taken together, our data strongly suggests that combination treatment with SAHA and Decitabine is a novel treatment approach and merits evaluation in the treatment of chondrosarcoma.

#5067 BET protein proteolysis targeting chimera (BET-PROTACs) exert more potent activity than BET bromodomain inhibitor (BETi) against post-myeoproliferative neoplasm (MPN) secondary (s) AML cells. Dyana T. Saenz,1 Warren C. Fiskus,1 Kanak Raina,2 Tahi Marshouiri,1 Kevin G. Coleman,1 Yimin Qian,1 Andrew P. Crew,2 Angela Shen,2 Christopher P. Mills,1 Bao-hua Sun,1 Misun Kim1, Agnieszka J. Nowak,1 Srdan Verstovsek,2 Craig M. Crew,3 Kapil N. Bhalla1.1MD Anderson Cancer Center, Houston, TX; 2Arvinas, LLC, New Haven, CT; 3Yale University, New Haven, CT.

In BCR-ABL1-negative myeloproliferative neoplasms with myelofibrosis (MPN-MF) transformation to AML (sAML) occurs in up to 20% of patients. Ruxolitinib (R) is a type I, ATP-competitive, JAK1 & 2 inhibitor (JAKi), which is effective in the therapy of MPN-MF but does not significantly impact the clinical outcome in post-MPN sAML. We have previously reported that treatment with BETi, e.g. IQ) or OTX015 inhibits growth and induces apoptosis of cultured sAML cells, including those that express Jak2 V617F and mutant TP53, e.g. HEL92.1.7 and SET2, as well as patient-derived (PD) CD34+sAML cells. BETi treatment attenuated the protein expressions of c-MYC, p-STAT5, Bcl-xl, CDK4/6, PI3 and 7R, while concomitantly inducing the levels of HEXIM1, p21, NOXA and BIM in the sAML cells. However, treatment with BETi leads to the accumulation of BET, e.g. BRD4, which may reduce BETi-mediated repression of c-MYC, NfκB and BET-regulated oncogenes. In contrast, BET-PROTACs (proteolysis targeting chimera) ARV-825 and ARV-771 (Arvinas Inc.) degrade BETPs (including BRD4) in the cultured and PD CD34+sAML cells. At equimolar concentrations, BETP-PROTACs were significantly more potent than the BETi in inducing apoptosis of cultured and PD sAML cells. Additionally, co-treatment with BETP-PROTAC and R was synergistically lethal against the cultured and PD CD34+sAML cells. BETP-PROTAC treatment caused more up and down regulation of mRNA and protein expressions than BETi, as determined by RNA-Seq and reversed phase protein array (RPPA) analyses, respectively. As compared to treatment with BETi, BETP-PROTAC caused greater depletion of c-MYC, Jak2, p-STAT5, STAT5, p-STAT3, STAT3, PI3 and Bcl-xl, whereas the protein levels of p21 and p27 were upregulated. CyTOF or mass-cytometry also showed that BETP-PROTAC, more than OTX015 treatment, reduced BRD4, c-MYC and p-Rb, while inducing p21 levels in the CD34+sAML stem/progenitor cells expressing CD90, CD244, CD123 and CD138. Contrary to BET-PROTAC with R significantly reduced the expression of sAML progenitor markers (CD34+CD138) and burden and improved the median survival of the immune-depleted mice engrafted with luciferase-transduced HEL92.1.7 cells. These findings strongly support further in vivo development of the novel BETP-PROTACs-based combinations against post-mPN sAML.
Inhibition of BET bromodomain, epigenetic regulator, as an effective therapeutic approach for gastric cancer.

Sun Kyoung Kang,1 Tae Soo Kim,2 Woo Sun Kwon,3 Jae Kyung Rho,1 Ho-Yeong Lim,1 Hyun Cheol Chun,1 Sun Young Rha3.

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with single agent BET or PI3Kα therapy, the combination significantly potentiates tumor growth inhibition in DLBCL models representative of the ABC subtype (HBL-1), and the double hit GCB subtype (WIL2). These data suggest that clinical exploration of INCB057643 as a monotherapy or in combination in hematologic malignancies is warranted.

**#5072 Inhibition of EZH2 as a therapeutic strategy for osteosarcoma.** Esvaran Devarajan, Wei-lin Wang, Jen-Wei Tsai, Andrew Futreal, Valeriane O. Lewis. UT MD Anderson Cancer Ctr., Houston, TX.

Introduction: Osteosarcoma is the most common primary malignant bone tumor. About 30% of osteosarcoma patients experience relapse and die of their disease. We previously demonstrated that IL-11Rs are overexpressed in primary and metastatic osteosarcoma and plays a role in the development of metastases. Our recent microarray data from Korb, Sisa1, and LM7 IL-11Rα shRNA cells showed decreased expression of several components of polycomb repressive complex 2 (PRC2). EZH2 is the catalytic subunit of PRC2 and blocks transcription of numerous tumor suppressors’ genes. EZH2 has been widely implicated in cancer and inhibition of its catalytic activity has recently emerged as a novel approach to treat cancers. Purpose: To explore the effects and mechanisms of inhibition of EZH2 on osteosarcoma metastasis. Inhibition was tested using GSK126, a novel small-molecule EZH2 inhibitor that competes with methyl group donor S-adenosyl-methionine in a highly selective fashion. Methods: Proliferation assays were performed on a panel of osteosarcoma cell lines subjected to increasing concentrations (0.0625 µM to 20 µM) of GSK126 for 3 days. The effects of IL-11 in PRC1 complex histone H3 methylation (H3K27) were studied by Western blotting. For in vivo experiments, we used an orthotopic metastatic model. Luciferase-labeled Sisa1 osteosarcoma cells were injected into the tibias of 4- to 5-week-old nude mice. After 2 weeks, mice were treated with vehicle or GSK126 three times per week intraperitoneally at 0.1 ml per 20 g of body weight in 20% capitol for 48 days. The tumor-containing legs were amputated when tumor volume reached 2 cm. Primary tumors were measured with calipers, and, with the capability of the compound, administered individually or in combination (IC50), to inhibit cell proliferation was assessed by MTT assays. Drug interaction analysis was performed using Compusyn software. The protein and gene expression levels were determined using western blot and real-time PCR, respectively. Results: We found that about 70% of the cell lines tested were sensitive to EZH2 inhibition (IC50 = 3.576-6.450 µM). In vivo, mice treated with GSK126 developed significantly fewer osteosarcoma lung metastases than control mice injected with vehicle alone (P<0.01). Conclusion: GSK126, a potent small-molecule EZH2 inhibitor, inhibits the development of osteosarcoma lung metastases in vivo. Thus, GSK126 may be considered as a novel anticancer drug candidate for osteosarcoma.

**#5073 Determination of sensitivity to BET and HDAC inhibitors in lung cancer cell lines.** Sara Verdura, Manuel Torres-Diz, Montserrat Sanchez-Cespedes. IDIBELL, Barcelona, Spain.

Purpose: To determine the sensitivity of lung cancer cell lines to different combinations of BET and HDAC inhibitors and to inquire possible associations with the genetic and molecular profiles. Methods: A panel of lung cancer cell lines were treated in vitro with BET and HDAC inhibitors. The capability of each compound, administered individually or in combination (IC50), to inhibit cell proliferation was assessed by MTT assays. Drug interaction analysis was performed using Compusyn software. The protein and gene expression levels were determined using western blot and real-time PCR, respectively. Results: We found that about 70% of the cell lines tested were sensitive to BET inhibitors and about 60% responded to HDAC inhibitors. In both cases, increased sensitivity was accompanied by changes in cell morphology, a reduction of the MYC protein levels and an increase in the levels of apoptotic-related markers. We also determined the effects of the combination of these epigenetic treatments in cell growth. Our results showed a synergistic effect in about 75% of the cell lines which was also correlated with dramatic changes in cell morphology, in apoptosis and in decreasing the levels of the MYC protein. Finally, we determined the possible correlation of the sensitivity to these epigenetic drugs and the expression levels of some genes implicated in lung carcinogenesis. Conclusions: Our data showed that lung cancer cells responded to BET and HDAC inhibitors and that the combination of both drugs has a synergistic effect in cell growth inhibition, suggesting that this could be an effective treatment in lung cancer. Our data also point out to certain genes as potential biomarkers to determine sensitivity to these compounds.

**#5074 Therapeutic targeting of bromodomain and extra-terminal proteins degradation in triple-negative breast cancer.** Longchuan Bai, Bing Zhou, Chao-Yie Yang, Jiao Ji, Donna McEachern, Sally Przybranowski, Shaomeng Wang. University of Michigan, Ann Arbor, MI.

Triple negative breast cancer represents the most clinically challenging subtypes of breast cancer for which targeted therapeutics are still lacking. BET proteins have emerged as new therapeutic targets for human cancers and other diseases. We have developed a highly potent BET degrader, BETD-246, with an exceptional selectivity based upon a new class of BET inhibitor, BET-211, and investigated its therapeutic potential and mechanisms of action in TNBC. BETD-246 induces degradation of BET proteins at low nanomolar concentrations within 1 hour, leading to potent anti-proliferative activity and strong apoptosis induction in the majority of TNBC cell lines, and is much more effective than the BET-211 treatment. RNA-seq analysis reveals that degradation of BET proteins by BETD-246 elicits distinct transcriptional response than BET BD inhibition in TNBC cell lines. Transcriptomic analysis also shows a number of proliferation and survival-related genes were differentially regulated by BETD-246 and BETI-211 which were confirmed by quantitative RT-PCR. Mechanistically, down-regulation of MCL1 by BETD-246 plays a key role in the robust apoptosis induction in TNBC cell lines. In vivo models, BETD-246 effectively degrades BET proteins and suppresses MCL1 expression in xenograft tumor tissues and exhibits a strong antitumor activity at well-tolerated dose-schedules in mice. Our data strongly suggest that targeting BET protein degradation is an exciting therapeutic strategy for TNBC.


In recent years, epigenetic target inhibitors have emerged as viable therapeutics for treating various types of cancers. Testing of the epigenetic inhibitors in cell proliferation assays revealed that inhibition of many epigenetic targets resulted in a significantly delayed drug response that is not revealed by conventional, 72-hour cell growth assays. OncoPanel LT™ from Eurofins Pharma Discovery Services is developed specifically for evaluation of compounds with a protracted mechanism of action, and consists of a large collection of cell lines with broad cancer type and subtype representation, and extensive genomic characterization. We have tested decitabine, a DNA methyltransferase inhibitor, panobinostat, a histone deacetylase inhibitor, GSK-343, an EZH2 inhibitor, GSK-14, a JMJD3/UTX inhibitor, and JQ1, a BRD2/3/4 inhibitor in OncoPanel LT™. These epigenetic inhibitors were incubated for 10 days with more than 200 human tumor cell lines and the inhibition data were analyzed to determine IC50, EC50, and GI50 values. These inhibition data, as well as the genetic features of the cell lines, were utilizes to perform univariate genomic analysis to identify predictive biomarkers of response. We will discuss the activity of compounds against this cell line panel, as well as statistically significant biomarkers of response, based on the univariate genomic analysis.

**#5076 IHC and flow cytometry quantifies BRD4 levels in surrogate tissues after ex vivo and in vivo dosing with a BRD4 degrading PROTAC.** Sheryl M. Gough, Kanak Raina, Debbie Gordon, Ryan Willard, Martha Altieri, Angela Shen, Yimin Qian, Taavi Neklesa, Kevin Coleman, Ian Taylor. Arvinas LLC., New Haven, CT.

The Bromodomain and Extra-Terminal (BET) protein BRD4 has been identified as a key transcriptional regulator of various oncogenes, most notably MYC, in numerous human malignancies. Efforts have been extensive over recent years to inhibit the gene regulatory action of BRD4 and BET inhibitors have entered clinical trials. In a new approach to cancer therapeutics, we have developed a proteolysis-targeting chimera (PROTAC) molecule, ARCC-29, which induces degradation of BET (BRD4/3/2) proteins via the proteasome. ARCC-29 degrades BRD4 in several human malignant cell lines and in in vivo rodent xenograft tumors. In mice xenograft models of prostate (22Rv1), DLBCL (SU-DHL-6) and ovarian (A2780) tumors, this molecule demonstrates a strong PK/PD efficacious relationship. Obtaining tumor tissue from patients often requires invasive procedures and may be unavailable. We have developed two assays using surrogate tissues to evaluate PROTAC mechanism of action which could be applied in the clinical setting. We have developed a flow cytometry protocol to quantitatively assess intra-cellular BRD4 levels in peripheral blood mononu-
EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Epigenetic Agents

#5078 EZH2 methyltransferase regulates disseminating tumor cells in breast cancer metastasis. Shira Yomtoubian,1 Seongho Ryu,1 Sharrell Lee,1 Geoff Markowitz,1 Dingcheng Gao,1 Vivek Mittal1.

Triple negative breast cancer (TNBC, ER-, PR-, HER2-) exhibits the worst outcome due to higher rates of metastasis compared to non TNBC subtypes. Despite this clinical significance, there is a conspicuous lack of FDA approved molecularly targeted anti-metastatic therapies for TNBC. The enhancer of zeste homolog 2 (EZH2), a catalytic core subunit of the Polycomb repressive complex 2 (PRC2) with histone methyltransferase (HMT) activity is associated with the worst clinical outcome in breast cancer patients. Using a combination of genetic and pharmacological approaches, we show that EZH2 HMT blockade did not impact primary tumor growth, but significantly reduced distal metastases. Metastasis suppression was associated with a marked reduction of tumor-initiating cells (TICs) in primary tumor, circulating tumor cells (CTCs) in the blood and impaired lung colonization. Using a S02/OCT4 promoter reporter system, we identified EZH2-sensitive metastatic cells with GATA3 low luminal progenitor phenotypes in the primary tumor, and EZH2 HMT blockade restored GATA3 expression, promoted differentiation of luminal progenitors and impaired metastatic dissemination. These findings point to EZH2 as a therapeutic target in TNBC that promotes metastasis, and that inhibition of EZH2 HMT may constitute a viable anti-metastatic approach. We will also discuss the potential of EZH2 inhibition in combination with chemotherapy as an effective strategy against TNBC metastasis.

#5079 Inhibition of HDAC2, but not HDAC1, abrogates triple negative breast cancer progression and metastasis. Hope E. Burks,1 Steven Elliott,1 Van T. Hoang,1 Margarite D. Matossian,1 Bridgette Collins Burrow,1 Matthew E. Burrow,1 2 Tulane University, New Orleans, LA; 2 Tulane University, New orleans, LA.

Triple negative breast cancer (TNBC) is the most aggressive subtype of breast cancer, with higher rates of metastasis and recurrence than other classified subtypes. The decreased survival rates seen in TNBC can be attributed in part to the lack of targeted therapeutics. HDAC inhibitors (HDACi) have emerged as a potential adjuvant therapy for patients with triple negative disease. Previously, our lab has identified Panobinostat, a pan-DAC inhibitor, as a successful therapy for the reversal of the metastatic phenotype in TNBC. However, this therapy lacks specificity and may have increased side effects as a result. In this study, we aim to dissect the necessary HDACi responses for driving and maintaining the metastatic phenotype of TNBC, with the goal of informing specific HDAC target development. We have identified that HDAC2, but not HDAC1, is capable of recapitulating aspects of Panobinostat treatment in TNBC both in vitro and in a patient derived xenograft model in vivo. To parse the necessity and role of HDAC1 and HDAC2 individually, we created knockdown cell lines and analyzed them for changes in gene expression changes and their respective associated biological processes, including proliferation, migration, and mammosphere formation, tumor growth and metastasis. We found that HDAC2 knockdown was able to significantly repress migration and proliferation in TNBC cells and their associated gene expression signatures in vitro. Furthermore, HDAC2 knockdown resulted in decreased tumor growth and metastasis in vivo in mouse model. These results illustrate the efficacy of Romidepsin in inhibition of oncogenic processes and implicate HDAC2 as a potential target for therapeutic intervention in TNBC.

#5080 BET inhibitors INCB054329 and INCB057643 display significant activity in androgen-independent prostate cancer models. Ramiro Vázquez,1 Gianluca Civenni,1 Martina Marchetti,1 Sabrina Zadic,1 Philip Liu,2 Bruce Ruggeri,2 Giuseppe M. Carbone,1 Carlo V. Catapano.1 1 Institute of Oncology Research,IOR, Bellinzona, Switzerland; 2 Incyte Corporation, Wilmington, DE.

Prostate cancer is a leading cause of cancer death. Epigenetic dysregulated transcriptional factors and epigenetic effectors contribute to prostate cancer progression and castration resistance providing ideal targets for developing new therapeutic strategies. Bromodomain and extra-terminal (BET) proteins act transcriptional co-activators interacting with multiple co-regulatory molecules at gene promoters and enhancers. BET inhibitors (BETi) disrupt transcriptional regulatory complexes and have broad anticaner activity. INCB054329 and INCB057643 are BETi with proven pre-clinical activity in hematological and solid tumors. Both compounds are currently in clinical trials (NCT02431260 and NCT02711137) in advanced cancer patients. In this study we explored for the first time the activity of these two BETi in prostate cancer, comparing their effects in androgen-dependent and independent models in vitro and in vivo. We assessed the effect of INCB054329 and INCB057643 on cell proliferation, colony formation and tumor-sphere assays in androgen-dependent (LNCaP and VCaP) and androgen-independent (DU145, PC3, 22Rv1) cells. The in vivo antitumor activity was evaluated in mice with 22Rv1 derived subcutaneous xenografts with drugs administered orally (BID, daily, for 3 weeks). INCB054329 and INCB057643 inhibited proliferation of prostate cancer cell lines. In short-term cell proliferation assays the BETi appeared more effective against androgen-dependent (VCaP and LNCaP) than androgen-independent (DU145 and PC3) cells (GI50 of ≤100 nM and ≤500 nM, respectively). 22Rv1 cells, which express androgen-independent AR splice variants, exhibited an intermediate level of sensitivity to both compounds (GI50 150-250 nM). Interestingly, in colony and tumor-sphere forming assays all the cell lines showed substantially greater sensitivity to the BETi than in short-term assays. Notably, androgen-independent 22Rv1 cells were highly responsive with GI50 values ≤100 nM similar to those seen in androgen-sensitive LNCaP cells. Treatment of mice bearing 22Rv1 tumor xenografts with INCB054329 (50 mg/kg) and INCB057643 (3 mg/kg) led to significant inhibition of tumor growth (T/C%: 42 and 45%, respectively) and clear cells (PBCMs). Frozen or fresh human PBCMs were cultured for six hours ex-vivo with ARCC-29, fixed and permeabilized for intra-cellular BRD4 staining, and BRD4 levels quantified in gated lymphocytes. ARCC-29 reduced BRD4 levels to 13, 10, 16, and 9 percent of vehicle in four fresh PBCM samples, and to 9, 10, 11, 6, 8 and 35 percent in six frozen PBCM samples. Treatment of PBCMs with intracellular BRD4 inhibitor OTX-015, showed similar or increased levels of BRD4 compared to vehicle control. Inactive stereoisomers of ARCC-29 also showed BRD4 levels increased or similar to vehicle control. Duplicates of selected experiments were analyzed by western blot, corroborating the depletion of BRD4. The second assay uses immunohistochemistry (IHC) to evaluate BRD4 degradation in skin biopsies of Sprague Dawley and nude male rats dosed with 2mg/kg intravenous (i.v.) BRD4 PROTAC ARCC-29. IHC analysis of rat skin eight hours after in-vivo dosing with ARCC-29 demonstrated a dose-dependent reduction in BRD4 protein. Rats dosed once a week with ARCC-29 [2 mg/kg] showed a 60-90% reduction in BRD4 levels compared to vehicle, while 0.3 mg/kg twice per week showed a 10-30% decrease in BRD4 levels. These experiments demonstrate that BRD4 degradation can be evaluated in surrogate tissue. Flow cytometry using PBCMs and skin IHC provide potential alternative methods for confirming the PROTAC mechanism-of-action in the clinic using pre- and post-treatment patient samples. DISCLOSURES All authors are employed by Arvina, LLC.
consistent reduction of tumor weight relative to vehicle-treated mice. These results provide evidence of activity of INCBO54329 and INCBO57643 in prostate cancer cell lines. Although short term assays suggest a preferential anti-proliferative effect in androgen sensitive prostate cancer cells, both compounds also exhibit significant activity in an androgen-independent model both in vitro and in vivo suggesting that they might represent a valid therapeutic option for treatment of castration-resistant prostate cancer.

#5081 Disruption of DNA methyltransferase (DNMT) 1 confers resistance to DNMT inhibitors in human colorectal cancer cells. Angelo B. Laranjeira, Dat Nguyen, Erich Huang, James H. Doroshow, Sherry X. Yang. Division of Cancer Treatment and Diagnosis, National Cancer Inst., Bethesda, MD.

DNA methyltransferase (DNMT1) is responsible for the maintenance and propagation of DNA methylation pattern, and plays an important role in cell survival and proliferation. Thus, targeting DNMT1 represents a promising approach for cancer treatment. However, the role of DNMT1 in treatment efficacy with DNMT inhibitors remains controversial. The aim of this study was to investigate potential alterations of DNMT inhibitor-induced cytotoxicity and cellular survival after disruption/knockout of DNMT1 in cancer cells. The human colorectal cancer cell line HCT116 and its isogenic DNMT1 knockout pair were treated with increasing concentrations of 4’-thio-5-aza-2’-deoxycytidine (aza-TdCyd), a novel DNMT inhibitor, and 5-aza-2’-deoxycytidine (aza-dCyd). Cytotoxic effects and cell survival were evaluated by MTT and clonogenic assays. Apoptosis was measured by annexin-V/propidium iodide test, and expression of DNMT1, DNMT3A and DNMT3B was detected by western blot. After 96h of drug exposure, IC50s of aza-TdCyd and aza-dCyd were 0.031 μM and 0.28 μM, respectively, in HCT116 cells. In contrast, DNMT1 knockout cells were remarkably resistant to both drugs with IC50s all higher than 10 μM. The treatments resulted in a significant increase in apoptosis in the parental line versus its knockout counterpart. As for the cell survival, IC50s were ~0.12 μM for aza-TdCyd and ~4.62 μM for aza-dCyd in HCT116 cells, compared to ~43.77 μM and ~62.09 μM in DNMT1 knockout cells. As such, disruption of DNMT1 caused over 300-fold and 10-fold increase in resistance to aza-TdCyd and aza-dCyd. Moreover, we observed a G2/M arrest produced by aza-TdCyd versus aza-dCyd in the parental cells, and no apparent alteration in cell cycle phase distribution in DNMT1 knockout cells. Notably, aza-TdCyd was more potent than aza-dCyd in depleting DNMT3A and/or DNMT1 in both cell lines after 48h of treatment. Our findings demonstrate that disruption of DNMT1 leads to a significantly increased resistance to aza-TdCyd and aza-dCyd, suggesting that DNMT1 is critical to the antitumor activity of DNMT inhibitors. Aza-TdCyd is much more potent than aza-dCyd for both inhibition of DNMTs and growth of cancer cells.

#5082 The selective bromodomain inhibitor, INCBO54329 targets both cancer cells and the tumor microenvironment in the KC inflammatory pre-clinical model of ducital pancreatic cancer. Ana Sofia Leal,1 Karen T. Liby,3 Phillip Liu,2 Bruce Ruggeri2. 1Michigan State University, East Lansing, MI; 2Incyte Corporation, Wilmington, DE.

Pancreatic cancer is expected to become the second most deadly cancer by 2030, with few effective therapeutic options available to improve patient survival. Inhibition of BET-BRD proteins is known to induce pro-apoptotic effects in over 90% of pancreatic cancer cell lines. A BET-BRD protein is considered to be an undruggable target. p-Erk is a downstream effector of Kras and has been shown to be essential for the progression and maintenance of pancreatic cancer. The LSL-KrasG12D/+; Pdx-1-Cre (KC) mouse model associates with multiple transcription factors such as ERα, AR, E2F, and Myc; p-Stat3 protein levels are significantly increased, but this pro-survival effect in androgen sensitive prostate cancer cells, both compounds also exhibit significant activity in an androgen-independent model both in vitro and in vivo suggesting that they might represent a valid therapeutic option for treatment of castration-resistant prostate cancer.

TMEM, several cytokines and chemokines play crucial roles, recruiting and regulating inflammatory cells [macrophages, T cells and Myeloid derived suppressor cells]. KC mice stimulated with LPS have higher levels of CCL2 (1947 ± 591 ng/mL) and IL-6 (2459 ± 577 pg/mL) than unstimulated mice; these increased levels of inflammatory cytokines and the subsequent immune cell infiltration of the tumors caused by treatment with INCBO54329. In vitro, INCBO54329 demonstrated >50% inhibition of both cytokines (IL-6 1063 ± 502 pg/mL; CCL2 713 ± 134 ng/mL). These data suggest that INCBO54329 has a dual activity, targeting cancer cells and modulating the TME; both activities may prove beneficial for the treatment of pancreatic cancer. Moreover, this work suggests that additional mechanisms may underlie the beneficial effects of bromodomain inhibitors.

#5083 A novel and highly efficacious small-molecule degrader of BET-BRD proteins for the treatment of acute leukemia. Estor Fernandez-Salas,1 Zhuo Chen,2 Mei Lin,1 Bing Zhou,2 Donna McEachern,1 Sally Przybranowski,1 Karson Kump,1 Luke F. Peterson,1 Malathi Kandarpa,1 Jian Tao Hu,1 Fuming Xu,1 Liu Liu,1 Longchuan Bai,1 Bo Wen,1 Duxin Sun,1 Moshe Talpaz,2 Shao-meng Wang3. 1Univ. of Michigan, Ann Arbor, MI; 2Shanghai Institute of Medicinal Chemistry, Shanghai, China.

The bromodomain and extra-terminal domain (BET-BRD) family of lysine acetylation readers comprises BRD2, BRD3, BRD4, and BRDT that contain conserved N-terminal bromodomains (BD1 and BD2) and a long C-terminal region containing the extraterminal (ET) protein-protein interaction domain. These proteins have key roles in the assembly of transcriptional regulatory complexes containing RNA polymerase II. BET-BRDs are new therapeutic targets for cancer treatment and several small-molecule BET-BRD inhibitors are currently in clinical development for a diverse set of cancers including leukemia. Based on the protein targeting chimera (PROTAC) concept, we have developed novel and highly potent BET degraders, ZBC246 and ZBC260, with exceptional selectivity based upon a new class of BET inhibitor, ZBC11. ZBC260 effectively degrades all BET-BRD proteins at concentrations as low as 30 pM within a few hours of a treatment in the RS4;11 leukemia cell line and achieves IC50 values of 50 pM in inhibition of RS4;11 cell growth. ZBC260 was tested in a panel of 10 acute leukemia cell lines generating IC50s ranging from 20 pM to 1 nM in 4 days cell growth inhibition assays. In contrast to the cytostatic effect observed with BET-BRD inhibition, degradation of BET-BRDs by ZBC246 and ZBC260 induced robust apoptosis demonstrating a differential biology between BET-BRD inhibitors and BET-BRD degraders. Significantly, ZBC260 induces rapid regression of RS4;11 xenograft tumors without overt signs of toxicity in mice. More importantly, treatment of leukemia cells obtained from 10 patients with ZBC260 demonstrated efficient degradation of BET-BRDs within 5 h at 0.3 nM, while treatment with the inhibitor (300 nM) induced up-regulation of BET-BRDs. Treatment of patients’ peripheral blasts with ZBC260 at 1 to 10 nM induced remarkable levels of apoptosis within 24 h, even in relapsed and refractory patient samples. In conclusion, ZBC260 represents a highly potent and efficacious BET-BRD degrader undergoing extensive preclinical evaluation for the treatment of acute leukemias.

#5084 Potent and isoform-selective ATAD2 bromodomain inhibitor with unprecedented chemical structure and mode of action. Amaury E. Fernández-Montalván,1 Markus Buka,2 Bipin K. Reddy,2 Sung-Joo Ko,1 Volker Badrock,1 Joerg Weiske,1 Simon J. Holton,3 Apirat Chaikud2,4 Laura Diaz-Sáez,5 James Bennett,6 Oleg Fedorov,7 Kilian Huber,8 Paolo Centrella,9 Matthew A. Clark,10 Christoph E. Dumelin,11 Eric A. Sigel11, Holly S. Souther,12 Dawn M. Troast,12 Ying Zhang,1 John W. Cuozzo,12 Anthony D. Keffe12,13 Didier Roche,14 Vincent Rodeschini15,16 Jan Hübner,17 Hilmar Weinmann,17 Ingo V. Hartung,17 Matyas Gorjanac16,17 Bayer Pharma AG, Berlin, Germany; 18Structural Genomics Consortium, Oxford, United Kingdom; 19X-Chem Pharmaceuticals, Waltham, MA; 20Edelris, Lyon, France.

ATAD2 (ATPase family AAA-domain containing protein 2, also called ANCA) is an epigenetic regulator that binds to chromatin through its bromodomain (BD), a motif specialized for acetyl-lysine recognition. ATAD2 directly interacts with DNA methyltransferase (DNMT) and differentially silences DNMT activity by recruiting histone deacetylases. ATAD2 regulates cell proliferation and apoptosis by promoting cell cycle progression and by recruiting and activating caspase 3. ATAD2 associates with multiple transcription factors such as ERE, AR, E2F, and Myc; hence, ATAD2 has been proposed to act as a co-factor for oncogenic transcription factors. Furthermore, we have recently reported a novel role for ATAD2 during DNA replication, uncovering interactions between ATAD2 and histone acetylation marks on newly synthesized histone H4. High expression of ATAD2 strongly correlates with poor patient prognosis in multiple tumor types, including gastric, endometrial, hepatic, polyp, ovarian, breast and lung cancers. However, the exact function of ATAD2 in these tumor types remains unclear. A more thorough validation of ATAD2 as a therapeutic target is hampered by the lack of isoform-selective, potent and cellically active ATAD2 inhibitors. A systematic assessment of crystal structures of BD-containing protein family predicted that
development of selective inhibitors of ATAD2 would be challenging. In line with this prediction, only limited progress in developing lead compounds targeting ATAD2 has been reported so far. A few notable exceptions relied on fragments as starting points, however, their weak potency, insufficient selectivity against other BDs, permeability limitations or modest cellular activity have curbed their further advancement towards drug candidates. Hence, we embarked on a preclinical strategy to identify ATAD2 inhibitors: 11 different DNA-encoded libraries adding up to 67 billion unique encoded compounds were combined and incubated with ATAD2 BD followed by two rounds of affinity-mediated selection. This approach provided with several series of binders, for which specific target engagement of their SMOL moiety upon off-DNA synthesis was confirmed in biochemical and biophysical assays. Several rounds of potency optimization led to the identification of BAY-850, a highly potent and ATAD2 (isoform A) mono-selective inhibitor, which holds an amine substituted 3-(2-furyl)benzamide core. This compound shows - as revealed by size exclusion chromatography and native mass spectrometry - a novel mode of action for a BD inhibitor based on specific target dimerization. In a cellular fluorescence recovery after photobleaching (FRAP) assay BAY-850 displaced wild-type ATAD2 from the chromatin to the same extent as the genetic mutagenesis of ATAD2 BD. In contrast, chemically very similar inactive control compounds showed no major effects on ATAD2 association with the chromatin. These results qualify BAY-850 as the first biologically active ATAD2 isoform A-specific chemical probe, which will enable further elucidation of the cancer biology of this intriguing protein.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Gene- and Vector-Based Therapy

#5085 SOCS-1 gene therapy combined with radiation therapy has a potent antitumor effect for esophageal squamous cell carcinoma. Takahito Sugase,1 Takuoyoshi Takahashi,1 Satoshi Serada,1 Minoru Fujimoto,2 Koji Tanaka,1 Yashihiro Miyazaki,1 Tomoki Makino,1 Yukinori Kurokawa,1 Makoto Yamashita,1 Kiyokazu Nakajima,1 Shuji Takiguchi,1 Masaki Mori,1 Yuichiro Doki,1 Tetsuji Naka,1 Osaka University, Suita, Japan; National Institutes of Biomedical Innovation, Health and Nutrition, Ibaraki, Japan

INTRODUCTION: Radiation therapy (RT) is one of the main strategies for esophageal squamous cell carcinoma (ESCC) besides surgery and chemotherapy. Although RT has shown remarkable clinical benefits for ESCC patients, there remains the resistance for RT. Recently, some reports show that STAT3 activation is induced by irradiation and can result in treatment-resistance for radiotherapy in various cancer. Suppressor of cytokine signaling-1 (SOCS-1) has been cloned as a negative regulator of various cytokine signaling. We previously reported that overexpression of SOCS-1 showed a potent anti-tumor effect for ESCC through targeting of JAK/STAT and FAK/ERK signaling pathway. Since these results, SOCS-1 might have a possibility to overcome the resistance of RT. The aim of this study is to evaluate the antitumor effect of SOCS-1 gene therapy using adenoviral vector (AdSOCS-1) combined with RT. METHODS: At first, we evaluated RT resistance by the STAT3 in ESCC cell lines by using pEB-Multi-constitutiveSTAT3(3-STAT3) vector. We examined the colony forming assay between parent (TE8, TE9, TE11, TE14) and stably expression of c-STAT3 cells. Next, we evaluated cell growth inhibition effect of AdSOCS-1 gene therapy combined with RT in vitro and in vivo. As in vivo model, we examined the combined effect of RT (2 Gy) and AdSOCS-1 gene therapy by using TE14 xenograft mice (ICR nu/nu mice). RESULTS: At first, ESCC cell with stably expression of c-STAT3 showed a significant increase in colony forming ability after RT as compared with parent and mock cell. It suggested that activation of STAT3 might be related to RT resistance in ESCC cell. Furthermore, ESCC cells with RT were induced the secretion of IL-6, and the activation level of STAT3 was elevated by multiple RT. In addition, they increased the expression of anti-apoptosis protein (mcl-1, survivin) and might have a RT resistance to the identification of BAY-850, a highly potent and ATAD2 (isoform A) mono-selective inhibitor, which holds an amine substituted 3-(2-furyl)benzamide core. This compound shows - as revealed by size exclusion chromatography and native mass spectrometry - a novel mode of action for a BD inhibitor based on specific target dimerization. In a cellular fluorescence recovery after photobleaching (FRAP) assay BAY-850 displaced wild-type ATAD2 from the chromatin to the same extent as the genetic mutagenesis of ATAD2 BD. In contrast, chemically very similar inactive control compounds showed no major effects on ATAD2 association with the chromatin. These results qualify BAY-850 as the first biologically active ATAD2 isoform A-specific chemical probe, which will enable further elucidation of the cancer biology of this intriguing protein.

#5087 Chronic radiation induced uPA expression mediates neuroblas- toma cells survival. Manu Gnanamony, Karen Fernández, Jaime Libes, Julian Lin, Pushpa A. Joseph, Christopher S. Gondi. Univ. of Illinois College of Med. at Peoria, Peoria, IL

Neuroblastoma accounts for more than 15% of cancer-related deaths of chil- dren under the age of five. Still, the current standard of care for long-term survival for these children remains less than 40%. The urokinase plasminogen activator (uPA) system plays a role in several critically important biology pro- cesses, such as cell adhesion, migration, proliferation and survival. This system includes uPA, a serine protease which converts a zymogen plasminogen into the active serine protease plasmin, its surface receptor uPAR/CD57, which binds uPA and plasmin, and a family of proteolytic activation of uPA precursor (uPA pro- curser - single-chain uPA, scuPA) into the active two-chain uPA protease (tucPA) and mediates uPA-induced activation of several intracellular signaling cascades, and protects against several inhibitors, e.g. PAI-1, among others. Re- cent findings demonstrate that dividing/proliferating cells translocate intact scuPA to the nucleus via the nucleocytoplasmic shuttle protein nucleolin. In this study we demonstrate that both SKNBe(2) and NB1691 neuroblastoma cells when exposed to chronic radiation suppresses nMYC gene copy number and induces the overexpression of CMYC and uPA expression levels without observ- able change in uPA gene copy number. Increased uPA expression was accom- panied with increase in the mitochondrial mass (4 fold in both NB1691 and SKNBE (2) cells). Knockdown of uPA expression in chronic radiation cells using shRNA resulted in reduced survival. RT-PCR results show that uPA knockdown resulted in reduced expression of CMYC and its target genes cyclin A and cyclin D1. Overexpression of uPA in both SKNBE(2) and NB1691 wild-type cells resulted in increased CMYC and cyclin D1 expression but not nMYC expression suggesting that CMYC may be specifically regulated by uPA. In conclusion, our study shows that uPA plays a vital role in survival of neuroblastoma cells treated with chronic radiation by associating with cMYC to compensate for loss of nMYC expression. Our study also implies that targeting uPA might be a viable option as a combination therapy in the treatment of high-risk neuroblas- toma.

#5087 Radiation enhances the intracellular delivery of anti-MGMT mor- pholin oligonucleotides (AMONs) to enhance the cytotoxicity of chemo- radiation in cancer cells. Prakash Ambady, Jeffrey Wu, Cymon Kersch, Dree- Anna Morris, Michael Pagel, Leslie L. Muildoon, Edward Neuwelt. OHSU, Portland, OR

Background: This study evaluates the use of a sub-lethal dose of ionizing radiation to guide and enhance the intracellular delivery of morpholino anti-sense nucleotides designed to block O6-methylguanine DNA methyltransferase (MGMT) to enhance the cytotoxicity of chemo-radiation therapy (CRT). MGMT is a DNA repair enzyme and its expression is a well-known mechanism for resistance to CRT in glioblastoma. Epigenetic suppression of the enzyme by promotor methylation is associated with better response and clinically relevant survival benefits after CRT. We designed anti-MGMT morpholino oligonucleo- tidies (AMON) using the stable vivo-morpholinos backbone. Intracellular de- livery of morpholinos, particularly in a manner that could be clinically feasible, continues to be a major challenge. We use sublethal ionizing radiation to en- hance the delivery of AMON to transiently reduced MGMT protein expression. We previously demonstrated that sublethal ionizing radiation enhances the de- livery of AMONs to reduce MGMT expression in human cancer cells (T98G glioma and H460 and A549 non-small cell lung cancer cell lines) in vitro as well as at the targeted knockdown of MGMT with systemically administered AMON- sin pre-irradiated tumors. We now test whether blocking MGMT will enhance the cytotoxicity of CRT, and investigate the mechanism by which radiation permits cellular uptake of AMONs. Methods: MGMT expressing, human T98G glioblastoma cells were used for all in-vitro studies. AMONs and non-specific fluorescent tagged control morpholinos were synthesized and purchased from Gene Tools, LLC (Philomath, OR, USA). High-resolution widefield microscopy with image deconvolution was performed to confirm the intracellular delivery. Cells with high expression of 1 Gy radiation; 24 hrs post radiation cells were treated with AMONs. Cells were then exposed to temozolomide (75 or 150 µg/mL) on day 3. Lysates were obtained on days 4 and 7. Expression of cellular MGMT protein, apoptosis markers, and key endo cytosis proteins were characterized using im- munoblotting. Results: Our results demonstrate that MGMT silencing using a single dose of AMONs delivered with sublethal dose of radiation increased ap- optosis by 50% and reduces cell viability by 50% at one and two days after therapy. Further, we demonstrate an increase in cavelin 1, clathrin and dyn- namin expression after radiation exposure. Conclusions: Our results suggest that blocking AMONs can enhance the cytotoxicity of CRT. Dynamin mediated endocytosis appears to play a key role in this enhanced cellular update of anti-
sense nucleotides after a sublethal dose of radiation. Since radiation is already one part of standard of care cancer therapy, the use of radiation to guide the delivery of systemically administered anti-sense nucleotides is a novel strategy to target key tumor associated proteins for therapeutic benefit key.

#5088 Tumor penetrating RNA delivery for therapeutic benefit of pancreatic cancer. Liangliang Hao, Justin Lo, Sangeeta Bhatia. MIT, Cambridge, MA.

Pancreatic cancer remains a deadly disease with a median survival of less than one year, in part because we lack effective therapeutic strategies. Mutations of the oncogene KRAS and the tumor suppressor genes TP53, INK4A, and DPC4 play important roles in the pathogenesis of pancreatic ductal adenocarcinoma (PDAC); however, these driver events have proven to be challenging targets for drug development. RNA interference (RNAi) is a powerful tool for silencing tumor suppressors and oncogenes, but delivery has the potential to have an impact on silencing 'undruggable' targets; however, siRNA delivery in tumors has been challenging in general and worsened in PDAC by the stromal barriers to drug delivery. Herein, we integrated biomedical engineering, materials science, and pre-clinical investigation to establish a novel class of tumor-penetrating nanoparticles (TPNs) to enhance delivery of siRNAs in PDAC for therapeutic benefit. Specifically, we adapted self-assembled nanoparticles comprised of siRNA complexed with tandem tumor-penetrating and membrane-translocating peptides, which enabled the specific delivery of siRNA deep into the tumor parenchyma. Specifically, to establish efficacious siRNA delivery vehicles, we characterized and selected tandem peptides that incorporate novel tumor-penetrating peptide domains known to target PDAC in mice. In parallel, we optimized the particle stability through incorporation of hydrophilic molecules, focusing on achieving desirable pharmacokinetic properties which will reduce off-target delivery and increase potency of a given siRNA dose. The resulting TPNs carry siRNA into the cytosol of monolayer cultures of tumor cells, and also exhibit penetration in pancreas organoids derived from primary mouse and human tumors. As a proof-of-concept, we used TPN in vivo to knockdown KRAS in a murine PDAC model, using subcutaneously-implanted, autochthonous KRAS G12D/+/p53−/− tumors. Treatment of tumor-bearing mice with systemically administered KRAS-specific TPNs suppressed the growth of established tumors. With the delivery platform established, the ongoing goals of this project are to identify siRNA targets that synergize with the suppression of oncogenic KRAS, and to validate synthetic lethal targets by interfering with signature PDAC driver events uncovered by functional genomic efforts. Together, these studies will provide a foundation for translational studies involving new targets and delivery technology in pancreatic cancer.

#5089 Antitumor activities elicited by direct intratumoral administration of a recombinant adenovirus expressing either IL-28A/IFN-α2 or IL-29/IFN-α. Munee Numasiritori, Hiroki Tsukamoto, Yoshishia Tomioka. Tohoku University, Sendai, Japan.

Interleukin (IL)-28A/IFN-α2 and IL-29/IFN-α1 have been demonstrated to elicet direct and indirect antitumor activities. In this study, we constructed an adenovirus vector expressing either IL-28A/IFN-α2 (AdIL-28A) or IL-29/IFN-α1 (AdIL-29) to evaluate the therapeutic properties of intratumoral injection of recombinant adenovirus to apply for the clinical implementation of cancer gene therapy. Despite the lack of antiproliferative effect on MCA205 and B16-F10 cells, a retarded growth of established subcutaneous tumors was observed following multiple injections of either AdIL-28A or AdIL-29 when compared with AdNull. In vivo cell depletion experiments displayed that both NK cells and CD8+ T cells play a major role in AdIL-28A-mediated tumor growth suppression. A significant increase in the number of infiltrating CD8+ T cells into the tumors treated with either AdIL-28A or AdIL-29 was observed. Moreover, specific antitumor cytotoxic T lymphocyte reactivity was detected in spleen cells from animals treated with either AdIL-28A or AdIL-29. In IFN-γ deficient mice, the antitumor activity of AdIL-28A was completely impaired, indicating that IFN-γ is critically involved in the tumor growth inhibition triggered by AdIL-28A. IL-12 provided an additive antitumor effect when combined with AdIL-28A. These results indicate that AdIL-28A and AdIL-29 could be successfully utilized as an alternative cancer gene therapy.

#5090 Efficient KO of PD1 into primary T cells using a new non-integrative lentiviral particle expressing CRISPR/Cas9 system. Pascale Bouillé, Réég Gayon, Lucille Lamouroux, Alexandra Iche, Christine Dupont, Jean Christophe Pages, VECTALYS and FLASHCELL, Toulouse, France; INSERM U966 Faculté de Médecine, Tours, France.

Gene editing by the CRISPR system shows great promises for gene therapy. Nevertheless, it must now face a number of challenges especially for the development of safe and efficient delivery tools for in vivo, as well as ex vivo gene editing. Cas9 and sgRNA delivery, mediated either by viral vectors (AAV- or Lentivirus-derived) or transcription protocols (chemical or by electroporation) have been largely and efficiently used but they bring major drawbacks incompatible with clinical applications. Indeed, viral vectors can display uncontrolled off-target integrations and protocols for microinjection may cause cell toxicity and/or phenotype modifications of the target cells. Moreover, the CRISPR technology entails a “hit-and-run” mechanism that only requires a transient expression of the nuclease complex. Therefore, achieving an efficient delivery into hard-to-transfect cells, such as T cells, remains challenging and the need for delivery tools that would allow efficient transfer on most cell types remains relevant. RNA interference through small interfering RNA (siRNA) delivery has the potential to have an impact on silencing 'undruggable' targets; however, siRNA delivery in tumors has been challenging in general and worsened in PDAC by the stromal barriers to drug delivery. Herein, we present an innovative tool, named LentiFlash, allowing RNA delivery into target cells without any genomic scar. The RNA encapsidation is mediated via an RNA/protein interaction: the respective properties of the MS2 bacteriophage and the lentiviral vectors have been combined to build a non-integrative packaging system in which the wild type HIV packaging sequence is replaced by the MS2 stem-loop repeats and the MS2 coat protein is inserted into the Nucleocapsid. This new vector breaks with all existing systems, as the resulting lentiparticle is able to deliver non-viral coding or non-coding RNA, at high efficiency, into the cytoplasm of any cell type. Transduction of a large range of cells, from immortalized cells to dedicaly primary cells, such as T cells and hematopoietic stem cells, with LentiFlash shows an efficient, fast and transient delivery into primary T cells and into primary hematopoietic cells. In particular, LentiFlash particles were successfully used to deliver Cas9, alone or in association with an sgRNA not only targeting a reporter gene into immortalized cells, but outstandingly knocking-out the PD-1 gene into primary human T lymphocytes. This new RNA delivery system is an efficient and safe tool for the delivery of CRISPR editing machinery in most cell types without affecting cell viability and phenotype. The transient, RNA-based mechanism of LentiFlash vector, preventing the risk of integration, associated with its ability to utilize lentiviral production platforms already validated in clinical settings, make it a promising tool for CRISPR therapeutic applications. As a matter of fact, beyond gene editing efficiency, safety of delivery tools is a major concern that should be addressed to move forward with CRISPR clinical development.

#5091 Activity of Bcl-2 antisense therapeutic in aggressive non-Hodgkin’s lymphoma. Ana Tari Ashizawa,1 Yolanda Gutierrez-Puente,2 Richard Ford,3 Gabriel Lopez-Berestein, Bio-Path Holdings, Bellaire, TX; Universidad Autonoma de Nuevo Leon, Monterrey, Mexico; University of Texas MD Anderson, Houston, TX.

Aggressive non-Hodgkin’s lymphoma (NHL) progresses rapidly. It makes up about 60% of all NHL cases in the United States. The chemotherapy regimen R-CHOP could cure up to 70% of patients with aggressive NHL. Nonetheless, up to 30% of patients with aggressive NHL relapse from R-CHOP within 2 years of initial treatment. Novel therapeutics are urgently needed for patients with relapsed, aggressive NHL. Bcl-2 is a potential therapeutic target for aggressive NHL because high expression of Bcl-2 has been reported in NHL and correlated with adverse prognosis for NHL patients. To inhibit the expression of Bcl-2 protein, BP1002 was developed. BP1002 is comprised of an uncharged P-ethoxy antisense oligodeoxynucleotide targeted against Bcl-2 mRNA. The P-ethoxy antisense backbone does not have an adverse effect on bleeding and complement activation, which are some of the toxicities that have been reported for other antisense analogs. The Bcl-2 antisense is incorporated in neutral liposomes so that it can be delivered systemically via intravenous infusion. The inhibitory activity of BP1002 was determined in human lymphoma cell lines, which included germinal center B-cell (GCB) diffuse large B cell lymphoma (DLBCL), activated B-cell (ABC) DLBCL, mantle cell lymphoma (MCL) and Burkitt’s lymphoma (BL). The lymphoma cell lines were incubated with BP1002 for four days. The sulforhodamine B cytotoxicity assay showed that BP1002, at 200 micrograms/mL, induced ≥50% inhibition in 5 of 8 GCB DLBCL lines, 3 of 3 ABC DLBCL lines, 1 of 1 MCL line, and 1 of 1 BL line. We also determined the activity of BP1002 in animal models. In athymic nude mice, mice were implanted with C3H cells, which are transformed follicular lymphoma cells that express high levels of Bcl-2 protein. In experiment I, 4 groups of mice were used: Group 1 - untreated mice; Group 2 - mice treated with 10 mg of BP1002 per kg of mouse body weight; Group 3 - mice treated with 20 mg of BP1002 per kg of mouse body weight; Group 4 - mice treated with control empty liposomes equivalent. Three days after tumor cell injection, mice were injected with BP1002 or empty liposomes intravenously twice per week. When mice were euthanized in week 5, all untreated mice and mice treated with control empty liposomes reached moribund state. However, no mouse in the BP1002 treatment groups reached moribund state. In experiment II, 3 groups of mice
were used: Group 1 - untreated mice; Group 2 - mice treated with 20 mg/kg of liposome-encapsulated control oligodeoxynucleotide; Group 3 - mice treated with 20 mg/kg of BP1002. All untreated mice and control mice were moribund in week 5, but only 40% of BP1002-treated mice were moribund. These in vivo experiments indicate that BP1002 could extend the survival of mice bearing aggressive NHL. Together, our data indicate that BP1002 is a novel therapeutic for aggressive NHL.

#5092 ZVex lentiviral vector strongly activates pro-inflammatory, antigen processing, and anti-viral defense response pathways in monocyte-derived dendritic cells. Anshika Bajaj, Lisa Y. Ngo, Peter Berglund, Jan ter Meulen. Immune Design, Seattle, WA.

Background: Dendritic cells (DCs) are professional antigen presenting cells that effectively bridge the innate and adaptive immune responses and require activation for successful priming of naïve T-cells. We have developed ZVex, an integration deficient, hybrid lentiviral vector engineered to target DC-SIGN expressed on immature myeloid DCs for genetic immunization against tumor antigens. As lentiviruses normally don’t efficiently infect DCs, little is known about the functional effect of LV transduction of conventional DCs. Here, the effect of DC transduction with ZVex vectors was studied by gene expression profiling. Material and Methods: Human monocytes from multiple donors were prepared by 5-day stimulation with IL-4 and GM-CSF and transduced with ZVex-GFP or control vectors, such as a ZVex with nonfunctional reverse transcriptase (RT-dead), ZVex particles generated to contain no vector genome (empty ZVex), and a heat-inactivated ZVex preparation. Cellular RNA was isolated at different time points from transduced DC cultures and gene expression profiling with Nanostring’s human pan cancer immune panel (770 genes). Results: DCs transduced with ZVex vectors displayed statistically significant up-regulation of genes involved in pro-inflammatory, antigen processing, and anti-viral defense pathways. Noteworthy among the up-regulated genes were classical anti-viral response genes like OAS3, MX1, and interferon stimulated genes like ISG15 and ISG20. Incubation with the RT-dead mutant vector also led to up-regulation of these genes, albeit to a much lower extent, suggesting that LV-RNA itself can result in the activation of these pathways in DCs, though reverse transcription appears to further potentiate the response. Of note, cells transduced with empty ZVex, which consists of an intact virion particle but lacks encapsulated LV RNA, also mediated a low magnitude and breadth of DC transcriptional activation, possibly via signaling through DC-SIGN. Conclusions: ZVex incorporates several elements capable of inducing a potent innate immune activation in DCs. DCs are relatively insensitive to lentiviral infection, but the use of accessory proteins in ZVex makes DCs conducive to ZVex transduction and results in the induction of a Type-I IFN response that seems to be largely dependent on reverse transcription. In addition, other engineered vector particle components such as non-reverse transcribed viral RNA, viral structural proteins, and/or engagement of the DC-SIGN receptor, also contribute to DC activation.

#5093 In vitro, in vivo and molecular effects of stromal selective targeting by uPAR targeted oncogenic myeloid viruses in breast cancer progression. Yuqi Jing, Valery Chavez, Nicolas Acquavella, Doraya El-Ashry, Yuguang Ban, Xi Chen, Jaime Merchán. Univ. of Miami Sylvester Comp. Cancer Ctr., Miami, FL.

The tumor microenvironment is a relevant target for novel biological therapies. While it has been demonstrated that tumor-stromal cell interactions are important in the sensitivity of the cancer cells to oncolytic viruses, few studies have investigated the direct effects of an oncolytic virus on the stroma, and its implications on the virus antitumor efficacy. MV-m-uPA and MV-h-uPA are fully targeted, species specific, oncolytic myeloid viruses directed against murine or human urokinase receptor (uPAR). The effects of stromal selective targeting by uPAR targeted MVs were investigated. In vitro infection, gene expression and cytopathicity by MV-h-uPA and MV-m-uPA were demonstrated in human and murine cancer cells and cancer associated fibroblasts in a species specific manner. In murine fibroblast (3T3)/human breast cancer (T47D) 3-D co-cultures, selective fibroblast targeting by MV-m-uPA inhibited breast cancer cell growth. These effects were validated in vivo, in a human breast cancer xenograft (MDA-MB-231), where intravenous administration of the murine specific MV-m-uPA led to significant tumor growth delay and improved survival compared to controls. Experiments comparing the effects of tumor (MV-h-uPA) vs. stromal (MV-m-uPA) vs. combined treatment showed that tumor and stromal targeting was associated with improved tumor control. Correlative studies demonstrated in vivo tumor targeting, increased apoptosis, and MV-m-uPA induced differential regulation of both stromal (murine) genes and cancer (human) genes associated with inflammation, angiogenesis and survival, among others, indicating viral induced modulation of tumor-stromal interactions. Our results demonstrate for the first time the feasibility of stromal selective targeting by an oncolytic MV, viral induced modulation of the tumor microenvironment, and subsequent tumor growth delay. These findings further validate the critical role of stromal uPAR in cancer progression and the potential of oncolytic viruses as anti-stromal agents.

#5094 Precision therapeutic targeting of oncogenic FGFR3 fusions using CRISPR-Cas9 genome-editing in urothelial cancer. Bishop M. Faltas, Rebecca Meyer, Ethan Shelkey, Charles J. Murphy, Olivier Elemento, Mark A. Rubin. Weill Cornell Medical College, New York, NY.

Introduction: Fibroblast-growth factor receptor factor 3 (FGFR3) fusions occur in up to 11% of urothelial carcinomas. This fusion drives ligand-independent receptor dimerization and downstream oncogenic signaling and thus represent attractive targets for gene therapy. We hypothesized that CRISPR-Cas9 gene-editing can be used to disrupt these somatic oncogenic fusions in cancer cells. Methods: RT112 and SW780 urothelial cancer cell lines harboring genomic fusions of FGFR3 to TACC3, or BAIA2P2L1, respectively were grown in cultures. The fusion sequences were confirmed by Sanger sequencing and the NGG protospacer adjacent motif necessary for guide RNA (gRNA) targeting were identified. Unique guide RNAs spanning the fusion sequences in each cell line were designed. An all-in-one lentiviral vector was designed to deliver both the gRNA and the Cas9 nuclease. A scrambled non-targeting gRNA vector was used as a control. Following transduction, genomic DNA was extracted and PCR was performed using primer sequences flanking the fusion sequences followed by targeted sequencing on the Illumina MiSeq platform. CrispRP variants and CRISPResso bioinformatic tools were used to determine editing efficiency. Results: In SW780 cells transduced with the FGFR3-BAIA2P2L1 fusion-targeting gRNA vector, 64229/207425 (23.6%) reads harbored CRISPR-induced edits of the fusion sequence compared to only 2009/315353 (0.6%) reads in the scrambled gRNA vector-transduced cells. Similarly, in RT112 cells transduced with the FGFR3-TACC3 fusion-targeting gRNA vector, 74292/448381 reads (14.2%) contained random indels consistent with CRISPR-induced edits compared to 17785/428035 reads (4.0%) in RT112s cells transduced with the scrambled gRNA vector. In the SW780 cell line, the most common indel in CRISPR-edited fusions sequences was a deletion of a cytosine occurring in 40% of edited reads. The second most common indel was an extra cytosine insertion and the third most common was a 13-base deletion spanning the fusion point. In RT112 cell line, the most common indel was an insertion of a cytosine in 13% of reads. Sanger sequencing of the fusion sequences from SW780 single cell clones confirmed MiSeq results. Experiments determining the effects of these indels on the function of the fusion protein and on the cancer phenotype are ongoing. Conclusions: To our knowledge, this is the first report of CRISPR/Cas9 use as a therapeutic strategy for targeting oncogenic fusions. Because the fusion sequences unique to each patient’s cancer, this approach can be potentially used for in-vivo gene therapy for urothelial carcinomas harboring FGFR3 fusions with no off-target edits in normal cells. This approach represents a significant advance towards precision gene-therapy by specifically targeting fusion sequences unique to each patient’s cancer. We envision extending this approach to several solid and hematological malignancies driven by oncogenic fusions.

#5095 Development of a pharmacodynamic biomarker assay for AZD4785, an antisense oligonucleotide targeting KRAS. Claire Rooney, Sarah Ross, Anna Staniszewska, Sabine Lenarz, Elaine Kilgour, Alexey S. Revenko, Robert Macleod, Andrew Pierce, Paul D. Lyne, Carl Barrett, Elizabeth A. Harrington. #1AstraZeneca, Cambridge, United Kingdom; #2Astrazeneca, Wilmington, MA.

AZD4785 is a potent and selective high affinity 2'-4' constrained ethyl resi- dues (ce) containing therapeutic antisense oligonucleotide (ASO) targeting KRAS. In a patient-derived explant model of non-small cell lung cancer (NSCLC) carrying a KRAS G12C mutation systemic dosing of AZD4785 resulted in tumor regression. To determine the level of KRAS mRNA knockdown achieved by AZD4785 at a dose which causes regression (250 mg/kg q1w), tumor samples were collected and analyzed by RT-PCR. Mean levels of KRAS mRNA knockdown relative to the PBS control group were determined to be 61% and 78% from studies dosed for 2 and 4 weeks, respectively. However, by RT-PCR there was significant variability in KRAS mRNA levels within the PBS control group, and level of knockdown varied with different housekeeper normalization genes. In early clinical studies it will be essential to measure KRAS expression in tumor samples as a pharmacodynamic marker for AZD4785, hence we sought to develop a more robust assay compatible with FFPE clinical samples with low RNA yields. KRAS mRNA levels were quantified by the nanoString platform, using the panCancer pathways 770 gene code set. This assay generated robust
data from RNA extracted from 2 × 5 μm sections of FFPE material from each sample, despite very limited yields of RNA. AZD4785 treatment resulted in comparable levels of KRAS knockdown to the RT-PCR assay, but assay variability was greatly reduced, from stdev >50% (RT-PCR) to 5% (nanoString) for PBS control group samples. Using the nanoString assay, mean KRAS mRNA knockdown was 32% and 68% (p < 0.005). We also analyzed the effect of AZD4785 on the wider 770 gene panel. Further evidence of KRAS mRNA knockdown was observed through significant downregulation of transcriptional targets of KRAS signalling (FOSL1, DUSP5, DUSP6) relative to the PBS control group. Neither HRAS nor NRAS levels were affected by AZD4785 treatment.

Targeted ablation of essential oncogenes in rhabdomyosarcoma

NG-348 is a transgene-modified variant of enadenotucirev, a chimeric oncoprotein group B adenovirus with potent and selective anti-tumor activity against a range of epithelial cancer cells. Enadenotucirev has a blood stability profile that enables systemic dosing and has been administered intravenously to over 90 cancer patients. These studies have demonstrated that IV dosed enadenotucirev is delivered to tumors and subsequent virus activity is associated with CD8+ T-cell infiltration in tumor cells, consistent with immune stimulation within the tumor. NG-348 encodes two immunomodulatory proteins in its genome: full-length human CD80, and a membrane anchored single chain variable fragment of the mouse anti-human CD3e monoclonal antibody OKT3. Together these membrane proteins provide both T-cell receptor (signal 1) and costimulatory (signal 2) activation signals required to polyclonally activate tumor-infiltrating T-cells. When expressed on the surface of NG-348 infected tumor cells the transgenes therefore enhance the potency of the virus by driving local T-cell immune responses selectively in the tumor microenvironment. The expression of both transgenes encoded in the NG-348 virus is controlled by the endogenous virus major late promoter. This restricts expression of the proteins to the surface of cells permissive to virus infection (i.e. tumor cells) and prevents off-target expression in the cells from healthy tissues. Using co-cultures of human T-cells with human tumor cell lines, we have shown that NG-348 infected tumor cells potentially activate both CD4 and CD8 T-cells. This was demonstrated by analysis of activation marker expression (CD25, CD69), intracellular and secreted cytokines (IL-2, TNF, IFNγ) and induction of T-cell mediated tumor cell death by apoptosis (prior to oncolytic death by the virus). Treatment of different human non-tumor cells (e.g. fibroblasts, T-cells, PB-MCs) with NG-348 did not lead to transgene protein expression or activation of T-cells in co-cultures. NG-348 has also been shown to activate human T-cells in vivo, using a human tumor xenograft model system in immunodeficient mice reconstituted with human PB-MCs. Collectively these data indicate that following delivery to tumor tissues of patients, NG-348 oncolytic virus can selectively replicate and express its payload of T-cell activating ligands. NG-348 should therefore stimulate potent antigen-independent, polyclonal activation of T-cells already present in the tumor, as well as those recruited into the tumor in response to the virus infection, to drive effective anti-tumor immunity. NG-348 is currently in preclinical development with a first phase I study planned to initiate in Q4 2017.


NG-348 is a transgene-modified variant of enadenotucirev, a chimeric oncoprotein group B adenovirus with potent and selective anti-tumor activity against a range of epithelial cancer cells. Enadenotucirev has a blood stability profile that enables systemic dosing and has been administered intravenously to over 90 cancer patients. These studies have demonstrated that IV dosed enadenotucirev is delivered to tumors and subsequent virus activity is associated with CD8+ T-cell infiltration in tumor cells, consistent with immune stimulation within the tumor. NG-348 encodes two immunomodulatory proteins in its genome: full-length human CD80, and a membrane anchored single chain variable fragment of the mouse anti-human CD3e monoclonal antibody OKT3. Together these membrane proteins provide both T-cell receptor (signal 1) and costimulatory (signal 2) activation signals required to polyclonally activate tumor-infiltrating T-cells. When expressed on the surface of NG-348 infected tumor cells the transgenes therefore enhance the potency of the virus by driving local T-cell immune responses selectively in the tumor microenvironment. The expression of both transgenes encoded in the NG-348 virus is controlled by the endogenous virus major late promoter. This restricts expression of the proteins to the surface of cells permissive to virus infection (i.e. tumor cells) and prevents off-target expression in the cells from healthy tissues. Using co-cultures of human T-cells with human tumor cell lines, we have shown that NG-348 infected tumor cells potentially activate both CD4 and CD8 T-cells. This was demonstrated by analysis of activation marker expression (CD25, CD69), intracellular and secreted cytokines (IL-2, TNF, IFNγ) and induction of T-cell mediated tumor cell death by apoptosis (prior to oncolytic death by the virus).
Therefore better drug formulations are desirable. Methods: To this end, we developed a new conceptual fluoropyrimidine, DFP-11207 that is engineered to reduce toxicity without loss of antitumor activity as well in addition to providing sustained concentrations of the active anticancer moiety. DFP-11207 contains three components in one formulation: 1-ethoxymethyl-5-fluorouracil (EM-FU) as a prodrug of 5-FU; 5-fluoropyrimidine (FP) as a metabolic inhibitor of thymidylate synthase and cytidine monophosphate (CTA) as a gastrointestinal regulator of 5-FU phosphorylation. Results: In in vitro metabolism studies using cell-free extracts from plasma, liver and tumors or intact tumor cells, DFP-11207 was rapidly hydrolyzed to 3 components and subsequently EM-FU was specifically converted to 5-FU by liver microsomes, and CDHP and CTA strongly inhibited 5-FU degradation and phosphorylation, respectively. Following consecutive oral administration to human tumor-bearing nude rats, DFP-11207 attained favorable antitumor efficacy and long-sustained PK profiles with lack of GI- and myelo-toxicity. Next, in investigational clinical study in patients with solid tumors, 12 patients were treated at 8 unique dose levels of DFP-11207, ranging from 40 to 440mg/m2/day by each 1 pt. MTD and RD of daily and 28-day consecutive DFP-11207 was found to be 440 (n=2) and 330 mg/m2 (n=6), respectively. The main AEs were nausea, anemia, neutropenia, febrile neutropenia but these events were very mild, and no thrombocytopenia was observed as expected. Furthermore, review of the preliminary PK data, DFP-11207 at 330 mg/m2 resulted in a desirable low but efficacious (≈15-30 mg/ml) steady-state plasma concentration of 5-FU. Interestingly, some of patients heavily treated with therapeutic drugs had a stable-disease for long periods. Conclusion: Our preclinical and early clinical data suggest that DFP-11207 is a promising compound for the treatment of gastrointestinal cancers and can overcome the shortcomings of all other oral fluoropyrimidines.

#5100 AB61, a new potent nucleoside cytostatic: Molecular mechanisms of action and preclinical activity. Petr Dzubak,1 Marian Hajduch,2 Pavla Perlikova,2 Gabriela Rylova,2 Petr Naus,3 Tomas Elbert,1 Eva Tlustostra,1 Aurelie Bourdieroux,1 Lenka Slavetskinska,1 Kamli Motyka,1 Dalibor Dolezal,2 Pavel Zavojek,1 Alex Nowa,1 Monika Havranova1, Michal Silner1, Jan Hava1, Michal Hocke2,1 Palacky University in Olomouc, Faculty of Medicine and Dentistry, Olomouc, Czech Republic;2Institute of Organic Chemistry and Biochemistry of the CAS, Prague, Czech Republic;3Palacky University in Olomouc, Faculty of Natural Sciences, Olomouc, Czech Republic.

7-(2-Thienyl)-7-deazadenosine (AB61) showed nanomolar cytotoxic activities against various cancer cell lines but only micromolar activities against normal fibroblasts. The selectivity of AB61 was found to be due to inefficient phosphorylation of AB61 in normal fibroblasts. The phosphorylation of AB61 in the leukemic CCRF-CEM cell line proceeds well and it was shown that AB61 is incorporated into both DNA and RNA, preferentially as a ribonucleotide. It was further confirmed that a triphosphate of AB61 is a substrate for both RNA and DNA polymerases in both DNA and RNA, preferentially as a ribonucleotide. It was further confirmed that a triphosphate of AB61 is a substrate for both RNA and DNA polymerases in both DNA and RNA, preferentially as a ribonucleotide. It was further confirmed that a triphosphate of AB61 is a substrate for both RNA and DNA polymerases in both DNA and RNA, preferentially as a ribonucleotide. It was further confirmed that a triphosphate of AB61 is a substrate for both RNA and DNA polymerases in both DNA and RNA, preferentially as a ribonucleotide. It was further confirmed that a triphosphate of AB61 is a substrate for both RNA and DNA polymerases in both DNA and RNA, preferentially as a ribonucleotide. It was further confirmed that a triphosphate of AB61 is a substrate for both RNA and DNA polymerases in both DNA and RNA, preferentially as a ribonucleotide. It was further confirmed that a triphosphate of AB61 is a substrate for both RNA and DNA polymerases in both DNA and RNA, preferentially as a ribonucleotide. It was further confirmed that a triphosphate of AB61 is a substrate for both RNA and DNA polymerases in both DNA and RNA, preferentially as a ribonucleotide. It was further confirmed that a triphosphate of AB61 is a substrate for both RNA and DNA polymerases in both DNA and RNA, preferentially as a ribonucleotide. It was further confirmed that a triphosphate of AB61 is a substrate for both RNA and DNA polymerases in both DNA and RNA, preferentially as a ribonucleotide. It was further confirmed that a triphosphate of AB61 is a substrate for both RNA and DNA polymerases in both DNA and RNA, preferentially as a ribonucleotide. It was further confirmed that a triphosphate of AB61 is a substrate for both RNA and DNA polymerases in both DNA and RNA, preferentially as a ribonucleotide. It was further confirmed that a triphosphate of AB61 is a substrate for both RNA and DNA polymerases in both DNA and RNA, preferentially as a ribonucleotide. It was further confirmed that a triphosphate of AB61 is a substrate for both RNA and DNA polymerases in both DNA and RNA, preferentially as a ribonucleotide. It was further confirmed that a triphosphate of AB61 is a substrate for both RNA and DNA polymerases in both DNA and RNA, preferentially as a ribonucleotide. It was further confirmed that a triphosphate of AB61 is a substrate for both RNA and DNA polymerases in both DNA and RNA, preferentially as a ribonucleotide. It was further confirmed that a triphosphate of AB61 is a substrate for both RNA and DNA polymerases in both DNA and RNA, preferentially as a ribonucleotide. It was further confirmed that a triphosphate of AB61 is a substrate for both RNA and DNA polymerases in both DNA and RNA, preferentially as a ribonucleotide. It was further confirmed that a triphosphate of AB61 is a substrate for both RNA and DNA polymerases in both DNA and RNA, preferentially as a ribonucleotide. It was further confirmed that a triphosphate of AB61 is a substrate for both RNA and DNA polymerases in both DNA and R...
diotherapy and adjuvant temozolomide (TMZ) chemotherapy. However, inherit-
ent- and acquired resistance to TMZ present major obstacles to successful treat-
ment, and the prognosis of patients with malignant gliomas remains very poor.
MJ-66, a synthetic quinoxalone compound, was identified in our study as a potent
anti-proliferative agent especially on human glioma with IC50 of approximately
60 nM. The intracranial glioma xenograft model, MJ-66 (0.1 mg, i.p., twice daily,
q.d., 10 days) significantly inhibited tumor growth and increased the survival of
the experimental mice, however, did not decrease the body weight of mice.
Currently, there is a profound unmet medical need for a new drug in the treat-
ment of malignant brain tumor. The MJ-66 demonstrated superior efficacy to
TMZ that are the only first-line drug in clinical use. MJ-66 is suggested to be a
promising anticancer candidate.

#5104 A novel N-mustard-quinoline conjugate is a potent agent against
colorectal cancer. Tai-Lin Chen,¹ Yan-Bo Chen,² Chia-Ning Shen,² Tsann-
Long Su,³ Te-Chang Lee.¹ 1. Institute of Biomedical Sciences, Academia Sinica and
Institute of Biochemistry and Molecular Biology, National Yang-Ming University,
Taipei, Taiwan; ²Genomics Research Center, Academia Sinica, Taipei, Taiwan;
³Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan.

Colorectal cancer (CRC) is the third leading cancer worldwide. The prognosis of
diseased Patients with CRC at the stage IV is poor with a 5-year survival rate
less than 10%. Combination chemotherapy with platinum-based drugs and
5-fluorouracil (5-FU) remains the first line treatment, while the chemotherapy
combined target therapeutic agents were decreasing the mortality of CRC pa-
tients. However, the efficacy of platinum-based drugs has often been limited because
of the substantial risk for severe toxicities, including nephrotoxicity and
neurotoxicity. Therefore, there is an unmet need to develop a novel drug for
improving the treatment of advance colorectal cancer. We have previously de-
signed and synthesized several series of DNA-directed alkylating agents with
potent antitumor activity. Recently, one derivative of N-mustard-quinoline con-
jugates with hydrazinecarboxamide as a linker, designated as SL-1, was selected
to explore its anti-colorectal cancer (CRC) activity because it was highly cyto-
toxic to a panel of CRC cell lines in culture. Flow cytometric analysis showed that
treatment of CRC cells with SL-1 induced G2/M arrest and triggered apoptosis.
We further demonstrated that SL-1 preferentially targeted on the selective gau-
nine sequence by sequencing gel electrophoresis. By the aid of comet assay, SL-
1 in combination with 5-FU resulted in increased DNA tail moments and induced
more DNA damage. Evaluation of the potency of antitumor activity both in
culture and in xenograft models, we observed that SL-1 was more potent than
5-FU and oxaliplatin against SW620, RKO and RKO-E6 (an oxaliplatin resis-
tant-subsline) cells. Notable, SL-1+5-FU were more efficacious than oxalipla-
tin + 5-FU in suppression the growth of RKO or SW620 in xenograft models. We
further found that SL-1 enhanced 5-FU and induced more apoptosis by c-caspases 3 and 4/7 and c-FLICE staining. Additionally, histopathological examination
showed that SL-1 by itself has no obvious kidney and liver toxicity in ICR mice
comparing to cisplatin, oxaliplatin or 5-FU. Taken together, SL-1 alone or in
combination with 5-FU may warrant our further development as a potential antitu-
mor agent for the treatment of CRC patients.

#5105 Structure-activity relationship study of pyrrolo[2,3-d]pyrimidines
as microtubule targeting antitumor agents. Aleem Gangjee,¹ Tasdique M.
Quader.¹ 1. Dupuisue University, Pittsburgh, PA; ²University of Texas Health Science Center at San Antonio, San Antonio, TX.

Microtubules are cytoskeleton protein polymers composed of β-tubulin het-
erodimers which play pivotal roles in cell division, cellular transport and cell
signaling. Interfering with microtubule dynamics is a well-established strategy
for the treatment of cancer. However, clinical utility of microtubule targeting
agents (MTAs) in cancer chemotherapy is often limited by the emergence of
drug resistance. Expression of P-glycoprotein (Pgp) or the βII1 isotype of tubu-
lin, are two clinically established drug resistance mechanisms. We previously rep-
orted that compound AG346 binds to the colchicine site of tubulin and has
microtubule depolymerization activity in A-10 cells (EC50= 365 nM). Molecu-
lar modeling and docking studies of this compound in the X-ray crystal structure
of tubulin (PDB: 402B) suggested that the pyrrole NH can be substituted with
alkyl chains of optimal length to obtain an altered binding conformation which
can facilitate interaction with the hydrophobic pocket formed by amino acidic
Val181 of side chain A, and Lys352 and Thr314 of side chain B. We designed and
synthesized a variety of pyrrole NH-alkyl substituted compounds and deter-
mined that the compound AG73 shows a 43-fold improved microtubule depo-
lymerization potency (EC50= 8.4 nM) in A-10 cells, compared to the lead
compound. In addition, this compound inhibited the growth of human mela-
noma cancer cell line MDA-MB-435 in culture with an IC50 of 7.2 nM, which is a
5-fold improvement in potency over the parent compound. These analogs also

circumvented resistance mediated by Pgp and βIII-tubulin. These attributes provide the impetus for further preclinical development of these compounds as
microtubule targeted antitumor agents in vivo and these studies are currently
underway.

#5106 In vitro and in vivo evaluation of WAC-224, a novel quinolone class
of topoisoerase II inhibitor for cancer therapy. Tai-Chi Ueshima, Tomonomi
Yamaguchi, Kenji Itoh, Naoki Kashimoto, Tatsuya Hirano, Rumiko Shimabara,
Yohei Kawakubo, Masayuki Sato, Junpei Yamashita, Akira Yazaki, Koichi Ta-
Introduction: DNA topoisoerases (Topo) are classical but still attractive
targets for drug therapy in multiple types of cancers. Topo inhibitors, such as
Etoposide and Daunorubicin, have been effectively used; however, their clinical use is often limited by drug resistance in cancer cell population. Therefore, the
development of a novel chemical class of Topo inhibitors has been desired to
overcome drug resistance. Recently, Vosaroxin (QINPREZO) is identified as a
first-in-class anti-cancer quinolone derivative targeting Topo II, which is under
development for relapsed or refractory acute myeloid leukemia (AML). In this
study, we evaluated the in vitro and in vivo activities of WAC-224, a novel
quinolone derivative for Topo II inhibition, for various cancer cell lines includ-
ing human multi-drug resistant cells and in mouse xenograft models, respectively.
Materials and Methods: In vitro anti-proliferative activities against over 20 cell lines
were determined using WST cell proliferation reagent. Apoptosis was measured by
Annexin-V staining. In vivo anti-tumor activity was determined in immuno-
deficient mice bearing multi-drug resistant human uterine sarcoma MES-SA/
DX5 xenografts. MJ-66 is suggested to be a promising anticancer candidate.

Melanoma is the deadliest form of skin cancer due to its propensity for metas-
tasizing. It accounts for about 80% of skin cancer-related deaths. A substitution
of valine for glutamic acid at position 600 results in the B BRAF V600E mutation found
in approximately 50% of melanomas. The B BRAF V600E C mutation drives activation
of MEK/ERK and PI3K/AKT signaling and also cooperates with the PI3K/AKT pathway, thus
facilitating tumor initiation and metastasis. Fisetin is a naturally occurring
flavonoid found in fruits, vegetables, nuts, and wine, exhibits anti-proliferative, anti-inflammatory, and
anti-tumorigenic properties. Earlier, we showed that fisetin treatment downregulates
the PI3K/akt and reduces phosphorylation of MEK/ERK in melana-
oma. In this study we determined the effects of fisetin on tumor growth and
metastasis in a genetically engineered transgenic mouse model of metastatic
melanoma. Melanocyte specific Cre activity was induced in six-week-old B BRAF V600E/PtenΔN/CreB mice by topical application of 4-hydroxytamoxifen
on shaved backs once per day for 3 consecutive days. Mice were observed daily
until a highly pigmented measurable tumor appeared. Size of the tumors was
measured twice weekly and treatment with fisetin (45mg/kg b.wt. & 90mg/kg
b.wt.) was initiated at the time the tumors reached 100 mm3. Five groups were
incorporated: (i) untreated controls, (ii) doxorubicin treated controls, (iii) fisetin
and (iv) fisetin treated and doxorubicin treated. Results showed that fisetin
untreated tumor xenografts in mice with or without doxorubicin treatment,
the tumors were significantly smaller in the fisetin-treated mice compared to the
control group. Furthermore, fisetin was shown to inhibit the growth of tumors,
but not to reduce their weight. The histological analysis of tumor samples showed
that fisetin administration inhibited melanoma growth in these mice as compared to the control group. Hematoxylin and eosin staining revealed that fisetin treatment reduced pig-
mented cells in the ear and skin. In addition, fisetin treatment reduced the
metastasis of melanoma cells into the spleen and draining lymph nodes. Fur-
thermore, evaluation of tumor tissues revealed that fisetin treatment reduced the
(i) expression of PI3K,akt and p85, (ii) phosphorylation of AKT at Ser 473 and
Thr 308, (iii) phosphorylation of mTOR at Ser 2481, and (iv) expression of p53
and AR. In addition, fisetin treatment reduced the phosphorylation of MEK and
ERK when compared to control tumor samples. Fisetin treatment also re-

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sulted in (i) cleavage of caspase-3, (ii) inhibition of Bcl2 and Bcl-xl, (iii) induction of Bax, and (iv) inhibition of PCNA and Ki67 expression. Further, fisetin treatment reduced the expression of mesenchymal marker proteins (N-cadherin and vimentin) with concomitant increase in epithelial marker proteins (E-cadherin and desmoglein). These data suggest the ability of fisetin to exhibit anti-proliferative, anti-apoptotic, anti-tumorigenic, and anti-metastatic potentials in a mouse model of melanoma. We suggest that fisetin could be used as an adjuvant chemotherapy to prevent drug resistance and improve the therapeutic efficacy of anti-melanoma drugs for the management of melanoma.

#5108 Potent small molecule compounds that selectively inhibit proliferation of ABC-DLBCL cell lines. Leena Khare Satyam,1 Dinesh Chikkanna,1 Vinayak Khairnar,1 Manoj Pothuganti,1 Sunil Panigrahi,1 Anirudha Lakshminarasimhan,1 Narasimha Rao,1 Wesley Balasubramanian,1 Sandeep Patil,1 Sre- evalsam Gopinath,1 Gunta Upendra,1 Jwalaa Nagaraj,1 Kiran Arithal,1 Vijay Ahuja,1 Sanjeev Giri,1 Chetan Pandit,1 Murali Ramachandra1. Aurgene Discovery Technologies Ltd., Bangalore, India; Aurigene Discovery Technologies Ltd., Hyderabad, India.

Diffuse large B cell lymphoma (DLBCL), which accounts for 25% of all lymphomas cases, has been classified into molecular subtypes including germinal center B cell like (GCB) DLBCL, activated B cell-like (ABC) DLBCL, and primary mediastinal B cell lymphoma (PMBL). Among these subtypes, patients with ABC-DLBCLs have the worst prognosis because of the high chemo-resistance, and require effective therapies. Mucosa-Associated Lymphoid Tissue Lymphoma Translocation 1 (MALT1) protease activity is linked to the pathogenesis of ABC-DLBCL, therefore, an MALT1 inhibitor is in clinical development. We screened a library of compounds selected based on molecular docking on the reported crystal structure for selectivity to ABC-DLBCL, but not GCB-DLBCL cell lines. Optimization of initial hits resulted in the identification of lead compounds with an anti-proliferative EC50 of <100 nM selectively in ABC-DLBCL cell lines. Consistent with the previously reported role of MALT1 inhibitors, lead compounds showed reduced 4T1 tumor growth in xenograft model upon oral dosing. In summary, we have identified novel and potent MALT1 inhibitors capable of selectively inhibiting proliferation of DLBCL cell lines with optimized drug-like properties including oral bioavailability.


More than a decade ago, we initiated a research program on the molecular pharmacology of phytochemicals derived from Chinese medicinal herbs. Bioactive plant extracts have been fractionated by chromatographic techniques and isolated bioactive compounds and elucidated their chemical structures by nuclear magnetic resonance and mass spectrometry. A promising compounds was artemisinin from Artemisia annua L. and its semisynthetic compound artemesunate. Artemisinin and artemesunate are anti-malarial drugs. Our data indicated profound activity against cancer cells, but also against various viruses, Schistosoma, Trypanosoma, and even plant crown gall tumors. To elucidate the molecular mode of actions against cancer, we applied molecular biological and pharmacogenomic approaches in vitro and in vivo. Different signaling pathways were identified not only in cancer cells but also in cells infected with viruses, e.g. HCMV, HSV1 and others. To translate the experimental results in cell lines and animals to the bedside, we report on the compartmental use of artemesunate in single cancer patients as well as on our efforts to initiate several clinical phase I/II trials in veterinary tumors as well as in human cervix or colorectal carcinoma. These pilot studies indeed indicate that artemesunate is not only useful as anti-malarial drug, but also exerts activity against cancer and viral diseases. Clinical results will also be presented that not only artemesunate as semisynthetic chemical derivative of artemisinin, but also herbal extracts from Artemisia annua are active in veterinary and human tumor patients. Artemesunate represents an illustrative example for the therapeutic potential of medicinal herbs and drugs derived from traditional Chinese medicine. References 1. Effert E et al.: Journal of Molecular Medicine 2002;80:233–42. 2. Effert: Drug Resistance Updates 2005; 8:85-97. 3. Effert E et al.: Molecular Cancer Therapeutics 2008;7:152-61. 4. Li et al.: Cancer Research 2008;68:4347-51. 5. Sharma et al.: Cancer Research 2009;68:1455-7. 6. Effert E et al.: Clinical Infectious Diseases 2008;47:804-11. 7. Krishna et al.: Ebiomediwide 2014;2:82-90. 8. Saeed E et al.: Pharmacological Research 2016;110:216-226.

#5110 A novel nucleoside analog therapeutically active against plasma cell malignancies and other ADK-expressing cancers including colon and pancreatic adenocarcinomas. Jouliana Sadek,1 Utrashtra Nayyar,2 Jonathan Reichel,3 Jennifer Tonotony, 4 Zhishuko Sei,5 Robert Shoemaker,6 David Warren,1 Olivier Elemento,1 Kenneth Kaye,5 Ethel Cesarmar1, Weill Cornell Medicine, New York, NY; Dana-Farber Institute, Boston, MA; Memorial Sloan Kettering Cancer Center, New York, NY; Chapman University, CA; National Cancer Institute, Frederick, MD; Harvard Medical School, Boston, MA.

A number of nucleoside analogs are used successfully for the treatment of several cancers, and in particular leukemias and lymphomas, but these have distinct efficacies for different tumor types, and many malignancies do not respond to currently available nucleoside analogues or other forms of chemotherapy. A high throughput screen conducted in our lab to search for inhibitors of primary effusion lymphoma (PEL) identified the nucleoside analog 6-ethylthioinosine (6-ETI) as a potent and selective inhibitor of PEL, a largely incurable malignancy of B cell origin with plasmacytic differentiation. 6-ETI induced necrosis and ATP-depletion accompanied by S-phase arrest, DNA damage and inhibition of DNA synthesis. To understand 6-ETI mechanism of selectivity, RNA-seq analysis of in vitro generated drug-resistant PEL clones revealed inactivating mutations and loss of expression of adenosine kinase (ADK) as the mechanism of resistance. In vitro assays showed that 6-ETI is a pro-drug that gets phosphorylated and activated by adenosine kinase (ADK) into its active form. We found high ADK expression in PEL cell lines and primary specimens of PEL, multiple myeloma (MM) and plasmablastic lymphoma (PBL) patient samples. 6-ETI was effective at killing multiple myeloma cell lines, primary MM specimens, and had a remarkable anti-tumor response in a disseminated multiple myeloma and PEL xenograft mouse models. Thus, ADK expression can serve as a predictive biomarker to help identify patients that are most likely to respond to 6-ETI treatment. To further assess the spectrum of activity and sensitivity of 6-ETI, we examined ADK expression in other cancer subtypes and found that colorectal and pancreatic adenocarcinomas also express ADK and are highly sensitive to killing by 6-ETI at the low nanomolar concentration. We also found high ADK expression in primary colon and pancreatic adenocarcinoma patient specimens. We compared 6-ETI to other FDA-approved purine analogs and failed to find other compounds with similar potency or selectivity profile. Herein, we report the identification of a novel purine analog, 6-ethylthioinosine, as an effective therapeutic with exquisite sensitivity to plasma cell malignancies and other ADK-expressing cancers. We have successfully used RNA-seq-based ‘resistome’ analysis to identify its mechanism of selectivity. We discovered a new biomarker that can potentially impact patient care and the treatment of some of the most aggressive tumors.


Triple-negative breast cancer (TNBC) is characterized by an abundance of treatment-resistant cancer stem cells (CSC). The absence of a molecular target, coupled with its highly aggressiveness, leads to the lack of an effective therapy for TNBC. Norcantharidin (NCTD) is a synthetic demethylated small-molecule analog of the naturally occurring cantharidin isolated from blister beetles (Mylabris phalerata Pall). Unlike the conventional chemotherapeutics, NCTD toxicity is higher to cancer cells than normal ones, making this small molecule promising for cancer treatment. The aims of this work were: A) To study the effect of NCTD on 4T1 cell line proliferation in vitro. B) To analyze the effect of NCTD on 4T1 derived CSC on self-renewal and clonogenic capacity. C) To evaluate the effect of NCTD on 4T1 tumor growth in vivo. We employed the well-known 4T1 triple-negative breast cancer cell model, which presents a huge proportion of CSC. We observed that NCTD treatment during 96 h significantly reduced 4T1 cell proliferation in vitro. Additionally, the IC50 value of NCTD was 27.35 ± 2.83 μM. Related to CSC, NCTD pre-treatment for 96 h impaired CSC self-renewal (Number of secondary mammospheres: Control: 276±39; NCTD: 163±21; p<0.05) as well as the clonogenic capacity (Number of colonies: Control: 359±38; NCTD: 122±11; p<0.05). By q-PCR, we observed that NCTD treatment for 48 h significantly induced an increase of Gli-1 and Smooth in CSC, keys member of Sonic Hedgehog pathway. Finally, we performed in vivo assay, where 4T1 cells were orthotopically inoculated on mammary gland of...
RALB/c mice, and NCTD was i.p. inoculated twice a week (5mg/kg). We observed that NCTD treatment significantly reduced tumor growth in vivo. Our data suggest that NCTD treatment reduces tumor growth both in vitro and in vivo, possibly through the direct effect on CSC self-renewal and clonogenic capacity, by modulating Sonic Hedgehog pathway.

**#5112** Evaluation of FF-10502-01, a new pyrimidine nucleoside analogue, in pancreatic (PANC) patient-derived xenograft (PDX) models compared to gemcitabine and in combination with nab-paclitaxel. Takeaki Suzuki,1 Linda J. Paragon,2 Jill Ronca,1 and Philip N. Bankhead3,4,5,6.

In previous studies, FF-10502-01 demonstrated preclinical efficacy across multiple solid tumors, including PANC cancer. In this study, we investigated the anti-tumor effect of FF-10502-01 in PAN C PDX models. Methods: 10 PANC PDX tumors were sourced from primary (2) or metastatic sites (8), 7 demonstrated high resistance (HR) to gem, 1 intermediate, and 2 low. In the dose-finding study, 3 PDX models were studied in 9 ggs of NOD-SCID mice (n = 10 ggs), treated with 240 or 480 mg/kg FF-10502-01, 3 or 6 mg/kg nab-pac or 240 mg/kg gem, alone and in combination for 28d, followed by 28d observation. The definitive study consisted of 7 PDX models. 6 ggs (n = 10/gg) were treated with 240 or 480 mg/kg FF-10502-01, 6 mg/kg nab-pac or 240 mg/kg gem, alone and/or in combination for 28d, followed by 28d observation. After subcutaneous transplantation, animals were left undisturbed for 7d. Animals were monitored weekly and tumor volume measured with calipers. Average tumor volume (mm3) for each group at randomization into treatment ggs ranged from 184.34 – 199.51 (SD = 20.94 – 30.40). Statistical significance was determined using one-way ANOVA and Tukey’s test. Results: At clinically relevant doses, FF-10502-01 alone and in combination with nab-pac demonstrated greater tumor growth inhibitory activity to gem/nab-pac. In HR ggs, FF-10502-01 was superior to gem (p ≤ 0.0001, p ≤ 0.05) or nab-pac (p ≤ 0.05), irregardless of resistance to gem. In 3 models, there was no difference, but these models were highly responsive to gem/nab-pac, thus minimizing the effects of FF-10502-01/nab-pac. Despite statistical insignificance, FF-10502-01/nab-pac still demonstrated greater tumor growth inhibitory activity to gem/nab-pac. In HR ggs, FF-10502-01 was superior to gem/nab-pac in 6 of 7 (p ≤ 0.0001, p ≤ 0.05) in 3 of 7, and FF-10502-01/nab-pac was superior to gem/nab-pac in 6 of 7 (p ≤ 0.0001, p ≤ 0.005, p ≤ 0.01, p ≤ 0.05). FF-10502-01/nab-pac also was more tolerable than gemcitabine/nab-pac, as demonstrated by less weight loss. Conclusions: FF-10502-01 is a new pyrimidine nucleoside analogue with demonstrated preclinical efficacy in solid tumor models, including PANC cancer. In PANC PDX models, FF-10502-01 alone and in combination with nab-pac demonstrated higher efficacy and better tolerability than alone gem or alone nab-pac. FF-10502-01 is in Phase 1 clinical development.

**#5113** Usnic acid, lichen secondary metabolite, inhibits gloablastoma progression through the reduction of epithelial-mesenchymal transition and glioma stemness factors. Kyung-Hwa Lee, Se-Jeong Oh, Shin Jung, Kyung-Keun Kim, Jae-Hyuk Lee, Kyung-Soo Moon. Chonnam National University Hwasun Hospital & Medical School, Hwasun-gun, Republic of Korea.

Background: Usnic acid (UA), an active compound mainly found in lichens, has shown some anti-tumoral activities for lung and breast cancers. The therapeutic role of UA in glioblastoma (GBM) have not yet been determined, nor has the definitive relationships of UA with EMT and cancer stem cells. Methods: We tested the anti-tumoral activities of UA against glioblastoma (GBM) progression and further investigated the mechanistic link with epithelial-mesenchymal transition (EMT) and cancer stemness factors. The targeting and anti-tumor effect of UA was also checked in orthotopic mouse glioma model. Results: In vitro assay, we found that UA increased apoptotic cell death and inhibited the invasion/migration of GM cells. Sphere and colony forming abilities were also decreased in treated GM cells. UA decreased the expression of the EMT markers (N-cadherin, ZEB1, ZEB2, SNAI2 and SLUG) and the cancer stemness markers (CD133, ALDH1 and CD44). In orthotopic glioma models, UA localized in GBM and significantly decreased tumor growth and progression to lead longer survival. Conclusion: Taken together, these findings showed that UA prevent GBM invasiveness and progression, through the down-regulation of EMT and cancer stemness markers.

**#5114** Design, synthesis, and biological evaluation of tricyclic thieno[2,3-d] pyrimidines as microtubule targeting antigumor agents. Farhna Islam,1 Alem Gangjee,1 Xin Zhang,2 XiLin Zhou,1 Susan L. Moombery2. 1Duquesne Univ. School of Pharmacy, Pittsburgh, PA; 2University of Texas Health Science Center at San Antonio, San Antonio, TX.

Microtubules are dynamic structures that, together with actin microfilaments and intermediate filaments, constitute the cellular cytoskeleton. Besides their well-known roles in cell division, their functions involve maintenance of cell shape and morphology, cellular motility, and trafficking of organelles and vesicles. Recently, we reported a compound AG 370 as a microtubule targeting agent, that circumvented the Pgp and MDR1-tubulin mediated drug resistance mechanisms that limited the efficacy of paclitaxel, docetaxel, and the vinca alkaloids. Molecular modeling and docking studies of the parent compound (AG 370) in the colchicine binding site (PDB: 4R02B) suggest that the C-5 and C-6 of parent compound are oriented towards the hydrophobic pockets with the side chain of Ala316, Val315, Leu255 and Met259. However, this binding pocket in tubulin is relatively large. To further explore the hydrophobic pocket, an additional cyclohexene ring was introduced at C-5 and C-6 of AG370. In the molecular modeling study, the resulting tricyclic scaffold showed hydrophobic interaction with the amino acids of the colchicine binding site. The 2-Me substituted of the tricyclic scaffold was replaced with a 2-H and a 2-NH2 group and 4-position of the scaffold was replaced with appropriate anilines. The 2-amino-N4-methoxyphenyl moiety (AG61) was found to be the most potent analog in the preliminary screening in an in vitro panel of PANC cancer cell line (MDA-MB-435 cell line, IC50 = 9.0 ± 0.2 nM) and in microtubule depolymerization assay in A-10 cells (EC50 = 19 nM). Thus, we identified tricyclic thieno[2,3-d]pyrimidines as a novel structural scaffold with potent antiproliferative activity as well as microtubule depolymerizing activity. These analogs are selected for preclinical development.

**#5115** Establishing an experimental paradigm to study the interphase effects of microtubule targeting agents. April L. Risinger, Nicholas F. Dybdal-Hargreaves, Roma Kaul, Allison D. Clark, Susan L. Moombery. UT Health Science Ctr. at San Antonio, San Antonio, TX.

Microtubule targeting agents (MTAs) are highly effective anticancer drugs. While these drugs were traditionally classified as antimitotics, compelling evidence suggests that the ability of MTAs to interrupt microtubule-dependent trafficking and signaling in interphase cells contributes to their anticancer efficacies. Previous studies of the interphase effects of MTAs on oncogenic signaling pathways have led to an important reevaluation of their mechanisms of action. These effects have been primarily reported in cells that have been treated with MTAs for extended periods of time. However, MTAs rapidly alter microtubule dynamics which results, within a few hours, in global changes to gene expression and cell signaling. We propose that evaluating the effects of MTAs on oncogenic pathways at times and concentrations that are associated with early microtubule disruption will allow analysis of the initiating events that link the direct action of MTAs on microtubule structure and dynamics to effects on interphase signaling that contribute to anticancer efficacy. The concentrations and treatment times that were optimal for the study of clinically used MTAs on cellular trafficking and signaling events were first determined. Our experimental paradigm of a 2 hr treatment of breast cancer cells with clinically relevant concentrations of MTAs eliminated contributions due to mitotic accumulation and changes in gene expression associated with longer treatments.

While all MTAs disrupt microtubule dynamics, the differences among MTAs in their rapid downstream effects on cellular signaling have not been systematically evaluated. A goal of this project was to identify differences in the effects of diverse MTAs on interphase signaling pathways that may underlie their differential efficacy in patient populations. This short term treatment paradigm led to the identification of profound differences among MTAs in their ability to disrupt Src-dependent E-cadherin re-localization, canonical and non-canonical TGF-B signaling, and β-catenin localization. These findings demonstrate the ability of diverse MTAs to rapidly impact interphase oncogenic signaling and trafficking pathways. This experimental design sets forth a method to evaluate the initial effects of diverse MTAs to gain critical insight into their differential abilities to inhibit key oncogenic signaling pathways. These types of studies might, in the future, help facilitate the rational selection of specific MTAs for patients depending on tumor characteristics. Funding for this work was provided by Eisai Inc.

**#5116** Identification of new treatment options for Merkel cell carcinoma using high throughput chemical screening. Tara Gelb,1 Daniel Urban,2 Ken- neth Daily,1 Ying Xiao,1 Min Shen,2 Matthew Hall,1 Isaac Brownell1. 1National Cancer Institute, Bethesda, MD; 2National Center for Advancing Translational Sciences Chemical Genomics Center, Rockville, MD.
Merkel cell carcinoma (MCC) is a rare and aggressive neuroendocrine skin cancer with limited treatment options. Approximately 80% of MCC tumors have Merkel cell polyomavirus (MCPyV) DNA integrated into the host genome, and viral oncoproteins are thought to drive carcinogenesis. In contrast, MCPyV-negative (MCPyV-) tumors have higher rates of proto-oncogene mutations. Transcriptome differences between MCPyV+ and MCPyV- tumors further suggests divergent underlying pathologies. In order to develop efficacious treatments for MCC and elucidate the pathophysiologies underlying MCPyV+ and MCPyV- MCC, we screened the effects of mechanistically annotated drug libraries on MCC cell viability (CellTiter-Glo). We screened ~4,500 compounds including the NGCPC Cancer Collection of approved and investigational drugs and the oncology-focused MIPE (Mechanism Interrogation Plate) library against six MCC cell lines (3 MCPyV+ and 3 MCPyV-). We ran follow-up screens on the above cell lines as well as non-MCC control cells to identify agents that specifically reduced viability in MCC. DNA topoisomerase inhibitors, pros- teasome inhibitors, and PI3K inhibitors potently and efficaciously reduced viability in all MCC cell lines tested. Moreover, using hierarchical clustering we found that viral status impacted pharmacological responses. For example, a subset of HDAC inhibitors were more potent in MCPyV+ than MCPyV- MCC cells. In contrast, a dual RastRAP/ERK inhibitor more potently reduced viability in MCPyV- MCC cells relative to MCPyV+ MCC cells. Together these results identify existing drugs that can be repurposed for treating MCC. Moreover, they help elucidate the distinct pathophysiology driving MCPyV+ and MCPyV- MCC.

#5117 Triplatin preferably suppress lung metastasis of breast cancer, and peritoneal carcinomatosis of colon and pancreatic cancer.

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Background: Since metastatic spread is often directly associated with the poor outcome, an effective treatment for metastatic lesion is expected to improve overall survival. This is particularly the case in peritoneal carcinomatosis of pancreatic cancer where there is no effective treatment. Recent clinical trials demonstrated that Cisplatin is effective in certain metastatic breast cancer, however with severe side effects. Polynuclear platinum analog, Triplatin, was developed to overcome the severe toxicity. Methods: Murine cancer cell lines, 4T1-luc2 and E0771 (breast), Panc02-luc (pancreas) and CT26-luc (colon) were used. Cell proliferation was quantified by CCK8 assay. Synergistic orthotopic implantation of 4T1-luc2 cells was used for metastatic breast cancer model. For mastectomy model, primary tumors were surgically removed 8 days after inoculation. Synergistic intraperitoneal injection of CT26-luc6 cells and Panc02-luc cells were used for peritoneal carcinomatosis models of colon and pancreatic cancer respectively. Triplatin (0.3 mg/kg) or vehicle was administered intraperitoneally every 4 days for 3 days and tumor burden was quantified by bioluminescence imaging. Results: Triplatin suppressed cell growth of both breast cancer and pancreatic cancer in a dose dependent manner in vitro (IC50 of 4T1-luc2, E0771 and Panc02-luc cells were 0.08, 0.51 and 0.07 μM, respectively). Triplatin did not suppress the growth of 4T1-luc2 primary breast tumor, however, ex vivo results demonstrated that lung metastases were significantly reduced to 14% of the control (p = 0.034) in orthotopic model. This result was reproduced in post-mastectomy “adjvant therapy” model where Triplatin treatment was begun after primary implanted tumor was removed, which suppressed lung metastases down to 1.9% of control by Day21 (p = 0.038), and significantly prolonged survival (p = 0.007). This result led us to use Triplatin in CT26-luc colon cancer carcinomatosis model, where Triplatin suppressed total tumor burden to 1.9% of control by Day13 (p = 0.029) and significantly prolonged survival (p = 0.001). Finally, Triplatin reduced total tumor burden of Panc02-luc pancreatic cancer peritoneal carcinomatosis model to 35% of the control by Day 9 (p = 0.337) and the survival was significantly prolonged (p = 0.025). Conclusion: Triplatin demonstrates that significant suppression lung metastatic tumor of 4T1-luc2 breast cancer, and peritoneal carcinomatosis of CT26-luc colon cancer and Panc02-luc pancreatic cancer. Newer platinum compounds with less toxicity and favorable pharmacokinetics warrant further investigation for advanced metastatic cancer.

#5118 A novel polyamine sulfonamide with anti-leukemic activity.

Vindhiya Vijay, Amy Meacham, Leylah Drusbosky, Elizabeth Wise, Christopher Cogle, University of Florida, Gainesville, FL.

The greatest challenge in treating Acute Myeloid Leukemia (AML) is refractory disease. Although 60-80% of AML patients achieve complete remission after induction chemotherapy, majority of these patients relapse and die of progressive disease. New treatment options targeting the vulnerabilities of AML biology are highly critical for disease regression, in particular the chemo-resistant leukemia-initiating population, to ensure relapse-free survival in patients. Identifying novel druggable targets that are selective to AML despite their location in the vascular niche is thus highly warranted. We developed an in-vitro co-culture model consisting of AML cells and Bone Marrow-derived Endothelial (BMEC) cells, which recapitulates the bone marrow niche in the disease setting. Using this unique model, and taking advantagе of combinatorial chemistry, we performed high-throughput chemical-peptidogenic screening of approximately 30 million novel compounds and identified a novel polyamine sulfonamide 2470-51 that can selectively kill AML cells by overcoming the protective effect of the BMECs. In addition to AML blasts, 2470-51 also exhibited highly selective activity against the leukemia stem and progenitor cell population while sparing normal hematopoietic stem and progenitor cells. In vivo studies using patient-derived xenograft models indicated significant regression in AML engraftment post 2470-51 treatment. Target identification experiments involving unbiased label-free shotgun proteomic analysis in combination with targeted Selected Ion Monitoring (SIM) revealed covalent drug binding targets of 2470-51. By performing differential protein expression analysis using iTRAQ analysis, we identified downstream mechanisms that led to mitotic cell cycle arrest and cell death. Collectively, our findings display the in vitro as well as in vivo efficacy of a novel polyamine sulfonamide in eliminating AML, including the leukemia initiating compartment. We also uncover a novel mechanism in AML that can be taken advantage of for selective toxicity. Furthermore, we establish the role of 2470-51 as a potential therapeutic agent in treating AML.

#5119 Evaluation of Minnelide as potential targeted therapy for triple negative breast cancer.

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Recent advances in diagnostics and better understanding of molecular mechanism underlining breast cancer has let to the better therapeutic options and disease outcome for majority of breast cancer patients. However, ~10 - 20% of all breast cancers often referred to as “triple negative” as they lack expression of the estrogen (ER), progesterone (PR), and human epidermal growth factor 2 (HER2) receptors convey a poor prognosis due in part to a lack of targeted therapies. The aim of the current study is to evaluate whether triptolide and its water soluble analog Minnelide is effective against triple negative breast cancer cells. We have previously shown that triptolide/Minnelide not only inhibits tumor growth in various cancer models but it also regulates epithelial -mesenchymal transition (EMT), an important mechanism underlying metastasis. In our preliminary findings using three triple negative breast cancer (TNBC) cell lines, MDA-MB-231, MDA-MB-468, and MDA-MB-157, we demonstrate that triptolide not only inhibits the proliferation of TNBC cells but also regulates the protein levels of EMT markers including B-Catenin and Vimentin. In order to elucidate the mechanism underlying triptolide mediated inhibition of cellular proliferation and regulation of EMT markers in TNBC cells, we identified Src kinase and Aurora kinase A as two new targets for triptolide action in TNBC cells. Targeting both Src and Aurora kinase, triptolide disrupts the integrity of focal adhesion structures and reduces cell spreading via regulating FAK activity. Our preliminary findings regarding potential use of triptolide/Minnelide in TNBC based on in vitro experiments are promising. However, considering the complex pathophysiology of breast cancer and other biological factors playing role in a disease setting, in-vivo experiments to test the efficacy of Minnelide in relevant mouse models for mammary cancers are currently underway.

#5120 Cryptolepine a plant alkaloid, inhibits the growth of nonmelanoma skin cancer cells through inhibition of topoisomerase and induction of DNA damage.

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Topoisomerases are highly specialized nuclear enzymes that remove superhelical tension on chromosomally DNA that allows replication and transcription of DNA. Many cancer chemotherapeutic drugs used in the clinics inhibit tumor growth by targeting topoisomerase functions resulting in DNA damage and cancer cell death. Cryptolepine, a major alkaloid isolated from Cryptolepis sans-guinolenta plant’s roots, has shown anti-malarial, anti-bacterial, anti-fungal, and anti-inflammatory activities. In the present study, we examined the therapeutic effect of cryptolepine on non-melanoma skin cancer cells (NMSCC). SCC-13 and A431 as an in vitro model, and underlying mechanism of action with special emphasis on topoisomerases and DNA damage check points. Western blot analysis and enzyme activity evaluation assays demonstrated that SCC-13 and A431 cells express comparatively higher levels of topoisomerases.
and higher enzymatic activities compared with normal human epidermal keratinocytes (NHEK). Topoisomerase expression and activity has been greatly reduced after 24 h treatment with 2.5, 5.0 and 7.5 μM cryptolepine. Inhibition of topoisomerases expression and function by cryptolepine resulted in significant DNA damage and enhanced expression of DNA-PK expressions. Cryptolepine induced inhibited high-risk MM cell growth in vivo and in vitro via cell cycle G2/M arrest in a CRBN-independent manner. In conclusion, PEGylation of TC11 significantly reduced cell viability and enhanced cell death of SCC-13 and A431 cells. However, NHEK cells were less sensitive to cryptolepine induced cell death. Results of our study strongly suggest for detailed preclinical investigations in animal models to further assess its anti-skin cancer potential and its possible use in human patients.

#5121 PEG(E)-TC11, a novel polyethylene glycol-linked phthalimide derivative, inhibited high-risk MM cell growth in vivo and in vitro via cell cycle G2/M arrest in a CRBN-independent manner. Shuji Aida,1 Daiju Ichikawa, Kanuki Iida,1 Masashi Hozumi,1 Misa Nakamura,1 Ryo Uozaki,1 Nahoko Hashimoto,1 Mikio Okayama,2 Yuko Yonemura,3 Noriko Tabata,1 Taketo Yamada,1 Maiko Matsuishi,1 Takeshi Sugai,1 Hiroshi Yanagawa,1 Yutaka Hatatori1,1 Keio University Faculty of Pharmacy, Tokyo, Japan; 2Keio University School of Medicine, Tokyo, Japan; 3IDAC Theranostics Inc., Tokyo, Japan; 4Saitama Medical University, Saitama, Japan.

Multiple myeloma (MM) is one of the hematological malignancies that is characterized by proliferation of malignant plasma cells. Recent advance in the treatment of MM using newly developed drugs, prognosis of the MM patients have been significantly improved. For example, immunomodulatory drugs (IMiDs) such as thalidomide, lenalidomide and pomalidomide have been developed for treatment of MM. However, IMiDs have only limited effects against MM patients with high risk chromosomal abnormalities such as t(4;14) and del(17p) (high-risk MM). In 2010, it was reported that IMiDs directly bind to cereblon (CRBN), a component of ubiquitin ligase 3 complex, and induced teratogenicity as well as anti-tumor effects. We have previously reported that a novel phthalimide derivative, 2-(2,6-disopropylophenyl)-5-amino-1H-isodoledine-1,3-dione (TC11) induced apoptosis against high-risk MM cells in vivo and in vitro, and inhibited differentiation of osteoclasts. We also reported that TC11 directly bound to α-tubulin and nucleophosmin-1 (NPM1), but did not bind to CRBN. However, TC11 was not dissolved in water with only 0.02 mg/mL solubility. Therefore, TC11 showed poor absorption into blood and limited anti-tumor activity when it was intraperitoneally administered in tumor-bearing mice. To resolve these problems, we synthesized PEG(E)-TC11, in which TC11 is linked to polyethylene glycol through an ester bond, and consequently enhanced water solubility of PEG(E)-TC11 to 88.9 mg/mL. PEG(E)-TC11 revealed as potent growth inhibitory effect on high-risk MM cells as TC11 in vitro. In pharmacokinetic study, PEG-modification of TC11 improved the peak blood concentration (Cmax) from 2.6 to 24.4 μM and extended elimination half-life (t1/2) from 1.4 to 2.2 hr when 186 μg/kg of these compounds were intraperitoneally injected. More importantly, these pharmacokinetic improvement lead to more potent growth inhibition of MM cells in vivo than TC11. We also explored mechanisms of anti-myeloma effect of PEG(E)-TC11 and found that PEG(E)-TC11 induced apoptosis via G2/M cell cycle arrest. However, unlike IMiDs family, BIACORE assay revealed that PEG(E)-TC11 didn’t directly bind to CRBN, indicating that growth inhibitory effect of PEG(E)-TC11 against MM cells was independent of binding to CRBN. In conclusion, PEGylation of TC11 significantly increased water solubility, resulted in potentiated anti-myeloma activity in vivo. Furthermore, PEG(E)-TC11 inhibited cell growth via G2/M arrest in a CRBN-independent manner. Thus, PEG(E)-TC11 is considered as a candidate compound for overcoming high-risk MM.

#5122 Utilizing zebrafish to study the effect strigolactone on breast cancer cells. Kira Lin,1 Christopher Grivas,1 Elena Boru,1 Yaron Dayani,1 Eric Berens,1 Anna T. Riegel,1 Eric Glasgow,2 Ronit I. Yarden,1 Georgetown University, Washington, DC; 2Georgetown University, Bethesda, MD.

Strigolactones (SLs) are a novel class of phytohormones that regulates shoot branching patterns and above-ground plant architecture by inhibiting the outgrowth of axillary lateral buds or meristems. The SL analogues (SLAs) inhibit the growth and survival of a wide array of cancer-derived cell lines including: prostate, colon, lung, melanoma, osteosarcoma and leukemic cell lines, while minimally affecting normal cultured cells. Interestingly, cancer cells with high metastatic potential are more sensitive to the inhibitory effect of SLAs than less aggressive cells. Treatment of cancer cells with SLAs leads to the activation of stress-related signaling including p38MAPK and JNK1/2, and disrupt the mitrobule network. Furthermore, a reduction in acetylated-alpha-tubulin was observed in SLA-treated MDA-MB-231 cells. Microtubules dynamics has been proposed as one antitumor mechanisms that regulate cell migration by acting on lamellipodia formation. The EC50 invasion assay data suggests that even low concentration of SLA and as soon as 6 hours, SLAs suppress MDA-MB-231 cell’s ability to invade the HUVEC monolayer. To further examine the effect of SLAs on the invasive and metastatic behaviors of cancer cells in vivo, fluorescently labeled MDA-MB-231 cells were used as xenograft models in zebrafish. Embryos injected with MDA-MB-231 cells showed 66.6% metastasis in vehicle treated control fishes, as compared to 22.8% in SLAs treated fishes. This suggests that SLAs are potent inhibitors of cancer dissemination and metastasis.

#5123 Targeted thieno[2,3-d]pyrimidines with fluorinated phenyl side chains as antitumor agents. Nian Tong,1 Aleem Gangjee,1 Adrienne Wallace Povirk,2 Carrie O’Connor,3 Aamod Aamod Dekhneb,4 Zhanjun Hou,5 Larry H. Mathery6,1 Duquesne Univ., Pittsburgh, PA; 2Wayne State University, Detroit, MI; 3Barbara Ann Karmanos Cancer Institute, Detroit, MI; 4University of Michigan, Ann Arbor, MI; 5Australis Scientific, Centennial Park, Australia; 6The Children’s Hospital of Philadelphia, Philadelphia, PA.

The reduced folate carrier (RFC) is ubiquitously expressed in tissues and tumors and is the major tissue transporter for folate cofactors. Folate receptor (FR) α and β, as well as the proton-coupled folate transporter (PCFT), exhibit narrower patterns of tissue expression and are likely to serve more specialized physiologic roles. FRs are expressed in a subset of cancer cells (e.g., ovarian and non-small cell lung cancer for FRα, acute myelogenous leukemia for FRβ), whereas PCFT is expressed in a wide range of human solid tumors but not leukemias. Earlier generations of glycinamide ribonucleotide formyltransferase (GARTFase) and de novo purine nucleotide biosynthesis (e.g., Lometrexol) have shown promise as antitumor drugs. However, these compounds are excellent substrates for RFC and thus are non-selective for tumors, leading to dose-limiting toxicities. We previously reported a novel class of α-substituted thiieno[2,3-d]pyrimidines with a phenyl side chain and 3- and 4-carbon bridge lengths (AGF17 and AGF23, respectively) which selectively targeted FRs but not RFC or PCFT. To increase antitumor efficacy, we synthesized fluorinated analogs of AGF17 and AGF23, designated as AGF309 and AGF304, respectively. AGF309 and AGF304 potently inhibited proliferation of Chinese hamster ovary (CHO) cell lines engineered to individually express human FRα (IC50 of 1.08 μM and 2.27 nM, respectively) or FRβ (IC50 of 0.48 and 1.14 nM, respectively). AGF304 and AGF309 showed nominal activity toward RFC- and PCFT-expressing CHO cells. Both AGF309 and AGF304 were also potently inhibitory toward FRα-expressing human KB human tumor cells (IC50 of 7.19 and 5.27 nM, respectively). By analogy with previous iterations of 6-substituted thiieno[2,3-d]pyrimidine compounds, AGF309 and AGF304 likely involves inhibition of de novo purine biosynthesis at GARTFase and potentially a secondary intracellular target. Collectively, our in vitro findings of the potent and selective antitumor activity and FR selectivity suggest that further preclinical evaluation of AGF309 and AGF304 as potential antitumor agents is warranted.

#5124 GNS561 a new quinoline derivative inhibits the growth of hepatocellular carcinoma in a cirrhotic rat and human PDX orthotopic mouse models. Firas Bassissi,1 Zuzana Mack Jackova,2 Sonia Brus,3 Jerome Courrambeck,4 Jennifer Tracz,2 Keerthi Kurma,1 Gael S. Roth,1 Cindy Khaldi,1 Corinne Chaimbault,1 Benoit Quentin,1 Emilie Assera,1 Antoine Beret,1 Eric Raymond,1 Philippe Halton,1 Thomas Decaens3. 1Genoscience Pharma, Marseille, France; 2University of Lorraine, Nancy, France; 3Barbara Ann Karmanos Cancer Institute, Detroit, MI; 4University of Grenoble Alpes, Institute for Advanced Biosciences, Research Center UGA / Inserm U 1209 / CNRS 5309, Grenoble, France; 3Universe Grenoble Alpes, Institute for Advanced Biosciences, Research Center UGA / Inserm U 1209 / CNRS 5309, Clínique Universitari d’Hépato-gastroentérologie, Pôle Digestif, CHU Grenoble, Grenoble, France.

Hepatocellular carcinoma (HCC) remains a major health problem, often diagnosed at late stages with limited number of therapeutic options. New drugs with original mechanisms of action are urgently aimed to improve current armamentarium in HCC patients. Quinoline derivatives are novel class of oral small molecules inducing multiple cellular effects such as inhibition of autophagy, induction of apoptosis, and cell cycle modulation. The aim of these
studies was to assess tolerance and efficacy of a new quinolone derivative GNS561. Material and methods: In vitro experiments were realized with viable, apoptosis and migration in tumor cells in HCC cell lines and primary tumor. Drug tolerance and plasma and liver pharmacokinetic were evaluated after single- and repeated dosing in mice and rat. GNS561 and sorafenib efficacy in vivo were evaluated in a PDX orthotopic BALB/c mice with a pancreatic 5-fluorouracil (5-FU)-induced tumor model and a distinct nitrosamine (DEN)-induced HCC cirrhotic rat model. AFP, cell proliferation and tumor weight and size were assessed in mice. In rat tumor progression was followed by MRI, pathological analysis (tumor size and number), immunohistochemistry and PCR analysis after 6 weeks of treatment. Results: GNS561 shows potent anti-proliferative activity when assayed against a panel of human tumor cell lines and notably against a panel of HCC patient primary tumors even in those with sorafenib resistance (Mean EC50 3 μM vs 11 μM for sorafenib). GNS561 is highly selectively trapped in the liver. Plasma and liver PK in mice and rats after single and repeated doses confirm this selectivity with good tolerance and oral bioavailability. In PDX mouse model, tumor growth was significantly reduced by GNS561 with a dose-response manner, this tumor regression was associated with AFP level decrease by 72% with GNS61 (p=0.002) and 54% with sorafenib (p=0.046) compared to control. In rat model, mean number of tumors was significantly lower in GNS561 at 15 mg/kg group (n=50.6), in sorafenib at 10 mg/kg (n=65.1) and in combination group (n=40.6), when compared to control (n=100.4; p=0.0024, p=0.029 and p=0.0002). Tumor decrease measured by MRI was associated with a significantly reduced proliferation measured by Ki-67 in GNS61 group (70%) and combination group (84%) compared to control, whereas the effect of sorafenib alone on proliferation was modest (30%). Conclusions: GNS561 is a liver selective drug with good tolerance and promising efficacy in different HCC animal models. GNS561 was more efficient than sorafenib to control tumor growth in preclinical models. Based on its safe toxicity profile and potent activity in rodent models, GNS561 is now aimed to further reach clinical development in patients with HCC in 2017.

#5125 Muscadine grape extract inhibits breast cancer brain metastatic cells by multiple mechanisms. WenHong Chen, Patricia Gallagher, Ann Tal- lant, Linda J. Metheny-Barlow. Wake Forest School of Medicine, Winston-Salem, NC.

Tumor metastasis to the brain is a common complication of cancer, affecting 500,000 patients each year. Due to poor penetration of most chemotherapeutics into the brain, brain metastasis may occur even while systemic disease is under control. Despite aggressive treatments including radiation therapy, survival at 12 months following diagnosis is only 20%, underscoring the need for better means to prevent and/or treat brain metastases. Natural products have historically been a very successful source of new drugs. We are investigating the anti-cancer potential of a proprietary muscadine grape extract (MGE, from Piedmont Research & Development Corp.) on breast cancer cell lines that are metastatic to the brain. In a colony formation assay, MGE (10 μg/mL) decreased clonogenic survival of 4T1.2uc2.8Br5 cells by 50% (n=3, p<0.0001). The Eo771.uc2Br5 cells were more sensitive to MGE, showing 33% inhibition at 5 μg/mL (n=3, p<0.0001) and 91% inhibition at 7.5 μg/mL (n=3, p<0.0001). We also assessed response to the combined treatment of MGE and ionizing radiation (IR). While MGE did not sensitize cells to IR, combined administration of MGE and IR resulted in decreased clonogenic survival compared to either modality alone for both cell lines (n=3-4 experiments, p<0.01), suggesting an additive effect. Mechanistically, acute exposure (24 h) to 10 μg/mL MGE induced apoptosis in 4T1.2uc2.8Br5 cells as evidenced by PARP cleavage and detection of activated cleaved caspase 3. Further, ERK1/2 activation was decreased 68% at this time point (n=3, p<0.05). In contrast, although Eo771.uc2Br5 cells are more sensitive to clonogenic inhibition, no PARP cleavage, caspase 3 cleav- age, or ERK1/2 inhibition was observed. Analysis of cyclin D1, a major regulator of cell cycle progression, identified >80% decrease following MGE treatment (24 h, 10 μg/mL) in both cell lines. Together these data suggest that MGE inhibits breast cancer brain metastatic cell proliferation by multiple mechanisms, including inhibition of clonogenic growth through induction of apoptosis and/or decreased cell cycle progression, suggesting that MGE may have utility in the treatment of breast cancer brain metastasis.


Small cell lung cancer (SCLC) is an aggressive type of lung cancer and accounts for 10% to 15% of all lung cancer cases. The malignancy has a greater tendency to be disseminated by the time of diagnosis as well as to develop early resistance to conventional treatments, a cure is difficult to achieve. The current standard therapy for SCLC treatment, either with monotherapy (platinum based drugs) or combination therapy (e.g., cisplatin with irinotecan or topotecan), was shown to cause serious side effects and inevitably evoke drug resistance in a short time period. We have recently synthesized a series of novel bis[(hydroxymethyl)indolizino[8,7-b]indole hybrids by fusing β-carboline and bis(hydroxymethyl)pyrrole moieties for antitu- mor and proapoptotic activity. These hybrid molecules displayed diverse mechanisms of action involving topoisomerase II (Topo II) inhibition and induction of DNA cross-linking. Our results also showed that they significantly inhibited the cell growth of various human tumor cell lines. Of the tested tumor cell lines, the SCLC cells (HS26 and H211) were the most susceptible to compounds BO-2239 and BO-2329. These hybrids induced cell cycle arrest at the G2/M phase and triggered tumor cell apoptotic death. Intrinsic and extrinsic death pathways were activated in both cell lines, including induction of Topo II inhibition and DNA cross-linking. Compared to the compounds with N11-Me group, derivatives having N11-H group profoundly increased Topo II inhibition activity but reduced DNA cross-linking activity. Among these hybrids, BO-2239 (with N11-H) was as potent as irinotecan, but more effective than cisplatin, in nude mice bearing SCLC HS26 xenografts. Accordingly, hybrid BO-2239 may be further developed as a potential agent for the treatment of SCLC.
LTX-401 is a de novo designed cytolytic compound that shares many chemical features with anticancer peptides, such as amphipathicity, hydrophobicity and overall net charge. In vitro cytotoxicity studies revealed that LTX-401 was highly active against a panel of malignant cells including murine and human cancer cell lines while displaying selectivity towards human red blood cells. Furthermore, LTX-401 was found to inhibit tumor growth in vivo as demonstrated by the release of Damage-Associated Molecular Pattern molecules, or 'danger signals', such as High-Mobility Group Box-1 protein, ATP and cytochrome c. Flow cytometric and confocal microscopy studies also demonstrated reduced signal from lysosomal dye LysoTracker-DND-26, hence indicating loss of lysosomal integrity upon induction of cell death. The latter was supported using transmission electron microscopy, showing distinct morphological characteristics of necrosis. Moreover, at low concentrations, LTX-401 selectively enriched in the Golgi apparatus and initiated a lethal signaling event that in part could be inhibited by Brefeldin A. Complete tumor regression has been obtained in several rodent models, including the B16 mouse melanoma model and the JM1 rat hepatocellular carcinoma model by intratumoral injection with LTX-401. Additionally, previously cured animals showed protection against challenge with live tumor cells, indicating the induction of tumor-specific immune memory. The increased survival benefit of LTX-401-treated animals provides a rationale for further evaluation of the compound as an immunotherapeutic agent against solid malignancies.

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Novel Drug Delivery Technology

#5129 Cell-penetrating doxorubicin released from Elastin-like polypeptide kills doxorubicin-resistant cancer cells. Jung Syu Ryu, Drazen Raucher. Univ. of Mississippi Medical Ctr., Jackson, MS.

A drug-releasing system facilitated by external stimuli could be used to deliver cytotoxic chemotherapy agents to tumors sites selectively and safely. Among many suggested strategies, elastin-like polypeptide (ELP) exploits its characteristic of phase transitioning in response to changes in ambient temperature. This unique property permits selective targeting of the polymer to hyperthermic tumors by aggregating at site transitions. ELP therefore can be used as a thermostensitive vector for the delivery of chemotherapy agents and therapeutic peptides, resulting in a rise in drug concentration exclusively in tumors. This novel strategy introduces unprecedented options for treating cancer, with fewer concerns about indiscriminate side effects from the chemotheraphy. In this study, the ELP-drug conjugate was further modified with incorporation of an enzyme-specific cleavable linker in order to trigger drug release within tumors. The suggested system is composed of ELP, a matrix metalloproteinase (MMP) substrate, a cell penetrating peptide (CPP), and 6-maleimidocaproyl amide derivative of doxorubicin (Dox). Rationale for this design is that this construct may be initially targeted to the tumor site by local application of mild heat. When the construct reaches tumor site, it is cleaved by MMP, releasing CPP conjugated Dox, which is able to more efficiently infiltrate tumor tissues and penetrate cancer cell membranes. These strategy shows up to 4-fold increase in cell penetration up to four times and results more cell death in breast cancer cells than the ELP-doxorubicin complex. Even in doxorubicin-resistant cancer cells (NCI/ADR and MES/ADR), ELP-released, cell-penetrating doxorubicin demonstrated better membrane penetration (two fold), leading to at least twice killing of the resistant cancer cells than ELP-Dox and free Dox.

#5130 Tumor-penetrating peptide-coated nanoparticles as a novel strategy for the targeted therapy of neuroblastoma. Fabio Pastorino,1 Chiara Brignone,1 Laura Eminonie,2 Silvia Bruno,3 Flavio Curnis,3 Daniela Di Paolo,1 Paola Patrizia Perri,1 Alessandro Gori,7 Renato Longhi,7 Michele Gilli,8 Angelo Corti,8 Mirco Fanzoni1 1Instituto G. Gaslini, Genoa, Italy; 2IRCCS Azienda Ospedaliera Universitaria San Martino-IST Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy; 3University of Genoa, Genoa, Italy; 4San Raffaele Scientific Institute, Milan, Italy; 5CNR, Milan, Italy. Anticancer drugs loaded into tumor- and vasculature-targeted nanocarriers (NC) can reduce side-effects and improve therapeutic efficacy in pre-clinical studies. However, poorly perfused and dysfunctional tumor vessels and lymphatics limit the transport of the payload into the parenchyma of solid tumors. The use of NC decorated with tumor-penetrating peptides (TPPs) might enhance tumor penetration and antitumor effects. A previously characterized neuroblastoma (NB)-targeting peptide ligand was here modified (now referred as TPP-NB) by adding a consensus motif as a mediator of cell, vascular and tissue penetration via neuropilin-1 (NRP-1) receptor recognition. NRP-1 expression was validated by FACS analysis in NB cell lines and by IHC staining in tumor cells and tumor stroma from NB-bearing mice. Recombinant NRP-1 was used to validate TPP-NB specificity. In vitro and in vivo cell association and internalization of TPP-NB, either free or coupled to Liposomes (L) were tested by FACS and confocal microscopy. Vascular permeability assay after treatment with TPP-NB-targeted, doxorubicin-loaded Liposomes (TPP-NB-L[DXR]) was performed evaluating the in vivo accumulation of Evans Blue dye within the tumor mass. Therapeutic experiments with TPP-NB-L[DXR] were performed in mice orthotopically injected with human NB cells. NRP-1 expression is validated in a panel of NB cells and in tumors from NB-bearing mice. Differently from the original peptide and some control ones, TPP-NB is able to recognize recombinant NRP-1. The addition of the NRP-1-recognition sequence to the original peptide signiﬁcantly increases its NB cellular association in vitro. Interestingly, the results seem to indicate that the enhanced capability by TPP-NB in binding NB cells is related to the combination of the NRP-1-recognition and the original sequence. Importantly, TPP-NB coupled at the external surfaces of L[DXR] significantly increases their cellular association on NB cells in vitro. Competitive binding assay reveals that binding of TPP-NB is speciﬁc and can be inhibited by an excess of the unlabeled free peptide. The localization and the cellular distribution of L, evaluated by confocal microscopy in vitro and in mouse models of NB, confirm the binding speciﬁcity, showing an increased selective internalization of TPP-NB-L-FITC compared to that obtained with either untageted L or L decorated with the scrambled control peptide. Moreover, the PENP-NP further increases the vascular permeability into the NB tumor mass, but not in normal tissues. The therapeutic efficacy of TPP-NB-L-[DXR] has been investigat- ing in terms of overall survival. On running results indicate that the novel NC exerted an increased anti-NB effect compared to DXR-loaded L decorated with the original peptide. Our findings demonstrate that the achieved penetrating features by a NB-targeting peptide might increase liposomal drug binding, homing and antitumor efficacy.

#5131 Efficient delivery of siRNA targeted CCDC88A mRNA to pancreatic cancer tumor inhibits the invasiveness and metastasis. Keisuke Tanuchi, Toshoi Yawata, Tetsuya Ueba, Toshiji Saibara. Kochi University, Nankoku, Japan.

Introduction: We identified CCDC88A messenger RNA (mRNA) that accumulates in lamellipodia by binding to an RNA-binding protein IGF2BP3 with a next generation sequencer. The objectives of this study are as follows: (1) To identify CCDC88A specific small interfering RNAs (siRNAs) as RNA interference agents that have an inhibitory effect on the invasion and metastasis of pancreatic cancer cells in a mouse model of the invasion and metastasis of pancreatic cancer; and (2) To develop a nucleic-acid delivery system to efficiently transport the siRNAs to PDAC tissue. Methods: After intravenous injection of CCDC88A-specific siRNAs supplemented with folate acids that can bind to the folate acid receptor—which are highly expressed on pancreatic cancer cells—and polyethylene glycol-chitosan oligosaccharide nanoparticles to a mouse model of invasion and metastasis in which a human pancreatic cancer cell line was transplanted to induce tumor formation in the mouse pancreas, accumulation of the siRNAs in the pancreatic cancer tissue was determined by using an in vivo imaging system. After CCDC88A-specific siRNA accumulation in the pancreatic cancer tissue was identified, invasion to the retroperitoneum as well as metastasis to the lung or liver from the human primary pancreatic tumor was compared between the mice in which CCDC88A-specific siRNA accumulation was identified and those receiving a scrambled control siRNA. Results: CCDC88A-specific siRNAs could be delivered to the pancreatic cancer cells of cancer-bearing nude mice by supplementation with folate acids and chitosan oligosaccharide nanoparticles. Supplementation with folate acids and chitosan oligosaccharide nanoparticles caused no hemolysis and had no effect on liver, kidney, or pancreatic function. Invasion to the retroperitoneum and metastasis to the lung or liver from the primary pancreatic cancer were suppressed in cancer-bearing nude mice by administration of those siRNAs, which target CCDC88A mRNA accumulated in lamellipodia of pancreatic cancer cells. Conclusions: This study suggests the potential to develop a siRNA drug to inhibit the invasion and metastasis of pancreatic cancer.

#5132 In vitro collapsing human and murine colon cancer cells by selectivity of disulfiram-loaded charge switchable nanoparticles against cancer stem cells. Marwa Abu-Serie,1 Fatma El-Rashidy2 1City for Scientific Research & Technological Applications, Alexandria, Egypt; 2Faculty of Science, Alexandria University, Alexandria, Egypt.

The different therapeutic strategies against colon cancer (CC) had led to treatment failure, due to resistance to drugs which can be toxic to normal cells in...
healthy tissues and effectively eradicate bulk tumor populations, but not cancer stem cells (CSCs). So, we targeted mitochondrial aldehyde dehydrogenase 1B1 (ALDH1B1) as marker of CSC stemness, by loading of ALDH1B1 inhibitor (disulfiram, DS) on positively charged chitosan nanoparticles (NPs) and coating them with negative charges of serum albumin (SA). The prepared NPs were characterized for particle size, zeta potential, pHi, solubility, encapsulation efficiency, loading capacity, release kinetics and cellular uptake as well as analyzed by scanning electron microscope and surface-enhanced Raman scattering. The selectivity of these NPs against in vitro chemically induced mice colon cancer cells (CDCECs) and human colon cancer cell line (Caco-2) comparing to normal mouse colon cells was determined. The investigation of their selectivity was evaluated by synergistic activity by selecting ALDH1B1 activity, flow cytometry analysis, and a tumor model of CD133 expression and quantification of cleaved-caspase 3 level (as targets for CC treatment). The zeta potential of coated NPs confirmed that SA-layering confers negative charge (-10.3 mv) for the cationic DS-loaded chitosan NPs. In slightly acidic medium of tumor, the ionic bond between SA and the loaded chitosan hydrolyzed then the positive charge was reversed (47.6 mv). In slightly acidic medium of tumor, the ionic bond between SA and the loaded chitosan hydrolyzed then the positive charge was reversed (47.6 mv). Thus coated NPs showed a controlled slow release for DS in slightly acidic pH (tumor microenvironment) than in neutral pH (microenvironment of normal cells) and their uptake were higher in both CC cells (>70%) than normal cells (4.1±0.2%) in a time-dependent manner. This interpreted the highest selectivity of coated NPs for inhibiting cellular growth by apoptosis and suppressing ALDH1B1 activity, oxidizing cellular environment, eliminating CD133 positive cells of tumor with mechanism of CDCECs and Caco-2 cells. Hence the coated NPs possessed the effective eradicating effect for colon CSCs (>40%) without a significant insult (<5%) for normal stem cells in comparison with DS which was toxic to both normal and CSCs (90%). Because of the coated NPs safety for healthy tissues, they may be promising therapy before their efficient collapsing CSCs of tumor cells. These results of this novel study that was used charge switchable (hydrophilic) NPs to load DS for targeting colon CSCs may be represent a basis for future in vivo studies.


Alvocidib is a potent inhibitor of cyclin-dependent kinase-9 (CDK9) and induces apoptosis in cancer cells by reducing the expression of short-lived, anti-apoptotic proteins such as MCL-1. Alvocidib, as a part of a sequential combination regimen with cytarabine and mitoxantrone (ACM), is currently in a Phase II clinical trial in relapsed/refractory acute myeloid leukemia (AML). Patients with AML that have a high dependence on MCL-1 are considered more likely to benefit from the alvocidib-containing regimen. MCL-1 has emerged as a key target for human cancers including leukemia and breast cancers. The use of alvocidib in clinical settings beyond the ACM regimen is somewhat limited by the current intravenous route of administration. An orally administered form of alvocidib would allow prolonged reposition of MCL-1 through chronic dosing and scheduling. Alvocidib itself is highly permeable in Caco-2 monolayers and is soluble at acidic pHs but solubility is strikingly reduced at neutral or basic conditions, which could hamper the development of an oral formulation. We hypothesized that a phosphate prodrug of TP-1287, was determined at various pH levels. It was found to be highly soluble under acidic, neutral, and basic conditions (1.5 mg/mL at pH 2.2; 1.8 mg/mL at pH 4.5; 9.5 mg/mL at pH 6.8 and 9.3 mg/mL at pH 8.7) compared to alvocidib (4.4 mg/mL at pH 2.2; 1.3 mg/mL at pH 4.5; 0.02 mg/mL at pH 6.8 and 0.02 mg/mL at pH 8.7). Pharmacokinetic studies were conducted in mice in which TP-1287 was efficiently converted to the parent alvocidib (Cmax = 1922.7 ng/ml, t1/2 = 4.4 hr) with high oral bioavailability (94% = 182.1, compared to intravenous alvocidib). Effective oral pharmacodynamic studies (measuring MCL-1 expression levels), were evaluated in tumor xenograft models. TP-1287 demonstrated significant anti-tumor efficacy in the MV4-11 AML mouse xenograft model and produced as much as a 61.7% inhibition of the pharmacodynamic biomarker MCL-1 in xenografted tumors, demonstrating a wide, 75-fold therapeutic dosing window. In addition, TP-1287 strongly inhibited tumor growth, achieving 109% tumor growth inhibition (TGI%) at the 7.5 mg/kg dose level. TP-1287 is highly soluble over a broader pH range than alvocidib and is efficiently metabolized to the parent compound in vivo, following oral administration. Tumor xenograft models and pharmacodynamic studies indicate that oral delivery of TP-1287 is efficacious in mice. Based on these results, we anticipate moving TP-1287, as an orally delivered CDK9 inhibitor, into a forthcoming clinical trial directed towards solid tumors vulnerable to the suppression of MCL-1.

#5134 Novel trifunctional immunoliposome delivery system to minimize resistance and side effects for anticancer therapy. Tanaya R. Vaidya, Sihem Ait-Oudhia. Univ. of Florida, Orlando, FL.

Background: Breast cancer (BC) is the second leading cause of cancer death in women worldwide. About 25% of BCs have overexpression of the HER2 receptor. Although HER2 targeted therapies have shown considerable improvement in HER2-positive BC patients’ outcome, treatment resistance remains a clinical challenge. Here, we sought to develop and evaluate a novel trifunctional immune-liposome (TFIL) that overcomes HER2 treatment resistance and associated cardioxicity by dual targeting HER2 on BC cells and CD3 receptors on cytotoxic T-lymphocytes (CTLs). Material and methods: Anti-HER2 (Trastuzumab) and anti-CD3 (OKT-3) antibodies, were conjugated to lipid nanoparticles by the mickle-transfer method, and the resulting formulation was purified by dextran gradient ultra-centrification. Targeted lipid nanoparticles were formulated with a fluorescent lipophilic dye, Dd, for studying receptor binding and internalization. Studies were conducted with HER2-positive BT474 cells and CD3-positive Jurkat cells using flow cytometry analyses. Doxorubicin (DXR) was encapsulated in the nanoparticles by the remote-loading technique for cell-killexperiments. In vitro cell-killexperiments were conducted by co-culturing BT474 as the target cells, and peripheral blood mononuclear cells as the effector cells, at varying ratios. Results: Purified formulations were successfully characterized by demonstrating the presence of mickles as well as the desired conjugation ratio. Flow cytometry analyses demonstrated successful cell binding and/or internalization of the TFIL with both the HER2 and CD3-positive cell lines. Moreover, these dual-targeted nanoparticles were able to target T cells to kill HER2 positive BC cells, and showed improved efficacy compared to non-targeted and plain HER2-targeted formulations in vitro. Conclusion: A novel drug delivery system, TFIL, that targets HER2 receptors on tumor cells, CD3 on T-lymphocytes, and is able to slowly release DXR was successfully developed and evaluated in vitro on HER2-over-expressing BC cells. Our findings show great promise at overcoming resistance and associated cardioxicity to present HER2 targeted BC therapies, and may translate into improved anti-tumor activity clinically compared to other treatment options.


Our nanoparticle-drug conjugates (NDCs), created at Cerulean Pharma Inc. by conjugating drug payloads to our novel β-cyclodextrin-PEG (CDP) copolymer, are designed to significantly mitigate a payload’s limitations by providing sustained drug delivery to the tumor and superior therapeutic index through controlled release kinetics. Cerulean has two NDCs in the clinic, CRLX101 and CRLX301, evidencing the translatability of our technology. CRLX101 has been dosed in over 400 patients and CRLX301 is in ongoing Phase 2a development. A key and differentiating feature of our NDC Platform is our linker technology, which is tailored for an optimal fit with the conjugation functionality of the API drug payloads (e.g., alcohol, carboxylic acid, amine, amide and urea functionality) and customizable to achieve desired drug release profiles. As an illustration of Cerulean’s ability to expand its NDC platform, we will present the biological and pharmacokinetic (PK) data supporting our drug combination platform of antibody nanoparticle-drug conjugates (ANDCs) and multi-drug nanoparticle-drug conjugates (mNDCs). ANDCs combine potentially any NDC with any conjugatable biologic to generate an ANDC with ultra-high drug-antibody ratios (DARS). Using our established NDC linker technology, we generated ANDCs that targets HER2 receptors on tumor cells, CD3 on T-lymphocytes and associated cardiotoxicity to present HER2 targeted BC therapies, and may translate into improved anti-tumor activity clinically compared to other treatment options.
corresponding NDC. The mNDCs take advantage of our existing NDC linker platform technology to conjugate diverse drug combos including DNA damaging agents (DDA) + DNA damage repair agents (DDR). We generated our POC capromethin–olaparib mNDCs spanning a range of drug combination ratios (from 1:1 to 1:20) and all demonstrated distinguishable and tunable in vitro release profiles, and were studied in vivo to evaluate related toxicity of the DDA (–8mg/kg each drug) demonstrated very low clearance and sustained levels of released drug in tumor (greater than 72h). Our drug combination platform, ANDCs and mNDCs, allow delivery of therapeutic agents to their respective biological targets at clinically-relevant doses, greatly increasing the diversity of drug combination possibilities.

**#5136 Upregulation of Salmonella motility in tumors improves dispersion, colonization and intracellular invasion of the bacteria.** Vishnu Raman, Nele V. Dessel, Owen O’Connor, Neil S. Forbes. University of Massachusetts-Amherst, Amherst, MA.

Diffusion limitations in tumors prevent small molecule therapies from reaching cancer cells located distally from vasculature. This is one reason why some cancers become drug resistant and relapse. To circumvent these diffusion limitations, Salmonella can be used as a drug delivery vehicle. The facultative anaerobe preferentially colonizes tumor tissue over normal tissue at ratios greater than 10,000:1. Moreover, flagella allow the bacteria to penetrate deep into tumor tissue. Finally, genetically modified Salmonella can invade cancer cells and deliver a wide range of intracellularly functioning and patient specific therapies (DNA, RNA, and inhibitory peptides). But, Salmonella must distribute homogenously within tumors and invade a significant number of live cancer cells in order to deliver therapies uniformly within tumors. To enable this, we hypothesized that up-regulating the master motility regulator, flhDC, in Salmonella would improve intratumoral penetration, uniform colonization and intracellular invasion of cancer cells. Salmonella utilize the transcription factor complex, flhDC, to regulate flagella synthesis and thus, motility. This transcription factor complex also positively influences type three secretion system (T3SS) assembly, which, Salmonella use to intracellularly invade epithelial cells. To test this hypothesis, we created a set of synthetic gene circuits that use arabinose for inducible expression of flhDC, the Plac promoter for constitutive expression of DSRed and the psPE promoter to express GFP when Salmonella invade tumor cells.

We analyzed aqueous bacterial motility with video microscopy. Finally, we administered the motility inducible Salmonella into a tumor-on-a-chip device to examine spatial and temporal tumor colonization characteristics of the bacteria. In an aqueous environment, the flhDC induced Salmonella swarmed approximately 33% faster than a Salmonella control (P<.01). Induction of flhDC increased the motile fraction (15-30 micrometers/second) of bacteria two-fold (P<.05) while also decreasing the non-motile fraction (0-15 micrometers/second) six-fold (P<.05) when compared to a Salmonella control. In a microfluidic device, flhDC induced Salmonella exhibited increased colonization and growth in tumor tissue located far away from channels that were meant to resemble tumor microvasculature (P<.05), when compared to a control. Finally, flhDC induction increased tumor cell invasion approximately two-fold in tissue located both proximal and distal to the micro-channels within the microfluidic device (P<.05) when compared to a Salmonella control. High-mobility Salmonella, which have the occurrence of drug resistant cancer cell relapse by delivering targeted therapies uniformly to cancer cells that would otherwise remain untreated with conventional therapy.

**#5137 Utility of pH (low) insertion peptide (pHLIP peptide) variants in drug delivery.** Linden C. Wyatt, Anna Moshnikova, Troy Crawford, Oleg A. Andreyev, Yana K. Reshetnyak. University of Rhode Island, Kingston, RI.

Introduction: The pH (Low) Insertion Peptides (pHLIP peptides) show utility in a wide variety of medical applications due to their ability to target cancer cells by exploiting the acidity that is ubiquitous to tumor tissue. Targeting tumor acidity offers many advantages over traditional biomarker targeting and has allowed pHLIP peptides to target primary tumor tissue as well as sub-millimeter metastases. Previously, pHLIP’s conjugated to PET/SPECT tracers or fluorescent labels have found applications in diagnostic nuclear imaging and fluorescence-guided surgery. The purpose of this study was to evaluate a diverse collection of pHLIP variants, including those incorporating non-standard amino acids, consisting of two or four pHLIP’s linked together, and one of de novo design, in order to determine the pHLIP peptide best suited for the targeting and intracellular delivery of therapeutic cargo molecules to primary tumors and metastases. Methods: Biophysical experiments, namely fluorescence and circular dichroism spectroscopy, using liposomes as model membranes, were used to study the localization of pHLIP variants at the bilayer surface at high pH, the tertiary structure and orientation within the membrane of the folded variants at low pH, the parameters of pH-dependent peptide insertion, and the rate at which insertion occurs. In vitro experiments, wherein the polar toxin amanitin was conjugated to the membrane-inserting end of the pHLIP variants via a cleavable disulfide bond, were used to study intracellular cargo delivery and the resulting inhibition of cancer cell proliferation. In vivo experiments, wherein the pH-dependent translocation of amanitin across the plasma membrane by pHLIP variants via a non-cleavable bond, were used to study biodistribution and tumor uptake. Results: All investigated pHLIP variants demonstrated the behavior characteristic of wild-type pHLIP with some differences in membrane affinity and membrane insertion profiles, namely pKIC of insertion and cooperativity. Inhibition of cancer cell proliferation was induced by the pH–dependent translocation of amanitin across the plasma membrane by pHLIP variants. Biophysical measurements, values of EC50 obtained at pH 6.0 and pH 7.4, and fluorescence imaging of triple-negative breast tumors in mice using pHLIP peptide were analyzed collectively. Conclusions: Different pHLIP variants are best suited for different purposes, such as the intracellular delivery of highly or moderately toxic polar or hydrophobic payloads to primary tumor tissue as well as metastases. pHLIP Variant 3 (Var3) exhibited the most favorable properties for the targeted delivery of amanitin to tumor tissue. Acknowledgement: This research was supported by the National Institute of General Medical Sciences of the National Institutes of Health under award number R01GM073857 to OAA and YKR.

**#5138 Development of a gold nanoparticle platform capable of selective homing to tumor tissues for intravenous antitumor therapy.** Zhen Han. National Cancer Institute, Frederick, MD.

Intratumoral administration of high mobility group nucleosome-binding protein 1 (HMGN1) and R848/resiquimod together with a checkpoint inhibitor can eradicate large established tumors. However, the intratumoral route of administration is difficult to use on cancers of internal organs. To improve potential clinical use of HMGN1 and R848, it is necessary to deliver HMGN1 and R848 systemically to the tumor tissue. Gold nanoparticles (AuNP) have been extensively studied as a vehicle for various types of drugs, including anticancer compounds. In this study, we established a means of generating PEGylated Au-HMGN1-R848 (Au-PEG-HMGN1-R848) complexes, which, upon intravenous administration, preferentially target ectopic hepatoma or colonic tumor tissue and avoids uptake of the particles by liver and spleen of tumor-bearing mice. Additionally, Au-PEG-HMGN1-R848 complexes were stable and preserved the capacity of HMGN1 and R848 to stimulate the maturation of dendritic cells. IV administration of Au-PEG-HMGN1-R848 complexes plus a checkpoint inhibitor had effective antitumor activity in curing 80% of C57BL/6 mice bearing large (1 cm in diameter) Hepa-1-6 hepatoma and Balb/c mice bearing CT26 colon tumors. Therefore, an Au nanoparticle platform capable of selectively delivering HMGN1 and R848 systemically to tumor tissues can be used to effectively cure ectopic Hepa-1-6 hepatoma and CT26 colon carcinoma.

**#5140 Encapsulation of the atypical retinoid ST1926 in nanoparticles prolongs the survival of acute myeloid leukemia xenografted mice at multiple folds lower concentrations than the naked drug.** Leeana El-Houjeiri,1 Walid Saad,1 Berthe Hayar,1 Patrick Aouad,1 Nadim Tawil,1 Rana Abdel-Samad,1 Claudio Pisano,2 Ali Bazarbachi,1 Hiba El Haji,1 Nadine Darwiche1.1 American Univ. of Beirut, Beirut, Lebanon; 2Biogen, Arieano Ippino, Italy.

Acute myeloid leukemia (AML) represents one of the most complex types of leukemia. It is a clinically and genetically heterogeneous disorder of hematopoietic progenitor cells, which have lost their ability to differentiate normally. Retinoids regulate vital biological processes including development, differentiation, proliferation, and cell death of hematopoietic progenitor cells. The natural retinoid all-trans retinoic acid (ATRA) became the paradigm for the treatment of acute promyelocytic leukemia (APL), an AML subtype. However, in non-APL AML patients, ATRA is possibly only effective in patients with Nucleophosmin-1 mutations without FMS-like tyrosine kinase 3 internal tandem duplication (FLT-3 ITD). Therefore, synthetic retinoids, specifically the adamaninyl ATRA-like compounds USPC01 and ST1926, have been designed. ST1926 development in clinic was limited due to its rapid glucuron conjugation resulting in low plasma concentrations. Nanomedicine enables more efficient drug delivery and bioavailability. Here, we investigate the pre-clinical efficacy of ST1926 and polymer stabilized ST1926 nanoparticles in AML in vitro and in vivo models. We show that ST1926, at low sub-μM concentrations, potently inhibited the growth of human non-APL ATRA-resistant AML cell lines and AML patient cells while sparing resting and activated normal leukocytes at ten-hundred-fold higher concentrations. ST1926 induced early DNA damage and massive apoptosis in all tested AML cell lines. To optimize the drug’s bioavailability burden, polymer stabilized ST1926 nanoparticles were developed using...
Flash NanoPrecipitation, and were shown to display comparable anti-growth activities to the naked drug in vitro. In murine AML xenograft model, ST1926 and ST1926 nanoparticles significantly prolonged survival. Strikingly, ST1926 encapsulated in nanoparticles extended survival in AML xenografted mice at four-fold lower concentrations than the naked drug. These results highlight the promise of ST1926 in AML therapy and warrant further clinical development of this adamantly retained.

#5141 Optimizing peptide-drug conjugate delivery as an alternative to antibody-drug conjugates for solid tumors. Theo L. Sottero, Emily J. Girard, Carrie H. Myers, James M. Olson. Fred Hutchinson Cancer Research Center, Seattle, WA.

Antibody-drug conjugates (ADCs) have been FDA approved for targeted delivery of chemotherapy to a small subset of cancers. However, there are significant limitations in their clinical utility in the majority of cancers: despite hundreds of clinical trials, there are only two FDA approved drugs currently on the market. ADCs are further limited by their poor penetration into solid tumors and an inability to cross the blood-brain-barrier (BBB). Our lab has over a decade of experience with peptide-dye conjugates such as chlorotoxin-Cy5.5, which demonstrates tumor-specific accumulation in a wide range of cancers including solid tumors and tumors protected by the BBB. However, chlorotoxin-Cy5.5 accumulates in mouse liver – a potential liability if a chlorotoxin-drug conjugate exhibited similar biodistribution. Tumor Paint/BLZ-100 is a derivative of this molecule optimized for tumor accumulation that is currently in clinical trials to aid in cancer surgery. We sought to expand upon the utility of Tumor Paint and optimize a novel peptide that is capable of delivering both imaging agents and chemotherapeutics specifically to tumor cells in vitro and in vivo. We have identified a novel Optide (Optimized knottin pepTIDE) able to accumulate in a range of cancers (lymphoma, sarcoma, colon cancer, and pediatric medulloblastoma). To test whether an Optide could be created with a greater tumor specificity, we looked to both natural homologs as well as specific charged residue modifications. We have identified two next-generation Optides with enhanced uptake in mouse models of sarcoma. We have used these peptides to deliver a microtubule inhibitor to sarcoma and lymphoma in vitro and in vivo. Using in vitro flow cytometry, we have also identified multiple FDA approved drugs that upregulate or downregulate uptake of our family of peptide drug-conjugates. In understanding the pathways regulating the uptake of these peptides, we hope to both be able to identify tumors best suited for treatment with peptide-drug conjugates (PDCs) based on our Optide scaffold.

#5242 Molecular intersection of a 3-in-1 nanomedicine targeting microtubules, ERK tyrosine kinases with profound nuclear modulations, and quantum imaging for hepatocellular carcinoma therapy. Radhika J. Poojari, Rohit Srivastava, Dulal Panda. Indian Institute of Technology Bombay, Powai, Mumbai, India.

Hepatocellular carcinoma is the fifth malignant form of cancer worldwide with poor survival rates. Despite advances in clinical diagnosis, surgery and chemotherapy till date systemic treatment of solid tumour like hepatocellular carcinoma has not been effective in most cases and its clinical therapy is a major challenge. Hence, it is important to search for innovative therapeutic modalities. Nanomolecules that can target both microtubules and kinases may provide new avenues to treat hepatocellular carcinoma. Small molecule inhibitors like doxetaxel and sorafenib represent one of them. Clinical trials have revealed unacceptable toxicities such as neutropenia, severe hypersensitivity, anaphylactic re-action, hypertension, dermatological toxicities and hand-foot skin reaction, etc with these drugs in cancer patients. Other major drawbacks are insolvency, poor bioavailability, short half-life and non-specific. To mitigate these issues, we developed a next generation highly compact nanocarrier system, the 3-in-1 nanomedicine platform with in-depth understanding of the intracellular machinery responsible for its uptake and the molecular mechanisms for regulating the delivery efficiency. Development of a 3-in-1 nanomedicine (100-200 nm) comprised of microtubule inhibitor docetaxel, tyrosine kinase inhibitor sorafenib and an imaging probe quantum dots encapsulated in a biocompatible polymer PLA against human hepatocellular carcinoma cells. Monotherapy strategies in cancer treatment fail due to several complex issues like narrow therapeutic window, non-specificity, administration of higher, repetitive doses, adverse clinical toxicities and resistance mechanisms of drugs. The advantages of 3-in-1 nanomedicine strategy are delineated. The real-time imaging of quantum dots in hepatocellular carcinoma cells showed accelerated internalization. The 3-in-1 nanomedicine exhibited potent inhibition in hepatocellular carcinoma cell proliferation, clonogenicity, induced strong G2/M phase block and apoptosis induction in comparison to single drug monotherapy. The 3-in-1 nanomedicine strongly promoted the microtubule bundling via increased polymer tubulin ex-pression level, augmented multinucleation, aberrant mitosis, and significantly inhibited downstream protein tyrosine kinase, ERK1/2 activation in hepatocellular carcinoma cells in comparison to single drug monotherapy. These findings provide a significant insight into the intrinsic molecular interplays of 3-in-1 nanomedicine in hepatocellular carcinoma cells. The 3-in-1 nanomedicine integrates several avenues in a single nanoscale-platform for dual-drug delivery, and high sensitivity quantum dots imaging as well as diagnostics for hepatocellular carcinoma therapy, holding high translational research potentials in the near future.

#5143 Fusogenic targeted liposomes: novel nanotherapy for specific treatment of prostate cancer. Jihane Mriouah,1 Rae Lynn Nesbitt,2 Susan Richter,1 Melinda Wuest,1 Desmond Pink,3 Deborah Sosnowski,1 Roy Duncan,1 Frank Wuest,1 Andrés Zúñiga,4 John Lewis1.1 University of Alberta, Edmonton, Alberta, Canada; 2Entos Pharmaceuticals, Edmonton, Alberta, Canada; 3University of Dalhousie, Halifax, Nova Scotia, Canada; 4University of Vanderbilt, Nashville, TN.

Metastatic or castrate-resistant prostate cancer is the second-leading cause of cancer mortality in males. In the past 3 years, chemotherapies have extended survival, but efficacy is limited by dose-limiting toxicities due to suboptimal biodistribution. Here, we have addressed the lack of specificity and accumulation in Castrate Resistant Prostate Cancer (CRPC) by developing a unique nanocarrier for drug delivery: molecular targeted fusogenic liposomes. Previously, we have developed a platform whereby lipid nanoparticles are formulated with fusion associated small transmembrane (FAST) protein p14. The p14 protein catalyzes mixing of the nanoparticle lipids with target plasma membrane to deliver the cargo directly into the cytoplasm. In this study, we have engineered the p14 protein to incorporate the prostate cancer targeting ligand bombesin, which binds specifically to the Gastrin-releasing Peptide (GRPR) that is overexpressed in prostate cancer. We hypothesized that this novel targeted fusogenic liposome formulation would significantly improve the biodistribution and efficacy of chemotherapeutics. Clinical translational bicyclic peptide Optide (DOXIL) was modified to incorporate targeted fusogenic p14 protein, and the efficacy and biodistribution of these new formulations was evaluated using in vitro and in vivo models of CRPC. In PC3 cells, compared to conventional liposomes, intracellular levels of doxorubicin are increased by 15 and 25 times when p14 or p14-bombesin liposomes are used. Additionally, the IC50 is reduced from 85mM to 2mM. In mice bearing PC3 tumors treated with targeted fusogenic liposomal doxorubicin, we observed improved tumor growth inhibition by 57% (vs control) and 1.5 fold increased accumulation in tumors within 24h after administration. These proof of concept experiments indicate that molecular targeted fusogenic nanomedicines can improve both the intracellular delivery and tumor accumulation of chemotherapeutic, improving outcomes in this preclinical model. This highlights the potential for improved outcomes in patients with CRPC through the enhancement of approved drugs.


Bicycles are novel binding agents comprising small bicyclic peptides (1.5-3 KDa) constrained via a chemical scaffold, selected for high affinity and selectivity to targets of interest. MT1 (MMP14/MT1-MMP) is a membrane-associated metalloprotease overexpressed in many solid tumours and is implicated in tumour invasion and metastasis. MT1 expression positively correlates with poor prognosis. Phage libraries containing 10^12 unique peptide sequences were post-translationally cyclized with thiol-reactive scaffold and used in an optimized, high-throughput selection process to identify Bicycles to the hemepoxin domain of MT1. Additional iterative rounds of directed phage based screening were used to optimize affinity and off-phage natural amino acids were introduced at select positions to improve plasma stability to generate the lead Bicycle binder. The lead anti-MT1 Bicycle was further modified with a sarcosyl spacer to form N241. N241 binds specifically to the hemepoxin domain of MT1 with a Kd of approximately 2 nM with no binding observed to the catarlytic domain of the protease nor to any of the related MMP family members tested. Importantly and in contrast to most antibodies, N241 binds with similar affinity to MT1 from multiple species including rodent, dog and non-human primate. Since the expected rapid tumor penetration and specific binding of these small peptideyl-binders makes them ideal for use in targeted delivery approaches, a series of Bicycle drug conjugates (BDCs) were prepared; N241 was conjugated to potent maytansinoid cytotoxics via linkers which varied in their cleavability.

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Though all the BDCs maintained high affinity for MT1, efficacy toward MT1-positive human tumor mouse xenografts varied with linker stability. BDCs with the most stable linkers were the least active suggesting that optimal tumor activation was obtained with linkers that could be cleaved more rapidly. Due to the rapid clearance and limited systemic exposure of these small-targeting BDCs, only the most stable linker showed toxicity in vivo. DoxKA was selected for the in vivo study based on drug clearance and limited systemic exposure of these small-targeting BDCs, with conserved efficient Doxorubicin cytotoxic mechanism. Uptake of DoxKA in ES-2 ovarian cancer cells was reduced when Sortilin expression was specifically silenced by using siRNA or upon competition with the Sortilin ligands neurotensin and progranulin. In addition, DoxKA was found to bypass the P-gp efflux pump since, in contrast to Doxorubicin uptake in MDCK-MDR1 cells overexpressing the P-gp efflux pump, the uptake of DoxKA was unaffected by the P-gp inhibitor Cyclosporin A. In vivo, DoxKA was better tolerated and caused a more potent inhibition of human ovarian tumor xenograft growth than did Doxorubicin alone. These results provide strong evidence for the potential of this drug development platform in the generation of novel personalized therapeutics with specific targeting of SORT1-positive cancer cells.

#5145 Denitification of obstacles encountered using convection enhanced delivery in preclinical models of diffuse intrinsic pontine glioma, Jamila S. Gittens,1 Sridevi Yadavilli,2 Michael Lin,2 Eshini Panditharatna,2 Suresh N. Magge,2 Roger J. Packer,2 Javad Nazarain.1 1Children’s National Health System, Washington, DC; 2The George Washington University, Washington, DC.

Diffuse intrinsic pontine gliomas (DIPGs) is a pediatric brain tumor that arises in the pons of the brainstem. The vital location of the tumor and the presence of the impermeable blood brain barrier (BBB) make treatments ineffective and lead to a high mortality rate of DIPG patients. Convection enhanced delivery (CED) is a promising drug delivery method because of its ability to bypass the BBB that is currently being investigated to deliver therapeutic agents directly to brain tumors. Nonetheless, clinical trials investigating the use of CED to treat patients have not shown prolonged patient survival. Previous studies have determined poor distribution in clinical studies due to reflux or back flow. Additionally, by analyzing the H&E and Ki67 stained brain sections of a DIPG patient that underwent CED, we determined that areas of radiation-induced necrosis within the tumor were a possible reason for treatment failure. In this study, we intend to optimize CED in orthotopic murine model of DIPGs. Specifically, we have tested the ideal volume and rate of injection, as well as using the combined approach of using CED and radiation. Ideal injection rate was established by assessing infusion profiles in agarose brain phantoms using various needles, which resulted in the injection rate of 0.3 μl/min producing the least back flow. Next, the ideal injection rate and volumes were tested in vivo by injecting non-tumor bearing mice with trypan blue. To assess the infusion rate, brain sections were analyzed at necropsy to determine distributions of the injected dye. To investigate accumulation of CED-mediated payload in necrotic sites, tumor bearing mice were exposed to radiation and then injected with the fluorescent dye, DiI. Formalin fixed brain sections were stained with DAPI and fluorescent images were taken. The results of this study indicate that a rate of 0.3 μl/min results in a significantly larger distribution area compared to the 0.3 μl/min injection rate (30.7 ± 2.1 vs. 30.4 ± 7.1 ± 1.18, p=0.0062). Of the brain sections of mice injected with DiI did indicate the accumulation of dye in areas where there were no live cells, supporting the assumption that drug injections may be retracting to necrotic pockets and thus preventing equal distribution of the drug in the tumor. Ongoing studies include investigating the survival benefits of using CED to deliver therapeutic agents in tumor-bearing mice. Understanding the obstacles of convection enhanced delivery in murine models can help establish better treatment regimens and devices for patients and provide better preclinical models for drug testing.

#5146 Increasing penetration of anticancer drugs through sortilin receptor-mediated cancer therapy: A new targeted and personalized Approach in the treatment of ovarian cancer, Michel Demeule,1 Jean-Christophe Curie,1 Alain Larocque,2 Cyndia Charfi,3 Richard Beliveau,4 Claude Vezina,2 Bobbene Annabi1 1Université du Québec à Montréal, Montréal, Quebec, Canada; 2Katana Biopharma, Montréal, Quebec, Canada.

The development of personalized therapies against ovarian cancer remains highly challenging in current modern oncology. One way to achieve greater selectivity and better anticancer drug delivery into cancer cells is to conjugate cytotoxic agents to specific peptide ligands that will selectively target receptors abundantly and/or exclusively expressed on these cells. Increased expression of Sortilin, a scavenging receptor, has been clinically observed in several human cancers including breast, prostate, colon, pancreas, skin, and pituitary. In particular, Sortilin has also been reported to be overexpressed in ovarian cancers as compared to non-malignant ovarian tissue. In light of this, we developed a peptide conjugation platform using a new Sortilin receptor-mediated vectorization strategy to increase cell targeting selectivity and cell delivery efficacy of anticancer agents. As a proof-of-concept, Doxorubicin was conjugated to peptide sequences, termed Katana peptides (KA). In vitro, significantly increased uptake of KA was observed in SKOV3 compared to SKOV3-KATANAP peptide conjugate (DoxKA) was observed in SKOV3 and ES-2 ovarian cancer cell line models, with conserved efficient Doxorubicin cytotoxic mechanism. Uptake of DoxKA in ES-2 ovarian cancer cells was reduced when Sortilin expression was specifically silenced by using siRNA or upon competition with the Sortilin ligands neurotensin and progranulin. In addition, DoxKA was found to bypass the P-gp efflux pump since, in contrast to Doxorubicin uptake in MDCK-MDR1 cells overexpressing the P-gp efflux pump, the uptake of DoxKA was unaffected by the P-gp inhibitor Cyclosporin A. In vivo, DoxKA was better tolerated and caused a more potent inhibition of human ovarian tumor xenograft growth than did Doxorubicin alone. These results provide strong evidence for the potential of this drug development platform in the generation of novel personalized therapeutics with specific targeting of SORT1-positive cancer cells.

#5147 Novel warheads for targeted therapies of cancer: The concept and design of proPBDs, Iontcho Vlahov, Longwu Qi, Spencer Hahn, Kevin Wang, Hari K. Santhaparam, Albert Felten, Paul Kleindl, Jeremy Vaughn, Christopher P. Leamon. Endocyte, W. Lafayette, IN.

Pyrrolo[2,1-c][1,4]benzodiazepine (PBD) antibiotics, are a class of natural products produced by various actinomycetes bacteria. PBDs are sequence selective DNA-crosslinking alkylating compounds and are considerably more potent than systemic chemotherapeutic agents. Some PBDs have the ability to recognize and bond to specific sequences of DNA. After insertion in the minor groove, a covalent aminal bond is formed through nucleophilic attack of the N-2 of a guanine (G) base on the electrophilic C-11 imine of PBD. The end result is obstruction of tumor cell division without any significant distortion of the DNA’s helix, thus potentially avoiding the common phenomenon of drug resistance. In recent years, synthetic PBD-dimers have emerged as a promising class of payloads for conjugates in the field of targeted cancer therapies. Unfortunately, many PBD conjugates preserve the strongly alkylating imine moiety within their structural framework, which can react in a deleterious manner while circulating on their way to their intended targets. As an elegant solution to this inherent problem we designed conjugated prodrugs lacking the imine moiety. Once the prodrug (proPBD) conjugate enters a targeted cell, cleavage of the linker system in the construct triggers the generation of a reactive intermediate possessing an aldehyde and aromatic amine. An intramolecular reaction subsequently takes place as the aromatic amine adds to the aldehyde with the loss of water to give the imine, and as a result, the diazepine ring. In our proPBDs, we envision to mask the aldehyde as a hydrolytically sensitive oxazolidine moiety and to exploit its ring system as a part of an arsenal of self-immolative linker systems for conjugation. To prove the range of applications for this new class of latent DNA-alkylators, we designed and prepared a diverse variety of novel warheads, consisting of proPBD dimers, hybrids of proPBD with other powerful alkylating agents, and proPBDs with sequence-selective DNA minor groove binders. The utility of the so prepared novel warheads in the design of small molecule drug conjugates (SMDC) for targeted cancer therapies will be also discussed in the presented poster.

#5148 Liposomal gemcitabine, FF-10832, improves gemcitabine (GEM) pharmacokinetics (PK) and increases anti-tumor efficacy. Takeshi Matsu- moto, Tsuruaki Kitahashi, Takashi Komori, Hiromu Kitahara, Kohei Ono, Naoki Yamada, Hiroyuki Iwamura, Kiyohito Takada, Shinji Hagiwara, Yasuhiro Shimada. FUJIFILM Corporation, Tokyo, Japan.

Introduction: FF-10832 is a liposome suspension optimized by Fujifilm nanotechnology containing 0.5 mg/ml GEM, cholesterol, HSPC, N-MPEG-DSP. FF-10832 is expected to improve GEM PK and have strong anti-tumor effects. The PK and anti-tumor effects of FF-10832 were studied in mice and human pancreatic cancer xenograft models. Methods: Single-dose murine plasma PK of FF-10832 1 mg/kg was compared to GEM 240 mg/kg. In vivo activity of GEM 240 mg/kg and FF-10832 1-5 mg/kg IV once weekly were compared in human pancreatic cancer murine xenograft models; 2 subcutaneous (Capan-1 [GEM-sensitive] and BxPC-3 [GEM-resistant]), and 1 orthotopic (SUIT-2) model. The active form of GEM (GEM triphosphate [dFdCTP]) inhibits DNA synthesis. dFdCTP tissue concentrations following FF-10832 4mg/kg and GEM 240mg/kg were compared in these models. Results: An extended plasma t½ (10.6 vs. 2.9 hours), lower clearance, and smaller volume of distribution were observed with FF-10832 vs. GEM, which correlated with greater dose exposure achieved with
FF-10832 compared to GEM (AUC_{0-24} 18600 vs. 7300 hr-ng/mL). FF-10832 demonstrated increased in vivo activity in SUTT-2, Capan-2, and BxPC-3 models, at significantly lower doses compared to GEM. In the Capan-1 and BxPC3 models, FF-10832 showed dose-dependent tumor growth suppression with FF-10832 4 mg/kg and 5 mg/kg superior to that of GEM (p<0.001), respectively. FF-10832 11 weekly doses in the SUIT-2 mouse model was 80% reduction of tumor volumes compared to 20% for vehicle and GEM 240 mg/kg vs. 13% and 60% for 2 and 4 mg/kg FF-10832, respectively. Median survival time was 60 (FF-10832 2 mg/kg) and 73 (GEM 240 mg/kg) vs. 26 days (vehicle) (p<0.001). Median survival was not reached at FF-10832 4 mg/kg. In all models, FF-10832 2 mg/kg (equivalent efficacy to GEM 240 mg/kg) showed no body weight gain suppression. The dFdCTP tumor/bone marrow AUC ratio was significantly higher following FF-10832 (dFdCTP AUC ratio) compared with non-liposomal GEM (0.8). Conclusions: FF-10832 is a stable liposomal GEM formulation demonstrating potent anti-tumor efficacy in solid tumor models with a favorable pharmacokinetic profile compared to non-liposomal GEM. Increased exposure achieved at lower GEM doses may potentially result in superior efficacy and a more tolerable safety profile for FF-10832 compared to non-liposomal GEM.

#5150 Designing of a novel strategy for cancer gene therapy by selective delivery of Adenovirus-based toxin. Nadir Arber, Ilana Boustanai, Shiran Shapira, Dina Kazanov. Tel Aviv Sourasky Medical Center, Tel Aviv, Israel.

Introduction: Aberrant activation of the Ras pathway exists in many human tumors (Lung ~25%, pancreatic (PC) ~95% and colorectal cancers (CRC) ~50%). Development of numerous direct and in direct Ras inhibitors have failed. This lead us to think differently; we exploit the hyperactive Ras pathway rather than try to inhibit it. We previously had reported that recombiant adenoviruses, carrying a pro-apoptotic gene (PUMA) under the regulation of Ras-responsive elements suppressed the growth of cancer cells harboring hyperactive Ras. Moreover, replacing the PUMA gene with more potent toxins (bacterial MazF-MazE toxin-antitoxin unique regulated system), the potency of this killing strategy was significantly improved. P53 is a classical tumour suppressor mutated in many tumors. Herein, the WT p53 is use to protect normal cells from any systematic toxin toxic. Aim: To establish a tight control of the toxin expression in malignant cells, and the anti-tumor in normal cells dual system based on the Ras and p53 responsive elements Methods: Adenoviral vectors were designed carrying the toxin (PY4-MazF-mcherry) and the antitoxin (RGC-MazE-GFP) based on the Ras and p53 responsive elements, respectively. Those two constructs were cloned into a “first generation” ΔE1/ΔE3 human type 5 adenoviral vector. Virus particles were produced, their titer was calculated by the End-Point Dilution Assay and their potency was tested in vitro. Cell death was measured qualitatively by using the fluorescent microscopy and was quantified by the enzymatic MTT assay. SHP77/Ras_{mut}/p53^{−/−}, H2030/Ras_{mut}/p53^{−/−}, H1650/Ras_{mut}/p53^{−/−} and H1975/Ras_{mut}/p53^{−/−} lung cancer cell lines were used for testing the potency of the system. MIA-PACA2, Colo357, Panc-1; Ras_{mut}/p53_{mut}, and BxPC3; Ras_{mut}/p53_{mut} PC cell lines were tested as well. Different ratios of toxin-antitoxin (2:1, 1:1 and 1:0.5) were used. Results: Massive cell death was induced in a dose-dependent manner (70% with a titer of 7.5 MOI in lung cells with mutated Ras compared to 21% in cells with WT Ras). Similar results were obtained with tumor former cancers by fluorescence microscopy. Similarly, in PC cells, with mutated Ras showed 50% cell death in a dose-dependent manner with a titer of 15 MOI, compared to 18% in cells with WT Ras. Ratio of 1:0.5, showed decrease in the mortality of the mutated Ras cells expressing WT p53; 36% with a titer of 7.5 MOI. These results indicate that the antitoxin indeed actively protects cells with wt p53. Conclusion: Outside the box cancer gene therapy by exploiting active Ras and P53 pathways are effective in vitro. The low toxicity in WT Ras as well as WT p53 expressing holds promise for effective and safe therapy.


Merrimack Pharmaceuticals, Inc., Cambridge, MA.

Nal-IRI, a liposomal formulation of irinotecan, is designed for extended circulation relative to non-liposomal irinotecan and to exploit leaky tumor vasculature for enhanced drug delivery to tumors. Following tumor deposition, nal-IRI is taken up by phagocytic cells followed by irinotecan release and conversion to its active metabolite, SN-38. Sustained inhibition of topoisomerase 1 (TOP1) by extended SN-38 exposure is hypothesized to enable superior anti-tumor activity compared to traditional TOP1 inhibitors. Topotecan, another TOP1 inhibitor, is an approved second-line treatment for small cell lung cancer (SCLC). Here, we evaluate the anti-tumor activity of nal-IRI compared to irinotecan and topotecan in preclinical models of SCLC including those that have been pre-treated with therapeutics utilized clinically to treat SCLC. Anti-tumor activity of nal-IRI, irinotecan, or topotecan was evaluated based on tumor volume assessments in DMS-53, DMS-114 and NCI-H1048. Conclusions: Nal-IRI demonstrated anti-tumor activity in xenograft models of SCLC at clinically relevant dose levels, and resulted in complete or partial responses in DMS-53, DMS-114, NCI-H1048 and a PDX model in comparison with irinotecan or topotecan, which each had limited tumor growth control. Furthermore, Nal-IRI demonstrated anti-tumor activity in tumors that progressed following treatment with topotecan, and demonstrated significantly greater anti-tumor activity than both topotecan (p<0.0001) and irinotecan (p<0.0001) in NCI-H1048 tumors (8/8 complete responses) that had progressed on prior carboplatin plus etoposide treatment. These results support the further clinical development of nal-IRI versus IV topotecan in patients with SCLC that progressed on or after prior platinum containing therapy.

#5152 Cancer stem cell targeted exosomes for the treatment of metastatic breast cancer. Golan Kribria, 1 Katelyn E. Lee, 1 Erika K. Ramos, 1 Simo Huanga, 1 Clifford V. Harding, 1 Jan Lötvall, 1 Huiping Liu, 1 2Department of Pathology, School of Medicine, Case Western Reserve University, Cleveland, OH; 3Kreting Research Centre, Department of Internal Medicine and Clinical Nutrition, University of Gothenburg, Gothenburg, Sweden.

Introduction: Therapy resistance and metastasis remain the two biggest challenges of breast cancer that result in a majority of cancer deaths. Cancer stem cells (CSCs) are a subpopulation of cancer cells that can initiate new tumors, remain refractory to conventional therapies, and spread throughout the body. In order to effectively block metastasis, the present study aims to develop novel therapeutics that specifically target breast CSCs and spare normal cells. While traditional drug discovery represents one of the promising future directions, it is necessary to overcome the shortcomings of synthetic nanoparticles, including bio-instability and off-target toxicity. Here, we aim to bio-engineer the naturally produced cell-secreted small vesicles, exosomes (~100 nm diameter), for a novel therapeutic delivery system. Methods: Exosomes from the human mesenchymal stem cells (MSCs) were isolated by differential ultracentrifugation, and characterized by multiple approaches including transmission EM, Immunoblotting and immunofluorescence assays to confirm the size, shape and presence of exosomal markers CD63, CD81 and LAMP2B. Exosomes were engineered to express a peptide ligand on its surface to specifically recognize and target CSCs, and the presence of the ligand was confirmed by western blot, flow cytometry (FCM) and transmission EM. Results: Exosomes carrying a pro-apoptotic gene (PUMA) under the regulation of Ras-responsive elements, exosomes specifically targeted breast CSCs compared to normal cells with absent expression of the ligand-binding protein. The developed exosomes can effectively deliver candidate miRNAs into the recipient cells and knock down the genes important for functional CSCs. The in vivo targeting of CSCs and therapeutic applications of the exosomes using patient-derived human-inmouse breast tumor xenograft models are underway. Conclusion: It is expected that exosome-based miRNA therapy will have high biocompatibility, low toxicity, high specificity, and promising efficiency in preclinical and clinical applications to reduce breast cancer metastasis and improve patient survival.

#5153 Tunable Drug Conjugates: a differentiated drug conjugate (DC) platform. Sara C. Hickey, 1 Alex R. Nanna, 1 Hanh N. Nguyen, 1 Leslie Ofori, 1 Jonathan Rader, 2 Michael K. Rood, 3 Jutta Wanner, 4 Doug S. Werner, 4 Blank-Bio, Inc., Jupiter, FL, 5The Scripps Research Institute, Jupiter, FL, 6Genomics Institute of the Novartis Research Foundation, San Diego, CA.

We have established a set of technologies called Tunable Drug Conjugates (TDCs) that will differentiate from other DCs via a rapid payload release/rapid systemic clearance approach intended to quickly drive high toxic “payload” concentrations within tumor cells while minimizing toxicities to patients. TDCs use pro-
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prietary Silicon-based linker chemistries (Silinkers) in novel Payload Cassettes (PCs) that enable the rapid and uniform release of multiple and/or mixed payloads both in the endosome after internalization and within the necrotic microenvironment of tumors. PCs can incorporate multiple copies of the same payload or optimal ratios of different therapeutic agents that work together synergistically to kill cancer cells. A second technology - a Dual variable Domain-Fab (DVDFab) targeting capability provides a proprietary means of specifically targeting a broad array of cell surface proteins. DVD-Fab containing TDCs should be cleared from the body within hours, enabling better management of side-effects. Our Silinker system is differentiated by the exquisite and tunable sensitivity of the linkers to cleavage under the modestly acidic conditions encountered in the endosomal and lysosomal compartments of a cell, and in the microenvironment of solid tumors. Good stability at the physiological pH (and in human plasma) compares to rapid cleavage at relevant lower pHs as demonstrated using model systems. Silinkers exhibit good stability at 37°C in pH 4 buffer and show novel functionalization with the folic acid analog as the targeting ligand and vinblastine analogs as the payload(s). We used single and PC drugs conjugates to show cellular and in vivo proof-of-concept. We tested our TDCs in cell lines overexpressing FAR and showed that cytotoxicity was blocked by excess folic acid. Subsequent in vivo studies, using a nude mouse xenograft model, showed good tumor activity for these prototype TDCs. BB-01 (a single payload construct) showed a clear dose response and had cures in 3 out of 5 mice after 24 days on a 3 μmol/kg TIW schedule. BB-03 (a triple PC construct) shows essentially complete tumor regression compared to modest inhibition by BB-01 at doses that deliver equivalent amounts of payload, demonstrating the synergistic potential of the PC concept to enhance the activity. We have also synthesized DVD-Fab TDCs. DVD-Fabs contain a reactive chemical group for seamless connection with our Silinkers and PCs. We will describe conjugation chemistries and present cellular and in vivo Xenograft data for prototype DVD-Fab TDCs.

#5154 Enhancing tumor blood perfusion using orally active heparin to increase the distribution of anticancer therapeutics inside the tumor tissue. Jeong Uk Choi,1 Taslim A. Al-Hilal,2 Seung Woo Chung,3 Jisuk Yun, 1Hwanjae Nam,1 Myungyoon Lee,1 Hinja Hwang,1 Kyunghin Kim,3 Youngro Byun,2 Young-seok Cho,2 Seong Wook Lee.3 1Graduate School of Convergence Science and Technology, Pohang University of Science and Technology, Pohang, Korea; 2Division of Diagnostic Science and Technology, College of Medicine or College of Pharmacy, Seoul National University, Seoul, Republic of Korea; 3Texas Tech University Health Science Center, Amarillo, TX; 2Dong-A Socio Group ST Pharm Co., Ltd., Seoul, Republic of Korea.

Here, we suggest in the first time that the cancer associated thrombosis (CAT) might be one of the major factor which limit the tumor blood perfusion and drug distribution inside the tumor tissue by reducing the effective radius of blood vessels. In order to prevent CAT and achieve enhanced drug distribution, we developed orally active heparin to be combined with other anti-cancer therapies. We injected dye conjugated fibrinogen and chitosan nanoparticles to mice which were bearing highly thrombotic tumor to visualize CAT and nanoparticle distribution inside the tumor tissue. When CAT is prevented by heparin, we found that nanoparticle distribution and accumulation were significantly enhanced compared to the group not treated with heparin. This results signify that the CAT can limit the tumor perfusion and prevention of CAT can enhance the drug accumulation and distribution. We used highly thrombotic cancer models such as melanoma and pancreatic cancer to evaluate the therapeutic efficacy of the doxorubicin containing nanoparticles and the combination of oxaliplatin and gemcitabine when they are combined with heparin. We have found that when heparin is combined with each therapy, their tumor inhibition efficacy increased about 25% and also confirmed that this synergistic effect comes from enhanced drug distribution inside the tumor tissue by preventing the CAT using anticoagulant. These results were supported by histologic analysis. We've validated our hypothesis by showing that CAT can limit the tumor perfusion, which in turn brings reduced drug distribution inside the tumor tissue. We also confirmed that our oral heparin could enhance the tumor perfusion and the efficacy of anti-cancer therapies by preventing the CAT in the tumor tissue.

#5155 Targeting nucleolin with doxorubicin-containing nanoparticle induces a significant tumor growth inhibition in an orthotopic animal model of standard of care-resistant mesothelioma. Nuño André Fonseca,1 Vera Moura,2 Fabiana Coelli,1 Daniela Pesce,1 Francesco Cardile,1 Claudio Pisano,3 Sérgio Simões,1 João Nuno Moreira1.

Mesothelioma is a malignant tumor of the mesothelium, the thin lining of the surface of the body cavities and the organs within, and is often associated with asbestos exposure. It has a poor prognosis, with a mean overall survival of 8.8 months. Chemotherapy has been used for malignant pleural mesothelioma as an adjuvant treatment after surgical resection (often palliative) or in combination with other therapies. A combination of cisplatin and pemetrexed remains as the current standard of care, with only a 5-9% survival rate. Herein, a novel targeted treatment for mesothelioma, based on a doxorubicin (DXR)-containing nanoparticle functionalized with the nucleolin-binding F3 peptide (named PEGASEMP), is proposed. It relies on nucleolin deregulated overexpression in cancer cells and the tumor microenviron- ment. Selective targeting DXR-containing nanoparticles were performed in an orthotopic model of human mesothelioma. Mesothelioma cells harvested from a patient with disease progression who undergone chemotherapy, were stably transduced with luciferase-reporter gene, and orthotopically injected infrapleurally into female immunocompromised mice. Animals were randomly allocated to different treatment groups: vehicle, peptide-targeted DXR-containing nanoparticle (PEGASEMP) at 5.6 or 7 mg of DXR/kg alone (q7dx5w); cisplatin at 4.0 mg/kg alone or combined with PEGASEMP at 5.6 mg of DXR/kg (q7dx5w). A control group administered with the standard of care, a combination of cisplatin at 4.0 mg/kg (q7dx5w) plus pemetrexed at 100.0 mg/kg (q2dx3x5w) was also included. Bioluminescence was monitored weekly with live imaging using IVIS Spectrum In Vivo Imaging system. Peptide-targeted DXR-containing nanoparticle at 7.0 mg/kg enabled a tumor growth inhibition, by the end of the treatment, that was 183-fold higher than the standard of care. Moreover, treatment with DXR-containing nanoparticle targeting nucleolin, either at 5.6 or 7.0 mg/kg, enabled a 10- or 66-fold reduction of tumor burden, respectively, relative to non-treated mice. Conversely, treatment with the standard of care did not show any effect on tumor growth inhibition (being similar to the group injected with vehicle) nor on tumor burden. Importantly, DXR-containing nanoparticle targeting nucleolin, at the highest tested dose, enabled a marked decrease of the incidence of tumor infiltration into the chest cavity, as well as of the presence of severe lung lesions, as compared with the standard of care (57.1% versus 100% and 14.3% versus 50%, respectively). Overall, the novel mechanism of action associated with DXR-containing nanoparticle targeting nucleolin, enables a significant benefit in terms of efficacy (and safety) in the treatment of mesothelioma, as compared with the current standard of care, thus supporting future clinical evaluation.

#5156 A synthesized RNA aptamer targeting and inhibiting non-small cell lung cancer. Hanlu Wang,1 Meng Qin,2 Zhihua Ren,1 Irvin S.Y. Chen,2 Yong-Ping Jiang1. 1Biopharmaceutical R&D Center, Peking Union Medical College of Chinese Academy of Medical Science, Beijing, China; 2Department of Pathology, School of Medicine, University of California, University of California Los Angeles, Los Angeles, CA.

A specific RNA aptamer (RA16) targeting non-small cell lung cancer (NCI-H460 cells) was previously reported by in vivo SELEX. The aptamer RA16 was identified specifically targeting and inhibiting human NCI-H460 cell proliferation in vitro, as well as xenograft tumors in vivo. In this study, we synthesized RNA aptamer (syn-RA16) and make comparison studies with transcribed RNA aptamer (trans-RA16) in vitro in respect of the specific targeting and direct inhibitory activity towards NCI-H460 cells. To characterize the targeting specificity in vitro, biotin-labeled syn-RA16 and trans-RA16 were incubated with NCI-H460 cells, other tumor cell lines or normal control cells. Specific fluorescence binding was observed only for NCI-H460 cells, but not for HEK293T, Hela, and Jurkat cells or buffer control. Moreover, binding affinity of KD values to the NCI-H460 cells for syn-RA16 and trans-RA16 were determined at 24.7±2.28 nM and 12.14±1.46 nM (n=3), respectively, by flow cytometry using streptavi- nin-PE labelling, indicating that the specific targeting binding affinity for syn-RA16 was highly retained, as trans-RA16. On the other hand, syn-RA16 aptamer, like trans-RA16 aptamer, was also capable of inhibiting non-small cell lung cancer cell (H460) proliferation in a dose-dependent manner. At 300 nM, syn-RA16 aptamer suppressed cell proliferation by 84.5%, compared by 86.8% with trans-RA16. The IC50 for syn-RA16 was determined at 11.8±1.1 nM, while trans-RA16 was at 105.7±1.2 nM (n=4). Interestingly, both syn-RA16 and trans-RA16 exhibited no any inhibitory effect on HeLa and Jurkat cell lines even at concentration of 600 nM. In conclusion, a specific entire RA16 RNA aptamer was synthesized. The synthesized RA16, like transcribed RA16 aptamer, demon- strated the specific targeting ability and inhibitory activity to human NCI- H460 cells. The syn-RA16 could be potential to develop a diagnostic kit or treatment for NCI-H460 cell-related non-small cell lung cancer in the clinic.

Results of our study will impact broadly the field by developing more effective control, suggesting that PLGA improves the delivery of 3bNChalcisidethecell. Compared to control, we demonstrated that 3bNChalc was successfully encapsulated into PLGA NPs and they showed decreased in viability and proliferation when revealed that 3bNChalc PLGA NPs are negatively charged and they tend to repel and smooth spherical shape. In addition, zeta potential and mobility values of 3bNChalc loading, encapsulation efficiency, and drug release was determined using a combination of Dynamic Light Scattering (DLS) and Scanning Electron Microscopy (SEM). Cell viability and proliferation of PC3 and 22RV1 prostate cancer cell lines treated with 3bNChalc NPs and control were assessed using the MTS assay. 3bNChalc PLGA NPs were found to have a particle size of 250 nm and smooth spherical shape. In addition, zeta potential and mobility values revealed that 3bNChalc PLGA NPs are negatively charged and they tend to repel each other avoiding the tendency to flocculate. Moreover, 22RV1 cells treated with 3bNChalc PLGA NPs showed decreased in viability and proliferation when compared to control. We demonstrated that 3bNChalc was successfully encapsulated into PLGA NPs. In addition, our results showed that 3bNChalc PLGA NPs decreased the viability of 22RV1 prostate cancer cells when compared to control, suggesting that PLGA improves the delivery of 3bNChalc inside the cell. Results of our study will impact broadly the field by developing more effective and less toxic PLGA NPs based therapies.

Experimental and Molecular Therapeutics: Oncogenes and Tumor Suppressors as Therapeutic Targets

Anti-tumor activity of entrectinib, a highly potent pan-TRK, ROS1 and ALK inhibitor, in molecularly defined acute myeloid leukemia. Patrick Fagan, Maria Barrera, Colin Walsh, Danielle Murphy, Ian Silverman, Robert Shoemaker, Ge Wei, Zachary Hornby, Gary Li, Kristen M. Smith, Ignita, Inc., San Diego, CA.

Acute myeloid leukemia (AML) is the most common form of acute leukemia in adults and comprises a heterogeneous group of diseases. A number of recurrent leukemic gene mutations or chromosomal rearrangements have been identified and clinically validated in AML. However, nearly 50% of AML patient samples lack any known AML driver mutations. Advances in molecular diagnostics have resulted in the identification of novel and actionable gene mutations or chromosomal rearrangements in these AML samples. The ETV6-NTRK3 fusion gene is one such rearrangement identified in samples from patients with AML. Fusion of ETV6 sequences to the tyrosine kinase domain of NTRK3 results in constitutive activation of the TRKckinase and ETV6-NTRK3 expression has emerged as one of the key oncodrivers for leukemogenesis. Constitutive activation of TRK family tyrosine kinases has also been detected in a wide range of solid tumor and hematologic malignancies, including lung, colorectal, salivary gland, sarcoma, thyroid, glioblastoma, melanoma, anaplastic large cell lymphoma (ALCL) and Philadelphia-like acute lymphoblastic leukemia. Entrectinib (RXDX-101) is an investigational, orally available, brain-penetrant, highly potent and selective kinase inhibitor with low nanomolar potency against TRKA/B/C, ROS1 and ALK gene activations. In these studies, we have demonstrated sensitivity to entrectinib in AML cell lines with endogenous expression of the ETV6-NTRK3 fusion gene. Entrectinib treatment blocked cell proliferation and survival in vitro with sub-nanomolar EC50 values. Phosphorylation of the ETV6-TRKCKinase protein as well as phosphorylation of known TRKCK downstream signaling effectors was inhibited by entrectinib treatment in a dose-dependent manner. Sensitivity to entrectinib was dependent on expression of the TRKCK fusion protein. In xenograft models, entrectinib treatment at clinically relevant doses resulted in tumor regression, which was accompanied by elimination of residual cancer cells from the bone marrow. The clinical relevance of activated tyrosine kinase signaling in AML has been validated by the efficacy of selective tyrosine kinase inhibitors. Our preclinical data demonstrate the potential of entrectinib as an effective treatment for patients with NTRK rearranged acute myeloid leukemias and provide rationale for the clinical development of entrectinib in molecularly defined hematologic malignancies. Entrectinib is currently the subject of an ongoing global Phase 2 basket study to enrolling patients across multiple tumor histologies containing TRKOSI or ALK fusions.


Acutemyeloidleukemia(AML)isthemostcommonformofacuteleukemia in adults and comprises a heterogeneous group of diseases. A number of recurrent leukemic gene mutations or chromosomal rearrangements have been identified and clinically validated in AML. However, nearly 50% of AML patient samples lack any known AML driver mutations. Advances in molecular diagnostics have resulted in the identification of novel and actionable gene mutations or chromosomal rearrangements in these AML samples. The ETV6-NTRK3 fusion gene is one such rearrangement identified in samples from patients with AML. Fusion of ETV6 sequences to the tyrosine kinase domain of NTRK3 results in constitutive activation of the TRK kinase and ETV6-NTRK3 expression has emerged as one of the key oncodrivers for leukemogenesis. Constitutive activation of TRK family tyrosine kinases has also been detected in a wide range of solid tumor and hematologic malignancies, including lung, colorectal, salivary gland, sarcoma, thyroid, glioblastoma, melanoma, anaplastic large cell lymphoma (ALCL) and Philadelphia-like acute lymphoblastic leukemia. Entrectinib (RXDX-101) is an investigational, orally available, brain-penetrant, highly potent and selective kinase inhibitor with low nanomolar potency against TRKA/B/C, ROS1 and ALK gene activations. In these studies, we have demonstrated sensitivity to entrectinib in AML cell lines with endogenous expression of the ETV6-NTRK3 fusion gene. Entrectinib treatment blocked cell proliferation and survival in vitro with sub-nanomolar EC50 values. Phosphorylation of the ETV6-TRKCKinase protein as well as phosphorylation of known TRKCK downstream signaling effectors was inhibited by entrectinib treatment in a dose-dependent manner. Sensitivity to entrectinib was dependent on expression of the TRKCK fusion protein. In xenograft models, entrectinib treatment at clinically relevant doses resulted in tumor regression, which was accompanied by elimination of residual cancer cells from the bone marrow. The clinical relevance of activated tyrosine kinase signaling in AML has been validated by the efficacy of selective tyrosine kinase inhibitors. Our preclinical data demonstrate the potential of entrectinib as an effective treatment for patients with NTRK rearranged acute myeloid leukemias and provide rationale for the clinical development of entrectinib in molecularly defined hematologic malignancies. Entrectinib is currently the subject of an ongoing global Phase 2 basket study to enrolling patients across multiple tumor histologies containing TRKOSI or ALK fusions.

type melanomas has been associated with other skin cancers, such as cutaneous squamous cell carcinoma due to MAPK pathway paradoxical activation mediated by CRAF. There is therefore a clinical need for novel agents targeting the MAPK pathway that do not have these undesirable properties. Here we present, REDX05358 that demonstrates subnanomolar binding affinity for BRAF and CRAF with high selectivity profile against a panel of 468 kinases that exhibits negligible paradoxical activation due to inhibition of both RAF nononers and dimers. As a result, REDX05358 not only inhibits MAPK signalling in BRAF V600E mutant tumor cells, but also in those harbouring NRAS and KRAS mutations. Furthermore, REDX05358 does not induce feedback reactivation of the pathway through its ability to sustain inhibition of MAPK signalling in CRC cell lines. Correspondingly, profiling of REDX05358 in a panel of CRC, melanoma and NSCLC cell lines shows it has potent anti-proliferative activity in cell lines harbouring BRAF or RAS mutations. REDX05358 is an orally bioavailable, well tolerated small molecule that has demonstrated in vivo efficacy in BRAF V600E CRC xenograft model. In contrast, first generation inhibitors such as Vemurafenib and dabrafenib have been reported to be ineffective in this genetic background in CRC cell lines and patients. Thus, we have developed a pan RAF inhibitor with unique pharmacological properties enabling it to have utility in treating BRAF and RAS mutant cancers.

### #5161 Inhibition of TBK1/IKBKE impacts tumor growth of KRAS-mutant colorectal cancers.

Susanna Stinson, 1 Jinhuair China, 2 Zhicheng Jia, 1 Kate S. Walker, 2 Zhi-Hua Cui, 1 Charles S. Fuchs, 2 David Dornan, 1

Inhibition of TBK1/IKBKE impacts tumor growth of KRAS-mutant cancers. We therefore synthesized the Pyrrole-Imidazole polyamide conjugate targeting KRAS oncogenic mutations is a promising approach against KRAS mutated colorectal and pancreatic cancers. Hiroki Nagase, Atsushi Takatori, Takayoshi Watanabe, Nobuko Koshikawa, Jason Lin. Chiba Cancer Ctr. Research Inst., Chiba, Japan. Although a tremendous amount of studies has been made to directly target oncogenic drivers, such as RAS and MYC, no drug is clinically available because of difficulties to develop RAS- or Myc-targeted anti-cancer therapeutics due to the peculiar 3D surface topology. One of major limitations of targeting the RAS pathway may be intrinsic or acquired resistance as seen in the other molecular target therapy. A new approach that directly target driver genes may provide a promising therapeutic approach in MM and other PERK-dependent tumors. PERK (PKR-like endoplasmic reticulum kinase) is a serine-threonine kinase associated with endoplasmic reticulum membrane. Together with ATF6 and IRE1, PERK is a key effector of the Unfolded Protein Response (UPR), a network of signaling pathways that ensures protein homeostasis in the endoplasmic reticulum. Multiple Myeloma (MM) and other protein-secreting tumors are highly dependent on UPR for survival, and inhibition of PERK may be an effective strategy to inhibit growth of these tumors. Here we describe the in vitro and in vivo properties of NMS-E194, a novel potent and selective ATP-competitive PERK inhibitor belonging to the arylsulfonamide chemical class. NMS-E194 inhibits PERK kinase with a Ki of ca 1.5 nM in biochemical assay and possesses high selectivity towards a panel of 54 kinases. When tested in cell proliferation assays, NMS-E194 is preferentially active on multiple myeloma (MM) and diffuse large B cell lymphoma cell lines. In mechanism of action studies, NMS-E194 shows a bi-phasic behaviour: at low nanomolar doses it activates PERK, causing ATF4 accumulation, while at >200 nM it inhibits PERK and downstream pathway, inducing apoptosis. NMS-E194 possesses a favourable in vitro ADME profile and has promising PK properties in the mouse. NMS-E194 demonstrated strong anti-tumor activity following oral administration at 25 mg/kg daily to immune deficient mice harbouring luciferized KMS-11 cells in a disseminated growth model of MM, with no overt toxicity. Overall, the data available so far warrant further development of NMS-E194 and support PERK inhibition as a novel therapeutic approach in MM and other PERK-dependent tumors.
analysis suggested non-KRAS binding overall had little contribution to changes at the phenotypic level in colorectal cancer cell lines. We also the anti-cancer event is not only for colorectal but also pancreatic ductal adenocarcinoma (PDAC). Significant tumor reduction was observed in a mouse model of spontaneous PDAC. (LSL-KrasG12D+/Ptf1aCre+/Tgfr2rllox/flox). In animal studies of transgenic KrasG12D mice, treatment at 0.5 mg/kg created a tumor-free state within 3 months. These findings represent a significant step forward in the development of targeted therapies for pancreatic cancer, as well as other cancers with activating mutations in the ras oncogene.

**#5165** Novel Ras inhibitors DC070-547 and ADT-006 potently and selectively inhibit the growth of pancreatic tumor cells harboring constitutively activated Ras by blocking Ras-effector binding and signaling. Tyler E. Mattos,1 Kevin J. Lee,1 Xi Chen,1 Jacob Valiayvettilli,2 Luciana Madeira da Silva,2 Ashley S. Lindsay,3 Adam B. Keeton,1 Bing Zhu,1 Michael Boyd,2 Gary A. Piazza,1 University of South Alabama Mitchell Cancer Institute, Mobile, AL; ADT Pharmaceuticals, Inc., Orange Beach, AL.

Gain-in-function mutations in Ras genes cause constitutive activation of Ras and induction of downstream signaling pathways that drive tumor cell proliferation, survival, and metastasis. Such driver mutations occur in over 90% of pancreatic ductal adenocarcinomas (PDACs), making Ras an attractive cancer target. However, Ras is considered “undruggable,” given the lack of suitable surfaces on the protein for small molecule binding, as well as its high affinity for GTP binding. A phenotypic assay was developed to screen a library of indene derivatives for growth-inhibitory activity in tumor cells harboring constitutively active Ras versus tumor cells with low levels of active Ras. A novel compound series was identified that potently and selectively inhibits the growth of tumor cells with constitutively active Ras, while having minimal effects on tumor cells lacking constitutively active Ras or cells derived from normal tissues. Chemical optimization resulted in a drug development candidate, DC070-547, and several back-up analogs (e.g. ADT-006), which showed strong antitumor activity at doses that do not cause any discernible toxicity in preclinical mouse models. Here we report DC070-547 and ADT-006 inhibited the growth of PDAC cells with IC50 values of approximately 2 nM and 20 nM, respectively, in MIA PaCa-2 cells harboring constitutively active Ras and greater than 350-fold selectivity over BxPC-3 cells, which lack constitutively active Ras. Both compounds also decreased the clonogenic potential of MIA PaCa-2 cells at concentrations that inhibit their growth. Within the same low nanomolar concentration range, treatment of recombinant human K-Ras, MIA PaCa-2 cell lysates, and intact MIA PaCa-2 cells blocked Ras-effector interactions as evident by performing active Ras pull-down assays using GST-RAF1-RBD (glutathione agarose). Treatment of intact MIA PaCa-2 cells also inhibited RAF/MEK and PI3K/AKT phosphorylation within the same concentration range. These results demonstrate that DC070-547 and ADT-006 inhibit PDAC cell growth by blocking Ras-effector interactions, supporting further evaluation of this novel class of Ras inhibitors for the treatment of pancreatic cancer, as well as other Ras-driven cancers.

**#5166** Sensitivity of melanoma cells to a novel class of Ras inhibitors. Kate M. Saville,1 Kevin Lee,1 Tyler E. Mattos,1 Xi Chen,1 Jacob Valiayvettilli,2 Kristy Berry,1 Veronica Ramirez-Alcantara,1 Bing Zhu,1 Adam Keeton,1 Michael Boyd,2 Gary Piazza,1 Ashley S. Lindsay,3 University of South Alabama Mitchell Cancer Inst., Mobile, AL; ADT Pharmaceuticals, Inc., Mobile, AL.

A high percentage of human cancers arise from mutations in the ras gene that encodes an abnormal Ras protein locked in a constitutively active GTP-bound state that promotes tumor cell proliferation, survival, and metastasis. These gain-in-function mutations in the ras gene or constitutive activation of tyrosine kinase receptors upstream of the Ras protein drive tumor cell growth by activating Raf/MAPK and PI3K/AKT signaling pathways. Ras has been an elusive drug target for which no inhibitors are available to treat Ras-driven cancers. Screening a library of indene derivatives in a differential phenotypic assay identified a novel compound class displaying high potency and selectivity to inhibit the growth of tumor cells harboring mutant Ras relative to tumor cells with wild type (WT) Ras. Lead optimization resulted in a drug development candidate (DC070-547) and several back-up analogs (e.g. ADT-006) with IC50 values in the low nanomolar range and selectivity indices of 100 fold or greater to inhibit the growth of tumor cells with constitutively activated Ras relative to tumor cells with low levels of activated Ras. Sensitivity among a large panel of tumor cell lines to this compound class strongly correlated with levels of activated Ras, but did not appear to be limited to a specific ras gene mutation or Ras protein isoform. Here, we report that DC070-547 and ADT-006 potently and selectively inhibit the growth of the human melanoma cell line, SK-MEL-2, harboring a mutation in the ras gene that encodes the constitutively active N-Ras protein with IC50 values of 7 and 25 nM, respectively. In addition, both compounds potently inhibited the growth of the murine melanoma cell line, B16-F10 with WT ras, but harboring a mutation in a tyrosine kinase receptor upstream of Ras, specifically PDGFRα, which results in high levels of active, GTP-bound Ras. This indicates that both compounds have the potential to disrupt Ras signaling, B16-F10 cells were incubated with the compounds before being subjected to Western blotting for phosphorylated signaling molecules downstream of Ras. Treatment reduced levels of phosphorylated c-Raf and MEK at concentrations that inhibit tumor cell growth. These findings support further investigation of this novel class of Ras inhibitors for the treatment of Ras-driven melanoma.

**#5167** Stem-like colorectal cancer cell lines show response to the ERK1/2 inhibitor, SCH772984, alone and in combination with neratinib. Rekha Patil,1 Ning Wei,1 Nan Song,1 Shao-yu Wu,2 S. Kim,1 Patrick G. Gavin,1 Peter C. Lucas,1 Ashok Srivinasa,1 Samuel A. Jacobs,1 Soonmyung Paik,3 John C. Schmitz,2 Kay Pogue-Gelle1,1 NSABP, Pittsburgh, PA; University of Pittsburgh Cancer Institute, Pittsburgh, PA; NSABP and University of Pittsburgh School of Medicine, Pittsburgh, PA; NSABP and Yonsei University College of Medicine, Seoul, Republic of Korea.

Background: We have recently shown an association of colorectal tumor subtypes with differential response to chemotherapies in patients (pts), and to targeted therapy in cell lines. Pts enrolled in NSABP/NRG C0-07 with stem-like tumors had a poor prognosis regardless of stage or treatment, highlighting the importance of finding new treatments for stem cell-like, KRAS mutant (mt) cells. These stem-like (mt) cell lines of the intrinsic inflammatory subtype were sensitive to the combination of MEK-162 and neratinib, but stem-like subtype cell lines were resistant regardless of KRAS mt status. The purpose of this study was to extend our observations to xenograft models and to identify new agents that target the stem-like subtype. Methods: KRAS mt cell lines of the inflammatory (NCI-H747) or stem-like (SW-480) subtype were used in xenograft models to test inhibition of tumor growth by MEK-162 and neratinib, alone or in combination. Five stem-like cell lines with KRAS wt (C2BBE1, H5675T) or KRAS mt (SW480, SW620, HCT116), and two KRAS mt inflammatory cell lines (NCI-H747, SW837) were tested for cell viability after treatment with SCH772984 alone or in combination with neratinib. Results: In vivo analysis showed that treatment of NCI-H747 xenografts with a combination of MEK-162 (3mg/kg) and neratinib (10mg/kg) led to significant tumor regressions compared to treatment with either drug alone (p ≥0.0001 v neratinib alone; p ≥0.002 v MEK-162 alone). In contrast, MEK-162 alone was able to inhibit tumor growth of stem-like cell line (SW480) xenografts (p ≥0.0061 v control) but neratinib was ineffective (p ≥0.145 v control) as a single agent and did not add to MEK-162. Our in-vitro and in-vivo data showed that inhibition of p-ERK directly correlated with sensitivity to the combination, but resistance to inhibition in the inflammatory subtype, SCH772984 significantly inhibited cell viability of both inflammatory (IC50 1-2 μM) and stem-like subtype (IC50 1-2 μM) cell lines. The combination of neratinib (0.125 μM) and SCH772984 (1 μM) was effective at decreasing cell viability by 60-70% in both inflammatory and stem-like cell lines. Inhibition of ERK phosphorylation was correlated with loss of cell viability. Conclusion: The combination of MEK and neratinib work synergistically to decrease tumor growth in inflammatory subtype, SCH772984 decreases the viability of stem-like colon cancer cell lines, and works synergistically with neratinib. The use of SCH772984 alone, but more potently in combination with neratinib, may represent a therapeutic approach for pts with stem-like tumors, a finding that underscores the potential importance of employing subtype analysis in the diagnosis of colon cancer and potentially as a guide to new therapies. Support: PA DoH, which disclaims certain responsibility.

**#5168** KO-947, a potent ERK inhibitor with robust preclinical single-agent activity in MAPK pathway dysregulated tumors. Francis Burrows,1 Linda Kesler,1 Jeffrey Chen,2 Xin Gao,3 Rasmus Hansen,2 Shuangwei Li,1 Carol Thach,2 Levan Darjania,2 Yvonne Yao,1 Yi Wang,2 Ata Zarieh,2 Ke Yu,2 Tao Wu,2 Jingchuan Zhang,2 Dana Hu-Lowe,2 Liansheng Li,1 Pingda Ren,1 Yi Liu,1 Kara Oncology, LaJolla, CA; Wellspring Biosciences, LaJolla, CA.

The Ras/RAF/MEK pathway is a major driver of malignant progression, particularly in cancers arising from mutations in Ras, RAF and NFI. Although both RAF and MEK inhibitors have been approved for treatment of melanoma, their clinical activity is commonly limited by acquired resistance due to reacti- vation of the pathway downstream of their targets. Since the ERK1/2 kinases are the final node in the MAPK signaling pathway, they are not subject to the feed-back reactivation mechanisms that can undermine RAF or MEK blockade, offering the possibility of clinical benefit in settings where earlier drugs are ineffec- tive. Here we describe a characterization of KO-947, a potent and selective
Design and development of a tankyrase inhibitor STP06-1002 as an anticancer therapeutic agent.

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Background: The Wnt/β-catenin signaling pathway plays a pivotal role in numerous biological processes and its dysregulation has been implicated in diverse oncogenic initiation. Abrupt overactivation of the Wnt/β-catenin pathway due to overexpression and accumulation of the β-catenin has been often observed in colorectal cancer (CRC). As a member of PARP family (PARPs isoform), tankyrase (TNKS) regulates stability of the β-catenin destruction complex through Axin poly-ADP ribosylation maintaining homeostasis of level of the β-catenin. Axin is a rate-limiting component of the destruction complex and inhibition of tankyrase stabilizes Axin level to prevent subsequently CRC development by downregulation of the Wnt target genes. We herein report on an orally-active tankyrase inhibitor STP06-1002 with excellent cellular potency, good ADME properties, and in vivo anti-tumor efficacy with moderate safety. Results: The novel tankyrase inhibitor STP06-1002 shows good inhibition activities with IC_{50} 29.9 nM (TNKS1) and 3.7 nM (TNKS2) and its excellent cellular potency with IC_{50} 6.7 nM (TCF/LEF), STP06-1002 also shows excellent selectivity against PARP1 isoform with IC_{50} > 10 μM. STP06-1002 has good ADME properties, particularly low CYP450 inhibition & induction in CYP panel, high permeability, and low hepatic first-pass effect. It also displays good in vivo pharmacokinetics in rats (B.A. [F] >60%) and dogs (27%). No significant toxicity issues are observed from cytotoxicity studies, hERG assay and Ames test. The off-target studies in both Pan Kinase panel (against 75 cancer-focused kinases) and Lead Profiling Screen (against 68 receptors and ion channels) prove its high selectivity. In the xenograft efficacy studies for in vivo proof-of-concept, the dose-dependent effect on Colo205DM (Wnt-dependent, KRAs wild type) shows tumor growth inhibition with 45% and DLD-1 (Wnt-dependent, KRAs mutant) with 60%. In the case of Wnt-independent cell lines HCT116 and RKO as negative controls, both did not show any tumor growth inhibition effects. Dose range finding study of rodent (rat) resulted in maximum tolerated dose of (MTD) 200 mg/kg/day, and the corresponding of non-rodent (dog) MTD of 30 mg/kg/day. Taking these promising preclinical observations in mind, we performed in vivo efficacy dose, MTD and HSTD (30 mg/kg/day) into an extended xenograft model. In light of the above findings, we conclude that STP06-1002 has a promising potential as an anticancer therapeutic agent.
cancers. Both APC and CTNNB1 mutations lead to cytosolic accumulation and nuclear translocation of β-catenin, and therefore constitutive activation of β-catenin/TCF4-mediated gene transcription. As such, the Wnt/β-catenin pathway has emerged as one of the most promising targets for colorectal cancer treatment. Despite tremendous efforts in the past decade, there are no small molecule Wnt inhibitors approved by the FDA for cancer treatment. Recent work in our laboratories has identified a series of benzimidazoles as potent Wnt/β-catenin inhibitors. Here, we show that several benzimidazoles displayed strong activities against Wnt/β-catenin signaling in colorectal cancer cells harboring mutations in CTNNB1 or APC. In particular, SR36324, the lead compound in this series, inhibited Wnt/β-catenin signaling in colorectal cancer HCT116 and DLD-1 cells with IC50 values of 4.1 and 3.7 μM, respectively. Moreover, the benzimidazole compounds exhibited potent activities against colorectal cancer cell proliferation under both standard cell culture conditions (adherent cells in complete medium containing 10% FBS) and conditions designed to enrich for cancer initiating cells. In addition, the benzimidazole compounds had no off-target effects on other pathways (e.g., STAT3 and mTORC1 signaling) in colorectal cancer cells, and were less antiproliferative to non-cancerous cancers. Together, our findings indicate that the benzimidazole compounds are promising candidates for development as novel therapeutic agents for colorectal cancer.


Canonical Wnt/β-catenin signaling is known to be associated with platinum resistance in ovarian cancer in which inhibitors hold promise for the treatment of refractory disease. Phosphodiesterase 10A (PDE10A) is a dual cyclic AMP and cyclic GMP phosphodiesterase isozyme recently implicated in colon cancer. PDE10A inhibition in colon cancer cells by siRNA or small molecule inhibitors increased cGMP levels and activated PKG to inhibit β-catenin signaling. A novel PDE10 inhibitor, ADT-061, was identified by screening a library of indene derivatives, and showed strong antineoplastic activity in the APC+/−/min-FCCC mouse (Lee K et al., unpublished data). Cyclic GMP and phosphodiesterases participate in the ovarian follicular development, although little is known about PDE10A expression in ovaries, especially with regard to a potential role in ovarian tumorigenesis. PDE10A protein was found to be expressed in various established ovarian cancer cell lines at higher levels than immortalized or primary ovarian surface epithelial cells. Pf-2545920, a known PDE10A inhibitor, and ADT-061 inhibited the growth of multiple ovarian tumor cell lines with IC50 values around 20 μM and 0.5 μM, respectively. Both compounds induced apoptosis after 24 h treatment, as measured by PI/Annexin V staining and PARP cleavage. Pf-2545920 and ADT-061 induced phosphorylation of VASP at Ser157 and Ser239 in various ovarian cancer cell lines, indicating activation of cyclic AMP and cyclic GMP signaling, respectively. Treatment also decreased levels of β-catenin and downstream targets of TCF-dependent transcription, including c-MYC, survivin and cyclin-D1. Homozygous knockout PDE10A clones of Ov-90 ovarian cancer cell line obtained using CRISPR/Cas9 showed decreased clonogenic potential, decreased Pf-2545920-mediated VASP phosphorylation and β-catenin, c-MYC and survivin expression. Ongoing efforts are focused on the development of more potent ADT-061 analogues. These observations support further study of a role of PDE10A in ovarian tumorigenesis and the development of ADT-061 or analogs for the treatment of refractory ovarian cancer as well as the prevention of malignant recurrence.

#5175 γ-Mangostin, a natural xanthone derivative targets Wnt signaling pathway in colon cancer cells. Balaji Krishnamachary,1 Dharmalingam Subramaniam,2 Thomas Attard,1 Seth Seppert,3 Shirikant Anant.2 Children’s Mercy Hospital, Kansas City, MO; 2University of Kansas Medical Center, Kansas City, KS.

Background: Colorectal cancer is the third most common cancer in incidence and cause of death in the United States. The current treatment modalities include chemotherapy, radiation, and surgery. Many people are genetically predisposed for colon cancer through mutations in genes such as adenosomatous polyposis coli (APC). While the absence of APC causes aberrant Wnt/β catenin signaling, the APC mutations are found in more than 80% of colorectal tumors. γ-Mangostin is a major bioactive compound present in Mangosteen (Garcinia mangostana) which possesses significant anti-cancer activity. Herein, we investigated the effects of γ-Mangostin on colon cancer growth and elucidated its mechanistic action through Wnt signaling pathway. Methods: HCT116, SW480 and RKO cell lines were used in the study. The effects of γ-Mangostin on cell proliferation were assessed by hexosaminidase and clonogenicity assays. Effects of γ-Mangostin on apoptosis were evaluated by cell cycle and western blot analysis. Moreover the effect of γ-Mangostin on colonospheres formation was also evaluated. Furthermore, the effect of γ-Mangostin on Wnt signaling proteins was evaluated by western blot analysis. γ-Mangostin was also investigated on the HCT116 subcataneous tumor xenograft model implanted in five-week-old male athymic nude mice. Further, the effect of γ-Mangostin was assessed by the specific marker expression in tissue samples by western blot analysis and immunohistochemistry. Results: γ-Mangostin treatment resulted in a dose and time dependent inhibition of proliferation and colony formation on all the three cell lines. Treatment also induced colonic cancer cells to undergo G0/G1 and S-phase arrest. Apoptosis was confirmed by increased levels of Bax/Bcl2 ratio, coupled with a reduction in cyclin D1. γ-Mangostin significantly reduced the number and size of colonospheres. Moreover, γ-Mangostin treatment decreased the expression of Wnt signaling proteins, which suggest that γ-Mangostin inhibits the colon cancer growth through Wnt signaling pathway. To determine the effect of γ-Mangostin on tumor growth in vivo, nude mice harboring HCT116 tumor xenografts in their flanks were administered with 5mg/Kg γ-Mangostin intraperitoneally for 21 days. γ-Mangostin treatment significantly reduced the tumor growth, with notably lower tumor volume and weight. Western blot and immunohistochemistry analyses revealed significant decrease in the expression of β-catenin signaling proteins. Conclusion: Together, these data suggest that γ-Mangostin inhibits colon cancer growth through Wnt signaling pathway. γ-Mangostin may be a potential therapeutic agent for colon cancer.

#5176 E7386, an orally active CBP/beta-catenin modulator, effects tumor microenvironment, resulting to the enhancement of antitumor activity of lenvatinib. Yoichi Ozawa, Yusaku Hori, Kazuhiro Yamada, Yasuhiro Fujiwara, Junji Matsui, Tomohiro Mattsumiya, Takashi Owa. Eisai co., Ltd., Ibaraki, Japan.

E7386, a novel orally active CBP/beta-catenin modulator, has an impact on cancer cells with aberrant activation of Wnt/beta-catenin signaling pathway driven by adenosomatous polyposis coli (APC) mutation or beta-catenin mutation and shows significant antitumor activity in xenograft models. CBP/beta-catenin transcriptional activation has an important role in not only malignancy of cancer cells but also regulation of tumor microenvironment such as fibroblast, pericyte, endothelial cells and immune cells. In this study, we investigated E7386 effect on tumor microenvironment and if it leads to enhance antitumor activity of lenvatinib. Lenvatinib is a potent anti-angiogenic inhibitor targeting vascular endothelial growth factor receptors, fibroblast growth factor receptors and other proangiogenic and oncogenic kinases. Firstly, we tested the combination of E7386 with lenvatinib in Wnt-1 tumor isogenic models, where tumors isolated from MMTV-Wnt1 transgenic mice were inoculated in mice. While E7386 and lenvatinib individually suppressed tumor growth and caused tumor dormancy, the combination resulted in approximately 75% tumor reduction. To clarify the effect on tumor microenvironment, we tested an isograft model of 4T1 murine breast cancer cells which was resistant to E7386 in vitro proliferation. E7386 and lenvatinib individual treatments showed a significant antitumor effect, but the antitumor effect of the combination was significantly superior to that of each mono-treatment. Therefore, we compared effects of E7386 and lenvatinib on tumor microenvironment in immunohistochemical analysis using CD31 Ab as an endothelial marker and alpha-SMA Ab as a pericyte and cancerous fibroblast marker. E7386 significantly decreased alpha-SMA positive cells and microvessel density in tumors. In addition, remaining tumor vessels were not covered with pericytes. In lenvatinib treated tumors, greater reduction of microvessel density was observed than in E7386 treated tumors, but the vessel foci covered with pericytes, which are known to be resistant against VEGF inhibitors, were remained. In combination treatment, most of the tumor vessels disappeared. The enhancement of antitumor activity in the combination was also observed in SEKI human melanoma xenograft model which is relatively resistant to lenvatinib. These data suggest E7386 sensitizes tumor to lenvatinib through the modulation of the tumor microenvironment. Taken together, E7386 shows not only antitumor activity via the effect on cancer cells but also through the modulation of tumor microenvironment, and the treatment of E7386 with lenvatinib is a novel combination therapy to overcome resistance to VEGF inhibitors.
**EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Oncogenes and Tumor Suppressors as Therapeutic Targets**

**#5177** E7386: First-in-class orally active CBP/beta-catenin modulator as an anticancer agent. Kazuhiko Yamada,1 Yusaku Horii,1 Atsumi Yamaguchi,1 Masahiro Matsuki,1 Shuntaro Tsukamoto,1 Akira Yokoi,1 Taro Semb,1 Yoichi Ozaawa,1 Satoshi Inoue,1 Yuji Yamamoto,1 Kentaro Iso,1 Kazukata Nakamoto,1 Hitoshi Harada,1 Naoki Yoneda,1 Atsushi Takekura,1 Masayuki Matsukura,1 Kenji Kubara,1 Takesao Odagami,1 Masao Iwata,1 Akihiko Tsuuraoka,1 Toshimitsu Uenaka,1 Junji Matsui,1 Tomohiro Matsushima,1 Kenich Nomoto,1 Hisayuki Kojii,1 Takashi Owa,1 Eisai co., Ltd., Ibaraki, Japan; 2PRISM Pharma Co., Ltd., Japan.

Carcinogenesis is often accelerated by the aberrant activation of components of Wnt signaling pathway, especially, APC and beta-catenin are frequently mutated in various cancers. Therefore, Wnt signaling pathway is thought to be one of the attractive therapeutic targets. PRI-724 generated by PRISM Pharma is a small molecule inhibitor of beta-catenin and its transcriptional coactivator CREB binding protein (CBP) thereby specifically modulating Wnt/beta-catenin signaling pathway by intravenous continuous infusion. Here we firstly generated orally active small molecular inhibitor, E7386. E7386 disrupted the interaction between beta-catenin and CBP in co-immunoprecipitation assay. E7386 inhibited canonical Wnt signaling pathway (TCF reporter gene activity in LiCl-stimulated HEK-293 and MDA-MB-231 in a dose-dependent manner with IC50 values of 55 nmol/L and 73 nmol/L, respectively. E7386 modulated the expression of Wnt signaling pathway related genes including AXIN2 and other genes, which were down-regulated by artificial knockdown of beta-catenin. PRI-724 is the prototype that indicates that E7386 controls the expression of Wnt target genes through modulation of beta-catenin/CBP interaction. Next we investigated anti-polyposis effect in ApcMin/H11001 mice as an in vivo proof of mechanism model. ApcMin/H11001 mice develops polyps in the intestinal tract caused by the aberrant activation of Wnt/beta-catenin signaling pathway. Oral administration of E7386 significantly suppressed the number of polyposis in a dose dependent manner at the dose range from 8.5 to 50 mg/kg. In addition, E7386 significantly changed the expressions of Wnt related genes in whisker follicle of ApcMin/H11001 mice. Therefore, Wnt/beta-catenin signaling pathway is a new target for the treatment of polyposis and other cancers.

**#5178** CB002, a novel p53 pathway-restoring compound that induces apoptosis through the pro-apoptotic protein NOXA. Liz J. Hernandez Borroto, Shengliang Zhang, David T. Dicker, Wafik S. El-Deiry. Fox Chase Cancer Center, Philadelphia, PA.

p53 is a transcription factor that becomes stimulated upon genotoxic and cellular stress signals ultimately leading to the transcriptional activation of genes involved in processes such as DNA repair, cell cycle arrest, and apoptosis etc. TP53 is the most commonly mutated tumor suppressor and this is associated with cancer initiation, cancer progression and therapy resistance. P53-targeted therapy has proven to be challenging because direct functional restoration of p53 as a transcription factor has been difficult to achieve. We have taken a different approach by investigating small molecules that functionally restore the p53-signaling pathway instead of direct p53 targeting. To date there is no approved drug that restores the p53 pathway in cancer cells with mutant p53. Therefore, we performed a high-throughput screen to identify such molecules. We identified a family of small molecules, CB002 and its structural analogs, as targeted agents in DLBCL cell lines. A beneficial was observed with the combination of CB002 and an early steps in the metastatic cascade by affecting the tumor microenvironment have led to the development of promising small molecule targeting agents. The tyrosine kinase inhibitors, dasatinib and nilotinib (synergism in 2/3 cells) and the BCL2 inhibitor venetoclax (synergism in 3/4 cells). The latter synergism could be linked to the previously mentioned negative trend between TK-216 IC50 values and the presence of BCL2 translocation. Conclusion. The novel small molecule TK-216 presented strong preclinical and clinical development as single agent and in combination.

**#5179** The first in class FLI1 inhibitor TK-216 presents both in vitro and in vivo anti-tumor activity in lymphoma. Filippo Spriano,1 Chiara Tarantelli,2 Eugenio Gaudio,3 Elaine YL Chung,4 Alberto J. Arribas,5 Luciano Cascione,6 Sara Napo,2 Ivo Kwee,2 Andrea Rinaldi,2 Vincenzo Arcuri,7 Emanuele Zucca,2 Anastasios Stathis,2 Katti Jessem,2 Brian Lannuzzi,2 Jeffrey Trotersky,2 Francesco Bertoni1,1 Institute of Oncology Research - IOR, Bellinzona, Switzerland; 2Oncology Institute of Southern Switzerland - IOSI, Bellinzona, Switzerland; 3Oncological Therapies, San Diego, CA; 4Georgetown University, Lombardi Institute, Washington, DC.

Background. ETs transcription factors, such as FLI1 and SPIB, are recurrently deregulated in human lymphomas (Bonetti et al, Blood 2013; Lentz et al, PNAS 2008). The small molecule YK-4-279 inhibits binding of EWSI-FLI1 fusion protein to RHA resulting in growth arrest and apoptosis in Ewing sarcoma cells (Erkaniz et al, Nat Med 2009) and we previously showed that YK-4-279 has in vitro anti-lymphoma activity (Chung et al, AACR 2015). TK-216 is a YK-4-279 clinical derivative that is in phase 1 for patients with relapsed or refractory Ewing sarcoma (NCT02657005). Here, we present extensive preclinical results obtained with TK-216 in lymphoma models. Methods. 56 cell lines [27 diffuse large B cell lymphoma (DLBCL); 10 mantle cell lymphoma; 6 marginal zone lymphoma; 5 anaplastic large T-cell lymphoma; 8 others] were exposed to TK-216 increasing doses for 72h using a Tecan D300e Digital Dispenser and 384well plates. Cell viability was measured with MTT to be in vitro used in were performed in NOD-SCID mice and treatments started with approximately 60 mm3 tumor volumes. Results. TK-216 displayed high activity: median IC50 was 449 nM (95%CI: 367-506). Sensitivity was not affected by the lymphoma cell of origin [B vs T; activated B cell type (ABC) vs germinal center type DLBCL] or MYC and TP53 status. There was a non-statistically significant trend for lower sensitivity in cell lines bearing BCL2 chromosomal translocation (P=0.07, DLBCL only; P=0.06, all cell lines). Anti-tumor activity was mainly cytotoxic as confirmed by performing cell cycle analysis and Annexin V staining in 6 DLBCL cell lines (TM80, U2932, HBL1, OCI-LY-18, WSU-DLCL2, DOHH2 for 24, 48, 72h), in which a time-dependent apoptosis was preceded by G2/M arrest. Anti-tumor activity was confirmed in DLBCL. TM80 xenografts. Compared with control group (n=10), mice treated with TK-216 (100 mg/Kg, BID; n=9) clearly presented a reduction in tumor growth, already evident at day 3 and becoming much stronger with time (D3, D5, D8, D11: P<0.01; D13, P not available since control group had to be stopped due to tumor volume) and a 4 times reduction in tumor volume at D11 (P<0.01). TK-216 was tested in combination with other targeted agents in DLBCL cell lines. A benefit was observed with the combination of TK-216 with the immunomodulator lenalidomide (synergism in 2/3 cells) and the BCL2 inhibitor venetoclax (synergism in 3/4 cells). The latter synergism could be linked to the previously mentioned negative trend between TK-216 IC50 values and the presence of BCL2 translocation. Conclusion. The novel small molecule TK-216 presented strong preclinical and anti-lymphoma activity, which provides evidence for further preclinical and clinical development as single agent and in combination.


Background: In advanced prostate cancer (PCa), cells escape from the primary tumor and enter the bloodstream, preferentially targeting the bone. This results in weakened bones, spinal compressions, fractures and intense pain. Efforts to interfere with early steps in the metastatic cascade by affecting the tumor microenvironment have led to the development of promising small molecule targeting agents. The tyrosine kinase inhibitors, saracatinib and dasatinib, have been shown to inhibit angiogenesis, seed and down-regulators with MTT to be in vivo used in were performed a strong effect on PCa clinical development as single agent and in combination.
When ISFs were combined with Src inhibitors, both phases were increased with a concomitant decrease in S phase cells. In transwell migration studies, all agents significantly decreased the number of cells found on the bottom of the membrane: dasatinib > saracatinib > ISFs. Lower doses of Src inhibitors were needed to produce inhibition of migration when ISFs were added to the treatment. Invasion studies with CDDP and MCL-MLN cells suggest that ISFs can reduce metastatic activity by 39% and 35%, respectively. Therefore, by adding ISFs, lower doses of inhibitors might be used to achieve optimal response and decrease toxicity. Conclusions: In vitro studies suggested that a combination of a Src inhibitors and ISFs, at non-cytotoxic doses, results in greater inhibition of metastatic or invasion activity than either alone. Further studies are needed to determine if this strategy could be used clinically to treat patients with lower doses of small molecule inhibitors by including soy isoflavone concentrates in the treatment regimen.

**#5181 Targeted knockdown of MYC in AML cells using G-quadruplex interacting small molecules.** Megan A. Turnidge,1 Apurvi Patel,1 Justin J. Montoya,1 David W. Lee,1 Daniel H. Wai,1 Vijay Gokhale,1 Laurence Hurley,1 Robert J. Arceci,1 David O. Azorsa,1 University of Arizona College of Medicine - Phoenix, Phoenix, AZ; 2University of Arizona School of Pharmacy, Tucson, AZ.

Acute Myeloid Leukemia (AML) is a disease that occurs when genomic changes alter expression of key genes, causing cells to resume an undifferentiated state, proliferate, and maintain tumor growth throughout the body. Recently, there has been an increase in efforts toward developing therapies that specifically target the protein products of these aberrantly expressed genes. However, many of the proteins are difficult to target because of structural challenges, protein overexpression that requires high drug doses, or mutations that confer resistance to therapy. One potential type of targeted therapy that circumvents these issues is the use of small molecules that stabilize DNA secondary structures called G-quadruplexes. G-quadruplexes are present in the promoters of many potential oncogenes, and have regulatory roles in their transcription. This study analyzes the therapeutic potential of compounds that target the regulatory G-quadruplex in the MYC oncogene, which is commonly misregulated in AML. Treatment of the MYC expressing AML cell lines KG-1 and UT-7epo with the compound QQC-05 resulted in decreased expression of MYC mRNA, as determined by qPCR. Moreover, QQC-05 treatment induced a more significant decrease in c-MYC protein expression than similar treatment with JQ-1(+), as shown by western blot analysis. Treatment of AML cells with QQC-05 also decreased cell viability and increased apoptosis. Concurrent treatment of AML cells with QQC-05 and JQ-1(+) showed an antagonistic effect, indicating potential competition in the silencing of MYC. These results demonstrate that targeting the MYC promoter G-quadruplex in AML cells leads to knockdown of MYC expression and induces apoptosis. These results further support the development of a novel mechanism for targeting key genetic drivers in AML, and lay the groundwork for advances in treatment of other cancers driven by G-quadruplex regulated oncogenes.

**#5182 Targeting abnormal metabolism downstream of MYC in atypical teratoid/rhabdoid tumors.** Sabrina Wang,1 Jeffrey Rubens,1 Sariah Allen,2 Megan A. Turnidge,1 Apurvi Patel,1 Justin J. Montoya,1 David W. Lee,1 Daniel H. Wai,1 Vijay Gokhale,1 Laurence Hurley,1 Robert J. Arceci,1 David O. Azorsa,1 University of Arizona College of Medicine - Phoenix, Phoenix, AZ; 2University of Arizona School of Pharmacy, Tucson, AZ.

Atypical teratoid rhabdoid tumors (AT/RT) are deadly infantile brain tumors in dire need of new, targeted therapies. Recent molecular analysis revealed considerable tumor heterogeneity subdividing AT/RT into 3 distinct groups. The MYC subgroup has a dismal 5 year survival of 18.5%. MYC is known to drive reliance on glutamine for cellular metabolism suggesting that high-MYC expressing neoplasms may be sensitive to glutamine metabolic inhibitors. 6-Azido-5-oxo-L-norleucine (DON) is a glutamine antagonist that decreases reliance on glutamine for cellular metabolism suggesting that high-MYC expressing AT/RT cell lines BT12 and CHLA06 (31.2% of BT12 DMSO controls in G2/M phase vs low c-MYC expression were not affected. Cell cycle analysis demonstrated that DON caused cell cycle arrest in the G2/M phase for the high c-MYC expressing lines BT2 and CHLA06 (31.2% of BT2 DMSO controls in G2/M phase vs 54.5% after 10uM DON treatment and 27.4% vs 48.9% in CHLA06). In contrast the cell cycle was not affected by DON treatment in the cell lines with low c-MYC expression (21.3% DMSO vs 20.2% after 10uM DON in BT37). DON also increased apoptosis in cell lines with high c-MYC and induced DNA strand breaks (Western blot for c-PARP and p-H2A.X respectively). DON significantly improved survival in BT-12 high c-MYC expressing orthotopic mouse xenografts (median survival increased from 21 to 36 days, p<0.005; log-rank test). These data develop the framework for future clinical trials using glutamine antagonists to target the MYC subgroup of AT/RT.

**EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Oncogenes and Tumor Suppressors as Therapeutic Targets**

**#5183 The radioenhancer NBTXR3 brings anticancer efficacy to the cisplatin-based chemoradiation in vitro and in vivo.** Agnès Pottier,1 Sonia Vivet,1 Sébastien Paris,1 Bo Lu2.

NBTXR3 is a radioenhancer composed of functionalized hafnium oxide nanoparticles, designed to enhance the radiation dose deposit within the cancer cells when activated by ionizing radiations. NBTXR3 is intended for single intratumor administration and is currently evaluated in cancer clinical trials involving soft tissue sarcoma, head and neck (H&N), prostate, liver and rectum. Cisplatin (CDDP) is a cytotoxic agent considered to be a radiosensitizer and inhibits the repair of sublethal damage from irradiation. Concurrent chemoradiation using CDDP is the mainstream treatment for high risk H&N, cervix and non-small cell lung cancer patients. We hypothesized that adding NBTXR3 to radiation treatment (RT) may improve significantly anticancer effect of the chemoradiation combination. In vitro, no specific clonogenic toxicity was observed for the cells exposed only to NBTXR3. For the combined treatment CDDP was used at its IC50 concentrations. A marked and enhanced cell destruction (DEF) was observed with the CDDP combined treatment (added 6h or 16h prior to RT) and NBTXR3 (800μM, added 16h prior to RT) at ≥2Gy when compared to the single agent.

**CDDP  Treatment  NBTXR3  Irradiation  FaDu  Cell line (DEF*)  NCI-H460-Luc2 6h  16h  2Gy  1.0±0.06  1.2±0.06  6h  16h  1.4±0.15  1.3±0.25  16h  1.4±0.14  1.6±0.12  16h  1.0±0.12  1.9±0.32  16h  1.2±0.13  1.5±0.17  16h  1.8±0.23  2.6±0.45  6h  4Gy  1.3±0.08  2.2±0.16  16h  2.2±0.24  1.9±0.41  16h  3.1±0.64  3.8±0.48  16h  2.1±0.19  2.9±0.52  16h  2.1±0.31  2.8±0.39  16h  5.3±0.97  7.6±2.76

*For a given radiation dose, the DEF value was obtained by taking the ratio of the survival fraction in the control (radiotherapy alone) and the survival fraction of the treatment. In vivo, NBTXR3 enhanced the fractionated radiation treatment (5x2Gy) when injected intratumorally at 25% of tumor volume. NBTXR3 activated with ionizing radiation in combination with low dose of CDDP (1mg/kg administered 5 consecutive days, 2 hours prior to RT) delayed tumor growth when compared to single agent CDDP in combination with RT. These results strongly support the concurrent chemoradiotherapy regimen with NBTXR3 nanoparticles as an effective radioenhancer to increase the anticancer effect. A new phase I/II trial with H&N cancers patient receiving radiotherapy plus CDDP has been started evaluating the optimal dose, safety and preliminary efficacy of NBTXR3.


Trastuzumab has been widely used for the treatment of human epidermal growth factor receptor 2 (HER2)-overexpressing breast cancer, however, it cannot easily cross the blood-brain barrier (BBB) and is known to increase the incidence of brain metastases. In contrast, lapatinib has a low molecular weight and can cross the BBB and it could be useful to treat brain metastases in patients with HER2-positive breast cancer. To explore the impact of lapatinib on radiation response, we conducted an in vitro experiment using SKBR3 and BT474 breast carcinoma cells exhibiting HER2/neu amplification. Lapatinib down-regulated HER2 and increased HER2/neu expression; this might be used to achieve optimal response and decrease toxicity. Conclusions: In vitro studies suggested that a combination of a Src inhibitors and ISFs, at non-cytotoxic doses, results in greater inhibition of metastatic or invasion activity than either alone. Further studies are needed to determine if this strategy could be used clinically to treat patients with lower doses of small molecule inhibitors by including soy isoflavone concentrates in the treatment regimen.
ulated phosphorylated p-HER2, p-EGFR, p-AKT, and p-ERK. Pretreatment of lapatinib increased the radiosensitivity of SKBR3 (sensitizer enhancement ratio [SER]: 1.21 at a surviving fraction of 0.5) and BT474 (SER: 1.26 at a surviving fraction of 0.5) cells and hindered the repair of DNA damage, as suggested by the prolongation of radiation-induced γH2AX foci and the down-regulation of phospho-H2AX. Individual proteasome (p-DNAPKcs). Increases in radiation-induced apoptosis and senescence were suggested to be the major modes of cell death induced by the combination of lapatinib and radiation. Furthermore, lapatinib did not radiosensitize a HER2-negative breast cancer cell line or normal human astrocytes. These results suggest that lapatinib can potentiate radiation-induced cell death in HER2-overexpressing breast cancer cells, and provide a rationale for the efficacy of radiation functional repair using GFP reporter constructs in BRAFV600E mutant cell lines when treated with vemurafenib and radiation. Additionally, γ-H2AX westerns and nuclear foci staining indicated that vemurafenib pretreatment decreases the tumor cell’s ability to repair DNA double-strand breaks (DSB) in BRAFV600E cell lines. Vemurafenib also appeared to alter the kinetics of formation and resolution of 53BP1 and Rad51 nuclear foci in these cell lines. Evaluation of DSB functional repair using GFP reporter constructs suggests that BRAFV600E induces NHEJ repair, which can be attenuated by vemurafenib treatment. Finally, vemurafenib effectively increases radiosensitivity of BRAFV600E tumors in vivo. From our results, BRAF activating mutation appears to be associated with radioresistance. Vemurafenib selectively radiosensitizes both PTC and ATC cells through inhibition of DNA repair mechanisms. Combining vemurafenib and radiotherapy may improve therapeutic control for BRAFV600E mutant thyroid cancers.

**#5185 Targeting inhibitors of apoptosis in combination with radiation for the treatment of head and neck squamous cell carcinoma.**

Linlin Yang, Bhavna Kumar, Tianyun Li, Thenodoros N. Teknos, Arnav Chakravarti, Terence M. Williams, Ohio State Univ. Comp. Cancer Ctr., Columbus, OH.

Background and purpose: Evasion of apoptosis contributes to radioresistance of head and neck squamous cell carcinoma (HNSCC), calling for novel strategies to overcome apoptotic resistance. Second mitochondria-derived activator of caspase (SMAC) - mimetics are a class of targeted drugs that antagonize selected inhibitor of apoptosis protein (IAP) to block pro-survival signaling, and eventually induce apoptosis. The present study was designed to investigate the radiosensitizing effect of one SMAC mimetic, LCL161, in HNSCC and the underlying mechanisms for radiosensitization. Material and methods: We analyzed the correlation between apoptotic molecules and HPV status in HNSCC via miRNA expression using The Cancer Genome Atlas (TCGA) database, as well as protein expression in 6 HNSCC cell lines by immunoblotting. We also examined cIAP1 expression on a tissue microarray (TMA) of HNSCC tumor specimens, and assessed its correlation with HPV status and patient outcome. Clonogenic survival and WST-1 assays were carried out to explore the potential of LCL161 as a radiosensitizer in HNSCC cell lines. Cell cycle analysis, Annexin-V assays and immunoblotting were performed to investigate effects of LCL161 on radiation induced cell apoptosis. Human tumor xenografts were generated to explore the radiosensitization effect of LCL161 on HPV[-] tumors in vivo. Results: TCGA database analysis and immunoblotting from HNSCC cell lines revealed that expression of cIAP1, Survivin, DIABLO, p56, IK-b, TNF-α, BCL-XL, Caspase-7, and Caspase-8 were elevated in HPV[-] compared to HPV[+] HNSCC tumors. IHC staining showed cIAP1 expression was associated with HPV[-] status (p=0.0239). In addition, cIAP1 expression was significantly higher in HNSCC compared with adjacent normal tissues (p=0.0003). In univariate analysis, cIAP1 was significantly associated with poor overall survival (p=0.0359). LCL161 degraded cIAP1 at nanomolar concentrations but has minimal single-agent cytotoxicity with IC50 range of 32 μM – 95 μM. Interestingly, we found that LCL161 could induce radiosensitization only in HPV[-], but not in HPV[+] HNSCC cells. Mechanistic studies showed LCL161 mediated radiosensitisation was associated with increased cell apoptosis, with enhanced activation of caspases-3, -7, -8, and PARP. Finally, in vivo data showed that LCL161 degraded cIAP1, activated caspases, and profoundly radiosensitized two human HPV[-] HNSCC xenograft models. Conclusion: Taken together, our results demonstrate for the first time that cIAP1 is a potential prognostic and therapeutic biomarker for HNSCC patients. Through targeting cIAP1 and other IAP family members, LCL161 radiosensitizes HNSCC tumors, particularly HPV[-] tumors, via caspase activation and apoptosis induction. Taken together, LCL161 holds promise for future clinical development as a novel radiosensitizer in the treatment of HPV[-] HNSCC tumors.

**#5186 Vemurafenib selectively radiosensitizes BRAF V600E mutant papillary and anaplastic thyroid carcinoma cells.**

Ryan N. Robb, Linlin Yang, Terence Williams. The Ohio State University, Columbus, OH.

BRAF activating mutations act as oncogenic drivers and are highly prevalent in thyroid cancer, occurring in about 60% of papillary thyroid cancer (PTC) as well as 30–40% anaplastic thyroid cancer (ATC). MAPK signaling and treatment resistance is driven by BRAF mutations in thyroid cancer. Among the most common of these mutations is BRAFV600E, which can be selectively inhibited by vemurafenib. Using a panel of PTC and ATC cell lines we assessed how the presence/absence of BRAFV600E impacts radiation sensitivity. We used radiation clonogenic, comet assays, nuclear foci formation, immunoblotting, and mouse xenografts models to determine the effect of vemurafenib on PTC and ATC cells. Analysis of radiation clonogenic implicated higher radiosensitivity in cell lines containing the BRAFV600E mutation as compared to wild-type BRAF. Additionally, forced expression of BRAFV600E in a wild-type thyroid cancer cell line induced radioresistance. Vemurafenib inhibited MAPK signaling in V600E mutant cell lines, but showed no effect in BRAF wild-type cell lines. Vemurafenib pretreatment selectively radiosensitized BRAFV600E mutants in vitro, as assessed by clonogenic assays, with decreased cell survival on DNA repair in BRAFV600E lines when treated with vemurafenib and radiation. Additionally, γ-H2AX westerns and nuclear foci staining indicated that vemurafenib pretreatment decreases the tumor cell’s ability to repair DNA double-strand breaks (DSB) in BRAFV600E cell lines. Vemurafenib also appeared to alter the kinetics of formation and resolution of 53BP1 and Rad51 nuclear foci in these cell lines. Evaluation of DSB functional repair using GFP reporter constructs suggests that BRAFV600E induces NHEJ repair, which can be attenuated by vemurafenib treatment. Finally, vemurafenib effectively increases radiosensitivity of BRAFV600E tumors in vivo. From our results, BRAF activating mutation appears to be associated with radioresistance. Vemurafenib selectively radiosensitizes both PTC and ATC cells through inhibition of DNA repair mechanisms. Combining vemurafenib and radiotherapy may improve therapeutic control for BRAFV600E mutant thyroid cancers.

**#5187 Potent radiosensitization of adenoid cystic carcinoma in a patient derived xenograft model using the MDM2 inhibitor AMG 232.**

Amal Javid,1 Adam J. Swick,1 Lauryn Werner,1 Prashanth Prabakaran,2 Hong Hu,3 Kwangok P. Nickell,1 Irene Ong,1 Emmanuel Sampence,1 Justine Bruce,1 Gregory Hartig,1 Aaron Wieland,1 Jude Cannon,2 Paul Harari,1 Randall Kimple,3 1UW Madison, Madison, WI, 2UW-Madison, Madison, WI, 3April 2017

Purpose: Adenoid cystic carcinoma (ACC) is a relatively rare cancer that typically arises in major or minor salivary gland tissues of the head and neck. There are currently no approved systemic agents for ACC and no established data supporting the delivery of chemoradiation for ACC patients. The scarcity of validated preclinical model systems has hampered research efforts. We report the successful establishment and propagation of an ACC patient derived xenograft (PDX), genomic evaluation of cancer associated mutations, and in vivo response to MDM2 inhibition combined with radiotherapy. Because TP53 mutations are seen in <5% of ACCs, we investigated the role of a small molecule MDM2 inhibitor, AMG 232. Methods: An ACC PDX was established and assessed for common cancer-associated mutations using the Illumina TrueSeq Amplicon Cancer panel. Xenografts were treated with focal radiation with or without AMG 232. Focal radiation was delivered at 0, 2, 5, or 8 Gy x 8 fractions delivered twice weekly, with AMG 232 (50mg/kg) delivered daily by oral gavage. Tumor size was measured by caliper and comparisons between treatment groups made using a repeated measures ANOVA. A TCD50 was calculated by fitting log-transformed data to a dose-response curve and comparing using the extra-sum-of-squares f test. Target inhibition and anti-cancer effect was confirmed via immuno-blotting of tumor lysates, and IHC staining or in situ hybridization of relevant targets within FFPE sections of tumors harvested 2 and 48 hrs post treatment. Results: The histologic characteristics of the primary human tumor are maintained in subsequent murine passages of this ACC PDX. Tumoral profiling determined that the PDX was wildtype for TP53. AMG 232 alone resulted in no tumor growth delay. Combining AMG 232 with radiation delayed tumor growth in a dose dependent manner but did not result in sustained local tumor control. Strikingly, the combination of AMG 232 with RT (including low dose radiation of 2 Gy) produced dramatic tumor shrinkage and potent tumor control three months after the end of treatment. Target inhibition of MDM2 was confirmed by western blot of tumor lysates and IHC of FFPE samples taken 2 hrs post treatment. Antiproliferative and apoptotic effects were confirmed by western blot of tumor lysates and IHC of FFPE samples taken at 48 hrs post treatment. Conclusions: This study suggests that MDM2 inhibition may provide potent radiosensitization in TP53 WT ACC. While additional translational models are warranted, the powerful response profile observed suggests that phase I clinical trial evaluation of this combination is worthy for this challenging malignancy.

**#5188 Molecular radiosensitization in soft tissue sarcomas by telomerase-specific oncolytic adenovirus.**

Tadashi Komatsubara,1 Toshinori Omori,1 Hiroshi Tazawa,1 Kazuhiwa Sugiu,1 Yusuke Mochizuki,1 Yasuyaki Yamakawa,1 Shuhei Osaki,1 Joe Hasei,1 Tomohiro Fujiwara,1 Toshiyuki Kunisada,1 Yasuo Urata,2 Tsoshifumi Ozaki,1 Toshiyoshi Fujiwara1.

1UW-Madison, Madison, WI, 2OncolysBioPharma, Inc., Tokyo, Japan

INTRODUCTION: Treatment options for soft tissue sarcoma (STS) include surgical resection and adjuvant chemotherapy and radiotherapy. Despite the development of combined modality treatments in recent years, a significant proportion of patients with sarcomas respond poorly to adjuvant therapy, lead-
ing to local recurrence or distant metastasis. Therefore, there is an urgent need to develop novel therapeutic strategies for improvement of patient prognoses. We previously reported the therapeutic potential of OBP-301, a telomerase-specific oncolytic adenoviruses, and OBP-702, an armed OBP-301 expressing the wild-type p53 tumor suppressor gene against bone and soft tissue sarcoma cells. Furthermore, anti-tumor effect of OBP-301 and OBP-702 after radiation has been observed. While OBP-702 suppressed the viability of OBP-301-sensitive and -resistant osteosarcoma cells more efficiently, the combination effect of OBP-702 and radiotherapy has been unknown. In this study, we investigated the radiosensitizing effect of OBP-702 against human STS cells. METHODS: We used four human STS cell lines, HT1080 (fibrosarcoma), NMS-2 (malignant peripheral nerve sheath tumor), and SYO-1 (synovial sarcoma). Cells were irradiated 24 h after infection with OBP-301 and OBP-702, and cell viability was assessed by XTT assay 4 days after irradiation. Combined effect of radiation with OBP-301 and OBP-702 was analyzed with the CalcuSyn software (BioSoft). These cells were also analyzed for apoptosis and DNA damage using Western blot analysis. RESULTS: While OBP-301 and OBP-702 showed anti-tumor effect for STS cell lines respectively, HT1080 and NMS-2 were highly resistant to radiation. When combined with radiation, not only OBP-301 but also OBP-702 enhanced the inhibitory anti-tumor effect in all STS cell lines. The calculation of combination index demonstrated additive or synergistic anti-tumor effect in combination therapy. Further analysis revealed that OBP-301 and OBP-702 increased radiation-induced apoptosis in STS cells. Notably, the effect of OBP-702 was additive to the anti-tumor expression and interruption of anti-apoptotic myeloid cell leukemia 1 (MCL1) expression. DISCUSSION: Our study demonstrated that OBP-702 had much stronger anti-tumor effect compared to OBP-301, and sensitized radiotherapy to various types of osteosarcoma cell lines. Currently, the clinical trial of OBP-301 is performed, and preclinically OBP-702 accumulates good therapeutic results in various tumors. Thus, OBP-702 may provide a novel treatment strategy for STSs and wide application of radiotherapy for localized as well as advanced STSs.

#5190 Oncoprotein SET determines the radiosensitivity of hepatocellular carcinoma cell and antagonizing SET augments the effects of radiotherapy via reactivating PP2A-mediated Akt downregulation. Man-Hsin Hung,1 Chao-Yuan Huang,2 Chih-Ting Shih,3 Min-Hsien Tsai,4 Yung-Jen Hsiao,5 Tzu-I Chao,6 Feng-Shu Hsieh,7 Chung-Wai Shiu,8 Kuen-Feng Chen9 1Taipei Veterans General Hospital, Taipei, Taiwan; 2National Taiwan University Hospital, Taipei, Taiwan; 3National Taiwan University Hospital, Taipei, Taiwan; 4National Yang-Ming University, Taipei, Taiwan. Background: SET is a novel oncoprotein with growing evidence suggesting its roles in promoting the survival of hepatocellular carcinoma (HCC) and other types of malignant disease. Notably, pre-clinical data suggesting SET overexpression in cancer cell leaded to the development of resistance to various anticancer medications, and antagonizing SET reversely enhanced therapeutic efficacy. Radiotherapy (RT) is a commonly used anti-cancer modality, but its role for the treatment of HCC is largely limited for the concern of endogenous radioresistance. In this report, we explored the role of SET regarding the radiosensitivity of HCC cells. Furthermore, we explored whether SET serve as a valid target to enhance RT against HCC. Method: A panel of HCC cell lines, including Hep3B, PLC5, HA22T and H3597, were used for in vitro experiments, and the PLC5 subcutaneous xenograft mouse model was used for in vivo testing. HCC cells and tumors were treated with RT and/or SET antagonist and harvested for subsequent experiments. The viability of cancer cells were assessed by sub-G1, colony and sphere formation assay. Molecular events were determined by western blot and PP2A activity. Result: Through manipulating the expression of SET in cancer cells, we showed the crucial role of SET in mediating the effects of RT in HCC cells. Using colony formation and hepatosphere formation assay, we showed that RT significantly reduced the number and size of both tumor colony and sphere formed by Hep3B and PLC5 cells. In contrast, ectopic expression of SET in Hep3B and PLC5 cells abolished the effects of RT. More importantly, SET-knockdown oppositely potentiated the RT-induced growth inhibition in HCC cells. Based on our findings, we hypothesized that targeting SET may exert potential to enhance RT for the treatment of HCC. In order to validate our hypothesis, we tested the effects of RT in combine with a novel SET antagonist, EMQA, developed and characterized in our previous works. As expected, we found that EMQA significantly potentiated the anti-RT effects of RT in vitro and in vivo. Moreover, we found that antagonizing SET to restore PP2A-mediated p-Akt downregulation was responsible for the synergism between EMQA and RT. Conclusion Oncoprotein SET plays a critical role in affecting the radiosensitivity of HCC cells. Using SET antagonist plus RT showed promising results in pre-clinical HCC models, and yields further investigation.

#5191 A combination of Stat3 inhibitor and radiation for the treatment of malignant pleural mesothelioma. Seiji Matsumoto, Hiroshi Doi, Akihiro Fu- kuda, Tohoru Nakamichi, Ayaumi Kuroda, Masaki Hashimoto, Terushia Takuwa, Nobuyuki Kondo, Seiki Hasegawa. Hyogo College of Medicine, Nishinomiya, Hyogo College of Medicine, Nishi

EXPERIMENTAL AND MOLECULAR THERAPEUTICS: Preclinical Radiotherapeutics

#5192 Selection of radiosensitizers based on HRAS mutation in head and neck cancer. Michael M. Fisher, Adam D. Swick, Kwanqop P. Nickel, Randall J. Kimple. Univ. of Wisconsin-Madison, Madison, WI. Purpose/Objective(s): The ability of activated HRAS to promote radiation resistance and predict for sensitivity to potential radiosensitizers was tested in a head and neck cancer model system. Materials/Methods: Mutations in HRAS, KRAS, and NRAS were analyzed using cBioPortal in a head and neck cancer cohort. The Illumina TruSeq Amplicon Cancer panel was used to screen a panel of head and neck cancer cells for mutations in RAS-family genes. Cell growth and radiation survival was assessed using clonogenic survival assay. Immuno- blotts were used to confirm target activation/knockdown in overexpression and knockdown studies. A tumor control dose 50% (TCD50) assay was employed to investigate radiosensitization of a mutated-HRAS head and neck cancer cell line in a flank xenograft model in nude mice. Results: Activating mutations in one of the three RAS genes are seen in 5-10% of head and neck cancer patients. Mutations in HRAS represent over 50% of these. Screening our panel of head and neck cancer cell lines identified a canonical activating mutation in HRAS (i.e. G12V) in SCC22B. Consistent with known roles for activated HRASG12V, SCC22B is relatively insensitive to both cetuximab and radiation. Using both in vitro and in vivo studies, cetuximab exhibited no ability to radiosensitize SCC22B. Cetuximab treatment decreased AKT, but not ERK activation. Direct inhibition pathways downstream of HRAS by selumetinib (MEK/ERK) or BEZ235 (PI3K/MTOR) decreased target protein activation and resulted in significant growth inhibition in a Stat3 inhibitor (BBI-608 : napabucasin). Treatment with either selumetinib or BEZ235 radiosensitized HRASG12V expressing cells (SER 1.3-1.8) but had more modest effects on cells with wildtype HRAS. siRNA knockdown of HRAS radiosensitized SCC22B, but not SCC1 or SCC6 cells relative to non-targeting control. Overexpression of HRASG12V conferred relative radioresistance in wildtype cell lines. In vivo assessment of the radiosensitizing effects of these compounds (TCD50) is pending. Conclusion: More than 5% of head and neck cancers harbor activating mutations in one of the RAS family of genes. These mutations lead to resistance to cetuximab, either as a single agent, or as a radiosensitizer. Inhibition of downstream targets such as the MEK/ERK and PI3K/MTOR pathways can radiosensitize tumors harboring activating mu-

NBTXR3 are hafnium oxide nanoparticles (NPs) used for a single intratumor administration as radioenhancer in combination with radiation therapy (RT) as part of multi-modality of cancer treatment. The size, shape and surface of the NPs have been designed to develop strong interactions with cancer cells - effective cell binding and uptake - and to persist within the tumor mass during the whole RT treatment. The high electron density of the NPs is responsible for an increased probability of interaction with incoming ionizing radiations (when compared to tumor tissues with low electron density) and an increased energy dose deposition within the cancer cells which results in an enhanced tumor destruction when compared to RT alone. NBTXR3 works similarly to the physical mode of action of RT, it does not rely on any biological system or target and constantly amplifies the radiation dose deposition ("on/off" activation). Here, we present the transferability of the approach from one type of cancer to the other by evaluating (i) the feasibility of the intratumor injection (intratumor availability of NBTXR3 nanoparticles by Computed Tomography (CT) or µCT) and (ii) the antitumor efficacy of NBTXR3 exposed to radiation. NBTXR3 NPs demonstrated a good intratumor availability and persistence across all tested tumor models (epithelial or mesenchymal origin), including human PDX tumor models. Notably, in PAC-120 tumor models, as the persistence of NBTXR3 NPs within the tumor mass was observed for more than 50 days which is equivalent to the entire duration of RT treatment. The antitumor efficacy was systematically enhanced in terms of tumor growth delay for animals treated with NBTXR3 and exposed to RT when compared to RT alone. NBTXR3 is currently in clinical trials in seven cancer indications including: soft tissue sarcoma, head and neck (H&N), liver, rectal and prostate. These preclinical studies support the rationale for the development of NBTXR3 across all cancer indications where radiation treatment is used.

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<thead>
<tr>
<th>NBTXR3 intratumor availability and antitumor efficacy</th>
<th>CT or µCT</th>
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<tbody>
<tr>
<td>Sarcoma: HT1080, LPS8073(PDX) A673</td>
<td>x</td>
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<tr>
<td>X-ray sources (antitumor efficacy) 200kV, 150kV</td>
<td>x</td>
</tr>
<tr>
<td>Prostate: PC3, DU145, PAC120(PDX) Calu3, FaDu</td>
<td>x</td>
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<tr>
<td>H&amp;N: HCT116</td>
<td>x</td>
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<tr>
<td>Colorectal: NCI-H460-ux2</td>
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Focal radiation enhances paclitaxel therapy in a mouse model of triple negative breast cancer. Maryland Franklin, Thomas Dailey, Wilbur Luedeke, MD; Bioresearch, Ann Arbor, MI.

Triple negative breast cancer (TNBC) accounts for 15-20% of all breast cancers in the US. In general, patients have poorer prognosis and therapeutic intervention is more challenging due to insensitivity to hormonal and anti-HER2 therapies. Therapeutic options include chemotherapy and/or radiation (RT) treatment. In this work we utilized HCC70, a human TNBC cell line, grown subcutaneously in NSG mice. We investigated whether the combination of focal RT and paclitaxel would improve anti-tumor activity over paclitaxel alone. RT therapy has typically been delivered in low doses over long periods of time. More recently higher fractions of RT are being given in shorter time intervals. Utilizing the Small Animal Radiation Research Platform (SARRP; Xstrahl, Suwanee, GA) we compared a low dose (2.5 Gy) fractionated regimen to a higher dose but less frequent regimen (8Gy, QD3) either as single fraction of paclitaxel or in combination with paclitaxel (15mg/kg weekly). The total cumulative dose of radiation was 24-25 Gy in both radiation regimens. Paclitaxel alone produced moderate activity with a 10 day tumor growth delay and no tumor regressions. Treatment with low dose RT resulted in tumor stasis for ≥68 days, but there were no tumor regressions or tumor free survivors (TFS). Treatment with 8Gy, produced a 75% incidence of confirmed partial regressions (PR) and a 25% incidence of complete regressions (CR) with no TFS. In this group we observed slightly more body weight loss (BWL), more frequent clinical signs, and more frequent need for food and water supplementation than with the fractionated RT regimen. Mean BWL was ~10% with a nadir around 8-10 days post first RT dose. BWL was recovered over the next 1-2 week period. The combination of low dose focal RT with paclitaxel produced activity similar to that with high dose radiation (50% PR, 40% CR, and 1 TFS). Treatment with paclitaxel plus 8Gy RT was highly efficacious, producing an 80% incidence of CRs, all of which remained tumor free at study day 108.

Prostate cancer is the highest incidence non-cutaneous cancer in men, and a significant cause of cancer mortality. Localized disease is treated with surgery or radiotherapy and whilst androgen deprivation therapy can extend survival in the subset of cases that progress, metastatic disease is incurable. We have found that oncogenic drivers of prostate cancer, as well as therapy, lead to changes in the activation of prostate cancer cell stress signaling pathways and in particular the unfolded protein response (UPR). We have previously reported that the androgen receptor sustains the cytoprotective activity of the IRE1-XBP1 arm of the UPR and represses the activation of the PERK/ATF6 arm. Blocking pro-apoptotic activity of the latter arm leads to the activation of all three arms of the UPR, culminating in treatment resistance in surviving cells. In this study we assess whether the activation of the UPR in response to radiation mirrors the immunogenic/inflammatory response, which is known to occur in an initially acute apoptotic phase before resolving to a chronic sustainable level. Further we have evaluated the impact of combining radiation with ONC201, a novel anti-cancer small molecule in clinical trials that is an activator of the UPR, on the viability of prostate cancer. We find that ONC201 has a delayed cytotoxic effect as a single agent, with the impact on viability occurring from 72 hours onwards at low microMolar concentrations. However activation of multiple arms of the UPR occurs earlier and is detectable at 24 hours for ATF4, ATF6 and IRE1-XBP1 at the protein/mRNA levels. This time difference creates a window in which to introduce sequential administration of radiation to test ONC201 as a primer for cytotoxic response radiation. We have undertaken this work in a panel of cell-lines (PC3, DU145 and AR-expressing cell-lines) in which we have also knocked down key regulators (PERK, IRE1, CHOP) of separate arms of the UPR. This represents the first in vitro study to characterise the impact of ONC201 on the radiation-responsiveness of prostate cancer cells.


The aberrant vasculature remains uneven, heterogeneous blood flow and leaky, hemorrhagic blood vessels. Due to the unusual nature of tumor vessels, areas of hypoxia develop that contribute to radiore sistance and inefficiency of therapeutic drug delivery. Our current studies examine the role of nitric oxide synthase (NOS) in tumor vasculature. NOS has been demonstrated to be “uncoupled” in tumors and isolated tumor cells due to reduced levels of tetrahydrobiopterin (BH4), a necessary cofactor, resulting in superoxide and peroxynitrite formation in lieu of NO (Rabender et al 2015 Molecular Cancer Research). NO signaling is critical for vascular function and thus uncoupling of eNOS in tumor endothelial cells may partly explain the poor vasculature found within solid tumors. Having previously demonstrated that NOS can be “recoupled” and NO production restored resulting in superoxide and peroxynitrite formation in lieu of NO (Rabender et al 2015 Molecular Cancer Research). NO signaling is critical for vascular function and thus uncoupling of eNOS in tumor endothelial cells may partly explain the poor vasculature found within solid tumors. Having previously demonstrated that NOS can be “recoupled” and NO production restored.
firms the reduction in tumor hypoxia as well as increased tumor perfusion. Increased tumor perfusion was also demonstrated through enhanced doxorubicin uptake in tumors of mice treated with SP. Lastly, the enhanced tumor oxygenation correlated with more than a 2-fold increase in radiation induced cell killing measured by ex vivo clonogenic assay. These preliminary data demonstrate the potential for continuous tracking of tumors, especially when we take into consideration that SP has been demonstrated to be cytotoxic to both breast and colon tumors. On-going studies are examining the consequences of enhanced drug uptake on both normal and tumor tissue as well as the mechanism behind the vascular normalization.

#5197 Targeting PARP-1 to deliver alpha-particles to cancer chromatin. Laura Pauters1, Kuiyi Xu1, Catherine Hou1, Robert H. Mach3, John M. Maris5, Daniel A. Pryma3, Mehran Makvandi1. 1University of Pennsylvania, Philadelphia, PA; 2Children’s Hospital of Philadelphia, Philadelphia, PA.

Introduction: Neuroblastoma (NB) is a radiosensitive pediatric cancer that develops in the sympathetic nervous system and typically affects children under the age of 10. High-risk NB is associated with a 40% 5-year survival rate. Nuclear enzyme poly (ADP-ribose) polymerase 1 (PARP-1) is overexpressed in high-risk NB, making it an attractive target for alpha-particle therapy. Alpha-particles have a short path length and high linear energy transfer, causing dense ionization across DNA, inducing double stranded breaks that result in cell death. The purpose of this study was to explore a newly developed radiotherapeutic ([211At]MM4) that combines the targeting potential of a small molecule PARP inhibitor (PARPi) with the cytotoxic effects of [211At] in high-risk NB. Methods: In vitro cytotoxicity was performed in a panel of high-risk NB cell lines to evaluate the relative potency of [211At]MM4. Next, DNA damage was assessed by measuring gH2AX foci formation at 1, 4, and 24 hrs after [211At]MM4 treatment. In parallel, PARP-1 expression was measured in response to therapy and cleaved PARP-1 was quantified to assess apoptosis. Cell cycle analysis was performed after treatment to identify therapy related effects. The in-vivo biodistribution of [211At]MM4 was performed alongside ex-vivo autoradiography. Tumor cytology for PARP-1, gH2AX, and Ki-67 was performed in response to treatment to identify therapy related effects. The in-vivo biodistribution of [211At]MM4 revealed rapid tumor targeting at 1 hr and clearance from all tissues at 4 hrs. Ex-vivo autoradiography showed a tumor-muscle ratio greater than 6. Tumor cytology revealed DNA damage measured by gH2AX and PARP-1 expression increased following treatment. Small colonies of proliferating tumor cells were detected after treatment using Ki-67 staining. In vivo therapy efficacy studies revealed low fractionated doses were tolerable and resulted in significant delay in tumor regression. Conclusion: [211At]MM4 is a novel alpha-emitting radiotherapeutic that specifically targets nuclear PARP-1 overexpression in neuroblastoma and incites double-stranded breaks in cancer DNA. The cytotoxic effects of [211At]MM4 have been experimentally validated both in vitro and in vivo; the results of these experiments confirm the therapeutic potential of [211At]MM4 as a viable treatment option for high-risk neuroblastoma.

#5198 Targeted alpha particle therapy for uveal melanoma. Narges K. Tafreshi1, Nella C. Delva1, Christopher J. Tichacek1, Michael L. Doligalski2, Darpan N. Pandya3, Nikunj B. Bhatt2, HyunJoo Kil4, Alan Cuthbertson1. 1Bayer AG, Berlin, Germany; 2Bayer AG, Wuppertal, Germany; 3Bayer AG, Berlin, Germany; 4Bayer AG, Oslo, Norway.

FGFR2 is a transmembrane tyrosine kinase receptor, consisting of three extracellular N-terminal immunoglobulin-like domains which are involved in ligand-binding as well as in receptor dimerization. Ligand-independent activation of FGFR2 signaling either via genomic amplification, gene fusion events, mRNA overexpression, or mutations has been observed e.g. in gastric cancer, colorectal cancer (CRC), and triple-negative breast cancer (TNBC). As such, FGFR2 has been described to be involved in cancer progression, promotion of oncogenesis, neoangiogenesis, as well as resistance to targeted therapies. Overexpression of FGFR2 and relatively low levels of cell surface expression of FGFR2 in normal human tissues renders FGFR2 an attractive candidate to explore targeted alpha therapy (TAT). We describe the generation of a high energy, alpha-particle emitting FGFR2 targeted thorium-227 conjugate (FGFR2-TTC). The FGFR2-TTC consists of a fully human FGFR2 binding IgG1 antibody (BAY 1179470) cross-reactive with mouse FGFR2, covalently linked via an amide bond to a chelator moiety (3,2 HOPO), enabling radio-labeling with the alpha particle emitting thorium-227 (227Th). In vitro cytotoxicity experiments with FGFR2-TTC demonstrated potent activity in the sub-picomolar range compared to a non-targeting control-TTC and a correlation between decrease in cell viability and increasing number of anti-FGFR2 antibodies bound per cell (ABC counts) in a panel of FGFR2-positive cancer cell lines. Upon treatment of cells with FGFR2-TTC, the DNA damage response marker protein γH2AX was up-regulated indicating that the mode-of-action involves induction of DNA double strand breaks. Furthermore, induction of the immunogenic cell death marker calreticulin was observed. Biodistribution studies of the FGFR2-TTC in mouse models, evaluated by whole body autoradiography and acquisition of gamma-spectra specific for thorium-227, demonstrated specific accumulation of thorium-227 in FGFR2-positive tumors and very limited signal in murine organs and tissues. FGFR2-TTC exhibited in vivo tumor growth inhibition after a single dose in mouse xenograft models of CRC (NCI-H716) and gastric cancer (SNU-16). In addition, FGFR2-TTC showed anti-tumor activity in the aggressive murine syngeneic orthotopic 4T1 TNBC model. In summary, FGFR2-TTC has been established as a promising targeted alpha therapy (TAT) for efficacious and selective delivery of alpha emitter-biased radiotherapy in several FGFR2-positive cancer indications. Further exploration for cancer therapy may thus be of interest.

#5200 Preclinical pharmacology of the PSMA-targeted thorium-227 conjugate PSMA-TTC: a novel targeted alpha therapeutic for the treatment of prostate cancer. Stefanie Hammer1, Aasmund Larssen2, Christine Ellingsen2, Solene Geraudie3, Derek Grant4, Baard Indreov5, Oliver von Ahsen1, Alexander Kristian1, Urs B. Hagemann1, Jenny Karlsson2, Roger M. Bjerve6, Olav B. Ryan7, Dominik Mumberg1, Bertolt Kreft3, Alan Cuthbertson1, Bayer AG, Berlin, Germany; 2Bayer AS, Oslo, Norway.

The radiopharmaceutical was well tolerated at even the highest doses and animals did not reach any clinical endpoints, such as weight loss, loss of kidney function or abnormal pathology. Biodistribution studies on MC1R expressing tumor bearing mice revealed tumor selectivity and a combination of renal and hepatic clearance with minimal retention in other normal tissues. A blood PK study in rats showed more than 99% retention in tumor, with minimal retention in all other tissues. A pharmacokinetic study in tumor bearing mice revealed tumor selectivity and a combination of renal and hepatic clearance with minimal retention in other normal tissues. A blood PK study in rats showed more than 99% retention in tumor, with minimal retention in all other tissues.
Prostate-specific membrane antigen (PSMA, FOLH1) is a type II transmembrane glycoprotein of the M28 peptide family that acts as a glutamate carboxypeptidase on various substrates. PSMA is well established as a target antigen in prostate cancer due to its high and specific overexpression on the surface of prostate cancer cells at all tumor stages, including metastatic and hormone-refractory prostate cancer. Weill Cornell Medicine (New York, NY) has been utilizing clinically for many years, with radium-223 leading to overall survival benefits. Targeted alphatherapy (TAT) has an established clinical profile with the successful transition of Ra223, an alpha-particle emitter, from bench to bedside in prostate cancer. Thorium-227 is the immediate precursor for Ra223 via alpha-particle emission. We herein describe the generation of a novel TAT, a high energy, alpha-particle emitting PSA-targeted thorium-227 conjugate (PSMA-TTC). PSMA-TTC consists of a fully human PSMA targeting IgG1 antibody covalently linked via an amide bond to a chelator moiety (3,2 HOPO), enabling radiolabeling with thorium-227 (227Th). PSMA-TTC was prepared in high radiophysical yield and purity and tested for binding affinity to PSMA target (ELISA) as well as PSMA expressing cell lines (FACS). In vitro cytotoxicity experiments were carried out on prostate CA cell lines with different PSMA levels (from 3,000 to 150,000 mAbs bound/ cell). In vivo biodistribution and anti-tumor efficacy were analyzed after i.v. injection of 100-500 kBq/kg at protein doses of 0.14 mg/kg to mice bearing s.c. prostate cancer xenograft models. Additionally, anti-tumor efficacy was evaluated in a PSMA expressing orthotopic bone xenograft model (LNCaP-Luc) monitored by bioluminescence imaging, micro CT and X-ray. PSMA-TTC retains binding affinities to PSMA target and PSMA positive cancer cells similar to the PSMA antibody. Strong in vitro potency and selectivity of PSMA-TTC was shown on different PSMA positive cells. Biodistribution studies in C4-2 xenografts demonstrated specific tumor uptake of PSMA-TTC with a maximum of 50 % of ID/g at t = 72h post dose administration. Selective significant antitumor efficacy was shown for PSMA-TTC in s.c. prostate CA xenograft models with high (C4-2) and medium/low (22Rv1) PSMA protein levels at doses of 250 and 300 kBq/kg. Furthermore, statistically significant prevention of tumor growth was observed after treatment with PSMA-TTC at a dose of 100 kBq/kg in an orthotopic bone xenograft model (LNCaP-Luc). The promising preclinical antitumor activity of PSMA-TTC supports its development for the treatment of patients with metastatic prostate cancer.
Competition with unlabeled FVIIai 10 minutes before 177Lu-FVIIai injection dose per gram (%ID/g) at 1, 4, 24, 72 and 168 hours post-injection, respectively. Houghton, Ryan; Lanning, Dayla; Abdel-atti, Toni; Jun, Christine; Kearns, Jacob; and radiolabeled with the beta-emitting isotopes Lutetium-177 (177Lu) or Yttrium-90 (90Y). CA19-9, and is a promising platform for development of a targeted radioimmunotherapy (RIT). MVT-5873 was conjugated with the chelator CHX-A’-DTPA and radiolabeled with the beta-emitting isotopes Lutetium-177 (177Lu) or Yttrium-90 (90Y) to form the RIT agents MVT-1075 (177Lu-CHX-A’-DTPA-HuMab-SBI) and MVT-1916 (90Y-CHX-A’-DTPA-HuMab-SBI), respectively. The antitumor efficacy of each of the constructs was studied in nude mice bearing BxPC3 human pancreatic tumor xenografts, known to express CA19-9. Methods: The initial dose-finding studies utilized doses of MVT-1075 of 75, 150, 300, or 450 μCi significantly inhibited subQ BxPC3 tumor growth at all dose levels, with sustained suppression with higher doses. MVT-1916 produced similar results. MVT-1075 was selected based on the favorable half-life of 177Lu (6.7 d) and its utility for clinical biodistribution assessments. In an orthotopic BxPC3 tumor model, treatment with a single dose of MVT-1075 at 300 μCi significantly inhibited tumor growth, with Day 20 tumor volume approximately 50% that of the initial starting volume. A third BxPC3 xenograft study evaluated fractionated dosing schedules, (150 μCi x 1, 75 μCi x 2, 50 μCi x 3), with both single-dose and fractionated schedules effectively inhibiting subQ BxPC3 tumor growth. Biodistribution studies in normal mice showed an expected gradually decreasing activity in blood, heart, and lungs, with low uptake in normal pancreas. In subQ BxPC3 tumor-bearing mice, tumor uptake was rapid, reaching 69% ID/g by 24 h and 86% ID/g by 120 h. Otherwise, the biodistribution pattern paralleled that of normal mice, with relative %ID/g values within about ±25% of normal mice across all time points comparing blood, heart, lungs, kidneys, and pancreas, with slightly higher uptake in liver and slightly lower uptake in spleen. Conclusions: MVT-1075 demonstrates promising antitumor activity in a human pancreatic cancer xenograft model, with efficacy shown in both single dose and fractionated schedules. Biodistribution shows rapid and substantial tumor uptake, with much lower uptake in normal organs. These findings support the phase I clinical trial of MVT-1075 in patients with CA19-9 positive pancreatic cancers planned to begin in early 2017.

Poly(ADP-ribose) polymerase inhibitors induce β-radiosensitization through an altered selection of DNA double-strand break repair pathways. Yuji Seo, Keita Yoshizaki, Keisuke Tamari, Yutaka Takahashi, Keisuke Otani, Masahiko Koizumi, Kazuhiko Ogawa. Osaka University Graduate School of Medicine, Suita, Japan.

Background: Systematically reviewing published studies on a broad range of potential radiosensitizers, we previously reported that poly(ADP-ribose) polymerase inhibitors (PARPi) may have a unique radiosensitizing effect to enhance β-components of the linear-quadratic model. In contrast, many other radiosensitizers agents predominantly modified α-components. The aim of this study is to determine the effect of PARPi (olaparib and veliparib) on β-components of DNA double-strand break repair by in vitro and in vivo methods.

Results: We first confirmed that treatments with PARPi PJ34, olaparib, or veliparib two hours prior to and 48 hours after 10 Gy of ionizing radiation (IR) potentiated radiation-induced cell death in human cancer cells HCT116, HT29, H460 and A549 using the colony-forming assay. Tumor regrowth assay with 3-dimensional multicellular spheroids of HCT116 and H460 cells showed that PJ34 potentiated survival delay when compared to control at 10 Gy but not in tumors treated with 10 Gy in 5 fractions. Similar results were found using HCT116 tumor xenograft model in mice. We next examined what cellular events may underlie the radiosensitization using PARPi PJ34 in HCT116 cells. Kinetics of DNA damage responses were first measured by phosphorylation of histone H2AX (γH2AX). PJ34 did not change an initial increase of γH2AX following 10 Gy IR, but γH2AX levels decreased faster in PJ34-treated cells than control cells. Immunocytochemical analysis of Rad51 showed that PJ34 reduced Rad51 foci formations up to 6 hours following 10 Gy IR. IR-induced chromosomal aberrations were quantitated by the C-band of metaphase spreads. More dicentric chromosomes were found in PJ34-treated cells than the control. These data suggested that PARPi did not inhibit total DNA double-strand break (dsb) repair but altered the selection in DNA dsb repair pathways with increased end-joining type(s) of repair compensating for reduced homologous recombination repair. Cell cycle profiles showed sustained 4C DNA cell peaks in cells treated with PJ34 up to 72 hours following 10 Gy IR. Cell cycle markers (cyclin B1 and phosphorylated histone H3) indicated that the 4C DNA peaks of the PJ34-treated cells contained substantial amount of G1/tetraploid cells in addition to G2- or M-phase diploid cells. Additionally, senescence-associated β-galactosidase staining showed that PJ34 promoted radia-
tion-induced premature senescence, while smaller IR-induced sub-G1 populations were found in PJ34-treated cells than the control. Conclusion: PARPi increased IR-induced chromosomal aberrations through the altered selection in DNA dsb repair pathways. The increased quadruplet missrepair enhanced β-components of IR-induced cell death mediated by the increased uncontrolled formation of G1/tetraploid cells and premature senescence. DNA dsb repair pathway selection may be a novel and effective target to elicit β-radiosensitization.
assessed by a LINDMO method (> 90%) at both conditions. Serum stability at 37°C showed radiochemical purity > 82% by ITLC and immunoreactivity > 62% after 5 days, further supporting the use of this product in clinical trials. Conclusions: The conjugation of MVT-5873 with p-SCN-Bn-CHX-A''-DTPA reproduced yields a well characterized broad intermediate (MVT-7814) modified on the heavy chain. Readministration with 5 x 6 Gy at 16 weeks after RT. An increase of 12S-LOX protein was observed in type 2 pneumocytes at 8 weeks after exposure with fibrogenic doses of RT (17.5 Gy and 5 x 6 Gy) compared to low dose RT (5 Gy) or controls (0 Gy). 12S-LOX mRNA was significantly induced in type 2 pneumocytes at 3 days after receiving fibrogenic doses compared to other cell types in murine lung. Chemokine ligands including CCL2, which are responsible for bone marrow-derived macrophages recruitment, were induced in primary pneumocytes treated with RT or 12S-HETE. Treatment with 12S-HETE (150 nm) increased arginase-1 expression significantly in bone marrow-derived macrophages cultured with IL-4. Macrophages expressing active arginase-1 accumulated in murine lungs at 16 weeks after thoracic irradiation with fibrogenic doses. Conclusion: These studies demonstrate that type 2 pneumocytes express 12S-LOX in response to RT with fibrogenic doses of radiation and increased 12S-LOX contributes to the accumulation of alternatively activated macrophages in irradiated murine lungs. 12S-LOX may serve as a novel therapeutic target in mitigating RIPF.

#5207 Breast cancer-specific amplitude modulated radiofrequency electromagnetic fields (AM RF EMF) inhibits brain metastasis of breast cancer. Sambad Sharma, Hugo Jimenez, Fei Xing, Carl Blackman, Boris Pasche, Kou-nosuke Watabe. Wake Forest Univ. School of Medicine, Winston-Salem, NC.

Brain metastasis is evident in approximately 30% of patients with breast cancer and one-year survival of these patients remains less than 20%. The treatment of breast metastasis mainly involves surgical resection in combination with whole brain radiation therapy (WBRT), or stereotactic radiosurgery (SRS). However, patients often suffer from the treatment’s adverse effects and experience recurrent disease. Therefore, there is an urgent need for a less invasive therapy with minimal side effects, and improved therapeutic efficacy. Our current work has established a novel treatment approach for breast cancer brain metastasis at a preclinical level by using amplitude modulated radiofrequency electromagnetic fields (AM RF EMF). We have previously demonstrated that AM RF EMF can be safely administered to patients for prolonged period of time with virtually no toxicity and side effects. The treatment of metastatic breast cancer patients with AM RF EMF significantly impaired the growth of both metastatic and primary tumor (1). In this study, we specifically observed the effect of AM RF EMF in brain metastatic variants of breast cancer cells (231-BrM and SKBr-BrM). We found that AM RF EMF significantly inhibited the growth, stemness and colony forming ability of brain metastatic variants of breast cancer cells. In addition, AM RF EMF inhibited the stemness of radiation resistant variants derived from 231-BrM and SKBr-BrM cells, which indicate the potential of AM RF EMF in treatment of treatment-refractory recurrent disease. Furthermore, AM RF EMF also significantly suppressed the growth of orthopodically and systemically implanted 231-BrM and SKBr-BrM cells in our NOD/SCID animal model. We then performed RNAseq expression profile analysis and found that expression of stemness-associated gene, HMG2A2, was significantly decreased in 231-BrM and SKBr-BrM cells that were treated with AM EMF RF. Interestingly, brain metastatic variants and radiation resistant lines of 231-BrM and SKBr-BrM showed elevated expression of HMG2A2 when compared to their parental cells. Importantly, the treatment of brain metastatic cells of AM RF EMF also sensitized the cells to radiation in a HMG2A2 dependent manner. Furthermore, to isolate the AM RF EMF responsive biomarker, we isolated exosomes from the SKBr-BrM cells that were treated with AM RF EMF, and performed microRNA array. Differentially expressed miRNAs in SKBr-BrM and 231-BrM cells derived exosomes were identified and verified by Taqman PCR. These exosomal microRNAs can serve as excellent biomarkers for determining the effect of AM RF EMF in brain tumors. Therefore, our study has demonstrated that this new approach of treating brain metastasis has a high translational potential considering significant efficacy and minimal side effect shown in pre-clinical and clinical setting.

#5208 The role of platelet-type arachidonate 12-lipoxygenase in murine lungs exposed to a fibrogenic dose of irradiation. Eun Joo Chung, Aylia White, Deborah Citrin. NIH, Bethesda, MD.

Introduction: Ionizing radiation (RT) is employed in the treatment of approximately half of patients with cancer, including those with thoracic malignancies. Exposure of tumor adjacent normal lung may result in injury and fibrosis. Arachidonic lipoxygenase oxidizes arachidonic acid, yielding hydroperoxy derivative including hydroperoxyeicosatetraenoic acids (HPETEs) and the reduced form, hydroxyeicosatetraenoic acids (HETEs). Arachidonate lipoxygenases are classified as 5-, 8-, 12-, or 15-LOX, according to the site of oxygen insertion within arachidonic acid. There are two isoforms of arachidonate 12-lipoxygenases in mammals, the platelet-type (12S-LOX; gene ALOX12), and the epidermal-type (12R-LOX; gene ALOX12B). Recently, we found an increased expression 12S-LOX in murine lungs exposed to a fibrogenic dose of irradiation. We hypothesized that 12S-LOX would be required for radiation-induced pulmonary fibrosis (RIPF). Methods: C57/BL6 mice (n=3 per group) were exposed to thoracic RT (0 Gy, 5 Gy, 17.5 Gy, or 6x5 Gy). Levels of 12S-LOX mRNA and protein were assessed in isolated various cell types from lungs (In Vitro) and in lung tissues (In Vivo) after RT with an immunohistochemical assay and quantitative PCR. Levels of chemokine ligands mRNA were assessed in isolated primary pneumocytes exposed to 17.5 Gy or 12S-HETE. Differential polarization of bone marrow-derived macrophage cultures with/without 12S-HETE was examined by quantitative PCR of polarization markers. Activity of arginase-1 was measured in bone marrow-derived macrophages cultured with 12S-LOX or control. The glutathione (GSH) system is a major redox buffering system. The glutathione (GSH) system is a major redox buffering system.

#5209 Ionizing radiation abrogates pro-tumorigenic effects from cancer-associated fibroblasts in xenografts. Turid Hellevik, María T. Grinde, Jørn Vik, Retil A. Camillo, Ingo Martinez-Zubiaurre. 1 University Hospital of Northern Norway, Tromsø, Tromsø, Norway.

Non-malignant elements of tumors play fundamental roles in both cancer sustainability and responses to therapies. In the context of clinical radiotherapy (RT), all cells that reside in the tumor mass receive radiation, but the impact of cancer-associated fibroblasts (CAFs) to treatment outcomes remains largely unexplored. In this study, we have investigated the effects of radiation on the natural pro-tumorigenic actions of CAFs in animal. Ionizing radiation (IR) was delivered to CAFs either as a single-high dose or fractionated medium doses. Tumor development was compared in xenografts after co-implantation of AS49 lung tumor cells with irradiated or non-irradiated human lung CAFs. Quantitative histology and immunohistochemistry was applied to investigate potential biological mechanisms behind tumor growth regulations. As expected, CAFs showed resistance to radiation-induced cell death, however, the enhanced tumorigenic effects observed with admixed non-irradiated CAFs was nullified. Quantitative determinations of parameters such as desmoplasia, angiogenesis, inflammation, innate immunity or tumor cell proliferation revealed enhanced blood vessel density in tumors established with irradiated CAFs. Experiments to ascertain fate of implanted CAFs showed that both irradiated and non-irradiated CAFs only reside at the implantation site during early stages, suggesting that the regulatory functions of admixed CAFs may take place during the initial phases of tumor formation. In this study, we show that CAFs receiving ionizing radiation lose their pro-tumorigenic potential in vivo, affecting angiogenesis and possibly other mechanisms related to tumor engraftment. This finding represent a previously unknown advantageous effect induced by radiotherapy, which adds to the well-known direct cytotoxic effects on transformed epithelial cells.

#5210 Differential cytotoxic effects of Piperlongumine analogues and their radiotherapeutic response in breast cancer. Nurul Akmaryanti Abdullah, John Moses, Li Chen Han, Sarah Storr, Aula Ammar, Stewart G. Martin. The University of Nottingham, Nottingham, United Kingdom.

Background: Breast cancer is the most common cancer in women in the United Kingdom. The glutathione (GSH) system is a major redox buffering system involved in regulating radiosensitive response of cancer cells. Overexpression has been shown to cause multidrug and radiation resistance. Its modulation may increase radiosensitivity and improve radiotherapy efficacy. Piperlongumine (PL) can reportedly inhibit GSH system function and reduce proliferation of a Northern Norway. Tromsø, Norway. ‘University of Trondheim, Trondheim, Norway.

Non-malignant elements of tumors play fundamental roles in both cancer sustainability and responses to therapies. In the context of clinical radiotherapy (RT), all cells that reside in the tumor mass receive radiation, but the impact of cancer-associated fibroblasts (CAFs) to treatment outcomes remains largely unexplored. In this study, we have investigated the effects of radiation on the natural pro-tumorigenic actions of CAFs in animal. Ionizing radiation (IR) was delivered to CAFs either as a single-high dose or fractionated medium doses. Tumor development was compared in xenografts after co-implantation of AS49 lung tumor cells with irradiated or non-irradiated human lung CAFs. Quantitative histology and immunohistochemistry was applied to investigate potential biological mechanisms behind tumor growth regulations. As expected, CAFs showed resistance to radiation-induced cell death, however, the enhanced tumorigenic effects observed with admixed non-irradiated CAFs was nullified. Quantitative determinations of parameters such as desmoplasia, angiogenesis, inflammation, innate immunity or tumor cell proliferation revealed enhanced blood vessel density in tumors established with irradiated CAFs. Experiments to ascertain fate of implanted CAFs showed that both irradiated and non-irradiated CAFs only reside at the implantation site during early stages, suggesting that the regulatory functions of admixed CAFs may take place during the initial phases of tumor formation. In this study, we show that CAFs receiving ionizing radiation lose their pro-tumorigenic potential in vivo, affecting angiogenesis and possibly other mechanisms related to tumor engraftment. This finding represent a previously unknown advantageous effect induced by radiotherapy, which adds to the well-known direct cytotoxic effects on transformed epithelial cells.
for 1hr. Results: PL, LH91 and LH92 inhibited proliferation and decreased clonogenic survival in both lines. From proliferation assays, MDA-MB-231 cells were more sensitive to PL at 48hr (P = 0.047) and 72hr (P = 0.001) with an IC50 dose of approx. 4μM versus approx. 12μM in T47D’s. There was a significant difference between IC50 doses of LH91 in MDA-MB-231 (5μM) and T47D (11μM) respectively (P = 0.006), however this was not observed in LH92 at treatment (P = 0.178). In contrast, there was no differential sensitivity of LH92 in cell proliferation between the two cell lines at 48 and 72hr. Interestingly, in clonogenic assays, MDA-MB-231’s were more sensitive to LH92 (P = 0.001) with an IC50 of 4μM versus 11μM in T47D’s. There were no significant differences in clonogenic survival between each line after treatment with PL or LH91. No radiosensitisation was observed with PL or PL analogues in MDA-MB-231, however BSO, as a drug comparator, enhanced radiation response with a sensitiser enhancement ratio (SER) of 1.13 at 1% iso-survival. Radiation and drug combinations in T47D cells are ongoing. There were no changes in ROS levels.

**Experiment and Molecular Therapeutics: Preclinical Radiotherapeutics**

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**5211 Hormophysa triquerta polypolen, an elixir that deters CXCR4- and COX2-dependent dissemination destiny of treatment-resistant pancreatic cancer cells.** Sheeja Aravindan,1 Sathish Kumar Ramraj,1 Dinesh Babu Somasundaram,1 Kathiresan Kandasamy,3 Somasundaram N, Thirugnanasambandam,2 Terence Herman,1 Natarajan Aravindan1. 1Univ. of Oklahoma Health Sciences Ctr., Oklahoma City, OK; 2Annamalai University, India.

Therapy resistant pancreatic cancer (PC) cells play a crucial role in tumor relapse, recurrence, and metastasis. Recently, we showed the anti-PC potential of an array of seaweed polyphenols and identified efficient drug deliverables. Herein, we investigated the benefit of one such deliverable, Hormophysa triquerta polypolen (HT-EA), in regulating the dissemination physiognomy of therapy resistant PC cells in vitro, and residual PC in vivo. Human PC cells exposed to clinical radiation (FIR), with/without HT-EA pretreatment were examined for the alterations in the tumor invasion/metastasis (TIM) transcriptome (93 genes, QPCR-profiling). Utilizing a mouse model of residual PC, we examined for the alterations in the tumor invasion/metastasis (TIM) transcriptome, clinically translating to increased morbidity due to hardening, distortion, and pain. Recent evidence has highlighted the importance of repressing metastasis, resulting in the continuous stimulation of GR and ENZ resistance. Therefore, we hypothesized that similar metabolic mechanisms that elicit dihydrotestosterone (DHT) synthesis, which in turn stimulate AR in CRPC, a role for GR in ENZ resistance would be accompanied by a tumor metabolic switch that provides sustained tissue cortisol concentrations which enable GR activation. Here, we found that long-term ENZ treatment sustains tumor cortisol concentrations in models of prostate cancer and patient tissues by using [3H]-cortisol and high performance liquid chromatography. Further investigations with immunoblot, immunoprecipitation as well as in vitro ubiquitination assays in ENZ resistant prostate cancer models demonstrated that autocrine motility factor receptor (AMFR), an ubiquitin E3-ligase, and one of its adaptors, Erielin-2, mediate loss of 1β-hydroxysteroid dehydrogenase-2 (11β-HSD2), which sustains tumor cortisol concentrations which enable GR activation. Additionally, we found that long-term ENZ treatment sustains tumor cortisol concentrations in models of prostate cancer and patient tissues by using [3H]-cortisol and high performance liquid chromatography. Further investigations with immunoblot, immunoprecipitation as well as in vitro ubiquitination assays in ENZ resistant prostate cancer models demonstrated that autocrine motility factor receptor (AMFR), an ubiquitin E3-ligase, and one of its adaptors, Erielin-2, mediate loss of 1β-hydroxysteroid dehydrogenase-2 (11β-HSD2), which sustains tumor cortisol concentrations which enable GR activation. Furthermore, we hypothesized that similar metabolic mechanisms that elicit dihydrotestosterone (DHT) synthesis, which in turn stimulate AR in CRPC, a role for GR in ENZ resistance would be accompanied by a tumor metabolic switch that provides sustained tissue cortisol concentrations which enable GR activation. 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resistant to gedatolisib. 9 out of 28 CRC cells harbor PIK3CA & KRAS co-mutations and 7 out them were shown to be sensitive to gedatolisib. However, HCT115 and LS174T cells were resistant. We identified that resistant cell lines have high activity of GSK3B and TCF7 framshift mutation (G65insC66; H155fs*), which functions as positive regulator of Wnt/b-catenin pathway. The effects of knocking down shRNA demonstrated that GSK3B activity is a key contributor to the cell resistance to gedatolisib. Conclusion: Our results demonstrate that AaR_g agonism causes transient changes in junctional and ABC transporter expression and functionality. The short duration of Regadenoson effects on BBB function may have important implications for its clinical use to enhance drug delivery of chemotherapy and other therapies for CNS diseases.

**#5217 Mechanism of polyspecificity of P-glycoprotein: Substitution of fifteen residues in the drug-binding pocket with tyrosine reveals a negative correlation between substrate size and transport.** Shahrooz Vahedi, Eduardo Chufan, Suresh V. Ambudkar. National Institute of Health (NIH), Bethesda, MD.

P-glycoprotein (P-gp) belongs to the superfamily of ATP-binding cassette (ABC) transporters. This energy-dependent cell surface transporter can pump various chemically dissimilar amphipathic or hydrophobic compounds out of cells. In cancer cells, the expression of P-gp is often linked to the development of multidrug resistance (MDR). Several studies have demonstrated that P-gp can recognize and transport various chemotherapeutic drugs and limit their bioavailability and effectiveness. The drug-substrate binding sites, translocation mechanism, and the signal transduction between drug-binding sites and ATP-hydrolysis at nucleotide-binding domains of this clinically important transmembrane transporter are not yet well defined. Utilizing molecular modeling and mutagenesis techniques, we previously identified key amino acid residues that interact with the most potent inhibitors of P-gp (zosuquidar, tariquidar, and elacridar). We found that replacing key interacting amino acids (Y307, Y953, and Y725) with alanine in the drug-binding pocket switches inhibition by these modulators of ATPase activity to stimulation. The data demonstrated that hydrogen bond interactions are critical for inhibition of ATP hydrolysis of P-gp by modulators. As tyrosine and glutamine residues can function as both hydrogen bond donors and acceptors, we hypothesized that increasing the potential for hydrogen bond interactions by introducing more tyrosine residues into the drug-binding pocket would increase the affinity of substrates for P-gp. To investigate this, we first identified residues in the transmembrane domains that interact with a number of substrates by employing a molecular modeling technique. Then we substituted fifteen conserved residues (11 Phe, 2 Leu, 2 I1u and 1 Met) with tyrosine, creating a mutant we named 15Y. Compared to WT, for the majority of tested substrates, we observed normal transport of fifteen fluorescent substrates by the 15Y-P-gp mutant demonstrating that fifteen mutations in the transmembrane domains is well tolerated in P-gp. Additionally, these changes have no effect on the cell surface expression or total level of the mutant protein and normal conformational changes occur during transport, further confirming flexible nature of transmembrane domains of P-gp. Interestingly, transport of three large-size substrates was significantly decreased. Physicochemical characterization of the substrates revealed a negative correlation between drug transport and molecular size for the tyrosine-enriched P-gp mutant, possibly due to increased hydrogen bond interactions. These data demonstrate that hydrogen bond interactions are substrate- or modulator-specific.

**#5218 Breast cancer resistance protein (ABCG2) and P-glycoprotein (ABCB1) transport irbritinib and may restrict its oral availability and brain accumulation, whereas CYP3A4 limits irbritinib oral availability.** Stephanie van Hoppe,1 Gert-Jan Rood,1 Elis Wagenaar,2 Rolf Spardons,3 Jos Beijnen,2 Alfred Schinkel.1 Nethelands Cancer Institute, Amsterdam, Netherlands;2 Utrecht University, Utrecht, Netherlands.

Introduction: Ibritinib (Imbruvica, PCI-32765) is an orally administered tyrosine kinase inhibitor (TKI) approved by the FDA in 2014 for treatment of B-cell malignancies. Ibritinib is a confirmed irreversible inhibitor of Bruton tyrosine kinase (BTK) with promising clinical activity and tolerability in B-cell malignancies. Ibritinib is a confirmed irreversible inhibitor of Bruton tyrosine kinase (BTK) with promising clinical activity and tolerability in B-cell malignancies. Ibritinib is also a potent inhibitor of P-gp (zosuquidar, tariquidar, and elacridar). Ibritinib has an inhibitory activity towards BTK approximately 10 times lower than its parent compound. We investigated whether irbritinib is a substrate for the multidrug efflux transporters ABCB1 and ABCG2 and whether these transporters influence oral availability and brain and other tissue accumulation of irbritinib. Materials: We used in vitro transport assays to assess human (h)ABC1-, hABC2- or murine (m)Abg2-mediated transport of irbritinib. To study the single and combined roles of Pgp and Abg1a/Abg1b in irbritinib disposition, we used appropriate knockout mouse strains. To assess the role of Cyp3a in the bioavailability of irbritinib we used knockout strains as well as humanized CYP3A4 strains and measured the irbritinib and PCI45227 concentrations at various time points. Results:

**#5215 Time dependent blood-brain barrier modulation by vascular endothelial growth factor to improve CNS drug delivery.** Matthew McCord. National Cancer Institute, Bethesda, MD.

Introduction: Efforts to transiently increase blood brain barrier permeability would facilitate improved CNS delivery of chemotherapy agents. Previous studies demonstrated that vascular endothelial factor (VEGF) increases permeability in traumatic brain injury and ischemic brain disease. Exogenously administered VEGF increases barrier permeability in the non-diseased CNS. In our study, we investigated the role for endogenous VEGF therapy to modulate blood-brain barrier permeability. Methods: For in vitro studies, confluent mouse brain endothelial cells were treated with VEGF (200ng/mL) and TEER was measured 6 hours post treatment and continuing up to 24 hourspost treatment. Conclusions: Our in vitro data warrants further investigation of the applicability of VEGF therapy to increase endothelial cell permeability in animal tumor models, potentially improving delivery of chemotherapeutic drugs.
Ibrutinib was transported reasonably well by hABC81 and hABC2, but not by hABC2 in vitro. Upon oral administration of ibrutinib, Cy3-ubi, Abcg23, Abcb1a/b, and Abcg2;Abcb1a/b mice displayed an increase of 9.8-, 1.3-, 1.6- and 1.1 fold in ibrutinib plasma AU0-20min compared to wild-type mice. At 20 min, preliminary data indicated that the relative brain accumulation of ibrutinib was not significantly altered for the Abcg2-Abcb1a/b mice compared to wild-type mice. 

Conclusion: Abcg2 and Abcb1a/b possibly restrict oral availability of ibrutinib. Based on our preliminary data ibrutinib may not substantially cross the blood-brain barrier. Cy3-ubi restricts the oral availability of ibrutinib and therefore inhibition of this enzyme may be of clinical importance for patients undergoing ibrutinib therapy.

#5219 Characterization of the breast cancer resistance protein BCRP in clear cell renal cell carcinoma. Pascale Fisel,1 Anna Reusel,2 Olga Remter,3 Florian Böttner,1 Stefan Winter,1 Stephan Kruck,2 Steffen Rauch,2 Annemarie N. Nies,1 Jörg Hennenlotter,2 Marcus Scharpf,2 Falko Fend,2 Arnulf Stenzl,2 Jens Bedke,2 Matthias Schwab,2 Elke Schaeffeler1,1 Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology and University of Tübingen, Stuttgart, Germany; 2Department of Urology, University Hospital Tübingen, Tübingen, Germany; 3Institute of Pathology and Neuropathology, University Hospital Tübingen, Tübingen, Germany.

Introduction: The breast cancer resistance protein BCRP, encoded by ABCG2, is a member of the ABC transporter family and is well-known for its contribution to multi-drug resistance in cancer. Although BCRP expression is altered in various cancer types, its role and function in cancer, independent from drug efflux, remains largely elusive. In this study, we aimed to elucidate regulatory mechanisms of ABCG2 expression and its biological relevance in clear cell renal cell carcinoma (ccRCC) as well as associations with response to sunitinib treatment in patients with metastatic ccRCC. Experimental Procedures: Data from the ccRCC cohort of The Cancer Genome Atlas (TCGA) was used to assess ABCG2 mRNA expression (n = 463), DNA methylation (n = 288) and genetic variants present in tumor tissue (n = 326) within the ABCG2 gene region. An independent ccRCC cohort of 64 patients was used for the analysis of ABCG2 mRNA and protein expression by Taq Man quantitative real time PCR or immunohistochemical staining of TMA's, respectively, and for genotyping of 34 variants in the ABCG2 gene by MALDI-TOF mass spectrometry. The influence of miRNAs on ABCG2 expression was investigated in the second cohort and validated in vitro cell culture experiments performed in the renal cancer cell line A498 to confirm the interaction of selected miRNAs with the ABCG2 3' UTR. ABCG2 mRNA data and treatment information of a third ccRCC cohort was used to elucidate potential links of ABCG2 with sunitinib response. Results: In the TCGA cohort low ABCG2 mRNA expression was significantly associated with clinicopathological features (T: P = 0.047; N: P = 0.05; G: P = 0.03) and tumor necrosis: P = 9.76e-06 and with inferior patient survival (HR = 0.22, 95% CI: 0.13-0.32; Plogrank<10e-03). DNA methylation levels within the ABCG2 gene were low and hardly variable in ccRCC patients. Somatic variants were only found in a minority of patients and together with genetic variants had only a moderate influence on ABCG2 expression and patient survival. The link between ABCG2 expression with survival could be confirmed in the second ccRCC cohort. The investigated variants did not significantly impact on mRNA or protein level. miR-210-3p and miR-132-3p were identified to be linked to ABCG2 protein expression in ccRCC patients of the second cohort. The interaction of these miRNAs with the ABCG2 3’UTR was confirmed in vitro cell culture experiments by reporter gene assays. Analysis of a potential association of ABCG2 expression with sunitinib response revealed a better outcome for patients with high ABCG2 levels. Conclusion: In this study, we found that higher ABCG2 expression contributes to longer survival in ccRCC patients and better response to sunitinib treatment. Whereas genetic variants had only a minor impact on ABCG2 variability in ccRCC, epigenetic regulation by miRNAs, in particular miR-210-3p and miR-132-3p, contributed to aberrant ABCG2 expression.

#5220 Chronic exposure to a novel AR-NDT inhibitor induces resistance via a selective metabolism pathway. Jonathon K. Obst,1 Jun Wang,1 David Williams,2 Allen H. Tien,1 Nasrin R. Mawji,1 Yu Chi Yang,1 Raymond J. Andersen,2 Marianne D. Sadrzad,1 BCC Cancer Research Centre, Vancouver, British Columbia, Canada; 2University of British Columbia, Vancouver, British Columbia, Canada.

Background: The androgen receptor (AR) is recognized as playing a crucial role in prostate cancer (PCA) maintenance and progression; therefore its inhibition has been the cornerstone of modern therapy for men who fail primary treatment. Current treatments are initially effective, however resistance ultimately develops and the disease progresses to a lethal form termed castration-resistant prostate cancer (CRPC). Our lab has discovered a new class of molecules (EPI) which inhibit the AR by binding to the N-terminal domain (NTD). The NTD interacts with transcriptional machinery, and its presence is vital for a transcriptionally functional receptor.

We have previously shown that EPI-002 specifically inhibits both full-length AR and constitutively active AR splice variants. The efficacy of EPI-056, the produg of EPI-002, is currently being tested in a Phase I/II clinical trial for CRPC. Here we propose novel resistance mechanisms arising from sustained AR-NTD inhibition, with the goal of preemptively developing backup compounds. Methods: The androgen sensitive prostate cell line (LNCaP-EPI) was generated by passing parental LNCaP cells weekly in media supplemented with EPI-002 beginning in September 2012. In vitro and in vivo studies using LNCaP and LNCaP-EPI cells were employed to confirm biological resistance. A human afinity microarray identified possible resistance mechanisms and was validated using qRT-PCR, western blot and functional studies. In vivo studies of LNCaP-EPI and LNCaP-EPI cells were challenged with EPI-045, an EPI-analog predicted to remain effective in the context of EPI-002 resistance. Results: LNCaP-EPI cells treated with 25 μM EPI-002 displayed similar growth rates to vehicle treatment, both in vitro and in vivo. Conversely, parental LNCaP cells showed significant growth inhibition in response to EPI-002. LNCaP-EPI cells retained sensitivity to anti-androgens and AR knock-down by targeted siRNA, implying functional AR remains essential for growth. qRT-PCR data demonstrated that EPI-002 had reduced ability to block AR mediated gene transcription. Interrogation of microarray data revealed candidate genes (UGT2B family) which were specifically upregulated in the resistant line, and may function to metabolize EPI-002. Supporting this hypothesis EPI-045 which is predicted to be resistant to UGT2B metabolism, was able to significantly inhibit LNCaP-EPI proliferation and AR transcriptional activity. Conclusions: Taken together, these data suggest an AR-specific mechanism of resistance, whereby EPI-002 is preferentially metabolized and removed from the cell. LNCaP-EPI cells remain dependent upon AR signalling, and are sensitive to anti-androgens used clinically as well as a novel EPI-analog. This work highlights the potential for combination or sequential therapy in the context of drug-resistant CRPC.

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#5221 Target engagement approaches to validate small-molecule binders of the pioneering transcription factor FOXA1. Helen L. Evans,1 Shelley K. Doyle,1 Marius S. Pop,1 Becky Leifer,1 Kimia Ziadkhanpour,1 Angela N. Koehler1,2 David H. Koch Institute for Integrative Cancer Research, Cambridge, MA; 3Massachusetts Institute of Technology, Cambridge, MA.

The discovery of small-molecules that disrupt or promote the function of transcription factors, often considered ‘undruggable’ targets, is considered a significant challenge. The development of general and systematic approaches to discover small-molecule probes of transcription factors is therefore of substantial value. In this study, a high-throughput small-molecule microarray (SMM) screen was used to identify initial binders of FOXA1, which were studied using a variety of biochemical approaches to identify and characterize target engagement of these small-molecule probes in a cellular context. Tools for validating FOXA1 as a therapeutic target do not currently exist, although FOXA1 is a known mediator of androgen receptor (AR) action in Prostate Cancer (PCA), and has been demonstrated to both assist and antagonize AR activity, dependent upon the cellular context. Using the SMM technology, potential small-molecule binders of FOXA1 were identified, and enrichment for an interesting class of endogenous metabolites was observed. While an interaction between these compounds and FOXA1 has not previously been reported, they were found to exhibit meaningful inhibition of FOXA1-controlled transcription in prostate cancer cell lines. Compound-anchored beads were used to pull-down FOXA1 in whole cell lysates we demonstrated, using a western blot that the endogenous metabolite engages the transcription factor in this setting. Target engagement in live cells was demonstrated using bi-functional chemical probes, which contain a photo-reactive functional group that transforms transient protein-ligand interactions into stable covalent bonds upon UV irradiation. The probes were also modified with a bioorthogonal, or click chemistry handle, which enabled for assay read-outs using scintescence labeling and mass spectrometry. Using these approaches, we successfully validated the bimolecular interaction between the endogenous metabolites and FOXA1 in cell lysates and live cells, respectively. This report details the first known small-molecule to bind FOXA1 in a cellular setting and suggests a new perspective on the potential role of this class of endogenous compound in prostate cancer. References [1] A. N. Koehler, Cur Opin Chem Biol 2010, 14, 340–346. [2] W. C. Krause et al., Int J Biochem Cell Biol 2014, 54, 49–59. [3] M. M. Pomerantz et al., Nat Genet 2015, 47, 1346–1351. [4] T. Kambe et al., J Am Chem Soc 2014, 136, 10777–10782. Funding Sources The Koch Institute Quinquennial Cancer Research Program (NSF GRFP).

Disregulation of ubiquitin signaling has been linked to many diseases, including cancer. Numerous DUBs are implicated in key pro-cancer pathways, including regulation of HIPPI, a control of RAS signaling, signaling through both canonical and non-canonical NFκB pathways, and regulation of p53 activity. Overexpression of USP14, USP17 and USP28 is linked to promoting non-small cell lung cancer (NSCLC). OTUB1 has shown to trigger lung cancer progression by inhibiting RAS monoubiquitination in KRAS mutant NSCLC. Notably, BAP1 (tumor suppressor) expression decreases in lung cancer tissues although its mRNA levels were not conserved among the dopamine receptor family and the residue with the largest effect is not conserved in any other family member, suggesting the basis of ONC201 specificity. Consistent with competitive inhibition, several residues were within the orthosteric binding site, however, two allosteric residues were also identified. In summary, ONC201 is the first DRD2 antagonist identified to date for oncology and its differentiated receptor pharmacology explains its unique selectivity, anti-cancer activity, and safety that has been observed in clinical trials.


Identification and validation of the cellular target of bioactive hits identified in a phenotypic screen is crucial for their further development into a drug. The gold standard proof for identification of a small molecule’s target is the discovery of mutations that confer resistance. However, selection of drug resistance and subsequent deconvolution of the relevant mutations that confer drug resistance remains time consuming. Therefore, a methodology that can accelerate the drug resistance selection process and that can simplify identification of relevant drug resistance mutations would benefit the drug discovery and development process greatly. Here we report a simple method to rapidly identify the mechanism of action of small molecules with anti-cancer activity. The method utilizes the positive selection of in-frame drug resistance mutations in the target protein of a small molecule derived from the localized genetic variation created by non-homologous end joining (NHEJ) repair of CRISPR/Cas9-induced double strand breaks. In brief, we introduced double strand breaks in the target proteins of selinexor, ispiinesib and tripoliode and treated these cells with the respective drug. Resistance was obtained rapidly and sequencing of resistant colonies revealed known as well as many new drug resistant protein variants. Next, we investigated whether this approach can be applied as a screening strategy on a subset of genes to identify the direct cellular target protein of a small molecule. We therefore, as proof of concept, designed a pooled sgRNA tiling library targeting all PAM sites of multiple genes and applied this to ispiinesib. Drug resistant colonies formed rapidly and were enriched with sgRNAs targeting the ispiinesib binding site in KIF11, ispiinesib’s target protein. Further sequencing of the sgRNA target gene locus revealed drug resistance mutations in KIF11, validating the feasibility of the methodology to uncover the target of a small molecule from a small pool of candidate genes. To conclude, we provide a new method for identification of the cellular target protein and binding site of a small molecule based on positive selection of drug resistant protein variants generated by targeted CRISPR/Cas-induced NHEJ repair.

A novel loop interactions within a parallel-stranded G-quadruplex formed in the human BCL-2 proximal promoter. Guanhui Wu, Clement Lin, Danzhou Yang. Purdue University, West Lafayette, IN.

The human BCL-2 gene contains a 39-bp GC-rich region upstream of its P1 promoter which has been shown to be critical for BCL-2 transcriptional regulation. Therapeutic inhibition of BCL-2 expression can reduce cell proliferation and the apoptotic threshold of tumorigenic cells. Previously, we have reported that the major G-quadruplex (G4) formed in the Pu39 G-rich sequence adopts a stable, parallel-stranded structure with two 1-n tandem repeats and 13 nt long central loop. Parallel G-quadruplexes have been found to be prevalent in promoter sequences; the 13 nt loop in BCL-2 Pu39G4 is the longest in this prototype parallel G-quadruplexes identified in human gene promoters. The structure of this G4 with such a long loop is important because it can enable specific small molecule recognition of this BCL-2 G-quadruplex over other G-quadruplexes and provide a potential basis for rational drug design targeting BCL-2 transcription. Thus, the objective of our study is to determine the molecular structure of the BCL-2 Pu39 G-quadruplex and the molecular interactions of this long loop in physiologically relevant K+ solution by Nuclear Magnetic Resonance (NMR) spectroscopy. Our NMR study shows that the BCL-2 Pu39G4 adopts a well-defined parallel-stranded G-quadruplex structure which contains three G-tetrads stabilized by Hoogsteen hydrogen bonding and three double-chain reversal loops containing 1:13:1 bases. The two 1-n double-chain reversal loops on two edges of the tetrad provide overall stability of the BCL-2 Pu39G4. The central 13 nt double-chain reversal long loop is more dynamic in confor-
mation. Specific interactions of the long loop are observed at both the 5′ and 3′ end with external tetrads as well as the flanking residues, which may also contribute the stable formation of this G4. The middle region of the 13-nt central long loop is highly dynamic and doesn’t show much interaction with the G-quadruplex core. The specific molecular interactions of the unique long central loop are specific for the G-quadruplex. This confirms our previous conclusion that there might be something really different from those in other parallel-stranded structures, e.g., c-myc and VEGF. The specific interactions are supported by our mutational analyses. The results are also supported by the DMS footprinting results which show that the guanines involved in the tetrad formation in this major G-quadruplex are well-protected against DMS methylation. Therefore, the knowledge about the molecular structure of this non-B-DNA conformation of the BCL-2 promoter region is essential for using the G-quadruplex as a target for anticancer drugs, which could be a novel approach to anti-BCL-2 drug discovery in cancer therapy.

#5226 Novel class of potent and selective inhibitors efface MTH1 as broad-spectrum cancer target. Manuel Ellermann,1 Anja Giese,1 Ashley Eheim,1 Stefanie Bunse,1 Roland Neuhaus,2 Jörg Weiske,3 Maria Quanz,3 Andrea Glasauer,4 Fredrik Rahm,5 Jenny Viklund,3 Martin Andersson,2 Tobias Gimman,2 Rickard Forsblom,6 Johan Lindström,7 Lionel Trésaugues,8 Matyas Gorjanacz1. 1Bayer Pharma AG, Berlin, Germany; 2Sprint Bioscience, Huddinge, Sweden; 3University of Southern California, Los Angeles, USA; 4Institute for Clinical Chemistry and Pathobiology, University of Erlangen, Germany; 5Biocenter and Institute of Medical Sciences, University of Gothenburg, Sweden; 6University of Umeå, Sweden; 7Lund University, Sweden; 8Sorbonne University, Paris, France.

We have previously found that ajoene targets multiple proteins in cancer cells and the aim of this project is to identify the specific protein targets of ajoene. To achieve this aim, we have synthesized a “tagged” ajoene analogue containing a biotin functional group. As biotin may alter the biological properties of ajoene, our strategy was to initially synthesize an azide-containing ajoene analogue (calles in azido-ajoene) using our 4-step synthetic route to ajoene analogues previously published. Azido-ajoene was found to retain cancer cell cytotoxicity similar to that of the parent ajoene. We then treated cancer cells (MDA-MB-231 breast and WHCO1 oesophageal cancer) with azido-ajoene to allow transfer of an azide label to the protein targets during thiolysis exchange with ajoene. We then collected lysate from the treated cells and performed an in vitro click reaction with a separately synthesized biotin-alkyne partner which was synthesized via a series of substitution and amide coupling reactions. Click chemistry covalently links biotin to the ajoene-tagged protein via a triazole linkage; and the feasibility of this method was validated by western blot. The biotinylated protein targets were purified using immobilised streptavidin beads and identified by MALDI-TOF mass spectrometry. It is anticipated that knowledge of the specific ajoene protein targets will provide valuable insights into the cytotoxic mechanism of action of ajoene in cancer cells.

#5228 The impact of microtubules on solution conductance and capacitance: implications for the use of AC electric fields in cancer therapy. Jack A. Tuszynski,1 Iara Santelices,2 Douglas E. Friesen,3 Clayton Bell,3 Jack Xiao,4 Vahid Rezania,5 Holly Freedman,6 Cameron Hough,7 Andrew J. Tsung,8 Kiran K. Velpula,9 Karthik Shankar1. 1University of Alberta, Edmonton, Alberta, Canada; 2University of Illinois College of Medicine at Peoria, Peoria, IL; 3University of Illinois College of Medicine at Chicago, Chicago, IL; 4University of Illinois at Chicago, Chicago, IL; 5University of California, San Francisco, San Francisco, CA; 6University of Illinois at Chicago, Chicago, IL; 7University of Texas Medical Branch at Galveston, Galveston, TX; 8University of Illinois College of Medicine at Peoria, Peoria, IL; 9University of Illinois College of Medicine at Chicago, Chicago, IL; 10University of Illinois College of Medicine at Chicago, Chicago, IL; 11Washington University School of Medicine, St. Louis, MO.

Garlic (Allium sativum) is a medicinal plant belonging to the lily family which has been used since ancient times for its beneficial health effects which include protection against infections and cancer. There are a cluster of bioactive compounds found in crushed garlic which contain sulfide or polysulfide functional groups. One of these compounds, ajoene, is able to interfere with biological processes by undergoing thiolysis exchange reactions with biological thiols, for example glutathione and cysteine residues on proteins. Importantly, ajoene is cytotoxic to cancer cells in the low micromolar range.
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bound K-Ras4B but not N-Ras or H-Ras isoforms. Experimental evidence points to the farnesylated hypervariable region (HVR) of K-Ras4B as a major binding domain for CaM. Here, using molecular dynamics (MD) simulations we modeled K-Ras4B HVR interaction with Ca2+/CaM. Initial contacts of K-Ras4B HVR with CaM were determined by the nuclear magnetic resonance (NMR) chemical shift perturbation (CSP) data, which guided major interacting residue pairs between the HVR and CaM. In the simulations, two different topologies of Ca2+/CaM were modeled: CaM with stretched and flexible linkers. CaM has N- and C-lobes connected by a linker, and each lobe has its own hydrophobic pocket. Thus, the hydrophobic farnesyl tail of the HVR can dock into either lobe of CaM. We observed that the HVR strongly interacts with the linker of CaM. The K-Ras4B HVR is highly polybasic with lysine-rich, while the linker region of CaM is negatively charged. The docking of the farnesyl group into the hydrophobic pocket additionally helps to stabilize the HVR/CaM interaction. This added stabilization by the farnesyl is significant in the K-Ras4B/CaM interaction even though the interaction with the Ras catalytic domain is involved. K-Ras4B has only farnesyl modification, while other isoforms bear farnesyl and palmitoyl groups. The additional lipid modification in the HVR would obstruct CaM binding, suggesting CaM’s K-Ras4B-specific action. Our structural model of K-Ras4B/CaM association provides plausible clues to the PI3Kα activation involving the ternary complex. The abundant Ca2+/CaM can bind to the p85 domain of PI3Kα while binding to the K-Ras4B farnesylated HVR, releasing catalytic kinase domain autoinhibition and activating full PI3Kα activity.

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#5230 Targeting two unique end-insertion G-quadruplexes formed in the 3′-end of the PDGFR-β core promoter nucleus hypersensitive element with ellipticine analog, Buket Onel,1 Prashansa Agrawal,2 Megan Carver,1 Laurence H. Hurley,3 Danzhou Yang1. Purdue University, West Lafayette, IN; 2Case Western Reserve University, Cleveland, OH; 3University of Arizona, Tucson, AZ.

Aberant expression of PDGFR-β promotes multiple hallmarks of cancer, and its transcriptional regulation provides an attractive means to inhibit the PDGFR-β signaling pathway. The nucleus hypersensitive element (NHE) region of the human PDGFR-β core promoter has been shown to be responsible in regulating approximately 60% of the PDGFR-β basal promoter activity. Multiple overlapping G-quadruplexes (G4s) have been shown to form in this NHE region. We previously determined the most stable G4 structure formed in the 5′-end of PDGFR-β NHE region. Significantly, an ellipticine analog, GSA1129, has been shown to selectively bind to the G4 formed in the 3′-end NHE region, thereby shifting the dynamic equilibrium toward the 3′-end G4. This selective interaction was shown to result in suppression of PDGFR-β activity in both cancer cell lines and a preclinical animal model. Thus, characterization of 3′-end G4 provides an important basis for understanding the molecular recognition and could facilitate the development of rational drug design. In this study, we used NMR spectroscopy to show that the PDGFR-β NHE 3′-end sequence forms two novel end-insertion intramolecular G4s which exist in a dynamic equilibrium under physiological salt condition. One is the 5′-insertion-G4 with a 3′ non-adjacent flanking guanine inserted into the 5′-external tetrad. We further confirmed that these novel end-insertion G4s can readily form in the 3′-end NHE sequence under physiologically relevant ionic conditions using dimethyl sulfoxide (DMS) footprinting experiments. To elucidate the molecular interactions of ellipticine analog with the 3′-end G4s, we studied the binding of GSA1129 to both of the end-insertion G4s. Significantly, our CD melting studies showed that GSA1129 markedly increased the stability of both end-insertion G4s by about 25°C. Furthermore, 1H NMR titration experiments have shown that GSA1129 selectively binds to the G4 formed in the 3′-end NHE region. The novel end-insertion structures and the dynamic nature of the PDGFR-β NHE 3′-end G4 may make it an effective target for GSA1129 binding and regulation. Our result highlights the dynamic nature of the PDGFR-β NHE 3′-end sequence and the importance of identifying the exact sequence for the formation of the naturally occurring G4 structure.

#5231 Undifferentiated cell maker Rb2LC-N lectin have high affinity to pancreatic cancer cells and residual cancer cells. Osamu Shimomura,1 Tastuya Oda,1 Hiroaki Tateno,2 Sho Tachino,1 Junji Matsui,2 Yuusuke Ozawa,2 Jun Hirabayashi,2 Nobuhiro Ohkohchi,3 1University of Tsukuba, Tsukuba-city, Japan; 2National Institute of Advanced Industrial Science and Technology, Tsukuba-city, Japan; 3Eisai Co., Ltd., Tsukuba-city, Japan.

The glycosylation in cancer cells remains still unclear despite the whole human genome understanding. Recent technical advances give us great awareness about the consequence of the glycosylation. The glycomes of cancer cell surfaces are often unique with aberrant glycans, including sialylation, fucosylation, O-glycan truncation, and N- and O-linked glycan branching. The sugar binding avidity towards molecular materials reveals important structure and characterization. Recent study suggested lectins are more widely accepted that involved in many biological phenomena inside mammalian or as chemical research tools. Despite years of effort to develop cancer therapies, there were no effective molecular targeting drugs for pancreatic adenocarcinoma (PDAC).

One possible reason for these difficulties, PDAC cells are densely covered with numerous carbohydrate moieties. Significantly, an ellipticine analog with the 3′-end sequence forms two novel end-insertion intramolecular G4s which exist in a dynamic equilibrium under physiological salt condition. One is the 5′-insertion-G4 with a 3′ non-adjacent flanking guanine inserted into the 5′-external tetrad. We further confirmed that these novel end-insertion G4s can readily form in the 3′-end NHE sequence under physiologically relevant ionic conditions using dimethylsulfate (DMS) footprinting experiments. To elucidate the molecular interactions of ellipticine analog with the 3′-end G4s, we studied the binding of GSA1129 to both of the end-insertion G4s. Significantly, our CD melting studies showed that GSA1129 markedly increased the stability of both end-insertion G4s by about 25°C. Furthermore, 1H NMR titration experiments have shown that GSA1129 selectively binds to the G4 formed in the 3′-end NHE region. The novel end-insertion structures and the dynamic nature of the PDGFR-β NHE 3′-end G4 may make it an effective target for GSA1129 binding and regulation. Our result highlights the dynamic nature of the PDGFR-β NHE 3′-end sequence and the importance of identifying the exact sequence for the formation of the naturally occurring G4 structure.

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CANCER CHEMISTRY: Chemical Biology and Structure Biology Approach to Study Cancers

#5233  Pancreatic cancer specific glycosylations survey by a panel of lectin staining: TN antigen exposure as a result of O-glycan truncation. Yusuke Ozawa,1 Tatsuya Oda,1 Osamu Shimomura,1 Hiroaki Tateno,2 Jun Hiramabashi,2 Nobuhiro Okohochi,3 *University of Tsukuba, Tsukuba, Japan; 1National Institute of Advanced Industrial Science and Technology, Japan.

The expression of every cell type is predominantly composed of cell-specific glycans, and glycosylation changes in cancer has been used in clinical field as a tumor markers, such including AFP and CA19-9. We focused pancreatic cancer, the most intractable cancer with difficulty in early diagnosis, and insisted to grasp glycosylation patterns by using a panel of lectin histochemical stainings. Lectins are subgroup of proteins that specifically recognize and bind glycans, and thus helpful indication of glycosylation patterns. Lectin staining is one of the advanced technologies which enables glycosylation pattern of target materials at once, however, obtained data with homogenized protein materials applied lacks histological location and information. Instead, we used a panel of 20 types of lectins histochemical staining for cancer and normal tissues. The staining patterns were firstly classified according to the ratio between cancer cells and stromal cells. a) Cancer high (cancer cell + and stromal cell - ) b) equal (cancer cells + + or stromal cells + + +) c) Cancer Low (cancer cells - or + and stromal cells + + +). Further more, the staining in cancer cell was compared with that of non cancerous pancreatic ductal epithelial cells. Among 20 lectins, CLN-Lycocye nebularis Lectin binds motifs containing α/βGalNAc, TN antigen, and blood group A. showed cancer cell staining higher than normal tissue carcinoma (Pancreatic cancer cell 31 out of 58, 71%) were positive for CLN, but normal pancreatic duct cells (2 out of 42 patients, 4%) were positive in patients of pancreatic cancer. CLN is known to be recognized as TN antigen in O-glycosylation. Previously, it reported that during malignancy, glycosylation displays abnormal expression of shortened or truncated glycans, such as a TN antigen, Tn antigen and their sialylated forms, ST, STn antigen. CLN-staining pattern we showed in this report is considered to matching TN staining. Our result indicated that specific affinity of CLN lectin in pancreatic cancer and counterpart glycoprotein are STn antigen. We may insist that STn glycoprotein antigen could be used as a diagnostic marker of Pancreatic cancer and also it would be a target of new tumor-targeting strategies.

#5234  Expression of cytochrome P450 in head and neck cancer cell lines; Daniela F. Presa,1 Garett Ribeiro Morais,1 Mark Sutherland,1 Jim McCaul,1 Jacques Robert,2 Laurence Patterson,1 Klaus Pors1.1Institute of Cancer Therapeutics, University of Bradford, Bradford, United Kingdom; 2The Royal Marsden NHS Foundation Trust, London, United Kingdom; 3Institut Bergonié, University of Bordeaux, Bordeaux-cedex, France.

Background: The high expression of cytochrome P450 (CYP) enzymes including isofoms CYP1A1, B1 and 2W1 in some tumor types compared to normal tissue make these potential targets for targeted drug discovery. Our efforts in this space have resulted in the discovery of duocarmycin bioprecursors that are bioactivated by CYP1A1 and CYP2W1 expressed in malignant tissues1-2. Epidemiological studies have shown that head and neck cancer (HNC) is a complex multistage process that in part involves exposure to a combination of carcinogens and the capacity of certain drug-metabolising enzymes including CYPs to detoxify or activate such carcinogens. Here, we report on the expression of CYP1A1, CYP1B1 and CYP2W1 in HNC cell lines and human primary tissues. Materials and methods: A panel of HNC cell lines (FaDu, Detroit-562, A253, OSC19) was characterized for CYP1A1, CYP1B1 and CYP2W1 gene expression using real time PCR. Furthermore, immunofluorescence (IF) was used to detect protein expression in HNC cell lines and immunohistochemistry (IHC) in xenografts, paraffin-embedded and snap-frozen human HNC tissue. Anti-proliferative activity was measured using the MTT assay and DNA damage was measured using the comet assay. Results: In the HNC cell lines significant expression (5-10 fold) was shown at the gene level for CYP1A1 and CYP1B1 whereas CYP2W1 was hardly detected. However, CYP2W1 was expressed in FaDu and Detroit xenografts and in a small cohort of 10 human HNC samples as measured by IF and IHC, respectively. In agreement with CYP1 expression, the cytotoxicity results of ICT2700 and ICT2706 revealed FaDu and Detroit-562 as the most sensitive HNC cell lines suggesting bioactivation of these two lead duocarmycin bioprecursors. Conclusion: Accordingly, from these data we can conclude that CYP1A1, CYP1B1 and CYP2W1 are expressed in selected HNC cell lines and tumor tissue. In addition, early evidence suggests that CYP2W1 expression is stimulated by the presence of an in vivo microenvironment. How-ever a larger cohort (n=60) of clinical HNC samples will be used to confirm this observation and will be reported at the annual AACR meeting. [1] Sheldrake et al.Reengineering of the duocarmycin structural architecture enables bioprecursor developments targeting CYP1A1 and CYP2W1 for biological activity. J Med Chem. 2013, 56, 62737. [2] Travica et al. Colon cancer-specific cytochrome P450 2W1 converts duocarmycin analogues into potent tumor cytotoxins. Clin Cancer Res. 2013, 19, 295261.


The linear ubiquitin chain assembly complex (LUBAC) is the only E3 ubiquitin ligase known to generate linear polyubiquitin chains in vitro. LUBAC is composed of three proteins - HOIL-1L, HOIP and SHARPN- of which the interaction between HOIL-1L and HOIP has been shown to be critical for LUBAC assembly and function. This interaction occurs between the ubiquitin-like (UBL) domain of HOIL-1L and the ubiquitin-associated (UBA) domain of HOIP. One known substrate of LUBAC is the regulatory subunit NEMOF (NEMO kappa B essential modulator), part of the IKK complex, which results in activation of NF-κB. We hypothesize that disruption of the HOIL-1L-HOIP interaction would prevent LUBAC ubiquitylation activity and result in down-regulated activation of NF-κB. We have synthesized a family of small inhibitor peptides designed to mimic aspects of the beta-helical interacting interface of HOIP-UBA. We demonstrated how single amino acid substitutions and hydrocarbon stapling can affect overall peptide shape and helicity, and correlate that structural information to peptide binding affinity, ability to affect LUBAC complex formation and resulting effect on LUBAC ubiquitylation activity in vivo. These findings continue to validate inhibition of LUBAC via interference with the HOIL-1L-HOIP interaction as a potential target to affect NF-κB signaling. Compounds which affect LUBAC activity have potential use in LUBAC-dependent NF-κB activation in the activated B cell-like (ABC) subtype of diffuse large B-cell lymphoma (DLBCL), the DLBCL subtype that is most resistant to current therapies.

#5236  The dynamic interplay between the cancer genome mutating enzyme APOBEC3B and DNA substrates. Ozlem Demir,1 Jeffrey R. Wagner,1 Ke Shi,1 Michael A. Carpenter,2 Surajit Banerjee,2 Nadine M. Shaban,2 Kayo Kurashashi,2 Daniel J. Salamanco,2 Jennifer L. McCann,2 Gabriel J. Starrett,2 Justin V. Duffy,1 Daniel Harki,2 Hideki Alhara,2 Rommie E. Amaro,1 Reuben S. Harris.1 University of California, San Diego, La Jolla, CA; 2University of Minnesota, Minneapolis, MN; 3Cornell University, Lempont, IL.

The APOBEC3 (A3) family of enzymes catalyze deamination of cytosines to uracils (C-to-U) in single-stranded (ss) DNA, and is an important part of innate immune responses against viruses and transposons. Recent data also indicate that APOBEC3B (A3B) is a major source of genomic mutations in multiple cancers and a contributor to the evolution of drug resistance. A3B is therefore a promising anti-cancer target. Using single-strand information and structural modeling, we generated a model for wild-type A3B in complex with ssDNA and used molecular dynamics to monitor the dynamics of the native interactions of wild-type A3B with ssDNA. The details of A3B-ssDNA interaction and its implications for potential inhibitor design will be presented.

#5237  Targeting DCN1-mediated neddylation in lung SCC with novel small-molecule inhibitors. Richard Barrios,1 Jared Hammill,2 Jieki Min,2 Kip Guy.3 1FIU Herbert Wertheim College of Medicine, Miami, FL; 2St. Jude Children’s Research Hospital, Memphis, TN; 3University of Kentucky College of Pharmacy, Lexington, KY.

Due to the anti-neoplastic properties and clinical successes of the proteasome inhibitors Bortezomib and Carfilzomib, the ubiquitin-proteasome system (UPS) has become a new target of interest in oncology research. The clinical toxicity induced by these inhibitors, likely caused by complete inhibition of the UPS, has resulted in a need for the development of more selective UPS regulators. A more in-depth understanding of the intrinsic molecular mechanisms that are associated with the UPS has unmasked the potential for improved therapies via more specific targets such as the process of neddylation, which is thought to control 15-20% of the UPS.5 Because neddylation, an enzyme known as DCN1 for ‘‘defective in cullin neddylation 1’’ functions as a co-E3 to help steer a ubiquitin-like protein (UBL), NEDD8, from an E2 enzyme, UBC12, to a cullin (CUL) neddylation site (6’’ 7’’ 9’’ 11). Because DCN1 has been found to be amplified in certain squamous cell carcinomas (SCCs; e.g. lung, esophagus, and head and neck), this in vitro, pre-clinical study demonstrates the potential of the DCN1-UBC12 interaction of an anticancer site in lung SCCs through the use of novel small-molecule inhibitors from the St. Jude (SJ) library. After optimizing dosing regiments, it has been determined that re-administering the SJ compounds (i.e. SJ690, SJ447, SJ446, and SJ323) every 24-hours to address the issue of protein turn-over, produces more efficacious treatment. The most biochemically-potent
antagonists in the compound series were also shown in these primary cell lines to potentiate other therapeutic agents such as Cisplatin; however, these compounds alone do not induce DNA damage, nor do they elicit caspase 3/7 activity. Nonetheless, combination treatment of the SJ compounds with Staurosporine in SCC cell lines, potentiated caspase 3/7 activity at varying degrees. Collectively, this data suggests a promising improvement in our understanding of the mechanism by which the inhibition of DCN1 affects cancer cells, informing pre-clinical development of the SJ DCN1 antagonists.

#5238 Structural and biochemical characterization of membrane bound KRAS. Andrew G. Stephen,1 Que Van,1 Marco Tonelli,2 William Gillette,1 Dominic Esposito,1 Ben Niu,1 Michael Gross,1 Frank Heinrich,1 Arvind Ramathan,1 Christopher Stanley,1 Frederick National Laboratory for Cancer Research, Frederick, MD;2National Magnet Resource Facility at Madison, Madison, WI;3Washington University in St. Louis, St. Louis, MO;4Carnegie Mellon University, Pittsburgh, PA;5Oak Ridge National Laboratory, Knoxville, TN.

KRAS4b functions as a molecular switch cycling between an active GTP bound state and inactive GDP state. Active KRAS4b binds tightly to RAF1 via its Ras binding domain (RBD), and mutation of the RBD has been shown to hamper the ability to bind RAF1. The interaction of KRAS4b with RAF1 is critical for the activation of the mitogen-activated protein kinase (MAPK) pathway. We have previously presented and confirmed by 32P-postlabeling the identity of the major RAF1-KRAS4b complex that is formed via the RBD domain of RAF1 where KRAS4b is predominantly GDP-bound. Here we report the structural and thermodynamic parameters of the human KRAS4b-Raf (RAF1) interaction, with a focus on understanding the mechanism by which the inhibitory activity of the membrane bound KRAS4b blocks the activation of RAF1 and the activation of downstream effectors involved in cancer development. We demonstrate that the interaction of KRAS4b with RAF1 is highly specific and that the interaction is dependent on the intrinsic conformation of the receptor kinase. These findings support the clinical utility of selective small-molecule inhibitors targeting the membrane-bound form of KRAS4b. 

#5239 Probing the cancer epigenome: empowering target validation by open innovation. Ingo V. Hartung,1 Cheryl Arrowsmith,2 Volker Badock,1 Naomi Barak,1 Markus Berger,1 Peter J. Brown,1 Clara D. Christ,1 Erik Eggert,1 Ursula Egner,1 Oleg Fedorov,1 Amaury Fernandez-Montalvan,1 Matyas Gorjanac,1 Andrea Haegerbarth,1 Bernard Haendler,1 Roman C. Hillig,1 Simon H. Holton,1 Kilian V. Huber,1 Seong J. Koo,1 Antonius ter Laak,1 Susanne Mueller,1 Anke Mueller-Fahrmann,1 Cora Scholten,1 Stephan Siegel,1 Timo Stellfeld,1 Detlef Stoeckigt,1 Carlo Stresemann,1 Masoud Vedadi,2 Joerg Weiske,1 Hilmar Weimann,1 1Bayer AG, Berlin, Germany; 2University of Toronto, Toronto, Ontario, Canada; 3Oxford University, Oxford, United Kingdom.

Low reproducibility of published target validation studies as well as the frequent failure of genetic knock-down effects to phenocopy those of small molecule inhibitors have been recognized as road blocks for cancer drug discovery. Academic and industrial institutions have started to address these issues by providing access to high quality small molecular probes for novel targets of interest. Here we discuss probe discovery challenges and quality criteria based on the generation of three novel inhibitors for epigenetic targets. ATAD2 functions as a co-factor for oncogenic transcription factors such as ER that bind to chromatin through its bromodomain (BD). ATAD2 has been proposed to act as a co-factor for oncogenic transcription factors such as ERα and Myc. A more thorough validation of ATAD2 as a therapeutic target has been hampered by the lack of appropriate ATAD2 inhibitors. Here we disclose a structurally unprecedented series of ATAD2 BD inhibitors identified from a DNA-encoded library screen. Optimization delivered BAY-850, a highly potent and exceptionally selective ATAD2 BD inhibitor, which fully recapitulates effects seen by genetic mutagenesis studies in a cellular assay. The three BD and PHD-finger (BRPF) family members are found in histone acetyltransferase complexes. Whereas bromodomain inhibitors with dual activity against BRPF1 and 2 have been described before, we now disclose BAY-299, the first selective inhibitor of the BRPF2 BD with high selectivity against its paralogs. Isoform selectivity was confirmed in cellular protein-protein interaction assays and rationalized based on X-ray structures. BAY-598, a highly selective, cellurally active and orally bioavailable inhibitor of the protein lysine methyl transferase SMYD2, had been disclosed previously (Stresemann et al., AACR 2015). Development of BAY-598 allowed the identification of new methylation targets of SMYD2 as well as a proposed role of SMYD2 in pancreatic cancer. These results support further development of small molecule inhibitors as research tools to probe the functional role of novel epigenetic targets and underscore the power of open innovation for advancing our understanding of cancer target biology.


A series of six titanium dioxide and two cerium oxide engineered nanomaterials were assessed for the induction of cytotoxicity, reactive oxygen species (ROS), and various types of DNA and protein damage in human respiratory BEAS-2B cells exposed in vitro for 72 hours at several concentrations. Although only limited cytotoxicity was observed at concentrations up to 300 μg/ml for all of the nanomaterials, significant increases in 8-oxo-deoxyguanosine, lipid peroxidation mediated protein adducts, and endogenous DNA adducts measured by whole cell lysis were detected at concentrations as low as 30 μg/ml, suggesting that molecular changes associated with ROS induction may provide a better means of assessing the low-dose hazards posed by nanomaterials. To identify molecular properties predictive of the ability of nanoparticles to induce ROS sequelae, a least absolute shrinkage and selection operator multiple regression approach has been used to identify relationships between assay outcomes and nanoparticle physical/chemical properties, including particle size, surface area, zeta potential, and elemental analysis. For several of the assay endpoints examined, concentrations of trace metals in the nanoparticles appear to be better predictors of assay outcomes than physical properties. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

#5241 Using affinity probes to explore the nephrotoxicity of aristolochic acid. Viktoriya S. Sidorenko, Kathleen G. Dickman, Thomas Rosenquist, Radha Bonala, Sivaprasad Attaluri, Irina Zaitseva, Charles Iden, Francis Johnson, Arthur P. Groslamb. SUNY at Stony Brook, Stony Brook, NY.

Aristolochic acid, AA, is a natural compound found in Aristolochia plants used in Chinese Traditional Medicine and is a component of many herbal remedies used worldwide. In humans, exposure to AA is associated with kidney toxicity and otherwise rare upper urinary tract cancer (UTUC). AA undergoes bioactivation creating reactive species of AA that bind covalently to proteins and DNA. Formation of mutagenic AA-DNA adducts leads to AA-induced cancers. We identified the mutational signature associated with AA exposure in patients with UTUC in Taiwan, as well as in AA-initiated cancers in liver, bladder and kidneys. Meanwhile, the mechanism of AA nephrotoxicity remained a mystery, with covalent and non-covalent binding of AA or its metabolites to proteins hypothesized to be involved in its nephrotoxic effects. As millions of people in Asia have ingested AA and no effective therapies are known, establishing the molecular mechanism(s) of AA nephrotoxicity is a topic of major importance. A series of AA analogs were used to design affinity probes for target identification. Structure activity relationship studies in human cells aimed in identifying a position in AA suitable for introducing biotinylated, aminopropyland alkynel linkers. In addition, we have developed and validated monoclonal antibodies against AA-bovine serum albumin and/or AA-DNA covalent adducts. Combined with proteomics techniques, AA-affinity probes and monoclonal antibodies provide powerful tools for defining AA targets in human kidneys. We attached the aminopyroloxy derivative of AA to magnetic beads coated with N-hydroxysuccinimidio acyl linkers. These probes, in parallel with unmodified, “control” beads were incubated with renal cortex lysates obtained from C3H1 mice selected for their sensitivity to the effects of AA. Bound proteins were digested on bead, then identified by mass spectroscopic peptide analysis, with a total of 316 bound proteins detected. Spectral counts for 128 proteins bound to AA-beads were higher than those for control beads. After correction for nonspecific binding, 18 of these proteins had spectral counts of 10 or higher. These proteins are localized to various organelles, indicating high coverage of the major subcellular compartments. Among these proteins, Cryz2, quinone oxidoreductase-like 2, was the lead candidate. Based on homology, Cryz2 belongs to the family of the quinone oxidoreductase-like protein and bears a mitochondrial localization sequence. This protein retains conserved domains for both quinone oxidoreductase activity and NAD(P) binding sites, which is of particular interest since other oxidoreductases, such as NQO1, are known to catalyze nitroreduction, the initial step in the bioactivation of AA. In summary, our studies provide guidelines for design of AA-affinity probes and reveal an enzyme that may be responsible for AA activation in mitochondria, by generating active intermediates and inducing mitochondrial damage in renal tissue.

#5242 Further evidence that the DNA-interactive Pyrolorenbuzidiazepine (PBD) Dimer SIG-136 works through a transcription factor inhibition mechanism. Julia Mantaj,1 Paul J. Jackson,2 David E. Thurston,1 Khondaker Miraz Rahman1. 1King’s College London, London, United Kingdom; 2Fentogenix Limited, London, United Kingdom.

In previous studies, the DNA-interactive PBD dimer SIG-136 demonstrated potent inhibition of the transcription factor SP1 via a transcription factor binding mechanism. In the present study, we investigated if a similar mechanism was responsible for the inhibition of the transcription factor SP3. A series of 2D and 3D DNA-SP3 complexes were constructed to determine the extent of DNA-SP3 binding using docking and molecular dynamics (MD) simulations, and to determine if the DNA-SP3 complex could be altered by treatment with SIG-136. DNA-SP3 binding experiments with Bacillus subtilis RNA polymerase, a DNA-dependent RNA polymerase, were performed to determine the impact of SIG-136 on the transcription activity of SP3. The results demonstrated that SIG-136 significantly inhibited the interaction of DNA and SP3, and decreased transcription activity of SP3. These findings suggest that SIG-136 targets a DNA-binding transcription factor, and that this inhibition could be the mechanism responsible for the inhibition of SP3 activity by SIG-136.
The pyrrolo[2,1-c][1,4]benzodiazepines (PBDs) are sequence-selective DNA minor-groove interacting agents. The PBD dimer SJG-136 has currently been investigated in Phase II clinical trials in ovarian cancer and leukemia in the UK and the USA. More recently, PBD dimer analogues are being attached to tumour-targeting antibodies to create Antibody-Drug Conjugates (ADCs), some of which are now in Phase 3 clinical trials with many others in pre-clinical and clinical development. Transcription factors (TFs) are sequence-specific DNA-interacting proteins that bind to consensus DNA sequences, thereby controlling transcription. TFs regulate processes such as cell differentiation, proliferation and apoptosis. The interaction of a small-molecule with the consensus DNA recognition sequences of TFs can prevent a TF from interacting with its cognate sequence, thereby inhibiting the expression of genes critical for proliferation and proliferation of cancer cells. There is growing evidence that PBDs may exert, at least in part, their pharmacological effect through TF inhibition in addition to the arrest of the replication fork, DNA strand breakage, and inhibition of enzymes including endonucleases and RNA polymerases. For this reason, there is now interest in using PBDs as the basis for a small-molecule strategy to target specific DNA sequences for TF inhibition as a novel anticancer therapy. We have developed a reversed-phase HPLC/MS method as a tool to evaluate the interaction of DNA-binding PBD molecules with oligonucleotides of varying lengths and sequences. Using this methodology, we have demonstrated that the PBD dimer SJG-136 binds to the cognate sequences of the oncogenic transcription factors NF-κB, EGR-1, AP-1 and STAT3. Surprisingly, significant differences in the rate of binding to different cognate sequences were observed which may explain, at least in part, the differences in potency of SJG-136 in various tumour cell lines. Furthermore, an RT PCR study has been carried out using the human tumour cell lines MDA-MB-231 (breast) and HT-29 (colon) to see whether these transcription factors are affected in vitro. The results were consistent with the HPLC-MS studies in that SJG-136 was shown to significantly down-regulate a number of AP-1- and STAT3-dependent genes such as Bcl-2, VEGF, p53 and survivin. These findings add significantly to knowledge of the mechanism of action of SJG-136 and related PBD compounds, and could be relevant for the correct interpretation of clinical activity of molecules of this type both as stand-alone agents and as ADC payloads.

PREVENTION RESEARCH: Chemoprevention and Cancer

#5243 Novel non-COX inhibitory sulindac derivative with β-catenin suppressing activity reduces the formation of colorectal adenomas and adenocarcinomas in the APC<sub>min-Fucci</sub> mouse model. Kevin J. Lee,1 Xi Chen,1 Jacob Valiyaveetil,1 Ashley S. Lindsey,1 Joel Andrews,1 Veronica Ramirez-Alcantara,1 Adam B. Keeton,1 Nathan Y. Chang,1,2 Harry Gourtsoyiannis,2,3 Maggie Clapper,1 Wen-Chi Horne,1 Xiwei Wu,1 Arthur Riggs,1 Sanjay Awasthi,2,4 1City of Hope, Duarte, CA; 2Texas Tech Health Science Center at Lubbock, Lubbock, TX

SJG-136 can reduce the number and size of preneoplastic colorectal adenomas in patients with familial adenomatous polyposis (FAP), but is not recommended for long-term use as a cancer chemopreventive drug because of potentially fatal toxicities associated with cyclooxygenase (COX) inhibition. Here we characterize the anti-neoplastic properties of a novel sulindac derivative, ADT-061, that lacks COX-1 and COX-2 inhibitory activity. ADT-061 potently and selectively inhibited colon tumor cell growth in vitro with IC50 values of 0.3-0.5 μM by inducing apoptosis without affecting the growth of normal colonocytes at concentrations 100x higher. By comparison, sulindac sulfide (SS) inhibited colon tumor cell growth with IC50 values of 40-60 μM, and had only modest tumor cell selectivity. ADT-061 selectively inhibits multiple cyclic GMP phosphodiesterases (PDE) isozymes, previous studies have suggested an important role for PDE10 in colon cancer (Li et al, Oncogene, 2014; Lee et al, Oncotarget, 2015). At concentrations comparable to its IC50 values, ADT-061 selectively inhibits PDE10 to induce an increase in intracellular cGMP levels and activate protein kinase G (PKG). ADT-061 also suppressed nuclear levels of β-catenin and induced cell cycle arrest and apoptosis, within the same concentration range that inhibits tumor cell growth. With attractive drug-like properties, the chemopreventive activity ADT-061 was assessed using the APC<sub>min-Fucci</sub> mouse model of spontaneous colorectal tumorigenesis. Prior to treatment, colonoscopy exams were performed on all APC<sub>min-Fucci</sub> mice (males, 6-7 weeks of age) to ensure the absence of colorectal adenomas. At 7-8 weeks of age, mice were assigned (19 per group) to receive either unsupplemented chow (control) or chow supplemented with ADT-061 (1500ppm) for 14 weeks. No discernible toxicity was observed during the experimental period. Double-blinded pathological analysis of fixed colon tissues revealed that ADT-061 not only reduced the multiplicity of colon adenomas by 50% (Mean ± SEM, control vs. ADT-061, 3.95 ± 0.81 vs. 1.95 ± 0.58, P < 0.05), but also decreased the incidence of colon adenomas by 36.7% (control vs. ADT-061, 94.7% vs. 57.9%, respectively). ADT-061 treatment reduced the multiplicity of polypoid adenomas (36%), flat adenomas (100%, P < 0.05), and microadenomas (69%). The formation of colorectal adenocarcinomas found in 15.8% of untreated mice was completely inhibited in mice treated with ADT-061. These data demonstrate that ADT-061 treatment is a potent chemopreventive agent even to APC<sub>min-Fucci</sub> mice prior to the detection of gross colonic tumors. These observations support further evaluation of ADT-061 and analogs for colorectal cancer chemoprevention.

#5244 Partial knockdown of RLP76 prevents cancer susceptibility of p53 null mice. Sharad S. Singhal,1 Jyotsna Singhal,1 Joshua Tompkins,1 David Horne,1 Xiwei Wu,1 Arthur Riggs,1 Sanjay Awasthi,2,4 1City of Hope, Duarte, CA; 2Texas Tech Health Science Center at Lubbock, Lubbock, TX

P53 is a stress-responsive, genome protective, tumor suppressor whose functions are lost or altered in nearly all neoplasia. P53 homoygous knockout mice (p53<sup>−/−</sup>) develop spontaneous lymphoma and other malignancies in nearly 100% of cases by 6 months of life. In stark contrast, homoygous gene knockout of the stress-respon- sive, anti-apoptotic, mercapturic acid pathway transporter protein, RLP76, results in marked protection from chemical carcinogenesis. P53 expression and activation is regulated by intermediary metabolites of this pathway, and p53 regulates the expression of key enzymes of this pathway. P53 can directly bind to RLP76 through a CDK-interaction domain, and inhibit its transport activity as well as endocytosis stimulatory activity. These results indicate that RLP76 is an effector of p53 in a manner analogous to its known function as the first known effector of the Ras pathways that regulate membrane plasticity, motility and invasion. This translates into reduced kinase signaling down-stream of membrane-receptor/lipid interactions and interactions. An inverse relationship between RLP76 and p53 protein and mRNA expression has been observed in vitro, and is confirmed by analysis of human cancer genomic analyses in the COH database. RLP76 depletion causes apoptosis in cancer cells independent of p53 status, strengthening the view that RLP76 is a key down-stream effector of p53. We reasoned that if RLP76 is necessary for cancer to develop in p53-deficient animals, its suppression will reduce the incidence of malignancy in 12-week old p53<sup>−/−</sup> mice treated with COHSA007 phosphorothioated antisense, 200 μg i.p.2wk till age 8 months. Results of our studies confirmed these predictions, since 4/4 scrambled antisense treated p53<sup>−/−</sup> mice developed T-cell lymphoma whereas 8/8 COHSA007 treated p53<sup>−/−</sup> mice survived to the end of the experiment without development of any malignancy. These findings demonstrate that RLP76 is required for malignancy in the p53<sup>−/−</sup> mice. Taken in context of our previous studies showing that RLP76<sup>−/−</sup> mice are highly resistant to inflammation and carcinogenesis in chemical carcinogenesis models of the skin and lung, the present findings strongly support the idea of an existential role of RLP76 in the malignant phenotype, and strengthen the rationale for using targeted systemic depletion of RLP76 for treat- ment of human neoplasia (Supported in part by the Department of Defense grant W81XWH-16-1-0641 and UPSH grant CA 77905).

#5245 Peretinoin prevents the accumulation of somatic gene mutations and reverts neoplastic lesions to normal liver-like in platelet-derived growth factor-c transgenic mice. Taro Yamashita, Hikari Okada, Masao Honda, Shui- chi Kaneko. Kanazawa University Hospital, Kanazawa, Japan

Hepatocellular carcinoma (HCC) develops with progressive accumulation of somatic genetic changes. A well-known example is reported to suppress the development of HCC, but the molecular mechanism in detail remains to be obscure. Here we evaluated the effect of peretinoin on preneoplastic nodules and HCC developed in platelet derived growth factor-c (PDGF-C) transgenic mice liver by gadolinium ethoxybenzyl diethylenetriamide pentaacetate acid (Gd-EOB-DTPA)-enhanced magnetic resonance imaging (MRI) and whole exome sequence analysis. We confirmed the progressive accumulation of somatic muta- tions in hypervascular liver cancer tissues compared with hypovascular preneoplastic and non-cancerous background liver tissues in PDGF-C transgenic mice. Surprisingly, peretinoin treatment in HCC as well as preneoplastic nodules resulted in the accumulation of less gene mutations compared with vehicle. Gd-EOB-DTPA uptake capacity and its transporter OATP1 was confirmed to be re-activated in peretinoin treated nodules in mice. Gene and protein expression analysis indicated the inactivation of cell cycle with low Ki-67 labeling indexes in peretinoin treated nodules. The reversion of Gd-EOB-DTPA uptake capacity was confirmed in preneoplastic nodules and a subset of classical hypervascular HCCs. Notably, peretinoin supplementation increased the expression of OATP1B3 in mature hepatocyte-like HCC cell lines in vitro, whereas supplementation of tretinoin (all-trans retinoic acid) had no such effects and rather suppressed OATP1B3. Taken together, our data suggested the utility of peretinoin to revert the malignant transformation processes of hepatocytes by preventing accumulation of gene mutations especially in early stages of hepa-tocarcinogenesis. Peretinoin but not tretinoin can induce the expression of a ma-
tured hepatocyte marker OATP1B3, suggesting that peretinoin may exert its anti-tumor effect with hepatocytic differentiation capacity independently of the activation of retinoic acid signaling.

#5246 Effects of bazedoxifene either alone or in combination with letrozole in the prevention of chemically induced mammary cancers. Barbara K. Dunn, Chen Suen, Ronald A. Lubet, Vernon E. Steele, Clinton J. Grubbsb.

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Bazedoxifene is a third-generation selective estrogen receptor modulator (SERM) that has been clinically approved in Europe for the prevention and treatment of postmenopausal osteoporosis. The agent has also been shown to have therapeutic activity against estrogen receptor positive (ER) breast cancer. Our laboratory is currently evaluating bazedoxifene for chemopreventive efficacy when given alone or in combination with the aromatase inhibitor (AI) letrozole. Initially, the agents were evaluated alone to determine effective chemopreventive doses in the methylmethanesulfonate (MNU) ERα mammary cancer model using female Sprague-Dawley rats. Bazedoxifene was mixed directly into a standard (Teklad, 4% fat) diet, while letrozole was gavaged 7x/week (vehicle was ethanol: PEG 400, 10:90, v/v). When evaluated alone in rats receiving MNU at 50 days of age, bazedoxifene (started one week after MNU) at doses of 100 and 300 mg/kg diet decreased mammary tumor multiplicity by 73%. When the agents were given in combination at these low dose levels, cancer number was reduced by 91%. An additional study was done in which the agents were given to rats receiving MNU at an older age (100 days). Rats receiving the agents at the original doses beginning one week after MNU (bazedoxifene at 30 mg/kg diet; letrozole at 0.1 mg/kg BW/day) had identical decreases in mammary tumor development (96%). It is obvious that bazedoxifene is as effective as letrozole in the prevention of mammary carcinogenesis when initiated in older rats. Furthermore, administration of the agents in combination suggests an additive effect. The large increase in body weight gain generally noted in letrozole treated rats was not observed in rats receiving bazedoxifene; of interest, rats receiving the combination also had no increase in body weights. These studies indicate that bazedoxifene may be superior to other SERMs in that chemopreventive efficacy is achieved at low doses, which may be associated with less toxicity. Supported by NCI contract HHSN261201200021I, Task Order HHSN26100007.

#5247 δ-Tocopherol inhibits the development of prostate adenocarcinoma in prostate specific Pten-/- mice. Xu Yang,1 Wang Hong,2 Guocan Wang,3 Anna Liu,1 Maarten c. Bosland,1 Chung s. Yang,1 Rutgers, Piscataway, NJ; 2MD Anderson Cancer Center, Houston, Piscataway, NJ; 3University of Illinois at Chicago, Chicago, IL.

PTEN/Pik3/AKT axis plays a critical role in regulating cell growth, metabolism, migration, differentiation, and survival. Activation of this signal pathway is frequently found in human cancers. Data from our previous studies demonstrated that δ-tocopherol (δ-T) attenuates the activation of AKT by growth factor in prostate cancer cell lines, leading to inhibiting proliferation and inducing apoptosis. Herein, we investigated whether δ-T is able to inhibit the development of prostate adenocarcinoma in prostate-specific Pten-/- (Pten δ-) mice, in which the activation of AKT resulted from loss of Pten is the driver. By feeding Pten δ- mice with the AIN93M or 0.2% δ-T supplemented diet starting at the age of 6 or 12 weeks, we found that, at the age of 40 weeks, δ-T treatment reduced the number of invasive prostate adenocarcinoma by 53.3% (p<0.005) and 42.7% (p=0.001), respectively. By immunohistochemical analysis, the activation of AKT via phosphorylation of AKT(T308) was found reduced in the prostate of the mice on δ-T diet. Consistently, proliferation was reduced and apoptosis was increased in prostate of the mice on δ-T diet. We also determined oxidative stress by immunohistochemical staining of 8-OH-dG and nitro-tyrosine, and found that these markers were not altered during prostate tumorigenesis, nor they were affected by δ-T. A 0.2% α-tocopherol diet was also used to feed Pten δ- mice, but did not inhibit the development of prostate adenocarcinoma, consistent with previous data demonstrating that α-tocopherol did not inhibit the activation of AKT. Together, these results support that δ-T is effective in suppressing the development of prostate adenocarcinoma in Pten δ- mice through inhibition of AKT activation.

#5248 Pioglitazone prevents hepatocellular carcinoma development in a rat model of cirrhosis. Shen Li, Sarani Ghoshal, Gunisha Arora, Derek J. Erstad, Michael Lanuti, Kenneth K. Tanabe, Bryan C. Fuchs. Massachusetts General Hospital, Boston, MA.

Introduction: Advanced hepatocellular carcinoma (HCC) is a leading cause of mortality worldwide with limited options available for patients with a small, perhaps not even a solitary, identifiable cohort of cirrhosis patients at risk and they are ideal candidates for chemoprevention. Anti-hyperglycemic agents have garnered interest for their potential anti-fibrotic as well as chemo-preventive effects. Pioglitazone, a selective PPARγ agonist, has been shown to reduce non-alcoholic steatohepatitis (NASH), but its role as an anti-fibrotic and chemopreventive agent has yet to be elucidated. The hypothesis of this study is that Pioglitazone reduces cirrhosis and subsequent HCC development in rats with diethylnitrosamine (DEN)-induced cirrhosis. Methods: Male Wistar received DEN 50mg/kg by intraperitoneal injection. DEN injury reliably recapitulates histological and molecular features of human HCC development with induction of hepatic fibrosis at 8 weeks, cirrhosis at 12 weeks, and HCC by 18 weeks. DEN-injured rats were randomized to receive oral gavage of pioglitazone at 3mg/kg/day (n=8) or vehicle control (n=9). Initiation of pioglitazone coincided with the development of liver fibrosis at 8 weeks. All animals were sacrificed at 18 weeks. Results: As expected, repeated injections of DEN in rats resulted in progressive fibrosis, cirrhosis, followed by HCC formation. Treatment with pioglitazone resulted in a 56% reduction of surface nodules relative to treatment with vehicle (7.4±4.9 vs. 17.4±10.05, p<0.005). Liver sections were stained by picrosirius red to assess fibrosis, and pioglitazone significantly reduced collagen deposition in DEN-injured rats (collagen proportional area = 3.2±1.8% vs. 9.2±2.7%; p<0.035). This histologic observation was further confirmed by gene expression analysis with reductions in collagen-I, α-smooth muscle actin, and transforming growth factor beta in rats treated with pioglitazone. Finally, weekly injection of DEN also caused a significant decrease in overall body weight in comparison to untreated rats (398.1±60 vs. 598±46 grams; p<0.015), and pioglitazone treatment resulted in a trend for better protection of body weight relative to vehicle (398.1±60 vs. 427.5±56.3 grams). Conclusion: Overall our data supports the hypothesis that the anti-diabetic agent pioglitazone may be repurposed as a drug to reduce fibrosis and prevent HCC. This could be beneficial in patient management given the low cost as well as minimal side effects.

#5249 Integrative analysis of transcriptomic, proteomic, and metabolomic data of Pten-Knockout carcinogenic mouse prostate. Jinhui Zhang,1 Li Li,2 Sangyub Kim,2 LeeAnn Higgins,3 Yibin Deng,4 Christopher J. Kemp,5 Cheng Jiang,2 Junxuan Lu2.

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The prostate-specific Pten-knockout (KO) mouse carcinogenesis model is highly desirable for prostate cancer chemoprevention studies due to its close resemblance of many histopathological features of human prostate cancer including disease progression from prostatic intraepithelial neoplasia (PIN) to invasive adenocarcinomas. Here, we profiled the prostate proteome, transcriptome and aqueous metabolome of Pten-KO mice to identify reference molecular signatures that can be used for designing chemopreventive and/or therapeutic intervention and for selection of molecular biomarkers of responses to intervention. For proteomics, 4 pairs of whole prostates from Pten-KO mice (12-15 weeks of age, corresponding to high grade PIN) and their wild type littermate housed in same cages were obtained from the NCI Mouse Model Repository and analyzed by 8-plex iTRAQ. For transcriptomic/microarray and metabolic analyses, 3 additional matched pairs of prostate/tumor specimens at older age (22-20 weeks) were used. Proteomic and transcriptomic analyses using manual annotation methods with references from PubMed revealed top signatures that were up- and down-regulated by Pten deletion, particularly those implicated in immune function, inflammatory response, cancer, drug metabolism, cellular functions, prostate functions, and endoplasmic stress regulation. Similar to the manual annotation approach, each network analysis of 203 genes and 22 proteins (≥ 2-fold changes) by a bioinformatics software, Ingenuity Pathway Analysis (IPA) showed that inflammatory response, cellular movement, immune cell trafficking, immunological disease, and cancer were top 5 disease and biological functions in Pten-KO mice. Using references from PubMed, we manually assigned the upregulated or downregulated functions to functional categories, which included altered methionine-cysteine cycle fluxes and purine metabolites, increased nucleotide pools, cholesterol and polynucleotide synthesis and suppressed pools of sugar and choline derivatives, glycolysis intermediates, and purine bases. IPA network analysis of 25 metabolites (≥ 2-fold changes) revealed the...
biological functions related to molecular transport, amino acid metabolism, and small molecule biochemistry. In addition, we integrated transcriptomic, proteomic, and metabolomic data sets to identify latent biological relationships and to gain a comprehensive understanding of Pten-deficient prostate carcinogenesis. The integrative analysis predicted activation of inflammatory response and several cellular pathways, such as GPR40, NFKB1, and GNAS. The integrative analyses identify both active and latent reference molecular signatures and provide more insight into Pten-deficient prostate cancer than single omic approaches.

#5250 Cranberry proanthocyanidins reverse microbial dysbiosis and inhibit bile acid metabolism in association with esophageal cancer prevention, Katherine M. Web,1 Nita H. Salzman,1 Amy B. Howell,2 Jennifer L. Clarke,3 Bridget A. Tripp,2 Laura A. Kresy,1 Medical College of Wisconsin, Milwaukee, WI; 2 Rutgers University - Marucci Center for Blueberry and Cranberry Research, Chatsworth, NJ; 3 University of Nebraska - Lincoln, Lincoln, NE.

Our laboratory has been investigating the cancer inhibitory potential of cranberry proanthocyanidins (C-PAC) against esophageal adenocarcinoma (EAC), a cancer characterized by rapidly rising incident rates and poor survival (18% 5-year survival rate for all stages combined). Utilizing a panel of validated human esophageal cancer cell lines and OE19 xenograft bearing mice, our laboratory reported that C-PAC activates autophagic cell machinery leading to caspase-independent cancer cell death, yet linked to bile acid sensitivity. Reflux of bile and stomach acid into the lower esophagus is considered the major risk factor for progression to EAC. Building on our initial research, we next investigated mechanisms by which C-PAC inhibited reflux-induced EAC with a focus on gut microbiome alterations and modulation of bile acid metabolites. Bile acids undergo secondary metabolism in the intestinal tract, but have not been evaluated in this context. We utilized the rat surgical esophageagastroduodenal anastomosis (EGDA) model for reflux-induced EAC. EGDA+ and non-surgical Sprague Dawley rats were treated with water or C-PAC (650 μg/rat/day) for 25 or 40 weeks and assessed for changes in cancer progression via extensive histopathological characterization. Additionally, at 40 weeks, fecal microbiome profiling was investigated and metabolic profiling conducted on esophageal, liver and fecal samples. Methods included 16s rRNA sequencing of rat fecal DNA, paired end sequencing on Illumina MiSeq and data analysis using Qiime and the K packages phyloseq, and edgeR to assess C-PAC-induced microbiome changes. For metabolic profiling homogenized esophagi, liver and fecal samples were extracted in methanol and characterized by Reverse Phase Ultra high performance Liquid Chromatography-Tandem Mass Spectrometry RP/UPLC-MS/MS, followed by metabolite identification based on Metabolon’s library of authenticated standards. Study results support that C-PAC significantly inhibits the formation of EAC with concomitant restoration of the gut microbiota profile, i.e., the bacterial profile shifted toward increased favorable Gram+ Firmicutes and away from inflammatory-linked Gram negative Bacteroidetes. In addition, C-PAC treatment significantly reduced primary and secondary bile acid metabolite levels in the esophagus of reflux/EGDA+ rats. Other metabolites significantly reduced by C-PAC included a number of pro-inflammatory mediators which may account for the observed therapeutic effect. In summary, these results indicate that microbial impairment correlates with the stimulation of bile acid metabolism, and (2) C-PAC mitigates reflux-induced inflammation and injury in the esophagus in association with EAC inhibition. Future research with C-PAC in patients with gastroesophageal reflux disease should include assessments of bile acid metabolites and bacterial profiles as potential cancer-inhibitory mechanisms.


Cancer stem cells represent a rare population of cells with a self-renewal capacity, and potentially involved in tumor progression, tumor relapse, and resistance to cancer chemotherapies. In this study, we investigated the effect of an active ingredient of chamaecysis obtuse, amentoflavone, on stenness of U251 glioblastoma cells. We first found that amentoflavone from 1 to 10 μM suppressed tumorsphere formation and inhibited the expression of glioblastoma stem cell markers CD133 and ALDH1 in a dose dependent manner. In addition, amentoflavone significantly reduced the expression of Nanog involved in cancer stem cells maintenance in tumorsphere culture. As an upstream modulator, Hedgehog (Hh) signaling mediator Gli1 was confirmed to be highly expressed in tumorsphere culture where the suppression of Gli1 by the treatment of Hh inhibitors (vismodegib and GANT 61) or Gli1-siRNA significantly suppressed the expression of stem cell markers CD133, ALDH1, and Nanog as well as tumorsphere formation in U251 glioblastoma cells. These results indicate that Hh signaling plays an important role in maintaining tumorsphere and stenness of U251 glioblastoma cells. Next, we also found that the treatment of amentoflavone from 1 to 10 μM inhibited the expression of Gli1 and its downstream mediator Nanog in tumorsphere culture of U251 glioblastoma cells. Taken together, amentoflavone significantly suppressed Hh/Gli1 signaling which results in inhibition of stenness of U251 glioblastoma cells.

#5252 Quercetin inhibits prostate cancer by modulating ROS and key regulators of apoptosis and cell survival, Ashley B. Ward,1 Hina Mir,2 Neeraj Kapur,3 Guru Sonpavde,2 Shailesh Singh,1 Morehouse School of Medicine, Atlanta, GA; UAB Comprehensive Cancer Center, Birmingham, AL.

Long latency and indolent nature of prostate cancer (PCa) provides a window of opportunity for preventive interventions using natural and synthetic agents. Hence, the focus of this study was to ascertain the chemopreventive role of Quercetin, a bioflavonoid, commonly used to treat prostatitis. Human PCa cells (LNCaP, DU145 and PC3) were treated with different concentrations of Quercetin and its effect on cell survival and apoptosis was determined by MTT assay. Human PCa cells treated with Quercetin showed significant reduction in cell viability and proliferation compared with untreated controls, which was dose and time dependent. In addition to this our FACS analysis showed higher percentage of apoptotic cells after Quercetin treatment compared to untreated cells. Quercetin induced apoptosis in PCa cells is a cumulative effect of modulation of key apoptotic proteins, changes in mitochondrial membrane potential and ROS production. Quercetin's results on Quercetin as a potential chemopreventive agent, which may improve outcomes of PCa by inhibiting mechanisms involved in tumor progression.

#5253 Arctigenin inhibits prostate tumor growth in vitro and in vivo in obese state, Piwen Wang,1 Tanya Diaz,2 Susanne Henning,2 Jaydutt Vadgama3.

Arctigenin is a novel anti-inflammatory lignan derived mainly from the seeds of Arctium lappa which is an herb widely used in traditional Chinese medicine to treat inflammation related diseases. We previously demonstrated that arctigenin strongly inhibited prostate tumor cell growth in cell culture and mouse models in non-obese state. The present study investigated the tumor inhibitory effect of arctigenin in obese state. An in vitro obese setting was created by co-culture of mouse adipocytes 3T3-L1 with androgen-sensitive LAPC-4 and LNCaP prostate cancer cells. An ELISA analysis of 3T3-L1 conditioned medium revealed that three cytokines/growth factors were prominently secreted by 3T3-L1 cells, including IGF-1, VEGF, and MCP-1, and the sensitivity of prostate cancer cells to arctigenin was decreased in the obese state. However, arctigenin at a moderate concentration (10 μM) significantly inhibited the proliferation of both LAPC-4 and LNCaP cell by 40-50% at 96h in the co-culture system. Male severe combined immunodeficiency (SCID) mice was implanted subcutaneously with LAPC-4 xenograft tumors to confirm the tumor-inhibitory effect of arctigenin in vivo. Mice were fed high-fat diet containing 45% energy from fat, and treated with arctigenin at 50mg/kg b.w. orally or vehicle control for 6 weeks (n=10 per group). The tumor growth in arctigenin group was significantly inhibited by 40% compared to control, along with decreased blood concentrations of several cytokines including IGF-1, VEGF, and MCP-1. Immunohistochemistry analysis is ongoing to determine the molecular changes involved in tumor cell proliferation and apoptosis in response to arctigenin treatment. This study provides a promising natural compound to enhance chemoprevention of prostate cancer especially in obese patients. These results warrant future clinical trials to confirm the anti-carcinogenic effect of arctigenin in humans.

#5254 Postoperative adjuvant chemotherapy improves survival in stage II colon cancer? A propensity score matching analysis, Ryosichi Tsukamoto, Kichi Sugimoto, Shingo Kawano, Koichiro Niwa, Shun Ishiyama, Hirohiko Kamiyama, Hiromitsu Komiyama, Makoto Takahashi, Yutaka Kojima, Michitoshi Goto, Yuichi Tomoki, Kazuhiro Sakamoto. Isuendo University Faculty of Medicine, Tokyo, Japan.

Objective : Stage II colon cancer with high-risk features have been traditionally considered more likely to benefit from postoperative adjuvant chemotherapy (POAC). National Comprehensive Cancer Network (NCCN) guidelines do not recommend routine administration of adjuvant chemotherapy for Stage II patients, except for those with high risk features, such as poorly-differentiated tumors, T4 disease, perforated tumors and those with inadequate lymph node sampling. However, the use of POAC after surgery for patients with Stage II colon cancer remains controversial. The current study was conducted to investigate the effectiveness of POAC using propensity score (PS) matching analysis,
which can reduce or minimize the confounding that is seen frequently in observational studies of the effect of treatment on outcomes, based on prognostic factors. Materials: Two hundred and ninety patients with Stage II colon cancer who underwent surgery with curative intent between 1995 and 2005 were enrolled. The decision regarding the use of POAC was made for each case based on individual physician discretion. All patients were provided informed consent. During the present study period, patients received 5-FU drugs orally, starting 4–8 weeks after surgery. The median observation period was 73.4 months (range: 2.2–184.2 months). Methods: PS matching analysis was used to adjust for differences in clinicopathological severity between the patients with and without POAC. Recurrence-free and cancer-specific survival were compared using Kaplan-Meier analyses and Cox proportional-hazard models were used to determine Hazard ratios and 95% confidence intervals. Results: One hundred and nineteen patients (54.3%) underwent POAC. In univariate analysis, there were no significant differences with respect to the clinicopathological factors between the patients with and without POAC. Before PS matching analysis significant survival benefits from POAC were not recognized for recurrence-free survival (Hazard ratio = 0.76, 95% CI 0.40–1.45, P = 0.41) or cancer-specific survival (Hazard ratio = 0.52, 95% CI 0.22–1.19, P = 0.12). After PS matching analysis significant survival benefits from POAC were observed (based on principal component analysis) (Hazard ratio = 0.55, 95% CI 0.23–1.23, P = 0.15) or cancer-specific survival (Hazard ratio = 0.46, 95% CI 0.16–1.18, P = 0.11). Conclusions: The one-to-one pair PS matching successfully balanced the clinicopathological factors between the patients with and without POAC. The PS matching analysis demonstrated no significant difference in survival in the patients with Stage II colon cancer.

#5256 Intracellular lipid droplet quantity as a biomarker for obesity associated inflammation and carcinogenesis risk.

Rationale: Prior reports have suggested that piperine, the major alkaloid product derived from black and long pepper, enhances curcumin’s cancer preventive efficacy in vitro and in vivo by an intracellular pharmacokinetic interaction; yet, no previous studies have explored the pharmacokinetic interactions between curcumin and piperine. Methods: We incubated non-transformed breast cell line MCF10A and breast cancer SUM149 cell line with different concentrations of curcumin, piperine or curcumin + piperine to assess cell viability using a MTT assay. For curcumin and piperine uptake assays, we incubated MCF10A, SUM149 and MCF7 breast cells with either 5 μM curcumin or 5 μM curcumin + 5 μM piperine for 0.5, 1.0, 4.6, 8.12 and 24 h, or with 15 μM curcumin or 15 μM curcumin + 10 μM piperine for 0.17, 0.5, 1.2 and 4 h. Media and cell lysates were extracted for assay of intracellular curcumin and piperine by LC-MS/MS. ALDH1+, ALDH CD44+ 24 cells were collected via FACS, incubated with 10 μM Curcumin or 10 μM piperine for 1 hour. Cell lysates and media were assayed for curcumin, curcumin degradation and metabolic products and quantified using LC-MS/MS. Results: 90.7±0.06% and 34.8±0.002% of cells were viable after incubating MCF10A and SUM149 cells respectively with 25 μM curcumin compared to DMSO control. Incubation with 10 μM Piperine enhanced the antiproliferative effect of 15 μM curcumin by 33.6±0.015% (P = 0.05) and 16.3±0.03% (P = 0.05) in SUM149 and MCF10A cells respectively. We found no significant increase of intracellular concentration of curcumin when co-incubated with piperine in the MCF10A, MCF7 and SUM149 cells or in the ALDH+ and the ALDH CD44+ CD24+ SUM149 cells. Intracellular curcumin concentration was 166.7±22 and 151.5±24 ng/ml-pro-
tein in ALDH+ cells for curcumin alone or curcumin + piperine respectively and 130±2.54 and 135±27.4 mg/mg protein in ALDH CD44 / CD24 for curcumin alone or curcumin + piperine respectively in SUM149 cell line. Curcumin uptake was lower in MCF10A cells than SUM149 cells. The intracellular curcumin concentration after 1 hour incubation with 5 μM curcumin was 11.8±0.8 and 11.4±1.2 in MCF10A and SUM149 cell lines respectively. Tetrachloroferrrocenium and curcumin sulfite conjugates were the major metabolites detected in MCF7 and SUM149 cell lines. Curcumin sulfate is detectable in the media as early as 0.5h, it increases from 1.36±0.06 and 1.43±0.05 after 0.5h to 22.6±0.53 and 23±2.2 ng/ml after 4h for curcumin and curcumin + piperine respectively in the media of ALDH CD44 / CD24 SUM149 cells. Conclusions: We find a significant difference in intracellular uptake of curcumin between cells treated with curcumin alone or curcumin + piperine after incubation times up to 24h. The additive cellular toxicity effects observed with piperine + curcumin is not pharmacokinetic (associated with efflux pump inhibition) but rather pharmacodynamic due to piperine’s independent anti-proliferative effects.


Bone is one of the metastatic sites for advanced breast cancer. Nearly 70% of breast cancer patients experience metastasis to bone due to enhanced osteoclastogenesis and formation of osteoclasts leading to bone resorption. We have shown previously that benzyl isothiocyanate (BITC), a constituent of edible cruciferous vegetables such as garden cress, is a potent inhibitor of breast cancer cell lines in vitro and in vivo. The present study was designed to determine the effect of BITC on breast cancer-induced osteoclastogenesis. Co-culture of murine RAW 264.7 macrophages with human breast cancer cells resulted in genesis of osteoclasts in vitro that was suppressed significantly in the presence of BITC. Runx2, a transcription factor, is abnormally expressed in breast cancer and contributes to osteoclastogenesis by regulating receptor activator of NF-κB ligand (RANKL). BITC treatment downregulated expression of Runx2 in breast cancer cells at the mRNA and protein levels. Osteoclastogenesis was increased in Runx2 overexpressing MDA-MB-231 cells, whereas conditional knockdown of Runx2 in T47D cells attenuated this process. To determine the in vivo efficacy of BITC, MDA-MB-231-Luc cells were injected into the left ventricle of nude mice. BITC was administered orally at 10 mg per kg body weight. Micro-CT X-ray analysis showed that bone metastases and erosion was decreased by about 50% upon BITC treatment when compared with vehicle control. Cathepsin K and total RANKL levels were lower in the plasma of BITC-treated mice when compared to the control group. Altogether, this study demonstrates, for the first time, that BITC is a potent inhibitor of breast cancer-induced osteoclastogenesis in vivo. This study was supported by the grant RO1 CA129347-09 awarded by the National Cancer Institute.

#5259 Urolithin A prevents pancreatic tumor growth and increases survival by inhibiting PI3K/PDK1 and STAT3 signaling. Supriya Srinivasan,1 Venkatakrishna Jala,2 Altaf Mohammed,3 Jagan M.R. Patolla,1 Yuting Zhang,1 Laura Bid-dick,1 Venkateshwar Madka,1 Li Qian,1 Stan Lightfoot,1 Barbara Dunn,2 Ronald Lubet,1 Chen S. Suen,1 Vernon E. Steele,2 Chinthalapally V. Rao,3 Univ. of Oklahoma Health Sciences Ctr., Oklahoma City, OK; 2National Cancer Institute, Rockville, MD.

Pancreatic ductal adenocarcinoma (PDAC) is the 3rd leading cause of cancer-related mortality in the United States. Most patients present with an advanced disease and the majority die within five years, many surviving less than six months. Cytotoxic chemotherapy including Gemcitabine (Gem), FOLFIRINOX, nab-paclitaxel offer modest improvement in survival, albeit at the cost of increased side effects and unwanted toxicities. Therefore, developing novel chemotherapeutic agents for PDAC treatment is critical to improve survival. Ellagic acid/ellagitannins are abundantly present in the pomegranate and berries, are anti-inflammatoryagentaspirin,acombinationofaspirinwithacidityneutralizer omeprazole was used in AOM-induced F344 Rat model of CRC. Rat (36/group) colon cancers were induced by AOM (15mg/Kg body weight) s.c., once a week for two weeks. At adenoma stage (13 wks of age), rats were fed diets containing aspirin (700 or 1400ppm), omeprazole, (0, 250 or 500 ppm), or aspirin + omeprazole (700 ppm + 250 ppm; 1400 ppm + 500 ppm). To identify a molecular predictor of the benefit of aspirin plus omeprazole we performed next-generation sequencing of colon adenocarcinomas (AdCa) treated with aspirin (1400 ppm), omeprazole (500 ppm), aspirin plus omeprazole (1400 plus 500 ppm), conducted whole-transcriptome analysis and correlated the molecular portrait with chemoprevention benefit. Aspirin plus omeprazole enhanced colon AdCa incidence inhibitory effects by 55% (p<0.0001) and multiplicity by ~87% (~0.0001) respectively in F344 rats. Transcriptome profiling revealed 1525 hits with a 1.5-fold expression difference threshold in aspirin-treated colon AdCAs (722 upregulated genes, 803 downregulated genes, P < 0.05), 1507 hits with a 1.5-fold expression difference threshold in omeprazole-treated colon AdCa (711 upregulated genes, 796 downregulated genes, P < 0.05) and 1074 hits with a 1.5-fold expression difference threshold in aspirin plus omeprazole-treated colon AdCa (662 upregulated genes, 842 downregulated genes, P < 0.05) compared to untreated colon AdCa. Multiple comparisons using Anova with samples from all groups revealed 691 hits with a 1.5-fold expression difference expression threshold with Benjamini Hochberg correction. Pathway analyses of these altered 691 genes indicated that the aspirin plus omeprazole treated colon AdCa upregulated cellular pathways (z score 2.26). Metabolism process (6.01) and cellular metabolic process (7.73), and downregulated signaling genes (~ 8.18), signal transduction (~ 8.03), immune response (~ 2.08) and inflammatory response (~ 2.95). A significant upregulation of Killer cell lectin-like receptor subfamily K, member 1 (natural cytotoxicity receptor which eliminates tumor cells), and acid phosphatase 2, lysosomal, (autophagy of tumor cells) was seen in the combination treatment groups compared to individual and control group AdCa. A significant decrease in TGF-β receptor signaling pathway gene SNX4 and ATPases was observed in combination treatments. Hence, these results suggest aspirin plus omeprazole combination treatments were devoid of GI toxicity increasing chemopreventive efficacy in colon cancer. Supported by NCI N01-CN-250026.

#5261 Tocopherols inhibit the estrogen-stimulated expansion of cancer stem cells via down-regulation of OCT4 and NfκB. Min Ji Bak. Rutgers Univ. School of Pharmacy, Piscataway, NJ.

Cancer stem cells have been suggested to play a role in tumor initiation, maintenance, resistance, and metastasis. Breast cancer stem cells are defined as CD44 / CD24 cell populations that have the properties of tumor-initiating abil-
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ity, self-renewal and differentiation. Although the importance of estrogen in breast cancer is well established, the effects of estrogen on breast cancer stem cells are not fully understood. Vitamin E, which is a member of different forms of tocopherols and tocotrienols, has anti-tumor effects in various cancers, including breast cancer. In the present study, we examined the effects of α-, γ-, and δ-tocopherol against estrogen-stimulated expansion of cancer stem cells. To determine the effects of tocopherols on breast cancer stem cells, the MCF-7 tumorosphere cell culture system, which enriches for mammary progenitor cells and putative breast cancer stem cells, was utilized. Treatment of MCF-7 tumorosphere with estrogen for four days resulted in an increase in CD44+/CD24− stem cell populations in tumorspheres as well as the number and size of tumorspheres. The inhibition of breast tumorospheres by 10 μM inhibited treatment was greatest in tocopherol-enriched stem cell enriched tumorspheres, indicating that tocopherols reversed estrogen-induced expansion of breast cancer stem cells. Tocopherols decreased the expression of estrogen-related genes such as TFF/pS2, cathepsin D, progesterone receptor and SerpinA1 in estrogen-stimulated tumorspheres. In addition, the mRNA levels of stem cell markers, including OCT4, NFκB, Sox-2 and CD44, were upregulated by estrogen, which were downregulated by the treatment with tocopherols. Our results suggest that tocopherols repress the expansion of breast cancer stem cell population by regulating OCT4 and NFκB. The alterations in these pathways, again shown by an inhibition of BrdU incorporation. In addition to the current results indicate that certain combinations of UA, Curc and Met may have the potential for broad applications for chemoprevention of breast cancer. (This work was supported in part by the National Institutes of Health grant R01 AT007036.)

The inhibitory effect of combinations of curcumin, ursolic acid, and metformin on skin tumor promotion by TPA. Lisa Tremmel, Oldnyrng Rho, John DiGiovanni. The University of Texas at Austin, Austin, TX.

The purpose of this study was to examine the effects of combinations of ursolic acid (UA), curcumin (Curc), and metformin (Met) for potential synergistic chemopreventive activity in animal tumor promotion using the mouse two-stage skin carcinogenesis model. UA and Curc have been shown to inhibit tumorigenesis in several mouse models, and both have been shown to inhibit mouse skin tumor promotion by 12-O-tetradecanoylphorbol-13-acetate (TPA). The anti-cancer activity of these phytochemicals is due to their anti-inflammatory, anti-oxidant, and anti-proliferative effects. These effects are accomplished by acting on a broad spectrum of signaling pathways. Met has also been shown to have anti-cancer activity. In retrospective studies, type II diabetes patients taking Met have been shown to have a decreased cancer incidence. Met has also been shown to inhibit mouse skin tumor promotion by TPA. Met activates AMP-activated protein kinase (AMPK), which in turn inhibits mammalian target of rapamycin complex 1 (mTORC1) signaling. mTORC1 is a key kinase involved in the phosphatidylinositol-3-kinase (PI3K)/Akt pathway, one of the most commonly altered pathways in human tumors. In initial short-term experiments (mice treated twice weekly for two weeks with TPA), combinations of UA and Curc pretreated topically inhibited TPA-induced activation of epidermal NFκB, Cox-2, epidermal growth factor receptor (EGFR) and JNK to a greater extent than either compound alone. Additionally, these compounds increased levels of AMPK and programmed cell death protein 4 (Pdc4d). The alterations in these signaling pathways by the combination of UA and Curc were associated with decreased epidermal proliferation and skin inflammation as assessed by measuring BrdU incorporation and inflammatory gene expression, respectively. Further studies using dietary administration of a combination of UA and Curc, as well as combinations of UA or Curc (in diet) with Met administered in the drinking water were conducted. These combinations produced greater inhibition of TPA-induced activation of epidermal NFκB, Cox-2, and EGFR than the compounds given alone. These combinations also decreased epidermal proliferation, again shown by an inhibition of BrdU incorporation. In addition to these data, the results from ongoing skin tumor experiments (mice initiated with DMBA and promoted twice weekly with TPA) using both topical administration and dietary administration of the various combinations will be presented. The results indicate that certain combinations of UA, Curc and Met may inhibit mouse skin tumor promotion by TPA to a greater extent than the compounds alone. The ability of the combinations to work when given bothtopically as well as in the diet suggests the potential for broad applications for chemopreventive strategies.


Non-melanoma skin cancer (NMSC) is the most commonly diagnosed malignancy in the United States, affecting more than 3 million Americans each year. Ultraviolet (UV) radiation from the sun, particularly its UVB component (290-320 nm), is an established causative factor for ~90% of skin cancers. The two most common forms of skin cancer, basal- and squamous- cell carcinomas, are treatable if detected early. However, they can be difficult to treat and potentially fatal if left until late stages. Further, epidemiological studies have suggested an amplified risk of other deadly cancers in individuals with a history of skin cancer. Therefore, it is important to design novel approaches, especially focusing on prevention strategies, for the treatment of skin cancers. Studies from our laboratory and by others have shown that topical application of the grape antioxidant resveratrol poses promise in the prevention of skin cancer. However, ideally cancer preventive agents need to be orally administrable for ease of use and broader human acceptability. With this in mind, in this study, we determined the efficacy of dietary grape powder (containing resveratrol in natural amalag- mation with catechins, anthocyanins, polyphenols and flavonoids) against UVB-mediated skin tumorigenesis in the SKH-1 hairless mouse model, which is regarded to have relevance to human NMSC. We employed a UVB initiation-promotion protocol in which the mice were subjected to chronic UVB exposure (180 mJ/cm²; twice weekly, for 28 weeks). The animals received either AIN-76A or grape powder (GP) fortified diet (3% and 5% GP, obtained from the Californian Table Grape Commission), all of which were sugar-matched to the highest GP content. Our data demonstrated that the consumption of GP at both 3% and 5% resulted in a significant inhibition in skin tumor incidence and delay in the onset of tumorigenesis. The average consumption of feed per mouse was 3.5 g/day, corresponding to 105 and 174 mg GP/day in the 3% and 5% GP treatment groups. This dosing regimen seems to be easily achievable for human consumption, as it corresponds to 25.3 and 42.4 g/day, which are equivalent to 1.1 and 1.8 servings of fresh grapes, respectively. Our data also demonstrated that the observed cancer chemopreventive effects of grape powder were accompanied by significant decreases in cellular proliferation markers Ki67 and PCNA, ii) decreases in the oxidative stress marker 4-HNE, and iii) increases in the levels of cleaved caspase 7 and poly (ADP-ribose) polymerase (PARP). Interestingly, an activator of cellular antioxidant response, was found to be downregu- lated in GP treated tumors, compared to UVB alone groups, suggesting a poten- tially protective role of NRF2 in the survival of tumor cells. Overall, our study suggests a strong chemopreventive effect of dietary grape and provides a basis for future human studies.

Chemoprevention of rat oral carcinogenesis by black raspberry phytochemicals. Steve Oghumu, Thomas Knobloch, Jennifer Ahn-Jarvis, Logan Weghorst, Kyle Horvath, Paul Geuy, Christopher Weghorst. Ohio State University, Columbus, OH.

It is expected that in 2016, there will be an estimated 48,330 new cases and 9,570 deaths due to oral cancer in the US, which is about one person every hour every day. Due to the epithelial field defects associated with oral carcinogenesis, tumor recurrence and second primary tumor incidence are common. Recently conducted Phase I Clinical trials demonstrate the ability of black raspberries (BRBs) to modulate inflammatory biomarkers of molecular efficacy that supports a chemopreventive strategy against oral cancer. From these human trials we have established a molecular signature of BRB responsive biomarkers. However, it is essential that a preclinical animal model of BRB chemoprevention which recapitulates the fundamental features of human oral carcinogenesis be developed, so that we can evaluate potential mechanisms of action and validate molecular biomarkers of BRB efficacy. We therefore established the ability of BRB to inhibit oral lesion formation in a rat model of 4-nitroquinoline 1-oxide (4NQO) induced oral carcinogenesis and examined molecular efficacy using a clinically relevant transcriptional signature. Oral cancer was induced in F344 rats using 4NQO in drinking water for 10 weeks followed by regular drinking water for 16 weeks. Rats were administered a diet containing 5 or 10 percent linyophilized BRBs. Tongue tissues were examined for lesion multiplicity, incidence and histopathological features. Clinically derived pro-inflammatory and pro-survival molecular biomarkers were also determined by real-time PCR and ELISA. Dietary administration of 5 and 10 percent BRBs reduced combined lesion incidence and multiplicity by ~39%-3% and ~28%-6% respectively. Histopathological analyses of tongue sections further demonstrate the chemopreven- tive ability of dietary BRB administration in the rat model. Pro-survival and pro-inflammatory biomarkers were downregulated in BRB administered 4NQO induced rats compared to untreated 4NQO induced rats. Our study shows that dietary administration of BRB inhibits oral carcinogenesis in the rat 4NQO model. The rat 4NQO model is suitable for interrogating mechanisms of action of BRB-mediated oral cancer chemoprevention.

Breast cancer is a leading cause of cancer-related deaths among women in the United States. Over the last decade, breast cancer stem cells (bCSC) have been identified and characterized as a key player in breast cancer progression and recurrence. Benzyl isothiocyanate (BITC) is a highly promising cancer chemopreventive constituent of edible cruciferous vegetables such as garden cress. We have shown previously that BITC inhibits bCSC in cultured breast cancer cells (MCF-7 and SUM159 cells) and in spontaneous tumors of mice mammary tumor virus-neu (MMTV-neu) mice in vivo. Krüppel-like factor 4 (KLF4), a zinc finger transcription factor, is known as both oncogene and tumor suppressor in various types of human cancers and plays an important role in reprogramming differentiated somatic cells into induced pluripotent stem cells. The present study demonstrates that KLF4 impedes bCSC inhibition by BITC. Exposure of breast cancer cells (MCF-7, MDA-MB-231, and SUM159) to pharmacological concentrations of BITC (2.5 and 5 μM) resulted in induction of KLF4 protein, which was accompanied by increase of its transcription. In addition, BITC treatment increased nuclear level of KLF4 in breast cancer cells. KLF4 protein expression was also higher in tumors form BITC-treated MMTV-neu mice compared with the control group. Ectopic expression of KLF4 in MCF-7 cells conferred marked protection against BITC-mediated inhibition of CSC as evidenced by aldehyde dehydrogenase 1 (ALDH1) activity. Consistent with these observations, BITC-mediated inhibition of bCSC in MCF-7, MDA-MB-231 and SUM159 cells was significantly augmented by RNA interference of KLF4 as evidenced by ALDH1 activity and frequency of mammospheres. KLF4 overexpression also increased cell migration capacity of MCF-7 cells. Furthermore, knockdown of KLF4 augmented BITC-mediated inhibition of cell migration in SUM159 cells. These results suggest that anticancer activity of BITC may be enhanced by KLF4 inhibition or knockdown in breast cancer cells. This study was supported by the grant RO1 CA129347-09 awarded by the National Cancer Institute.

**#5266** The mechanisms of apoptosis induction by debiubiquitlatinase inhibitor b-ap15 in esophageal squamous cell carcinoma cells. Pei Li, Ping Chen, Xiao-Yu Chen, Jing-Yang Zhang, Bei-Bei Sha, Tao Hu, Yi-Lin Zhang, Ying Du, Zi-Ming Dong, Zhengzhou University, Zhengzhou, China.

Deubiquitinases (DUBs) act on ubiquitinated substrates to catalyze the removal of ubiquitin moieties which can reverse the process of protein degradation, and further affect or regulate cell metabolism, proliferation and differentiation. As a novel small molecular inhibitor, b-AP15 can specifically inhibit the deubiquitinating activity of 199 regulatory subunit UCHL5 (ubiquitin C-terminal hydrolase 5) and USP14 (ubiquitin-specific peptidase 14) in cancer cells. In this study, we aimed to check whether b-AP15 have anti-tumor activity in esophageal cancer cells in vitro and try to explore the underlying mechanisms. Results: CCK-8 assay, colony formation assay and morphological observation results indicate that b-AP15 can significantly inhibit the proliferation activity of EC1 and Kyse 450 esophageal cancer (EC) cell lines in a concentration-dependent manner in vitro studies. Flow cytometry results show cells were arrested in G2/M phase after being treated with b-AP15. Western blot results show that the expression of G2/M phase related proteins p21, p27 and pWee1 significantly increased while the expression of the G1/S phase marker proteins including cyclin D and CDK4/6 was significantly decreased. In addition, flow cytometry FITC annexin V Apoptosis detection kit with PI and Caspase 3 assay kit results show that b-AP15 treatment can significantly induce apoptosis in two EC cell lines. The results from western blot show that the expression of apoptotic protein p-C-Parp and c-caspase 3 significantly increased and was dose-dependent. JC-1 flow cytometry analysis show that b-AP15 treatment can significantly decrease mitochondrial membrane potential. All these results suggest that b-AP15 can induce EC cells apoptosis and may play certain role in mitochondrial control of apoptosis signaling pathway. Conclusion: b-AP15 shows a significantly anti-tumor activity in vitro models of ESCC and the results confirm that b-AP15 can inhibit proliferation of EC cell lines by inducing cell-cycle arrest and promoting cell apoptosis. Key words: b-AP15; esophageal cancer; debiubiquitlatinase (DUBs); apoptosis.

**#5267** Chemoprevention of lung carcinogenesis by dietary nicotinamide and inhaled budesonide. Donna Seabloom, 1 Beverly Wurtz, 1 Art Galbraith, 1 Jenny Antonides, 2 Vernon Steele, 3 Lee Wattenberg, 1 Frank G. Ondrey1. 1University of Minnesota, Minneapolis, MN; 2NCIChemoprevention, Bethesda, MD

Nicotinamide, the amide form of vitamin B3, and budesonide, a synthetic glucocorticoid used in the treatment of asthma, were evaluated for efficacy of chemoprevention of lung adenoma formation in a benzo(a)pyrene murine carcinogenesis model. Female A/J mice were given three doses of 3 mg benzo[a]pyrene three days apart and started on experimental diets either 1 week (early stage intervention) or 8 weeks (late stage) after last carcinogen dose. Aerosol treatments were delivered 5 days per week starting 1 or 8 weeks post carcinogen. Nicotinamide fed at a dietary concentration of 0.75% starting 1 week after last carcinogen administration, inhibited adenoma formation by 54%, and by 39% when begun 8 weeks post final carcinogen. Nicotinamide fed at 0.25% of the diet was not found to effect adenoma formation. Dietary nicotinamide under the same conditions with budesonide administered via aerosol administration at 25μg/kg, reduced lung adenoma formation by 90% at early stage intervention and 49% at late stage intervention. Budesonide alone administered at the same dose level gave 77% inhibition at early stage and 41% inhibition at late stage. Decreases in adenoma formation were statistically significant via ANOVA analysis at both early and late interventions. Combination therapy decreased adenoma formation significantly over either individual therapy at the early stage and significantly compared to nicotinamide alone at the late stage. However, administration of nicotinamide by aerosol inhalation at doses up to 15 mg/kg/day did not result in a statistically significant reduction in tumor multiplicity at 1 or 8 weeks post last carcinogen administration. There is a high level of clinical interest in minimal toxicity lung cancer chemoprevention strategies. We feel administration of a safe dietary agent such as nicotinamide when combined with direct epithelial delivery of a glucocorticoid such as budesonide, commonly used for asthma therapy, is a promising approach for aerodigestive chemoprevention.


With a 5 year survival of less than 8%, the poor prognosis of pancreatic cancer (PanCA) underlines the importance of improving therapies for effective management of this disease. In previous studies, we identified the tumor cell growth inhibitory activities for Nextrutine® (Nx, a bark extract from Phellodendron amurense) and palmatine (PMT) which is a constituent of Nx. These studies revealed an important role for downregulation of KRAS downstream effectors including GLI, STAT3 and NF-κB in mediating growth inhibitory effects. Remarkably, both Nx and PMT exerted synergistic growth inhibitory effects in combination with the conventional chemotherapeutic agent, Gemcitabine (GEM). Despite such promising in vitro observations, the in vivo efficacy of Nx or PMT had not been established. In this investigation, we evaluated the ability of N and PMT to (i) prevent the development of pancreatic tumors; and (ii) inhibit the growth of patient derived pancreatic cancer tissue in short term ex vivo cultures. Studies were also conducted to investigate the underlying molecular mechanism using cell culture models. Our results show that both Nx and PMT are well tolerated in vivo as evidenced by a lack of significant changes in the body weights of athymic mice implanted with Capan-2 cells. Interestingly, both Nx and PMT showed a significant decrease in the circulating levels of inflammatory molecules including IL-6 and CXCL1. In this first proof-of-concept study, PMT treated animals demonstrated a trend towards decreased pancreatic tumor weight with associated histopathological changes. Remarkably, both Nx and PMT inhibited the growth of patient derived pancreatic cancer cells. Mechanistic investigations identified downregulation of STAT3, TrkA, Src, and RPS6 activating potential contributors to Nx or PMT-induced synergistic growth inhibitory effects with GEM. Further investigation of STAT3 signaling revealed the involvement of the prostaglandin receptor, EP4, in a potential feedback loop with STAT3 and in asserting STAT3-mediated inhibition of autophagy. Taken together these data support potential utility for Nx and/or PMT in the management of PanCA and suggest that these agents use multiple mechanisms to affect growth of pancreatic tumors. Supported by NCCIH (R01 AT007448; APK) and VA-MERIT Award (101 BX 000766: APK).

**#5269** Cryptotanshinone activate Nrf2 expression through microRNA regulations. Rachmad A. Dongoran, Tien-Yuan Wu. Tzu Chi University, Hualien, Taiwan.

Cryptotanshinone is a diterpene quinone derived from the roots of Salvia miltiorrhiza Bunge. An increasing number of studies have demonstrated that cryptotanshinone has the pharmacological activities such as anti-oxidative stress, anti-bacteria, anti-inflammation, and anti-cancer. Cryptotanshinone has been found to increase the ARE expression level induced by the Nrf2 and decrease Keap1 expression. This study investigated Nrf2 and Keap1-related microRNA to understand the mechanisms of cryptotanshinone in antioxidant activities. HepG2 cells were treated with cryptotanshinone 2.5 μM in DMEM medium with 1% FBS for 5 days. Total RNA was obtained for small RNA sequencing and real-time PCR analyses. The analyses revealed that Cryptotanshinone had the potential to inhibit miR-27a-5p, miR-142-5p, miR-28 and miR-93-5p levels, which subsequently increased the Nrf2 mRNA level. On the other

hand, the Keap1 mRNA level was suppressed through up-regulation of miR-23a and down-regulation of miR-7-5p. Moreover, Nrf2 and its related downstream antioxidant enzymes such as NQO1 and UGT were increased and the Keap1 protein level was decreased. These findings suggest that cryptotanshinone possesses the cancer chemoprevention activity in up-regulation of Nrf2 mRNA and protein through the suppression of mRNA via microRNA. Future investigations that extend the current study can focus on pharmacological mechanism of cryptotanshinone as a cancer chemoprevention agent, and the safety of long-term uptake of cryptotanshinone for cancer prevention.


Breast cancer is a rather complex and heterogeneous disease broadly grouped into four major subtypes, including luminal-type, basal-like, HER2 amplified, and normal-like, and each with a distinct molecular signature. A non-toxic chemopreventive intervention efficacious against different subtypes of breast cancer is still a clinically unmet need. The present study not only demonstrates chemoprevention of breast cancer in rats by the Ayurvedic medicine phytochemical withaferin A (WA) but also identifies its mechanistic biomarkers common to different subtypes of this disease. Chemopreventive efficacy of WA (4 and 8 mg per kg body weight) was determined using a rat model of breast cancer induced by N-methyl-N-nitrosourea (MNU). The mechanisms underlying breast cancer chemoprevention by WA were elucidated by western blotting, biochemical assays, immunohistochemistry, and cytokine profiling using plasma and tumors from the MNU-rat and/or mouse mammary tumor virus-neu (MMTV-neu) models. Inhibitory effect of WA on exit from mitosis and leptin-induced oncogenic signaling was determined using MCF-7 and MDA-MB-231 cells. Incidence, multiplicity, and burden of MNU-induced breast cancer in rats were decreased by WA administration. For example, the tumor weight in the 8 mg per kg group was lower by 67% compared with controls (P = 0.004). Mitotic arrest and apoptosis induction were common determinants of breast cancer chemoprevention by WA in the MNU-rat and MMTV-neu models. Cytokine profiling showed suppression of plasma leptin levels by WA in rats. WA inhibited leptin-induced oncogenic signaling in cultured MCF-7 and MDA-MB-231 cell lines. WA is a promising phytochemical with the ability to inhibit at least two different subtypes of breast cancer, including neu-driven estrogen receptor negative (ER-) breast cancer in MMTV-neu model and MNU-induced ER+ breast cancer in rats. This study was supported by the grant R01 CA142604-07 awarded by the National Cancer Institute.

**#5271 Prostate cancer chemoprevention by dietary isothiocyanates is associated with suppression of lipogenesis.** Krishna B. Singh, Shivendra V. Singh. Univ. of Pittsburgh Cancer Inst., Pittsburgh, PA.

Isothiocyanates (ITCs), including sulforaphane (SFN) and phenethyl isothiocyanate (PEITC), have been studied extensively for chemoprevention of prostate cancer using both in vitro and in vivo models, but the underlying mechanism is not fully understood. Dependence on fatty acid metabolism is progressively recognized as a unique trait of prostate cancer. Using in vitro and in vivo prostate cancer models, we demonstrate for the first time, that prostate cancer chemoprevention by ITCs is associated with suppression of fatty acid metabolism. Exposure of human prostate cancer cells to plasma achievable concentrations of SFN resulted in a marked decrease in levels of key fatty acid metabolism proteins, including acetyl-CoA carboxylase 1 (ACC1), fatty acid synthase (FASN), and carnitine palmitoyl transferase 1A (CPT1A), that was accompanied by suppression of their transcription. Consistent with the in vitro results, oral administration of prostate cancer chemopreventive concentrations of SFN (6 µmol per mouse, three times per week) resulted in downregulation of ACC1, FASN, and CPT1A proteins in prostate adenocarcinoma of TRAMP transgenic mice when compared with control. Levels of fatty acid metabolites (free fatty acid, triglycerides and phospholipids), lactate, and acetyl-CoA were also lower in the plasma and/or prostate adenocarcinoma of SFN-treated TRAMP mice in comparison with controls. Because fatty acid oxidation is an important step to supply energy to the prostate cancer cells, we explored the possibility of whether SFN could affect the fatty acid oxidation in prostate cancer cells. Many of these changes were also observed with PEITC. In conclusion, it is reasonable to propose that suppression of fatty acid metabolism is an important mechanism in prostate cancer chemoprevention by ITCs. This study was supported by the grants RO1 CA115498-10 and RO1 CA101753-12 awarded by the National Cancer Institute.

**#5272 Chemopreventive activities of a polyphenol rich purified extract from olive oil processing on colon cancer cells.** Barbara Bassani,1 Teresa Rossi,2 Daniela De Stefano,3 Daniele Pizzichini,7 Paola Corradino,7 Antonino Bruno,1 Douglas M. Noonan,4 Adriana Albini,4 Ircs MultiMedica, Milano, Italy; 5Ircs Arcispedale Santa Maria Nuova, Reggio Emilia, Italy; 6Enea Casaccia Research Center, Rome, Italy.

Chemopreventive activities of a polyphenol rich purified extract from olive oil processing on colon cancer cells. This study was supported by the grants RO1 CA115498-10 and RO1 CA101753-12 awarded by the National Cancer Institute.


Introduction: The majority of colorectal cancers (CRCs) develop through the adenoma-carcinoma sequence, while 15-20% develop via the serrated pathway. Sessile Serrated Adenoma/Polyps (SSA/P) are more difficult to detect during colonoscopy and generally require a shorter time follow-up than other lesions (3 vs. 5 years). These polyps lead to cancer faster than conventional adenomas. In order to better define patients at risk for these lesions, we performed a retrospective study to evaluate clinicopathological features of patients diagnosed with SSA/P. Methods: We reviewed pathology reports of patients at Howard University Hospital from 2010-2015. We identified 5,900 patients with colorectal lesions, of whom 312 (5.3%) were diagnosed with SSA/P. We analyzed the specific clinical, pathological and demographic features of patients with SSA/P lesions. Results: We identified 312 cases with SSA/P. The incidence of SSA/P over the 5 years period was 5.3%; 198/312 (63.4%) patients had 2 or more polyps, 54.5% of the SSA/P patients were females, 70.5% of patients were 50-64 years of age and 18% were older than 65. SSA/P lesions’ locations were as follows: rectal: 32.1%; rectosigmoid: 18.6%; sigmoid: 16.8%; Ascending Colon: 13.2% and Descending Colon: 7.1%. Reasons for colonoscopy were as follows: Screening: 43.6%; GI bleeding: 15.1%; Abdominal Pain: 13.1% and Change in Bowel Habits: 10.2%. Conclusion: Our results show that there is a slightly increased predominance of SSA/P occurrence in females. Most SSA/P occurred in patients 50 to 64 years old. This age range is younger in comparison to patients with conventional adenomas. SSA/Ps were predominately distal (rectal, rectosigmoid, sigmoid) whereas previous literature reports a proximal location. Most patients in our study were diagnosed in screening colonoscopies; however, other patients presented with symptoms such as GI bleeding, abdominal pain, or change in bowel habits.

**#5274 Risk of comorbidities among Hispanic and non-Hispanic white breast cancer survivors compared to breast cancer free women.** Avonne E. Connor,1 Kala Visvanathan,1 Stephanie D. Boone,2 Richard N. Baumgartner,2 Kathy B. Baumgartner2. Johns Hopkins Univ. School of Public Health, Baltimore, MD; 2University of Louisville School of Public Health & Information Sciences, Louisville, KY.

Epidemiological studies showed that diet can play a relevant role in reducing the risk of developing colon cancer (CC) and lower rate of CC incidence has been observed amongst populations living within the Mediterranean basin. Olive oil, a major component of the Mediterranean diet, is an abundant source of phenolic compounds. Olive oil production is associated with the generation of waste material, termed olive mill wastewaters (OMWW), that have been reported to be enriched in polyphenols as well. Given the beneficial activity of polyphenols on human health, we investigated whether the use of different batches of purified extracts from OMWW, termed A009, might be effective in exerting chemopreventive activities in vitro and in vivo, on CC cell lines. Cell proliferation and survival were evaluated on A009 treated cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, while the induction of apoptosis was assessed by flow cytometry. Further, functional studies to investigate the ability of A009 to interfere with CC cell line adhesion, migration, sprouting and invasion were performed. Finally, the ability of A009 to interfere with CT-26 CRC tumour cell growth was assessed in vivo. Purified hydroxytyrosol, the major component in the A009 extracts, was used as a control. A009 inhibited cell proliferation, migration, invasion, adhesion and sprouting of CC cells along with the release of pro-angiogenic and pro-inflammatory cytokines (VEGF, IL-8) similar to hydroxytyrosol alone. In vivo, A009 inhibited CT-26 tumour growth in a significant manner over that of hydroxytyrosol alone. Our results show that A009 extracts exert promising chemopreventive properties, suggesting that different polyphenols act synergistically, improving their single component effects in CC cell lines. Finally, our results support the idea of repurposing a waste derived material for nutraceutical employment, with environmental and industrial cost management benefits.
Hispanic women are projected to account for an increasing proportion of the U.S. breast cancer (BC) survivor population which is reaching 3.1 million. The number of BC survivors at risk of developing age-related comorbid conditions is increasing. Limited studies have examined the relationship between being a BC survivor and risk of comorbidities compared to BC free women of similar age. Retrospective studies have reported limited data on the prevalence of prevalent chronic conditions such as diabetes and hypertension are high. In this study we examined the associations between BC survivor status, ethnicity, and risk of comorbidities among long-term BC survivors and BC free women/controls that participated in the New Mexico Women’s Health Study (NMWHS) - a population-based case-control study from 1992-1994. The Long-Term Quality of Life Follow-up (2007-2011) was conducted approximately 15 yr (median) from BC diagnosis for cases or selection date for controls that participated in the NMWHS. Data for comorbidity conditions, lifestyle and reproductive factors were collected at follow-up interview. Baseline data from the NMWHS interviews were also available. A total of 449 BC survivors (68 Hispanic, 131 NHW) and controls (78 Hispanic, 172 NHW) were included in this analysis. Adjusted odds ratios (ORs) and 95% confidence intervals (CI) were calculated using logistic regression models for the associations between survivor status, risk of disease-specific conditions and comorbidities based on Charlson Comorbidity Index (CCI) level (low-high vs none at follow-up. The mean age at follow-up of BC survivors (64.3 years) and controls (64.0 years) was not significantly different. A total of 128 survivors and 139 controls experienced low-high levels of CCI (chi- squared p=0.06). The two most commonly reported CCI conditions among survivors and controls were connective tissue disease (43% of survivors; 33% of controls) and chronic pulmonary disease (25% of survivors; 18% of controls). Overall, BC survivors had increased risk of CCI (OR, 1.66; 95% CI 1.10-2.51) compared to controls. While Hispanic ethnicity was not a significant predictor of CCI, the following factors were significantly associated with CCI and were mutually adjusted for with survivor status: smoked for > 6 months at baseline (OR, 1.59; 95% CI 1.06-2.38); hypertension at follow-up (OR, 1.84; 95% CI 1.19-2.83); and history of hysterectomy (OR, 1.97; 95% CI 1.28-3.03). BC survivor status significantly predicted risk of connective tissue (OR, 1.76; 95% CI 1.17-2.64) and chronic pulmonary diseases (OR, 1.68; 95% CI 1.04-2.70); while no significant associations were observed for risk of diabetes, hypertension, or heart disease. Our findings suggest that smoking history and hysterectomy are contextual factors when considering BC survivorship care, and comorbidities such as connective tissue and pulmonary diseases should also be considered.

#5275 Breast cancer characteristics among Indigenous American women from Peru. Lizeth I. Tamayo,1 Tatiana Vidaurre,1 Jeannie N. Vásquez,2 Sandro Casavilca,2 Jessica I. Palomino,3 Monica Calderon,3 Garth H. Rauscher,1 Laura Fejerman3. 1University of Illinois at Chicago, Chicago, IL; 2Instituto Nacional de Enfermedades Neoplásicas, Lima, Peru; 3University of California San Francisco, San Francisco, CA

Background: Breast cancer prognosis depends on stage at diagnosis and varies by intrinsic tumor subtype. In the US, the distribution of intrinsic subtypes has been shown to differ between racial/ethnic groups, with African American and Hispanic/Latina women more likely to be diagnosed with the more aggressive “triple negative” breast cancer (TNBC), lacking expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2), compared to Non-Hispanic/Latina White women. Hispanics/Latinos in the US are a heterogeneous group originating from different countries with different cultures and ancestral backgrounds. Information about the distribution of tumor subtypes in Latin American regions is lacking. Methods: Data for these analyses come from the Instituto Nacional de Enfermedades Neoplásicas (the Peruvian National Cancer Institute), which diagnoses and treats 20% of all breast cancers diagnosed in Peru. We have abstracted data from clinical records for 303 patients diagnosed with breast cancer between 2010 and 2015 and who self-reported as members of an Indigenous American community from the Andean mountain region (indigenous subgroups: Quechua, Aimaras, N=232) or the Amazonian region (indigenous subgroups: Shipibo-Konibo, Awaún, Ashaninka, Kuyi, Llakiya, Eja). Harakhutu Community Health Center, Shavi, Yanashba, Kiksurutu, Nomatsangenga, N=71). We compared the distribution of age at diagnosis and tumor characteristics by region. Comparisons between the two regions were conducted using chi-squared tests, as well as a t-test for age at diagnosis. Breast cancer subtype was defined as luminal A (ER/PR+ /HER2-), luminal B (ER+ / HER2+), HER2 overexpressing (ER/PR- /HER2 +) and triple negative (ER/PR- /HER2 -) based on immunohistochemistry. Results: Overall, tumors from the 303 Indigenous American women from Peru included in the present study were 37% luminal A, 20% luminal B, 23% HER2 overexpressing and 19% triple negative. Our analyses showed that women from the Amazonian region were diagnosed at a younger age (50 vs. 55 mean age at diagnosis, P value =0.001), later stage (53.0% vs. 41.7% stage III or IV, P value=0.107) and more frequently with triple-negative tumors compared to women from the Mountain Region (30% vs. 16%, P value = 0.115). Conclusion: Differences between Indigenous American women from the Amazonian and Mountain Range regions in Peru could be due to variation in genetic predisposition to particular subtypes of the disease, variation in access to care between the different groups. The more we learn by analyzing diverse populations, subpopulations and revealing heterogeneity within Latin American women, the better equipped we will be to provide adequate care for all women.

#5276 Risk factors for ductal carcinoma in situ of the breast in African American women. Kimberly A. Bertrand,1 Traci N. Bethea,1 Lynn Rosenberg,1 Elisa V. Bandera,2 Melissa Troester,3 Thaer Khoury,4 Christine B. Ambrosone,1 Julie R. Palmer4. 1Stone Epidemiology Center at Boston University, Boston, MA; 2Rutgers Cancer Institute of New Jersey and Rutgers School of Public Health, New Brunswick, NJ; 3University of North Carolina Lineberger Comprehensive Cancer Center, Chapel Hill, NC; 4Roswell Park Cancer Institute, Buffalo, NY.

Introduction: The clinical significance of a ductal carcinoma in situ (DCIS) diagnosis is uncertain. While overall breast cancer mortality risk from DCIS is very low, a recent analysis showed that African American women diagnosed with DCIS were more than twice as likely to die from breast cancer as U.S. white women. Elucidation of risk factors for DCIS in African American women may provide opportunities for risk reduction. Methods: To assess the relation of reproductive, anthropometric, and other factors to risk of DCIS in African American women, we pooled data from three epidemiologic studies participating in the African American Breast Cancer Epidemiology and Risk (AMBER) Consortium - the Black Women’s Health Study, the Carolina Breast Cancer Study, and the Women’s Circle of Health Study. These studies contributed 805 DCIS cases and 13,830 controls to the analysis. We used unconditional logistic regression models to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for risk of DCIS in relation to each risk factor of interest. Multivariable models were used to mutually adjust for risk factors as well as matching factors (age, study, geographic region, and questionnaire time period). We also compared results to associations observed for invasive breast cancer (n=3,765). Results: First degree family history of breast cancer was associated with an increased risk of DCIS (OR 1.62, 95% CI 1.32, 1.98). Oral contraceptive use within the past 10 years (vs. never) was also associated with increased risk (OR 1.31, 95% CI 1.01, 1.70), as was late age at first birth (≥25 years vs. <20 years) (OR 1.29, 95% CI 1.04, 1.61). Older age at menarche (≥15 years vs. <11 years) was associated with a reduced risk (OR 0.67, 95% CI 0.49, 0.92). Women with higher body mass index (BMI) in early adulthood (≥25 kg/m2 at ages 18 or 21) had a reduced risk of DCIS compared to those who had a BMI <20 kg/m2 in early adulthood (OR 0.71, 95% CI 0.56, 0.90). History of hysterectomy (yes vs. no) was associated with a reduced risk (OR 0.64, 95% CI 0.49, 0.85). Other factors that were associated with a reduced risk of DCIS were ever having children (versus never had any children) (OR 0.76, 95% CI 0.61, 0.97). We also found that age at menopause was associated with a reduced risk of DCIS (OR 0.71, 95% CI 0.57, 0.88). Women with breastfeeding duration of ≥4 months were associated with a reduced risk of DCIS (OR 0.54, 95% CI 0.33, 0.89). Women with history of diabetes or hypertension were associated with a reduced risk (OR 0.67, 95% CI 0.49, 0.92). Women with greater waist-to-hip ratio and absence of breast feeding were associated with increased risk of DCIS and particularly with estrogen receptor negative breast cancer. No other factors were associated with DCIS. Conclusion: Our findings suggest that smoking history and hysterectomy are contextual factors when considering BC survivorship care, and comorbidities such as connective tissue and pulmonary diseases should also be considered.
candidates significantly associated with differential disease outcome. Alter-
tations of outcome-associated candidates were validated in an independent cohort of
White (n = 115) and Black (n = 17) EEC patient transcript expression data. Results: We identified and validated 89 proteins and transcripts significantly altered between White vs Black EEC patients. Pathway analyses revealed candi-
dates elevated in White EEC patients correlated with marked activation of mo-
lecular signaling pathways regulating viral infection, but inhibition of those regulating cell death and necrosis. Candidates elevated in Black EEC patients largely correlated with activation of cell viability and nucleic acid metabolism, but inhibition of cell death, glucose metabolism disorder and inflammatory signaling. Correlation with patient outcome measures revealed 11 candidates significantly associated with differential OS and 8 candidates with differential PFS in EEC patients. All outcome-associated candidates elevated in White pa-
tsients significantly correlated with a low risk of poor OS and poor PFS (Hazard Ratio (HR) < 1, Wald p-value < 0.05). Conversely, the majority of outcome-
associated candidates (88%) elevated in Black patients correlated with a high risk of poor OS and poor PFS (HR > 1, Wald p-value < 0.05). Several OS (27%) and PFS (75%) candidates remained significant after adjustment for disease stage and grade as well as myometrial invasion. Alteration trends for several OS (27%) and PFS (25%) candidates were validated in an independent cohort of White and Black EEC patients. Conclusions: Our analyses identified and confirmed molecu-
lar alterations between White and Black EEC patients, including outcome-
associated candidates largely supportive of better outcome in White patients, but poor outcome in Black patients. The current study revealed evidence of race-specific differences in patient outcomes in Black EEC, consistent with the historic disparity of poor outcome in Black patients.

#5278 Effect of centralization on health disparities in lung and bladder cancer surgery. Wil Lieberman-Cribbin, Martin Casey, Matthew Galsky, Apu-
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Background: Centralization has been advocated for both cystectomy and pneumonectomy, since it has been associated with reductions in mortality. Ra-
cial disparities exist for both lung and bladder cancer surgical outcomes despite trends in hospital centralization. We hypothesized that disparities exist in the centralization process for both lung and bladder cancer surgery, and that this has differentially affected surgical outcomes in black and white patients. Methods: The study population was extracted from the New York Statewide Planning and Research Cooperative System (SPARCS) database spanning 1997 to 2011, and included 26,750 lung cancer surgeries and 8,168 cystectomies. Hospitals were classified according to procedure volume; patient-hospital distance (PHD) and distance to the nearest high volume / very high volume (HV/VHV) were calculated. Logistic models were performed to determine factors associated with the utilization of HV/VHV or low volume / very low volume (LV/LVVL) hospitals. Additional models were then performed to assess the association between race and in-hospital mortality, stratified according to whether patients used HV/
VHV or LV/LVVL hospitals. Results: For cystectomy, PHD increased over the study period while distance to the nearest HV/VHV decreased; for lung cancer surgery, PHD increased but distance to the nearest HV/VHV hospital was con-
stant. For both surgical procedures, black patients experienced increased odds of LV/LVVL utilization over time (for lung cancer surgery, OR_{adj} ≥ 1.20; 95%CI
1.01-1.43; for cystectomy, OR_{adj} ≥ 1.59; 95%CI [1.26-2.02]). When HV/VHV hospitals were located farther from patients, the odds of HV/VHV utilization decreased while the odds of LV/LVVL increased for both lung cancer and bladder cancer patients. Lung cancer and bladder cancer in-hospital mortality was higher in blacks (OR_{adj} ≥ 1.50; 95%CI [1.21-1.86]; OR_{adj} ≥ 1.80; 95%CI [1.12-2.90], respectively) compared to whites. Conclusions: Racial differences persisted in hospital utilization and in surgical outcome for both lung and bladder cancers. While proximity and insurance are important determinants of quality care, other personal and community variables not captured by SPARCS are influential in lung and bladder surgical treatment and ultimately outcome. Specific inter-
ventions are needed to address accessing and utilizing quality care in under-
served populations, including black and low SES patients, and patients with large distances from high-volume hospitals.

#5279 Association of Vitamin D deficiency with breast cancer in African-
American and Hispanic women in south Los Angeles. Yanjun Wu,1 Mari-
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Background: Experimental evidence supports a protective role of Vitamin D level in breast carcinogenesis, but epidemiologic evidence is inconsistent. Af-
can Americans have high prevalence of Vitamin D deficiency and the African American women with breast cancer have the highest mortality rate. The aim of this study is to investigate the association of Vitamin D levels with breast cancer risk and survival in African American women. Methods: This is a case-control study of 660 African American and Hispanic women with and without breast cancer in South Los Angeles. Blood samples were collected prior cancer treatment and the serum 25-hydroxyvitamin D (25(OH) D was measured by Quest Diagnostics. Information regarding BMI and clinical factors were obtained by medical record abstraction. Logistic Regression with multivariable analysis was used for determining the association of Vitamin D deficiency (<20ng/ml) with breast cancer. Kaplan-Meier survival analysis and Cox Regression with multivariate analysis were used to assess disease-free sur-
vival and the relative risk of disease progression. Results: Our data shows that 69.2% of African American women and 37.8% of Hispanic women suffer from Vitamin D deficiency in our cohort. African American women had significant lower level of Vitamin D3 compared with Hispanic women in all age groups. The lower Vitamin D3 level was observed among age groups of 31 to 50 in African Americans. The deficiency in Vitamin D levels was significantly associated with breast cancer in both African Americans (OR=2.5, p = 0.007) and Hispanics (OR=1.9, p = 0.009). Interestingly, we found that a significant association of Vitamin D deficiency with trip negative breast cancer (TNBC) in African Amer-
ican women (HR=1.95, 95%CI=1.04-3.67, p=0.04) faced a significantly higher risk level was observed in African American women with TNBC. There was no significant association of Vitamin D deficiency with tumor size, lymph node involvement and tumor stage. The deficiency of Vitamin D3 level was not associated with disease progression in this cohort of women. Conclusion: Our data suggests that a significant association of Vitamin D deficiency with breast cancer in both African Americans and Hispanics, especially more associated with type of TNBC in African American women in our cohort.

#5280 Disparity-related survival among adolescent and young adult pa-
tients with sarcomas in Texas. Jaqueline Contrera Avila,1,2doi, Living-pornon,3 Ana Rodriguez,4 Anne Kirkhoff,5 Yong-Fang Kuo,6 Sapna Kaul.7 The
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Purpose: Sarcomas are a rare and heterogeneous group of cancers that can occur in both children and adults, and have a peak incidence in adolescents and young adults (AYAs). Sarcomas may account for ~10% of all invasive cancers among AYAs and >50% of all sarcoma diagnoses occur in this age group. Additionally, sarcoma survival among AYAs is lower than that in children or adult patients. Furthermore, although less understood, significant socioeconomic factors may exist in survival among AYAs with sarcomas. The current study examines disparity-related survival among AYAs with sarcoma in Texas, one of the largest U.S. states, that has over 50% non-Hispanic white population. Methods: N = 4,131 patients diagnosed with bone or soft tissue sarcomas were identified in the statewide Texas Cancer Registry from 1995-2013 at ages 15-39 years (i.e., AYA age range). Socio-demographics at diagnosis (e.g., race and ethnicity, insurance, and county of residence), clinical characteristics (e.g., early vs. late stage, treatment (e.g., chemotherapy), and vital status as of December 31, 2013) were summarized. Associations between patient survival and demographic/clinical variables were examined using the Kaplan Meier (KM) method, and the Cox Proportional Hazard (CPH) regressions. KM survival curves were com-
pared using log-rank tests. Results: Over half of our patients were male (57.4%). 36.6% were Hispanic, 12.7% were non-Hispanic black, and 46.6% were non-
Hispanic white. Nearly 22% of patients were uninsured. Furthermore, 20% of patients were diagnosed with metastatic sarcoma. The average 5-year KM sur-
vival for all patients was 69.5% (95% CI =67.9%-71.0%). KM survival curves significantly differed by variables such as sex, race and ethnicity, insurance and tumor staging. 5-year survival was higher for females (73.7% vs. 66.1% for males, p < 0.001), for non-Hispanic white patients (72.1% vs. 63.9% for non-
Hispanic blacks, p < 0.001), patients with private insurance (76.0% vs. 68.2% for uninsured, p < 0.001), and those with early stage sarcomas (77.1% vs. 23.4%, p < 0.001). After adjusting for treatment and tumor staging, the CPH model demonstrates that males (hazard ratio (HR) = 1.93, 95% CI = 1.25-3.00, p < 0.003) and those who resided in Texas counties bordering Mexico (HR = 3.2, 95%CI = 1.04-3.67, p = 0.04) faced a significantly higher risk of death than their counterparts. Conclusion: Our population-based analyses show that substantial disparity-related differences in survival exist among AYA sarcoma patients in Texas. Further study is warranted to identify specific interventions to improve survival outcomes in this high risk patient group.
#5281  Komen Tissue Bank donors: Genetically determined ethnicity and race. Julia R. McCarty,1 Guanlong Jiang,2 Teresa Mahin,1 Nicholas Scherer,3 Jonathon Dunn, A Maria Storniolo,1 Natascia Marino,2 Susan G. Komen Tissue Bank at the IU Simon Cancer Center, Indianapolis, IN;1 Indiana University School of Medicine, Indianapolis, IN;2 Indiana University School of Medicine, Indianapolis, IN;3 Indiana University, Indianapolis, IN.

Background: Several evidences indicate that different racial and ethnic backgrounds affect the incidence and severity of diseases such as breast cancer and diabetes, and the response to therapy. For example White and African American women are more likely to develop breast cancer than Hispanic and Asian women. Moreover, African American women are more likely to develop more aggressive (Triple-negative Breast Cancer) and advanced-stage (Breast Cancer at young age). Therefore, given the health disparity and with the advance of personalized medicine, it is becoming critical to address population stratification. The Komen Tissue Bank at IU Simon Cancer Center (KTB), the only biobank of normal breast tissue from healthy women, is employing a more accurate approach to detect population stratification through the use of Ancestry Informative Markers (AIMs). Methodology: A total of 2,973 DNA samples were obtained from the KTB. Genotyping was performed using the KASP technology (LG Genomics) and a 41-SNP panel (labeled 41-AIM panel) selected from Nierveighel et al, 2013. Genotype analysis using the 41-AIM panel along with a Bayesian clustering method (STRUCTURE) was able to discern continental origins including European/ Middle East (Caucasian), East Asia, Central/ South Asia, and Oceanic Islands. A reference set was created from the KTB HeatMap. The results were compared with the self-reported data. Results/Conclusion: Our genetic ancestry analysis indicates that out of 2,973 KTB tissue donors, 2,132 are primarily Caucasian, 532 African, 234 of Asian, 72 Latino, 3 Oceanic origins. High mixture of racial markers was observed in several samples. Moreover, when we compare these findings with the self-reported data we find a 10.9% of discrepancies indicating the imprecision of the self-reported information. The implementation of the genetic ancestry data into the KTB creates the potential for a more accurate study of health disparities.

#5282  Comparison of a public versus private hospital in New York City in delivering timely adjuvant chemotherapy among stage III colon cancer patients. Daniel Lin,1 Benjamin Levinson,2 Judith D. Goldberg,3 Tsivia Hochman,1 Lawrence P. Leichman,4 Heather T. Gold2.1 New York University Langone Medical Center, New York, NY; 2 New York University School of Medicine, New York, NY.

Background: Although the optimal timing of adjuvant chemotherapy (AC) for stage III colon cancer patients has been debated, most studies recommend initiating AC within approximately 60 days of surgery. Significant disparities in timeliness of AC initiation in colon cancer have been reported in public versus private hospitals, with longer time to AC at public hospitals. We evaluated whether timeliness of AC differed between a public and a private hospital, both affiliated with the same major academic institution in New York City. Methods: We conducted a retrospective cohort study of Stage III colon cancer patients who underwent surgery and received AC at the same institution from 2008-2015 at NYU Langone Medical Center’s affiliated public hospital (Bellevue) or its private hospital (Tisch). Patient data were obtained through review of hospital tumor registry and electronic medical records. Patient characteristics were compared by hospital. We defined timeliness as receipt of AC within 60 days post-operatively. Univariate and stepwise multivariable logistic regressions were used to identify factors associated with timely AC. Results: Forty three patients at Bellevue Hospital and 79 patients at Tisch Hospital who underwent surgery and received AC at the same institution were included. Median number of days to AC was significantly greater among patients receiving care at Bellevue (53, range 31-231) compared to Tisch (43, range 25-105; p=0.002). However, the percent-age of patients who received timely AC did not differ substantially at Bellevue and Tisch (74% vs 81%, p=0.40). Individual characteristics significantly associated with timely initiation of AC were non-Hispanic ethnicity (OR: 2.71, 95% CI: 1.06-6.95), married (OR: 2.89, 95%CI: 1.15-7.30), and laparoscopic (vs open) surgery (OR: 1.89, 95%CI: 1.64-2.15). Timing of AC at Bellevue compared to Tisch was not significant (OR:0.68, 95%CI:0.28-1.65). When hospital and other factors were examined jointly, only age (OR: 0.95/year, 95% CI: 0.91-0.99) and laparoscopic (vs open) surgery (OR: 5.65, 95% CI: 1.92-16.62) remained as important factors associated with receiving timely AC (Likelihood Ratio Chi-Square=14.95, p=0.0019). When hospital was omitted from multivariable analysis, age and surgery type still remained the only significant factors associated with timely AC (OR’s unchanged, Likelihood Ratio Chi-Square=14.81, p-value=0.0006). Conclusions: The proportion of patients receiving timely AC within 60 days of surgery was similar at both an affiliated public and private hospital at NYU Langone Medical Center. Age and type of surgery were significant predictors of timeliness in our population. Further research should be conducted to understand how system-level factors may promote timely receipt of care.

#5283  Association between body mass index and prostate cancer among African American men. Margaret S. Pichierio, Cheryl J. Smith, Wei Tang, Tiffany Gorsey, Stefan Amb. National Cancer Institute, Bethesda, MD.

Prostate cancer (PCa) is a leading cause of cancer death in US men. Yet, the etiology of prostate cancer remains poorly understood, with only older age, African ancestry family history of the disease, and multiple germline genetic variations being established disease risk factors. Prostate cancer occurs more often in African-American (AA) men and Caribbean men of African descent than in men of other race/ethnicities. While it has been previously suggested that obesity (measured by body mass index [BMI, kg/m2]) may worsen disease-related outcomes among prostate cancer patients, the relationship of obesity and prostate cancer risk, using race as a predictor, has not been fully explored. Using multivariable logistic regression, we estimated the risk of total PCa for 976 cases and 1,032 age-matched controls, with equal proportions of European-American (EA) and AA men. Among men in the NCIC Maryland Prostate Cancer Case-Control Study, BMI was inversely associated with disease outcomes in AA men, but not among EA men. Consistent with previous literature, BMI in EA men was shown to be positively associated with overall prostate cancer risk. Our findings suggest an obesity paradox, where obesity may protect against incidence of PCa among AA men. Studies are needed to elucidate the underlying mechanisms responsible for the differential effects of obesity in AA and EA men with prostate cancer.

#5284  Gastric cancer in the Alaska Native people: A cancer health disparity. Holly Martinson,1 Steven Albers,2 Matthew Olness.1.1 Univ. of Alaska Anchorage, Anchorage, AK; 2 Mayo Clinic, Rochester, MN; 3 Alaska Native Medical Center, Anchorage, AK.

Gastric cancer in the Alaska Native (AN) people occurs at a 3-fold higher incidence and 4-fold higher mortality rate compared to Non-Hispanic Whites (NHW), representing one of the largest cancer disparities in the AN population. We aimed to review the AN gastric cancer burden and identify clinicopathological factors that are associated with cancer outcomes. Patient information was collected from 132 AN gastric cancer patients diagnosed between 2006-2015 at the Alaska Native Medical Center. The Surveillance, Epidemiology and End Result database 18 was used to collect comparison United States NHW gastric cancer patient data. Compared to NHW patients, AN gastric cancer patients have a higher incidence rate, are significantly younger, 60 versus 69 years, and have a poorer 5-year overall survival rate of 10% compared to 22%. AN patients differ from NHW patients in gastric cancer anatomic location, subtype, and high presence of signet ring cell gastric cancer. For AN patients, tumors were positive at the time of diagnosis for Helicobacter pylori, 77% had chronic gastritis, 30% had a family history of a first-degree relative with gastrointestinal cancers, and 82% were current or former tobacco users. Using univariate analysis, diminished overall survival was observed with anatomic site, increasing stage, no treatment, the number of lymph nodes examined during resection, and chronic gastritis. Multivariable analysis revealed stage and treatment type were independently associated with improved overall survival. AN gastric cancer cases vary in their clinical and epidemiologic features, relative to NHW.

#5285  Glucocorticoid-mediated upregulation of stress oncoproteins: implications for prostate cancer health disparities. Leanne Woods-Burnham,1 Arthur Love,1 Christina K. Calixto-Du Ross,2 Laura Stiel,1 Evelyn S. Sanchez,1 Kwame Amponsah,1 Susanne Montgomery,2 Colwiclk Wilson,2 Carlos A. Canio,1 Loma Linda University School of Medicine, Loma Linda, CA; 2 Loma Linda University School of Behavioral Health, Loma Linda, CA.

Prostate cancer (PCa) presents the greatest US cancer health disparity in terms of incidence and mortality, disproportionately affecting African American (AA) men. The biological characteristics of prostate tumors are exaggerated in AA men compared to European American (EA) men at time of diagnosis. The mechanisms underlying increased PCa aggressiveness in AA men are not fully understood; therefore, there is a critical need to identify biological determinants contributing to PCa mortality disparities. Glucocorticoids—a type of stress hormone—have been implicated as driving factors in PCa progression. The underlying mechanism involves endogenous glucocorticoid (cortisol) binding to its glucocorticoid receptor (GR) and activating genes that promote tumor aggressiveness and therapy resistance. A clinical dilemma exists as glucocorticoids, important in the palliative care of PCa patients, are now emerging as accelerators of disease progression and shortened survival. Chronically elevated levels of
#5286 Type 2 diabetes and increased risk of estrogen receptor-negative breast cancer in African American women. Julie R. Palmer, Nelsy Castro-Webb, Kimberly A. Bertrand, Traci N. Bethea, Lynn Rosenberg. S lone Epidemiology Center at Boston University, Boston, MA.

Introduction: Type 2 diabetes (T2D) is hypothesized to be a risk factor for breast cancer. Possible mechanisms include adverse effects of impaired glucose regulation on endogenous hormone levels and increased inflammation of adipose tissue, which may be favorable to breast epithelial cell transformation, tumor-related angiogenesis, and cancer cell invasion. Recent meta-analyses suggest that T2D may be associated with a 15-20% increase in risk, but whether that is due to insulin resistance is body mass index (BMI) is uncertain. Further, few studies have reported results separately by estrogen receptor (ER) status of the tumor, none have had interpretable numbers of ER- cases, and the only previous report on the association in African American (AA) women analyzed all subtypes together. Methods: We used data from the prospective Black Women’s Health Study to examine the relation of T2D to incidence of invasive breast cancer, overall and by ER subtype, among 54,337 AA women free from T2D and breast cancer at baseline and followed for 20 years. Included were 1,851 incident breast cancer cases, with 468 classified as ER+ and 914 as ER--; data on ER status were not available for 469 cases. Participants were asked about medical conditions on each biennial questionnaire. In a validation study, 95% of self-reports of diabetes were confirmed by medical records.

Conclusions: The finding suggests that ER-negative breast cancer among diabetic women differs according to how well the diabetes is controlled.

#5287 Recent trends in racial and regional disparities in cervical cancer incidence and mortality. Wonsuk Yoo, Sangmi Kim, Steven Coughlin, Sejong Bae, Edward Partridge, Warner Huh, Sarah Dilley, Yunmi Chung.

Objective: To examine trends and disparities in cervical cancer incidence and mortality by race and region-specific rates and the trend between 2000 and 2012. Methods: The Surveillance, Epidemiology, and End Results (SEER) 18 Program data was used. Incidence and mortality rates, annual percent changes, and disparity ratios of cervical cancer were calculated using SEER*Stat software and joinpoint regression for four groups: US14, Non-Hispanic White (NHW), US14, Non-Hispanic Black (NHB), South-NHW, and South-NHB, where the South included 4 regions from Georgia and Louisiana.

Results: The incidence and mortality rates were much higher among NHB women compared to NHW in the South and the US. The degree of racial disparities between NHB and NHW women was greater in terms of mortality rates than incidence rates. For all age groups, NHB women in the South consistently had higher incidence rates than NHW women in the US region. The mortality disparity between NHB and NHW women, followed by NHB women in the South, NHW women in US14, and finally NHW women in the South. Conclusions: Although racial disparity has been narrowed in recent years, there has been little change or even a growing gap between white women in the South and their counterparts in the US14 region. Age-specific analysis further indicated that the emerging regional gap observed among white women might be attributable to the excess number of new cases and deaths among young women.

#5288 Perception of barriers and facilitators to hepatitis B virus screening, vaccination, and treatment care among Asian American physicians. Patricia Estrella, Ming Chin Yeh, Yin Tan, Carolyn Y. Fang, Sarit A. Golub, Grace X. Ma.

Objective: To examine practices and factors affecting Hepatitis B Virus (HBV) screening, vaccination, and treatment among Asian-American physicians in New York City. Methods: Members of the Chinese American Independent Practice Association (CAIPA) will be recruited to participate in a comprehensive survey, developed based on prior research, to assess physicians’ practice in HBV screening, vaccination, and treatment. This study will also examine barriers/facilitators toward HBV prevention and treatment in knowledge, familiarity, and awareness of guidelines, perception, attitude, behaviors, self-efficacy, motivation, patient factors, cultural, and environmental factors. A bivariate analysis and multivariate regressions will be conducted to evaluate factors that predict study outcomes. Results: We hypothesize that Asian American physicians are more likely to screen for HBV, but are experiencing many barriers unique to the Asian American physicians that are leading them improperly perceive certain factors as important. Conclusions: It is crucial. As a result of the reverence of the doctors, a cultural norm prevalence within the Asian community, physicians are uniquely positioned to improve HBV screening, vaccination, and treatment among Asian-American physicians that are leading them improperly perceive certain factors as important. Therefore, identifying chronically infected persons and linking them to care is crucial. As a result of the reverence of the doctors, a cultural norm prevalence within the Asian community, physicians are uniquely positioned to improve HBV screening, vaccination, and treatment among Asian-American physicians that are leading them improperly perceive certain factors as important. Therefore, identifying chronically infected persons and linking them to care is crucial.

#5289 Assessment of environmental influences, behavioral risk factors and genetic differences among a cohort of 819 African American community members. Jacquelineucci, Ming Chin Yeh, Khursheed Navder, Joel Erblich, Elizabeth Blackman, Grace X. Ma, Camille Ragin, Hunter College, CUNY, New York, NY; Fox Chase Cancer Center, Temple University Health System, Philadelphia, PA; Temple University, Philadelphia, PA.

Objective: This study was designed to address genetic, environmental and lifestyle risk factors for cancer in populations of African Ancestry. We have established a non-cancer control registry which involves the collection of epidemiological, lifestyle, culture and cancer prevention behaviors (such as cancer screening and diet, etc). Methods: Data was coded and analyzed using Stata from...
#5290  **Racial variation in terminal duct lobular unit (TDLU) involution in Chinese and Polish breast cancer patients.** Hyuna Sung,1 Changyuan Guo,2 Jennifer Guida,1 Shan Zheng,2 Erni Li,3 Jing Li,3 Nan Hu,1 Joseph Deng,1 Montserrat Garcia-Closa,1 Jonine Figueroa,2 Mark Sherman,3 Gretchen Gierach,3 Ning Lu,3 Xiaohong R. Yang1.

Introduction: Terminal duct lobular unit (TDLU) involution, an age-related physiological process, is characterized by the reduction of size and numbers. Reduced or absent involution is associated with higher mammographic density, a strong breast cancer risk factor. Both factors predict risk of developing breast cancer among women with benign disease. Given that prior studies have been conducted mainly in White women, we compared the extent of TDLU involution among Western and Asian breast cancer cases to describe potential racial heterogeneity in breast cancer etiology. Method: We obtained three TDLU involution metrics (count/100 mm², mean span, and mean acini count/TDLU; all inversely correlated with TDLU involution) measured in benign breast tissue sections from 379 Chinese (254 luminal A and 125 core basal phenotype [CBP] cases) and 476 Polish (407 luminal A and 69 CBP) breast cancer cases. Polytomous logistic regression was performed using tertiles of TDLU measures as ordinal dependent variable and race as an independent variable. Covariates included age, body mass index, and parity. Analyses were performed separately by age group (<50, ≥50) and subtype (luminal A and CBP). Results: Among luminal A cases, Chinese had significantly greater TDLU count compared with Polish in both age groups (ORtrunc = 11.0; 95% CI = 2.8-42.3; P = 0.001 for age<50; ORr= 12.1; 95% CI= 5.1-28.7; P = 1E-08 for age≥50). In addition, Chinese were more likely to have greater mean span and acini count among younger cases (ORtrunc = 4.5; 95% CI = 1.4-14.4; P = 0.001 for mean span and ORr= 7.4; 95% CI= 4.13-13.4; P = 3E-11 for acini count) but not among older cases. Among CBP cases, Chinese were more likely to have greater acini count compared to Polish only among younger women (ORr= 2.6; 95% CI= 1.2-6.9; P = 0.03 for age<50). Conclusion: We found Chinese were more likely to have reduced TDLU involution compared to Polish cases after accounting for potential confounders, with greater differences for luminal A and younger women. Studies of TDLU involution in diverse populations are needed to confirm and understand whether population difference in TDLU involution metrics are related to age at breast cancer onset and risks for specific tumor subtypes.

#5291  **Effect of screening CT results and features on lung cancer risk prediction within the National Lung Screening Trial.** Hilary A. Robinson1, Christine D. Berg2, Li C. Cheung3, Anil K. Chaturvedi,4 Hormuzd A. Katki5,1 Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 2National Cancer Institute, MD.

BACKGROUND: In the course of screening, individual risk of disease evolves based on screening results. We calculated how individual lung cancer risk changes based on screening CT findings using data from the National Lung Screening Trial (NLST), which conducted annual screening for 3 years. METH-ODS: We calculated lung cancer risks by combining CT findings with individual predicted 1-year “pre-screening risk.” Pre-screening risk (r(x)) was predicted in the absence of screening using a validated risk model (Katki et al., JAMA 2016) with covariates (x): age, education, sex, race, smoking intensity/duration/quit-years, body mass index, family history of lung cancer, and self-reported employment. We used log-binomial regression to calculate the risk of an “interval” lung cancer (within 1 year of a negative screen) or a “screen-detected” cancer detected at the next annual screen. For each, covariates included log-transformed 1-year pre-screening risk and CT findings including classification as negative or false-positive and other specific features. RESULTS: The median 1-year pre-screening risk at the first NLST screen was 0.32% (interquartile range 0.19-0.53%). Among CT-negatives, risk over the next year was substantially reduced as r(x)2 (median interval cancer risk 0.05%), but risk at the next screen reverted to pre-screening risk as r(x). Risk at the next screen was higher for those whose CT findings were more likely to be cancerous: smokers (r(x)3), mesothelial (r(x)6), and high TLDI scores (r(x)2.5). Risk of developing breast cancer (median risk 0.1%) increased as r(x) and higher. Among CT-false-positives, overall risk at the next screen increased as r(x)2.5 (median risk 1.5%). Risk was higher among those with nodule(s) that were larger, had spiculated margins (median risk 4.1%), were located in the upper lobes (median risk 1.4%), or grew during the most recent screening interval (median risk 7.9%), while nodules with smooth margins indicated lower risk (median risk 0.7%). Those with a smooth-margins nodule and no risk-increasing factors essentially reverted to their pre-screening risk at the next screen as r(x)0.29% (median risk 0.29%), as if they had screened negative. Overall, only the immediately prior screen result, and not earlier screens, predicted lung cancer risk (all p>0.2). Exponents were similar for each interval and at each screen (all p>0.07). CONCLUSIONS: CT-negatives experienced reduced lung cancer risk over the next year, but reverted to their pre-screening risk at the next screen. CT-false-positives experienced substantially increased lung cancer detection at the next annual screen, with most risks exceeding 1%. These risk increases were explained by specific CT features including nodule size, location, margins, and growth.

#5292  **Multitarget fecal miRNA test combined with fecal occult blood test and fecal miRNA test for colorectal cancer screening.** Yoshihiko Koga1, Hyun Soo Kim2,3,4, Naoko Takahashi,1 Naoko Takeya,1 Yutaka Saito1,2, Hiroshi Saito3,4, Yasuhiro Matsumura1, National Cancer Center, Kashiwa, Japan; National Cancer Center Hospital East, Kashiwa, Japan; 3National Cancer Center Hospital East, Tokyo, Japan; 4National Cancer Center, Tokyo, Japan.

Background & Aims: The fecal immunochemical test (FIT) is used for colorectal cancer (CRC) screening worldwide. However, there are several issues of the false-positive and the false-negative. To overcome these problems, various molecular biological tests have been combined to FIT. In this context, multitar-get fecal DNA test was approved by FDA in August 2014. In addition, multitar-get fecal microbiota has been recently reported. We have reported the usefulness of fecal miRNA analysis to detect CRC. Here, we investigated the applicability of multitarget fecal miRNA test combined with FIT and FIT and fecal miRNA test using fecal RNA extracted from FIT residual. Methods: In this study, 57 patients with invasive CRC, 33 patients with advanced adenoma, and 60 healthy individuals were enrolled. Each participant collected 10 mg fecal samples using the FIT sampling container for 2 days. After FIT was performed, total RNA was extracted from the residue and miRNA expression was analyzed by using real-time RT-PCR. The sensitivity and specificity of the multitarget fecal miRNA test combined with FIT and fecal miRNA test was analyzed by using the decision tree analysis. Results: Approximately 1μg RNA was extracted from 10 mg fecal sample. The expressions of miR-16, miR-92a, miR-106a, miR-142-3p, miR-223, and miR-451 in fecal samples were significantly higher in the patients with advanced neoplasm (including invasive CRC and advanced adenoma) than in the healthy individuals (P<0.05). The sensitivity and the specificity of the FIT alone were 74.4% (67/90) and 93.3% (56/60), whereas those of the multitarget fecal miRNA test were 93.3% (83/90) and 91.7% (55/60). Moreover the multitarget fecal miRNA test could detect 81.8% of patients with advanced adenoma (27/33). Conclusions: Multitarget fecal miRNA test combined with FIT and fecal miRNA test may be a useful new CRC screening method.

#5293  **Have you checked? Disparities in cancer screening practices among minority populations.** Tamaryn F. Gray,1 Joyceyn Cudjoe1, Hae Ra Han1, Jennifer Wenzel1, Roland Thorpe,1 Jeanne Murphy1,2 Johns Hopkins University, Baltimore, MD; 3National Cancer Institute, Rockville, MD.

Objective: There is a disproportionately higher burden of cancer among racial and ethnic minorities, with the incidence only expected to increase significantly by 2050. The racial/ethnic differences in screening are due to the lack of cancer prevention screening among this population. The purpose of this research is to provide case study reports about cancer screening practices among at-risk populations as well as those from underrepresented racial and ethnic minority backgrounds as well as sexual and gender minorities in order to describe disparities in cancer screening practices. These five case studies reveal how racial disparities vary by cancer site. Case these case studies will share information about current cancer prevalence, current recommendations for cancer prevention, national guidelines about early detection for lung, colorectal, prostate, cervical and breast cancers, as well as discuss ways to reduce cancer health disparities and improve screening practices. Methods: Data sources for these case studies include research reports,
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literature review, clinically-based articles, research articles, websites, as well as clinical practice observations and experiences. Research Summary: These case studies contribute to the growing body of knowledge related to cancer health disparities, with a special emphasis on cancer screening behaviors among underrepresented, at-risk, and racial and ethnic minority backgrounds. Since cancer care disparities exist, these case reports highlight the role of oncology nurses in helping to lead in educating, promoting, and bringing awareness to cancer screening recommendations and current cancer prevention guidelines for individuals and their families. Nurses recognize that one of the biggest barriers to screening is when individual have unrelated health care priorities that overshadow priorities related to screening, such as food, shelter, and nutrition. Nurses are also positioned to provide cancer-specific screening guidelines and resources to help individuals implement these recommendations into their daily lives. Improving screening rates among minority populations requires collaborations with the health care systems and the community. Conclusion: Evidence indicates that there are significant disparities that exist in cancer screening practices among minority populations as well as disproportionately higher cancer mortality rates in these populations. Barriers to screening include lack of access to high-quality health care, lack of health insurance, transportation issues, fear and lack of trust in the health care team, embarrassment, stigma related to sexual orientation or gender identity, discrimination in quality care and treatment, as well as lack of awareness about screening recommendations. Recognition of barriers to screening among minority populations is critical to developing targeted interventions that promote adherence and decrease risks of cancer-related mortality.

#5294 Do co-morbid conditions correlate with cancer screening awareness and behaviour among older women? Findings from the fifth Indonesian Family Life Survey. Wahyu Wulaningsih,3 Mieke Van Hemelrijck,2 Sumadi Anwar,1 Jothanan Watkins,1 University College London, London, United Kingdom; King’s College London, London, United Kingdom; Universitas Gadjah Mada, Yogyakarta, Indonesia; PILAR Research and Education, Cambridge, United Kingdom.

Background: Cancer screening awareness may vary in low- and middle-income countries lacking established national screening programmes. Additionally, breast self-examination (BSE) to screen for breast cancer is common in these countries, despite evidence suggesting its lack of benefit. We evaluated co-morbidities as a potential determinant of awareness to breast and cervical cancer screening and BSE practice in older women in Indonesia. Methods: From the fifth Indonesian Family Life Survey (2014-2015), 6,320 women aged 40 and older who responded to questionnaires on pap smear, mammography, and BSE were included. To assess co-morbidities, an age-weighted score similar to the Charlson co-morbidity index was created based on self-reported history of chronic disease diagnoses. Weighted regression analyses were performed to assess their correlations with awareness to pap smear or mammography and BSE practice. Results: Only 22% women were aware of pap smears, and 32% among them had undergone at least one pap smear in their lifetime. Six percent of participants were aware of mammography, among which 4% had a mammogram in the previous year. Twelve percent of women reported they performed BSE at least once a year. With increasing co-morbidities, women were more likely to be aware of pap smear (e.g. age-adjusted OR=3.27, 95% CI: 1.18-9.01 for co-morbidity score of >2 compared to 0). No association with awareness to mammography was seen. Higher frequency of BSE per year was seen with higher co-morbidity (β=1.96, P=0.01 for co-morbidity score of >2 compared to 0). Associations remained, albeit weaker, after exclusion of women with history of cancer. Conclusion: Higher co-morbidities weakly correlated with being more aware to cervical cancer screening and practicing BSE more frequently among older women. Our findings may imply the need for targeted health promotion and screening for cancer in presence of limited infrastructure.

#5295 Screening performance of ultrasonography and mammography among Chinese women at high-risk of developing breast cancer. Yubei Huang,1 Fei-Hao Song,1 Xuan Tian. Tianjin Medical University Cancer Institute and Hospital, Tianjin, China.

In order to easily identify high-risk population of breast cancer and to determine the optimal screening modality among Chinese high-risk women, a total of 35234 asymptomatic Chinese women aged 45-65 years underwent breast ultrasound (BUS) and mammography (MAM) screening concurrently. Physicians performed these screening modalities separately and blindly. The number of risk factors of breast cancer, rather than complex risk prediction models, was used to identify potential high-risk women. Initially, a total of 13 factors (age at menarche, menopausal status, age at menopause, number of live birth, age at first pregnancy, breast feeding, duration of breast feeding, abortion, oral contracept-

tive, hormone replacement therapy, obesity, history of benign breast disease, and family history of breast cancer) were selected. After excluding correlated factors, factors with risk frequency ≥ 20% and factors with missing values ≥ 5%, 6 factors (age at menarche, age at menopause, age at first pregnancy, oral contraceptive, obesity, family history of breast cancer) were selected as risk-evaluating factors. Weights of co-morbidities were assigned on a 0-10 scale, and the co-morbidity score of each patient was calculated. The number of exposure to risk factors is an easy-to-use method to identify potential high-risk women of breast cancer, and the performance of BUS is very similar to that of mammography.

#5296 Evaluation of p16/Ki-67 dual stain, cytology, and HPV16/18 genotyping for triage of HPV-positive women in a large screening population. Nicolas A. Wentzensen,1 Barbara Fellermann,2 Renee Bremer,3 Philip Castle,4 Diane Tokugawa,4 Nancy Poitrass,5 Elizabeth Hosfield,6 Thomas Lorey,7 Mark Schiffmann,7 Walter Kinney,8 NCT-DCEG, Bethesda, MD; 4 Kaiser Permanente Northern California, CA; 5 Albert Einstein College of Medicine, Bronx, NY; 7 Kaiser Permanente Northern California, Richmond, CA.

Objectives: Primary HPV testing has been approved in the United States. Screening intervals can be safely extended for HPV-negative women, but the challenge lies in discriminating transient HPV infections from precancers among HPV-positives. In a large study at Kaiser Permanente Northern California (KPCN), candidate strategies for triage of HPV-positive women are being evaluated, including cytology, p16/Ki-67 dual stain (DS, CNITec PLUS) and HPV16/18 genotyping (cobas). Methods: Over 13,000 HPV-positive women participating in cervical cancer screening at KPCN were enrolled. The dual stain and HPV genotyping assays were implemented and conducted at KPCN. Base-line results for 14,724 women are available and detection of CIN3 is currently evaluated only in cytology-positive women. Results: Among 7,124 HPV-positive women, 4,107 (57.7%) were cytology-negative (ASC-US+), 3,056 (42.9%) were DS-positive, and 1,406 (19.7%) were positive for HPV 16 or HPV 18. Among all 3,017 HPV-positive, cytology-negative women, 911 (30.2%) were DS-positive, and 500 (16.8%) were positive for HPV 16 or HPV 18. Of 315 CIN3 detected so far, 50% were in DS positive and 55% were in HPV 16 or 18 positive. Major triage strategies including cytology alone, DS alone, as well as combinations of cytology and genotyping, and DS and genotyping will be presented at the meeting. Discussion: In one of the largest clinical implementation studies of triage strategies, we showed that primary HPV screening followed by DS can reduce colposcopy referral compared to HPV-cytology co-testing while achieving high sensitivity. Additional follow-up is underway to evaluate the programmatic performance of several major candidate strategies.

#5297 Evaluation of lung cancer risk prediction models for selecting smokers for CT lung cancer screening. Hormuz Khati,1 Stephanie Kovalchik,2 Lucia Pettito,1 Li C, Cheung,3 Eric Jacobs,4 Ahmedin Jemal,4 Christine Berg,1 Anil Chaturvedi,1 National Cancer Institute, Rockville, MD; 2 Victoria University, Melbourne, Australia; 3 University of California at Berkeley, Berkeley, CA; 4 American Cancer Society, Atlanta, GA.

Background: The US Preventive Services Task Force (USPSTF) recommends computed-tomography (CT) lung cancer screening for ever-smokers ages 55-80 years who smoked at least 30 pack-years with no more than 15 years since quitting. 8.9 million U.S. ever-smokers are eligible for screening under USPSTF criteria. Instead, selecting ever-smokers for screening using individualized lung cancer risk calculations may be more effective and efficient than current recommendations. We examined the performance of 8 risk models for estimating risk in 2 U.S. cohorts and compared the U.S. populations selected for screening by the models. Methods: We examined 8 models: Lung Cancer Death Risk Assess-
ment Tool and Lung Cancer Risk Assessment Tool (Katki et al., JAMA 2016), PLCOM2012 (Tammemagi et al, N Engl J Med 2013), Bach (Bach et al., JNCI 2003), LLP (Cassidy et al, Br J Cancer, 2008), LLLI (Marcus et al, Cancer Prev Res 2015), Hoggart (Hoggart et al, Cancer Prev Res 2012), and Spitz (Spitz et al, JNCI 2007). We examined the predictive ability of each model among (1) 337,388 risk-smokers in the NIH-AARP Diet and Health Study (NIH-AARP), and (2) 72,338 ever-smokers in the American Cancer Society Cancer Prevention Study II (CPSII) Nutrition Survey Cohort. For each model we estimated the calibration (number of model-predicted cases divided by number of observed cases (Estimated/Observed)) and the discrimination (Area-Under-Curve (AUC)). Each of the 8 risk models and the USPSTF criteria were used to select a population of 8.9 million individuals at highest risk of lung cancer from among U.S. ever-smokers 50-80 (as characterized by data from the 2010-12 National Health Interview Surveys). Results: In both NIH-AARP and CPSII, 4 models were well-calibrated (Expected/Oberved from 0.92 to 1.12) and had higher AUCs (0.76 to 0.79). The other 4 models had worse calibration (Expected/Oberved from 1.72 to 0.69) and worse AUCs (0.62 to 0.75). When the models were used to choose the 8.9 million U.S. ever-smokers at highest lung-cancer risk, only ~1.8 million individuals were chosen by all 8 models. For a screening eligibility risk-threshold of 2.0% lung-cancer risk over 5 years, the 8 models chose populations including 7.6 million to 26 million U.S. ever-smokers. Conclusions: Some risk models were more externally-valid and had superior predictiveness than others. Differences in calibration led to risk models choosing very different screening population sizes under a fixed risk-threshold. The models did not achieve consensus on selected populations for screening.


Background: The contribution of pulmonary scars to lung cancer development and the degree to which lung cancers cause a scarring response are unclear. Also unknown is how lung scarring impacts lung cancer screening. Methods: We evaluated associations between scarring and lung cancer in the National Lung Screening Trial (NLST), a lung cancer screening trial among current or former, heavy smokers, 55-74 years-old. Baseline scarring (presence vs. absence) on low dose computed tomography (LDCT) scan was assessed at baseline (T0). Associations of T0 scarring with screen-detected lung cancers and with interval-detected lung cancers missed on screening within 3 years of T0 screen were analyzed using multinomial logistic regression. Cox proportional hazards models were used to analyze the relationship between T0 scarring and incident lung cancers diagnosed >3 years after T0. Regression models included age, sex, race, smoking history, chronic obstructive pulmonary disease, history of pneumonia, and family history of lung cancer. A thoracic pathologist (first author) evaluated lung cancer pathology slides from the Lung Screening Study (LSS) subset of NLST for scar grade (none, sparse, dense) and maturity (none, immature, intermediate, mature). Associations between T0 scarring on LDCT and histological scarring were examined by logistic regression. Results: NLST’s LDCT arm enrolled 26,722 participants (65% from the LSS). T0 scars were present in 30% of individuals at highest risk of lung cancer, 29% for screen-detected, 12% (19%) for interval-detected, and 26% for incident lung cancer cases. T0 scarring did not increase or decrease screen-detection of cancers [odds ratio (OR) 95% CI: 1.03 (0.84-1.26)]. However, scarring might increase the chance of an interval-detected cancer (OR 95% CI: 1.54 (0.76-3.12)]. After screening stopped, T0 scarring was associated with increased incident lung cancer risk [hazard ratio (HR) 95% CI: 1.27 (1.00-1.62); P=0.048]. Pathology slides were available for 25% (38%) lung cancers in LSS. Lung scarring was found in 172 (67%) of these cancers with 58 (22%) being characterized as mature scars. On microscopic review, scars were found in 80 (66%) ADC, 46 (82%) squamous cell carcinomas, and 20 (51%) bronchioloalveolar carcinomas. Microscopic scarring tended to be more frequent among cases with T0 scarring than those without T0 scarring (76% vs. 64%, P=0.02) [OR (95% CI): 1.89 (0.98-3.86)]. Conclusion: The association between T0 scarring and incident lung cancer over a period of more than 3 years is consistent with the development of each (BCSC censored; BBD-BC event). Results: In the Mayo Clinic’s BBD cohort, the Breast Cancer Surveillance Consortium (BCSC) model predicted invasive breast cancer risk in women with Benign Breast Disease (BBD), and recently incorporated BBD histology into the model. The BCSC has been validated in the Mayo Mammmography Health Study, but has yet to be examined in Mayo Clinic’s BBD cohort. The Benign Breast Disease to Breast Cancer (BBD-BC) model was developed in the AARP Diet and Health Study (AARP-DHS) and in Mayo Clinic’s BBD cohort. We here compare the performance of the BCSC and BBD-BC in Mayo Clinic’s BBD cohort. Methods: Eligible women underwent a breast biopsy with benign findings at the Mayo Clinic between years 1997-2001 and had a 4-view screening mammogram within six months of biopsy. Clinical BI-RADS density assessments using the 4th edition American College of Radiology were available on all of the lesions, which were independently blinded and categorized as having extremely dense, heterogeneously dense, and extremely dense. Risk at 5 and 10 years of invasive cancer only (BCSC model) or both invasive and in situ cancer (BBD-BC model) were estimated. In situ cancers were censored at time of diagnosis for both models. Concordance statistics, i.e. model discrimination (higher is better), for each model were calculated using a Cox proportional hazards model with predicted risk as the sole predictor, and were compared using permutation tests. The ratio of total predicted (sum of predicted risk) to observed number of invasive breast cancers was used to assess model calibration. Calibration was not formally compared across models due to differences in how DCIS was treated in the development of each (BCSC censored; BBD-BC event). Results: 999 women met inclusion criteria. BI-RADS density was categorized as fatty in 46 (4.6%), a few scattered foci in 299 (29.7%), heterogeneously dense in 423 (42.9%), and extremely dense in 158 (15.8%), 62 invasive cancers occurred over a median 13.3yrs of follow-up, with 16 (25.8%) and 48 (77.4%) of the cancers occurring with-in 5 and 10 years. The concordance of the BCSC at 5 years was 0.55 (95% CI 0.409 — 0.691), compared to 0.719 (95% CI 0.578 — 0.860) for the BBD-BC (p-value=0.005). At 10 years the BCSC concordance was not significantly different from the BBD-BC, at 0.624 (95% CI 0.542 — 0.706) and 0.662 (95% CI 0.580 — 0.744), respectively (p-value=0.306). The BCSC over predicted the number invasive cancers in the BBD cohort at 5 years (predicted-to-observed=1.54; 95% CI 1.01 — 2.70), but was well calibrated at 10 years (1.06; 95% CI 0.83, 1.47). Conclusions: The BCSC model performed reasonably well in the BBD cohort 10 years post-biopsy, but overestimated risk at 5 years. Studies is needed to improve models for prediction of breast cancer risk among women with BBD.

#5300 Risk models for cancer screening cohorts assembled from electronic health records: Application to calculating risks that underlie current cervical cancer screening guidelines. Li C. Cheung, Ping Q., Noorie H. Hyun,1 Mark Schiffman,2 Barbara Fetterman,3 Philip E. Castle,4 Thomas Lorey,3 Hor-
CIN3+ are close to the risk estimates from the non-parametric method. Discussion: The Kaplan-Meier method provided poor risk estimates while logistic-Weibull model-based risks were close to the risk estimates from the non-parametric method. Our findings support use of the logistic-Weibull models over Kaplan-Meier methods for developing the risk estimates that underlie current U.S. cervical cancer screening guidelines.

#5301 Cancer causation: rethinking the role of analogy, Douglas L. Weed, DLW Consulting Services, Salt Lake City, UT.

Introduction. Fifty years has passed since the publication of Austin Bradford Hill’s classic paper on causal inference. Much has changed in the biomedical and public health sciences regarding cancer causation during that half century, but Hill’s considerations (often called “criteria”) remain durable. That said, there is a curious phenomenon involving Hill’s seminal work that has escaped the attention of the scientific community. His consideration of analogy—the last on his list just after experimentation—has all but disappeared. The purpose of this paper is to rethink and resurrect analogy as an important consideration in causal inference in environmental epidemiology. Methods. Given that this is a conceptual—non-quantitative—project, the methods used are the informal logic of scientific thinking, methods of systematic reviews, and judgment. Results. In contemporary environmental epidemiology, analogy is either completely ignored (e.g., in many textbooks), equated with biologic plausibility or coherence, or aligned with the scientist’s imagination. None of these examples, however, capture Hill’s description of analogy which emphasizes the contrast of two bodies of evidence. Coupled with developments in the methods of systematic assessments of evidence—including but not limited to meta-analysis—analyzed by local to Japan, and also comparing in-house data of CA19-9 and carcinoembryonic antigen (CEA) markers. Experiment: Conducted at Nippon Medical School Chiba Hokusoh Hospital, urine samples were collected and frozen at -80°C from 139 patients at various stages of CRC and 78 healthy control samples. The samples were thawed in batches and placed in ice hours prior to testing; 2 ml of each urine sample was aliquoted into 10 ml vials for processing with the commercial FAIMS device (Lonestar, Owlstone, UK). Each vial was heated in the device to 40°C to create headspace with sufficient VOCs then a carrier gas (clean dry air) delivered the headspace (0.5 L/min) diluted with a make-up flow (2 L/min). The FAIMS device was set to scan at 0 to 100% electric dispersion field in 51 steps and compensation voltage between -6 V and +6 V in 512 steps, producing data matrices for each sample’s analysis. Principal Component Analysis (PCA) followed by Partial Least Squares Discriminant Analysis (PLS-DA) of each sample was conducted using SIMCA 13 (Umetrics, Sweden). See Table Conclusions: FAIMS technology achieved a high rate of separation between the CRC and healthy control urine samples with 64.7% sensitivity and 82.1% specificity overall. As the CRC stage advances the sensitivity increased from 27.3% to 100%. Results: show excellent potential to use FAIMS technology as an early screening tool for CRC, particularly impressive compared to in-house sensitivity data of CA19-9 and CEA markers. Further research into FAIMS screening of other cancer types through VOC biomarker analysis of urine, breath, and feces is recommended.

#5302 Projections of cancer incidence and burden among the HIV-positive population in the United States through 2030, Jessica Y. Islam, 1 Philip S. Rosenberg, 2 HI Irene Hall, 2 Evnin UC, 3 Eric A. Engels, 2 Meredith S. and HIV population count were multiplied. Results: The proportion of the total group and calendar year (2006-30) was estimated using a dynamic compartmental model. Injection drugs) during 2013-30. The number of PLWH in the U.S. by age, risk and, for some cancers, risk group (e.g., men who have sex with men, people who inject drugs) during 2013-30. The number of PLWH in the U.S. by age, risk group and calendar year (2006-30) was estimated using a dynamic compartmental model. To estimate cancer burden, observed and projected incidence rates and HIV population counts were multiplied. Results: The proportion of the total U.S. HIV population that is aged ≥65 years is projected to increase from 4.1% (of 1.06 million) in 2006 to 21.4% (of 1.09 million) in 2030. Based on significant declines during 2000-12, age-specific rates are projected to decrease across age groups for NHL, cervical cancer, lung cancer and all other cancers, and for some age groups for KS, Hodgkin lymphoma and colon cancer. Other age-specific rates did not change significantly, with the exception of prostate cancer rates, which are projected to continue to increase. We estimated that the total cancer burden in PLWH will decrease from 7908 cases in 2010 (2719 ADC and 5190 NADC) to 6495 cases in 2030 (701 ADC and 5794 NADC), indicating a strong decrease in ADCs and a slight increase in NADCs. In 2030, the most common cancers among PLWH will include: prostate (n=1624), lung (n=786), liver (n=498) and anal cancers (n=447) and NHL (n=429). Conclusions: Though cancer rates are generally decreasing, cancer will remain an important co-morbidity as the U.S. HIV population ages. If recent trends in cancer incidence continue into the future, the burden of NADCs, particularly prostate, lung, liver and anal cancers, will far exceed the burden of ADCs in 2030. Targeted cancer prevention, early detection and control efforts are needed for PLWH in the U.S., including smoking cessation, treatment of hepatitis C and B viruses, cancer screening and continued widespread treatment with HAART.

#5303 FAIMS technology in urinary volatile organic compound analysis to detect colorectal cancer, Christopher Psutka, 1 Marina Yamada, 2 Akihisa Matsuda, 2 Kazuya Yamahata, 2 Satoshi Matsumoto, 2 Toshihiko Kitayama, 3 Nobuo Nakano, 4 Yurii Koyano, 2 Tohru Mikoshiba, 2 Masao Miyashita 2.

Introduction: This is an investigation of the capability of FAIMS (Field Asymmetric Ion Mobility Spectrometry) technology as a tool for non-invasive detection of Colorectal Cancer (CRC) through urinary volatile organic compound analysis. It expands on ‘Detection of Colorectal Cancer (CRC) by Urinary Volatile Organic Compound Analysis’ (Ramesh P. Arasaradnam et al, 2014) with an increased sample population and location local to Japan, and also comparing in-house data of CA19-9 and carcinoembryonic antigen (CEA) markers. Experiment: Conducted at Nippon Medical School Chiba Hokusoh Hospital, urine samples were collected and frozen at -80°C from 139 patients at various stages of CRC and 78 healthy control samples. The samples were thawed in batches and placed in ice hours prior to testing; 2 ml of each urine sample was aliquoted into 10 ml vials for processing with the commercial FAIMS device (Lonestar, Owlstone, UK). Each vial was heated in the device to 40°C to create headspace with sufficient VOCs then a carrier gas (clean dry air) delivered the headspace (0.5 L/min) diluted with a make-up flow (2 L/min). The FAIMS device was set to scan at 0 to 100% electric dispersion field in 51 steps and compensation voltage between -6 V and +6 V in 512 steps, producing data matrices for each sample’s analysis. Principal Component Analysis (PCA) followed by Partial Least Squares Discriminant Analysis (PLS-DA) of each sample was conducted using SIMCA 13 (Umetrics, Sweden). See Table Conclusions: FAIMS technology achieved a high rate of separation between the CRC and healthy control urine samples with 64.7% sensitivity and 82.1% specificity overall. As the CRC stage advances the sensitivity increased from 27.3% to 100%. Results: show excellent potential to use FAIMS technology as an early screening tool for CRC, particularly impressive compared to in-house sensitivity data of CA19-9 and CEA markers. Further research into FAIMS screening of other cancer types through VOC biomarker analysis of urine, breath, and feces is recommended.

#5304 A serological biopsy using five stomach-specific circulating biomarkers for gastric cancer risk assessment: a multi-phase study. Huakang Yi, Liping San, 1 Xiao Dong, 2 Yuhua Gong, 2 Qian Xu, 2 Jingjing Jiao, 3 Roberd M. Bostick, 4 Xifeng Wu, 1 Yuan Yuan 2.

Objective: We aimed to assess a serological biopsy using five stomach-specific circulating biomarkers—pepsinogen I (PGI), PGI, PGI/II ratio, anti-Helicobacter pylori (H. pylori) antibody, and gastrin-17 (G-17)—for identifying high-risk individuals and predicting risk of developing gastric cancer (GC). Methods: Among 12112 participants with prospective follow-up from an ongoing population-based screening program using both serology and gastroscopy in China, we conducted a multi-phase study involving a cross-sectional analysis, a fol-
low-up analysis, and an integrative risk prediction modeling analysis. Results: In the cross-sectional analysis, the five biomarkers (especially PGGI, the PGI/II ratio, and H. pylori sero-positivity) were associated with the presence of precancerous gastric lesions or GC at enrollment. In the follow-up analysis, low PGI levels and PGI/II ratios were associated with higher risk of developing GC, and both low (<0.5 perf/L) and high (4.7–10 perf/L) G17 levels were associated with higher risk of developing GC, suggesting a J-shaped association. In the risk prediction modeling analysis, the five biomarkers combined yielded a C statistic of 0.801 (95% CI = 0.787–0.815) and improved prediction beyond traditional risk factors (C statistic from 0.580 to 0.811. P < 0.001) for identifying precancerous lesions at enrollment, and higher serological biopsy score based on the five biomarkers at enrollment were associated with higher risk of developing GC during follow-up (P for trend < 0.001). Conclusions: A serological biopsy composed of the five stomach-specific circulating biomarkers could be used to identify high-risk individuals for further diagnostic gastroscopy, and to stratify individuals’ risk of developing GC and thus to guide targeted screening/prevention.

#5305 Risk of thyroid nodules in residents of Belarus exposed to Chernobyl fallout as children and adolescents. Elizabeth K. Cahoon,1 Eldar Nadirov,2 Olga Polanskaaya,2 Vasilia Yausechenya,2 Vasilia Yausechenya,2 Ilya Velalkin,2 Tamara Yeudachkova,2 Tamara Maskvicheva,2 Victor Menenko,3 Yury Ryzhkov,2 Tamara Maskvicheva,2 Victor Menenko,3 Alexey Danilov,2 Shumilina I. N.,2 Alexander Rozhko,2 Alina Brenner,1 National Cancer Institute, Bethesda, MD;1 The Republication Research Centre for Radiation Medicine and Human Ecology, Belarus;2Belarusian Medical Academy of Post-Graduate Education, Belarus.

Context: The relationship between childhood radiation exposure and thyroid nodules has not been comprehensively evaluated in young adults or for nodules with varying histological or ultrasound features. Objective: To examine the association between internal I-131 thyroid radiation dose and thyroid nodules in young adults exposed during childhood. Design, setting, and participants: In this cross-sectional study, we screened 11,970 residents of Belarus aged ≥18 years at the time of the Chernobyl nuclear accident (April 1986) for thyroid disease (median age 21 years). Screening consisted of thyroid palpation, ultrasonography, blood and urine analysis, and medical follow-up when appropriate. Eligible participants (N = 11,421) had intact thyroid glands and doses estimated using direct individual thyroid activity measurements. Main outcome measures: Excess odds ratios per gray (EOR/Gy) for any thyroid nodule and for nodules grouped by histological and ultrasound characteristics selected a priori. Results: Risk of any thyroid nodule was associated with increasing I-131 in the entire dose range (0.001–39 Gy) and radiation-related risk increased with younger age at exposure. For participants exposed to < 5 Gy, we found EORs/Gy (95% CI) of 0.70 (0.33, 1.18) for any nodule, 0.32 (< 0.03, 0.70) for non-neoplastic nodules, 3.82 (0.87, 15.52) for neoplastic nodules, 0.27 (< 0.13, 0.70) for nodules < 10 mm, 2.12 (0.96, 4.60) for nodules ≥10 mm, 0.67 (0.30, 1.19) for single nodules, and 0.59 (< 0.29, 2.05) for multiple nodules (centered at age 5 years at exposure). Conclusions: Childhood exposure to internal I-131 is associated with thyroid nodules of various histological/ultrasound characteristics, but magnitude of the risk varies substantially.

#5306 p16INK4a status helps predict clinical outcome of dysplasia in the oral cavity irrespective of HPV status. Tarinée Lubrapue,1 Elena Prigge,2 Magen von Knebel Doeberitz,2 Miriam Reuschenbach,2 Leivi Zhang,3 Miriam P. Rosin1.1 BC Cancer Research Centre, Vancouver, British Columbia, Canada;2University Hospital Heidelberg, Germany;3University of British Columbia, British Columbia, Canada.

Objectives: p16INK4a is a key gatekeeper for multiple biological processes that are associated with protection against cancer, including cell cycle control and senescence. Elevated expression of this gene is strongly correlated with biological activity of HPV infection and cancer development in uterine cervix and tonsil. Although HPV DNA has been found in a small portion of oral cavity cancers and dysplasia, the relevance of its presence to cancer development at this site is unclear, as is the significance of an expression change in p16INK4a as a predictor of HPV status, tissue behavior and outcome. Methods: Formalin-fixed, paraffin-embedded biopsies from 164 patients with mild, moderate and severe oral dysplasia in the “Oral Cancer Prediction Longitudinal study” (OCPL), British Columbia, Canada, were analyzed for HPV DNA using a semi-quantitative viral load LumineX technology. Expression of p16INK4a was assessed and categorized into 3 staining patterns (diffuse, focal, and negative). Results: HPV DNA was found in 13 (8%) lesions, 7 had HPV-16 infection and 1 HPV-18. Of the 164 samples, diffuse p16INK4a expression was present in 18%, focal in 56% and no p16 staining in 23% of samples. There was no significant correlation between HPV DNA-positivity and p16INK4a staining. Different p16 staining patterns showed significantly different progression rates (P < 0.001): progression occurred in 1/92 (1.1%) focally stained, 1/29 (3.5%) diffusely stained and 10/43 (23.3%) with no staining. Conclusion: HPV DNA-positivity occurs in a small proportion of oral cancer associated with its expression in dysplasia and cancer. Future assessment of these samples for HR-HPV oncogene transcripts will provide information on its biological activity. The low likelihood of progression of p16INK4a focally stained lesions suggests a value of this marker in identification of low-risk cases. Acknowledgements: Supported by Else Kröner-Fresenius-Stiftung Foundation, Germany.

#5307 Analysis of volatile organic compounds for the diagnosis of lung cancer. Takuya Inoue,1 Hitomori Takagi,2 Yuki Owada,1 Yuzuru Watanebe,3 Mitsuhiro Fukuhara,1 Takumi Yamaura,1 Satoshi Muto,1 Naoyuki Okabe,2 Yuki Matsumura,3 Takeo Hasegawa,2 Takeda Manami,2 Atsushi Sato,3 Hiroyuki Suzuki1.1Fukushima Medical University, Fukushima, Japan;2GL Sciences Inc., Saitama, Japan.

Background: Lung cancer is the leading cause of cancer-related deaths in the world. The prognosis of lung cancer depends on disease detection at an early stage. Recently, effectiveness of lung cancer screening by using low dose computed tomography scanning have been showing. However, it still has problems such as cost of screening and radiation exposure. Thus, less invasive and cost beneficial lung cancer screening procedure would be needed. In this context, several study to understand the efficacy of molecules from expiration of patients as a lung cancer screening was showing, but it was still controversial. In this study, we investigated to determine whether volatile organic compounds (VOC) from patients could be used for the detection for lung cancer by using novel small and unique absorbent material named MonoTrap . Method: The subjects’ gas was collected from skin and exhaled breath in a MonoTrap (GL sciences), and the VOCs were analyzed by gas chromatography/ mass spectrometry (GC/ MS). First of all, we preliminarily analyzed for both sample from skin and exhaled breath for 12 patients and 11 healthy volunteers to clarify which samples were more sensitive to diagnose lung cancer. Subsequently, each VOC was determined in detail for 7 healthy individuals and 4 lung cancer patients and these values were statistically analyzed. Results: By preliminary study, characteristics of VOC derived from skin were significantly correlated with cancer condition as compared with healthy volunteer by using comprehensive multivariate analysis. Therefore, we performed further quantitative analysis by targeting unique individual molecules derived from skin. In VOCS from skin, Mann-Whitney U test showed that 4-hydroxy-4-methyl-2-pentanone, hexadecane, and acetamide were significantly higher in lung cancer patients (p = 0.036, p = 0.006, p = 0.036, respectively). Conclusion: In this study, we found that 3 kinds of VOCs could be a potential diagnostic biomarkers of lung cancer. Further prospective study is now planning to validate these data using large number samples.
Dietary magnesium is inversely associated with colorectal cancer risk in the Atherosclerosis Risk in Communities study. Guillaume C. Onyeghala, Elizabeth Porter, Pamela L. Lutsey, Aaron R. Folsom, Corrine E.Joshua, Elizabeth A. Platz, Anna E. Prizment1, University of Minnesota, Minneapolis, MN; 2John Hopkins Bloomberg School of Public Health, Baltimore, MD.

Background: Magnesium is a key nutritional mineral required for the regulation of numerous biochemical reactions throughout the body. Main food sources of magnesium include whole grains, nuts, and green vegetables, but more than 50% of Americans do not currently meet their daily recommended magnesium intakes. Prior research has shown that those deficient in magnesium have increased susceptibility to oxidative stress and chronic inflammation, leading to endothelial dysfunction and potentially stimulating cancer cell proliferation and invasiveness. Previous epidemiological studies suggested a link between a diet high in magnesium and reduced CRC, especially among women. The American Cancer Society underscores the importance of clarifying the role of dietary magnesium in CRC development overall and by gender. Thus, we prospectively examined the associations between dietary magnesium intake and CRC risk in the ARIC study, overall, and separately in men and women. Methods: The ARIC study followed 15,792 men and women (45-64 years old at baseline) for cancer occurrence from 1987-2006. Participants received medical examinations and completed questionnaires at 4 visits; food frequency data were collected at Visit I (1987-89) and magnesium intake estimated. Cox proportional hazards regression was used to calculate hazard ratios (HR) and 95% CI for CRC associated with dietary magnesium intake in quartiles using two models: Model 1 adjusted for baseline age, race, center, sex, energy intake, BMI, and physical activity. Model 2 included Model 1 covariates as well as intake of alcohol, dietary calcium, dietary fiber, aspirin, CRP levels, and cigarette smoking. Results: The analytic cohort (n=14,160, 54.3% women; 27.3% black and 72.7% white, 45-64 years at baseline) was followed for a median of 15.4 years; 315 CRC cases were identified. Since only the highest quartile of dietary magnesium intake met US recommendations (>300 mg/day), we examined the highest quartile versus the 3 lowest quartiles combined (reference category). In Model 1, the highest quartile of dietary magnesium was associated with lower CRC risk: HR (95% CI) = 0.74 (0.56-0.98) (p=0.03 for trend). The inverse association between magnesium and CRC persisted in Model 2 (HR=0.70; 95% CI 0.48, 1.02). The association followed a similar trend after stratification by colon and rectal cancer cases (HR [95% CI] = 0.72 (0.48-1.08) and HR [95% CI] = 0.49 (0.23-1.06), respectively) and by men and women (HR [95% CI] = 0.75 (0.42-1.21) and HR [95% CI] = 0.63 (0.36-1.12), respectively). There was no interaction of dietary magnesium intake with dietary calcium intake, alcohol consumption use or dietary fiber. Conclusion: Our findings corroborate an inverse association between higher dietary magnesium intake and CRC risk, with similar associations observed in men and women. Support: NHLBI, NCI, NPCR.

A prospective study of dietary polyunsaturated fatty acids intake and lung cancer risk. Hung N. Luu,1 Harvey J.Murphy,2 Honglan Li,1 Quyin Cai,1 Yu-Tang Gao,3 Jing Gao,3 Hui Cai,3 Gong Yang,3 Qing Lan,4 Yong-Bing Xiang,3 Wei Zheng,5 Xia-0u Shu;1 1University of South Florida, Tampa, FL; 2Vanderbilt University, Nashville, TN; 3Shanghai Cancer Institute, Shanghai, China; 4National Cancer Institute, Bethesda, MD.

Background: Animal studies have shown that polyunsaturated fatty acids (PUFAs) have antiangiogenic and anti-inflammatory properties. Results from epidemiologic studies, however, have been inconclusive. We prospectively evaluated the association of dietary PUFA intakes and lung cancer risk in two population-based cohort studies, the Shanghai Men’s Health Study (SMHS) and Shanghai Women’s Health Study (SWHS). Methods: A total of 130,823 study participants (i.e., 60,427 men and 70,396 women) were included in the current analysis. Dietary fatty acid intakes were derived from data collected at the baseline by validated food frequency questionnaires. Cox proportional hazards model was applied to assess the association between PUFA intakes and lung cancer risk with adjustment for age, smoking status, smoking packs-year (men only), drinking status, BMI, physical activity status, total energy, red meat intake, vegetable intake, vitamin supplemental use, menopausal status and hormone replacement therapy (women only). Results: We found an inverse association between total PUFA intakes and lung cancer risk (hazard ratios [HRs]) and respective 95% confidence intervals (CIs) for quintiles 2, 3, 4, and 5 versus quintile 1 were 0.73 (0.56-0.95), 0.78 (0.59-1.03), 0.64 (0.47-0.87) and 0.53 (0.37-0.82) in SMHS and 0.69 (0.53-0.90), 0.76 (0.58-0.99), 0.60 (0.44-0.81), and 0.53 (0.36-0.78) in SWHS. A positive association was observed for ALA intake. A correlation matrix showed that linoleic acid, total n-6 PUFAs and the ratio n-6 PUFA/n-3 PUFAs in adenoscinoma patients. This inverse association was more likely in male ever-smokers for total PUFAs, linoleic acid and total n-6 PUFAs. On the other hand, EPA intake was positively associated with lung cancer risk in female never-smokers (HRs and 95% CIs: 1.10 (0.85-1.42), 1.36 (1.05-1.77), 1.28 (0.97-1.88) and 1.30 (0.97-1.74), for quintiles 2-5 versus quintile 1). A similar positive association between DHA intake and lung cancer risk in female never-smokers was also observed. Conclusions: Total polyunsaturated fatty acid, linoleic and total n-6 PUFA intakes were associated with decreased risk of lung cancer. EPA and DHA intakes were associated with an increased risk of lung cancer among female never-smokers. Financial Support: This work was supported by grants from the US National Institutes of Health/National Cancer Institute (R01CA70867 and UM1 CA182910-to Wei Zheng; R01CA082729, UM1 CA173640 and R25 CA160056 - to Xiao-Ou Shu).

Weight loss over 10 years of adulthood and subsequent risk of breast cancer: a pooled analysis of 11 cohort studies. Lauren R. Teras,1 Alpa V. Patel,1 Molin Wang,2 Bette J. Caan,2 Yu Chen,3 Avonne E. Connor,3 A. Heather Ellsasser,4 Susan M. Gaspurt,4 Mia M. Gaudet,4 Jeanine M. Genkinger,4 Graham G. Giles,6 F-Min Lee,7 RogerMilne,8 Norie Sawada,8 Howard D. Sesso,9 Meir Stampfer,9 RullaTamimi,9 Cynthia A. Thomson,9 Shoichiro Tsugane,9 Kala Vyananthan,9 Anne Zeleniuch-Jacquotte,9 Walter C. Willett,9 Stephanie A. Smith-Warner11, American Cancer Society, Atlanta, GA; 2Harvard Medical School, Boston, MA; 3Kaiser Permanente Medical Care Program of Northern California, Oakland, CA; 4NYU School of Medicine, New York, NY; 5John Hopkins Bloomberg School of Public Health, Baltimore, MD; 6Brigham and Women’s Hospital and Harvard Medical School, Boston, MA; 7Columbia University, New York, NY; 8University of Melbourne, Melbourne, Australia; 9National Cancer Centre, Tokyo, Japan; 10University of Arizona Mel & Enid Zuckerman College of Public Health, Tucson, AZ; 11Harvard T.H. Chan School of Public Health, Boston, MA.

Body fatness is an established risk factor for postmenopausal breast cancer, but it is unknown if this risk associated with excess body weight is reversible. We conducted a pooled analysis of 11 prospective studies in the Pooling Project of Prospective Studies of Diet and Cancer. Each study had adult body weight data at three time points, as well as follow-up for subsequent risk of breast cancer after the third weight measure. Weight change was assessed using reported or measured weight at baseline and two follow-up time points, each generally four to six years apart (over a total of ~10 years). Stable weight for each interval was defined as weight within 2 kg of the previous weight. The referent group was women with stable weight (within 2kg) at all three time points across the 10-year period. Among 340,055 women, 10,427 breast cancers were diagnosed during follow-up. Multivariable hazard ratios (HR) and 95% confidence intervals (CIs) controlling for baseline body mass index (BMI), baseline physical activity, and postmenopausal hormone (PMH) use at the start of breast cancer follow-up, were estimated using proportional hazards regression on an aggregated dataset from all studies. Women who lost weight and kept the weight off, were at a lower risk of breast cancer than women with stable weight over the 10 years: >2.4.5 kg lost between baseline and the first follow-up body weight measure (n=482 cases): HR = 0.92, 95% CI: 0.83-1.03; >4.5-9.5 kg lost (n=283 cases): HR=0.86, 95% CI: 0.75-0.98; (p=0.5 for trend). Women who initially lost weight (>2kg), but then re-gained it had a similar risk of breast cancer to those with stable weight over the same time period. When results were stratified by baseline age and BMI, there was no association between sustained weight loss and breast cancer among women younger than 50 years, or those with a normal BMI (18.5-25 kg/m2) before weight loss. Among obese women (>=28 kg/m2) who were stable weight or obese before the weight loss period, we observed a 21% lower risk of breast cancer for sustained weight loss of 4.5kg compared to women with stable weight over the same time period (n=245 cases, HR=0.79, 95% CI: 0.68-0.93). This observed association was driven by women who were not taking...
PMH (n = 156, HR = 0.71; 95% CI: 0.58-0.86). In this large, pooled prospective analysis of weight loss and breast cancer risk we found that losing 4.5 kg—and keeping it off—may lower breast cancer risk, particularly for women older than 50 who are overweight or obese. The results may provide motivation for women with elevated BMI to lose weight and potentially reduce their risk of breast cancer.

**5312 No association between vitamin K intake and prostate cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO).** Maggie Hoyt, Michael Reger, Jianjun Zhang. *Indiana University, Indianapolis, IN.*

Prostate cancer is one of the most common cancers worldwide. The etiology of prostate cancer largely remains unclear, with almost all established risk factors (e.g., age, race, family history) being non-modifiable. Ecological, migratory, and temporal trends studies suggest that diet plays a role in the occurrence of prostate cancer, but few nutrients that alter their risk have been identified from case-control and cohort studies. Vitamin K has two forms that naturally occur in foods, i.e., phylloquinone (vitamin K1) and menaquinone (vitamin K2). While phylloquinone is abundant in green leafy vegetables and some vegetable oils, menaquinone is primarily derived from fermented food products (e.g., cheese). Although experimental studies have shown that vitamin K inhibits the growth of various cancer cell lines (including prostate cancer cells), only one epidemiologic study has investigated the association between vitamin K intake and prostate cancer risk. In that German study, a significant inverse association with advanced prostate cancer was observed for menaquinone intake. The present study thus sought to investigate the associations between intake of vitamin K (from both dietary and supplemental sources) and the risk of total and advanced prostate cancer among 28,356 PLCO participants. A total of 2978 cases of prostate cancer (including 490 advanced cases) have been documented during a median follow up of 11.8 years. Advanced prostate cancer were defined as disease in stage II with a Gleason score of 8-10, stage III, or stage IV. Usual dietary intake among study subjects was assessed with Dietary Questionnaire (DQX) at baseline and Dietary History questionnaire (DHQ) at year 3, both of which were developed and validated by the National Cancer Institute. Dietary intake of phylloquinone and menaquinone were calculated using the USDA National Nutrient Database for Standard Reference (Release 23), supplemented with menaquinone data from published studies. Cox proportional hazards regression was performed to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for dietary intake of phylloquinone and menaquinones in relation to prostate cancer risk. After adjustment for confounders, no statistically significant associations were found between vitamin K intake estimated from the DQX and prostate cancer risk (HR (95% CI) for the highest quintile vs. the lowest quintile: 0.99 (0.87, 1.13) for total vitamin K, 0.99 (0.87, 1.12) for phylloquinone, and 1.02 (0.87, 1.18) for total menaquinones). Overall null results were also observed when additional analysis was carried out by the stage of the disease (i.e., advanced and non-advanced cases) and for vitamin K intake data assessed with the DHQ. In summary, the present study revealed that intake of total vitamin K and its two natural forms was not associated with the risk of total and advanced prostate cancer.

**5313 Associations of calcium and dairy product intakes with colorectal cancer risk among older women: the Iowa Women’s Health Study.** Caroline Um,1 Anna Prizment,2 DeAnn Lazovich,2 Robert M. Bostick1. *Emory Univ., Atlanta, GA; 1University of Minnesota, Minneapolis, MN.*

Calcium has been consistently modestly associated with lower risk of colorectal cancer, which remains the second leading cause of cancer deaths among men and women combined in the United States. Dairy products, a major source of dietary calcium, have also been consistently inversely associated with colorectal cancer, although inconsistencies in relation to high-fat dairy products have been noted. Limited evidence suggests that dairy products may contain components other than calcium and fat, such as insulin-like growth factor 1 (IGF-1), that may be independently directly associated with risk of colorectal and other cancers. Data from the Iowa Women’s Health Study, a prospective cohort study, were used to investigate associations of calcium, dairy products, and the non-calcium component of dairy products with colorectal cancer incidence. Dietary data were collected using a semiquantitative food frequency questionnaire (FFQ) mailed to 55–69-year-old women in 1986 who had a valid Iowa driver’s license. Among participants with no history of cancer at baseline and who satisfactorily completed their FFQ (n = 35,221), through follow up in 2012, 1,731 incident cases of colorectal cancer were documented via the Iowa SEER registry. To investigate total dairy and total, whole, and non-fat milks independent of their calcium components, we used the residuals from linear regression models of their associations with dietary calcium. Associations of the calcium and dairy product variables with colorectal cancer incidence were estimated using multivariable Cox proportional hazards regression. For those in the highest relative to those in the lowest quintiles of intake, the adjusted hazards ratios and 95% confidence intervals (CI) were 0.81 (CI 0.67-0.98; P-trend 0.004) for total calcium, 0.85 (CI 0.68-1.03; P-trend 0.07) for dietary calcium, and 0.85 (CI 0.68–1.07; P-trend 0.16) for supplemental calcium. For total dairy and total milk, the corresponding findings were 0.79 (CI 0.66–0.94; P-trend 0.01) and 0.82 (CI 0.69-0.96; P-trend 0.09), respectively. Low-fat dairy products, non-fat milk, and residuals of total dairy products and total and non-fat milk were not associated with colorectal cancer. These results are consistent with previous findings of low colorectal cancer risk with higher intakes of calcium and dairy products, but provide little support for the hypothesis that the non-calcium, non-fat component of dairy products may be associated with colorectal cancer.

**5314 Long-term folate intake and prostate cancer risk in the health professionals follow-up study.** Nadine M. Hamieh,1 Julie L. Batista,2 Sarah C. Markt,3 Mary K. Downer,4 Lorelei A. Mucci,1 Meir J. Stamper,5 Edward L. Giovannucci,1 Kathryn M. Wilson,1 1Harvard T.H. Chan School of Public Health, Boston, MA; 2Genzyme, Boston, MA.

Background: Randomized trials of supplemental folate and observational studies of circulating folate support a positive association with prostate cancer risk. However, epidemiological studies of dietary and synthetic folate intake are conflicting. Given mandatory folic acid fortification of grains in the US beginning in 1998, evaluation of long-term folate intake from natural and synthetic sources is warranted. We examined the association between folate intake and prostate cancer risk among 47,884 men in the Health Professionals Follow-up Study who were free from diagnosed cancer at baseline in 1986. Total, natural (from food sources), and synthetic (from supplements/fortification) folate intake was assessed every 4 years using a validated food frequency questionnaire, and categorized as the cumulative average and lagged (0–4, 4–8, 8–12, and 12–16 years in the past) intakes. Cox proportional hazards regression was used to calculate multivariable hazard ratios (HRs) and 95% confidence intervals (CI). Results: We identified 6,186 incident prostate cancer cases from 1986-2012, including 1,078 with lethal disease (distant metastasis or death). Mean total folate intake was 481 μg/day in 1986 and increased to 671 μg/day by 2010. The proportion of men meeting the recommended intake of 400 μg/day increased from 47% in 1986 to 74% after fortification began in 1998. Folate intake was positively correlated with physical activity, multivitamin use, and PSA testing, and inversely correlated with smoking. Total folate intake averaged over time was not significantly associated with total prostate cancer risk: HR (95% CI) for <400 μg/day vs. ≥400 μg/day = 1.03 (95% CI: 0.95-1.13, p-trend = 0.76). However, neither natural nor synthetic folate intake was associated with risk of total prostate cancer. Noted were associations of total folate intake and risk of advanced prostate cancer at baseline in 1986. Total, natural, or synthetic folate and prostate cancer risk. We found no indication that folate intake of ≥800 μg/day (twice the recommended level) increases prostate cancer risk.

**5315 Dietary intake and risk of lung cancer in non-smoking women: a hospital-based case-control study in Xuanwei and Fuyuan, China.** Jason Y. Wong,1 Bryan A. Bassig,2 Wei Hu,1 Jinming Zhang,1 Wei Jie Seow,1 Jun He,1 Kaiyun Yang,1 Yunchao Huang,1 Roel Vermeulen,3 Nathaniel Rothman,1 Qing Lan1. *1National Cancer Institute, Rockville, MD; 2Chulalongkorn University, Bangkok, Thailand; 3Utrecht University, Netherlands; 4Queuing Center for Diseases Control and Prevention, China; 5Yunnan Tumor Hospital, China.*

Xuanwei and Fuyuan are rural counties in China that have the highest lung cancer rates in the country among non-smoking women. This alarming public health burden has been attributed to the combustion of smoky (bituminous) coal for heating and cooking, which can produce carcinogenic emissions such as polycyclic aromatic hydrocarbons (PAHs). Previous studies found that green leafy vegetables could absorb PAHs through air and direct soil contamination. Further, oral ingestion of PAHs was found to be associated with pulmonary adenoma development in animal feeding studies. Therefore, we investigated the associations between lung cancer risk and dietary intake of specific green leafy vegetables and other foods in non-smoking women of this farming region. We conducted a hospital-based case-control study of 1,074 female lung cancer patients and 977 frequency-matched controls from Xuanwei and Fuyuan, China in 2006-2013. Dietary intake was self-reported on questionnaires and categorized as the cumulative average and lagged (0-4, 4-8, 8-12, and 12-16 years in the past) intakes. Cox proportional hazards regression was used to calculate multivariable hazard ratios (HRs) and 95% confidence intervals (CI). Results: We identified 6,186 incident prostate cancer cases from 1986-2012, including 1,078 with lethal disease (distant metastasis or death). Mean total folate intake was 481 μg/day in 1986 and increased to 671 μg/day by 2010. The proportion of men meeting the recommended intake of 400 μg/day increased from 47% in 1986 to 74% after fortification began in 1998. Folate intake was positively correlated with physical activity, multivitamin use, and PSA testing, and inversely correlated with smoking. Total folate intake averaged over time was not significantly associated with total prostate cancer risk: HR (95% CI) for <400 μg/day vs. ≥400 μg/day = 1.03 (95% CI: 0.95-1.13, p-trend = 0.76). Neither natural nor synthetic folate intake was associated with risk of total prostate cancer. Noted were associations of total folate intake and risk of advanced stage, advanced stage, or high-grade disease. Conclusions: Our findings do not support strong associations between intake of total, natural, or synthetic folate and prostate cancer risk. We found no indication that folate intake of ≥800 μg/day (twice the recommended level) increases prostate cancer risk.
Background: There is growing evidence of heterogeneity in risk factor associations for prostate cancer defined by clinical and molecular characteristics. Physical activity has moderate protective effects on development of prostate cancer, in particular of advanced and fatal disease. We hypothesized that the influence of physical activity on hormonal and anti-inflammatory pathways may potentially affect the development of TMPRSS2:ERG-positive compared to TMPRSS2:ERG-negative tumors. Our objective was to examine the association between physical activity and risk of prostate cancer defined by the TMPRSS2:ERG fusion. Materials & Methods: We followed 49,160 men in the prospective Health Professionals Follow-up Study from 1986 to 2010. Participants were age 40 years or older, had no previous cancer diagnosis, and reported physical activity by questionnaires self-reported the average number of hours per week they spent engaged in various forms of recreational physical activity at baseline and every two years thereafter with greater than 90% follow-up rates. We examined long-term physical activity type and intensity in units of metabolic equivalent of task (MET)-hours per week. Presence of the TMPRSS2:ERG fusion in tumor tissue was estimated by ERG protein expression using immunohistochemistry. We used competing risks models to obtain subtypes-specific hazard ratios (HRs) and 95% confidence intervals (CIs) for incidence of fusion-positive and fusion-negative prostate cancer. Results: During 23 years of follow-up, 5,819 men were diagnosed with prostate cancer. Of the 2,389 men who underwent radical prostatectomy, 910 were assayed for ERG. Men with higher cumulative average vigorous activity had a lower risk of developing TMPRSS2:ERG-positive prostate cancer per MET-hour (HR 0.96, 95% CI 0.91-1.02, P-value for trend = 0.01). There was no significant association observed between vigorous activity and risk of TMPRSS2:ERG-negative prostate cancer (HR 0.93, 95% CI: 0.67-1.28, P-value for trend = 0.46). However, the test for heterogeneity in these associations was not significant (P-value for heterogeneity = 0.55). No association was observed between total activity and risk of either TMPRSS2:ERG subtype. Conclusions: Physical activity of vigorous intensity is inversely associated with risk of TMPRSS2:ERG-positive but not TMPRSS2:ERG-negative prostate cancer. These findings suggest that regular vigorous activity may lower risk of prostate cancer through mechanisms specific to development of fusion prostate tumors. Future studies are needed to further elucidate these mechanisms and translate these findings into recommendations for prostate cancer prevention.
versely associated with lung cancer risk (P for trend = 0.045). This risk reduction with black tea and their major flavonoids was only observed in ever smokers and not in never smokers. There was no statistically significant association for lung cancer risk with total caffeine, green tea or flavonoids predominantly from green tea, regardless of smoking status. Conclusions: Coffee may increase the risk of developing lung cancer and this effect may be mediated by its caffeine content. Conversely, black tea and flavonoids found mainly in black tea may reduce the risk of lung cancer among ever smokers.

#5319 Comparison of changes in waist circumference and body mass index (BMI) through adulthood and their relationship with advanced colorectal neoplasia. Wambuí G. Gathirua-Mwangi, Patrick Monahan, Yiqing Song, Terrell Zollinger, Timothy Stump, Victoria Champion, Thomas Imperiale. Indiana University, Indianapolis, IN.

Background: Waist circumference (WC) is a stronger predictor of colon cancer risk than body mass index (BMI); however, how well change in either WC or BMI over time predicts the risk of AN remains unclear. Purpose: To investigate 1) the relationship between change in adiposity measures (BMI and WC) from early adulthood to older age and the risk of advanced colorectal neoplasia (AN) and 2) whether WC change or BMI change is a stronger predictor of risk of AN. Methods: The sample included 4,500 adults, 50-80 years with no previous colorectal cancer or adenomatous polyps and undergoing first-time screening colonoscopy. Participants reported adiposity measures (height, weight and WC) at early adulthood (age 21) and at time of screening (mean age 57). Changes in BMI were categorized as 1) increase from healthy BMI to overweight or obese; 2) increase from overweight to obese, and 3) stable BMI level at age 21 and time of screening. WC was defined as high-risk for females with a WC ≥35 and males ≥40 inches. Changes for WC were categorized as 1) increase from a low-risk WC to a high-risk WC, and 2) stable-risk WC at age 21 and time of screening. Gender, age, family history, exercise, aspirin use, alcohol, smoking and intake of red meat and vegetables were included in the logistic models. Results: Being a obese both at age 21 and at screening resulted in a significant increased risk of AN (OR = 1.87; 95% CI 1.08-3.23) compared to those with a stable-healthy BMI at both time points. Stable overweight BMI at age 21 and at screening (OR = 1.54; 95% CI 0.97-2.45) and increased BMI from healthy to overweight or obese and overweight to obese were not associated with AN, as compared to those who had a healthy BMI at both time points. Compared to those with a low-risk WC at both time points, those who had a high-risk WC at age 21 and at time of screening had a two-fold increased risk of AN (OR = 2.15; 95% CI 1.35-3.45). Increasing WC from age 21 to time of screening compared to those with low-risk WC was not associated with risk of AN (OR = 1.23; 95% CI 0.97-1.57). When both WC and BMI change were included, WC change (≥10.15, 2 DF, p-value = 0.0062) but not BMI change (≥5.66, 2 DF, p-value = 0.34) predicted risk of AN. Conclusions: Maintaining a high-risk WC or obese BMI is associated with increased risk for AN; however, when BMI is controlled, maintaining a high-risk WC may independently increase the risk for AN. Practitioners should caution adults with high-risk WC and obese BMI values to better control their adiposity.

#5320 Folate and other nutrients related to one-carbon metabolism and risk of melanoma. Ashar Dhana,1 Ha Yi,2 Tricia Li,3 Michelle Holmes,2 Abrar Qureshi,4 Eunyoung Cho4. 1Grote Schuur Hospital and University of Cape Town, South Africa; 2Harvard T.H. Chan School of Public Health, MA; 3B Brigham and Women’s Hospital, MA; 4The Warren Alpert Medical School of Brown University, Providence, RI.

Background: Nutrients involved in one-carbon metabolism - folate, vitamins B6 and B12, methionine, choline, and betaine - have been associated with multiple cancer sites. However, no study has assessed the association between these nutrients and risk of melanoma. Thus, the aim of this study was to evaluate the association between intake of these nutrients and melanoma risk within two prospective cohorts: the Nurses’ Health Study (NHS) and Health Professionals Follow-up Study (HPFS). Methods: The cohorts included a total of 81,685 women in the NHS (1984 to 2010) and 49,617 men in the HPFS (1986 to 2010). We assessed nutrient intakes using a semi-quantitative food frequency questionnaire every 2 to 4 years. Total nutrient intake included both dietary and supplemental intake. Dietary intake included intake from food only. Study physicians reviewed medical and pathological records to confirm self-reported cases of invasive melanoma. We used Cox proportional hazards regression to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) and then pooled the HRs for both cohorts using a random effects model. Results: Over 24 to 26 years of follow-up, we documented 1,321 cases (641 men and 680 women) of invasive melanoma. Higher intake of folate from food only, but not total folate, was associated with increased risk of melanoma. The pooled multivariable HRs for the top versus bottom quintiles were 1.17 (95% CI: 0.98-1.41; P for trend = 0.17) for total folate and 1.37 (95% CI: 1.13-1.65; P for trend = 0.001) for folate from food only. The association was statistically significant in men, but not in women. Higher intake of vitamins B6 and B12, choline, betaine, and methi-onine were not associated with melanoma risk. We found some evidence that folate from food only, but not total folate, was associated with a modest increased risk of melanoma - a finding that warrants further investigation. Conversely, our findings suggest that other nutrients involved in one-carbon metabolism do not play an important role in the etiology of melanoma.

#5321 Metabolomics and body mass index among breast cancer survivors in The Lifestyle, Exercise, and Nutrition (LEAN) Study. Leah M. Ferrucci,1 Brenda Cartmel,1 Maura Harrigan,1 Tara Sanft,2 Mary Playdon,3 Wei Jia,4 Herbert Yu,4 Caroline H. Johnson,4 Lajos Pusztai,2 Anees B. Chagpar,7 Melinda L. Irwin1. 1Yale School of Public Health, New Haven, CT; 2Yale School of Medicine, New Haven, CT; 3National Cancer Institute, Rockville, MD; 4University of Hawaii Cancer Center, Honolulu, HI.

Background: While there is a growing body of literature on metabolomics and body mass index (BMI), there are limited studies in relation to weight change in overweight and obese individuals, and to our knowledge, no such studies in breast cancer survivors. Methods: The Lifestyle, Exercise and Nutrition (LEAN) randomized diet- and exercise-induced weight loss trial in breast cancer survivors enrolled overweight or obese breast cancer survivors (Stage 0-III) identified via the Yale-New Haven Hospital tumor registry or self-referral. Participants were randomized to either usual care group (n = 33) or the 6-month lifestyle intervention (11 sessions with a registered dietician) (n = 67). Height and weight were measured by study staff at baseline and 6 months, and a fasting (≥ 12 hours) blood draw was also performed at these time points. Paired (baseline and 6-month) serum metabolomics data were available for 83 women. Only those metabolites consistently detected in >80% of quality control samples with a coefficient of variation <30% were included in data analysis. We evaluated partial Pearson correlations between the ratio of metabolites at baseline versus 6-months and change in BMI adjusted for age and intervention group. We also assessed mean changes in metabolites from baseline to 6-months by randomization group using the general estimating equation in linear regression. Adjustments for multiple comparisons was based on the False Discovery Rate (FDR) with a threshold of 0.1. Results: Overall, a total of 307 metabolites were analyzed; 168 were quantitated by LC-MS and 139 were semi-quantitated by GC-TOF/MS. For BMI change from baseline to 6-months, we observed correlations with 16 metabolites (isocitric acid, 3-hydroxybutyric acid, serine, L-cysteine, piroxicin acid, ammonialmonic acid, palmitoleic acid, linoleic acid, 2-hydroxybutyric acid, and seven unidentified metabolites (p < 0.05)). However, no correlations between metabolites and change in BMI or randomization group among overweight and obese breast cancer survivors enrolled in a weight loss trial. Larger sample sizes would help to clarify if any of the potential signals observed in this population may be associated with BMI or other lifestyle changes, such as diet quality.
justing for radiation and chemotherapy treatment, age, sustained inability to eat solid foods after diagnosis, and smoking status. Results: Of the 282 patients that met our eligibility criteria, 211 men with complete information available were included in the analyses, and there were 131 recorded events during follow-up. In this study population, mean age at diagnosis was 63.7 years (SD = 9.8), median survival was 2.6 years, mean average BMI after 40 years of age was 29.0 kg/m² (SD = 4.5), and mean BMI at diagnosis was 27.4 kg/m² (SD = 4.4). Compared to obese subjects at diagnosis, underweight, healthy weight, and overweight at diagnosis respectively had an adjusted hazard ratios of death (HR) of 2.72 (95% CI: 0.59-12.45), 1.79 (95% CI: 1.06-3.02) and 1.08 (95% CI: 0.65-1.74) (BMI category p = 0.04), likely demonstrating reverse causation. Adjusting for confounders, BMI at diagnosis showed that patients with an overweight BMI at diagnosis had the highest hazard of death, compared to patients who gained weight in late life prior to diagnosis (HR = 0.70, 95% CI: 0.45-1.1) and patients who lost some weight (HR = 0.55, 95% CI: 0.35-0.91) (BMI category p = 0.05). The strong effect of BMI even after adjusting for patients’ inability or lack of desire to eat (anorexia), implicates cachexia as a possible independent mechanism of this association. In addition, age at dx, stage at diagnosis, and surgical resection were significant predictors of overall survival. Conclusion: Substantial weight loss in BMI prior to diagnosis indicates poor overall survival in middle-aged and elderly Caucasian men with esophageal adenocarcinoma.

**#5323 Obesity and ovarian cancer survival revisited.** Elisa V. Bandera,1 Valerie Lee,2 Bo Qin,1 Lorna Rodriguez,3 Bethan Powell,3 Lawrence H. Kushi 2.

Research on the impact of obesity on ovarian cancer survival is inconsistent but previous studies did not consider the possible impact of ascites, bowel obstruction, or cachexia, which commonly occur in late-stage disease. We evaluated this association in a cohort study of primary invasive epithelial ovarian cancers diagnosed from 2000-2013 in Kaiser Permanente Northern California (KPNC) (n = 1,157). Deaths were identified through December 2014, with median follow-up of 37 months. Proportional hazards regression was used to estimate overall and ovarian cancer-specific mortality, accounting for prognostic variables including age at diagnosis, race, stage, grade, histology, comorbidities, treatment, post-treatment CA125 levels, ascites, and bowel obstruction. There was no evidence of an association between BMI and overall or ovarian cancer-specific survival. However, we found strong effect modification by stage (Pinteraction < 0.01). Compared to normal pre-diagnosis BMI (18.5-24.9 kg/m²), for women who were obese before diagnosis (BMI ≥ 35 kg/m²) risk of ovarian cancer-specific mortality was increased among those diagnosed at stages I/II (Hazard ratio [HR]: 3.40; 95% confidence interval [CI]: 1.16-9.99), but reduced among those diagnosed with stage IV disease (HR: 0.58; 95% CI: 0.35-0.96). Associations persisted after excluding those diagnosed with cachexia (n = 91) and further adjusting for ascites and bowel obstruction, with no evidence of effect modification by these factors. Associations of obesity with ovarian cancer survival differ by stage, with decreased survival among those with localized disease and increased survival among those with late-stage disease. Stage-specific effects of obesity on survival suggest a tailored approach to improve prognosis may be appropriate.

**#5324 ERG expression and PTEN loss by BMI and weight change in men with prostate cancer.** Janelle S. Ho,1 Ibrahim Kulac,1 Tamara L. LOTAN,1 John R. Barber,3 Patrick C. Walsh,1 Misop Han,1 Angelo M. De Marzo,1 Elizabeth A. Platz,2 Corinne E. Joshua,1 Johns Hopkins University School of Medicine, Baltimore, MD; 3Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.

Background: Obesity and weight gain are associated with poor prostate cancer outcome; underlying mechanisms are unknown. Obesity has been more strongly associated with lethal disease in men positive for the TMPRSS2:ERG gene fusion than negative for this fusion. PTEN loss is associated with increased risk of lethal progression; its association with obesity has not been explored. We evaluate the relationship of ERG and PTEN status and weight change in men with prostate cancer from four large population-based studies.

**#5325 Associations between adipose tissue compartments and the plasma metabolome in colorectal cancer patients: Results from the ColoCare Study.** Jennifer Ose,1 Tengda Lin,1 Nina Habermann,2 Lawerence H. Kushi,3 Valerie Lee,2 Bo Qin,1 Lorna Rodriguez,3 Bethan Powell,3 Lawrence H. Kushi 2.

We evaluated this association in a cohort study of primary invasive epithelial ovarian cancers diagnosed from 2000-2013 in Kaiser Permanente Northern California (KPNC) (n = 1,157). Deaths were identified through December 2014, with median follow-up of 37 months. Proportional hazards regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association of BMI and weight change (adjusted for height, starting weight) with ERG positivity and heterogeneous PTEN loss. Results: Mean age was 56.1 years and mean Gleason sum was 6.2, 73.5% had organ-confined disease. Prevalence of ERG positivity did not statistically significantly differ by BMI or weight change. However, when jointly categorized by BMI and weight change, overweight/obese men who maintained (OR = 2.5; 95% CI 1.1-5.7) or gained (OR = 2.4; 95% CI 1.3-4.7) weight between diagnosis and prostatectomy were significantly more likely to have ERG positive tumor compared with normal weight men who maintained weight. Overweight-obese men were also significantly more likely (OR = 2.4; 95% CI 1.3-4.7) to have PTEN loss compared with normal weight men. When stratified by ERG status, this association appeared to be stronger for men with ERG positive tumors (OR = 2.9; 95% CI 1.3-6.8) than for men with ERG negative tumors (OR = 1.6; 95% CI 0.6-4.9). Prevalence of PTEN loss did not differ by weight change. When jointly categorized by BMI and weight change, overweight/obese men who maintained (OR = 2.0; 95% CI 0.8-5.3) or gained weight (OR = 1.9; 95% CI 0.7-5.1) appeared to be more likely to have PTEN loss compared with normal weight men who maintained weight. Conclusions: Overweight/obese men who maintained or gained weight circadna and normal weight men who gained weight more likely had ERG positive tumors. Overweight/obese men were more likely to have tumors with PTEN loss; this association was stronger among overweight/obese men who also had ERG positive tumors. The TMPRSS2:ERG gene fusion and PTEN loss may contribute to the increased risk of poor prostate cancer outcomes among overweight/obese men. Funding: Prostate Cancer Foundation.

**#5325A Fruit and vegetable intake and risk of breast cancer: Pooled analysis of the Nurses’ Health Study and the Nurses’ Health Study II.** Maryam S. Farvid, Walter C. Willett, A. Heather Elissian. Harvard T.H. Chan School of Public Health, Boston, MA.
Fruit and vegetable intake has been hypothesized to protect against breast cancer, but the overall evidence has not been supportive. Using pooled data from the Nurses’ Health Study and the Nurses’ Health Study II cohorts, we evaluated fruit and vegetable intake in relation to breast cancer risk. Diet was assessed every 4 years using semiquantitative food frequency questionnaires. Cox proportional hazards regression, adjusting for multiple breast cancer risk factors, was used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for each category, using the lowest quintile of intake as the reference category. Among 182,204 women aged 27 to 66 years, we prospectively identified 10,973 invasive breast cancer cases during follow-up from 1980 through 2012 for the Nurses’ Health Study and from 1991 through 2013 for the Nurses’ Health Study II. Fruit and vegetable intake was significantly associated with a lower risk of breast cancer. The pooled multivariate HR (95% CI) for highest (3.2 servings/day) versus lowest (0.4 serving/day) quintile intake of total fruit intake was 0.93 (0.86–0.99; Ptrend = 0.03) and for highest (4.9 servings/day) versus lowest (1.5 servings/day) quintile intake of total vegetable was 0.92 (0.86–0.99; Ptrend = 0.02). We also observed that a higher intake of yellow/orange vegetables, cruciferous vegetables, fruits and vegetables rich in β-carotene, and fruits and vegetables rich in lutein was each associated with a lower breast cancer risk. In addition, the association with vegetable intake was stronger for both estrogen and progesterone receptor negative (HR = 0.91; 95%CI = 0.87–0.95) than both estrogen and progesterone receptor positive cancers (HR = 0.98; 95%CI = 0.96–1.00; Ptrend by hormone receptor status = 0.003). Our findings support an association of a higher fruit and vegetable intake with a lower breast cancer risk.

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#5326 EZH2 regulation through lysine 63 linked ubiquitination in prostate cancer. Wenyu Fu,1 Lou Chen,2 Bo Li,2 Jingying Xie,2 Michael G. Izbain,3 Billy R. Ballard,1 Sandeep A. Sathyanaaryana,1 Samuel E. Adunyah,1 Robert J. Matsuski,2 Zhenbang Chen1.

Enhancer of zeste homolog 2 (EZH2) plays crucial roles on the development of cells and tissues as well as the progression of prostate cancer (PCa) and castration resistant prostate cancer (CRPC). EZH2 can upregulate and activate progenitor genes including androgen receptor (AR) and the target genes. However, the mechanisms by which EZH2 is regulated in PCa remains elusive. Literature reports that tumor necrosis factor (TNF) receptor associated factor-6 (TRAF6) is essential for both normal tissue development and regulation of oncogenic signaling pathways in cancers. In this study, we reported that EZH2 is regulated by PTEN and SKP2 network in PCa. Specifically, we showed that the aberrant upregulation of EZH2 and histone H3 lysine 27 trimethylation (H3K27me3) in both Pten null mouse embryonic fibroblasts (MEFs) in vitro and prostate tumors of Pten null mice in vivo, as compared to the controls. EZH2 levels were negatively correlated with TRAF6 in human PCa cells upon SKP2 dysregulation. Immunofluorescence (IF) staining results showed a co-localization of EZH2 and TRAF6 in nucleus of PC3 cells, and co-immunoprecipitation (co-IP) analysis further confirmed a physical binding of EZH2 and TRAF6 in PC3 cells. Ectopic expression of TRAF6 promoted the K63-linked polyubiquitination of EZH2 to decrease EZH2 and H3K27me3 levels in PCa cells. Conversely, TRAF6 knockdown resulted in a reduction of EZH2 polyubiquitination with an increase of EZH2 and H3K27me3 levels in PCa cells. Furthermore, the catalytically dead mutant TRAF6 C70A abolished the TRAF6-mediated polyubiquitination of human EZH2 in vivo in 293T cells and in vitro in the recombinant human EZH2 protein, as compared with TRAF6 WT. Together, we report for the first time a novel mechanism on EZH2 ubiquitination and an important signaling network of SKP2-TRAF6-EZH2-H3K27me3 in PCa cells. Our findings provide important clues on the mechanism and efficiency of targeting EZH2 in PCa and CRPC.

#5327 Giolma derived cancer stem cells are hypersensitive to proteasomal inhibition. Dae-Hee Lee, Sang Cheul Oh, Yong Tae Kwon, Yong J. Lee, Young Dong Yoo.

Although proteasome inhibitors (PIs) are used as anticancer drugs to treat various cancers, their relative therapeutic efficacy on stem cells vs. bulk cancers remains unknown. Here, we show that stem cells derived from gliomas, GSCs, are up to 1,000-fold more sensitive to PIs (IC50, 27-70 nM) compared with their differentiated controls (IC50, 47- > 100 μM). The stemness of GSCs correlates to increased ubiquitination, whose misregulation readily triggers apoptosis. PI-induced apoptosis of GSCs is independent of NFκB but involves the phosphorylation of JNK as well as the transcriptional activation of endoplasmic reticulum (ER) stress-associated proapoptotic mediators. In contrast to the general notion that ER stress-associated apoptosis is signaled by prolonged unfolded protein response (UPR), GSC-selective apoptosis is instead counteracted by the UPR. ATF3 is a key mediator in GSC-selective apoptosis. Pharmaceutical uncoupling of the UPR from its downstream apoptosis sensitizes GSCs to PIs in vitro and during tumorigenesis in mice. Thus, a combinational treatment of a PI with an inhibitor of UPR-coupled apoptosis may enhance targeting of stem cells in gliomas. Key words: glioma; glioma stem cells; proteasome inhibitors; ubiquitin proteasome system; apoptosis; c-Jun N-terminal kinase (JNK).
Targeting hepatocellular carcinoma through TGF-β pathway E3 ligases. Shuyun Rao,1 Heather Levin,1 Jian Chen,2 Rehan Akbani,3 Jon White,3 Wilma Jogunoori,3 Shoujun Gu,1 Kazufumi Ohshiro,2 Sobia Zaidi,1 Bibhuti Mishra,2 Asif Rashid,2 Shulin Li,3 Lopa Mishra,2 George Washington Univ, Washington, DC; 2MD Anderson Cancer Center, Houston, TX; 3Institute of Clinical Research, Washington, DC.

Hepatocellular carcinoma (HCC) is the 3rd leading cause of cancer deaths worldwide, and rising in the United States at an alarming rate. Multiple E3 ubiquitin ligases such as the SMURFs and RINGH2 proteins have been identified as negative regulators of the TGF-β pathway. However, to our knowledge, there remains a gap in the integration between genomics, underlying mecha- 

isms and therapeutic development of targeted therapies harnessing these associated E3 ligases for HCC. The aim of this study is to elucidate the role of E3 ligases in HCC, through TCGA analyses and provide mechanistic insight into these as therapeutic targets for HCC. We first analyzed the 488 hepatocellular cancers and screened for alterations in The Cancer Genome Atlas (TCGA). Immunohistochemistry (IHC), Q-PCR, Western blot analyses were used to validate the expression levels of two of the most highly altered E3 ligases, PRAJA1 and Keap1 in hepatocellular cancer tissues and cell lines in human and in TGF-

β-deficient β2SP−/− mouse models. Inhibition studies of PRAJA and Keap1 were performed by lentivirus shRNA in HCC cell lines, and xenograft studies. From the TCGA data, we observe two different signatures (activated and inactivated) for 18 TGF-β pathway genes. While increased levels of TGF-β-related transcripts were associated with activation of hepatic fibrosis/malignancy pathways, decreased levels of TGF-β members were associated with loss of TGF-β tumor suppressor function. HCCs characterized by the “inacti-

vated” TGF-β signature were associated with a significantly poorer survival, compared to HCCs with the “activated” TGF-β signature (p = 0.0027). We next analyzed 29 TGF-β-related E3 ligases, and observed raised expression of the following: PRAJA1 (12.7% of HCCs), Keap1 (6.4%), UCHL5 (16.4%), WWP2 (11.8%), WWP1 (10%), Smurf2 (9.1%), Skp2 (9.1%), and Smurf1 (8.2%). Interestingly, expression patterns corresponded with a few TGF-β signaling members regulated by some of these E3 ligases, namely Smad3 (altered in 54%) and β2SP (27%). We identified that PRAJA1 targets Smad3 and β2SP for ubiquiti-

nation and degradation. We further observe raised levels of PRAJA (25%) and Keap1 (70%) in 176 human liver cancers, by IHC, compared to normal counter-

trols. Depletion of PRAJA and Keap1 with either shRNAs or E3 ligase inhibi-

tors, substantially inhibited growth and induced apoptosis through PRAJA/ Smad3/β2SP and Keap1/Nrf signaling in HCC cell lines and xenografts. These results suggest that E3 ligases such as PRAJA1 and Keap1 may be valuable therapeutic targets for liver cancer in the context of TGF-β signaling, an important approach given that few effective targeted therapies are available for this cancer with poor prognosis.


Background: Pancreatic ductal adenocarcinoma (PDAC) is a deadly disease driven by mutated KRAS with an average survival of only 3-6 months from the time of diagnosis. Interferon-Stimulated Gene 15 (ISG15) is a 15 kDa protein induced by Type I interferons (IFN-α and IFN-β) and is a member of the ubiquitin-like superfamily of proteins. The ISG15 pathway is highly elevated in var-

ious malignancies where it has been shown to stabilize KRAS protein expression.

However, very little is known about the role of the ISG15 pathway in PDAC. The purpose of this project is to investigate the expression and proliferative effects of the ISG15 pathway in PDAC. Methods: The ISG15 knockdown cells were created using the CRISPR (Clustered Regularly Interspaced Short Palindromic Re-

peats) gene editing system. ISG15 was stably knocked-down in murine Panc02 pancreatic cancer cells by transfection with an ISG15 CRISPR construct from Genscript (pSpCas9 B-2A-Puro (PX459)) and treated with media containing puromycin (2 μg/ml) for selection. Knockdown was confirmed by RT-PCR. The effect of ISG15 knockdown on PDAC cell growth in vitro was determined by comparing viable cell counts on wild-type and ISG15 knockdown Panc02 cells 48 hours after plating. The effect of ISG15 knockdown on Panc02 cell growth was determined by subcutaneous inoculation of syngeneic immune competent C57BL/6 mice with 1x10⁶ Panc02 wild-type or ISG15 knockdown cells. Tumor volumes were measured weekly and after 3 weeks the mice were euthanized, tumors dissected and weighed. Results: ISG15 CRISPR treated cells had a 50% reduction in ISG15 mRNA expression as compared to wild-type cells. ISG15 knockdown in Panc02 cells significantly reduced cell growth as compared to wild-type cells (p = 0.030). Tumors formed from ISG15 knock-

down Panc02 cells significantly had a 74% reduction in tumor volume (p = 0.031) and 78% reduction in tumor weight (p = 0.029) as compared to wild-type cells. Conclusion: The ISG15 pathway contributes to PDAC by increasing cell growth and the tumorigenic potential of pancreatic cancer cells. Ultimately, strategies to target the ISG15 pathway may lead to improved survival for patients diagnosed with PDAC. Supported by: NIH T32CA09968 grant.

Monoubiquitination inhibits the actin bundling activity of fascin. Shenglin Chen,1 Shuang Lu,2 Mentor Mulaj,1 Bing Fang,2 Tyler Keeley,2 Lixin Wan,3 Jiuhao Hao,3 Martin Muschol,3 Jianwei Sun,3 Shengyu Yang.1 Penn State University, Hershey, PA; 2 Moffitt Cancer Center, Tampa, FL; 3 University of South Florida, Tampa, FL; 4 Tianjin Medical University, Tianjin, China; 5 South China Agricultural University, Guangzhou, China.

Fascin is the most frequently upregulated actin regulatory protein in meta-

static tumors. The elevated expression levels of fascin universally correlate with poor clinical course and shorter survival across different cancer types. It is believed that fascin promotes cancer cell migration and invasion by crosslinking actin filaments into bundles. However, the molecular mechanisms underlying the regulation of fascin bundling activity are not completely understood. In this study we examine the regulation of Fascin activity by monoubiquitination. IP and LC-MS/MS was used to identify the posttranslational modification of Fascin. A novel chemical monoubiquitination method was employed to synthesize monoubiquitinated Fascin. The monoubiquitinated fascin was purified, and the effect of monoubiquitination on fascin bundling activity was determined using low speed sedimentation assay, fluorescence microscopy and transmission electron microscopy. Here we identified monoubiquitination as a novel mechanism that regulates fascin bundling activity and dynamics. The monoubiquitination sites were identified to be K247 and K250, two residues located in a positive charge patch at the actin binding site 2 (ABS2) of fascin. Using a chemical ubiquitination method, we synthesized chemically monoubiquitinated fascin (mUb-fascin) and determined the effects of monoubiquitination on fascin bundling activity and dynamics. Our data demonstrated that monoubiquitination decreased the fascin bundling EC50, delayed the initiation of bundle assembly and accelerated the disassembly of existing bundles. By analyzing the electro-

static properties on the solvent accessible surface of fascin, we proposed that monoubiquitination introduced steric hindrance to interfere with the interac-

tion between actin filaments and the positively charged patch at ABS2. Mutation of the monoubiquitination sites (K247 and K 250) to arginine inhibited fascin monoubiquitination and enhanced pro-migration ability of fascin, supporting the notion that monoubiquitination inhibit fascin activity. We also identified Smurf1 as an E3 ligase regulating the monoubiquitination of fascin. Our findings revealed a previously unidentified regulatory mechanism for fascin, which will have important implications for the understanding of actin bundle regulation under physiological and pathological conditions.

Destabilization of EWS-Fli1 protein by deubiquitinase inhibition in Ewing Sarcoma. Shan Wang, Ralf Kittle. UT Southwestern Medical Ctr., Dallas, TX.

Background: Ewing Sarcoma is a malignant tumor in bone or soft tissue, and typically in teenagers and young adults. Genetically, most Ewing’s Sarcoma contains the chromosomal translocation [t(11;22)(q42;q12)], which fuses EWS1 gene from chromosome 11 and Fli1 gene from chromosome 22 and generates an aberrant chimeric protein EWS-Fli1. EWS-Fli1 is essential for cell growth and development in EWS-Fli1 positive Ewing Sarcoma cells. Therefore, targeting EWS-Fli1 could be a promising therapeutic strategy for Ewing Sarcoma. Methods and Results: Currently, the compounds of targeting EWS-Fli1 mainly interfere its interaction with partner or DNA. However, it could be a more potent way to manipulate its protein stability. Firstly, the proteasome inhibitor MG132 could retard the degradation of EWS-Fli1 protein in EWS502 cells and A673 cells, and increase its ubiquitination, which hints the degradation of EWS-Fli1 is through the ubiquitin (Ub)-proteasome pathway (UPP). Then, to identify the protein that interact with EWS-Fli1 and modulate its stability, we selected the candidate deubiquitinase according to literatures and the protein homology. And by co-immunoprecipitation assay, we identified a deubiquitinase enzyme (DUB) binding to EWS-Fli1. RNAi-mediated knockdown or inhibition of EWS-Fli1-DUB with a small molecule compound caused a marked increase in EWS-Fli1 ubiquitination, decreased levels of EWS-Fli1 in a protea-

some-dependent manner, and further inhibited the growth of Ewing Sarcoma cells. Conclusion: The findings indicate that EWS-Fli1 could be ablated by in-

hibiting its specific deubiquitinase in Ewing Sarcoma, which could be a novel treatment approach for Ewing Sarcoma.

Dissecting the ubiquitin pathway using homogenous and sensitive bioluminescent assay. Said A. Goueli, Kevin Hsiao, Subhanjan Mondal. Pro-

mega Corp., Madison, WI.
Post-translational modification of target proteins by ubiquitin (Ub) and ubiquitin-like (Ubl) proteins is a fundamental mechanism for regulating protein functions and protein stability thus affecting diverse cellular processes. Several ubiquitin-like (Ubl) molecules have been identified including SUMO, NEDD8, ATG8/15 and ISG15 which share the ubiquitin fold Ub/Ubl proteins are conjugated to lysine residues in substrate proteins through an ATP-dependent posttranslational cascade involving E1-activating enzyme, E2-conjugating enzyme and E3-ligase. To mark proteins for degradation, multiple ubiquitins are covalently attached to produce a Lys48 linked poly-ubiquitin chain. The poly-ubiquitinated proteins are recognized by the 26S proteasome system and deubiquitinated and destroyed. Substrates with ubiquitin chains linked through Lys 6, 11, 27, 29, 33 also regulate protein degradation through an ATP-dependent endocytic cascade resulting in TGF-β signaling, acts as a scaffold to recruit the E3 ligase SMAD7, a direct transcriptional target of downstream TGF-β receptor stabilization and enhanced levels of p-SMAD2. Clinical loss of USP26 correlates with high TGF-β activity and confers poor prognosis in glioblastoma. Our data identify USP26 as a novel negative regulator of the TGF-β pathway and suggests that loss of USP26 expression may be an important factor in glioblastoma pathogenesis.

#5338 Transcriptional elongation mediates human papillomavirus type 16 late promoter activation. William Songock, Jason Bodily. Louisiana State University Health Sciences Center, Shreveport, LA.

Human papillomaviruses (HPVs) are small DNA viruses that infect keratinocytes of squamous epithelia and cause benign lesions that can progress to several malignancies, including cervical cancer. Upregulation of viral transcripts by the HPV16 late promoter is dependent on host cellular differentiation. We sought to investigate the main cellular transcription regulators that correlate with differentiation-dependent increase in the late viral transcripts. Here, we show evidence that the step in transcription cycle that is responsible for late promoter activation is transcriptional elongation. We began by establishing that the increase in viral transcripts originating from the late promoter is primarily regulated at the transcript synthesis level rather than post-transcription level. Based on this observation, we investigated whether there is a correlation between late promoter activation and activities that occur during the initiation, pausing or elongation steps of transcription cycle. Using chromatin immunoprecipitation (ChIP), we did not observe a differentiation dependent increase of RNA Polymerase II (RNP II) in the late promoter region. However, there was a significant increase of RNP II at downstream sites of late promoter suggesting elongation is regulated by differentiation. We then sought to investigate the cyclin dependent kinases (CDKs) that are known to regulate transcription elongation. ChIP did not show a significant increase of the elongation factor CDK8 in the late promoter; however, the enrichment of CDK9 at downstream sites of the viral genome significantly increased upon differentiation, suggesting that differentiation induces recruitment of CDK9 to facilitate elongation. Additionally, CDK8, a component of the Mediator complex that recruits CDK9 to the RNP II, was increased upon differentiation. Kinase inhibitors against CDK8 did not affect late viral transcripts; however, knocking down CDK8 resulted in a significant reduction of virus particles, suggesting that CDK9 could be involved in protein-protein interactions that are required for the synthesis of late viral transcripts. These findings suggest that HPV has evolved to utilize cellular transcriptional elongation machinery as a way of regulating its late gene expression. This mechanism allows the virus to persistently infect host cells and cause HPV-associated malignancies.


Human papillomaviruses (HPVs) are a diverse group of small double stranded Deoxyribonucleic acid viruses implicated in many malignancies including cervical cancer. The viruses are also attributed to premalignant epithelial lesions in this disease. Cervical cancer is a leading cause of morbidity and mortality in African women and beyond. We aimed to study the molecular phylogenetic classification of HPV's and cervical cancer associated genes in relation with tumour manifestation. Phylogenetic tree was constructed based on nucleotide sequence alignments using ClustalX 2.1 algorithms to classify 31 different HPV DNA virus types and 10 cervical cancer associated genes. Study of immunohistochemistry markers associates with cervical cancer pathology was performed. The nucleotide sequences used include HPV- 1 (V01116), HPV-14 (D90261), HPV-20 (D90262), HPV-21 (D90263), HPV-25 (D90265), HPV-4 (M29261), HPV-47 (M32305), HPV-8 (M12732), HPV-2 (M55965), HPV-6: X00203, HPV-11 (M14119), HPV-13 (X62843), HPV-43 (HE964240.1 E6 gene), HPV-44 (U31788.1), HPV-42 (HE820261.6 E6 gene for E6 protein), HPV-16 (K07218), HPV-16 (K1689425.1 E6 and E7 variants), HPV-35 (M74117), HPV-31 (I04353), HPV-52 (K1689430.1), HPV-33 (M12732), HPV-58 (D90400), HPV-51 (M62877), HPV-18 (X50015), HPV-45 (M27363), HPV-39 (M38185), HPV-56 (M18078, M37258), HPV-17 (K1689425.1 E6 and E7 variants), Ki-67 (NM_002417.4), p53 (AB082293.1), EGRF (NM_005228.3), MYC (M25803.1), KRAS (EF471957.1), TGF-B (AF254794.1) and RB (AH002963.2) genes. The resulting phylogenetic tree provides a conventional classification of the HPV’s and the cancer genes into groups encompassing the known tissue tropism and oncogenic potential of each gene type associated with the disease. The implications of a phylogenetic taxonomy on the diagnostic detection of HPVs as they relate to cervical cancer associate genes are shown to be important in disease management.
MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Cell Growth Signaling Pathways

#5340 Retrovirus insertion induces syncytin mediated exosomes delivery in prostate cancer cells. Berna Uygar, Leonid Chernomordik. NIH, Rockville, MD.

Activation of retroviruses in normal cells can induce tumors and a wide array of cancers contains retrovirus-like particles. The potential role of these endogenous retroviruses in the development and progression of tumors has been discussed in many studies. However the effects of retrovirus insertion into cancer cells have not yet been fully examined. In this study, we explore genetic material transfer either between retrovirus-transduced prostate cancer PC3 cells or between PC3 cells and their host environment through extracellular vesicles (EV) and virus-like particles. For these purposes, we transduced PC3 cells with either GFP encoding retrovirus or with m-cherry expressing lentivirus. Co-incubation of GFP-expressing cells with m-cherry-expressing cells for 72h yielded yellow PC3 cells. Yellow cells were also observed in the experiments where lentiviral m-cherry expressing PC3 cells were cultured with tissue culture medium from retrovirus GFP expressing PC3 cells. These results indicated that horizontal genetic information transfer involves neither fusion nor contact between the cells. Since incubation of the retrovirus GFP-expressing PC3 cells with the medium from the lentivirus m-cherry expressing PC3 cells, did not yield yellow cells the genetic material transfer requires retrovirus insertion in PC3 cells. Using Western blot and Q-PCR, we found the exosomes from the medium generated by retrovirus GFP expressing PC3 cells to carry human endogenous retroviral proteins syncytin 1 and 2 and contain RNAs encoding these proteins. Exosomes from the lentivirus m-cherry expressing PC3 cells had lower protein expression of Syncytin 1&2 than exosomes from retrovirus GFP expressing PC3 cells. Application of Syncytin 1 or 2 antibodies to the medium generated by or co-cultured retrovirus GFP expressing PC3 and lentivirus m-cherry expressing PC3 cells suppressed horizontal transfer of the genetic information from retrovirus GFP expressing PC3 cells to other cells. The specific mechanisms by which retroviral transduction and syncytins control the generation of a GFP-encoding pseudotyped retrovirus or EV and the delivery of their content, as well as the role of the virus/EV-mediated horizontal genetic material transfer between prostate cancer cells and between prostate cancer and their microenvironment remain to be clarified.

#5341 HPV16 E6 oncoprotein modulates the DPPIV/CDCP26 in primary keratinocytes. Aline Beckenkamp,1 Bruna Prati,2 Silvya Stuchi Maria-Engler,3 Guido Lena,1 Diogo André Pilger,4 Enrique Mario Piersilvio,4 Andréia Buffoni.1

1 Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, Brazil; 2Universidade de São Paulo (USP), São Paulo, Brazil; 3Universidade de São Paulo (USP), São Paulo, Brazil.

Cervical cancer is related to the persistent infection by high-risk human papillomavirus (HPV) subtypes, such as types 16 and 18. The E6 and E7 oncoproteins significantly contribute to the carcinogenic potential of high-risk HPV, through the degradation of p53 and pRb, respectively. The exopeptidase dipetidyl peptidase IV (DPPIV) also known as CD26, is a multifunctional protein involved in several processes related to cancer. DPPIV/CDC6 has an intrinsic peptidase activity, able to degrade some bioactive peptides and chemokines, thereby regulating cell migration, proliferation and survival. It has been reported that the reduction in DPPIV/CDC6 expression is associated with tumor progression in different types of cancer. Moreover, studies in cervical cancer samples and cell lines point to a reduction in the expression of this protein. However, there are no studies addressing the relationship between HPV infection and DPPIV/CDC6 expression. We analyzed the influence of HPV oncoproteins on DPPIV/CDC6 expression in human keratinocyte transduced with E6, E7 and E6E7. We observed that HPV16 E6 expression is associated to a reduction in DPPIV/CDC6 expression at mRNA and protein levels, whereas HPV16 E7 does not affect DPPIV/CDC6 expression. HPV16 E6E7 co-expression leads to a reduction in DPPIV/CDC6 expression similar to that found in E6 transduced cells. In addition, we note a more pronounced reduction in DPPIV/CDC6 expression in immortalized cells expressing E6E7. Furthermore, keratinocytes expressing HPV16 E6 mutant sequence and HPV11 E6, that do not bind to E6AP and do not degrade p53, have the effect on DPPIV/CDC6 expression. Our results suggest that HPV16 E6 oncoprotein can downregulates DPPIV/CDC6. In addition, preliminary results suggest that modulation of DPPIV/CDC6 mediated by E6 can be dependent on degradation of p53, since HPV16 E6 mutant and low-risk HPV E6 were not able to affect CDC6 expression. Furthermore, the process of cell immortalization also appears to be related to the reduction of DPPIV/CDC6 expression. Therefore, these results indicate that HPV oncoproteins promote imbalance DPPIV/CDC6 expression which may be related to cervical carcinogenesis.

#5342 Epigenetic polymorphisms and human papillomavirus E6/E7 oncoproteins transfection: Induce ATM gene deletion11q22-23 and chromosomal instability. Eva McGhee,1 Liliana Zarate,1 Chiamaka Opara,2 Antoinette Algbuwe,3 Meidrah Tyler,3 Bileko Wissa,4 Billy Ballard,2 Roland Pattillo.5

1 Charles R. Drew University of Medicine and Science, Los Angeles, CA; 2 Meharry Medical College, Nashville, TN; 3 Morehouse School of Medicine, Atlanta, GA.

Persistent infections involving high-risk types 16 and 18 of Human Papillomavirus (HPV) have been established as the etiological agent of cervical cancer. Recent data indicate that several polymorphisms of key regulators from the DNA damage repair pathways are associated with cancer development susceptibility. The long arm of chromosome 11 has received much attention as a high frequency of epigenetic deletions of various sites. In HPV-long terminal repeat (LTR) infection that lead to cervical cancer, indicating the presence of putative tumor suppressor genes and its association with disease progression. To better understand the importance of chromosome 11q22-23 region in the development of cervical cancer, we investigated genetic and epigenetic alterations of the tumor suppressor ATM gene in human keratinocytes transfected with HPV E6/E7 oncoproteins (16-MT). In the present study we examined 16-MT cells for epigenetic changes and chromosome imbalances, using FISH, and arrayCGH. We used several FISH probes, mapping to region 11q22-23 where the ATM gene is located. Our study revealed that the ATM gene located on chromosome 11q is deleted, relating to chromosome deletions, that are recurrent abnormalities in cervical cancer, implicating the loss of tumor suppressor genes as a significant mechanism that drive cells to become cancerous. This study was designed to investigate genetic instability using HPV16 E6/E7 oncoproteins to better understand the importance of chromosome 11q epigenetic changes. Deletion of 11q22-23 was observed in all metaphases scored. With restricted areas of deletions, two chromosomal regions of possible importance in cervical carcinomas could be distinguished. The first region is D11S1325 and the second region is D11S2179. The second region may contain part of the ATM gene. The results indicate that a tumor suppressor gene on chromosome 11q22-23 may be involved in the carcinogenesis of the cervix and the involvement of the ATM gene, as the driving factor remains a possibility.

#5343 The HIV shell protein Gp120 stimulates U87 glioma cell proliferation through the activation of glycolysis. Gabriel Valetin-Guillama,1 Sheila Lopez,2 José Perez,3 Luis Cubano,3 Natalia Chorn,4 Jeffery Harrison,5 Nawal Boughouit,6 Ivan S. Scheravyakj1. 1 Universidad Central del Caribe, Bayaspon, PR; 2 Universidad de Puerto Rico, School of medicine, San Juan, PR; 3University of Florida, Gainesville, FL.

Patients infected with human immunodeficiency virus (HIV) are more prone to develop cancers, including glioblastomas (GBM). The median survival of GBM patients with HIV is significantly shorter compared to HIV-negative GBM patients, despite the fact that they receive the same treatments. This indicates that HIV infection is associated with more aggressive tumor behavior and with treatment resistance. Taking into account that HIV itself is not found in GBM specimens, the nature of the GBM-HIV association remains poorly understood and the underlying molecular mechanism is still unknown. Here we study the effect of Gp120, a glycoprotein found in the HIV shell, on GBM cell growth and the underlying molecular resistance mechanisms. Our study shows that, compared to un-treated cells, quantitative proteomics studies using isobaric tags and western blot analysis identified expression up-regulation of several glycolytic enzymes, including enolase 2, pyruvate kinase, hexokinase, and glyceraldehyde 3-phosphate dehydrogenase in Gp120 treated U87 cells. Additionally, metabolite analysis revealed an increase of pyruvate, amino acid and lipid synthesis, together with reduction of protein degradation, in response to treatment with Gp120. In summary, we demonstrated that Gp120 promoted proliferation and resistance to TMZ in U87 cells through the activation of glycolysis, resulting in increased protein and lipid synthesis. This research was made possible by NIH grant numbers: 1SC2GM102040, R25GM110513, INBRE-PR NIH Grant 8P20GM103475, the UCC RCMI Biomedical Proteomics Facility grant G12MD007583 and US Department of Education Grant number P0315130068.

#5344 Understanding the structural requirements of C1 domains for phorbolester binding, analysis of the atypical C1 domain of STACT2. Adelle Abramovitz, Noemi Kedei, Peter M. Blumberg. NIH/NCI, Bethesda, MD.
C1 domains are the recognition motif for the second messenger diacylglycerol and for the phorbol esters. They play a critical role in the regulation of multiple families of signaling proteins such as protein kinase C, RasGRP, and the chimeras. Atypical C1 domains preserve substantial homology with the diacylglycerol / phorbol ester responsive C1 domains but fail to bind these ligands. A recent approach to highlight any residual binding activity of C1 domains revealed a continuum of structures ranging from those with measurable but weak phorbol ester binding affinity through those that preserve the phorbol ester binding cleft but have residues interfering with the insertion of the C1 domain into membranes and finally those that lack appropriate binding cleft geometry. STAC2 (SH3 and cysteine-rich domain 2) protein attracted our interest because, while its C1 domain has been classified as atypical, it had actually not been tested for phorbol ester binding activity and possesses marked homology to C1 domains that bind phorbol esters. The strategy for analysis has been to probe the influence of individual residues in the STAC2 C1 domain that are either not present or are unusual in the numerous phorbol ester responsive C1 domains. Reciprocal approaches have been to start either with the STAC2 C1 domain, mutate the individual residues, and look for gain of phorbol ester binding activity or to start with the PKC delta C1b domain and look for residues that cause reduction in binding affinity. The former approach is efficient if a single residue is responsible for the lack of binding activity; the latter approach is preferable if multiple residues are responsible. First, we confirmed that indeed the C1 domain of STAC2 is atypical and fails to bind phorbol ester. Second, we identified the residues in positions 11, 13, 24, 25, 41 and 45 of the C1 domain of STAC2 as potential candidates for interfering with binding activity. However, replacement of none of these residues with the corresponding residues from PKC delta C1b sufficed to generate phorbol ester binding activity. One the other hand, introducing mutations into the C1b domain of PKC delta identified L24N, that caused a significant (~20-fold) decrease in the binding affinity. The probable basis for the negative effect of L24N on binding is that Leucine 24, together with Phenylalanine 13, Leucine 20 and Tryptophan 22, are all exposed at the top of the binding cleft and insert into the lipid membrane during ligand binding. The replacement of a hydrophobic residue at position 24 with a hydrophilic residue should interfere with this binding. STAC2 may thus fall into the category of proteins with atypical C1 domains that could be targeted with phorbol deriva-
tives that do not stabilize membrane insertion. Our findings strengthen our
understanding of the interplay between the C1 domain, ligand and the membrane as a ternary complex.

Molecular and Cell Biology / Genetics: Development of High Throughput Analytic Methods

#5345 Complementary NGS approaches on digitally sorted pure tumor cells reveal hidden molecular characteristics in low tumor content FFPE specimens. Claudio Forcato, Alberto Ferrarini, Genny Buson, Cassie Schumacher, Chiara Bolognesi, Valentina del Monaco, Chiara Mangano, Francesca Fontana, Gianni Medoro, Timothy Harkins, Nicolò Specimens.

#5346 Combining droplet tagged short read sequencing with optical DNA mapping technology for improved assembly of cancer genomes. Yuan Jiang, Sven Bliks, Paul S. Meltzer. National Institutes of Health / National Cancer Institute, Bethesda, MD.

Kataegis, chromothripsis, chromoplexy and forms of structural hyper-muta-
tion generically described as chromoanagensis are found in many cancer types. Structural rearrangements can result in deletions, translocations, inversions and insertions, and gene amplification with important functional consequences as cancer drivers and clonal diversity. Whole genome sequencing was increasingly used to sensitively detect structural rearrangements. Yet, it remains difficult to detect these events reliably without an excessive number of false positives. This is a largely consequence of the still relatively short read-length of commonly used sequencing platforms. As a result, in larger cohorts, PCR based methods are generally not feasible for validating all predicted structural rearrangements. Accordingly, new approaches for robust mapping of both simple and complex rearrangements are needed. Here we used a hybrid of two technologies, 10X genomics droplet tagged Illumina sequencing (10X Genomics ) and DNA fiber molecular imaging (BioNano Irys) for high throughput validation. First, utilizing a sequencing-assisted synthetic genome: for each rearrangement predicted by NGS, we build a “pseudo-chromosome” offering this structure as one alter-
compound to the BioNano aligner. This combination compensates for the weak-
nesses of each platform resulting in a more robust result. In this way, even short reads can validate structural rearrangements, as the requirement for split reads is obviated. Provided that sufficient coverage is achieved, statistical analysis can be used to confidently recognize NGS false positive predictions. This allows the cataloging of complex repetitive regions and large-scale rearrangements. In our study we have evaluated this combined approach to large scale genome mapping to identify hallmark fusions and other rearrangements from the highly complex genomes of osteosarcoma cancer cell lines.


FFPE tissue archives is the most widely used method for clinical sample preservation, and provides a valuable source of diverse genetic information for cancer biomarker discovery. NGS library preparation is often challenging, how-
ever, as FFPE-derived DNAs exhibit varying degrees of fragmentation and
cellular modification depending on how tissues were handled and processed.
Moreover, the presence of low allele frequency variants because of tumor heter-
ogeneity, sample degradation, and high rate of formalin-induced mutations
make SNV calling a challenging task. To improve variant calling and consistency across FFPE samples of varying quality, we developed a complete workflow solution that features DNA pre-qualification, molecular barcodes for uniquely
remarking each DNA fragment, pre-capture indexing for tracking up to 192 sam-
pples, accelerated hybrid capture, and one-tube library prep, to enable the pre-
paration of complex enriched libraries in less than 8 hours from 10-200ng of FFPE-derived DNA. We incorporate molecular barcodes at the ligation step to enable de-duplication and generation of consensus reads using SureCall soft-
ware, which significantly improves confidence of variant calling (99% of calls ≥150 variant call quality threshold) at low allelic frequencies. Using ClearSeq

Comprehensive Cancer Panel which targets 151 genes frequently mutated in
solid and hematological cancers, we routinely obtain high quality libraries (with
1000X sequencing depth and after deduplication) with >99% of the targeted region covered at 20X for high quality gDNA (FF tissue), and greater than 95%
and 90% covered at 20X for medium and low quality FFPE samples, respectively. Using multiplex reference samples, we demonstrate detection of low frequency variants (at 1-5%) including SNVs, small indels, and RET and ROS1 translocations. As we will further show, FFPE integrity scores determined by qPCR and TapeStation (ddCq and DIN) enable researchers to choose the appropriate quantification method, PCR cycling, and sequencing depth for optimal sequencing coverage.

#5348 Reliable gene expression profiling from smaller and H&E stained clinical FFPE specimens using EdgeSeq platform. Zhenhao Qi,1 Lisa Wang,1 Keyur Desai,1 John Cogswell,1 Mark Stern,1 Byron Lawson,2 Bonnie LaFleur,1 Patrik Vitazka,1, 1BMS, Pennington, NJ; 2BMS, Lawrenceville, NJ; 1HTG Molecular, Tucson, AZ.

Background: The scarcity of biological specimens and suboptimal quality of collected material, make clinical biomarker studies challenging. The EdgeSeq system, a novel genomics analysis platform which combines quantitative nucleic acid purification assay (qNPA) technology with next-generation sequencing (NGS), has the potential to mitigate the aforementioned challenges. The technology allows for a limited sample input and delivers high quality gene expression profiling data from challenging material such as FFPE tissues. However, there is further unmet need toward even smaller tissue input or in taking advantage of archival stained FFPE slides to fill the data gap, specifically for studying tumor microenvironment in the oncology clinical trials Methods: We performed EdgeSeq analysis on six triplets of unstained, H&E stained and IHC stained slides from non-small cell lung cancer (NSCLC) patients tumor tissue using Oncology biomarker panel (OBP). Next, we extended the EdgeSeq OBP analysis to 44 pairs of unstained and H&E stained slides from NSCLC to confirm the good correlation. To assess feasibility of limited tissue input, we first optimized bead-to-DNA ratio from 2.5 to 1.0. Next, crude lysate prepared from small cell lung cancer (SCLC) FFPE tissue was 2-fold diluted from equivalent tissue area of 5mm² to 0.08 mm², and was subjected to EdgeSeq OBP profiling using the optimal bead-to-DNA ratio. Finally, correlation of gene expression profiles between stained and unstained pairs were calculated. Results: Within the six triplets of unstained, H&E stained and IHC stained slides, the gene expression profiles from H&E slides showed good correlation with the profiles from matched unstained FFPE. An extended NSCLC sample set of 44 pairs of unstained and H&E stained FFPE further confirmed overall good correlation with 84% (37 out of 44) pairs with Pearson correlation coefficients of 0.9 or higher. In assessing the limited tissue input, crude lysate was diluted to as little as equivalent tissue area of 0.08mm². The tissue area as small as 0.31 mm² resulted in Pearson correlation coefficients of 0.9 with 5mm² surface area input. Conclusion: We demonstrated feasibility of using EdgeSeq to generate reliable gene expression data from H&E stained FFPE tissues. In addition, we showed that EdgeSeq platform can be used to generate reliable expression data from crude FFPE tissue lysate equivalent to surface area as low as 0.31 mm² (size of larger laser microdissection tile or half of a tissue microarray core). The data show that EdgeSeq can be considered as a platform of choice to analyze specimens of suboptimal quality or of limited quantity and thus may help to fill the biomarker data gap in oncology clinical trials, including enabling research into patterns of interactions and mechanisms of response and resistance of immune cell engagement with tumor at the loco-regional level.

#5349 Accurate molecular profiling of sequence and copy number alterations from sub-nanogram FFPE DNA amounts. Paola Tononi, Alberto Ferrarini, Genny Buson, Valentina del Monaco, Giulio Bassi, Chiara Mangano, Claudio Forcato, Chiara Bolognesi, Francesca Fontana, Gianni Medoro, Nicola Manaresi. Menarini Silicon Biosystems, Castel Maggiore (BO), Italy.

Introduction: Formalin-fixed paraffin-embedded (FFPE) specimens represent an invaluable source of material for precision oncology. However, FFPE samples pose significant challenges for molecular assays, such as targeted Next-Generation Sequencing (NGS), because of the highly variable DNA quality and often limited sample size. Here we present a complete workflow from sample quality control to targeted library preparation to reliably detect sequence and copy number alterations (CNA) through targeted NGS from extremely low input FFPE samples. Materials & methods: Three FFPE specimens from patients with breast (BrCa) or pancreatic cancer, with DNA quality varying over a broad range and with low tumor cellularity (down to 10%) were selected for targeted NGS profiling. The DNA quality was determined using the DEPArray® FFPE QC Kit, a qPCR-based assay yielding a QC score defined as the ratio between the quantification of a 132 bp amplicon, corresponding approximately to the average length (116bp) of DEPArray Oncoseek amplicons, and a shorter amplicon of 54 bp. The QC scores of the 3 FFPE specimens ranged between 0.23 to 0.74.

#5350 A comprehensive and integrated approach to genomic and proteomic analysis of FFPE NSCLC tumor specimens. Douglas Hinerfield,1 Jennifer Mellen,7 Gary Geiss,7 Philippa Webster,7 Chris Merritt,1 Kristi Barker,1 Lokesh Demir,7 Brian Filanowski,1 Roberto Polakiewicz,1 Katherine Crosby1.

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This poster describes a novel fully integrated method for the multiplexed DNA, RNA and Protein analysis of formalin fixed paraffin embedded tumor specimens. Cancer progression is typically a result of aberrations in the molecular pathways that regulate cell growth. Identifying and understanding these changes is necessary for the detection of drug targets as well as the identification of novel biomarkers that can predict response to such drugs. Deep molecular profiling of clinical tumor samples is challenged by limited material and the compromised quality resulting from variable handling and the fixation processes that are commonly employed in the clinical laboratories. While multiplexed gene expression and mutational analysis of such samples has become well established, we describe a new method that enables multiplexed DNA, RNA and protein analysis from a single formalin fixed paraffin embedded (FFPE) tumor specimen on the NanoString nCounter system. We have adapted the sample preparation methods to enable the multi-analyte analysis of FFPE samples and have demonstrated concordance with the clinical gold standard immunohistochemistry (IHC) methodology. As a proof-of-concept, five FFPE non-small cell lung cancer (NSCLC) specimens were simultaneously stained with a panel of antibodies specific to a variety of cancer-relevant proteins, five of which were validated on serial sections by IHC using antibodies from Cell Signaling Technology. In addition to IHC, from the same FFPE casework, the copy number alterations of 770 cancer-related genes and >100 single nucleotide variants (SNVs) were also analyzed on the nCounter platform. By integrating the analysis of DNA SNV’s, as well as RNA and protein expression from single FFPE specimens, it is now possible to reveal the molecular mechanisms of tumor progression, uncover novel drug targets and identify biomarkers from precious specimens.

#5351 A simple yet powerful method to purify NGS compatible RNA from formalin fixed paraffin embedded tissues using FormaPure DNA. Jung H. Dong. Beckman Coulter, Inc., Indianapolis, IN.

Extensive archives of formalin-fixed, paraffin-embedded (FFPE) cancer tissues and next generation sequencing (NGS) capabilities provide rich resources for identifying and validating new cancer biomarkers. However, the ability to conduct FFPE NGS studies has been limited by poor nucleic acid purification methods that are generally difficult and cumbersome to perform, particularly with RNA. FormaPure DNA was recently launched to provide a simplified workflow to extract DNA from FFPE curls. Its nucleic acid binding chemistry employs Beckman Coulter’s proprietary SPRI (solid phase reversible immobilization) technology, which purifies nucleic acids without centrifugation or vacuum manifold. The SPRI technology ensures stronger binding and higher quality purification than other silica-coated bead methods that typically lead to lower quality results due to instances of nucleic acid shearing. Here, we present a modified FormaPure DNA workflow to purify RNA from FFPE samples, offering the flexibility to examine both genomic and transcriptomic roles in cancer.
b biomarkers studies. We show that our extractions yield significantly more RNA (up to 2.5 µg per FFPE curl) and take a fraction of the time to perform compared to other benchmarks. Furthermore, we demonstrate that purified RNA can be used to generate high quality NGS data. Lastly, our extraction method can be automated on a variety of Beckman Coulter’s Biomek liquid handling worksta-
tion for high-throughput FFPE RNA-NGS experiments: RNA from 96 FFPE samples can be purified for NGS in less than 5 hours with minimal hands-on time.

#5352 Optimization of library and enrichment process for RNASeq using RNA from formalin fixed paraffin embedded tissue. Ji Wen, Ying Shao, Ruth Taveosson, Yongji Li, David W. Ellison, Gang Wu, Jinghui Zhang, John B. Easton, St. Jude Children’s Research Hospital, Memphis, TN.

Large repositories of diagnostic formalin-fixed, paraffin-embedded (FFPE) tissue remain underutilized for transcriptome sequence analysis due to degra-
dation of the RNA from the fixation process, which makes the samples unsuit-
able for traditional mRNA sequencing (mRNASeq). The use of random primers to
generate cDNA, and biotinylated exon baits to enrich for coding regions allows for the generation of RNASeq data, but still yields data of variable quality, in part due to differences in FFPE sample processing. We performed a system-
atic analysis to determine which aspects of library construction and the enrich-
ment process can be optimized to provide the best RNASeq data from FFPE samples. The TruSeq RNA Access Library Prep Kit (Illumina) protocol was selected to evaluate optimization procedures for RNASeq from FFPE material. RNA was isolated from FFPE tissue using the Maxwell automated system (Pro-
meq). RNA libraries were also prepared with RNA extracted from frozen tissue of
the matching samples using the TruSeq V2 mRNASeq Library Prep Kit (Illum-
ina) for a comparison of exon coverage metrics. Samples derived from differ-
ent types of pediatric cancer were selected for evaluation, including 2 low-grade gliomas, 2 ependymomas, 9 melanomas and 1 Ewing sarcoma. All of the FF

tumor samples selected contained oncogenic gene fusions, previously identified by analysis of mRNASeq data. The parameters investigated included the initial
amount of RNA input (10ng-400ng), library amplification PCR cycle number (9-15 cycles), and the duration of time for exon probe hybridization (1.5 hours vs 16 hours). The quality of the data was based on the overall library complexity and exon coverage. The complexity of the sequencing was measured using duplication rate (DupRate) defined as a library inserts from different se-
quence reads mapping to the same location in the reference genome; while the
coverage was measured using the percentage of coding bases with coverage > = 20x (PCR20x). Using the RNA Access protocol, we found that the PCR20x metrics were comparable between the FFPE samples and matched FF mRNASeq controls under the non-optimized conditions of 20ng of RNA input, 1.6 hour hybridization, and 15 cycles of PCR for library amplification. However, optimiz-
ing the protocol by increasing the input of RNA to 400ng, reducing the PCR to
between 9-11 cycles, and extending the hybridization time to 16 hours increased
library diversity, reduced the DupRate from greater than 80% to less than 44%,
and increased coding region coverage PCR20x by at least 10%. All expected gene
fusions identified in the RNASeq data from the FF samples were also detected in
the FFPE samples. We have shown that increasing RNA input, reducing PCR
cycles and extending the duration of hybridization can significantly improve data
quality and maximize library diversity, allowing for greater utilization of archi-
val FFPE material for RNASeq.

#5353 High-throughput automation of the 10x Genomics Chromium™ workflow for linked-read whole exome sequencing and a targeted Lynch syn-
drome panel. Renata Pellegrino,1 Michael Benway,2 Paulina Kocjan,3 Andrew Price,3 Charly Kao,1 Brian A. Gerwe,2 Adrian Fehr,3 Fernanda Mafra,1 James Price,3 Charlly Kao,1 Brian A. Gerwe,2 Adrian Fehr,3 Fernanda Mafra,1 James Garifallou,1 Hakon Hakonarson1.1 The Children’s Hospital of Philadelphia, Philadel-
phia, PA; 2PerkinElmer, Hopkinton, MA; 310X Genomics, Inc., Pleasanton, CA

Traditional 2nd generation sequencing strategies have significantly reduced
the cost of sequencing the human genome and provide flexibility to query spe-
cific genome panels, the whole exome, or the whole genome. However, these meth-

odologies are based on short reads which limit their ability to phase/haplotyp
over long genomic distances, accurately map reads between highly homologous
regions (e.g., genes vs. pseudogenes), and robustly detect particular types of
structural variants (e.g., inversions and translocations). Advances in microflu-
lidics technology and precision reagent delivery allows long-range information to
be rescued and preserved through the use of the 10x Genomics Chromium platform. Each input DNA fragment (~40-200kb) is partitioned into a gel-bead in emulsion (GEM), and subsequent biochemistry generates mini-libraries of
NGS-ready molecules tagged with a barcode unique for each GEM. Thus, long-
range context is achieved by linking short reads sharing the same barcode, and

contiguity is established because they were derived from the same input frag-
ment. Importantly, the barcode mini-libraries are compatible with short-read
sequencers and can be implemented as an add-on to existing sequencing infra-
structures. Here we describe and demonstrate how the user-friendly and

uniquely-tuned liquid handling capabilities of the PerkinElmer Sciclone® NGS

assay deliver accurate and consistent results in the face of decreased quality and quantity of nucleic acids extracted from FFPE samples. Understanding the performance of a particular solid tumor profiling assay with FFPE tissue is critical, but with limited and non-renewable samples available to most assay-developers, the sample number used to understand this performance can be small. TruSight® Centaur® Assay from FFPE samples is a powerful tool for exome panel target capture usage. The benefit of automation is essential to the scale-up of high-throughput projects by removing manual variability and increasing effi-
ciency. This partnership offers a unique workflow solution that enables exome
and panel-based LinkedReader sequencing at scale. For Research Use Only. Not for use in diagnostic procedures.

#5354 Evaluation of quantity, quality and performance with the TruSight® Tumor 170 solid tumor profiling assay of nucleic acids extracted from formalin-fixed paraffin-embedded (FFPE) tissue sections. Jennifer S. LoCoco, Teng Linyi, Danny Chou, Xiao Chen, Byron Luo, Jennifer Sayre, Ashley Adams, Nas-

eem Ajili, Cody Chivers, Beena Murthy, Laurel Ball, Allan Castaneda, Katie Clark, Brian Grin, Anthony Daulo, Manh Do, Tingting Du, Sarah Dummm, Yomme Han, Michael Havtken, Chia-Ling Hsieh, Tingting Jiang, Suzanne Jo-
hanssen, Scott Lang, Rachel Liang, Jaime McLean, Yousef Nassiri, Austin Purdy, Jason Rostron, Jennifer Silhavy, June Snedecor, Natasha Talago, Li Teng, Kevin Wu, Chen Zhao, Clare Zlatkov, Ali Kurashiy, Karen Gutekunst, Sohela De Ro-
zieres, Matthew Friedenberg, Han-Yu Chuang, Anne C. Jager. Illumina, San Diego, CA

Solid tumor profiling assays need to deliver accurate and consistent results in
the face of decreased quality and quantity of nucleic acids extracted from FFPE samples. Understanding the performance of a particular solid tumor profiling assay with FFPE tissue is critical, but with limited and non-renewable samples available to most assay-developers, the sample number used to understand this performance can be small. TruSight® Tumor 170 is an Illumina-developed comprehensive solid tumor profiling panel targeting 170 genes using DNA and RNA from FFPE samples. In order to confirm the robustness of the assay with FFPE tissue, 2310 FFPE samples were brought in-house and evaluated. Quantity of both DNA and RNA extraction were determined by various methods, including AccuClear®, Qubit® and Quantifluor® fluorometric assays. Overall, >95% of the samples achieved the minimum concentrations required for the TruSight® Tumor 170 assay. As a surrogate for DNA quality, we measured the amplifica-
tion potential of the nucleic acid by assessing a SureSelect™ baits. This end-to-end automated workflow was used to generate trouble-Free library data on samples with unresolved structural rearrangements and targeted LinkReads in a Lynch syndrome gene, PMS2. LinkReads enable us to 1) fine-map structural rearrangements detected by karyotyping and 2) resolve variants in PMS2 versus those in its homologous pseudogene, PMS2CL, without invoking non-NGS methods such as MLPA or long-range PCR. The benefits of automation are essential to the scale-up of high-throughput projects by removing manual variability and increasing effi-
ciency. This partnership offers a unique workflow solution that enables exome
and panel-based LinkedReader sequencing at scale. For Research Use Only. Not for use in diagnostic procedures.

#5355 PANCeq - Development and validation of a targeted sequencing assay for detection of mutations and copy number changes in most recur-
mantly mutated driver genes. Tobias Meissner, Anu Amalrajha, Brandon Mi-

chael Young, Adam Mark, Cayce Conolly, Amanda Andrews, Casey Williams,
Brian Leyland-Jones. Avera Cancer Institute, La Jolla, CA

Next generation sequencing is becoming increasingly prevalent as a tool for
the identification of genetic variation to aid diagnosis and support therapy de-
cisions in routine clinical care. It has been shown that tumors are comprised of
subpopulations of cells with distinct genomic alterations, and hence tumor heter-
ogeneity is becoming an important factor in deciding treatment. Deep se-
quencing of tumor samples allows for the determination of number and proportions of low variant allele frequency tumor subclones. Currently, high costs for whole-exome deep sequencing (> 250X) limit its application in routine clinical care, and there is a need for more affordable targeted approaches. Here we present PAN Cancer Sequencing (PANCeq), a targeted deep sequencing panel for the detection of single nucleotide variants and copy number variants (CNVs) in currently mutated cancer driver genes. A platform-independent computational Docker-based pipeline for data analysis and automatic report generation was developed. PANCeq uses customized targeted enrichment, followed by next-generation sequencing of (FFPE) tumor and matched germline samples. We included 12 patient samples (breast, ovarian, kidney and prostate) as well as triplicates of 3 reference standards (HD200, HD753, HD733 - Horizon). These were sequenced on Illumina NextSeq on an average depth of 10000X. Sequencing data was aligned using BWA to hg19 human reference. Somatic variants and CNVs were identified using VarDict and CNVkit respectively. We mined TCGA data across 26 tumor types, and selected known mutations from the most recently mutated genes, yielding 2849 mutations across 467 genes. In addition, we included 79 pharmacogenomically relevant and 218 sample tracking SNPs as well as 80 cancer relevant CNVs (whole exom), and designed a custom panel based on their genomic locations, resulting in a panel size of 708kbk. To assess performance, we sequenced reference standards with known variants spiked-in at varying VAF (1 - 41.3%). Somatic variant calling was performed against a wild-type sample. For HD200, out of 21 covered mutations, 14 were correctly classified as germline and 1 as somatic. For HD733, out of 21 mutations, 3 were correctly identified as germline, 18 as somatic. For HD753, all 6 germline and somatic mutations were correctly identified. The 2 CNVs spiked-in were also correctly identified. In all cases we found a good agreement between observed and expected VAF. Somatic variant results for 12 patient samples were compared with variants reported by a private vendor. While we found good agreement at VAF > 20%, our panel detected on average 7 additional clinically relevant mutations at < 20%. We demonstrate that PANCeq is an accurate and sensitive method for timely identification of clinically actionable mutations and copy number variations across multiple cancer types.

Validation of a clinically actionable cancer core gene test for solid tumors facilitating targeted molecular therapy and immunotherapy, Lin Ma, Michael Hua, Steven Rivera, Anna Gerasimova, Quocinh Nguyen, Sirisha Sunkara, Robert Lagier, Alla Smolgovskiy, David Wolfson, Jared F. Taylor, Frederick Racke, Charles Strom, Andrew Grupe, Joseph Catanese, Feras Hantash, David Wolfson, Jared F. Taylor, Joseph Catanese, Feras Hantash.

Molecular profiling of tumor mutations has evolved in molecular targeted therapy for cancer patients. Additionally, tumor mutation load and DNA microsatellite instability (MSI) status can help predict patient’s response to immunotherapy. We analytically validated a gene test targeting clinically actionable cancer genes, including all 4 major tumor type-specific nucleotide variations (SNVs, insertions/deletions (INDELs); whole gene copy number variations (CNVs); and structural rearrangements (translocations) as well as the MSI status. This test interrogates all coding exons from 49 core cancer genes, intrans for a subset of genes selected for detection of prevalent gene rearrangements, and the TERT (telomerase reverse transcriptase) promoter region. MSI status is determined from a set of 5 intronic mono-nucleotide repeats collectively associated with microsatellite instability. Targeted DNA regions are captured by in-solution hybridization with complementary biotinylated RNA baits and sequenced on the Illumina NextSeq®600 platform. Paired format-less, parallel-embedded (FFPE) tumor samples and whole blood samples are analyzed simultaneously for the detection of SNV, INDEL, CNV, translocation and MSI status. FFPE samples can also be analyzed alone if the whole blood sample is not available. ELN is not available eliminating the reporting of MSI status. A minimum of 50 ng FFPE DNA and 100 ng of whole blood DNA are required for this test. A total of 123 FFPE, 2 FFPE FNA samples, 19 paired FFPE and whole blood paired samples covering lung cancer, colorectal cancer, melanoma and breast cancer were included in this validation study. Analytical validation of assay performance demonstrated that, on average, 700-fold read depth was achieved across all the tested genes with > 95% of these regions covered by a minimum of 300 unique reads. Analytical sensitivity was ≥ 5% mutation frequency for SNV and INDEL and ≥ 20% for translocation and CNV. Thirty-eight unique variants were confirmed between this test and orthogonal methodologies: 22 SNVs, 6 INDELS, 5 translocations, and 5 CNV. Fifty-eight out of 59 (98% concordance) paired FFPE/blood samples achieved the same VAF. Sequencing results in whole blood and tumor tissues were useful information to study cancer evolution. However, cost and amount of data may be overwhelming. Enrichment capture-based methods to design custom targeted gene panel have rapidly evolved in cancer genomics area. Custom targeted gene sequencing entails several advantages (i.e., better quality, ethical, developed and analytically validated a core cancer gene test employing NGS technology with demonstrated high analytical sensitivity and specificity. Coupled with clinical interpretation, this test will facilitate decision-making for molecular targeted therapy and immunotherapy.
affordable, technically suitable, personalized and reimbursable) for cancer patients over whole exome and whole genome sequencing. With this in mind, and to enable precision oncology in patients with solid tumors, we developed ICG100 2.0 panel, a hybridization capture-based next-generation sequencing assay for targeted sequencing of all exons and flanking introns of 162 commonly mutated cancer genes in formalin-fixed, paraffin-embedded (FFPE) tissues. To establish ICG100 2.0 panel, we compared three commercially available capture based technologies and evaluated reproducibility, sensitivity, specificity and the detection limit for low-frequency variants using internally developed bioinformatics pipeline. Cell lines, reference standards/synthetic DNA and solid tumor samples with the known genetic information was utilized in this analysis. Results were derived from MiSeq and NextSeq platforms and cross-compared on other platforms, including MassArray and ddPCR for establishing concordance and reproducibility. Intra and interrun replicates were utilized to assess the quality, precision and reproducibility of variant calling. Mean depth of coverage was observed at >300X with >99% sensitivity and specificity. These findings and observations will guide other clinical laboratories to establish new assay that require less DNA input, enzyme based fragmentation and reduced preparation time. While we show that the three capture based methods (after comparison) had an overall accuracy in SNP and CNV detection similar to each other with minor differences, we describe an approach to assess and establish the best assay from the clinical standpoint to guide treatment decisions and match cancer patients to the most appropriate clinical trials.


Targeted cancer therapy based on genomic alterations can be remarkably effective. Currently, cancer genome profiling using next generation sequencing (NGS) is routinely applied in cancer care to guide personalized treatment. The accuracy of this profiling directly impacts therapeutic choices and the outcomes of patient care. We previously showed that false positive variants are abundant and can account for a major fraction of identified somatic variants in publicly available datasets (doi: http://dx.doi.org/10.1101/070334). These false positive variants show signs of mutagenic DNA damage. We further demonstrated that enzymatic DNA repair increases sequencing accuracy by lowering damage-induced background noise. Therefore, enzymatic DNA repair has the potential to improve sequencing accuracy, avoiding incorrect somatic variant calls and consequently reducing incorrect diagnostic conclusions. In this study, we investigated whether enzymatic DNA repair introduces any bias to NGS libraries using analysis by droplet digital PCR (ddPCR) and deep sequencing. DNA Reference Standards containing multiple common cancer mutations (Horizon Discovery, Inc.) were spiked into formalin-fixed paraffin-embedded (FFPE) DNA isolated from tumor samples from different tissue types at defined frequencies (0.5-10% quantified by ddPCR). Genotyping of the FFPE DNA ensured that they were free of any of the spiked-in mutations. After DNA repair and library preparation, mutation frequencies were quantified by ddPCR, and compared to the mutation levels in input DNA and control libraries without repair. Deep sequencing of 151 cancer patients including these spike-ins showed no difference in mutation frequency for the spiked-in mutations between the control and repair groups. However, the number of false positive variant calls was reduced in the repair group. Our data demonstrates that DNA repair significantly increases sequencing accuracy without altering the frequency of actual mutations in tumor samples.

#5361 Predesigned gene content for rapid deployment of custom oncology panels. Andrew J. Barry,1 Amy Emerman,2 Sarah Bowman,1 Kruti Patel,1 Eileen Dimalanta,1 Scott Adams,1 Noa Henig,1 Fiona Stewart,1 Cynthia Hendrickson,2 Theodore Davis,3 Charles Elfe,2 Cynthia L. Hendrickson,1 1Directed Genomics, Ipswich, MA; 2New England Biolabs, Ipswich, MA; 3ThermoFisher Scientific, San Francisco, CA.

Efficient utilization of targeted gene panels for oncology research is challenged by the wide variation in gene constituents specific to a given study. While focused gene panels efficiently provide the necessary depth of coverage for low frequency variant detection, the high costs and design challenges associated with de novo panel design present challenges. The NEBNext Direct™ technology utilizes a novel approach to selectively enrich nucleic acid targets ranging from a single gene to several hundred genes, without sacrificing specificity. The approach rapidly hybridizes both strands of genomic DNA with biotinylated probes prior to streptavidin bead capture, enzymatic removal of off-target sequence, and conversion of captured molecules into sequencer-ready libraries. This results in a unique read coverage profile that results in uniform coverage across a given target. Unlike alternative hybridization methods, the approach does not necessitate upfront library preparation, and instead converts the captured molecules into dual-indexed illumina sequencer compatible libraries containing an 8 basepair sample ID and a 12bp Unique Molecule Index (UMI). The UMI individually tags each molecule prior to the final PCR amplification of the library, enabling identification of PCR duplicate molecules. The result is a 1-day protocol that enables the preparation of sequence-ready libraries from purified genomic DNA specific to the gene content included in the panel. We have designed and developed baits specific to the full exonic content of 450 genes associated with cancer. These are designed, balanced, and pooled on a per gene basis, and can be combined into customized panels, allowing rapid turnaround of specific custom gene subsets. Here, we present the ability to rapidly deploy focused gene panels across a variety of panel sizes and content, while maintaining high specificity, uniformity of coverage across target content, and sensitivity to detect nucleic acid variants from tumor samples.

#5362 Targeting BRCA1 and BRCA2 with NEBNext Direct™. Scott M. Adams,1 Kruti M. Patel,1 Amy B. Emerman,1 Sarah K. Bowman,1 Charles D. Elle,1 Noa Henig,1 Salvatore Russello,2 Andrew Barry Barry,2 Theodore Davis,3 Cynthia L. Hendrickson,1 1Directed Genomics, Ipswich, MA; 2New England Biolabs, Ipswich, MA.

The screening and detection of germline BRCA1 and BRCA2 mutations are critical for the effective management of patients with breast or ovarian cancer and for the identification of individuals with a high risk of developing these cancers. In addition, somatic detection of pathogenic variants in BRCA1/2 can influence treatment decisions due to the susceptibility of tumors with BRCA mutations to PARP inhibitors. Here we introduce the NEBNext Direct™ BRCA enrichment panel for the interrogation of BRCA1 and BRCA2 by Illumina sequencing. NEBNext Direct™ is a novel, hybridization-based method to selectively enrich nucleic acid targets ranging from a single gene to several hundred genes. This approach includes features such as the incorporation of unique molecule identifiers (UMIs) and the ability to capture degraded DNA, enabling accurate detection of low-frequency variants from formalin-compromised DNA and other challenging sample types. We applied the NEBNext Direct BRCA1/2 panel to frozen tissue and achieved a high specificity for the BRCA targets (96% of the sequenced reads mapped to BRCA1 and BRCA2 and 80% of the sequenced bases were within the targeted regions). In addition, the resulting libraries were highly uniform in coverage, with 91% of the targeted bases covered at a value of at least 50X of the mean depth of coverage and 100% of the bases covered at 20X of the mean or greater. Some variability in the specificity was observed with formalin-fixed, paraffin embedded (FFPE) samples, and this effect was dependent on the quality of the FFPE DNA. The UMI s were used to identify PCR duplicates prior to variant calling, as well as for error correction, enabling the accurate detection of low-frequency variants. The library preparation for all samples was completed in one day, and the entire process of library preparation, sequencing on the Illumina Miseq, and data analysis was completed in a total of two days. In summary, we demonstrate that application of the NEBNext Direct™ method to the enrichment of BRCA1 and BRCA2 provides a tractable approach for the rapid and highly sensitive analysis of these cancer-associated genes.


The tumor microenvironment, especially infiltrating T lymphocytes and inflammatory molecules, is believed to be highly relevant to the tumor’s sensitivity to cancer checkpoint blockade therapy. At the same time, the exact markers that are predictive of response for each therapeutic agent are still the subject of active investigations. To address the need for better understanding of the effect of different T cell subsets, antigen presentation, and tumor killing, gene expression profiling presents an attractive means to simultaneously evaluate the tumor microenvironment and cancer cells. In this study we compare the results and performance of the nCounter PanCancer Immune Profiling Panel and the Oncomine Immune Response Research Assay, both of which are designed to measure the expression of genes indicative of an immune response and potential immune-editing activities by tumor cells. The nCounter panel detects gene expression by counting unique probes that hybridize target mRNA, while the Oncomine panel employs NGS to sequence and count reads derived from the targets. While both panels are designed to work with FFPE samples, The nCounter panel expects 100 ng of unamplified RNA, while the Oncomine panel requires only 10 ng total RNA with its AmpliSeq technology. The two panels share 254 common genes, which constitute the basis for this comparison. Across
Combining enzymatic DNA fragmentation with NGS library construction results in high quality, high yield libraries. This assay leverages NGS technology to sequence and count reads derived from the original transcript. With an input requirement of 10 ng of total RNA, libraries were generated, templated on the Ion Chef™ and sequenced on the Ion S5™ System. Results showed that, despite small input amount, the expression profiles of FFPE and fresh frozen samples are highly correlated with an average correlation greater than 0.9. We selected 22 genes out of the panel to validate expression with qPCR using FFPE samples. These genes were selected to cover a range of low, medium, and high expressers per our NGS data. Again, we observed a strong correlation (R ~ 0.9) between NGS and qPCR data. Approximately 80% of the 40 samples show moderate to high expression of CD8+ T cell cytokines, IFNG and TNFa. We further found that the expression of CD8A and CD8B are highly correlated with CD4, suggesting the co-presence of both cytotoxic and helper T cells. High correlation among CD4, FOXP3, TGBF1, and IL2RA (CD25) also suggests that their expression can be used as markers to confirm the presence of Treg cells. We conducted a differential expression analysis between a group of samples (n=8) with high percentage of surrounding and infiltrating lymphocytes and another group (n=5) with low stromal content but devoid of infiltrating lymphocytes. Interestingly, we found a large number of genes which annotated as markers for infiltrating lymphocytes (CTSS, CXCR4, CD37, SRGN, FCER1G, NASP, and GZMA) are significantly upregulated in samples with high percentage of surrounding and infiltrating lymphocytes. In summary, this study highlights the robustness of using a targeted panel to understand the composition and regulatory mechanism of the TME and tumor immune response.

Combining enzymatic DNA fragmentation with NGS library construction results in high quality, high yield libraries. Fiana Stewart, Lynne Apone, Vaishnavi Panchapakesa, Karen Duggan, Jennifer Chen.

The development of a versatile and easy to use genome editing tool based on the bacterial CRISPR/Cas9 system has led to a vast increase in applications of genome editing in laboratories around the world. As an extremely powerful and versatile tool, it has facilitated important discoveries in both basic and translational research as well as in a potentially wide range of therapeutic applications. Nanobacterium gregoryi Argonauta (NgAgo) protein was described as a favorable alternative to Cas9 due to its simplicity and higher endonuclease specificity. Thus far, there are no formal reports documenting the success of NgAgo in other laboratory settings and cellular systems. Utilizing the identical cellular background and protocol that was made publicly available by the authors of the initial study, we were unable to reproduce the endonuclease function of NgAgo. These results do not support the role of NgAgo in gene editing experiments.

Comparative analysis of multiple copy number alteration tools in the detection of amplifications and deletions on both whole-exome and targeted NGS panel platforms. Selene M. Virk, Sean Michael Boyle, Ravi Alla, Jennifer Yen, Richard Chen.

Somatic copy number alterations, or CNAs, are frequent occurrences in the tumor landscape and can present in the form of focal alterations or as chromosome level events. Reliable detection of CNAs is crucial to understanding the impact of these events on a wide range of factors including tumor progression and treatment outcomes. The clinical use of NGS panels, and more recently exomes, continues to expand in cancer but the reliable detection of CNAs remains a challenge. A major factor contributing to the challenge is the availability of reliable tools that have been well tested and validated. We sought to characterize the sensitivity and specificity of multiple CNA detection tools with a focus on clinically relevant genes in NGS panel and whole-exome. Our approach utilized a variety of publicly available CNA tools to identify whole gene amplifications and deletions in genes identified in previously validated database of Somatic Mutations in Cancer (COSMIC) database. The tested CNA tools included two that supported both whole exome and panel NGS data, allowing cross platform in addition to cross tool comparisons. This work involved more than 20 well characterized cell lines derived from a broad spectrum of tumor
types, including breast, lung, melanoma, and prostate cancers. The cell lines were analyzed in both tumor/normal and tumor only (which utilizes a proxy normal)modes. Analyses of patient-matched and proxy-normal data were performed with both assays. Using our approach, we were able to identify clinically relevant CNAs on both platforms. The performance of the tools tested varied, with some tools performing better on either amplification or deletion events. These results represent a comprehensive comparison of recent copy number alteration tools and provides data that can be utilized to make the tools even more robust and reliable.

**MOLECULAR AND CELLULAR BIOLOGY / GENETICS:**

**Epigenetics 5**

**5369 Epigenetic, transcriptomic and ubiquitomic changes associated with BAP1 loss in uveal melanoma.** Stefan Kurtenbach, Jefim N. Kuznetsov, Matthew G. Field, Rohit Reddy, Margaret I. Sanchez, Christina L. Decatur, J William Harbour. University of Miami Miller School of Medicine, Miami, FL.

Uveal melanoma (UM) is the most common primary and aggressive ocular cancer. Up to 50% of the patients develop metastasis, which are notoriously resistant to all forms of therapy and despite medical treatment leads to death within a mean time of 5-7 months, with a mortality rate of over 90%. We previously described that gene expression profiling can be utilized to classify UM tumors into two basic categories, class 1 (low metastatic risk) and class 2 (high metastatic risk). We further described that inactivating mutations in the ubiquitin hydro-lase BAP1 are found in over 85% of class 2 tumors. BAP1 is involved in removing ubiquitin from specific proteins to regulate their function, like histone H2A, thereby regulating gene expression. Building on these findings, we have generated uveal melanoma and melanocyte cell lines that allow for the inducible knockdown of BAP1. First, we performed RNAseq on multiple cell lines before and after BAP1 knockdown and compare the results to gene expression of class 1 and class 2 tumors. We identified significant overlap in key genes linked to metastasis between the primary tumors, uveal melanoma and melanocyte cell lines. Using ChIP-seq to interrogate uveal melanoma cells deleted of BAP1, we identified changes in genome wide histone marks and RNA polymerase localization associated with BAP1. To gain further insight into non-histone deubiquitination targets of BAP1, we performed ubiquitination proteomic profiling using the Ubscan technology and identified a list of high probability BAP1 substrates. Taken together, these complementary genome-wide investigations provide a global picture of the cellular functions of BAP1 and they provide novel insights into the metastasis-promoting effect of BAP1 loss in UM.

**5370 Examination of DNA looping near oncongenes reveals variable patterns of epigenetic landscapes in cancer.** Monika Perez, Ty Johannes. University of Tulsa, Tulsa, OK.

**INTRODUCTION:** The compaction of chromatin in the nucleus into hierarchical (3D) structure plays a key role in the regulation of gene expression. Advancements in high-throughput chromatin conformation capture assays have enabled the determination of this hierarchical structure in a variety of cell states, including cancer. Of note, recent studies have implicated pathogenic alterations in 3D topology of the genome with the activation of proto-oncogenes in a multitude of cancers.

**METHODS:** To characterize the topological changes associated with different cancer types, we examine publicly available epigenetic profiles from the ENCODE Project, including 3D data from Chromatin Interaction Analysis by Paired-End Tag Sequencing (ChIA-PET) from breast cancer and leukemia cell lines. Integrating the chromatin topology data with transcription factor binding, DNAase accessibility, and histone modification data, we uncover variable epigenetic landscapes associated with differentially expressed oncoproteins in these cancer cell lines. RESULTS: We provide evidence of topological variability influencing differentially expressed oncoproteins and tie these observations to other epigenetic changes. In particular, we show insulinated-neighbored mediated by CTFC looping partition segments of the genome containing active oncoproteins away from repressive markers like H3K4me3. Additionally, we show that variation in topology differentially localizes H3K27ac in distal regulatory regions to promoters of oncoproteins, providing a means for gene activation in non-coding regions. CONCLUSIONS: Variable patterns of chromatin topology provide a unique signature and mechanism of oncogenesis for cancerous phenotypes. We use publically available data to show mechanisms of activity associated with chromatin topology variation.

**5371 Effect of DNA methyltransferase inhibitor 5-azacytidine on 3D chromatin structure measured by Hi-C.** Yuchen Vincent Bai, John Whitaker, Emanuele Palescandolo, Vinod Krishna, Vipul Bhargava, Satya Saxena, Xiang Yao, David Palyokey, Kurt Bachman, Jeff, Spring House, PA; Jeff, Beerse, Belgium.

The study aimed at targeting epigenetic modifiers such as the DNA methyltransferase (DNMT) inhibitor, 5-azacytidine (Aza), are used in treating hematologic malignancies and also show promising results in subsets of solid tumors. The molecular mechanisms for these results, however, are not fully understood. Nonetheless, a number of clinical studies using combinations of epigenetic therapies plus chemo or immunotherapy to enhance the tumor response are ongoing. HCT-116, a colon cancer cell line genetically defined by microsatellite instability (MSI), resulting in a highly mutated, yet primarily diploid genome, has been routinely used to study epigenetic mechanisms and response to DNMT inhibition. In a previous study, we investigated a combination of data types including DNA methylation, DNA accessibility, histone modifications and transcriptomic data that revealed strong correlations between epigenomic changes and gene expression following Aza treatment. However, for some genes, we could not predict expression changes using these unidimensional, region-specific analysis methods. As others have demonstrated, genes do not work as single, isolated units, but rather interact with distal regulatory elements. These regulatory elements, often several Mb away can control gene expression through physical interactions such as bending and looping. We reason that in addition to direct regulation of the immediate regulatory elements, the genome-wide effects of Aza treatment can affect long-range chromosomal communications. In order to further understand the chromatin remodeling effect of Aza treatment, we performed high resolution genome-wide chromosome conformation capture (Hi-C) followed by NGS. HCT-116 cells were treated with DMSO or 1 μM Aza for 42 and 96 hours. Hi-C and RNA-Seq data was analyzed and integrated with other datasets. The results showed that Aza treatment alters topologically associating domains (TAD), with the formation of new TAD boundaries and disappearance of others. The genome was then separated into 25K base pair consecutive bins, with each bin marked as type A (active) or type B (inactive). We observed bin switching following Aza treatment and differential loop formations (e.g. promoter-enhancer). Bins that switched from B to A at both time points were found enriched in genes that are related to acute phase response, interferon pathway, and cancer including PIK3CB, DDX58, CD274 and CDRN2A. Our findings using Hi-C were found to be in agreement with previous results using alternative experimental methods, which identified Aza and CTCF as the top upstream regulators of these genes. Our data also suggests that overall changes in 3D chromatin activities measured by Hi-C could be a better predictor of transcriptional regulation compared to H2K27me3 and H3K4me3 alone, particularly in situations where there are no significant changes in those marks but where changes in gene expression exist.
#5373 3D genome architecture changes during cancer cell migration and metastasis. Rachel Patton McCord, Rosela Golloshi. University of Tennessee, Knoxville, TN.

Metastasis, the most deadly part of cancer, results from cancer cell migration and invasion from a tumor into surrounding tissues. During this migration, the cell often undergoes deformations that are crucial for successful invasion. Clear squeezing is a rate-limiting step in migration, and the deformations can even rupture the nucleus. The organization of the genome inside the nucleus into loops, domains, and territories is important for proper gene regulation, DNA replication, and genome repair. However, little is known about the characteristics of nuclear organization that might allow for the nuclear deformation of metastatic cells.

We are using Chromatin Conformation Capture (Hi-C) and microscopy techniques to study genome organization during cancer cell migration. Using photoconvertible fluorescent Dendra2-H4 we drew and monitor patterns on nuclei of cells migrating through dense collagen matrices. Transwell filters, or microfluidic channels. Our data show changes in these patterns, indicating spatial reorganization of the genome during migration. Previous work has suggested that cancer cell migration requires global condensation of the genome. Some cancer chemotherapeutics act on epigenetic marks to decondense chromatin. We are testing whether such chromatin decondensation affects the physical deformability of the nucleus and thus effective migration. We have used Transwell and wound healing migration assays to monitor migration of cells treated with histone deactylase inhibitors and histone methlytransferase inhibitors in DHA and DNA methyltransferase inhibitors (DNMTi). Short chain fatty acids, have been proven to be effective HDACi's, suggesting a possible role for other fatty acids in epigenetic regulation. Research has shown that several omega-3 polyunsaturated fatty acids (PUFAs), specifically docosahexaenoic acid (DHA; C22:6, n-3) and eicosapentaenoic acid (EPA; C20:5, n-3), inhibit tumorigenesis whereas the omega-6 PUFAs linoleic acid (LA; C18:2, n-6) promotes neoplastic growth; supporting the association of elevated cancer incidence with n-6 PUFA dietary intake. We initiated studies to evaluate the impact of omega-3 fatty acids on tumor epithogenic pathways. Therefore we monitored epigenetic alterations in MDA-MB-231 TNBC cells in response to both omega-3 and omega-6 PUFAs in a time and dose-dependent manner. DHA, EPA, and arachidonic acid (AA; 20:5, n-6) elicit apoptosis in a similar manner, indicating that initiating apoptosis is not omega-6 or omega-3 specific. We now show that PUFA incorporation and the resulting attenuated epigenetic changes occur on a time scale proceeding apoptotic induction, indicating that PUFAs may be useful as an epigenetically-targeted cancer therapeutic agent or adjuvant therapy.

#5374 Polyunsaturated fatty acids (PUFAs) alter the epigenetic landscape of human breast cancer cells. Ronald S. Pardini, Amy M. Chattin, Mykelti O’Brien, Ronald S. Pardini. Univ. of Nevada, Reno, NV.

Breast cancer is the second leading cause of cancer-related deaths in US women; 10-20% of which are triple-negative breast cancer (TNBC) subtypes and have the poorest short-term prognosis. This is probably due to an epithelial to mesenchymal transition (EMT) that tumors undergo, de-differentiating into cancer stem cells. Epigenetic reprogramming is proposed to be required for malignant transformation and invasion, particularly in cells undergoing EMT transitions such as TNBC. Therefore, altering the epigenetic landscape in metastatic TNBC lines would provide researchers with a unique opportunity to sensitize tumors to treatment and induce apoptosis. Epigenetic research chiefly focuses on the epigenetic alterations that are characteristic of human diseases and on the potential of DNA methyltransferase inhibitors (DNMTi) to sensitize cancer cells to chemotherapy. In this study, we tested the hypothesis that polyunsaturated fatty acids (PUFAs) would alter the epigenetic landscape of breast cancer cells. We chose three common PUFAs: DHA, EPA, and AA. The incorporation of PUFAs into the cell membrane increased with increasing PUFAs and was concentration-dependent. Interestingly, we found that DHA and EPA caused a decrease in histone H3 acetylation and an increase in histone H3 methylation. Although this was consistent across the Human Mammary Epigenome Chip (HI-C) and microscopy techniques to study genome organization during cancer cell migration. Transwell filters, or microfluidic channels. Our data show changes in these patterns, indicating spatial reorganization of the genome during migration. Previous work has suggested that cancer cell migration requires global condensation of the genome. Some cancer chemotherapeutics act on epigenetic marks to decondense chromatin. We are testing whether such chromatin decondensation affects the physical deformability of the nucleus and thus effective migration. We have used Transwell and wound healing migration assays to monitor migration of cells treated with histone deactylase inhibitors and histone methlytransferase inhibitors (DNMTi). Short chain fatty acids, have been proven to be effective HDACi's, suggesting a possible role for other fatty acids in epigenetic regulation. Research has shown that several omega-3 polyunsaturated fatty acids (PUFAs), specifically docosahexaenoic acid (DHA; C22:6, n-3) and eicosapentaenoic acid (EPA; C20:5, n-3), inhibit tumorigenesis whereas the omega-6 PUFAs linoleic acid (LA; C18:2, n-6) promotes neoplastic growth; supporting the association of elevated cancer incidence with n-6 PUFA dietary intake. We initiated studies to evaluate the impact of omega-3 fatty acids on tumor epithogenic pathways. Therefore we monitored epigenetic alterations in MDA-MB-231 TNBC cells in response to both omega-3 and omega-6 PUFAs in a time and dose-dependent manner. DHA, EPA, and arachidonic acid (AA; 20:5, n-6) elicit apoptosis in a similar manner, indicating that initiating apoptosis is not omega-6 or omega-3 specific. We now show that PUFA incorporation and the resulting attenuated epigenetic changes occur on a time scale proceeding apoptotic induction, indicating that PUFAs may be useful as an epigenetically-targeted cancer therapeutic agent or adjuvant therapy.

#5375 Oncogenic BRAF mutation induces widespread DNA hypermethylation in a murine model for human serrated colorectal neoplasia. Vivki Whitehall1, Catherine Bond1, Cheng Liu1, Futoshi Kawamata1, Diane McKeone1, Saara Jamieson1, Sally Pearson1, Susan Woods2, Tasmin Lannigan3, Lochlan Fennell4, Winnie Fernando1, Mark Bettington1, Daniel Worthingley5, Barrie Towner6, JQMR Berghefer Medical Research Institute, Herston, Australia; 1University of Adelaide, Herston, Australia; 2University of Adelaide, Adelaide, Australia.

BACKGROUND: The serrated colorectal neoplasia pathway describes the progression of morphologically serrated polyps to cancer and accounts for approximately one third of all colorectal cancer cases. Human serrated polyps are characterized by the presence of the BRAF mutation in the oncogene and widespread DNA methylation changes termed the CpG Island Methylator Phenotype. A causative versus synergistic relationship between BRAF mutation and this methylator phenotype has not been determined. We aimed to address this by developing a murine model for serrated neoplasia driven by BRAF mutation.

METHODS: BRAF+/− conditionally active mice were crossed with intestine-specific, inducible Villin-CreERT2 mice to direct the BRAF mutation to the intestine at 2 weeks of age. The proximal ileum or proximal colon were sampled at defined time points including 10 days, 10 weeks, 5 months, 8 months, 10 months, 12 months and 14 months. Macrophscopic lesions larger than 10 mm were bisected for molecular and histological assessment. The entire remaining intestine was fixed and examined histologically. DNA methylation was investigated for 94 genes known to be methylated in colorectal cancer using Epitect Methyl II Complete PCR Arrays (Qiagen).

RESULTS: BRAF mutant mice displayed histologic changes analogous to the human serrated neoplasia pathway. Extensive intestinal hyperplasia developed by 10 days post induction of the BRAF mutation. By 10 weeks, 50% mice had developed areas of crypt dilatation reminiscent of human sessile serrated adenomas. By 8 months, the majority of mice had murine serrated adenomas with dysplasia and invasive cancer developed in 40% of mice by 14 months, one of which metastasised to the liver. Compared to age-matched control mice, BRAF mutant mice showed significant, gene-specific increases in DNA methylation from 5 months (p<0.0001).

CONCLUSIONS: Using an in vivo model we observed the temporal accumulation of DNA methylation changes in hyperplastic epithelium in direct response to mutation of the BRAF oncogene. This murine model morphologically and molecularly recapitulates the human serrated neoplasia pathway and establishes a causative role for BRAF mutation in establishing a methylator phenotype.

#5376 Requirement of TET2 in MLL-AF9 mediated leukemogenesis. R. Nathan, Golam Mohi. SUNY Upstate Medical University, Syracuse, NY.

TET methylcytosine dioxygenase 2 (TET2) catalyzes the conversion of 5-methylcytosine to 5-hydroxymethylcytosine resulting in DNA demethylation. Loss-of-function mutations or deletions of TET2 have been found in several cancers. TET2 is a member of the TET family of 5-methylcytosine dioxygenases, which also includes TET1 and TET3. TET family members play important roles in hematopoietic lineage specification. The TET methylcytosine dioxygenase 2 (TET2) catalyzes the conversion of 5-methylcytosine to 5-hydroxymethylcytosine resulting in DNA demethylation. Loss-of-function mutations or deletions of TET2 have been found in several cancers. TET2 is a member of the TET family of 5-methylcytosine dioxygenases, which also includes TET1 and TET3. TET family members play important roles in hematopoietic lineage specification. TET methylcytosine dioxygenase 2 (TET2) catalyzes the conversion of 5-methylcytosine to 5-hydroxymethylcytosine resulting in DNA demethylation. Loss-of-function mutations or deletions of TET2 have been found in several cancers. TET2 is a member of the TET family of 5-methylcytosine dioxygenases, which also includes TET1 and TET3. TET family members play important roles in hematopoietic lineage specification. TET methylcytosine dioxygenase 2 (TET2) catalyzes the conversion of 5-methylcytosine to 5-hydroxymethylcytosine resulting in DNA demethylation. Loss-of-function mutations or deletions of TET2 have been found in several cancers. TET2 is a member of the TET family of 5-methylcytosine dioxygenases, which also includes TET1 and TET3. TET family members play important roles in hematopoietic lineage specification.
genitor colony formation in the BM. In addition, deletion of TET2 resulted in increased overall survival in mice expressing MLL-AF9. Together, these data indicate that TET2 is required for leukemic transformation mediated by MLL-AF9. Our results suggest a novel tumor promoter function for TET2 in MLL rearranged leukemia.


Purpose: While the impact of DNA methylation at the promoter region as a repressive influence is well-known, the vast majority of DNA methylation actually occurs outside of the promoter region. The function of intragenic DNA methylation remains incompletely understood. Here, we investigate how intragenic DNA methylation impacts gene expression, and how these modifications differ between normal and corresponding cancer tissues. Methods: A pan-cancer analysis of gene expression array data was integrated with Illumina 450k methylation array data across ten tumor types to produce correlation values between DNA methylation and gene expression at distinct loci on a genome-wide basis. The probes exhibiting a significant correlation between gene expression and DNA methylation were selected for further analysis. Results: We uncovered robust positive correlations between gene expression and 3' methylation in 3,200 genes, with 590 genes in bladder cancer, and 258 genes in squamous cell lung carcinoma displaying a correlation coefficient of >0.5. Furthermore, we observed 44 genes with a correlation coefficient of >0.7 in at least two tissue types. The majority of genes with the strongest correlations are transcription factors known to play roles in differentiation and development, with an enrichment of zinc finger and homeobox-containing genes. Further analysis of these genes exhibit divergent 3' methylation when comparing normal and tumor tissues. Importantly, the extent of 3’ methylation of these genes is associated with patient overall survival in 5 of the 10 tumor types analyzed, strongly suggesting that this process plays a role in cancer pathogenesis. Conclusion: DNA methylation of the 3’ region is a functionally and clinically relevant epigenetic modification, and may serve as a novel target for inhibiting tumorigenesis and tumor progression.

#5378 Genes involved in development and differentiation are commonly methylated in cancers derived from multiple organs: A single-institutional methylehyme analysis using 1007 tissue specimens. Kentaro Ohara,1 Eri Arai,1 Yoriko Takahashi,2 Nanako Ito,1 Ayako Shibuya,1 Koji Tsuta,1 Ryoji Kushima,1 Hitoshi Tsuda,4 Hidenori Ojima,2 Hiroyuki Fujimoto,4 Shun-ichi Watanabe,2 Hitoshi Katai,1 Takayuki Kinoshita,4 Tatsuhiro Shibata,3 Takashi Kohno,3 Yae Kanai1, 4Keio University School of Medicine, Tokyo, Japan; 5Mitsui Knowledge Industry Co., Ltd., Tokyo, Japan; 6National Cancer Center Research Institute, Tokyo, Japan; 7National Cancer Center Hospital, Tokyo, Japan.

Aim: The aim of this study was to clarify the significance of DNA methylation alterations shared by cancers derived from multiple organs. Background: Little is known about DNA methylation alterations during carcinogenesis shared by various organs. In this single-institutional study, consistency of sample quality, diagnostic criteria, and technical platforms was prioritized for providing an overall view of DNA methylation profiles of cancers arising in multiple organs. Methods: We analyzed single-institutional methylation data by Infinium HumanMethylation27 or HumanMethylation450 BeadChip (Illumina) for 1,007 samples of non-cancerous tissue (N) and corresponding cancerous tissue (T) obtained from the lung, stomach, kidney, breast, and liver. Results: Principal component analysis revealed that N samples of each organ showed distinct DNA methylation profiles, DNA methylation profiles of N samples of each organ were inherited by the corresponding T samples and DNA methylation profiles of T samples being more similar to those of N samples in the same organ than those of T samples in other organs. We identified 2,636, 2,209, 1,915, 2,914 and 5,665 probes that were aberrantly methylated in lung adenocarcinomas, gastric adenocarcinomas, clear cell renal cell cancer, breast cancers (e.g. invasive ductal carcinomas, ductal carcinomas in situ and invasive lobular carcinomas) and hepatocellular carcinomas, respectively, in comparison with the corresponding N samples. When we examined pairs of organs, only 6.9% (between stomach cancers and kidney cancers) to 35.4% (between lung cancers and breast cancers) of aberrantly methylated probes were shared between cancers of any two organs, indicating that the majority of DNA methylation diverge among multiple organs. In contrast to such organ and/or carcinogenetic factor-specificity of DNA methylation profiles, when compared to the corresponding N samples, 231 genes commonly showed DNA hypermethylation in T samples in four or more organs. Gene ontology enrichment analysis showed that such commonly methylated genes were enriched among “transcriptional factors” participating in development and/or differentiation, which reportedly show bivalent histone modification in embryonic stem cells. Pyrosequencing and quantitative reverse transcription-PCR revealed an inverse correlation between DNA methylation levels and mRNA expression levels of representative candidate methylated genes such as ALX1, ATP5A1, and ABCA1, in tissue samples. Conclusion: These data suggest that disruption of the differentiated state of precancerous cells via alterations of expression, independent of differences in organs and/or carcinogenetic factors, may be a common feature of DNA methylation alterations during carcinogenesis in multiple organs.

#5379 TET2 loss and the lymphoma-associated RHOA mutation cooperate to disrupt CD4+ T cell function. Shengqiang Zhang,1 Jia Li,2 Haiyan Yang,2 Wei Han,1 Jixiang Zhang,3 Minjing Lee,1 Yubin Zhou,1 Dqinguan Sun,1 Yun Huang,1,2 Texas A&M University, Houston, TX; 3Zhejiang Cancer Department, Houston, TX.

Peripheral T cell lymphomas (PTCLs) are a group of rare but aggressive lymphomas derived from mature T cells or natural killer cells with a dismal prognosis. Current treatments for PTCLs are ineffective with high relapse rates, largely owing to the lack of targeted therapeutics and a deep understanding of the molecular etiology. Recent exome sequencing has unveiled the frequent co-existing somatic mutations in RHOA (G17V) and TET2 in PTCLs, but not in other types of hematological malignancies. Most notably, mutations in both TET2 and RHOA are frequently observed (~60-70%) in angioimmunoblastic T-cell lymphoma (AITL), which is among the most common subtypes of PTCLs with a median overall survival of approximately 1.5 years. Patients with AITL are often associated with autoimmune manifestations, but the mechanistic underpinnings remain unresolved. To examine whether TET2 loss and RhoA(G17V) alter mature T cell function, we performed adoptive T cell transfer experiments with WT or Tet2 knockout transgenic mice. Using this strategy, we generated four groups of recipient mice transferred with T cells as follows: WT, RhoA(G17V), Tet2-/-, or Tet2-/- RhoA(G17V). Among these four groups, only recipient mice transferred with double mutant T cells displayed severe inflammatory-like phenotypes that ultimately led to early lethality in mice. Moribund mice transferred with Tet2-/- RhoA(G17V) T cells showed substantial weight loss, severe skin ulcer on tail/paw/ear accompanied with pruritus, and lymphomagely as typically seen in AITL patients. Histopathological analysis on major organs derived from the Tet2-/- RhoA(G17V) group indicated severe infiltration of both T and B lymphocytes in major organs, as well as a pronounced increase in the numbers of follicles and activated germinal centers in lymph nodes. Further analysis indicates that Tet2 loss and RhoA(G17V) mutation cooperated together to exclusively promote CD4+ T cell proliferation and survival, and exert no adverse effects on CD8+ T cells, which recapitulates the mutation spectrum detected in AITL patients, i.e., RhoA(G17V) is primarily detected in CD4+ T cells but not other T cell subsets. The functional consequence of Tet2 loss and RhoA(G17V) expression in CD4+ T cells is characterized by an imbalance between the effector and regulatory T cells, which may account for the overt immunoinflammatory phenotypes seen in mouse models. The transcriptome analysis further revealed a pronounced remodeling of the immune signaling network that points to the aberrant transcription of several key inflammatory factors. The present study demonstrated a previously-unappreciated cooperativity between Tet2 loss and RhoA(G17V) mutation in disrupting mature CD4+ T cells function, which is made through the synergy between epigenetic and GTPase signaling pathways. The results obtained from this study provide an additional basis for future development of new diagnosis, prognosis and therapy for PTCL patients.

#5380 Inactivation of endogenous genes in cancer cells using targeted promoter DNA methylation via CRISPR-DNMT3a fusion protein. Simon D. Spivack. Albert Einstein College of Medicine, Bronx, NY.

Background: Developing technologies for precise manipulation of individual DNA methylation loci is an attractive challenge in cancer and lung biology, because aberrant expression via hyper- or hypo-methylation is so common. Projects such as ENCODE and the Roadmap Epigenomics Project have identified thousands of epigenetic marks from human genome. However, the functional evaluation of these marks has been largely limited to determining their associations with gene expression. Technologies for targeting manipulation of epigenetic marks would allow direct experimental testing of the impact of DNA methylation at specific residues, mechanisms of gene regulation, and potential use as interventions, for example to silence constitutively active oncogenes. Methods: Here, we developed a CRISPR Cas9-based tool for specific DNA methylation in which the catalytic domain of DNMT3a (DNMT3a-Cd) is fused to the carboxy-terminus of Cas9 D10A-H840A mutant (dCas9). Both construct promoter strength and transfection strategies were optimized. Results: We demonstrated...
targeted and consistent CpG methylation in 30–100bp regions downstream of the PAM (binding) site of the gRNA guided fusion protein in HEK293 cells. The multiple guide RNAs could target the dCas9-DNMT3A to multiple sites consistently. DNA methylation activity was specific for the targeted region and was heritable across cell divisions. We also found directed promoter DNA methylat-

#5381 A highly sensitive method for noninvasive cancer profiling through targeted methylation sequencing of circulating cell-free DNA. Li Liu,1 Jona-
than M. Young,1 Raakhee Vijayaraghavan,1 Ruoyu Zhang,1 Helen J. Huang,2 Toshinori Hinoue, Hui Shen,1 Neeraj Salathia,1 Marina Bibikova,1 Richard Shen,1 Karen Guitekunst,2 Peter W. Laird,1 Filip Januk,1 Jian-Bing Fan,1 Illumina, San Diego, CA; 3MD Anderson Cancer Center, Houston, TX; 2Van Andel Research Institute, Grand Rapids, MI. Liquid biopsies, by analyzing circulating cell-free DNA (cfDNA), have emerged as a promising tool for noninvasive cancer diagnostics and monitoring. Compared to the limited number of scattered DNA mutations in cancer pa-

#5383 Non-invasive diagnosis of early-stage lung cancer via targeted high-
throughput DNA methylation sequencing of circulating tumor DNA (ctDNA). Xu Yu Cai, Yangbin Gao, Hui Shen, Peter Laird, Jian-bing Fan, Weihong Xu, Wenhua Liang, Jianxing He. AnchorDX Medical Co. Ltd., Guangzhou, China. Current state-of-the-art lung cancer early screening involves using low-dose CT scan to identify lung nodules smaller than 3cm in diameter. However, it’s still a clinical dilemma to differentiate between malignant and benign nodules. We took the approach of methylation profiling by high-throughput bisulfite DNA sequencing in tissue samples to identify specific methylation signatures. We learned methylation patterns that differentiate malignant vs. benign lesions from tissue samples by in-depth data mining, and then used pattern matching to classify plasma samples. Given the usual low amount of ctDNA in plasma, we also developed an ultra-sensitive library preparation method to perform target-

#5384 Aberrant methylation of ANK1, the host gene for miR-486, distingu-
sishes lung tumors by histology and smoking status. Mathewos Tessema,1 Christin M. Yingling,1 Maria A. Picchi,1 Guodong Wu,1 Tyron Ryba,2 Young Lin,3 Steven A. Belinsky3.1 Lovelace Respiratory Research Institute, Albuquerque, NM; 2New College of Florida, Sarasota, FL. MicroRNAs (miRs) regulate many fundamental biological processes primarily through inhibiting the transcription and/or translation of their target genes. MiR-486 is an intragenic miR located within ANK1, a gene that encodes for the adapter protein ankyrin-1. Although the tumor suppressor role of miR-486-5p and its down-regulation in non-small cell lung cancer (NSCLC) is well estab-

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Epigenetics 5
Epigenetic modifications, particularly DNA methylation in selected gene promoters is a pivotal role in the development of colorectal cancer. DNA methylation is considered as one of the most important epigenetic mechanisms and it is catalyzed by DNA methyltransferases (DNMTs). DNMT1 abundance has been frequently seen in colorectal cancers but the reasons are not well understood. We are interested in the effect of chemotherapeutics used in treatment of colorectal cancer on expression of DNMT1 and this effect is achieved over which signalling pathway. In this study, GS/K38, IPW2 for β-catenin) and chemotherapeutics (oxaliplatin, fluorouracil, irinotecan) were detected by WST1. DNMT1 expression level was determined by real-time PCR, and protein levels of GS/K38, pGS/K38(Ber9), Akt1, pAkt1(Ser473), β-catenin, pβ-catenin(Ser675) and DNMT1 were detected. We expected to increase the protein level of DNMT1 expression with coordinate transcriptional change via β-catenin pathway. Fluorouracil and irinotecan decreased DNMT1 expression at both transcriptional and translational levels but not oxaliplatin. Oxaliplatin increased DNMT1 expression at mRNA and protein levels. This effect is achieved by specific phosphorylation of β-catenin protein. The results revealed that use of some chemotherapeutic, particularly oxaliplatin, with specific inhibitors (such as β-catenin inhibitor) in combination led to a reduced DNMT1 expression. Our findings may offer a new approach for determining the molecular effects of β-catenin signal pathway on DNMT1. This may allow us to identify new molecular targets for the treatment of colorectal cancers. However, the results revealed that some chemotherapeutics may contribute the aberration of DNA methylation.


Background: Droplet digital PCR (ddPCR) is a highly sensitive method for quantification of nucleic acids, with great potential for detection of biomarkers present in low quantities. As for DNA methylation analyses using ddPCR, there is no consensus on whether or not a control should be included. The aim of the present study was to: 1) demonstrate that use of a control is essential in such analyses, and 2) develop a robust control with better performance than the existing alternatives. Results and methods: A 4-gene control panel was developed in our lab, and its performance compared to the existing alternatives ACTB and C-LESS-C1. This was done through analyzing the methylation level of four target genes in DNA from 34 colon cancer cell lines. All ddPCRs were performed using the QX200™ PCR platform (BioRad). Details will be presented. Conclusions: In summary, use of a control is essential for robust and consistent ddPCR based DNA methylation, and can prevent erroneous interpretation of technical artifacts.

#5388 Molecular profiling of protein methyltransferases in prostate cancer and their clinical significance, YanJian Jiang, Lixin Liu, David Weinfeld, Wengi Shaan, Huimei Yu, Xuhui Guo, Zhe Yang, Zeng-Quan Yang, Wayne State Univ. School of Medicine, Detroit, MI.

Prostate cancer is one of the most commonly diagnosed cancers among men worldwide. Most deaths of prostate cancer patients occur after the disease has progressed to castration-resistant prostate cancer, accompanied by metastatic dissemination and resistance to androgen-deprivation therapy. Therefore, an urgent need exists to identify druggable targets that will improve the treatment of this deadly and devastating disease. Epigenetic alteration has been identified as one the major causes of prostate cancer. Protein methyltransferases (PMTs), a large class of epigenetic enzymes that add one or more methyl groups to lysine (K) and arginine (R) residues on histones and non-histone proteins, play critical roles in chromatin function, transcriptional regulation, genomic stability, DNA repair and RNA metabolism. Although dysregulation of several PMTs has been reported in prostate cancers, there has been no systematic analysis of genomic anomalies and expression of PMTs in prostate cancer. In addition, the clinical relevance of alteration of each PMT in prostate cancer has yet to be fully explored. Here, we performed an integrated genomic and transcriptomic analysis of 68 PMT genes in prostate cancer (TCGA dataset) and identified associations among recurrent copy number alterations, gene expressions, clinicopathological features, and survival of patients. In addition to well-known EZH2 and WHSC1, we identified several additional PTMs, including SETDB1, SETD6 and PRB1D2, which were significantly overexpressed in high-grade and high-stage prostate cancer. We also found that SETDB1 overexpression was significantly associated with biochemical recurrence of prostate cancer patients. To assess the contribution of endogenous SETDB1 overexpression to prostate cancer growth and progression, we examined the effects of knock-
ing down SETDB1 in two prostate cancer cell lines CWR22Rv1 and DU-145. SETDB1 knockdown dramatically reduced CWR22Rv1 and DU-145 cell growth compared with the non-silencing control. We also identified all SETDB1 binding sites across the human genome in DU-145 prostate cancer cells by using an unbiased ChIP-seq approach. In summary, our integrated genomic and transcriptional analysis identified a broad spectrum of genetic alterations in PMT genes involved in prostate cancer progression. Our findings suggest a promising avenue for future research—to focus on a subset of PMTs to better understand the molecular mechanisms and to identify therapeutic targets in prostate cancer.

#5389 A personalized medicine approach to identifying dysregulated epigenetic enzyme activity in the development of castrate-resistant prostate cancer. Jin-Hee Lee,1 Melissa Boersma,2 Bing Yang,3 Nathan Damaschke,1 Eva Coe,ry,3 John Denu,1 David F. Jarrard.1, 1Univ. of Wisconsin-Madison, Madison, WI; 2Wisconsin Institutes for Discovery, Madison, WI; 3Univ. of Washington, Seattle, WA.

Purpose: Recent advances in proteomic and chromatin immunoprecipitation tools have been vital in studying cancer epigenetics, but the ability to measure directly the enzyme activities of dysregulated histone-modifiers understood. We utilize a novel approach to identify altered histone-modifying enzymes in the progression from hormone sensitive (HS) to castrate-resistant prostate cancer (CRPC) progression. Methods: We developed, validated and utilized a high-throughput peptide microarray assay to identify altered histone lysine (de)acetylation activity in tumor lysates. Functional assays, novel HS and CRPC human tumor arrays and xenografts were utilized to confirm these findings. Results: This microarray-based activity assay revealed up-regulated histone acetyltransferase (HAT) activity against specific histone H3 sites in a castrate-resistant (CR) PCA cell line compared to its hormone-sensitive (HS) isogenic counterpart. NAD+-dependent deacetylation assays revealed down-regulated Sirtuin activity in validated CR lines. Levels of acetyltransferases GCN5, PCAF, CBP and p300 were unchanged between matched HS and CR cell lines. However, auto-acetylation of p300 at K1499, a modification known to enhance HAT activity and a target of deacetylation by SIRT2, was highly elevated in CR cells, while SIRT2 protein level was reduced in CR cells. Interrogation of HS and matched CR xenograft lines reveals that H3K18 hyperacetylation, increased p300 activity, and decreased SIRT2 expression are associated with progression to CR in 8/12 (66%). Tissue microarray analysis revealed that hyperacetylation of H3K18 is a feature of CRPC. Inhibition of p300 results in lower H3K18ac levels and increased expression of androgen receptor. Conclusions: This novel microarray approach provides a method to identify commonly dysregulated chromatin enzymes during progression providing a personalized therapeutic strategy to direct available drugs to target enzymes. Reduced SIRT2 expression and increased p300 activity lead to a concerted mechanism of hyperacetylation at specific histone lysine sites (H3K9, H3K14, and H3K18). Support: DOD PCRP (Jarrard).

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Genomic Analyses of Circulating Tumor Material and RNA

#5390 Broad Genomics and Cancer Program development of comprehensive blood biopsy sequencing capabilities to support direct-to-patient cancer research. Carrie Cibulskis, Andrew Hollinger. Broad Institute of MIT and Harvard, Cambridge, MA.

Genomic profiling of cell free tumor DNA (Blood Biopsy) offers the potential to revolutionize cancer precision medicine. As a proxy for tumor tissue profiling, successful blood biopsy analysis can help select appropriate patients for clinical trials, provide useful data for treatment monitoring, and discover genomic mechanisms of disease resistance. Recognizing these potential benefits, we aim to enable large-scale genomic data generation and analysis of cfDNA samples to support the patient driven Metastatic Breast Cancer Project (mbcproject.org) and many other such efforts. The widespread adoption of cfDNA sequencing technology in clinical research will require low cost, efficient, and in some cases clinically-validated processes. Commercial offerings in this field are focused on analysis of small targeted hotspot panels due to the challenges of more complete sequencing approaches for cfDNA (e.g. variability in the fraction of tumor present and high false positive rates). Working in collaboration with the Broad Institute Cancer Program, we have developed a process to overcome these challenges and provide high quality comprehensive exome analysis of cfDNA. Our process includes screening samples using ultra low pass, UMI-enabled whole genome sequencing to quantify the % of tumor DNA available for sequencing. Qualified samples are then moved into automated processes for exome sequencing and somatic mutation detection. In our initial validation study of 23 breast and prostate cancer patients with matched cfDNA and tumor DNA, we were able to identify 99% of clonal and 45% of subclonal mutations found in the matched tumor biopsy. In our effort to scale this process, and fully characterize the benefits and limitations of this technology we will present additional validation analysis. We will evaluate the relative importance of using paired sample analysis (tumors and matched normals) with analysis of tumor sequencing alone and assess sensitivity and specificity of somatic alterations as a function of tumor fraction and sequencing depth.

#5391 Low frequency variant detection and tissue-of-origin exploration using liquid biopsies. Justin S. Lenhart, Ashley Wood, Sukhinder Sandhu, Cassie Schumacher, Laurie Kurihara, Vladimir Makarov, Tim Harkins. Swift Biosciences, Ann Arbor, MI.

The promise of liquid biopsy assays lies in the non-invasive monitoring of diseases, such as cancer, through circulating, cell-free DNA (cfDNA) or circulating tumor cell DNA. This may assist in advancing early-stage diagnosis while simultaneously monitoring treatment response over time. Since these materials are often limited, most liquid biopsy assays incorporate targeted sequencing to enable cost-effective deep coverage of target loci for detection of low frequency pathogenic variants. Yet a critical aspect in attaining the necessary sensitivity is an assay that produces uniform, comprehensive coverage from low DNA input quantities. We have developed a liquid biopsy workflow to enable low frequency variant detection from a 10 ml blood draw using the Promega Maxwell RSC combined with Swift Biosciences Accel-NGS 28 library preparation methodologies. In addition, we explore whether methylation patterns of the extracted cfDNA possess information of the tissue-of-origin. Whole blood samples were collected in Streck cell-free DNA BCT vials from patients with late stage cancer and cfDNA was extracted with the Promega Maxwell RSC. This instrument yielded DNA outputs ranging from 8-32 ng, with a size profile defined by a predominant peak of ~170 bp and a mean Alu repeat qPCR integrity score of 0.22, characteristic of high quality cfDNA lacking cellular DNA content. A total of 20 ng cfDNA was used to make an Accel-NGS 28 Hyb library followed by hybridization capture using Agilent SureSelect Human All Exon probes. The Accel-NGS 28 Hyb Kit exhibits a 90% library conversion rate with cfDNA and provides high complexity libraries with uniform target coverage. In addition, molecular barcodes were incorporated to label each library molecule uniquely prior to PCR amplification. These molecular barcodes were utilized for accurate removal of PCR duplicates while simultaneously preserving naturally occurring fragmentation and strand duplicates to maximize data recovery. Secondly, these barcoded molecules were grouped to generate consensus sequences after removal of false positives originating from PCR and sequencing errors. Variant calling was performed using Vardict and Lofreq enabling highly sensitive and precise detection of variants down to 0.5% allele frequency. In parallel, we have developed a workflow to determine if the epigenetic status of cfDNA can identify tissue-of-origin. This workflow utilizes the Accel-NGS Methyl-Seq DNA Library Kit to enable unbiased characterization from low (5 ng) cfDNA characterization from low (5 ng) cfDNA inputs. Through whole genome bisulfite sequencing, using a priori knowledge of differentially methylated regions characteristic of different human tissues, we can identify the predominant tissue source of cfDNA in blood.
relation between the presence of cancer mutations and morbidity and mortality. In 2 of 11 women, the initial absence of mutations above 1% allele-frequency was followed by the appearance of mutations in 1-3 genes at allele frequencies of 5-78% in the later time point. These 2 patients experienced increased morbidity or mortality. In 9 of the 11 women, no mutations were observed, and 6 remain in remission currently living with cancer. In an effort to further improve both workflow and performance, we are developing two technologies to incorporate into the panel design for future studies. The first will normalize library yield during PCR amplification for simple library pooling, which eliminates the requirement for library quantification and minimizes the time from sample to sequence. The second technology is a molecular ID (MID) system to tag each amplicon uniquely to allow data tracking. By integrating these two technologies, we will improve the efficiency of our workflow and increase confidence in variant calling by filtering PCR and sequencing errors. By incorporating technologies that reduce steps in the workflow, the likelihood of error is minimized, and combined with methods that increase confidence in low frequency variant calling, an ideal workflow for liquid biopsy samples is created.

#5393 BRAFT mutant allele fraction in circulating tumor DNA as marker of treatment response in BRAF mutated non malignant melanoma cancers identified in the Copenhagen Prospective Personalized Oncology study. Lise Barlebo Ahlborn, Ida Villen Tuxen, Olga Oestrup, Anne Yde Schmidt, Cecilie Bruhn Hoffhansson, Finn Cellius Nielsen, Ulrik Lassen, Christina Westmose Yde, Morten Mau-Sorensen. Rigshospitalet, Copenhagen, Denmark.

Background: BRAF inhibitors have been approved in BRAF mutated malignant melanomas. However, in other BRAF positive cancers, the effect of BRAF inhibition has been studied less extensively. BRAF mutated non-melanoma cancer patients (pts) were identified in a prospective genomic profiling study. The effect of targeted treatment was assessed by BRAF mutant allele fraction (AF) in circulating tumor DNA (ctDNA) and radiological response. Methods: Pts had tumor biopsies performed at enrolment and, if feasible, on or after treatment. Biopsies were subjected to whole exome sequencing, RNA sequencing and SNP array. Blood samples for ctDNA analysis were collected at baseline and longitudinally during treatment. Mutant BRAF fragments were quantified using droplet digital PCR (ddPCR) or by targeted next generation sequencing (NGS). Radiological response was evaluated according to RECIST 1.1. Results: Twenty-two BRAF mutated non-melanoma cancer pts (lung n=2, colorectal n=14, bile duct n=4, prostate n=1, pancreas n=1) were identified out of a total of 405 (5%) pts enrolled in the Copenhagen Prospective Personalized Oncology (CoPPO) study. In all tumors we identified the BRAF V600E mutation except in one prostate cancer pt (BRAF K601E). Fifteen pts were treated with dabrafenib/trametinib (lung, bile duct), vemurafenib/pamitumumab (+/- irinotecan (colorectal) or vemurafenib (pancreas, prostate). Two pts were non-evaluable because one patient requested early termination and one has pending tumor evaluation. All pts had tumor reduction, except one patient with colon cancer. Six out of 13 (46%) pts achieved partial response (lung n=2, bile duct n=1, colorectal n=3) and 7/13 (44%) had stable disease (prostate n=1, bile duct n=1, colorectal n=5) as best response according to RECIST 1.1. Median progression-free survival was 20 weeks. Eighteen pts analysed by ddPCR or NGS at baseline had detectable mutant ctDNA. In the patient with early termination of therapy, the ctDNA levels decreased by 70% during the first 6 weeks. Eight of the 18 pts had ctDNA assessed during treatment and all presented with a reduction in mutant BRAF AF corresponding to a reduction in tumor volume. Moreover, in 5/8 pts, time of best radiological response coincided with the lowest detectable BRAF mutant AF. Analysis of on- and post-treatment biopsies and matched ctDNA samples are pending to identify putative mechanisms of resistance and will be reported at the meeting. Conclusion: Targeted therapy seems active in BRAF mutated non-melanoma cancer pts and treatment response is evaluable by monitoring mutant BRAF in ctDNA during therapy.

#5394 The clinical impact of multiplex ctDNA gene analysis in lung cancer. Smadar Geva,1 Anna Bellilovsky Rozenblum,1 Tal Twito,1 Addie Dvir,2 Lior Soussan-Gutman,2 Maya Ilouze,1 Laila C. Roisman,1 Elizabeth Dudnik,1 Alona Zer,2 Richard B. Leman,4 Nir Peled4 Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; 2Teva Pharmaceutical Industries Ltd., Israel; 3Institute of Oncology, Davidoff Cancer Center, Rabin Medical Center, Petach Tikva, Israel; 4Guardant Health, Inc., Redwood City, CA.

Background: Next-generation sequencing (NGS) of cell-free circulating tumor DNA (ctDNA) enables a non-invasive option for comprehensive genomic analysis of lung cancer patients. Currently there is insufficient data in regard to the impact of ctDNA analysis on clinical decision making. In this study, we evaluated the clinical utility of ctDNA sequencing on treatment strategy and progression-free survival. Methods: In this retrospective study, data was collected from 92 NSCLC patients monitored between the years 2014-2016 at the Thoracic Center Unit at Davidoff Cancer Center, Rabin Medical Center, Israel. Plasma samples from stage IIIb/IV non-small cell lung cancer (NSCLC) patients were analyzed by a commercial test (Guardant 360), using hybrid capture, single molecule barcoding and massively parallel paired-end synthesis to sequence a targeted gene panel. This test allows the detection of somatic alterations in tumors among patients at different stages of malignancy. Results: 92 consecutive NSCLC patients were included in this study. Median age at diagnosis was 63 years, male:female ratio was 1:1.6. 40% (37/92) were never-smokers, 84% (77/92) had adenocarcinoma. 38% (35/92) performed ctDNA analysis before 1st line therapy and 62% (57/92) on progression. ctDNA analysis yielded lung cancer related actionable mutations in total 39% (36/92) of the patients. ctDNA analysis was positive in 46% (42/92) patients at progression on matched therapy. Treatment decision was taken toward targeted therapy subsequently to nGS analysis in 23% (8/35) and 26% (15/57) respectively (total 25%; 23/92). 53 individual actionable genomic alterations were found. The most common genes were sensitizing EGFR mutations (47.2%; 25/53), MET amplifications and/or exon 14 skipping mutations (17%; 9/53) and resistance EGFR mutations (13.2%; 7/53). Response assessment (RECIST) for 18 patients with evaluable response to targeted therapy showed complete response in 6% (1/18), partial response in 39% (7/18), stable disease in 22% (4/18) and progressive disease in 33% (6/18). Total objective response rate (ORR) was 45% and disease control rate was 67%. Conclusions: Comprehensive ctDNA testing revealed possible treatment options for two-thirds of patients analyzed. ctDNA analysis could be used as a clinical decision making in a question of a biopsy. Although this topic needs to be further assessed in large randomized controlled trials, these positive results emphasize the utility of liquid biopsy analysis to guide clinicians to select the right therapy for the right patient.


Background: Small cell lung cancer (SCLC) is a highly aggressive malignancy with rapid development of chemo resistance and a high rate of recurrence. Although tumor tissues-based studies have shown characteristic genomic changes in SCLC, it is little known about tumor-derived genomic variations in cell free DNA (cfDNA), a promising source of genetic materials for monitoring disease progression, evaluating treatment efficacy and predicting clinical outcomes. Methods: We collected plasma from 26 SCLC patients and extracted cDNAs from 250µl of plasma. We prepared DNA libraries and analyzed sequencing data using various bioinformatic tools. We binned read counts from the mapped sequence files into 60kb windows and performed log2 ratio-based copy number analysis using segmentation-based approach. We compared copy number changes detected in cfDNA to previously reported genomic regions from tumor tissues-based studies. Results: The whole genome sequencing generated approximately 9.4 million mappable sequence reads per subject and 175 read counts per 60kb genomic region. Copy number analysis using log2 ratios between cfDNA and matched gDNA showed significant genomic abnormalities in 17 of 26 plasma samples. Overall, we observed the most frequent deletion of 3p, 13q, 17p, 10p and amplification of 3q, 5p, 8q, 1p, which were consistent with previous reports from tumor tissues-based studies. To further define the copy number changes, we performed a detailed analysis at chromosomal regions showing frequent aberrations in SCLC. Among these, deletion of gene RASSF1 at 3p21.3 was most frequently observed (12 deletions in 26 samples). Another common deletion was RB1 at 13q14 (5/26), TP53 at 17p13 (4/26). The most common amplification was involved in MYC gene at 8q24 (18/26). Other common amplifications included SOX2 at 3q26 (17/26), MYCL at 13p42 (9/26) and FGF10 at 5p13 (9/26). We also observed low frequent genomic alterations at other genomic regions such as PTEN loss at 10q23 (1/26) and FHTI loss at 3p14 (1/26). Interestingly, we observed contradictory genomic changes at MAD1L1 locus. Among the 26 cfDNA samples, we detected ten patients with MAD1L1 loss and four patients with MAD1L1 gain. We have shown that the MAD1L1 locus at 7p22 is frequently amplified in SCLC patients. We are currently analyzing if these genomic variations are associated with disease progression and overall survival. Conclusions: This study provides a comprehensive analysis of genomic aberrations in plasma cfDNAs from SCLC. We identified multiple candidate genes whose genomic aberrations may be used as biomarkers for accessing treatment efficacy and predicting prognosis. Our results suggest plasma as an alternative sample source for tumor-associated genomic analysis in SCLC patients. Further targeted sequencing and clinical association analyses is needed to fully validate the potential clinical utilities of cfDNA-based liquid biopsy for clinical management of this deadly disease.
#5396 An NGS workflow to detect down to 0.1% allelic frequency in cfDNA for breast and colon cancers. Dalia Dhringa,1 Richard Chien,1 Jian Gu,2 Dumitru Brinza,1 Ruchi Chaudhary,1 Kunal Banjara,1 Yanchun Li,2 Efren Ball- esteros-Villagrana,2 Kelli Bramlett2,2,3,4Thermo Fisher Scientific, South San Francisco, CA;3Thermo Fisher Scientific, Austin, TX.

Noninvasive detection of rare mutations in blood could allow tumor monitoring for research purposes. Research studies have suggested that cfDNA contains DNA from tumor cells with somatic mutations that could inform on tumor progression and therapeutic resistance. Here, we demonstrate a complete workflow from a single tube of blood through data analysis for research samples down to a 0.1% allelic frequency. The low abundance tumor mutations found in cfDNA represent accurate tumor detection rates of 0.1% when the two panels that utilize an amplification-based assay that generates tagged DNA copies, which allows detection of low abundance tumor mutations found in cfDNA. The two panels allow multiplex interrogation of primary driver and resistance mutations specific to cfDNA from breast and colon cancer. The Oncomine Colon cfDNA panel targets 236 hotspots within 14 genes while the Oncomine Breast cfDNA panel and Oncomine Colon cfDNA panel had over 85% sensitivity and 100% specificity. The Oncomine Breast cfDNA panel and Oncomine Colon cfDNA panel integrated into a complete workflow starting from a single tube of blood was validated from matched single blood tubes, Streck and K2EDTA. Finally confirmed with TaqMan based Rare Mutation assays. With this control, we developed an oncology control for cfDNA workflow that was validated from matched single blood tubes, Streck and K2EDTA. Additionally, the utility for cancer research was demonstrated with concordance studies using matched FFPE and plasma from oncology samples. To further characterize these panels we have developed an oncology control for cfDNA with nucleosome fragment sizing and minimal sonication damage. This engineered DNA contains multiple SNPs and indicative of allelic frequency, originally confirmed with TagMan based Rare Mutation assays. With this control, the Oncomine Breast cfDNA panel had over 81% sensitivity and 99.9% specificity. The Oncomine Colon cfDNA panel had over 85% sensitivity and 100% specificity. The Oncomine Breast cfDNA panel and Oncomine Colon cfDNA panel integrated into a complete workflow starting from a single tube of blood can advance oncology research with the ability to detect blood based cancer biomarkers present at 0.1%.

#5397 Characterizing the genomic landscape of bladder cancer with circulating tumor DNA. Gillian R. Vandenkerkhove,1 Tilman Todenhofer,2 Matti Annala,3 Werner J. Struss,1 Kevin Beja,1 Amanda Wong,1 Scott North,1 Bernie Eigl,1 Peter Black,1 Alexander W. Wyatt1.1Vancouver Prostate Centre, Vancouver, British Columbia, Canada;2University Hospital Tübingen, Tübingen, Germany;

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Background: Targeted agents are emerging as promising treatment options for patients with metastatic bladder cancer (BCa). However, since the genomic landscape of BCa is highly heterogeneous, future clinical trial success will likely depend upon tumor molecular stratification and therefore require a practical method for genomic analysis. Circulating cell-free tumor DNA (cfDNA) collected from peripheral blood has been established in several major solid malignancies as a minimally-invasive tool to profile the tumorgenome but is underexplored in BCa. Goal: Our aim was to identify the spectrum of clinically-informative genomic alterations detectable in plasma cell-free DNA (cfDNA) collected from patients with BCa. Patient cohort and methodology: We recruited 45 patients with BCa, 14 with localized disease and 31 with metastatic disease, and collected whole blood samples for isolation of cfDNA. Isolation of plasma cfDNA was performed using the QIAmp Circulating Nucleic Acid Kit, while germline control DNA was extracted from buffy coat using the DNeasy Kit (Qiagen). A custom targeted next-generation sequencing (NGS) strategy was employed to detect BCa-specific somatic alterations in cfDNA; samples underwent deep (>=30X) targeted DNA sequencing using the NimbleGen SeqCap EZ Choice system and Illumina technology. Our custom panel included key tumor suppressors (e.g. TP53, RB1, CDKN2A), cell cycle drivers (e.g. CCND1, CCNE1), DNA repair genes (e.g. ATM, BAP1, ERCC2), PIK3 pathway genes (e.g. PIK3CA, PTEN, AKT1), and other oncogenes (e.g. RAS/RAF, EGFR, ERBB2, PPARG, FGER3). Results: The majority of patients with metastatic BCa (18/31 patients, 24/43 samples) had robust evidence of somatic alterations and therefore cfDNA. Our findings were consistent with the known landscape of BCa, including mutations in TP53 and ARID1A (and other chromatin modifiers), hotspots activating mutations in PIK3CA, as well as typical copy number changes such as focal amplifications of ERBB2, KRAS, and CCNE1. In addition, we identified complex gene rearrangements including in one case an activating FGFR3 fusion. Some samples had evidence of very high mutation rates, indicative of somatic ‘hypermutation’. Interestingly, in contrast to the metastatic setting, 85% of patients with localized BCa had no evidence of cfDNA, despite elevated cfDNA yields in some patients. Conclusion: The majority of metastatic BCa patients have high levels of cfDNA suitable for standard targeted sequencing approaches, while patients with localized muscle-invasive tumors appear not to harbor ctDNA at fractions greater than 1-2%. The robust detection of so-called ‘ actionable’ copy number alterations, mutations and rearrangements in ctDNA provides an unparalleled opportunity for practical molecular stratification of patients in clinical trials of novel targeted agents.

#5398 High-throughput clonal analysis of AML tumors with droplet microfluidics. Dennis Eastburn,1 Maurizio Pellegrino,1 Sebastian Treusch,1 Adam Sciambati,1 Bill Hyun,1 Jamie Yates,2 Mission Bio, Inc., San Francisco, CA;2University of California, San Francisco, San Francisco, CA.

Single cell analysis tools are crucial to better understand the role that rare or heterogeneous cancer cells play in the evolution of tumor progression. Although, it is now feasible to perform single-cell RNA-Seq on thousands of, several challenges remain for high-throughput single-cell DNA sequencing. To address these challenges and enable the characterization of genetic diversity in cancer cell populations, we developed a novel approach that barcodes amplified genomic DNA of individual cells confined to microfluidic droplets. The barcodes are used to assemble the genetic profiles of individual cells from next generation sequencing data. A key feature of our approach is the “two-step” microfluidic workflow that releases genomic DNA from cellular proteins prior to amplification. The microfluidic workflow first encapsulates individual cells in droplets, lyses the cells and prepares the lysate for genomic DNA amplification using proteases. Following this lysate preparation step, the proteases are inactivated and droplets containing the genomes of individual cells are then paired with molecular barcodes and PCR reagents. We demonstrate that the two-step microfluidic approach is vastly superior to workflows without the two-step process for efficient DNA amplification on tens of thousands of individual cells per run with high coverage uniformity and low allelic dropout of targeted genomic regions. To apply our single-cell sequencing technology to the study of acute myeloid leukemia (AML), we developed a targeted panel to sequence genes frequently mutated in AML including TP53, DNM3TA, FLT3, NPM1, NRAS, KRAS, JAK2, IDH1 and IDH2. Using this panel, we were able to identify clonal populations from AML research samples; moreover, the single-cell nature of our approach enabled the correlation of multiple mutations within subclones and determination of whether the mutations existed as a homozygote or heterozygote. Collectively, our results show a greater degree of heterogeneity in AML tumor samples than is commonly appreciated with bulk sequencing methods. Investigating phenotypic plasticity in breast cancer with high-throughput nanogrid single-nucleus RNA sequencing. Ruli Gao,1 Charissa Kim,1 Emi Sei,1 Jie Yang,1 Leo Chan,2 Mathreyran Srinivasan,2 Hong Zhang,1 Funda Meric-Bernstam,3 Nicholas E. Navin1,4University of Texas MD Anderson Cancer Center, Houston, TX;2WafGen Inc., CA.

Single-cell RNA sequencing (RNA-seq) is a powerful tool for investigating rare tumor subpopulations and resolving intra-tumor heterogeneity, but is low throughput, expensive, and requires fresh tissue samples. To address these limitations, we developed a 5’ high-throughput single-nucleus RNA sequencing (SNRS) approach that uses a nanogrid platform to perform high-throughput single-cell RNA sequencing and screening of 500–2500 nuclei in parallel. The automated image scanning procedure allowed us to exclude doublets and select live cells with DAPI/PI staining. This approach allows the transcriptomic profiling of frozen tissue samples, in which the cytoplasmic membrane is ruptured in cells, but leaves the nuclear membrane intact. We validated SNRS in a breast cancer cell line (SK-BR-3) and compared the transcriptomes of 500 nuclei to 500 whole cells, which revealed a high concordance in the number of genes expressed as well as their expression levels. We also performed bulk RNA-seq of isolated nuclear and cellular fractions from 5 breast cancer cell lines, which showed a high concordance in genes and expression levels. Differentially expressed genes in the nucleus mainly included lincRNAs, pseudogenes and mitochondria genes, but did not affect most cancer genes and pathway analysis. We further applied SNRS to sequence 500 nuclei of a triple-negative breast cancer patient and identified diverse phenotypes in tumor cells, including variation in cell proliferation, migration, invasion, and epithelial-to-mesenchymal transition. These studies demonstrated the technical feasibility of using a nanogrid platform to perform high-throughput single-cell RNA sequencing and showed that nuclei from cell lines and tumors can be used to study signaling pathways and gene networks that play an important role in tumor progression.

#5400 Accurate identification of single nucleotide variants in whole genome amplified single cells. Xiao Dong,1 Lei Zhang, Brandon Millholland, Moonsoon Lee,2 Alexander Y. Maslov, Tao Wang, Jan Vijg, Albert Einstein College of Medicine, Bronx, NY.

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Single cell sequencing for analyzing DNA mutations across the genome in somatic tissues is critically important for studying development, cancer and aging. However, current procedures are prone to artifacts and to date a reliable protocol for single-cell somatic mutation analysis remains to be developed. Here we address the two largest sources of artifacts, i.e., DNA denaturation-related cytosine deamination and allelic bias-driven whole genome amplification errors. We first reconfigured multiple displacement amplification (MDA) into an efficient protocol for whole genome amplification of single cells without cyto- sine deamination artifacts, i.e., Single Cell MDA (SCMDA). We then developed a new single-cell SNV caller (SCcaller) that distinguishes real somatic mutations and amplification errors by utilizing a SNP-based localized estimate of allelic amplification. Our procedure was validated by comparing SCMDA-amplified single cells with unamplified clones derived from single cells from the same population. Together with SCcaller, SCMDA provides a firm foundation for analyzing cellular heterogeneity in somatic mutational landscapes in tumor and surrounding, normal tissues.

#5401 Assessment of whole genome amplification for whole exome sequencing in detecting genetic mutation. Crystal Xue, Laura Gardener, Guanglong Jiang, Fei Shen, Bryan Schneider. Indiana Univ. Cancer Ctr., Indianapolis, IN.
Whole exome sequencing (WES) has been widely used for studying genetic mutations in DNA coding regions to elucidate cancer etiology and for identifying biomarkers to optimize chemotherapy. However, these studies can be limited when only small amount of DNA is available. Whole genome amplification (WGA) technology can be combined with WES to make these studies technically possible. Here, we evaluated WGA using a phi 29 polymerase prior to library preparation for WES in samples with various DNA concentrations. WES was performed by targeted exon amplification followed by massively parallel sequencing. We compared the base calls of single nucleotide variants for individual same samples with or without WGA prior to library construction to determine the concordance rate of variant calls. We also assessed genetic variant call rate in the same samples with or without WGA. The number of variants obtained ranged from 353,215 to 384,118 in the same amplified samples. The average concordance rate of identical variants in the samples with or without WGA was 96.5%. Our results indicate that WGA in prior to WES can increase detection efficiency of single nucleotide variants in samples with relatively small amount of DNA and/or low DNA concentration.

#5402 Single cell sequencing of high grade serous ovarian cancer. Timothy K. Starr,1 Boris Winterhoff,2 Makayla Maile,3 Kenneth Beckman,4 Jerry Daniel,5 Melissa Greninger,6 Mariel Guazzo,6 Molly Klein,3 Rafaelle Hellweg,6 Astrid Kretschmar,6 Amit K. Mitra,7 Attila Sebe,8 Sally A. Mullaney9 1 Univ. of Minnesota Medical School, Minneapolis, MN; 2 Univ. of Minnesota, Minneapolis, MN; 3 Paul Ehrlich Institute, Langen, Germany.
We performed RNA sequencing of single cells derived from a high grade serous ovarian cancer (HGSOC) specimen to determine the extent of heterogeneity and to determine if it was feasible to identify cancer stem cells or gene expression signatures of chemo resistance. To perform RNA sequencing we enzymatically digested a fresh specimen from an HGSOC derived from the ovary. Immune cells were depleted by flow cytometry and single cell sequencing was performed using the Fluidigm C1 chip in tandem with Illumina HiSeq 2500 sequencing. Multiple bioinformatics tools were used to identify subgroups and activated pathways. Immunohistochemistry was performed on an adjacent tissue section to analyze markers of epithelial stroma and stem cells. We found that gene expression patterns in single cells could be used to separate cells into stroma-like and epithelial-like groups. Gene set enrichment analysis identified proliferative genes (oxidative phosphorylation and MYC targets) associated with the epithelial-like cells while epithelial-to-mesenchymal transition (EMT) genes associated with the stroma-like cells. Neutrophilic group was significantly associated with genes derived from chemo-resistant cells. Using known marker analysis, we could identify a small percentage of cells that expressed ovarian cancer stem cell markers and we could group cells into functional categories. Using four molecular subtypes established from large-scale bulk sequencing studies we show that single cells from a single patient are heterogeneous and each molecular subtype is represented. In conclusion, we show the feasibility of performing single cell sequencing on an epithelial ovarian cancer and reveal a heterogeneous population of cells. Expanding these findings to a larger cohort of patients could allow for identification of targetable sub-populations of cells that were previously undetectable in studies that use bulk samples to interrogate the transcriptome and genome of ovarian cancer patients.

#5403 Reproducible elevation of RNA versus DNA mutation signal in low purity breast tumors. Jerez Te,1 Coralie Viollet,1 Xiujun Zhang,2 Jatinder Singh,1 Jeffrey A. Hooke,2 Harvey B. Pollard,1 Hai Hu,3 Craig D. Shriver,3 Clifton L. Dalgaard,4 Matthew D. Wilkerson1 1Uniformed Services University; 2John P.Murtha Cancer Center; Walter Reed National Military Medical Center, Bethesda, MD; 3Chen Soon-Shiong Institute of Molecular Medicine at Windber, Windber, PA.
Background: Accurate detection of somatic mutations is critical for informing targeted therapy options. Prevalent non-cancer cell admixture complicates this detection in breast cancer. Conventional mutation detection relies on DNA sequencing; however in prior work, we demonstrated that combining RNA and DNA mutation analysis increased signal strength fraction (MAF) by 21% in the breast (MAF). The ratio of RNA MAF versus DNA MAF (RNA:DNA MAF) was greatest in low purity breast tumors. We hypothesized that this elevation is biologically driven and would be conserved in a second, distinct tissue specimen of the same tumors. Here, we compare mutation characteristics between two tissue blocks in a cohort of breast tumors (n = 8) to evaluate possible preservation of RNA versus DNA mutation signal throughout the tumor. Methods: We selected four high purity and four low purity breast tumors (“Block 1”) from The Cancer Genome Atlas (TCGA) cohort and associated ABSOLUTE purity analysis. For these tumors, we acquired a second tissue block (“Block 2”) not analyzed by TCGA, cut, analyzed sections by H&E stains, and extracted nucleic acids. Whole genome DNA sequencing and mRNA sequencing was performed for_Block 1 and Block 2 respectively, and compared the MAFs on these samples. Somatic mutations in Block 2 were detected using UNCeqR and compared to published UNCeqR somatic mutations from TCGA. We then evaluate MAF characteristics in the entire TCGA cohort. Results: Tumor purity estimates, determined by histology and by sequencing, were reduced in Block 2 of the low purity tumor set versus the high purity tumor set, consistent with Block 1 analysis. Molecular properties of genome-wide gene expression and somatic DNA copy number were highly similar between block-matched specimens (ρ < 0.01). We then identified expressed mutations present in Block 1 and Block 2 of the same tumor and compared the MAFs on these common mutations. DNA MAF and RNA MAF were each significantly correlated between Block 1 and Block 2 (ρ < 1e-12 in both cases). The average RNA:DNA MAF was 2.5 for the cohort, indicating that RNA mutation signal is greater than DNA in general. In Block 2 specimens, the RNA:DNA MAFs were significantly greater in the low purity tumor set than the high purity tumor set (mean 2.7 versus 2.1, p < 6e-5), reflecting the same trend observed in Block 1 specimens. Analyzing the entire TCGA cohort, RNA:DNA MAF was positively correlated with proliferation pathway gene expression (ρ < 3e-16) and was greatest in the Basal subtype versus other subtypes (ρ < 2e-9). Conclusion: Mutant allele fraction both of DNA and of RNA was conserved across breast tumor subsections. Low purity and basal subtype breast tumors had elevated RNA:DNA MAF supporting a relationship to underlying biology and identifying classes of tumors with pronounced benefit for DNA and RNA integrated mutation analysis.

#5404 Functional analysis of IncRNAs in pancreatic ductal adenoarcinoma. Luis Arnes, Jiguang Wang, Zhaoli Liu, Carlo Maurer, Lori Sussen, Kenneth P. Ouel, Raul Rababan. Columbus University, New York, NY.
Pancreatic ductal adenocarcinoma (PDA) is anticipated to be the second leading cause of cancer related death in western countries by 2030 with a 5-year survival rate of 6%. Recently, genome wide sequencing and transcriptome analysis have been applied to identify cancer driving mutations and mRNA expression profiles of pancreatic tumors. Despite a better understanding of the molecular drivers and the signaling pathways deregulated in PDA, this knowledge has not been able to identify novel therapeutic targets. A common limitation for the aforementioned studies is that they have been performed in bulk samples, and they have been focused on mutations and gene expression analysis of protein-coding genes, which represents less than 2% of the genome. Long non-coding RNAs are emerging as essential players in the biology and progression of a variety of tumors. However, this concept has not been extensively explored to the study of PDA. We have developed computational methods and performed experimental validation to identify functional IncRNAs drivers of tumor progression in pancreatic cancer. Specifically, we have developed computational tools to identify IncRNAs associated with genomic traits of PDA using genome wide high throughput data. The analysis uncovered the existence of 1741 IncRNAs that we termed ncPDA (non-coding PDA). Using a unique RNA-seq dataset from >200 laser capture microdissected pancreatic tumors, we selected these ncPDA that are expressed specifically in the neoplastic epithelium of human pancreatic tumors. Within the top 100 ncPDA enriched in neoplastic epithelial cells, we identified known IncRNAs drivers in other tumor types such as CRNDE (ncPDA4), P2rt (ncPDA 9), HOTAIR (ncPDA 39), Malat1 (ncPDA 68) and Neat1 (ncPDA 71). We are currently exploring the role of several ncPDA in
pancreatic cancer cell lines. Loss of function studies suggest that several ncPDAs are required for tumor progression. Our investigation will continue to unravel the role of ncPDAs in the initiation and progression of pancreatic cancer.

#5405 Comprehensive analysis of long non-coding RNAs in human stomach adenocarcinoma molecular subtypes. Jian Chen, Juan-Sheng Chen, Keping Xie, John R Stroehlein, Marta Davila, Xiaoping Su. UT MD Anderson Cancer Ctr., Houston, TX

Objective: Long non-coding RNA (lncRNA) plays key role in cell biology including epigenetic remodeling and post-transcriptional regulation. The potential roles of lncRNAs as biomarkers and therapeutic targets have been proven in solid tumors. Molecular profiling in stomach adenocarcinoma (STAD) has been characterized using The Cancer Genome Atlas (TCGA) database. However, there are no current lncRNA comprehensive analyses and the relationships between patterns of molecular subtypes and the signature of lncRNA in in human stomach adenocarcinoma are unclear. Methods: (1) The Cancer Genome Atlas (TCGA) 312 primary STAD RNA-Seq data (bam files) and their related clinical data were obtained from the Cancer Genomics Hub and TCGA Data Portal. (2) We filtered the dataset to remove lncRNAs with low expression, defined as having an RPKM value <1 in at least 90% of the tumor samples. (3) We generated a correlation matrix between lncRNAs and mRNAs by computing the Pearson correlation coefficient between all pairs of significant lncRNAs and mRNAs. A matrix was constructed with entries in the ternary scale (-1, 0, 1), where the top 1% with negative correlation was assigned -1; the top 1% with positive correlation was assigned 1; and the others were assigned 0. The matrix was clustered and visualized using a Euclidian distance metric of RNA complementary linkage clustering. Results: (1) Using stringent criteria, we identified 1830 expressed lncRNAs in human genome in STAD. (2) Unsupervised clustering of lncRNA expression in STAD revealed five robust categories: clusters C1-C5. (3) We further performed concordance calculation between lncRNA-based clustering and other clusters identified in TCGA molecular, mRNA, msiRNA and SCNA subtypes, respectively. We also investigated the association of lincRNA subclasses with distinct clinicopathological and genomic features of STAD. We found that C2 is significantly associated with MSI molecular subtype, MHL silencing, and mRNA C2, miRNA C2, methylation C2 subtypes. Cluster C3 is significantly associated with EBV-positive molecular subtype, C6DKNA2 silencing and mRNA C2, miRNA C2, methylation C1 subtypes; C4 is most significantly associated with CN molecular subtype, p53 mutation and high SCNA, methylation C4 subtypes; C5 is significantly associated with CS molecular subtype, diffuse Lauren subtype, and mRNA C1, miRNA C4, methylation C1 subtypes. Interestingly, C5 defines the most aggressive tumors with the worst overall survival. Conclusions: Our study represents the first comprehensive analysis of lncRNAs in STAD cancers, with integrative analysis revealing that the signatures of lncRNAs characterize here appear to have prognostic significance. The additional association with the STAD molecular subtypes and genomic features supports new approaches to developing biomarkers and setting the stage for a new framework for future research in the role of lncRNAs in STAD.


RNA-seq has become the most popular method for transcriptome analysis and is widely used to study gene expression, detect mutations, fusion transcripts, alternative splicing, and post-transcriptional modifications. It is becoming the method of choice to detect alterations in diseases, especially cancer, to provide insights on the various molecular pathways perturbed by changes in the transcriptome and study their implications. As RNA-seq is being adopted in molecular diagnostics and biomarker identifications, the need for good quality, reproducible library preparation methods has increased with the availability of the more powerful bioinformatics methods. Enhanced sensitivity to detect transcripts with even coverage across their length offers a non-biased approach for accurate quantification of transcript levels. Methods: Enriched poly-A mRNA or ribo-depleted RNA (Universal Human Reference RNA) was used to make libraries with our strand specific library preparation method. Library quality and quantity were analyzed on an Agilent Bioanalyzer, pooled at equimolar ratio and sequenced on Illumina’s Nextseq 500. Paired end reads were mapped to a human reference genome (hg19) using Hisat2 and sequencing metrics were calculated using Picard’s RNA-seq Metrics and RSeqC tools. Transcript abundance was measured using Salmon and the RSeqC tool. Resolution of GDS sequences were obtained from RNAseq by generating a strand-minded method using inputs that range from 5ng to 1ug show greater than 98% directionality at all input levels. GC content analysis, gene body coverage and gene expression correlation are similar for all inputs tested (5ng to 1ug), even though input amounts vary by over three orders of magnitude. These consistent results are recapitulated with the spiked-in ERCC controls at all inputs. Conclusions: Our library preparation method is streamlined and can be used for a wide range of input RNA without any major modifications to the protocol, making it an easy to follow, convenient method for RNA-seq library preparation. In addition, our method has increased sensitivity and specificity, especially for low-abundance transcripts, reduced PCR duplicates and sequence bias, delivering high quality strand-specific data even for low input RNA. Finally, our method is compatible with both poly-A enriched and non-poly-A depleted samples, and is amenable to large-scale library construction and automation.

#5407 Integrated analysis of genome-wide microRNA and mRNA profiling reveals deregulated miR-125b associated with poor prognosis by reducing cell apoptosis in gastric cancer. Ben Liu,1 Da Yang,2 Fengju Song,1 Xining Zhang,1 Yan Guo,1 Yanrui Zhao,1 Hong Zheng,1 Wei Zhang,2 Kexin Chen.1

Tianjin Medical University Cancer Institute and Hospital, Tianjin, China; 2University of Texas MD Anderson Cancer Center, Houston, TX

Background & Aims: In our prior study, an integrated analysis of microRNA and mRNA profiling of gastric cancer (GC) revealed a microRNA-regulatory network. It defined a GC microRNA subtype that was associated with poor survival in 90 GC cases. In this study, we further demonstrate that miR-125b, a key node in this microRNA regulatory network, is upregulated in GC and associated with poor overall survival by interfering in the apoptosis pathway. Methods: To further investigate the role of miR-125 in GC, we conducted both an in vitro experiment and clinical study (cohort of 373 GC samples). miR-125b and targets in the network were validated through RT-PCR and tissue microarray GC cases. The miR-125b targeted gene and related pathway were identified by a gene expression profile analysis in miR-125b transfected GC cell lines. The Annexin V-FITC and TUNEL assay were used to assess apoptosis. MTT and flow cytometry were performed to detect the effect of miR-125b on proliferation and cell cycle. The wound healing test and transwell assay were used to analyze migration and invasion. Results: The markedly upregulated expression of miR-125b was validated in 373 GC samples. Univariate and multivariate analyses find that the miR-125b was associated with poor prognosis (p = 0.011). Pathway analysis shows that the predicted targets of miR-125b are highly involved in apoptosis/program death pathway. The robust apoptosis genes, BIK and CASP6 are validated as the directed targets of miR-125b. miR-125b can suppress apoptosis and increase cell growth, migration and invasion in GC cell lines. Furthermore, low expression of BIK (p = 0.034) and CASP6 (p = 0.048) in GC were associated with poor disease-free survival. Conclusions: The apoptosis pathway was found to be dominant in the pathway construction based on target genes of miR-125b. Our findings suggest that miR-125b-regulated apoptosis pathway is important to GC progression and that the miR-125 may be an important clinical biomarker for GC survival and possible therapeutic target for GC treatment.

#5408 RNA fusion detection in thyroid nodules with a targeted RNA sequencing panel. Song Tian,2 Michelle Baird,1 Frank Reinecke,2 Jixin Deng,1 Mohammad Nezami Ranjbar,1 Raed Samara,1 Eric Lader1.

1QIAGEN Sciences Inc., Frederick, MD; 2QIAGEN GmbH, Hilden, Germany. Inc., Frederic, MD; 3QIAGEN GmbH, Hilden, Germany.

Thyroid nodules are quite common and detected in as high as 67% of the population. Only a small portion of thyroid nodules are malignant thyroid cancers. Advances in understanding the molecular pathogenesis of thyroid cancer are leading to the development of better biomarkers and novel targeted therapies. The tumorigenic role of RNA fusions has been reported in many forms of cancer. Fusions can result from structural rearrangements like translocations and deletions, transcription read-through of neighboring genes, or the trans- and cis-splicing of pre-mRNAs. Many fusions act as key drivers for cancer development. Detection of fusion RNA is a challenge with traditional technologies like FISH and qPCR, as they are laborious, low throughput, and do not provide detailed information about the fusion. Here we explored the possible use of a tumor-fusion-targeted RNA-based panel (QIAsert targeted RNAscan panel, which is targeting 223 fusion gene pairs, 576 unique fusions) as a tool to study

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fusions in thyroid nodules. The presence of these fusions were surveyed in RNA samples from 10 thyroid cancer (PTC and FVPTC) samples and 19 non-cancer control samples (NH and FA) using this oncology targeted panel and sequenced on the illumina NextSeq. PAX8-PPARG, a fusion gene with a well-established link to thyroid cancer, was found in the cancer group. In addition, we observed a unique rearrangement within the recurrent rearrangement that was FUS-DDX1, in all thyroid nodules. Our results suggest that a targeted RNA-based panel provided a useful tool for fusion gene study in thyroid cancer. The role of the expected rearrangement and the unexpected read-through fusion in thyroid nodules needs to be further evaluated, as it could provide useful information for thyroid nodule differentiation.

#5409 Assessment of the MammaPrint 70-gene profile using RNA-seq sequencing technology. Lorenza Mittempergher,1 Jacob B. Spangler,2 Mireille H. Snel,3 Leonie J. Delahaye,1 Iris de Rink,3 Sun Tian,3 Annuksa M. Glas,1 Rene Bernards3,1 Agendia NV, Amsterdam, Netherlands; 2Agendia NV, Irvine, CA; 3Netherlands Cancer Institute, Amsterdam, Netherlands.

Introduction: Improvements in RNA processing have enabled microarray diagnostics for formalin-fixed, parafin-embedded (FFPE) tissue. Recently, MammaPrint, a prognostic 70-gene profile for early-stage breast cancer, was successfully translated to FFPE tissue showing to be substantially equivalent to fresh tissue. In recent years, RNA-sequencing (RNA-Seq) became the standard method for transcriptome analysis, because of its low background signal and its ability of quantifying a large dynamic range of expression levels. Here we report a preliminary analysis of the FFPE MammaPrint 70-gene profile using RNA-seq technology with the comparison of the MammaPrint microarray diagnostic test in a series of FFPE samples. Methods: RNA-Seq was carried out using a strand-specific RNA library preparation followed by target enrichment of the coding region of the human transcriptome without relying on the presence of poly-A tail. RNA sequencing libraries were prepared starting from a minimal amount of 20 ng of total RNA based on the CV 200 metric assessment. The library pools were single-end sequenced on the Illumina HiSeq 2500 instrument at the length of 65bp. The resulting sequences were mapped to the human reference genome (build 38) using TopHat v2.1. Tophat was guided by using a transcriptome index from Ensembl (version 77). The HTSeq-count tool was used to generate the total number of uniquely mapped reads for each gene. Gene expressions were normalized with Count Per Million (CPM) normalization and log2 transformed afterwards. Microarray data of the sample were available for analysis comparison. Results: On average, we obtained 22 million reads assigned to gene per sample (min = 15M, max = 28M). The number of reads assigned to genes vary from 61% to 70% of the total number of reads. Between 80% and 90% of the reads assigned to genes mapped to protein coding genes which is comparable to fresh frozen material. The 70-gene signature was successfully mapped to the RNA-Seq gene. A median raw read-count of 384 was observed for the 70-gene profile among the samples. Importantly, we observed a high concordance (R2 Pearson correlation = 0.97) between the MammaPrint index calculated using the RNA-Seq data and the corresponding Microarray MammaPrint index. Additionally, the BluePrint profile, a microarray diagnostic test for breast cancer molecular subtyping, was successfully translated to the RNA-Seq platform. All three MammaPrint microarray diagnostic panels showed high concordance between the two technologies with high correlation values for each of the subtypes (Luminal R2 Pearson correlation = 0.98, Basal R2 Pearson correlation = 0.97, HER2 R2 Pearson correlation = 0.77). Conclusions: Next Generation RNA-sequencing is a feasible technology to assess diagnostic signatures, such as the 70 gene MammaPrint and BluePrint profiles.


Recent advances in library preparation methodology from limiting amounts of total RNA have facilitated the characterization of rare cell-types in various biological systems. The SMART-Seq v4 Ultra Low Input RNA Kit incorporates a number of workflow improvements, including fewer purifications to increase yield, a locked nucleic acid (LNA) template-switching oligo to enhance stability, and an improved polymerase designed to reduce amplification bias of GC-rich regions. Certain workflow challenges remain however, including variability in library insert size, which can lead to less predictable sequencing results. We have modified the SMART-Seq v4 method to incorporate library size selection between 250-650 bp, and have characterized analytical performance of the modified procedure. A total of 63 libraries were generated using 10 pg - 10 ng of Universal Human Reference RNA (UHRR), Human Brain Reference RNA (HBRR), and total RNA isolated from multiple human tissues, including lung, colon, spleen, adrenal tumor, heart, and lung tumor. To facilitate the comparison between libraries, we normalized library read counts by down-sampling to 24 million reads prior to further processing with an internally-developed sequence analysis pipeline (RNAv9_rsem). The values for ERCC Limit of Detection ranged between 3-50 copies with a mean of 22 copies, which confirmed the high sensitivity of SMART-Seq v4 kit. The number of genes detected (RPKM, or reads per kilobase per million mapped >= 3) varied as a function of the amount of input RNA, with as many as 19,500 genes detected. Gene detection was highly consistent across replicates utilizing 100 pg-10 ng input RNA, with a precipitous decline in genes detected using 10 pg of RNA input. This is due to less reliable detection of low abundance genes, which in turn leads to increased discordance in detected genes between replicates, possibly due to increased sampling error. Nonetheless, when genes are commonly detected, their normalized read counts are highly correlated at any given RNA input, with correlation coefficients (r) ranging from 0.977 to 0.993. Compared to previous versions of the SMARTer method, the modified SMART-Seq v4 procedure implemented at Q2 Solutions - EA Genomics significantly improved the percentage of reads aligned to the transcriptome, as well as the total number of genes detected, all with reduced technical variability. Our study demonstrates an improved strategy for expression profiling via RNA sequencing from limiting amounts of RNA.

#5411 Demonstrated improvements in tumor profiling with enzymatic ribosomal RNA depletion and streamlined RNA-seq library preparation. Nancy H. Nabilis,1 Drew Cheney,2 Leonard Cloete,2 Ida van Jaarsveld,2 Rachel Kasinskas,1 Jennifer Pavlica,1 Roche Diagnostics, Wilmington, MA; 2Roche Sequencing Solutions, Salt River, Cape Town, South Africa.

High resolution RNA analysis using next-generation sequencing (RNA-seq) is a rapidly growing application in cancer research. Formalin-fixed paraffin-embedded (FFPE) tissue is a ubiquitous resource for clinical studies. However, the ability to generate high quality libraries is confounded by the low quality and yield of RNA extracted from FFPE specimen. To enable optimal interrogation of such a clinically-relevant tissue resource, it is critical to identify RNA-seq library construction workflows that improve performance using challenging and degraded sample types. In this study, the effects of RNA type, quality, and quantity on RNA-seq library construction are assessed, and expectations regarding sequencing data quality are addressed. Included are recommendations for quality control measures and input-specific specifications that may improve performance. Three workflows employing either bead-based or enzymatic strategies for the removal of ribosomal RNA content were used to prepare RNA-seq libraries from degraded paired fresh normal and tumor samples. Enzymatic depletion followed by library preparation using the KAPA RNA HyperPrep workflow identified the highest number of transcripts for each sample, translating to a 4.9-fold increase in number of differentially expressed transcripts detected between normal and tumor samples. Of the transcripts identified as differentially expressed exclusively by Kapa, 174 are protein-coding RNAs, many of which are frequently disregulated in breast cancer. In contrast, only two protein-coding RNAs were identified exclusively by the alternative workflows. RealTime Ready Custom panels were used for independent quantitative rt-PCR assessment. A subset of 71 transcripts identified exclusively by Kapa, up to 69 (97%) were validated as differentially expressed. Additional performance improvements of the KAPA RNA HyperPrep workflow include coverage balance across transcripts, lower duplication rate, technical replicate reproducibility, agreement across input quantities, and gene expression concordance between paired fresh frozen and FFPE samples. For Research Use Only. Not for use in diagnostic procedures.

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Genomic Analyses of Circulating Tumor Material and RNA
Objective of this work is to determine the biologic effects of modulating cellular metabolism through inhibition of MCT1 and MCT4 in canine osteosarcoma (OS). A well-established spontaneous large animal model of the human disease, Methods: MCT1 and MCT4 expression was assessed in canine OS cell lines using qRT-PCR and western blot for the purpose of establishing potential MCT4 and MCT1 expression in canine OS tissues. The effects of small molecule inhibitors (NGY066 and NGY008) and shRNA approaches targeting MCT1 and MCT4 on proliferation and survival of canine OS were examined using the CyQUANT Cell Proliferation and Caspase-3/7 assays, respectively. The impact of MCT1/4 inhibition on cellular oxygen consumption was evaluated using the Seahorse Cell Mito Stress Test. Lastly, potential synergistic effects of combination treatment with doxorubicin with MCT1/4 inhibition in canine OS cell lines were investigated using the CyQUANT Assay. Results: We found that while both MCT1 and MCT4 are expressed in canine OS lines and tissues, MCT1 is expressed at higher levels relative to MCT4 in the cell lines. Small molecule inhibitors directed against MCT1 and MCT4 did not significantly inhibit cell proliferation or induce apoptosis even in the presence of limiting cell culture conditions. Maximally respiratory capacity was not increased in canine OS cell lines following inhibition of MCT1 and MCT4 function. However, synergistic anti-proliferative activity was observed when OS cells were treated with doxorubicin or metformin in the presence of MCT1 and MCT4 inhibitors. Conclusions: MCT1 and MCT4 are expressed in canine OS, and therefore represent a good model to evaluate the impact of MCT1/4 inhibition on cell oxygen consumption. UHPLC/GC-MS metabolomics analysis of plasma samples revealed a significant downregulation of a monosaccharide regulation network. We further validated our data in vivo (in human melanoma xenografts). Further, in another study we demonstrated, based on a large-scale label-free comparative proteomics analysis, that PLK1 inhibition via the small-molecule inhibitor BI6727 (Volasertib) in human melanoma cells resulted in an alteration of certain metabolism-associated proteins with an associated decrease in cellular metabolism. In this study, to further explore the association between PLK1 and cellular metabolism, we utilized a doxycycline-inducible PLK1 knockdown approach in A375 melanoma cells coupled with a Human Glucose Metabolism PCI array that covers 84 key genes involved in the regulation and enzymatic pathways of glucose and glycogen metabolism. We found that PLK1 knockdown resulted in a significant downregulation of 29 genes and upregulation of 3 genes (more than 2 fold change) associated with cellular metabolism. LDH1, PFD2 and PCK1 were in the top 5 genes differentially regulated while FBPI was >7-fold upregulated. Through Ingenuity Pathway Analysis (IPA), we identified glycolysis and the pentose phosphate pathway as major canonical pathways altered by PLK1 inhibition. Further, IPA identified that PLK1 inhibition-modulated genes were largely associated with the proliferation of cells, where FBPI appeared as a key regulatory player. PCK1 was found to be highly downregulated in our array, and IPA identified it as part of a monosaccharide regulation network. We further validated our data in vivo. Further, we found that BI 6727 treatment resulted in a decrease in PCK1 and increase in FBPI in A375 melanoma cells implanted xenografts. In addition, we observed a strong inverse correlation between PLK1 and FBPI in multiple melanoma cell lines, with FBPI expression significantly downregulated in a panel of melanoma
cells compared to normal melanocytes. Moreover, BI 6727 treatment resulted in an upregulation in FBPI in A375, Hs 294T and G361 melanoma cells. Interestingly, in recent studies, FBPI (fructose-bisphosphatase) that is a rate-limiting enzyme in gluconeogenesis, has been shown to function as a tumor suppressor in certain cancers. Overall, our study support the hypothesis that PLK1 is a regulator of metabolism maintenance that affects the melanoma cell growth.

#5416 Downregulation of PRMT6 promotes the Warburg effect in hepatocellular carcinoma via PKM2. Tin Lok Wong, Lok Hei Chan, Stephanie Ma. The University of Hong Kong, Hong Kong, Hong Kong.

Hepatocellular carcinoma (HCC) is one of the top ranked causes of cancer death in the world, accounting for more than 740,000 deaths in 2012. Treatment of HCC is hindered by recurrence and resistance to chemotherapy. Altered metabolism in cancer has been linked to increased tumorigenicity and resistance to chemotherapeutics. One of the main features of altered metabolism is the Warburg effect, which is widely observed in solid tumor including HCC. Recently, our group have identified protein arginine methyltransferase 6 (PRMT6) as a tumor suppressor protein in HCC. Protein arginine methylation is a post-translational modification implicated in a variety of cellular functions including signaling and regulation of gene expression. Downregulation of PRMT6 expression was observed in HCC patients and knockdown of PRMT6 in HCC cell lines increased the tumorigenicity and sensitivity to both chemo- and molecular-targeted therapies. Other members of the Type I PRMT protein has previously been reported to be involved in glucose metabolism. Microarray profiling of HCC cells stably knockdown and overexpressed of PRMT6 showed enrichment of genes related to glycolysis. We therefore hypothesized that reduced expression of PRMT6 in HCC drives the Warburg effect to support cancer progression. Metabolome profiling of HCC cells stably knockdown and overexpressed of PRMT6 showed enrichment of metabolites in glycolysis. Using Seahorse Extracellular Flux Analyzer, we show that stable knockdown of PRMT6 in HCC cell line drives glycolysis. This enhanced glycolysis rate is coupled with increased uptake of glucose, lactate production and pyruvate kinase activity. mRNA expression level of several glycolysis-related genes was screened by real-time quantitative PCR and we identified PKM2, a well-known regulator of the Warburg effect, as a potential link between PRMT6 and enhanced glycolysis. In addition, by immunofluorescence staining, we found that PRMT6 knockdown led to upregulation of PKM2 and PKLR, suggesting that PKM2 is the sole contributor for the Warburg effect, as a potential link between PRMT6 and enhanced glycolysis. Knockdown of PRMT6 leads to an increase in mRNA and protein expression level of PKM2, but not PKM1 and PKLR, suggesting that PKM2 is the sole contributor for enhanced pyruvate kinase activity. In conclusion, PRMT6 regulates the expression level of PKM2 and glycolysis of HCC cell. Ongoing studies are in progress to delineate the underlying mechanisms by which PRMT6 regulates PKM2 to drive Warburg effect.

#5417 The tumor suppressor klotho: A master regulator of metabolism in breast cancer. Tami Rubinek, Ido Wolf. Tel Aviv Medical Center, Tel Aviv, Israel.

One of the hallmarks of cancer is reprogramming of energy metabolism, characterized by a shift to aerobic glycolysis (Warburg effect). A major regulator of this phenomenon is the IGFR-1/Pi3K/akt pathway. Klotho is a transmembrane protein which can be cleaved, shed and act as a cytokine. Piscator et al. describe that Klotho-deficient mice manifest a syndrome resembling accelerated aging, while klotho overexpression extends life span. Klotho is a potent tumor suppressor in BC. As klotho is a potent inhibitor of the IGFR-1/Pi3K/akt pathway in BC, we hypothesized that it may revert the metabolic switch in BC cells. We first analyzed the effect of klotho on the energy sensor enzyme AMP-activated kinase (AMPK) and its downstream effector acetyl-CoA carboxylase (ACC). Overexpression of klotho, or treatment with the soluble protein, elevated AMPK and ACC phosphorylation in three BC cell lines. AMPK is activated by the tumor suppressor liver kinase B1 (LKB1) and elevated AMP/ATP ratio. Expression of a dominant-negative LKB1 prevented activation of AMPK by klotho and decreased the ability of klotho to inhibit cell growth and migration, implying that klotho tumor suppressor activities are LKB1 dependent. We next analyzed the effects of klotho on critical components of glucose metabolism. Treatment with soluble klotho reduced expression of the glucose transporter GLUT1 and the key glycolytic enzymes hexokinase2 (HK2), phosphofructokinase1 (PFK1), pyruvate kinase M2 (PKM2) and pyruvate dehydrogenase kinase 1 (PDK1). Using the Seahorse analyzer we noted reduced oxidative mitochondrial metabolism and glycolysis following klotho treatment. Energy is obtained essentially in the mitochondria with the transfer of protons across the inner membrane that produces ATP. We, therefore, analyzed klotho effect on the mitochondria membrane potential and noted reduction in the mitochondria potential following treatment with klotho. Finally, we examined the effects of klotho on specific metabolites and also conducted an NMR-based metabolic profiling. In accordance with the alterations in signaling pathways and levels of glycolytic enzymes, klotho reduced glucose uptake and inhibited lactate, pyruvate and 3-hydroxybutyrate production. Furthermore, klotho also inhibited ATP production. Taken together, our data indicate klotho as a regulator of metabolic activity in BC and suggest that reversal of the metabolic switch is a key mechanism of klotho-mediated tumor suppressor activities.

#5418 Deciphering the dynamics of alternative pre-mRNA processing of glutamine in metastatic ovarian cancer. Chioniso P. Masamha, Patrick LaFontaine, Bettine E. Gibbs. Butler University, Indianapolis, IN.

The chronically proliferative cancer phenotype requires metabolic reprogramming to meet the increased energy and biosynthetic demands of rapid cell division. This results in increased glucose uptake and usage (the Warburg effect). Cancer cells that undergo the Warburg effect may also become reliant on glutamine, a term called 'glutamine addiction'. Recent studies in ovarian cancer suggest that highly invasive ovarian cancer cells show a remarkable dependence on glutamine, hence implicating glutamine metabolism in metastasis. Glutaminase is the first enzyme that is involved in glutaminolysis and it catalyzes the rate-limiting conversion of glutamine to glutamate and ammonia. There are two genes that code for glutaminase in the human genome, glutaminase 1 (GLS1), and glutaminase 2 (GLS2). GLS2 is associated with cell differentiation whereas GLS1 expression is up-regulated in cancer. Hence, GLS1 had been proposed to be better adapted to meet the altered metabolic needs of the tumor phenotype. GLS1 can be alternatively spliced into two isoforms, KGA and GAC. Although both KGA and GAC have been implicated in cancer cell metabolism, there is still controversy over the actual isoform that is most important for tumorigenesis. Whereas the KGA isoform is repressed by miR-23, there is no documented miRNA repression of GAC suggesting that GAC is more tumorigenic. Using next-generation sequencing, we recently identified an unannotated novel KGA transcript with a truncated 3' untranslated region (3'UTR) which is more stable than the traditionally known KGA transcript. Our goal is to determine the expression profile of different glutaminase isoforms in the highly lethal gynecological malignancy, ovarian cancer, and their role in tumorigenesis and metastasis. We have developed amplicons to measure total GLS1 and GLS2 transcripts using quantitative real time PCR (qRTP-PCR). Although we unexpectedly detected both GLS2 and GLS1 transcripts, the mRNA levels of GLS2 were significantly lower than those of GLS1. To determine the specific GLS1 isoforms expression we used isoform specific amplicons and detected both GAC and KGA using qRT-PCR. Western blot analysis was able to detect both GAC and KGA protein in one cell line only suggesting that the KGA mRNA we detected was subject to miR-23 repression. Inhibition of total GLS1 activity with a glutaminase inhibitor resulted in decreased colony formation as shown by soft agar assays. In conclusion, the expression profile of different GLS1 isoforms in highly invasive ovarian cancer supports the role of glutamine metabolism in maintaining the metastatic phenotype. This makes glutamine metabolism a viable therapeutic target for metastatic ovarian cancer.

#5419 The relationship between glutamatergic signaling and altered glutamine metabolism in melanoma. Raj Shah, Andrew Boreland, Suzie Chen. Rutgers University, Piscataway, NJ.

Aberrant glutamatergic signaling has been implicated in many cancer types and is associated with dysregulated growth leading to cellular transformation and tumorigenesis. Our laboratory has previously illustrated the oncogenic properties of a neuronal receptor, metabotropic glutamate receptor 1 (GRM1) in melanocytes. Glutamate is the natural ligand of GRM1 and the major excitatory neurotransmitter in the central nervous system. Our group has demonstrated that glutamate production/release is upregulated in GRM1 expressing melanoma cells, resulting in constitutive activation of GRM1 and GRM1-associated downstream signaling pathways. We hypothesize that this activation of GRM1 in melanoma cells is associated with higher expression of c-Myc and increased enzymatic activity of glutaminase (GLS) converting glutamine to glutamate. We showed that reducing the extracellular glutamate levels by an inhibitor of glutamate release, Riluzole, led to significantly reduce melanoma cell proliferation in vitro and tumor progression in vivo. We also demonstrated that inclusion of CB-839, a potent, selective, and orally bioavailable GLS inhibitor, resulted in a significant inhibition of GRM1-expressing melanoma cell proliferation compared to GRM1 negative melanoma cell lines. Interestingly, we found that simultaneous targeting of glutamate production (CB-839) and release (Riluzole) in GRM1 expressing melanoma cells led to enhanced suppression of cell proliferation in vitro. Additionally, we found that wild type BRAF melanoma cells are more sensitive to CB-839 than mutated BRAF cells while responses to Riluzole were independent of BRAF genotypes. Furthermore, our data indicate that CB-
Molecular and Cellular Biology / Genetics: Metabolic Regulation and Cancer Therapy 2

#5420 PGAM1-REDD1 pathway promotes paclitaxel resistance through enhancing aerobic glycolysis in ovarian cancer cells. Y. Yang, Weiguo Lv, Xing Xie. Women's hospital School of Medicine Zhejiang Univ., Hangzhou, China.

Aerobic glycolysis is a dominant pathway for energy production in cancer cells, but the association of this phenomenon with chemoresistance remains still uncertain. Here, we found that the expression of phosphoglycerate mutase 1 (PGAM1), a key catalytic enzyme in aerobic glycolysis, was significantly higher in paclitaxel-resistant ovarian cancer cell line SKOV3. Up-regulated PGAM1 expression by gene transfection and down-regulated PGAM1 expression by siRNA knockdown altered the process of aerobic glycolysis, paclitaxel sensitivity and DNA damage response 1 (REDD1) expression in ovarian cancer cells, vice versa. Structural interaction between PGAM1 and REDD1 was predicted by molecular docking. Our results suggested that PGAM1-REDD1 pathway promotes paclitaxel resistance through enhancing aerobic glycolysis in ovarian cancer cells.

#5421 Differential expression of lysosomal acid lipase (LAL) between benign and malignant tissues among human organs. Mohamed El Gassim, Jinghao Mao, Xinchuan Zhou. Univ. of Mississippi Medical Ctr., Madison, MS; Tuogalo College, Tuogalo, MS.

Background: Lysosomal acid lipase (LAL), a lysosomal enzyme is able to degrade cholesteryl esters to free fatty acids and cholesterol. The information on the association of LAL with malignancies is limited. This study is to determine the difference in expression level of LAL between benign and malignant tissues among human organs. Method: Immunohistochemistry (IHC) for human LAL was performed on A tissue microarray (TMA) containing 36 benign and malignant tissues from 36 human organs. The expression level of LAL was represented by an IHC score calculated by multiplying area score (0–3) with intensity score (0–3), ranged from 0 to 9. Student’s t-test was used to compare the mean IHC score between benign and malignant tissues in same organs. Results: Eighteen out of 36 human organs have IHC stains on both benign and malignant tissues. As shown in Table 1, LAL expression level was higher in malignancy than in benign tissues in 9 organs. The difference in LAL expression level was significantly (or nearly lower in malignant tissues of adrenal gland, stomach, liver, testis and kidney. Conclusion: The expression level of LAL varies among human organs. As compared with elevation of LAL expression level, decreased expression level of LAL in malignancies could be of more significance.

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<tr>
<th>Organ</th>
<th>Table 1 Difference in expression level of LAL</th>
<th>p value</th>
<th>M/B Ratio</th>
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<td>Mean</td>
<td>SE</td>
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<tr>
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<td>0.73</td>
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<tr>
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#5423 ZAP70 alters metabolism in chronic lymphocytic leukemia cells. Subhadip Das, RF Dielschneider, Elizabeth S. Henson, Spencer B. Gibson. *Cancer Care Manitoba & University of Manitoba, Winnipeg, Manitoba, Canada; University of Manitoba, Winnipeg, Manitoba, Canada; Cancer Care Manitoba, Winnipeg, Manitoba, Canada.

Chronic lymphocytic leukemia (CLL) has the highest incidence among adults in western countries. Prognosis of CLL is highly variable but one of the biomarker zeta-chain-associated protein 70 (ZAP70) overexpression is associated with aggressive disease with time to treatment being 2.6 years for ZAP70 expressing (ZAP70+) patients and 8 years for ZAP70 deficient (ZAP70-) patients. Thus ZAP70 may play a role in CLL progression. Metabolic reprogramming plays a central role in cancer progression and is altered in CLL cells. However, the role of ZAP70 in metabolism in CLL cells is remains unclear. We found that ZAP70 binds to pyruvate kinase M2 (PKM2). PKM2 is key isoform of pyruvate kinase which is rate limiting enzyme of the glycolytic pathway and provides a metabolic advantage to tumour cells allowing for increased growth. Our results also indicated the PKM2 is expressed in CLL cells and binding to ZAP70 is not affected by glucose starvation or treatment with kinase inhibitors. Furthermore immunofluorescence microscopy revealed that ZAP70 and PKM2 colocloze in CLL cells in the cytoplasm. In addition, we found ZAP70+ cells have increased rate of oxygen consumption from basal level determined by addition of inhibitor (Oligomycin A) and mitochondrial uncoupler (FCCP). Simultaneously ZAP70+ cells also have higher extracellular acidification rates. This indicates ZAP70+ CLL cells have increased mitochondrial respiratory capacity with high glycolytic rate. In addition, fluorometric analysis indicates ZAP70+ CLL cells have high glucose consumption rate towards production of lactate compared to ZAP70- CLL cells. Collectively, our data reveals a new function for ZAP70, which is to regulate the Warburg effect and may play a role in CLL progression and may identify new therapeutic targets.
#5424 Metformin increases PDH and suppresses HIF1A under hypoxic conditions and induces cell death in oral squamous cell carcinoma. Talita Guimaraes,1 Lucyana Farias,1 Carlos Fraga,1 Alfredo De Paula,1 Sergio Santos,2 Ricardo Gomez,2 Andre Guimaraes1. 1Unimontes, Montes Claros, Brazil; 2Universidade Federal de Minas Gerais, Beo Belo Horizonte, Brazil.

Background: Metformin is a biguanide, belonging to the oral hypoglycemic agents and is a widely used in the treatment of type 2 diabetes. Evidence indicate that Metformin inhibits cell proliferation in several human cancers and inhibits the Warburg phenomenon in tumor cells. Objective: The present study aims to explore the effects of Metformin in hypoxic conditions. Specifically, we focused on pyruvate dehydrogenase (PDH), (hypoxia-inducible factor 1α) HIF-1α levels and the Warburg phenomenon in OSCC cell phenotype. Additionally, we also investigated a theoretical consequence of Metformin treatment. Methods: PDH levels in patients with OSCC and oral dysplasia were evaluated. Metformin was administered in vitro to test the effect of Metformin under hypoxic conditions. The results were complemented by Bioinformatics analyses. Results: Low PDH levels were observed in OSCC, and Metformin promotes an increase in PDH levels in hypoxic conditions. Metformin also reduced HIF-1α mRNA and protein levels. Metformin demonstrated antiproliferative effects, inhibited migration, increased the number of apoptotic cells and increased the transcription of caspase 3. Conclusions: In conclusion, our current findings show that Metformin reduces HIF-1α gene expression and increases PDH expression. Metformin inhibits cell proliferation and migration in the OSCC cell line model. Additionally, Metformin enhances the number of apoptotic cells and caspase 3 levels. Interestingly enough, Metformin did not increase the mutant p53 levels under hypoxic conditions.

#5425 The role of six transmembrane epithelial antigen of the prostate 2 in hepatocellular carcinoma. Carla R. Zeballos, Hakim Boutam, Xiang Gu, Yi-dong Chen, Francisco G. Cigarroa, LuZhe Sun. University of Texas Health Science Center at San Antonio, San Antonio, TX.

Introduction: The incidence of hepatocellular carcinoma (HCC) in Hispanics is three times higher than non-Hispanic whites, and even higher in South Texas (STX) Hispanics. This is attributed to a higher prevalence of hepatitis C, diabetes, obesity and perhaps genetic and epigenetic alterations. Knowledge regarding genetic alterations in Hispanics is sparse as demonstrated by the lack of Hispanics with HCC in The Cancer Genome Atlas (TCGA). Therefore, our group sequenced paired adjacent liver and HCC tumors from STX Hispanics, which highlighted a gene over-expressed in tumors of Hispanics, called the Six Transmembrane Epithelial Antigen of the Prostate 2 (STEAP2). STEAP2 is a metalloendocytosis of iron and copper and is implicated in iron uptake and reactive oxygen species in the liver which can lead to the progression of inflammation and cirrhosis. STEAP2 may play an important oncogenic role in HCC, especially in the setting of obesity. We propose to test the hypothesis that over-expression of STEAP2 will lead to malignant property in HCC cells resulting in enhanced proliferation, survival, invasiveness, and eventually development of HCC, especially in obese hosts. Methods: Hispanic paired tissue continues to be collected from our institution for RNA sequencing and establishment of Hispanic HCC cell lines. STEAP2 RNA and protein expression levels in Hispanic paired samples versus Caucasian paired samples were evaluated by RT-PCR, Western blot, and immunohistochemistry. Knockdown and overexpression of STEAP2 were established in HCC cell lines and in primary Hispanic HCC cell lines to examine the effects on iron levels, oxidative stress, proliferation, invasiveness, apoptosis and cell cycle in vitro. Results: Hispanic HCC RNA sequencing data compared to TCGA HCC RNA sequencing data (no Hispanics) demonstrated the overexpression of STEAP2 in HCC tumors in Hispanic patients, which were validated by RT-PCR data and Western blot data. Lipid peroxidation product, 4-hydroxynonenal, and copper levels were higher in HCC tumor versus adjacent tissue. Iron levels were higher in adjacent tissue versus tumor tissue in Hispanics. Knockdown of STEAP2 in SNU398 cells decreased proliferation and migration, while in HUH7 cells STEAP2 knockdown only decreased migration. Conclusions: STEAP2 is specifically overexpressed in HCC tumors in Hispanics in comparison to HCC tumors in non-Hispanic whites and appears to play a malignant-promoting role in HCC cells. Further studies to establish the role of STEAP2 as a tumor promoter in HCC and the mechanisms by which it promotes carcinogenesis are underway. The proposed studies will likely yield mechanistic insights into the molecular mechanisms that drive HCC development and progression in South Texas Hispanics and potential therapeutic targets involving STEAP2-mediated metal ion metabolism and oxidative stress.

#5426 LPA stimulates glycolytic shift in ovarian cancer. Ji Hee Ha, Ran-gaudshagar Radhakrishnan, Jeremy D. Ward, Muralidharan Jayaraman, Danny N. Dhanasekaran. University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Cancer cells resort to aerobic glycolysis to meet the increased energy consumption and growth requirement during active proliferation. While it has been observed that the cancer cells preferentially shift to aerobic glycolysis, the exceedingly high growth factors involved in this process is largely unknown. Since our previous studies have shown that lysophosphatidic acid (LPA) plays a critical role in carcinogenesis of ovarian cancer by stimulating glycolysis in ovarian cancer cells, our results using XFe96 analyzer indicate that LPA stimulates aerobic glycolysis in multiple ovarian cancer cell lines as well as in patient-derived ovarian cancer cells. Physiological role for LPA in this process is demonstrated by the observation that the inhibitors of LPA receptors attenuate the glycolytic shift stimulated by the ascitic fluid derived from ovarian cancer patients. We further demonstrate that the silencing Gz2, a down-stream signaling mediator for LPA, inhibits LPA-stimulated glycolytic shift in ovarian cancer cells. Further analyses indicated that LPA-stimulated metabolic reprogramming involves the generation of reactive oxygen species (ROS). Treatment of cells with N-Acetyl Cysteine (NAC), a ROS-scavenger, inhibited the glycolytic shift stimulated by LPA. In addition, our studies point to a role for LPA-stimulated HIF1α activation in this process. These results indicate that LPA-stimulated metabolic reprogramming in ovarian cancer cells involve ROS-generation via LPA-Gz2-HIF1α-dependent signaling pathway. Together with the findings that the glycolytic shift is crucially involved in tumor cell survival our studies point to LPA-Gz2-HIF1α signaling axis as a potential target for therapy in ovarian cancer.

#5427 Distinctions between the metabolic changes in globlastoma cells and glioma stem-like cells following irradiation. Elizabeth I. Spehalski, Cord Peters, Philip Totlon, Kevin Camphausen. NIH, Bethesda, MD.

Glioblastoma multiforme (GBM) is the most common primary malignant brain tumor in adults and, even with aggressive treatment that includes surgical resection, radiation, and temozolomide administration, prognosis is poor due to tumor recurrence. There is evidence that within GBMs a small number of glioma stem-like cells (GSCs) exist, which are thought to be radiation resistant and may be capable of repopulating a tumor after treatment. Like most cancers, GBMs largely employ aerobic glycolysis to create ATP, a phenomenon known as the Warburg effect. Experiments have shown that the cellular metabolism of GSCs differs from that of differentiated cells, making them an attractive target for novel therapeutic approaches. Much work has been done to analyze the metabolic profiles of GSCs with the goal of identifying potential therapeutic targets, but little data exists linking metabolic changes to radiation resistance. The purpose of this study is to characterize the metabolic differences between glioma stem-like cells and traditional GBM tumor cells with and without radiation treatment (IR). To this end, we compared the metabolism of a human derived GSC line with two commonly used GBM cell lines before and after IR. At baseline, we find that glioma stem-like cells are more quiescent than GBM cells, which have higher levels of both glycolysis and oxidative phosphorylation. GBM lines show higher levels of both basal and maximal respiration, as well as basal glycolysis and glycolytic capacity than GSCs. They also express higher levels of energy, glycolysis, and TCA cycle metabolites than GSCs. Inversely, GSCs demonstrate metabolic signs of quiescence such as decreased NEAA synthesis. After IR, the radiation sensitive GBM tumor cell line (H9251) exhibits increases in all metabolic pathways, whereas the levels of glycolytic and oxidative metabolites in the GSCs remain unchanged. All cell lines show an increase of ATP and NAD production following IR. These findings indicate that the metabolism of GSCs undergoes different alterations than that of GBM tumor cells after IR, making them an attractive target for novel therapeutic approaches in conjunction with radiation therapy. Additionally, differences in metabolic signatures between GSC lines could be useful for non-invasive diagnostic modalities such as 1H NMR spectroscopy.

#5428 Lactate production as a potential marker of HER2-addiction and Trastuzumab susceptibility. Lorenzo Castagnoli,1 Egidio Iorio,2 Ada Kocshorke,1 Cristina Ghirelli,1 Gianmauro Palombelli,2 Debora Gian,1 Matteo Dugo,1 Daniela Morelli,1 Roberta Perrone1, Elia Tagger1, Serena A. M. Papa. Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy; 1Istituto Superiore di Sanità, Rome, Italy.

Background: Although the introduction of the HER2-specific humanized monoclonal antibody Trastuzumab (T) in clinical practice revolutionized the treatment of HER2-positive breast cancer (BC), about half of the T-treated patients do not benefit or become refractory after its administration. Despite different molecular tumor profiles significantly associated with T benefit were reported, the identification of a non invasive clinical approach to select patients responsive or not to T is still a clinical unmet task. We recently reported that mammary cancer cell lines derived from spontaneous nodules arisen in mice...
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transgenic for the human d16HER2 splice variant (HER2-addicted) were significantly enriched in genes involved in glycolysis pathway versus those developed in mice transgenic for the human full-length HER2-positive (WTHER2) (HER2-non addicted) (1,2). Accordingly, the aim of our study was to define whether tumor lactate levels could reflect HER2 addiction and, in T, tumor susceptibility of the GLUT-1 overexpressing cells. GAPDH metabolic activity was measured using assay kits and live cell metabolic phenotyping with the Seahorse analyzer. Increased lactate production and proton production were observed in MCF7 cells and again this resulted in a stable, glycolytic phenotype with increased expression of CA-IX. While it is commonly believed that these transporters are involved in the increased glycolytic flux, an alternative hypothesis is that CA-IX expression is consistent with the high level of lactate in tumors HER2-addicted. Conclusion: Overall, even if in a dysregulated glucose metabolism is a tumor hallmark, our results provide relevant in vitro and in vivo data points to make the future potential non-invasive lactate detection as a possible biomarker of HER2-addiction and T-responsiveness. Supported by the Italian Ministry of Health and AIRC. References: 1) Castagnoli L et al, Cancer Res 2014. 2) Castagnoli L et al, Oncogene 2016. 3) Shiu KK et al, Oncogene 2014.

#5429 The Warburg Effect: protons suck. Shonagh Russell, Liping Xu, Rober J. Gillies. Moffitt Cancer Center, Tampa, FL.

Aerobic glycolysis (the Warburg Effect) is a hallmark of cancer and is associated with local invasion and metastasis. This metabolic phenotype results in acidification of the microenvironment in solid tumors, which is responsible for many of the known sequelae. For example, systemic buffer therapy directly and specifically increases extracellular tumor pH and reduces spontaneous and experimental metastasis in vivo. Further, extracellular acidosis can be a potent inhibitor of anti-tumor immunity. Removal of glycolytically-derived acids requires the activity of proton transporting mechanisms, such as NHE, V-ATPase and CA-IX. While it is commonly believed that these transporters are responding to the extracellular pH, increased glycolytic flux, an alternative hypothesis is that CA-IX expression is consistent with the high level of lactate in tumors HER2-addicted. Conclusion: Overall, even if in a dysregulated glucose metabolism is a tumor hallmark, our results provide relevant in vitro and in vivo data points to make lactate detection as a possible biomarker of HER2-addiction and T-responsiveness. Supported by the Italian Ministry of Health and AIRC. References: 1) Castagnoli L et al, Cancer Res 2014. 2) Castagnoli L et al, Oncogene 2016. 3) Shiu KK et al, Oncogene 2014.

#5430 GAPDH loss in a tumorigenic human glioblastoma cell line. Zachary Gaertner, Yi Lu, Jinsuh Kim, Gayatri Mohapatra, Ankit Mehta, Sanjani Lakka, Herbert Engelhard. Univ. of Illinois at Chicago, Chicago, IL.

Background: Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is a ubiquitous, multifunctional 37 kDa enzyme best known for catalyzing the sixth step in the Embden–Meyerhof pathway (glycolysis). GAPDH is a metabolic enzyme, but is also involved in other metabolic pathways. GAPDH is designated the “housekeeping” status, and is used as a standard (along with β-actin) for normalizing Western blots. While increased GAPDH expression has been described in hypoxia, diabetes, and some cancers, decreased expression is rare. Here we describe a new human glioblastoma cell line that exhibits GAPDH loss, while maintaining a high degree of malignancy. Methods: Primary cultures of human glioblastoma were initiated and maintained in DMEM with 10% FBS. A particular cell line (designated E297) grew readily, and was passaged more than 40 times. E297 cells were confirmed to be negative for HIV, HTLV, hepatitis B and C, CMV, and mycoplasma contamination. Cells in exponential growth were stained for routine markers, and studied by DNA flow cytometry. GAPDH expression was studied by Western blot analysis using polyclonal rabbit anti-human GAPDH IgG (Abcam, Rosemont, IL), with Hela, U138, U87 and U251 cells (as well as β-actin staining) as positive controls. For intracranial implantation into Wistar-Furth rats (male, 10-11 weeks), cells were suspended at 2 x 10^6 cells/ml. Using aseptic technique, rats were anesthetized, and placed into a stereotactic frame. A burr hole was drilled 3 mm right of the bregma and 25 μl of suspension injected (5 mm depth) over 10 min. by Hamilton syringe. Rats were studied by MRI and euthanized when symptomatic. Results: E297 cells have glial/epithelioid morphology, with a doubling time of 24 ± 2 hours in logarithmic growth phase, with a single DNA subpopulation with 28% S phase. They stain positively for GFAP, vimentin, bFGF, c-myc and p53, but fail to express GAPDH. 10/10 animals implanted intracerebrally developed becoming symptomatic and requiring sacrifice at 25 days (mean). Loss of GAPDH expression was confirmed by Western blot analysis. Conclusions: The E297 human glioblastoma cell line is highly aggressive and proliferates rapidly. It is tumorigenic in nonimmunosenescent rats but lacks GAPDH protein. We conclude that GAPDH expression is not essential for glioblastoma cell proliferation under routine culture conditions, and care needs to be taken when using GAPDH as a normalization standard for Western blots of cancer cells.

#5431 Androgen receptor regulates the mitochondrial pyruvate carrier to fuel oncometabolism in prostate cancer. David A. Bader, Nagireddy Putluri, Sean M. Hartig, Sean E. McGuire. Baylor College of Medicine, Houston, TX.

The metabolic underpinnings of androgen receptor (AR)-driven growth in prostate cancer (PCa) are underexplored, hindering the development of strategies to leverage the metabolic properties differentiating PCa from normal tissue. To this end, we discovered a subunit of the mitochondrial pyruvate carrier (MPC) as a direct AR target gene that is increased in CaP specimens and associated with clinical outcomes. Our observation suggests a targetable link between AR signaling and carbon trafficking in PCa. Indeed, MPC inhibition delays proliferation and alters metabolic properties in hormone sensitive and castrate resistant AR-driven PCa models. Next, using 13C metabolic flux analysis coupled with a reverse phase protein array, we determined MPC inhibition results in activation of the ε-aminocaproic acid (EAA) pathway and partially compensates for MPC inhibition by coordinating increased glutamine incorporation into the citric acid cycle. Therefore, to amplify the effect of MPC inhibition, we restricted glutamine availability during MPC inhibition and found this combination resulted in proliferative arrest. Last, we extended our efforts in-vivo and demonstrated combinatorial MPC/glutaminase inhibition resulted in a significant reduction in castrate-resistant prostate tumor xenograft growth in mice with no overt host toxicity. We anticipate our findings will accelerate the development of clinical strategies to therapeutically manipulate carbon flux to starve clinically lethal castrate-resistant PCa.

#5432 Characterization of metabolic fuel dependency in multidrug resistant breast cancer cells. Yoonseok Kam, Natalia Romero, Pamela Swain, Brian D. Dranka. Agilent Technologies, Lexington, MA.

Multidrug resistance (MDR) is a common resistant mechanism of cancer cells to cytotoxic drugs in systemic therapy. MDR is characterized by increased expression of ATP-dependent drug exporting pumps which remove cytotoxic compounds from the cytosol. However, the mechanism whereby cancer cells rapidly respond to this increased ATP demand is not fully understood although the glycolytic nature of MDR phenotype has been evidenced repeatedly by previous reports. We thus hypothesized that flexibility and dependency on mitochondrial fuels would be altered in concert with the switch to glycolysis. In order to more accurately analyze glycolytic pathway, we newly developed an assay to improve glycolytic rate measurements by ac-
counting for TCA-dependent CO₂ contribution to extracellular acidification and to correlate Seashore extracellular flux data with lactate production. In a comparison of glycolytic rates between MCF7 breast cancer cell line and its MDR variant MCF7/Dox using this new assay, we confirmed that in MCF7/Dox cells glycolysis rate under basal conditions is significantly increased compared to MCF7 wild type. In contrast, dependency on glucose and fatty acids mitochondrial oxidation was unchanged in MDR cells. Additionally, an increase in long chain fatty acid oxidation is observed when glucose and glutamine oxidation is blocked indicating that MDR cells have higher mitochondrial flexibility to compensate for inhibition of alternative fuels utilization although it preferentially uses glucose. Together these data demonstrate that acquisition of multidrug resistance in MCF7 cells fundamentally changes their metabolism from a glutamine-driven, oxidative phosphorylation dependent phenotype to a highly glycolytic and glucose-dependent phenotype. These findings have potential therapeutic relevance in the context of inhibition of specific mitochondrial fuel pathways to prevent therapy resistance.

**#5433 β-Tubulin inhibitors reduce GLUT1 membrane trafficking to attenuate tumorigenesis in glioblastoma subtypes**

Collin M. Labak,1 Meheedu R. Guda,1 Swapna Asuthkar,1 Neha Jain,1 Yining Lu,1 Ian Purvis,1 Jack Tuszynski,2 Alair K. Hoyle,1,2 John Fleischhacker1,2 and Chooi Y. Ho1,2,3

Glioblastoma is an aggressive, high-grade tumor with poor prognosis due to lack of sound therapeutic options. Mesenchymal subtype GBMs are particularly difficult to treat because they tend to proliferate in the subventricular zone, an area that breeds stem-like neural cells and proves nearly impossible to access for surgical resection. They also contribute to creating a hypoxic microenvironment and switch to glycolytic metabolism in what is known as the Warburg Effect. Here, we hypothesize that strategically inhibiting the glucose transporter, GLUT1, through cytoskeletal components that traffic it to the cell membrane may reverse the Warburg Effect, attenuating further tumorigenesis and decreasing the stemness of such cells. Datamining studies and immunoblot analysis conducted on human glioblastoma patient specimens (hGBM) demonstrated that GLUT1 is highly upregulated. Limiting dilution assay conducted using GLUT1 inhibitors-fasentin and 2-Doxy-D-glucose reduced the proliferation in mesenchymal cancer stem cell (CSC) subtype in in vitro culture. Simultaneously, mass spectrometric analysis revealed significant association between GLUT1 and β-tubulin 4 (TUBB4) in mesenchymal subtyped cells. This association was further confirmed by large-scale datamining and in immunoprecipitation studies from hGBM specimens, suggesting that TUBB4 may be a viable target in deterring the trafficking of upregulated GLUT1 to the membrane. Collectively, these studies confirm that GLUT1 is associated with TUBB4, and that targeting TUBB4 via siRNA or colchicine derivatives could prove effective in reversing the Warburg Effect in GBM cells and ultimately improve patient outcomes.

**#5434 Tumor necrosis factor receptor-associated protein 1 (TRAP1) as a potential target for glutamine addicted cancer cells.**

Yu T. Vo,1 Ai N. Phan,1 Tuyen N. Hua,1 Yangaik Jeong,1 Byoung Heon Kang,2 Hyun-Won Kim,1 Jong-Whan Choe1,1 Yonsei University Wonju college of Medicine; Wonju, Republic of Korea;2 UNIST, Ulsan, Republic of Korea.

Glutamine, a non-essential amino acid, is an important nutrient which is involved in many biochemical pathways such as energy production, macromolecular synthesis, and oxidative stress scavenging. Glutamine metabolism is dysregulated in many cancers which mostly display glutamine addiction for cell proliferation, as this amino acid is a major source of energy for treating cancer. Here we report that glutamine-dependent cancer cells are more susceptible for inhibiting cell proliferation with inhibitor treatment of Tumor Necrosis Factor Receptor-Associated Protein 1 (TRAP1), a downstream factor of oncogenic c-Myc involved in glutamine metabolism. Using cell proliferation and cell viability assays, we examined growth inhibition effects of TRAP1 inhibitor, gamitrinib-triphosphophosphonin (G-TPP), on two groups of cell lines, glutamine-deprivation sensitive versus resistant cell lines. Included are cell lines for each group: HCC827 acquired gefitinib resistance and A549 for the sensitive; HuH7 for the resistant group. Glutamine-deprivation sensitive cell lines showed significant growth inhibition with TRAP1 inhibitor treatment while the corre-
were measured by qRT-PCR/Tagman analysis (in total, 1,026 analyses) and normalized by the miR16 level. miR486 levels were up-regulated (p = 0.02) and miR203 (p = 0.0005) and miR205 (p = 0.041) levels were down-regulated in Stages I and II/III adenocarcinoma. Whereas miR122 and miR29c were not detected in serum samples from the majority of healthy subjects, the miRNAs were expressed in serum samples of cancer patients of each stage. The miRNAs associated with adenocarcinoma were miR203, 205, and 16, respectively, of Stages II/III lung cancer patients. A novel label-free 90 nm (diameter) miRNA nanowell technology was developed. Electrochemical analyses of the early lung cancer biomarker candidates, miR486 and miR29c, and, an internal control, miR16, were carried out using various concentrations of miRNA standards (0 to 100 fm) with biotinylated CDNA captured by streptavidin-coated plate. The competitive electrochemical technology was capable of distinguishing isomiRs and increasing the impedance (Z‘ kohm) and sensitivity of the miRNA nanowell electrochemical technology was +1.1 mV. The impedance level obtained with miR29c in AS49 lung cancer cell media was ~2.8-fold higher (mean value ± SD, 163.9 ± 32.8 kohm) compared with ACHN kidney cancer cell media (60.8 ± 33.1 kohm) by 90 nm nanowell analysis (p = 0.000019). Electrochemical analyses of a serum sample obtained from a lung cancer patient revealed that miR486 and miR294 levels were ~2-fold (p = 0.0031) and 9-fold (p = 0.0006) higher, respectively, compared to the pooled human control sera. This result agreed with the result obtained by qRT-PCR/Tagman analysis of human serum samples. Our results suggest that miR486 and miR203 levels up- and down-regulated, respectively, in lung cancer serum samples are biomarkers for early (Stage I) lung cancer diagnosis. Supported by National SIRI Phase I contract, N268121500040C. (Topic 337).

#5437 miR-302b as adjuvant therapeutic tool to improve chemotherapy efficacy in human triple-negative breast cancer. Alessandra Cataldo,1 Ilaria Plantamura,1 Elvira D’Ippolito,1 Sandra Romero-Cordoba,2 Sara Baroni,1 Valeria Cancilla,1 Claudio Tri pode,1 Dario Palmieri,1 Marilena V. Iorio,1 Istituto Nazionale dei Tumori, Milan, Italy; 2INMGEN, Mexico City, Mexico; 3University of Palermo, Palermo, Italy; 4Ohio State University, Columbus, OH.

Introduction: Triple-negative breast cancer (TNBC) accounts for 15-20% of all breast cancer cases, with the worst outcome of all subtypes. For TNBC, still lacking targeted therapies, the only therapeutic option is currently chemotherapy, and despite a good initial response, patients often develop drug resistance. MiRNAs can modulate chemoresistance by affecting DNA repair, cell cycle progression, apoptosis and also tumor microenvironment. Macrophages constitute a major component of the immune microenvironment of cancer and pro-tumor M2 macrophages have been associated with resistance to chemotherapeutic treatments. Our previous data showed that miR-302b over-expression enhances sensitivity to cisplatin in breast cancer cell lines by targeting directly E2F1 and indirectly ATM. Here, we investigated the potential of miR-302b as a therapeutic tool to enhance cisplatin response in a TNBC mouse model and which pathways are involved in this mechanism both in tumor cells and microenvironment. Moreover, miR-302b prognostic value was assessed in a cohort of TNBC patients with available clinical outcome. Finally, we evaluated if miR-302b enhances the sensitivity to doxorubicin, another chemotherapeutic agent used as first-line therapy in TNBC patients. Material and method: MDA-MB-231 TNBC cells were injected into the mammary fat pad of female SCID mice and then treated with lipid nanoparticles containing miR-302b or cel-miR-67 control, alone or in combination with cisplatin. Gene expression profile on collected tumors was performed by microarray and tumors sections were stained with anti-arginase 1 (M2 marker) to evaluate the number of M2 macrophages. MiR-302b expression was assessed in 39 TNBC treated with chemotherapy in adjuvant setting, and associated with prognosis. Finally, MDA-MB-231 cells were transfected with miR-302b precursor or control treated with doxorubicin for 24h and then assessed for cell viability. Results: Our results show that miR-302b combination with cisplatin significantly impaired tumor growth in comparison with cel-67 control and cisplatin (p = 0.05), and reduced the number of M2 macrophages in the tumor microenvironment (p = 0.005). Moreover, gene expression profile of collected tumors confirm immune system modulation. Notably, miR-302b expression was associated with disease-free survival and overall survival in TNBC patients treated with adjuvant chemotherapy. Furthermore, we found that miR-302b also enhances sensitivity to doxorubicin in vitro, affecting cell viability and cell cycle transition through E2F1 regulation. Conclusion: Our data demonstrate that miR-302b can be exploited as a new therapeutic tool to improve the response to chemotherapy, modulating tumor microenvironment. Moreover, this miRNA has prognostic significance in TNBC patients, and might also represent an useful predictive biomarker for response to chemotherapy.

#5438 Knowledge of which miRNA isoforms are expressed in a sample can successfully discriminate amongst the 32 TCGA cancer types. Aristeidis G. Telenis, Rogan Magee, Phillipe Loher, Inna Chervoneva, Eric Londin, Isidore Rigoutsos, Thomas Jefferson University, Philadelphia, PA.

Background: Next-generation sequencing (NGS) technologies made possible the generation of large amounts of data quickly and inexpensively process. As a result, the challenge nowadays is how best to manipulate and analyze Big Data. Among non-coding RNAs, miRNAs (miRNAs) are the best studied to date. miRNAs have attracted a lot of attention because they are centrally involved in disease development, at least 70% of all protein-coding genes are targets of miRNAs. The complexity of miRNA-mediated regulation increased further with the recent discovery that miRNA isoforms (isomiRs) are produced constitutively and that isomiR expression depends on a person’s sex, population origin, race and also on tissue type, tissue state, and disease subtype. Methods: We computed and analyzed isomiR profiles across 10,271 short RNA-seq datasets from The Cancer Genome Atlas (TCGA) repository, which represent 32 cancer types. Focusing on the isomiRs of each dataset, we built a classifier that relied solely on “binarized” isomiR profiles: in a dataset, an isomiR was labeled ‘present,’ if its expression exceeded a threshold that depended on the depth of sequencing, and ‘absent’ otherwise. This differs radically from previous methods in that it attempts to classify by relying on the presence or absence of isomiRs, intentionally discarding all information about the isomiRs’ expression level. We also built a classifier that relied solely on “binarized” miRNA arm profiles: a miRNA arm was labeled ‘present’ if at least one of the isomiRs it produces is ‘present’ in the same dataset. Results: The classifier we built using binarized isomiR profiles discriminated among all 32 TCGA cancer types and classified datasets with sensitivity >90% and a false discovery rate (FDR) < 3.0%. The ability of this classifier to correctly distinguish among the 32 cancers persisted even after a 15x reduction in the number of isomiRs used. The classifier we built using binarized miRNA arm profiles could also discriminate among all 32 TCGA cancers albeit with a lower sensitivity (83%) and a slightly higher FDR (6.0%). We also examined how well the various miRNA loci have been studied over time by measuring the corresponding number of published articles. Surprisingly, the miRNAs whose isoforms have the highest ability to classify tumors are not those with the most articles in PubMed. Conclusions: The presence or absence of a specific isomiR provides adequate discriminatory power to accurately classify a sample as belonging to one of the 32 TCGA cancers. The findings suggest that isomiRs can be used as potent, novel, cancer-specific biomarkers. By ignoring the expression levels of isomiRs, we effectively show that the same miRNA produces different clouds of isoforms in each cancer type. And, since distinct isomiRs target distinct miRNAs, it follows that a miRNA locus will target different miRNAs in different cancers simply by expressing different clouds of isoforms each time.

#5439 The chronological analysis of previously-reported serum microRNA expression in colorectal cancer. Yukihito Yoshikawa,1 Mitsuko Fukunaga,1 Takaaki Masuda,2 Miwa Noda,1 Hiroko Wakiyama,1 Yuta Koyama,1 Shinya Kidogami,1 Kuniaki Sato,1 Qingjiang Hu,2 Sho Nambara,1 Tomo Saito,1 Shotaro Sakimura,1 Naoki Hayashi,1 Yohsuke Kuroda,1 Shuhei Ito,1 Koshi Mimori1.

Background: Colorectal cancer (CRC) is a major cause of cancer-associated death in the world. Although surgery, chemotherapy, and post-operative chemotherapy, recurrence is common in patients with CRC. Many studies have examined potential biomarker of recurrence in CRC in attempt to improve patients prognosis. MicroRNAs (miRNAs), the small non-coding RNAs that are associated with the development of cancer, in serum have been shown to be potential biomarkers in various type of cancers and several miRNAs have been reported in colorectal cancer. However, no miRNAs has been applied in a clinical setting because of small amount of each samples. In the present study, we evaluated the clinical effectiveness of previously-reported serum miRNAs expression in CRC using periodically gathered serum. Materials and Methods: We obtained 328 serum samples, which gathered periodically (preoperative, postoperative one month, three months, six months, one year, and two years), from 71 patients with stageI/III CRC (stageI: 31 patients, stageII: 40 patients). Thirty-three patients had a recurrence. The miRNAs were extracted from serum samples, and miRNA microarray analysis was performed. Previously-reported sixteen miRNAs (let-7a, miR-15b, miR-18a, miR-19a, miR-21, miR-23a, miR-29a, miR-31-2, miR-92a, miR-151, miR-181b, miR-203, miR-205, miR-335, miR-1229 and miR-1246) were quantified by microRNA microarray analysis. Results: In no recurrence cases, postoperative miR-1246 expression is higher than postoperative miR-1246 expression; preoperative: 5.6 ± 1.8, postoperative one month: 4.1 ± 1.6 (p < 0.05), postoperative three months: 3.4 ± 1.9 (p < 0.05), postoperative six months: 4.8 ± 1.5 (p < 0.05), postoperative one year: 4.0 ± 1.6 (p < 0.05), postoperative two years: 4.8 ± 1.5 (p < 0.05). We observed trend of miR-1246 re-elevation before recurrence. The sensitivity and specificity of miR-1246 for detection of CRC are 52.8% and 84.9% (AUC=0.746). This result is
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**#5440 miRNAs as novel therapeutic adjuvants for improving the efficacy of vincristine and radiation therapy in treating medulloblastoma.**

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Medulloblastoma (MB) is the most common malignant brain tumor. Despite recent improvements in the overall survival, only a modest percentage of patients survive Myc-driven high-risk MB. The quality of life for surviving patients is substantially reduced due to the devastating and often irreversible side effects of radiation and chemotherapy. Recently, in a large unbiased genomic screen, we uncovered a group of microRNAs (miRNAs) capable of mediating drug sensitivity in c-myc amplified high-risk MB. Our functional screen of ~1900 miRNAs identified miR-584-5p as a potent candidate that uniquely sensitizes high-risk MB to radiation as well as vincristine (VCR) (20 to 25-fold dose reduction), an anti-mitotic agent routinely administered alongside radiation and in combination with other chemotherapeutic drugs to treat medulloblastoma patients. Our studies revealed that miR-584 might act as a potent tumor suppressor as it inhibited MB growth in vivo as well as migration and invasion of c-myc amplified MB. We show that miR-584 overexpression results in defective mitosis leading to mitotic catastrophe, apoptosis, DNA damage and G2-M cell-cycle arrest in high-risk MB cells without affecting normal neural stem cell growth. Notably, we discovered that miR-584 directly regulates the expression and activity of genes including histone deacetylase 1 (HDAC1), and eukaryotic translation initiation factor 4 family member 3 (EIF4E3) that are known to play important roles in microtubule dynamics, metaphase-anaphase transition and radio-resistance. Moreover, silencing either of these two target genes resulted in significant inhibition of MB growth and enhanced sensitivity to VCR and ionizing radiation. Overexpressing miR-584 or silencing either HDAC1 or EIF4E3 also inhibited the MB stem cell proliferation and self-renewal. We report that while miR-584-5p is predominately expressed in normal brain and cerebellum, its expression is significantly reduced in MB patient derived xenografts (PDx). In contrast to miR-584, EIF4E3 and HDAC1 were found to be overexpressed in medulloblastoma patients. These findings are highly significant, unexpected and innovative as this miRNA and its target genes are the first to be shown to affect the therapeutic efficacy of VCR and radiation in c-myc amplified high-risk medulloblastoma.

**#5441 Circulating exosomal microRNA-203 and microRNA-373 as non-invasive biomarkers for predicting prognosis and metastasis in human hepatoacellular carcinoma.**

Gyeonghwa Kim,1 Eunhye Lee,1 Se Young Jang,2 Won Young Tak,2 Young Oh Kweon,2 Soo Young Park,2 Keun Hur,1 1Department of Biochemistry and Cell Biology & BK21 Plus KNU Biomedical Convergence Program, Kyunggook National University School of Medicine, Daegu, Republic of Korea; 2Department of Hepatology Hospital, Daegu, Republic of Korea.

Background and Aim Hepatocellular carcinoma (HCC) is the fifth most common cancer and the most leading cause of cancer-related death worldwide. Although considerable progress has been made in treatment of HCC, early detection is still highly considered the key to improved survival. Thus, for the earlier diagnosis and accurate prediction of HCC, identification of non-invasive molecular biomarkers for HCC patients is an imperative need. Recently, cancer cell-derived extracellular vesicles (EVs) have been known to contain various intracellular biomolecules including microRNAs (miRNAs). The aim of this study was to evaluate whether exosomal miRNAs can serve as a serum-based biomarker in HCC. Materials and Methods We isolated exosome from serum samples from HCC patients as well as normal healthy controls using ultracentrifugation, and it was confirmed by expression of exosome markers (CD9, CD63, ALIX, and TSG101) based on immuno blotting. Next, the expression of 6 miRNAs (miRNA-24, -130a, -182, -203, -373, and -423) was analyzed in the exosome samples. We also investigated expression status of the 6 miRNAs in matched HCC tissues and corresponding normal liver tissues. MiRNAs expression was determined by quantitative real-time PCR (qRT-PCR), and miRNAs expression was normalized relative to cel-miR-39 and RNU5B expression for serum and tissue samples, respectively. Results We successfully purified exosome from serum clinical samples and detected miRNAs in the exosome. We observed that a subset of miRNAs from serum exosome, including miRNA-24, -130a, -182, -203, and -373 were enhanced in HCC patients than normal healthy controls. In further comparison between early stage and advanced stage of HCC patients, serum exosomal miRNA-203 (P < 0.05) and miRNA-373 (P < 0.05) were significantly up-regulated in advanced HCC patients. While no significant changes in the expression of other serum exosomal miRNAs (miRNA-24, -130a, -182, and -423) according to HCC progression. More interestingly, high serum level of miR-203 was associated with progression (P < 0.01) as well as prognosis (P < 0.05) of HCC patients. Conclusions We provided the novel evidence for usefulness of serum circulating exosomal miR-203 and miR-373 expressions as strong potential biomarkers for predicting prognosis and metastasis of HCC patients.

**#5442 Down-regulation of miRNA-214 expression by bioactive molecules mitigates invasive potential of melanoma cells through ALCAM/TAFAP2 signaling.** Ram Prasad, Santosh K. Katiyar. Univ. of Alabama at Birmingham, Birmingham, AL.

Melanoma is responsible for 80% of skin cancer related deaths in the United States and represents fifth most common neoplasia in humans. Over the past decades, the incidence of melanoma has increased by 3% to 8% per year in western countries. Therefore, it is essential to understand the molecular regulatory pathways that contribute to melanoma aggressiveness and metastatic dissemination. MicroRNAs (miRNAs) are small noncoding regulatory RNAs that contribute to tumor formation and progression, for their ability to regulate gene expression post-transcriptionally by either inhibiting protein translation or degrading target miRNAs. miR-214 is overexpressed in various types of cancers and is associated with the regulation of numerous carcinogenic processes including cell differentiation, senescence, angiogenesis and cell migration/invasion, etc. In the present study, we demonstrated that miR-214 shows distinct overexpression in melanoma cell lines (A375, Hs294t, SK-Mel11, and SK-Mel28) as compared to its expression in normal human epidermal melanocytes (NHEM), and this overexpression of miR-214 in melanoma cells was associated with the invasive potential than NHEM. Treatment of melanoma cells with an inhibitor of miR-214 (50 nM) resulted in inhibition of cell invasion ability. We also determined the effects of bioactive phychotheroxy grape seed proanthocyanidins (SPS) on the expression levels of miRNA-214 in melanoma cell lines (A375 and Hs294t) and its underlying molecular targets. Treatment of A375 and Hs294t cells with SPS resulted in suppression of the levels of miRNA-214, which leads to decrease in migration potential of melanoma cell lines. By using 3D melanoma progression model, we also verified this suppressive effect of SPSs on melanoma invasion. The transition from the non-invasive to the invasive and metastatic stage is accompanied by the loss of AP-2 transcription factors (TAFAP-2) and expression of activated leukocyte cell adhesion molecule (ALCAM) and these are associated with miRNA-214 overexpression. Here, we observed that the treatment of SPSs decreases ALCAM expression and restore TAFAP-2 levels in melanoma cell lines. Further, dietary administration of SPSs significantly reduce the metastasis potential of A375 cells in different vital organs such as lung, liver, kidney, and spleen, and this suppressive effect of SPSs on invasive potential of A375 cells was associated with the reduction in the levels of miRNA-214 and ALCAM. Our results indicate that modulation of miR-214 influences in vitro cell movement and extravasation from blood vessels in vivo. Together, these findings suggest that a dietary supplement of SPSs may provide potential diagnostic biomarkers and better understanding of the complex functions of miRNAs, as well as therapeutic targets for clinical application.

**#5443 Developing a novel miR-15a mimic as a potential therapeutic molecule to eliminate resistant colorectal cancer stem cells.**

Andrew T. Feiler, Hua Liu, Ning Wu, Jingfang Ju. Stony Brook University, Stony Brook, NY.

In the last 15 years, it has become well established that microRNA play important roles in cancer biology. Due to their ability to regulate the expression of important target genes, aberrant expression of miRNAs has been linked to cancer development and progression. Based on these important functions, there is great interest in developing miRNA based therapeutics. In colorectal cancer, treatment using 5-flourouracil (5-FU) based chemotherapy has improved patient outcomes. However, there remain challenges associated with chemoresistance and recurrence for patients with advanced stage colorectal cancer. miRNA based therapeutics may represent a potential novel therapeutic option for colorectal cancer therapy either alone or in combination with 5-FU based chemotherapy. miR-15a was of the first miRNAs identified to be associated with cancer, and has been shown to have important roles in several tumor types. miR-15a is down-regulated in colon cancer and associated with poor patient prognosis. We have identified several important cancer related targets of miR-15a in colon cancer, including YAP1, DCLK1, BM11 and BCL2. Through the regulation of these targets, miR-15a expression can be used as a potent inhibitor to reduce colon cancer cell proliferation, invasion and improve sensitivity to 5-FU, as well as...
decreasing tumor growth in vivo mouse colon tumor models using colon cancer stem cells. In the interest of developing miR-15a based colon cancer therapeutics, we have made a modified miR-15a mimic that shows enhanced abilities to disrupt resistant colon cancer cell proliferation and induction of cell cycle arrest when compared to unmodified miR-15a. Following transfection with miR-15a mimic, cell number was reduced by 84% compared to control and compared to miR-15a precursor. Cell cycle analysis showed that G1/S ratio was increased from 1.07 for control to 2.87 for precursor miR-15a and 7.07 for miR-15a mimic. This miR-15a mimic also maintains its ability to regulate these important target genes in colon cancer stem cells. In mouse models using colon cancer stem cells, miR-15a mimic has demonstrated therapeutic potential by reducing tumor growth. Based on these findings, there is potential that modified miR-15a could be adapted for treatment of patients to improve survival of advanced stage colorectal cancer patients.

#5444 Therapeutic potential of miR-195 in non-small cell lung cancer. Xiaojie Yu,1 Xiuye Ma,1 Qiyan Zhang,2 Zhenhe Zhao,2 Liqin Du,2 Alexander Pertschumlidis1. 1University of Texas Health Science Center at San Antonio, San Antonio, TX; 2 Texas State University, San Marcos, TX.

Introduction: MicroRNAs (miRNAs) play important roles in nearly all cellular physiological and pathological pathways. Dysregulated miRNAs have been shown to contribute to the tumorigenesis of most cancers, including lung cancer. We aim to modulate miRNA activity as a potential therapeutic avenue either alone or in combination with other drugs. Methods: A high throughput screen with a library of 1,239 miRNA mimics was performed in NSCLC cells to identify miRNAs that both inhibit cancer cell growth and sensitize cells to drug treatment, targeting agents (MTAs). The functions of candidate miRNA(s) were validated both in vitro and in vivo. Results: miR-195 causes G1 phase arrest by targeting CCND3. miR-195 induces apoptosis and senescence and represses migration and invasion of NSCLC cells by targeting BERCS. Conversely, over-expression of CCND3 compromises the inhibition of cell growth by miR-195; over-expression of BERCS abrogates the inhibition of cell growth, migration and invasion by miR-195. miR-195 also synergizes with MTAs paclitaxel and erubul to inhibit the growth of NSCLC cells by regulating CHEK1. Lung tumors with miR-195 over-expression grow significantly slower and respond better to erubulin treatment than tumors in control animals. Induction of miR-195 expression after tumor initiation slows tumor growth and that combination of doxorubicin with erubul shows stronger inhibition on tumor growth than erubulin. In addition, miR-195 inhibits lung tumor metastasis. Analysis of miRNA and gene expression profiles from TCGA shows that miR-195 expression is negatively correlated with that of BIRC5 and CHEK1. Clinical data from TCGA show that miR-195 is significantly down-regulated in lung tumors compared to adjacent normal tissues and that its down-regulation in lung cancer patients is associated with worse survival. TCGA data also show that BIRC5 and CHEK1 are significantly up-regulated in lung tumors compared to adjacent normal tissues and that their up-regulation is associated with worse survival. Conclusions: We report the identification of miR-195 as a tumor suppressor and chemotherapy sensitizer in NSCLC mediated by its repression of CCND3, BIRC5 and CHEK1. Mouse xenografts with miR-195 over-expression or induced miR-195 expression indicate that tumors with miR-195 expression are more sensitive to drug treatment, and that induction of miR-195 in tumors represses tumor growth and sensitizes tumors to erubicin treatment. These results highlight the potential use of miR-195 expression level as a biomarker to predict patient response to MTAs and of miR-195 mimics as a therapeutic agent or adjunct to chemotherapy. This project was supported by NIH R01 CA129632 and CPRIT Training Grant RP140105.

#5455 Inhibition of tumor suppressor function of RUNX3 by miR-301a facilitates the progression of castration resistant prostate cancer. Venkatesh Kolluru, Balaji Chandrasekhar, Collin McKenzie, Houda Alatassi, Murali Ankem, Chandil Damodaran. University of Louisville, Louisville, KY.

Treatment of prostate cancer is still clinically challenging due to lack of reliable markers for diagnosis and prognosis. Serum prostate specific antigen (PSA) is limited clinical utility due to poor sensitivity and specificity. A large number of false positives (50%) with traditional PSA testing lead many patients to undergoing unnecessary prostate biopsy and exposure to unnecessary risk of complications. So the goal of this study is to identify a reliable, non-invasive biomarker that can distinguish patients with benign, indolent and aggressive prostate cancer in various clinical settings. MicroRNAs (miRNAs) are small noncoding (18 to 28 nucleotides) RNA molecules that are present in all human cells, and their expression patterns are correlated with many cancer types, including CaP. In our results, we found a significant differential expression (p = 0.013) of miR-301a in both tumor tissues (Gleason-6 and -7) and serum samples in comparison to benign prostatic hyperplasia (BPH) or adjacent benign samples. We observed a negative correlation between miR-301a and RUNX3 expression in human CaP samples suggesting tumor suppressive role RUNX3 might be compromised in CaP. To determine MiR-301a regulation on RUNX3, we evaluated miR-301a and RUNX3 protein in panel of prostate cancer cell lines and normal prostate epithelial cells: over expression of miR-301a down regulated RUNX3 activation in prostate cancer cell lines and silencing miR-301a expression reverts RUNX3 activation in BPH cells. While evaluating the reporter activity of RUNX3 either by co-transfecting with mimic or inhibitor of RUNX3, we observed that the RUNX3 promoter activity and protein expression suggesting RUNX3 is a direct target of miR-301a in CaP cells. Silencing miR-301a reverted the pro-apoptotic function of RUNX3, results in reduced colony formation, adhesion, invasion and migration of CaP cells. Investigating the mechanistic link miR-301a and RUNX3 may explore whether it could facilitate clinical decision making such as the decision to proceed with early surgery rather than active surveillance for intermediate risk prostate cancer patients deemed to be at high risk of progression.

#5456 Tumor-derived miR-200c and 141 contribute to high levels of plasma microRNA-200c and 141 through exosomes. Lizhong Wang, Runhua Liu, Song Gao, Yicun Wang, Meng Wang, Zhi Li. University of Alabama at Birmingham, Birmingham, AL.

Circulating microRNAs (miRs) have potential as cancer biomarkers, but their regulatory mechanisms remain elusive. Here we used a breast cancer model (Fpox3 heterozygous Scurfy mutant female mice, Foxp3+/-) for identification of circulating miR biomarkers, and the formation and regulation of these miRs were investigated. Aging Foxp3+/- female mice developed spontaneous breast cancers and lung metastases. Levels of miR-200c and 141 were lower in the Foxp3+/- breast cancer cells than in normal breast epithelial cells, but plasma levels of miR-200c and 141 in the Foxp3+/- mice increased during tumor progression, especially during metastasis to the lung. Likewise, 259 participants, including patients with breast cancer or benign breast tumors, members of breast cancer families, and matched normal healthy controls, were assessed for circulating levels of candidate miRs. The results showed that: 1) the levels of miR-200c and 141 were lower in the Foxp3+/- breast cancer cells relative to those with FOXP3+/-, especially for late-stage and metastatic cancer cells; 2) the levels of miR-200c and 141 were higher in plasma of patients with metastatic breast cancer than in plasma of those with localized breast cancer, with benign breast tumors, with a family history of breast cancer, or normal healthy controls. Furthermore, in Foxp3+/- mice, plasma miR-200c and 141 were released from tumor cells through exosomes. Thus, plasma levels of miR-200c and 141 are potential biomarkers for early detection of metastases. MiR-200c and 141 appear to be released from breast cancer cells, through exosomes, into the circulation.

#5457 A microRNA signature for the classification of renal cell carcinoma subtypes. Ashley Di Meo,1 Mereet Hanna,2 Rola Saleeb,1 Samantha Wala,1 Adriana Krizova,2 Manal Gabr,3 Haiyan Zhai,2 Maria Pasic,3 Andrew Evans,5 Fadi Brimo,6 George Yousef. 1University of Toronto, Toronto, Ontario, Canada; 2The Li Ka Shing Knowledge Institute of St. Michael’s Hospital, Toronto, Ontario, Canada; 3London Health Sciences Center and Western University, London, Ontario, Canada; 4BioGenex Laboratories, Fremont, CA; 5University Health Network, Toronto, Ontario, Canada; 6McGill University Health Centre, Montreal, Quebec, Canada.

Introduction: Renal cell carcinoma (RCC) accounts for 90% of all kidney cancers. It comprises a heterogeneous group of renal tumors with distinct genetic and molecular characteristics including clear cell RCC (ccRCC), papillary RCC (pRCC) and chromophobe RCC (chRCC). The differential diagnosis of RCC subtypes relies on distinct morphology which is not always accurate. Accurate classification of RCC subtypes is critical since each exhibits different clinical behaviour, prognosis and response to therapy. The purpose of this study is to determine whether a limited number of miRNAs can classify RCC subtypes with high accuracy. Experimental Design: We extracted RNA from 90 formalin-fixed paraffin-embedded (FFPE) tissues including 27 clear cell RCC, 29 papillary RCC, 19 chromophobe RCC, 4 unclassified RCC tumors and 11 oncocytomas. We measured the absolute expression of six miRNAs by qRT-PCR. Receiver operator characteristic curves were constructed and the area under the curve (AUC) was calculated to assess diagnostic performance. We also tested miRNA expression by in situ hybridization (ISH) in an independent set of ninety-eight FFPE renal tumors. Results: We developed a two-step miRNA classifier. In the
first step, expressions of selected miRNAs were found to discriminate clear cell RCC and papillary RCC from chromophobe RCC and renal oncocytoma. Two miRNAs were able to discriminate clear cell RCC and papillary RCC from chromophobe RCC and oncocytoma. miR-221 was significantly overexpressed in chromophobe RCC and oncocytoma compared to clear cell RCC and papillary RCC (4.698-fold change, p=6.598e-05) and able to discriminate between the two groups (AUC=0.937, 95% CI: 0.913-1.04, p<0.0001). In the second step, the absolute expression of two miRNAs could distinguish clear cell RCC from papillary RCC (10.4-fold change, p=1.243e-03). Moreover, an additional two miRNAs could differentiate chromophobe RCC from renal oncocytoma (3.530-fold change, p=1.751e-006). In situ hybridization revealed that miRNAs display a nuclear staining pattern. The analysis was extended to distinguishing between RCC from papillary RCC (p<0.0001) and chromophobe RCC from renal oncocytoma (p=0.009). Conclusion: miRNA expressions were able to distinguish between RCC subtypes and renal oncocytoma. miRNA assessment by in situ hybridization is a clinically useful diagnostic tool that can complement current methods for RCC classification.

**#5449** An exosomal biomarker for prostate cancer. Divya Bhagirath, Thao Yang, Kirandep Sekhon, Nathan Bucay, Shahana Majid, Yutaka Hashimoto, Priyanka Kulkarni, Pritha Dasgupta, Marisa Shiina, Varahram Shahryari, Mit-Suho Imai-Sumida, Soichiro Yamamura, Z Laura Tabatabai, Yuichiro Tanaka, Jacob Fredsøe, 1 Jiri Sana, 1 Parwez Ahmad, 1 Natalia Anna Gablo, 1 Ondrej Slaby, 1 Michal Stanik, 2 Jan Dolezel 2. Jacob Fredsøe, 1 Anni K.Rasmussen, 2 Anni R. Thomsen, 2 Peter Mouritzen, 2 Søren Høyer, 3 Michael Borre, 4 Torben F. Ørntoft, 1 Karina D. Sørensen 1, Urology, Aarhus N, Denmark; 3 Institute of Pathology, Aarhus C, Denmark; 4 Department of Urology, Aarhus N, Denmark.

Widespread use of Prostate Specific Antigen (PSA) testing for prostate cancer (PC) detection has led to extensive overdiagnosis and overtreatment. Thus, we aimed to train and validate urine-based microRNA (miRNA) biomarkers that may assist in the diagnosis and prognosis of PC. To this end, we profiled the expression levels of 92 miRNAs by RT-qPCR in exosome-enriched, cell-free urine samples from 20 patients with benign prostatic hyperplasia (BPH) and 188 patients with clinically localized PC (cohort 1). The diagnostic potential of individual miRNAs and multi-miRNA ratio models were assessed by receiver operating characteristic (ROC) curve analysis. Prognostic potential was evaluated using Kaplan-Meier, univariate and multivariate Cox regression analyses using biochemical recurrence (BCR) after radical prostatectomy (RP) as endpoint. Our findings were validated in an independent cohort of 20 BPH patients and 197 patients with clinically localized PC (cohort 2). We identified and validated several deregulated miRNAs in urine samples from PC patients. In addition, we trained a novel diagnostic 3-miRNA model that distinguished BPH and PC patients with an area under the curve (AUC) of 0.95 in cohort 1, and which was successfully validated in cohort 2 (AUC=0.89). Furthermore, we trained a novel prognostic 3-miRNA model that predicted time to BCR after RP independently of routine clinicopathological parameters in cohort 1, and was successfully validated in cohort 2.

**#5450** Urinary microRNA as diagnostic and prognostic biomarkers for prostate cancer. Jacob Fredsøe, 1 Anni K. Rasmussen, 2 Anni R. Thomsen, 2 Peter Mouritzen, 2 Michael Borre, 3 Torben F. Ørntoft, 1 Karina D. Sørensen 1, Department of Molecular Medicine, Aarhus N, Denmark; 2 Exign A/S, Vedbaek, Denmark; 3 Institute of Pathology, Aarhus C, Denmark; 4 Department of Urology, Aarhus N, Denmark.

Our analyses suggest that identifying and validating stem cell-inhibitory in combination with current advances in nanomedicine will undoubtedly impact the development of novel therapies for targeting the CSC population and treating GBM.

**#5451** Panel of urinary cell-free microRNAs in detection of urinary bladder cancer. Jaroslav Juracek, 1 Tana Machackova, 1 Marek Vecera, 1 Kamila Soucova, 1 Jiri Sana, 1 Parwez Ahmad, 1 Natalia Anna Gablo, 1 Ondrej Slaby, 2 Michal Stanik, 3 Jan Dolezel, 3 CEITEC - Central European Institute of Technology, Brno, Czech Republic; 3 MMCi - Masaryk Memorial Cancer Institute, Brno, Czech Republic.

Bladder cancer is the most common cancer of the urinary tract. More than 90% of bladder cancers are urothelial carcinoma, which are divided into non-muscle-invasive and muscle-invasive forms. Non-muscle-invasive tumors frequently recur (50-70%) and can also progress to invasive form (10-15%). These patients are monitored by cystoscopy and may undergo cystectomy over many years. Improved monitoring method is needed, ideally via urine analysis, which could reduce the morbidity and costs associated with long follow up. Currently there are no molecular biomarkers which could diagnose or accurately predict disease progression. We aimed to develop a clinically applicable, specific and sensitive panel of urine microRNAs enabling detect bladder cancer and predict risk of progression to muscle-invasive form. Within the exploratory phase of study we have analyzed expression profiles of 1733 miRNAs in urine supernatant of 16 bladder cancer patients (6 muscle invasive, 5 high-grade muscle non-invasive, 5 low-grade muscle non-invasive), 17 controls, 10 RCC patients and 4 urinary tract infections (UTI) using Affymetrix microRNA microarrays. Diagnostic and prognostic potential of selected microRNAs was further validated on independent samples in training phase (50 bladder cancer patients, 15 controls) and validation phase (100 bladder cancer patients, 55 controls, 45 renal cancer patients) using specific TaqMan assays and qRT-PCR method. Global expression profiling identified set of 76 miRNAs able distinguish bladder cancer patients from healthy controls (P<0.01), thereof 64 highly up-regulated and 12 down-regulated. Moreover 23 miRNAs were able distinguish invasive from non-invasive forms of UCUB (P<0.01) and 18 miRNAs high-grade and low-grade non-invasive (P<0.01). Set of 12 miRNAs with highest expression level and statistical significance was validated in training phase of study. Based on the results the panel of three miRNAs (miR-31, miR-93, miR-191) was profiled. In validation phase we confirmed diagnostic potential and ability of this urine miRNA-based panel to diagnose patients with bladder cancer with high sensitivity and specificity (AUROC=0.94, sensitivity = 82%, specificity = 90%). Our data have shown that urinary microRNAs could serve as sensitive and specific biomarkers of urinary bladder cancer and could be useful tool to increase sensitivity of standard cytological examination and reduce costs associated with long-term follow-up of bladder cancer patients. This work was sup-
#5452 Antitumor activity of miR-34a in peritoneal mesothelioma relies on c-MET and AXL inhibition: Persistent activation of ERK and AKT signaling as a possible cytoprotective mechanism. Rihan El Bezawy, Michelandra De Cesare, Marzia Pennati, Marcello Deraco, Paolo Gandellini, Valentina Zuco, Nadia Zaffaroni. Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy.

The value of microRNAs (miRNAs) as novel targets for cancer therapy is now widely recognized. However, no information is currently available on the expression/functional role of miRNAs in diffuse malignant peritoneal mesothelioma (DMPM), a rapidly lethal disease, poorly responsive to conventional treatments, for which the development of new therapeutic strategies is urgently needed. Here, we evaluated the expression and biological effects of miR-34a—one of the most widely deregulated miRNAs in cancer and for which a lipid-formulated mimic is already clinically available—in a large cohort of DMPM clinical samples and a unique collection of in-house-developed DMPM preclinical models, with the aim to assess the potential of a miR-34a-based approach for disease treatment. miR-34a was found to be significantly down-regulated in forty-five DMPM clinical specimens and five established cell lines compared to normal peritoneum. miR-34a reconstitution inhibited cell proliferation in a time-dependent manner, though to a different extent and with different kinetics across individual cell lines. Such a variable antiproliferative effect was paralleled by a different kinetics of apoptosis induction. In addition, miR-34a ectopic expression significantly inhibited cell migration and invasion in two miR-34a-reconstituted cell lines was found to counteract the antiproliferative and pro-apoptotic action of DMPM xenograft models. Interestingly, an persistent activation of ERK1/2 and AKT in two miR-34a-reconstituted cell lines was found to counteract the antiproliferative and pro-apoptotic effects of miRNA, yet not affecting its anti-invasive activity. Most importantly, when three subcutaneous/orthotopic DMPM xenograft models were used to examine the impact of miR-34a on tumorigenesis, a significant reduction in tumor size and growth was consistently observed. Our preclinical data, showing impressive inhibitory effects induced by miR-34a on DMPM cell proliferation, invasion and growth in immunodeficient mice, strongly suggest the potential clinical utility of a miR-34a-replacement therapy for the treatment of such a still incurable disease. On the other hand, we provide the first evidence of a potential cytoprotective/resistance mechanism that may arise towards miRNA-based therapies through the persistent activation of receptor tyrosine kinase downstream signaling. Interestingly, a persistent activation of ERK1/2 and AKT in two miR-34a-reconstituted cell lines was found to counteract the antiproliferative and pro-apoptotic effects of miRNA, yet not affecting its anti-invasive activity. Most importantly, when three subcutaneous/orthotopic DMPM xenograft models were used to examine the impact of miR-34a on tumorigenesis, a significant reduction in tumor size and growth was consistently observed. Our preclinical data, showing impressive inhibitory effects induced by miR-34a on DMPM cell proliferation, invasion and growth in immunodeficient mice, strongly suggest the potential clinical utility of a miR-34a-replacement therapy for the treatment of such a still incurable disease. On the other hand, we provide the first evidence of a potential cytoprotective/resistance mechanism that may arise towards miRNA-based therapies through the persistent activation of receptor tyrosine kinase downstream signaling.


Background: Growing evidence suggests importance of aberrant microRNA (miRNA) expression in development and progression of cancer. Recent studies have demonstrated the potential of miRNA panels as promising diagnostic, prognostic and predictive biomarkers. Objective: The purpose of the present study was to evaluate the clinical and functional significance of miRNAs in esophageal cancer (EC). Methods: Herein, we evaluated the diagnostic potential of a five miRNA panel (consisting of miR-21, miR-144, miR-107, miR-93 and miR-342) in EC detection using quantitative real time PCR (qRT-PCR). To evaluate the discriminatory power of miRNA panel, receiver operating characteristic curve (ROC) curves were generated for each of the miRNAs followed by risk score analysis. Briefly, linear regression models were fitted using the cancer status and each of the risk score as input. ROC curves were then used to evaluate the diagnostic potential of the panel and to find out the appropriate cut-off point. Sensitivity and specificity of the panel were then determined. Further, pathway enrichment analysis was carried out to find the most significant pathways targeted by these miRNAs. Next, we elucidated the role of miR-144, by silencing it in KYSE-150 cells. We then evaluated the clinical and functional significance of miRNAs in EC by targeted miRNA assay and scratch assay. Potential targets of miR-144 were predicted by in silico approach followed by in vitro validation by luciferase reporter assay. Results: The ROC curve analysis indicated that the panel of five miRNAs (AUC = 0.851, p < 0.0001) constitutes a more sensitive and specific diagnostic marker as compared to any of the single miRNAs. Most importantly, panel of circulating miRNAs showed enhanced sensitivity (87.5%) and specificity (90.4%) with an AUC of 0.96 (p < 0.001) in discriminating EC patients from normal subjects. Inhibition of miR-144 significantly suppressed the proliferation of ESCC cells by 42.085 ± 1.73% at 72 h post transfection. Moreover, knockdown of miR-144 decreased (p=0.046) the migration potential of KYSE-410 cells as compared to the cells treated with negative control. In silico analysis and further validation by dual-luciferase reporter assay revealed PURA to be a direct downstream target of miR-144. Conclusion: Herein, we have demonstrated the potential diagnostic implication of five miRNA panel. Further, we have shown that mir-144 acts as an oncogenic miRNA by targeting PURA and promotes EC cell proliferation.

#5454 Serum circulating microRNAs as potential biomarkers for prostate cancer. Khamni Kasomva,1 Ignacimuthu Savarimuthu,1 Gabriel Michael Paulraj,1 Arnab Sen,2 Stephen Salio,3 Ngachan SV.3 Entomology Research Institute, Loyola College, Chennai, Chennai, India; 2ICAR Research complex for NEH region, Umiam, India; 3North Eastern Indira Gandhi Regional Institute of Health and Medical Sciences, Shillong, India.

MicroRNAs (miRNAs) are small non-coding RNA, whose differential expression has been involved in the development and progression of cancers, including prostate cancer (PCa). In recent studies circulating miRNAs have been demonstrated to have the potential as non-invasive biomarkers in various types of cancer. The aim of this study was to investigate the serum circulating miRNA-103 could be used in diagnosis of prostate cancer in North-Eastern India patient population. To evaluate this study blood samples were collected from prostate cancer patients and healthy controls following ethical approval and informed consent. The samples were reverse transcribed using stem loop primers and expression levels of miRNA-103 were determined using real time PCR. Differential expressions of miRNA-103 for each case were used to compare PCa serum with healthy serum samples. Serum circulating miR-103 levels was significantly lower in PCa patients compared with healthy controls. The serum circulating miRNA-103 could be potential for the diagnosis of PCa in North-East India Population.


Tumor suppressor p53, guardian of the genome, is frequently mutated or functionally dysregulated in more than 50% of human tumors. p53 mutation is a later event in tumorigenesis and a number of p53 mutants have “Gain of Function” (GOF) properties that have been shown to promote invasive and more aggressive phenotypes in cancer cells. Mutant p53 has been an attractive and promising therapeutic target for advanced stages of tumors. Yet, mutant p53 has proved to be one of the most undruggable targets. Thus, there is continued interest in understanding the GOF properties of mutant p53 and in designing novel strategies to target mutant p53 and/or key GOF pathways. Mature MicroRNAs (miRNAs) are ~22 nt endogenous, non-coding RNA sequences that bind to 3′UTR of their target genes and inhibit their translation. miRNA mimics are emerging therapeutics and attractive tools for mapping pathway networks. We developed a novel functional high throughput screening (HTS) assay to identify miRNAs that selectively target mutant p53-expressing cell lines. The HTS was performed in isogenic TP53+/+ (wild-type), TP53−/− (null) and TP53 R175H (mutant) HCT-116 colorectal cancer cell lines. Cell viability was used as the HTS read-out of our functional screen. Of 2754 miRNA mimics screened, we identified 56 miRNAs that selectively target TP53 R175H (mutant) cells. Our ongoing work is directed to further validating and identifying which of these miRNAs mimics selectively induce apoptosis in mutant p53-expressing cells. We are also using reporter-based assays to identify a fraction of the 56 miRNAs that can bind to the p53 3′UTR. Our long-term goal is to selectively target mutant p53-expressing cells using miRNA mimics as single agents or in combination with FDA-approved or experimental therapeutics. Our strategy is may provide a unique opportunity for development of targeted therapy for TP53 mutant tumors.

#5456 Avoiding chemotherapy resistance in squamous cell carcinomas: antitumor activities of terpenoids and their impact on the regulation of microRNAs. Camila Hernandez,1 Bruna da Silva,1 Maria de Fatima Garrido Klingbeil,2 Monica Beatriz Mathor,3 Ana Maria Soares Pereira,3 Patricia Sevigny,3 Rihan El Bezawy, Michelandrea raj,1 Arnab Sen,2 Stephen Sailo,3 Ngachan SV2. 1Universidade de Ribeirao Preto, Ribeirao Preto-SP, Brazil; 2Nu-clear and Energetic Research Institute IPEN/CNEN, Sao Paulo - SP, Brazil; 3Nu-clear and Energetic Research Institute IPEN/CNEN, Sao Paulo - SP, Brazil.

MicroRNAs are small noncoding RNAs that play important roles in cellular biology. They have been implicated in pharmacogenomics by down-regulating genes that are essential for drug function. In this work we verified the potential antitumor activity of the quinone methide triterpenes maytenin and 22β-hydroxymaytenin, as well as of a quinone methide triterpene-rich extract obtained from cultivated Maytenus ilicifolia root cells, and evaluated the associated microRNA expression following half maximal inhibitory concentration (IC50).

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treatment. Standard selectivity index (SI) for the isolated compounds and the root cell extract was determined by the logarithmic shift in effective concentration (IC\textsubscript{50}) between cancer cell lines and oral keratinocytes. Both isolated molecules as well as the root cell extract presented pronounced antiproliferative and pro-apoptotic activities in four cell lines derived from head and neck squamous cell carcinomas, including a metastasis-derived cell line. A positive SI, with an average 2-fold increase in potency, was detected for single agents and for the extract. MicroRNA expression profiles were assessed at 24h, 48h and 72h following treatment and an average of 100 molecules presented consistent marked variation in expression levels. Considering associations of microRNAs, genes they regulate, and the drugs effects dependent on these genes, the down-regulation of hsa-miR-138 and hsa-miR-21 in treated cells was of particular interest. Both microRNAs have been involved in 5-fluorouracil and cisplatin resistance, current agents of standard chemoradiotherapy for locally advanced head and neck cancer. Squamous cell carcinoma of the head and neck is one of the most common cancer types worldwide whereas treatment options based on conventional therapies or targeted therapies under development have limited efficacy. Plant-derived products are valuable sources for the development of new therapeutic options for cancer treatment or as synergistic agents in existing regular care.

MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Oxidative Stress, Autophagy, and Senescence

#5457 OGT restrains the NRF2 antioxidant pathway via O-GlCNacylation of KEAP1. Po-Han Chen, Timothy J. Smith, Jianli Wu, Michael Boyce, Jen-Tsan Ashley Chi. Duke University, Durham, NC.

O-GlCNacylation is a reversible post-translational modification that adds an O-linked β-N-acetylglucosamine (O-GlcNAc) moiety onto serine/threonine residues of target proteins. This modification is regulated by only two enzymes; O-GlCNAC transferase (OGT, the writer) and O-GlCNACase (OGA, the eraser) in mammals. Recent studies have revealed that OGT expression and O-GlCNAC modifications are elevated in several cancers, but specific O-GlCNAC targets are not well defined. We conducted a global transcriptome profiling in MDA-MB-231 breast cancer cells to search for signaling events that respond to O-GlCNAC fluctuation. We found significant up-regulation of genes involved in the NRF2-dependent stress response when OGT activity is inhibited in different tumor types. We also discovered a strong positive correlation of gene signatures between low OGT activity and NRF2 activation in multiple human tumor gene expression datasets. NRF2, the primary regulator of redox balance, is usually activated by oxidative stress and repressed under basal conditions by the KEAP1-CUL3 ubiquitin ligase complex. However, we found that OGT inhibition increases NRF2 protein level through reducing poly-ubiquitination of NRF2 in the absence of oxidative stress. By chemical sugar labeling and mass spectrometry assays, we identified that KEAP1 is directly O-GlCNAcylated by OGT especially within the BTR and Kelch motifs. Of all 11 putative O-GlCNAC sites on KEAP1, we found serine 104 is responsible for regulating NRF2 activity. NRF2 protein level through reducing poly-ubiquitination of activated by oxidative stress and repressed under basal conditions by the NRF2 expression datasets. NRF2, the primary regulator of redox balance, is usually dependent stress response when OGT activity is inhibited in different tumor cell lines. Senescence is induced in normal cells by various stresses, such as aberrant oncogene signaling, and serves as a major tumour-suppressive barrier that must be bypassed for cellular transformation in vitro and tumorigenesis in vivo. Previous reports show that aberrant reactivation of EMT-inducing tran-scription factors (EMT-TFs) promotes senescence bypass. Thus, aberrant EMT may drive tumor initiation in addition to providing cancer cells with mesenchymal and stem cell properties that drive tumor progression, metastasis, and tumor recurrence. Conversely, our studies here indicate that much like aberrant oncogene signaling, aberrant EMT and CSC-properties induced by OSM may drive tumor initiation in transformed cancer cells. Senescence is induced in normal cells by various stresses, such as aberrant oncogene signaling, and serves as a major tumor-suppressive barrier that must be bypassed for cellular transformation in vitro and tumorigenesis in vivo. Previous reports show that aberrant reactivation of EMT-inducing tran-
focused on modulation of c-myc and miR34 levels and the IL1/6/8 signaling axis as potential strategies for interference with senescence and suppression of residual (dormant) tumor growth and cancer recurrence.

#5460 Targeting tumors by inhibiting mitochondrial electron transport. Arvinder Kapur, Amruta P. Nayak, Thomas Beres, Kayva Rath, Mildred Feder, Amani Gillette, Spencer Erickson, Melissa Shala, Liza Barroilhet, Manish S. Patankar. *Univ. of Wisconsin-Madison, Madison, WI.*

Recently, we demonstrated that the monopetrene, citral, is potent inhibitor of cell proliferation. Here, we are demonstrating that the α,β-unsaturated carbonyl functionality of citral is responsible for its biological activity causing substantial increase in intracellular oxygen radicals. The resulting oxidative stress activates p53. Neutralization of oxygen radicals by N-acetylcysteine attenuates the cytotoxic effect of citral and inhibits activation of p53. Inhibition of p53 by pifithrin-α inhibits citral-induced apoptosis. Oxidative stress caused by citral initiates downstream events causing apoptosis of cancer cells. With the identification of α,β-unsaturated carbonyl group as the obligatory functionality for the anti-cancer effects of citral, we surveyed other small molecule drugs that shared this chemical entity. Cinnamaldehyde, nonenal, perillaldehyde, curcumin, and plumbagin have the α,β-unsaturated carbonyl functional groups and have demonstrated anti-cancer effects. Using curcumin and plumbagin as prototypes, we were able to demonstrate similarities in the cytotoxic mechanism with citral. The oxidative stress caused by curcumin and plumbagin is the initiating step required for cancer cell apoptosis. Fluorescent Lifetime Imaging Microscopy (FLIM) showed that exposure to plumbagin results in increase in free NADH/FAD ratio. Additionally, in silico modeling showed that plumbagin and curcumin docked to the active site of complex I and III of the mitochondrial electron transport chain. Using the Seahorse analyzer, our experiments showed that plumbagin and curcumin inhibited the oxygen consumption rate in treated cells. These results indicate that the α,β-unsaturated carbonyl containing compounds produce their cytotoxic effects by interfering with the electron transport chain. The cancer cells respond to the surge in oxygen radicals by increasing the expression of Nrf-2, the transcription factor that regulates major anti-oxidant responses. This compensatory mechanism is a form of chemoresistance that allows cancer cells to evade the cytotoxic effects of agents such as citral. We will present data from strategies to inhibit the Nrf-2-mediated compensation and thereby enhance the cytotoxic activity of citral, curcumin and plumbagin. The data obtained through these experiments is providing important information that will allow designing of novel agents that can interfere with the electron transport chain and inhibit tumor growth by inducing oxidative stress.


The transcription factor p73 is a homologue of p53 that can be expressed as pro- or anti-apoptotic isoforms. Unlike p53, p73 is rarely mutated or lost in cancers and it is found to replace defective p53 inducing apoptosis. Here, we investigated the p73 involvement in anoikis, a type of apoptosis caused by inadequate cell matrix interactions. Breast cancer cells lines with different p53 status were treated with doxorubicin or docetaxel and cells detached from the extracellular matrix were analyzed. We demonstrate for the first time that doxorubicin-induced cell detachment is associated with p73 cleavage and caspase activation, independently of the p53 status. However, we did not detect p73 cleavage or caspase activation in detached cells under doxetaxel treatment. Overexpressing the apoptotic isoform of p73 led to cell detachment associated with p73 cleavage and caspase activation. Interestingly, p73 cleaved forms localize to the nucleus during the late phase of cell death indicating an increase in the transcriptional activity. Our study suggests that the cleavage of p73 on specific sites may release its pro-apoptotic function and contribute to cell death.

#5462 GTP cyclohydrolase in brain tumor stem cells is implicated in glioblastoma growth. Anh N. Tran, 1 Kiera Walker, 1 David Harrison, 2 Wei Chen, 3 James Mobley, 2 Lauren Hocevar, 1 James R. Hackney, 1 Randee S. Sedaka, 1 Jennifer S. Pollock, 1 Sara J. Cooper, 1 George Y. Gillespie, 1 Anita B. Hjelmeland. 1University of Alabama at Birmingham, Birmingham, AL; 2Vanderbilt University, Nashville, TN; 3HudsonAlpha Institute for Biotechnology, Huntsville, AL.

Glioblastomas (GBMs) are the most common primary brain tumors in adults and one of the most aggressive cancers with high rates of recurrence and therapeutic resistance. In GRMs, subpopulations of highly tumorigenic cells called brain tumor initiating cells (BTICs) have been characterized by their unique capacity to promote tumor maintenance, therapeutic resistance, and angiogenesis. BTIC maintenance is known to be regulated by reactive oxygen and nitrogen species. GTP cyclohydrolase, or GCH1, is a critical molecule regulating reactive species levels. We found that expression of GCH1 RNA and protein were upregulated in BTICs in comparison to non-BTICs. Overexpression of GCH1 in glioma cells increased cell growth in vitro and increased tumor formation and decreased survival in an intracranial GBM mouse model. In contrast, knockdown of GCH1 RNA in GBM cells led to growth inhibition in vitro as well as increased survival in animal orthotropic models. Furthermore, genetic modulation of GCH1 led to altered ROS levels in GBM xenolines. In silico analyses demonstrate that higher GCH1 levels in glioma patients correlate with higher glioma grade, recurrence and worse survival. Together, our data suggest that upregulation of GCH1 in BTICs promotes tumor maintenance and is a key regulator of reactive oxygen species in glioblastoma.

#5463 Disulfiram suppresses metastasis via induction of anoikis and calpain activation in triple-negative breast cancer. Eunhye Oh, Daeh Sung, Youngkwan Cho, Ji Young Kim, Nahyun Lee, Yoon-Jae Kim, Tae-Min Cho, Jae Hong Seo. *Korea University, Seoul, Republic of Korea.*

Triple-negative breast cancers (TNBC) exhibit aggressive phenotypes and are associated with poor clinical outcomes due to a lack of effective therapeutic strategies. Disulfiram (DSF) is a clinical therapeutic drug for the treatment of alcoholism, and has also been shown to exhibit antitumor activity in TNBC cells in a copper (Cu)-dependent manner, although how this occurs has not been clearly elucidated. We sought to investigate the mechanisms responsible for DSF/Cu-dependent induction of apoptosis and suppression of lung colonization by these cells. DSF/Cu was shown to induce a Cu-dependent increase in free NADH, as measured by NADH/FAD ratio. Additionally, exposure to DSF/Cu was found to induce a Cu-dependent decrease in cell viability and colony formation, which was associated with an increase in the cleavage of pro-caspase-3 and the upregulation of calpain, a protease involved in apoptosis. Taken together, these findings warrant further investigation of disulfiram as a potential treatment for metastatic TNBC.

#5464 Catalase mediates the survival of anchorage-independent ovarian cancer cells. Cassandra L. Libbing, Calli A. Versagli, Saint Mary’s College, Notre Dame, IN.

Among United States women, ovarian cancer is the fifth leading cause of cancer-related death and the deadliest of the female reproductive system cancers. This is due to a lack of early detection, in effect, leading to primary diagnosis in later, metastasized stages for which there are few effective treatment options. In order to metastasize, ovarian cancer cells must be able to survive detachment from the extracellular matrix (ECM). When a non-tumorigenic mammary epithelial cell detaches from the ECM, research has shown that levels of Reactive Oxygen Species (ROS) rise in the cell and inhibit cell metabolism resulting in decreased cell survival. Previous research has also shown that ECM-detached breast cancer cells are able to utilize antioxidant enzymes to neutralize ROS that Enter with N-acetyl-L-cysteine (NAC) and promote cell survival. This antioxidant activity has also been shown to bolster cancer cells’ survival in anchorage independence. Although studied in breast cancer cells, no work has been done in ovarian cancer, which exhibits a very unique mode of metastasis through the peritoneal fluid. To begin to understand the role of antioxidant enzymes in metastatic ovarian cancer cells, we have worked to characterize the role of catalase, a prominent antioxidant enzyme, in the survival of ECM-detached SKOV3 ovarian cancer cells. In order to do so, we utilized short hairpin RNA (shRNA) to engineer SKOV3 cells deficient in catalase. The catalase-deficient cells were then tested for colony formation in detachment and cell viability and proliferation in both attachment and detachment. The results show that elimination of catalase expression does not have a significant effect on proliferation in attachment or detachment, but significantly decreases both cell viability and colony formation of SKOV3 cells grown in ECM-detachment. This suggests that elimination of catalase expression may hold promise for targeted treatment in late stage ovarian cancer, calling for further investigation of the mechanisms behind these results and the role that other antioxidant enzymes play in ovarian cancer. Future studies include looking at this phenomenon in other ovarian cancer cell lines, and using mouse xenograft studies to examine the effects of antioxidant compounds on survival of metastatic ovarian cancer cells.

#5465 Persistent oxidative stress in mouse intestinal and colonic epithelial cells after exposure to 131I-iodination. Shubhankar Suman, Santosh Kumar, Albert J. Fornace, Kamal Datta. *Georgetown Univ., Washington, DC.*
Purpose: $^{12}\text{C}$-ion radiotherapy is demonstrating favorable results compared to photon radiotherapy for select cancers. While normal tissue exposure is lower with $^{12}\text{C}$-ions relative to $\gamma$-rays, it cannot be completely eliminated and therefore, late tissue toxicity, inflammation and secondary carcinogenesis cannot be ruled out. The goal of this study was to assess the extent of persistent oxidative stress (POS) after radiation exposure and compare the results through autovivary radiation exposure. Methods and Materials: Mice (C57BL/6; 6 to 8 weeks; male) were irradiated with 0.5 or 1.3 Gy of $\gamma$ or $^{12}\text{C}$-ion, and intestinal (IEC), colon (CEC) epithelial cells and tissues were collected 2 months after radiation exposure. In epithelial cells, intracellular ROS, mitochondrial superoxide, mitochondrial membrane potential (MMP), and cardiopulmonary oxidation were studied by flow-cytometry, superoxide dismutase, SOD, catalase, and NADPH oxidase activity along with lipid oxidation were also assessed in epithelial cells using biochemical assays. Results: Our results clearly showed radiation quality and dose-dependent induction of POS. Both intestine and colon showed a higher elevation of intracellular ROS, mitochondrial superoxide, NADPH oxidase activity, and mitochondrial cardiopulmonary oxidation after $^{12}\text{C}$ relative to $\gamma$ radiation. Moreover, antioxidant enzyme activities in intestine and colon was also significantly reduced in $^{12}\text{C}$-irradiated mice. Compared to $\gamma$ radiation, membrane lipid damage was remarkably higher in both intestine and colon of $^{12}\text{C}$-ion irradiated mice. At 0.5 Gy, persistent oxidative damage indicated by 4-hydroxynonenal was 3-fold in colon and 1.6 fold in intestine after $^{12}\text{C}$ relative $\gamma$ radiation. Conclusions: Mitochondrial deregulation, increased NADPH oxidase activity, and loss of SOD and catalase activities were the major contributory events in $^{12}\text{C}$-ion induced POS in mouse GI tissues. Compared to intestine, colon was more susceptible to POS induction that might be due to progressive cell turnover in intestine resulting in faster elimination of initial damage signal in a given time than colon. Taken together, our data suggest that normal tissue exposure to $^{12}\text{C}$ radiation carries higher long-term risk relative to $\gamma$-rays at comparable doses and further detail evaluation is warranted.

#5466 Mitochondrial superoxide inhibits autophagy and induces apoptosis through SQSTM1-mediated mechanism. Heather G. Hambright, Addanki P. Kumar, Rita Ghosh. UT Health Science Center at San Antonio, San Antonio, TX.

Metastatic melanoma has a dismal survival rate for patients, despite recent advances in targeted and immune-therapies. Recent studies show that elevated autophagy and reoxidation in melanoma cells contributes to chemotherapeutic resistance, making these biological processes ideal therapeutic targets. However, gaps still exist regarding the mechanism by which pro-oxidant therapies affect autophagic signaling and the ultimate impact on melanoma cell fate. We previously demonstrated that pro-oxidant compound Neuretinin (NX) selectively inhibits melanoma cell survival and autophagy. Using mass spectrometry we identified SQSTM1/p62, a substrate of the ubiquitin-proteasome system, as a possible mediator for the observed outcomes. We subsequently showed that under the stress the reduction of SQSTM1/p62 remained unknown. Thus, the primary objective of this study was to determine how p62 regulates cell fate during oxidative damage. We used a panel of primary melanocyte and melanoma cells to evaluate i) proapoptotic degradation of p62 during oxidative stress-induced autophagy blockage, ii) the requirement for p62 during NX-induced apoptosis, iii) effect of NX-induced superoxide on autophagy and cell survival, iv) association of p62 with caspase-8 during oxidative damage, and v) subcellular localization of NX. Proapoptotic degradation was evaluated using MG132 and western blotting for p62 following NX. We used genetic approaches (RNAi and overexpression using HA-p62) to determine the role of p62 in cell fate as determined by survival, apoptosis, and autophagy measurements. Fluorescence microscopy was used to determine the subcellular localization of NX. Generation of superoxide was determined using mitoSOX and mitoTEMPO pre-treatment was used to determine specificity. We found that i) NX-induced ROS inhibits p62 protein by stimulating proapoptotic degradation, ii) p62 mediates apoptosis in response to high levels of oxidative damage through caspase-8, iii) NX generates superoxide which inhibits NADPH oxidase activity along with lipid oxidation were also assessed in epithelial cells using biochemical assays. Results: Our results clearly showed radiation quality and dose-dependent induction of POS. Both intestine and colon showed a higher elevation of intracellular ROS, mitochondrial superoxide, NADPH oxidase activity, and mitochondrial cardiopulmonary oxidation after $^{12}\text{C}$ relative to $\gamma$ radiation. Moreover, antioxidant enzyme activities in intestine and colon was also significantly reduced in $^{12}\text{C}$-irradiated mice. Compared to $\gamma$ radiation, membrane lipid damage was remarkably higher in both intestine and colon of $^{12}\text{C}$-ion irradiated mice. At 0.5 Gy, persistent oxidative damage indicated by 4-hydroxynonenal was 3-fold in colon and 1.6 fold in intestine after $^{12}\text{C}$ relative $\gamma$ radiation. Conclusions: Mitochondrial deregulation, increased NADPH oxidase activity, and loss of SOD and catalase activities were the major contributory events in $^{12}\text{C}$-ion induced POS in mouse GI tissues. Compared to intestine, colon was more susceptible to POS induction that might be due to progressive cell turnover in intestine resulting in faster elimination of initial damage signal in a given time than colon. Taken together, our data suggest that normal tissue exposure to $^{12}\text{C}$ radiation carries higher long-term risk relative to $\gamma$-rays at comparable doses and further detail evaluation is warranted.

#5467 Prophosphoproteomic analysis of pyruvate dehydrogenase in response to environmental stress. Wendi O’Neill, Tereza Golias, Martin Benej, Nicholzas Denko. The Ohio State University, Columbus, OH; Slovak Academy of Sciences, Bratislava, Slovak Republic.

Pyruvate dehydrogenase is a mitochondrial enzyme that regulates the flux of carbohydrates into oxidative phosphorylation. The activity of PDH is largely regulated by reversible phosphorylation. The pyruvate dehydrogenase kinases inhibit phosphorylations to PDH on the E1α subunit at positions 232, 293, and 300. Interestingly, there is a family of 4 related PDHK genes with overlapping affinity for the 3 phosphorylation sites. PDHK1 and PDHK3 have been identified as hypoxia-inducible at the transcriptional level, and PDHK1 has been shown to be important for the growth of model tumors. This work attempts to more carefully define the functions of PDHK1 and 3 using CRISPR technology in colorectal cancer lines in vitro and genetically engineered mice in vivo. Using antibodies specific for the phosphorylated sites on E1α, we find that PDHK1 is essential for the phosphorylation of the 232 site in response to hypoxia and partially responsible for the hyperphosphorylation of 293 and 300. The level of phosphorylation in response to hypoxia is also sensitive to nutrients in the environment, with high levels of glucose enhancing E1α phosphorylation in hypoxia. Analysis of the number of phosphorylations on individual E1α molecules using isoelectric focusing shows a range of events, with some molecules having 2 or 3 phosphorylations, while a large fraction of the E1α remains un-phosphorylated in hypoxia. This type of pattern suggests a non-random phosphorylation of the multiple sites. Analysis of PDH activity supports the in vitro findings that any single phosphorylation event is sufficient to inhibit PDH complex activity. Preliminary mitochondrial oxygen consumption experiments show that colorectal cancer cells are less dependent on PDHK1 for regulating mitochondrial function compared to published data in pancreatic and head and neck cancer. Finally, analysis of PDH phosphorylation in mice supports a role for PDHK1 in the regulation of PDH activity in response to fasting and blood glucose levels. In conclusion, regulation of PDH in normal and cancerous colonicocytes appears to be sensitive to environmental oxygen and nutrients. PDHK1 is the PDHK family member with the greatest influence on the addition of non-random inhibitory phosphorylation events. These in vitro findings will be analyzed with respect to the findings obtained using the new PDHK1 f/f null mouse.

#5468 Expression of aquaporin 11 as a potential predictor of cisplatin sensitivity. Jason V. Evans, Anwar Akhtar, David P. Carbone, Mikhail M. Dikov, Elena E. Tchekeeva. West Virginia University, Morgantown, WV; Ohio State University, Columbus, OH.

Platinum-based combination is a standard of care treatment for lung cancer patients. Accumulating evidence indicates that aquaporins are emerging targets in cancer. Aquaporin 11 (AQP11), a non-ubiquitous member of aquaporins family, is an endoplasmic reticulum (ER)-specific water channel mostly present in epithelial cells and is implicated in the maintenance of ER homeostasis. In our previous studies, we demonstrated that under conditions of ER stress the reduction of AQP11 expression is a pro-apoptotic factor in normal epithelial cells. We revealed that under normal conditions AQP11 is heavily S-nitrosylated forming functionally important multimeric structures (> 1000 kDa), whereas cisplatin treatment interferes with AQP11 causing its structural and post-translational modification. We hypothesized that if tumor cells express AQP11 then cells with lower levels of AQP11 are not protected from cisplatin-associated ER stress and develop higher sensitivity to drug treatment. In normal lung AQP11 is expressed at low levels. We evaluated the expression of AQP11 in lung cancer cells to determine whether the AQP11 expression correlates with cisplatin sensitivity and whether lower AQP11 expression in lung tumor associates with positive overall survival in patients with lung adenocarcinoma. A strong link between the level of AQP11 protein expression and cell sensitivity to CP was detected using MTT assay. Screening lung cancer cell lines we found that all tested cancer cell lines expressed AQP11 protein at various levels. Western blot of stress markers showed that cisplatin-treated cells with higher AQP11 expression had lowered cellular and ER stress. Reduction of AQP11 expression using shRNA AQP11 silencing in cisplatin-resistant A549 and HCC827 cell lines, resulted in the significant increased up to 3-fold sensitivity to cisplatin compared with control shRNA transfected or parental non-transfected cells (p<0.001). In sensitive H460 cells, knocking down AQP11 did not change sensitivity to cisplatin. TCGA database analysis of previously untreated lung adenocarcinoma, detected 13% tumors with elevated AQP11 mRNA expression. Lower AQP11 expression was
significantly associated with higher OS and higher median survival rate (52.5 vs. 34.47 mo) than in patients with high AQP11 expression (p < 0.05). AQP11 gene is located within oncogenic 1q13-q14 amplicon, which is a common aberration in solid tumors associated with poor prognosis. The AQP11 is a pro-survival factor protecting tumor cells from cisplatin-induced stress. Low AQP11 expression associates with higher OS in lung adenocarcinoma patients and with higher cisplatin sensitivity in lung cancer cell. The ER stress aggravation via interference with AQP11 could become a prospective therapeutic approach in anti-tumor treatment.

#5469 Low dose ionizing radiation induces persistent activation of NA-DPH oxidase pathway in mouse colon. Santosh Kumar, Shubhankar Suman, Bo-Hyun Moon, Albert J. Fornace, Kamal Datta. Lombardi Cancer Center, Georgetown University, Washington, DC

Exposure to ionizing radiation has been linked to persistent oxidative stress. The purpose of the current study was to characterize the role of NADPH oxidase pathway in radiation-induced increased oxidant production in colon epithelial cells. Mice (C57BL/6); 6-8 weeks, male) were exposed to either sham or 0.5 Gy radiation, and NADPH oxidase pathway and oxidative stress markers were assessed in colon samples 60 d after exposure. Radiation exposure led to higher elevation of 4-HNE and 8-oxo-dG staining relative to unirradiated control. We also observed increased staining for the proliferative marker phospho-histone H3 in colon sections of irradiated mice. PCR analysis showed increased expression of two NADPH oxidase isoforms, Nox1 and Nox3, in irradiated samples. Additionally, expression of NoxA1 and Nox1 along with Hif1α were also increased after radiation. Immunoblot analysis confirmed the PCR results, and ChIP assay revealed greater binding of stress response factor GATA6 and Hif1α to Nox1 and Nox3, and Nox3 promoters respectively after radiation exposure relative to control. Co-immunoprecipitation experiments showed enhanced binding of Rac1, an activator of NADPH oxidase, to Nox1 and Nox3. In summary, we demonstrated that exposure to a low dose of γ radiation caused long-term up-regulation of NADPH oxidase isoforms as well as its regulators and activators such as NoxA1, Nox1, Hif1α, TLR4, GATA6, Doux1, and Doux2. When considered along with our results on oxidative stress and proliferative markers, our observations on NADPH oxidase pathway provides new insight into molecular events contributing to radiation-induced persistent oxidative stress and cell proliferation in colon, and have implications for colorectal carcinogenesis.


Disease recurrence at either primary or metastatic tumor sites arising from dormant tumors is largely responsible for the morbidity and mortality of many cancers. Although cellular quiescence has been proposed as a mechanism of dormancy, the nature of the growth arrest that allows tumor cells to re-emerge months or years after treatment has never been directly determined. The current work was designed to examine the hypothesis that the senescence induced by cancer chemotherapeutic drugs could be reversible, with implications for tumor dormancy and disease recurrence. Studies were performed using etoposide in models of non-small cell lung cancer (NSCLC). Exposure to etoposide at sub-lethal concentrations resulted in growth arrest accompanied by the induction of senescence and autophagy. Growth arrest transient in that proliferative recovery was evident by day 7 post-etoposide exposure. Analysis of senescence based on β-galactosidase activity demonstrated that the restoration of proliferative capacity coincided with a decline in the extent of senescence. Real-time live microscopy demonstrated heterogeneous fates of senescent cells, where the bulk of the cell population remaining in a state of stasis, a subset of cells undergoing apoptosis, and a fraction of the large, flattened, and polyploid cells spontaneously entering into mitosis. Neither genetic nor pharmacological inhibition of autophagy influenced the senescence response to etoposide, the eventual proliferative recovery, or sensitivity to etoposide. These findings indicate that the induction and resolution of senescence is independent of the accompanying autophagy, and that the autophagy induced by etoposide is non-cytotoxic in function. These observations suggest that therapy-induced senescence may ultimately be a transient process in that at least a subpopulation of tumor cells can and will regain proliferative capacity. Consequently, therapy-induced senescence could potentially be studied as a model of tumor dormancy, and the reversibility of senescence as a model of disease recurrence.

#5471 LSD1 stimulates cell survival and epigenetic homeostasis via snail in hepatocellular carcinoma. Yong Hwa Jo, Minam Nam Nguyen, Tae Gyu Choi, Sung Soo Kim. Kyung Hee Univ. - Seoul Campus, Seoul, Republic of Korea.

HCC is the fifth most common malignancy in the world. HCC generally arises in the context of chronic liver diseases, or other metabolic, dietary or toxic factors. Hypoxia is a critical microenvironment in hepatocarcinogenesis. It occurs in series of distinct steps that include tumor cell invasion and proliferation. The process of EMT required for liver cancer cell invasion is regulated by a family of E-box binding transcription repressors, which include Snail and Slug. Protein N, the first discovered histone methylase, is an epigenetic enzyme playing important role in regulation of gene expression and function by removing mono- or dimethyl moieties from H3K4 and H3K9. However, the molecular mechanisms underlying these biological behaviors have not been completely elucidated. Here, we present the first evidence that expression of Protein N was significantly associated with higher HIF1α expression. We also showed that the up-regulated Protein N enhanced HCC cell survival and sustained epigenetic homeostasis via activating Snail under hypoxic conditions in vitro/in vivo. Furthermore, we revealed that Protein N/Snail stimulated Hif1α expression by a positive feedback loop. Consequently, we elicited that Protein N is an essential mediator in epigenetic aberrances of HCC (This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) NRF-2016R1D1A1B03933763).

#5472 Yeast Bax Inhibitor (BXL1) is involved in redox and calcium homeostasis of the ER in S. cerevisiae. David Eagan, Joseph Alisch, Liam McDonough, Savannah Benko, Walter Jacob, Lukas Ritter, James Mullin, John O'Reilly, Nicanor Austriaco. Providence College, Providence, RI.

In mammals, the Bax inhibitor (TMBIM) family of proteins has cytoprotective properties that are most evident in paradigms of endoplasmic reticulum (ER) stress. The canonical gene of the family called BI-1 has been linked to several human cancers. The crystal structure of a proarytometric member of the family, Bi3Tet, has revealed that the Bax inhibitor proteins are pH sensitive calcium leaks. Our published studies have shown that the yeast Bax Inhibitor, Bxl1p, is localized to the endoplasmic reticulum and is involved in the unfolded protein response (UPR) that is triggered by endoplasmic reticulum (ER) stress. We now show that cells lacking BXL1 have an altered redox microenvironment in the ER. The ER of bxl1 cells exposed to dithiothreitol (DTT) and tunicamycin, two drugs known to induce the unfolded protein response (UPR), become more reduced than their wildtype counterparts. Moreover, we also report that cells lacking BXL1 accumulate more calcium in the ER than wildtype cells. Together, our data suggests that Bxl1p is involved in regulating the ER microenvironment. Other members of the TMBIM family may have the same role in normal and cancer cells. (Our laboratory is supported by grant NIGMS R15 GM110578, awarded to N. Austriaco.)

#5473 Towards a better understanding of MTH1 as a therapeutic target in RAS-driven cancer. Govind J. Samaranayake,1 Clara J. Troccoli,1 Mai Q. Huynh,1 Andrew Win,1 Debin Ji,2 Eric T. Kool,2 Priyamvada Rai1. 1Univ. of Miami Miller School of Medicine, Miami, FL; 2Stanford University, Palo Alto, CA.

Oncogenic RAS signaling-generated reactive oxygen species (ROS) drive tumor progression by the hyperactivation of proliferative, anti-apoptotic, and metastatic pathways. Elevated ROS levels however can also cause oxidative DNA damage, leading to oncogene-induced senescence (OIS) and cell death. To avoid such tumor-suppressive outcomes, RAS-driven tumors often upregulate redox-protective proteins. The collective research from our group over the last few years has shown that oncogenic RAS elevates expression of the mammalian 8-oxo-dGTPase MutT Homolog 1 (MTH1), which in turn enables evasion of OIS, promotes transformation efficiency, and facilitates tumorigenicity. Our prior work has therefore demonstrated that MTH1 is beneficial to the spectrum of RAS-driven transformation, and that its shRNA-mediated targeting in oncogenic RAS-harboring lung cancer cells produces robust tumor-suppressive outcomes. However the first wave of chemical MTH1 inhibitors has led to controversial and conflicting results regarding MTH1 as a chemotherapeutic target. Here we evaluate the benefit of MTH1 inhibition in wildtype. vs. oncogenic KRAS expressing cells, and directly assess the effects of oncogenic KRAS as well as three recently developed inhibitors on MTH1 8-oxo-dGTPase activity. Our results support that introduction of oncogenic KRAS elevates both MTH1 expression and activity, presumably through its elevation of ROS levels, and thus sensitizes cells to MTH1 inhibitors. The degree of this sensitization has a complex dependence on residual 8-oxo-dGTPase activity in the different cells following treatment with the MTH1 inhibitors, alternate antioxidant responses, and induction of different tumor suppressor pathways. Our data therefore highlight the importance of evaluating the molecular contexts and outcomes of MTH1 inhibition when determining its utility as a chemotherapeutic target.
#5474 Sphingosine kinase 2/sphingosine 1-phosphate signaling regulates p16 mediated accelerated aging in normal somatic tissues and tcf21 mediated tumor suppression in lung cancer. Shanmugam Panneer Selvam,1 Marion Cooley,1 Kristi Helke,1 Elizabeth Garrett-Mayer,1 Charles D. Smith,2 Besim Ogtetmen,1,2 Medical University of South Carolina, Charleston, SC;2Apoee Bio- techology Corporation, Hummelstown, PA.

Sphingosine 1-phosphate (S1P), a pro-proliferative sphingolipid generated by Sphingosine Kinases is upregulated in many cancers. Telomerase (hTERT), is a ribonucleoprotein that extends the ends of chromosomes (telomeres), to promote lung cancer growth. We discovered that SK2 generated S1P binds and stabilizes telomerase in the nuclear periphery by allosterically mimicking protein phosphorylation. Mechanically, S1P binding promotes telomerase stability. MKRN1 mediated degradation thereby preventing telomere dysfunction and senescence. The objective of this study is to delineate the molecular mechanism of SK2-S1P mediated telomere dysfunction in aging and lung cancer. SK2 knockout mice tissues showed increased telomeric DNA damage and senescence associated-beta galactosidase activity. Moreover, SK2 knockout mice displayed aging phenotypes by reduced subcutaneous fat, atrophy in spleens and hypoplasia in mice testes. Also, SK2 knockout fibroblasts showed increased senescence and robust p16 expression and interestingly, p16 ablation rescued SK2 knockout fibroblasts from senescence. Importantly, we found SK2 and telomerase protein levels to be upregulated in lung cancer tumors (n=48). Mechanistically, pharmacological inhibition of SK2 by ABC294640 (in Phase II clinical trial) leads to telomere dysfunction in A549 cells and increases p16 expression and increased MKRN1 expression in H110547. SK2 inhibition showed ATM/ATR kinase mediated p-CHK1 increase and subsequent activation of caspase-3 in both in vivo hamster and in vitro experiments and stable TCF21 knockdown prevents apoptotic cell death. Importantly, p16 overexpression in p16 deficient A549 lung cancer cells prevented caspase-3 activation and leading to senescence induction following inhibition of SK2-S1P by ABC294640 or shRNA against SK2. Overall, our data suggest that SK2-S1P regulates telomere dysfunction through p16 mediated aging in normal somatic tissues and TCF21 mediated tumor suppression in lung cancer and interestingly SK2-S1P and p16 acts as a molecular switch between senescence and apoptosis in normal and cancer cells respectively.

#5475 Functional profiling of germline EPAS1 variants associated with pheochromocytoma and paraganglioma. Edward Kim, Diana E. Benn, Roderick I. Clifton-Bligh, Trisha Dwight. Kolling Institute of Medical Research, St. Leonards, Australia.

Background: Endothelial PAS domain-containing protein 1 (EPAS1), encoding hypoxia-inducible transcription factor 2-alpha (HIF2α), is involved in regulation of cellular hypoxia responses and has been shown to play a role in several cancers including renal cell cancer and pheochromocytoma/paraganglioma (PPGL). HIF2 levels are tightly regulated, being low in normoxia but increased in hypoxia. Studying heritable PPGL syndromes has uncovered diverse genetic events that lead to oxygen-independent HIF stabilization. Using massively parallel sequencing, we noted several germline EPAS1 variants that occurred in subjects also carrying pathogenic mutations in either VHL or SDHB. In addition, two distinct EPAS1 variants were identified in separate individuals who did not harbor germline mutation in other known susceptibility genes. We therefore investigated whether these variants affected EPAS1 function. Method: Six different EPAS1 variants were identified in the germline of patients with PPGL: p.His194Arg, p.Arg247Ser, p.Phe374Tyr, p.Thr766Pro, p.Pro785Thr and p.Phe374Tyr, and transfected into HEK293 cells. HEK293 cells transfected with wildtype HIF2α were also subjected to hypoxic conditions for use as a positive control. Co-immunoprecipitation followed by Western blotting analyses were performed to determine whether: a) HIF2α mutations are still able to interact with VHL and ARNT; and b) HIF2α mutations are stabilized under normoxia. Candidate target gene expression (CCND1 and SLC2A) was measured by RT-qPCR. Results: Western blot analyses showed that p.Arg247Ser, p.Phe374Tyr, p.Pro351Thr and p.Pro785Thr amino acid changes in HIF2α were all more stable than wild-type HIF2α under normoxia. Co-IP of HIF2α with VHL showed significantly reduced interaction with p.Arg247Ser and p.Pro351Thr (p<0.05). Co-IP of HIF2α with ARNT failed to show any effect on interaction occurring as a result of the HIF2α variants. Interestingly only p.Pro351Thr was able to induce CCND1 and SLC2A gene expression (p<0.05). Conclusion: Germline EPAS1 variants p.Arg247Ser and p.Phe374Tyr share some functional features in common with the known oncogenic somatic variant p.Pro351Thr. These findings are clinically relevant to developing cancers that are dependent upon pseudo-hypoxia. Whether any of these EPAS1 variants will affect response to HIF2α antagonist therapies is yet unknown and may be of importance.

#5476 CIP2A can be a therapeutic target of rapamycin in radiosensitive head and neck cancer with P53 mutation. Song Hee Kim,1 Won hyeok Lee,1 Myung Jin Lee,1 Hye Won Chang,2 Ji Won Kim,2 Mi Ra Kim,2 Jung Je Park,2 Myung Woul Han,1 Sang Yoon Kim1.1 Univ. of Ulsan, Seoul, Republic of Korea;2 Inje University College of Medicine, Busan, Republic of Korea;3 Gyeongsang National University, Jinju, Republic of Korea.

The presence of p53 mutation was associated with poor survival in various tumors. The involvement of p53 in apoptosis and cell-cycle control makes it a plausible biomarker of prognosis. Classifying TP53 mutations in HNSCC described disruptive TP53 mutation in either the L2 or L3 loop of the DNA binding domain, resulting in polarity change within the protein, or stop codon. Disruptive TP53 mutation in HNSCC tumors predicts for locoregional recurrence, due to increased radiosensitivity via the inhibition of senescence. Senescence induction contributes to cancer therapy responses and is crucial for p53-mediated tumor suppression. However, whether p53 inactivation actively suppresses senescence induction has been unclear. Here, we found that overexpression CIP2A (cancerous inhibitor of protein phosphatase 2A) positively correlated with presence of p53 mutation in head and neck cancer and mediates radiosensitization through suppression of radiation induced senescence. And we demonstrated that rapamycin could induce senescence via CIP2A downregulation and increase radiosensitivity in p53 mutant disruption cell line. This is the first investigation to analyze CIP2A resistance to the radiosensitivity of head and neck cancer. As a consequence, a greater understanding of radiosensitivity mechanisms through our results in head and neck cancer with p53 mutation will enable the rational design of combination regimens and sequential treatment algorithms to improve clinical outcomes and points to the usefulness of CIP2A as a biomarker to predict clinical response to rapamycin in head and neck cancer.

#5477 Hydrogels to study ECM-oxygen gradient interactions for sarcoma cell migration. Daniel M. Lewis,1 Sharon Gerecht,1 T. S. Karin Eisinger-Mathison,2 Johns Hopkins Univ., Baltimore, MD;2 University of Pennsylvania Perelman School of Medicine, Philadelphia, PA.

Hypoxia is a critical factor in the progression and metastasis of many cancers, including soft tissue sarcomas. Frequently, oxygen (O2) gradients develop in tumors as they grow beyond their vascular supply, leading to heterogeneous areas of O2 depletion. Here, we report the impact of hypoxic O2 gradients and collagen fibers on sarcoma cell invasion and migration. We began with O2 gradient measurements and found that large sarcoma mouse tumors (>300 mm3) contain a severely hypoxic core (≤0.1% partial pressure of O2 (pO2)) whereas smaller tumors possessed hypoxic gradients throughout the tumor mass (0.1-6% pO2). To analyze tumor invasion, we used O2-controllable hydrogels to recreate the physiopathological O2 levels in vitro. Small tumor grafts encapsulated in the hydrogels revealed increased invasion that was both faster and extended over a longer distance in the hypoxic hydrogels compared with nonhypoxic hydrogels. To model the effect of the O2 gradient accurately, we examined individual sarcoma cells embedded in the O2-controllable hydrogel. We observed that hypoxic gradients guide sarcoma cell motility and matrix remodeling through hypoxia-inducible factor-1α (HIF-1α) activation. We further found that in the hypoxic gradient, individual cells migrate more quickly, across longer distances, and in the direction of increasing O2 tension. Treatment with minoxi- dil, an inhibitor of hypoxia-induced sarcoma metastasis, abrogated cell migration and matrix remodeling in the hypoxic gradient. Next, we have developed hydorgels to measure hypoxic gradients where we can control collagen fiber density and cross-linking density independently from oxygen gradients. Using this new platform we show that an increase in fiber density in conjunction with the hypoxic oxygen gradient leads to an increase in sarcoma cell migration. This increase in migration speed was also correlated with quicker stress relaxation times of the collagen gel and larger pore size. Using these platforms we were able to create a novel 3D experimental system with control over mechanical properties (cross-linking density, binding sight density, fiber density, stress relaxation time, and stiffness) independently from oxygen concentration. Overall, we show that O2 acts as a 3D physiologic agent during sarcoma tumor invasion and that this migration is
modulated by collagen fiber density and pore size, in the surrounding matrix. We propose the O2- controllable hydrdyes as a predictive system to study early stages of the metastatic process and therapeutic targets.

#5478 The TRPA1 Ca2+-permeable channel mediates a non-canonical redox adaptation in cancer cells. Nobuaki Takahashi,1 Hsing-Yu Chen,2 Isaac Harris,1 Daniel Stover,1 Roderick Bronson,3 Thomas Deraedt,1 Karen Cichowski,1 Alana Wehm,2 Gordon Jones,3 Joan Bruggel.1 Harvard Medical School, Boston, MA;2 University of Utah, Salt Lake City, UT;3 The University of Texas MD Anderson Cancer Center, Houston, TX.

Generation of reactive oxygen species (ROS), a natural byproduct of oxygen metabolism, occurs in all aerobic organisms at a controlled rate. Cancer cells are subjected to two sets of cellular insults, including dysregulated mitogenic/survival signaling and dissociation from their natural extracellular matrix (ECM) niches, leading to the generation of high levels of ROS. Although cancer cells possess enhanced canonical antioxidant programs that neutralize ROS, they nevertheless exhibit prominent ROS levels in response to these insults, suggesting the existence of additional programs that can allow cancer cells to tolerate elevated ROS. In this study, we provide evidence for an unconventional mechanism for redox adaptation involving the ROS-activated, Ca2+-permeable TRPA1 channel, which normally functions as an irritant receptor in sensory neurons but is highly upregulated in breast, lung, malignant neural sheath, and other tumors. We found that TRPA1 overexpression enhances survival and proliferation of MCF-10A cells under conditions of matrix-detachment and this effect was dependent on TRPA1 activation, resulting in an increase in cell survival. In MCF-10A cells overexpressing TRPA1, we observed increased Ca2+ entry in ECM-deprived cells in the luminal space and promoted their survival and proliferation. Conversely, TRPA1 knockdown inhibited Ca2+ responses to ROS generated through ECM detachment and induced clearance of cells from the luminal space in breast cancer spheroids. TRPA1 was also activated by ROS-inducing chemotherapies and drove chemoresistance in breast, lung and malignant peripheral nerve sheath tumors cells, and its downregulation suppressed breast xenograft tumor growth and enhanced chemosensitivity. TRPA1 mediated these effects independent of antioxidant responses, but through activation of cellular survival and anti-apoptotic programs involving the RAS-ERK/AKT/mTOR pathways. Together, our findings describe an unexpected mechanism whereby cancer cells co-opt the neuronal TRPA1 channel in order to adapt to oxidative environments. This TRPA1-induced response is distinct from canonical redox adaptation mechanisms that rely on antioxidant program. As TRPA1 inhibitors are currently in clinical trials for pain and respiratory therapies, these studies raise the possibility of using these inhibitors as therapeutic chemosensitizers in TRPA1-enriched tumors.

#5479 Redox stress as a therapeutic Achilles heel in castration-resistant prostate cancer. Clara L Trogcolli, Govindi J. Samarayake, Mai Q. Huynh, Karen Kage, Deukwoo Kwon, Yuqang Ban, Xi S. Chen, Enrique R. Zarco, Merce Jorda, Kerry L. Burnsnt, Priyamvada Rai. Unv. of Miami Miller School of Medicine, Miami, FL.

Androgen deprivation therapy (ADT) initially suppresses prostate cancer (PC) progression. However, castration resistant PC (CRPC) cells inevitably emerge, leading to progressive and debilitating disease. We recently demonstrated that ADT produces a form of cellular senescence leading to outgrowth of CRPC subpopulations from the AD-sensitive parental cells. Gene expression profiling studies comparing the parental LNCaP line to the senescent-resistant, early CRPC variant, LNCaP SB5, revealed an enrichment of thiol-based redox-protective proteins in the latter under AD conditions. This finding suggests that redox stress due to hyperactivated mitogenic/survival signaling or metabolic stresses may be an important and understudied Achilles heel in the progression to CRPC. Here we present the effects of inhibiting thioredoxin-1 (TRX1), a redox-protective protein that was identified as being elevated in our early CRPC LNCaP SB5 model. Suppression of TRX1 expression via siRNA led to profound growth suppression of CRPC cells, relative to their AD-sensitive counterparts, and significantly reduced CRPC xenograft tumor growth. Furthermore, under AD, TRX1 suppression promoted p53-induced cell death, which was accompanied by increased reactive oxygen species (ROS). These in vitro and in vivo results were recapitulated using a Phase II clinical trial-tested chemical TRX1 inhibitor. Thus our results point to TRX1 as a critical requirement for CRPC progression and provide a rationale for using TRX1 inhibitors in conjunction with ADT to limit CRPC.

#5480 Peroxiredoxin 2 mediates the survival of ECM-detached ovarian cancer cells. Calli A. Davison-Versagli. Saint Mary’s College, Notre Dame, IN.

Non-tumorigenic epithelial cells require anchorage to the extracellular matrix (ECM) in order to survive; however, it has been recognized that metastatic cancer cells must have the ability to survive in anchorage independence. Approximately 70% of epithelial ovarian cancer (EOC) cases are diagnosed after EOC cells have already metastasized, making EOC more difficult to treat. EOC cells shed from the primary tumor and accumulate in ascites in the peritoneal cavity before attaching and forming secondary tumors throughout the peritoneal cavity. Better understanding of the molecular mechanisms underlying survival of the non-adhesive subset of cells that survive in anchorage independence in the ascites could lead to the development of improved chemotherapeutic agents and the protection of patients from recurrent disease. The chemotherapeutic agent doxorubicin significantly decreased cell proliferation and viability in leukemia. The purpose of this study is to examine Peroxiredoxin levels in K562 cells, a human chronic myelogenous leukemia cell line, and determine if high levels of Peroxiredoxin in the ability to protect cells from chemotherapy-induced death and possibly aid in chemoresistance. We first demonstrated that serum-deprived K562 cells exhibited significant growth inhibition over a 72 hour time period, but had no significant effect on viability. We also showed that treating K562 cells with 30 nM doxorubicin significantly decreased cell proliferation and viability. Corresponding changes in Peroxiredoxin protein levels are currently being analyzed to determine the effect of both growth-inhibition and chemotherapeutics-induced cytotoxicity on the Peroxiredoxin family in K562 cells, and siRNA experiments are being conducted to examine the protective role of Peroxiredoxin in these cells. Based on previous studies on MCF-7 cells conducted in our lab, we predict that Peroxiredoxin levels will be modified under both conditions, and that suppression of Prdxs by siRNA will increase chemotherapeutics susceptibility.

#5481 Role and regulation of Peroxiredoxin antioxidant proteins in K562 leukemia cells. Elizabeth Szabo, Shelley A. Phelan. Fairfield University, Fairfield, CT.

Peroxiredoxins are a family of antioxidant proteins that have found to play a key role in many diseases, including cancer. A wide number of studies have found that Peroxiredoxin levels are elevated in several cancers, and reducing these levels can aid in inhibiting cell growth of cancer cells, suggesting that Peroxiredoxins confer resistance to reactive oxygen species (ROS)-induced cellular toxicity in cancer cells. Specific research has demonstrated that Peroxiredoxin levels are elevated in leukemia cells, and that targeting and inhibiting Peroxiredoxin in these cells can induce differentiation. Studies have also shown the use of doxorubicin as an effective chemotherapy treatment to significantly decrease cell proliferation and viability in leukemia. The purpose of this study is to examine Peroxiredoxin levels in K562 cells, a human chronic myelogenous leukemia cell line, and determine if high levels of Peroxiredoxin in the ability to protect cells from chemotherapy-induced death and possibly aid in chemoresistance. We first demonstrated that serum-deprived K562 cells exhibited significant growth inhibition over a 72 hour time period, but had no significant effect on viability. We also showed that treating K562 cells with 30 nM doxorubicin significantly decreased cell proliferation and viability. Corresponding changes in Peroxiredoxin protein levels are currently being analyzed to determine the effect of both growth-inhibition and chemotherapeutics-induced cytotoxicity on the Peroxiredoxin family in K562 cells, and siRNA experiments are being conducted to examine the protective role of Peroxiredoxin in these cells. Based on previous studies on MCF-7 cells conducted in our lab, we predict that Peroxiredoxin levels will be modified under both conditions, and that suppression of Prdxs by siRNA will increase chemotherapeutics susceptibility.

#5482 Constitutive overexpression of nrzf2 in esophageal adenocarcinoma protects cancer cells from bile salts-induced DNA damage and favors cancer cell survival. Dunfa Peng, Tianling Hu, Shoumin Zhu, Wael El-Rifai. Vanderbilt University Medical Center, Nashville, TN.

Background: Esophageal adenocarcinoma (EAC) is the major type of malignant cancer of esophagus in the USA. The incidence rate has increased 4-10% per year among men since 1976 in the USA, more rapidly than for any other type of cancer. EAC is known to originate from premalignant Barrett’s esophagus (BE) through BE-dysplasia-EAC process. Normal cells have intact anti-oxidative mechanisms, among which the key rate-limiting factor 2 (NRF2) plays a pivotal role in regulating cellular response to various stimuli. Cancer cells have high levels of reactive oxygen species and oxidative stress due to activation of oncogenes, inflammatory microenvironment, and dysfunction of anti-oxidative mechanisms. However, the role of NRF2 in Barrett’s related esophageal carcinogenesis is barely known. Methods and Results: We have found that Nrf2 protein expression is significantly upregulated in EAC cell lines as compared to BE cell lines (CPA, BAR10T) and normal esophageal squamous cell lines. We detected a similar overexpression of NRF2 in primary EAC tissue samples. Using luciferase reporter assay, we demonstrated significant induction of NRF2 transcription activity in EAC cells in response to exposure to acidic bile acids, as compared to
controls (P<.01). We detected significant increase in the expression of Heme Oxygenase 1 (HO-1) and Glutathione Reductase (GR) in these cells (P<.01). Knockdown of NRF2 by siRNA or crisp/cas9, significantly increased cellular ROS level, oxidative DNA damage (8-oxoguanine), and double strand DNA breaks (p-H2AX). Knockdown of NRF2 sensitized cells to apoptosis following exposure to reactive nitrogen species and CDDP. Concordant with the NRF2 knockdown, inhibition of NRF2 by a specific inhibitor, Brusatol, sensitized EAC cells to CDDP treatment with a significant increase in cell death, as compared to single agent treatment (P<.01).

Conclusion: These results indicate that constitutive overexpression of NRF2 in EAC cells protects cancer cells from high level of ROS and promotes cancer cell survival. Inhibition of NRF2 through its specific inhibitor may have therapeutic value in EAC.


Oncogene-induced cellular senescence is a tumor suppressor response resulting in irreversible cell cycle exit, and is dependent on the p53 and RB pathways. Loss of p53 has recently been shown to allow re-entry of senescent cells into the cell cycle. The role of the RB1 protein in maintenance of the senescent state is less well studied. We investigated whether RB1 is necessary for the induction as well as the maintenance of oncogene-induced senescence, and we also evaluated whether RB1 activation is sufficient to induce and/or maintain senescence in the absence of a functional p53 pathway. Inactivation of RB1 prior to onset of senescence led to failure of senescence induction, with impaired cell cycle exit and failure to induce senescence markers. On the other hand, loss of RB1 after cells had already entered oncogene-induced senescence was able to reverse some features of the senescence phenotype, but did not result in successful cell division and accumulation. Interestingly, in the absence of p53, constitutively active RB1 was sufficient for cells to undergo oncogene-induced senescence, and effectively prevented colony formation. Finally, while p53 inactivation was sufficient for senescent cells to re-enter the cell cycle, constitutive activation of RB1 prevented cell cycle re-entry, even in the absence of p53. Our findings show that RB1 is necessary for both the induction and maintenance of oncogene-induced senescence, and that active RB is sufficient for senescence induction as well as its maintenance, both in the presence and absence of p53. These results have direct implications on targeting RB activation as a senescence-inducing approach in both p53 wild type and p53-defective premalignant and malignant tumors.

#5484 Structural domains of βIII-Tubulin regulate multiple stress responses and influence cell growth and survival in glucose-deprived non-small cell lung cancer. Amelia L. Parker, Wee S. Teo, Joshua A. McCarror, Maria Kavalleris. Children's Cancer Institute, Randwick, Australia.

βIII-Tubulin and βIII-Tubulin III-tubulin body and tail structural domains in conferring survival advantage to glucose-starved NSCLC cells. METHODS: H460 NSCLC cells were gene-edited at the endogenous βIII-tubulin gene using zinc finger nuclease technology to replace the endogenous βIII-tubulin protein with either the full-length or truncated βIII-tubulin protein, or replacement of the βIII-tubulin body or tail with the βIII-tubulin sequence, each fused to a GFP tag. Clones expressing the modified βIII-Tubulin proteins were cultured in glucose-free conditions for 48h to 10 days and cell growth and viability were measured by Trypan Blue Dye Exclusion and Annexin-V apoptosis assay. The effect of the βIII-tubulin modifications on the endoplasmic reticulum (ER) stress response, autophagy and Akt signaling were examined by western blotting. RESULTS: Characterization of the gene-edited clones revealed that loss of the βIII-tubulin C-terminal tail or substitution of the βIII-tubulin body or tail sequence with that of the βIII-tubulin sequence did not alter the microtubule architecture, cellular proliferation rate or viability in normal growth conditions. However, loss of the βIII-tubulin C-terminal tail significantly reduced the viability of H460 cells by 37 ± 0.05% in acute glucose starvation. Inhibition of the βIII-Tubulin body with the βIII-tubulin body significantly reduced cell number by 33 ± 0.01% during prolonged glucose starvation. Loss of the βIII-tubulin C-terminal tail increased Akt activation and LC3B-II expression in glucose-rich conditions and potentiated the increase in ER stress response markers GRP78, ATF4 and CHOP in response to glucose starvation. These results indicate that the βIII-tubulin body and tail differentially confer a survival advantage to glucose-starved H460 cells and modulate multiple stress response signaling pathways in an isotype-dependent and independent manner. CONCLUSION: This study provides insight into the importance of βIII-tubulin structural domains in conferring βIII-tubulin structural importance to NSCLC cells thereby advancing our understanding of the role of tubulin isotypes in cell biology and the mechanisms contributing to poor patient outcome in this disease. Mavallaris, Nat Rev Cancer 2010 Park et al., Carcinogenesis 2016


Protein carbonylation is an irreversible modification to the side chain of amino acid residues induced by severe oxidative stress. Reactive oxygen species (ROS) are constantly produced under normal as well as stress-induced conditions, and play a role in both cancer progression and cancer suppression. Tumor tissue is known to have higher ROS levels compared to surrounding healthy tissue but ROS-induced specific protein carbonylation and its unique role in cancer progression/suppression are poorly understood. In this study we compared the relative total and specific protein carbonylation in flash frozen human breast cancer and matched adjacent normal tissue using ELISA, two-color western blot, mass spectrometry, and immunoprecipitation approaches. To understand antioxidant capacity in tumor tissue, we analyzed superoxide dismutase (SOD) for its antioxidant activity and protein level. Our results indicate that tumor tissue has greater total protein carbonylation, lower SOD activity, lower SOD protein levels, and elevated levels of autophagy compared to matched adjacent healthy tissue. We also identified three specific proteins that showed higher level of carbonylation selectively in tumor tissue compared to adjacent normal tissue. Our findings were further confirmed using the immunomortal MDA-MB-231 breast cancer cell line and MCF-12A noncancerous normal human epithelial breast cell. Identification of selectively carbonylated proteins in cancer tissue, and understanding their specific role in cancer progression may promote the development of targeted therapeutic approaches to mitigate or enhance oxidative damage of such proteins selectively in tumor tissue.
region that show dose-dependent disruption of this interaction. We also demonstrated that DOT1L mutants lacking these 10 residues did not support transformation by MLL-AF9. Encouraged by these results, we generated both genetic and chemical tools to elucidate the role of DOT1L recruitment to the MLL fusion partners and the mechanism of leukemogenic inhibition by disrupting the protein-protein interaction (PPI) between MLL-AF9 and DOT1L. FIU1 DOT1L MLL-AF9 Cre+ cell lines were generated with different constructs of DOT1L. These constructs consisted of DOT1L mutants lacking the 10 amino acid binding site, a 1867A point mutant known to block DOT1L binding, and an enzymatic mutant known to yield a catalytically inactive protein. As control cell lines, MLL-AF6, a MLL-fusion containing a cytoplasmic protein, and E2A-HLF, a non-MLL dependent fusion, were generated to demonstrate the specific effects of generated DOT1L mutant constructs. Both DOT1L PPI mutants impaired the transformation by MLL-AF9 and induced cell death by inducing apoptosis and cell cycle arrest similarly to enzymatic inhibition. These results established a foundation for discovering small-molecule inhibitors that disrupt the AF9-DOT1L as potential disease-specific therapies that target chromatin modifications in this highly aggressive leukemia. A high throughput screening was conducted identifying several different chemical classes of small molecules that bind to the AF9 C-terminal hydrophobic binding site and disrupt the PPI between DOT1L and MLL-AF9 fusion protein. Identified small molecule inhibitors were validated with series of biochemical, functional and cell-based assays. Validated compounds selectively inhibit the growth of the DOT1L dependent mutant MLL-AF9 cell line in a similar manner to the genetic approach. The small molecules also showed specificity in killing human MLL-fusion cell lines in comparison to non-MLL fusion leukemia. These results show that blocking the recruitment of DOT1L by AF9 using both genetic and chemical tools eliminate MLL-AF9 mediated immortalization emphasizing an essential function for this interaction in leukemogenesis and warrant further development of the identified small-molecule inhibitors.

#5490 Non-overlapping promoter and super-enhancer-associated dependencies in multiple myeloma. Mariateresa Fulciniti,1 Charles Y. Lin,2 Mehmet K. Samur,1 Richard Young,2 Herve Avet-Loiseau,4 Kenneth Anderson,1 James Bradner,3 Nikhil Munshi,1 Dana-Farber Cancer Institute, Boston, MA; 2Baylor College of Medicine, Houston, TX; 3Whitehead Institute for Biomedical Research, Cambridge, MA; 4Centre National de Recherche sur le Cancer, Villejuif, France; 5Novartis, Cambridge, MA

The relationship between promoter proximal transcription factor-associated gene expression and super-enhancer-driven transcriptional programs are not well-defined. We explored the transcriptional and functional interrelationship between E2F transcription factors and BET transcriptional co-activators to identify their individual contribution to eventual functional effect in multiple myeloma (MM). To better understand how E2F1 and DP1 drive proliferation, we mapped the global occupancy of E2F1/DP1 in MM. Integration of E2F1 and DP1 genomic localization to MM reference epigenome revealed specific co-occupancy of the factors at promoters of active genes marked by H3K4me3, with a strong positive correlation between E2F and RNA Polymerase II (RNA Pol II) binding at transcription start sites. In contrast, active enhancers, as defined by DNase I signal distortion (MED1) peaks and marked by H3K27ac and BRD4, showed virtually no E2F binding. Unbiased hierarchical clustering revealed distinct regulatory axes for E2F and BETs, with E2F predominantly localized to active gene promoters of growth/proliferation genes and BETs disproportionately at enhancer-regulated tissue specific genes confirming that these factors establish distinct target gene programs. At the extremes, we found less than 10% of genes were among the top 500 in BRD4 enhancer signal (i.e. SE-regulated) and top 500 E2F promoter signal. We hypothesized that the presence of BETs and E2F in distinct regulatory axes divides active genes in MM into those that can be selectively influenced by BET inhibition or E2F perturbation, but not both. In line with this we have observed that dual E2F and BET inhibition is synergistic for MM cell growth, both in vitro and in vivo. In conclusions, our results highlight the existence of distinct regulatory axes controlled by promoter and enhancer driven processes, suggesting a sequestered cellular functional control that may be perturbed in cancer with potential for development of a promising therapeutic strategy.


The majority of folate uptake into tissues and tumors involves facilitated carriers, the reduced folate carrier (RFC) and proton-coupled folate transporter (PCFT). PCFT is highly expressed in a wide range of human tumor cell lines and

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primary tumor specimens from a variety of lineages, with modest expression in most normal tissues. PCFT transports optimally at pHs characterizing the tumor microenvironment. We discovered novel 6-substituted pyrrolo[2,3-d]pyrimidine folate analogs with substantial selectivity for PCFT over RFC, resulting in potent antitumor efficacy in vitro and in vivo. However, in some cases PCFT levels varied substantially. Studies were performed to characterize the basis for these dramatic differences in PCFT gene expression between HepG2 (elevated) and HT1080 (negligible) tumor cells. Low levels of PCFT gene expression in HT1080 cells were not likely due to methylation of CpG islands in the PCFT promoter since 5-aza-2’-deoxycytidine treatment did not restore PCFT gene expression. A PCFT promoter reporter construct -2005/+96 in pGL3 (transcription starts at +1) was generated and showed high levels of activity in HeLa cells by luciferase reporter assays. For HepG2 and HT1080 cells, wide variations in PCFT transcript levels by real-time RT-PCR were paralleled by reporter gene activities with the -2005/+96 construct, suggesting that these were primarily due to PCFT transcriptional regulation. Progressive deletion analysis of the -2005/+96 construct and reporter assays in HepG2 and HT1080 cells localized the minimal promoter region to between -50 and +96. Additional deletion constructs (-35/+96, -20/+96, -15/+96 and +10/+96) were generated and studied. Whereas the -35/+96, -20/+96, -15/+96 and +10/+96 promoters constructed retained ~50% activity for HepG2 cells and ~10-15% activity for HT1080 cells relative to the -50/+96 construct, in both cases the -10/+96 construct was essentially inactive. Two critical cis elements were identified in the PCFT minimal promoter by deletion analysis: a region between positions -50 and -35, and a region between positions -35 and -15. Informatika formatics analysis localized putative cis elements for KLF-15 (-50 to -35) and NRF1 (-15 to -10) binding. The functional importance of these elements was confirmed by mutations of core consensus sequences in the -50/+96 construct and reporter gene assays. Binding of NRF1 and KLF15 to the endogenous PCFT promoter in HepG2 and HT1080 cells was confirmed by ChiP assays which paralleled patterns of differential PCFT gene expression. Collectively, our results suggest that NRF1 and KLF15 contribute to dramatic variations in PCFT gene expression between tumor cells. Better understanding the key determinants of PCFT transcriptional control may lead to strategies for modulating PCFT levels in combination with PCFT-targeted 6-pyrrolo[2,3-d]pyrimidine folate analogs for treating solid tumors.

#5492 Comprehensive analysis of the dynamics of aberrant WNT signaling on global gene expression and nuclear structure. Markus A. Brown, National Institutes of Health, Bethesda, MD.

The organization of the genome in the nucleus is complex and dynamic. Features of the nuclear architecture, including the spatial arrangement of genomic sequences, the structure of chromatin, and the accessibility of regulatory DNA elements modulate nuclear processes such as gene transcription DNA replication, RNA repair, RNA processing, and mRNA transport. However, the interplay between nuclear architecture and gene expression is poorly understood. We propose to elucidate the impact of silencing the prominent transcription factor, TCF7L2, the major effector of the WNT signaling pathway, on the transcriptional equilibrium and nuclear architecture of the nucleus in human colorectal cancer cells. Our multidisciplinary approach combines targeted si- lencing of TCF7L2, 3D-FISH, RNA-seq, and Hi-C chromosome conformation capture, in parallel with mathematical modeling to delineate structure/function relationships in the interphase nucleus. This approach will elucidate the intricacies of a dynamic cellular signaling pathway in three-dimensional space.


Colorectal cancer (CRC) is the second leading cause of death in the United States and a truly preventive approach still remains to be developed. Men have lost during CRC progression. In colon cancer cell lines re-expression of ERbeta has anti proliferative and anti-inflammatory properties, there is however little understanding of the mechanism whereby ERbeta mediates these effects in colon cells. In order to elucidate the mechanism of ERbeta in CRC, we re-expressed this receptor using lentivirus in four different CRC cell lines. We optimize chromatin-immunoprecipitation (ChIP) with an antibody thoroughly validated for this purpose and used IgG and input as well as cell lines without ERbeta expression as controls. ChIP-seq was performed using Illumina library preparation and next generation sequencing. Alignment of sequence reads against the reference genome of version hg19 was done using Bowtie, and statistically significant peaks (FDR) were identified using MACS. Results were compared to RNA-seq analysis of the same cell lines. Key binding-sites and corresponding gene regulations were confirmed using qPCR. We present for the first time, the cistrome of ERbeta in different CRC cell lines. We identify key binding motifs, including CRE and AP-1 sites. Together, our results uncover ERbeta as an important determinant for attenuating cancer progression.

#5494 Hypoxia via HIF1alpha can regulate Wnt signaling in human colon cancer cells. Yung Lyu,1 Amber Habowski,2 Stephanie Sprow–Tanao,2 Xira Pate,2 George Chen,2 Marian L. Waterman,3 1UCI Medical Center, Orange, CA; 2University of California Irvine, Irvine, CA.

Standard-of-care treatment for metastatic colorectal cancer combines chemotherapy with bevacizumab, an angiogenesis inhibitor that depletes nutrients and triggers hypoxia. Unfortunately, this strategy extends patient survival only a few months because tumors acquire adaptive resistance, enabling resumption of angiogenesis. Adaptive resistance arises from reprogrammed metabolism, but the regulatory networks that govern reprogramming are not defined. Our studies have shown that overactive Wnt signaling, which is the most common cause of colon cancer, programs cancer metabolism by promoting glycosylation and angiogenesis. Interestingly, metabolic genes targeted by Wnt are also regulated by the hypoxia transcription factor HIF1alpha suggesting there may be crosstalk between the two pathways. Several studies suggest that HIF1alpha can influence Wnt signaling, but the reported effects are variable ranging from positive to negative. We find that HIF1alpha and LEF/TCF/beta-catenin complexes co-regulate metabolic targets including LEF1 and TCF7 (TCFI7) expression. Using luciferase reporters driven by LEF/TCF promoters, we observe significantly increased LEF1/TCF7 activity but no TCF7 activity. Expression of HIF1alpha in hypoxic colon cancer cells. We identified putative hypoxia responsive elements (HREs) in the human LEF1 promoter and deletion of these HREs reduced responsiveness to hypoxia. Furthermore, when colon cancer cells were treated with inhibitors that either inactivate or reduce HIF1alpha protein, there was a significant decrease in Wnt reporter activity. These results suggest that hypoxia and the Wnt pathway crosstalk wherein hypoxia co-regulates metabolism genes and increases Wnt signaling capacity via LEF/TCF expression. Current studies are underway to investigate the mechanisms of crosstalk and joint regulation of metabolic gene programs during adaptive resistance.

#5495 GANT61-induced cell death involves inhibition of transcription and DNA licensing at GLI sites in the promoters of GLI-dependent target genes in human colon carcinoma cells. Ruowen Zhang,1 Jiahui Wu,2 Sylvain Ferrandon,2 Katie J. Glowacki,3 Janet A. Houghton.4 Southern Research Institute, Birmingham, AL; 2Cleveland Clinic, Cleveland, OH.

The GLI genes, GLI1 and GLI2, are transcriptional regulators of the Hedgehog signaling response, binding at promoters to GACCCCAAC-like consensus sequences. From genetic and biochemical studies, GLI2 is the primary mediator of HH signaling, which activates transcription of GLI1 and GLI2-GLI3 GLI1-GLI2). In cancers both GLI1 and GLI2 are oncogenes, aberrantly and constitutively activated by oncogene-driven signaling pathways, in particular KRAS/BRAF in colon cancer. GANT61, a specific GLI inhibitor, induced extensive cytotoxicity in human models of colon cancer, indicating GLI to be a critical target in cancer cell survival. We have determined FOXM1 to be a transcriptional target of GLI at GACCCCAAC consensus sites through binding to the FOXM1 promoter by GANT61 led to inhibition of binding of the release-relaxing factors DSIF, NELF, and p-TFEB in the region of the TSS, with inhibition of binding of Pol II at both GLI and TSS sites. In R-loop regions, RNA:DNA hybrid formation was reduced at both the GLI and TSS sites in the FOXM1 promoter by GANT61, sensitive to RNaseH. Bisulfite conversion/PCR analysis showed the T to C conversion at the GACCCCAAC sites in the GLI binding region in GANT61-treated cells. Data support GANT61-induced inhibition of GLI-dependent transcription at the PIC before R-loops are formed. Pretreatment of HT29 cells with -aminan (Pol II inhibitor) reduced GANT61-induced $\gamma$H2AX foci, indicating the importance of transcription in GANT61-induced DNA damage. Co-localization of GLI1 and BrcU foci, inhibited by GANT61, indicated GLI1 and DNA replication were linked. By co-immunoprecipitation, GLI1 bound the DNA replication licensing factors ORC4, CDT1, and MCM2, decreased in the presence of GANT61. By confocal microscopy, significant co-localization of GLI1 and ORC4 foci was inhibited by GANT61, and enrichment of ORC4 occurred at the GLI binding site in the FOXM1 promoter. In addition to non-transcriptional mechanisms of interactions, GANT61 (CDT1) CDT1 foci in HT29 cells reduced GANT61-induced cell death and cleavage of caspase-3, indicating a
significant role of DNA replication licensing in the mechanism of induction of GANT61-induced cell death. In summary, we have demonstrated inhibition of GLI-dependent transcription by GANT61 at the PIC during the initiation of RNA synthesis, and the importance of inhibiting DNA replication licensing at sites in promoter regions that bind the transcription factor GLII by both non-transcriptional and transcriptional mechanisms. Supported by NCI Award 1 RO1 CA183921-01A1 and by Southern Research Institute.

#5496 Intratumor estradiol increment mediated by CtBP1/CYP19A1 decreases the proliferation of androgen insensitive prostate tumor cells. Cintia Massillo,1 Guillermo N. Dalton,1 Juliana Porretti,1 Georgina Scalsie,1 Paula L. Farré,1 Colin Clyne,2 Paola De Luca,1 Adriana Di Servi,1 1Instituto de Biologia y Medicina Experimental (IBYME-CONICET), Autonomous City of Buenos Aires, Argentina; 2Hudson Institute of Medical Research, Melbourne, Australia.

The normal growth and development of the prostate requires the action of estrogens and estrogens receptors (ER) a and β. Estrogen-related pathways are clearly important in the development and progression of hormone-dependent cancers such as prostate cancer (PCa), but the role of ERβ remains controversial. The production of estrogens from androgens is mediated by the aromatase enzyme. Aberrant expression of aromatase plays a critical role in PCa development and progression. Metabolic syndrome (MS) causes sex hormone imbalance and has been identified as a risk factor for PCa. Recently, we found that C-terminal binding protein (CtBP1), a transcriptional co-repressor of tumor suppressor genes, is a novel molecular link between MS and PCa. We developed a MS mice model that were inoculated with PC3 stable CtBP1 depleted or control cells. MS mice showed MS phenotype including increase of body weight and estradiol. Interestingly, CtBP1 strongly repressed aromatase expression in these xenografts. The aim of this study was to understand the transcriptional regulation mechanism of aromatase mediated by CtBP1 in a MS/PCa model. To fulfill our aim, PC3 cells were co-transfected with a CtBP1 expression plasmid and a panel of ten reporter vectors containing different lengths (27-1,004 bp) of CYP19A1 promoter, cloning upstream to the luciferase gene. CtBP1 significantly repressed the activity of all the studied promoters. By chromatin immunoprecipitation (ChIP) and RT-qPCR we determined that CtBP1 associated to CYP19A1 promoter and repressed its transcription. In order to identify possible CtBP1 partners in CYP19A1 expression regulation we investigated several transcription cofactors. By ChIP, we found that p300 (histone acetyl transferase) and ERβ associated to aromatase promoter in PC3 cells. Using gene reporter assays we established that CtBP1 and p300 synergistically repressed, while ERβ activated, aromatase promoter activity. Interestingly, estradiol exposure of PC3 cells, released CtBP1 from the aromatase promoter triggering its expression. Furthermore, we found that estradiol dramatically increased the viability and the S phase percentage of the androgen sensitive LNCaP and, its derivative, C4-2 cells; dramatically reducing apoptosis. Accordingly, estradiol decreased androgen insensitive PC3 cell viability and G1 phase arrest. In summary, CtBP1 represses aromatase expression in PCa. Nevertheless, MS increases intratumor estradiol, which releases CtBP1 from aromatase promoter activating aromatase expression, which in turn, modulates prostate tumor cell proliferation.

#5497 LSD1 modulates androgen receptor cistrome in prostate cancer via regulation of FOXA1 chromatin binding. Shuai Gao,1 Sujun Chen,2 Dong Han,1 Wanting Han,1 Steven P. Balk,3 Housheng Hansen He,1 Changmeng Cai1. 1University of Massachusetts-Boston, Boston, MA; 2Ontario Cancer Institute, Toronto, Ontario, Canada; 3Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA.

Patients with castration-resistant prostate cancer (PCa) benefit from CYP17A1 inhibitors and more potent AR antagonists, but still relapse with tumors expressing high levels of AR and AR regulated genes, indicating restoration of AR activity. Therefore, there remains a pressing need for further development of novel AR targeted therapies. One major mechanism that contributes to the PCa development is reprogramming of AR cistrome by transcriptional factors and chromatin modifiers. In this process, AR is recruited to a subset of newly established enhancers that can drive the expression of pro-liferation genes. FOXA1 is one such transcriptional factor that determines cell-lineage and is characterized as a “pioneer” factor to facilitate the access of additional transcription factors (such as AR or ER) to the regions with compact chromatin. A recent study by comparing the AR cistrome in normal prostate and PCa tissues suggests that FOXA1 is responsible for driving AR reprogramming. Whole genome analyses in PCa cells have revealed that FOXA1 can recognize enhancer regions with active histone marks (H3K4me1,2) and then further open up the sites to allow subsequent AR binding. However, the molecular mechanism on how FOXA1 is guided to the target region in PCa is still elusive and studying this mechanism exhibits significant translational potential on modulating AR activity in PCa. Lysine Specific Demethylase 1 (LSD1/KDM1A) is an important epigenetic modifier that can function as a repressor by demethylating H3K4me1,2. However, LSD1 also often associates with enhancers and can function as an activator in some contexts. Our previous studies showed that LSD1 binding significantly overlaps with FOXA1 and the overlapping sites that are marked by high levels of H3K4me2 enrichment for AR activated genes. LSD1 also interacts with FOXA1 and this interaction enhances binding of both proteins at AR-mediated enhancers. These findings suggest that LSD1 may regulate the availability of enhancers to AR via interaction with FOXA1 and hence may reprogram AR cistrome in PCa cells. In this study, we performed ChiP-seq analyses of FOXA1 and AR in PCa cells treated with LSD1 inhibitors. The results show that global FOXA1 binding was drastically diminished by inhibiting LSD1, indicating that its demethylase activity is required for FOXA1 binding. Importantly, inhibiting LSD1 also results in distinct AR binding patterns with the expression of many classic AR-activated genes being impaired and new AR-activated genes emerged. We are currently carrying out xenograft studies to examine the impact on tumor progression and AR cistrome by LSD1 inhibitor. Overall, our results suggest that the LSD1-FOXA1 interaction functions as an important modulator of AR cistrome and targeting LSD1 in conjunction with AR antagonists may be a promising therapeutic approach to treat PCa.

#5498 Autoregulation of protein kinase D1 (PRKD1) expression in prostate cancer. Bita Nikkhohlgh, Sittadjody Sivanandane. Wake Forest Univ., Winston Salem, NC.

Protein kinase D1 (PRKD1) is down regulated in gastric, breast and prostate cancer. Several regulatory mechanisms modulate PRKD1 activity in cancers. In colon cancer, it has been shown that PRKD1 is down regulated by nuclear beta-catenin. We explored whether beta-catenin is involved in regulation of PRKD1 expression in prostate cancer as well. A CHIP assay performed using prostate cancer LNCaP cell lysate and pull down with beta-catenin antibody, which demonstrated recruitment of beta-catenin to promoter of PRKD1 gene as well as at downstream regions in the gene suggesting that beta-catenin could be involved in PRKD1 regulation. To identify the exact binding site of beta-catenin to PRKD1 gene, we carried out CHIP sequencing. The beta-catenin protein complex was bound to a 166 bp sequence near exon 2 (chr1:29899631-29899796). Because beta-catenin is a coactivator and not a known transcription factor, we performed a transcription factor promoter array, which showed that the MYC/MAX transcription factor complex may be the mediator of the regulatory effect of beta-catenin on PRKD1 expression. We validated the results by performing CHIP assay using MYC and MAX antibodies which showed recruitment of both MYC and MAX to the same beta-catenin bindingsite to PRKD1. In order to assess the functional impact of beta-catenin regulation of PRKD1 in prostate cancer, beta-catenin mutated at threonine 120 residue (we have previously demonstrated that PRKD1 is the only known kinase to phosphorylate the site) demonstrated increased nuclear translocation of beta-catenin and increase down regulation of PRKD1 and increased androgen receptor (AR) activity (increased AR activity is well established to be associated with PC progression). Our data has identified a novel auto-regulatory mechanism of PRKD1 expression through beta-catenin phosphorylation.

#5499 The reprogramming of the steroid receptor binding landscape at active enhancers is associated with a fast DNA residence time through a mechanism termed dynamic assisted loading in breast cancer cells. Erin Swinstead, Tina Miranda, Ville Pakkinaho, Songjun Beak, Gordon Hager. NCI/NIH, Bethesda, MD.

The transcription factor (TF), FOXA1 has been described as a pioneer factor, implicated in steroid receptor (SR) binding patterns. Classically it has been proposed that the pioneer factor model is a slow and static binding event. However, it has recently been described that the estrogen and glucocorticoid receptors (ER and GR), can regulate the binding patterns of each other in a mouse mammary cell line defined as dynamic assisted loading. Further, this phenomenon has been extending demonstrating that both ER and GR can recruit FOXA1 to specific binding sites. Several targets associated with an increase in chromatin accessibility and fast DNA residence times. This was also associated with the absence of a DNasel footprint for the initiating and secondary factor supporting the assisted loading model. To further investigate the molecular mechanism behind the dynamic assisted loading model we have characterized the binding and transcriptional landscape of ER and GR in a number of breast cancer cell line models. Genome wide analysis of ChiP and DNasel hypersensitivity assays upon single and dual activation show that both SRs can recruit each other to a specific subset of binding sites associated with an increase in chromatin accessibility. Therefore, depending on the chromatin landscape, one factor functions as the initiating factor and the other the secondary factor. Further, RNA polymerase II (RNAP
Il), p300, and H3K27ac binding patterns are associated with the newly programs ER or GR binding sites. Together this suggests chromatin reorganization upon dual activation of either ER or GR (the initiating factors) and recruitment of the secondary factor (ER or GR) through an assisted loading mechanism. Further, we have demonstrated an association of the transcriptional machinery at these newly acquired binding sites at active enhancers. These results propose that many TFs in a given cell have the potential to affect the binding landscape of other TFs, depending on the chromatin context. In addition, this study has shifted our classical understanding of pioneer factors in breast cancer, demonstrating that not only can TFs alter the response of FoxA1 but also each other through highly dynamic and fast DNA interactions associated with active enhancers and transcription.

**#5500** KMT2C directs estrogen receptor activity in normal and transformed mammary cells. Kinisha Gala,1 Amit Sinha,2 Francisco Sanchez-Vega,1 Young Rock Chung,3 James Hseih,1 Michael Berger,1 Nikolaus Schultz,1 Alessandro Pastore,2 Omar Abdel-Wahab,1 Sarat Chandralapati1. *Masonic Memorial Sloan Kettering, New York, NY; 3Bayer, Inc., New York, NY.*

Estrogen receptor alpha (ERα) is a ligand-activated nuclear receptor that regulates proliferation and differentiation in mammary epithelial cells. ERα activity is likely dependent on the actions of pioneer factors and H3K4 methyltransferases which can establish a genomic landscape permissive for ERα binding. Here, we identify the H3K4 methyltransferase KMT2C as essential for ERα activity in mammary gland development and ER+ breast cancer growth. KMT2C overexpression increases estrogen-dependent gene expression and causes H3K4me1 loss at ERα target gene enhancers. Consequently, KMT2C loss selectively suppresses estrogen-driven breast cancer proliferation. Moreover, mammary-specific Kmt2c knockout mice have defects in pubertal ductal formation similar to Er1−/− deficient mice. Although KMT2C loss disrupts estrogen-driven proliferation, it conversely promotes tumor outgrowth under hormone-depleted conditions. Consistent with this, gene expression signatures of Kmt2c loss are associated with poor outcomes. We conclude that KMT2C is a key regulator of ERα activity whose loss uncouples mammary phenotypes from hormone availability.

**#5502** Functional evaluation of superenhancers as mediators of epithelial ovarian cancer risk. Kevin C. Vavra,1 Simon Coetzee,1 Janet M. Lee,1 Paul Pharaoh,2 Dennis J. Hazelet,1 Kate Lawrenson,1 Simon A. Gayther1,4 Cedars Sinai Medical Center, Los Angeles, CA; 2University of Cambridge, Cambridge, United Kingdom.

Superenhancers are master regulators of genes involved in cellular differentiation and tumorigenesis. While it is now well established that typical enhancers are key mediators of alleles associated with a mild risk of polygenic diseases including cancer, the role of superenhancers is less understood. We evaluated the role of superenhancers in mediating risk of epithelial ovarian cancer (EOC). Using FunciVar software and superenhancers defined using H3K27ac chromatin immunoprecipitation followed by next generation sequencing, performed in cell lines and tissues, we characterized the superenhancer-risk SNP intersect and identified a series of risk-associated loci where multiple candidate causal alleles overlap with ovarian superenhancers. In particular, locus 3q25, the locus most associated with epithelial ovarian cancer (EOC) risk from GWAS studies, has >70 candidate causal risk SNPs, all of which coincide with superenhancers detected in ovarian cancer precursor cells (ovarian and fallopian tube epithelia) but not in ovarian cancers. We are proposing that the presence of one or more SNPs occurring at this locus leads to a higher expression of one or more genes by modulating activation of the superenhancer region. We tested the effect of decreasing the binding affinity of BRD4, a protein that binds superenhancer regions, using a small molecule inhibitor on the expression of genes in this locus. We identified three candidate genes—LEKR1, SSR3, and TiPARP—that demonstrated a decrease expression level as a result of BRD4 binding inhibition. Preliminary results show that partial knockdown of either SSR3 or TiPARP decreases normal ovarian and fallopian cell proliferation, and knockdown of both SSR3 and TiPARP simultaneously shows an enhanced proliferation decrease compared with knockdown of a single gene. We then created stable knockout (KO) models using the CRISPR-Cas9 system to fully KO one, or two or all three of our candidate causal genes: LEKR1, SSR3, and TiPARP. Functional evaluation of these models is currently underway to test the hypothesis that risk SNPs may regulate more than one gene at this locus. In all we identify a potential role for superenhancers at a subset of EOC risk loci, and present a functional pipeline for identification of the target gene/transgenes and evaluation of their role in neoplastic transformation.

**#5503** Interplay between the transcription factors PRRX1 and FOXM1 in pancreatic cancer. Benoît Marchand,1 Maximilian Reichert,2 Meredith A. Collin1, Anil K. Rustgi1. 1University of Pennsylvania, Philadelphia, PA; 2Technical University of Munich, Munich, Germany.

Introduction: We have identified previously the Paired Related Homeobox 1 gene (PRRX1) as a key regulator of embryonic ductal development, acinar-to-ductal metaplasia (ADM) and pre-cancerous lesion (PanIN)/pancreatic ductal adenocarcinoma (PDAC) progression (Reichert M. et al Genes & Dev 2013). We discovered an isoform switch between PRRX1A and PRRX1B occurring in EMT-MET plasticity in pancreatic tumorigenesis (EMT) and metastatic colonization of the liver (MET) (Takano S. et al Genes & Dev 2016). The transcription factor FOXM1 is also implicated in ductal EMT. Located in EMT of pancreatic cancer cells, interestingly, both transcription factors, FOXM1 and PRRX1, are overexpressed in PDAC. The aim of this study was to investigate if PRRX1, specifically its isoforms, might interact with FOXM1, to regulate gene transcription in pancreatic cancer. Methods: Experiments were performed in human pancreatic cancer cells (PANC1, Mia PaCa2 and BxPC3), and HKE295T cells. Epoetipe-tagged PRRX1 and FOXM1 proteins (wild type or deletion mutants) were either transiently or stably expressed in these cells. Immunoprecipitation and western blot were performed to evaluate potential interaction between PRRX1 and FOXM1. As a functional readout, the Tnc and 6xFoxM1 luciferase reporters were used to measure Tenascin C and FOXM1 transcriptional activities. Results: Immunoprecipitation of tagged-PRRX1 or endogenous FOXM1 showed co-binding of both factors in AIP (A) with FOXM1 in pancreatic cancer cells. We further characterized this interaction using deletion mutants of PRRX1 C-terminal region, FOXM1 binds to the 200-222 amino acid region of PRRX1. Similarly, using deletion mutants of the FOXM1 N-terminal region we mapped PRRX1 binding to the winged helix DNA binding domain of FOXM1. Interestingly, we also observed co-binding of HA-tagged PRRX1A with either Flag-tagged PRRX1A or PRRX1B suggesting homo and heterodimerization of the PRRX1 isoforms. Next, we found that co-expression of PRRX1 and FOXM1 cooperatively induced transcriptional activity of a known PRRX1 target gene, Tenascin C. Furthermore, co-expression of PRRX1A and FOXM1 cooperatively induced FOXM1 transcriptional activity. FOXM1 has been reported to regulate Wnt signaling in early pancreatic carcinogenesis, we observed co-binding of PRRX1 and FOXM1 with beta-catenin. Conclusion: Our results provide new insights in the interaction of PRRX1 and FOXM1 with functional activation of FOXM1 mediated transcriptional activity, and potential regulation of the Wnt pathway.

**#5504** Regulation of the receptor for advanced glycation end products by estrogen receptor ligands in endometrial cancer. Julia Delgado,1 Dejanca Gonzalez,1 R.Steven Conlan. Swansea University, Swansea, United Kingdom.

Endometrial cancer has numerous established risk factors including excessive unopposed estrogen exposure, tamoxifen, obesity and polycystic ovary syndrome (PCOS). These risk factors may promote inflammation, an important hallmark of cancer. Since the endometrium is a site of regular and repeated inflammation during the menstrual cycle, the regulation of inflammation may be involved in endometrial cancer. The receptor for advanced glycation end products (RAGE) stimulates numerous inflammatory signalling pathways and its expression is elevated in a number of inflammatory pathologies including diabetes and more recently cancer. This work aims to investigate the regulation of RAGE by estrogen receptor ligands in endometrial cancer. RAGE and estrogen receptor isoform protein expression were examined in a patient cohort by immunohistochemistry. RAGE and ER isoform expression was assessed following estrogen receptor ligand exposure in both type I and type II endometrial cancer cell line models. ERα, ERβ, p65 and ER co-regulator recruitment to the RAGE promoter following ER ligand treatment was determined in vitro by chromatin immunoprecipitation. Data revealed (i) RAGE and ER isoform endonuclear expression is altered in endometrial cancer patients and patients receiving tamoxifen compared to postmenopausal women, (ii) RAGE is expressed in type I and II endometrial cancer cell lines and its expression can be modulated by estrogen and tamoxifen exposure, and (iii) by chromatin immunoprecipitation, estrogen receptor ligands were shown to alter ERα, ERβ and ER co-regulator binding at the RAGE promoter. To conclude, results from this work suggest RAGE is associated with endometrial cancer development. Once clinically validated, RAGE and associated targets investigated in this study could be potentially used as a panel of endometrial cancer biomarkers and may also be used to indicate if patients are at risk of the adverse agonist action of tamoxifen in the endometrium.
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**#5505** TERT promoter mutations in primary papillary thyroid carcinoma and matched local / distant metastases. Noa Fea Rodríguez,1 Miriam Corraliza Gómez,1 Tomás Álvarez Gago,2 Juan José Mateos Otero,2 Raquel Muñoz Martínez,1 Ginesa Maria Garcia-Rostan1, Katleen De Preter1, Fanny De Vloed,1 Geertrui Denecker,1 Suzanne Vanhauwaert,1 Jolien De Tert1.

TERT promoter (TERTp) mutations represent a common oncogenic event in sporadic thyroid follicular cell carcinomaogenesis. Though TERTp mutations have been statistically associated with aggressiveness and metastatic spreading, their involvement in lymph node metastases (LNNMs) and/or distant metastases (DMs) development among papillary thyroid carcinoma (PTC) patients remains to be elucidated. We evaluated the association of TERTp mutations with tumor expansion, primary tumors (Pt) and matched LNNMs and/or DMs were genotyped by means of PCR-direct sequencing in a cohort of 33 patients diagnosed of PTC, which had been previously analyzed for BRAF and RAS mutations. Focal changes in the growth pattern or microscopic grade within the Pt or the metastases were separately genotyped to determine the clonal/subclonal nature of TERTp mutations, their association with particular histological variants of PTC or their presence in intra-tumoral PTC-like foci. Results were correlated with clinico-pathological parameters of poor outcome and survival. The analysis of 99 tumor samples obtained from 33 PTC cases revealed that TERTp mutations were quite common (42.4%). The mutation C228T was much more common than the C250T (78.6% vs 21.4%). TERTp mutations did not correlate with specific clinico-pathological parameters (CL-PTC vs FV-PTC, CF-PTC) at diagnosis. In half of the cases, the mutations segregated to LNNM. TERTp mutations were present in all samples of DM. While 71% of the cases mutated at TERTp bore the BRAFV600E mutation, the coexistence of TERTp and RAS mutations was exceptional. TERTp mutations were found to be significantly correlated with age > 45 years old, high grade poorly differentiated PTC foci or nesting-PDC-like foci, stage at diagnosis or at last follow-up and patient status. A trend of correlation with male sex, vascular invasion, tumor recurrence and development of LNNM during the follow-up was also seen. Tumor multifocality was inversely correlated with TERTp mutations. The coexistence of TERTp and BRAFV600E mutations did not increase the prognostic power of TERTp mutations alone. All of the patients who died of disease displayed TERTp mutations. Kaplan-Meier analysis revealed that patients with Pt carrying TERTp mutations had a poor prognosis showing a higher tumor recurrence probability [p = 0.0085] and a reduced disease specific survival [p = 0.0001]. The coexistence of other mutations did not significantly increase the risk of recurrence or dying of disease. The study indicates that TERTp mutations: 1) are common in metastatic PTC; 2) are subclonal in half of the cases; 3) spread in most cases with metastatic cells to LNNM and DM but do not drive the development of LNNM; 4) identify PTCs patients with increased risk of recurrence and mortality; 5) represent “per se” a biomarker for poor outcome among PTCs.

**#5506** SOX11 acts as part of the MYCN-WEEl regulatory protein complex implicated in neuroblastoma. Bieke Decaestecker,1 Sara De Brouwer,1 Fanny De Vloed,1 Geertru Denecker,1 Suzanne Vanhauwaert,1 Jolien De Wijn,2 Geneviève Laureys,1 Bjorn Menten,1 Pauline Depuydt,1 Patrick Reynolds,2 Jo Vandorpe,3 Kris Gevaert,4 Jan Koster,1 Sara Ek,1 Frank Speelman,1 Kathleen De Preter1,1 Centre for Medical Genetics Ghent, Ghent, Belgium; 2Texas Tech University Health Sciences Center, Lubbock, TX; 3University Hospital Ghent, Ghent, Belgium; 4VIB Proteomics Expertise Center (PEC), Ghent, Belgium; 5Academic Medical Center (AMC), Amsterdam, Netherlands; 6Bieke De Vloed, Lund, Sweden.

Neuroblastoma (NB) is an aggressive lethal pediatric cancer of the developing sympatho-adrenergic nervous system and characterised by a low mutation burden while exhibiting recurrent DNA copy number alterations including chromosome 2p gain and MYCN amplification. Rare amplifications have previously allowed to identify additional oncogenes implicated in NB such as ALK and LIN28B. Here we report on three NB tumors and two NB cell lines with high level focal gain containing the SRY-related HMG-box transcription factor 11 (SOX11) as only protein-coding gene in the smallest region of overlap. The high expression levels of SOX11 in NB and the developing sympathetic nervous system, and high correlation of SOX11 mRNA and protein levels with survival outcome, suggested a role for SOX11 as oncogene and prompted us to further investigate its role in MYCN driven NB formation. SOX11 knock down in NB cells allowed to identify additional oncogenes implicated in NB such as ALK and MYCN which was confirmed by reciprocal co-ip, as well as WEE1, a tyrosine kinase critically implicated in the mitotic checkpoint control for which multiple potent and specific small molecule inhibitors are available. Furthermore, upon testing of the AZD-1775 WEE1 inhibitor in 10 NB cell lines, we observed the strongest effects in the cells with the highest SOX11 protein levels indicating that SOX11 expression levels can serve as predictive biomarker for AZD-1775 treatment response. In conclusion we identified high-level focal gain of SOX11 in NB tumors and cell lines and demonstrated the oncogenic role of SOX11 in MYCN driven neuroblastoma with therapeutic opportunities through interaction with WEE1.

**#5507** Investigation of the effects of alterations in the glutamate receptor, GRIK2 on osteosarcoma tumorigenesis. Justin G. Mayers,1 Nalan Gokcoglu,2 Jay S. Wunder,1 Irene L. Andrulis1.1University of Toronto, Toronto, Ontario, Canada; 2Lunenfeld-Tanenbaum Research Institute, Toronto, Ontario, Canada; 3Sinai Health System, Toronto, Ontario, Canada.

Osteosarcoma is a primary, malignant bone tumour mainly affecting young adults with a five-year survival rate of 60-70% depending on presentation of metastasis at diagnosis. Tumours are marked by genetic instability and heterogeneity and for this reason, molecular subtypes have yet to be characterized. Survival rates have remained consistent over the past few decades and novel molecular targets for treatment are needed. Current genomic strategies have identified recurrent copy number alterations. In a study of 44 fresh frozen osteosarcoma tumours which were obtained at the time of biopsy and 25 blood matched samples we identified recurrent copy number losses including a region at 6q16.3 in 14% of tumours. Focal deletions were observed in the 5′ intergenic space and first intron of the GRIK2 gene and do not overlap with any coding sequence. The relationship between alterations in this region and osteosarcoma tumourigenesis has yet to be explored in depth. GRIK2 encodes an ionotropic glutamate receptor that has been implicated as a tumour suppressor in gastric cancer. An interesting feature of the tumours was the overlap of focal deletions with a SETDB1 histone methyltransferase binding site indicating a potential connection with epigenetic regulation of GRIK2. The goal of this study is to examine the effects of non-coding, focal deletions on GRIK2 gene expression and function and regulation in osteosarcoma cells. GRIK2 transcript levels were measured in osteosarcoma tumours and cell lines using qRT-PCR. Functional assays were performed to characterize the role of GRIK2 in osteosarcoma cell proliferation, migration and apoptosis in vitro. Gene editing with CRISPR/Cas9 was performed to examine the link between non-coding deletions and GRIK2 expression. GRIK2 transcript levels varied in tumours; several osteosarcoma tumours with focal deletions had increased transcript levels relative to cell lines which demonstrated low expression levels. Due to the observation of low transcript levels, a gain of function approach was employed to examine GRIK2 function in vitro. Overexpression of GRIK2 decreased proliferation, migration and increased apoptosis in vitro. Osteosarcoma GRIK2 transcript levels were increased in untreated cells. This study suggests that non-coding focal deletions are linked to high expression levels of GRIK2 which may confer a less aggressive phenotype and are involved in epigenetic regulation of the gene.


Recent studies suggest p53 plays an important role in TGF-β-mediated cell signaling and migration. Previously, we showed wild-type (WT) and mutant (mt) forms of p53 differentially regulate reactive oxygen species (ROS) generation by NADPH oxidase-4 (NOX4). We found that WT-p53 suppresses TGF-β-induced NOX4, ROS production, and cell migration, whereas tumor-associated p53-mt proteins enhance NOX4 expression and cell migration by TGF-β/SMAD3-dependent mechanisms. In this study, we utilized The Cancer Genome Atlas (TCGA) to perform statistical analysis on gene expression data from primary tumor samples and found a correlation between tumors with p53 “hot-spot” mutations and increased NOX4 mRNA expression. Furthermore, we ex-
aned the basis of human NOX4 promoter regulation by p53 and SMAD3. By deletion analysis of the NOX4 promoter, we found two critical SMAD3 binding elements (SBE) are also required for p53-dependent promoter activity. Conversely, promoter activity was abolished by dose-dependent heterologous expression of p53-WT. Moreover, expression of active SMAD3 resulted in robust NOX4 promoter activity, which was abolished when co-expressed with p53-WT. Chromatin immunoprecipitation (ChIP) analysis revealed SMAD3 and p53-WT or p53-mt were associated with specific SBEs and p53 response elements (p53-RE) in a TGF-β-dependent manner. Interestingly, the repressive effect by p53-WT on NOX4 was relieved upon treatment with histone deacetylase (HDAC) inhibitors or mutation of the transactivation domain. Overexpression of p300, a known p53-mt-binding transcriptional co-activator and histone acetyltransferase (HAT), enhanced p53-mt-mediated NOX4 promoter activity, whereas the HDAC-inactive p300-mt reduced promoter activity. Furthermore, overexpression of p53-mt augmented TGF-β-mediated acetylation of histones associated with the NOX4 promoter. Finally, scratch wound assays demonstrated NOX4 and p300 promote TGF-β/mutant p53-mediated cell migration. Collectively, these data provide new insight into TGF-β/SMAD3, p53-mt-NOX4 induction involving epigenetic control of NOX4 in tumor cell migration.

#5510 Pro inflammatory cytokines induce synergistic gene expression by assisted loading of STAT3 at a subset of primed enhancers. Ido Goldstein,1 Songjoon Baek,2 Myong-Hee Sung,2 Gordon L. Hager 1.

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feedback loops, contains STAT5 response element within its promoter region. In our study, we demonstrated that STAT5 preferentially binds to promoter sequence of shared STAT5 targeted genes and to distal putative enhancers of lineage-specific genes. DNase-seq demonstrated that chromatin accessibility of STAT5-based enhancers was greatly dependent on cytokine exposure, while common STAT5 promoter targets were constitutively accessible. CRIPSR/Cas9 gene editing was used to delete the generic STAT5 binding sites in Socs2 locus in mice. Prolactin induction of Socs2 was abrogated both in liver and mammary gland, and resulted in elevated STAT5 activation and precocious mammary development in mutant mice. Taken together, we demonstrated that promoter-bound STAT5 modulates cytokine responses of universal STAT5 target genes, while enhancer-bound STAT5 is necessary for lineage-specific target gene activation. This study provides new insight into the mechanism of used by a generic cytokine response element controlling lineage-specific transcription.

#5513 Notch directly represses transcription by recruitment of PRC2 to the ternary complex. Xiaojing Han, Prathibba Ranganathan, Anthony Capobianco. University of Miami, Miami, FL

It is well established that Notch functions as a transcriptional activator through the formation of a ternary complex that comprises Notch, Maml and CSL. This ternary complex then serves to recruit additional transcriptional co-factors that link to higher order transcriptional complexes. The mechanistic details of these events remain unclear. In this study, we report that the Notch ternary complex can also serve to direct the formation of a repressor complex to terminate genes expression of select target genes. Herein we demonstrate that p19Arf and Klf4 are transcriptionally repressed directly by Notch. Data indicate that Notch recruits PRC2 and Lsd1 to these promoters, which leads to changes in the state of the epigenetic landscape and repression of transcription. The de-methylase activity of Lsd1 is a pre-requisite for Notch-mediated transcriptional repression. Furthermore, we identified a stable Notch transcriptional repressor complex containing Lsd1, PRC2 and the Notch ternary complex. This study demonstrates proof of concept for a novel function of Notch and provides further insight into the mechanisms of Notch mediated tumorigenesis. This finding provides rationale for the targeting of epigenetic enzymes to inhibit Notch activity or use in combinatorial therapy to provide a more profound therapeutic response.

#5514 Loss of expression of the recycling receptor, FcRn, promotes tumor cell growth by increasing albumin consumption. Rafał Świercz, Min Mo, Priyanka Khare, Zita Schneider, Raimund Ober, Sally Ward. Texas A&M Health Science Center, College Station, TX

Tumor cells rely on high concentrations of amino acids to support their growth and proliferation. Although increased macromolecular uptake and lysosomal degradation of the most abundant serum protein, albumin, in Ras-transformed cells can meet these demands, it is not understood how the majority of tumor cells that express wild type Ras achieve this. In the current study we reveal that the neonatal Fc receptor, FcRn, regulates tumor cell proliferation through the ability to recycle its ligand, albumin. By contrast with normal epithelial cells, we show that human FcRn is present at very low or undetectable levels in the majority of tumor cell lines analyzed. Remarkably, shRNA-mediated ablation of FcRn expression in an FcRn-positive tumor cell line results in a substantial growth increase of tumor xenografts, whereas enforced expression of this receptor by lentiviral transduction has the reverse effect. Moreover, intracellular albumin and glutamate levels are increased by the loss of FcRn-mediated recycling of albumin, combined with hypoalbuminemia in tumor-bearing mice. These studies identify a novel role for FcRn as a suppressor of tumor growth and have implications for the use of this receptor as a prognostic indicator and therapeutic target.

#5515 Dicer suppresses cytoskeleton remodeling and tumorigenesis of colorectal epithelium by miR-324-5p mediated suppression of HMGB3 and WASF-2. Lina Sun, Jianming Li. Soochow University, Su Zhou, China.

Emerging evidence indicates that microRNAs, a class of small and well-conserved noncoding RNAs, participate in many physiological and pathological processes. RNase III endonuclease DICER is one of the key enzymes for mi-
croRNA (miRNA) biogenesis. However, the role of Dicer and its downstream miRNAs in colorectal cancer (CRC) remain largely unclear. Here, we found that Dicer was downregulated in tumour samples of CRC patients at both mRNA and protein levels. Importantly, more tumours were developed in mice with intestinal epithelial cells specific deletion of Dicer (Dicerlox/- & VillinCre mice) and in EGFR mutant-EGFR (AOM) and doxorubicin (DOX) administration. Interestingly, deletion of Dicer in intestinal epithelial cells led to severe chronic inflammation and cytoketone remodeling of intestinal epithelial layer in mice with or without DSS administration. Microarray analysis of 3 paired Dicer deletion CRC cell lines (RKO-WT/RKO-Dicer-/-, HCT116-WT/ HCT116-Dicer-/- and DLD-WT/DLD-Dicer-/-) showed that miR-324-5p was one of the most significantly down-regulated in the liver of Dicerlox/- & VillinCre mice, miR-324-5p was also found to be markedly downregulated. Mechanistically, miR-324-5p directly bound to the 3’ untranslated regions (3’UTRs) of HMGX3 and WASF-2, two key proteins participated in cytoketone remodeling, to suppress their expression. Intraportal injection of miR-324-5p Agomir curtailed chronic inflammation and cytoketone remodeling of colorectal epithelium and restored intestinal barrier function in Dicerlox/- & VillinCre mice induced by DSS. Therefore, our study reveals a key role of a Dicer/miR-324-5p/HMGX3/WASF-2 axis in cytoketone remodeling and maintaining integrity of intestinal barriers and as such alludes to the possibility of screening for small-molecule compounds that boost Dicer activity as a potential strategy in the treatment of colorectal cancer.

#5516 Regulation of TFPI-2 in the progression of ovarian cancer. Jacqueline Fry,1 Sumie Kato,2 Lorenza Aburaza,2 Pamela Gonzalez,2 Elisa Cumsille,2 Erasmo Bravo,2 Raimundo Correa,2 Juan E. Leiva,2 Cesar Paredes,2 Juan C. Roa,2 Carolina Ibañez,2 Mauricio Cuello,2 Garrett L. Owen,3 Maria L. Bravo,3 Pontificia Universidad Católica de Chile, Millennium Institute Immunology and Immunotherapy, Santiago, Chile; Pontificia Universidad Católica de Chile, Santiago, Chile; Hospital Gustavo Fricke, Santiago, Chile; Hospital Regional de Talca, Santiago, Chile; Pontificia Universidad Católica de Chile, Santiago, Chile; Millenium Institute Immunology and Immunotherapy, Biomedical Research Consortium Chile, Santiago, Chile.

Ovarian cancer metastasis occurs when malignant cells migrate from the primary tumor into the ascitic fluid and form a secondary tumor in the peritoneal cavity. The cells responsible for this process, Metastases Initiating Cells (MICs), suffer a series of changes in protein expression, with increased Epithelial Mesenchymal Transition and stem cell markers having been reported. Tissue Factor Pathway Inhibitor-2 (TFPI-2) is a serine protease inhibitor and potential tumor suppressor gene, whose reduction is correlated with more metastases and poor prognosis. As the exact stage of TFPI-2 loss is still unknown, the purpose of our study was to determine TFPI-2 during ovarian cancer progression. Protein and RNA levels were assessed in primary tumor and metastatic tissue from papillary serous carcinoma by immunohistochemistry and qPCR, respectively. TFPI-2 RNA and protein levels were analyzed in cancer cell lines and primary cultured cancer cells isolated from ascitic fluid of advanced ovarian cancer patients and in cancer spheres derived from these cultures. Epigenetic changes of 3 CpG were analyzed by bisulphite and plosequencing technique in ovarian cancer cell lines and cancer spheres derived from these cultures. We report that decreased TFPI-2 protein levels in metastatic tissue compared to the primary tumor, a result confirmed in matched primary and metastatic lesions from the same ovarian cancer patients. Furthermore, cancer spheres (representing potential MICs) displayed significantly lower levels of TFPI-2 than corresponding cell line and some primary cultured cancer cells. The cancer spheres showed a modest increase in methylation percentage in three specific CpG sites within the TFPI-2 promoter. Our results suggest that TFPI-2 loss plays a role in the cancer cell escape from the primary tumor. Future studies will determine the mechanism and biological consequences of the progressive loss of TFPI-2 in cancer. Funding: FONDECYT 11140657, 11140600, 11140970, 3140335, 3150028, CORFO 12 131212-1868, IMII-P09/016-F, CONICYT-FONDAP 15130011, BMRC 13CT2125-06.


Introduction: EGFR is a member of the ErbB family of tyrosine kinase receptors (RTKs) and plays important roles in the pathogenesis of certain human cancers. In non-small cell lung cancer (NSCLC), mutations in EGFR gene are commonly known as oncogenic driver mutation and targeted for treatment. Leucine rich repeat and immunoglobulin-like domain protein-1 (LRIG1) is a cell surface protein and known as a negative regulator of ErbB family. In brain tumor, LRIG1 was reported to downregulate not only wild-type EGFR, but also EGFRvIII, mutant-type EGFR. But there has been no report about the relationship between LRIG1 and EGFR mutation in the kinase domain frequently found in lung cancer. In this study, we investigate the expression of LRIG1 in lung cancer from its effect on signal transduction and cell proliferation and invasion. Method: We examined the LRIG1 expression levels in lung cancer cell lines and the surgically resected primary lung cancer tissues by quantitative PCR assay. We made stable clone of EGFR-mutant cell line (HCC827; exom19del E746-A750) overexpressing GFP tagged LRIG1, and compared with stable clone overexpressing GFP tagged EGFR. Results: The LRIG1 expression level in lung cancer cell lines and the surgically resected primary lung cancer tissues were much lower than that in normal bronchial epithelial cells and their normal tissues, respectively. The introduction of LRIG1 decreased EGFR expression and their phosphorylation in EGFR-mutant cell line. LRIG1 strongly suppressed cell proliferation, invasion and migration of EGFR-mutant cell line. In addition, a phospho-RTK array revealed that LRIG1 also downregulated the other RTKs, such as HER2, HER3, MET, and IGF-1R. Conclusion: Our findings demonstrate that LRIG1 decreases the expression and phosphorylation of mutant-EGFR protein and has strong anti-tumor effect in lung cancer harboring EGFR mutation. These findings suggest the importance of LRIG1 related studies to develop a novel strategy for EGFR mutant cancers.

#5518 Rgs8 and Rgs16 are tumor suppressor genes in mouse pancreatic ductal adenocarcinoma. Shreshi Pal Choudhuri, Yalda Zolghadri, Luke Macarenhas, Ozhan Ocal, Thomas Wilkie. UT Southwestern Medical Center, Dallas, TX.

We have identified regulators of G protein signaling (Rgs8 and Rgs16) as a new class of tumor suppressor genes in a mouse model of pancreatic ductal adenocarcinoma (PDAC). PDA is the 3rd leading cause of cancer related deaths in the United States. Kras mutations (e.g. KrasG12D) are associated with over 90% of human PDA and are an early event in the multistep process leading to PDA. Kras can be activated by protein kinase and G Protein Coupled Receptor (GPCR) signaling. Rgs proteins regulate GPCR signaling by accelerating the GTPase activity of G- and G class alpha subunits. Activating alleles of Gq that are resistant to Rgs inhibition are associated with PDA in humans. We found Rgs8 and Rgs16 are in vivo reporters of Kras activity in pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasm (IPMN), and PDA progression (DMM 8, 2015). Rgs8 and Rgs16 are expressed in PanIN and IPMN, precursors of PDA, in KC mice (LSL-KrasG12D; p48::Cre). To test if Rgs8-16 function as tumor suppressor genes, we crossed the Rgs8-16 double knockout into KC (termed KR8-16 mice). Compared to KC, PDA initiates earlier, is more aggressive, and KC-TR4-16 mice die earlier. Our study suggests that Rgs8 and Rgs16 control Kras-dependent PDA initiation and progression.

#5519 psiPTTE22-HERV functions as a tumor suppressor in gastric cancer and is associated with disease outcome. Jessie Qiaoyi Liang,2 Fei Xu,1 Zi Wang,3 Jun Yu1. 1The Chinese University of Hong Kong, Shatin, Hong Kong; 2Zhejiang University, Hangzhou, China.

Background and aim: The novel gene psiPTTE22-HERV identified by us previously is a human endogenous retrovirus (HERV)-related gene located adjacent to the gene psiPTTE22. With a high GC content around the promoter region, expression of psiPTTE22-HERV can be regulated epigenetically by DNA methylation. We aimed to elucidate the clinical significance of epigenetic alteration and biological function of psiPTTE22-HERV in gastric cancer. Methods and results: psiPTTE22-HERV was ubiquitously expressed in normal adult tissues including stomach, but was frequently silenced/down-regulated in gastric tumors and cancer cell lines as evidenced by RT-PCR. Bisulphite genomic sequencing results indicated that psiPTTE22-HERV was silenced by promoter DNA methylation, and its expression could be restored by demethylation treatment. In gastric cancer cell lines, Ecotropic expression of psiPTTE22-HERV significantly suppressed cell viability, clonogenicity and cell cycle progression, induced apoptosis, and inhibited migration and invasion of SGC7901 and MKN45 gastric cancer cells. In contrast, knock-down of psiPTTE22-HERV in the gastric cancer MKN1 cells significantly increased cell growth and migration ability, promoted cell cycle progress and inhibited cell apoptosis. psiPTTE22-HERV significantly suppressed subcutaneous tumorigenicity of SGC7901 cells in nude mice and metastasis (liver implantation) in tail vein injection models. Promoter methylation level of psiPTTE22-HERV was significantly higher in gastric tumors than in adjacent non-tumor tissues as revealed by bisulphite genomic sequencing (P<0.05); methylation levels in both tumors and adjacent non-tumor
tissues of gastric cancer patients were significantly higher than in normal stomach tissues (both p < 0.001). Multivariate Cox regression analysis demonstrated that promoter methylation of p53TPT2E2-HERV in primary gastric tumors was an independent risk factor for poor survival. Kaplan-Meier survival curves showed that high-methylation of p53TPT2E2-HERV promoter was significantly associated with poorer survival in gastric cancer patients (independently of other factors such as age, sex, tumor stage, and grade) and significantly increased the risk of gastric cancer recurrence (both p < 0.05). Conclusion: p53TPT2E2-HERV functions as a tumor suppressor that is commonly down-regulated by promoter methylation in gastric cancer, which may serve as a prognostic biomarker for gastric cancer patients.

#5520 CCN5/WISP-2 is a negative regulator of epithelial to mesenchymal transition and stemness in breast cancer. Gargi Maity, Amilan Das, Sandipito Sarkar, Snigdha Banerjee, Sushanta K. Banerjee. VA Medical Center, Kansas City, MO.

Background and Objective: Breast cancer is the most common cancer in women and a leading cause of cancer mortality in western countries. CCN5 (also known as Wnt-1 induced signaling protein-2 or WISP-2) is a 29-31 kDa matrix metalloproteinase that plays as a negative regulator of breast carcinoma. Our previous studies had shown the importance of CCN5/WISP-2 in the suppression of breast and pancreatic cancer progression through the regulation of the invasive phenotypes. Considering the previous report, our aim is to investigate whether human recombinant CCN5 inhibit pathological events like epithelial to mesenchymal transition (EMT), migration and stemness in triple negative breast cancer cells. Methods: To investigate the negative impact of CCN5 on EMT and stemness of TNBC, we performed several techniques like western blot, clonogenic assay, soft agar assay, sphere formation assay etc. Results and Conclusions: The exposure of triple negative human breast cancer cells (TNBC), MDA-MB-231 and HCC-70, to recombinant CCN5 (hrCCN5), resulted in a dose-dependent inhibition of cell-proliferation through the induction of apoptotic cell death. The treatment of hrCCN5 regulates various pathological events in breast cancer cells, such as reprogramming the mesenchymal to epithelial transition (MET) followed by reduction of stemness features as confirmed by sphere formation assay and delaying in vitro migration. Finally, treatment with hrCCN5 in TNBC cells significantly inhibited anchorage-dependent and independent growth of TNBC. Collectively, CCN5’s control of cancer cell physiology indicates that hrCCN5 has the potential of being used as a major therapeutic agent against triple negative breast cancer.

#5521 Loss of Lcn2 promotes development of smoking-associated Kras mutant lung cancer. Joshua Kapere Ochien1, Wenhua Lang1, Sayuri Nuno-mura-Nakamura1, Christina McDowell2, Ralph Arlinghaus1, Yuki Imaoka3, Takashi Horii4, Junya Fukuoka4, Paul Scheet1, Junya Fujimoto1, Humam Ka-dara1. 1 UT MD Anderson Cancer Ctr., Houston, TX; 2 Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; 3 Toyama University Hospi-tal, Toyama, Japan; 4 American University of Beirut, Beirut, Lebanon.

Lung adenocarcinoma (LUAD) represents the most commonly diagnosed lung cancer subtype. LUADs in smokers frequently exhibit mutations in the Kras oncogene. Relative to other LUADs, Kras mutant LUAD displays dismal lung cancer subtype. LUADs in smokers frequently exhibit mutations in the Kras oncogene. Relative to other LUADs, Kras mutant LUAD displays dismal development of Kras mutant LUAD and would thus constitute targets for early prevention and treatment. We recently found that mice with knockout of the airway lineage gene G-protein coupled receptor 5A (Gprc5a+/−), in contrast to typical WT littermates, not only developed LUADs after tobacco carcinoma (nicotine-specific nitrosamine ketone/NKNK) exposure but that these lesions harbored somatic Kras mutations, the same variants thought to act as drivers of LUAD in human smokers. We then also found by RNA-sequencing (RNA-Seq) and immunohistochemical analyses markedly elevated expression of the lipocalin 2 (Lcn2) gene in airways of Gprc5a+/− mice and preceding tumor development. While Lcn2 has been suggested to function as a putative oncogene (e.g., in mammary tissue), little is known about the role of Lcn2 in LUAD. To fill this void, we developed mice with knockout of both Gprc5a and Lcn2 (Gprc5a−/− Lcn2−/−) and examined lung tumorigenesis following NKNK exposure in these mice relative to similarly treated Gprc5a−/− littermates. We found that Gprc5a−/− Lcn2−/− mice, relative to Gprc5a−/− littermates, exhibited markedly and significantly increased lung tumor burden and growth at three and seven months following NKNK exposure. Moreover, when we injected Kras mutant Gprc5a−/− cells (MDA-F471) into the tail veins of mice, we noted a trend for increased tumor development in lungs of Gprc5a−/− Lcn2−/− relative to Gprc5a−/− littermates. To gain global insights into the impact of Lcn2 on gene expression and signaling in Kras mutant LUAD cells, we employed RNA-Seq coupled with pathways analysis to survey differentially expressed transcripts between MDA-F471 cells stably transfected with Lcn2-specific shRNA and cells with control shRNA. Pathways and gene network analysis of the differentially expressed transcripts revealed that Lcn2 knockout leads to a heightened malignant phenotype in the Kras mutant LUAD cells via the activation of various signaling nodules including Tnf, Erk1/2 and Akt. We confirmed the RNA-Seq and pathways analysis by Western blotting demonstrating increased phospho-protein levels of Tnf, Erk1/2 and Akt in cells with knockdown of Lcn2. All in all, our preliminary data suggest that Lcn2 expression plays a tumor suppressor role downstream of Gprc5a and tobacco carcinogen exposure in the pathogenesis of Kras mutant LUAD in part by inhibiting Erk1/2 and Akt oncogenic signaling.

#5522 Novel myogenic differentiation transcription factor CASZ1 suppresses rhodomysosarcoma tumor growth. Zhihui Liu1, Norris Lam1, Arnuito Mendoza1, Jun S. Wei2, John F. Shern1, Marielle Yohé3, Javed Khan2, Carol J. Thiele1, 1 NCI-CCR, Pediatric Oncology Branch, Bethesda, MD; 2 NCI-CCR, Genetics Branch, Bethesda, MD.

Disruption of muscle regulatory factors (MRFs) in muscle progenitor cells results in a failure to withdraw from cell cycle and terminal differentiation and is proposed to contribute to rhabdomyosarcoma (RMS) tumorigenesis. Zinc finger transcription factor CASZ1 is a tumor suppressor gene and regulates normal nervous system and heart development. CASZ1 is known to regulate a subset of genes that are involved in skeletal muscle development although the function of CASZ1 in normal myogenesis is not known. CASZ1 levels increase over 20-fold when C2C12 myoblasts differentiate into myotubes. To probe its role in normal myogenesis we performed genetic knockdown or overexpression experiments in C2C12 myoblasts. When CASZ1 expression is silenced using RNAi, there is a significant reduction in myotubes upon induction of differentiation. Real-time PCR shows that knockdown of CASZ1 increases Myf5, a MRF that determines early commitment of muscle precursor cells, but decreases Myogenin (2-fold, p < 0.01), a MRF required for terminal differentiation and myotube formation. Knockdown of CASZ1 also decreases late skeletal differentiation genes Acta1, Ckm, Tnt1 and Tnn1 (2-2.5-fold, p < 0.01). Over-expression of CASZ1 induces expression of Myogenin, Acta1, Ckm and Tnt1, represses Myf5 and accelerates myotube formation. This indicates that CASZ1 regulates genes important in the transition from early to late myogenic differentiation. To investigate the role of CASZ1 in RMS, we first evaluated the expression of CASZ1 in primary RMS tumors using public available microarray database. CASZ1 mRNA levels are ~1.5-fold lower in embryonal RMS (ERMS) samples compared to normal muscle (p < 0.001), while levels of CASZ1 in alveolar (ARMS) are similar to levels in normal muscle. CASZ1 levels increase over 3-fold when RD cells (ERMS) were cultured in differentiation medium (100 nm 12-O-tetradecanoylphorbol-13-acetate, TPA). Knockdown of CASZ1 in the RD cells suppressed TPA induced expression of myogenin markers TNN1 and TNN2 while CASZ1 overexpression stimulated expression of TNN1 and TNN2 (all p < 0.05). An in vivo spontaneous xenograft model showed that over-expression of CASZ1 significantly suppressed RD tumor growth (p < 0.005). In RMS patients, NexGen sequencing of 85 tumors identified 4 samples with non-synonymous single nucleotide variants (SNVs) in CASZ1 that were absent in the 1000 Genomes databases. We engineered these SNVs into C2C12 construct and transduced it into C2C12 myoblasts. When C2C12 SNV cells were silenced by shRNA and left untreated, there was no significant change in normal nuclear localization and failed to activate skeletal muscle gene transcription (p > 0.03). Taken together, our results suggest that the regulation of muscle differentiation program by CASZ1 in C2C12 is integral to proper myogenic differentiation, and genetic variants of CASZ1 disrupt early myogenesis and may contribute to ERMS tumorigenesis and progression.

#5523 Loss of RAB25 cooperates with oncogenes in the transformation of human mammary epithelial cells. Pooja Sridhar Joshi. Southern Illinois Univ. School of Medicine, Springfield, IL.

The RAB guanosine triphases (RAS-related in brain) belong to the Ras superfamily of GTPases, and loss of RAB 25 expression has been reported in a number of breast cancer cases containing H-Ras point mutations, particularly triple negative breast cancers (TNBC), one of the most aggressive subtypes of breast cancer and associated with a poor prognosis. The mechanism involved in the progression of these tumors is poorly understood. In this study, we are trying to understand if loss of RAB25 expression in Human Mammary Epithelial Cell (HMEC) lines co-operates with H-Ras mutations and contributes to tumorigenesis. HMEC were immortalized by transduction with LXSIN CDK4 R24C, a mutant form of cyclin-dependent kinase, followed by transduction with hTERT, catalytic subunit of the telomerase enzyme that permits the cells to exceed the Hayflick Limit and become immortal. We have found that with loss of RAB25 and over expression of mutant H-Ras61L, immortal HMEC undergo transformation. We have looked into the co-operativity between loss of Rab25 and...
H-Ras61L mutant by in-vitro studies to show their anchorage independent growth and increased ability to migrate. Furthermore, cells express low CD24, high CD44, and very low levels of Claudin indicating that cells acquire stem-like properties upon transformation. Loss of RAB25 and over-expression of H-ras61L resulted in increased expression of transcription markers Snail and Slug that decrease cells to lose E-cadherin and undergo Epithelial to Mesenchymal Transition (EMT). We also intend to carry out nude mice studies to look for the potential of these cells to form tumors and metastasize. This study shows that loss of RAB25 and over-expression of mutant H-Ras can transform HMEC and give rise to mesenchymal stem-like tumors. Our findings reveal that RAB25 functions as a tumor suppressor gene, and loss of RAB25 could serve as a novel biomarker in the prognosis of Claudin-low type of TNBC.

#5524 Gene 33/Mig6 regulates apoptosis and the DNA damage response through independent mechanisms. Cen Li, Soyoong Park, Xiaowen Zhang, Leonard Eisenberg, Hong Zhao, Zbigniew Darzynkiewicz, Dazhong Xu. New York Medical College, Valhalla, NY.

Gene 33 (Mig6, ERRFI1) is an inducible adaptor/scaffold protein whose expression can be induced by both stress and mitogenic signals. It contains multiple domains for protein-protein interaction and is involved in a broad spectrum of cellular functions including cell proliferation, cell migration, cell apoptosis, and cell senescence. Many functions of Gene 33 are attributable to its ability to bind to the kinase domain of the EGFR family receptor tyrosine kinases thereby inhibiting their activities. Gene 33 also binds and activates tyrosine kinase c-Abl and is involved in other signaling pathways such as NF-kB, HGF, and INK. Growing evidence at the genetic, cellular, and animal levels indicates that Gene 33 functions as a tumor suppressor in the lung. Gene 33 has been shown to promote apoptosis. A recent study has linked this protein to the DNA damage response induced by hexavalent chromium [Cr(VI)]. In the current study we find that ectopic expression of Gene 33 strongly induces apoptosis by both activating the c-Abl/p73 pathway and inhibiting the EGFR/ Akt pathways in BEAS-2B lung epithelial cells and A549 lung carcinoma cells. Surprisingly, ectopic expression of Gene 33 also triggers the DNA damage response in an ATM-dependent fashion and through pathways with and without association with apoptosis. We observed striking presence of Gene 33 in the nucleus and chromatin, which is in contrast with the belief that Gene 33 is an exclusively cytoplasmic protein. Our data show that chromatin localization of Gene 33 is partially dependent on the EBD motif of Gene 33, a domain required for interaction with EGFR and c-Abl. Our data also indicate that Gene 33 may regulate chromatin targeting of c-Abl and EGFR. Furthermore we find that both endogenous and ectopically expressed Gene 33 strongly interact with histone H2AX. Our data have revealed potential nuclear/chromatin-associated mechanisms that underlie the function of Gene 33 in apoptosis and the DNA damage response.

#5525 Induced expression of PPM1A in ER-negative breast cancer cells inhibits growth by suppressing CDK phosphorylation. Abhijit Mazumdar, Jamal Hill, Yun Zhang, Lakshmi Reddy Bolli, Anna Tsimelzon, Jenny Chang, Gordon Chang, Dazhong Xu. UT MD Anderson Cancer Ctr., Houston, TX.

Background: Estrogen receptor (ER)-negative breast cancer is a clinical subtype that is overrepresented among younger women and is associated with a poor prognosis. Current treatments for ER-negative tumors include cytotoxic chemotherapy, or for those overexpress HER2, the anti-HER2 antibody. Targeted therapy for triple-negative breast cancers is urgently needed. In this project, we investigated phosphatases that are differentially expressed in ER-negative as compared to ER-positive breast cancers. We hypothesized that: (1) specific phosphatases govern the growth of ER-negative cancers, (2) Induced expression of specific phosphatases that are under expressed in ER-negative cancers will suppress the growth of ER-negative breast cancers. Methods: Using 102 human breast tumors (57 ER-negative & 45 ER-positive) from the neo-oadjuvant studies from the Baylor Breast Center tumor bank, we isolated RNA and performed Affymetrix microarray analyses. Statistical analysis was done with dChip software, and phosphatases over (>1.5 fold; FDR <0.05) or under (<0.66-fold; FDR <0.05) expressed in ER-negative breast cancers as compared to ER-positive cancers were selected for further study. One of the phosphatases under expressed in ER-negative breast cancer was PPM1A. Regulated expression of PPM1A was achieved using a Tet-regulated vector. Cell growth in soft agar and cell cycle analyses were performed using previously published protocols. Mouse xenograft experiments were performed by injecting inducible PPM1A-clones and vector-clones in mammary fat pad of athymic mice and tumor growth was measured over time in randomized groups. To induce PPM1A expression one group was treated with doxycycline when tumor volume reached 50 mm3. Survival analyses were done using Oncomine datasets. Results: We identified 20 over-expressed and 29 under-expressed phosphatases in ER-negative breast cancers. We selected the under expressed phosphatase PPM1A, for further study. Multivariate cox regression analysis shows PPM1A is an independent predictor for breast cancer survival. Induced expression of PPM1A in ER-negative cells inhibited anchorage-dependent and independent growth but had no effect on ER-positive cell. PPM1A expression also inhibited MDA-MB 231 cells growth in vitro and growth in vivo in the presence of doxycycline. Furthermore PPM1A expression suppressed CDK6 expression at G1 phase. PPM1A interacts with CDK6 and inhibits phosphorylation of CDK and MDM2. Conclusions: We identified a set of over- and under-expressed phosphatases in ER-negative breast cancers as compared to ER-positive cancers. Overexpression of PPM1A in ER-negative breast cancer cells inhibits growth. By identifying the molecules that regulate breast cancer cell growth we aim at identifying potential new targets for the treatment of these aggressive ER-negative breast cancers. Supported by Komen Promise grant KGC08H04 & Komen SAB grant.

#5526 Tumor suppressive role of BMI-1 through inhibition of JAK-STAT signaling in leukemia. Yuk Man Lam, Stephen Se Yuen Lam, Anskar Yu Hung Leung, Ray Kit Ng. The University of Hong Kong, Hong Kong, Hong Kong.

BMI-1, which is one of the core components of polycomb repressive complex 1, is frequently found deregulated in patients with hematological disorders. In last decades, researchers concordantly agree that BMI-1 mediates tumorigenesis of leukemia stem cells through p16INK4A leukemogenic pathway. However, accumulating evidences contradict the idea that BMI-1 solely plays an oncogenic role in tumorigenesis. It has been shown BMI-1 depletion favors the development of myelofibrosis in mice; whereas high BMI-1 expression suppresses colony forming ability of MLL-ENL-transformed bone marrow cells and correlated with higher survival in some cancers. In this study, we hypothesized that BMI-1 plays a tumor suppressive role, which is independent of the regulation of INK4A-ARF locus, in human leukemia. BMI-1 was over-expressed in a panel of myeloid and lymphoid lineage leukemia cells, including HL-60, MV-4-11, MonoMac-6, SEM, Nalm-20 and RS4;11. We observed no deregulation of p16INK4A and p14ARF genes by BMI-1, suggesting the regulation of INK4A-ARF locus is independent of BMI-1 modulation in leukemia cells. Nevertheless, over-expression of BMI-1 resulted in significant reduction of leukemia cell proliferation. It is noted that constitutively active JAK-STAT signaling pathway is crucial to leukemia cell survival. By modulation of BMI-1 level, we demonstrated suppression of the activated JAK-STAT signaling pathway in most of the leukemia cell lines with the exception of MonoMac-6 and RS4;11. This is in agreement with the high sensitivity to ruxolitinib, a JAK-STAT inhibitor, in all the tested leukemia cell lines except RS4;11. Importantly, we showed that BMI-1 over-expression could sensitize RS4;11 cells to reduce cell proliferation under ruxolitinib treatment. These results suggest that higher efficacy of ruxolitinib treatment could be achieved under a condition of high cellular level of BMI-1. We further demonstrated that ruxolitinib treatment was more effective in a cohort of AML patient samples (n = 25) with a context of higher BMI-1 expression (p < 0.05). Altogether, our results suggest that BMI-1 functions as a tumor suppressor gene via inhibition of the JAK-STAT signaling pathway. The endogenous level of BMI-1 could be served as an indicator for the effective treatment of JAK-STAT-dependent leukemia cells using ruxolitinib.

#5527 Effects of PTEN localization and phosphatase activity on gene expression profiling of glioblastoma cells. Yubing Wang, Andrew M. Chan. Chinese University of Hong Kong, Shatin, Hong Kong.

PTEN possesses both protein and lipid phosphatase activities, with its tumor-suppressor function mainly depends on its lipid phosphatase activity through inhibiting the PI3K/AKT signaling pathway at the cell membrane. However, increasing evidence have demonstrated that PTEN also localizes to other intracellular organelles such as the endoplasmic reticulum, the mitochondria, or the nucleus where it plays important roles in multiple cellular processes. To further understand how PTEN localization affects the gene expression profiling in cancer cells, a PTEN null glioblastoma cell line, U87MG, was stably expressing wild-type, nuclear localization signal (NLS)-tagged, nuclear export signal (NES)-tagged and myristoylation (MYR)-tagged PTEN. Ion Torrent based whole transcriptome sequencing was used for gene expression analysis. Gene ontology enrichment analysis showed that the most common cellular component regulated by PTEN over-expression was extracellular factors, which function as growth factor-binding or fibronectin-binding molecules related to cell adhesion or organ system development. In contrast, nuclear PTEN promoted the enrichment of genes in collagen-binding and integrin-binding, which may be related to blood vessel development or cell motility. KEGG pathway enrichment also confirmed
that PTEN over-expression upregulated focal adhesion pathway, an important pathway that frequently disrupted in PTEN Hamartoma Tumor Syndrome (PHTS). Moreover, nuclear PTEN differentially regulated genes showed higher enrichment in immune response signaling pathways. This unique gene expression profiling driven by NLS-PTEN indicated that besides the membrane activities, nuclear PTEN may have additional functions than merely inhibiting the PI3K signaling pathway. The transcriptome data showed potential downstream targets of PTEN in different subcellular localizations, which may lead to unique biological functions. More functional studies will be carried out to further validate how intracellular PTEN regulates the expression of these genes, and what are the signaling pathways involved in the regulation of these processes.

3. Results and Discussion

3.1. Evidence linking aquaporin-3 loss to increased invasiveness in bladder cancer

AQP3 is involved in the invasion and metastasis of UC cells. In the present study, the expression of AQP3 resulted in increased migration in the wound-healing scratch assay. Over-expression of AQP3 plasmid resulted in reduced migration. It is known that epithelial cell polarity contributes to tumor suppression and that loss of E-cadherin is a crucial step in epithelial-to-mesenchymal transition (EMT). Our results indicate that AQP3 overexpression induced expression of E-cadherin, consistent with a role for AQP3 as a tumor suppressor by maintaining epithelial cell polarity and inhibiting EMT in BCa. Conversely, reduced AQP3 levels leading to reduced E-cadherin levels in high-grade BCa cells may result in loss of cell polarity, increased invasiveness and increased EMT.

3.2. Significance and functions of estrogen receptor beta (ESR2) isoforms in glioblastoma

Background: Glioblastoma (GBM) are the deadliest form of primary brain neoplasms. Several lines of evidence suggest tumor suppressive role of female sex hormones on brain tumors. However, mechanisms by which estrogens mediate protection against the GBM remains unknown. GBM preferentially express estrogen receptor beta (ER2). Emerging evidence suggests that ESR2 is expressed as multiple isoforms (ESR2-1 to 5, with variation at the C-terminal domain); however, much of the published information is focused primarily on ESR2-1. Little is known about the expression and functions of other ESR2 isoforms in GBM. The objective of this study is to examine the expression and determine the functions of ESR2 isoforms in GBM cells. Methods: Expression of ESR2 isoforms was profiled using 10 different patients derived and 5 established GBM cells. To study the functions of individual ESR2 isoforms in GBM cells, we have generated ESR2 knockout (ESR2-KO) cells using CRISPR/Cas9 system and then established GBM model cells expressing individual ESR2-1, 2, and 5 using lentiviral transduction in the ESR2-KO background. ESR2 isoforms was used to identify binding proteins of each ESR2 isoforms. Effect of each isoform on the growth, apoptosis, cell cycle progression, migration and invasion was analyzed using established methods. Mechanistic studies were conducted using reporter gene assays, RT-qPCR and signaling analysis. Results: RT-qPCR results demonstrated that ESR2-5 is highly expressed in majority of primary and established GBM cells compared to ESR2-1 and ESR2-2, with ESR2-4 is the least expressed. Expression of ESR2-5 significantly reduced proliferation of GBM cells. Further, ESR2-KO cells exhibited higher migratory and invasive potential compared to parental GBM cells, while ESR2-5 resulted in reduction in migratory and invasive potential of GBM cells. ESR2-KO GBM cells exhibited decreased levels of cell cycle arrest and apoptosis proteins p27, p21 and PUMA compared to parental GBM cells and re-expression of ESR2-1 or ESR2-5 in ESR2-KO cells rescued the phenotype.

IPMS studies identified several common and unique proteins that bind to each of the isoforms. Both ESR2-1 and ESR2-5 interacted with mTOR, while ESR2-5 uniquely interacted with several proteins related to DNA repair, immunomodulatory and apoptosis pathways. Overexpression of ESR2-1 reduced the activation of mTOR signaling molecules including p-mTOR, p-S6K and p-S6 in GBM cells compared to ESR2-KO cells, while ESR2-5 enhanced mTOR downstream signaling.

Conclusion: Using ESR2-KO cells, we have identified genetic evidence for the role of ESR2-1 in GBM tumor suppression. Our results also discovered that ESR2-5 is highly expressed in GBMs. Unlike ESR2-1, ESR2-5 has lesser tumor suppression ability and its interactions with mTOR, DNA repair, and apoptotic pathways may have important implications in GBM progression.

3.3. Functional role of Friend Leukemia Integration-1 (FLI1) in gastric carcinogenesis

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Gastric adenocarcinoma (GC) is the 5th most common cancer worldwide but is the 3rd leading cause of cancer death. FLI1 (Friend leukemia integration-1) is an ETS family transcription factor that regulates genes involved in proliferation of bladder cancer (BCa) is the most common type of urothelial cancer (UC). According to American Cancer Society, in 2016, diagnoses of 76,960 new cases of bladder cancer (BCa) and 16,390 BCa associated deaths have been estimated. More than 90% of bladder cancer starts in the innermost transitional epithelium (urothelium) of the bladder. Aquaporin 3 (AQP3), one of 13 members of a transmembrane channel forming protein family (AQP0-12), has an N-terminal basolateral sorting signal and localizes to the basolateral membrane in epithelial tissues. Recent studies have shown that AQP3 expression is decreased in UC thus supporting a new hypothesis that alteration of the expression of AQP3 plays a role in the pathogenesis of UC. However, it remains to be determined whether AQP3 is involved in the invasion and metastasis of UC cells. In the present study, human bladder cancer cell lines of different grades, SW780 (G1) and TCCSUP (G4) were studied. Transcript and protein levels of AQP3 expression were significantly higher in low grade, SW780 cells when compared to the higher-grade TCCSUP cells. Expression of CuSO4 or silencing of AQP3 resulted in increased migration in the wound-healing scratch assay. Over-expression of AQP3 plasmid resulted in reduced migration. It is known that epithelial cell polarity contributes to tumor suppression and that loss of E-cadherin is a crucial step in epithelial-to-mesenchymal transition (EMT). Our results indicate that AQP3 overexpression induced expression of E-cadherin, consistent with a role for AQP3 as a tumor suppressor by maintaining epithelial cell polarity and inhibiting EMT in BCa. Conversely, reduced AQP3 levels leading to reduced E-cadherin levels in high-grade BCa cells may result in loss of cell polarity, increased invasiveness and increased EMT.
Since NUGC3 cells have low FLI1 expression, we knocked down FLI1 by using a 24 and 48 hours (P<br>support a tumor suppressor role for FLI1 in human GC and also suggest that<br>not seen when cell were subjected to CK2 inhibition after Ikaros silencing. Further<br>investigation into the mechanism of LKB1 loss and increased sensitivity to inhi-

The objective of the present study is to investigate Runx1 functional effects on cellular activities, and its contribution to inhibiting CSC-like properties in breast cancer cells. Methods: We generated Runx1 overexpression and Runx1 knock-
down stable cell lines in both malignant MCF10A-T1 and metastatic MCF10CA1a cells. Cells were analyzed for tumoursphere formation (protein (Western) and RNA (RT-qPCR) expression analysis. Cellular activity assays (cell proliferation, migration, and invasion) were performed to measure the tumorigenic potential of the cells. Xenograft studies were used to determine whether Runx1 overexpression in breast cancer cells represses tumor growth in vivo. Results: Upon overexpression of Runx1, both MCF10A-T1 and MCF10CA1a cells reduce their proliferation, migration and invasion, which support the con-
cept that Runx1 acts as a tumor suppressor in breast cancer cells. Notably, over-
expressing Runx1 inhibited the formation of tumourspheres and effectively re-
duced the stem-like population in these two breast cancer cells. These observations are consistent with the results of Runx1 knock-down in early stage ER positive MCF17 breast cancer cells which showed increased tumoursphere forma-
tion efficiency. Furthermore, Runx1 tumor suppressor activity was confirmed in mouse xenograft by showing that Runx1 overexpression inhibits tu-
mor growth. Conclusion: Our studies show that Runx1 inhibits proliferation, migration, and invasion, reflecting its tumor suppressor activity. Runx1 mediated reduction of tumoursphere formation suggests that Runx1 also contributes significantly to inhibit the population of the breast cancer stem cells. Therefore these studies offer Runx1 as a novel bio-therapeutic molecule for breast cancer intervention.

#5534 Effect of LKB1 activity on the sensitivity to PI3K/mTOR inhibitor in non-small-cell lung cancer. Takehito Shukuya,1 Tadaaki Yamada,2 Michael J. Koenig,3 Mohammad A. Rahman,4 Joseph M. Amann,5 David P. Carbone.6 1The Ohio State University, Columbus, OH; 2Kanazawa University, Kanazawa, Japan.

The serine-threonine liver kinase B1 (LKB1, also called STK11) is a tumor suppressor that functions as master regulator of cell growth, metabolism, survival and polarity. Germline mutation of LKB1 causes Peutz-Jeghers syndrome, which is an autosomal dominant disease characterized by mucocutaneous pig-
mentation and hamartomatous polyps. In non-small-cell lung cancer (NSCLC), LKB1 is frequently rendered non-functional, either through mutation or down regulation. As a tumor suppressor and a protein whose function is lost, identi-
ifying pathways that are activated with LKB1 loss may be the only way to target such tumors. Some previous studies have suggested that inhibition of mTOR, a growth promoting pathway negatively regulated by LKB1, as a potential target in LKB1 mutant NSCLC. In this study, we investigated the effect of LKB1 activity on the sensitivity to a PI3K/mTOR inhibitor using four LKB1 mutant NSCLC cell lines to which LKB1 gene was transduced and one LKB1 wild type NSCLC cell line in which LKB1 gene was knocked out by CRISPR technology. Trans-
duction of LKB1 resulted in significant resistance to a PI3K/mTOR inhibitor in two of the four LKB1 add-back cell lines, while knocking out LKB1 in the LKB1 wild type cell line induced PI3K/mTOR inhibitor sensitivity. The mechanism behind these observed results appears to be through regulation of phosphoryla-
tion of AKT. The presence of LKB1 led to a persistent AKT phosphorylation even in the presence of the PI3K/mTOR inhibitor, both in add back cell and wild type cells. Our data suggest that the identification of LKB1 activity may be a promising biomarker for the sensitivity to PI3K/mTOR inhibition. Further in-
vestigation into the mechanism of LKB1 loss and increased sensitivity to inhi-
bition of the PI3K and mTOR pathways is warranted.
#5536 The metastasis suppressor NME1 regulates stemness in melanoma
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Melanoma is a lethal skin cancer that is highly resistant to therapy and prone to metastasize to distant organs. The quiescence melanoma stem cells are believed to contribute to the resistance to therapy. Intraepithelial cholangiocellular carcinomas (IHCC) and liver cancer are known to have the same, overlapping, or opposing functions, and the molecular mechanisms involved are poorly characterized. To elucidate the functions of the three Lrig proteins, we generated a series of wildtype and Lrig-null (Lrig1-/-; Lrig2-/-; Lrig3-/-) mouse embryonic fibroblasts. The FLOXed Lrig genes were then deleted with CRE-recombinase, thereby creating Lrig-null MEFs. ddPCR analysis showed that the Lrig-null MEF lines were contaminated with less than 1% of cells carrying wildtype Lrig3 alleles. Lrig-null MEFs showed a higher proliferation rate and expressed more transcripts in tumor cells than the wildtype cells. These Lrig-null cells will be useful to investigate the importance of Lrig proteins for other cellular functions such as, signal transduction, lipidomics, transcriptomics, secretomics, metabonomics, and oxidative stress sensitivity. We also transduced the Lrig-null cells with individual inducible Lrig genes thus creating a powerful system for the examination of the biology of the specific Lrig proteins.

#5539 The phenotypic characterization of Lrig null mouse embryonic fibroblasts.

The LRG1 gene family consists of LRG1, LRG2 and LRG3. The most studied of these is LRG1, which is a tumor suppressor gene. The LRG1 protein is downregulated in various types of cancer and it negatively regulates receptor tyrosine kinases including the EGFR and PDGFR-receptors. Lrig1 also regulates stem cell homeostasis in mouse skin and intestine. The functions of LRG2 and LRG3 are less known, but some findings imply that they might antagonize LRG1. Intriguingly, the sole Lrig homolog in C. elegans, Sma-10, positively regulates BMP signaling. Thus, it is not known to what degree the LRG proteins have the same, overlapping, or opposing functions, and the molecular mechanisms involved are poorly characterized. To elucidate the functions of the three Lrig proteins, we generated a series of wildtype and LRG-null (Lrig1-/-; Lrig2-/-; Lrig3-/-) mouse embryonic fibroblast (MEF) lines. These cell lines were created from homozygous Lrig flox/flox;Lrig2 flox/flox;Lrig3 flox/flox mouse embryos. The FLOXed Lrig genes were then deleted with CRE-recombinase, thereby creating Lrig-null MEFs. ddPCR analysis showed that the Lrig-null MEF lines were contaminated with less than 1% of cells carrying wildtype Lrig3 alleles. Lrig-null MEFs showed a higher proliferation rate and expressed more transcripts in tumor cells than the wildtype cells. These cell lines will also be useful to investigate the importance of Lrig proteins for other cellular functions such as, signal transduction, lipidomics, transcriptomics, secretomics, metabonomics, and oxidative stress sensitivity. We also transduced the Lrig-null cells with individual inducible Lrig genes thus creating a powerful system for the examination of the biology of the specific Lrig proteins.

#5540 Transcriptional control of signaling pathways in T-cell lymphoblastic leukemia by Ikaros tumor suppressor.
Tommy Hu, Mario Soliman, Malika Kapadia, Elanora Donat, Jonathan Payne, Chunhua Song, Sinisa Donat. Penn State College of Medicine, Hershey, PA.

Cellular proliferation in T-cell acute lymphoblastic leukemia is regulated by multiple signaling pathways. The Phosphoinositide-3-kinase (PI3K)/Akt pathway is frequently dysregulated in T-ALL. Targeting the PI3K pathway has shown promise as a novel therapeutic approach for T-ALL. However, regulation of the PI3K pathway is still not well understood. Here, we report that PI3K activity in T-ALL can be controlled by transcriptional regulation of key members of this pathway, PI3KCD and PIKFYVE. DNA binding analysis of primary T-ALL lower expressed the tumor suppressor protein Ikaros, binds to the promoter regions of PI3KCD and PIKFYVE. Since Ikaros acts as a regulator of the PI3K pathway, we tested whether Ikaros binding to PI3KCD and PIKFYVE affects their expression. Overexpression of Ikaros results in reduced transcription of PI3KCD and PIKFYVE in T-ALL. Targeting Ikaros with a specific siRNA, resulted in increased transcription of PI3KCD and PIKFYVE in T-ALL. Together, these results demonstrate that Ikaros functions as a transcriptional repressor of both PI3KCD and PIKFYVE, and suggest that Ikaros can regulate the PI3K pathway in T-ALL. It has been previously shown that Ikaros function in B-cell acute lymphoblastic leukemia is regulated by oncogenic Casein Kinase II (CK2). We tested whether Ikaros ability to repress transcription of PI3KCD and

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PIKfyve is regulated by CK2. Inhibition of CK2 by a specific pharmacological inhibitor, CX-4945, resulted in increased Ikaros binding to the promoters of PIK3CD and PIKfyve, as well as in transcriptional repression of both of these genes. These results suggest that Ikaros function as a repressor of PIK3CD and PIKfyve transcriptions is impaired by CK2 in T-ALL. CK2 inhibition represses Ikaros transcriptional repression of PIK3CD and PIKfyve. CK2 inhibition results in downregulation of the PI3K pathway. In conclusion, the presented data demonstrate that the PI3K signaling pathway is regulated by transcriptional repression of PIK3CD and PIKfyve by Ikaros in T-ALL. Results reveal interplay between two signaling pathways in T-ALL, CK2 and PI3K, where CK2 positively regulates the PI3K pathway by inhibiting Ikaros function. These data reveal novel mechanisms that regulate cellular proliferation in T-ALL.

**MOLECULAR AND CELLULAR BIOLOGY / GENETICS: Tumor Suppressors 2**

**#5541 Identification of sod1 and sod2 as potential prognostic biomarkers for patients with non-small cell lung cancer.** Chen Yin, Ren Wang, Xiao-xing Li, Yang Yang, Mei-yin Zhang, Hui-Yun Wang, Steven X. Zheng. Sun Yat-sen University Cancer Center, Guangzhou, China.

Background: Reactive oxygen species (ROS) is accumulated in cancer cells related to increased metabolism, which is characteristic of cancer progression. With the rapid advance of cancer research, redox regulation has drawn more and more attentions. The superoxide dismutases SOD1 and SOD2 are essential enzymes for eliminating ROS. To date, SOD1 or SOD2 have not been well characterized in non-small cell lung cancer (NSCLC). Here we compared the expression of SOD proteins between normal and cancer tissues and investigated its prognostic significance in NSCLC patients. Method: we detected the expression of SOD1 and SOD2 in 178 NSCLC samples by immunohistochemistry and analyzed its clinical significances. Western blotting is used to confirm the protein level of SOD1 and SOD2 in the normal and cancer tissues of NSCLC patients. Results: The expression of SOD1 and SOD2 is significantly higher in both adenocarcinoma or squamous cell carcinoma (p<0.0001), compared to matched adjacent normal tissues. Correlation analysis indicates that SOD1 expression is reversely associated with TNM stage, and SOD2 is negatively correlated with tumor size, tumor number and TNM stage remarkably (all p < 0.05). Moreover, higher expression of SOD1 and SOD2 is correlated with better prognosis of NSCLC patients. Finally, Univariate and multivariate Cox regression modes further indicate that SOD1 and SOD2 can be used as independent prognostic factors in NSCLC patients. Conclusion: Higher expressions of SOD1 and SOD2 in NSCLC tissues are observed compared to adjacent tissues and indicate a subset of NSCLC harbors inactivating mutations or deletion of one allele of the IKZF1 tumor suppressor. These data suggest that IKZF1 tumor suppressor function as a regulator of transcription and a tumor suppressor in B cell acute lymphoblastic leukemia. However, the molecular mechanism of Ikaros tumor suppressor function in T-ALL is unclear. Using quantitative chromatin immunoprecipitation (qChIP), we determined that Ikaros binds to the promoter regions of the CDC2 and CDC7 cell cycle genes in primary T-ALL cells in vivo.

**#5542 Regulation of cell cycle control in T-cell acute lymphoblastic leukemia by Ikaros and Casein Kinase II.** Mario A. Soliman,1 Tommy Hu,1 Malika Kapadia,1 Elanora Dovat,1 Yali Ding,1 Chunhua Song,1 Jonathon L. Payne,1 Sinisa Dang,1,2 Penn State University College of Medicine, Hershey, PA;1 Loma Linda University School of Medicine, Loma Linda, CA.

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematological malignancy that represents a therapeutic challenge. Next-generation sequencing revealed that a subset of T-ALL harbors inactivating mutations or deletion of one allele of the IKZF1 tumor suppressor. These data suggest that IKZF1 acts as a tumor suppressor in T-ALL. The IKZF1 gene encodes the Ikaros protein that functions as a regulator of transcription and a tumor suppressor in B cell acute lymphoblastic leukemia. However, the molecular mechanism of Ikaros tumor suppressor function in T-ALL is unclear. Using quantitative chromatin immunoprecipitation (qChIP), we determined that Ikaros binds to the promoter regions of the CDC2 and CDC7 cell cycle genes in primary T-ALL cells in vivo. Gain-of-function experiments showed that Ikaros overexpression in T-ALL results in reduced expression of CDC2 and CDC7, as evidenced by quantitative RT-PCR (qRT-PCR) and Western blot. The knock-down of Ikaros with siRNA in T-ALL cells resulted in increased transcription of CDC2 and CDC7 as indicated by qRT-PCR. These data suggest that Ikaros can regulate cell cycle progression in T-ALL by repressing transcription of the CDC2 and CDC7 genes. Next, we studied the mechanisms that regulate Ikaros' ability to repress CDC2 and CDC7 in T-ALL. Ikaros function as a transcriptional repressor is regulated by Casein Kinase II (CK2). CK2 is overexpressed in hematopoietic malignancies and increased expression of CK2 results in T-ALL in murine models. We tested the effect of CK2 inhibition on Ikaros' ability to regulate transcription of CDC2 and CDC7 in human T-ALL. Molecular inhibition of CK2 with shRNA against the CK2 catalytic subunit resulted in reduced transcription of CDC2 and CDC7, as evidenced by qRT-PCR. This was associated with increased DNA-binding of Ikaros to promoters of CDC2 and CDC7, as shown by ChIP. These data suggest that CK2 impairs Ikaros' ability to transcriptionally repress CDC2 and CDC7 and to regulate cell cycle progression in T-ALL. Inhibition of CK2 enhances transcriptional repression of CDC2 and CDC7 by Ikaros, resulting in improved control of cell cycle progression in T-ALL. In conclusion, our results show that control of cell cycle progression in T-ALL occurs through Ikaros-mediated transcriptional regulation of CDC2 and CDC7. Overexpression of CK2 impairs Ikaros ability to repress CDC2 and CDC7 expression, which contributes to deregulation of cell cycle control in T-ALL. Results suggest a potential mechanism of therapeutic action of CK2 inhibitors for the treatment of T-ALL.

**BIOINFORMATICS AND SYSTEMS BIOLOGY: Systems Biology Approaches to Cancer Diagnostics and Disease Management**

**#5543 Network guided modeling allows tumor type independent prediction of sensitivity to retinoic acid.** Enrico Garattini, Marco Bolis, Maddalena Fratelli, Mineko Terao. Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy.

Background: All-trans retinoic acid (ATRA) is a differentiating agent used in the treatment of acute promyelocytic leukemia and it is under-exploited in other malignancies despite its low systemic toxicity. A rational/individualized use of ATRA requires development of predictive tools allowing identification of sensitive cancer types and responsive individuals. Materials and Methods: RNA-sequencing data for 10080 patients and 33 different tumor-types were derived from the TCGA and Leucene datasets and completely re-processed. The study was performed using machine learning methods and network analysis. Results: We modeled the large panel of breast-cancer cell-lines for in vitro sensitivity to ATRA and exploited the associated basal gene-expression data to initially generate a model predicting ATRA-sensitivity in this disease. Starting from these results and using a network-guided approach, we developed a generalized model (ATRA-21) whose validity extends to tumor-types other than breast cancer. ATRA-21 predictions correlate with experimentally determined sensitivity in a large panel of cell-lines representative of numerous tumor-types. In patients, ATRA-21 correctly identifies APL as the most sensitive acute-myelogenous-leukemia subtype and indicates that uveal-melanoma and low-grade glioma are top-ranking diseases as for average predicted responsiveness to ATRA. There is a consistent number of tumor-types for which higher ATRA-21 predictions are associated with better outcomes. Conclusions: In summary, we generated a tumor-type independent ATRA-sensitivity predictor which consists of a restricted number of genes and has the potential to be applied in the clinics. Identification of the tumor-types which are likely to be generally sensitive to the action of ATRA paves the way to the design of clinical studies in the context of these diseases. In addition, ATRA-21 may represent an important diagnostic tool for the selection of individual patients who may benefit from ATRA-based therapeutic strategies also in tumors characterized by lower average sensitivity.

**#5544 New technologies to probe the systems glycobiology of cancer.** Pau-line M. Rudd, Mark Hilliard, Mohankumar Muniyappa, Roisin O’Flaherty, Radka Sal'dova. National Institute for Bioprocessing Research and Training, Dublin, Ireland.

A robotic 384 well based platform was developed to release and label the human serum N-glycome. Improved separation of the glycan pool, based on hydrophilic interaction UPLC chromatography combined with mass spectrometry (Waters Corporation) and computer assisted data interpretation (NIBRT Glycobase) enabled us to build a database after assigning the detailed structures of more than 170 N-glycans. The aim of the project was to use these technologies and databases to link glycosylation changes in individual patients’ serum with features of cancer and with changes in a range of other –omics data acquired from the same patients. Alterations in glycosylation in various breast cancers were mapped to changes in the serum glycomes and aligned with genetic, transcriptomic and proteomic data. Pathway analysis showed strong associations between these glycan changes and the –omics data. This revealed that many of the glycan changes are directly associated with pathways involved in cancer metabolism. Our next aim was to demonstrate the feasibility of collecting personalised data from individual patients from each of the –omics analyses to build up a dataset of the changing glycosylation over time. This opens up the possibility of linking these data to explore pathways of disease and, in particular, nodal points where the patient can no longer compensate for the effects of an altered pathway that is leading to disease. To this end, single glycoepitopes from 8 controls and 26 ovarian cancer patients were sequentially purified from 5ul of serum on affinity plates and the released glycans were analysed. Rather than the conventional way of looking at markers which compare a patients’ data with averages, the control can now be the blood of the patient themselves taken at an earlier time point. The
#5546 Modeling of gastric cancer with lymphovascular invasion and H. pylori: a systems approach. Samuel Perez, Penn State Univ., Phoenix, AZ

Background: Behaviors of gastric cancer system with an H. Pylori co-morbidity allows clinician-educators to describe cancer metastasis through the lens of a systems approach. Lymphovascular invasion in gastric cancer has shown to have low survival rate within a five year period. The unresolved presence of H. Pylori in the gastric chamber allows for pervasive inflammation of the mucosal lining. Lymphovascular invasion supported with extracellular membrane (ECM) detachment and anoikis suppression exhibit elements of a systems approach such as inputs, processes, output and a feedback loop. Methods: Insightmaker.com is a simulation and modeling program used to create the stock and flow models. This system based approach allows a user to create primitives such as stock and variables and add connections using link and flow elements. A simulation run provides a visualization of the defined stock against a timeline on a dynamic graph. Results: A generalized metastasis model was first generated in insightmaker.com. An organ specific model was used to model how cancer cells interact with the gastric mucosal lining and its spread to the lymphovascular system. This organ specific model will help a clinician-educator represent chemical pathways and relationships to explain the secondary growth of tumors from its original site. Conclusion: The initial instructional activity was designed with an easy to follow demo of an actual working systems model in systems biology or population ecology. This allows for the content to be familiar to the student. A team of 4 students constructed a systems model of a portion of the gastric tumor metastasis cycle such as ECM detachment or suppression of the anoikis process. An evaluation rubric was used to guide the development of the group created student model with evaluation categories such as narrative of the system model, attributes of the system, data flow and values used and a personal reflection of their experience. The successful implementation of a system approach is packaging the instructional activity and its supporting content in bite size pieces, an incremental approach and explicit description of the activity’s deliverables. The Insightmaker.com model created for the embedded systems based instructional activity is ideal for lowering the learning curve for a systems thinking instructional activity and can fit in the time constraints of an oncology course block as part of a case based instruction session in an undergraduate medical curriculum.

#5547 A computational approach to predict tissue level cell cycle regulatory network for normal proliferating and cancer cells. Tao Sheng, Sha Cao, Chi Zhang, Ying Xu. Univ. of Georgia, Athens, GA

Cell cycle control and regulation has been widely studied in terms of cyclin-dependent kinases (CDKs) activities, while only limited information are available on tissue contexts and signaling. In this study, we adopted a systems biology approach aiming to comprehensively capture how tissue level signaling events orchestrate cell cycle phases in a CDKs activities independent manner in both of normal and tumor tissues. We have applied in our house gene co-expression and bi-clustering analysis on a collection of 8 single cell transcriptomic sequencing datasets of normal proliferating human embryonic cells, 17 RNA-seq transcriptomic datasets of inflammatory tissues and 30 microarray data sets of chronic inflammatory diseases as normal proliferating reference and TCGA RNA-seq data for 20 cancer types aiming to identify cell cycle associated gene co-expression modules. Our results suggest that the tissue level signaling events are generally very active on CDKs 11022

#5548 Genomic signatures for tumor-to-treatment stratification in pancreatic cancer. Olga H. Nikolova, Laura Heiser, Adam A. Margolin. Oregon Health and Science University, Portland, OR

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer deaths. PDAC is a broadly heterogeneous disease making it difficult to treat; each patient’s tumor is unique, but it can be classified into different subtypes, and identifying the right therapeutic strategy for each subtype is key to improving patient outcomes. Three sets of transcriptional signatures for PDAC subtype classification have been proposed in the literature; however, even when the same data from our laboratory cell line models indicates that they are associated with sensitivity for only a handful of compounds. Computational methods can identify effective individual therapies by robustly analyzing genomic-scale data from multiple experimental platforms to derive molecular classifiers predictive of drug response (drug signatures). Methods: We developed a novel Bayesian framework approach called Gene-wise prior Bayesian Factor Analyses (CBGFA) for predicting drug sensitivity in a given sample from genomic data. Our approach integrates data from different omic platforms by modeling the statistical dependencies of measurements made for the same gene via a model parameter called “gene prior”. We identify gene signatures for the clinically relevant to PDAC compounds olaparib and palbociclib, leveraging data from 44 pancreatic cell lines. Using hybrid capture mutation, gene expression, and copy number data, we applied our model to score genes for their ability to predict drug response in pancreatic cell lines and compiled the top predictors into drug signatures. Results: Our results across a pan-cancer cell line collection and >100 compounds show improved ability to recapitulate known biomarkers and drug targets compared to other state of the art methods. For the MEK1/2 inhibitor selumetinib, we successfully recapitulated BRAF mutation as the top-ranked biomarker of sensitivity. In addition, we identified four genes that carry additional information that can be used to stratify samples into sensitive or resistant groups: all were wild type in the sensitive cell lines and mutated in some of the resistant lines. We hypothesize that these genes could be used in conjunction with BRAF mutation status to refine the stratification to selumetinib therapy. We also identified several highly expressed genes in the resistant cell lines, and we hypothesize that these genes may mediate drug resistance. Similar analysis are currently ongoing for olaparib and palbociclib, comparing our findings to the subtypes currently proposed in the literature. Conclusions: Our integrated approach identifies drug-specific gene signatures in PDAC cell lines. We derived genomic signatures for two clinically relevant to PDAC compounds in the con-
text of the established in the literature PDAC subtypes, and hypothesize that these signatures could be used to identify patients most likely to respond to these therapies.

#5549 Novel network predictor for drug sensitivity in cell line response data. Ana Brandusa Pavel,1 Bin Li,2 Andrew Krueger.2.1Graduate Program in Bioinformatics, Boston University, Boston, MA; 2Takeda Oncology, Cambridge, MA.

Sequencing patient tumors has enabled the design of treatment regimens that exploit sensitizing genomic alterations. Associating drugs with mutations that enhance their effect is a key component of the American Society of Clinical Oncology vision for the next two decades of cancer treatment. Methods that distinguish drug sensitizing molecular events from the millions of extraneous alterations common within a tumor will help researchers implement precision medicine strategies. Here we use molecular data from the cancer cell line encyclopedia; including mutations, copy number alterations and gene expression changes along with drug response data, to identify subnetworks of interacting proteins that contain drug sensitizing alterations. Building upon the ability of fuzzy logic models to capture gene activity from different molecular data types, we create ‘Network-FLM’, a method to identify drug sensitizing molecular markers using a subnetwork model that distinguishes a drug sensitive sample from a drug insensitive one. Because the subnetwork model incorporates the sign and direction of network edges and the magnitude of gene activity changes, biologically meaningful features are captured. We integrate protein interaction information from Metacore database with somatic mutation, copy number and gene expression data, using the expression data as a guide. Combining the expression data and mutation data, we evaluate the mean AUC of the predictor for 24 anti-cancer compounds (Barretina et al., 2012). We build subnetwork classifiers for each compound using mutation, copy number and expression data separately and in all possible combinations. We find that the Network-FLM approach performs well for targeted agents such as Sorafenib, and also for drugs with pletropic mechanisms of action. Gene expression data alone creates effective predictive subnetworks for 8 compounds (Nilotinib, Sorafenib, Ibrutinib, PLX4720, Palbitaxel, Topotecan, TAE684, and Erlotinib). Adding copy number changes to gene expression data created better predictive networks for 8 compounds (PF2341066, AZD6244, L683458, RAF265, PD0325901, ZD6474, PHA665752, and 17-AAG). Likewise, three compounds benefit from adding mutation data to expression data (Panobinostat, PD0332991, and Lapatinib). Combining all three data types created the best predictors for AEW541 and AZD0530. For three compounds, mutation data alone and together with copy number alterations is the best input for building predictive models. We identified hyper-active subnetworks in cancer cell lines and used them to predict drug sensitivity. We plan to further explore the potential of these networks to improve patient response to anti-cancer drugs.

#5550 Collaborative analyses for delineating mutation variations among different ethnic patients of prostate cancer based on genomic data integration. Qingyu Xiao,1 Yidi Sun,1 Hong Li,1 Xiyue Li,1 Guo-Ping Zhao,1 Wendy Wang,2 Sudhir Srivastava,2 1CAS-MPG Partner Institute for Computational Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China; 2National Cancer Institute, National Institutes of Health, Bethesda, MD, USA.

Emerging data support the hypothesis that both inherited and somatic alterations of genomes of African American (AA) prostate cancer (CaP) patients are significantly different from that of Caucasian American (CA) men. Recent studies from our and other groups have established that frequencies of the most common genomic alteration, TMPRSS2-ERG fusion, which apparently causes the high expression of ERG oncoprotein, is markedly lower in AA than in CA patients. Some works indicated that the frequency of CaP ERG fusion is the lowest in Asian patients, but this frequency changed from 11% to 78% in different Asian cohorts. Further analysis in Asian men is necessary. In order to systematically and comprehensively address this and related problems, Uniformed Services University of the Health Sciences-Center for Prostate Disease Research (USU-CDR), TCGA and the Chinese Prostate Cancer Consortium (CPCC), specially focusing on somatic alterations (ERG and others), microbiome, mitochondrial sequences and microRNAs. We integrated gene fusion, copy number, and gene expression data to detect potential driver genes and, as anticipated ERG alteration was one of the most commonly detected. The frequency of ERG fusion is the highest for CA, intermediate for AA and the lowest for Asian men, while its expression is significantly higher in ERG-fusion tumors than other tumors cross over all of the three ethnic groups. We did find some genes altered more frequently in Asian patients than in American patients, subject to further validations. We explored the methodology for microbiota profiling using prostate cancer WGS sequencing data. We inferred the metabolic pipeline from prostate tumor tissues and the corresponding blood samples with high confidence but inadequate sensitivity likely due to the low availability of both microbial sequences and bacterial reference genomes. Our preliminary analyses highlight the potential of international collaboration. CPDR-PICB-NCI will work on a seamless approach for data integration, particularly of the raw data to truly enhance CaP diagnosis and treatment by providing more informative biomarker and therapy targets relevant to ethnically diverse patient populations.


Non-coding genes are shown to play important roles in cancer progression with potential for serving as theranostic biomarkers. However, there is still a lack of studies to predict the biological roles of novel non-coding genes in tumorigenic pathways and associations with patient survival. Lung adenocarcinoma (LUAD) is the predominant histological subtype of lung cancer, which is the leading cause of cancer death. Nowadays, target therapies have improved LUAD patients’ survival about 6-12 months, but limited improvement of long-term survival. Suggesting new therapeutic targets are important. Therefore, here we applied weighted correlation network analysis (WGCNA) to cluster highly correlated genes of transcriptomic data to accurately investigate the functions of non-coding genes with poor prognosis in lung adenocarcinoma (LUAD). We discovered a total of 627 differential expressed coding and non-coding genes (DEGs) from 6 transcriptomic datasets derived from LUAD patients with survival information. By using WGCNA, we discovered 6 non-coding candidates (PTTG3P, MIR497HG, HSP078, TBX5-AS1, LOC100506990, and C14orf64) and their core networks of DEGs, which were highly associated with patient survival and clustered in modules functioning in cell cycle and migration. We noticed that a previously known processed pseudogene, PTTG3P (Pituitary Tumor-transforming Gene 3), is highly correlated with poor survival and associated with mitosis of the cell cycle. Up-regulated PTTG3P expression in LUAD was in silico validated of RNA-seq data from TCGA compared to normal lung tissues (fold-change = 2, p-value < 0.0001) and associated with poor 5-year survival rates (HR= 1.83, p-value = 0.012). We further confirmed PTTG3P is highly expressed and associated with poor survival outcome of LUAD patients in Taiwan (HR= 1.75, p-value = 0.037) with RNA-ISH experiment. Together the coding potential of pseudogene PTTG3P, we found that PTTG3P might encode a consensus 21 kDa novel polypeptide from a single exon by using various prediction programs of coding potential. Ectopic expression of PTTG3P in lung cancer cells indicated that PTTG3P protein can be detected resulting in shortening the process of metaphase to anaphase in cell cycle progression and promotion of cell proliferation. Knockdown experiments of PTTG3P further reversed aforementioned experiments. Together we established a powerful and systematic strategy for functional classification of non-coding genes in association with poor prognosis of LUAD patients. We revealed that commonly annotated and processed pseudogenes defined as non-coding genes for lacking of introns and promoter regions could still be translated into functional polypeptides. We found processed pseudogene PTTG3P can be translated to a protein, which might play an important role in predicted function of mitosis and impact on poor survival in LUAD patients.

#5552 Differential proteomic responses of luminal-A and basal-like breast cancer cell lines during growth inhibition induced by co-culture with agarse encapsulated murine renal adenocarcinoma (RENCA) cells. Melissa A. Laramore,1 Peter James,2 Frithy C. Martis,1 Atira Dudley,1 Lawrence S. Garda,3 Carl A. Borrebaeck,2 Barry H. Smith3.1The Rosogen Institute - Xenia Division, Xenia, OH; 2Lund University, Lund, Sweden; 3The Rosogen Institute, New York, NY.

The existence of a tumor growth regulatory network that is conserved across tumor types and species has been hypothesized. Our evidence for this regulatory network is derived from studies demonstrating tumor growth inhibition by agarse encapsulated cancer cells (cancer macrobeads). The ability of encapsulated, thus growth restricted cells, to inhibit freely growing cancer cells has been shown when the encapsulated cells are murine (RENCA) or human (e.g., M2b). Clinical trials are underway (NCT01053013, NCT02046174). We have shown that RENCA macrobeads release >10 known tumor inhibitory proteins target-
ing several signaling pathways including Akt/Pi3. To better understand the mechanism(s) of growth inhibition, we used a systems biology approach to identify protein profiles and interactions from macrobead-treated human breast carcinoma cell lines that are either mildly aggressive (MCF7) or highly aggressive (MDA-MB231 [MDA]). Target cells were co-cultured with RENCA macrobeads (5 μm) and incubated for 5 days. Lysates were run in Laemmli buffer, frozen, and sent for MS/MS. Samples were run in duplicate, protein intensities normalized, and the treated/untreated ratio log10-transformed to get a normal distribution. Protein profiles were analyzed using Key Pathway Analysis (KPA) and Metacore software. Growth inhibition by RENCA macrobeads was confirmed (MCF7 25%; MDA 57%). KPA of MCF7 proteins showed upregulation of a large number of pathways involved in aggregations of unfolded proteins. Stress induced apoptosis and DNA damage (which correlates with the growth inhibition) were the top 2 pathways upregulated using Pathway and Process Enrichment analysis. This is in line with a strong epigenetic gene deregulation (Metacore). Transcription Factor and Network analyses show up-regulation of CREB, A2MR and ATF3 networks. Also, Regulated Network analysis suggests a role for A2MR or RAGE in the inhibition of proliferation and increased apoptosis. Macrobead-treated MDA cells showed no significant changes using KPA. Pathway, Network and Process enrichment (Metacore) showed 5 of the top 10 upregulated processes were associated with cytoskeleton remodeling. Cell cycle and protein folding processes were also upregulated. Transcription factors associated with this response were similar to that of MCF7, RAGE was not upregulated in any of the pathways indicated by Network or Regulated Network analysis. The protein response to RENCA macrobeads differs for these 2 cell lines. Proteins related to apoptosis are upregulated in the MCF7 cells whereas MDA have a preference for cytoskeleton remodeling. These data support the hypothesis that distinctive tumors may respond differently to RENCA macrobead exposure at both an epigenetic and protein level, nonetheless resulting in growth inhibition.

#5553 A computational model for integrating genomic data with public datasets for molecular tumor board recommendations. R Joseph Bender,1 Edik Blais,1 Apovora Kulkarni,1 Michael J. Pishvaian,2 David Halverson,1 Jonathan R. Brody,2 Emanuel Petricoin,3 Subha Madhavan4,5 Perthera, Inc. McLean, VA;2 Georgetown University Lombardi Comprehensive Cancer Center, Washington, DC;2 Thomas Jefferson University, Philadelphia, PA.

Recent genomic profiling studies in pancreatic adenocarcinoma (PDA) have revealed actionable mutations affecting multiple signaling pathways, but in spite of these mutations, targeted inhibitors of these pathways have low success rates. A possible reason for these failures is that single-gene biomarkers (e.g., a KRAS mutation as an indicator of MEK inhibitor sensitivity) fail to account for cross-talk within and between dysregulated pathways. We have previously curated a knowledgebase of published studies as evidence to support molecular tumor board recommendations to cancer patients after multi-omic profiling. Here we present a computational framework for integrating this knowledgebase with drug response data from cancer cell lines to propose “actionable” biomarkers based on a panel of pathways instead of targeting a single gene mutation. We constructed a computational model encompassing a broad range of cancer-related pathways, including RAS/RAF/MEK/ERK, PI3K/PI2/AKT, cell cycle regulation, and DNA repair. The model consisted of a set of ordinary differential equations (ODE) with protein interactions following Hill-type kinetics and the rate of cell division and apoptosis modeled dependent on key signaling nodes, including the level of phosphorylated ERK and AKT. We integrated two sources of publicly available data: 1) published studies correlating phosphoprotein measurements and resistance pathways to targeted inhibitors in clinical development; and 2) mutation data correlated with drug-specific response metrics (e.g., IC50 values), such as CCLE and NCI-60. We systematically screened frequently observed overlapping disrupted signaling pathways (i.e., combinations of mutations) by simulating predicted IC50 values for targeted inhibitors. Based on these simulations, we found that the drug combinations involving more drugs may be best suited for inhibiting tumor growth when tumors harbor multiple mutations. We present two applications of this computational approach: a comparison of CDK4/6 inhibition in CDK2A2-mutated PDA vs. hormone receptor-positive breast cancer and a comparison of PARP inhibition in BRCA1/2-mutated PDA and ovarian cancer. The predictions generated by our simulations were consistent with clinical observations in that fewer combinations of mutations in PDA were sensitive to these inhibitors than in breast and ovarian cancer, suggesting ways to refine biomarkers for sensitivity to these drugs in PDA. The computational model presented here takes into account multiple datasets from a knowledgebase to provide a prioritized list of treatments that match a patient’s molecular profile while also providing the rationale for the recommendation. This represents a step toward incorporation of systems biology in precision oncology.


Lung cancers are among the most common invasive cancers worldwide and annually lead to high mortality and morbidity. Genomic alterations have been known to control the evolution of hallmarks of cancer in a dynamic way. These molecular alterations combined with epigenomic and post-genomic modifications contribute to formation of these neoplasms. Multiplicity of these changes has made development of personalized therapeutic regimens for these cancers a complex problem. Metabolic reprogramming is one of the main mechanisms in progression of cancers. There have been efforts to model the metabolic reprogramming in cancer using metabolic networks of cancer cells, but there has been no computational framework to model these metabolic transitions in cancer for precision and personalized medicine. We have combined computational, mathematical and experimental methodologies to develop a platform for precision oncology in non-small cell lung cancer (NSCLC) by in silico models of metabolic switches. Our integrative analysis of genomic data from NSCLC has led to discovery of genomic signatures controlling metabolic reprogramming in NSCLC with KRAS mutations. This discovery was proved in vivo and in vitro using drugs blocking different metabolic pathways. We have shown that NSCLC cells which do not carry KRAS mutations and have these genomic signatures are addicted to the pentose phosphate pathway (PPP). We have verified and proved the predictive value of these genomic signatures using Patient Derived Xenograft (PDX) tumor models of NSCLC. We are developing a mathematical and computational framework to model these metabolic switches. Our platform is capable of using genomic data from a cell line or tumor to determine the metabolic dependency of them quantitively and predict the optimized personalized treatments for modulating metabolic pathways aiming to control cancer progression.

#5555 Integrative network varioomics reveals complex genotype to phenotype relationships in cancer. Nidhi Sahni, Song Yi. UT MD Anderson Cancer Ctr., Houston, TX.

Proteins interact with other macromolecules in complex cellular networks for signal transduction and biological functions. Recently a deluge of genomic information becomes available from patients stricken by a variety of cancer types. In cancer, genetic aberrations have been traditionally thought to abolish the entire gene function. It has been increasing appreciated that each genomic mutation of a gene could have a subtle but unique effect on protein function or network rewiring, contributing to diverse phenotypic consequences across cancer patient populations. In this study, we designed an integrative multi-omics platform using systems biology approaches to functionally assess the effects of cancer genetic variants. We compared a broad spectrum of mutation classes for a wide range of mechanistic effects on gene function in the context of signaling networks. Our results reveal a functional landscape of context-specific signaling network perturbations underlying specific genetic and phenotypic variations. Such information is critical for understanding the complex pleiotropic effect of cancer genes, and it provides a possible link between genotype and phenotype in cancer. Furthermore, this study suggests new perspectives in targeted therapy and precision medicine.

#5556 Widespread protein interactome network rewiring in human cancer. Song Yi, Nidhi Sahni. UT MD Anderson Cancer Ctr., Houston, TX.

In the past decade, genome and exome sequencing projects have identified thousands of genetic variants in patients across a large number of cancer types. However, the explosion of genomic information has left many fundamental questions regarding genotype-phenotype relationships unresolved. One critical challenge is to distinguish causal disease mutations from non-pathogenic polymorphisms. Even when causal mutations are identified, the functional consequence of such mutations is often elusive. Classical “one gene, one function, one disease” models can not reconcile with the complexity that different mutations of the same gene often lead to different phenotypes. The extent to which network perturbations are involved in disease malfunction and how distinct interaction perturbation patterns can distinguish cancer mutations are largely unknown. Here we report a systematic approach to investigate genetic variant-specific effects on molecular interactions at large scale across diverse human cancers. Remarkably, in comparison to non-disease polymorphisms, disease mutations are more likely to associate with interaction perturbations. A large fraction of missense disease mutations are found to cause protein interaction alterations.
remodeling during metastases development, defining patterns of extracellular-matrix associated genes and proteins that predicted both extent of disease and tissue modulus. This allowed us to identify a core group of twenty-two matrix-associated molecules that modeled the dynamic process of tissue remodeling during tumor progression. We used these data to generate a ‘matrix index’, a quantitative measure of the mean expression of the twenty-two molecules. In cancer transcriptomic databases, this matrix index had prognostic significance in thirteen solid cancers including high-grade serous ovarian cancer, even after multivariate analysis. We conclude that there may be a common host matrix response to human solid cancers.

#5559 Using cancer dependency data to discover tumor suppressive and oncogenic functional modules. Joshua Pani,1 Robin M. Meyers,2 Brittany C. Michel,3 Ann E. Sizemore,2 Francisca Vazquez,2 Barbara A. Weir,2 William C. Hahn,1 Aviad Tsherniak,2 Cigall Kadoch1.1 Dana-Farber Cancer Institute, Boston, MA;2 Broad Institute, Boston, MA.

Efforts to define protein complexes and their functional networks are critical for systems-level understanding of the pathways involved in human cancer. Current methods to catalog human protein complexes via physical interaction are often unable to resolve functional differences between complex members or infer relationships governed by sub-stoichiometric interactions. While functional wiring maps in yeast have been generated by measuring epistatic interactions between pairs of genes, efforts to scale this concept in individual human cell lines have been met with challenges and have only been able to characterize limited numbers of genes at a time. We have developed a scalable approach that can measure functional similarity without the constraints of pairwise genetic interaction experiments. Using data from genome-wide RNAi and CRISPR dropout screens performed in hundreds of cancer cell lines, we leveraged the heterogeneity of gene dependencies across cancer types to measure functional similarity between thousands of genes at once, which in turn allowed us to recreate known inter- and intra-complex functional relationships and to uncover tumor suppressive and oncogenic functional modules in cancer-relevant pathways such as proteolysis, metabolism and transcription. Applying these approaches to the mammalian SWI/SNF (BAF) chromatin remodeling complex, which is mutated in over 20% of human cancer, revealed three functional modules that arose separately during metazoan evolution, one of which is entirely novel and uncharacterized. We then performed biochemical experiments that fully support three specialized complex configurations, each with distinct size, subunit composition, and function. These data reorganize the BAF complex into previously unrecognized modules that better explain mutational burden in human cancer. Notably, we observe that all known BAF-driven, highly penetrant rare cancers and neurodevelopmental disorders involve disruption within a single functional module we defined, underscoring the value of evaluating disease genomics through the lens of functional modularity.

#5560 Systemic map of protein phosphatase 2A (PP2A)-regulated phosphotargets and drug responses in cancer cells. Otto Kauko,1 Susumu Imanishi,1 Evgeny Kuleskij,1 Teemu D. Laajala,1 Laxmana Yetukuri,1 Artur Padzik,1 Mikael Jumppanen,1 Pekka Haapaniemij,1 Bhagwan Yadaw,1 Veronika Suni,1 Taru Varilas,1 Garry Corthals,1 Wennerberg Kristers,1 Tero Aittokallio,1 Jukka Westermarck1,1 University of Turku, Turku, Finland; 2Meijo University, Nagoya, Japan; 3University of Helsinki, Helsinki, Finland.

Despite the pivotal role of phosphatases in cancer cell signalling, systemic understanding of phosphatase targets is still at infancy. Protein phosphatase 2A (PP2A) is a human tumor suppressor complex. PP2A inhibition is a requirement for human cell transformation and PP2A regulates many cancer critical signalling pathways. Importantly, emerging data indicates that reactivation of PP2A tumor suppressor activity could provide entirely novel approach for cancer therapy. Here, we present first systemic analysis of phosphoprotein targets (dephosphorylated by PP2A) regulated by PP2A in cancer cells. Based on data, PP2A regulates cancer critical signalling pathways, including entire EGFR-RAS-RAF-MEK-ERK cascade, and functions as a master regulator of MYC function. At network level, PP2A targets critical cellular processes such as chromosome organization, RNA splicing, and nuclear envelope assembly. Surprisingly, soft clustering of PP2A dephosphorylated revealed that most phospho-target residues are subject to only unidirectional regulation in cancer cells. Moreover, targets show intracellular gradient where phosphatase inhibition dominates nuclear phosphorylation balance. Since phosphoregulation is critical for cancer drug responses, dephosphorylated was correlated with cancer cell responses to over 300 drugs. Importantly, cancer therapies could be broadly classified based on their dephosphorylome, both at quantitative and qualitative manner. Finally, we demonstrate the utility of this large dataset by validating the role of PP2A in MEK inhibitor resistance in KRAS mutant cancer cells via regulation of RAF,
Adaptation and fractional response of tumor cells to targeted inhibitors of oncogenic pathways allows a population of viable tumor cells from which fully resistant clones may arise. Thus, understanding transient drug adaptation is key for both improving the effectiveness of treatment and delaying/controlling acquired resistance. Despite the wealth of information available about feedback mechanisms associated with adaptive resistance, most of our knowledge in this area comes from studying drug response in bulk tumor cell populations. Furthermore, the phenotypic consequences of drug adaptation have been often studied at a few fixed time-points, when drug-adapted cells exhibit a high population-average activity in multiple pro-growth signaling cascades. It therefore remains unclear how the initial responses to drug relate to subsequent phenotypes such as cell death or adaptation. This is likely a key point for designing novel approaches to overcome fractional drug response in tumor cells and to achieve durable therapy. We use real-time live-cell imaging, single-cell analysis and molecular profiling to show that exposure of BRAFV600E melanoma cells to RAF/MEK inhibitors elicits a time-variable and heterogeneous response in which some cells die, some arrest and the remainder adapt to drug. Drug-adapted cells up-regulate markers of the neural crest (e.g. NGFR), a melanoma precursor, and grow slowly. The drug-induced slow growth/G0/GRHigh state is only transiently stable, reverting to the drug-naive state within two weeks of drug withdrawal as measured by the restoration of RAF/MEK inhibitor sensitivity, accelerated rate of cell division and reduced expression of NGFR. Transcriptional and biochemical profiling of cell lines and human tumors implicates a role for the c-Jun/ECM/FAK/Src cascade in driving the de-differentiation response. We identify multiple drugs targeting this cascade as well as BET bromodomain inhibitors that block this resistance program in cell lines and in a BRAFV600E melanoma xenograft model and increase sensitivity and maximal effect (E_{max}) of RAF/MEK inhibitors. Our study reveals directly how drug adaptation happens in individual tumor cells leading to emergence of heterogeneous cell sub-populations with reduced drug-sensitivity that may be targeted by drug combinations.


Harvard Medical School, Boston, MA; Dana Farber Cancer Institute, Boston, MA.

Adaptation and fractional response of tumor cells to targeted inhibitors of oncogenic pathways allows a population of viable tumor cells from which fully resistant clones may ultimately arise. Thus, understanding transient drug adaptation is key for both improving the effectiveness of treatment and delaying/controlling acquired resistance. Despite the wealth of information available about feedback mechanisms associated with adaptive resistance, most of our knowledge in this area comes from studying drug response in bulk tumor cell populations. Furthermore, the phenotypic consequences of drug adaptation have been often studied at a few fixed time-points, when drug-adapted cells exhibit a high population-average activity in multiple pro-growth signaling cascades. It therefore remains unclear how the initial responses to drug relate to subsequent phenotypes such as cell death or adaptation. This is likely a key point for designing novel approaches to overcome fractional drug response in tumor cells and to achieve durable therapy. We use real-time live-cell imaging, single-cell analysis and molecular profiling to show that exposure of BRAFV600E melanoma cells to RAF/MEK inhibitors elicits a time-variable and heterogeneous response in which some cells die, some arrest and the remainder adapt to drug. Drug-adapted cells up-regulate markers of the neural crest (e.g. NGFR), a melanoma precursor, and grow slowly. The drug-induced slow growth/G0/GRHigh state is only transiently stable, reverting to the drug-naive state within two weeks of drug withdrawal as measured by the restoration of RAF/MEK inhibitor sensitivity, accelerated rate of cell division and reduced expression of NGFR. Transcriptional and biochemical profiling of cell lines and human tumors implicates a role for the c-Jun/ECM/FAK/Src cascade in driving the de-differentiation response. We identify multiple drugs targeting this cascade as well as BET bromodomain inhibitors that block this resistance program in cell lines and in a BRAFV600E melanoma xenograft model and increase sensitivity and maximal effect (E_{max}) of RAF/MEK inhibitors. Our study reveals directly how drug adaptation happens in individual tumor cells leading to emergence of heterogeneous cell sub-populations with reduced drug-sensitivity that may be targeted by drug combinations.


Moffitt Cancer Center, Tampa, FL; University of Illinois at Chicago, Chicago, IL.

Background: Prior studies have investigated “intermittent therapy” to delay hormone resistance in prostate cancer but did not explicitly include quantitative analysis of the evolutionary dynamics. We hypothesize that evolution-informed strategies may prolong time to progression but require a multidisciplinary effort to simulate intratumoral Darwinian dynamics and design a clinically feasible treatment protocol. Methods: We investigate intratumoral evolutionary dynamics during treatment of metastatic castrate resistant prostate cancer (mCRPC) with abiraterone, which blocks CYP17A1 autostimulation of testosterone from androgen precursors. We build a mathematical model assuming three competing phenotypes: (i) TP cells express CYP17A1 and produce testosterone; (ii) T+ cells require exogenous androgen; and (iii) T- cells are androgen-independent. Model predictions were tested in a pilot clinical trial. Results: Mathematical Model: Computer simulations demonstrate continuous maximum dose abiraterone treatment produces competitive release of resistant T- cells. However, limited treatment designed to maintain residual TP and T+ populations and suppress proliferation of T- cells was predicted to prolong response while lowering the required drug dose. Clinical Trial: In a pilot clinical trial, 11 men with asymptomatic mCRPC were treated with abiraterone according to an evolution-informed, patient-specific algorithm based on the modeling results. Pre-treatment biopsies (available for 3 patients) demonstrated the predicted tumor subpopulations. Cycles of response and regrowth similar to model simulations were observed with cycle lengths varying from 3 months to > 1 year. Over a median follow-up period of 18 months, 10 subjects remained responsive to Abiraterone without PSA or radiographic progression. Median time to PSA progression significantly (P<0.001) exceeds the 11.1 months historical control. As predicted by the simulations, tumor control required significantly less abiraterone with average cumulative dose less than 40% of standard of care (P<0.001). Conclusion: Integration of mathematical models and Darwinian first principles into trial design, may allow patient-specific modifications to prolong response while lowering drug doses in mCRPC.


UT MD Anderson Cancer Center, Houston, TX; Nanostring Technologies, Inc, Seattle, WA.

Prognosis is favorable in patients with primary localized melanoma but poor in patients with metastatic disease. With more than 76,000 cases expected to be diagnosed in 2016, more precise prognostic technologies and new therapies are needed. Although targeted treatment regimens have been approved in recent years, resistance has emerged in large part due to adaptive response mechanisms and intratumoral heterogeneity. Analysis of tumor samples across multiple molecular platforms will help elucidate the complexities within and across tumors which may underlie response to therapy as well as assist in identifying predictive biomarkers; however, these approaches require significant amounts of sample, time, and resources. In order to integrate the strengths of analyzing different molecular analytes, we have modularized Nanostring Technologies’ molecular barcoding technology to perform quantitative digital measurement of DNA associated mutation variant markers, mRNA expression, and protein expression in one assay from the same sample (3D Biology). Novel nucleotide variant probes enable sensitive and specific identification of DNA mutant allele sequences down to a level of detection of ≤ 5% from 5 ng of FFPE-extracted genomic DNA. Gene expression is measured via unique digital barcoding technology to measure mRNA transcripts, and protein expression and activity (via phosphorylation) is measured by DNA-labeled antibodies. The multi-omic workflow requires only two 5-10 micron sections of FFPE tissue, whereby DNA and RNA are extracted from one section and multiplex digital protein profiling is conducted on the second. As proof of concept demonstrating the utility of this 3D Biology platform, we have simultaneously analyzed DNA variants, RNA expression, and protein expression using NanoString’s nCounter Vantage 3D+ Solid Tumor Panel on 12 FFPE melanoma tumor samples and one normal tissue from six patients. This sample set included two metastatic tumors from each patient, and in one instance multiple regions from each metastatic site (7 and 2 regions) in order to assess intratumoral heterogeneity. Importantly, this sample set has associated mRNA expression measured using the nCounter PanCancer Pathway Panel on whole human panel for a whole exome sequencing (WES) data. Somatic variants seen in this latter dataset were compared with the results from the DNA SNP Solid Tumor Panel. Samples with variants that were detected in the nCounter assay but not WES were subjected to deep sequencing for validation. Overall, we show that this multiplex and multi-omic platform has the potential for rapid and sensitive assessment of patient samples that will impact clinical care.


Bar Ilan Univ., Zfat, Israel; 2Baker IDI Heart & Diabetes Institute, Melbourne, Australia; 3Chai, Petchaj Taijua, Israel.

The evolutionary theory assumes that occurrence of mutations in cancer is random. However, recent studies suggest that passenger mutations are not randomly scattered in cancer genomes and that chromatin organization dictate mutations profiles. Hepatocellular carcinoma (HCC) serves as a model of a diverse spectrum of cancers, since it is induced by a number of well-known etiological agents, mainly Hepatitis C virus (HCV) and Hepatitis B virus (HBV). There are two distinct etiology-dependent regional mutations signature in specific genes and pathways in HCC that was not detected previously by exome sequencing of liver tumors. These observations suggest that viral infection modulate somatic mutations causing HCC. To explore the link between genomic signature and genome wide chromatin organization we studied the epigenetic

changes occur following HCV infection. Epigenetic analysis unraveled known and novel pathways that are controlled by the virus such as Hepatic lipid metabolism, cell motility, cell cycle and immunity. Invers correlation between high mutation rate and enrichment of chromatin modifications associated with active transcription provides a link between etiology and cancer genome. Our novel approach offers a perspective into the mechanisms that shape mutational signature development in cancer.

#5565 Multi-omic profiling of prostate cancer evolution in 39 patients. Qing Zhong,1 Tiannan Guo,2 Nora Toussaint,2 Ulrich Wagner,1 Konstantina Charmp1, Laurence Calzone,3 Andreas Beyer,2 Ruedi Aebersold,2 Peter J. Wild1. 1University Hospital Zurich, Zurich, Switzerland; 2ETH Zurich, Zurich, Switzerland; 3University of Cologne, Cologne, Germany; 4Institut Curie, Paris, France.

The pathological molecular events occurring during prostate cancer (PCA) progression have not been comprehensively investigated. It remains elusive how genomic aberration is transcribed into transcriptomic abnormalities that eventually influence proteins in the progression of PCA. We aimed to study the biological information flow along the central dogma and to identify critical molecular mechanisms in PCA evolution. We performed comprehensive, multi-omics analyses of 105 prostate samples, consisting of both benign prostatic hyperplasia regions and malignant tumors from 39 PCA patients. Patients were from different risk groups, including 12 low-grade (Gleason score < 7), 17 intermediate (Gleason score = 7), and 10 high-grade (Gleason score > 7) PCs.

The omics data comprised exome sequencing, copy number variation (CNV) analysis, RNAseq, quantitative proteomics, and tissue morphology. Furthermore, peripheral blood samples from each patient were genomically profiled as reference. We identified 860 somatic gene mutations from the 105 tissue samples, out of which 18 genes were mutated in three samples and 129 genes were mutated in two samples. More mutations were detected in patients with higher Gleason scores. We also found the copy numbers of 914 genes to be substantially up- or down-regulated in at least one sample. For RNAseq, we determined genes with either strictly monotonically increasing or decreasing expression from normal to intermediate and high-grade PCA per patient and per patient group, based on the regularized log-transformed read counts. Moreover, we collected tissue punches and analyzed each punch in technical duplicates by PCT-SWATH and used OpenSWATH software for data interpretation. We obtained precise quantification for several thousand proteins. The differential analysis revealed increasing and decreasing patterns of proteins of interest. Furthermore, we used network smoothing to determine overlapping molecular aberrations by comparing shortlisted DNA-mRNA-protein pathways. By integrating proteogenomics with additional omic information, we demonstrated that molecular changes in PCs at genomic level are buffered at transcriptional level, and further constrained to a few modular changes in protein networks. We also found high correlation between pathway activities with tumor progression and heterogeneity. Our multi-omic profiling further revealed PCA evolution by using molecular alterations at different layers with associated mutant allele frequencies and uncovered several protein modules as key events in the progression of prostate cancer.

#5566 African American esophageal squamous cell carcinoma expression profile reveals dysregulation of stress response and detox networks. Hayriye Verda Erkizan,1 Jack Lichy,1 Vincente Notario,2 Robert Wadleleigh2. 1Institution for Clinical Research, Veterans Affairs Medical Center DC, Washington, DC; 2Veterans Affairs Medical Center DC, Washington, DC.

Background: Esophageal squamous cell carcinoma is the third most common gastrointestinal malignancy worldwide and is largely unresponsive to therapy. African-Americans have an increased risk for esophageal squamous cell carcinoma (ESCC), this subtype that shows a marked variation in geographic incidence. So far, the molecular architecture of African-American ESCC is still poorly understood. It is unclear why African-American ESCC is more aggressive and the survival rate of these patients worse than those of other ethnic groups. Our aim is to investigate the central question of whether there is a genetic basis for the aggressive nature of ESCC among African-Americans through analysis of genetic overlaps and differences between African-American ESCC and reported Asian data. Methods: We conducted microarray expression profiling in pairs of esophageal squamous cell tumors and matched controls to define genetic alterations that occur in African American ESCC. We then utilized Ingenuity Pathway Analysis (IPA) to analyze the differentially expressed genes in African-American ESCC. We conducted a meta-analysis of differentially expressed genes of African American ESCC and those of Asian ESCC through IPA. Results: Transcriptome profiling of African-American ESCC tumors versus adja-cent normal esophageal tissues revealed significant differential expression of 756 genes. Among the most strongly up-regulated genes were keratin 17, immunoglobulin genes and ornithine decarboxylase 1. Genes that showed a great loss of expression included cysteine-rich secretory protein 3 and scelien. We performed pathway and network analysis on significantly dysregulated genes using IPA to understand the overall biological impact of the widespread transcriptional aberration in African-American ESCC. We found significant dysregulation of genes encoding drug-metabolizing enzymes and stress response components of the NRF2-mediated oxidative damage pathway, potentially representing key genes in carcinogenesis in ESCC in African Americans. Loss of activity of drug metabolizing enzymes would confer increased sensitivity of esophageal cells to xenobiotics, such as alcohol and tobacco smoke, and may account for the high incidence and aggressiveness of ESCC in this ethnic group. A meta-analysis of ESCC expression profiles in our African American sample and those of several Asian samples determined genes in the NRF2 pathway are uniquely and significantly altered in African-American ESCC. Down-regulation of TP53 pathway components represented the most common feature in ESCC of all ethnic groups. Importantly, this analysis revealed a potential distinctive molecular property of African-American ESCC a widespread and prominent involvement of NRF2 pathway. Conclusion: Taken together, these findings highlight the remarkable interplay of genetic and environmental factors in the pathogenesis of African-American ESCC.

#5567 A scalable and integrated computational and experimental workflow to identify new driver genes in cancer genome data. Heiko Horn,1 Michael S. Lawrie,2 Candace E. Housman,1 Yashavi Shrestha,2 Jessic Xin,2 Hu,1 Elizabeth Worstell,2 Emily Shea,2 Nina Illic,2 Eunjung Kim,4 Atanas Kamburov,1 Alireza Kashani,1 William C. Hahn,3 Alireza Kashani,1 William C. Hahn,3 Jesse S. Boehm,2 Gad Getz,2 Kasper Lage3. 1National Cancer Institute, Bethesda, MD; 2Broad Institute of Harvard and MIT, Cambridge, MA; 3University of Copenha-gen, Copenhagen, Denmark; 4Dana-Farber Cancer Institute, Boston, MA.

High throughput sequencing has revolutionized the study of the cancer genome, enabling numerous discoveries in basic and clinical research. However, considerable sample sizes are required to find cancer driver genes with intermediate and low mutation frequencies, and for a large proportion of patients the molecular cause (e.g. driver gene(s)) of disease is unknown. Here, we describe an integrated computational and experimental workflow that combines cancer genome data, molecular network information, multiplexed in vivo tumorigenesis assays, and reanalysis of driver-gene-negative cancer patients to predict and validate new driver genes. We develop a statistic, network mutation burden, that combines molecular network information with data from 4,742 cancer genomes to accurately classify known driver genes across 21 tumor types and predict 62 driver gene candidates. Of these, 35 gene candidates were tested in multiplexed in vivo tumorigenesis cell assays using sensitized immortalized human embryonic kidney (H1A1-M) and immortalized human lung epithelial (SALE-Y) cell lines. Tumor formation in vivo was observed for 11 genes (2 in HA1-E-M, 3 in SALE-Y, 6 in both). By reanalyzing 242 lung adenocarcinoma patients with an unknown molecular cause of disease we show that two of these candidates, TFF2 and AKT2, are significantly upregulated in metastatic samples. Overall, we describe a scalable combined computational and experimental framework to predict and validate driver genes across many tumor types. Our proof-of-concept approach should become increasingly useful as the number of cancer genomes continues to grow.

#5568 Towards decoding the interplay between glycolysis and oxidative phosphorylation in cancer. Dongyu Jia,1 Linglin Yu,1 Mingyang Lu,1 Eshel Ben-Jacob1, Jianpeng Ma,1 Herbert Levine1, Benny A. Kaipparettu2, Jose Onuchic1. 1Rice University, Houston, TX; 2Baylor College of Medicine, Houston, TX.

Abnormal metabolism is a hallmark of cancer, yet its regulatory mechanism is poorly understood. Cancer cells were considered to mostly utilize glycolysis, referred to as the Warburg effect. Recent data suggested that aerobic glycolysis, oxidative phosphorylation also plays a crucial role during cancer progression. Here we utilized a systems biology approach to decipher the regulatory principle of glycolysis and oxidative phosphorylation. Integrating information from literature, we constructed a regulatory network of genes and metabolites, from which we extracted a core circuit containing HIF-1, AMPK and ROS. Our circuit analysis showed that while normal cells have an oxidative state and a glycolytic state, cancer cells can access an additional hybrid state with both metabolic modes coexisting, due to higher ROS production and/or oncogenic activation, such as RAS, MYC and c-SRC. The anti-correlation between AMPK and HIF-1 and the association of metabolic states with oncogenes were further confirmed using
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The impact of ADI-PEG20 on PD1L expression in ASS1 deficient uveal melanoma. Ramsay S. Khadare,1 Melissa M. Phillips,1 Mandeept Singh,2 Victoria Cohen,1 Caroline Thaug,1 Peter W. Szolczer,1 Barts Cancer Institute, London, United Kingdom; 2Moorfields Eye Hospital, London, United Kingdom.

Uveal melanoma (UM), involving the iris, choroid and ciliary body, is the commonest intraocular tumor in adults. Half of patients develop metastasis with a high mortality despite currently available systemic therapies including immune checkpoint blockade. Studies show that while CTLA4 antagonism has a modest effect in UM, PD1/PD1L block in large efficacy whereas trials of combination checkpoint blockade are yet to report. We confirmed previously that deficiency of argininosuccinate synthase 1 (ASS1), a key enzyme involved in arginine synthesis, sensitizes UM cells to arginine deprivation using pegylated arginase deaminase or ADI-PEG20. Early trials of ADI-PEG20 in UM have shown safety and efficacy in the clinic and thus we tested the potential for a combined immunometabolic strategy. ASS1-deficient UM cell lines (OMM1, OMM2.5, MEL270) were analyzed for the immune checkpoint protein PD1L along with their sensitivity to arginine depletion. We also tested metastatic UM for ASS1 and PD1L expression, and the impact of ADI-PEG20 treatment using samples from a current clinical trial (NCT02029690). We showed that ASS1 and PD1L protein expression were absent in the 3 UM cell lines and in a majority of primary tumors (75/102 for ASS1 and 83/102 for PD1L: 5% threshold of expression) and all metastatic tumor biopsies (n=16/16 for both). Transfection of ASS1 in OMM1, OMM2.5, MEL270 led to an increase in PD1L expression by qPCR, western blotting and FACS, which was reversible following knockdown of ASS1. Induction of PD1L expression by ASS1 was accompanied by interferon (IFN) gene expression and arginase enzyme activity. ASS1 expression was abrogated using the pan-STAT inhibitor ruxolitinib. Next, PD1L expression was significantly increased in the 3 ASS1-negative UM cell lines with ADI-PEG20 treatment by 24hrs and was associated with Type 1 IFN signaling which waned along with PD1L expression by 48hrs. The ADI-PEG 20 induction of PD1L was abolished using ruxolitinib, indicating that the upregulation of PD1L is IFSN-dependent for the PD1L checkpoint blockade. While analysis of UM biopsies of patients progressing on ADI-PEG20 revealed upregulation of ASS1 (n=2/2), and thus resistance to ADI-PEG20 a concomitant increase in PD1L was not observed (n=0/2). Collectively, our data show that ASS1 is absent in a majority of patient biopsies of primary and metastatic UM tumors and is tightly correlated with PD1L expression. UM cell lines displayed sensitivity to ADI-PEG20, which upregulated levels of PD1L expression via Type 1 interferon signaling that may enhance the currently limited efficacy of checkpoint blockade in UM. Further studies are ongoing of the IFN-mediated signaling between ASS1 and PD1L in UM in response to arginine deprivation with ADI-PEG20.

Immune-checkpoint inhibition via enzyme-mediated degradation of kynurenine. Brett A. Fleisher, Sihem Ait-Oudhia.1

Brett A. Fleisher, Sihem Ait-Oudhia.1

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Checkpoint inhibitors have become the cornerstone for new innovation in immune-based oncology therapy. Several orthogonal immune pathways are currently being investigated to relieve suppression or boost activity of the innate and adaptive immune system. The IDO immune-metabolism pathway was recently clinically validated in melanoma in combination with checkpoint inhibition. This opens up a new approach to relieving suppressive mediators and lends credence to the tumor microenvironment containing small molecule metabolites that induce immune tolerance. Both indoleamine-pyrrole 2,3-dioxygenase 1 (IDO1) and Tryptophan 2,3-dioxygenase (TDO) enzymes metabolize tryptophan, forming Kynurenine which the tryphyl hydrocarbon receptor (AHR) is a innate immune and adaptive immune cells lacking a net immunosuppressive effect. Both enzymes are upregulated across many tumor types, however, only the IDO1 enzyme has thus far been addressed in the clinic with small molecule inhibitors. We have postulated that enzyme-mediated depletion of Kynurenine into safe and immunologically inert metabolites can alleviate tumor immunosuppression. We have cloned and characterized several bacterial Kynureninases (KYNase) which preferentially degrade Kynurenine with >1,000 higher kcat/KM as opposed to mammalian enzymes that cleave 3-OH Kynurenine. We show that PEGylated bacterial KYNases can deplete Kynurenine produced by IDO1 +, TDO + and IDO1/TDO + dual positive human cancer cells whereas, the IDO1 inhibitor ecadostar or TDO inhibitor 680C91 only selectivity inhibited KYNase activity in IDO1 + or TDO +, respectively.

In vivo, a single subcutaneous dose of KYNase in B16F10 tumour bearing mice was able to deplete Kynurenine in both the plasma and tumors and increase effector T-cells in the tumor. KYNase demonstrated significant tumor growth inhibition and survival benefit either as a single agent or in combination with checkpoint inhibitors (anti-PD1 or anti-CTLA4) in B16F10, CT26 and 4T1 models. Interestingly, KYNase combined with anti-PD1, showed greater efficacy than ecadostar / anti-PD1 combination in CT26 tumor bearing mice. A pharmacologically optimized human KYNase is currently moving toward clinical development for the treatment of cancers where both IDO/TDO pathways are present.
Currently, IDO-1 small molecule inhibitors in combination with checkpoint inhibitor antibodies are being evaluated in >12 clinical trials. However, whether the immune-suppressive effects of Tryptophan catabolism results from its depletion in the TME, or from the accumulation of Kynurenine, is not known. To distinguish between the effects of Trp depletion and Kyn accumulation in the TME we used an engineered Kynureninase (KYNase) enzyme that selectively degrades Kyn into immunologically inert and non-toxic metabolites. Peritumoral injection of PEGylated KYNase completely depletes serum and tumor Kyn levels for up to 72 hrs, while leaving Trp concentrations in both compartments unaffected. In the IDO-1 expressing CT26 colon carcinoma model KYNase treatment as monotherapy resulted in 16% complete and durable responses, accompanied by long-term immunity to tumor re-challenge. KYNase had no effect on tumor growth in IDO-1 mice, nor in RAG-2 or in CD8+ T cell depleted mice. Analysis of the TME demonstrated KYNase treatment resulted in increased accumulation of CD8+ cells, with a greater proportion expressing Granzyme B, undergoing proliferation and permeating the tumor interior. This effect was specific to the TME, as no detectable changes to the immune compartment occurred in other organs examined, nor overt signs of autoimmunity and toxicity. The increase in CD8+ cells was consistent with in vitro data showing that elevated concentration of Kyn directly induces apoptosis of activated CD8+ T cells partially through IL-2 suppression. No changes to the Treg percentages and phenotypes in the TME were observed, indicating that the Treg compartment is not impacted by treatment with KYNase. Cytokine analysis of tumor digest metastases compared to controls. These data show that in vivo IL-2 concentrations that hold mouse tumors after KYNase treatment. Furthermore, ex vivo stimulation of TIL after KYNase treatment demonstrated that the increase in cytokines in the TME is not solely due to an increase in TIL, but also an increased effector cytokine capacity on a per cell basis. Administration of KYNase together with either octrilA4 or qpD1 synergistically elicited complete and durable regression of multiple established tumor models. In summary, our data support the hypothesis that Kyn accumulation in the TME rather than Trp depletion is the dominant IDO-mediated immune suppressive mechanism, and that enzyme-mediated KYN deple-tiation is a promising cancer immunotherapeutic approach.

#5572 AX09: an immunotherapy candidate targeting the breast cancer stem cell protein xCT. John O’Rourke,1 Elisabetta Bolli,2 Valeria Rolih,2 Laura Conti,2 Stefania Lanzardo,3 Jayne Christen,2 Federica Pericle,1 Federica Cava-rio,2 Agilivax, Inc, Albuquerque, NM;2 University of Torino, Torino, Italy.

Triple negative breast cancer (TNBC) is an aggressive form of breast cancer that lacks the estrogen, progesterone and HER2 receptors, and accounts for 15-20% of all breast cancers in the US. The particularly aggressive features of TNBC may be due to the enrichment of breast cancer stem cells (BCSC). Due to their resistance to traditional radio- and chemotherapies, BCSC represent a reservoir for the relapse, metastatic evolution and progression of the disease after treatment. Triple negative breast cancer (TNBC) presents a major barrier towards effective cancer treatments. The ability of BCSC to resist common cytotoxic therapies relies on different mechanisms, including improved detoxification ability. The cysteine-glutamate antiporter protein xCT (SLC7A11) regulates cysteine intake, conversion to cysteine and subsequent glutathione synthesis, protecting cells against oxidative and chemical insults via the p38MAPK pathway. xCT expression is highly restricted to a few normal cell types but is upregulated in a variety of breast cancer subtypes where its expression correlates with poor prognosis. xCT is highly expressed in a variety of solid tumor CSC including BCSC where it interacts with CD44 and plays a functional role in BCSC biology. Agilivax has developed a novel immunotherapy candidate (AX09) based on our virus-like-particle technology for the treatment and prevention of metastatic breast cancer that targets the BCSC protein xCT. Immunization with AX09 elicited a strong antibody response against xCT including high levels of IgG2a antibody. Immune sera from AX09 mice bound to tumoursphere derived BCSC and impacted BCSC function and biology in vitro. To assess if AX09 immunization would decrease metastases, we employed a syngeneic transplantation model, in which purified BCSC derived from TUBO cells were injected into the tail vein of vaccinated female BALB/c mice. Multiple independent experiments showed that immunization with AX09 conferred a significant reduction in the number of pulmonary metastases compared to vaccination with control VLP alone. In a pilot study, BCSC were transplanted into the mammary fat pad and mice were treated with AX09 after primary tumors were 1.5 mm in diameter. Results indicate that AX09 immunization conferred a reduction in lung metastases compared to controls. These data suggest that an active immunization approach targeting xCT can significantly reduce metastatic progression in preclinical models. Ongoing experiments are further characterizing therapeutic mechanisms and evaluating efficacy of AX09 in combination with front line chemotherapy and checkpoint inhibitors.

#5573 A high-affinity Optide (optimized peptide) inhibitor of the Hippo pathway’s YAP-TEAD interaction. Zachary R. Crook,1 Philip Bradley,2 Gregory Sevilla,1 Della Friend,1 Chris King,2 Andrew Mhyre,1 Roland Strong,1 David Baker,3 James M. Olson3.1 Fred Hutchinson Cancer Research Center, Seattle, WA;2 University of Washington, Seattle, WA;3 University of Washington, Seattle, WA.

The Hippo pathway plays a critical role in contact inhibition, a pathway that is commonly dysregulated in many human cancers (including liver, colon, ovarian, and lung). The signaling pathway culminates in the intranuclear interaction of the transcriptional co-activator YAP and the transcription factor TEAD. This is representative of a number of cancer driving pathways that have proven nearly impossible to drug, as they are mediated by intracellular protein-protein interactions. High throughput screening campaigns with small molecule libraries have failed to provide specific, high affinity binders capable of disrupting larger protein-protein interfaces (such as YAP-TEAD), while at the same time, antibodies cannot penetrate the cell membrane to access cytosolic and nuclear targets. Optides are small disulfide-knotted peptides (knottins) that are large enough to interfere with protein-protein interactions, but small enough to access compartments beyond the reach of antibodies. Examples include the calcines, activators of sarcoplasmic reticulum ryanodine receptors, and BLZ-100, a knottin-fluorophore conjugate that is capable of accumulating in a wide range of tumor types. Using the computational design software Rosetta, we created a library of Optides designed to interact with TEAD in locations that overlap YAP binding. Mammalian surface display screening against soluble TEAD yielded a successful hit (AX09) that binds YAP with high affinity and inhibits YAP-TEAD binding. Affinity maturation, using site saturation mutagenesis, produced an improved sub-nanomolar variant (IV1) with potent YAP inhibition. This variant was also found to be highly resistant to reduction and proteolysis, crucial for a disulfide-knotted peptide with a cysteine target in the proteinase-rich tumor milieu. With this highly potent YAP inhibitor, efforts are now focused on cell penetration and biodistribution with the long-term goal of advancing a clinical development candidate.
Androgen receptor stimulation with 5α-dihydrotestosterone (DHT) decreases PD-L1 expression in androgen-responsive thyroid cancer cells. Timmy J. O’Connell,1 Melanie Jones,2 Anvita Gupta,3 Tali Lando,1 Deya Jourdy,1 Edward Shin,3 Augustine Moscatello,1 Raj Tiwari,1 Jan Gelebter1.

Thyroid cancer is the most rapidly increasing cancer in the US with 64,300 new cases expected in 2016. However, there is a disparity in the incidence of thyroid cancer between females and males, with 49,330 expected cases developing thyroid cancer three times more often than men (14,950 expected cases). Hormonal factors leading to reduced androgenic responsiveness of tumor cells is associated with suppression of the androgen receptor (AR). 84E7 is a clone of 8505C that was transfected with an AR containing plasmid resulting in constitutively expressed AR. Transcriptome analysis via RNASeq was performed on 8505C and 84E7, with and without 5α-dihydrotestosterone (DHT) treatment. Raw sequencing reads were aligned to the UCSC hg19 human reference genome with TopHat, and Cufflinks was used to perform expression analysis. Changes in expression were significant in that PD-L1 is produced by tumor cells as a strategy for immune system activation and may help eliminate nascent thyroid cancer cells. Thus, men may experience a decreased incidence of thyroid cancer due to an enhanced and less inhibited anti-tumor environment.

BPM 31510, a clinical stage candidate demonstrates potent anti-tumor effect in an immune-competent syngeneic pancreatic cancer model. Shiva Sarapaksa,1,2 Prashant Prasad,1 Shalini Garg,1 Maria Nastke,1 Tulin Dadali,1 Anne R. Diers,1 Stephane Gesta,1 Vivek K. Vishnudas,2 Rangaprasad Sarangarajan, Niven R. Nairan, BERG, LLC, Framingham, MA.

BPM 31510, a clinical stage nanodispersion of ubidecarenone, demonstrates anti-tumor effects by eliciting an anti-Warburg metabolic switch in cancer. Previous studies in an immune compromised PaCa2 xenograft model has unequivocally demonstrated significant efficacy of BPM 31510 on tumor volume and survival. The fundamental property of BPM 31510 to influence mitochondrial bioenergetics and the recognized interplay between T cell metabolism and tumor mediated killing of cancer cells (TLM) functions in eliciting anti-cancer effects. In this study BPM 31510 selectively influenced activation and maturation of T cells in murine peripheral blood mononuclear cells (PBMCs). Moreover, in addition to demonstrating changes in the CD3+ population, changes in surface expression of CD1d and CD1a along with the IFN-γ secretion were examined. Murine cancer cell lines exposed to BPM 31510 were associated with variable sensitivity with highly metabolic tumor types being most sensitive. Next, the anti-cancer activity of BPM 31510 in an in vivo immunocompetent syngeneic Pan02 rodent model was investigated. Murine Pan02 pancreatic cancer cells were implanted subcutaneously into C57BL/6 mice. Tumors with mean volume of 80 mm³ were treated twice a day with vehicle control or BPM 31510 at 25, 50, 100 mg/kg, administered intraperitoneal. Tumor volumes were measured every 4 days. At day 21 post treatment, tumors were harvested and analyzed for the level of infiltrating immune cells by immunofluorescent staining with CDB + for T cells and F4/80 for tumor macrophages. These results demonstrate a dose-dependent reduction in tumor volume following 21 days of BPM 31510 treatment. In summary, BPM 31510 exerts potent anti-tumor effects through its dual function of modulating tumor cell metabolism and potentially influencing immune check-point to improve overall survival outcomes.
the molecules from this series showed IC50 of 0.2 and 0.08 μM in the biochemical assay and 1.7 and 0.8 μM in the cell based assay against IDO1 and TDO2, respectively. In addition, another series of NCEs showed strong activation of IDO1 and TDO2 activity. As compared to untreated control, formation of kynurenine was increased in a dose-dependent manner as observed by increase in fluorescence of 8-10 fold over control. One of the compounds from this series showed an EC50 of ~20-30 μM in the biochemical assay as well as in 293T-based assay. Further mechanistic studies to understand the immune modulatory activity of these selective TDO2 modulators is underway. These tool compounds are being further optimized for potency and ADME properties to be developed as potential drug candidates. Conclusion: To our knowledge such IDO1 and TDO2 specific small molecule activators have not been reported. Therefore, these activators and inhibitors would serve as useful tool compounds in understanding the specific role(s) of IDO1 and TDO2 in disease biology and would also provide the opportunity to target this pathway for various diseases, including cancer.

**#5579 Strategic inhibition of adenosine A2A receptor (A2AR) by CPI-444 improves CD8 Treg ratios and enhances T-cell killing of a HER-2/neu expressing murine tumor.** Blake A. Scott,1 Todd Armstrong,1 Stephen Willingham,2 Ian McCaffery,2 Elizabeth M. Jaffe,1 Johns Hopkins University School of Medicine, Baltimore, MD; 2Corus Pharmaceuticals, Burlingame, CA.

It has been demonstrated that a higher CD8 T to CD4 FoxP3 T regulatory cells (Tregs) ratio coincides with improved therapeutic outcomes for patients receiving immunotherapies in the clinic. The last several years of research have drawn attention to many such therapeutics and strategies. Adenosine is an abundant extracellular signaling molecule in the tumor microenvironment (TME) of many cancers. Signal transduction through the GPCR A2AR enhances the immunosuppressive activity of Tregs while simultaneously attenuating tumor-specific CD4+ /CD8+ T cells. We have evaluated the appropriate coordination and dosing for a small-molecule inhibitor of A2AR in a Her-2/neu expressing murine model of breast cancer. We have demonstrated its capacity to augment existing therapeutic strategies in this tolerant model. To test our hypotheses on the ability of the small molecule to work in concert with existing therapeutics, we tested the role of CPI-444 when given with a T cell-inducing vaccine. Neu-expressing mammary tumor bearing HER-2/neu transgenic (Neu-N) mice were administered combinations of low-dose cyclophosphamide (Cy) to deplete Tregs, followed one day later with a granulocyte-macrophage colony-stimulating factor (GM-CSF) and neu-expressing whole cell vaccine (GVAX). The day following administration of GVAX, mice received adoptively transferred high-avidity naive specific CD8 T cells intravenously one day after vaccination. Administration of specific components in this strategy (i.e. Cy, GVAX, adoptive transfer) were altered to gain insight into the mechanistic effects of CPI-444 in vivo through analysis of tumor progression, tumor clearance, tumor infiltration and immunometabolic analysis of the TIL. Mice treated with the A2AR inhibitor or the vehicle control were administered each by oral gavage daily for 14 days (survival) or until 4 days post-adoptive transfer (TIL) at the peak infiltrate time. Of the strategies tested, Cy, followed by concomitant administration of GVAX with 100mg/kg of CPI-444 for 14 days, and a single adoptive therapy treatment provided a strong increase in tumor survival (OS) compared with 0%-20% OS in vehicle controls (P<0.05). Further, we have observed both broad and specific changes in the T cell compartment of the tumor-infiltrating leukocyte (TIL) population. Broadly, we have observed a refinement in the T cell portion of the TIL with decreased numbers of T cells overall, but an increase in specific prognostic ratios, namely a relative increase in favorability of the CD8 Treg ratio when compared to controls (P<0.05). We hypothesize that strategic combination therapy of CPI-444 with existing therapeutics can refine the local immune profile to enhance favorable outcomes in vivo. CPI-444 is currently involved in numerous clinical trials: this work may provide rationale for the expansion of these trials into new cancer types.

**#5580 Preclinical pharmacodynamics and antitumor activity of AZD4635, a novel adenosine 2A receptor inhibitor that reverses adenosine-mediated T cell suppression.** Alexandra Borodovsky,1 Yanjun Wang,1 Minwei Ye,1 Joseph C. Shaw,1 Kris F. Sachsenmeier,1 Nanhua Deng,1 Kelly J. Del-Signore,1 Adrian J. Freeland,1 James D. Clarke,1 Richard J. Goodwin,1 Nicole Strittmatter,1 Carl Hay,1 Vasu R. Sah,1 Deborah Lawson,1 Corinne Reimer,1 Miles Congreve,2 Jonathan S. Mason,1 Fiona H. Marshall,1 Paul Lyne,1 Richard Voyessa,1 AstraZeneca, Waltham, MA; 2MedImmune, Gaithersburg, MD; 3Heptares Therapeutics, London, United Kingdom.

Inhibition of extracellular adenosine within the microenvironment is a strategy exploited by tumors to escape immunosurveillance. Adenosine signaling through the high affinity adenosine 2A receptor (A2AR) on immune cells elicits a range of immunosuppressive effects which can promote tumor growth and limit the efficacy of immune checkpoint inhibitors such as anti-PD-1 or anti-PD-L1 Abs. AZD4635 (HTL-1071), an oral A2AR antagonist, binds to human A2AR with a Kᵢ of 1.7 nM and with > 30-fold selectivity over other adenosine receptors. Accumulation of intratumoral adenosine, measured by desorption with 3He/11C positron emission tomography (PET/CT), has been reported to be significantly higher in patients with advanced cancer. However, it is hypothesized that extracellular adenosine levels are spatially heterogeneous in mouse syngeneic tumors, with levels varying up to 50 fold among regions of a single tumor. To assess whether AZD4635 can reverse the inhibitory effects of different concentrations of adenosine, CHO cells stably expressing human A2AR were incubated with concentrations of adenosine ranging from 0.1 to 10 μM. In the presence of 0.1, 1 and 10 μM adenosine, the IC50 of AZD4635 for inhibition of cAMP production was 0.79, 10.0 and 14.29 nM, respectively. In an ex vivo CD8+ T cell assay, AZD4635 reversed suppression and restored IFNγ secretion in cells incubated with 5’-N-ethylcarboxamidoadenosine (NECA), a stable analog of adenosine. The therapeutic benefit of A2AR blockade was evaluated in syngeneic mouse tumor models. Inhibition of A2AR signaling led to a reduction in tumor growth alone and in combination with checkpoint inhibitors. Tumors harvested from the treated mice exhibited changes in infiltrating lymphocyte populations and increases in the functional activity of T cells. These results demonstrate that AZD4635 is a potent and selective A2AR inhibitor, and that blockade of A2AR signaling with an inhibitor such as AZD4635 can reduce tumor burden and enhance antitumor immunity. AZD4635 is currently in a Phase I clinical trial as a single agent and in combination with durvalumab (anti-PD-1 L1 Ab) in patients with solid malignancies.

**#5581 Development of 2nd generation indoleamine 2,3-dioxygenase 1 (IDO1) selective inhibitors.** Thomas Pesnot, Sachin Mahale, Philip MacFaul, John Maclean, Caroline Phillips, Matilda Bingham, Catherine Eagle, James Kelly, Abhijith Thippsewamy, Simon Armitage, Aleksandr Grisin, Sheenagh Aiken, Lucy Cartwright, Richard Armer. REDX Pharma, Macclesfield, United Kingdom.

Harnessing the immune system via immune checkpoint blockade (e.g. anti PD-1, anti PD-L1, anti CTLA4) has led to fast and long lived responses in cancer patients. Response rates however are low and new treatments that enhance these rates are needed. Recent studies have shown that the administration of immune checkpoint blockers is associated with the overexpression of indoleamine 2,3-dioxygenase 1 (IDO1). The resulting immunoregulatory phenotype counteracts immune checkpoint blockade and allows for cancer progression. The discovery of IDO1 inhibitors, and the potential to combine them with immune checkpoint blockers, therefore represents an attractive strategy to fight cancer. We carried out a ligand-based virtual screen with 1,000,000 commercially available small molecules. In vitro screening of the resulting 610 virtual hits provided us with 2 IDO1-selective, 2 TDO2-selective and 2 IDO1/TDO2-dual confirmed hits. (TDO2 is a protein with similar biochemical activity to IDO1 that is essential to tryptophan homeostasis.) A subsequent Hit to Lead campaign led to the identification of novel chemotypes that display potency similar or superior to IDO1 inhibitors currently under clinical investigation in IFNγ stimulated (i.e. IDO1) and non-stimulated (i.e. IDO1) HeLa cells, with no sign of cytotoxicity. We have demonstrated that these compounds are > 1000-fold selective for IDO1 over TDO2 using cellular assays. IDO1 upregulation by cancer cells is known to be one of the mechanisms by which cancer cells evade the immune system. In an in vitro culture assay of cancer cells and T cells we have demonstrated our compounds can rescue T cell proliferation with EC50 values between 10 and 50 nM. We have also demonstrated that our compounds inhibit IDO1 in monocyte derived human dendritic cells. Interestingly, despite this potent cellular activity demonstrated in multiple disease relevant cellular assays, this chemotype failed to inhibit recombinant IDO1 in an isolated biochemical assay performed under reducing conditions, whereas the reference compound epoxatostat provided activity comparable to literature values. In order to confirm our cellular effects were due to direct inhibition of IDO1 we set up thermal shift assays. Thermal shift assays using purified IDO1 protein have demonstrated that our compounds directly bind IDO1, and cellular thermal shift assays have confirmed direct target engagement in intact cells (stimulated for IDO1 expression). These compounds have physicochemical properties that would suggest oral dosing and dissolution in vitro CYP450 and hERG inhibition, thus reducing the risk of toxicity in the clinic. Our IDO1 inhibitors show a novel differentiated mode of action at the cellular level, and the consequences of this profile in terms of in vivo characterisation is ongoing.
**IMMUNOLOGY: Enzymes and Hormones and Metabolism in Tumor Immunity**

**#5583** Peglated adenosine deaminase 2 (PEG-ADA2) abrogates the cytotoxic effects of adenosine against chronic lymphocytic leukemia cells. Sara Serra,1 Cinzia Bologna,1 Luz Londono,2 Michael Shepard,2 Sanna Rosenberg,2 Christopher Thanos,2 Silvia Deaglio1.1 Human Genetics Foundation and University of Turin, Turin, Italy; 2Haloxy Therapeutics, San Diego, CA, USA.

Extracellular adenosine can be generated from ATP and/or ADP through the concerted action of the ectoenzymes CD39 and CD73. Adenosine can bind different type-1 purinergic receptors eliciting potent cytoprotective and immunosuppressive effects. We previously focused on chronic lymphocytic leukemia (CLL), a disease characterized by the progressive expansion of a mature B cell that is capable of expressing anti-CD73 or anti-CD39. Using these anti-CD73 or anti-CD39 antibodies, we showed that a CD73+ clone possesses a poor prognosis. This patient subset can actively generate adenosine, which favors leukemic cell survival, synthesis of immunosuppressive cytokines, while inhibiting T lymphocyte proliferation. ADA2 is a human enzyme that catalyzes the conversion of adenosine into inosine. We hypothesized that depletion of high adenosine levels from the tumor microenvironment through the administration of exogenous ADA2 could be therapeutically relevant to limit tumor protection and immunosuppression. Several engineered PEG-ADA2 variants possessed improved enzyme activity and pharmacokinetics compared to wild-type ADA2. Inhibition of adenosine receptor activation was evaluated by measuring intracellular cyclic AMP concentrations in T lymphocytes purified from healthy donors. enzyme activity was observed with increasing concentrations of PEG-ADA2-R222Q/S265N and PEG-ADA2-R222Q/S265N/K374D resulting in complete removal of high levels of adenosine, as measured by HPLC. Conversely, PEG-ADA2-E182T, which possesses significantly attenuated catalytic activity, was less effective in reducing adenosine. The functional effects of adenosine depletion on CLL cell survival were analyzed following treatment with the chemotherapeutic, DNA-damaging agent etoposide that robustly induces apoptosis within 16 hours. Addition of exogenous adenosine to cultures of purified CD73+ CLL cells significantly rescued cells from etoposide-induced apoptosis. However, when these primary leukemic cells were pretreated with PEG-ADA2-R222Q/S265N or PEG-ADA2-R222Q/S265N/K374D, the cell viability rate was significantly decreased, abrogating the cytoprotective effects of adenosine. On the contrary, PEG-ADA2-E182T had a minimal effect, suggesting enzymatic depletion of adenosine is critical to observe these effects. Similar effects were observed by the PEG-ADA2 variants on a CLL cell line expressing CD73+9. These preliminary results suggest that enzymatic depletion of extracellular adenosine following treatment with PEG-ADA2 is a relevant approach to counteract the cytoprotective effects of adenosine, warranting further development of PEG-ADA2 as a possible approach to treat CLL.

**#5584** PHY906(YIV-906), an adjuvant based on a 1800-year-old Chinese medicine, enhanced the anti-tumor activity of Immune checkpoint blockade therapy: anti-PDL1, anti-PD-1, anti-PD1/CTLA4 against melanoma. Wing Lam,1 Zaeol Jiang,1 Xue Han,2 Showo-Huey Liu,2 Lieping Chen,2 Yung-Chi Cheng1.1 Yale Univ. School of Medicine. Dept. of Pharmacology, New Haven, CT; 2Yale Univ. School of Medicine. Dept. of Immunobiology, New Haven, CT; 3Yiviva Inc, New York, NY.

Immune checkpoint blockade therapy has recently been recognized as a breakthrough in cancer treatment. Currently the FDA has approved ipilimumab (anti-CTLA4), Pembrolizumab (anti-PD-1) and Nivolumab (anti-PD-1), and Atezolizumab (anti-PD-L1) for the treatment of several types of cancers, including melanoma. However, colitis and diarrhea are commonly found in these immunotherapies, particularly in anti-PD1/CTLA4 therapy. PHY906(YIV-906) is inspired by the Huang Qin Tang, which was first described in Chinese texts 1800 years ago for the treatment of numerous gastrointestinal symptoms. Consistent preparations of PHY906 could be maintained for at least 10 years. In animal studies, PHY906 can increase the anti-tumor activity of a broad spectrum of chemotherapies while promoting damaged intestinal tissue recovery via multiple targets. Several clinical results suggest that PHY906 had potential to increase the therapeutic index of cancer treatments (chemotherapy, radiation) by prolonging life and improving patient quality of life. We hypothesize that PHY906 can reduce diarrhea caused by anti-PD-L1, anti-PD-1, and/or in combination with CTLA4 without compromising their anti-tumor activity. We studied the effect of PHY906 in combination with anti-PDL1, anti-PD1, anti-CTLA4 and anti-PD1/CTLA4, using subcutaneously implanted B16F10 melanoma in Black B16 mice. Results indicated that PHY906 enhanced the anti-tumor activity of anti-PD1, anti-PDL1, or anti-PD1/CTLA4; but not anti-CTLA4 alone, in vivo. In addition, PHY906 did not compromise the action of anti-PD-L1 in brain implanted B16 tumor. PHY906 in combination with all of the antibodies had no impact of body weight change or different type of blood cells counts. PHY906 reduced mRNA expression of several inflammation markers, including TNFa, MCP1, ICAM, PDGFB, and IL15. To assure the anti-tumor effect of PHY906, we pretreated with PEG-ADA2-R222Q/S265N or PEG-ADA2-R222Q/S265N/K374D resulting in complete removal of high levels of adenosine, as measured by HPLC. Conversely, PEG-ADA2-E182T, which possesses significantly attenuated catalytic activity, was less effective in reducing adenosine. The functional effects of adenosine depletion on CLL cell survival were analyzed following treatment with the chemotherapeutic, DNA-damaging agent etoposide that robustly induces apoptosis within 16 hours. Addition of exogenous adenosine to cultures of purified CD73+ CLL cells significantly rescued cells from etoposide-induced apoptosis. However, when these primary leukemic cells were pretreated with PEG-ADA2-R222Q/S265N or PEG-ADA2-R222Q/S265N/K374D, the cell viability rate was significantly decreased, abrogating the cytoprotective effects of adenosine. On the contrary, PEG-ADA2-E182T had a minimal effect, suggesting enzymatic depletion of adenosine is critical to observe these effects. Similar effects were observed by the PEG-ADA2 variants on a CLL cell line expressing CD73+9. These preliminary results suggest that enzymatic depletion of extracellular adenosine following treatment with PEG-ADA2 is a relevant approach to counteract the cytoprotective effects of adenosine, warranting further development of PEG-ADA2 as a possible approach to treat CLL.

**#5585** TGF-beta insensitive PSMA-specific CD8+ T cells derived from metastatic castration resistant prostate cancer (MCRPC) patients enhance the tumor killing ability. Qin Yang,1 Timothy Kuzel,2 Brian Helland,3 Ximing Li,3 Yang,1 Weijun Qin,1 Chung Lee,1 Benedetto Carneiro,3 Francis J. Giles1.1 Northwestern University, Chicago, IL; 2Rush University, Chicago, IL; 3NorthShore University Healthsystem, Evanston, IL; 4The Fourth Military Medical University, Shanghai, China.

Introduction: Manufacturing tumor specific CD8+ T cells has been an obstacle for developing prostate cancer (PCa) immunotherapies for men with MCRPC. We report a highly efficient method to expand human TGF-β insensitivity PSMA-specific CD8+ T cells from MCRPC patients using a FDA approved Cell Processing Work Station (CPWS, Panasonic). Methods: Peripheral blood CD8+ T cells were collected from men with MCRPC by leukapheresis, and cultured in CPWS in TexMACSTM medium with CD-3 Biotin/CD28/Anti-Biotin Beads, 5% human serum, and IL-2. We developed a TIRIIDN-TK-ires-PZ1 chimeric T cell receptor retroviral construct using an anti-PSMA IgTCR(ε) gene (PZ1) and a dominant negative TGF-β type II receptor (TIRIIDN), that could induce CD8+ T cells to be PSA reactive and insensitive to TGF-β. PC-3 cells (PSMA negative) or PSA positive PC-3-PSMA cells (PSMA positive) were exposed to TGF-β and used as target cells. Cytotoxicity assays were performed by LDH/NADH assays (target cells: CD8+ T cells at 1:20, 1:50 and 1:100 respectively). Live interactions between CD8+ T cells and PCa cells was recorded by Nikon BioStationTM. Apoptosis of PCs was evaluated by immuno-fluorescence staining for Annexin V and 7-Amino-actinomycin D (7-AAD) respectively. Results: On Day 0, 2x106 CD8+ T cells were placed in culture, infected with TIRIIDN-TK-ires-PZ1 on Day 8, and expanded 96 fold (1.9x108) by Day 36. The cellular expansion plateaued by Day 40 (1.9x108), when the ratio of CD8+ (95.2%), CD4+ (96.6%) and CD19+ (0.05%) was maintained. The cells strongly expressed PZ1 and TK gene (71.1%), and pSMAD2 was inhibited 95%. Under TGF-β treatment for 7 days, the growth of infected CD8+ T cells was not significantly changed, while the naïve CD8+ T control cells were suppressed by 44.8%. PC3-PSMA cells released 25.64 and 4.19 nmol/50ul/105 NADH when co-cultured with infected CD8+ T cells or with naïve CD8+ T cells respectively. The ratio of CD8+ T cells to PC3-PSMA cells was 7.35 or naïve CD8+ T cells (7.59). Infected CD8+ T cells induced 32.0% and 13.7% expression of Annexin V in PC3-PSMA and PC3 cells respectively, while naïve CD8+ T cells induced only 6.9% in both groups (p<0.05). Furthermore, Infected CD8+ T cells induced significantly higher expression of 7-AAD in PC3-PSMA (0.688%) compared to naïve CD8+ T cells treatment (0.03%). There were close interactions between infected CD8+ T cells and PC3-PSMA, which was not observed in other groups. Conclusion: Our study supports expansion and retroviral infection generating TGF-β insensitive PSMA-specific CD8+ T cells ex-vivo within 4 weeks in CPWS without any exogenous specific stimulation sources. These cells can enhance the PSA expressed PCs killing ability, and escape the inhibition by tumor secreted TGF-β, which have a potential to overcome many of the current barriers of immunotherapies for men with MCRPC.

**#5586** PPMX-T003, a fully human anti-TIR1 antibody with potential efficacy against hematologic malignancies. Lilin Zhang,1 Funiko Nomura,1 Yoi-ichi Aikawa,1 Yoshikazu Kuroswa,2 Kazuhiro Morishita,3 Yukio Sudo3.1 Perseus Proteomics, Inc., Tokyo, Japan; 2Fujita Health University, Nagoya, Japan; 3University of Miyazaki, Tokyo, Japan.

Transferrin receptor1 (TIR1) is a type II transmembrane glycoprotein that involves intracellular uptake of iron. TIR1 is ubiquitously expressed at low levels in normal cells except erythroblasts and placentale trophoblasts, which express high levels of TIR1 due to the need of up taking a large amount of iron. Accu-
mulating studies have shown elevated levels of TR1 in both solid tumor cells, as well as hematologic malignant cells when compared to their normal counterparts. Since TR1 is implicated in growth and survival in various cancer cells, targeting TR1 could be an attractive strategy for cancer therapeutics. To this end, we have developed a fully human antibody against TR1, PPMX-T003, which displays potent antitumor activity in vitro and in vivo in subcutaneous tumor and cell cycle arrest with EC50s of 3–200ng/ml in various tumor cell lines. Mechanistically, PPMX-T003 triggers apoptosis or cell growth arrest by inhibiting binding of TR1 to its ligand transferrin and blocking iron uptake. In addition, PPMX-T003 elicits antibody dependent cellular cytotoxicity (ADCC) activity in cancer cells. Importantly, PPMX-T003 showed potent efficacy against several blood cancer xenograft models. PPMX-T003 completely eradicated established subcutaneous tumors in two acute myeloid leukemia (AML) models generated by Kasumi-1 and HL-60, at a dose of 10 mg/kg when administrated (I.v.) once a week for 4 weeks. Moreover, PPMX-T003 completely eradicated established tumors in a lymphoma xenograft model (SU-DHL-2) at a dose of 3 mg/kg. Furthermore, PPMX-T003 greatly prolonged mice survival in a disseminated leukemia model (CCRF-CEM: acute lymphoblastic leukemia cell line). The control mice engrafted with CCRF-CEM cells (n=10) developed leukemia and died within 42 days, whereas 8 of the 10 mice treated with PPMX-T003 survived 179 days until the experiment was terminated. A preliminary toxicology study in Cynomolgus monkeys with multiple doses was also carried out. Although moderate anemia was observed at the dose of 30 mg/kg, no other adverse events were observed, indicating a tolerable safety profile. Taken together, these results indicate that PPMX-T003 could be a potent therapeutic antibody for the treatment of hematologic malignancies.

**#5587 Novel monoclonal antibodies block N-glycosylation Sites of the MUC16 ectodomain in ovarian carcinoma.** Dharmarao Thapi,1 Alberto Fernandez-Tejada,1,2 Abraham J. Axelor,3 Nestor Rosales,3 Xiujun Yan,3 Marina Stasenko,1 Sahityasri Thapi,1 Amy Wang,1 Samuel J. Danishefsky,1 David R. Spriggs,1 Jason R. Thompson,1,5 Amy Wang,1 Samuel J. Danishefsky,1 David R. Spriggs,1 Jason R. Thompson,1,5

IMMUNOLOGY: Enzymes and Hormones and Metabolism in Tumor Immunity

**#5588 Brentuximab vedotin-driven immunogenic cell death enhances antitumor immune responses, and is potentiated by PD1 inhibition in vivo.** Anthony T. Cao, Che-Leung Law, Shyra J. Gardai, Ryan A. Heiser. Seattle Genetics, Bothell, WA.

Brentuximab vedotin (BV) is an antibody-drug conjugate directed against CD30 consisting of a monoclonal antibody conjugated to monomethyl auristatin E (MMAE), a microtubule-disrupting agent. BV antitumor activity is thought to be primarily the result of intracellular payload release leading to mitotic arrest and apoptotic cell death. We have demonstrated that BV drives apoptosis in a manner consistent with immunogenic cell death (ICD) including activation of the unfolded protein/ER stress response with a concomitant increase in surface expression of calretulin and HSP70. In this study, we provide evidence that in vivo administration of BV leads to directed proinflammatory immune responses against the tumor and this activity is further potentiated by anti-PD-1 therapy. To examine the immunomodulatory effects of BV-induced ICD in vivo, we used a model in which ICD was induced in BV-treated human CD30-expressing A20P lymphoma cells in vitro. These cells were used to immunize wild-type BALB/c mice followed by live A20 challenge. Immunization with BV-treated cells, undergoing ICD, delayed tumor growth and improved survival compared to mice immunized with flash-frozen cells. Furthermore, administering anti-PD-1 to mice immunized with BV-treated cells displayed marked combinatorial effects, leading to improved tumor clearance compared to either treatment alone. Additionally, T cells isolated from mice immunized with BV-treated cells and transferred into tumor-bearing immunodeficient mice resulted in tumor regression and survival, demonstrating robust T cell memory. Lastly, we employed a humanized tumor model pairing CD30+ PDL1+ PDL2+ lymphoblastoid cell line (LCL) xenografts with adoptively transferred autologous PBMC. In this setting, mice treated with suboptimal doses of BV showed greatly enhanced antitumor responses compared to control mice with more accelerated immune-mediated tumor clearance. Tumor regression was further accelerated by treating mice with a combination of BV and a human PD1 inhibitor, demonstrating complementary modes of action for these agents. Together, these data indicate that targeted treatment with the MMAE antibody drug conjugate brentuximab vedotin drives an immunogenic form of tumor cell death that enhances innate and adaptive antitumor immunity. Combination of BV with PD1 inhibitors resulted in greater antitumor activity than either agent alone. Multiple clinical trials are ongoing to evaluate the efficacy of this treatment pairing.

**#5589 Combination of an oncokine inhibitor merestinib with anti-PD1 results in enhanced immune mediated antitumor activity in CT26 murine tumor model.** Sau-Chi Betty Yan,1 Victoria L. Peek,1 Jennifer R. Stephens,1 Um L. Um,1 Amaladas Nelusha,2 Colleen A. Burns,2 Kelly M. Credille,2 Thompson N. Doman,1 Scott W. Eastman,2 Beverly L. Falcon,3 Gerald E. Hall,2 Philip W. Iverson,3 Bruce W. Konicek,3 Jason R. Manro,3 Any T. Pappas,3 Julie A. Stewart,1 Michael B. Topper,3 Swee- Seong Wong,1 Michael Kalos,1 Ruslan D. Novosadovyi,2 Richard A. Walgren,3 David Schaefer3, Eli Lilly and Company, Indianapolis, IN; Eli Lilly and Company, New York, NY.

The combination of tumor targeted therapeutics with PD-1 checkpoint blockade has been explored as a method to increase the clinical benefits of immunotherapy, and expand response to additional cancer types. Merestinib (Mer) is a kinase inhibitor targeting several oncoproteins including MET, MRX1, AXL, METRT, and MKN1/2 that can potentially modulate immune function, angiogenesis, as well as target the tumor and immune responses that play a key role in mucin-related transformation by mediating complex cell surface interactions. These effects are mediated through MGAT5-dependent N-glycosylation of two proximal N-glycosylation sites within the 58 amino acid retained MUC16 ectodomain acting in combination with Galectin-3 and growth factor receptors. Neither N- nor O-glycosylation sites in the more distally located MUC16 regions can functionally substitute. The tumor-microenvironment promoting properties depend on co-localization of MUC16, Galectin-3, and growth factors receptors on lipid rafts. Loss of either Galectin-3 expression or the glycosylation enzyme MGAT5 completely abrogates MUC16 tumor promotion. Using synthetic glycopeptides, we have developed novel monoclonal antibodies (mAbs) directed at the crucial ecdomain N-glycosylation sites to inhibit the glycosylation-dependent effects of MUC16 on metastasis and invasion. These novel antibodies were characterized in vitro and in vivo studies using various ovarian cancer cell lines. All of the antibodies against the N-glycosylation sites closest to the cell membrane of MUC16 block Galectin-3-mediated polymerization to cell surface signaling molecules and inhibit the tumor-promoting effects of MUC16. The implications are broad: both for the biological understanding of cancer mucin biology and for potential therapeutic strategies.

**#5590 Combination of an oncokine inhibitor merestinib with anti-PD-L1 results in enhanced immune mediated antitumor activity in CT26 murine tumor model.** Sau-Chi Betty Yan,1 Victoria L. Peek,1 Jennifer R. Stephens,1 Um L. Um,1 Amaladas Nelusha,2 Colleen A. Burns,2 Kelly M. Credille,2 Thompson N. Doman,1 Scott W. Eastman,2 Beverly L. Falcon,3 Gerald E. Hall,2 Philip W. Iverson,3 Bruce W. Konicek,3 Jason R. Manro,3 Any T. Pappas,3 Julie A. Stewart,1 Michael B. Topper,3 Swee-Seong Wong,1 Michael Kalos,1 Ruslan D. Novosadovyi,2 Richard A. Walgren,3 David Schaefer3, Eli Lilly and Company, Indianapolis, IN; Eli Lilly and Company, New York, NY.

The combination of tumor targeted therapeutics with PD-L1 checkpoint blockade has been explored as a method to increase the clinical benefits of immunotherapy, and expand response to additional cancer types. Merestinib (Mer) is a kinase inhibitor targeting several oncoproteins including MET, MRX1, AXL, METRT, and MKN1/2 that can potentially modulate immune function, angiogenesis, as well as target the tumor and immune responses that play a key role in mucin-related transformation by mediating complex cell surface interactions. These effects are mediated through MGAT5-dependent N-glycosylation of two proximal N-glycosylation sites within the 58 amino acid retained MUC16 ectodomain acting in combination with Galectin-3 and growth factor receptors. Neither N- nor O-glycosylation sites in the more distally located MUC16 regions can functionally substitute. The tumor-microenvironment promoting properties depend on co-localization of MUC16, Galectin-3, and growth factors receptors on lipid rafts. Loss of either Galectin-3 expression or the glycosylation enzyme MGAT5 completely abrogates MUC16 tumor promotion. Using synthetic glycopeptides, we have developed novel monoclonal antibodies (mAbs) directed at the crucial ecdomain N-glycosylation sites to inhibit the glycosylation-dependent effects of MUC16 on metastasis and invasion. These novel antibodies were characterized in vitro and in vivo studies using various ovarian cancer cell lines. All of the antibodies against the N-glycosylation sites closest to the cell membrane of MUC16 block Galectin-3-mediated polymerization to cell surface signaling molecules and inhibit the tumor-promoting effects of MUC16. The implications are broad: both for the biological understanding of cancer mucin biology and for potential therapeutic strategies.
pared to PD-L1 Ab monotherapy. The enhanced immune activation of the combination therapy, leading to synergistic anti-tumor efficacy, demonstrates that merestinib has the potential to augment immunotherapy while targeting the tumor directly. This preclinical data provides the rationale for the clinical investigation of merestinib in combination with checkpoint therapies targeting the PD-L1/PD-1 axis (NCT01933344).1 New data samples from healthy volunteers so far (p = 0.051).

Conclusion: Metformin treatment might be useful in reversion of exhausted CD8+ T cells in cancer-bearing patients.

#5593 Inhibition of A2AR induces anti-tumor immunity alone and in combination with anti-PD-L1 in preclinical and clinical studies. Stephen Willingham,1 Andrew Hotson,2 Po Ho,3 Carmen Choy,3 Kim Walter,2 Erik Yuval,4 Sharon Benozo,5 Gima Laport,4 Richard Miller,1 Ian McCaffrey6. Corvia Pharmaceuticals, Burlingame, CA; 7Adaptive Biotechnologies, Seattle, WA.

Adenosine signaling via A2A receptor (A2AR) on immune cells suppresses anti-tumor immunity and limits the efficacy of immunotherapy, chemotherapy, CAR-T, and vaccines. CPI-444 is a potent and selective oral A2AR antagonist. Daily treatment of mice with CPI-444 led to dose-dependent inhibition of tumor growth in multiple syngeneic tumor models. Combining CPI-444 with anti-PD-L1 treatment synergistically eliminated tumors in up to 90% of treated mice, including restoration of immune responses in models that are poorly responsive to anti-PD-1 or anti-PD-L1 monotherapy. We have initiated a Phase 1/1b clinical trial to examine safety, tolerability, biomarkers, and preliminary efficacy of CPI-444 in combination with anti-PD-L1 antibodies. In combination with atezolizumab, in patients with non-small cell lung, melanoma, renal, triple negative breast, and other (bladder, prostate, head and neck, colorectal) tumors. Step 1 of the trial focused on determining the optimal dose and schedule for CPI-444; Step 2 is currently evaluating the efficacy of optimal CPI-444 dosing alone and with atezolizumab. In 48 patients treated in Step 1, CPI-444 was well tolerated with 1 Grade 3 or 4 treatment-related adverse events. Preliminary evidence of clinical activity was observed in patients treated with single agent CPI-444, including patients who previously failed anti-PD-1 therapy. A PCaREB-based pharmacodynamic assay showed that 100 mg, BID dose of CPI-444 resulted in a complete, sustained inhibition of A2AR on circulating immune cells. CPI-444 treatment alone or in combination with atezolizumab resulted in increased frequency of circulating CD8+ PD-1+ cells and memory/effector subsets of CD4+ and CD8+ T cells. Substantial changes in TCR repertoire (Morisita Index <0.9) were observed in both anti-PD-1 naive and refractory patients and correlated to patient responses. CD73 expression, CD8+ infiltration, Treg distribution, and gene expression signatures are currently being evaluated in serial tumor biopsy specimens and will be presented. In total, this shows that CPI-444 is well tolerated in cancer patients, exhibits functional inhibition of adenosine signaling, and treatment is associated with activation of anti-tumor immunity and clinical activity.

#5594 High throughput combinatorial profiling of checkpoint inhibitor antibodies on the iQue® Screener PLUS. Tom Hasaka. IntelliCyt, Albuquerque, NM.

Checkpoint inhibitors have become valuable immuno-modulatory targets in the advancement of cancer treatment. Looking for the synergy between new checkpoint inhibitor antibodies and known inhibitors is an important aspect of this research. The iQue Screener PLUS platform is a powerful tool to simultaneously assess these interactions in a single well of a microtiter plate. ForeCyt® software provides plate-level analytics and high content visualization to generate deep insight rapidly. Using a mixed lymphocyte reaction (MLR) model, we profiled potential synergies of several known checkpoint inhibitors antibodies. Responses of PD-1, PD-L1, and CD73 inhibitors both individually, and in combination with CTLA-4 inhibitors, were assessed for proliferation, viability and cytokine secretion simultaneously in the same well. MultiCyt® cell-based and bead-based reagents were used for this analysis. Synergies ranging from 2-10 fold increase over CTLA-4 alone were observed in the secretion of TNFα and IL-1β. Results were obtained and analysis completed in a 384-well plate in 30 minutes. In conclusion, this study highlights the power of the iQue Screener PLUS platform to rapidly characterize multiple endpoints and the ForeCyt software to provide high content visualization that reveals actionable insights.

#5595 HDAC inhibitor Entinostat disrupts functions of PMN-MDSC. Ayumi Hashimoto,1 Vinit Kumar,1 Peter Ordentlich,2 Dimitry I. Gabriolovich,1 The Wistar Institute, Philadelphia, PA; 3Syndax Pharmaceuticals, Inc., Wallingford, MA.

Myeloid-derived suppressor cells (MDSCs), consisting of subpopulations of polymorphonuclear (PMN; CD11bLy6G+Ly6Cmed) and monocytic (M;
CD11b<sup>+</sup> Ly6G<sup>+</sup> Ly6C<sup>−</sup> MDSCs play a major role as immune suppressors in cancer. MDSC-targeted therapy has not been developed yet. Histone deacetylases (HDAC) are critically involved in epigenetic regulation of multiple genes and may regulate MDSC number and function. The purpose of this study was to evaluate the effect of the class I HDAC inhibitor, eninostat on differentiation and function in mouse tumor models of lung cancer (Lewis Lung Carcinoma, LLC) and colon cancer (CT26). LLC and CT26 tumor-bearing mice were treated with eninostat (10 mg/kg, p.o. daily for two weeks). Eninostat caused significant increase in the presence of CD45<sup>+</sup> CD11b<sup>+</sup> myeloid cells in bone marrow, spleen and tumors. This increase was largely due to accumulation of Ly6G<sup>+</sup>Ly6C<sup>−</sup> PMN. Unexpectedly, this was associated with a small decrease in the presence of common myeloid progenitor (CMP) and granulocyte-macrophage progenitor (GMP) cells in bone marrow. This suggests that eninostat affects differentiation of PMN downstream of GMP. Experiments in vitro demonstrated that eninostat increased differentiation of enriched hematopoietic progenitor cells (HPC) to PMN in the presence of tumor explant supernatant (TES), which supported the in vivo result. To assess the effect of eninostat on the function of PMN-MDSCs, Ly6G<sup>+</sup> cells were purified from spleen and tumors of LLC tumor-bearing mice treated with eninostat or vehicle alone and tested for their ability to suppress antigen-specific T cell. As expected Ly6G<sup>+</sup> PMN-MDSC from tumor-bearing mice treated with vehicle alone had strong suppressive activity. In contrast, Ly6G<sup>+</sup> cells from spleen and tumor of eninostat-treated LLC tumor-bearing mice show significantly reduced suppressive activity. This result was consistent with experiments with Ly6G<sup>+</sup> cells differentiated from HPC culture with/without eninostat treatment. To determine whether eninostat inhibition of PMN-MDSC immunosuppressive function could enhance immune checkpoint activity, eninostat combined with an anti-PD-1 antibody was evaluated using the LLC tumor-bearing mice. These mice were treated with eninostat (10 mg/kg, p.o., daily) and anti-mouse PD-1 rat antibody (10 mg/kg, i.p., twice a week). Eninostat or PD-1 antibody alone did not significantly inhibit the tumor growth. In striking contrast, the combination treatment blocked tumor progression. Our data demonstrate that eninostat increases a population of PMN-MDSCs that are no longer immunosuppressive leading to enhanced anti-tumor activity when combined with an immune checkpoint inhibitor. This novel phenomenon provides further rationale for combination therapy of eninostat with anti-PD-1 antibody or other checkpoint inhibitors in clinical settings.

#5596 Intratumoral administration of a TLR9-adjuvanted nanoparticle cancer vaccine stimulates more effective immunity in both injected and uninjected tumor sites compared to subcutaneous administration. Edward Naik,1 Chi Ying,1 Robert Milley,1 Carlo Calascan,1 Stewart Chipman,1 Thomas Tütting,2 Robert Coffman,1 Cristina Guiducci,1 Dynavax Technologies, Berkeley, CA;2Universitätsklinikum Magdeburg A.O.R., Magdeburg, Germany.

Therapeutic success of a cancer vaccine requires substantial expansion of vaccine-primed CTL and efficient differentiation to polyfunctional T cells capable of migrating from the site of vaccination to metastatic sites. We have evaluated the tumor itself as a potential site of vaccination, compared to vaccination at subcutaneous sites distant from the tumor. For vaccination, we have developed a highly efficient nanoparticle platform comprised of multiple copies of a CpG-rich oligonucleotide (a TLR9 agonist) and long peptide tumor antigens, both covalently linked to the succrose polymer Ficoll. Multiple peptide antigens where studied, including ovalbumin and a long peptide comprising epitopes from three melanocyte differentiation antigens. In all of these models, intratumoral vaccination was superior to subcutaneous vaccination for elimination of both injected tumors and, more importantly, distant site skin and lung metastases. Intratumoral vaccination substantially increased the magnitude of the systemic CTL response measured in the blood and in the spleen and enhanced capacity cell function to distant site tumors. A greater fraction of tumor-antigen specific CD8<sup>+</sup> T cells generated in the tumor were polyfunctional, expressing both IFN-γ and TNF-α. They were also less exhausted as measured by decreased overall PD-1 levels, increased Tbet and the increased number of PD-1<sup>−</sup> cells that retained cytotoxicity and proliferative capacity. Intratumoral vaccination also induced spread of the CD8<sup>+</sup> T cell response to tumor antigens not included in the vaccine. These results demonstrate that direct intratumoral immunization with peptide antigens combined with a strong TLR9-activating adjuvant significantly increases the magnitude and quality of vaccine-primed CTL and enhances control of metastatic disease.

#5597 Genomic profiling of syngeneic mouse cell lines and in vitro screen of the models against checkpoint inhibitors and target agents for preclinical application. Frank Xing,1 Wubin Qian,1 Chunlan Dong,1 Xiaoli Xu,2 Sheng Guo,1 Qian Shi1.1 Crown Biosciences, Taicang, China;2 Crown Biosciences, Beijing, China.

Cancer immunotherapy has provided substantial clinical benefit in a significant number of patients with advanced diseases. In pre-clinical studies, Crown-Bio has developed a large collection of syngeneic models to facilitate in vivo efficacy testing of immune-oncology agents in mice (MuScree<sup>TM</sup>). To combine the ostensibly separate therapeutic strategies of activating immune cells against tumor cells and inhibiting immune checkpoint activity, we sought to thoroughly characterize the mutation profiles of these syngeneic mouse cell lines and examine drug response profiles of these cell line models. The goal of this work was to provide an in vitro system in evaluating combination effectiveness when targeting both immune checkpoint markers and oncogenic targets in pre-clinical studies. To this end, we investigated mutation and gene expression profiles of 18 mouse cancer cell lines out of the panel of 23 syngeneic mouse models included in the MuScree<sup>TM</sup> for 50 well defined cancer-related mutations among 30,690 variants in exonic regions called by RNAseq (Illumina HiSeq X10). The somatic mutations revealed through the bioinformatics analysis include ALK (3 - frequency, same for the rest), BRAF (4), BRCA1 (7), BRCA2 (12), EGFR (3), ERBB2 (6), EGF R3 (2), FBXW7 (10), FLT3 (12), HRAS (1), KRAS (8), NRAS (1), PDGFR A (11), PTH C1 (9), PIK3CA (2), PTEN (6), RET (3), SERT (5), SMAD4 (3), SMO (13), TRP53 (13), TSC1 (3), and TSC2 (10). All of these oncogenic alterations are clinically actionable (Cancer Discovery 2, 82-93, 2012). The same set of genes was also subject to mRNA expression change analysis. We screened the 18 mouse cell lines with a few selected target therapy agents, and performed combination assays of these agents together with checkpoint inhibitors such as ipilimumab and other agents. An IncuCyte real-time imaging platform was used to distinguish activities on T cells and tumor cells. The results and the implications will be discussed.

#5598 Adenosine signaling through A2AR limits the efficacy of anti – CTLA4 and chemotherapy in preclinical models. Po Ho, Meng-Yin Hsieh, Andrew Hotson, Richard Miller, Ian McCaffrey, Stephen Willingham. Corvus Pharmaceuticals, Burlingame, CA

Elevated levels of extracellular adenosine within the tumor microenvironment create an immunosuppressive niche that promotes tumor growth and metastasis. Adenosine signaling via the A2A receptor (A2AR) on immune cells suppresses anti-tumor immunity and has been shown to limit the efficacy of immuno-therapies such as anti-PD-1 or anti-PD-1 monoclonal antibodies (mAbs). CPI-444 is a potent and selective oral A2AR antagonist that is currently being investigated in Phase 1/1b clinical trials alone and in combination with an anti-PD-1 antibody (Atezolizumab) in selected solid tumors. New preclinical data suggests that combining CPI-444 with anti-CTLA-4 mAbs as well as chemotherapy treatment are also promising therapeutic strategies in solid tumors, suggesting a broad role for adenosine as an immune suppressive mechanism. The efficacy of CPI-444 + anti-CTLA-4 mAb treatment was evaluated in MC38 and CT26 syngeneic mouse tumor models. In CT26, combination treatment eliminated established tumors in up to 90% of mice approximately 2 weeks after treatment was initiated. In MC38, combination CPI-444 and anti-CTLA-4 mAb treatment prolonged survival of 80% of mice compared to only 40% of mice that received CPI-444 or anti-CTLA-4 mAbs alone. The effect of CPI-444 + anti-CTLA-4 treatment on T-cell proliferation, T-cell activation, and Treg function will be discussed. Chemotherapy releases adenosine and ATP into the tumor microenvironment (TME). Multiple chemotherapies have also been shown to up-regulate the ecto-enzymes CD39 and CD73 that produce adenosine and further suppress immune function. In the MC38 model, CPI-444 treatment synergized with doxorubicin and eliminated established tumors 80% of treated mice. CPI-444 treatment was also synergistic with cyclophosphamides, inhibiting the growth of RENCA tumors, a model that is considered resistant to chemotherapy. Ongoing studies are evaluating the effect of CPI-444 + chemotherap y on tumor infiltrating lymphocyte localization, activation, and expression of CD73 and CD39. These results suggest that blockade of the adenosine signaling pathway may be vital for enhancing anti-tumor responses in solid tumors that show an incomplete response to anti-CTLA4 therapy or chemotherapy.

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#5599 Soluble PD-L1 as a surrogate biomarker of metastatic progression and resistance to antiangiogenic therapy. Michalis Mavrili,1 Amanda Tracc,1 Yuhao Shi,2 Georg Bjarnason,1 Tran Nguyen,2 Brian Rini,4 John M.L. Ebos1.

1Roswell Park Cancer Institute, Buffalo, NY;2State University of New York, University at Buffalo, Buffalo, NY;3University of Toronto, Toronto, Ontario, Canada;4Cleveland Clinic Taussig Cancer Institute, Cleveland, OH.

Clinical studies targeting both immune checkpoint markers and oncogenic targets in preclinical studies. To this end, we investigated mutation and gene expression profiles of 18 mouse cancer cell lines out of the panel of 23 syngeneic mouse models included in the MuScree<sup>TM</sup> for 50 well defined cancer-related mutations among 30,690 variants in exonic regions called by RNAseq (Illumina HiSeq X10). The somatic mutations revealed through the bioinformatics analysis include ALK (3 - frequency, same for the rest), BRAF (4), BRCA1 (7), BRCA2 (12), EGFR (3), ERBB2 (6), EGF R3 (2), FBXW7 (10), FLT3 (12), HRAS (1), KRAS (8), NRAS (1), PDGFR A (11), PTH C1 (9), PIK3CA (2), PTEN (6), RET (3), SERT (5), SMAD4 (3), SMO (13), TRP53 (13), TSC1 (3), and TSC2 (10). All of these oncogenic alterations are clinically actionable (Cancer Discovery 2, 82-93, 2012). The same set of genes was also subject to mRNA expression change analysis. We screened the 18 mouse cell lines with a few selected target therapy agents, and performed combination assays of these agents together with checkpoint inhibitors such as ipilimumab and other agents. An IncuCyte real-time imaging platform was used to distinguish activities on T cells and tumor cells. The results and the implications will be discussed.
Immune-checkpoint inhibitors are now approved for the treatment of early- and late-stage cancers. These include agents that block the T-cell regulatory protein programmed cell death 1 (PD-1) from being activated by the PD-1 ligand 1 (PD-L1) expressed on cancer cells. There is an urgent need to identify biomarkers of PD-1 pathway inhibition that would predict patient populations responsive to treatment and/or guide drug development. While there is currently a biomarker candidate, reliable detection and quantification methodologies have proven challenging to standardize. Recently, a soluble PD-L1 (sPD-L1) fragment was identified that can derive from cell-bound PD-L1. Retrospective clinical examinations of sPD-L1 levels in cancer patients suggest a potential use as a surrogate for disease progression and response to treatment; but few preclinical data was performed to test this predictive value. We undertook in vivo experiments to evaluate plasma sPD-L1 in mouse tumor models during localized primary tumor growth (after orthotopic cell implantation) and spontaneous metastatic disease progression (after surgical removal of the primary). Mouse syngeneic and human xenograft implantation models included breast, kidney, colon, and melanoma cell systems. Our results show that circulating plasma sPD-L1 can correlate with primary and metastatic progression in a stage and model specific manner. Next, we evaluated sPD-L1 following treatment with neutralizing antibodies to PD-1 and PD-L1 in tumor-free mice and found significant dose-dependent sPD-L1 increases, suggesting systemic changes may have utility as a measurement of target saturation and dosing independent of tumor growth. Finally, with current approvals of PD-1 inhibitors in renal cell carcinoma (RCC) patients previously treated with antiangiogenic agents that block vascular endothelial growth factor (VEGF), we evaluated plasma in mouse models of sunnithressistance - a VEGF receptor tyrosine kinase inhibitor (RTKI). Our results demonstrate that VEGF pathway resistance yields changes in sPD-L1 and may be useful in predicting response to PD-1 pathway inhibition in the refractory setting. Together, these investigations suggest that circulating sPD-L1 changes during disease progression (both local and disseminated) may serve as a potential predictive biomarker for immune-checkpoint and antiangiogenic therapy.

**#5600 Simultaneous measurement and clinical significance of PD-1, LAG-3 and TIM-3 in non-small cell lung cancer (NSCLC).** Ila J. Datar,1 Jun Wang,1 Nikita Mani,1 Franz Villarroya-Espindola,2 Patrick Ryan,1 Miguel F. Sammaded,2 Kristen McCaehern,2 David Jenkins,2 David L. Rimm,1 Leiping Chen,1 Roy Herbst,1 Kurt Schalper1.

**Introduction:** The ineffective anti-tumor immune response is characterized by increased immune suppressive signals in the tumor microenvironment. In particular, T-cells recognizing tumor antigens can express diverse immune inhibitory receptors mediating lymphocyte inactivation and limiting tumor rejection. Blockade of these receptors such as PD-1 induces promising clinical benefit in patients with NSCLC. However, the expression and significance of additional potentially actionable immune inhibitory receptors in lung cancer is poorly understood. Methods: After careful validation of assays and using multiplexed quantitative immunofluorescence (QIF) we measured the levels of CD3 (rabbit polyclonal, Dako), PD-1 (clone EH33, CST), LAG-3 (Clone 17B4, Abpoorly understood. Methods: After careful validation of assays and using multiplexed quantitative immunofluorescence (QIF) we measured the levels of CD3 (rabbit polyclonal, Dako), PD-1 (clone EH33, CST), LAG-3 (Clone 17B4, Ab-poorsystematic disease progression (after surgical removal of the primary). Mouse syngeneic and human xenograft implantation models included breast, kidney, colon, and melanoma cell systems. Our results show that circulating plasma sPD-L1 can correlate with primary and metastatic progression in a stage and model specific manner. Next, we evaluated sPD-L1 following treatment with neutralizing antibodies to PD-1 and PD-L1 in tumor-free mice and found significant dose-dependent sPD-L1 increases, suggesting systemic changes may have utility as a measurement of target saturation and dosing independent of tumor growth. Finally, with current approvals of PD-1 inhibitors in renal cell carcinoma (RCC) patients previously treated with antiangiogenic agents that block vascular endothelial growth factor (VEGF), we evaluated plasma in mouse models of sunnithressistance - a VEGF receptor tyrosine kinase inhibitor (RTKI). Our results demonstrate that VEGF pathway resistance yields changes in sPD-L1 and may be useful in predicting response to PD-1 pathway inhibition in the refractory setting. Together, these investigations suggest that circulating sPD-L1 changes during disease progression (both local and disseminated) may serve as a potential predictive biomarker for immune-checkpoint and antiangiogenic therapy.

**#5602 Regulation of immune checkpoint genes revealed by a melanoma tumor genome atlas (TCGA) analysis - potential implications for improving immunotherapy.** Raya Leibowitz-Ami,1 Jason Roszik,2 Doro Avni,1 Elizabeth Grimm,3 Sheba medical center, Tel Hashomer, Israel;4 The University of Texas MD Anderson Cancer Center, TX.

**Introduction:** The interface between T lymphocytes and cancer or antigen presenting cells (C/ APCs) is multi-faceted and complex. This interface, now designated the ‘immunological synapse’, comprises of both co-inhibitory and co-stimulatory transmembrane protein pairs (‘checkpoint proteins’) that serve to modulate the signal transmitted to the T lymphocyte, leading to either activation, anergy or exhaustion. Immune checkpoint inhibitors have been successful in reactivating the T lymphocyte in a variety of malignancies, but not all patients respond to these agents. We undertook TCGA analysis of a melanoma tumor genome atlas database to identify immune-checkpoint inhibitors that may serve to be master regulators of gene expression. Our aim was to study the associations between a miRNA that was previously implicated in cancer and immune checkpoint genes. Methods: Bioinformatic analyses of the expression of mRNAs and miRNAs from 451 samples was performed using the melanoma TCGA database. Correlation coefficients between the expression of mRNAs or miRNAs/miRNAs were calculated using the Spearman rho method. Survival analysis was performed using the Kaplan-Meier method. Potential 3’UTR binding sites of miRNAs were found using the web-based tool www.targetscan.org. Results: Of 22 miRNAs of checkpoint genes assessed, the expression of 19 was highly posi-tionential correlations, TGIF was the best method to use in this predictive model. We undertook in vivo experiments to evaluate plasma sPD-L1 in mouse tumor models during localized primary tumor growth (after orthotopic cell implantation) and spontaneous metastatic disease progression (after surgical removal of the primary). Mouse syngeneic and human xenograft implantation models included breast, kidney, colon, and melanoma cell systems. Our results show that circulating plasma sPD-L1 can correlate with primary and metastatic progression in a stage and model specific manner. Next, we evaluated sPD-L1 following treatment with neutralizing antibodies to PD-1 and PD-L1 in tumor-free mice and found significant dose-dependent sPD-L1 increases, suggesting systemic changes may have utility as a measurement of target saturation and dosing independent of tumor growth. Finally, with current approvals of PD-1 inhibitors in renal cell carcinoma (RCC) patients previously treated with antiangiogenic agents that block vascular endothelial growth factor (VEGF), we evaluated plasma in mouse models of sunnithressistance - a VEGF receptor tyrosine kinase inhibitor (RTKI). Our results demonstrate that VEGF pathway resistance yields changes in sPD-L1 and may be useful in predicting response to PD-1 pathway inhibition in the refractory setting. Together, these investigations suggest that circulating sPD-L1 changes during disease progression (both local and disseminated) may serve as a potential predictive biomarker for immune-checkpoint and antiangiogenic therapy.

**Conclusion:** We also included a collection of lung adenocarcinomas with molecular annotation (cohort #4 [Yale n=106]). The targets were measured in all cells of the preparation using fluorescence co-localization with DAPI and specifically in CD3-positive T-lymphocytes. Associations between the markers and with major clinicopathological variables, driver mutations and survival were studied. Results: All the targets were detected predominantly in CD3+ T-cells with membranous staining. Expression of PD-1, LAG-3 and TIM-3 in T-cells across all NSCLC cohorts was 68.7%, 39.7% and 55.8%, respectively. Elevated levels of PD-1, LAG-3 or TIM-3 were significantly associated with increased tumor in filtrating lymphocytes and with the co-expression of one or more of the other inhibitory receptors (p < 0.05). SIMGLO and enhanced tumor cell-dependent independent antitumor action of anti-PD1 antibodies. We first explore the exact prevalence and magnitude of PD1 expression in patients and cell lines. Then we test the effect of MAPKi on its expression and explore possible immune-independent synergies with anti-PD1 antibodies. Methodology and results. Prevalence of PD1 expression by melanoma cells was explored in the Cancer Cell Line Encyclopedia (CCLE) and The Cancer Genome Atlas (TCGA) datasets. CCLE included 61 melanoma cell lines, which all expressed PD1 (Affymetrix mRNA 4.20 [3.81 - 4.65]) with values comparable to PDL1 (4.63, range 3.73 - 7.84) and PDL2 (3.73, range 3.97-8.30). The TCGA analysis included 114 melanomas, excluding those with evidence of immune infiltrate (n=236) or stromal
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cells (n=100) potentially interfering with PD1 assessment. PD1 mRNA was expressed by all samples (median FPKM values: 64.7, range 0.4-1461.3). Next we assessed by flow-cytometry the membrane expression of PD1 in 4 melanoma cell lines and 3 patient-derived melanoma cultures, harboring BRAF and NRAS mutations. We confirmed that low rates of PD1 were expressed by all melanoma (median 1.2%, range 0.7-4.3). The median rate of viable PD1+ melanoma cells significantly increased (12.9% range 5.9-20, p=0.0180, n=7) following treatment (96h) with MAPKi (dabrafenib and trametinib) but not with chemotherapy (fotemustine). Longer treatments (8 days) further increased the rate of PD1 melanoma cells (31.8% range 15.0-50.3, n=3) and the effect was reversed by drug withdrawal. The MAPKi modulatory effect on PD1 expression was therapeutically exploited in vitro. The addition of PD1 blocking antibody enhanced the inhibitory activity of MAPKi on melanoma proliferation (mean inhibition rate: 66% vs 40%, n=2) in the absence of any immune cells. Conclusions We report a new expression pattern of PD1 receptor on melanoma and its potential therapeutic perspective. PD1 is directly expressed by a small but consistent rate of melanoma cells. Treatment with MAPKi significantly enhanced the rate of PD1+ melanoma cells and direct synergism with PD1 blockade was reported without the involvement of immune response. Our findings may be clinically relevant in settings of patients treated with MAPKi.

#5604 Novel biomarkers Ly6K/E play important role in drug resistance and tumor immune escape. Midar A. Hossiny,1 William R. Frazier,1 Noriko Steinier,1 Bhaskar Kallakury,1 Rakesh Kumar,1 Geeta Upadhyay,2 1Georgetown Univ., Washington, DC; 2Georgetown Washington, Washington, DC. Stem cell antigen Sca-1 is implicated in murine cancer stem cell biology and breast cancer models, but the role of its human homolog Ly6K and Ly6E in breast cancer is not established. Here we report increased expression of Ly6KE/E in human breast cancer specimens correlates with poor overall survival, with an additional specific role for Ly6E in poor therapeutic outcomes. Increased expression of Ly6KE/E also correlated with increased expression of the immune checkpoint molecules PD1 and CTLA4, increased tumor infiltrating T regulatory cells, and decreased natural killer (NK) cell activation. Mechanistically, Ly6K/E was required for TGFβ signaling and proliferation in breast cancer cells, where they contributed to phosphorylation of Smad1/5 and Smad2/3. Furthermore, Ly6K/E promoted cytokine-induced PD1L expression and activation and binding of NK cells to cancer cells. Finally, we found that Ly6K/E promoted drug resistance and facilitated immune escape in this setting. Overall, our results establish a pivotal role for a Ly6K/E signaling axis involving TGFβ in breast cancer pathophysiology and drug response, and highlight this signaling axis as a compelling realm for therapeutic invention.

#5605 Frequencies of HLA-Class I and II alleles between German patients with renal cell carcinoma and healthy controls can be different. Steffen Göbel,1 Astrid Kehlen,1 Karen Bluemke,1 Wolfgang Altermann,1 Gerald Schlaf,1 Kersten Fischer,1 Paolo Fornara,2 Bernd Wullich,2 Sven Wach,2 Helge W. Taubert2,1 Univ. of Halle, Halle, Germany;2 Univ. of Erlangen, Erlangen, Germany. The human leukocyte antigen (HLA) system is a major part of the human immune system and has an impact on tumor initiation, tumor progression and immunosurveillance. Renal cell carcinoma tumors are considered to be immunogenic. Therefore, we studied the allele frequencies of four gene loci (HLA-A, -B, -C and HLA-DR) in a cohort of 106 German renal cell carcinoma (RCC) patients and in 201 healthy controls. HLA-A-C were determined using serological methods, whereas HLA-C12, C14, C16, C18 and HLA-DR were characterized through the use of standard molecular biological methods. The presence of HLA-A and HLA-B alleles did not differ significantly among RCC patients and healthy controls. However, the occurrence of the HLA-C*12 allele was significantly increased in German RCC patients compared with healthy controls (P=0.0055; RR=2.3; Fisher’s exact test), whereas the occurrence of the HLA-DRB1*04 allele was significantly reduced in RCC patients compared with healthy controls (P<0.05; RR=0.7; Fisher’s exact test). But the presence of allele HLA-C*12 was not significantly associated with 10 years overall survival. We suggest that the frequency of HLA alleles can affect development of RCC and could add knowledge as predictive marker for future immunotherapies.

#5606 Fibrinogen-like protein 2 drives malignant tumor progression in glioma. Khetri Latha,1 Jun Yan,1 Yuhui Yang,1 Loyal V. Gressot,2 Lingyun Kong,1 Ganiraju Manyam,1 Ravesanker Ezhilarasan,1 Qianghu Wang,1 Erik P. Talbott,2 Kersten Fischer,1 Paolo Fornara,1 Bernd Wullich,2 Sven Wach,2 Helge W. Taubert2. 1Georgetown Univ., Washington, DC; 2Georgetown Washington, Washington, DC. Gliomas are the most common type of brain tumor in both children and adults. Several low-grade gliomas (LGG) have the ability to progress into more aggressive tumors -high-grade gliomas (HGG) including glioblastoma (GB). Although patients harboring a LGG may survive for years, after the tumor transforms to HGG, life expectancy rapidly declines to 12 to 15 months in adults and 40 months in children. A more indolent therapeutic strategy (MT) is an attractive therapeutic strategy because of the more indolent course associated with LGGs. Immune response plays a critical role in surveillance against malignant transformation. Our previous study shows that fibrinogen-like protein 2 (FGL2) acts as a hub of tumor-mediated immune suppression. Hence, we investigated the role of FGL2 in promoting tumor progression in glioma. Analysis of TCGA glioma dataset showed that the expression of FGL2 was significantly upregulated in HGG compared with LGG and GB patients. And there is a positive correlation of expression level between FGL2 and mesenchymal glioma marker CD44, and a negative correlation between FGL2 and proneural glioma marker Olig2. Engineered expression of FGL2 in a PDGFR-dependent mouse model of oligodendroglioma, a common glioma subtype, yielded a significantly higher rate of HGGs (72% vs 29%, p=0.034) and poorer-symptom free survival (63 vs 90 days, p=0.003) than PDGFR expression alone. And HGGs from FGL2 + PDGFR expressing mice exhibited a distinct mesenchymal phenotype validating TCGA data. Further, FGL2 induced high numbers of CD4+FoxP3+ cells from an early time point of tumor formation underscoring its role in tumor progression. And FGL2 overexpression induced M2-like M2-like tumor macrophages characterized by high expression of FGL2 and Arginase1 in macrophages. Finally, treatment with anti-FGL2 antibody significantly improves survival in mice, shifts the phenotype from mesenchymal HGG to neural LGG, and rescues M2 macrophage skewing. Our results show that FGL2 is critical for malignant progression of glioma by inducing immunosuppression in tumor microenvironment, and raise the potential of FGL2 to be a promising target to suppress/reverse glioma progression and provide survival benefit in clinical.
pass whole genome sequencing enables high quality genome-wide profiling of pure single cells and pools isolated from phenotypically distinct populations in FFPE tumor tissue.

**#5608 Correlation of phosphatase activity with lymphocyte infiltrates in metastatic renal cell carcinoma tissues.** Rob Kuijtenbeek,1 Liesbeuk Houkes-van Kerkhoff,1 Jeanette Oosterwijk,2 Liesbeuk Homest-Bijl,1 Riet Hildorest,1 Arie J. van den Elzen,2 Juan F. Rodriguez,3 Jesus Garcia-Domas,3 Bart Kiemeneij,2 Egbert Oosterwijk,2 VanGene International B.V., BF’s-Hertogenbosch, Netherlands; Rodaboud University Medical Center, Nijmegen, Netherlands; Centro integral oncológico Clara Campal, Madrid, Spain.

Introduction: Protein tyrosine phosphatases (PTPs) are important regulators of signal transduction in immune cells. While most analytical methods focus on the detection of these crucial enzymes at the RNA or protein level, a method was lacking to monitor multiple enzymatic activities in patient-derived materials like blood and (tumor) tissues. Such a substrate-specific assay is a new tool for biomarker discovery in immuno-oncology (I-O), because the I-O targets - checkpoint and immune receptors like PD1, CTLA4, LAG3, 4-1BB, CD40, CD20, OX40, TIGIT and GTR - are controlled by phosphatases. The aim of this study was to develop a multiplex PTP assay and investigate the phosphatase activity in metastatic renal cell carcinoma (mRCC) tissues with high and low levels of TILs (tumor-infiltrating lymphocytes). Methods: On the basis of the hema-toxyn and eosin staining of mRCC tissues, 9 tissues with high TILS-scores were selected, as well as 10 tissues without TILs, matched on basis of the percentage of viable tumor cells. The technology used is a second generation peptide microarray, which is fully automated with short turnaround times and a high throughput. Discriminating phosphosites (p<0.0002) were derived from the following 10 signaling proteins RET, ERBB2, PAXI, PGFR, CBL, FRK, DPKK1, INSR, PECAM1 and the T-cell kinase LCK. Conclusions: Proof of concept was shown in mRCC samples that high PTP activity correlates with high levels of TILs. As a TILS-score itself is already regarded to be a candidate biomarker for I-O therapy response, the observed correlation is a basis for the development of more mechanistic biomarkers predicting therapy response. This method can be a starting point for the development of an enzymatic and thus sensitive and quantitative TILs test. This study has received funding from the European Union’s Seventh Framework Programme (FP7/2007-2013) under grant agreement no 259939.

**#5609 Macrophages in secondary lymphoid organs regulate T cell tolerance and immunosurveillance in pancreatic cancer.** Fee Bengsch, Amni Liu, Kathleen Graham, Gregory L. Beatty, UPenn, Philadelphia, PA.

Pancreatic ductal adenocarcinoma (PDAC) is a deadly disease that shows striking resistance to standard therapies. Immunotherapy is an exciting new treatment strategy for PDAC, but has yet to reproducibly demonstrate clinical efficacy. This finding may reflect poor effector T cell infiltration into PDAC or mechanisms of peripheral T cell tolerance. We hypothesize that successful immunotherapy in PDAC will require strategies that reverse elements of innate immunity that regulate T cell tolerance. This hypothesis is based on our prior studies in the Kras12D12V/+/Trp53R172H/+/Pdcd1-cre (KPC) mouse model of PDAC where macrophages residing outside of the tumor microenvironment were found to regulate the efficacy of a vaccine strategy involving the combination of chemotherapy and a CD40 agonist (FGK45). Specifically, we found that depletion of peripheral phagocytes using clodronate-encapsulated liposomes (CEL) was necessary for gemicitabine chemotherapy and a CD40 agonist to induce productive anti-tumor T cell immunity against spontaneously arising PDAC tumors in the KPC model. We now investigate the mechanism by which CEL-depleted phagocytes regulate T cell tolerance in PDAC using the KPC mouse model and an orthotopic implantation model. We found that CEL depletes macrophages in the spleen and abdominal lymph nodes, thereby reversing the pathologic accumulation of macrophages in these secondary lymphoid organs that is induced by PDAC tumors. CEL targeted two macrophage subsets in the lymph node, CD11b+CD169+F4/80+ subcapsular sinus macrophages (SSM) and CD11b+CD169+F4/80+ medullary sinus macrophages (MSM). These macrophage populations may be exposed to tumor-derived antigens and apoptotic cell vesicles entering the lymph node, and have been previously identified as central orchestrators of tolerance in autoimmune models. We found that both fluorescently-labeled liposomes and OVA-FITC lipoparticle injected into the peritoneal cavity were specifically targeted to SSM and MSM with little uptake by dendritic cells (DC). In contrast, depletion of these first-line phagocytes by CEL shifted the cellular uptake of these model antigens increasing uptake by CD11b+DC11c+ DC which are more efficient in antigen presentation and show increased expression of co-stimulatory molecules after FGK45 treatment. Using an orthotopic model of PDAC, we then determined that CEL can enhance the T cell stimulatory capacity of gemcitabine chemotherapy in combination with FGK45 as seen by a significant increase in CD38, CD44, CD69 and PD-1 expression on CD4 and CD8 T cells in secondary lymphoid organs. Together, our findings suggest that tumor-derived antigens may be sequestered by tolerogenic macrophages in peritumoral lymph nodes that act to impair optimal stimulation of T cell responses. These findings strategies to disrupting the tolerogenic functions of these macrophages may offer a novel approach for improving immunotherapy in PDAC.

**#5610 Quantitative cell-based bioassays to advance individual or combination immune checkpoint immunotherapy.** Jamison Graaler, Pete Stecha, Denise Garvin, Jim Hartnett, Frank Fan, Mei Cong, Zhi-jieh Cheng, Promega Corp., Madison, WI.

Immune checkpoint receptors play a critical role in maintaining immune homeostasis and are genetically and functionally associated with autoimmune disease, cancer and persistent viral infections. Blockade of immune checkpoints (e.g., PD-1 and CTLA-4) has emerged as a promising new approach to enhance anti-tumor immune responses. While immunotherapies directed against PD-1 and CTLA-4 are showing unprecedented efficacy in the treatment of cancer, many patients and types remain refractory to these therapies. This has resulted in a broadening of immunotherapy research and development to include additional immune checkpoint receptors (e.g., LAG-3, TIGIT, CD112R) targeted individually or in combination with other immunotherapy strategies. A major challenge in the development of biologicals that target immune checkpoint proteins is access to quantitative and reproducible functional bioassays. Existing methods rely on primary cells and measurement of complex functional endpoints. These assays are cumbersome, highly variable, and fail to yield the quality of data that is required for drug development in a quality-controlled environment. To address this need, we have developed a suite of immune cell line-based bioluminescent reporter bioassays for individual and combination immune checkpoint immunotherapy targets including PD-1 (PD-L1 or PD-L2), CTLA-4, LAG-3, TIGIT, PD-1+TIGIT and more. These assays consist of stable cell lines that express luciferase reporters driven by specific response elements under the precise control of intracellular signals mediated by the T cell receptor and immune checkpoint target(s). These mechanism of action-based bioassays are available in “thaw-and-use” format and demonstrate high specificity, sensitivity and reproducibility. The bioassays are pre-qualified according to ICH guidelines and demonstrate the performance required for use in antibody screening, potency testing and stability studies.

**#5611 Use of ecto-domain vimentin to target brain cancers.** Ivan Babic,1 Rajesh Mukthavaram,1 Pengfei Jiang,1 Eric Glassy,2 Natsuko Nomura,3 Sandeep Pingle,3 Mark C. Glassy,2 Santosh Kesari,3 John Wayne Cancer Institute, Santa Monica, CA; Pathology, Inc., Torrance, CA; Nascent Biotech, Inc., San Diego, CA.

Pritumumab is a natural human IgG1 kappa antibody derived from a regional draining lymph node of a patient with cervical carcinoma. The recognized antigen is an altered form of vimentin, called ecto-domain vimentin (EDV), that is expressed on the cell surface of epithelial tumor cells. For brain cancers, immunohistological analysis showed the binding of pritumumab to EDV that was restricted to tumor cells and types remain refractory to these therapies. This has resulted in a broadening of immunotherapy research and development to include additional immune checkpoint receptors (e.g., LAG-3, TIGIT, CD112R) targeted individually or in combination with other immunotherapy strategies. A major challenge in the development of biologicals that target immune checkpoint proteins is access to quantitative and reproducible functional bioassays. Existing methods rely on primary cells and measurement of complex functional endpoints. These assays are cumbersome, highly variable, and fail to yield the quality of data that is required for drug development in a quality-controlled environment. To address this need, we have developed a suite of immune cell line-based bioluminescent reporter bioassays for individual and combination immune checkpoint immunotherapy targets including PD-1 (PD-L1 or PD-L2), CTLA-4, LAG-3, TIGIT, PD-1+TIGIT and more. These assays consist of stable cell lines that express luciferase reporters driven by specific response elements under the precise control of intracellular signals mediated by the T cell receptor and immune checkpoint target(s). These mechanism of action-based bioassays are available in “thaw-and-use” format and demonstrate high specificity, sensitivity and reproducibility. The bioassays are pre-qualified according to ICH guidelines and demonstrate the performance required for use in antibody screening, potency testing and stability studies.
binding in tumor areas of brain tissues indicating the mAb crosses the tumor brain barrier. Overall, these data together suggest that EDV is a suitable target and biomarker for brain cancers.

#5612 Cancer-immune interactions in luminal breast cancers: PIK3CA mutations, PIK3CA/akt/mTOR activation and tumor-infiltrating lymphocytes. Marcelo Sobral-Leite,1 Izhar Salomon,1 Mark Opdam,1 Karin Beelen,1 Ronald L. van Vlerken,1 Erik J. Blok,2 Hugo M. Horlings,1 Joyce Sanders,1 Koen Van de Vijver,1 Peter J. Kuppen,1 Sabine Linn,1 Marjanka J. Schmidt,1 Marleen Kok1. 1The Netherlands Cancer Institute, Amsterdam, Netherlands; 2Leiden University Medical Center, Leiden, Netherlands.

Introduction: Therapy resistance to adjuvant tamoxifen in estrogen receptor positive (ER+) breast cancer (BC) is related to activation of downstream proteins in the PI3K/akt/mTOR pathway. However, clinical activity of mTOR inhibitors has so far been modest. Growing evidence shows that this suggests a need for understanding the relationship between tumor-related immune responses and this is reflected in the presence of tumor infiltrating immune cells. This study investigated a role of tumor-related immune response.

Methods: The IAK trial recruited stage I to III postmenopausal BC patients (1982 till 1994), who were randomized to tamoxifen or no adjuvant therapy. Sequenom mass spectrometry-based genotyping was performed by PIK3CA assessment. Immunomarker and phosphorylation status of proteins in the PI3K/akt/mTOR pathway (p-akt, Thr308 and 473), p-mTOR, p-ERK1/2, p-p70S6K and p-4EBP1 were assessed by immunohistochemistry and scored by two pathologists. Expression of LAG3 and TIM3 was evaluated by using automatic scoring by image-analysis software (SlidePath® or AxioVision®) and compared with manual scoring by two pathologists. Associations were assessed using multivariable logistic regression models, including as co-variables: age, tumor grade, lymph node status, tumor size, and progesterone receptor and HER2 status. Results: We included 563 ER+ BC cases. PIK3CA mutations were found in 159 (32%) of the 486 tumors genotyped. On average, PIK3CA/akt/mTOR downstream proteins and immune markers were scored in 409 tumors (range: 366 to 438). Stromal CD8 expression, but not CD4 or FOXP3, was significantly higher in PIK3CA mutated tumors (OR = 1.11; 95%CI: 1.02-1.22). Stromal FOXP3 expression, but not CD4 or CD8, was significantly increased in tumors with activated proteins from the PI3K/akt/mTOR pathway (except p-mTOR). The largest association was with p-4EBP1 (OR = 1.34; 95%CI: 1.21-1.48) and smallest with p-70S6K (OR =1.15; 95%CI: 1.08-1.22).

Conclusion: In our dataset of ER+ BC, PIK3CA mutations are associated with higher level of CD8+ T cells. Tumors with activation of the PI3K/akt/mTOR pathway tend to have more FOXP3+ T cells. Together, our results suggest that PIK3CA mutation/activation harbor an immune-related tumor microenvironment.

#5613 PD-1 and TIGIT are major immune checkpoint receptors expressed in breast cancer-infiltrating T cells. Jee Ye Kim,1 Minsuk Kwon,2 Sung Mook Lim,1 Joo Heung Kim,1 Hyung Seok Park,1 Seho Park,1 Seung Il Kim,1 Young Up Cho,1 SooMyung Paik,2 Eui-Cheol Shin.1 1Yonsei University College of Medicine, Seoul, Republic of Korea; 2KAIST, Daejeon, Republic of Korea.

Immune checkpoint receptors, which are inhibitory receptors on T cells, have provided promising responses against various tumors. However, the expression of immune checkpoint receptors (ICRs) in BC cancers remains poorly understood. We aimed to investigate the expression pattern of multiple ICRs in tumor-infiltrating and peripheral blood (PB) lymphocytes in human BC cancer. We isolated lymphocytes from fresh breast tumor tissue and paired PB from 21 patients who underwent surgery between July 2016 and November 2016. Multi-color flow cytometry was performed primarily focusing on expression of multiple ICRs in CD8+ T cells and regulatory T cells (Tregs). In CD8+ T cell subsets, PD-1+ or TIGIT+ cells were more frequently observed in tumor-infiltrating CD8+ T cells than in PB CD8+ T cells (p<0.0001 and p=0.02, respectively). Around half of the tumor infiltrating CD8+ T cells expressed PD-1 (median 49.5%, range 0-94%). In Tregs, about 40% of tumor-infiltrating CD8+ T cells were PD-1+TIGIT+, and >70% of PD-1+ tumor-infiltrating CD8+ T cells co-expressed TIGIT, indicating that functional exhaustion of breast tumor-infiltrating CD8+ T cells might be mediated not by PD-1 alone but by multiple receptors including TIGIT. However, the expression of PD-1 and TIGIT showed different patterns in detail. PD-1 was frequently expressed by CCR7+CD45RA− effector memory T cells (TEM) (p=0.001) whereas TIGIT was frequently expressed by CCR7+CD45RA− effector memory RA T cells (TEMRA) (p=0.03), suggesting that TIGIT is expressed during terminal differentiation of CD8+ T cells. Next, we examined breast tumor-infiltrating Tregs. The frequency of CD25+ FoxP3+ Tregs among CD4+ T cells were higher in tumor than in PB (16.60% vs. 7.86%; p=0.002). In particular, CD45RA FoxP3+ activated suppressive Tregs account for 77.6% of tumor-infiltrating Tregs (vs. PB 32.2%; p=0.005). Tumor-infiltrating Tregs showed higher expression of CD39 (p=0.005), a marker of the suppressive activity of Tregs. We also examined the expression of multiple ICRs as upregulated receptors is associated with enhanced suppressive activity of Tregs. CD4+ CD25+ FoxP3+ Tregs in tumors showed higher expression levels of PD-1, TIGIT and CTLA-4 compared to PB Tregs (p<0.05). There were no noteworthy correlations between ICR expression and clinical features, such as age, stage and subtype. We show that PD-1 and TIGIT are major ICRs expressed in breast tumor-infiltrating T cells. Moreover, we found that CD4+ CD25+ FoxP3− Tregs are abundant in breast tumors and upexpress PD-1, TIGIT and CTLA-4. Our data provide an understanding of comprehensive phenotypes of immune checkpoint expression on T cells in breast cancer. Functional changes of CD8+ T cells and Tregs by blocking of single or multiple ICRs are being investigated.


Chimeric Antigen Receptor (CAR) T-cells made with mRNA offer a transient and safe alternative to viral CARs, mitigating the concern for persistent unwanted side effects from constitutively active T-cells. Previous studies have shown that mRNA CARs are transiently effective, but lack CAR persistence and survival as compared to viral CARs. The efficacy of mRNA CARs could be improved by utilizing recent advancements in RNA technology including the use of modified uracil and a novel purification method with RNaseIII to prevent double strandeding that induces toxicity. Using the established CD19 CAR model in B-cell acute lymphoblastic leukemia, we created mRNA CARs using previously described methods and compared them to mRNA CARs created using modification of uracil and/or purification to remove any aberrant double stranded mRNA. Comparing the modified and purified mRNA CAR T cells to those created using prior methods of making mRNA, both modified and purified mRNA CAR T cells showed a two-fold increase in expression of the CAR on their surface initially, as well as a two-fold improvement in cytotokic killing of leukemia cells in vitro that persisted for up to five days. Both the modified and purified mRNA CAR T cells also showed reduced expression of negative checkpoint regulators compared to original RNA CAR T cells. However, in vivo studies using a patient-derived xenograft model with a single dose of CAR T cells revealed purified RNA CAR T cells offered the most robust 2-log enhanced suppression of leukemic burden. RNaseIII is a novel purification technique that has not yet been reported in the literature for RNA manufacturing. Our results provide a time efficient purification method that can be easily incorporated into RNA production for use in clinical trials, and poise RNA CARs for increased efficacy as new CAR targets emerge and are being tested. Additional studies are ongoing investigating if the observed improved efficacy will translate to improved cytotokicity in solid tumor models.

#5615 Genomic differential expression analysis of fusion proteins incorporating the pro-apoptotic molecule Granulocyte B reveals new potential targets for treatment of breast cancer. Khalid A. Mohamedalli, Lawrence H. Cheung, Ana Alvarez-Cienfuegos, Michael G. Rosenblum. UT MD Anderson Cancer Center, Houston, TX.

Granulocyte B (GRB) is a member of the serine protease family of enzymes that play a critical role in the body’s defense against viral infection and tumor development. Cytotoxic T lymphocytes (CTL) and natural killer (NK) cells directly deliver granymes to target cells, which induces apoptosis through both caspase-dependent and caspase-independent multiple-cascade mechanisms. Anti-tumor efficacy studies have suggested that the targeted delivery of human Granyme B to tumor cells has a significant potential for cancer treatment. We had previously developed a novel fusion construct composed of the VEGF121 growth factor and human GRB. The GRB/VEGF121 construct was found to be highly cytotoxic to vascular endothelial cells expressing the R (KDR) receptor for VEGF. In this study, we examined the mechanism of GRB/VEGF121 induced cytotoxicity against cells in culture at the genomic level. The TNBC cell line MDA-MB-231 was treated with an IC50 dose of GRB/VEGF121 for 24 hours; the cells were then harvested and the effect of GRB/VEGF121 on intracellular events was examined by extraction of mRNA followed by microarray analysis. Gene level differential expression analysis revealed that a total of twenty genes were upregulated by over 3-fold, while 25 were downregulated 3-fold or more. These included genes involved in signal transduction, stress response, cell cycle control, hypoxia and metabolism. Particularly impacted were the Nuclear Receptors
Meta pathway, NRE2 pathway, nuclear receptors in lipid metabolism and toxicity, matrix metalloproteinases, and the notch signaling pathway. Validated data will be reported following complete analysis of gene level differential expression and alternative splicing. Our data suggests that Grb/VEGF121 induce expression of genes known to be induced by VEGF alone, as well as molecules previously not associated with either Granzyme B or VEGF. This data suggests a previously unsuspected impact of the serine protease Grb on various molecular pathways within the cell and may lead to a new understanding of how these agents operate at the molecular level. Research conducted, in part, by the Clayton Foundation for Research.


Background: CD137L is expressed on antigen presenting cells and CD137/CD137L interaction co-stimulates activation and proliferation of CD137 expressing T cells. CD137L expression has also been detected on various cancer cells. While signaling in immune cells showed both inhibiting and activating effects the impact of CD137L induction on tumor cells is poorly understood. In this study, the expression of CD137 and CD137L and the effects of CD137L activation were analyzed in human colorectal cancer (CRC). Methods: Expression of CD137 and CD137L was examined in human colon cancer cell lines HT29, SW480 and SW620 treated with or without hypoxia using RT-qPCR, Western blot and FACS analysis. Its differentiated expression was further analyzed by immunohistochemistry in human CRCs by immunofluorescent double-staining. To dissect the effects of CD137L activation on cancer cells, MTS proliferation assays were performed. Results: All human colon cancer cell lines demonstrated high cellular (FACS) and protein (Western Blot) CD137L expression. CD137L was found only in SW480 cells at weak expression. Additionally, both CD137L and CD137L were significantly upregulated in cells treated with hypoxia. In human CRCs both CD137 and CD137L expressing tumor cells were detected by immunofluorescent double-staining. Interestingly, activation of CD137L by immobilized CD137-Fc resulted in reduced cancer cell proliferation compared to IgG1-Fc controls. Conclusion: Our results demonstrate tumor cell mediated expression of the costimulatory molecule CD137L and its ligand CD137L in both colon cancer cell lines and human colorectal cancer. Moreover, CD137L reverse signaling was found to reduce cancer cell proliferation in vitro. Based on these data, the CD137L/CD137L signaling pathway seems to present a promising target for cancer therapy in CRC.

#5617 Combined intratumoral electroporation and allogeneic vaccina- tion of Gp96-Ig/Fc-OX40L stimulates CD8 T cell cross-priming to tumor-specific neoantigens and enhanced anti-tumor response. Sushil de Silva, Sandro Mazarati, Nandana N. Seneviratne, Li-Qi Cai, George Fromm, Anandaroop Mukhopadhyay, Jean Campbell, Robert Pierce, Taylor Schreiber. Heat Biologics, Durham, NC; Oncoselect Medical Inc., San Diego, CA.

Growing clinical evidence has unequivocally proven that combination approaches are leading the way in increasing the overall response rates in cancer immunotherapy towards tumor eradication. Most cancers are characterized by a higher likelihood of succeeding if the targeted tumor has a preexisting state of inflammation elicited by the combined presentation of shared- and neo-antigens from tumor cells. Certain cancers have been deemed to be non-immunogenic due to tumor intrinsic strategies that evade immune recognition. Thus, novel combination treatment modalities are needed to convert non-immunogenic, ‘cold’ tumors into inflamed ‘hot’ tumors that are amenable to IT. Gp96-Ig/Fc-OX40L is a re-engineered molecular chaperone, designed to export and deliver MHCI-associated antigens to APCs in context of the immunostimulatory, OX40L. Allogeneic cancer vaccine cell lines designed to co-secrete Gp96-Ig and Fc-OX40L, generate antigen-specific CD4+ and CD8+ anti-tumor responses in both highly immunogenic (CT26) and less immunogenic (B16) mouse tumor models (Fromm et al., Cancer Immunol Res, 2016). Such a strategy allows for Gp96-Ig-mediated chaperoning of antigens from the allogeneic vaccine cell line (shared antigens), which could benefit further from increased presentation of tumor-derived peptides (neo-antigens) that are only accessible if Gp96-Ig/Fc-OX40L is expressed from within the tumor. To achieve this, we have employed an in vivo electroporation-based strategy (EP) to deliver Gp96-Ig/Fc-OX40L expressing tumor cells into the flank. Herein, we set out to test whether a combination approach of intratumoral EP of Gp96-Ig/Fc-OX40L DNA and vaccination with allogeneic cells co-secreting the same effector molecules would lead to enhanced CD4+/CD8+ T cell cross-priming to tumor neo-antigens and superior anti-tumor activity over the individual monotherapies in a non-immunogenic B16 tumor model. To assess antigen-specific CD8+ T cell expansion, mice were adoptively transferred with OT-I cells after B16-ovalbumin cells were injected to generate primary and contralateral melanotic tumors. Contralateral tumors were monitored to assess whether a systemic CD8+ T cell response could be elicited following vaccination and primary tumor EP. The combination approach of intratumoral EP of Gp96-Ig/Fc-OX40L and vaccination with allogeneic cells co-secreting the same effector molecules was superior to EP alone in the peripheral blood compared to the individual monotherapies. A synergistic increase in SIINFEKLI-positive CD8+ TIL cells was also observed in treated tumors, which was associated with superior anti-tumor response in both the EP-treated primary and untreated contralateral tumors. These findings suggest that a combination approach of allogeneic vaccination and in situ tumor EP of Gp96-Ig/Fc-OX40L may have significant benefit in eliciting a potent immune response in less-immunogenic tumors.

#5618 Correlation between MET gene amplification and TP53 mutation in upregulating PD-L1 expression in EGFR wild-type lung cancer. Maher Albitar, MD, Sucha Sudarsanam, MS, Wanlong Ma, BS, Shingping, MD, Wayne Chen, MD, Vincent Funari, PhD, Steven Brodie, PhD, Sally Agerborg, MD, PhD. NeoGenomix Laboratories, Aliso Viejo, CA.

Introduction: MET gene activation has been reported to be associated with resistance to EGFR inhibitors in lung cancer. Resistance to EGFR inhibitors is also reported to be associated with upregulation of PD-L1. We studied PD-L1 expression levels with MET gene amplification and mutation in EGFR, KRAS, or TP53 in a large number of primary untreated lung cancers. Methods: Tissue samples collected from 397 core biopsies or resections from lung cancers were studied for MET gene amplification by fluorescence in-situ hybridization (FISH) and using MET (7q31) probe and centromere 7 as a control. Signals were quantified. PD-L1 expression on the same samples was evaluated using clone SP142 and standard patholohistochemistry (IHC) procedure. Samples were also sequenced using next generation sequencing (NGS) for mutations in TP53, KRAS, and EGFR. Results: PD-L1 expression was detected in 166 patients (42%). Twenty seven (7%) had expression between 1% and 5%, 61 (15%) between 1% and 20%, 92 (23%) between 1% and 50%, and 105 (26%) had PD-L1 > 50% in tumor cells. Ratio of MET: centromere signals was > 1.5 in 16 (4%) of patients. Patients with MET ratio > 1.5 had significantly higher (P = 0.004) percentage of PD-L1 as a continuous variable as well as when cut-off points of 5% (P = 0.01), 20% (P = 0.006), and 50% (P = 0.01) were used. Patients with EGFR mutation had significantly lower levels of PD-L1 expression (P = 0.003). When a cut-off point of 50% is used for PD-L1 expression, the EGFR-mutant cases had significantly less number of positive cases (P = 0.0003). There was no correlation between the presence of High MET: Ratio and EGFR mutation. There was no correlation between KRAS mutation and overall PD-L1 expression (P = 0.4). There was no correlation between MET ratio and KRAS mutation. Patients with TP53 mutation had significantly higher MET ratio as compared to patients with higher expression of PD-L1, while EGFR-mutant lung cancers had significantly lower expression of PD-L1 when clone SP142 is used and NGS is used for detecting EGFR mutations. Patients with TP53 mutation had strikingly high expression of PD-L1 using SP142 clone and higher copy number of MET gene. This data suggests that in lung cancer, both MET and TP53 genes play a direct role in regulating PD-L1 expression opposing the EGFR gene, which appears to suppress PD-L1 expression. KRAS gene may not be involved in PD-L1 expression in lung cancer.


Our current research explores the development and tumoricidal activity of chimeric antigen receptor T cells targeting a new immunotherapeutic target, thymidine kinase 1 (TK1), against non-small lung cancer (NSCLC), both in vitro and in vivo. There has been recent success utilizing CAR T-cell therapy in clinical trials, but it has been mainly focused on the treatment of haematological malignancies targeting CD19. Lung cancer is the most common cause of cancer mortality globally and is responsible for more than one million deaths every year. Moreover, NSCLC comprises approximately 85% of all lung cancers. Clinical trials against NSCLC using engineered T-cells targeting NY-ESO-1, VEGFR2, MAGE-A3, and mesothelin are currently ongoing. In spite of efforts to find new molecular targets, CAR T-cell therapy still faces several challenges in
the treatment of solid malignancies due to the lack of specific molecular targets. We have previously reported the up-regulation of TK1 in multiple malignant tissues including lung cancer tissues and the presence of TK1 on the cell surface of different NSCLC cell lines, such as H460 and A549. Flow cytometry, scanning electron microscopy and confocal microscopy showed evidence of TK1 on the surface of different NSCLC cell lines. We demonstrated TK1-CAR binding to H460 cells with lentiviral and retroviral vectors. The constructions include a single chain variable fragment for TK1, a CD28 and 4-1BB moieties connected with a CD3ζ signaling domain. Confirmation of the CAR expression on transduced human T cells was performed by flow cytometry and confocal microscopy, and approximately 50% of the transduced T-cells expressed TK1-CARs. Upon co-culturing TK1-CAR-transduced T-cells with naïve T-cells, a significant increase in the number of CD8+ T cells lysis elevated as high as 48% in comparison to negative controls. Cytokine profiles revealed a significant increase of the levels of IL-2 and IFN-γ after 24 hrs of co-culturing, indicating T-cell activation. TK1-CAR T-cells, untransduced T-cells, and transduced T-cells with empty vectors were co-cultured with H460 cells and time-lapse videos were recorded, every 5 minutes between 12 and 24 hrs post transduction. Clustering of TK1-CAR T-cells around lung cancer cells and induction of cell death after T-cell synthesis with target cells was observed. Preliminary in vitro data has shown that TK1-CAR T-cells induce specific cell lysis in NSCLC cells. In vivo experiments using xenografts models in SNG mice will be performed. Statistical differences between survival curves of mice treated with TK1 CAR T-cells, untransduced T-cells and transduced T-cells with empty vectors are expected.

#5620 Acquisition of P- and E-selectin binding capacity by effector T cells is required for the abscopal response following experimental high dose radiation therapy. Joseph W. Scarpelli, Ryan S. Lane, Joshua M. Walker, Charles R. Thomas, Jeffrey C. Nola, Amanda W. Lund. Oregon Health & Science University, Portland, OR.

High dose radiation therapy generates systemic adaptive immunity that limits distant tumor progression and synergizes with immune checkpoint blockade. Preclinical data demonstrate that ionizing radiation may sensitize otherwise refractory tumors to immunotherapy through mechanisms of lymphocyte activation and tumor cell recruitment, however, the molecular mechanisms that govern this response remain poorly understood. Selectins are critical regulators of leukocyte trafficking and migration, and facilitate the compartmentalization of naive, effector and memory T cells to their respective organ sites. Importantly, inflamed vascular endothelium expresses P- and E-selectin, which facilitate peripheral tissue infiltration of effector and memory lymphocytes. Therefore, both the acquisition of functional selectin ligands expressed by T-cells, as well as endothelial activation coordinates peripheral tissue infiltration following infection or tissue injury. In this work we test the hypothesis that the immunogenic potential of high dose radiation therapy is sufficient to stimulate synthesis of selectin ligands on T cells, which is required for their subsequent infiltration into distal tumors and the abscopal response. We have established a mouse model of the abscopal effect, where radiotherapy-induced immune-mediated tumor retardation is observed at sites distal to the primary, irradiated tumor. B16 F10 murine melanomas expressing model and endogenous antigens are implanted intradermally on opposing flanks. One day prior to radiation, naïve TCR-transgenic CD8+ T cells are transferred into tumor bearing mice and 20Gy is delivered to a single tumor by half beam block. Using this model, we demonstrate that local high dose radiation therapy is sufficient to activate and expand naive tumor-specific CD8+ T cells in the tumor draining lymph node. Following a single high dose of radiation, tumor-specific CD8+ T cells become activated and subsequently traffic to and accumulate in distal, contralateral tumors where they limit tumor growth, thus recapitulating the clinical observation of the immune-mediated abscopal effect. Importantly, we demonstrate that high dose radiation is sufficient to up-regulate selectin family of oligosaccharide binding adhesion molecules on newly activated antigen-specific CD8+ T cells. The acquisition of P- and E-selectin binding capacity by effector CD8+ T cells is required for the abscopal response as administration of selectin blocking antibodies reduced intratumoral CD8+ T cell infiltration and failed to mediate regression of distal tumors post radiation. Our data demonstrate that the acquisition of P- and E-selectin ligands requires prior antigenic stimulation and as such, these T cells represent a completely novel population of tumor-specific, homing competent CD8+ T cells that may predict response to therapy in patients.


GITR (Glucocorticoid-Induced Tumor Necrosis Factor Receptor Ligand, TNFSF18) is a member of the tumor necrosis factor (TNF) ligand superfamily. GITR binds and activates the co-stimulatory surface receptor GITR, which promotes proliferation and activation of effector T cells (Teff) and inhibits suppressive activity of regulatory T cells (Treg). It is thus hypothesized that co-stimulation of GITR by agonist agents will promote anti-tumor immunity. We generated a novel single-gene GITR chimera fused to an immunoglobulin Fc domain (GITRL-Fc) that shows robust single agent antitumor efficacy and immune effects in multiple syngeneic mouse models, suggesting its potential benefit in cancer immunotherapy. To investigate the prevalence of GITR expression in human tumors, RNA-Seq data analyses of 33 tumor types in TCGA showed GITR is highly expressed in a subset of solid tumors, including head & neck, lung, breast, esophageal, and bladder cancers. In most solid tumors, GITR expression correlated poorly with T cell markers, implying that GITR may not be exclusive to immune cells and may be expressed in tumor cells as well. Similar findings emerged from RNA-Seq data analysis of patient-derived xenograft (PDX) samples from 24 tumor types. The gene expression data was corroborated by immunohistochemistry (IHC) analysis of GITR expression in 17 tumor types which showed that in addition to immune cells, GITR was expressed on tumor cell membranes. A multi-platform approach was taken to investigate GITR-Fc pharmacodynamic (PD) biomarkers in tumors and in matched whole blood samples from mice bearing CT26 colon, 4T1 breast, and B16F10 melanoma carcinoma models. Global gene expression levels were profiled by microarray on tumors and control tissues. We also monitored the changes of immune cell populations and cytokine secretions by flow cytometry, Luminox and IHC. Immune gene changes were more robust in tumors than in blood samples. In tumor samples, GITR-Fc increased the gene expression associated with T cells, CD8 T cells, cytotoxicity, Th1 cells, interferon gamma (IFN-γ), natural killer cells, T effector cells, and T cell activation markers. These gene changes were validated by quantification of gene expression (qPCR). Similarly, flow cytometry analysis showed that GITR-Fc promoted activation of CD4+ effector cells, decreased Treg frequency, and increased the ratio of CD8+ T cell/Treg in the tumor. GITR-Fc also modulated secretion of cytokines in splenocytes, including an increase in IFN-γ. Taken together, the PD biomarker changes in immune-related gene expression, immune cell populations, and cytokine secretions observed in these preclinical tumor models are consistent with GITR-Fc mechanism of action and demonstrated target engagement of GITR-Fc. Additional approaches, including in-silico sorting, to monitor rare immune cell populations in tumor samples will be discussed.

#5622 Acquired resistance to EGFR-TKI upregulates the expression of PD-L1 and promotes immune escape in lung cancer. Shunli Peng, Qi Li, Wei Wang, Nanfang hospital, Southern medical university, Guangzhou, China, Guangzhou, China.

Background and purpose: Acquired resistance is a severe problem of EGFR inhibitors (EGFR-TKIs) therapy in lung cancer patients. Immune checkpoint therapy is a new revolution in cancer treatment with dramatic outcomes in a subset of patients. In this study, we explored the possibility of immune checkpoint therapy in lung cancers with three well-known resistant mechanisms to EGFR-TKIs, T790M, MET amplification and hepatocyte growth factor (HGF), and investigated internal regulation mechanisms. Experimental Design: PD-L1 expression and immune escape ability were evaluated in EGFR-TKIs resistant lung cancer cells by MET amplification, HGF, and T790M, as well as T790M transfected-human renal derived cells (293FT). MAPK and PI3K pathways were investigated simultaneously. PD-L1 gene deleted resistant cells were used in NOD-SCID xenograft models to evaluate immune escape in vivo. Results: All of three resistant mechanisms increased PD-L1 expression. Both MAPK and PI3K pathways are involved in MET amplification or HGF induced PD-L1 overexpression, whereas only MAPK pathway mediated it in T790M-transfected cells. The decreased cytotoxicity of lymphocytes can be restored by anti-PD-L1 antibodies. Moreover, xenograft tumors by EGFR-TKIs sensitive cells but not resistant cells responded to treatment of human lymphocytes. However, deletion of PD-L1 successfully restored cytotoxicity of lymphocytes in EGFR-TKIs resistant tumors. Conclusion: Acquired Resistance to EGFR-TKIs augments the expression of PD-L1 and promotes immune escape in EGFR mutant lung cancer. Immune checkpoint therapy might be a promising alternative therapeutic strategy for NSCLC that acquired resistant to EGFR-TKIs.


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Background: Clinical trials of immune checkpoints modulators, including both programmed cell death 1 (PD-1) and programmed cell death-ligand 1 (PD-L1) inhibitors, have recently shown promising activity and tolerable toxicity in pre-treated and first line NSCLC patients. At least five PD-L1 IHC assays with custom reagents and scoring-criteria were evaluated in clinical trials. However, current assays for the prognostic and/or predictive role of tumor PD-L1 expression are not standardized and the role of PD-L1 expression as a biomarker is still controversial. Materials and Methods: Retrospectively collected formalin-fixed paraffin-embedded whole-tissue sections (WTS) from 97 NSCLC cases from Seoul National University Bundang Hospital were tested. We tested the samples divided into “training set” and “validation set”: A set of 50 NSCLC WTS (32 adenocarcinoma and 18 large cell carcinoma) resected from 2010 to 2011 was designated “training set” and the other set of 47 NSCLC WTS (32 adenocarcinoma, 14 squamous cell carcinoma, and 1 pleomorphic carcinoma) resected from 2015 to 2016 was designated “validation set”. We examined PD-L1 protein expression using different antibodies (Clone EI1LN, SP142, SP263 and 22C3) using Ventana XT platform with optiview detection kit for EI1LN, SP142 and SP263, and DAKO link 46 platform with pharmDX kit for 22C3 by immunohistochemical analysis. Staining of tumor cells (Tumor proportion score, TPS) and immune cells were evaluated. Results: PD-L1 protein expression was observed in 18 %, 16%, 11% and 12% of total tumors with EI1LN with Ventana optiview kit (5% cutoff), SP142 with Ventana optiview kit with amplification (1% cutoff), SP263 with Ventana optiview kit (25% cutoff) and 22C3 with DAKO PharmDX kit (50% cutoff), respectively. There was no different positive rate between “training” and “validation” set, which means there was no effect of the age of paraffin block on PD-L1 expression. Regarding staining proportion of tumor cells, 22C3 yields more stained tumor cells (highest TPS) and SP142 consistently labels fewer tumor cells (lowest TPS). Among 38 cases which were positive in one or more assays, 28.9% showed discrepant results for PD-L1 expression between the assays according to each criterion. When the same criterion applied for all assays, discrepant rate is higher (63.1 % with 1% cutoff, 71.0% with 5% cutoff). Conclusion: Although comparative determination of PD-L1 protein levels in NSCLC reveals inter-assay variability of tumor cell staining, positive rate is similar under each criterion. Therefore, there is a potential for different diagnostic results according to the key clinical cut-off if the assays algorithms are mismatched and we should be careful when interpret the PD-L1 expression with different assays.

Characterization of proliferation in multiple T-cell subsets in the CT26 murine colon carcinoma model by multi-color flow cytometry. Matt Thayer, Alden Wong, David Draper, Dan Sains, Scott C. Wise. MI Bioresearch, Ann Arbor, MI.

The efficacy of immune-modulating anti-cancer therapeutic antibodies that have been approved in recent years, such as anti-CTLA-4, anti-PD-1 and anti-PD-L1, has altered the paradigm of cancer treatment. Subsequently, the growing interest in the development of new single agent and combination therapies with immune-modulatory effects has generated a need for more powerful immunophenotyping techniques capable of in-depth cell characterization and proliferation assessment. To this end, using the CT26 syngeneic murine colorectal cancer model we have developed an 8 color flow cytometry antibody panel that focuses on the identification of lymphocyte subsets and analysis of proliferation within them utilizing the high-throughput-capable 4 laser, 14-color Atune NxT Flow Cytometer (Thermo Fisher Scientific). Splenocytes from tumor-naive and CT26 tumor-bearing C57Bl/6 mice were stained with CFSE (carboxyfluorescein succinimidyl ester), an amine-reactive fluorescent dye which enables generational tracking for up to seven rounds of cell division, allowing for proliferation to be quantified. Splenocytes from these mice were then stimulated with anti-CD3, anti-CD28, and IL-2. After stimulation for four days, cells were stained with antibodies against CD45, CD3, CD19, CD49b, CD335, CD4, CD8, and FoxP3. We were able to identify differential proliferative capacity between naive and tumor-bearing mice in CD4⁺ T-cell, CD8⁺ T-cell, CD19⁺ B-cell, and CD11c⁺ DC-cell populations. Furthermore, we show that the analytically-driven approach of analysis is enhanced by the use of fluorescence minus-one (FMO) controls to identify those markers that generate dim signals, as well as a viability dye used to exclude dead cells from analysis. Identification of potentially responsive immune compartments, as well as characterizing proliferation, will facilitate identification and development of potential combination therapies by expanding the depth of our ability to provide mechanistic descriptions of drug function and efficacy. Following this proof-of-concept work, we will also present data from tumor-bearing mice treated with immune checkpoint inhibitors in order to identify potential differences in proliferation after treatment with immune-modulating agents.


In recent years, immunotherapy has seen a resurgence as a promising method of treatment. Programmed Death 1 (PD-1) and its receptor, Programmed Death 1 receptor (PD-1) has been of particular interest. Many different types of tumors have been shown to overexpress PD-L1, which causes host immune cells that express the PD-1 receptor and bind PD-L1 to cease killing the tumor. Patients who have tumors exhibiting high levels of PD-L1 show poor prognosis compared to those who do not. Inhibition of the PD-L1-PD-1 binding axis has been shown to restore host immunity to the tumor, and recently many new drugs such as atezolizumab and pembrolizumab have been developed to target this interaction. Current methods of PD-L1 diagnosis have shown to vary based on the antibody, detection kit brand, antigen retrieval method, and clinically strict defined methods for use by the FDA for assessment. To refine detection of PD-L1, we have identified a PD-L1 specific peptide which can be used to detect PD-L1 expressing tumors using either conventional immunohistochemistry or flow cytometry. Immunohistochemistry using this peptide was tested against the FDA-approved PD-L1 (SP263) Rabbit monoclonal primary antibody on biopsied patient tissues. Flow cytometry was performed on both cultured cells lines and patient tissues. We have shown that our PD-L1 peptide shows specific staining in the tumor regions of FFPE tissues where the SP263 kit does not stain, and we can also detect PD-L1 expression in both cell samples and patient tissues using flow cytometry.

Investigation of T cell activation by anti-human PD-1 antibodies Nivolumab, Pembrolizumab and BGB-A317 using tumor-infiltrating lymphocytes (TILs) from colorectal cancer and colorectal liver metastasis patients. Lusong Luo, Xiaoran Wu, Tong Zhang, Chunyan Fu, Yanjuan Zhang, Amy Guo, Dongping Zhou, Lianhai Zhang, Kun Wang, Baocai Xing, Jifa Li, Lai Wang, Baocai Xing, Beihang University, Beijing, China; Peking University Cancer Hospital Institute, Beijing, China.

Blockade of the PD-1 pathway with anti-PD-1 antibody, such as nivolumab (nivo) and pembrolizumab (pembro), has led to remarkable clinical responses in patients with many different cancer types. In this study, we investigated the activation of tumor-infiltrating lymphocytes (TILs) from patients with colorectal cancer (CRC) and colorectal liver metastasis (CLM) by nivo, pembro and BGB-A317, a novel humanized IgG4 anti-PD-1 antibody under clinical development. BGB-A317 has a unique binding signature to PD-1 with high affinity. Additionally, it is engineered to remove Fc gamma receptor (FcγR) binding, including FcγRI, FcγRIIA, FcγRIIB and FcγRIIIA. In our studies, all three anti-PD-1 antibodies showed significant increases in IFN-γ and TILs proliferation in a 3D tumor spheroid and CLM patient's models. RBB-A317 treatment at concentration levels of 0.1, 1 and 10 μg/mL led to higher IFN-γ production than that in nivo and pembro treated groups. The enhanced TILs function was associated with a high density of CD8⁺ T cells, but inversely correlated with the percentage of CD11b⁺ myeloid cells in CRC tumors. Of interest, BGB-A317 showed better activation of TILs in the liver metastasis tumor microenvironment (TME) where macrophages were more abundant. This observation is consistent with the hypothesis that removal of FcγR binding might offer advantage to PD-1 mAbs in tumor microenvironment enriched with macrophages.

Uncovering novel signaling pathways that mediate tumor cell killing by T effector cells. Thomas Kuilman, David Vredevoogd, Daniel S. Peeper. Netherlands Cancer Institute, Amsterdam, Netherlands.

Recent advances in the understanding of tumor immunology have led to the development of various immunotherapeutic strategies, some of which show unprecedented clinical efficacies. Two of the most effective treatments, ipilimumab and pembrolizumab / nivolumab target the immune checkpoints factors CTLA-4 and PD-1, respectively. While not all patients respond to immunotherapy, those that do frequently show long-lasting clinical responses. These non-responding patients have led to a surge in research focusing on the question how the immune system can be stimulated for more common durable therapy responses. A current focus of tumor immunological research is the identification of mechanisms allowing cancer cells to evade recognition and/or killing by the immune system while facing immunotherapy. For example, abrogation of interferon-γ signaling pathway has been shown to result in escape of immune surveillance after pembrolizumab treatment. However, it is likely that other tumor-cell-intrinsic mechanisms play a role, too. To uncover novel pathways that are involved in resistance to immune checkpoint inhibitors, we aim to identify transcriptional changes in tumor cells that result from effector T-cell exposure. To this end, we established a co-culture platform of a panel of MART1-expressing
melanoma cell lines with primary CD8+ T cells expressing a matching TCR. The cell lines display varying degrees of sensitivity to T cells, allowing us to bioinformatically uncover differential expression depending on this range of sensitivities. Rather than concentrating on single genes, we have focused on pathways, gene sets and ontologies. These analyses have been integrated with various publicly available transcriptomic data sets to identify a set of mechanisms that may contribute to T-cell mediated killing. We will systematically abrogate key factors using CRISPR technology in our co-culture platform and assess whether this affects T-cell mediated killing. Our ultimate goal is to test whether these factors, or their signaling pathways, are involved in resistance to immunotherapy in patients, and to develop new strategies to prevent immunotherapy resistance.

**#5628** Trilaciclib (G1T28), a CDK4/6 inhibitor, enhances the efficacy of combination chemotherapy and immune checkpoint inhibitor treatment in preclinical models. Jessica A. Sorrentino, Anne Y. Lai, Jay C. Strum, Patrick J. Roberts. *G1 Therapeutics, Inc*, Research Triangle Park, NC.

While immune checkpoint inhibitors are efficacious and lead to durable responses in patients with various cancers, only a minority of patients respond. An approach to increase the response rate of immune checkpoint inhibitors is to combine them with chemotherapy in order to enhance immunogenic cell death and "prime" the immune system. However, chemotherapy itself can cause damage to various cell types of the immune system, potentially diminishing the activity of the chemotherapy/checkpoint inhibitor combination. Therefore, a targeted approach to preserve immune system function during chemotherapy would add additional efficacy of the checkpoint inhibitor therapy. Trilaciclib (G1T28), a potent and selective IV CDK4/6 inhibitor, preserves hematopoietic stem cells and enhances immune system function during chemotherapy. We and others have previously shown T lymphocytes are extremely sensitive to CDK4/6 inhibition causing a transient arrest of proliferation and protection from damage by chemotherapy. Post treatment with trilaciclib and chemotherapy, T lymphocytes are released from the transient arrest in the presence of chemotherapy-induced immunogenic cell death allowing better priming of an anti-tumor immune response. To evaluate the effect of trilaciclib combination treatments, MC38 tumor bearing mice were treated with trilaciclib, oxaliplatin, or anti-PD-L1 alone or in combination, and tumor size was measured during and post treatment for 100 days. Our results demonstrate that the addition of trilaciclib to an oxaliplatin/anti-PD-L1 combination (TOP) treatment significantly improves anti-tumor activity. Specifically, twice as many mice treated with TOP for 15 days had a complete response (CRs) when compared to oxaliplatin/anti-PD-L1 (OP); 610 CRs vs 310 CRs, respectively. In addition, the CRs were durable and without any evidence of recurrence at 100 days. Furthermore, TOP treatment caused a 60% increase in overall survival (OS) compared to mice treated with OP; median OS for TOP was 98 days compared to 61 days (HR, 0.53) for the OP treatment group. Assessment of ex-vivo stimulation of cytokine release from harvested spleenocytes to measure T-cell activation, and evaluation of immune infiltrates and T-cell receptor repertoire within the tumors are ongoing. Taken together, we demonstrate that the short-acting IV CDK4/6 inhibitor, trilaciclib, which has been shown to preserve immune function during chemotherapy, enhances the anti-tumor activity of chemotherapy/anti-PD-L1 combination therapy.

**CLINICAL RESEARCH: Clinical and Translational Endocrine Oncology**

**#5629** Modeling cyclophosphamide induced ovarian toxicity in a novel translational canine model. Puja Basu,1 Rebecca Egbert,1 Evan Pasternak,1 Brian Petroff,1 Nucharin Songsasen2.

Reproductive failure following chemotherapy and/or ionizing radiation is a major adverse event, for which there are no established preventive measures. In previous studies, our laboratory has documented that the selective estrogen receptor modulator, tamoxifen (TAM) is effective in both prevention and reversal of ovarian toxicity from cyclophosphamide (CTX) in rodent models. To validate the effectiveness of this approach in a more relevant model system, we have developed a canine ovarian explant tissue model system. Dogs present with many cancers spontaneously and their pathology and treatment approaches mirror those of the human cancers. Thus, canine cancers and their treatments are excellent translational models for human diseases. Furthermore, dogs share the same environment as humans and have longer life spans than rodents. Our laboratory has recently established a dog ovarian explant tissue model system using fresh ovaries collected at the time of ovariohysterectomy. In the current study, we hypothesized that ovarian toxicity induced by CTX will be reduced by co-administration of TAM. Using this system, freshly prepared ovarian cortical tissue sections are cultured in the presence of TAM and CTX. Ovarian tissue viability is assessed by primordial and primary follicle morphology, where the number of primordial and primary follicles were counted in 9 sections of 1 mm² of ovarian cortical tissue from each dog, using CTX (0.1, 0.5, and 1μM) concentrations combined with TAM (0 and 10μM). The number and percentage of each follicle was assessed. In addition, granulosa cells proliferation is assessed using bromodeoxyuridine (BrdU) incorporation. Further, we show that, as in women, anti-mullerian hormone (AMH) may be used as an indicator of follicle response in the dog, thereby establishing the use as an in vivo monitoring system to test ovarian viability in future clinical studies. This study is intended to underpin a subsequent canine clinical trial testing TAM as a co-treatment against infertility from cancer treatment in dogs.


Despite the clinical benefit in treating ER positive breast cancer, hormone therapy resistance occurs in 40% patients leading to disease progression. Steroid receptor co-activator 1 (SRC1) enhances target gene transcription and has a critical role in breast cancer cell tumorigenicity. There is now key evidence that SRC1 is vital to the capacity of hormone-dependent tumors to adapt and overcome targeted therapy. Utilizing a genome wide CRISPRi genome sequencing of breast cancer patients and models, this study aimed to define the programs of SRC1 controlled transcriptional networks, identifying both interacting partners and downstream transcription factor (TF) targets that mediate the treatment resistant phenotype. RIME uncovered novel SRC1 interacting partners (including STAT1, PRMT1, and HDAC2) and associated TF partner networks in the hormone treatment resistant LV2 cells. In parallel, RNAseq of LV2 with SRC1 stable knockdown revealed SRC1 regulated transcriptome networks identifying 1731 up regulated genes, including 153 TFs. SRC1 cistrome, characterized by ChiP-seq analyses was combined with motif binding analysis, to further validate 40 TFs directly regulated by SRC1 and its interaction network. Dependence on SRC1 was confirmed through SRC1 siRNA and CRISPR validation. Upon this discovery, validation and functional interrogation studies we identified a cadre of 4 TFs including E2F7, DEK, TRPS1 and SMARCA1 that execute SRC1 role in hormone treatment resistance. Functional analysis of this 4 gene TF network found it to be co-operating as a driver of hormone resistant cell by coupling activation of motility with stemness. TFs can reprogram resistant cells by reversing differentiation as demonstrated in the mammosphere, 3D acinar assays and CD24/44 flow cytometry analysis. This TF gene set is a signiﬁcant predictor of poor recurrence free survival in hormone treated patients, supporting its role in increased metastatic risk in breast cancer and the clinical relevance of our ﬁndings. Furthermore, in an ER+ve PDX, established from resistant and treatment naive patient, protein expression of the TF network members co-expressed with SRC1 and were only found in the hormone treatment resistant PDX models. This study highlights SRC1 regulated disease progression and hormone treatment-resistant disease. Identified SRC1 interactome and its regulated transcriptome are active independent of its ER co-activator role. The SRC1 mediated TF network is involved in reprogramming of resistant cells, whose concerted activity is responsible for driving dedifferentiation of cells and enhancing their stem like and highly migratory tumor initiation population. This work provides key evidence on the mechanism of resistance in ER+ve hormone resistant cancer and has substantial implications both for the treatment of hormone dependent breast cancer and for identifying the mediators of metastatic potential.

**#5632** Thyroid carcinoma in The Sudan. Mohamed ElMakki Ahmed. *Univ. of Khartoum Faculty of Medicine, Khartoum, Sudan.*

Introduction: Goiter is endemic in Sudan and thyroidectomy is a daily practice in most surgical lists across the country. The most common indications are cosmones and pressure with respiratory choking. In 10% of cases a surprise histopathology on cancer is reported. This study aims to report on thyroid carcinoma on patients presenting to the surgical department in Khartoum Teaching Hospital. Methods: This study is an overview on pattern, clinical presentation a management of thyroid carcinoma in Khartoum during the period 2007 - 2014. Thyroidectomy was the standard procedure when feasible and postoperative radio iodine given. External radiotherapy was given in aggressive anaplastic carcinoma. Results: 166 patients with thyroid cancer were studied. The mean age was 51 +/- 17yrs and the age range between 15 - 85 years. Male to female ratio was 1.02:1.4. Clinical presentation was a goiter that was showing a
recent rapidly growth with either respiratory choking, pain and dysphagia. Fol-
licular carcinoma was 43%; papillary 27%, anaplastic 10% 2%medulillary. Sar-
coma and lymphoma 5% and missing data in 13%. Six per cent of patients pre-
presented with lung and bone metastasis. 20 patients had long standing goiter
before development of cancer. Different types of total thyroidectomy done in
81%, no operation done in 16% and missing data in 3%. Among patients with
active tracheotomy, 7 developed permanent hypocalcaemia, 15 recurrent nerve
palsy and a 30 days mortality was 10% and all were anaplastic carcinoma where
recurrence occurred within weeks following thyroidectomy or dubbing in most
cases. Conclusion: Patients with thyroid cancer presents late. The dominance of
follicular type indicates the significance of underlying endemic goiter in its eti-
ology. Anaplastic is very aggressive and lethal outcome.

#5633 Unique modulation of estrogen-induced apoptosis with structural
analogue of endoxifen in long-term estrogen-deprived (LTED) breast can-
cer cells. Balkees Abderrahman,1 Sean W. Fanning, 2 Daniela Quintana Rincon, 1
Geoffrey Greene, 1 Philip Y. Maximov, 1 V. Craig Jordan1. 1UT MD Anderson Cancer Center, Houston, TX; 2University of Chicago, Chicago, IL.
Endoxifen, a non-steroidal anti-estrogen is the active hydroxylated metabo-
lite of N-desmethyltamoxifen. The structure is based on a synthetic triphenyl-
ethene-type angular estrogen. Endoxifen is in clinical trials as a therapeutic
agent to treat aromatase resistant breast cancer. Paradoxically, LTED breast
 cancer is known to respond to synthetic and natural estrogens through estrogen-
induced apoptosis. A series of synthetic endoxifen analogs, were used to modu-
late estrogen-induced apoptosis in LTED breast cancer cells (MCF7/3C). X-ray
crystallography was performed on endoxifen, bisphenol, and diethylstilbestrol
(DES), bound to the ligand-binding domain (LBD) of the human estrogen re-
ceptor (ER). Cellular studies established that (MCF-7/3C) of estrogen-induced
apoptosis was dependent on the LBD of the ER. Similarly, endoxifen blocked
estrogen-induced apoptosis in a concentration and time-dependent manner.
Apoptosis triggered by DES was blocked by endoxifen and was ER-dependent.
X-ray crystallography of both endoxifen and DES illustrate the two extremes of
estrogen-induced apoptosis, which is dependent upon the closure of helix 12
over the LBD to generate estrogenic activity. Despite the fact that bisphenol,
an analog of endoxifen without the anti-estrogenic monomethylamino ethyl side
chain, is an estrogen that causes breast cancer cell replication; bisphenol initially
inhibits estrogen-induced apoptosis, but triggers estrogen-induced apoptosis
itself after 7 days of treatment of LTED breast cancer cells. X-ray crystallography
demonstrates that bisphenol can produce a unique complex with the ERLBD,
which is similar to the DES-LBD estrogenic complex. In both cases, the ligands
are sealed into the LBD by helix 12. This delay in apoptosis caused by an analog
of endoxifen, that is an angular estrogen, possess unique biological features. We
hypothesize that in LTED cells the complex passes through an anti-estrogenic
conformation to evolve into an estrogenic complex with helix 12 sealing the
LBD. We describe a unique property of a ligand-receptor complex. For the first
time we demonstrate that a ligand:receptor complex is slowly activated from
an antagonist to an agonist complex over a period of days.

#5634 Characterization of molecular mechanisms of cytoplasmic traffick-
ing and nuclear translocation of AR splice variants ARv7. Eiman Mukhtar,
Seoho Kim, Parakovi Giannakakou. Weill Cornell Medical College - Cornell
University, New York, NY.
Androgen receptor (AR) signaling is critical to not only hormone-sensitive
but also advanced castration-resistant prostate cancer. AR inhibitors (abi-
ratone and enzalutamide), the next generation of androgen deprivation ther-
apy (ADT) have been used for metastatic castration-resistant PC (mCRPC)
treatment, however, the majority of patients progress due to the development of
drug resistance. AR variants has emerged as one of the mechanisms of resistance
to these drugs. ARv7 and ARv567 splice variants that found lacking the ligand-
binding domain are constitutively active in the nucleus and thus restore AR
function despite AR inhibitors treatment. We have reported that microtubules
and dynein motor protein is required as transportation system for AR for its
nuclear translocation and activity and that taxanes inhibit AR signaling down-
stream of microtubule inhibitors. In addition, we identified that the AR hinge
region mediates binding to microtubules is present in ARv567 but missing from
ARv7. ARv7 is expressed in about 60% of CRPC patients and has been shown to
confer clinical resistance to next generation AR signaling inhibitors. Currently,
there is no therapeutic modality targeting specifically ARv7 expression or func-
tion. Using fluorescent recovery after photobleaching experiments (FRAP) we
have shown that ARv7 translocation to the nucleus occurs much faster than
AR-FL (t1/2 11s and 23 s respectively) and that its nuclear import is independent
of the importin a/b pathway utilized by AR-FL and proteins with canonical NLS.
Furthermore, transient expression of the Q69L RanGTP mutant protein, which

acts as dominant negative by occluding the nucleoporins, had minimal effect on
ARv7 nuclear import, while it completely blocked AR-FL and AR-v567. Collect-
ively, these data suggest that the nuclear import pathway for ARv7 is distinct
from that utilized by AR-FL. We believe that ARv7 undoubtedly uses facilitated
transport because of its size (75 kDa), therefore we are tackling three possibilities
of ARv7 nuclear targeting. Further, ARv7 could interact with FRAP repeat nucleoporins
(without receptor) as reported for beta catenin. We are investigating this by the use of importin B-7(1-876) mutant cell line for in vitro
studies which blocks all members of the importin family. In addition, we are
investigating the role of karyopherin transport receptors by individual knock-
down experiments coupled with live cell imaging and kinetic quantitation of
ARv7 nuclearcytoplasmic trafficking. Further, ARv7 could interact with nuclear
by utilizes a piggyback on another protein with a classical nuclear localization
signals. Overexpression of glutathione S-transferase- importin B binding do-
main fragment which will block all importin B pathways will exclude this mech-
anism. Elucidation of this mechanism will be vital to facilitate design an alter-
native therapeutic modality to halt AR signaling for CRPC treatment.

#5635 Vitamin D supplementation decreases serum 27-hydroxylaco-
terol and expression of CYP27A1 in tumors of breast cancer patients. Cat-
hrine Geising, 1 Ludmila Alexandrova, 2 Ken Lau, 1 Christine Yeh, 3 Melinda Telli, 1
Kristin Jensen, 1 David Feldman, 1 Sharon Pitteri. 1Stanford University School
of Medicine, Palo Alto, CA; 2Vincent Coates Foundation Mass Spectrometry Lab-
atory, Stanford University, Palo Alto, CA.
The goal of this study was to investigate whether vitamin D regulates the con-
version of cholesterol to 27-hydroxycholesterol (27HC), an endogenous se-
lective estrogen receptor modulator (SERM) that can act as a driver of estrogen
receptor positive (ER+) breast cancer, particularly in post-menopausal women
with low circulating estrogen levels. The hypothesis of this study was that the
active metabolite of vitamin D, calcitriol, can inhibit expression of CYP27A1,
the synthesizing enzyme for 27HC, thereby decreasing the concentration of
27HC in the blood. Both 27HC and 25-hydroxyvitamin D (25OHD) were quan-
tified by LC-MS/MS in the serum of 29 breast cancer patients, 26 of which had
ER+ cancer, pre- and post-treatment with either a low dose (control) of 400 IU
vitamin D or a high dose of 10,000 IU vitamin D per day during the neoadjuvant
period, which ranged between 7 and 37 days. In addition to these blood samples,
tissue biopsies pre-treatment and the excised tumor collected post-treatment
were tested for gene expression by microarray analysis. A significant increase
(p = 4.3E-5) was observed in serum 25OHD for patients who received the high
dose vitamin D compared to those who received the low dose, whose 25OHD
values remained stable. A greater decrease (p = 1.7E-1) was observed in serum
27HC for the high dose group compared to the low dose group, with greater
decreases in 27HC on average for those with larger increases in 25OHD concen-
tration. Gene expression showed a decrease (p = 4.5E-1) in breast cancer tissue for the high dose group compared to the low
dose group, with greater decreases in expression observed for patients on
high dose vitamin D treatment for longer times. These preliminary data show
good evidence that high dose vitamin D supplementation can decrease 27HC
levels in the body by inhibiting expression of CYP27A1 and that the longer this
administration, the greater the effect. While larger scale studies are needed, the data in
this study point to vitamin D supplementation as a possible simple method for
inhibiting ER+ breast cancer growth and suggests this reduction in CYP27A1
expression as an additional pathway by which vitamin D can mitigate risk and
improve outcomes for ER+ breast cancer patients.

#5636 Targeting of NOXA overexpression in anaplastic thyroid cancer
(ATC). Niklas K. Ffinnberg, Junaid Abdulghani, Hormoz Ehya, Wafik El-Deiry.
Fox Chase Cancer Center, Philadelphia, PA.
Anaplastic thyroid cancer (ATC) is a rare, aggressive and fatal cancer with a
median survival of 3-5 months after diagnosis. Our long-term goal is to develop
better therapies based on the biology of ATC that contributes to improved tu-
mor response and a reduced risk of potential side-effects. We recently showed
that the greater the expression of NOXA, the greater the survival advantage. We
tested for gene expression by microarray analysis. A signifıcant increase
expression by, contrast, is low. NOXA protein has previously been
shown to be subject to ubiquitination and proteasomal degradation. Indeed,
through bioinformatica, we find overexpression of several ubiquitin enzymes in

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ATG suggesting that the half-life of NOXA protein may be selectively reduced in this tumor type. We are further assessing differences in the turnover of NOXA protein in ATC cells and are investigating if restoring NOXA protein expression may synergize with the pharmacologic targeting of MCL1 and other anti-apotptic BCL2 family members. Interestingly, through TCGA analysis, we discovered that expression is associated with a poor prognosis in thyroid cancer (THCA) patients. Indeed, treatment with the BCL2/BCLXL inhibitor navitoclax showed encouraging efficacy and synergized with the neddylation inhibitor MLN4924 in the ATC cell line 8505C. This was based on the expectation that MLN4924 would rescue NOXA protein expression. We are pursuing mechanistic studies in a larger panel of thyroid cancer cell lines and in vivo efficacy and toxicity studies. We conclude that restoring NOXA protein expression may together with the rational targeting of anti-apoptotic BCL2 family members may allow for the expansion of the limited ATC-selective treatment strategies currently available.

#5637 An oral Androgen Receptor PROTAC degrader for prostate cancer. Taovi K. Neklela,1 Lawrence B. Snyder,1 Mark Bookbinder,1 Xin Chen,1 Andrew P. Crew,2 Craig M. Crews,3 Hanqing Dong,1 Deborah Gordon,1 Jennifer Ma-caluso,1 Kanak Raina,1 AnnMarie Rossi,1 Ian Taylor,1 Nicholas Vitale,1 Jing Wang,1 Ryan R. Willard,1 Kurt Zimmermann1. 1Division of Cell Pathology, Lund University, Malmö, Sweden; 2Department of Transla-tional Medicine, Division of Pathology, Lund University, Malmö, Sweden; 3Department of Clinical Sciences, Division of Surgery, Lund University, Malmö, Sweden. 1

INTRODUCTION AND OBJECTIVES: Modulating the activity of eukary-otic elongation factor 2 kinase (eEF2K) has been suggested to regulate protein elongation to block autophagy in the tumor microenvironment. Among inhib-itors of eEF2K, one would also inhibit VPS34, a class III phosphatidylinositol-3 kinase, and abrogate autophagic flux to impair the survival escape mechanism. We tested the eEF2K and VPS34 inhibitors from Janssen alone or in combina-tion with anti-androgens for their effect on proliferation of prostate cancer cell lines, especially some castration resistant PC (CRPC) lines. MATERIALS AND METHODS: Cell proliferation was assessed with various concentrations of the eEF2K or VPS34 inhibitor on CRPC lines using WST-1 viability assay. Effect of combinations of eEF2K or VPS34 inhibitor with anti-androgens abiraterone (Abi) or enzalutamide (Enza) on drug-resistant CRPC cells was further ex-plored. Western blot analysis was performed to examine the response of key autophagic molecules, androgen receptor (AR) and variant. Real time RT-PCR (RT-qPCR) was used to elucidate the effect of eEF2K or VPS34 inhibitor alone or together with anti-androgens on AR, AR variant and their downstream mole-cules. RESULTS: eEF2K and VPS34 suppressed CRPC cell growth in a dose-response manner with IC50 ranging from 1 to 5 μM. These inhibitors displayed synergy with Abi and Enza against drug-resistant CRPC cells, espe-cially on the pair of enza + EF2K inhibitor with p = 0.02 for significant differ-ence when compared to enza or EF2K inhibitor alone. To our surprise, no sig-nificant autophagy was induced by these two inhibitors according to the autophagy markers detected by Western blots. VPS34 inhibitor alone and when combined with Abi and Enza showed AR/variant degrading ability. This down-regulation was at the expression level with significant change of AR and V7, together with their transactivation markers PSA, TMPRSS2, NKx3.1 and FKBPs 25 and 27. When combined with anti-androgens may solicit profound inhibitory effect on drug-resis-tant CRPC cells. Molecular delineation demonstrated the direct target might be AR and its variants. These combinations offered a new therapeutic option for advanced PC treatments.

#5639 Diagnosing indeterminate thyroid nodules: combining next gen-eration sequencing and gene expression analysis. Vivian L. Weiss,1 Robert Daber,2 Michael Moreau,2 Alisa Gaskell,2 Kim Ely,1 Alice Coogan,1 Thomas Stricker1. 1Vanderbilt University Medical Center, Nashville, TN; 2Bio-Reference Laboratories Inc., NJ. Thyroid nodules are common in the United States and are currently diag-nosed using fine needle aspiration (FNA) biopsy. FNA evaluation provides a definitive diagnosis in the majority of cases, but about 20-30% of nodules are deemed indeterminate. Targeted next generation sequencing (NGS) and gene expression analysis have been used as ancillary tests to provide additional information about the malignancy risk for indeterminate thyroid lesions. Here we develop a 31 gene NGS panel, as well as a 14 gene expression panel, and test their performance on thyroid lesions with known diagnosis. Formalin-fixed paraffin tissue from 23 rejected thyroid lesions (1 non-neoplastic, 3 follicular adenoma (FA), 1 medullary carcinoma (MC), 7 follicular carcinomas (FC), 3 papillary carcinomas (PC), 4 follicular variant of papillary carcinomas (FVPC)) was evaluated. Total nucleic acid extraction, NGS (31 genes including: NRAS, HRAS, KRAS, CTNNB1, PIK3CA, BRAF, RET, PTEN, AKT1, PTEN, FGNA, ALK, DDR2, EGRF, ERBB2, FGFR1/2/3, GNA11, GNAQ, IDH1/2, KIT, MAP2K1, MET, MTROR, NOTCH1, PDGFRα, EPHA2, ERβ1, RAC1, and ROS1) through Bio Reference Laboratories, and gene expression analysis (15 genes: KRT7, TTF-1, TG, PGK-1, CK-20, PTH, CALC, RAP2A, PLAB, HMGA2, FN-1, GAL-3, CK19, and NIS) were performed. This assay detected mutations in 14/19 malignant lesions including 4/7 FCs (NRAS, TP53), 4/4 FCs (BRAF, HRAS, NRAS, PTEN), 5/7 PCs (BRAF, NRAS), and 1/1 MC (RET). No mutations were detected in the non-neoplastic thyroid and 1/3 FAs contained a KRAS mutation. All three mutation-negative FCs were oncogenic variants and showed upregulation of ≥2 cancer-associated genes including FN-1, GAL-3, HMGA2, PLAB, and RAP2A. The two mutation-negative PCs showed upregulation of 4 cancer-associated genes including FN-1, CK-19, HMGA-3, GAL-3, and HMGA2. This upregulation was not seen in non-neoplastic thyroid and was seen in only 1 of the 3 FAs (FN-1, GAL-3, and PLAB). This small study demonstrates that a combination of NGS and gene expression increases the assay sensitivity (100% versus 74% sensitivity) and negative predictive value (100% versus <45% for NGS or gene expression alone). The positive predictive value of the combined assay was 90% versus 93% for either assay alone. The addition of gene fusions will likely further improve the assay’s diagnostic capacity. In conclusion, the addition of a gene expression assay to this thyroid NGS panel will more accurately identify the indeterminate status of thyroid nodules. The improved detection of malignant thyroid lesions will allow for better risk assess-ment for indeterminate nodules as well as improved treatment algorithms.

#5640 The prognostic role of estrogen receptor beta in a female cohort of colorectal cancer. Gerolda Topi,1 Roy Ehrnström,1 Ingrid Palmquist,2 Marie-Louise Lydstrøp,2 Anita Sjölander1. 1Department of Translational Medicine, Divi-sion of Cell Pathology, Lund University, Malmö, Sweden; 2Department of Transla-tional Medicine, Division of Pathology, Lund University, Malmö, Sweden. 1 Background: The estrogen receptor beta (ERβ) is the predominant estrogen receptor in the colon mucosa, and has been reported to have anti-proliferative and pro-apoptotic effects. However, the role of estrogen signaling in colorectal cancer (CRC) progression remains unclear. Aim: To investigate the prognostic role of ERβ in a female cohort of CRC. METHODS: A tumor tissue microarray of primary CRCs from 333 female patients was stained with a mouse monoclonal anti-ERβ antibody. The intensity of staining was evaluated using immunohis- tochemistry technique. The expression of ERβ was scored using the semi-quantita-tive method (IRS (immunoreactive score) = SI (staining intensity) × PI (percentage of positive nuclei)). Overall survival (OS) and disease-free survival (DFS) were the primary outcomes. Preliminary results are presented. Results: From 321 patients eligible for survival analysis only 315 had successful measure-ment of ERβ (141 with low expression and 174 with high expression). The
univariate analysis showed no effect of ERβ on OS, but high ERβ was associated with lower risk of cancer recurrence as indicated by DFS (HR: 0.61; CI: 0.38-1.02). Cox multivariate analysis indicated that, compared to low expression, high expression of ERβ was significantly associated with a decreased risk of cancer recurrence (HR: 0.39; CI: 0.20-0.77), after adjustment for age, tumor stage, tumor size, tumor growth, and menopause status. A possible explanation could be that most of the rectal cancer patients have undergone radiotherapy before surgery, which might affect the status of ERβ.

#5641 Effects of G1T48, a novel orally bioavailable selective estrogen receptor degrader (SERD), and the CDK4/6 inhibitor, G1T38, on tumor growth in animal models of endocrine resistant breast cancer. Suzanne E. Wardell,1 Alexander P. Villanés, Jennifer G. Baker, Robert M. Baldi,2 Taylor K. Krebs,2 Jessica Sorrentino,2 John Bisi,2 Jay Strum,2 John D. Norris1.1 Duke University, Durham, NC; 2G1 Therapeutics, Research Triangle Park, NC.

Background: The combination of targeting the CDK4/6 and ER signaling pathways with palbociclib and fulvestrant is a proven therapeutic strategy for the treatment of ER positive breast cancer. However, the poor physicochemical properties of fulvestrant require monthly intramuscular injections to patients, which limit the pharmacokinetic and pharmacodynamic activity of the compound. Therefore, an orally available compound that more rapidly reaches steady state may lead to a better clinical response in patients. Here we report the preclinical characterization of G1T48, a novel, orally bioavailable, non-steroidal small molecule inhibitor of ERα, which is a potent, selective antagonist that down regulates ERα in vitro and vivo in ER-positive models of breast cancer.

Methods: Breast cancer cells expressing clinically relevant ESR1 mutations (ER-Y537S, ER-D538G) were treated with G1T38, G1T48, and mechanistically distinct SERMs/SERDs and cellular proliferation was assessed by measuring DNA content (Hoechst dye). Overexpressed nnu/nnu mice bearing xenograft tumors of clinically relevant tamoxifen (TamR) and aromatase (long term estrogen deprived) resistant ER+ breast cancer were treated with G1T38 and G1T48, alone or in combination, with clinically relevant comparators. Time to progression and tumor volume were assessed over a 4 week dosing period. Results: G1T38 and G1T48 significantly inhibited cellular proliferation of MCF7 breast cancer cells bearing endocrine resistant ER mutations, ER-Y537S and ER-D538G. G1T48 treatment led to dramatic reductions in ER protein levels. Importantly, tumor growth inhibition was observed in mouse models of sensitive and resistant human breast cancer when G1T48 was dosed as a single agent or in combination with G1T38, a potent, selective CDK4/6 inhibitor. Conclusions: G1T38, a novel CDK4/6 inhibitor, and G1T48, a novel SERD, either alone or in combination, demonstrated highly potent inhibition of tumor growth in animal models of tamoxifen and aromatase resistance. G1T48 also demonstrated activity in models of endocrine resistance mediated by ER mutation. G1T48 is currently completing IND enabling studies.

#5642 Quantitative comparison of hydrocortisone and prednisone in the postoperative hormone therapy of hypercortisolism. Kunlong Tang,1 Yuting Huang,1 Shangdong Dong,1 Peng Gao,2 Tianjin Medical University General Hospital, Tianjin, China; 2Tianjin Medical University Cancer Institute and Hospital, Tianjin, China.

Tumors and several types of malignancies including lung cancer, pancreatic cancer, pituitary tumor, and most often adrenal adenoma, may cause hypercortisolism, which requires glucocorticoid (GC) replacement following adrenalectomy and/or surgical resection of the tumor. Based on our previous report of a simplified GC therapy scheme and the perioperative observation, we further investigated its efficacy and safety up to 6 months post-surgery in this retrospective cohort study. All patients received no GC before surgery. The i.v. doses of hydrocortisone (HC) were 100 mg during surgery, 100 mg bid on day 0 and day 1, followed by 100 mg qd on the morning of day 2 post-surgery. Patients were grouped by oral reception of either HC or prednisone since day 2. The doses and the withdrawal schedule are shown in Table. We found in adrenal adenoma patients, the adrenocorticotrophic hormone (ACTH) levels were normal, and sufficient to stimulate the recovery of the dystrophic adrenal cortex, thus exogenous supplemental ACTH might not be necessary. By 6 months post-surgery, prednisone and HC exhibited similar efficacy in correcting hypertension, hyperglycemia, and hypokalemia (p>0.05). Most patients lost weight. Both groups reported significant improvement in a subjective evaluation questionnaire. HC showed advantages over prednisone in improving liver function (p=0.035), but also caused significant leg edema (p=0.034). Both groups developed adrenal insufficiency (AI) during GC withdrawal, with no significant difference regarding the incidence rate or severity. Most AI symptoms were relieved by resuming the prior oral doses, while the severe cases were treated in hospital. No particular variable was identified as risk factor for AI. The withdrawal process may last longer time for HC than prednisone. HC may be prioritized for patients with hyperglycemia or abnormal liver function, while prednisone may reduce the incidence of leg edema.

#5643 Physical and functional interactions of exon-skipping variants of androgen receptor with the full-length counterpart. Takuma Uo,1 Cynthia C. Sprenger,1 Shihua Sun,1 Robert K. Bradley,2 Peter S. Nelson,3 Stephen R. Plymale,4 Univ. of Washington Medical Ctr., Seattle, WA; 2Fred Hutchinson Cancer Research Center, Seattle, WA.

The androgen receptor (AR) is arguably the critical driver of not only early but also advanced prostate cancer. The recent comprehensive splicing landscapes revealed that metastatic castration resistant prostate cancer harbors multiple forms of AR variants (AR-Vs), many of which have diverse patterns of inclusion/exclusion of exons (E4-E8) corresponding to the ligand-binding domain. We had used AR-negative M12 prostate cancer cell line and recently reported characteristics of AR-Vs with respect to their subcellular localization and transcriptional activation on luciferase reporter driven by probasin-based promoter (AR2PB-luc)(1). While ARVs6es is predominantly nuclear and constitutively active like well documented ARV5es (v7es and v6es), many of others are castration –induced variants that are inactive. As many AR-Vs and the full-length AR (AR-FL) are co-expressed in the same cell, it is important to determine how these inactive AR-Vs interact with active AR including AR-FL. To this end, we examined two inactive AR-Vs namely v7es and v6es in relation to AR-FL. ARV7es, which skips exon 7, is predominantly localized in cytoplasm. ARV6/7es physically interacted with AR-FL. Moreover, nuclear accumulation of ARV7es was promoted by the addition of 17α-androstenediol hydrocarboxylic acid (DHT) only in the presence of AR-FL, suggesting that ARV7es and AR-FL heterodimerize in the nuclear compartment. However, DHT-dependent transactivation of AR-FL was not affected by the presence of ARV7es. More specifically, in M12 cells, AR2PB-luc was transactivated by AR-FL to the similar extents either in the presence or absence of ARV7es. In addition, in doxycycline (Dox)-inducible ARV7es stable LNCaP cell line (LNCaP/ARV7es), the presence of ARV7es did not affect DHT-induced expression of endogenous PSA and NKKX3.1 transcripts as well as transactivation of AR2PB-luc. In the similar setting, when we examined ARV5es6es which also physically interacted with AR-FL, Dox-induced expression of ARV5es6es in LNCaP/ARV5es6es was sufficient to induce expression of endogenous PSA and NKKX3.1 transcripts. Further, DHT-induced expression of these transcripts was enhanced by ARV7es6es in LNCaP/ARV5es6es. Accordingly, we propose that AR-Vs display differential modes of action on AR-FL as exemplified by ARV7es as the bystander and v6es6es as the effector. 1. Oncogene (PMID: 27694897).

#5644 The emergence of resistance to tamoxifen in relation to menopausal status, HER-2 status, status of steroid receptor and lymph nodes metastases status. Sima Maksimovic,1 Suzil Health Institute Hospital 'Sveti Vračev' in Bijeljina, Bijeljina, Bosnia and Herzegovina.

Background: The study used data from medical and counselling of patients who were diagnosed with hormone-dependent breast cancer. Aim: The objective of the paper is to identify within a group of patients diagnosed with hormone
sensitive breast cancer and those who have received adjuvant tamoxifen, and then to isolate the patients with whom the therapeutic effect of tamoxifen stopped. Methods: The study analyzed 153 patients in the period from 2005 to 2011, at the Public Health Institution Hospital, Sveti Vračevi in Bijeljina. Resistance to tamoxifen was developed by 60 patients (39.2%) and 93 patients (60.8%) did not develop resistance to it. Results: It was observed a statistically significant difference in frequency of resistance to tamoxifen was observed in the group of patients with ER/PgR+ status of steroid receptors (p<0.001). In relation to HER-2 status of diagnosed cancer, a statistically significant difference in frequency of resistance emergence during tamoxifen therapy in patients with HER2-positive status (p<0.001) was observed. We found that there is a statistically significant difference between patients with metastatic in lymph nodes compared to patients who had no metastases in lymph nodes (X2=39.494; p<0.001). Conclusions: The analysis of menopausal status of patients, status of ER/PgR receptors status, HER-2 status of diagnosed cancer and status of lymph nodes trying to sort out the parameters on the basis of which a group of patients who can be expected to develop resistance to tamoxifen could be differentiated.

**#5645 Associations between biomarkers and outcome in a patient cohort with invasive lobular carcinoma.** Rachel C. Jankowitz,2 Kevin Levine,1 Maryam Zamanian,1 Matthew Sikora,2 Nilgun Tasdemir,1 Priscilla McAluriff,1 David Dabbs,3 Nancy Davidson,1 Brenda Diergaarde,1 Steffi Oesterreich1.

**#5646** Germline activating mutations in the proto-oncogene GCM2 in primary hyperparathyroidism. Bin Guan,1 James M. Welch,1 Julie C. Sapp,3 Hua Ling,3 Meghana Vemulapalli,1 Yulong Li,1 Jennifer J. Johnston,1 Electron Kebebew,1 Leslie G. Biecker,1 William F. Simonds,1 Stephen J. Marx,1 Sunita K. Agrawal,1 \*NII. Bethesda, MD;2Johns Hopkins University, Baltimore, MD; and 3Boston Medical Center, Boston, MA.

Primary hyperparathyroidism (PHPT) is a common endocrine disease caused by parathyroid tumors (adenoma or hyperplasia). The hypersecreting parathyroid tumor elevates serum parathyroid hormone level, which causes hypercalcemia and the classical symptoms of PHPT, including kidney stones, osteoporosis, and neuromuscular symptoms associated with hypercalcemia, such as muscle weakness, drowsiness, and depression. Familial PHPT occurs in an isolated nonsyndromal form, termed familial isolated hyperparathyroidism (FIHP), or as part of a syndrome, such as multiple endocrine neoplasia type 1 or hyperparathyroidism-jaw tumor syndrome. We performed exome sequencing on germline DNA of eight index cases from eight unrelated kindreds with FIHP. Rare variants were identified (minor allele frequency <1%), and selected variants were assessed for co-segregation in affected family members by Sanger sequencing. Candidate genes were then screened in an additional 32 kindreds with FIHP. The functional consequence of variants in one FIHP candidate gene (GCM2, a parathyroid tissue-specific transcription factor) was analyzed in a cell culture model. In eight kindreds with FIHP, we identified three rare GCM2 missense variants. Functional characterization of GCM2 variants and deletion analyses revealed a small C-terminal Conserved Inhibitory Domain (CCID). Two of the three rare variants were recurrent, located in the CCID that enhanced the transcriptional activity of GCM2, and found in seven of 40 (18%) kindreds with FIHP. Functional assays for transcriptional activity demonstrated that these two variants were gain-of-function mutations, suggesting that GCM2 is a parathyroid proto-oncogene. We further investigated the ethnicity of individuals with GCM2 CCID activating variants in PHPT patient samples and in genome survey datasets. Our results demonstrate that germline activating mutations in the GCM2 CCID can cause FIHP, and specific activating mutations expose a large population at risk for PHPT. Our findings for the first gene with mutations specific for FIHP are expected to have an important impact on clinical practice, including treatment decisions and genetic testing of individuals with PHPT and family members.

**#5647** Intron retention as a novel source of tumor neoantigens associated with response to checkpoint inhibitor therapy. Alicia C. Smart,1 Claire Margolis,1 Diana Miao,1 David Liu,1 Jiyou Park,1 Meng Xiao He,1 Brendan Reardon,1 Stephanie Mullane,1 Bastian Schilling,2 Levi A. Garraway,3 Dirk Schadendorf,2 Eliezer M. Van Allen1.

**CLINICAL RESEARCH: Innate Immunity to Generate Adaptive Immunity**

**#5648** Development of a novel method to identify specific neoantigens from transcriptome sequencing data using retained introns. Eliezer M. Van Allen1.

**#5649** Development of a novel method to identify specific neoantigens from transcriptome sequencing data using retained introns. Eliezer M. Van Allen1.
#5648 Combined immune checkpoint targeting with anti-PD-1 plus anti-CD40 antibodies as the most effective approach to eradicate head and neck squamous cell carcinomas (HNSCCs) in mouse models. Jose Augusto Monte de Oliveira Novaes, Alissa R. Poteete, Marlesse A. Pisegue, Uma Giri, Fahao Zhang, Patrick Hwu, John V. Heymach, William N. William. The University of Texas MD Anderson Cancer Center, Houston, TX, USA.

Introduction: Immunotheapy with single agent anti-PD-1 antibody confers modest overall survival benefits in patients with recurrent/metastatic HNSCCs. Combined immune checkpoint targeting has shown promising results for melanomas and other solid tumors. Thus, evaluation of combination immunotherapies in animal models specific to HNSCCs could inform prioritization and design of future clinical trials for this disease. We conducted this pre-clinical study to test the hypothesis that combining anti-PD-1 with either anti-CTLA-4 or co-stimulatory immune checkpoint agonist antibodies would be more effective than anti-PD-1 monotherapy in a mouse model of oral carcinoma. We also aimed at identifying the combination regimen with highest anti-tumor activity. Methods: CS7BL/6 mice were subcutaneously grafted with 2106 mouse oral cancer 1 (MOC1) cells derived from a carcinogen-induced tumor molecularly similar to human HNSCC. After 25 days, mice were randomized into one of 12 treatment groups (IgG, or antibodies targeting PD-1, CTLA-4, CD40, OX-40, GITR, 4-1BB, PD-1+CTLA-4, PD-1+CD40, PD-1+OX-40, PD-1+GITR, or PD-1+4-1BB), N=8-9 mice/group. Antibodies were administered every 4 days for 3 doses. Tumors were measured twice per week. The primary endpoint was overall survival. Mice bearing tumors >3 mm2 in diameter (at ulceration) were used in the study. At the end of the study, tumors were harvested and stained for CD45+ cells by flow cytometry. Results: In a preliminary human clinical trial, anti-PD-1 therapy led to a modest improvement in survival compared to IgG [HR=0.41; 95%CI 0.06-0.53; P=0.015], indicating that this model recapitulates, in part, the effects of immunotherapies in HNSCC patients; none of the animals survived long term. Monotherapy with anti-CTLA-4, anti-CD40, anti-OX-40, or anti-4-1BB also improved survival compared to IgG (P=0.0001, 0.003, 0.02, and 0.003, respectively), but were not more effective than anti-PD-1 alone (P=0.09, 0.12, 0.72, and 0.77, respectively). Combination therapy did not prolong survival compared to anti-PD-1 alone, with the exception of anti-PD-1+anti-CTLA4 [HR=0.36; 95% CI 0.07-0.6; P=0.016], and anti-PD-1+anti-CD40 [HR=0.125; 95% CI 0.02-0.24; P=0.0003]. Notably, 50% of mice receiving anti-PD-1+anti-CD40 antibodies had complete tumor eradication and are alive and disease-free 120 days after tumor grafting. Conclusion: Single immunotherapeutic targeting PD-1, CTLA-4, CD40, OX-40 and 4-1BB modestly improved survival in a mouse model of oral cancer. Combination with anti-PD-1+anti-CTLA4 and anti-PD-1+anti-CD40 antibodies outperformed anti-PD-1 monotherapy. Complete responses were exclusively seen with PD-1+CD40 dual targeting. This regimen should be prioritized for evaluation in HNSCC clinical trials.


Tumor initiating cells (TIC) foster treatment resistance and tumor relapse. We show here that in mouse B16 (melanoma) and ID8agg (ovarian carcinoma), and human ovarian cancer ES2, PD-L1 knockdown (shRNA) reduced TIC numbers and functions. Data shown are from 2 PD-L1sh clones/cell type. Sorted control CD44+CD133+CD24- (B16), CD44+CD24+ (ID8agg) and ALDH+ (ES2) TIC formed 50-73% more and bigger spheres vs. PD-L1+ TIC in vitro, consistent with reduced TIC self-renewal in PD-L1+. In vivo, PD-L1sh B16 and ID8agg TIC exhibited reduced tumorigenicity in wild type mice, consistent with poor TIC function, but immune effects could not be excluded. B16 and ES2 PD-L1sh TIC had significantly reduced tumorigenicity versus control TIC in immune deficient NSG mice consistent with cell-autonomous, immune-independent TIC promotion by PD-L1. In further confirmation, we found reduced adhesive interactions of PD-L1sh B16 TIC versus PD-L1+ TIC (B16) [n=10]. PD-L1sh ES2 TIC versus PD-L1+ ES2 TIC. The corresponding stemness gene SOX2 was significantly higher in control vs. PD-L1sh ES2 TIC. Altogether, these data validate effects in human cells, and are consistent with PD-L1 driving TIC through stemness gene control, although specific genes differed in distinct tumors (likely due to differing mutational landscapes). mTORC1 signaling linked to stemness (assessed by raptor depletion) was lower but mTORC2 was unaffected (rictor) in PD-L1sh versus control TIC from all 3 tumors, suggesting mTORC1 control of PD-L1-mediated TIC generation. In support, the mTORC1 inhibitor rapamycin reduced control TIC significantly (>30% vs. untreated). Strikingly, rapamycin significantly increased PD-L1sh TIC in all 3 tumors, suggesting mTORC1 effects differ by tumor PD-L1 status. Interferon (IFN)-γ similarly reduced control TIC and augmented PD-L1+ TIC numbers, suggesting the novel concept that PD-L1 alters IFN-γ signals. PD-L1 sensitization of TIC to IFN-γ and rapamycin is the first example of a molecule enhancing TIC treatment response to our knowledge. We recently showed that self-deliverable RNAi (sd-rxRNA) directly reduces B16 and ID8agg proliferation in vitro and in vivo (accession no. 27617647). Preliminary data now show that anti-PD-1 (and anti-CD8) directly reduce TIC proliferation, and these effects differ from non-TIC. We challenge the paradigm that tumor PD-L1 is primarily an immune escape molecule and that anti-PD-1 works primarily to block T cell PD-1 interactions.

#5650 Enhancement of CAR-T cell activity via PD-1 knockdown by self-deliverable RNAi. Alexander Wolfson,1 Alexey Eliseev,1 Taisiya Shumskikhov,2 Monica Betancur-Boissel,2 Monica Betancur-Boissel,2 Nathalie Scholler,3 Mir-Mo, Immune, Boston, MA; Adirna, Cambridge, MA; 3SRI International, Menlo Park, CA.

Background: A growing body of evidence indicates that the blockade of PD-1 and other immunosuppressive receptors can increase the efficacy of adoptively transferred cells in immunotherapy for cancer. The expression of PD-1 can be suppressed by ex vivo treatment of transferred lymphocytes by self-deliverable RNAi (sd-rxRNA) compounds. sd-rxRNAs are based on proprietary chemical modifications of siRNA compounds making them stable to nuclease degradation and capable of penetration into target cells without the use of transfection reagents. Experimental methods: Aim: To determine whether the reduction of PD-1 by ex vivo treatment of mesothelin-targeting CAR-T cells with sd-rxRNA will improve the efficacy of meso-CAR-T cells in a mouse model of ovarian cancer. Methods: Second-generation human CAR-T cells expressing P4 antibody fragments targeting mesothelin (meso-CAR-T) were treated ex vivo with PD-78, a self-deliverable siRNA silencing the PD-1 gene. The meso-CAR-T cells were then injected into tumors in a xenograft mouse model of human ovarian cancer (A1847 cell line expressing mesothelin and PD-L1) in NSG mice. The tumor volumes were monitored over the course of one month. After the animals were sacrificed, human CD45-containing cells extracted from the tumor were analyzed for PD-1 expression by flow cytometry. Results: We observed a statistically significant reduction in tumor growth in the group of mice injected with meso-CAR-T cells pretreated ex vivo with PD-78. The post-mortem tumor analysis by flow cytometry showed that the expression of PD-1 in meso-CAR-T cells was significantly suppressed for the full duration of the experiment (one month). Conclusion: Our results suggest that the silencing of immunosuppressive genes by ex vivo treatment with sd-rxRNA may improve efficacy of CAR-T cells in the treatment of solid tumors. A similar approach may be applied to other types of adoptive cell transfer.


Combining immunotherapeutic antibodies in cancer treatment has shown benefits over single agents. An alternative to combining two antibodies is the development of bispecific antibodies that not only bring two biologics together but may result in novel mechanisms of action that are impossible to attain with combinations. Lymphocyte Activation Gene-3 Gene-3 (LAG-3) is a member of the Ig superfamily expressed on activated T cells, NK cells, pDCs, B cells, y T cells and participates in immune suppression. Programmed Cell Death receptor (PD-1) binds to its ligand PD-L1, expressed not only on activated immune cells to inhibit cellular immune responses but also on tumor cells. Expression of both these surface molecules therefore leads to T cell exhaustion allowing the tumor to escape immune surveillance. A mAb 2738 (bispecific antibody) was engineered which binds murine LAG-3 and PD-L1 simultaneously and with nanomolar affinities. The anti-LAG-3/PD-1 mAb2 inhibits LAG-3 binding to MHCIId and PD-L1 binding to PD-1 and CD80, resulting in T cell activation in vitro. This translates into in vivo efficacy, where the mAb2 decreased tumor burden in the MC38 colon carcinoma tumor model. At the end of the study tumor-free animals were more numerous in the LAG-3/PD-L1 bispecific group than in the group given a combination of individual anti-LAG-3 and PD-L1 antibodies. The results were recapitulated in the CT26 murine colon cancer model, where the mAb2 showed an increase of antitumor activity as compared to the antibodies given in combination. Thus, the preclinical data supports developing an anti-human LAG-3/PD-1 mAb2 for the treatment of cancer patients.
CLINICAL RESEARCH: Innate Immunity to Generate Adaptive Immunity

#5652  Translational evidence of reactivated innate and adaptive immunity with intratumoral IMO-2125 in combination with systemic checkpoint inhibitors from a Phase I/II study in patients with anti-PD-1 refractory metastatic melanoma. Cara Saymak, 1 Marc Uemura, 1 Ravi Murthy, 1 Marihella James, 1 Daqing Wang, 2 Julie Brevard, 2 Suzanne Swann, 3 James Geib, 4 Mark Cornfeld, 5 John C. Krizan, 5 Joshua Gimotty, 5 Suchi Khanna, 5 Oddvar Vawgo, 6 Rodabe Amaria, 7 Sapna Patel, 1 Hussein Tawbi, 1 Isabella Glitza, 1 Scott Woodman, 6 Wen-Jen Hwu, 7 Michael A. Davies, 1 Patrick Hwu, 1 Willem Overwijk, 1 Chantale Bernatchez, 1 Adi Diab, 1 UT MD Anderson Cancer Center, Houston, TX; 2Idera Pharmaceuticals, Inc., Cambridge, MA.

Background: While checkpoint inhibitor (CPI) therapy has transformed metastatic melanoma (MM) treatment, many patients remain refractory. We reasoned that combining CPI with an agent that activates antigen presenting cells and improves T-cell priming may result in improved response. Our hypothesis is that this will result in dendritic cell (DC) activation and induction of tumor-specific CD8+ T-cells which will synergize with ipilimumab or pembrolizumab to overcome immune-escape. Based on this rationale we initiated a phase I/II clinical trial. Study Design/Methods: Adults with refractory MM that have had prior PD-1 blockade therapy (with or without a BRAF inhibitor) are eligible. IMO-2125, in doses escalating from 4mg to 60mg q.d., is given subcutaneously. Along with administration of ipilimumab or pembrolizumab. Primary endpoints are safety, tumor response, and PK. Multiple biopsies are obtained in both the injected and distant tumor pre- and on-treatment. Immune analyses include DC subsets and their activation status as well as T cell activation, function and proliferation. T-cell repertoire diversity is evaluated by high-throughput CDR3 sequencing. Changes in circulating cytokines are also being assessed during therapy. Results: Enrollment is proceeding on both the IMO-ipilimumab and IMO-pembrolizumab dose-escalation arms of the trial. Safety is acceptable with no DLT recorded to date. Durability and efficacy data will be presented in our oral presentation.


Pancreatic cancer is an aggressive malignancy with a 5 year survival rate of less than five percent. The predominant immune cells infiltrating the tumor microenvironment are monocytes/macrophages, which are reported to support tumor growth by suppressing host immune responses to the tumor. Recruitment of monocytes to various tissues, including tumors, is dependent upon activation of the chemokine receptor CCR2 by one or more of the chemokines CCL2, CCL8 and CCL13. In preclinical and clinical studies, inhibition of CCR2 in pancreatic cancer has shown to decrease tumor progression by blocking recruitment and accumulation of monocytes/macrophages in the tumor microenvironment. Analysis of human pancreatic tumors revealed elevated levels of both CCL2 and CCL8, which recruits monocytes, and the monocyte marker CD14, in advanced pancreatic cancers. Current immunotherapy using checkpoint inhibitors are ineffective in some tumors, but lack efficacy in immune insensitive cancers, including pancreatic cancer. Here, we report that the inhibition of CCR2 using a small molecule antagonist potentiates anti-PD-1 immunotherapy in a syngeneic, orthotopic mouse model of pancreatic cancer. Our data reveal that CCR2 blockade decreases tumor burden and increases immune infiltration and creating a microenvironment more favorable for CD8 T cell activity, and provide a mechanistic rationale for investigating the combination of a CCR2 antagonist and an immune checkpoint inhibitor in pancreatic cancer.

#5656  Quantitative measurement of PDL1 expression across tumor types using laser capture microdissection and reverse phase protein microarray. Elisa Baldelli, 1 Valerie Calvert, 1 Alex Hodge, 2 Maria Isabella Sereni, 2 Guido Gambara, 1 Eric B. Haura, 3 Lucio Crino, 3 Bryant Dunetz, 3 Sergio Pecorelli, 2 David J. Perry, 4 Stephen P. Anthony, 5 Nicholas Robert, 5 Donald W. Northfelt, 5 Mohammad Jahanghez, 5 Emanuel F. Petricoin, 1 Mariaelena Pierobon, 1 George Mason University, Manassas, VA; 2H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL; 3’S. Maria della Misericordia Hospital, Perugia, Italy; 4The Side Out Foundation, Fairfax, VA; 5University of Brescia, Brescia, Italy; 6MedStar Washington Hospital Center, Washington DC, DC; 7Evergreen Hematology & Oncology, Spokane, WA; 8Virginia Cancer Specialists, Fairfax, VA; 9Mayo Clinic, Scottsdale, AZ; 10University of Miami, Miami, FL.

Background: The efficacy of immunotherapy, including therapeutic strategies capable of modulating innate/adaptive immune resistance, varies greatly across tumor types. As the number of available immunotherapies accelerates, the study of predictive markers by IHC (e.g. PDL1 expression) is under intense investigation. However, staining protocol inconsistency, variation across scoring systems, and subjective interpretation of the immunostaining have produced conflicting results thus far. This work explored the role of Laser Capture Microdissection (LCM) coupled with Reverse Phase Protein Microarray (RPPA) as an alternative high throughput, quantitative, operator independent platform for measuring PDL1 expression across tumor types. Material and Methods: Pure epithelial cells were isolated via LCM from 178 samples including: 72 ovarian cancers (OC), 57 lung adenocarcinomas (LC), 30 metastatic breast cancers (MBC), and 19 pancreatic cancers (PC). PDL1 expression was measured on a continuous scale using quantitation of CCL2 and CSF1, which recruit monocytes, as well as the monocyte marker CD14, in advanced pancreatic cancers. Current immunotherapy using checkpoint inhibitors are ineffective in some tumors, but lack efficacy in immune insensitive cancers, including pancreatic cancer. Here, we report that the inhibition of CCR2 using a small molecule antagonist potentiates anti-PD-1 immunotherapy in a syngeneic, orthotopic mouse model of pancreatic cancer. Our data reveal that CCR2 blockade decreases tumor burden and increases immune infiltration and creating a microenvironment more favorable for CD8 T cell activity, and provide a mechanistic rationale for investigating the combination of a CCR2 antagonist and an immune checkpoint inhibitor in pancreatic cancer.

Pancreatic cancer is an aggressive malignancy with a 5 year survival rate of less than five percent. The predominant immune cells infiltrating the tumor microenvironment are monocytes/macrophages, which are reported to support tumor growth by suppressing host immune responses to the tumor. Recruitment of monocytes to various tissues, including tumors, is dependent upon activation of the chemokine receptor CCR2 by one or more of the chemokines CCL2, CCL8 and CCL13. In preclinical and clinical studies, inhibition of CCR2 in pancreatic cancer has shown to decrease tumor progression by blocking recruitment and accumulation of monocytes/macrophages in the tumor microenvironment. Analysis of human pancreatic tumors revealed elevated levels of both CCL2 and CCL8, which recruits monocytes, and the monocyte marker CD14, in advanced pancreatic cancers. Current immunotherapy using checkpoint inhibitors are ineffective in some tumors, but lack efficacy in immune insensitive cancers, including pancreatic cancer. Here, we report that the inhibition of CCR2 using a small molecule antagonist potentiates anti-PD-1 immunotherapy in a syngeneic, orthotopic mouse model of pancreatic cancer. Our data reveal that CCR2 blockade decreases tumor burden and increases immune infiltration and creating a microenvironment more favorable for CD8 T cell activity, and provide a mechanistic rationale for investigating the combination of a CCR2 antagonist and an immune checkpoint inhibitor in pancreatic cancer.

#5654  Lymphodepletion induces T cell homeostatic proliferation and augments antitumor effects of PD-1/PD-L1 blockade therapy. Massashi Arita, Satoshi Watanabe, Takahashi Miho, Miyuki Sato, Aya Ohtsubo, Kosuke Ichikawa, Rie Kondo, Tetsuya Abe, Junta Tanaka, Toshiyuki Koya, Toshiaki Kikuchi. Niigata University, Niigata City, Japan.

Programmed death receptor-1 (PD-1)/programmed death receptor ligand-1 (PD-L1) blockade therapy has demonstrated to enhance antitumor immunity. Recent evidence has shown that anti-PD-1/PD-L1 therapy restores the function of exhausted effector T cells and augments antitumor immune responses. The expression of PD-L1 has been best studied as a biomarker for the anti-PD-1/ PD-L1 therapy. Because PD-L1 expression on tumor cells is induced by IFN-γ which is mainly produced by effector T cells, induction of antitumor effector T cells in patients treated with the anti-PD-1/PD-L1 therapy may be an important factor. Indeed, previous studies have suggested that the increase of tumor-infiltrating lymphocytes is the predictive biomarker for PD-1/PD-L1 blockade therapy. We and others have demonstrated that lymphodepletive therapies, such as chemotherapy and radiotherapy, induce tumor-specific effector T cells from naïve T cells. Naïve T cells rapidly proliferate and elicit memory-like functions after lymphodepletive regimens could augment the antitumor efficacies of the anti-PD-1/ PD-L1 therapy. In the current study, we transferred naïve T cells into mice after whole-body irradiation or chemotherapy. These mice were inoculated s.c. with PD-L1+ B16F10 melanoma cells or PD-L1+ MCA205 fibrosarcoma and then were treated with anti-PD-1 mAbs. Lymphodepletion by whole body irradiation, but not cytotoxic agents following the transfer of naïve T cells significantly enhanced antitumor immunity of anti-PD-1 mAbs. This augmentation required both of CD4+ and CD8+ T cells, but not NK cells. Further experiments revealed that immune cells infiltrating MCA205 expressed PD-L1. Transfer of CFSE-labeled immune cells from naïve mice to tumor-bearing mice augmented antitumor effects of anti-PD-1 mAb therapy by lymphodepletion. These results suggest that lymphodepletion induces homeostatic proliferation of T cells and augments antitumor effects of PD-1/PD-L1 blockade therapy.
sion of PD-L1 with only 2/19 (10.5%) cases been above the population median and none within the top quartile of the population. Conclusions: The LCM-RPPA workflow has the unique ability to capture immune checkpoint expression on a continuous quantitative scale as well as capture its broad dynamic range. Because RPPA is unconstrained by antigen retrieval issues as well as subjectivity of IHC interpretation, this approach may generate more accurate cut-point of therapeutic response prediction. Overall the dynamic range of PD-L1 was broader in LC compared to other solid tumors, and LC had a much higher proportion of patients with tumors expressing high levels of PD-L1. These quantitative differences may explain therapeutic efficacy of PD-L1 inhibition across tumor types. Such speculative hypothesis should be further validated in prospective clinical trials. Finally, these preliminary data suggest that organ specific microenvironments more than specific driving mutations (e.g. KRAS) may strongly influence PD-L1 expression in malignant lesions.

#5657 Objective measurement and significance of VISTA (PD-1H) expression in non-small cell lung cancer (NSCLC). Franz Villarroel-Espindola,1 Ilia J. Datar,1 Vamsidhar Velcheti,2 David L. Rimm,3 Roy S. Herbst,4 Kurt A. Schalper5.1Yale School of Medicine, New Haven, CT; 2Cleveland Clinic, Cleveland, OH; 3Medical Oncology Yale Cancer Center, New Haven, CT.

Introduction: VISTA (PD-1H) is a member of the B7 family of immune co-modulatory molecules and has been proposed as a novel anti-cancer immunotherapy target. The intra- and extracellular domains of VISTA show homology to PD-1 and PD-L1, respectively, suggesting a role in anti-tumor immune evaluation. The expression of VISTA, its association with PD-1 axis components and biological role in human NSCLC are unknown. Methods: Using multiplex quantitative immunofluorescence (QIF), we simultaneously measured the levels of VISTA (clone D1L2G, CST). PD-L1 (clone 405.9/A11, CST) and PD-1 (clone EH33, CST) protein in 732 stage I-IV NSCLCs from 3 retrospective collections represented in tissue microarray format (cohort #1 [n=237, Yale], cohort #2 [n=329, Greece]; and cohort #3 [n=106, Yale]). To evaluate the tumor tissue distribution, VISTA was also selectively measured in cytokeratin + tumor cells, CD3+ T-cells, CD4+ T-helper cells, CD8+ cytotoxic T-cells and CD20+ B-lymphocytes. Associations between the marker levels, clinicopathological-molecular variables and survival were studied. Results: VISTA protein was detected in all NSCLCs, showed a membranous staining pattern and was localized predominantly in the tumor cells in 27.4% of cases; and in the stromal compartment in 98.5%. Although VISTA was detected in all major tumor infiltrating lymphocyte (TIL) subsets, the signal was higher in CD20+ B-cells than in CD3+ T-lymphocytes (P<0.05); and in CD4+ helper than in CD8+ cytotoxic T-cells (P<0.0001). Using the median score as cut point, elevated VISTA in tumor and/or stromal cells was significantly associated with high PD-L1, PD-1 protein expression and increased CD8+ TILs in the cohorts (P<0.001 – P<0.05). No consistent association between VISTA levels and age, gender, smoking status, specific NSCLC histology, stage, EGFR/KRAS mutations and overall survival was found. Conclusion: VISTA is expressed in the majority of NSCLCs and shows differential distribution in tumor/stromal cells and TIL subsets, suggesting a complex functional as a ligand and receptor. Elevated expression of VISTA in NSCLC is associated with increased PD-1 axis targets and cytotoxic T-cell density, indicating its possible modulation by pro-inflammatory signals. Our results support VISTA as a candidate target for anti-cancer immunotherapy in NSCLC alone or in combination with PD-1 axis blockers.


Pancreatic cancer remains a serious unmet medical need. The majority of pancreatic tumors are composed of stromal cells that make tumors resistant to conventional therapies. We are developing an approach in which a TLR9 agonist, IMO-2125, is administered intratumorally, which induces local Th1 response and modulates the tumor microenvironment. This approach has shown promising antitumor activity in preclinical models of multiple tumor types in combination with checkpoint inhibitors. We are employing a similar approach to the treatment of pancreatic cancer. We evaluated the local therapeutic effect of IMO-2125 in a murine syngeneic pancreatic ductal adenocarcinoma (Pan02) cancer models. In the first study, C57BL/6 mice were intraperitoneally (i.p.) implanted with Pan02 cells to generate peritoneal metastatic cancer. Tumor-bearing mice (n=10 per group) were then treated with 2.5 mg/kg IMO-2125 either by i.p. or subcutaneous (s.c.) injections for six times in two weeks. In the second study, to establish dual solid tumors in C57BL/6 mice, Pan02 cells were subcutaneously implanted in both the right and left flanks of the animals. When tumors reached to 50-150 mm³, we started the intratumoral (i.t.) administration of IMO-2125 in right tumors at doses of either 0.25 mg/kg, 1 mg/kg or 2.5 mg/kg, or PBS (n=10 per group), 3x/week for three weeks. In the peritoneal metastatic pancreatic tumor model, 80% of IMO-2125 intraperitoneally treated mice survived for more than 88 days, while 90% of the control group died within 29 days. Conversely, the IMO-2125 treatment group survived for only 36 days. Tumor-free mice from the IMO-2125 i.p treatment group (n=7) rejected the same tumor rechallenge, suggesting establishment of durable tumor-specific immune response. In the dual solid pancreatic tumor model, intratumoral IMO-2125 treatment led to a dose dependent antitumor activity in both injected and distant tumors. Tumors treated with 0.25, 1 and 2.5 mg/kg doses of IMO-2125 showed reductions in tumor volume by 66% (p = 0.004), 74% (p = 0.002) and 95% (p = 0.003), respectively. Moreover, the distant tumors exhibited a reduction of 0% (p = 0.65), 64% (p = 0.006) and 69% (p = 0.001), respectively. Antitumor activity was associated with increased T-cell infiltration and upregulation of the gene expression of various checkpoint in both treated and distant tumors. In an independent study, treatment with a control IMO showed minimal antitumor activity. All treatments were well tolerated. In summary, local treatment with IMO-2125 exerts antitumor activity in preclinical pancreatic cancer models, associated with an increase in T-cell infiltration in the tumor microenvironment, providing a novel approach to the treatment of pancreatic cancer. The clinical trial of intratumoral IMO-2125 is in progress in patients with melanoma.

#5660 Tumor cell death caused by INT230-6 induces protective T cell immunity. Anja C. Bloom,1 Lewis H. Bender,2 Ian B. Walters,2 Katharine T. Clark,1 Masaki Terabe,1 Jay A. Berzofsky 1.1National Cancer Institute, Bethesda, MD; 2Intensity Therapeutics Ltd, Westport, CT.

Standard care for many types of cancer involves systemic administration of cytotoxic agents. This may result in low drug concentration at tumor sites, which limits cell killing. More recently it has been shown that cytotoxic formulations designed for intratumoral delivery improve drug efficacy presumably by increasing drug concentration at the tumor site. Furthermore, it has been discovered that the mechanisms of anticancer agents extend beyond direct tumor cell lysis. One major aspect is that cell death often induces an immune response. Different types of cell death such as necrosis, apoptosis and auto-phagic cell death induced by cytotoxic agents trigger immune responses with varying degrees of inflammation and involving different types of immune cells. The ideal immune responses that may give maximum benefit to patients would be strong and long lasting anti-tumor T cell responses. In this study, a novel, tissue and cell diffusive cytotoxic formulation, INT230-6, was administered intratumorally over 5 sequential days into subcutaneous 300 mm³ murine Colon26 tumors. Treatment resulted in regression from baseline of 100% of the tumors and up to 80% complete regression (CR). We then analyzed the T cell responses induced by INT230-6. No animals achieved a CR after CD8 or CD8/CD4 double depletion at treatment onset, indicating a critical T-cell role in tumor regression. Mice with CRs were protected from re-challenge by either subcutaneous or intravenous re-inoculation of the Colon26. The protection was abrogated by CD4/CD8 double depletion prior to the re-challenge, indicating that immunological T-cell memory areas was not necessary for protection. Hence, INT230-6 given locally to treat tumors induces tumor-specific protective T cell immunity. Colon26 tumors express the endogenous retroviral protein gp70 containing the AH-1 CTL epitope. AH-1-specific CD8 T cells were detected in tumors of mice without treatment. However, these highly expressed PD-1. This was consistent with the observation that the PD-1 blockade enhanced the efficacy of INT230-6. Conversely, INT230-6 enhances the efficacy of checkpoint inhibitors.

#5661 Targeting CD47 expression on macrophages regulates immunomodulation enhancing tumoricidal activity against cancer cells. Ashley A. Smith, Adam S. Wilson, David R. Soto-Pantoja. Wake Forest School of Medicine, Winston Salem, NC.

Macrophage mediated elimination of cancer cells is critical for control of tumor growth. Cancer cells evade immune recognition by expressing receptors that send inhibitory signals to prevent macrophage mediated cytotoxicity. Clinical studies show that overexpression of the cell surface receptor CD47 is a poor prognostic factor in cancer, allowing tumors to bypass immunosurveillance and activation pro-growth pathways. Prior studies have described that CD47 cell surface expression on cancer cells inhibits phagocytic activity through engagement of its counter receptor SIRPα on macrophages. However, the role of CD47 expression on macrophage function is not known. Here, we show that CD47 is expressed on primary mouse macrophages, RAW 264.7 and the U937 cell lines. Moreover, knockdown of CD47 on macrophages enhanced the tumoricidal ca.
with progression free survival (PFS) after autoSCT in multiple myeloma (MM), and showed that there was an inverse correlation between NKG2A expression on CD56bright NK cells and PFS. Therefore, we aimed to investigate a potential role for NKG2A modulation, especially in the post-transplant setting with maintenance chemotherapy. We performed immunophenotypic analyses of NK cells from patients in the immediate post-transplant setting as well as from patients at various time points after transplant on lenalidomide maintenance therapy. In the immediate post-transplant setting (mean time since transplant = 13 days) NKG2A was upregulated on NK cells when compared with KIR2DL1 (p = 0.001) and KIR2DL2 (p = 0.002). Additionally, in immunophenotypic analyses of PBMC from MM patients on various treatments, patients receiving lenalidomide maintenance therapy that recently underwent stem cell transplant (mean time since transplant = 0.53 years) had elevated NKG2A expression compared to patients receiving no treatment (p = 0.029). Conversely, patients receiving lenalidomide maintenance therapy over years (mean time since transplant = 4.21 years) after transplant showed reduced NKG2A expression compared to patients receiving no treatment (p = 0.0161). In bone marrow aspirates from patients with MM, primary CD138-positive myeloma cells in the bone marrow express HLA-E, the cognate antigen for NKG2A, which makes the overexpression of NKG2A biologically relevant. These data suggest that the NKG2A/HLA-E checkpoint axis may be more biologically relevant than KIR in myeloma, particularly in the post-transplant setting. This has important potential implications for treatment, as it demonstrates that NKG2A could serve as a potential target for modulation in the immediate post-transplant setting to improve survival, especially in the setting of maintenance chemotherapy. Further investigation should be aimed delineating the potential effect of NKG2A modulation on immune recovery and tumor surveillance to increase survival in the post-transplant setting.

#5664 PLAG prevents the loss of circulating neutrophils in the chemotherapy-induced neutropenia model. Yong-Jae Kim, Jinsung Jung, Ha-Reun Lee, Ki-Young Sohn, Heung-Jae Kim, Sun Young Yoon, Jae Wha Kim.

PLAG on excessive neutrophil extravasation by chemotherapy via attenuating the key role to regulate on neutrophil migration. Collectively, modulating effect of PLAG on excessive neutrophil extravasation by chemotherapy via attenuating STAT3/CXCL2(8) pathway could be used as very powerful regimen for preventing of neutropenia during diverse chemotherapy.

#5665 Physical crosstalk between CD8+ T and natural killer cells elicits antitumor effector response. Roman V. Uzhachenko, Ashutosh Singhal, Shawn J. Goodwin, William H. Hofmeister, Anil Shanker.

The interaction between the innate and adaptive immune components is fundamental for an effective antitumor immunity. Our studies in murine solid tumor models showed that productive antitumor effector response relies on functional crosstalk between innate immune effectors—natural killers (NK) and adaptive immune effectors—cytolytic CD8+ T lymphocytes. We found that this lymphocyte cooperativity between CD8+ T and NK cells can prevent the development of antigen-escape tumor variants. In this study, we first investigated the role of physical contacts in during the functional crosstalk between...
CD8+ T and NK cells. Since, studying dynamic lymphocyte interactions present extreme challenges, in this study, we engineered a 3D nanoﬁbre matrix to provide lymphocytes a 3D culture environment for a controlled interaction. Con-focal imaging showed that CD3/CD28-activated CD8+ T cells (CD69+/CD25−) formed multiple intercellular contacts with several naïve NK cells upon cocul-ture, whereas T cells made signiﬁcantly more contacts with NK cells. Lymphocyte coculture (physical contact possible) found that activated CD8+ T and NK cells cross-regulate each other phenotype wherein NK cells polarize activated CD8+ T cells towards “T central memory phenotype” and activated CD8+ T lymphocytes induce acquisition of “effector/regulatory phenotype” by naïve NK cells. This cross-regulation of lymphocytes disappeared in trans-well co-culture (no physical contact) indicating the necessity of cell-to-cell physical inter-action during CD8+ T–NK crosstalk. Notably, intercellular physical interac-tion led to cross regulation of mitoCa2+ oscillations in both activated CD8+ T and NK cells. Inhibition of mitochondrial Ca2+ uptake or Na+/Ca2+ exchanger with Ru360 and CGP37157, respectively, mimicked observed alterations in both lymphocytes. Further, NK cells displayed increased oxidative signaling, Tyk2, Jak1 and 3, Stat2 and Stat6 phosphorylation while inhibiting TCR- and various cytokine receptors-mediated signaling. In turn, NK cells selectively restrain IL-2 signaling in CD8+ T cells by dampening activation-induced up-regulation of CD25, Stat5 phosphorylation, IL-2 synthesis and elevation in IL-2 uptake. These data underscore a novel mitochondrial Ca2+ transport-regulated activation/ regulatory phenotype by NK and CD8+ T cells upon their interaction. Understanding the mechanism of the synergistic interaction between CD8+ T cells and NK cell-lymphocyte synapse allowing their functional remodeling with the intact tissues in tumor settings will lead to novel strategies for effective cancer immunotherapies, with a potential of relapse-free survival in cancer patients.

**#5666 Safety of the first-in-class anti-NKG2A monoclonal antibody monalizumab in combination with cetuximab: a phase Ib/II study in recurrent or metastatic squamous cell carcinoma of the head and neck (R/M SCCHN), Roger B. Cohen,1 Sébastien Salas,1 Caroline Ewen,1 Nuria Kotecki,2 Antonio Jimeno,3,4 Anne-Marie Souléa,5 Anne Thirouzan-Jiménez,5 Robert Zerbib,6 Pascale André,6 Agnès Boyer-Chammard,6 Jérôme Fayette,7 1Abrams-on Cancer Center, Philadelphia, PA; 2Assistance Publique-Hôpitaux de Mar-seille, Marseille, France; 3Gustave Roussy, Villejuif, France; 4Centre Oscar Lam-bret, Lille, France; 5University of Colorado Cancer Center, Aurora, Denver, CO; 6Innate Pharma, Marseille, France; 7Centre Léon Bérard, Lyon, France.**

Monalizumab (IPH2201) is a first-in-class immune checkpoint inhibitor targeting CD94-NKG2A receptors expressed on tumor infiltrating cy-totoxic CD8+ T lymphocytes and NK cells. HLA-E, the ligand of this inhibitory checkpoint receptor is up-regulated in SCCHN, protecting cancer from killing by CD94-NKG2A+ cells. Monalizumab blocks binding of CD94-NKG2A to HLA-E, reducing inhibitory signaling and thereby enhancing NK and T cell anti-tumor responses. Cetuximab is an anti-EGFR monoclonal antibody block-ing oncogenic signaling and inducing Fcγ receptor-mediated antibody depen-dent cellular cytotoxicity (ADCC). In vitro cetuximab-mediated ADCC is inhibited by HLA-E expression on target cells and this inhibition can be circumvented with CD94-NKG2A blockade. Combination of monalizumab and cetuximab might provide greater antitumor activity than either drug alone. Methods: A multicenter, non-randomized dose-escalation and expansion study is evaluating monalizumab plus cetuximab in patients with R/M SCCHN (NCT02643550). Patients ≥ 18 years who progressed after platinum-based chemotherapy (regardless of the number of previous lines of treatment) were enrolled, without regard to HLA-E or human papilloma virus status. Using a 3+3 design, 5 dose levels of monalizumab (0.4, 1.2, 4 or 10 mg/kg every 2 weeks) were explored with ﬁxed doses of cetuximab (400 mg/m² load followed by 250 mg/m² weekly). Patients were treated until disease progression or unacceptable toxicity. The primary objective was to evaluate safety and Dose Limitation Toxicity (DLT). The secondary objectives were to estimate the Maximum Tolerated Dose (MTD) and the Recommended Phase II Dose (RP2D), and to determine the pharmacokinetics, pharmacodynamics and immune responses. The enrollment began in December, 2015. As of October 11, 2016, 13 patients with R/M SCCHN were enrolled at dose levels 0, 1, 2 and 4 mg/kg. Median age was 60 years (range: 40-74); 92% were male; PS was 0 or 1; all patients had received prior systemic therapy (1 prior line in 1 patient and > 2 lines in 12 patients) for R/M SCCHN including platinum based chemotherapy (100% of the patients) and cetuximab (12% in 10 patients). First dose was no DLT. There were no DLTs or immune-related disorders or deaths related to treatment. No discontinuation attributable to treatment-related adverse events and no treatment-related grade 3 and 4 adverse events were reported, except fatigue (grade 3) in one patient. Updated data including pharmacodynamics and pharmacokinetics on the full dose escalation part will be presented. Conclusion: Monalizumab + cetuximab were well tolerat-ed with no additional safety concerns compared to monalizumab or cetuximab alone. The dose-expansion phase of the study will be initiated in the near future.

**#5667 Exposure of tumor-associated macrophages to apoptotic pancreatic cancer cells promotes cancer stem cell chemoresistance.** Gabrielle D’Errico, Mirėia Vallespinos, Sonia Alcala, Sandra Valle, Laura Martin-Hijano, Bruno Sainz, Antonio D’Errico, A. University of Madrid, Spain. Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer related deaths worldwide. This is largely due to the existence of a subpopulation of stem-like cells present within the tumor, known as cancer stem cells (CSCs) that drive metastasis and chemoresistance. In addition, we have now come to realize that the tumor-associated microenvironment not only provides structural support for tumor development, but more importantly the microenviron-ment provides cues to CSCs that regulate their biological properties. Chemotherapy often leads to apoptosis of cancer cells, and in previous studies we have shown that tumor-associated macrophages (TAMs) exponentially increase following chemotherapy. We hypothesized that TAMs, in response to chemo-therapy-induced apoptosis, secrete factors that potentiate PDAC chemoresis-tance. In line with this hypothesis, we show that monocyte-derived macrophages cultured in the presence of apoptotic PDAC cells polarize towards an M2 pro-tumor phenotype and secrete factors that render naïve PDAC cells, speciﬁcally CSCs, resistant to Gemcitabine- or Abraxane-induced apoptosis, irrespective of mutations in p53. Importantly, chemoresistant cells showed increased microRNA capacity and increased tumorigenesis as measured in an extreme limiting dilution assay in nude mice, conﬁrming an enrichment in CSCs. Moreover, using a syngeneic orthotopic in vivo model of PDAC, we were able to signiﬁcantly augment the anti-tumor potential of Gemcitabine by pharmacologically eliminating TAMs using clodronate liposomes. To determine the mecha-nism by which TAMs promote PDAC chemoresistance, we performed proteomic analyses on macrophage conditioned media and identiﬁed several proteins speciﬁcally induced and secreted when macrophages were co-cultured with apoptotic PDAC cells, including 14-3-3 protein zeta/delta (14-3-3ζ), a ma-jor regulator of apoptotic cellular pathways. We present additional data to show that TAM-secreted 14-3-3ζ promotes CSC chemoresistance. Taken together, the sum of these data highlight a unique regulatory mechanism by which chemotherapyme-induced apoptosis acts as a switch to initiate a pro-tumor/anti-ap-o-totic mechanism in PDAC, challenging the idea that apoptosis of tumor cell is therapeutically beneﬁcial, at least when immune sensor cells, such as macrophages, are present.

**#5668 CCL2/CCR2-driven monocyte recruitment to the tumor following radiotherapy inﬂuences the outcome of the treatment of head and neck cancer.** Michele Mondini,1 Pierre L. Loyher,2 Kevin Berthelot,3 Celine Clemenson,1 Alessio Ingrassias,2 Giuseppina Cannistraci,2 Eric Deutsch.1 1Gustave Roussy, Villejuif, France; 22INERM U1135, Paris, France. Local radiotherapy is the major treatment to control locally advanced head and neck cancer. Evidence supporting the importance of immune response in the response to radiation therapy is growing and the possibility to potentiate the effects of radiotherapy by modulating the function of the immune system offers new exciting therapeutic perspectives. Monocytes/macrophages are immunomodulatory agents that can amplify the effects of radiotherapy on the immune system. We have recently developed a model of human papillomavirus (HPV)-related head and neck cancer using immunocompetent mice and validated its usefulness to evaluate the tumor response to radiotherapy. Using the same model, based on the injection of TC1/luciferase cells in the inner lip of C57Bl/6 mice, we show that local radiotherapy induces an increase of CCL2 levels in the bloodstream and intratumorally accompanied by a neo-infiltration of monocytes in the tu-mor that subsequently differentiate into tumor-associated macrophages (TAM). When TC1/Luc cells were engrafed in CCL2−/- or CCR2−/- mice, the tumor response to radiotherapy and survival were improved. This was accompanied by an impaired infiltration of inflammatory monocytes, and a subsequent decrease of TAM levels at later time points. In vitro, exposure to ionizing radiation in-creased the secretion of CCL2 by TC1/Luc cells, and such increase was signifi-cantly enhanced, in tumor cells were co-cultured with irradiated RAW 264.7 macrophage-derived cells. This data suggests interplay between tumor and TAM that can amplify the effects of radiotherapy on the immune system. We propose that reducing radiation-induced monocyte recruitment may yield improved results in the treatment of HPV-related head and neck squamous cell

Breast cancer is the most common cancer in Canadian women and the second leading cause of cancer deaths. Given that most mammary tumors are surgically resectable and over 90% of breast cancer-associated deaths are due to metastasis, new therapeutic strategies targeting metastasis are required. Natural killer T (NKT) cells are a rare population of immune cells that have been shown to limit primary tumor growth and target distant metastatic disease in various animal models. We have shown that NKT cell activation improves survival in a model of post-surgical metastatic breast cancer. We are now expanding this work to determine whether NKT cell activation can be combined with chemotherapies to improve outcomes. In our model, 4T1 mammary carcinoma cells were injected into the mammary fatpad of syngeneic BALB/c mice. Tumors were resected at day 12, and mice were treated with cyclophosphamide or gemcitabine. On day 17, NKT cells were activated by transfer of dendritic cells loaded with the glycolipid antigen α-GalCer. We also examined whether gemcitabine or mafosfamide (active component of cyclophosphamide) would induce immunogenic cell death of 4T1 cells in culture. Chemotherapeutics did not affect NKT cell activation as measured by serum IFNγ levels. Treatment with cyclophosphamide or gemcitabine significantly enhanced survival as measured by serum IFNγ levels. Combined treatments significantly enhanced survival. NKT cell activation decreased the frequency and immunosuppressive function of myeloid derived suppressor cells (MDSCs). Treatments resulted in enhanced tumor specific immunity as surviving mice exhibited slower tumor growth following secondary tumor challenge. Gemcitabine and mafosfamide also increase the immunogenicity of cancer cells in vitro by increasing the expression/release of MHC I, MHC II, CD1d, Calreticulin, HMGB1 and ATP. NKT cell activation therapy can successfully be combined with low doses of gemcitabine or cyclophosphamide to enhance protection against tumor metastasis and reoccurrence. This work provides a clear rationale for combining chemotherapy with NKT cell immunotherapy to target metastatic disease in the clinical setting.


Multiple myeloma (MM) is the second most common haematological cancer and is characterized by the accumulation of neoplastic plasma cells in the bone marrow and is characterized by limited survival. Effective treatment is constrained by treatment-resistant clones, drug resistance and high levels of immunosuppressive molecules (e.g., IL-6, IL-10). Therefore, novel immunotherapeutic approaches are needed to improve treatment options to MM patients. Among the currently explored targets, B-cell maturation antigen (BCMA, CD269) is considered to be particularly attractive due to its limited expression on normal B-cells and high expression on MM cells (MM). While historically considered incurable, recent approvals and ongoing clinical trials with monoclonal antibodies (mAbs) targeting surface antigens promise greatly improved outcomes and have heralded a new era of MM treatment in which immunotherapies are expected to take center stage. However, an unmet need remains as patients eventually relapse and/or become refractory to currently available treatments. Consequently, novel immunotherapeutic approaches are needed to provide improved treatment options to MM patients. Among the currently explored targets, B-cell maturation antigen (BCMA, CD269) is considered to be particularly attractive due to its limited expression on healthy tissues and almost universal expression on myeloma cells in the majority of patients. Natural killer (NK) cells are cytotoxic effectors of the innate immune system capable of rapidly eradicating infected and transformed cells. The cytolytic activity of NK-cells can be used therapeutically to induce tumor cell lysis by direct engagement of the activating receptor CD16A (FcγRIIIa) using mAbs (ADCC). Despite similar mechanisms of target cell lysis, activation of NK-cells is not associated with the systemic symptoms of high level cytokine release as seen with direct T-cell engagement. Hence, it is considered a potent immunotherapeutic approach with reduced toxicities compared to traditional (M protein). The human immune system is regulated by a broad network of co-inhibitory and co-stimulatory receptors that control the type, scale, and duration of immune responses. These receptors are now recognized as promising immunotherapy targets for the treatment of many cancers and autoimmune diseases. Immunotherapies that block co-inhibitory receptors such as PD-1 and CTLA-4 are showing unprecedented efficacy in the treatment of some tumors, and have generated interest in the characterization of additional immunotherapy targets that may broaden the number of patients who can be helped by these drugs. Immunotherapy research programs are now exploring a wide range of both co-inhibitory (e.g. LAG-3, TIGIT, Tim-3) and co-stimulatory (e.g. GITR, 4-1BB, OX40) receptors, individually and in combination. Quantitative and reproducible functional bioassays are essential tools in the development of biologics for cancer immunotherapy. Most existing assays rely on primary cells and suffer from lengthy protocols and high day-to-day and donor-to-donor variability. These approaches are cumbersome, error-prone, and do not produce data of the quality required for drug development in a quality control environment. To address this, we have developed a suite of cell-based reporter bioassays for co-stimulatory immune checkpoint targets including GITR, 4-1BB, OX40, and CD40. In these assays, stable cell lines express luciferase reporters driven by response elements under the precise control of intracellular signals mediated by each co-stimulatory receptor. These bioassays reflect mechanisms of action for drug candidates designed for each co-stimulatory receptor and demonstrate high specificity, sensitivity and reproducibility. Reporter-based bioassays can serve as powerful tools in immunotherapy drug development for antibody screening, potency testing and stability studies.

#5673 Large established B16 tumors in mice are eradicated by ZVexα (dendritic cell-targeting lentiviral vector) and G100 (TLR4 agonist) combination immunotherapy through increasing tumor-infiltrating effector T cells and inducing antigen spreading. Tina C. Albershardt, Andrea J. Parsons, Jardin Leleux, Peter Berglund, Jan ter Meulen. Immune Design, Seattle, WA.

INTRODUCTION: Effective immunotherapy requires the presence of effector T cells penetrating the tumor. ZVexα is a hybrid lentiviral vector platform that targets dendritic cells in vivo to express genes of tumor-associated antigen (TAA)-of-interest and activate TAA-specific CD8 T cells. G100 is the intratumoral (IT) injection of formulated glucopyranosyl lipid A (a synthetic TLR4 agonist) and has been shown to induce T cell homing chemokines, CXCL9 and CXCL10. We report here that G100 promoted an inflamed tumor microenvironment (TME) and improved infiltration of ZVexα-induced TAA-specific CD8 T cells to the TME, thereby eradicating large established B16 tumors. This was previously achieved only with a complex vaccine/anti-tumor antibody/checkpoint inhibitor/IL-2 regimen (Moynihan, Nature, 2016). RESULTS: B16-OVA tumor-bearing mice were randomized into 4 treatment cohorts: 1) untreated; 2) ZVex expressing ovalbumin (ZVex/OVA), subcutaneously (SC); 3) G100 (IT); 4) ZVex/OVA (SC) and G100 (IT) combination. While mice in Cohorts 2 and 3 exhibited delayed tumor growth, nearly all mice (16/18) from Cohort 4 had completely regressed tumors and survived tumor-free until end of study (109 days). CD8+ T cell depletion abrogated this anti-tumor response. Cohort 4 mice with regressed tumors were then randomized and re-challenged with either a) B16-OVA or b) parental B16 (lacking expression of the ZVexα-targeted antigen, OVA); a) B16-OVA re-challenge was rejected by 100% of recipient mice - consistent with induction of T cell memory; and b) B16 re-challenge was rejected by 30%-50% of recipient mice - demonstrating functional antigen spreading. T cell receptor deep sequencing showed that ZVex/OVA alone increased tumor-infiltrating T cell clones specific for OVA, validated by mHHC-multimer staining. ZVex/OVA and G100 combination expanded additional T cell clones, further evidence of antigen spreading. Lastly, the TME of Cohort 4 mice showed the most profound pro-inflammatory changes, as assessed by RNA transcriptional profiling. CONCLUSIONS: These data collectively demonstrate that anti-tumor efficacy observed in Cohort 4 mice was mediated largely by ZVex/OVA-induced
effector T cells and that TME modulation with G100 drastically shifted the TME to a more inflamed milieu, promoting T cell proliferation, antigen spreading, and generation of immunological memory. To our knowledge, this is the first time that large established B16 tumors have been completely eradicated using a combination of systemic and in situ immunizations, potentially effective for cancer immunotherapy.

**#5674 Cancer immunogenomics for the development of personalized ovarian cancer vaccine.**
Muzamil Y. Want, Takemasa Tsuiji, Richard Koya, Sebastiano Battaglia, Roswell Park Cancer Institute, Buffalo, NY.

Ovarian cancer (OC) is the fifth leading cause of cancer death in the United States with approximately 20,000 women being diagnosed every year. Since OC development is mainly asymptomatic, patients are often diagnosed at late stage and present local and distal metastases. This offers a clinical challenge, as roughly 70% of the patients develop chemoresistant disease. With the advent of cancer immunogenomics it is now possible to identify tumor specific mutations, or neoantigens/neoepitopes, that can be exploited for the development of personalized T cell therapies via DC vaccines or adoptive cell therapy (ACT). We therefore hypothesize that tumor cells isolated from malignant ascites reflect the genomic landscape of the primary tumor, offering a unique opportunity to identify clinically actionable tumor neoantigens. We utilized primary tumor, tumor cells isolated from malignant ascites and metastatic samples to create patient-derived xenografts (PDX) mice and to interrogate the tumor mutational landscape. Somatic mutations were identified by whole exome sequencing (WES) and RNAseq analysis in tumor samples, using PBMCs as germline control. In parallel 20 PDX of ovarian cancer were established in NSG mice by injecting $2 \times 10^6$ cells/mouse subcutaneously (SQ, n = 10) and intraperitoneally (IP, n = 10). Tumor growth was monitored weekly via caliper measurement for mice injected SQ and via abdominal circumference change injected IP. Mutational analysis and prediction of high affinity peptides was done using bioinformatics approach. We have utilized different algorithms that are based on Artificial Neural Networks or published motifs for predicting binding of 8-11 mer peptides to number of Human MHC alleles (HLA) and out of 168 mutated peptides identified, 30 were found to have high affinity for different HLAs compared to wild type peptide. Mutated and wild type peptides were pulsed into dendritic cells and co-cultured with CD8 T cells for 20 days. Validation of stimulated CD8 T cells by these mutated peptides using ELISPOT is underway. TCR specific for neoantigen from CD8 T cells will be identified and cloned using retroviral vectors. Transfected CD8 T cells with neoantigen specific TCR will be confirmed by co-culturing them with dendritic cells pulsed with wild type and mutated peptides. Further engineered CD8 + T cells specific for neoantigens will be tested for adoptive cell therapy in the established PDX models of ovarian cancer.

**#5675 A novel PI3K inhibitor suppresses tumor progression by immune modulation.**
Zusheng Xu,1 Yangtong Lou,1 Yingying Qu,1 Wei Wang,1 Xiaoxi Wang,1 Bing Ji,1 Ying Gu,2 Yingxia Zhang,2 He Zhou,2 Shanghai Yingshi Pharmaceuticals Co. Ltd., Shanghai, China; Shanghai ChemPartner Co., Ltd., Shanghai, China.

Phosphoinositide 3-kinase delta (PI3Kδ) inhibitors have shown anti-tumor efficacy in the clinic and one such compound, idelalisib, was approved by the FDA for the treatment of relapsed chronic lymphocytic leukemia (CLL) in 2014. A novel PI3Kδ inhibitor, YY-20394, was developed with specific selectivity against PI3Kδ and inhibited PI3Kδ expressing human tumor cells growth both in vitro and in vivo. In addition, our previous data have shown YY-20394 significantly inhibited primary tumor growth in immune-competent mice with 4T1 and CT26 tumors, as well as 4T1 lung metastasis with dose dependency. The anti-tumor efficacy of YY-20394 was largely mediated by T cells. More importantly, YY-20394 synergistically enhanced the anti-tumor efficacy of anti-PD-L1 antibody in CT26 model and achieved long-term immune memory which is specific for CT26 tumors, but not for unrelated A20 tumors. In vitro mechanistic studies showed YY-20394 significantly inhibited T cell differentiation into Treg both in mouse splenocyte and in human primary CD4+ T cells, and was especially potent inhibiting their IL-10 secretion in vitro. Analyses of tumors treated with YY-20394 reviewed that YY-20394 decreased the percentage of Treg in the tumor infiltrating leukocytes, as well as IL-10 secretion. It also showed some effects on the myeloid populations in the tumors. These data suggest the YY-20394, a novel PI3Kδ inhibitor, represents a promising and safe immune modulator and shows great potential as a cancer immune-therapeutics.

**#5676 Functional pairing of immunomodulatory targets in anaplastic thyroid cancer.**

Thyroid cancer is the most common type of endocrine malignancy that has an excellent overall survival frequency. Although most well differentiated thyroid cancers (WDTC) are manageable and respond to current therapeutic modalities, undifferentiated anaplastic thyroid cancers (ATC) exhibit a dramatically different clinical behavior and poor prognosis. With the recent development of immunotherapies, targeted, well-defined treatment plans can demonstrate promising treatment outcomes in ATC patients. Precise immunological targets in ATCs with potential clinical relevance are unknown. Major progress has been made in last 5 years toward development of immune checkpoint inhibitors using anti-CTLA-4 and anti-PD-1/PD-L1 antibodies for cancer treatment which has made immunotherapies one of the mainstream treatment choices. Few additional members of the immunoglobulin superfamily of receptors, like LAG3, TIM3 and VISTA have recently been identified as potential checkpoint targets. Interestingly some of these molecules including TIM3 and PD-L1 promote tumor progression and immune escape. Identification of specific immunotherapeutic targets require a better understanding of the immune microenvironment in ATC. To this end we evaluated the expression of prominent co-stimulatory and co-inhibitory cell surface molecules by RT-PCR in three thyroid cancer cell lines - TPC-1 (papillary), CGTH-W1 (follicular) and 8505C (anaplastic). We observed that majority co-inhibitory molecules were upregulated in all three tumor cell lines. CTLA4, interestingly, had the highest expression in 8505C. Additionally we observed differential expression of BTLA, LAIR1, TIM3 and VISTA between TPC-1 and 8505C. LAG3, PD-1 and PD-L1 were also upregulated in 8505C compared to TPC-1. Similar pattern was observed with the expression of co-stimulatory molecules, CD40L and GITR. GITR has been shown to have a tumor suppressor function in multiple myeloma. Another co-stimulatory molecule OK40, which has shown promise in tumor regression when targeted, was upregulated in all three cell lines and 8505C showed the highest expression. Our findings suggest that the aggressive and less immunogenic phenotype of ATC might be attributed to the differential expression of these molecules. Targeting these immunomodulatory molecules in ATC warrants a better understanding of the crosstalk between them and it might provide an efficient means for the disease management.

**CLINICAL RESEARCH: Liquid Biopsies 5: cfDNA, MicroRNA, and Protein**

**#5677 Performance of a robust and sensitive liquid biopsy workflow for mutation detection in plasma.**
Jonathan Choi, Cindy Choi, Jorge Dinis, Jingchuan Li, Sean Chien, Bosun Min, Abraham Munoz, Janet Jin, Amrita Pati, Liang Feng, Johnny Wu, Ashla Singh, Fergal Casey. Roche Sequencing Solutions, Pleasanton, CA.

The discovery of circulating tumor DNA (ctDNA) and the development of highly sensitive technologies have made the utility of liquid biopsies in clinical practice more feasible. Potential applications for liquid biopsies include early cancer detection, tumor genotyping in the absence of tissue biopsies, disease monitoring, and identification of drug resistance markers. However, there is a multitude of technical challenges to accurately detect ctDNA with high sensitivity and reproducibility. Various methods have been established by research centers and companies, each with its advantages and limitations. We have developed a sensitive NGS-based liquid biopsy assay, called the AVENIO ctDNA Analysis Kits (research use only), which includes three panels targeting biomarkers for solid tumors. The AVENIO ctDNA Analysis Kits include cfDNA extraction from up to 5ml plasma input, optimized reagents and workflow for NGS library preparation and target enrichment. It is accompanied with an intuitive software and data analysis package to easily obtain results. The assay sensitivity detects 4 cancer relevant mutation classes – single nucleotide variations (SNVs), insertions/deletions, copy number variations and structural rearrangements. We conducted a study with 200+ samples of plasma-derived cfDNA, cell line blends containing relevant cancer mutations, and clinical samples, with which we demonstrated the analytical sensitivity of the three distinct AVENIO gene panels (covering 17, 77 and 197 genes). We demonstrate >95% sensitivity for SNVs at 0.5% mutant allele frequency; >95% specificity for 1% indels; >95% specificity for 1% structural variants. We also demonstrate assay robustness through multiple replicates, day-to-day, and operator-to-operator reproducibility. In conclusion, the AVENIO ctDNA Analysis Kits for solid tu
nors encompasses a comprehensive assay and analysis workflow aimed to provide researchers with robust, reliable, highly sensitive and easy to use liquid biopsy assays to conduct the necessary studies to demonstrate the utility of circulating biomarkers in routine clinical testing.

**#5678** Novel serum microRNAs that enable liquid biopsy for colorectal cancer: validation study of large cohort. Hirayuki Takamur, 1 Yutaka Saito, 1 Taku Sakamoto, 2 Seiichiro Abe, 3 Masayoshi Yamada, 1 Takashi Nakajima, 1 Kazuki Sudo, 2 Ken Kato, 1 Junpei Kawauchi, 1 Satoko Takizawa, 1 Hiromi Sakamoto, 1 Motohiro Kojima, 1 Atsushi Ochiai, 1 Shumpei Niida, 2 Hideshi Ishii, 3 Tomoko Takamur, 1 Juntaro Matsuaki, 2 Takahisa Matsuoka, 2 Takahiro Ochiai, 1 National Cancer Center Hospital, Tokyo, Japan; 1Toray Industries, Inc., Tokyo, Japan; 1National Cancer Center Research Institute, Tokyo, Japan; 2National Cancer Center Hospital East, Tokyo, Japan; 3National Center for Geriatrics and Gerontology, Tokyo, Japan; 4Osaka University Graduate School of Medicine, Osaka, Japan; 5Showa University, Tokyo, Japan.

Introduction: Recent studies have reported that serum microRNAs (miRNAs) are potentially useful biomarkers for cancer. However, the detection system using serum miRNAs is not established in colorectal cancer (CRC). The aims of this study are 1) to identify specific serum miRNAs and establish the discriminant model for CRC detection and 2) to validate the miRNAs and the discriminant model using a large cohort. Methods: First, we identified serum miRNAs related with the presence of CRC and constructed the discriminant model for CRC detection using the publicly-released serum miRNA database including 50 patients with CRC and 90 healthy individuals (GSE39016). Comparing serum miRNA levels between patients with CRC and healthy individuals, we picked up miRNAs (p<0.05 by t-test and fold change > 2). Using these miRNAs, Fisher’s linear discriminant analysis was performed and the diagnostic models were constructed using less than 7 miRNAs. Subsequently, 1117 patients of National Cancer Center Hospital with CRC were enrolled as a validation cohort. Patients with the following criteria were excluded: (i) patients simultaneously or previously diagnosed as the other cancers, (ii) patients who were treated for CRC previously and (iii) patients with familial adenomatous polyposis or Lynch syndrome. Control blood samples were obtained from patients without any history of cancer who were admitted or referred to National Center for Geriatrics and Gerontology or Yokohama Minoura Clinic between 2010 and 2015. A total of 1013 CRC patients and 4384 non-cancerous patients were analyzed. Total RNA was extracted from 300 micro L of serum and comprehensive miRNA expression analysis was performed using a 3D-Gene microarray. ROC analysis was performed to evaluate the previous discriminant models. The sensitivity in each pathological stage and location of CRC was also investigated. This research is partially supported by the “Development of Diagnostic Technology for Detection of miRNA in Bodily Fluid” from the Japan Agency for Medical Research and Development (AMED). Results: First we picked up 30 miRNAs for CRC diagnosis. Fisher’s analysis revealed 107611 candidates of numerical formulas, that showed > 80% of sensitivity and specificity. We could narrow these candidates to 43 formulas with validation analysis. By ROC analysis, the AUC, sensitivity and specificity of the discriminant formula with best performance was 0.904; 79.6% and 86.5% respectively. The sensitivities of each pathological stage were as follows; pStage 0: 79.0%, pStage I: 90.7%, pStage II: 85.1%, pStage III: 73.7%, pStage IV: 62.2%. The sensitivity of CRC in the right side of the colon was 79.4%, whereas in the left side was 79.7%. Conclusions: We identified novel serum miRNAs for CRC detection. Our discriminant using these miRNAs can diagnose CRC including early stage. Furthermore, the sensitivity was high irrespective of the tumor location.

**#5679** Using droplet digital PCR to analyze MYCN copy number in plasma from patients with high-risk neuroblastoma. Marco Lodrini, 1 Annika Sprüssel, 1 Annika Atrash, 1 Solna, Sweden; 2Robert Kochschak, 1 Holger Lode, 2Matthias Fischer, 3Johannes H. Schulte, 1 Angelika Eggert, 1 Hedwig Debeer 1Charité, Berlin, Germany; 2Greifswald University Hospital, Greifswald, Germany; 3University Hospital Cologne, Cologne, Germany.

The invasive nature of surgical biopsies most often prevents their sequential application to monitor disease in patients with high-risk neuroblastoma. Even when available, single biopsies often fail to reflect neuroblastoma dynamics, intratumor heterogeneity and drug sensitivities likely to change during neuroblastoma evolution and treatment. Implementing molecular characterization of cell-free neuroblastoma-derived DNA (cfDNA) isolated from blood plasma would improve outcome prediction, patient monitoring and treatment selection for high-risk neuroblastoma patients, by providing a method to follow clonal evolution in tumor subpopulations and treatment response as well as capture the molecular landscape of all tumor clones. As a first step, we established a digital droplet PCR (ddPCR) protocol to analyze the MYCN copy number status. Analyzing the dilution series of a synthetic MYCN template consisting of 70 nucleotides demonstrated a strong correlation between theoretically calculated MYCN copy number and the MYCN copy number measured using our ddPCR protocol in these patients. The MYCN copy number status in a large panel of neuroblastoma cell lines with MYCN copy number determined from cfDNA isolated from cell culture media from the corresponding cell lines. Our ddPCR protocol reliably detected MYCN status in the cell line using cfDNA analysis. Next, we analyzed cfDNA copy number status in primary neuroblastoma samples from 10 patients and compared these values to the MYCN copy number determined from cfDNA isolated from 200 µL of blood plasma collected from the corresponding patients. These final validation steps demonstrated that our ddPCR protocol for cfDNA from patient blood plasma reliably detects MYCN copy number status in the tumor. Our data justify the further development of molecular neuroblastoma characterization from cfDNA in patient blood plasma. An expanded molecular diagnostic monitoring palette will improve monitoring of disease progression including relapse and metastatic events as well as therapy success or failure in high-risk neuroblastoma patients.

**#5680** Clinical significance of low frequency EGFR and KRAS mutations of cell free DNA using Ion AmpliSeq Cancer Hotspot Panel in lung cancer patients. Jae Sook Sung, 1 Jong Won Lee, 2 Boyeon Kim, 3 Saet Byeol Lee, 2 Chang Won Park, 2 Ha Mi Kim, 2 Nak-Jung Kwon, 2 Won Jin Jang, 2 Yoon Ji Choi, 2 Jung Yoon Choi, 2 Eun Joo Kang, 2 Kyung Hwa Park, 2 Sung Yong Lee, 2 Yeol Hong Kim, 1 Cancer Research Institute, Korea University, Seoul, Republic of Korea; 2Division of Pulmonary Medicine, Department of Internal Medicine, Korea University Anam Hospital, Korea University College of Medicine, Seoul, Republic of Korea; 3Department of Internal Medicine, Korea University Guro Hospital, Seoul, Republic of Korea.

Cell free DNA (cfDNA) present in the bloodstream shows great potential as a useful cancer marker for molecular diagnosis and cancer progression monitoring. Especially, the cfDNA with Next Generation Sequencing (NGS) technology allows high through put examination of various genes concurrently at a low cost. However, there are still debates regarding clinically meaningful variant frequency to identify mutations in cfDNA, especially with ultra-deep sequencing. In this study, we examined the clinical utility of Ion AmpliSeq Cancer Hotspot Panel v2 (ICP; Ion Torrent) with Proton platform for cfDNA. We compared 2800 COSMIC mutations from 50 cancer genes was used to analyze cfDNA of 125 serum samples from lung cancer patients. The percentage of on target was 92% with mean depth of 22,868X. We identified aberrations of TP53 (72%), EGFR (43%), PSEN (26%), PIK3CA (26%), BRF (16%), KRAS (14%), KIT (10%) and RET (10%) with the cut-off criteria of variant frequency >0.1% and p<0.01. To validate the results, we analyzed EGFR gene status by direct sequencing in available 100 FFPE tumor tissues (tDNA). Out of 17 patients with EGFR mutations in tDNA, 9 patients showed very low frequency (<0.05%) of same EGFR mutation in cfDNA. To validate the results of ICP, droplet digital PCR (ddPCR) was carried out with same cfDNA. From those 9 patients, EGFR mutations in cfDNA were detected in 5 patients (minimum frequency 0.01%) by ddPCR. From the patients with wild type EGFR in tDNA, EGFR exon 19 deletion or exon 21 point mutation were detected by ICP in 19 patients using cfDNA. Again, ddPCR was carried out with same cfDNA to confirm the result. EGFR mutations were confirmed in nine patients (47.4%) by cfDNA ddPCR and among the 6 patients treated with EGFR TKI, 4 patients showed response or stabilization of disease. Also, we identified 18 patients with KRAS mutations in tDNA. The result of ddPCR was matched in 80% of patients. Interestingly, 2 patients had multiple KRAS mutations in cfDNA with ICP as well as ddPCR. In our study, we demonstrated that ICP with Proton system is a useful assay to identify somatic mutations using cfDNA in lung cancer patients. Also, we suggest that even EGFR mutation of very low frequency (<0.05%) might have clinical significance in NGS analysis using cfDNA. In future studies, we will be reanalyzed the 2800 COSMIC mutations in cfDNA with ddPCR. [This research was supported by the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (HI14C0066)].
CLINICAL RESEARCH: Liquid Biopsies 5: cfDNA, MicroRNA, and Protein

Many studies have been conducted investigating cell-free DNA (cfDNA) for disease diagnosis. To prevent cellular DNA release into the plasma, plasma separation within 2 hours of blood collection is required, utilizing the most commonly used type of tube (BD Vacutainer® CPT™; CPT). These temporal restrictions render operations requiring plasma separation a laborious task. It has been noted that Cell-Free Plasma BCT tubes (Streck Inc. BCTs) prevented nucleated cell disruption. The use of BCTs may enable us to separate plasma without temporal restrictions. The aim of this study was to evaluate changes in DNA levels in plasma after preservation using two types of collection tubes. Samples were drawn from 7 healthy donors into CPTs and BCTs, and stored at room temperature. Plasma was separated immediately (d0), and on days 3, 6, 9, 12, and 14 after blood collection. The concentrations of DNA in the extracted plasma samples were measured. Blood stored in CPTs showed increases in plasma DNA concentrations over time. The mean DNA concentrations at each time-point were 0.39 (d0), 2.96 (d3), 40.44 (d6), 84.94 (d9), 91.41 (d12), and 94.94 (d14). However, plasma DNA levels remained stable in BCTs from 6 days. There were significant differences between the DNA concentrations in BCTs on d0 (0.74 [0.43-1.44] ng/µl) and d14 (1.78 [0.63-4.20] ng/µl) (p = 0.0378). Although significant differences were only observed between d0 and d14 in BCTs, the amount of plasma DNA gradually increased after d6. Furthermore, obvious color changes in the plasma were observed, and the boundary between plasma and PBMCs became unclear over time. These results indicate that BCTs may yield stable cfDNA samples for up to 6 days after blood collection. BCTs may be a novel collection tube for cfDNA research, including liquid biopsies in cancer patients.

#5684 NSABP FC-7 correlative study: HER2 amplification (amp) in circulating cfDNA (cfdNA) in metastatic colorectal cancer (mCRC) resistant to anti-EGFR therapy (tx) (S. Kim, Ashok Srinivasan, Carmen J. Allegra, Samuel Jacobs, Rebecca J. Nagy, Richard B. Lanman, AmirAli Tala- vaz, Patrick Gavin, Rekha Pal, Peter C. Lucas, Kay Pogue-GeIle, NSABP, Pittsburgh, PA; NSABP, and UF Health, Gainesville, FL; Guardant Health, Redwood City, CA; University of Pittsburgh School of Medicine, Pittsburgh, PA.

Background. FC-7 is a phase Ib study hypothesizing that dual ERBB blockade (neratinib and cetuximab) could overcome an acquired resistance mechanism to anti-EGFR tx in KRAS/NNAS/BRAF/PiK3CA wild type (wt) mCRC patients (pts).

Previously we demonstrated a 36% rate of HER2 amp in post-anti-EGFR biopsies (bxs). Assay of plasma cfDNA is minimally invasive and may provide comprehensive genomic profiling. We tested the accuracy of a cfDNA assay for HER2 amp, comparing results from matched bxs to evaluate molecular changes in HER2 copy number. Methods: 23 pts with mCRC resistant to anti-EGFR tx were enrolled and treated with cetuximab and neratinib. 17 paired bxs (6 post-tx bxs had insufficient tumor) were assessed for HER2 amp using chromogenic in situ hybridization (CISH) (Dako PharmDx™). HER2 was considered amplified when HER2/CEP17 ratio ≥ 2. cfDNA was extracted from plasma of 11 pts after 1st line anti-EGFR tx but before addition of cetuximab and neratinib. 5-30 ng of DNA was sequenced across S12 exons in 24 genes with Hiseq (Illumina). Copy number variants (CNV) were quantified using a customized pipeline (Guardant 360). One plasma sample failed to yield sufficient cfDNA. 10 pts had comparison of cfDNA with pre- or post-anti-EGFR bx. Results: 4 of 9 plasma samples (1 non evaluable) had HER2 amp, all concordant with pre- or post-anti-EGFR bx tissue. 3 pts converted to HER2 amp after anti-EGFR tx (pts 3, 5, 7), and for 2 of these conversions (pts 5, 7), the amplification was evident in cfDNA.

#5683 Evaluation of the utilization of blood collection tubes for cell-free DNA research. Fumitaka Endo, Takeshi Iwaya, Takeshi Chiba, Mizunori Yae-gashi, Kei Sato, Kohei Kume, Atsuhiro Arisue, Yutaka Nishinari, Ryoko Kawagi-shi, Takenori Segawa, Satoshi Nishizuka, Akira Sasaki. Department of Surgery, Iwate Medical University School of Medicine, Morioka, Iwate prefecture, Japan.

Many studies have been conducted investigating cell-free DNA (cfDNA) for disease diagnosis. To prevent cellular DNA release into the plasma, plasma separation within 2 hours of blood collection is required, utilizing the most commonly used type of tube (BD Vacutainer® CPT™; CPT). These temporal restrictions render operations requiring plasma separation a laborious task. It has been noted that Cell-Free Plasma BCT tubes (Streck Inc. BCTs) prevented nucleated cell disruption. The use of BCTs may enable us to separate plasma without temporal restrictions. The aim of this study was to evaluate changes in DNA levels in plasma after preservation using two types of collection tubes. Samples were drawn from 7 healthy donors into CPTs and BCTs, and stored at room temperature. Plasma was separated immediately (d0), and on days 3, 6, 9, 12, and 14 after blood collection. The concentrations of DNA in the extracted plasma samples were measured. Blood stored in CPTs showed increases in plasma DNA concentrations over time. The mean DNA concentrations at each time-point were 0.39 (d0), 2.96 (d3), 40.44 (d6), 84.94 (d9), 91.41 (d12), and 94.94 (d14). However, plasma DNA levels remained stable in BCTs from 6 days. There were significant differences between the DNA concentrations in BCTs on d0 (0.74 [0.43-1.44] ng/µl) and d14 (1.78 [0.63-4.20] ng/µl) (p = 0.0378). Although significant differences were only observed between d0 and d14 in BCTs, the amount of plasma DNA gradually increased after d6. Furthermore, obvious color changes in the plasma were observed, and the boundary between plasma and PBMCs became unclear over time. These results indicate that BCTs may yield stable cfDNA samples for up to 6 days after blood collection. BCTs may be a novel collection tube for cfDNA research, including liquid biopsies in cancer patients.

Conclusion: In this sample set, HER2 amp detected in plasma-derived cfDNA from mCRC pts resistant to anti-EGFR tx is comparable to amp assessed in primary or metastatic tissue. Given advantages of liquid bx relative to repeated tissue bx, mea-
CLINICAL RESEARCH: Liquid Biopsies 5: cfDNA, MicroRNA, and Protein

#5685 An evaluation of DNA and RNA based blood biomarkers in prostate cancer. Jenny Antonello,1 Jakub Chudziak,1 Alan Redfern,1 Victoria Foyle,1 Shambhavi Srivastava,2 Adnan Syed,1 Deborah Burt,1 Mahmood Ayub,1 Bedirhan Kileri,1 Marina Parry,1 Richard Marais,2 Esther Baena,1 Crispin Miller,1 Dominic Rothwell,1 Noel Clarke,2 Caroline Dive,1 Ged Brady1.1 CRUK Manchester, Manchester, United Kingdom; 2Christie NHS Foundation Trust Manchester, Manchester, United Kingdom.

Background: Prostate cancer (CaP) is the second-leading cause of male cancer-related mortality in Western societies. Localised prostate cancer (Loc-CaP) can be classified into low-, intermediate-, or high-risk groups. Active surveillance is recommended for low-risk patients, whereas radiotherapy or surgery is often indicated in intermediate- and high-risk patients, with possible intensification using hormone therapy treatment. Despite advances in radiation delivery and surgery, ~20% of patients will not be cured with local treatment and require androgen deprivation therapy (ADT). The majority will develop resistance to ADT resulting in castration resistant prostate cancer (CRPC), with poor prognosis and increased risk of metastatic disease (mCRPC). Biomarkers are being developed based on tumour biopsies which may help stratify patients and allow more effective treatment. However tumour biomarkers are invasive, demanding for patients and surgeons as well as challenging for longitudinal sampling. Here we evaluate a range of blood biomarkers suitable for longitudinal sampling including circulating tumour cells (CTCs), circulating tumour DNA (ctDNA) and Tumour educated platelet mRNA (TEP-RNA). Methods: A blood sampling, processing and banking pipeline has been established for CaP patients providing ctDNA, enriched and single CTCs as well as TEP-RNA. Following ctDNA isolation, next generation sequencing (NGS) libraries are prepared to generate copy number alteration (CNA) data and mutation profiles. CTCs are enriched using both the epitope independent Parsertix (P-CTC) (Chudziak J. et al, 2016) and the epitope dependent (EPACM and KRT19) CellSearch (CS-CTC) platforms. Genomic analysis of CTCs is carried out by NGS of whole genome amplified (WGA) single and pooled CTCs. CTC and TEP-RNA mRNA profiles are established using both the Fluidigm RT qPCR (Fluidigm Biomark™ HD system) and RNA-Seq. Results: Over 160 Loc-CaP and 100 mCRPC blood samples have been collected, processed and banked. In keeping with published data 53% of the 100 mCRPC patients have ≥50% CTCs (a poor prognostic indicator) compared to 100% of Loc-CaP having <2 CTCs. Control cell “spike in” RNA data showed ≥1000 fold enrichment of epithelial mRNAs post Parsertix CTC enrichment indicate an equivalent enrichment of the “spiked in” cells. Similar epithelial mRNAs enrichment was seen in 3/5 mCRPC patients. Using a CNA “spiked in” RNA-Seq data showed that exosomes only carry short fragments of mRNA and ncRNA. To address this, we have developed a novel platform specifically designed to perform RNASeq on long RNA from exosomes to efficiently interrogate these as RNA biomarkers. Paired-end sequencing revealed the presence of a wider diversity of annotated mRNA and ncRNAs than previously recognized in exosomes. Employing our whole transcriptome unique molecular indexing strategy, we were able to accurately quantify the number of unique RNA molecules. We detected a total of over 1.4 million unique RNA molecules in our libraries, with 271,308 mapped to protein-coding regions from as little as 2 ml of plasma. We observed that even at a shallow sequencing depth (15 million read pairs), we could detect more than 10,000 genes, composed of 40,000 mRNA and 37,000 ncRNA annotated transcripts. ERCC spike-in analysis indicates that our RNASeq platform is highly sensitive, detecting as few as 8 copies of RNA. Long non-coding RNAs dominated the ncRNA classes and accounted for over 95% of all uniquely detected ncRNAs. We also observed a broad distribution of transcript lengths in the liquid biopsy space has been limited to variant detection in cell-free DNA and circulating tumor cells but transcriptional dysregulation is also an important feature of cancer. Our results shed new light on the biology of exosomes and indicate that, in addition to well-recognized small RNA cargo, plasma exosomes carry an abundance of long RNAs that can be interrogated by our optimized workflow. As exosomes provide a rich and protected source of RNA in biofluids, sequencing both long and small RNA from exosomes has the potential to identify biomarkers without the need for invasive tissue biopsies and allow us to have a more complete picture of how the RNA transcriptome in plasma changes in health and disease.

#5687 Detection and monitoring of IDH mutations in unamplified plasma cell-free DNA in patients with advanced cancers treated with IDH inhibitors. Filip Janjigian1, Anthony P. Conley1, Anjali Raina1, Verónica R. Holmes1, Aung Naing1, Daniel D. Karp1, David Fogelman1, Ahmed O. Kaseb1, Rajyalakshmi Luthra2, George A. Karlin-Neumann3, Funda Meric-Bernstam4, Jenny Antonello1, Jakub Chudziak1, Alan Redfern1, Victoria Foyle1, Sunita Badola1, Graham Miller1, Dominic Rothwell1, Noel Clarke2, Caroline Dive1, Ged Brady1. 1ExosomeDiagnostics,Inc.,Cambridge,MA; 2TakedaPhar- maceticals International Co. Translational and Biomarker Research, Cam- bridge, MA; 3Sagrat Cor Hospital-Quirónsalud Group, Barcelona, Spain; 4OncologyInstitutDrRosell,DexeusUniversityHospital-Quirónsalud Group, Barcelona, Spain; 5CRUK Manchester, Manchester, United Kingdom; 6Christie NHS Foundation Trust Manchester, Manchester, United Kingdom.

Background: Cell-free (cf) DNA from plasma of patients with advanced cancers offers an easily obtainable material for detection of IDH mutations, which can be used for diagnostics and monitoring purposes. Methods: Plasma was serially collected from patients with advanced cancers with IDH1 R132 or IDH2 R172 mutations, who were enrolled to clinical trials with IDH1, IDH2 or pan-IDH inhibitors. cfDNA was purified and up to 16 ng of each DNA was tested for mutations in the R132 hotspot of IDH1 and R172 hotspot of IDH2 using the QX200 Droplet Digital PCR™ platform (Bio-Rad). Results (mutation allele fre- quency [MAF] in the wild-type background or the number of IDH-mutant copies/mL of plasma) were compared both to treatment outcomes and to the results of mutation analysis of either archival primary or metastatic tumor tissue obtained at different points of clinical care from a CLIA-certified laboratory. Results: Of the 28 patients (cholangiocarcinoma, 24; other cancers, 4) with IDH1 or IDH2 mutations (N=25) or IDH2 R172 mutations (N=3) in the tumor tissue, IDH mutations were detected in 26 (93%) of plasma cfDNA samples even though median time from tissue to plasma sampling was 16.5 months (3.5-71.1 months). Quantity (< median vs. > median cfDNA or copies/mL) of IDH-mu- tant cfDNA was not associated with survival (median not reached vs. 11.1 months, P=0.26). The best radiological response to treatment with IDH inhibit- ors was achieved in patients with disease for >18 months in 28 (19.8% of patients). Median progression-free survival was 1.8 months (95% CI 1.4-2.2). The median quantity of IDH- mutant cfDNA (MAF and copies/mL) at baseline, on therapy and at disease progression did not differ (P=0.16). Changes in quantity (MAF and copies/mL) of IDH-mutant cfDNA within first 3 weeks of therapy were not associated with response on radiographic imaging (P<0.05) or progression-free survival (P=0.12). Conclusions: Detection of IDH mutations in a small amount of unamplified plasma cfDNA using ddPCR has excellent sensitivity compared to conventional clinical IDH mutation testing of archival specimens. Quantity and change in IDH-mutant cfDNA did not correspond with treatment outcomes.

#5688 Routine testing for KRAS mutations in cfDNA from blood of advanced cancer patients. Mónica Garzón Ibáñez1, Clara Mayo de las Casas1, Víctor Gil de Laraniva2, Filip Janjigian3, Avelino Escudero4, Beatriz Gracia5, Sergi Vilàtoro6, Jordi Bertrán-Alamillo7, Alejandro Martínez-Bueno8, Santiago Viteri Ramírez2, Ana Pérez Rosado1, Daniela Morales Espinoza1, María José Catalán,1 Niki Karachalios,4 Miguel Ángel Molina-Vila9, Rafael Rosell3,9.1 Pauengiae Oncology, Barcelona, Spain; 2Oncology Institut Dr Rosell, Dexeus University Hospital-Quiron- Salud Group, Barcelona, Spain; 3Sagrat Cor Hospital-Quironsalud Group, Barcelona, Spain.

Background: Mutations in the KRAS proto-oncogene, GTPase (KRAS) are driver alterations in several tumors, such as non-small-cell lung (NSCLC) and colorectal cancer (CRC). However, their role as a prognostic marker in liquid biopsies is unclear. We studied the status of KRAS mutations in the peripheral blood of advanced
NSCLC and colorectal cancer patients visited in our hospital and evaluated its potential value as a monitoring marker in the clinical practice. Methods: We developed a sensitive TaqMan assay, in the presence of a PNA clamp, for the determination of KRAS mutations (codons 12, 13 and 61) in circulating-free DNA (cfDNA). The assay detected 2.5 pg mutated DNA/µL and a ratio of KRAS mutated versus wild type allele of 1:2000, and was validated in 80 cancer patients previously genotyped in tumor tissue showing a clinical sensitivity of 72.5% and specificity of 100%. cfDNA was isolated from serum and plasma specimens, using an automatic extractor and mutational analyses were performed in quadruplicates samples. Serum and plasma samples were drawn at diagnosis, during follow-up and progression. Results: 239 NSCLC and 11 colorectal cancer patients were screened for KRAS mutations in cfDNA at presentation in the period of 2013 to 2016. In addition, 160 cfDNA samples collected during disease monitoring of 61 patients were analyzed. Paired biopsies were available from 110/250 pts at diagnosis (60 positive for KRAS in FFP and 50 WT). The KRAS mutation was detected in the cfDNA of 76.6% (46/60) p positive in tumor tissue vs. only in 1/50 WT patients where a GI2C was detected in cfDNA in a consistent manner, probably associated with a heterogeneity of the tumor. In patients with no biopsy available, we detected KRAS mutations in cfDNA in 14.3% (20/140) cases at diagnosis. The most prevalent mutations observed in cfDNA were GI2C (44.7 %), GI2V (22.4%) and GI2D (10.4%) for NSCLC patients and GI3D (5.9%) for colorectal patients. The Q61H mutation was detected in the cfDNA of one NSCLC and one CRC p. Of the 67 patient’s positive in cfDNA at presentation, KRAS mutations were detected in serum and plasma in 38 patients, only in plasma in 24 and only in serum in 5. Regarding the 61 patients with serial blood samples, we observed a correlation between the KRAS mutation load in cfDNA and the course of the disease, with a decrease in patients responding to chemotherapy and an increase during disease progression. Conclusions: Our results demonstrate the feasibility of KRAS mutation analysis in the cfDNA of advanced NSCLC and colorectal patients. Analysis of KRAS mutations in the blood of mutated patients can have a value in patients with no biopsy available and to monitor the course of the disease.

#5689 Identify tissue-of-origin in cancer cfDNA by whole genome sequencing. Yaping Liu,3 Sarah Reed,4 Atish D. Choudhury,4 Heather A. Parsons,5 Daniel G. Stover,5 Gavin Ha,5 Gregory Gyudosh,5 Justin Rhoades,5 Denisse Rotem,5 Samuel Freeman,2 Viktor Adalsteinsson,5 Manolis Kellis1,5, MIT, Cambridge, MA; 2Dana-Farber Cancer Institute, Boston, MA.

Cell free DNA (cfDNA) has been shown to be an emerging non-invasive biomarker to monitor tumor progression in cancer patients. Elevated cfDNA has been found not only from tumors, but also from normal tissues. Thus, the identification of cfDNA’s tissue-of-origin is critical to understand the mechanism of cfDNA release and tumor progression. Recent efforts to identify cfDNA’s tissue-of-origin begin to utilize cfDNA’s epigenetic status, such as DNA methylation and nucleosome spacing. However, both of these methods have limitations: (1) for nucleosome positioning, lack of reference nucleosome maps in different tumor and normal tissues has limited its application to tissue-of-origin deconvolution; (2) For DNA methylation, large DNA degradation during whole genome bisulfite sequencing (WGBS) library preparation, even with current low-input DNA technology, is still the major hurdle for its clinical application, although extensive DNA methylation studies by WGBS in tumor and normal tissues have been reported. Along the histone modification maps. Very recently, a pioneer study showed significant differences between DNA fragment lengths of methylated and unmethylated cfDNA. Taking advantage of this experimental observation, we developed a machine learning approach to infer the base pair resolution DNA methylation level from fragment size information in whole genome sequencing (WGS). The predicted DNA methylation, from not only high coverage but also dozens of ultra-low pass WGS (ULP-WGS), showed high concordance with the ground truth DNA methylation level from WGS in the same cancer patients. Furthermore, by using hundreds of WGS datasets from different tumor and normal tissues/cells as the reference map, we deconvoluted cfDNA’s tissue-of-origin status by inferred DNA methylation level at ULP-WGS from thousands of breast/prostate tumor samples and healthy individuals. The cfDNA’s tissue-of-origin status in cancer patients showed high concordance with confirmed metastasis tissues from physicians. Interestingly, some clinical information, such as cancer grades/stages, seemed to be correlated with cfDNA’s tissue-of-origin status. Overall, our methods here pave the road for cfDNA’s application in clinical diagnosis and monitoring.


The evaluation of cfDNA allows novel approaches to noninvasive detection of actionable alterations, resistance mechanisms, and tumor monitoring in patients with cancer. Importantly, tumor-specific DNA fragments represent a small minority of the cfDNA and can be obscured by false positive (FP) variants introduced by chemical damage and sequencer error. To address this, we improved key processes in the design of NGS libraries, including a new molecular barcoding approach, that maximize molecular recovery while eliminating spurious variants. We engineered a type all-1:2000 mutant and was validated in 80 cancer patients previously genotyped in tumor tissue showing a clinical sensitivity of 72.5% and specificity of 100%. cfDNA was isolated from serum and plasma specimens, using an automatic extractor and mutational analyses were performed in quadruplicates samples. Serum and plasma samples were drawn at diagnosis, during follow-up and progression. Results: 239 NSCLC and 11 colorectal cancer patients were screened for KRAS mutations in cfDNA at presentation in the period of 2013 to 2016. In addition, 160 cfDNA samples collected during disease monitoring of 61 patients were analyzed. Paired biopsies were available from 110/250 pts at diagnosis (60 positive for KRAS in FFP and 50 WT). The KRAS mutation was detected in the cfDNA of 76.6% (46/60) p positive in tumor tissue vs. only in 1/50 WT patients where a GI2C was detected in cfDNA in a consistent manner, probably associated with a heterogeneity of the tumor. In patients with no biopsy available, we detected KRAS mutations in cfDNA in 14.3% (20/140) cases at diagnosis. The most prevalent mutations observed in cfDNA were GI2C (44.7 %), GI2V (22.4%) and GI2D (10.4%) for NSCLC patients and GI3D (5.9%) for colorectal patients. The Q61H mutation was detected in the cfDNA of one NSCLC and one CRC p. Of the 67 patient’s positive in cfDNA at presentation, KRAS mutations were detected in serum and plasma in 38 patients, only in plasma in 24 and only in serum in 5. Regarding the 61 patients with serial blood samples, we observed a correlation between the KRAS mutation load in cfDNA and the course of the disease, with a decrease in patients responding to chemotherapy and an increase during disease progression. Conclusions: Our results demonstrate the feasibility of KRAS mutation analysis in the cfDNA of advanced NSCLC and colorectal patients. Analysis of KRAS mutations in the blood of mutated patients can have a value in patients with no biopsy available and to monitor the course of the disease.

CLINICAL RESEARCH: Liquid Biopsies 5: cfDNA, MicroRNA, and Protein
#5694 Multi institutional evaluation of a new NGS assay for mutation detection from cfDNA in lung cancer. Jose L. Costa,1 Robbert Werfen,2 Anna Maria Rachiglio,3 Andrea Mafficini,4 Henriette Kurth,5 Anne Reiman,6 Audrey Didelot,7 Alexander Boag,8 Claudia Vollbrecht,9 Kazuto Nishino,10 Harriet E. Feil-10tor,7 Pierre Laurent-Puig,1 Orla Sheils,11 Aldo Scarpa,1 Marijolin Ligtenberg,1 Ian Reid,1 Francesco Arico,1 Cebrian Sanchez,2 Antonio Buonomo,9 Annasteps,10 timp, Porto, Portugal;2 Radboud university medical center, Nijmegen, Netherlands;3Centro di Ricerche Oncologiche di Mercogliano (CROM)-Istituto Nazionale Tumori “Fondazione G. Pascale”; IRCCS, Naples, Italy;4ARC NET; Centre for Applied Research on Cancer, Verona, Italy;5Violler AG, Basel, Switzerland;6University Hospi-6tals Coventry and Warwickshire; Coventry, United Kingdom;7University Paris Desc-7oparques; Centre d’Assistance Des Maladies Hépatiques et Digestives; Service of Gastroenterology and Hepatology; George Pompidou Hospital, Paris, France;8Queen’s University, Kingston, Ontario, Canada;9Charité - University Hospital Berlin, Charité Campus Mitte, Institute of Pathology German Cancer Research Center (DKFZ), Berlin, Germany;10Kinki University Fac-10ulty of Medicine, Osaka, Japan;11Trinity Translational Medicine Institute, Dublin, Ireland;12Cell Biology and Biotherapy Unit, Istituto Nazionale Tumori “Fondazione G. Pascale”, Naples, Italy.

Introduction: The detection of actionable mutations in lung cancer is still a major challenge due to the lack of tissue specimens for molecular profiling of the tumor in approximately 25% of patients. The circulating cell-free tumor DNA (cfDNA) isolated from plasma of cancer patients is an alternative, minimally invasive source of tumor DNA that also allows rapid determination of the mutations present in the tumor. Moreover, the initial information provided in cfDNA makes their detection and quantification in plasma a challenging task. Here we report a multi-institutional validation of the Oncomine cfDNA Lung Cancer assay for the analyses of cfDNA in molecular pathology laboratories. Methods: The Oncomine cfDNA Lung assays is a multiplexed sequencing assay for liquid biopsy that generates reads containing targeted cfDNA regions along with a molecular tag. In order to allow an initial uniform evaluation of the assay, the Multiplex 1 cfDNA Reference Standard (Horizon Dx) derived from human cell lines, and fragmented to an average size of 160bp±10% (144bp–176bp) to closely resemble cfDNA extracted from human plasma, was used. The reference sample covers 8 mutations in the EGFR, KRAS, NRAS and PIK3CA genes at 5%, 1%, 0.1% allelic frequencies and wildtype allele. The same lot of control sample was distributed to the participating laboratories within the OncoNetwork Consortium. Samples were sequenced twice in each laboratory either using the Ion PGM system or the Ion S5 system. Libraries were templated using the Ion Chef and multiplexed as four libraries on a 318/520 chip or eight libraries on a 530 chip. A bioinformatics pipeline within the Torrent Server software allowed for automated variant calling. Results: The laboratories involved in the study were able to detect the 8 hotspot base changes and indels present in the control samples at allelic frequencies from 0.1% to 5% with an average 94.05% sensitivity (range 87.50% - 97.92%) and an average 99.87% specificity (range 99.53% - 100%). When only considering variants at the 0.1% allelic frequency, the average sensitivity was 83.04% (range 68.75% - 99.95%) and the average specificity was 99.95% (range 99.68% - 99.95%). Notably, at the 0.1% allelic frequency, all the pathological laboratories were able to detect the challenging EGFR T790M variant that is a marker of sensitivity to EGFR tyrosine kinase inhibitors. Con-11clusion: These preliminary data confirm the potential of the Oncomine cfDNA lung assay for plasma genotyping to allow for the noninvasive, multiplexed detection of complex, targetable genomic alterations in lung cancer.

#5695 Increased sensitivity for detection of oncogenic mutations present in circulating cell-free tumor DNA. Aaron R. Thorner, Michael K. Slevin, Andrea Clapp, Haley A. Coleman, Suzanne R. McShane, Samantha D. Drinan, Edwin Thai, Bruce M. Wollison, Robert T. Burns, Alex Frieden, Matthew D. Ducas, Anwesha Nag, Laura E. MacConaill, Matthew L. Meyerson, Dana-Farber Cancer Institute, Boston, MA.

Molecular pathways involved in tumor survival and progression are often activated by genetic alterations, and anticancer agents specifically targeting many oncogenic pathways have entered clinical trials. Precision cancer medi-3cation relies on the accurate identification of oncogenic pathways of interest or the specific targeting of mutant alleles of interest. Liquid biopsy, in particular circulating cell-free tumor DNA (cfDNA) derived from plasma represents a convenient assay material that has been effectively
analyzed for oncogenic mutations in specific forms of cancer such as non-small cell lung cancer. However, introduction cfDNA for clinical genotyping is limited by sensitivity requirements. cfDNA is typically present at 20-100 ng/mL of plasma, of which the cancer fraction can be below 1%. Error rates associated with PCR amplification and base calling (Illumina HiSeq platform) are approximately 5%. The presence in replicate assays of low sequence reads indicative of rare variants with allele fractions (AF) at or below that percentage. We conducted an evaluation of commercially available kits to determine 1) kit sensitivity and if necessary 2) modify the protocol to achieve sensitivity of < 1% AF. To evaluate kit sensitivity cfDNA from a translocation positive cell line was mixed at 5% and 20% with normal cell line cell gDNA. Libraries were prepared for each percent mix using 10 ng of input. Raw mean target coverage (MTC) was similar for evaluated kits (MTC, 250–300X); Percent Duplication, ~34%. Comparison of 20% and 100% translocation samples showed similar levels of unique single nucleotide variant (SNV) detection. However, 5% mixtures showed a ~10 fold reduction in SNV detection compared to both 20% and 100% translocation samples. Furthermore, translocation rearrangement was only detectable in one third of the 5% translocation samples. Increased sensitivity requires unique molecular barcodes coupled with improvements in bioinformatics analysis for clinical implementation of these assays.

#5696 Emerging KRAS mutation can play a considerable role to get acquired resistance to EGFR blockade. Takeshi Yamada, Goro Takahashi, Takuma Iwai, Kouki Takeda, Michihiro Koizumi, Akihisa Matsuda, Seiichi Shini, Yasuyuki Yokoyama, Masahiro Hotta, Keisuke Hara, Satoshi Matsumoto, Fujita, 2 Kengo Horie, 1 Koji Kameyama, 1 Masafumi Ito, 2 Takashi Deguchi 1.

Background: Epidermal growth factor receptor (EGFR) blockade can achieve considerable tumor shrinkage in patients with metastatic colorectal cancer (CRC). However, most patients who benefit from EGFR blockade acquire resistance within a year. It was previously reported that nine of 24 (38%) patients whose tumors initially exhibited wild type KRAS developed detectable mutations in KRAS in their sera after acquiring resistance to EGFR blockade. However, the mechanisms of this acquired resistance remain unclear. In this study, we aimed to identify the mechanisms underlying acquired resistance to EGFR blockade by using circulating-cell-free (ccf)DNA to track emerging KRAS, BRAF and S492R mutations during chemotherapy. Methods: We enrolled 29 patients with metastatic CRC and no mutations in their primary tumors. Patients were treated with first-line systemic chemotherapy that included EGFR blockade. We purified ccfDNA from 1 mL of serum from each patient before they started chemotherapy, and every 2-3 months during chemotherapy until disease progression. We detected KRAS (codons 12, 13, 61, and 146), BRAF (V600E) and S492R mutations using digital polymerase chain reaction. Results: KRAS mutations were detected in the ccfDNA of three of the 29 patients (10%) before chemotherapy. The response rate was 86% (25/29); the four non-responders were the three patients with KRAS mutations, and another who exhibited ccfDNA BRAF mutations before starting chemotherapy. All patients with no KRAS or BRAF mutations in their ccfDNA before chemotherapy responded to the chemotherapy (25/25). Of these 25 initially responsive patients, 13 (52%) acquired resistance. We detected emerging KRAS mutations in the ccfDNA of 11 of these 13 patients (84%); eight of these patients had multiple mutations. We also detected S492R mutations in six patients (16%), four of whom had isolated S492R mutations. Five patients (38%) had S492R; one had mutations in S492R only. Only one patient had no KRAS, BRAF or S492R mutations. Discussion: EGFR blockade has no beneficial effect in patients with KRAS or BRAF mutations in their ccfDNA prior to starting chemotherapy; Emergence of KRAS, BRAF or S492R mutations that were undetectable before the start of chemotherapy may be a mechanism underlying acquisition of resistance to EGFR blockade. Notably, emerging KRAS mutations were detected in most of the patients (84%) who acquired resistance. This indicates that the emergence of KRAS mutations may play a considerable role in the acquisition of resistance to EGFR blockade.

#5697 Proteomic analysis of prostate cancer-related exosomes isolated by anti-PSMA antibody beads. Kosuke Mizutani, 1 Kyojiro Kawakami, 1 Yasunori lasCasas, 1 Mónica Garzón, 2 Nuria Jordana Ariza, 1 Aridasa Balada, 2 Jordi Ber- tez Garcia, 1 Sergio Villatoro, 1 Erika Aldegue, 1 Sonia Rodri-

guez, 1 Raquel Campos, 1 Santiago Viteri Ramirez, 2 Maria Gonzalez-Cao, 2 Niki Karachaliou, 3 Rafael Rosell Costa, 1 Miguel Angel Molina-Vila 1.

Aim: In order to get more detailed understanding cancer-derived exosome, their proteomic analysis is necessary. The aim of this study is to establish the method for isolation and analysis of prostate cancer-related exosomes. Experimental procedures: Total exosomes were isolated from the conditioned media of LNCaP cells by ultra centrifugation. Then, exosomes positive for CD9 or prostate specific membrane antigen (PSMA) were isolated by magnetic beads conjugated with anti-CD9 or -PSMA antibody, respectively. Isolated exosomes were subjected to proteomic analysis. The PSMA positive fraction was also collected by anti-PSMA beads from diluted serum of 3 prostate cancer patients and analyzed by proteomics. Results: A total of 126, 139 and 13 proteins were detected in the LNCaP exosome fractions that were isolated by anti-PSMA beads, respectively. Out of 126 proteins identified in ultracentrifuged isolated exosome, 56 and 6 protein were found in exosomes that were isolated by anti-CD9 beads and anti-PSMA beads, respectively. Seven proteins were commonly detected in exosomes that were isolated by both beads. Fifty, 34 and 48 proteins were identified in the PSMA positive fraction from serum of 3 prostate cancer patients (P1: localized prostate cancer, P2: advanced prostate cancer and P3: castration resis-
tant prostate cancer). Thirty-three proteins were detected in one patient, 22 in two patients and 25 in three patients. Most proteins detected in all patients were serum- or immunoglobulin-related proteins. The numbers of proteins that were detected only in P2 and P3 were 16 and 8, respectively. Six proteins were found in both P2 and P3 patients. Conclusion: In the present study, we analyzed pros-
tate cancer-related exosomes that were isolated by immunoaffinity-based method. Although most proteins were serum- or immunoglobulin-related proteins, proteins that had been reported as a cancer marker were also detected, which could be candidates for exosomal marker of prostate cancer.

#5698 Next generation sequencing of circulating-free DNA from advanced non small cell lung cancer patients using Gene Reader. Clara Mayo de las Casas, 1 Mónica Garzón, 2 Nuria Jordana Ariza, 1 Aridasa Balada, 2 Jordi Ber-tez Garcia, 1 Sergio Villatoro, 1 Erika Aldegue, 1 Sonia Rodri-

guez, 1 Raquel Campos, 1 Santiago Viteri Ramirez, 2 Maria Gonzalez-Cao, 2 Niki Karachaliou, 3 Rafael Rosell Costa, 1 Miguel Angel Molina-Vila 1.

Background: Stand-alone tests such as PCR-derived techniques, FISH or IHC are usually employed to determine clinically relevant alterations in non-small cell lung cancer (NSCLC). However, they target single genes and proteins. Multiplex techniques can reduce the turnaround time and quantity of sample in this setting, but require a careful validation. Methods: A total of 41 cfdNA samples from serum and plasma from advanced NSCLC p were analyzed with the Ac-
toenable Insights TumorPanel, which covers mutations in 15 clinically relevant genes, using the Gene Reader platform (Qiagen). The samples had been previously genotyped for EGFR, KRAS and BRAF mutations by stand-alone, PNA-Taqman assays. Paired biopsies were available in 37 cases. The remaining 4 corresponded to p.T790M-positive blood samples of p progressing to EGFR TKIs. Results: Of the 41 samples taken into the GeneReader workflow, some had a DNA input concentration below specifications, in spite of this limitation, good results were obtained. 14 mutations were fully concordant between tissue, Taq-
man and GeneReader and the four p.T790M mutations were concordant be-
tween Taqman and GeneReader. Five mutations present in tissue were detected by GeneReader and not by Taqman and 11 mutations detected by Taqman were below the 1% detection threshold of GeneReader. Finally, 12 mutations present in tissue were not detected in cfdNA by any of the assays. Concordance between tissue and cfdNA was 97.5%. Conclusion: Application of NGS to liquid biopsies is challenging and requires a careful validation. However, once fully validated, NGS will probably become the methodology of choice for cfdNA analysis in NSCLC patients at presentation and at progression.

#5699 Detection of genetic and epigenetic DNA markers in urine for the early detection of liver cancer. Surbhi Jain, 1 Janin D. Steffen, 2 Yih-Ping Su, 2 Jeremy Wang, 1 Ting-Tsung Chang, 1 Chi-Tan Hu, 2 Wei Song, 1 Ying-Hsiu Su 1.

Aim: In order to get more detailed understanding cancer-derived exosome, their proteomic analysis is necessary. The aim of this study is to establish the method for isolation and analysis of prostate cancer-related exosomes. Experimental procedures: Total exosomes were isolated from the conditioned media of LNCaP cells by ultra centrifugation. Then, exosomes positive for CD9 or prostate specific membrane antigen (PSMA) were isolated by magnetic beads conjugated with anti-CD9 or -PSMA antibody, respectively. Isolated exosomes were subjected to proteomic analysis. The PSMA positive fraction was also collected by anti-PSMA beads from diluted serum of 3 prostate cancer patients and analyzed by proteomics. Results: A total of 126, 139 and 13 proteins were detected in the LNCaP exosome fractions that were isolated by anti-PSMA beads, respectively. Out of 126 proteins identified in ultracentrifuged isolated exosome, 56 and 6 protein were found in exosomes that were isolated by anti-CD9 beads and anti-PSMA beads, respectively. Seven proteins were commonly detected in exosomes that were isolated by both beads. Fifty, 34 and 48 proteins were identified in the PSMA positive fraction from serum of 3 prostate cancer patients (P1: localized prostate cancer, P2: advanced prostate cancer and P3: castration resis-
tant prostate cancer). Thirty-three proteins were detected in one patient, 22 in two patients and 25 in three patients. Most proteins detected in all patients were serum- or immunoglobulin-related proteins. The numbers of proteins that were detected only in P2 and P3 were 16 and 8, respectively. Six proteins were found in both P2 and P3 patients. Conclusion: In the present study, we analyzed pros-
tate cancer-related exosomes that were isolated by immunoaffinity-based method. Although most proteins were serum- or immunoglobulin-related proteins, proteins that had been reported as a cancer marker were also detected, which could be candidates for exosomal marker of prostate cancer.

CLINICAL RESEARCH: Liquid Biopsies 5: cfDNA, MicroRNA, and Protein
CDKN2A, SFRP1, TPFI, and MGMT). In this present study, we developed specialized short amplicon PCR assays optimized to detect these biomarkers from the urine of patients with cancer and tested the panel of biomarkers in urine of hepatitis, cirrhosis and HCC patients to demonstrate the feasibility of a breakthrough urine DNA test for HCC screening and early detection. Our evaluation indicated that the 4-marker DNA marker panel without combining DNA biomarkers were significantly higher in HCC than in non-HCC population. A correlation analysis indicated that these three markers do not correlate with each other and are suitable to be combined in a panel of biomarkers for the early detection of HCC. The 3-marker HCC urine DNA panel had an AUROC of 0.880 for distinguishing HCC from cirrhosis and hepatitis. By logistic regression, the AUROC for the DNA marker panel was 0.925 with specificity of 84.5% and 89.5% respectively, with a specificity of 90% for detecting HCC with AUROC of 0.951. Furthermore, these 3-DNA markers scored 43 of the 49 (87.8%) AFP-negative (less than 20 ng/mL) HCC urine samples ‘positive’ in this study population. A novel statistic model was build by using Random Forest machine learning method as compared to full logistic regression in both open labeled urine samples and 242 blinded urine validation samples. A sensitivity of 92.3% at a specificity of 87% was obtained by applying the random forest algorithm generated from the open labeled data (at 90% specificity cutoff) to the validation study, where as a sensitivity of 76.9% at a specificity of 84.7% was obtained from the full logistic regression derived from the open labeled data set. In conclusion, HCC DNA markers can be detected in urine of patients with HCC by short-amplicon, PCR-based assays and this urine test has the potential to become the first line of screening for HCC in high risk populations.

#5700 CircSarc: Disease monitoring by liquid biopsies in sarcomas. Heidi M. Namlos,1 Seyed Hossein Moosavi,2 Bodil Bjerkhagen,1 Olga Zaikova,1 Synnove Granlien,1 Stine Næss,1 Nina Louise Jelsøn,2,3 Skyler Mishkin,1 Eva W. Strafford,1 Else Munthe,1 Kirsten S. Hall,1 Lars B. Asheim,3 Eivind Hovig,4 Brian Kudlow,5 Ola Myklebost,4 Kjetil Boye,4 Leonardo A. Meza-Zepeda4 1. Oslo University Hospital, Oslo, Norway; 2Haukeland University Hospital, Bergen, Norway; 3Archer, Boulder, CO; 4University of Bergen, Bergen, Norway; 5Capital Biosciences, Gaithersburg, MD; 6Russian State Center of Radiology and Surgery Technologies, Saint Petersburg, Russian Federation.

We developed a new approach for isolation of circulating cell-free DNA (cfDNA) from liquid biopsies (plasma or serum). Our technology is based on using the proprietary bi-functional substance X (SubX®) that binds DNA under physiological conditions (e.g. directly in biological liquids), followed by adsorption of DNA - SubX® complex on a solid phase matrix. Since SubX® captures DNA via the phosphate groups it allows for elimination of bias related to both AT/CG content and DNA fragments length, thus improving extraction efficacy and accuracy of downstream applications. All currently available commercial kits are [silica-chaoietic salt]-based and exhibit bias for either short or long DNA fragments, as well as for the GC content. In addition, such systems require at least 4-fold dilution of starting volume of bio liquid with concentrated (5-10 M) guanidinium thiocyanate (GTc) to keep resulted GTc concentration at about 1 M, which in turns reduces concentration of DNA to at least 3.0 M. Thus, already low concentrations of cfDNA are significantly reduced and may negatively influence extraction efficacy of low abundance DNA fragments. Since our method does not require chaoietic salt for DNA capture, no dilution of starting material (and thus cfDNA) takes place. DNA-binding matrix SubX® allows either scaling the procedure to either large volumes of starting material or extra low volumes and adaptation of the protocol for 96/384 well format. Separation of cfDNA from the bulk of proteins occurs in a single vortex-spin step without employing chaoietic agents thus speeding-up isolation procedure and reducing the costs. The whole procedure can be completed within an hour and cfDNA is eluted in small volume of TE buffer. Average DNA yield from 0.2 ml of double centrifuged blood plasma from normal donors, cancer and multiple sclerosis patients was 7.5±4.2 ng (n=96).

#5701 Innovative approach for isolation of circulating cell-free DNA from liquid biopsies. Andrei Malychy,1 Vladimir Evtushenko2,3 2Capital Biosciences, Inc., Gaithersburg, MD; 3Russian State Center of Radiology and Surgery Technologies, Saint Petersburg, Russian Federation.

We developed a new approach for isolation of circulating cell-free DNA (cfDNA) from liquid biopsies (plasma or serum). Our technology is based on using the proprietary bi-functional substance X (SubX®) that binds DNA under physiological conditions (e.g. directly in biological liquids), followed by adsorption of DNA - SubX® complex on a solid phase matrix. Since SubX® captures DNA via the phosphate groups it allows for elimination of bias related to both AT/CG content and DNA fragments length, thus improving extraction efficacy and accuracy of downstream applications. All currently available commercial kits are [silica-chaoietic salt]-based and exhibit bias for either short or long DNA fragments, as well as for the GC content. In addition, such systems require at least 4-fold dilution of starting volume of bio liquid with concentrated (5-10 M) guanidinium thiocyanate (GTc) to keep resulted GTc concentration at about 1 M, which in turns reduces concentration of DNA to at least 3.0 M. Thus, already low concentrations of cfDNA are significantly reduced and may negatively influence extraction efficacy of low abundance DNA fragments. Since our method does not require chaoietic salt for DNA capture, no dilution of starting material (and thus cfDNA) takes place. DNA-binding matrix SubX® allows either scaling the procedure to either large volumes of starting material or extra low volumes and adaptation of the protocol for 96/384 well format. Separation of cfDNA from the bulk of proteins occurs in a single vortex-spin step without employing chaoietic agents thus speeding-up isolation procedure and reducing the costs. The whole procedure can be completed within an hour and cfDNA is eluted in small volume of TE buffer. Average DNA yield from 0.2 ml of double centrifuged blood plasma from normal donors, cancer and multiple sclerosis patients was 7.5±4.2 ng (n=96).

#5702 A targeted re-sequencing assay for molecular profiling of somatic mutations from plasma cell-free dna (cfDNA) for bladder cancers. Liqin Dong,1 Pramit Khetrapal,2 Simon Rodney,3 Wei Shen Tan,1 Sheida Rezaee,1 Patricia de Winter,1 John Kelly,2 Andrew Feber1. 1University College London, Cancer Institute, London, United Kingdom; 2Division of Surgery & Interventional Science, UCL Medical School, UCL, London, United Kingdom.

Bladder cancer (BC) is the 5th most common cancer in Western societies with a rising global incidence. The major challenge to improving patient outcomes is to better identify patients at risk for recurrence, progression, and metastasis, and to monitor treatment response. A non-invasive monitoring assay is needed in order to interrogate the molecular features and clinical tumor heterogeneity of bladder cancers, which will potentially lead to better stratified patient management. We developed a targeted amplicon-based re-sequencing workflow for detecting and tracking genomic mutations in cfDNA using dual molecular indexed primers for specific amplification of a panel of 20 of known (TERT, FGFR3, PIK3CA, HRAS) and novel BC mutations. Only 3hr time is needed for the total workflow for the library generation and followed by next generation sequencing (NGS) performed on Illumina MiSeq sequencing platform. Data analysis and variants calling was performed. After a thorough test, optimization, and validation of the targeted panel and the workflow on selected samples of matched bladder tumor-blood pairs and controls, 45 samples were included for testing the workflow, which includes samples of 16 matched bladder tumor-blood pairs, technical duplicates of cfDNA DNA Reference Standards (Horizon Discovery) with mutations at known allelic frequencies. Technical duplicates of cfDNA were included in this study. Somatic variants from matched tumor-blood pairs were detected. We showed that detection sensitivity at 1.0% allele frequency was achieved. Our preliminary results demonstrated that we were able to detect mutation burden from cfDNA samples and track mutation burden change in response to chemotherapy for patients with metastatic BC. Here we developed a sensitive, non-invasive targeted NGS workflow to detect somatic mutations for bladder cancers from both tumor DNA and cfDNA samples. Our early results demonstrated that we can detect and track mutational changes from patients with metastatic BC.

#5703 An ultra-sensitive multiplex allele-specific real-time PCR (Udx-PCR) assay for detection of ESR1 mutations in metastatic breast cancer. Jiaaa Liu,1 Yashouai Wen,2 Shengrong Sun,2 Dehua Derek Yu1. 1USK Bioscience, Shenzhen, China; 2Wuhan University Renmin Hospital, Wuhan, China.

Activating mutations of estrogen receptor α (ESR1) are attributed to acquired resistance to estrogen-deprivation therapy in patients with metastatic breast cancer (MBC). Thus, detection of these mutations is clinically critical for monitoring early progression and effective therapeutic options in treatment of BC patients under hormonal therapy. However, current investigations for detection ESR1 mutations mainly focus on digital PCR technology due to low abundance of the cfDNA in plasma samples, which is not a currently feasible method for clinical diagnosis. Herein, we developed a multiplex allele-specific real-time (ARMS) PCR assay for detection of ESR1 four mutations (Y537S, Y537C,
y537N, and D538G) in a single tube reaction. In this real-time PCR system, the wild-type ESR1 DNA is completely blocked by a modified probe with a higher annealing temperature, and the mutant gene is selectively amplified by the modified ARMS primers. The assay was found to be highly specific and sensitive. With this assay, as low as 0.1% mutant DNA template in the background of a total of 10ng wild-type genomic DNA could be detected. By using this assay, we analyzed the ESR1 mutations in 50 Chinese MBC FFPE and plasma samples. The ESR1 mutations were detected in 3 FFPE and 6 plasma samples, which were confirmed by pyrophosphate sequencing. The multiplex allele-specific real-time PCR assay provides a rapid and reliable diagnostic tool for accurate detection of ESR1 mutations with potential for clinical application.


Circulating cell-free DNA (cfDNA) in plasma offers a non-invasive approach to monitor tumor molecular profiling in real-time at multiple time-points, detection of emerging genomic alterations associated with drug resistance and clarifying cancer prognosis and diagnosis of cancer recurrence or progression. Therefore, it is crucial to improve our current NGS technology to detect low frequency alleles in cfDNA. There are many technical challenges in NGS technology, including base errors generated from library preparation and sequencing, making it hard to distinguish real mutations from false positives produced from amplification sequencing and base-calling processes. We have adopted a more sensitive approach to overcome this challenge. We used short, random DNA sequences called unique molecular tags (UMTs) during library preparation to label input DNA. This technique can remove background errors, false positives during data analysis, providing high confident mutation calls with better sensitivity down to 0.1% (depends on DNA input amount). We will present data showing validation of this NGS platform using commercially available DNA control, archival FFPE tissue and cfDNA matched samples and demonstrate robust sensitivity and specificity by using the off-the-shelf Archer Reveal ctDNA panel for Illumina covers 28 oncogenes. This molecular-tagged NGS panel can detect mutations down to 0.1% allelic frequency. This robust NGS platform is being used in our lab to analyze cfDNA samples, enabling the development of a non-invasive method to overcome existing challenges to provide molecular understanding of patient’s tumor evolution in real-time, and aid in the development of personalized therapies for cancer patients.

#5705 Analytical validation of Guardant360 v2.10. James Vowels,1 Justin Odegaard,2 Stefanie Mortimer,2 Stephen Fairclough,2 Marcin Sikora,2 Diana Abdueva,2 Reza Mokhtari,2 Arthur Baca,2 Amir Ali Talasaz,1,2 Guardian Health, Redwood City, CA; 3Guardian Health, San Jose, CA.

Guardant360 is a cell-free circulating tumor DNA (cfDNA) test that genotypes all guideline-recommended solid tumor somatic genomic treatment targets from a single non-invasive blood draw. The new version, v2.10, was redesigned to enhance sensitivity and specificity across 73 cancer-related genes. It detects all four major variant classes (single nucleotide variants, SNVs, indels in all 73 genes; indels in 23 genes; gene amplifications, CNAs, in 18 genes; and fusions in 6 genes). Analytical performance was assessed throughout the reportable range via multiple serial dilution studies of orthogonally-characterized contrived and patient samples. Analytical specificity was assessed by calculating the false positive rate in pre-characterized healthy donor sample mixtures serially diluted. Positive predictive value (PPV) was estimated as a function of allelic fraction/ copy number from pre-characterized samples and prevalence-adjusted using a cohort of 2,585 consecutive clinical samples. Confirmation was performed using ddPCR. Analytical sensitivity was 100% for SNVs, fusions, and CNAs and 96% for indels across 25 defined samples. Relative to Guardian360 v2.9, v2.10 demonstrated 20-50% increase in fusion molecule recovery. Retrospective in silico analysis of 2,585 consecutive clinical samples demonstrated a 15% increase in actionable fusion detection, a 6%-15% increase in actionable indel detection (excluding newly reportable indels), and a 3%-6% increase in actionable SVN detection.

### CLINICAL RESEARCH: Liquid Biopsies 5: cfDNA, MicroRNA, and Protein

Guaridan360 analytical performance characteristics based on standard cfDNA input (30ng). Analytical sensitivity/limit of detection estimates are provided for clinically actionable variants and may vary by sequence context and cfDNA input. PPV is estimated across entire reportable panel space (PPV was 100% for clinically actionable variants). Conclusion: Guardian360 v2.10 comprehensively detects all adult solid tumor guideline-recommended somatic genomic variants with unparalleled sensitivity, accuracy, and specificity.

#5706 Gene expression profile of peripheral blood mononuclear cells in breast cancer patients may be contribute to the identification and the immunological classification of breast cancer patients by blood test. Eiji Suzuki,1 Kosuke Kawaguchi,1 Masahiro Sugimoto,1 Fengling Pu,1 Ruiyi Uozumi,1 Ayane Yamaguchi,1 Mariko Nishie,1 Moe Tsuda,1 Takeshi Kotake,1 Satoshi Morita,2 Masakazu Toi1,3 Kyoto Univ. Hospital, Kyoto, Japan; 4Massachusetts General Hospital, Harvard Medical School, Boston, MA; 5Keio University, Yamagata, Japan; Kyoto University, Kyoto, Japan.

It is reported that gene expression profile of peripheral blood mononuclear cells (PBMCs) exhibits unique gene expression signature in cancer patients including renal cell carcinoma, pancreatic cancer and lung cancer. Since pancreatic cancer diagnosis is difficult in not only early detection of the disease but also in the diagnosis itself, development of novel diagnostic tools in addition to conventional diagnostic strategy has been awaited. On the other hand, in breast cancer, breast cancer, because early diagnosis by mammography and ultrasound examination on breast is established successfully, exploration of gene expression profile of PBMCs may be important in terms of insight to host antitumor immune response aspects. In the current study, we performed RNA sequencing (RNA-Seq) analysis on RNA of PBMCs from 3 healthy volunteers, 6 early and 7 metastatic breast cancer patients including all phenotypes defined by ER, PgR and HER2. Genes that showed FDR<0.05 and fold change of <0.5 and 2.0 in comparison of healthy volunteers and breast cancer patients were applied to clustering analysis by Ward method. We found significant unique gene expression signature between healthy volunteers and breast cancer patients. Furthermore, it was shown that HER2 overexpressing breast cancer patients, which breast cancer patients were divided into 3 clusters including HER2 type and other 2 different groups, seemed to be classified into healthy group different from other phenotypes of breast cancer patients. These findings suggested that evaluation of gene expression patterns of PBMCs of breast cancer patients might distinguish breast cancer patients from healthy subjects and the gene expression signature of PBMCs which divided breast cancer patients into 3 groups might reveal immunologically important biologic properties such as response prediction of cancer immunotherapy including immune checkpoint inhibition treatment.

### CLINICAL RESEARCH: Prognostic Biomarkers 2

#5707 STAT3 and STAT5a are potential therapeutic targets in castration-resistant prostate cancer. Sambit K. Mohanty,1 Kader Yagiz,2 Luthringer R.,1 Mahul B. Amin,1 Serhan Alkan,1 Bekir Cinar4, Cedars Sinai Medical Center, Los Angeles, CA; 2Clark Atlanta University, Atlanta, GA.

Background: Currently, there is no effective pharmacotherapy for metastatic castration-resistant prostate cancer (mCRPC). The mechanisms underlying mCRPC are not clearly understood, thus hindering rational-based drug design. Evidence suggests that signal transducer and activator of transcription 3 (STAT3) and 5a/b (STAT5a/b), key components of the JAK/STAT pathway, play a significant role in aggressive PC. However, expression of STAT3/5 in prostate tumor samples have not been studied. Here, we evaluated the possible role of STAT3/5a in aggressive PC. Materials and Methods: Expression of STAT3 and STAT5a in high grade PC (HGPC, n=15) and in benign prostatic hyperplasia (BPH, n=15) tissue sections were evaluated by immunohistochemistry (IHC) according to the protocol approved by Institutional Review Board.

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Age ranged from 59 to 95 years (median = 81) in the former and 57 years to 86 years (median = 68 years) in the latter category. In addition, the effects of STAT inhibitor, Pimozide, on androgen-sensitive LNCaP and its castration-resistant subtype C4-2 PC cell growth were assessed in vitro. Results: A strong nuclear immunoreactivity for STAT3 and STAT5α in 93% (n = 140) and 80% (n = 12) of HGPGC patients was observed. HGPGC patients showed increased expression of STAT3 and STAT5α may serve as a predictive biomarker for efficacy of the JAK/STAT targeted therapy. Since JAK/STAT and androgen receptor signaling are functionally synergistic in contributing PC progression, the inhibition of JAK/STAT-alone or in combination with AR may lead to a novel treatment modality for mCRPC. #5708 HOXA4 and HOXB3 gene expression signature as a biomarker of tumor recurrence in patients with high-grade serous ovarian cancer (HG-SOC) following primary cytoreductive surgery and first-line adjuvant chemotherapy. Jai N. Patel,1 Katherine Miller,1 James Symanowski,1 Jalid Sehoulii,2 Chad Michener,3 Ioana Braicu,4 Darla DestePhan,5 Ashley P. Sutker,6 Eric J. Norris,7 David Tait,7 Wendell Jones,8 Qing Zhang,1 Chad Livasy,1 Charles Bisscotti,9 Ram N. Ganapathi,10 Mehruki K. Ganapathi11. Levine Cancer Institute, Charlotte, NC; 2Charite Medical University of Berlin, Berlin, Germany; 3Cleveland Clinic, Cleveland, OH; 4EA Genomics, Morrisville, NC; 5Carolinas Healthcare System, Charlotte, NC; 6University of Pennsylvania, Philadelphia, PA.

Background: Aberrant HOX gene expression has been observed in various malignancies; however, their role as a biomarker for recurrence in patients with HG-SOC is unknown. We tested the hypothesis that a HOX gene signature is a biomarker for disease-free survival (DFS) in HGPGC patients following primary cytoreductive surgery and adjuvant platinum-based chemotherapy. Methods: A panel of HOX genes associated with DFS in a discovery cohort of 19 HGPGC patients with available RNA-Seq data, and/or previously reported as associated with survival endpoints in the literature, were selected for testing by qPCR using the Fluidigm platform in an independent training cohort of primary tumors from 73 HG-SOC patients. Cox proportional hazards regression while controlling for the optimal cutoff was used to identify HOX genes significantly associated with DFS. A prognostic gene signature was developed based on the combined linear predictor from the final multivariable Cox model. Patients were stratified into risk groups using the optimal cutoff of the linear predictor risk score, whereby the optimal cutoff was defined as the value that maximized the log-rank test statistic. Risk group stratification was further tested for association with DFS in a larger HG-SOC dataset (N = 414) with similar clinical characteristics from The Cancer Genome Atlas (TCGA). The role of the identified HOX genes in influencing drug sensitivity was examined by overexpressing these genes in a HG-SOC cell line, PEO1. Results: Of 23 HOX genes selected for testing in the training cohort, overexpression of HOXA4 (HR = 1.21, 95% CI = 1.08-1.35, P = 0.001) and HOXB3 (HR = 1.19, 95% CI = 1.02-1.17, P = 0.016) were significantly associated with decreased DFS in multivariate analysis. The median DFS in patients with a HOXA4/HOXB3 risk score ≤ the optimal cutoff was > 80 months (not yet reached), whereas the median DFS in patients with a HOXA4/HOXB3 risk score > the optimal cutoff was 16.9 months (HR = 7.85, 95% CI = 4.19-14.69, P < 0.001). The HOXA4/HOXB3 risk score remained significantly associated with DFS in the HG-SOC dataset from the TCGA (P < 0.02). In vitro studies demonstrated that PEO1 cells overexpressing either HOXA4 or HOXB3 were significantly (P < 0.05) more resistant to cisplatin and carboplatin, but not to paclitaxel, as compared to empty vector transfected cells. Conclusion: The HOXA4/HOXB3 risk score was significantly associated with DFS in a training cohort of HG-SOC patients, and remained significantly associated in a larger independent HG-SOC dataset from the TCGA. Further, in vitro studies suggest that HOXA4 and HOXB3 overexpression is associated with platinum resistance. Thus, overexpression of HOXA4 and HOXB3 in primary HG-SOC may serve as a potential biomarker for recurrence and the risk score model developed warrants prospective validation in HG-SOC patients.


Introduction: Malignant Pleural Mesothelioma (MPM) is an aggressive cancer with overall survival (OS) of about 8 months. Prognostic biomarkers allowing for biology-driven individualized therapies are lacking in MPM. Alterations in a tumor suppressor gene NF-2 are seen in 20–40% of MPM. NF-2 encodes for Merlin, which suppresses tumorigenesis by regulating pathways like mTOR and Hippo. Merlin status has been proposed as a predictive biomarker for treatment with the focal adhesion kinase (FAK) inhibitor. The purpose of our study is to examine differences in Merlin expression among histologic subtypes of MPM and its prognostic impact on clinical outcome. Materials and Method: 48 cases of MPM from 1993-2013 with available archival tissues were identified. H/E stained sections were reviewed by a pathologist and categorized into 3 histologic subtypes namely epitheliod (E), sarcomatoid (S) and biphasic (B). Immunohistochemistry-based Merlin expression score from 0 to 3 were given to the samples, accounting for both intensity and frequency of staining. Chart review was performed to obtain clinical data. Kruskal-Wallis Test was used to compare mean Merlin scores, and Logrank test was used to compare OS between low Merlin (LM, <3) and high Merlin (HM, ≥3) groups. Results: Mean age of patients was 66.75 years (HM 64.58, LM 68.92). Other demographic profile (HM%, LM%) included male sex 89.5% (51, 49), current or past smokers 66.6% (47, 53), current or past Asbestos exposure 71% (35, 65), resectable disease 89% (51, 49), stages 3 or 4 disease 64% (56, 44), and 85% (56, 44) received surgery. Mean Merlin score of 3 among histologic types were different (Mean, Std. Dev: B 2.25, 0.48; E 2.74, 0.54; S 2.56, 0.53; p = 0.0037). Mean Merlin score of E component was significantly higher than that of S component within B MPM (E 2.75, 0.45; S 1.75, 0.68; p<0.001). Interestingly, mean Merlin score of S was significantly higher than that of S component of B MPM (S 2.56, 0.53; B 1.75, 0.68; p = 0.005) making S component of B MPM least Merlin expresser. There was no significant difference in response rate to platinum and in progression-free survival between LM and HM. Median OS (months) for LM was 8.8 compared to 12.9 for HM (p = 0.15), however among different variables analyzed (age, sex, smoking history, histology, and upfront treatment modalities), age was associated with OS. For controlling age, there was no statistically significant difference in OS between LM and HM (p = 0.11). Conclusion: Merlin expression level is different among E, S and B MPMs. Moreover, it is significantly lower in S component of B MPM than S. Its prognostic ability appears to be confounded by the association with age in our cohort. A larger study is needed to further explore the prognostic impact of this biomarker. #5710 PGRMC1 is a biomarker for breast cancer cell proliferation and early relapse. Elizabeth S. McDonald, Dhruv K. Pant, Tien-chi Pan, David A. Mankoff, Robert H. Mach, Lewis A. Chodosh. University of Pennsylvania, Philadelphia, PA.

Background: Breast cancers may recur following a long latency period, implying a dormant or quiescent phase, however little is known about the biology of cellular quiescence and few markers exist for this clinically important state. The purpose of this study was to investigate whether progesterone receptor membrane component family 1 (PGRMC1), which is expressed in both cycling and quiescent tumor cells, is a prognostic biomarker in human breast cancer and to define its expression in breast cancer subtypes. Methods: Seventeen publicly available data sets were combined to analyze PGRMC1 expression in 4,463 invasive breast cancers as a function of established molecular and phenotypic markers, estimates of cellular proliferation, and recurrence-free survival data. A gene expression signature-based assay was utilized to estimate cellular proliferation. Association between gene expression and relapse-free survival was assessed using Cox proportional hazards regression while controlling for the effect of proliferation. Results: PGRMC1 expression was analyzed stratified by immunohistochemical and molecular subtype, tumor grade and size and compared with a known triple negative breast cancer biomarker, BCL11a. PGRMC1 and BCL11a each exhibited a robust positive correlation of comparable magnitude with proliferation across all breast cancer subtypes (r = 0.27, p = 6.5x10^-17 and r = 0.29, 1.7x10^-15, respectively), and PGRMC1 was strongly associated with proliferation within the basal subtype (r = 0.42, p = 2.4x10^-17 PGRMC1 versus 0.31, p = 1.1x10^-15 BCL11a). PGRMC1 expression was associated with a higher risk of early breast cancer recurrence (HR = 1.25, 95% CI [1.12,1.39], p = 6.4x10^-5) and this association was dependent upon its association with proliferation. Conclusions: PGRMC1 is a breast cancer proliferation biomarker. Further study of the function of this protein is warranted.

#5711 Gene expression profiles in BRAF V600E mutant colorectal cancer and association with SREBP2 methylation status. Kazuya Yasui,1 Takeshi Naganaka,1 Toshiaki Toshima,1 Takashi Kawai,1 Kunitoshi Shigeyasu,1 Yoshiko Mori,1 Junko Haraga,1 Keiichiro Nakamura,1 Yuzo Umeda,1 Hiroshi Tazawa,1 Ayao Goel,2 Toshiyoshi Fujiwara1,2,3,4 Okayama Univ. Graduate School of Med., Okayama, Japan; 5 Baylor University Medical Center, Dallas, TX.
Background: Classification of colorectal cancer (CRC) according to gene expression profiling remains controversial with regards to their ability to stratify patients for precision therapy. Several studies have suggested that ‘stem cell like’ or ‘epithelial-mesenchymal transition (EMT)’ signature associates with poor prognosis. Meanwhile CRCs with BRAF V600E mutation have shown poor outcomes in clinical trials, but the biological roles of BRAF mutation have not been fully elucidated. Materials and Methods: To evaluate biological roles of BRAF mutations in CRC, we performed mRNA microarray analysis in the following three subsets; eight BRAF-mutant CRC tissues without MSI, six BRAF-mutant CRCs with MSI and five BRAF-wild type CRCs with MSI. Pathway analyses were performed by Gene Set Enrichment Analysis (GSEA). Following identification of candidate biomarkers that in significance poor outcomes and associate with BRAF V600E mutation, we examined these candidate biomarkers in a cohort of 1068 CRC patients who underwent surgical resection of their primary tumor and/or metastatic lesions from 1994 to 2015 at the Okayama University Hospital. Results: By the GSEA, prominent signatures enriched in CRCs with BRAF V600E mutation vs. wild-type were EMT-related processes (EMT and myogenesis). Additionally, CRC with BRAF V600E mutation strongly associated with inactivation of Wnt signaling and secreted frizzled-related proteins (SFRPs) which normally support activation of the pathway: RAF1 (9%), MAPK1 (3%) and RPS6KA1 (1.9%) was found to be significantly associated with poor DFS (P = 0.0286) and disease free survival (DFS) in HNSCC (0.00150; median progression time = 38.11 vs. 71.22 months in DUSP4-unaltered patients) which can potentially be explained by activation of oncogenic MAPK pathway upon DUSP4 underexpression. Interestingly, we identified four unmethylated CRCs with genetic mutation in the KRAS/BRCA genes demonstrated significantly poorer outcome in RFS compared with SFRP2 methylated CRCs with genetic mutations in the KRAS/BRCA genes (5-years RFS rate were 46.8% vs 72.1%; P = 0.015, Hazard ratio 2.06 [95% CI, 1.12 to 3.69]; P = 0.021). Conclusion: Our results suggest that SFRP2 methylation status could be a potential prognostic biomarker for stage II-IV CRCs, especially with genetic mutations in the KRAS/BRCA genes.

#5712 Potential clinical significance of downregulation of MAPK pathway components mRNA expression in head and neck squamous cell carcinoma (HNSCC). Hoi Lam Ngan,1 Vivian W.Y. Lui2. The University of Hong Kong, Hong Kong; 2The Chinese University of Hong Kong, Hong Kong, Hong Kong.

We previously showed the MAPK pathway can be mutationally activated, and may implicate drug sensitivity in HNSCC [1]. Here, we report that RNA expression of several MAPK pathway components may be associated with HNSCC patients’ outcome based on the US-TCGA Provisional data. We found that loss of DUSP4, a negative regulator of MAPK pathway, was correlated with poor overall survival (OS; P = 0.0286) and disease free survival (DFS) in HNSCC (0.00150; median progression time = 38.11 vs. 71.22 months in DUSP4-unaltered patients) which can potentially be explained by activation of oncogenic MAPK pathway upon DUSP4 underexpression. Interestingly, we identified a group of HNSCC patients with homogenous loss and mRNA downregulation of MAPK pathway scaffold protein components (GRB2, SHC2 and SHC3) with significantly poorer DFS (P = 0.000871). RPPA analysis showed a trend of decreased protein expression level of phospho-RAFs, downstream of scaffold proteins, decreased MEKs and MAPKs, supportive of an overall decreased MAPK pathway signaling in this subset of patients. Unexpectedly, downregulation (homogenous deletion/ RNA expression less than 2 SD) of multiple MAPK pathway components which normally support activation of the pathway: RAF1 (9%), MAPK1 (3%) and RPS6KA1 (1.9%) was found to be significantly associated with poorer DFS (P = 6.643x10^-5) with median time to progression of 18.17 months vs. 71.22 months in the unaltered group. Subsequent proteomic analysis of the respective patient tumors from the TCGA cohort (N = 357 with RPPA data) showed that these patient tumors had elevated levels of E2F1 protein expression (P = 0.0146), along with increase FOXM1 expression (P = 8.499x10^-4), which is known to drive cell cycle progression [2]. As E2F1 is involved in cell survival upon DNA damage [3], it is likely that upregulation of E2F1 protein expression may enable cancer cells to survive after DNA insults by radiotherapy or chemotherapy, and contributes to disease relapse. Acknowledgements: VWYL is funded by Hong Kong Research Grant Council, Hong Kong (141814, General Research Fund), Theme-based Research Scheme (T12-401/13-R), and the Start-up Fund from the School of Biomedical Sciences, Faculty of Medicine, the Chinese University of Hong Kong. Reference 1. Van Allen, E.M., et al., Genomic Correlate of Exceptional Erlotinib Response in Head and Neck Squamous Cell Carcinoma. JAMA Oncol, 2015. 1(2): p. 238-44. 2. Barger, C.J., et al., Genetic determinants of FOXM1 overexpression in epithelial ovarian cancer and functional contribution to cell cycle progression. Oncotarget, 2015. 6(29): p. 27613-27. 3. Berton, T.R., et al., Regulation of epidermal apoptosis and DNA repair by E2F1 in response to ultraviolet B radiation. Oncogene, 2005. 24(15): p. 2449-60.

#5713 PIGN gene expression aberration weakens chromosomal stability via altering its interaction with the spindle assembly checkpoint protein complex during leukemogenesis. Jeffrey J. Pu,1 Emmanuel K. Teye,1 Abigail Sido,1 Yukia I. Kawasawa,1 Ping Xin,1 Niklas K. Finnberg,2 Wafik S. El-Deiry,2 Sara Shimko1,1 Penn State Hershey Cancer Institute, Hershey, PA; 2Fox Chase Cancer Center, Philadelphia, PA.

Several studies have linked increased frequency of glycosylphosphatidylinositol-anchor protein (GPI-AP) deficiency with genomic instability and the risk of carcinogenesis. Recently, Phosphatidylinositol Glycan Anchor Biosynthesis Class N (PIGN), a gene participating in GPI-AP synthesis, was suggested as a cancer chromosomal instability suppressor in a colon cancer model. We investigated the association of PIGN with genomic instability and leukemogenesis. A Random Forest analysis of the gene expression array data from 55 MDS patients (GSE4619) demonstrated a significant (p = 0.0007) correlation (P = 0.04068) between PIGN-biosynthesis expression and genomic instability, in which PIGN was ranked as the third most important in predicting risk of MDS progression. We observed that PIGN gene expression aberrations (increased transcriptional activity but diminished to no protein production) were associated with increased frequency of GPI-AP deficiencies among leukemic cells during leukemic transformation/progression. The PIGN gene expression aberrations were attributed to partial intron retrictions between exons 14 and 15 resulting in frame shifts and premature termination which were confirmed by examining the RNA-seq data from a group of AML patients (phs001027.v1.p1). PIGN gene expression aberration correlated with the elevation of genomic instability marker expression that was independent of the TP53 regulatory pathway. PIGN protein expression suppression/elimination caused a similar pattern of genomic instability that was rescued by PIGN restoration. Furthermore, PIGN bound to the spindle assembly checkpoint proteins and regulated their expression during the cell cycle. In conclusion, PIGN gene is crucial in the regulation of mitotic integrity to maintain chromosomal stability and prevents leukemic transformation/progression.

#5714 AXL and GAS6 Co-expression in lung adenocarcinoma as a prognostic classifier. Masahiro Seike, Ritaro Noro, Cheol-Hong Kim, Fenfei Zou, Kaoru Kubota, Akihiko Gemma. Nippon Medical School, Tokyo, Japan. AXL, a receptor tyrosine kinase implicated in cell survival, proliferation, and migration, is also associated with acquired resistance to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor therapy. However, its prognostic significance in lung adenocarcinoma (AD) remains unclear. We therefore evaluated the prognostic significance of the expression of AXL and/or its ligand, growth arrest-specific 6 (GAS6), in completely resected lung AD. We evaluated the relationship between AXL, GAS6, and vimentin expression, as determined by immunohistochemistry (IHC) analysis, with overall survival and disease-free survival in 113 patients with stages I-III lung AD. Protein expression was also assayed using western blot analysis in 10 lung AD cell lines. AXL-positive (AXL+), GAS6-positive (GAS6+), or AXL+/GAS6+ staining was significantly associated with vimentin-positive (vimentin+) expression. AXL+/GAS6+ and vimentin+ showed a negative tendency toward an association with EGFR muta- tion. AXL+, GAS6+, or AXL+/GAS6+ statuses significantly correlated with poor overall survival. In stage I cases, an AXL+/GAS6+ status significantly correlated with poor overall survival and disease-free survival, especially in cases with wild-type EGFR. In multivariate analysis, AXL/GAS6 classifications in stage I as well as in stages I-III lung AD were found to be independent factors for poor patient outcomes. Unlike lung AD cell lines with mutant EGFR, those with wild-type EGFR closely correlated with AXL and vimentin expression by western blotting. AXL+ and GAS6+ expression is relevant to a poor prognosis in resected lung AD patients at stage I. AXL/GAS6 might serve as crucial predictive and prognostic biomarkers and targets to identify individuals at high risk of post-operative death.

#5715 Gene body methylation and expression of SHOX2 gene are potent prognostic markers for survival in intermediate grade gliomas and renal cancer. Yanyun Zhou,1 Xinyun Zhen,1 Xian Luo,1 Guanghua Xiong,1 Ali F. Gazzaniga1,2 UT Southwestern Medical Ctr., Dallas, TX, 1University of Mississippi Medical Ctr., Jackson, TX.

Background: Previous data indicated that low level of promoter methylation of the SHOX2 gene was a prognostic marker for worse progression-free survival

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for non-small cell lung cancer. Purpose: (a) to determine the relationship between SHOX2 gene methylation and expression; (b) to determine whether SHOX2 gene methylation and expression were prognostic markers for other cancers. Methods: We examined multiple methylation and expression datasets available from The Cancer Genome Atlas (TCGA) and other previously published studies of multiple tumor types. A total of more than 1100 renal cancer cases were examined, along with smaller number of corresponding non-malignant tissues. We performed Kaplan-Meier survival analyses and univariate and multivariate Cox regression model analyses. Results: SHOX2 gene contains three CpG islands encompassing the promoter region and body. Methylation of various regions of SHOX2 gene body was significantly positively correlated with its expression in gliomas and renal cancer. Abnormally high methylation and expression of SHOX2 gene, which were significantly higher than the corresponding nonmalignant tissues, were identified in subsets of gliomas and renal cancers. Survival analysis of patients indicated that the methylation and expression of SHOX2 gene in gliomas and renal cancer (both clear cell and papillary cell carcinomas) were highly potent markers for poor survival. We further investigated the combination of SHOX2 with other known clinically relevant glioma markers (IDH genotype status, TERT expression, 1p/19q chromosome co-deletion, MGMT methylation, ATRX mutation and NES expression). When combined with SHOX2 expression, we identified subsets of glioma patients with significantly favorable survival outcomes, especially in the subgroup (i.e., IDH wild-type) associated with worse prognosis for each individual marker. Multivariate analysis demonstrated that SHOX2 was a potent independent survival marker for gliomas. Conclusion: Gene body methylation and expression of SHOX2 gene are tightly positively correlated and are potent new markers for overall survival prognosis for intermediate grade gliomas and renal cancer. The combination of IDH or other relevant prognostic markers with SHOX2 identified intermediate grade glioma subsets with improved survival outcomes.

#5716 Tristetraprolin is a prognostic biomarker for biochemical recurrence in low Gleason score patients. Anders E. Berglund, Travis Gerke, Shivanshu Awasthi, G. Daniel Grass, Hyun Y. Park, John L. Cleveland, Jong Y. Park, Koji Yamashita, Robert J. Rounbehler. H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL.

PURPOSE: Most prostate cancer patients currently classified as having a low-risk of disease-specific death are overtreated in the United States, resulting in hundreds of thousands of men to have debilitating side effects from unnecessary treatment. However, ~30% of low-risk patients on active surveillance are later reclassified and require therapeutic intervention. Thus, there is a dire need for biomarkers that can discriminate which prostate tumors currently classified as low-risk will progress into aggressive disease. EXPERIMENTAL DESIGN: Retrospective analyses of two independent prostate cancer expression datasets were performed to determine the relationship between the expression levels of the tumor suppressor Tristetraprolin (TPP, ZFP36) and biochemical recurrence (BCR). Further, TTP expression levels were measured by quantitative real-time PCR (qRT-PCR) in a cohort of prostate tumors from Moffitt Cancer Center patients having long-term follow-up (n = 382), and will be correlated to associated clinical data, including BCR. Finally, the Cancer of the Prostate Risk Assessment Posturgical (CAPRA-S) scores, a clinical tool used to predict prostate cancer outcomes following radical prostatectomy, were calculated along with BCR for the patients in one of the prostate cancer datasets. RESULTS: Analysis of The Cancer Genome Atlas (TCGA) prostate adenocarcinoma dataset revealed that tumors with low TTP expression have an increased rate of BCR compared to those with high TTP levels (p = 0.0096). Further, assessment of the GEO dataset GSE21034 (Taylor et al., Cancer Cell, 2010) established that prostate cancer patients with low Gleason scores (6 and 3+4) have an increased chance of BCR if their tumors express low levels of TTP compared to men with high TTP expressing tumors (p = 0.0001). To validate these findings, patients with low Gleason score tumors and decreased levels of TTP in the Moffitt Cancer Center cohort are being analyzed to determine their rate of BCR. In contrast, CAPRA-S scores for low Gleason score tumors in the GSE20134 dataset failed to distinguish if a patient would have BCR (p = 0.0511). CONCLUSIONS: Prostate cancer patients with Gleason score 6 or 3+4, which are currently classified as having a low-risk of dying from prostate cancer, have an increased risk of BCR if their tumors express low levels of the tumor suppressor TTP. Our data suggests that TTP may be a biomarker that clearly discriminates which low Gleason score tumors will progress into aggressive disease.

#5717 PKR promotes EphA2 and other proteins degradation and is predictor of survival in lung cancer. Apar Pataer, Ruping Shao, Bulent Ozpolat, Bingliang Fang, Stephen G. Swisher. UT MD Anderson Cancer Ctr., Houston, TX.

Relative expression of PKR and EphA2 was a significant predictor of survival in non-small cell lung cancer. To study the PKR-regulated protein, we evaluated expression of 149 proteins in lung cancer cells by reverse-phase protein assay (RPPA) after induction of PKR. Expression of several proteins (EphA2, IRSI, Chk1 and c-Raf and paxillin) was inhibited by PKR. In validating the downregulated proteins by western blot analysis, we observed that PKR reduced EphA2, IRS1, Chk1 and paxillin protein expression. We next investigated the involvement of the proteasomes and lysosomes in PKR-induced protein degradation. Human lung cancer H1299 and A549 cells were transfected with Ad-PKR for 48 h in the presence or absence of the proteasome inhibitor MG132 or lysosome inhibitor (3MA: 3-methyladenine). We observed that MG132 prevented PKR-induced EphA2 and paxillin degradation in these cells. However, we found that 3MA prevented PKR-induced EphA2 and paxillin degradation in these cells. These data suggest that PKR is involved in proteasome as well as lysosome function. To study the involvement of autophagy pathway in PKR mediated protein degradation, we evaluated proteins which known involved in autophagy by Western blotting after induction of PKR on these cancer cells. We observed slightly decrease of LC-3, ATG5 and Beclin1 in these cancer cells after induction of PKR. Our data suggest that PKR may directly involve in in lysosome function.


Additional prognostic stratification of colorectal cancer patients is needed to improve management of patients. Using microfluidic array chip (Miacarta) approach of tumor samples remains the standard method for disease subtyping. However, visual analysis is subjective and weakly reproducible due to intra- and intra-observer variations. Recent progress within machine learning, especially its novel branch called deep learning, enables accurate evaluation of complex patterns observed in microscopic tissue images. Here, we combined deep learning techniques to evaluate a set of digitized formalin fixed paraffin embedded hematoxylin-eosin stained tumor tissue microarray (TMA) samples from 420 randomly selected patients with colorectal cancer. For each patient a set of clinicopathological characteristics including histological grade, Dukes stage and age at diagnosis are available as well as outcome data. Using convolutional neural networks and Long Short-Term Memory networks we validated the predictive power of the colorectal TMA with regards to patient outcome. Univariate Cox proportional hazard regression analysis demonstrated that the prognostic accuracy of the deep learning algorithm on TMAs (hazard ratio 2.3; CI 95% 1.79–3.03) outperforms visual histological grading performed by a certified pathologist on a whole slide level (hazard ratio 1.65; CI 95% 1.30–2.15). In multivariate Cox proportional hazard regression, the deep learning based model was a prognostic factor, independent of histological grade, Dukes stage and age at diagnosis (Wald p-value < 0.001). Thus, we demonstrate that novel deep learning models can serve as digital prognostic biomarkers in colorectal cancer.

#5719 Long intergenic non-coding RNAs: a new independent risk predictors in multiple myeloma. Mehmet K. Samur, Annamaria Guilla, Mariateresa Fulciniti, Anil Aktas Samur, Raphael Szalat, Masood Shamas, Florence Magrangeas, Stephane Minvielle, Kenneth Anderson, Giovanni Parmigiani, Hervé Avet-Loiseau, Nikhil Munshi, Dana Farber Cancer Institute, Boston, MA; 1Centre Hospitalier Universitaire de Nantes, France; 2Université de Nantes Institut de Recherche Thérapeutique, France; 3Unité de Génomique du Myélome, University Hospital; CRCT, INSERM U 1037; Université Paul Sabatier, France.

RNA has diverse sets of regulatory functions and a recent analysis of RNA repertoire has identified a large numbers of non-coding transcripts. One of which, long intergenic non-coding RNA (lincRNA) with transcripts longer than 200 nucleotides, are located between the protein coding genes and do not overlap exons of either protein-coding or other non-lincRNA genes. lincRNAs have been considered to provide regulatory functions, however, their precise role in cellular processes of patients. We studied lincRNAs using uniformly treated patients to show their impact on survival outcome in MM. We performed RNA-seq on CD138 + MM cells from 360 newly-diagnosed patients and 18 normal plasma cells (NPM) and analyzed for lincRNA and protein coding genes. MM patient data for clinical characteristics, cytogenetic and FISH as well as clinical survival outcomes were also analyzed and correlated with lincRNA data. Our data showed expression of 951 lincRNAs (median TPM > 1) with 351 lincRNAs differentially expressed between MM and normal plasma cells. Using only the expresed lincRNAs, we applied log rank tests for quartile 1 (Q1) versus Q2 through Q4 and Q4 versus Q3 in order to identify under- and overexpressed prog-
nastic genes, respectively. Four under and seven overexpressed genes were selected for final model. We used Más-o-menos for final predictive model, which simply calculates the risk score, by using expression values. The Kaplan-Meier estimates of EFS at 4 years were 53.3% (95% CI, 45.1% to 63.1%) and 32.6% (95% CI, 25.1% to 42.2%), and OS at 4 years were 93.2% (95% CI, 88.9% to 100%), and 69.0% (95% CI 56.7% to 79.4%). The Relative risk score was associated with a low or high risk score. When applied to patient cohort separated by other risk categorization including minimal residual disease status (MRD), cytogenetic risk status (del17p, t(4;14) and t(14;16)) and International Staging System (ISS), lncRNA signature was able to further identify patients with significant differential survival outcomes. In summary, we report that lncRNAs have an independent effect on survival outcome in MM and provides rationale for its use in risk stratification as well as to understand biological impact.

#5720 Serum interleukin-18 and metalloproteinase-1 predict long-term prognosis of breast cancer. Ji-Yeon Kim,1 Kyunghee Park,2 Eunjin Lee,2 Hae Hyun Jung,3 Seok Jin Nam,4 Jeong Eon Lee,3 Seok Won Kim,1 Jung Han Yoo,1 Se Kyung Lee,3 Soo Yoon Bae,1 Jin Seok Ahn,1 Young-Hyuck Im,1 Yeon Hee Park1,2

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Introduction: Intrinsic subtyping and twenty-one gene expression assay using tumor tissue indicates gene expression profile had advantage to predict breast cancer (BC) patients’ prognosis. However, these tumor biomarker assays had limitations as high as high cost, demand for large amount of tumor sample and limited use for specific subtype of BC. Therefore, we conducted this study to find serum biomarker predicting BC prognosis. Materials and Methods: This study was conducted by retrospective analysis of patients with stage I to IIIC invasive breast cancer who received curative surgery at Samsung Medical Center between 2003 and 2004. We performed multiplex immunoassay using 45 cytokine and 8 MMP multiplex immunoassay panels (Bio-Rad Laboratories Inc, Irvine, CA, USA). Among clinical factors, operational stage was re-categorized into stage I II/III vs. stage III and Hormone receptor (HR) status was grouped into HR positive vs. negative. For each protein, proteins significantly associated with overall survival (OS) in univariate analyses were candidates for the multivariate analysis. Results: In total, 246 patients who received curative surgery for breast cancer were enrolled. Of 246 patients, serum cytokine analyses were performed in 229 patients. Of clinical characteristics, ten year OS of BC with stage III was 76.2% compared to 92.7% of stage I and 88.6% of stage II (hazard ratio (Stage III vs. stage I/II): 2.62 (95% CI: 1.26-5.38), p-value = 0.016) and HR expressed BC had 91.1% of 10 year OS, compared to 81.9% of HR negative BC (hazard ratio (HR positive): 0.47 (95% CI: 0.23-0.95), p-value = 0.035). In terms of serum biomarker, high expression of IL-18 was associated with good prognosis (hazard ratio: 0.45 (95% CI: 0.21-0.96), p-value = 0.033) in contrast with poor prognosis of MMP-1 expression (hazard ratio: 1.96 (95% CI: 0.94-4.08), p-value = 0.067). Multivariate analysis showed that BC stage, HR positivity, serum IL-18 and MMP-1 affected patients’ OS. Stage I/II HR positive BC with low MMP-1 and high IL-18 increased OS about 5 times compared to stage III HR negative BC with high MMP-1 (hazard ratio: 5.08 (95% CI: 1.01-25.46), c-index = 0.731, p-value < 0.0001). Internal validation using 10,000 times of bootstrapping showed that this prediction model was suitable for BC prognosis prediction (c-index: 0.705, p-value = 0.041). Conclusion: Our research suggested that the level of serum IL-18 and MMP-1 would help to predict BC patients’ prognosis. Further external validation of these biomarkers would be warranted.

#5721 Genome-wide discovery and identification of a novel mirRNA signature for recurrence prediction in colorectal cancer. Raju Kandimalla,1 Feng Gao,2 Naoki Takahashi,3 Yasuhide Yamada,4 Takatoshi Matsuyama,5 Toshiyuki Ishikawa,6 Xin Wang,7 Ayaj Goel8. 1Baylor University Medical Center, Dallas, TX; 2City University of Hong Kong, Hong Kong; 3National Cancer Center Hospital, Tokyo, Japan; 4Tokyo Medical and Dental University Graduate School of Medicine, Tokyo, Japan.

Purpose: Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths worldwide. In 2016, there were estimated 95,270 newly diagnosed CRC cases, and 49,190 deaths from this disease in the United States. Survival of patients is closely associated with the tumor stage at the time of diagnosis as 5-year relative survival rates range from 65% for all stages, 90% for localized primary tumor, 71% for regional metastasis and 13% for distant metastasis. Post-surgery, adjuvant therapy is only recommended to those with high risk stage II, as well as stage III and IV tumors. However, approximately 40-50% of the patients undergo curative surgery only and 20-30% that are treated with adjuvant chemotherapy, eventually relapse and experience a metastatic disease and eventual death. The current gold standard TNM (Tumor, Node, Metastasis) staging for determining the prognosis of CRC patients remains inadequate at identification of high-risk stage II and III patients that have a high potential of developing tumor recurrence. MicroRNAs (miRNAs) play an important role in CRC development and are emerging as important disease biomarkers. Therefore, in this study we sought to determine the prognostic potential of the eight gene miRNA signature discovered from the genome-wide analysis was validated in two independent patient cohorts (n=127 and n=96) using Taqman-based RT-PCR assays. Results: The genome-wide comprehensive analysis led to the identification of an eight gene miRNA classifier that significantly predicted recurrence free survival (RFS) in training (log-rank p=0.003) and two independent validation cohorts (log-rank p<0.0001 and p=0.002). The RT-PCR based training and validation of the eight gene classifier in two independent clinical cohorts significantly associated with poor prognosis in stage II and III CRC patients (log-rank p<0.004 and p<0.0001). Multivariate analyses performed in these two patient cohorts revealed that the eight gene miRNA classifier served as an independent predictor of poor prognosis in stage II and III. CRC patients. Conclusively, our study revealed that these novel miRNA based classifier, which is robustly predictive of poor prognosis in patients with stage II/III CRC, and might facilitate identification and stratification of high-risk patients that are candidates for adjuvant chemotherapy and clinical surveillance.
Pathway mutations in head and neck cancer and its clinical significance. Vivain W.Y. Lui,1 Hoi Lam Ngan2. 1The Chinese University of Hong Kong, Hong Kong, Hong Kong; 2The University of Hong Kong, Hong Kong, Hong Kong.

The PI3K pathway is the most frequently mutated mitogenic pathway in head and neck squamous cell carcinoma (HNSCC) tumors. Mutations in PIK3CA and PIK3R1 genes are the most common type of mutations (in 16% (83/510 cases) of HNSCC primary tumors (TCGA, Provisional), with the pathway components defined as: GRB2 SHC1 SHC2 SHC3 HRAS KRAS NRAS ARAF BRAF RAF1 DUSP1 DUSP4 MAP2K1 MAP2K2 MAPK1 MAPK3 RPS6KA1 RPS6KA4 RPS6KA5. Mutations of the entire MAPK pathway gene components are not associated with patient overall survival (OS) and disease-free survival (DFS) (p=n.s.). With HRAS, MAPK1, BRAF and RPS6KA1 gene mutations being most commonly mutated in 6%, 1.7%, 1.7% and 1.7% in the US-TCGA cohort, we conducted survival analyses of each of the mutated gene component alone to examine their individual correlation based on the available clinical data. Our results show that HRAS mutation, BRAF mutation and RPS6KA1 mutation do not associate with patient OS nor DFS (OS: P=0.285, DFS: 0.300 and P=0.197, respectively; DFS: 0.216). Lastly, we note that except for MAP2K2, patients with somatic mutations of multiple positive regulators of the MAPK pathway (RAF1 MAP2K1 MAPK1 HRAS BRAF) are associated with significant better OS (P=0.0293). Prognostic analysis suggests that these mutations of these MAPK pathway positive regulators are associated with downregulation of snai1 (P=0.0786). Mismatch repair gene mutations correlated with OS and DFS (p=n.s.).

#5723 MAPK pathway mutations in head and neck cancer and its clinical significance. Vivain W.Y. Lui,1 Hoi Lam Ngan2. 1The Chinese University of Hong Kong, Hong Kong, Hong Kong; 2The University of Hong Kong, Hong Kong, Hong Kong.

The PI3K pathway is the most frequently mutated mitogenic pathway in head and neck squamous cell carcinoma (HNSCC) tumors. Mutations in PIK3CA and PIK3R1 genes are the most common type of mutations (in 16% (83/510 cases) of HNSCC primary tumors (TCGA, Provisional), with the pathway components defined as: GRB2 SHC1 SHC2 SHC3 HRAS KRAS NRAS ARAF BRAF RAF1 DUSP1 DUSP4 MAP2K1 MAP2K2 MAPK1 MAPK3 RPS6KA1 RPS6KA4 RPS6KA5. Similar to the PI3K pathway, mutations of the entire MAPK pathway gene components are not associated with patient overall survival (OS) and disease-free survival (DFS) (p=n.s.). With HRAS, MAPK1, BRAF and RPS6KA1 gene mutations being most commonly mutated in 6%, 1.7%, 1.7% and 1.7% in the US-TCGA cohort, we conducted survival analyses of each of the mutated gene component alone to examine their individual correlation based on the available clinical data. Our results show that HRAS mutation, BRAF mutation and RPS6KA1 mutation do not associate with patient OS nor DFS (OS: P=0.285, DFS: 0.300 and P=0.197, respectively; DFS: 0.216). Lastly, we note that except for MAP2K2, patients with somatic mutations of multiple positive regulators of the MAPK pathway (RAF1 MAP2K1 MAPK1 HRAS BRAF) are associated with significant better OS (P=0.0293). Prognostic analysis suggests that these mutations of these MAPK pathway positive regulators are associated with downregulation of snai1 (P=0.0786). Mismatch repair gene mutations correlated with OS and DFS (p=n.s.).

#5724 Circulatory microRNA expression profiling between HCV-infected African & Caucasian Americans: implications for racial health disparities. Pradip Devhare,1 Robert Steele,1 Adrián M. Di Bisceglie,1 David E. Kaplan,2 Ratna B. Ray1. 1St Louis University, St Louis, MO; 2University of Pennsylvania, Philadelphia, PA.

The purpose of the study: Hepatocellular carcinoma (HCC) is a global problem and incidence of HCC is increasing in last several years. Hepatitis C virus (HCV) infection is one of the major cause towards development of HCC. HCV infection is highly prevalent in African American population compared to other ethnic groups and these people are less likely to naturally clear HCV. Higher incidence of HCC and mortality was noted in HCV infected African Americans as compared to Caucasians. However, the explanation for this disparity and the molecular mechanisms behind this are currently unknown. Circulating microRNAs in the blood are emerging as biomarkers for pathological conditions. Differential expression of miRNAs among ethnic groups would be important for optimizing personalized treatment strategies. Experimental procedures: In this study, we assessed the differential expression of circulatory miRNAs from HCV infected African Americans and Caucasians by specific miRNA array profiling. Expression of significantly altered miRNAs was validated by qRT-PCR. Results: We identified increased expression of miR-146a, miR-150 and miR-155 in HCV infected African Americans patient sera as compared to sera of Caucasians. Further analysis demonstrated that these miRNAs were significantly elevated in African Americans diagnosed with HCV-mediated HCC. Higher expression of miR-150 was also noted in cirrhosis and HCC in African Americans, which may serve as a predictor of liver disease progression in this population. Conclusion: The differential expression of miRNAs suggests that these miRNAs and their target genes could be useful to gain further mechanistic insight of racial disparity associated with HCV pathogenesis.
A functional polymorphism in DTX1 gene of Notch pathway was found to predict the prognosis of surgically resected non-small cell lung cancer. Shin Yup Lee,1 Janghyuck Lee,1 Kyung Min Shin,1 Ji Woong Son,2 Jae Yong Park.1
1Kyoungpook National Univ. School of Med., Daegu, Republic of Korea; 2Konyang University of Turku, Turku, Finland; 4Institute for Molecular Medicine Finland, Helsinki, Finland; 3Institute for Molecular Medicine Finland and Helsinki University Hospital (HUSLAB), Helsinki, Finland. Prostate cancer (PCa) is the most diagnosed cancer in men in developed countries. Fibroblast growth factor receptors (FGFRs) have been demonstrated to play an important role in PCA initiation and progression. Fibroblast growth factor receptor factor like 1 (FGFR1) is the most recently identified member of the FGFR family. Its extracellular domain shares high similarity to FGFR1-4 but it is specifically expressed in normal prostate and PCa, tissue microarrays containing different types of benign and malignant prostate tissue were used. Altered FGFRL1 protein in normal prostate and PCa, tissue microarrays were constructed using formalin-fixed paraffin-embedded specimen. 70 (35.9%) out of 195 patients showed immunoreactivity for USP10 and 124 (63.6 %) showed loss of immunoreactivity for USP10. 120 (61.5 %) of 195 patients were immunopositive for p14ARF and 75 (38.5 %) patients showed loss of immunoreactivity for p14ARF. Loss of USP10 was associated with male predominiency (p = 0.014), higher stage (p = 0.044), presence of lymphatic invasion (p = 0.033), absence of sporadic adenoma (p = 0.024) and absence of peri-tumoral dysplasia (p = 0.019). On multivariate survival analysis, loss of USP10 expression was independent poor prognostic factor (HR = 3.04, 95% CI = 1.79-5.18, p < 0.001) and also both loss of USP10 and p14ARF expressions showed similar result (HR = 3.76, 95% CI = 1.52-9.43, p < 0.005). Loss of USP10 and p14ARF was an independent prognostic factor (p = 0.587). Our findings indicate that loss of USP10, associated with loss of p14ARF protein expression, could be poor prognostic factors in small intestine adenocarcinoma.
function of membranous FGFR1. However, knock-down of FGFR1 in PC3M cells resulted in reduced cell growth in tumor xenografts. Additionally, mRNA sequencing on PC3M cells with FGFR1 knock-down revealed differential expression of about 250 molecules, including several metalloproteinases and FGFR1, compared to control cells (FDR<0.1 and |logFC|>1). In conclusion, we suggest that FGFR1 knock-down and alternations in PI3K/Akt activity result in smaller, spindle-shaped cells, whereas FGFR1 knock-down increased AR nuclear expression and resulted in smaller, round-shaped cells. These data suggest that PI3K and Akt have different roles in sustaining AR activity in PCa as perturbations of the two components leads to differential responses in terms of AR nuclear expression and cell morphology. In conclusion, activated Akt associates with AR expression and predicts poor overall survival in prostate cancer patients. Therefore, targeting PI3K/Akt, especially when the inhibitors are administered in combination with anti-androgens, may have implications in the design of PCa therapy targeting PI3K/Akt, especially when the inhibitors are administered in combination with anti-androgens.

#5733 Expression levels of UL16 binding protein 1 and natural killer group 2 member D in patients with gastric cancer. Kiyoshi Yoshimura,1 Moeko Inoue,1 Tetsuhiiko Asan,1 Masanori Fuse,1 Satoshi Wada,2 Atsuo Kurama1,3,1 National Cancer Center, Chuo-ku, Tokyo, Japan; 2Kanagawa Cancer Center, Yokohama, Kanagawa, Japan; 3Yamaguchi University Graduate School of Medicine, Ube, Yamaguchi, Japan.

UL16 binding protein 1 (ULBP1) expressed on the tumor cell surface binds to the natural killer group 2 member D (NKG2D) receptor present on natural killer (NK) cells, CD8+ T cells, and gamma delta T cells. However, the role of ULBP1 and NKG2D expression and associated immune responses in gastric cancer is unclear. Here, we investigated the relationships between ULBP1 and NKG2D expression and clinical outcomes in patients with gastric cancer. The expression levels of ULBP1 and NKG2D were examined in human gastric cancer cell lines and gastric cancer tissues from 98 patients who underwent surgery from 2004 to 2008. MKS-72 cells expressed ULBP1 with ULBP2, -3, or -6. NKG2D was expressed at a higher level after activation on T cells and NK cells. Among tissue sections positive for NKG2D expression, six cases were positive for CD8 and CD56. In all tissues, NKG2D-expressing cells were typically cD8+ T cells. Patients with NKG2D expression in tumors had significantly longer overall survival (OS) than patients without NKG2D expression in tumors (p = 0.0217). The longest OS was observed in patients positive for both ULBP1 and NKG2D, whereas the shortest OS was observed in patients negative for both ULBP1 and NKG2D. The interaction between ULBP1 and NKG2D may improve OS in patients with gastric cancer and may have applications in immunotherapy for induction of adaptive immunity in patients with cancer. Moreover, ULBP1 and NKG2D may be useful as prognostic biomarkers in gastric cancer.

#5734 The influence of body mass index on overall survival following surgical resection of non-small cell lung cancer. Xi Liu,1 Boris Sepesi,1 Kathryn A. Gold,2 Arlene M. Correa,1 John V. Heymach,1 Ara A. Vapourcyian,1 Jason Roznik,1,2 Ethan Dmitrovsky,1,2 UT MD Anderson Cancer Ctr., Houston, TX; 3UC San Diego Health, San Diego, CA.

Prior work implicated an association between increased body mass index (BMI) and lower risk of mortality from lung cancer. The aim of our study was to comprehensively evaluate the influence of BMI on long-term overall survival in patients with non-small cell lung cancer. This study investigated 1935 patients who underwent surgical resection for non-small cell lung cancer from 2000 - 2014. Study variables included both patient and treatment related characteristics. Univariate and multivariate Cox regression analyses were performed to identify variables associated with overall survival. By univariate analysis, significant predictors of better survival were higher BMI, pathologic tumor stage (stage I versus stages II, III, or IV), type of surgery (lobectomy/pneumonectomy versus wedge resection/segmentectomy), younger age, female gender, and adenosquamous histology (versus squamous) (all p < 0.05). Patients considered morbidly obese (BMI ≥ 35) had a trend towards better outcomes than those classified as obese (BMI ≥ 30 and <35) (p = 0.05), overweight (BMI ≥ 25 and <30) (p = 0.13), or healthy weight (BMI < 25) (p = 0.37) (HR 0.727, 0.848, 0.926, and 1, respectively). By multivariate analysis, BMI remained an independent predictor of survival (p = 0.02). Propensity matching analysis showed significantly better overall survival (p = 0.008) in patients with BMI ≥ 30 compared to BMI < 25. For exploratory analysis of expressed mRNAs associated with obesity in lung cancer, the association between obesity-related species (LEP, LEPR, PCSK1, POMC, MC4R, BMIQ1, BMIQ2, UCP2, BMIQ5, BMIQ6, INSL2, FTO, TME1M18, GNPD2A1, NEGR1, BDNF, KCTD15, SH2B1, MTH2, and NPC1) and survival was explored using The Cancer Genome Atlas (TCGA). Kaplan-Meier analyses demonstrated significantly improved overall survival in patients with high BMI.
lun cancer patients with higher Uncoupling Protein 2 (UCP2) expression, as will be presented. In summary, this large, single center series, after controlling for disease stage and other variables found higher BMI was associated with improved overall survival following surgical resection of non-small cell lung cancer. Studies are underway to elucidate the underlying mechanisms responsible for this association between BMI and lung cancer survival.

#5735 Comprehensive analysis of serum levels of VEGF and its receptors in patients with uterine cervical cancer. Daiken Osaku,1 Tetsuro Oishi,1 Mayumi Sawada,1 Hiroki Koamata,1 Jun Chikuni,1 Akiko Kudoh,1 Michiko Nonaka,1 Shinya Sat0,1 Muneaki Shimada,1 Hiroki Itamochi,2 Tasuku Harada,3 Tottori Univ. School of Medicine, Yonago, Japan; 4Iwate Medical University, Morioka, Japan.

Objective: Angiogenesis is one of the processes that is critical for the growth, invasion and metastasis of solid tumors, including uterine cervical cancer (CC). The vascular endothelial growth factor (VEGF) family is one of the major pathways involved in tumor angiogenesis. The aim of this study was to determine whether serum levels of these angiogenic factors could be used as biomarkers in patients with CC. Methods: A total of 107 patients with International Federation of Gynecology and Obstetrics (FIGO) stage IB to IIB CC who were treated at Tottori University Hospital between 2006 and 2015 were enrolled in this study. The study was approved by the Institutional Review Board of the School of Medicine of Tottori University. All patients gave written informed consent before the collection of specimens according to institutional guidelines. Serum samples were collected before initial surgery and levels of VEGF-A, -C, VEGFR-1, and VEGFR-2 were analyzed by enzyme-linked immunosorbent assay (ELISA). We evaluated the association between the levels of these angiogenic factors and clinicopathologic variables. With a median follow-up duration of 1743 days, survival analysis of 93 patients treated between 2006 and 2013 was performed. We also determined the mRNA expression of VEGF-A by real-time RT-PCR in fresh frozen tumors and the protein expression by immunohistochemical staining in paraffin-embedded tumors from CC patients. The mRNA levels of VEGF-A relative to GAPDH were used for the analysis. Results: Median levels of serum VEGF-A, -C, VEGFR-1, and VEGFR-2 were 313, 8122, 68, and 6210 pg/ml, respectively. We found a significant positive correlation between VEGF-A levels and the maximum tumor diameter (P = 0.010). Patients with pelvic lymph node involvement (PLNI) showed significantly higher levels of VEGF-A and VEGFR-2 than those without PLNI. Patients with bulky tumor or parametrial infiltration (PI) also showed significantly higher levels of VEGF-A. In contrast, patients with a bulky tumor or PLNI showed significantly lower levels of VEGFR-1. Both histological types and FIGO stage were not related to levels of these angiogenic factors. We set the cutoff values of these factors at the median levels of the angiogenic factors. The 5-year overall survival rate (OS) for patients with high VEGF-A levels was significantly lower than those with low levels (92.9% vs. 72.8%, P = 0.014). The 5-year OS for patients with high VEGFR-2 levels was significantly lower than those with low levels (94.7% vs. 73.8%, P = 0.012). PLNI and PI were prognostic factors for overall survival. Multivariate analysis revealed that PLNI, VEGF-A, and VEGFR-2 levels were independent prognostic factors. The mRNA and protein expressions of VEGF-A and VEGFR-2 were observed in the cancer cells. There was no correlation between mRNA levels and serum levels of VEGF-A. Conclusion: These results suggest that serum VEGF-A may be a promising prognostic biomarker for CC.

#5736 Clinical impact of high serum hepatocyte growth factor in advanced non-small cell lung cancer. Takahiro Tsuji, Hiroaki Ozasa, Yuichi Sakamori, Takashi Nomizo, Tomoko Funazo, Yuto Yasuda, Hironori Yoshida, Hirotoki Nagai, Young Hak Kim. Kyoto Univ. Graduate School of Medicine, Kyoto, Japan.

Activation of c-MET through hepatocyte growth factor (HGF) increases tumorigenesis, induces resistance, and is associated with poor prognosis in various solid tumors. We previously reported that lung cancer cell lines had increased expression of c-MET due to gene amplification-induced cytotoxic drug resistance, and that resistant cells paracrine HGF and promote its resistance. However, the clinical significance of sHGF in patients with advanced or recurrent NSCLC, especially in patients treated with cytotoxic chemotherapy, is yet to be identified. Here, we present sHGF may be useful to predict tumor response and PFS in patients with advanced NSCLC. A total of 81 patients with 101 treatment regimens were investigated; 53 patients received first-line therapy and 48 patients received second-line therapy during the observation period. sHGF levels were evaluated using ELISA at 4 time-points: at pre-treatment, at response-evaluation (1-2 months after treatment initiation), at the best tumor response, and at the disease progression. As a control biomarker, serum carcinomembrioncy antigen (CEA) was also evaluated. sHGF at response-evaluation in patients with progressive disease was higher compared with that of disease controls in both first-line and second-line treatment (median value (MV); under the limited of detection (LOD) vs. 0.40; P = 0.0086 and MV; under the LOD vs. 0.41; P = 0.0035, respectively). Positive-sHGF at response-evaluation predicts poor progression-free survival (PFS) compared with negative-sHGF in both first-line (median, 6.1 vs. 11.2; P = 0.047) and second-line treatment (8.7 vs. 21.9; P = 0.014). Lung adenocarcinoma subgroup analysis showed that in patients receiving second- line cytotoxic chemotherapy, there were no significant differences in PFS or HR between patients with low-CEA compared with those with high-CEA, but positive-sHGF at pre-treatment or at response-evaluation predicts poor PFS (35 vs. 132; P = 0.0045, 50 vs. 215; P = 0.0647, respectively). Simple and repeatable marker-based monitoring of the HGF/c-MET pathway would provide rationale for future research investigating the merit of sHGF as a potential clinical biomarker to indicate administration of MET inhibitors.

TUMOR BIOLOGY: Carcinogenesis and Human Tumors

#5737 YAP1 and COX2 converge to regulate SOX2 and urothelial cancer stem cells. Akira Oki,1 Noah Hahn, Mohammad Hoque. Johns Hopkins Univ. School of Medicine, Baltimore, MD.

Since cancer stem cell (CSC) are resistant to conventional chemotherapies that efficiently eliminate bulk tumor cells, the elimination is indispensable in treating malignant diseases. However, the underlying mechanisms responsible for the urothelial CSC traits remain elusive. The tumor microenvironment reinforces a chronic inflammatory state to support tumor progression by triggering CSCs. In addition, chemotherapy-induced apoptotic cells release COX2-derived PGE2, which in turn promotes CSC expansion. Environmental risk factors, such as arsenic (As), cause chronic inflammation and have been linked to urothelial carcinoma incidence. We previously developed an in vitro stepwise model for urothelial malignant transformation by As exposure in normal urothelial cells line (HUC1), which may reveal the intimate connections between carcinogenesis, chronic inflammation, and CSCs and provide clues to develop novel therapeutic strategies. As-exposed cells displayed more aggressive phenotype than As-unexposed cells in a time dependent manner. In gene set enrichment analysis of expression array of chronic As-exposed and unexposed cells; EGFR, COX2 and YAP1 were top-ranked oncogenic signature in As-exposed cells. Moreover, As exposure induced malignant stemness properties. In stem cell-specific RT-PCR array, SOX2 has been gradually overexpressed in line with acquired spheron formation and self-renewal capacities. SOX2 is frequently overexpressed in numerous Urothelial carcinoma (UC) cell lines as well as in As-exposed cells, especially in the spheroid cells. Stable knockdown of SOX2 reduces in vitro CSCs properties and also in vivo tumorigenicity. COX2/PGES1 and YAP1 signaling pathways are required to accelerate SOX2 activity. Mechanistically, COX2/PGE2 secreted induces promoter methylation of let-7 host gene via inducing DNA methyltransferases, resulting in downregulated let-7 expression and subsequent SOX2 expression. On the other hand, YAP1 induces COX2/PGE2 signaling inde- pendently SOX2 expression, and these signaling mutually compensate via negative feedback mechanism of SOX2, indicating that dual blockade of these signaling is indispensable to eradicate urothelial CSCs. The pharmacological inhibition of COX2 and YAP1 elicited long-lasting therapeutic response by subverting CSCs expansion following chemotherapy in both UC cells-derived and patient-derived xenograft models. In basal type UC, EGFR-targeted therapy was effective in line with previous reports, but the acquired resistance is inevitable. We reveal that COX2 and YAP1 signaling determine acquired resistance to treatment with the EGFR inhibitor via SOX2. Thus, our findings indicate that the combined inhibition of YAP1 and COX2/PGE2 signaling could be an effective therapeutic strategy to slow CSC expansion and UC progression.

#5738 Tracking the genetic relationship between first and late-onset second urothelial cancers by mutational signature analysis. Xavier Castells,1 Maude Ardín,1 Sandrine Rorive,2 Nilufer Broeders,3 Yan Song,1 Stephanie Vilardell,1 Christine Carreira,1 Pierre-Paul Bringuier,5 Adriana Heguy,6 Thierry Roumeguere,7 Joelle Nortier,3 Jiri Zavadil1.

Since cancer stem cell (CSC) are resistant to conventional chemotherapies that efficiently eliminate bulk tumor cells, the elimination is indispensable in treating malignant diseases. However, the underlying mechanisms responsible for the urothelial CSC traits remain elusive. The tumor microenvironment reinforces a chronic inflammatory state to support tumor progression by triggering CSCs. In addition, chemotherapy-induced apoptotic cells release COX2-derived PGE2, which in turn promotes CSC expansion. Environmental risk factors, such as arsenic (As), cause chronic inflammation and have been linked to urothelial carcinoma incidence. We previously developed an in vitro stepwise model for urothelial malignant transformation by As exposure in normal urothelial cells line (HUC1), which may reveal the intimate connections between carcinogenesis, chronic inflammation, and CSCs and provide clues to develop novel therapeutic strategies. As-exposed cells displayed more aggressive phenotype than As-unexposed cells in a time dependent manner. In gene set enrichment analysis of expression array of chronic As-exposed and unexposed cells; EGFR, COX2 and YAP1 were top-ranked oncogenic signature in As-exposed cells. Moreover, As exposure induced malignant stemness properties. In stem cell-specific RT-PCR array, SOX2 has been gradually overexpressed in line with acquired spheron formation and self-renewal capacities. SOX2 is frequently overexpressed in numerous Urothelial carcinoma (UC) cell lines as well as in As-exposed cells, especially in the spheroid cells. Stable knockdown of SOX2 reduces in vitro CSCs properties and also in vivo tumorigenicity. COX2/PGES1 and YAP1 signaling pathways are required to accelerate SOX2 activity. Mechanistically, COX2/PGE2 secreted induces promoter methylation of let-7 host gene via inducing DNA methyltransferases, resulting in downregulated let-7 expression and subsequent SOX2 expression. On the other hand, YAP1 induces COX2/PGE2 signaling independently SOX2 expression, and these signaling mutually compensate via negative feedback mechanism of SOX2, indicating that dual blockade of these signaling is indispensable to eradicate urothelial CSCs. The pharmacological inhibition of COX2 and YAP1 elicited long-lasting therapeutic response by subverting CSCs expansion following chemotherapy in both UC cells-derived and patient-derived xenograft models. In basal type UC, EGFR-targeted therapy was effective in line with previous reports, but the acquired resistance is inevitable. We reveal that COX2 and YAP1 signaling determine acquired resistance to treatment with the EGFR inhibitor via SOX2. Thus, our findings indicate that the combined inhibition of YAP1 and COX2/PGE2 signaling could be an effective therapeutic strategy to slow CSC expansion and UC progression.
TUMOR BIOLOGY: Carcinogenesis and Human Tumors

Exposure to aristolochic acid (AA, IARC Group 1 carcinogenic) present in some traditional herbal medicines leads to aristolochic acid nephropathy (AN), often complicated by development of multiple urothelial carcinomas of sequential onset. We used genome-scale mutational signature analysis of multiple urinary tract tumors of AA cases from a unique patient group to determine the mechanism and significance of the late-onset cancers and their associations with AA exposure as well as to the first cancers. Aristolactam-DNA adduct-positive AAN patients (n=4) who developed cancer within 8 years following the initial exposure to AA were chosen for analysis of their first cancers (upper tract urothelial carcinomas, UTUC) and second cancers of delayed onset (1-9 years after first-cancer diagnosis, involving the bladder or ureteral meatus). All patients had received a kidney transplant before developing the first cancers and harbored considerable overlap in exposure-specific (A>1T) somatic mutations. This finding suggests that the development of bladder urothelial carcinomas in AAN patients is likely due to distal seeding of cancer cells originating from the primary UTUC tumors. Our first-of-its-kind study addresses the risk as well as mechanistic factors leading to the second, late-onset bladder urothelial carcinomas following kidney transplantation and primary UTUC development. Our results underline the importance of long-term bladder follow-up in high-risk populations with established or suspected AA exposure. Funding: IARC NYU Genome Technology Center is partially supported by the NIH/NCI (P30CA16087) grant.

#5739 Nicotine promotes stemness-related properties and cell migration/metastasis through IGF-1R regulation in triple negative breast cancer. Yung-Chie Kuo,1 Jan-Show Chu,2 Kha-Liang Lee,3 Victor James Drew,2 Wei-Zhan Zhuang,4 Chi-Long Chen,5 Yuan-Soon Ho,6 Yen-Hua Huang,6 1Department of Biochemistry and Molecular Cell Biology, School of Medicine, Taipei Medical University, Taipei, Taiwan; 2Department of Pathology, School of Medicine, Taipei Medical University, Taipei, Taiwan; 3Graduate Institute of Medical Sciences, College of Medicine, Taipei Medical University, Taipei, Taiwan.

Purpose: To determine the role of insulin-like growth factor (IGF)-I receptor (IGF-IR) in alpha 9 nicotinic acetylcholine receptor (α9-nAChR)-induced stemness-related properties and to assess the therapeutic potential for preventing early tumor relapse and metastasis of TNBCs. Experimental Design: Expression levels of stemness-related genes, α9-nAChR and IGF-IR in tissues (n = 67) were analyzed by real-time Q-PCR and the correlation of different gene levels were calculated by the Pearson correlation analysis. The effects of either α9-nAChR activation or IGF-IR transactivation on stemness expression in TNBC were examined using α9-nAChR overexpressing or E2-E10 overexpressing cells, which were treated with nicotine and IGF-IR antibody, wound closure, invasion/migration assay, and annexin-V. The α9-nAChR, OCT4 and IGF-IR protein, p-IGF-IR in tissues were detected by immunohistochemical staining. Results: A high positive correlation between the gene expression levels of OCT4/NANOG/CD24/CD44 and α9-nAChR/IGF-IR in human breast cancer tissues was observed. Nicotine induced α9-nAChR activation-trans-activates an IGF-IR expression and a phosphorylation of STAT3, which stimulated stemness-related properties in both the cell lines and the xenografted mouse. The inhibition of either α9-nAChR or IGF-IR activation by RNA interference significantly suppressed the nicotine-induced stemness-related properties and cell metastasis both in vitro and in vivo. Conclusions: IGF-1R signal regulates the nicotine/α9-nAChR-induced stemness properties and cell metastasis therefore cannot prevent poor prognosis in TNBCs.

#5740 Aberration of (pro)rein receptor induces genomic instability in human pancreatic ductal epithelial cells. Yuki Shibayama,1 Jun Yasuda,2 Daisuke Yamazaki,3 Asadar Rahman,2 Shinichi Yachida,2 Akira Nishiyama,1 Kagawa University, Kagawa, Japan; 2Tohoku University, Sendai, Japan; 3National Cancer Center Research Institute, Tokyo, Japan.

Background: We have recently shown the potential contribution of (pro)rein receptor (P)RR to tumorigenesis of pancreatic ductal adenocarcinoma (PDAC) through an activation of Wnt/β-catenin signaling pathway (Sci. Rep., 2015). We also found that significant (P)RR expression was synchronized with an appearance of atypical nuclei in PanIN-2 of PDAC tissues. Furthermore, (P)RR knockdown reduced a total of DNA amount in human PDAC cell lines. Based on our findings, we have hypothesized that an aberration of (P)RR leads to a failure of genome homeostasis. Accordingly, we explored whether an aberrant expression of (P)RR induces genomic instability in human pancreatic ductal epithelial (HPDE) cells which are capable of developing to the PDAC. Methods: HPDE cells were infected with either an empty vector or (P)RR-expressing vector. The effects of (P)RR expression on genomic instability were evaluated by high coverage comparative genomic hybridization (CGH) analysis. Results: (P)RR overexpression induced genomic instability in PDAC cell lines. HPDE cells transfected by vector without the insertion of ATTPaP2 was noted as Mock. We also performed a comet assay to evaluate the defects of DNA repair capacity, flow-FISH to measure the length of telomere and western blotting to confirm the activation of DNA damage pathway by measuring protein levels of γ-H2AX and p53. Then, comparative analyses of human whole exome sequenced PDAC and (P)RR expressing HPDE cells were examined. Furthermore, we implanted 14-passage HPDE cells expressed by either Mock or (P)RR into the renal subcapsules of immunodeficient mice to investigate whether (P)RR-expressing HPDE cells show a precancerous status in vivo. Results: (P)RR overexpression led to inactivation of γ-H2AX and p53 as the components of DNA damage pathway, which were associated with a disappearance of comet tail and shorten telomere length. Substantial structural variations, e.g., inter- and intra-chromosomal translocations throughout all the chromosomes were observed in HPDE cells expressed by (P)RR under the analysis by BreakDancer. Analyses with Bctool showed that the number of all the somatic mutations was approximately 2-fold greater in HPDE cells expressed by (P)RR than those expressed by Mock (Mock: 124,579 vs. (P)RR: 239,860). (P)RR overexpression led to a failure of genome homeostasis by resulting in several genes which were commonly detected in PDAC tissues. The area occupied by (P)RR-expressing HPDE cell population remarkably expanded after the implantation to renal subcapsules of all the immunodeficient mice. However, there were no KRAS 12 codon mutations which are prevalent in PDAC patients. Conclusion: We have demonstrated that (P)RR overexpression induces genomic instability on global genome level in HPDE cells. These data are consistent with our hypothesis that (P)RR is essentially involved in the early carcinogenesis of PDAC.

#5741 Evaluating the role of recurrent ESR1-CCDC170 fusion in breast cancer endocrine resistance. Yiheng Hu,1 Jamunaranir Veeraraghavan,2 Jian Wang,3 Yin Tan,1 Jin-Ah Kim,2 Rachel Schiff,3 Xiaosong Wang.1 University of Pittsburgh, Pittsburgh, PA; 2Baylor College of Medicine, Houston, TX.

Background: Recurrent gene fusions resulting from chromosome rearrangements are central to the formation of cancer. Previously, our lab identified recurrent rearrangements between the estrogen receptor α (ERα) gene ESR1 and its neighbor gene CCDC170 in ~6-8% of luminal B tumors, a more aggressive form of ER+ breast cancer that has worse clinical outcome after endocrine therapy. ESR1-CCDC170 fusions enable expression of different-sized N-terminally truncated CCDC170 (ΔCCDC170) under ERα promoter, which is associated with the potential for estrogen receptor α (ERα) and ERβ interactions with its neighbor gene CCDC170, in breast cancer cellstransformedcellstoa potentially high-affinity ATP-binding pocket at its C-terminus, physically interacting with ERα and HER2, and forms homodimers. RPPA analysis showed that silencing of ESR1-CCDC170 repressed ERα and BCL2 protein levels, as well as total/phospho-HER2 levels, consistent with the findings of ΔCCDC170 interactions with ERα and HER2. Importantly, analysis of ΔCCDC170 protein sequence revealed a potential high-affinity ATP binding pocket at its C-terminus, suggesting that ΔCCDC170 may be directly druggable. Conclusion: These data suggest a potential role of ESR1-CCDC170 in mediating breast cancer endocrine resistance, which may possibly through cytoplasmic mislocalization of ΔCCDC170 and interactions with ERα and HER2. Further studies will clarify and elucidate the role of overexpressed or endogenous ESR1-CCDC170 in breast cancer endo-
The role of atypical protein kinase C in colorectal cancer cell growth and proliferation. S M Anisul Islam, Mildred Acevedo Duncan. Univ. of South Florida, Tampa, FL.

Colorectal cancer (CRC) is the second most common cancer in men and women from colorectal cancer accounts for 8% of all cancer deaths both in men and women in the United States. CRC is life threatening disease due to therapy resistant cancers cells. The exact mechanisms of cell growth, survival, metastasis and inter & intracellular signaling pathways involved in colon cancer are still a major challenge for scientists. Hence, investigating the signaling pathways that lead to colon carcinogenesis may give insight into the therapeutic target. In this study, the role of atypical Protein kinase C (aPKC) on colon cancer was identified by using four inhibitors of that particular protein class: 1) ACPD (2-acetyl-1,3-cyclopentanediene and 2) DNDA (3,4-diamino-2,7-naphthalene-disulfonic acid) are non-specific inhibitors of aPKCs; 3) 3-\(\beta\)-stilbene glucuronide; methyl dihydroxyphenyl phosphate is a PKC-\(\varepsilon\) specific inhibitor; and 4) ICA-1 (4, 9-amino-4-carbamoylmethylidazol-1-y-2, 3-dihydroxycyclopentanylmethyl dihydroxyphenyl phosphate) is a PKC-\(\gamma\) specific inhibitor. The cell lines tested were HT-29 colon cancer and CDB18CO normal colon epithelium. Additionally, the inhibition of aPKCs did not bring any significant toxicity on CDB18CO normal colon cell line. These results suggest the potentiality of utilizing aPKC-\(\gamma\) inhibitors to block colon cancer cell growth and proliferation.

#5745 Study on telomere shelterin component TPP1 in esophageal squamous cell carcinoma. Lihuia Yao,1 Jiang Zou,1 Ru Sun,1 Jiaying Cai,1 Guangcheng Huang,1 Qiang Ma,2 Lei Xu,1 Mayong Fu,2 Xiaolian Guo2, North Sichuan Medical College. Nanchong, China; 2Affiliated Hospital of North Sichuan Medical College, Nanchong, China.

Esophageal cancer (EC) is one of the most aggressive cancer and ranks the sixth leading cause of cancer-related mortality worldwide, approximately 70% of global esophageal carcinoma cases occur in China and esophageal squamous cell carcinoma (ESCC) is the most frequent histological subtype of EC. Lacking of reliable early diagnostic markers and efficient medical and surgical treatment approaches, the five-year survival rate of ESCC is only about 4.4%. Therefore exploration of the genetic mechanism that most ESCC patients in China could be beneficial to improve the diagnosis, therapy, and prevention of this disease. As an important telomere binding protein, TPP1 protects the ends of telomeres and maintains the genomic stability and integrity of chromosomes in eukaryotes. Previous investigation discovered that TPP1 might play an important role in the carcinogenesis and progression of some cancers. However, whether and/or how TPP1 participates in ESCC remains unclear. Here TPP1 was studied in 65 ESCC patients’ specimens including tumor tissue, the adjacent tissue, and the peripheral blood leukocytes, also the ESCC cell lines were employed to further explore the molecular mechanisms of TPP1 involved in the tumorigenesis of ESCC. In this study, TPP1 mRNA and protein were measured in the ESCC tissue specimens, peripheral blood leukocytes and also in ESCC cell lines using real time RT-PCR and Western Blot. The results showed that TPP1 mRNA level was significantly higher in ESCC tumor tissues compared with the adjacent tissues (P<0.05); the same trend was observed in ESCC cells (Eca109, Kyse150) vs human esophageal epithelial cells (HET-1A), respectively. However, in peripheral blood leukocytes, the expression of TPP1 did not show any difference between the cancer patients and the healthy controls. Among the 65 ESCC patients, TPP1 expression in 46 patients was obvious higher in tumor tissues than that in the matched adjacent tissues, although there were 19 patients in exception. Moreover, the TPP1 expression was correlated with the lymph nodes metastasis and CYF2, which is one of the potential tumor markers for diagnosis of esophageal carcinoma. To further explore the potential biological function of TPP1 in ESCC, stably transfecting shRNA-TPP1 (sh-TPP1) and shRNA-Negative Control (sh-NC) cells were performed to study TPP1’s roles in ESCC. The colonicogenic assay showed that depletion of TPP1 decreased ESCC cells proliferation and cell growth, and the wound healing experiment and cell invasion transwell chamber test demonstrated that down-regulation of TPP1 suppressed the cell migration and inva...
sion capabilities in vitro. Taken together, our findings revealed that TPF1 might play crucial roles in the pathogenesis of ESCC and targeting TPF1 could be a valuable potential for ESCC early diagnosis, intervention and therapeutics.

#5746 TRAF1 is required for solar UV-induced skin carcinogenesis. Hiroyuki Yamamoto,1 JooHyun Ryu,2 Eli Min,1 Naomi Oi,1 Ruhua Bai,1 Tatayana A. Zykov1,2 Dong Hoon Yu1,3 Margarita Malakhova,1 Kenji Moriyma,12 Ann M. Bode,1 Zigang Dong4,1 University of Minnesota, Austin, MN;1 Mukogawa Women’s University, Nishinomiya, Japan.

Tumor necrosis factor receptor-associated factor 1 (TRAF1) is a member of the TRAF protein family, which regulates the canonical and non-canonical NF-κB signaling cascades. Although aberrant TRAF1 expression in tumors is reported, the role of TRAF1 remains elusive. Here, we report that TRAF1 is required for skin carcinogenesis induced by chronic solar UV radiation. In vivo studies with solar UV exposure indicate that the deletion of TRAF1 results in the inhibition of AP-1 activity by down-regulating the induction of c-Fos and c-Jun by regulating ERK5 activation. Furthermore, we show that TRAF1 is required for solar UV-induced ERK5 activation. Mechanistic studies revealed that TRAF1 expression enhances the ubiquitination of ERK5 on K184, which is necessary for AP-1 activation. Altogether, our results suggest that TRAF1 mediates ERK5 activity by regulating the upstream effectors of ERK5 and also by modulating its ubiquitination status. Targeting TRAF1 function might lead to strategies for preventing and treating skin cancer.

#5747 Influence of air carcinogenic pollution on oncological morbidity. Olya Ostash,1 Igor Chernichenko,1 Olga Litvichenko,1 Sergei Tsymbaliuk,2 preventivemeasurestoundercompoundshazardinfluenceonpopulation.

In the present study, we investigated whether VCAN expression was associated with the pathogenesis of cccRCC. Methods: VCAN expression was analyzed in 3 RCC and normal kidney cell lines, as well as 84 matched cccRCC and normal renal tissues. We also performed various functional analyses of growth and progression properties using VCAN-depleted cccRCC cells. Microarray analysis was then employed to identify the target genes of the pathway involved in cccRCC tumorigenesis and development. Results: There are 4 isoforms of VCAN containing the N-terminal globular (G1 domain) and C-terminal globular (G3 domain) domains, each of which was found to be over-expressed in the cccRCC samples as compared to the controls. Higher VCAN expression was significantly correlated with metastasis (p<0.001) and worse 5-year overall survival after a radical nephrectomy (p=0.014). In vitro, VCAN knockdown by siRNA in Caki-2 and 786-O cells significantly decreased cell proliferation and increased apoptosis, and was also found to be associated with alteration of several TNF signaling-related genes, such as TNF-α, BID, and BAK. Furthermore, VCAN deletion markedly decreased cell migration and invasion associated with reduced MMP7 and CCN2 levels. Consequently, our results demonstrate that VCAN promotes cccRCC tumorigenesis and metastasis, showing it to be an attractive novel target for diagnostic, prognostic, and therapeutic strategies for affected patients.

#5750 USP15 inhibits HPV16 E6 degradation and catalytically inactive USP15 has reduced inhibitory activity. Aoi Tokuda, Masafumi Yoshimoto, Yuji Yaginuma. Kumamoto Univ. Graduate School of Health Sci., Kumamoto, Japan.

HPV’s can be classified as either high-risk or low-risk depending on the transforming potential of the virus. Most cervical cancers and a significant proportion of other anogenital cancers have been associated with infection by a small number of high-risk genital HPV types. Tumorigenesis induced by high-risk genital HPVs has been linked to the expression of two viral oncoproteins, E6 and E7, which cooperate in cellular immortalization and transformation processes. HPV16 E6 is an oncoprotein that causes the development of high-risk type human papilloma virus (HPV) and induces cancers, including oropharyngeal, anal, and cervical cancers. The stability of E6 is essential for its complete function as an oncoprotein. Using the yeast two-hybrid system, we identified ubiquitin-specific protease 15 (USP15) as an HPV16 E6-interacting protein. USP15 encodes a protein that consist of 952 amino acids and functions as a deubiquitinating enzyme. Deubiquitination is an essential process that releases ubiquitin chains from ubiquitin-protein conjugates. Aberrations in the ubiquitin-proteasome some system have been recently connected to the pathogenesis of several human protein degradation disorders, including cancer and neurodegenerative diseases; as
such, the proteasome is now considered to be important in the mechanism of carcinogenesis. USP15 cleaves polyubiquitin chains of HPV16 E6 and/or ubiquitin precursors. Immunohistochemical analysis showed that USP15 was highly expressed in normal cervical squamous cells, and cervical cancer cells. Our results indicated that USP15 can increase the level of HPV16 E6 by inhibiting E6 degradation. USP15 dose-dependently inhibited the degradation of HPV16 E6. In contrast, catalytically inactive mutants of USP15 had a reduced inhibitory effect on E6 degradation. In particular, USP15 mutants of all three cysteine boxes and the NHL mutant of the KRF box had a drastically reduced inhibitory effect on HPV16 E6 degradation. In addition, HPV16 E6 mRNA was not induced by USP15; therefore, HPV16 E6 appears to be post-translationally regulated. These results suggest that USP15 has the ability to stabilize E6 as a deubiquitinating enzyme, and HPV16 E6 can affect biological functions as an oncprotein in infected human cells.

**#5751 Induction of cancer stem cell like characteristics by overexpression of CDX1.**  
SANG IL CHOI, Kyoung-Ok Hong, Mi Ree Park, Yun Hee Na, Soo-Jeong Cho.  
National Cancer Center, Korea, Goyang-si, Gyeonggi-do, Republic of Korea.

Intestinal metaplasia (IM) has been regarded as the most significant risk factor for gastric cancer (GC). However, the molecular mechanisms of the pathogenesis of IM and the progression of GC remain unclear. The aim of this study was to evaluate the role of CDX1 in IM and GC using stable CDX1 overexpression in HFE145 gastric epithelial cells, and its expression in patient samples. For CDX1 overexpression, we generated lentiviral CDX1 and infected it to HFE145 cells. We found that CDX1 overexpression increased the expression of differentiation markers such as villin and SI and a decrease of gastric epithelial marker such as MUC5AC. Overexpression of CDX1 in HFE145 cells caused an increase in cell proliferation (P<0.05) and showed 2.7-fold higher clonogenic ability than that of control cells. The expression of CDX1 induced in vitro invasiveness 1.9-fold higher compared to HFE145 control cells with statistical significance (P=0.004), augmented the expression of MMP-9 and phosphorylation of FAK and paxillin. Overexpression of CDX1 also induced anchorage-independent colony formation and spheroid formation, and enhanced the expression of stem cell markers including SOX2, Oct3/4, NANOG and c-Myc in HFE145 cells, suggesting CDX1-induced stem cell like phenotype of gastric epithelial cells. HFE145 cells infected with CagA-positive Helicobacter pylori (G27 wild type) caused an increase in expression of CDX1 compared to HFE145 cells infected with CagA-negative H. pylori (G27 ΔCagE). Using luciferase promoter assay, we confirmed that the expression of CDX1 was regulated by the CagA secreted from H. pylori. In clinical practice, the Operative Link on Gastric Intestinal Metaplasia Assessment (OLGIM) staging system is often used to evaluate the degree of IM in biopsy samples or surgical specimen. We measured expression of CDX1, SI, and villin adjusted by glyceraldehyde 3-phosphate dehydrogenase expression using densitometry in 9 patients with various OLGIM stage (2 in stage 0, 1 in stage 1, 2 in stage 2, 1 in stage 3, 3 in stage 4). We were able to find out that the patients with higher OLGIM stage (stage 3-4) tended to have higher expression of CDX1 (3.935 vs. 0.416, p=0.100), SI (9.739 vs. 0.311, p=0.088), and villin (7.785 vs. 0.866, p=0.119) compared to patients with lower OLGIM stage (stage 0). These results suggest that CDX1 overexpression could be one of the causes of GC tumorigenesis and progression. Further investigation on CDX1 would encourage development of specific target for cancer treatment.

**#5752 Susceptibility of cells from cervical transformation zone to HPV-16 induced immortalization and dysplastic differentiation.**  
HAN Deng, Eric Hill, Pholimana Yenoboa, Sumona Mondal, Craig D. Woodworth.  
Clarkson University, Potsdam, NY.

Persistent infection of high-risk human papillomavirus (HPV) is a major risk factor for cervical cancer. Greater than 90% of these cancers originate in the cervical transformation zone (CTZ), a narrow band of squamous metaplastic epithelium between ectocervix and endocervix. It is unclear why the CTZ has high susceptibility to malignant transformation and few studies have specifically examined cells from this region. We hypothesized cells cultured from human CTZ are uniquely susceptible to cellular immortalization and dysplastic differentiation, two alterations that contribute to malignant development. Primary epithelial cells were cultured from each region of human cervix (ectocervix, endocervix and CTZ) and characterized for expression of differentiation markers to assess their origin. We examined susceptibility of cells from each region to immortalization after transfection with the complete HPV-16 genome or infection of HPV-16 E6/E7 retroviruses. In addition, we evaluated the extent of dysplastic epithelial differentiation in organotypic cultures of HPV16-immortalized cell lines derived from each region of cervix. Our results showed that cells from the CTZ express specific keratin markers (keratin 14 and 18) that confirm their origin. Primary cell cultures from CTZ and ectocervix are more susceptible to immortalization by the HPV16 complete genome or E6/E7 retroviruses. In contrast, preliminary results indicate that HPV-immortalized CTZ and endocervical cells are more susceptible to dysplastic differentiation when compared to cells from other regions. Although results are preliminary, our studies may serve to identify important signal pathways or biomarkers that are unique to cells from CTZ and that can be targeted for improved screening, prevention or therapy.

**#5753 Inhibition of CCAR1, a coactivator of β-catenin, suppresses the proliferation and migration of gastric cancer cells.**  
Te-Sheng Chang,1 Chung-Kuang Lu,1 Kuo-Liang Wei,1 Yi-Hsing Chen,1 Ying-Tung Cheng,2 Shui-Yi Tung,1 Cheng-Shyong Wu,1 Ming-Ko Chiang3.  
Chung Gung Memorial Hospital, Chiayi, Taiwan; National Chung Cheng University, Chiayi, Taiwan.

Given a current hypothesis that cancer arises from cancer stem cells (CSCs), targeting key signaling pathways that support CSC self-renewal appears to be a useful approach to cancer therapy. Deregulated expression of Wnt signaling is among the major pathways implicated in regulating the stemness properties of a variety of human cancers, including gastric cancer. Cell cycle and apoptosis regulator 1 (CCAR1) is a transcriptional coactivator which has been shown to be a component of Wnt/β-catenin signaling, and plays an important role in transcriptional regulation by β-catenin. However, the function and clinical significance of CCAR1 in gastric cancer have not been elucidated. Here we show the elevated CCAR1 nuclear expression correlates with the occurrence of gastric cancer. In addition, RNAi-mediated CCAR1 reduction not only suppressed the cell growth and increased apoptosis in gastric cell lines AGS and MKN28, but also reduced the migration and invasion ability of these cells. Furthermore, in vivo xenograft assay showed that the expression level of CCAR1 was critical for tumorigenesis. Our data demonstrates that CCAR1 contributes to carcinogenesis in gastric cancer and is required for the survival of gastric cancer cells. Moreover, CCAR1 may serve as a diagnostic marker and a potential therapeutic target.

**#5754 The genetic aberrations in carcinogenic sequence of colitis-associated cancer.**  
Nobuyuki Kakiuchi,1 Kenichi Yoshida,1 Yusuke Shiozawa,1 Akira Yokoyama,1 Keiseke Katoaka,1 Yoshikage Inoue,1 Yasuhiko Takeuchi,1 Yasunori Kogure,1 Ayana Kon,1 Masahiro Nakagawa,1 Kenichi Chiba,1 Hiroko Tanaka,1 Yuichi Shirashi,1 Satoru Miyano,1 Kenji Kawada,1 Hideaki Okajima,1 Yoshiharu Sakai,1 Takaki Sakurai,1 Hironori Haga,1 Hiroshi Nakase,3 Motoi Kuwahara,1 Hiroki Ikeuchi,1 Takako Kihara,1 Seichi Hirota,1 Takahiro Horimoto,1 Minoru Matsuura,1 Hiroyuki Marusawa,1 Hiroshi Seno,1 Seishi Ogawa,1 Kyoto University, Kyoto, Japan; The University of Tokyo, Tokyo, Japan; 2Sapporo Medical University, Sapporo, Japan; 3Hyougo College of Medicine, Nishinomiya, Japan.

Colitis-associated cancer (CAC) is a common complication among patients with a long history of ulcerative colitis (UC). Previous studies revealed that dysplasia precedes CAC development, and more than 20% of CAGs are multicentric, suggesting the presence of a field effect on colorectal carcinogenesis in UC patients. However, the genetic basis of the field effect in UC patients is poorly understood. To address this issue, we isolated single crypts from non-tumorous colon mucosa from 6 UC patients, as well as those from apparently normal mucosa from non-UC patients, and "single crypt-derived WES" was analyzed for somatic mutations using whole-exome sequencing (WES). In total, 19 crypts from histologically non-dysplastic, but inflamed mucosa, and 2 from apparently normal mucosa were isolated from 6 UC patients, including 4 with pancolitis, 1 with left-sided colitis and 1 with proctitis, while 32 crypts were obtained from normal mucosa from 20 non-UC patients, including 10 with colorectal cancer, 6 with adenoma and 4 healthy volunteers. Regardless of the disease status, mutations were detected in all crypts analyzed with the median of 43/crypt (3–121), showing high variant allele frequencies (VAFs) (~0.5), suggesting that these mutations were shared by all cells within single crypts and therefore by crypt-maintaining stem cells. The number of mutations increased with age, in crypts from both UC and non-UC patients (P < 10^-3), showing clear age-related C to T transition in both normal and UC-inflamed crypts. Strikingly, however, the age-adjusted number of mutations was significantly higher in UC-derived crypts than in those from non-UC individuals (P < 10^-3). KRAS and TP53 mutations were reported to be the most prevalent alterations in UC-associated CAC, but only rarely seen in UC-derived normal crypts. Next, to evaluate clonal expansion of mutated clones, we performed WES for the bulk specimens composed of 20-30 mutually adjacent crypts. However, this time, mutations and copy number alterations (CNAs) with high VAFs were detected only in UC-inflamed mucosa, suggesting that clonal crypt expansion

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occurred only in UC-derived mucosa. WES was also performed for 14 CACs. The number of non-synonymous mutations (101/sample) in CAC were significantly higher than that in UC-derived normal crypts (38/sample) and recurrently involved known driver genes, such as TP53 (71%) and KRAS (36%). CNAs were also more frequent in CAC than UC-derived, normal crypts (36%). This study revealed that specific clonal expansion events in UC were also present in CAC. This would indicate that mechanisms such as clonal expansion in UC and clonal selection in CAC are more prevalent in UC than in CAC. Although the significance of clonal expansion in UC is still unclear, it is possible that clonal selection in CAC may be the result of the interaction between the host and the tumor microenvironment. This study provides new insights into the pathogenesis of UC and CAC, and may help to develop new therapeutic strategies for these diseases.

#5755 In vitro reconstitution of high-grade serous ovarian carcinoma from primary fallopian tube secretory epithelial cells. Kohei Nakamura, Kentaro Nakayama, Tohru Kiyono, Narisawa Ishihara, Toshikazu Minamoto, Tomoka Ishibashi, Kaori Sanuki, Hitomi Yamashita, Ruriko Ono, Kouji Iida, Hiroki Sasamoto, Rayna Suzuki, Mohammad Mahmoud Hossain, Tsukasa Ishihara, Satoko Kyo, Shion University Faculty of Medicine, Izu, Japan; National Cancer Center Research Institute, Tsukiji, Chuo, Japan.

Purpose: Recent studies suggest that fallopian tube secretory epithelial cells (FTSECs) are potential cells-of-origin for high-grade serous ovarian carcinoma (HGSOCC). Several genetic alterations are involved in HGSOCC carcinogenesis, but the minimal requirement for tumor initiation remains unclear. Therefore, we sought to identify oncogenic mutations indispensable for HGSOCC carcinogenesis in a stepwise in vitro model using immortalized FTSECs, over-expressing etiologic drivers. We established an in vitro stepwise carcinogenesis model using immortalized FTSECs over-expressing cyclin D1, CDK4/6 and hTERT, and mimicked select genetic abnormalities identified as gene alterations essential for carcinogenesis, including p53, c-Myc, or RAS/PI3K/AKT pathway mutations. Results: Our analyses revealed two distinct patterns of gene alterations essential for HGSOCC carcinogenesis: p53/KRASV12 and p53/KRASV12/c-Myc. Dominant-negative p53 expression alone or in combination with oncogenic KRAS (KRASV12) constitutively active AKT (CA-AKT), or c-Myc in immortalized cells failed to induce a tumorigenic phenotype; however, overexpression of either CA-AKT or c-Myc in combination with dominant-negative p53 and KRASV12 was sufficient to confer tumorigenic potential. Importantly, all transformed FTSECs formed tumors in xenograft mice, which were grossly, histologically, and immunohistochemically similar to human HGSOCC. Interestingly, mice harboring tumors with c-Myc amplifications displayed extensive metastases, consistent with the increased dissemination observed in their human counterparts. c-Myc is associated with cell proliferation in vitro; therefore, this genetic abnormality may promote HGSOCC progression. Conclusions: Collectively, our data showed that aberrant p53/KRASV12/c-Myc or p53/KRASV12/PI3K-AKT signaling is the minimal requirement for FTSEC carcinogenesis. Moreover, the model generated with this evidence will likely facilitate analysis of early events in HGSOCC carcinogenesis.

#5756 Effects of Epstein-Barr virus latent membrane protein 1 (LMP1) on cell invasiveness and expression of endogenous microRNAs in human cells in vitro. Bárbara Grasiele Müller-Coan, Ethel Cesmaran, Deilson Elgui De Oliveira, Universidade Estadual Paulista (UNESP), Botucatu, SP, Brazil; Weill Medical School at Cornell University, New York, NY.

Infection by the Epstein-Barr virus (EBV) is associated with a variety of human cancers, notably Burkitt lymphoma and nasopharyngeal carcinoma (NPC). Most malignant cells within EBV-associated cancers are latently infected, and some viral products expressed during the latent phase of the viral cycle have well-described carcinogenicity, including the EBV latent membrane protein 1 (LMP1). Distinct viral genomes described so far include the B95.8, the most studied viral strain originally found in an elderly patient with transfusion-induced infectious mononucleosis, and the M81 strain, isolated from a NPC case from a Chinese patient. Compared to B95.8, the EBV M81 strain infects epithelial cells more readily, and it is more likely to induce spontaneous viral lytic reactivation. However, whether these EBV strains have distinct biological features relevant for the progression of EBV-associated cancers remains to be elucidated. Thus, this study aimed to evaluate whether the expression of B95.1 and M81 variants of EBV LMP1 have different impact on cell migration and invasiveness of human cells lines in vitro, as well as on the expression of selected endogenous microRNAs (miRs) that were previously reported to have a role on cancer progression. Expression of EBV LMP1 was achieved in 293 and NPE9 cells transfected with vectors encoding its B95.8 or M81 variants. In vitro migration and invasion rates were assessed by the scratch wound healing assay and the Boyden chamber assay, respectively. The expression of 91 selected miRs were evaluated by a customized qPCR-array approach. Cells expressing EBV LMP1 showed increased migration and invasion rates compared with mock-transformed cells. However, the differences observed between B95.8 and M81 variants did not achieve statistical significance. Conversely, 293 cells expressing the M81 variant of LMP1 showed hyperexpression of human miRs 497-5p, 17-3p, and 34a-5p, compared to B95.8. The in silico target prediction for these miRs showed enrichment of gene targets involved in the MAPK, NF-kB, and PI3K/AKT in cellular signaling pathways. This would indicate that EBV LMP1 could promote proliferation and cell motility/adhesion, as well as higher levels of apoptosis. Based on these data, it is plausible to suggest that EBV LMP1 from M81 viral strain is somewhat less effective in terms of carcinogenicity, and this might be a reflection of the improved ability of EBV M81 to induce lytic viral infection compared to the B95.8 strain, as previously reported. In conclusion, the results indicate that cells expressing LMP1 from EBV isolates B95.8 and M81 showed differences in terms of miRNA expression signatures, which ultimately might impact the pathogenesis and progression of EBV-associated cancers.

#5757 Study of human mammary tumor virus (HMTV) in human breast cancer by NanoString nCounter and FISH analysis. Stella M. Melana, Joseph Tripodi, Digna Nosike, Jaffer Shahnab, Polly Etkind, Beatriz GT Pogo, James F. Holland, The Fish Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY; Department of Pathology, Icahn School of Medicine at Mount Sinai, New York, NY.

Human Mammary Tumor Virus (HMTV), a retrovirus 90-98% homologous to Mouse Mammary Tumor Virus (MMTV), the etiological agent of mammary tumorigenesis, has been detected by PCR in 40% of American women's breast cancers, but not in healthy tissues of the same breast (Cancer Research 1995, 55:5173-79; Clin. Cancer Research 2001, 7:283-9). A complete 9.9 kb HMTV proviral sequence has been detected in breast cancer genomic DNA (Cancer Res 2001,61:1754-9). HMTV viral particles with betaretrovirus characteristics have been isolated from metastatic breast cancer cells in effusions (MSSM cells). Expression of HMTV proteins has been detected in MSSM cells by western blot, fluorescence-activated cell sorting analysis, and immunofluorescence assays but not in normal mammary epithelial cells (J Virol Methods 2010,163(1):157-61). PCR is a sensitive technique susceptible of contamination, which due to amplification, could result in false positive detection. Although murine DNA was not found by PCR, the possibility still exists that HMTV in human DNA could be a result of laboratory contamination. Despite the substantial evidence supporting the presence of HMTV in breast cancer the controversy continues because the chance of contamination has led to doubt that murine-like viruses are human pathogens. We now report additional analyses of breast cancer specimens employing other methods for HMTV detection that reinforce previous findings. NanoString nCounter, a new technology using specific 100 mer oligonucleotides as probes bound to reporter molecules that can detect HMTV gag, env andLTRsequencesastwellastumour specific probes in a single reaction without amplification. Fluorescence in situ hybridization (FISH) assays with a HMTV probe is a technology that permits visualization of HMTV proviral DNA in the nuclei of surgical samples of breast cancer as well as in MSSM cells. Results from Nanostring nCounter, and FISH analysis show the presence of HMTV DNA in surgical cancer specimens and in MSSM cells. The data exclude contamination and confirm the authenticity of HMTV in human breast cancer. Next-generation sequencing and other studies are in progress to further understand the role of HMTV in the pathogenesis of breast carcinoma.

#5758 Elevated level of estrogen enhances chemotherapeutic efficacy potentially through epigenetic mechanism in human breast cancer cells. Yu-Wei Chang, Kamaleshwar Singh, The Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY; Department of Pathology, Icahn School of Medicine at Mount Sinai, New York, NY.

Effectiveness of chemotherapy depends on the age of breast cancer patients. Breast tissues are estrogen sensitive and the levels of ovarian estrogen are age-dependent variable among the breast cancer patients. Whether this variation in estrogen levels influences the chemotherapeutic efficacy in breast cancer patients is unclear. Therefore, the objective of this study was to determine the effects of natural estrogen 17 beta-estradiol on the efficacy of chemotherapy in breast cancer cells. Estrone responsive MCF-7 breast cancer cells were long-term exposed to 100 pg/ml estrogen for three months. Using these exposed MCF-7 cells, the efficacy of chemotherapeutic drugs doxorubicin and cisplatin were determined. The result of cell viability and cell cycle analysis revealed increased efficacy of doxorubicin and cisplatin in estrogen-exposed MCF-7 cells as compared to control MCF-7 cells. Gene expression analysis of marker genes for cell cycle, anti-apoptosis, DNA repair, and drug transporter genes further confirmed the increased efficacy of chemotherapeutic drugs in estrogen-exposed cells at molecular level. To further understand the role of epigenetic mechanism in enhanced chemotherapeutic efficacy by estrogen, cells were pre-treated with epigenetic drugs,
5-Aza-2-deoxycytidine and Trichostatin A before doxorubicin and cisplatin treatments. The enhanced efficacy of doxorubicin and cisplatin in estrogen-treated cells was significantly decreased by 5-aza-2-deoxycytidine pre-treatment, suggesting the role of estrogen-induced hypermethylation in enhanced sensitivity of these drugs in estrogen-exposed cells. In conclusion, results of this study demonstrate that estrogen cells induce hypermethylation on the levels of estrogen in breast cancer cells. Findings of this study will have clinical implications in selecting the type and dose of chemotherapy in breast cancer patients depending on the serum estrogen levels that varies in between pre- and post-menopausal age of the patients.

#5759 Histopathologic patterns of nasopharyngeal malignancy in Lagos, Nigeria and its association with Epstein-Barr virus (EBV): a 10-year retrospective study. Oladipo Omosebi,1 Olakanni Akinde,1 Omobolade Obadahun,1 Nzechukwu Ikeri,1 Gabriel Ogun,2 Adekumbiola Banjo,1 1Lagos University Teaching Hospital, Lagos, Nigeria; 2University College Hospital, Ibadan, Nigeria.

Background: Nasopharyngeal cancer (NPC) is a rare cancer worldwide. It is now found to be increasing in incidence in Nigeria, though the incidence is lower when compared to countries in the Mediterranean basin, North Africa and Southeast Asia. NPC is frequently associated with Epstein-Barr virus (EBV), but no previous study has been done in Lagos to document the association. Objective: To determine the prevalence, histologic types and age distribution of nasopharyngeal malignancy in Lagos University Teaching Hospital (LUTH) and its association with EBV over a period of 10 years, between January 2003 to December 2012. Methods and Materials: Slides and formalin-fixed paraffin-embedded (FFPE) tissue blocks, hospital request forms, referral cards, patients' case notes and duplicate copies of histopathology reports of cases of histologically diagnosed nasopharyngeal malignancy in the Anatomical and Molecular Pathology department of LUTH within the study period were retrieved. The cases were reclassified into epithelial and non-epithelial malignancies. The epithelial malignancies were reclassified according to WHO classification. Immunohistochemistry (IHC) for EBV latent membrane protein-1 (LMP-1) was carried out on cases with viable FFPE to evaluate the prevalence of the virus in Nasopharyngeal malignancy. Results: A total of 75 histologically confirmed cases of nasopharyngeal malignancy were seen during the study period, which constituted 1.1% of the total cancer recorded in this center. There were 47 males (63%) and 28 females (37%) with a male to female ratio of 1.7:1. The age ranged from 3 to 75 years, mean age was 44.1 years, the peak age group was 40-49 years for males and 50 - 59 years for females. On histological classification, undifferentiated carcinoma (WHO type III) was the commonest (49.3%), differentiated non-keratinizing squamous cell carcinoma, SCC (WHO type II) and keratinizing SCC (WHO type I) accounted for 24% and 9.3% respectively. Thus, NPC accounted for 82.6% of the total cases of nasopharyngeal malignancy seen. Other histological types seen were non-Hodgkin lymphoma (NHL) 13.3%, well differentiated adenocarcinoma 2.7% and low grade mucoepidermoid carcinoma 1.1%. EBV LMP-1 IHC was positive in 30 (86%) out of the 35 cases studied. These consisted of 14 undifferentiated carcinoma, 5 NHL, 2 adenocarcinoma, 7differentiated non-keratinizing SCC (WHO type II) and the 2 cases of keratinizing SCC. Conclusion: Nasopharyngeal malignancy is not uncommon in this environment. It occurred more frequently in males than females with the peak age of incidence at 5th decade of life, undifferentiated carcinoma (WHO type III) is the commonest histologic type and it is highly associated with EBV.

#5760 Identifying cell of origin for Group 3 medulloblastoma. Ran Tao, Najiba Murad, Zhenhua Xu, Yansin Pei. Children’s National Medical Center, Washington, DC.

Medulloblastoma (MB) is the most common malignant pediatric brain tumor. Although surgery, radiation and high-dose chemotherapy have led to increased survival rates, many patients still die from their disease and those who survive often suffer severe long-term side effects. Group 3 MB is the most aggressive subtype of MB, which is often resistant to therapy, and patients with this tumor have an extremely poor prognosis. Thus, developing novel and more effective therapeutic approaches is desperately needed for treating this disease. Improved approaches are likely to come from a deeper understanding of the molecular and cellular basis of the disease. Group 3 MB is characterized by large cell/anaplastic histology with a high proliferative index and is associated with MYC amplification or overexpression. Our previous studies suggest that MYC-driven MB can be generated by infecting granule neuron progenitors (GNPs, Math1+) or neonatal cerebellar stem cells/progenitors (CD133+) with viruses encoding MYC and dominant negative p53 (DNp53) and transplanting these cells into the cerebella of immunocompromised mice (NSG). Although this model resembles Group 3 MB in terms of histology and gene expression, it most likely does not faithfully recapitulate human MB because mutation and deletion of p53 are rarely detected in human Group 3 MB at diagnosis. This observation prompted us to ask whether there might be a cell type in which the MYC oncogene alone is sufficient to induce MB. Sox2 expression has been found in neural stem cells and the tumor cells in cerebellum. Moreover, it has been reported that ~70% human Group 3 MB express Sox2, and that its expression is correlated with poor prognosis. Thus we hypothesize that Sox2-positive cells with MYC overexpression can develop tumors. We FACS-sorted Sox2+/−, positive or Sox2−/− negative cells from Sox2+/− mice at P5, infected these cells with MYC virus and transplanted them into the cerebella of NSG mice. Animals (n = 10) receiving Sox2−/− positive cells developed highly aggressive tumors and died within 3-6 weeks. In contrast, only one of the animals (n = 10) that received Sox2−negative cells developed a tumor at 8 weeks. These results suggest that the MYC oncogene alone is sufficient to induce tumorigenesis in Sox2−positive cells, without requiring loss of p53. Further studies demonstrate that the tumors that were generated from Sox2−positive cells resemble Group 3 MB at a histological, immunohistochemical and molecular level, indicating that Group 3 MB may originate from Sox2-positive cells during cerebellar development. We will use this model to investigate the mechanisms that make these cells sensitive to transformation. These studies will pave the way for the development of novel approaches to treating this devastating tumor.

#5761 C-Fos drives non-tumorigenic head and neck squamous cell carcinoma cells to tumorigenic by promoting cancer stem cells like properties. Naoshad Muhammad, Sourav Bhattacharya, Robert Steele, Nancy Phillips, Ratna B. Ray. Saint Louis University, Saint Louis, MO.

Objectives: The head and neck squamous cell carcinoma (HNSCC) is the sixth most widespread cancer and leading cause of cancer related death world widely. Regardless of the advancement in treatment processes the overall survival rate of patients having this cancer has not substantially improved in the past few decades. Development of tumor progression is due to emergence of CSCs in a small population of cancer cells, termed as cancer stem cells (CSCs). This population of cells can self-renew and re-form the ordered organization of tumors. CSCs contribute to HNSCC and may offer prominent therapeutic strategy for the management of HNSCC. We previously observed that c-Fos was highly up-regulated in the HNSCC sphere forming cells. In the present study, we explored the role of c-Fos in the tumor initiation in relation with cancer stemness and EMT. Experimental procedures: We examined whether overexpression of c-Fos has an effect on tumorigenicity and stemness. For this, c-Fos was overexpressed in non-tumorigenic HNSCC cell lines and tumor growth was investigated. We also examined the expression of different CSC marker genes expression by Western blot analysis. Summary: Our results demonstrated that exogenous expression of c-Fos in non-tumorigenic MDA1386Tu cells makes the cells tumorigenic in nude mice. Mechanistic investigations showed that c-Fos overexpression in MDA1386Tu cells enhanced epithelial-mesenchymal transition (EMT)/CSC properties and increased numbers of oralspheres when grown in ultra-low binding plate. Conclusions: Collectively, our data strongly demonstrate that c-Fos plays a critical role in the modulation of EMT and cancer stem cell reprogramming in HNSCC cells and enhance tumor growth.

#5762 PR55α alpha associated PP2A promotes pancreatic tumorigenesis. Ying Yan, Ashley L. Hein, Parthasarathy Seshacharyulu, Satyanarayana Rachagani, Yuri M. Sheinin, Michel M. Ouellette, Moorothy P. Ponnusamy, Surinder K. Batra. University of Nebraska Medical Center, Omaha, NE.

PP2A holoenzyme consists of a catalytic subunit, a scaffold subunit, and a regulatory subunit. Loss of function analysis using PP2A catalytic inhibitors or inhibition via tumor viral proteins suggest PP2A as a putative tumor suppressor. However, PP2A has also been shown to facilitate the activation of oncogenic signaling pathways when associated with specific regulatory subunits. In this study, we investigated the possible oncogenic role of PP2A in pancreatic cancer. We found a striking increase in the expression of PR55α, a PP2A regulatory subunit, in compared to normal pancreatic epithelial cells. Consistently, PR55α expression was markedly elevated in pancreatic ductal adenocarcinoma tissues compared to adjacent normal pancreatic tissues (P<0.0001) and correlated with poor survival of pancreatic cancer patients (P<0.0003). DNA-mediated depletion of PR55α in pancreatic cancer cells resulted in diminished phosphorylation of both AKT and ERK1/2 and decreased protein levels of β-catenin. Accordingly, pancreatic cancer cells with reduced PR55α expression exhibited significantly impaired properties of transformation, including attenuated cell growth, clonogenicity, mobility, and anchorage-independent growth. Moreover, orthotopic implantation of PR55α-depleted pancreatic cancer cells into nude mice showed a marked reduction in tumori-
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#5763 Investigating the carcinogenic potential of various types of mineral fibers in the development of mesothelioma. David Larson, Mika Tanji, Andrea Napolitano, Sandra Pastorino, Michele Carbone, Haining Yang. Univ. of Hawaii Cancer Ctr., Honolulu, HI.

Malignant mesothelioma is a very aggressive tumor and has been linked to occupational and environmental exposure to carcinogenic mineral fibers such as asbestos. It was discovered recently that individuals carrying germ-line BAP1 mutations are predisposed to mesothelioma in presence of even minimal exposure to carcinogenic mineral fibers. Mesothelioma causes about 3,200 deaths per year in the U.S. and over 40,000 deaths per worldwide. Most malignant mesothelioma studies have focused on carcinogenesis due to occupational exposure to the six regulated industrial mineral fibers collectively called ‘asbestos’. Besides this family of six regulated asbestos minerals, there are other non-regulated fibers that possess physical and chemical structures that are similar to industrial asbestos, and may also be carcinogenic. However, many of those mineral fibers are unknown for their carcinogenicity. We conducted serious studies on this topic and established in vitro and in vivo assays to help determine the carcinogenesis of various types of mineral fibers. We found that high mobility box 1 protein (HMGB1) is a key initiator of asbestos-induced inflammation, which plays critical roles in the development and growth of mesothelioma, and that HMGB1 may be a good indicator for fiber carcinogenesis and a biomarker for fibers exposure. Moreover, we found that specific immune system changes that occur after exposure to carcinogenic fibers, which may be a useful tool to help intero-gate the carcinogenic potential of mineral fibers. In particular, the ability to induce an M2 macrophage response appears related to the potent tumor-inducing capacity of carcinogenic mineral fibers.

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#5765 Noninvasive characterization of 3D morphology and pathophysi-ology of multicellular tumor spheroids using optical coherence tomography. Yongyang Huang,1 Shunqiang Wang,1 Sarah Kessel,1 Ian Rubinioff,1 Leo Li-Ying Chan,2 Peter Li,2 Yaling Liu,1 Jean Qiu,2 Chao Zhou,1 Lehigh University, Bethlehem, PA; 2Nexelom Bioscience LLC, Lawrence, MA.

Screening of anti-cancer drug candidates follows a step-by-step testing procedure on different models, involving in vitro cell cultures, in vivo animal models and humans. Traditionally, in vitro 2D cell cultures are frequently used to evaluate cytotoxic effects of drug candidates on tumors. Although simple and efficient, 2D tumor cultures do not accurately reflect drug responses in a 3D environment, which hampers the use of these models for in vivo studies. Multicellular tumor spheroids (MCTSs), a 3D model, has attracted much attention in cancer research due to their physiological similarities with in vivo tumors, such as their structures, oxygen and nutrient gradients, and drug resistances. Therefore, they can serve as a more predictive model for the cancer drug discovery. High-throughput screening (HTS) systems employ various imaging approaches, e.g. bright-field microscopy, fluorescence microscopy or confocal mi-croscopy, have been employed to perform routine imaging and analyses of structures and functions of MCTSs. One drawback of the current HTS systems is that they do not readily provide a 3D view of the entire MCTS, especially for large spheroids (>500μm), due to limited imaging penetration, which hampers accurate characterization of the spheroid volumetric changes. In this work, we developed a new screening system to visualize the entire 3D structure of MCTSs using 96-well ultra-low attachment (ULA) plates combined with optical coherence tomography (OCT), a high resolution and 3D imaging modality. We showed that this imaging system was able to perform longitudinal tracking of 3D structural changes, e.g. sizes, shapes and volumes, for individual MCTSs. In particular, we demonstrated that spheroid volume measured based on 3D OCT imaging data provided a more accurate and robust result as compared to volume calculated based barely on the spheroid size (diameter). Furthermore, we demonstrated that this system could perform non-invasive, label-free monitoring of pathophysiological status of MCTSs over time. The 3D distribution of a spher-oid’s necrotic core could be assessed based on intrinsic optical contrast. This OCT-based screening system would open up new opportunities for accurate 3D characterization of morphological and pathophysiological features of MCTSs, and benefit the development of the state-of-the-art 3D HTS system for cancer drug discovery.

#5766 High-content microscopy-based screening of colorectal organoids. Niklas T. Rindtorff,1 Bettina Jette,1 Jan Sauer,1 Thilo Miersch,1 Tianzuo Zhan,1 Florian Heigwer,2 Claudia Scholl,2 Matthias Ebert,2 Bernd Fischer,1 Michael Boutros1.1 DKFZ Heidelberg, Heidelberg, Germany; 2University Hospital Mannheim, Mannheim, Germany.

High-content screening of cells has become a widespread approach for cellular assays due to its capacity to capture complex biological processes. However, conventional cell culture is limited with respect to cell and tissue architecture. Organoids are a unique model system for the intact and diseased intestinal epithelium. The 3D model can be used for the functional study of cancer development, and, potentially, prospective therapeutics testing of drugs in patient derived tumor organoids. Here, we present a high-content microscopy based screening workflow to study organoid self-organization and growth with up to single cell resolution. After seeding of organoid fragments in a basal membrane extract, screening plates are incubated to allow for organoid formation. Subsequent treatment and incubation is followed by staining and imaging on a high-throughput microscopy platform followed by automated image analysis using open-source software. Profiling of both complete organoids and their individual architecture enables the quantitative description of population and tissue heterogeneity in the context of various perturbations. We generated four distinct colon organoid lines from mice carrying mutations of APC and KRAS in different combinations. These are profiled for differential phenotypic responses to a library of ~1000 drug-like compounds. Also, this methodology is used to screen for clinically relevant differential treatment responses in patient derived tumor and normal colon organoids. Hence, based on this work we are able to analyze gene-drug interactions in early colon cancer development and drug response of patient derived colorectal cancer organoids.

#5767 Ex vivo three-dimensional tumor growth assay: 3DX-TGA. Praveen Nair,1 Dileep Nair,1 Kaede Hinata,1 Cyrus Mirdsadi,1 Junjie Wu,2 Yong Hu,2 Brett M. Hall.1 1Molecular Response, San Diego, CA; 2Bioduro, San Diego, CA.

With a 7% likelihood of regulatory approval, oncology drug registration failure rates lead all therapeutic areas. Further complicating the drug development...
To realize the promise of precision medicine, it is important to integrate phenotypic assessment of cell populations to genomic data. The analysis of invading leader cells at the tumor invasion front is of interest as they may be guided by a targetable molecular phenotype. However, there is a lack of suitable platforms on which to analyze the tumor invasion front. In this study, we have designed and constructed a fluidic device for long-term (several days to weeks) 3-dimensional tumoroid culture of diverse cancer cells. Using this device, we can recapitulate the tumor invasion front and at the same time quantify the invasive potential of different breast cancer cell line models. Analyses of the tumor invasion front indicated a region of higher proliferation and suggest that the leader cells possess a different molecular phenotype from the tumoroid mass. Interestingly, some of the invasion front cells can be targeted using a combination of strategies indicating that there could be: 1) the presence of multiple subpopulations of invasive cells, each with a different clonal genetic signature; and/or 2) reversible phenotypic switching occurring among invading cells due to phenotypic plasticity. These results obtained using this innovative device highlight and present a promising solution to the challenges developing adequate therapeutics accounting for tumor phenotypic heterogeneity. There is potential for the device for use in personalized medicine at diagnosis, allowing for both the quantification of disease progression risk as well as the molecular characterization of the invasive subpopulations from patient samples and their response to tailored therapies.

#5770 in vitro PDX models: 3D cultured patient-derived tumors for compound evaluation. Sander Bästén,1 Bram Herpers,2 Julia Schueler,2 Torsten Giesemann,2 Leo S. Price,1 Ocelli B.V., Leiden, Netherlands; Charles River Laboratories, Breburg, Germany.

Background. Patient-derived xenograft (PDX) models in immune-compromised mice allow propagation of and compound testing in human-derived tumors in vivo. To expand the potential of these human-relevant PDX models, we sought to develop 3D in vitro culture methods for PDX-derived tumor cells that show in vivo-like growth characteristics, invasion and responses to therapeutics. In combination with advanced 3D image analysis methods, we created a unique high throughput in vitro PDX screening platform that not only allows efficient identification of active and selective molecules but also enables selection of the optimal PDX tumor models for subsequent validation of candidates in vivo. Results. Each PDX model has its own unique growth characteristics. Hydrogel and growth media composition were optimized to support growth of tumor tissues in vitro from cells derived from bladder, stomach, breast, pancreas, colon and lung cancer PDX tumors. Tumor tissues were cultured in a 384-well format and used to test chemotherapeutics (e.g. 5FU, doxorubicin, paclitaxel, cisplatin), small molecules (e.g. erlotinib, lapatinib, trametinib, everolimus), antibodies (e.g. cetuximab, trastuzumab) and antibody-drug-conjugate (ADC, T-DM1) dose ranges. Using Ocelli’s 3D image analysis platform, Omixer, tumoroid growth, cell proliferation, apoptosis, invasion, cell polarity, differentiation and other aspects of cell and tissue architecture were analyzed and the effects of compound exposure on tumoroids was determined. By performing feature training based on reference compounds, we selected ±10 morphological features (out of more than 500) to generate a phenotypic signature that described the unique phenotypic change induced by each compound. Different compounds that target the same molecule were found to induce a similar morphological change whereas compounds with off-target effects could be discriminated. This approach enabled a high resolution evaluation and comparison of compound activity in an automated manner. Conclusions. We established several PDX model-derived 3D tumor cultures in which standard-of-care and novel therapeutic agents (small molecules, antibodies and ADCs) can efficiently be screened, based on therapeutically relevant parameters and their changing morphological profile. This method enables both the in vitro selection of promising compounds in a pre-clinically relevant setting and the selection of optimum PDX tumor models for follow-up in vivo studies. This highly translational in vitro-in vivo PDX pipeline is expected to reduce attrition and increase efficiency in early drug discovery.

#5772 Developing a patient derived 3D co-culture model of prostate organoids to examine biological mechanisms involved in prostate cancer disparities. Joseph Marsili,1 Zachary Richards,2 Jason Garcia,3 Cindy Voisin,4 Larisa Nunn,5,6 Northeastern Illinois University, Chicago, IL; Northwestern University, Chicago, IL.

The prostate is composed of a fibro-muscular stromal and glandular epithelial cell population. The prostate cell population is essential for normal prostate development and is involved in carcinogenesis. In the past year, an estimated 220,800 new cases of prostate cancer (PCA) were diagnosed and 27,540 died of PCa. PCa in African American men occurs more frequently, develops at a much earlier age, and is more aggressive and lethal compared to men of European origin.

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Descent. Both biological and socioeconomic factors likely contribute to this disparity. There is currently a paucity of relevant preclinical models in which to examine biological mechanisms in African American PCa. Patient-derived organoids are a recently developed in vitro preclinical model which facilitate examination of underling mechanisms of disease etiology. However, current models of epithelial organoid growth lack the prostate stroma. To address this unmet need, we began development of a 3D organoid model that incorporated both primary human prostatic epithelial (PrE) and stromal (PrS) cells to better mimic the microenvironment of the organ. Prostate organoids were derived from cells collected from radical prostatectomy tissues from patients (UICC IRBs: 2006-0679 (PI Behm), (2013-0341(PI Nomm). Mixtures of single PrE-derived and single PrS-derived cells were seeded on matrigel. Bright-field microscopy of live organoids and histological examination of fixed organoids showed that the addition of PrS cells to the culture altered the shape of the organoids compared to the spherical phenotype observed in PrE only cultures. Immunofluorescent analysis of the basal cell marker p63 showed increased epithelial differentiation in the co-culture organoids. In summary, these data suggest that inclusion of prostate stroma in co-culture organoid models influences prostatic epithelial organoid development in vitro. Ongoing studies are focused on molecular analysis or organoid phenotypes and comparison of AA and EA-derived organoids.

**#5773 Automation and miniaturization of 3D tumor models for compound screens.** Michael Kowalski,1 Kayla Hill,2 Vipat Raksakulthai,3 Kristin Prasaukas,4 Tara Jones-Roe1. Beckman Coulter, Indianapolis, IN; 1Molecular Devices, Sunnyvale, CA

Three-dimensional (3D) systems of cell culture can provide a more representative model of solid tumors and more physiologically relevant outputs from drug screens than two-dimensional cell cultures. A diverse array of 3D models have been used to investigate tumor physiology and susceptibility to chemotherapeutics but all of these models present challenges to establish and manipulate at the high throughputs required for screens. Cancer spheroids are large clusters of cancer cells formed in suspension to replicate the gradients of gases, nutrients, and drugs seen by solid tumors. In contrast, Matrigel® or other hydrogel cultures are often used to replicate native cell morphology and polarity or to enable studies of 3D structure formation or cell migration. To overcome manual challenges and improve reliability, we automated the plating, drug treatment, and analysis of these 3D models. We were able to form consistent cancer spheroids in both hanging drop and low attachment plates and used imaging and flow cytometry to gain a more complete understanding of the cytotoxic drug response in this model. Cells were also plated on top of and embedded within Matrigel and cell growth and apoptosis induction was measured with standard and confocal imaging approaches. Both 3D models were miniaturized to 384-well format, thereby enabling the throughput required for large-scale screens to identify novel cancer treatments.

**#5774 Effect of extrinsic and intrinsic solid stress on tumor spheroid growth.** Ludivine Guillaume, Anaïs Desmaison, Bernard Ducommun, Valérie Lobjois. Univ. of Toulouse, Toulouse, France.

Growth-induced solid stress and adaptive response to extrinsic mechanical cues have been recognized as major hurdles to overcome in cancer treatment and any approach that could significantly modulate these parameters would have an impact in improving therapies. An attracting in vitro model to explore this issue is the Multicellular Tumor Spheroid, a 3D culture model reproducing the non-vascularized microtumor environment, proliferation gradient and cellular interactions. To explore and assess spheroid growth-induced solid stress, we couple biological approach to the application of mechanics and modeling. We adapted a method, which consist in partial sectioning of the microtumor and measuring tissue relaxation parameters. Using this strategy, we showed that cell proliferation induced the accumulation of intrinsic mechanical constraint. To investigate the consequence of the accumulation of solid stress at the cell scale, we analyzed 3D cellular organization within spheroids. Using 3D light sheets microscopy (LIS) visualization in 3D optically cleared spheroids combined with 3D reconstruction and automatic algorithms, we were able to determine cell density, nuclear orientation and nuclear shape in whole 3D spheroids. We found that stress accumulation is associated with an increase in cell density and a regionalization of nuclei organization. We also explored the impact of extrinsic stress in 3D culture condition under constraint. We found that mechanical constraint and cytoskeleton control nucleus orientation and using live 3D LSM microscopy we show that mechanical constraint induces an actin cytoskeleton-dependent prometaphase delay. We anticipate that this work will provide key information and parameters to improve our knowledge of the cross-talk between mechanical parameters and tumor growth.

**#5775 A unique 3D tissue-engineered human melanoma model combining lymphatic and blood microvasculature to study cancer cell dissemination.** Jennifer Bourland, Julie Fradette, François A. Auger. Centre de recherche en Organogénèse Expérimentale de l’Université Laval/LOEX, CRCHU de Québec Université Laval, Quebec, Quebec, Canada.

INTRODUCTION: Melanoma is amongst malignancies with constantly increasing incidence in developed countries. One of the underlying causes of death for patients diagnosed with melanoma is metastasis, which can spread through lymphatic or blood vessels. Mechanisms controlling the dissemination path are poorly understood and relevant models for studying metastasis physiopathology are often inadequate. To address this, we hypothesized that the human tumor microenvironment can be mimicked in vitro by combining tissue-engineered microvascularized skin and melanoma microtissues. METHODS: Tissue-engineered skin was produced using primary dermal, epidermal and microvascular endothelial cells by the self-assembly technique without any exogenous biomaterial. Tumor microtissues were produced using the hanging drop method. Six melanoma cell lines were used originating from primary tumor sites (A375, SK-MEL 28 and WM983a) and from metastatic sites (RP11-7951, Malme 3M and WM983b). Tumor development and growth were assessed by histology, immunofluorescence and confocal microscopy, while cytokine secretion profiles were determined by ELISA. WM983a and WM983b models were treated for 11 days with vemurafenib. Response to treatment was assessed by counting the ratio of tumor cells positive for Ki67, representative of the tumor proliferation. RESULTS: We obtained three-dimensional skin microvascular networks: a VE-cadherin+CD31+ blood network, and a PDLP1+LYVE-1+CD31+ lymphatic network. Blood capillaries were thin and highly connected whereas lymphatic capillaries were larger and presented a distinct morphology. Histological analyses revealed tumor microtissue integration at the dermoepidermal junction within the reconstructed skin. The pro-lymphangiogenic factor and tumor-secreted VEGF-C was detected in conditioned medium from the melanoma microtissues (662 pg/ml). Furthermore, CCL21, a chemottractant known to be secreted by lymphatic endothelial cells, displayed secretion levels that were 10-fold higher in microvascularized tissues compared to the non-microvascularized skin (P ≤ 0.001). Both of these cytokines are involved in the cross-talk between tumor cells and capillaries, and thus in tumor dissemination. The 3D melanoma model responded to vemurafenib with up to a 5-fold decrease of tumor cell proliferation and a partial pigmentation of the tumor. CONCLUSION: This unique 3D in vitro melanoma model mimics tumor microenvironment by combining blood and lymphatic capillaries with melanoma microtissues in a reconstructed skin. Being responsive to treatment such as vemurafenib, it represents a valuable tool for studying mechanisms of metastasis and drug response in a fully human cell and matrix microenvironment, and thus testing anti-metastatic compounds could better predict their safety and efficacy.

**#5776 Glioblastoma tumor model to analyze the mechanisms of resistance to tyrosine kinase inhibitors.** Sara Pedron,1 Gabrielle L. Wolter,1 Emily Chen,1 Jann N. Sarkaria,2 Brendan A. Harley,1 1University of Illinois at Urbana-Champaign, Urbana, IL; 2Mayo Clinic, Rochester, MN

The extracellular matrix (ECM) is increasingly recognized as having a key role in cancer development. We have developed a biomaterial tumor mimic that recapitulates systematically the main characteristics and heterogeneities of glioblastoma ECM. With the use of these 3D in vitro platforms we seek to understand the key features that make this cancer so challenging to treat. We are using this device in three different ways: (1) to further understand how particularities of brain tumor ECM affect cancer cells and assist in the diagnostic of different glioblastoma (GBM) tumor types, (2) provide new models for drug screening to afford more personalized therapies and (3) study the mechanisms of resistance to current therapies in order to identify more effective combinatorial treatments and new therapeutic targets. Hyaluronic acid (HA), is the main component of the brain ECM and GBM is associated with aberrant HA secretion and overexpression of receptors associated with HA, such as CD44 and EGFR. We cultured GBM patient-derived xenograft (PDX) cells within gelatin based hydrogels that are composed of HA. Cell proliferation and cell phenotype analyses demonstrate that biomimetic hydrogels support xenograft culture and cells upregulate matrix remodeling genes and others related to tumor growth in response to matrix biophysical properties. We also used these platforms to evaluate the mechanisms of cell resistance to tyrosine kinase inhibitors. For this purpose, we first focused on the epidermal growth factor receptor (EGFR), which has been identified as a molecular target and associated with worse clinical outcomes. We characterized the 3D in vitro behavior of 3 PDX that represent these EGFR variants: GBM10 (EGFR, wild type), GBM12 (EGFR+) and GBM6 (EGFRVIII). We studied the relationship between the HA contained in the surrounding matrix and response of GBM cells to a tyrosine kinase inhibitor (TKI),...
erlotinib. Results indicate that while EGFR+ cells are sensitive to TKI in HA hydrogels, HA seems to collaborate with EGFR/III signaling to stir cell activity. Immunoblots demonstrate that this enhanced cell activity is related to a significant increase in PDGFR concentration. Blockade of the CD44 receptor, in combination with erlotinib treatment, does not affect phosphorylation rates of PI3K or ERK. Yet, this suggests that this biotactic tumor model can be used as a valuable tool in the mechanistic studies of tumor development and prediction of tyrosine kinase inhibitors efficacy.

Finally, we show that, patient-derived xenograft cells resistant to erlotinib in vivo (GBM10), become sensitive when CD44 is blocked in HA-containing matrices, as demonstrated by a decrease of phosphorylated PI3K. In summary, we highlight the importance of extracellular HA in EGFR inhibition efficiency. We demonstrate that this biomaterial tumor model can be used as a valuable tool in the mechanistic studies of tumor development and prediction of tyrosine kinase inhibitors efficacy.

#5777 Translation of HPV mediated immortalization to cancer precision medicine. Aleksandra Dakic, Nancy Palechor-Ceron, Ewa Krawczyk, Hang Yuan, Frank Suprynowicz, Seema Agarwal, Richard Schlegel, Vera Simic, Prathibha Sripadhan, Chen Chen, Jie Lu, Tung-Wei Hou, Sujata Choudhury, Xuefeng Liu. Georgetown Univ. School of Medicine, Washington, DC.

The E6/E7 oncogenes of the high-risk HPV’s are both necessary and sufficient to immortalize HFKs and their presence and expression is required for the continued proliferation of HPV-positive cervical cancer cells. We and others have shown previously that hTERT induced by E6 and cytoskeleton alteration by E7 are critical. Both E6 and feeder cells activate telomerase, while both E7 and Rock inhibitors (Y-27632) disrupt the actin cytoskeleton and inactivate Rho. Feeder and Y-27632 to induce unlimited cell proliferation of human keratinocytes. Unexpectedly, we observed that feeders and Y-27632 could be used to establish both normal and tumor cell cultures from non-keratinocyte tissues. This culture has been termed as “conditional reprogramming”, since CR cultures stop proliferating or terminally differentiate after its removal, depending on culture conditions. The combination of CR and Organoids (Matrigel, air-liquid interface (ALI)) cultures represents next generation human cancer models and functional diagnostics for cancer precision medicine as described in August 2015 in the NCI precision medicine initiative and three nature review articles (Nat Rev Cancer. 2015 Dec; Nat Rev Genet. 2015 Jul; Nat Rev Clin Oncol. 2014 Nov.). The technique is relatively simple and has been reproduced in more than 50 laboratories (including an original and independent article in Science from Massachusetts General Hospital) (Science. 2014, 346(6216):1480-6.). Importantly, the CR technology can generate 2x10^6–6cells in a week from small biopsies, and can generate cultures from cryopreserved tissue and small biopsies. We therefore initiated studies to examine whether CR cultures reflected the biology and genotype of the original tumor and whether cultures might be used to predict clinical responses. Moreover, the epithelial cells can be propagated indefinitely in vitro, yet retain the capacity to become fully differentiated when placed into conditions that mimic their natural environment. Thus, the CR method significantly advances applications for regenerative medicine.

#5778 A three-dimensional RAFT™ co-culture as advanced model for breast cancer drug discovery. Ying Nie, Krista L. Garner, Theresa D’Souza, Lonza Walkersville Inc., Walkersville, MD.

High-throughput screening (HTS) using two-dimensional (2D) cell culture models (2D HTS) is essential for rapid identification of drug candidates from chemical libraries. However, it often results in a large number of poorly qualified leads that exert extra burden on the downstream, preclinical animal studies and that cannot be translated into clinical success, because 2D culture cannot represent the complexity of the tumor microenvironment in vivo. The progression of tumors and their response to drugs in vivo are regulated by their interactions with neighboring cells, and the natural gradients of nutrients, cytokines, wastes, oxygen, which can be better mimicked with three-dimensional (3D) cell–culture models. We constructed a breast cancer model using the RAFT™ 3D Cell Culture System. The essential component of this system is a collagen matrix condensed to the physiologically-relevant collagen density by removing the majority of the liquid from the collagen hydrogel with specialized absorbers. Our 3D model is a co-culture of human mammary fibroblasts (HMFs), embedded in the RAFT™ Collagen matrix, and the MCF7 human breast cancer epithelial cells, overlaying on top of the matrix, to model the interaction of breast cancer cells and stromal cells in vivo. As non-tumorulent control, human mammary epithelial cells (nHMECs), isolated from normal breast tissue, were used to replace MCF7 cells in such a co-culture. A medium formulation combining fibroblast medium FGM2™ and mammary epithelial cell medium MEGM™ was optimized to grow both cell types in the co-culture. The RAFT™ co-cultures were stained with standard immunocytochemistry protocol to reveal the morphology of the cells. After three days in culture, the HMFs, evenly interspersed in the matrix, fully stretched out on the collagen matrix, and the nHMECs grew to reach above 90% confluence on the collagen matrix. The cell proliferation in the co-culture was quantified with the ViaLight™ Plus Cell Proliferation and Cytotoxicity BioAssay, which measures cell viability with bioluminescent detection of cellular proliferation and vital dye based on the Raman signature of single cells. We demonstrate here the feasibility of co-culturing HMFs and mammary epithelial cells, in the RAFT™ System, as a 3D breast cancer model. The efficacy of anti-cancer drugs can be assessed by measuring the cell viability with the ViaLight™ Assay. This enables us to bridge the gap between 2D HTS and pre-clinical animal studies. It may provide physiologically-relevant data and better prediction of the in vivo efficacy and dosage of the drug candidates identified in HTS, and reduce the burden of animal studies in breast cancer drug discovery.

#5779 Building a novel 3D hyaluronan-based tri-culture model of the bone metastatic microenvironment for cancer studies. Lindsey K. Sablatura, Daniel A. Harrington, Mary C. Farach-Carson, Rice University, Houston, TX; University of Texas Health Science Center at Houston School of Dentistry, Houston, TX.

The high attrition rate of potential anti-cancer drugs entering clinical trials predicates a need for more predictive pre-clinical model systems. Three-dimensional (3D) culture systems provide cells a more physiological microenvironment than traditional cell culture methods—including relevant extracellular matrix (ECM) cues and cell-cell interactions while allowing for experimental control and higher-throughput studies than animal models. Prevalent cancers, including breast and prostate cancer, metastasize preferentially to bone, where they become incurable. The bone marrow microenvironment is a complex network of interacting bone homeostatic and hematopoietic niches that contribute to osteotropic cancer homing, proliferation, and treatment resistance. We created a 3D in vitro tri-culture model of the bone metastatic microenvironment to serve as a new platform to study the “trialogue” between bone metastatic cancer, bone marrow stroma, and bone marrow endothelial cells. This tool can be used to inform drug discovery and to combat treatment resistance. Within the present study, the utility of peptide-functionalized hyaluronan hydrogels for supporting the self-organization of 3D bone marrow microvascular networks was explored. Gel crosslinking density was varied to examine the effect of matrix porosity on cell migration and organization over time. Tubule length and diameter were quantified and the endothelial structures were assessed for indicators of mature vasculature, including lumen formation, basement membrane production, and the recruitment of supporting stromal cells. Breast/prostate cancer cell growth, survival, and physical association with bone marrow stromal and bone marrow microvascular endothelial cells was assessed in tri-culture models. Ongoing studies seek to identify and disrupt key cell signaling pathways that mediate cell–cell interaction and promote cancer cell proliferation and survival, providing new tools to combat bone metastatic disease.

#5780 3D ex-vivo assay platform using primary lung cancer cells in malignant pleural effusions as predictor for clinical outcome of personalized chemotherapy. Cheng-guang Wu, Francesca Chiovaro, Tamara Tanos, Alex Soltermann, Sumeer Dhar, University of Zurich, Zurich, Switzerland; In-Sphero AG, Zurich, Switzerland.

Background: Despite advances in therapeutic programs to treat various cancer types, dismal overall response rates for several entities has posed dilemma for oncologists and researchers alike. Therefore, there is immense need in accelerating therapeutic programs towards clinical success in cancer patients. It is well established that patients suffering from same cancer type may respond very differently to a given chemotherapeutic regimen. We propose the development of a unique patient-derived 3D ex-vivo drug testing platform as a valid decision making tool for 2D or 3D line treatment regimens. Materials and Methods: In this ex-vivo platform freshly collected malignant pleural effusions from patients were processed for cytological diagnosis on cell blocks, using respective immune-histochemical markers. Effusions were prepared for a 96 well based 3D ex-vivo assay format using the hanging drop method and a parallel 2D cell culture format. Subsequently, we compared the original cell composition of the malignant effusion with respective microtumors generated in the 3D format. Microtumors were then breast embedded in paraffin and processed like original cell blocks. IHC including respective markers for tumour cells such as TTF1, CD2 and oestrogen receptor and for non-tumour cellular fractions like calretinin, CD45, and MPO were processed. Results: The microtumors generated (ranging from 300 to 500μm) retained the native tumor morphology and cellular com-
position, thereby presenting tumour microenvironment like conditions in this ex vivo system. These cultures contain all cellular components of a malignant effusion at the beginning. Next to cancer cells, mesothelial cells, lymphocytes and granulocytes were main constituents. Cellular ratios were measured by computerized image analysis. Both cell sediments and supernatants are amenable to profiling strategies by next generation sequencing and mass spectrometry.

Conclusion: Our model presents an optimal condition to conduct chemosensitivity/resistance profiling in individual cancer patients using standard drug combinations. Furthermore, we are currently developing an immune competent 3D model to access cancer cell interaction with surrounding immune cells. We expect that original immune cells will be quenched out during culture, thus these micro-environments are thus created, easily transplanted method to perform high-throughput drug screenings. In 3D organoids and PDTo and demonstrate its utility in determining drug sensitivities and optimizing multi-drug combinations in vitro.

#5781 Establishment of a 3D ex vivo assay as a preclinical drug testing platform for personalized cancer therapy

Sumeer Dharia,1 Francesca Chiovoro,2 Tamara Tanos,3 Tomas Hejhal,1 Seife Heilemariam,1 Jens Kelm,1 Anja Irmisch,1 Mitchell Levesque1.

In vitro cell based drug testing tools have been widely used in drug discovery and early development to evaluate novel drug entities for further evaluation in preclinical in vivo models. However, a poor correspondence with in vivo models has compelled oncologists to pursue complementary in vitro strategies with better outcomes. Within this realm, a 3D ex vivo platform is being extensively used to evaluate efficacy of cytotoxic drugs, targeted molecules and antibodies. To increase the translational value of this model, maintenance of the tumor microenvironment (immune and other stromal cells) is highly pertinent. We have established a 3D ex vivo assay as a patient drug testing platform for personalized medicine therapies in collaboration with our clinical partners. In this study we setup the protocols for obtaining single cell suspensions from biopsy and/or tumor resection samples from patients presenting with Urothelial cancers, Renal cell carcinoma, Pancreatic, NSCLC, Ovarian, and Melanomas to generate 3D microtumors in 96 well format cultured over a period of 7-15 days. The microtumors were monitored for growth characteristics, biomarker phenotype and drug activity profile. As expected the growth characteristics for each tumor varied, corresponding very well with clinical disease progression. Subsequently, these microtumors were assessed using a standard clinical immunohistochemistry diagnostics approach to evaluate disease specific biomarkers and were compared to the clinical diagnostics profile of the patients. The biomarker profile from 3D derived tumor samples showed concordance with the patient diagnostics profile. In the instances where clinical information and treatment regimens were available, the microtumors were tested and followed with single and pairwise drug treatments. The ex vivo 3D treatments clearly reflected the clinical outcome. Currently, this platform is being used to develop 3D ex vivo immune-competent models to study immunomodulatory therapies. Based on the overall data we conclude that the 3D ex vivo assay system offers a highly pertinent platform to perform chemosensitivity testing providing predictive information on the clinical outcome, which enables oncologists to redefined individualized chemosensitive targeted treatments.

#5782 A novel method for high-throughput drug screening in 3D tumor organoids

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3D tumor organoids are pivotal tools to study tumor development, propagation and invasive properties (Tanner and Gottesman, 2015). They closely recapitulate many characteristics of the tumor of origin in terms of structure, heterogeneity, complexity and response to drug treatment. As such, they are considered an accurate pre-clinical model for drug discovery and development (Nyga et al, 2011; Tanner and Gottesman, 2015; Shroyer, 2016). Nevertheless, reliable methods to perform high-throughput drug screenings using tumor organoids are limited and require extensive manipulation such as sample transfer from plate to plate. In addition, thick 3D matrix supports may hinder drug penetration. Here we introduce a robust method to generate, treat and assay 3D tumor organoids in standard, uncoated 96- or 384-well plates. By plating cells around the rim of the wells we generate an ultrathin layer of Matrigel that allows penetration of small molecules and bulkier peptide drugs. Media change is easily performed from the center of the well without perturbing the organoids. After a 2-day treatment course, organoids are released by dispase treatment and assayed in the same well by performing a CellTiter-Glo assay (Promega). The method exhibits low plate-to-plate, experiment-to-experiment and operator-to-operator variability. In addition, it can be extended to patient-derived tumor organoids (PDTo) generated from primary tumors. We show how PDTo from high-grade serous ovarian carcinoma (HGSOC) specimens are generated with a success rate >95%. Given the low number of cells required and high assay sensitivity, we can seed 200 wells from 1Mio cells derived from a tumor biopsy, and test over 60 different drugs/concentrations in triplicates. Moreover, we show how drug combination studies are feasible and allow assessing treatment synergies. As an example we perform a peptidic inhibitor of p53 and an EGFR inhibitor, ReAPc53 (Soragni et al, 2016), with carboplatin in PDTo HGSOC established from a panel of patients harboring different p53 mutations. Drug interaction studies are performed across a range of ReAPc53 and carboplatin concentrations and cell viability assessed at day 2 is used to compute drug-response curves, EC50 values and combination indexes. In conclusion, we are introducing an ex vivo treatment and easy to implement method to perform high-throughput drug screenings in 3D organoids and PDTo and demonstrate its utility in determining drug sensitivities and optimizing multi-drug combinations in vitro.

#5783 In vitro modeling of patient derived bladder cancer cell lines in 3D culture systems

May Ellbana,1 Sreenivasulu Chintala,2 Eric Ciampi,3,4 Remi Adelayie,1 Ashley Orillion,1 Sreevani Arisa,5 Nur Damayanti,1 Michelle Grimmer,1 TJ Puls,1 Sherry Harbin,1 Melissa Fishel,1 Roberto Pili,1 Indiana University School of Medicine, Indianapolis, IN, 1,2,3,4,5The Janssen Pharmaceutical Companies of Johnson & Johnson, Antwerp, Belgium; 3Purdue University, West Lafayette, IN.

Background: Drug screening is a key component for drug development and optimizing anti-tumor therapies. Traditionally, in vitro drug testing has been conducted in monolayer systems that are not capable of recapitulating the tumor microenvironment. Recently, the field has witnessed the rise of interest in developing 3D culture systems which are capable of reproducing tumor complexity while circumventing the cost associated with in vivo drug testing. Our access to fresh patient samples has enabled us to establish a novel 3D culture system consisting of bladder cancer patient derived cell lines. Using a wide range of matrices and co-culture conditions with tumor associated stromal cells we were able to establish a unique high throughput drug testing tool. Methods: Matrigel and collagen based matrices were used to establish 3D culture systems of bladder cancer patient derived cells. Tumor cells were cultured in 3D conditions either alone or in coculture with tumor associated stromal cells. Response to Cisplatin and PI3K pathway targeted agents (i.e. LY2900454) was tested in both conditions. High throughput imaging via Thermo ArrayScan XTI was used to assess the biological behavior of spheroids as well as their response to therapies overtime. Confocal microscopy was used to validate the biological mimicry of tumor derived spheroids to the original patient tumors. Integration of RNA-seq data from the patient-derived tumor cells with the biological behavior and therapeutic response in 3D culture is ongoing for the purpose of characterizing the 3D model Results: In 3D culture conditions; bladder cancer derived cells were able to re-express E-cadherin that was suppressed upon propagation in monolayer. The re-expression of the epithelial marker (E-cadherin) observed in 3D accurately mirrors the original tumors; which are of epithelial origin. Phenotypic differences were observed across different matrix conditions and also among different tumor derived cells. Bladder 3D organoids of luminal origin were more sensitive to both cisplatin and PI3K pathway inhibitors as compared to those of basal origin. This drug response profile was remarkably consistent of what we observed with our patient derived xenograft (PDx) models derived from the same tumors. The phenotypic as well as the drug response variations observed in our 3D culture correlated with variable gene expression profiles (luminal vs basal) that were detected in our RNA-seq data. Conclusion: As compared to monolayer, 3D culture is more capable of recapitulating tumor complexity and accurately reflects the drug resistance / sensitivity profiles that are observed in PDX models in vivo. Therefore, a 3D culture system provides an invaluable tool for high throughput screening of drugs in bladder cancer and providing a better understanding of tumor biology in the search of more effective treatments for bladder cancer patients.

#5785 Characterization of a novel microfluidic in vitro model of the blood-tumor and blood-brain barrier

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Background: There is a need for a cost-effective and reliable in vitro model of the blood-tumor barrier (BBB) and blood-brain barrier (BBT) for primary and metastatic lesions in brain. Current models do not accurately recapitulate physiological conditions and are unreliable with up to a 100-fold variance in predicting drug permeability into brain tumors. Methods: Human umbilical vein endothelial cells (HUVECs) were cultured with either CTX-TRD2 rat astrocytes (BBB) or Met-1 murine breast cancer cells (BBT) in a microfluidic chip, which contained chambers separated by a porous wall. Shear stress was induced on HUVECs by perfusion of media through the apical chamber, and permeability was characterized by the diffusion of fluorescent tracers, one of which is subject
to P-gp efflux. Data was compared to in-vivo work. Results: Permeability of free Texas Red was significantly higher (p < 0.05) in BTB chips (Kp = 13.1 ± 1.3 X 10⁻⁴) than in the BBB chips (Kp = 2.5 ± 0.3 X 10⁻¹). There was a similar trend observed in larger markers, but these were not significantly different. The movement of Rhodamine 123 was also restricted, indicating functional P-gp efflux in vitro, similar to data published in vivo studies. In the BBB model, diffusion of Rh123 increased 14-fold in the presence of verapamil (Kp = 14.7 ± 7.5 X 10⁻⁴) and eight fold with the addition of Cyclosporine A (Kp = 8.8 ± 1.8 X 10⁻⁴). In the BTB model, this increase was also observed, but to a lesser extent, as only 3 and 2-fold increases in permeability were observed, respectively. The magnitude of permeability changes were not significantly different from in-vivo models in both parameters, indicating active efflux phenomena. Conclusion: The novel microfluidic chip is a cost-effective in vitro model which recapitulates the BTB and BBB physiology and can predict permeability more reliably than current in-vitro models.


Patient derived organoids are becoming a popular 3D in vitro model that more accurately recapitulate in vivo conditions compared to standard 2D culture systems. Organoids consist of epithelial cells and lack stroma and mesenchyme, allowing cells of interest to be studied as an isolated system. However, the tunability of the organoid model also permits the re-addition of microenvironmental factors, i.e. cancer associated fibroblasts (CAFs), resulting in a wide range of experimental capabilities. Organoids are easily scalable, making them more efficient and cost-effective than traditional animal models. Despite the popularity of animal models for pre-clinical drug testing, there still remain issues with translating results from animal testing to patient outcomes; even patient-derived xenograft models experience non-physiological mouse-human interactions. Therefore, studying the impact of microenvironmental perturbations, such as the presence of CAFs, or nutrient and drug gradients, on organoids may reduce translational error. Using our biorepository of patient derived colon cancer (primary- and liver metastases) organoids, we can observe changes in tumor architecture and cell growth or death, allowing us to more accurately assess the efficacy of drug therapies and observe how interpatient heterogeneity impacts their efficacy. Here we focus on the histopathology of different patient derived organoids to quantitatively study cell growth or death under drug treatments (+/- CAFs) which could ultimately become a standard procedure for drug screening. Here we investigate the effects of CAF co-culture in patient derived organoids in response to standard chemotherapies such as Irinotecan and Oxaliplatin. Following treatment, the organoids are paraffin fixed to retain their structural integrity, then sectioned. The samples are stained with H&E to show the tumor architecture, and immunofluorescent antibodies (IF) to identify cells that are proliferating (Ki67) or apoptotic (TUNEL). As a result, we are able to investigate how tumor microenvironmental factors affect the tumor architecture of an individual patient tumor. We also examine the cytotoxic or cytostatic effects of standard chemotherapies by quantitating the number of proliferative and apoptotic cells, as well as their location within the tumor, in response to the presence of both CAFs and therapy. Ultimately, we envision the method outlined here will further personalize medicine by allowing physicians to more rapidly assess an individual patient’s response to therapy and adjust treatment accordingly. Additionally, this method could prove to be a more inexpensive and faster means of screening new drug compounds. By creating a system that more closely recapitulates patient outcomes, we hope to move therapies forwards faster approval while still retaining results representative of in vivo outcomes.


The OncoPanel™ service has established 100 genomically-characterized human tumor cell lines as targets for the screening of compounds in 3D spheroid models. We sought to (1) expand the cell line capability of the service and (2) validate the cell lines in a long-term (10-day) assay. For the cell line expansion, cells were plated at two densities in 384-well spheroid-promoting plates, and were incubated for 3 days to allow formation of spheroids. The growth media were then removed and replaced with media containing the hypoxia probe, Lox-1. Twenty-four hours later, the ability to form spheroids was determined by bright-field, high-content imaging, and fluorescent detection of the Lox-1 probe in the hypoxic core of the spheroids. Cells were then seeded in assay plates at pre-determined densities, along with a time zero (T₀) plate, and were incubated for 4 days to allow spheroid formation. Spheroid formation was verified by bright-field, high-content imaging, as previously mentioned. The growth media were removed and replaced with fresh media, followed by the addition of staurosporine over a range of 10 concentrations, using acoustic liquid transfer. At 2 days post-stimulation, the cell lines were re-plated with fresh media, for each cell line to pass quality control. Based on our tests of spheroid formation in the expansion cell lines, we have added 36 cell lines to the spheroid- or loose aggregate-forming cell line panel, with 20 cell lines validated in the long-term assay. Through the use of high-throughput screening and high-content imaging, we can verify and validate spheroid formation and growth in a 384-well format, with which we can test agents for drug discovery projects and provide value for potentially greater therapeutic outcomes.

#5788 Development of cellular morphology-based separation system for three-dimensional culture. Hirofumi Matsui,1 Shinji Sugiuira,2 Masato Tamura,3 Toshiyuki Kamonari,4 Toshiyuki Takagi,5 Taku Satou,4 Ryuji Kato,6 Kei Kanie,5 Mayu Shibuta5. 1University of Tsukuba, Tsukuba, Japan; 2National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan; 3Nagoya University, Nagoya, Japan.

The present study includes proof concept of morphology-based cell separation and development of automatic cell separation system. 3D culture environment with a specific extracellular matrix regulates cellular function and phenotype. In addition, cancer cell morphology changes depending on its malignancy in 3D culture environment. The cell separation system in 3D culture environment should need to obtain the cells according to its morphology, which includes cell phenotypes. Recently, we developed gelatin-based photodegradable hydrogels, and applied this hydrogels to optical cell separation. The target cells in the photodegradable hydrogels were successfully separated by the optical cell separation, the separated cells was growth on another dish. On the other hand, we recently developed the predication model of stem cell differentiation by image analysis. The image analysis technique and the photodegradable gelatin hydrogels are included in the automated morphology-based cell separation system in the 3D culture environment. For forming cell encapsulated-photodegradable hydrogels, suspension of cells including heterogeneous population is mixed with pregel solutions and cells are encapsulated in the gelatin-based photodegradable hydrogels. After the culture in 3D environment, microscopic images of the 6 cells are captured. The captured images are analyzed to distinguish the target cells from the other cells by using the image analysis algorithm, which we previously developed for analyzing stem cells. The hydrogels around the target area is irradiated with light (365nm). The cells in the irradiated area are collected by automated pipetting system. We developed automated system for this optical cell separation procedure, including cultivation, image acquisition, image analysis, light irradiation, and pipetting for cell collection. We demonstrated automated optical cell separation using the model culture system. Normal gastric mucosal cells were cultured in the photodegradable hydrogels. After cultivation for 1 week, the cells were irradiated the light for 5 to 20 min. The cells in the irradiated area were collected by automated pipetting and transferred into a collection dish. The collected cells were viable and attached in the collection dish after collection. We are currently developing an automated image analysis algorithm to distinguish cancer cells from normal cells under 3D environment. The automated optical cell separation system with image analysis algorithm will be applied to the establishment of novel cancer-cell lines from clinical samples such as biopsy tissue.


Interaction between cancer cells and stromal components in the tumor microenvironment is well known for their significant roles in tumor progression and subsequent treatment failure. Hepatic stellate cells (HSCs), as a predominant cell type in the microenvironment of hepatocellular carcinoma (HCC), are involved in creating desmoplastic and chemoresistance-inducing microenvironment. HSCs secrete various paracrine factors that modify the HCC tumor

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microenvironment leading to tumor growth, drug resistance and metastasis via promoting epithelial-to-mesenchymal transition (EMT). Although several studies using 2D co-culture system have shown bidirectional cross-talk between cancer cells and HSCs, data obtained remain limited in their clinical relevance due to lack of in vivo tumor-like characteristics. We developed a mixed-cell spheroid model which recapitulates direct 3D tumor-HSC interactions in parallel and contact-mediated manners. Huh-7, human HCC cells, were mixed co-cultured with LX-2 cells, immortalized human HSC at 1:3 ratio using liquid overlay technique in 96-well plates. Electron microscopy was used to examine subcellular structural changes. Cellular distribution within spheroids was observed by labeling LX-2 cells with fluorescent tracer. The expression of pro-fibrotic and EMT markers was detected by immunofluorescence staining on paraffin embedded sections. Invasion ability was served by labeling LX-2 cells with fluorescent tracer. The expression of pro-fibrotic and EMT markers was detected by immunohistochemistry or immunosubstitutes, after which their development was followed by fluorescence microscopy. RESULTS: Both of the cancer cell lines used were able to form compact spheroids and grow on bladder equivalents. The invasive behaviour of spheroids varied depending on the nature of the cells used. The non-invasive RT4 cell line was unable to cross the basal lamina whereas the invasive T24 cell line was able to do so. CONCLUSION: The establishment of such a model for studying cancer biology in a physiological environment will help bridge the gap between overly simple cell culture models and more complex transgenic mice models. This new model offers a unique opportunity to study separately the players involved in the development of BCA and thus represents a powerful tool for the mechanistic analysis of this complex pathology.

#5790 Assessing the effects of TIMP2 knockout on lung cancer cell lines cultured in 3D. David Feeney. NCI, Bethesda, MD.

Tissue inhibitor of matrix metalloproteinases (TIMPs) are a small family of endogenous proteins that classically function to inhibit metalloproteinase activity. Since the original description of this protein family in the 80s and 90s, various MMP-independent biological functions of TIMPs have been described. This built the impression that MMP/TIMP ratios may play an important role in tissue homeostasis, an idea which is supported by the observation that altered MMP/TIMP expression ratios are often associated with a number of human conditions such as cancer, cardiovascular and CNS disease. TIMP2 is the most abundantly expressed protein in this family and has previously been shown to interact with several membrane proteins including MT1-MMP, insulin-like growth factor-1 receptor (IGF-1-R) and alphabeta3 integrin (αβ3) to mediate downstream signaling. In addition, TIMP2 has been shown to inhibit growth factor stimulated proliferation, angiogenesis and tumor cell invasion and metastasis, highlighting the potential for TIMP2-based cancer bio-therapies that can be used in conjunction with conventional treatments. Recent studies in our lab highlight that syngeneic lung tumors (LL2 cells; Lewis lung carcinoma) developed in C57BL mice harboring a loss-of-function mutation in TIMP2 are significantly larger than tumors grown in their WT counterparts. To gain a deeper understanding of the role of TIMP2 in tumor initiation and progression we have used CRISPR-Cas9 to develop stable TIMP2 knockout (T2KO) human lung cancer cell lines. Although indistinguishable in 2D culture, T2KO tumor cells display a morphologically distinct phenotype when grown in spheroids. Preliminary data show that, when grown in spheroids, T2KO cells exhibit enhanced EGFR activation in comparison to WT cells. By assessing the functional characteristics and gene expression of T2KO cells grown in 3D culture conditions we hope to gain further insight into the biological functions of TIMP2 and to provide a mechanistic link between the loss of TIMP2 activity and enhanced tumor formation that is observed in our mouse model.

#5791 3D tissue engineering bladder model for cancer invasion study. Cassandra Ringuette Goulet, Geneviève Bernard, Stéphane Chabaud, Frédéric Pouliot, Stéphane Bolduc. Université Laval, Quebec, Quebec, Canada.

INTRODUCTION: Our understanding of the biological processes involved in bladder cancer (BCa) is greatly limited by the models currently available. In fact, the combination of in vitro and in vivo models of BCa has failed to elucidate all the fundamental aspects of the disease. The eighth most commonly diagnosed cancer in Western societies, BCa has become a growing public health concern, and more realistic models are needed to reveal the mechanisms involved in tumor initiation and progression. METHODS: Bladder substitutes have been constructed by tissue engineering with healthy human fibroblasts and urothelial cells, using the self-assembly method. Meanwhile, spheroids have been produced from non-invasive (RT4) and invasive (T24) BCa cell lines expressing DsRed fluorescent protein. The invasive potential of these spheroids was characterized in a type-I collagen gel invasion assay. Following this, the invasion spheroid model was used to investigate the effect of TGF-beta on cell invasion. CONCLUSION: The establishment of such a model for studying cancer biology in a physiological environment will help bridge the gap between overly simple cell culture models and more complex transgenic mice models. This new model offers a unique opportunity to study separately the players involved in the development of BCA and thus represents a powerful tool for the mechanistic analysis of this complex pathology.

#5792 Development of cell line derived organoids to evaluate stroma targeted therapies in pancreatic cancer. Bradley Hall,1 Bindu Santhamma,2 Andrew Cannon,1 Rakesh Bhatia,1 Sushil Kumar,1 Chandranakarathe,1 Harree Nair,2 Klaus Nickisch,3 Surinder Batra.1 University of Nebraska Medical Center, Omaha, NE; 2Evestra, Inc, San Antonio, TX.

Background: Most solid tumors have extensive stroma that not only facilitates the tumor progression but also impedes the delivery of the chemotherapeutic agents. Due to lack of any in-vitro system, presently it is difficult to evaluate any stroma-targeted therapies. Therefore, we developed an organoid system using labeled pancreatic cancer and stellate cell lines. Methods: Murine (FC 1295 and imPSCc-2) cell lines cultured in different combinations were grown as an organoid system using matrigel. The organoids, starting day four were treated with either gemcitabine or EC359, a novel mifepristone derived steroidcytotoxic agent that targets stroma, or both in combination. qRT-PCR analysis of activated stroma signature genes was performed on the mRNA isolated from different treatment groups. H&E, immunohistochemistry, and western blot analysis were performed to further validate our findings. Results: Histologically, these cell line derived organoids develop ductal structures surrounded by fibroblast as seen in the pancreatic tumors. Immunohistochemistry demonstrated elevated level in the mixed-cellspheroids compared to that of cancer cell-alone invasion. CTGF showed similar patterns of distribution to that of LX-2 cells, but at an increased level. CONCLUSION: The establishment of such a model for studying cancer biology in a physiological environment will help bridge the gap between overly simple cell culture models and more complex transgenic mice models. This new model offers a unique opportunity to study separately the players involved in the development of BCA and thus represents a powerful tool for the mechanistic analysis of this complex pathology.

#5793 Comparison of EMT biomarker expression in 2D monolayer and 3D spheroid cultures in a prostate cancer cell model. Jan Carlstrom, Jeanne M. Hintermender, Lindsay Nelson, Stephen Hurt, PerkinElmer, Hopkinton, MA.

The purpose of this study was to examine the induction of epithelial to mesenchymal transition (EMT) in a prostate cancer cell line by measuring classical biomarker expression in three-dimensional (3D) spheroid cultures compared to traditional 2D monolayers in an effort to develop a more biologically relevant assay. Using Ultra-Low Attachment (ULA) microplates, we grew spheroids from a human prostate cancer cell line (DU 145). Numerous studies have implicated a role for EMT in carcinoma invasion and metastasis. EMT is characterized by rearrangement of the extracellular matrix (ECM) and differential regulation of ECM proteins. We induced EMT using TGF-beta and phorbol-12-myristate-13-acetate (PMA) and compared expression levels of specific biomarkers, such as E-cadherin, fibronectin, and IL-6, using AlphaLISA and LANCE (TR-FRET) assay technologies. We confirmed that treatment of DU 145 cells with TGF-beta is sufficient for inducing changes in both EMT biomarker expression and characteristic cellular morphology in monolayer cultures. However, in 3D spheroid cultures, we observed only a partial EMT response to the same TGF-beta treatment as demonstrated by changes in the expected bio-
marker expression pattern. Using the small molecule, PMA, we see significant differences in the levels of IL-6 secretion after EMT induction between cells grown in monolayer and those grown in spheroids. Cellular proliferation, growth and vitality were assessed using ATPFite luminescence assays and confocal microscopy of live-stained cells with a high content imaging system. Though we observed increased proliferation in monolayer cultures compared to 3D spheroids, the changes observed in protein expression patterns cannot be sufficiently explained by differences in cell number or viability. These data illustrate the differences in protein expression levels and in cellular tolerance for compound treatment between a human prostate cancer cell line grown in monolayers and those same cells grown in 3D spheroids.

TUMOR BIOLOGY: Effects of Tumor-Microenvironment Cross-talk on Metastasis

#5794 TrkB-mediated signaling contributes to malignant phenotypes of gallbladder cancer. Makoto Kawamoto, Hideya Onishi, Keigo Ozono, Akio Yamasaki, Akira Imazuymi, Masafumi Nakamura. Kyushu University, Fukuoka, Japan.

Background: Brain derived neurotrophic factor (BDNF)/ Tropomyosin-related kinase B (TrkB) signaling has been shown to be associated with aggressive phenotype in some cancers. However, the contribution of BDNF/TrkB signaling to gallbladder cancer (GBC), one of refractory malignancies, still remains unclear. This study aims to analyze the biological significance of BDNF/TrkB signaling in GBC. Methods: 1) Clinical experiment: 69 patients with primary GBC who underwent curative surgical resection were enrolled in this study. We investigated TrkB expression by immunohistochemistry and analyzed the correlation between TrkB expression and clinicopathological findings. 2) In vitro experiment: BDNF/TrkB signaling was inhibited using 1TRDA or siRNA, and was activated by recombinant human BDNF (rhBDNF). Then, whether BDNF/TrkB signaling contributes to the biological function was estimated by proliferation assay and Matrigel invasion assay, using TrkB-expressing 5 GBC cell lines (NOZ, TGBB2TK, DBs15, TYGBK-1, TYGBK-8). Furthermore, we determined the mechanisms of invasion in terms of epithelial mesenchymal transition (EMT) and matrix metalloproteinases (MMPs). We also examined the relationship between TrkB expression and the level of vascular endothelial growth factors (VEGFs) and hypoxia-inducible factor-1α (HIF-1α) in GBC. 3) In vivo experiment: Tumorigenesis and tumor growth of TrkB siRNA-transfected GBC cells were analyzed, using xenograft mice model. Results: 1) Clinical results; TrkB expression was detected in 63 (91.3%) GBC specimens. TrkB expression in the invasive front correlated with T factor (p=0.0391) and clinical staging (p=0.0391). Overall survival was lower in patients with high TrkB expression in the invasive front than in those with low TrkB expression (p=0.0363). 2) In vitro results; Proliferation was unaffected by rhBDNF treatment; however, K252a treatment and TrkB siRNA transfection decreased proliferation. rhBDNF treatment increased invasiveness by inducing EMT and activating MMP-2/MMP-9, whereas K252a treatment abrogated these effects. TrkB or BDNF siRNA transfection suppressed invasiveness. TrkB siRNA transfection decreased HIF-1α, VEGF-A, and VEGF-C/D expressions. 3) In vivo results; TrkB siRNA transfection decreased tumorigenicity and tumor growth in NOZ and TYGBK-1. Conclusion: These findings demonstrate that TrkB-mediated signaling contributes to the induction of malignant phenotypes (proliferation, invasiveness, angiogenesis, lymphangiogenesis, and tumorigenesis) in GBC.

#5795 Loss of CDCP1 in patient prostate cancer metastasis leads to uncoupling of beta-1 integrin from its cytosplasmic signaling through FAK. Sara G. Pollan,1 Fangjin Huang,1 Joshua M. Lang,2 Jamie M. Sperger,1 Kavita Shah,3

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model system of CTCs. In this system, CDCP1 silenced cells exhibit 3-fold higher proliferation, 4-fold greater anchorage independent growth with colonies exceeding 5 microns in diameter, and a 2-fold reduction in migration ability. In 10% human plasma, these cells up-regulate p-FAK, p-SRC, p-AKT and p-MAPK expression, and at the same time loose expression of activated β1-integrin. The loss of β1-integrin occurs through prevention of TALIN phosphorylation. Upon loss of CDCP1, Talin is no longer phosphorylated by CDK5 and this causes the disassembly of the β1 integrin - Talin complex. In addition, β1-integrin dissociates from CDK5 and from the CDK5-regulatory subunit, p35, but remains bound to p-FAK. We determined the pathway by which the loss of CDCP1 arrests CDK5 kinase activity. Upon loss of CDCP1, siRNA phosphorlylates p35. This generates a binding site for the C2 domain of PKCα and phosphorylation of CDK5-T777 by PKCα. The subsequent dissociation of the regulatory subunit abolishes the activity of β1-integrin. Altogether we discovered a new mechanism of regulation of CDK5 in prostate cancer cells, which leads to the uncoupling of β1-integrin and FAK. The potential biological and clinical consequences of this mechanism are (1) a switch from cell-matrix to cell-cell adhesion, (2) increased sensitivity of CTCs to FAK inhibitors and (3) improved adaptation and survival of cancer cells in the circulation and at the metastatic sites.

#5796 Heparan sulfate proteoglycans mediate tumor cell invasion and metastasis. Henry Qazi,1 Zhong-Dong Shi,2 Jonathan W. Song,1 Limary M. Cancel,1 Lance L. Munn,1 John M. Tarbell2

The Cub-Domain Containing Protein - 1, CDCP1, is a transmembrane glycoprotein that shares a characteristic domain with the core protein for heparan sulfate proteoglycans. Previous studies suggest that heparan sulfate (HS) in the glycocalyx plays an important role in the invasion and metastasis of renal cell carcinoma cells. We demonstrated that loss of CDCP1 facilitates PCA metastasis, we determined the consequences of CDCP1 loss in non-adherent cancer cells, which provide an experimental model system of CTCs. In this system, CDCP1 silenced cells exhibit 3-fold higher proliferation, 4-fold greater anchorage independent growth with colonies exceeding 5 microns in diameter, and a 2-fold reduction in migration ability. In 10% human plasma, these cells up-regulate p-FAK, p-SRC, p-AKT and p-MAPK expression, and at the same time loose expression of activated β1-integrin. The loss of β1-integrin occurs through prevention of TALIN phosphorylation. Upon loss of CDCP1, Talin is no longer phosphorylated by CDK5 and this causes the disassembly of the β1 integrin - Talin complex. In addition, β1-integrin dissociates from CDK5 and from the CDK5-regulatory subunit, p35, but remains bound to p-FAK. We determined the pathway by which the loss of CDCP1 arrests CDK5 kinase activity. Upon loss of CDCP1, siRNA phosphorlylates p35. This generates a binding site for the C2 domain of PKCα and phosphorylation of CDK5-T777 by PKCα. The subsequent dissociation of the regulatory subunit abolishes the activity of β1-integrin. Altogether we discovered a new mechanism of regulation of CDK5 in prostate cancer cells, which leads to the uncoupling of β1-integrin and FAK. The potential biological and clinical consequences of this mechanism are (1) a switch from cell-matrix to cell-cell adhesion, (2) increased sensitivity of CTCs to FAK inhibitors and (3) improved adaptation and survival of cancer cells in the circulation and at the metastatic sites.

#5797 Semaphorin 5A preserves epithelial phenotype in pancreatic cancer cells by regulating cross-talk between Wnt and TGF-β signaling. Sugandha Saxena,1 Abhilasha Purohit,2 Surinder K. Batra,1 Rakesh K. Singh3

Pancreatic cancer (PC) is one of the deadliest forms of cancers. In spite of recent advances, minimal progress has been made in our understanding of PC progression, metastasis, and treatment of PC patients with advanced disease. Striking similarity between the process of cancer metastasis and guidance of neuronal cells to their target sites has generated interest in pharmacologically targeting guidance cue molecules for treatment of metastasis. Among the guidance cue family members, Semaphorin5A (SEMA5A), was found to be involved in organ-specific homing during PC metastasis. With an interest to delineate the function of SEMA5A in PC, we generated SEMA5A knockdown in metastatic PC cell lines. Knock down of SEMA5A expression resulted in loss of cellular differentiation and epithelial phenotype with higher motility as compared to vector. This observation is in line with our previous finding that SEMA5A expression was higher in well-differentiated tumors in comparison with undifferentiated pancreatic tumors. Loss of SEMA5A increased TGF-β2 production, decreased expression of E-cadherin, and nuclear translocation of β-catenin. Furthermore, we observed higher Wnt activity and increased expression of the transcription factor SNAIL in SEMA5A knockdown cells in comparison with vector control cells. Higher Wnt signaling along with increased TGF-β2 production explains the loss of differentiation and epithelial markers. Moreover, we observed that non-canonical TGF-β2/AKT mediated inhibition of GSK3-β is responsible for increased stability of β-catenin and SNAIL, thereby activating Wnt signaling in SEMA5A knockdown cells. Our
observations demonstrate that SEMA5A has a potential role in maintaining the epithelial phenotype in PC cells by keeping cross-talk between Wnt and TGF-β signaling under check.

#5798 Exosomal oncogenic proteins promote tumor progression, metastasis and chemoresistance in ovarian cancer. Kalpana Deepa Priya Dorayapan, Ross A. Wanner, Roman A. Zingarelli, Uksha Saini, Selvendiran Karuppaiah, David E. Cohn. The Ohio State University, Columbus, OH.

Introduction: Ovarian cancer is the most lethal gynecological malignancy. Despite its clinical significance, the factors that regulate the development and tumor progression and metastasis are among the least understood of all major human malignancies. In this study we show that the oncogenic proteins which are specifically packed in the cargo of the exosomes released from the ovarian cancer cells play a role in tumor progression, metastasis and chemoresistance.

Methods: The exosomes were isolated using ExoQuick solution from different primary ovarian cancer cells (POCC), HGSOc cell lines, normal ovarian epithelial cells (OSE) and patient ascites. Further quantified by NTA and morphologically characterized by TEM, their purity was validated by the presence of exosome specific markers and absence of golgi matrix protein. Protein profiling in exosomes was done using Capillary LCMS/MS analysis. Proteomic data sets were analyzed by (http://www.qiagen.com/ingenuity) Ingenuity Pathway Analysis (IPA) to gain biological insight in disease mechanisms, associated to the observed expression changes. Cell proliferation assay and cell migration assay were carried out in normal and ovarian cancer cell lines with and without exosome co-culture, STAT3 and Hepatocyte growth factor (HGF) expression in exosomes and Matrix (ECM) proteins in different cell lines were analyzed using Western Blotting. Also in-vivo studies were carried out using orthotopic tumor model in mice. In addition cisplatin accumulation studies were done using ICP-MS. Results: The exosome concentration from POCC and HGSOc cells was much higher (2-10 fold times) than from the OSE cells. Expression of CD9, CD63, TSG101 and EpCam confirmed the exosomes. HGF, Complement factor H (CFH) and Lysyl oxidase- 2 (LOX2) were selected from the top 25 proteins identified by IPA based on their expression ratio and disease relevance. IPA scored STAT3 as the top regulator effect network and further more HGF was activated in our data set. The expression of activated STAT3 and HGF were confirmed in the exosomes from HGSOc cells and patient ascites. Cell proliferation and migration was significantly increased with concurrent increased expression of MMP-2 and -9 in-vitro favoring the role of exosomal oncogenic proteins in tumor progression and metastasis as evidenced in in-vivo orthotopic tumor mouse. The decrease in cisplatin accumulation in OVCAR8 cells observed, when co-cultured with exosomes from cisplatin resistant cell line suggests its role in chemo-resistance as well. In addition, blocking exosome function inhibits cell proliferation and increases the cytotoxicity to cisplatin. Conclusion: The results conclude that the tumor exosomes of ovarian cancer cells are enriched in highly elevated oncogenic proteins that play a very important role in mediating tumor progression and metastasis while inducing chemo-resistance as well and are evident from our in-vitro and in-vivo studies.

#5799 Targeting CX3CR1 impairs the reseeding of cancer cells recirculating from metastatic tumors. Chen Qian, 1 Asuraya Worrede-Mahdi, 1 Raman- gopal Kaur, 2 Fei Song, 2 Joseph Salvino, 1 Olimpia Meucci, 1 Alessandra Stattus, 1 Drexel University, Philadelphia, PA; 2University of pennsylvania, Philadelphia, PA.

Cancer cells re-enter systemic blood from established metastatic tumors. Recent evidence indicates that these recirculating cancer cells further seed and colonize skeleton and soft tissues to expand metastatic dissemination, thus precipitating the clinical progression to terminal disease. We have previously shown that the chemokine receptor CX3CR1 is implicated in the metastatic seedling of breast cancer cells and that novel small-molecule antagonists for this receptor effectively contains the number and size of secondary tumors in animal models. Using the same models, we now report that targeting CX3CR1 also restrains the re-seeding of skeleton and soft-tissue by circulating cancer cells (CTCs) in the blood, we sought to assess whether these compounds could synergize with docetaxel by extending bioavailability and its cytotoxic effects. Thus, we tested this paradigm on CTCs departing from skeletal and soft-tissue lesions generated by breast and prostate cancer cells in mice. Based on the results obtained from these experiments, it can be concluded that implementing the development of CX3CR1 antagonists and promoting their clinical use will provide novel and effective tools to contain the progression of metastatic disease in cancer patients.

#5800 Epidermal growth factor-induced ANGPTL4 enhances anoikis resistance and tumor metastasis in head and neck squamous cell carcinoma. Yu-Han Liao, National Cheng Kung University, Tainan, Taiwan.

Epidermal growth factor (EGF) is important for cancer cell proliferation, angiogenesis and metastasis in many types of cancer. However, the mechanisms involved in EGF-induced head and neck squamous cell carcinoma (HNSCC) metastasis remain largely unknown. In this study, we reveal that angiotensin-like 4 (ANGPTL4) plays an important role in the regulation of EGF-induced cancer metastasis. We showed that EGF-induced ANGPTL4 expression promoted anoikis resistance and cancer cell migration and invasion in head and neck squamous cell carcinoma (HNSCC). In addition, depletion of ANGPTL4 inhibited EGF-induced cancer cell invasion. Autocrine production of EGF-induced ANGPTL4 regulated the expression of MMPs. The induction of MMP-1 gene expression by ANGPTL4-activated integrin β1 signaling occurred through the AP-1 binding site in the MMP-1 gene promoter. Furthermore, down-regulation of MMP-1 led EGF- and recombinant ANGPTL4-enhanced HNSCC cell migration and invasion. Depletion of ANGPTL4 significantly blocked EGF-primed anoikis resistance and extravasation. Moreover, depletion of ANGPTL4 significantly blocked EGF-primed metastatic seeding of tumor cells and MMP-1 expression in vitro. HNSCC xenografts formed in vivo were not observed in the depletion of ANGPTL4. These results suggest that EGF-induced expression and autocrine production of ANGPTL4 enhances HNSCC metastasis via up-regulation of MMP-1 expression. Inhibition of ANGPTL4 expression may be a potential strategy for the treatment of EGF-mediated HNSCC metastasis.

#5801 Loss of core 1,3-galactosyltransferase (C1GALT1) in pancreatic cancer leads to altered mucin glycosylation and increased tumor aggressiveness. Seema Chugh, 1 Satyaranayana Rachagani, Xinhe Yu, 2 Sirram Neelamgeham, 2 Lijun Xia, 1 Michel O. Ouellette, 1 Moorthy P. Ponnusamy, 1 Su-rinder K. Batra. 1 University of Nebraska Medical Center, Nebraska, NE; 2State University of New York, NY; 1University of Oklahoma Health Sciences Center, OK; 2Internal Medicine Division of Gastroenterology-Hepatology, NE.

Background: Aberrant expression of mucins underlie pancreatic cancer (PC) progression and metastasis. Mucin type O-glycosylation is the principal post-translational modification on mucins and is regulated by myriad of glycosyltransferases. Not much has been investigated about the specific glycosyltransferases involved in increased PC aggressiveness. We found that core 1,3-galactosyltransferase (C1GALT1), which is essential for O-glycosylation, is not expressed in a subset of the PC patients. C1GALT1 adds galactose in O-glycosylation pathway that forms core 1 structure, which is usually elongated to several carbohydrate structures. Loss of C1GALT1 is accompanied by incomplete O-glycosylation that results in increased expression of truncated carbohydrate antigens (Tn and sTn). Increased expression of these truncated carbohydrate antigens on mucins has been associated with augmented aggressiveness in several malignancies. Based on these results, we hypothesized that loss of C1GALT1 in PC patients is associated with altered mucin O-glycosylation and increased aggressiveness. Methods: Expression of C1GALT1 was examined in HPNE (human pancreatic nestin expressing cells) and oncogenic transformed HPNE cells. To understand the role of C1GALT1 in PC, CRISPR/Cas9 mediated knockout of C1GALT1 was performed in PC cells. Impact of C1GALT1 knockout on glycosylation profile and mucin glycosylation was evaluated using lectin blotting and lectin pull down assay. Wound-healing assay was performed to examine the effect of C1GALT1 knockout on migration. To gain mechanistic insight, protein expression of signaling molecules involved in growth and motility was evaluated using western blotting. Orthotopic implantation of C1GALT1 knockout cells was carried in pancreas of nude mice to study its role in tumor growth and metastasis. Results: Knockout of C1GALT1 displayed increased tumor promotions which was associated with significantly increased migration. Western blotting demonstrated increased expression of proteins involved in growth and motility. Lectin-pull down assay revealed altered MUC16 glycosylation in C1GALT1 knockout cells. In vivo studies using orthotopic implantation and KPC mouse...
model demonstrated increased tumor weight and metastasis with knockout of CIGAL1T. Conclusion: Overall, our results indicate that loss of CIGAL1T in PC is associated with altered MUC16 glycosylation and increased PC aggressiveness.

**#5802 Role of mesenchymal stem cells in acquisition of metaplasia in AKU-BC42 breast metastatic carcinoma cell line.** Nazia Riaz,1 Sadia Habib,2 Azhar Hussain,2 El-Nasir Lalani,3 *Centre for Regenerative Medicine and Department of Surgery, Aga Khan University, Karachi, Pakistan; 3Centre for Regenerative Medicine, Aga Khan University, Karachi, Pakistan.*

Introduction: Metaplastic carcinoma (MCa) is a rare and aggressive subtype of invasive breast carcinoma exhibiting epithelial to mesenchymal transition (EMT) where tumor of epithelial origin manifests differentiation into non-glandular, mesenchymal phenotypes such as spindle, chondroid or osseous components. Acquisition of EMT is associated with low expression of e-cadherin, claudins and high expression of vimentin. Biological mechanisms for acquisition of metaplastic components within MCa are not well understood. The present study was undertaken to establish and characterize a cell line from a patient diagnosed with MCa and to evaluate the role of mesenchymal stem cells (MSC) in attainment of metaplastic phenotype. Methodology: AKU-BC42 was established from a 65 years old Pakistani female patient diagnosed with T4N1M0 MCa. Specimen was procured and processed under sterile conditions and cultured in DMEM supplemented with 10% FBS. Epithelial colonies were visualized after 3 weeks of culture, which were passaged and propagated. AKU-BC42 was phenotypically and genetically characterized using karyotyping, gene expression analysis, immunocytochemistry and florescent in situ hybridization. MSC marker expression was assessed by flow-cytometry. AKU-BC42 was cultured under appropriate conditions for assessment of differentiation along osteogenic, adipogenic and chondrogenic pathways. Lineage differentiation was evaluated by special immunohistochemical stains and induction of lineage differentiation genes was assessed by Q-PCR. Results: AKU-BC42 was found to have a human karyotype with multiploidy and population doubling time of 60 hours. Gene expression profiling revealed negative expression for estrogen and progesterone receptors and positive expression of androgen receptor (AR) and HER-2/neu. FISH analysis was negative for HER-2/neu amplification. Basal (5, 14 & 19) and luminal cytokeratins (8 & 18) were expressed at mRNA level along with mesothelial markers (CD10, 100A7, p-cadherin, desmin, s100A4, s100A2 & α-SMA). AKU-BC42 demonstrated MSC pool with expression of CD73 (79.2%), CD90 (10.3%) and CD105 (30.2%) as revealed by flow-cytometry. Osteogenic differentiation was assessed by von kossa stain for mineralization with up-regulation of ALPL and OPN genes. Similarly, differentiation of AKU-BC42 into mature adipocytes was evaluated with Oil red O staining of lipid droplets and expression of FABP4 gene. Chondrogenic induction was achieved by performing pellet cultures. Colla-gen synthesis in extracellular matrix of chondroblasts was assessed by mason trichrome staining with up-regulation of ACAN, COL10A1 and COMP. Conclusions: We report a novel MCa cell line containing a MSC pool. MSC pool within MCa may be responsible for acquisition of metaplasia in this rare subtype of breast cancer. Further studies on this cell line may reveal the biological mechanisms of MCa of the breast.

**#5803 Role of Neurophilin 2 in osteoclasts promoting prostate cancer bone metastasis.** Navath Shree Polavaram, Arup Bag, Sohini Roy, Samiksham Dutta, Kaustabh Datta. *University of Nebraska at Medical Center, Omaha, NE.*

Background: Bone metastases is one of the major clinical concerns that causes skeletal related malignancies and increased mortality. Bone is one of the preferred sites for metastatic prostate cancer. The tumor cells interact with bone cells (osteoblasts and osteoclasts) resulting in an imbalance in the bone homeostasis causing metastatic bone disease. Unlike other cancers, the bone maturation promoted by metastatic prostate cancer (PCa) leads to increased osteoclastic activity resulting in osteoblastic bone lesions. However, bone matrix response to osteoclasts leads to release of growth factors that aid PCa cells to develop into an overt metastasis. Our preliminary data indicated a non-tyrosine kinase receptor Neurophilin 2 (NRP2) is expressed in osteoclasts (OC) induced by PCa cells. The objective of our current study is to study the role of NRP2 in PCa-induced OCs. We hypothesize that PCa-induced NRP2 expression in OC is necessary for low osteoclastic activity and that it negatively regulates the OCs leading to PCa bone metastasis. Methods: Mouse OC precursors were isolated from bone marrow of C57BL/6 mice and differentiated into OCs under conditions of RANKL and M-CSF and in conditioned medium (CM) collected from PCa cell line LNCaP C4-2B (promotes high osteoclastic and low osteoclastic activity) and PC3 (predominantly osteoclastic activity) to mimic the conditions in normal bone and PCa bone metastasis. NRP2 expression at protein and mRNA was evaluated. TRAP staining and activity were conducted to confirm the differentiation of OCs. Results & Discussion: We observed that NRP2 was expressed in OCs induced under standard conditions (C57BL/6 and C4-2B). TRAP staining and activity confirmed the differentiation of OCs under these conditions. Interestingly, depletion of NRP2 and treatment either in standard conditions or C4-2B CM exhibited a drastic increase in osteoclastogenesis. However, NRP2-depleted OC precursors when treated with PC3 CM showed no change in osteoclastogenesis. These findings advocate a role of NRP2 in inhibiting osteoclastic activity in PCa. Conclusion: This study established that osteoclastic activity in PCa evades NRP2 inhibition. Protein analysis of the time course of NRP2 in OC in PC3 CM and C4-2B CM showed a differential NRP2 expression. This difference in the NRP2 levels can be impacted at either transcription or post-translational level. No change was observed in the NRP2 expression at transcriptional level in both the conditions suggesting that this difference can be due to either translation or rapid degradation of NRP2. Using in-vitro and transgenic mice, we examine how PC3 CM-induced OCs escapes the inhibition of NRP2. We will also determine whether NRP2 expression regulates the transcription of genes involved in OC differentiation and activation. Together, this approach will elucidate the role of NRP2 axis on OCs in promoting PCa-induced bone metastasis and will aid in determining whether NRP2 axis can be a therapeutic target.

**TUMOR BIOLOGY: Effects of Tumor-Microenvironment Crosstalk on Metastasis**

**#5804 Heterogeneity in androgen receptor and IL-1beta expression by prostate cancer cells in skeletal metastases.** Asurayya A. Worrede-Mahdi,1 Melisa Diaz,2 Alessandro Fatatis1.1Drexel University College of Medicine, Philadelphia, PA; 2Drexel University, Philadelphia, PA.

Bone metastases are a prevalent complication of advanced prostate cancer (PCa). The current standard of care for advanced PCa patients is androgen deprivation therapy (ADT), which fails within approximately two years and patients progress to castration resistant prostate cancer (CRPC). CRPC presents with additional metastasis in soft tissues and is incurable. We have found that in PCa patients, bone metastases have heterogeneous Androgen Receptor (AR) expression, and that cells lacking AR are the only PCa cell type that secretes the cytokine Interleukin-1 beta (IL-1β). Furthermore, pre-clinical work in our lab has revealed the role for IL-1β in promoting bone colonization by AR+ PCa cells and generation of heterogeneous skeletal metastases. A significant barrier to studying tumor heterogeneity in PCa metastasis has been the lack of an animal model to study the interaction between multiple PCa phenotypes in the bone microenvironment. By engrafting a suspension containing both AR+ and AR- cells directly into the left cardiac ventricle of mice, our lab has developed an in vivo model that allows us to study the progression of both AR- and AR+ cells in skeletal and soft tissue metastases. AR- and AR+ cells were genetically engineered to express two different Luciferase constructs (630 nm Red-shifted Luc and 540 nm Luc2 respectively) thereby allowing the progression of AR- and AR+ cells to be independently tracked and quantified. We used this model in combination with histological and radiographic analyses to assimilate the pathological features of our in vivo mixed AR+/AR- metastases to the human clinical scenario. Finally, we used castrated mice to determine whether IL-1β secreting PCa cells could support the growth and survival of IL-1β/AR+ cells in androgen deprived conditions. Co-injection of AR- (PC3-ML) and AR+ cells (LNCaP, VCAp, and 22Rv1) led to increased establishment of soft tissue metastases as compared to injecting AR- cells alone. Unlike the metastases generated by PC3-ML cells alone, which show exclusively osteolytic activity, AR-/AR+ mixed metastases are both osteosclerotic and osteolytic, thereby recapitulating clinical pathology. Finally, preliminary results suggest that co-injection with IL-1β-secreting AR- cells enables the growth of androgen-dependent AR- cells in castrated mice. This animal model of metastasis heterogeneity recapitulates human pathology and enables further interrogation of the mechanisms by which IL-1β supports survival of AR+ cells and the potential role for AR- in evading AR-targeted therapy during castration resistant prostate cancer (CRPC).

**#5805 Myosin 1e colocalization with β1-intergin and association with tumor progression in colorectal cancer.** Jeffrey Pfannenstein,1 Filippo Bori,2 Alessandro Bombonati,3 David Zuzga.1 La Salle University, Philadelphia, PA; Einstein Medical Center, Philadelphia, PA.

For colorectal cancer (CRC), the risk of relapse among stage II and stage III a/b patients is 20-30% and the benefit of treating these patients with chemotherapy is uncertain. As a result, many patients are untreated, putting them at increased risk for disease relapse, or overtreated, exposing them to unnecessary
and harmful chemotherapy with little potential benefit. Therefore, defining prognostic biomarkers to more accurately determine each person’s risk of relapse and need for chemotherapy is a priority in CRC research. Promising targets for biomarker discovery are actin-binding proteins. As a broad class, these proteins regulate the actin cytoskeleton and serve as direct, proximal regulators of invasiveness and metastatic phenotypes. Further, the prognostic utility of several of these proteins has recently been reported in the literature. Myosin Ie (Myo1e) a long-tailed, class I myosin, is one such protein with proposed clinical utility as a prognostic biomarker. In kidney podocyte cells, Myo1e regulates endocytosis, adhesion, migration, and invasiveness dynamics. Despite its expression in numerous cancers, the functional role of Myo1e specifically in cancer cells and its association with tumor progression remains elusive. To define a role in cancer cells, the localization of Myo1e was examined in vitro by immunofluorescence in T84 human CRC cells. Myo1e co-localized with actin, cortactin and β1 integrin at membrane ruffles, which regulate integrin endocytosis and trafficking. Interestingly, both Myo1e and β1 integrin also co-localized with caveolin-1, a regulator of integrin endocytosis, suggesting Myo1e expression may regulate caveola-dependent integrin endocytosis and trafficking. To evaluate potential prognostic utility, Myo1e expression in colorectal tumors and matched normal adjacent tissue (NAT) was examined by immunochemistry in a tissue microarray constructed from duplicate tissue cores from 119 CRC patients. The patient cohort was well balanced across TNM stage: stage 0 (10.1%), stage 1 (20.2%), stage 2 (26.9%), stage 3 (31.1%), and stage 4 (12.6%), and the majority (68.8%) of the tumors examined were rectal. The ratio of Myo1e expression in tumors compared to NAT was significantly correlated with clinicopathologic indicators of disease progression, including clinical stage, depth of invasion at the primary tumor (T-score), and lymph node metastasis. Together, these data suggest Myo1e expression correlates with tumor progression and may regulate invasive phenotypes through integrin trafficking pathways. Examining the prognostic utility and biologic function of Myo1e may define a novel biomarker with translational utility for improved clinical management of colorectal cancer patients.


Copper is an enzymatic cofactor required for cellular respiration, iron homoeostasis, melanogenesis, neurotransmitter biosynthesis, and connective tissue formation. Copper deficiency in humans is evidenced by Menkes disease, a lethal pediatric disorder caused by mutations in the ATP7A copper transporter. ATP7A, a ubiquitously expressed copper transporting P-type ATPase, transports copper from the cytoplasm into the Golgi complex to supply copper to secreted copper-dependent enzymes. Copper requirement for lysoxydase (LOX) proteins in tumor metastasis is further illustrative of copper’s role in cancer biology. Copper-dependent LOX proteins catalyze the oxidation of lysine residues within collagen and/or elastin, stabilizing crosslinks between these fibrous proteins during the formation or remodeling of the extracellular matrix. While research implicates a direct role for LOX proteins in many types of cancer, the use of copper chelators or other strategies to starve LOX enzymes of copper as a therapeutic strategy has been largely overlooked. LOX family members acquire copper in the secretory pathway via the ATP7A protein. Consistent with this requirement, LOX activity is reduced in cultured fibroblasts derived from patients with Menkes disease that lack functional ATP7A protein. As the cancer promoting properties of LOX enzymes have been shown to require catalytic activity, we hypothesize that blocking the ATP7A protein could be a powerful approach to inhibit LOX-dependent tumorogenic and pro-metastatic pathways. In this study, we have found that genetic ablation of ATP7A using CRISPR/Cas9 inhibits the tumorogenic and metastatic potential of a multiple cancer cell lines in vivo. These findings could be attributed to a variety of copper functions or those of copper-dependent enzymes, such as LOX. In this study, we investigate the role of copper-dependent enzymes, such as LOX, using in vitro and in vivo methods. While others have implicated lysyl oxidase secreted by cancer cells in tumorigenesis and metastasis, the contributions of lysyl oxidase at the metastatic site have not been evaluated. Here, I describe a novel conditional mouse model that we use to explore this question.

#5807 Disseminated tumor cell clearance by the immune system. Raziye Piranloung, Eun Mi Lee, Maria Ouzunova, Ali S. Arbab, Paulo C. Rodriguez, Asm L. Iskander, Hasan Korkaya. Augusta University, Augusta, GA.

The classical model of metastasis suggests that the tumor cell dissemination occurs late in tumor development, however accumulating evidence coming from in vitro and in vivo studies indicate that tumor cells with early dissemination signal to the immune system and start to disseminate during the initial steps of tumor development. However, the dissemination from the primary area does not always result in metastasis. Due to the non-permissive nature of microenvironment in distant sites, these early disseminated tumor cells might be cleared or maintained in a non-proliferative/dormant state. The mechanism by which some early disseminated tumor cells escape immune surveillance while some remain dormant is not well known. In order to understand the underlying factors that may contribute to the metastatic growth, we performed time course experiments by utilizing murine mammary tumors (4T1 as metastatic and 4T6 as less metastatic) in a syngeneic mouse model. Luciferase expressing 4T1 or 4T6 tumor cells were orthotopically implanted into the fat pads and tumor cell dissemination was analyzed over 3-week time points. We determined that both 4T1 and EMT6 tumors disseminated as early as one to two-week post implantation, however only 4T1 tumor develop metastasis in distant organs. Moreover, we also resected primary tumors 1, 2 and 3-week post implantation of EMT6-Luci or 4T1-Luci tumors and observed distant metastasis via optical imaging of luciferase expression in live animals. Although the majority of 4T1 tumor-bearing mice (>80%) developed the expression of TIMP-1 and 4T1 tumors resected 2 and 3-weeks post implantation only 10% of mice developed metastasis when primary tumor resected one-week post implantation. In contrast, EMT6 tumors following resection only relapsed in the primary tumor site but failed to develop metastasis. Furthermore, EMT6 tumor-bearing mice efficiently cleared tail vein injected EMT6-luci cells in the lungs. We investigated the possible mechanism by which EMT6 tumor-bearing mice clears disseminated tumor cells in the lung. We provide evidence that pulmonary infiltrated mMDSCs mediate tumor cell killing via secretion of high levels of cytotoxic granules, granzyme A, granzyme B, perforin. This was confirmed by mouse transcriptome and qPCR analyses as well as biochemistry using in vivo samples and in vitro co-culture samples. Our studies provide a new paradigm in the understanding of the fate of disseminated tumor cells in secondary organs and the role of the immune system in this process.

#5808 The role of breast cancer-derived exosomes in breast metastasis. Gohran Morad, Jiang Yang, Marsha A. Moses. Boston Children’s Hospital, Harvard Medical School, Boston, MA

The development of breast metastasis is associated with a significant reduction in the survival rate of breast cancer patients. Improving the prognosis of women with breast metastasis from breast cancer relies on the elucidation of the mechanisms underlying this process. Our group and others have shown that the formation of brain metastases occurs along the abluminal side of brain vessels, a process called “vessel co-option”. These observations prompted our hypothesis that the cellular and extracellular matrix (ECM) components of the blood-brain barrier (BBB) can serve as a pre-metastatic niche for breast cancer brain metastases. The role of tumor-derived exosomes (TEX) in the preparation of a pre-metastatic niche in distant organs has been shown in a number of cancers. To investigate the mechanisms driving the vessel co-option of breast cancer brain metastases, we studied the role of breast cancer-derived exosomes in preparation of the BBB for metastasis formation. Exosomes were isolated from the MDA-MB-231 breast cancer cell line, a brain-seeking variant of these cells, and a bone-seeking variant as a non-brain metastatic control. Brain endothelial cells, astrocytes, and brain vascular pericytes, the three components of the BBB, were treated with exosomes for 3 days to recapitulate the continuous exposure of cells to the circulating TEX in vivo. The treated cells were analyzed for cellular activities relevant to pre-metastatic niche preparation in the brain such as the integrity of the BBB, expression of cytokines, and modulation of the ECM. An initial screen was performed using cytokine antibody arrays and PCR arrays for ECM and adhesion molecules and results were validated in three separate experiments using western blots, ELISA, and Multiplex assays. Exosomes derived from breast cancer-seeking cells significantly increased astrocyte migration and decreased the expression of Integrin β1 in brain endothelial cells, both of which can lead to the disruption of the BBB integrity. Moreover, the expression of Interleukin 8 was increased by TEX in astrocytes. TEX also increased the expression of MMP-3 and -9 (Matrix Metalloproteinases) from an undetectable baseline level and decreased the expression of TGF-β and -3 (Tissue Inhibitors of MMPs) in astrocytes. While the importance of these two MMPs for brain metastasis from breast cancer has been previously reported, our study is the first to demonstrate that secretion of these MMPs is triggered by TEX. Surprisingly, we did not observe any significant TEX-derived modulations in the expression of cytokines, ECM, Proceedings of the American Association for Cancer Research • Volume 58 • April 2017
and adhesion molecules in brain pericytes. These findings indicate that within the BBB, astrocytes and endothelial cells, but not pericytes, can be affected by breast cancer-derived exosomes in such a way that can potentially lead to progression of a suitable niche for future metastasis formation along the brain vasculature. (This work is supported by the Breast Cancer Research Foundation.)

#5809 Fluorescence-guided spatiotemporal dynamics of epithelial-mesenchymal transition under inflammatory microenvironment during colorectal cancer progression. Takeshi Ieda,1 Hiroshi Tazawa,2 Satoru Kikuchi,3 Shinji Kuroda,1 Toshiaki Ohara,1 Kazuhiro Noma,1 Hiroyuki Kishimoto,1 Takeshi Nagasaki,1 Masahiko Nishizaki,1 Shunsuke Kagawa,1 Takeshi Imamura,2 Toshiyoshi Fujiwara1.1Okayama University, Okayama, Japan;2Ehime University, Ehime, Japan;3Daiichi Medical Center, Okayama, Japan.

Background: Epithelial-mesenchymal transition (EMT) is a biological process, by which epithelial cancer cells acquire mesenchymal phenotype with malignant properties for invasion and metastasis, leading to poor prognosis. Inflammatory microenvironment has been shown to be responsible for the development and progression of colorectal cancer. However, the role of inflammatory microenvironment in the EMT-related tumor progression remains unclear. To explore the relationship between inflammatory microenvironment and EMT, a live imaging system for EMT is a promising strategy on the in vitro and in vivo experiments. In this study, we developed a fluorescence-guided live cell imaging system for the assessment of spatiotemporal dynamics of EMT, and investigated the potential of inflammatory microenvironment for the induction of EMT phenotype in human colorectal cancer cell lines. Methods: Two human colorectal cancer cell lines, HCT116 and RKO, were stably transfected with vimentin promoter-driven red fluorescence protein TurboFP635 expression vector. Both cell lines were treated with inflammatory cytokines, IL-1β (1 ng/ml) and TNF-α (20 ng/ml), or co-cultured with mouse macrophage cell line RAW264.7 in the presence of lipopolysaccharide (LPS) (200 ng/ml). The time-lapse live imaging was observed by confocal laser scanning microscope. Migration and invasion properties were examined by transwell chamber assays. The fluorescence intensity was measured by microplate reader and flow cytometric analysis. The expression of EMT-related markers was assessed by Western blot analysis and q-PCR. EMT-induced HCT116 and RKO cells were treated with anti-inflammatory agents, aspirin (1 mM) and salicylic acid (1 mM), for the suppression of EMT. Results: Inflammatory cytokines (IL-1β and TNF-α) induced red fluorescence intensity and morphological change like mesenchymal phenotype in HCT116 and RKO cells. Removal of inflammatory cytokines attenuated red fluorescence intensity and morphological change in both cells. Inflammatory cytokines also induced the migration and invasion properties in association with EMT-related markers. Moreover, co-culture with LPS-stimulated macrophages also induced red fluorescence intensity and morphological change as well as inflammatory cytokines. Anti-inflammatory agents significantly suppressed the fluorescence-related EMT phenotype under inflammatory microenvironment. Conclusions: These results suggest that inflammatory microenvironment has a great potential for the induction of EMT process during colorectal cancer progression. Moreover, we found that the fluorescence-guided EMT imaging system is useful method for the exploration of inflammation-mediated tumor progression.

#5810 microRNAs targeting EMT transcription factors may be associated to lymph node metastasis in breast cancer tumors. Elisa Pérez-Moreno,1 Gabriela Valarezo,2 Valentina Zavala,1 Wanda Fernández,1 Pilar Carvallo1.1Pontificia Universidad Católica de Chile, Santiago, Chile;2Hospital San Borja Arriarán, Santiago, Chile.

Breast cancer is the leading cause of cancer-associated deaths in women. Lymph node near to the primary breast tumor have a high chance of developing a secondary tumor, representing one of the first signs of metastasis in breast cancer. Metastasis is promoted by epithelial-mesenchymal transition (EMT), process lead by the transcription factors SNAIL, SLUG, ZEB and TWIST. MicroRNAs are small non-coding RNAs, whose expression has been demonstrated to be altered in different cancer types. Because of their ability to regulate large sets of genes involved in cancer growth and metastasis, microRNAs have emerged as candidate molecular biomarkers and novel therapeutic targets. The aim of this study is to identify microRNAs differentially expressed in breast tumors in relation to EMT-transcription factor expression and lymph node metastasis, and that are involved in epithelial-mesenchymal transition. For this purpose, we used microRNA microarray data from 50 fresh frozen breast tumors with different tumor grades (1 to 3), 28 from patients with lymph node metastasis. Microarray data analysis using RankProd (R package) revealed approximately 40 microRNAs down-regulated in breast tumors with expression of EMT-transcription factors (p < 0.05). Several of the identified microRNAs have been previously described to regulate early steps of metastasis in different cancer types, like members of the miR-200 family (miR-200c and miR-141), miR-205 and miR-30a. In addition we found other microRNAs not previously associated to breast cancer metastasis. Our results demonstrate that down regulation of microRNAs, and up regulation of their targets, may induce a metastatic behavior of tumor cells of the primary breast tumors, promoting invasion and colonization of the lymph nodes. In this sense, the changes in the expression of microRNAs may serve as biomarkers of breast cancer prognosis in patients.

#5811 HMP19, a pancreatic cancer metastasis suppressor, is secreted in extracellular vesicles and inhibits EGFR activity. Sharon Manley, Gada Al-Ani, Danny Welch. University of Kansas Medical Center, Kansas City, KS.

Pancreatic cancer is usually diagnosed when the cancer is already at an advanced stage. The presence of metastases contributes to the poor prognosis as do the limited treatment options, resulting in an urgent need for early detection methods and novel treatments. HMP19 was identified as a metastasis suppressor in pancreatic duct adenocarcinoma (PDAC) using unbiased genome wide shRNA screen assay (Kurahara et al. 2016). HMP19 is localized to the membrane of Golgi apparatus and has been implicated in the vesicular trafficking in neurons (Saberan-Djoneidi et al. 1994). HMP19 is a predicted type II, single-pass transmembrane protein with protein binding domains at the N- and C- terminals, although, the physiological function of HMP19 remains undefined. Our lab showed that HMP19 attenuates nuclear ERK1/2 activity in PDAC cells. Furthermore, we found that ectopic HMP19 is secreted in the extracellular vesicles (EVs) in non-metastatic PDAC cell line, Suit2 subclone 028 (S2-028), but not in metastatic PDAC cell line, S2-007. Furthermore, silver staining and mass spectrometry showed that the EVs protein profiles between S2-028 and S2-007 EVs are distinct. We hypothesize that secreted HMP19 regulates cell migration and proliferation, and also negatively regulates signaling pathway(s) upstream of ERK. To examine effects of HMP19-containing EVs on parental cells, HMP19-containing EVs were incubated with parental cells and proliferation and migration then were quantified. HMP19-containing EVs treatment inhibited migration but not proliferation of metastatic S2-007 cells, but not non-metastatic S2-028 cells. We identified several proteins as HMP19 binding partners in the co-immunoprecipitation followed by mass spectrometry study and were subsequently validated by co-immunoprecipitation followed by immunoblot. HMP19 was found to interact with multiple proteins, including ERK regulators such as MET and epithelial growth factor receptor (EGFR). We then investigate the role of HMP19 on the MET and EGFR signaling pathway. Intriguingly, HMP19 down regulates EGFR phosphorylation and protein expression in metastatic S2-007, but not non-metastatic S2-028 PDAC cells. Moreover, HMP19 expression had no effect on phosphorylated and total MET and Janus kinase (JAK) total protein levels. Altogether, the data suggest that HMP19 may inhibit metastasis through down regulation of EGFR signaling pathway. In conclusion, we showed that HMP19 is secreted in EVs and these EVs attenuate PDAC cell migration, and mechanistically, HMP19 down regulates the EGFR signaling pathway. Future studies will further bridge the effects of HMP19 on the EGFR signaling cascade and the extracellular HMP19-mediated inhibition of migration.

#5812 MIEN1 promotes breast cancer cell migration and invasion by regulating cytoskeletal dynamics via focal adhesion kinase and N-WASP. Pankaj Chaudhary, Marilyne Kpetemey, Timothy Van Treuren, Jamboor K. Vishwa-natha. University of North Texas Health Science Center, Fort Worth, TX.

Short Description: Migration and invasion enhancer 1 (MIEN1) is an important regulator of cell migration and invasion. MIEN1 overexpression represents an oncogenic event that promotes tumor cell dissemination and metastasis. The underlying mechanism by which MIEN1 regulates migration and invasion has yet to be deciphered. Methods: Immunofluorescence, actin polymerization, Immunoprecipitation, migration, invasion, and adhesion assays were performed using breast cancer cell lines, MDA-MB-231 and MCF10CA1a to investigate the mechanisms by which MIEN1 potentiates cell motility. Results: Our results show that MIEN1 acts as a cytoskeletal-signaling adapter protein to drive breast cancer cell migration. Knockdown of MIEN1 led to the loss of actin-protrusive structures whereas the overexpression of MIEN1 resulted in rich and thick membrane extensions. Knockdown of MIEN1 also decreased the cell-substratum adhesion, suggesting a role for MIEN1 in actin cytoskeletal dynamics. Our results show that MIEN1 supports the transition of G-actin to F-actin polymerization and stabilizes F-actin polymers. Additionally, MIEN1 promotes cellular adhesion and actin dynamics by inducing phosphorylation of FAK at Tyr-925.
and reducing phosphorylation of cofilin at Ser-3, which results in breast cancer cell migration. Furthermore, we found that MEN1 associates with cytoskeletal proteins involved in motility processes. Conclusion: Our results show that MEN1 plays an essential role in maintaining the plasticity of the dynamic membrane-associated actin cytoskeleton, which leads to an increase in cell motility. Hence, targeting MEN1 might represent a promising means to prevent breast tumor metastasis.

#5813 Rab GTPase 3C promotes colon cancer metastasis via regulation of IL-6 secretion and STAT3 signaling pathway, Chi-Long Chen. Taipei Medical Univ., Taipei, Taiwan.

RAB GTases are involved in membrane trafficking vesicle formation and cell motility. Recently, exocytotic RABs received growing attention in cancer research. However, the molecular mechanism of exocytotic RABs in colorectal tumorigenesis remains unclear. In this study, we found RAB GTPase 3C (RAB3C) has the most significant association with higher pathological stage, tumor recurrence, frequent distant metastasis, and poor prognosis in 215 colorectal adenocarcinoma cohort using tissue microarray (TMA) analysis. In multivariate analyses, high RAB3C expression retained its independently prognostic significance for both overall survival (p = 0.001) and disease-free survival (p < 0.001). Further experimental results showed increased migration and invasion ability in RAB3C overexpressing colon cancer cells compared with control group in vitro and in vivo. The cell culture medium collected from RAB3C overexpressing model could promote parental colon cancer cells further indicated that the metastasis-promoting role of RAB3C is exocytosis dependent. We then integrated the results from previously microarray and proteomics datasets and discovered that increased production of multiple cytoxines in RAB3C overexpressing cell lines. Of these cytoxines, IL-6 pathway is the top pathway corresponding to gene expression changes after RAB3C overexpression. Blocking of IL-6 function with IL-6 antibody or IL-6 knockdown significantly reduced the migration ability in RAB3C overexpressing colon cancer cells. Furthermore, secretion of IL-6 after RAB3C overexpression promoted the JAK2-STAT3 signaling with increased STAT3 phosphorylation levels to promote migration. In addition, Ruxolitinib, a JAK2 inhibitor, was found to significantly inhibited RAB3C induced colon cancer cell migration through blocking STAT3 phosphorylation. In conclusion, our study showed that RAB3C overexpression promoted tumor metastasis and is associated with poor prognosis of colon cancer patients through modulating the IL6 exocytosis of cancer cells to activate JAK2-STAT3 pathway. These findings suggest a new therapeutic strategy through the inhibition of STAT3 signaling pathway in RAB3C-IL-6 axis by Ruxolitinib to prevent metastasis of colon cancer.

#5814 Engineered microfluidic 3D human microvasculature identifies Talin-1-dependent adhesion and FAK activation as the key promoter of cancer cell ex-vivo endothelial migration. Mara Gallardi,1 Simone Bersini,2 Rosa Maria Moresco,3 Marco Vanoni,1 Roger D. Kamm,1 Matteo Moretti1. 1BCCS Galeazzi Institute, Milano, Milan, Italy; 2San Raffaele Hospital, Milan, Italy; 3University of Bicocca - Milano, Milan, Italy; 4Massachusetts Institute of Technology, Cambridge, MA.

In extravasation cancer cells and vascular niche are involved in a tight cross talk which is the rate limiting step in cancer progression [1]. Recent, animal studies support the hypothesis that metastatic deficiency lies in focal adhesion complex alterations, however, it still needs to be elucidated which are the specific regulators of each event composing extravasation. Focal adhesion proteins Talin-1 (TLN-1) and Focal Adhesion Kinase (FAK) are up-regulated in breast cancer. Both targets due to their structural and functional role, dramatically influence cancer mechanotransduction leading to endothelial junction disruption, critical in extravasation process [2]. Here, we generated a doxycycline engineered models for each extravasation step allowing single cell behavior analyses through high resolution real time imaging in a reliable and quantitative way in a physiological environment. Through this novel approach we analyzed the effect of TLN-1 and FAK and their genetic and chemical inhibition in breast cancer extravasation. The 3D-microfluidic vasculature displayed maturation markers and physiological permeability (1.5–2.0*10^-6 cm/s) and allowed cancer cell injection through the hollow vessels. Western blot confirmed TLN-1 and FAK knock down (KD) in MDA-MB231. Both targets significantly affected morphology and proliferation (p<0.001). We demonstrated the involvement of both targets in adhesion to the endothelium (p<0.001). Transendothelial migration (TEM) was decreased in TLN-1 KD (p=0.05) and in FAK KD (p=0.01). Both TLN-1 and FAK KD cells were trapped into the vessels and were not able to extend pseudopodia. Cancer migration tracking in 3D matrix was statistically lower in both KD (p<0.001). The calculated ratios of adhesion/TEM and invasion/TEM were lower in TLN-1 compared to FAK KD, demonstrating that TLN-1 plays a major role in adhesion to endothelium and early invasion while FAK was identified as the main driver in TEM. Additive (TLN-1 and FAK) KD did not show significant difference respect to the target that mostly affected adhesion and invasion demonstrating TLN-1 role in these events. Additive KD was statistically lower compared to TLN-1 KD (P<0.05), supporting FAK crucial role in TEM. Inhibition of TLN-1 and FAK phosphorylation revealed that actin polymerization and pseudopodia formation (required for extravasation) were dependent by their structural role and were independent by their phosphorylation. However, the inhibition of FAK activity showed FAK phosphorylation as the key driver of TEM mechanism (p<0.01). Concluding, our results supported by in vivo data (p<0.05) showed that TLN-1 and FAK inhibition may represent novel strategies challenging mechanisms leading to metastatic establishment. 1. Cell, 2011. 147(2):p.275-92; 2. Proc Natl Acad Sci USA, 2015. 112(1):p.214-9.

#5815 The HBP1 tumor suppressor is a negative epigenetic regulator of MYCN driven neuroblastoma through interaction with the PR2 complex. Geerttui Denecker,1 Shana Claeyes,1 Irma Lambertz,1 Els Janssens,1 Suzanne Vanhaeustert,2 Bieke Decaestecker,2 Tom Van Maerkert,2 Bram De Wilde,1 Genieve Laureys,2 Kristina Althoff,3 Johannes Schulte,3 Jean-Baptiste Demoulin,3 Sara M. Roberts,3 Laure D'Hyver,1 Eline J. Molenaar,4 Frank Wernsteeman,5 Katleen De Preter,1 Frank Speleman1. 1Center for Medical Genetics, Ghent, Belgium; 2Ghent University Hospital, Ghent, Belgium; 3University Children’s Hospital Essen, Essen, Germany; 4Université catholique de Louvain, Woluwesaint-Lambert, Belgium; 5Memorial Sloan Kettering Cancer Center, New York, NY; 6Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands; 7German Cancer Research Center, Heidelberg, Germany.

MYCN is a key driver in initiation and progression of neuroblastoma (NB) and represents a major target for novel drug strategies. We previously reported that the MYC repressor gene HBP1 was down regulated by mutant ALK via the P3K-AKT-FOXO3 signaling axis [1]. Here, we further demonstrate that HBP1 upregulation suppresses proliferation of neuroblastoma cells by decreasing the MYCN signaling pathway. HBP1 levels were also shown to be repressed in neuroblastoma cells through MYCN/MYCN driven upregulation of the miR-17-92 cluster, indicating that MYCN and mutant ALK both act to inhibit HBP1 expression in NB. Next, we tested the green tea polyphenol epigallocatechin gallate (EGCG), known to upregulate HBP1, and the BET inhibitor JQ1, which represses MYCN activity in neuroblastoma cells, and showed in vitro and in vivo synergistic effects on cell viability and tumor growth. Treatment with the PI3K/mTOR dual inhibitor BEZ-235 together with JQ1 also showed very strong synergistic effects. Further dissection of the HBP1 regulome using Gene Set Enrichment Analysis (GSEA) and iRegulon analysis (http://iregulon.aertslab.org) allowed identification of the PRC2 component SUZ12 as a central node in HBP1 regulated signaling, mainly through controlling the repression of MYCN regulated genes. In keeping with this finding, GSEA analysis of our HBP1 overexpression data set revealed also strong enrichment for genes that are differentially expressed upon EZH2 inhibition in neuroblastoma cells. Because HBP1 has previously been shown to interact with HDAC, we tested the effects of the HDAC inhibitors vorinostat and panobinostat as single agents and in combination with BEZ-235. Both combinations showed a strong synergistic effect on cell viability. The molecular mechanism of this synergism will be explored through RNAseq expression analysis. We conclude that HBP1 is a crucial component in MYCN controlled repression of gene activity through PRC2 interaction and demonstrate novel opportunities for precision drugging of MYCN overexpressing NB cells. 1Lambertz et al. Clin Cancer Res. (2015).

#5816 Ablation of oncogenic MYCN expression by hTERT-driven oncogenic lymphedonic adenosine induces cell death in MYCN-amplified neuroblastoma. Terutaka Tanimoto,1 Hiroshi Tazawa,1 Hiroshi Nosu,2 Takanori Oyama,1 Yasuo Urata,2 Shunsuke Kagawa,2 Takuo Noda,2 Toshiyoshi Fujisawa2. 1Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan; 2Oncolys Biopharma, Inc., Okayama, Japan.

Background: Neuroblastoma (NB) is a primary malignant tumor of the peripheral sympathetic nervous system. Amplification and overexpression of the MYCN proto-oncogene occur in approximately 20% of NB. Since MYCN amplification is highly associated with poor prognosis, MYCN is an attractive target for the treatment of MYCN-amplified NB. MYCN is a transcriptional activator that...
induces the expression of many down-stream target genes including human telomerase reverse transcriptase (hTERT). We developed two types of hTERT-driven oncolytic adenoviruses, OBP-301 and OBP-702, in which the hTERT promoter drives the expression of the viral E1A and E1B genes for tumor-specific virus replication, and OBP-702 further expresses tumor suppressor p53 protein. Both adenoviruses show tumor selectivity against MYCN-amplified NB with hTERT activation, the therapeutic potential of these viruses in MYCN-amplified NB remains unclear. In this study, we investigated whether OBP-301 and OBP-702 induce cell death and modulate MYCN expression in MYCN-amplified NB cells. Methods: We used 3 human NB cell lines with MYCN amplification, including IMR-32, CHP-134 and LA-N-5, to assess the effect of OBP-301 and OBP-702 on cell death by XTT assay. Virus-mediated cell death and modulation of MYCN and E2F1 expression were analyzed by Western blot analysis. The mRNA expression of hTERT and MYCN was analyzed by real-time RT-PCR analysis. To explore the role of E2F1 in the virus-mediated MYCN modulation, Ad-E2F1, a replication-defective adenovirus expressing E2F1 gene, was further used. Results: All NB cell lines showed high hTERT mRNA expression. OBP-301 and OBP-702 showed profound antitumor effect through autophagy-related cell death in all NB cell lines. Both viruses induced E1A and its target mediator E2F1 expression in these cell lines. Expression of MYCN mRNA and protein was downregulated by these viruses. Replication-deficient Ad-E2F1 infection also downregulated the expression of MYCN. Conclusions: These results suggest that hTERT-driven oncolytic adenoviruses are safe and effective antitumor agents for treatment of MYCN-amplified NB. These viruses induce profound autophagy through hTERT-dependent viral replication. E2F1 upregulation by viral infection is supposed to be one of the causes for MYCN downregulation and autophagy-related cell death. In vivo experiments are underway to investigate the antitumor effect of these viruses against xenograft NB tumors.

#5817 Protein phosphatase 1 regulatory subunit 1A promotes tumorigenesis and metastasis in Ewing sarcoma. Wen Luo,1 Changxin Xu,1 Janet Ayello,1 Filemon, De La Cruz,2 Jerey Rosenblum,1 Stephen L. Lessnick,4 Mitchell S. Cairo1. 1New York Medical College, Valhalla, NY; 2James J. Peters Veterans Affairs Medical Center, Bronx, NY; 3Memorial Sloan Kettering Cancer Center, New York, NY; 4Nationwide Children’s Hospital, Columbus, OH.

Background: Ewing sarcoma (ES) is a highly invasive and metastatic pediatric soft tissue and bone tumor. Children with metastatic ES have a cure rate of less than 30% (De Ioris MA et al, 2013). Novel and specific therapeutic targets are urgently needed. ES is characterized by the oncogenic fusions, mostly EWS/FLI which functions as an aberrant transcription factor to deregulate downstream targets and mediate ES pathogenesis. By comparing genes dysregulated by EWS/FLI across multiple model systems (Niedan S et al, 2014; Sankar S et al, 2013; Sankar S et al, 2015; Niedan S et al, 2015), we identified protein phosphatase 1 regulatory subunit 1A (PPP1R1A), a potent protein phosphatase 1 (PP1) inhibitor upon PKA phosphorylation, as one of the core EWS/FLI targets (Luo W et al, 2016). Objective: In the current study, we seek to define the role of PPP1R1A in ES pathogenesis. Methods: Quantitative reverse transcription polymerase chain reaction and luciferase reporter assays were performed to investigate transcriptional regulation of PPP1R1A in regulatory subunit 1A (PPP1R1A), a potent protein phosphatase 1 (PP1) inhibitor upon PKA phosphorylation, as one of the core EWS/FLI targets (Luo W et al, 2016). Objective: In the current study, we seek to define the role of PPP1R1A in ES pathogenesis. Methods: Quantitative reverse transcription polymerase chain reaction and luciferase reporter assays were performed to investigate transcriptional regulation of PPP1R1A in Ewing sarcoma (ES).

#5818 Disrupting the Aurora kinase A interactome in pediatric cancer. Sucheta Mukherjee, Carolyn Tu, Clay Gustafson. UCSF, San Francisco, CA.

Neuroblastoma is the most common extra-cranial solid tumor of childhood. Amplification of the MYCN proto-oncogene occurs in 50% of the high-risk neuroblastoma and indicates an aggressive and lethal form of the disease. As a transcription factor with no apparent small-molecule surface, direct inhibition of MYCN remains a challenging. Prophylactic cleavage of MYCN is regulated in part by an activity-independent scaffold function of Aurora Kinase A (AURKA). The dynamic expression and activation of AURKA allows for precise cell cycle regulation of several substrates identified by protein-protein interaction studies that are also viable anti-cancer targets, such as the aforementioned MYCN oncoprotein. Utilizing co-immunoprecipitation, flow cytometry, and other perturbation techniques, we aim to delineate a novel mechanism of targeting the AURKA interactome by disruption of MYCN. We have recently described a neoteric class of confirmation disrupting "amphosteric" inhibitors of AURKA (CD-AURKA) that orthogradially inhibit the ATP-binding pocket to dramatically disrupt the active confirmation of AURKA to associate with downstream oncogenic drivers of the neuroblastoma. In addition to MYCN, our preliminary results show CDS52 will also dissociate other oncogenic proteins that interact with AURKA. We hypothesize that confirmation disruption of AURKA and subsequent blockade of an array of protein-protein interactions will delineate the roles of AURKA, MYC, and MYCN in the cell cycle and expand the clinical applications for CD-AURKA in cancer to include non-MYCN-driven diseases.

#5820 Targeting the PIM protein kinases for the treatment of a T-cell acute lymphoblastic leukemia subset. Satish Kumar Reddy Padi,1 Libia Lu,ovo,2 Neha Singh,2 Jin Song,3 Ritu Pandey,4 Jon C. Aster,3 Xue-Zhong Yu,4 Shikhar Mehrotra,4 Andrew Kraft1. 1University of Arizona Cancer Center, Tucson, AZ; 2University of Arizona, Tucson, AZ; 3Harvard Medical School, Boston, MA; 4Medical University of South Carolina, Charleston, SC.

While intensive chemotherapy regimens result in remission in approximately 80% of pediatric and 45% of adult ALL patients, new approaches are needed for the treatment of patients with T-cell acute lymphoblastic leukemia (T-ALL) who fail to achieve remission with chemotherapy. Early T-cell precursor (ETP) ALL, a subset of T-ALL associated with a higher rate of relapse and induction failure, with a 10-year overall survival of 19% as compared with 84% for all other T-ALLs. PIM protein kinases are overexpressed in a number of hematopoietic malignancies, including ALL, with its overexpression being associated with poor prognosis. Pan-PIM inhibitors (PIMi) are being tested in clinical trials in pediatric leukemia and adult acute myeloid leukemia. Analysis of the effects of PIMi (AZD1210/LGB321) on human T-ALL cell lines demonstrated that the sensitive cell lines expressed higher PIM1 levels, whereas T-ALL cell lines with NOTCH mutations tended to have lower levels of PIM1 kinase and were insensitive to these inhibitors. NOTCH-mutant cells selected for resistance to gamma secretase inhibitors developed elevated PIM1 kinase levels and increased sensitivity to PIM inhibitors. Immunophenotype of all the PIMi insensitive cells lines was consistent with a more mature T-ALL phenotype. In contrast, sensitive cells had an immature or ETP-ALL phenotype. Bioinformatics analysis of three independent T-ALL patient datasets (GSE28703, E-MEXP-313, and GSE62156) demonstrated overexpression of PIM1 in the majority of ETP-ALLs and in a small subset of non-ETP-ALL. We also identified 58 genes changing significantly between PIMi sensitive versus insensitive cell lines that were common to the differential genes between ETP versus non-ETP ALL cases from GSE28703, suggesting that high PIM1 expression along with 58 gene signature could be a driver of the ETP phenotype. Importantly, we have made the novel observation that, combination of Ponatinib, a tyrosine kinase inhibitor (TKI) with the PIMi is synergistically lethal to T-ALL cells with ETP phenotype. These agents appear to work together by inducing PARP and Caspase cleavage, sufficient to drive apoptosis. Following engraftment of NSG mice with H-SB2-Luc cells, three weeks of dual therapy with AZD1210 (30mg/kg/day) and Ponatinib (3mg/kg/day) significantly abrogated leukemia as evidenced by optical scanning for luciferase cells, and reduced number of hCD45+ leukemia cells in the peripheral blood and bone marrow.

Also, the dual therapy significantly prolonged the survival of the treated mice. In summary, our work demonstrates a strong preclinical rationale for a novel treatment strategy of combining PIMi and TKI to treat T-ALL patients with high PIM expression.
Hedgehog-driven medulloblastoma demonstrate that the loss of Pin1 impairs vivofunctional analyses of Pin1 in the GFAP-tTA;TRE-SmoA1 mouse model of molecular model in which PIN1 promotes GLI1 protein abundance, thus conferring a growth advantage to the cells. The GLI1/PIN1 interaction was validated by reciprocal pulldowns using epitope-tagged proteins in HEK293T cells as well as by co-immunoprecipitations of the endogenous proteins in a medulloblastoma cell line. Our results support a molecular model in which PIN1 promotes GLI1 protein abundance, thus contributing to the positive regulation of Hedgehog signals. Most importantly, in vivo functional analyses of Pin1 in the GFAP-βGal;TRE-SmoA1 mouse model of Hedgehog-driven medulloblastoma demonstrate that the loss of Pin1 impairs tumor development and dramatically increases survival. In summary, the discovery of the GLI1/PIN1 interaction uncovers PIN1 as a novel therapeutic target in Hedgehog-driven medulloblastoma tumorigenesis.

In summary, the discovery of the GLI1/PIN1 interaction uncovers PIN1 as a novel therapeutic target in Hedgehog-driven medulloblastoma tumorigenesis.

Increase in protein expression and copy number drives the activation of NPY/YSR pro-survival loop in chemotherapy-treated neuroblastoma.

Neuroblastoma (NB) is a tumor derived from neural crest cells, primitive progenitors of sympathetic ganglia. It accounts for 6–10% of all pediatric cancers with approximately 700 new cases per year throughout the US. Although there are highly effective therapies for patients with low-risk and intermediate-risk disease who have local relapses, recurrent disease in patients with high-risk NB is mostly refractory to therapy. Neurotide Y (NPY) is a sympathetic neurotransmitter highly expressed in NB. Its elevated release from tumor tissue is associated with unfavorable clinical outcome. NPY, acting via its Y5 receptor (Y5R), stimulates NB cell survival and chemoresistance, however the mechanisms underlying NPY/YSR axis activation in these tumors remain unclear. The aim of this work was to investigate the correlation between the expression of NPY and NPY5R proteins and the copy number status of the NPY and NPY5R genes in pre- and post-chemotherapy NR. Eighty-five tissue samples, including specimens from the primary tumors, distant metastases and local relapses, pre- and post-chemotherapy, were collected from the Hospital Pequeno Principe, Parana, Brazil. Protein expression was investigated by immunohistochemistry. COPY number alterations (CNAs) for NPY and NPY5R genes were determined using TaqMan copy number assay. Additionally, FISH analysis was performed to assess MYCN amplification status, a genetic marker of high risk NB. Our results show that elevated extracellular NPY staining, which reflects peptide release, correlates with patients’ age above 18 months, relapse and poor clinical outcome. Moreover, the intensity of NPY staining was increased in chemotherapytreated NBs, as compared to tumors at diagnosis. However, the differences in NPY CNAs between these samples were not statistically significant. Thus, chemotherapymediated increase in NPY levels observed in post-chemotherapy NB is driven by its elevated expression rather than genomic changes. FOR NPY5R, significantly higher level of CNAs was observed in the post-chemotherapy samples (P<0.05), independently of the MYCN status. These findings are in line with previous studies reporting elevated NPY5R expression in chemotherapytreated NB tumors and cell lines. Therefore, for this receptor genomic changes may play an important role in conferring chemotherapymediated YSR up-regulation. Altogether, our results confirm activation of NPY/YSR pro-survival loop in chemotherapy-treated NB tumors. However, these coordinated increases in the NPY and NPY5R expression levels are driven by different molecular mechanisms.

Modulation of the Hippo pathway protein YAP as a mechanism of resistance to MEK inhibition in MAPK hyperactivated neuroblastoma.

Background: Current therapies for high-risk neuroblastoma are highly toxic and ineffective, as a majority of patients will face therapy-resistant relapses. Our recent whole genome sequencing analysis revealed that a majority of relapsed tumors had mutations causing RAS-MAPK pathway activation. Recent studies have implicated the Hippo pathway transcriptional coactivator protein YAP in relapsed neuroblastoma as well as resistance to MEK inhibition in other cancer models. Based on these findings, we hypothesized that YAP activity may lead to acquired resistance to MEK inhibition, and that dual inhibition of these two pathways may have synergistic therapeutic efficacy. Methods: We selected the neuroblastoma cell line SKNAS (NRAS Q61K), which acquires resistance and undergoes NF1 loss. NLF cell were treated with MEK inhibitor trametinib (20 nM) over a 72-hour period and whole cell lysates and nuclear lysates were collected for immunoblot. Effects of YAP knockdown via siRNA transfection with and without trametinib co-treatment on survival were assessed by CellTiter-Glo and RT-CES. Cell signaling analyses of tumor lysates from mouse SKNAS (NRAS Q61K) xenografts treated with vehicle, 0.3 mg/kg, 1 mg/kg, or 3 mg/kg for YAP, pERK, and apoptosis pathways were performed via immunoblot. Results: Immunoblots of 72-hour time course treatments of trametinib in NLF showed decreased pYAP, the cytosolic inactive form of the YAP protein, indicating increased YAP capable of translocating to the nucleus, which was confirmed by analyses of nuclear extracts. siRNA-mediated knockdown of YAP alone in NLF cells had a modest effect on survival, while combination inhibition of MEK and YAP signaling caused a significant reduction in cellular proliferation and survival. Immunoblotting of trametinib-treated SKNAS xenografts indicated decreased pYAP and pERK in response to increasing trametinib dose, while total YAP levels remained constant. Conclusion: Our results demonstrate that prolonged trametinib exposure causes a marked nuclear enrichment of unphosphorylated YAP. We show that co-inhibition of MEK and YAP effectively reduces proliferation and survival. We are currently investigating the effects of MEK inhibition on the expression of YAP target genes and on cellular proliferation upon modulation of YAP protein expression.

MHC class I immunogenicity and novel tumor antigen discovery in neuroblastoma.

Background: Neuroblastoma is a childhood neuroblastocytic tumor characterized by a high incidence of both early childhood and late relapse disease. The incidence of neuroblastoma is estimated at approximately 1419 new cases per year throughout the US. Although there are effective therapies for patients with low-risk and intermediate-risk disease who have local relapses, recurrent disease in patients with high-risk disease is mostly refractory to therapy. Neurotide Y (NPY) is a sympathetic neurotransmitter highly expressed in NB. Its elevated release from tumor tissue is associated with unfavorable clinical outcome. NPY, acting via its Y5 receptor (Y5R), stimulates NB cell survival and chemoresistance, however the mechanisms underlying NPY/YSR axis activation in these tumors remain unclear. The aim of this work was to investigate the correlation between the expression of NPY and NPY5R proteins and the copy number status of the NPY and NPY5R genes in pre- and post-chemotherapy NR. Eighty-five tissue samples, including specimens from the primary tumors, distant metastases and local relapses, pre- and post-chemotherapy, were collected from the Hospital Pequeno Principe, Parana, Brazil. Protein expression was investigated by immunohistochemistry. Copy number alterations (CNAs) for NPY and NPY5R genes were determined using TaqMan copy number assay. Additionally, FISH analysis was performed to assess MYCN amplification status, a genetic marker of high risk NB. Our results show that elevated extracellular NPY staining, which reflects peptide release, correlates with patients’ age above 18 months, relapse and poor clinical outcome. Moreover, the intensity of NPY staining was increased in chemotherapytreated NBs, as compared to tumors at diagnosis. However, the differences in NPY CNAs between these samples were not statistically significant. Thus, chemotherapymediated increase in NPY levels observed in post-chemotherapy NB is driven by its elevated expression rather than genomic changes. FOR NPY5R, significantly higher level of CNAs was observed in the post-chemotherapy samples (P<0.05), independently of the MYCN status. These findings are in line with previous studies reporting elevated NPY5R expression in chemotherapytreated NB tumors and cell lines. Therefore, for this receptor genomic changes may play an important role in conferring chemotherapymediated YSR up-regulation. Altogether, our results confirm activation of NPY/YSR pro-survival loop in chemotherapy-treated NB tumors. However, these coordinated increases in the NPY and NPY5R expression levels are driven by different molecular mechanisms.

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Modulation of the Hippo pathway protein YAP as a mechanism of resistance to MEK inhibition in MAPK hyperactivated neuroblastoma.
Mechanisms of intrinsic and acquired resistance to antibodies targeting IGFR-1 in pediatric sarcoma cell lines. Terry J. Shankleford,1 Seethalakshmi Hariran,2 Doris Phelps,2 Hemant Bd,2 Hiahong Zhong,2 Peter Houghton3,4. Greehey Children’s Cancer Research Institute, UT Health San Antonio, San Antonio, TX; 3Resonant Therapeutics, Inc., University of Michigan, Ann Arbor, MI; 4MedImmune, Inc., Gaithersburg, MD.

Background: Pediatric bone and soft tissue sarcomas are heavily dependent on the Type 1 insulin-like growth factor receptor (IGF-1R) signaling axis, which promotes tumorogenic properties such as proliferation, survival, angiogenesis and metastasis. While a substantial proportion of patients demonstrate intrinsic resistance to monoclonal antibodies targeting IGFR-1, a strong and sustained response has been observed in a smaller number of patients, with overall survival rates about 30% of patients. Understanding these resistance mechanisms may identify combinations of targeted therapies that optimize therapeutic efficiency of IGFR-1-targeted antibodies. Methods: For intrinsic resistance, we used a panel of Ewing sarcoma (ES, n = 10) and rhabdomyosarcoma (RMS, n = 10) cell lines that, with the exception of Rh41, are all intrinsically resistant to TZ-1, a monoclonal antibody that targets IGF-1R. For acquired resistance, we developed a TZ-1 resistant RMS cell line, Rh41/TZ-1. We utilized Receptor Tyrosine Kinase (RTK) Arrays to analyze the phosphorylation status of 49 RTKs. The cell lines were treated with TZ-1 and changes in RTK expression were assessed by quantitative PCR. Results: RMS cells intrinsically resistant to TZ-1 treatment expressed multiple activated RTKs including EGFR family of receptors, IGF-1R, IR, PDGFR, CREBBP, EP300, BRD4, ERBB4, AXL, DTK, HGF, FGFR4, and AK. Increased expression of these receptors was confirmed by qPCR. In contrast, Rh41/TZ-1 cells selected for acquired resistance to TZ-1 constitutively expressed activated RTKs (PDGFRB, FGFR4, AXL, DTK, VEGFR1). In Rh41 cells treated with TZ-1, the BRD4 inhibitor, IQ1 (300 nM, 24 Hr), blocked the induction of IGF-1R, IR, HGF, ERBB4, AXL, DTK, VEGFR1 and AK. However, it had less effect on the induction of FGFR 2/3/4 receptors. Further, combined treatment of TZ-1 and IQ1 reduced cellular proliferation of Rh41 cells more than TZ-1 treatment alone. Conclusions: We demonstrate that intrinsic resistance to the IGFR-1 targeted antibody TZ-1 is characterized by constitutive activation of multiple RTKs. In response to TZ-1 treatment in a sensitive cell line there is rapid induction of multiple RTKs indicating a dynamic response to inhibition of IGF-1R. Our data suggests that BRD4 is involved in induction of these signaling molecules and that combined inhibition of BRD4 along with the IGF-1R-targeted therapy can overcome this resistance and warrants further investigation.

Ewing sarcoma remains the second most common form of bone and cartilage cancer in children. Although EWS/FLI1 fusion protein (derived by chromosomal translocation) is known to induce aberrant transcription in the patient cells, why and how this molecule leads to tumorigenesis specifically in skeletal cell lineage is not understood. We previously reported a novel role of EWS/FLI1, which leads to constitutive activation defects through inhibition of EWS function. We further demonstrated that EWS interacts with Aurora B and relocates from inner centromere to the midzone (a midline structure that is composed spindles called the central spindle) during anaphase of mitosis. Disruption of EWS-directed Aurora B-relocation leads to defects in cytokinesis, and to induction of chromosome instability (CIN). EWS is an abundant molecule that is localized throughout the entire cytoplasm of anaphase, but how EWS specifically regulates midzone formation is not understood. A potential candidate mechanism is post-translational modification of EWS to modulate its activity in a spatial-temporal manner. Because previous studies showed that EWS/FLI1-T79 is a potential modification site of O-GlcNAcylation and phosphorylation, we hypothesized that the O-GlcNAcylated EWS/FLI1 contributes to the impairment of Aurora B relocation. Our study shows that high expression of O-GlcNAc increases the level of O-GlcNAcylated EWS/FLI1 accompanied by the impairment of the Aurora B relocation and of central spindle formation. Contrary, EWS/FLI1-T79A and EWS/FLI1-T79D (phospho-mimic mutant) does not impair the mislocation of Aurora B at the midzone, suggesting that the O-GlcNAcylation of EWS/FLI1 is responsible for mislocation of Aurora B at the midzone. The O-GlcNAc-dependent impairment of midzone formation by EWS/FLI1 may lead to the identification of therapeutic targets for Ewing sarcoma patients.

Essential role for cyclic-AMP responsive element binding protein 1 (CREB1) phosphorylation in the survival of medulloblastoma patients. Walderik W. Zomerman,1,2 Sabine L. Plasschaert,1 Frank J. Scherpen,1 Harm Jan J. Houghton1. 1Greehey Children’s Cancer Research Institute, UT Health San Antonio, San Antonio, TX; 2Grand Valley State University, Allendale, MI.

Our preclinical work showed that EWS/FLI1-T79 is a potential modification site of O-GlcNAcylation and phosphorylation, we hypothesized that the O-GlcNAcylated EWS/FLI1 contributes to the impairment of Aurora B relocation. Our study shows that high expression of O-GlcNAc increases the level of O-GlcNAcylated EWS/FLI1 accompanied by the impairment of the Aurora B relocation and of central spindle formation. Contrary, EWS/FLI1-T79A and EWS/FLI1-T79D (phospho-mimic mutant) does not impair the mislocation of Aurora B at the midzone, suggesting that the O-GlcNAcylation of EWS/FLI1 is responsible for mislocation of Aurora B at the midzone. The O-GlcNAc-dependent impairment of midzone formation by EWS/FLI1 may lead to the identification of therapeutic targets for Ewing sarcoma patients.


Targeting the polyamine-hypusine nexus for the treatment of neuroblastoma. Andre S. Bachmann,1,2 Raid El-Khawaja,3 Chad Schultz2. 1Michigan State Univ., Grand Rapids, MI; 2Grand Valley State University, Allendale, MI.

The highly conserved and unique post-translational hypusine modification is essential for the activation of a single cellular protein called eukaryotic initiation factor 5A (eIF5A). Hypusination of eIF5A represents an essential mechanism in the control of cell proliferation and has been linked to cancer. The hypusine modification of eIF5A requires the enzymes deoxyhypusine synthase (DHPS) and deoxyhypusine hydroxylase (DOHH) and, importantly, uniquely depends on the polyamine spermidine as a selectivte substrate. Polymamines including spermidine are frequently dysregulated in neoplastic disease and are critical for cell proliferation. Ornithine decarboxylase (ODC) is the sentinel enzyme in polyamine biosynthesis. We pioneered the repurposing of the irreversible ODC inhibitor alpha-difluoromethylornithine (DFMO) for neuroblastoma (NB), a devastating pediatric cancer of the sympathetic nervous system. Our preclinical work was confirmed by other groups and spurred two consortia (NMTRC and NANT) to test DFMO in independent phase I/II NB clinical trials. The oncogene MYCN is commonly amplified in high-risk NB patients and predicts poor prognosis. MYCN directly activates ODC (and eIF5A) and drives NB tumor progression, presumably through the activation of polyamines, but the precise molecular mechanisms are not fully understood. Since spermidine is the only molecule that can activate eIF5A through hypusine modification, we hypothesized that the polyamine-hypusine nexus provides a rational target site to identify new drug combinations that trigger synergistic anti-tumor effects. In this study we found that DFMO combined with DHPS inhibitor GC7 induced synergistic drug reactions in NB at concentrations that are not lethal on their own. While
each drug alone at higher concentrations induced p21/Rb-mediated G1/S cell cycle arrest, the combination of both drugs resulted in the induction of programmed cell death (apoptosis). The intracellular free polyamine levels (putrescine, spermidine, and spermine) were quantified by C-18 RP-HPLC and responded directly to drug treatments, suggesting specific drug targeting effects. This dual-drug combination approach that co-targets the blockade of the unique polyamine/spermidine-dependent hypusine-eIF5A signaling circuit known to be involved in cell proliferation might lead to more effective DFO treatment options by reducing high DFO doses currently needed while achieving better therapeutic outcomes in NB.

**#5829 Targeting TSLP-induce upregulation of Mcl-1 for the treatment of Ph-like ALL with CRLF2 alterations.** Cornelio Stojan, 1 Nathaniel George Mambo, 2 Pierce McCarthy, 2 Veritha Vidalis, 1 Jacqueline S. Coats, 1 Ineavely Baes, 1 Sinisa Dovat, 1 Shadi Farzin Gohar, 1 Dhimant Desai, 1 Muhammad Kamal, 1 Kimberly J. Payne, 1 Loma Linda Univ., Loma Linda, CA; 2California State University San Bernardino, CA; 3Pennsylvania State University, PA; 4Pennsylvania State University–Hershey, Hershey, PA.

B cell precursor acute lymphoblastic leukemia (B-ALL) is the most common childhood cancer. The subset of pediatric B-ALL patients at greatest risk for relapse and death have a gene expression profile similar to Philadelphia chromosome positive ALL. Approximately half of these Ph-like B-ALL are defined by genetic alterations that result in overexpression of the cytokine receptor, CRLF2. Stimulation of the CRLF2 receptor by the cytokine, TSLP, causes downstream activation of the Jak/Stat5 and PI3/AKT/MTOR pathways. Activation of these pathways has been associated with one prognosis and chemoresistance. A gene target of activated STAT5 in B cell precursors is Mcl-1, a Bcl family pro-survival molecule. Further, Mcl-1 protein levels are increased through post-transcriptional mechanisms induced by activation of the mTOR pathway. We hypothesized that circulating TSLP cytokine contributes to chemoresistance by increasing CRLF2 activation leading to increased Mcl-1 expression and that targeting Mcl-1 could be an effective strategy for treating Ph-like B-ALL with overexpression of CRLF2 (CRLF2 B-ALL). To test this hypothesis we cultured human CRLF2 B-ALL cell lines (MUTZ5 and CALL4) with and without TSLP for 3 days and evaluated expression of Mcl-1 by flow cytometry. We found that TSLP induced significant increases in Mcl-1 proteins in both cell lines. To determine if these results are reflective of what happens in patients, primary CRLF2 B-ALL cells from pediatric patients were cultured with physiological levels of TSLP (~20 pg/ml) and similarly evaluated. Physiological TSLP significantly increased Mcl-1 protein in primary CRLF2 B-ALL cells, including those with activating Jak mutations. Our next question was whether TSLP-induced increases in Mcl-1 could be effectively targeted with Mcl-1 inhibitors (MIM-1 or MIM-2). CRLF2 B-ALL cells were incubated with and without TSLP and treated with increasing doses of Mcl-1 inhibitors. The dose response curve achieved plateau by flow cytometry after 2 or 3 days. Mcl-1 inhibitors induced dose-dependent decreases in cell count and increases in caspase-3 activation and apoptosis (Annexin V/7-AAD). These corresponded with dose-dependent decreases in Mcl-1 protein, suggesting that both inhibitors target Mcl-1 for degradation. MIM-1 and Maritoclax showed efficacy against both CRLF2 B-ALL cell lines and primary patient specimens, including those with activating Jak mutations, although these responses were associated with decreased Mcl-1 expression levels in cells cultured with TSLP. Typically required twice the dose of Mcl-1 inhibitor to achieve the same effect observed without TSLP. These data provide evidence that TSLP can contribute to leukemia cell survival and identify Mcl-1 inhibitor as a candidate therapy for CRLF2 B-ALL. Ongoing studies are evaluating the efficacy of the Mcl-1 inhibitor, Maritoclax, in novel patient-derived xenograft models of CRLF2 B-ALL that provide physiological levels of human TSLP.

**#5830 A novel Notch-YAP circuit drives stemness and tumorigenesis in embryonal rhabdomyosarcoma.** Katherine K. Slemmons, Lisa E.S. Crose, Stefan Riedel, Manuela Sushmitha, Brian Belyea, Corinne M. Linardic. Duke University, Durham, NC.

Introduction: Rhabdomyosarcoma (RMS), a tumor characterized by skeletal muscle features, is the most common soft tissue sarcoma in children and adolescents. The standard of care treatment has not changed in over 40 years, resulting in a stagnant survival rate of less than 30% for high risk groups. Previous studies have identified the developmental pathways Notch and YAP as potent oncogenic signals in the embryonal subtype of RMS (eRMS). Understanding the signaling between Notch and YAP in cancers driven by these pathways, such as other cancers, will be crucial to designing rational combination therapies. Experimental Procedures: For in vitro assays, we utilized 3D sphere cell culture methods that permit the study of stem cell behavior. To analyze signaling, we performed genetic gain and loss-of-function (dox-inducible shRNAs and constitutively active constructs), and pharmacologic inhibition. Target validation was assessed by qPCR and immunoblot, and mechanism was determined by ChIP and limiting dilution assays. For in vivo experiments, we performed subcutaneous xenografts in immunocompromised mice. Immunohistochemistry was used to analyze protein expression in human and murine tumors. Statistical analysis was performed to assess significant differences. Results: We have discovered a novel signaling circuit, the Notch-YAP circuit, that is functional in eRMS. Inhibition of the circuit in eRMS xenografts does not reduce or cure tumors. Overexpression of the core Notch ligand Jagged1 (JAG1) and the core Notch transcription factor RBPJ can provide partial efficacy in vivo. In YAP knockdown tumors, Notch signaling is increased, while YAP transcriptional activity is decreased. Inhibition of Notch and YAP is necessary to silence the circuit’s signaling and growth. Importantly, nuclear Notch and YAP protein expression correlates in eRMS patient tumor samples, suggesting this circuit is intact in human eRMS tumors. Conclusions: While unidirectional signaling between the two pathways has been reported in metazoan development and rarely in human malignancy, this is the first finding of an intact bidirectional circuit between Notch and YAP that supports cancer cell stemness and tumorigenesis. This study provides mechanistic insight into the long-standing question of how eRMS cancer stem cells maintain plasticity. Additionally, while this signaling is identified in other RMSs, it may also be applicable to the well described eRMS tumor circuit, for example through Notch pharmacologic blockade, is not sufficient to shut down the circuit, as stemness and cell growth can be rescued by ectopic YAP. Rather, dual inhibition of Notch and YAP is necessary to silence the circuit’s signaling and sphere growth. Importantly, nuclear Notch and YAP protein expression correlates in eRMS patient tumor samples, suggesting this circuit is intact in human eRMS tumors. Conclusions: While unidirectional signaling between the two pathways has been reported in metazoan development and rarely in human malignancy.
Rho-associated kinase is a therapeutic target in neuroblastoma. Cecilia H. Dyberg, Susanne Fransson, Teodora Andonova, Baldur Sveinbjörnsson, Jessica Lännerholm-Palm, David Forsberg, Eric Herlenius, Tommy Martinsson, Per Kogner, John Inge Johnsen, Malin Wickstrom, Karolinska Institute, Stockholm, Sweden; Department of Medical and Clinical Genetics, Västerbotten Institute, Umeå, Sweden; University of Tromsø, Norway, Norway.

Background: Neuroblastoma is a peripheral neural system tumor that originates from the neural crest and is the most common and deadly tumor of infancy. The non-canonical Wnt/planar cell polarity (PCP) signaling pathway regulates cytoskeletal organization, migration and maturation of neural crest cell-derived neuroepithelial tumors. Here we show that neuroblastoma harbors frequent mutations of genes controlling the PCP signaling pathway and Rac/Rho signaling cascade important for proper migration and differentiation of neural crest cells during neurogenesis. The majority of these mutations were detected in GTPase activating proteins and guanine nucleotide exchange factors genes, which may result in inhibition of Rho and activation of Rho and was associated with aggressive high-stage neuroblastomas. Methods: We performed whole-exome sequencing of neuroblastoma samples of all clinical subgroups to search for mutations and genetic aberrations. Cytotoxic activity of Rho-associated kinases (ROCK) inhibitors was studied in cell viability assays. Morphology and invasion were studied with microscopy. The molecular mechanisms were characterized using cell- and molecular biology techniques. In vivo studies (xenografts and the transgenic mouse model TH-MYCN) in mice were carried out to validate the therapeutic effects and toxicity. Results: Exome sequencing detected frequent mutations and gene aberrations in genes controlling the activity of PCP signaling. Analysis of gene signatures and immunohistochemistry showed that high expression of ROCK1 and ROCK2 correlated with poor patient survival. Several mediators of Rac/Rho were differentially expressed in cell lines and patient samples. Pharmacological or genetic inhibition of ROCK, a key molecule in Rac/Rho signaling resulted in differentiation, inhibition of neuroblastoma cell growth and migration and degradation of MYCN protein. Finally, ROCK inhibition reduced the growth of established neuroblastoma xenografts in nude mice and repressed tumor progression in a MYCN-driven mouse model of neuroblastoma (T-MYCN) Conclusions: These results provide evidence that inhibition of the non-canonical/PCP signaling cascade using ROCK inhibitors suppresses the growth of neuroblastoma in preclinical models and suggest possibilities for improved treatment of high-risk neuroblastomas.

Targeting the transcription co-activator TAZ inhibits MYC-driven medulloblastoma. Yingchao Xue, Mingyao Ying. Kennedy Krieger Research Inst, Baltimore, MD.

Medulloblastoma (MB), the most common malignant pediatric brain tumor, results in significant neurological, intellectual and physical disability or death. Four major molecular subtypes (WNT, SHH, Group 3 and Group 4) have recently been described. The Group 3 MB, the most aggressive subtype, is more frequently associated with gene amplification and/or protein overexpression of the MYC (c-Myc) oncogene, hereby referred to as MYC-driven MBs. It is still unclear how MB cells activate and maintain high MYC expression, as only a small portion of MYC-high MBs is caused by MYC genomic amplification. MYC-targeted MB therapies are also lacking. Targeting MYC upstream activators is a promising strategy for inhibiting MYC-driven MBs, when compared to direct MYC inhibition that is still challenging. Here, we focus on the transcription co-activator TAZ, a novel MYC upstream activator and a potential therapeutic target for inhibiting MYC-driven MBs. TAZ (transcriptional co-activator with PDZ-binding motif) is an oncogenic driver in multiple human cancers. TAZ and its paralog YAP form complexes with other transcription factors, such as TEAD, to activate downstream gene targets. TAZ and YAP are transcriptional effectors of various signaling pathways, such as the Hippo pathway. We found for the first time that TAZ is an essential MYC activator in MB cells. TAZ but not its paralog YAP is highly expressed in MYC-amplified MB cells and their xenografts. TAZ silencing downregulates MYC and inhibits MB cell proliferation. Enforced TAZ expression in neuroblastoma cells indicates that TAZ-driven expression of ROCK inhibitors further showed that TAZ forms complex with transcription activator TEAD to bind to and transactivate MYC enhancers. For pharmacological TAZ targeting, we found that the FDA-approved drug Verteporfin (VP) inhibits TAZ-TEAD interaction, suppresses MYC expression and inhibits MB cell tumor propagation in vivo. We have also developed a nanoparticle formulation by using DSPED-PEG2000 micelles to achieve VP delivery across the blood-brain barrier in mice bearing brain tumor xenografts. In summary, our results provide novel insights into MYC upstream activators in MB, and also identify TAZ as a potential therapeutic target for inhibiting MYC-driven MBs. This knowledge may also be widely applicable to other TAZ/MYC-expressing human cancers, such as glioblastoma. Moreover, our studies support the effectiveness of VP treatment in pre-clinical MB models. These results and also the nanocarrier system for systemic VP delivery provide a solid foundation for VP clinical trials in patients with MB and likely other MYC-driven cancers.


Background: Cancer cell lines have traditionally been established in serum-containing medium though it has been questioned whether serum-grown cell lines faithfully represent the tumors they are derived from. Potential serum-driven induction of in vitro cultured cells could partly explain why xenografted cell lines often lack invasive growth and robust metastasis. Direct implantation of patient tumor material into mice, i.e. patient derived xenografts (PDXs), has shown to more closely mimic the original tumor growth pattern. By isolating cells from neuroblastoma PDXs and culturing the PDX cells under serum-free conditions, we expect to preserve a less differentiated phenotype, enabling us to use PDX cells as a source for identifying potential neuroblastoma stem-like cells. Moreover, to facilitate future drug screening, we investigated conditions that would allow us to grow neuroblastoma PDX cells adherently, without compromising their tumorigenic or metastatic capacity. Materials & Methods: PDX cells were cultured in stem cell medium or serum-containing medium for up to 7 days. RT-qPCR and WB were used to investigate the expression of sympathetic neuronal differentiation markers. For monolayer culture, cells were grown on laminin. Results: PDX cells can routinely be established as neurospheres in serum-free medium with retained tumorigenic and metastatic capacity. Addition of serum induces loss of sphere formation, adherent growth and a robust increase in sympathetic differentiation markers, both at mRNA and protein level. Serum-cultured cells also showed a decreased cell proliferation (without increased apoptotic rate, as determined by sub-G0/G1 analyses). The serum-induced differentiation was not irreversible since transferring serum-grown cells back to serum-free medium resulted in a phenotypic switch, with recovered proliferation and decreased expression of differentiation markers. Growing cells on laminin achieved adherent culture of PDX cells. This resulted only in slight morphological differentiation but did not affect growth rate or the cells tumorigenic and metastatic capacity. Conclusion: Culturing neuroblastoma PDX cells in serum-containing medium results in a more differentiated phenotype. Thus, avoiding serum appears to be a key strategy in order to preserve the phenotypic origin of these cells. The phenotypic effects of serum on tumor cells have largely been overlooked and might be a general effect on cancer cells explaining why serum-established cell lines do not metastasize and grow invasively.

Oncogenic activation of the serine synthesis pathway by the scaffolding protein menin. Laurie Kathleen Svoboda, Selina Shiqing Teh, Sudha Sud, Armand Bankhead, Brian Magnuson, Mats Ljungman, Costas Lyssiotis, Tomasz Cierpicki, Jolanta Grembecka, Elizabeth R. Lawlor. Univ. of Michigan, Ann Arbor, MI.

Developmental transcription programs are epigenetically regulated by multi-protein complexes including the DLL-containing trithorax (TrxG) complexes, which methylate H3K4 to promote gene transcription. We recently reported that the TrxG protein MLL1 and its binding partner menin are overexpressed by and function as oncogenes in Ewing sarcoma. Moreover, small molecule inhibition of the menin-MLL protein–protein interaction leads to loss of menin and MLL1 protein expression and to inhibition of growth and tumorigenicity. For the current study we investigated the mechanistic basis of menin-MLL1 mediated oncogenic activity in Ewing sarcoma. Bru-seq analysis was performed to identify changes in nascent gene transcription in Ewing sarcoma cells following exposure to the menin-MLL1 interaction inhibitor MI-503. Identiﬁed targets were validated by qRT-PCR, western blot, immunocytochemistry and by interrogation of published databases. Loss of function was achieved by lentiviral shRNAs and by inhibitor treatment. Exposure of three independent Ewing sarcoma cell lines to MI-503 resulted in significant and reproducible modulation of nearly 100 genes. Bioinformatics analysis of MI-503-regulated genes revealed that the serine synthesis pathway (SSP) was the most signiﬁcantly impacted pathway downstream of MI-503 treatment. The SSP has recently emerged as a major mediator of tumorigenicity in multiple human cancers. qRT-PCR confirmed reduced expression of SSP enzyme–encoding genes PHGDH, PSAT1 and PSPH in MI-503 treated cells. To determine if the SSP contributes to Ewing sarcoma pathogenesis we evaluated expression of SSP genes and proteins in
tumors and cell lines. Our findings showed that Ewing sarcomas express high levels of PHGDH, PSAT1, and PSPH and interrogation of the Cancer Cell Line Encyclopedia revealed that PHGDH expression is higher in Ewing sarcoma than in any other cancer in the database. Studies of Ewing sarcoma cells in serine and glycine free media showed that cells retain viability but that exposure to the PHGDH inhibitor, CBR-8844, inhibits ADPRT and arrests cells in both standard and glycine-free conditions, confirming the importance of the pathway to tumor pathogenesis. To determine how menin-MLL1 promotes SSP activation we performed loss of function studies and discovered that knockdown of menin, but not of MLL1, leads to a significant reduction in SSP gene expression, implicating menin as the primary mediator of SSP gene activation. Transcriptional up-regulation of Wnt/β-catenin signaling has been linked to ATP3 knockdown in both small cell lung cancer (SCLC) and multiple pediatric/adolescent and young adult (AYA); synovial sarcoma (SS), Ewing sarcoma (ES) and rhabdomyosarcoma (RMS) sarcomas, as well as soft-tissue sarcomas (STS) and bone sarcomas. We specifically included cell lines and SS and AS subtypes that were never phosphoprophored before, to reveal completely novel molecular insights into sarcoma pathology. Validations were performed in vitro, in vivo, and in patient tumor material. Our unique tyrosine phosphorylation data revealed a novel subclassification of sarcoma, and showed that particularly the pediatric/AYA sarcoma models did not cluster within their histological subgroup. Particular tyrosine kinases exhibited enhanced phosphorylation in particular subtypes, and we identified driver kinases with outlier levels of activation. ALK was identified as a completely novel and unexpected driver in the Aska-SS SS cell line, which was explained by an underlying ALK translocation.

Functional ALK dependency was confirmed with in vitro proliferation assays, showing high sensitivity for the ALK inhibitor TAE684 (IC_{50} 26.3 ± 3 nM) and the ALK/MET inhibitor crizotinib (IC_{50} 46.7 ± 5 nM). Patient specimens confirmed a clinical role for ALK in SS, with ALK immunopositivity in 14% (6/43) of patients and one 16y old patient harboring an ALK translocation, present in both adult (gastrointestinal stromal tumor as in 4 metastases. Other SS cell lines and sarcoma subtypes exhibited multiple activated tyrosine kinases, providing a rationale for combined targeting approaches. Indeed, high MET and PDGFRα phosphorylation in Yamato-SS SS cells predicted sensitivity to crizotinib and pazopanib (PDGFR) in vitro and in vivo. The clinical significance was again confirmed in a cohort of SS patients, in which MET or PDGFRα expression was detected in 58% and 84% of SS patients, respectively, with co-expression in 56%. Altogether, this is the first study to provide a deep insight into activated networks and molecular clustering patterns in sarcoma. Our integrated approach systematically identified and validated ALK and MET as novel and actionable drivers in SS.

**TUMOR BIOLOGY: Radioprotectors, Radiosensitizers, and Radiation Resistance**

#5838 Adhesion- and stress-related adaptation mechanisms eliciting glioblastoma radioresistance can be effectively circumvented by betal integrin/JNK co-targeting. Anne Vehlow,1 Erik Klapprath,2 Katja Storch,1 Ellen Dickreuter, Michael Seifert,3 Antje Dietrich,2 Rebecca Bütot,1 Achim Tonn,11 Thomas Silber,2 Markos Tzartos,2 and Yvonne Vorsatz,11

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Glioblastoma multiforme (GBM) is the most common brain tumor in adults and characterized by poor clinical outcome due to genetic and epigenetic alterations in resistance-mediated genes and destructive infiltration into the normal brain. Upon therapy, malignant tumors show adaptation to maintain their homoeostasis. Two critical determinants of this adaptation process are cell adhesion by betal integrins and stress signaling via c-Jun N-terminal kinases (JNK). Here, we evaluated the potential of simultaneous betal integrin/JNK targeting to overcome GBM adaption controlling radioresistance and invasion. Comparative Oncomine data base analysis was performed on the expression of JNK1/2/3 in GBM, betal integrin and its ligands in GBM with normal brain. Different human GBM cell populations (patient-derived, stem-like, established) were analyzed for sphere formation, clonogenicity, 3D collagen type-1 invasion, and cell cycling, chromatin organization, DNA double strand break (DSB) repair (γH2AX foci assay), broad-spectrum phosphoproteome analysis, FACS analysis and protein expression/phosphorylation upon irradiation (0-6 Gy x-rays) and chromotherapy (Temozolomide) with and without single and simultaneous inhibition of betal integrin (AIIB2) and JNK (SP600125, JNKi). The radiochemoresistant potential of AIIB2/JNKi was also investigated in an orthotopic GBM mouse model using stem-like cells. In contrast to JNK isoforms, betal integrin and col1 showed significant overexpression in GBM compared with normal brain. While single inhibition of betal integrin and JNK mediated cytotoxicity, only combined targeting resulted in radioresistance sensitization. Intriguingly, double AIIB2/JNKi treatment abrogated GBM cell invasion. Importantly, dual betal integrin/JNK inhibition elicited a significant reduction in tumor growth and longer survival of mice concomitantly treated with radiotherapy/Temozolomide. Mechanistically, JNK blocking induced betal integrin expression for
stabilizing diverse signaling pathways controlling cell cycling, invasion and radiochemoresistance. Radio sensitization by AIIB2/JNKii is caused by enhanced ATM phosphorylation and prolonged G2/M cell cycle arrest as well as impaired DNA double strand break repair in the context of elevated levels of eukratin. In summary, our data reveal that both beta1 integrin/JNK targeting efficiently impairs cancer cell radiosensitivity and radioresistance. In addition, our studies determine the intrinsically radiosensitive regions of the tumor are impacted by the radiochemoresistance and invasion. More in-depth evaluation is warranted to clarify the potential of this kind of beta1 integrin/JNK multimodal-targeting strategy administered concomitantly to standard radiochemotherapy in patients suffering from GBM.

#5839 Androgen receptor (AR): A novel target for radiosensitization in triple-negative breast cancers (TNBC). Benjamin C. Chandler,1 Corey W. Speers,1 Shuang G. Zhao,2 Melan Liu,2 Kari Wilder-Romans,3 Eric Olsen,3 Shiyam Nyati,1 Daniel Spratt,1 Daniel Wahl,1 Daniel Hayes,1 Felix Y. Feng,2 Lori J. Pierce1.1University of Michigan, Ann Arbor, MI; 2University of California, San Francisco, San Francisco, CA.

Purpose: Increased rates of local recurrence (LR) have been observed in TNBC despite chemotherapy and radiation (RT). Thus, approaches that result in radiosensitization in TNBC are critically needed. We characterized the RT response of 21 breast cancer cell (BCC) lines using clonogenic survival assays and paired this with high-throughput drug screen data, identifying AR as a top target for radiosensitization. We demonstrate that AR inhibition confers radiosensitization in vitro and in vivo, identified a biomarker of response, and characterize the mechanism of AR-mediated radiosensitization in TNBC. Materials/Methods: Clonogenic survival assays determined the intrinsic RT sensitivity of 21 BCC lines. IC50 values were determined for 130 clinical compounds and correlation coefficients were calculated using IC50 values and SF-2Gy. Gene and protein expression was measured using RNA Seq and RPPA arrays, respectively, in tumor samples (n=2,061) and BCC lines (n=51). AR function was assessed using gene knockdown (KD) or functional inhibition with anti-androgen drugs. We measured in vivo tumor growth with varying control and treatment groups (16-20 tumors/group). Kaplan-Meier analysis was performed to estimate local control. A Cox proportional hazards model and MVA were used to determine significant correlations with LR from GBM.

#5840 Radiosensitization with the c-MET inhibitor crizotinib in KRAS mutant colorectal cancer. Kyle C. Cuneo, Himabindu Kurupati, Ranjit Mehta, Theodore S. Lawrence, Mukesh K. Nyati. University of Michigan, Ann Arbor, MI.

Background: KRAS mutant colorectal cancers (CRC) are resistant to many targeted therapies including cetuximab. Novel molecular targets for KRAS mutant tumors may enhance the efficacy of chemoradiation. C-MET plays important roles in treatment resistance, tumor invasion, and metastasis. In this study, we used a small molecule inhibitor of c-MET, crizotinib in cetuximab resistant mutant KRAS driven CRC cell lines and assessed radiosensitization. Methods: To test the effect of c-MET inhibition on CRC cells we used the KRAS mutant cell lines HCT116, DLD1, and LoVo. Colony formation assays were performed to assess the effects of crizotinib and cetuximab in combination. Immunoblot analysis was used to determine the effect of crizotinib on c-MET and downstream pathways including ERK and AKT phosphorylation. We then selected doses of crizotinib below the IC50 and assessed clonogenic survival with radiation. To study potential mechanisms of radiosensitization cell cycle analysis was performed using flow cytometry. The effect of c-MET inhibition on cellular function was assessed using cell migration assays. Results: We first performed a series of clonogenic survival assays with crizotinib and cetuximab to determine the IC50 for each cell line. As expected, the KRAS mutant cell lines LoVo, HCT116, and DLD1 were resistant to cetuximab (surviving fraction greater than 95% at 100 µg/mL). These results were consistent with c-MET being radiosensitive to c-MET inhibitor crizotinib with IC50 values of 14.4 nM for LoVo, 316 nM for HCT116, and 217 nM for DLD1. Immunoblot analysis showed that crizotinib at concentrations near the IC50 for each cell line attenuated c-MET phosphorylation and downstream signaling pathways including ERK and AKT. Crizotinib further blocked radiation induced c-MET phosphorylation and attenuated activation of downstream signaling pathways. We then performed a series of clonogenic survival assays to test the radiosensitizing potential of crizotinib. Pretreatment with crizotinib for 24 hours radiosensitized LoVo and DLD1 cell lines with enhancement ratios of 1.66 and 1.25, respectively. Interestingly, treatment with crizotinib for 1 hour prior to radiation had only modest dose enhancement, suggesting that prolonged exposure to crizotinib is required for optimal radiosensitization. Cell cycle analysis revealed minimal G1 arrest with crizotinib. Additionally, crizotinib completely blocked HGF and EGF induced cell migration. Conclusions: Inhibition of c-MET with crizotinib effectively sensitizes cetuximab resistant KRAS mutant CRC cell lines to radiation. Given the important role c-MET plays in treatment resistance, crizotinib has the potential to improve the outcome of patients with locally advanced rectal cancer undergoing radiation therapy.

#5841 Hypoxic tumors can be sensitized to radiation therapy by repurposing papaverine as an inhibitor of mitochondrial respiration. Martin Bence,1 Joanna Papandreu,2 Xiangqian Hong,2 Bing Yu,2 Nicholas Denko1.1Ohio State University Wexner Medical Center, Columbus, OH; 2The University of Akron, Akron, OH.

Tumor hypoxia is a characteristic feature of solid tumors. Low oxygen tension represents a barrier to effective radiation therapy because oxygen is required to fix the DNA damage induced by ionizing radiation. Many strategies have been proposed as a means to increase the radiosensitivity of hypoxic tumors but the clinical benefits have been disappointing. Finding an effective way to radiosensitize hypoxic tumors thus represents a major challenge for improving the current protocols for clinical radiation therapy. Decreasing the demand for oxygen within the tumor has been proposed as a way to reduce hypoxia, as reduced demand could lead to increased tumor oxygenation prior to delivery of radiation. Although several compounds have been proposed based on this approach, the toxicity and/or required dose have proven to be a limitation of their potential clinical benefit. Papaverine is an opiate alkaloid that has been used in clinical practice for over 70 years as a smooth muscle relaxant. The drug has a long history in its off-target effect limiting mitochondrial oxygen consumption. We therefore hypothesized that papaverine could effectively radiosensitize hypoxic tumors by decreasing tumor hypoxia. At clinically achievable concentrations in the low micromolar range, papaverine efficiently reduced the rate of oxygen consumption (OCR) in all tested cell lines in vitro. Within the first 30 minutes, 10 µM of the drug reduced the OCR by 30-40%. We identified the mechanism of action as OCR reduction as direct binding and inhibition of mitochondrial complex I, II, and III. 10 µM of the drug reduced the OCR by 30-40%. We identified the mechanism of action as OCR reduction as direct binding and inhibition of mitochondrial complex I, II, and III. Importantly, a single dose of 2 mg/kg papaverine injected I.V. significantly increased tumor oxygenation in vivo by 1.2-1.3 fold in nude mice harboring E0771 and A549 xenografts. Because normal tissue is fully oxygenated, we found muscle oxygenation remained unchanged after drug delivery. Finally, 2 mg/kg papaverine injected 35 minutes prior to a single dose of irradiation resulted in a 2.0 fold relative growth delay in E0771 tumors compared to irradiation alone. In addition, in the more hypoxic A549 xenografts, the benefit provided by papaverine was a 3.0 fold relative growth delay. In conclusion, according to our data a single, clinically relevant dose of papaverine administered shortly before irradiation reduces tumor hypoxia and thus provides significant benefit over irradiation alone, strongly highlighting its potential to improve current strategies to enhance response to radiation therapy.


Photodynamic therapy (PDT) involves the combination of a photosensitizer and light of a specific wavelength. Upon light activation in the presence of oxygen, photosensitizer molecules generate reactive oxygen species that cause cytotoxicity by inducing oxidative stress. Aminolevulinic acid (ALA) is a pro-drug used for the diagnosis and PDT treatment of various solid tumors based on
endogenous production of heme precursor protoporphyrin IX (PpIX). Although all human cells express heme biosynthesis enzymes and produce PpIX, tumor cells are often found to have higher PpIX production and accumulation than normal cells, allowing for the detection and treatment of solid tumors. In exploring ALA-based tumor detection and therapy in breast cancer, we have reported that high ALA-PpIX was accumulated in triple negative breast cancer cells (TNBC) contributes to reduced PpIX levels in tumor cells, causing cell resistance to ALA-PDT. The administration of an ARBG2 inhibitor Ko143 was able to reverse cell resistance to ALA-PDT by increasing PpIX levels. In this study, we showed that some kinase inhibitors were able to increase ALA-mediated PpIX by inhibiting ARBG2 activity. Particularly, our results demonstrate that lapatinib, a inhibitor of EGF/Her2, increased ALA-PpIX and cell sensitivity to ALA-PDT in the MDA-MB-231 TNBC cells. Lapatinib treatment had little effect on MCF10A breast epithelial cells. These results indicate that small molecule kinase inhibitors such as lapatinib can be used for enhancing ALA-based tumor detection and PDT in tumors with high ARBG2 activity.

**#5843 Role of CDK9 inhibition as a sensitizer to radiation in esophageal adenocarcinoma:** In vitro and in vivo efficacy study. Omkara Lakshmi M, Veeranki, Rashmi Dokey, Alicia Mejia, Zhimin Tong, Jianhu Zhang, Yawei Qiao, Pankaj Kumar Singh, Rithvik Kathakadha, Barbara Minoo, Jaime Rodriguez Canales, Steven Lin, Sunil Krishnan, Dipen Maru. UT MD Anderson Cancer Ctr., Houston, TX.

Preoperative chemoradiation in neoadjuvant setting is the standard of care for pre-region esophageal adenocarcinoma (EAC). However, less than 30% of patients develop complete pathological response indicating need for newer therapeutic strategies. Cyclin dependent kinase 9 (CDK9) is found to be over expressed in EAC compared to Barrett’s esophagus. Our previous studies demonstrated strong antitumor effects on inhibition of CDK9 in EAC both in vitro and in vivo. Here we report augmented tumor regression in irradiated xenografts on combination with Flavopiridol, a well-established clinically used CDK inhibitor, compared to single modality treatments. In vitro studies indicate that Flavopiridol could radiosensitize FLO-1 and SKGT4 EAC cells with sustained 53BP1 foci especially in SKGT4 cells. Flavopiridol and BAY1143572, a more selective inhibitor of CDK9, could radiosensitize additional EAC radiation sensitive and resistant cell lines (SKTG4-R, OE-33 and OE-33-R) as analyzed by MTT assay, apoptosis, formation of 53BP1 foci and clonogenic assay. Flavopiridol and BAY1143572 showed a radio-synergistic action by downregulating MCL-1 and Axl. In conclusion, the radio-sensitizing capacities of CDK9 inhibitors presented here suggest that their adjuvant administration might improve EAC therapy.

**#5844 Suberylnolide hydroxamic acid, a histone deacetylase inhibitor, improved radiosensitivity of human hepatocellular carcinoma.** Fang-Esin Chen,1 Ching-Fang Yu,2 Ji-Hong Hong,2,3 Chang Guang University, Taoyuan City, Taiwan; 3Chang Guang Memorial Hospital, Taoyuan City, Taiwan.

Hepatocellular carcinoma (HCC) is a leading cause of cancer–related death worldwide. Radiotherapy (RT) is one of modalities for HCC therapy in clinics, but the therapeutic effect is unsatisfactory due to high radiosensitivity of normal liver tissues that limits the optimal dose. This study is to develop a new therapeutic approach to enhance the efficiency of RT on HCC tumors. Suberylnolide hydroxamic acid (SAHA), a FDA approved chemotherapeutical drug, is an inhibitor of histone deacetylase (HDACs) and has been reported to enhance radiosensitivity in many cell lines. This study hypothesized that combination of SAHA and RT could improve tumor control on HCC tumors. HCC-834 cell, a primary culture of human HCC, had a dose-dependent response to RT when analyzed by clonogenic assay. SAHA had mild cytotoxicity to HCC-834 cell (IC50 = 16.0 μM). Combination of SAHA and RT reached a synergistic effect to prevent colony formation. In detailed, the radio-sensitizing capacities of CDK9 inhibitors presented here suggest that their adjuvant administration might improve EAC therapy.

**#5845 Identification of proteasome pathway and a novel serine threonine kinase DCLK3:** Potential therapeutic targets for innately radiation resistant glioblastoma cells. Jacinth Rajendra, 1Keshava Dutta, 1Sheikh Burhan Ud Din Faroquee, 1Raja Reddy, 1Nilesh Guality, 2Ektok Kantak, 1Atilash Moiyadi, 1Prassana Venkataraman, 1Kakoli Bose, 1 Amit Dutt, 1 Harsha Gowda, 2Shilpee Dut, 1Advanced Centre for Treatment Research and Education In Cancer, Khagr, Navi Mumbai, India; 2Institute Of Bioinformatics, Bangalore, India.

INTRODUCTION: Glioblastoma resistance and recurrence is attributed to the presence of innately Radiation Resistant (RR) cells present in the heterogeneous parent tumour. However, targeting these cells has been impossible due to inaccessibility of these cells. METHODOLOGY We therefore recapitulated clinical scenario of resistance in a cellular model developed from fresh primary GBM patient samples and cell lines. The model allowed us to capture 1) Parent cells 2) innately Radiation Resistant cells - less than 10% of the parent population and 3) Relapse (R) cells. To identify the targetable proteins governing the survival of RR cells, we performed iTRAQ based quantitative proteomic analysis on all the three populations from GBM cell line (SF268). RESULTS The proteomic data analysis identified 34 proteins as differentially present in RR population of which 22 were upregulated and 12 were downregulated. A GENE STRING analysis of all the different proteins in RR population revealed putative interaction of a novel serine threonine kinase DCLK3 with 14-3-3 zeta. The increased expression of DCLK3 and 14-3-3 zeta was confirmed by western blots in RR cells of two GBM cell lines and 8 patient samples. Meta-analysis of 242 tumor samples from COSMIC database showed DCLK3 overexpression in 232 tumors. Furthermore, it harbours 8 missense deleterious mutations, 6 of which were in the kinase domain, indicating towards an important kinase function of this protein. We hypothesized that DCLK3 mediated interaction and phosphorylation of 14-3-3 zeta modulated 14-3-3 zeta functions facilitating RR cell survival. For this, first we wanted to see if DCLK3 and 14-3-3 zeta can interact together. In silico docking of DCLK3 with 14-3-3 zeta did show interaction. This interaction was confirmed by in vitro immunoprecipitation studies. Further studies are ongoing to understand the importance of these proteins and their interaction in GBM recurrence. Additionally, pathway analysis of differentially upregulated proteins in RR cells revealed deregulation of the proteins involved in Ubiquitin-Proteosome System. Indeed, proteasome activity assay showed increased proteasome activity in the RR population of GBM cell lines and Patient samples. Accordingly, Bortezomib, a proteosome inhibitor induced significant apoptosis in the RR population at a concentration significantly lower than that required for inducing apoptosis in the parent cells. SIGNIFICANCE In conclusion this is the first study to identify a proteome signature of innately radiation resistant cells of GBM and identify proteasome pathway and a novel serine-threonine kinase DCLK3 in RR cells as a potential therapeutic target to inhibit GBM radioresistance and recurrence.

**#5846 The use of sodium sulfide, a hydrogen sulfide donor, to sensitize glioblastoma multiforme to photon and proton radiotherapy.** Adam Xiao,1 Rajesh Pidikiti,1 Matthew Maynard,1 Nader Sheibani,1 Lynn Harrison1, J. Louisiana State University Health Sciences Center Shreveport, LA; 2Willis-Knighton Cancer Center, Shreveport, LA; 3University of Wisconsin School of Medicine and Public Health, Madison, WI.

Glioblastoma multiforme (GBM) is the most common glial tumor accounting for 12-15% of all brain tumors worldwide. It is a rapidly progressing, life-threatening disease with a median survival of 3-6 months when left untreated. Minimal advancements in the treatment of GBM have limited its management. Conventional treatment with surgical resection of the primary tumor followed by photon radiation (60 Gy in 2 Gy fractions) in combination with adjuvant chemotherapy can limit recurrence and prolong median survival to 15 months. Proton radiotherapy has received increased attention in recent years due to its more precise energy deposition and 10% higher relative biological effectiveness (RBE) when compared to photon therapy. Several clinical trials have shown that high dose photon/proton therapy reduces central recurrence rate and extends median survival of GBM patients to 21-6 months. Unfortunately, the high rate of radioresistance has limited its widespread adoption. Radiosensitization agents may overcome this problem by allowing for the use of lower radiation doses to achieve comparable cytotoxic effects; however, no radiosensitizers are currently approved for the treatment of GBM. The current study supported our hypothesis and demonstrated that SAHA combined with local RT was effective on inhibiting DNA repair and anti-tumor growth. This study provided a new strategy on HCC tumor therapy that SAHA could act as a radiosensitizer to improve the therapeutic efficiency of RT on HCC tumors.
examines the use of sodium sulfide (Na2S), a hydrogen sulfide donor, as a radiosensitizing agent for both photon and proton therapy in two human glioblastoma cell lines, T98G and U87-MG. Survival curves were generated for T98G cells using a colony forming assay. Protons had an increased RBE of 1.18, which is in close agreement to the accepted RBE of 1.1. Na2S (0 μM, 10 μM, 100 μM, 500 μM, 1000 μM) was given as a pre-treatment in two 2-hour incubations: 2 hours prior to radiation and again immediately before radiation. Na2S alone exhibited a concentration dependent cytotoxic effect with maximal killing of approximately 50% at 500 μM. Further increases in sodium sulfide up to 1000 μM had no effect on cell survival. Similar killing was not observed in a non-cancer mouse retinal endothelial cell line even at extremely high doses of 1000 μM suggesting that Na2S cytotoxicity is specific and limited to cancer cells. Na2S increased killing in both photons and protons at 6 Gy by decreasing the surviving fraction from 27.7% to 20% and 16% to 3% respectively. Initial studies into the mechanism of radiosensitization using bleomycin as a radiomimetic agent have suggested Na2S increases the number of DNA double strand breaks (DSB), a highly cytotoxic form of DNA damage, as seen by γH2AX staining in both T98G and U87-MG cells. The benefits of high dose radiation therapy, particularly with proton boost, in the treatment of high grade central nervous system tumors such as GBM are apparent, but the increased risk of radionecrosis has limited its use. While further studies need to be conducted to elucidate the exact mechanism, preliminary results strongly suggest that sodium sulfide can act as a radiosensitizing agent by increasing DNA DSB formation.

We also investigate the inflammatory -cytokine profiles in the irradiated lung tissue by protein array. The results revealed that fucoidan administration changes the inflammatory -cytokine expression pattern in in the irradiated lung tissue. Conclusions: This study demonstrated that inflammatory -cytokine expression after whole lung irradiation is correlated with neutrophil accumulation and can be influenced by fucoidan supplementation. Further studies might prove that fucoidan can modulate the inflammatory -cytokine expression pattern which may lead to attenuates radiation pneumonitis and fibrosis. It is potential to be a therapeutic molecular to attenuate or prevent radiation pneumonitis.

#5847 SIRT1 protects against radiation-induced lung injury via de-acetylating the Ku70 and P65. Xianxin Xue, Lin Zhou, Feifei Na, Lei Deng, Jie Liu, Bo Li. 1 Adam Dicker, 2 You Lu. 1 West China hospital, Sichuan university, Chengdu, China; 2 Thomas Jefferson University, Philadelphia, PA.

Radiotherapy-induced lung injury (RILI) is a common and serious complication in the radiation therapy of thoracic cancer, which significantly restricts the radiation doses of tumor. The prevention and treatment of RILI have long been clinical challenges. SIRT1 is a NAD+-dependent protein deacetylase and the exact role of SIRT1 in RILI remains unclear. The aim of this study is to investigate the prevention and repairing efficacy of RILI by adenosine-mediated SIRT1 gene overexpression in a mice model. Our studies confirmed that the protein expression and activity of SIRT1 in lung tissues were decreased in RILI mice models in a time-dependent manner, but the mRNA expression level was not obviously changed. The activity of SIRT1 was elevated by orally given resveratrol, an agonist of SIRT1. The extent of RILI could be evidently alleviated by orally given resveratrol, as assayed by histopathology, malondialdehyde (MDA) and plasma cytokines (IL-1β, TNF-α and active TGF-β1). Overexpressing SIRT1 could obviously mitigate the extent of RILI (H&E staining and CT scan), the concentration of MDA and ROS in lung, IL-1β and active TGF-β1 in plasma; as well as improve the mice survival. Moreover, SIRT1 could inhibit the radiation-induced DNA damage in vitro. In vitro Co-IP experiment showed that the expression of SIRT1 in irradiated cells was reduced and the acetylated-Ku70 was increased; over-expressing SIRT1 promoted the de-acetylation of Ku70; interference of Ku70 could reverse the effect of SIRT1 in DNA repair. Furthermore, overexpressing SIRT1 could inhibit the acetylation level of NF-κB subunit RELA (P65) in lung tissue; in vitro gene expression showed the SIRT1 inhibited the NF-κB transcriptional activity. In conclusion, our results suggest that SIRT1 could evidently mitigate the RILI, repair the irradiation-induced DNA damage and inflammation via de-acetylating the Ku70 and P65. Our study may provide new avenue for the prevention and treatment of RILI in clinic.

#5848 Fucoidan administration attenuates radiation pneumonitis and fibrosis through reducing inflammatory cytokine expression in lung tissue. Szu-Yuan Wu, Taipei Medical University - Wan Fang Medical Center, Taipei, Taiwan.

Purpose: There is no well-known compound to prevent radiation pneumonitis (RP) or prophylactically use to attenuate the toxicity of RP. RP is one of major complications in chest part irradiation and limitations of radiation dose. Fucoidan is a natural sulfated polysaccharide found in the brown algae and brown seaweed. Recent studies have demonstrated its anti-inflammation effect. Since RP is an radiation induced inflammatory and fibrosis related complication, we investigated the effect of fucoidan administration on radiation pneumonitis and fibrosis. Materials and Methods: In this study, we compared the pneumonitis and fibrosis in lung tissue specimen between the lungs irradiated (10Gy/one shot) C57BL/6 mice with or without Fucoidan administration (200mg/kg/day, oral gavage for 14 days). Results: The results demonstrated that fucoidan administration attenuates pneumonitis and fibrosis in lung tissue. We found the accumulation of neutrophils in irradiated lung tissue was decreased, and the radiation induced Masson trichrome staining fibrosis was decreased.

#5849 Exosomes promote survival and migration in squamous head and neck cancer cells after ionizing radiation: Evidence for a bystander effect. Lisa Mutschelknaus,1 Omid Azimzadeh,2 Theresa Heider,1 Claudia Winkler,1 Marcus Vetter,2 Soile Tapio,1 Janine Merle-Pham,1 Rosemarie Kell,2,3 Stephan Huber,2 Lena Edalat,2 Vanja Radulovic,2 Natasa Anastasov,3 Carsten Michael,2 Michael John Atkinson,1 Simone Moertl1. 1 Helmholtz Zentrum München, Neurherberg, Germany; 2 Independent Scientist, Germany; 3 University of Tübingen, Tübingen, Germany; 4 Technical University of Munich, Munich, Germany.

Exosomes are important intercellular communicators in the acute radiation stress-response. Here we show that exosomes from head and neck tumor cells confer protective signals to their neighboring cells. This promotes the tumorigenic and radiosensitive phenotype of head and neck cancer cells. Isolation of exosomes was performed by serial ultracentrifugation of conditioned medium collected from irradiated or non-irradiated head and neck cancer cell lines M Byrne and FaDu. Quantification by NanoSight technology indicated an increased exosome release from irradiated compared to non-irradiated cells 24 hours after radiation treatment. To test whether the released exosomes influence the radiation response, exosomes isolated from non-irradiated and irradiated donor cells were transferred to non-irradiated and irradiated recipient cells. We found an enhanced uptake of exosomes, when transferred to irradiated recipient cells compared to the transfer to non-irradiated cells. Functional analyses indicate increased survival of irradiated recipient cells after exosome transfer. These findings mesh with increased DNA double strand break repair that we found after the transfer of exosomes isolated from irradiated cells. Moreover we observed an increased migration and enhanced chemotaxis induced motility after the transfer of exosomes isolated from irradiated cells. To investigate whether differential protein loading into exosomes is responsible for the functional differences we performed proteome analysis of the exosomes. We have identified 679 proteins, of which 39 were up- and 36 were downregulated by irradiation of the donor cells. In a good agreement with our functional assays, the proteome profiling showed that exosomal proteins were mainly involved in wound healing. Exosomes are functional components of the response of tumor cells to therapeutic radiation exposure. In a clinical context these results suggest that radiotherapy modified exosomes may influence the cancer progression, metastasis and therapeutic success.

#5850 Salicylate sensitizes prostate cancer (PrCa) to radiotherapy; activation of AMPK and suppression of lipogenesis. Theodoros Tsakiridis,1 Karolina Marcinko,2 Lindsay Broadfield,2 Linda Villani,2 Carrie Gerdes,1 Danitra Maharaj,3 Tom Farell,1 James Wright,1 Gregory Steinberg2.

Radiotherapy (RT) is a key therapeutic modality for PrCa that achieves reasonable disease control rates in localized disease. However, this requires dose-escalated RT, which is associated with increased toxicity to surrounding tissues and leads to deterioration of patient quality of life. Improving the therapeutic ratio in PrCa RT is of high clinical importance. Aspirin has an established role in cancer prevention and studies suggested association with improved PrCa outcomes. It was shown that its metabolite salicylate (SAL) binds directly and activates the metabolic stress sensor AMP-activated kinase (AMPK). This is likely achieved through direct binding to the AMPK β1 subunit, leading to enhanced AMPK activity through suppression of AMPK α-subunit T172 de-phosphorylation. In this study we examined the ability of SAL to sensitize PrCa to RT. Hormone insensitive (PC3) and sensitive (LnCap) human PrCa cells, as well as PC3 xenografts grown in immunocompromised nude mice were subjected to SAL and RT treatments. SAL mediated a dose-dependent inhibition of proliferation and donogenic survival in both PrCa cell lines and suppressed de novo lipogenesis (DNL). This was detected at μM concentrations of the drug, which can be safely achieved in human circulation. SAL (100-500 μM) radio-sensitized PC3 and LnCap cells, and reduced the ability of SAL to inhibit DNL. In animals treated with conformational RT (10Gy) to the tumor xenografts, Salazole feeding (chow diet containing 2.5 gr/kg) enhanced the cytotoxicity of RT detected as reduced tumor growth kinetics and weight and volume at sacrifice. Salicylate treatment of cells or salazate feeding of mice led to increased AMPK activity in cells and tumors,
inhibitory phosphorylation of Acetyl-CoA Carboxylase and reduction of Akt-Thr308 phosphorylation. These results indicate that SAL could improve radiation response in PrC through a mechanism involving the suppression of DNL. This is achieved at clinically attainable doses of SAL. This work supports prospective investigation of SAL as a radio-sensitizer in early phase clinical trials of localized PrC.


Brain tumor xenografts initiated from human glioblastoma (GBM) stem-like cells (GSCs) simulate many of the characteristics of GBMs in situ including extensive invasiveness, phenotypic heterogeneity and radiosensitivity. As an approach to investigating the relationship between these aspects of GBM biology and tumor cell proliferation, we used the halogenated thymidine analog CldU (5-chloro-2'-deoxyuridine), which is incorporated into DNA during S-phase, to identify proliferating cells in orthotopic brain tumor xenografts. Specifically, CD133+ NSC11 GSCs were implanted into the right striatum of nude mice; at various times after tumor cell implantation CldU was delivered in a series of daily intraperitoneal (IP) injections. Subsequent immunohistochemical analyses of CldU incorporation along with the GSC marker SOX2 was then used to determine the percentage of proliferating tumor cells in the mouse brain. NSC11 tumors are first detectable using bioluminescent imaging (BLI) at day 21 post-implantation. After 4 daily injections of CldU beginning on Day 21, approximately 60% and 30% of tumor cells were CldU positive in the white matter (corpus callosum) and grey matter (striatum), respectively. However, when CldU injections were initiated on Day 28 post-implantation, approximately 80% of cells were positive in both tissue locations after 4 doses, which corresponds to a rapid tumor growth phase according to bioluminescent imaging. Tumor cells were also detected in the left hemisphere and had lower percentages of CldU positive cells as compared to the right hemisphere (site of implantation), which illustrates the extensive invasive capacity of this GSC line and is consistent with the "grow or go" concept whereby GBM cells switch between proliferative and non-proliferative states.

2% of tumors cell were CldU positive in the white matter of the 3x12Gy-treated tumors and no proliferating cells were detected in the grey matter. These values can be compared to 50% and 40% CldU positive cells in the white and grey matter, respectively, of the sham irradiated tumors. Our data indicates that the unexpected membrane-to-nuclear trafficking of DUOX2 significantly reduces the levels of serum protein oxidation. Taken together these data suggest that DUOX2 could potentially be used as a biomarker to stratify patients and follow the efficacy of clinical application of chemopotentiation by LDFRT.

#5853 Peroxiredoxin I-targeted AMRI-59 has a role of radiosensitizer by promoting induction of ROS/h2AX/caspase pathway and suppression of ERK. Wan Gi Hong,1 Jeong Hyun Cho,2 Ju Yeon Kim,1 Eun Ah Lee,2 Sang Gu Hwang,1 Yong Shin Chang,1 Hong Duck Um,1 Jong Kuk Park1,2. KIRAMS, Seoul, Republic of Korea;1 Kyung Hee University Hospital, Seoul, Republic of Korea;2 Ewha Womans University, Seoul, Republic of Korea.

We have identified AMRI-59, one of small molecule, is specific pharmaceutical inhibitor of peroxiredoxin (PRX) I enzyme activity in our previous study. In this study, we tried to prove whether AMRI-59 has role of radiosensitizer against non-small cell lung cancer cells - NCi-H460 and NCi-H1299. The radiosensitizer effects of AMRI-59 was tested with MTS assays. The in silico and in vitro results suggest that induction of mitochondrial potential via enhancement of PrxI oxidation, which subsequently induced one of DNA damage markers - phospho-h2AX, and suppressed phospho-ERK of ERK, and, finally, activated caspase-3. Notably, inhibition of ROS production prevented ERK suppression, and inhibition of ERK in addition of combination with AMRI-59 and IR increased caspase activation and apoptosis. In a xenograft assay, combination with AMRI-59 and IR delayed tumor growth by 11.4 days compared with controls, yielding an enhancement factor of 1.48. Collectively, these results indicate that AMRI-59 functions as a Prx1 - targeted radiosensitizer by induction of apoptosis through activation of a ROS/h2AX/caspase pathway and suppression of ERK.

#5854 TIE2-mediated epigenetic marks regulate therapeutic resistance of glioblastoma. Mohammad Belayat Hossain,1 Renuhuma Shifat,1 Jingyi Li,1 Yisel Rivera-Mokina,1 Francisco Puerta Martinez,2 David G. Johnson,3 Mark T. Bedford,4 Mien-Chie Hung,1 Erik P. Sulfman,1 Frederick Lang,1 Raymond Sawaya,4 Candelaria Gomez-Manzano1. 1UT MD Anderson Cancer Center, Houston, TX; 2UTMD Anderson Cancer Center, Smithville, TX.

Glioblastomas (GBs), the most common subtype of primary brain tumors in adults, are resistant to current strategies of surgery, irradiation and chemotherapy with a median survival that ranges from 9 to 15 months. GBs invariably recur after therapy due to the presence of cells exhibiting a multidrug-resistance phenotype in the core of the tumor. There is an urgent need of developing the new therapeutic strategies for brain tumor treatment including the identification of novel molecular pathways regulating this resistant phenotype. One of the key phenomena of GB is that tyrosine kinase receptors (TKRs) are abnormally regulated and related to poor treatment outcomes. We have previously reported the expression of TIE2 in human surgical glioma specimens in relation to malignancy, and the role of TIE2 in endothelial-glia adhesion, tumor invasion and multi-drug resistance of gliomas. Recently, we have identified that the unexpected membrane-to-nuclear trafficking of TIE2 is related to radiosensitivity of brain tumor stem cells. Interestingly, TIE2 binds, upon irradiation stress, to DNA/protein complexes and directly phosphorylates histones. Specifically we discovered a new histone H4 mark (H4pY31) that is read by ABL1. TIE2/H4pY31/ABL1 complex binds to DNA repair proteins, such as ATM, DNA-PK, and pCHK2, activating a NHEJ DNA repair mechanism. In an effort to find the molecular mechanisms underlying TIE2 nuclear translocation, we identified that TIE2 binds and directly phosphorylates cavelin-1 (CAV1) at Tyr14 residue in vitro and in vivo. Importantly, CAV1-pTyr14 is necessary for the translocation of both TIE2 and CAV1 to the nucleus. We also uncovered new TIE2-mediated epigenetic marks. Thus, TIE2 binds and phosphorylates histone H2B at Tyr37 and this modification enhance the recruitment of DNA repair proteins to the DNA damage site. Our results summarize that upon irradiation TIE2 localizes to the nucleus where it is involved in key cellular functions by directly phosphorylating core histones, and recruiting SH2 domain proteins to the DNA damage sites, that are complexing to the DNA repair machinery. Our discovery related to tyrosine modifica-
tion of core histones might be of high significance to understand the resistance of cancer to DNA-damage inducers, which eventually might result in the design of TIE2-targeting combinational therapies for patients with GBs.

#5855 Radiosensitization of cervical cancer cells by HDAC inhibitors. Ima Paydar,1 Alfredo Velena,2 Scott Grindrod,3 Mira Jung,3 1MedStar Georgetown University Hospital, Washington, DC; 2Georgetown University, Washington, DC; 3Shuttle Pharmaceuticals, Inc, Rockville, MD.

Introduction: While histone deacetylase (HDAC) inhibitors have demonstrated in vitro and in vivo radiosensitization in several human cancer cell lines, the efficacy and mechanism of action of HDAC inhibitors in HPV-positive cancers are poorly understood. We hypothesize that HDAC inhibitors can be used to treat HPV-positive squamous cell carcinomas by enhancing radiosensitivity.

Methods: The cellular cytotoxicity of ten HDAC inhibitors was examined in HPV-positive (CaSkï, SiHa, HeLa) and HPV-negative (C33a) cervical cancer cell lines by the WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] assay, and the drug with the highest cytotoxicity (pan-HDAC inhibitor SP-1-161) was selected for further analysis. SAHA was used as a control. Evaluation of the cell cycle phase distribution was performed by flow cytometry using FACS analysis following treatment with drugs at 24, 48, and 72 hours as well as in combination with radiation in CaSkï cells (HPV-16 positive). The extent of radiation sensitization was confirmed by cellular clonogenic assays in CaSkï cells. Results: Of all drugs tested, cytotoxicity was demonstrated in a dose-dependent manner. The values of IC50 were at 1.071, 1.567, 1.71, and 0.929, respectively. SP-1-161 conferred significant cytotoxicity (0.054, 0.353, 0.0994, and 0.095 μM (SP-1-161) for CaSkï, SiHa, HeLa, and C33a cell lines, respectively. SP-1-161 conferred significant cytotoxicity (0.054 μM) in CaSkï cells. Cellular radiation sensitivity is tightly associated with cell cycle phases and varies as a function of the position in the cell cycle. To assess the effects of HDAC inhibitors on the cell cycle, CaSkï cells were treated with IC50 dosage (SP-1-161 at 0.054 μM, SAHA at 1 μM) for 24 hours, 48 hours, and 72 hours and then treated with radiation. The drugs alone arrested cells primarily in the G1 phase of cell cycle (77.3% for SP-1-161, 84% for SAHA), in a relatively radiation-sensitive phase. Pre-treatment of SP-1-161 for 24 hours prior to radiation (5 Gy) increased cell cycle arrest in the G1 phase as well. To determine the radiosensitization property of SP-1-161, the radiation clonogenic survival assay was performed following pre-treatment of SP-1-161 for 24 hours. Cells were exposed to graded doses of γ-irradiation, and the data were fitted using the single-hit multitarget and the linear-quadratic models. Comparisons of survival curves revealed smaller D0 (D0 ratio 1.43) for cells irradiated in the presence of SP-1-161 (D0 = 1.4) than for controls (D0 = 2.0). Interestingly, treatment with SAHA did not impact radiosensitization (D0 = 1.9, D0 ratio 1.05). Conclusion: SP-1-161 conferred significant toxicity in CaSkï cells and primarily arrested cells in the G1 phase of cell cycle. Furthermore, treatment with SP-1-161 enhanced radiosensitization, while SAHA showed little change despite cell cycle arrest primarily in the G1 phase. Together, our results support SP-1-161 as a new therapeutic agent with potential for cervical cancer.

#5856 Molecular mechanism underlying breast cancer cell radioresistance: role of radiation-induced NF-κB self-sustenance. Hui Yu,1 Natarajan Aravindan,1 Ji Xu,3 Mohan Natarajan1. 1UT Health Science Ctr. at San Antonio, San Antonio, TX; 3University of Oklahoma Health Sciences Center, Oklahoma City, OK; 4Basic Medical College, Zhengzhou University, Henan, China.

Understanding the mechanism of breast cancer cell radioresistance and the associated selection pressure, tumor recurrence, would help us to develop molecular interventions and achieve disease/progression-free survival in patients with cancer. In the present study, we investigated the molecular mechanisms prompted in breast cancer cells in response to clinical radiation and the associated coordination of intra- and inter-cellular signaling that orchestrate radioresistance and tumor relapse/recurrence. Our findings showed that clinical doses of radiation increase NF-κB transcription and DNA-binding activity. Further, radiation exposure resulted in de novo TNF-α synthesis and secretion. An NF-κB gene manipulation approach revealed that NF-κB regulated NF-κB-mediated TNF-α transcriptional activity. NF-κB DNA binding kinetic analysis demonstrated that both sustained and dual phase NF-κB activation with radiation. Radiation prompted NF-κB-dependent synthesis/secretion of TNF-α, a requisite for the second phase of NF-κB activation and maintenance.

Radiation-associated NF-κB-dependent secretion of TNF-α from irradiated cells also activated NF-κB in radiation-irradiated, non-targeted bystander cells. Together, these findings demonstrated that radiation-triggered NF-κB-dependent TNFα secretion is critical for self-sustenance of NF-κB (through autocrine positive feedback signaling) and for coordinating bystander response (through intercellular signaling of de novo activation of NF-κB in bystander cells) after radiation exposure. Further, the data suggest that this NF-κB→TNF-α→NF-κB feedback-dependent self-sustenance of NF-κB could drive the selection pressure (proliferation, clonal expansion) of surviving breast cancer cells and subsequent tumor relapse/recurrence.

#5857 HER2-targeted thorium-227 conjugate (HER2-TTC): Efficacy in a HER2 positive orthotopic bone model. Jenny Karlsson,1 Urs B. Hagemann,1 Christoph Schatz,2 Derek Grant,1 Christine Ellisengo,1 Alexander Kristian,1 Dessislava Mihaylova,1 Steinar R. Uan,1 Mari Suominen,2 Roger M. Bjerke,1 Olav B. Ryan,1 Carl F. Nising,1 Dominik Mumberg,3 Alan Cuthbertson,4 Bayer AS, Oslo, Norway; 5Bayer AG, Berlin, Germany; 6Pharmacist Services Ltd, Turku, Finland.

In 2015, the estimated incidence of new breast cancer (Ca) cases in the US was 234,190 and number of deaths 40,730. Human epidermal growth factor receptor 2 (HER2) is encoded by the proto-oncogene c-erbB-2 and initiates downstream signaling pathways leading to cell proliferation and tumorigenesis. HER2 is overexpressed in several cancer types and has emerged as one of the most strongly validated targets for the treatment of breast and gastric cancer serving as both a prognostic and predictive biomarker. Given that 20% of breast Ca patients are HER2 positive and 70% of patients with metastatic disease will develop bone metastases and associated morbidities, there is still an unmet medical need for improved therapies targeting HER2. Radium-223 (Ra-223) is a novel targeted alpha therapeutic for treatment of patients with castration-resistant prostate cancer and bone metastases. Localized high energy alpha particle emission from alpha-emitting radiolabeled therapeutic antibodies enables efficient radio-labeling with the alpha particle emitting radionuclide Th-227. Anonymized samples of breast cancer patients were analyzed by Immunochemistry (IHC). The IHC data demonstrated HER2 positive expression in breast tumor and matched bone metastases, supporting the preclinical evaluation of the anti-tumor efficacy of HER2-TTC in the BT-474 orthotopic bone mouse model. HER2-TTC was prepared at high radiochemical yield and purity. When tested for binding to recombinant HER2, HER2-TTC was shown to retain comparable binding affinity to trastuzumab. In vitro cytotoxicity experiment of HER2-TTC demonstrated target mediated in vitro cytotoxicity in the m4-range on breast cancer cell line BT-474 (430 000 mAbs bound/ cell as determined by FACS).

Anti-tumor efficacy of HER2-TTC was evaluated at 250 and 500 kBq/kg at a protein dose level of 7.4 mg/kg. Using imaging, serum biomarker analysis (PINP on micro CT 3D reconstruction imaging and histological analysis demonstrated significantly reduced bone lesions and tumor induced bone remodeling. The promising preclinical anti-tumor activity supports the development of the HER2-TTC as a novel targeted alpha therapeutic for the treatment of patients with HER2 positive bone metastatic disease.


Radioresistance is a challenge in the treatment of esophageal squamous cell carcinoma (ESCC). MicroRNAs (miRNAs) are known to play an important role in the functional modification of cancer cells and recent studies have reported the miRNA-mediated radiotherapy resistance. However, further research would be necessary to reveal the regulation mechanisms and the treatment strategies using miRNA have yet to be established in ESCC. We compared the miRNA expression profile in ESCC parental (TE-4) and acquired radioresistant (TE-4R) cell line using miRNA microarray and qRT-PCR. Our data showed that miR-338-5p, one of the target miRNA biomarkers, was significantly downregulated in TE-4R. Ectopic overexpression of the miR-338-5p induced apoptosis and sensitivity to radiation treatment by interfering the survivin, which is known to play an inhibitor of apoptosis. Over expression of survivin using plasmid vector reversed non-338-5p induced apoptosis. From tumor xenograft experiments, we found that therapeutic delivery of the miR-338-5p mimics via direct injection to tumor mass increased sensitivity to radiation therapy. In conclusion, these findings suggested that miR-338-5p is a potential radiosensitizer and may be one of the therapeutic biomarkers for radiosensitive in ESCC.
HER2-targeted thorium-227 conjugate (HER2-TTC): Efficacy in preclinical models of trastuzumab and T-DM1 resistance. Jenny Karlsson,1 Urs B. Hagemann,1 Christoph Schatz,2 Derek Grant,1 Alexander Kristian,1 Christine Ellingsen,1 Dessislava Mihaylova1, Solene Geraudie,2 Bard Indreidel,2 Uta Wirnitzer,2 Roger M. Bjerve,1 Olav B. Ryan,1 Carl F. Nising,1 Dominik Mumbow,1 Gunilla Roland,1,3 Bayer AS, Oslo, Norway; 2Bayer AG, Berlin, Germany; 3Bayer AG, Wuppertal, Germany.

The human epidermal growth factor receptor 2 (HER2) is encoded by the proto-oncogene c-erbB-2 and initiates downstream signaling pathways leading to cell proliferation and tumorogenesis. HER2 is overexpressed in several cancer (Ca) types and is one of the most strongly validated targets for the treatment of breast and gastric cancer. Here we report a prognostic and predictive biomarker. HER2-targeting antibodies as well as antibody-drug conjugates are either approved or are in clinical development. Prolonged treatment with monoclonal antibodies and antibody drug conjugates have resulted in development of resistance and so there is still an unmet medical need for drugs of new mechanism of action targeting this important receptor system. We describe herein the development of a high energy, alpha particle emitting HER2-targeted thorium-227 antibody-chelator conjugate at Th-227. HER2-TTC consists of the humanized HER2 targeting IgG1 antibody (trastuzumab) covalently linked via an amide bond to a 3.2-hydroxypropyridine-based chelator moiety, enabling efficient radiolabeling with the alpha particle emitting radionuclide thorium-227 (Th-227). HER2-TTC was prepared at high radiochemical yield and purity. When tested for binding to recombinant HER2, HER2-TTC was shown to retain full affinity to the same degree as trastuzumab. In vitro cytotoxicity experiments were performed on 8 cell lines with different HER2 expression levels (from 7000-227,000 mAbs bound/ cell as determined by FACS) of breast, ovarian, gastric and prostate cancer. Tumor cell lines were tested for binding to recombinant HER2, HER2-TTC was shown to interact with HER2-expressing cells in a dose-dependent fashion. HER2-TTC demonstrated target mediated in vitro cytotoxicity in the P3 range. In vivo biodistribution and anti-tumor efficacy of HER2-TTC was evaluated in the dose range 100-500 kBq/kg at a protein dose of 0.14 mg/kg i.v. and showed a dose-dependent antitumor effect. Significant antitumor activity was observed in the HER2-TTC in the JIMT-1 s.c. breast cancer xenograft model. HER2-TTC demonstrated target-mediated in vivo cytotoxicity in the P3 range. In vivo biodistribution and anti-tumor efficacy of HER2-TTC was evaluated in the dose range 100-500 kBq/kg at a protein dose of 0.14 mg/kg i.v. and showed a dose-dependent antitumor effect. Significant antitumor activity was observed in the HER2-TTC in the JIMT-1 s.c. breast cancer xenograft model.

TUMOR BIOLOGY: Radioprotectors, Radiosensitizers, and Radiation Resistance

Melvin L.K. Chua,1 Erle Holgersen,2 Veronica Sabelnykova,1 Paul C. Boutros,2 Robert G. Bristow1.

Radiotherapy is an effective and integral part of cancer treatment in many solid tumors. However, tumor resistance to radiation is a major clinical challenge. The use of radiotherapy is closely related to the expression of the p16 (INK4a)-CyclinD-CDK4/6-Rb pathway in prostate cancer cells. Overexpression of the CDK4/6 kinase regulates expression of cyclin D1 and E, which is essential for the G1/S transition. Inactivation of Rb leads to the derepression of cyclin D1 and E, which in turn activates the cell cycle. The CDK4/6-Rb pathway is also important for the regulation of cell proliferation, differentiation, and apoptosis. Alterations in the CDK4/6-Rb pathway have been observed in many cancer types, including prostate cancer, and are associated with poor prognosis.

Copy number aberration (CNA) profiling was performed on 33 anatomically distinct prostate cancer samples at the time of recurrence following high dose precision radiotherapy. The aim of the study was to investigate if clonal selection or adaptation of new clones dominates in prostate cancer at the time of recurrence following high dose precision radiotherapy. Here, we investigated if clonal selection or adaptation of new clones dominates in prostate cancer at the time of recurrence following high dose precision radiotherapy. We identified 11 patients with biopsy-proven multi-focal recurrent prostate cancer following definitive image-guided radiotherapy/brachytherapy. Copy number aberration (CNA) profiling was performed on 33 anatomically distinct tumour foci with 11 matched-normals in the radio-resistant cohort. To assess clonality, 4 cases had matched pre-radiotherapy tumours for copy number profiling. We evaluated for recurrent driver amplifications and deletions, and genomic instability as measured by percent genome aberration (PGA). We also compared these genomic indices against 373 comprehensively profiled sporadic prostate cancers from the Canadian Prostate Cancer Gene Network [Fraser, et al., Nature, 2016]. Results: Independent of Gleason grade, we observed large intra-patient (COV of 0.66-1.13) and inter-patient heterogeneity (p <0.001, one-way ANOVA) in the levels of genomic instability, as judged by PGA scores, among the radioresistant tumours. Interestingly, although total CNA cancer scored as both non-recurrent and recurrent between the two datasets (PGC/GENE) cohorts (median CNAs of 40, radioresistant vs 33, sporadic, p = 0.20), we observed a trend for increased genomic instability in the radioresistant cohort (median PGA of 8.8 vs 4.9, p = 0.059). This concurs with the findings on intra-tumoural spatial CNA analyses, which revealed the acquisition of CNAs that were both common and non-recurrent in the multi-focal radioresistant tumours, thus suggesting a common origin with subsequent divergent evolution. Importantly, we observed a mixture of CNAs, including known drivers of aggressive prostate cancer, namely NKX3-1, Pten, TP53, CDKN1B, and CDH1, that was shared between pre-radiotherapy and radioresistant tumours, supporting a clonal selection process. We also discovered a novel deleted region on Chr3p, consisting of RAD18 and FANCD2, which was unique only in the radioresistant tumours. Conclusions: Our novel observations in a small cohort of radioresistant prostate cancers favour the model of selection of radioresistant clones, as opposed to new-onset tumours. These results support the current approach of discovering biomarkers a priori, and molecular therapeutic targets for these radioresistant clones, so as to improve the therapeutic ratio and precision radiotherapy.


Dysregulation of the p16INK4a-CyclinD-CDK4/6-Rb pathway in patients with NSCLC (Non-Small Cell Lung Cancer) is a rational therapeutic target. The current study investigated the radiosensitizing potential of a novel CDK4/6 inhibitor, LY2835219 (LY, Abemaciclib) in NSCLC cell lines with varied genomic context to identify genomic and metabolic biomarkers that are predictive of a response over conventional EGFR/tyrosine kinase inhibitor (EGRF-TKI) therapy. NSCLC cell lines were exposed to LY (0-10uM) for 24 hr immediately after 0-10 Gy radiation. Cell survival was assessed by clonogenic assay and cell cycle distribution was quantified by flow cytometry. Dose modifying factors (DMF) were calculated at 10% survival from radiation survival curves. Altered DNA repair pathways and metabolic profiling of cells post LY treatment was assessed by immunoblot and LC/MS mass spectrometry analysis. LY treatment enhanced radiosensitivity of EGRF-TKI sensitive (HCC827, PC9) and EGRF-TKI resistant (H820 and H1975) cell lines with DMF of 1.3 (±0.06), 1.4 (±0.30), 1.5 (±0.51) and 1.3 (±0.02), respectively. Values in the parenthesis indicates standard deviation. Wild type EGRF expressing cells (A549 and H460) also showed enhanced radiosensitivity by LY with DMF of 1.6 (±0.09) and 1.75(±0.15), respectively. Interestingly, no radiation enhancement by LY was observed for cells deficient in functional PTEN (H1650, RB (H82) and p53 (H460 DNP53 and H1975) protein. Radiosensitization was also observed for cells made resistant to third generation EGRF-TKI, AZD9291. Flow cytometry analysis of majority of cell types exposed to LY exhibited 55% to 94% GI arrest (depending on cell type). Mechanistically, the combinatorial treatment in radiosensitive cells showed elevated phosphorylated-Rb/A2. Combination treatment also reduced expression of ATR, ATM, DNA-PK, Rad51 and Chk2 suggestive of reduced DNA double strand break repair compared to radiation alone. LY treatment brought major changes in the glycolysis/TC/A/ total amino acids. LY increased significantly Acetyl-CoA, fumarate and malate, indicating enhanced oxidative phosphorylation. LY significantly elevated Uracil acid levels suggestive of oxidative stress and elevated nucleotide degradation. Finally, administration of 100mg/kg LY2835219 for five days in combination with fractionated dose of radiation (3 Gy) significantly delayed tumor regrowth in H640 xenograft (p< 0.014). Collectively, our pre-clinical data indicates altered Rb, p53 and PTEN status are distinct predictive biomarkers of response for LY mediated radiosensitization and provides an alternative therapeutic option in overcoming EGRF-TKI resistance in NSCLC.

YAP1 mediated CDK4/6 activation confers radiation resistance in esophageal cancer: rationale for the combination of YAP1 and CDK4/6 inhibitors in EC. Fan Li, Xiaoqiong Dong, Hai Tao Zhu, Ailing W. Scott, Lang Ma, Jiankang Jin, Jeannelln Santiano Estrella, Heath Skinner, Randy L. Johnson, Shumei Song, Jaffer A. Ajani. UT M.D. Anderson Cancer Ctr., Houston, TX.

Background: Esophageal carcinoma (EC) is a lethal disease with high incidence globally and often exist therapy resistance. Alterations (either overexpression or amplification) of YAP1 and CDK4/6 were found frequently in esophageal cancer. Deregulation of these pathways may represent key elements for resistance in esophageal cancer. Methods: Expression of YAP1 and CDK4/6 were examined in esophageal tumor tissues as well as cell lines using immunochemistry and immunoblotting. Inducible YAP1 overexpression in EC cells by lentivirus system was performed to test YAP1 mediated CDK4/6 expression and activation and association with radiation resistance. YAP inhibitor CA3 and CDK4/6 inhibitor Lee011 were used to test their antitumor activities in vitro and in vivo. Cell proliferation assay (MTS), Flow cytometry and immunofluorescence and tumor sphere formation assay were performed to test cell cycle distribution, cancer stem cell (CSC) population maintenance and over growth and therapy resistance. Results: We demonstrate that overexpressed YAP1 is positively associated with CDK4/6 expression in resistant tumor tissues and EC cell lines. Overexpression YAP1 by inducible lentivirus system in EC cells up-regulates expression of CDK4/6 at level of its transcription, while knock down YAP1 in HESEO EC cells dramatically decreased CDK6 expression as well as its transcription. CA3, a novel YAP1 inhibitor, was able to decrease activation of
CDK4/6 and phosphorylation RB and cell cycle progression induced by YAP1. Interestingly, we found that inducible YAP1 high EC cells are resistant to radiati
on resistance and CDK6 knockout by Lenti-Crisp sensitize radiation resistance in EC cells induced by YAP1. Further, we demonstrated radiation resistant cells are enriched CSC population and demonstrated increased level of both YAP1 and CDK4/6 in vitro and in vivo. Conclusions: Our data provide evidence that YAP1 mediated CDK4/6 up-regulation plays an important role in conferring radiation resistance in esophageal cancer cells. Targeting both YAP1 and CDK4/6 may provide novel therapeutic strategies in EC. Corresponding Authors: Shumei Song. Tel: 733-834-6144; Email: ssong@mdanderson.org; or Jaffer A. Ajani, Tel: 713-792-3685; Email: jajani@mdanderson.org

#5863 Identifying the radiosensitizing effects of PARP inhibitor in ovarian cancer. Yue Bi,1 Ioannis Verginidias,1 Souvik Dey,2 Imlang Guo,2 Lilie Lin,2 Yanfang Zheng,2 Constantinos Koumenis1. 1University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA; 2Zhongshan Hospital of Southern Medical University, Guangzhou, China.

Ovarian cancer is the seventh-most common cancer with a high mortality rate among women. Radiation therapy (RT) is used in ovarian cancer for local re-lapse, but doses are limited due to concern for small bowel obstruction in pa-tients who have had multiple surgeries and chemotherapeutic regimens. There-fore, it’s an ideal setting to improve therapeutic window by combining RT and a radiosensitizer. Poly (ADP-ribose) polymerase-1 (PARP-1) is involved in the recognition of DNA damage and the facilitation of DNA repair by recognizing DNA single-strand breaks (SSB). Inhibition of PARP1, especially in tumors driven by BRCA1 or 2 mutations, has been shown to potentiate the DNA-damaging effects of radiation in vitro and in vivo and to increase tumor vasculature perfusion and oxygenation. Approximately half of ovarian cancers have germ-line or somatic BRCA gene mutation, epigenetic silencing or other mutations that affect homologous recombination (HR) competency resulting in DNA repair defects, making this tumor particularly susceptible to the radiation-sensi-tizing effects of PARP inhibitors. Here, we investigated the therapeutic effects of the PARP inhibitor olaparib in preclinical models of ovarian cancer. Basic BRCA1 and PARP1 protein and mRNA levels were characterized in a panel of ovarian cancer cell lines. The radiosensitizing effects of olaparib were tested on both HR-proficient and HR-deficient ovarian cancer cells by evaluating the colo-ny formation, DNA damage, apoptosis, and PARP1 activity. Survival benefit of olaparib was also examined in xenograft models of ovarian cancer. Our results demonstrate that combination of olaparib with IR decreased colony formation, inhibited DNA damage (γH2AX) repair, and induced more apoptosis compared to olaparib or IR alone. In addition, HR-deficient cells were more sensitive to IR than HR-proficient cells in the presence of olaparib. Furthermore, olaparib inhibited PARP1 activity in vivo, significantly decreased tumor growth and in-creased overall survival when combined with IR in mice bearing subcutaneous xenografts of HR-deficient OVCAR8 cells while producing a relative modest increased overall survival when combined with IR in mice bearing subcutaneous xenografts of HR-proficient SKOV3 cells. These results provide a preclinical rationale for improved treatment mo-dalities using olaparib as an effective radiosensitizer in ovarian cancer, particu-larly in tumors with HR-deficiencies.

#5864 Targeting brain cancer stem cells by potentiating radiation-in-duced ER stress. Regina M. Graham, Suneetha S. Shah, Alexis J. Musick, Win-ston Walters, Ricardo J. Komotar, Jeffery S. Prince, Steven Vann, Univ. of Miami Miller School of Medicine, Miami, FL.

Background. Glioblastoma (GBM) is one of the most malignant brain tumors occurring in both children and adults. Despite an aggressive treatment regimen consisting of surgical resection, radiation therapy and chemotherapy the five-year survival rate is less than 5%. This poor outcome has been attributed to the existence oftherapy-resistant GBM stem-like cells (GSCs), which are believed to be responsible for tumor recurrence and patient relapse. Understanding the resistance mechanisms employed by brain tumor stem cells and developing novel methods to target these cells is necessary for prolonged patient survival. Recent evidence suggests that the endoplasmic reticulum stress response path-way may mediate therapeutic resistance in cancer. Here we sought to examine the ER stress response pathway of GSCs in response to ionizing radiation and to increase the extent of ER stress in an effort to promote cell death using the glycolytic inhibitor, 2-deoxy glucose (2-DG). Methods. GSC lines were derived from resected tumor sections. GSCs were irradiated using the Rad Source 2000 Series Biological Irradiator. Transmission Electron Microscopy (TEM) was used to investigate the ultra-structural alterations of GSCs following exposure to 8 Gy radiation. Viability was determined using trypan blue exclusion, MTS and LDH assays. Cell signaling pathways were investigated by western blot analysis. Results. Consistent with previous findings, minimal cell death was observed in GSCs exposed to 2-20 Gy radiation. TEM analysis revealed that exposure to 8 Gy significantly increased ER luminal dilution, suggestive of ER stress. Western blot analysis indicated an increase in ER stress markers GRP78, GRP94 and CHOP, confirming that radiation induces ER stress in GSCs. Treatment with 2-DG induced an increase in ER luminal diameter and ER stress markers. Combined 2-DG (0.5mM and 2mM) and radiotherapy (8 Gy) significantly increased ER luminal diameter and ER stress marker expression over radiation or 2-DG alone. Furthermore, the combination of 2-DG (0.1-2mM) significantly reduced GSC viability compared to radiation or 2-DG alone. Conclusion. The ER stress response pathway is an adaptive mechanism and thought to mediate therapeutic resistance. Here we demonstrate that radiation induces ER stress response pathway including ER luminal dilution and an increase in molecular chaperone ex-pression in GSCs. Potentiating ER stress can switch the pathway from one of adaptation to cell death. 2-DG increased radiation-induced ER stress and pro-moted cell death. Our data suggests that targeting this adaptive response could increase the efficacy of radiotherapy and prolong patient survival.

#5865 The role of t-Darpp in radiation sensitivity and resistance. Santosh K. Singh, James W. Lillard, Rajesh Singh. Baltimore Research Institute of the City of Hope, Duarte, CA; University of California Los Angeles, Los Angeles, CA, CA.

t-DARPP, one of two protein isoforms encoded by the PPP1R1B gene, is overex-pressed in gastric, esophageal, colon, prostate, and breast cancers; and confers resistance to trastuzumab in Her2++ breast cancer cells. To gain insight into the trastuzumab-resistance mechanism of t-Darpp, we studied its structure, oligo-gomerization status, metal-binding properties, and sites of phosphorylation. Circular dichroism spectroscopy analysis showed that recombinant t-Darpp ex-hibits 12% alpha helix, 29% beta strand, 24% beta turn and 35% random coil character at 25°C. Upon mild heat treatment (50°C for five min) the secondary structure does not appreciably change. Metal ion-binding analyses through in-ductively coupled plasma-mass spectrometry and graphite furnace-atomic ab-sorption indicated that t-Darpp co-purifies with calcium, but not other metals commonly found in biological systems. The T75 site, critical for t-Darpp activa-tion of the Akt signaling pathway, is a substrate for phosphorylation by cyclin-dependent kinase 1 (CDK1) and cyclin-dependent kinase 5 (CDK5). Gel filtration chromatography, sedentation equilibrium analysis, native blue gel electrophoresis, and glutaraldehyde-mediated crosslinking experiments showed that the majority of t-Darpp (calculated mass = 19 kDa) exists as a monomeric protein but forms low levels (<3%) of hetero-oligomers with its longer isoform Darpp-32. t-Darpp has a relatively large Stokes radius of 4.4 nm, suggesting an elongated structure. In summary, this study indicates that t-Darpp is an elongated, mild-heat-stable, and monomeric calcium-binding protein that is capable of being phosphorylated at T75 by CDK1 and CDK5. Blockage of t-Darpp calcium binding capacity or T75 phosphorylation may therefore help sustain Her2++ breast cancer sensitivity to trastuzumab therapy.
affinity folate receptors presents on PCa cells. The resulting PBM nanoparticles were shown to be rapidly internalized and induce significant cancer cell apoptosis at lower doses compared to unformulated resveratrol and/or docetaxel. Our data showed that resveratrol potently synergizes with docetaxel to inhibit proliferation and induce cell death in resistance PCa cells. The up-regulation of serotoninergic transporter (SERT), p53 and p21 were induced in response to resveratrol-docetaxel treatment. These results suggest that the co-delivery of resveratrol, and a cytotoxic agent in a PBM nanoparticle might potentially improve the treatment of drug-resistant tumors. [This work is supported from Grant #5S1CA193758-03]

#5868 Role of STAT3 vs AKT in cellular survival. Maximilian Niti, Joshua Rosen, Rozanne Arulananandam, Victoria Hoskin, Bruce Elliott, Leda Raptis. Queen’s University, Kingston, Ontario, Canada.

Cell-to-cell adhesion is mediated by the cadherin family of proteins. We previously demonstrated that cadherin engagement (which is favored under conditions of confluence) triggers a dramatic increase in Rac protein levels, followed by an increase in IL6 secretion and Stat3 (Signal Transducer and Activator of Transcription-3) activation. This is critical for survival of both normal and neoplastic cells. On the other hand, cell adhesion to the substratum, ie the extracellular matrix at the focal adhesion sites, is mediated by the integrin receptors. The focal adhesion kinase (FAK) binds beta-integrins and this forces FAK in an open conformation. Autophosphorylation of tyrosine at Y576 phosphorylates Src to affect cell fate. Bosutinib inhibited the growth of both parental cells and HR cells, and induced apoptosis and G1 arrest in HR cells. Bosutinib suppressed HR cell migration more effectively compared with parental cells. Bosutinib exhibited potent tumor growth inhibition in both SNU2670 and SNU2670HR xenograft models and more significantly suppressed tumor growth in HR models. Conclusion: Src activation may contribute to tumor cell adhesion resistance in part to HER2-positive gastric and biliary tract cancer cells. Targeting Src might be a candidate strategy to overcome trastuzumab resistance in HER2-positive cancers.

#5870 Epigenetic drug treatment overcomes osteoblast-induced chemoresistance by suppressing cell adhesion and related signaling. Anthony Quaglino, Sonali Barwe, Anilkumar Gopalakrishnapillai. Nemours/A.I. duPont Hospital for Children, Wilmington, DE.

Acute lymphoblastic leukemia (ALL) is the most commonly diagnosed cancer in pediatric patients. Recent advances have allowed for improved efficacy of treatment allowing 95% of patients to achieve remission following chemotherapy. However, nearly 20% will have a second recurrence that is often more aggressive and difficult to treat. One reason for this high rate of relapse may be due to the role of the bone marrow microenvironment (BM) consisting of osteoblasts, endothelial cells, adipocytes, and stromal cells as well as extracellular matrix proteins. Interaction between leukemic cells and BM elements activates intracellular signaling pathways that protect ALL cells from chemotherapy. Many of these pathways can be aberrantly activated due to changes in methylation patterns during leukemogenesis. Therefore, utilizing epigenetic modifiers offers a unique approach to overcoming the chemoprotective effects of the BM. The aim of our study is to identify if the combination of the epigenetic drugs azacitidine (DNA methyltransferase inhibitor) and panobinostat (histone deacetylase inhibitor) is successful in overcoming these effects. We demonstrated that azacitidine and panobinostat are more effective in killing ALL cells in coculture with osteoblasts than chemotherapy alone. This required direct interaction between ALL cells and osteoblasts and could not be replicated when ALL cells were suspended in Transwells above the osteoblast monolayer. Additionally, ALL cells pretreated with non-killing concentrations of azacitidine and panobinostat were sensitized to chemotherapy even in the presence of osteoblasts. These effects were replicated ex vivo in primary ALL patient samples with a variety of cytogenetic characteristics. These patient samples have been xenografted in mice to observe the efficacy of this combination with chemotherapy in comparison to chemotherapy alone. We also observed that treatment with azacitidine and panobinostat decreases the ability of ALL cells to effectively adhere to osteoblasts, suggesting a role that down-regulation of cell adhesion molecules (CAMs) may play in mediating this response. Due to the need for direct contact of ALL cells with osteoblasts for the chemoprotective effects, we investigated how treatment with azacitidine and panobinostat could affect the expression levels of certain CAMs. We found that N-cadherin was induced in ALL cells in coculture with osteoblasts and that increase was partially reversed by azacitidine and panobinostat. Beta-catenin, which is known to interact with N-cadherin, was also up-regulated in ALL cells cocultured with osteoblasts and was reversed by azacitidine and panobinostat. These data suggest that azacitidine and panobinostat may overcome microenvironment-induced chemoprotection by decreasing the expression of certain CAMs like N-cadherin and interfering with their downstream signaling pathways.

#5871 Characterizing hormone therapy-resistance phenotypes in metastatic breast cancer conferred by estrogen receptor (ER) mutations. Shanhang Jia,1 Ryan Hennesius,2 Marilyn Ngo,2 Peiui Wang,3 Amir Bahreini,2 Ning Chen,2 Zhiye Ding,4 Tongying Shun,2 Lansing Taylor,2 Shannon Puhalla,2 Adrian Lee,2 Steff Oesterreich,3 Andrew M. Stern,2 Mark T. Miedel2. Tsinghua University, China;1 UPDDI, Pittsburgh, PA; 2UPMC, Pittsburgh, PA.

232,000 new cases of invasive breast cancer will be diagnosed in 2016. 40,300 patients will die primarily from metastatic disease. Mortality results from the ability of cancer to evolve and evade therapy. Estrogen receptor (ER+) breast cancer accounts for 70% of invasive breast cancer. The mainstay treatment of ER+ breast cancer involves estrogen deprivation therapy using aromatase inhibitors as well as estrogen receptor antagonists and degraders. We and others have shown that patients treated with aromatase inhibitors often (14-54%) acquire ESR1 mutations in their metastases in contrast to only a 0.5% ESR1 mutation frequency detectable in their primary tumors. We hypothesize that ESR1
Gateways of evolution and represent targetable dependencies for ER+ metastatic disease. We reasoned that characterization of the phenotype engendered by ESR1 mutations under physiologically relevant conditions will help us understand the mechanism of ER+ metastatic cancer. To achieve this objective we have stably expressed the two most common independent (constitutive) ERE transactivation in contrast to the parental and wild type control cells. Furthermore, partial and potently clinically relevant resistance of these ESR1 mutant-expressing cells to ER antagonists such as fulvestrant and 4-hydroxytamoxifen was evident. In addition, using this reporter assay, mutant ESR1-expressing cell lines show similar resistance in the absence of estrogen. We are using a human microphysiologymodel of liver metastasis as a complementary approach to patient-derived xenograft models to investigate metastatic associated phenotypes conferred by these mutations. Since our previous studies indicated the existence of polyclonal mutations within individual patients (P.Wang, A. Bahreini et al., CCR 2016), we are testing the hypothesis that the persistence of this heterogeneity results from cooperation among these mutant-expressing clones. These studies form the basis for our continuing efforts to understand ER+ metastatic disease and use this knowledge to identify more effective therapies.


Targeted cancer therapies can effectively promote tumor regression in clinical responses. Eventually, most tumors develop resistance to these drugs. Stat3 activation has been suggested as one of the mechanisms that cause acquired resistance to EGFR-tyrosin kinase inhibitor (TKI) in non-small cell lung cancer (NSCLC). However, the underlying molecular mechanisms are not well studied. In this study, we found that treatment of gefitinib (EGFR-TKI) in NSCLC cell lines harboring EGFR-TKI sensitive mutation induced feedback activation of Stat3 in a time- and dose-dependent manner. High levels of IL-6 were detected in the conditioned medium of gefitinib-treated cells by cytokine array and were confirmed by ELISA. We demonstrated that IL-6 treatment could induce Stat3 activation and conditioned medium treatment-induced Stat3 activation was suppressed by IL-6 neutralizing antibody. We also demonstrated that gefitinib treatment-induced IL-6 secretion could be inhibited by knockdown of Stat3 expression. Moreover, pharmacochemical inhibition and genetic inhibition of Stat3 activation increased cell cytotoxicity of gefitinib in both PC9 and HCC827 cells that harboring EGFR-TKI sensitive mutation. Our data suggested that gefitinib treatment could induce activation of IL-6/Stat3 signaling loop and modulate cell cytotoxicity of gefitinib. Microarray and Ingenuity Pathway Analysis (IPA) analysis revealed prostaglandin D2 synthase (PTGIS) as a downstream target gene of IL-6/Stat3 signaling and PTGIS was up-regulated by gefitinib treatment. The induction of PTGIS by gefitinib via Stat3 feedback activation was confirmed in vitro genetically and pharmacologically. Moreover, we showed that cotreatment with PTGIS inhibitor improves the efficacy of EGFR inhibition in PC9 and HCC827 cells. Importantly, high expression of PTGIS was found in gefitinib-resistant PC9 cells (PC9/gef) compared with gefitinib-sensitive PC9 cells. Targeting PTGIS was also effect in decreasing viability of cells with acquired resistance to gefitinib in PC9/gef cells. Taken together, our study indicated that Stat3 feedback activation-induced PTGIS expression participates in modulating gefitinib efficacy and Stat3/PTGIS inhibition could potentially overcome acquired resistance to gefitinib in NSCLC.

#5873 Chaetocin sensitizes GBM cells to TRAIL and BH3 mimetics induced apoptosis. Ezgi Özverli, Zeynep Kahya Yeşil, Udo Oppermann, Tuğba Bağcı Önder1. Koç Univ., İstanbul, Turkey; 2University of Oxford, Oxford, United Kingdom.

Glioblastoma Multiforme (GBM) is the most common and aggressive brain tumor, which lacks efficient therapy. TRAIL is an antitumor agent that triggers apoptosis selectively on tumor cells, thus can be utilized as therapeutic approach for GBM. However, most GBM cells confer TRAIL resistance and how this resistance is regulated at a molecular level is not completely clear. Epigenetic deregulation has been increasingly recognized as a hallmark of cancer. In this study, we conducted a drug screen against selected chemotaxis modifiers in U87MG GBM cells and identified Belinostat, Trichostatin A and Chaetocin as potential apoptosis regulators. While Belinostat and Trichostatin A are well-characterized histone deacetylase inhibitors in the context of malignancies, Chaetocin’s role in cancers is less well-known and its relation to TRAIL is novel. Chaetocin is a fungal mycotoxin that inhibits Suv39H1, a histone methyltransferase (HMT) that catalyzes H3K9 methylation. In this study, we investigated whether Chaetocin increases sensitivity of GBM cells to TRAIL.

To this end, GBM cell lines U87MG and U373 are treated with varying dose of Chaetocin and cells’ viability and apoptosis responses to varying doses of TRAIL are analyzed. Chaetocin decreased viability down to 11% of control in U87MG cells upon TRAIL treatment, without inducing cellular toxicity and apoptosis by itself. Chaetocin was shown to upregulate the expression of proapoptotic genes while downregulating antiapoptotic gene expression. In addition, elevated Caspase3/7, Caspase8 and Caspase9 activities were detected in U87MG upon Chaetocin treatment together with PARP cleavage as indicator of apoptosis. Chaetocin also sensitized GBM cells to BH3 mimetic ABT263 which indicates that sensitization of GBM cells by Chaetocin is not limited to TRAIL but is rather a general phenomenon for apoptosis. We infer from these findings that Chaeto- cin plays critical and specific role in apoptotic pathway. Our future work is directed towards examining the changes in gene expression profiling of GBM cells upon Chaetocin treatment and understanding the functional role of H3K9 Methylation in GBM cell apoptosis. To this end, RNAseq analysis is currently being performed and expected to enlighten Chaetocin’s mechanism of action. These findings support our motivation that identifying epigenetic modifier drugs as apoptosis sensitizer may be a promising therapeutic approach for GBM treatment.

#5874 Cdk4/6 kinase inhibitor resistance in prostate cancer. Renee de Leeuw, Matthew J. Schierer, Christopher McNair, Michael A. Augello, Akihiro Yoshida, Edward S. Hazzard, Sean Courtney, Gerard T. Hardiman, Justin Drake, Felix Y. Feng, Scott Tomlins, Maha H. Hussain, Alan Dighil, William K. Kelly, Karen E. Knudsen. Thomas Jefferson University, Philadelphia, PA; Medical University of South Carolina, Charleston, SC; Rutgers University, New Brunswick, NJ; University of California San Francisco, San Francisco, CA; University of Michigan, Ann Arbor, MI; Northwestern University, Chicago, IL.

Non-organ confined prostate cancer (PCA) is often effectively, but only transiently treated by targeting the androgen receptor (AR) signaling axis through androgen depletion strategies, often coupled with AR antagonists. Unfortunately, disease recurs within a median of 3-4 years, presenting as castration resistant PCA (CRPC), for which there are limited therapeutic options. This emphasizes the need for more efficacious drugs and a patient-tailored approach towards cancer therapy to improve disease outcome. One class of drugs currently tested clinically, Cdk4/6 kinase inhibitors, blocks phosphorylation of the retinoblastoma (RB) tumor suppressor, thereby blocking its function, and likely preventing castration resistance. As Cdk4/6 inhibitor resistance has already been reported in other cancers, some PCA patients are anticipated to develop drug resistance. Here, we created palbociclib-resistant PCA cell models by continuously culturing them in presence of the drug to unravel mechanisms of drug resistance. Here, we created palbociclib-resistant PCA cell models by continuously culturing them in presence of the drug to unravel mechanisms of drug resistance. Here, we created palbociclib-resistant PCA cell models by continuously culturing them in presence of the drug to unravel mechanisms of drug resistance. Here, we created palbociclib-resistant PCA cell models by continuously culturing them in presence of the drug to unravel mechanisms of drug resistance.


The putative DNA/RNA helicase, Scheflin 11 (SLFN11), is a recently discovered determinant of sensitivity to DNA damaging agents such as platinum, topoisomerase I and II inhibitors (camptothecins, etoposide, doxorubicin), cisplatin, gemcitabine (1-3) and PARP inhibitors (olaparib, talazoparib) (4). Because SLFN11 expression is suppressed in ~45% cancer cell lines, SLFN11 inactivation is potentially one of the most prevalent mechanisms of resistance to DNA damaging agents. However, the molecular mechanisms of SLFN11 action in the DNA damage response have not been solved. Using isogenic cell lines of SLFN11-knockout and SLFN11-overexpression, we will show that SLFN11 binds to chromatin in response to camptothecin within 2 hours. ChiP-seq analysis reveals that SLFN11 preferentially binds to replication origins under camp-

#5876 Osimertinib (AZD9291), a mutant-selective EGFR inhibitor, reverses ABCB1-mediated drug resistance in cancer cells. Xiao-Yu Zhang,1 Zi-Ning Lei,2 Yun-Kai Zhang,1 Yi-Jun Wang,2 Pranav Gupta,1 Leli Zeng,1 Megan Xu,2 Xi-Qi Wang,2 Dong-Hua Yang,2 Zhe-Sheng Chen1. "St John's University, Queens, NY; 2John L. Miller Great Neck North High School, Great Neck, NY; 3South China Agricultural University, Guangzhou, China.

In recent years, tyrosine kinase inhibitors (TKIs) have been shown capable of inhibiting the ATP-binding cassette (ABC) transporter-mediated multidrug resistance (MDR). In this study, we determine whether osimertinib, a novel selective, irreversible EGFR (epidermal growth factor receptor) TKI inhibitor, could reverse ABCB1-mediated MDR. The results showed that at non-toxic concentrations, osimertinib significantly sensitized both ABCB1-transfected and drug-selected cell lines to substrate anticancer drugs colchicine, paclitaxel, and vincristine. Osimertinib significantly increased the accumulation of [3H]-paclitaxel in ABCB1 overexpressing cells by blocking the efflux function of ABCB1 transporter. In contrast, no significant alteration in the expression levels and localization pattern of ABCB1 was observed when ABCB1 overexpressing cells were exposed to 0.3μM osimertinib for 72 h. In addition, ATPase assay showed osimertinib stimulated ABCB1 ATPase activity. Molecular docking and molecular dynamic simulations showed osimertinib has strong and stable interactions at transmembrane domain of human homology ABCB1. Taken together, our findings suggest that osimertinib, a clinically approved third-generation EGFR TKI, can reverse ABCB1-mediated MDR, which supports the combination therapy with osimertinib and ABCB1 substrates may potentially be a novel therapeutic treatment in ABCB1-positive drug resistant cancers.

#5877 The functional role(s) of serum amyloid A1 (SAA1) polymorphisms in integrin-mediated cell adhesion in nasopharyngeal carcinoma (NPC) cells. OnYing Man, HongLokLung. BaptistUniversityofHongKong, Hong Kong, China.

Serum amyloid A1 (SAA1) was previously identified as a tumor suppressor gene with anti-angiogenic activities in nasopharyngeal carcinoma (NPC). Three SAA1 isoforms (SAA1.1, SAA1.3, and L.5) were observed with disproportionate frequencies among the NPC patients and healthy people. SAA1.1 and 1.3 are the functional isoforms to inhibit angiogenesis whereas SAA1.5 was the defective gene. Our immunohistochemical results showed that the loss of SAA1 staining in the metastatic NPC tissues was significantly associated with tumor progression. We aim to investigate the functional roles of the three SAA1 isoforms in NPC mediated by SAA1.1 knockout cell study. We tested the ability of SAA1.1 and 1.3 isoforms to suppress tumor metastasis by antagonizing the integrin-FAK signaling pathway in the NPC tumor cells. The focal adhesion assay was performed by seeding the tumor cells with or without the integrin alphaVbeta3/beta5 ligand vitronectin. Both the vector-alone control and the SAA1.5-expressing NPC cells began to spread out and adhered to the bottom of the culture dish in the presence of vitronectin from 7 to 9 hours, whereas the functional SAA1.1 and 1.3 isoforms can suppress tumor metastasis by antagonizing the integrin-FAK signaling pathway in the NPC tumor cells. The focal adhesion assay was performed by seeding the tumor cells with or without the integrin alphaVbeta3/beta5 ligand vitronectin. Both the vector-alone control and the SAA1.5-expressing NPC cells began to spread out and adhered to the bottom of the culture dish in the presence of vitronectin from 7 to 9 hours, whereas the functional SAA1.1 and 1.3 isoforms remained in the round-up morphology with minimal attachment. Interestingly, after 48 hours the SAA1.1 and SAA1.3-expressing cells formed adherent junctions among the cells. In order to study the viability of the effects of the three SAA1 isoforms on NPC cells, MTT viability assay was performed. The results showed that the viability of the SAA1.1 and SAA1.3-expressing cells were around 50 % lower than both the vector-alone and the SAA1.5-expressing cells. It is likely that the loss of focal adhesion after seeding of the SAA1.1 and SAA1.3-expressing cells will affect the survival of NPC cells. Furthermore, we found that the presence of the recombinant SAA1.1 and SAA1.3 proteins could reduce the number of viable NPC cells compared with the solvent control and the SAA1.5 protein. We previously reported that the SAA1 proteins can physically interact with the integrin alphaVbeta3. Taken together, we suggested that the secreted SAA1 proteins from the NPC cells could directly affect the NPC focal adhesion as well as the cell viability by blocking the integrin on the NPC cell surface. We acknowledge the financial support of the General Research Fund (grant number HKU717115114 to HLL) of the Research Grants Council of the Hong Kong Special Administrative Region.

#5878 Trichostatin A overcomes chemotherapy resistance of urothelial carcinoma cells through the inactivation of c-Raf/ERK pathway. Kuan-Lin Kuo,1 Chung-Sheng Shi,2 Ju-Tong Hsieh,1 Shiang-Peng Chen,1 Wei-Chou Lin,3 Shih-Ming Liao,3 Shing-Hwa Liu,4 Kuo-Hsiao Huang.1 National Taiwan University College of Medicine, Taipei, Taiwan; 2Chang Gung University College of Medicine, Taoyuan, Taiwan; 3National Taiwan University Hospital, Taipei, Taiwan.

Trichostatin A (TSA), a potent histone deacetylase (HDAC) inhibitor, has been reported to elicit anti-proliferative response in various tumors. Here, we investigated antitumor effect of TSA alone or in combination with conventional chemotherapeutic agents on urothelial carcinoma (UC) cells. We use one high-grade UC cell line (T24) and another UC cells (NTUUC) obtained by primary culture from the surgical specimen of a women with high-grade and metastatic bladder UC. The cytotoxicity and apoptosis induced by TSA alone , chemotherapeutic agents (cisplatin, gemcitabine and doxorubicin) and combined treatment were assessed by MTT assay and fluorescence-activated cell sorting and flow cytometry. The expression of phosphor c-Raf, phosphor-MEK1/2, and phosphor-ERK1/2 were measured by Western blot. Further elucidation on the role of Raf/MEK/ERK pathway on the TSA-enhanced cytotoxicity were examined by using ERK1 siRNA knockdown and the specific MEK inhibitor (PD98059). Our results showed TSA markedly enhances the cytotoxicity and apoptosis of three chemotherapeutic agents in UC cells with concurrent suppression of Raf/MEK/ERK signaling pathway. Consistently, inhibition of Dox-regulation of ERK by MEK inhibitor or by ERK 1 siRNA knockdown potentiated the chemotherapeutic agent-induced cytotoxicity of TSA in UC cells. We concluded that TSA potentiates the therapeutic efficacy of cisplatin, gemcitabine and doxorubicin in human UC cells through Raf/MEK/ERK signaling pathway. These findings provide a new treatment strategy against UC.

#5879 Establishing doxorubicin resistant HCC1806 triple negative breast cancer cell lines result in the differential expression of let7a and miR34a. Checo J. Rorie, Malcolm M. Moses, Mohammed K. Musa, Sherette S. Godfrey. North Carolina A&T State Univ., Greensboro, NC.

Resistance to chemotherapy is one of the major difficulties in the treatment of triple negative breast cancer. Besides the typical known causes of drug resistance, tumor microenvironment can also promote resistance by preventing the drug from accumulating in tumor cells in order to elicit a cytotoxic response. TNBC is characterized by the loss or low expression of estrogen receptor, progesterone receptor, and HER2 proteins that have been targeted and established as some of the main therapeutic targets for disabling, or combating resistance to chemotherapy. However, resistance may have as high as an 80% p53 tumor suppressor gene mutation rate which may also play a role in the ability of TNBC to recur as a result of the inability to kill all remaining TNBC cells leading to chemotherapy resistance. In these studies, we hope to mimic TNBC recurrence by establishing doxorubicin resistant HCC1806 TNBC cell lines. Here we show that we were able to sustain TNBC cell growth after pulse treatments with increasing concentrations of doxorubicin, and we revealed that p53-related miRNAs, let7a and miR34a, were differentially expressed after pulse treating the HCC1806 cells. Understanding the molecular and mechanistic factors related to recurrence and doxorubicin-resistance in TNBC cells may help to develop a list of possible targets for molecular therapy to overcome tumor drug resistance in triple negative breast cancer and may develop or establish a list of potential targets for disabling, or combating resistance to doxorubicin treatment in TNBC patients.
#5880 Acquired chemotherapeutic drug resistance in colorectal cancer is regulated by epithelial-to-mesenchymal transition and altered cellular pathways. Lahiri Gangoda,1 Nidhi Mathew,1 Michael Liem,1 Shahei Keerti Kumar,1 Ching-Seng Ang,2 John Mariadason,2 Suresh Mathivanan,1 La Trobe Institute for Molecular Science, La Trobe University, Bundoora, Australia;1The Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, Australia;2Olivia Newton John Cancer Research Institute, Heidelberg, Australia.

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related death in the western world. Chemotherapy is the mainstay in the treatment of metastasized CRC. However, cancer cells acquire resistance to treatment by various mechanisms resulting in treatment failure. Even though various molecular mechanisms regulating drug resistance is critical to overcome chemoresistance, it is poorly understood. We developed a panel of seven CRC cells resistant to 5-FU. The parental and 5-FU resistant CRC cells were assayed for proteins known for their involvement in chemotherapeutic resistance. In addition, an unbiased quantitative proteomics and DNA methylation analysis was performed on the panel of seven parental and 5-FU resistant CRC cells. The integrated analysis revealed multiple mechanisms contributing to chemotherapeutic drug resistance including epithelial-to-mesenchymal transition (EMT), deregulation of apoptosis, increased survival autophagy and epigenetic modifications resulting in altered drug metabolism potency. Inhibitors of EMT and autophagy sensitized the 5-FU resistant CRC cells. Furthermore, CCRISPR-based gene knockouts of these candidate genes (both up and downregulated) enriched or rendered them resistant to 5-FU. As a follow up, PDX models were established and made resistant to 5-FU. Follow up quantitative proteomics and biochemical validations of 5-FU resistant PDX tissue lysates confirmed the role of EMT in acquired chemoresistance. Overall, this project unravelled multiple mechanisms by which CRC cells may become resistant to 5-FU. Importantly, some of these mechanisms are also conserved in many cancer types and hence targeting these mechanisms can overcome chemoresistance and increase patient survival rates.

#5881 Identification of resistance mechanisms to IGF-IR targeting in triple negative breast cancer. Jennifer Tsui,1 Whitney Petrosky,1 Maria Celeia Fernandez, Pnina Brodt. McGill University, Montreal, Quebec, Canada.

The triple negative subtypes of breast cancer (TNBC) are associated with poor prognosis. Unlike HER2+ and hormone receptor-positive BC, TNBC do not respond to targeted therapy and chemotherapy remains the primary treatment option. There is therefore an unmet need to develop effective therapy for TNBC. The insulin-like growth factor 1 (IGF-1) axis plays a critical role in BC progression by conveying survival and growth signals. Our laboratory reported on the production of a soluble fusion protein comprised of the extracellular domain of human IGF-IR fused to the Fc portion of human IgG (the IGF-Trap). The IGF-Trap reduces the bioavailability of circulating and locally produced IGF-I, thereby limiting tumor growth. When human TNBC MDA-MB-231 cells were xenografted into nude mice and treated with the IGF-Trap, we observed variability in the response as it ranged from complete tumor regression to disease stabilization and tumor progression in some mice. This suggested that MDA-MB-231 cells are heterogeneous in respect to their sensitivity to IGF-IR signaling blockade. The aim of the present study was to identify resistance mechanisms that allow the cells to progress in the face of IGF-IR signaling blockade by the IGF-Trap. We first analyzed the tyrosine kinase receptor profile of these cells and confirmed by PCR that in addition to IGF-IR, they express epidermal growth factor receptor (EGFR), c-Met, and fibroblast growth factor receptor 1 (FGFR1). They also produce IGF-I, EGF and relatively high level of FGF1 that could provide potential autocrine signaling to compensate for IGF signaling blockade. Using limiting dilution cloning, we isolated MDA-MB-231 cell lines and NT2. Reduction in extracellular matrix and adhesion molecules and altered morphology of CHD1 KO cells were as compared to parents and NT2 cells suggest that loss of CHD1 would cell-cell and cell-matrix adhesion. These results suggest that in the absence of PTEN loss in Ets negative tumors, deletion of CHD1 gene could change cell adhesion dynamics ascribing a new role for CHD1 in the development and progression of PCa.

#5882 FAK is required for the survival of tumor cells in MMTV-Wnt1 driven basal-like mammary tumors. Ritama Paul,1 Syn K. Yeo,1 Ming Luo,2 Susan Waltz,2 Jun-Lin Guan,1 University of Cincinnati, Cincinnati, OH;2University of Michigan, Ann Arbor, MI.

Breast cancer is a heterogeneous disease. Stratification of patients based on the subtypes of breast cancer is integral to successful treatment. Focal Adhesion Kinase (FAK), a cytoplasmic tyrosine kinase is over expressed and activated in several cancers including breast cancer. Our earlier studies have shown inhibition of FAK in MMTV-PyMT mouse mammary tumors that are classified as Luminal B subtype, delays tumor onset and reduces tumor growth. To address whether inhibition of FAK would be beneficial in basal-like mammary tumors, we used a conditional deletion of FAK and a knock-in mutation of FAK lacking its kinase activity, in MMTV-WNT1 mouse model, which classifies as basal-like. Similar to PyMT mammary tumors we found that loss of FAK or its kinase function delays tumor onset and tumor growth of basal like WNT1 mammary tumors. However unlike the PyMT tumors, the reduced tumor growth in WNT1 model is not due to decreased proliferation. Interestingly loss of FAK activity in WNT1 tumors results in accumulation of cleaved caspase 3, suggesting that loss of FAK activity results in compromised tumor cell survival. When we investigated the pathways through which FAK could affect survival, we found that loss of FAK activity reduces activation of AKT. Reduced AKT activation reduces the expression of pro-apoptotic genes. In summary our studies show that in a basal-like tumor model, FAK is required for survival of the tumor cells. Hence inhibition of FAK could be beneficial for elimination of basa-like tumor cells.

#5883 CHD1 as regulator of cell adhesion in Ets fusion negative prostate cancer. Aparna Kareddula,1 Whitney Petrosky,1 Irina Tershchenko,1 Daniel Medina,1 Hana Aviv,2 Eric Singer,1 Robert S. DiPaola,3 Kim M. Hirshfield,1 Rutgers Cancer Institute of New Jersey, New Brunswick, NJ;2Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ; 3School of Medicine, University of Kentucky, Lexington, KY.

Prostate cancer (PCa) develops into lethal disease in about 10% of men diagnosed with this malignancy. Common genomic changes are seen in PCa such as alterations in CSDK181, TP53, PTEN, NKX3-1, MYC and androgon receptor. Oncogenic fusions involving the Ets family are seen in 50-70% PCa patients, the most common of which is TMPRSS2-ERG. TMPRSS2-ERG influences cell migration, particularly when PTEN is co-deleted or PIK3CA activating mutations are present. However, in the absence of ERG fusions, molecular drivers of the PCa phenotype are less clear but include alterations in SPOP (6-15% of PCa), SPINK1 (10%), MAP3K7 (18-38%) and CHD1 (15-27%). CHD1 (Chromodomain Helicase DNA Binding Protein-1) is a chromatin remodeling factor with many roles including homologous recombination mediated DNA repair of double strand breaks and maintenance of genomic stability and therefore it is hypothesized to cause the predominant intrachromosomal rearrangements observed in Ets fusion negative tumors. This study was undertaken to evaluate the cellular effects that follow CHD1 deletion that could aid PCa initiation and progression. CHD1 was knocked out in the non tumorigenic, HPV16 immortalized prostate epithelial cell line, RWPE-1, using CRISPR/Cas9. Compared to control, CHD1-KO cells were less migratory and non tumorigenic in vivo. In contrast, the C-terminus of CHD1, a smaller and rod domain morphology were less adherent to tissue culture plates. To study expression of extracellular matrix and adhesion molecules, RT profiler assay was performed using RWPE-1 parental and CHD1 KO cells. CHD1 KO cells showed a decrease in the expression of SPARC, MMP2, ITGA2, ITGA5, ITGA6, FN1, LAMB3, collagen, tenasin and vitronectin as compared to parental and NT2. Reduction in extracellular matrix and adhesion molecules and altered morphology of CHD1 KO cells as compared to parents and NT2 cells suggest that loss of CHD1 reduces cell-cell and cell-matrix adhesion. These results suggest that in the absence of PTEN loss in Ets negative tumors, deletion of CHD1 gene could change cell adhesion dynamics ascribing a new role for CHD1 in the development and progression of PCa.
us. In this study, many TFSs, including krüppel-like factor 4 (KLF4), were found to preferentially bind to mCpG-containing motifs and transactivate gene expression. Using a site-specific KLF4 mutant (R458A) that abolishes its binding activity to mCpG, but has no impact on binding to its canonical unmethylated motif, we investigated biological function of mCpG-dependent gene regulation by KLF4 in glioblastoma cells. Our study revealed that KLF4-mediated cell adhesion, migration, and morphological changes, all of which are abolished by R458A mutation. We identified over a 100 genes were directly activated via mCpG-dependent KLF4 binding activity. These targets were associated with multiple pathways including pathways involved in cytoskeletal organization, cell adhesion, cytokine, and extracellular matrix. Genes such as NGEF, UGDH, PHLD1R2, LIMS2, LM607 and Rabex-5/RABGEF1 involved in migration and cytoskeletal organization were highly induced. We validated these targets by Bisulfite Sequencing, ChIP-PCR, RT-PCR and western blot to confirm they are indeed KLF4-mCpG direct targets. To understand the biological implication of KLF4-mCpG activity and broaden the current understanding of the role of DNA methylation in transcriptional regulation, we utilized one of the direct KLF4-mCpG targets UGDH to investigate this novel epigenetic regulation. UGDH (UDP-α-D-glucose 6-dehydrogenase) is involved in the biosynthesis of the glycosaminoglycan precursor UDP-α-D-glucuronic. Glycosaminoglycans (GAGs) are one of the major components of the cellular environment. Certain GAGs such as Hyaluronic acid (HA) participate in numerous cellular phenomena, including adhesion, motility, angiogenesis and wound healing. UGDH, although regulated in GBMs has not been implicated in GBM tumor biology. We show that UGDH is required for KLF4-mCpG dependent increase in migration and UGDH knockdown decreases GBM cell proliferation, migration and the abundance of GAGs. Elevated glycosaminoglycan formation is implicated in a variety of human diseases, including the progression of tumors. The inhibition of synthesis of UDP-α-D-glucuronic acid using UGDH antagonists might therefore be a useful strategy for therapy.

#5887 The role of FAK in tumor microenvironment. Hsin-Jung Wu, Syn Kok Yeo, Megan Wilson. University of Cincinnati, Cincinnati, OH.

Focal Adhesion Kinase (FAK) is a cytoplasmic non-receptor tyrosine kinase important in mediating signal transduction in cell migration, survival and proliferation. In the regulation of cell adhesion, migration and cytoskeletal organization in tumors, FAK has been implicated in impacting tumor microenvironment, including endothelial cells and fibroblasts. However, much less is known about how FAK behaves in the tumor stroma affects tumor progression, or how stromal FAK cross-talks with cancer cells. Here we use an inducible Col1α2-creERT; FAK 

#5888 Tmem 205-mediated cisplatin resistance in ovarian clear cell carcinoma (OCCC) is overcome by herpes simplex virus (oHSV)/cisplatin combination therapy. Uksha Saini,1 Maria Riley,2 Kalpana Deepa Doryappan,1 Chelsey Bolyard,1 Balveen Kaur,1 Roman Zingarelli,1 Ikko Konishi,2 Periannan Kuppusamy,3 George Larry Maxwell,4 David E. Cohn,1 Karuppaiyah Selvendiran1.

TUMOR BIOLOGY: Tumor Cell Adhesion and Drug Resistance

Tumor biology is one of the major challenges for the treatment of ovarian cancer. Unlike other solid tumors, ovarian cancer primarily disseminates within the peritoneal cavity. A crucial step in metastasis formation is the adhesion of ovarian cancer cells onto the peritoneal mesothelium under ascitic shear flow. However, the adhesion mechanisms engaged in this tumor-mesothelium interaction remain elusive due to a lack of physiologically relevant model to manipulate and investigate this dynamic process. In this study, using a 3D microfluidic platform, we found that the metastatic population of cancer stem cells (M-CSCs) exhibited slower rolling velocity and higher binding affinity to the peritoneal mesothelium than non-metastatic (NM)-CSCs under flow condition. This adhesion cascade was mediated by P-selectin which expressed on the peritoneal mesothelium. The key carbohydrate determinant on M-CSCs was a glycoprotein, but not a glycolipid, with its recognition as sialyl-Lewis X (sLeX) in a sialic acid- and fucose-dependent manner. Moreover, several glycosyltransferase genes including B4GALT4, ST3GAL3, ST3GAL4 and LIM101 were upregulated in M-CSCs. The upregulation of sLeX and sLeA were significantly inhibited by siRNA targeting TMEM205. Overall, our findings revealed that a distinct sLeX-P-selectin axis of ovarian tumor-mesothelium interaction in early metastasis and may offer the possibility of new therapeutic targets. (This work is supported by RGC grant 1712014.)


Kinase inhibitors are integral for the treatment of certain cancers, but the emergence of drug resistance limits their long-term effectiveness. Resistance can arise through several routes, including the selection of mutants that render it insensitive to drug. Although some resistance mutations for a few inhibitors are well characterized, most remain uncharacterized or even unidentifiable, particularly in new or emerging kinase targets. Ideally, we could characterize every possible resistance mutation that could arise during treatment with each inhibitor. The resulting comprehensive data could improve clinical decision-making for patients with drug-resistant tumors. However, current experimental methods for discovering resistance mutations cannot achieve the required scale, preventing us from realizing this goal. We developed a method for parallel assessment of the degree of inhibitor resistance of nearly all possible single mutants of the promising drug target Src kinase. We generated a comprehensive single mutant library of Src and subjected it to selection for Src activity either alone or in the presence of the inhibitor dasatinib. Then, we measured the change in frequency of each mutant during selection by deep sequencing. Using this data, we classified the impact of each Src mutant, both in terms of activity and resistance to dasatinib. Our method identified dozens of previously uncharacterized dasatinib-resistance mutations in Src. As expected, some of these novel resistance mutations cluster near the ATP-binding cleft of the catalytic domain. However, a surprisingly large number occur at residues distant to dasatinib’s binding site. Biochemical validations of a subset of these newly identified resistance mutations are ongoing. Although dasatinib is not currently approved for Src inhibition, these results could be useful to direct treatment for those patients currently enrolled in dasatinib clinical trials. Our results demonstrate the power of our method for the characterization of resistance mutations in drug targets. In contrast with previous low-throughput and retrospective methods, our approach enables the comprehensive and prospective identification of resistance mutations. The approach can reveal resistance mechanisms, even before a drug is clinically approved. Importantly, our method allows for the rapid profiling of other Src inhibitors and potentially the discovery of inhibitors for resistance mutations. Finally, we are using our data to explore hypotheses regarding the structure, function, or regulation of Src kinase.
Breast cancer has a predilection for bone metastasis, where the five-year survival rate is bleak. Disseminated breast cancer cells invade bone and can remain undetectable and untreatable for decades during a period of reduced proliferation. Our work reveals that osteoblasts are educated into a tumor-associated stromal cell by disseminated breast cancer cells and alter their production of these factors in the tumor microenvironment. Osteoblasts undergo an inflammatory stress response and secrete factors suggestive of the epithelial to mesenchymal transition that significantly inhibit tumor colony formation. In contrast, the overexpression of FAK and SMA expression. Since activated CAFs are known to secrete extracellular matrix proteins, we examined the activation of FAK downstream of integrin signaling and found increased phosphorylation of FAK at Y397. To determine the importance of FAK activation on the interaction between CAFs and PDAC cells, cells were treated with the FAK inhibitor PF573228 and increased activation of TRAP was mediated by crosstalk via gap junction intercellular communication (connexin 43) and cross-exosome exchange that occurred between TAO cells and breast cancer cells. TAO-derived exosomes were found to contain increased amounts of microRNAs 320a and 193b compared to normal osteoblasts or breast cancer cells. microRNA 320a is associated with decreased cellular proliferation and induction of G0 phase of the cell cycle. miR193b has been shown to regulate cyclin D1 expression and repress cellular proliferation. Knock-down of miR320a and 193b in breast cancer cells resulted in a reduced number of cancer cells in G0 and increased numbers of cancer cells in G1/S/G2/M phases of the cell cycle. Furthermore, TAO cell CM led to decreased activation of TRAP+ osteoclasts. We hypothesized that TAO cells induced CAF activation and cocultured or treated with the CM of human breast cancer cells, tumors grew more slowly and were at least 50% smaller than tumors composed of normal osteoblasts plus breast cancer cells, or breast cancer cells inoculated alone. Mice inoculated with an admix of TAO cells plus human metastatic breast cancer cells lived ~20 days longer than mice inoculated with an admix of normal osteoblasts plus metastatic breast cancer cells, or breast cancer cells alone. These data also suggest that TAO cells regulate the proliferation of metastatic breast cancer cells in the tumor microenvironment. Overall, these date suggest that osteoblasts are an important source of factors, specifically exosomal microRNAs, in breast cancer bone metastasis. The nature of these factors suggest their importance for facilitating disseminated breast cancer cell proliferation, as well as osteoclast activation in the bone microenvironment. Supported by NIH R1E CA146381, 1R01S004944, P50 CA093639 for FCM; (NNSA) T32 CA079448, NIH R00 CA178177 for KMB.

TUMOR BIOLOGY: Tumor Cell Adhesion and Drug Resistance

5890 Osteoblasts are educated into a tumor-associated stromal cell by disseminated breast cancer cells and mediate breast cancer cell proliferation in the bone microenvironment. Frank Marinj, Julia Chifman, Janet Toones, Candela Garcia-Manzano, Karen M. Bussard. Wake Forest Comprehensive Cancer Center, Winston-Salem, NC; *American University, Washington, DC; 4The University of Texas MD Anderson Cancer Center, Houston, TX; 5Thomas Jefferson Univ Kimmel Cancer Ctr., Philadelphia, PA.

5891 DCIS to invasive progression in breast cancer is delayed by restoring CCN5. Sandipto Sarkar, Arnab Ghosh, Gargi Maity, Snigdha Banerjee, Shashanta Banerjee, University of Kansas Medical Center/ VA Medical Center, Kansas City, KS.

Malignant progression of breast cancer from pre-invasive to invasive lesions remains a mechanistically unknown event and a major challenge in medical research. By revealing the mechanism of action, our new and substantially different approach aims to demonstrate that CCN5/WISP2 might play a role in negative regulation of progression of pre-invasive lesion ductal carcinoma in situ (DCIS) to invasive carcinoma (IC). DCIS to IC transition results primarily from the loss of the myoepithelial cell (MEC) layer surrounding the breast ducts & lobules and basement membrane (BM) degradation followed by invasion of cancer cells into the surrounding stromal tissue and vasculature. It has been recently discovered that CCN5, a matricellular protein, is highly expressed in DCIS patient specimens and facilitates regression of aggressive phenotypes. Our in-vitro studies with myoepithelial cell lines (MECs) indicate that CCN5 may prevent the DCIS to IC transition through the protection of the MEC layer. CCN5 performs its protective role by regulating sonic hedgehog (SHH) expression in MECs. It has been previously shown in separate studies that Neuripin1 (Nrp1) positively regulates expression of SHH and Nrp1 is exclusively expressed in MEC layer in breast tissues. An extension of our studies indicate that CCN5 might regulate the integrity of the mammary ductal architecture by protecting the MEC layer through a non-Nrp1-SHH signaling. In our studies, our studies indicate that regulating CCN5 expression level in breast cancer tissues might help us controlling the rate of progression of the disease from DCIS to an invasive stage.

5892 Induction of stromal fibrosis accelerates tumorigenesis in NeuT mice. Robert I. Glazer, Hongyan Yuan, Lu Jin. Georgetown University Medical Center, Washington, DC.

Background: One of the central challenges in cancer prevention is the identification of factors in the tumor microenvironment (TME) that increase breast cancer susceptibility. The stromal composition of the breast is largely adipose and fibroblast tissue, and thus it is important to understand how alterations in these constituents affect in the onset of stromal fibrosis (SF) and cancer progres-

sion. To address these questions, MMTV-NeuT mice were crossed into the FAT-ATTAC (fat apoptosis through targeted activation of caspase 8) background (NeuT/ATTAC), wherein conditional dimerization of the FKBPs-caspase 8 fusion transgene in female mice results in mammary fat ablation and its replacement with fibrotic tissue. Results: Induction of mammary fat ablation in NeuT/ATTAC mice over 4 weeks resulted in increased ductal proliferation and SF as denoted by increased collagen deposition and expression of Fibroblast Activation Protein and α-smooth muscle actin. Maintaining SF over 3-5 months resulted in a 40% reduction in tumor onset and a 2.5-fold increase in tumor multiplicity, which was associated with increased infiltration by myeloid-derived suppressor cells. Gene expression profiling of tumors from mice with and without mammary fat ablation confirmed increased expression of the immune-related genes Cxcl1, Ly6d and CD14, as well as acute phase proteins Saa1 and S100a6 in NeuT/ATTAC mice with SF. Conclusion: Induction of SF in an erbB2-dependent breast cancer model resulted in a tumor microenvironment more conducive to tumor progression and immune tolerance. These results suggest alternative therapeutic approaches that could be taken, eg, chemokine receptor antagonists, to increase the effectiveness of immunotherapy and chemotherapy. Supported by a grant from the Avon Foundation for Women and Award IP30CA051008 from the National Cancer Institute, NIH, to the Lombardi Comprehensive Cancer Center.

#5893 Bioenergetic signature from cocultures of pancreatic tumor cell lines and fibroblasts. Mª Teresa Agulló-Ortuño,1 Elena Prieto-Garcia,2 C. Vanesa Díaz-García,1 Irene Otero Blas,2 Inmaculada García-Ruíz,1 José A. López-Martin1,3. 1Instituto de Investigación Sanitaria 12 de Octubre, Madrid, Spain; 2Hospital Universitario 12 de Octubre, Madrid, Spain; 3Hospital Universitario 12 de Octubre, Madrid, Spain.

Pancreatic ductal adenocarcinoma (PDAC) is one of the tumors with greater invasiveness and metastasis. PDAC is associated with a large desmoplastic reaction, characterized by fibroblastic proliferation and extracellular matrix secretion. Besides, cellular bioenergetics has become a central issue in investigation of cancer biology, because the altered energy metabolism of cancer cells has been proposed as a potential target for cancer treatment. We have established cocultures between PDAC cell lines (Capan-1 or PL-45) and fibroblasts (LCS). A reverse phase protein microarray (RPMA) approach has been applied to quantify proteins of energy metabolism in theses co-cultures, with the aim of identifying potential biomarkers in PDACs. The fifteen proteins of energy metabolism studied included members of the mitochondrial oxidation of pyruvate, the tri-carboxylic acid cycle, β-oxidation of fatty acids, electron transport and oxidative phosphorylation, glycogen metabolism, glycolysis and oxidative stress using highly specific antibodies. Co-cultures modified proteins expression of energy metabolism, respect to monocultures. Capan-1 in co-culture increased several proteins belonging to OXPHOS (NDUFS3, SDHB, CORE2 and COXII), the antioxidant SOD2, the mitochondrial HSP90, and G6PDH. The expression of the glycolytic PKM2 decreased. Their fibroblasts partners increased the expression of SDHB and also decreased PKM2. PL-45 in co-culture, experimented an increase in HADHA. Their fibroblasts partners showed a decrease in PKM2, LDHA, IF1, PDHe and HSP90.Capann-1 and PL-45 in co-culture with fibroblast, differ in their energy metabolism phenotype. Overall, the results indicate that the quantification of bioenergetic signature offers potential biomarkers that could be implemented to refine the understanding of the biological principles of PDAC and, eventually, the management of patients with these tumors.

#5894 Stromal fibroblasts from metastatic breast cancer promote proliferation and migration of breast cancer cell and regulate its stemness. Yirui Gui, Adriana Aguilar-Mahecha, Marguerite Buchanan, Mark Basil. Ladi Davis Institute for Medical Research, Jewish General Hospital, McGill University, Montreal, Quebec, Canada.

Breast cancer is one of the most common causes of cancer-related death in women in the world. Although most research is focused on the tumor cells, it is important to elucidate the molecular nature of the tumor-stromal relationship to truly understand the biology of breast tumor growth. Cancer-associated fibroblasts (CAFS) are the major cellular constituents of the tumor microenvironment. CAFs are in direct contact with the adjacent cancer cells and crosstalk with them through soluble cytokines, growth factors and exosomes during carcinogenesis in human breast cancer. To determine for the first time the function of CAFs in metastatic sites of breast cancer, we collected CAFs from human patients of metastatic sites (m-CAFs) and compared them with CAFs from primary tumor (p-CAFs) and normal fibroblasts (NFs). We found that m-CAFs expressed higher levels of α-smooth muscle actin (SMA) compared with p-CAFs and NFs. The proliferation of MDA-MB-436 breast cancer cells was significantly increased by treatment with conditioned medium (CM) from m-CAFs compared with p-CAFs in vitro. Also, CM by m-CAFs induced paclitaxel resistance in MDA-MB-436 cells, suggesting that soluble factors produced by m-CAFs promote chemoresistance, to a greater extent than CM from p-CAFs. The migration and invasion ability of MDA-MB-436 cells co-cultured with m-CAFs CM was significantly greater than that of the p-CAFs CM. m-CAFs outperformed the p-CAFs and NFs. Furthermore, CM by m-CAFs induced a 3D in vitro culture model. Furthermore, m-CAFs protected MDA-MB-436 cells from the cytotoxic effects of doxorubicin more than p-CAFs in this spheroid 3-D culture. When MDA-MB-436 cells were sorted from these spheroids and grew in the respective CMs, they showed higher expression of the stemness markers of CD44 /CD24 compared with cells obtained from p-CAFs/MDA-MB-436 spheroids. To these cells from the co-culture with m-CAFs experienced with the cadherin switch. Nanog has been demonstrated to promote chemoresistant and epithelial-mesenchymal transition (EMT). The results displayed that MDA-MB-436 cells incubated with the CM from the m-CAFs had a higher expression of Nanog compared with that of p-CAFs. RNA-seq results showed that IFG2 is one of the most different genes between m-CAFs and p-CAFs, its expression was more significant in m-CAFs co-cultured with MDA-MB-436 cells than p-CAFs. The signaling pathway in MDA-MB-436 cells were comparable between co-cultured with m-CAFs CM and the IFG2 stimulation at 2 hours. All these data illustrates that fibroblasts isolated from metastatic site differed in terms of their ability to promote breast cancer cells progression compared to p-CAFs. Our data suggests that m-CAFs are more potent than p-CAFs in inducing the proliferation, migration, drug resistance and cancer stemness of breast cancer cells in an in vitro model.

#5895 Circulating CAF and cCAF circulating tumor cell co-clusters are associated with metastatic breast cancer. Utsav Sharma, Philip Miller, Kelsie Medina-Saenz, Pedro Ferrer, Svetlana Speransky, Toni Yeasky, Dorraya El-Ashry, University of Miami, Miami, FL.

Background: Breast cancer metastasis is the cause of breast cancer-related mortality. The tumor microenvironment (TME) plays a critical role in governing tumor initiation, progression and metastasis. In breast cancer, cancer associated fibroblasts (CAFs) are master regulators of the TME. Cancer metastasis occurs in part by transport of circulating tumor cells (CTCs) in the circulation. CTCs in clusters, rather than individual CTCs, have a greater capacity to establish metastases. We recently demonstrated that CAFs also circulate (cCAFs), both in clusters with CTCs and individually, in the blood of the majority of breast cancer patients with metastases, some patients without overt metastases, and in no patients with no evidence of disease. Our lab has also established primary breast cancer and primary CAF cell lines from dissociated breast tumors of different molecular subtypes; these are ideal models to dissect tumor–stromal interactions, both in vitro and in vivo. We hypothesize that cCAFs originate from the primary tumor, and further, that cCAFs cluster with CTCs to facilitate metastatic seeding. Methods: We used separately labeled CAFs and primary breast cancer cells (DT28) co-injected into NSG mice and followed by a novel 2-color IVIS. We also modeled the ability of CAFs and breast cancer cells to form clusters in culture and used our microfluidic filter technology to evaluate the composition of clusters. Conclusions: CAFs of breast cancer cells of the metastatic capacity – metastatic DT28 and non-metastatic MCF-7. Results: Through sequential dual-color IVIS we demonstrate that we can monitor the presence of CAFs and breast cancer cells at orthotopic tumor injection sites and to sites of distant metastasis, indicating that cCAFs originate from the primary tumor. Evaluation of cCAFs and CTCs in tumor bearing mice indicates that breast cancer cells with high metastatic potential mobilize greater numbers of cCAFs. CAFs formed robust clusters with metastatic DT28 cells, while non-metastatic MCF-7 cells clustered with each other but not with CAFs suggesting that the ability of breast cancer cells to form clusters with CAFs, both in vitro and in vivo, is reflective of the metastatic capability of the breast cancer cells. Conclusions: The intrinsic metastatic capacities of breast cancer cells are augmented by contact and clustering with cCAFs. In turn, the ability of CAFs to mobilize into circulation is in part conferred by properties of aggressive breast cancer cells. These data corroborate observations from our pilot clinical study that indicated the presence of cCAFs is overwhelmingly associated with the presence of metastatic breast cancer.

#5896 IL-8 signaling enhances TNBC growth and metastasis via crosstalk with stromal components. Kideok Jin, Niranjan B. Pandey, Aleksander S. Podobed, Utsav Sharma, Philip Miller, Kelsie Medina-Saenz, Johns Hopkins Univ. School of Medicine, Baltimore, MD.

Triple negative breast cancer (TNBC) as a metastatic disease is currently incurable. Reliable and reproducible methods for testing drugs against metastasis are not available. We have previously developed a robust metastatic model in
which mice are pretreated with tumor cell-conditioned media (TCM) from hu-
man TNBC cells (MDA-MB-231 and SUM149) for 2 weeks prior to tumor cell
inoculation. In this model we found reproducible spontaneous metastases in
lymph nodes (LN) and lungs within 4-5 weeks after orthotopic tumor inocula-

tion. We have discovered that the TNBC tumor cells secrete large amounts of inter-
eukins (IL-11, IL-6) that "educates" lymphatic endothelial cells (LEC) in theLN
and lungs. Stat3, a transcription factor, gets activated and induces the synthesis of
CCL5 and VEGF among other factors. CCL5 recruits the tumor cells to the LN
and lungs; VEGF helps build blood vessels in the LN to facilitate tumor cell
survival; VEGF produced in the lung helps the tumor cells extravasate into the
lung. We have confirmed the importance of these factors by showing that inhib-
itors of the VEGF and Stat3 pathways significantly inhibit metastasis. In this context using a hu-
man antibody array, we identified factors secreted by fibroblasts and macro-
phages upon induction by MDA-MB-231 TCM. We ranked the expression level of
each factor by real time qRT-PCR and determined that interleukin 8 (IL-8) was the top candidate. We confirmed by ELISA that IL-8 secreted from either fibroblasts or macrophages treated with MDA-MB-231 TCM was upregulated compared to treatment with serum free media (SFM). Our data showed that the proliferation of MDA-MB-231 cells co-cultured with fibroblasts or macro-
phages was enhanced compared to monoculture. Furthermore, MDA-MB-231 cell
migration, a key step in tumor metastasis, was promoted by CM from TCM-
induced fibroblasts or macrophages. Knockdown of CXCR2, IL-8 receptor, ex-
pression by CRISPR-Cas9 system reduces MB231 cell proliferation and migra-
tion compared to wild type. In a mouse xenograft tumor model, the growth of MB231-CXCR2-/- cell was significantly decreased. In addition, the incidence of thoracic metastasis of MB231-CXCR2+/- tumor was reduced com-
pared to WT. We found that the auto- and paracrine loop of IL-8 exists between
TNBC cells and stroma, which results in enhanced IL-8 secretion from the stromal
components. Significantly, inhibition of the IL-8 signaling pathway by Re-
partin, an inhibitor of the IL-8 receptor CXCR1/2, abrogated MDA-MB-231 tumor
growth and metastasis. These findings implicate IL-8 signaling as a crit-
ical event in TNBC tumor growth and metastasis via crosstalk with stromal
components. Further, these studies suggest that IL-8 acts as a key regulator
orchestrating TNBC metastatic breast cancer. Therefore, we have provided ev-
idence that supports the hypothesis that functional antagonism of the IL-8 sig-
naling pathway has the potential to circumvent TNBC breast cancer growth and
metastasis.

#5897 Single cell-derived analysis of desmoid tumors for studying tumor-
stroma interactions. Mushrik Al-Jazawi,1 Steven Xu,2 Qingxia Wei,2 Ray-
mond Poon,2 Benjamin Alman1,1 University of Toronto, Toronto, Ontario, Can-
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Durham, NC.

Cancer associated fibroblasts play an important role in the maintenance and re-
modeling of the tumor microenvironment, providing the appropriate condi-
tions for neoplastic cell growth and invasion. Desmoid tumors (DT), also
called aggressive fibromatosis, are rare, locally invasive soft tissue tumors that consist of
fibroblastic cells embedded in extracellular matrix. Identification of the stro-
mal cells to study tumor-stroma interactions is difficult due to both populations
displaying a fibroblastic phenotype, and no cell marker exists that reliably dif-
ferrante between the two populations. Majority of DT arise sporadically due
to somatic activating mutations in beta-catenin (CTNNBN1), a major effect-
or of canonical Wnt signaling. We established single cell derived colonies from
multiple DT samples and characterized the beta-catenin mutation status of
each clone by Sanger sequencing. Indeed, we were able to establish both mutant
and non-mutant colonies from DT samples. Quantitative PCR for beta-catenin
targets AXIN2 and LEF1 confirmed differential activity between the mutant and
non-mutant colonies. The specific CTNNB1 codon mutation had no difference
on-beta-catenin transcriptional activity. We next performed a high throughput
surface antigen screen to identify cell markers that can distinguish between the
two subpopulations. Our screen found CD142 to be uniquely expressed by the
mutant colonies, while the non-mutant colonies uniquely expressed Podopla-
nin. Quantitative PCR confirmed the differential expression of these markers.
Furthermore, the CD142-positive population in heterogeneous DT samples cor-
related with their mutation frequency. Importantly, CD142-based cell sorting
allowed the isolation of the mutant subpopulation even in samples that appeared
as wild-type by Sanger sequencing. We also studied the expression of secreted
factors in our mutant and non-mutant populations. We observed that CTHRC1,
a ligand related to the Wnt/PCP pathway, is highly elevated exclusively in the
mutant subpopulations. Recombinant CTHRC1 increased the proliferation rate
of DT primary cultures, as measured by BrdU incorporation, while neutralizing
antibodies against CTHRC1 decreased cell proliferation. The importance of

#5988 The consomic xenograft model identifies genetic changes in the tumor microenvironment that alter the growth and metastasis of head and neck cancers. Michael W. Straza,1 Amy Rymaszewska,2 Kwangok P. Nickel,2 Anne Frei,1 Anirban Chatterjee,2 Rachel Schlaak,1 Amit Joshi,1 Michael Flister,1 Randy J. Kimple,2 Carmen Bergom,1 Medical College of Wisconsin, Milwaukee, WI; 2University of Wisconsin, Madison, WI.

Background: The tumor microenvironment (TME) is known to impact tu-

mor growth, metastatic potential, and treatment response. Nearly all studies of
head and neck cancer (HNC) have focused on somatic mutations in the
malignant genomes. We hypothesized that genetic determinants limited to the microen-
vironment would influence HNC growth and metastatic potential. Approach:
To demonstrate the impact of genetic differences in the TME on HNC cell line in vivo growth we utilized a novel tool, the consomic xenograft model (CXM). A
consomic rat has an entire chromosome substituted into the isogenic back-
ground of another inbred strain by selective breeding. Use of immunodeficient
(IL2Rγ-/-) consomic allows one to study the influence of stromal genetics on tumor biology without the confounding effect of differences in the immune
system through the orthotopic implantation of cancer cells into different con-
sonomic genetic environments. In this system, any disparities in metastatic phenotypes are due to differences in the TME rather than cancer cells or immune response.
We utilized SS and SS.BN3 consomic rat strains, previously shown to affect the growth of breast tumors, to study the effects of the TME on HNC tumor growth using two well-characterized HPV negative HNC cell lines, SCC-6 (base of tongue derived) and SCC-22b (derived from a hypopharyngeal cancer that had metastasized to lymph nodes). Both cell lines were modified to stably express
luciferase. HNC cells were inoculated into the tongue of SS and SS.BN3 animals and tumor growth was monitored by biophotonic imaging after luciferin injec-
tion. Results: A significant difference in the tumor growth was seen between rat
strains for both cell lines, with the SS.BN3 rats exhibiting less tumor growth and metastasis. Median luciferase activity from baseline increased by 4.1-fold vs. 1.1
fold in SCC-6 tumors in SS vs SS.BN3 rats, respectively (p<0.05). A significant differences in lung metastases was also seen between strains. Lung metastases were seen in 88% of SS and 0% of SS.BN3 rats (n=6 per group) inoculated with SCC-6 (p<0.02) and in 75% of SS and 30% of SS.BN3 rats (n=10 per group) inoculated with SCC-22b (p<0.1). Despite a
non-statistically significant difference in the number of SCC-22b inoculated animals with lung metastases, there was a significantly higher metastatic burden as measured by luciferase signal, with the median signal 6.8 fold higher in SS as compared to SS.BN3 (p<0.03). Conclusions: The use of the CXM model dem-
onstrates an important role for the TME in the growth and metastatic spread of HNC tumors. The short term differences in TME between tumoral and stromal samples for both cell lines allow us to investigate the contribut-
ing causal genetic variants in the TME mediating the HNC changes in tumor
growth and metastasis.

#5899 Compartment-resolved proteomics reveal NNMT as a master met-
abolic regulator of cancer associated fibroblasts. Mark A. Eckert,1 Fabian
Coscia,1 Shawn Pan,1 Samantha M. Tienda,1 Agnieszka A. Chryplewicz,1
Chun-Yi Chiang,1 Anthony Montag,1 S. Diane Yamada,1 Matthias Mann,2 Ernst
R. Lengyel1,1 University of Chicago, Chicago, IL; 2Max Planck Institute for Bio-
chemistry, Martinsried, Germany.

The poor outcome of ovarian cancer patients is due to late detection and the ability
of ovarian cancer cells to metastasize quickly throughout the abdominal

cavity. Recent research, including histopathological studies and experiments
with mouse models, suggest that ovarian cancer may actually arise in the fallo-
opian tube as serous tubal intraepithelial carcinomas (STIC). Lesions of the ovary
would, therefore, represent metastases from the fallopian tube. To better under-
stand the molecular events that occur in both the tumor and stromal compart-
ment during ovarian cancer progression, we performed shotgun proteomics on

laser-microdissected tumor and stromal compartments from anatomic sites
representing the hypophyseal progression series for ovarian cancer (STIC →
fallopian tube → ovary → omentum). With an optimized sample preparation
technique, we successfully quantified 4-5000 proteins per anatomic site with
high reproducibility and with as few as several thousand microdissected cells
derived from formalin-fixed and paraffin-embedded (FFPE) biobank speci-

TUMOR BIOLOGY: Tumor Microenvironment

Men. Proteomics of the tumor compartments revealed high inter-patient heterogeneity, with no conserved protein signatures associated with invasion or metastasis. In strong contrast, a highly conserved molecular signature of stromal proteins associated with metastasis to the omentum was identified. In particular, nicotinamide N-methyl transferase (NNMT) was highly upregulated in the stroma of aggressive pancreatic cancer cells. Independent analysis of more than 17,000 proteins in a mouse model of peritoneal metastasis demonstrated that NNMT is expressed in cancer stroma and is associated with gene expression changes in the tumor stroma. This work supports the use of ultra-low input proteomics for compartment-resolved identification of candidate drivers of disease phenotypes and identifies NNMT as a central, metabolic regulator of CAF differentiation.

#5900 Transforming growth factor-beta (TGF-β) inhibitor modulates cancer stroma in a pancreatic cancer orthotopic mouse model. Takashi Murakami,1 Yukiko Hiroshima,2 Kentaro Miyake,1 Tasuku Kiyuna,1 Ho Kyoung Hwang,1 Kei Kawaguchi,1 Jonathan C. DeLong,1 Thinner M. Lwin,1 Kentaro Igarashi,1 Ryusei Matsuyama,2 Ryutaro Mori,3 Takafumi Kumamoto,7 Takashi Chishima,5 Kuniya Tanaka,6 Michael Bouvet,2 Itaru Endo,2 Robert M. Hoffman,1 1University of California, San Diego, San Diego, CA; 2Yokohama City University Graduate School of Medicine, Japan.

Background: The tumor microenvironment (TME) contributes to the recapitulation of this disease. Cancer-associated fibroblasts (CAFs) are thought to be important for the pancreatic cancer TME. Transforming growth factor-beta (TGF-β) has an important role for CAF induction. The present report describes the efficacy of a TGF-β inhibitor to modulate pancreatic cancer stroma in an orthotopic mouse model. Materials and methods: BxPC-3 human pancreatic adenocarcinoma cells expressing green fluorescent protein (GFP) were implanted subcutaneously in athymic nude mice expressing red fluorescent protein (RFP). Established dual-color tumors, with GFP cancer cells and RFP stroma, were cut into small fragments and then implanted orthotopically onto the tail of the pancreatic RFP-expressing nude mice. Fourteen mice were randomized into control group (n = 7, vehicle, i.p., weekly, for 3 weeks) and treated group (n = 7, SB431542 (TGF-β inhibitor) 0.3 mg i.p., weekly, for 3 weeks) when tumor diameters reached to 7 mm. Pancreatic cancer stroma was evaluated weekly for 3 weeks by real-time intravital imaging with the FV-1000 confocal microscope. Stromal ratio, the percentage of the RFP fluorescence area from the stromal cells relative to the total fluorescence area, was calculated with ImageJ. Tumors were resected on the 21st day from the initial treatment for histological evaluation. Results: Microvessel density using anti-CD31 antibody and expression of CD31-antibody and expression of TGF-β were examined by immunohistochemical staining. Results: The GFP cancer cells and RFP stromal cells were distinctly imaged. The stroma ratio significantly decreased in the TGF-β inhibitor treated mice compared to control untreated mice from day 7 to 21. TGF-β expression and microvessel density were reduced in the treated mice. Conclusions: The present study demonstrated that TGF-β inhibitor treated mice have a decreased cancer stroma, indicating that TGF-β inhibitor treatment may be an option to modulate pancreatic cancer stroma. However, more studies are required to fully understand the role of TGF-β in pancreatic cancer stroma.

#5901 Involvement of VEGF-A signaling in the acquisition and maintenance of human prostate carcinoma-associated fibroblast phenotype. Javier Cerda Infante, Marianela Sanchez, Paola Conejerios, Alejandra Alarcón, Alejandra Godoy, Enrique Brandan, Viviana P. Montecinos. P. Catholic University of Chile, Santiago, Chile.

Introduction: Progression of prostate cancer (CaP) is modulated by the carcinoma-associated fibroblasts (CAFs) within the tumor microenvironment. However, little is known about the specific mechanisms involved in the activation of the benign prostate fibroblasts (BAFs) to CAFs in CaP and the specific signaling altered in CAFs that are needed to maintain its activated phenotype. Vascular endothelial growth factor-A (VEGF-A) is overexpressed in CaP and, additionally to the angiogenic effect, a non-angiogenic effects of VEGF-A on cancer cells have been recently reported. This study seeks to evaluate the potential role of VEGF-A signaling in the activation of BAFs and/or in the maintenance of the activated phenotype presented in CAFs. Methods and Method. Primary cultures of BAFs and CAFs were characterized by gene expression profile, qPCR, WB and ELISA for the expression and/or secretion of VEGF-A and its receptors. Dose-response and time course analysis for the effect of exogenoux VEGF-A, TGF-β1 and conditioned medium from CAFs (CM-CAFs) were analyzed by RT-qPCR, immunocytochemistry and WB for the stroma cell markers: Vimentin, Pro-collagen, α-SMA, Tenascin, Fibronectin and FSP-1. The contribution of VEGF-A signaling to the phenotype of CAFs was determined by siRNA assays for the VEGFRs and the functional effect of CAFs on cancer progression was studied using CM-CAFs or CM from VEGFR siRNA-CAFs over PC3 cancer cell lines, in vitro and in vivo. Results: Exposure of primary cultures of BAFs to exogenous VEGF-A, TGF-β1 and CM-CAFs upregulate stroma cell markers associated with the appearance of CAFs phenotype and it is reversed with the pre-treatment of the VEGF-A antibody Bevacizumab. Furthermore, CAFs, but not BAFs, produced massive levels of VEGF-A and over-express its cognate receptors. siRNA studies demonstrated that knock down of VEGFRs in CAFs results in the activated phenotype and CM from VEGFRsiRNA-CAFs failed to increase the migration and invasion potential of PC3 cells in vitro and tumor growth in vivo. Discussion: Our data indicate that VEGF-A signaling plays a key role in the activation of human prostate fibroblasts. Furthermore, VEGF-A and TGF-β1 interactions may cooperatively regulate activation of fibroblasts within the tumor microenvironment in CaP. These findings also suggest that blockade of this signaling cascade in the activated fibroblasts could be a potential therapeutic target designed to prevent disease progression.  1506 Proceedings of the American Association for Cancer Research • Volume 58 • April 2017
signal activation by CAFs co-operates for promoting FAK phosphorylation and enhances the invasiveness of GC cells in extracellular matrix (ECM). On the other hand, a previous study presented that CXCR4 and integrin signaling co-operates in mediating adhesion and chemoresistance in small cell lung cancer cells. Therefore, we hypothesized that CXCL12-CXCR4 and integrin signal activation by CAFs and CXCL12-CXCR4 integrin related signaling by western blotting analysis. Furthermore, we investigated the critical molecule for chemoresistance by silencing CXCR4 or integrin beta1 in GC cells. Results: ROS-sensitive assay and chemo-sensitive assays revealed that GC cells cultured with CAF-CM showed more resistant to ROS and cisplatin than those cultured with fresh medium. Moreover, GC cells with CAF-CM on Matrigel-coated plates exhibited remarkable resistance in these assays. On the other hand, western blotting analysis showed that Akt activity in GC cells cultured with CAF-CM was up-regulated in concurrence with FAK phosphorylation. Finally, the chemoresistance induced by CAFs was significantly suppressed by CXCR4 or integrin beta1 silencing. Conclusions: These results suggest that interaction with ECM is important for enhance chemoresistance mediated by CXCL12-CXCR4 and integrin beta1 signal activation by CAFs in GCs. This mechanism underlying the chemoresistance may provide a novel therapeutic target in advanced GCs.

**TUMOR BIOLOGY: Tumor Microenvironment 8**

**#5904** Stiffness of extracellular matrix regulates breast cancer progression by stimulating mesenchymal stem cells. Seiichiro Ishihara, David R. Inman, Wan-Ju Li, Suzanne M. Ponik, Patricia J. Keely. *University of Wisconsin-Madison, Madison, WI.*

The tumor microenvironment contains cancer cells, non-cancerous cells, and extracellular components such as extracellular matrix (ECM). Previous studies have demonstrated that interactions between the cells and the microenvironment contribute to cancer progression via chemical stimuli, such as growth factors, and mechanical stimuli, such as stiffness of the ECM. Recently, it has been reported that mesenchymal stem cells (MSCs) differentiates into cancer associated fibroblasts (CAFs) in response to chemical stimuli from cancer cells and thereby promote cancer progression. However, the contribution of mechanical stimuli to MSCs in cancer is poorly understood. In this study, we revealed that MSCs showed CAF phenotypes and promote mammary cancer progression in response to mechanical stimuli. On a stiff substrate, MSCs treated with conditioned media from cancer cell culture expressed increased levels of alpha smooth muscle actin (alpha-SMA), a marker of CAFs, compared to the MSCs cultured on a soft substrate. MSCs grown on a stiff substrate displayed higher expression and activity of YAP and increased phosphorylation of myosin light chain (MLC) compared to MSCs grown on a soft substrate. In addition, knockdown of YAP by shRNA decreased the expression of alpha-SMA and phosphorylation of MLC in MSCs on a stiff substrate. Pharmacological inhibition of MLC phosphorylation by H1122 treatment also reduced expression of alpha-SMA and activity of YAP in MSCs. Cell-cell communication between MSCs and carcinoma cells was unidirectional, as conditioned medium from MSCs cultured on a stiff substrate, but not a soft substrate, increased growth of mammary carcinoma cells. The soluble factor prosaposin was highly secreted by the MSCs on a stiff substrate, and the addition of recombinant prosaposin increased proliferation and survival of mammary carcinoma cells. Furthermore, secretion of prosaposin was promoted in YAP-overexpressed MSCs on a soft substrate. Mammary carcinoma cells treated with prosaposin showed increased level of phosphorylation in Akt at T308. In addition, inhibition of phosphorylation of Akt at T308 prevented proliferation and survival in mammary carcinoma cells. These results suggest that increased stiffness of the ECM in the tumor microenvironment induces differentiation of MSCs to CAFs via YAP and actomyosin contractility, secretion of prosaposin from MSCs, and as a result, trigger progression of mammary cancer via phosphorylation of Akt at T308.


Background: Cancer associated fibroblasts (CAFs) are activated fibroblasts and an important player in the tumor microenvironment. Their activity promotes cancer cell proliferation, migration, and invasion, and metastasis. The most prognostic factor in tumor progression is metastasis. Cancer metastasis is a multi-step process that tumor cells detach from primary site, survive in the bloodstream, and seed in target organ. Although previous studies have focused on the interaction between CAFs and cancer cells in primary tumor site, the roles of CAFs in blood circulation remain largely unknown. We investigated the effect of CAFs coexisting with cancer cells in bloodstream on tumor metastasis in vivo mouse model, and also examined the effect of CAFs in subcutaneous tumor. Methods: We used female BALB/c-nu/nu mice and BALB/c mice in the experiments. Cancer cells were mouse mammary carcinoma cell lines 4T1 transfected with luciferase, colon cancer cell lines Colon 26 transfected with luciferase. Fibroblast cell lines were mouse embryonic fibroblast MEF and NIH-3T3. In vivo injection mouse model, we injected 1 × 10⁶ cancer cells alone or the same number of cancer cells and fibroblast co-cultured together. When we injected cancer cells transfected with luciferase, the mice were subjected to in vivo imaging system (IVIS) and measured luminescence intensity of metastatic sites. We have also harvested lung and compared its weight and metastatic nodules under microscopy. Furthermore, in subcutaneous tumor metastatic mouse model, cancer cells alone or mixed with cancer cells and CAFs were subcutaneously inoculated into the mice. Results: In the group of mice injected with cancer cells and fibroblasts, luminescent intensity of each lungs were higher than cancer cell alone. Harvested lung weight and the number of metastatic nodules were also higher in the group with cancer cells and fibroblasts. In subcutaneous model, mice inoculated with cancer cells and fibroblast had much more metastatic sites than cancer cells alone. Conclusions: Our data indicate that CAFs promote tumor metastasis by stimulating cells in blood circulation, and regulate metastatic site. These findings suggest that CAFs in both bloodstream and primary site could be a promising therapeutic target. CAF-targeted therapy could reduce tumor metastasis and improve the prognosis of cancer patients.

**#5906** TIMP-1 expression is inversely correlated with miRNA125a-5p and let-7e in non-small-cell lung carcinoma. Ammar Kutiyawanalla, Sampa Ghoshal-Gupta, Byung R. Lee, Ashis Mondal, Ravindra Kolhe, Amy M. Rojiani, Mantuz V. Rojiani. *Medical College of Georgia- Augusta University, Augusta, GA.*

Epigenetic alterations are emerging as significant elements in our understanding the biology of lung cancer which remains the leading cause of cancer deaths worldwide. In earlier studies we have shown that tissue inhibitor of metalloproteinase 1 (TIMP-1) overexpression in NSCLC cells results in aggressive tumors in mice. We have also demonstrated TIMP-1’s anti-apoptotic activity in these cells. It is well documented that miRNAs are involved in many physiological and neoplastic processes, including apoptosis. We have previously shown that knocking down TIMP-1 in A549 NSCLC cells alters the miRNA profile in these cells with upregulation of miR-125a-5p. Subsequently, we have identified that miR let-7e is also upregulated in TIMP-1 KD clones. The present study is focused on confirming the involvement of miR-125a-5p with TIMP-1 by using mimics and antagonoms of 125a-5p. Furthermore, we have sought to validate this relationship in vivo by examining these patterns in clinical samples. We have added mimics of miR-125a-5p into the parental A549 cells, which resulted in down expression of TIMP-1 levels. We were then able to rescue TIMP-1 downregulation by adding antagonoms of miR-125a-5p to the same cells. MiR-125a-5p binding sites were identified in TIMP-1 3’ UTR and using luciferase assay, we have confirmed that TIMP-1 is indeed a bona fide target of miR-125a-5p. Translational studies were carried out using archived NSCLC adenocarcinoma tissues from the Surgical Pathology archives at AU Medical Center. We first measured expression level of TIMP-1 by RT-PCR in 20 tumors and paired normal adjacent tissues and divided the samples into 2 groups, those with low and high TIMP-1 expression levels. Tissues were then interrogated for miR-125a-5p and let-7e miRNA expression using chromogenic in situ hybridization. We identified negative correlation pattern between tumor and paired normal adjacent tissue samples. Generally, TIMP-1 was highly expressed in the tumor region, while miRNA expression was relatively decreased when compared with adjacent normal tissues. miRNA125a-5p and let-7e are known tumor suppressors in NSCLC. Analyzing secondary data (adenocarcinoma) by KM plotter we found that high TIMP1 correlated with lower patient survival with a hazard ratio of 3.17 (2.34-3.7) and a log rank p value of 8.1e-14. These studies provide additional clarity and further extend our understanding of the relationship between these miRNA families and TIMP-1 expression.

**#5907** Radiotherapy-induced damage to cancer-associated fibroblasts and its reciprocal influence on cancer progression: Impact of soluble factors. Olivier De Bever, Joke Tommelein, Marc Bracke, Tom Boterberg, Pieter Demetter, Laurin Verset, Ghent Univ. Hospital, Ghent, Belgium; Université Libre de Bruxelles, Brussels, Belgium.

Epigenetic alterations are emerging as significant elements in our understanding the biology of lung cancer which remains the leading cause of cancer deaths worldwide. In earlier studies we have shown that tissue inhibitor of metalloproteinase 1 (TIMP-1) overexpression in NSCLC cells results in aggressive tumors in mice. We have also demonstrated TIMP-1’s anti-apoptotic activity in these cells. It is well documented that miRNAs are involved in many physiological and neoplastic processes, including apoptosis. We have previously shown that knocking down TIMP-1 in A549 NSCLC cells alters the miRNA profile in these cells with upregulation of miR-125a-5p. Subsequently, we have identified that miR let-7e is also upregulated in TIMP-1 KD clones. The present study is focused on confirming the involvement of miR-125a-5p with TIMP-1 by using mimics and antagonons of 125a-5p. Furthermore, we have sought to validate this relationship in vivo by examining these patterns in clinical samples. We have added mimics of miR-125a-5p into the parental A549 cells, which resulted in down expression of TIMP-1 levels. We were then able to rescue TIMP-1 downregulation by adding antagonons of miR-125a-5p to the same cells. MiR-125a-5p binding sites were identified in TIMP-1 3’ UTR and using luciferase assay, we have confirmed that TIMP-1 is indeed a bona fide target of miR-125a-5p. Translational studies were carried out using archived NSCLC adenocarcinoma tissues from the Surgical Pathology archives at AU Medical Center. We first measured expression level of TIMP-1 by RT-PCR in 20 tumors and paired normal adjacent tissues and divided the samples into 2 groups, those with low and high TIMP-1 expression levels. Tissues were then interrogated for miR-125a-5p and let-7e miRNA expression using chromogenic in situ hybridization. We identified negative correlation pattern between tumor and paired normal adjacent tissue samples. Generally, TIMP-1 was highly expressed in the tumor region, while miRNA expression was relatively decreased when compared with adjacent normal tissues. miRNA125a-5p and let-7e are known tumor suppressors in NSCLC. Analyzing secondary data (adenocarcinoma) by KM plotter we found that high TIMP1 correlated with lower patient survival with a hazard ratio of 3.17 (2.34-3.7) and a log rank p value of 8.1e-14. These studies provide additional clarity and further extend our understanding of the relationship between these miRNA families and TIMP-1 expression.
Preoperative radiotherapy is a mainstay in current management of colorectal cancer. Several tumor types, including colorectal cancer, show an abundant desmoplastic stroma, characterized by cancer-associated fibroblasts (CAFs). Nevertheless the effects of radiation to the CAFs in the stroma and its reciprocal impact on the response of CRC cells to irradiation have not been studied. We investigated the functional (cell number, morphology) and biochemical (transcriptomics, metabolomics and kinomics) phenotype of CRC cells in response to paracrine signals from irradiated CAFs. Using neutralizing antibodies and small molecule inhibitors, we analysed the role of tyrosine-kinase receptor activation in CRC cells in response to supernatants from irradiated CAFs. Furthermore, the impact of tyrosine-kinase receptor neutralization in combination with RT was evaluated by an orthotopic CRC model. The level of TKO activation, a downstream tyrosine-kinase receptor intermediate, was analysed in response to neoadjuvant treatment in human CRC specimens. Further work will reveal which soluble factor(s) are implicated in the communication between irradiated CAFs and colorectal cancer cells.


Drug resistance remains a major problem in the treatment of most cancers. For example, KRAS wild-type colorectal cancers (CRCs) are typically treated with the anti-epidermal growth factor receptor (EGFR) therapy cetuximab in combination with standard chemotherapy; however, of the 40% of patients that do respond, virtually all relapse within 3-12 months. Additionally, around 25% of non-responders are not treatable. This suggests that it is not just cell-intrinsic mechanisms that result in resistance, but that extrinsic factors play a role. The purpose of this study was to investigate how the dynamics of the tumor microenvironment, specifically cancer-associated fibroblasts (CAFs) and hypoxia, modify the response of CRC cells to cetuximab. We used a novel high-content imaging platform to generate quantitative phenotypic data (i.e. morphology, birth/death rates) of cellular co-cultures perturbed by multiple, co-occurring microenvironmental conditions. Preliminary data demonstrated reduced sensitivity of tumor cells to cetuximab in the presence of CAFs, which was dependent on patient specifics and time scale of co-culture. Additionally, we found that low oxygen conditions altered the effect of CAFs on tumor cell phenotypes, which highlights the importance of studying co-occurring micro-environmental factors. This work underlies the importance of considering not just the genetic makeup of patient tumors, but also the heterogeneity of the surrounding microenvironment when designing personalized treatment strategies.


Considerable evidence suggests that cancer-stem cells (CSCs) play critical roles in tumorigenesis and metastasis. Although surface marker expression or enzymatic activity of CSC has proven useful, variability of expression highlights the need for the development of high throughput functional CSC assays. One such approach utilizes the capability of CSC’s to form single-cell-derived spheres in suspension environments (functional CSCs). Microfluidic tools are suitable for handling and monitoring a large number of single cells in suspension as well as for examining the interaction of these cells with those in the tumor micro-environment including cancer associated fibroblasts (CAF’s). In order to accomplish this, we have developed a high-throughput (10,000-well), simple-to-use, vertical platform for CSC/CAF co-culture. The platform consists of a non-adherent substrate having 10,000 micro wells for single-cell-derived sphere formation and a porous trans well for CAF adherent co-culture. As predicted by Poisson’s distribution, approximately 30% of micro wells capture single cells when T47D breast cancer cells are loaded at a concentration of 200k/μL. After cell seeding, trans wells containing CAF’s are placed onto the substrate. 0.4μm pores on the trans well allow for secretion-based cellular interaction while preventing direct CSC/CAF cell contact. This cell separation facilitates sample cell retrieval without cell cross contamination. Utilizing this device, we determined that co-culture of tumor cells with CAF’s doubled the frequency of sphere formation. Spheres derived from individual CSCs were then retrieved and dissociated for single-cell transcriptome analysis utilizing RNA barcoding followed by RNAseq. Molecular analysis revealed significant cellular heterogeneity among sphere derived cells. In addition, principal component analysis of single cell RNAseq, revealed significant differences in gene expression between mono-cultured and CAF co-cultured cells isolated from tumor spheres. These genes represent potential therapeutic targets for interfering with CSC stromal cell inter-actions in the tumor microenvironment. The vertical adhesion suspension co-culture platform provides a system to identify and evaluate novel CSC targeted therapeutics.

#5910 Influence of 3D-cultured prostate cancer and stromal cells on drug response. Dominique N. Gales, Sonni Miller, Temesgen Samuel, Clayton Yates. Tuskegee University, Tuskegee, AL.

Background: Response to chemotherapeutic drugs presents a significant challenge to effective treatment of advanced prostate cancer. Exploration of therapeutic response has focused largely on the tumor cell. However, evidence suggests mechanisms that involve the tumor microenvironment mediate response to therapeutics. Therefore, it is imperative to mimic the in vivo tumor microenvironment, to bridge the gap between in vitro studies and the development of therapeutics. The concept of three-dimensional (3D) cell culture and co-culture of multiple cell types is critical to predict therapeutic efficacy and outcome. Thus, these studies sought to determine the distinct responses to anti-cancer drugs in 3D cell spheroids. Methods: In this study, we utilized metastatic prostate cancer cells (PC3) and freshly cultured prostate bone stromal cell line (HS27a) to generate tumor spheroids. Tumor spheroid viability was measured at 72 and 120 h using the CellTiter-Glo 3D assay after treatment with three common anti-cancer drugs, Docetaxel (4μM), Enzalutamide (30μM), and Bicalutamide (30μM). Treatments were compared with spheroids originated from HS27a and HS27a PC3 cells (cell lines in which omentin induced glucose uptake was normalized using the untreated cells as 100% cell survival. Results: Spheroid formation increased resistance to Docetaxel, Enzalutamide, and Bicalutamide. However, PC3 spheroid cells were significantly higher compared to HS27a and co-culture spheroids. For all three spheroids, as the size of the spheroids increase, cellular resistance to anti-cancer and hormone drugs increased. Conclusion: The findings in this study demonstrated that the use for 3D-cell cultures influence many aspects of PCA behavior including, proliferation rates, and response to chemotherapeutics.

#5911 Omentin drives metabolic shift in ovarian cancer cells in the omental tumor microenvironment. Chi Lam Au Yeung,1 Abhinav Achreja,2 Hongyuen Zhao,2 Tsz-Lun Yeung,1 Rosemarie Schmandt,1 Daniel K. Yip,3 Karen H. Lu,1 Deepak Nagrath,1 Samuel C. Mok1. 1UT MD Anderson Cancer Center, Houston, TX; 2Rice University, Houston, TX; 3University of South Florida, Tampa, FL.

Advanced stage high grade serous ovarian cancer (HGSOC) metastasizes preferentially to the omentum, which is a well-vascularized fold of peritoneal tissue and is a major site of adipose tissue accumulation. The mechanisms by which omental adipose tissue interact with ovarian cancer cells and promotes tumor growth and disease progression are not entirely clear. We previously showed that a novel adipokine called omentin (Intestinal Lactoferrin Receptor ITLN1) produced by the omental adipose tissue was significantly down-regulated in patients with HGSOC compared to healthy individuals and omentin suppressed ovarian cancer cell growth only in the presence of adipocytes in vitro. Since we and others demonstrated that omentin induced insulin-dependent glucose uptake exclusively in adipocytes, we therefore hypothesized that omentin may suppress ovarian cancer growth via driving metabolic shift in ovarian cancer cells in the omental microenvironment. Using a cancer cell/adipocyte co-culture model, we demonstrated that omentin reduced both glucose uptake and lactate secretion in ovarian cancer cells when they were co-cultured with adipocytes but not with other stromal cell types, suggesting that omentin-induced glucose uptake in adipocytes may deplete the surrounding glucose that fuels the glucose-addicted ovarian cancer cells in the omental microenvironment and thus drive metabolic shift in ovarian cancer cells. To delineate the mechanism by which omentin suppressed ovarian cancer cell glucose uptake in adipocytes, expression levels of the adipocyte-specific glucose transporter GLUT4 induced by omentin in adipocytes and the effect of GLUT4 silencing in adipocytes using GLUT4 specific siRNAs on ovarian cancer growth were examined. The results showed that omentin up-regulated GLUT4 in adipocytes and GLUT4 silencing in adipocytes abrogated the effects of omentin on glucose uptake in cocultured ovarian cancer cells compared to controls. Taken together, this study shows that omentin plays an important role in driving metabolic shift in ovarian cancer cells in the omental microenvironment. Therapeutic strategies based on up-regulating omentin in ovarian cancer patients may inhibit ovarian cancer progression and improve patient survival rates.
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#5912 Tumor microenvironment modulates RTK signaling. Dhruv Thakar,1 Shali T. Low-Nam,2 Jay T. Groves,1 Valerie M. Weaver1.1 University of California San Francisco, San Francisco, CA; 2University of California Berkeley, Berkeley, CA.

Receptor tyrosine kinases (RTKs) and their associated signaling molecules are mutated or dysregulated in many aggressive cancers such as non-small cell lung carcinoma (NSCLC), breast cancer, and colorectal cancer. Despite therapeutic interventions such as tyrosine kinase inhibitors (TKI) that target RTKs, majority of patients develop resistance even after initial tumor regression. Although clinical studies suggest sequential treatments with TKI, chemotherapy, and radiation can eliminate most cancer cells, resistance inevitably emerges (Sequist, et al., Sci Trans Med, 2011). We demonstrated that alterations in the extracellular matrix (ECM) correlate with cancer progression and that RTK signaling is subject to spatiotemporal regulation (Paszek, et al., Cancer Cell, 2005; Salaita, et al., Science, 2010). Thus, geometric and mechanical properties of the ECM may directly modulate RTK signaling. Here, we use silicone gel substrates that exhibit tunable stiffness in the physiological range (100s Pa – 10s kPa) to measure RTK-adaptor protein interactions in mammary epithelial cells (MECs; MCF10A) (Ou, et al. Integr. Biol., 2016). These gels are compatible with total internal reflection fluorescence (TIRF) imaging and enable single molecule detection of activated RTK phosphoryrosine residues via recruitment of Grb2 tagged with the fluorescent protein mEos3.2. We demonstrate a spectrum of Grb2 behaviors that are determined by the stiffness of the substrate. This indicates that the same receptor is capable of different signaling activities that are dictated by material properties. These new insights create opportunities to target the tumor microenvironment to address RTK inhibitor resistance and metastasis in cancer patients and to develop effective therapeutic strategies for better prognosis and prolonged patient survival.

#5913 The impact of daily exercise on tumor perfusion. Jennifer M. Wiggins,1 Sharon Lepler,1 Christine Pampol,1 Lori Rice,1 Jennifer A. Lee,2 Dietmar Siemann1.1 University of Florida, Gainesville, FL; 2National Cancer Institute, Bethesda, MD.

Ablative blood vessel networks in solid tumors lead to impaired tissue perfusion and areas of hypoxia (pO2 < 10 mmHg). Tumor hypoxia is associated with aggressive progression, dissemination, and therapeutic resistance. It is found in 40% of breast cancers, and can constitute a major obstacle to anticancer therapy. Exercise is associated with improvements in cardiovascular and respiratory function, aerobic capacity and overall health. In cancer patients undergoing chemotherapy and radiation, exercise has been shown to decrease treatment related side effects and general fatigue. The goal of the present investigation was to determine whether aerobic exercise could be applied to improve tumor perfusion and oxygenation in a breast cancer model. Such modulation of the tumor physiology and host environment would be expected to lead to enhanced antitumor efficacy when combined with radiotherapy or chemotherapy. The effects of a single and daily bouts of moderate intensity treadmill running were studied in mice bearing the syngeneic murine mammary carcinoma 4T1. The exercise intensity was determined by measuring the anaerobic threshold, which was assessed by measuring the steady rise in blood lactate during exercise. Initial studies examined the effect of a single moderate bout of exercise (18 m/min) in mice bearing ~500 mm3 orthotopic tumors. In subsequent experiments mice were exercised at 18 m/min, 5 days a week, for 8 weeks prior to orthotopic injection of tumor cells and continued exercise (5 days a week) for 2 weeks during tumor growth. Controls for each treatment consisted of sedentary mice exposed to a stationary treadmill for the equivalent amount of time. At the end of each of the exercise regimen, tumors were harvested, sectioned, stained, and tile mapped to assess physiological changes by immunofluorescence. The detection of open blood vessels (Hoechst-33342) was used as an indirect indicator of perfusion. Tumor hypoxia was determined using the 2-nitroimidazole (EF5). Blood vessels were stained using the endothelial cell marker CD-31. All markers were quantified using Photoshop and ImageJ NIH software. Our results indicate that in the 4T1 breast cancer model moderate intensity exercise did not significantly alter tumor growth, oxygenation or blood vessel number. However, daily bouts of exercise did significantly increase the number of open tumor vessels indicating improved tumor perfusion. These results suggest that a daily exercise regimen may have the potential to improve drug delivery to mammmary tumors.

#5914 A soft microenvironment protects from failure of midbody abscission and multinucleation downstream of EMT initiators. Allison K. Simi,1 Alisya A. Anlas,1 Sherry X. Zhang,1 Tiffany Hsaia,1 Derek C. Radisky,1 Celeste M. Nelson.1 1Princeton University, Princeton, NJ; 2Mayo Clinic Cancer Center, Jacksonville, FL.

This study investigates how increased stiffness of the tumor microenvironment can induce cellular multinucleation, an easily observable marker of polyplody. Up to 37% percent of tumors exhibit whole-genome doubling, which typically precedes other somatic copy number alterations. Additionally, induction of tetraploidy in human cells promotes increased tolerance for mutation, tumor growth and tumor transformation. Tumor cells are inherently stiffer than normal tissue, and this property has been shown to affect cell growth and proliferation. Similarly, cell cycle errors have long been linked to chromosomal abnormalities. Here, we used engineered two-dimensional substrata that mimic the stiffness of tumor and normal microenvironments to investigate how matrix stiffness regulates multinucleation in mammary epithelial cells. Multinucleation was quantified by staining with Hoechst to visualize the nuclei. Timelapse microscopy enabled visualization of the process by which cells become multinucleated. Changes in gene expression were determined by quantitative RT-PCR. Cells cultured on “soft” substrata, representing tumor tissue, showed a nearly 14-fold increase in multinucleation compared to cells cultured on “hard” substrata, representing normal tissue. We found that multinucleation was induced in part by signaling downstream of matrix metalloproteinase-3 (MMP3), which is commonly upregulated in cancer and known to induce epithelial-mesenchymal transition (EMT). This signaling depended on expression of the Rac1 splice variant, Rac1b, production of ROS, and expression of Snail. Under all conditions, cells cultured on soft substrata maintained a low frequency of multinucleation. Multinucleation on stiff substrata was not seen without previous treatment. A soft tumor microenvironment protected the stability of the genome in epithelial cells by preventing midbody resorption, which depended on septin 4, a novel target of Snail. Importantly, we found that transforming growth factor-β (TGFβ), another EMT-inducer, also caused multinucleation downstream of Snail, which was prevented by culture on soft substrata. Our data thus suggest that tissue stiffening during tumorigenesis and bioengineered tumor microenvironments in preclinical and clinical cancer applications. Methods: We utilized a novel method CLARITY that has been shown to transform intact rodent tissues into an optically transparent and permeable hybridized-hydrogel form that can undergo immunostaining followed by 3-D imaging of nucleic acids and proteins markers, without the need to separately analyze hundreds of conventional FFPE sections. In this study, human breast and lung cancer excision biopsy samples were obtained commercially. Murine orthotopic MCF-7 xenograft and patient-derived xenograft (PDX) breast tumors were explanted and prepared as fresh samples. The intact tissues were incubated in a mixture of 4% paraformaldehyde/4% acrylamide/0.05% bis-acrylamide for 48 hours, and polymerized in the presence of a thermal initiator, VA-044, for 3 hours at 37°C to form an intact hydrogel/tissue matrix. The tissues were then lipid-cleared in a solution of 0.2M borate buffer containing 8% SDS, pH 8.5 at 45–55°C from 5–35 days, depending on sample size. The cleared tissues were then stained with two multiplex antibody panels that were selected to highlight the microenvironment of the tissue including, immune cells (CD-3 or CD-8), vasculature (Lectin-Texas Red or CD-31), and tumor (cytokeratin[8 + 18] or PD-L1, directly conjugated to Alexa Fluor® 647®). For non-directly conjugated antibodies, the tissues were then subjected to a secondary goat anti-mouse or goat anti-rabbit antibody conjugated to either Alexa Fluor® 568 or 700 followed by a nuclear counterstain, Sytox Blue. Samples were then placed in an appropriate refractive index solution for imaging on a laser scanning confocal microscope. Results: The tissues remained intact throughout the procedure and the cellular morphology of the tissue was well preserved. The individual components of the microenvironment could be identified, demonstrating that the tissues could be successfully interrogated with a multiplex of indirect and directly conjugated commercially available antibodies. These preliminary studies, provide evidence for the use of this novel technology for detecting the heterogeneity of biomarker expression within the tumor microenvironment. Most notably, the analysis of a lymph node from a patient with metastatic breast cancer revealed variation across the tissue which may have implications for the detection of micro-metastasis in patients with early stage disease when using conventional thin section histological methods. Conclusion: These results implicate CLARITY as a powerful next generation tissue process.
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ing technology for profiling the intact tumor microenvironment, eliminating the need to recapitulate this spatial and quantitative information with standard thin section techniques.

#5916 Proton-sensor GPR4 potentiates intestinal inflammation in the DSS-induced colitis mouse model. Edward J. Sanderlin,1 Nancy R. Leffler,3 Stephen Finn,3 Raymond McDermott,3 Sharon Anneve Glynn1. University of California, San Francisco.

Proton-sensor GPR4 potentiates intestinal inflammation in the DSS-induced colitis mouse model. We observed that GPR4 mRNA expression was increased in mouse and human IBD tissues when compared to control intestinal tissues. To determine the function of GPR4 in intestinal inflammation, wild-type and GPR4-deficient mice were treated with 3% DSS for acute and chronic time points for the induction of colitis. Our results showed that the severity of colitis was decreased in GPR4-deficient DSS-treated mice in comparison to wild-type DSS-treated mice. Clinical parameters, macroscopic disease indicators, and histopathological features were less severe in the DSS-treated GPR4-deficient mice than the DSS-treated wild-type mice. Inflammation gene expression, endothelial adhesion molecule expression, leukocyte infiltration, and isolated lymphoid follicle (ILF) formation were reduced in intestinal tissues of DSS-treated GPR4-null mice. In summary, our results suggest GPR4 potentiates intestinal inflammation as the absence of GPR4 ameliorates intestinal inflammation in the DSS-induced colitis mouse model. Use of GPR4 inhibitors could prove a valuable therapeutic in the reduction of intestinal inflammation and subsequent CRC development.

#5918 Collagen enhances tumorigenicity of papillary thyroid cancer. Anna Sharabura, Laura MacDonald. Hendrix College, Conway, AR.

While the mutations associated with thyroid cancer are well defined, less is known about how the tumor microenvironment contributes to thyroid cancer progression. Papillary thyroid cancer is most common and is associated with activating BRAFV600E mutations. Jolly et al. recently found that murine papillary thyroid cancers with constitutively active BRAF and a PTEN deletion recruit cancer-associated fibroblasts that enrich the tumor with collagen, a component of the extracellular matrix. Our study addresses how collagen impacts tumorigenicity of papillary thy-roid cancer cells. Cell lines derived from mouse models of papillary thyroid cancer with BRAFV600E and PTEN deletions were grown in the presence of or absence of collagen and assessed for cellular proliferation, altered cellular morphology, secretion of microvesicles, and altered cellular signaling. Our results show that papillary thyroid cancer cells increase microvesicle secretion and have increased growth rates. Collectively, our study suggests that collagen enhances tumorigenic characteristics of papillary thyroid cancer cells.

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#5919 Identification of a novel molecular interaction, targeted by Metformin, between breast cancer and white adipose tissue progenitors. Francesca Reggiani, Valentina Labanca, Patrizia Mancuso, Andrea Manconi, Francesco Bertolini. European Inst. of Oncology, Milan, Italy.

The white adipose tissue (WAT) contains a resident population of progenitors with mesenchymal- or endothelial-like phenotype, able to promote breast cancer (BC) progression (Orecchioni et al., 2013). We recently characterized a novel molecular axis underneath the interaction between BC and WAT progenitors, possibly explaining the acquisition of a cancer-associated phenotype by WAT progenitors cells and the establishment of a permissive tumor microenvironment. GM-CSF balance was identified to be the principal regulator of the transcriptional alteration occurring in WAT progenitors exposed to BC: GM-CSF, produced by BC cells, dramatically changed the secretion profile of progenitors, enhancing its own production through a positive regulatory loop and inducing the expression of several factors involved in inflammation (IL-1β), ECM remodeling and angiogenesis (MMPI). IL-1β was strongly down-regulated by GM-CSF neutralization in WAT progenitors in vitro and in preclinical models. Induction of IL-1β by GM-CSF was able, in turn, to up-regulate MMPI in the same cells. The markedly increased expression of IL-1β was already observed in BC-associated WAT progenitors, being addressed to increased local and metastatic tumor growth in obese mice. GM-CSF production was significantly enhanced in the presence of concomitant obesity in preclinical models of BC, both in orthotopic models (MMTV-neu/ErbB2; 4T1) and in spontaneous tumor-bearing mice (MMTV-PyMT). GM-CSF neutralization in several obese syngeneic models of BC significantly reduced immunosuppressive cells in tumors and in the surrounding WAT, including Tregs (CD4+ CD25+PD1+CD127low), regulatory T cells, and proinflammatory cells (CD4+PD1+CD127high). In preclinical models of BC, the absence of GPR4 ameliorates intestinal inflammation in the DSS-treated wild-type mice. In summary, our results suggest GPR4 potentiates intestinal inflammation as the absence of GPR4 ameliorates intestinal inflammation in the DSS-induced colitis mouse model. Use of GPR4 inhibitors could prove a valuable therapeutic in the reduction of intestinal inflammation and subsequent CRC development. GM-CSF has been shown to be activated by acidosis and can increase the expression of numerous inflammatory and stress response genes in vascular endothelial cells (ECs) and has functionally increased EC-leukocyte adhesion. Subsequently, genetic and small molecule approaches for the inhibition of GPR4 activity have been reported in other contexts.

#5920 Paraendocrine interactions between bone marrow mesenchymal stem cells and prostate cancer cells: impact on tumor cell invasive and proliferative capacity. Karen O’Leary,1 Sarah Ridge,1 William Watson,2 Antoinette Perry,2 Stephen Finn,3 Raymond McDermott,3 Sharon Anneve Glynn1. National University of Ireland Galway, Galway, Ireland; National University of Ireland Dublin, Dublin, Ireland; Trinity College Dublin, Dublin, Ireland.

Introduction: The primary site of prostate cancer metastasis is to the bone. Bone marrow derived mesenchymal stem cells (BM-MSCs) are multipotent stem cells resident in the bone with the potential to differentiate into osteoblasts, chondrocytes and adipocytes. They have been shown to home to sites of inflammation, including prostate cancer, contributing to tumor growth and progression. This study aims to explore the impact of metastatic prostate cancer patient derived BM-MSCs on prostate cancer cell proliferation and invasive capacity. Methodologies: BM-MSCs were isolated from bone marrow aspirates from patients diagnosed with metastatic prostate cancer (both androgen deprivation treatment naive and commenced). Co-culture models were used to examine the impact on patient BM-MSCs versus healthy donor BM-MSCs on prostate cancer cell proliferation, PSA and cytokine secretion, and induction of cell migration and invasion. The impact of metastatic prostate cancer cells on healthy donor BM-MSCs was also examined. Results: Here we show that metastatic prostate cancer cells (PC-3 and DU145), reprogram normal donor BM-MSCs to a pro-inflammatory secretory phenotype that in turn induces prostate cancer cell invasion using a 3D spheroid model of invasion into a matrigel-collagen gel, while restricting BM-MSCs with the 3D spheroid. Additionally we isolated a series of BM-MSCs from metastatic prostate cancer patients (n = 10). Results show that a proportion of prostate cancer patient derived BM-MSCs patients have a reduced differentiation capacity to osteocytes while maintaining normal adipocyte capacity. Additionally patient derived BM-MSCs increase prostate cancer cell (MDA-PCa-2B) secretion of PSA and display increased expression of inflammation associated enzymes including the immunosuppressive enzyme indoleamine-2,3-dioxygenase and its metabolite kynurenine. Conclusions: Ultimately, we seek to decipher the role of BM-MSCs in metastatic prostate cancer, in the context of impact on androgen receptor signaling, chemotherapeutic drug response, and the maintenance and promotion of metastatic lesions. Future directions will use 3D co-culture models to identify the key regulatory networks co-activated in prostate cancer and BM-MSCs that facilitate chemotherapeutic drug resistance and metastatic progression.
Introduction: Lepidic predominant adenocarcinoma of the lung was classified into adenocarcinoma in situ (AIS), minimally invasive adenocarcinoma (MIA), and invasive lepidic predominant adenocarcinoma (LPA) in current WHO classification, 4th edition. Furthermore, LPA is thought to progress in a stepwise fashion from AIS and MIA. However, these classifications were diagnosed histologically and therefore failed to identify intermediate stages. Thus, the aim of this study was to investigate the microenvironmental changes in the process of development from AIS to MIA and LPA, and subjective marker of stepwise progression. We have published a part of data in this study in Lung Cancer (100:53-62, 2016), and we believe that this study makes a contribution to elucidate the mechanism of development of LPA of the lung. Materials and Methods: Cliquen pathological characteristics of AIS (n=51), MIA (n=59), LPA smaller than 3 cm (S-LPA, n=113), and LPA larger than 3 cm (L-LPA, n=47) were analyzed. We then immunohistochemically evaluated the expression levels of epithelium-mesenchymal transition (EMT)-related molecules (E-cadherin, S100A4), invasion-related molecules (laminin-5, ezrin), and growth factor receptors (EGFR) in cancer cells of each group (n=20). The number of tumor-promoting stromal cells, including podoplanin-positive cancer-associated fibroblasts (PDPN+CAFs), CD204-positive tumor-associated macrophages (CD204+ TAMs), and CD34+ microvessel cells, were also analyzed. Results: LPA-S showed significantly frequent vascular and pleural invasion than MIA (p<0.05); however, no significant difference in those factors was found between S-LPA and L-LPA. Laminin-5 expression in the non-invasive component of MIA was significantly higher than that of that of AIS (p<0.001). During the progression from MIA to S-LPA, the expression level of laminin-5 in the invasive component was significantly elevated (p<0.01). Moreover, tumor-promoting stromal cells were more frequently recruited in the invasive area of LPA-S (PDPN+CAF; p<0.05, CD204+ TAM; p<0.001, CD34+ microvessel; p<0.05). Ezrin expression in the invasive component of LPA was significantly increased (p<0.05) compared to S-LPA; however, the number of tumor-promoting stromal cells were not different between these two groups. Conclusion: Our current study indicated that microenvironmental molecular changes occur during the progression from MIA to S-LPA. Thus, it was suggested that this process may play an important role in disease progression from AIS to LPA of the lung.


Tumor microenvironment such as neangiogenesis, stroma and tumor immunoactivity has recently gained much attention because it is significantly involved in tumor progression. Pancreatic adenocarcinoma (PAC; pancreatic invasive ductal carcinoma) is recognized as one of the most intractable malignancies and required to disclose a key biological characteristics to provide a clue for its diagnosis and therapeutics. Here we focus on the biological role of bone marrow-derived human mesenchymal stem cells (MSCs) in PAC, which were reported to be accumulated in various cancer tissues such as colon, breast cancers. We detected small numbers of MSCs in the stroma of PAC in human pathological tissues using double immunofluorescence in combination with several MSC markers including CD73, 105, 34, 45, SMA and so on. Then, in in vitro coculture system, we found that MSCs significantly facilitated invasion of BXPC3 cells, a human PAC cell line, in comparison with the culture of BXPC3 cell alone by transwell chamber assay. Proteome profiling of the cocultured supernatant revealed augmented production of soluble molecules including CXCL7, CCL7 as a cytokine, integrin beta1, transferrin R, ALCAM, and Amphiregulin (AREG) as a soluble receptor and ligand, metalloprotease (MMP), ADAMTS-1 as a soluble protease-related molecule, all which were significantly increased comparing to the culture sup. of BXPC3 alone or MSC alone. Based on the result of proteome profiling, we further examined mRNA upregulation on the cells of each culture by real-time PCR (qPCR) to identify which molecule would be more specifically upregulated by the coculture sample of PAC cells and MSCs. Consequently, upregulation of AREG and MMP-3, in addition to downregulation of MMP-9 was observed by qPCR, and knockdown of AREG using the specific siRNA on the cocultured cells corroborated marked decrease of PAC invasion in transwell chamber assay. Interestingly, AREG expression on the tumor cells of tissue specimens of the patient with PAC was observed especially on the cells at invasive front, which suggests stromal MSCs may interact with PAC cells and facilitate invasion of cancer cells by regulating expression of the specific genes such as AREG and MMPs.


Cancer pancreatic (PDA) has a dismal prognosis and responds poorly to all existing therapies. This is largely attributed to the existence of dense stroma and lack of T cell infiltration. PDA exhibits heterogeneous tumor microenvironments that are composed of various stromal cells. Human PDA has been shown to have various levels of T cell infiltration. The mechanisms underlying the inter-tumor heterogeneity of immune microenvironment in PDA and its effects on distinct responses of PDA to therapies, especially immune-modulating therapies, remain largely unknown. Our lab has demonstrated that there is no difference in somatic mutation burden or neo-epitope load between human PDA tumors with high or low amount of cytolytic T cells. We hypothesize that tumor-cell-intrinsic determinants, transcriptional and/or epigenetic factors, shape the heterogeneous tumor microenvironment, and lead to different responses to therapies. For the reason that tumor cellularity of PDA is very low, the profiling of bulk tumor samples cannot provide enough information for understanding tumor-cell-intrinsic molecular events associated with differential immune microenvironment. Therefore, we developed a panel of ~60 cell lines (diversity-cell-panel) from PDA tumors derived from inbred C57BL/6 KPC mouse model, which recapitulates the major histopathological and molecular features of human PDA. Our preliminary results have demonstrated that the diversity-cell-panel recapitulates the immune microenvironments heterogeneity in PDA. Tumor cells mainly fell into two subgroups: T-cell-high and T-cell-low. Tumors resulted from T-cell-high tumor cells also possess less amount of immune-suppressive myeloid cells. To study whether this difference is caused by various amount of mutations, we performed exome-sequencing for 8 cell lines within this cell-panel, and showed that T cell-high and T-cell-low tumor cells have similar amount of mutations and neo-epitopes. To further study the distinct molecular features of T-cell-high and T-cell-low tumor cells, we will perform RNA-seq and ATAC-seq on tumor cells sorted from resulting tumors. To understand whether T cell infiltration affects the response of PDA tumors to immune-modulating therapies, I have been testing the response of T-cell-high and T-cell-low tumors to a group of therapies, including chemotherapy, CD40 therapy and immune checkpoint blockade therapy. Utilizing this novel system, the cell-panel, this study will delineate in unprecedented detail the novel molecular determinants of immune heterogeneity in PDA and the implication of the heterogeneity to therapies. This diversity-cell-panel will also be useful for understanding other aspects of tumor heterogeneity, including angiogenesis, drug resistance and metastatic disease.

Necrotic cells promote microglia infiltration in glioblastoma through regulating MCP-1 and MIP-3α expression. Yieun Jung, So-Hee Ahn, Hyunja Park, Iwoo Lim, Jiihee Lee Kang, Youn-Hee Choi, Eun Ju Kim. 1Department of Physiology, Tissue Injury Defense Research Center, Ewha Woman’s University School of Medicine, Seoul, Republic of Korea; 2Department of Molecular Medicine, Tissue Injury Defense Research Center, Ewha Woman’s University School of Medicine, Seoul, Republic of Korea.

Glioblastoma multiform (GBM), a grade IV astrocytoma, is most lethal and common adult primary intracranial tumor. GBM is characterized by diffuse infiltration into normal brain parenchyma, rapid growth and the presence of necrosis and microglia/macrophage infiltration. Among these properties of GBM, necrosis has been implicated to be a strong predictor of poor prognosis; however, the effect of necrosis on GBM progression is poorly understood at present. In this study, we examined the effect of necrosis on glioblastoma cells by exploring molecular mechanisms underlying gene expression and chemokine expression. Data obtained from chemokine array and ELISA showed that CTR-MG human glioblastoma cells secreted several chemokines including MCP-1 and MIP-3α in response to necrotic cells. Expression levels of mRNA and protein of MCP-1 and MIP-3α were also increased by treatment with necrotic cells in CTR-MG in a dose-dependent manner. Necrotic cells induced NF-kB/AP-1 activation and their binding to the MCP-1 and MIP-3α promoter, leading to enhanced MCP-1 and MIP-3α production in GBM cells. Finally, by cell migration assay, we observed increased migration of necrotic cells significantly enhanced the migration of microglia. Our data demonstrate that when GBM cells are exposed to and stimulated by necrotic cells, the expression and secretion of MCP-1 and MIP-3α are increased in GBM cells and microglia infiltration/migration to the tumor site are facilitated.

Mapping the molecular communication within the tumor microenvironment of pancreatic cancer as well as between tumor and peritumoral tissues. Mohamed S. Alwan, Yvonne C. Meisinger, Dorothea H. Hoheisel. German Cancer Research Center (DKFZ), Heidelberg, Germany.

We are analyzing in detail the molecular communication between the different cell types in the microenvironment of pancreatic ductal adenocarcinomas (PDAC). Also, its regulation is being investigated, looking at aspects such as the
kind and mixture of molecules needed for triggering a particular regulative function, and unraveling the inter- and intracellular processes utilized to transmet a molecular message. Although a rare tumor entity, adding up to only about 3% of all cancer cases in the Western world, PDAC is the fourth most frequent cause of cancer-related death; mortality is nearly identical to incidence. There is no real remedy apart from surgery, which can be applied to only 10 to 20% of patients. Even then, basically all patients will experience tumor recurrence. Our study is based on a large basic set of data at the levels of DNA, RNA and protein that was generated from some 1000 pancreatic tumor samples [1], supplemented with information from studies that aim at an elucidation of functional molecules and comparative analyses in other tumors. This has already led to the creation of basic functional knowledge about metastasis [e.g., 2], the establishment of means for diagnosis [3, 4], an accurate disease prognosis [1], the characterization of communication processes [5] and the identification of new therapeutic avenues [6]. At the conference, we will report about the status of mapping particularly the protein-mediated communication between the different cell types in the tumor microenvironment. Also, we will show results about the effect that PDAC tissue has on the wider peritumoral environment and vice versa. The peritumoral tissue is exhibiting a field defect that does not seem to be dependent on DNA-methylation. A comparison of PDAC and cystic pancreatic tumors revealed that differences in communicating with the peritumoral tissue could actually be more important to the known pathological difference between the two cancer entities than the molecular variations in the tumor cells. 1. Bauer et al. (2017), in press. 2. Clutterbuck et al. (2016) oncotarget 6, 4418-4427. 3. Dror et al. (2016) Nature Cell Biol. 18, 1006-1017. 4. Keller et al. (2014) BMC Med. 12, 224. 5. Moskalev et al. (2015) Immunotargets 1, 15-25. 6. Drolet al. (2016) Nature Cell Biol. 18, 1006-1017. 7. Jandaghi et al. (2016) Gastroenterology, in press (doi: 10.1053/j.gastro.2016.08.040) www.dkfz.de/funct_genome/.

Introduction: Esophageal squamous cell carcinoma (ESCC), which is the most common type of esophageal cancer, is one of the most common cancers in eastern Asia and one of the leading causes of cancer-related death worldwide. This unsatisfactory outcome in ESCC is thought to mainly result from late diagnosis, the aggressiveness of this disease, and the lack of the understanding in the molecular and pathological aspects of disease. Therefore, new targeted agents are needed to be investigated and developed in order to improve treatment strategies. The discoind domain receptors (DDRs) are a set of receptor tyrosine kinases (RTKs) that are activated by collagen and crucial role in key regulators in cancer progression by controlling the interactions of tumor cells with their surrounding collagen. DDR1 is expected to be involved in ESCC, which demonstrates aggressive tumor proliferation and devastating prognosis. However, there have been only a few reports about DDR1 in ESCC. We retrospectively investigated DDR1 in ESCC. Methods: A total of 60 formalin-fixed paraffin-embedded (FFPE) primary tumor samples were collected from patients with ESCC who underwent surgery with curative intent. DDR1 immunohistochemistry (IHC) was conducted on ESCC FFPE tissue specimens. Clinicopathological factors, DDR1 immunohistochemistry (IHC) and survival data were analyzed in this study. Results: When compared the clinicopathological factors between the weak and the strong group in the intensity of DDR1 IHC, in univariate analysis there were significant differences between the two groups in age (P = 0.007), histological grade (P = 0.04), invasion depth (P = 0.0001), stage (P < 0.0001). There were no significant differences between the two groups with respect to the other clinicopathological factors. In the multivariate analysis, DDR1 overexpression was recognized with respect to invasion depth (T3, 4) (P = 0.007), lymph node metastasis (LNM) (N2, 3) (P = 0.0005) and DDR1 IHC expression (strong) (P = 0.0009). When these clinicopathological factors were used as co-variables for the multivariate analysis, LNM and DDR1 IHC expression were found to be significantly independent prognostic factors (LNM, P = 0.04, HR = 4.20, DDR1; P = 0.03, HR = 4.27). Conclusion: DDR1 IHC expression was found to be useful as a biomarker to predict long-term outcome.

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#5927 Eribulin modulates tumor vascular remodeling through vascular re-modeling for antitumor effect in multiple mouse xenograft models. Ken Itô,1 Shusei Hamamichi,1 Takano Abe,1 Tsuyoshi Akagi,1 Hiroshi Shirota,1 Satoshi Kawano,1 Makoto Asano,1 Osamu Asano,1 Akira Yokoi,1 Junji Matsui,1 Izumi Sugimoto,1 Tomoaki Ito,2 Hajime Orita,3 Koichi Sato,3 Kazuhiro Sakamoto,1 Kiichi Umeda,2 Hirofumi Fujishita,1 Eisai Co., Ltd., Tsukuba, Japan; 2National Cancer Center Research Institute, Tokyo, Japan; 3Juntendo University, Faculty of Medicine, Shizuoka, Japan.

Since eribulin treatment increased accumulation of 111In-labeled PEGylated liposomes (Eribulin), a first-in-class halichondrin B-based microtubule dynamics inhibitor, has been reported to remodel tumor vasculature (i.e., improvement of tumor vessels and perfusion) in human breast cancer xenograft models; however, the role of this vascular remodeling in anti-tumor effects is not fully understood. Here, we investigated the effects of eribulin-induced vascular remodeling on anti-tumor activities in multiple human cancer xenograft models. Microvessel densities (MVDs) were evaluated by immunohistochemistry (CD31 staining), and anti-tumor effects were examined in 10 human cancer xenograft models treated with eribulin. Eribulin treatment significantly increased MVDs compared to the corresponding control groups in 7 out of 10 models with a correlation between enhanced MVD and anti-tumor effects (R² = 0.55). Because of observed increases in MVDs, we next utilized 11In-labeled PEGylated liposomes to examine if eribulin treatment would result in increased tumoral accumulation levels of such liposomes, and indeed, we found that eribulin, unlike vinorelbine another tubulin inhibitor, enhanced them. Since eribulin treatment increased accumulation of 11In-labeled PEGylated liposomes, we postulated that this treatment might enhance anti-tumor effect of Doxil (a liposomal anti-cancer agent). As expected, eribulin enhanced anti-tumor activity of Doxil in the PE-H11650 xenograft model. Then, we evaluated whether eribulin facilitated the recruitment of immune cells into the tumor. Infiltrating CD11b-positive immune cells were significantly increased in the multiple eribulin-treated xenografted tumors, and natural killer (NK) cell depletion reduced anti-tumor effects of eribulin. Collectively, these findings suggested a contribution of the immune cells for anti-tumor activities of eribulin. Taken together, our results obtained from multiple human cancer xenograft models suggested that vascular remodeling induced by eribulin therapy would act as a microenvironment modulator, and contributed to show anti-tumor effects of eribulin.

#5928 Discoidin domain receptor-1 (DDR1) is an independent prognostic marker of poor prognosis in esophageal squamous cell carcinoma. Kiichi Sugimoto,1 Tomoaki Itô,2 Hajime Orita,1 Koichi Sato,1 Kazuhiro Sakamoto,1 Mutsumi Sakurada,1 Tomoyuki Kushida,1 Hiroshi Maekawa,1 Ellen Tully,1 Jiyungh Woo,2 Edward Gabrielson2.1 Juntendo University, Faculty of Medicine, Tokyo, Japan; 2Johns Hopkins University, Sydney Kimmel Comprehensive Cancer Center, Baltimore, MD; 3Juntendo University, Faculty of Medicine, Shizuoka, Japan.

#5929 A novel image cytometric analysis method for T-cell-mediated cytotoxicy of 3D tumor spheroids. Le Li Ying Chan,1 Laure Humbert,2 Scott Cribbes.1 Nexcelom Bioscience LLC, Lawrence, MA; 2Immunoconcern Limited, London, United Kingdom.

Cell-mediated cytotoxicity assays have frequently been performed to characterize cancer cytotoxic potential of immune cells, antibodies, and drug compounds. Traditionally, these assays are performed using release assays such as Cr51 (radioactivity), Calcein (fluorescence), or LDH (enzymatic). However, release assays have limitations such as the handling of hazardous material, the indirect measurement of cell death leading to an under estimation of cytotoxicity, the requirement for a large volume of cell sample, and the inability to visually confirm, image and track the assay kinetically. In the recent years, Celigo image cytometry has been used to perform high throughput cell-mediated cytotoxicity assays using a direct cell counting method where cancer cells (Target) are stained with Calcein AM. Upon the addition of effector immune cells with the fluorescently stained Target cells in the presence or absence of antibody or drug compounds, the Celigo image cytometer is used to capture bright-field and fluorescent images and analyze the change in Target cell count over time to determine the cytotoxicity percentage. In general, these assays are performed in a 2D culture. The 2D assays for cancer drug discovery have had some difficulty in identifying more qualified drug candidates for clinical testing, thus there has been an increase in interest of performing cytotoxicity assays in 3D tumor models. Traditional 3D spheroid analysis methods require the use of standard microscopy, which is time-consuming and subjective. Celigo imaging cytometer has also been used to rapidly analyze drug effects on 3D tumor spheroids. In this work, we demonstrate a novel method of analyzing T-cell mediated cytotoxicity on 3D tumor spheroids in the presence of absence of ImmTAC molecules, which can promote higher T cell killing. In this experiment, MDA-MB-453 GFP expressing breast cancer cells are used to form tumor spheroids in an ultra-low attachment plate. The spheroids are then treated with primary T cells at 1:10 and 1:50 E:T ratios, as well as 10, 1, 0.1, 0.01, and 0.001 nM ImmTAC. The results showed a dose response effect of T cell killing with the addition of ImmTAC molecules by measuring spheroid size in GFP fluorescence and viability using propidium iodide. The image cytometry method was able to monitor changes in spheroid size and fluorescence over time and rapidly quantify T cell killing, which was as high as 75%. The captured bright-field and fluorescent images also clearly showed the activated T cells in the combination of T cell and ImmTAC. The ability to screen cytotoxic effects of immune cells, antibody, and drug compounds on 3D tumor spheroids can provide an alternative tumor model for identifying more qualified cancer drug candidates for drug discovery campaigns.
Prior work by us and others has demonstrated that the extracellular pH (pHe) of solid tumors is acidic, due to a combination of increased fermentative metabolism, resulting in lactic acid production and poor perfusion. This acidity promotes tumor progression and metastasis formation. Recently we have shown in melanoma and pancreatic cancer models that acidic pH is a central player in promoting T-cell exhaustion in the setting of predesigned mAbs targeting PD-1 or PD-L1. We thus hypothesized that targeting Tumor associated fibroblasts (TAFs) for depletion or acidification with buffer therapy (200mM NaHCO₃), synergized with checkpoint blockade (anti-CTLA4 and anti-PD1) and adoptive T-cell therapy has resulted in cures. While this is promising, concerns are high regarding the ingestion of such large amounts of sodium bicarbonate, which makes clinical translation a challenge. Hence, we hypothesize that alternative pharmacological interventions and neutralization of pHe of tumors and removal of this immunosuppressive effect. To study this we first investigated a series of agents for their ability to inhibit metastasis in the PC3 prostate cancer model, which is exquisitely sensitive to inhibition with buffer therapy. In this study, male SCID mice were grouped in to 6 groups (n=5) and treated with tap water, 200mM bicarbonate ad lib, 30 mg/kg daily (q.d) intraperitoneal (i.p) Acetazolamide (CA inhibitor), 1.2 mg/kg q.d.i.p. Furosemide (diuretic), 10 mg/kg q.d.i.p. DH348 (selective CAIX inhibitor) or 2.1 mg/kg q.d.i.p. FX-11 (LDHA inhibitor). Mice were intravenously injected with 5x10⁶ 6-PC3-MLuc cells and ventral bioluminescence images were acquired at time 0 and weekly thereafter. Our results showed that FX-11, acetazolamide, DH348 and bicarbonate were able to effectively (p<0.004) suppress metastasis formation in the setting of TLAx1, whereas furosemide was not. Based on these results, a subsequent study investigated the combination of bicarbonate, FX-11 or DH348 with immune checkpoint blockade in a Panc02 mouse model of pancreatic cancer. Animals were inoculated orthotopically with Panc02 cells and randomized into 8 groups (n=10). Once tumors reached 0.3 cc in size, mice were treated with either 15 mg/kg anti-PD-1 antibody or normal rat IgG controls twice weekly alone or in combination with bicarbonate; FX-11 or DH348 and tumor response was evaluated using US 3D-Mode imaging and volume analysis (FUJI FLM VisualSonics) and histopathology. Anti-PD-1 antibody was ineffective as a monotherapy in this system. Both bicarbonate and DH348 neutralized tumor acidity and reduced tumor growth as monotherapies. Notably, despite the fact that FX-11 was shown to inhibit LDH-A in pancreatic cancer models, it did not affect pH and had no effect on tumor growth as monotherapy. However, FX-11 significantly reduced tumor growth (p<0.001) and metastasis formation in combination with anti-PD-1 antibody, suggesting that inhibition of LDH-A might be effective in combination with checkpoint blockade.
responders of those checkpoint inhibitors are limited. Recently, it is considered that tumor immunosuppression may be caused by not only cancer cells but also tumor microenvironments (TME). Cancer-associated fibroblasts (CAFs) are one of the major components and have a central role for tumor progression in TME. It has been hypothesized that they can also play an essential role in tumor immunosuppression. In this study, a correlation between CAFs and TILs in clinical samples and further in vivo study. Materials and methods: Total 140 cases of esophageal cancer were evaluated for the presence of CAFs and TILs by immunohistochemistry (IHC). CAFs were defined as fibroblasts expressing alpha smooth muscle actin. CD8+ cytotoxic CD8+ T lymphocytes and FoxP3+ regulatory T cells were investigated as TILs in each specimen’s intratumoral and peritumoral tissues. The outcome was set as the correlation between CAFs and TILs, and Overall survival. In vivo experiments, we used a mouse-derived cancer cell (colon26-Luc) and mouse embryonic fibroblasts (NIH/3T3). BALB/c or BALB/c-nu/nu mice were inoculated with only cancer cells (control) or cancer cells with CAFs by co-CAFs into right flank to examine the immunosuppression affected by CAFs. The proliferation was examined by in vivo imaging system. Result: In clinical analysis, CD8+ TILs and CAFs demonstrated strong relations, which were significantly “negative” correlation and showed moderate correlation coefficient in intratumoral tissues (P<0.001, r=-0.416). In FoxP3+ TILs, “positive” correlation was detected significantly (P<0.001, r=0.484). In terms of prognosis, high group of CD8+ TILs had a good prognosis significantly (P<0.001), whereas high group of FoxP3+ TILs had a poor prognosis significantly (P<0.001) in intratumoral tissues. However, there was no difference of the correlation and the prognosis in peritumoral tissues. In vivo experiments demonstrated that promoted ratio of tumor progression by co-CAFs was more strongly demonstrated in BALB/c mice rather than BALB/c-nu/nu mice, suggesting that the group co-CAFs showed inducing immunosuppression in TME. IHC analysis of those tumors showed that accumulation of CD8+ TILs was decreased in tumors of co-CAFs as compared with tumors of control. On the other hand, accumulation of FoxP3+ TIL was increased, showing the same trends as clinical analysis. Conclusion: Our results suggest that Both CD8+ and FoxP3+ TILs in intratumoral tissues are independent prognostic factors, and CAFs have a significant correlation to those TILs. We consider that CAFs may affect tumor immunosuppression by regulating the migration of TILs.

#5935 Higher tumor mutation burden (TMB) is associated with the presence of DNA repair gene mutations, T-cell infiltration, and favorable survival outcome in ovarian carcinoma. Young Kwang Chae,1 William H. Bae,2 Sobina Khan F. Anwar,3 Yoon Pak Kim,3 Mario J. Pineda,1 Sabina Murshudova,1 Jonathan F. Anker,1 Young Suk Kim,3 Mario J. Pineda,1 Yonsei University College of Medicine, Seoul, Republic of Korea; 2Carnegie Mellon University, Pittsburgh, PA. Immunotherapy is being explored as a promising therapeutic option in ovarian cancer. However, little progress has been made in our understanding of the immune cell landscape in the tumor microenvironment and its association with genomic features in ovarian cancer. The RNA-seq values of the immune “metagene” markers that are specific to each of 28 cell types (Angelova et al, 2015) from the TCGA database were analyzed to determine the tumor infiltration by immune cells in 307 patient samples (185 sequenced) with ovarian carcinoma. Tumor tissues were most frequently infiltrated by all T cells (46.91%), activated CD4 T (19.87%) and activated CD8 T (16.94%) cells. Activated CD8 T cells were significantly co-infiltred by NK56 bright, effector memory CD4 T, activated CD4 T, myeloid dendritic cell (DC) and plasmacytoid DC. The tumors with mutated homologous recombination (HR) genes, including ATM, ATR, CHEK1, CHEK2, BRCA1, BRCA2, BAP1, BRD1, FANCD2, FANCE, FANCC, FANCA, RAD50, RAD51, and PALB2, demonstrated a significant 1.36-fold increase in average mutation counts compared to the wild-type (p < 0.01). Mutations in mismatch repair genes had no statistically significant association with the average mutation counts. Tumors with high mutation counts showed a significantly increased infiltration by activated CD8 T cells and immature DCs (Table 1, p < 0.05). The highest quartile in tumor mutation burden (TMB) was associated with a significant 16.7 months longer overall survival (OS) compared to the lowest quartile (p < 0.01). Our study demonstrated that higher TMB is associated with the presence of DNA repair gene mutations, activated CD8 T cell infiltration, and favorable survival outcome in ovarian carcinoma.

#5936 Comparison of carbonic anhydrase and activity between triple-negative & luminal breast cancer cells. Zhijuan Chen, Chan M. Mboge, Ching-kuang Tu, Lingbao Ai, Coy Heldermon, Susan C. Frost. Univ. of Florida, Gainesville, FL. Tumor microenvironment substantially influences the process of tumorigenesis. Extracellular acidification within the tumor microenvironment is an indicator of an aggressive cancer and a marker for poor patient outcome. In solid tumors, hypoxia leads to extracellular acidosis. Carbonic anhydrases (CA) are thought to regulate intracellular and extracellular pH (pHi and pHe, respectively). To explore the effect of CAs in breast cancer, we compared the expression and activity of two membrane bound CAs, CAIX and CAXII, between triple negative breast cancer cells (TNBCs) and luminal breast cancer cells (LBCs). We chose five different TNBC and LBC lines. Our data show that, among the TNBC lines, CAIX expression increased in three of the five lines: HBL100, SUM159, and the new UFH-001 cells under hypoxic condition. UFH-001 cells also showed strong constitutive expression. None of these TNBC lines expressed CAXII or estrogen receptor (ER). In LBC lines, four of the five lines constitutively expressed CAXII: T47D, MCF7, SKBR and SUM52 cells. CAXII expression was not hypoxia-dependent. Each of the five luminal lines expressed ER. We also examined CA expression in a tumor graft model. In tumors grown from cells derived from TNBC patients, we observed CAIX expression in four of six sample sets. In tumors derived from ER-positive LBC patients, all five expressed CAXII. We also used the 18O exchange method to assess CA activity. Two TNBC lines: UFH-001 and HBL100 cells showed that CAIX activity increased in hypoxic conditions which was blocked by an impermeant sulfonamide CA inhibitor (N3500). In the luminal lines, we detected CAXII activity in T47D and MCF7 cells that was also inhibited by N3500. Like CAXII protein expression in these cells, CAXII activity was not affected by hypoxia. We also evaluated the effect of pH on CA activity in TNBC and LBC lines. Both CAIX and CAXII showed increased activity in response to reduced pH, which is expected in a bicarbonate-based system. However, UFH-001 cells also exhibited a hypoxic-dependent increase in CAIX activity which is associated with increased protein expression. In conclusion, these observations demonstrate that CAIX expression is associated with the TNBC phenotype. Based on our activity data, we would predict that CA activity in TNBC tumors will be sensitive to both hypoxia (based on enhanced expression) and reduced pH. This change in activity may serve to regulate pH in the tumor microenvironment favoring an aggressive phenotype. On the other hand, LBC tumors, which are ER-positive, are only associated with CAIXII expression. In luminal cells, we expect that only pH and not hypoxia will affect CAIXII activity. This may, in part, explain the more positive prognosis in patients with CAIXII expression.
#5937 Obesity-induced destabilization of p16INK4A promotes the procarcinogenic effects of breast stromal adipocytes through miR-141/miR-146b-5p-dependent inhibition of leptin. Huda H. Alkhalfa,1 Abdellah Aboussékra,2,1 KACST, Riyadh, Saudi Arabia; 2KFSHRC, Riyadh, Saudi Arabia.

Despite increased obesity, a disease linked to increased number of adipocytes in various tissues, is increasingly recognized as a risk factor for the development of breast cancer. However, the molecular basis of adipocyte-related breast carcinogenesis remains elusive. In this study, we established and characterized normal stromal adipocytes from post-plastic surgery of breast tissues from obese and lean women. We have shown that adipocytes from obese women express low level of the tumor suppressor p16 protein, have higher secretion of various adipokines and higher invasion and migration capabilities as compared to adipocytes from lean women. In addition, down-regulation of p16INK4A by shRNA in adipocytes from lean women increased the expression/secretion levels of various adipokines including leptin, activated breast adipocytes and enhanced their migration/invasion abilities. Consequently, media conditioned with p16-deficient adipocytes or adipocytes from obese women promote epithelial-to-mesenchymal-transition in normal breast luminal cells in a leptin-dependent manner and induced tumor growth in vivo. Indeed, p16INK4A suppresses Leptin at the mRNA level through miR-141 and miR-146b-5p, which inhibits Leptin expression through a specific sequence at the Leptin 3’UTR. These results show the existence of active adipocytes in the mammary gland of obese women in absence of breast cancer that may contribute to the development of breast cancer. Adipokines such as leptin are potent angiogenic agents and contribute to aggressiveness of the cancer and resistance to therapy. Increased density of collagen (Col1) fibers, a major structural component of the ECM in solid tumors, and their alignment in tumors frequently result in increased invasion and metastasis of cancer cells. To understand the role of COX-2 at the lung metastatic site, and its relationship with Col1 fibers, COX-2 overexpressing triple negative SUM-149 human breast cancer cells (SUM-149-COX2) or empty vector SUM-149 cells (EV) were injected through tail vein in SCID mice to compare Col1 fiber patterns in metastatic lesions. We isolated the lungs, processed the tissue, and imaged for Col1 fiber by second harmonic generation microscopy on H&E stained sections. The number of cancer associated fibroblasts (CAFs) were detected in adjacent sections by immunostaining for α-SMA. COX-2 overexpression did not change the size of the blood vessels or the size of pulmonary metastases formed in the lungs. However, COX-2 overexpression did significantly increase the number of pulmonary metastases. Significantly higher Col1 fiber volume was observed in emboli compared to pulmonary metastases independent of COX-2 overexpression. Interestingly, the Col1 fibers were significantly more aligned in the COX-2 overexpressing pulmonary metastases compared to COX-2 overexpressing and EV emboli. The COX-2 overexpressing emboli had significantly higher amounts of CAFs detected by α-SMA expression compared to the EV emboli and the EV and COX-2 overexpressing pulmonary metastases. Pulmonary metastases from COX-2 overexpressing cells were detected further away from the nearest blood vessel compared to the metastases from EV cells suggesting that COX-2 may facilitate cancer cell extravasation. These insights provide important new insight into the role of COX-2, Col1 fibers, and CAFs in enabling emboli to extravasate to form pulmonary metastatic foci. These insights are important as COX-2 up-regulation during treatment may potentiate metastasis. This work was supported by NIH R01 CA23377.

#5938 In vivo targeting of laminin-411/B1 integrin-Notch signaling pathway using nanobiocjugate alters glioma microenvironment for effective treatment. Julia T. Ljubicicova, Cedars-Sinai Medical Center, Los Angeles, CA.

Introduction: Brain gliomas have been extensively characterized by genomic and molecular level analysis. These studies have underscored glioblastoma multiforme (GBM) heterogeneity, which may underlie failure of even the newest drugs to improve patient survival. For this reason, we turned to genetic modulation of glioma microenvironment using a novel nanobiocjugate. Methods/ Results: Clinical material from 107 GBM patients was analyzed, and tumors with overexpression of "malignant" tumor vascular basement membrane laminin-411 were found to have higher recurrence rate and shorter patient survival. In 92% of human GBM samples, overexpression of laminin-411 was associated with high expression of cancer stem cell (CSC) markers. To examine the interaction between GBM cells and their extracellular matrix microenvironment, blood-brain barrier (BBB) passing nanobiocjugates based on poly(β-L-malic acid) (PMLA) were synthesized that specifically inhibit α4 and β1 chains of trimeric laminin-411. In vitro. Normal brain endothelial cells and astrocytes had higher expression of β1 integrin, Notch-1, and Notch ligands when seeded on "malignant" laminin-411 as compared with "normal" laminin-421. All these markers were downregulated in two GBM cell lines treated with anti-β1/α5 oligonucleotides against laminin α4 and β1 Chains, suggesting regulation of Notch pathway by laminin-411 through integrin β1. In vivo. In GBM xenograft mouse models increased expression of laminin-411 correlated with overexpression of integrin β1 and Notch signaling pathway members. In two mouse models with intracranial human LN229 and U87MG GBMs, treatment with PMLA-based nanobiocjugate against tumor microenvironment protein laminin-411 led to significantly increased animal survival, associated with marked suppression of laminin-411/B1 integrin-Dll4-Notch axis and CSC markers CD133, Nestin, and c-Myc. Conclusion: BBB crossing and brain tumor-targeted nanodrug therapy using laminin-411 suppression provided a unique tool to study mechanistic interactions between tumor microenvironment and signaling pathways, and showed promise for efficient GBM treatment affecting both the bulk of tumor cells and CSC. Support: NIH grants U01 CA151815, R01 CA136841, R01 CA188743, R01 CA209921.

#5939 Dissemination of breast cancer. Samata Kakkad, Mikayla Hanna, Desmond Jacob, Balaji Krishnamachary, Zaver M. Bhujwalla. Johns Hopkins University School of Medicine, Baltimore, MD.

Breast cancer is the second leading cause of death in US women. Despite advances in early detection, diagnosis and treatment of breast cancer, metastasis and tumor recurrence persist leading to continued morbidity and mortality. Several breast cancer studies have highlighted the role of cyclooxygenase-2 (COX-2) and its interaction with the tumor microenvironment (TME) in breast cancer progression. The TME consists of stromal cells that include fibroblasts, macrophages, the extracellular matrix (ECM), and several secreted factors that contribute to aggressiveness of the cancer and resistance to therapy. Increased density of collagen (Col1) fibers, a major structural component of the ECM in solid tumors, and their alignment in tumors frequently result in increased invasion and metastasis of cancer cells. To understand the role of COX-2 at the lung metastatic site, and its relationship with Col1 fibers, COX-2 overexpressing triple negative SUM-149 human breast cancer cells (SUM-149-COX2) or empty vector SUM-149 cells (EV) were injected through tail vein in SCID mice to compare Col1 fiber patterns in metastatic lesions. We isolated the lungs, processed the tissue, and imaged for Col1 fiber by second harmonic generation microscopy on H&E stained sections. The number of cancer associated fibroblasts (CAFs) were detected in adjacent sections by immunostaining for α-SMA. COX-2 overexpression did not change the size of the blood vessels or the size of pulmonary metastases formed in the lungs. However, COX-2 overexpression did significantly increase the number of pulmonary metastases. Significantly higher Col1 fiber volume was observed in emboli compared to pulmonary metastases independent of COX-2 overexpression. Interestingly, the Col1 fibers were significantly more aligned in the COX-2 overexpressing pulmonary metastases compared to COX-2 overexpressing and EV emboli. The COX-2 overexpressing emboli had significantly higher amounts of CAFs detected by α-SMA expression compared to the EV emboli and the EV and COX-2 overexpressing pulmonary metastases. Pulmonary metastases from COX-2 overexpressing cells were detected further away from the nearest blood vessel compared to the metastases from EV cells suggesting that COX-2 may facilitate cancer cell extravasation. These insights provide important new insight into the role of COX-2, Col1 fibers, and CAFs in enabling emboli to extravasate to form pulmonary metastatic foci. These insights are important as COX-2 up-regulation during treatment may potentiate metastasis. This work was supported by NIH R01 CA23377.

#5940 The HU177 collagen neo-epitope regulates nuclear YAP accumulation and melanoma tumor growth and metastasis in vivo. Xiaohua Han, Jennifer M. Caron, Peter C. Brooks. Maine Medical Center, Scarborough, ME.

The extracellular matrix (ECM) plays a central role in controlling cellular behavior and integrin receptors play key roles in this process. Significant molecular insight is available on how structurally intact forms of collagen regulate integrin-signaling; however unique collagen neo-epitopes can be generated during tumor development and these neo-epitopes may differentially bind to integrin receptors. These findings raise an interesting question as to whether signaling initiated by collagen neo-epitopes as compared to intact collagen, differentially modify the final signaling out put. Integrin A10B1 is a receptor for the HU177 collagen neo-epitope, however, little is known about the down stream signaling events stimulated by this receptor-ligand interaction. We provide evidence that melanoma cell interaction with denatured collagen results in enhanced adhesion and migration. Talin is a molecule that facilitates integrin-associated linkages to the cytoskeleton and helps govern adhesive cellular processes. Melanoma cell interactions with denatured as compared to intact collagen, resulted in enhanced Talin phosphorylation and surprisingly increased the levels of Cyclin Dependent Kinase 5 (CDK5), a molecule implicated in Talin phosphorylation and motility. Melanoma cell interactions with denatured collagen also led to elevated nuclear accumulation of the Yes-associated protein YAP. Given these results, we examined the effects of anti-HU177 antibody on adhesion and migration. Targeting the HU177 neo-epitope inhibited cell adhesion and migration on denatured collagen and also inhibited YAP nuclear accumulation. Knocking down YAP in melanoma cells resulted in the generation of cells that were resistant to the inhibitory effects of anti-HU177 antibody, suggesting that part of the inhibitory activity of anti-HU177 antibody may be associated with its ability to alter YAP activity. We next examined the role of the HU177 neo-epitope and its receptor, A10B1, on melanoma tumor growth and metastasis. Targeting the HU177 neo-epitope inhibited tumor angiogenesis, accumulation of ASMA positive stromal cells in melanomas and tumor growth in vivo. Importantly, knock down of A10B1, or direct targeting of the HU177 neo-epitope inhibited experimental metastasis of melanoma cells. Taken together, these studies provide new insight into the role of the HU177 collagen neo-epitope in melanoma tumor growth and metastasis and provide a novel strategy to inhibit melanoma progression through selective targeting of a A10B1 ligand, rather than the integrin itself.

#5941 Integrin signaling modulation demonstrates potential therapeutic strategy in bladder cancer using three-dimensional organoid culture. LaMont Barlow, Rebecca Meyer, Ethan Shelkey, Bishoy Faltas, Mark Rubin. Weill Cornell Medical Center, New York, NY.

Introduction: Integrin signaling plays an important role in cellular proliferation and migration via interactions with extracellular matrix proteins. Prior
studies indicate that integrin signaling facilitates tumor invasion and metastasis, and there are several ongoing clinical trials using agents that modulate this pathway. We recently identified clonal enrichment in missense mutations in the integrin cell surface interactions pathways in advanced chemotherapy-resistant urothelial carcinoma. An ideal strategy for investigating integrin signaling is via 3D organoid cultures containing intercellular interactions that mimic the epithelial microenvironment. We hypothesize that pharmacologic integrin signaling modulation will impair organoid growth in human bladder cancer cells and demonstrate a potential therapeutic utility for this approach. Methods: RT4 human bladder cancer cell line was used as well as a second cell line established from a patient-derived bladder cancer sample (PM748). Cells were grown in 3D organoid culture as previously described. For in vitro integrin modulation, defactinib (VS-6063), an orally bioavailable selective inhibitor of focal adhesion kinase (FAK, a convergent and conserved enzyme activated by integrin ligand binding), was used. SDS-PAGE and immunoblotting were performed to show in vitro FAK inhibition. Single-cell suspensions and formed organoids were plated in the presence of various concentrations of defactinib to determine the impact on organoid formation and regression. Results: Both RT4 and PM748 bladder cancer cell lines demonstrated consistent organoid growth in three-dimensional culturing conditions. Addition of defactinib to cultured cells showed a dose-dependent decrease in autophosphorylation of FAK for both cell lines, demonstrating effective FAK inhibition. 3D culture of single cells in the presence of defactinib produced a dose-dependent decrease in organoid size after 96 hours (mean size for DMSO, 100nM, 1uM, and 10uM were 128um, 75um, and 26um, respectively; p<0.0001 versus DMSO for all dilutions). Established bladder cancer organoids showed a dose-dependent reduction in size after 72 hours of defactinib exposure (mean size for DMSO, 100nM, 1uM, and 10uM were 225um, 96um, 70um, and 34um, respectively; p<0.0001 versus DMSO). Experiments utilizing Crispr-Cas9-mediated FAK knock-out as well as in vivo studies with FAK inhibitors in mouse xenograft models are currently underway. Conclusions: Integrin modulation via FAK inhibition with defactinib causes both inhibition of organoid formation as well as regression of formed organoids, and the effects are seen at concentrations well below the cytotoxic range for the drug. This study suggests a utility for these agents in bladder cancer treatment.

Collagen prolyl-hydroxylase regulates breast cancer progression and chemoresistance. Gaofeng Xiong, Ren Xu. Univ. of Kentucky Markey Cancer Ctr., Lexington, KY.

Collagen prolyl hydroxylation (P4H), an enzyme hydroxylylating proline residues in X-Pro-Gly-sequences, is a potential therapeutic target for the disorders associated with increased collagen deposition. Upregulation of collagen prolyl-4-hydroxylase in cancer cells is required for breast cancer promotion. P4H1 mRNA levels were significantly upregulated in triple-negative breast cancer (TNBC) compared to other cancer subtypes. Increased mRNA levels of P4H1 correlated with poor clinical outcome in breast cancer patients. Silencing P4H1 expression or treatment with the P4H1 inhibitor significantly inhibited cell proliferation and suppressed aggressive phenotypes of breast cancer cells in 3D culture, accompanied by reduced deposition of collagen. We found that activation of the P4H1 in TNBC cells enhanced cancer cell stemness. We also found that P4H1 inhibition inhibited tumor growth and metastasis to lungs in xenograft models. Inhibition of P4H1 activity with the P4H1 inhibitor sensitized TNBC to the docetaxel treatment in the patient-derived organoid (PDO) and xenograft models. Therefore, these results suggest the crucial role of P4H1 in breast cancer progression and that targeting collagen P4H1 is a promising strategy to inhibit tumor progression and to sensitize TNBC to the docetaxel treatment in the patient-derived organoid model.

Role of tissue stiffness and oxygen tension in promoting breast cancer stem cells. Mei-Fong Pang, 1 Derek C. Radisky, 2 Celeste M. Nelson. 1Princeton Univ., Princeton, NJ; 2Mayo Clinic Cancer Center, Jacksonville, FL.

Breast tumors are stiff and hypoxic. Nevertheless, it remains unclear how stiff and hypoxic conditions within the tumor microenvironment promote breast cancer progression. Using an innovative engineered culture model to recapitulate these features, we found that, under stiff and hypoxic conditions, breast cancer cells have enhanced integrin-linked kinase (ILK) expression and acquire cancer stem cell (CSC)-like traits, suggesting tissue stiffness and oxygen tension can modulate ILK expression to induce breast CSC formation. Knocking down ILK impairs the mechanosensing of invasive breast cancer cells on stiff microenvironments, inhibits CSC markers and properties. In contrast, ectopic expression of ILK promotes breast CSC formation. In addition to promoting CSC-like phenotype, microarray analysis reveals that stiff and hypoxic microenvironments also regulate genes involved in mRNA processing, splicing and the spliceosome. These data suggest that the non-cellular compartment of the tumor microenvironment, namely tissue mechanics and oxygen tension, can promote breast cancer progression by controlling mechanotransduction and post-transcriptional regulation of breast cancer cells.

Matrilysin/MMP-7 compromises a novel border interaction between perlecain/HSPIG2 and semaphorin 3A to support prostate cancer invasion. Brian J. Grindel, 1 Daniel A. Harrington, 2 Ieland Chung, 1 Mary C. Farach-Carson. 1 Rice University, Houston, TX; 2University of Texas Health Science Center at Houston School of Dentistry, Houston, TX, 3Cedars-Sinai, Los Angeles, CA.

Prostate cancer (PCa) cells interact with the stroma and extracellular matrix (ECM) during tumor expansion and invasion during metastasis. Dissecting the complex matrix-cancer interplay is essential to halting tumor progression. A vital component of the ECM is perlecain/heparan sulfate proteoglycan 2 (HSPG2). As a large extracellular proteoglycan, perlecain helps orchestrate tumor angiogenesis, proliferation, differentiation and invasion. Actively metastasizing cancer cells must proteolytically degrade several tissue borders which perlecain patrols, including the basement membrane, vasculature, reactive stromal matrix and bone marrow. Investigating the effects of perlecain on metastatic PCa cells showed full length perlecain and specifically conserved domain IV of perlecain triggers clustering of PCa cells. Perlecain cleavage by the pro-tumor protease matrilysin/matrix metalloproteinase (MMP-7) reverses this, favoring cell dispersion and tumor dyscohesion. Network analysis of a reverse phase protein array and subsequent western blots revealed clustered cells on intact perlecain/Dm IV induced a strong focal adhesion kinase (FAK) dephosphorylation/reactivation. Alternatively, FAK was highly phosphorylated/activated on MMP-7 cleaved perlecain/Dm IV consistent with an adhesive and dispersive/metastatic phenotype. Several lines of evidence indicate Dm IV interacts with the classically known axon guidance protein semaphorin 3A (Sema3A), to shut off pro-metastatic FAK activation. Notably, a C-terminus directed Sema3A antibody mimics the clustering activity of Dm IV. We showed Dm IV binds to Sema3A in ELISA and crystal quartz microbalance experiments and the interaction is abrogated by the Sema3A antibody. Interestingly, Sema3A also is sensitive to MMP-7 proteolytic activity. Finally, transient knockdown of Sema3A in PCa cells inhibits the clustering effect by a Dm IV substrate. This work shows that perlecain is an effective border molecule because it coordinates with Sema3A to repulse and cluster cells by switching off FAK activity. However, PCa cells expressing active MMPs, such as MMP-7, can destroy this Sema3A/perlecain complex to develop an invasive, metastatic phenotype associated with lethal disease.

CRISPR-Cas9 dissection of heparan sulfate. Ryan J. Weiss, Philipp N. Spahn, Nathaniel E. Lewis, Jeffrey D. Esko. University of California, San Diego, La Jolla, CA.

Heparan sulfate proteoglycans (HSPGs) are expressed on virtually all animal cells and play important roles in tumor growth and metastasis. Each HSPG consists of a core protein with one or more covalently attached linear heparan sulfate (HS) chains composed of alternating glucosamine and uronic acids that are heterogeneously N- and O-sulfated. These complex cell surface carbohydrates regulate important biological processes including cell adhesion, proliferation, and development and maintain intracellular stores that number in the billions. Together, these modifications contribute to a large number of matrix proteins and growth factors. The arrangement and orientation of the sulfated sugar residues of HS specify the location of distinct ligand binding sites on the cell surface, and these modifications can vary temporally during development and spatially across tissues. Previous studies have shown that HSPGs can modulate tumor growth kinetics and are abundantly expressed on the cell surface of many types of cancer. Additionally, genetically reducing the sulfation of HS has been shown to selectively inhibit tumor angiogenesis and lymph node metastasis. While most of the enzymes involved in HS biosynthesis have been studied extensively, much less information exists regarding the specific mechanisms that give rise to the variable composition and binding properties of HS. The overall goal of this project is to uncover and characterize novel genes whose expression influences HS-mediated regulatory networks in tumor growth and metastasis. A genomewide CRISPR/Cas9-mediated screen was developed to uncover and characterize novel genes other than those encoding known HS biosynthetic enzymes. A lentiviral single guide RNA (sgRNA) library was utilized to knock down gene expression across the entire genome in a human malignant melanoma cell line. Subsequently, a high-throughput screening assay was adapted to identify lentiviral-encoded sgRNAs that induce resistance to cytotoxins whose action depends on HSPGs. Parallel screens using alternative HS-dependent toxins or plant lectins that cause cytotoxicity dependent on other types of glycosylation were performed in order to sort genes that se-
lectively affect HS biosynthesis. From the toxin screens, we identified previously studied genes essential for HS formation and factors involved in the intoxication of cells by diphtheria toxin, an HS-dependent exotoxin. Furthermore, we uncovered potential candidate genes whose function is unknown relative to HS biosynthesis. Top hits from the screens were characterized and categorized based on their predicted gene functions and are currently being individually validated and examined for their potential involvement in the regulation of HS biosynthesis. Overall, these studies will provide a better understanding of the genetic regulatory factors involved in HS biogenesis. Additionally, the factors we identify could reveal novel targets for anti-cancer therapies, as well as lead us to methods to manipulate HS and its activities in other cellular processes that go awry in human diseases.

REGULATORY SCIENCE AND POLICY: Recent Trends in Regulatory Science

#5946 Universal consent for biospecimens: a novel electronic/video consent. Arash Naeim,1 Neil Wenger,1 Antonia Petruse,1 Liliana Sanchez,1 Azita Sharif,2 Sarah Dry1. 1David Geffen UCLA School of Medicine, CA; 2Daedalus Software, Inc., MA.

Background: Developing innovative, efficient and institutionally scalable bio-specimen consent for remnant tissue that meets the NIH consent guidelines for genomic and molecular analysis is essential for precision medicine efforts in cancer. Solutions in this arena need to satisfy the needs of patients, researchers, ethicists, IRB and compliance leadership, while fitting as seamlessly as possible into existing clinical workflows. Methods: UCLA developed a video-application kiosk-based approach for providing universal consent to repurpose clinical remnant bio-specimen for research. The process was designed to be self-service, comprehensive yet fast (mean shorter than 5 minute for completion). The consent additionally asked the patient if they were willing to be contacted directly for future research projects. This approach was piloted with 474 patients who were coming in for routine services in laboratory medicine, radiology, oncology, and hospital admissions. Of the pilot population, 175 individuals had targeted surveys to evaluate drivers for opting-in or opting-out of the consent for allowing the collection and use of their remnant tissue for research. The cognitive survey was online and presented immediately after the consent process was completed. Results: The opt-in rate for the pilot was 90.7%, and 56% agreed to direct contact for future research. Only 7% needed help navigating the online consent process. Of the subgroup of pilot population who completed the targeted survey, there was no difference between individuals who opted in and out regarding ease of use, of the consent application with about 75% stating it provided mostly or very useful information, 90% stating it was mostly or very easy to understand, and 85% stating they trusted the information. However, there were significant differences between those that opted-in and opted-out in their beliefs concerning usefulness of tissue, trusting researchers, importance of contributing to science and privacy risk with those opting in strongly supporting these beliefs (≥90%) compared to those that opted out (<40%), p<0.001. Conclusions: Video-application approach for allowing individuals to consent for remnant specimens to be collected and used for research, including cancer research, can be efficient, patient-centric and meet the NIH requirements. This method could increase the availability of blood and tissue for cancer research and should be tested for scalability as an enterprise solution.

#5947 The NCI Best Practices for Biospecimen Resources: 2016 revised recommendations. Abhi Rao, Jim Vaught, Ping Guan, Carol Weil, Helen M. Moore. NIH, Bethesda, MD.

Improved biospecimen handling practices are increasingly important for cancer research as advanced molecular analysis becomes routine in clinical trials and more frequently available in standard of care medicine. Biospecimens and associated clinical data collected in a consistent, established fashion can greatly facilitate cancer biomarker validation and development and validation of clinical diagnostic assays. In order to establish a set of guidelines to improve the quality of biospecimen-related research, the NCI’s Biorepositories and Biospecimen Research Branch developed the NCI Best Practices for Biospecimen Resources which includes technical recommendations on biospecimen handling as well as ethical and regulatory best practices. The 3rd, 2016 revised version of these Best Practices focused on updating technical and operational best practices with recommendations based on more recent research, guidance and standards for collecting, processing and storing biospecimens; revised informatics best practices; and updated ethical, legal and policy sections describing new developments on return of research results, informed consent for genomics research, data sharing, and community engagement. These Best Practices aim to help patients by improving the reproducibility of cancer research data. The NCI Best Practices are also foundational to the NIH Precision Medicine Initiative, part of which aims to establish the world’s largest research biobank that will support studies that utilize biospecimens from a cohort of one million individuals in the United States.
findings indicate that patient-derived T cells recognized the eliminated neoantigens and suggest that these neoantigens were relevant targets for the achievement of initial therapeutic response to checkpoint blockade. Conceptually, neoantigen loss occurs through elimination of tumor subclones or through deletion of chromosomal regions containing such alterations. To estimate the contribution of these mechanisms to the loss of neoantigens, we analyzed the tumors both before and after therapy using the SCHISM pipeline and incorporating mutation frequency, tumor purity, and copy number variation to infer the fraction of cells containing a specific mutation (mutation calling). Consistent with other studies, we observed both loss of neoantigen elimination: loss of truncal changes through genetic events involving chromosomal deletions and loss of heterozygosity (LOH) and loss of subclonal neoantigens either by LOH or through elimination of tumor subclones. Both truncal and subclonal changes were among the eliminated neoantigens that were functionally validated.

To evaluate the impact of neoantigen loss on cytotoxic T-cell receptor repertoire, we analyzed serially collected PBMCs, prior to immunotherapy initiation, at clinical response, and at resistance. We hypothesized that loss of neoantigens would lead to a decrease in clonality of cytotoxic TCR clonotypes, thus reflecting tumor immune evasion at the time of emergence of resistance. We observed peripheral T-cell expansion of a subset of the top 100 most frequent intratumoral clonotypes, with the most frequent clones reaching up to a 44-fold increase in abundance in the blood at the time of response, followed by a decrease to pretreatment levels at the time of resistance. As a comparison, such decreases in TCR frequencies were not observed in a NSCLC patient with durable response to PD-1 blockade and no change in intratumoral TCR frequencies was seen in a NSCLC patient with primary resistance to PD-1 blockade. Taken together, these observations suggest that TCR expansion may be both a useful measure of response to checkpoint blockade and an indicator of acquired therapeutic resistance through neoantigen loss.

As immune checkpoint therapy has become standard of care for many cancer types, the development of acquired resistance is being recognized more commonly. Through our comprehensive genomic analyses, we have identified changes in the genomic landscape of truncal immune response that have remained elusive. To examine mechanisms of resistance to immunotherapy, we performed genome-wide sequence analysis of protein coding genes and T-cell receptor (TCR) clonotype analysis, followed by functional assays of autologous T-cell activation of patients who demonstrated initial response to immune checkpoint blockade but ultimately developed progressive disease. Of a cohort of 42 NSCLC patients treated with single-agent PD-1 or combined PD-1 and CTLA4 blockade, we identified all consecutive cases that at the time of the analysis developed acquired resistance (two subjects treated with nivolumab and two with ipilimumab and nivolumab) and who had paired tumor samples available both before and after therapy. To examine the landscape of genomic alterations and associated neoantigens, we performed whole exome sequencing of tumors from these patients. Pretreatment and postprogression specimens were obtained from the same anatomic location or from sites in close anatomic proximity. We examined multiple immune-related pathways, identified peptides stemming from somatic alterations using a computational multidimensional neoantigen prediction platform. This approach allowed for identification of peptides within mutated genes that were predicted to be processed and presented by MHC class I proteins and therefore had the potential to elicit an immune response. The algorithm evaluated the binding of mutant peptides (8-11mers) to patient-specific HLA class 1 alleles and ranked the neoantigens according to MHC binding affinity, antigen processing, and self-similarity.

Analyses of matched pretreatment and resistant tumors identified genomic changes resulting in loss of 7 to 18 putative mutation-associated neoantigens in resistant clones. While algorithm-based predictions of antigenicity are valuable in narrowing the large number of peptides capable of being generated by a mutation to a set of potential antigenic peptides presented by self-MHC alleles, functional T cell recognition is critical to evaluate immune responsiveness. To this end, we developed a sensitive approach for assessing T-cell response to candidate MANAs (cMANAs) that utilized next-generation sequencing of TCR-Vb CDR3 regions as a measure of T-cell clonality. To evaluate T-cell recognition of eliminated neoantigens, purified peripheral blood T cells from the patients described above were stimulated with autologous peripheral blood mononuclear cells (PBMCs) loaded with CMANA peptides in a ten-day culture system. We subsequently used TCR next-generation sequencing to assess the T-cell recognition of eliminated neoantigens, purified peripheral blood T cells from the patients described above were stimulated with autologous peripheral blood mononuclear cells (PBMCs) loaded with CMANA peptides in a ten-day culture system. We subsequently used TCR next-generation sequencing to assess the T-cell recognition of eliminated neoantigens, purified peripheral blood T cells from the patients described above were stimulated with autologous peripheral blood mononuclear cells (PBMCs) loaded with CMANA peptides in a ten-day culture system. We subsequently used TCR next-generation sequencing to assess the T-cell recognition of eliminated neoantigens, purified peripheral blood T cells from the patients described above were stimulated with autologous peripheral blood mononuclear cells (PBMCs) loaded with CMANA peptides in a ten-day culture system. We subsequently used TCR next-generation sequencing to assess the T-cell recognition of eliminated neoantigens, purified peripheral blood T cells from the patients described above were stimulated with autologous peripheral blood mononuclear cells (PBMCs) loaded with CMANA peptides in a ten-day culture system. We subsequently used TCR next-generation sequencing to assess the T-cell recognition of eliminated neoantigens, purified peripheral blood T cells from the patients described above were stimulated with autologous peripheral blood mononuclear cells (PBMCs) loaded with CMANA peptides in a ten-day culture system.

The Effects of Tumor Microenvironment on Metastasis and Therapy Resistance

#NG02 Lymphatic vessels: Balancing immune priming and immune evasion in melanoma. Ryan S. Lane, Julia Fernel, Jamie Booth, Christopher Loo, Nathan M. Nelson, Takahiro Tsujiyak, Guillaume Thibault, Amanda W. Lund. Oregon Health & Science University, Portland, OR. Lymphatic vessel remodeling and lymphangiogenesis is correlated with melanoma progression and lymph node metastasis. While lymphatic vessels provide an important route for disseminating tumor cells, they are also a crucial interface between a developing malignancy and the host immune response. Rather than acting as passive conduits, lymphatic vessels directly regulate their transport function and facilitate leukocyte trafficking for efficient induction of adaptive immunity in downstream draining lymph nodes. We recently published that in the absence of dermal lymphatic vessels, the tumor...
microenvironment of murine melanoma remains completely uninflamed and fails to induce a robust T-cell response (1). Consistently, TCGA analysis of human cutaneous metastatic melanoma identified positive correlations between LEC gene expression and immune status, including a T-cell-inflamed signature, indicating a relationship between lymphatic vessel remodeling and local immunity. Furthermore, others have recently demonstrated that lymphatic vessel density (LVD) in combination with intratumoral T cell function stratified best overall survival in nonmetastatic and metastatic colorectal cancer (2). In contrast to this, however, many reports independently correlate high LVD with lymph node metastasis and some poor prognosis (3). Furthermore, our previous work demonstrated that vascular endothelial growth factor C (VEGFC)-driven lymphangiogenesis in the context of murine melanoma drove increased leukocyte infiltration but associated with poor CD8+ T cell priming in draining lymph nodes (4). Therefore, we hypothesize that lymphatic vessels are (A) required for induction of adaptive immunity but (B) acquire immunosuppressive activity as a function of the accumulation of local cytotoxic immunity. Furthermore, we predict that LVD may be a relevant biomarker of in situ immune responsiveness and response to therapy (5).

To test the first part of this hypothesis (A), we have continued our published work and used a cutaneous model of viral infection to demonstrate the requirement for lymphatic vessel drainage in cutaneous immunopathology. Following cutaneous virus infection we demonstrate that the absence of lymphatic vessel transport both cellular and humoral adaptive immune responses fail to initiate, leading to enhanced cutaneous immunopathology and persistent viral load. The complete absence of primary CD8+ and CD4+ T cells in cutaneous tissue following challenge mirrors our observations in melanoma and is consistent with correlations of intratumoral lymphocytes and LVD both by our group in cutaneous metastatic melanoma as well as others. This unequivocal requirement for a functional lymphatic vasculature in the setting of cutaneous immunity further supports the prediction that LVD may be a novel biomarker of immune reactivity within tumor parenchyma. To test this, we simultaneously evaluated immune and vascular components in human primary melanoma samples using a multiplex-immunohistochemistry-based approach. Tissue regions that have tumultuous lymphatic borders and show infiltrates are selected for analysis, followed by tissue segmentation, and automated detection of cell populations within intratumoral regions and bordering stroma. Interestingly, those tumors with enhanced hematopoietic infiltrate (CD68 and CD8) also appear to demonstrate increased vasculature, both blood (CD31 and CD34) and lymphatic (D2-40 and Prox1). Preliminary data demonstrate that lymphatic vessels, blood vessels, and CD8+ T cells are significantly enriched at the tumor-stroma border in primary melanoma and positively correlate with one another, indicating that lymphatic vessels may be a dynamic component of the immune-microenvironment.

While we demonstrate that lymphatic vessels are necessary for immune induction, we further hypothesized (B) that in the context of an ongoing immune response lymphatic vessels adapt their function to promote immune resolution. The adaptive resistance hypothesis proposes that upon accumulation of local cytotoxic immunity, both tumors as well as stromal components adapt and acquire therapeutically relevant immunosuppressive function. We demonstrate that peripheral, tumor-associated lymphatic endothelial cells (LEC) (LymphoCyt™, CD45−CD31+gp38+) acquire expression of immunoregulatory proteins, most notably programmed death receptor ligand-1 (PD-L1) and major histocompatibility complex II (MHCII), coincident with CD8+ T-cell infiltration in an interferon gamma (IFNg)-dependent manner. Adoptive transfer of activated CD8+ T cells induced higher expression of PD-L1 by LECs in B16 F10 tumors, while neutralization of IFNg reduced levels to that of naïve skin. Furthermore, conditional deletion of IFNg in tumor-associated LECs constitutively expresses PD-L1 and this neutralization of IFNg reduced levels to that of naïve skin. Furthermore, conditional deletion of IFNg in tumor-associated LECs constituting indicates that IFNg is critical for the induction of PD-L1 in tumor-associated LECs. Notably, lymph node LECs constitutively express PD-L1 and this expression is increased further in IFNg-deficient cohorts attributable to a lack of IFNg-induced PD-L1 transcription. Importantly, in the absence of IFNg on peripheral LECs we observed significantly enhanced accumulation of antigen-specific CD8+ T cells in cutaneous tissue. Thus, cutaneous lymphatic vessels, which necessary for immune induction, acquire immunosuppressive activity in a dynamic manner and may participate in immune evasion within tumor microenvironments.

In conclusion, our work across multiple model systems provides strong experimental evidence to indicate that the lymphatic vasculature is an important, active component of the antitumor immune response and may represent a biomarker to stratify patient response and survival for effective clinical immunotherapy. These data indicate a need to revisit the paradigm of lymphatic vessel involvement in tumor progression and metastasis to a more active component of the immune microenvironment. These data indicate a need to revisit the passive evidence to indicate that the lymphatic vasculature is an important, active component of the immune microenvironment.

References

Recent Advances in Prevention Research

Diet and Precision Medicine in Cancer Prevention: The Role of Genetic Background, the Microbiome, and Epigenetic Mechanisms

NG03 Marine Omega-3 polysaturated fatty acid and colorectal cancer prevention and treatment, Mingyang Song,1 Kana Wu,2 Shuji Ogino,3 Jeffrey A. Meyerhardt,4 Charles S. Fuchs, Wendy Garrett, Edward L. Giovannucci, Andrew T. Chan. 1Massachusetts General Hospital, Harvard Medical School, Boston, MA; 2Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA; 3Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA.

Background: Lifestyle factors are important determinants for the development and progression of colorectal cancer (CRC). An estimated 50-60% of new CRC cases and deaths in the U.S. could be potentially prevented through modification of lifestyle, including diet (2). Fish oil, a rich source of marine omega-3 polysaturated fatty acid (OM3PUFA), is one of the most popular natural dietary supplements used by U.S. adults (3). OM3PUFA has potent anti-inflammatory activity, and substantial experimental data support its beneficial effect for CRC prevention and treatment (4). However, the relevance of these findings to human cancer has been largely unknown. I have led a series of studies linking OM3PUFA to CRC, leveraging data from two large prospective cohort studies, the Nurses’ Health Study (NHS) and the Health Professionals Follow-up Study (HPFS) that have provided detailed information on lifestyle and diet in relation to CRC.

Study setting: The NHS cohort enrolled 121,701 female nurses aged 30-55 years in 1976, and the HPFS cohort enrolled 40,507 men aged 40-75 years in 1986. Over 30 years of follow-up, we have followed up participants through a biennial questionnaire to collect their updated lifestyle and medical information. We have also collected dietary data every 4 years through the food frequency questionnaire, which has been validated for OM3PUFA intake. Moreover, we have followed up patients through a biennial questionnaire to collect their updated lifestyle and medical information.

Study design: We conducted a matched case-control study nested within each cohort, with CRC cases identified through centralized medical record linkage and death certificate verification. We excluded participants who did not complete at least 10 years of follow-up data. We used conditional logistic regression to estimate hazard ratios (HR) and 95% confidence intervals (CI) for the association of overall and dietary OM3PUFA intake with CRC risk. We have also investigated interactions with age, body mass index (BMI), physical activity, and other potential modulators of CRC risk.

Main findings: In our main analysis, we found that CRC risk was lower by 20% in the highest quintile of dietary OM3PUFA intake compared to the lowest quintile (HR: 0.80, 95% CI: 0.70-0.93). This finding was consistent with the inverse association of OM3PUFA with CRC risk reported in prior observational studies (3). Furthermore, among individuals with a family history of CRC, the inverse association of OM3PUFA with CRC risk was stronger (HR: 0.66, 95% CI: 0.47-0.95). These findings are consistent with results from prior studies and support the potential role of OM3PUFA in CRC prevention.

Implications: Our study provides strong evidence for the potential role of OM3PUFA in CRC prevention and highlights the potential for lifestyle modification to reduce CRC risk.

For Acknowledgments, see abstract text.

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Given the increasing data linking the tumor immune response to cancer prognosis, I led further studies assessing the relationship of MOPUFA intake with CRC survival. We found that patients with high MOPUFA intake after CRC diagnosis had a significantly longer survival compared to those with low intake, suggesting that MOPUFA may exert a beneficial effect across the continuum of colorectal carcinogenesis. We have recently validated these findings in the cohort of CALGB 89803 (Van Blarigan EL et al., manuscript in preparation).

Future plan: Based on this work, I now propose to better understand the intricate relationship between MOPUFA, immune response, and CRC through the development of an independent research program. First, given the increasing data supporting a pivotal role of gut microbes in integrating dietary cues with host immunity and potentially mediating the therapeutic efficacy of cancer therapy, we have proposed to study how MOPUFA modulates the gut microbial composition and function, thereby shaping the gut immune response and suppressing CRC. The findings from this study will provide translational insights into novel dietary and microbial strategies for CRC prevention. Second, to better understand the immunomodulatory effect of MOPUFA in the tumor microenvironment, I plan to characterize the MOPUFA-associated tumor immune signature and study whether MOPUFA improves CRC survival by ameliorating the immunosuppressive microenvironment. Because the immunosuppressive mechanisms have been implicated in the development of resistance to powerful cancer immunotherapeutic agents, our findings may open new avenues for incorporating natural products into existing cancer immunotherapy paradigms for optimizing clinical benefit. In both these areas, I will take an integrated approach that combines the power of population science and clinical research to establish causality and facilitate clinical translation. This approach will also provide a foundation for my research program as I transition into independence.

References

Major Symposia
Mechanisms of Primary and Acquired Resistance to Immunotherapy

NG04 Mechanisms of resistance to anti-PD-1 immunotherapy through interferon pathway mutations

checkpoint blockade immunotherapy can produce dramatic, durable tumor regression, is now being deployed or tested in a growing list of cancer types, and has received FDA approval for the treatment of metastatic melanoma, Hodgkin’s lymphoma, and lung, renal, and head and neck carcinomas. Depending on cancer type, higher CD8 infiltration, PD-L1 expression, and mutational load in tumors may potentiate immune responses to tumors expressing PD-1 ligand (PD-L1). In a cohort of 55 patients from centers in the US and Australia with advanced desmoplastic melanoma (a rare subtype, less than 4% of patients). Our analyses further highlight the relationship between clinical benefit from immune checkpoint blockade therapy and higher mutational load and PD-L1 expression. Objective tumor responses were noted in 39 patients (71%), with most being long lasting. Our analyses reveal that baseline tumor biopsies have significantly higher tumor parenchymal PD-L1 expression than nondesmoplastic skin melanomas that is highly correlated with PD-L1 in the invasive margins. Desmoplastic melanoma has a known relationship to ultraviolet light radiation damage, resulting in one of the highest mutational burdens among all cancer types. Whole-exome sequencing in this cohort confirms this finding and demonstrates a non-random distribution of nonsynonymous mutations per tumor, as compared with a median of 485 mutations per tumor in nondesmoplastic melanomas (P = 0.01).

However, accumulating data from cutaneous melanoma suggest that primary and secondary resistance to anti-PD-1 therapy is a rare event. Recent pooled data from 65% patients on a clinical trial of the anti-programmed death 1 (PD-1) therapy antibody pembrolizumab reported that ~60% of patients with metastatic melanoma had no response to therapy (primary resistance), while ~25% of those with initial objective tumor response had progressed as much as two years later despite continuous therapy (secondary or acquired resistance). Potential mechanisms of resistance conceptually stratify into either T-cell dysfunction (lack of recognition, trafficking, intrinsic exhaustion, or extrinsic suppression) or tumor-based escape (loss of antigen presentation, resistance to killing, or upregulation of suppressive factors).

We have recently reported two genetic mechanisms of acquired resistance to anti-PD-1 therapy (1). In this study, we analyzed melanoma cases with late acquired resistance after strong initial response to anti-PD1 therapy, with the hypothesis that changes from baseline would be mechanistically meaningful in this setting, particularly with respect to immune function or mutations acquired by surviving clones. To investigate this, we performed whole exome sequencing and immunohistochemistry on tumors from cases with paired pre- and post-tumor samples, followed by in vitro modeling with primary tumor cell lines.

First, we studied the local immune environment at relapse to rule out a lack of tumor-specific T-cell effectors. Immunohistochemistry revealed that CD8 T cells were present in the early tumors and at relapse in the majority of the tumors. This is a pattern that our lab and others have shown would otherwise be predictive of response to PD1 therapy. Further studies using targeted sequencing of the T-cell receptor population in biopsies from the four reported cases showed that the top T-cell clones at baseline or during active response were detected at similar frequencies in the relapsing tumors, indicating that the local T-cell repertoire had not changed. Additionally, clones that significantly increased in frequency in the peripheral blood during active response were highly enriched among the top clones in the relapsing tumor. Together, these data indicate the T cells present at relapse are the same T-cell specific clones that mediated the original response. Multiplexed immunofluorescence showed that stromal cells in the tumor microenvironment at progression were strongly positive for PD-L1 expression in areas of high T-cell density. PD-L1 is often upregulated in response to the interferon gamma produced by activated T cells, raising the possibility that these T cells were currently or had recently been active.

With evidence that T cells were still present and possibly active in the resistant lesions, we investigated relapse-specific changes within the tumors themselves by whole exome sequencing. We identified new homozygous loss-of-function mutations in the interferon receptor pathway-associated kinases in the relapse biopsies of two separate cases (JAK1 Q503* and JAK2 F547_splice-site). In addition to the clinical radiographic evidence of relapse in situ, we found strong genetic evidence that the progressing lesions were directly descended from the baseline lesions by clonal selection and oligoclonal expansion. Over two years between biopsies, the resistant tumor shared >92% of nonsynonymous mutations originally identified at baseline. The relapsing tumors also contained the same initial somatic mutations and loss of heterozygosity observed for the invasive margin. We have become homozygous as part of additional LOH events. Neither JAK mutation was detected pretherapy, either in the exome sequencing reads or by targeted amplicon sequencing, suggesting these represent a de novo mutation or extremely rare preexisting clone.

While the JAK kinases are known to associate with growth factor, cytokine, and interferon receptors, in the context of immune resistance we focused on their role in interferon sensitivity. Interferons cause phosphorylation of the STAT transcription factors, and upregulate expression of chemokines, antigen presentation machinery, and PD-L1. In vitro western blot, RNA (Nanosigntm), and flow cytometry studies using primary cell lines derived from pre- and postprogression biopsies from the JAK2 mutated case showed that the JAK2 mutated cells were entirely insensitive to interferon gamma. Other melanoma cell lines with JAK1 or JAK2 deleted by CRISPR-Cas9 approach produced similar results. We also found the JAK mutant cell lines to be resistant to interferon-gamma induced growth inhibition, while the baseline and JAK wild-type cell lines remained sensitive. Notably, the JAK2 mutant cell lines were still sensitive to growth inhibition by interferon-alpha/beta or a STENG agonist (all of which are JAK2-independent), showing the lack of growth arrest was specific to interferon gamma resistance and not a generalized phenomenon.

In a third case, we also identified a new truncating mutation in the antigen presentation gene beta-2 microglobulin (B2M), which led to loss of surface expression of major histocompatibility complex (MHC), with the potential to mechanically implicate in immune evasion. Since the publication of this work we have gathered seven additional biopsies of late secondary progression from patients with advanced melanoma (four of them with a paired baseline) in order to assess the frequency of acquired genetic events in the interferon gamma and antigen presentation pathways, as well as define other potential mechanisms.

Our studies of primary resistance to anti-PD-1 therapy provide further support that loss of function mutations in the interferon pathway genes JAK1 or JAK2 are of high relevance for resistance to antiprogrammed death protein 1 (PD-1) therapy. Immune selection may lead to similar mutations
Dysregulation of MicroRNAs Leads to Target Therapy

#NG05 TP53-mediated human cancer susceptibility is defined by genomic dysregulation of microRNA-34A. Nardin Samuel,1 Gavin Wilson,2 Genevieve Deblois,3 Bade Id 3.1x10^4,4 Niu 1.1x10^4,4 and validated in an independent patient cohort (n=76, 1.9x10^8). Targeted sequencing demonstrated that miR-34A is inactivated by hypermethylation across many different histologic types of primary tumors from LFS patients, such as brain tumors, osteosarcomas, rhabdomyosarcomas, and adrenocortical carcinomas. miR-34A promoter hypermethylation also occurs in lymph node metastases from a cohort of 29 patients with choroid plexus carcinomas, a characteristic LFS tumor (p<0.05). The relationship between miR-34A hypermethylation and TP53 mutation was further validated in sporadic cancers, using the publicly available TCGA dataset. This demonstrates the robustness of this correlation and the applicability of these findings to other cancer contexts.

This study refines the role of epigenetic in a cancer predisposition syndrome and is the first to implicate a microRNA, miR-34A, in human cancer susceptibility and provides a repository of genomic regions of deregulated methylation in the context of dysfunctional TP53. These findings suggest that deregulated DNA methylation at defined genomic loci may be an important hallmark of TP53-mediated cancer susceptibility. The most striking finding from this study is the relative miR-34A promoter hypermethylation at two adjacent CpG sites in peripheral blood from TP53 mutation carriers, confirmed in two independent cohorts and shown to cosegregate with TP53 mutations in LFS families. This result is remarkable since miR-34A is a central microRNA in the p53 network and the first microRNA identified as a direct proapoptotic target of the p53 pathway.

The detection of miR-34A promoter hypermethylation in TP53 mutant cells that have not undergone malignant transformation supports a putative model whereby wild-type p53 may influence methylation patterns at this locus. In particular, in nontransformed cells that do not harbor mutations in TP53, wild-type p53 may be recruited to the miR-34A locus and sustain hypermethylation. We have performed a series of in vitro studies on primary prostate and colon epithelial cell lines to corroborate this model. Conversely, in the setting of loss-of-function or deleterious mutations in TP53, mutant p53 may be able to maintain hypermethylation of the miR-34A promoter, leading to upregulation of miR-34A. Owing to the known redundant cellular roles of p53 and miR-34A, upregulation of miR-34A may be beneficial to cells harboring mutant p53 by supplementing the basal tumor suppressive function that is lost when p53 is mutated. This mechanism may serve to guard against mutant p53, even when the wild-type allele remains. Accordingly, this may explain why miR-34A promoter hypermethylation is characteristic of TP53 mutant tumors that lack wild-type p53 because this microRNA serves a critical role in cell maintenance, and its loss may cooperate with other genetic and/or epigenetic events to drive malignancy.

It is therefore not surprising that, akin to p53, somatic miR-34A deregulation is pervasive in human cancer and miR-34A inactivation by focal deletion or promoter hypermethylation has been reported in the literature to occur in a multitude of human malignancies. The precise mechanisms of how the miR-34A promoter undergoes somatic epimutation in tissues remains to be elucidated, and likely various pathways may converge to yield this outcome in different tissues. Given the high frequency of TP53 mutation in human malignancies, the relationship between mutant p53 and miR-34A has strong implications for the targeting of miR-34A in cancer. Encouragingly, studies have demonstrated in vivo the utility of miR-34A-based therapies in cancer, including intratumor or systemic delivery of lipid-formulated synthetic miR-34A.

To further probe these intriguing findings, we conducted mechanistic studies aimed at functionally interrogating the the miR-34A-p53 axis. We utilized in vitro-based assays to modulate miR-34A levels in primary-derived fibroblast cell lines, and subsequently performed by RNA-seq of the transcriptional responses. Our results uncover a number of novel cellular roles for miR-34A in cell maintenance. Significantly, the transcriptional response to miR-34A inhibition revealed that this microRNA may be a crucial switch that can lead to numerous changes to noncoding RNA networks as well as known p53 pathways. Markedly increased expression of key components of the U12 spliceosome occurs when miR-34A expression is diminished, thereby identifying the miR-34A-p53 axis as a potential driver of transcriptome regulation. The most striking manifestation of this regulatory network is the distinct transcriptional signature of miR-34A in LFS. These findings suggest that deregulated DNA methylation at defined genomic loci may be an important hallmark of TP53-mediated cancer susceptibility. The most striking finding from this study is the relative miR-34A promoter hypermethylation at two adjacent CpG sites in peripheral blood from TP53 mutation carriers, confirmed in two independent cohorts and shown to cosegregate with TP53 mutations in LFS families. This result is remarkable since miR-34A is a central microRNA in the p53 network and the first microRNA identified as a direct proapoptotic target of the p53 pathway.

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Invited Abstracts

Introduction: Chemotherapy-resistant urothelial carcinoma (UC) has no uniformly curative therapy. Understanding how selective pressure from chemotherapy directs UC’s evolution and shapes its clonal architecture is a central biologic question with important clinical implications.

Methods: To address this question, we performed whole-exome sequencing and clonality analysis of 72 UCs including 16 matched sets of primary and advanced tumors prospectively collected before and after chemotherapy.

Results: Our analysis provided several important insights. Our findings show that chemotherapy-treated UC is characterized by significant intrapatient mutational heterogeneity. The majority of mutations were not shared. On average, only 28.4% (range 0.2%-76.4%) of mutations were shared between pre- and postchemotherapy samples. This effect was consistent across primary-primary tumor pairs and primary-metastatic tumor pairs (p=0.17, Wilcoxon test). Surprisingly, even mutations in previously reported driver genes, including PIK3CA, KMT2D (MLL2), ATM, and TP53, were not consistently shared between matched prechemotherapy and postchemotherapy tumors. We confirmed these findings with targeted sequencing of 250 common driver genes achieving an average coverage of 400x and an excellent concordance with variant allele frequencies obtained from whole exome sequencing (Pearson correlation coefficient = 0.93, P<10^-171). Phylogenetic analysis revealed that both clonal evolution and metastatic spread were very early events in the natural history of UC. At the copy number level, our analysis revealed limited intrapatient heterogeneity with respect to interpatient heterogeneity, suggesting that each patient’s cancer is relatively stable during evolution at the copy number level. We discovered two distinct copy-number-based clusters. Cluster A was defined by 9p21 (CDKN2A, CDKN2B, and MTAP) deletions in the setting of euploid copy number background. Cluster B was characterized by several enriched amplifications, including 1q21.1 (SETDB1 and MLLT11) amplifications (P=0.0002, Fisher’s exact test) and 6p22.3 (E2F3) amplifications (P=0.001, Fisher’s exact test). This cluster was also enriched with TP53 mutations (P=0.0001, Fisher’s exact test). We validated the presence of these clusters in the TCGA dataset of untreated UC and confirmed that these copy-number alterations persisted as a relatively static feature during the lifetime of UC tumors. On the other hand, the mutational landscape of UC tumors evolved significantly after chemotherapy. We observed a significant increase in the number of clonal mutations in the postchemotherapy samples across the study cohort (P=0.0134, Fisher’s exact test). Chemotherapy-treated UC was enriched with clonal mutations involving L1-cell adhesion molecule (LICAM) and integrin-signaling pathways. To understand the mutational mechanisms driving the evolution of UC, we compared the patterns of single base transitions between chemotherapy-naive and chemotherapy-treated tumor samples. We observed a significant increase in C to A nucleotide substitutions in tumors treated with cisplatin-based chemotherapy consistent with previously described specific mutagenesis signature induced in C. elegans genome after cisplatin treatment. Further analysis of context motifs of various base substitutions showed enrichment of C>T or G changes at the TCW motifs (where W=A or T), which is highly suggestive of APOBEC-induced mutagenesis. APOBEC-induced mutations were clonally enriched in chemotherapy-treated UC and continued to shape UC’s evolution throughout its lifetime.

Conclusions: Our findings have several potential clinical implications: First, genomic divergence between untreated and treated clones suggests that clinically actionable molecular targets in metastatic chemotherapy-treated tumors will be potentially missed when relying only on analyses of untreated primary tumors at the time of diagnosis, and that repeat mutative biopsies during the course of clinical care would be needed to detect the most recent version of the rapidly changing molecular landscape of a given patient’s UC. Second, further study of the functional role of LICAM and integrin signaling in mediating chemotherapy resistance in UC could lead to a potential strategy for reversing or preventing chemotherapy resistance by targeting these pathways. Third, despite its initial effectiveness in eliminating cancer cells, platinum-based chemotherapy is associated with unintended significant mutagenic editing of the genomic landscape of postchemotherapy tumors. Our insight into the nature of these edits is crucial towards a complete understanding of the basis of chemotherapy resistance in advanced UC, which lays the foundation for the development of rational therapeutic strategies for preventing the emergence or reversing the chemotherapy-resistant state of UC. In summary, our results demonstrate that adjuvant chemotherapy-treated UC undergoes extensive and dynamic clonal evolution throughout the lifetime of the tumor with significant genetic editing that continues during and after chemotherapy. Our findings lay the foundation for an evolutionary understanding of advanced chemotherapy-treated UC and present opportunities for advancing cancer precision medicine.

Drug Development Sessions

New Drugs on the Horizon

#DDT01-02 AZD5991: A potent and selective macrocytic inhibitor of Mcl-1 for treatment of hematologic malignancies, Alexander W. Hirud,1 J. Paul Secrist,1 Annmar Adam,1 Matthew E. Belmonte,1 Eric Gang,1 Frank Gibbons,1 David Hargreaves,1 Jeffrey W. Johannes,1 Stephen L. Kazmirk,1 Jason G. Kettle,1 Stephen E. Kurtz,1 Michelle L. Lamb,1 Martin J. Packer,1 Bo Peng,1 Craig R. Stewart,1 Jeffrey W. Tyner,1 Wenzhan Yang,1 Qing Ye,1 XiaoLan Zheng,1 Edwin A. Clark 1, AstraZeneca, Cambridge, United Kingdom; 2AstraZeneca, Macclesfield, United Kingdom.

Mcl-1, a member of the Bcl/Mcl family, is a key protein involved in evasion of apoptosis in a wide variety of tumors. Its amplification and overexpression have also been implicated in innate and acquired resistance to anticancer drugs. Mcl-1 is capable of preventing induction of apoptosis, both by binding and inactivating the pro-apoptotic executioner Bcl-2 protein, Bak, as well as by sequestering other pro-apoptotic Bcl-3-only proteins. Given the role of Mcl-1 in hematologic malignancies, such as multiple myeloma, acute myeloid leukemia, and non-Hodgkin lymphoma, are particularly sensitive to Mcl-1 inhibition with the expression of an Mcl-1 inhibitor. In addition, cell line profiling data revealed an activity profile distinct from a selective Bcl-2 inhibitor, further underscoring the unique therapeutic opportunity for an Mcl-1 inhibitor. Synergistic combinations were identified with therapies selectively targeting Bcl-2, the MAPK pathway, and proteasome inhibitors. In vivo, AZD5991 demonstrated robust inhibition of tumor xenograft growth with a clear PK/PD relationship, exhibiting on-mechanism activity as measured by Bak and caspase activation on a discontinuous dosing schedule of once or twice weekly. An ongoing phase 1 clinical trial is currently evaluating AZD5991 in multiple myeloma.

#DDT01-03 ABBV-621: A best-in-class TRAIL-receptor agonist fusion protein that enhances optimal clustering for the treatment of solid and hematologic tumors, Susan E. Morgan-Lappe. AbbVie, North Chicago, IL.

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a member of the TNF superfamily of proteins that play diverse roles in the activation of several intracellular signaling pathways that control cell proliferation, survival, and apoptosis. Activation of the TRAIL pathway has been viewed as an attractive therapeutic approach for the treatment of cancer because of its distinct role in the induction of tumor cell apoptosis. TRAIL can bind as a trimmer to membrane-bound or soluble receptors, and only the two closely related cell surface death receptors TRAIL-R1 (DR4) and TRAIL-R2 (DR5) can preferentially trigger the extrinsic pathway of tumor cells, leading to apoptotic cell death. The BCL-2 family member Mcl-1 is an essential component of the extrinsic apoptotic pathway since Mcl-1 acts as a gatekeeper to cell death signaling. The BCL-2 family member Mcl-1 is a key player in the extrinsic apoptotic pathway, serving to amplify the extrinsic pathway through further downstream activation of caspases. Given the crossstalk between the extrinsic and intrinsic cell death pathways, several resistance mechanisms have emerged to escape TRAIL-induced apoptosis. These include altered receptor regulation, overexpression of cellular FLICE-like inhibitory protein (cFLIP) that inhibits the proapoptotic activity of the DISC, overexpression of the prosurvival BCL-2 family member proteins, and the inhibitor of apoptosis proteins (IAPs) that directly inhibit caspases. In addition, TRAIL can induce non–cell death signaling pathways (e.g., NF-κB, MAPK, P38) to promote tumor cell growth as a potential resistance mechanism. Results of several clinical trials that targeted DR4 and DR5 with first-generation agonistic anti-TRAIL antibodies or human recombinant TRAIL indicated that these agents failed to demonstrate significant improvement in patient progression-free survival compared to standard-of-care therapy. The major contributing factor in this lack of clinical activity is believed to be the suboptimal TRAIL receptor clustering by these agonists.
ABBV-621 is a novel, second-generation TRAIL receptor agonist comprising a human IgG1Fc linked to native single-chain TRAIL-receptor binding domain (sc-TRAIL-RBD) monomers that are covalently connected by glycosylated linkers, resulting in a plethora of trimeric RBDs. ABBV-621 is designed to maximize receptor clustering but does not require FCyR-mediated crosslinking for optimal in vivo efficacy, which has been deemed an activity-limiting step for the competitor anti-DR4 and anti-DR5 antibodies in the clinic. In-house studies have demonstrated the requirement for enhanced caspase-8 aggregation into the death-inducing signaling complex (DISC) to induce potent tumor cell death, providing mechanistic insights as to why death receptor agonists originally failed and further distinguishing ABBV-621 from the first-generation agents.

ABBV-621 induces dose-dependent apoptotic cell death at sub- to single-digit nanomolar potencies across a large panel (>100) of human hematologic and solid tumor cell lines in vitro. ABBV-621 activity is on-target and mechanism-based as demonstrated by a rapid activation of downstream apoptotic signaling events (cleavage of caspase-3, caspase-8, and PARP). In human tumor xenograft models, ABBV-621 exhibits potent antitumor activity in vivo as a monotherapy and in combination with targeted agents or chemotherapy using xenograft tumors derived from colorectal, lung, leukemia, and lymphoma cell lines. This activity is on-target, mechanism-based, and dose-dependent as indicated by rapid activation of tumor caspase-8 and caspase-3 after a single ascending dose of ABBV-621 in a colorectal carcinoma xenograft model. ABBV-621 exhibits a dose-proportional pharmacokinetic (PK) profile in mice and cynomolgus monkeys, and the projected human half-life is estimated to be ~2 days. PK/pharmacodynamic (PD) assessments estimate that achieving exposure above an efficacious concentration for 4-7 days is sufficient to maintain tumor regressions up to 4-7 weeks.

Screening of 55 patient-derived xenograft (PDx) models comprising 12 different solid tumor types uncovered single-agent activity (regressions) in >50% of all PDx tested. PDx models can be more reflective of human disease by providing estimation of effects of tumor heterogeneity on ABBV-621 activity. Models that were partially sensitive or completely resistant were also identified. These responses are being utilized with associated pre- and post-treatment genomic/proteomic information to further inform potential patient stratification markers. To demonstrate on-target biologic activity in the clinic, proximal and distal target engagement markers will be pursued. In a GLP nonmonkey toxicity study, ABBV-621 was well tolerated with no adverse test item–related findings. The first-in-human (FIH) phase 1/1b study with ABBV-621 will enroll subjects with previously treated advanced solid tumors and hematologic malignancies. The phase 1 objectives will be to establish the safety and tolerability of ABBV-621, as well as to understand the PK properties. Secondary objectives will be to explore target engagement and efficacy biomarkers and to evaluate clinical activity.

**#DIT01-04 Discovery and development of H3B-6545: A novel, oral, selective estrogen receptor covalent antagonist (SERCA) for the treatment of breast cancer.**

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H3B-6545 is being developed for the treatment of breast cancer. In preclinical studies H3B-6545 demonstrated several key properties, including: (a) a high degree of selective activity, with robust antitumor activity in xenograft models of estrogen receptor (ER) positive breast cancer including models carrying ER mutations; (b) high intrinsic cell potency in biochemical and cell-based assays. In vitro H3B-6545 shows potent activity and superior efficacy to fulvestrant in the MCF-7 xenograft model with maximal antitumor activity at doses >10x below the maximum tolerated dose in mice. In addition, H3B-6545 shows superior antitumor activity to tamoxifen and fulvestrant in patient derived xenograft models of estrogen receptor positive breast cancer including models carrying ER mutations. In a clinical safety study in both humans and monkeys, H3B-6545 is well tolerated across a broad dose range and at exposures that significantly exceed those required for efficacy in mouse xenograft models.

In summary, H3B-6545 is a first-in-class, orally available and potent selective estrogen receptor covalent antagonist with a compelling pre-clinical efficacy and safety profile that is being developed for the treatment of breast cancer.
advances, however, and the knowledge gap between cultured cell models and in vitro (2). The relevance of these reprogrammed pathways to bona fide human cancer is an essential component of malignancy (1). In particular, pathologic differences between human and rodent models of cancer—such as the NIH or the Food and Drug Administration. is solely the responsibility of the authors and does not necessarily represent the official views of the NIH or the Food and Drug Administration. Drug Abuse and FDA Center for Tobacco Products (CTP) (U54 DA031659). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH or the Food and Drug Administration.

Cancer Development and Outcomes Related to Tobacco Use

#SY01-03 Reducing harm by targeting the addictiveness of combusted tobacco products through regulated reductions in nicotine content. Eric Donny. University of Pittsburgh, Pittsburgh, PA.

Nicotine is the primary addictive constituent in cigarettes, driving chronic dependence in smokers. New tobacco products are marketed in the US with approximately half the nicotine content of traditional cigarettes. The impact of such reductions on smoking behavior and harm reduction potential is unclear. In this study, we compared smoking behavior and harm reduction potential in human smokers before and after using reduced nicotine content cigarettes compared to standard nicotine content cigarettes. Cigarettes were delivered in a double-blind, randomized design to 30 smokers in a factorial 2 x 2 x 2 design (2 nicotine content x 2 delivery methods x 2 orders of presentation). The results showed that smokers who received standard nicotine content cigarettes smoked significantly more cigarettes per day, which is associated with reduced harm reduction potential. These findings suggest that reduced nicotine content cigarettes may not be an effective harm reduction strategy.

References

Major Symposia

Cancer Metabolism: Moving towards the Clinic

#SY02-02 Exploring the lung cancer metabolism, in vivo and ex vivo, for individualized cancer. Teresa W. Fan, 1 Ramon Sun, 2 Marc Warwomes, 1 Qiushi Sun, 1 Yuan Song, 1 Angela Mahan, 1 Jeremiah Martin, 2 RIchard M. Higashi, 2 Andrew N. Lane, 1

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Lung cancer is a leading cause of cancer death worldwide. This disease is of serious concern in Kentucky, which leads the nation in both lung cancer incidence and mortality. The past decade of cancer research has focused on the downstream value of exploring human metabolism for the discovery of novel therapeutic and diagnostic biomarkers for human cancers and other diseases. To better understand metabolic reprogramming in individual tumors of lung cancer patients, we have developed stable isotope tracers (e.g., [13 C]glucose) coupled with NMR and MS-based stable isotope-resolved metabolomics (SIRM) analysis directly in patients and in patient-derived ex vivo and in vivo models. In the in vivo patient study, we mapped differential metabolic network between paired cancerous (CA) and noncancerous (NC) tissues procured from non–small cell lung cancer (NSCLC) patients infused with [13 C]-glucose and snap-frozen immediately after resection. In particular, we uncovered elevated anaplerotic pyruvate carboxylase (PC) activity in CA versus paired NC tissues. Proliferating cancer cells require active Krebs cycle for generating metabolic fuels, intermediates for anaplerotic intermediates to meet anabolic demands cannot be sustained without anaplerotic. Pyruvate carboxylation represents one of the two major anaplerotic pathways that...
replenish the Krebs cycle intermediates; the other involves glutaminolysis as a feature of more aggressive, less differentiated tumors (5,6).

At the same time, the variability of tumor FDG uptake seen in clinical imaging has driven applications of aberrant glycolysis as a feature of more aggressive, less differentiated tumors (5,6). This has been most widely exploited using the elevated rate of glycolysis observed in most tumors as a target for imaging (2)–in particular, positron emission tomography (PET) imaging of glucose analog \(^{18}F\)-fluorodeoxyglucose (FDG). PET, now combined with anatomic imaging in the form of PET/CT, has become an important tool for cancer detection, staging, and response assessment, widely used in oncologic clinical practices around the world (3,4). Clinical use of FDG PET has increased our appreciation of aberrant glycolysis as a feature of more aggressive, less differentiated tumors (5,6). At the same time, the variability of tumor FDG uptake seen in clinical imaging has heightened opportunities to exploit cancer metabolism as a target for diagnosis. This has been most widely exploited using the elevated rate of glycolysis observed in most tumors as a target for imaging (2)–in particular, positron emission tomography (PET) imaging of glucose analog \(^{18}F\)-fluorodeoxyglucose (FDG). PET, now combined with anatomic imaging in the form of PET/CT, has become an important tool for cancer detection, staging, and response assessment, widely used in oncologic clinical practices around the world (3,4). Clinical use of FDG PET has increased our appreciation of aberrant glycolysis as a feature of more aggressive, less differentiated tumors (5,6). At the same time, the variability of tumor FDG uptake seen in clinical imaging has heightened opportunities to exploit cancer metabolism as a target for diagnosis. This has been most widely exploited using the elevated rate of glycolysis observed in most tumors as a target for imaging (2)–in particular, positron emission tomography (PET) imaging of glucose analog \(^{18}F\)-fluorodeoxyglucose (FDG). PET, now combined with anatomic imaging in the form of PET/CT, has become an important tool for cancer detection, staging, and response assessment, widely used in oncologic clinical practices around the world (3,4). Clinical use of FDG PET has increased our appreciation of aberrant glycolysis as a feature of more aggressive, less differentiated tumors (5,6). At the same time, the variability of tumor FDG uptake seen in clinical imaging has heightened opportunities to exploit cancer metabolism as a target for diagnosis. This has been most widely exploited using the elevated rate of glycolysis observed in most tumors as a target for imaging (2)–in particular, positron emission tomography (PET) imaging of glucose analog \(^{18}F\)-fluorodeoxyglucose (FDG). PET, now combined with anatomic imaging in the form of PET/CT, has become an important tool for cancer detection, staging, and response assessment, widely used in oncologic clinical practices around the world (3,4). Clinical use of FDG PET has increased our appreciation of aberrant glycolysis as a feature of more aggressive, less differentiated tumors (5,6).

Supporting information can be found as supplementary material online.}

**References**


Emerging Targets in Immunotherapy

##SY03-02 Preclinical assessment of JTX-2011, an agonist antibody targeting ICOS, supports evaluation in ICONIC clinical trial. Jennifer S. Michaelson,1 Chiron Harvey,1 Katlu Elpjo,1 Ellen Doong,1 Lindsay Shulberg,1 Matthew Wallace,1 Robert Mabry,1 Jennifer Sho,1 Amit Dey,1 Tom Foss,1 Stephen Safzinsky,1 Joshua Aggar,2 Barbara Mouhou-Zamora,3 Michael Briskin,4 Elizabeth Trehu,1 Jason Reeves,1 Heather Hirsch,2 Siriram Sathyanarayanan,1 Deborah Law1,7. 1Sounce Therapeutics, Cambridge, MA; 2Applied Biome, MA; 3ToxStrategies, CA.

ICOS (the inducible T-cell co-stimulator) is a co-stimulatory molecule expressed on the surface of T cells and a member of the CD28 family, which includes clinically validated targets of cancer immunotherapies, such as PD-1 and CTLA-4. Clinical data identified ICOS as a potentially key molecule in providing optimal antitumor benefit following anti-CTLA-4 therapy. We have developed a species cross-reactive humanized IgG1 agonist antibody, JTX-2011, that binds ICOS and is designed to induce an antitumor immune response. Our preclinical data suggest that JTX-2011 functions through a dual mechanism of action, by stimulating T effector cells (Teff) and by depleting intratumoral T regulatory cells (Tregs). The ICOS antibody is efficacious as a single agent in mouse syngeneic tumor models and demonstrates enhanced activity when administered in combination with anti-PD-1. Single-agent activity in the preclinical models appears to correlate with ICOS expression, with greater efficacy observed in tumor models that exhibit a higher percentage of ICOS-expressing immune cell infiltrate. An integrated expression analysis of human tumors identified non-small cell lung cancer (NSCLC) and head and neck squamous cell carcinoma (HNSCC) as indications with higher percentages of ICOS-expressing cell infiltrate. Preclinical studies performed in rodent and monkeys evaluated safety, pharmacokinetics, and pharmacodynamics of JTX-2011 to inform the first in-human study. The ICONIC phase I/I clinical trial is currently ongoing for evaluation of JTX-2011 alone or in combination with the anti-PD-1 antibody Nivolumab in patients with advanced solid tumors and incorporates a patient enrichment strategy design based on the preclinical and translational findings.
Inherited risk for cancer has been well established from twin studies and family studies. For example, a large, population-based study in Scandinavian countries of more than 200,000 same-sex twin pairs found that heritability was 33% for overall cancer and 57% for prostate cancer (1). To determine, family history is the most commonly used method for evaluating an individual’s inherited risk for cancer. For those individuals with a strong family history, typically defined as multiple affected relatives or relatives who died of cancer at an early age, genetic testing of specific genes may also be recommended. However, recent data suggest that this standard of care is insufficient and inadequate for identifying individuals with higher inherited risk.

Several recent studies suggest that inherited mutations in a number of high-penetration genes (HPGs) are more common in cancer patients than previously estimated. In one study based on The Cancer Genome Atlas (TCGA) data, 4-19% of cancer patients harbored an HPG mutation, with 8% of prostate cancer patients found to be HPG mutation carriers (2). Furthermore, mutation carriers do not have a positive family history. In our recent study of patients with prostate cancer, 60% of pathogenic mutation carriers of BRCA1/2 and ATM had a negative family history of prostate cancer (3). Therefore, it is necessary and relevant to include HPGs in cancer risk assessment among all subjects, regardless of family history.

Another major component of inherited risk is cancer risk-associated SNPs. Many cancer risk-associated SNPs have been identified through genome-wide association studies. The validity of these SNPs has been well established, as they are statistically significant (P<5E-08) and have been replicated among independent study populations. Although each SNP has a small individual effect on cancer risk, they have a strong cumulative effect. The cumulative effect of these SNPs can be measured using several methods, including genetic risk score (GRS) (4). Because GRS is based on individuals’ genotypes and is a continuous variable, it is objective and informative in stratifying inherited risk of developing specific cancers. More importantly, GRS can identify considerably more high-risk subjects in the general population than family history.

For example, in the Prostate Cancer Prevention Trial (PCPT), 24% of men in the study population had a GRS >1.4 and the prostate cancer detection rate during the 7 years of follow-up in these men was 33% (5). In contrast, 17% of men in the cohort had a positive family history and the prostate cancer detection rate in these men was 29%.

Taken together, the latest data suggest that a comprehensive assessment of inherited cancer risk should include family history, HPGs, and GRS. These measures are complementary and, used together, can better identify subjects in the general population at elevated inherited risk for cancer. Using prostate cancer as an example, approximately 7-17% of men in the U.S. population have a positive family history of the disease and are therefore considered to have elevated inherited risk for prostate cancer (6,7). In addition, approximately 2% of men in the general population have a considerably higher inherited risk for prostate cancer due to harboring at least one pathogenic mutation in an HPG for prostate cancer (8). Many of these men do not have a positive family history. Finally, approximately 24% of men in the general population have higher GRSs (>1.4) and have an estimated prostate cancer risk equivalent to or even higher than a positive family history (5). Considering all three risk factors, approximately 56% of men in the general population are estimated to be at higher inherited risk for prostate cancer using this comprehensive risk assessment strategy, more than twice that of the current standard of care risk assessment.

The more comprehensive assessment of inherited cancer risk has important clinical implications, both for asymptomatic subjects in the general population as well as for cancer patients. The most notable clinical utility is for developing individualized cancer screening strategies. A personalized cancer screening approach can maximize benefits and minimize harms associated with cancer screening. For example, offering PSA screening among men with higher inherited risk based on family history, HPGs, and GRS is a rational approach to address the current debate on prostate cancer screening. It is important to note that individualizing cancer screening is the most impactful component of precision medicine, as it can effectively reduce cancer-related mortality and improve quality of life.

Myelomonocytic Cells and Stroma as Therapeutic Targets

The tumor microenvironment (TME) is a complex network, which includes soluble factors and components of the extracellular matrix as well as stromal, endothelial, and immune cells. Immune cells and, among them, myeloid cells play important roles in cancer development and can promote or inhibit cancer initiation and progression (4).

Among tumor-infiltrating immune cells, macrophages are well-known determinants of cancer-related inflammation and are typically characterized by their remarkable plasticity. This consists of the ability to acquire a wide spectrum of activation states in response to various signals derived from the microenvironment. Classical M1 and alternative M2 macrophages represent the paradigm of this property. Tumor-associated macrophages (TAMs) usually display a so-called “M2-like” phenotype that can foster tumor progression in different ways, namely by promoting genetic instability, angiogenesis, and metastasis and by restraining antitumor adaptive immunity. Notably, TAMs can also play a dual role in the response to conventional antitumor therapies: they can enhance the antineoplastic effect or, in contrast, they can sustain a tumor-promoting response and so foil the antitumor power of these drugs (5).

Given their roles in tumor development and the number of macrophage-targeted anticancer approaches are currently being evaluated. They include inhibition of macrophage recruitment and/or survival at tumor sites, functional reprogramming of TAMs to the antitumor M1-like phenotype, and enhancement of killing and/or phagocytosis of cancer cells. Moreover, TAMs express checkpoint proteins that modulate T-cell activation in such a way that they can be targeted by checkpoint blockade immuno-therapies. Also, neutrophils can affect the TME. However, unlike macrophages, they do so by releasing stored and newly synthesized inflammatory mediators. In the “classic,” albeit now obsolete, point of view, neutrophils were viewed as terminally differentiated effector cells that play major roles in the acute inflammatory response and antimicrobial defense. This limited standpoint was challenged by the finding that neutrophils can infiltrate tumors, interact with a number of microvascular sprouts, and produce a wide array of cytokines and effector molecules that exert a plethora of effects on tumor behavior (6,7). Therefore, macrophages and neutrophils are both involved in the regulation of the innate and adaptive immune responses in various inflammatory situations, including cancer.

Besides immune cells, innate immunity comprises a humoral arm that includes a variety of molecules, namely complement components, collectins, ficolins, and pentraxins. The role of the humoral arm of the innate immune system in cancer-related inflammation is still being evaluated. PTX3 deficiency was recently found to increase susceptibility to mesenchymal and epithelial carcinogenesis in mice (8). In detail, tumor-infiltrating leukocytes and endothelial cells were found to be a major source of PTX3 and to contribute to PTX3-mediated protection against carcinogenesis. PTX3 deficiency was associated with enhanced macrophage recruitment, profibrogenic cytokine production, angiogenesis, complement C3 deposition, and C5a levels, which suggests exacerbated cancer-related inflammation. Moreover, genetic inactivation of...
C reverts the increased susceptibility to 3-MCA-induced carcinogenesis and macrophage recruitment. PTX3 regulated C3 deposition on sarcoma cells by recruiting the negative complement regulator factor H. In addition, CCL2 inhibition was sufficient to revert this increased susceptibility to PTX3 deficiency in mice to the M2-like phenotype of TAMs. Thus, in 3-MCA sarcomas, unleashed complement activation and increased C5a production associated with PTX3 deficiency is likely to increase the production of CCL2, which in turn recruits tumor-promoting macrophages and favors M2-like polarization. PTX3 deficiency was also associated with increased DNA damage, which is in accordance with the theory that cancer-related inflammation contributes to the genetic instability of tumors. Moreover, the PTX3 gene was found to be highly methylated in some human mesenchymal and epithelial tumors, and its demethylation might sensitize TAMs to therapeutic intervention. Thus, an essential component of the humoral arm of innate immunity and regulator of complement activation works as an extrinsic oncosuppressor gene in mice and humans by modulating complement-mediated, macrophage-enhanced, tumor-promoting inflammation (8).

In an increasingly more personalized treatment approach, the more accurate understanding of cancer-related inflammation has led to new therapeutic options that target the TME. These strategies include inhibition of inflammatory mediators or of their downstream signaling molecules, blockage of recruitment/activation of myeloid cells, modulation of their immunosuppressive properties, and re-education of TME. These novel therapeutic strategies could synergize with conventional anticancer treatment and so significantly improve the patient’s clinical outcome and follow-up.

Mechanisms Regulating Immune Checkpoint Therapies

**#SY09-01 Next-generation anti-CTLA-4 antibodies.** Alan J. Korman,1 John Engelhardt,1 John Loffredo,2 Jose Valle,1 Rahima Akter,1 Raja Vayyuru,2 Natalie Bezman,1 Paula So,1 Robert Graziano,1 Kimberly Tipton,1 James West,1 Bryan Irving,1 Mark Schulz,2 Bristol-Myers Squibb Co, Redwood City, CA; Bristol-Myers Squibb Co, Lawrenceville, NJ; CytomX Therapeutics, South San Francisco, CA.

The activity of ipilimumab as a single agent and in combination with nivolumab (anti-PD-1) in melanoma, as well as the use of ipilimumab/nivolumab combinations in other malignancies, has confirmed the importance of CT-L cell blockade in immunotherapy. The antitumor effect of this treatment also results in significant immune-related adverse events that limit dosing and result in patient discontinuation. We have taken two approaches to alter the activity of ipilimumab so as to improve its potency and its safety profile. One approach is to enhance the antibody-dependent cellular cytotoxicity (ADCC) activity of ipilimumab in order to increase the potential for Treg depletion at the tumor site; this would be expected to increase the activity of the antibody. The second approach is to produce a produg form of ipilimumab (an anti-CTLA-4 Probody therapeutic) that will have reduced activity systemically, but will become proteolytically cleaved at the tumor site to produce the fully functional antibody; the goal of this approach is to reduce the adverse event profile while retaining the antitumor activity of ipilimumab. It has previously been shown that antitumor activity of anti-CTLA-4 antibodies in mouse models of cancer is dependent on the ability of the antibody to bind activating FcγRs and mediate ADCC against Tregs at the tumor site (1, 2). Although human IgG1 Abs have been shown to be effective mediators of ADCC in patients with hematologic malignancies, it is still unclear whether ipilimumab mediates Treg depletion in solid tumors. Using in vitro ADCC assays, we have found that a nonfucosylated (NF) version of ipilimumab (ipilimumab-NF) has increased activity compared to ipilimumab. Ipilimumab-NF also demonstrates fucosylated IL-2- and IL-15-independent ADCC activity.

In addition, ipilimumab-NF was tested for its ability to enhance a vaccine response in 3-MCA sarcoma-bearing mice. As previously demonstrated, the vaccination of mice with SIV antigens has been shown to induce a CD4+ T-cell response. In colorectal cancer patients coexpression of human CSF1R and CD40 on TAMs was detected, providing the basis to explore this combination in the clinic.

**#SY06-03 Reprogramming the tumor microenvironment to improve responses to therapy.** David G. Denardo. Washington Univ. School of Medicine, Saint Louis, MO.

Immunotherapeutics represent highly promising agents with the potential to improve patient outcomes in a variety of cancer types. Unfortunately, single-agent immunotherapy has achieved limited clinical benefit to date in patients suffering from pancreatic ductal adenocarcinoma (PDAC). This may be due to the presence of a uniquely immunosuppressive tumor microenvironment (TME) present in PDACs, which creates a barrier to effective immune surveillance. Critical obstacles to immuno-therapy in PDAC tumors include the dense desmoplastic stroma that acts as a barrier to T-cell infiltration and the high numbers of tumor-associated immunosuppressive cells. We have identified hyperactivated focal adhesion kinase (FAK) activity in neoplastic PDAC cells as a significant regulator of the fibrotic and immunosuppressive TME. We found that FAK activity was elevated in human PDAC tissues and correlates with high levels of fibrosis and poor CD8+ cytotoxic T-cell infiltration. Single-agent FAK inhibition (VS-4718) significantly limited tumor progression, resulting in a doubling of survival in the p53-Cre/LSL-KrasG12D/;P53Flox;LoxPTX mouse model of human PDAC. This alteration in tumor progression was associated with dramatically reduced tumor fibrosis, decreased numbers of tumor-infiltrating immature myeloid cells, and immunosuppressive macrophages. We postulated that these beneficial effects of FAK inhibition in the TME might render PDAC tumors more sensitive to immunotherapy. Accordingly, we found that FAK inhibition rendered the previously unresponsive KPC mouse model responsive to anti-PD1 and anti-CTLA4 antagonists, leading to a near tripling of survival times. These data suggest that FAK inhibition increases immune surveillance by overcoming the fibrotic and immunosuppressive PDAC TME and thus renders tumors responsive to immu

# Obesity, Inflammation, and the Adipose Microenvironment in Cancer

Prostate cancer (PCa) is promoted by white adipose tissue (WAT) overgrowth in obesity, and underlying mechanisms for this enhancement have been discovered in the recruitment of adipose stromal cells (ASC) by tumors. We reported that CXC1L1 expressed by cancer cells chemotacticlly activates CXC1R1, a candidate upstream mechanism in which we identified interleukin 22 secreted by WAT-infiltrating leukocytes and signaling through IL-22R in cancer cells. Our findings in Hi-Myc mice indicate that obesity promotes epithelial-mesenchymal transition (EMT). We show that ASC directly induce EMT in adenocarcinoma cells. To test whether cancer progression can be blocked by ASC targeting, we have used a human-killed peptide D-WAT composed of an ASC-homing and a pro-apoptotic domain. D-WAT administration depleted prostate stromal cells expressing a chemokine SDF-1α and suppressed the EMT. These results indicate that ASC promote the EMT, at least in part, via SDF-1α paracrine signaling and validate ASC as a therapeutic target.

**#SY07-02 Adipose stromal cells: A player in cancer progression and a therapeutic target.** Mikhail Kolonin. The University of Texas Health Science Center at Houston, TX.

Prostate cancer (PCa) is promoted by white adipose tissue (WAT) overgrowth in obesity, and underlying mechanisms for this enhancement have been discovered in the recruitment of adipose stromal cells (ASC) by tumors. We reported that CXC1L1 expressed by cancer cells chemotactically activates CXC1R1, a candidate upstream mechanism in which we identified interleukin 22 secreted by WAT-infiltrating leukocytes and signaling through IL-22R in cancer cells. Our findings in Hi-Myc mice indicate that obesity promotes epithelial-mesenchymal transition (EMT). We show that ASC directly induce EMT in adenocarcinoma cells. To test whether cancer progression can be blocked by ASC targeting, we have used a human-killed peptide D-WAT composed of an ASC-homing and a pro-apoptotic domain. D-WAT administration depleted prostate stromal cells expressing a chemokine SDF-1α and suppressed the EMT. These results indicate that ASC promote the EMT, at least in part, via SDF-1α paracrine signaling and validate ASC as a therapeutic target.
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reduced activity of the Probody Tx outside the tumor microenvironment. The development of next-generation anti-PD-L1 antibodies holds promise for improving the utility of ipilimumab for single-agent or combination therapy. The two improvements to ipilimumab outlined above could each lead to a superior therapeutic outcome and merit further investigation.

References


#SV09-02 A novel genetic mechanism of evading antitumor immunity in multiple human cancers. Seishi Ogawa. Kyoto Univ. Graduate School of Medicine, Kyoto-shi Sakyoku-Ka, Japan.

Immune checkpoint blockade therapy using antibodies against programmed cell death 1 (PD-1) and PD-L1 ligands (PD-L1) is revolutionizing cancer treatment. Surprising therapeutic responses to these agents are obtained in many advanced cancer patients with different tumor types, such as melanoma, non-small-cell lung cancer, kidney cancer, and Hodgkin lymphoma, suggesting that cancer cells depend critically on evading immune surveillance for their malignant growth. Accumulating evidence suggests that various modulatory factors and molecular pathways contribute to modulating the PD-1/PD-L1 immune checkpoint to unleash antitumor immune responses. However, the genetic basis for the PD-1/PD-L1-mediated immune escape has not been fully elucidated, with the exception of enhanced PD-L1 expression by gene amplification and utilization of an ectopic promoter by translocation, which were reported in Hodgkin and other B-cell lymphomas as well as stomach adenocarcinoma. Only a few genetic markers are currently available to reliably predict response to anti-PD-1/ PD-L1 therapy as well. I will show a unique genetic mechanism of immune escape caused by terminal variations (SVs) commonly disrupting the 3′-UTR region of the PD-L1 gene. Widely affecting multiple common human cancer types, including adult T-cell leukemia/lymphoma (27%), diffuse large B-cell lymphoma (8%), and stomach adenocarcinoma (2%), these SVs invariably lead to a marked elevation of aberrant PD-L1 transcripts that are stabilized by truncation of the 3′-untranslated region (UTR). Disruption of PD-L1 3′-UTR in mice enables immune evasion of EG7-OVA tumor cells with elevated PD-L1 expression in vivo, which is effectively inhibited by PD-1/PD-L1 blockade, supporting the role of relevant SVs in clonal selection through immune evasion. Our findings not only unmask a novel regulatory mechanism of PD-L1 expression, but also suggest that PD-L1 3′-UTR disruption could serve as a genetic marker to identify cancers that actively evade antitumor immunity through PD-L1 overexpression.

Pan-cancer Genomic Analysis

#SY10-02 Pan-cancer study of recurrent and heterogeneous RNA aberrations and association with whole-genome variants. Samikumar Amin, Philip Awadalla, Andrew Biemond, Paul Boutros, Alviz Brazma, Angela Norie Brooks, Claudia Calabrese, David Chang, Aurélien Chateligne, Ken Chen, Zichen Chong, Brian Craft, Chad Creighton, Deniz Demircioğlu, Nuno Fonseca, Mihalya Frenkel-Morgenstern, Gad Geit, Jonathan Goe, Mary Goldman, Liliana Gregor, Syed Haider, Yao He, Katherine Hoadley, Yuan Ji, Andre Kahles, Jan Korbel, Katherine Hoadley, Yau He, Andre Kahles, Jan Korbel, Kjong Lehmann, Han Liang, Fenglin Liu, Maximillian Marin, Matthew Meyerson, Akinyemi Ojesina, Francis Ouellette, Chandra Pedamallu, Marc Perry, Gunnar Rathsch, Roland Schwarz, Yuichi Shiraishi, Cameron Soulouli, Olivier Stegle, Patrick Tan, Alfonso Valencica, Linda Xiang, Christina Yung, Junjun Zhang, Fan Zhang, Zemin Zhang, Jinchun Zhu. The UT MD Anderson Cancer Center, TX; University of Toronto, Ontario, Canada; Glasgow Royal Infirmary; University of Toronto; European Molecular Biology Laboratory; UC Santa Cruz; Santa Cruz; CA; OICR; MD Anderson; Baylor College of Medicine; National University of Singapore; Bar-Ilan University; Massachusetts General Hospital; Peking University; UNC; The University of Chicago; Eidgenössische Technische Hochschule Zürich; Weill Cornell Medical College; European Molecular Biology Laboratory (EMBL); ETHZ; Broad Institute; HudsonAlpha Institute for Biotechnology; Swiss Federal Institute of Technology; Max Delbrück Center for Molecular Medicine; University of Tokyo; European Bioinformatics Institute; Spanish National Bioinformatics Institute.

To more comprehensively catalogue cancer-associated gene alterations, we have extended our catalog of recurrent somatic mutations that contribute to cancer pathogenesis; however, these studies limit our ability to identify cancer-associated mutations to those that cause protein-coding changes. To more comprehensively catalogue cancer-associated gene alterations, we have extensively characterized tumor transcriptions from 1,200 donors with matched whole-genome sequence data to identify recurrent RNA-level aberrations. Specifically, we created a unified RNA-Seq analysis pipeline including sequence alignment and quality control and subsequently identified gene alterations through outlier detection from estimated gene expression levels, alternative splicing, alternative transcription start, and allele-specific expression and through identified RNA-edited sites and gene fusions. Our data represent an extensive catalog of RNA aberrations for each gene across 27 cancer types. We have also provided genetic associations with these RNA phenotypes. Using an integrative analysis approach, we have mapped genome-wide cis and trans effects on individual RNA phenotypes, considering both common germline variants as well as somatic SVs in gene promoters, enhancers, and intronic and intergenic regions. Many of the regulatory associations we identify are not accessible by exome sequencing, underlining the importance of whole-genome sequence data. Utilizing this RNA-centric view, we have identified genes that are recurrently altered, yet have not been previously characterized as cancer genes or identified through DNA-level driver gene analysis. To identify further supporting evidence that these recurrent alterations are potential drivers, we identified genes with mutually exclusive RNA-level alterations. Our findings reveal new insights into selective advantages of somatic changes and molecular mechanisms of cancer. This work is by the Transcriptome Working Group of the Pan-Cancer Analysis of Whole Genomes (PCAWG) consortium and authors are listed in alphabetical order.

The Unfolded Protein Response in Tumor Biology

#SY13-03 GRP78/BiP: Cancer’s aide in crime. Amy S. Lee, Jeli Shen, Daisy Rangel, Chun-Chih Tseng, Yuan Li Tsai, Dat P. Hu, He Zhao, Louis Dubeau. USC Norris Comprehensive Cancer Center, Los Angeles, CA.

The 78 kDa glucose-regulated protein (GRP78), also referred to as BiP/HSIP5, is a major endoplasmic reticulum (ER) chaperone with antiapoptotic properties and also serves as a regulator of ER stress signaling (1). Tumor cells are subjected to ER stress due to intrinsic factors such as genetic mutations, altered metabolism and hyperproliferation, as well as extrinsic factors in the tumor microenvironment including oxygen and nutrient deprivation (2). As an adaptive measure, cancer cells turn on the unfolded protein response (UPR), and ER stress induction of GRP78 in cancer cells represents a major prosurvival response, suppressing apoptosis while promoting proliferation and invasive-ness. Thus, GRP78 is an emerging target for therapy to blunt cancer development, progression, and drug resistance (3). GRP78 conditional knockout mouse models further established the requirement of GRP78 in solid and blood tumorigenesis driven by mutation of the tumor suppressor gene PTEN and that GRP78 is an upstream regulator of the P38/ERK/S6 pathway (3,4). KRAS is commonly mutated in various cancers, including pancreatic adenocarcinoma (PDAC) and lung cancer, and thus far is deemed “undruggable”. We discovered that haploinsufficiency of a single moiety, GRP78, while having no effect on the normal pancreas, is sufficient to impede acinar-to-ductal metaplasia, block oncogenic signaling, curb PDAC progression, and prolong survival. Similarly, GRP78 haploinsufficiency is able to suppress mutant KRAS-driven lung cancer development. Further, we recently established that ER stress can actively promote cell surface localization of GRP78, where it assumes novel co-receptor functions with cell surface protein partners in regulating signal transduction pathways (5). Our studies uncover that cGRP78 interacts with specific cell surface signaling partner proteins and through those interactions impacts cancer cell adherence, polarity, migration, and survival.

References


Presidential Select Symposium: Cancer Health Disparities

#SY14-01 Colon cancer screening and genomics. John M. Carethers. Univ. of Michigan, Ann Arbor, MI.

Colon cancer (CRC) is the third most prevalent and second deadliest cancer in the U.S. in 134,490 cases and 49,190 deaths in 2016. Its pathogenesis stems from genetic...
susceptibility coupled with environmental interactions in the colon that synergize ideal conditions for neoplastic growth, initially from benign adenomatous polyps that might progress to carcinoma over several years. Adenoma initiation and progression to carci-
mona may take one of a few identified genetic pathways that determine the tempo of progression as well as morbidity and mortality of the patient. Each patient’s CRC is genetically unique with variable numbers of passenger mutations and propelled by 2-8 driver mutations. CRC’s can be classified as hypermutated, possessing hundreds to thousands of somatic mutations and including CRCs driven by defects in DNA mismatch repair and POLE mutations; and non-hypermutated, with tens of somatic mutations containing multiple copy number alterations and aneuploidy with oncogenic activation of KRAS and PIK3CA coupled with mutations and loss of heterozygosity of tumor suppressors APC and TP53. Epigenetic events as well as inflammatory cellular alterations in DNA mismatch repair further contribute to CRC pathogenesis and metastasis. Because CRC is deadly, prevention through screening (and/or surveillance in high-risk patients) is an ideal and effective approach. Screening provides the opportunity to identify and remove the adenoma precursor before it becomes CRC, and can also utilize the somatic genetic knowledge to effectively screen patients noninvasively. There are seven CRC screening tests suggested by the USPSTF and a Multi-society Task Force for effective screening, with varying abilities to detect significant colonic neoplasia and various specificities; even the most noninvasive and least sensitive test has shown a durable reduction in CRC mortality and is cost effective. Thus, any of the seven CRC screening tests is better than no test. Noninvasive strategies include guaiac-based fecal occult blood testing, fecal immunochemical testing, fecal DNA testing, and CT colonog-
raphy. Luminol-immunoassay tests include flexible sigmoidoscopy and colonoscopy and their combinations with a noninvasive test. If any of the noninvasive tests shows a positive result, a colonoscopy should be performed to identify the cause of the positive test. For high-risk populations, or if there is a chance of patients who have a poor prognosis, it may be used. Knowledge of the patient’s age and family history and personal adenoma or CRC history can influence when to commence screening; additionally, multiple lines of evidence suggest that race should be included in the algorithm influencing the timing and tempo of screening and/or surveillance.

#SY14-04 Cancer health disparities in American Indian and Alaskan Native pop-
ulations, Cheryl L. Willman. University of New Mexico School of Medicine and University of New Mexico NCI Comprehensive Cancer Center, Albuquerque, NM.

American Indian and Alaskan Native communities constitute 562 federally recognized independent sovereign nations within the United States, including 229 located in Alaska and the remainder in 33 other states. These tribal nations and communities are highly diverse in ethnicity, language, customs, modes of tribal governance, and cultural perspec-
tives regarding cancer and health care. American Indian and Alaskan Natives are a diverse group with varying experiences of colonization, contact, and the conduct of health care or community interventions. These nations have very significant cancer health disparities, disproportionately low rates of cancer screening, and more limited access to state of the art cancer diagnosis and treatment and cancer clinical trials when compared to other Americans. Cancer incidence and mortality rates vary, often dramatically, among different tribal nations in different geographic regions of the U.S. (see American Indian and Alaskan Native Cancer Profiles at surveillance.cancer.gov and http://statecancerpro-
files.cancer.gov). These variations may result from differing cancer etiologies, environ-
mental exposures, social behaviors (including diet and tobacco use), spectra of cancers associated with somatic mutations and genetic susceptibilities to cancer and pre-cancerous conditions (such as diabetes), limited rates of community-based cancer screening, and limited access to health care. American Indian and Alaska Natives are often diagnosed at later stages of disease. Disparities may have a poorer prognosis as compared to other racial/ethnic groups. Regional variations in cancer staging and survival within specific cancers are also observed. Highlighting specific examples of cancer health disparities, lung cancer incidence rates for American Indians and Alaska Natives in the Northern Plains and Alaska are currently among the highest in the world. In contrast, lung cancer rates in American Indians in the Western United States are among the lowest in the country. Kidney and hepatobiliary cancers are increasing in incidence in many tribal communities and occur at a significantly higher frequency than observed in other racial and ethnic groups. American Indians and Hispanics, particularly in the Southwest, have the nation’s highest rates of hepatocellular carcinoma, gall bladder cancer, and cholan-
giocarcinoma. Colorectal cancers are also seen at higher rates in American Indian and Alaska Native communities, which may be a result of exceedingly low rates of colorectal cancer screening. Tribal women and girls are also disproportionately impacted by higher rates of cervical cancer, despite implementation of more robust cervical cancer screening programs. Hispanic and American Indian children and adolescents affected by acute lymphoblastic leukemia (ALL) also have significantly poorer survival compared to non-Hispanic and ethnic groups in the U.S. Strikingly, Hispanic children now have the highest ALL incidence in the United States, increasing from 8% of all pediatric ALL cases reported in 1990 to nearly 25% in 2005. Our genomic studies, supported by the NCI TARGET / TCGA (cancer.gov) programs, discovered that American Indian children affected by ALL, and Hispanics with a significant degree of American Indian genetic ancestry, have a high frequency of unique leukemia-causing mutations compared to other racial/ethnic groups (such as those in CRLF2/JAK signaling pathways or novel fusions involving genes encoding epigenetic modifiers and tyrosine kinase signaling pathways (2)). These mutations can be targeted by tyrosine kinase inhibitors (TKIs) or novel therapeutic strategies that are now being tested in national clinical trials, focused on improving overall outcomes in these diseases.

In recognition and respect of the sovereign nation status of American Indian and Alaska Native communities, of tribal laws, of requirements for tribal membership, and of unique cultural perspectives and beliefs, meeting and overcoming cancer care delivery challenges and significant cancer health disparities through research and engagement requires unique, sustained, collaborative, community-engaging, partnership models. Such partnerships must address the concerns of American Indian and Alaska native communities regarding cancer health research and care intervention, including joint oversight of collaborative projects; regular communication of data and results; genetic privacy; appropriate and potentially more limited informed consent for specific studies; appropriate oversight of biospecimen acquisition and utilization of samples collected and stored for specific research projects; intellectual property; and access to data and modes of data sharing and utilization. The National Cancer Institute, The Centers for Disease Control, and the Indian Health Service have supported several projects within tribal communities, largely focused on cancer control. Such activities include the provision of educational materials (such as Native Circle) to communities, the development of high quality cancer registries for American Indians and Alaska Natives including 12 Tribal Epidemiology Centers (tribealpecenter-
s.org), and training and education programs targeted to American Indian and Alaska Native students. The NCI SEER/New Mexico Tumor Registry at the University of New Mexico Comprehensive Cancer Center, which holds cancer registry data from American Indian communities in New Mexico and Arizona and supports the Cherokee Native Cancer Registry and the Alaska Native Tumor Registry, is working with the NCI to leverage the American Indian Cancer Registry and facilitate the registries to collection to allow American Indian and Alaska Native communities to track their cancer data and monitor the incidence and outcome of cancers in their communities. New collaborative partnership models (such as The Southcentral Foundation of Alaska, the TGEN/Pima Indian Nation Partnership in Arizona, and evolving partnerships in New Mexico, Utah, and other regions of the U.S.) with full engagement of tribal nations and collaborating universities, research entities, and health systems are providing new opportu-
nities for American Indians, can and Alaska Native communities to engage in and benefit from cancer research focused on gene-environment interactions and cancer causation, cancer precision medicine and discovery of the spectrum of cancer-associated mutations in cancers disproportionately affecting these communities, cancer education and screening interventions, and cancer clinical trials.

#SY17-03 Can a change in diet change your cancer risk? Stephen J. O’Keefe. University of Pittsburgh, Pittsburgh, PA.

There is a remarkable variation in gastrointestinal cancer risks around the world, best illustrated by colon cancer, where the incidence varies 20-fold between Westernized and less-developed countries. Overwhelming experimental and human study evidence shows that the variation can be accounted for by environmental factors rather than genetic constitution. For example, studies have shown that migration from a low-incidence country to a high-incidence country results in a change in risk to that of the host country population. This is in contrast to the genetic mutation theory for the etiology of colon cancer. Less than 10% of cancers are due to inherited genetic aberrations, and GWAS studies have struggled to identify mutations that strongly influence risk for developing sporadic colon cancer. On the other hand, there is convincing evidence, based on observation and interventions, that cancer risk is increased by consuming加工和/or processing meat and suppressed by the consumption of fiber-rich foods, suggesting that the primary environmental factor that drives risk is what we eat. Recent studies of ours in African Americans, who suffer the highest rates of colon cancer incidence and death in the U.S., showed that a change to a traditional ‘African’ high-fiber (55 gm/day), low-meat diet suppressed mucosal biomarkers of colon cancer risk in their colons within 2 weeks (1). Furthermore, these biomarkers were increased when rural Africans were given a ‘Western’ diet for only 2 weeks. We obtained evidence that these diet effects were mediated by the American microbiota, which break down undiseged food into metabolites that either support mucosal health and are anti-neoplastic, for example short-chain fatty acids such as butyrate, or are inflammatory and carcinogenic, such as conjugated bile acids (which increase in a high-fat diet) and nitroso-compounds (which increase with a high-meat diet). Butyrate has a remarkable array of colonic health-

Diet, Microbiome, and Cancer

References
Large-scale genomic and transcriptomic interrogation of cancer has underscored the complexity of its genetic landscape, revealing a massive number of genetic alterations that results in the impossibility of distinguishing “driver” from “passenger” mutations. This understanding has sparked unprecedented interest in pursuing strategies to achieve the preferential targeting of cancer cells. This talk will focus on recent advances in the field of metabolic reprogramming of cancer metabolism.

Invited Abstracts

Inflammation and Cancer

Michael Karin, UCSD, La Jolla, CA

The majority of sporadic human colorectal cancer is initiated by genetic loss or inactivation of tumor suppressor genes like APC (Aberrant in Polyposis Colon). In addition to activation of beta-catenin signaling, we found that loss of APC results in upregulation of IL-6 signal transducer (IL6/gp130), thereby leading to activation of STAT3 and YAP, two transcription factors that make important contributions to colorectal tumorigenesis. In addition, APC loss results in deterioration of the intestinal epithelial barrier and immune homeostasis, barrier loss due to downregulation of genes encoding tight junction proteins, and suppression of mucin production. Together, such changes promote the invasion of inflammation-inducing colitogenic bacteria and their products. Loss of immune homeostasis is due to defective luminal transport of IgA dimers caused by loss of polymunoglobin receptor (PlgR) and CCL28, a chemokine responsible for recruitment of IgA-producing plasma cells. Defective luminal transport of IgA results in dysbiosis and over-representation of inflammation-causing bacteria that can accelerate colorectal tumor genesis through upregulation of IL-17 production.

Kingdom et al. (2011) observed that germ-free mice treated with intraperitoneal Lactobacillus reuteri elicited lower levels of colonic IL-17 production than conventional mice. Inflammation-driven cancer: Host and microbial pathways. Fiona Powrie, Sarah McCuaig, Nathan West, Grigory Ryzhakov, University of Oxford, Oxford, United Kingdom

The gastrointestinal (GI) tract is home to a large number and vast array of bacteria that play an important role in nutrition, immune system development, and host defense. In inflammatory bowel disease (IBD) there is a breakdown in this mutualistic relationship, resulting in aberrant inflammatory responses that can progress to colon cancer. Our studies in model systems have implicated innate lymphoid cells and production of IL-22 as key drivers of neoplasia and cancer in the intestine. In this presentation I will discuss new checkpoints that control bacteria-driven innate inflammation in the intestine as well as the functional effects of IL-22 on intestinal epithelial cells and interactions between host genetics, gut microbiota, and deranged inflammatory pathways will yield new approaches to patient stratification and personalized therapy.

Targeting Residual Disease

Alberto Bardelli, Univ. of Turin School of Medicine, Candiolo, Italy

When metastatic cancers are challenged with targeted agents, almost invariably a subset of cells insensitive to the drug emerges. As a result, in most instances targeted therapies are only transiently effective in patients. Strategies to prevent or overcome resistance are therefore needed to delay the next generation of resistance. How can we overcome the near-certainty of disease recurrence following treatment with targeted agents? Addressing this question means considering as a target not “only” individual oncogenes but also the evolving nature of human tumors. We used colorectal cancer (CRC) as a model system to test the hypothesis that by understanding tumor evolution, the emergence of drug resistance can be controlled. We find that clonal dynamics can be monitored in real time in the blood of patients, and liquid biopsies can be used to intercept the emergence of resistant clones before relapses are clinically manifest. We discovered that a multiphase clonal evolution process driven by progressive increases in drug fitness underlies the development of resistance in cells and patient avatars. To have long-term efficacy, the use of targeted therapies must take into account the continuous evolution of cancer cells; that is to say, therapies must adapt to tumor evolution. One possibility is to anticipate the changes the tumors will make. For example, by knowing in advance how CRC cells overcome resistance to EGFR blockade, we devised further rounds of therapy. Another approach is to unleash the ability of the immune system to recognize drug-resistant cells. We tested this possibility in syngeneic mouse models of CRC sensitive to targeted therapies. Our findings indicate that manipulation of the mutational loads can trigger prolonged therapeutic responses that are not observed when cancer cells are challenged with targeted drugs alone. We postulate that rationally combined targeted therapies and immunotherapies can restrain tumor evolution and can limit the emergence of drug resistance, thus leading to long-term responses.

AACC-JCA Joint Symposium: Cancer Metabolomics

Sofia M. Settimi, University of Turin, Turin, Italy

AIB1 is required for the viability of tumor-suppressor hepatocellular carcinoma (HCC) cells in culture. AIB1 knockdown reduces the viability of HCC cells, not only by apoptosis but also by autophagy. AIB1 is a pleiotropic regulator of metabolism; however, its role in the metabolic regulation of HCC cells remains to be fully elucidated. In this study, we aimed to investigate the role of AIB1 in the metabolic regulation of HCC cells. We found that AIB1 knockdown decreased the viability of HCC cells by inducing autophagy and apoptosis. AIB1 knockdown also decreased the expression of ATF4, a key transcription factor in the regulation of autophagy, indicating that AIB1 knockdown induced autophagy by inhibiting the expression of ATF4. These findings suggest that AIB1 knockdown may be a novel strategy for the treatment of HCC by inducing autophagy and apoptosis.
levels of most metabolites were unaltered in tumor tissues. This raises the question of which molecules contribute to the regulation of metabolic reprogramming of colorectal cancer metabolism.

Host Immunity to Oncogenic Viruses

#SY23-01 KSHV: Immune evasion and oncogenesis. Blossom A. Damania. Univ. of North Carolina at Chapel Hill, Chapel Hill, NC.

Infection of cells with oncogenic DNA viruses, including herpesviruses, triggers innate immune responses mediated by DNA sensors. Cyclic GMP-AMP synthase (cGAS) is a key DNA sensor that upon activation produces the cyclic dinucleotide cGAMP, which binds and activates Stimulator of Interferon Genes (STING), leading to interferon production and an antiviral response. Kaposi’s sarcoma-associated herpesvirus (KSHV) is an oncogenic herpesvirus that is linked to several human malignancies including Kaposi’s sarcoma (KS), primary effusion lymphoma (PEL), and multicentric Castleman’s disease (MCD). We have found that KSHV infection activates the cGAS-STING pathway and that cGAS and STING also play an important role in regulating KSHV reactivation from latency. Furthermore, we have identified several KSHV viral proteins that inhibit the cGAS-STING pathway. It appears that modulation of this pathway is important for viral transmission and the lifelong persistence of oncogenic herpesviruses in the human population.

#SY23-02 Host immune responses to HPV and HPV vaccines. Lrigia Pinto. Leidos Biomedical Research, Inc., Frederick National Laboratory, Frederick, MD.

Human papillomavirus (HPV) infection is the cause of multiple cancers in both women and men. There are three different licensed HPV prophylactic vaccines. Currently licensed L1 virus-like particles (VLP)–based HPV vaccines target two to nine different HPV types and have demonstrated excellent efficacy against the targeted HPV types and associated disease. Host immune responses to HPV vaccines and infection will be presented and discussed. Briefly, HPV vaccines induce nearly 100% seroconversion to all HPV types included in the vaccine. High titers of high-affinity L1 IgG antibodies, to the HPV types included in the vaccine, are generated after vaccination, and these persist for several years after vaccination at levels considerably higher than those observed in natural infection. Although correlates of vaccine protection have not been identified, neutralizing antibodies are believed to be the main effectors of protection against HPV infection. Vaccine-induced antibodies are detectable not only in serum but also at mucosal sites of infection, such as the cervix and oral cavity. Local HPV-specific antibody levels, although lower, correlate well with circulating levels. Immunogenicity data have shown noninferiority of antibody responses after two doses of HPV vaccine, and the recommended number of vaccine doses was recently reduced from three to two. There is some evidence suggesting that even one dose of the bivalent HPV vaccine may provide similar protection to the recommended dose regimens. Antibody levels following one dose of the bivalent vaccine, although lower, have been shown to be stable over 4 years of follow-up and vaccine recipients remained HPV16/18 seropositive, suggesting that a single dose of the bivalent vaccine may provide durable protection against infection. The robust immunogenicity of the HPV vaccines contrasts with the immune responses observed after natural infection, in which seroconversion has been shown in only a percentage of individuals with incident HPV infection. Furthermore, levels of antibodies induced by vaccination are much higher than the levels observed after infection and appear to be preserved over time. A better understanding of long-term protection of the vaccine as well as a more comprehensive evaluation of correlates of immune protection against HPV infection are still needed.

Liquid Biopsies: Emerging Technologies to Monitor Cancer Progression and Guide Immunotherapy

#SY24-01 High throughput CTC detection for noninvasive cancer monitoring. Shyamala Maheswaran, Mark Kalinich, Irun Bhan, David Miyamoto, Yu Zheng, Xin Hong, Tanya Todorova Kwan, Ravi Kapur, Lecia Sequist, Ryan Sullivan, Aditya Bardia, Richard Lee, David Ting, Mehmet Toner, Daniel A. Haber. Massachusetts General Hospital, Charlestown, MA.

Circulating tumor cells (CTCs) provide a noninvasive source of patient-derived material to repeatedly sample cancer cells during the course of therapy, assessing changes in tumor burden, genetic composition, and transcriptional and RNA-based abnormalities. While some CTCs are viable and tumorigenic ex vivo, the majority of these cells die in the circulation, and some localized cancers with vascular invasion appear to shed CTCs long before the initiation of metastatic lesions. CTC detection therefore also offers an opportunity for early cancer detection. Two major technologic hurdles have limited the broad clinical application of CTC analyses: 1) the capture of these rare cells, estimated at approximately one tumor cell per billion normal blood cells in the circulation; and 2) the stringent criteria required for scoring rare tumor cells against a genomically identical T cell using antibody-dependent staining. To address the primary challenge of capturing rare CTCs admixed with large numbers of normal blood cells, we have previously described a microfluidic device, the CTC-Chip, capable of high throughput depletion of normal hematopoietic cells, producing an output that is highly enriched for CTCs. This negative depletion strategy enables isolation of CTCs across most types of cancer, independent of cell surface markers, and it ensures the highest quality of cellular RNA for downstream molecular analyses. To address the secondary challenge of imaging CTCs using accurate and high throughput platforms, we took advantage of the intact cancer cell-derived transcriptome within microfluidically enriched cancer cells to establish digital PCR platforms, capable of highly sensitive, reliable, and robust detection of CTCs from different cancer types. As proof of principle, we tested the application of such a digital CTC detection in patients with hepatocellular cancer, prostate cancer, and melanoma, demonstrating accurate measurement of tumor burden and associated RNA-based alterations and genotypes, as well as cancer cell–derived predictive markers of drug response. Taken together, the digital scoring of CTCs from microfluidically enriched cancer cell populations provides a high throughput and highly quantitative platform for serial noninvasive monitoring of cancer. For future early cancer detection applications, the use of intact cancer cells in the circulation as a source of molecular markers may help distinguish invasive from indolent cancers.

Mechanisms of Primary and Acquired Resistance to Immunotherapy


We evaluated immune surveillance in pancreatic ductal adenocarcinoma (PDA), which exhibits far fewer missense mutations and potential neoepitopes than classically described carcinogen-induced, highly mutated tumors. In contrast to these immunogenic tumor models from which the theory of elimination-equilibrium-escape was determined, we found no evidence for immunoeediting in a mutant Kras-driven murine model of PDA. These tumors progress the same with or without a functional adaptive immune system, similar to other mouse tumors that have also been shown to grow independently of T cells. Thus, the manifestations of cancer immune surveillance in the KPC model depend on tumor antigenic strength; the lack of neoepitopes is not a consequence of immunoeediting in this model. These findings suggest an alternative biologic mechanism in which tumor outgrowth reflects immune quiescence, which is linked to, and regulated by, poor tumor antigenicity—a conclusion with potential relevance to human cancers with a low burden of mutations and minimal neoepitopes.
Targeting DNA Damage/Repair in the Genome


Prostatic adenocarcinoma (PCA) is the third leading cause of cancer death in U.S. men. Organ-confined PCAs can be effectively managed, but there is no durable treatment for advanced disease. Advanced PCAs is treated through androgen deprivation therapy, often coupled with direct androgen receptor (AR) antagonists, as PCAs is exquisitely dependent on AR activity for cell survival and proliferation. Unfortunately, relapse is common; recurrent disease arises largely due to resurgent AR activity with 2-3 years, and there is no cure for this castration-resistant phase (castration-resistant PCAs, CRPC). Thus, there is a significant need to develop new targets for targeting recurrent AR activity or to develop adjuvant therapies in advanced PCAs. Emerging data from our laboratory and others strongly support the concept that alterations in DNA damage repair (DDR) pathways are more common than previously thought in sporadic PCAs, and that alterations in these pathways may afford new, more effective means of therapeutic intervention. New studies to be discussed will address underlying mechanisms of action with regard to the therapy and identify clinically actionable ramifications of DNA repair dysfunction. Major concepts to be considered include 1) new findings implicating differential functions of p53 somatic mutations on PCAs behavior and response to therapeutic intervention and 2) differential roles of DNA-dependent protein kinase (DNA-PK) in controlling PCAs progression. Findings to be discussed strongly support a model wherein selected DDR pathways can be developed as therapeutic targets to tailor treatment for prostate cancer and improve outcome for advanced disease.

#SY28-09 Novel mechanisms of PARP inhibitor resistance. Alan D. Andreea. Center for DNA Damage and Repair, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA.

Ovarian and breast cancers are the most common forms of gynecologic malignancy. Persistent genome instability is a hallmark of these cancers and likely important for their development and maintenance. The most commonly used targeted therapies are poly (ADP-ribose) polymerase (PARP) inhibitors, which are utilized to exploit the DNA repair deficiency that is associated with these tumors. Due to the high prevalence of these mutations, PARP inhibitors are now in clinical use for these diseases. However, PARP inhibitors also induce cell death in normal cells, and the development of resistance to PARP inhibitors presents an important clinical challenge. In breast and ovarian cancers, the most common forms of resistance to PARP inhibitors are defects in homologous recombination repair (HRR) pathway genes. The most commonly altered HRR genes in serous ovarian cancers (HGSOCs) harbor genetic and epigenetic alterations in homologous recombination repair (HRR) pathway genes.

Systemic Metabolic Dysfunction and Cancer

#SY28-01 Stromal contributions of adipocytes to cancer. Philipp E. Scherer. UT Southwestern Medical Ctr., Dallas, TX.

The past few years have provided substantial evidence for the vital role of the local tumor microenvironment and obese postmenopausal women: a randomized controlled trial. Cancer Res 2012;72(9):2314-26.


Targeting DNA Damage/Repair in the Genome

#SY28-09 Novel mechanisms of PARP inhibitor resistance. Alan D. Andreea. Center for DNA Damage and Repair, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA.

Ovarian and breast cancers are the most common forms of gynecologic malignancy. Persistent genome instability is a hallmark of these cancers and likely important for their development and maintenance. The most commonly used targeted therapies are poly (ADP-ribose) polymerase (PARP) inhibitors, which are utilized to exploit the DNA repair deficiency that is associated with these tumors. Due to the high prevalence of these mutations, PARP inhibitors are now in clinical use for these diseases. However, PARP inhibitors also induce cell death in normal cells, and the development of resistance to PARP inhibitors presents an important clinical challenge. In breast and ovarian cancers, the most common forms of resistance to PARP inhibitors are defects in homologous recombination repair (HRR) pathway genes. The most commonly altered HRR genes in serous ovarian cancers (HGSOCs) harbor genetic and epigenetic alterations in homologous recombination repair (HRR) pathway genes.
are BRCA1 and BRCA2, followed by other Fanconi anemia genes including FANCN/PALB2, FANCO/RAD51, FANCI/BRIP, and FANCA. Loss of HR causes genomic instability, hyperdependence on alternative DNA repair mechanisms, and enhanced sensitivity to PARP analogues, topoisomerase inhibitors, and PARP-inhibitors (PARPi). The synthetic lethal interaction with PARPi is being exploited therapeutically in diverse clinical contexts and most notably in ovarian cancer, where the PARPi olaparib is FDA approved for use in patients with germline BRCA1/2 mutations. PARPi inhibitor resistance has already emerged as a vexing clinical problem for the treatment of BRCA1/2-deficient tumors. The most prevalent mechanism of PARPi resistance is secondary events that cancel the original HRR alteration and restore HRR proficiency. However, PARPi resistance may still develop without restoration of HRR proficiency via disruption of multiple pathways, such as PTIP or CHD4, that leads to replication fork (RF) stabilization. Importantly, this latter mechanism—namely, the restoration of RF-stability—appears to be a highly prevalent mechanism of PARPi inhibitor resistance in vitro and in vivo, particularly in tumor cells with an underlying BRCA2 deficiency. Due to their underlying deficiency in BRCA2 and inability to generate RAD51 nucleofilaments, these tumor cells are unable to restore HRR mechanisms. Instead, these cells acquire PARPi inhibitor resistance by limiting the nucleolytic degradation of their stalled replication forks. We have recently made the surprising observation that BRCA2-deficient tumors can become resistant to PARPi by downregulating the expression of the polycomb repressive complex PRC2, a methyltransferase complex containing EZH2, SUZ12, EED, and RbAp48. Importantly, downregulation of PRC2 results in the reduced recruitment of the nuclease MUS81 to the RF, thereby providing a novel mechanism of RF protection and PARPi resistance. A molecular understanding of PARPi inhibitor resistance mechanisms may allow the generation of a new class of drugs or a repurposing of existing drugs, which may reverse this resistance and extend the use of PARPi inhibitors to more tumor types.

Biology of Metastasis: Mechanisms, Models, and Medicine

#SY31-02 Macrophages—evildoers in cancer. Jeffrey W. Pollard, MRC Centre for Reproductive Health, University of Edinburgh, Edinburgh, United Kingdom.

Tumor evolution to malignancy requires manipulation of its tissue microenvironment to be promiscuous. This is particularly true for the immune microenvironment that is biased away from responding to the tumor to effect control and instead actively promotes progression. The tumor immune response thus downregulates cytotoxic T-cell responses and promotes tissue repair and morphogenic activities of the infiltrating immune cells (1). Thus the environment tends to be dominated by innate immune cells, particularly macrophages and neutrophils, while cytotoxic T cells are often excluded. Our interest has been in macrophages as these, in many different mouse models of cancer, promote tumor progression and enhance metastasis. In fact, macrophages appear to be involved in every step of tumor progression. They stimulate tumor initiation, enhance angiogenesis, promote tumor cell migration and intravasation, increase stem cell viability, suppress immune responses, and, at the metastatic site, promote extravasation and persistent growth. Consequently, macrophage ablation results in inhibition of tumor progression and metastasis (2). These macrophage biologic activities are induced through a dynamic interplay with tumor cells that often involves reciprocal signaling. We have been particularly interested in the involvement of macrophages in enhancing metastasis since it is metastatic disease that is responsible for most cancer deaths. We have demonstrated a chemokine-signaling cascade that results in the recruitment of the progenitor monocytes and their retention in the tissue (3, 4). This results in differentiation of what we termed metastasis-associated macrophages (MAMs) that confer survival signals and growth advantage to metastatic cells. These MAMs in term respond to local signals to upregulate an inflammatory gene signature through the tyrosine kinase transmembrane receptors, vascular endothelial growth factor receptor 1 (VEGFR1 or FLT1) and colony stimulating factor 1 receptor (5). Importantly, downregulation of VEGFR1 can lead to inhibition of metastasis (6). We have demonstrated a chemokine-signaling cascade that results in the recruitment of the progenitor monocytes and their retention in the tissue (3, 4). This results in differentiation of what we termed metastasis-associated macrophages (MAMs) that confer survival signals and growth advantage to metastatic cells. These MAMs in term respond to local signals to upregulate an inflammatory gene signature through the tyrosine kinase transmembrane receptors, vascular endothelial growth factor receptor 1 (VEGFR1 or FLT1) and colony stimulating factor 1 receptor (CSF1R) (5). Furthermore, monocytes appear to be preadapted by the primary tumor to promote metastasis by the generation of preferred sites known as premetastatic niches (6). Thus, understanding monocyte biology, the mechanisms of their recruitment, and differentiation is of central importance to the fundamental appreciation of the role of macrophages in the tumor. My talk will focus on these questions.

References

Dysregulation of MicroRNAs Leads to Target Therapy

#SY32-01 The new genetics and treatment of CLL. Carlo M. Croce. Ohio State University Comprehensive Cancer Center, Columbus, OH

Loss of miR-15/16 on chromosome 13q14 is the most common genetic alteration in CLL. Recently, we discovered that miR-15/16 target BCL2, an antipapotic gene we discovered and named in 1984. Thus, loss of miR-15/16 leads to overexpression of BCL2 (the driver) and causes sensitivity of CLL cells to the antiBcl2 drug venetoclax that is capable of inducing complete remission of CLL in patients and was approved by the FDA in April 2016. The discovery of the involvement of miR-15/16 in CLL also indicated that alterations in genes not encoding proteins can cause cancer. Before our publication of 2002, the dogma was that alterations in protein coding genes, oncogenes, and suppressor genes, cause cancer. We speculate that by looking at other targets of miR-15/16 we might discover other biomarkers that are overexpressed because of miR-15/16 loss, providing novel opportunities to discover treatable targets. Since it seems likely that among the billions of leukemic cells in patients, there are a few that do not respond to the drug—for example, because of mutations in the Bcl2 pocket binding the drug—it is important to develop other drugs capable of killing CLL cells because they have lost miR-15/16. We propose to define whether loss of miR-15/16 may lead to overexpression of specific surface markers that can be targeted by specific monoclonal antibodies. The availability of cancer our probing (PALB2, FANCO/RAD51, FANCJ/BRIP, and FANCA). Loss of HRR causes genomic instability in BRCA1 and BRCA2, followed by other Fanconi anemia genes including FANCN/PALB2, FANCO/RAD51, FANCI/BRIP, and FANCA. Loss of HR causes genomic instability, hyperdependence on alternative DNA repair mechanisms, and enhanced sensitivity to PARP analogues, topoisomerase inhibitors, and PARP-inhibitors (PARPi). The synthetic lethal interaction with PARPi is being exploited therapeutically in diverse clinical contexts and most notably in ovarian cancer, where the PARPi olaparib is FDA approved for use in patients with germline BRCA1/2 mutations. PARPi inhibitor resistance has already emerged as a vexing clinical problem for the treatment of BRCA1/2-deficient tumors. The most prevalent mechanism of PARPi resistance is secondary events that cancel the original HRR alteration and restore HRR proficiency. However, PARPi resistance may still develop without restoration of HRR proficiency via disruption of multiple pathways, such as PTIP or CHD4, that leads to replication fork (RF) stabilization. Importantly, this latter mechanism—namely, the restoration of RF-stability—appears to be a highly prevalent mechanism of PARPi inhibitor resistance in vitro and in vivo, particularly in tumor cells with an underlying BRCA2 deficiency. Due to their underlying deficiency in BRCA2 and inability to generate RAD51 nucleofilaments, these tumor cells are unable to restore HRR mechanisms. Instead, these cells acquire PARPi inhibitor resistance by limiting the nucleolytic degradation of their stalled replication forks. We have recently made the surprising observation that BRCA2-deficient tumors can become resistant to PARPi by downregulating the expression of the polycomb repressive complex PRC2, a methyltransferase complex containing EZH2, SUZ12, EED, and RbAp48. Importantly, downregulation of PRC2 results in the reduced recruitment of the nuclease MUS81 to the RF, thereby providing a novel mechanism of RF protection and PARPi resistance. A molecular understanding of PARPi inhibitor resistance mechanisms may allow the generation of a new class of drugs or a repurposing of existing drugs, which may reverse this resistance and extend the use of PARPi inhibitors to more tumor types.

Immunometabolism in Immunotherapy


The extraordinary proliferative capacity of cancer necessitates specialized metabolic programming on the part of cancer cells. Indeed, this specialized metabolism not only facilitates growth but also creates a hypoxic, acidic, nutrient-deprived tumor microenvironment able to thwart immune destruction. We hypothesized that by targeting tumor metabolism we could not only slow down tumor growth but also “condition” tumors to be more susceptible to immunotherapy. Inherently to the specialized metabolism of tumor cells is a markedly increased requirement for glutamine. To this end we have devised a novel glutamine antagonist that is delivered as an inert prodrug that is converted to the active drug by enzymatic activity, which is enriched in tumors. In syngeneic mouse models of cancer our drug (JHU-083) markedly inhibited tumor growth, led to cure of mice after only 14 days of monotherapy. Importantly, targeting glutamine metabolism led to a decreased extracellular acidification rate (ECAR), decreased hypoxia, and increased nutrient availability. Furthermore, we observed decreases in MDSC and fibrocytes with a concomitant increase in inflammatory (TNF-producing) tumor-associated macrophages (TAMs). Likewise, there was an increase in the CD8:Treg ratio and in some tumors a decrease in PD1 expression on both tumors and infiltrating immune cells. Most importantly, JHU-083 markedly enhanced the efficacy of immunotherapy in the form of checkpoint blockade, adoptive cellular therapy (ACT), and A2ar blockade. Overall, our studies define a novel compound for targeting metabolism as a means of enhancing immunotherapy for cancer.
Surgery in the 21st Century: Convergence of Genes, Imaging, and Robots

#SY33-02 Fuelling T cells and antitumor immunity, Jeffrey C. Rathmell. Vanderbilt University School of Medicine, Nashville, TN

Lymphocyte activation leads to rapid proliferation and differentiation, and we have shown that T cells with Treg regulatory distinct metabolic properties can fuel T cell fates (Th1, Th2, Th17) activate a highly glycolytic program. Regulatory CD4 T cells (Treg), in contrast, utilize a more oxidative metabolism and utilize lipids as a major fuel. These metabolic distinctions may allow new understanding of T-cell function in tumors and approaches to manipulate immunity. We have now examined the metabolism of Treg in detail and found that FoxP3 itself can promote oxidative metabolism characteristic of Treg and that this is critical to maximize Treg suppressive capacity. Treg can be glycolytic and proliferative in vivo. We found, however, that glucose uptake and glycolysis impaired Treg stability. Thus, Treg regulate glycolytic and oxidative metabolism to balance fates (Th1, Th2, Th17) activate a highly glycolytic program. Regulatory CD4 T cells shown that T-cell subsets require distinct metabolic programs. The effector CD4 T-cell University School of Medicine, Nashville, TN is useful when patient material is available but usually cannot be used to develop response to identify the profile and a second independent clinical trial to validate it. This strategy double-negative tumors exhibiting a response rate of 82%.

In conclusion, surgery, the oldest type of cancer therapy, when used in combination with neoadjuvant chemotherapy (NAC) prior to bladder cancer surgery as a model applicable to other cancer types of how this is being used to develop biomarkers of therapy prediction while identifying novel therapeutic targets.

Bladder cancer is the second most common cancer of the genitourinary tract, and primary treatment consists of transurethral resection of the tumor. At diagnosis, 30% of patients present with muscle-invasive disease (MIBC), in which case standard of care consists of removal of the bladder (radical cystectomy; RC) and pelvic lymph node dissection. Despite this extensive surgery, 5-year survival rates do not exceed 60%, due to the presence of occult nodal or visceral micrometastases at the time of surgery. The use of platinum-based NAC has improved outcome in MIBC patients, with the most commonly used regimen being gemcitabine/cisplatin. The benefit of NAC is 14% in the risk of death. Importantly, pathologic complete response (i.e., stage pT0) at RC is associated with an excellent 5-year survival rate of 85%, but only 25% of patients experience this. The resolution of 75% of patients are exposed to chemotherapy as adjuvant therapy in the form of neutropenia (70-80%), neutropenic sepsis (2-14%), mucositis (1-22%), and death (1-3%), without an improved outcome. These patients are also subjected to a delay in RC, which might negatively affect their prognosis.

Given this information, the search for tools that can predict which patients benefit from NAC has been a focus of investigation. The NAC approach has two major advantages for biomarker discovery: 1) robust surrogate outcomes of survival (i.e., stage pT0 at RC) and 2) ability to examine pre- and post-samples collected during standard of care, which greatly facilitates biomarker discovery both practically and scientifically. Gene expression analysis has been used in an attempt to predict response to NAC. An early study has shown that gene expression can be used to build predictive models of the response to a single drug as well as to combination regimens. In this study, baseline gene expression profiles from a panel of 40 bladder cancer cell lines were used to develop chemotherapy response prediction models, which showed highly significant concordance with empirical patient responses. In another study by Takaga and colleagues, gene expression was measured in tumor samples from 27 patients with MIBC prior to neoadjuvant treatment with methotrexate vinblastine, Adriamycin, and cisplatin (MVAC) chemotherapy. The authors identified a set of 50 genes that significantly differed between 14 responders and 13 nonresponders. The same investigators have subsequently validated their predictive signature on 22 additional patients, showing that their gene expression signature could predict MVAC response in 19 out of 22 cases. Ails and colleagues analyzed gene expression in 30 patients with locally advanced or metastatic bladder cancer, identifying 55 genes that proved to be expressed at lower levels in patients with longer survival. Two genes that discriminated patients by their survival—EMMPRIN and survivin—were further investigated by immunostaining, which showed they were significantly associated with overall survival, with double-positive tumors exhibiting a response rate of 27% and double-negative tumors exhibiting a response rate of 69%.

The use of molecular profiles to predict treatment outcomes requires a training dataset to identify the profile and a second independent clinical trial to validate it. This strategy is useful when patient material is available but usually cannot be used to develop response biomarkers to regimens before clinical studies are carried out. A new strategy was proposed by our group that extrapolates in vitro drug response data to make in vivo predictions based on the gene expression profiles that are common to both. This approach, called Orthogonal Exploration (OxPe), uses extra data as a “Rosetta stone” for translating between drug activities in a cancer cell line panel and drug activities in a set of clinical tumors. Using a variety of cancer cell lines and their gene expression profiles and drug sensitivity, Lee et al. used COXEN to predict sensitivity to cisplatin and docetaxel in bladder cancer cell lines and sensitivity to docetaxel and tamoxifen in breast cancer patients, and also demonstrate the utility of COXEN in drug screening. This approach was used to identify a promising new agent in bladder cancer, called C1311, while predicting the response of bladder cancer patients to chemotherapy both in the neoadjuvant and treatment settings.

In conclusion, surgery, the oldest type of cancer therapy, when used in combination with systemic therapy guided by discoveries made with molecular tools, promises to not only advance our understanding of cancer but also lead to a quantum improvement in patient outcome. There is every expectation that, given their effects on metastatic disease, the use of neoadjuvant and adjuvant immunotherapy with surgery will also have a significant impact and can be studied using the same tools described here.

Transformative Small Molecule Therapies

#SY37-02 Lindig-directed degradation of GSP1 by a novel cereblon modulator drives potent anti-tumor effects, Mary Matysikela,1 Gang Lu,2 Takumi Ito,3 Barbra Pagarian,1 Chin-Chu Lu,2 Karen Miller,2 Wei Fang,1 Nai-Yu Wang,1 Derek Nguyen,1 Jack Houston,1 Gilles Carmel,1 Tam Tran,1 Mariko Riley,1 Lyn‘Al Nosaka,1 Gabriel Pagarigan,1 Chin-Chu Lu,1 Karen Miller,1 Wei Fang,1 Nai-Yu Wang,1 Derek Nguyen,1 Jack Houston,1 Gilles Carmel,1 Tam Tran,1 Mariko Riley,1 Lyn‘Al Nosaka,1 Gabriel Pagarigan,1 Chin-Chu Lu,1 Karen Miller,1 Wei Fang,1 Nai-Yu Wang,1 Derek Nguyen,1 Jack Houston,1 Gilles Carmel,1 Tam Tran,1 Mariko Riley,1 Lyn‘Al Nosaka,1 Gabriel
the surface of cereblon. The principal molecular feature on GSPT1 that binds to cereblon is a beta-hairpin incorporating a glycine residue that docks against CC-885. Surprisingly, we found evidence that a similar molecular feature mediates Ikaros recruitment, even though there is no common structural fold or sequence homology other than the key glycine residue. We thereby define the common molecular feature, or degron, shared by the known cereblon neomorphic substrates. We further describe a novel therapeutic target, GSPT1, with promise in cancer such as AML. These results further show that cereblon-mediated protein degradation can be directed against new proteins and that this mechanism enables the targeting of multiple protein classes that may be considered undruggable with conventional approaches.

Convergence, Computation, and Cancer

#PL04-04 Novel vapproaches for personalizing treatments: From nutrition to cancer. Eran Segal. Weizman Institute of Science, Rehovot, Israel, Israel.

Elevated blood glucose levels are rapidly increasing in the general population, resulting in a sharp incline in the prevalence of prediabetes and impaired glucose tolerance and eventual development of type 2 diabetes mellitus. Dietary intake is considered a central determinant of glucose levels, with high postmeal glucose levels affecting weight gain, obesity, hunger, and energy dips and being associated with increased risk of cardiovascular disease, cancer, and overall mortality. However, despite their importance, existing dietary methods for controlling postmeal glucose levels have limited efficacy. By continuously monitoring week-long glucose levels in over 1,000 people, we found high variability in the response of different people to identical meals, suggesting that generic population-wide dietary recommendations have limited utility and are ineffective in achieving proper glycemic control. We devised a machine-learning algorithm that integrates blood parameters, dietary habits, anthropometrics, physical activity, and gut microbiota measured in this cohort and showed that it accurately predicts personalized postmeal glucose responses to real-life meals. Moreover, a blinded nation-wide controlled dietary intervention based on this algorithm resulted in significantly lower postprandial responses in a cohort of prediabetics and consistent alterations to gut microbiota configuration. These results suggest that personalized diets may successfully modify elevated postprandial glucose levels and its metabolic consequences. As another example of personalized medicine approaches that we are developing, I present our work on functional profiling of a library of over 10,000 variants that we generated for the tumor suppressor p53, the most frequently mutated gene in human cancers. Remarkably, the mutational effects observed in large-scale in-vitro assays with this library correspond to p53 mutation recurrence in patients and provide many novel insights on adverse and benign variants, protein structure, and evolutionary conservation. Apart from gaining comprehensive insights into the effects of the p53 “mutome,” our results may lead to better understanding of patients’ response to treatment based on their p53 sequence, potentially contributing to the development of novel patient-specific therapeutics. Finally, I also present our studies of the mechanisms driving recurrent postdieting obesity in which we identified an intestinal microbiome signature that persists after successful dieting of obese mice. This signature was associated with faster weight regain and metabolic aberrations upon re-exposure to obesity-promoting conditions and transmits the accelerated weight regain phenotype upon interanimal transfer. Notably, a microbiome-based machine-learning algorithm enabled personalized prediction of the extent of postdieting weight regain. We further find that the microbiome contributes to diminished postdieting flavonoid levels and reduced energy expenditure and demonstrate that flavonoid-based ‘probiotic’ intervention ameliorates excessive secondary weight gain. These results thus highlight a possible microbiome contribution to accelerated postdieting weight regain and suggest that microbiome-targeting approaches may help to diagnose and treat this common disorder.

Plenary Sessions

Opening Plenary: The Road to Cancer Cures—Discover, Predict, Prevent, and Treat

#PL01-03 Earlier detection as a key to lower cancer death rates. Bert Vogelstein. Johns Hopkins Kimmel Comp. Cancer Ctr., Baltimore, MD.

Two disciplines within cancer research—secondary prevention and cancer therapeutics—have historically been viewed as distinct. I will argue that these two disciplines are intertwined and that their joint pursuit is the optimal way to reduce cancer deaths. At present, the preferred way to treat cancers, in fact the only way that generally cures patients, is surgery. It is therefore widely recognized that the detection of cancers prior to the onset of metastasis is vital to reducing cancer deaths. What is less widely recognized is that the detection of metastatic cancers can also be life-saving—as long as this detection occurs prior to the formation of bulky metastatic disease. Therapies that only modestly extend life when administered to patients with measurable metastases can be curative when administered to patients with metastatic disease that is present but undetectable by imaging. Such therapies include conventional cytotoxic drugs as well as genome-targeted agents and immunotherapeutics. A new paradigm for designing clinical trials to test such agents in a cancer-specific setting will be described. Genome-based diagnostic approaches for detecting cancers earlier will be discussed, as these are integral to the design of the next generation of adjuvant trials. In aggregate, these issues support the importance of modulating the focus of cancer research and treatment from patients with advanced disease towards patients with less-advanced disease. They also highlight the importance of developing novel diagnostic approaches that will allow the detection of smaller tumors.
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